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Update section

Sequence

Nucleotide sequence of cDNA clones encoding PSI-D2 protein of photosystem I in *Nicotiana sylvestris*

Yoshiharu Yamamoto[1,2], Hideo Tsuji[1], Nobuaki Hayashida[3], Kazuhito Inoue[4] and Junichi Obokata[2]*

[1] *Department of Botany, Faculty of Science, Kyoto University, Kyoto 606, Japan;* [2] *Department of Botany, Faculty of Science, Hokkaido University, Sapporo 060, Japan (*author for correspondence);* [3] *RIKEN Institute, Tsukuba 305, Japan;* [4] *Department of Biological Sciences, School of Science, Kanagawa University, Hiratsuka, Kanagawa 259–12, Japan*

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Photosystem I (PSI) consists of at least eleven subunits [6, 10] in the thylakoid membrane, and mediates photosynthetic electron transfer from plastocyanin to ferredoxin. PSI-D subunit which is located at the stromal surface of the PSI [7] is thought to be the docking site of ferredoxin [11, 12], and encoded by the nuclear gene *psaD*. Complementary DNA clones encoding PSI-D have been isolated in several plant species [2, 3, 4, 5]. In *Nicotiana* species, two types of PSI-D subunits are present per genome, and they are alternatively integrated into each PSI complex [6]. The amount of the high-molecular-mass type isoprotein, PSI-D1, is smaller than that of the low-molecular-mass type one, PSI-D2, but the ratio between them changes during leaf development [6]. Here we present the nucleotide sequence of cDNA clones encoding the PI-D2 protein in *Nicotiana sylvestris*.

A λgt10 cDNA library was constructed from poly(A)$^+$ RNA purified from whole young plants of *N. sylvestris*, and screening was carried out in a novel procedure. First, cDNA fragments were amplified from the library by the polymerase chain reaction (PCR) [8] using a universal λgt10 primer (5′-AGCAAGTTCAGCCTGGTTAAG-3′) and a synthetic primer (5′-GA$^{A}/_{G}$GCICCIGTIGGI-TTIACICCICCICAI$^{T}/_{C}$TIGA$^{T}/_{C}$CCIAA$^{T}/_{C}$AC--3′) which corresponds to the N-terminal amino acid sequence of the PSI-D2 protein, AEEAAT-KEAEAPVGFT [6]. The amplified cDNA fragments were then labelled with [α-^{32}P]-dCTP by a random primer method [9] and used for a probe in subsequent plaque hybridization under high stringency conditions. The screening gave several positive clones, among which three clones, yaDC12, yaDC17, and yaDC60, were isolated and subjected to DNA sequencing (Fig. 1). They are overlapping clones derived from an identical gene, and contain one large open reading frame in which a stretch from amino acid 49 matches the N-terminal sequence of the PSI-D2 protein. From these results, we conclude that these cDNA clones encode the PSI-D2 protein of *N. sylvestris*,

The nucleotide sequence data repeated will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases.

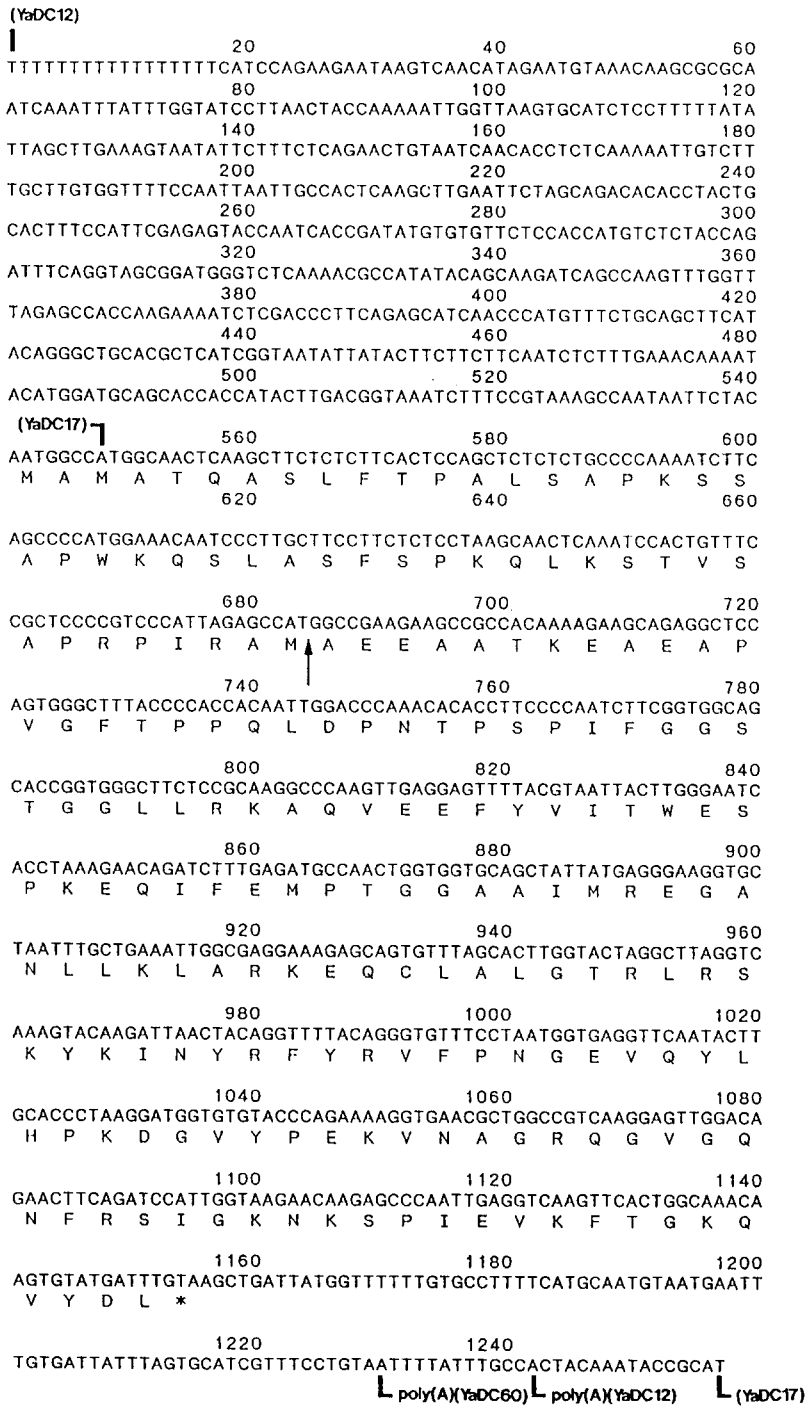

Fig. 1. Nucleotide sequence of cDNA clones, yaDC12, yaDC17, and yaDC60. These are overlapping clones with their termini indicated. As for yaDC60, nucleotide sequence was determined only for 200 bases from the 3′ terminus, and the position of its 5′ terminus was not determined. The deduced amino acid sequence is shown underneath. The arrow indicates the junction between the transit peptide and the mature protein.

and we designate the nuclear gene from which these cDNAs are derived as *psaDa*.

The putative precursor of the PSI-D2 consists of 204 amino acids with a calculated molecular mass of 22.4 kDa, and it is cleaved between methionine at 48 and alanine at 49 to give the mature protein of 156 amino acids with a predicted molecular mass of 17.4 kDa.

Amino acid sequences of PSI-D precursor proteins are compared among *N. sylvestris*, tomato [2], cucumber [3], and spinach [5] in Fig. 2. The sequences are conserved in the mature protein regions, but divergent in the transit peptides and around the processing sites. Amino acids which promote α-helical structure are shown as shaded in Fig. 2. Secondary structure prediction [1] revealed that α-helical structure is conserved around the processing sites rather than the primary sequence in the PSI-D proteins (data not shown).

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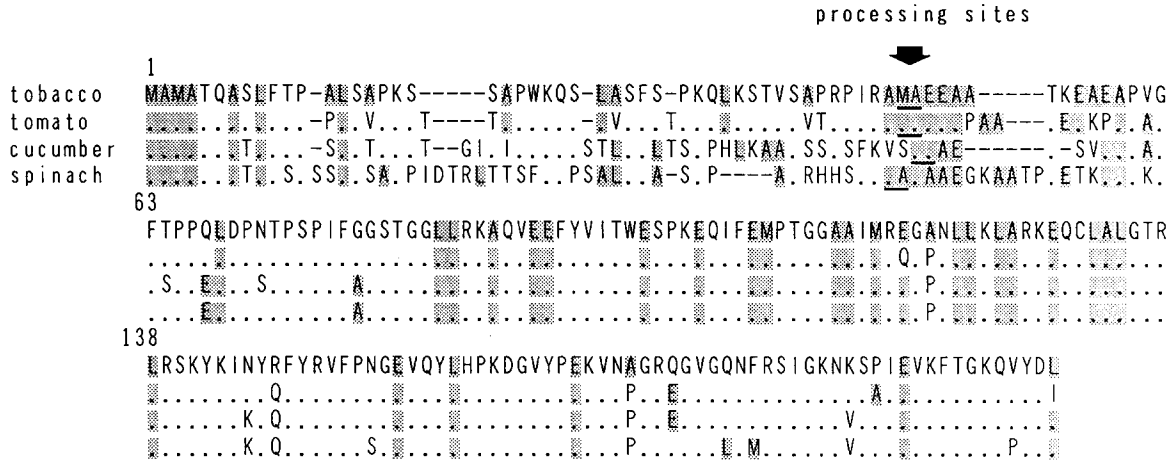

Fig. 2. Amino acid sequences of the PSI-D precursor proteins from tobacco (*Nicotiana sylvestris*), tomato [2], cucumber [3], and spinach [5]. Amino acid residues promoting α-helical structure, A, M, L, and E [1], are shaded. The processing sites are underlined.

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