Molecular and Cellular Biology, Apr. 1996, p. 1543–1549 Vol. 16, No. 4
0270-7306/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Protein Polymorphism Generated by Differential RNA Editing of a Plant Mitochondrial *rps12* Gene

BINGWEI LU,[1] ROBIN K. WILSON,[1] CHRISTINA G. PHREANER,[2]
R. MICHAEL MULLIGAN,[2] AND MAUREEN R. HANSON[1]*

Section of Genetics and Development, Cornell University, Ithaca, New York 14853,[1] and Department of Developmental and Cell Biology, University of California, Irvine, California 92717[2]

Received 27 October 1995/Returned for modification 27 November 1995/Accepted 9 January 1996

The *rps12* gene transcripts encoding mitochondrial ribosomal protein S12 are partially edited in petunia mitochondria. Different petunia lines were found vary in the extent of *rps12* transcript editing. To test whether multiple forms of RPS12 proteins are produced in petunia mitochondria as a result of partial editing, we probed mitochondrial proteins with specific antibodies against edited and unedited forms of a 13-amino-acid RPS12 peptide spanning two amino acids affected by RNA editing. Both antibodies reacted with mitochondrial proteins at the expected size for RPS12 proteins. The amounts of unedited RPS12 protein in different petunia lines correlate with the abundance of unedited transcripts in these plants. Unedited *rps12* translation products are also detected in other plant species, indicating that polymorphism in mitochondrial *rps12* expression is widespread. Moreover, we show that RPS12 proteins recognized by both edited-specific and unedited-specific antibodies are present in a petunia mitochondrial ribosome fraction. These results demonstrate that partially edited transcripts can be translated and that the protein product can accumulate to detectable levels. Therefore, genes exhibiting incompletely edited transcripts can encode more than one gene product in plant mitochondria.

The discovery of RNA editing represents another challenge to the central dogma of molecular genetics regarding genetic information flow. The posttranscriptional modification of specific nucleotides by RNA editing alters the coding capacities of certain transcripts and makes it impossible to predict amino acid sequences of corresponding proteins directly from DNA sequences. Transcripts that are subject to RNA editing have been found in a wide range of organisms, including trypanosomes, higher plants, mammals, *Physarum polycephalum*, and certain viruses (for reviews, see references 3 and 4). Nucleotide modification, insertion, and/or deletion may occur, depending on the organism and gene.

Higher plant organellar transcripts are edited by a C-to-U-type alteration (2, 9, 15, 18, 22, 35). While only a few editing events have been reported for chloroplast transcripts, numerous editing sites have been documented for plant mitochondrial transcripts. In plant mitochondria, RNA editing sites occur mainly in the protein-coding regions and are often located within the first and second positions of codons, therefore changing the encoded amino acids (16, 44). RNA editing can create new start (5) and stop (24, 45) codons and often though not always increases amino acid sequence conservation (8, 39). That RNA editing is a posttranscriptional RNA processing or maturation step is supported by the findings that RNA editing extent correlates temporally with splicing (43, 47) and that a single nuclear locus specifies both RNA editing extent and transcript abundance in petunia mitochondria (27). Many transcripts are shown to be partially edited in plant mitochondria (10, 17, 21, 24, 27, 28, 38, 40, 43, 47). In some cases, partially edited transcripts are more abundant than fully edited transcripts (28, 40).

In certain other editing systems in which differential RNA editing was detected, both edited and unedited transcripts have been shown to be translated, giving rise to proteins with different biological properties. For example, differential editing of apolipoprotein B (*apoB*) mRNA in mammals results in the production of ApoB-100 and ApoB-48, two proteins with distinct properties in lipid transport and metabolism (6, 33). The extent of editing of mammalian glutamate receptor subunit gene transcripts was found to be regulated developmentally and neuron specifically in the brain (26, 31, 42). Electrophysiological studies demonstrated that edited and unedited forms of the receptor proteins differ in Ca^{2+} permeability and kinetic aspects (23, 27). RNA editing of the Wilms' tumor susceptibility gene may also help produce functionally distinct gene products (41).

The presence of abundant partially edited transcripts of some plant mitochondrial genes raises the possibility that multiple forms of certain mitochondrial proteins exist. Very limited analysis of plant organelle DNA-encoded mitochondrial proteins has been carried out, partly because of the hydrophobic nature of many such proteins, which makes characterization difficult. It was shown previously that only a single form of ATP6 (subunit 6 of ATPase) protein accumulates in petunia mitochondria, although transcript analysis showed that partially edited transcripts are more abundant than fully edited transcripts (28). The observation that partially edited *atp6* transcripts are associated with mitochondrial polysomes suggests posttranslationally acting mechanisms prevent the accumulation of proteins specified by partially edited *atp6* transcripts (28).

To determine whether partially edited transcripts can be translated in plant mitochondria, it is necessary to analyze additional mitochondrial genes that display partially edited transcripts. Epitope-specific antibodies against edited and un-

* Corresponding author. Phone: (607) 254-4833 or (607) 254-4814. Fax: (607) 255-6249. Electronic mail address: mrh5@cornell.edu.

† Present address: USDA Plant Gene Expression Center and Department of Plant Biology, University of California, Berkeley, Albany, CA 94710.

edited RPS12 peptides have recently shown that both fully and partially edited *rps12* transcripts are translated in maize mitochondria (32). Products of the partially edited maize *rps12* transcripts were found in the soluble rather than the ribosome fraction (32). We have analyzed mitochondrial RPS12 protein by using these anti-maize RPS12 antibodies in a unique set of petunia genotypes differing in *rps12* editing extent. Here we present evidence for the translation of both edited and unedited petunia *rps12* transcripts and incorporation of the corresponding RPS12 proteins into petunia mitochondrial ribosomes.

MATERIALS AND METHODS

Plant material. For nucleic acid analysis, either green leaves or suspension culture cells of different petunia lines were used. Only suspension culture cells were used to purify petunia mitochondria for immunoblot analysis and polysome preparation.

Isolation of RNA, RT-PCR, and cDNA sequence analysis. Total RNAs were prepared as described previously (1). The *nad3-rps12* transcripts were amplified by using a reverse transcription (RT)-PCR technique essentially as described previously (27). A downstream primer, *rps12-1* (5′CAGAGGCATCTTCCATT CATATCG3′), was used for first-strand cDNA synthesis. First-strand *rps12* cDNAs from fertile lines were PCR amplified by using primers *rps12-1* and *nad-fer* (5′GAAGGAGCAGGCTCTCTTGGATATG3′). Those from sterile lines were amplified by using primers *rps12-1* and *nad-ste* (5′GCCTTGACAAGTTAGT ACGGGTACTG). The *nad* primers differ because of some sequence divergence in the 5′ untranslated regions. RT-PCR products were cloned into TA cloning vectors (InVitrogen) according to the manufacturer's protocol. The double-stranded plasmid DNA sequencing procedure was used in cDNA sequencing analysis.

Fusion protein production. An *Xho*I-*Eco*RI fragment of the *rps12* cDNA sequence was cloned in frame into *Sma*I-*Eco*RI-digested pGEX-2T vectors following Klenow fill-in of the *Xho*I cohesive end (29). The resulting constructs that contain *Xho*I-*Eco*RI fragments derived from fully edited, partially edited, and unedited *rps12* cDNAs were named pGEX cDNA-1, pGEX cDNA-2, and pGEX cDNA-3, respectively. Codon position 95 is not edited in pGEX cDNA-2. Fusion constructs were transformed into *Escherichia coli* BL20 cells. Fusion proteins were produced as described previously (28).

Affinity purification of antibodies and immunological detection of proteins. Crude rabbit antisera were raised against edited RPS12 peptide (VKDLPGVK FHCIR) and unedited RPS12 peptide (VKDSPGVKSHRIR) (32). These antisera were affinity purified by using fusion proteins expressing edited and unedited RPS12 forms according to published procedures (28). Total petunia mitochondrial proteins or *E. coli* proteins were subjected to electrophoresis, transferred onto nitrocellulose filters, and probed with antibodies as described previously (20).

Fractionation of mitochondria and analysis of petunia mitochondrial ribosomes. Petunia mitochondria were purified from suspension culture cells as described previously (19). Purified mitochondria were lysed in mitochondrial lysis buffer containing 25 mM Tris-HCl (pH 7.5), 25 mM (low salt) or 500 mM (high salt) KCl, 25 mM $MgCl_2$, 5 mM dithiothreitol, 1% Triton X-100, 100 μg of chloramphenicol per ml, and 0.5 mg of heparin per ml. After incubation for 10 min on ice, the lysate was centrifuged for 15 min in a microcentrifuge at 4°C to remove membranes. The supernatant was then layered onto cushions of 1.5 ml of 1.75 M sucrose overlaid with 1 ml of 0.5 M sucrose. Both sucrose buffers were prepared in mitochondrial lysis buffer. After ultracentrifugation in a Beckman 75 Ti rotor at 54,000 rpm for 3 h, the supernatant was carefully removed. The polysome pellet was briefly rinsed with ice-cold water and then resuspended in sodium dodecyl sulfate (SDS)-gel loading buffer. Proteins in the supernatant fraction were precipitated with trichloroacetic acid and dissolved in SDS-gel loading buffer. Equal amounts of proteins from both fractions were subjected to gel electrophoresis, transferred onto nitrocellulose membranes, and probed with antibodies.

RESULTS

Identification of RNA editing sites in petunia *rps12* transcripts. As in other plant species, the *rps12* gene is cotranscribed with the *nad3* (subunit 3 of NADH dehydrogenase) gene in petunia mitochondria (36). An RT-PCR method was used to amplify *nad3*/*rps12* transcripts from five different petunia lines, 3688, 3699, 3704, 11127, and S10636. Lines 3699 and 3704 are male fertile, while lines 3688, 11127, and S10636 are male sterile. Lines 3688 and 3699 carry the *Petunia parodii* nuclear genome; 3704 and 11127 carry the same *P. hybrida* nuclear genomes. Comparisons of cDNA sequences with genomic DNA sequences allowed the identification of edited nucleotides. Nineteen edit sites were found in the *nad3* coding region (data not shown), and five edit sites were found in the *rps12* coding region (Fig. 1). RNA editing changes 14 and 5 amino acids in petunia NAD3 and RPS12 proteins, respectively. It has been shown that wheat (17) and maize (32) mitochondrial *rps12* transcripts are edited at six identical sites. Comparison of *rps12* cDNA sequences from petunia, maize, and wheat mitochondria shows that they share three editing sites (codon positions 66, 90, and 95). The edit sites at codon positions 35 and 49 in petunia are genomically encoded in maize and wheat, whereas edit sites at codon positions 24, 74, and 97 in maize and wheat are genomically encoded in petunia (Fig. 2).

```
                                                  M  P  S  L  N
    CCACTAGTGAGAGGGCAAAAATAGGGGGAAGGACAAAGGAAAGACGATGCCATCACTAAA

  6  Q  L  I  R  H  G  R  E  E  K  R  R  T  D  R  T  R  A  L  D
    TCAATTGATTCGTCATGGTAGAGAAGAAAAACGGCGCACGGACCGTACTCGAGCTTTGGA

                                L
 26  Q  C  P  Q  K  Q  G  V  C (P) R  V  S  T  R  T  P  K  K  P
    TCAATGTCCCCAGAAGCAAGGAGTATGCCCGCGTGTTTCAACGAGAACACCGAAAAAACC

              L
 46  N  S  A (P) R  K  I  A  K  V  R  L  S  N  R  H  D  I  F  A
    TAATTCAGCTCCGCGTAAGATAGCCAAAGTACGGTTGAGCAATCGACATGATATATTTGC

     Y
 66 (H) I  P  G  E  G  H  N  L  Q  E  H  S  M  V  L  I  R  G  G
    TCACATTCCAGGCGAAGGTCATAATTTGCAGGAACATTCTATGGTCTTAATAAGAGGAGG

                 L              F
 86  R  V  K  D (S) P  G  V  K (S) H  C  I  R  G  V  K  D  L  L
    TAGAGTGAAAGATTCGCCAGGTGTGAAATCCCATTGTATTCGAGGAGTCAAGGATTTGCT

106  G  I  P  D  R  R  R  G  R  S  K  Y  G  A  E  K  P  K  S  I
    GGGAATTCCGGATCGAAGAAGAGGCAGATCAAAATATGGTGCGGAAAAACCCAAATCGAT

     End
    ATGAATGGAAGATGCCTCTGGAACTTGTTTTTCTCGGTAAGGATAGGTACGAAGTCACTC
```

FIG. 1. Nucleotide and deduced amino acid sequences of the petunia mitochondrial *rps12* gene. Edited nucleotides are underlined. The amino acids that are affected by RNA editing are enclosed in parentheses, with the amino acids encoded by edited codons written above. The numbers refer to codon positions relative to the start codon.

Variation in editing extent of *rps12* transcripts among different petunia lines. By sequencing a large number of cDNA clones derived from RT-PCR products, we found that the editing status of *rps12* transcripts varies greatly among different petunia lines. For example, fertile line 3704 and cytoplasmic male sterile line 11127 show higher degrees of editing, while cytoplasmic male sterile line 3688 and fertile line 3699 show lower degrees of editing (Fig. 3). Given that the high-editing lines 3704 and 11127 and the low-editing lines 3688 and 3699 are isogenic pairs, the extent of editing of *rps12* transcripts is evidently influenced by the nuclear background, as was previously reported for the cotranscribed *nad3* gene (27).

Specific antibodies against edited and unedited forms of RPS12 proteins. To test whether partially edited *rps12* transcripts were translated, we made use of antibodies raised against 13-amino-acid long peptides (amino acids 87 to 99) specified by fully edited and unedited maize *rps12* cDNA sequences (32). The peptide sequences span codons 90 and 95 of petunia RPS12 protein that are altered by RNA editing. The RPS12 proteins encoded by edited petunia and maize *rps12* transcripts are identical at this 13-amino-acid region. There is a one-amino-acid mismatch (at codon 97) in this region between petunia and maize RPS12 proteins encoded by unedited transcripts (Fig. 2). For convenience, protein specified by unedited transcripts will be referred as unedited peptide or un-

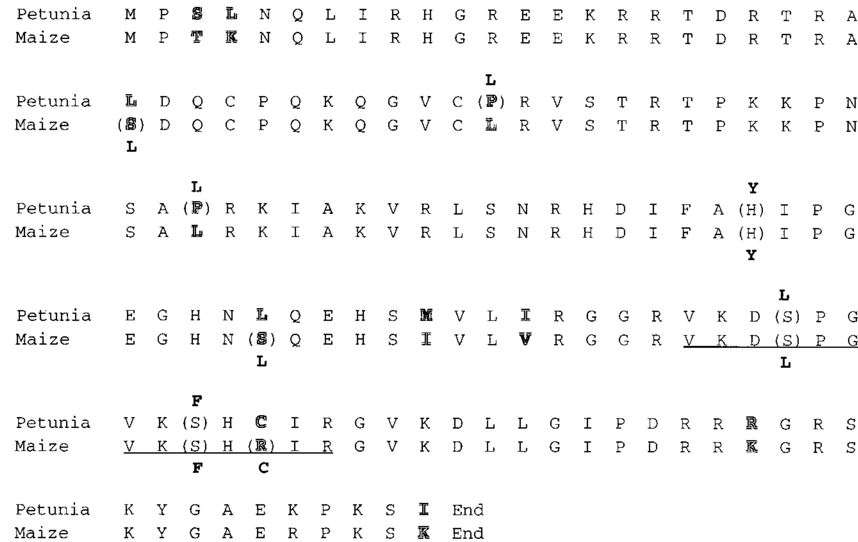

FIG. 2. Comparison of petunia and maize RPS12 amino acid sequences. Petunia and maize RPS12 sequences deduced from genomic DNA sequences are aligned. The amino acids affected by RNA editing are enclosed in parentheses, with the amino acids encoded by edited codons written above. Amino acid differences between petunia and maize sequences are outlined. The 13-amino-acid peptide sequence used to generate RPS12 peptide antibodies is underlined.

edited protein, and protein encoded by edited transcripts will be termed edited peptide or edited protein.

We produced specific antibodies against unedited and edited peptides by affinity purification using pMAL-unedited maize RPS12 (32) and glutathione *S*-transferase-edited petunia RPS12 fusion proteins, respectively. The specificity of each purified antibody is shown in Fig. 4. The anti-edited peptide antibody reacts strongly with fully edited petunia RPS12 fusion protein (pGEX cDNA-1). It also reacts with partially edited petunia RPS12 fusion protein in which codon position 95 remains unedited (pGEX cDNA-2) but does not react with unedited petunia RPS12 fusion protein (pGEX cDNA-3). The anti-unedited peptide antibody reacts strongly with unedited petunia RPS12 fusion protein. It does not react with either partially edited or fully edited petunia RPS12 fusion protein (Fig. 5). These results show that the two antibodies have specificities sufficient to distinguish RPS12 proteins specified by edited and unedited RNAs.

Detection of edited and unedited RPS12 proteins in petunia mitochondria. When total mitochondrial proteins from petunia lines 3688, 3699, 3704, and S10636 were transferred to nitrocellulose filters and probed with anti-edited and anti-unedited RPS12 peptide antibodies, mitochondrial proteins that react with each antibody were detected (Fig. 5). The apparent molecular mass of the reactive proteins is 15 kDa, which is very close to the expected size of petunia RPS12 protein (14.5 kDa). Moreover, the amount of the unedited RPS12 protein differs greatly among the petunia lines tested (Fig. 5). Such proteins were readily detected in lines 3688 and 3699 but could barely be detected in lines 3704 and S10636. This variation of unedited RPS12 protein levels correlates with the proportion of partially edited *rps12* transcripts in these different petunia lines. In lines S10636 and 3704, in which unedited RPS12 proteins are barely detected, only 1 of 15 and 1 of 22, respectively, of the sequenced cDNA clones could encode RPS12 protein that can be detected by the unedited antibody (Table 1). In contrast, in line 3699 and 3688, in which unedited RPS12 proteins are readily detected, 15 of 39 and 15 of 23, respectively, of the sequenced cDNA clones encode RPS12 proteins that react with the unedited antibody (Table 1). This correlation further affirms that the protein recognized by the anti-unedited RPS12 peptide antibody represents unedited RPS12 protein. The anti-edited RPS12 antibody recognizes proteins from all four petunia lines (Fig. 5). The lower amount of the

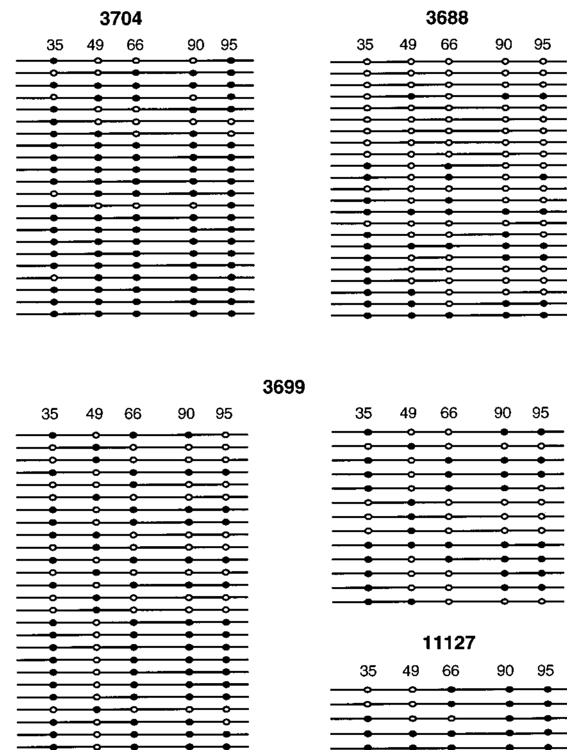

FIG. 3. Summary of sequencing analysis of *rps12* cDNA clones derived from RT-PCR products from different petunia lines. Total RNAs from petunia lines 3704, 3688, 3699, and 11127 were amplified by the RT-PCR technique. RT-PCR products were cloned into TA cloning vectors and sequenced. The numbers indicate codon positions of the editing sites. Filled circles represent sites that are edited; open circles represent unedited sites.

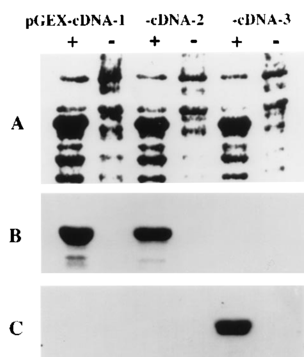

FIG. 4. Immunoblot analysis showing specificities of the anti-edited RPS12 and anti-unedited RPS12 antibodies. Constructs that express edited *rps12* cDNA (pGEX cDNA-1), partially edited *rps12* cDNA (pGEX cDNA-2), and unedited *rps12* cDNA (pGEX cDNA-3) were transformed into *E. coli* BL21. (A) Total proteins made from isopropylthiogalactopyranoside (IPTG)-induced (+) or uninduced (−) cells were transferred to nitrocellulose filters and stained with Ponceau S. (B) Filter immunodecorated with anti-edited RPS12 peptide antibody. (C) Filter probed with anti-unedited RPS12 peptide antibody.

edited RPS12 protein in line 3704 is reproducible and is not due to underloading, as all four lanes contain comparable amounts of ATPA (ATPase subunit α) protein (Fig. 5).

Identification of unedited *rps12* translation products in other species. Translation of unedited *rps12* mRNAs could be a general phenomenon or one that is restricted to a few isolated systems. Heterogeneity of *rps12* cDNAs has been confirmed for wheat (17), maize (32), and petunia. Editing sites at codon positions 90 and 95 are conserved in all known dicot *rps12* sequences (*Panax ginseng* and *Brassica napus*), and editing sites at codon positions 90, 95, and 97 are conserved in all known monocot sequences (*Triticum aestivum*, *Zea mays*, and *Oryza sativa*). Thus, the unedited antibody should detect the presence of unedited translation products from these plant mitochondrial systems. To test whether RPS12 protein products specified by partially edited transcripts accumulate in other plant species, we probed mitochondrial proteins from zucchini, wheat, squash, and maize. The unedited immune serum recognized ribosomal S12 polypeptides from isolated mitochondria from all four species (Fig. 6A), and the edited immune serum also recognized S12 polypeptides from these mitochondrial samples (Fig. 6B). These data suggest that transcript heterogeneity as a result of incomplete C-to-U editing exists and that these transcripts are utilized as templates for translation in these species.

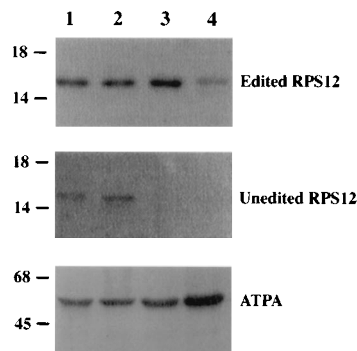

FIG. 5. Immunoblot analysis of petunia mitochondrial proteins with anti-RPS12 antibody. Approximately 50-μg aliquots of total mitochondrial proteins from petunia lines 3688 (lane 1), 3699 (lane 2), S10636 (lane 3), and 3704 (lane 4) were separated by electrophoresis, transferred to nitrocellulose filters, and probed with anti-edited RPS12 peptide antibody, anti-unedited RPS12 peptide antibody, and anti-ATPA antibody. Sizes are indicated in kilodaltons.

TABLE 1. Degree of RNA editing at codon positions 90 and 95 based on sequencing analysis of *rps12* cDNAs from four different petunia lines[a]

Strain	No. of cDNA clones edited at indicated site/total no. analyzed			
	90 edited, 95 unedited	95 edited, 90 unedited	Both 90 and 95 edited	Both 90 and 95 unedited
3704	1/22	3/22	17/22	1/22
3688	1/23	1/23	6/23	15/23
3699	2/39	0/39	21/39	15/39
S10636	0/15	0/15	14/15	1/15

[a] Summary of data obtained from sequencing analysis of *rps12* cDNAs from petunia lines 3688, 3699, 3704 (shown in Fig. 2), and S10636 (data not shown) are summarized.

Mitochondrial fractionation analysis indicates assembly of edited and unedited RPS12 proteins into ribosomes. To test whether the RPS12 proteins specified by edited and unedited transcripts are both ribosomal proteins in petunia mitochondria, we fractionated purified petunia mitochondria into soluble supernatant and ribosome pellet and probed the localization of RPS12 proteins. Ribosomes were prepared under both low-salt and high-salt conditions. Equal amounts of proteins from each fraction were separated on an SDS-polyacrylamide gel and transferred to nitrocellulose filters. As shown in Fig. 7A and B, probing of identical filters with anti-edited and anti-unedited RPS12 peptide antibodies detected both the edited and unedited RPS12 proteins specifically in the polysome pellet under low- and high-salt conditions. When an identical filter was probed with an antibody against petunia mitochondrial aspartate aminotransferase (28), a soluble mitochondrial matrix enzyme, mitochondrial aspartate aminotransferase was found mainly in the supernatant fraction (Fig. 7C), demonstrating efficient separation by the fractionation procedure. This result indicates that both edited and unedited RPS12 proteins can be found in the ribosome pellet in petunia mitochondria.

Comparison of RPS12 amino acid sequences at the codon positions affected by RNA editing. RPS12 protein sequences from plants and other organisms were compared. Eight residues affected by RNA editing with RPS12 proteins in plants are completely conserved after RNA editing. However, comparison of plant sequences with sequences from *Drosophila melanogaster* (37), *Paramecium tetraurelia* (34), and *Eikenella*

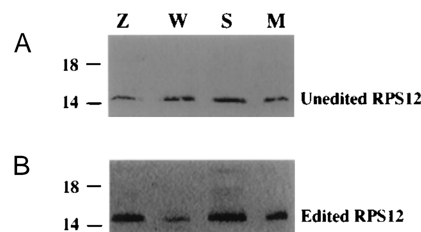

FIG. 6. Unedited and edited antisera recognize S12 polypeptides from various dicot and monocot species. Total proteins from isolated mitochondria were blotted to nitrocellulose and probed with S12-specific immune serum as described by Phreaner et al. (32). Total mitochondrial protein (30 μg) from the following sources was used: lane Z, *Cucumis pepo* (zucchini); lane W, *Triticum aestivum* (wheat); lane S, *Cucumis pepo* (squash); and lane M, *Zea mays* (maize). (A) Protein blots probed with unedited antisera; (B) protein blots probed with edited antisera. Sizes are indicated in kilodaltons.

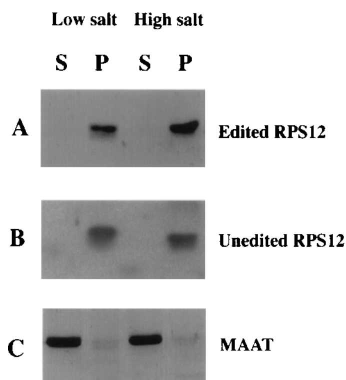

FIG. 7. Fractionation analysis showing assembly of RPS12 proteins into mitochondrial ribosomes. Purified mitochondria from petunia line 3699 were lysed under low-salt and high-salt conditions and sedimented through sucrose cushions to prepare polysome pellets and soluble supernatants. Approximately 10 μg of proteins from the supernatant (S) and the ribosome pellet (P) were subjected to gel electrophoresis and transferred onto filters. Identical filters were probed with anti-edited RPS12 peptide antibody (A), anti-unedited RPS12 peptide antibody (B), and anti-petunia mitochondrial aspartate aminotransferase (MAAT) antibody (C).

corrodens (Swiss-Prot database number P35643) showed that the amino acids are less conserved at certain positions (Fig. 8). For example, radical amino acid changes at codon positions 35, 49, and 97 were found. Interestingly, although codon 24 is conserved among plants, *D. melanogaster*, and *P. tetraurelia*, it is missing in *E. corrodens*, suggesting that this amino acid is not absolutely required for RPS12 function. The variations revealed by this comparison suggest that the RPS12 proteins encoded by partially edited plant mitochondrial *rps12* transcripts may still be functional as ribosomal protein components.

DISCUSSION

By analysis with epitope-specific antibodies, we have shown that multiple forms of RPS12 exist in petunia and several other species. Even more diversity may exist in the RPS12 protein population in the mitochondrion than our experiments could detect. Not all residues at sites of partial editing have been assayed in this study. There could be divergence in petunia RPS12 at sites in addition to divergence at residues specified by codon 90 and 95.

rps12 genes are found to be cotranscribed with *nad3* genes in the mitochondria of several plant species (17, 32, 36, 40). Like *rps12* transcripts, *nad3* transcripts are also partially edited. In petunia mitochondria, a correlation between transcript abundance and RNA editing extent of *nad3* transcripts has been established. A single nuclear locus that determines both traits was identified (27). It is possible that this nuclear gene directly controls *nad3* and *rps12* transcript stability and that the observed RNA editing extent difference is a secondary effect. Because of the cotranscription of *nad3* and *rps12* transcripts, the same nuclear locus that determines *nad3* RNA abundance also controls *rps12* RNA abundance (27). The variation in *rps12* RNA editing extent between the two nuclear backgrounds shown in this study also correlates with *rps12* transcript abundance, as is the case for *nad3* transcripts. Whether similar correlations between transcript abundance and editing extent variation exist in other plant species remains to be examined.

Finding multiple forms of RPS12 in maize, petunia, and other species raises the question of whether the different forms have different functions. In maize, the unedited RPS12 translation products apparently fail to assemble into ribosomes (32) and therefore evidently do not function in the same way as the RPS12 protein that is produced from edited transcripts and integrated into ribosomes. Whether the maize RPS12 protein from incompletely edited transcripts is merely a nonfunctional waste product or has another function is unknown. RPS12 may have more than one function in *E. coli*. *E. coli* RPS12 is incorporated into the ribosome and forms a component of the streptomycin binding site. Purified RPS12 can facilitate in vitro splicing of phage T4 introns, suggesting that RPS12 may have the capacity to act as an RNA chaperone (7).

In contrast to the maize protein, petunia RPS12 protein from incompletely edited transcript is found in the ribosome pellet. The variation in the amount of unedited RPS12 among male fertile and male sterile petunia lines does not correlate with male fertility, and so our data do not implicate abnormal *rps12* transcript editing in sterility. Moreover, the presence of abundant unedited RPS12 protein in petunia fertile line 3699 indicates that this protein does not have toxic effects on plant mitochondrial function. The mitochondrial RPS12 residue which is homologous to the residue altered in streptomycin resistance in *Chlamydomonas reinhardtii* and *E. coli* is not affected by editing, and so it is not likely that petunia ribosomes containing the different RPS12 forms differ in streptomycin binding (13, 14, 25). Whether petunia ribosomes containing unedited RPS12 protein and those containing edited protein differ in some other function is not known.

Circumstantial evidence is consistent with the functionality of certain protein products specified by partially edited transcripts in plant organelles. Species-specific RNA editing patterns of mitochondrial transcripts have been reported in several cases. For example, *rps13* (which encodes ribosomal protein S13) and *coxII* (encoding cytochrome *c* oxidase subunit II) transcripts show differences in editing at homologous sites such that certain sites are edited in one species but are not edited in another species (10, 46). This finding suggests that RNA editing at these sites is not absolutely required for the function of the protein products, as a result of differential RNA editing of the *coxII* transcripts, even though the existence of editing affects the encoded amino acid. For example, codon 5 encodes serine in wheat but leucine in maize, and codon 31 encodes leucine in wheat but phenylalanine in maize. A similar situation was found in chloroplast *rpoB* (encoding the β subunit of chloroplast RNA polymerase) transcripts, in which one site was edited in maize but not edited in barley (48). Lack of RNA editing at this particular site does not seem to affect chloroplast function in barley. Further evidence comes from

Codon positions	24	35	49	66	74	90	95	97
Petunia unedited	L	P	P	H	L	S	S	C
Maize unedited	S	L	L	H	S	S	S	R
Wheat unedited	S	L	L	H	S	S	S	R
Plants edited	L	L	L	Y	L	L	F	C
Drosophila	L	L	N	Y	L	V	L	A
Paramecium	L	L	R	H	L	L	H	C
Eikenella		T	L	Y	L	L	Y	T

FIG. 8. Alignment of RPS12 amino acid sequences at the seven codon positions affected by RNA editing in plants. RPS12 amino acid sequences of plants, *D. melanogaster*, *P. tetraurelia*, and *E. corrodens* were used for this comparison. Only the eight amino acids corresponding to the codons affected by RNA editing in plants are shown for each sequence.

studies of mitochondrial genes that have been transferred to the nucleus during evolution, such as the *coxII* genes of cowpea (30) and soybean (11). The nuclear *coxII* sequences more closely resemble edited mitochondrial *coxII* RNA sequences than mitochondrial DNA sequences. While most of the edited C's identified in mitochondrial *coxII* transcripts are found in the nuclear *coxII* gene sequences as T's, certain editing sites remain as C's in the nuclear *coxII* sequences. Because the transferred *coxII* genes are single copy in the nucleus, and the mitochondrial copies were either lost or silent in both species, it can be inferred that the nucleus-encoded COXII proteins, corresponding to proteins encoded by partially edited mitochondrial transcripts in other species, function properly in plant mitochondria.

RNA editing may be a process that was introduced into the mitochondrial gene expression system and has added an extra RNA processing step in the expression of mitochondrial genes. C-to-U editing in plant mitochondria is apparently an acquired trait that is prevalent in higher plant mitochondria (12, 16). Incomplete editing makes possible the creation of protein polymorphism. A single gene such as *rps12* may direct the synthesis of a family of polypeptides and thereby allow the system to test numerous changes in the *rps12* polypeptide sequence. The situation is comparable to that for genes that have been duplicated and allowed to diverge. The subsequent steps in evolution of duplicated genes have occurred in other systems, with one member of the duplicated gene set taking on additional and unique functions.

On the basis of our studies of ATP6 and RPS12 in maize and petunia, we propose that translation is not a barrier to the accumulation of products of partially edited transcripts in plant mitochondria. Whether such proteins can accumulate inside mitochondria is probably determined posttranslationally. Because differential RNA editing can result in accumulation of more than one protein form encoded by a single gene, RNA editing may play a regulatory role in plant mitochondrial gene expression.

ACKNOWLEDGMENTS

This work was supported by NIH grant R01GM50723 to M.R.H. and by USDA grant 94-37301-0498 to R.M.M. B.L. received a Cornell Graduate School A. D. White Fellowship and a fellowship from the Cornell NSF/DOE/USDA Plant Science Center, a unit in the Cornell Center for Advanced Technology. We thank Claudia Sutton, Martha Reed, and Su Guo for helpful discussions and Rob Last and Tom Fox for critically reading the manuscript.

REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1989. Current protocols in molecular biology. Greene Publishing Associates and Wiley Interscience, New York.
2. **Begu, D., P.-V. Graves, C. Domec, G. Arselin, S. Litvak, and A. Araya.** 1990. RNA editing of wheat mitochondrial ATP synthase subunit 9: direct protein and cDNA sequencing. Plant Cell **2:**1283–1290.
3. **Cattaneo, R.** 1991. Different types of messenger RNA editing. Annu. Rev. Genet. **25:**71–78.
4. **Chan, L.** 1992. RNA editing: exploring one mode with apolipoprotein B mRNA. Bioessays **15:**33–41.
5. **Chapdelaine, Y., and L. Bonen.** 1991. The wheat mitochondrial gene for subunit 1 of the NADH dehydrogenase complex: a *trans*-splicing model for this gene-in-pieces. Cell **65:**465–472.
6. **Chen, S.-H., G. Habib, C.-Y. Yang, Z.-W. Gu, B. R. Lee, S.-A. Weng, S. R. Silberman, S.-J. Cai, J. P. Deslypere, M. Rosseneu, A. M. Gotto, Jr., W.-H. Li, and L. Chan.** 1987. Apolipoprotein B-48 is the product of a messenger mRNA with an organ-specific in-frame stop codon. Science **23:**363–366.
7. **Coetzee, T., H. Herschlag, and M. Belfort.** 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.
8. **Conklin, P. L., R. K. Wilson, and M. R. Hanson.** 1991. Multiple *trans*-splicing events are required to produce a mature *nad1* transcripts in a plant mitochondrion. Genes Dev. **5:**1407–1415.
9. **Covello, P. S., and M. W. Gray.** 1989. RNA editing in plant mitochondria. Nature (London) **341:**662–666.
10. **Covello, P. S., and M. W. Gray.** 1990. Differences in editing at homologous sites in messenger RNAs from angiosperm mitochondria. Nucleic Acids Res. **18:**5189–5196.
11. **Covello, P. S., and M. W. Gray.** 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.
12. **Covello, P. S., and M. W. Gray.** 1993. On the evolution of RNA editing. Trends Genet. **9:**265–268.
13. **Funatsu, G., and H. G. Wittmann.** 1972. Location of amino acid replacements in protein S12 isolated from *Escherichia coli* mutants resistant to streptomycin. J. Mol. Biol. **68:**547–550.
14. **Galili, S., H. Fromm, D. Aviv, M. Edelman, and E. Galun.** 1989. Ribosomal protein S12 as a site for streptomycin resistance in *Nicotiana* chloroplasts. Mol. Gen. Genet. **218:**289–292.
15. **Graves, P. V., D. Begu, J. Velours, E. Neau, F. Belloc, S. Litvak, and A. Araya.** 1990. Direct protein sequencing of wheat mitochondrial ATP synthase subunit 9 confirms RNA editing in plants. J. Mol. Biol. **214:**1–6.
16. **Gray, M. W., and P. S. Covello.** 1993. RNA editing in plant mitochondria and chloroplasts. FASEB J. **7:**64–71.
17. **Gualberto, J. M., G. Bonnard, L. Lamattina, and J. M. Grienenberger.** 1991. Expression of wheat *nad*3-*rps*12 transcription unit: correlation between editing and mRNA maturation. Plant Cell **3:**1109–1120.
18. **Gualberto, J. M., L. Lamattina, G. Bonnard, J. H. Weil, and J. M. Grienenberger.** 1989. RNA editing in wheat mitochondria results in the conservation of protein sequences. Nature (London) **341:**660–662.
19. **Hanson, M. R., M. L. Boeshore, P. E. McClean, M. A. O'Connell, and H. T. Nivison.** 1986. The isolation of mitochondria and mitochondrial DNA. Methods Enzymol. **118:**437–453.
20. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Hiesel, R., B. Wissinger, and A. Brennicke.** 1990. Cytochrome oxidase subunit II messenger RNA in *Oenothera* mitochondria are edited at 24 sites. Curr. Genet. **18:**371–376.
22. **Hiesel, R., B. Wissinger, W. Schuster, and A. Brennecke.** 1989. RNA editing in plant mitochondria. Science **246:**1632–1634.
23. **Hume, R. I., R. Dingledine, and S. F. Heinemann.** 1991. Identification of a site in glutamate receptor subunits that controls calcium permeability. Science **253:**1028–1031.
24. **Kempken, F., J. A. Mullen, D. R. Pring, and H. V. Tang.** 1991. RNA editing of sorghum mitochondrial *atp*6 transcripts changes 15 amino acids and generates a carboxyl terminus identical to yeast. Curr. Genet. **20:**417–422.
25. **Liu, X.-Q., N. W. Gillham, and J. E. Boynton.** 1989. Chloroplast ribosomal protein gene *rps12* of *Chlamydomonas reinhardtii*. J. Biol. Chem. **264:**16100–16108.
26. **Lomeli, H., J. Mosbacher, T. Melcher, T. Hoger, J. R. P. Geiger, T. Kuner, H. Monyer, M. Higuchi, A. Bach, and P. H. Seeburg.** 1994. Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science **266:**1709–1713.
27. **Lu, B., and M. R. Hanson.** 1992. A single nuclear gene specifies the abundance and extent of RNA editing of a plant mitochondrial transcript. Nucleic Acids Res. **20:**5699–5703.
28. **Lu, B., and M. R. Hanson.** 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.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Nugent, J. M., and J. D. Palmer.** 1991. RNA-mediated transfer of the gene *coxII* from the mitochondrion to the nucleus during flowering plant evolution. Cell **66:**473–481.
31. **Nutt, S. L., and R. K. Kamboj.** 1994. Differential RNA editing efficiency of AMPA receptor subunit GluR-2 in human brain. Neuroreport **5:**1679–1683.
32. **Phreaner, C. G., M. A. Williams, and R. M. Mulligan.** 1996. Incomplete editing of *rps12* transcripts results in the synthesis of polymorphic polypeptides in plant mitochondria. Plant Cell **8:**107–117.
33. **Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott, and J. Scott.** 1987. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. Cell **50:**831–840.
34. **Pritchard, A. E., J. J. Seilhamer, R. Mahalingam, C. L. Sable, S. E. Venuti, and D. J. Cummings.** 1990. Nucleotide sequence of the mitochondrial genome of *Paramecium*. Nucleic Acids Res. **18:**173–180.
35. **Rajasekhar, V. K., and R. M. Mulligan.** 1993. RNA editing in plant mitochondria: α-phosphate is retained during C-to-U conversion in mRNAs. Plant Cell **5:**1843–1852.
36. **Rasmussen, J., and M. R. Hanson.** 1989. A NADH dehydrogenase subunit gene is co-transcribed with the abnormal petunia mitochondrial gene associated with cytoplasmic male sterility. Mol. Gen. Genet. **215:**332–336.
37. **Royden, C. S., V. Pirrotta, and L. Y. Yan.** 1987. The *tko* locus, site of a behavioral mutation in *D. melanogaster*, codes for a protein homologous to

prokaryotic ribosomal protein S12. Cell **51:**165–173.

38. **Salazar, R. A., D. R. Pring, and F. Kempken.** 1991. Editing of mitochondrial *atp9* transcripts in two sorghum lines. Curr. Genet. **20:**483–486.
39. **Schuster, W., and A. Brennicke.** 1991. RNA editing makes mistakes in plant mitochondria: editing loses sense in transcripts of a *rps19* pseudogene and in creating stop codons in *coxI* and *rps3* mRNAs in *Oenothera* mitochondria. Nucleic Acids Res. **19:**6923–6929.
40. **Schuster, W., B. Wissinger, R. Unseld, and A. Brennicke.** 1990. Transcripts of the NADH-dehydrogenase subunit 3 gene are differentially edited in *Oenothera* mitochondria. EMBO J. **9:**263–269.
41. **Sharma, P. M., M. Bowman, S. L. Madden, F. J. Rauscher III, and S. Sukumar.** 1994. RNA editing in the Wilms' tumor susceptibility gene, *Wtl*. Genes Dev. **8:**720–731.
42. **Sommer, B., M. Kohler, R. Sprengel, and P. H. Seeburg.** 1991. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell **67:**11–19.
43. **Sutton, C. A., P. L. Conklin, K. D. Pruitt, and M. R. Hanson.** 1991. Editing of pre-mRNAs can occur before *cis*- and *trans*-splicing in petunia mitochondria. Mol. Cell. Biol. **11:**4274–4277.
44. **Walbot, V.** 1991. RNA editing fixes problems in plant mitochondrial transcripts. Trends Genet. **7:**37–39.
45. **Wintz, H., and M. R. Hanson.** 1991. A termination codon is created by RNA editing in the petunia mitochondrial *atp9* gene transcript. Curr. Genet. **19:** 61–64.
46. **Wissinger, B., W. Schuster, and A. Brennicke.** 1990. Species-specific RNA editing patterns in the mitochondrial *rps13* transcripts of *Oenothera* and *Daucus*. Mol. Gen. Genet. **224:**386–395.
47. **Yang, A. J., and R. M. Mulligan.** 1991. RNA editing intermediates of *cox2* transcripts in maize mitochondria. Mol. Cell. Biol. **11:**4278–4281.
48. **Zeltz, P., W. R. Hess, K. Neckermann, T. Borner, and H. Kossel.** 1993. Editing of the chloroplast *rpoB* transcripts is independent of chloroplast translation and shows different patterns in barley and maize. EMBO J. **12:** 4291–4296.