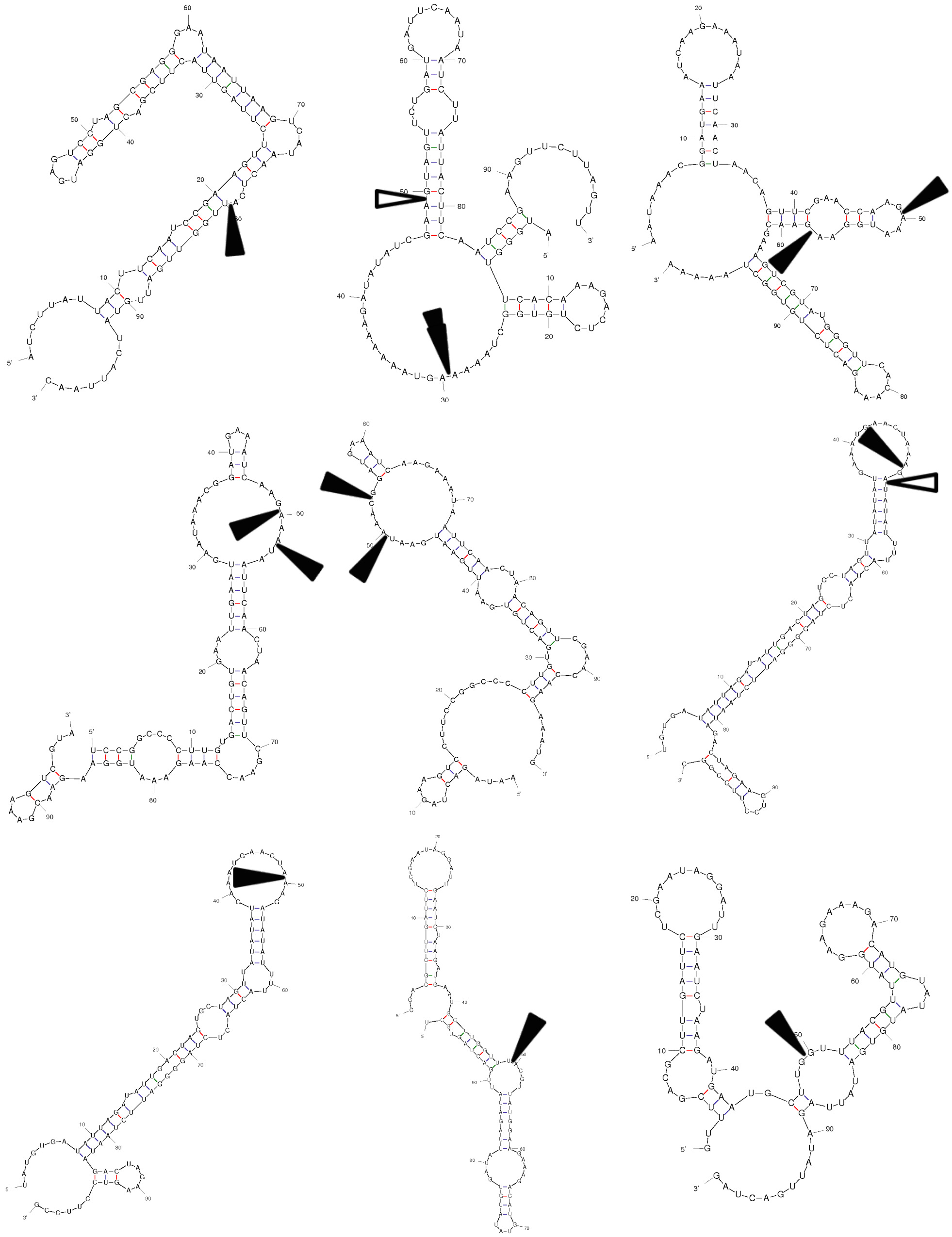
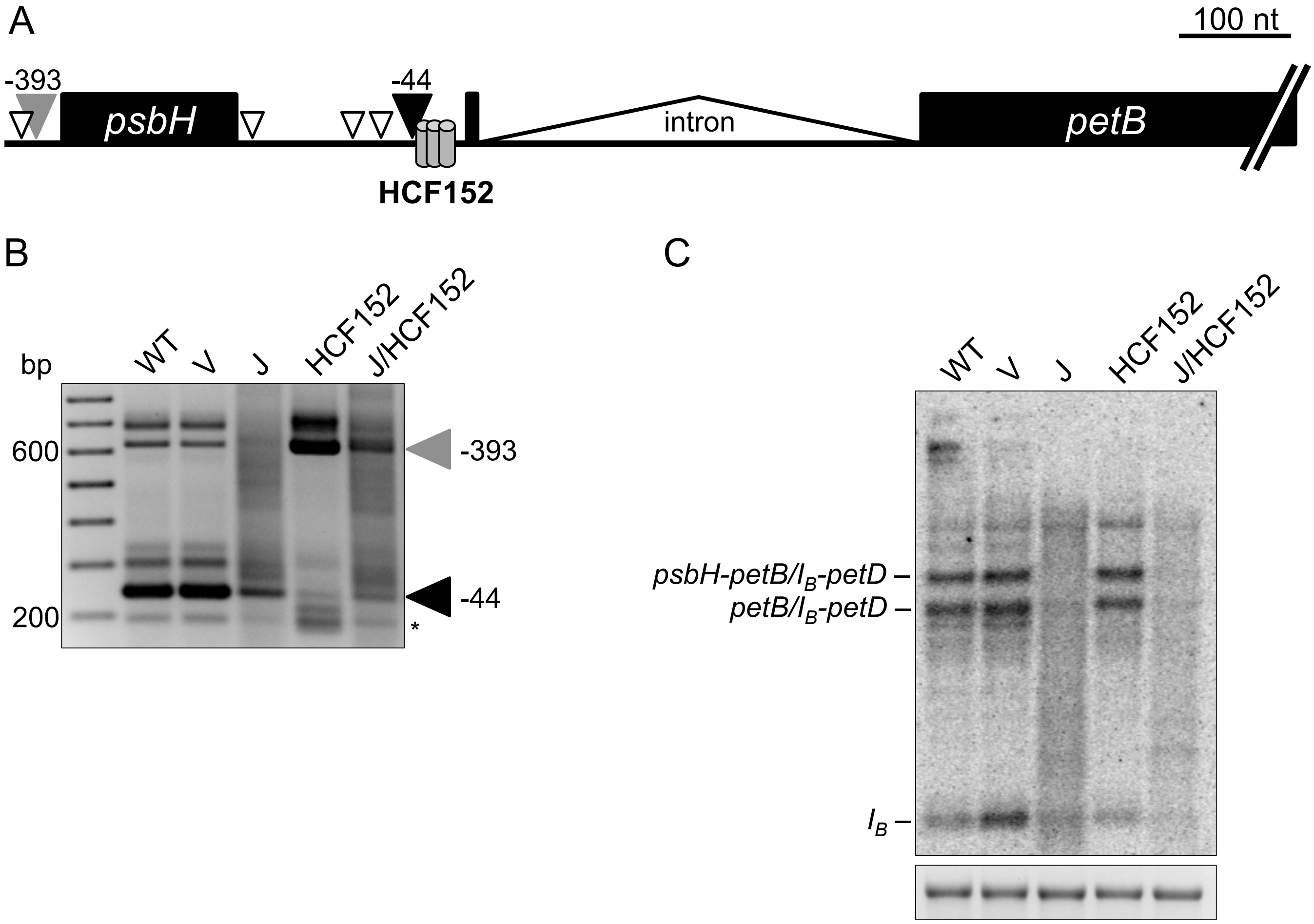
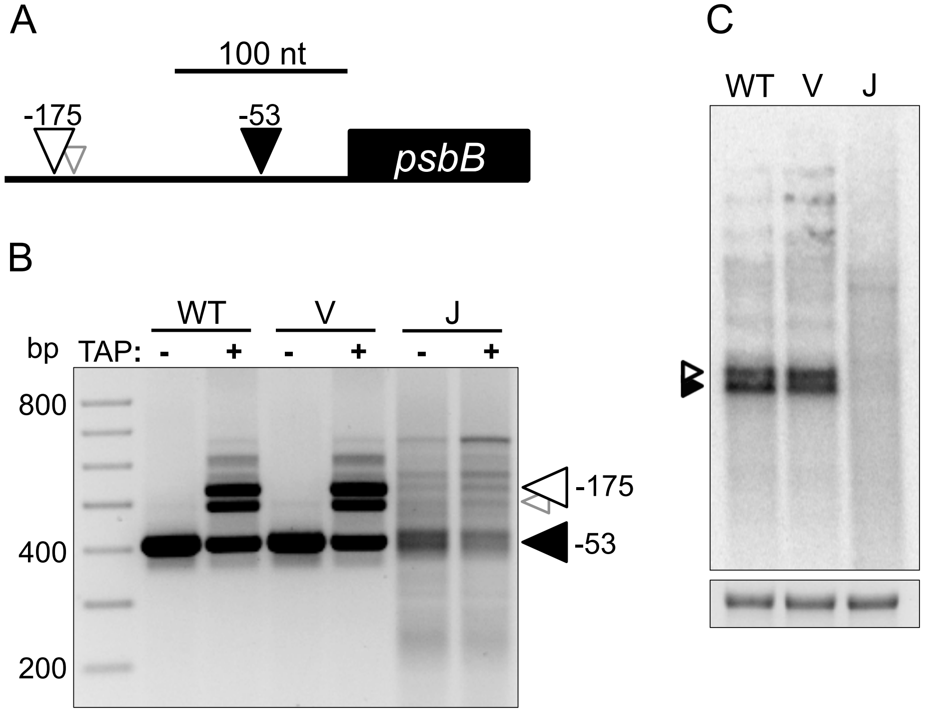


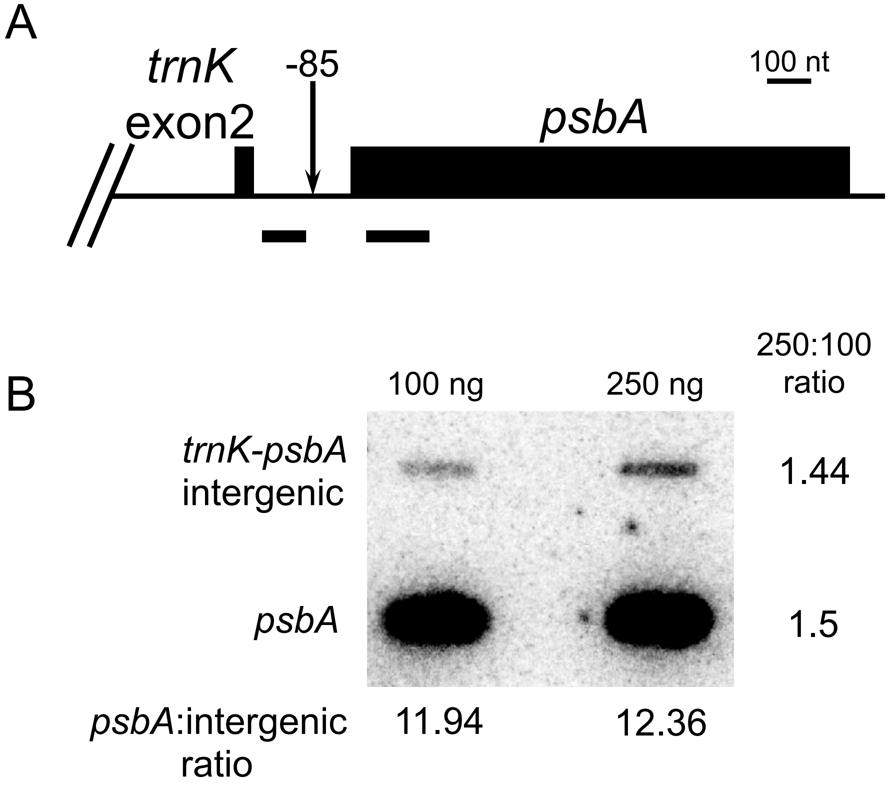
Supplementary Figure 1.
The majority of *atpH* 5’ ends in RNase J-deficient material map to RNA regions with predicted single-stranded character. Segments of the *atpI*-*atpH* intergenic sequence flanking (+/- 50 nucleotides) each mapped RNase J-dependent *atpH* 5’ extension (Figure 2B) were folded by the mfold web server (http://mfold.rna.albany.edu/?q=mfold) with standard parameters at 37°C. Predicted local secondary structures for each *atpH* 5’ end are shown. Filled arrowheads indicate termini mapping to unstructured regions (13 out of 15 clones), and open arrowheads designate ends mapping to structured segments.



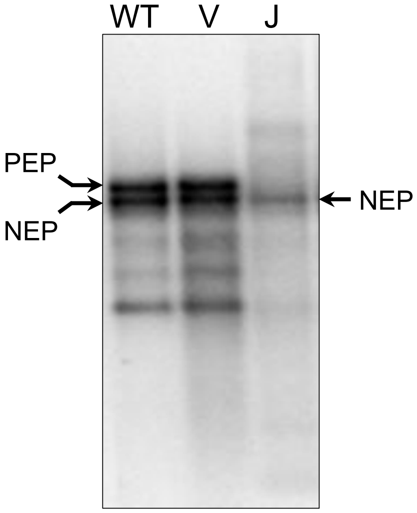
Supplementary Figure 2.
5’ RACE and RNA gel blot analyses of unspliced *petB* transcripts. (**A**) Schematic of the major bands detected by 5’ RACE. The filled gray and black arrowheads correspond to the similarly-labeled bands in (B). Open arrowheads represent estimated 5’ ends from products of intermediate sizes. HCF152, conserved minimal HCF152 binding site (1). (**B**)Gel separation of 5’ RACE products for *petB* intron-containing RNAs. The filled arrowhead designates the processed 5’ end of unspliced *petB* (2), the open arrowhead marks an unspliced transcript terminating upstream of *psbH*, and the asterisk indicates the linearized intron. Positions relative to the *petB* translation initiation codon are shown. (**C**) RNA gel blot hybridized with a α^{P32}-UTP-labeled RNA probe for the intron of *petB*. I_B indicates the *petB* intron. An ethidium bromide-stained image of 28S rRNA is shown below to reflect loading.



Supplementary Figure 3.
5’ RACE and RNA gel blot analyses of *psbB*. (**A**) Schematic of the major 5’ RACE products generated for *psbB*. Arrowheads correspond to the labeled bands described in (B). (**B**) Gel separation of *psbB* 5’ RACE products. TAP treatment is designated as in Figure 4. The filled arrowhead marks the processed *psbB* 5’ end (3); the open black arrowhead specifies the major PEP-derived primary transcript (3); and the open grey arrowhead indicates a second minor primary transcript (4). Numbers are positions relative to the *psbB* translation initiation codon. (**C**) RNA gel blot hybridized with a α^{32P}-UTP-labeled RNA probe for *psbB*. Arrowheads indicate the major primary (open) and processed (filled) forms of *psbB*. An ethidium bromide-stained image of 28S rRNA is shown below to reflect loading.



Supplementary Figure 4.
Run-on transcription in WT chloroplasts. 100 or 250 ng of PCR products from the regions labeled in red (A) were fixed to nylon membrane. Primers are in Supplementary Table S1. Run-on transcription (B) was conducted as previously described (7). The ratios were calculated after quantification using a Storm scanner (GE Healthcare).



Supplementary Figure 5.
DNA-probed RNA gel blot for *atpB*. NEP- and PEP-transcribed *atpB* transcripts are indicated (6).

Supplementary Figure 6.
Mature mRNA 5’ ends in *N. benthamiana* (filled arrowheads) closely resemble the 5’ ends of orthologous sRNA (open arrowheads) that serve as markers for PPR or PPR-like protein binding sites (1). Highlighted regions represent alignments to sRNA from *Arabidopsis* (5), or in the case of *psbB* from *H. vulgare* (1).

Supplementary references

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Supplementary Table 1. Primer sequences for VIGS target amplification, 5’ RACE analysis, and RNA gel blot probe synthesis.

Primer name	Sequence (5’-3’)
PPR10(1) – for	TAGGATCCGCTGTGGTTTTGAGCCTGAT
PPR10(1) – rev	GTTCCCTCCCTTGGAATAGC
PPR10(2) – for	TAGGATCCTCATGGGTATGGAGAGAGCA
PPR10(2) – rev	ATTCTTACGGCCTCCTCCAT
HCF152 – for	TAGGATCCGATGCGAGCGAGAGGTATTG
HCF152 – rev	GGCTTCCTAGCCAAAGCAAT
MRL1(1) – for	TAGGATCCTATGATTCCCGTGCCTTTTC
MRL1(1) – rev	CTTGATAAGGCAAGCCAAGC
MRL1(2) – for	TAGGATCCACTGGACTGAAACCCGATTG
MRL1(2) – rev	ATTGGTCGTGCTTCTGCTTT
RNJ(1) – for	ACGAATGGCACTATGGGAAG
RNJ(1) – rev	GGGCCGTCGCCTCCAAATC
RNJ(2) – for	GATTTGGAGGCGACGGCCC
RNJ(2) – rev	CTTGGAACCTGCTGTGGAGT
atpH sense – for	TTGTAATACGACTCACTATAGGGTCGCAAATAAAAGTGCTAATGC
atpH sense – rev	TTCCGTTATTGCTGCTGGAT
petB.exon sense – for	CCGTTACTGAGGCTTTTGCT
petB.exon sense – rev	TTGTAATACGACTCACTATAGGGGGCAGTAAGAAGCGGCAATA
rbcL sense – for	TTGTAATACGACTCACTATAGGGATGTCACCACAAACAGAGAC
rbcL sense – rev	TTACTTATCCAAAACGTCCACTG
psbA sense – for	TTGTAATACGACTCACTATAGGGGGTTGAAAGCCATAGTGCTGA
psbA sense – rev	GTATGCGACCTTGGATTGCT
petB.intron sense – for	TGATAGAGATGGTTCTACTTCGTCA
petB.intron sense – rev	TTGTAATACGACTCACTATAGGGTCTCTGGAAAATCCGAAAGC
psbB sense – for	GCTTTGGTTTTGGTGCATTT
psbB sense – rev	TTGTAATACGACTCACTATAGGGTATTCCATCCCCGTTATCCA
atpB – for	TATTTGGCGGAGTGGGTGAACGTA
atpB – rev	AGTTCATAGCCTTCGCGGTAGCTT
5’RACE adapter oligo (RNA)	AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA
5’RACE oligo primer	AATTCCTGTACGAACACTAG
atpH – 5’RACE GSP	GTGCTAATGCTACAACCAGTCC
petB.exon – 5’RACE GSP	ACGTGGGGAGGAACGTATTT
rbcL – 5’RACE GSP	TCCTTGGTTTGGTACTCAGGA
psbA – 5’RACE GSP	AACCATTCATCAACGGATGC
petB.intron – 5’RACE GSP	CCGGACACGAGGTCTGAATA
psbB – 5’RACE GSP	GCCAAAAAGCATAAGCCAGA
qPCR atpH-5’	CTCGCGATACCCTCTACAGC
qPCR atpH-3’	ATTTCTGCCGCTTCCGTTAT
qPCR petB-5’	GTATCTCACCGGCGGATTTA
qPCR petB-3’	CACTGCCCAATAACCGACTT
trnK-psbA inter-run-on-5’	CATGACTTATATACTCGTGTCAACCA
trnK-psbA inter-run-on-3’	TTCTAATTTTCTGTAGAGAAGTCCGTA
psbA-run-on-5’	TGTCTACTGGAGGAGCAGCA
psbA-run-on-3’	TGTCTACTGGAGGAGCAGCA