Specific Ribonuclease Activities in Spinach Chloroplasts Promote mRNA Maturation and Degradation*

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We have used an in vitro system to characterize ribonuclease activities present in spinach chloroplasts. We show that 3' end maturation of petD mRNA, which encodes subunit IV of the cytochrome b_{6}/f complex, is affected by a 33-kDa protein that binds to a hairpin structure at the 3' end of the mature mRNA. Binding of the 33-kDa protein to the petD hairpin structure decreases the efficiency of 3' end maturation, probably by impeding the progress of the processive 3'-5' exonuclease activity involved in chloroplast mRNA processing. A two-base mutation in the stem of the petD hairpin structure creates a novel recognition site for a ribonuclease which competes with the normal processing exonuclease activity. This mutation results in a very low 3' end processing efficiency for mutant petD transcripts, and instead generates a second processing product that lacks a complete hairpin structure. An endonuclease activity which is biochemically distinct from the previously characterized exonuclease activities has also been identified. This endonuclease activity is EDTA-insensitive, and cleaves petD RNA both at the termination codon and at the mature RNA 3' end. Cleavage of petD mRNA at the termination codon leads to rapid degradation of upstream RNA. The possible roles of these ribonuclease activities in chloroplast mRNA decay in vivo are discussed.

The expression of plastid genes is regulated by complex developmental, organ-specific, and light-dependent mechanisms (1-3). The control of mRNA decay plays an important role in regulating chloroplast mRNA accumulation. Not only do the stabilities of certain individual transcripts appear to be differentially modulated (4-6), but mRNA stability regulation is also of fundamental importance in establishing basal levels for all chloroplast RNAs.

The control of mRNA decay is achieved by the interaction of specific RNA sequences and structures with factors such as ribonuclease and RNA-binding proteins. For example, the interaction of a RNA-binding protein with a sequence capable of forming a hairpin structure present in the 3'-untranslated region of transferrin receptor mRNA (7), is required for RNA stability (8, 9). RNA:protein interactions also regulate the stabilities of histone (10, 11) and protooncogene (12) mRNAs. In plastids, it has been previously shown that the inverted repeat sequences (IRs) present in the 3'-untranslated regions of mRNAs are required for RNA accumulation in vivo and in vitro (13-16). These IRs are presumed to form hairpin structures that play a protective role against exonucleolytic degradation in both prokaryotes and eukaryotes (for a review, see Ref. 17). It has been proposed that the function of spinach plastid mRNA 3' IRs is modulated by RNA:protein interactions (15), since some plastid mRNAs accumulate only to low levels in dark-grown plants despite constitutive transcription and the presence of an IR (4). Although several reports have described RNA-binding proteins in chloroplasts, none of these proteins has been directly implicated in RNA stability regulation (15-20).

To further our understanding of how chloroplast mRNA accumulation is controlled, we have begun to study ribonuclease activities present in spinach chloroplasts. We have focused on the petD gene, which encodes subunit IV of the cytochrome b_{6}/f complex (21), and for which we have previously reported a detailed analysis of the interactions between chloroplast proteins and the mRNA 3'-untranslated region (22). We report that the binding of a 33-kDa protein to petD 3' IR-RNA affects the efficiency of 3' end processing. We have also identified endonuclease activities that cleave petD 3' IR-RNA within or near the 3' protective hairpin structure. These data suggest a possible model for petD mRNA decay, in which rate-limiting endonucleolytic cleavages generate unstable mRNA decay intermediates.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis—Single-stranded (ss) DNA was obtained, and mutations were introduced as described previously (15). Most of the base-change and deletion mutants were constructed using ssDNA of the petDA50 deletion mutant (15), except the compensatory mutants for which the template was petD ssDNA.

Soluble Chloroplast Protein Extracts—Soluble chloroplast protein extracts were prepared from leaves of hydroponically grown spinach by the method of Gruissem et al. (23). Briefly, total soluble proteins were prepared from a clarified chloroplast lysate, passed over a DE-52-cellulose column, and precipitated with ammonium sulfate at 60% saturation. The proteins were dialyzed against buffer E containing 20 mM HEPES, pH 7.9, 60 mM KCl, 12.5 mM MgCl_2, 0.1 mM EDTA, 2 mM dithiothreitol, and 17% glycerol (23). For heparin-agarose chromatography, total soluble proteins in buffer E were loaded onto a heparin-agarose column (250 µg of protein/ml heparin-agarose, obtained from Bethesda Research Laboratories) pre-equilibrated with buffer E at a flow rate of 0.5 ml/min. After washing with buffer E until no further A_{260} was eluting (flow-through fraction), bound proteins were eluted with buffer E containing 1 M KCl (bound fraction) and dialyzed against buffer E, then concentrated using Centricon filtration devices (Amicon). The concentration of each protein fraction was determined by using the Bio-Rad protein assay.

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1 The abbreviations used are: IR, inverted repeat sequence; ss, single-strand; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; bp, base pair(s); EndoC1 and EndoC2, chloroplast endonucleases 1 and 2, respectively; FT, flow-through.
and aliquots were stored at -70°C.

Preparation of Synthetic RNAs and in Vitro Processing—Synthetic RNAs were prepared as described by Stern and Gruissem (14). Trace-labeled RNAs were synthesized using 8 nM [α-32P]UTP and 0.5 mM unlabeled UTP. RNA processing reactions were carried out as described (14). The time points taken for the processing reactions were 0, 5, 10, 20, 30, 45, and 60 min, except as noted. The RNA:protein ratios were 1 fmol of RNA:2.4 μg of total soluble proteins, 1 fmol of RNA:0.8 μg of flow-through proteins, and 1 fmol of RNA:1 μg of bound fraction proteins.

RNase T1 partial digestions were carried out as described previously (15). Briefly, trace-labeled wild-type and mutant petD 3′ IR-RNAs were treated with calf intestinal phosphatase and then 5′ end-labeled in the presence of [γ-32P]ATP and T4 polynucleotide kinase. The 5′ end-labeled RNAs were purified from polyacrylamide gels and subjected to RNase T1 digestion. The digestion products were analyzed in 6% sequencing gels.

An RNA ladder was generated by boiling 5 fmol of 5′ end-labeled RNA for 5 min in a solution containing 45 mM NaHCO3, 5 mM Na2CO3, and 1 mM EDTA in a final volume of 6 μL, followed by rapid cooling on ice.

RESULTS

Efficiency of petD 3′ IR-RNA Processing Is Reduced by a Hairpin-specific petD RNA-binding Protein—We have previously shown that in vitro-synthesized RNA corresponding to the precursor form of petD 3′ mRNA is correctly processed to a mature form in a spinach chloroplast protein extract, by a processive exonuclease activity (15). The mature form, like petD mRNA isolated from chloroplasts, terminates in a hairpin structure. Previously we have characterized a 33-kDa protein (15, 22). The mutant petD 3′ IR-RNAs that are indicated by arrows. Previously we have shown that in vitro-synthesized RNA corresponding to the precursor form of petD 3′ mRNA is correctly processed to a mature form in a spinach chloroplast protein extract, by a processive exonuclease activity (15). The mature form, like petD mRNA isolated from chloroplasts, terminates in a hairpin structure. Previously we have characterized a 33-kDa protein (15, 22). The mutant petD 3′ IR-RNAs that are indicated by arrows.

To test whether the 33-kDa protein binding to the petD hairpin plays a role in RNA processing, we used these mutant petD 3′ IR-RNAs in RNA processing assays. Fig. 2A shows a kinetic analysis of in vitro processing for wild-type and mutant petD 3′ IR-RNAs. All the petD7 mutants showed similar rates of precursor disappearance when compared to the wild-type 3′ IR-RNA precursor; however, each mutant accumulated an increased amount of product relative to the wild-type. We also tested several other mutant petD 3′ IR-RNAs containing point mutations or deletions that do not affect the binding of the 33-kDa protein. None of these mutant 3′ IR-RNAs showed significant differences in RNA processing when compared to wild-type RNA (data not shown), except one mutant RNA termed petD2 (15), whose properties will be described below.

Since increased accumulation of the petD 3′ IR-RNA product could be correlated with the lack of 33-kDa protein binding, we wished to determine whether this increase resulted from greater stability of the product and/or from enhanced enzymatic activity. First, we measured the efficiency of processing (14). This parameter measures the molar conversion of precursor to product; i.e. the percentage of degraded precursor that is represented as mature product at a given time point. Efficiency takes into account both the processing rate and product stability, since only full-length molecules are counted as precursor, and only fully processed molecules are counted as mature product. When processing efficiency was measured at different time points, we found that the processing efficiency for all the petD7 mutant 3′ IR-RNAs was several-fold higher than that of the wild-type (Fig. 2B). This result suggests that the mutant 3′ IR-RNA precursors are more efficiently converted into a correctly sized product, instead of being incorrectly processed or degraded nonspecifically, and/or that the mutant 3′ IR-RNA products are more stable than the wild-type product. However, when the in vitro half-lives of petD7 mutant 3′ IR-RNA products were compared to the wild-type, no significant differences were found (data not shown). This suggests that more effective processing rather than increased product stability underlies the higher processing efficiency.

petD 3′ IR-RNA 3′ Processing Is Altered by Modified RNA Secondary Structure—We previously showed that petD2 3′ IR-RNA, which contains a mutation of two nucleotides in the stem of the hairpin structure (Fig. 1A), has a low rate of precursor degradation and low amount of product RNA accumulation in in vitro processing assays (15). In addition, we have recently identified a second RNA processing product (product 1) that can be detected when using petD2, but not wild-type 3′ IR-RNA (Fig. 3A). We did not detect product 1 when using mutant petD 3′ IR-RNAs containing only one (petD2.2 and petD2.1) of the petD2 mutations, or when using compensatory (petD2.C1, petD2.C2, and petD2.C3; Fig. 1A) mutant RNAs of petD2 (data not shown). However, when the petD2 mutation was combined with other mutations (petD65, petD66, and petD10; Fig. 1B) that are close to the 5′ ends of the product 2 RNA (Fig. 3B; see below), product 2 RNA was always detected (Fig. 3A). Since the appearance of product 2 was uniquely correlated with the presence of the petD2 mutation, we infer that this mutation may have created a novel recognition site for a ribonuclease activity present in the chloroplast protein extract. To determine the 5′ and 3′ ends of petD2 product 2 RNA, we compared the products of 5′-labeled and uniformly labeled RNAs in RNA processing assays. We found that product 2 RNA possesses the same 5′ end as mature and precursor 3′ IR-RNAs and that its 3′ end lies within the loop region of the hairpin structure (Fig. 3B).

To explore a possible basis for the altered nuclease sensitivity of petD2 3′ IR-RNA, we used RNase T1 partial digestions of 5′-end-labeled RNA to determine its secondary structure. Several regions of petD2 3′ IR-RNA showed a reduced degree of secondary structure (Fig. 4), predicting the confor-
FIG. 2. Kinetic analysis of petD wild-type and mutant 3’ IR-RNA in vitro processing. A, the left panel shows the degradation of precursor 3’ IR-RNAs during the 60-min processing reaction. The right panel shows the accumulation of mature product 3’ IR-RNA during the 60-min processing reaction. B, efficiency of precursor 3’ IR-RNA conversion to mature product RNA after 10 min (left) and 60 min (right) of the processing reactions. Efficiency is calculated according to the formula $E = \frac{\text{Pro}_t}{\text{Pre}_0}$, where $\text{Pro}_t$ is the molar amount of product at time (10 min or 60 min), and $\text{Pre}_0$ and $\text{Pre}_t$ are the molar amounts of precursor at times 0 and 10 min or 60 min, respectively (16).

null

FIG. 3. Identification of petD2 product 2 RNA. A, processing assays of wild-type, petD2, and double-mutant 3’ IR-RNAs. From left to right: wt, wild-type; D2, petD2 mutant; D2D10, petD2 and petD10 double mutant; D2Dd5, petD2 and petDd5 double mutant; D2Dd6, petD2 and petDd6 double mutant. The processing assays were carried out using total soluble proteins as described under “Experimental Procedures.” Reactions were analyzed in a 6% denaturing polyacrylamide gel. The RNA bands corresponding to the 3’ IR-RNA precursor, the mature 3’ IR-RNA product (in vivo 3’ end) and the novel product 2 RNAs are indicated. B, the 3’ ends of petD2 mutant product 2 RNA are indicated within the petD2 3’IR-RNA hairpin structure, as determined by RNase T1 secondary structure analysis (Fig. 4 and text). The dashed arrows represent the less abundant products, and the solid arrow represents the major product.

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nucleotides longer than product 2 RNA and may represent the precursor form of product 2 RNA generated by endonuclease cleavage. This product appears in gels as a weak band (data not shown). If endonuclease cleavage is the first step of product 2 formation, the double-stranded stem of the initial hairpin structure would be maintained, and subsequent exonuclease digestion would pause upon reaching the double-stranded stem structure to generate the mature product 2 RNA.

**Fig. 4.** RNase T1 partial digests of wild-type and petD2 mutant 3′ IR-RNAs. A, left, gel analysis of petD2 mutant (D2) and wild-type (ut) RNAs following RNase T1 partial digestion (see "Experimental Procedures"). The positions of RNase T1 cleavage are numbered according to Fig. 4B. Right, an RNase T1 partial digestion of petD2 mutant RNA was analyzed by higher resolution gel electrophoresis to determine the size of each product. D2, petD2 RNA. B, RNase T1 cleavage sites of wild-type (left) and petD2 mutant (right) were used to infer the hairpin structures as shown. Solid arrows represent major cleavage sites, and dashed arrows represent weak cleavage sites.

**Identification of an Endonuclease Activity (EndoC1)** Since endoribonuclease cleavages are often specifically separated from exonuclease activities, however, its identification must remain tentative.

**TABLE I**

<table>
<thead>
<tr>
<th>Mutant</th>
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<td></td>
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<tr>
<td>petD2.C3</td>
<td>-</td>
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<td>Wild-type</td>
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*The symbols used are: +, RNase T1 cleavage; -, No RNase T1 cleavage; ±, weak cleavage; *, no guanosine residue in the RNA sequence.

**Fig. 5.** In vitro stabilities of petD product 2, Δ24, and Δ63 RNAs. In vitro-synthesized RNAs were incubated with total chloroplast proteins at an RNA:protein ratio of 1 fmol of RNA:4.2 μg of protein. Aliquots were removed after 0, 5, 20, and 45 min and analyzed by gel electrophoresis and radioactive imaging (PhosphorImager, Molecular Dynamics, Inc., Sunnyvale, CA). The 0-min time point was taken by removing an aliquot immediately after mixing the RNA sample with the protein. The actual reaction time was approximately 10–15 s.

RNA 3′ end. This hypothetical processing pathway is diagramed in Fig. 6.

Although we were unable to find reaction conditions that clearly distinguished the proposed endonuclease activity (termed EndoC1; "chloroplast endonuclease 1") from exonuclease activity, there are other data suggesting that an endonuclease activity is involved in the production of product 2 RNA. If product 2 RNA is generated by the same exonuclease activity as for 3′ IR-RNA precursor processing (e.g. Fig. 1; see Ref. 14), we would expect the petD2 3′ IR-RNA precursor to show a similar or even higher degradation rate when compared to wild-type RNA in processing assays. However, the petD2 3′ IR-RNA precursor has a much lower rate of degradation and only a small amount of mature RNA accumulation. This suggests that the petD2 3′ IR-RNA precursor has become a substrate for another ribonuclease activity that competes with the normal processing activity and results in the appearance of product 2 RNA. The model shown in Fig. 6 invokes the participation of two ribonuclease activities, one an endoribonuclease activity (EndoC1) that recognizes either specific RNA sequences or structures, and the other an exonuclease that also may be involved in normal 3′ end formation. Until the putative endonuclease can be biochemically separated from exonuclease activities, however, its identification must remain tentative.
candidates for the rate-limiting steps in mRNA decay, we have attempted to identify such activities in spinach chloroplast protein extracts. One such activity is a nonspecific endonuclease activity that degrades petD 3' IR-RNA in vitro (16). Although preliminary evidence for an endonuclease activity (EndoC1) that cleaves a mutant petD 3' IR-RNA was found, as discussed above, we were also interested in endonuclease activities that direct efficient cleavages of wild-type petD 3' IR-RNA in vitro.

Following heparin-agarose chromatography of total soluble chloroplast proteins, we found that the exonuclease activity involved in 3'-end processing could be eluted with 1 M KCl along with most petD 3' IR-RNA-binding proteins [bound fraction (see Fig. 9A) (22)]. We have now characterized an endonuclease activity (termed EndoC2) in the flow-through (FT) fraction from these columns. This endonuclease activity is different from the putative EndoC1 which generates product 2 RNA from petD2 mutant 3' IR-RNA (see above), since we did not observe product 2 RNA when incubating petD2 mutant 3' IR-RNA with FT proteins (data not shown). When the petD 3' IR-RNA precursor was incubated with FT proteins, we observed two major products as shown in Fig. 7A. Because the size of the upper band varied when using different petD 3' IR deletion mutant RNAs (Fig. 7A, 3' and data not shown), while the lower band (Fig. 7A, 5') remained the same, the upper band must include the deletion in the hairpin structure. When using 5'-end-labeled 3' IR-RNA in processing reactions with FT proteins, we did not observe the upper band, indicating that the upper band, as expected, does not include the 5' end of the 3' IR-RNA precursor. We subsequently determined that the two RNA species are most likely generated by a single endonucleolytic cleavage of petD precursor 3' IR-RNA at the petD termination codon (Fig. 7B).

Since EndoC2 cleavage separates the downstream hairpin structure from the coding region of petD RNA, we would expect the upstream coding region portion to be destabilized. We therefore measured the stability of the 5' endonuclease cleavage product in the total chloroplast protein extract. To do so, we used an in vitro-synthesized RNA (DraI-RNA) transcribed by T7 RNA polymerase from a DraI-linearized DNA template, since the DraI restriction site on the DNA
template fortuitously coincides with the endonuclease cleavage site (Fig. 7B). Fig. 8 shows that when incubated in the chloroplast protein extract, Dral-RNA was degraded rapidly, whereas Δ18 RNA, which corresponds to the mature form of petD3' IR-RNA, was not significantly degraded after similar period of incubation. We conclude that EndoC2 activity plays an important role in establishing the rate of mature petD 3' IR-RNA degradation in vitro.

To differentiate biochemically EndoC2 activity from the exonucleolytic 3' IR-RNA processing activity, we carried out a series of reactions under different conditions. Fig. 9A shows that the EndoC2 activity was not affected by the addition of poly(U) to the reaction, under conditions that inhibit most

![Graph showing RNA cleavage over time](image-url)

**Fig. 8.** In vitro stabilities of endonucleolytic cleavage 5' product RNA and mature petD 3' IR-RNA. In vitro-synthesized RNAs were incubated with total chloroplast proteins at an RNA:protein ratio of 1 fmol of RNA:4.2 µg of protein. Aliquots were removed after 0, 5, 20, 45, and 90 min, and analyzed by gel electrophoresis and radioactive imaging. Δ18 RNA corresponds to a mature form of petD 3' IR-RNA (Fig. 1B), and Dral RNA corresponds to the 5' upstream product of the EndoC2 cleavage at the termination codon (see Fig. 7B). The error bars for Dral RNA samples are not visible at this scale.

**Fig. 9.** Analysis of endonuclease activities in chloroplast protein extracts. A, the wild-type petD precursor 3' IR-RNA was incubated in the absence of poly(U) with total proteins (T), flow-through fraction proteins (FT), and bound fraction proteins (B), or in the presence of 100 ng of poly(U) with flow-through fraction proteins [FT/poly(U)] or bound fraction proteins [B/poly(U)]. B, the bands corresponding to precursor and mature product 3' IR-RNAs are indicated on the left. The major products resulting from endonucleolytic cleavage are indicated as in Fig. 7A. B, the wild-type petD precursor 3' IR-RNA was incubated with FT proteins in the absence (−) or presence (+) of EDTA.

We have shown that binding of a 33-kDa protein that interacts specifically with the hairpin structure of petD 3' IR-RNA reduces the efficiency of 3' end processing in vitro. Interestingly, we have previously characterized two other spinach chloroplast petD RNA-binding proteins of 57 and 100 kDa, which seem to be involved in the regulation of the 33-kDa protein binding to petD 3' IR-RNA. We have shown that binding of the 57-kDa protein is required for 33-kDa protein binding, while binding of the 100-kDa protein to petD 3' IR-RNA prevents binding of the 33-kDa protein (22). It is therefore possible that regulation of the binding of the 33-kDa protein to petD 3' IR-RNA by the 57- and 100-kDa proteins regulates the petD mRNA maturation process in vivo. We have shown that a 2-base change in the stem of the petD3' IR-RNA hairpin structure creates a novel recognition site for a ribonuclease activity which is most likely an endonuclease (EndoC1). Cleavage by EndoC1 activity disrupts the stabilizing hairpin structure of petD 3' IR-RNA. In vivo, cleavage within the IR could be an early step in mRNA decay. For example, the newly created 3' end could serve as an initiation site for rapid exonucleolytic degradation by nonspecific enzymes analogous to Escherichia coli RNase II. Since we have been unable, using several approaches, to consistently separate this putative endonuclease activity from exonuclease activities, we cannot state conclusively that petD2 product 2 RNA actually results from combined endonuclease and exonuclease activities rather than from exonuclease alone. Furthermore, the petD2 sequence represents a mutated variant of the petD 3' IR which is not found in vivo. Thus, the in vivo function of the nuclease activity that forms product 2 may be unrelated to mRNA decay. Interestingly, however, substitution of the petD2 3' IR for the Chlamydomonas chloroplast atpB 3' IR in vivo results in extremely low mRNA accumulation, whereas substitution with the wild-type petD IR re-

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**D. B. Stern and K. L. Kindle, unpublished results.**
sults in normal mRNA accumulation (13).

We have also identified an endonuclease activity (EndoC2) that is biochemically distinct from the previously characterized exonuclease activities of spinach chloroplasts (24). EndoC2 activity is insensitive to EDTA, suggesting that it does not require Mg$^{2+}$ ions. An EDTA-insensitive endonuclease termed RNase I has been recently purified from tomato cells (25).

It is generally thought that endonucleolytic cleavages constitute the rate-limiting step in mRNA decay. In prokaryotes, ribonucleases such as RNase III (26), RNase E (27–29), RNase M (30), and RNase K (31) have been shown to initiate the degradation of different mRNA species. In comparing the activity that we have identified in spinach chloroplasts most closely resembles E. coli RNase E, which cleaves in the vicinity of RNA hairpin structures (27, 29, 32, 33). The cleavage of petD 3′ IR-RNA upstream of the hairpin structure, resulting in rapid degradation of the upstream RNA, is reminiscent of the decay of ribosomal protein S15 (rpsO) mRNA of E. coli (29). The decay of rpsO mRNA is initiated by a RNase E–dependent endonucleolytic cleavage upstream of the 3′hairpin structure of the message, that is followed by rapid mRNA degradation. Interestingly, RNase E also cleaves downstream of the hairpin, which generates a mRNA nearly identical to the monocistronic attenuated transcript (29). Spinach chloroplast EndoC2 also cleaves downstream of the petD hairpin structure, generating an RNA comigrating with mature petD 3′ IR-RNA (Fig. 9B).

It is interesting to note that spinach chloroplast EndoC2 cleaves petD 3′ IR-RNA at the termination codon. Other reports of site-specific endonucleolytic cleavages of mRNA include the endonuclease activity that cleaves specifically the Shine-Dalgarno sequence of T4 motA mRNA (34). It appears that the cleavage at the Shine-Dalgarno sequence does not lead directly to mRNA degradation, but prevents ribosome loading by removing the signal sequence. If spinach chloroplast EndoC2 cleaved specifically mRNA at the termination codon in vivo, the biological function of this activity might be to preclude complete translation. Such untranslatable mRNAs might be rapidly degraded either because they lacked the protective hairpin structure, and/or because translation itself prolongs mRNA lifetime.

Previously we have shown that a petD 3′ IR-RNA binding protein of 57 kDa (15) (previously estimated at 55 kDa) possesses two binding sites on petD 3′ IR-RNA: one upstream and one downstream of the hairpin structure (22). EndoC2 activity described here also possesses two cleavage sites; one at the termination codon is only five nucleotides away from the upstream binding site, and the other site downstream of the hairpin lies within the downstream binding site of the 57-kDa protein. We do not know if the 57-kDa protein is itself EndoC2. However, we are in the process of purifying this endonuclease activity. Using a poly(U)-agarose column, we have been able to obtain protein fractions that contain only the petD 3′ IR-RNA binding protein of 57 kDa, as detected by UV-cross-linking assays, although additional proteins of unknown function are also present.5 When petD 3′ IR-RNA was incubated with these protein fractions in processing assays, a high level of EndoC2 activity was detected.4 It therefore appears that this specific endonuclease activity is associated with or copurifies with a petD 3′ IR-RNA binding protein. A potential role for such associations would be to provide a basis for selective chloroplast mRNA decay.

It appears that EndoC2 activity may also be involved in petD RNA maturation, since it possesses a cleavage site at the maturation site of petD RNA. Previous reports on histone mRNA 3′ end formation have shown the involvement of an endonucleolytic cleavage (35). This endonuclease activity is sensitive to the Mg$^{2+}$ ion concentration, and is insensitive to EDTA. Taken together with our previous results, we have shown that the downstream region of petD 3′ IR, including the RNA maturation site, is involved both in endonucleolytic cleavage and interactions with mRNA-binding proteins. A possible model for petD mRNA decay is that protein binding to the 3′ IR would control the rate of endonuclease cleavage, thereby liberating unstable RNA products that contain the petD coding region. In this way, both ribonucleases and RNA-binding proteins could influence the process of petD mRNA maturation and decay.

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