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Structure and expression of a nuclear gene for the PSI-D subunit of photosystem I in *Nicotiana sylvestris*

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Abstract

The PSI-D subunit is the ferredoxin-binding site of photosystem I, and is encoded by the nuclear gene *psaD*. We isolated a *psaD* genomic clone from *Nicotiana sylvestris*, by screening a genomic library with a *psaD* cDNA which we previously cloned from *N. sylvestris* (Yamamoto *et al.*, Plant Mol Biol 17: 1251, 1991). Nucleotide sequence analysis revealed that this genomic clone contains a *psaD* gene, which does not correspond to the *psaD* cDNA, so we designated these genes *psaDb* and *psaDa*, respectively. The *psaDb* clone encodes a protein of 214 amino acids uninterrupted by introns. The N-terminal sequence determined for the *N. sylvestris* PSI-D protein encoded by *psaDb* begins at the 49th residue. The products of *psaDa* and *psaDb* share 82.7% and 79.5% identity at the amino acid and nucleotide levels, respectively. Genomic Southern analysis showed that two copies of *psaD* are present in the *N. sylvestris* genome. Ribonuclease protection assays and immunoblot analysis in *N. sylvestris* indicate that both genes are expressed in leaves, stems and flower buds, but neither is expressed in roots. During leaf development, the ratio of *psaDb* to *psaDa* mRNA increases from 0.12 in leaf buds to 0.36 in mature leaves. The relative abundance of the corresponding proteins decreased over the same developmental period. These results indicate that differential regulation mechanisms control *psaDa* and *psaDb* expression at both the mRNA and protein levels during leaf development.

Introduction

Photosystem I (PSI) mediates photosynthetic electron transfer from plastocyanin to ferredoxin. PSI is a multiprotein complex in the thylakoid membranes of chloroplasts, composed of at least 12 subunits, designated PSI-A through PSI-L. The genes encoding these proteins are designated *psaA* through *psaL*, respectively [26, 31]. PSI-A, PSI-B, PSI-C, PSI-I and PSI-J are encoded in the chloroplast genome, while the remaining seven subunits are nuclear-encoded [11, 26, 31].

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number D13718.

PSI-D is located on the stromal surface of PSI [18, 25], and is the binding site of ferredoxin [39, 40]. The primary structure of the PSI-D subunit has been determined in several plant species by cDNA analysis [12, 13, 17, 22, 38]. *Nicotiana* species have two PSI-D isomers per genome, which differ from each other in N-terminal (partial) amino acid sequence as well as in apparent molecular mass, according to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis [23]. *Nicotiana sylvestris*, a diploid species, has two PSI-D isoforms with apparent molecular masses of 19 kDa and 17.5 kDa. We have designated these PSI-D1 and PSI-D2, respectively [23]. The relative abundance of PSI-D1 to PSI-D2 is very low, hence most PSI complexes in the plant are thought to contain PSI-D2 instead of PSI-D1. We previously isolated *psaD* cDNA clones which encode PSI-D2 of *N. sylvestris*, and designated the corresponding gene *psaDa* [38].

It is well known that the small subunit of ribulose 1,5-bisphosphate carboxylase (*rbcS*) and the chlorophyll *a*/*b*-binding protein (*Cab*) are encoded by multi-gene families in the nuclear genome [4, 5, 21, 29]. The expression of each gene in a given family is differentially regulated with respect to the others in response to plant development or environmental stimuli [34]. Ferredoxin and the 33 kDa protein of the oxygen-evolving complex are also encoded by multi-gene families in the nuclear genome [10, 37]. However, little is known about the organization of the photosystem gene family within the nuclear genome. Clarification of the structure and organization of the nuclear genes encoding the PSI subunits is a prerequisite for analysis of the molecular mechanisms which control PSI biogenesis.

Many studies of thylakoid protein structure and photosynthetic gene expression have been carried out in *Nicotiana tabacum*. However, *N. tabacum* is an allotetraploid, with a very large genome and multiple copies of most genes, which complicates molecular analysis and studies of gene structure. *N. sylvestris* is an autogamous diploid species, which is the maternal ancestor of *N. tabacum* [24] and is readily transformable. Therefore, we have concentrated our studies in *N. sylvestris*. We have isolated a genomic clone containing the second *psaD* gene from *N. sylvestris*, and characterized this gene in comparison with the *psaDa* gene, mentioned above.

Materials and methods

Plant material

Nicotiana sylvestris was grown at 25 °C in a greenhouse. Root tissues were harvested from hydroponically grown plants.

DNA and RNA preparation

Nuclear DNA was isolated and purified by CsCl-EtBr ultracentrifugation according to Jofuku and Goldberg [14].

Total RNA was prepared according to the method of Piechulla *et al.* [28], from leaf buds of less than 3 cm in longitudinal axis, developing leaves of 5–10 cm, mature leaves of 20–30 cm, stems, flower buds and roots. These organs were harvested in the afternoon on fine days.

Genomic cloning and sequence analysis

An *N. sylvestris* lambda dash genomic library [20] was screened with the insert of a *psaDa* cDNA clone, yaDC17 [38], which was labeled with [α-^{32}P]dCTP by the random hexanucleotide-priming method [30]. A 5.7 kb *Xba* I fragment of the positive clone was subcloned into pBluescript SKII$^+$, and subjected to DNA sequence analysis.

Mapping of the transcription start site

Primer extension mapping was carried out essentially according to Sambrook *et al.* [30]. An end-labelled primer was hybridized with 20 μg of total RNA from *N. sylvestris*, and elongated with

MMLV reverse transcriptase (BRL, Superscript) for 90 min at 45 °C. The gene-specific primer, 5′-GGGCGACGACACGGGGATCGGCTG-TTTTCGAGGTG-3′, is complementary to the *psaDb* coding strand (Fig. 2, +68 to +102) but does not hybridize with *psaDa* mRNA.

Genomic Southern hybridization

A 10 μg portion of nuclear DNA was digested with restriction endonucleases, electrophoresed on 0.7% agarose gels, transferred to nylon membranes (Amersham, Hybond-N^+), and hybridized to the *psaDa* cDNA probe mentioned above. Hybridization was carried out overnight at 65 °C in a solution containing 6 × SSC, 5% Irish cream liqueur (Original Irish Cream, R & A Bailey's) [6], 0.5% SDS, 20 mM Na_2HPO_4, 20 μg/ml heat-denatured salmon sperm DNA, and 2 μCi/ml of the ^{32}P-labelled probe. The membrane was subsequently washed several times in 2 × SSC at room temperature, then once at 65 °C in 1 × SSC, 0.1% SDS for 15 min under continuous agitation. The membrane was then subjected to autoradiography.

RNase protection assay

The riboprobes for the *psaDa* and *psaDb* mRNAs were prepared according to a standard protocol [1]. Fragments of yaDC17 (18–279 bp downstream from the ATG initiation codon) and yaDG20 (18–263 bp downstream from the ATG) were subcloned into pBluescript II, and the antisense transcripts were produced by T7 RNA polymerase in the presence of [α-^{32}P]-UTP.

RNase protection assays were carried out using a RPAII kit (Ambion, Texas) according to the manufacturer's protocol. The samples digested with RNase A/T1 were subjected to electrophoresis in 6% polyacrylamide gels containing 8 M urea, followed by autoradiography. The radioactivity of each band was analyzed by AMBIS radioanalytic imaging system (K & M, Torrance, CA) or Fujix BAS2000 (Fuji Photo Film, Japan). The riboprobes for the *psaDa* and *psaDb* mRNAs incorporated 61 and 55 [α-^{32}P]-UTPs per molecule, respectively. Hence, the ratio of *psaDb* to *psaDa* mRNA was estimated using the following formula: (61/55) × (cpm of Db background)/(cpm of Da background).

Immunoblot analysis

Plastid membranes were prepared from leaves, stems, flower buds and roots. These tissues were homogenized with a polytron homogenizer in isolation medium (0.35 M sorbitol, 2 mM EDTA, 2 mM sodium isoascorbate and 25 mM HEPES-NaOH, pH 7.6), filtered through eight layers of gauze, and centrifuged at 7000 × ***g*** for 10 min at 0 °C. The plastid precipitates were resuspended in isolation medium and subsequently layered onto 50% sucrose cushions containing 2 mM sodium isoascorbate and 25 mM Tris-HCl pH 7.5, followed by centrifugation at 7000 × ***g*** for 30 min at 0 °C. The colored bands on the sucrose cushions were recovered, washed with 5 mM Tris-HCl pH 7.5, and subjected to immunoblot analysis. Protein quantity was estimated according to Smith *et al.* [33]. The proteins in plastid membrane preps containing equal amounts of protein were resolved by SDS-PAGE, and blotted onto PVDF membranes [24]. Western blots were probed with rabbit antiserum raised against spinach PSI proteins [25], or with an anti-PSI-D antibody purified according to the protocol of Kelly *et al.* [16]. The membranes were then incubated with a biotinylated donkey antibody raised against rabbit immunogloblin (Amersham, UK). The positive proteins were visualized using a streptavidin-alkaline phosphatase conjugate (Amersham, UK).

Results

Isolation of a psaD genomic clone

Using a *psaDa* cDNA clone of *N. sylvestris* [38] as a probe, we screened about 3 × 10^6 clones of

an *N. sylvestris* genomic library, and obtained one positive clone, which we named yaDG20. A physical map of yaDG20 is shown in Fig. 1. We determined the nucleotide sequence of part of a 1.9 kb *Hin*d III-*Xba* I fragment which hybridized to the *psaDa* cDNA, and part of the adjoining 2.2 kb *Hin*d III-*Hin*d III fragment (Fig. 2). Nucleotide sequence analysis revealed that yaDG20 has an open reading frame of 214 amino acids which is uninterrupted by introns. Within this reading frame the N-terminal sequence of the mature protein (previously determined by partial amino acid sequencing of PSI-D1 from *N. sylvestris*) starts from the 54th residue (Fig. 2, wavy line). Figure 3 shows a comparison of the primary structure of the protein encoded by yaDG20 with that of the PSI-D2 which is encoded by the *psaDa* cDNA. These two proteins are strikingly homologous, with amino acid identity of 82.7% and 79.5% identity between the nucleotide sequences (data not shown). From these results, we conclude that this open reading frame encodes the PSI-D1 protein of *N. sylvestris*, and have designated the corresponding gene *psaDb*. The predicted molecular masses of the precursor and the mature form of PSI-D1 are 23.5 kDa and 17.9 kDa, respectively. The latter value is somewhat smaller than the apparent molecular mass of 19 kDa determined by SDS-PAGE [23].

Structural characteristics of psaDb

The transcription start sites of *psaDb* were determined by primer extension analysis (Fig. 4). The extension products produced two signals, one intense and one faint, at +1 and +9, respectively (Fig. 4). Similar results were obtained with RNase protection mapping (data not shown). These results indicate that the adenine nucleotide located 23 bases upstream of the ATG initiation codon is the major transcription start site of *psaDb*. No TATA-like sequence was found immediately upstream of this site.

Upstream of the transcription start site there are sequences homologous to the GT-1 box (GGTTAA) [8], −335 to −330 (Fig. 2a, the TGA1a binding sequence (TGACGT) [15], −257 to −252 (Fig. 2b), and the light-responsive element (LRE) of Grob and Stüber (CCTTATCCT) [9], −78 to −70 (Fig. 2d). The GATA motif [3] also is repeated three times in the reverse orientation between −83 and −65 (Fig. 2, TATC indicated by c). Finally, three repeated sequences are present upstream of *psaDb* and are shown in Fig. 2. We have designated them R1 (CATCATCAGCT, −1495 to −1484 and −1397 to −1386), R2 (TTGATTCATTAACTCACTTT, −433 to −413 and −369 to −349), and R3 (TAGTATATTA; −304 to −295 and −246 to −237).

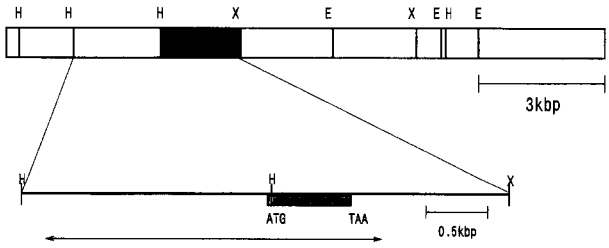

Fig. 1. A physical map of genomic clone, yaDG20. The letters H, X and E represent the restriction sites of *Hin*d III, *Xba* I and *Eco* RI, respectively. The area indicated by an arrow was subjected to sequence analysis.

psaD gene copy number in N. sylvestris

We determined the number of copies of *psaD* present in the *N. sylvestris* (a diploid) genome by digesting nuclear DNA with *Eco* RI, *Eco* RV, and *Hin*d III, followed by Southern blot hybridization with the *psaDa* cDNA probe under high stringency conditions (Fig. 5). Both a strong and a weak signal were detected in each digest. A 15 kb *Eco* RI fragment and a 7.8 kb *Hin*d III fragment both hybridized weakly to the *psaDa* cDNA. The sizes of these fragments are in agreement with the size of the restriction fragments of yaDG20 (Fig. 1), suggesting that these signals are derived from the *psaDb* gene. A 1.9 kb *Eco* RI fragment produced a strong hybridization signal, implying that it encodes *psaDa*. Since this fragment appears to be too small to encode more than one *psaD* gene, it appears likely that *N. sylvestris* has no more than two copies of *psaD*.

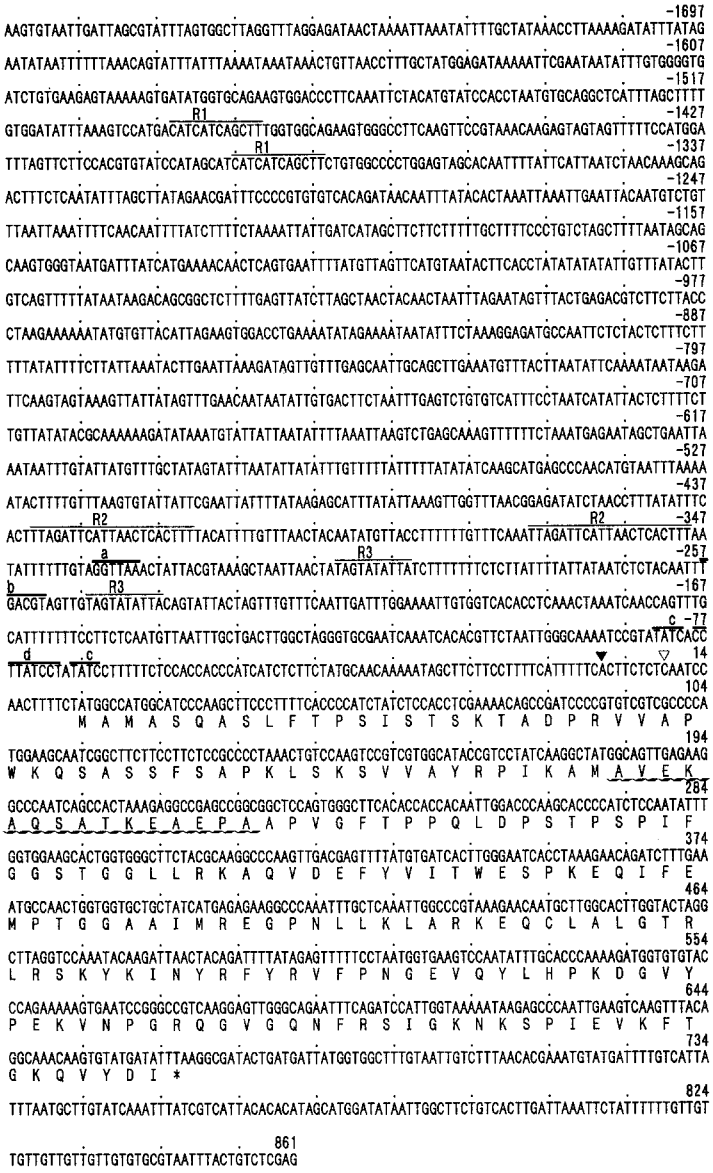

Fig. 2. Nucleotide sequence of *psaDb* of *N. sylvestris*. The sequence of part of yaDG20 is shown, with the deduced amino acid sequence underneath. The N-terminal sequence of PSI-D1 was directly determined [23] and is indicated by a wavy line. The transcription start site (solid arrowhead) is denoted + 1. An open arrowhead indicates the transcription start site of a weak band obtained by primer extension analysis (Fig. 4). Sequences homologous to the GT-1 box [8], TGA1a binding sequence [15], GATA box [3] and the LRE of Grob and Stüber [9], are indicated by a, b, c and d, respectively. R1, R2, and R3 represent repeated sequences.

Expression of psaD *gene family*

The steady-state mRNA levels of *psaDa* and *psaDb* were examined by RNase protection analysis in several tissues, including leaf buds, developing leaves, mature leaves, stems, flower buds, and roots (Fig. 6). In all these tissues except for roots, both of the mRNAs were detected, with *psaDa* mRNA three to eight times more abundant than that of *psaDb*. We found no signal for either *psaD* mRNA in roots. The concentrations of both mRNAs are highest in developing leaves, how-

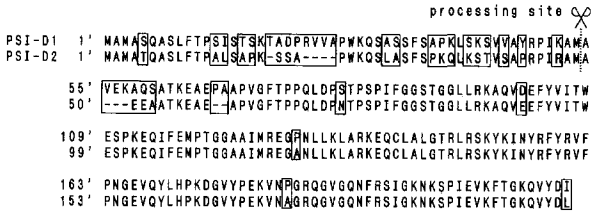

Fig. 3. Amino acid sequences of the PSI-D proteins in *N. sylvestris*. The sequence of PSI-D1 is deduced from the *psaDb* genomic sequence (Fig. 2). The sequence of PSI-D2 is deduced from the *psaDa* cDNA [38]. Amino acids which differ between the D1 and D2 proteins are boxed. Scissors indicate processing sites.

ever, their abundance differ substantially during leaf development. The *psaDa* mRNA is abundant in leaf buds but scarce in mature leaves; the reverse is true for *psaDb* mRNA. Consequently, the

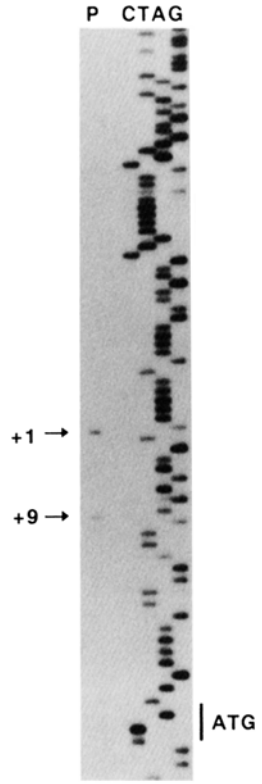

Fig. 4. Mapping of the 5′ end of the *psaDb* mRNA by primer extension. The primer extension product was electrophoresed on a 5.3% sequencing gel (lane P) in parallel with a sequencing ladder of the corresponding genomic DNA. The nucleotide position of the major signal is denoted by + 1.

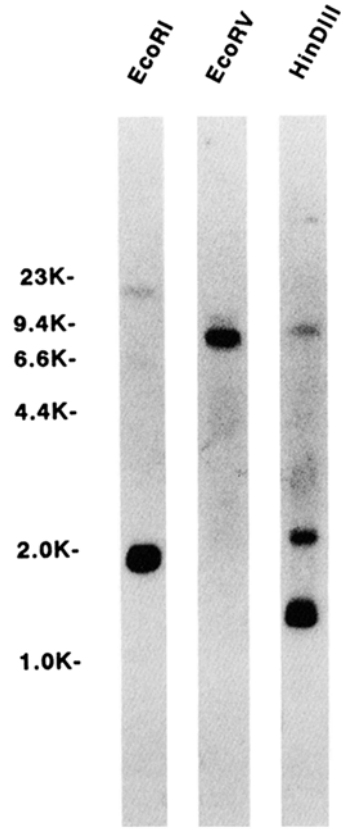

Fig. 5. Genomic Southern analysis of *psaD*. Restriction enzymes used for the digestion of *N. sylvestris* genomic DNA are shown above the lanes. The blot was hybridized with the *psaDa* cDNA probe.

ratio of *psaDb* to *psaDa* mRNA increased from 0.12 to 0.36 during leaf development (Fig. 6, lower panel). The ratios of *psaDb* to *psaDa* mRNA in stems and flower buds were similar, 0.18 and 0.17, respectively (Fig. 6, lower panel), implying that neither gene is differentially expressed in these tissues.

In order to examine whether the changes in mRNA levels corresponded to protein abundance, immunoblot analysis of PSI-D proteins was carried out using antiserum raised against spinach PSI (Fig. 7). The translation product of *psaDa*, PSI-D2, is more abundant than the *psaDb* product, PSI-D1, in leaves, stems and flower buds. Neither gene product was detected in roots. The change in steady-state levels of PSI-D1 and PSI-D2 during leaf development is the reverse of

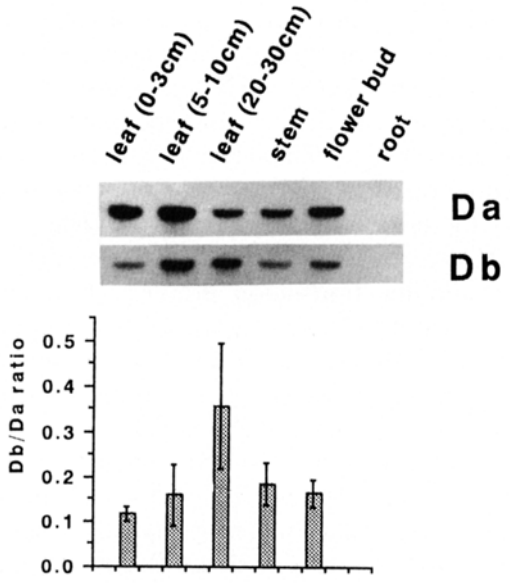

Fig. 6. Messenger RNA levels of the *psaDa* and *psaDb* genes. Twenty two micrograms of total RNA extracted from the indicated tissues of *N. sylvestris* were subjected to RNase protection assays using antisense RNA probes for the *psaDa* and *psaDb* transcripts. The ratio of *psaDa* to *psaDb* mRNA represents a mean ± SD of three independent experiments.

the steady-state mRNA profiles. The abundance of PSI-D1 is highest in developing leaves, and markedly decreases during leaf maturation. In contrast, PSI-D2, is abundant in developing leaves and is present at even higher levels in mature leaves. Thus, the ratio of PSI-D1 to PSI-D2

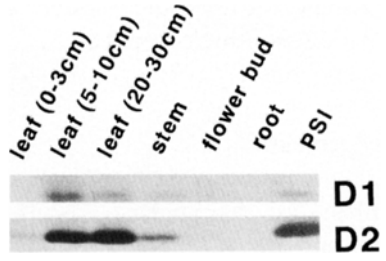

Fig. 7. Accumulation profiles of PSI-D1 (19 kDa) and PSI-D2 (17.5 kDa). PSI-D1 is encoded by *psaDb*, and PSI-D2 by *psaDa* [38]. Plastid membrane proteins (225 ng for the detection of PSI-D1, 45 ng for PSI-D2) from the indicated tissues of *N. sylvestris* were subjected to immunoblot analysis using antiserum raised against spinach PSI proteins, and visualized with a color-generating reaction of alkaline phosphatase. PSI, PSI purified from *N. sylvestris* leaves.

(the ratio of the *psaDb* to *psaDa* gene products) decreased during leaf development. These results were confirmed with immunoblots using an anti-PSI-D antibody.

Discussion

This is the first report of the structure of a nuclear gene encoding the ferredoxin-binding subunit of PSI. This *N. sylvestris* gene, *psaDb*, has 5′ flanking sequences homologous to the GT-1 box [8] (Fig. 2a), which is conserved among photoregulated genes such as *rbcS*, *Cab* and ferredoxin genes [7], and mediates light responsiveness [19]. It also contains a sequence similar to the LRE of Grob and Stüber [9] (Fig. 2d), which is shared by many phytochrome-dependent, light-inducible genes [9]. The *Cab* genes are known to have tandem repeats of a third motif, GATA, 110–140 nucleotides upstream of the ATG codon [3]. A similar motif is present in reverse orientation in *psaDb* (Fig. 2c). Thus *psaDb* has at least three *cis*-regulatory elements in common with other light-regulated genes, suggesting that the expression of *psaDb* may also be light-regulated. Actually, Brunner *et al.* reported that, in etiolated spinach seedlings, mRNAs of the nuclear-encoded PSI genes such as *psaD*, *psaE*, *psaF*, *psaG* and *psaH* accumulated in response to light stimuli, and phytochrome is involved in this response [2]. We are currently analyzing the regulatory elements of the PSI genes in transgenic tobacco in order to determine the extent to which PSI genes share signal transduction pathways with the genes encoding the light-harvesting complex or the enzymes involved in the dark reactions of photosynthesis.

A genomic clone of *psaH* was isolated in rice [27], and this is the only other nuclear-encoded PSI gene whose structure has been reported to date. The rice *psaH* and *psaDb* of *N. sylvestris* have homologues of as-2 site [27] and GATA motif (this paper, Fig. 2), respectively, and both motifs are known to be bound by a protein factor, GA-1, which interacts with the *cab-E* gene of *Nicotiana plumbaginifolia* [32]. The rice *psaH* has

a sequence, AGTATATT, which is a core of the R3 motif, TAGTATATTA (Fig. 2), in the reverse orientation about 920 bases upstream of the translation start codon. These are the only traits found to be shared by these two PSI genes, and *cis*-regulatory elements of PSI genes may be somewhat diverged between monocots and dicots.

Genomic Southern analysis revealed that there are two copies of *psaD* in the *N. sylvestris* genome (Fig. 5). This result is consistent with immunoblot analysis, which showed that the PSI-D subunit of *N. sylvestris* consists of two isoforms [23]. Immunoblot analysis of tomato, *Arabidopsis*, maize and rice have also demonstrated that there are no more than a few isoforms of PSI-D in the nuclear genome of each of these species [23]. A similar result was obtained in the spinach genome [11]. Taking these results together with the results described here, it appears that *psaD* gene organization occurs in two to three copies in many higher plants. This is quite different from the organization of the *rbcS* and *Cab* gene families, which comprise four to twelve [4] and at least twelve genes [5, 21, 29] in higher plants, respectively.

The proteins encoded by *psaDa* and *psaDb* are so homologous (Fig. 3) that we expect only minimal conformational differences between them if any exists at all. Thus it seems likely that there is little functional difference between PSI-D1 and PSI-D2. This question cannot be fully resolved without functional analysis of the two proteins.

The PSI-D subunit is located on the stromal surface of PSI, and is the ferredoxin binding site [39, 40]. There are at least two isoforms of chloroplastidic ferredoxin, Fd I and Fd II, and the relative abundance of these isoforms changes during leaf development [35]. The relationship between the isoforms of PSI-D and ferredoxin remains to be analyzed.

The finding that two copies of *psaD* are present in the *N. sylvestris* genome raises a question as to the difference in expression of the individual genes. Both genes appear to be expressed in all photosynthetic tissues, and neither is specific to flowers, stems or roots (Fig. 6). Thus, these genes probably did not diverge due to organ specific expression. During leaf development, the ratio of the *psaDb* to *psaDa* mRNA increases (Fig. 6), however, the ratio of the corresponding proteins decreases (Fig. 7). These results indicate that differential regulatory mechanisms are operating at both the mRNA and protein levels. The *psaDa* mRNA is abundant in developing leaves (Fig. 6, Da), and the translation product accumulates during leaf development to reach a maximum in mature leaves (Fig. 7, D2). This accumulation profile suggests that the *psaDa* gene product is present throughout leaf development, once it is stably integrated into the PSI complex.

The accumulation profiles of the *psaDb* mRNA and gene product are more complicated. Although *psaDb* mRNA is present at a high level in mature as well as developing leaves (Fig. 6, Db), the relative abundance of the *psaDb* protein, PSI-D1, markedly decreases during leaf maturation (Fig. 7, D1). It is likely that this phenomenon results from translational and/or post-translational activity. Translational and post-translational regulation is thought to provide a more immediate response to environmental stimuli than transcriptional regulation [36]. The high level of *psaDb* mRNA in conjunction with the relatively low abundance of the corresponding gene product, PSI-D1, may suggest that expression of *psaDb* is rapidly induced by one or more environmental factors. Further analysis of expression in the *psaD* gene family will provide us with interesting insights into the role of molecular diversity in the photosynthetic apparatus.

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References

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K: Current Protocols in Molecular Biology. John Wiley, New York (1987).
2. Brunner H, Thümmler F, Song G, Rüdiger W: Phytochrome-dependent mRNA accumulation for nuclear coded photosystem I subunits in spinach seedlings. J Photochem Photobiol B: Biol 11: 129–138 (1991).
3. Castresana C, Staneloni R, Malik VS, Cashmore AR: Molecular characterization of two clusters of genes encoding the type I CAB polypeptides of PSII in *Nicotiana plumbaginifolia*. Plant Mol Biol 10: 117–126 (1987).
4. Dean C, Pichersky E, Dunsmuir P: Structure, evolution, and regulation of *RbcS* genes in higher plants. Annu Rev Plant Physiol Plant Mol Biol 40: 415–439 (1989).
5. Dunsmuir P, Smith SM, Bedbrook J: The major chlorophyll *a*/*b* binding protein of petunia is composed of several polypeptides encoded by a number of distinct nuclear genes. J Mol Appl Genet 2: 285–300 (1983).
6. Elbrecht A, Rowley DR, O'Malley BW: Irish cream liqueur as a blocking agent for DNA dot blots. BMBiochemica 4: 12–13 (1987).
7. Gilmartin PM, Sarokin L, Memelink J, Chua N-H: Molecular light switches for plant genes. Plant Cell 2: 369–378 (1990).
8. Green PJ, Yong M-H, Cuozzo M, Kano-Murakami Y, Silverstein P, Chua N-H: Binding site requirements for pea nuclear protein factor GT-1 correlate with sequences required for light-dependent transcriptional activation of the *rbcS-3A* gene. EMBO J 7: 4035–4044 (1988).
9. Grob U, Stüber K: Discrimination of phytochrome dependent light inducible from non-light inducible plant genes. Prediction of a common light-responsive element (LRE) in phytochrome dependent light inducible plant genes. Nucl Acids Res 15: 9957–9973 (1987).
10. Hase T, Kimata Y, Yonekura K, Matsumura T, Sakakibara H: Molecular cloning and differential expression of the maize ferredoxin gene family. Plant Physiol 96: 77–83 (1991).
11. Herrmann RG, Oelmüller R, Bichler J, Schneiderbauer A, Steppuhn J, Wedel N, Tyagi AK, Westhoff P: The thylakoid membrane of higher plants: genes, their expression and interaction. In: Herrmann RG, Larkins B (eds) Plant Molecular Biology 2, pp. 411–427. Plenum Press, New York (1991).
12. Hoffmann NE, Pichersky E, Malik VS, Ko K, Cashmore AR: Isolation and sequence of a tomato cDNA clone encoding subunit II of the photosystem I reaction center. Plant Mol Biol 10: 435–445 (1988).
13. Iwasaki Y, Sasaki T, Takabe T: Sequencing and expression of the gene that encodes a 20-kDa polypeptide of the PSI complex from cucumber cotyledon. Plant Cell Physiol 31: 871–879 (1990).
14. Jofuku KD, Goldberg RB: Analysis of plant gene structure. In Chaw CH (ed), Plant Molecular Biology, pp. 37–42. IRL Press, Oxford (1988).
15. Katagiri F, Chua N-H: Plant transcription factors: present knowledge and future challenges. Trends Genet 8: 22–27 (1992).
16. Kelly JL, Greenleaf AL, Lehman IR: Isolation of the nuclear gene encoding a subunit of the yeast mitochondrial RNA polymerase. J Biol Chem 261: 10348–10351 (1986).
17. Lagoutte B: Cloning and sequencing of spinach cDNA clones encoding 20 kDa PSI polypeptide. FEBS Lett 232: 275–280 (1988).
18. Lagoutte B, Vallon O: Purification and membrane topology of PSI-D and PSI-E, two subunits of the photosystem I reaction center. Eur J Biochem 205: 1175–1185 (1992).
19. Lam E, Chua N-H: GT-1 binding site confers light responsive expression in transgenic tobacco. Science 248: 471–474 (1990).
20. Li Y, Ye L, Sugita M, Sugiura M: Tobacco nuclear gene for the 31 kd chloroplast ribonucleoprotein: genomic organization, sequence analysis, and expression. Nucl Acids Res 19: 2987–2991 (1991).
21. McGrath JM, Terzaghi WB, Sridhar P, Cashmore AR, Pichersky E: Sequence of the fourth and fifth photosystem II type I chlorophyll *a*/*b*-binding protein genes of *Arabidopsis thaliana* and evidence for the presence of a full complement of the extended CAB gene family. Plant Mol Biol 19: 725–733 (1992).
22. Münch S, Ljungberg U, Steppuhn J, Schneiderbauer A, Nechushtai R, Beyreuther K, Herrmann RG: Nucleotide sequence of cDNAs encoding the entire precursor polypeptides for subunits II and III of the photosystem I reaction center from spinach. Curr Genet 14: 511–518 (1988).
23. Obokata J, Mikami K, Hayashida N, Nakamura M, Sugiura M: Molecular heterogeneity of photosystem I: *psaD*, *psaE*, *psaF*, *psaH*, and *psaL* are all present in isoforms in *Nicotiana* species. Plant Physiol, in press.
24. Obokata J, Mikami K, Hayashida N, Sugiura M: Polymorphism of a photosystem I subunit caused by alloploidy in *Nicotiana* species. Plant Physiol 92: 273–275 (1990).
25. Oh-oka H, Takahashi Y, Matsubara H: Topological consideration of the 9-kDa polypeptide which contains centers A and B, associated with the 14- and 19-kDa polypeptides in the photosystem I complex of spinach. Plant Cell Physiol 30: 869–875 (1989).
26. Okkels JS, Scheller HV, Svendsen I, Møller BL: Isolation and characterization of a cDNA clone encoding an 18-kDa hydrophobic photosystem I subunit (PSI-L) from barley (*Hordeum vulgare* L.). J Biol Chem 266: 6767–6773 (1991).

27. de Pater S, Hensgens LAM, Schilperoort RA: Structure and expression of a light-inducible shoot-specific rice gene. Plant Mol Biol 15: 399–406 (1990).
28. Piechulla B, Pichersky E, Cashmore AR, Gruissem W: Expression of nuclear and plastid genes for photosynthesis-specific proteins during tomato fruit development and ripening. Plant Mol Biol 7: 367–376 (1986).
29. Piechulla B, Kellmann JW, Pichersky E, Schwartz E, Förster HH: Determination of steady-state mRNA levels of individual chlorophyll a/b binding protein genes of the tomato cab gene family. Mol Gen Genet 230: 413–422 (1991).
30. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).
31. Scheller HV, Møller BL: Photosystem I polypeptides. Physiol Plant 78: 484–494 (1990).
32. Schindler U, Cashmore AR: Photoregulated genes expression may involve ubiquitous DNA binding proteins. EMBO J 9: 3415–3427 (1990).
33. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC: Measurement of protein using bicinchoninic acid. Anal Biochem 150: 76–85 (1985).
34. Sugita M, Gruissem W: Developmental, organ-specific, and light-dependent expression of the tomato ribulose-1,5-bisphosphate carboxylase small subunit gene family. Proc Natl Acad Sci USA 84: 7104–7108 (1987).
35. Takahashi Y, Hase T, Wada K, Matsubara H: Ferredoxins in developing spinach cotyledons: the presence of two molecular species. Plant Cell Physiol 24: 189–198 (1983).
36. Thompson WF, White MJ: Physiological and molecular studies of light-regulated nuclear genes in higher plants. Annu Rev Plant Physiol Plant Mol Biol 42: 423–466 (1991).
37. Wales R, Newman BJ, Pappin D, Gray JC: The extrinsic 33 kDa polypeptide of the oxygen-evolving complex of photosystem II is a putative calcium-binding protein and is encoded by a multi-gene family in pea. Plant Mol Biol 12: 439–451 (1989).
38. Yamamoto Y, Tsuji H, Hayashida N, Inoue K, Obokata J: Nucleotide sequence of cDNA clones encoding PSI-D2 protein of photosystem I in *Nicotiana sylvestris*. Plant Mol Biol 17: 1251–1254 (1991).
39. Zanetti G, Merati G: Interaction between photosystem I and ferredoxin. Eur J Biochem 169: 143–146 (1987).
40. Zilber AL, Malkin R: Ferredoxin cross-links to a 22 kD subunit of photosystem I. Plant Physiol 88: 810–814 (1988).