PROGRESS REPORT

Differential Regulation of Plastid mRNA Stability

This Progress Report describes research activities during the first 20 months of the funding period. Our rationale and research objectives, as stated in the original funded grant application, were the following:

The long-term goal of our work is to understand how the stability and expression of higher plant organelle-encoded mRNAs are developmentally regulated, and the role of the nuclear genome in this process. The goal of this proposal is to identify *cis*-acting sequences and *trans*-acting factors that function in plastid mRNA maturation, stabilization, and/or decay through an *in vitro* and *in vivo* analysis of mRNA:protein interactions. We have built on our previous results which have emphasized the study of 3' end inverted repeat sequences (IRs) that serve both as mRNA processing elements and stability determinants *in vitro*, and associate with plastid proteins that potentially play enzymatic, structural and/or regulatory roles. The specific goals of the project are:

1. To define, by single base and internal deletion mutagenesis, the sequence and structural requirements for protein binding to the 3' IRs of *petD* and *psbA* mRNAs.

2. To purify RNA-binding proteins that demonstrate gene- or sequence-specific binding, or that are implicated in RNA stabilization or decay. The purified or partially purified proteins will be used in functional assays.

3. To investigate the native form of mRNA in the plastid, by attempting to purify ribonucleoprotein (RNP) particles from organelles.

Below, our progress on these objectives is summarized, emphasizing our unpublished data.

Objective 1: Mutagenesis of 3' IRs

To address the role of RNA sequence and structure in RNA:protein interactions and RNA stability, we have mutagenized the *petD* and *psbA* 3' IR regions, and tested the mutant RNAs in a variety of *in vitro* experiments.

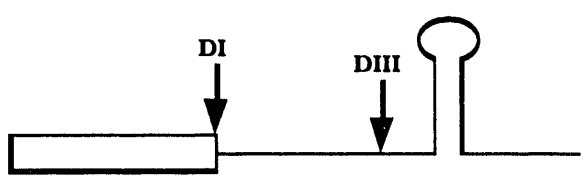

Figure 1. EndoC2 cleavage sites in the *petD* 3' region. The box represents the protein coding region, and DI and DIII represent the major and minor endoC2 cleavage sites, respectively.

petD

To date, we have obtained and studied approximately 40 mutant forms of the *petD* 3' IR (Stern *et al.* 1989; Chen and Stern 1991a; 1991b). Approximately two-thirds of the mutations have no major effects on RNA stability or protein binding, as judged by *in vitro* RNA decay assays and UV-crosslinking, respectively. A new approach, described below, will allow us to re-examine these mutants for protein-binding effects that cannot be detected by UV-crosslinking. Fifteen of the mutations, however, did affect *petD* 3' IR-RNA stability and/or protein interactions. Their properties are described in the Table below.

From the group of petD2 mutants, we have identified an endoribonuclease activity that we call endoC1 (chloroplast endoribonuclease 1). EndoC1 efficiently cleaves only petD2.x 3' IR-RNAs. This susceptibility is correlated with altered RNA secondary structure. EndoC1 may therefore represent a chloroplast endoribonuclease activity with a selectivity for certain sequences and/or RNA structures. EndoC1 is described in detail in Chen and Stern (1991b).

Mutants petD11 and petD12 contain mutations in two cleavage sites recognized by an endoribonuclease that we call endoC2 (also described in Chen and Stern, 1991b). These cleavage sites are located in the 3' UTR, as shown in Figure 1. As discussed below, we hypothesize that endoC2 cleavage at the DI site of *petD* mRNA occurs *in vivo*, and that endoC2 cleavages at site

Mutant designation	Class of mutation	Alteration in IR	*In vitro* effect of mutation
petD2	RNA stability	double base change	unstable; endoC1-sensitive
petD2.D10	RNA stability	petD2 + stem mutation	endoC1-sensitive
petD2.Dd6	RNA stability	petD2 + lower loop Δ	endoC1-sensitive
petD2.Dd5	RNA stability	petD2 + upper stem Δ	endoC1-sensitive
petD11	RNA stability and protein binding	14 nt changed proximal to IR at DI site/55 RBP consensus binding site	no cleavage at endoC2 site DI; no binding to 55 RBP
petD12	RNA stability and protein binding	14 nt changed proximal to IR at DIII site	no cleavage at endoC2 site DIII; reduced binding to 29 RBP
petD7	protein binding and 3' processing	single base change in stem of IR	reduced binding to 29 RBP; more efficient 3' processing
petD7.1	similar to mutant petD7		
petD7.2	similar to mutant petD7		
petD7.3	similar to mutant petD7		
petD7.4	protein binding and 3' end processing	double base change in stem of IR	reduced binding to 29, 55 RBPs; more efficient 3' processing
petD8	protein binding	double base change in IR	reduced binding to 29 RBP
petD7.C2	compensatory	restores stem to petD7	restores 29 RBP binding
petDΔ24	RNA stability	lacks 3' half of IR	RNA rapidly degraded
petDΔ63	RNA stability	lacks part of IR stem 2	RNA rapidly degraded
Mutants are described in Stern *et al.*, 1989; Chen and Stern, 1991a,b and Chen *et al.*, submitted. "Δ" indicates a deletion.			

DI (DI is at the termination codon/DraI site) may be an initial and possibly rate-limiting step in mRNA decay. Mutations at the DI and DIII sites independently prevent endoC2 cleavage *in vitro*, demonstrating a role for RNA sequence and/or structure.

The Table above also lists a series of petD7 mutations, and petD8, as possessing defects in the binding of 29 kd and/or 55 kd RNA-binding proteins (RBPs). The RBPs are discussed under objective 2. These mutants also undergo more efficient 3' end processing, as described below. Finally, as we have previously shown, partial deletions of the IR destabilize RNA *in vitro*.

psbA

Site-directed mutants of the *psbA* 3' IR are described in Adams and Stern (1990). To summarize, we have measured the processing and stability of wild-type and eight mutant RNAs corresponding to the 3' UTR of *psbA* mRNA. Wild-type and mutant 3' IR-RNA precursors were processed at similar rates *in vitro*, but RNAs with either a mutant loop sequence CUUCGG or specific base substitutions in the IR exhibited an enhanced accumulation of mature product. Incubation of mature products in the *in vitro* system demonstrated that this was due to an increased stability of the product. These mutant RNAs displayed the same order of stabilities when their decay was measured following electroporation into intact chloroplasts.

We found that the *in vitro* system contains an endonuclease activity that cleaves the wild-type 3' IR-RNA within the loop and also in other single-stranded regions, suggesting a possible role for the loop sequence in determining RNA longevity *in vitro*. Interestingly, the altered loop sequence CUUCGG, which enhances RNA stability in bacteria, prolonged the half-life of *psbA* 3' IR-RNA *in vitro* and also resulted in an altered endonuclease cleavage pattern. Such nucleases could potentially play an important role in plastid mRNA decay *in vivo*.

Objective 2: Analysis of RNA-binding proteins

Our studies of spinach chloroplast RBPs have taken two directions: analysis of RBP function, and purification of RBPs. Because many RBPs can be detected by UV-crosslinking and other techniques, we have been selective in choosing which proteins to purify.

RBP function

We have studied RBP function in two ways. The first is to conduct *in vitro* RNA processing/stability assays on RNA substrates in which mutations alter the protein binding pattern. In this way we may obtain correlative evidence between the lack of protein binding and a processing effect. The second analytical tool is to use partially or fully-purified RBPs in RNA-binding and/or RNA processing assays. This strategy allows us a) to determine a potential enzymatic function for the protein; and b) to use footprinting techniques to study RNA:protein interactions.

The Table on p. 2 shows that the petD7.x and petD8 mutations allow more efficient RNA processing, and do not bind 29 RBP. 29 RBP binds to the *petD* stem/loop (Stern *et al.*, 1989; Chen and Stern, 1991a), and we hypothesize that lack of binding in petD7.x and petD8 mutants allows the processing exonuclease to approach the mRNA maturation site more efficiently (Chen and Stern, 1991b). We have also investigated a potential role for 29 RBP in transcription termination and RNA stability, and so far have obtained negative results. Our current model is that 29 RBP is involved in 3' end formation as described above, and also in the assembly of RNA:protein complexes (see Objective 3).

As shown in Figure 2, we have purified a poly-U-sensitive (i.e. RNA binding does not occur in the presence of poly-U) RBP of approximately 100 kd to apparent homogeneity, with the final step being poly-C-agarose affinity chromatography. Using much larger amounts of protein, the 100 kd band was transferred to nitrocellulose and N-terminal sequencing was attempted. However, an ambiguous sequence was obtained, possibly due to insufficient protein. UV-crosslinking and gel shift analyses show that 100 RBP binds to the 3' end of the *petD* coding region in the absence of poly-U and in the presence of other IR binding proteins such as 41 RBP (see below) and 29 RBP (Chen and Stern, 1991a). In the absence of these other proteins 100 RBP will also bind to the IR. It does not bind to RNA under gel shift conditions (pH 9 Tris-glycine). Preliminary enzymatic assays do not show any obvious nucleolytic activity associated with this protein. Its biochemical properties have some interesting parallels with a core mRNA-binding (hnRNP) protein found in animal cells, as described in the Experimental Plan.

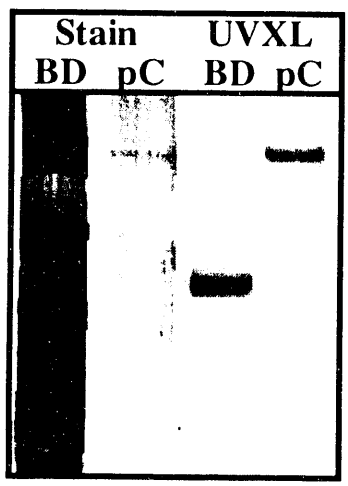

Figure 2. A silver-stained gel (left two lanes) and UV-crosslinking (right two lanes) are shown of the 1M KCl eluate from a heparin agarose column (see also Fig. 8, lane BD) and a 0.3M KCl eluate from a poly-C agarose column (pC). The proteins were crosslinked with wild-type *petD* 3' IR-RNA. The strongly crosslinking band in the BD lane is 55 RBP.

A third protein that we have devoted considerable effort to is 55 RBP, for which there are three binding sites in the *petD* 3' region (Fig. 3; in Chen and Stern 1991a it is referred to as a 57 kd protein). Two lines of evidence indicate that 55 RBP represents a group of two or more related proteins. First, ammonium sulfate fractionation yields two distinct 55 RBP-binding activities. The 30-45% fraction 55 RBP (55' RBP) binds to the most distal *petD* binding site, and is not labeled by *psbA* or *rbcL* 3' RNAs in crosslinking experiments. The 45-60% fraction 55 RBP binds to the two proximal sites, and possibly to all three sites, and is also labeled by *psbA* and *rbcL* 3' IR-RNAs in crosslinking experiments. A second line of evidence for two types of 55 RBP comes from a partially purified 55 RBP fraction that we obtained from Wilhelm Gruissem's laboratory. The 55 RBP in this fraction corresponds to 55' RBP. It was isolated by heparin FPLC of proteins that had not been ammonium sulfate fractionated, and eluted at high salt (~0.7M). In the same column, the other 55 RBP activity or activities eluted in a broad peak at lower salt concentrations.

Although all 55 RBPs are labeled strongly in crosslinking experiments, gel shift/SDS-PAGE using 55' RBP demonstrates that this protein does not bind stably to RNA. However, 55 RBP is involved in RNA:protein complex formation, as shown below. When the 55' RBP binding site is deleted (e.g. in petDΔ50), complex formation is not affected. RNAse-H generated fragments containing either of the two remaining sites are also capable of complex formation (Chen and Stern, 1991a), but an RNA containing both sites, with the proximal site destroyed by deletion mutagenesis (petD11), does not label 55 RBP in crosslinking experiments and forms complexes poorly (Fig. 4). This suggests that in the presence of two sites, complex formation initiates at the proximal site.

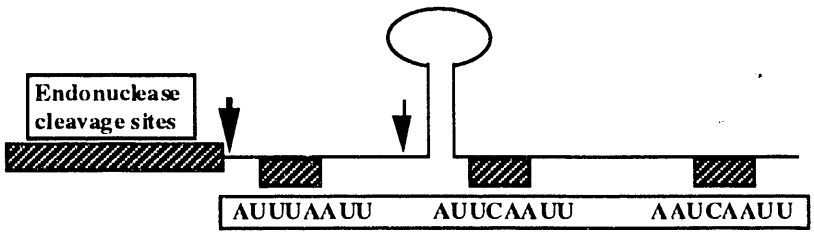

Figure 3. The consensus sequences at the apparent binding sites of 55 RBP are shown. The protein called 55' RBP binds to the most distal site. EndoC2 cleavage sites are shown (see Fig. 1).

55 RBP has co-purified with the endoC2 activity described above using three different methods. These are 1) a 0.7M KCl fraction from a heparin-agarose column; 2) a 60 mM KCl fraction from a poly-U agarose column; and 3) a 60 kd fraction from a gel filtration column of native chloroplast proteins. We are therefore pursuing the idea that 55 RBP is the same as, or closely associated with, endoC2. As a first step, we separately mutated the two endoC2 cleavage sites DI and DIII, creating mutants petD11 and petD12, respectively. As shown in the Table and in Figure 4, endoC2 does not cleave the mutated sites. Interestingly, Figure 4 shows that the petD11 mutation also eliminates 55 RBP binding, and inhibits RNA:protein complex formation. On the protein level, the only affect of the petD12 mutation appears to be reduced binding of 29 RBP, consistent with the apposition of this mutation to the IR.

Our interpretation of these results is that endoC2 cleavage site DI and one of the 55 RBP recognition sites are very close or overlap, suggesting a potentially interesting regulatory scenario. The additional effects on UV-crosslinking and protein complex formation are surprising, since a) there are multiple 55 RBP binding sites; and b) *petD* 3' IR-RNA shifted complexes do not contain exclusively 55 RBP (see below). The simplest explanation is that the 55 RBP site mutated in DI serves as a nucleation site for complex formation, including binding at other 55 RBP sites. This model is diagrammed in Figure 5. There are also alternative explanations for these data, which have been omitted from this discussion in the interest of brevity. To address the relationships between 55 RBP binding and endoC2 activity, and to understand the role of

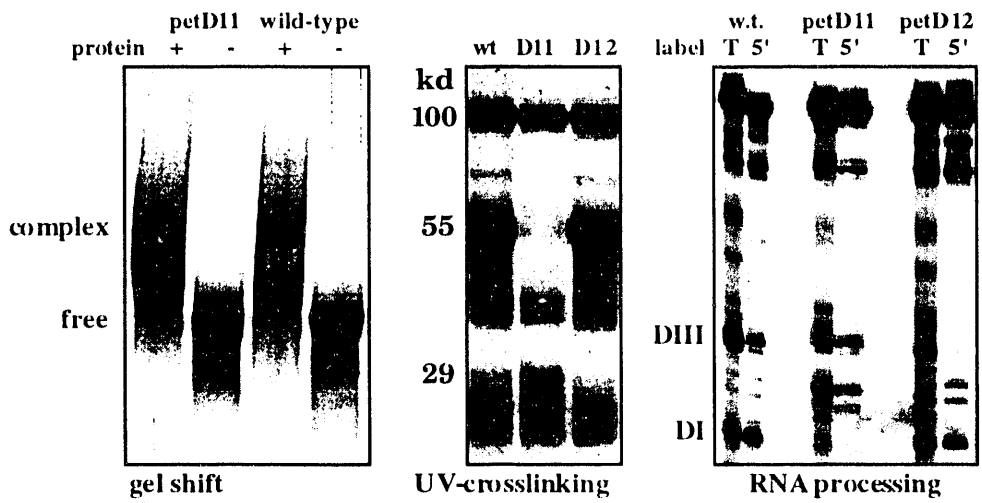

Figure 4. ^{32}P-labeled wild-type or mutant *petD* 3' IR-RNAs were incubated with total soluble chloroplast proteins and electrophoresed in a 50 mM Tris-glycine gel without T1 nuclease treatment (left). The mobility shift is much more pronounced for wild-type RNA. The center panel shows UV-crosslinking of the same RNAs and protein fraction. In the right panel, body-labeled (T) or 5' end-labeled (5') 3' IR-RNAs were incubated in a gel filtration fraction containing endoC2 activity. In petD11, cleavage does not occur at site DI; in petD12, cleavage does not occur at site DIII.

different 55 RBP binding sites in complex formation and RNA degradation, additional site-directed mutants are being constructed, as shown in Figure 6.

A second approach has been to elute 55' RBP from SDS gels and use the purified protein in endoC2 assays. Protein was isolated from fraction 67 (Fig. 8), which when overloaded showed bands at 100 kd, 79 kd, 55 kd, 51 kd, 41/39 kd, 30 kd and 22 kd. These seven fractions were tested for endoC2 activity, and Figure 7 shows that there is activity in the 79 kd and 55 kd fractions. The 41 kd/39 kd fraction contained non-specific nuclease activity. These results are consistent with 55' RBP and endoC2 being equivalent, although the previously undetected 79 kd protein appears to also have activity, with a slightly different cleavage pattern. Since there may be multiple proteins in these fractions, we consider these results to be suggestive, but not conclusive. Confirmatory experiments are in progress.

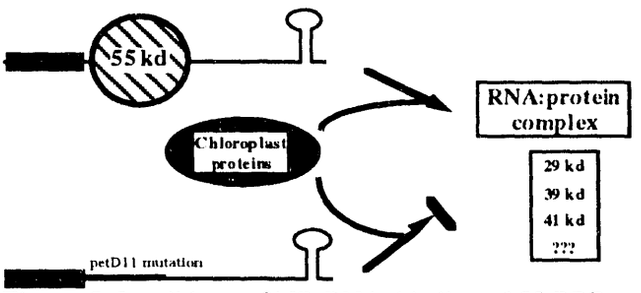

Figure 5. In wild-type 3' IR-RNA, binding of 55 RBP to the most proximal site is a first step in complex formation that involves other chloroplast RBPs (see also Fig. 8). The petD11 mutation prevents binding of 55 RBP to the proximal site, thus affecting subsequent steps of complex formation. Since other proteins can still be labeled by UV-crosslinking (Fig. 4), their associations with petD11 3' IR-RNA must be largely transient.

RBP purification

As described above, 100 RBP has been purified by affinity chromatography, and will be further characterized. We have come to recognize that 55 RBP represents a group of similarly-sized, but biochemically distinct RNA-binding proteins, as described above. One of the 55 RBPs, namely 55' RBP, has been partially purified by W. Gruissem's laboratory. Two-dimensional gels reveal co-migration with two non-RBP proteins of 55 kd. This partially purified fraction contains endoC2 activity and a 55 RBP that binds to *petD*, but not *psbA* or *rbcL* 3' IR-RNAs. We are collaborating with W. Gruissem's laboratory on further studies of this protein (see below and Experimental Plan).

An important advance that we have recently made involves the purification of RBPs from RNA:protein complexes formed *in vitro*. We were inspired to try this technique by papers from Danon and Mayfield (EMBO J. 10:3993; 1991) and Brewer (MCB 16:2460; 1991). Briefly, *in*

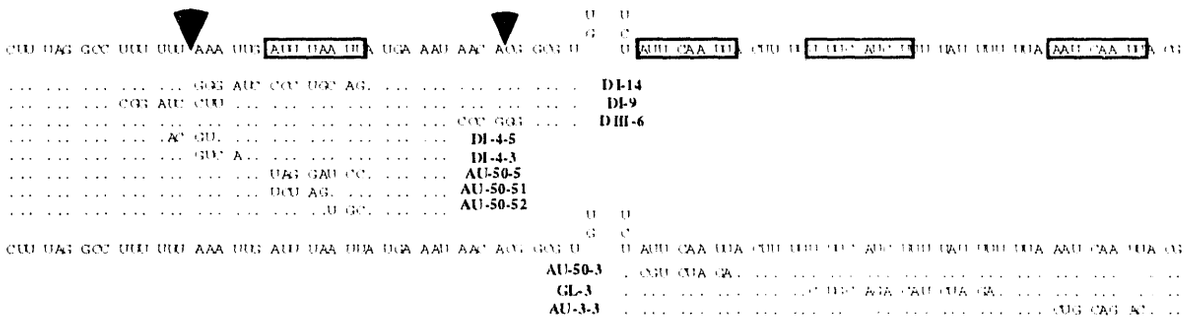

Figure 6. Oligonucleotide-directed mutagenesis of the *petD* 3' region. The bottom only of the stem/loop is shown. Mutagenesis is planned for the two endoC2 cleavage sites (DI and DIII), the IR-proximal 55 RBP binding site (AU-50-5/51/52), the IR-distal 55 RBP binding sites (AU-50-3 and AU-3-3), and a sequence similar to the 54 RBP binding site in mustard chloroplast RNA (GL-3). The 54/55 RBP binding sites are boxed; DI and DIII are indicated by large and small arrows, respectively.

vitro-synthesized ^{32}P-labeled RNA is mixed with chloroplast proteins and treated with RNAse T1, and unbound RNA is separated from RNA:protein complexes in a native gel (gel shift). The RNA:protein complex-containing band is excised from the native gel and electrophoresed directly into a denaturing gel (SDS-PAGE). When this technique was scaled up using 0.5-2 pmol of *in vitro*-synthesized RNA, silver staining of the SDS gel revealed 1-6 proteins (depending on the RNA and protein fractions used), as shown in Figure 8. Among these proteins is one

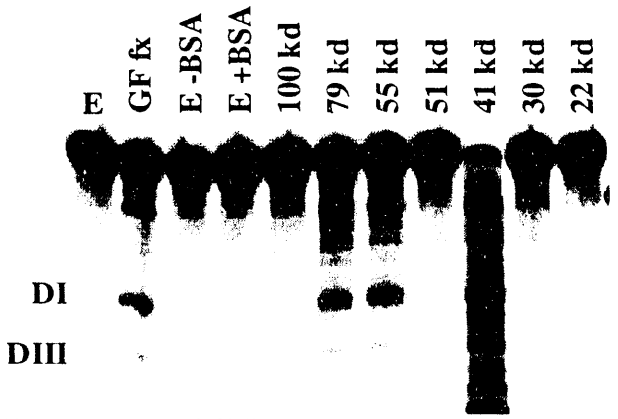

Figure 7. petDΔ3 3' IR-RNA was incubated in buffer alone (E), buffer containing BSA, a gel filtration fraction containing endoC2 activity (GF), and fractions containing proteins eluted from an SDS gel and precipitated with BSA as a carrier (except 55 kd and 41/39 kd fractions). The faint band near the DI position in control lanes represents a hypersensitive site in the RNA.

tentatively identified as 29 RBP, and others that are undetectable by UV-crosslinking, presumably due to the nature of the RNA:protein or protein:protein interaction.

One novel protein that we have identified is 41 RBP. This protein co-purifies with 55' RBP on heparin columns (fraction 67 in Fig. 8), and is also detected when HA-bound proteins are used in the gel shift/SDS-PAGE assay. Since T1 nuclease is used to treat complexes before shifting, we recovered the protected RNA fragment and subjected it to T1 fingerprinting. Figure 9 shows that the IR and 3' flanking region is protected. Since only 41 RBP is present in the complex, this suggests that 41 RBP binds to the *petD* 3' IR. One of our goals for the next granting period is to study 41 RBP.

We are confident that the gel shift/SDS-PAGE procedure can be scaled up to yield sufficient quantities for antibody production, biochemical assays, and gene isolation. This work is currently in progress.

Objective 3: Ribonucleoprotein particles from chloroplasts and *in vivo* studies

We have obtained extensive information on RNA:protein interactions, plastid RNA processing and decay pathways by using *in vitro* assays. We feel it is important, however, to compare our *in vitro* results with data obtained from native chloroplasts or intact plants. Although in many cases such direct comparisons are problematic, we have taken several approaches to address this issue, as described below.

Native RNA:protein complexes

We made considerable efforts to isolate RNA:protein complexes from chloroplasts using cesium sulfate equilibrium centrifugation, FPLC gel filtration, sucrose density centrifugation, and combinations of methods. Although we have been able to obtain high molecular weight material containing mRNA, and although in several cases RNA-binding proteins and mRNA have co-purified, we are not satisfied that these represent *bona fide* RNP particles. The reason is that control preparations of deproteinized RNA have fractionation properties too similar to the native preparations. For this reason, we are not pursuing this line of investigation.

Native protein complexes

Through several approaches, including some of those used to search for RNA:protein complexes, we have observed co-fractionation of RNA-binding proteins (Stern *et al.*, 1990). As an example, heparin-agarose fraction 67 (see Fig. 8), which contains 55 kd, 41 kd and 39 kd proteins, was further fractionated by gel filtration. The purpose was to purify 55' RBP for endoC2 studies. Figure 10 shows, however, that the 39 kd and 55 kd proteins cannot be

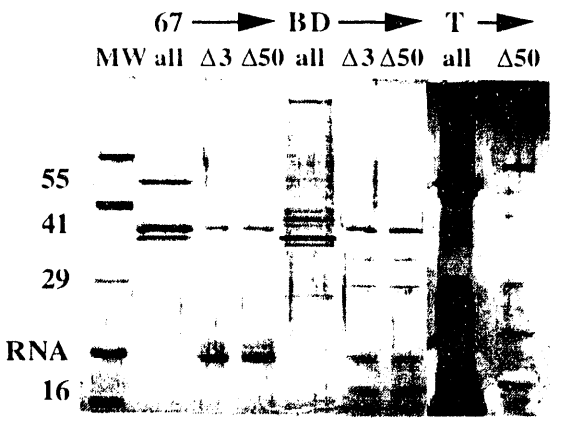

Figure 8. Gel shift/SDS-PAGE analysis of chloroplast protein fractions. 67, BD and T are fraction 67 proteins from a heparin-agarose column, obtained from W. Gruissem's laboratory, which include 55' RBP, 41 RBP, and a 39 kd protein, total spinach proteins bound to heparin-agarose in low salt and eluted with 1M KCl, and total soluble proteins, respectively. petDΔ3 and/or petDΔ50 3' IR-RNA were incubated with the respective protein fractions, treated with RNAse T1 and electrophoresed in a non-denaturing gel. The shifted complex was excised and electrophoresed into an SDS-polyacrylamide gel which was stained with silver. MW are molecular weight markers; RNA represents the silver-stained and largest RNAse T1 product.

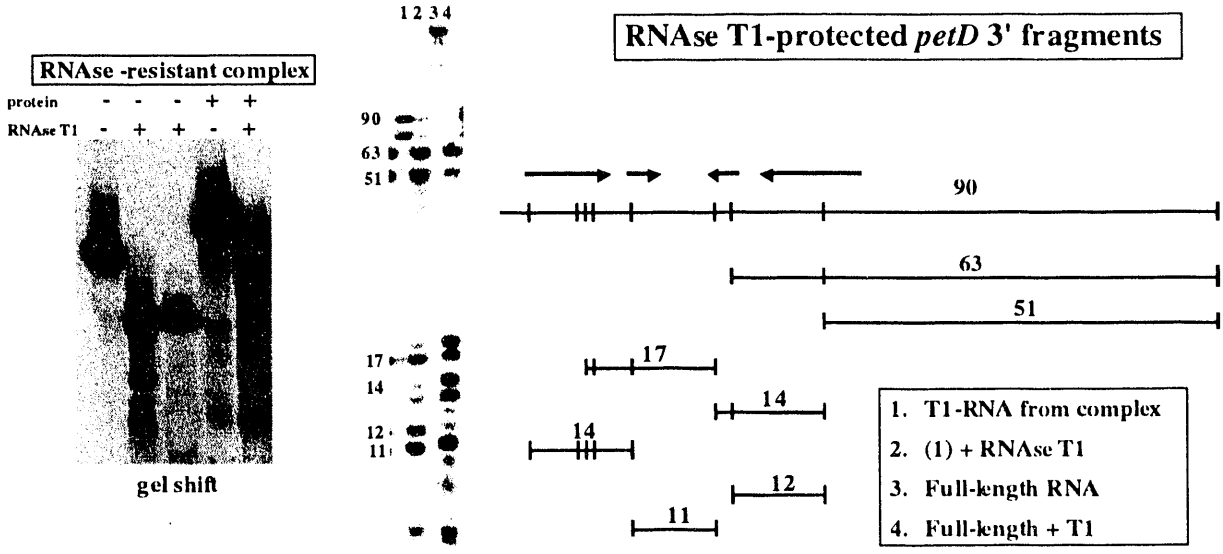

Figure 9. Wild-type ^{32}P-labeled *petD* 3' IR-RNA was incubated in the absence or presence of fraction 67 proteins (see Fig. 8), treated in some cases with RNAse T1, and electrophoresed in a 50 mM Tris-glycine gel (left). A mobility shift is seen both with and without RNAse T1 treatment. RNA was eluted from untreated and treated complexes, part of each sample was digested with RNAse T1 under partially denaturing conditions, and the products were electrophoresed in a 20% polyacrylamide/urea gel (center). The major T1-protected fragment is 90 nt, as shown in the map (right) and confirmed by secondary digestions (compare lane 2 to lane 4).

separated by this method. A second example is another gel filtration fraction, which we call fraction 9 (Chen *et al.*, submitted). Incubation of fraction 9 proteins with RNA does not result in any RNA processing. However, if this fraction is pre-incubated with EDTA, endoC2 activity is observed. Control experiments suggest that endoC2 is activated by dissociation of a large protein complex (>700 kd) in which endoC2 activity is suppressed. These data, and others, strongly argue that RBPs and ribonucleases are found in macromolecular complexes.

Finally, as shown above, *in vitro*-formed RNA:protein complexes identify RNA-binding proteins that are not detected by UV-crosslinking experiments. As we develop antibody probes for these proteins in the future, we will be able to use them to immunoprecipitate the native proteins, which will presumably co-purify with other components of their complexes such as proteins and perhaps RNA. Thus, we plan to develop new tools to analyze the *in vivo* form of plastid mRNA.

Electroporation

As we have shown (Adams and Stern, 1990), RNAs can be electroporated into living chloroplasts and their decay rates measured. For the three *psbA* 3' IR-RNAs tested, we observed decay rates in electroporated chloroplasts that had the same rank order as they did *in vitro*. Although the applications of electroporation to our experiments are limited by the inefficiency of the process (>90% of chloroplasts are ruptured by the electrical pulse), we will use it selectively in the future, as we are currently for endoC2 assays.

Analysis of RNA decay intermediates

We have recently obtained strong evidence for endoC2-mediated mRNA cleavage *in vivo*. By using S1 nuclease protection, we have identified *petD* mRNA in several RNA preparations with 3' ends corresponding exactly to the endoC2 DI cleavage site as shown in Figures 1 and 6. The discovery of these decay intermediates lends credence to our proposal that endoC2 cleavages form part of the *petD* mRNA decay pathway *in vivo*. As we study other nuclease activities, we will apply this technique.

Chloroplast transformation

Pal Maliga's laboratory at Rutgers University has made great strides in transforming tobacco chloroplasts. We are collaborating with his laboratory to analyze 3' end mutations *in vivo*. To do

this, we are creating transgenic plants with wild-type or altered *petD* 3' regions on reporter genes. It is worth noting that the *petD* 3' UTR's of spinach and tobacco are nearly identical. These experiments are an important component of our renewal proposal.

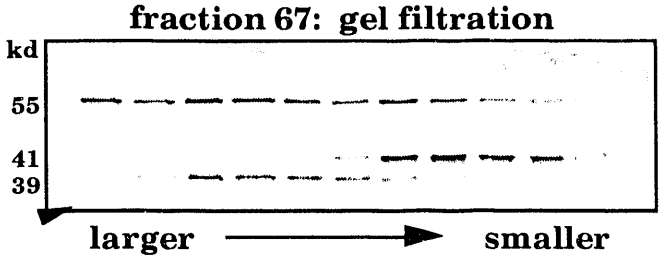

Figure 10. Fraction 67 proteins (see Fig. 8) were separated on a Pharmacia FPLC gel filtration column in 60 mM KCl. Portions of each fraction were examined by silver staining of an SDS-polyacrylamide gel. The 55 kd band spreads out over the column; we have since learned that this band consists of three proteins separable by two-dimensional electrophoresis. The co-elution of the 55 kd and 39 kd bands suggests that a complex may include these two proteins.

Summary

Our view of mRNA decay is that it is regulated by three interactive components: RNA structure, ribonucleases and RNA-binding proteins. We have used mutagenesis to study the role of RNA structure in regulating RNA decay rates, and to identify protein binding and endonuclease recognition sites. We have identified at least three endonuclease activities; one that cleaves *psbA* RNA; and two whose cleavage patterns with *petD* 3' IR-RNA has been studied (endoC1 and endoC2). Additionally, we have continued to analyze the properties of the major RNA processing exoribonuclease.

We have concentrated our efforts on three RNA-binding proteins. A 100 kd protein with properties suggestive of a mammalian RNP component has been purified. A protein of 55 kd that may also be an endonuclease has been partially purified. We have studied the interaction of a 29 kd protein with the *petD* stem/loop, and its role in RNA processing. Recently, we have used a novel gel shift/SDS-PAGE technique to identify new RNA-binding proteins that form complexes of as-yet unknown function.

Reviewed publications from DOE funding

Adams, C.C. and Stern, D.B. (1990). Control of mRNA stability in chloroplasts by 3' inverted repeats: Effects of stem and loop mutations on degradation of *psbA* mRNA *in vitro*. *Nucleic Acids Res.*, **18**, 6003-6010.

Hsu-Ching, C. and Stern, D.B. (1991a). Specific binding of chloroplast proteins *in vitro* to the 3' untranslated region of spinach chloroplast *petD* mRNA. *Molec. Cell. Biol.*, **11**, 4380-4388.

Hsu-Ching, C. and Stern, D.B. (1991b). Specific ribonuclease activities in spinach chloroplasts promote mRNA maturation and degradation. *J. Biol. Chem.*, **266**, 24205-24211.

Chen, Q., Hamblin, M., Adams, C.C. and Stern, D.B. Characterization of a specific endoribonuclease activity that cleaves the 3' end of spinach chloroplast *petD* mRNA. *J. Biol. Chem.*, (submitted 6/10/92).

Invited publication

Stern, D.B., Chen, H.C., Adams, C.C. and Kindle, K.L. (1990). Post-transcriptional control of gene expression in chloroplasts. In: Post-transcriptional Control of Gene Expression, J.E.G. McCarthy and M.F. Tuite, eds., NATO-ASI series on Cell Biology, Springer-Verlag, Heidelberg, Vol. H 49, pp. 73-82.

Other reference cited

Stern, D.B., Jones, H. and Gruissem, W. (1989). Function of plastid mRNA 3' inverted repeats: RNA stabilization and gene-specific protein binding. *J. Biol. Chem.*, **264**, 18742-18750.

DISCLAIMER

END

DATE
FILMED
10 15 93