



Regulation of ion homeostasis under salt stress

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When under salt stress, plants maintain a high concentration of K^+ and a low concentration of Na^+ in the cytosol. They do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport. Although salt-stress sensors remain elusive, some of the intermediary signaling components have been identified. Evidence suggests that a protein kinase complex consisting of the myristoylated calcium-binding protein SOS3 and the serine/threonine protein kinase SOS2 is activated by a salt-stress-elicited calcium signal. The protein kinase complex then phosphorylates and activates various ion transporters, such as the plasma membrane Na^+/H^+ antiporter SOS1.

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Abbreviations

ABA abscisic acid
abi1 *ABA-insensitive1*
HKT high affinity K^+ transporter
NHX Na^+/H^+ exchanger
Snf3 Sucrose non-fermenting3
SOS1 SALT OVERLY SENSITIVE1

Introduction

The homeostasis of intracellular ion concentrations is fundamental to the physiology of living cells. Proper regulation of ion flux is necessary for cells to keep the concentrations of toxic ions low and to accumulate essential ions. Plant cells employ primary active transport, mediated by H^+ -ATPases, and secondary transport, mediated by channels and co-transporters, to maintain characteristically high concentrations of K^+ and low concentrations of Na^+ in the cytosol. Intracellular K^+ and Na^+ homeostasis is important for the activities of many cytosolic enzymes, and for maintaining membrane potential and an appropriate osmoticum for cell volume regulation.

Under salt stress, the maintenance of K^+ and Na^+ homeostasis becomes even more crucial. Thus, the regulation of ion transport by salt-stress signaling provides a model

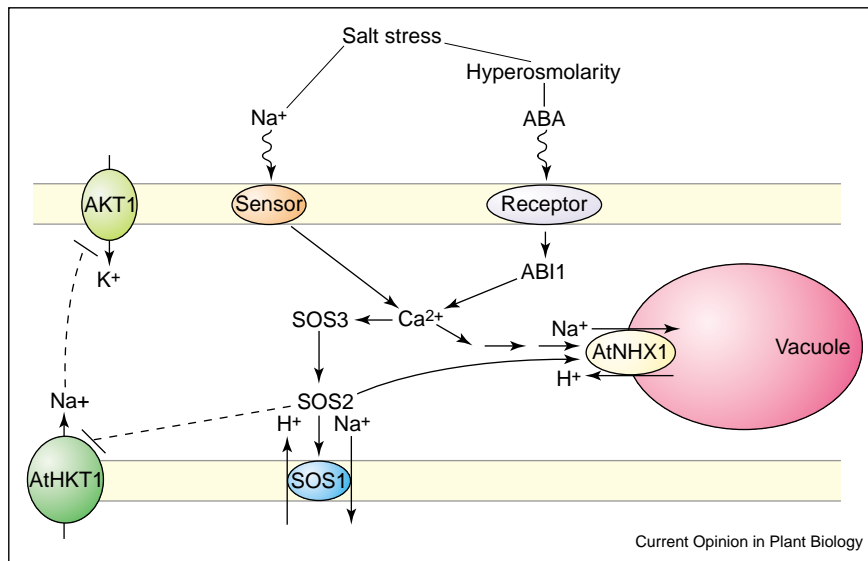
case for understanding the general regulation of ion homeostasis in plant cells. In addition, understanding how plants cope with excessive Na^+ in the environment is of great agricultural importance as soil salinity accounts for large yield losses in crops worldwide. Na^+ stress disrupts K^+ uptake by root cells [1]. When Na^+ enters cells and accumulates to high levels, it becomes toxic to enzymes [1]. To prevent growth cessation or cell death, excessive Na^+ has to be extruded or compartmentalized in the vacuole [1]. Unlike animal cells, plant cells do not have Na^+ -ATPases or Na^+/K^+ -ATPases, and they rely on H^+ -ATPases and H^+ -pyrophosphatases to create a proton-motive force that drives the transport of all other ions and metabolites [1]. Many of the transporters of H^+ , K^+ and Na^+ have now been identified. The regulatory mechanisms that control the expression and activity of the transporters are beginning to be elucidated. This review focuses on recent progress in understanding the cellular transduction of the salt-stress signal to regulate Na^+ transport in plants.

Sensing salt stress

Presumably, both hyperosmolarity and ion-specific signals of salt stress are sensed by plant cells. Although ion-specific signals are probably more important than hyperosmolarity in the regulation of Na^+ transport, osmotic stress also plays a role (Figure 1). Osmotic stress activates the synthesis of abscisic acid (ABA), which can upregulate the transcription of *AtNHX1*, the gene encoding the vacuolar Na^+/H^+ exchanger [2]. Osmotic stress may be sensed in part by stretch-activated channels and by transmembrane protein kinases, such as two-component histidine kinases [3] and wall-associated kinases [4]. At present, genetic evidence to support the role of any of these proteins in plant osmotic-stress responses is lacking.

Little is known about how Na^+ is sensed in any cellular system. Theoretically, Na^+ can be sensed either before or after entering the cell, or both. Extracellular Na^+ may be sensed by a membrane receptor, whereas intracellular Na^+ may be sensed either by membrane proteins or by any of the many Na^+ -sensitive enzymes in the cytoplasm. The plasma-membrane Na^+/H^+ antiporter SOS1 (SALT OVERLY SENSITIVE1) is a possible Na^+ sensor [5]. The SOS1 protein has 10–12 transmembrane domains and a long tail (of more than 700 amino acids) that is predicted to reside in the cytoplasm [5]. SOS1 has Na^+/H^+ exchanger activity, and this transport activity is essential for Na^+ efflux from *Arabidopsis* cells [6•,7•]. However, the unusually long cytoplasmic tail of SOS1 suggests that this protein may not only transport Na^+ but

Figure 1



Signaling pathways that regulate the expression and activities of ion transporters to maintain a low cytoplasmic concentration of Na⁺ under salt stress. Excessive Na⁺ and hyperosmolarity are each perceived by unknown sensors. The Ca²⁺-responsive SOS3–SOS2 protein kinase pathway mediates Na⁺ regulation of the expression and activities of Na⁺ transporters. Hyperosmolarity is proposed to induce the synthesis of ABA, which in turn upregulates the transcription of *AtNHX1* and other ion-transporter genes. The potential negative regulation of AtHKT1 by SOS3–SOS2 and of AKT1 by intracellular Na⁺ is also indicated by broken lines ending in T-bars.

also sense this ion. Several transporters with long cytoplasmic tails or loops have been demonstrated to be sensors. For example, the glucose transporters Snf3 (Sucrose non-fermenting 3) and Rgt2 (Regulator of glucose transporter 2) in yeast function as low- and high-glucose sensors, respectively [8]. Although the yeast proteins Snf3 and Rgt2 do not have a significant glucose-transport activity [8], studies of other proteins have demonstrated that sensing and transport are not mutually exclusive functions. For instance, the sugar permease BglF in *Escherichia coli* has a dual role in sensing and transporting β -glucosides [9]. The yeast ammonium transporter Mep2p also functions in both sensing ammonium and transporting it into cells, initiating nutritional signals that regulate filamentous growth [10,11]. It is conceivable, therefore, that SOS1 may be both a transporter and a sensor of Na⁺.

Na⁺ entry

The enormous negative membrane potential across the plasma membrane of plant cells favors the passive transport of Na⁺ into cells. Na⁺ enters plant cells through the high-affinity K⁺ transporter HKT1 [12,13**] and through non-selective cation channels [14]. Additionally, in some plant species such as rice, Na⁺ leakage into the transpiration stream via the apoplast can account for a major part of Na⁺ entry into plants [15]. Na⁺ uptake through the apoplastic pathway is affected by root development and silica deposition in the cell wall. The precise molecular identities of non-selective cation channels are still

unknown. Na⁺ currents that are mediated by non-selective cation channels are partially sensitive to calcium, and this correlates with the inhibition of Na⁺ entry into roots by calcium [16*]. It is unclear whether calcium's regulation of the activity of non-selective cation channels is direct or indirect via intracellular regulatory proteins.

The *Arabidopsis* AtHKT1 protein mediates Na⁺ influx when expressed in heterologous systems such as *Xenopus* oocytes and yeast [17]. A screen for suppressor mutations of the salt-hypersensitive *Arabidopsis* mutant *sos3* identified mutant alleles of *AtHKT1* [12]. *athkt1* suppression of *sos3* is due to reduced Na⁺ accumulation. In wheat, the K⁺–Na⁺ co-transporter HKT1 also functions in Na⁺ influx under salt stress [18*]. It is possible that in wildtype cells the Na⁺-influx activity of AtHKT1 is negatively regulated by SOS3 (a calcium-binding protein) and by other components of the SOS regulatory pathway (Figure 1). Alternatively, the SOS pathway may not regulate AtHKT1 and the suppressive effect of *athkt1* may be due simply to reduced Na⁺ entry. An intriguing question is why Na⁺-influx transporters such as AtHKT1 have been maintained during evolution, given the toxic effect of intracellular Na⁺. *athkt1* mutants do not have obvious growth or developmental defects. They are more tolerant of Na⁺ stress than wildtype plants when grown in culture media but are more sensitive when grown in soil. The essential role of AtHKT1 in saline soil is likely explained by its potential involvement in long-distance Na⁺ transport between the root and the shoot [13**].

Na⁺ efflux

The role of cellular efflux of Na⁺ is not intuitive in multicellular plants, as Na⁺ transported out of one cell would present a problem for neighboring cells. So the role of Na⁺ efflux has to be considered in specific tissues and in the context of whole plants. In *Arabidopsis*, Na⁺ efflux is catalyzed by the plasma-membrane Na⁺/H⁺ antiporter encoded by the *SOS1* gene [5,6^{••},7^{••},19[•]]. *SOS1* activity is detected in salt stressed but not in unstressed plants [6^{••}]. It is an electroneutral Na⁺/H⁺ exchanger that is specific for Na⁺ and cannot transport Li⁺ or K⁺ [6^{••},20]. Activity of the *SOS1* promoter is detected ubiquitously in virtually all tissues, but its greatest activity is found in root epidermal cells (particularly in epidermal cells at the root tip) and in cells bordering the vascular tissue throughout the plant [19[•]]. This *SOS1* expression pattern, together with the results of ion analysis in *sos1* mutant plants, suggests that *SOS1* has several roles. First, Na⁺ efflux into the root medium; second, buying time for Na⁺ storage in the vacuole by slowing down Na⁺ accumulation in the cytoplasm; and third controlling long-distance Na⁺ transport between roots and leaves by loading Na⁺ into and unloading Na⁺ from the xylem and phloem. *SOS1*'s role in long-distance transport is important for coordination between transpirational Na⁺ flow and the vacuolar sequestration of Na⁺ in leaves. Increased expression of *SOS1* results in improved salt tolerance in transgenic *Arabidopsis* [21].

The transcript level of *SOS1* is upregulated by salt stress [5]. This upregulation appears to be at the posttranscriptional level, as *SOS1* promoter activity is not upregulated by salt stress but *SOS1* expression driven by the constitutive Cauliflower mosaic virus 35S promoter is [21]. The salt-stress upregulation of *SOS1* is partly under the control of *SOS2* and *SOS3* [5]. Plasma-membrane H⁺-ATPases generate the driving force for Na⁺ transport by *SOS1*. Disruption of the root-endodermis-specific plasma-membrane H⁺-ATPase, *AHA4*, in mutant *Arabidopsis* plants causes increased salt sensitivity [22]. The transcript levels of some H⁺-ATPases have been shown to increase in response to salt stress [23]. Overexpression of the *Arabidopsis* H⁺-pyrophosphatase, *AVP1*, was shown to improve salt as well as drought tolerance [24]. Whether *SOS3* and *SOS2* are involved in this regulation is not known.

Activation of the Na⁺/H⁺ antiport activity of *SOS1* by salt stress is controlled by *SOS3* and *SOS2* ([6^{••},7^{••}]; Figure 1). *SOS3* is a myristoylated calcium-binding protein that is capable of sensing the cytosolic calcium signal elicited by salt stress [25,26]. *SOS2* is a serine/threonine protein kinase that has a unique carboxy-terminal regulatory domain and an amino-terminal catalytic domain similar to that of the yeast protein *SNF1* and animal AMP-activated kinase (*AMPK*) [27]. The amino-terminal kinase catalytic domain of *SOS2* interacts with the carboxy-terminal regulatory domain [28]. The carboxy-terminal regulatory domain of *SOS2* also interacts with *SOS3*,

and this interaction is mediated by a 21-amino-acid sequence, the FISL motif [28]. In the presence of calcium, *SOS3* activates the substrate phosphorylation activity of *SOS2* [29]. The FISL motif is autoinhibitory and its deletion results in constitutive activation of *SOS2* [6^{••},28]. Constitutive activation of *SOS2* can also be achieved by introducing mutations into the kinase activation loop [28,30]. These mutations substitute Ser-156, Thr-168 or Tyr-175 with the acidic Asp to mimic phosphorylated residues [30]. The data suggest that *in vivo* *SOS2* may be activated by phosphorylation in the activation loop by an upstream protein kinase.

In *sos3* or *sos2* mutant plants, *SOS1* activity cannot be induced by salt stress [6^{••}]. *In-vitro* addition of constitutively active *SOS2* recombinant protein to plasma-membrane vesicles that were isolated from *sos2* or *sos3* mutant plants restores *SOS1* activity to near the wildtype level [6^{••}]. The activation of *SOS1* by *SOS3* and *SOS2* has also been demonstrated in yeast, in which co-expression of the three genes could restore salt tolerance to a mutant that was defective in all endogenous Na⁺ transporters [7^{••}]. Yeast cells with the reconstituted *SOS* pathway were used to show that *SOS1* could be phosphorylated by the *SOS3*–*SOS2* protein kinase complex [7^{••}].

Na⁺ compartmentation

Vacuolar sequestration of Na⁺ not only lowers Na⁺ concentration in the cytoplasm but also contributes to osmotic adjustment to maintain water uptake from saline solutions. Other organelles, such as plastids and mitochondria, may also accumulate some Na⁺ and thus contribute to the overall subcellular compartmentation of Na⁺. In *Arabidopsis*, the *AtNHX* family of Na⁺/H⁺ antiporters function in Na⁺ compartmentation [31]. *AtNHX1* and *AtNHX2* are localized in the tonoplast membrane, and their transcript levels are upregulated by ABA or osmotic stress [32]. The transcript levels of vacuolar H⁺-ATPase components also increase in response to salt stress [33]. Overexpression of *AtNHX1* in various plants [34[•]], of an *Atriplex* homolog of *AtNHX1* in rice [35] or of the vacuolar H⁺-pyrophosphatase in *Arabidopsis* [36] was reported to enhance plant salt tolerance substantially.

Salt-stress regulation of *AtNHX1* expression is not impaired in the *Arabidopsis sos1*, *sos2* or *sos3* mutants. However, mutations that cause ABA deficiency or the *ABA-insensitive1 (abi1)* (but not the *abi2*) mutation partially disrupt *AtNHX1* upregulation by salt stress [2,32]. This suggests that an *SOS*-independent, ABA-dependent pathway regulates the expression of the vacuolar antiporter in response to salt stress (Figure 1). However, the *SOS* pathway appears to regulate the activity of vacuolar Na⁺/H⁺ antiporters [37].

K⁺ homeostasis

A high cytosolic K⁺/Na⁺ ratio is important for maintaining cellular metabolism. Under salt stress, Na⁺ competes

with K^+ for uptake into roots. The transcript levels of several K^+ transporter genes are either down- or upregulated by salt stress, probably reflecting the different capacities of plants to maintain K^+ uptake under salt stress. Salt stress increases the transcript level of the *Arabidopsis* root K^+ -transporter gene *AtKCI* [38]. In the common ice plant, salt stress upregulates the expression of *KMT1* (a AKT/KAT family member) and various HAK/KUP (high affinity K^+ transporter/ K^+ uptake transporter)-type genes, whereas it downregulates the expression of *MKT1* (another AKT/KAT family member) [39,40]. The significance of this transcript-level regulation is difficult to determine because the transport characteristics and *in-vivo* roles of the transporters are unclear. At the activity level, K^+ channels are regulated by protein kinases [41] and phosphatases [42]. Whether salt stress regulates the activities of K^+ -uptake transporters through these or other protein kinases or phosphatases remains to be determined. A particularly novel mode of activity regulation has been found for two HKT1 homologs from *Eucalyptus camaldulensis* [43]. These Na^+ - K^+ co-transporters display intrinsic osmosensing capabilities when expressed in *Xenopus* oocytes. Their Na^+ - and K^+ -transport activities are enhanced by a downshift in extracellular osmolarity.

The *Arabidopsis sos* mutants have a growth defect under K^+ -limiting conditions [44]. *Atthk1* mutations suppress not only the salt-hypersensitivity but also the K^+ -acquisition defect of the *sos3* mutant [12]. The involvement of the SOS pathway could be indirect. A defect in Na^+ efflux in the *sos* mutant may lead to excessive cytoplasmic Na^+ that is inhibitory to K^+ -uptake transporters such as AKT1 (E Spalding, personal communication; Figure 1). Under K^+ -limiting conditions, inhibitory levels of cytoplasmic Na^+ may arise in the *sos* mutants, even when grown in media that is not supplemented with extra NaCl.

Conclusions

Many of the transporters for H^+ , K^+ and Na^+ have been identified from various plant species. It is clear that salt stress regulates the expression level as well as the activities of some of these transporters. Evidence suggests that the SOS pathway plays a central role in coordinating the activities of several of the transport systems (Figure 1). Future efforts should be directed towards discovering the elusive salt-stress sensors and identifying additional signaling components that mediate the salt-stress regulation of the expression and activities of ion transporters.

Update

Berthomieu *et al.* [45] have recently shown that *Arabidopsis sos2* mutations, which cause overaccumulation of Na^+ in the shoot, are allelic to *AtHKT*. On the basis of *AtHKT*'s strong expression in phloem tissues, they propose that *AtHKT1* is involved in Na^+ recirculation from shoots to roots.

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