The Plant Cell, Vol. 8, 107–117, January 1996 © 1996 American Society of Plant Physiologists

Incomplete Editing of *rps12* Transcripts Results in the Synthesis of Polymorphic Polypeptides in Plant Mitochondria

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C-to-U editing causes specific nucleotide changes in plant mitochondrial mRNAs that are required for the restoration of the evolutionarily conserved amino acid sequence. Transcripts for the ribosomal protein S12 gene (*rps12*) have six C-to-U editing sites and are highly heterogeneous as a result of incomplete editing. Immunological analysis demonstrated that unedited or partially edited transcripts as well as edited mRNAs are translated. The edited *rps12* translation products accumulate as ribosomal subunits, but the unedited *rps12* translation products are present as unassembled subunits and are not detected in the ribosomes. Thus, gene expression is polymorphic as a result of incomplete C-to-U editing, and aberrant polypeptides are present from the translation of these mRNAs. However, because only the edited translation products accumulate in mitochondrial ribosomes, the overall expression of *rps12* is rendered coherent by the selection of edited translation products for ribosomal biogenesis.

INTRODUCTION

RNA editing in plant mitochondria is a prevalent RNA processing step and frequently results in C-to-U changes in the coding sequences of mRNAs (Covello and Gray, 1989; Gualberto et al., 1989; Hiesel et al., 1989; Schuster et al., 1991). RNA editing in plant mitochondria appears to be a genetic correction mechanism that occurs at the RNA level. Two observations support this view: first, comparison of the deduced amino acid sequences from unedited and edited mRNAs demonstrates that the edited sequence corresponds to the evolutionarily conserved information (Gualberto et al., 1989); second, C-to-U changes usually result in a change in the amino acid specified by the codon, whereas "silent" changes in the wobble nucleotide occur at reduced frequency (Bonnard et al., 1992; Gray and Covello, 1993). Thus, RNA editing plays a genetic role in gene expression in plant mitochondria.

Plant mitochondrial transcripts are edited with various degrees of efficiency. Transcripts for some genes are essentially completely edited, and only infrequently are incompletely edited transcripts detected. Examples of transcripts that show homogeneously edited populations include spliced cytochrome *c* oxidase subunit 2 (*cox2*) transcripts (Sutton et al., 1991; Yang and Mulligan, 1991), ATP synthase subunit 9 (*atp9*) transcripts (Bégu et al., 1990; Rish and Breiman, 1993), and NADH dehydrogenase subunit 4 (*nad4*) transcripts (Lamattina and Grienenberger, 1991). In contrast, many plant mitochondrial transcripts exhibit pronounced heterogeneity as a result of incomplete RNA editing (Covello and Gray, 1990; Schuster et al., 1990; Gualberto et al., 1991; Kempken et al., 1991; Salazar et al., 1991; Lu and Hanson, 1992, 1994).

The genetic consequences of heterogeneity of mRNAs that results from incompletely edited transcripts are unclear. Incompletely edited transcripts would encode polypeptides with radical amino acid substitutions. The amino acid sequence of the wheat ATP9 polypeptide has been shown to reflect the fully edited mRNA sequence; however, the corresponding region of the RNA exhibits little to no heterogeneity from incomplete C-to-U editing (Bégu et al., 1990). Amino acid sequence analysis of NADH dehydrogenase subunit 9 polypeptide (NAD9) from potato demonstrated that the subunit isolated from purified complex 1 reflects the fully edited translation product, even though the corresponding mRNAs exhibited incomplete editing (Grohmann et al., 1994). In addition, a single homogeneous form of ATP synthase subunit 6 polypeptide (ATP6) accumulated, despite the presence of incompletely edited *atp6* mRNAs in petunia mitochondria (Lu and Hanson, 1994). Thus, analysis of polypeptides from assembled ATP synthase or NADH dehydrogenase complex indicates that only edited translation products accumulate, even though the corresponding mRNAs are heterogeneous. Some investigators have suggested that unedited transcripts are not translated in plant mitochondria based on amino acid sequence analysis of mature, assembled polypeptides (Grohmann et al., 1994) or based on reduction of the frequency of unedited transcripts in polysomal RNA (Gualberto et al., 1991).

To determine whether unedited transcripts are translated, we have developed epitope-specific antibodies to the ribosomal

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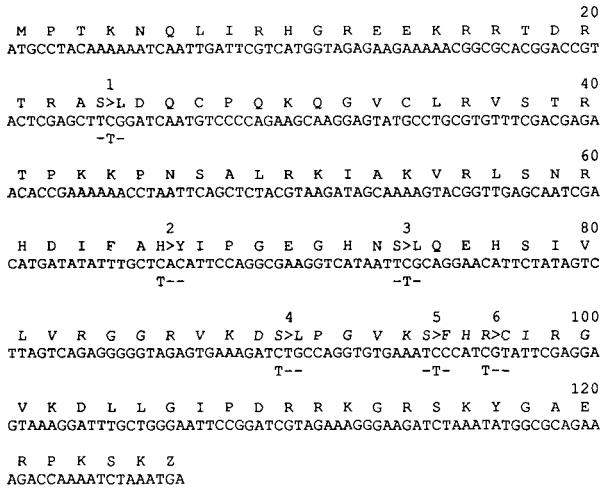

Figure 1. *rps12* DNA Sequence and Editing Sites.

The DNA sequence of the maize mitochondrial *rps12* gene is shown under the deduced amino acid sequence. The edited nucleotides are shown as T under the genomic sequence, and editing sites are identified by numbers 1 to 6. Amino acid changes directed by C-to-U editing are shown as the unedited > edited amino acid residues. The numbering of amino acid residues is indicated at the end of each line.

protein S12 polypeptide (RPS12) that reflect unedited and edited translation products. Immunological analysis demonstrated that unedited or partially edited transcripts as well as edited *rps12* mRNAs are translated; however, only the edited translation product accumulates in mitochondrial ribosomes.

RESULTS

rps12 Transcripts Are Incompletely Edited

The maize mitochondrial *rps12* gene and edited mRNA sequence are shown in Figure 1. Six C-to-U editing sites exist in *rps12* mRNAs, and each changes the amino acid specified by that codon. Table 1 compares the deduced amino acid sequence at maize mitochondrial *rps12* editing sites with RPS12 sequences from other organisms. The edited sequence is the evolutionarily conserved sequence, whereas the unedited transcript encodes an RPS12 polypeptide with radical amino acid substitutions at the editing sites.

DNA sequence analysis of 24 cDNA clones is shown in Table 2 and indicates that the population of cDNAs exhibited a pronounced degree of heterogeneity from incomplete editing. Only 17% (four of 24) of the cDNAs were completely edited at all six editing sites, whereas 21% (five of 24) of the cDNAs examined were edited at none of the sites. The majority of the cDNAs were at some intermediate stage of editing. In summary, 144 editing sites were examined, with only 56% (80 of 144) exhibiting C-to-U conversion.

The heterogeneity of *rps12* mRNAs was confirmed by RNA gel blot hybridization. Figure 2A shows the results of hybridization of an unedited-specific oligonucleotide probe for *rps12* transcripts in the region of editing sites 4, 5, and 6 (oligonucleotide 3). The unedited-specific probe hybridized to RNA prepared by in vitro transcription from an unedited cDNA clone (lane 1) or DNA amplified from an unedited cDNA clone (lane 3). The unedited-specific probe did not hybridize to RNA or DNA derived from edited cDNA clone (lanes 2 and 4, respectively). Thus, the unedited-specific oligonucleotide probe specifically recognized the unedited RNA or DNA sequences and clearly detected transcripts of ~900 nucleotides and a larger transcript from maize mitochondrial RNA (lane 5).

Figure 2B shows the results of hybridization of an edited-specific oligonucleotide probe for *rps12* transcripts in the region of editing sites 4, 5, and 6 (oligonucleotide 4). The edited-specific probe hybridized to in vitro–transcribed RNA and polymerase chain reaction (PCR) product from the edited clone (lanes 2 and 4, respectively). The edited probe hybridized very weakly to the in vitro–transcribed RNA prepared from the unedited clone (lane 1) and did not hybridize to the PCR product from the unedited clone (lane 3). The edited-specific oligonucleotide selectively recognized the edited mRNA and strongly hybridized to a maize mitochondrial transcript of ~900

Table 1. Evolutionary Conservation of Amino Acid Residues by C-to-U Editing

	Amino Acid Residue No.						
Ribosomal Protein S12 Source	24	66	73	90	95	97	GenBank Locus
Maize mitochondria (unedited RNA)	S	H	S	S	S	R	MIZMNAD3
Maize mitochondria (edited RNA)	L	Y	L	L	F	C	This report
Acanthamoeba castellanii mitochondria	L	Y	L	L	Y	L	ACU12386
Drosophila mitochondria	L	Y	L	V	L	A	DROTKO
Maize plastid	L	Y	L	L	Y	I	CHZMXX
Tobacco plastid	L	Y	L	L	Y	I	CHNTXX
Chlamydomonas reinhardtii plastid	L	Y	L	L	Y	I	CRECPRPS12
Marchantia polymorpha plastid	L	Y	L	L	Y	I	CHMPXX
E. coli	L	Y	L	L	Y	T	ECOSTR1

Amino acid residues encoded by unedited and edited maize mitochondrial transcripts are compared with amino acid residues in RPS12 polypeptides from other systems.

Table 2. Heterogeneity of *rps12* cDNAs

Clone Type	No. Observed	Edit Site No. 1	2	3	4	5	6
A	4	+[a]	+	+	+	+	+
B	1	−[b]	+	+	+	+	+
C	2	+	−	+	+	+	+
D	1	+	+	−	+	+	+
E	1	+	+	+	−	+	+
F	1	+	+	+	+	+	−
G	1	−	+	+	+	−	+
H	1	+	−	+	+	−	+
I	1	+	−	+	+	+	−
J	1	+	+	−	+	+	−
K	1	+	+	−	−	−	+
L	1	−	+	−	+	−	−
M	2	−	+	−	−	+	−
N	1	+	−	−	−	−	−
O	5	−	−	−	−	−	−

[a] (+) indicates the presence of a T at the editing site.
[b] (−) indicates the presence of a C at that position.

nucleotides (lane 5), although the larger transcript was only weakly recognized. Thus, the heterogeneity of *rps12* transcripts was confirmed by RNA gel blot hybridization and is not the result of a PCR artifact such as differential amplification of transcripts or amplification of DNA.

Characterization of Epitope-Specific Antisera

Most of the cDNAs were either completely edited (33%) or completely unedited (25%) at editing sites 4, 5, and 6, and the remaining cDNAs reflected partially edited forms, as indicated in Table 2. Epitope-specific antisera were prepared to distinguish between the unedited and edited translation products of *rps12* mRNAs. Peptides were synthesized that corresponded to a 13-residue region (residues 87 to 99); this region was deduced from the completely unedited or the completely edited sequences, and each included a C-terminal cysteine residue. This region included three changes directed by C-to-U editing at amino acid residues 90, 95, and 97 (editing sites 4, 5, and 6), as shown in Figure 1.

Edited-specific antibody was purified by retention and elution from a peptide column prepared with the edited peptide. The affinity-purified antibody specifically hybridized to edited translation products that were expressed as a fusion with maltose binding protein, as shown in Figure 3A (lane 2), and did not recognize the unedited translation product fused to maltose binding protein (lane 1). The specificity of the antibody was demonstrated further by the ability of 1 or 10 μg of edited peptide to attenuate strongly or eliminate completely the ability of the edited antibody to detect the edited fusion protein (lanes 4 and 6, respectively). In addition, the immune recognition was tested by peptide competition with the alternative form, the unedited peptide, as shown in Figure 3B. The unedited peptide was unable to compete for recognition of the edited fusion protein at 1, 10, or 100 μg of unedited peptide (lanes 4, 6, and 8, respectively).

Antiserum that was raised against the unedited peptide was rendered specific by hybridization of the immune serum to a protein gel blot of the edited fusion protein such that cross-reacting antibodies would be depleted. The unedited-specific antiserum specifically hybridized to the unedited fusion protein, as shown in Figure 3C (lane 1), and did not recognize the edited translation product (lane 2). The specificity of the immune reaction was demonstrated further by the ability of 1 μg of unedited peptide to eliminate completely the ability of the unedited-specific antiserum to detect the unedited fusion protein (lane 3). In addition, the edited peptide was unable to compete effectively for recognition of the unedited fusion protein at 1, 10, or 100 μg of edited peptide, as shown in Figure 3D (lanes 3, 5, and 7, respectively). A cross-reacting polypeptide was detected just below the fusion protein, and a doublet is observed in Figures 3C (lane 1) and 3D (lanes 1, 3, 5, and 7). The faster migrating polypeptide was also noted in the other lanes, and this polypeptide was detected by the preimmune serum.

Each antibody preparation was specific for the unedited or edited fusion protein and was sensitive to competition by the homologous peptide, but the recognition was insensitive to competition by the alternative epitope. Thus, these antibody

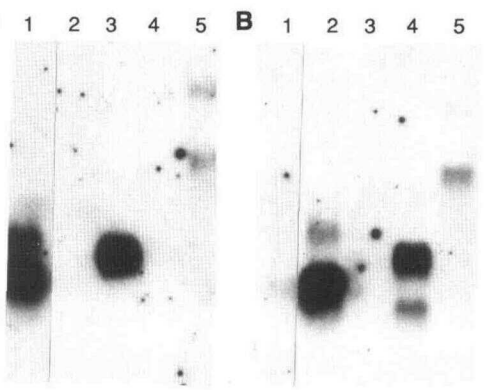

Figure 2. *rps12* mRNAs Are Heterogeneous as a Result of Incomplete Editing.

RNA gel blots were probed with 20 ng of end-labeled oligonucleotide 3 or 4 (antisense for unedited or edited mRNAs in the edit site 4, 5, and 6 region) and 200 ng of unlabeled competitor oligonucleotide that was specific for the alternative form. The following samples were loaded: lanes 1, in vitro–transcribed sense RNA from an unedited *rps12* clone; lanes 2, in vitro–transcribed sense RNA from an edited *rps12* clone; lanes 3, PCR product amplified from an unedited *rps12* clone; lanes 4, PCR product amplified from an edited *rps12* clone; lanes 5, total mitochondrial RNA.

(A) Blot probed with unedited-specific oligonucleotide 3.

(B) Blot probed with edited-specific oligonucleotide 4.

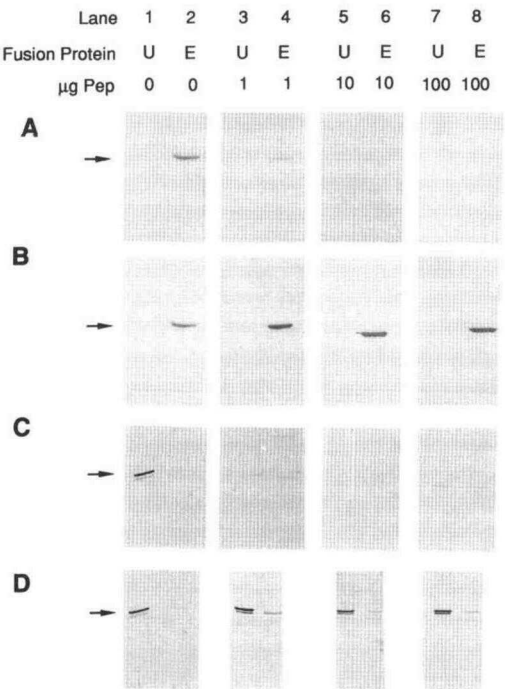

Figure 3. Epitope-Specific Antibodies Specifically Recognize Edited and Unedited Fusion Proteins.

Fusion proteins from pMALS12U (lanes labeled U) or pMALS12E (lanes labeled E) were probed with immune serum in the presence of various quantities of unedited or edited peptide (μg Pep). The arrows indicate the position of the MalE–ribosomal protein S12 fusion.

(A) Protein gel blots of bacterial extracts expressing fusion proteins from pMALS12U (lanes 1, 3, 5, and 7) or pMALS12E (lanes 2, 4, 6, and 8) were probed with the edited-specific antibody. The edited-specific antibody specifically recognized the edited fusion protein (lane 2) in the absence of the edited peptide, but 1, 10, or 100 μg of the edited peptide (lanes 3 and 4, lanes 5 and 6, and lanes 7 and 8, respectively) competed for recognition of the edited epitope.

(B) Protein gel blots of bacterial extracts expressing fusion proteins from pMALS12U (lanes 1, 3, 5, and 7) or pMALS12E (lanes 2, 4, 6, and 8) were probed with edited-specific antibody. The edited-specific antibody specifically recognized the edited fusion protein (lanes 2, 4, 6, and 8) in the presence of 0, 1, 10, or 100 μg of the unedited peptide (lanes 1 and 2, lanes 3 and 4, lanes 5 and 6, lanes 7 and 8, respectively).

(C) Protein gel blots of bacterial extracts expressing fusion proteins from pMALS12U (lanes 1, 3, 5, and 7) or pMALS12E (lanes 2, 4, 6, and 8) were probed with unedited-specific antiserum. The unedited-specific antiserum specifically recognized the unedited fusion protein (lane 1) in the absence of the unedited peptide, but 1, 10, or 100 μg of the unedited peptide (lanes 3 and 4, lanes 5 and 6, lanes 7 and 8, respectively) competed for recognition of the unedited epitope.

(D) Protein gel blots of bacterial extracts expressing fusion proteins from pMALS12U (lanes 1, 3, 5, and 7) or pMALS12E (lanes 2, 4, 6, and 8) were probed with unedited-specific antiserum. The unedited-specific antiserum specifically recognized the unedited fusion protein (lanes 1, 3, 5, and 7) in the presence of 0, 1, 10, or 100 μg of the edited peptide (lanes 1 and 2, lanes 3 and 4, lanes 5 and 6, lanes 7 and 8, respectively).

preparations specifically discriminate among fully edited or fully unedited *rps12* translation products and do not react with the alternative epitope.

Immune Reaction with Incompletely Edited Forms

The antisera were prepared against a region with three amino acid changes that are directed by C-to-U editing; therefore, eight forms of the epitope are possible by translation of the forms of the transcripts. Indeed, the cDNA sequence analysis shown in Table 2 identified examples of all eight types of clones (types A, E, F, G, K, L, M, and O). Thus, all eight forms of the mRNAs were present in maize mitochondria and, in principle, might be translated. Therefore, it was important to analyze the interaction of the antibody preparations with fusion proteins that are heterogeneous with respect to editing in this region. Seven different forms of *rps12* were expressed as fusion proteins with maltose binding protein and probed with unedited-specific antiserum or edited-specific antibody. Figure 4A shows that the unedited-specific antiserum recognized forms of the fusion protein in which any one site was unedited (lanes 1 to 5 and lane 7), although recognition of the polypeptide with a single unedited site at residue 90 (lane 5, −++ form) was sub-

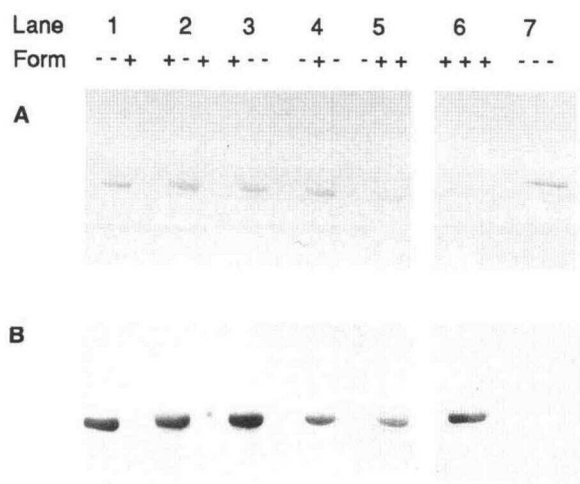

Figure 4. Immune Specificity for Incompletely Edited RPS12 Fusion Proteins.

Fusion proteins were expressed as MalE fusions with cDNAs that were at various stages of completion with respect to the editing at sites 4, 5, and 6. Each lane shows a distinct form of the ribosomal protein S12 fusion, with the editing status indicated by the three pluses or minuses for the editing status at sites 4, 5, and 6, respectively. (+) indicates the edited condition at that site; (−) indicates the unedited condition at that site.

(A) The protein gel blot was probed with unedited-specific antiserum.

(B) The protein gel blot was probed with edited-specific antibody.

stantially weaker than the other forms. Figure 4B shows that the edited-specific antibody recognized forms of the fusion protein in which any one site was edited (lanes 1 to 6).

The edited-specific antibody and unedited-specific antiserum recognized polypeptides translated from any mRNA that had any individual site edited or unedited, respectively. Each immune fraction specifically detected unedited or edited translation products because recognition required at least one editing site that was appropriately represented in the epitope. Thus, a positive reaction with either unedited-specific antiserum or edited-specific antibody indicated that some polypeptides in that fraction were translated from mRNAs with unedited or edited sites. The inability of the unedited-specific antiserum or edited-specific antibody to recognize a polypeptide fraction suggests the absence of polypeptides translated from unedited or edited mRNAs, respectively.

Unedited *rps12* Transcripts Are Translated

The unedited-specific serum specifically recognized a 14-kD polypeptide from maize mitochondria, as shown in Figure 5A (lane 8). The preimmune serum recognized a polypeptide migrating very slightly faster than the fusion protein (lane 1) and an ~35-kD polypeptide from the maize mitochondrial lane (lane 3). Hybridization to these polypeptides by immune serum was also detected in lanes 6 and 8. The unedited translation products were readily detectable in maize mitochondria (lane 8), demonstrating that unedited *rps12* transcripts are translated. Figure 5B shows that the edited-specific antibody also specifically recognized a 14-kD polypeptide from maize mitochondria (lane 8), and the preimmune sera recognized a 35-kD polypeptide (lane 3).

To determine whether both unedited and edited translation products assembled into ribosomes, maize mitochondria were lysed and fractionated into a ribosomal pellet and a postribosomal supernatant. Figure 5B shows that the edited *rps12* translation product was depleted in the postribosomal supernatant (lane 9) and enriched in the ribosomal fraction (lane 10). In contrast, Figure 5A shows that the unedited *rps12* translation product was retained in the postribosomal supernatant (lane 9) and did not sediment with ribosomes (lane 10). Thus, both unedited and edited *rps12* transcripts are translated, but only the edited translation products accumulate in mature ribosomes. The unedited translation products either failed to assemble or resulted in ribosomes that were selectively degraded.

Unedited *rps12* Translation Products Are Mitochondrial Gene Products

To demonstrate that the unedited RPS12 epitope is detectable only in systems that edit *rps12* transcripts, the *Escherichia coli* protein was probed with the edited-specific antibody or unedited-specific antiserum. The corresponding epitope of the RPS12 polypeptide from *E. coli* (VKDLPGVRYHTVR) has nine residues of sequence identity and four conservative changes from the edited form of the maize mitochondrial RPS12 polypeptide, and recognition of this epitope by the edited-specific antibody can be observed in Figures 5B (lane 7) and 6A (lanes 1 and 2). However, the unedited-specific antiserum did not detect a 14-kD polypeptide from *E. coli*, demonstrating that a related polypeptide is not present in the bacterium. Thus, the unedited epitope is not represented in a prokaryotic source, suggesting that the unedited epitope detected in plant mitochondria does not represent a related polypeptide found in prokaryotes.

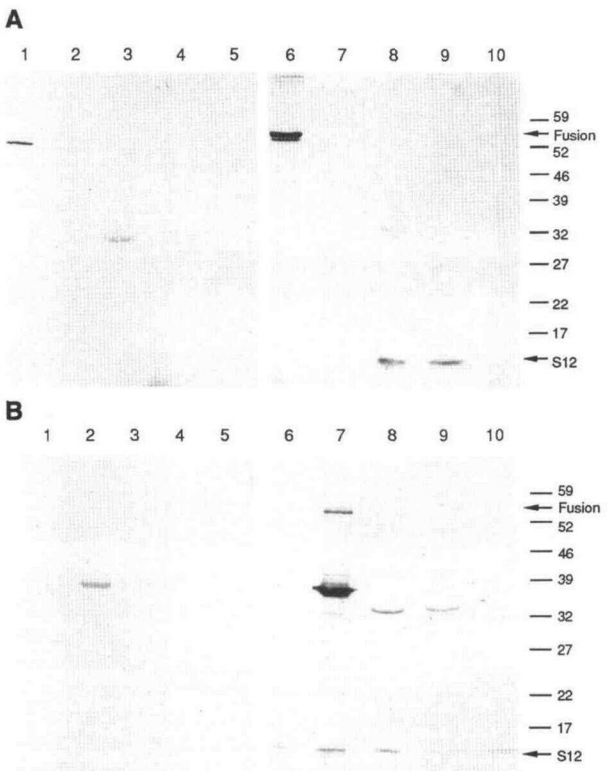

Figure 5. Unedited and Edited *rps12* Translation Products Differentially Fractionate in the Ribosomes and Postribosomal Supernatant.

Protein gel blots were prepared with the following protein samples: lanes 1 and 6, unedited fusion protein (80 ng); lanes 2 and 7, edited fusion protein (80 ng); lanes 3 and 8, maize mitochondrial lysate (30 μg); lanes 4 and 9, postribosomal supernatant (30 μg); lanes 5 and 10, ribosomal fraction (10 μg). The mobility of prestained protein ladder (6 to 60 kD) from Gibco BRL is indicated by lines and apparent sizes in kilodaltons. Arrows indicate the mobility of the fusion protein or ribosomal protein S12.

(A) Preimmune serum (lanes 1 to 5) or unedited-specific antiserum (lanes 6 to 10) was probed against the protein gel blot.

(B) Preimmune serum (lanes 1 to 5) or edited-specific antibody (lanes 6 to 10) was probed against the protein gel blot.

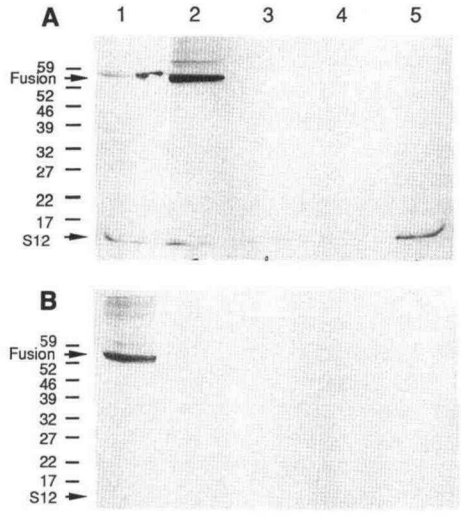

Figure 6. Unedited-Specific Antiserum Does Not Recognize a 14-kD Polypeptide from Chloroplasts or *E. coli*.

Protein gel blots were prepared with the following protein samples: lane 1, unedited fusion protein (80 ng); lane 2, edited fusion protein (80 ng); lane 3, total chloroplast protein (30 μg); lane 4, postribosomal supernatant (30 μg); lane 5, chloroplast ribosome fraction (10 μg). The mobility of prestained protein ladder (6 to 60 kD) from Gibco BRL is indicated by lines and apparent sizes in kilodaltons. Arrows indicate the mobility of the fusion protein or ribosomal protein S12.

(A) Protein gel blots were hybridized with edited-specific antibody. A loading artifact caused some of the edited fusion protein from lane 2 to spill into lane 1.

(B) Protein gel blots were hybridized with unedited-specific antiserum.

Plastids contaminate plant mitochondrial fractions, and it was important to demonstrate that the unedited immune reaction was not derived from chloroplast contamination. Intact maize chloroplasts were fractionated into a postribosomal supernatant and ribosomal fractions and probed with edited-specific antibody. Figure 6A shows that the edited-specific antibody weakly detected RPS12 in the total chloroplast fraction (lane 3) and strongly in the ribosomal pellet (lane 5). The epitope from the maize chloroplast RPS12 polypeptide (VKDLPGVRYRIIR) has nine residues of amino acid sequence identity and four conservative changes compared with the edited peptide antigen. Figure 6B shows that the unedited-specific antiserum did not recognize any polypeptide from total chloroplast protein, postribosomal supernatant fraction, or the ribosomal pellet (lanes 3, 4, or 5, respectively). Thus, the polypeptide recognized by the unedited-specific antibody did not result from a chloroplast contamination of the mitochondrial fraction. In addition, the maize chloroplast system does not produce a polypeptide recognized by the unedited-specific antiserum. Thus, neither a bacterial nor a chloroplast system produces a polypeptide recognized by the unedited-specific antiserum, suggesting that the unedited *rps12* translation product is not a ubiquitously expressed polypeptide in bacteria and organelles but is specifically expressed in the mitochondrial system that edits RNA.

Mitochondrial genes are known to migrate to the nucleus during the course of evolution (Nugent and Palmer, 1991; Covello and Gray, 1992; Brennicke et al., 1993), and it is possible that the unedited translation product detected in these analyses resulted from an unedited *rps12* gene that moved to the nucleus. To investigate this possibility, maize mitochondria were radiolabeled with ^{35}S-cysteine and ^{35}S-methionine, and the edited and unedited *rps12* translation products were immunoprecipitated. Figure 7A shows that the edited-specific antiserum specifically immunoprecipitated the edited fusion protein (lane 5) and did not immunoprecipitate the maltose binding protein or unedited fusion protein (lanes 1 and 3). In addition, the immunoprecipitation of the edited fusion protein was effectively competed by 100 μg of edited peptide (lane 6). The edited-specific antiserum also immunoprecipitated a 14-kD polypeptide that was radiolabeled in intact mitochondria (lane 7) and was slightly competed by preincubation of the antiserum with edited peptide (lane 8).

Figure 7B shows that the unedited-specific antiserum specifically immunoprecipitated the unedited fusion protein (lane 3) and did not immunoprecipitate the maltose binding protein or edited fusion protein (lanes 1 and 5). In addition, the immunoprecipitation of the unedited fusion protein was effectively competed by 100 μg of unedited peptide (lane 4). The unedited-specific antiserum immunoprecipitated an ~14-kD polypeptide that was radiolabeled in intact mitochondria (lane 7) and was partially competed by preincubation of the antibody with unedited peptide (lane 8). These results demonstrate that polypeptides recognized by both the edited-specific and unedited-specific antisera are maize mitochondrial gene product and not derived from chloroplast or nuclear gene expression.

DISCUSSION

Unedited Transcripts Are Translated in Plant Mitochondria

The prevalence of incompletely edited transcripts in plant mitochondria varies widely, depending on the specific transcript. The editing status of plant mitochondrial transcripts can be broken down into three classes: nearly homogeneously edited transcripts, such as *atp9* and spliced *cox2* from maize; highly heterogeneous transcripts that are nascent transcripts and may reflect RNA editing intermediates (pre-*cox2*) (Sutton et al., 1991; Yang and Mulligan, 1991); and highly heterogeneous transcripts that are prevalent in the mature-sized transcripts (*rps12*).

The degree of heterogeneity for a given transcript may simply reflect the relative kinetics of transcription and editing such that slowly transcribed sequences may tend to be more highly edited and rapidly transcribed sequences may tend to be less completely edited. Run-on transcription analyses indicated that *atp9* transcripts are transcribed at very low relative rates but

accumulate to very high levels, suggesting a very long half-life of the transcripts (Mulligan et al., 1991). Thus, the slow rate of synthesis and longevity of *atp9* transcripts may facilitate the editing system to edit completely the *atp9* RNAs to homogeneity. In contrast, *rps12* appears to be very highly transcribed (Muise and Hauswirth, 1992), and the relatively incomplete status of C-to-U conversion in the mRNAs may be the result of a high rate of synthesis but slower rate of editing. This speculation is consistent with the highly heterogeneous status of incompletely edited transcripts in the transient pool of pre-mRNAs for *cox2*, yet nearly homogeneously edited population in the longer lived spliced *cox2* transcripts (Sutton et al., 1991; Yang and Mulligan, 1991). This speculation is also consistent with the interpretation that incompletely edited forms are intermediates in the editing process (Yang and Mulligan, 1991).

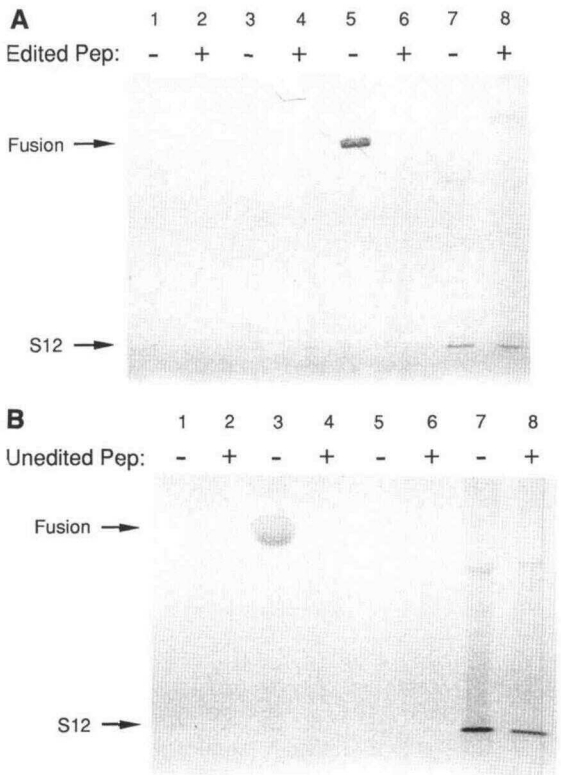

Figure 7. Immunoprecipitation of Edited and Unedited *rps12* Translation Products from Maize Mitochondria.

Maize mitochondrial and *E. coli* proteins were radiolabeled with ^{35}S-methionine and ^{35}S-cysteine and immunoprecipitated in the absence (odd lanes) or presence (even lanes) of 100 μg of competing homologous peptide. The immune complexes were collected with protein A Sepharose and subjected to SDS-PAGE and autoradiography. The following samples were immunoprecipitated: lanes 1 and 2, protein from *E. coli* expressing maltose binding protein (vector control); lanes 3 and 4, protein from *E. coli* expressing unedited fusion protein; lanes 5 and 6, protein from *E. coli* expressing edited fusion protein; lanes 7 and 8, protein from maize mitochondria. Treatments in lanes with a (−) were performed without competing peptide, and treatments in lanes with a (+) were performed with competing peptide. Arrows indicate the mobility of the fusion protein or ribosomal protein S12. **(A)** Immunoprecipitation was performed with edited-specific immune sera.

(B) Immunoprecipitation performed with unedited-specific immune sera.

C-to-U editing in plant mitochondria causes dramatic changes in the amino acids specified by modified codons but does not result in changes that are likely to affect the ability of these transcripts to be translated. Start codons are rarely created by editing, and editing is infrequently observed in 5′ flanking sequences that might change a ribosome binding site (Bonnard et al., 1992). In the case of *rps12*, editing causes no change that would be expected to affect the translation of the *rps12* transcripts.

Previous investigations have failed to detect unedited translation products in plant mitochondria (Grohmann et al., 1994; Lu and Hanson, 1994), or investigators have speculated that unedited RNAs were not translated (Gualberto et al., 1991). Furthermore, several investigators have proposed that a mechanism may exist by which unedited transcripts are either sequestered or screened to ensure that aberrant polypeptides cannot be synthesized from incompletely edited RNAs (Gualberto et al., 1991; Grohmann et al., 1994). However, incompletely edited RNAs have been detected in polysomal RNA fractions from every reported analysis (Gualberto et al., 1991; Yang and Mulligan, 1991; Lu and Hanson, 1994). The authors have concluded either that unedited transcripts are probably not translated (Gualberto et al., 1991) or that unedited transcripts might be translated (Yang and Mulligan, 1991) but that these polypeptides may not accumulate (Lu and Hanson, 1994). Analysis of polysomal RNA as a criterion of translation usually suffers the equivocation that transcripts have been reported in polysomal fraction that are not actively translated (Berry et al., 1988; Klein et al., 1988), and problems with the purity of the polysomal fractions may exist. However, incompletely edited *atp6* transcripts analyzed by Lu and Hanson (1994) were shown to be released from polysomes by puromycin, strongly suggesting that these transcripts were engaged in translation.

Physical analyses of mitochondrial polypeptides have also been used to analyze the translation of unedited *nad9* transcripts. Amino acid sequence analysis of the NAD9 polypeptide from potato has been performed with a peptide that was derived from an incompletely edited portion of the transcript (Grohmann et al., 1994). The amino acid sequence of the peptide from NAD9 reflects the edited translation product but failed to indicate any heterogeneity in the amino acid sequence of the NAD9 polypeptide. However, the NAD9 polypeptide analyzed was prepared from purified complex 1 such that only mature, functional polypeptides would be detected in this analysis (Grohmann et al., 1994). These researchers concluded that translation "is restricted to completely edited transcripts"; however, no attempt was made to analyze directly unincorporated polypeptides or nascent translation products.

The editing of *atp6* RNAs results in the conversion of a glutamine codon (CAA) to a termination codon (UAA) in petunia mitochondria, and *atp6* transcripts are highly heterogeneous at this site as a result of incomplete editing (Lu and Hanson, 1994). Unedited transcripts would fail to terminate translation, and the unedited translation product would have a 13–amino acid C-terminal extension. An antibody raised against the 13–amino acid extension does not detect the ATP6 polypeptide on a protein gel blot or immunoprecipitate a radiolabeled polypeptide from a mitochondrial translation reaction. In addition, purified ATP6 in petunia mitochondria is homogeneous with respect to mass. Thus, unedited *atp6* transcripts are apparently incorporated into polysomes, although the unedited polypeptide is not detectable from total protein or nascent translation products. The failure to detect unedited translation products in this system may result from rapid turnover of the polypeptide. Alternatively, translational control may be exerted over the expression of unedited transcripts. For example, assembly of yeast mitochondrial membrane complexes occurs cotranslationally with the assistance of specific assembly proteins (Costanzo and Fox, 1990), and aberrant polypeptides may fail to assemble properly. Failure to assemble could result in dissociation of these ribosomes and failure to complete translation. In addition, complexes with unedited translation products may be nonfunctional and selectively degraded.

Conclusion

These results demonstrate that the expression of *rps12* is polymorphic in plant mitochondria as a result of incomplete editing. Epitope-specific antibodies distinguished among edited and unedited translation products when analyzed as fusion proteins with maltose binding protein and were sensitive to the identity of individual amino acid residues within the epitope. Edited *rps12* translation products were detected in plant mitochondria and were depleted from the postribosomal supernatant but accumulated in the ribosomal fraction. Thus, the edited translation product is assembled into ribosomes and provides a functional polypeptide for the small subunit of the ribosome. The unedited *rps12* translation product was also detectable in maize mitochondria, conclusively demonstrating that unedited transcripts can be translated by this system. The unedited translation product accumulated in the postribosomal supernatant and failed to accumulate in the ribosome fraction. It is not known whether the unedited ribosomal S12 polypeptides failed to assemble or produced ribosomes that were unstable. In either case, the unedited transcript was translated, but it failed to function in the ribosome.

It is possible that the unedited ribosomal S12 polypeptide has taken or may eventually take on a separate function. The ribosomal S12 polypeptide from *E. coli* has been shown to be a nonspecific RNA binding protein and to facilitate intron splicing and RNA folding in vitro (Coetzee et al., 1994). Maize mitochondrial RPS12 polypeptides have an isoelectric point of 11, and the unedited translation products would be likely to function as RNA binding proteins. It is conceivable that unedited *rps12* translation products act as an RNA chaperone, or they could evolve to perform a required RNA binding function.

METHODS

Preparation of Mitochondria

Mitochondria were isolated from dark-grown shoots of 8-day-old maize (*Zea mays* B37N; Pioneer Hi-Bred International Inc., Johnston, IA) seedlings as described previously by Mulligan et al. (1991). Mitochondria were purified by centrifugation on a discontinuous sucrose density gradient (20, 36, 40, and 52%) as described by Kemble et al. (1980).

DNA, RNA, and Oligonucleotides

Total nucleic acids were extracted from lysed mitochondria and extracted with phenol and chloroform as described previously (Mulligan et al., 1991). Mitochondrial DNA was prepared by treatment of the total nucleic acid fraction with 10 units of DNase-free RNase (Boehringer Mannheim). Mitochondrial RNA was prepared by digestion of the total nucleic acid fraction with 3 units of RNase-free DNase for 30 min at 37°C (Boehringer Mannheim).

Genomic DNA or cDNAs for *rps12* were amplified by polymerase chain reaction (PCR) with oligonucleotides 1 and 2 from total mitochondrial DNA or RNA, respectively. PCR products were digested with XbaI and HindIII and cloned in pBluescript SK+ (Stratagene) for sequence analysis. Expression constructions were prepared by ligation of XbaI-HindIII fragments into the vector pMal-c2 to prepare in-frame fusion of maltose binding protein with desired forms of the maize mitochondrial ribosomal protein S12 (*rps12*) DNAs. The fully edited and unedited *rps12* cDNAs were cloned into the pMal-c2 vector and designated pMALS12E and pMALS12U, respectively.

Oligonucleotide 1 (5′-AAAGGAAGCTAGAAGCTTCCATATCG-3′) is a 3′ antisense oligonucleotide for amplification of *rps12*. Oligonucleotide 1 corresponds to the nucleotide sequence from +443 to +418 relative to the translation start codon. These sequences are 41 nucleotides beyond the translation stop codon for *rps12*. Oligonucleotide 2 (5′-GGGAAGGACATAGTCTAGAGGGATGCCTACA-3′) is a 5′ sense oligonucleotide for amplification of *rps12*. Oligonucleotide 2 corresponds to the nucleotide sequence from −22 to +9 relative to the translation start codon for *rps12* and was designed to eliminate an upstream termination codon in the genomic sequence so that the PCR products could be readily expressed as fusion proteins. Prior cDNA sequence analysis demonstrated that no editing occurs in the first nine nucleotides. Oligonucleotide 3 (5′-GATTCGCCAGGTGTGAAATCCCATCGTAT-3′) is an antisense oligonucleotide that is complementary to the unedited transcript in the region with edit sites 4, 5, and 6. Oligonucleotide 4 (5′-GATTTGCCAGGTGTGAAATTCCATTGTAT-3′) is an antisense oligonucleotide that is complementary to the edited transcript in the region with edit sites 4, 5, and 6.

Reverse Transcriptase and PCR

cDNAs were prepared by reverse transcription of mitochondrial RNA (1 μg) in PCR buffer (10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1 mM each

deoxynucleotide triphosphate) and 0.75 μM 3′ antisense oligonucleotide 1 and 5 units of Moloney murine leukemia virus reverse transcriptase (Gibco BRL) in a final volume of 20 μL. The reaction was incubated at 42°C for 15 min, heated to 99°C for 5 min, and cooled to 4°C. Control reactions included omission of reverse transcriptase from the reactions, and these reactions failed to amplify any detectable PCR products, indicating that the products are in fact amplified cDNAs and not genomic DNA contamination of the RNA preparations.

Genomic DNA and cDNAs were amplified by PCR in PCR buffer with 0.5 mM $MgCl_2$, 2.5 units of *Taq* polymerase (Gibco BRL), 0.15 μM oligonucleotides 1 and 2, and 1 μg of genomic mitochondrial DNA or the reverse transcription reaction in a final volume of 100 μL. The reaction was incubated at 94°C for 3 min. A cyclic temperature regime for 26 cycles was performed with the temperature held at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. The final products were incubated at 72°C for 10 min to ensure completion of the 3′ ends.

DNA Sequence Analysis

DNA was sequenced by dideoxy termination with Sequenase Version 2.0 (U.S. Biochemicals). DNA fragments were separated on a 6% acrylamide–bisacrylamide/8 M urea gel and visualized by autoradiography.

RNA Gel Blot Analysis

A 1% denaturing agarose gel was prepared with formaldehyde gel running buffer (20 mM 3-[*N*–morpholino]propanesulfonic acid–NaOH, pH 7.0, 8 mM sodium acetate, 1 mM EDTA) and 2.2 M formaldehyde (Sambrook et al., 1989). Mitochondrial RNA (10 μg) was electrophoresed and transferred to a nylon membrane (Hybond N+; Amersham). Hybridizations were performed as described by Sutton et al. (1991), except that SSPE (1 × SSPE is 150 mM NaCl, 1 mM EDTA, 11.5 mM sodium phosphate, pH 7.4) was substituted for SSC (1 × SSC is 0.15 mM NaCl, 0.015 M sodium citrate). The membrane was prehybridized for 3 hr in hybridization solution (6 × SSPE, 0.1% SDS, 2 × Denhardt's solution [1 × Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% BSA], and 50 μg of denatured herring sperm DNA) at 42°C. Fresh hybridization solution was added with 20 ng of end-labeled oligonucleotide (oligonucleotide 3 or 4; specific activity 2.5 × 10^8 cpm/μg) and 200 ng of cold competitor oligonucleotide and hybridized overnight at 42°C. Filters were washed twice with 2 × SSPE and 0.5% SDS at room temperature and then twice with 6 × SSPE and 0.1% SDS for 10 min at 60°C.

Expression of Fusion Proteins

Bacteria carrying the pMAL expression vector, pMALS12U, or pMALS12E were inoculated into 1 mL of Luria-Bertani broth with 50 μg/mL ampicillin and grown overnight at 37°C. A fresh culture was inoculated and grown for 2 hr at 37°C with constant agitation. Isopropylthiogalactopyranoside was added to a final concentration of 1 mM, and the bacteria were grown for an additional 2 hr at 37°C with constant agitation.

Peptide Synthesis and Antiserum Production

Peptides were synthesized on an automated peptide synthesizer (Milligen model 9050; PerSeptive Biosystems, Framingham, MA). The amino acid sequences of the edited and unedited peptides were deduced from residues 87 to 99 of RPS12 with the addition of a C-terminal cysteine and were VKDLPGVKFHCIRC and VKDSPGVKSHRIRC, respectively. Peptides were purified by HPLC and coupled to ovalbumin (Sigma) using *N*-succinimidyl-3-(2-pyridyldithio)propionate (Boehringer Mannheim). Each rabbit was immunized with 500 μg of ovalbumin conjugated to edited or unedited peptide with 1 mL of Freund's complete adjuvant (Sigma). The immune response was boosted with peptide antigen (0.5 μg of free peptide per rabbit) at ~30-day intervals.

Protein Gel Blots

Total mitochondrial protein (30 μg) or *E. coli* protein (80 ng) was electrophoresed on 12% SDS–polyacrylamide gels and transferred to nitrocellulose. Protein gel blots were incubated with blocking solution (blocking solution is 3% BSA, 10 mM Tris-HCl, pH 7.6, 50 mM NaCl, 0.02% NaN_3) at 4°C overnight. Edited-specific antibody (100 μg/mL affinity purified) or unedited-specific antisera (1:1000 dilution of cross-hybridized) was added to the protein gel blots and incubated at room temperature for 1.5 hr. The membranes were washed with wash solution (10 mM Tris-HCl, pH 7.6, 50 mM NaCl, 0.2% Nonidet P-40 [Sigma], 0.02% NaN_3) every 5 min for 15 min. Five milliliters of blocking solution was added to the blots, and the blots were incubated for 1 hr at room temperature. An alkaline phosphatase–coupled monoclonal antibody against the constant region of rabbit IgGs (Sigma) was added to the membranes (1:9000 dilution) at room temperature for 1 hr. Membranes were washed every 5 min for 15 min with wash solution. Immune recognition was visualized using the alkaline phosphatase substrate 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

Affinity Purification of Edited-Specific Antibody

Crude edited antisera were affinity purified by antigen immobilization on a peptide column (ImmunoPure Ag/Ab Immobilization SulfoLink Agarose; Pierce). The edited peptide (7 mg) was coupled to the agarose support according to the manufacturer's instructions. Crude edited antisera were diluted 1:10 with 10 mM Tris-HCl, pH 7.5, and were recirculated over the column for 16 hr. The column was washed with 50 bed volumes of 10 mM Tris-HCl, pH 7.5. The epitope-specific antibody was eluted from the column with 100 mM glycine, pH 2.7. The absorbance of the eluate was measured at 300 nm, and the major peak was collected into 330 mM Tris-HCl, pH 8.8. The affinity-purified antibody was dialyzed against 4 L of PBS (PBS is 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, 1.8 mM KH_2PO_4) overnight at 4°C and stored at −20°C. The affinity-purified edited-specific antibody was cross-hybridized to the unedited-specific antigen before use in protein gel blots as described below.

Cross-Hybridization of Antisera

Unedited- or edited-specific antisera were rendered specific by prior hybridization against the alternative antigen. Edited or unedited fusion protein (50 μg) was loaded on a preparative gel and transferred to nitrocellulose. Unedited-specific antisera (1:100 dilution in blocking solution) were incubated overnight on membranes with the edited fusion protein. Edited-specific antibody (100 μg/mL in blocking solution) was incubated overnight on membranes with the unedited fusion protein.

Peptide Competition

Protein gel blots were performed as described above except that peptide (1, 10, 100, and 1000 μg) was incubated with the antiserum (1:1000 dilution) for cross-hybridized unedited-specific antisera (100 μg/mL affinity-purified edited-specific antibody) for 2 hr before addition to the blocked membranes.

Mitochondrial Ribosome Isolation

Maize mitochondria were purified by sucrose density gradient centrifugation and lysed with lysis buffer (200 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 20 mM potassium acetate, 5 mM DTT, 2% polyoxyethylene 10 tridecyl ether; Sigma). The lysate was cleared by centrifugation in a microcentrifuge for 10 min. The supernatant was diluted to 3.3 mL with lysis buffer, and ribosomes were centrifuged through 1.5 mL of 1.7 M sucrose in lysis buffer in an SW50.1 rotor (Beckman) at 43 K rpm (200,000*g*) for 2 hr. The ribosomal pellet was resuspended in 100 μL of 1 × Tris-EDTA with 0.1% SDS. The postribosomal supernatant was aliquoted and frozen at −20°C.

Isolation of Ribosomes from Maize Chloroplasts

Chloroplasts were isolated from 14-day-old light-grown maize seedlings (Kunst et al., 1988). Approximately 20 g of leaves was homogenized in 200 mL of extraction buffer (450 mM sorbitol, 20 mM Tricine-KOH, pH 8.4, 10 mM EDTA, 0.1% BSA) with a Polytron homogenizer (Brinkmann) at an intermediate setting. The homogenate was filtered through Miracloth (Calbiochem, La Jolla, CA) and centrifuged at 270*g* for 90 sec. The chloroplast pellet was resuspended in buffer A (300 mM sorbitol, 20 mM Tricine-KOH, pH 7.6, 5 mM $MgCl_2$, 2.5 mM EDTA), transferred to a 50-mL centrifuge tube, and underlayed with 8 mL of Percoll pad (40% Percoll in buffer A). Chloroplasts were collected by centrifugation in a swinging bucket HB-4 rotor (Beckman) at 5000*g* for 4 min. Chloroplast ribosomes were prepared by the method described for mitochondrial ribosomes.

^{35}S Labeling of RPS12 Fusion Proteins in *E. coli*

RPS12 fusion proteins were ^{35}S-labeled with Translabel (ICN, Irvine, CA), a mixture of ^{35}S-methionine and ^{35}S-cysteine (Sambrook et al., 1989). A 5-mL culture of SOB (SOB is 2% bacto-tryptone, 0.5% bacto-yeast extract, 0.5% NaCl) with 50 μg/mL ampicillin was inoculated with bacteria harboring pMALS12U or pMALS12E and grown for 2 hr at 37°C. Isopropylthiogalactopyranoside (1 mM) and 20 μCi/mL ^{35}S-Translabel (11.1 μCi/μL) were added to the suspension. Cultures were grown for 2 hr at 37°C with constant shaking. The culture was dispensed into 1-mL aliquots, and bacteria were collected by centrifugation for 5 min at 12,000*g* and resuspended in 50 μL of PBS.

^{35}S Labeling of Mitochondrial Proteins

Intact mitochondria were prepared from 7-day-old dark-grown maize seedlings (~300 g). The mitochondrial pellet was resuspended in 1 mL of homogenization buffer (0.4 M mannitol, 1 mM EGTA, 10 mM 3-(*N*-morpholino)propanesulfonic acid–NaOH, pH 7.2). Mitochondrial protein (680 μg) was diluted to a final volume of 250 μL in translation mix (400 mM mannitol, 18 mM KCl, 2 mM $MgCl_2$, 2 mM Tricine, pH 7.2, 0.2 mM EGTA) with 125 μM each amino acid (cysteine and methionine omitted), 50 mM DTT, 40 mM sodium succinate, 10 mM GTP, 8 mM ADP, and 10 μCi/μL of Translabel. The reaction was incubated at 25°C for 1 hr with constant rotation. The reaction was terminated by dilution with 1 mL of 10 mM Tricine, pH 7.2, with 1 mM EGTA and 400 mM mannitol, and the mitochondria were collected by centrifugation and stored at −80°C.

Immunoprecipitation of Mitochondrial and *E. coli* Proteins

^{35}S-labeled proteins from mitochondria or *E. coli* were resuspended in 50 μL of PBS; 150 μL of 10% trichloroacetic acid (w/v) was added to 48 μL of ice-cold bacterial suspensions. One-ninth of the original volume (48 μL) of 100% trichloroacetic acid was added to cold samples and incubated on ice for 30 min. Proteins were collected by centrifugation at 12,000*g* for 5 min. Pellets were washed four times with 300 μL of acetone and allowed to air dry. Pellets were resuspended in 40 μL of 100 mM Tris-HCl, pH 11.0, with 3% SDS and 3 mM DTT.

Fifty microliters of 20% (v/v) protein A Sepharose was mixed with 3% (w/v) radioimmune assay grade BSA. Edited-specific or unedited-specific antibody (1:500 dilution of crude sera) was added to each sample. For peptide competition experiments, 100 μg of free peptide was also added. Samples were rotated overnight at 4°C.

Sepharose beads were collected by centrifugation for 5 min at 12,000*g*. The supernatant was aspirated, and the protein A Sepharose beads were washed four times with Tris-buffered saline–Nonidet P-40 (50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.2% Nonidet P-40, 0.02% NaN_3). Sepharose beads were resuspended in 15 μL of 1 × Laemmli loading dye (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol, 10% glycerol) and heated to 95°C. Samples were electrophoresed on 15% SDS–polyacrylamide gels and visualized by autoradiography.

ACKNOWLEDGMENTS

Maize seed was a gift from Pioneer Hi-Bred Seed Company (Johnston, IA). This research was supported by U.S. Department of Agriculture Grant No. 94-37301-0498.

Received August 9, 1995; accepted November 3, 1995.

REFERENCES

Bégu, D., Graves, P.-V., Domec, C., Arselin, G., Litvak, S., and Araya, A. (1990). RNA editing of wheat mitochondrial ATP synthase subunit 9: Direct protein and cDNA sequencing. Plant Cell **2,** 1283–1290.

Berry, J.O., Carr, J.P., and Klessig, D.F. (1988). mRNAs encoding ribulose-1,5-bisphosphate carboxylase remain bound to polysomes but are not translated in amaranth seedlings transferred to darkness. Proc. Natl. Acad. Sci. USA **85,** 4190–4194.

Bonnard, G., Gualberto, J.M., Lamattina, L., and Grienenberger, J.M. (1992). RNA editing in plant mitochondria. Crit. Rev. Plant Sci. **10,** 503–524.

Brennicke, A., Grohmann, L., Hiesel, R., Knoop, V., and Schuster, W. (1993). The mitochondrial genome on its way to the nucleus: Different stages of gene transfer in higher plants. FEBS Lett. **325,** 140–145.

Coetzee, T., Herschlag, D., and Belfort, M. (1994). *Escherichia coli* proteins, including ribosomal protein S12, facilitate in vitro splicing of phage T4 introns by acting as RNA chaperones. Genes Dev. **8,** 1575–1588.

Costanzo, M.C., and Fox, T.D. (1990). Control of mitochondrial gene expression in *Saccharomyces cerevisiae.* Annu. Rev. Genet. **24,** 91–113.

Covello, P.S., and Gray, M.W. (1989). RNA editing in plant mitochondria. Nature **341,** 662–666.

Covello, P.S., and Gray, M.W. (1990). Differences in editing at homologous sites in messenger RNAs from angiosperm mitochondria. Nucleic Acids Res. **18,** 5189–5196.

Covello, P.S., and Gray, M.W. (1992). Silent mitochondrial and active nuclear genes for subunit 2 of cytochrome *c* oxidase (*cox2*) in soybean: Evidence for RNA-mediated gene transfer. EMBO J. **11,** 3815–3820.

Gray, M.W., and Covello, P.S. (1993). RNA editing in plant mitochondria and chloroplasts. FASEB J. **7,** 64–71.

Grohmann, L., Thieck, O., Herz, U., Schroder, W., and Brennicke, A. (1994). Translation of *nad9* mRNAs in mitochondria from *Solanum tuberosum* is restricted to completely edited transcripts. Nucleic Acids Res. **22,** 3304–3311.

Gualberto, J.M., Lamattina, L., Bonnard, G., Weil, J.H., and Grienenberger, J.M. (1989). RNA editing in wheat mitochondria results in the conservation of protein sequences. Nature **341,** 660–662.

Gualberto, J.M., Bonnard, G., Lamattina, L., and Grienenberger, J.M. (1991). Expression of the wheat mitochondrial *nad3-rps12* transcription unit: Correlation between editing and mRNA maturation. Plant Cell **3,** 1109–1120.

Hiesel, R., Wissinger, B., Schuster, W., and Brennicke, A. (1989). RNA editing in plant mitochondria. Science **246,** 1632–1634.

Kemble, R.J., Gunn, R.E., and Flavell, R.B. (1980). Classification of normal and male-sterile cytoplasms in maize. II. Electrophoretic analysis of DNA species in mitochondria. Genetics **95,** 451–458.

Kempken, F., Mullen, J.A., Pring, D.R., and Tang, H.V. (1991). RNA editing of sorghum mitochondrial *atp6* transcripts changes 15 amino acids and generates a carboxy-terminus identical to yeast. Curr. Genet. **20,** 417–422.

Klein, R.R., Mason, H.S., and Mullet, J.E. (1988). Light-regulated translation of chloroplast proteins. I. Transcripts of *psaA-psaB, psbA,* and *rbcL* are associated with polysomes in dark-grown and illuminated barley seedlings. J. Cell Biol. **106,** 289–301.

Kunst, L., Browse, J., and Somerville, C. (1988). Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast glycerol-3-phosphate acyltransferase activity. Proc. Natl. Acad. Sci USA **85,** 4143–4147.

Lamattina, L., and Grienenberger, J.M. (1991). RNA editing of the transcript coding for subunit 4 of NADH dehydrogenase in wheat mitochondria: Uneven distribution of the editing sites among the four exons. Nucleic Acids Res. **19,** 3275–3282.

Lu, B.W., and Hanson, M.R. (1992). A single nuclear gene specifies the abundance and extent of RNA editing of a plant mitochondrial transcript. Nucleic Acids Res. **20,** 5699–5703.

Lu, B.W., and Hanson, M.R. (1994). A single homogeneous form of ATP6 protein accumulates in petunia mitochondria despite the presence of differentially edited *atp6* transcripts. Plant Cell **6,** 1955–1968.

Muise, R.C., and Hauswirth, W.W. (1992). Transcription in maize mitochondria: Effects of tissue and mitochondrial genotype. Curr. Genet. **22,** 235–242.

Mulligan, R.M., Leon, P., and Walbot, V. (1991). Transcriptional and posttranscriptional regulation of maize mitochondrial gene expression. Mol. Cell. Biol. **11,** 533–543.

Nugent, J.M., and Palmer, J.D. (1991). RNA-mediated transfer of the gene *coxII* from the mitochondrion to the nucleus during flowering plant evolution. Cell **66,** 473–481.

Rish, S.Y., and Breiman, A. (1993). RNA editing of the barley (*Hordeum vulgare*) mitochondrial ATP synthase subunit 9. Plant Mol. Biol. **22,** 711–714.

Salazar, R.A., Pring, D.R., and Kempken, F. (1991). Editing of mitochondrial *atp9* transcripts from two sorghum lines. Curr. Genet. **20,** 483–486.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory).

Schuster, W., Wissinger, B., Unseld, M., and Brennicke, A. (1990). Transcripts of the NADH-dehydrogenase subunit 3 gene are differentially edited in *Oenothera* mitochondria. EMBO J. **9,** 263–269.

Schuster, W., Ternes, R., Knoop, V., Hiesel, R., Wissinger, B., and Brennicke, A. (1991). Distribution of RNA editing sites in *Oenothera* mitochondrial mRNAs and rRNAs. Curr. Genet. **20,** 397–404.

Sutton, C.A., Conklin, P.L., Pruitt, K.D., and Hanson, M.R. (1991). Editing of pre-mRNAs can occur before *cis-* and *trans*-splicing in petunia mitochondria. Mol. Cell. Biol. **11,** 4274–4277.

Yang, A.J., and Mulligan, R.M. (1991). RNA editing intermediates of *cox2* transcripts in maize mitochondria. Mol. Cell. Biol. **11,** 4278–4281.