Plant Molecular Biology **36:** 307–314, 1998.

The sequence and structure of the 3′-untranslated regions of chloroplast transcripts are important determinants of mRNA accumulation and stability

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Received 27 February 1997; accepted in revised form 15 September 1997

Key words: atpB, Chlamydomonas reinhardtii, chloroplast, inverted repeat, 3′-untranslated region

Abstract

A general characteristic of the 3′-untranslated regions (3′ UTRs) of plastid mRNAs is an inverted repeat (IR) sequence that can fold into a stem-loop structure. These stem-loops are RNA 3′-end processing signals and determinants of mRNA stability, not transcription terminators. Incubation of synthetic RNAs corresponding to the 3′ UTRs of *Chlamydomonas* chloroplast genes *atpB* and *petD* with a chloroplast protein extract resulted in the accumulation of stable processing products. Synthetic RNAs of the *petA* 3′ UTR and the antisense strand of *atpB* 3′ UTR were degraded in the extract. To examine 3′ UTR function *in vivo*, the *atpB* 3′ UTR was replaced with the 3′ UTR sequences of the *Chlamydomonas* chloroplast genes *petD*, *petD* plus *trnR*, *rbcL*, *petA* and *E. coli thrA* by biolistic transformation of *Chlamydomonas* chloroplasts. Each 3′ UTR was inserted in both the sense and antisense orientations. The accumulation of both total *atpB* mRNA and ATPase β-subunit protein in all transformants was increased compared to a strain in which the *atpB* 3′ UTR had been deleted. However, the level of discrete *atpB* transcripts in transformants containing the antisense 3′ UTR sequences was reduced to approximately one-half that of transformants containing the 3′ UTRs in the sense orientation. These results imply that both the nucleotide sequences and the stem-loop structures of the 3′ UTRs are important for transcript 3′-end processing, and for accumulation of the mature mRNAs.

Introduction

Chloroplast genes are often organized into clusters which are transcribed into precursor transcripts that undergo complex processing events, including splicing and intercistronic cleavages. Most plastid mRNAs contain inverted repeat (IR) sequences in their 3′-untranslated regions (UTRs), which are believed to fold into stem-loop structures. These IR sequences do not function as transcription terminators, but are necessary for maintaining message stability and correct 3′-end processing [7, 13, 19, 22, 23]. Plastid mRNA 3′ end processing has been modeled *in vitro* using chloroplast protein extracts and synthetic RNAs. Results from these experiments suggest that like some bacterial mRNAs [12], precursor mRNAs containing stem-loops with 3′ extensions can be processed efficiently by a 3′→5′ exonuclease activity [19, 20]. However, several lines of evidence indicate that in chloroplasts, the exonuclease(s) works in concert with RNA-binding proteins (RBPs) [3, 6, 15, 21, 26]. These RBPs may direct the exonuclease(s) to precursor 3′ ends, and/or stabilize the stem-loop to prevent 'breathing' and subsequent digestion of the body of the transcript. There is also evidence for another 3′-end processing pathway in which a specific endonuclease cleaves the primary transcript downstream of the stem-loop, and a 3′→5′ exonuclease trims the processed intermediate to create the mature 3′ end of the mRNA [6, 22].

The ability to introduce modified genes into the chloroplast of the green alga *Chlamydomonas reinhardtii* provides the opportunity to analyze the function of 3′ IR sequences *in vivo*. When the 3′ IR of the *Chlamydomonas* chloroplast *atpB* gene was deleted,

no discrete *atpB* mRNA accumulated [23]. The *atpB*-containing transcripts of this strain were heterogeneous in size, accumulated to reduced levels, and resulted in decreased protein accumulation and weak phototrophic growth. The spinach *petD* 3′ UTR or a polyguanosine tract can substitute for the *atpB* 3′ UTR *in vivo*, apparently by forming structures that impede 3′→5′ exonuclease digestion [4, 23]. It has also been shown that the nucleotide sequence of the *rbcL* 3′ UTR is important for the accumulation of a correctly 3′-end-processed chimeric reporter gene transcript [2].

We have recently tested the abilities of *Chlamydomonas* chloroplast 3′ UTRs to function as transcription termination signals *in vivo*. The UTRs were inserted between a promoter and a reporter gene, and the rate of transcription of the reporter gene was determined. None of the 3′ UTRs tested were able to efficiently terminate transcription [13], providing additional evidence that the 3′ ends of chloroplast transcripts are formed by RNA processing events. In this work we use *in vitro* processing and chloroplast transformation to analyze the function of 3′ UTRs in mRNA 3′-end formation. The 3′ UTR of the *atpB* gene was replaced by several 3′ UTRs to generate a chimeric *atpB* transcripts, and the level of accumulation of total transcripts and discrete transcripts with processed 3′ ends was determined.

Materials and methods

Plasmids

Construction of the plasmids pB17BS (*atpB* 'wild-type') and pΔ26 have been described [23]. The plasmid pΔ26 contains a deletion immediately downstream of the *atpB* coding region, extending from position 1490 (the stop codon is at position 1474, numbering as in Woessner *et al.* [25]) to position 3807, and the deleted sequences are replaced by a 7 bp linker that includes a *Bgl*II site (Figure 2B). The 3′ UTR sequences of *petD*, *petD-trnR*, *petA*, *rbcL*, and the *Escherichia coli thrA* genes were previously described [13]. The 3′ UTR sequences were inserted into the *Bgl*II site of pΔ26 in both the sense and antisense orientations.

Chloroplast protein extract and in vitro *processing*

A *Chlamydomonas* chloroplast protein extract was prepared from cell wall-deficient CC406 cells as described by Stern and Kindle [22]. Uniformly ^{32}P-UTP-labeled RNAs were transcribed from linearized pBluescript plasmids as described by Lisitsky *et al.* [8]. The full-length labeled transcripts were gel-purified and processed in a chloroplast protein extract at a ratio of 2 fmol of RNA to 10 μg of protein at 25 °C [22].

Chloroplast transformation

The nonphotosynthetic *Chlamydomonas* strain CC373 [17] was transformed to photosynthetic competence using the DuPont particle delivery system with 2 μg of the chimeric *atpB* plasmids as described by Stern *et al.* [23]. Transformants were selected on high-salt minimal medium and subcloned to generate homoplasmic strains.

Isolation of nucleic acids, filter hybridization, and PCR

RNA and DNA were isolated from cell cultures grown in 50 ml acetate-containing medium (HSA) as described by Drager *et al.* [4]. For RNA filter hybridizations, 15 μg total RNA was fractionated in 0.8% agarose, 2.2 M formaldehyde gels, transferred onto Amersham Hybond-N nylon membranes and cross-linked by UV irradiation. Prehybridization and hybridization were conducted in 50% formamide, 5× SSC, 10× Denhardt's solution, 0.1% SDS and 0.1 mg/ml salmon sperm DNA at 42 °C. The blots were washed in 0.1× SSC, 0.1% SDS at 65 °C. RNA slot blots were prepared as described by Stern *et al.* [23], and hybridized as described above. Hybridization probes were generated by random priming from double-stranded DNA fragments in the presence of α^{32}[P]ATP [5]. The *Bgl*II/*Eco*RI fragment of pΔ26, and a 5.8 kb *Eco*RI fragment containing the *psbA* gene were used to generate *atpB* and *psbA* probes, respectively [23]. Hybridization signals were quantified using the Fuji Imaging Analyzer. Quantitative data represent the average of five independent experiments in which the hybridization signal of the *atpB* probe was normalized to that of the *psbA* probe of the same blot.

Protein isolation and immunoblots

Total proteins were prepared from the same cell cultures from which RNA and DNA were isolated. Cells were collected by centrifugation and resuspended in SDS denaturing sample buffer. Proteins were fractionated by SDS-PAGE (12% polyacrylamide), transferred

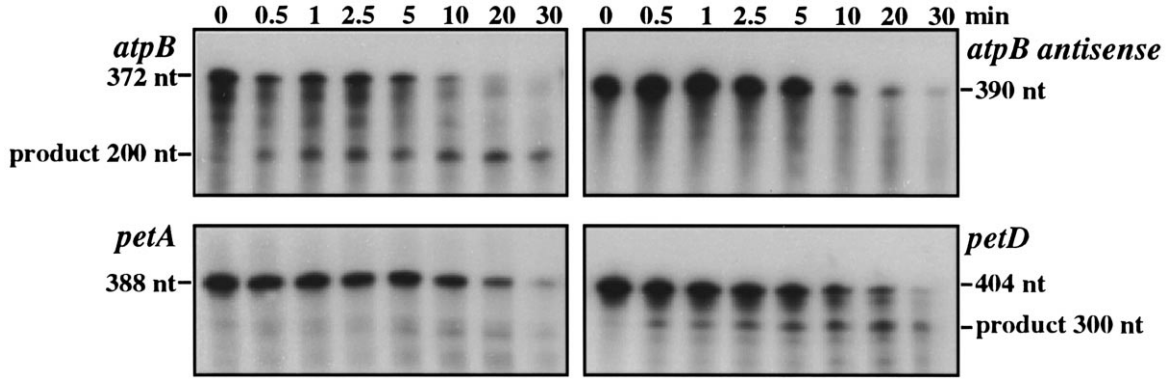

Figure 1. *In vitro* processing of 3′ UTRs in a chloroplast protein extract. ^{32}P-labeled RNAs synthesized from the cloned 3′ UTRs of the *Chlamydomonas* chloroplast genes *atpB*, *petA*, *petD* and the antisense strand of *atpB* as indicated, were incubated in a *Chalmydomonas* chloroplast soluble protein extract. Aliquots were removed at the indicated times and analyzed by denaturing polyacrylamide gel electrophoresis. The sizes of the RNA substrates and products, based on markers of known sizes, are shown.

to nitrocellulose membranes and reacted with antisera directed against the chloroplast ATPase β-subunit [13, 14] and the D1 protein of photosystem II [16] as described in Stern *et al.* [23]. Antigenic proteins were visualized using chemiluminescent detection and quantified by densitometric analysis as described in Lisitsky *et al.* [9].

Results

In vitro *processing of chloroplast mRNA 3′ UTRs*

One model of 3′-end processing of chloroplast mRNAs is endonucleolytic cleavage followed by 3′→5′ exonuclease trimming of the processed mRNA to generate the mature 3′ end, that is located at or several nucleotides downstream of the stem-loop structure [6, 22]. This mechanism implies that the stem-loop structure, in concert with protein factors, impedes exonuclease digestion, and is required for the formation of the mature 3′ end. To determine whether 3′ UTR sequences can impede exonuclease activity and direct 3′-end formation *in vitro*, synthetic ^{32}P-labeled RNAs corresponding to the 3′ UTRs of *atpB*, *petD* and *petA* were incubated in a chloroplast soluble protein extract. The *atpB* and *petD* 3′ UTRs contain IR sequences that potentially can form stable stem-loop structures with the free energies of −88 and −107 kJ/mol, respectively [13, 23]. The *petA* 3′ UTR was chosen to represent genes that accumulate discrete mRNAs *in vivo* without apparent IR sequences [13]. The *atpB* 3′ UTR was analyzed previously in this system, and products accumulated with 3′ ends coincident with the 3′ ends of the mature mRNA [22]. In this work, we analyzed the synthetic RNA transcribed from the antisense strand of the *atpB* 3′ UTR to determine whether it is the stem-loop structure, or the structure plus the correct nucleotide sequence, that is required for 3′-end formation *in vitro*. As shown in Figure 1, when the *atpB* and *petD* sense strand 3′ UTRs were processed, products of 200 and 300 nt accumulated, respectively. RNase protection analysis of the *petD* processing product determined that the *in vitro* processing product corresponded to the 3′ end of mature *petD* mRNA (data not shown). No stable product accumulated when the *petA* 3′ UTR or the *atpB* antisense 3′ UTR RNAs were incubated in the chloroplast extract. Similar results were obtained for the *rbcL* 3′ UTR; the sense, but not the antisense transcripts were processed to stable products (data not shown). These results demonstrate that both the stem-loop structure and the correct nucleotide sequence are required for correct 3′-end processing of chloroplast 3′ UTRs in this *in vitro* system.

Testing of modified atpB *genes* in vivo

To compare the ability of the sense and antisense 3′ UTR sequences to direct 3′-end formation *in vivo*, chloroplast transformants were constructed containing the experimental 3′ UTR sequences in place of the *atpB* 3′ UTR. The recipient strain CC373 harbors a deletion of the *atpB*-coding region and 3′ UTR and is nonphotosynthetic. Photosynthetic colonies are expected to be recovered from transformation with the modified *atpB* genes even if the introduced sequences do not permit

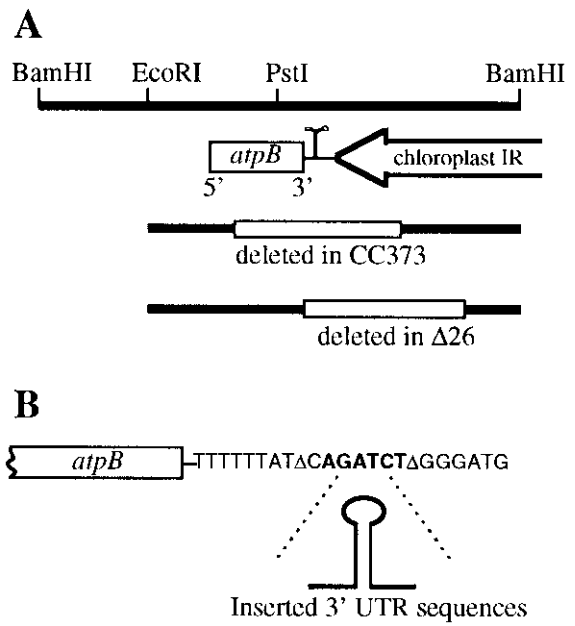

Figure 2. Strategy for testing 3′ UTR function. **A.** Map of the 7.6 kb *Bam*HI fragment of the *Chlamydomonas* chloroplast genome. A portion of the large inverted repeat of the chloroplast genome is shown as an open arrow. The inverted repeat of the *atpB* 3′ UTR is shown as a stem-loop structure. The extents of the deletions in the chloroplast genomes of strains CC373 and Δ26 are indicated. **B.** Detailed view of the sequence of the *atpB* 3′ UTR in Δ26. The *Bgl*II site, into which the experimental 3′ UTRs were inserted, is shown in bold-face type. The endpoints of the deletion in Δ26 are indicated by triangles.

the formation of discrete transcripts, because plasmids that lack the IR (e.g., pΔ26) are able to transform CC373 to photosynthetic competence [23].

Maps of the *atpB* regions of the wild-type and deleted chloroplast genomes are shown in Figure 2, and plasmids and strains are described in detail in Materials and methods. As shown in Figure 2B, a *Bgl*II linker has replaced the *atpB* 3′ IR in plasmid pΔ26. The experimental 3′ UTR sequences were inserted into the *Bgl*II site. These constructs are expected to produce chimeric transcripts *in vivo* containing either the sense or antisense 3′ UTRs of *petA*, *petD*, *rbcL*, *petD* plus the downstream *trnR* gene, or the sense strand of *thrA* (Table 1). The *E. coli thrA* terminator was used as a non-chloroplast stem-loop structure. Biolistic transformation of CC373 cells with each plasmid resulted in photosynthetic colonies. After several rounds of subcloning, DNA filter hybridization and PCR analyses confirmed the identity and homoplasmicity of the transformants (data not shown). These strains were subsequently used for molecular analysis.

Table 1. **Chlamydomonas chloroplast *atp*B transformants.**

Transformant	3′ UTR (orientation)	Length of insert (bp)
Δ26	none (N/A)	none
D+	*petD* (sense)	320
D−	*petD* (antisense)	320
Dt+	*petD-trnR* (sense)	538
Dt−	*petD-trnR* (antisense)	538
A+	*petA* (sense)	254
A−	*petA* (antisense)	254
R+	*rbcL* (sense)	437
R−	*rbcL* (antisense)	437
T+	*E. coli thrA* (sense)	227

Accumulation of atpB *mRNA from modified genes*

To determine the level of accumulation of *atpB* mRNA, total RNA was fixed to filters using a slot-blot apparatus, and identical filters were hybridized with probes for *atpB* or *psbA*. Results from a typical hybridization are shown in Figure 3A, and quantification of 5 independent experiments is shown in Figure 4 (empty bars). The *atpB* transcripts in Δ26 accumulated to 30% of the wild-type level, which is somewhat higher than the 21% previously reported [23]. Transformants in which the *atpB* gene was flanked by *petD-trnR* (in either orientation), or by the *E. coli* threonine attenuator (in the sense orientation) accumulated approximately wild-type levels of *atpB* transcripts. Transformants in which *atpB* was flanked by the other 3′ UTRs, including *petA* 3′ UTR which is not predicted to form a stable stem-loop structure, accumulated *atpB* transcripts at 60–75% of the wild-type level. These results show that elements in these 3′ UTRs promote increased *atpB* transcript accumulation relative to the Δ26 deletion mutant. Since the 3′ UTRs function with similar efficiencies in the sense and antisense orientations, it is likely that structural features rather than specific nucleotide sequences are important for total transcript accumulation under the conditions tested. It is also likely that a portion of these transcripts are non-functional because of truncation of the *atpB*-coding region.

To determine whether the introduced 3′ UTRs can direct 3′-end processing resulting in accumulation of discrete *atpB* transcripts, total RNA from each transformant was fractionated in formaldehyde agarose gels, transferred to nylon filters and hybridized with probes for *atpB* and *psbA*. The results of a representative filter hybridization are shown in Figure 3B, and

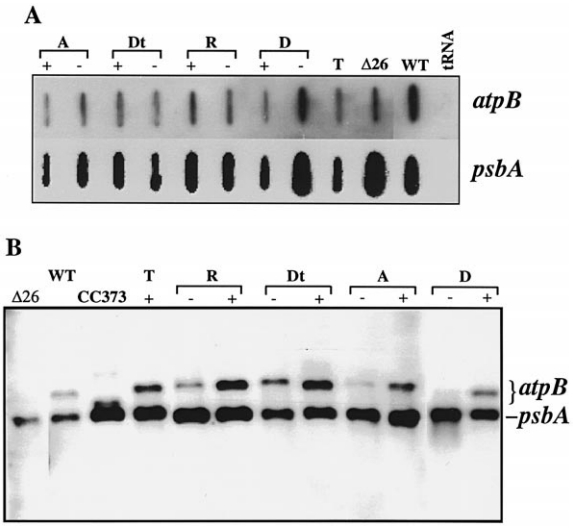

Figure 3. Accumulation of transcripts from chimeric *atpB* genes. **A.** Total RNA from the indicated transformants (5 μg) or yeast tRNA (10 μg) was fixed to nylon membranes with a slot-blot apparatus and hybridized with ^{32}P-labeled *atpB* and *psbA* probes. **B.** Total RNA (15 μg) from the indicated transformants was fractionated in an agarose gel, transferred onto a nylon membrane and hybridized sequentially with ^{32}P-labeled *atpB* and *psb*A probes.

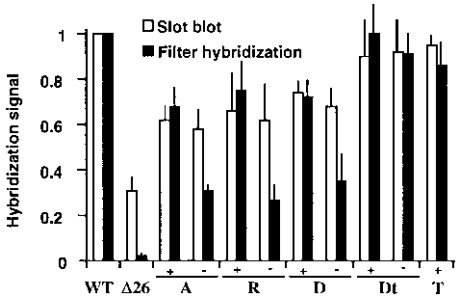

Figure 4. Quantification of *atpB* transcript accumulation. Accumulation of total (slot blot) and discrete (filter hybridization) *atpB* transcripts in the indicated transformants is displayed as a fraction of the accumulation in wild-type cells. The hybridization signals of slot blots and filter blots such as those shown in Figure 3 were quantified using a Fuji Imaging Analyzer, and *atpB* transcript accumulation was normalized to *psbA* transcript accumulation.

the average of 5 independent experiments is shown in Figure 4 (filled bars). Increased levels of discrete *atpB* mRNAs, relative to Δ26, accumulated in all of the transformants in which *atpB* was flanked by an inserted 3′ UTR. Transformants containing the sense orientation of the 3′ UTRs accumulated higher levels of discrete *atpB* transcripts than those in which *atpB* was flanked by the antisense sequences. In transformants in which *atpB* was flanked by antisense 3′ UTRs, discrete *atpB* transcripts with processed 3′ ends accumulated to about 30% of the wild-type level. Additionally, in all transformants containing a 3′ UTR in the sense orientation, the accumulation of discrete *atpB* transcripts was very similar to the level of total *atpB* transcripts as measured by slot blot analysis. This result suggests that when the transcript is correctly processed, most, if not all, of the transcript accumulates as a homogeneous population of a single length. However, the reduced accumulation of discrete *atpB* transcripts in strains containing the antisense 3′ UTRs implies that when the primary transcript is incorrectly processed, the accumulating transcripts are more heterogeneous in size and have variable 3′ ends.

Accumulation of the ATPase β-subunit in the different transformants

To determine the effects of the inserted 3′ UTR sequences on the accumulation of the ATPase β-subunit, total proteins from each strain were fractionated by SDS-PAGE, transferred to nitrocellulose filters, and incubated with antisera directed against the chloroplast ATPase β-subunit and the D1 protein of photosystem II. A representative immunoblot is shown in Figure 5A. The amount of ATPase β-subunit protein was normalized to the amount of the D1 protein, and the average of 3 independent experiments is shown in Figure 5B. As expected, no ATPase β-subunit accumulated in CC373, and ca. 30% of the wild-type level accumulated in Δ26 (Figure 5B). The reduced accumulation of D1 in CC373 may result from a reduction in total chloroplast proteins in this non-photosynthetic strain. The ATPase β-subunit accumulated to at least 70% of the wild-type level in the other transformants. In some cases in which *atpB* was flanked by antisense 3′ UTRs (i.e. A$-$ and R$-$), the level of discrete *atpB* transcripts but not the ATPase β-subunit was significantly reduced. In other strains (i.e. R$+$ and Dt$-$), accumulation of the ATPase β-subunit was decreased even though these strains accumulate ca. 75% and 95% of the wild-type level of discrete *atpB* transcripts, respectively. These results suggest that *atpB* transcript accumulation and the level of ATPase β-subunit are not necessarily related.

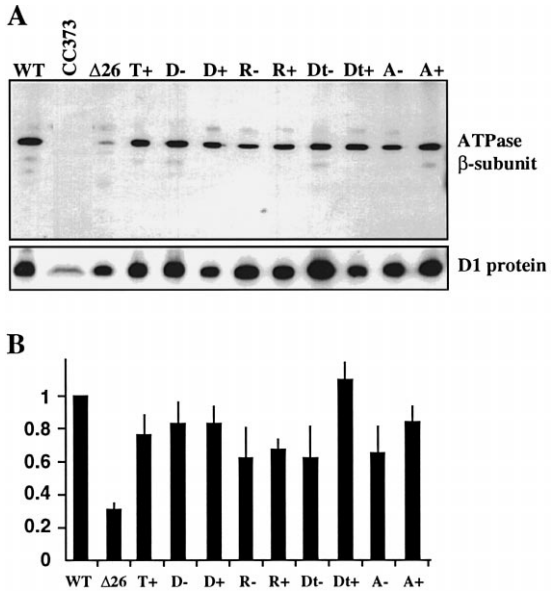

Figure 5. Accumulation of the ATPase β-subunit. **A.** Total proteins from the indicated transformants were fractionated by SDS-PAGE, transferred to a nitrocellulose membrane, and incubated sequentially with antibodies directed against the ATPase β-subunit (top panel) and the D1 protein of photosystem II (lower panel). Antigenic proteins were visualized by chemiluminescence. **B.** Densitometric quantification of autoradiograms of immunoblots such as those shown in A. Accumulation of the ATPase β-subunit was normalized to D1 protein, and displayed as a fraction of the accumulation in wild-type cells.

Discussion

The mechanisms of chloroplast mRNA 3′-end formation have been unraveled largely through the use of *in vitro* RNA processing systems [6, 19, 23]. In this work, we have extended the previous analysis of *Chlamydomonas* chloroplast *atpB* mRNA 3′-end formation by showing that 3′ UTR of the *petD* can also be processed *in vitro*. However, processing of the *petA* 3′ UTR transcript, which does not contain IR sequences, did not result in accumulation of a product, even though a *petA* transcript of discrete size accumulates *in vivo* [13]. It is likely that either factor(s) required for transcript processing are missing from the chloroplast extract, or that the substrate provided does not form the requisite structure. Antisense *atpB* 3′ UTR RNA, which can form a stem-loop structure similar to that of the sense strand, was degraded without the accumulation of a stable product. These results demonstrate that a stem-loop structure is required, but not sufficient for mRNA 3′-end processing *in vitro*.

Our studies also address the importance of 3′-end processing in chloroplast gene expression. Nucleus-encoded mRNAs, for example, must undergo 3′ cleavage and polyadenylation in order to reach the cytosol and be efficiently translated [18, 24]. Elaborate mechanisms have also evolved to process the cell cycle-regulated histone mRNAs at their 3′ stem-loop [10]. Using chloroplast transformation, we have tested whether the 3′ UTRs of the *Chlamydomonas reinhardtii* chloroplast *petD*, *petA* and *rbcL* genes, or the *E. coli* threonine attenuator, can substitute for the wild-type *atpB* 3′ UTR *in vivo*. Compared to a transformant lacking the *atpB* 3′ IR (Δ26), transformants containing *atpB* genes flanked by any of the 3′ UTRs in either the sense or antisense orientation, accumulated increased levels of *atpB* transcripts and ATPase β-subunit. However, there were significant differences in the accumulation of discrete *atpB* transcripts between transformants harboring the 3′ UTRs in sense or antisense orientations. Transcripts of *atpB* genes flanked by the sense orientations of the 3′ UTRs tested accumulated almost entirely as homogeneous populations of discrete RNA molecules. Although the accumulation of total *atpB* transcripts flanked by the antisense orientations of the 3′ UTRs was similar to that of transcripts flanked by the sense orientations, the proportion of transcripts which accumulate as discrete RNA molecules was reduced by approximately one-half. The remainder, which was detected by slot-blot analysis, could either be shorter (and untranslatable) or longer than the discrete molecules. The exception to this observation was the antisense orientation of the *petD-trnR* sequences, which resulted in accumulation of the wild-type level of discrete *atpB* transcript. The reason for this is not clear, but it is possible that the antisense strand fortuitously contains efficient processing signals.

Since the chimeric *atpB* genes are identical except for the 3′ UTR sequences, we interpret our results as evidence that the antisense 3′ UTRs are less efficient at forming 3′ ends resistant to exonuclease degradation. Although the antisense UTRs (except *petA*) contain inverted repeats that can form stem-loop structures, the nucleotide sequences of the loop and regions flanking the stem are different. These results, as well as those of Blowers *et al*. [2], are consistent with the idea that the loop and/or IR flanking sequences influence the efficiency of 3′-end processing. For example, they may contain binding site(s) for proteins and/or nucleases that perform the 3′-end processing of the transcript. Both *Chlamydomonas atpB* and spinach *petD* 3′-end

processing are initiated by specific endonucleolytic cleavages just downstream of the mature 3′ end [6, 22]. Comparisons of the nucleotide sequences of several *Chlamydomonas* 3′ UTRs did not reveal a consensus sequence downstream of the inverted repeat, although few mRNA 3′ ends have been mapped precisely in *Chlamydomonas* chloroplasts. We note, however, that the sequence UGUCAU, which is the site of endonucleolytic cleavage of the *atpB* 3′ primary transcript [22], is also found 10 nucleotides downstream of the mature 3′ end of *petD* 3′ mRNA [13]. *In vitro* analysis of *atpB* transcripts containing mutations in this sequence demonstrated that in some cases, 3′-end processing was affected (V. Liveanu, R. Rott and G. Schuster, unpublished). Therefore, the lack of specific endoribonuclease target site sequences could be one reason for the inability of the antisense sequences to direct efficient 3′-end processing of the *atpB* transcript.

The relationship between chloroplast transcript 3′-end processing and translation is not yet understood. It may be that only 3′-end-processed mRNAs which accumulate as discrete transcripts are efficiently translated. Alternatively, it is possible that any transcript which includes a complete coding sequence can be translated regardless of the transcript length and 3′-end position. Strains Δ26, R−, R+, Dt− and A− accumulate significantly less ATPase β-subunit than the wild-type strain. Because strains R− and A− accumulate only 30% of the wild-type level of discrete *atpB* mRNAs, it is possible that the lack of translatable mRNA is limiting ATPase β-subunit accumulation in these strains. However, strain Δ26, which lacks the *atpB* 3′ IR and does not accumulate discrete mRNAs, accumulates 30% of the wild-type level of the ATPase β-subunit, although it is possible that only a subpopulation of transcripts that have 3′ ends at, or near, the normal 3′ end is translated. Since the chloroplast ATPase complex is quite stable [11], the rate of β-subunit synthesis in these strains may be less than the level of accumulation. Interestingly, strain R+ and Dt−, which accumulate 75% and 95% of the wild-type level of discrete *atpB* mRNAs, respectively, also showed reduced accumulation of the ATPase β-subunit. The reasons for these results remain unclear, however these data suggest that the structure and/or sequence of the 3′ UTRs may affect translational efficiency in *Chlamydomonas* chloroplasts. Association of unspliced and unprocessed transcripts with translating polysomes has been reported in maize chloroplasts [1], and analysis of the interaction of *Chlamydomonas atpB* transcripts containing the inserted 3′ UTRs with polysomes may address whether 3′-end processing is required for, or facilitates, translation of chloroplast transcripts.

Acknowledgements

This work was supported by United States-Israel Binational Agricultural Research and Development Fund grant US-2207-92. R.G.D. was supported by NRSA Postdoctoral fellowship 1-F32-GM17616-01.

References

1. Barkan A: Proteins encoded by a complex chloroplast transcription unit are each translated from both monocistronic and polycistronic mRNAs. EMBO J 7: 2637–2644 (1988).
2. Blowers AD, Klein U, Ellmore GS, Bogorad L: Functional *in vivo* analyses of the 3′ flanking sequences of the *Chlamydomonas* chloroplast *rbcL* and *psaB* genes. Mol Gen Genet 238: 339–349 (1993).
3. Chen Q, Adams CC, Usack L, Yang J, Monde R-A, Stern DB: An AU-rich element in the 3′ untranslated region of the spinach chloroplast *petD* gene participates in sequence-specific RNA-protein complex formation. Mol Cell Biol 15: 2010–2018 (1995).
4. Drager RG, Zeidler M, Simpson CL, Stern DB: A chloroplast transcript lacking the 3′ inverted repeat is degraded by 3′→5′ exoribonuclease activity. RNA 2: 652–663 (1996).
5. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6–13 (1983).
6. Hayes R, Kudla J, Schuster G, Gabay L, Maliga P, Gruissem W: Chloroplast mRNA 3′-end processing by a high molecular weight protein complex is regulated by nuclear encoded RNA binding proteins. EMBO J 15: 1132–1141 (1986).
7. Lee H, Bingham SE, Webber AN: Function of 3′ non-coding sequences and stop codon usage in expression of the chloroplast *psaB* gene in *Chlamydomonas reinhardtii*. Plant Mol Biol 31: 337–354 (1996).
8. Lisitsky I, Klaff P, Schuster G: Addition of destabilizing poly(A)-rich sequences to endonuclease cleavage sites during the degradation of chloroplast mRNA. Proc Natl Acad Sci USA 93: 13398–13403 (1996).
9. Lisitsky I, Liveanu V, Schuster G: RNA-binding characteristics of a ribonucleoprotein from spinach chloroplasts. Plant Physiol 107: 933–941 (1995).
10. Marzluff WF: Histone 3′ ends: essential and regulatory functions. Gene Expr 2: 93–97 (1992).
11. Merchant S, Selman BR: Synthesis and turnover of the chloroplast coupling factor in *Chlamydomonas reinhardtii*. Plant Physiol 75: 781–787 (1984).
12. Nierlich DP, Murakawa GJ: The decay of bacterial messenger RNA. In: Cohn WE, Moldave K (eds) Progress in Nucleic Acid Research and Molecular Biology, pp. 153–216. Academic Press, San Diego, CA (1996).
13. Rott R, Drager RG, Stern DB, Schuster G: The 3′ untranslated regions of chloroplast genes in *Chlamydomonas reinhardtii*

do not serve as efficient transcriptional terminators. Mol Gen Genet 252: 676–683 (1996).
14. Rott R, Nelson N: Purification and immunological properties of proton-ATPase complexes from yeast and rat liver mitochondria. J Biol Chem 256: 9224–9228 (1981).
15. Schuster G, Gruissem W: Chloroplast mRNA 3′ end processing requires a nuclear-encoded RNA binding protein. EMBO J 10: 1493–1502 (1991).
16. Schuster G, Timberg R, Ohad I: Turnover of thylakoid photosystem II proteins during photo inhibition of *Chlamydomonas reinhardtii*. Eur J Biochem 177: 403–410 (1988).
17. Shepherd HS, Boynton JE, Gilham NW: Mutations in nine chloroplast loci of *Chlamydomonas* affecting photosynthetic functions. Proc Natl Acad Sci USA 76: 1353–1357 (1979).
18. Standart N, Jackson RJ: Regulation of translation by specific protein/mRNA interactions. Biochimie 76: 867–879 (1994).
19. Stern DB, Gruissem W: Control of plastid gene expression: 3′ inverted repeats act as mRNA processing and stabilizing elements, but do not terminate transcription. Cell 51: 1145–1157 (1987).
20. Stern DB, Gruissem W: Chloroplast messenger RNA 3′ end maturation is biochemically distinct from prokaryotic messenger RNA processing. Plant Mol Biol 13: 615–625 (1989).
21. Stern DB, Jones H, Gruissem W: Function of plastid mRNA 3′ inverted repeats. RNA stabilization and gene-specific protein binding. J Biol Chem 264: 18742–50 (1989).
22. Stern DB, Kindle KL: 3′ end maturation of the *Chlamydomonas reinhardtii* chloroplast *atpB* mRNA is a two-step process. Mol Cell Biol 13: 2277–2285 (1993).
23. Stern DB, Radwanski ER, Kindle KL: A 3′ stem/loop structure of the *Chlamydomonas* chloroplast *atpB* gene regulates mRNA accumulation *in vivo*. Plant Cell 3: 285–297 (1991).
24. Wahle E, Keller W: The biochemistry of 3′-end cleavage and polyadenylation of messenger RNA precursors. Annu Rev Biochem 61: 419–440 (1992).
25. Woessner JP, Gillham NW, Boynton JE: The sequence of the chloroplast *atpB* gene and its flanking regions in *Chlamydomonas reinhardtii*. Gene 44: 17–28 (1986).
26. Yang J, Schuster G, Stern DB: CSP41, a sequence-specific chloroplast mRNA binding protein, is an endoribonuclease. Plant Cell 8: 1409–1420 (1996).