

The Salt Overly Sensitive (SOS) Pathway: Established and Emerging Roles

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ABSTRACT Soil salinity is a growing problem around the world with special relevance in farmlands. The ability to sense and respond to environmental stimuli is among the most fundamental processes that enable plants to survive. At the cellular level, the Salt Overly Sensitive (SOS) signaling pathway that comprises SOS3, SOS2, and SOS1 has been proposed to mediate cellular signaling under salt stress, to maintain ion homeostasis. Less well known is how cellularly heterogeneous organs couple the salt signals to homeostasis maintenance of different types of cells and to appropriate growth of the entire organ and plant. Recent evidence strongly indicates that different regulatory mechanisms are adopted by roots and shoots in response to salt stress. Several reports have stated that, in roots, the SOS proteins may have novel roles in addition to their functions in sodium homeostasis. SOS3 plays a critical role in plastic development of lateral roots through modulation of auxin gradients and maxima in roots under mild salt conditions. The SOS proteins also play a role in the dynamics of cytoskeleton under stress. These results imply a high complexity of the regulatory networks involved in plant response to salinity. This review focuses on the emerging complexity of the SOS signaling and SOS protein functions, and highlights recent understanding on how the SOS proteins contribute to different responses to salt stress besides ion homeostasis.

Key words: auxin; ion homeostasis; root system architecture; salt stress; the SOS signaling pathway.

INTRODUCTION

Soil salinity is a major environmental constraint to agricultural productivity (Greenway and Munns, 1980; Rhoades and Loveday, 1990). High concentrations of different types of salts, including chlorides, carbonates, and sulfates of magnesium, calcium, potassium, and sodium, characterize different saline soil areas around the world. Moreover, for each type of naturally occurring salinity, constantly changing environmental conditions, such as temperature and precipitation, as well as agricultural practices cause rapid modifications in levels of salinity and salt distribution patterns. Sodium and chloride are the predominant ions in the vast majority of saline areas. During evolution, various species of plants, known as halophytes, readapted to life in high-salinity environments, but a large majority of plant species grown in non-saline areas are salt-sensitive (referred to as glycophytes). These glycophytic plants, including the majority of crop species, differ greatly in their tolerance to salt stress (Greenway and Munns, 1980; Flowers and Colmer, 2008).

The salt tolerance trait is controlled by many genes through complex genetic regulatory networks. Salinity affects growth

by challenging plants with both osmotic and ionic stress. The presence of high concentrations of salt lowers soil water potential, thus making it harder for roots to take up water, while the ionic stress is associated with the gradual accumulation of salts in plant tissues over time (Munns and Tester, 2008). In the long term, the ability to detect changes in ion levels and provide an appropriate response is a requisite for plant survival in saline environments. One of the key responses to salt stress is to maintain cellular ion homeostasis by restricting the accumulation of toxic sodium (Na⁺) (Clarkson and Hanson, 1980; Tester and Davenport, 2003). A well-defined signaling pathway known to be required for control of ion homeostasis is the Salt Overly Sensitive (SOS) signaling pathway. Activation of the SOS signaling pathway has long

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been recognized as a key mechanism for Na⁺ exclusion and ion homeostasis control at the cellular level (Zhu, 2000). Less well known is how salt signals are integrated with intrinsic developmental cues to ensure appropriate plant growth in a saline environment. More recently, combinatorial approaches have been used to unravel both the novel roles of the SOS pathway and the functions of other complex regulatory networks that regulate ion homeostasis in *Arabidopsis*. This review focuses on the currently known and suggested functions of the SOS pathway in ion homeostasis and salt tolerance at cellular, tissue, and organ levels, and discusses the complexity of a coordinated response to salt stress.

THE SOS SIGNALING PATHWAY AND NA⁺ EXTRUSION FROM THE CYTOSOL

Multiple independent Na⁺-permeable transporters are thought to mediate Na⁺ uptake in plant cells (Tester and Davenport, 2003). Under non-saline conditions, nutritional Na⁺ uptake is mediated by dedicated ion transporters of the HKT family. Biophysical transport studies and phylogenetic analyses showed that HKT transporters can be classified into two subgroups, depending on whether they show a preference for Na⁺ uniport (class 1) or Na⁺-K⁺ symport (class 2) (Horie et al., 2007, 2009; Jabnour et al., 2009; Yao et al., 2010; Ali et al., 2012). When Na⁺ concentration in the surrounding environment increases, Na⁺ is thought to enter the plant symplast through plasma-membrane non-selective cation channels (NSCCs), whose functional molecular identities are still uncertain, or Na⁺ may also passively enter via anatomical 'leaks' in the root endodermis (Davenport and Tester, 2000; Hasegawa et al., 2000; Demidchik and Tester, 2002; Munns and Tester, 2008). Pronounced elevation in cytoplasmic Na⁺ disrupts enzymatic functions and is toxic to both cells and the whole plant. Three major processes have long been considered to participate in protection against high cytosolic Na⁺: (1) minimize Na⁺ entry into cells; (2) maximize compartmentation of Na⁺ into the vacuole; and (3) increase efflux of Na⁺ out of the cell. Of these, an efficient Na⁺ extrusion mechanism has been demonstrated to be a key homeostatic process for keeping a low concentration of cytosolic Na⁺ and maintaining a dynamic equilibrium of essential ions such as K⁺ and Ca²⁺. Since roots are the site of perception of salt in the environment, their responses and adaptive behavior form the first line of defense against stress damage. In the 1990s, a forward genetic approach was used to dissect the molecular mechanisms regulating salt tolerance. Isolation and characterization of several mutants showing root growth hypersensitivity under salt stress led to the identification of SOS3, SOS2, and SOS1, the key components of the SOS signaling pathway involved in Na⁺ extrusion (Zhu et al., 1998). Loss of function of SOS genes results in different levels of hypersensitivity to NaCl, with *sos1* mutants being the most sensitive identified to date (Wu et al., 1996).

It has been recognized for many decades that excess Ca²⁺ can 'protect' plants from Na⁺ toxicity. There is compelling

evidence gathered over the years that, after perception of salt stress, a Ca²⁺ spike generated in cytoplasm of root cells activates the SOS signal transduction cascade to protect the cells from damage due to excessive ion accumulation (Knight, 1999; Halfter et al., 2000; Zhu, 2002; Guo et al., 2004; Chinnusamy et al., 2005). SOS3 encodes a myristoylated calcium-binding protein that appears to function as a primary calcium sensor to perceive the increase in cytosolic Ca²⁺ triggered by excess Na⁺ that has entered the cytoplasm. Upon binding with Ca²⁺, SOS3 is able to interact with and activate the serine/threonine protein kinase SOS2, which belongs to the SnRK3 family of protein kinases (sucrose non-fermenting-1-related protein kinase-3) (Liu and Zhu, 1998; Halfter et al., 2000; Liu et al., 2000; Hrabak et al., 2003). More recently, SOS3-like Calcium Binding Protein 8 (SCaBP8, also known as Calcineurin B-like CBL10) has been shown to be an alternative regulator of SOS2 activity that functions primarily in the shoot of *Arabidopsis*, whereas SOS3 is more prominent in roots (Quan et al., 2007). SOS2 phosphorylates SCaBP8 and this event stabilizes the protein complex (Lin et al., 2009). Phosphorylation of SOS3-like proteins by their interacting protein kinases appears to be a common regulatory mechanism of CBL/SCaBP-CIPK/PKS modules (Du et al., 2011). SOS3-SOS2 or SCaBP8-SOS2 interactions recruit SOS2 to the plasma-membrane leading to activation of the downstream target SOS1, a Na⁺/H⁺ antiporter. This causes subsequent extrusion of excessive Na⁺ from the cytosol (Shi et al., 2000; Qiu et al., 2002; Quintero et al., 2002; Quan et al., 2007; Quintero et al., 2011).

The role of SOS1 in controlling ion homeostasis has been shown through a combination of biochemical, genetic, and physiological analyses. Using yeast mutant strains and isolated plasma-membrane vesicles, SOS1 was first shown to be able to specifically transport Na⁺ out of cells under salt stress (Qiu et al., 2002; Shi et al., 2002). Physiological analyses of *sos1* mutant plants together with gene expression studies in *Arabidopsis* demonstrated that SOS1 is involved in ion efflux from the cytosol to the surrounding medium in epidermal cells and to the vascular tissues from surrounding parenchyma, thus maintaining low concentrations of Na⁺ in root cells (Shi et al., 2002).

Studies in several species have now shown that SOS1 is conserved in higher plants including both monocots and dicots (Martínez-Atienza et al., 2007; Olías et al., 2009; Tang et al., 2010). Interestingly, the activity of SOS1 has been reported to be required for halophytic characteristics of *Thellungiella salsuginea* (also known as *T. halophila*), a naturally salt-tolerant plant species closely related to *Arabidopsis* (Oh et al., 2009). A 50% or greater reduction in SOS1 expression using RNA interference results in high levels of ion accumulation and causes loss of halophytism of *T. salsuginea* (Oh et al., 2009). Therefore, SOS3/SCaBP8-SOS2-SOS1 signaling is a paramount regulatory mechanism in Na⁺ exclusion and cellular ion homeostasis.

Recent studies, however, indicate that the homeostatic regulation of root functions that occur through SOS1 is not

as simple as initially thought. Electrophysiological analysis of roots revealed that K^+ and H^+ flux and accumulation in *sos* mutants in response to salt stress are greatly different from wild-type, and that SOS signaling is branched. It appears that Na^+ -induced activity of SOS1 does not solely depend on the SOS3–SOS2 complex (Shabala et al., 2005). Indeed, SOS1 is a target of the phospholipase D (PLD) signaling pathway in ion sensing and dynamic equilibrium adjustment under salt stress (Yu et al., 2010). Exposure to salt stress causes an increase in enzyme activity of PLD α 1 in *Arabidopsis*, resulting in rapid and transient accumulation of the lipid second messenger phosphatidic acid (PA). PA in turn activates Mitogen-Activated Protein Kinase 6 (MPK6), which can directly phosphorylate SOS1 (Yu et al., 2010). Loss-of-function mutants of PLD α 1 and MPK6 show altered Na^+ accumulation in the shoot and increased sensitivity to salt stress. Although the PLD signaling pathway mediates active ion exclusion and homeostatic maintenance through SOS1, it appears to operate in parallel to the SOS3–SOS2 complex. Thus, the regulatory mechanisms of Na^+ flux and ion homeostasis appear far more sophisticated than our current understanding, and the linear SOS3/SCaBP8–SOS2–SOS1 signaling pathway is not the only cellular regulatory process for Na^+ exclusion.

SOS SIGNALING AND TISSUE/ORGAN PROTECTION AGAINST EXCESSIVE Na^+

When plants are exposed to salt stress, in addition to the rapid osmotic effect, the ion-specific response occurs in two phases. First, Na^+ exclusion by root cells is initiated and, second, tissue protection against excessive Na^+ in leaf tissues occurs (Munns and Tester, 2008). Sodium exclusion by root cells is the primary protecting response in plants that delays the toxic effects of high cytoplasmic Na^+ . The comparison of unidirectional Na^+ uptake fluxes and the rates of net accumulation of Na^+ in roots indicate that the vast majority of the Na^+ taken up into the root symplast is extruded back to the apoplast and soil solution. In both *A. thaliana* and its halophytic relative *T. salsuginea*, this Na^+ cycling amounts to 77–78% of the Na^+ taken up by roots (Amtmann and Beilby, 2010). Thus, it was concluded that the major difference in Na^+ tolerance between *A. thaliana* and *T. salsuginea* was due to a much higher Na^+ influx in *A. thaliana*. This observation is consistent with others' findings that SOS1 is required for Na^+ tolerance but overexpression of SOS1 from either *A. thaliana* or *T. salsuginea* does not result in a major improvement in Na^+ tolerance (Shi et al., 2003).

The SOS signaling transduction pathway is a major regulatory mechanism for ion homeostasis that originally was considered to regulate Na^+ exclusion in roots. However, when external levels of salt are high and persistent, and this initial line of defense fails to protect roots from accumulating excess salt, Na^+ entering the root eventually reaches vascular tissues and starts accumulating in the shoot. Plants tend to maintain their root Na^+ content within narrow limits. The rate of Na^+

accumulation in plant shoots is determined by the net uptake of Na^+ into roots and its translocation rate from roots to shoots. The critical role of the SOS signaling pathway in shoot salt tolerance and the mechanisms of ion homeostatic regulation in protecting different tissues and organs against salt stress are now emerging. A reverse genetic approach in *T. salsuginea* showed that interference with SOS1 transcript caused a Na^+ accumulation in the pericycle of RNAi-suppressed transgenic lines and increased the Na^+/K^+ ratio in root xylem parenchyma cells by 12-fold (Oh et al., 2009). A similar approach in the agronomically relevant species tomato confirmed the role of SOS1 in the loading of Na^+ in the xylem. As most glycophytes, tomato plants exclude salts from the photosynthetic tissues in leaves by removing Na^+ from the root while retaining it mainly in the stem. A decrease in the abundance of *S/SOS1* transcript altered the Na^+ accumulation pattern in tissues of RNAi transgenic plants, causing an excessive accumulation of Na^+ in roots and leaves, while decreasing the stem Na^+ content, thus confirming the role of SOS1 in the partitioning of the toxic ion between plant organs (Olías et al., 2009).

Expression of all three SOS genes was induced by salt stress in roots and, in particular, high levels of SOS1 expression were detected in root epidermal cells of *A. thaliana* (Shi et al., 2002). It is conceivable that, in the case of low concentrations of NaCl, SOS1-mediated Na^+ exclusion to the growth environment by root epidermal cells may be sufficient for maintaining ion homeostasis and proper cell functions. This particular ion exclusion process in root epidermal cells is likely to be regulated by both SOS-dependent and -independent signaling pathways. When plants are subjected to higher concentrations of NaCl, Na^+ enters the cortex, endodermis, and xylem of roots. Although we have learned that each type of cell contains different concentrations of Na^+ (Kiegle et al., 2000; Tester and Davenport, 2003), much less is known about the control of tissue-specific maintenance of Na^+ exclusion and ion homeostasis. A recent study has shown that, in addition to a small core set of genes conserved in a specific cell type, the majority of unique physiological and biochemical processes of specific types of cells are highly responsive to salt stress in roots (Dinneny et al., 2008). Microarray analysis showed that SOS3 expression displayed differential induction in various types of root cells located in different developmental root zones in response to salt stress. Higher levels of SOS3 expression are detected in the cortex and endodermis, whereas expression in the epidermis of stressed roots is low (Figure 1; <http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>). Stress responses and overall maintenance of ion homeostasis are finely tuned at the cellular and tissue levels in stressed *Arabidopsis* roots in different layers of root cells. Although previous reports suggested that SOS1 also plays critical roles in controlling Na^+ compartmentalization in the vacuole and regulating Na^+ loading to the xylem (Shi et al., 2002; Oh et al., 2010), it is not clear how many other processes are involved in tolerance of root tissue to excessive Na^+ .

It is also not clear whether the activity of the SOS signaling pathway is required for Na^+ exclusion from leaf blades

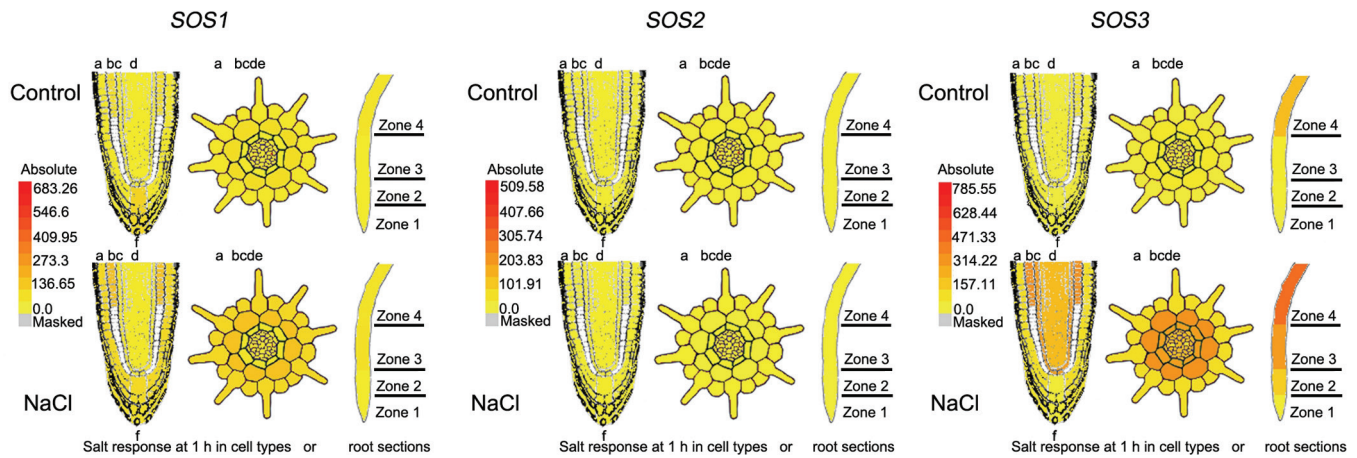


Figure 1. Salt Stress-Induced Transcriptional Changes of *SOS1*, *SOS2*, and *SOS3* in Different Types of Root Cells of 5-Day-Old *Arabidopsis* when Treated with 140 mM NaCl for 1 h.

Absolute gene expression data in control and NaCl stress conditions are shown. The cell type- and section-specific microarray data were obtained and processed using the eFP browser (Winter et al., 2007), using ATH1 microarray data. In 'Absolute', the expression level for the gene in each tissue is directly compared to the highest signal recorded for the given gene, with low levels of expression colored in yellow and high levels colored in red. a, epidermis and lateral root cap; b, cortex; c, endodermis and quiescent center; d, stele; f, columella root cap.

and subsequent salt tolerance in leaf tissues. Previous results showed that *SOS1*, *SOS2*, and *SOS3* have different spatial expression profiles. The former two genes are expressed in both roots and shoots, while the latter is mainly expressed in root tissues (Liu et al., 2000; Shi et al., 2002; Quan et al., 2007). This suggests that an alternative regulatory mechanism or an unrecognized component replacing *SOS3* in *SOS* signaling may exist for Na^+ exclusion and compartmentalization. Two recent studies using genetic and biochemical approaches have uncovered a novel component of the *SOS* signaling pathway that is specifically involved in leaf Na^+ exclusion. The *SOS3*-like *SCaBP8* (synonym *CBL10*) has been identified as a new Ca^{2+} sensor being an alternative to *SOS3* in aerial tissues. Loss of function in *SCaBP8* resulted in a more prominent increase in salt sensitivity of shoots compared to *SOS3* (Kim et al., 2007; Quan et al., 2007). A biochemical study has demonstrated that, like *SOS3*, *SCaBP8* interacts with and recruits *SOS2* to the plasma-membrane, thus activating *SOS1* Na^+/H^+ antiporter activity. Recruitment and activation of *SOS2* to the plasma-membrane requires myristoylation of *SOS3* in roots and phosphorylation of *SCaBP8* in shoots, respectively (Lin et al., 2009; Xie et al., 2009). *SCaBP8* may have additional functions related to Na^+ sequestration into vacuoles (Kim et al., 2007). These studies provide direct evidence for the *SOS3*- and *SCaBP8*-mediated regulation of organ-specific protection from Na^+ toxicity. The possibility exists that additional *SOS3*-like Calcium Binding Proteins (*SCaBPs*) capable of interaction with *SOS2* (Guo et al., 2001) may also act through the *SOS2*-*SOS1* module to reduce excessive Na^+ damage and maintain ion homeostasis in a tissue- and organ-specific fashion not thus far recognized. It is conceivable that different sensors recognize specific Ca^{2+} signal magnitudes and amplitudes, and activate specific

downstream signaling cascades leading to specific responses after exposure to salt stress.

NOVEL CELLULAR FUNCTIONS OF THE *SOS* PROTEINS IN CYTOSKELETON DYNAMICS

The regulation of *SOS1* by *MPK6* evokes similarities with one of the regulatory outputs of the High Osmolarity Glycerol (*HOG*) *MAPK* pathway in *Saccharomyces cerevisiae*. In budding yeast, hyperosmotic stress causes the activation of the *HOG* pathway, in which the *Hog1* *MAPK* is the crucial signaling component (Saito and Posas, 2012). *Hog1* is the closest *MAPK* homolog in yeast of the *Arabidopsis* *MPK6*. Upon hyperosmotic stress, *Hog1* is activated and translocated to the nucleus to phosphorylate stress-related transcription factors. *Hog1* also appears to directly recruit the RNA polymerase (Pol) II machinery to stress promoters and to regulate translation efficiency during osmotic stress. Additional phosphorylation targets of *Hog1* *MAPK* include two transport proteins located at the plasma-membrane, the Na^+ , K^+ H^+ antiporter *Nha1*, and the K^+ channel *Tok1*. Surprisingly, the *Hog1*-dependent activation of *Nha1* not only precedes, but it is required for, the early *HOG*-dependent activation of gene expression and glycerol synthesis (Proft and Struhl, 2004). The resulting increase in intracellular glycerol concentration allows the cell to achieve osmotic balance with the external environment. The mechanism underlying this unexpected connection between alkali cation transporters and gene expression is unclear but it presumably involves reducing ion concentrations, specifically in the nucleus, thus permitting transcriptional regulatory proteins to associate with their genomic targets.

It can be envisaged that the regulatory role of plasma-membrane transporters may also involve the reorganization of the cytoskeleton upon stress imposition, since the actin cytoskeleton of yeast cells rapidly disassembles in response to hyperosmotic stress and is induced to reassemble only after osmotic balance is re-established. The HOG pathway is required for the repositioning of the actin cytoskeleton (Brewster and Gustin, 1994; Yuzyuk et al., 2002). In plants, the cell cortex is a specialized layer of the cytoplasm underlying the plasma-membrane that is composed of a network of microtubules and actin filaments. Cortical microtubules are highly dynamic and remodeled by numerous stimuli. Salt stress induces dynamic cytoskeletal changes, with initial depolymerization of microtubules at the onset of stress followed by repolymerization (Dhonukshe et al., 2003; Wang et al., 2007). Both depolymerization and reorganization of the cortical microtubules are important for the plant's ability to withstand salt stress (Wang et al., 2007). A phospholipase D (PLD) was shown to associate strongly with microtubules and membrane microtubules in plant cells. Upon addition of *n*-butanol, a potent activator of PLD, cortical microtubules were released from the plasma-membrane and partially depolymerized (Gardiner et al., 2001; Dhonukshe et al., 2003). Tobacco cells treated with NaCl underwent microtubule reorganization and PLD activation within the same time frame. PLD was also activated under microtubule-stabilizing (taxol) and microtubule-depolymerizing (oryzalin) treatments, suggesting that PLD activation triggers microtubular reorganization and not vice versa. Notably, activation of PLD by hyperosmotic or dehydration stress elevates the level of PA (Katagiri et al., 2001; Munnik et al., 2001), which is a direct activator of MPK6 that, in turn, phosphorylates SOS1 (Yu et al., 2010). MPK6 has also been implicated in the organization and dynamics of mitotic and cortical microtubules (Müller et al., 2010).

Additional and more direct functional connections between members of the SOS pathway and the dynamics of the cytoskeleton have been established. Salt stress induces wild-type *Arabidopsis* seedling roots to display right-handed skewed growth and depolymerization of the cortical microtubules (Wang et al., 2007). The low amount of salt used (50 mM) and the enhanced response in the *sos1* mutant devoid of a Na⁺-specific efflux protein, indicated that Na⁺ is the main effector of the depolymerization of cortical microtubules under salt stress (Wang et al., 2007). The reorganization of the cytoskeleton, however, was not a detrimental consequence, but a necessary component of adaptation to salinity, since paclitaxel, a drug that stabilizes the cortical microtubules, reduced the ability of *Arabidopsis* seedlings to withstand salt stress. Very recently, SOS3 has also been shown to play a key role in mediating Ca²⁺-dependent reorganization of actin filaments during salt stress (Ye et al., 2013). Abnormal actin assembly and arrangement under salt stress was more pronounced in *sos3* plants, and this phenomenon

was partially complemented by addition of external Ca²⁺ or low concentrations of latrunculin A, an actin monomer-sequestering agent that inhibits actin polymerization. These findings indicate that the salt-hypersensitivity of the *sos3* mutant partially results from its disordered actin filaments.

Further genetic evidence supports the idea that the cytoskeleton has a pivotal function in plant tolerance to salt stress. The prefoldin (PFD) complex, a hetero-oligomer composed of six different subunits, facilitates the correct folding of actin and tubulin proteins. *Arabidopsis* mutants in prefoldins 3 and 5 are more sensitive to NaCl but not to LiCl or mannitol (Rodriguez-Milla and Salinas, 2009). Microtubule organization is partly disrupted in the *spiral1* (*spr1*) mutant of *Arabidopsis*, which displays a right-handed helical growth phenotype in the root elongation zone. SPR1 is a plant-specific protein of unknown function that localizes to microtubules. Mutations in *sos1* or *sos2* suppressed both microtubule disruption and helical growth phenotypes of *spr1* (Shoji et al., 2006). Incorporating a null mutation in *HKT1*, which encodes a Na⁺ influx protein, abrogated the suppression of *spr1* by *sos1* and *sos2* mutations, suggesting that the suppressive effect was linked to altered Na⁺ content. In fact, addition of NaCl also suppressed the *spr1* phenotype. Genetic suppression, however, was specific to the *spr1* mutation because the *sos1* mutation had no effect on the right-handed and left-handed phenotypes of *spiral2* and *lefty2* mutants. On the other hand, *sos1* and *sos2* were more sensitive to microtubule-interacting drugs in the absence of salt stress and to the NaCl-induced reorganization of microtubules. Together, these results support the notion that SOS1 functionally interacts with the cortical cytoskeleton, but they do not provide sufficient information to determine the mechanistic details of this interaction. In animal cells, plasma-membrane-localized ion channels interact with cortical cytoskeletons (Fujita et al., 2001). The cytoplasmic C-terminal tail of NHE1, a ubiquitous Na⁺/H⁺ antiporter at the plasma-membrane that regulates the cytosolic pH and volume of animal cells, anchors actin filaments of the cortical cytoskeleton, controlling its integrity independently of the ion transport function (Denker et al., 2000). In plants, however, the interplay between SOS1 and the cytoskeleton appears to involve the exchanger activity of SOS1, since the effects on the remodeling of microtubules were Na⁺-specific. What aspects of microtubule functions are sensitive to salt is not clear, since NaCl does not affect microtubule assembly or stability *in vitro*. An intriguing possibility is that the protein(s) responsible for the Na⁺-specific reorganization of the cytoskeleton is a constituent of the salinity sensor.

SOS SIGNALING AND THE ROOT SYSTEM

Although much work has been done to model the regulatory cascades that emerge during salt response and sodium

extrusion in plants, many questions remain to be answered. Critical remaining questions include whether the Ca^{2+} /SOS3–SOS2-mediated ion extrusion is sufficient for ionic stress tolerance, and whether control of ion extrusion is by itself the major biological process of plant adaptation to saline conditions. This is doubtful, since the presence of passive uptake of sodium causes the use of extrusion alone to result in a futile cycle (Wang et al., 2006).

The plasticity of the postembryonic development of roots under adverse conditions appears to be an additional component to salt tolerance that is controlled by both initiation and growth of lateral roots (Huh et al., 2002; Zolla et al., 2010). The role of the SOS signaling pathway in the regulation of plastic root development during adaptation to salt stress has been examined in recent reports. In a natural saline environment, levels of salinity in the soil are highly variable. In response to non-uniform salinity, a novel mechanism by which root growth direction is dynamically regulated in order to help avoid salt damage to plant roots has been identified (Li and Zhang, 2008; Sun et al., 2008). This avoidance tropism is achieved by interrupting the gravitropic response and altering auxin distribution in roots. The study by Sun and colleagues (2008) showed that, under salt stress, both gravitropic signal perception and asymmetric distribution of auxin in roots are altered in *sos* mutants. Loss of function in *SOS* genes resulted in the failure of rapid amyloplast degradation in columella cells and a greater negative gravitropic growth in response to salt stress (Sun et al., 2008). In a uniform saline environment, plant roots have evolved alternative adaptive strategies of protection, including a reduction in the number and length of root hairs, which leads to a lower surface area exposed to external Na^+ . Recent results revealed that the SOS signaling pathway plays a role in the plastic development of root hairs under salt stress (Wang and Li, 2008; Wang et al., 2008). Single mutants of *SOS* genes exhibited apparent developmental-defective phenotypes for root hairs, indicating that SOS signaling activities are required for reprogramming root epidermal cell development in response to salt stress and for maintenance of root hair homeostasis (Wang et al., 2008).

It is well known that manipulation of root system architecture (RSA) is another important way to minimize the negative effects of abiotic stresses on plants. Remodeling of root distribution in soil allows plants to optimize water and nutrient uptake (Benjamin and Nielsen, 2006; de Dorlodot et al., 2007; Postma and Lynch, 2011). In response to NaCl stress, primary root elongation is inhibited whereas the effect on lateral root (LR) formation seems to vary depending on the severity of the stress, with high concentrations of NaCl inhibiting LR formation, whereas lower concentrations of salt stimulate LR proliferation in an auxin-dependent manner (Wang et al., 2009; Zolla et al., 2010). In a recent study, the role of the SOS pathway in lateral root development and RSA under mild salt stress was revealed, showing the role of SOS3 as a positive

regulator of developmental plasticity of LR in *Arabidopsis* under mild salt stress (Zhao et al., 2011).

SOS3 is a key positive regulator of developmental plasticity of LR in *Arabidopsis* under mild stress. Loss of function of SOS3 resulted in an approximately 50% reduction in activation of lateral root primordia (LRP), whereas lateral root emergence was completely abolished, suggesting that SOS3 is critical for LRP initiation and required for LR emergence (LRE) under salt stress. Molecular and physiological analyses revealed that SOS3 is required for auxin biosynthesis, polar movement to stressed roots, and for the formation and maintenance of an auxin gradient. In *sos3-1* mutants, the accumulation of auxin at approximately 50% of sites where LRPs occur was found to be insufficient for cell specification of pericycle founder cells. Among the remaining 50% of LR initiated under mild salt stress, substantially reduced auxin accumulation in the newly formed LRPs caused defects in cell cycle progression and the eventual cessation of LR growth (Zhao et al., 2011).

The findings described above have uncovered a novel function for SOS3 beyond its role in regulating the activity of SOS1 and thereby Na^+ extrusion. It remains to be determined, however, whether regulation of lateral root development by SOS3 requires SOS2, and how SOS3 mediates auxin biosynthesis and its distribution pattern in LRI and LRE in response to mild salt stress. Considering the nature of SOS3 as a Ca^{2+} sensor, it is possible that Ca^{2+} spikes induced by salt stress activate not only SOS2, but also unrecognized components in Ca^{2+} signaling cascades that lead to the control of a diverse range of cellular processes in stressed plants. Recently, new interactors of SCaBP/CBL proteins have been identified in addition to SnRK3 kinases. For example, an enzyme in the methionine salvage pathway, the 5'-methylthioadenosine nucleosidase (AtMTAN), was recently identified as a new partner of calcineurin B-like protein CBL3 (Oh et al., 2008), whereas several other CBLs were found to interact with the protein phosphatase PP2CA (Lan et al., 2011), thus showing that SnRK3s may not be the only targets of plant CBL calcium sensors as originally thought (Halfter et al., 2000; D'Angelo et al., 2006; Li et al., 2006; Xu et al., 2006; Fuglsang et al., 2007).

DIVERSE FUNCTIONS OF SOS GENES IN CELL PROTECTION

The role of SOS proteins in Na^+ exclusion and salt tolerance at cellular, tissue, and organ levels is now well established. A growing amount of evidence further implicates the SOS proteins in diverse cellular functions that are also crucial for salt tolerance. In 2007, Verslues and colleagues demonstrated that SOS2 interacts with Nucleoside Diphosphate Kinase 2 and with Catalases 2 and 3, suggesting that SOS2 is part of a signaling node connecting salt stress response with ROS signaling (Verslues et al., 2007). In addition, SOS2 has been shown to interact with and modulate the activity of proteins located on the tonoplast such as the $\text{Ca}^{2+}/\text{H}^+$ antiporter CAX1

and the Vacuolar proton ATPase (V-ATPase), indicating that SOS2 plays a central role as a regulator of transport activities (Cheng et al., 2004; Batelli et al., 2007). SOS2 was also shown to interact with ABI2, suggesting a crosstalk between the ABA pathway and the SOS pathway (Ohta et al., 2003). Several lines of evidence suggest that SOS2 also regulates the activity of the tonoplast Na⁺/H⁺ exchanger. Ion transport assays using *Arabidopsis* tonoplast vesicles have shown that the tonoplast Na⁺/H⁺ exchange activity originating from AtNHX proteins is greatly reduced in *sos2* mutants and that the addition of activated SOS2 protein to vesicles increased this activity, demonstrating that the tonoplast Na⁺/H⁺ exchanger in *Arabidopsis* is a target of the SOS regulatory pathway (Qiu et al., 2004). These results were recently confirmed in tomato, with transgenic plants overexpressing the *Solanum lycopersicum* homolog of SOS2. A higher Na⁺/H⁺ and K⁺/H⁺ antiport activity in root intracellular membranes was shown in *SISOS2*-overexpressing plants, as well as a higher expression of *LeNHX2* and *LeNHX4*, suggesting that the regulation of NHX activity by the SOS pathway may be a conserved mechanism (Huertas et al., 2012). In a recent report, the photoperiodical and circadian clock switch *Gigantea* (*Gl*) was shown to be a negative regulator of the SOS pathway. By physical interaction, *Gl* effectively sequesters SOS2, thus preventing the activation of SOS1 in the absence of stress (Kim et al., 2013).

Microarray analyses under salt stress revealed that *sos2* and *sos3* mutants displayed dramatically different expression profiles. Expression of several genes implicated in defense, auxin, and ethylene signaling was affected by the *sos2* mutation, suggesting multiple novel roles of SOS2 in plant growth, development, and protection from stress injury. In contrast, very few genes in *sos3* mutant plants were specifically affected compared to wild-type under salt stress (Kamei et al., 2005). It is also possible that SOS2 is regulated by several SCaBPs (Guo et al., 2001). Comparative genomics of the proximal 2 Kb sequences upstream regulatory region of SOS genes using the AthaMap software program (www.athamap.de/) showed that both *SOS2* and *SOS3* share some *cis*-elements in their promoter regions, indicating that these sequences could be involved in common features of transcriptional regulation. The number of *cis*-elements predicted in the region upstream of the *SOS3* gene transcription initiation site is much less lower that of the *SOS1* and *SOS2* promoters (Figure 2). In particular, many small RNA target sites were found in the *SOS2* promoter region. Together with the previous microarray results, this finding suggests that the *SOS2* gene is regulated by several upstream transcription factors and is involved in other outputs besides Na⁺ transport by *SOS1*.

The most compelling evidence for *SOS* genes acting as regulators in multiple functionally diverse targets has been obtained by phenotypic analysis of *sos1* mutant root tips. In comparison to wild-type cells, *sos1* mutants had altered endocytosis, abnormal vacuolar functions, and different

intracellular pH under salt stress, indicating unrecognized functions of *SOS1* in membrane traffic, vacuolar function, and pH homeostasis (Oh et al., 2010). Further analysis of the 2 Kb upstream of the *SOS1* transcription initiation site revealed that the *SOS1* promoter region contains several binding elements for transcription factors of the bZIP, NAC, and WARY classes (Figure 2). This supports the suggestion that *SOS1* function is enhanced by unknown signaling cascades, which is consistent with the conclusions drawn by Shabala and colleagues (2005). It is not clear yet which transcription factor(s) mediate promoter activities of *SOS* genes, and which genes function as their downstream components.

CONCLUSIONS AND PERSPECTIVES

Since the discovery of the SOS signaling pathway in *Arabidopsis*, rapid progress has been made in our understanding of the molecular mechanisms of signaling in response to salt stress (Figure 3). Originally, the SOS pathway, like other signal transduction pathways, was thought to function in a linear system from an unknown Na⁺ sensor through *SOS3* and *SOS2* to mediate the Na⁺/H⁺ antiport activity of *SOS1*. The evidence that has accumulated over the years, however, has shown that the SOS pathway is branched, incorporates additional functions, and is a point of crosstalk with other signaling pathways (Katiyar-Agarwal

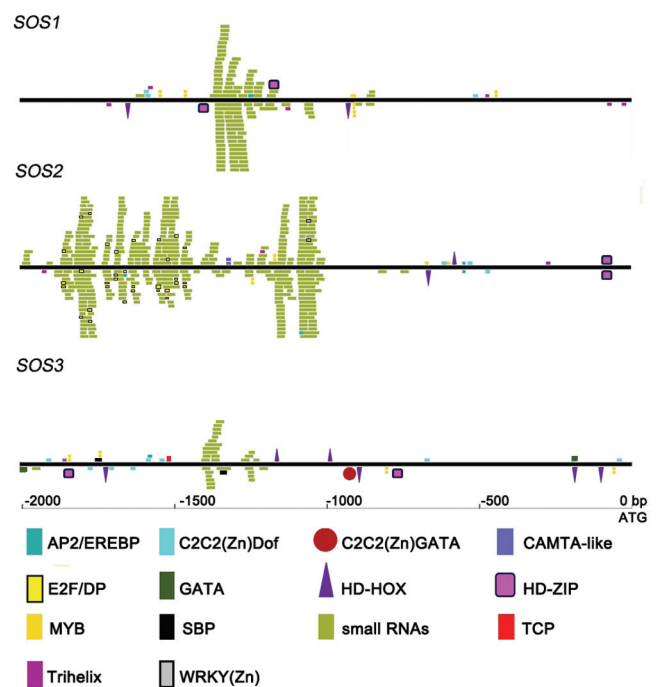


Figure 2. Promoter Sequence Analyses of *SOS1*, *SOS2*, and *SOS3* Genes. The promoter regions from translation start (ATG) to 2000 bp upstream (–2000) were analyzed using the AthaMap software program (Bülow et al., 2010).

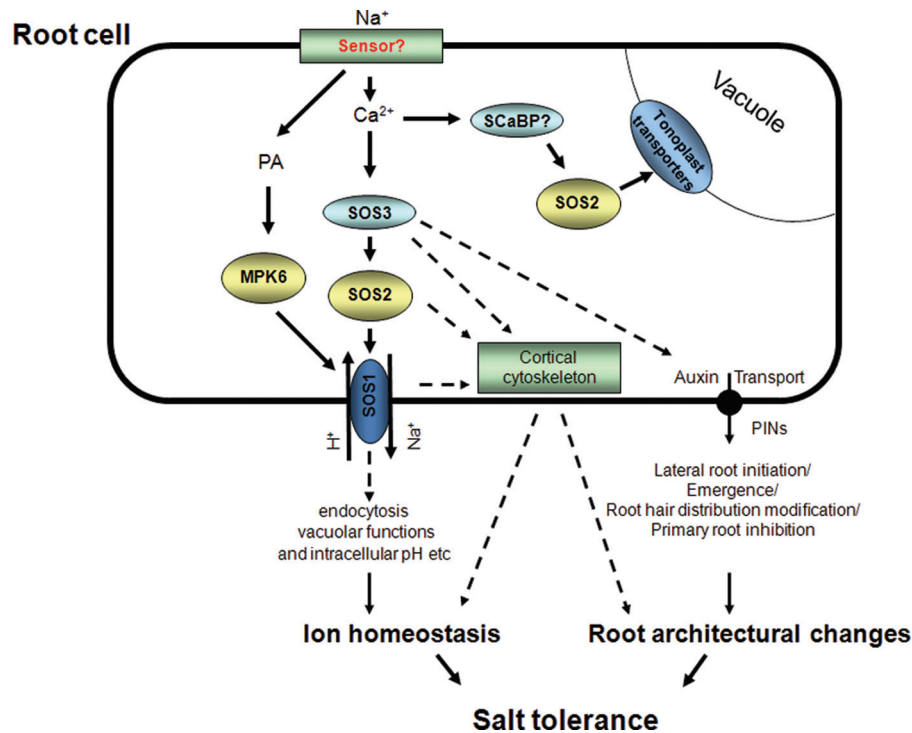


Figure 3. The Current Model of SOS Signaling in Root Cortex/Epidermal Cells for Maintenance of Ion Homeostasis, Regulation of Various Cellular Processes, and Lateral Root Development Based on Current Knowledge.

Solid arrows indicate established, direct regulations, while dashed lines indicate suggested links between the components represented.

et al., 2006; Jaspers et al., 2010). The SOS signal transduction more closely resembles a web of signaling components allowing input from many stress sensors and modulation by several components at once. The final output of the network of signaling may be multidimensional and the product of integration of several inputs. Such signaling networks function to allow many specific simultaneous changes in cell, tissue, organ, developmental, and chronological features. Genetic studies using overexpressing lines and knockout and knockdown mutants of *SOS1* have shown that *SOS1* is critically required for salt tolerance, but is not sufficient, since overexpression does not result in a major improvement in tolerance. Additional entities must be involved in the signaling process whose molecular identities remain elusive. However, it has become clear that these novel components must be linked somehow to *SOS1*, whose function is a prerequisite for salt tolerance. It has long been recognized that responses to environmental challenges can become partially stabilized and persist through mitotic events—a process called adaptation (Baldwin, 1902; Binzel et al., 1985). The most well-known example of an epigenetic change that is induced by the environment and persists through mitotic events is vernalization. Environmental adaptive phenotypes may also become stabilized over somatic generations (Binzel et al., 1985). In a few known instances, epigenetic changes can pass through sexual generations—a process referred to as transgenerational inheritance of epigenetic changes. Epimark

transgenerational inheritance is seen in a number of genetic phenomena such as imprinting and paramutation. The inheritance of epimark changes through mitotic generations and eventually meiotic generations was widely postulated in the past by several geneticists (Baldwin, 1902; Waddington, 1953; West-Eberhard, 2003) and was originally called genetic assimilation. Although clear evidence for the participation of genetic- and epigenetic-mediated phenotype changes in the process of environmental adaptation is largely lacking, several earlier reports have demonstrated that features of salt and temperature adaptation are stabilized mitotically and/or meiotically in protozoans, animals, and plants (Gause, 1942; Waddington, 1953; Binzel et al., 1985; LaRosa et al., 1989; Watad et al., 1991). These concepts remain an important part of the reawakening of epigenetics. It will be interesting to see if and how the activated SOS pathway may also be stabilized by epigenetic changes in NaCl-adapted cells and plants.

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