The Spinach Chloroplast Endoribonuclease CSP41 Cleaves the 3'-Untranslated Region of petD mRNA Primarily within Its Terminal Stem-Loop Structure*

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3'-Untranslated region stem-loop structures are major determinants of chloroplast mRNA stability. The 3' stem-loop region of spinach petD precursor mRNA (pre-mRNA), a chloroplast gene encoding subunit IV of the cytochrome b$_{6}$f complex, forms a stable RNA-protein complex in vitro with chloroplast stem-loop binding proteins (CSPs) of 55, 41, and 29 kDa. We have previously purified CSP41 and cloned the corresponding cDNA. In vitro studies demonstrated that CSP41 is a bifunctional protein that displays both endoribonuclease and RNA-binding activities. In this work, the RNase activity of CSP41 is further characterized using the bacterially expressed protein. Our data show that CSP41 cleaves both single-stranded and double-stranded RNAs but not DNA. However, it exhibits a preference for stem-loop-containing RNAs. When the 3'-untranslated region of petD pre-mRNA is provided as a substrate, CSP41 specifically cleaves it within the stem-loop region, implying that CSP41 has an important role in the control of petD mRNA stability. Our data also show that the sequence-specific RNA-binding activity of CSP41 affects the rate, but not the specificity, of its RNase activity, suggesting that CSP41 is probably involved in other events of chloroplast RNA metabolism in addition to RNA degradation. By analyzing C-terminal deletions of CSP41, the RNase domain was located between amino acid residues 73 and 191.

Most chloroplast precursor mRNAs (pre-mRNAs) and mature mRNAs contain an inverted repeat sequence in their 3'-untranslated region (UTR) that can fold into a stable stem-loop structure. Both in vitro and in vivo studies have demonstrated that these 3' stem-loop structures are required for correct 3' processing of pre-mRNAs and for stabilization of mature mRNAs, by impeding processive 3' to 5' exonucleolytic degradation. In the absence of a stable secondary structure, or by readthrough of the stem-loop structure, mRNA becomes heterogeneous and generally unstable (1–7). Therefore, the 3' stem-loop structure has a primary role in controlling chloroplast mRNA accumulation through pre-mRNA processing and the protection of upstream sequences. Removal of the 3' stem-loop structure, therefore, is likely to be a key regulatory step in chloroplast mRNA degradation.

Bacterial pre- and mature mRNAs are another major group of mRNAs containing 3'-inverted repeats. The 3' stem-loop structures of bacterial mRNAs function in the processing of pre-mRNAs and the stabilization of mature mRNAs by a mechanism similar to that found in chloroplasts (8). Extensive studies have shown that at least three groups of proteins are involved in the control of 3' to 5' degradation of bacterial mRNAs: 1) endoribonucleases, such as RNase E and RNase III, which cleave the 3'-UTR within or upstream of the stem-loop structure to overcome the secondary structure barrier; 2) exoribonucleases, such as RNase II and polynucleotide phosphorylase, which bind to the free 3' end created by the endonucleases and degrade mRNA in the 3' to 5' direction; and 3) stem-loop-binding proteins, which modulate degradation by interacting with RNA and/or RNases (8–11). Each of these classes of protein can be found within a single multiprotein complex, termed the “degradosome” (12–14).

Several reports have documented proteins that may be involved in the maturation and/or 3' to 5' degradation of chloroplast mRNA (6, 15, 16). It was recently reported that a multiprotein complex containing a polynucleotide phosphorylase homologue and a putative RNase E-like protein could function in spinach chloroplast mRNA 3' processing and degradation (17). We have previously identified a stable stem-loop-protein complex in the 3'-UTR of spinach petD pre-mRNA, a chloroplast gene encoding subunit IV of the cytochrome b$_{6}$f complex (18). Complex formation requires not only the stem-loop but also an AU-rich element (box II) immediately downstream of the stem-loop. Protein components of the complex include chloroplast stem-loop-binding proteins (CSPs) of 55, 41, and 29 kDa. CSP41 was purified and the corresponding nuclear gene was cloned (19). Bacterially expressed and purified CSP41 displayed both endoribonuclease activity and sequence-specific RNA-binding activity. A detailed characterization of the RNA-binding activity indicated that CSP41 can bind to the stem-loop and to box II and may interact with other CSPs. CSP41's dual functions raised the question of how these activities were interrelated and regulated. Here, we report a detailed biochemical characterization of CSP41 endonuclease activity. Of particular significance is that CSP41 primarily cleaves the 3'-UTR of petD mRNA within the stem-loop structure, consistent with a key role in the control of chloroplast mRNA stability.

EXPERIMENTAL PROCEDURES

Nucleic Acid Substrates for Nuclease Assays—Four stem-loop-containing RNAs were used. As shown in Fig. 1, the 208-nt petD50 wild-type (WT) and box II mutant (BIIa) RNAs include 84 nucleotides of the 3' end of the petD coding region and 107 nucleotides of the petD pre-mRNA 3'-UTR, encompassing the stem-loop structure and its upstream and downstream AU-rich elements (box I and box II). The

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The abbreviations used are: UTR, untranslated region; Ni-NTA, nickel nitritotriacetatetrapotassium; FPLC, fast protein liquid chromatography; CSPs, chloroplast stem-loop binding proteins; nt, nucleotide(s); WT, wild-type.

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Transcribed with T7 RNA polymerase in the presence of EcoRI sites. The resulting plasmids were linearized into pBluescript KS psbA135 and rbcL115 RNAs, the corresponding DNAs were amplified downstream of the stop codons and encompassing their stem-loop structures. The rbcL115 RNA also includes a box II-like AU-rich element.

Both petD50 RNAs were synthesized as described previously (4). For procedures.” petD50BIIa include 78 nt of the pre-mRNA 3′-UTR, respectively, beginning immediately downstream of the stop codons and encompassing their stem-loop structures. The rbcL115 RNA also includes a box II-like AU-rich element. Both petDΔ50 RNAs were synthesized as described previously (4). For psbA135 and rbcL115 RNAs, the corresponding DNAs were amplified from spinach total DNA by the polymerase chain reaction and cloned into pBluescript KS+ (Stratagene, La Jolla, CA) between the XhoI and EcoRI sites. The resulting plasmids were linearized with EcoRI and transcribed with T7 RNA polymerase in the presence of [γ-32P]UTP. For 5′-end labeling, nonradioactive petD50 RNA was dephosphorylated and then labeled with [γ-32P]ATP and polynucleotide kinase (4). Before use, all transcripts were purified from 6% denaturing polyacrylamide gels.

To make uniformly labeled single- and double-stranded RNAs, pBluescript SK+ was separately linearized with SacI and KpnI and transcribed with T7 and T3 RNA polymerases, respectively, yielding a 118-nt RNA from the T7 promoter and a 121-nt RNA from the T3 promoter. These RNAs were gel-purified, mixed, boiled for 5 min, and then cooled to room temperature. The resulting double-stranded RNA was purified from an 8% native polyacrylamide gel.

Preparation of homogeneous HCSP41 with reproducible and robust endoribonuclease activity, cleaving the 3′-UTR of spinach petD pre-mRNA into multiple fragments of poly- and oligoribonucleotides (19).

To further characterize the RNAse activity of HCSP41, we expressed the N-terminal 6 × histidine-tagged HCSP41 fusion protein (HCSP41) in E. coli and purified it through a Ni-NTA column under denaturing conditions. HCSP41 was re-purified under optimized conditions and further purified by heparin-FPLC (see “Experimental Procedures”). This protocol yielded a preparation of homogeneous HCSP41 with reproducible and robust endonuclease activity, Fig. 2A shows that when uniformly labeled 208-nt petDΔ50WT RNA (Fig. 1) was provided except that to recover both RNase and RNA-binding activities of HCSP41, refolding was carried out in a buffer system containing 0.02 mM oxidized and 2 mM reduced glutathione for a minimum of 72 h. The initial concentration of denatured protein was limited to less than 0.5 μM. Homogeneous HCSP41 was obtained by purification on a HiTrap-heparin FPLC cartridge (Pharmacia Biotech Inc.) (19), whereas the mutant proteins were only purified through the Ni-NTA column. Using the oligonucleotide ATCCCCCTCATAAGCAGGAGGTGCAGCTGTAGAATT, pQE30-csp41A126 was created by site-directed mutagenesis (20). HCSP41A126 mutant protein was produced and prepared as described above. Protein profiles were examined in a Bio-Rad mini-SDS-polyacrylamide gel electrophoresis system and visualized by Coomassie Blue staining.

**Results**

CSP41 Cleaves Single- and Double-stranded RNAs, but Not DNAs—We have previously reported that spinach CSP41, a nuclear-encoded chloroplast RNA-binding protein, can specifically bind to the 3′ stem-loop region of petD pre-mRNA. Surprisingly, we found that highly purified CSP41 also exhibited robust endoribonuclease activity, cleaving the 3′-UTR of spinach petD pre-mRNA into multiple fragments of poly- and oligoribonucleotides (19).
as substrate, HCSP41 cleaved it into smaller fragments identical to those we have reported previously, although in different relative amounts. In this standard assay, 0.4 μg of refolded HCSP41 cleaved approximately 90% of the full-length substrate in 25 min.

Since petDΔ50WT RNA contains a stable 46-nt stem-loop structure (see Fig. 1), it was unclear whether the endonuclease activity of CSP41 was specific for single-stranded or double-stranded RNA. It was also unknown whether CSP41 could digest DNA, as other endonucleases do (21). To test CSP41 specificity, uniformly labeled 121-nt single- and 116-base pair double-stranded RNAs and 5’-end-labeled 89-nt single- and 94-base pair double-stranded DNAs were provided as substrates under our standard conditions. Fig. 2, B and C, shows that HCSP41 can cleave both single- and double-stranded RNAs, but neither single- nor double-stranded DNAs, demonstrating that CSP41 is an RNA-specific endonuclease. However, quantification of the ribonuclease activities using a PhosphorImager revealed that only 39% of the single-stranded RNA and 36% of the double-stranded RNA was shortened, a much lower amount than that seen with petDΔ50WT RNA under the same conditions (Fig. 2A). The higher degradation rate of petDΔ50WT RNA implies either that petDΔ50WT RNA contains preferred target sequences or structures for the RNase activity of CSP41 but the shorter RNA substrates do not or that the sequence-specific binding of CSP41 to petDΔ50WT RNA enhances its RNase activity (see below).

To characterize more completely the properties of the RNase activity, we performed RNA degradation assays under a variety of conditions. Table I summarizes the results, where we define the initial cleavage of petDΔ50WT RNA in 25 min under standard conditions (Fig. 2A) as 1.0 unit of activity. The data show that 1) CSP41 is active up to at least 55 °C and has a temperature optimum between 37 and 45 °C; 2) the pH opti-
The Endoribonuclease Activity of CSP41 Is Partially Decreased by Its RNA-binding Activity—The RNA-binding activity of CSP41 requires an AU-rich element immediately downstream of the petD 3′ stem-loop region. Mutations in this element, such as the BIIa mutation shown in Fig. 1, abrogate CSP41 binding (19). To investigate whether the RNA-binding activity of CSP41 enhances the RNase activity of CSP41 when petD50 RNA is the substrate, we utilized uniformly labeled petD50WT, petD50BIIa, psbA135, and rbcL115 RNAs. As shown in Fig. 1, these RNAs contain parts of the 3′-UTRs of wild-type petD pre-mRNA, mutant petD pre-mRNA, psbA pre-mRNA, and rbcL pre-mRNA, respectively. Each of these RNAs contains a 3′ stem-loop structure and flanking sequences, but only petD50WT and rbcL115 possess an immediately downstream AU-rich element.

To examine their interactions with CSP41, the RNAs were first used in a gel mobility shift assay. As expected, in a standard assay containing 0.4 μg of HCSP41 and 4 fmol of RNA, HCSP41 formed a complex with petD50WT RNA but not with either petD50BIIa or psbA135 RNA, both of which lack the downstream AU-rich element (Fig. 3). HCSP41 also formed a complex with rbcL115 RNA, but the signal was much weaker. This difference may be due to the one-nucleotide difference between the AU-rich elements or to other sequence or structural differences. In contrast, when used as substrates in standard RNase assays, we found that each RNA was rapidly cleaved into smaller fragments. Even in the relatively slow reactions, such as those with psbA135 or rbcL115 RNAs, less than 20% of the precursor remained after 25 min, as shown in Fig. 4A. It is especially notable that HCSP41 cleaves petD50WT and petD50BIIa nearly identically, although the protein binds stably only to petD50WT RNA under these conditions. When samples were taken over the 25-min time course, the profiles of petD50WT and petD50BIIa cleavages were similar in each case (data not shown). However, it took nearly twice the time for HCSP41 to degrade 50% of the petD50WT precursor (~8 min) than to degrade 50% of the petD50BIIa precursor (~4 min, Fig. 4B). Taken together, these data suggest that the RNA-binding activity of CSP41 does not alter the specificity but partially decreases the efficiency of its RNase activity, probably due to conformational changes of bound CSP41, and/or a limitation of accessible substrate for free CSP41. The RNase activity clearly shows a
preference for stem-loop-containing RNAs over other single- and double-stranded RNAs, which strongly suggests that some specific sequences or structures in the RNAs are efficiently recognized.

CSP41 Initially Cleaves the 3' -UTR of Spinach petD Pre-mRNA in the Stem-Loop Region—Although HCSP41 cleaves petDΔ50 RNA into multiple shorter fragments, we noted in experiments such as the one shown in Fig. 2 that certain products were much more prevalent than others. To identify the initial cleavage products of petDΔ50WT RNA, a time course of RNase digestion was carried out, as shown in Fig. 5. The group of bands marked with a single asterisk in Fig. 5 comprises the earliest products, appearing as early as the 0-min time point, with the cleavage apparently occurring during the mixing of the reaction. Two other groups of bands, marked with two or three asterisks in Fig. 5, were generated by HCSP41ΔS and HCSP41ΔP within 2 min. Subsequently, additional fragments were generated, either transiently or stably. At the end of the 120-min reaction, all the larger products were completely degraded and accumulated as very short RNA fragments seen near the bottom of the gel.

To determine the locations of the initial cleavage sites, 5' -end-labeled petDΔ50WT RNA was synthesized and used as a substrate. A parallel assay used uniformly labeled petDΔ50WT RNA. Fig. 6 shows that by comparing the patterns generated by the two substrates, it could be concluded that the group of initial cleavage products of petDΔ50WT RNA marked with a single asterisk in Fig. 5, and one of the groups of the secondary cleavage products of petDΔ50WT RNA marked with two asterisks, retained the original 5' -terminus. When calibrated using an alkaline RNA ladder and a 47-nt RNA marker, the initial cleavage sites were determined to be at nucleotides 133–137 and 141–142. The secondary cleavage sites were at nucleotides 114–115 and 119–120. Therefore, as shown in Fig. 6, CSP41 initially cleaves petDΔ50 RNA within the 3' stem-loop and subsequently upstream. Another group of secondary products, marked with three asterisks in Fig. 5, and other shorter products, were not seen in the assay with 5' -end-labeled substrate, indicating that they are internal or 3' -end-containing products.

Using the same approach, we found that CSP41 initially cleaved 5' -end-labeled petDΔ50BIIa RNA at identical sites to those indicated in Fig. 6, demonstrating again that the RNA-
were used to calculate similarity. The proposed active site of nuclease P1 is underlined boldface. B, partial elimination of RNA-binding activity of CSP41 and involvement in the RNA-binding activity. In this hypothesis, rewild-type HCSP41 may imply that the C terminus of CSP41 is with vertical bars using the BESTFIT program of the Genetics Computer Group software package (University of Wisconsin, Madison, WI), showing 50% similarity, with vertical bars and dots indicating identical and similar amino acid residues, respectively. Only identical or highly similar (two dots) residues were used to calculate similarity. The proposed active site of nuclease P1 is underlined boldface. B, 3 μg of wild-type HCSP41 (WT) and HCSP41A126 (A126) were prepared and examined in a 15% SDS-polyacrylamide gel. Protein molecular mass markers in kilodaltons are indicated at the left. A 30-kDa contaminant is indicated by the asterisk at the right. C, 3 μg of wild-type HCSP41 and HCSP41A126 were assayed for RNase activities as described in the legend to Fig. 7C. The inset shows the initial degradation of petD50 RNA by wild-type HCSP41 (WT) and HCSP41A126 (A126). RNA remaining during the 25-min time course was quantified and plotted as described in the legend to Fig. 4B. D, wild-type HCSP41; C, HCSP41A126.

**Fig. 9. RNase activity of HCSP41A126.** A, an alignment between Glu127-Trp176 of CSP41 and Gln123-Trp172 of nuclease P1 was performed using the BESTFIT program of the Genetics Computer Group software package (University of Wisconsin, Madison, WI), showing 50% similarity, with vertical bars and dots indicating identical and similar amino acid residues, respectively. Only identical or highly similar (two dots) residues were used to calculate similarity. The proposed active site of nuclease P1 is underlined boldface. B, 3 μg of wild-type HCSP41 (WT) and HCSP41A126 (A126) were prepared and examined in a 15% SDS-polyacrylamide gel. Protein molecular mass markers in kilodaltons are indicated at the left. A 30-kDa contaminant is indicated by the asterisk at the right. C, 3 μg of wild-type HCSP41 and HCSP41A126 were assayed for RNase activities as described in the legend to Fig. 7C. The inset shows the initial degradation of petD50 RNA by wild-type HCSP41 (WT) and HCSP41A126 (A126). RNA remaining during the 25-min time course was quantified and plotted as described in the legend to Fig. 4B. D, wild-type HCSP41; C, HCSP41A126.

**The Ribonuclease Domain of CSP41 Is Located Between Amino Acids 73 and 191**—Although it exhibits strong RNase activity, the primary amino acid sequence of CSP41 does not show significant overall similarity to any nuclease in the database. To begin locating domains of CSP41 required for RNase activity, we constructed 3' deletion mutants of CSP41 and examined RNase activities of the resulting proteins. For this purpose, increasingly long C-terminal deletions of CSP41 were generated by restriction enzyme digestion of pQE30-csp41. Wild-type and mutant proteins (Fig. 7A) were expressed in E. coli, purified through an Ni-NTA column, and then refolded, but were not subjected to further heparin-FPLC purification as in other experiments described above, since the mutant proteins have very low affinity for heparin. Their purities were examined in an SDS-polyacrylamide gel (Fig. 7B), showing that there is only one major contaminant (marked with an asterisk) in each preparation. These protein preparations were then tested for RNase activity in standard assays, as determined by quantification of the remaining petD50WT RNA over a 25-min time course. The results shown in Fig. 7C indicate that HCSP41ΔS, ΔE, and wild-type HCSP41 quickly degrade petD50 RNA, but much less RNase activity was displayed by HCSP41ΔP. A comparison of their degradation profiles, shown in Fig. 8, reveals that both HCSP41ΔS and ΔE degrade petD50 RNA in the same manner as wild-type HCSP41 but that HCSP41ΔP does not. Therefore, an essential domain for HCSP41 RNase activity is located between amino acid residues 73 and 191. Based on the results shown in Fig. 4B, the fact that HCSP41ΔS and ΔE degrade petD50 RNA more quickly than wild-type HCSP41 may imply that the C terminus of CSP41 is involved in the RNA-binding activity. In this hypothesis, removal or partial removal of the C terminus would eliminate or partially eliminate the RNA-binding activity of CSP41 and thus enhance the efficiency of its RNase activity.

Although no overall similarity was noted between CSP41 and other proteins, careful examination of the search results revealed a 50-amino acid stretch from Glu127 to Trp176 of CSP41 which has 50% similarity to a sequence from Gln123 to Trp172 of nuclease P1, a bacterial endonuclease. Furthermore, this region contains the proposed active site of nuclease P1 (Pro\(^{128}\)Leu\(^{129}\)His\(^{130}\)), which can be aligned with Pro\(^{128}\)Pro\(^{129}\)His\(^{130}\) of CSP41 (Fig. 9A, 22). Because the 50-amino acid sequence lies within the region containing the RNase domain of CSP41, we decided to test whether this sequence contributed to CSP41 RNase function. We used the alanine-scanning approach (23) to make mutant HCSP41A126, changing the wild-type sequence Asp\(^{126}\)Glu\(^{127}\)Pro\(^{128}\)Pro\(^{129}\)His\(^{130}\) to Ala\(^{126}\)Ala\(^{127}\)Pro\(^{128}\)Pro\(^{129}\)Ala\(^{130}\). In this way, all of the hydrophilic residues in the immediate vicinity of the putative active site were changed. HCSP41A126 was prepared in the same way as the deletion mutant proteins. To avoid any variation in the RNase activity due to expression and purification conditions, especially to efficiency of refolding, a wild-type HCSP41 preparation was concomitantly made as a control. The purities of the preparations were examined in an SDS-polyacrylamide gel (Fig. 9B). In contrast to our expectations, HCSP41A126 still exhibited strong endoribonuclease activity (Fig. 9C), similar to that of wild-type HCSP41. We conclude that RNase active site of CSP41 is not contained within this sequence.

**DISCUSSION**

In this paper we have shown that CSP41, a spinach chloroplast endoribonuclease, is able to cleave multiple single- and double-stranded RNA molecules but not DNA. CSP41 appears to exhibit a preference for stem-loop structures found at the 3' termini of chloroplast mRNAs, and we have shown that for petD it cleaves initially within the stem and subsequently proximal to it. Since the stem-loop is known to stabilize chlo-
roplast transcripts (1–7), this cleavage could be a rate-determining step in petD mRNA degradation.

3’ to 5’ exonucleolytic hydrolysis is a major pathway for mRNA degradation in eukaryotes, prokaryotes, and organelles. Initially, an endoribonuclease is usually required to overcome a 3’ barrier, such as a stem-loop structure or poly(A) tail, to generate free 3’ ends susceptible to exonucleolyses (11, 24, 25). RNase III and RNase E are two of the best-characterized endoribonucleases in bacteria (9), and eukaryotic homologues have been reported (26, 27). In bacteria, RNase III and RNase E can cleave mRNAs within and upstream, respectively, of mRNA 3’ stem-loop structures (28–30). Since CSP41 is a chloroplast endoribonuclease and cleaves initially within a stem-loop structure, it is more similar to RNase III than to RNase E. However, RNase III typically makes staggered, double-strand cleavages in duplicated regions, whereas CSP41 cleaves only one strand in the duplex region of petDΔ50 RNA. In addition, RNase III has a K’ optimum of 100–300 mM, conditions under which CSP41 RNAse activity is partially inhibited (Table I).

Several endoribonuclease activities, namely EndoC1, EndoC2, and a putative RNase E-like protein, have been identified in spinach chloroplasts by our laboratory and others (15, 17). Although each activity can cleave the 3’-UTR of petD mRNA in vitro, a comparison of cleavage sites and optimal conditions for their activities suggests that CSP41 is distinct from them. It is important to note, however, that each of these other spinach chloroplast activities was examined only in a complex mixture of proteins. CSP41 also does not appear to be related to nuclease P1, despite 50% similarity over a 50-amino acid region (Fig. 9). Therefore, CSP41 could represent a new class or subclass of ribonucleases.

Our data show that CSP41 degrades both single- and double-stranded RNAs but prefers RNAs containing a stable stem-loop structure (Figs. 2 and 4). These data suggest that CSP41 could recognize certain special nucleotide sequences or structural features of stem-loop regions. We have noted that the two groups of initial cleavage sites are located immediately upstream of two bulges in the petD stem-loop structure (Fig. 6B). However, further characterization of CSP41 RNAse activity, especially using other stem-loop-containing RNAs as substrates, is necessary to define its specificity. The 3’ stem-loop structure of chloroplast mRNA has been demonstrated to function as a determinant of both correct 3’ processing of pre-mRNA and stabilization of mature mRNA by impeding the activities of 3’ to 5’ processive exonucleases (1–7). Our study suggests that CSP41 may be a candidate to overcome the 3’ secondary structure and initiate degradation of upstream RNA sequences, possibly through the polyadenylation-mediated pathway shown to function in bacteria (31, 32) and chloroplasts (33, 34).

Although some RNases have RNA-binding activities to target their substrates (35), the RNA-binding activity of CSP41 does not enhance specificity or efficiency of its RNAse activity in our in vitro assay (Fig. 4), indicating that this RNA-binding activity could be involved in a second function. CSP41 possesses two important properties as an RNA-binding protein. First, it binds only to the 3’-UTRs of certain chloroplast pre-mRNAs, such as those of petD and rbcL, because it recognizes both the 3’ stem-loop structure and a downstream AU-rich element (Fig. 3); and second, it interacts with CSP55 and CSP29 to form a more stable complex containing the 3’-UTR of the selected pre-mRNA and multiple protein subunits (18, 19). Although the functions of CSP55 and CSP29 are still unknown, it is possible that the function of CSP41 could be altered in this larger complex by suppression of its RNAse activity. In fact, our data suggest that bound CSP41 may cleave substrate RNAs less efficiently than free CSP41 (Fig. 4). Therefore, our data support one of our previous (19) models that CSP41 could have two functions in chloroplast mRNA metabolism. First, free CSP41 could directly cleave chloroplast mRNA and pre-mRNA in the 3’ stem-loop structure to initiate bulk mRNA degradation, and second, as CSP41, along with CSP55 and CSP29, forms a stable RNA-protein complex with the 3’ stem-loop structure of a pre-mRNA, its RNAse activity could be suppressed and the entire complex could serve as a steric hindrance to 3’ to 5’ processive exonucleolyses, and thus ensure efficient 3’ end maturation.

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