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In vivo analysis of *Chlamydomonas* chloroplast *petD* gene expression using stable transformation of β-glucuronidase translational fusions

(post-transcriptional control/particle gun/foreign gene expression)

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ABSTRACT **We have used the *Escherichia coli* β-glucuronidase (*uidA*) gene as a reporter gene to localize the promoter and analyze the function of the 5′ untranslated region (UTR) of the *Chlamydomonas* chloroplast *petD* gene. Using particle bombardment, *petD–uidA* transcriptional and translational fusion genes were introduced into the chloroplast genome in the large inverted repeat flanking the *atpB* gene. In transformants carrying a *petD–uidA* transcriptional fusion, *uidA* mRNA accumulated but was not translated. However, in a translational fusion that included the entire *petD* 5′ UTR, *uidA* mRNA accumulated and a high level of β-glucuronidase activity was detected. When ≈70% of the *petD* 5′ UTR was deleted from the translational fusion, *uidA* mRNA accumulation and β-glucuronidase activity decreased 4- to 6-fold and 8-fold, respectively. Run-on transcription assays demonstrated that all strains transcribe the *uidA* gene at equivalent rates. Our results show that sequences essential for translation reside in the *petD* 5′ UTR and also that sequences within the 5′ UTR directly or indirectly affect mRNA stability. The expression of β-glucuronidase under the control of chloroplast transcriptional and translational signals will facilitate further studies of chloroplast gene regulatory mechanisms.**

Protein subunits of photosynthetic complexes are encoded in the chloroplast and nuclear genomes, and the expression of these genes is coordinately regulated. The green alga *Chlamydomonas reinhardtii* is an excellent system to study such regulatory pathways. Mutations in nuclear genes that alter the expression of chloroplast-encoded components of photosystem I, photosystem II, ATP synthase, and the photosynthetic electron transport chain have been identified (1–5). These genes presumably encode proteins that regulate the splicing, stability, or translation of specific chloroplast RNAs.

Recently, it has become possible to define cis-acting chloroplast gene regulatory sequences using biolistic transformation (6, 7). Mutations generated *in vitro* are introduced into the chloroplast genome by homologous recombination. Transformed cells can be selected directly or by cotransformation with antibiotic resistance genes as selectable markers (8). However, cotransformation efficiencies can be low, and the resultant strains are heteroplasmic, requiring several cycles of single colony isolation to become homoplasmic (9). An alternative and more rapid method is to use reporter genes fused to DNA sequences that govern transcription, translation, or RNA stability. In higher plants, the *Escherichia coli uidA* gene, which encodes β-glucuronidase (GUS), is widely used as a reporter gene (10). However, no GUS expression has been reported in chloroplast transformation experiments in *Chlamydomonas*, although *uidA* transcription has been used to define putative *atpA*, *atpB*, and *rrn16* promoter elements (11, 12).

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In this paper, we show that *petD–uidA* translational fusions result in GUS expression and thus can be used to study the transcriptional and translational control elements of the *petD* gene, which encodes subunit IV of the cytochrome b_6/f complex. The expression of GUS in stably transformed chloroplasts provides a simple method to analyze elements that regulate chloroplast gene expression.§

MATERIALS AND METHODS

Culture Conditions and Transformation. *C. reinhardtii atpB* deletion mutant strains CC373 (ac-u-c-2-21; ref. 13) and CC373Δ31 (8) and a strain containing wild-type chloroplast DNA (*nit-1*-305; ref. 14) were used in this study. Cell growth and chloroplast transformation were performed as described (8).

Plasmids, DNA Sequencing, and DNA Amplification. The 11.6-kilobase (kb) chloroplast *Bam* 7 DNA fragment, which contains the *petD* gene (15), was isolated from a *Bam*HI library in the Bluescript vector, using a *Chlamydomonas eugametos petD* probe (16). The DNA sequence of the *petD* upstream region was determined by the dideoxy chain termination method, using a series of exonuclease III-deleted clones derived from a 2.3-kb *Eco*RV–*Hin*dIII subclone of the *Bam* 7 fragment.

Specific fragments of the *petD* 5′ region were amplified by PCR using primer WS4 (*Sac* I–*Xba* I-GGAATCTCCTATTTTGTAGGATG), which anneals to nucleotides (nt) +985 to +1007 relative to the mRNA 5′ end, and other primers as shown in Fig. 1*B*. To create pDG1, a 659-base-pair (bp) fragment containing the sequence from −635 to +25 relative to the *petD* mRNA 5′ end was amplified from pRV4 (a clone that is deleted 1.2 kb from the *Eco*RV site), using a T3 promoter primer and WS2 (Fig. 1*B*). This fragment was cloned into *Bgl* II/*Xba* I-digested pIC20R (17), and the resultant plasmid was designated pCD12. pCrc34, which contains the *uidA* gene flanked by the *atpB* promoter and the *rbcL* 3′ untranslated region (UTR) (11), was digested with *Xho* I and *Sma* I to remove the *atpB* promoter. A blunt-ended *petD* upstream fragment obtained by *Eco*RI digestion of pCD12 and subsequent repair by the Klenow fragment was inserted into this site, yielding pDG1.

pCD34 and pCD84 were created by cloning fragments amplified from pRV4 using primers WS3 and WS4 or WS8 and WS4, respectively, into pIC20R. The *petD* upstream region was released from pCD12 with *Bgl* II and *Sma* I, and inserted into *Bgl* II/*Eco*RV-digested pCD34 and pCD84, creating plasmids pCD1234 and pCD1284, respectively. To construct translational fusions to *uidA*, the *petD* upstream region and 5′ UTR were amplified with primers WS9 and WS11 using either

Abbreviations: UTR, untranslated region; GUS, β-glucuronidase; MUG, 4-methylumbelliferyl glucuronide; nt, nucleotide(s).

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L05506).

pCD1234 or pCD1284 as templates, digested with *Xho* I and *Sma* I, and then inserted into *Xho* I/*Sma* I-digested pCrc34. The resultant plasmids were designated pDG2 and pDG3, respectively. These translational fusions extend the N terminus of GUS by nine amino acids (MSVPGGNSL, derived from the *petD* coding region and the polylinker).

Analysis of Transformants. Isolation of total DNA and RNA, agarose gel electrophoresis, and filter hybridizations were carried out as described (18). A 1.9-kb fragment containing the entire GUS coding region was used as a *uidA* probe, and a 1.6-kb *Eco*RI–*Pst* I fragment containing the 5′ half of the *atpB* coding region was used as an *atpB* probe. Hybridizing bands were quantified using a Phosphor Imager (Molecular Dynamics). Permeabilized cells for run-on transcription were prepared by the freeze/thaw method as described by Gagné and Guertin (19). Of the pelleted permeabilized cells (5×10^7 cells), 30 μl was mixed with 30 μl of 2× transcription buffer [1 M sucrose/60 mM $MgCl_2$/50 mM Hepes, pH 7.5/15 mM dithiothreitol/50 mM NaF/0.25 mM GTP/0.5 mM ATP/0.25 mM CTP/250 μCi of [^{32}P]UTP (1 Ci = 37 GBq)/80 units of RNasin]. The mixture was incubated at 0°C for 1 min and then at 26°C for 15 min. The reactions were terminated by the addition of 10 μl of 20% SDS and 130 μl of H_2O, and nucleic acids were extracted twice with phenol/chloroform and precipitated with ethanol. The pellet was washed once with 70% ethanol, dissolved in 100 μl of H_2O, and used in filter hybridizations.

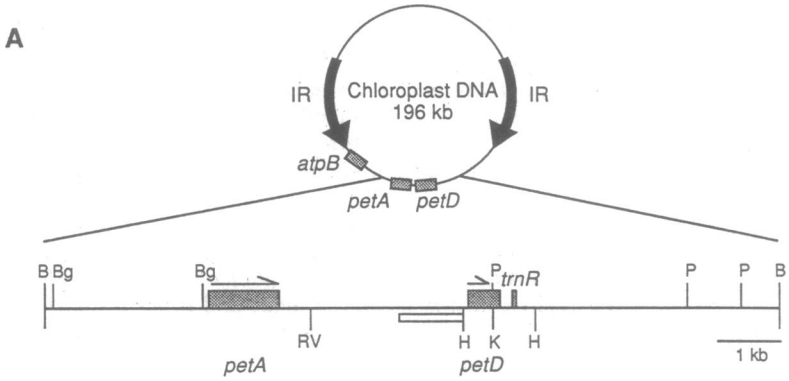
Primer Extension and S1 Nuclease Protection. To map the *petD* mRNA 5′ end, primer WS5 was extended using total RNA and reverse transcriptase. To map the 3′ terminus of *petD* mRNA, primer WS4 was annealed to a plasmid containing the 1.1-kb *Hin*dIII fragment and extended with the Klenow fragment in the presence of [α-^{32}P]dCTP. The labeled DNA was digested with *Kpn* I, and the WS4-*Kpn* I fragment was recovered from a 5% polyacrylamide gel. This probe was hybridized with total RNA at 30°C for 16 hr and the hybrids were treated with S1 nuclease as described (20).

GUS Assays. Histochemical staining of GUS activity was carried out according to Jefferson *et al.* (21). Cells were incubated with the staining solution for 1–2 days and then decolorized by washing with 70% ethanol for 1 day or longer. Decolorized cells were viewed using a bright light microscope, without further treatment. The fluorescence assay using 4-methylumbelliferyl glucuronide (MUG) was performed as described by Jefferson *et al.* (21).

RESULTS

Mapping of *petD* mRNA. The *Chlamydomonas petD* gene has been previously localized to the 11.6-kb *Bam* 7 fragment (15). The physical map in Fig. 1*A* shows that *petA*, which encodes cytochrome *f*, is located upstream of *petD* (15) and that a *trnR* gene lies just downstream of *petD* (22). Hybridization of *Bam* 7 subclones with filter blots of total RNA identified three major transcripts from this region: 1.3 kb for *petA*, 0.9 kb for *petD*, and <0.1 kb for *trnR* (data not shown).

To map the ends of *petD* mRNA precisely, the upstream DNA sequence was determined and primer extension was carried out using oligonucleotide WS5 (Fig. 1*B*). As shown in Fig. 2*A*, we detected one major product whose intensity was



B

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ATAAATATTAGTGGCAGTTGCCTGCCAACTGCTCCTTCGGAGTATTAAAATAGGATATTT  -575
ATATACTGCGAACGGCAGTGGCGGTACCACTGCCTGCTTCGGTTATATAAATATCCACTA  -515
ATATTTATATTCCGTAAGGACGTCCTAATATAAATATTGGGCAACTAAAGTTCGGGCAGT  -455
ATATAAATATCGGAGTTGGCGGGCAACAAATTTATTTATTGTATATAAATATCGGCAGTT  -395
GGCAGGCAACAAATTTATTTATTGTATATAAATATAGGATGTTAATACTCCGAAGAGCAG  -335
TTCGCAGGCAACTCACTAAAATTCATTTGCCCGAAGGACGTCCACTAATATTTATATTAG  -275
GACAAATTAATTTATTGTGGTACCGCCACTGCCTATTTTAATACTCCGAAGAGCAGTGGC  -215
                                    X   WS9
GGTACCACAATAAATTAATTTGTCCTCCTTCCCCTTCGGCAAGTAAACTTAGGAGTATGT  -155
AAACATTCTATTTTAATACAATAAATAAATTTGTTGGCAGGCAACTGCCACTGACGTCCC   -95
GTCAGGGGAAGGGGAAGGGGACGTCCTAATATAAATATATTTTAAACAATAAATCCCGCA   -35
                                  +1  Transcription
CAAAATATATAAATATATAATATATATTAAAAATTTTTAGCATGTAAACATTAGAAATAC   +26
                                                 WS2      BgBg
AGCATAATTGGAGTAAAAGAAAAATATTAAACTTTTACATTGAAAAGTTTATGGCGTTTT   +86
      WS3                                          WS5
GGCTTTATAAAATAAAAAACTTTTCGGAACGGCTAAACCATATTTATTATCATTAAAATT  +146
  Bg
TATTTGCCCGAAGGGGACGTATCCGAAATAGAACAAATGCCAAAATCTACTAAATTAGAT  +206

TAAAATAGTTTTAAAAATGGATAGATTTAAATAAAAAACAGAAGTAAAATGTAATTCTGT  +266
                                                      HindIII
CCCTTTTTACAGGGTGGTATCTCTAAAAACCAGGGCTTGCCCAATCAACAATTTAAAGCT  +326
    Bg      WS8                          Translation
TATTTAGTTTTATTGAAAATTAACGGATAAATAAATATGTCAGTTACTAAAAAACCTGAT  +386
                          WS11          S
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FIG. 1. (*A*) Restriction map of the *Bam* 7 fragment. The chloroplast genome and the locations of *petA*, *petD*, and *atpB* are shown above the map. The open box indicates the position of the sequence determined in this study. Selected restriction enzyme recognition sites are as follows: B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; K, *Kpn* I; P, *Pst* I; RV, *Eco*RV. (*B*) DNA sequence of the *petD* upstream region. The mRNA 5′ end (+1) and translation initiation codon (+363) are indicated. The locations of primers used in this study are indicated; restriction enzyme recognition sites included at the 5′ ends of primers are shown.

proportional to the amount of total RNA added. Based on this analysis, we conclude that *petD* mRNA has a 362-nt-long 5′ UTR (Fig. 1*B*). We determined the 3′ terminus of *petD* mRNA by S1 nuclease protection. As shown in Fig. 2*B*, a single protected fragment of 220 nt was detected, indicating that *petD* mRNA has a 3′ UTR of ≈70 nt. Our 5′ and 3′ end mapping data predict an RNA of 909 nt, consistent with the size observed in RNA filter hybridizations. To study the putative promoter region and the 5′ UTR, reporter genes with these elements were constructed and tested *in vivo* using chloroplast transformation.

A Promoter Is Located Immediately Upstream of the *petD* mRNA End. Higher plant chloroplast promoters often contain prokaryotic-like −35 (TTGACA) and −10 (TATAAT) elements. A −10 like element, TATATT, is found 12 bp upstream of the *petD* 5′ end mRNA (Fig. 1*B*). However, the high (A+T) content of noncoding *Chlamydomonas* chloroplast DNA precludes identification of promoters by inspection of sequences. Indeed, since *petD* is linked to *petA*, and since chloroplast genes from higher plants are often arranged in operons, the question arises as to whether *petD* is transcribed from its own promoter in *Chlamydomonas* or, alternatively, if *petD* mRNA is generated by processing of a primary *petA–petD* cotranscript.

To determine whether a promoter lies immediately upstream of the 5′ terminus of *petD* mRNA, we fused a 659-nt fragment from the *petD* 5′ region (−634 to +25) to the *uidA* gene, flanked by the *rbcL* 3′ region. This chimeric gene was inserted in the large inverted repeat, downstream of *atpB* and in the opposite orientation, as shown in Fig. 3*A*, and the resultant plasmid was designated pDG1. pDG1 was used to transform the nonphotosynthetic recipient strains CC373 and CC373Δ31, which lack functional *atpB* genes (Fig. 3*A*). Phototrophic transformants were selected on HS plates and screened for the presence of *uidA* sequences by colony hybridization (23). Genomic DNA was isolated from *uidA*-positive transformants and analyzed by DNA filter hybridizations. When DNA was digested with *Bam*HI and probed with *uidA*, a single 2.6-kb fragment, representing the *petD* upstream region fused to *uidA*, was detected in each transformant (Fig. 3*B Left*). When the filter was reprobed with *atpB*, the 5.2-kb fragment from the recipient strain was replaced by a fragment of 5.6 kb, the expected size if the *uidA* cassette was inserted downstream.

In addition to the expected 5.6-kb fragment, however, we detected a faint 7.6-kb *Bam*HI fragment corresponding to a wild-type *atpB* region in some transformants (Fig. 3*B Left*,

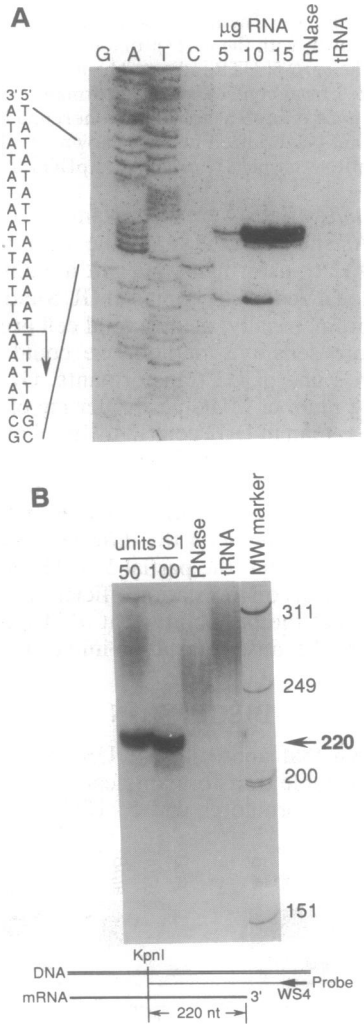


FIG. 2. Mapping of *petD* mRNA. (*A*) The 5′ end was mapped by primer extension and analysis in a 5% sequencing gel. The sequence surrounding the mRNA 5′ end is indicated to the left. Control lanes include pretreatment of total RNA with RNase (RNase) and annealing with tRNA instead of *Chlamydomonas* RNA (tRNA). (*B*) Mapping of the 3′ end. A uniformly labeled 480-nt single-stranded probe initiated by WS4 (below) was annealed to total RNA, digested with 50 or 100 units of S1 nuclease, and analyzed in a 5% sequencing gel. The 220-nt fragment protected from S1 digestion is shown schematically at the bottom. Control lanes are as described for *A*. MW, molecular weight.

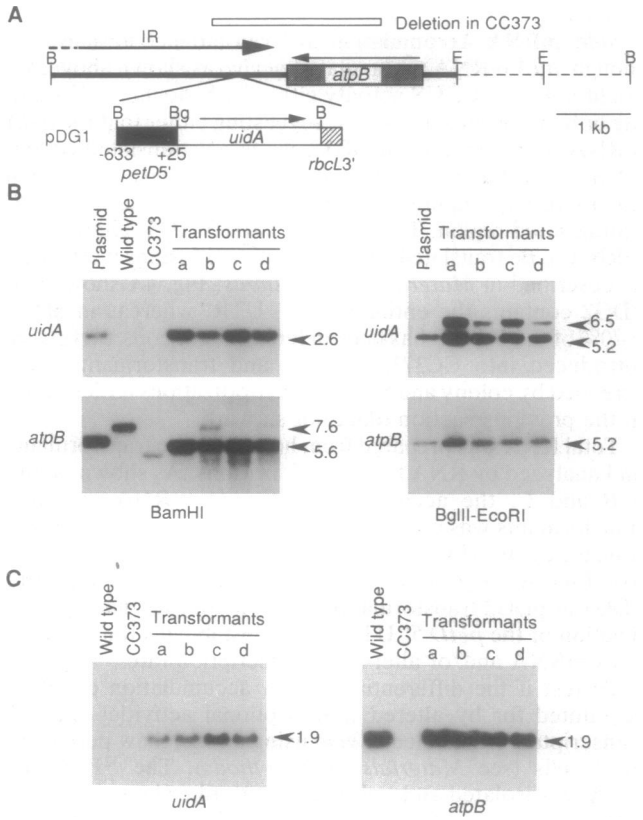


FIG. 3. Characterization of pDG1 transformants. (*A*) Restriction map of the chloroplast genome *atpB* region, with the pDG1 insert shown as a thick line, and restriction enzyme recognition sites as in Fig. 2. The region deleted in the recipient strain CC373 is indicated by an open box. (*B*) DNA filter hybridizations. Genomic DNA was isolated from wild type, CC373, and pDG1 transformants, digested with the enzymes indicated below the panels, separated in 0.7% agarose gels, blotted to nylon membranes, and hybridized with either *uidA* (top) or *atpB* (bottom) probes. (*C*) RNA filter hybridizations. Total RNA from wild type, CC373, and transformants was separated in 1.2% agarose/formaldehyde gels, blotted to nylon membranes, and hybridized with either *uidA* (left) or *atpB* (right) probes.

lanes b and d). This result cannot be explained by residual untransformed molecules, since the recipient strain does not possess a wild-type *atpB* gene (Fig. 3*A*). Instead, it appears that the *uidA* cassette has been deleted from some copies of the genome by recombination with an untransformed copy of the large inverted repeat (IR). Thus, following integration of *atpB* and *petD–uidA* by homologous recombination, intra- and/or intermolecular recombination between IRs has created heteroplasmic cells with a wild-type *atpB* region in some copies of the genome. In stable homoplasmic transformants, the *uidA* gene is duplicated within the other IR. When DNA is digested with *Bgl* II and *Eco*RI and probed with the *uidA* coding region, two hybridizing fragments are seen in each transformant: a 5.2-kb fragment, which corresponds to the *atpB* flanking region, and a 6.5-kb fragment, derived from the other IR (Fig. 3). The hybridization signals of the 6.5- and 5.2-kb fragments were equivalent in transformants a and c, suggesting that these transformants, which also lack a wild-type 7.6-kb *Bam*HI fragment, are homoplasmic.

To test if the region upstream of the *petD* mRNA 5′ end promotes transcription of *uidA in vivo*, total RNA was prepared from transformants. RNA filter hybridizations using the *uidA* probe identified a single band of ≈1.9 kb, as shown in Fig. 3*C*. Primer extension analysis using a *uidA* primer and total RNA from these transformants showed that the 5′ end of *uidA* mRNA was at the same position as for endogenous *petD* mRNA (data not shown). This demonstrates that the 5′ upstream region of *petD* confers promoter activity.

***uidA* mRNA Accumulation in Translational Fusions.** Although *uidA* mRNA accumulated *in vivo* as shown above, we could not detect GUS activity either by histochemical staining or by fluorometric assays, suggesting either that the *uidA* mRNA transcribed from DG1 cells is not translated or that GUS is unstable in chloroplasts. Chloroplast translation initiation may require a sequence element in the mRNA 5′ untranslated region. To test if the *petD* 5′ UTR allows *uidA* mRNA to be translated, two translational fusions were made as described in *Materials and Methods*. Fig. 4*A* shows that pDG2 contains the entire *petD* 5′ UTR, whereas in pDG3 ≈70% of the 5′ UTR has been deleted. These constructs were introduced into CC373Δ31 cells, and transformants were screened by colony and Southern hybridizations as described in the previous section (data not shown).

Total RNA was prepared from homoplasmic transformants and analyzed by RNA filter hybridizations. As shown in Fig. 4 *B* and *C*, the accumulation of *uidA* mRNA in pDG2 transformants was comparable to that in homoplasmic pDG1 transformants. However, *uidA* mRNA accumulation was 4- to 6-fold lower in pDG3 transformants when compared to pDG1 or pDG2 transformants. Thus, it appears that a partial deletion of the *petD* 5′ UTR either destabilizes the chimeric *uidA* mRNA and/or alters the transcription rate.

To test if the differential mRNA accumulation could be accounted for by altered transcriptional activities, run-off transcripts were labeled *in vivo* using freeze/thaw permeabilized cells (see *Materials and Methods*). The ^{32}P-labeled RNA was isolated and hybridized with filter-bound plasmid DNA as shown in Fig. 5, and the relative signals for *petD*, *uidA*, and *atpB* were quantified. Because the relative *uidA*/*atpB* transcription activities were similar in each case, we conclude that the partial 5′ UTR deletion in pDG3 transformants does not significantly alter the *uidA* transcription rate, and therefore that the difference in *uidA* mRNA accumulation is probably caused by differential mRNA stabilities.

A 5′ UTR Is Required for *uidA* Translation. We tested each of the transformants for GUS activity by histochemical staining and fluorescent assays. As shown in Fig. 6*A*, we observed intense blue staining with X-glucuronide only in pDG2 transformants, which contain the entire *petD* 5′ UTR

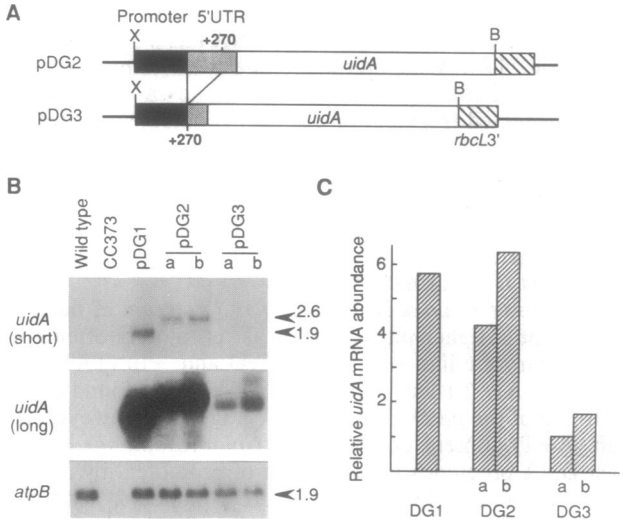
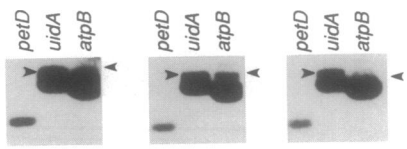


FIG. 4. Differential accumulation of *uidA* mRNA in pDG2 and pDG3 transformants. (*A*) The translational fusion constructs pDG2 and pDG3 are shown, with the *petD* promoter region indicated by a black box, and the *petD* 5′ UTR indicated by a shaded box. (*B*) Total RNA was isolated from control and transformant cells and hybridized with either *uidA* or *atpB* as for Fig. 3. Short and long exposures of the resulting *uidA*-hybridized blot are shown. (*C*) Relative abundance of *uidA* mRNA in pDG1, pDG2, and pDG3 transformants.

in frame with *uidA*. Cells were also examined by bright field microscopy. As shown in Fig. 6*B*, blue staining can clearly be seen in pDG2 transformants but not in control cells and appears to be localized to chloroplasts. GUS activity was also quantified fluorometrically, using a total cell protein extract. Using this more sensitive method, we could detect GUS activity in pDG2 and pDG3 transformants. GUS activity in DG3 cells (4.38 nmol of MUG per hr per mg of protein) was about 8-fold less than in DG2 cells (34.4 nmol of MUG per hr per mg of protein) but significantly higher than control wild-type or DG1 cells (<0.4 nmol of MUG per hr per mg of protein). The 8-fold difference in GUS activity between DG2 and DG3 cells correlates with the difference in RNA accumulation, suggesting that the partial 5′ UTR in pDG3 probably has a small effect on translation efficiency. However, we have not ruled out the possibility that *uidA* mRNA in DG3 cells is less stable because translation initiation is inefficient.

DISCUSSION

We have shown that appreciable GUS enzymatic activity accumulates in stable transformants carrying the *uidA* gene fused to the *petD* promoter and 5′ UTR. Transcriptional



	DG1	DG2	DG3
petD/*atpB*	0.26	0.29	0.27
uidA/*atpB*	0.42	0.57	0.39
uidA/*petD*	1.62	1.97	1.44

FIG. 5. Transcriptional activity in transformed cells. RNA was pulse-labeled in freeze/thaw-treated cells and hybridized with a DNA blot of gel-purified *petD*, *uidA*, and *atpB* restriction fragments. The ratios of hybridization signals are indicated at the bottom. The weak bands indicated by arrowheads represent cross-contamination of the *atpB* and *uidA* fragments, recovered from digests of pCrc34 that contains both genes.

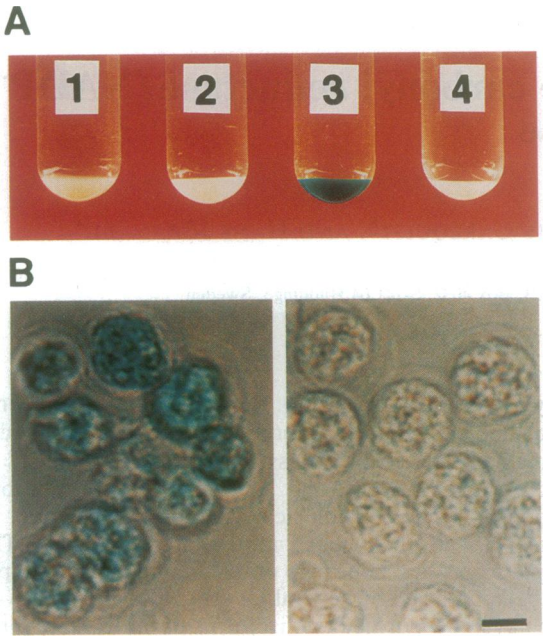


FIG. 6. Histochemical staining of transformants for GUS activity. (*A*) Histochemically stained and decolorized cells from *nit* 1-305 (1) (wild-type chloroplast genome; equivalent results were obtained with CC373 cells), DG1 (2), DG2 (3), and DG3 (4) are shown. (*B*) Samples of the cells shown in *A* for DG2 (*Left*) and DG1 (*Right*) were examined by bright light microscopy. (Bar = 12 μm.)

fusions to the *uidA* gene have previously been used to define transcriptional control regions in *Chlamydomonas* chloroplasts (11, 12). Expression of active GUS enzyme will allow the identification of sequence elements required for translational regulation of chloroplast genes, as shown here for the *petD* gene. The *E. coli aadA* gene, which encodes aminoglycoside adenyltransferase, has also been fused to chloroplast DNA sequences and can be used as a selective marker in chloroplast transformation (24, 25).

Although transformants carrying *petD–uidA* fusions accumulated similar amounts of *uidA* mRNA when either none or all of the *petD* 5′ UTR was present, we observed a 4- to 6-fold reduction in mRNA when the *uidA* transcript was preceded by a truncated 5′ UTR. Since the decline in mRNA was not due to reduced transcription, the results suggested three possibilities. First, the deletion in pDG3 vs. pDG2 might have removed an element required for mRNA stability. Kuchka *et al.* (1) suggest that the target site for the nuclear factor affecting the stability of *psbD* mRNA is a stem/loop structure found in the *psbD* 5′ UTR. In yeast mitochondria, a nuclear factor, CBP1, has been shown to interact with a potential stem/loop in the 5′ UTR of *cob* mRNA and stabilize the transcript (26). However, this first explanation cannot account for the high level of *uidA* mRNA in pDG1 transformants, which lack a 5′ UTR. A second possibility is that the deletion in pDG3 altered an RNA secondary structure in the 5′ UTR, thus creating a destabilizing element. Neither pDG1 nor pDG2 would contain this element. A third possibility to explain instability of pDG3 mRNA is that the translation of *uidA* mRNA is inefficient, and therefore the RNA is rapidly degraded at some time during or after the formation of a preinitiation complex. In spinach chloroplasts, the association of mRNA with polysomes has been shown to decrease the stabilities of mRNAs (27). The interaction of mRNA with polysomes also decreases the stability of animal histone and tubulin mRNAs (28, 29). Our data are consistent with a model in which decreased mRNA stability is correlated with ribosome association. However, we cannot rule out that pDG1 RNA associates with polysomes but generates nonfunctional translation products from short open reading frames upstream of *uidA*.

Taken together, our results from translational fusions suggest that the region from +26 to +272 in the *petD* 5′ UTR plays a role in RNA stability and/or translation initiation efficiency and that the region from +273 to +362 contains sufficient information for the formation of a translation initiation complex. Experiments to identify cis elements required for efficient translation initiation can now be undertaken in *Chlamydomonas* chloroplasts using quantitative GUS activity measurements in transformed cells.

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