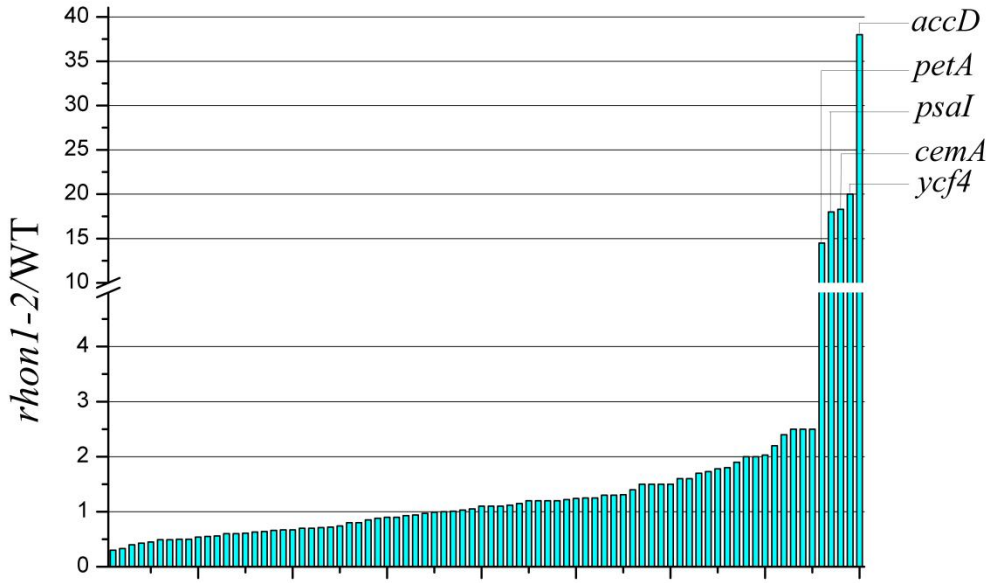
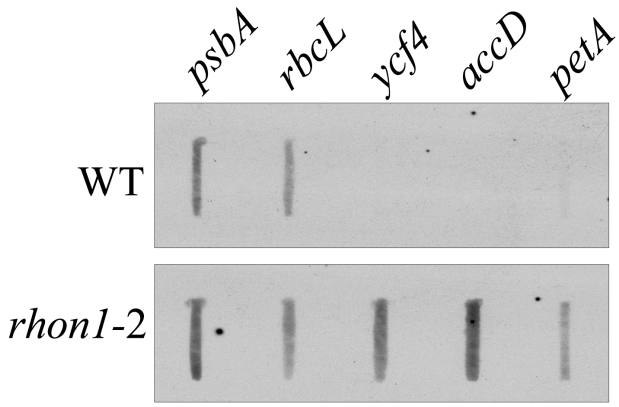
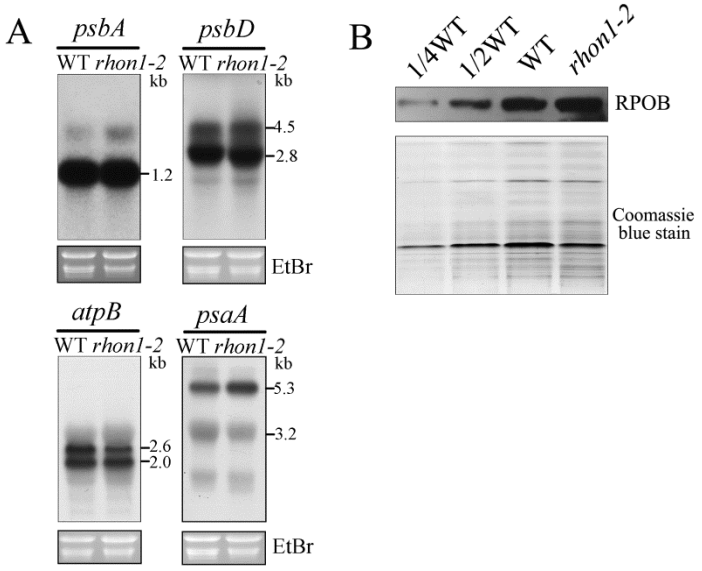


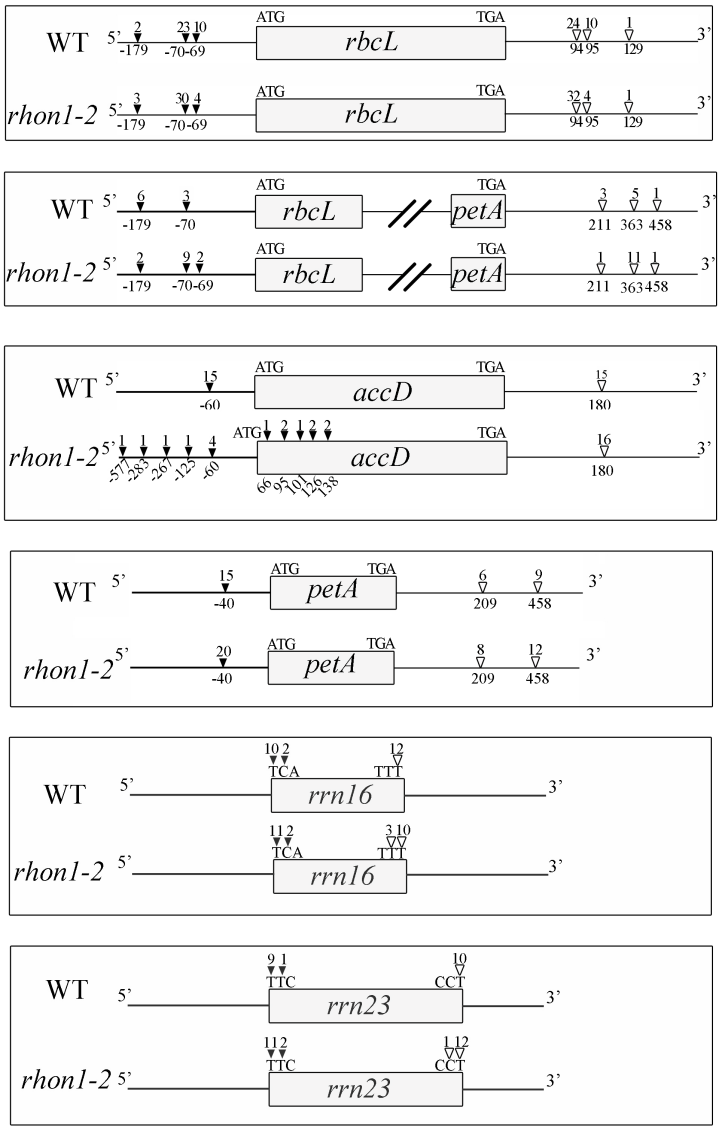
Supplemental Figure 1. Microarray analysis of plastid gene transcripts in *rhon1-2* compared with wild-type.
Values have been obtained from three independent experiments, each one performed in two replicates. The plastid genes are sorted as fellows (from left to right): *rpl22,rps3,rps14,psaB,atpE,psbJ,rpl14,rpl36,rps11,psbB,psbH,psbI,psbE,atpI, psbL,psbN,psbT,psbA,atpF,ndhA,rps8,ndhE,atpA,rbcL,psbF,ndhH,rps19,psaA,rps2, ycf1-1,rpl16,ndhK,psbC,rpL2,ndhJ,ndhC,psbK,atpH,petL,ndhI,ycf3,orf77,atpB,rps12,rpoC1, ndhD,psaJ,rps4,petN,rpl23,matK,rpl33,rps16,rps15,psbZ,rpoB,ndhG,rpoA, psbD,ycf2,psbM,ccsA,petB,ycf1,rpoC2,ndhB,rpl20,psaC,rps7,rpl32,rps18,clpP,petD, petG,ndhE,petA,psaI,cemA,ycf4* and *accD.*



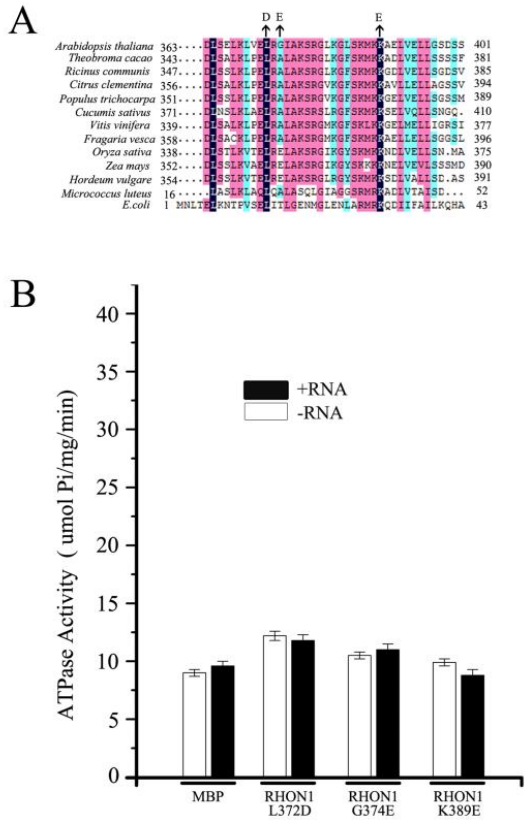
Supplemental Figure 2. Run-on transcription assay of plastid genes in *rhon1-2* and wild-type plants.
The procedure is same to that of Figure 2B but a lower amount of DNA probes (100ng) was used for hybridization with labeled transcripts.



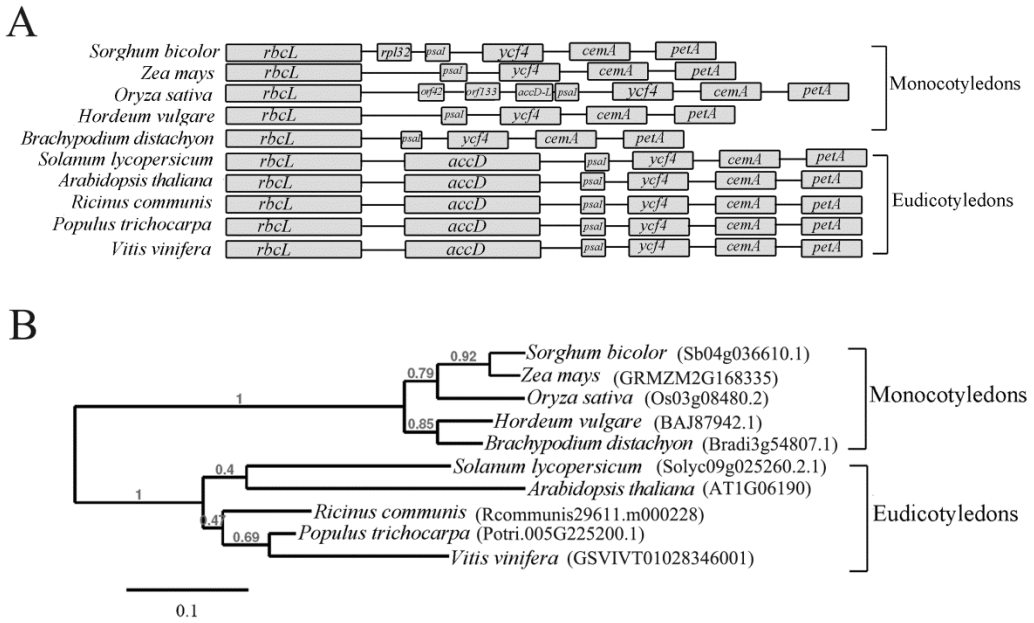
Supplemental Figure 3. Analysis of PEP-dependent transcription in *rhon1-2.*
(A) RNA gel blot analysis of the transcript levels of plastid genes in wild-type and *rhon1-2* plants. Total RNA (5 μg) were size-fractionated by agarose gel electrophoresis, transferred to nylon membranes, and probed with ^{32}P-labeled DNA probes. The sizes of the transcript (in kb) are shown. The 25S rRNA stained with ethidium bromide is shown as a loading control.
(B) Immunoblots of rpoB in wild-type and *rhon1-2* plants.



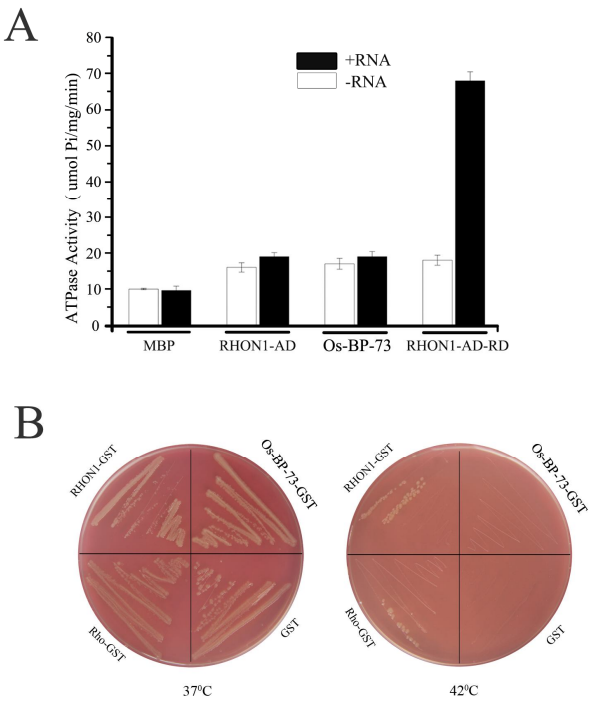
Supplemental Figure 4. Mapping of 3' and 5'ends of plastid transcripts in wild-type and *rhon1-2* plants by circularized RNA RT-PCR.
The 5' and 3' ends of transcripts for the indicated genes in wild-type and *rhon1-2* are shown by closed and open arrowheads, with numbers below indicating the positions of the nucleotide with respect to the initiation and termination codon respectively. For *rrn 16* and *rrn 23*, the nucleotides of the 5' and 3' ends are directly shown. Numbers of corresponding clones obtained are shown at each position.



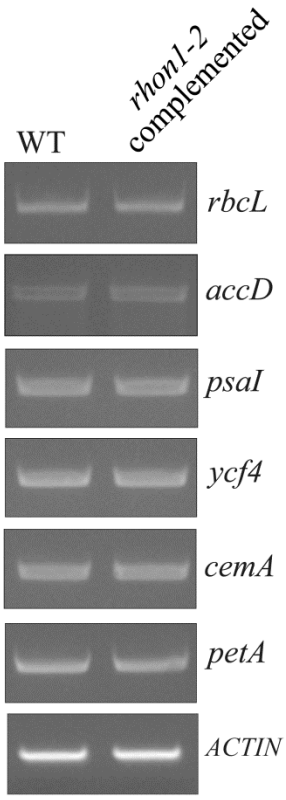
Supplemental Figure 5. The ATPase activities of mutated RHON1 proteins.
(A) The sequence alignment of RNA-binding domain of RHON1 homologs and Rho from *E. coli*. The conserved resides are shown in blue.
(B) The ATPase activities of three RHON1 proteins with site-directed mutations in conserved resides. Error bars represent the standard error of the mean (n=6)



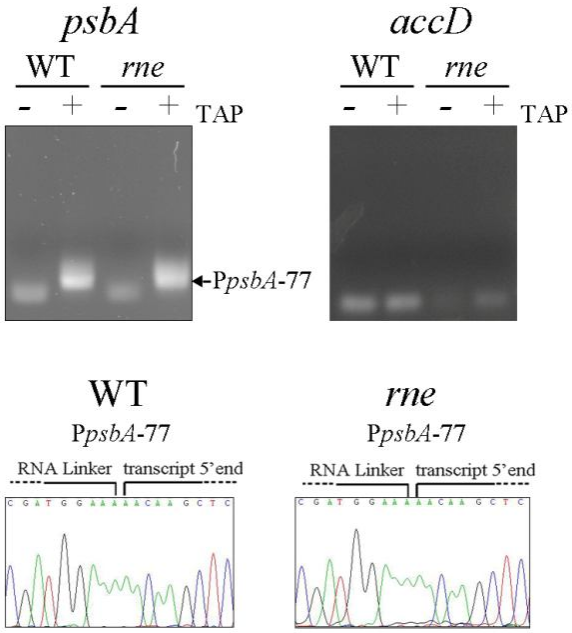
Supplemental Figure 6. Phylogenetic analysis of RHON1 homologs in vascular plants.
(A) Schematic illustrations for *rbcL*-associated gene locus of eudicotyledonous and monocotyledonous species.
(B) Phylogenetic analysis of RHON1 homologs of eudicotyledonous and monocotyledonous species. Phylogenetic tree was constructed on the Phylogeny.fr web tool (http://www.phylogeny.fr; Dereeper et al., 2008). Posterior probabilities are indicated near nodes. The bar indicates the branch length that corresponds to 0.1 substitutions per position.



Supplemental Figure 7. Characterization of RHON1 homolog of rice, Os-BP-73.
(A) The ATPase activity of rice Os-BP-73 protein. Error bars represent the standard error of the mean (n=6)
(B) Os-BP-73 is not able to complement the *rho* mutant of *E. coli.*



Supplemental Figure 8. The accumulation of *rbcL*, *accD*, *psaI, ycf4, cemA* and *petA* transcripts in RHON1- complemented plants assayed by RT-PCR.



Supplemental Figure 9. The transcription initiation sites of *accD* in *rne* mutant.
The transcription initiation site in green seedlings was identified by 5'- RACE as shown in Figure 3A. Chromatograms showed the sequences at the ligation sites of the cloned RT-PCR product. The transcription initiation site of *psbA*, P*psbA*-77 was used as a control.

Supplemental Table 1. List of Primers Used in This Study

Name	Sequence 5' → 3'	Experiment / Figure
rbcL-f	TGTCACCACAAACAGAGACTAAAGC	probe/1B,1C
rbcL-r	TCTACTCTTGGCCATCTAATTTATC	probe/1B,1C
accD-f	ATGTCACCACAAACAGAGACTAAAG	probe/1B,1C
accD-r	CTACTCTTGGCCATCTAATTTATCG	probe/1B,1C
psaI-f	TCAGTAAACCTCTATCAACAAGCTA	probe/1B,1C
psaI-r	ATTTTGTTGTGAACCTTTACCTTAG	probe/1B,1C
Ycf4-f	ATGAGTTGGCGATCAGAATCTATAT	probe/1B,1C
Ycf4-r	TCAAAATACTTCAATTGGTACACGC	probe/1B,1C
cemA-f	GGCAAAAAAGAAAGCATTCATTCCT	probe/1B,1C
cemA-r	GTCGTTTATTGCATGATAAATCACT	probe/1B,1C
petA-f	GCAAACTAGAAATACCTTTTCTTGG	probe/1B,1C
petA-r	CGGATAATTGAACCTTCTCAAACTG	probe/1B,1C
psbA-f	ATGACTGCAATTTTAGAGAGACGCG	probe/1C
psbA-r	TTATCCATTTGTAGATGGAGCCTCA	probe/1C
rbcL-race-outer	GATACCATGAGGTGGTCCTTGGA	RACE/2A
rbcL-race-inner	GCTGGTAAGCCCATCGGTCCACACA	RACE/2A
accD-race-outer	AGAGTAAGCAAAACATATCGATGCAA	RACE/2B
accD-race-inner	AGAGTAAGCAAAACATATCGATGCAA	RACE/2B
psbA-race-outer	TCCAGTTACAGAAGCGACCCCATAG	RACE/S5
psbA-race-inner	TCCAGTTACAGAAGCGACCCCATAG	RACE/S5
c-*accD*-rt-5	GGATCATAGTGCAGATCGTTGTCA	(CR) RT PCR/3
c-*accD*-5	AGAGTAAGCAAAACATATCGATGCAA	(CR) RT PCR /3
c-*accD*-3	GGTTCACAAGCGGCTGAATCTTTAT	(CR) RT PCR /3
c-*rbcL*-rt-5	CACAGTTGTCCATGTACCAGTAGA	(CR) RT PCR /3
c-*rbcL*-5	CTTGCTTTAGTCTCTGTTTGTGGT	(CR) RT PCR /3
c-*rbcL*-3	GATCTTGCAGTCGAGGGTAATGAA	(CR) RT PCR /3
c-*petA*-rt-5	CCTCAATATCCACGGGCTTATTAGC	(CR) RT PCR /3
c-*petA*-3	CTAGAACTTCTTGTTTCAGAGGGC	(CR) RT PCR /3
c-*cemA*-3	GCCTCACGGTTGGGAACTAATGATT	(CR) RT PCR /3
RHON1-f	ATGGCGATGTCGGGAACTTTCCATT	PCR/4C
RHON1-r:	TCAGCTGGAATCACTACCAAGCAAC	PCR/4C
*rbcL*42-88:	TGCACTCGGCTCAATCTTTTTTTTACTAAAA AAGATTGAGCCGAG	EMSA/5A
*rbcL*89-133:	GTTATCTGTTGTATATACTATTTTTTTTGATA GATACATACTTAAATTT	EMSA/5A
*rbcL*134-178	AGATAGAAAAAAAACTCTTCAATAAAAA AAAGAAGATTAAACACAACTA	EMSA/5A
*rbcL*179-235	CAATTTTGTTATTGTAGTGTTGTGTCCACA AGAAATCCTATACGAAACA	EMSA/5A
rrn16S-f	TGGGCGTAAAGCGTCTGTAGGTG	probe/S7
rrn16S-r	GGCTACCTTGTTACGACTTCACTCC	probe/S7
rrn23S-f	AGGAGAGCACTCATCTTGGGGTGGG	probe/S7
rrn23S-r	TTCAAACGAGGAAAGGCTTACGGTG	probe/S7
psbD-f	ATGACTATAGCCCTTGGTAAATTTA	probe/S2
psbD-r	TTAAAGAGCGTTTCCACGTGGTAGA	probe/S2
atpB-f:	ATGAGAACAAATCCTACTACTTCAA	probe/S2
atpB-r	TCATTTCTTCAATTTACTCTCCATT	probe/S2
psaA-f	ATGATTATTCGTTCGCCGGAACCAG	probe/S2
psaA-r	TTATCCTACTGCAATAATTCTTGCT	probe/S2
accD-p1:	CGAGATTTTACTAAAAAAGTTCTTAATATT CTTATATTCATAAGC	EMSA/S5
accD-p2:	AAGAACAAATATTTCTTTTTTTTTTATGAG AATTTTACACAATAT	EMSA/S5

Supplemental Table 1. List of Primers Used in This Study (Continued)

Name	Sequence 5' → 3'	Experiment / Figure
*rps16*i-f	GCAATGAGACAAACAAAAAAAGGGC	QPCR/S8
*rps16*i-r	CCATAAAAAGCATTCATAACATTCG	QPCR/S8
trnQ-f	CGCTATTCGGAGGTTCGAATCCTTC	QPCR/S8
trnQ-r	GAAAGATGGTGTACAAAGGCAATAG	QPCR/S8
trnT.1-f	TAGACTTTCAAATGAAATTTTGACT	QPCR/S8
trnT.1-r	GTAAGTCATCGGTTCAAATCCGATA	QPCR/S8
ndhD-2-f	ATAGCTCCATTAAGTCCAGGATCCG	QPCR/S8
ndhD-2-r	GTATGCTTCTCGCTGGAATCTTATT	QPCR/S8
ndhF-5'-2-f	TACTAAGAAAAGTCCACATGCGGCG	QPCR/S8
ndhF-5'-2-r	GGATCATCCCTTTCATTCCACTTCC	QPCR/S8
Ycf1.2-4-f	CCCAAATAACAGTTTTACGCCTTTG	QPCR/S8
Ycf1.2-4-r	CGATGAAATGGCTTTGATCCGTTAT	QPCR/S8
Ycf2.1-1-f	GTTAATACGATATAGAAGGGCCGCT	QPCR/S8
Ycf2.1-1-r	GTATCCTACTTTTGCGCAATTCACA	QPCR/S8
4.5S-5S-f	TGCTTTTCTCGCATGCCTTTCTTCG	QPCR/S8
4.5S-5S-r	CACCAAGTTCGGGATGGATTGGTGT	QPCR/S8
*psbL-psb*J-f	GTAATTTTATTATCCCATTCGGATG	QPCR/S8
psbL-psbJ-r	TATCCCGCTTCCCTCCACATTTAAT	QPCR/S8
PsaJ-rpl33-f	GTAGAATAAATTAGAAAAGGTGGGG	QPCR/S8
PsaJ-rpl33-r	GTATCTGATAGGTTCCCTAGCAACA	QPCR/S8
P1	ACCTGGTGCAGCAGCTAATCGTGTG	RTPCR/S9
P2	CAATTGCCGGAAATACTAAGCCTAC	RTPCR/S9
P3	CAGTAGTAGGTAAGTTAGAAGGGGA	RTPCR/S9
P4	GCGCGGTAAAGGAGGGAAAATAGCA	RTPCR/S9
P5	GAAGTGGAATAGAATAACCCGGTTA	RTPCR/S9
P6	GTCACTTGTTGACTCACGATTGTGC	RTPCR/S9
P7	GGGCTTTGTTGATTTACTGCGTGAT	RTPCR/S9
P8	CTGTCACCGTTACTCTCACTACCAC	RTPCR/S9
P9	GTAGGTAAACTTGAAGGAGACAGGG	RTPCR/S9
P10	CTATCCATTGCTTTACTTAGCTCAC	RTPCR/S9
P11	GTGATCTTGCTCGCGAAGGTAATGA	RTPCR/S9
P12	GTCTTAGTGAATCCCGATTCGACAT	RTPCR/S9
RHON1-PSN1301-S	GCCGGATCCATGGCGATGTCGGGAACTTTC	cDNA/1A
RHON1-PSN1301-A	GGCGGTACCTCAGCTGGAATCACTACCAAG	cDNA/1A
RHON1-ADRD-S	GCCGAATTCTATTCTAGCGAGGCAACTTTT	cloning/6B
RHON1-ADRD-A	GGCCTCGAGTCACTCCACTAATTCGGCTTT	cloning/6B
RHON1-AD-A	GGCCTCGAGTCATTTCACGGCTTCTTCCTC	cloning/6B
RHON1-GST-S	GAAGAATTCCCGCAAGTTCTGGCGGCTATA	cloning/6E
RHON1-GST-A	GGCCTCGAGTCAGCTGGAATCACTACCAAG	cloning/6E
OS73-GST-S	GCCGGATCCTGCAGTGCTAATCCCAACAAC	cloning/6E
OS73-GST-A	GGCGTCGACTCAAGCCATGTTGCTCAGCAG	cloning/6E
Rho-GST-S	GCCCCCGGGATGAATCTTACCGAAT	cloning/6E
Rho-GST-A	GCCCTCGAGTTATGAGCGTTTCATCATT	cloning/6E
OS73-1301-S	GCCGGATCCATGCTGCTTCCGCCGCATCCA	cDNA/7C
OS73-1301-A	GGCGGTACCTCAAGCCATGTTGCTCAGCAG	cDNA/7C
RHON1-GST-KE-A	GGCCTCGAGTCAGCTGGAATCACTACCA AGCAACTCCACTAATTCGGCGGCCTTC	cDNA/S5B
RHON1-GST-GE-A	CTACCAAGCAACTCCACTAATTCGGCTTTCTTCATCT TTGACAACCCTTTTAGTCCCCGTGACTTTGCTATCTC	cDNA/S5B
RHON1-GST-LD-A	GGCCTCGAGTCAGCTGGAATCACTACCAAGCAACTC CACTAATTCGGCTTTCTTCATCTTTGACAACCCTTTTA GTCCCCGTGACTTTGCTATGCCTCTGTC	cDNA/ S5B

Supplemental Methods

Microarray

The microassay was performed using Uniplastomic Macroarray Kit as describe (Zghidi et al. 2007). The synthetic oligonucleotide probes in sense-and antisense direction (60-mers) for the whole transcriptome of the plastid chromosome were spotted on nylon membranes. Each DNA sample was spotted two times on a nylon membrane. 2 µg of total RNA from *rhon1-2* mutant and wild-type seedlings was labeled using accompanied cDNA Labeling Kit in the in the presence of 100 µCi of [α-^{32}P]dATP. The cDNA samples were treated with RNase H at 37°C for 15 min and non-incorporated deoxyribonucleotides were removed by passage through Sephadex G50. Hybridization was carried out at 65 °C for 12 h. After washing microarrays were scanned and analyzed using ImageMaster TM 2D Platinum software version 5.0.
uminescent substrate.

Circularized RNA RT-PCR

The precise 5' and 3' ends of the plastid transcripts were determined using cRT-PCR as described in Pfalz et al (2009). Briefly, 10 µg RNA was circularized with T4 RNA ligase at 37°C for 1 h and then subjected to AMV reverse transcription with 2 pmol primer (mapping within ~100 nucleotides of the anticipated 5' end) for the synthesis of cDNA spanning the junction of the 5' and 3' ligated ends. This junction region was amplified by PCR using gene-specific primers, cloned into pGEM-T (Promega) and sequenced.

Phylogenetic analysis

A multiple alignment of RHON1 homologs and a phylogenetic tree were constructed using the A la Carte mode (Muscle 3.7 for multiple alignment; Gblocks 0.91b for alignment refinement; MrBayes 3.1.2 for phylogeny using maximum likelihood 6 number of substitution types, default substitution model, invariable + γ rates variations, MCMC 10,000 generations; TreeDyn 198.3 for Tree rending) of the Phylogeny.fr program online (Dereeper et al., 2008). The multiple sequence alignment used to construct the phylogeny was shown in Supplemental Data Set 1 online.

Supplemental References

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Zghidi, W., Merendino, L., Cottet, A., Mache, R., and Lerbs-Mache, S. (2007). Nucleus-encoded plastid sigma factor SIG3 transcribes specifically the *psbN* gene in plastids. Nucleic. Acids Res. **35:** 455-464.

Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., and Gascuel O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic. Acids Res. **36:** W465–469.