Translation of partially overlapping *psbD-psbC* mRNAs in chloroplasts: the role of 5′-processing and translational coupling

Yuka Adachi[1], Hiroshi Kuroda[1], Yasushi Yukawa[1] and Masahiro Sugiura[1,2,*]

[1]Graduate School of Natural Sciences, Nagoya City University, Yamanohata, Mizuho, Nagoya 467-8501 and [2]Center for Gene Research, Nagoya University, Nagoya 464-8602, Japan

ABSTRACT

The chloroplast *psbD* and *psbC* genes encode the D2 and CP43 proteins of the photosystem II complex, and they are generally cotranscribed. We report studies on the basic translation process of tobacco *psbD-psbC* mRNAs using an *in vitro* translation system from tobacco chloroplasts. The primary transcript has an unusually long 5′-UTR (905 nt). We show that it is translatable. Processing of the 5′-UTR greatly enhances the translation efficiency of the *psbD* cistron. A striking feature is that *psbD* and *psbC* cistrons overlap by 14 nt. Removal of the *psbD* 5′-UTR plus the start codon and introduction of a premature termination codon in the *psbD* cistron considerably reduce the translation efficiency of the downstream *psbC* cistron. These results indicate that translation of the *psbC* cistron depends largely on that of the upstream *psbD* cistron and thus shows translational coupling; however, a portion is independently translated. These observations, together with the presence of monocistronic *psbC* mRNAs, suggest that the *psbD* and *psbC* cistrons are translated via multiple processes to produce necessary amounts of D2 and CP43 proteins.

INTRODUCTION

The chloroplast DNA in flowering plants is a circle ~150 kb long, and includes approximately 80 different protein-encoding genes (1,2). Chloroplast gene expression is regulated in part at the transcriptional level (3,4), whereas posttranscriptional steps such as RNA editing, splicing, cleavage, trimming, translation and control of mRNA stability are more important for expression of most genes (5–11). Many chloroplast genes are clustered and are cotranscribed to polycistronic transcripts that are then processed into mature RNAs (12–15). Some gene clusters consist of functionally unrelated genes; for example, the *psaC-ndhD* operon encodes a photosystem I subunit and an NADH dehydrogenase subunit (16). To express functionally unrelated cistrons from a polycistronic mRNA, regulation at posttranscriptional steps for each cistron would be required to supply proper amounts of products during development and under different environmental conditions. The photosystem II complex consists of approximately 20 different subunits, of which 14 are encoded in the chloroplast genome of flowering plants (17,18). Many of these genes are also clustered. One example is the clustered *psbD* gene for D2 protein and *psbC* gene for CP43 protein. A striking feature is that these two genes overlap by 14 nt in flowering plants (19–21), algae (22) and cyanobacteria (23). A notable exception is that these two genes are separated in *Chlamydomonas reinhardtii* (24).

In flowering plants, the overlapping *psbD* and *psbC* genes (*psbD/C*) are cotranscribed with the downstream *psbZ* gene (formerly ORF62 or *ycf9*) to produce multiple overlapping transcripts (25,26). Transcription of monocot *psbD/C*s starts from the preceding *psbK* and *psbI* gene cluster to give longer transcripts (27–29). The promoter for *psbD/C* is blue-light responsive (30,31) and is recognized by *E. coli*-type RNA polymerase with sigma factor 5 (32,33). However, little is known about the translation process of *psbD/C* mRNAs. In contrast, translation of separated *psbD* and *psbC* mRNAs in *Chlamydomonas* has been well studied using a biolistic chloroplast transformation system and heterologous reporter systems. Translation and stability of *Chlamydomonas psbC* and *psbD* mRNAs are controlled by interactions between 5′-UTRs and nucleus-encoded proteins (7–10).

We previously reported that the tobacco monocistronic *psbC* mRNA is translatable (34). However, translation of the *psbC* cistron from the dicistronic *psbD/C* mRNA has not been investigated. In addition to being overlapping, the primary *psbD/C* transcript has an unusually long 5′-UTR. A portion of the primary transcript is processed

into mRNA with a shorter 5'-UTR (25). Hence, chloroplasts contain a mixture of primary and processed *psbD/C* mRNAs. *In vitro* translation systems are a useful tool to study the basic translation process for single mRNA species by eliminating possible effects of mRNA cleavage or stability, translation of other mRNAs, assembly-dependent control and levels of necessary components (e.g. ATP, GTP, amino acids, ribosomes).

Using our *in vitro* translation system from tobacco chloroplasts, we show here that the *psbD* cistron is translatable from the primary transcript. Processing of 5'-UTRs of both *psbD/C* dicistronic and *psbC* monocistronic mRNAs significantly enhances translation efficiency. We then show that translation of the *psbC* cistron occurs from the dicistronic mRNA and it depends highly on translation of the upstream *psbD* cistron (indicating translational coupling). We discuss how translation of the downstream cistron proceeds in the overlapping *psbD/C* mRNA.

MATERIALS AND METHODS

The coding region for Cerulean (35) was prepared from the EGFP-coding region (36) and that for Citrine was obtained as described (36). Procedures for plasmid construction and primers are in Supplementary Figures S1 and S2. Necessary regions from *psbD/C* were amplified by PCR from tobacco chloroplast DNA (37) and PCR products were confirmed by DNA sequencing. Synthesis and purification of test mRNAs were as described (38). Test mRNA sequences are shown in Supplementary Figure S3. Isolation of intact tobacco chloroplasts and preparation of chloroplast extracts (S30 fractions) were as described (38). *In vitro* translation was performed at 28°C for 1 h as described (38). Protein products were separated by 12.5% native polyacrylamide gel electrophoresis (PAGE). Fluorescent signals were detected

and quantified with a Typhoon 9400 system (GE Healthcare, Little Chalfont, UK). The 526SP and 555BP20 filters were used to detect Cerulean and Citrine bands when excited by 457-nm and 532-nm actinic light, respectively.

RESULTS

Measurement of translation from *psbD* and *psbC* cistrons

Figure 1 shows a schematic of the tobacco chloroplast *psbD-psbC-psbZ* operon (17,37). These genes are cotranscribed to produce various mRNAs and the *psbD/C* mRNA is one of the major transcripts accumulated in chloroplasts (25,26). *In vitro* capping assays have identified two transcription initiation sites: the main site is located 905-bp upstream from the *psbD* ATG start codon and the additional site is 230-bp upstream from the *psbC* GTG start codon (25,34). The primary *psbD/C* transcript has a 905-nt 5'-UTR with a processing site at position −132 relative to the A (+1) of the AUG start codon (25).

To differentially monitor translation of the *psbD* and *psbC* cistrons in *psbD/C* mRNA, we utilized Cerulean (a cyan-fluorescent protein) and Citrine (a yellow-fluorescent protein). A large portion of the *psbD* cistron was replaced with the Cerulean-coding sequence (*cerulean*, 236 codons without start and stop codons), and that of the *psbC* cistron with a Citrine-coding frame (*citrine*, 237 codons without start codon) (Figure 2A). We left the last 75 nt of the *psbD* cistron and the first 24 nt of the *psbC* cistron in the mRNA, because these sequences include the Shine–Dalgarno (SD)-like sequence (GAGGAGGU), the *psbC* start codon (GUG) and the *psbD* stop codon (UAA), and because sequences downstream from start codons are often required for efficient translation (39). The tobacco chloroplast *rps16*
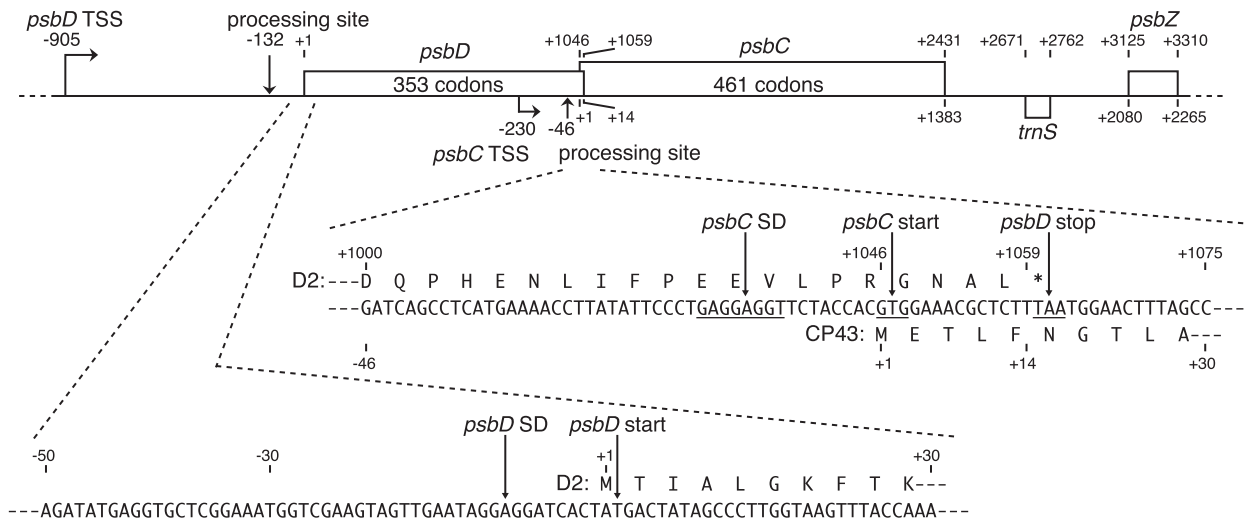

Figure 1. The tobacco chloroplast *psbD/C/Z* operon. Positions relative to the *psbD* ATG start codon (A is +1) are shown above, and positions relative to the *psbC* GTG start codon (the first G is +1) are shown below. Bent arrows indicate transcription start sites, and vertical arrows indicate processing sites. mRNA sequences around the *psbD* and *psbC* start codons are shown below. SD-like sequences, start codons and a stop codon are underlined. Deduced amino acid sequences from the *psbD* and *psbC* cistrons (encoding the D2 and CP43 proteins) are respectively shown above and below the mRNA sequences.
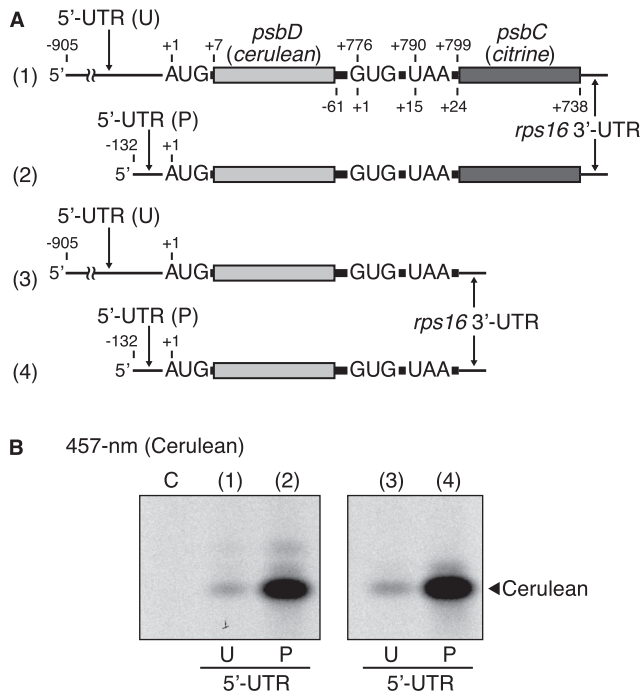
Figure 2. Translation of the *psbD* cistron with primary and 5′-processed UTRs. (**A**) mRNA templates in which the *psbD* and *psbC* cistrons were respectively replaced with Cerulean (*cerulean*) and Citrine (*citrine*) coding regions. Positions are as in Figure 1. (template '1') mRNA with the primary unprocessed (U) 5′-UTR. (template '2') mRNA with processed (P) 5′-UTR. (templates '3' and '4') mRNAs lacking the downstream cistron with U and P 5′-UTRs. (**B**) Gel patterns of translation products. After *in vitro* translation, the synthesized Cerulean products were detected by fluorescence under 457-nm light. Lanes labeled 'C', no-mRNA control. Faint bands above the main Cerulean band are probably Citrine products (top, lane 2) or loosely folded Cerulean (middle, lanes 2 and 4).

3′-UTR was linked after *citrine* to standardize test mRNAs (38).

Translation efficiencies of test mRNAs were measured using the *in vitro* translation system under template-limiting and linear progression (1 h) conditions, which enable estimation of the relative rate of translation (38). We confirmed by primer extension analysis that 5′-processing or cleavage of test mRNAs was hardly detectable during a 1-h incubation, probably due to the presence of an RNase inhibitor (38) (Supplementary Figure S3). After reaction, protein products were resolved by native PAGE. The fluorescent intensity of Cerulean and Citrine products was measured (see Figure 4B). Hence, this assay can monitor either *psbD* translation or *psbC* translation from the *psbD/C* mRNA by changing the excitation wavelength. The Cerulean product migrates faster in native gels than the Citrine product due to charge and size differences, which further helps differential monitoring (see Figure 4B).

Roles of 5′-processing on translation efficiency of the *psbD* cistron

The tobacco *psaC-ndhD* transcript is processed into functional mRNA species (16). It is hence possible that the primary *psbD/C* transcript is simply a precursor and not functional in translation because it has an extremely long

5′-UTR. We first examined whether translation of the *psbD* cistron occurs from the primary *psbD/C* mRNAs. Two test *psbD/C* mRNAs were prepared: one contains the primary (unprocessed) 5′-UTR of 905 nt and the other the processed 5′-UTR of 132 nt (Figure 2A, lanes 1 and 2). These mRNAs are expected to synthesize Cerulean products when translation starts from the *psbD* start codon. As shown in Figure 2B (lane 1), 457-nm excitation gave a single major band, indicating that the primary transcript is translatable. However, processing at position −132, removing 85% of the primary 5′-UTR, greatly enhanced its translation efficiency (10-fold, lane 2).

The long 5′-UTR of the primary transcript may interact with the downstream cistron, thereby interfering with *psbD* translation. To exclude this possibility, we prepared additional test mRNAs that lack the *psbC* (*citrine*) cistron, namely, artificial monocistronic *psbD* mRNAs (Figure 2A, lanes 3 and 4), and assayed their translation. Figure 2B (lanes 3 and 4) shows that their translation efficiencies were similar to the original dicistronic mRNAs, indicating that the downstream cistron does not affect translation of its upstream cistron.

The tobacco *psbD* mRNA contains a typical SD-like sequence, GGAGG, in its 5′-UTR (see Figure 1). The distance between the SD-like sequence and the AUG codon is 6 nt, a proper spacing (40). Changing GGAGG to AAGAA nearly abolished *psbD* translation, indicating a crucial role of the SD-like sequence on *psbD* translation initiation (Supplementary Figure S5).

Translation of the *psbC* cistron from dicistronic and monocistronic mRNAs

The second promoter located within the upstream *psbD* cistron produced 1.5- to 2.6-kb transcripts, of which two are monocistronic *psbC* mRNAs (25). We previously showed that the monocistronic *psbC* mRNA is translatable and its start codon is GUG, not the upstream in-frame AUG (34). The *psbC* start codon is located 14 nt upstream from the preceding *psbD* stop codon, and this feature may interfere with *psbC* translation initiation in the dicistronic *psbD/C* mRNA. We then examined whether translation of the *psbC* cistron occurs from the dicistronic mRNA. We again used the primary unprocessed and 5′-processed mRNAs (Figure 3A). As shown in Figure 3B (labeled 'Di'), excitation with 532-nm light enabled detection of Citrine products from the *psbC* start codon in both mRNAs, indicating that the *psbC* cistron can be translated from the dicistronic mRNA. Again, the translation efficiency of the *psbC* cistron was much higher with the 5′-processed mRNA than with the primary transcript. This difference parallels that observed in translation efficiencies of the upstream *psbD* cistron (Figure 2B), which suggests that translation of the downstream *psbC* cistron depends on translation of the preceding cistron.

We prepared two monocistronic *psbC* mRNAs: one from the second transcription initiation site (the primary 230-nt 5′-UTR) and another from the processed site (the processed 46-nt 5′-UTR), as shown in Figure 2A, and assayed as above. Unexpectedly, the translation efficiency of the *psbC* cistron was much higher from the dicistronic

mRNA than the monocistronic mRNA (Figure 3B, compare lanes labeled 'Di' and 'Mono'). The 5'-processed mRNA was again high in translation efficiency (lanes labeled 'Mono'). Both the primary and 5'-processed mRNAs accumulate to fairly high levels in tobacco chloroplasts (25). Hence, the results in Figures 2 and 3 strongly suggest that processing of the 5'-UTRs has a role in controlling translation efficiency.

To compare translation efficiencies for the *psbD* and *psbC* cistrons, we modified the above test mRNAs (labeled 'Di'), replacing *cerulean* with *citrine* in the *psbD* cistron) to minimize variables, because quantitative assays are difficult to compare between different fluorescent proteins. The Citrine products from the *psbD* start codon migrate faster in native gels than those from the *psbC* start codon due to charge and size differences; however, minor cross-contamination is inevitable between the upper and lower bands. We estimated that the translation efficiency of the *psbC* cistron is roughly one-third that of the *psbD* cistron from the primary mRNA, and roughly one-sixth that of the 5'-processed mRNA (Figure 3C, compare lanes labeled 'U' and 'P'). The reason for such a difference remains unclear.

Translational coupling of *psbD* and *psbC* in *psbD/C* mRNA

If translation of the *psbC* cistron is coupled with that of the upstream *psbD* cistron, the translation efficiency of the *psbC* cistron should depend on translation of the upstream cistron. To examine this possibility, we removed the 5'-UTR and the following AUG start codon from the processed *psbD/C* mRNA (Figure 4A). As expected, this deletion led to no *psbD* translation (Figure 4B, lane labeled '−' in the left panel). Consequently, the translation efficiency of the downstream *psbC* cistron decreased dramatically (to ∼20% of the wild-type), though did not disappear completely (lane '−' in the right panel).

Next, we introduced a G-to-A point mutation at position −70 relative to the G (+1) of the *psbC* GUG codon, creating a premature stop codon (UAA) in the 3'-region of *cerulean* (Figure 5A). Consequently, the *psbD* and *psbC* cistrons no longer overlap and are
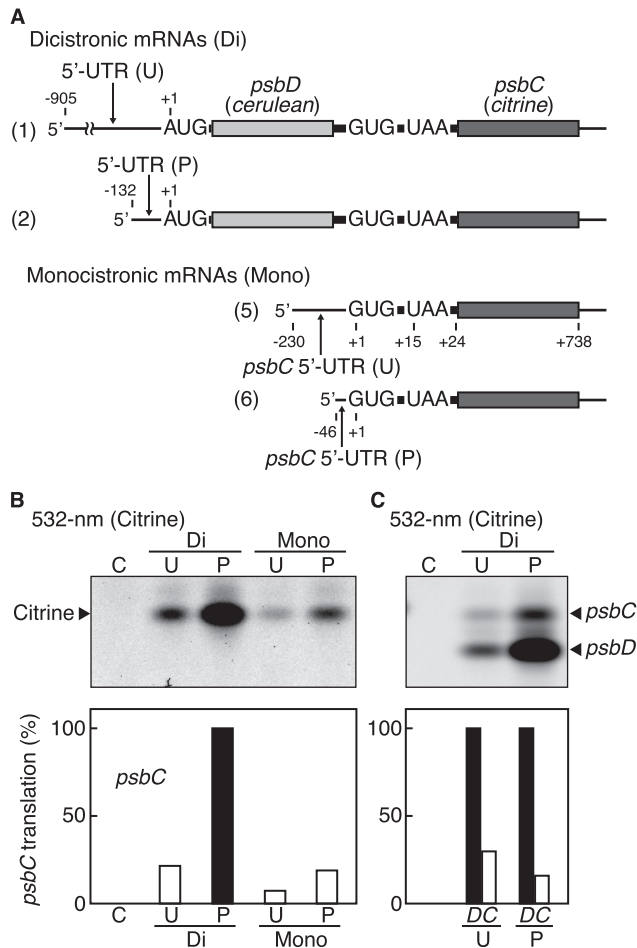

Figure 3. Translation of the *psbC* cistron. (**A**) mRNA templates. Dicistronic mRNAs (labeled 'Di') with primary/unprocessed (labeled 'U') and processed (labeled 'P') 5'-UTRs as shown in Figure 2A (template '1') and (template '2'). Monocistronic mRNAs (labeled 'Mono') with primary/unprocessed ('U') and processed ('P') 5'-UTRs. (**B**) Gel patterns of translation products. Synthesized Citrine products were detected by fluorescence under 532-nm light. Lanes labeled 'C', no-mRNA control. Faint bands above the main Citrine bands could be loosely folded Citrine. (**C**) Templates are the dicistronic mRNAs above with *cerulean* replaced with *citrine*. Gel patterns of translation products. Synthesized Citrine products from both cistrons were detected by fluorescence under 532-nm light. Lanes labeled 'C', no-mRNA control. Quantified efficiencies are shown in the bar graphs below ('P'-*psbC* value defined as 100% in (B), and *psbD* value as 100% in (C), mean values from ≥3 independent assays).
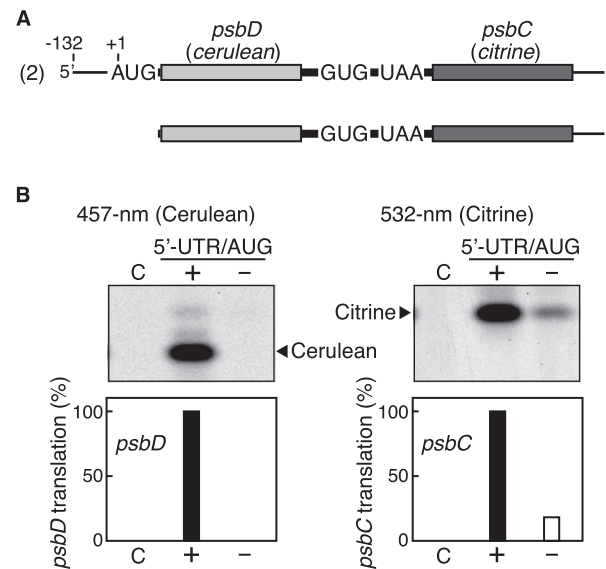
Figure 4. Effect of *psbD* 5'-UTR/AUG deletion on *psbC* translation. (**A**) mRNA templates. The processed dicistronic mRNA is as in Figure 2A (template '2'), and the mRNA after deletion of its 5'-UTR and the following AUG. (**B**) Gel patterns of translation products. Cerulean products were detected by fluorescence under 457-nm light (left panel) and Citrine products under 532-nm light (right panel). Lanes labeled 'C', no-mRNA control. Lanes labeled '+' and '−' respectively represent mRNAs with and without the sequence for the *psbD* 5'-UTR and the following AUG. Faint bands are as in the legend for Figure 2. Quantified efficiencies of *psbD* and *psbC* translation are shown in the bar graphs below ('2' mRNA value defined as 100%, mean values from ≥3 independent assays).

separated by a 70-nt spacer, which prevents translational coupling (41). As shown in Figure 5B (left panel), no significant change in *psbD* translation was observed, whereas the translation efficiency of the *psbC* cistron was greatly reduced, to 20% (the right panel). On the basis of these results, we concluded that translation of the *psbC* cistron largely depends on translation of the preceding *psbD* cistron, though there is some independent *psbC* translation.

DISCUSSION

In *psbD/C* transcripts of tobacco, spinach and pea (25,26,31), a striking feature is that the primary transcript has an unusually long 5′-UTR; for example, it is 905 nt in tobacco *psbD*, 75 nt in *Chlamydomonas psbD* (24) and 85 nt in tobacco *psbA* (encoding D1 protein) (38). This study was the first to demonstrate that the *psbD* cistron with its long 5′-UTR is translatable, indicating that the primary transcript is not merely a precursor for functional mRNAs. We then showed that the 5′-processed mRNA with a 132 nt 5′-UTR has very high translation efficiency for the *psbD* cistron and that the SD-like sequence is essential for its translation. Both primary and processed mRNAs are abundant in chloroplasts of mature tobacco leaves (25). Hence, we hypothesize that *psbD/C* expression is regulated at least in part via 5′-processing. This hypothesis could be applied for translation of the monocistronic *psbC* mRNA, because its 5′-processing greatly increases *psbC* translation efficiency. The 5′-UTR processing of *psbD/C* and *psbC* transcripts is presumably catalyzed

Figure 5. Effect of premature termination of the upstream cistron on *psbC* translation. (**A**) The mRNA template is as in Figure 2 (template '2'). Arrow indicates the point mutation from G to U, creating a premature termination codon. (**B**) Gel patterns of translation products. Cerulean products were detected by fluorescence under 457-nm light (left panel) and Citrine products under 532-nm light (right panel). Lanes labeled 'C', no-mRNA control. Lanes labeled 'wt' and 'mut' represent wild-type and mutant mRNAs, respectively. Faint bands are as in the legend for Figure 2. Quantified efficiencies of *psbD* and *psbC* translation are shown in the bar graphs below ('wt' value defined as 100%, mean values from ≥3 independent assays).

or mediated by nucleus-encoded proteins. The amount or activity of these proteins could be modulated in response to light or developmental cues. Specific RNA-binding proteins are involved in mRNA processing and mRNA stabilization or translation (7–10,42–46). For example, maize PPR10 defines the processing site of *atpH* mRNAs and thereby might enhance *atpH* translation (46). Our previous *in vitro* study indicated that the effect of 5′-UTR processing on translation varies from mRNA to mRNA (38). Translation efficiencies from primary and processed *rbcL* and *atpH* 5′-UTRs were comparable, whereas processing of *atpB* and *psbB* 5′-UTRs enhanced translation efficiency (38). It is interesting to note that 5′-processing of tobacco *psbD/C* mRNAs leads to a 10-fold increase in translation efficiency (Figure 2B).

Another striking feature is the partial overlap of *psbD* and *psbC* cistrons. We demonstrated that translational coupling does occur between *psbD* and *psbC* cistrons. As expected, the translation efficiency of the *psbC* cistron was much lower than that of its upstream cistron (Figure 3C). Blocking *psbD* translation still supported 20% of *psbC* translation (Figure 4B), indicating a contribution from independent *psbC* translation, probably in a manner similar to translation of the monocistronic *psbC* mRNA.

Four pairs of overlapping genes have been reported in the tobacco chloroplast: *ndhC-ndhK*, *atpB-atpE*, *psbD-psbC* and *rpl22-rps3* (37). The *atpB* and *atpE* cistrons have an overlap (AUGA) of the *atpB* stop codon (UGA) with the *atpE* start codon (AUG). We recently revealed that translation of the downstream *atpE* cistron is independent of that of the upstream *atpB* from the dicistronic *atpB/E* mRNA and that the *atpE* cistron is translated via its own *cis*-element (36). In contrast, we found that the *ndhC* and *ndhK* cistrons overlap by 7 nt (the *ndhK* start codon is located 4 nt upstream from the *ndhC* stop codon) and *ndhK* translation is strictly dependent on the upstream termination codon (47). It has been suggested that the *ndhK* cistron is exclusively translated by the ribosomes that complete translation of the upstream cistron; that is, some proportion of the ribosomes participating in *ndhC* translation move 4 nt upstream after termination and resume translation of the *ndhK* cistron; the remainder are released at the termination codon. It has been suggested that in *E. coli*, the ribosome could undergo bidirectional diffusion for a very short time following termination (48). Generally, increasing the distance between the stop codon of an upstream cistron and the start codon of the downstream cistron reduces or arrests translation of the downstream cistron. When the distance between the *ndhC* stop codon and the *ndhK* start codon is increased artificially, translation of the downstream *ndhK* cistron is reduced or abolished; for example, a separation of 11 nt between the stop and start codons arrests *ndhK* translation (47). Because the distance between the *psbD* stop codon and the *psbC* start codon is 11 nt, it seems unlikely that the *psbC* cistron is translated exclusively by ribosomes participating in *psbD* translation. We favor the hypothesis that the *psbC* cistron becomes capable of being translated by ribosomes from the free ribosome pool through the action of the ribosome translating the *psbD* cistron.
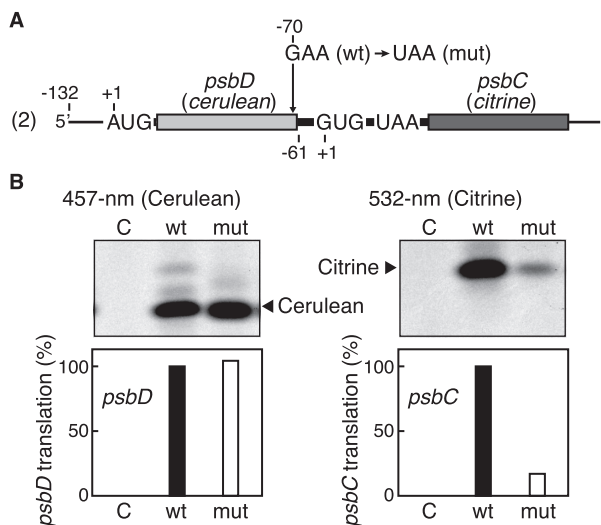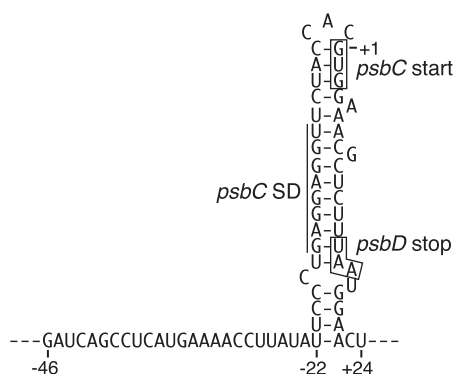
Figure 6. Predicted secondary structure of the mRNA region around the *psbC* start site. Positions are relative to the GUG start codon. The SD-like sequence, *psbC* start codon and *psbD* stop codon are marked.

A rigid stem–loop structure can be predicted from the region including the SD-like sequence, the *psbC* start codon and the *psbD* stop codon (Figure 6). Because the SD-like sequence and the *psbC* start codon are embedded in the hairpin, loading of ribosomes (or 30S subunits) could interfere with initiation of *psbC* translation. The ribosome translating the upstream cistron could unwind the hairpin to facilitate initiation of translation of the *psbC* cistron. Both primary and processed *psbC* 5′-UTRs are also predicted to form this hairpin, which could be one reason why translation of the monocistronic *psbC* mRNAs is inefficient (Figure 3B). The translation efficiency of the *psbC* cistron from the dicistronic mRNA is apparently insufficient to assemble a functional photosystem II complex. Chloroplasts accumulate a considerable amount of the monocistronic *psbC* mRNAs in tobacco (25) and other plants (27,49,50). Translation of monocistronic *psbC* mRNAs may compensate for a shortage of CP43.

In conclusion, D2 and CP43 are produced via multiple translation processes, from primary and 5′-processed mRNAs and by translational coupling and uncoupling pathways. The sequences encompassing *psbC* start/*psbD* stop codons are well conserved among various land plants, algae and cyanobacteria (Supplementary Figure S6), suggesting that the basic translation process of *psbD/C* mRNAs is also conserved. Information on the basic translation process, such as reported here, should help to understand more about the translational control mechanisms operating within the chloroplast.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Figures 1–6.

ACKNOWLEDGEMENT

REFERENCES

1. Sugiura,M. (1992) The chloroplast genome. *Plant Mol. Biol.*, **19**, 149–168.
2. Bock,R. (2007) Structure, function, and inheritance of plastid genomes. In: Bock,R. (ed.), *Cell and Molecular Biology of Plastids*. Springer, Berlin Heidelberg, pp. 29–63.
3. Shiina,T., Tsunoyama,Y., Nakahira,Y. and Khan,M.S. (2005) Plastid RNA polymerases, promoters, and transcription regulators in higher plants. *Int. Rev. Cytol.*, **244**, 1–68.
4. Liere,K. and Börner,T. (2007) Transcription and transcriptional regulation in plastids. In: Bock,R. (ed.), *Cell and Molecular Biology of Plastids*. Springer, Berlin Heidelberg, pp. 121–174.
5. Gruissem,W. and Tonkyn,J.C. (1993) Control mechanisms of plastid gene expression. *Crit. Rev. Plant Sci.*, **12**, 19–55.
6. Sugiura,M., Hirose,T. and Sugita,M. (1998) Evolution and mechanism of translation in chloroplasts. *Annu. Rev. Genet.*, **32**, 437–459.
7. Rochaix,J.-D. (2006) The role of nucleus- and chloroplast-encoded factors in the synthesis of the photosynthetic apparatus. In: Wise,R.R. and Hoober,J.K. (eds), *The Structure and Function of Plastids*. Springer, Dordrecht, pp. 145–165.
8. Marín-Navarro,J., Manuell,A.L., Wu,J. and Mayfield,S.P. (2007) Chloroplast translation regulation. *Photosynth. Res.*, **94**, 359–374.
9. Peled-Zehavi,H. and Danon,A. (2007) Translation and translational regulation in chloroplasts. In: Bock,R. (ed.), *Cell and Molecular Biology of Plastids*. Springer, Berlin Heidelberg, pp. 249–281.
10. Wobbe,L., Schwarz,C., Nickelsen,J. and Kruse,O. (2008) Translational control of photosynthetic gene expression in phototrophic eukaryotes. *Physiol. Plant.*, **133**, 507–515.
11. Tangphatsornruang,S., Birch-Machin,I., Newell,C.A. and Gray,J.C. (2011) The effect of different 3′ untranslated regions on the accumulation and stability of transcripts of a *gfp* transgene in chloroplasts of transplastomic tobacco. *Plant Mol. Biol.*, **76**, 385–396.
12. Tanaka,M., Obokata,J., Chunwongse,J., Shinozaki,K. and Sugiura,M. (1987) Rapid splicing and stepwise processing of a transcript from the *psbB* operon in tobacco chloroplasts: determination of the intron sites in *petB* and *petD*. *Mol. Gen. Genet.*, **209**, 427–431.
13. Barkan,A. (1988) Proteins encoded by a complex chloroplast transcription unit are each translated from both monocistronic and polycistronic mRNAs. *EMBO J.*, **7**, 2637–2644.
14. Westhoff,P. and Herrmann,R.G. (1988) Complex RNA maturation in chloroplasts. The *psbB* operon from spinach. *Eur. J. Biochem.*, **171**, 551–564.
15. Stern,D.B., Goldschmidt-Clermont,M. and Hanson,M.R. (2010) Chloroplast RNA metabolism. *Annu. Rev. Plant Biol.*, **61**, 125–155.
16. Hirose,T. and Sugiura,M. (1997) Both RNA editing and RNA cleavage are required for translation of tobacco chloroplast *ndhD* mRNA: a possible regulatory mechanism for the expression of a chloroplast operon consisting of functionally unrelated genes. *EMBO J.*, **16**, 6804–6811.
17. Shinozaki,K., Ohme,M., Tanaka,M., Wakasugi,T., Hayashida,N., Matsubayashi,T., Zaita,N., Chunwongse,J., Obokata,J., Yamaguchi-Shinozaki,K. *et al.* (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. *EMBO J.*, **5**, 2043–2049.
18. Hiratsuka,J., Shimada,H., Whittier,R., Ishibashi,T., Sakamoto,M., Mori,M., Kondo,C., Honji,Y., Sun,C.-R., Meng,B.-Y. *et al.*

(1989) 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**, 185–194.

19. Holschuh,K., Bottomley,W. and Whitfeld,P.R. (1984) Structure of the spinach chloroplast genes for the D2 and 44 kd reaction-centre proteins of photosystem II and for tRNA^{Ser} (UGA). *Nucleic Acids Res.*, **12**, 8819–8834.

20. Alt,J., Morris,J., Westhoff,P. and Herrmann,R.G. (1984) Nucleotide sequence of the clustered genes for the 44 kd chlorophyll *a* apoprotein and the "32 kd"-like protein of the photosystem II reaction center in the spinach plastid chromosome. *Curr. Genet.*, **8**, 597–606.

21. Rasmussen,O.F., Bookjans,G., Stummann,B.M. and Henningsen,K.W. (1984) Localization and nucleotide sequence of the gene for the membrane polypeptide D2 from pea chloroplast DNA. *Plant Mol. Biol.*, **3**, 191–199.

22. Wakasugi,T., Nagai,T., Kapoor,M., Sugita,M., Ito,M., Ito,S., Tsudzuki,J., Nakashima,K., Tsudzuki,T., Suzuki,Y. *et al.* (1997) Complete nucleotide sequence of the chloroplast genome from the green alga *Chlorella vulgaris*: the existence of genes possibly involved in chloroplast division. *Proc. Natl Acad. Sci. USA*, **94**, 5967–5972.

23. Golden,S.S. and Stearns,G.W. (1988) Nucleotide sequence and transcript analysis of three photosystem II genes from the cyanobacterium *Synechococcus* sp. PCC7942. *Gene*, **67**, 85–96.

24. Rochaix,J.-D., Dron,M., Rahire,M. and Malnoe,P. (1984) Sequence homology between the 32K dalton and the D2 chloroplast membrane polypeptides of *Chlamydomonas reinhardtii*. *Plant Mol. Biol.*, **3**, 363–370.

25. Yao,W.B., Meng,B.Y., Tanaka,M. and Sugiura,M. (1989) An additional promoter within the protein-coding region of the *psbD-psbC* gene cluster in tobacco chloroplast DNA. *Nucleic Acids Res.*, **17**, 9583–9591.

26. Swiatek,M., Kuras,R., Sokolenko,A., Higgs,D., Olive,J., Cinque,G., Müller,B., Eichacker,L.A., Stern,D.B., Bassi,R. *et al.* (2001) The chloroplast gene *ycf9* encodes a photosystem II (PSII) core subunit, PsbZ, that participates in PSII supramolecular architechture. *Plant Cell*, **13**, 1347–1367.

27. Sexton,T.B., Christopher,D.A. and Mullet,J.E. (1990) Light-induced switch in barley *psbD-psbC* promoter utilization: a novel mechanism regulating chloroplast gene expression. *EMBO J.*, **9**, 4485–4494.

28. Kawaguchi,H., Fukuda,I., Shiina,T. and Toyoshima,Y. (1992) Dynamical behavior of *psb* gene transcripts in greening wheat seedling. I. Time course of accumulation of the *psbA* through *psbN* gene transcripts during light-induced greening. *Plant Mol. Biol.*, **20**, 695–704.

29. Chen,S.-C.G., Lu,J.-H., Cheng,M.-C., Chen,L.-F.O. and Lo,P.-K. (1994) Four promoters in the rice plastid *psbK-psbI-psbD-psbC* operon. *Plant Sci.*, **99**, 171–182.

30. Gamble,P.E. and Mullet,J.E. (1989) Blue light regulates the accumulation of two *psbD-psbC* transcripts in barley chloroplasts. *EMBO J.*, **8**, 2785–2794.

31. Christopher,D.A., Kim,M. and Mullet,J.E. (1992) A novel light-regulated promoter is conserved in cereal and dicot chloroplasts. *Plant Cell*, **4**, 785–798.

32. Tsunoyama,Y., Ishizaki,Y., Morikawa,K., Kobori,M., Nakahira,Y., Takeba,G., Toyoshima,Y. and Shiina,T. (2004) Blue light-induced transcription of plastid-encoded *psbD* gene is mediated by a nuclear-encoded transcription initiation factor, AtSig5. *Proc. Natl Acad. Sci. USA*, **101**, 3304–3309.

33. Nagashima,A., Hanaoka,M., Shikanai,T., Fujiwara,M., Kanamaru,K., Takahashi,H. and Tanaka,K. (2004) The multiple-stress responsive plastid sigma factor, SIG5, directs activation of the *psbD* blue light-responsive promoter (BLRP) in *Arabidopsis thaliana*. *Plant Cell Physiol.*, **45**, 357–368.

34. Kuroda,H., Suzuki,H., Kusumegi,T., Hirose,T., Yukawa,Y. and Sugiura,M. (2007) Translation of *psbC* mRNAs starts from the downstream GUG, not the upstream AUG, and requires the extended Shine-Dalgarno sequence in tobacco chloroplasts. *Plant Cell Physiol.*, **48**, 1374–1378.

35. Rizzo,M.A., Springer,G.H., Granada,B. and Piston,D.W. (2004) An improved cyan fluorescent protein variant useful for FRET. *Nat. Biotechnol.*, **22**, 445–449.

36. Suzuki,H., Kuroda,H., Yukawa,Y. and Sugiura,M. (2011) The downstream *atpE* cistron is efficiently translated via its own *cis*-element in partially overlapping *atpB-atpE* dicistronic mRNAs in chloroplasts. *Nucleic Acids Res.*, **39**, 9405–9412.

37. Yukawa,M., Tsudzuki,T. and Sugiura,M. (2005) The 2005 version of the chloroplast DNA sequence from tobacco (*Nicotiana tabacum*). *Plant Mol. Biol. Rep.*, **23**, 359–365.

38. Yukawa,M., Kuroda,H. and Sugiura,M. (2007) A new *in vitro* translation system for non-radioactive assay from tobacco chloroplasts: effect of pre-mRNA processing on translation *in vitro*. *Plant J.*, **49**, 367–376.

39. Kuroda,H. and Maliga,P. (2001) Sequences downstream of the translation initiation codon are important determinants of translation efficiency in chloroplasts. *Plant Physiol.*, **125**, 430–436.

40. Hirose,T. and Sugiura,M. (2004) Functional Shine-Dalgarno-like sequences for translational initiation of chloroplast mRNAs. *Plant Cell Physiol.*, **45**, 114–117.

41. Jackson,R.J., Kaminski,A. and Pöyry,T.A.A. (2007) Coupled termination-reinitiation events in mRNA translation. In: Mathews,M.B., Sonenberg,N. and Hershey,J.W.B. (eds), *Translational Control in Biology and Medicine*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 197–223.

42. Meierhoff,K., Felder,S., Nakamura,T., Bechtold,N. and Schuster,G. (2003) HCF152, an Arabidopsis RNA binding pentatricopeptide repeat protein involved in the processing of chloroplast *psbB-psbT-psbH-petB-petD* RNAs. *Plant Cell*, **15**, 1480–1495.

43. Sane,A.P., Stein,B. and Westhoff,P. (2005) The nuclear gene *HCF107* encodes a membrane-associated R-TPR (RNA tetratricopeptide repeat)-containing protein involved in expression of the plastidial *psbH* gene in Arabidopsis. *Plant J.*, **42**, 720–730.

44. Schmitz-Linneweber,C., Williams-Carrier,R. and Barkan,A. (2005) RNA immunoprecipitation and microarray analysis show a chloroplast pentatricopeptide repeat protein to be associated with the 5′ region of mRNAs whose translation it activates. *Plant Cell*, **17**, 2791–2804.

45. Johnson,X., Wostrikoff,K., Finazzi,G., Kuras,R., Schwarz,C., Bujaldon,S., Nickelsen,J., Stern,D.B., Wollman,F.-A. and Vallon,O. (2010) MRL1, a conserved pentatricopeptide repeat protein, is required for stabilization of *rbcL* mRNA in *Chlamydomonas* and *Arabidopsis*. *Plant Cell*, **22**, 234–248.

46. Prikryl,J., Rojas,M., Schuster,G. and Barkan,A. (2011) Mechanism of RNA stabilization and translational activation by a pentatricopeptide repeat protein. *Proc. Natl Acad. Sci. USA*, **108**, 415–420.

47. Yukawa,M. and Sugiura,M. (2008) Termination codon-dependent translation of partially overlapping *ndhC-ndhK* transcripts in chloroplasts. *Proc. Natl Acad. Sci. USA*, **105**, 19550–19554.

48. Adhin,M.R. and van Duin,J. (1990) Scanning model for translational reinitiation in eubacteria. *J. Mol. Biol.*, **213**, 811–818.

49. Meurer,J., Berger,A. and Westhoff,P. (1996) A nuclear mutant of Arabidopsis with impaired stability on distinct transcripts of the plastid *psbB*, *psbD/C*, *ndhH*, and *ndhC* operons. *Plant Cell*, **8**, 1193–1207.

50. Gamble,P.E., Sexton,T.B. and Mullet,J.E. (1988) Light-dependent changes in *psbD* and *psbC* transcripts of barley chloroplasts: accumulation of two transcripts maintains *psbD* and *psbC* translation capability in mature chloroplasts. *EMBO J.*, **7**, 1288–1297.