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## SOS1 and halophytism

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Much is already known about the function and functioning of the three genes that make up the SOS (Salt-Overly-Sensitive) pathway in plants, but recent studies indicate that the linkage between external increases in salinity and stress protection provided by genes SOS1, SOS2 and SOS3 is more complex than previously appreciated. It has recently been shown that the engineered reduced expression of the sodium/proton antiporter SOS1 affected several pathways indicating a role for SOS1 that exceeds its known function as an antiporter. Interference with expression of SOS1, characterized as a sodium/proton antiporter in the halophyte *Thellungiella salsuginea* converted *Thellungiella* into an essentially glycophytic species.

The SOS1 gene product encodes a protein specifying a plant plasma membrane Na<sup>+</sup>/H<sup>+</sup>-antiporter that is characterized by a N-terminal domain with 12-transmembrane helices that constitutes the antiporter and an intracellular domain of largely unknown function(s). When knocked out or mutationally inactivated in *Arabidopsis thaliana*, SOS1 has been identified to generate a most severe salt stress-sensitive phenotype.<sup>1,2</sup> The protein is unusually large, larger than other cation/proton antiporters contained in the *Arabidopsis* genome, owing to the C-terminal region that comprises more than 60% of the entire coding region.<sup>3</sup> Further genetic and biochemical studies revealed SOS1 to be part of a pathway, in which it is activated by a protein kinase, SOS2, in a complex with SOS3, a calmodulin-binding calcium sensor.<sup>4-6</sup>

It has been speculated that an increase of Na<sup>+</sup> is recorded by a yet unidentified sensor/receptor, leading to an increase in intracellular Ca<sup>2+</sup> that in turn activates the SOS pathway resulting in the extrusion of Na<sup>+</sup> ions from the cytosol.<sup>7-9</sup> Measurements of ion contents in plant tissues suggested that SOS1 is indeed a part of the a pathway that functions in extruding Na<sup>+</sup> ions under salt stress conditions.<sup>10</sup> Ectopic expression of the protein confers some salt stress tolerance in *Arabidopsis* and yeast cells.<sup>4,11,12</sup>

Although much work has been devoted to outlining the working of the pathway in *Arabidopsis*, and to modeling the sodium extrusion activity in yeast,<sup>4</sup> several tantalizing questions remain. The gaps are both mechanistic and conceptual. Such an ion-exclusion strategy may be sufficient for relieving ionic stress in unicellular organisms. However, explaining how a single membrane protein might dramatically alter the tolerance in a multicellular context is less easily explained. A major lack of understanding concerns the necessity and function of the long C-terminal extension that has, apparently, no relationship to sodium transport as other Na<sup>+</sup>/H<sup>+</sup>-antiporters of NHX family lack this C-terminus although the N-terminal domains are conserved.<sup>3</sup> Interestingly, NHX8, most closely related to SOS1 at the N-terminus but lacking the C-terminal region, encodes a Li<sup>+</sup>/H<sup>+</sup> antiporter.<sup>13</sup> In the category of unknown facts is how SOS1-dependent removal of Na<sup>+</sup> from the cytosol of one cell would have to be orchestrated with both vacuolar, sequentially apoptotic, and external

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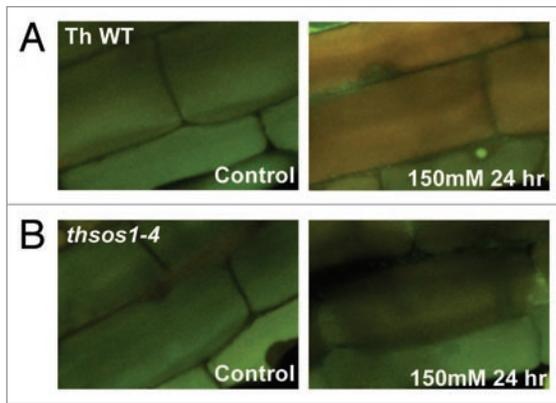
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**Figure 1.** Compromised vacuolar alkalization in the root cortex cells under salt stress by inhibition of SOS1 expression in *Thellungiella salsauginea*. Root cells were loaded with carboxyl-SNARF, a fluorescence pH indicator whose emission spectrum shifts from 580 nm (green) to 640 nm (red) as the pH increases. Confocal images were collected at 580 nm and 640 nm separately and merged. Seedlings of five day-old *Thellungiella* wild type (A) and the *thsos1-4* line (B) were subjected to 150 mM NaCl. Root cortex cells are shown.

sodium partitioning and transport mechanisms, so as not to increase stress for the entire organism. Considering the characterization of sodium extrusion based on SOS1 in the highly sodium-sensitive, glycophytic *Arabidopsis*, it remained to be studied whether the SOS pathway, and particularly SOS1, had any function and relevance for the sodium tolerance of halophytic species. We have now characterized the function of SOS1 in the *Arabidopsis*-relative *Thellungiella salsauginea*, an extremely salt-tolerant species<sup>14-17,27</sup> with special emphasis on intracellular processes that might be altered by the inhibition of SOS1 expression. The study identified SOS1 as an intrinsic part of the halophytic nature of this species because the down-regulation of SOS1 transcript expression converted *Thellungiella* into a glycophytic species.<sup>18</sup>

SOS1 is critical for the halophytic nature of *Thellungiella* by protecting predominantly cells of the root under salt stress in the zone of maximum cell expansion. In wild type plants, root cell elongation slowed by application of NaCl to the medium while cell death is not observed but, rather, the inhibition of cell expansion is gradually overcome leading to slower but normal expansion growth. Cells newly emerging from the division zone, which is not affected by sub-lethal salt stress, accumulated sodium in vacuoles indicating successful adaptation. In contrast, the reduction of SOS1

transcript amounts to less than 50% by RNAi expression in *Thellungiella* precipitated several intra-root and intracellular responses not observed in wild type plants. Sodium ions accumulated under salt stress to a much higher degree first in vacuoles and over time also in the cytosol. Prolonged exposure to NaCl changed the shape of the vacuoles, eventually leading to fragmentation. Also, endocytosis became increasingly inhibited, culminating in the rupture of plasma membranes and cell death beginning in a segment of the root adjacent to the cell division zone.<sup>18</sup>

The observations suggested a novel function for SOS1, extending beyond its role as an antiporter. However, caution is advised for distinguishing SOS1 functions from pleiotropic consequences of the lack of SOS1 under stress condition. Mutations of the SOS pathway in *Arabidopsis* are already known to affect proton flux in root cells, both under normal condition and in the presence of Na<sup>+</sup>.<sup>19</sup> Disruption of intracellular pH homeostasis by a mutation of AVPI inhibited endocytosis and auxin transport,<sup>20</sup> hence the observed inhibition of endocytosis in *thsos1-4* under salt stress<sup>18</sup> might be a result of disrupted pH homeostasis.

Observations from transcript profiling that compared wild type and *sos1*-RNAi lines have provided indications for the disturbance of expression of several ion channels, including Ca<sup>2+</sup>/H<sup>+</sup> antiporters, CNGCs, NHXs, and many

proton-ATPases, by SOS1 knockdown.<sup>21</sup> Activities of CAX1 and CAX2, the vacuolar Ca<sup>2+</sup>/H<sup>+</sup>-antiporters are regulated by pH<sup>22</sup> suggesting that changes into an intracellular pH could be converted to Ca<sup>2+</sup> signal. Additional observations revealed changes of intracellular pH under salt stress (Fig. 1). In wild type, the root cell vacuole became alkalized as Na<sup>+</sup> accumulated (Fig. 1A). The vacuolar alkalization upon salt stress was observed also in *Arabidopsis* wild type,<sup>23</sup> but demolished in both *thsos1-4*, a representative transgenic *Thellungiella* line expressing the ThSOS1-RNAi (Fig. 1B) and *atsos1-1*, an *Arabidopsis* SOS1 knockout mutant (Oh DH, unpublished). A function of SOS1 in signaling even in the absence of Na<sup>+</sup> ions has recently been suggested. SOS1 might be activated by different proteins in shoot and root.<sup>24</sup> Different from other Na<sup>+</sup>/H<sup>+</sup> antiporters (NHX family), the long cytosolic C-terminal domain of SOS1 includes a cyclic nucleotide (cNMP) binding domain.<sup>21</sup> In this respect, the different regulation of CNGCs in the *Thellungiella sos1*-RNAi line might be significant.<sup>21</sup> Members of the CNGC family are involved in different pathways that affect ion homeostasis, defense and developmental pathways.<sup>25</sup>

Unlike inhibition of endocytosis,<sup>18</sup> alkalization of the vacuoles was observed only when SOS1 expression was intact, but not in *Thellungiella* RNAi lines and the *Arabidopsis* KO-line. This could suggest a more direct involvement of SOS1 in the control of the vacuolar pH. Consequently, studies are required aimed at identifying whether SOS1 is directly involved in membrane trafficking, for example, by regulating the pH of endosomes as has been observed in some animal Na<sup>+</sup>/H<sup>+</sup>-antiporters.<sup>26</sup> Also, it must be ascertained whether the observed inhibition of endocytosis is a manifestation of imbalanced ion homeostasis caused by SOS1 deficiency or whether SOS1 has a role in the process. As well, SOS1 could function as the speculative plasma membrane Na<sup>+</sup> sensor<sup>7-9</sup> by either directly or indirectly, via regulation of other H<sup>+</sup> pumps,<sup>19</sup> changing the intracellular pH in the presence of Na<sup>+</sup> ions, which in turn induce Ca<sup>2+</sup> signals and activate the protein itself by the pathway involving SOS2/SOS3 complex.

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