

Article Addendum

Signaling control of *SOS1* mRNA stability

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Regulation of mRNA decay rates appears to be an important control point in determining the abundance of gene transcripts. Rapid change in decay rates of mRNAs could provide prompt responses of the plants to environmental fluctuations. *SOS1* is a plasma-membrane Na^+/H^+ antiporter crucial for salt tolerance in Arabidopsis. In our recent paper in *The Plant Journal*, we have shown that *SOS1* mRNA is inherently instable at normal growth conditions, but its stability is substantially elevated by salt and oxidative stress treatments. Salt stress-induced *SOS1* mRNA stability is mediated by reactive oxygen species (ROS) produced, at least in part, through NADPH oxidases. We proposed a hypothetical model for the signaling pathway controlling *SOS1* mRNA stability. In this addendum, we discuss the possible involvement of other components in conferring inherent instability and stress-induced stability of *SOS1* mRNA.

Genome-wide analysis of mRNA decay rates in Arabidopsis has indicated that mRNA half-lives vary from minutes to >24 hours.¹ This research also concluded that transcripts that are miRNA targets generally have shorter half-lives.¹ To determine if the inherent instability of *SOS1* mRNA is attributed to mi- or si- RNA mediated mRNA degradation, we searched the Arabidopsis Small RNA Project database (<http://asrp.cgrb.oregonstate.edu/db/>)² for possible small RNA target sequences in the 500 nt region of *SOS1* mRNA shown essential for *SOS1* mRNA instability. Although perfect complementary small RNA target sequences was not found in the 500 nt region, several hits with minimum free energy (MFE) over 60% were identified (Table 1). Mismatches between the small RNAs and the sequences in the 500 nt region raise the question as to whether these putative small RNAs could really direct degradation of *SOS1* mRNA. Studies on recognition and regulation of target transcripts by miRNAs have suggested that ~7 nt sites matching with the seed region at the 5' end of the miRNA is important for target recognition and posttranscriptional gene repression.³⁻⁵ Mismatch studies revealed

Table 1 Putative small RNA target sequences in the 500 nt region of *SOS1* mRNA

DBE#	Name	Sequence	Location	MFE %
<u>874</u>		query: CGCTCATCTTCATGATGCTGTCC small RNA: ACGAGUAGAAGUCGUACGAAAGG	96 - 117	65.08
<u>178607</u>		query: CTTTGGAGAAACCCCTCAATAGTTA small RNA: GAAUACUCUUUGGG-GGUUAUCUCU	135 - 156	68.15
<u>9342</u>		query: CTCCAATAGTTAAACTTCCAAA small RNA: AAGGUUUUCAUUUGUAGGUAA	149 - 167	66.33
<u>175061</u>		query: ATCACCTCACATCCGTTATCG : small RNA: AAGUGGAGUGUAGGCAGAGAC	184 - 198	82.45

that efficiency of gene repression by miR-7 was strongly reduced by mismatches in positions 2 to 8. However, some of the mismatches between miR-278 and the target sequence in positions 2-7 still allowed repression of the reporter EGFP up to 50%. Even as few as four base pairs in positions 2-5 conferred efficient target regulation. Moreover, strong base pairing at 3' end has compensatory role for insufficient 5' end pairing for miRNA function.³ Thus, although mismatches appear between the target sequences and all four small RNAs, it is plausible that these four putative small RNAs could, at least at some extent, confer *SOS1* mRNA instability.

SOS1 mRNA stability was substantially increased upon salt treatment. Salt-induced *SOS1* mRNA stability was impeded by the treatments using ROS scavengers and NADPH oxidase inhibitor. However, treatment using NADPH oxidase inhibitor DPI only partially inhibited salt-induced *SOS1* mRNA accumulation, while treatments with ROS scavengers nearly abolished salt-induced *SOS1* mRNA accumulation. These results suggested that the plasma membrane-bound NADPH oxidase might not be the sole source of ROS production mediating salt-induced *SOS1* mRNA stability. Other possible sources for extracellular ROS production are pH-dependent cell wall peroxidases, germin-like oxalate oxidases and amine oxidases.^{6,7} These enzymes may also contribute to the stress induced apoplastic production of ROS that mediates salt-induced *SOS1* mRNA stability. In addition to extracellular ROS, intracellular ROS produced in the mitochondria, chloroplast and peroxisome is also likely to play a role in salt-induced *SOS1* mRNA stability. In fact, treatments with intracellular ROS-producing chemicals such as diuron, methyl viologen, menadione, or Rose Bengal induced *SOS1* mRNA accumulation (data not shown). Diuron and methyl viologen are two chemicals that cause ROS production in chloroplast.

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Menadione causes ROS generation in mitochondria. Rose Bengal is a cell-permeable generator of singlet oxygen. Therefore, both extracellular and intracellular ROS may play important role in mediating salt-induced *SOS1* mRNA stability.

The intermediate signaling components transducing ROS signal to modulate *SOS1* mRNA stability are remained to be identified. Since salt induction of *SOS1* mRNA stability is a rapid process requiring only a few minutes, signal transduction events may solely occur in all existing molecules, including Ca^{2+} channels responsible for cytosolic Ca^{2+} elevation, membrane-bound NADPH oxidases, protein kinases and phosphatases and their substrate proteins, and the mRNAs to be stabilized. RNA binding proteins might be also involved. In yeast, RNA-binding protein Csx1 stabilizes the transcripts of the MAPK-regulated transcription factor Atf1 and mediates global control of gene expression in response to oxidative stress.⁸ In Arabidopsis, MAPK signaling cascade is modulated by ROS in response to various biotic and abiotic stresses.⁹ Several Csx1 homologs in Arabidopsis have been identified by sequence similarity search. The involvement of MAPKs and Csx1 homologous RNA-binding proteins in salt-induced *SOS1* mRNA stability is currently under investigation.

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