Ribosomal protein S19 is encoded by the mitochondrial genome in *Petunia hybrida*

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Received February 5, 1991; Revised and Accepted April 12, 1991

EMBL accession no. X57283

ABSTRACT

The rps19 ribosomal protein gene, which has not been previously reported in any mitochondrial genome, was identified by sequence analysis in the mitochondrial DNA of the higher plant *Petunia hybrida*. According to the sequence of eight rps19 cDNAs, seven C to U conversions with respect to the genomic sequence are present in rps19 transcripts. Not all transcripts are fully edited at these seven sites. Six of the seven C to U conversions change the encoded amino acid sequence by altering four codons. The rps19 gene is located entirely within a repeat sequence which is present in three copies on the 443 kb genome. Due to intragenomic recombination across these repeats, *Petunia rps19* is present in nine different genomic environments.

INTRODUCTION

Mitochondrial ribosomal RNA genes are located in the mitochondria (mt) in all eukaryotes that have been studied (1). In contrast, the cellular location of the mt ribosomal protein genes varies among different groups of eukaryotes. In vertebrates, the entire set of ribosomal proteins required by the mitochondria are encoded by the nucleus (2,3). One ribosomal protein gene has been found in the mitochondrial genome of yeast (var1) and *Neurospora* (S5), but the majority of the ribosomal proteins in the fungi are imported from the cytoplasm (4).

In contrast to the relatively small mitochondrial genomes of vertebrates and fungi, higher plant mt genomes are quite large, and vary in size from 208 kb (*Brassica hirta*) to approximately 2200 kb (muskmelon) (5,6). The large plant mitochondrial genome can potentially accommodate many more protein-coding genes than other eukaryotic mitochondrial genomes. Within the past few years, coding regions similar to prokaryotic ribosomal protein genes have been found in plant mt genomes; rps12 in wheat, maize, and *Petunia* (7, 8), rps13 in tobacco, maize, wheat, and *Petunia* (9,10,11), rps14 in broadbean, soybean, and *Oenothera* (12,13), and rps3 and rpl16 in maize (14) and *Petunia* (15). The transcripts from several of these plant mt ribosomal protein genes (S12, S13, S14) have been shown to undergo the process of RNA editing (13,16,17).

We have found a gene in the mitochondrial genome of *Petunia hybrida* line 3704 which is identified as the ribosomal protein S19 (RPS19) by virtue of its similarity to bacterial and chloroplast S19. The rps19 gene is located within a repeated sequence. The 443 kb mitochondrial genome of *Petunia hybrida* line 3704 contains three copies of this repeat, which appears to undergo recombination (11). By cDNA analysis, we have determined that transcripts containing rps19 undergo the process of RNA editing.

MATERIALS AND METHODS

DNA preparation and Southern analysis

Total DNA enriched for mitochondrial DNA from *Petunia hybrida* line 3704 suspension cells was isolated by differential centrifugation following the procedure previously described (18), but the DNA was harvested prior to sucrose gradient centrifugation. The DNA blot hybridization analysis was carried out using standard procedures (19) except that the gel was subjected to short-wave UV for 5 minutes prior to blotting to aid in the transfer of large DNA fragments. After hybridization, the blot was washed at 65°C in 0.2×SSC, 0.1% SDS. The probe used for this analysis was the same as that described below for cDNA library screening.

Genomic and cDNA clone isolation

DNA preparation and Southern analysis

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A cDNA library was made by C. Sutton using random hexamers as primers to 3704 suspension cell total mitochondrial RNA which was prepared by conventional methods. The cDNAs were cloned into the Lambda Zap vector supplied by Stratagene. S19 cDNAs were isolated from this library with a random hexamer labelled 255 bp BglII fragment containing 177 bp of the coding sequence and 76 bp of the 3' flanking sequence from a genomic subclone of rps19 from line 3688. Bluescript plasmids used in further manipulations were excised in vivo from the lambda cDNA clones of interest using the procedure supplied by Stratagene.

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Figure 1. Restriction map of the rps19 locus in *Petunia hybrid* line 3704 mitochondria. rps19 is located within a repeated region upstream of coxH-1. The two coxH-1 exons are shown as open boxes. The exact boundaries of this repeat are not known and are shown as dashed lines. Restriction sites are indicated for PstI (P), Sall (S), XbaI (X), and BamHI (B). The map is oriented with the 5′ terminus of S19 to the left in the figure.

Figure 2. Nucleotide sequence of the rps19 locus beginning from the Sall site upstream of rps19 and extending 91 bp beyond the stop codon. The S19 amino acid sequence deduced from the genomic DNA sequence is shown. From cDNA analysis, the rps19 transcript appears to be edited. The seven edited sites are shown as thymidines below the genomic sequence. Amino acid changes which result from this editing are shown in bold type above the S19 protein sequence.

**RESULTS**

**Isolation and sequence of the rps19 gene**

Physical mapping of the entire 443 kb circular mitochondrial genome of *Petunia hybrid* line 3704 by cosmid walking revealed the presence of three copies of a single recombination repeat (8.9P, 19.6P, and 26.0P). Intramolecular recombination between these three repeat copies causes the recombination repeat to exist in nine different environments (11). Cosmid 2H3 contains a copy of this recombination repeat on an 8.9 kb PstI fragment (the 8.9P repeat) (11). Sequence analysis of a 2.0 kb Sall-BamHI subclone from this cosmid revealed the presence of an open reading frame with the potential to encode a 94 amino acid protein. This open reading frame was identified as the ribosomal protein gene rps19 by computer analysis. A gene for cytochrome oxidase subunit II (coxH-1,21) is located approximately 4.0 kb downstream of the ATG for rps19. A restriction map of the rps19 locus is shown in Figure 1, with the recombination repeat and coxH-1 shown for reference. The sequence of the *Petunia hybrid* line 3704 mitochondrial DNA that encodes rps19 is presented in Figure 2.
The sequence of the rps19 coding region contained in two additional repeat copies present in line 3704, the 19.6P and 26.0P repeats, are identical to the presented rps19 sequence from the 8.9P repeat (data not shown). In addition, the coding sequence of rps19 from a truncated recombination repeat in Petunia parodii line 3688 is identical to that from line 3704 (data not shown).

Genomic environments of the rps19 gene

Since the rps19 gene lies entirely within the recombination repeat, it was predicted to also exist within the other copies of the repeat present in the Petunia mitochondrial genome. This concept is shown schematically in Figure 3 with the Psfl sites adjacent to the recombination repeat, and the rps19 locus shown for clarity. To test this prediction, a DNA hybridization analysis was performed. Figure 4(A) shows the autoradiogram of a DNA blot of Psfl-digested Petunia hybrida 3704 DNA probed with a BgIII clone containing 177 bp of coding sequence and 76 bp of 3' flanking sequence from the rps19 gene cloned from Petunia parodii line 3688. The restriction map of the rps19 locus from line 3688 is shown in Figure 4 (B) along with the BgIII fragment used as a probe. As seen in Figure 4(A) the rps19 probe hybridized to nine Psfl fragments whose sizes correspond to the nine fragments which contain the recombination repeat in nine different genomic environments. The difference in intensity between the nine different bands is likely to be a result of uneven transfer and is not an indication of the relative abundance of the different repeats.

cDNA analysis of the rps19 gene

Recently, RNA editing has been shown to occur in higher plant mitochondria. cDNAs from plant mitochondrial RNAs contain thymidine at selective positions in which cytidine is present in the genomic DNA sequence (17,22,23). This editing can be quite extensive, e.g., 10 cytidines edited in 75 codons for Petunia hybrida atp9 (24), or quite limited, e.g., 2 cytidines edited within 99 codons of Oenothera rps14 (13).

Eight rps19 cDNAs were isolated from a lambda cDNA library of Petunia hybrida line 3704 suspension cell mt RNA using the rps19 probe shown in Figure 4B. Four of these cDNAs (2,3,4,8) cover the entire rps19 coding sequence while 3 cDNAs (1,5,6) end between 34 and 44 bp upstream of the TAA stop codon. cDNA # 7 begins 116 bp downstream of the ATG start codon and ends at the stop codon. One strand of the coding region from each of these rps19 cDNAs was sequenced. A schematic of the sequencing results is shown in Figure 5. Seven RNA editing sites were found within the rps19 coding region. cDNA # 1 was edited at all seven sites while the remaining seven cDNAs were partially edited.

The process of RNA editing often changes the amino acid sequence of the proteins encoded by the edited RNA (13,16,17,22-27). The seven RNA edits in rps19 are shown in Figure 2 as thymidine residues below the genomic sequence. Six of these edits change four amino acids at positions 39, 46, 55, 74 as shown above the S19 protein sequence deduced from the genomic sequence. The seventh RNA edit, at +15 bp relative to the ATG, does not change the amino acid at that position because of the degeneracy of the genetic code.

S19 protein identification and comparison

The amino acid sequence shown in Figure 2 was identified as the ribosomal protein S19 based on its similarity to known S19 proteins using the FASTA search (28). An alignment of the Petunia hybrida line 3704 mitochondrial S19 protein with six other S19 proteins is shown in Figure 6. Amino acids which are conserved between eight of the nine S19 proteins are highlighted with an asterisk. The Petunia S19 protein is 65 %, 37 %, and 35 % identical to S19 from Mycoplasma capricolum, maize chloroplast, and tobacco chloroplast respectively.

The alterations in amino acids predicted as a result of RNA editing are shown in bold above the genomic sequence in Figure 6. In several cases, RNA editing increases the amino acid sequence conservation between plant mitochondrial proteins and their counterparts in the prokaryotes and the chloroplast (13,23,26,27). Two of the four amino acid changes resulting from RNA editing in Petunia S19 increase its conservation with other S19 proteins. In Petunia S19, a serine at position 39 is changed to a leucine, which conserves a hydrophobic amino acid at this position. Leucine is also present at the analogous position in

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**Figure 4.** Panel A: DNA blot hybridization analysis of mitochondrial enriched DNA from Petunia hybrida line 3704 suspension cells. The DNA was cut with Psfl. Lambda DNAs which were digested with ClaI,Sall, and EcoRI/HindIII were used as size standards (stds). Panel B: This is a restriction map of the rps19 locus from line 3688. The coding sequence of this rps19 gene (open box) is identical to that of rps19 in line 3704 (data not shown). The 253 bp BgIII fragment was used as a probe for the DNA blot in Panel A. The rps19 probe hybridized to nine Psfl fragments, the sizes of which are shown in Panel A. These Psfl fragments correspond to the recombination repeat described previously (11).

**Figure 5.** A detailed map of the rps19 locus is shown with restriction sites for HincII (Hc), Sall (S), Xbal (X), BgIII (Bg), EcoRI (E), and EcoRV (RV) indicated. Below this map are schematic line drawings of the eight rps19 cDNAs isolated from a lambda cDNA library of Petunia hybrida line 3704 suspension cell mitochondrial RNA using the probe shown in Figure 4 (B). The seven RNA editing sites within the rps19 coding region deduced from the cDNA sequences are shown as circles on the line drawing. Filled circles indicate sites where editing occurred in individual cDNAs, while open circles indicate sites where editing did not occur.
Cyanophora paradoxa cyanelle, Halobacterium halobium and maize chloroplast S19. At position 55 in Petunia S19, a proline is changed to a phenylalanine. Mycoplasma capsicum and Halobacterium S19 also contain phenylalanine at the same position. The two other amino acid changes resulting from RNA editing in S19 (positions 46 and 74) do not increase the protein conservation of this sequence relative to other S19 proteins.

**DISCUSSION**

We have identified a mitochondrial gene in Petunia which has the potential to encode the ribosomal protein, S19. The rps19 DNA sequence predicts a protein with a molecular weight of 11,222. The molecular weight of E. coli S19 is similar (10,430) (29). 37% of the Petunia S19 amino acid residues are basic, and this highly basic composition is expected for ribosomal proteins. In addition, the Petunia S19 protein is 37% identical to E. coli S19. This degree of similarity is enough to suggest the predicted 94 amino acid protein as S19.

Rps19 was found on the 8.9P repeat which lies upstream of atp9 (24). In Oenothera nad3 transcripts were found in wheat (25) or in Petunia atp9 (30). The Petunia mitochondrial rps19 gene is located entirely within the recombination repeat, with approximately 1 kb of repeated sequence upstream and 3 kb downstream of the coding region. It is therefore unlikely that repeat recombination would alter the expression of this gene.

From an analysis of eight rps19 cDNAs, seven RNA editing sites (C to U conversions) were found within the rps19 coding region. Sequence information gained from these cDNAs revealed no evidence for RNA editing in the sequence flanking the rps19 coding region. Only one of the sequenced cDNAs was fully edited at all seven sites. The partially edited transcripts may represent intermediates in the editing process. The proportion of fully edited versus partially edited transcripts appears to vary widely between different plant mitochondrial genes. No partially edited coxI III transcripts were found in wheat (25) or in Petunia atp9 (24). In contrast, all of the examined Oenothera nad3 transcripts are partially edited (26). If these partially edited transcripts are translated, proteins with variations in amino acid sequence would be synthesized.

RNA editing of plant mitochondrial genes has been shown to result in the increased conservation of protein sequences (13,15,22,23,26,27). In Oenothera NAD3, nine of the twelve amino acid changes which result from RNA editing clearly increase the Oenothera NAD3 conservation with other NAD3 proteins (26). RNA editing at the seven specified nucleotides in Petunia S19 results in amino acid changes at four positions. At these four sites, the amino acid predicted from the DNA sequence is not found in any other known S19 protein; editing at two of these sites results in the encoding of an amino acid which increases the amino acid conservation of Petunia S19 with other S19 proteins. However, in two cases, the edited transcript encodes an amino acid different from the ones observed in all other known S19 proteins. At position 46, the Petunia transcript encodes phenylalanine while threonine is present in every other S19. The
amino acid predicted from the genomic sequence at this position (serine) is a conservative change from threonine while the phenylalanine predicted at this position from two of the rps19 cDNAs is non-conservative. At position 74, the Petunia transcript encodes phenylalanine while other S19 proteins have proline, leucine or glutamine at this position.

In vertebrates and lower eukaryotes, the mitochondrial ribosomal proteins are encoded by the nucleus (with the exception of varI(S5) in the fungi) (3,31). Because a full-length rps12 is not present in the Oenothera mitochondrial genome (26) and rps14 has not been detected in maize mitochondria (12), certain plant mitochondrial ribosomal proteins are also likely to be encoded by the nucleus. However, genes with homology to rps12, rps13, rps14, rpl16 (7-15) have been found in plant mitochondrial DNA. Although immunological evidence for the synthesis of ribosomal proteins in plant mitochondria is presently lacking, Petunia rps19 and the other plant mitochondrial ribosomal protein genes whose transcripts undergo processing in the form of RNA editing are likely to be functional genes.

ACKNOWLEDGEMENTS

We would like to thank Otto Folkerts for repeat-containing cosmids from the Petunia line 3704 and 3688 mitochondria cosmid libraries, Kim Pruitt for a repeat-containing subclone from line 3688, Claudia Sutton for the Petunia line 3704 mitochondrial cDNA library and Kristy Richards for photography. This work was supported by the Cornell NSF Plant Science Center, a unit in the USDA-DOE-NSF Plant Science Centers Program, which is sponsored by the New York State Science and Technology Foundation, a consortium of industries, and the U.S. Army Research Office, and by the USDA Competitive Grants Program (Genetic Mechanisms) and Hatch NYC #186418.

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