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Ribosomal protein S14 genes in broad bean mitochondrial DNA

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ABSTRACT

Broad bean (Vicia faba) mtDNA contains an open reading frame with a predicted amino acid sequence that is 41% homologous to the ribosomal protein S14 (RPS14) of Escherichia coli, and which is located 1232 ntp upstream from a gene for cytochrome b (*cob*). A second putative *rpS14* gene occurs in broad bean mtDNA, 344 ntp upstream from a gene for ATPase subunit 9 (*atp9*). However, the *atp9*-linked *rpS14* gene is 12 codons shorter than the cob-linked *rpS14* gene. Sequence homology is found upstream (for 218 ntp) but not downstream from the two *rpS14* genes. Transcripts were detected in broad bean mtRNA only for the cob-linked *rpS14* gene. All RNA molecules that include a transcript of the *rpS14* gene also include a transcript of the *cob* gene. Sequences homologous to the broad bean mitochondrial *rpS14* gene were detected in soybean mtDNA, but not in corn mtDNA. Relationships between the amino acid sequences of RPS14s encoded in broad bean mtDNA, in chloroplast DNAs of various angiosperms, and in E. coli are consistent with the view that the ancestral lines of these three kinds of DNA diverged from each other within a relatively short time period.

INTRODUCTION

The mitochondrial DNAs (mtDNAs) of higher plants examined to date have a size that varies within the approximate range of 165 to 2500 kb (1-5). These values are greater than the sizes of multicellular animal mtDNAs (14.2 to 39.3 kb; 6,7) and fungal mtDNAs (8). As only 5 to 10% of plant mtDNAs consist of repeated sequences (1) it seems plausible that these mtDNAs encode considerably more protein genes than animal mtDNAs and that the mitochondrial (mt-) genetic information content of different plants is highly variable.

In mtDNAs of different species (including corn, wheat, rice, Oenothera, pea, soybean, broad bean, tobacco, and petunia) the following protein genes which also occur in animal and fungal mtDNAs, have been identified and sequenced: cytochrome *c* oxidase subunits I, II and III (*coxI*, 9,10; *coxII*, 11-15; *coxIII*, 10); cytochrome b (*cob*; 16-18); F_o-ATPase subunit 6 (atp6; 19); F_o-ATPase subunit 8 (*atp8*; 20). A gene for F_o-ATPase subunit 9 (*atp9*) that occurs in some fungi is also found in plant mtDNA (21-23). Two genes have

been located in plant mtDNAs that are not found in the mtDNAs of other organisms: a gene for the α-subunit of the F_1-ATPase complex (*atpA*; 24-27) and the mt-ribosomal protein S13 (*rpS13*; 22,28).

In this paper we report the finding of a gene for ribosomal protein S14 (*rpS14*) in mtDNA of broad bean, Vicia faba. Two versions of the gene occur in broad bean mtDNA, a complete, transcribed copy, and a truncated copy that is apparently not transcribed.

MATERIALS AND METHODS

The broad bean (Vicia faba) seed used in this work was FAVA BROAD WINSOR (Musser Seed Co., Twin Falls, ID).

Nucleic acid preparations. Mitochondria were isolated from 12 to 14 day old, dark grown, etiolated hypocotyls as previously described (29) and DNA was isolated from SDS lysates of mitochondria by preparative cesium chloride centrifugation. Total mtRNA was obtained from isolated mitochondria as given in (30).

Restriction enzyme digestions and cloning. Details regarding restriction enzyme digestions, electrophoresis, cloning of restriction fragments into pUC9, M13mp18 and M13mp19, and preparation of single-stranded and double-stranded M13 DNAs are given or referenced in (31).

Construction of mtDNA libraries. Total broad bean mtDNA was digested with either EcoRI, HindIII, SalI, or BamHI, ligated into plasmid pUC9 (32) digested with the same enzyme, and the products transformed into competent E. coli host strain JM103. More than 300 colonies representing each cloning experiment were stored in 15% glycerol at -20°C (33).

Heterologous probes. The DNA probe used to detect a cytochrome b (*cob*) gene-containing fragment of broad bean mtDNA was the Zea mays plasmid pZmEH680 (derived from pBR328; 16) that contains a 680 ntp, HindIII-EcoRI fragment of the Z. mays *cob* gene. The DNA probe used to detect an ATPase subunit 9 (*atp9*) gene-containing fragment of broad bean mtDNA was a pUC9-derived plasmid that includes an *atp9* gene-containing, 2.2 kb, XbaI fragment of Z. mays mtDNA (21).

Oligonucleotide probes. Two, 21 nt sequences (the complements of nt 752-772, Fig. 2, and of nt 755-775, Fig. 4) were synthesized using an Applied Biosystems Synthesizer 380B.

DNA probe labelling. Each DNA probe derived from an M13-mtDNA clone was ^{32}P-labelled by extension synthesis, using the Klenow fragment of E. coli DNA polymerase I and [α-^{32}P]dATP. Double-stranded DNA probes were ^{32}P-labelled by either nick translation or end-labelling as described previously (30).

Synthetic, 21 nt sequences were ^{32}P-end-labelled using T4 polynucleotide kinase (34).

Nucleic acid hybridizations. The procedure for the capillary transfer of electrophoretically separated RNAs and DNAs from agarose gels to the hybridization support Gene Screen Plus was that given in New England Nuclear Catalog No. NEF0976 (Jan. 1984). All DNA:RNA and DNA:DNA hybridizations were carried out in 5 x Denhardt's solution, 5 x SSC, 50 mM sodium phosphate (pH 6.5), 0.1% SDS and 100 mg/ml salmon sperm DNA. DNA:RNA and DNA:DNA hybridizations to detect identical sequences were carried out at 60°C overnight. Heterologous DNA:DNA hybridizations (broad bean mtDNA probe to restriction enzyme-digested corn or soybean mtDNA) were carried out at 45°C overnight. Hybridizations involving synthetic 21 nt probes were carried out at 40°C overnight. All hybridization products were subjected to four washes, 5 min each in 0.1% SDS and 2 x SSC at 21°C, followed by two washes, 20 min each in 0.1% SDS, 0.1 x SSC at 42°C, or when oligonucleotide probes were used, at the hybridization temperature.

In order to determine the sizes of electrophoretically separated RNA molecules to which radioactively labelled probes hybridized, RNA molecules of known size (RNA ladders 0.24-9.5 kb and 0.16-1.77 kb (Bethesda Research Laboratories)) were coelectrophoresed in lanes adjacent to mtRNA, stained with ethidium bromide and visualized by UV light.

Colony hybridizations were carried out as described in (33).

Sequencing. pUC9-cloned restriction fragments of the mtDNAs were recloned into M13mp18 and M13mp19. Deletion clones, containing overlapping sequences representing the entire nucleotide sequence of both complementary strands of the fragment were obtained using modifications of the methods described in (35,36). Details concerning DNA sequencing, computer assembly and analysis of sequences are given in (30).

RESULTS AND DISCUSSION

Nucleotide sequences of *cob*, *atp9* and *rpS14* genes. By hybridization of a labelled DNA clone that contains 680 ntp of the corn *cob* gene (pZmEH680; 16) to a blot of electrophoretically separated EcoRI fragments of broad bean mtDNA, we identified a single fragment of 3.1 kb containing the *cob* gene. The 680 ntp corn mtDNA fragment purified from pZmEH680, was used as a probe to identify a colony in our EcoRI-pUC9 library of broad bean mtDNA that included the *cob* gene-containing 3.1 kb fragment. This latter fragment was isolated and transferred to M13mp18 and M13mp19 and its nucleotide sequence determined.

A clone containing a section near the 3' end of the 3.1 kb EcoRI fragment was used to identify an overlapping HindIII fragment in a colony of our broad bean HindIII-pUC9 library. This HindIII fragment was also isolated and transferred to M13mp18 and M13mp19 DNAs, and its nucleotide sequence determined. A continuous sequence of 4351 ntp was obtained (Figs. 1 and 2) that contains the entire *cob* gene (1179 ntp) and 2023 ntp upstream and 1149 ntp downstream from this gene. The broad bean *cob* gene is 6 ntp shorter and 12 ntp longer than the corresponding genes of Oenothera berteriana and corn (17,16). Homology of the nucleotide sequences and predicted amino acid sequences between the broad bean and Oenothera *cob* genes are 97.36% and 96.94%, respectively, and between the broad bean and corn *cob* genes are 95.53% and 94.33%, respectively.

Within the sequence upstream from the *cob* gene and separated from this gene by 1232 ntp is an open reading frame of 300 ntp (Fig. 2). As the amino acid sequence predicted from this open reading frame is 41.2% homologous to the amino acid sequence of the ribosomal protein S14 (RPS14) of Escherichia coli (Fig. 3, Table 1; 37,38), we have interpreted the broad bean open reading frame as a mt-ribosomal protein S14 (*rpS14*) gene. The encoded protein would be highly basic (arg = 17, lys = 11, his = 1, asp = 5, glu = 3) as has been

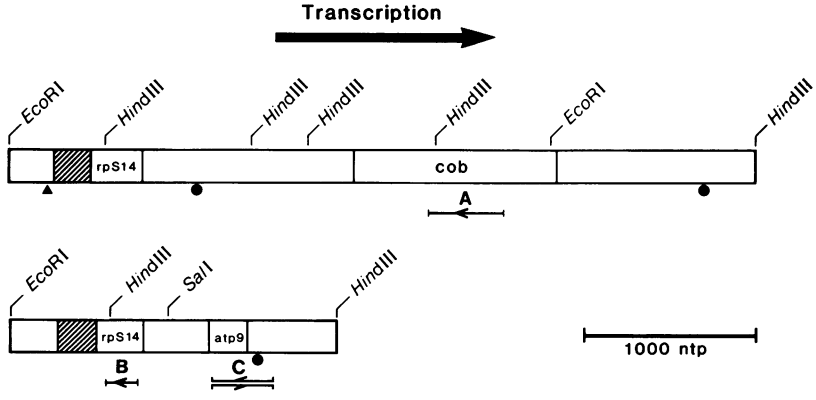


Figure 1. Maps of the two sequenced segments from broad bean mtDNA that contain the *cob* gene, the *atp9* gene and the two copies of the *rpS14* gene. The *cob*, *atp9* and *cob*-linked *rpS14* genes are transcribed in the direction shown. The shaded regions upstream from the two *rpS14* genes represent sequences that differ by only one nt insertion-deletion. Restriction enzyme sites are those used for sequencing. Below each map the locations and directions of DNA probes used to test for transcription (bars A, B and C) are indicated. The solid circles indicate the locations of double stem and loop structures (Fig. 7). The solid triangle indicates the location of a 13 nt sequence that is complementary to the first 13 nts of the minor transcript of broad bean mt-plasmid 2.

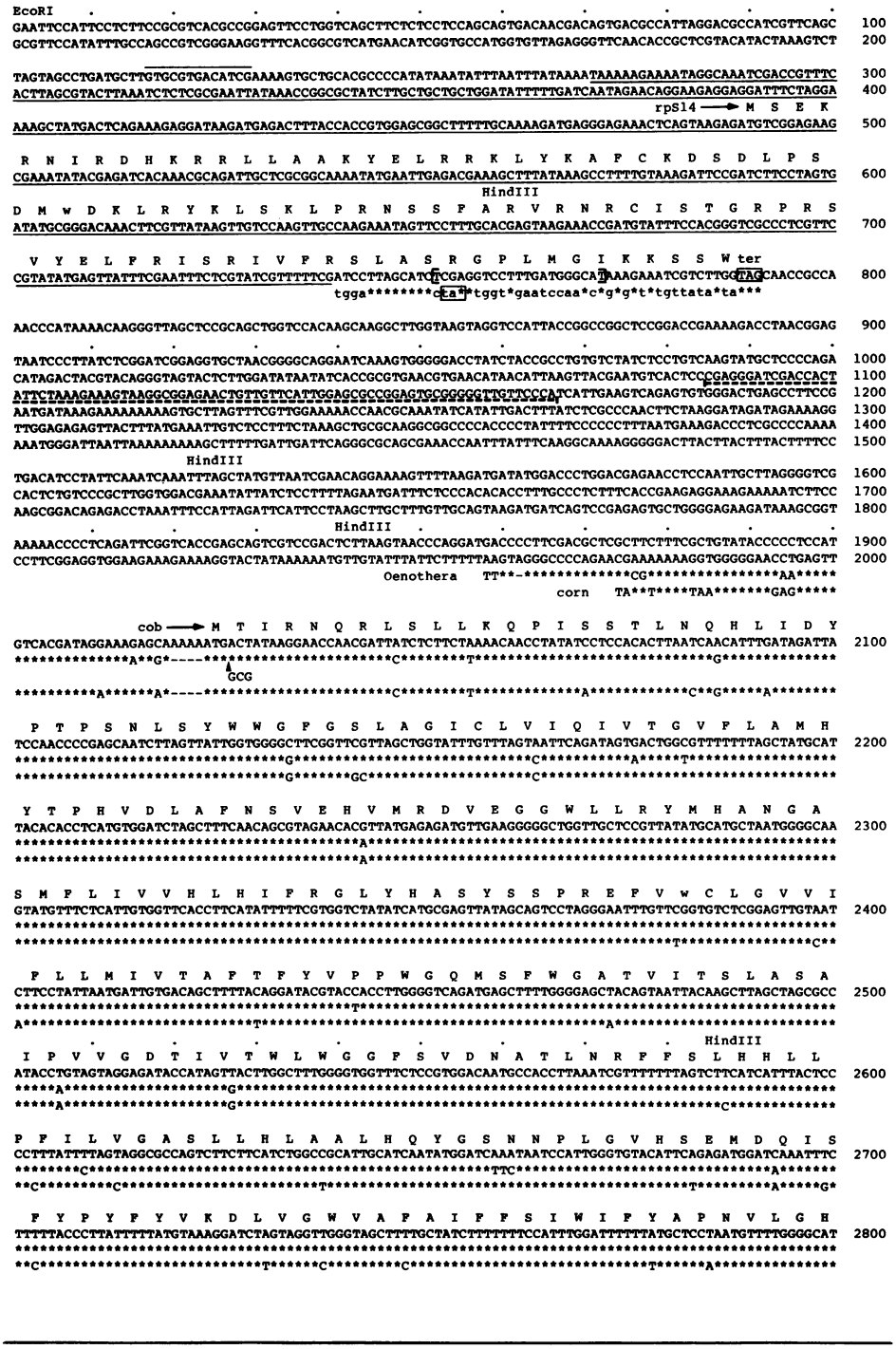
noted for the mt-RPS13 of tobacco and corn (22) and as would be expected for a protein in close association with RNAs.

Beginning with a probe that contained the corn *atp9* gene, we used a strategy similar to that used for the isolation of broad bean mtDNA fragments containing the *cob* gene to isolate fragments containing a broad bean *atp9* gene. These fragments were then sequenced: a 1.3 kb HindIII fragment and an overlapping 0.9 kb EcoRI-SalI fragment. The resulting nucleotide sequence (1879 ntp) contained the entire *atp9* gene (225 ntp) and 1154 ntp upstream, and 500 ntp downstream from this gene (Figs. 1 and 4). The broad bean *atp9* gene is the same size as the corn *atp9* gene (21) but 9 ntp shorter than *atp9* gene of Oenothera (22). Nucleotide and amino acid sequence homologies between the broad bean and Oenothera *atp9* genes are 86.49% and 90.54%, respectively, and between the broad bean and corn *atp9* genes are 85.14% and 90.54%, respectively (Fig. 4). Upstream from the broad bean *atp9* gene and separated from it by 384 ntp is a second copy of the *rpS14* gene (Fig. 4). However, this *atp9*-linked *rpS14* gene lacks the twelve 3' terminal codons of the *cob*-linked *rpS14* gene. The two genes are identical for the first 251 ntp, but there is then a 4 ntp divergence followed by an 8 ntp identical section that ends 1 ntp before the terminal triplet of the *atp9*-linked *rpS14* gene. There is no convincing homology downstream from the two *rpS14* genes. However, there is complete homology (except for an extra adenosine residue 28 ntp upstream from the *atp9*-linked gene) for 218 ntp upstream from the two broad bean *rpS14* genes (Figs. 2 and 4).

Transcription of the *cob*, *atp9* and *rpS14* genes. When a single-stranded probe containing an internal 421 nt segment of the *cob* gene (Fig. 1, probe A) was hybridized to blots of electrophoretically separated mtRNAs, two main bands were observed indicating transcripts of 3600 and 2850 nt (Fig. 5). There were also four lighter bands (2550, 2300, 1900 and 1700 nt) and at least nine bands of very low intensity indicating complementary RNA molecules in the range 740-1550 nt.

Following hybridization to broad bean mtRNA of a sequence complementary to an internal segment of the *rpS14* gene (derived from a region that is identical to the corresponding region of the *cob*-linked *rpS14* gene, Fig. 1, probe B), bands were seen all of sizes similar to some of the bands representing *cob* gene transcripts: two major bands of 3600 and 2850 nt and the two lesser bands of 1900 and 1700 nt (Fig. 5).

To determine whether one or both copies of the *rpS14* gene are transcribed we obtained two, 21 nt probes that were complementary to the non-homologous


EcoRI
rpS14 ⟶ M S E K
HindIII
R N I R D H K R R L L A A K Y E L R R K L Y K A F C K D S D L P S
D M W D K L R Y K L S K L P R N S S F A R V R N R C I S T G R P R S
V Y E L F R I S R I V F R S L A S R G P L M G I K K S S W ter
HindIII
HindIII
cob ⟶ M T I R N Q R L S L L K Q P I S S T L N Q H L I D Y
GCG
P T P S N L S Y W W G F G S L A G I C L V I Q I V T G V F L A M H
Y T P H V D L A F N S V E H V M R D V E G G W L L R Y M H A N G A
S M F L I V V H L H I F R G L Y H A S Y S S P R E F V w C L G V V I
HindIII
F L L M I V T A F T F Y V P P W G Q M S F W G A T V I T S L A S A
I P V V G D T I V T W L W G G F S V D N A T L N R F F S L H H L L
P F I L V G A S L L H L A A L H Q Y G S N N P L G V H S E M D Q I S
F Y P Y F Y V K D L V G W V A F A I F F S I W I F Y A P N V L G H

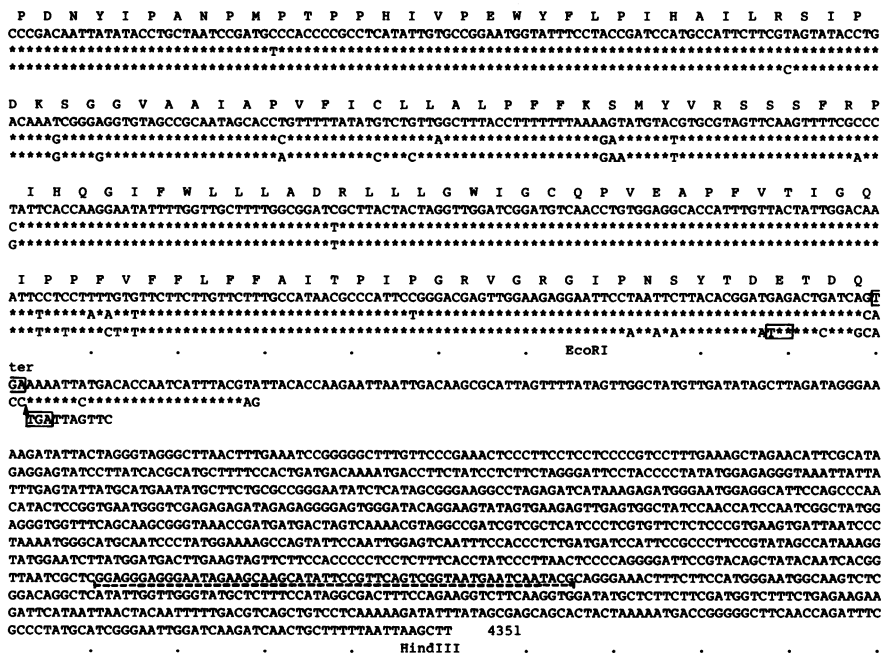


Figure 2. The nucleotide sequence of the 4351 nt fragment of broad bean mtDNA that contains the *cob* and *rpS14* genes. Restriction sites used in sequencing are shown. For each gene the direction of transcription is indicated by an arrow and the predicted amino acid sequence is shown. CGG codons are translated as w to indicate the putative assignment of tryptophan (11,12). The nucleotide sequences of the *cob* genes of corn and Oenothera and portions of the 5′ and 3′ flanking sequences of these genes (16,17) are aligned beneath the broad bean cob gene: an asterisk indicates a nucleotide which is conserved relative to the broad bean sequence; a dash indicates a nucleotide which is absent. Termination codons in the Oenothera and corn genes are boxed. Insertions in the Oenothera sequence are indicated by vertical arrowheads. The sequence (nt 271-739) underlined is that which is identical (with a 1 nt exception: inverted arrow, Fig. 4) to a sequence upstream from the *atp9* gene (Fig. 4). The nucleotide sequence of the 3′ end of the *atp9*-linked *rpS14* gene is shown in lower case letters beneath the *cob*-linked *rpS14* gene. The bracketed sequence (nt 752-772) is the complement of the 21 nt sequence used to test for transcription of the *cob*-linked *rpS14* gene. The 13 nt sequence (nt 217-229) that is identical to 13 nt at the 5′ end of a broad bean mt-plasmid 2 transcript is overlined. Sequences that can fold into double stem and loop structures (Fig. 7) are identified with broken underlines.

sequences at the 3′ ends of the two *rpS14* genes (Figs. 2 and 4). The two probes were end-labeled and hybridized to mtRNA. Hybridization of the probe comprising the nucleotide sequence of the end of the *cob*-linked *rpS14* gene resulted in a band pattern identical to the band pattern obtained by

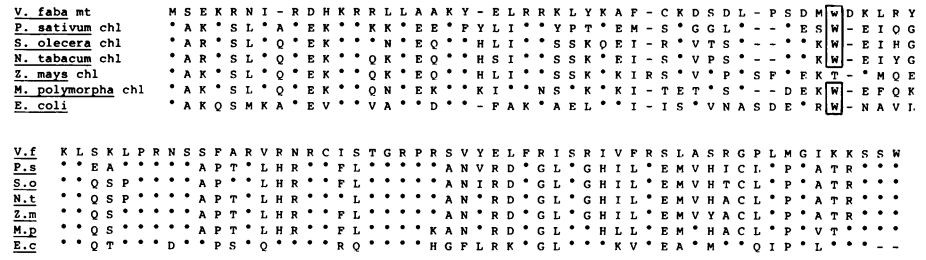


Figure 3. Comparisons of the amino acid sequence predicted from the putative *rpS14* gene of broad bean (Vicia faba) mtDNA with the amino acid sequence of RPS14 of Escherichia coli (37,38) and the predicted amino acid sequences of the *rpS14* genes of chloroplast DNAs of pea (Pisum sativum; 39), spinach (Spinacia oleracea; 40), tobacco (Nicotiana tabacum; 41), corn (Zea mays; 42), and a liverwort (Marchantia polymorpha; 43). The box indicates the location in the broad bean *rpS14* nucleotide sequence of a CGG codon (shown as w) that corresponds in position to a tryptophan in the E. coli amino acid sequence and to a TGG codon in each of the chloroplast sequences, except that of Z. mays.

hybridization of the probe derived from an internal segment of the *rpS14* gene (Fig. 6). In contrast, no bands representing complementary RNA molecules were detected when hybridization was attempted using the probe comprising the sequence at the end of the *atp9*-linked *rpS14* gene (Fig. 6). Data from control hybridization experiments in which each 21 nt probe was separately incubated with M13 DNAs containing the complementary strand of the cob-linked *rpS14* gene and of the *atp9*-linked *rpS14* gene, confirmed the specificity of the two probes (Fig. 6). These results are clearly consistent with the view that only the *cob*-linked *rpS14* gene is transcribed.

The 3600 nt and 2850 nt classes of RNAs to which the *cob* gene probe and the *rpS14* gene probe hybridized are large enough to include transcripts of the

Table 1.

	P. s	S. o	N. t	Z. m	M.p	E. coli
V. faba mt	37.3	39.2	40.2	39.4	41.2	41.2
P. sativum chl	-	75.0	77.0	74.1	67.0	37.3
S. oleracea chl		-	89.0	80.4	71.0	37.3
N. tabacum chl			-	84.5	74.0	38.2
Z. mays chl				-	74.8	38.8
M. polymorpha chl					-	41.2

Homologies (as percentages) among amino acid sequences of RPS14 proteins of broad bean (Vicia faba) mitochondria, E. coli and chloroplasts of various species (37-43). The box includes comparisons of angiosperm chloroplast RPS14s.

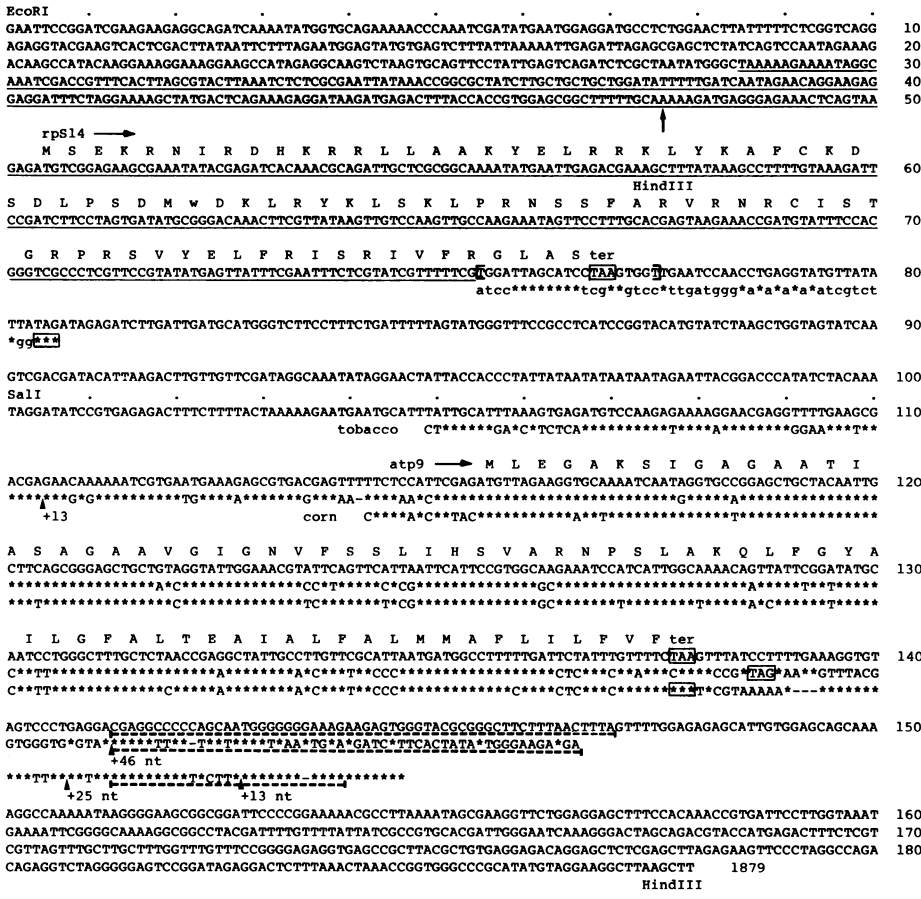


Figure 4. The nucleotide sequence of a 1879 nt fragment of broad bean mtDNA that contains the *atp9* gene and a truncated copy of the *rpS14* gene (Fig. 2). The nucleotide sequences of the *atp9* genes from tobacco and corn, and portions of the 5′ and 3′ flanking sequences (22) of these genes are aligned beneath the broad bean *atp9* gene. Insertions in the corn sequence are indicated by vertical arrowheads beside which the number of nucleotides inserted are given. The nucleotide sequence at the end of the *cob*-linked *rpS14* gene is shown in lower case letters beneath the *atp9*-linked *rpS14* sequence. The bracketed sequence (nt 755-775) is the complement of the 21 nt sequence used to test for transcription of the *atp9*-linked *rpS14* gene. The inverted arrow indicates the only nucleotide (nt 476: an insertion) that distinguishes nt 285-754 (underlined) from nt 270-739 of the sequence shown in Fig. 2. All other details are as given in the legend to Fig. 2.

rpS14 and *cob* genes and the sequence between these genes. The 1900 nt and 1700 nt classes of RNAs to which the same two probes hybridized could also contain transcripts of the *rpS14* and *cob* genes, but neither RNA is large

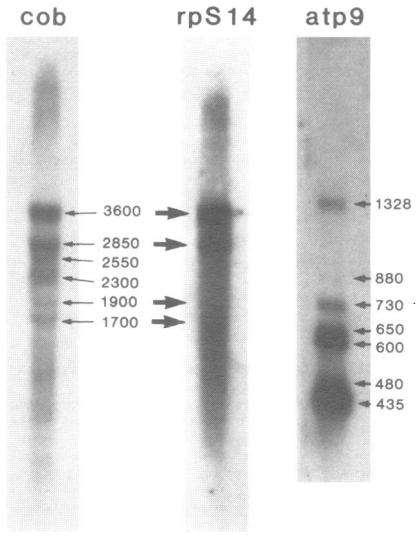


Figure 5. Autoradiographs resulting from hybridization experiments to detect transcripts of the *cob*, *rpS14* and *atp9* genes of broad bean mtDNA. Each lane contains similar portions of a single preparation of whole mtRNA separated by electrophoresis through a 1.5% agarose gel containing 2.2 M formaldehyde and transferred to Gene Screen Plus. The lanes were probed with the following: *cob*, an M13 single-stranded clone containing a 421 nt sequence that is complementary to an internal section of the sense strand of the *cob* gene (probe A, Fig. 1; nt 2485-2905, Fig. 2); *rpS14*, an M13 single-stranded clone containing a 179 nt sequence that is complementary to an internal section of the sense strand of the *rpS14* gene (probe B, Fig. 1; nt 573-751, Fig. 4); *atp9*, an M13 double-stranded clone containing a 347 ntp sequence that includes the 3' terminal 196 nt of the *atp9* coding region (probe C, Fig. 1; nt 1184-1530, Fig. 4). The numbers indicate the sizes of fragments contained in the various bands, determined by coelectrophoresis of RNA molecules of known sizes. Identical band patterns were observed when these hybridization experiments were repeated using three other, separately isolated preparations of broad bean whole mtRNA.

enough to contain both genes and the intervening sequence. This suggests either that the 1900 nt and 1700 nt RNAs detected in the two experiments are, in fact, different sequences, or that each RNA represents a molecule that contains transcripts of both the *cob* and *rpS14* genes from which all or part of the transcript of the intervening sequence has been excised.

Hybridization to mtRNA of a 347 nt probe containing the 196 terminal nt of the *atp9* gene and 151 nt 3' to this gene (Fig. 1, probe C) resulted in seven labeled bands, a predominant band representing an RNA molecule of 435 nt and six lighter bands representing RNA molecules of 480, 600, 650, 730, 880 and 1320 nt. As the *atp9*-linked *rpS14* gene is not transcribed, then the six

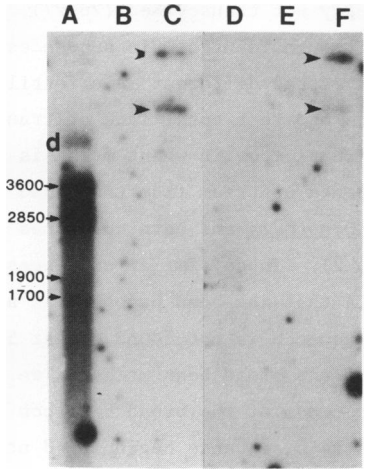


Figure 6. Autoradiographs resulting from hybridization experiments to detect transcripts of the two broad bean mt-*rpS14* genes. The lanes contain the following: A and D, total mtRNA. B and F a single-stranded M13 clone that includes the sense strand of the *atp9*-linked *rpS14* gene. C and E a single-stranded M13 clone that includes the sense strand of the *cob*-linked *rpS14* gene. Lanes A, B and C were probed with a ^{32}P-labelled 21 nt fragment complementary to the 3′ end of the *cob*-linked *rpS14* gene (nt 752-772, Fig. 2). Lanes D, E and F were probed with a ^{32}P-labelled 21 nt fragment complementary to the 3′ end of the *atp9*-linked *rpS14* gene (nt 755-775, Fig. 4). Arrowheads indicate the positions of circular and linear single-stranded M13 cloned molecules. d indicates contaminating DNA in this RNA preparation. Other details are as in the legend to Fig. 5.

RNA molecules detected must result from transcription initiation downstream from this gene. The extent to which the six RNAs might be produced as separate transcripts, or result from processing of one or more primary transcripts is not known.

Sequence relationships. As noted above, the first 251 ntp of the coding regions of the two broad bean *rpS14* genes are identical, and there is only one ntp difference between the 218 ntp upstream from the two *rpS14* genes. However, outside these DNA segments, both upstream and downstream there is a striking dissimilarity of sequences (Figs. 2 and 4). These observations, together with the apparent lack of transcription of the *atp9*-linked *rpS14* gene suggests that the sequence that contains this latter gene originated from a relatively recent rearrangement within broad bean mtDNA. Two or more copies of other mt-protein genes have been reported. Multiple copies of the *atpA* gene have been found in corn, Oenothera and pea mtDNAs. In Oenothera and pea some of these copies, like one of the broad bean *rpS14* genes, are incomplete

(pseudogenes) and apparently not transcribed (26,27). The multiple copies of the *atpA* gene of corn, although identical in sequences, occur in unequal abundance in mitochondria of the different male fertile/male sterile lines of this species (25,44). The apparent total lack of transcription of the four copies of the *rpS13* gene that occur in wheat mtDNA is unusual (28).

Homology of the sequence upstream from the broad bean *cob* gene to the upstream sequence of the Oenothera and corn *cob* genes extends for only 62 and 48 nt, respectively (Fig. 2). These similar sequences include the octanucleotide 5′ AGTTGTCA that has been proposed as a ribosome binding site (16,17). However, this sequence is not found either 5′ to the wheat *cob* gene (18) or 5′ to any other of the broad bean mt genes we have sequenced. Following the termination codon of the broad bean *cob* gene is a sequence of 25 nt that is 96% homologous to a sequence beginning 8 nt downstream from the termination codon of the Oenothera *cob* gene (Fig. 2).

The 104 nt sequence upstream from the broad bean *atp9* gene has sequence homology to the upstream sequence of the tobacco *atp9* gene. In contrast, sequence homology extends for only a short distance upstream from the broad bean and corn *atp9* genes. Segments of homology between sequences downstream from the broad bean and tobacco *atp9* genes, and from the broad bean and corn *atp9* genes are found, but they are separated by insertions/deletions (Fig. 4).

Beginning 272 nt upstream from the coding region of the transcribed *cob*-linked *rpS14* gene (and upstream from the homologous segment that precedes both copies of the *rpS14* gene) is a sequence of 13 nt (5′ GTGCGTGACATCG) that is complementary to the first 13 nt of the minor transcript of mt-plasmid 2 (Figs. 1 and 2). This finding raises the interesting possibility that the mt-plasmid transcript might be associated in some way with the control of transcription of at least the *rpS14* gene.

Secondary structures. At various distances downstream from the broad bean *cob* gene, *atp9* gene and *cob*-linked *rpS14* gene (but not the *atp9*-linked *rpS14* gene), there occurs a sequence that can be folded into a double stem and loop structure (Figs. 2, 4 and 7), and that is preceded in each case by 5′ GAGG. A similar double stem and loop, also preceded by 5′ GAGG, has been found in the sequences downstream from the Oenothera *coxII* and *atpA* genes, and the corn *coxI* and *T-urf13* genes. For each of the two Oenothera genes it was demonstrated that transcription termination occurred within a few nucleotides of the 3′ end of the second stem and loop (46). Each of these four corn and Oenothera genes has a simple transcript pattern (46). Clearly this is not the case in broad bean mtDNA. Sequences that could be folded into single stem and

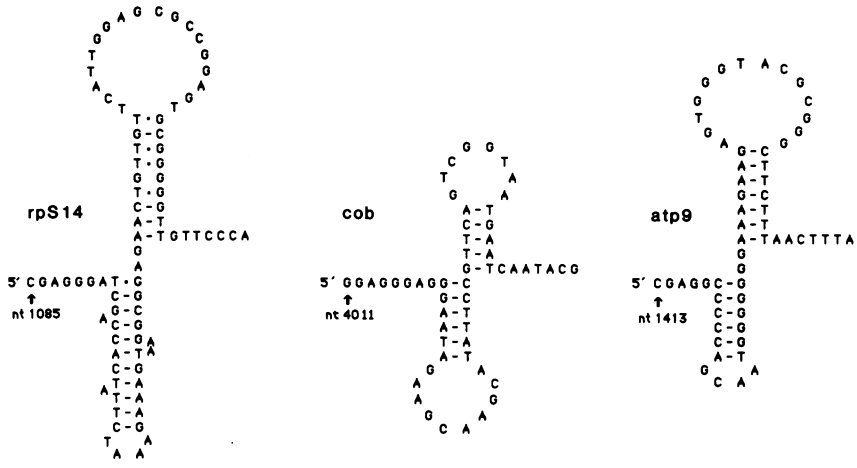


Figure 7. Double stem and loop structures that can be formed by sequences downstream from the *cob*, *atp9* and *cob*-linked *rpS14* genes of broad bean mtDNA. The numbers identify nucleotides in the sequences in Figs. 2 and 4. Calculations made using free energy increments for predicting RNA secondary structure stability reported recently (45: which include negative free energy increments for G·U pairs) suggest that the formation of each of the stem and loops shown would be plausible in the corresponding RNA transcripts.

loop structures, again preceded by 5' GAGG are located downstream from the *atp9* genes of tobacco and corn. It was suggested that these secondary structures may be associated with transcript processing rather than termination (22). From considerations of the sizes of RNA molecules that contain transcripts of the broad bean *rpS14* and *cob* genes discussed above, it seems more likely that the double stem and loop sequence that lies downstream from the *cob*-linked *rpS14* gene functions in transcript processing rather than transcription termination. In the absence of transcript end mapping data the presently available information regarding the sizes of *atp9* gene-containing, and *cob* gene-containing transcripts are equally consistent with either a transcript processing or a transcription termination role for the double stem and loop sequences found downstream from these genes.

Evolutionary considerations - The predicted amino acid sequence of the broad bean RPS14 is between 37.3% and 41.2% homologous to the amino acid sequences of the RPS14s of chloroplast DNAs of pea, spinach, tobacco, corn and the liverwort Marchantia polymorpha (Fig. 3, Table 1). These values are close to the homology of broad bean and E. coli RPS14s (41.2%, Fig. 3, Table 1). Homologies of the E. coli RPS14 and each of the chloroplast RPS14s are also in

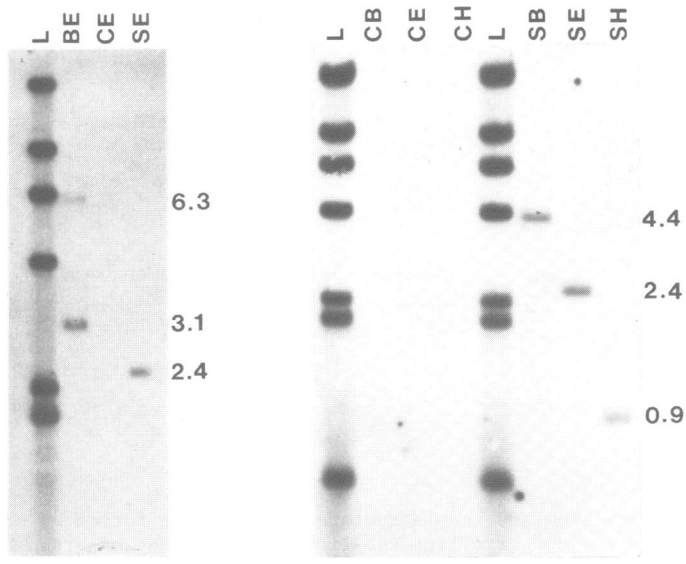


Figure 8. Autoradiographs resulting from hybridization experiments to determine the distribution of sequences homologous to the broad bean *rpS14* gene in broad bean, corn and soybean mtDNAs. The autoradiographs shown result from electrophoresis of restriction enzyme digested DNAs through a 1% agarose gel, transfer of the restriction fragments to Gene Screen Plus, and probing with a ^{32}P-labelled internal 179 nt sequence of the *rpS14* gene (probe B, Fig. 1). Lane BE contains EcoRI digested broad bean mtDNA. Lanes SE, SH and SB contain soybean mtDNA digested with EcoRI, HindIII and BamHI, respectively. Lanes CE, CH and CB contain corn mtDNA digested with EcoRI, HindIII and BamHI, respectively. The numbers indicate estimated fragment sizes (kb). Lanes L contain ^{32}P-labelled, HindIII digestion products of bacteriophage lambda: 23.1 kb, 9.4 kb, 6.7 kb, 4.6 kb, 2.3 kb, 2.0 kb and 0.56 kb.

the range 37.3% to 41.2% (Table 1). In contrast, amino acid sequence homologies among angiosperm chloroplast RPS14s are in the range, 74.1% to 89.0%. These findings are consistent with the view that the three major lines of DNA molecules that led to present day plant mtDNAs, chloroplast DNAs and *E. coli* DNA all diverge from each other at about the same time. Also, the difference in the range of homologies of the broad bean mt-RPS14 and each of the chloroplast RPS14s (37.3% to 41.2%), and the range of homologies among the different angiosperm chloroplast RPS14s (74.1% to 89.0%) suggests that the presence of the RPS14 genes in broad bean mtDNA is not due to a recent transfer of DNA from the chloroplast to the mitochondrion.

rpS14 gene sequences in other plant mtDNAs. The same *rpS14* gene probe (Fig. 1, probe C) that was used to detect RNA transcripts, was hybridized to

blots of electrophoretically separated restriction fragments of broad bean, soybean, and corn mtDNAs. Resulting autoradiographs are shown in Fig. 8. The probes hybridized to two bands representing fragments of 3.1 and 6.3 kb in the EcoRI digest of broad bean mtDNA. This and data from hybridization experiments using broad bean mtDNA digested with other restriction enzymes (not shown) are consistent with the view that the *cob*-linked *rpS14* and the *atp9*-linked *rpS14* genes are the only ones present in the broad bean mt-genome. The *rpS14* gene probe hybridized to a single band of 0.9 kb, 2.4 kb and 4.4 kb in the HindIII, EcoRI and BamHI digests, respectively, of soybean mtDNA, suggesting that this DNA contains a single copy of the *rpS14* gene. The broad bean *rpS14* gene probe did not hybridize to corn mtDNA indicating that corn mtDNA lacks an *rpS14* gene. With regard to this latter finding it is pertinent to note that sequences homologous to the *rpS13* gene of tobacco, which are present in corn and wheat mtDNAs, could not be detected in the mtDNAs of either broad bean or pea (22). This suggests that of the total presently undefined number of proteins present in plant mt-ribosomes, there may be considerable variation in partitioning of the genes encoding these proteins between the mtDNA and nuclear DNA of different plants. Also, the recent report that in wheat *rpS13* gene sequences are not transcribed raises the possibility that in some plants copies of this and other mt-ribosomal protein genes could be present in both mtDNA and nuclear DNA (28).

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* atp9-rpS14, X07236; cob-rpS14, X07237

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