Alkali cation exchangers: roles in cellular homeostasis and stress tolerance

José M. Pardo*, Beatriz Cubero, Eduardo O. Leidi and Francisco J. Quintero

Instituto de Recursos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, Reina Mercedes 10, Sevilla 41012, Spain

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Abstract

Uptake and translocation of cations play essential roles in plant nutrition, signal transduction, growth, and development. Among them, potassium (K$^+$) and sodium (Na$^+$) have been the focus of numerous physiological studies because K$^+$ is an essential macronutrient and the most abundant inorganic cation in plant cells, whereas Na$^+$ toxicity is a principal component of the deleterious effects associated with salinity stress. Although the homeostasis of these two ions was long surmised to be fine tuned and under complex regulation, the myriad of candidate membrane transporters mediating their uptake, intracellular distribution, and long-distance transport is nevertheless perplexing. Recent advances have shown that, in addition to their function in vacuolar accumulation of Na$^+$, proteins of the NHX family are endosomal transporters that also play critical roles in K$^+$ homeostasis, luminal pH control, and vesicle trafficking. The plasma membrane SOS1 protein from Arabidopsis thaliana, a highly specific Na$^+$/H$^+$ exchanger that catalyses Na$^+$ efflux and that regulates its root/shoot distribution, has also revealed surprising interactions with K$^+$ uptake mechanisms by roots. Finally, the function of individual members of the large CHX family remains largely unknown but two CHX isoforms, AtCHX17 and AtCH23, have been shown to affect K$^+$ homeostasis and the control of chloroplast pH, respectively. Recent advances on the understanding of the physiological processes that are governed by these three families of cation exchangers are reviewed and discussed.

Key words: Endosomes, intracellular localization, ion exchangers, membrane transport, mineral nutrition, pH regulation, potassium, salinity, sodium, vacuole, vesicle trafficking.

Introduction

Plant nutrition depends on the activity of membrane transporters that translocate minerals from the soil into the plant and mediate their intra- and intercellular distribution. The genome of Arabidopsis thaliana appears to encode >800 membrane transport proteins, 65% of which are secondary active transporters. Classification of these co-transporters has been based on both phylogeny and their known or predicted functions as transporters for cations, anions, and organic compounds, including sugars and amino acids (Saier, 2000). In plants, most co-transporters are energized by the proton electrochemical gradient generated by primary proton pumps working in all cell membranes, but alternative couplings also exist. Multigene families, whose members often exhibit overlapping expression patterns and a high degree of sequence homology, encode most types of plant membrane transporters. Furthermore, more than one transporter family transports many inorganic nutrients. Their extensive array of membrane transporters may provide plants with flexible strategies to cope with fluctuations in their environment and to minimize the adverse effects of nutrient deficiency and an excess of toxic ions.

Computer-assisted phylogenetic analyses of the completed Arabidopsis genome sequence have revealed a large family of putative cation/H$^+$ antiporters (Mäser et al., 2001). Based on the relative electrochemical gradients of their substrates, cations, and protons, most if not all of these exchangers are thought to extrude cations from the cytosol to the outside across the plasma membrane or into intracellular compartments, including the vacuole. The best examples of these exchangers are the Ca$^{2+}$/H$^+$ and Na$^+$/H$^+$ exchangers at the vacuole membrane that extrude Ca$^{2+}$ and Na$^+$, respectively, from the cytosol to maintain low cytosolic concentrations (Blumwald, 2000; Hepler, 2005).
In addition, similar transport activities are present in the plasma membrane and organelles (Pardo and Quintero, 2002; Hepler, 2005). Up to four phylogenetic subfamilies of cation/H+ exchangers have been identified within the Arabidopsis genome that may exchange Ca2+, Na+, and K+ with H+ (Mäser et al., 2001). The CaCA gene subfamily of Ca2+/H+ exchangers contains 11 members that have been named CAX1 to CAX11. CAX1 and CAX2, but not CAX3 or CAX4, suppressed defective vacuolar Ca2+ transport in a yeast vcx1 yeast mutant (Cheng et al., 2002). Accumulated evidence indicates CAX1, CAX2, and CAX3 may play a central role in Ca2+ and metal (Mn2+, Cd2+) sequestration into the vacuole, but the other family members remain to be characterized. AtMHX, a protein related phylogenetically to the CAX family, facilitates H+-coupled transport of Mg2+ and Zn2+ into plant vacuoles (Shaul et al., 1999). There are two members of the NhaD subfamily (NHD1 and NHD2) that have similarity to Na+/H+ antiporters previously found in bacteria, both of which remain uncharacterized. In Arabidopsis, there are eight members of the CPA1 family, including the best characterized proteins, AtNHX1 and SOS1, that catalyse Na+/H+ exchange at the tonoplast and plasma membrane, respectively (Blumwald, 2000; Qiu et al., 2002). The large CPA2 subfamily has 33 members that include 28 CHX proteins thought to mediate cation/H+ exchange and five homologues of the K+/H+ antiporter AtKEA1. Members of this family are just beginning to be characterized and the progress made so far will be discussed in this review.

The focus of this review is on the major alkali cation exchanger families for which initial characterizations have been achieved for individual members with respect to K+ and Na+ homeostasis. K+ is an indispensable macronutrient and the most abundant cation in plants. K+ is essential for many metabolic processes and a major contributor to cell turgor. In Arabidopsis, K+ transport can occur through members of the HAK/KUP/KT transporter family, KAT and AKT channel families, NHX exchangers, and possibly also via other non-characterized transporters such as those belonging to the CHX and KEA families. In spite of these apparent redundancies, individual transporters are thought to achieve diverse and specialized functions, both at the subcellular and organismic levels. By contrast, Na+ is normally a non-essential element that is toxic to many plant species when present in high concentrations. Na+ stress often results in deficiency of K+ because of the physicochemical similarities between Na+ and K+ (Maathuis and Amtmann, 1999). For instance, K+ uptake by HAK/KUP/KT transporters is inhibited by Na+ (Santa-Maria et al., 1997), and inhibition of the Arabidopsis AKT1 K+ channel by intracellular Na+ has also been reported (Qi and Spalding, 2004). By contrast, at low K+ availability, moderate levels of Na+ actually promote plant growth by replacing K+ in its role as provider of turgor (Rodriguez-Navarro, 2000). In low K+ conditions, Na+ can stimulate high-affinity uptake of K+ in Arabidopsis (Spalding et al., 1999). In barley, high-affinity Na+ uptake was detected in roots of plants exposed to a K+-free medium. This Na+ unipor activity was insensitive to external K+ at the beginning of K+ starvation and inhibited by K+ several hours later (Haro et al., 2005). Besides their roles in regulating the uptake and distribution of K+ and Na+, plant alkali cation exchangers are emerging as critical regulators of basic cellular processes such as the regulation of luminal pH regulation in endosomes, vesicle trafficking, and protein sorting. Moreover, genetic studies with knockout mutants and transgenic lines with ectopic overexpression of these transporters are revealing unexpected implications on ion nutrition. In this review, recent developments on the current understanding of the function of alkali cation exchangers are summarized and discussed.

Endosomal exchangers of the NHX family

Phylogeny and subcellular localization

The Arabidopsis AtNHX1 protein was the first Na+/H+ exchanger identified in plants (Gaxiola et al., 1999). Since then, the number of homologous NHX transporters identified has grown dramatically. DNA sequences encoding NHX proteins from >60 plant species, including gymnosperms and dicotyledonous and monocotyledonous angiosperms, have been deposited in the different datasets of GenBank. Following the phylogenetic classification of Saier (2000), plant NHXs are members of the NHE/NHX subfamily of Na+/H+ exchangers, a subgroup of the cation antiporter CPA1 family that is present across all taxonomic groups of eukaryotes. With the sole known exception of yeast, where a single NHX1 gene exists, NHE/NHX exchangers are present as multiple isoforms in all genomes sequenced to date. Plant NHX exchangers are proteins of about 550 residues in length and have the typical transporter topology of 10–12 transmembrane domains with a hydrophilic C-terminal tail that is thought to be cytosolic. Recently, however, luminal localization of the C-terminal domain of the Arabidopsis vacuolar exchanger AtNHX1 has been reported (Yamaguchi et al., 2003). The highest sequence homology among NHXs occurs in the N-terminal part that forms the membrane pore, whereas C-terminal domains are more dissimilar. In animals, where the NHE family has been studied in great detail, the C-terminal domain regulates ion translocation by the membranous region of the molecule. Variation in amino acid sequences of the C-terminus probably reflects differential regulation of each isoform (Putney et al., 2002). In addition to the conserved domain of the CPA superfamly ( Pfam00999), NHE/NHX exchangers have a small conserved stretch of 14 residues in the fourth transmembrane segment, with the consensus FF(I/L)(Y/F)LFLLPPI thought to be the binding site of the drug amiloride and its derivatives, which are inhibitors of this family of exchangers (Putney et al., 2002).
On the basis of protein sequence similarity, the NHE/NHX subfamily can be classified in two major groups that have been named plasma membrane (PM) and intra-cellular (IC) according to their subcellular localization (Brett et al., 2005a). The PM group is exclusively present in animal cells, whereas members of the IC group can be found in animals, plants, and fungi, with the exception of NHE8-like exchangers that are found only in animals and would have appeared later in evolution to fulfill specific physiological needs of animal cells (Brett et al., 2005a) (Table 1). All plant NHXs characterized to date are assigned to the IC group and can be further divided into two main groups that will be denoted herein as class I and class II. In Arabidopsis, members of the class-I category (AtNHX1–4) are 56–87% similar to each other, whereas AtNHX5 and 6 (class II) are 79% similar but only 21–23% similar to class-I isoforms (Yokoi et al., 2002). All NHX proteins of class I characterized to date are localized in the vacuolar membrane and form a separate clade within the IC group that is composed exclusively of plant exchangers (Fig. 1). By contrast, class-II members are found in endosomal vesicles of plants (Table 1) and homologous proteins with various endosomal localizations are also present in animals and fungi (Table 1). In Arabidopsis and rice, where the whole genomic sequence is known, the size of the NHX gene family is almost identical. Arabidopsis contains six NHX genes and rice has five, which are distributed in a similar way: AtNHX1–4 and OsNHX1–4 constitute class I, and AtNHX5–6 and OsNHX5 constitute class II (Fig. 1). Members of the class-I category of Arabidopsis and rice show 54–87% similarity. Similarity among class-II members is 72–79%, but they are only 21–23% similar to class-I isoforms. These data indicate that divergence between class-I and class-II exchangers in plants occurred before the separation of dicotyledons and monocotyledons.

The selectivity for ion substrate seems to be an additional distinctive feature of each subgroup. Vacular exchangers of class-I catalyse Na⁺/H⁺ or K⁺/H⁺ exchange with equal affinity (Venema et al., 2002; Apse et al., 2003), whereas the endosomal class II shows a preference for K⁺ over Na⁺ as substrate (Venema et al., 2003). All these differential characteristics suggest that vacuolar and endosomal exchangers play distinct roles in planta as discussed below.

### Ion specificity

Plant antiporers were originally described exchanging Na⁺/H⁺ at the tonoplast of beetroots and they were consequently related to Na⁺ sequestration into the vacuole, a critical feature for salt tolerance (Blumwald and Poole, 1985; Niemetz and Willenbrink, 1985). The Na⁺/H⁺ exchange was electroneutral and driven by the vacuolar proton gradient established by the activity of the proton pumps V-ATPase and V-PPase (Blumwald, 1987). Tonoplast vesicles from beetroot were able to exchange Na⁺ and K⁺ by H⁺, but amiloride inhibited Na⁺ transport activity and not K⁺ exchange, suggesting that separate entities mediated Na⁺/H⁺ and K⁺/H⁺ exchanges (Blumwald and Poole, 1985). In tonoplast vesicles from salt-treated barley roots, a Na⁺/H⁺ antiporter was induced showing no significant activity with other monovalent cations (K⁺, Li⁺, Cs⁺, Rb⁺) (Garbarino and DuPont, 1988). By contrast, other studies performed with cotton or sunflower roots found antiporter activity exchanging both K⁺ and Na⁺ at similar rates (Hassidim et al., 1990; Ballesteros et al., 1997). In the halophytes Atriplex numularia, Plantago maritima, and

### Table 1. NHX/NHE homologues for which the subcellular localization has been experimentally determined

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Type</th>
<th>Subcellular localization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>NHX1</td>
<td></td>
<td>Prevacuolar compartment</td>
<td>Nass and Rao, 1998</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>NHX1</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Yokoi et al., 2002</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
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<td>Class I</td>
<td>Vacuole</td>
<td>Hordeum vulgare</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>NHX4</td>
<td>Class I</td>
<td>Vacuole</td>
<td>B Cubero and JM Pardo, unpublished results</td>
</tr>
<tr>
<td>Atriplex numularia</td>
<td>NHX1</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Hamada et al., 2001</td>
</tr>
<tr>
<td>Ipomoea nil</td>
<td>NHX1</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Yamaguchi et al., 2001</td>
</tr>
<tr>
<td>Ipomoea nil</td>
<td>NHX2</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Ohnishi et al., 2005</td>
</tr>
<tr>
<td>Ipomoea tricolor</td>
<td>NHX1</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Yoshida et al., 2005</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>NHX1</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Fukuda et al., 2004</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
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<td>Class I</td>
<td>Vacuole</td>
<td>Vasekina et al., 2004</td>
</tr>
<tr>
<td>Beta vulgaris</td>
<td>NHX1</td>
<td>Class I</td>
<td>Vacuole</td>
<td>Xia et al., 2002</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>NHX5</td>
<td>Class II</td>
<td>Endosomal compartment</td>
<td>B Cubero and JM Pardo, unpublished results</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>NHX2</td>
<td>Class II</td>
<td>Endosomal compartment</td>
<td>B Cubero and K Venema, unpublished results</td>
</tr>
<tr>
<td>Homo sapiens</td>
<td>NHE6</td>
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<td>Brett et al., 2002</td>
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<td>Homo sapiens</td>
<td>NHE7</td>
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<td>Numata and Orlowski, 2001</td>
</tr>
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<td>NHE9</td>
<td>Class II</td>
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<td>Nakamura et al., 2005</td>
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<td>NHX5</td>
<td>Class II</td>
<td>Intracellular</td>
<td>Nehrke and Melvin, 2002</td>
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<td>Homo sapiens</td>
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<td>Mid- to trans-Golgi</td>
<td>Nehrke and Melvin, 2002</td>
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<td>Caenorhabditis elegans</td>
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<td>Intracellular</td>
<td>Nehrke and Melvin, 2002</td>
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<td>Intracellular</td>
<td>Nehrke and Melvin, 2002</td>
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<tr>
<td>Caenorhabditis elegans</td>
<td>NHX9</td>
<td>Intracellular</td>
<td>Nehrke and Melvin, 2002</td>
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intracellular compartmentalization of Na⁺ (Gaxiola et al., 1999; Quintero et al., 2000). Experiments with vacuolar vesicles isolated from yeast expressing AtNHX1 showed low-affinity electroneutral Na⁺/H⁺ exchange (Darley et al., 2000). Although initial reports with leaf vacuoles from transgenic *Arabidopsis* overexpressing AtNHX1 indicated that Na⁺/H⁺ exchange activity was much higher than in control plants without affecting K⁺/H⁺ exchange (Apse et al., 1999), subsequent work with transgenic tomato overexpressing AtNHX1 showed enhanced antiprot activity with both Na⁺ and K⁺, albeit with lower specificity for K⁺ (Zhang and Blumwald, 2001). To study further its substrate selectivity, a histidine-tagged AtNHX1 protein was purified to homogeneity and reconstituted into artificial lipid vesicles where AtNHX1 was the only transport protein present (Venema et al., 2002). The AtNHX1 protein catalysed Na⁺ and K⁺ transport with similar affinity in the presence of an imposed pH gradient. Li⁺ and Cs⁺ ions were also transported with lower affinity, but organic cations were not. This is in agreement with the ability of AtNHX1 to increase the Na⁺ and Li⁺ tolerance of yeast (Quintero et al., 2000; Yokoi et al., 2002). Indirect evidence from restored tolerance of yeast *nhx1* mutants to NaCl and KCl suggests that vacuolar proteins OsNHX1 from rice, and InNHX1 and InNHX2 from *Ipomoea nil* (morning glory), also have dual Na⁺ and K⁺ specificity (Fukuda et al., 2004; Ohnishi et al., 2005). Collectively, the evidence gathered suggests that NHX proteins of class I, which are resident in the tonoplast, function as (Na⁺,K⁺)/H⁺ exchangers that transport alkali cations with relatively low selectivity. Interestingly, topological studies with AtNHX1 showed that deletion of the C-terminus modified the cation specificity of the antiprot when expressed in yeast, increasing the transport rate of Na⁺ over K⁺ in exchange for vacuolar H⁺ (Yamaguchi et al., 2003). These results imply that Na⁺/K⁺ selectivity may be under physiological control through a regulatory C-terminal domain.

The only class-II NHX antiprot of plants whose activity has been analysed in any detail is LeNHX2 from tomato (Venema et al., 2003), which by amino acid sequence comparison is most similar to AtNHX5 of *Arabidopsis* (Fig. 1). The LeNHX2 protein co-localizes with prevacuolar and Golgi markers in both yeast and plants. LeNHX2 complemented the salt- and hygromycin-sensitive phenotype of *nhx1* yeast mutant and affected the accumulation of K⁺, but not Na⁺, in intracellular compartments. Purified LeNHX2 reconstituted in proteoliposomes catalysed K⁺/H⁺ exchange but only minor Na⁺/H⁺ exchange *in vitro*. Indirect evidence suggests that AtNHX5 shares with LeNHX2 the same K⁺ preference. The enhanced K⁺ and Na⁺ accumulation in *nhx1* yeast mutant expressing the vacuolar class-I isoforms AtNHX1 and AtNHX2 indicates that both were able to transport K⁺ and Na⁺ (Yokoi et al., 2002). By contrast, an *nhx1* yeast mutant transformed with AtNHX5 (class II) accumulated *Mesembryanthemum crystallinum*, the Na⁺/H⁺ antiprot activity was greater than the K⁺/H⁺ exchange (Hassidim et al., 1990; Staal et al., 1991; Barkla et al., 1995). Monitoring by NMR of luminal pH in vacuoles of *Catharanthus roseus* showed that Na⁺, K⁺, and Ca²⁺ produced vacuolar alkalinization, but also that Na⁺ was more effective than equimolar K⁺ concentrations to induce luminal alkalinization (Guern et al., 1989). These studies using biochemical approaches with whole vacuoles or tonoplast vesicles are of limited use for the assignment of discrete ion exchanges to specific transport entities because an array on different exchangers may contribute additively to the recorded ion fluxes and, consequently, the substrate specificity of individual transporters cannot be discerned with confidence.

The molecular cloning of genes encoding ion transporters has permitted a systematic, case-by-case approach to the problem of substrate specificity. The first plant Na⁺/H⁺ antiprot of the NHX family was cloned in *Arabidopsis* (Gaxiola et al., 1999). Expression of AtNHX1 in an *nhx1* yeast mutant suppressed its NaCl sensitivity and enhanced...
comparatively less Na⁺ than AtNHX1 and AtNHX2, and conveyed negligible tolerance to Li⁺ (Yokoi et al., 2002). Moreover, a translational AtNHX5:GFP fusion localized to the prevacuolar compartment of onion cells (B Cubero and JM Pardo, unpublished results). Similar results were reported for the human NHE7 exchanger, a mammalian homologue of class-II exchangers. NHE7 localizes in trans-Golgi vesicles and catalyses K⁺ or Na⁺ exchange (Numata and Orlowski, 2001).

Taken together, these data suggest that diverse subcellular localizations of class-I and class-II NHX proteins in plants (Table 1) correlate with distinct substrate-ion specificities and physiological roles. Vacuolar isoforms (class I) are low-selectivity transporters exchanging alkali cations and protons. In physiological terms, this dual specificity implies that under normal growth conditions class-I NHX proteins would mediate the accumulation of K⁺ and Na⁺ into vacuoles, thereby contributing to osmotic regulation and the generation of turgor essential for cell expansion. Indeed, Arabidopsis plants bearing a knockout in gene NHX1 show impaired leaf expansion, although this phenotype has not been unequivocally linked to vacuolar dysfunction (Apse et al., 2003). Under salinity stress, Na⁺ transport would be preferred simply because of the greater abundance of this ion within cells or by modulation of ion specificity through a regulatory mechanism involving the C-terminal part of the protein (Yamaguchi et al., 2003). In this regard, it would be of interest to test the ion selectivity of NHX proteins from salt-tolerant species. The capability for alkali cation transport in exchange for protons also poses NHX proteins as prime candidates for pH regulation, like their animal NHE counterparts. Presumably, the localization of class-II NHX proteins in endosomal compartments other than the vacuole imposes restrictions on ion selectivity since proper functioning of the endomembrane system must rely on K⁺/H⁺ exchange for pH regulation to prevent undue accumulation of potentially toxic Na⁺ into the endosomal lumen. For instance, K⁺ and, to a much lesser extent, Na⁺ and Li⁺ act as modulators of the activity of the Kex2/furin family of endoproteases required for the maturation of proteins along the secretory pathway in yeast and animal cells (Rockwell and Fuller, 2002).

Roles in stress responses

Selective ion uptake and differential ion compartmentalization are main features that explain disparities in tolerance to salinity between glycophytes and halophytes (Flowers et al., 1977; Greenway and Munns, 1980; Jeschke, 1984). Salinity affects nutrient acquisition by interfering with K⁺ uptake by carriers and channels (Maathuis and Amtmann, 1999). At the cellular level, intracellular ion sequestration into vacuoles for osmotic adjustment, strong ion selectivity in the cytosol (preference of K⁺ over Na⁺), and accumulation of compatible (non-toxic) organic solutes in the cytosol to equilibrate water potential across the tonoplast are widely accepted mechanisms contributing to salt tolerance (Greenway and Munns, 1980; Gorham et al., 1985). The sequestration of ions that are potentially damaging to cellular metabolism (e.g. Cl⁻, Na⁺) into the vacuole while maintaining high K⁺/Na⁺ ratios in the cytosol would provide the osmotic driving force required for water uptake in saline environments and, at the same time, provide plants with an efficient instrument for ion detoxification (Greenway and Munns, 1980; Yeo, 1983).

The biochemical characterization of Na⁺/H⁺ antiporters in the tonoplast supplied the evidence for a mechanism of Na⁺ accumulation in the vacuole (Blumwald and Poole, 1985). Salt treatment of sugar beet suspension cells led to an increase in the Vₘₐₓ of Na⁺/H⁺ antiport activity (Blumwald and Poole, 1987). The induction of vacuolar ATPase (protonmotive force for the cation exchanger) and Na⁺/H⁺ antiport activity by salt stress has been recorded in glycophytes and halophytes (Garbarino and DuPont, 1988; Barkla et al., 1995, 1999; Ballesteros et al., 1997; Parks et al., 2002). In some salt-sensitive species (Plantago media), no antiport activity could be recorded after salt treatment (50 mM NaCl) (Staal et al., 1991). However, in glycophytes bearing limited salt tolerance, like cotton and sunflower, a detectable (Na⁺,K⁺)/H⁺ antiport activity was shown in the absence of salt (Hassidim et al., 1990; Ballesteros et al., 1997). Thus, biochemical and physiological data support the role of Na⁺/H⁺ antiporters regulating cytoplasmic Na⁺ concentration by vacuolar sequestration when Na⁺ trespasses on the plasma membrane selectivity barrier. Differences between glycophytes and halophytes in the basal and inducible antiport activity and ion selectivity might be related to their degree of salt tolerance.

Osmotic stress rather than ion-specific stress seems to activate NHX activity at the vacuole since mannitol and KCl induced AtNHX1, AtNHX2, and OsNHX1 mRNA abundance as much as NaCl (Gaxiola et al., 1999; Shi and Zhu, 2002; Yokoi et al., 2002; Fukuda et al., 2004). In yeast, SacNHX1 provided resistance to hyperosmotic shock (Nass and Rao, 1999). After exposure to sudden hyperosmotic stress, wild-type cells showed rapid shrinkage of their vacuoles but resumed growth earlier than nhx1 mutant cells. Abscisic acid (ABA), a drought- and osmotic stress-related hormone, enhanced expression of AtNHX1 and AtNHX2 in Arabidopsis (Quintero et al., 2000; Yokoi et al., 2002), LeNHX2 in tomato (Venema et al., 2003), and GhNHX1 in cotton (Wu et al., 2004), but it failed to modify antiport activity in Mesembryanthemum crystallinum (Barkla et al., 1999). Differences in the ABA responsiveness of NHX genes may provide further clues to elucidate the different mechanisms of regulation of antiport activity in salt-sensitive and -tolerant species. Gene up-regulation of AtNHX1 and AtNHX2 in Arabidopsis was dependent on both ABA biosynthesis and signalling, since the stress...
response was greatly reduced in aba2-1, aba3-1, and abii1-1 mutants but not in abi2-1 (Shi and Zhu, 2002; Yokoi et al., 2002). Transcript abundance of AtNHX1 and AtNHX2 was greater in sos1, sos2, or sos3 mutants, presumably as a result of their exacerbated Na⁺ uptake (Yokoi et al., 2002).

Overexpression of NHX antiporters has been used to improve salt tolerance in several plant species (Apse et al., 1999; Zhang and Blumwald, 2001; Zhang et al., 2001; Ohta et al., 2002; Fukuda et al., 2004; Xue et al., 2004; Wu et al., 2004, 2005). Transformed plants showed improved plant survival and increased shoot growth over the control lines under salt stress. Arabidopsis overexpressing AtNHX1 grew better than the wild type and accumulated Na⁺ in the shoot, but the extent to which ectopic expression of AtNHX1 increased Na⁺ concentration in tissues relative to controls was not determined (Apse et al., 1999). Rice has been transformed to overexpress NXH antiporters from a halophyte (AgNHX1, Atriplex gmelini) (Ohta et al., 2002) or its own (OsNHX1) (Fukuda et al., 2004). The AgNHX1-plants showed greater survival rates after an osmotic shock (300 mM NaCl, 3 d) (Ohta et al., 2002). Unfortunately, only leaf Na⁺ contents on a dry-matter basis were reported, offering no relevance for physiological interpretation as K⁺ content and/or plant water status parameters were not presented. Cells derived from OsNHX1-overexpressing plants and growing at 100–200 mM NaCl showed greater growth and Na⁺ concentration but lower K⁺ content than controls (Fukuda et al., 2004). Surprisingly, K⁺ and Na⁺ concentrations in leaves of transformed plants did not show significant differences (Fukuda et al., 2004). Ryegrass plants transformed with OsNHX1 showed greater leaf Na⁺, K⁺, and proline contents than control plants (Wu et al., 2005). By contrast, leaves from transgenic wheat expressing AtNHX1 accumulated lower levels of Na⁺ but higher levels of K⁺ than non-transgenic plants (Wu et al., 2005). These conflicting reports call for a cautionary note. Heterologous expression of proteins could modify the ion selectivity of NHX antiporters, particularly if ion selectivity by individual NHX proteins is under physiological control that could be distorted by ectopic expression. Heterologous expression of other ion exchangers has been reported to result in dominant phenotypes of distorted ion homeostasis (Hirschi, 1999). Furthermore, relevant information is still missing about the way NHX expression affects ion compartmentalization within the cell, overall ion homeostasis, and osmotic adjustment, in order to fulfil the basic physiological premises that sustain the increase in salt tolerance.

Transient shifts of intracellular and apoplastic pH are essential steps in several signal transduction processes. Cytoplasmic acidification, combined with alkalinization of the apoplasm, loss of cellular K⁺, influx of external Ca²⁺, and an oxidative burst are rapidly induced by microbial elicitors. The increase in external pH originates from an influx of protons, which contributes to cytoplasmic acidification. In addition, lysophosphatidylcoline (LPC) generated by phospholipase A2 at the plasma membrane triggers the release of vacuolar protons via the activation of a Na⁺-dependent proton efflux that was inhibited by amiloride (Viehweger et al., 2002). In the presence of 1 μM LPC, the proton efflux rate saturates at 10 mM Na⁺, a physiological concentration of Na⁺ in the cytosol. These data suggest the involvement of an NHX-like exchanger at the vacuolar membrane that is stimulated by LPC, and have broader implications for pH-controlled signal relay by this family of proteins. The current availability of Arabidopsis knockout mutants in genes encoding vacuolar NHX isoforms should allow the unequivocal assignment of function in biotic-stress signalling to specific NHX exchangers.

Endosomal pH regulation

Cellular functioning requires the maintenance of pH optima for the enzymes that participate in the different metabolic pathways through mechanisms of pH stabilization often called pH-stat. Plant metabolism generates H⁺ or OH⁻ ions that should be balanced to preserve intracellular pH (Raven, 1985). Biotic or abiotic stresses also induce cytoplasmic pH changes triggering adaptive metabolic reactions (Sakano, 2001). The concerted transport of ions across membranes may compose a biophysical pH-stat. Electrophysiological studies suggest that tonoplast antiporters are among the mechanisms responsible for the regulation of cytoplasmic and vacuolar pH and of ion gradients induced by salt stress (Carden et al., 2003).

The critical role of NHX exchangers in regulating vacuolar pH is best illustrated by the flower colour transition in Ipomoea spp. The petal colour of Ipomoea changes from red-purple in flower buds to blue in the fully open flower, and the same anthocyanin pigment accumulated in vacuoles is responsible for both colours. Anthocyanins give a blue colour in alkaline solution and a red colour in an acidic environment. The flower colour transition from red to blue is accompanied by an increase in the pH of petal vacuoles from 6.6 to 7.7 in one of the few instances in which vacuolar pH is alkaline relative to the cytosol (Yoshida et al., 1995). Also during the colour transition there is a simultaneous increase in protein levels and activities of V-PPase, V-ATPase, and PM-ATPase, and the de novo appearance of NHX1, whose polypeptide and activity (measured as Na⁺/H⁺ exchange) was still absent 6 h before the fully open stage was reached (Yoshida et al., 2005). Insertion of a transposable element in the InNHX1 gene of Ipomoea nil partly abrogated the vacuolar pH shift and flower colour transition (Fukuda-Tanaka et al., 2000; Yamaguchi et al., 2001). InNHX2, another isoform that is also expressed in vegetative tissues may co-operate with InNHX1 to control the alkaline pH shift in petal vacuoles (Ohnishi et al., 2005).
Vesicle trafficking

Eukaryotic cells are spatially structured in a complex and functionally co-ordinated system of internal membranes that serve to carry proteins to their site of action. These internal membranes subdivide the cell into a series of membrane-bound compartments, defined by their lipid and protein composition, and comprise the organelles of the secretory and endocytic pathways: the endoplasmic reticulum (ER), the Golgi apparatus, the trans-Golgi network (TGN), prevacuolar compartments (PVC), lytic compartments (vacuoles or lysosomes), and endosomes (Fig. 2). Exchange of material between them is thought to be achieved by small vesicles which bud from one membrane and fuse with another in a highly controlled fashion. The basic mechanisms involved in membrane-exchange reactions appear to be conserved in eukaryotes, from microorganisms to mammals. Newly synthesized proteins are transported through the secretory pathway to the ER lumen or membrane, pass from the ER through the Golgi apparatus, and in late Golgi enter the secretory pathway or are diverted to other cellular compartments: the vacuole in yeast and plant cells, or the lysosome in animal cells. In yeast and animals the material destined for the vacuole is sorted away from that destined for the plasma membrane in the TGN, whereas in plants the sorting can start as early as at the cis-Golgi stacks (Hillmer et al., 2001) or bypass the Golgi and be directed from the ER to storage vacuoles (Hara-Nishimura et al., 1998). The vacuole also receives material from the cell surface delivered by the process of endocytosis. These two pathways overlap at a set of membrane vesicles called the multivesicular body (MVB) in animal cells and prevacuolar compartment (PVC) in yeast and plants (Fig. 2). Besides the anterograde transport to the vacuole, retrograde transport of proteins exists between the vacuole and earlier compartments: the MVB, Golgi, or ER (Gotte and Lazar, 1999).

Fusion of transport vesicles with the target membranes depends on SNARE (soluble N-ethylmaleimide-sensitive factor adaptor protein receptor) molecules in the vesicle (v-SNARE) and target (t-SNARE) membranes (Sollner et al., 1993; Ungermann et al., 1998; Weber et al., 1998). The interaction of these proteins is regulated by small GTPases (RAB/YPT), belonging to the Ras superfamily. RAB/YPT proteins cycle between active GTP-bound and inactive GDP-bound states. The guanine nucleotide exchange factor catalyses GDP/GTP exchange and RAB-GTP remains anchored in the donor membrane where it intervenes in the v- and t-SNARE interaction. A new cycle starts with the hydrolysis of GTP enhanced by a GTPase-activating protein, GAP (Strom et al., 1993). ScNHX1, the endosomal yeast Na+/H+ exchanger, was originally defined as an ion homeostasis determinant mediating vacuolar sequestration of Na+, coupling Na+ movement to the proton gradient established by the vacuolar H+-ATPase (Nass et al., 1997; Nass and Rao, 1998). Besides sodium sensitivity, nhx1 mutants show hygromycin sensitivity (Gaxiola et al., 1999; Quintero et al., 2000), acidification of the vacuolar lumen and impaired growth at low pH (Ali et al., 2004; Brett et al., 2005b), and a ‘class E’ vacuolar protein-sorting phenotype characterized by enlargement of the prevacuolar compartment and missorting of vacuolar carboxypeptidase Y to the cell surface (Bowers et al., 2000). Ali et al. (2004)

Fig. 2. The plant endomembrane system. The connecting membrane exchange routes are represented by arrows. ER, Endoplasmic reticulum; GA, Golgi apparatus; TGN, trans-Golgi network; Vac, Vacuole; EE, early endosome; PVC, prevacuolar compartment; PM, plasma membrane.
demonstrated that the carboxy-terminal tail of ScNHX1 binds to GYP6, a GTPase-activating protein that also regulates the YPT6-mediated vesicle fusion in the retrograde PVC to Golgi pathway (Fig. 3). It has been proposed that NHX1 regulates the luminal pH of PVC and that GYP6 acts as a negative effector on ScNHX1 activity. When ScNHX1 is inhibited by GYP6, a relative acidification of the compartment would lead to relatively more acidic vesicles that would be targeted to the vacuole. The relief of ScNHX1 inhibition by competitive binding of YPT6 to GYP6 would result in less-acidic vesicles that could be directed to retrograde delivery to the TGN/Golgi (Fig. 3).

Recently, Brett et al. (2005b) have shown that the trafficking defects of nhx1 strains, but not their salt sensitivity, can be mimicked by the addition of weak acids or ameliorated by the addition of weak bases, providing further evidence that pH regulation by ScNHX1 is critical for trafficking pathways out of the late endosome.

There is also a critical requirement for a specific luminal ionic concentration and pH in vesicular trafficking of proteins in animal cells. Several pH-dependent processes are: ligand–receptor dissociation (Dautry-Varsat et al., 1983), endocytosis (Aniento et al., 1996; Chapman and Munro, 1994; Maranda et al., 2001), post-translational processing and sorting of newly synthesized proteins (Halban and Irminger, 1994), binding of coat proteins to early endosomes (reviewed in Stevens and Forgac, 1997), and the association of vesicles with an ARF GTPase and its GTP/GDP exchanger in pancreatic cells (Zeuzem et al., 1992; Maranda et al., 2001). The abnormal lysosomal/endosomal morphologies and associated defective trafficking observed in a class of lysosomal storage disorders are associated with abnormal changes in luminal pH (Futerman and van Meer, 2004). Changes in cellular pH alone severely alter morphology and movement of organelles (Heuser, 1989). The plasma membrane Na⁺/H⁺ exchangers of the NHE family are clearly associated with cellular pH regulation in mammalian cells (Orlowski and Grinstein, 2004), but much less is known about the properties of the intracellular subgroup despite the recent discovery of numerous candidate genes from model animal organisms and higher vertebrates, including human NHE6–NHE9 (reviewed by Brett et al., 2005a). Their intracellular location within the endomembrane system and the remarkable conservation of protein trafficking mechanisms in eukaryotes brings up the possibility that each may regulate pH-mediated trafficking to or from different organelles. NHE6 is found in endosomes (Brett et al., 2002; Nakamura et al., 2005), NHE7 is located in the trans-Golgi network of brain cells where it is thought to play a significant role in organelle pH and volume homeostasis (Numata and Orlowski, 2001), and NHE9 is found in the late recycling endosomes (Nakamura et al., 2005) when expressed in mammalian cell culture models. By regulating the pH within these compartments, they are likely to influence numerous pH-mediated trafficking events in the endocytic

Fig. 3. Model for endosomal traffic regulation in yeast. GYP6 binds to NHX1 at the PVC, inhibiting its activity and promoting anterograde transit (left side) of relatively acidic vesicles to the vacuole (Vac). In retrograde traffic (right side) YPT6:GTP competes with NHX1 for GYP6 binding, relieving its inhibition. The relative alkalization of the endosome due to NHX1 activity promotes vesicular retrograde traffic to the trans-Golgi network and the hydrolysis of GTP. YPT6:GDP returns to the TGN and is reactivated by a guanine nucleotide exchange factor protein.
pathway of cells such as linking ligand–receptor dissociation to membrane recycling or lysosomal protein degradation.

The above precedents in yeast and animal cells, together with endosomal localization and a strong preference for K⁺/H⁺ exchange (Yokoi et al., 2002; Venema et al., 2003) suggest that class-II NHX proteins are likely to fulfil essential roles in the control of endosomal pH and vesicle trafficking in plants (Fig. 2). By the criteria of cation specificity and subcellular localization, class-II NHX transporters are the ScNHX1 counterparts in the endosomal compartment of plant cells, and they are likely candidates to intervene in vesicular trafficking by mechanisms equivalent to those described by Ali et al. (2004) for ScNHX1. It is tempting to hypothesize that class-II NHX transporters would thus play a role in the regulation of vesicle trafficking by contributing to the maintenance of the required pH and K⁺ gradients in the endosomal compartment. Class-I NHX transporters could also be involved in protein sorting and vesicular trafficking processes since a T-DNA insertional nhx1 mutant of Arabidopsis shows, among other phenotypic traits, reduced leaf area which correlates with the overall reduction of epidermal cell size. This phenotype could be attributed not only to the loss of ability of the cells to maintain a proper ion homeostasis in the vacuole, leading to loss of vacuolar turgor, but also to a defective vacuole biogenesis as it has been described for nhx1 mutants of yeast (Bowers et al., 2000; Apse et al., 2003). Although neither vacuole biogenesis nor vesicle trafficking has been experimentally analysed in nhx mutants of plants, the transcriptome of wild-type and nhx1 mutant lines of Arabidopsis does in fact show significant differences in the expression of transcripts associated with protein processing and vesicular trafficking (Sottosanto et al., 2004). Whether vacuolar NHX isoforms have a specific role in vesicle trafficking or transcriptome alterations result indirectly from a putative defect in vacuole formation is presently unknown.

Mineral nutrition

The vacuole occupies as much as 80–90% of the cell volume in mature plant cells and quantitatively locates most of the cell K⁺ (Leigh and Wyn Jones, 1984). Functionally, vacuoles are K⁺ reservoirs that ensure adequate supply to the cytosol. K⁺ concentration in vacuoles of barley root varied proportionally with the overall tissue K⁺ concentration, whereas cytosolic K⁺ remained constant over a wide range of tissue K⁺ concentrations. At diminishing K⁺ availability, the cytosolic K⁺ concentration was higher than the vacuolar concentration (Walker et al., 1996). Cytosolic K⁺ concentrations declined only when vacuolar K⁺ concentrations decreased to values around 20 mM (Leigh and Wyn Jones, 1984).

In several studies performed on salt tolerance provided by the overexpression of NHX genes, a greater leaf K⁺ concentration was found associated with the tolerant phenotype (Zhang and Blumwald, 2001; Xue et al., 2004; Wu et al., 2005). Considering that NHX antiporters of class I may exchange K⁺ as well as Na⁺ for vacuolar H⁺ (see above), overexpression might affect cytosolic K⁺ by directing the active transport of K⁺ into the vacuole when K⁺ availability is high (Walker et al., 1996). In fact, electrophysiological studies suggest the suitability of tonoplast antiporters for the homeostatic regulation of cytosolic K⁺ when cells are provided with surplus concentrations of K⁺ (Walker et al., 1996). Thus, NHX antiporters may play a significant role in K⁺ nutrition together with ion channels by capturing K⁺ into vacuoles for cellular storage and turgor generation at physiological growth conditions (Leigh and Wyn Jones, 1984; Hsiao and Läuchli, 1986) besides their protective function under salt or osmotic stress (Nass and Rao, 1999; Blumwald, 2000). Under K⁺ deficiency, cytoplasmic acidification occurs and it may act as a signal affecting K⁺ transport activity at the plasma membrane and the tonoplast (Walker et al., 1996). Animal NHE homologues are controlled by cytosolic pH through C-terminal regulatory domains (Putney et al., 2002), and a similar mechanism may exist for plant NHX proteins. Potassium uptake seems affected in Arabidopsis nhx1 mutants as both a lower shoot K⁺ content and a lower root K⁺ (Rb⁺) uptake rate than in the wild type have been observed (Leidi et al., 2005). Thus, the reduced leaf expansion in nhx1 mutants (Apse et al., 2003) may be linked to a role of AtNHX1 in net K⁺ uptake and the gain in cell turgor required for growth (Mengel, 1996). By contrast, AtNHX1-overexpressing tomato showed greater K⁺ (Rb⁺) uptake than control lines but were nevertheless prone to showing K⁺ deficiency symptoms at leaf K⁺ concentrations greater than the control line (Leidi et al., 2005). This paradoxical phenotype is likely to be due to exacerbated activity of the AtNHX1 antiporter in the transgenic lines which could increase, unduly, the vacuolar pool at the expense of the cytoplasmic K⁺ pool, thereby inducing a K⁺ starvation signal and eliciting greater K⁺ uptake by roots.

Plasma membrane exchangers

SOS1 phylogeny and function

The SOS1 antiporter belongs to the NhaP subfamily of the CPA1 family, which is related but is clearly different from the NHE/NHX subfamily. In a previous phylogenetic classification of Arabidopsis membrane transporters, SOS1 was renamed NHX7 and included in the NHX family (Mäser et al., 2001). This was probably due to the fact that plant NhaP is a very small family, with only two members, and the analysis method may have failed to differentiate between NHX/NHE and SOS1/NhaP clusters. When the phylogenetic analysis was performed with a larger number of NhaP proteins from several organisms,
its sensitivity was increased and the two different clades could be easily distinguished (Fig. 4) (Brett et al., 2005a).

For this reason and to avoid confusion, it is suggested that the NHX7 name should be discontinued in favour of the original name SOS1.

The NhaP group of exchangers is present in bacteria, protozoa, and plants (Brett et al., 2005a). The predicted structure of NhaP-like exchangers consists of an N-terminal transmembrane region followed by a hydrophilic C-terminal extension that, in eukaryotic NhaP homologues, is remarkably long, with >600 residues. Since C-terminal regions in many other transporters have been shown to interact with regulatory proteins (Putney et al., 2002), a complex regulation of eukaryotic NhaP should be expected. Subcellular localization and biochemical characterization of NhaP in eukaryotic organisms is not available, with the sole exception of Arabidopsis SOS1. By contrast to NHX proteins, SOS1 has been shown to be localized at the plasma membrane (Shi et al., 2002), where it catalyses an electroneutral Na+/H+ exchange (Qiu et al., 2002; Shi et al., 2002). In addition, SOS1 is highly specific for Na+ and cannot transport other monovalent cations, such as K+ or Li+. Since biochemical data supporting the presence of plasma membrane Na+(K+)/H+ antiport activity were found in only a limited number of plant species (Mennen et al., 1990), the very existence of Na+/H+ exchangers in plasma membranes was questioned. The cloning of the Arabidopsis SOS1 gene and the presence of >120 ESTs (expressed sequence tags) encoding SOS1-like proteins from 31 different species demonstrates not only the existence of this family of transporters but also its wide distribution in plants. The size of plant NhaP gene families within individual species is not clear because very few full-length sequences are available. Only one SOS1-like gene has been reported in Physcomitrella patens and in Cymodocea nodosa (Benito and Rodriguez-Navarro, 2003). In the fully sequenced genomes of rice and Arabidopsis, one (Os12g44360) and two (SOS1 and At1g14660) genes, respectively, have been found. The protein encoded by locus At1g14660 is very similar to SOS1 (72% identity) but is about 400 residues shorter than SOS1 at the C-terminus. The function of the protein encoded by locus At1g14660 is still unknown. The strong phenotype of sos1 plants suggests that protein At1g14660 probably plays a non-overlapping role with SOS1 despite their high similarity at the sequence level. By contrast, the rice SOS1 protein has been shown to be the functional equivalent of the Arabidopsis SOS1 since it shows Na+/H+ antiport activity and partially complements the Arabidopsis sos1 mutation (J Martínez-Atienza and JM Pardo, unpublished results).

Based on the expression pattern of SOS1 and the physiological characterization of sos1 mutant plants, it has been suggested that SOS1 controls long-distance Na+ transport in Arabidopsis (Shi et al., 2002). The expression pattern of SOS1 was examined using a SOS1 promoter:GUS fusion. GUS activity was mainly detected in the inner tissues surrounding the vasculature throughout the plant. In the root, cross-sectional analysis revealed that GUS activity was localized primarily in the pericycle and in parenchyma cells bordering xylem vessels. In stem and petiole cross-sections, GUS activity was also restricted to parenchyma cells at the xylem/symplast boundary. Besides its association with the vasculature, SOS1 expression was observed at the epidermal cells of the root tip. As undifferentiated cells at the root tip lack vacuoles large enough for significant Na+ compartmentalization, SOS1 might be critical to prevent Na+ from accumulating in these cells by actively extruding Na+ back to the soil solution.

The preferential expression of SOS1 in cells surrounding the vasculature suggests a role of this transporter in long-distance Na+ transport in plants, since Na+ is transported from the root to the shoot via the xylem. When grown in the presence of a high Na+ concentration (100 mM NaCl), the roots and aerial parts of sos1-mutant plants accumulated more Na+ than wild-type plants. The Na+ concentration in the xylem sap of both sos1-mutant and wild-type plants increased substantially over time, but the Na+ concentration was always higher for sos1. By contrast, sos1 mutant plants accumulated less Na+ than the wild type in response to low levels (25 mM NaCl) of salt stress (Ding and Zhu, 1997; Shi et al., 2002). These contradictory results have been explained by suggesting that SOS1 might function in loading Na+ into the xylem for controlled delivery to the

**Fig. 4.** Phylogenetic tree of plant NHE/NHXs and NhaP/SOS1 exchangers. Sequence alignment of the Arabidopsis and rice NHXs families and all the plant full-length SOS1-like proteins was performed using Clustal X. GenBank accession numbers: AtSOS1, AAF76139; AtSOL1, NP_172918; OsSOS1, AAW33875; CnSOS1, CAD20320; Pp, CAD91921.
Regulation of the SOS system

Significant progress has been made towards the understanding of SOS1 regulation. Na⁺/H⁺ exchange by SOS1 is detected in plasma membrane vesicles from salt-stressed Arabidopsis plants but not in control plants, indicating that SOS1 activity is induced by stress (Qiu et al., 2002). The up-regulation of SOS1 occurs at two levels at least: mRNA stability and ion exchange rate. Key intermediaries in the regulation of SOS1 are the protein kinase SOS2 and its associated Ca²⁺-sensor protein SOS3. Mutant plants deficient in either SOS2 or SOS3 share the salt-sensitive and K⁺-deficiency phenotype of sos1 plants (Zhu, 2000, and references therein). SOS3 is a calcium-binding protein capable of sensing calcium transients elicited by salt stress. SOS2 is a serine/threonine protein kinase whose catalytic domain is evolutionarily related to that of the yeast protein SNF1 and the animal AMP-activated kinases (Liu et al., 2000). The C-terminal part of SOS2 is unique to plants and physically interacts with SOS3 in a Ca²⁺-dependent manner through the FISL domain located at the boundary between the regulatory C-terminus and the catalytic domain of SOS2 to relieve the kinase from auto-inhibition (Guo et al., 2001). The FISL domain is itself inhibitory and its deletion results in constitutive activation of SOS2 (Guo et al., 2004). The crystal structure of SOS3 has been resolved recently (Sánchez-Barrena et al., 2005). Upon Ca²⁺ binding, SOS3 undergoes dimerization and hydrophobic domains are exposed. This change in surface properties may facilitate interaction with SOS2 and the transmission of the Ca²⁺ signal elicited by stress. Adjacent to the SOS3-binding domain of SOS2 is the PPI domain for interaction with ABI2, a type-2C protein phosphatase that is a negative intermediary in ABA signalling (Ohta et al., 2003). Although this interaction may be a cross-talk point between ABA signalling and the SOS pathway for ion homeostasis under salinity stress, there is no direct evidence of the regulation of SOS1 by ABA. Moreover, sos2 and sos3 mutants are specifically defective in salt tolerance but not in ABA responses (Ohta et al., 2003). Conversely, mutants deficient in the SOS2-like kinase PKS3 or the SOS3-like protein SCaBP5, whose complex also interacts with ABI2, are hypersensitive to ABA but not significantly affected in salt tolerance (Guo et al., 2002). The dominant negative protein phosphatase encoded by the abi2-1 allele, which brings about ABA insensitivity rather than hypersensitivity, cannot interact with SOS2 or PKS3 and permits greater tolerance to salt shock, suggesting that dissociation of the ABI2/SOS2 complex is a requisite for the progression of the salt-stress signal. Whether or not activation of SOS1 is a signalling output of the stress phenotype associated with the abi2-1 allele is presently unknown.

Biochemical activation of Na⁺/H⁺ exchange of SOS1 by salt stress is controlled through protein phosphorylation by the SOS2/SOS3 kinase complex. Co-expression of the three proteins in a yeast strain lacking endogenous Na⁺ transporters restored salt tolerance to a much greater extent than SOS1 alone, whereas SOS2 or SOS3 individually failed to stimulate SOS1 activity (Quintero et al., 2002). SOS2 and SOS3 had no effect per se in the absence of SOS1. The SOS2/SOS3 kinase complex promoted the phosphorylation of SOS1 in yeast plasma membrane fractions and purified SOS2 phosphorylated SOS1 in vitro, demonstrating that SOS1 is a genuine substrate for SOS2 kinase. Consistent with these interactions, SOS1 activity in the plasma membrane was not induced by salt stress in sos2 or sos3 mutant plants (Qiu et al., 2002). However, the addition of a constitutively active, SOS3-independent form of SOS2 to plasma membrane vesicles isolated from sos2 or sos3 plants, but not from sos1, restored SOS1 activity. Besides kinase activation, SOS3 was also shown to recruit SOS2 to the plasma membrane to achieve efficient interaction with SOS1 (Quintero et al., 2002). N-myristoylation of SOS3 is necessary for its functionality in planta (Ishitani et al., 2000) and for the targeting of the SOS2/SOS3 complex to the plasma membrane (Pardo et al., 2005). Nonetheless, the evaluation in planta of an array of mutant alleles of SOS2 has revealed that the kinase may reach the plant plasma membrane in the absence of a functional SOS3 protein, perhaps as a result of interactions with other proteins such as alternative SCaBP proteins or even ABI2 (Guo et al., 2004). The SOS2/SOS3 kinase phosphorylates SOS1 at the C-terminus and stimulates its Na⁺ extrusion activity in yeast, and C-terminally truncated SOS1 mutants become hyperactive and independent of SOS2/SOS3 (Pardo et al., 2005). These results indicate that SOS1 is released from auto-inhibition by its C-terminal through protein phosphorylation.

The transcript level of SOS1 is up-regulated by salt stress by a mechanism that is partly dependent on SOS2 and SOS3 (Shi et al., 2000). Regulation of mRNA abundance seems to be post-transcriptional because it was observed even when SOS1 transcription was driven by the constitutive CaMV 35S promoter (Shi et al., 2003). The low level of transcript accumulated in transgenic lines under standard growth conditions suggests that the SOS1 transcript is
unstable in the absence of salt and that stress causes a post-transcriptional stabilization of the transcript. Nothing is known about the factor(s) that regulate SOS1 mRNA stability.

**Interaction of the SOS pathway with K⁺ nutrition**

*Arabidopsis* mutants lacking the SOS1 transporter or one of its regulatory proteins, SOS2 and SOS3, manifest symptoms of K⁺ deficiency on culture medium with low K⁺ (<200 μM K⁺) implying that the Ca²⁺-regulated SOS pathway has a positive regulatory effect on K⁺ acquisition. SOS1 is the only known target of the SOS2/SOS3 kinase complex and it has been shown to mediate Na⁺/H⁺ exchange but to lack any detectable K⁺ transport capacity, both in its basal state and after activation by the SOS2/SOS3 kinase (Qiu et al., 2002; Quintero et al., 2002; Shi et al., 2002). High Na⁺ concentration in the soil solution is thought to disturb K⁺ acquisition by competing with binding sites in transport systems that mediate K⁺ uptake (Hasegawa et al., 2000), but the K⁺-deficiency phenotype of sos mutants is perplexing and raises the question of how a disturbed Na⁺ homeostasis impinges on K⁺ nutrition beyond the competition for common transporters.

The K⁺-deficiency phenotype of sos mutants is conditional as it is exacerbated by low Ca²⁺ (0.15 mM) and suppressed by high Ca²⁺ (3 mM). Ca²⁺ has long been known to improve K⁺/Na⁺ selectivity, which effectively reduces Na⁺ uptake and increases salinity tolerance. Based on biochemical and electrophysiological studies, three co-existing pathways for Na⁺ influx have been proposed (Tester and Davenport, 2003). Two pathways are transporter-mediated components that can be distinguished by their relative sensitivity to extracellular Ca²⁺. The third pathway (bypass flow) is attributed to leakage through the apoplastic and to discontinuities in the endodermis, for instance at the points of emergence of lateral roots. The relative contribution of each pathway varies with species and growth conditions. Bypass flow is particularly apparent in rice (Yeo et al., 1987). In excised *Arabidopsis* roots incubated with 50 mM NaCl at low Ca²⁺ activity (0.2 mM), the three components of Na⁺ influx were of similar magnitude (≈0.6 μmol g⁻¹ min⁻¹; Essah et al., 2003), although bypass flow might have been underestimated by the use of detached roots lacking transpirational water flux. Electrophysiological work suggests that non-selective cation channels (NSCCs) at the plasma membrane account for at least part of the transporter-mediated Na⁺ uptake that is Ca²⁺-sensitive. NSCCs are characterized by their poor discrimination among inorganic monovalent cations. In addition, some NSCCs are also permeable to divalent cations like Ca²⁺ and Mg²⁺, but many are, instead, inhibited by Ca²⁺ (reviewed by Demidchik et al., 2002). The *Arabidopsis* genome has revealed the existence of two large families of NSCCs with sequence similarity to animal counterparts, namely the cyclic nucleotide-gated and calmodulin-regulated channels (CNGCs) and the glutamate receptor-like channels (GLRs), some of which may correspond to the NSCC activities characterized electrophysiologically. Maathuis and Sanders (2001) described in the plasma membrane of *Arabidopsis* root protoplasts the presence of CNGC activity that was deactivated by cAMP and cGMP, by contrast to animal CNGCs and other plant CNGCs which are activated by ligand (cAMP or cGMP) binding to the channel protein (Demidchik et al., 2002; Talke et al., 2003). Moreover, when supplemented into the growth medium, both cyclic nucleotides effectively restricted Na⁺ uptake by seedlings and increased salt tolerance (Maathuis and Sanders, 2001). At the molecular level, the inward-rectifier and non-inactivating channel AtCNGC1, which is expressed in plant roots and conducts Na⁺ equally as well as K⁺, may provide a physiologically significant Na⁺ entry from the soil solution in the Ca²⁺-sensitive pathway (Sunkar et al., 2000; Leng et al., 2002; Hua et al., 2003). Presumably, the inhibition of NSCC channels (AtCNGC1 and others) by Ca²⁺ diminishes the Na⁺ load of sos mutants and alleviates the K⁺-deficiency phenotype in low K⁺.

Remarkably, hkt1 mutations that were initially isolated as extragenic suppressors of the Na⁺ hypersensitivity of the sos3 mutant also suppressed the K⁺-deficiency phenotype of sos mutants completely, whereas moderate overexpression of AtHKT1 exacerbated K⁺ deficiency in wild-type and sos3 seedlings (Rus et al., 2001, 2004). Heterologous expression in yeast and *Xenopus* oocytes has shown that plant HKT transporters are all permeable to Na⁺ and, in some cases, to K⁺ as well. The wheat transporter TaHKT1 functions as a high-affinity Na⁺/K⁺ symporter at low external K⁺ or Na⁺ concentrations but, in the presence of sufficient amounts of both ions, K⁺ transport by TaHKT1 was blocked and only low-affinity Na⁺ uptake occurred (Rubio et al., 1995; Gassman et al., 1996). In rice, OsHKT1 and OsHKT4 isoforms are specific high- and low-affinity Na⁺ transporters, respectively, whereas OsHKT2 showed Na⁺/K⁺-coupled transport (Horie et al., 2001; García de Blas et al., 2003). High-affinity Na⁺ transport by OsHKT1 was inhibited by 1 mM K⁺ but K⁺ transport itself was marginal (García de Blas et al., 2003). In *Arabidopsis*, genetic and electrophysiological analyses indicate that its single AtHKT1 protein performs several physiological functions all pertaining to specific Na⁺ transport. Mutants lacking AtHKT1 failed to show significant reduction in short-term Na⁺ uptake by roots (Essah et al., 2003) but manifested a consistent increment of Na⁺ in aerial parts with a concomitant reduction in Na⁺ root content (Mäser et al., 2002; Berthomieu et al., 2003; Rus et al., 2004). *AtHKT1* gene expression has been localized to the root stele and leaf vasculature, with preferential accumulation in phloem tissues (Mäser et al., 2002; Berthomieu et al., 2003). Na⁺ content in the phloem sap of hkt1 mutants was reduced...
7-fold, whereas Na⁺ content was slightly greater in the xylem sap (Berthomieu et al., 2003). These results indicate that AtHKT1 controls root/shoot Na⁺ partition, reducing leaf Na⁺ accumulation by Na⁺ re-circulation from shoots to roots. The current model posits that AtHKT1 mediates Na⁺ loading into the phloem sap in leaves and unloading in roots, and that influx or efflux through AtHKT1 would be determined by the local electrochemical gradient of Na⁺ (Berthomieu et al., 2003). Although export from leaves could help to maintain low salt concentrations in the shoot, it should be kept in mind, however, that there appears to be comparatively little retranslocation of salt from leaves relative to the amount imported in the transpiration stream (Munns, 2005). Plants transpire about 50 times more water that they retain in their leaves. Estimates of xylem and phloem fluxes of Na⁺ and Cl⁻ indicated that, in barley, phloem export from a leaf was only 10% of the import from the xylem. As exemplified by rice (Ren et al., 2005), retrieval of ions from the xylem sap could conceivably be a more practical strategy to ward off excessive accumulation of salts in the shoot (see below) than export in the phloem.

The evidence gathered from the genetic analysis of the Na⁺ transporters SOS1 and AtHKT1 establishes that K⁺ nutrition is linked to Na⁺ homeostasis in more complex ways than the widely accepted model that Na⁺ competes with K⁺ for uptake through transport systems mediating K⁺ acquisition. In the sos mutants extrusion of Na⁺ to the soil solution is impaired due to insufficient SOS1 activity at the root epidermis (Qiu et al., 2002; Shi et al., 2002). SOS1 is also preferentially expressed in xylem parenchyma cells where it contributes to Na⁺ loading of the xylem and subsequent translocation to the shoot along the transpiration stream (Shi et al., 2002). Consequently, Na⁺ is accumulated in the root of sos mutants (Shi et al., 2002; Rus et al., 2004). In this scenario of compromised xylem loading, incremental Na⁺ deposition in the root stele by Na⁺ re-circulation and phloem unloading would be detrimental and averted by inactivation of AtHKT1. Indeed, single sos³ and hkt1 mutations promote the undue accumulation of Na⁺ in roots and shoots, respectively, whereas a double sos³ hkt1 mutant achieves a more balanced partition of Na⁺ that is closer to the profile of wild-type plants (Rus et al., 2004). Excessive retention of Na⁺ in cells of the root stele of sos mutants in a AtHKT1 wild-type background may impede transverse flux of K⁺ to the stele and/or K⁺ loading of the xylem sap under low K⁺ conditions. Reduction of stelar Na⁺ by mutation of AtHKT1 may restore sufficient K⁺ flux from the root cortex towards the xylem. It appears that the transport function of the SOS system and AtHKT1 are co-ordinated tightly and together they achieve Na⁺ (and K⁺) homeostasis. Dysfunction of either system alters long-distance transport and adequate partition of Na⁺, thereby resulting in salt-sensitive phenotypes.

Recent evidence implicates the K⁺ inward-rectifying channel AKT1 as a target for Na⁺-induced failure of K⁺ uptake (Qi and Spalding, 2004). In growth medium with millimolar NH₄⁺ concentrations, which block non-channel K⁺ uptake systems, Arabidopsis seedlings rely on the AKT1 channel for physiologically relevant K⁺ uptake. In Na⁺-free media with high NH₄⁺, the AKT1-dependent K⁺-uptake ability of the sos mutant root cells, measured electrophysiologically, was normal and the growth rates of these mutants displayed wild-type K⁺ dependence. Addition of 50 mM NaCl strongly inhibited root-cell K⁺ permeability and growth rate in K⁺-limiting conditions of sos1 but not wild-type plants. Moreover, addition of 10 mM NaCl into the cytoplasmic side of patch-clamped wild-type root cells inhibited AKT1 K⁺ channel activity completely. Therefore, it appears that elevated cytoplasmic Na⁺ levels resulting from loss of SOS1 function impaired K⁺ permeability in root cells and compromised K⁺ nutrition. It is also conceivable that excessive retention of Na⁺ in the root stele of sos mutants due to reduced xylem loading (Shi et al., 2002; Rus et al., 2004) may result in the inhibition of additional K⁺ transporters involved in long-distance transport of this nutrient, such as SKOR (shaker-like K⁺ outward-rectifying channel), a channel expressed in the stele of the root that mediates K⁺ efflux into the xylem for distribution from root to shoot (Gaymard et al., 1998). However, growth measurements showed that akt1 seedlings were salt sensitive during early seedling development, but skor seedlings were normal, suggesting that the detrimental effect of Na⁺ on K⁺ transport through channels is probably more important at the uptake stage than at the xylem-loading stage (Qi and Spalding, 2004).

Non-channel targets for Na⁺ inhibition in the long-distance transport pathway for K⁺ may nevertheless exist. Lacan and Durand (1996) have shown that excised soybean roots treated with NaCl reabsorbed Na⁺ from the xylem vessels in exchange for K⁺. This net Na⁺/K⁺ exchange at the xylem/symplast interface was strongly linked because Na⁺ in the xylem sap enhanced K⁺ release, whereas increased xylematic K⁺ prompted Na⁺ reabsorption. Na⁺ removed from the xylem sap was subsequently excreted to the external medium. Lacan and Durand (1996) suggested that reabsorption of Na⁺ from the acidic xylem sap by a Na⁺/H⁺ antiporter was energetically enabled through H⁺ cycling by K⁺/H⁺ antiporter and H⁺/anion symport. Based on the expression pattern of SOS1 and the ion profile of sos1 plants at various salinity regimes, Shi et al. (2002) have suggested that SOS1 has a dual role in loading and reabsorption of Na⁺ from the xylem stream. When salinity is moderate, SOS1 functions to load Na⁺ into the xylem for controlled delivery to the shoot and storage in leaf mesophyll cells. Under severe salt stress, Na⁺ might quickly accumulate in the shoot, exceeding the capacity of vacuoles in mesophyll cells to compartmentalize ions. Under these conditions, SOS1 would function to restrict net Na⁺ uptake at the root tip and to retrieve Na⁺ from the xylem in the mature root, both processes helping to limit...
rapid accumulation of Na+ in the shoot. If Na+ and K+ fluxes at the xylem/symplast boundary of Arabidopsis were coupled to produce the net Na+/K+ exchange as proposed by Lacan and Durand (1996), a functional SOS1 protein might be required to enable K+ loading to the xylem when plants were growing in the presence of Na+. In other organisms, transporters of the CPA2 family are well characterized and they show very different catalytic properties. KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997). KefB and KefC mediate K+ transport, whereas KefA, a member of the CPA2 family, is involved in Na+ transport into the cytoplasm of cyanobacteria (Millard et al., 1997).

Control of long-distance transport of Na+ may function differently in rice plants. Fine mapping of a major QTL for shoot K+ content and salt tolerance in rice identified SKC1 (Ren et al., 2005), an HKT-type protein corresponding to gene OsHKT8 (Garcia-Deblas et al., 2003), as a Na+-selective transporter preferentially expressed in the parenchyma cells surrounding xylem vessels. SCK1/HKT8 transcripts were more abundant in roots and up-regulated by salt stress (Ren et al., 2005). In electrophysiological tests, the SCK1/HKT8 isoform from the relatively salt-tolerant variety Nona Bokra was more active in the facilitation of Na+ transport across the plasma membrane than its counterpart from the salt-sensitive Koshihirakari variety, suggesting that greater capacity for Na+ retrieval from the xylem by SCK1/HKT8 in Nona Bokra plants was the basis for their salt-tolerance. Conversely, increased Na+ concentration in the xylem sap and leaves was associated with the weaker SKC1 allele of the salt-sensitive variety. Whether SCK1/HKT8 functions co-ordinately with the rice SOS1 homologue to regulate the net Na+ load of the xylem remains to be determined. Also unknown is whether or not in rice there is a functional homologue of AtHKT1 from Arabidopsis permitting the recirculation of Na+ via the phloem and contributing further to the restriction of Na+ accumulation in the leaves.

**CHX family**

**Phylogeny and classification**

The CHX transporters, along with the related KEA subfamily, constitute the CPA2 family of cation/proton antiporters. This is probably the largest group of cation exchangers in plants. According to Sze et al. (2001) there are 28 CHX isoforms in Arabidopsis and 17 in rice. Arabidopsis CHXs are subdivided into five groups (I–V). Rice CHXs can be assigned to groups I, IV, and V; there are no rice orthologues of groups II and III. The functional meaning of this classification is unknown, but it could be related to substrate specificity or subcellular targeting as illustrated by the NHX family. All CHXs have a similar size of about 800 amino acids and, so far, there are no biochemical data demonstrating the transport mechanism or substrate specificity of any of the CHX family members. In other organisms, transporters of the CPA2 family are well characterized and they show very different catalytic properties. KefB and KefC mediate K+ efflux in Escherichia coli, and they seem to act as ligand-gated channels (Miller et al., 1997). NapA from Enterococcus hirae and NhaS3 from Synechocystis sp. are Na+/H+ antiporters (Waser et al., 1992; Inaba et al., 2001), and GerN from Bacillus cereus has a dual exchange capacity as an Na+/H+ or Na+/K+ antiporter (Southworth et al., 2001). Finally, the yeast KHA1 antiporter, the only reported eukaryotic CPA2 member, localizes in an intracellular compartment (Maresova and Sychrova, 2005), probably the mitochondria (Sickmann et al., 2003), and indirect evidence suggests that it could transport K+. Any or several of the transport modes described for homologous proteins can be expected in the plant CHX family.

**Function of CHX exchangers**

Numerous transcriptome analyses have been carried out in Arabidopsis but, unfortunately, the CHX gene family seem to be expressed at a very low level and no functional hint for any member of this family can be drawn from its expression pattern. Changes in transcript levels were detected only for AtCHX10 and AtCHX15, which in both cases were down-regulated by salt stress (Maathuis et al., 2003). Recently, a detailed gene expression analysis of the complete Arabidopsis CHX family was performed using sensitive techniques (Sze et al., 2001). Most CHX genes were specifically expressed during male gametophyte development. Important changes in K+ and H+ concentrations have been reported to occur throughout the gametophyte differentiation process in tobacco, and these oscillations have been associated with the regulation of biosynthetic reactions. Moreover, the whole anther is an important K+ sink, having almost twice the concentration of apical leaves (Andreyuk et al., 2001). Potassium transport is also essential during pollen tube elongation, and disruption of the Arabidopsis SPIK K+ channel results in impaired pollen tube growth (Mouline et al., 2002). CHX exchangers with K+ transport capacity may play different roles in any of those complex processes taking place during gametophyte differentiation, but the large number of individual CHX genes apparently expressed in these organs is nevertheless surprising.

Only two CHX isoforms, AtCHX17 and AtCH23, have been studied to date following a genetic approach (Cellier et al., 2004; Song et al., 2004). AtCHX17 encodes a protein, 820 residues long, that is most similar to the nhaS4 protein in Synechocystis sp. Expression of nhaS4 in an E. coli mutant strain, deficient in several endogenous antiporters, could not restore Na+ tolerance but it allowed growth in medium with low K+ concentration. Transcripts of AtCHX17 were detected in roots only when the plants were salt stressed, K+ starved, or upon treatment with acidic pH or the hormone ABA. In two transcriptome analysis reports, AtCHX17 was also up-regulated by Ca2+ deprivation and by osmotic- and cold-stress (Kreps et al., 2002;
Maathuis et al., 2003). A more detailed analysis using GUS reporter activity and in-situ RT-PCR showed that AtCHX17 transcription was restricted to epidermal and cortical cells of roots. GUS activity was also detected in anthers of flowering plants. Transgenic plants containing the AtCHX17 promoter-GUS gene did not show a significant change when they were K⁺ starved or challenged with acidic pH, and the up-regulation caused by salt stress was much lower. This discrepancy was explained as the result of post-transcriptional regulation or the absence of regulatory sequences in the AtCHX17 promoter fragment used in the GUS fusion construct. Attempts to express AtCHX17 in Saccharomyces cerevisiae were unsuccessful. To gain information about AtCHX17 function, two independent homozygous T-DNA insertion lines within the gene were phenotypically characterized. No anatomical differences with the wild type were found under all conditions tested, including salt stress and K⁺ starvation, although salt-stressed or K⁺-starved roots of chx17 plants contained ~20% less K⁺ than wild type. No significant changes were observed in the shoot K⁺ content. More research data will be necessary to confirm the putative function of AtCHX17 in K⁺ homeostasis and elucidate its substrate specificity.

The AtCHX23 exchanger localizes to the chloroplast envelope and plays an essential role in chloroplast development (Song et al., 2004). Plants with reduced expression of AtCHX23 by RNAi showed leaf chlorosis and were smaller in size. Chloroplast number was lower in comparison with the wild type and their structure was altered. A similar phenotype was observed in a chx23 mutant plant obtained by TILLING. Interestingly, extracellular alkaline pH exacerbated the observed phenotype but acidic pH alleviated the symptoms of the mutant. These results indicate the involvement of CHX23 in chloroplast pH regulation. Stromal pH oscillates depending on the photosynthetic conditions (Jagendorf and Uribe, 1966) and a cation/H⁺ antiporter located in the chloroplast envelope was suggested to be, in part, responsible for these pH changes (Demming and Gimmler, 1983). In agreement with the involvement of AtCHX23 on the control of stromal pH, it was demonstrated that cytoplasmic pH in guard cells of the mutant was higher than in the wild type, presumably due to malfunctioning of the chloroplast pH-stat (Song et al., 2004). The effects of Na⁺ and K⁺, the likely substrates of AtCHX23, were also investigated. Mutant chx23 plants were more sensitive to NaCl, and Song et al. (2004) suggested that Na⁺ sequestration into the chloroplast could be a possible mechanism contributing to salt tolerance. However, the correlation between salt sensitivity and chloroplast disorganization possibly points to chloroplast function as one of the targets of Na⁺-toxicity. Recently, Liska et al. (2004) found that enhanced photosynthesis might be one of the processes contributing to the halotolerance of the green alga Dunaliella salina. Therefore, preservation of chloroplast pH-stat and functionality would be important for salt-stress adaptation. By contrast, addition of KCl produced better growth of the mutant roots and the guard cell aperture was increased, presumably because the absence of K⁺ antiport activity at the chloroplast envelope could be compensated by increases in the cytosolic K⁺ concentration. Taken together, these observations strongly suggest that CHX23 is a K⁺(Na⁺)/H⁺ antiporter.

Concluding remarks

During evolution, plants have accrued a large array of ion transporters, many of which are exchangers of monovalent cations. Emerging data indicate that members of the latter group play relevant roles in mineral nutrition, vesicle trafficking and protein sorting, plant development, ion accumulation into the vacuole, resistance to salinity, and response to pathogens. Most of these functions have been unravelled by genetic data and the trend will continue in the near future with forward, reverse, and quantitative genetics contributing to the unequivocal assignment of specific functions to, hopefully, each and every member of this large group of ion transporters. This may seem an enormous task but, considering the physiological processes impinged by cation exchange at the various cell membranes, their importance cannot be overstated.

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