Supplemental Material S1, Zhang et al. (2009)

Cloning of PS small RNA fragments.

PS RNA labeled with [γ^{32}P]-ATP (NEN) of a length from 30 to 90 nucleotides were isolated from the gel after denaturing PAGE. The gel was washed twice with RNAse-free H2O, gel slabs containing the small RNA were subjected to thaw/freeze cycles, and submitted to elution in 1x TE by incubation for 12h at 4˚C, the sample was centrifuged 1000g for 2 min, filtered with 0.2 µm filter syringes (VIVAscience AG), and butanol extracted to obtain approx 500µl aqueous RNA solution. The RNA solution was precipitated by adding 1 vol. isopropanol, 1/10 vol. 3M NaOAC (pH 5.2) and 25µg linear acrylamide (Ambion), de-phosphorylated with shrimp alkaline phosphatase (NEB), 3'-OH linked (T4 RNA Ligase, 10U/µl, Fermentas) with 10 pmol 5' phosphorylated and 3` biotinylated DNA linker (5' P-dCdTdGdTdAdGdGdCdAdCdCdAdTdCdAdAdAd-3'biotin), phosphorylated with T4 kinase (Ambion), partially labeled with [γ^{32}P]-ATP, and the expected linker-ligated fragments appearing on a denaturing PAGE were isolated from gel slabs described above. After incubation at 90˚C for 30 sec. in 1x RNA ligase buffer (Fermentas) and 20% (v/v) DMSO, and transfer on ice for 20 sec., the 5' phosphorylated and 3'OH linked RNA was ligated to 10 pmol 5' biotinylated DNA/RNA adaptor (5'biotin - dAdGdAdTdCdGdTrArArGrGrCrArCrCrTrGrArArA–OH) with 0.5 U/ul T4 RNA ligase (Fermentas) and 0.5 mM ATP, at 37˚C for 1 hour. Next resulting fragments carrying the 5' and 3' linkers were submitted to an RT reaction with 10 pmol linker specific 3` RT primer (5-ATTTGATGGTGCCTACAG-3'OH) using 1 U/µl AMV-RT (Promega) followed by a PCR reaction (Advantage PCR Kit, Clontech) with 20 pmol 5` linker primer (5'-ATCGTAGGCACCTGAAA-3'OH) and 3` RT primer. The produced cDNA was digested with BanI

(NEB), isolated from a 4% agarose gel (Gel Extraction Kit, QIAgen), concatamerized using 1 U/µl T4 Ligase (NEB), and resulting ligated fragments (> 300bp) were extracted from a 2% agarose gel, and, after a Taq-polymerase fill-in reaction, cloned into TOPO-TA vector (Invitrogen).

List of oligonucleotides used in the study.

PCR primers to detect phloem specific RNAs and to create templates for T7 *in vitro* transcription/translation:

Name	Sequence (5' to 3')	Template Acc.# / target sequence
FK281	TAATACGACTCACTATAGATGGAGGAGATCACCCAAC	Zea mays KN1 GB acc.# AY312169 forward primer
FK185	CGGCGGATCCGCGCCGAGCCGGTACAG	Zea mays KN1 GB acc.# AY312169 reverse primer
FK334	TAATACGACTCACTATAGACAAATTAAAGAAGCAGAAACAA	Arabidopsis FT TAIR acc. # AT1G65480 forward primer
FK335	AAAGTATAAATATAACACTTCATTTCATG	Arabidopsis FT TAIR acc. # AT1G65480 reverse
FK284	TAATACGACTCACTATAGATGTATGAGCAGCAGCAAC	AtMPB2C TAIR acc. # At5G08120 forward primer
FK228	ATAATATGTAAAGGCTAGTGATTG	AtMPB2C TAIR acc. # At5G08120 reverse primer
FK323	TAATACGACTCACTATAGATCTTTGCGCTTGGGGCAATG	AtU4.2/CmU4.2 snRNA TAIR acc. # AT3G06900 forward primer
FK322	CCGCTCGAGTATGCATGATGCATCATTTAGACTAGTTATTGTAG	AtU4.2/CmU4.2 snRNA TAIR acc. # AT3G06900 reverse primer
FK406	TAATACGACTCACTATAGATCAGAGTGGCGCAGCGG	Met-tRNA forward primer
FK408	TATCAGAGCCAGGTTTCGAT	Met-tRNA reverse primer
FK268	atgggtttgaagaagccaagccactta	CmPP16 GB acc.# AF079170 reverse primer (Ruiz-Medrano et al., 1999)
FK267	gtggtaaaggacttcaagcccacgacc	CmPP16 GB acc.# AF079170 forward primer (Ruiz-Medrano et al., 1999)
FK270	ttgtcgaagccaatgactctgatgaa	C.max RUBISCO small SU reverse (Ruiz-Medrano et al., 1999)
FK269	atggcttccatcgtctcatccgcc	C.max RUBISCO small SU forward (Ruiz-Medrano et al., 1999)

Oligonucleotides used to detect small PS RNAs in northern blot assays:

Name	Sequence (5' to 3')	Template Acc.# / target sequence
FK311	CACGCTTAACTGCGGAGTTCTGATG	5S rRNA antisense probe
FK310	CATCAGAACTCCGCAGTTAAGCGTG	5S rRNA sense probe
FK439	AATCAAGAAAGAGCTCTCAG	18S rRNA 3' end antisense probe
FK438	CTGAGAGCTCTTTCTTGATT	18S rRNA 3' end sense probe
FK458	TTCACAGTCTGAATTAGTTC	18S rRNA 5´end antisense probe
FK457	GAACTAATTCAGACTGTGAA	18S rRNA 5´end sense probe
FK456	ACGAATCGGAGCGACGAAGGGC	26S rRNA 3´end antisense probe
FK459	GCCCTTCGTCGCTCCGATTCGT	26S rRNA 3´end sense probe
FK305	TTAATGCCCCCAATTGAGTTACAAG	7S SRP RNA >42_31_2s antisense probe
FK304	CTTGTAACTCAATTGGGGGCATTAA	7S SRP RNA >42_31_2s sense probe
FK309	GGGAGCCCTTCCAGAAATTCTCGAA	U4 snRNA >42up1_2s antisense probe
FK308	TTCGAGAATTTCTGGAAGGGCTCCC	U4 snRNA >42up1_2s sense probe
FK307	GCGCCAGGCCCATGCCGTAGTGCAA	U2 snRNA >85_95_6_2S antisense probe
FK306	TTGCACTACGGCATGGGCCTGGCGC	U2 snRNA >85_95_6_2S sense probe
FK362	GGAACGAACCCGGGTCACCC	tRNA-Asp (GAC) antisense probe (41-60)
FK363	GGGTGACCCGGGTTCGTTCC	tRNA-Asp (GAC) sense probe (41-60)
FK368	AACCCACGACCACAAGGTTA	tRNA-Lys (CTT) antisense probe (36-55)
FK367	TAACCTTGTGGTCGTGGGTT	tRNA-Lys (CTT)sense probe (36-55)
FK370	ATCCTAACCGTTGGACTACA	tRNA-Gly (GGA) antisense probe (6-25)
FK369	TGTAGTCCAACGGTTAGGAT	tRNA-Gly (GGA) sense probe (6-25)
FK358	CTTCCGCTGCGCCACTCTGA	tRNA-Met (ATG) antisense probe (8-27)
FK359	TCAGAGTGGCGCAGCGGAAG	tRNA-Met (ATG) sense probe (8-27)
FK361	TGGTATCAGAGCCAGGTTTC	tRNA-Met (ATG) antisense probe (53-72)
FK360	GAAACCTGGCTCTGATACCA	tRNA-Met (ATG) sense probe (53-72)
FK372	GGGTGAGAGCCGAGTATCCT	tRNA-Glu (GAG) antisense probe (22-41)
FK371	AGGATACTCGGCTCTCACCC	tRNA-Glu (GAG) sense probe (22-41)

FK376	TTCCGAGAATCGAACTCGGG	tRNA-Pro (CCA) antisense probe (47-66)
FK375	CCCGAGTTCGATTCTCGGAA	tRNA-Pro (CCA) sense probe (47-66)
FK374	TGAGAGATTCGAACTCTCGC	tRNA-Ser (AGC) antisense probe (56-75)
FK373	GCGAGAGTTCGAATCTCTCA	tRNA-Ser (AGC) sense probe (56-75)
FK460	TTCCACTGGGGATCGAACCC	tRNA-Arg (CGT) antisense (31-50)
FK494	GGGTTCGATCCCCAGTGGAA	tRNA-Arg (CGT) sense (31-50)
FK415	CAGGTCGCTGGATTCAAAGT	tRNA-Gln (CAA) antisense (31-50)
FK506	ACTTTGAATCCAGCGACCTG	tRNA-Gln (CAA) sense (31-50)
FK399	CGTACCTCTCGCATGCTAAG	tRNA-Ala (GCT) antisense probe (31-50)
FK492	CTTAGCATGCGAGAGGTACG	tRNA-Ala (GCT) sense probe (31-50)
FK411	GCAACCTTCTGATCTGGAGT	tRNA-Trp (TGG) antisense probe (30-49)
FK498	ACTCCAGATCAGAAGGTTGC	tRNA-Trp (TGG) sense probe (30-49)
FK413	CCTCCGCTATACCAACAGAG	tRNA-Tyr (TAC) antisense probe (11-30)
FK504	CTCTGTTGGTATAGCGGAGG	tRNA-Tyr (TAC) sense probe (11-30)
FK377	CTGACGCTCTCCCAACTGAG	tRNA-Phe (TTC) antisense probe (11-30)
FK485	CTCAGTTGGGAGAGCGTCAG	tRNA-Phe (TTC) sense probe (11-30)
FK414	GGTGACCTATTGATCTGCAG	tRNA-Cys (TGC) antisense probe (31-50)
FK505	CTGCAGATCAATAGGTCACC	tRNA-Cys (TGC) sense probe (31-50)
FK403	CAGGTCCCTTCGGCCTCAAC	tRNA-Leu (CTC) antisense probe (31-50)
FK497	GTTGAGGCCGAAGGGACCTG	tRNA-Leu (CTC) sense probe (31-50)
FK401	CAGGTCTCCACGGCCACAAC	tRNA-His (CAC) antisense probe (31-50)
FK495	GTTGTGGCCGTGGAGACCTG	tRNA-His (CAC) sense probe (31-50)
FK436	TCTAACCAGCTGAGCTATTC	tRNA-Asn (AAC) antisense probe (5-24)
FK507	GAATAGCTCAGCTGGTTAGA	tRNA-Asn (AAC) sense probe (5-24)
FK437	AGAAGGAGGGTTCGAACCTC	tRNA-Asn (AAC) antisense probe (51-70)
FK508	GAGGTTCGAACCCTCCTTCY	tRNA-Asn (AAC) sense probe (51-70)
FK402	AGACCTTCAGTGTGTTAGAC	tRNA-Val (GTT) antisense probe (31-50)
FK496	GTCTAACACACTGAAGGTCT	tRNA-Val (GTT) sense probe (31-50)
FK412	AGACCTCCCGCTTACTAAAC	tRNA-Thr (ACT) antisense probe (31-50)
FK501	GTTTAGTAAGCGGGAGGTCT	tRNA-Thr (ACT) sense probe (31-50)

FK487	**ACCAACTGAGCTACGGGACC**	**tRNA-Ile (ATA) antisense probe (1-20)**
FK486	**GGTCCCGTAGCTCAGTTGGT**	**tRNA-Ile (ATA) sense probe (1-20)**
FK357	**GTGACCTTCGCGTTATTAGC**	**tRNA-Ile (ATT) antisense probe (32-51)**
FK490	**TGCTAATAACGCGAAGGTCG**	**tRNA-Ile (ATT) sense probe (31-50)**

Reference:

Ruiz-Medrano R, Xoconostle-Cazares B, Lucas WJ (1999) Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants. Development **126:** 4405-4419