Supplemental Data

Endogenous siRNAs Derived from a Pair of Natural *cis*-Antisense Transcripts Regulate Salt Tolerance in Arabidopsis

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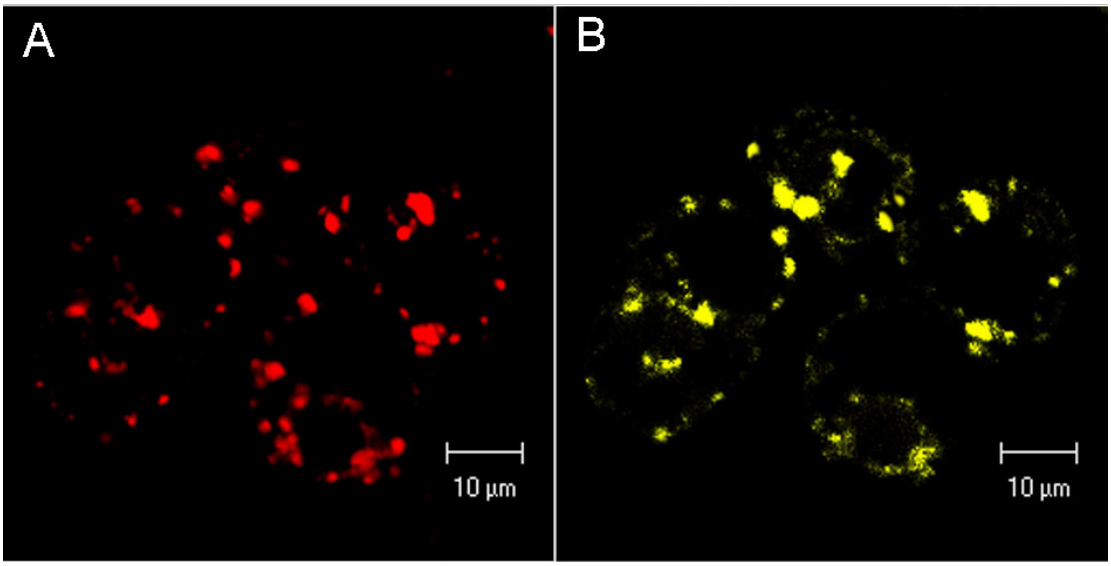

Figure S1. Mitochondrial Localization of SRO5-YFP Fusion Protein in Cell Suspension Cultures of *A. thaliana*

Plasmid pEG101 harboring the SRO5-YFP fusion was used to transform *A. thaliana* cell cultures. Extraction and transformation of *A. thaliana* protoplasts was performed following the method of Doelling and Pikaard (1993).
Three days after transformation, mitochondria were stained using Mito Tracker Deep Red 633 (Molecular Probes) by incubating cells in solution containing 200 nM Mito Tracker Deep Red 633® (Molecular Probe) for 4 h. YFP and Mito Tracker were visualized simultaneously under a Zeiss LSM510 confocal microscope. Mito Tracker was detected using emission and excitation conditions recommended by the manufacturer with excitation at 644 nm and emission at 665 nm. YFP was detected using excitation at 458 nm and emission at 514 nm.

(A) Mitochondria stained with Mito Tracker.
(B) Subcellular localization of SRO5-YFP fusion protein. Comparison of images A and B shows that SRO5 is localized in the mitochondria. Note that many mitochondria appear clustered together, and some mitochondria do not have

SRO5-YFP, likely because of limited expression of the SRO5-YFP construct in the transient assay.

Reference

Doelling, J.H. and Pikaard, C.S. (1993).Transient expression in *Arabidopsis thaliana* protoplasts derived from rapidly established cell suspension cultures. Plant Cell Reports *12*, 241-244.

Table S1. Verification of the 24-nt *SRO5-P5CDH* nat-siRNA Sequence

Sequence	Number of clones
TATCCCGGCCCCTCTCGggtcccc (3' end)	9
tatcCCGGCCCCTCTCGGGTC (5' end)	6

Upper case letters indicate forward and reverse primer sequences designed based on the previously cloned *P5CDH* siRNA. Small RNAs of 18–26 nt were isolated from salt stressed plants and ligated to 5'- and 3′-adaptors. PCR was carried out using cDNAs of the ligation products, and primers corresponding to the cloned 21-nt *P5CDH* siRNA sequence (clone # P96-F02; Sunkar and Zhu, 2004) and the 5'- or 3′-adaptor. The resulting PCR products were cloned and the indicated number of clones sequenced to determine the additional nucleotides (lower case letters) present in the 24-nt *SRO5-P5CDH* nat-siRNA.

Table S2. Sequences of Oligonucleotides Designed to Detect siRNAs 5’ or 3’ of the 24-nt nat-siRNA-Directed *P5CDH* mRNA Cleavage Site

Sequential 21-nt oligonucleotide probes 5’ of and in phase with the 24-nt *SRO5-P5CDH* nat-siRNA.
5’-GCTCCUCAGAATCACUGGTTT-3’
5’-CTAAGAGGAAGAACAACTGGA-3’
5’-AATGGGACTACATATGCTGGA-3’
5’-GAAGTAATAGGGAACTCAGTG-3’
5’-GGACCTGCGGGGGACCCGAGA-3’

Sequential 24-nt oligonucleotide probes 5’ of and in phase with the 24-nt *SRO5-P5CDH* nat-siRNA but out of phase with the 21-nt *P5CDH* nat-siRNAs.
5’-CAGGAAGUAATAGGGAACTCAGTG-3’
5’-AATGGGACTACATATGCTGGACTA-3’
5’-AGAGGAAGAACAACTGGAGCTCCT-3’
5’-CAGAATCACTGGTTTGGACCTGCG-3’

Sequential 24-nt oligonucleotide probes 3’ of and in phase with the 24-nt *SRO5-P5CDH* nat-siRNA but out of phase with the 21-nt *P5CDH* nat-siRNAs.
5’-GGGACACCAGAGGCTATAAAGCTG-3’
5’-GTTTGGTCATGCCACAGAGAAGTC-3’
5’-ATCTACGATTATGGTCCGGTTCCA-3’
5’-CAAGGTTGGGAACTTCCTCCATCT-3’

Small RNA blots were probed with each oligonucleotide as described in Material and Methods except that each set of oligonucleotide probes was first labeled separately and then mixed in the hybridization solution.