3'-Processed mRNA Is Preferentially Translated in Chlamydomonas reinhardtii Chloroplasts†

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3'-end processing of nucleus-encoded mRNAs includes the addition of a poly(A) tail that is important for translation initiation. Since the vast majority of chloroplast mRNAs acquire their 3' termini by processing yet are not polyadenylated, we asked whether 3' end maturation plays a role in chloroplast translation. A general characteristic of the 3' untranslated regions of chloroplast mRNAs is an inverted repeat (IR) sequence that can fold into a stem-loop structure. These stem-loops and their flanking sequences serve as RNA 3'end formation signals. Deletion of the Chlamydomonas chloroplast atpB 3' end in strain Δ26 results in reduced accumulation of atpB transcripts and the chloroplast ATPase β-subunit, leading to weakly photosynthetic growth. Of the residual atpB mRNA in Δ26, approximately 1% accumulates as a discrete RNA of wild-type size, while the remainder is heterogeneous in length due to the lack of normal 3' end maturation. In this work, we have analyzed whether these unprocessed atpB transcripts are actively translated in vivo. We found that only the minority population of discrete transcripts of wild-type size is associated with polysomes and thus accounts for the ATPase β-subunit which accumulates in Δ26. Analysis of chloroplast rbcL mRNA revealed that transcripts extending beyond the mature 3' end were not polyadenylated associated. These results suggest that 3'-end processing of chloroplast mRNA is required for or strongly stimulates its translation.

Chloroplast genes are often organized into operons and gene clusters, which are transcribed into precursor transcripts that undergo complex processing events including splicing and intercistronic cleavages (reviewed in references 35 and 49). While intercistronic cleavages form some mRNA 5' and 3' termini, these can also be formed by other types of events. For example, 5' ends are often formed by endonucleolytic processing of primary transcripts, and this may be the exclusive mode of 5' end formation in chloroplasts in the green alga Chlamydomonas reinhardtii (reviewed in reference 12). Most plastid mRNAs contain inverted-repeat (IR) sequences in their 3' untranslated regions, which are believed to fold into stem-loop structures. These IR sequences do not function as efficient transcription terminators but instead are thought to stabilize upstream sequences and mediate correct 3'-end processing (36, 37, 44, 47, 48). In most cases, the 3' termini of mature transcripts lie immediately downstream of the IR.

Plastid 3' IR sequences act to stabilize upstream mRNA segments in vitro and in vivo. When RNA molecules containing the IR sequences were incubated in spinach chloroplast protein extracts, they were correctly processed at their 3' ends and the products were stable for several hours. However, when the IR sequences were deleted from the same RNA molecules and incubated in an identical protein extract, the RNA molecules were rapidly degraded (17, 37, 44, 46). The ability to introduce altered genes into the chloroplast of the green alga C. reinhardtii presented the opportunity to test the in vitro results in an in vivo context. When the 3' IR of the chloroplast atpB gene was deleted in strain Δ26, atpB mRNA became heterogeneous and unstable, and the resulting decrease in protein accumulation limited photosynthetic growth (48). In addition, the nucleotide sequence of the 3' untranslated regions (UTRs) can influence the accumulation of a correctly 3'-end-processed transcript, since the functionality of some Chlamydomonas 3' UTRs is orientation dependent in vivo (5, 37).

Evidence for possible involvement of the 3' UTR in the initiation of translation has accumulated from studies of the poly(A) binding protein in the yeast Saccharomyces cerevisiae as well as in other systems (21, 39). In yeasts and plants, this protein was found to stimulate binding of the 40S ribosomal subunit to mRNA by association with the translation initiation factor eIF-4G, which also binds to eIF-4E and the 5' cap of the mRNA (16, 40, 50). In mammalian cells, a protein called PAIP, an eIF4G homolog, binds the poly(A) binding protein and enhances translation (10). A model invoking mRNA circularization has been proposed, in which the mRNA 5' and 3' ends can interact via this association, which in turn is required for the initiation of translation (10, 16, 18, 40, 50). Although polyadenylation of mRNA has recently been described for spinach chloroplasts, it occurs primarily on degradation products and not at the 3' end of the intact transcript (27, 30–32). As in Escherichia coli (41), chloroplast polyadenylation is transient and seems to target mRNA for rapid degradation.

The generally accepted finding that the poly(A) tails of eukaryotic, nucleus-encoded mRNAs stimulate translation initiation prompted us to look for a related phenomenon in chloroplasts, which share many features of prokaryotic mRNA metabolism (45). We used the green alga C. reinhardtii as a model system, since the chloroplast genome can be modified by biolistic transformation. Several strains in which the 3' end processing elements of the atpB gene differed or were lacking were utilized. Analysis of these strains revealed that correctly
processed atpB mRNA was highly enriched in polyosomal fractions, whereas heterogeneous mRNA was poorly associated with polysomes regardless of its size. These results suggest that 3′ end processing of mRNA in the chloroplast may stimulate its translational activation.

MATERIALS AND METHODS

Plasmids and strains. Construction of the plasmids pB17BS (atpB [wild-type]) and pΔ26 and of the corresponding Chlamydomonas strains P17 and Δ26 has been described previously (48). pΔ26 carries a deletion immediately downstream of the atpB coding region, extending from position 1490 (the stop codon is at position 1474) to position 3807, and the deleted bases are replaced by a 7-bp linker that includes a BglII site (Fig. 1B) (48). A fragment containing the 3′ UTR and flanking sequences of rbcL was previously described (36); this sequence was inserted in the BglII site of pΔ26, creating strain R+ (37).

Strain Δ26S was isolated during transformations used to create Δ26. Unlike Δ26, Δ26S grows robustly under photoautotrophic conditions and survives under high light (23). Δ26S was found to contain a recessive nuclear suppressor mutation, resulting in the accumulation of discrete 1.9- and 2.1-kb atpB transcripts that are 3′ end processed at cryptic sites and wild-type levels of the ATPase β-subunit (29).

Isolation of nucleic acids, filter hybridization, and PCR. For nucleic acid preparations, cells were grown in 50 ml of HSA (high-salt medium containing acetate) to midlog phase. RNA and DNA were isolated as described previously (13, 36). For RNA filter hybridizations, 10 μg of total RNA was fractionated in 0.8% agarose–2.2 M formaldehyde gels, transferred to Amersham Hybond-N nylon membranes, and cross-linked by UV radiation. Prehybridization and hybridization were conducted in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt’s solution, 0.1% sodium dodecyl sulfate (SDS), and 0.1 mg of salmon sperm DNA per ml at 42°C. The blots were washed in 0.1× SSC–0.1% SDS at 65°C. Hybridization probes were generated by random priming in the presence of [α-32P]dATP, or with both [α-32P]dATP and [α-32P]dCTP for the experiment shown in Fig. 7. The BglII/EcoRI fragment of pΔ26 was used as an atpB probe, and a 5.8-kb EcoRI fragment was used to identify the psbA transcript (48). For rbcL, a PCR product covering nucleotides 2407 to 3000 (shown in Fig. 1A) was used as a probe to detect 3′ end-processed mRNA. A PCR product extending from nucleotides 2775 to 2930 was used to identify unprocessed rbcL mRNA. The 3′ end of the mature mRNA is located at nucleotide 2677 (14). All quantification of 32P-labeled blots was carried out with a Fuji-Imaging Analyzer.

Protein isolation and immunoblots. Total proteins were resuspended in SDS denaturing sample buffer, fractionated in SDS–12% polyacrylamide gels, transferred to nitrocellulose membranes, and decorated with antibodies as described elsewhere (46). Antibodies directed against the chloroplast ATPase β-subunit (38) and the D1 protein of photosystem II (42) were used. Antigenic proteins were visualized by chemiluminescence and were quantitated by densitometric analysis (33).

Polysome fractionation. To isolate total polyosomes (4, 24) from Chlamydomonas cells, 30 ml of log-phase cells (2 × 10^6 cells/ml) was broken in a buffer containing 0.2 M Tris-HCl (pH 9.0)–0.2 M KCl–35 mM MgCl2–25 mM EGTA–0.2 M sucrose–1% Triton X-100–2% polyethylene glycol ether–0.5 mg/ml heparin–0.1 mg/ml chloramphenicol with a French press cell. Following centrifugation at 3,000 × g for 5 min, the supernatant was adjusted to 0.5% sodium deoxycholate, incubated for 5 min on ice, and centrifuged at 15 min at 10,000 × g. Aliquots (1 ml) were layered onto four of 15 to 35% sucrose gradients in 40 mM Tris-HCl (pH 8.0)–20 mM KCl–10 mM MgCl2–0.5 mg/ml heparin–0.1 mg/ml chloramphenicol and centrifuged for 65 min at 45,000 rpm in a Beckman SW30 rotor. Ten fractions of 0.5 ml each were collected. The RNA of each fraction was purified by the addition of SDS to 0.5%, EDTA to 20 mM, phenol extraction, and precipitation with ethanol. Aliquots of each fraction were subjected to RNA blot analysis as described above. Since fractions 1 and 2 represent the buffer remaining from the sample loaded onto the gradient and are thus identical, only fraction 2 and subsequent fractions are presented in the figures. In control samples, polysomes were dissociated by the addition of EDTA (20 mM) to the algal lysates prior to gradient loading. In these gradients, 1 mM EDTA was substituted for 10 mM MgCl2.

RESULTS

Chlamydomonas strains containing altered atpB 3′ UTR sequences. In order to test the relationship between 3′ end processing of mRNA and translational efficiency, we took advantage of four strains with unique 3′ processing properties for the atpB gene. The generation of these strains has been described previously (see Materials and Methods). The wild-type control strain was P17, which was created by transformation of the atpB deletion mutant CC373 (Fig. 1A) with a wild-type atpB gene. Strain Δ26 lacks nearly the entire atpB 3′ UTR and downstream sequences; these were replaced by a BglII linker, as shown in Fig. 1. This led to the accumulation of a heterogeneous and unstable set of atpB transcripts, weak photosynthetic growth, and sensitivity to high light. When the rbcL 3′ UTR was inserted into the BglII site of Δ26, normal photosynthetic growth as well as the accumulation of the atpB transcript was restored (36). Compared to the 1.9-kb length of the atpB transcript in wild-type cells, the atpB transcript harboring the 3′ UTR of the rbcL mRNA is 2.1 kb long (36). This strain was used to determine whether atpB sequences per se were required for 3′ UTR function or whether those of another gene could function equally well for the assays described in this paper. Finally, strain Δ26S was used. This strain is a derivative of Δ26 in which the chloroplast genome is unaltered, but there is a single, recessive nuclear mutation that permits the accumulation of reduced amounts (compared to wild-type cells) of a discrete atpB transcript, in spite of the deletion of the atpB 3′ UTR. We have hypothesized (29) that the gene mutated in Δ26S, CRP2, encodes a general chloroplast mRNA processing factor.

Variation of atpB transcript and ATPase β-subunit accumulation with 3′ UTR structure and function. To measure the accumulation of all atpB transcripts in each strain described above, total RNA was fixed to filters by using a slot blot apparatus, and identical filters were hybridized with probes for atpB or with psbA as a loading control. Results from a typical hybridization are shown in Fig. 2A, and averaged results from several such experiments are shown in Fig. 3 (hatched bars). In agreement with previously obtained results (37, 47), atpB transcript accumulations relative to the wild-type strain were approximately 30% in Δ26, 45% in Δ26S, and 100% in R+. However, because of the technique used, it should be noted

FIG. 1. The Chlamydomonas chloroplast atpB region and constructs used in this work. (A) Map of the 7.6-kb BamHI fragment of the Chlamydomonas chloroplast genome. A portion of the large IR of the chloroplast genome is shown as an open arrow. The inverted repeat downstream of the atpB coding region is shown as a stem-loop structure. The extents of the deletions in the chloroplast genomes of strains CC373, Δ26 and Δ26S are indicated by hatched boxes. (B) Detailed view of the sequence of the atpB 3′ UTR in Δ26 and Δ26S. The BglII site into which the rbcL 3′ UTR was inserted in strain R+ is shown in boldface type. The endpoints of the deletion in Δ26 and Δ26S are indicated by triangles.
that hybridizing transcripts might not contain the entire atpB coding region or other parts of the message.

Δ26S was isolated as a spontaneous mutant that allowed rapid and high-light-tolerant photosynthetic growth (29). Since photosynthetic growth in Δ26 was limited by the synthesis of the ATPase β-subunit, we postulated that the restoration of rapid photosynthetic growth in Δ26S resulted from increased accumulation of the ATPase β-subunit. To measure the accumulation of the β-subunit in Δ26, Δ26S, and R+, total proteins were isolated from the same number of logarithmically growing cells and were subjected to immunoblot analysis with antibodies raised against the ATPase β-subunit or the D1 protein of the photosystem II reaction center as a loading control. As shown in Fig. 2B and 3 (stippled bars) and as reported previously (37, 48), the ATPase β-subunit accumulated to approximately 20% of the wild-type level in Δ26, fluctuating between 10 and 30%, depending on growth conditions (data not shown).

However, in Δ26S and R+, the protein accumulated at or near the wild-type level. These higher levels of the ATPase β-subunit account for the wild-type photosynthetic growth characteristics of these strains.

Given these data and the fact that the two strains Δ26 and Δ26S have an identical atpB gene structure, it was of interest to determine the mechanism by which β-subunit accumulation was augmented in Δ26S relative to Δ26. Since we knew already that Δ26S, as opposed to Δ26, accumulates a population of homogeneous discrete transcripts (29), we used RNA gel blots to measure the relative amounts of these processed atpB transcripts in Δ26S. We use the term processed in this paper to indicate accumulating atpB transcripts of an approximately wild-type size; we infer that these transcripts are similarly 3′ end processed, since all atpB mRNA in each strain used in this study has no alterations at the atpB 5′ end.

Figures 2C and 3 (filled bars) show that the accumulation of processed atpB transcripts in Δ26S is 35 to 40% of the wild-type level, but only 1% or less in Δ26. This processed atpB RNA is of a length similar to that of the wild-type atpB transcript, although the 3′ end is located at a cryptic processing site inside the large chloroplast genome IR, since the normal atpB 3′ UTR has been deleted (Fig. 1) (29). A longer exposure of this gel (middle panel of Fig. 2C) shows that for Δ26, some discrete transcripts do accumulate, as well as a smear of transcripts both shorter and longer than wild-type atpB mRNA (this result is also observed with Δ26 cells suppressed by chloroplast gene amplification [23]). All of the data of Fig. 2 and 3 taken together suggest that the formation of atpB transcript with a distinct 3′ end is important for the accumulation of the ATPase-β subunit, which in turn facilitates photosynthetic growth. A hypothesis for the molecular mechanism of this phenomenon supported by these data is that 3′-end-processed atpB transcripts are more efficiently translated than their unprocessed and heterogeneous counterparts. An alternative explanation is that the formation of the mRNA 3′ end is not related to the translation efficiency and that the formation of approximately 35% more 3′-end-processed atpB transcript in Δ26S (Fig. 2 and 3) (28) is correlative rather than causal. In the second explanation, the suppression acts by increasing the translational efficiency of processed and unprocessed...
$\text{atpB}$ transcripts compared to both wild-type and $\Delta 26$ cells. In order to distinguish between these two possibilities, we decided to determine the degree to which chloroplast polysomes are loaded with heterogeneous $\text{atpB}$ transcripts versus 3'-end-processed transcripts.

**Preferential polysome association of normally 3'-processed $\text{atpB}$ transcripts.** The central question at this point of the work was that $\Delta 26$ and $\Delta 26S$ accumulated similar amounts of $\text{atpB}$ mRNA as measured by slot blots, yet $\Delta 26S$ accumulated 4- to 10-fold more protein. While $\Delta 26S$ does accumulate a greater amount of correctly processed $\text{atpB}$ mRNA, as judged by RNA gel blots, $\Delta 26$ also accumulates a significant level of heterogeneous transcripts longer than the wild-type size, and these transcripts presumably include the entire $\text{atpB}$ coding region. Thus, it was possible that certain types of $\text{atpB}$ mRNA were preferentially translated.

To measure the polysome association of $\text{atpB}$ mRNAs in different strains, polysomal fractions derived from total cell lysates made in the presence of heparin, nonionic detergents, and chloramphenicol (to prevent runoff chloroplast translation) were sedimented in analytical sucrose gradients. RNA was isolated from 10 fractions and analyzed with RNA gel blots. Figure 4A shows the distribution of various rRNAs as revealed by ethidium bromide staining of each fraction and the total lysate (T). Since fractions 1 and 2 are identical and correspond to the 1 ml of cell lysate that was loaded on the gradient, results are presented only for fractions 2 to 9. The plastidic rRNAs (23S, 16S, and 23S*, an in vivo breakdown product of 23S rRNA [2, 28]) peak in fractions 4 and 5, indicating that a majority of polysomes are in these fractions. To verify that the rRNAs observed in fractions 4 to 7 are derived from polysomes, cell lysates were treated with EDTA and fractionated through EDTA-containing gradients. EDTA causes dissociation of polysomes into monosomes (2, 24). Indeed, when treated with EDTA, the rRNAs were no longer observed in fractions 5 to 7. Instead, they were concentrated in fractions near the top of the gradient (compare Fig. 5A and B).

We then analyzed the distribution of $\text{atpB}$ transcripts between the nonpolysomal (fractions 2 to 4) and polysomal (fractions 4 to 7) fractions. In wild-type cells (Fig. 4B), most of the $\text{atpB}$ transcripts were in fractions 4 to 6 and therefore polysome associated. In contrast, the majority of $\Delta 26 \text{atpB}$ transcripts, irrespective of size, appeared as a smear in fraction 2 and were therefore nonpolysomal (Fig. 4C). The prolonged exposure of the blot in this panel (see also Fig. 2C) revealed the presence of two processed $\text{atpB}$ transcripts of 1.9 and 2.1 kb in the polysomal fractions. These two 3'-end-processed $\text{atpB}$ transcripts in $\Delta 26$ and $\Delta 26S$ have been recently characterized (29). This result suggested that the ATPase $\beta$-subunit is translated in $\Delta 26$ cells from the 1.9- and 2.1-kb transcripts, rather than from the ones of variable size. As a control, EDTA treatment was used, and this treatment resulted in all $\text{atpB}$ transcripts migrating in the nonpolysomal region of the gradient (Fig. 5C and D).

It was formally possible that the reduced amount of ATPase $\beta$-subunit in $\Delta 26$ cells resulted from the deletion of 2 kb downstream of the $\text{atpB}$ gene, rather than from the low level of processed transcripts. To address this issue, we analyzed the polysome distribution of $\text{atpB}$ transcripts in $\Delta 26S$ and $\Delta 26$. $\Delta 26S$ contains the same deletion as $\Delta 26$ but an increased amount of processed RNA, while $\Delta 26$ contains the deletion but also the inserted 3’ UTR of rbcL. Due to the high instability of the $\text{atpB}$ transcripts in $\Delta 26S$ cells, we repeatedly obtained a poor yield of polysomal transcripts. Figure 4D shows that in $\Delta 26S$, only processed (and some degraded) $\text{atpB}$ transcripts were detected in the polysomal fractions, much as in $\Delta 26$.
EDTA treatment caused slower sedimentation of these transcripts (Fig. 5E and F). In these two related strains, therefore, processed atpB transcripts are preferentially associated with polysomes. We conclude that although the total amounts of atpB transcript are similar in Δ26 and Δ26S, the increased amount of processed transcript in Δ26S is responsible for its wild-type protein level and normal photosynthetic growth.

R+ was used to see whether the presence of 3' end processing signals from another gene could fully restore polysomal localization of atpB mRNA. Figure 4E shows that most atpB transcripts (which are 2.1 kb long in R+[36]) are polysome-associated in R+, similar to the distribution in wild-type cells (Fig. 4B), although slightly skewed toward more slowly migrating fractions. However, the nonpolysomal fraction 2 contained very little atpB mRNA. This result suggests that 3' end processing strongly enhances polysome association of atpB transcripts.

Lack of nonspecific RNA degradation in polysomal fractions. In order to verify that the heterogeneity of atpB transcripts in cell lysates of Δ26 and Δ26S is due to the lack of a stem-loop structure in the 3' UTR and not because of general degradation activity, the gradient of Δ26S shown in Fig. 4D was reprobed with a psbA-specific fragment (Fig. 4F). As described before, the 1.1-kb psbA transcript is distributed between polysomal and nonpolysomal fractions (2, 11, 24, 25). A very small amount of RNA degradation was observed for the psbA transcript, or from the sequences immediately downstream of the mature 3' end, which should detect only unprocessed RNA. These probes are indicated as A and B, respectively.

To ascertain independently that unprocessed transcripts not detected by RNA blot analysis were localized at the top of the gradient, total RNA from the polysome gradient fractions was extracted and analyzed by slot blot hybridization, as shown in Fig. 6A. These data clearly show that the vast majority of Δ26 atpB mRNA is found at the top of the gradient, while for wild-type cells it is primarily in the faster-sedimenting fractions. To quantify these results, the total amount of atpB mRNA in all fractions was set at 100%, and the distribution was calculated accordingly. The results presented in Figure 6B demonstrate that more than 98% of the atpB transcripts in Δ26 were found in fractions 1 to 3, with the remaining 1% in the polysomal fractions 4 to 6. In contrast, in wild-type cells more than half of the transcripts were found in fractions 4 and higher. These results further confirm our interpretations derived from the data shown in Fig. 4 and 5.

Polysomal distribution of processed and unprocessed rbcL transcripts. To determine whether the preferential polysomal association of 3'-end-processed mRNA also occurs with other chloroplast transcripts in Chlamydomonas, we examined the distribution of wild-type rbcL mRNA. To detect possible unprocessed transcripts, hybridization probes were prepared either from the 3' end of the coding region, which should detect all rbcL transcripts, or from the sequences immediately downstream of the mature 3' end, which should detect only unprocessed RNA. These probes are indicated as A and B, respectively.
Partially processed mRNAs can also be loaded onto ribosomes, as has been shown for \( \text{psbB} \) operon mRNAs in maize (3).

The partitioning of mature chloroplast transcripts between polysomal and nonpolysomal fractions has been noted previously and can vary with growth conditions and the gene which is analyzed (25). In particular, \( \text{psbA} \) mRNA can be present to a large degree in nonpolysomal fractions. In the soluble phase of barley plastids, for example, nearly all \( \text{psbA} \) mRNA is non-polysomal, with a higher proportion on polysomes isolated from membrane fractions (25). Spinach amyloplast ribosomes discriminate among mRNAs, with \( \text{psbA} \) being among those remaining nonpolysomal (11). In \( \text{Chlamydomonas} \), the distribution of \( \text{psbA} \) and \( \text{rbcL} \) mRNAs between thylakoids and stroma was found to fluctuate during the cell cycle (6). Thus, ribosome loading of mRNAs in chloroplasts appears to be a tightly regulated process.

The interaction of 3' end processing with the translation machinery could ensure translation of only mature and full-length transcripts. Indeed, it is now well-established for nucleus-encoded transcripts that the poly(A) tail together with the poly(A)-binding protein is essential for translation initiation on 80S ribosomes (10, 16, 18, 40, 43). To explain this phenomenon, recent models have been presented in which the mRNA is drawn as a circle with the poly(A)-tail connected via the poly(A) binding protein to the translation initiation complex (10, 16, 18, 40). In fact, electron micrographs of cells actively synthesizing secreted peptide hormones show that the great majority of membrane-bound polysomes are circular (8).

In prokaryotes, transcription and translation are often coupled. The chlororolast translation apparatus in many respects resembles the prokaryotic system but also has eukaryotic characteristics (45). If an equivalent to the poly(A)-tail-poly(A)-binding protein-mediated translation activation mechanism exists in chloroplasts, it must involve elements other than the poly(A) tail, which actually destabilizes chloroplast transcripts (27, 30–32). One candidate element would be the 3'-end stem-loop structure and/or proteins which bind in this region. However, the results presented here do not favor such an hypothesis, since in \( \Delta 26S \) cells, which produce wild-type levels of the ATPase \( \beta \)-subunit, the \( \text{atpB} \) gene lacks 2 kb of the 3' UTR, including the stem-loop-forming sequences and the authentic 3'-end processing site (29). This observation can be reconciled in two ways: either the nuclear \( \text{crp} \) mutation in \( \Delta 26S \) cells overcomes the need for the wild-type 3' UTR in terms of translation, or it is transcript length and/or the 3' processing mechanism per se that confers translatability to \( \text{atpB} \) transcripts. In vitro chloroplast translation systems (19, 20) may help in resolving the role of 3' end processing in \( \text{atpB} \) translation.

At least one case in which 3' end processing is required for translation has been documented in prokaryotes. The \( E. \ coli \) R1 plasmid \( \text{hok} \) mRNA, which mediates plasmid stabilization by killing of plasmid-free segregants, is translated only following 3' end processing (51). \( \text{hok} \) mRNA is folded in such a way that the unprocessed 3' end and the 5' end hybridize, inhibiting ribosome binding. Following 3' end processing, the 5' end becomes available to ribosomes (15). Whether this is a special or a more general mechanism in bacteria remains to be determined, and no information is available on whether long-range intramolecular interactions occur in chloroplast mRNAs. Chloroplast transformation in \( \text{Chlamydomonas} \) or tobacco offers a
promising methodology for testing these and related possibilities.

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