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Regulation of expression of the vacuolar Na^+/H^+ antiporter gene *AtNHX1* by salt stress and abscisic acid

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Abstract

The *Arabidopsis thaliana AtNHX1* gene encodes a vacuolar Na^+/H^+ antiporter that is important in salt tolerance. We report here the tissue distribution and regulation of *AtNHX1* expression by salt stress and abscisic acid (ABA). The steady-state level of *AtNHX1* transcript was up-regulated by treatment with NaCl, KCl or ABA. *AtNHX1* promoter-GUS analysis in transgenic *Arabidopsis* showed that *AtNHX1* was expressed in all tissues except the root tip. Strong GUS expression was detected in guard cells, suggesting that AtNHX1 may play a role in pH regulation and/or K^+ homeostasis in the specialized cells. *AtNHX1* promoter activity was substantially up-regulated by NaCl, KCl or ABA, demonstrating that salt and ABA regulation of *AtNHX1* expression occurs at the transcriptional level. Strong induction of GUS activity in root hair cells was observed, which suggests a role of AtNHX1 in storing Na^+ in the enlarged vacuoles in root hair cells. The up-regulation of *AtNHX1* transcript levels by NaCl was reduced in *abi1-1*, *aba2-1* and *aba3-1*, but not in *abi2-1*, *sos1*, *sos2* or *sos3* mutants. ABA-induced *AtNHX1* expression was also decreased in *abi1-1* but not in *abi2-1*. These results suggest that salt stress up-regulates *AtNHX1* expression transcriptionally and the up-regulation is partially dependent on ABA biosynthesis and ABA signaling through ABI1.

Introduction

Na^+/H^+ antiporters are ubiquitous membrane proteins that play major roles in cellular pH and Na^+ homeostasis throughout the biological kingdom. They catalyze the exchange of Na^+ for H^+ across membranes. In animals, Na^+/H^+ exchangers of the NHE family are implicated in a variety of important physiological functions, including regulation of cell volume, intracellular pH, and Na^+ homeostasis (for review, see Counillon and Pouyssegur, 2000). NHE are present in various tissues and cells, and six isoforms (NHE1-6) have been reported. NHE1-5 are localized to the plasma membrane and NHE6 has a mitochondrial distribution. The plasma membrane NHEs have been extensively studied, and much is known about their expression and regulation (Counillon and Pouyssegur, 2000). In yeast, SOD2 was identified from *Schizosaccharomyces pombe* as a Na^+/H^+ antiporter on the plasma membrane involved in salt tolerance (Jia *et al.*, 1992). A homologue of *SOD2*, *NHA1*, was cloned from *Saccharomyces cerevisiae* and found to contribute to Na^+ extrusion, although Na^+-ATPases have a dominant role in reducing intracellular Na^+ concentration (Prior *et al.*, 1996). A novel antiporter, NHX1, was identified from *S. cerevisiae* and was localized to a late endosomal/prevacuolar compartment where it mediates intracellular sequestration of Na^+ in a pH-dependent manner (Nass *et al.*, 1997; Nass and Rao, 1998). This finding indicates a role for intracellularly localized Na^+/H^+ antiporters in mediating NaCl tolerance through prevacuolar compartmentation of Na^+. In addition, control of Na^+ influx by modulation of the TRK1 and TRK2 K^+ uptake system also provides a mechanism for yeast to adapt to salt stress (Gomez *et al.*, 1996). In *Escherichia coli*,

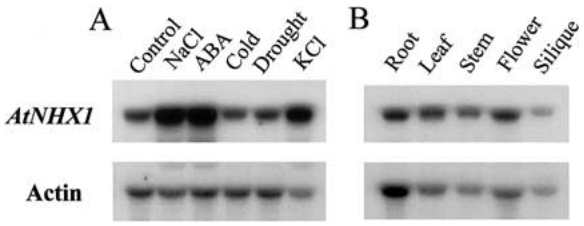

Figure 1. RNA blot analysis of *AtNHX1* expression. Twenty micrograms of total RNA from each sample was hybridized with a ^{32}P-labeled *AtNHX1* probe. Actin is shown as loading control. A. *AtNHX1* expression is up-regulated by NaCl, ABA and KCl treatments. NaCl, 300 mM NaCl for 5 h; ABA, 100 μM ABA for 3 h; Cold, 0 °C for 24 h; Drought, dehydration for 30 min; and KCl, 300 mM KCl for 5 h. RNA was isolated from 10-day old seedlings of the Ler ecotype. B. Expression of *AtNHX1* in different plant parts. Different parts were collected from wild-type Col plants.

two genes, *NhaA* and *NhaB*, encoding Na^+- and Li^+-specific Na^+/H^+ antiporters were identified (Goldberg *et al.*, 1987; Pinner *et al.*, 1992). Deletion of these genes from the bacterial genome revealed a critical role of the Na^+/H^+ antiporters in salt tolerance and pH homeostasis (Padan *et al.*, 1989; Pinner *et al.*, 1993). The expression, regulation, and functional domains of the *E. coli* antiporters have been extensively studied, which also contributes to our understanding of Na^+/H^+ antiporters from other organisms (Padan *et al.*, 2001).

The presence of Na^+/H^+ antiporter activities has been detected in both plasma membrane and tonoplast vesicle preparations from different plant species (Blumwald *et al.*, 2000). Physiological studies have suggested a role of Na^+/H^+ antiporters in plant salt tolerance (Blumwald and Poole, 1985; Ballesteros *et al.*, 1997). Facilitated by large-scale EST sequencing and the *Arabidopsis* genome project, much progress has been made recently on the molecular characterization of Na^+/H^+ antiporters from plants (Zhu, 2001). A significant advance in understanding the role of Na^+/H^+ antiporters in ion homeostasis in plants under salt stress was made by the cloning of the *SOS1* gene from the *salt overly sensitive Arabidopsis* mutant (Shi *et al.*, 2000). *SOS1* encodes a plasma membrane Na^+/H^+ antiporter and functions to control long distance Na^+ translocation from root to shoot in *Arabidopsis* (Shi *et al.*, 2002a). Over-expression of *SOS1* in *Arabidopsis* conferred the transgenic plants with increased tolerance to NaCl stress (Shi *et al.*, 2002b). The vacuolar Na^+/H^+ antiporter has long been proposed to play important roles in salt tolerance. However, its molecular nature was not revealed until recently. Gaxiola *et al.* (1999) found that *AtNHX1*, an *Arabidopsis* homologue of the yeast *NHX1*, was able to suppress some of the phenotypes of the yeast *nhx1* mutant. The level of *AtNHX1* mRNA was increased in plants treated with 250 mM NaCl or 250 mM KCl for 6 h (Gaxiola *et al.*, 1999). Quintero *et al.* (2000) have also demonstrated functional substitution of *AtNHX1* for yeast *NHX1* and the up-regulation of *AtNHX1* transcripts by NaCl and ABA. Apse *et al.* (1999) provided evidence demonstrating that AtNHX1 is localized in tonoplasts and functions as a Na^+/H^+ antiporter in *Arabidopsis*. Using a yeast expression system, Darley *et al.* (2000) also provided direct evidence of AtNHX1 for pH-gradient-energized Na^+ accumulation into vacuoles of yeast cells. Moreover, overexperession of AtNHX1 improved salt tolerance in both *Arabidopsis* and tomato plants (Apse *et al.*, 1999; Zhang and Blumwald, 2001), further supporting a role of the vacuolar Na^+/H^+ antiporters in salt tolerance. Intriguingly, Apse *et al.* (1999) did not find any up-regulation of *AtNHX1* mRNA by NaCl treatment. Aside from *Arabidopsis*, vacuolar Na^+/H^+ antiporter genes have also been identified from other plant species (Fukuda *et al.*, 1999; Fukuda-Tanaka *et al.*, 2000; Hamada *et al.*, 2001). Cloning and analysis of a putative vacuolar Na^+/H^+ antiporter gene from a morning glory mutant have revealed a critical role of the vacuolar Na^+/H^+ antiporter in pH regulation of vacuoles in floral tissues (Fukuda-Tanaka *et al.*, 2000).

Although the molecular identification and biochemical characterization of Na^+/H^+ antiporters in plants is advancing rapidly, their basic functions in cells and whole plants are still not fully understood. In particular, the expression pattern and regulation of vacuolar Na^+/H^+ antiporter genes have not been vigorously investigated. Here we report on the pattern of expression and salt stress regulation of *AtNHX1* in *Arabidopsis*. *AtNHX1* expression was detected in all tissues except the root tip. *AtNHX1* promoter activity was up-regulated by NaCl, KCl or ABA, suggesting that *AtNHX1* expression was regulated by salt and ABA at the transcriptional level. Salt stress up-regulation of *AtNHX1* transcript levels was reduced in *abi1-1*, *aba2-1* and *aba3-1*, but not in *abi2-1*, *sos1*, *sos2* or *sos3* mutants. ABA induction of *AtNHX1* was also reduced in *abi1-1* but not in *abi2-1*. Our results show that salt stress transcriptionally up-regulates *AtNHX1* expression and the up-regulation is partially dependent on ABA biosynthesis and ABA signaling through ABI1.

Materials and methods

Plant materials and growth conditions

The *Arabidopsis thaliana* genotypes used in this study were Columbia (Col) and Landsberg (Ler) wild-types, and *abi1-1*, *abi2-1*, *aba2-1*, *aba3-1*, *sos1-1*, *sos2-1* and *sos3-1* mutants. The *abi* and *aba* mutant seeds were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH). The *sos* mutants were generated in our laboratory (Wu *et al.*, 1996; Zhu *et al.*, 1998). Seeds were surface-sterilized in a solution of Clorox plus 0.05% Triton X-100 for 10 min, washed with sterilized water for three times, and suspended in sterile 0.3% low-melting-point agarose. Suspended seeds were treated at 4 °C for three days to synchronize germination and then planted onto agar medium containing Murashige and Skoog (MS) salts (Murashige and Skoog, 1962), 3% sucrose and 0.6% agar, pH 5.7, with or without antibiotics. Plates were then incubated at 22 °C under continuous illumination. For the growth of mature plants, 10-day old young seedlings were transferred from MS agar plates to soil in pots and grown under normal growth conditions (Wu *et al.*, 1996).

Plant treatments and RNA blot analysis

Ten-day old seedlings grown in MS agar plates were used for different treatments. For NaCl, KCl, ABA and cold treatments, the seedlings were transferred to a filter paper soaked with MS salts solution containing 300 mM NaCl or 300 mM KCl for 5 h, or sprayed with 100 μM ABA and kept for 3 h, or stored at 4 °C for 24 h as described before (Shi *et al.*, 2000). For dehydration treatment, seedlings were transferred from MS agar medium to a filter paper and kept in a flow hood for 30 min. For the collection of plant parts, wild-type plants were grown in Turface soil (Profile Products LLC) to facilitate root harvesting. Roots and leaves were collected from 3-week old seedlings, and the stems, flowers and siliques were collected after plants flowered. Total RNA was prepared and northern analysis was performed as described previously (Zhu *et al.*, 1998). An *AtNHX1*-specific probe containing the 3′-untranslated region was used for northern hybridization.

Construction of AtNHX1 *promoter-GUS*

A ca. 1.9 kb promoter region just upstream of ATG start codon of *AtNHX1* gene was amplified from genomic DNA by PCR and verified by sequencing. The PCR fragment was cloned into the *Hin*dIII/*Pst*I site of binary vector pCAMBIA 1391Z to obtain a transcriptional fusion of the *AtNHX1* promoter and the GUS coding sequence. The constructs were introduced into *Agrobacterium* and transferred into *Arabidopsis* Columbia wild-type plants using the vacuum infiltration method (Bechtold *et al.*, 1993). Twenty independent transgenic lines in the T_2 generation were grown on MS medium containing 25 mg/l hygromycin and subjected to β-glucuronidase (GUS) assays.

GUS assays

Seeds and young seedlings at different developmental stages and different parts from mature transgenic plants were collected and used for histochemical detection of GUS expression. For general detection of GUS expression patterns in seedlings and different cells and organs, materials were stained at 37 °C overnight in 1 mg/ml X-gluc, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 0.03% Triton X-100, and 0.1 M sodium phosphate buffer (pH 7.0). To test the induction of GUS expression by NaCl, ABA or KCl, 10-day old transgenic seedlings were subjected to the same treatments as described above (Plant treatments and RNA blot analysis). The treated and control transgenic seedlings were stained in 1 mg/ml X-gluc, 0.03% Triton X-100, 20 mM HEPES buffer pH 7.0 for 5 h for histochemical detection. For quantitative assays, GUS activities in control and treated transgenic seedlings were determined by measuring 4-methylumbelliferone (4-MU, Sigma) produced from the glucuronide precursor 4-methylumbelliferyl-β-D-glucuronide as described by Jefferson (1987). The GUS activity was calculated in units of μmol MU per mg protein per minute.

Results

AtNHX1 *expression is up-regulated by NaCl, KCl and ABA*

NaCl treatment is known to up-regulate vacuolar Na^+/H^+ antiporter activities and enhances Na^+ compartmentation into vacuoles (Blumwald *et al.*, 2000). This up-regulation is either due to the activation of existing proteins, and/or increase in the level of gene transcripts. Conflicting data have been reported regarding the induction of *AtNHX1* transcripts in *Arabidopsis* (Gaxiola *et al.*, 1999; Apse *et al.*, 1999).

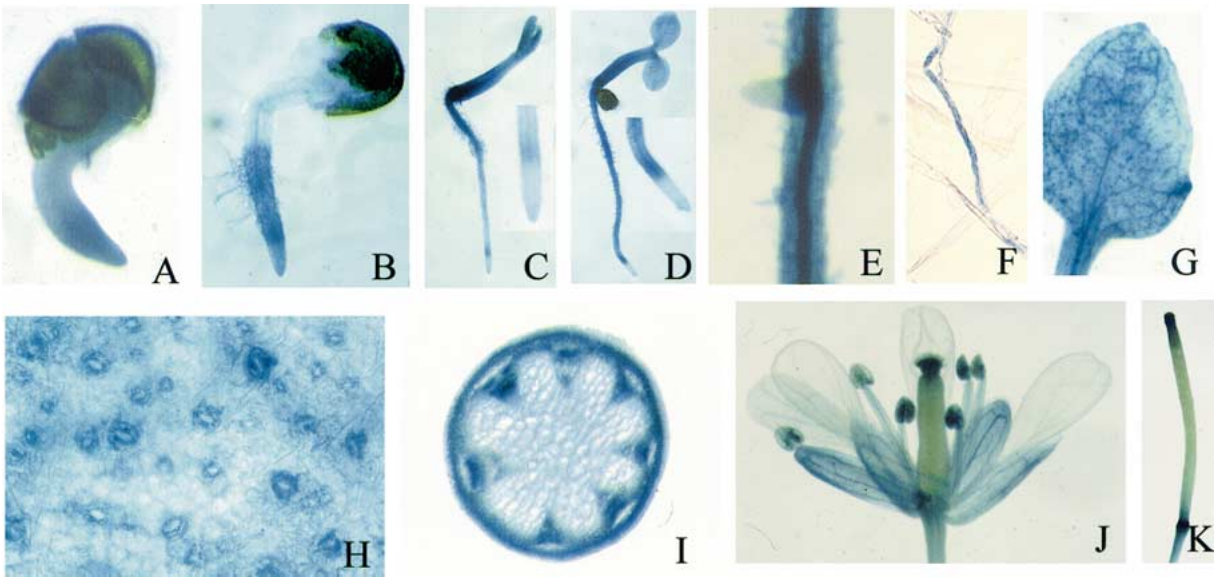

Figure 2. AtNHX1 promoter-GUS expression pattern in transgenic *Arabidopsis* plants. A. One-day old germinating seed. B. Two-day old germinating seed. C. Three-day old seedling; insert: enlarged picture of root tip. D. Four-day old seedling; insert: enlarged picture of root tip. E. Young root with an emerging lateral root, showing no GUS activity in the root tip; there was also no GUS activity in the main root tip (not shown). F. Root hair. G. Leaf. H. Strong GUS activity in guard cells. I. Hand section of an inflorescence stem. J. Flower. K. Silique.

We carried out RNA blot analysis to verify whether *AtNHX1* expression is regulated by different stresses. Ten-day old *Arabidopsis* seedlings of the Columbia (Col) or Landsberg (Ler) background were subjected to different treatments. As shown in Figure 1A, the transcript level of *AtNHX1* in the Ler background was up-regulated by NaCl, ABA and KCl, but not by cold or dehydration treatments. The *AtNHX1* transcript was detected in the root, leaf, stem, flower and silique (Figure 1B), indicating ubiquitous expression of *AtNHX1* in *Arabidopsis* plants. In the Col background, AtNHX1 transcript levels in both shoots and roots were also increased upon NaCl treatment (see Figure 5B).

AtNHX1 *is expressed throughout plant development*

To determine the tissue expression pattern of *AtNHX1* during plant development, a promoter-GUS analysis was employed. A ca. 1.9 kb promoter region upstream of the *AtNHX1* ATG start codon was fused with the β-glucuronidase reporter gene (GUS) and the resulting construct was introduced into wild-type *Arabidopsis* plants. GUS expression was monitored throughout plant development by histochemical staining. As depicted in Figure 2, GUS expression was detected at all stages tested, from seed germination to flowering and seed setting, confirming that *AtNHX1* is expressed at all developmental stages and throughout the *Arabidopsis* plant. GUS expression was detected in one-day old germinated seed, with stronger GUS staining in the emerging radicle (Figure 2A). Two days after germination, GUS staining appeared to be stronger in the root including the root hair, but relatively weaker in the hypocotyl (Figure 2B). The root tip at this stage had GUS expression but the expression was relatively lower than that in the other parts of the root (Figure 2B). In three- and four-day old seedlings, GUS expression was detected throughout the plants except the root tip (Figure 2C, D). Similarly, no GUS activity was detected in the root tip of two-week old seedlings (Figure 2E). Strong GUS expression in the root hair of young seedlings suggests possibly an important role of AtNHX1 in Na^+ compartmentalization into the enlarged vacuoles of root hair cells (Figure 2F). Interestingly, a high level of GUS expression was detected in guard cells when visualized under high magnification (Figure 2H), which appeared as dark blue spots of GUS staining in the leaf (Figure 2G). GUS activity was also observed in the inflorescence stem, with strong staining in parenchyma cells of the cortex and vascular strands (Figure 2I). In flowers, strong GUS staining was observed in sepals and in pollens within anthers (Figure 2J). In siliques, GUS staining was evident at the tip and base (Figure 2K).

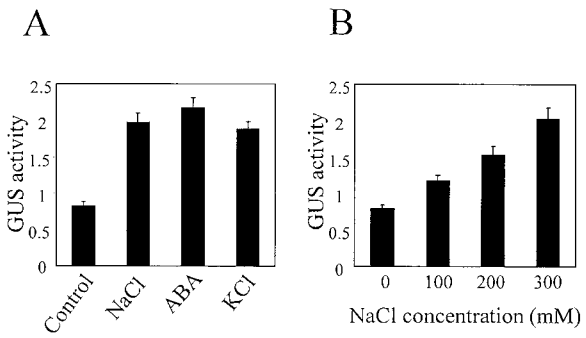

Figure 3. Salt and ABA up-regulation of GUS expression driven by the *AtNHX1* promoter. Results are the average of 6 replicates with standard deviation. A. Effects of NaCl, ABA and KCl treatments on the GUS activity in transgenic seedlings. B. NaCl dose response of *AtNHX1* promoter-GUS in the transgenic seedlings.

AtNHX1 *promoter activity is enhanced by NaCl, KCl and ABA treatments*

Since the steady-state level of *AtNHX1* transcript is up-regulated by NaCl, KCl and ABA (Figure 1), the regulation may be transcriptional and conferred by *cis* elements in the promoter region of *AtNHX1*. Transcriptional fusion of *AtNHX1* promoter with GUS reporter gene provides a tool to test if the promoter is responsive to NaCl, KCl, or ABA treatment. Figure 3A shows that GUS expression driven by the *AtNHX1* promoter was increased by treatment with NaCl, ABA or KCl. The GUS activity after NaCl, ABA and KCl treatments was 2.4-fold, 2.6-fold and 2.3-fold higher, respectively, than that in untreated control samples. The GUS activity increase was correlated with the NaCl concentration used in the treatments (Figure 3B). Higher GUS activity was obtained in response to higher concentrations of NaCl treatment (Figure 3B). Histochemical GUS staining indicated that the GUS activity increased in both the leaf and root after treatments with 300 mM NaCl for 5 h (Figure 4A). Similar increases in GUS expression were observed for the ABA and KCl treatments (not shown). Without NaCl treatment, GUS expression in the root was relatively stronger in the vascular tissue (Figure 4B). After NaCl treatment, the GUS activity in roots was dramatically increased (Figure 4C). Observations under higher magnification revealed that the GUS activity increase occurred mainly in epidermal cells with root hairs (Figure 4D). Histochemical staining revealed that GUS activity increases in the leaf upon NaCl treatment was mainly in leaf mesophyll cells (data not shown). No substantial increase in GUS activity was observed in guard cells after NaCl, ABA or KCl treatments (data not shown).

Up-regulation of AtNHX1 *is partially dependent on ABA but not the SOS signal transduction pathway*

To elucidate the genetic control of *AtNHX1* regulation by NaCl and ABA, *AtNHX1* transcription levels in wild type, *abi* and *aba* mutants were determined and compared. RNA samples shown in Figure 1 and Figure 5A were loaded onto the same gel and transferred to a single blot. The RNA blot was subjected to hybridization and signals from all samples were detected under identical conditions to facilitate comparison. As shown in Figure 5A, the increases of *AtNHX1* transcript upon both NaCl and ABA treatments were reduced in the *abi1-1* mutant compared with those in the wild type (Figure 1A). Interestingly, the up-regulation of *AtNHX1* expression in *abi2-1* was similar to that in wild-type plants (Figure 5A). In the ABA-deficient *aba* mutants, the transcript level of *AtNHX1* after ABA treatment was, as expected, similar to that in the wild type (Figure 5A). However, the up-regulation of *AtNHX1* transcript by NaCl treatment in both the *aba2-1* and *aba3-1* mutants was substantially reduced (Figure 5A). These results show that the up-regulation of *AtNHX1* expression by NaCl is partially mediated through ABA, and is partially dependent on the ABI1 branch of ABA signal transduction pathways.

The SOS signal transduction pathway controls ion homeostasis under salt stress in *Arabidopsis* (Zhu, 2001). To determine if the induction of *AtNHX1* expression by NaCl is under control of the SOS signaling pathway, *AtNHX1* transcript levels in the shoot and root of wild-type, *sos1*, *sos2* and *sos3* mutant plants were analyzed. Figure 5B shows that the expression levels of *AtNHX1* in *sos* mutants are generally similar to that in the wild-type, except for somewhat higher basal levels in untreated *sos1* and *sos3* mutant roots. The results indicate that *AtNHX1* gene expression is not significantly regulated by the SOS pathway.

AtNHX1 *promoter contains a putative ABA-responsive element*

A number of *cis* elements containing the consensus ABA-responsive element (ABRE) were identified in various ABA-responsive genes either by functional analysis or by sequence similarities. Most functionally defined ABREs contain the core sequence, ACGT (Shinozaki and Yamaguchi-Shinozaki, 2000). The up-regulation of *AtNHX1* mRNA and GUS expression

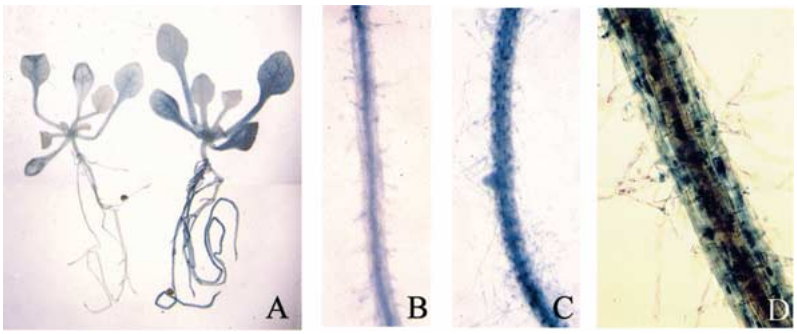

Figure 4. Tissue localization of enhanced GUS expression in *AtNHX1* promoter-GUS transgenic seedling treated with NaCl. A. Increased GUS activity in both roots and leaves; left, control without NaCl treatment; right, treated with 300 mM NaCl for 5 h. B. GUS expression in the root without treatment. C. GUS expression in the root treated with NaCl. D. Enlarged image of the root in C, showing GUS expression induced in root hair cells.

driven by the *AtNHX1* promoter in response to NaCl and ABA indicate that the *AtNHX1* promoter may contain *cis* elements responsible to ABA and stresses. Sequence analysis of the *AtNHX1* promoter revealed that the sequence between −736 to −728 (ATG as position 0), ACACGTCT, is conserved in ABA responsive genes in different plant species. This sequence contains the ACGT core sequence that has been shown to be essential for ABA response for a number of genes. Future studies will determine whether this putative ABRE is important in the regulation of *AtNHX1* expression.

Discussion

Although AtNHX1 is the first and most intensively studied plant vacuolar Na^+/H^+ antiporter to date (Blumwald *et al.*, 2000), its *in planta* function has only been inferred from ectopic overexpression studies and the regulation of its gene expression is largely unknown. Conflicting data regarding salt stress regulation of *AtNHX1* expression in *Arabidopsis* have been reported (Gaxiola *et al.*, 1999; Apse *et al.*, 1999; Francisco *et al.*, 2000). Under our experimental conditions, the *AtNHX1* transcript level was up-regulated by NaCl, KCl or ABA, but not by cold or dehydration treatments. Similar levels of up-regulation were observed by treatments with the same concentrations of NaCl and KCl, suggesting a non-specific salt stress response. Interestingly, unlike *SOS1* (Shi *et al.*, 2000), *AtNHX1* was also induced by ABA treatment, which suggests that different signal transduction pathways may be involved in the regulation of plasma membrane Na^+/H^+ antiporter (i.e. SOS1) and vacuolar Na^+/H^+ antiporter (i.e. AtNHX1) genes. Indeed, salt stress up-regulation of *SOS1* (Shi *et al.*, 2000) but not *AtNHX1* (Figure 5B) was affected by mutations in the *SOS2* and *SOS3* regulatory genes.

Salt stress signaling in plants occurs via ABA-dependent and ABA-independent pathways (Shinozaki and Yamaguchi-Shinozaki, 2000). The observation of reduced salt stress up-regulation of *AtNHX1* in *abi1-1*, *aba2-1* and *aba3-1* mutants suggests a partially ABA-dependent regulation of *AtNHX1* expression. The presence of a putative ABA-responsive element within the promoter region of the *AtNHX1* gene, and the up-regulation of *AtNHX1* promoter-GUS reporter gene expression by NaCl, KCl and ABA further supports our notion of a partially ABA-mediated transcriptional regulation. The ABA-dependent part of the salt stress regulation appears to be mediated by the ABI1 and not the ABI2 pathway.

AtNHX1 was expressed at a high level in leaves and the expression was significantly increased upon NaCl stress, particularly in older leaves (Figure 4). This is consistent with the fact that Na^+ compartmentalization into the vacuoles of leaf cells is an important salt tolerance mechanism (Apse *et al.*, 1999). *AtNHX1* expression in root hair cells was strongly up-regulated by salt stress as suggested by promoter-GUS analysis. The sequestration of Na^+ into the vacuole of hair cells would decrease the cellular water potential and facilitate water uptake through the root hairs under NaCl stress. Since root hair cells contain enlarged vacuoles, a high level of *AtNHX1* expression would increase the storage of Na^+ in these cells, which would attenuate Na^+ entry into the cortex and then the xylem, and thus reduces Na^+ transport from root to shoot. In the context of whole plant function under salt stress, it is likely that SOS1 and AtNHX1 coordinate functionally

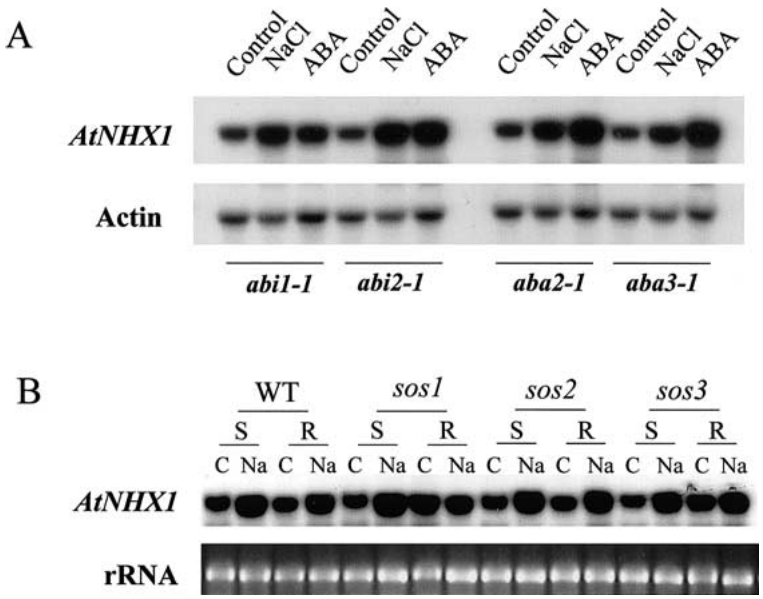

Figure 5. The up-regulation of *AtNHX1* expression is partly under control of the ABA signaling pathway. A. *AtNHX1* expression in *abi* and *aba* mutants. NaCl and ABA treatments were the same as in Figure 1A; actin is shown as loading control. B. *AtNHX1* expression in wild-type (WT), *sos1-1*, *sos2-2*, and *sos3-1* mutants. Ethidium bromide-stained rRNA is shown as loading control. S, shoot; R, root; C, control; Na, 300 mM NaCl for 5 h.

to confer salt tolerance. *SOS1* is expressed preferentially in the parenchyma cells surrounding the vascular tissue to control the amount of Na^+ to be transported to the shoot for compartmentalization in leaf vacuoles through vacuolar antiporters such as AtNHX1 (Shi, 2002a). Strong expression of *SOS1* but lack of expression of *AtNHX1* in root tips suggests that the meristematic tissues rely on Na^+ efflux rather than compartmentation to maintain low cytoplasmic Na^+ concentration, due perhaps to the fact that these cells lack large developed vacuoles.

Besides a function under salt stress, the role of AtNHX1 under normal growth conditions remains to be answered. Ubiquitous and a relatively high basal level of expression indicate that AtNHX1 has important physiological functions in *Arabidopsis* even in the absence of stress. The expression of *AtNHX1* in guard cells revealed by promoter-GUS analysis suggests the possibility that it is involved in stomatal regulation. It has been shown that AtNHX1 can mediate both Na^+ and K^+ coupled transport in vacuoles (Zhang and Blumwald, 2001) and the vacuolar Na^+/H^+ antiport has been shown to regulate vacuolar pH (Fukuda-Tanaka *et al.*, 2000). Moreover, Venema *et al.* (2002) reported that AtNHX1 reconstituted in artificial liposomes was able to mediate low affinity Na^+ as well as K^+ transport. Therefore, AtNHX1 may be involved in K^+ accumulation in the vacuole and in pH regulation in guard cells and other cells, in addition to Na^+ transport. More information from genetic, physiological and biochemical investigations will be needed to address the question of AtNHX1 function *in vivo*. For instance, future identification and characterization of *Arabidopsis* knockout mutants of *AtNHX1* could provide valuable insights into AtNHX1 function.

Acknowledgements

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