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# Expression of the rice chloroplast *psa*A-*psa*B-*rps*14 gene cluster

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The rice plastid genes *psa*A and *psa*B encoding the two apoproteins of P700 chlorophyll *a* protein complex of photosystem I reaction center, and the putative *rps*14 gene for ribosomal protein S14, are organized into a transcription unit. Northern blot analyses revealed that the polycistronic mRNA is transcribed in leaves of illuminated and dark-grown rice seedlings. However Western immunoblot analysis detected the accumulation of P700 chlorophyll *a* protein complex only at 3 h after illuminating the dark-grown seedlings. These observations suggest the expression of *psa*A and *psa*B gene products during rice plastid development is light-regulated primarily at translational or post-translational level. S1-nuclease mapping and primer extension located the 5' end of the transcript predominantly at 175 bp upstream of *psa*A, and the 3' end at 116 bp downstream from *rps*14. The transcript is calculated to be 5230 nucleotides long. A heterologous in vitro transcription assay with spinach transcriptionally active high salt extract, followed by S1-nuclease mapping, also located the 5' end of in vitro transcript at around 175 bp upstream of rice *psa*A.

*Key words:* transcription; P700 chlorophyll *a* protein; ribosomal protein S14; plastid genes; rice

## Introduction

Photosystem I (PS I) is the protein complex of the thylakoid membranes that utilizes light energy to mediate electron transfer from plastocyanin to ferredoxin during photosynthesis [1,2]. In green plants more than 13 individual polypeptides are present in the PS I complex, which bind the reaction center P700, electron acceptors $A_0$, $A_1$, iron-sulfur centers ($F_X$, $F_A$, $F_B$) and light-harvesting chlorophylls [3,4]. The two largest polypeptides, carrying P700 and approximately 40–60 molecules of chlorophyll *a* are believed to dimerize through leucine zipper to form the (4 Fe–4 S) center $F_X$ [5]. The genes for the two polypeptides, *psa*A and *psa*B, are located adjacent to each other in the large single copy region of the circular plastid genome in higher plants [6,7], spaced by a distance of 25–26 bp. Gene *psa*B is followed by *rps*14 encoding the putative chloroplast ribosomal protein S14 [8]. In monocotyledonous rice, the translation products of *psa*A and *psa*B (83.2 and 82.6 kDa) deduced from the nucleotide sequences exhibit 48% sequence identity. Eleven to thirteen membrane-associated helices are predicted in both rice *psa*A and *psa*B polypeptides [9].

We have isolated the two largest polypeptides from rice thylakoid membranes as P700 chlorophyll *a* protein complex (CP I, 105 kDa) by a modified lithium dodecyl sulfate-polyacrylamide gel electrophoresis (LDS-PAGE) [10]. Further clear-cut separation of the two polypeptides was not very successful due to high hydrophobicity and the substantial structural similarities between the two polypeptides. It was reported that the biosynthesis and accumulation of P700 chlorophyll *a* protein complex is light-dependent in plants including pea, wheat and barley [11,12]. Whereas the studies in oat, bean and spinach showed the accumulation of P700 chlorophyll *a*

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protein in etiolated leaves [13]. Earlier transcription studies in maize [14] and barley [12] indicated that *psa*A and *psa*B are cotranscribed, and significant transcript level was observed in dark-grown barley seedlings [12]. Recent reports in tobacco [15] and rice [16] clearly demonstrated that *psa*A and *psa*B are cotranscribed together with the downstream *rps*14 gene. In this paper, we study the expression of rice *psa*A-*psa*B-*rps*14 operon in more detail. The transcript of the operon was characterized by nuclease S1 mapping and primer extension. Moreover, the 5′ end of the transcript was confirmed with a heterologous in vitro transcription assay.

## Methods

*Plant growth*

Rice (*Oryza sativa* L. cv. Tainong 67) seeds were germinated at 28°C in the dark for 4 days. It was then planted in vermiculite and either transferred to a chamber under a 12-h photoperiod (10 000 lux, white light) at 28°C for 1–3 weeks or maintained in complete darkness for 1 week followed by illumination for various lengths of time before harvesting.

*Rice RNA isolation and Northern blot analysis*

To isolate rice total RNA, tissues (root, stem, sheath and leaf) of rice seedlings were ground to fine powder in liquid nitrogen, followed by acid guanidinium thiocyanate-phenol-chloroform extraction according to Chomczynski and Sacchi [17]. To isolate rice chloroplast RNA, intact chloroplasts were prepared from green leaves by brief homogenization, low speed centrifugation and gradient centrifugation through Percoll step gradient (20/40%). The green chloroplast band at 20–40% Percoll interface was then subjected to RNA isolation by phenol extraction as described above.

Samples of total RNA were electrophoresed on 1.0% agarose gel after denaturation with glyoxal and transferred to nitrocellulose filter (Hybond-C extra, Amersham International plc). Hybridization was carried out with $^{32}$P-labeled specific gene probes for *psa*A, *psa*B, *rps*14 and *rbc*L at 42°C in the presence of 50% formamide for 16 h. Hybridization with heterologous probe for 25S rRNA was carried out at 37°C. The blot was washed and autoradiographed essentially at the same conditions as described in the previous report [16].

The gene-specific fragments used as probes were: 0.7-kb *Bgl*II/*Nde*I fragment of plasmid p15 for *psa*A (probe A, see Fig. 1), 1.5-kb insert of plasmid I9 for *psa*B (probe B), 141-bp fragment amplified from plasmid I4 (probe C, equivalent to amino acid residues 14–60 of *rps*14) by polymerase chain reaction (PCR) according to the procedures of Maniatis et al. [18], 537-bp fragment amplified from chloroplast DNA by PCR (equivalent to amino acid residues 70–248 of *rbc*L), and 4.8-kb *Eco*RI/*Sal*I fragment of plasmid I19 for 25S rRNA. They were labeled with [$\alpha$-$^{32}$P]dCTP by random primed synthesis developed by Feinberg and Vogelstein [19]. The overlapping genomic clones p15, I9 and I4 covering rice *psa*A, *psa*B and *rps*14 regions were described in the previous report [16]. Plasmid I19 is mouse 28S rDNA clone kindly provided by Dr. Selina Chen-Kiang (Mt. Sinai Medical School, NY).

*Rice leaf protein isolation and Western blot analysis*

To isolate leaf total protein of rice seedlings after exposure to light for various times, leaves were ground in liquid nitrogen and then extracted with buffer containing 0.5 M sucrose, 2.5% sodium dodecyl sulfate, 40 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, 1 mM phenylmethyl sulfonylfluoride, 10 $\mu$M leupeptin, 2 $\mu$M aprotinin, 1 $\mu$M pepstatin and 50 mM Tris-glycine (pH 8.3). It was vigorously stirred at 30°C for 10 min. The protein extract was collected by centrifugation at 10°C for 20 min followed by fractionation on a modified LDS-PAGE gel (7.5% acrylamide) as described by Chen et al. [10]. The protein bands were then electroblotted onto nitrocellulose filter (Schleicher & Schnell BA85) and examined by enzyme immunoassay with mouse antiserum against rice P700 chlorophyll *a* protein complex at 1:500 dilution [16]. Sheep anti-mouse Ig conjugated to horseradish peroxidase (Amersham International plc.) was used to detect the immunocomplexes.

*S1-nuclease mapping and primer extension analysis*

For S1 protection of the 5′ end of *psa*A-*psa*B-*rps*14 transcript, the 5′end-labeled single-stranded DNA was used as probe. For this purpose, the *Hin*dIII/*Bam*HI fragment of p15 covering −715 to +48 of *psa*A gene was subcloned into *Hin*dIII-*Bam*HI site of M13mp19 to get plasmid pM19–15HB. The synthetic oligonucleotide (primer A1) corresponding to the sequence complementary to +20–+49 of the noncoding strand of *psa*A (Fig. 2) was 5′ end-labeled with [γ-$^{32}$P]ATP and then annealed to single-stranded pM19–15HB. It was then extended using Klenow fragment of DNA polymerase I (Boehringer Mannheim), cut with *Hin*dIII, and the resulting 760-nt probe (Fig. 1) isolated on an alkaline low-melting agarose gel (1.2%). This 760-nt probe was hybridized with rice leaf RNA (40 μg) at 34°C in a 80% formamide solution, and then digested with nuclease S1 according to a published procedure [18]. The S1 protected DNA was analyzed by electrophoresis through 6% polyacrylamide-8 M urea DNA sequencing gel and subsequent autoradiography. Products of sequencing reaction of single-stranded pM19-15HB with primer A1 were run in parallel with S1-nuclease-treated sample to generate DNA sequence ladders.

The 5′ end of transcript was also determined by primer extension [18]. The 5′ end-labeled A1 primer was annealed with 40 μg of rice leaf total RNA or 5 μg of rice chloroplast RNA and extended using AMV reverse transcriptase (Promega Corporation). The sequence ladders were the same as that in S1 mapping.

To prepare 3′ end-labeled DNA probe for S1 protection of 3′ end of the transcript, *Bam*HI/*Eco*RV fragment of plasmid I4 covering *rps*14 gene and downstream region was isolated. The 1161-bp fragment was specifically labeled at 3′ end of the coding strand by filling reaction with [α-$^{32}$P]dGTP and Klenow fragment. The resulting double-stranded 1162-nt probe (Fig. 1) was denatured and hybridized with rice leaf total RNA (40 μg) at 52°C in a 80% formamide solution according to the method of Dean [20]. It was followed by S1-nuclease digestion and analysis with sequencing gel.

*In vitro transcription assay*

The *Hin*dIII/*Bam*HI fragment of p15 was subcloned into *Hin*dIII-*Bam*HI site of pUC19 to get plasmid pU19–15HB for serving as in vitro transcription template. Transcriptionally active high salt extract was prepared from intact spinach

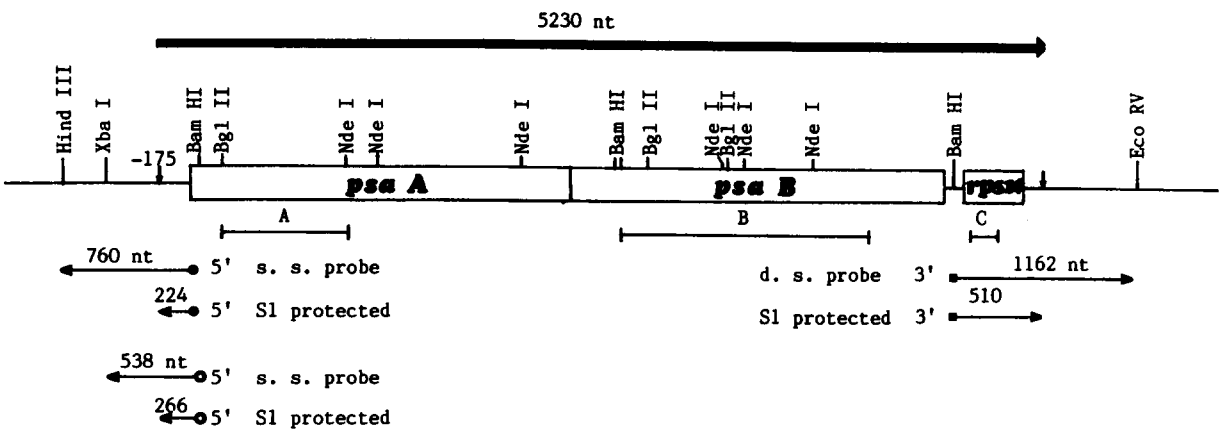


**Fig. 1.** Restriction map of rice plastid *psa*A-*psa*B-*rps*14 gene cluster and the location of the probes used in the study. The thicker long arrow indicates the transcript of the operon. The open rectangles indicate the coding regions of *psa*A, *psa*B and *rps*14. The vertical short arrows indicate the 5′ and 3′ termini of the transcript. A,B,C, represent the three specific gene probes for *psa*A, *psa*B and *rps*14, respectively, in Northern studies. The 5′ end-labeled single-stranded probes (760 nt, 538 nt) and the 3′ end-labeled double-stranded probe (1162 nt) for S1-nuclease mapping, as well as the resulting S1 protected fragments (224 nt, 266 nt and 510 nt), were indicated by thin horizontal arrows.

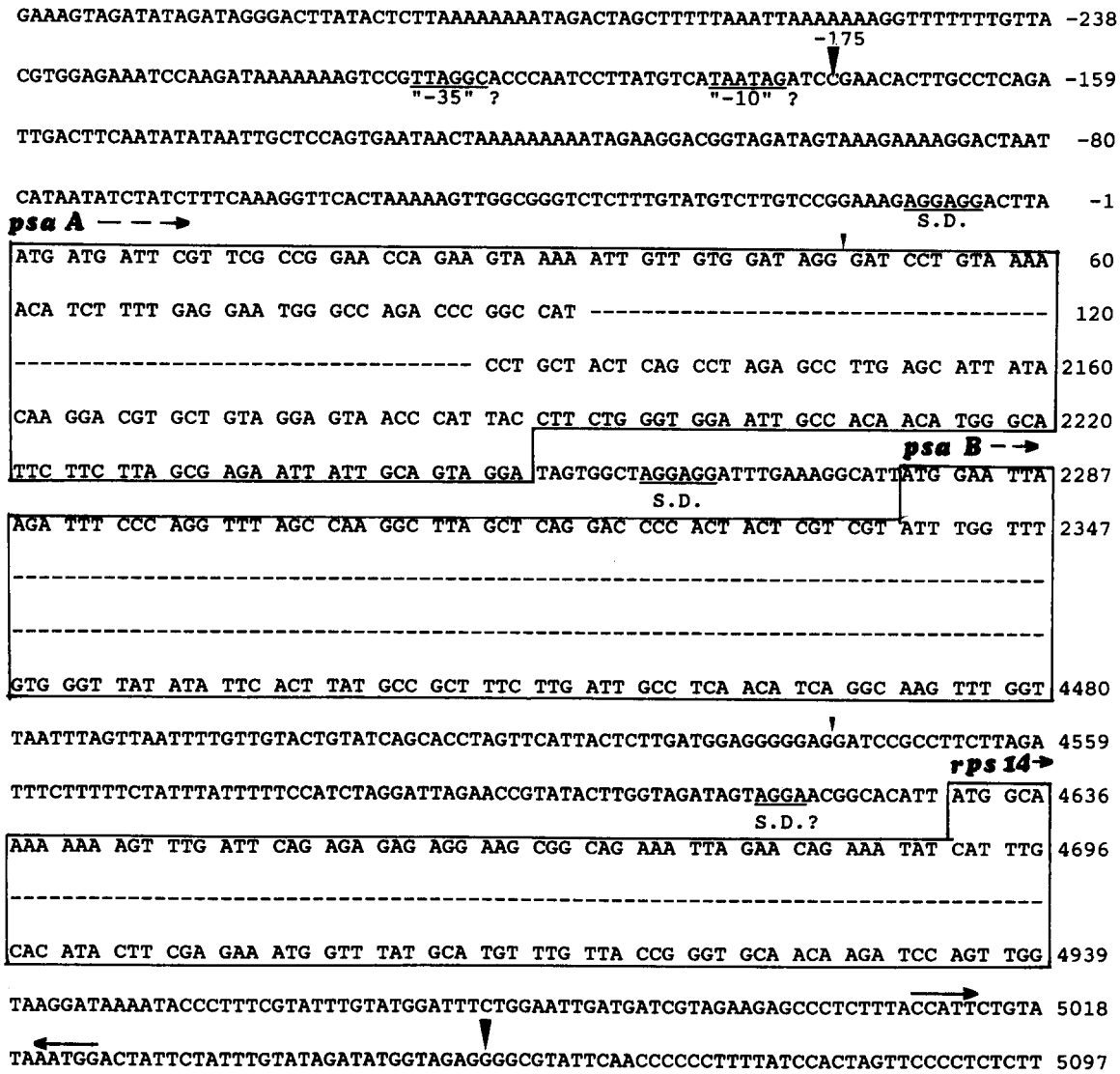


**Fig. 2.** Nucleotide sequence of the non-coding strand of rice *psa*A-*psa*B-*rps*14 operon. Coding region are boxed. Thick vertical arrows indicate the 5′ and 3′ termini of the transcript determined by S1-nuclease mapping and primer extension. The putative promoter and *E. coli* like Shine-Dalgarno sequences are underlined. The DNA sequences that form inverted repeats are overlined. The short arrows mark the *Bam*HI site. Numbering of nucleotides begins with the first A of the first methionine codon of *psa*A.

chloroplasts as described by Orozco [21]. The transcription reaction (40 $\mu$l) containing 4 $\mu$l of spinach high salt extract (approx. 50 $\mu$g of protein) and 400 ng of supercoiled template pU19–15HB was performed at 28°C for 1 h. The transcription products were then examined by S1-nuclease analysis as described above. A single-stranded DNA probe of 538 nt containing a short segment of vector DNA sequence at the $^{32}$P-labeled 5′ end was designed to use. For this purpose, the M13 universal primer (17 bases) was 5′ end-labeled and annealed with pM19–15HB, extended using Klenow fragment, cut with *Xba*I, and the resulting 538-nt probe (Fig. 1) isolated on an alkaline gel.

## Results and Discussion

*Expression of psaA-psaB-rps14 operon*

To study the expression of rice *psa*A-*psa*B-*rps*14 operon, total RNA from vegetative organs including root, short stem and leaf (with sheath) of 3-week-old green rice seedling was denatured with glyoxal and electrophoresed on 1.0% agarose gel. Northern blot hybridization was performed sequentially with specific gene probes for *psa*A, *psa*B and *rps*14. Figure 3 shows the three probes hybridized to a common RNA band of 5.2 kb, indicating the cotranscription of *psa*A, *psa*B and *rps*14 in leaf tissue. No *psa*A-*psa*B-*rps*14 transcripts were detected in root and stem RNA. An internal fragment of *rbc*L was used to probe the same Northern blot. Two *rbc*L transcripts (1.8 and 1.6 kb), similar to that reported in maize [22], were revealed in leaf tissue. 25S rRNA was abundantly observed in all the organs with mouse 28S rDNA as probe. The leaf-specific expression of *psa*A operon raises the question whether *rps*14 mRNA is transcribed separately in root or stem. However no RNA band of other size was detected to hybridize to *rps*14 probe (data not shown). The open question is whether the rice plastid *rps*14 is functional and whether rice plastid ribosomes do not contain an S14-like protein. Immunochemical study of rice plastid ribosomal proteins with antiserum specific to bacterial S14 protein is to be performed to answer this question in the near future.

In order to investigate the effect of light on the

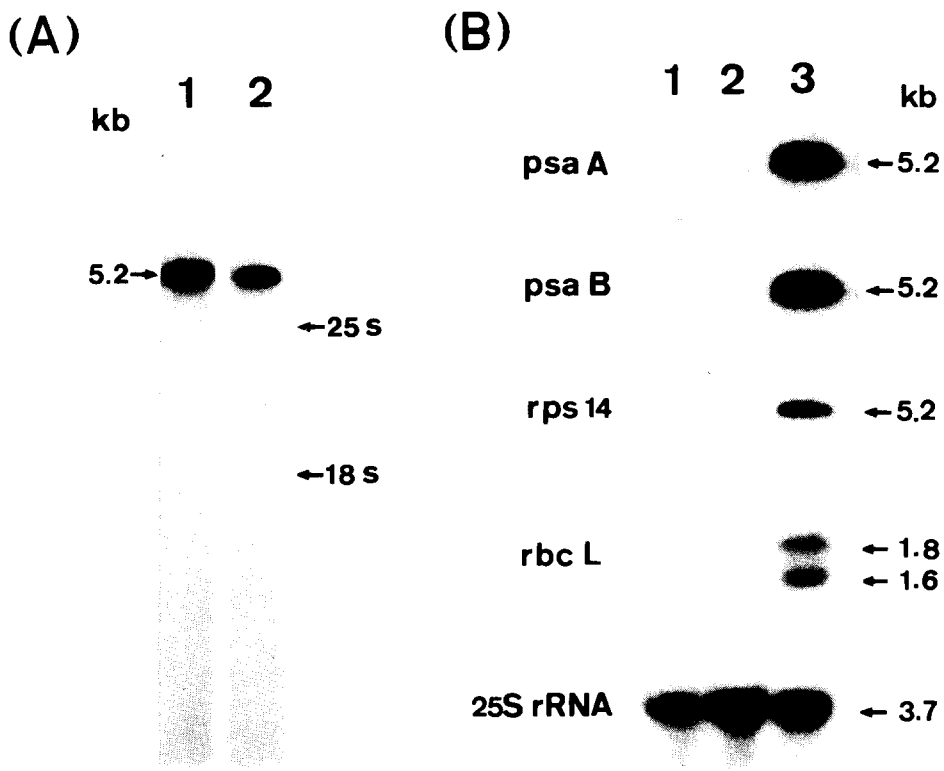


**Fig. 3.** Northern blot analyses of the expression of plastid *psa*A-*psa*B-*rps*14 in rice tissues. (A) Ten μg of total RNA from leaves of green rice seedlings were separated on 1.0% agarose gel after denaturation with glyoxal, and blotted to nitrocellulose filter. The same filter was hybridized sequentially with specific gene probes for *psa*A (lane 1) and *rps*14 (lane 2). Arrows at the right mark the position of 25S rRNA and 18S rRNA. (B) Equal amounts (10 μg) of total RNA from roots (lane 1), stems (lane 2) and leaves (including sheaths, lane 3) of 3-week-old green rice seedlings were separated on 1.0% agarose gel and blotted as described in (A). The same filter was hybridized sequentially with gene probes for *psa*A, *psa*B, *rps*14, *rbc*L and 25S rRNA as described in Methods. Arrows indicate the size of transcript in kb.

expression of *psa*A operon, 1-week-old rice seedlings grown in the dark were exposed to white light for various lengths of time (1, 3, 6, 12 and 24 h). The second leaf of seedling was then excised, and leaf total RNA and protein were prepared. The leaf RNA was subjected to Northern blot analysis for *psa*A-*psa*B-*rps*14 transcript. Figure 4A demonstrates that high level of 5.2-kb transcript was already present in dark-grown seedlings. The transcript remained fairly constant throughout the 24-h illumination period except for a slight transient increase after 1 h of illumination. Quantitative dot-blot hybridization has been carried out to confirm this result (data not shown). On the other hand, when leaf protein was fractionated by LDS-PAGE followed by immunoblot analysis with mouse antiserum against rice CP I, no CP I complex was detected in dark-grown plants (Fig. 4B). The accumulation of CP I complex of 105 kDa was observed after 3 h of illumination, and increased gradually during the 24-h period. These observations indicate that the expression of rice *psa*A and *psa*B gene products during plastid development is light-regulated primarily at translational or post-translational level. Klein et al. reported that in barley transcripts for *psa*A-*psa*B are associated with polysomes in dark-grown and illuminated seedlings [23]. Furthermore, it has been shown that light-induced chlorophyll synthesis triggers chlorophyll *a* apoprotein accumulation [24,25]. These data suggest that chlorophyll acts by binding to and stabilizing the P700-apoprotein.

*Mapping of psaA-psaB-rps14 transcript*

The 5′ end of the 5.2-kb transcript was finely mapped by S1-nuclease protection and primer extension analysis. For S1-nuclease protection, the 5′-$^{32}$P-labeled 760-nt probe of the coding strand (positions −711 to +49, Fig. 2) was hybridized with green leaf total RNA or etiolated leaf total RNA, and digested with S1 nuclease. The S1 protected DNA fragment was electrophoresed in parallel with the sequence ladders of the coding strand obtained by dideoxy chain termination method with A1 primer. A1 primer is complementary to the *psa*A coding region from +20 to +49. A major protected band of 224 nt corresponding

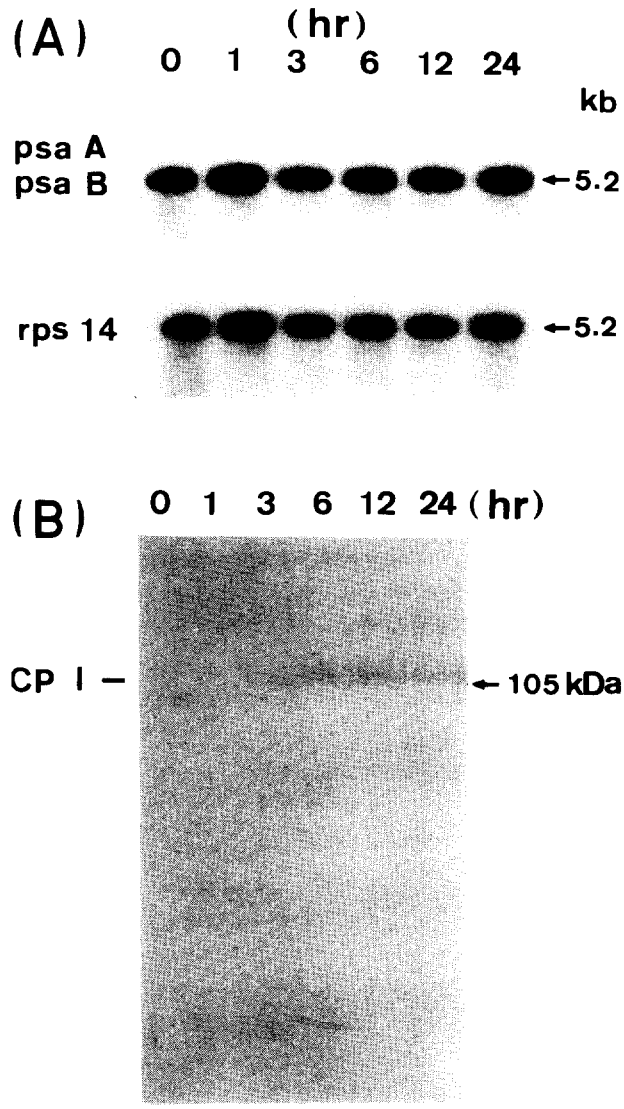


**Fig. 4.** Effect of illumination on the expression of rice *psa*A-*psa*B-*rps*14. (A) Northern blot analyses of transcript for *psa*A-*psa*B-*rps*14 in dark-grown and illuminated rice seedlings. Equal amounts (10 μg) of leaf total RNA from dark-grown and illuminated rice seedlings (1, 3, 6, 12 and 24 h) were separated on a 1.0% agarose gel, blotted to nitrocellulose filter, and hybridized to probes for *psa*A and *psa*B (upper panel) and probe for *rps*14 (lower panel) as described in Fig. 3. (B) The light-induced accumulation of P700 chlorophyll *a* protein complex (CP I) in rice seedlings. Equal amounts (30 μg) of leaf protein extracted from dark-grown and illuminated rice seedlings (1, 3, 6, 12 and 24 h) were subjected to LDS-PAGE (7.5% acrylamide) and immunoblot analysis with mouse specific antiserum against rice CP I as described in Methods. Arrow indicates CP I of 105 kDa.

to position −175 (G) of the sequence ladders was observed, along with three less intense bands corresponding to positions −176, −174 and −169 (Fig. 5A). Both RNA preparations from green leaf and etiolated leaf gave the same protection pattern. The presence of several additional bands could reflect nibbling by S1 nuclease or represent 5′ heterogeneity of the transcripts. To confirm the 5′ end, the primer extension analysis was performed using 5′ end-labeled A1 primer to hybridize to green leaf total RNA or chloroplast RNA. The primer was then extended by reverse transcription, followed by ribonuclease digestion. The extended DNA was electrophoresed in parallel with the sequence ladders. A major extended fragment and a minor fragment, corresponding to positions −175 and −174 respectively, were obtained for both RNA preparations (Fig. 5B). Based on these results, it is suggested that the 5′ end of *psa*A-*psa*B-*rps*14 transcript is predominantly at 175 bp upstream the start codon of *psa*A.

The 3′ end of the transcript was also mapped by S1-nuclease analysis. The *Bam*HI/*Eco*RV fragment was specifically labeled at 3′ end of the coding strand (Fig. 1) and hybridized to RNA from green leaf or etiolated leaf, followed by digestion with S1 nuclease. The S1 protected DNA fragment was also analyzed with sequencing gel. Figure 5C shows a single protected fragment of 510 nt. The 3′ end of the transcript is located at 116 nt downstream from the *rps*14 stop codon. The *psa*A-*psa*B-*rps*14 transcript is thus calculated to be 5230 nt long based on the 5′ start site at −175.

*In vitro transcription assay*

Since the above S1-nuclease protection and primer extension analyses locate the 5′ end of *psa*A-*psa*B-*rps*14 transcript predominantly at 175 bp upstream of *psa*A, it is interesting to see if the transcript synthesized in vitro also shows the same result with S1 nuclease analysis. To examine this problem, supercoiled plasmid pU19–15HB containing −715 to +48 region of *psa*A subcloned in pUC19 was utilized as DNA template. Due to the lack of homologous in vitro transcription system for rice plastid genes at present, the in vitro transcription reaction was performed with the transcriptionally active high salt extract from spinach chloroplasts [21]. The transcription products were then examined by S1-nuclease analysis. The single-stranded DNA probe of 538 nt contained −447 to +48 of the coding strand and 43 nt of vector sequence at the $^{32}$P-labeled 5′ end. Thus only in vitro synthesized RNA was protected by S1-nuclease analysis. Fig. 6 shows a major protected fragment of 266 nt, which locates the 5′ end of the in vitro transcript at −175 position. The less intense bands were again observed at positions corresponding to −174 and −169. These observations imply that the chloroplast transcription machinery of spinach is able to recognize the promoter of rice *psa*A operon, and accurately initiate transcription as that in rice chloroplasts in vivo. Attempts are being made to develop a good in vitro homologous transcription system for rice plastid genes. It is hoped that transcriptionally active extract from rice chloroplasts to be obtained in the near future to fulfill this purpose.

In barley, two transcripts of *psa*A operon (5.3 kb) of roughly equal amounts were identified by S1 mapping, which have a common 5′ end at approximately 215 bp upstream of *psa*A but differ at their 3′ ends by approximately 26 nt [26]. In maize, a 4.9-kb transcript of *psa*A operon was identified to have 5′ end at a site approximately 183 bp upstream of *psa*A [14]. In tobacco [15], a 5207-nt long transcript of *psa*A operon was identified to have transcriptional initiation site at 194 bp upstream of *psa*A and 3′ end at 105 bp downstream of *rps*14. Furthermore, two stem-loop secondary structures are proposed in the region immediately preceding the 3′ end of tobacco transcript. We have searched for potential stem-loop structure, which may act as chloroplast RNA processing and stabilizing elements [27,28], in the 3′ end region of rice *psa*A operon. As shown in Fig. 2, only a short inverted repeat sequences were observed at approximately 30 bp preceding the 3′ end. The possible function of this structure remains to be elucidated.

It is known that chloroplast promoters have many prokaryotic characteristics [29,30], including −10 and −35 promoter elements separated by a defined distance. In rice *psa*A operon, the putative −10 element TAATAG (positions −184 to −179) and −35 element TTAGGC (positions −207

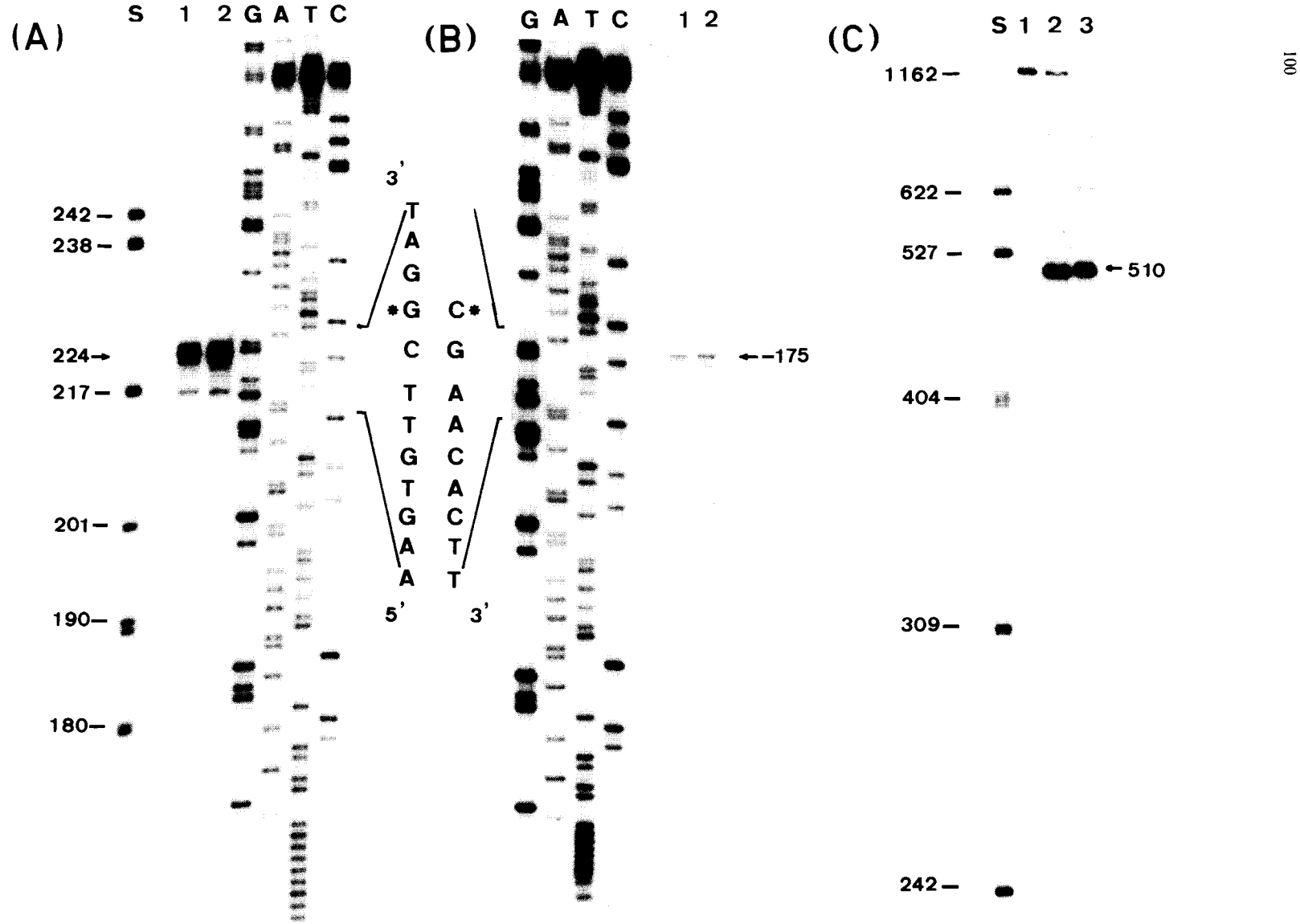


**Fig. 5.** Mapping of the transcript for rice plastid *psa*A-*psa*B-*rps*14 operon. (A) 5′-End mapping by nuclease S1 protection. The 5′-$^{32}$P-labeled 760-nt probe was hybridized to etiolated leaf RNA (lane 1) or green leaf RNA (lane 2) and digested with nuclease S1. Arrow indicates the predominant 5′ end. Lane S indicates the sizes (bp) of pBR322/*Hpa*II standards. (B) 5′-End mapping by primer extension. Primer A1 was using chloroplast RNA (lane 1) or green leaf total RNA (lane 2). Arrow indicates the major extension product. (C) 3′-End mapping by nuclease S1 protection. The *Bam*HI/*Eco*RV fragment of plasmid I4 labeled at 3′ end (lane 1) was hybridized to green leaf RNA (lane 2) or etiolated leaf RNA (lane 3). Arrow indicates the protected fragment.

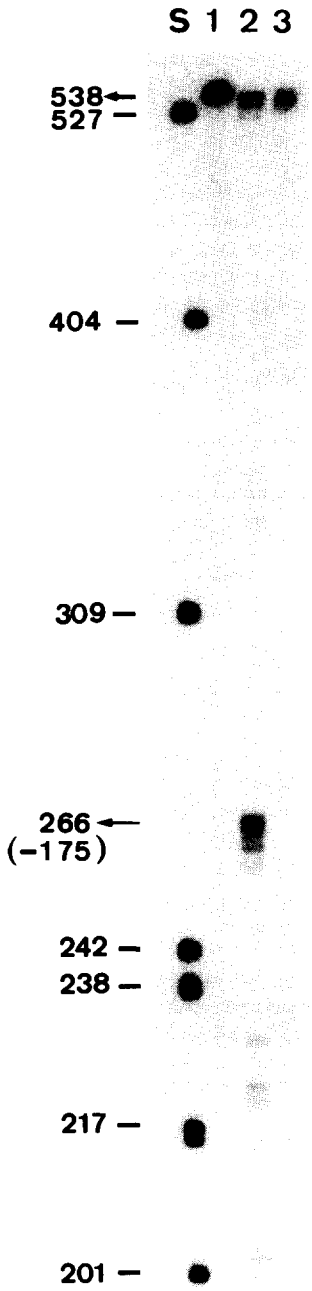


**Fig. 6.** S1-nuclease protection analysis of in vitro transcription products of rice *psa*A gene by spinach crude high salt extract. The transcription reaction is described in text. The 538-nt probe (lane 1) containing −477 to +48 of the coding strand and a stretch of vector sequence was 5′ end-labeled to protect the in vitro synthesized RNA. The protected fragments are shown in lane 2, with arrow to mark the major band of 266 nt. Transcription reaction without plasmid DNA template was run as control (lane 3).

to −202) were demonstrated in Fig. 2. There are 17 bp between the two promoter elements. The typical Shine-Dalgarno sequence AGGAGG was observed before both *psa*A and *psa*B coding sequences, which is complementary to the sequence near the 3′ end of rice plastid 16S rRNA [7]. It is thus believed to be the ribosome binding site. Although there is shorter stretch of consensus sequence AGGA before *rps*14, it is not clear at present if transcript for *rps*14 is translated in rice plastids in vivo.

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## References

1 R. Malkin, Photosystem I. Annu. Rev. Plant Physiol., 33 (1982) 455–479.
2 W. Haehnel, Photosynthetic electron transport in higher plants. Annu. Rev. Plant Physiol., 35 (1984) 659–693.
3 E. Vierling and R.S. Alberte, P700 chlorophyll *a* — protein. Plant Physiol., 72 (1983) 625–633.
4 K.G. Parrett, T. Mehari, P.G. Warren and J.H. Golbeck, Purification and properties of the intact P700 and $F_x$ containing photosystem I core protein. Biochim. Biophys. Acta, 973 (1989) 324–332.
5 A.N. Webber and R. Malkin, Photosystem I reaction-center proteins contain leucine zipper motifs. A proposed role in dimer formation. FEBS Lett., 264 (1990) 1–4.
6 K. Shinozaki, M. Ohme, M. Tanaka, T. Wakasugi, N. Hayashida, T. Matsubayashi, N. Zaita, J. Chunwongse, J. Obokata, K. Yamaguchi-Shinozaki, C. Ohto, K. Torazawa, B.Y. Meng, M. Sugita, H. Deno, T. Kamogashira, K. Yamada, J. Kusuda, F. Takaiwa, A. Kato, N. Tohdoh, H. Shimada and M. Sugiura, The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J., 5 (1986) 2043–2049.
7 J. Hiratsuka, H. Shimada, R. Whittier, T. Ishibashi, M. Sakamoto, M. Mori, C. Kondo, Y. Honji, C.R. Sun, B.Y. Meng, Y.Q. Li, A. Kanno, Y. Nishizawa, A. Hirai, K. Shinozaki and M. Sugiura, The complete sequence of the rice (*Oryza sativa*) chloroplast genome: Intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. Mol. Gen. Genet., 217 (1989) 185–194.
8 W. Kirsch, P. Seyer and R.G. Herrmann, Nucleotide sequence of the clustered genes for two P700 chlorophyll *a*

apoproteins of the photosystem I reaction center and the ribosomal protein S14 of the spinach plastid chromosome. Curr. Genet., 10 (1986) 843–855.

9 S.C.G. Chen, J. Chen, N.J. Yu and M.C. Cheng, Comparison of chloroplast-encoded *psa*A gene sequences between two rice cultivars. Rice Genet. Newslett., 7 (1990) 140–142.

10 S.C.G. Chen, N.J. Yu, J. Chen and M.C. Cheng, Electrophoretic separation and characterization of rice chlorophyll *a* protein complexes. Agric. Biol. Chem. (Tokyo), 54 (1990) 2455–2457.

11 T. Takabe, T. Takabe, and T. Akazawa, Biosynthesis of P700-chlorophyll *a* protein complex, plastocyanin, and cytochrome $b_6$/f complex. Plant Physiol., 81 (1986) 60–66.

12 K. Kreus, K. Dehesh and K. Apel, The light-dependent accumulation of the P700 chlorophyll *a* protein of the photosystem I reaction center in barley. Evidence for translational control. Eur. J. Biochem., 159 (1986) 459–467.

13 R. Nechushtai and N. Nelson, Biogenesis of photosystem I reaction center during greening of oat, bean and spinach leaves. Plant Mol. Biol., 4 (1985) 377–384.

14 L.E. Fish, U. Kück and L. Bogorad, Two partially homologous adjacent light-inducible maize chloroplast genes encoding polypeptides of the P700 chlorophyll *a* protein complex of photosystem I. J. Biol.Chem., 260 (1985) 1413–1421.

15 B.Y. Meng, M. Tanaka, T. Wakasugi, M. Ohme, K. Shinozaki and M. Sugiura, Cotranscription of the genes encoding two P700 chlorophyll *a* apoproteins with the gene for ribosomal protein CS14: determination of the transcriptional initiation site by in vitro capping. Curr. Genet., 14 (1988) 395–400.

16 S.C.G. Chen, M.C. Cheng, J. Chen and L.Y. Hwang, Organization of the rice chloroplast *psa*A-*psa*B-*rps*14 gene and the presence of sequence heterogeneity in this gene cluster. Plant Sci., 68 (1990) 213–221.

17 P. Chomczynski and N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 162 (1987) 156–159.

18 T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, 1989.

19 A.P. Feinberg and B. Vogelstein, A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 132 (1983) 6–13.

20 M. Dean, Determining the hybridization temperature for S1 nuclease mapping. Nucleic Acids Res., 15 (1987) 6754.

21 E.M. Orozco, Jr., J.E. Mullet and N.H. Chua, An in vitro system for accurate transcription initiation of chloroplast protein genes. Nucleic Acids Res., 13 (1985) 1283–1302.

22 L.D. Crossland, S.R. Rodermel and L. Bogorad, Single gene for the large subunit of ribulosebisphosphate carboxylase in maize yields two differentially regulated mRNAs. Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 4060–4064.

23 R.R. Klein, H.S. Mason and J.E. Mullet, Light-regulated translation of chloroplast proteins. I. Transcripts of *psa*A-*psa*B, *psb*A, and *rbc*L are associated with polysomes in dark-grown and illuminated barley seedlings. J. Cell Biol., 106 (1988) 289–301.

24 L.A. Eichacker, J. Soll, P. Lauterbach, W. Rüdiger, R.R. Klein and J.E. Mullet, In vitro synthesis of chlorophyll *a* in the dark triggers accumulation of chlorophyll *a* apoproteins in barley etioplasts. J. Biol. Chem., 265 (1990) 13566–13571.

25 J.E. Mullet, P.G. Klein and R.R. Klein, Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability. Proc. Natl. Acad. Sci. U.S.A., 87 (1990) 4038–4042.

26 T. Berends, P.E. Gamble and J.E. Mullet, Characterization of the barley chloroplast transcription units containing *psa*A-*psa*B and *psb*D-*psb*C. Nucleic Acids Res., 15 (1987) 5217–5238.

27 D.B. Stern and W. Gruissem, Control of plastid gene expression: 3′ inverted repeats act as mRNA processing and stabilizing elements, but do not terminate transcription. Cell, 51 (1987) 1145–1157.

28 D.B. Stern and W. Gruissem, Chloroplast mRNA 3′ end maturation is biochemically distinct from prokaryotic mRNA processing. Plant Mol. Biol. 13 (1989) 615–625.

29 S.D. Kung and C.M. Lin, Chloroplast promoters from higher plants. Nucleic Acids Res., 13 (1985) 7543–7550.

30 L. Hanley-Bowdoin and N.H. Chua, Chloroplast promoters. Trends Biochem. Sci., 12 (1987) 67–70.