Characterization of three cDNA species encoding plastid RNA polymerase sigma factors in *Arabidopsis thaliana*: evidence for the sigma factor heterogeneity in higher plant plastids

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Received 18 June 1997; revised version received 7 July 1997

Abstract By database search analysis, we identified three *Arabidopsis* EST (Expression Sequence Tag) entries having similarity to eubacterial RNA polymerase sigma factors. cDNA clones corresponding to these partial sequences were isolated, and the complete nucleotide sequences were determined. All three sequences encode proteins highly homologous to cyanobacterial and plastid sigma factors, and the gene products have N-terminal extensions which are assumed to function as plastid-targeting transit peptides. Thus we have concluded that the gene products are RNA polymerase sigma factors of plastids, and named *sigA*, *sigB* and *sigC*, respectively. Expression of these genes was analyzed by RNA gel-blot analysis and shown to be induced by illumination after a short-term dark adaptation. This strongly suggests that light regulation of the nuclear encoded sigma factor genes is involved in light-dependent activation of plastid promoters.

Key words: Expression sequence Tag; Sigma factor; *Arabidopsis thaliana*; Plant plastid; Gene expression

1. Introduction

Plant plastids are semi-automonous organella containing their own genetic system, such as DNA, RNA polymerase and ribosome. Although considerable amounts of genetic information required for various plastid functions are encoded by the plastid genome [1–3], many nuclear gene products are targeted into plastids to support and control the organella. Most macromolecular constituents, such as ribosome and photoreaction centers, are composits of both nuclear and plastid gene products.

Plastids of higher plants are indicated to possess two independent RNA polymerases. One of them appears to be a nuclear gene product of 110 kDa which is abundant in the early stage of plastid development [4]. Another is a multisubunit enzyme of the eubacterial type and four subunits corresponding to the catalytic core enzyme are encoded by the plastid genome [1–3,5,6]. Sigma subunits, which donate the core enzyme promoter recognition specificities, were identified biochemically and immunologically from plastids [7–9]. However, the corresponding structure genes were not found on the plastid genome and have been assumed to be encoded by the nuclear genome without direct evidence. Recently, we as well as another group have demonstrated that a plastid sigma subunit is actually encoded by the nuclear genome in primitive red algae [10,11]. Therefore, it is highly probable that higher plant nucleus also encodes for plastid sigma factor genes and targets sigma factors into plastids to control the plastid gene expression. In this report, we describe the characterization of three cDNA species encoding plastid sigma factors from a higher plant, *Arabidopsis thaliana* Columbia. This is the first identification of plastid sigma factor sequences in higher plants.

2. Materials and methods

2.1. DNA and RNA techniques

Recombinant DNA technology was basically performed as described [12]. *Escherichia coli* strains DH5α and LE392 were used for hosts for plasmids and phage vectors, respectively. DNA sequencing was performed with SequiTherm Cycle-sequencing kit LC (Epicenter Technology) and LI-COR Model 4000 DNA sequencer (LI-COR). *Arabidopsis* total DNA was prepared as described [13]. To prepare RNA, plant tissues were harvested and stored at −70°C. Total RNA was prepared by RNeasy Plant Mini Kit (QIAGEN), and poly(A$^+$) RNA was prepared by Micro-FastTrack Kit (Invitrogen).

2.2. cDNA isolation and analysis

Based on EST (Expression Sequence Tag) sequences, three sets of DNA primers were synthesized with Beckman Oligo1000M DNA synthesizer, and used for PCR amplification of *A. thaliana* Columbia total DNA. The oligonucleotides used were:

T88387 5′-CGGCGGGAATTCAGGGTGGTCTTATCGGAC-3′
5′-GGCGGCGGATCCTCAATGGAGGGTGTGATTCC-3′
N65838 5′-GGCCGGATCCGGCAACGTATACATCATGGC-3′
5′-CGCACAAGCTTGCTTGACCCACCAATGCGC-3′
N97044 5′-GGCCGGATCCACACAAGGGGTTACAAGTT-3′
5′-CGCCAAGCTTCTTGTCAATTGAACCGACC-3′

The underlines indicate attached restriction sites. The reaction was performed with TAKARA Taq DNA polymerase (Takara Shuzo, Japan) for 30 cycles at 95°C for 1 min, 55°C for 2 min, and 72°C for 3 min. The amplified fragments were digested with *Eco*RI and *Bam*HI (for T88387) or *Bam*HI and *Hin*dIII (for N65838 and N97044), and cloned in the multi-cloning site of the pBluescriptKS M13$^+$ plasmid (Toyobo, Japan).

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PII S0014-5793(97)00906-X

The sequences of the amplified fragments were

T88387 5′-GAATTCAGGGTGGTCTTATCGGACTTTTGCGGG-
GAATAGAGAAATTTGATTCTTCCAAAGGTTTCA-
GAATTTCAACTTATGTATATTGGTGGATTCGA-
CAGgtgagaataaccatttttgtgcttgccaatatggctagaatgtaaaacctga-
caagtgatactctatgtttcaaatatctataagGGTGTCTCAAGAG-
CACTAGTGGACAACTCAAGAACCTTGAGGTTACC-
TACTCACCTACATGAAAGACTCGGTTTAATCC-
GAAATGCAAAGCTTAGACTTCAAGAGAAAGGAAT-
CACACCCTCCATTGAGGATCC-3′

N65838 5′-GGATCCGGCAACGTATACATCATGGCACAC-
TATGCAAAGACAAAATGATCAAAAGCAACATTC-
GACTCGTTATTTCGATTGCAAAGAATTATCAAG-
GAGCTGGGATGAACCTCCAAGATCTTGTCCAGgttt-
gagcttttctctccttatttttttttgtaataacacatctgtaagcagtagtgatgaagt-
gatagatattgaacaccttgctcaaatattttcgaaatggtcacag-
GAAGGGTGCAGAGGGCTTGTGAGGGGAGCAGA-
GAAGTTTGATGCTACAAAGGGTTTTAAATTTTC-
GACTTACGCGCATTGGTGGATCAAGCAAG-
CAAGCTT-3′

N97044 5′-GGATCCACACAAGGGGTTACAAGTTTTCAACA-
TATGTGCAGTATTGGATAAGAAAATCAATGTC-
TACGATGGTGTCACGGCATGCAAGAGGCGTCCA-
TATTCCTgtttgtgcatttgatttttggccctttttgtctcattttcaacaaagcta-
gaacctcacttgtttttccttattgtttctgttttttttggcagTCATCAA-
TAATCCGAACTATCAATCATATACAAAAGGCTCG-
TAAGACCCTGAAAACGAGCCATGGTATAAAA-
TATGCGGCTGATGAGGAGATTGCAAAACTAA-
CAGGCCACTCGGTGAAGAAGATTCGAGCAGC-
TAACCAATGCCTAAAAGTGGTCGGTTCAATTGA-
CAAGAAGCTT-3′

where the lowercases denote putative introns.

After the sequence analyses of the cloned fragments, each fragment was purified, ^{32}P-labeled by the random priming method, and used for the screening of an *A. thaliana* Columbia cDNA library by the plaque hybridization method. The cDNA library was a kind gift from Dr. Kazuko Yamaguchi-Shinozaki (Japan International Research Center for Agricultural Sciences (JIRCAS)). In brief, this library was constructed from poly(A$^+$) RNA of mixed tissues. After the synthesis of the double-strand complementary DNA, fragments were ligated with an *Eco*RI–*Not*I–*Bam*HI adaptor (Takara Shuzo, Japan) and cloned in the λgt11 phage vector. The identified cDNA fragment was isolated by *Bam*HI digestion, and subcloned into pBluescriptKS M13$^+$. The nucleotide sequence was determined on both strands following appropriate subclonings.

2.3. 5′-RACE analysis

5′-RACE was performed with TAKARA 5′-Full RACE Core Set (Takara Shuzo, Japan) as described by the supplyer. Poly(A$^+$) RNA from *A. thaliana* Landsberg *erecta* was hybridized with a 5′-phosphorylated olgonucleotide primer (5′-CTCTCTCCCTGCTCACTCC-CATCATCTC-3′) complementary to the *sigB* mRNA, and reverse transcribed. The cDNA was circularized by RNA ligase, and the 5′-region of the *sigB* mRNA was amplified by two successive PCR reactions, first with 5′-CAGACGGAATACCTGATGC-3′ and 5′-ACAGAGCGTAGTGGACGTC-3′, and then with 5′-CCGGGC-CCGGGTTGCTCCTCCAGAAGC-3′ and 5′-GGCCGGATCCGG-CAACGTATACATCATGGC-3′. The reactions were performed with TAKARA Taq DNA polymerase (Takara Shuzo, Japan) for 25 cycles at 95°C for 1 min, 55°C for 2 min, and 72°C for 3 min. The amplified fragment was digested with *Bam*HI and *Sma*I, cloned in pBluescriptKS M13$^+$, and the nucleotide sequence was determined.

2.4. Phylogenetic analysis

Phylogenetic tree was constructed from the evolutionary distance data [14] using the neighbor joining method [15] with a computer software, Clustal W [16]. Bootstrap resampling was used to estimate the reliability of the inferred tree.

2.5. RNA gel-blot analysis

For detection of the *sigA* transcripts, an 842 bp *Hin*dIII fragment of the *sigA* cDNA corresponding to positions 424–1505 (D89993) was purified. Fragments for the *sigB* and *sigC* transcripts were the same as those used for isolation of the corresponding cDNA clones. They were ^{32}P-labeled by Random Primer DNA Labeling Kit Ver.2 (Takara Shuzo, Japan) as described by the supplier. Poly(A$^+$) RNA from *A. thaliana* Landsberg *erecta* corresponding to 30 μg of total RNA were loaded for each lane and analyzed on RNA gel blots as described previously [17]. Washing was performed in 1×SSC for 1 h, and then in 0.2×SSC for 1 h. The results were analyzed with a Bioimage analyzer BAS1000 (Fuji Film, Japan).

3. Results

3.1. Identification of EST clones and cDNA analysis

Recent *Arabidopsis* genome analysis projects accumulate a vast number of cDNA sequences in EST databases [18], and such sequences were successfully used for identification of important genes for cellular processes [19,20]. To identify plastid sigma factor genes, we performed an amino acid sequence similarity search on the TIGR *A. thaliana* Database (http://www.tigr.org/tdb/at/at.html) using a highly conserved sequence, 'G–F–K–F–S–T–Y–A–T–W–W–I–R–Q–A' [21], and found three cDNA sequence entries (GenBank accession numbers: T88387, N65838 and N97044) which may encode eubacteria-type RNA polymerase sigma factors. To make clear whether these sequences actually encode the eubacterial sigma factor homologs, genomic DNA fragments corresponding to these sequences were PCR amplified (see Section 2), and used as probes to screen an *Arabidopsis* cDNA library constructed from poly(A$^+$) RNA of mixed tissues.

Out of 200 000 plaques, 17 clones corresponding to T88387, 3 clones to N65838, and 2 clones to N97044 were identified. Since partial nucleotide sequence analyses indicated that these clones encode eubacterial sigma factor homologs, we named these genes *sigA* (T88387), *sigB* (N65838) and *sigC* (N97044), respectively, and determined the complete cDNA structures. They were termed just after the order of characterization, and the names do not indicate a special relationship with the corresponding genes of other species having the same name.

3.2. Structure of cDNAs and predicted gene products

Several cDNA clones corresponding to each *sig* gene were analyzed. The *sigA* cDNA was comprised of 1821 bp with a 3′-poly(A) tail, and encodes an open reading frame (ORF) of 502 amino acids (Fig. 1A). Of the three *sigB* cDNA clones obtained, the longest clone contained a 1.5-kb sequence with a 3′-poly(A) extension. Judged from the length of the *sigB* transcripts (see Fig. 3), this sequence appears to be a 5′-truncated version of the full-length cDNA. Therefore, the 5′-terminal structure of *sigB* cDNA was determined by 5′-RACE, and the cDNA was predicted to be a 1925 bp sequence with a 3′-poly(A) sequence. This cDNA encodes an ORF of 572 amino acids (Fig. 1B). The *sigC* cDNA was a 2010 bp sequence with a 3′-poly(A) tail, and encoding a 571 amino acid ORF (Fig. 1C). The molecular weights of each gene product were calculated as 56 398 (SigA), 64 053 (SigB) and 64 901 (SigC), respectively.

The predicted gene products of the *sigA*, *sigB* and *sigC* genes were compared with representative RNA polymerase sigma factors of red algal plastid, cyanobacteria and *E. coli*. The amino acid sequences of the *Arabidopsis* gene products revealed strong similarity to the principal sigma factors of eubacteria, and the sequences were aligned in Fig. 2. Recent

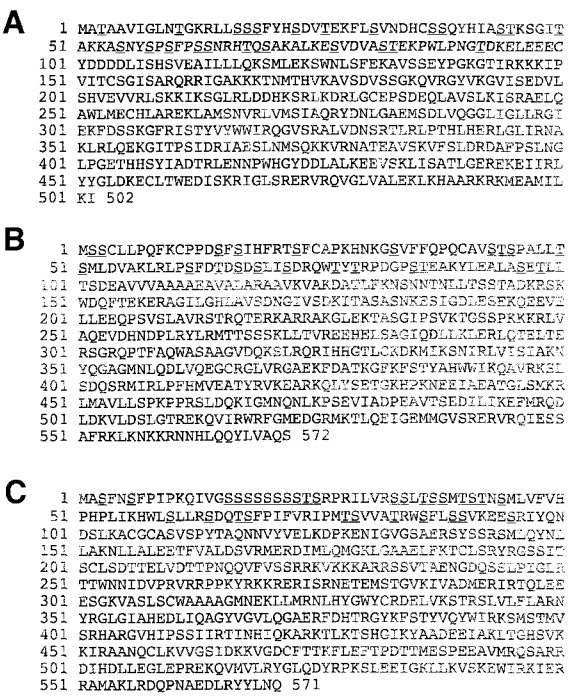

Fig. 1. Structure of the predicted *sig* gene products. The predicted amino acid sequences of *sigA* (A), *sigB* (B) and *sigC* (C) gene products are described. For the N-terminal first 100 amino acids, serine and threonine residues, which are abundant in plastid targeting signals, are underlined. Nucleotide sequence data of cDNA clones will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the following accession numbers, D89993 (*sigA*), AB004293 (*sigB*) and D89994 (*sigC*), respectively.

phylogenetic analyses have suggested that eubacterial sigma factors (σ^{70} family) are classified into three categories: group1; the principal sigma factors, group2; sigma factors closely related to the principal sigma factors, but non-essential for the cell viability, and group3; other alternative sigma factors [22]. The *Arabidopsis* sigma proteins were found to be structurally close relatives of group1 and group2 sigma factors. Conserved domain structures among sigma proteins [22,23] are well conserved except for the N-terminal region 1.1, and sequences corresponding to the regions 1.2, 2.1, 2.2, 2.3, 2.4, 3, 4.1 and 4.2 are indicated in Fig. 2. Among the sigma factors of cyanobacterial and plastid origin, an amino acid sequence motif (typically E–W–A–A–A) is frequently found between regions 1.2 and 2.1. This motif is also observed in the three *Arabidopsis* sequences as shown in Fig. 2, indicating the close relationship of these proteins.

A phylogenetic tree was constructed using the neighbor-joining method [15] (Fig. 3). The three *Arabidopsis sig* gene products were clustered with plastid sigma factors of red algae, strongly suggesting that these gene products are RNA polymerase sigma factors of *Arabidopsis* plastids. This branch is subsequently clustered with cyanobacterial group2 sigma factors, consistent with the close relationship between cyanobacteria and plastids.

For plastid proteins encoded by the nuclear genome, an N-terminal extension sequence called 'transit peptide' is known to direct the targeting of proteins into plastids. The N-terminal region of the *sigC* gene product is abundant in serine and threonine and poor in aspartic and glutamic acids, which are typical characteristics of transit peptides (Fig. 1C) [24]. As for the *sigA* and *sigB* gene products, the N-terminal regions are also rich in serine and threonine residues, although not so apparent as compared with the *sigC* product (Fig. 1A,B). Therefore, these regions may direct the *sig* gene products into the plastid compartment.

Based on such lines of evidence described above, we have concluded that the three *sig* gene products of *Arabidopsis* are RNA polymerase sigma factors of plastids.

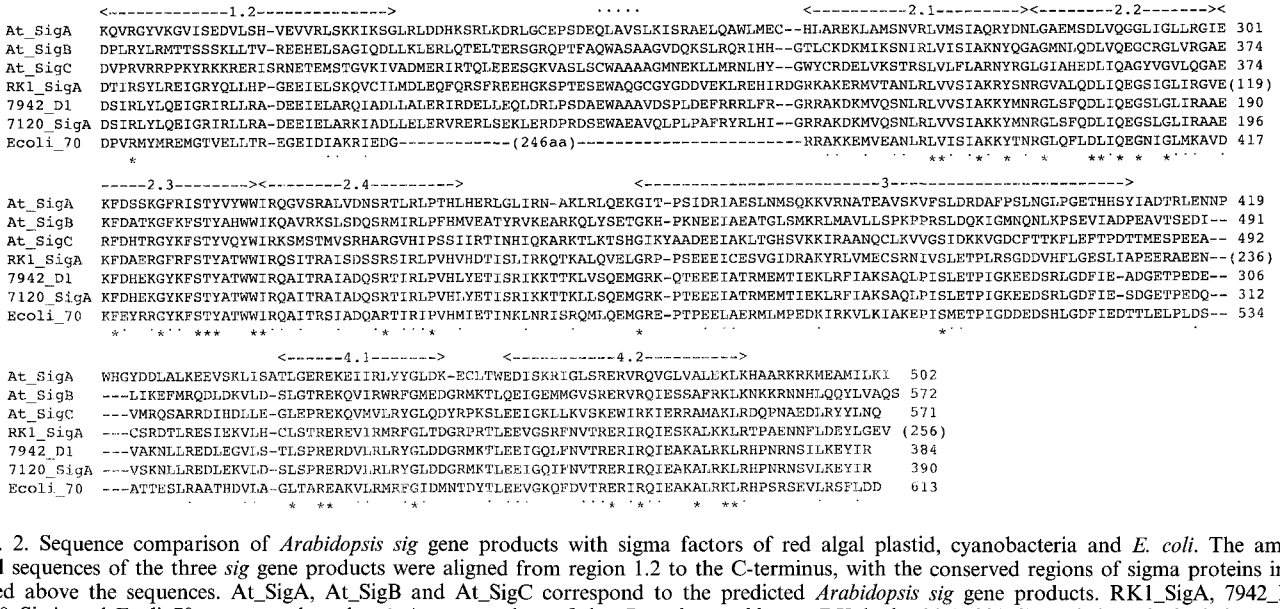

Fig. 2. Sequence comparison of *Arabidopsis sig* gene products with sigma factors of red algal plastid, cyanobacteria and *E. coli*. The amino acid sequences of the three *sig* gene products were aligned from region 1.2 to the C-terminus, with the conserved regions of sigma proteins indicated above the sequences. At_SigA, At_SigB and At_SigC correspond to the predicted *Arabidopsis sig* gene products. RK1_SigA, 7942_D1, 7120_SigA and Ecoli_70 correspond to the *sigA* gene product of the *Cyanidium caldarium* RK-1 plastid (D83179), and the principal sigma factors of *Synechococcus* sp. PCC 7942 (D10973), *Anabaena* sp. PCC 7120 (M60046) and *E. coli* (J01687). The amino acid positions of RK1_SigA were counted from the N-end of the region 1.2 and described in brackets, since the N-terminus of this protein was not confirmed. Identical and conserved amino acids are marked with asterisks and dots under the sequences, respectively. The position of the E–W–A–A–A motif specifically conserved among the cyanobacterial and plastid sigma proteins are indicated by dots above the sequences.

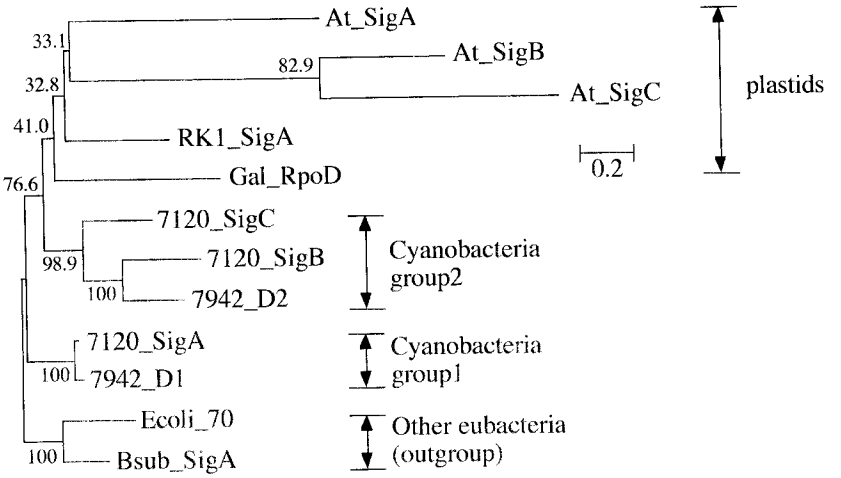

Fig. 3. Phylogenetic tree of the *Arabidopsis sig* gene products and principal sigma factors of cyanobacteria and plastids. The unrooted tree was constructed from evolutionary distance data using the neighbor-joining method [15]. A total of 315 positions were considered. Here the bootstrap procedure sampled 1000 times with replacement by Clustal W [16]. The number at each node represents the percentage of trees supporting the specific branching pattern in the bootstrap analysis. The bar indicates the distances corresponding to 20 changes per 100 amino acid positions. At_SigA, At_SigB and At_SigC represent the predicted *sig* gene products of *Arabidopsis* (this study). The other sequences are RK1_SigA (the *sigA* gene product of *Cyanidium caldarium* RK-1, D83179), Gal_RpoD (the *rpoD* gene product of *Galdieria sulphraria*, L42639), 7120_SigC (the *sigC* gene product of *Anabaena* sp. PCC 7120, M95759), 7120_SigB (the *sigB* gene product of *Anabaena* sp. PCC 7120, M95760), 7942_D2 (the *rpoD2* gene product of *Synechococcus* sp. PCC 7942, D78583), 7120_SigA (the principal sigma factor of *Anabaena* sp. PCC 7120, M60046), 7942_D1 (the principal sigma factor of *Synechococcus* sp. PCC 7942, D10973), Ecoli_70 (the principal sigma factor of *Eschericha coli*, J01687) and Bsub_SigA (the principal sigma factor of *Bacillus subtilis*, X03897), respectively.

3.3. Expression of sig genes

The expression of the *Arabidopsis sig* genes were analyzed by RNA gel-blot analysis. Initially, total RNA was isolated from aerial parts of adult plants grown under continuous light and used for gel-blot analysis. However, expression of the genes was so weak that we could not detect it (data not shown). Therefore, we modified the light conditions and repeated the experiments. The timings of the harvests were as follows. The plants were grown under continuous light for 4 weeks (lanes 1, 4 and 7), dark adapted for 24 h (lanes 2, 5 and 8), and re-illuminated for 24 h (lanes 3, 6 and 9). The results are shown in Fig. 4. In each lane, transcripts of a similar size were detected, and the size was estimated to be about 2 kb as calculated from the rRna sizes. Although amounts of transcripts differ significantly depending on the genes in the order of *sigA* ≫ *sigC* > *sigB*, all three transcripts were similary induced by illumination after dark adaptation (lanes 3, 6 and 9).

4. Discussion

In this report, we described the first isolation of cDNA species for RNA polymerase sigma factors of higher plant plastids. At present, we have no direct evidence that the gene products function as RNA polymerase sigma factors of plastids. However, the sequence similarity to cyanobacterial and plastid sigma factors, as well as the presence of N-terminal plastid targeting sequences, strongly suggests that all of them are nuclear encoded plastid sigma factors. In a previous work, we have demonstrated that a plastid sigma factor is encoded by the nuclear genome, and the gene product is targeted into the plastid in a primitive red alga, *C. caldarium* RK-1 [10]. Therefore, the basic architecture of plastid RNA polymerase is probably conserved among divergent plastids, and sigma proteins appear to link the nuclear gene expression to the transcriptional regulation in plastids.

In eubacteria, sigma factors are responsible not only for basic promoter recognition but also for control of cellular developmental processes, such as phage development and sporulation, through modulation of transcription specificities. Plastids of higher plants transform to proplastids, chloroplasts, etioplasts, amyloplasts and so on, depending on the environments and tissues to which the cells belong. Therefore, it is tempting to speculate that change of the transcription specificity through plastid sigma substitution is, at least in part, responsible for development of each plastid under control of the nucleus. In fact, transformation of plastids is demonstrated to accompany the change of transcripts abundance in plastids [25]. Identification of multiple plastid sigmas clearly opened a way to answer these questions.

Three gene products deduced from the cDNA sequences belong to group1 or group2 sigma factors of eubacteria. The structures of the promoter recognition domains, regions 2.4 and 4.2, are highly similar to each other, indicating that these sigma factors recognize similar promoter sequences. In *E. coli*, group1 σ^{70} and group2 σ^{38} proteins have been shown to recognize similar promoter structures sharing the consensus 'TATAAT' type-10 box [26,27]. Since most plastid promoters also contain a similar structure, involvement of group1 or group2 sigma proteins has been suggested. Thus, it coincides well with the presence of the highly homologous sigma genes in higher plants.

Transcripts of the three *sig* genes were similarly induced by illumination after 24 h dark adaptation. Some promoters have been shown to be induced by light in plastids, and among them, the p3 promoter of the *psbD/C* operon is best characterized [28,29]. Since this promoter also contains the 'TATAAT' type-10 box, the sigma factor(s) described in this paper may involve in the activation. Although expression of these genes appears not to be simply directed by light, some relationship between gene expression and the light condition

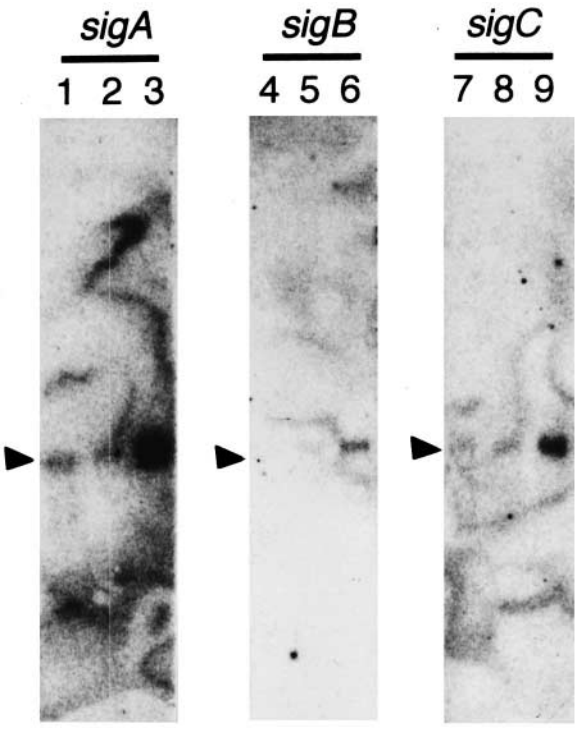

Fig. 4. RNA gel-blot analysis of *sigA*, *sigB* and *sigC* transcripts. Adult plants grown under continuous light (a) were dark-adapted for 24 h (b), and then illuminated for 24 h (c). At each time point (a, b and c), poly(A^+) RNAs were purified from the aerial parts of the plants, and used for gel-blot analysis. Arrowheads indicate the position of the transcripts. Lanes 1, 4 and 7: RNA at (a), lanes 2, 5 and 8: RNA at (b), and lanes 3, 6 and 9: RNA at (c). Lanes 1–3, 4–6 and 7–9 were hybridized with the *sigA*, *sigB* and *sigC* probes, respectively.

should be present. The photosignal transduction system regulating sigma genes is another problem to be solved.

Acknowledgements: The authors thank Dr. Kazuko Yamaguchi-Shinozaki (JIRCAS) for providing the *Arabidopsis* cDNA library. This work was supported by Grants-in-aid for Scientific Research in Priority Areas (No. 09274207) to K.T. from the Ministry of Education, Science, and Culture of Japan, by Grants for Research Program for the Advanced Recombinant DNA Techniques to K.W. and Y.T. from Ministry of Agriculture, Forestry and Fisheries, and by the Biodesign Research Program of RIKEN.

References

[1] Shinozaki, K. et al. (1986) EMBO J. 5, 2043–2049.
[2] Ohyama, K. et al. (1986) Nature 322, 572–574.
[3] Hiratsuka, J. et al. (1989) Mol. Gen. Genet. 217, 185–194.
[4] Lerbs-Mache, S. (1993) Proc. Natl. Acad. Sci. USA 90, 5509–5513.
[5] Hu, J. and Bogorad, L. (1990) Proc. Natl. Acad. Sci. USA 87, 1531–1535.
[6] Hu, J., Troxler, R.F. and Bogorad, L. (1991) Nucl. Acids Res. 19, 3431–3434.
[7] Tiller, K., Eisermann, A. and Link, G. (1991) Eur. J. Biochem. 198, 93–99.
[8] Tiller, K. and Link, G. (1993) Plant Mol. Biol. 21, 503–513.
[9] Troxler, R.F., Zhang, F., Hu, J. and Bogorad, L. (1994) Plant Physiol. 104, 753–759.
[10] Tanaka, K., Oikawa, K., Ohta, N., Kuroiwa, H., Kuroiwa, T. and Takahashi, H. (1996) Science 272, 1932–1935.
[11] Liu, B. and Troxler, R.F. (1996) Proc. Natl. Acad. Sci. USA 93, 3313–3318.
[12] J. Sambrook, E.F. Fritsch, and T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
[13] Edwards, K., Johnstone, C. and Thompson, C. (1991) Nucl. Acids Res. 19, 1349.
[14] Kimura, M. (1980) J. Mol. Evol. 16, 111–120.
[15] Saitou, N. and Nei, M. (1987) Mol. Biol. Evol. 4, 406–425.
[16] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Nucl. Acids Res. 22, 4673–4680.
[17] Mochizuki, N., Susek, N. and Chory, J. (1996) Plant Physiol. 112, 1465–1469.
[18] Cooke, R. et al. (1996) Plant J. 9, 101–124.
[19] Osteryoung, K.W. and Vierling, E. (1995) Nature 376, 473–474.
[20] Smith, F.W., Ealing, P.M., Dong, B. and Delhaize, E. (1997) Plant J. 11, 83–92.
[21] Tanaka, K., Shiina, T. and Takahashi, H. (1988) Science 242, 1040–1042.
[22] Lonetto, M., Gribskov, M. and Gross, C.A. (1992) J. Bacteriol. 174, 3843–3849.
[23] Helmann, J.D. and Chamberlin, M.J. (1988) Annu. Rev. Biochem. 57, 839–872.
[24] Keegstra, K. and Olsen, L.J. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 471.
[25] Mullet, J.E. (1993) Plant Physiol. 103, 309–313.
[26] Tanaka, K., Takayanagi, Y., Fujita, N., Ishihama, A. and Takahashi, H. (1993) Proc. Natl. Acad. Sci. USA 90, 3511–3515.
[27] Tanaka, K., Kusano, S., Fujita, N., Ishihama, A. and Takahashi, H. (1995) Nucl. Acids Res. 23, 827–834.
[28] Wada, T., Tunoyama, Y., Shiina, T. and Toyoshima, Y. (1994) Plant Physiol. 104, 1259–1267.
[29] Allison, L.A. and Maliga, P. (1995) EMBO J. 14, 3721–3730.