Supporting Information

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SI Results and Discussion

Identification of Insertion Sites of T-DNA in Transgenic Plants. DNA prepared from the transgenic lines was subjected to Southern hybridization and thermal asymmetric interlaced (TAIL)-PCR (1) to characterize how and where T-DNA harboring *SIG1* and its derivative are inserted. The introduced gene in the *SIG1*-transformed line named "S1-1", was integrated at a region outside the ORF of At3g47530, which has not been investigated except for its homology to pentatricopeptide repeat (PPR) and absence of a predicted transit peptide for chloroplast transport. We obtained several different lines transgenic with *sig1(T170V)*. The most intensively studied line named "m2-1", was analyzed by Southern hybridization and TAIL-PCR, and the introduced *sig1(T170V)* gene was detected at a single locus in a stretch of repetitive sequences near the centromere of chromosome 5, where any ORFs have not been reported.

SI Materials and Methods

Transformation of *Arabidopsis*. Constructs for ectopic expression of *SIG*s were made by replacement of the DNA fragment for sGFP (S65T) in the 35S-sGFP(S65T) binary vector (2) with the sequence of the ORFs of *SIG1*. To construct *sig1(S55L)* and *sig1(T170V)*, the *Pst*I/*Eco*RV fragment of the cDNA for *SIG1* was ligated into *Pst*I-digested and *Sma*I-digested pKF1914 (Takara). The mutated DNA fragments were amplified with the primers listed in Table S1 and selected by using Mutan-Super Express Km13 (Takara). The *Hin*dIII fragment harboring S55L was replaced with the corresponding wild-type region of the plasmid of *sig1(T170V)* to generate *sig1(S55L/T170V)*. The mutated constructs were replaced with their wild-type constructs in pUC18 to generate "CaMV 35S promoter-sig-NOS terminator" constructs and finally introduced into pSMAB701 (3). All of the constructs were confirmed by nucleotide sequencing. *Arabidopsis thaliana* (Columbia, Col-0) and its transformants were grown as described (2) except for a 16-h light/8-h dark cycle. Four transgenic lines were developed both for the wild-type *SIG1* and for *sig1(T170V)* and were named S1-1 to S1-4 and m2-1 to m2-4 respectively. S1-1 and m2-1 were the main subjects of the analysis, and the others were used supplementally.

Quantitative Reverse Transcription-PCR (RT-PCR). Total leaf RNA was reverse transcribed with a first-strand cDNA synthesis kit for RT-PCR (AMV) (Roche) by using random hexamer primers. Real-time PCR was performed with a LightCycler (Roche), with primers (Table S1) designed to generate products of <200 base pairs, and detected with LightCycler DNA Master SYBR Green I (Roche).

Preparation of Antibodies. Recombinant SIG1 and the recombinant C-terminal peptide of RpoB were made by using the pBAD/Thio-TOPO expression system (Invitrogen). The recombinant SIG1 and RpoB peptide were used to immunize rabbits (4), which were treated according to the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka. The anti-SIG1 and anti-RpoB were affinity purified from the IgG fraction with the recombinant products immobilized on Hybond-P (Amersham Biosciences), followed by elution with 0.1 M glycine-HCl (pH 2.5).

Protein Preparation. Leaves, unless noted, were ground by using mortars and pestles at 0 °C with 0.1 M K-phosphate (pH 7.0) containing the protease inhibitor mixture for plant cell and tissue extracts (Sigma) and the phosphatase inhibitor mixture (Sigma). Extracts were centrifuged at 10,000 × *g* for 15 min, and the resulting supernatants were used for analysis.

Chloroplast Preparation and Run-On Transcription. Chloroplasts were purified by Percoll (GM Healthcare) gradient centrifugation and aliquots (2.5×10^7 to 3.0×10^7 chloroplasts) were subjected to run-on assays as described (5). The DNA probes prepared by PCR with each primer set shown in Table S1 were immobilized on Hybond-N+ (GM Healthcare).

Thermal Asymmetric Interlaced (TAIL)-PCR. Total DNA was extracted from leaves of *Arabidopsis*, transgenic with wild-type *SIG1* (line S1-1) and *sig1(T170V)* (line m2-1), and subjected to TAIL-PCR (1). Primers specific to the T-DNA left borders were LB1, LB2, and LB3 (Table S1). The degenerate primer used was AD (Table S1). After tertiary PCR, fragments were separated by gel electrophoresis and purified from gels. Purified fragments were directly sequenced.

1. Liu YG, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J* 8:457–463.
2. Niwa Y, Hirano T, Yoshimoto K, Shimizu M, Kobayashi H (1999) Non-invasive quantitative detection and applications of non-toxic, S65T-type green fluorescent protein in living plants. *Plant J* 18:455–463.
3. Igasaki T, Ishida Y, Mohri T, Ichikawa H, Shinohara K (2002) Transformation of *Populus alba* and direct selection of transformants with the herbicide bialaphos. *Bull FFPRI* 1: 235–240.
4. Kobayashi H, Akazawa T (1982) Biosynthetic mechanism of ribulose-1,5-bisphosphate carboxylase in the purple photosynthetic bacterium, *Chromatium vinosum*. *Arch Biochem Biophys* 214:531–539.
5. Deng XW, Gruissem W (1995) *Methods in Plant Molecular Biology: A Laboratory Course Manual*, eds Maliga P, Klessig DF, Cashmore AR, Gruissem W, Varner JE (Cold Spring Harbor Laboratory Press, Plainview, NY), pp 191–207.
6. Isono K, et al. (1997) Leaf-specifically expressed genes for polypeptides destined for chloroplasts with domains of σ^{70} factors of bacterial RNA polymerases in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 94:14948–14953.

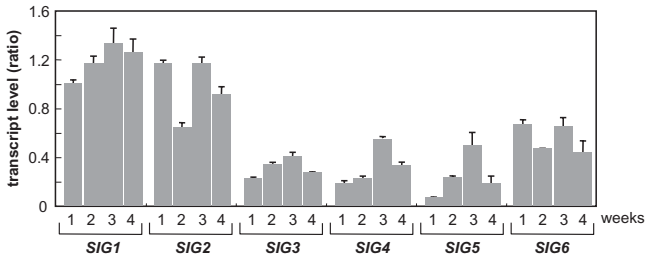

Fig. S1. Levels of transcripts of *SIG* genes in leaves harvested in 1–4 wk after germination. These were determined by real-time RT-PCR after treatment with RNase-free DNase and standardized with transcript for actin 2. Transcript levels of the *SIG* genes relative to the level for *SIG1* in 1-wk-old cotyledons grown in the dark are shown. The data presented are means with SDs of four individual experiments.

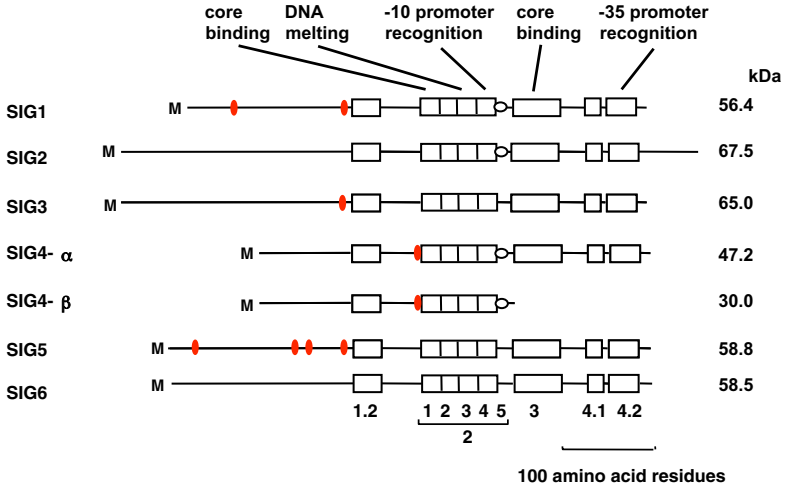

Fig. S2. Conservation of Ser/Thr phosphorylation sites among *Arabidopsis* SIG1 orthologs. The amino acid sequences were deduced from the nucleotide sequences of *SIG1*, *SIG2*, and *SIG3* reported by (6) and those of *SIG4* (GenBank accession no. AAC97954), *SIG5* (GenBank accession no. BAA78109), and *SIG6* (GenBank accession no. BAA92288). The open boxes indicated by 1.2, 2.1, 2.2, 2.3, 2.4, 3, 4.1, and 4.2 are regions conserved in the *Escherichia coli* sigma^{70} family. The open circles indicate conserved region 2.5. Red-filled circles are sites predicted to be phosphorylated by Ser/Thr protein kinases.

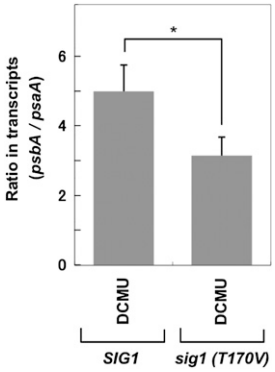

Fig. S3. Effect of phosphorylation of SIG1 on transcript levels of genes for PS-II (*psbA*) and PS-I (*psaA*) at an oxidized state of PQ in multiple transgenic lines. Transcript levels in three transgenic lines for both *SIG1* (lines S1-2 to S1-4) and *sig1(T170V)* (lines m2-2 to m2-4) were determined by real-time RT-PCR. The redox status of PQ was manipulated with the inhibitor DCMU. Ratio of transcripts of *psbA* to *psaA* in each plant is subjected to statistical analysis. Error bars represent the variations of three independent experiments. The asterisk indicates statistically significant differences ($P < 0.05$).

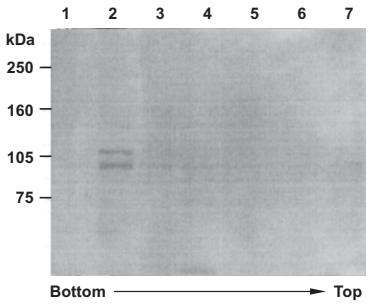

Fig. S4. The β-subunit (*rpoB* product) detected by immunoblotting of SDS/PAGE after fractionation by sucrose density-gradient centrifugation. The experiments were done as described for Fig. 3*A*, except for the employment of anti-RpoB, which has been prepared as shown in *SI Text*.

Table S1. Primers used in PCR for cloning, in real-time PCR, and in TAIL-PCR

Gene	Template	Sequence
For cloning transgenic plants		
SIG1	*SIG1* cDNA	5′-AAAGAGTG<u>GTCGAC</u>CatgGCTACTGCAGCTGTTATAGGA-3′
		5′-AGGAAACAGCTATGACCATGA-3′ (M13 reverse)
sig1(S55L)	*SIG1* cDNA	5′-GCGAAAAAGGCGT***T***GAACTATAGTCCG-3′
		5′-AACCAGACCGTTCAGCTGGA-3′ (selection primer)
sig1(T170V)	*SIG1* cDNA	5′-GGTGCTAAGAAGAAA***GT***TAATATGACTCATG-3′
		5′-AACCAGACCGTTCAGCTGGA-3′ (selection primer)
For cloning recombinant product		
SIG1	*SIG1* cDNA	5′-ATGGCTACTGCAGCTGTTATAGG-3′
		5′-ATTCTTAAGGATCATTGCCTCC-3′
For real-time PCR		
rbcL		5′-TGTACTACAATTCGGTGGAGGAAC-3′
		5′-TTGTACACATGCTTCCAGAGCTAC-3′
ACT2		5′-CGCTATGTATGTCGCCATCCAAGC-3′
		5′-CATTGGCACAGTGTGAGACACACC-3′
cab		5′-TGGTAAGGGACCGATAGAGAACC-3′
		5′-TTCTCCTCTCACACTCACGAAGC-3′
psaA		5′-CATGTCTTCTTAGGACTATTC-3′
		5′-AAGAACCATAAGACTGAATTACCTG-3′
psbA		5′-CCAATCCGTAGTTGATAGTCAAGG-3′
		5′-AACTTCCATACCAAGGTTAGCACG-3′
SIG1		5′-ATAAGTGCAACACTCGGAGAAC-3′
		5′-CTCTCTCTCGATAATCCTATCC-3′
SIG2		5′-AAGAGACTCATGGCGGTTCTACTC-3′
		5′-CTGACGTTACTGCTTCTGGATCTG-3′
SIG3		5′-AATGTCTACGATGGTGTCACGGC-3′
		5′-TTGCAATCTCCTCATCAGCCGCA-3′
SIG4		5′-TGTTGTTGGAGCTTCACGATCTGG-3′
		5′-CCACTGGCTAACAAAACTGATACC-3′
SIG5		5′-AACACCTGAGAGATACCGTGAAG-3′
		5′-CCGTACCTCTGTCGAATAACCAGAC-3′
SIG6		5′-CAGCAAACCTACGTCTGGTAGTCC-3′
		5′-CCAGTAAGCGTAAGTAGCGAATCTGCATC-3′
For preparing run-on probes		
psaA		5′-CCTACATGCTGATGCTCACGATTTTGATAG-3′
		5′-GAAGGTAATGGGTTACTCCTACAGCACG-3′
psbA		5′-ATGACTGCAATTTTAGAGAGACGCGA-3′
		5′-AACTTCCATACCAAGGTTAGCACG-3′
psbD		5′-CTGAATAATGAGTCATCCGACAATTC-3′
		5′-GAACCTCCTCAGGGAATATAAGG-3′
rbcL		5′-ATGTCACCACAAACAGAGACTAAAGC-3′
		5′-CGATGGTTGGGAACTTAAATGTG-3′
rpoB		5′-ACAAATTTTAGCGGATGGTGCTGC-3′
		5′-AACTTCCTTCCTATTAATCTGGAAAT-3′
rrn16		5′-GCTGAGGAGCAAAAGGAGGAATC-3′
		5′-CAAGCCCTGGTAAGGTTCTTCG-3′
accD		5′-TGTCGACAAGAAATCCTATACGAAACATGG-3′
		5′-CTTCAAGCTTTGGTGCTCCTCCGGAAGAAC-3′
SIG1		5′-ATGGCTACTGCAGCTGTTATAGG-3′
		5′-ATTCTTAAGGATCATTGCCTCC-3′
For TAIL-PCR		
LB1		5′-GAAGTTGACCGTGCTTGTCTC-3′
LB2		5′-TCGATGTAGTGGTTGACGATG-3′
LB3		5′-GGATAAGGTAATTGCCATTGCAG-3′
AD		5′-(A/T)GTGNAG(A/T)ANCANAGA-3′

Italics indicate nucleotides not complementary (additional or exchanged) to the templates used. Underlining indicates unique restriction enzyme sites. Lowercase indicates original initiation codons. Bold indicates nucleotides substituted.