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Short Communication

Early Light-Response of *psaD, psaE* and *psaH* Gene Families of Photosystem I in *Nicotiana sylvestris*: PSI-D has an Isoform of Very Quick Response

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Light response of the *psaD, psaE* and *psaH* gene families of photosystem I was studied in etiolated seedlings and dark-adapted mature leaves of *Nicotiana sylvestris* using RNase protection assays and immunoblot analysis. The results suggest that the initial light-response consists of at least two types; a very quick response found only in the *psaDb* gene, and a slower response common to all the examined genes.

Key words: Gene expression — Greening — Light-response — *Nicotiana sylvestris* — Photosystem I.

Photosystem I (PSI) is a multi-protein complex in the thylakoid membrane of chloroplasts which mediates light-driven electron transfer from plastocyanin to ferredoxin. In higher plants, PSI consists of at least 13 subunits, including PSI-A through PSI-L and PSI-N. Their genes are designated *psaA* thorough *psaL* and *psaN*, respectively, and are encoded by the chloroplast or nuclear genome (Bryant 1992, Golbeck 1992, Knoetzel and Simpson 1993). The chloroplast-encoded subunits, PSI-A, PSI-B and PSI-C constitute the integral part of the reaction center core, while the periphery is made up of the nuclear encoded subunits, including PSI-D, PSI-E, PSI-F, PSI-G, PSI-H, PSI-K, PSI-L and PSI-N (Bryant 1992, Golbeck 1992, Knoetzel and Simpson 1993). PSI-D and PSI-F are docking sites for ferredoxin (Zanetti and Merati 1987, Zilber and Malkin 1988) and plastocyanin (Wynn and Malkin 1988), respectively. PSI-E is located very close to PSI-D, and is thought to be involved in cyclic electron flow (Yu et al. 1993). The function of the other peripheral subunits remains to be elucidated.

In higher plants, etiolated seedlings do not have any detectable level of PSI activity, but activity appears during the greening process following light stimulation. Recent studies revealed that phytochrome turns on the nuclear-encoded PSI genes in response to light (Brunner et al. 1991), and G proteins and cGMP were shown to be involved in this signal transduction (Neuhaus et al. 1993, Bowler et al. 1994). The nuclear-encoded PSI genes generally constitute small multi-gene families and encode sets of isoproteins (Obokata et al. 1993). However, since these previous studies did not pay attention to the multi-gene organization nor isoprotein composition of PSI, we do not know whether all the PSI genes are controlled through the same signal transduction pathway or not. Organization of the PSI genes and isoproteins has been well studied in *Nicotiana sylvestris* (Obokata et al. 1993), and the *psaD, psaE* and *psaH* gene families of this plant consist of two, two and three gene copies, respectively (Yamamoto et al. 1991, 1993, Obokata et al. 1992, 1994, Nakamura and Obokata 1994). In the present study, we examined the expression of these gene families of *N. sylvestris* with special reference to the individual gene copies and isoproteins.

Plants were grown at 25°C in all cases. Seeds of *Nicotiana sylvestris* were surface-sterilized and sown on 0.8% agar plates containing half-strength MS salts (Murashige and Skoog 1962). These plates were initially placed under white fluorescent light (70 μE m^{-2} s^{-1}) for 48 h, and then kept in darkness for 5 days. This 48 h light treatment was necessary for the *N. sylvestris* seeds to fully germinate in

Abbreviations: AGPC, acid guanidinium thiocyanate-phenol-chloroform; LDS, lithium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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darkness. The resulting etiolated seedlings were exposed to fluorescent white light (50 μE m^{-2} s^{-1}) for up to 72 h. At the indicated times, liquid N_2 was directly poured onto the Petri plates, and total RNA was isolated by the AGPC method (Chomczynski and Sacchi 1987) from the portions of the seedlings not embedded in the agar. Ten μg RNA samples were subjected to a standard RNase protection assay (Ausubel et al. 1987), and the radioactivity in each protected fragment was quantitated using Fujix BAS2000 Imaging Analyzer (Fuji Photo Film, Tokyo). Riboprobes for *psaDa*, *psaDb*, *psaHa*, *psaHb* and *psaHc* were prepared as previously described (Yamamoto et al. 1993, Nakamura and Obokata 1994). Riboprobes for *psaEa* (from +20 to +265, relative to the ATG initiator codon) and *psaEb* (from +18 to +335) were prepared from the cDNA fragments (Obokata et al. 1994) subcloned into pBluescript II vector (Stratagene). As the riboprobe for *psaHa* produces several protected fragments derived from

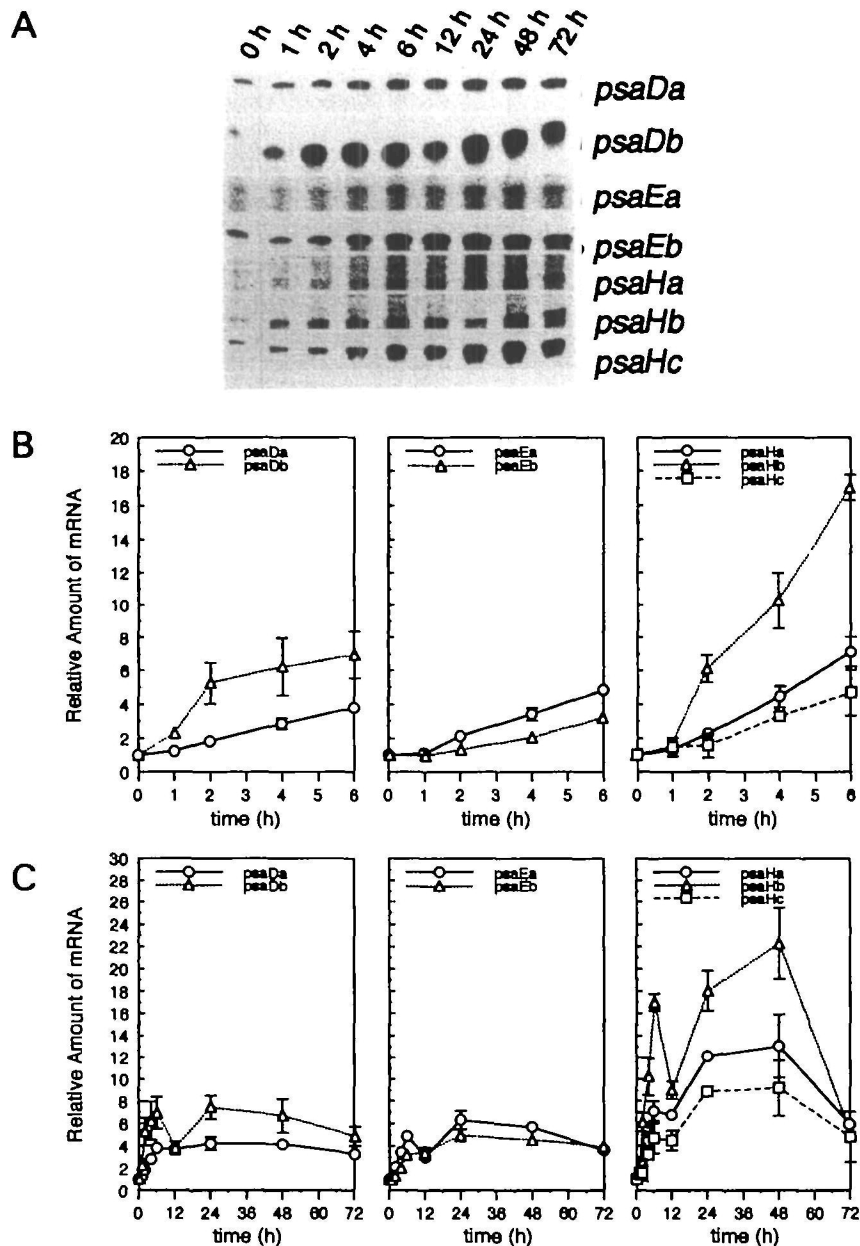


Fig. 1 Light response of *psaD*, *psaE* and *psaH* during the greening process of *N. sylvestris*. Ten μg samples of total RNA from greening seedlings were subjected to a RNase protection assay. (A) Autoradiograph. (B) and (C) Time course of mRNA accumulation during greening. The RNA level of etiolated seedlings (0 h) was shown as 1.0. The average value and standard deviation were calculated from three experiments.

distinct poly(A) sites (Nakamura and Obokata 1994), radioactivities of these fragments were taken together when the *psaHa* mRNA abundance was determined.

Membrane protein fractions were prepared from an equal number of plastids, then subjected to LDS-PAGE and immunoblot analysis (Obokata et al. 1993). PSI proteins were purified as previously described (Obokata 1986). Protein blots on nitrocellulose filters were probed with rabbit anti-barley PSI antibody, and visualized by a chemiluminescence method (Amersham, ECL Detection Kit). X-ray film was exposed to each membrane for 15 s to one night at 37°C, and the resultant fluorogram was analyzed using a computerized densitometer (Molecular Dynamics, Model 300SXE). The relative amount of protein in each band was determined using standard curves made using a series of dilutions of 72 h-irradiated samples.

Figure 1 represents the light-induced accumulation profiles of the *psaD*, *psaE* and *psaH* mRNAs in greening *N. sylvestris* seedlings. All the mRNAs examined here were already present in etiolated seedlings (Fig. 1A, 0 h). After the onset of light illumination, levels of all these mRNAs prominently increased, and then varied following a common motif, though individual mRNAs showed different magnitude of change (Fig. 1B, C). The mRNA levels increased for the first 6 h (Fig. 1B), then decreased to certain levels by the next time point (Fig. 1C), and subsequently increased again until 24 to 48 h, followed by another decrease (Fig. 1C). All the mRNAs, except for *psaDb*, had a lag phase for 1 h preceding a linearly increasing phase (Fig. 1B). In this respect, *psaDb* is unique in exhibiting a very quick response without the initial lag phase, and its mRNA level increased at the highest rate during the first two hours.

Does the quick response of the *psaDb* gene within the first 2 h occur specifically in etiolated tissues? To answer this question, we examined the light-response of the above mentioned genes in dark-adapted plantlets, which had been grown with light for 1 to 2 months and then kept in darkness for 5 days just before the experiment. This dark treatment reduced the mRNA levels of the above mentioned genes to more than 20 fold (data not shown). The light-induced accumulation profiles to the *psaD*, *psaE* and *psaH* mRNAs in mature leaves (Fig. 2) are very similarly to those in etiolated seedlings (Fig. 1B) except for the magnitudes of increase. As is evident in Figure 2, the quick response of *psaDb* occurs not only in etiolated tissues but also in green tissues.

Next, we examined the light-responses of the above mentioned genes in terms of protein accumulation. Figure 3 shows the accumulation profiles of PSI proteins during the greening process, determined by immunoblot analysis. The antibody used here reacts with PSI-A/B, C, D, E, H, and L subunits. In *N. sylvestris*, PSI-D, H, and L subunits each have two types of isoproteins, while PSI-E has four (Obokata et al. 1993). Isoproteins of PSI-D reacted equally with the antibody; the same is true for the PSI-E isoproteins. Hence, the apparent ratio of the isoproteins in Figure 3A reflects the actual protein abundance. PSI-H1 protein, the higher molecular mass isoform of PSI-H (Obokata et al. 1993), could not be detected, probably because of its paucity. The relationships between the genes in Figure 1 and their protein products in Figure 3 are as follows: *psaDa*

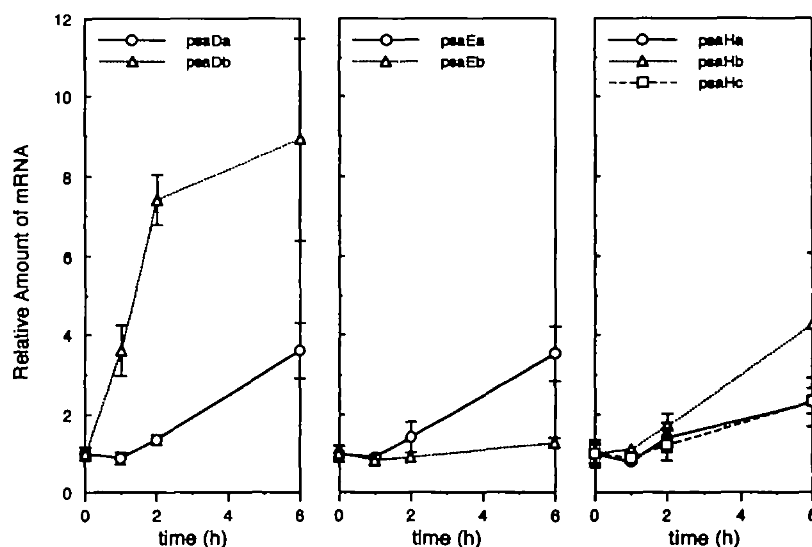


Fig. 2 Light-response of *psaD*, *psaE* and *psaH* in green leaves. Plantlets at 1–2 months grown in Petri plates under a light regime of 16 h light/8 h dark were transferred to darkness for 5 days and re-exposed to white light (50 μE m^{-2} s^{-1}) for 0, 1, 2 and 6 h. Total RNA samples from leaves were subjected to a RNase protection assay. The RNA level at 0 h is defined as 1.0. The average and standard deviation were calculated from three experiments.

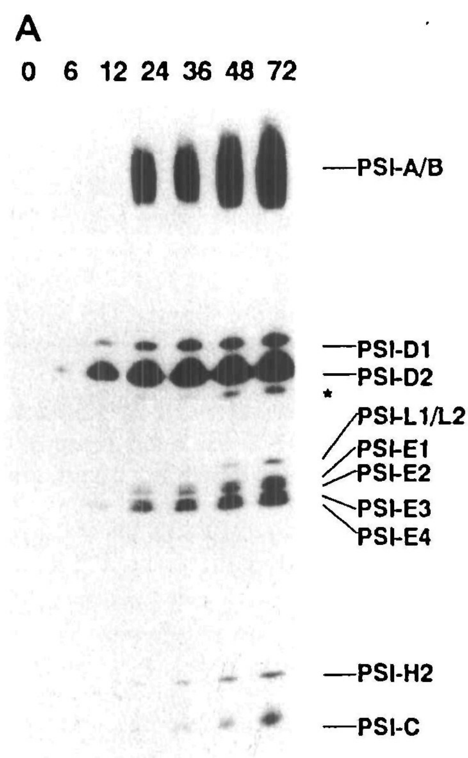
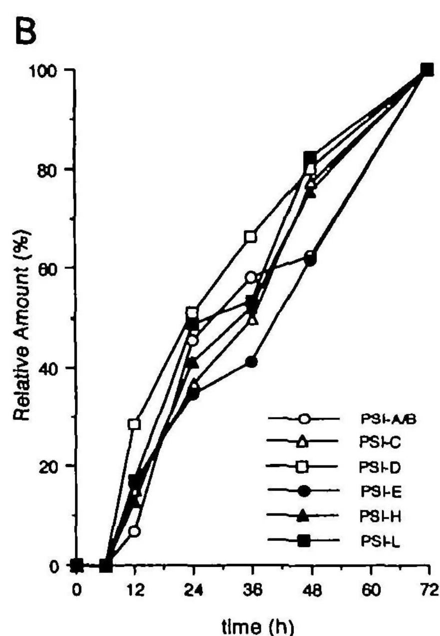
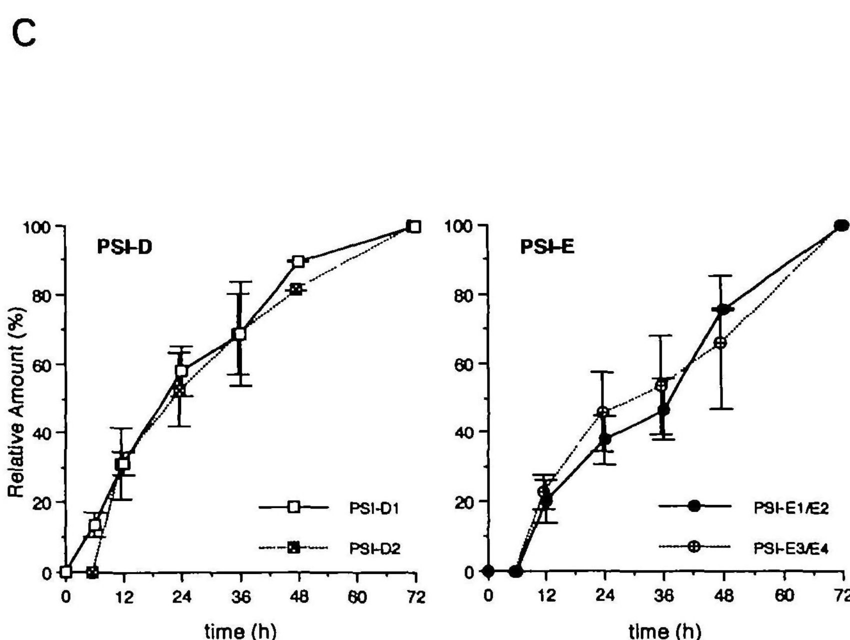


Fig. 3 Accumulation profiles of PSI subunits during greening. Etiolated seedlings were exposed to white light for 0, 6, 12, 24, 36, 48 and 72 h and plastid membrane fraction proteins from 5 × 10⁸ plastids were subjected to PAGE, and then immunoblot analysis. Authentic PSI proteins purified from *N. sylvestris* were used for identifying each protein band. (A) Fluorograph of PSI proteins. An asterisk indicates an unidentified band. (B) Quantitative presentation of the accumulation profiles of PSI subunits. The 72 h illuminated sample amount is defined as 100%. The relative amounts are the average of two experiments. Quantitative analysis was possible only when protein levels were above 3.6% for PSI-A/B and 10.8% for the others. Hence, protein levels less than these are expressed as 0%. (C) Accumulation profiles of PSI-D isoproteins (left) and PSI-E isoproteins (right). The relative protein amounts are the average of two experiments.





encodes PSI-D2 (Yamamoto et al. 1991); *psaDb*, PSI-D1 (Yamamoto et al. 1993); *psaEa*, PSI-E1/E2 (Obokata et al. 1994); *psaEb*, PSI-E3/E4 (Obokata et al. 1994); *psaHa*, *psaHb* and *psaHc* all encode PSI-H2 (Nakamura and Obokata 1994). Isoproteins of PSI-L were not separated by the LDS-PAGE method used here.

PSI subunits were detected after 6 to 24 h of light illumination (Fig. 3A). In Figure 3B, abundances of individual subunits at the indicated times are presented relative to the amount at 72 h. Quantitative examination of the fluorographic images revealed that these subunits accumulate in a relatively synchronous fashion. No PSI protein was detected in etiolated seedlings (0 h). After 6 h of initial lag phase, amounts of every subunit linearly increased at least until

72 h. Various dilutions of the 72 h samples were made to determine the least detectable amount of protein as a percentage of the 72 h samples (100%). PSI-A/B and PSI-D2 were detected at 3.6%, but not at 1.2%, while the other subunits were detected at 10.8%, but not at 3.6%. In etiolated seedlings, PSI protein levels were less than 3.6% or 10.8% of the 72 h-illuminated samples. It should be noted that the mRNAs of the *psaD*, *psaE* and *psaH* genes were already significantly present (greater than 10% of the 72 h value) in etiolated seedlings (Fig. 1A, 0 h), although their translation products could not be detected (Fig. 3). This indicates that without light, the expression of these genes is arrested at translational and/or post-translational steps in etiolated tissues.

In Figure 3C, the light-induced accumulation profiles are compared between the isoproteins of PSI-D (Fig. 3C, left) and PSI-E (Fig. 3C, right). In these cases, the isoproteins accumulated similarly. However, one notable finding is that the light-induced accumulation of PSI-D1 occurred earlier than PSI-D2, and that at 6 h its abundance was 14% of the 72 h-illuminated level. This early appearance of PSI-D1 coincides with the quick response of its mRNA (*psaDb*) in the initial greening phase (Fig. 1B).

This study revealed two aspects of the light-induction kinetics of the PSI genes. The first aspect is that all the PSI genes examined here change their mRNA levels following a common motif in both etiolated seedlings (Fig. 1) and mature leaves (Fig. 2). This finding clearly shows that a set of PSI genes, including those for the isoproteins, are subjected to a common regulatory mechanism at least for mRNA light-induction. The second aspect is that the nuclear genes for PSI are composed of two types in respect to their early light-responses. *psaDb*, which encodes the minor isoform of the PSI-D subunit (Obokata et al. 1993), is characterized by its very quick response to light both for mRNA (Fig. 1, 2) and protein (Fig. 3) levels, while the other genes were devoid of this quick response (their mRNA and protein levels began to increase after a lag phase, Fig. 1, 2, 3). Hence, it appears that *psaDb* responds to an additional induction pathway other than those shared by all the genes of the *psaD*, *psaE* and *psaH* families. The physiological significance of this quick response of *psaDb* is not known at present. In the initial phase of greening, PSI activity is known to appear earlier than PSII (Wellburn and Hampp 1979, Ohashi et al. 1989), raising the question as to how PSI works in the absence of PSII. It is an interesting subject whether the *psaDb* protein, the PSI-D isoform of very small abundance (Obokata et al. 1993), is used for cyclic electon flow or some specialized function of unknown identity.

In this study, we also examined the time dependent accumulation of PSI subunits during greening. In Figure 3A, individual PSI subunits appear to accumulate sequentially during this process. However, this sequential profile is due to the antibody specificity. Quantitative analysis with the aid of standard curves of individual proteins revealed that all the subunits accumulate in a synchronous fashion (Fig. 3B). Previous workers have proposed a 'sequential synthesis model' of PSI subunits during greening on the basis of their immunoblot analyses (Nechushtai and Nelson 1985, Herrmann et al. 1991). However, these previous reports did not refer to the titers of the antibodies nor what isoproteins they examined. Thus, we could not evaluate their conclusions. Our quantitative studies of the immunoblots analyzed here support synchronous accumulation rather than sequential accumulation during the greening process. However, we do not exclude the possibility that a sequential assembly occurs for each complex in a time scale of minutes or less, or in the very beginning of the greening period (Fig. 3, 0–6 h) during which PSI protein levels were too low to be detected in the present study.

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