Review

Sodium transport in plant cells

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Abstract

Salinity limits plant growth and impairs agricultural productivity. There is a wide spectrum of plant responses to salinity that are defined by a range of adaptations at the cellular and the whole-plant levels, however, the mechanisms of sodium transport appear to be fundamentally similar. At the cellular level, sodium ions gain entry via several plasma membrane channels. As cytoplasmic sodium is toxic above threshold levels, it is extruded by plasma membrane Na^+/H^+ antiports that are energized by the proton gradient generated by the plasma membrane ATPase. Cytoplasmic Na^+ may also be compartmentalized by vacuolar Na^+/H^+ antiports. These transporters are energized by the proton gradient generated by the vacuolar H^+-ATPase and H^+-PPiase. Here, the mechanisms of sodium entry, extrusion, and compartmentation are reviewed, with a discussion of recent progress on the cloning and characterization, directly in planta and in yeast, of some of the proteins involved in sodium transport. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Na^+ transport in plants has largely been studied in the context of salinity stress or tolerance responses. Although there are some plants for which there is a growth requirement for Na^+, particularly halophytes [1], there is little biological relevance in the study of Na^+ as a limiting micronutrient because it is an abundant element in soils and soil-solutions. Rather, an overabundance of sodium is a limiting factor to plant growth over large terrestrial areas of the world. The detrimental effect of salt on plants is the result of a combination of factors and can be observed at the whole-plant level as a decrease in growth rate, leaf damage, and an increase in root/shoot ratio [1].

Moreover, the variability of plant growth responses to salinity defines a wide spectrum of salinity tolerance from the salt-sensitive glycophytes to the salt-tolerant halophytes.

Salinity imposes two stresses on plant tissues: (1) a water-deficit that results from the relatively high solute concentrations in the soil; and (2) ion-specific stresses resulting from altered K^+/Na^+ ratios and Na^+ and Cl^- ion concentrations that are inimical to plants. The alteration of ion ratios in the plant is due to the influx of sodium through pathways that function in the acquisition of potassium. The stealth of sodium entry is due to the similarity between the hydrated ionic radii of sodium and potassium, which makes difficult the discrimination between the two ions by transport proteins. This discrimination problem is also the basis for Na^+ toxicity. In vitro protein synthesis depends on physiological potassium (100–150 mM) and is inhibited by sodium concentrations

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above 100 mM [2] because of the competition by Na^+ for potassium binding sites. Moreover, halophyte cytosolic enzymes are not adapted to high salt levels and display the same sensitivity to salt as enzymes from glycophytes [3]. Thus, the net result of plant responses to elevated Na^+ concentrations is the maintenance of low cytosolic Na^+ concentrations and a high cytosolic K^+/Na^+ concentration ratio.

The strategies for maintaining a high K^+/Na^+ ratio in the cytosol include sodium extrusion and/or sodium compartmentation. The entry of Na^+ into the plant cell is essentially a passive process: the negative electrical potential difference at the plasma membrane and low cytosolic Na^+ concentrations strongly favor the movement of Na^+ into the cell. In contrast, Na^+ extrusion and compartmentation are, although indirectly, active processes. Na^+/H^+ antiporters mediate the compartmentation of Na^+ within the vacuole and the extrusion of Na^+ from the cell. These are two key processes in the concerted action of cytosolic Na^+ detoxification and cellular osmotic adjustment that are necessary to tolerate salinity stress.

In this review, the different mechanisms of Na^+ transport at the cellular and molecular level will be discussed. Ancillary mechanisms of salt extrusion, such as salt glands and salt bladders, and the transport of Na^+ between the different plant tissues are beyond the scope of this review.

2. Sodium uptake

Under typical physiological conditions, plants maintain a high K^+/Na^+ ratio in their cytosol with relatively high K^+ (100–200 mM) and low Na^+ concentrations (1–10 mM) [4]. Given the negative electrical membrane potential difference at the plasma membrane (-140 mV) [5], a rise in extracellular Na^+ concentrations will establish a large Na^+ electrochemical potential gradient that will favor the passive transport of sodium from the environment into the cytosol.

Although the mechanisms for Na^+ influx across the plant plasma membranes have not yet been established, it is evident that Na^+ ions can be transported into the cell through K^+ carriers (Fig. 1). Plant cells utilize low- and high-affinity transporters to take up K^+ from the extracellular medium. Low-affinity K^+ carriers (non-saturating at physiological K^+ concentrations in the external solution), such as AKT1 [6], are inward rectifying channels that activate K^+ influx upon plasma membrane hyperpolarization. They display a high K^+/Na^+ selectivity ratio at physiological K^+ and Na^+ external concentrations. Nevertheless, they could mediate a significant Na^+ uptake with an increase in external Na^+ concentrations. High-affinity K^+ carriers, such as HKT1 [7], are active at external K^+ concentrations in the micromolar range. Although originally characterized
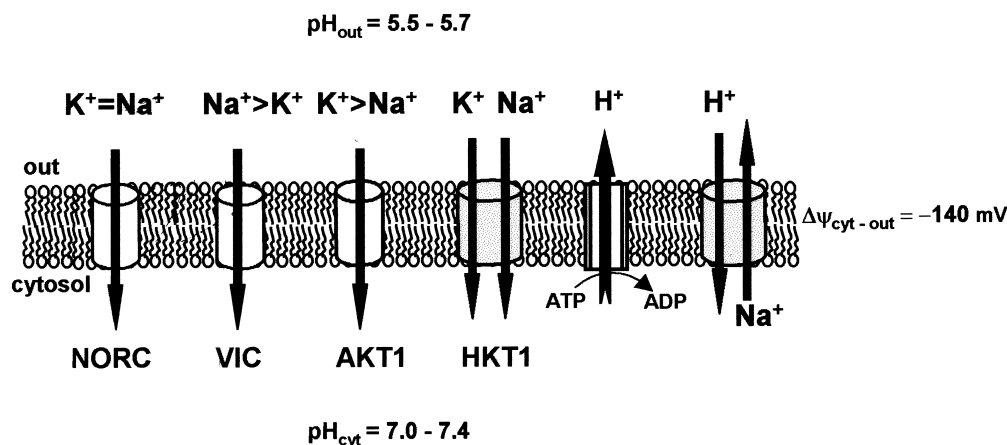

Fig. 1. Na^+ uptake and extrusion at the plant plasma membrane. Different plasma membrane potassium carriers can mediate the influx of Na^+ into the cells. AKT1 is a low-affinity K^+ channel with a higher K^+/Na^+ selectivity. HKT1 is a high-affinity K^+/Na^+ symporter. Outward rectifying channels, such as NORC, are activated by Ca^{2+} and do not discriminate between Na^+ and K^+. Voltage-independent channels (VIC) have a higher Na^+/K^+ selectivity. The main mechanism for Na^+ extrusion is powered by the plasma membrane H^+-ATPase. The electrochemical gradient for H^+ is used by a Na^+/H^+ antiporter that couples the downhill movement of H^+ with the active extrusion of Na^+.

as a H^+/K^+ symporter [7], it has been shown that HKT1 may function as a Na^+/K^+ symporter [8]. Rubio et al. [8] showed that low Na^+ concentrations stimulated K^+ transport by HKT1 expressed in heterologous systems. The influx of Na^+ was stimulated by external K^+ concentrations. High external Na^+ concentrations inhibited the influx of K^+ into oocytes [8], suggesting that under high external Na^+ concentrations HKT1 could provide an important pathway for the influx of Na^+ into plant cells. More recently, Schachtman et al. [9] identified a gene from wheat, *LCT1* that encodes a low-affinity cation transporter that could also mediate the influx of Na^+ into plant cells.

Outward-rectifying ion channels could play a role in mediating the influx of Na^+ into cells. These channels, which open during the depolarization of the plasma membrane (i.e., shift of the electrical potential difference to more positive values), could mediate the efflux of K^+ and the influx of Na^+. A number of outward-rectifying channels have been identified [10–12]. One of these channels, known as NORC (nonselective outward-rectifying conductance) does not discriminate between cations and is activated by increased cytosolic Ca^{2+} concentrations [12]. In the Characean alga *Nitella obtusa*, an increase in external Na^+ concentrations (100 mM) induced the depolarization of the plasma membrane with a concomitant decrease in cytosolic K^+ concentration and an increase in Na^+ content [13]. These effects were not seen in presence of Ca^{2+} in the growth medium. Schachtman et al. [14] suggested that the exposure of wheat roots to high NaCl concentrations could induce the depolarization of the root plasma membranes. This depolarization would activate outward-rectifying potassium channels, thus providing a pathway for the diffusion of Na^+ into the cells down its electrochemical gradient. Cramer et al. [15] suggested that the displacement of Ca^{2+} ions by Na^+ at the plasma membrane surface could induce the depolarization of the plasma membrane, inducing the leakage of cytosolic K^+ from the cell.

A number of studies have reported the presence in plant plasma membranes of voltage-independent cation channels (VIC) [16–20]. These channels have a relatively high Na^+ to K^+ selectivity and in contrast to the voltage-dependent (inward-rectifying and outward-rectifying) channels, they are not gated by volt-

age. In a recent review on the mechanisms of Na^+ uptake by plant cells, Amtmann and Sanders [21] integrated into a simple model the characteristics and properties of different cation channels and concluded that voltage-independent channels constitute the main pathway for Na^+ uptake in high salt conditions.

The relationship between intracellular Ca^{2+} homeostasis and Na^+ transport has been suggested [22–24]. In maize root protoplasts, external NaCl induced an increase in cytosolic Ca^{2+} concentration [22] and increases in external Ca^{2+} can ameliorate the effects of NaCl on plant growth [23]. A Na^+-induced depolarization of the plasma membrane would induce the activation of depolarized-activated Ca^{2+} channels [24] and the resulting increase in cytosolic Ca^{2+} will activate the influx of Na^+ through the outward-rectifying channels. Recently, a genetic locus that is necessary for salt tolerance has been identified in *Arabidopsis* [25,26]. A mutation in this locus, *sos3*, resulted in the hypersensitivity of the mutant plants to NaCl and increased Ca^{2+} in the growth medium suppressed the mutant phenotype. *SOS3* codes for a protein that contains Ca^{2+}-binding domains and a sequence similarity to the yeast calcineurin B subunit [27]. In vitro studies showed that ion transport in plants can be modulated by bovine brain calcineurin, a Ca^{2+}-dependent protein phosphatase 2B-type [28]. In yeast, there is evidence demonstrating that the perception of NaCl stress and the tolerance to salt involve Ca^{2+}-dependent processes that regulate ion homeostasis [29]. The operation of similar mechanisms in plants has been suggested [27].

3. Sodium extrusion

The extrusion of Na^+ from the cell is an active process, since Na^+ ions have to be transported against their electrochemical potential. The existence of an ATP-driven Na^+ transport, mediated by a Na^+-ATPase at the plasma membrane has been suggested in algae. A Na^+-activated ATPase was identified in plasma membrane preparations from the unicellular Chrysophyte *Heterosigma akashiwo* [30, 31]. The formation of a Na^+-dependent phosphate intermediate [30] and the immunological cross-reactivity with antibodies raised against the animal

Na^+,K^+-ATPase [31] suggested a similarity of the *H. akashiwo* plasma membrane ATPase with the P-type ATPase involved in K^+-uptake and Na^+-efflux from animal cells. Studies in perfused *Chara* cells showed that the energy contained in the protonmotive force was not enough to maintain steady state values of Na^+ in the cytosol at alkaline external pH [32,33] and the operation of a Na^+-ATPase at the plasma membrane of *Chara* has been suggested.

In higher plants, the main mechanism for Na^+ extrusion is powered by the operation of the plasma membrane H^+-ATPase [34] (Fig. 1). The H^+-ATPase uses the energy of ATP hydrolysis to pump H^+ out of the cell, generating an electrochemical H^+ gradient. This protonmotive force generated by the H^+-ATPase allows the operation of plasma membrane Na^+/H^+ antiporters that couple the downhill movement of H^+ into the cell along its electrochemical gradient to the extrusion of Na^+ against its electrochemical gradient. Evidence consistent with the operation of plasma membrane Na^+/H^+ antiporters has been obtained in different plant species [35] and algae (Table 1). Na^+/H^+ antiporter activity has been reported to occur across the plasma membrane of barley [36], tobacco [37], red beet [38], *Atriplex num-mularia* [39], tomato [40,41], wheat [42], blue-green algae [43], Chara [44,45], and the halotolerant alga *Dunaliella salina* [46]. The plasma membrane Na^+/H^+ antiporter activity increased in *Dunaliella salina* [46] and in the halophyte *Atriplex nummularia* [39] when the NaCl concentration of the external medium was increased. In *Atriplex*, the NaCl-dependent increase in Na^+/H^+ antiporter activity was correlated with an increase in plasma membrane H^+-ATPase activity [47]. An increase in steady state transcript levels indicated an up-regulation of the *Atriplex* plasma membrane H^+-ATPase in plants treated with 400 mM NaCl [48]. In a comparative study, the effects of NaCl on the plasma membrane H^+-ATPase activities from glycophytic (*Lycopersicon esculentum*) and halophytic (*Lycopersicon cheesmanii*) tomato species were measured [40]. Both species displayed a NaCl-dependent increase in their plasma membrane H^+-ATPase activity. Contrasting results were obtained with *Plantago*, where no difference in the plasma membrane H^+-ATPase activity from salt-tolerant and salt-sensitive plants was observed [49,50]. A correlation between the extrusion of Na^+ from the cell via a plasma membrane Na^+/H^+ antiporter and an enhanced energy requirement via an increased H^+

Table 1
Na^+/H^+ antiporter activity measured in plants, algae, and fungi

Organism	Membrane	Ref.
Saccharomyces cerevisiae	Plasma membrane	[88,89]
Saccharomyces cerevisiae	Mitochondria/prevacuole	[83,91]
Schizosaccharomyces pombe	Plasma membrane	[90]
Zygosaccharomyces rouxii	Plasma membrane	[91]
Atriplex gmelini	Microsomes	[65]
Tobacco	Plasma membrane	[37]
Blue-green algae	Plasma membrane	[43]
Dunaliella salina	Plasma membrane	[46]
Chara	Plasma membrane	[44,45]
Atriplex nummularia	Plasma membrane	[39]
Red beet	Plasma membrane	[38]
Barley	Plasma membrane	[36]
Tomato	Plasma membrane	[40,41]
Corn	Plasma membrane	[53]
Red beet	Tonoplast	[56,60]
Barley	Tonoplast	[57,64]
Catharantheus roseus	Tonoplast	[58]
Plantago maritima	Tonoplast	[63]
Messembryanthemum crystallinum	Tonoplast	[69]
Sunflower	Tonoplast	[66]
Arabidopsis thaliana	Tonoplast	[95]

$pH_{vac} = 5.5 - 5.7$

vacuolar lumen

$\Delta\psi_{cyt-vac} = -40$ mV
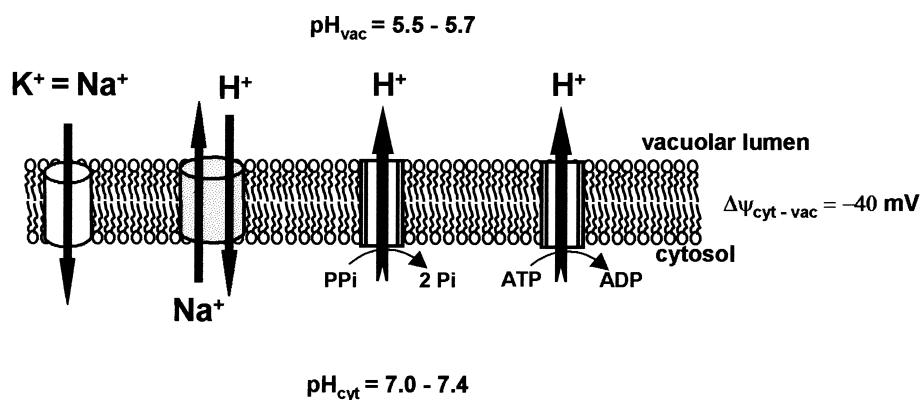
cytosol

$pH_{cyt} = 7.0 - 7.4$

Fig. 2. Na^+ compartmentation at the tonoplast. The transport of Na^+ into the vacuoles is mediated by a vacuolar Na^+/H^+ antiporter that is driven by the electrochemical gradient of protons generated by the H^+-ATPase and the H^+-PPiase. At physiological conditions, the vacuolar ion channels will favor the efflux of cations out of the vacuole.

extrusion by the plasma membrane H^+-ATPase would be expected. Nevertheless, increased plasma membrane H^+-ATPase activity has also been reported in plant cells challenged by non-ionic osmoticum such as sorbitol and PEG [51–53]. Rausch et al. [54] concluded that the scanty information available precluded a proper assessment of both plasma membrane H^+-ATPase and plasma membrane Na^+/H^+ exchange in salt tolerance and questioned the validity of comparisons between different species, even within the functional group of halophytes.

4. Intracellular sodium compartmentation

In spite of the considerable influx of Na^+ into the cytosol of all plants, the cytosolic Na^+ concentration is maintained at non-toxic levels by both halophytes and glycophytes. The compartmentation of Na^+ into the vacuoles provides an efficient mechanism to avert the deleterious effects of Na^+ in the cytosol. Moreover, the compartmentation of Na^+ (and chloride) into the vacuole allows the plants to use NaCl as an osmoticum, maintaining an osmotic potential that drives water into the cells. The transport of Na^+ into the vacuoles is mediated by a Na^+/H^+ antiporter that is driven by the electrochemical gradient of protons generated by the vacuolar H^+-translocating enzymes, the H^+-ATPase and the H^+-PPiase [55] (Fig. 2). While salt-sensitive plants depend mainly on exclusion of Na^+ ions at the plasma mem-

brane, salt-tolerant species accumulate large amounts of Na^+ in the vacuoles.

A vacuolar Na^+/H^+ antiporter activity was first directly measured in purified tonoplast vesicles from red beet storage tissue [56]. Since then, the presence of a vacuolar Na^+/H^+ exchange activity has been shown in different plant species (see Table 1). Short-term in vivo ^{23}Na nuclear magnetic resonance (NMR) spectroscopy studies of barley roots exposed to salt revealed evidence that supported the involvement of a Na^+/H^+ antiporter in the vacuole [57]. Using the same methodology, Guern et al. [58] demonstrated the operation of an Na^+/H^+ antiporter in intact vacuoles from *Catharanthus roseus* cells grown in a medium enriched in phosphate. The movement of H^+ out of the vacuole was driven by an initial vacuolar gradient of 1.5 pH-units, and Na^+ accumulation into the vacuole was achieved against a four- to fivefold Na^+ concentration gradient. This accumulation was both a saturable process, with respect to extravacuolar Na^+ concentrations, and a selective process, as K^+ was less effective than Na^+ in inducing the vacuolar alkalinization [58]. Using ^{23}Na- and ^{31}P-NMR, the alkalinization of the vacuole was observed upon the treatment of maize root tips with NaCl [53]. The data suggested the operation of a vacuolar Na^+/H^+ antiporter, nonetheless, as the authors suggested, an alternative explanation of the salt-induced alkalinization of the vacuoles could be also due to the inhibition of the vacuolar PPiase by high cytosolic

Na$^+$ concentrations [59]. In the salt-tolerant *Beta vulgaris*, an Na$^+$/H$^+$ exchange mechanism was demonstrated in intact vacuoles from root storage tissue [60] and cell suspensions [61]. In both cases, the ATP-dependent acidification of the vacuoles, measured by the fluorescence quenching of acridine orange, was reversed upon addition of external Na$^+$. In similar studies, using isolated tonoplast vesicles, Na$^+$/H$^+$ antiporter activity was reported in membranes isolated from sugar beet cell suspensions [62], from roots of the salt-tolerant *Plantago maritima* [63], from salt-grown barley roots [64], from the halophyte *Atriplex gmelini* [65] and from sunflower roots [66]. In addition to Na$^+$-dependent H$^+$-efflux, a few studies have used H$^+$-dependent ^{22}Na$^+$ influx measurements in tonoplast vesicles isolated from red beet storage tissue [67] or sugar beet cell suspensions [68]. A tenfold Na$^+$ accumulation was measured when a pH gradient of 1.5 units was established across the tonoplast vesicles and the influx was negligible in the absence of a pH gradient [68].

In some species the vacuolar Na$^+$/H$^+$ antiporter appeared to be constitutive, while in others, such as *Beta vulgaris*, the constitutively active antiporter was also upregulated by high NaCl concentrations. Increasing concentrations of NaCl in the growth medium of sugar beet cell suspensions did not change the K_m but doubled the V_{max} of the antiporter [61]. An increase in V_{max} for the antiporter with no change in apparent K_m suggested the addition of more antiporter molecules to the tonoplast in response to NaCl in the growth medium. A similar increase in tonoplast Na$^+$/H$^+$ antiporter activity has been also reported after the exposure of *Mesembryanthemum crystallinum* to high NaCl concentrations [69]. An inducible Na$^+$/H$^+$ antiporter activity was also demonstrated in tonoplast from barley roots grown in the presence of NaCl [64]. The induction of the Na$^+$/H$^+$ antiporter activity was very fast and appeared to be due to the activation of an existing protein rather than to de novo synthesis, since induction was observed in the presence of protein synthesis inhibitors [70]. Salt-treated plants lost their Na$^+$/H$^+$ antiporter activity when transferred to a solution lacking Na$^+$ with a similar time course to that seen during the induction by salt [70]. In *Plantago* species the vacuolar Na$^+$/H$^+$ antiporter activity is only present in the salt-tolerant *Plantago maritima*,

but not in the more salt-sensitive *Plantago media* [63]. The absence of vacuolar Na$^+$/H$^+$ antiporter activity may be related to a general property observed in salt-sensitive plants that rely on extrusion of Na$^+$ ions at the plasma membrane and not in the accumulation of Na$^+$ in the vacuoles [71]. Nonetheless, mechanisms that regulate the activity of the vacuolar Na$^+$/H$^+$ antiporter and/or its expression under normal growth conditions and during salt stress have not yet been elucidated.

The activity of the vacuolar primary H$^+$-pumps, that provide the driving force for the operation of the vacuolar Na$^+$/H$^+$ antiporters, is regulated by sodium. Increases in tonoplast H$^+$-ATPase activity in response to NaCl has been reported for barley roots [72], *Nicotiana* cell suspensions [73], mung bean roots [74], carrot cell suspensions [75], *Messembryanthemum* leaves [76], sunflower roots [77], and others [54,78]. Less information is available on the effect of Na$^+$ ions on the PPiase activity. Nevertheless, it would appear that while the vacuolar H$^+$-ATPase is stimulated by Na$^+$, the vacuolar H$^+$-PPiase is inhibited by increased NaCl concentrations [79]. This effect of Na$^+$ on the PPiase activity could be due to the competition of Na$^+$ for K$^+$ binding sites in the enzyme [80].

The molecular analysis of the vacuolar Na$^+$/H$^+$ antiporter and the characterization of factors affecting its activity have been hampered by the slow progress in cloning the gene(s) coding for the antiporter in plants. The recent molecular characterization of genes coding for putative plant Na$^+$/H$^+$ antiporters (see below) will facilitate the investigation of the transcriptional and post-translational controls regulating the Na$^+$/H$^+$ antiporter activity.

5. Molecular characterization of the Na$^+$/H$^+$ antiporter: from yeast to plants

The relatively well-detailed genetics of yeast, easy transformation protocols, and the homology between numerous plant and yeast genes has allowed for the identification and characterization of a number of plant transporters. Recent evidence suggests that the mechanisms of Na$^+$ detoxification in yeast may be quite similar to those in plants. This is particularly true for the role of calcium-dependent signal trans-

duction in Na^+ transport, salinity stress response [27,81] and the cloning of putative Na^+/H^+ antiporters from both organisms [82–85]. Therefore, understanding the mechanisms of Na^+ transport in yeast may well facilitate the characterization of similar mechanisms in plants, as well as the molecular characterization of specific plant genes.

Sodium extrusion in *Saccharomyces cerevisiae* relies mainly on a plasma membrane efflux mechanism whose characterization was based on increased lithium tolerance in a lithium-sensitive strain [86]. This system is encoded by the *PMR2/ENA* locus, which consists of a tandem array of four to five similar genes (*ENA1–ENA4*) that are homologous to the P-type ATPases. Expression of the *ENA1* gene can be induced by Na^+, Li^+ or high pH in the extracel-

lular medium, while the *ENA2–4* are expressed constitutively at low levels [87] (Fig. 3).

In addition to the plasma membrane Na^+-ATPase activity that functions mainly at alkaline pH, extrusion of Na^+ at acidic pH is achieved in yeast via a plasma membrane Na^+/H^+ antiporter. This antiporter, encoded by the *NHA1* gene, was identified by selection based on increased NaCl tolerance [88,89]. This antiporter is highly similar to the *SOD2* gene from *Schizosaccharomyces pombe* [90] and the *Z-SOD2* from *Zygosaccharomyces rouxii* [91]. In addition, sodium- and lithium-tolerance in cells overexpressing *NHA1* appeared to be pH-dependent, increasing at low pH values (i.e., 5.5) [88,89] (Fig. 3).

Recently, the salt-sensitive calcineurin mutants (*cnb1*), which lack the ability to increase expression
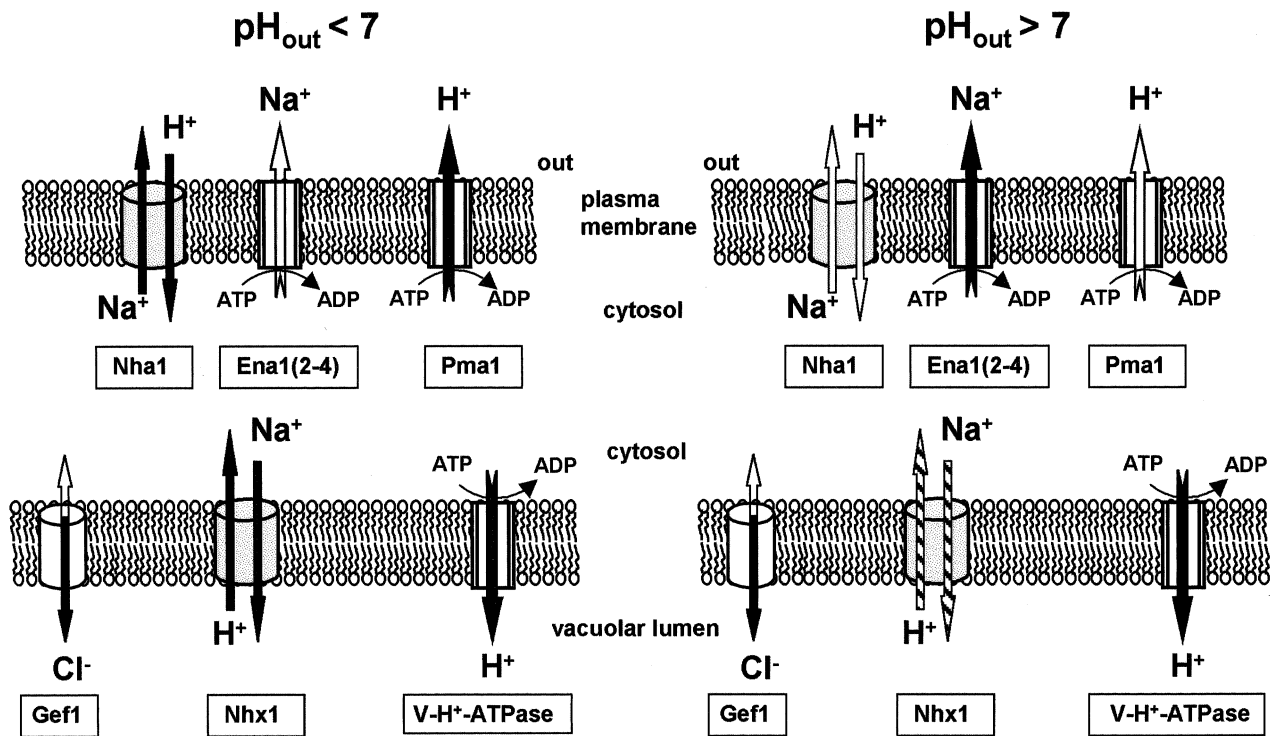

Fig. 3. Na^+ transport proteins in the yeast *Saccharomyces cerevisiae*. At alkaline extracellular pH (pH > 7), Na^+ extrusion at the plasma membrane is mediated mainly by the plasma membrane Na^+-ATPases (Ena1(2–4)). At these conditions, the plasma membrane H^+-ATPase (Pma1) and the Na^+/H^+ antiporter Nha1 are less active. Black arrows represent activation of a transporter in the indicated direction while reduced activity under particular conditions is represented by white arrows. At acidic extracellular pH, (pH < 7), Na^+ extrusion at the plasma membrane is mediated mainly by the Na^+/H^+ antiporter (Nha1) energized by the electrochemical proton gradient generated by the plasma membrane H^+-ATPase (Pma1). At these conditions, the Na^+-ATPase is less inactive. Intracellular sequestration of Na^+ may be mediated by a Na^+/H^+ antiporter (Nhx1) in a prevacuolar/vacuolar compartment. In this endosomal compartment, an electrochemical proton gradient is generated by the V-type H^+-ATPase (V-H^+-ATPase) and drives Na^+ and Cl^- transport into the lumen through Nhx1 and a chloride channel (Gef1), respectively. Although evidence to date suggests that Nhx1 is more active at low cytosolic pH, its role under neutral or alkaline pH is yet unknown (indicated by the striped arrows).

of the *PMR2A/ENA1* gene and modulate K^+ transport to high affinity, were used in screening for genetic suppressors [82]. Interestingly, loss of function mutations in the plasma membrane H^+-ATPase (*PMA1*) were observed to confer Na^+ tolerance. The mutant cells (*pma1-α4*) had a number of interesting phenotypes that included reduced Na^+ influx, increased Na^+ tolerance in response to low extracellular pH, and increased intracellular Na^+ levels. These results suggested that in addition to the limited Na^+ influx, a mechanism for intracellular sequestration of Na^+ may exist. The authors identified a novel gene coding for a Na^+/H^+ exchanger (*NHX1*) by in silico analysis of the yeast genome and examined its possible intracellular role. Disruption of the *NHX1* gene in the *pma1-α4* mutant nullified its Na^+-tolerant phenotype at neutral pH. Furthermore, disruption of the *NHX1* gene in wild-type strains showed significant decrease in Na^+ tolerance when cells were grown at pH 4.0 compared to those grown at pH 7.0. These results indicated that cytosolic pH may be a contributing factor in Na^+-tolerance. Reduced proton pumping in the *pma1-α4* mutant seems to lead to a reduction in cytosolic pH, which in turn facilitates the action of an intracellular Na^+/H^+ antiporter. In wild-type cells, the activation of the intracellular Na^+ sequestration mechanism could be achieved by reduction of the extracellular pH [82].

Since the first report of this novel Na^+/H^+ antiporter in *Saccharomyces cerevisiae* there have been conflicting reports regarding its intracellular localization [92,93]. Numata et al. [92] tagged the *NHX1* gene (referred to as *NHA2*) with GFP at its C-terminus, and used fluorescence microscopy to monitor the localization of the Nhx1-GFP chimera. GFP fluorescence co-localized with the DAPI DNA-binding dye for mitochondrial DNA staining. In addition, the authors demonstrated that both Li^+ and the amiloride analogue, benzamil (which preferentially inhibits mammalian mitochondrial Na^+/H^+ antiporter activity) inhibited $^{22}Na^+$ influx into isolated mitochondria. In yeast cells that were disrupted for the *NHX1* gene (*Δnhx1* cells), the benzamil-inhibitable $^{22}Na^+$ influx was absent. Furthermore, when *Δnhx1* cells were grown on non-fermentable carbon sources such as galactose, succinate or lactate, the authors observed growth retardation when compared to wild-type cells. The effects were even more noticeable on long term survival of *Δnhx1* cells, in stationary phase, under conditions of nutrient depletion. These results taken together suggested a role for Nhx1 in mitochondrial function [92].

Nass and Rao [93] reported the localization of Nhx1 to a late endosomal compartment. The authors tagged the *NHX1* gene with either the triple HA epitope or the GFP at the C-terminus. *Δnhx1* cells expressing either the HA- or GFP-tagged Nhx1, under the transcriptional control of the endogenous *NHX1* promoter, no longer exhibited the pH-dependent and salt-sensitive phenotype, which suggested that the Nhx1 chimeras were fully functional. Furthermore, the expression of the epitope-tagged gene was induced by NaCl treatments, suggesting a role for Nhx1 in salt-tolerance. Subcellular fractionation studies showed co-localization of Nhx1 with markers for the vacuole and prevacuolar compartment for both HA- and GFP-tagged proteins. Confocal laser microscopy, used to monitor fluorescence emission by Nhx1-GFP, revealed a bipolar perivacuolar compartmentalization. The emission increased with the increase of NaCl in the growth medium. Moreover, the fluorescence emitted by the Nhx1-GFP chimera was distinct from that of the two mitochondrial-specific dyes $DiOC_6$ and Mito-Tracker Red CMXRos [93].

The conflicting results reported by Nass and Rao [93] and Numata et al. [92] may be due in part to subtle experimental differences. Nass et al. [82] expressed the epitope-tagged Nhx1 in *Δnhx1* cells under the control of the *NHX1* promoter, while Numata et al. [92] used an Nhx1-GFP chimera under the control of the constitutive, methione-repressible *MET25* promoter in wild-type cells. This differential expression may have contributed to the conflicting localization of the *NHX1* product observed. Unfortunately, Numata et al. [92] did not assess possible contamination by other membranes, in their mitochondrial preparations. And, as noted by the authors, assaying for the benzamil-inhibitable Na^+/H^+ activity had its inherent difficulties, being detected in only three of five preparations. In addition, functionality of the chimera was not assessed by rescue of the *Δnhx1* phenotype [92].

The strong phenotype of *Δnhx1* cells, observed in response to Na^+-stress, does seem to favor the prevacuolar/vacuolar compartmentalization of Na^+ via

Nhx1 in yeast [93] (Fig. 3). This model is supported by the requirement for the yeast Cl^- channel, Gef1, and Nhx1, in Na^+ sequestration, which were both recently co-localized to the pre-vacuolar compartment [85]. But, the observation that Δnhx1 cells experience retarded growth on nonfermentable carbon sources [92] is still puzzling, and remains to be addressed; this is especially enigmatic as the mitochondrial membranes examined by Nass and Rao [93] strongly suggest the absence of the tagged Nhx1 chimera. The proposed pre-vacuolar bipolar distribution of Nhx1 raises the possibility of its role in the regulation of vesicle volume and pH, which may, in turn, be of importance in the process of vacuole biogenesis. However, the effects of Δnhx1 on the pre-vacuolar compartment and/or vacuole biogenesis are yet to be examined. These cells appear to grow well under normal conditions or neutral pH. In addition, *NHX1* has not been identified in any of the genetic screens designed to investigate vacuolar biogenesis in the past two decades [94]. It therefore remains to be seen to what extent Nhx1 is involved in processes such as vesicle fusion in vacuolar assembly.

The current model being proposed for intracellular Na^+ sequestration in yeast relies on the action of an endosomal H^+-ATPase to establish a H^+ gradient that can drive Na^+ and Cl^- influx via a Na^+/H^+ antiporter and chloride channels, respectively [85]. According to this model, increased H^+ influx into an endosomal compartment will enhance cation sequestration via the Nhx1 Na^+/H^+ antiporter. In testing this model, Gaxiola et al. [85] overexpressed the *Arabidopsis* vacuolar H^+-pyrophosphatase (*AVP1*) in the salt-sensitive *ena1* mutant of yeast. As mentioned above, these cells do not express the plasma membrane Na^+-ATPase, which serves as a primary Na^+ extrusion mechanism, thus leading to a significant increase in the levels of cytosolic Na^+. Under these conditions, cytosolic detoxification can only be achieved by the compartmentalization of Na^+ ions into an endosomal organelle. Yeast cells overexpressing *AVP1-D*, a mutant H^+-pyrophosphatase with an enhanced H^+-pumping ability, appeared to suppress the salt-sensitive phenotype of the *ena1* mutant. Although the restoration of the salt-tolerant phenotype seems to confirm the author's hypothesis, measurements of intracellular Na^+ in these cells contradicted the original rationale behind the experiment.

While the intracellular Na^+ content in the *ena1* mutant was measured to be eightfold higher than in the wild-type strain, in the salt-resistant strain *ena1 AVP-D*, intracellular Na^+ content was only fourfold higher than wild type (or twofold less than in the *ena1* cells) [85]. These results do not support the targeting of the *AVP-D* gene product to the yeast vacuole. Enhanced Na^+ sequestration through the action of a vacuolar pyrophosphatase would be expected to result in an equivalent, if not an increased, intracellular Na^+ content to that observed in the *ena1* strain. Instead, the decreased intracellular Na^+ content would seem to suggest an exclusion or extrusion of Na^+ at the plasma membrane upon expression of the *AVP-D* product. Unfortunately, the authors have neither attempted to localize the *AVP-D* product, nor have they addressed their puzzling observations.

The *Arabidopsis thaliana* genome-sequencing project has allowed for the identification of plant genes with significant similarity to the *Saccharomyces cerevisiae NHX1* gene product [83–85]. These genes may well be the first plant Na^+/H^+ antiporters cloned. As mentioned before, there is overwhelming biochemical evidence implicating the role of plant Na^+/H^+ antiporters in salt tolerance. Nonetheless, the lack of progress in the molecular characterization of these antiporters has hindered progress in our understanding of the cellular and molecular bases of salt tolerance. The present availability of plant genes coding for Na^+/H^+ antiporters, in particular from *Arabidopsis thaliana*, a model plant amenable for genetic analysis, allows for the direct probing of their function. Gaxiola et al. [85] have described the cloning of *ATNHX1* and its expression in the yeast *Saccharomyces cerevisiae* as an heterologous expression system. The predicted *ATNHX1* gene product is a protein of 538 amino acids with a putative amiloride-binding domain. It shows a high degree of similarity to Na^+/H^+ antiporters from *C. elegans*, human (mitochondrial, NHE6) and to the yeast ScNhx1, except within the N- and C-terminal regions [83–85]. Gaxiola et al. [85] expressed *AtNHX1* in hygromycin/ NaCl-sensitive Δnhx1 cells in an attempt to suppress these phenotypes. Plate assays suggested partial suppression of the cation-sensitive phenotype. Specifically, while hygromycin sensitivity appeared to be efficiently suppressed in the Δnhx1/ATNHX1 cells,

suppression of NaCl sensitivity required reduced K^+ availability. Furthermore, the authors remarked that the presence of amiloride in culture did not appear to inhibit either Nhx1 or AtNHX1 activity. Additional evidence suggesting the role of *AtNHX1* in cation homeostasis arises from Northern blot analyses from plants exposed to either 250 mM NaCl or 250 mM KCl for 6 h. *AtNHX1* mRNA transcripts appeared to increase by 4.2-fold and 2.8-fold in response to Na^+ and K^+, respectively. These results seem to indicate that AtNHX1 is functionally equivalent, in some respects, to the yeast Nhx1. Nevertheless, this study should be considered preliminary since the authors have not provided a thorough analysis of the strains used in their experiments and did not show the localization of the *AtNHX1* product, as well as the intracellular Na^+ and K^+ content. Suppression of the NaCl-sensitivity under reduced K^+ availability would seem to indicate that AtNHX1 is a low-affinity Na^+/H^+ antiporter.

Recently, Apse et al. [95] provided evidence demonstrating that *AtNHX1* codes for a vacuolar Na^+/H^+ antiport. In order to assess the Na^+/H^+ exchange function, Na^+-dependent H^+ movement was measured in intact vacuoles isolated from both wild-type plants and plants overexpressing AtNHX1. While Na^+/H^+ exchange rates were very low in vacuoles from wild-type plants, much higher rates were observed in vacuoles from transgenic plants [95]. The higher vacuolar Na^+/H^+ antiporter activity in the transgenic plants correlated with an increase in the amount of AtNHX1 protein. Moreover, transgenic plants overexpressing AtNHX1 were able to grow in the presence of 200 mM NaCl, supporting the role of the vacuolar Na^+/H^+ antiport in salt tolerance [95].

6. Conclusions

The identification of proteins mediating sodium transport and their biochemical characterization in yeast and plants is advancing rapidly. An understanding of the regulation of these pathways of Na^+ movement is incomplete. For example, the yeast plasma membrane plasma membrane Na^+/H^+ antiporter activity is identified with the *NHA1* gene product, but the regulation of its activity remains

to be dissected. There is no doubt that the use of yeast as a model system for sodium transport in plants facilitates the molecular identification of transporters. The cloning of the *Arabidopsis* antiporters was certainly expedited by the identification of the homologue in yeast. Nonetheless, the plant system is inherently more complex and at least four antiporters in *Arabidopsis*, similar to the yeast endomembrane Na^+/H^+ antiporter, have been identified (Blumwald, unpublished data). The number of putative plant Na^+/H^+ antiporters can be accounted for, a priori, by the activities measured in different tissues and membranes, not to mention the possible developmental and stress-specific requirements for Na^+ transport. However, the yeast model may point to a Na^+/H^+ antiporter function other than Na^+ detoxification. Volume and osmotic regulation in organellar biogenesis and ion homeostasis may be roles fulfilled by Na^+/H^+ antiporters. These roles have yet to be investigated in plants.

It is noteworthy that plant Na^+/H^+ antiporter genes have been cloned first from a species that is salt-sensitive. This seems contrary to the notion that Na^+/H^+ antiporters are present primarily in plants that tolerate salinity, a view that is implied by the set of species for which Na^+/H^+ antiporter activity has been reported. However, this view has not been substantiated by a methodical survey of glycophytic and halophytic species. Moreover, the differences between salt-sensitive and salt-tolerant species in whole-plant Na^+ distribution have not been attributed to Na^+/H^+ antiporter activity. Mennen et al. [40] concluded that Na^+/H^+ antiporters were not a ubiquitous characteristic of all plant cells, based on the lack of pH-dependent $^{22}Na^+$ uptake in excised roots. Their conclusion supported the underlying assumption that some plant species, both tolerant and non-tolerant to sodium, simply do not have Na^+/H^+ antiporters at either the plasma membrane or tonoplast (or both). All plants must deal with sodium and cytosolic Na^+ toxicity. In the absence of Na^+/H^+ antiporters, how will plants cope with the inevitable influx of Na^+? Mechanisms that reduce sodium uptake might include K^+ channels and/or transporters that are more selective for K^+. Another alternative is sodium efflux via a Na^+-ATPase, but this has not been detected in higher plants.

Certainly, a survey for Na^+/H^+ antiporters in a

wide variety of plants is more amenable to molecular techniques and the identification of other types of Na^+ transporters is being facilitated by the progress of the different genomic sequencing projects.

References

[1] R. Munns, A. Termatt, Aust. J. Plant Physiol. 13 (1986) 143–160.

[2] R.G. Wyn Jones, A. Pollard, Proteins, enzymes and inorganic ions, in: A. Lauchli, A. Person (Eds.), Encyclopedia of Plant Physiology, New Series, vol. 15B, Springer, New York, 1983, pp. 528–562.

[3] T. Flowers, P.F. Troke, A.R. Yeo, Annu. Rev. Plant Physiol. 28 (1977) 89–121.

[4] M.L. Binzel, F.D. Hess, R.A. Bressan, P.M. Hasegawa, Plant Physiol. 86 (1988) 607–614.

[5] N. Higinbotham, Annu. Rev. Plant. Physiol. 24 (1973) 25–46.

[6] H. Sentenac, N. Bonneaud, M. Minet, F. Lacroute, J.M. Salmon, F. Gaymard, C. Grignon, Science 256 (1992) 663–665.

[7] D.P. Schachtman, J.I. Schroeder, Nature 370 (1994) 655–658.

[8] F. Rubio, W. Gassmann, J.I. Schroeder, Science 270 (1995) 1660–1663.

[9] D.P. Schachtman, R. Kumar, J.I. Schroeder, E.L. Marsh, Proc. Natl. Acad. Sci. USA 94 (1997) 11079–11084.

[10] L.H. Wegner, K. Raschke, Plant Physiol. 105 (1994) 799–813.

[11] S.K. Roberts, M. Tester, Plant J. 8 (1995) 811–825.

[12] L.H. Wegner, A.H. De Boer, Plant Physiol. 115 (1997) 1707–1719.

[13] M. Katsuhara, M. Tazawa, Protoplasma 135 (1986) 155–161.

[14] D.P. Schachtman, S.T. Tyerman, B.R. Terry, Plant Physiol. 97 (1991) 598–605.

[15] G.R. Cramer, A. Lauchli, V.S. Polito, Plant Physiol. 79 (1985) 207–211.

[16] J.T.M. Elzenga, E. Van Volkenburgh, J. Membr. Biol. 137 (1994) 227–235.

[17] P.J. White, F. Lemtiri-Clieh, J. Exp. Bot. 46 (1995) 497–511.

[18] A. Amtmann, S. Laurie, R. Leigh, D. Sanders, J. Exp. Bot. 48 (1997) 481–497.

[19] S.K. Roberts, M. Tester, J. Exp. Bot. 48 (1997) 431–440.

[20] S.D. Tyermann, M. Skerrett, A. Garrill, G.P. Findlay, R.A. Leigh, J. Exp. Bot. 48 (1997) 459–480.

[21] A. Amtmann, D. Sanders, Adv. Bot. Res. 29 (1998) 76–112.

[22] J. Lynch, V.S. Polito, A. Lauchli, Plant Physiol. 90 (1989) 1271–1274.

[23] A. Lauchli, Calcium, salinity and the plasma membrane, in: R.T. Leonard, P.K. Hepler (Eds.), Calcium in Plant Growth and Development. The American Society of Plant Physiologists Symposium Series, vol. 4, 1990, pp. 26–35.

[24] J.W. Huang, D.L. Grunes, L.V. Kochian, Proc. Natl. Acad. Sci. USA 91 (1994) 3473–3477.

[25] J. Liu, J.-K. Zhu, Proc. Natl. Acad. Sci. USA 94 (1997) 14960–14964.

[26] J.-K. Zhu, L. Liu, L. Xiong, Plant Cell 10 (1998) 1181–1191.

[27] J. Liu, J.-K. Zhu, Science 280 (1998) 1943–1945.

[28] G.J. Allen, D. Sanders, Plant Cell 7 (1995) 1473–1483.

[29] R.A. Bressan, P.M. Hasegawa, J.M. Pardo, Trends Plant Sci. 3 (1998) 411–412.

[30] M. Wada, S. Satoh, K. Kasamo, T. Fujii, Plant Cell Physiol. 30 (1989) 923–928.

[31] M. Wada, O. Urayama, S. Satoh, Y. Hara, Y. Ikawa, T. Fujii, FEBS Lett. 309 (1992) 272–274.

[32] E.A. Kiegle, M.A. Bisson, Plant Physiol. 111 (1996) 1191–1197.

[33] J. Whittington, M.A. Bisson, J. Exp. Bot. 48 (1994) 657–665.

[34] M.R. Sussman, Annu. Rev. Plant Physiol. Plant Mol. Biol. 45 (1994) 211–234.

[35] L. Reinhold, Y. Braun, M. Hassidim, H.R. Lerner, The possible role of various membrane transport mechanisms in adaptation to salinity, in: J.H. Cherry (Ed.), Biochemical and Physiological Mechanisms Associated with Environmental Stress Tolerance, Springer, Berlin, 1989, pp. 121–130.

[36] A. Ratner, B. Jacoby, J. Exp. Bot. 27 (1976) 843–852.

[37] A.A. Watad, P. Pesci, L. Reinhold, H.R. Lerner, Plant Physiol. 81 (1986) 454–489.

[38] B. Jacoby, S. Teomi, Plant Sci. 55 (1988) 103–106.

[39] M. Hassidim, Y. Braun, Y.H.R. Lerner, L. Reinhold, Plant Physiol. 94 (1990) 1795–1801.

[40] H. Mennen, B. Jacoby, H. Marschner, J. Plant Physiol. 137 (1990) 180–183.

[41] C. Wilson, M.C. Shannon, Plant Sci. 107 (1995) 147–157.

[42] G.J. Allen, R.G.W. Jones, R.A. Leigh, Plant Cell Environ. 18 (1995) 105–115.

[43] E. Blumwald, J.M. Wolosin, L. Packer, Biochem. Biophys. Res. Commun. 122 (1984) 452–459.

[44] E.A. Kiegle, M.A. Bisson, Plant Physiol. 111 (1996) 1191–1197.

[45] G.M. Clint, E.A.C. MacRobbie, Planta 171 (1987) 247–253.

[46] A. Katz, U. Pick, M. Avron, Plant Physiol. 100 (1992) 1224–1229.

[47] Y. Braun, M. Hassidim, H.R. Lerner, L. Reinhold, Plant Physiol. 81 (1986) 1050–1056.

[48] X. Niu, M.L. Narasimhan, R.A. Salzman, R.A. Bressan, P.M. Hasegawa, Plant Physiol. 103 (1993) 713–718.

[49] W. Bruggemann, P. Janiesch, J. Plant Physiol. 130 (1987) 395–411.

[50] W. Bruggemann, P. Janiesch, J. Plant Physiol. 134 (1989) 20–25.

[51] L. Reinhold, A. Seiden, M. Volokita, Plant Physiol. 75 (1984) 846–849.

[52] M. Reuveni, R. Colombo, H. Lerner, A. Pradet, A. Poljakoff-Mayber, Plant Physiol. 85 (1987) 383–388.

[53] C.M. Spickett, N. Smirnoff, R.G. Ratcliffe, Plant Physiol. 102 (1993) 629–638.

[54] T. Rausch, M. Kirsch, R. Löw, A. Lehr, R. Viereck, A. Zhigang, J. Plant Physiol. 148 (1996) 425–433.

[55] E. Blumwald, Physiol. Plant. 69 (1987) 731–734.

[56] E. Blumwald, R.J. Poole, Plant Physiol. 78 (1985) 163–167.

[57] T.W.M. Fan, R.M. Higashi, J. Norlyn, E. Epstein, Proc. Natl. Acad. Sci. USA 86 (1989) 231–236.

[58] J. Guern, Y. Mathieu, A. Kurkdjian, P. Manigault, J. Manigault, B. Gillet, J.C. Beloeil, J.Y. Lallemand, Plant Physiol. 89 (1989) 27–36.

[59] R.A. Rea, R.J. Poole, Plant Physiol. 77 (1985) 46–52.

[60] C. Niemietz, J. Willenbrink, Planta 166 (1985) 545–549.

[61] E. Blumwald, R.J. Poole, Plant Physiol. 83 (1987) 884–887.

[62] E. Blumwald, E.J. Cragoe Jr., R.J. Poole, Plant Physiol. 85 (1987) 30–33.

[63] M. Staal, F.J.M. Maathius, T.M. Elzenga, J.H.M. Overbeek, H.B.A. Prins, Physiol. Plant. 82 (1991) 179–184.

[64] J. Garbarino, F.M. Dupont, Plant Physiol. 86 (1988) 231–236.

[65] T. Matoh, T. Ishikawa, E. Takahashi, Plant Physiol. 89 (1989) 180–183.

[66] E. Ballesteros, E. Blumwald, J.P. Donaire, A. Belver, Physiol. Plant. 99 (1997) 328–334.

[67] P.A. Rea, D. Sanders, R.A. Leigh, Wash-activated Na^+/H^+ exchange in tonoplast vesicles isolated from *Beta* storage root disks, in: M.J. Beilby, N.A. Walker, J.R. Smith (Eds.), Proceedings of the 7th International Workshop on Plant Membrane Transport, University of Sydney, Sydney, 1990, pp. 277–291.

[68] B.J. Barkla, J.H.M. Charuk, E.J. Cragoe Jr., E. Blumwald, Plant Physiol. 93 (1990) 924–930.

[69] B.J. Barkla, L. Zingarelli, E. Blumwald, J.A. Smith, Plant Physiol. 109 (1995) 549–556.

[70] J. Garbarino, F.M. Dupont, Plant Physiol. 89 (1989) 1–4.

[71] B.J. Barkla, M.P. Apse, M.F. Manolson, E. Blumwald, The plant vacuolar Na^+/H^+ antiport, in: M.R. Blatt, R.A. Leigh, D. Sanders (Eds.), Membrane Transport in Plants and Fungi: Molecular Mechanisms and Control, The Company of Biologists, Cambridge, 1994, pp. 141–153.

[72] H. Matsumoto, G.C. Chung, Plant Cell Physiol. 33 (1992) 139–149.

[73] M. Reuveni, A.B. Bennett, R.A. Bressan, P.M. Hasegawa, Plant Physiol. 94 (1990) 524–530.

[74] Y. Nakamura, K. Kasamo, N. Shimosato, M. Sakata, E. Ohta, Plant Cell Physiol. 33 (1992) 139–149.

[75] R. Low, T. Rausch, J. Exp. Bot. 47 (1996) 1725–1732.

[76] I. Struve, A. Weber, U. Luttge, E. Ball, J.A.C. Smith, J. Plant Physiol. 117 (1985) 451–468.

[77] E. Ballesteros, J.P. Donaire, A. Belver, Physiol. Plant. 97 (1996) 259–268.

[78] U. Luttge, R. Ratajczak, Adv. Bot. Res. 25 (1997) 253–296.

[79] C. Bremberger, H.-P. Haschke, U. Luttge, Planta 175 (1988) 465–470.

[80] R.-G. Zhen, E.J. Kim, P.A. Rea, Adv. Bot. Res. 25 (1997) 297–337.

[81] I. Mendoza, F.J. Quintero, R.A. Bressan, P.M. Hasegawa, J.M. Pardo, J. Biol. Chem. 271 (1996) 23061–23067.

[82] R. Nass, K.W. Cunningham, R. Rao, J. Biol. Chem. 272 (1997) 26145–26152.

[83] M.P. Apse, G.S. Aharon, W.A. Snedden, E. Blumwald, Annual Meeting of the American Society of Plant Physiologists, Madison, WI, 1998, p. 636.

[84] C.P. Darley, O. van Wuytswinkel, K. van der Woude, P. Mager, B. de Boer, 11th International Workshop on Plant Membrane Biology, Cambridge, UK, 1998, p. 89.

[85] R.A. Gaxiola, R. Rao, A. Sherman, P. Grisafi, S.L. Alper, G.R. Fink, Proc. Natl. Acad. Sci. USA 96 (1999) 1480–1485.

[86] R. Haro, B. Garciadeblas, A. Rodriguez-Navarro, FEBS Lett. 291 (1991) 189–191.

[87] B. Garciadeblas, F. Rubio, F.J. Quintero, M.A. Banuelos, R. Haro, A. Rodriguez-Navarro, Mol. Gen. Genet. 236 (1993) 363–368.

[88] C. Prior, S. Potier, J.-L. Souciet, H. Sychrova, FEBS Lett. 387 (1996) 89–93.

[89] M.A. Banuelos, H. Sychrova, C. Bleykasten-Grosshans, J.-L. Souciet, S. Potier, Microbiology 144 (1998) 2749–2758.

[90] Z.P. Jia, N. McCullough, R. Martel, S. Hemminngsens, P.G. Young, EMBO J. 11 (1992) 1631–1640.

[91] Y. Watanabe, S. Miwa, Y. Tamai, Yeast 11 (1995) 829–838.

[92] M. Numata, K. Petrecca, N. Lake, J. Orlowski, J. Biol. Chem. 273 (1998) 6951–6959.

[93] R. Nass, R. Rao, J. Biol. Chem. 273 (1998) 21054–21060.

[94] N.J. Bryant, T.H. Stevens, Microbiol. Mol. Biol. Rev. 62 (1998) 230–247.

[95] M.P. Apse, G.S. Aharon, W.A. Snedden, E. Blumwald, Science 285 (1999) 1256–1258.