Symbiosis, 9 (1990) 3–17
Balaban Publishers, Philadelphia/Rehovot

Transports in roots*

CLAUDE GRIGNON

Biochimie et Physiologie Végétales ENSA-M/INRA
CNRS URA 573.34060 Montpellier cedex 1, France

In terrestrial ecosystems, the mineral elements essential to life enter the living world by crossing the plasma membrane of cells at the periphery of plant roots, in some cases after passing through mycorrhizal fungi. This absorption step depends on the functioning of membrane proteins. These proteins constitute the first of the two sets of membrane transport systems which cooperate to provide the shoots with water and nutrients essential for growth. Most of the absorbed solutes diffuse through the root tissues, and reach the stele, where they are secreted into the xylem by a second set of transporters. Only a few of the membrane transport systems are known at the molecular level. Most of the knowledge has been obtained with simple systems, convenient for genetic approaches (yeasts), or adapted to "molecular" electrophysiology (naturally isolated cells, e.g. yeasts, fungi, and guard cells of higher plants). However, roots are complex organs made of cells with different physiological properties, connected by plasmodesmata. Thus, they are not as adapted to molecular approaches as is, for instance, the guard cell, which is today the most detailed model of regulation and integration of transports at the cell level (105). Indeed, there is still a large gap between our knowledge at two levels. Physiological studies indicate that root transports are highly integrated in the whole plant, so as to respond to the specific demands of shoot growth. On the other hand, biochemical features of the mechanisms and controls of membrane transports are just beginning to be documented at the cell level.

This review begins with a presentation of the transports in roots as an integrated activity, regulated by (unknown) signals emitted by the plant in response to its needs in specific elements. Thereafter, the state of our knowledge on the nature of the root

*Invited paper

transport systems will be presented through the example of the "master" system responsible for the energization of the membrane, which uses ATP to drive a proton extrusion, often electrically compensated by K^+ absorption. It is important to realize that this presentation combines results obtained with roots, and data extrapolated from other models.

Integration of Root Transports in the Whole Plant

There is physiological evidence of two kinds of control mechanisms acting on the root transport systems. The first one corresponds to the responses to signals which come from the root itself, or from its environment. For instance, the capacity of roots for NO_3^- uptake is low in plants grown on reduced N, and it is progressively revealed after transfer of the plants on solutions containing NO_3^-. The presence of this ion in the medium, or in the cytoplasm (8), seems to be the primary local signal which commands the augmentation of the NO_3^- transport capacity of the roots, perhaps through induction of new transporters (43). When the internal contents in K^+ (61, 72, 83), SO_4^{2-} (71, 131), $H_2PO_4^-$ (82), Cl^- and NO_3^- (40–41, 130) increase in roots, the influx of the corresponding ion decreases. This inhibition of the influx seems to result from a direct retroinhibition of the transporters, because it occurs without any lag, and the kinetic relation between the influx and the internal concentration (62) resembles the classical kinetics of allosteric negative cooperativity (61, 71).

The second kind of regulation which is at work in roots corresponds to the transmission of signals from the shoots to the roots. Briefly, it is clear that the roots receive some information about the needs of the shoots, and that they adjust the transports to answer this demand. This demand depends on the growth rate of the shoots, and on deficiencies in specific elements. For instance, in *Lolium perenne*, the internal content in nitrogen was observed to remain almost constant in shoots, in spite of a large variation of the nitrogen concentration in the medium (36), or of growth rate and shoot/root ratio (35). This indicates that the transports in roots have been controlled, so as to be exactly proportional to the shoot growth. Other examples of the effect of the shoot growth demand on root transports may be found in experiments with barley. Controlling the growth rate by using various photoperiods (102), or surgically modifying the root/shoot ratio (73), made it possible to show that the K^+ influx into the cell, and the secretion into the xylem, were dependent on the shoot demand.

Other evidence for the control exerted by the shoots upon root transports has been obtained with mineral starvation experiments. When grown in the absence of phosphate in the medium, barley and tomato plants exhibited a reduction of their growth rate after four to six days (33). The plants were periodically sampled, and challenged with radioactive phosphate, in order to measure the phosphate influx. This

revealed that the capacity to absorb phosphate had been augmented well before the growth rate had been inhibited. Similar results have been obtained with SO_4^{2-} (34). These kinds of responses are thought to involve changes in the level of expression of the genes of the transporters.

The deficiency signal responsible for the induction of high rate uptake systems in roots is emitted by shoots. This has been shown for K^+, Cl^-, and $H_2PO_4^-$ (45). A good illustration of this point is offered by the experiments of Cogliatti and Clarkson (38), with potato grown in the absence of phosphate, except in the medium around one root isolated from the main root system. This root could not transport enough phosphate to avoid starvation of the shoots, but it was not deficient in P. However, the high rate influx characteristic of starved plants was present. Thus, in this root, the transports have been stimulated by the starvation of shoots, in spite of the high nutritional status of the root. The conclusion is that the local retroinhibition mechanisms have been overridden by other regulating mechanisms, controlled by the shoots. Another example of the priority of other controls upon local satiety signals is given by the effect of Na^+ on K^+ transport in barley roots, in which the classical retroinhibition of K^+ uptake by internal K^+ is suppressed when Na^+ is present in the medium (29).

The nature of the signals emitted by the shoots is not identified. The response of the root is specific: if the needed element is, for instance, nitrogen, only the nitrate transport is increased, and not the transports of phosphate, sulfate or chloride (81). It is possible that several different membrane systems cooperating in the uptake of the same element are elicited by the deficiency in this element. This situation is documented for Fe (118). It is unlikely that the signals are ordinary plant hormones going down in the phloem, one for each element. The information must be sent by more specific signals. Recent advances in the field of long distance transports support an interesting alternative hypothesis. It is well established today that there is a continuous cycling of elements between roots and shoots (74, 136). In barley with a split-root system, tracers of N or K supplied to one part of the root system were transported to the shoots, and then, back to the other part of the root system (39). When a root of this part, which was not in the medium containing the tracer, was excised, and allowed to exude, the tracer was observed to be in the xylemic sap: it was cycling. The important point is that the specific activity in the sap returning to the shoots was *higher* than in the root tissues. Thus, there is a cycling pool, which is not at isotopic equilibrium with the root. This means that, at the root level, the cycling pool is relatively isolated, and thus its specific composition possibly conveys informations. In the cycling pool, the composition of the phloem sap returning to the roots represents the difference between the amount of nutrients reaching the shoots by the xylem, and the amount of nutrients actually used by the shoots. For instance, when the nitrogen input is sufficient, one may imagine that the phloem sap is enriched in

specific aminoacids, known to be inhibitors of nitrate uptake (17, 44).

General Organization of the Transports on the Plasma Membrane of Plants

The transporters in the plasma membrane have two functions. The first one is selective uptake of nutrients needed for the nutrition of the plant. The second function is the maintenance of physical and chemical conditions in the cytoplasm, compatible with normal functioning of the metabolism. For instance, a continuous extrusion of H^+ and Ca^{2+} is always necessary to maintain the activities of these ions at a very low level in the cytoplasm (51–52). In some cases, Na^+ extrusion is also necessary.

Many transport systems on the plasma membrane of the outer cortical cells in roots are known from *in vivo*, (electro)physiological studies. Measurements of ion fluxes, ions concentrations in cells, membrane potential and resistance, using a large variety of more or less specific pharmacological effectors of metabolism and transports, have resulted in a coherent model of the transports. The transport systems on the plasma membrane are organized according to the general principle of the chemiosmotic theory. Three subsets are to be distinguished.

(i) In roots, as in the other organs of higher plants, and in yeast and fungi, metabolic energy (ATP) is directly used by a membrane (H^+)ATPase to extrude protons (125, 128–129). While doing this, the ATPase hyperpolarizes the membrane. Thus, the "primary" active transport system converts chemical energy of ATP into two other forms, namely a pH gradient, and an electrical gradient. Such active transport systems, which directly use metabolic energy are called "primary" systems. Another transport ATPase has been demonstrated and characterized in the plasma membrane of root cells, both *in vivo* and *in vitro*. It extrudes Ca^{2+} ions, by exchanging them for H^+ ions (57, 107, 115).

(ii) The pH gradient and the electrical gradient are used for the energetic coupling of the so called "secondary" systems, which transport various cations, anions, aminoacids and sugars, together with H^+ (103, 110). These H^+ cotransporters are responsible for active transports, since they provide the transported solutes with a supplement of free energy, transferred from that lost by the protons while entering the cell down their electrochemical gradient (which is maintained by the primary system).

(iii) The third set of transport systems contains the ion channels (69). They do not provide the ions with energy: they simply allow them to cross the permeability barrier, by opening specific gates for them. They are secondary, passive systems.

The Master System for the Energization of the Plasma Membrane

In the eighties, successful experiments in preparing tight vesicles from purified plant plasma membrane of higher plants have authorized approaches of the transport systems at the biochemical level (42, 56, 86, 106). However, only the master system (the

plasma membrane (H^+)ATPase, which functions as an electrogenic proton pump) has been fully characterized at the molecular level. The root enzyme has been reconstituted in artificial membrane vesicles (75, 99, 119, 127, 138). The plasma membrane (H^+)ATPase belongs to the class of E1:E2 transport ATPases (26, 124), which are characterized by a phosphorylation step in their catalytic cycle (20–21, 120). This class includes the metal transporting ATPases of pro- and eucaryotes. The (H^+)ATPase hydrolyses Mg-ATP, with a K_M of *ca* 0.25 mM. This means that it is generally not limited by its substrate in normal conditions, since the ATP concentration in the cell is normally one order of magnitude higher than the K_M. The activity of the (H^+)ATPase is very sensitive to the pH at the cytosolic face of the membrane. This is partly because its pH optimum is one pH unit lower than the normal cytosolic pH. As judged from the electrogenic H^+ extrusion observable *in vivo*, it does not seem to be sensitive to the external pH. This is probably due to the fact that only a very small part of the protein protrudes at the external face of the membrane (see below). The (H^+)ATPase is made of one peptidic chain, with a relative molecular weight of *ca.* 100 000 dalton (3, 75, 127). But there are indications that the (H^+)ATPase of root plasmalemma may form a trimer (from cross linking study) (3), and radiation inactivation data (22–23) suggest that the native functional form in the membrane is a dimer.

The genes of the plasma membrane (H^+)ATPase have been cloned in yeasts (55, 126), in a fungus (1), in a protozoan (91), and in higher plants (9, 68, 93, 100). They are present as members of small multigene family. Some details of the structure of the enzyme have been deduced from sequence analysis and mutational analysis (in yeast). Ten transmembrane helices as predicted, with the most part of the remaining peptidic chain located at the cytosolic face. Six domains present strong homologies with other proton or metal transporting ATPases from bacteria and animals. Three of them are involved in the binding of Mg-ATP. A fourth domain may be responsible for the kinase activity, which results in the phosphorylation of the aspartic acid, in the fifth conserved domain. Finally, the last conserved domain probably has a phosphatase activity, responsible for the dephosphorylation of the phosphorylaspartate.

Potassium Transport

The active H^+ extrusion must be electrically compensated. In physiological situations, K^+ absorption is one of the major charge compensating transports. It is easy to devise experimental conditions leading to observation of a net H^+/K^+ exchange in roots with a 1/1 stoichiometry (7, 135). However, the mechanisms underlying this coupling of H^+ and K^+ transports are multiple, and not fully characterized.

Potassium channels. None of the secondary systems has been isolated. However, in the case of ion channels, new methods give access to characteristic electrical signatures of

single molecules. The discovery, and the study of the ion channels in plants has been due to recent methodological advances in electrophysiology. The patch-clamp techniques (70) make it possible to identify the characteristic electrical signature of the channels at two levels. Firstly, it is possible to isolate a single channel at the tip of the microelectrode, and thus to record the microscopic current across this channel. This is a true molecular approach, in spite of the fact that the molecule is not physically isolated in the biochemical sense. Secondly, it is possible to observe the macroscopic electrical current resulting from the flow of ions across the whole set of channels in a cell. It is possible to select the channels selective for the studied ion by using specific effectors, or controlling the ionic composition of the medium.

Macroscopic currents, characteristic of two of the classical K^+ channels, have been observed in protoplasts from root cells (77). These channels are controlled by the membrane potential. Channels of the first kind open when the membrane is depolarized. They conduct an outwardly directed K^+ current, which opposes the initial depolarization. These channels may be involved in the regulation of the membrane potential. Potassium channels of the second kind are activated in case of membrane hyperpolarization: when the internal potential becomes more negative than about minus one hundred millivolts, the opening of these channels authorizes the passive entry of potassium into the cell. These channels seems to be responsible for the absorption of K^+ when this ion is present at high concentration in the soil (78).

The active uptake of K^+. When the concentration of K^+ is low in the medium (in the micromolar range), it is possible to measure a net uptake of K^+ by plant cells. From the measured membrane potential, and reasonable estimates of the K^+ concentration in the cytoplasm, one may calculate that this absorption corresponds to an increase in the electrochemical potential of K^+ (in contrast to the absorption through K^+ channels, from solution with high K^+ concentrations, which corresponds to a decrease in the free energy of the ion, and thus is passive). Such observations (16, 30–32, 37, 97, 135, 137) reveal that the plasma membrane of root cells possesses active transport systems with a high affinity for K^+ (half saturated at less than 10μM K^+)(97). For some time, it has been believed that the (H^+)ATPase itself was this system: it was thought that the hydrolysis of ATP by this system was directly coupled to K^+ transport, as described in *E. coli* (48) and in yeast (54, 139–140).

In plants, the evidence for this is essentially physiological. As indicated above, the extrusion of H^+ is coupled with the uptake of K^+ with a 1 to 1 stoichiometry. Also, the (H^+)ATPase may be activated by K^+ *in vitro* (96). But the hypothesis of a direct involvement of the (H^+)ATPase in K^+ transport has not been confirmed by the biochemical studies with purified ATPase reinserted in liposomes. The enzyme may be indirectly stimulated by K^+, when this cation is responsible for the charge compensation of the H^+ transport (60). In some cases, it also may be stimulated by

K^+ via an aspecific ionic strength effect (58). Finally, it is directly and selectively stimulated by K^+, probably upon the binding of this cation on a specific site. This binding induces changes in the V_{max} (60), in the vanadate inhibition constant (59), in the turn over of the phosphoenzyme (18), and also a shift in steady state concentrations of the phosphorylated intermediates (19). However, it does not seem that this stimulation is the consequence of the participation of K^+ in a direct H^+/K^+ exchange, because the K^+ site seems to be located on the cytosolic side of the membrane, and its apparent affinity for K^+ is three orders of magnitude lower than that estimated *in vivo* for the K^+ transport system (97). Furthermore, the purified (H^+)ATPase is capable of ATP hydrolysis, and H^+ transport, in the absence of K^+ (65, 138).

Another system for active uptake of K^+ has ben suggested in yeast (27), and described in detail in *Neurospora crassa*, through its electrical properites (116). It is a $1\,H^+/1\,K^+$ *symport*. Thus, it is very sensitive to the changes of the membrane potential which result from variations in the electrogenic proton pump activity (14), and conversely, the pump is itself stimulated by the membrane depolarization which results from the activation of the H^+/K^+ symport, and by cytosol acidification (14, 16). The coupled operations of the (H^+)ATPase and the H^+/K^+ symport results in an equimolecular H^+/K^+ net exchange (50% of the extruded H^+ reenter the cell together with K^+). The H^+/K^+ symport of *Neurospora* is currently known only through its electrical signature. As emphasized by Hedrich and Schroeder (69), there are striking similarities between the latter, and that of K^+ channels of *Vicia faba* guard cells, which suggest that K^+ uptake in *Neurospora* could also be explained by cooperation of the electrogenic (H^+)ATPase and K^+ channels. However, it is not clear how such a system could drive *active* uptake of K^+.

In higher plants, the H^+/K^+ symport, is considered as an obvious alternative to the primary H^+/K^+ exchange to explain the active uptake of K^+ at a low external concentration. But the evidence for it remains meagre. Addition of K^+ to the medium at micromolar concentrations results in a strong membrane depolarization (79, 97), which suggests that a system transfering a positive net charge into the cells is activated. Also, in contrast to what is observed at the macroscopic level, an unbalanced H^+/K^+ stoichiometry is observed at the microscopic level with surface ion-selective microelectrodes. Other indications of the absence of a direct link between the proton extrusion and the K^+ active uptake have been obtained by using inhibitors of the (H^+)ATPase. In roots (135) as in some bacteria (4, 112), it is possible to block the net uptake of K^+ in two different ways. Inhibitors of ATP production totally suppress the H^+ extrusion and the high affinity K^+ net transport, as well as the K^+ tracer influx. However, if the H^+ extrusion is suppressed by inhibitors specific for the (H^+)ATPase (not affecting the ATP level in the cell), the K^+ tracer influx is not perturbed. Only the net K^+ transport is inhibited in this condition. Thus, this maintaining of the K^+ isotopic exchange through the high affinity transporter in the

absence of active (H^+)ATPase does not support the hypothesis of a direct responsibility of this enzyme in K^+ transport. These data suggest that the high affinity K^+ transport is energized by the proton electromotive force, and mediated by a system kinetically controlled by ATP. An ATP-dependent K^+ channel, in close relation to the (H^+)ATPase has been described in a mutant of yeast (104).

Regulation of the H^+ Pump of the Plasma Membrane

Physiological data indicate that the *in vivo* activity of the proton pump is under the control of many effectors. A first set of effectors corresponds to the deviation of several important parameters from their normal values: membrane potential (14–16), cytosolic pH (10, 25, 66), cytosolic Ca^{2+} (84), and turgor pressure (111). Another basic effector is K^+ in the external solution, which is necessary to activate the proton extrusion. But probably, external K^+ activates the proton pump simply by depolarizing the membrane. A second set of effectors corresponds to several of the plant growth regulators (5, 95, 117), and a third set, to bacterial or fungal toxins (2, 11, 87), and elicitors (101). Also, we know that various stresses inhibit the proton pump, for instance, mechanical or thermal shocks (28, 64). These treatments have something in common: they depolarize the membrane, and this results in an acceleration of the Ca^{2+} influx (113, 143), perhaps through voltage-gated Ca^{2+} channels (63, 142, 143). Thus, the increase in cytosolic Ca^{2+} may be the true effector of the pump. Finally, there are indirect indications than an essential cystein (141) near the phosphorylated residue in the active site (76) has to be maintained in a reduced state, perhaps by a membrane associated NADH reductase (132; see also 13).

Since the pH optimum of the ATPase is one pH unit lower than the normal cytoplasmic pH, any acidification of the cytoplasm results in acceleration of the proton pumping, and in hyperpolarization of the membrane. For instance, both auxin and the fungal toxin fusicoccin rapidly induce a pH decrease in the cytoplasm, observable with selective microelectrodes (10, 24–25, 53). The simultaneous activation of the (H^+)ATPase which is then observed may result from this shift of the cytosolic pH towards the pH optimum of the enzyme. However, longer term determinations of cytosolic pH with permeant probe and/or ^{31}P–NMR do not confirm the acidification (133), or indicate an alkalinization (80, 88–89, 109). One explanation of this discrepancy could be that, after the extrusion of H^+ has opposed the initial pH shift, there is an over correction leading to an alkalinization of the cytoplasm. But the fact that the electrogenic H^+ extrusion persists in these conditions indicates that the initial, transient pH shift, had activated the proton pump by another effect, more permanent than its direct kinetic effect. In *Neurospora* (84), Ca^{2+} injected into the cell by ionophoresis caused changes in membrane hyperpolarization and pump current, indicating a stimulation of the (H^+)ATPase at moderately increased Ca^{2+} level, and

an inhibition at higher levels. The stimulatory effect was thought to be mediated by cytosolic factors, since it was not observed on isolated plasma membrane. These regulations by pH and Ca^{2+} may correspond to phosphorylations of the (H^+)ATPase. We know that the activity of the plant root (H^+)ATPase may be modulated *in vitro* by phosphorylations under the control of protein kinases (different from the self-phosphorylation which is involved in the catalytic cycle). Biochemical data indicate that the protein kinases which modulate the plant plasma membrane (H^+)ATPase are activated by three of the classical secondary messengers: cytosolic pH and cytosolic Ca^{2+} (which are regulated in a coordinated manner: 50, 51), and perhaps degradation products of phosphatidylinositol. However, protein kinases *specific* for the (H^+) ATPase have not yet been identified.

There is evidence for the operation of different protein kinases. In plasma membrane vesicles from oat roots (122), the ATPase is phosphorylated on several serine and threonine residues, by a membrane associated protein kinase, the activity of which is pH dependent, and Ca^{2+} dependent.

Calcium/calmoduline dependent phosphorylation of the membrane proteins was also observed in microsomes from corn roots (142). In this case, the ATPase was inhibited under phosphorylation, and reactivated by dephosphorylation. In red beet (12), the plasma membrane ATPase is stimulated by syringomycin, a peptidic bacterial toxin, which promotes the phosphorylation of the enzyme, in the presence of Ca^{2+}. If this kinase activity is eliminated from the membrane with surfactants, the ATPase no longer responds to syringomycin. Finally, there are some indications that a third kind of protein kinase is present in plants, and acts on the ATPase. Protein kinase C is activated by diacyl glycerol (DG), a product of the degradation of phosphatidylinositol 4,5-biphosphate (PIP_2) by phospholipase C (95), and by Ca^{2+}. Phospholipase C activity also produces inositol 1,4,5-triphosphate (IP_3), a mediator which releases of Ca^{2+} from internal stores (45, 108, 114, 123) (except, perhaps, from the endoplasmic reticulum (85)).

Phospholipase C activity has been found associated with the plasma membrane in several plants (92, 134). In contrast to the cytosolic form (90), the membrane associated form is stimulated by micromolar Ca^{2+}. Protein kinase C-like activity is known in plants (47, 67, 98, 121), but the enzyme has not been isolated. However, the various events which follow the reception of auxin on the membrane strongly suggest that the resulting activation of the (H^+)ATPase is mediated by protein kinase C: (i) auxin stimulates the turn over of inositol phospholipids (49), increasing the level of IP_3, and probably, producing DG, the first activator of protein kinase C; (ii) IP_3 induces a leak of Ca^{2+} (the other activator of protein kinase C) from the internal stores (see above), which may be the cause of the increase in cytosolic Ca^{2+} observed *in vivo* (50); (iii) the electrogenic H^+-pumping is stimulated (5–6, 50).

In summary, the (H^+)ATPase activity may be modulated by various growth

regulators, physical stress, and chemicals signalling pathogene attack. The action of these effectors is thought to be mediated by changes in membrane polarization, cytosolic Ca^{2+} and pH, and phosphatidylinositol degradation products, which activate various protein kinases. The final step of this transduction process is phosphorylation(s) of the (H^+)ATPase. However, both stimulating and inhibiting phosphorylations have been observed, and virtually nothing is known about the mechanisms which ensure the specificity of these regulations.

Conclusion

The picture which is emerging, is that of a continuous exchange of information between shoots and roots, resulting in an integration of root transports in the functioning of the whole plant. At the physiological level, we are beginning to perceive the global organization of this system, without detail. On the contrary, at the biochemical level, we are beginning to accumulate fine details. However, the available biochemical data may not yet be integrated in a coherent model of the regulation of the transports of the plasma membrane at the cell physiology level. The study of the mechanisms which adjust the ion transport capacity of the roots to the growth demand in mineral elements, will make necessary that both the proteic equipment of the membrane, and the corresponding genes to be identified. For this reason, the obtention of molecular probes of "secondary" transport systems and their genes is of primary importance.

REFERENCES

(1) Addison, R. 1986. *J. Biol. Chem.* **261:** 14896–14901
(2) Aducci, P., Ballio, A., Blein, J.P., Fullane, M.R., Rossignol, M. and Scalla, R. 1988. *Proc. Natl. Acad. Sci. USA.* **85:** 7849–7851.
(3) Anton, G.E., Spanswick, R.M. 1986. *Plant Physiol.* **81:** 1080–1085.
(4) Bakker, E.P., Harold, F.M. 1980. *J. Biol. Chem.* **255:** 433–440.
(5) Barbier–Brygoo, H., Ephritikhine, G., Klämbt, D., Ghislain, M., and Guern, J. *Proc. Natl. Acad. Sci. USA.* **86:** 891–895.
(6) Bates, G.W. and Goldsmith, M.H.M. 1983 *Planta.* **159:** 231–237.
(7) Behl, R. and Raschke, K. 1987. *Planta.* **172:** 531–538.
(8) Behl, R., Tischner, R., and Raschke, K. 1988. *Planta.* **176:** 235–240.
(9) Bennett, A.B., Ewing, N.N., Wimmers, L.E., and Meyer, D.J. 1989. In Plant Membrane Transport: The current position. J. Dainty et al. eds, Elsevier, Amsterdam. pp. 449–454.
(10) Bertl, A. and Felle, H. 1985. *J. Exp. Bot.* **36:** 1142–1149.

(11) Bidwai, A.P. and Takemoto, J.Y. 1987. *Proc. Natl. Acad. Sci. USA.* **84:** 6755–6759.
(12) Bidwai, A.P., Zhang, L., Bachmann, R.C., and Takemoto, J.Y. 1987. *Plant Physiol.* **83:** 39–43.
(13) Bienfait, F. and Lüttge, U. 1988. *Plant Physiol. Biochem.* **26:** 665–671.
(14) Blatt, M.R., Rodriguez–Navarro, A., and Slayman, C.L. 1987. *J. Memb. Biol.* **98:** 169–189.
(15) Blatt, M.R. 1987. *J. Memb. Biol.* **98:** 257–274.
(16) Blatt, M.R. and Slayman, C.L. 1987. *Proc. Natl. Acad. Sci. USA.* **84:** 2737–2742.
(17) Breteler, H. and Arnozis, P.A. 1985. *Phytochem.* **24:** 653–658.
(18) Briskin, D.P. 1986. *Physiol. Plant.* **68:** 159–163.
(19) Briskin, D.P. 1988. *Plant Physiol.* **88:** 84–91.
(20) Briskin, D.P. and Leonard, R.T. 1982. *Proc. Natl. Acad. Sci. USA.* **79:** 6922–6926.
(21) Briskin, D.P. and Poole, R.J. 1983. *Plant Physiol.* **72:** 1133–1135.
(22) Briskin, D.P. and Reynolds–Niesman, I. 1989. *Plant Physiol.* **90:** 394–397.
(23) Briskin, D.P., Thornley, W.R., and Rotti–Rotti, J.L. 1985. *Plant Physiol.* **78:** 642–644.
(24) Brummer, P., Felle, H., and Parish, R.W. 1984. *FEBS Lett.* **174:** 223–227.
(25) Brummer, B., Bertl, A., Potrykus, I., and Felle, H. 1985. *FEBS Lett.* **189:** 109–114.
(26) Bowman, B.J. and Bowman, E.J. 1986. *J. Memb. Biol.* **94:** 83–97.
(27) Boxman, A.W., Dobbelmann, J., and Borst–Pauwels G.W.F.H. 1984. *Biochim. Biophys. Acta.* **772:** 51–57.
(28) Chastain, C.J. and Hanson, J.B. 1982. *Plant Sci. Lett.* **24:** 97–104.
(29) Cheeseman, J.M. 1985. *Physiol. Plant.* **64:** 243–246.
(30) Cheeseman, J.M. and Hanson, J.B. 1979. *Plant Physiol.* **63:** 1–4.
(31) Cheeseman, J.M. and Hanson, J.B. 1979. *Plant Physiol.* **64:** 842–845.
(32) Cheeseman, J.M. and Hanson, J.B. 1980. *Plant Sci. Lett.* **18:** 81–84.
(33) Clarkson, D.T. and Scattergood, C. 1982. *J. Exp. Bot.* **33:** 865–875.
(34) Clarkson, D.T., Smith, F.W., and Vanden Berg, P. 1983. *J. Exp. Bot.* **34:** 1463–1483.
(35) Clarkson, D.T., Hopper, M.J., and Jones L.H.P. 1986. *Plant Cell. Environ.* **9:** 535–545.
(36) Clement, C.R., Hooper, M.J., and Jones, L.H.P. 1978. *J. Exp. Bot.* **29:** 453–464.
(37) Clint, G.M. and Blatt, M.R. 1984. *Planta.* **178:** 495–508.
(38) Cogliatti, D.H. and Clarkson, D.T. 1983. *Physiol. Plant.* **58:** 287–294.
(39) Cooper, H.D. and Clarkson, D.T. 1989. *J. Exp. Bot.* **40:** 753–762.
(40) Cram, W.J. 1973. *J. Exp. Bot.* **24:** 328–341.
(41) Cram, W.J. 1980. *Aust. J. Plant Physiol.* **7:** 41–49.
(42) De Michelis, M.I. and Spanswick, R.M. 1986. *Plant Physiol.* **91:** 542–547.
(43) Dhugga, K.S., Waines, J.G., and Leonard, R.T. 1988. *Plant Physiol.* **87:** 120–125.

(44) Doddema, H. and Otten, H. 1979. *Physiol. Plant.* **45:** 339–346.
(45) Drew, M.C. and Saker, L.R. 1984. *Planta.* **160:** 500–507.
(46) Drøbak, B.K. and Ferguson, I.B. 1985. *Biochem. Biophys. Res. Commun.* **130:** 1241–1246.
(47) Elliot, D.C. and Skinner, J.D. 1986. *Phytochem.* **25:** 39–44.
(48) Epstein, W., Wieczorek, L., Soeners, A., and Karlheinz, A. 1984. *Biochem. Soc. Trans.* **12:** 235–236.
(49) Ettlinger, C. and Lehle, L. 1988. *Nature.* **331:** 176–178.
(50) Felle, H. 1988. *Planta.* **174:** 495–499.
(51) Felle, H. 1988. *Planta.* **176:** 248–255.
(52) Felle, H. 1989. *Plant Physiol.* **91:** 1239–1241.
(53) Felle, H., Brummer, B., Bertl, A., and Parish, R. 1986. *Proc. Natl. Acad. Sci. USA.* **83:** 8992–8995.
(54) Gaber, R.F., Styles, C.A., and Fink, G.R. 1988. *Mol. Cell. Biol.* **8:** 2848–2859.
(55) Ghislain, M., Schlesser, A., and Goffeau, A. 1987. *J. Biol. Chem.* **262:** 17549–17555.
(56) Giannini, J.L. and Briskin, D.P. 1987. *Plant Physiol.* **84:** 613–618.
(57) Giannini, J.L., Ruiz–Cristin, J., and Briskin, D.P. 1987. *Plant Physiol.* **85:** 1137–1142.
(58) Gibrat, R., Grouzis, J.P., Rigaud, J., and Grignon, C. 1985. *Biochim. Biophys. Acta.* **816:** 949–957.
(59) Gibrat, R., Grouzis, J.P., Rigaud, J., Galtier, N., and Grignon, C. 1989. *Biochim. Biophys. Acta.* **979:** 46–52.
(60) Gibrat, R., Grouzis, J.P., Rigaud, J., and Grignon, C. 1990. *Plant Physiol.* **93:** 1183–1189.
(61) Glass, A.D.M. 1976. *Plant Physiol.* **58:** 33–37.
(62) Glass, A.D.M. 1977. *Aust. J. Plant Physiol.* **4:** 313–318.
(63) Graziana, A., Fosset, M., Ranjeva, R., Hetherington, A.M., and Lazdunski, M. 1988. *Biochemistry.* **27:** 764–768.
(64) Gronewald, J.W. and Hanson, J.B. 1980. *Plant Sci. Lett.* **18:** 143–150.
(65) Grouzis, J.P., Gibrat, R., Rigaud, J., Ageorges, A., and Grignon, C. 1990. *Plant Physiol.* **93:** 1175–1182.
(66) Hager, A. and Moser, I. 1985. *Planta.* **163:** 391–400.
(67) Harmon, A.C., Putnam–Evans, C., and Cormier, M.J. 1987. *Plant Physiol.* **83:** 830–837.
(68) Harper, J.F., Surowy, T.K., and Sussman, M.R. 1989. *Proc. Natl. Acad. Sci. USA.* **86:** 1234–1238.
(69) Hedrich, R. and Schroeder, J.I. 1989. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40:** 539–569.
(70) Hedrich, R., Schroeder, J.I., and Fernandez, J.M. 1987. *TIBS.* **12:** 49–53.
(71) Jensén, P. and König, T. 1982. *Physiol Plant.* **55:** 459–464.

(72) Jensén, P. and Pettersson, S. 1978. *Physiol Plant.* **42:** 207–213.
(73) Jeschke, W.D. 1982. *J. Exp. Bot.* **33:** 601–618.
(74) Jeschke, W.D., Atkins, C.A., and Pate. J.S. 1985. *J. Plant Physiol.* **117:** 319–330.
(75) Kasamo, K. 186. *Plant Physiol.* **80:** 818–824.
(76) Katz, D.B. and Sussman, M.R. 1987. *Plant Physiol.* **83:** 977–981.
(77) Ketchum, K.A., Shrier, A., and Poole, R. 1989. *Plant Physiol.* **89:** 1184–1192.
(78) Kochian, L.V., Xin–Zhi J., and Lucas, W.J. 1985. *Plant Physiol.* **79:** 771–776.
(79) Kochian, L.V., Schaff, J.E., and Lucas, W.J. 1989. *Plant Physiol.* **91:** 1202–1211.
(80) Kurkdjian, A. and Guern, J. 1989. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **40:** 271–303.
(81) Lee, R.B. 1982. *Ann. Bot.* **50:** 429–449.
(82) Lefebvre, D.D. and Glass, A.D.M. 1982. *Physiol Plant.* **54:** 199–206.
(83) Leigh, R.A. and Wyn Jones R.G. 1973. *J. Exp. Bot.* **24:** 787–795.
(84) Lew, R.R. 1989. *Plant Physiol.* **91:** 213–216.
(85) Lew, R.R., Briskin, D.P., and Wyse, R.E. 1989. *Plant Physiol.* **82:** 47–53.
(86) Lew, R.R., Buchunow, N., and Spanswick, R.F.M. 1985. *Biochim. Biophys. Acta.* **821:** 341–347.
(87) Marré, E. 1980. *Progr. Phytochem.* **6:** 253–284.
(88) Marré, E., Beffagna, N., and Romani, G. 1988. *Bot. Acta.* **101:** 24–31.
(89) Marré, M.T., Romani, G., Bellando, M., and Marré, E. 1986. *Plant Physiol.* **82:** 316–323.
(90) McMurrary, W.C. and Irvin, R.F. 1988. *Biochem. J.* **249:** 877–881.
(91) Meade, J.C., Shaw, J., Gallagher, G., Lemaster, S., and Stringer, J.R. 1987. *Molec. Cell. Biol.* **7:** 3937–3946.
(92) Melin, P., Sommarin, M., Sandelius, A.S., and Jergil, B. 1987. *FEBS Lett.* **223:** 87–91.
(93) Michelet, B., Perez, C., Goffeau, A., and Boutry, M. 1989. *In* Plant Membrane Transport: The current position. J. Dainty et al. eds., Elsevier, Amsterdam, pp. 455–460.
(94) Miller, A.L. and Gow, N.A.R. 1989. *Plant Physiol.* **89:** 1198–1206.
(95) Morse, M.J., Satter, R.L., Crain, R.C., and Coté, G.G. 1988 Physiol Plant. **76:** 118–121.
(96) Nagao, T., Sasakawa, H., and Sugiyama, T. 1987. *Plant Cell Physiol.* **28:** 1181–1186.
(97) Newman, I.A., Kochian, L.V., Grusak, M.A., and Lucas, W.J. 1987. *Plant Physiol.* **84:** 1177–1184.
(98) Olah, Z. and Kiss, Z. 1986. *FEBS Lett.* **195:** 33–37.
(99) O'Neill, S.D. and Spanswick, R.M. 1984. *J. Memb. Biol.* **79:** 231–243.
(100) Pardo, J.M. and Serrano, R. 1989. *J. Biol. Chem.* **264:** 8557–8562.
(101) Pélissier, B., Thibaud, J.B., Grignon, C., and Esquerré Tugayé, M.T. 1986. *Plant Sci.* **46:** 103–109.

(102) Pitman, M.G. 1972. *Aust. J. Biol. Sci.* **25:** 905–919.
(103) Poole, R.J. 1978 *Annu. Rev. Plant. Physiol.* **29:** 437–460.
(104) Ramirez, J.A., Vacata, V., McCusker, J.H.M., Haber, J.E., Mortimer, R.K., Owen, W.G., and Lecar, H. 1989. *Proc. Natl. Acad. Sci. USA.* **86:** 7866–7870.
(105) Raschke, K., Hedrich, R., Reckmann, U., and Schroeder, J.I. 1988. *Bot. Acta.* **101:** 283–294.
(106) Rasi–Caldogno, F., Pugliarello, M.C., and De Michelis, M.I. 1985. *Plant Physiol.* **77:** 200–205.
(107) Rasi–Caldogno, F., Pugliarello, M.C., and De Michelis, M.I. 1987. *Plant Physiol.* **83:** 994–1000.
(108) Reddy, A.S.N. and Poovaiah, B.W. 1987. *J. Biochem.* **101:** 569–573.
(109) Reid, R.J., Field, L.D., and Pitman, M.G. 1985. *Plant Physiol.* **166:** 341–347.
(110) Reinhold, L. and Kaplan, A. 1984. *Annu. Rev. Plant Physiol.* **35:** 45–83.
(111) Reinhold, L., Seiden, A., and Volokita, M. 1984. *Plant Physiol.* **75:** 846–849.
(112) Rhoads, D.B. and Epstein, W. 1977. *J. Biol. Chem.* **252:** 1394–1401.
(113) Rincon, M. and Hanson, J.B. 1986. *Physiol. Plant.* **67:** 567–583.
(114) Rincon, M. and Boss, W.F. 1987. *Plant Physiol.* **83:** 395–398.
(115) Robinson, C., Larsson, C., and Buckhout, T.J. 1988. *Physiol. Plant.* **72:** 177–184.
(116) Rodriguez–Navarro, A., Blatt, M.R., and Slayman, C.L. 1986. *J. Gen. Physiol.* **87:** 649–674.
(117) Romani, G., Marré, M.T., Bonetti, A., Cerana, R., Lado, P., and Marré, E. 1983. *Physiol. Plant.* **59:** 528–533.
(118) Römheld, V. 1987. *Physiol. Plant.* **70:** 231–234.
(119) Rossignol, M., Kervella, V., and Blein, J.P. 1989. *Plant Physiol. Biochem.* **27:** 437–441.
(120) Scalla, R., Amory, A., Rigaud, J., and Goffeau, A. 1983. *Eur. J. Biochem.* **132:** 525–530.
(121) Schäfer, A., Bygrave, F., Matzenauer, S., and Marme, D. 1985. *FEBS Letts* **187:** 25–28.
(122) Schaller, G.E. and Sussman, M.R. 1988. *Planta.* **173:** 509–518.
(123) Schumaker, K.S. and Sze, H. 1987. *J. Biol. Chem.* **262:** 3944–3946.
(124) Serrano, R. 1988. *Biochim. Biophys. Acta.* **947:** 1–28.
(125) Serrano, R. 1989. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40:** 61–94.
(126) Serrano, R., Kielland–Brandt, M.C., and Fink, G.R. 1986. *Nature.* **319:** 689–693.
(127) Singh, S.P., Sudershan Kesav, B.V., and Briskin, D.P. 1987. *Physiol. Plant.* **69:** 617–626.
(128) Slayman, C.L. 1985. *BioScience.* **35:** 34–37.
(129) Slayman, C.L. 1987. *J. Bioenerg Biomembr.* **19:** 2–20.
(130) Smith, F.A. 1973. *New Phytol.* **72:** 769–782.
(131) Smith, I.K. 1975. *Plant Physiol.* **55:** 303–307.

(132) Staal, M., Prins, H.B.A., Elzenga, J.T.M., and de Beer, A.H. 1989. *In* Plant Membrane Transport: The current position. J. Dainty et al. eds, Elsevier, Amsterdam. pp. 121–122.
(133) Talbott, T.D., Ray, P.M., and Roberts, J.K.M. 1988. *Plant Physiol.* **87:** 211–216.
(134) Tate, B.F., Schaller, G.E., Sussman, M.R., and Crain, R.C. 1989. *Plant Physiol.* **91:** 1275–1279.
(135) Thibaud, J.B., Soler, A., and Grignon, C. 1986. *Plant Physiol.* **81:** 847–853.
(136) Touraine, B., Grignon, N., and Grignon, C. 1989. *Plant Physiol.* **88:** 605–612.
(137) Ullrich, C.I. and Guern, J. 1990. *Planta.* **180:** 390–399.
(138) Vara, F. and Serrano, R. 1982. *J. Biol. Chem.* **257:** 12826–12830.
(139) Villalobo, A. 1982. *J. Biol. Chem.* **257:** 1824–1828.
(140) Villalobo A. 1984. *Can. J. Biochem.* **62:** 865–877.
(141) Walderhaug, M.O., Post, R.L., Saccomani, G., Leonard, R.T., and Briskin, D.P. 1985. *J. Biol. Chem.* **260:** 3852–3859.
(142) Zocchi, G. 1985. *Plant Sci.* **40:** 153–159.
(143) Zocchi, G. and Hanson, J.B. 1982. *Plant Physiol.* **70:** 318–319.
(144) Zocchi, G., Rogers, S.A., and Hanson, J.B. 1983. *Plant Sci. Lett.* **31:** 215–221.