

Plant Gene Register

Cloning of a Nuclear-Encoded Photosystem I Gene, *psaEb*, in *Nicotiana sylvestris*¹

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PSI is a multiprotein pigment complex in thylakoid membranes and mediates light-driven electron transport from plastocyanin to ferredoxin. PSI consists of at least 13 subunits, designated PSI-A through PSI-L and PSI-N, which are encoded in the nuclear or plastid genome (Bryant, 1992). The gene for the PSI-E subunit is designated as *psaE* and is located in the nuclear genome in higher plants and green algae (Bryant, 1992) and in the plastid genome in red algae (Reith, 1992). *psaE* cDNAs have been isolated from several plant species (Bryant, 1992), whereas genomic clones have not been isolated as yet. In this study, we isolated what to our knowledge is the first nuclear-encoded *psaE* gene.

We screened a *Nicotiana sylvestris* genomic library in λ DASH vector using *psaE* cDNA clones (Obokata et al., 1994) as probes and isolated a genomic clone named kuEG3. This clone has a 14.7-kb insert, and a 2.2-kb region containing a *psaE* gene was sequenced (Table I). Comparison of the nucleotide sequences of kuEG3 with the *psaE* cDNA clones revealed that this genomic clone contains the *psaEb* gene. The protein-coding region of *psaEb* is interrupted by two introns of 456 and 198 bp. These introns have the consensus dinucleotides, GT and AG (Hanley and Schuler, 1988), at their 5' and 3' borders, respectively. According to the exon-shuffling hypothesis, introns were present in the most ancient genes (Gilbert et al., 1986). If an ancient *psaE* gene originally located in the plastid genome were transferred to the nuclear genome during plant evolution, as for the genes *rpl22* (Gantt et al., 1991) and *tufA* (Baldauf and Palmer, 1990), these introns would have been maintained in the nuclear genome of land plants but lost during the subsequent evolution of red algae genomes.

The *psaEb* gene isolated here has several sequence elements homologous to well-defined *cis*-elements of other photoregulated genes, such as the GT-1 box of *rbcS* (Green et al., 1988) and the GATA motif of *Lhca* and *Lhcb* genes (Castresana et al., 1987). In addition, the R3 and R5 motifs previously found in the tobacco genes *psaD* (Yamamoto et

Table I. Characteristic of *psaEb* gene from *N. sylvestris*

Organism:	<i>Nicotiana sylvestris</i> .
Gene Copy Number:	In <i>N. sylvestris</i> , genomic Southern blot and cDNA analyses indicate that <i>psaE</i> has two gene copies, which we designated <i>psaEa</i> and <i>psaEb</i> (Obokata et al., 1994).
Techniques:	We screened an <i>N. sylvestris</i> genomic library in λ DASH vector by using <i>psaE</i> cDNA probe, which was a mixture of <i>psaEa</i> and <i>psaEb</i> cDNA.
Method of Identification:	Sequence comparison was made with previously identified <i>psaEa</i> and <i>psaEb</i> cDNA clones.
Structural Features of Genes:	<i>psaEb</i> has two introns of 456 and 198 bp.

al., 1993) and *psaH* (Nakamura and Obokata, 1994) are also present in *psaEb*. It remains to be analyzed whether these sequence elements are involved in any regulatory mechanisms of *psaEb* expression.

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LITERATURE CITED

- Baldauf SL, Palmer JD (1990) Evolutionary transfer of the chloroplast *tufA* gene to the nucleus. *Nature* **344**: 262–265
- Bryant DA (1992) Molecular biology of photosystem I. In J Barber, ed, *The Photosystems: Structure, Function and Molecular Biology*. Elsevier Science Publishers, Amsterdam, The Netherlands, pp 501–549
- Castresana C, Staneloni R, Malik VS, Cashmore AR (1987) Molecular characterization of two clusters of genes encoding the type I CAB polypeptides of PSII in *Nicotiana plumbaginifolia*. *Plant Mol Biol* **10**: 117–126
- Gantt JS, Baldauf SL, Calie PJ, Weeden NE, Palmer JD (1991) Transfer of *rpl22* to the nucleus greatly preceded its loss from the chloroplast and involved the gain of an intron. *EMBO J* **10**: 3073–3078
- Gilbert W, Marchionni M, McKnight G (1986) On the antiquity of introns. *Cell* **46**: 151–154
- Green PJ, Yong MH, Cuzzo M, Kano-Murakami Y, Silverstein P, Chua NH (1988) Binding site requirement for pea nuclear protein factor GT-1 correlate with sequences required for light-

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- dependent transcriptional activation of the *rbcS-3A* gene. *EMBO J* **7**: 4035–4044
- Hanley BA, Schuler MA** (1988) Plant intron sequences: evidence for distinct groups of introns. *Nucleic Acids Res* **16**: 7159–7176
- Nakamura M, Obokata J** (1994) Organization of the *psaH* gene family of photosystem I in *Nicotiana sylvestris*. *Plant Cell Physiol* **35**: 297–302
- Obokata J, Mikami K, Yamamoto Y, Hayashida N** (1994) Micro-heterogeneity of *psaE* subunit of photosystem I in *Nicotiana sylvestris*. *Plant Cell Physiol* **35**: 203–209
- Reith M** (1992) *psaE* and *trnS* (CGA) are encoded on the plastid genome of the red alga *Porphyra umbilicalis*. *Plant Mol Biol* **18**: 773–775
- Yamamoto Y, Tuji H, Obokata J** (1993) Structure and expression of a nuclear gene for the PSI-D subunit of photosystem I in *Nicotiana sylvestris*. *Plant Mol Biol* **22**: 985–994