Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 14948–14953, December 1997
Plant Biology

Leaf-specifically expressed genes for polypeptides destined for chloroplasts with domains of σ^{70} factors of bacterial RNA polymerases in *Arabidopsis thaliana*

(tissue specificity/photosynthesis/transit peptides/green fluorescent protein/cDNA)

KYOICHI ISONO*, MASANORI SHIMIZU†, KOHKI YOSHIMOTO†, YASUO NIWA†, KIMIYUKI SATOH‡, AKIHO YOKOTA*, AND HIROKAZU KOBAYASHI†§¶

*Laboratory of Plant Molecular Physiology, Research Institute of Innovative Technology for the Earth, 9-2 Kizugawadai, Kizu-cho, Soraku-gun, Kyoto 619-02, Japan; †Laboratory of Plant Cell Technology, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422, Japan; ‡Department of Biology, Faculty of Science, Okayama University, 3-1-1 Tsushimanaka, Okayama 700, Japan; and §Laboratory of Biological Regulation and Photobiology, National Institute for Basic Biology, Okazaki, 444, Japan

Edited by Lawrence Bogorad, Harvard University, Cambridge, MA, and approved October 29, 1997 (received for review June 12, 1997)

ABSTRACT **Genes for σ-like factors of bacterial-type RNA polymerase have not been characterized from any multicellular eukaryotes, although they probably play a crucial role in the expression of plastid photosynthesis genes. We have cloned three distinct cDNAs, designated *SIG1*, *SIG2*, and *SIG3*, for polypeptides possessing amino acid sequences for domains conserved in σ^{70} factors of bacterial RNA polymerases from the higher plant *Arabidopsis thaliana*. Each gene is present as one copy per haploid genome without any additional sequences hybridized in the genome. Transient expression assays using green fluorescent protein demonstrated that N-terminal regions of the *SIG2* and *SIG3* ORFs could function as transit peptides for import into chloroplasts. Transcripts for all three *SIG* genes were detected in leaves but not in roots, and were induced in leaves of dark-adapted plants in rapid response to light illumination. Together with results of our previous analysis of tissue-specific regulation of transcription of plastid photosynthesis genes, these results indicate that expressed levels of the genes may influence transcription by regulating RNA polymerase activity in a green tissue-specific manner.**

The chloroplast is a semi-autonomous organelle whose genetic information is encoded in the nuclear and plastid genomes. The plastid genome encodes genes for photosynthesis, as well as genes for housekeeping functions such as protein synthesis. There is evidence that photosynthesis genes are transcribed by a multimeric *Escherichia coli*-type RNA polymerase (RNAP), and that housekeeping genes are transcribed by a monomeric T7 or T3 bacteriophage-type RNAP (1). The −10 and −35 sequences, 5′-TATAAT-3′ and 5′-TTGACA-3′, respectively, in promoters of many plastid genes (2) are recognized by the *E. coli*-type RNAP (3). The *E. coli* RNAP is composed of a core complex of α, β, and β' subunits and one of a variety of σ factors, the principal one being σ^{70}, which is capable of binding to the −10 and −35 sequences (4–6). Determination of the complete nucleotide sequences of plastid genomes from liverwort (7), tobacco (8), rice (9), and other plants has resulted in finding of genes, *rpoA*, *rpoB*, and *rpoC*, probably encoding α, β, and β' subunits, respectively, of a plastid RNAP. In higher plants, *rpoC* is duplicated, *rpoC1* for β' subunit and *rpoC2* for β'' subunit. Amino acid sequences deduced from maize plastid genes, *rpoC2*, *rpoB*, *rpoC1*, and *rpoA*, have proved to correspond to those determined chemically of 180-, 120-, 78-, and 38-kDa polypeptides, respectively, of highly purified maize plastid RNAP (10, 11).

No homolog for the bacterial-like σ factor of RNAP has been detected in the plastid genomes so far sequenced. However, some reports indicate that plastid σ-like factors (SLFs) exist in higher plants. Antibodies against an *E. coli* σ^{70} homolog from the cyanobacterium *Anabaena* sp. PCC7120 have cross-reacted with polypeptides in purified plastid RNAPs from maize and rice (12). In spinach 90- and 33-kDa polypeptides have been identified in a plastid RNAP that are immunologically related to the σ^{70} factor of *E. coli* RNAP, and these proteins bound to the promoter of gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (*rbcL*) and correctly initiated transcription in association with RNAP core enzyme from spinach (13). Functionally distinct 67-, 52-, and 29-kDa SLFs have been identified in mustard, and none of them alone bind to DNA, but they confer enhanced binding to promoters and transcriptional activity on RNAP core enzyme (14, 15). These experiments suggest that genes for homologs of the *E. coli* σ^{70} factor exist and are encoded in the nuclear genome. Nuclear genes for such σ factors have recently been reported in the red alga *Cyanidium caldarium* (16, 17) but not in any multicellular eukaryotes.

We have demonstrated that expression of plastid photosynthesis genes is reduced predominantly at the level of transcriptional initiation in nonphotosynthetic tissues of *Arabidopsis thaliana*, indicating the importance of regulation through RNAP activity in plastids (18). Therefore, we thought that cloning of genes for SLFs from multicellular eukaryotes was needed for complete understanding of the regulatory mechanism. We report here three cDNAs for putative σ factors from the higher plant *A. thaliana*. Amino acid sequences deduced from the cloned cDNAs have high homology to conserved regions of the bacterial σ^{70} factor family.

MATERIALS AND METHODS

Plant Materials. *A. thaliana* ecotype Columbia was grown on vermiculite for 4 weeks at 22°C under 16-hr light/8-hr dark. *A. thaliana* was also grown on a Murashige–Skoog (MS) agar medium (19) without sugar at 22°C in continuous light at 3,000

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviations: RNAP, RNA polymerase; SLF, σ-like factor; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RT, reverse transcription; EST, expressed sequence tag; GFP, green fluorescent protein.
Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AB004820, AB004821, and AB004822 for *SIG1*, *SIG2*, and *SIG3*, respectively).
¶To whom reprint requests should be addressed. e-mail: hirokazu@u-shizuoka-ken.ac.jp.

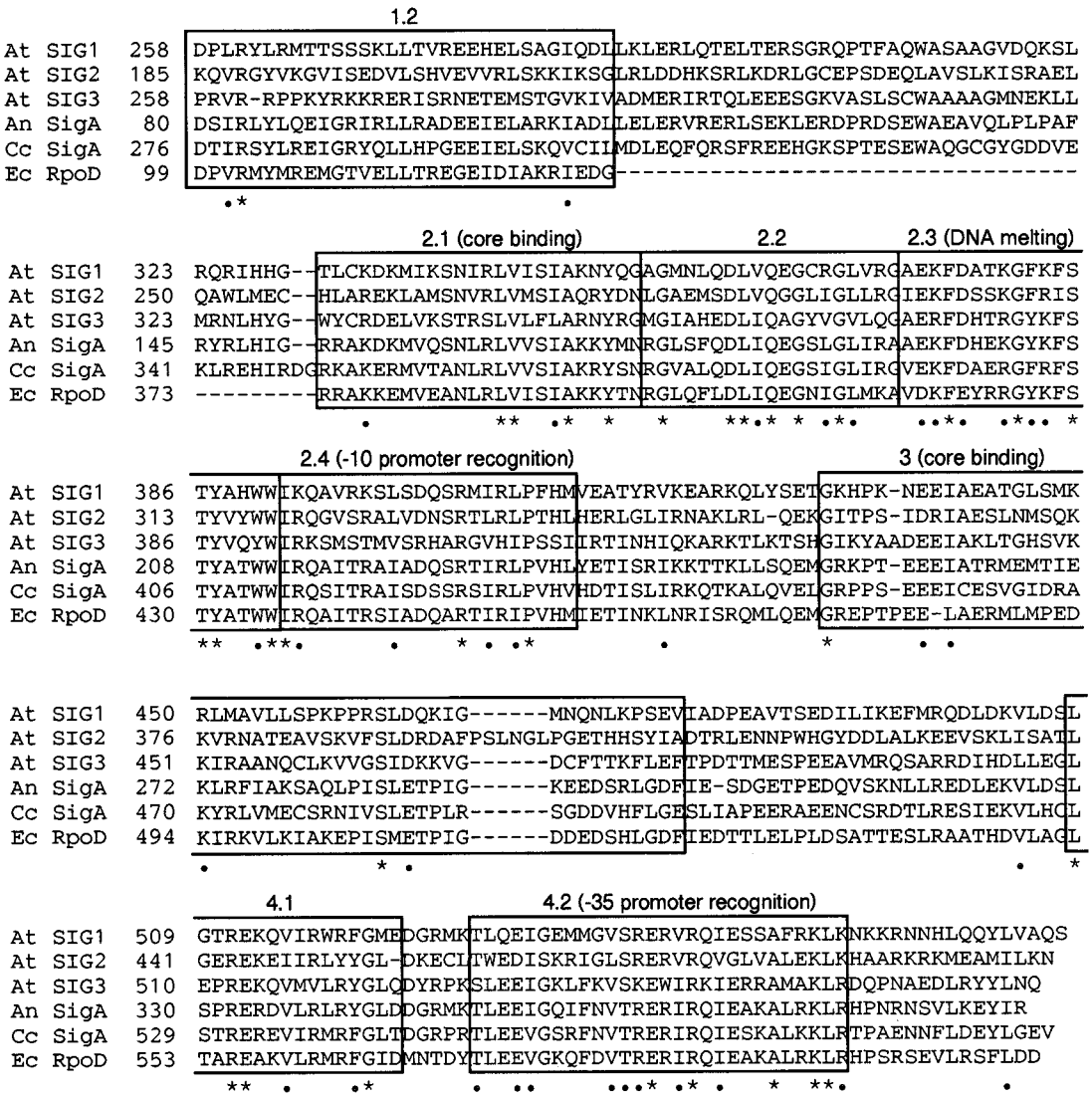

FIG. 1. Alignment of conserved domains in the deduced amino acid sequences of *A. thaliana* SIG1 (At SIG1), SIG2 (At SIG2), and SIG3 (At SIG3) with those of SigA from *Anabaena* sp. PCC 7120 (An SigA), SigA from *C. caldarium* (Cc SigA), and σ^{70} factor encoded in *rpoD* from *E. coli* (Ec RpoD). Regions 1–4 in bacterial σ factors (5, 26) are boxed. Residues marked with an asterisk (*) or a dot (•) are identical or similar, respectively, in all six sequences. The numbers between the product designations and the amino acid sequences are those of amino acid residues. There are 246 amino acids in a gap between subregion 1.2 and subregion 2.1 in *E. coli* RpoD.

lux for 3 weeks until the growth stage of generation of 8 rosette leaves, and employed for reverse transcription (RT)–PCR. Tobacco (*Nicotiana tabacum* cv. Petit Havana) was grown on a MS agar medium (19) containing 3% sucrose at 28°C under continuous light at 4,000 lux.

Preparation of Nucleic Acids. Total cellular DNA was prepared from leaves with cetyltriethylammonium bromide (CTAB) (20), and RNA was isolated from leaves and roots with a Total RNA Separator Kit (CLONTECH) according to the manufacturer's instructions. Poly(A)$^+$ RNA fraction was recovered from the total RNA by using Oligotex-dT30 (Takara, Otsu, Japan). Total cellular RNA was also prepared by Isogen (NipponGene, Toyama, Japan) and treated with RQ1 (RNase-free DNase, Promega) following the suppliers' instructions for employment for RT-PCR.

Screening of cDNAs for σ^{70} Homologs. The amino acid sequence GYKFSTYAMWWIRQAITRSIAD, which is responsible for DNA melting and recognition of the −10 sequence in bacterial promoters, was highly conserved in σ^{70} factors in bacteria. The sequence was subjected to homology search in the database of *A. thaliana* expressed sequence tags (ESTs). Three EST clones were found with accession numbers [stock numbers at the *Arabidopsis* Biological Resource Center (ABRC), number of nucleotides reported, including unidentified ones], N65838 [240C23T7, 538 bp], T88387 [155H23T7, 389 bp], and N97044 [242P3T7, 535 bp]. DNA fragments corresponding to the first two of these clones were amplified by PCR from total cellular DNA from *A. thaliana* with primers designed on the basis of their nucleotide sequences in the EST database. The PCR products were inserted into pT7Blue-T (Novagen) and sequenced to confirm the inserts. These two inserts, as well as a *Sal*I-*Xho*I fragment from 242P3T7A, were labeled with [α-^{32}P]dCTP by using a Random Primer DNA Labeling Kit (Takara), and used to screen a λ ZAP cDNA library (4×10^5 plaque-forming units) of *A. thaliana* leaves, which was constructed by using the ZAP-cDNA Synthesis Kit (Stratagene). The inserts of positively hybridizing clones were sequenced by an Applied Biosystems 373A DNA sequencer.

Hybridization of Nucleic Acids. Total cellular DNA digested with *Pst*I or *Eco*RI was electrophoresed in 1% agarose gels, and poly(A)$^+$ RNA was electrophoresed in 1.2% agarose gels containing 0.66 M formaldehyde (21). The nucleic acids were transferred to nylon membranes (Hybond-N+, Amersham) with 0.4 M NaOH for DNA and 20× SSC for RNA (1× SSC =

0.15 M NaCl/0.015 M sodium citrate, pH 7.0). The prehybridization and hybridization were performed at 65°C (21). Gene-specific DNA probes were labeled with [α-^{32}P]dCTP by using a Random Primer DNA Labeling Kit (Takara). Membranes were washed with 2× SSC containing 0.1% SDS for 30 min at 65°C, and with 0.5× SSC plus 0.1% SDS under the same conditions. Radioactivity on the membranes was detected by BAS2000 Bio-Imaging Analyzer (Fujix, Tokyo).

RT-PCR. Total cellular RNA prepared from *A. thaliana* was treated with RQ1 DNase, following the supplier's instructions. RT-PCR was performed with ≈1 μg of the total RNA and oligo(dT)$_{12-18}$ in 21 μl of reaction mixture by using the SuperScript Preamplification System for First Strand cDNA Synthesis (GIBCO/BRL). An aliquot (2 μl for *SIG* genes, described in *Results*, or 1 μl for *ACT2* encoding actin 2) from the reaction mixture was further subjected to PCR with AmpliTaq (Perkin–Elmer). Primers for PCR of *SIG1*, *SIG2*, and *SIG3* (see *Results*) were designed to amplify 395-, 254-, and 342-bp DNA fragments, respectively, on the basis of nucleotide sequences of cDNAs determined in this investigation: 5′-CCTCCGAGGTCGCTAGACCAG-3′ and 5′-GCTCAGGTGGGTGGGTTCTATGC-3′ for *SIG1*, 5′-GGTCTCCCTGGAGAAACTCATC-3′ and 5′-CTCCAGCGCCACAAGCCCTACC-3′ for *SIG2*, and 5′-GATTCGAGCAGCTAACCAATGCC-3′ and 5′-GAGGTCTTCGGCGTTGGGTTGG--3′ for *SIG3*. *ACT2* (22) was also examined as an internal standard with primers, 5′-GAAGATTAAGGTCGTTGCACCACCTG-3′ and 5′-ATTAACATTGCAAAGAGTTTCAAGGT-3′, to amplify the 477-bp DNA fragment with cDNA or the 563-bp one with genomic DNA, if any, contaminating the RNA fractions.

Transient Expression of Chimeric Genes. DNA fragments encoding the first 83 and 89 amino acid residues of the ORFs of *SIG2* and *SIG3* cDNAs, respectively, were amplified by PCR with pairs of synthetic oligonucleotides containing new *Nco*I sites (italicized): 5′-TT*CCATGG*CTACTGCAGCTG-3′ and 5′-TT*CCATGG*TAGAAGCAACATCATC-3′ for *SIG2*, and 5′-TT*CCATGG*CTTCCTTCA-3′ and 5′-TT*CCATGG*ATAGAAACGACC-3′ for *SIG3*. Two PCR products were digested with *Nco*I and inserted into the *Nco*I site of CaMV35S-sGFP(S65T)-nos [pUC18, harboring a synthetic gene for improved green fluorescent protein sGFP(S65T), hereafter referred to as "GFP"] driven by cauliflower mosaic virus 35S promoter and *NOS* terminator (23), resulting in two chimeric GFP constructs, SIG2-GFP and SIG3-GFP. The synthetic GFP gave ≈100 times higher fluorescent signal upon excitation with 488-nm light in comparison with that of the native GFP (23). The chimeric constructs were introduced into tobacco leaf cells by using Biolistic PDS-1000/He (Bio-Rad). Gun parameters employed were as follows: pressure rupture disks rated at 1,100 psi (7.58 MPa), a vacuum at 27 inches of Hg (reaching 9.83 kPa), distance to target tissues 6 cm, and gold particles 1.0 μm in diameter. After bombardment, cells were incubated for 20 hr at 28°C under continuous light, and observed by using the Bio-Rad MRC-1024 Confocal Imaging System (480×).

RESULTS

Three Distinct cDNAs for Polypeptides with Conserved Domains in σ Factors. Three cDNA clones (reported partial sequences of 538 bp, 389 bp, and 535 bp, including unidentified nucleotides) in the EST database of *A. thaliana* contain animo acid sequences that are highly homologous to regions for melting DNA and recognizing the −10 promoter sequence of bacterial σ factors. The sequences in these clones were employed as probes to screen a leaf cDNA library from *A. thaliana*, and positively hybridizing clones, 6, 9, or 7, respectively, for these three ESTs, were obtained from the library of 4×10^5 plaque-forming units. Three cDNAs, corresponding to stock numbers 240C23T7, 155H23T7, and 242P3T7, were designated *SIG1*, *SIG2*, and *SIG3*, respectively. The largest ORFs of *SIG1*, *SIG2*, and *SIG3* coded for polypeptides with 572, 502, and 571 amino acids, respectively.

Similarity of Functional Domains in σ Factors. Amino acid sequences deduced from *SIG1*, *SIG2*, and *SIG3* were compared with σ factors from *E. coli* (24), *Anabaena* sp. PCC7120 (25), and *Cyanidium caldarium* (17) (Fig. 1). The comparison showed that all ORFs of *SIG* genes are highly conserved in two major regions (designated regions 2 and 4) in bacterial σ factors (26). Subregions 2.1, 2.3, 2.4, and 4.2 are involved in interaction with core enzyme, DNA melting, recognition of −10 promoter sequence, and recognition of −35 promoter sequence, respectively. The subregion 4.1 can be modeled as an amphipathic α-helix. Region 3 may contribute to binding to core RNAP. The functions of subregions 1.2 and 2.2 are unclear. Table 1 shows comparisons of SIG1, SIG2, and SIG3 with the highly conserved subregions 2.1, 2.3, 2.4, and 4.2 from *E. coli*, *Anabaena*, and *C. caldarium*. Among three SIGs, SIG1 has the highest similarity to bacterial σ factors, and SIG3 has the least similarity. Among the subregions of the SIGs, 2.3 is the most conserved, while 2.1 and 2.4 are the least conserved, especially in SIG3 (18–29%). The subregion 4.2 in SIG2 is more diverse from bacterial σ factors, showing 43% in spite of 57–61% in SIG1 and 57–64% in SIG3.

Numbers and Expression of the *SIG* Genes. Total cellular DNA from *A. thaliana* was digested with *Pst*I or *Eco*RI and subjected to Southern hybridization using *SIG1*, *SIG2*, and *SIG3* as probes (Fig. 2*A*). One band was generated in each case except for *SIG2* and *SIG3* digested with *Pst*I (lanes 4 and 6). Multiple bands in these lanes resulted from the presence of *Pst*I sites in these two *SIG* genes. Therefore, *SIG1*, *SIG2*, and *SIG3* are probably single-copy genes in *A. thaliana*.

We have already demonstrated that suppressed expression of the plastid genome-encoded photosynthesis genes in roots of *A. thaliana* is due predominantly to transcriptional regulation (18). To examine the possibility that there is an involvement of SIGs in the transcriptional regulation, levels of transcripts for the *SIG* genes in RNAs from leaves and roots were investigated by Northern hybridization. All transcripts for *SIG1*, *SIG2*, and *SIG3* were detected in leaves, but barely in roots (Fig. 2*B*). This finding suggests that expressed levels of *SIG* genes correlate with transcriptional activities of plastid photosynthesis genes evaluated by run-on transcription (18).

Light induction of *SIG* transcripts in leaves of dark-adapted plants was examined by RT-PCR. Transcripts for *ACT2* (encoding actin 2), which is reported to be constitutively expressed

Table 1. Identity of *A. thaliana* SIG1, SIG2, and SIG3 to other factors in the σ^{70} family in major conservative regions

	Identity, %		
σ factor subregion	SIG1	SIG2	SIG3
Escherichia coli			
2.1	50	42	25
2.3	61	44	56
2.4	55	45	27
4.2	57	43	57
Anabaena			
2.1	58	50	29
2.3	78	61	67
2.4	55	64	18
4.2	61	43	57
Cyanidium caldarium			
2.1	46	50	21
2.3	78	67	56
2.4	50	55	27
4.2	57	43	64

See Fig. 1 for sequences of the σ factors.

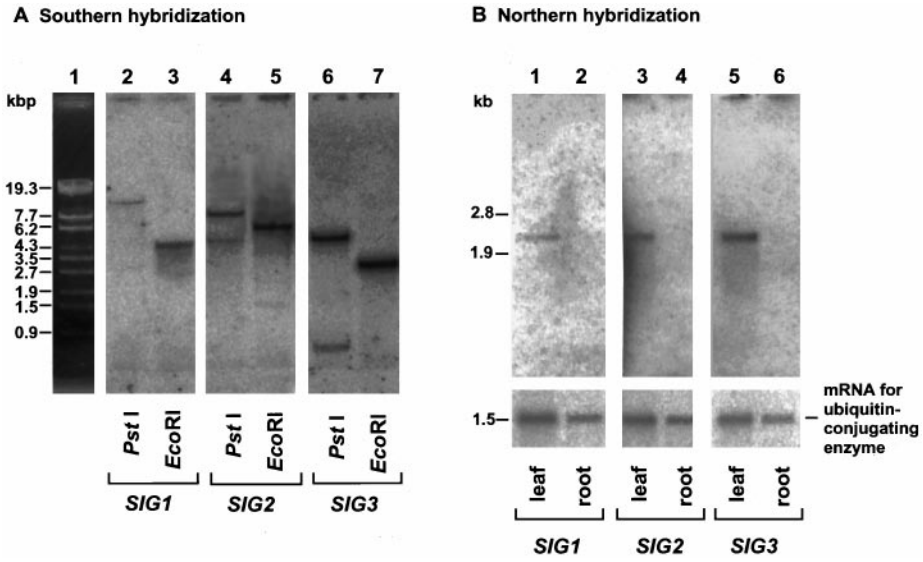

FIG. 2. Southern and Northern blot analyses of *SIG* genes. (*A*) Southern hybridization with *SIG* gene probes of total cellular DNA (8 μg per lane) digested with *Pst*I (lanes 2, 4, and 6) and *Eco*RI (lanes 3, 5, and 7). DNA in lanes 2 and 3, lanes 4 and 5, and lanes 6 and 7, was hybridized with 1.7-kb *Eco*RI fragment from *SIG1* cDNA, 1.6-kb *Xba*I fragment from *SIG2* cDNA, or 1.2-kb *Xho*I fragment from *SIG3* cDNA, respectively. (*B*) Northern hybridization with *SIG* probes of total poly(A)$^+$ RNA (0.5–1.0 μg per lane) from leaves (lanes 1, 3, and 5) and roots (lanes 2, 4, and 6). The cDNA for ubiquitin-conjugating enzyme, which is constitutively expressed (M.S., Y.N., and H.K., unpublished results), was used as a probe as an internal control for equivalence of amounts of RNA loaded into lanes. Each of the *SIG* genes gives rise to ≈2.3-kb mRNA.

(22), were determined as internal standards both for proving no contamination with genomic DNA in the RNA fractions and estimating the amounts of *SIG* transcripts. Expression of all three *SIG* genes was obviously under light control (Fig. 3). *SIG1* and *SIG2* transcripts were induced during 3-hr illumination (Fig. 3*B*) and seemed to reach a plateau between 7 and 15 hr in the light induction (Fig. 3*A*).

Destination of *SIG* Gene Products for Chloroplasts. Transit peptides can be predicted by PSORT, a program for protein sorting (http://psort.nibb.ac.jp/index.html) (27). The N-terminal regions of SIG2 and SIG3 (Fig. 4) were predicted to be transit peptides at probability higher than 90%. These N-terminal sequences are enriched with Ser and Thr, a property of transit peptides (28). However, the N-terminal region of *SIG1* ORF (Fig. 4) was less likely to be a transit peptide.

We have tested whether N-terminal regions of SIGs can function as transit peptides for plastid-targeting by transient expression assays with an improved GFP. We made two constructs in which peptides composed of the first 83 amino acids of *SIG2* ORF and of the first 89 residues of *SIG3* ORF (Fig. 4) were fused to the N terminus of GFP (SIG2-GFP and SIG3-GFP) and placed under the control of cauliflower mosaic virus 35S promoter. Tobacco leaves were bombarded with one of these constructs. The GFP alone (23) and a chimeric construct of GFP with the transit peptide of small subunit of Rubisco [RBCS-GFP, as a positive control (23)] were used. Fig. 5 shows results with tobacco guard cells, in which it is easier to visualize GFP accumulated in chloroplasts. Green fluorescence was observed only in chloroplasts of the guard cells (Fig. 5 *B*, *C*, and *D*), where chloroplasts were identified by red fluorescence of chlorophyll (Fig. 5 *F*, *G*, and *H*). Without these N-terminal sequences, GFP alone was not localized in chloroplasts (Fig. 5 *A* and *E*). We obtained similar results with leaves of *A. thaliana* (data not shown). These data suggest that the N-terminal regions of *SIG2* and *SIG3* ORFs can function as transit peptides for import into chloroplasts. We could not confirm the function of N-terminal region of SIG1 as a transit peptide by transient expression with GFP (data not shown).

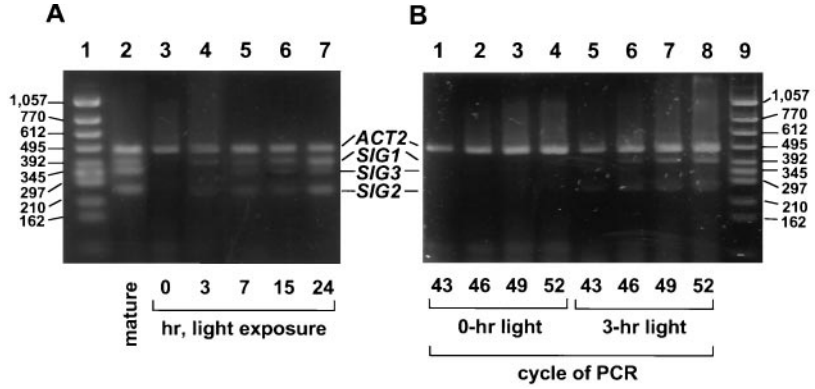

FIG. 3. Time-sequential changes of transcripts for *SIG* genes in dark-adapted plants after exposure to light. (*A*) *A. thaliana* grown for 3 weeks was incubated in the dark for 3 days or maintained under the same light conditions for plants indicated as "mature." Leaves of individual dark-adapted plants were harvested at 0, 3, 7, 15, and 24 hr after initiation of the exposure to light at 3,000 lux. RT-PCR products were subjected to electrophoresis after PCR cycles when intensities of signals for *ACT2* were unsaturated and comparable among RNA fractions harvested at different illumination times. RT-PCR products (5 μl from 50 μl of reaction mixture) were electrophoresed in 3% agarose gel (Agarose H, NipponGene), stained with SYBR green I (FMC BioProducts), and observed by FluorImager SI (Molecular Dynamics). (*B*) RT-PCR products of 0- and 3-hr illumination after indicated PCR cycles were electrophoresed and stained with SYBR green I.

```
SIG1  1 MSSCLLPQFKCPPDSFSIHFRTSFCAPKHNKGSVFFQPQCAVSTS
        PALLTSMLDVAKLRLPSFDTDSDSLISDRQWTYTRPDGPSTEAKY 90

SIG2  1 MATAAVIGLNTGKRLLSSSFYHSDVTEKFLSVNDHCSSQYHIAST
        KSGITAKKASNYSPSFPSSNRHTQSAKALKESVDVASTEKPWLPN 90

SIG3  1 MASFNSFPIPKQIVGSSSSSSSSTSRPRILVRSSLTSSMTSTNSM
        LVFVHPHPLIKHWLSLLRSDQTSFPIFVRIPMTSVVATRWSFLSS 90
```

FIG. 4. N-terminal sequences of SIG1, SIG2, and SIG3. The first 90 amino acids in the ORFs of *SIG1* (SIG1), *SIG2* (SIG2), and *SIG3* (SIG3) cDNAs are represented. Ser and Thr residues are indicated in boldface.

DISCUSSION

When the complete nucleotide sequence of tobacco plastid genome was determined in 1986 (8), genes for all subunits of *E. coli*-type RNAP except for SLFs were found in the plastid genome. In spite of thorough effort to identify genes for SLFs in plants, they were not cloned until 1996, when the genes were isolated from the unicellular red alga *Cyanidium caldarium* by two research groups (16, 17). In the investigation reported here, putative genes for σ factors from multicellular eukaryotes have now been characterized. It took over a decade to obtain clones for SLFs from higher plants for the following reasons: (*i*) low contents of mRNA, one in 40,000–70,000 molecules of total poly(A)$^+$ RNA in leaves of *A. thaliana* as estimated from the frequency of cloning in this investigation; (*ii*) homology conserved but at degree nonhybridizable to bacterial ones; and (*iii*) possible interference by the gene products with the growth of the *E. coli* used for cloning (data not shown).

The ORFs of *SIG1*, *SIG2*, and *SIG3* encode polypeptides consisting of 572, 502, and 571 amino acids, respectively, with calculated molecular masses of 64, 56, and 65 kDa. Although these are precursors with transit peptides, they are close to 67 kDa (SLF67) and 52 kDa (SLF52) of mustard SLFs associated with the activity of σ factors (16, 29) and to 64-kDa peptides from maize and rice that immunochemically cross-reacted with antibodies against a σ factor from *Anabaena* (12). SIG1 among the three SIGs most resembles bacterial σ factors and has more than 50% identity to four major conservative subregions—2.1, 2.3, 2.4, and 4.2—in each of the σ factors from *E. coli*, *Anabaena*, and *C. caldarium* except for the subregion 2.1 in *C. caldarium*. SIG2 has lower identity in its subregion 4.2 for -35 promoter recognition, and SIG3 has significantly low identity in both its subregions 2.1 and 2.4 for DNA melting and -10 promoter recognition, respectively. The differences in amino acid sequences among the subregions of the three SIGs may contribute to fine regulation of transcription of distinct target genes.

We have already demonstrated that expression of plastid photosynthesis genes, *rbcL* for large subunit of Rubisco, *psbA* for D1 protein in photosystem II reaction center, and *atpB/E* for β and ε subunits of coupling factor 1, is remarkably suppressed by transcriptional regulation in roots in *A. thaliana* (18). Northern analysis of *SIG1*, *SIG2*, and *SIG3* gene expression showed that their transcripts were few in roots but were present in leaves (Fig. 2*B*). This observation suggests that changes of SIG levels could regulate the expression of plastid photosynthesis genes in a tissue-specific manner. The light induction of transcripts for *SIG* genes (Fig. 3) probably precedes induction of transcripts of plastid photosynthesis genes such as *rbcL* (data not shown). Therefore, it is very probable that generation of all *SIG* gene products results in the initiation of transcription of photosynthesis genes after their

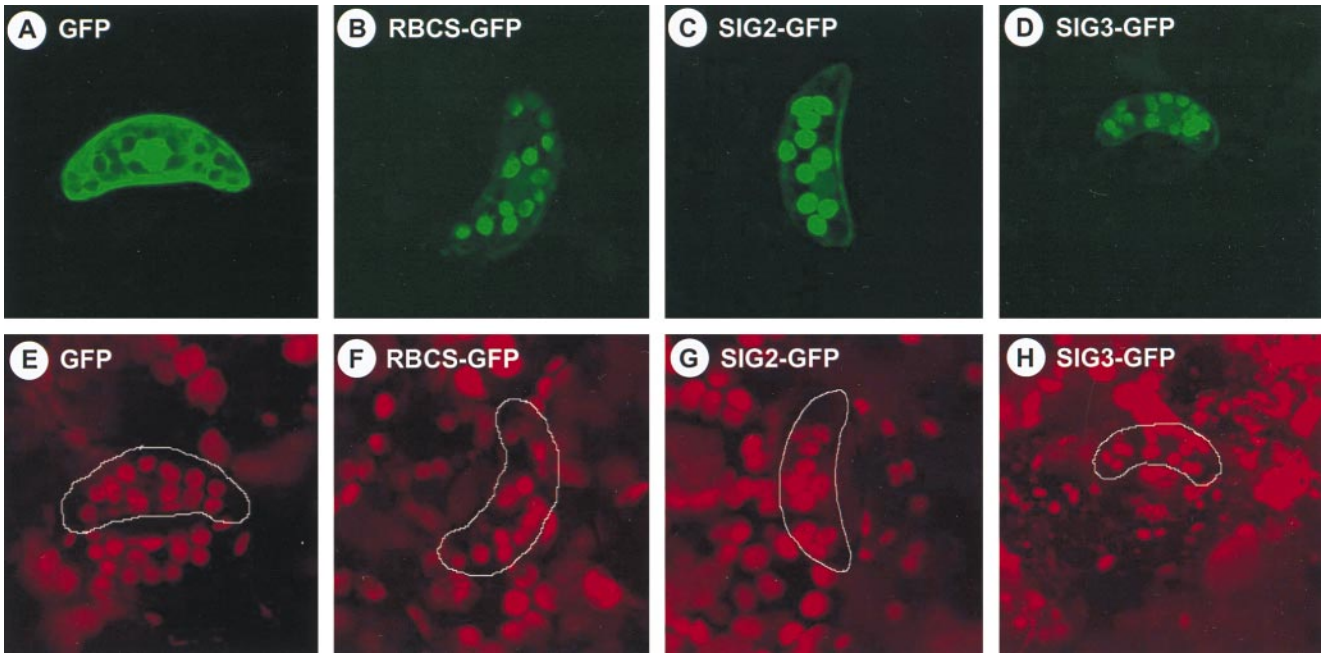

FIG. 5. Localization of GFPs fused to N-terminal regions of SIG2 and SIG3. GFP fusion constructs with the N-terminal regions of *SIG2* ORF (SIG2-GFP, *C* and *G*) and *SIG3* ORF (SIG3-GFP, *D* and *H*), and the transit peptide of the small subunit of Rubisco (23) (RBCS-GFP, *B* and *F*), as well as GFP alone (23) (GFP, *A* and *E*), were introduced into tobacco leaves by particle bombardment. Guard cells were observed by using the MRC-1024 Confocal Imaging System (480×) with excitation at 488 nm and emission at 520 nm (*A–D*), as well as excitation at 647 nm and emission at 666 nm (*E–H*). The same objects are shown in each pair of upper and lower panels.

transport into plastids and association with RNAP core enzyme in plastids. We think that the present investigation has opened a way to study not only the biological significance of each of the three distinct SIGs but also the regulation of their tissue-specific expression.

We are indebted to Thomas Newman and the *Arabidopsis* Biological Resource Center for the EST database and clones and to Jen Sheen for GFP. We thank Steven R. Rodermel for critical reading of the manuscript. The research was supported by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan (Monbusho) and by grants from the New Energy and Industrial Technology Development Organization (NEDO)/the Research Institute of Innovative Technology for the Earth (RITE), and the Toray Science Foundation.

1. Allison, L. A., Simon, L. D. & Maliga, P. (1996) *EMBO J.* **15,** 2802–2809.
2. Kung, S. D. & Lin, C. M. (1985) *Nucleic Acids Res.* **13,** 7543–7549.
3. Igloi, G. L. & Kel, H. (1992) *Crit. Rev. Plant Sci.* **10,** 525–558.
4. Burgess, R., Travers, A., Dunn, J. & Bautz, E. (1969) *Nature (London)* **221,** 43–46.
5. Helmann, J. D. & Chamberlin, M. J. (1988) *Annu. Rev. Biochem.* **57,** 839–872.
6. Busby, S. & Ebright, R. H. (1992) *Cell* **79,** 743–746.
7. Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H. & Ozeki, H. (1986) *Nature (London)* **322,** 572–574.
8. Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. & Sugiura, M. (1986) *EMBO J.* **5,** 2043–2049.
9. Hiratsuka, J., Shimada, H., Whittier, R., Ishibashi, T., Sakamoto, M., Mori, M., Kondo, C., Honji, Y., Sun, C.-R., Meng, B.-Y., Li, Y.-Q., Kanno, A., Nishizawa, Y., Hirai, A., Shinozaki, K. & Sugiura, M. (1989) *Mol. Gen. Genet.* **217,** 185–194.
10. Hu, J. & Bogorad, L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1531–1535.
11. Hu, J., Troxler, R. F. & Bogorad, L. (1991) *Nucleic Acids Res.* **19,** 3431–3434.
12. Troxler, R. F., Zhang, F., Hu, J. & Bogorad, L. (1994) *Plant Physiol.* **104,** 753–759.
13. Lerbs, S., Bräutigam, E. & Mache, R. (1988) *Mol. Gen. Genet.* **211,** 459–464.
14. Bülow, S. & Link, G. (1988) *Plant Mol. Biol.* **10,** 349–357.
15. Tiller, K., Eisermann, A. & Link, G. (1991) *Eur. J. Biochem.* **198,** 93–99.
16. Liu, B. & Troxler, R. F. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 3313–3318.
17. Tanaka, K., Oikawa, K., Kuroiwa, H., Kuroiwa, T. & Takahashi, H. (1996) *Science* **272,** 1932–1933.
18. Isono, K., Niwa, Y., Satoh, K. & Kobayashi, H. (1997) *Plant Physiol.* **114,** 623–630.
19. Murashige, T. & Skoog, F. (1962) *Physiol. Plant.* **15,** 473–497.
20. Rogers, S. O. & Bendich, A. J. (1985) *Plant Mol. Biol.* **5,** 69–76.
21. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
22. An, Y. Q., Mcdowell, J. M., Huang, S. R., Mckinney, E. C., Chambliss, S. & Meagher, R. B. (1996) *Plant J.* **10,** 107–121.
23. Chiu, W. L., Niwa, Y., Zeng, W., Hirano, T., Kobayashi, H. & Sheen, J. (1996) *Curr. Biol.* **6,** 325–330.
24. Burton, Z., Burgess, R. R., Lin, J., Moore, D., Holder, S. & Gross, C. A. (1981) *Nucleic Acids Res.* **9,** 2889–2903.
25. Brahamsha, B. & Haselkorn, R. (1991) *J. Bacteriol.* **173,** 2442–2450.
26. Lonetto, M., Gribskov, M. & Gross, C. (1992) *J. Bacteriol.* **174,** 3843–3849.
27. Nakai, K. & Kanehisa, M. (1992) *Genomics* **14,** 897–911.
28. Keegstra, K. & Olsen, L. J. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40,** 471–501.
29. Tiller, K. & Link, G. (1993) *Plant Mol. Biol.* **21,** 503–513.