

The mitochondrial gene encoding ribosomal protein S12 has been translocated to the nuclear genome in *Oenothera*

Lutz Grohmann, Axel Brennicke* and Wolfgang Schuster

Institut für Genbiologische Forschung, Ihnestr. 63, D-1000 Berlin 33, Germany

Received August 17, 1992; Revised and Accepted October 7, 1992

EMBL accession no. X68040

ABSTRACT

The *Oenothera* mitochondrial genome contains only a gene fragment for ribosomal protein S12 (*rps12*), while other plants encode a functional gene in the mitochondrion. The complete *Oenothera rps12* gene is located in the nucleus. The transit sequence necessary to target this protein to the mitochondrion is encoded by a 5'-extension of the open reading frame. Comparison of the amino acid sequence encoded by the nuclear gene with the polypeptides encoded by edited mitochondrial cDNA and genomic sequences of other plants suggests that gene transfer between mitochondrion and nucleus started from edited mitochondrial RNA molecules. Mechanisms and requirements of gene transfer and activation are discussed.

INTRODUCTION

Higher plant chloroplast and mitochondrial genomes code for several components of their respective genetic translation system (1, 2, 3). Among these are genes for the ribosomal RNAs, most of the tRNAs and a number of ribosomal proteins. However, many of the ribosomal proteins are encoded by nuclear genes and have to be imported into the organelles from the cytoplasm. According to the endosymbiont theory most of the nuclear genes coding for organellar proteins have been transferred to the nucleus during establishment of the eukaryotic cell.

Such gene translocations from the mitochondrion require the addition of a mitochondrial target sequence to the open reading frame, the correct positioning of cytoplasmic expression signals such as promoters and regulatory elements and the elimination of features specific to the organelle. These latter include organellar introns and RNA editing, which has so far not been documented for nuclear transcripts in higher plants.

RNA editing in plant mitochondria alters numerous cytidines to uridines in the mRNAs of all higher plants investigated (4–6) resulting in different polypeptides to be encoded by the edited mRNAs. The proteins deduced from unedited sequences often differ in otherwise evolutionary highly conserved amino acids and may thus not be functionally competent. A gene translocation from the mitochondrion to the nucleus will have to compensate

somehow for these RNA editing sites. An investigation of one such comparatively young sequence transfer of the gene for cytochrome oxidase subunit II (*coxII*) from the mitochondrial genome to the nucleus in legumes (7) showed the nuclear gene to correspond essentially to the edited mRNA sequence of the mitochondrial genes in other plants. This observation was interpreted to support suggestions of RNA-mediated sequence transfer between the organelles of plant cells (8).

To gather more information about the mode of transfer we chose to investigate the gene for ribosomal protein S12 (*rps12*) in the higher plant *Oenothera*. The *rps12* genes in the mitochondrial genomes of all other plants investigated to date, including ginseng (9), maize (10), *Petunia* (11) and wheat (10), appear to be intact genes. The sole exception is found in *Oenothera* where only a small part of the reading frame is retained in the mitochondrial DNA (12). Genes for mitochondrial ribosomal proteins appear to be particularly affected by gene inactivation and loss from the organelle since already four examples have been described for genes incomplete or missing in some plant species. These include besides the *Oenothera rps12* gene (12) the genes for *rps19* in the same species (13), the *rps14* gene in maize (14) and the *rps13* gene in bean and pea (15). The functional genes for these ribosomal proteins are likely to have been transferred to the nucleus in these plants during evolution.

Our investigation of the intracellular location of the *rps12* gene in *Oenothera* now shows that while part of the coding region is still present in the mitochondrial DNA the functional gene has indeed been translocated to the nuclear genome. The nuclear gene is colinear with the mitochondrial edited mRNA of other species suggesting the transferred molecule to have originated from the mature mitochondrial RNA. We discuss requirements and probabilities of such gene transfers between organelles.

MATERIALS AND METHODS

Purification of nucleic acids

Genomic DNA from *Oenothera* was isolated according to (16) and further purified by CsCl centrifugation. *Oenothera* mitochondrial nucleic acids were obtained as described previously (17). Total cellular RNA from *Oenothera* was isolated using the guanidinium isothiocyanate procedure (18). Poly(A)⁺ mRNA

* To whom correspondence should be addressed

was purified by use of Hybond-mAP paper (Amersham) according to the protocol of the manufacturer, unbound RNA was taken as the poly(A)⁻ fraction. The *Oenothera* nuclear cDNA library, constructed in lambda gt11 was a kind gift of Prof Dr R.G.Herrmann and Dr N.Wedel.

cDNA cloning, hybridizations and sequencing

About 5×10^5 plaques of the cDNA library were screened with a ³²P labelled wheat *rps12* probe (284 bp *Bgl*III/*Xho*I fragment) in successive rounds of decreasing plaque density until clear correlations between hybridization signals and individual plaques were possible. cDNA inserts from positive phages were subcloned into Bluescript SKII+ plasmids (Stratagene) and sequenced using the T7 sequencing kit (Pharmacia) according to the manufacturer's protocol.

For Northern analysis RNAs were electrophoresed through a 1.4% formaldehyde agarose gel and blotted onto Nylon membranes (Pall Biodyne A). The filter membrane was hybridized under stringent conditions with the ³²P radiolabelled *Bam*HI/*Pst*I fragment of cDNA clone 26-1. Southern blot analysis of genomic DNA was performed by standard methods under stringent conditions as described (19).

Radiolabelling of probes, hybridization and washing of membranes were conducted according to standard methods (19).

PCR amplification

Amplification of the genomic *rps12* coding sequence was done with 2.5 U Taq-polymerase (Boehringer) in the supplier's incubation buffer under standard conditions (19). About 1 μg genomic DNA and two primers (1 μM each) deduced from the cDNA sequence (underlined in Fig. 3) were amplified in 30

cycles. The 100 μl reaction mixture was initially incubated 3 min at 94°C, followed by 30 rounds of incubation for 1 min at 94°C, 2 min at 60°C and 4 min at 72°C in a Bio-med thermocycler. For nucleotide sequencing the PCR product was cut with *Sac*I and the resulting restriction fragments were subcloned into Bluescript SKII⁺.

Sequence analysis

Nucleic acid and protein sequences were analysed with the Wisconsin GCG programs (version 7.1). Sequence similarity comparisons were done with the Pileup algorithm of this program package.

RESULTS

Mitochondrial RPS12 is encoded by a nuclear gene

To identify the complete open reading frame of the mitochondrial RPS12 protein which is not encoded by the mitochondrial genome a lambda gt11 cDNA library from poly(A)⁺ RNA from *Oenothera* was probed with the mitochondrial *rps12* coding sequence of wheat (10). Three of ten positive clones were further investigated and clone 26-1 was found to contain the complete *rps12* open reading frame (Fig. 1).

Northern experiments identify the *rps12* transcripts in the poly(A)⁺ fraction and confirm the absence of complete *rps12* transcripts in the mitochondrial RNA preparation of *Oenothera*. These hybridizations thus clearly show the mRNA to be polyadenylated confirming the nuclear location of the intact *rps12* gene (Fig. 2). A polyadenylation signal sequence typical for nuclear encoded mRNAs is located near the 3'-end of the cloned cDNA.

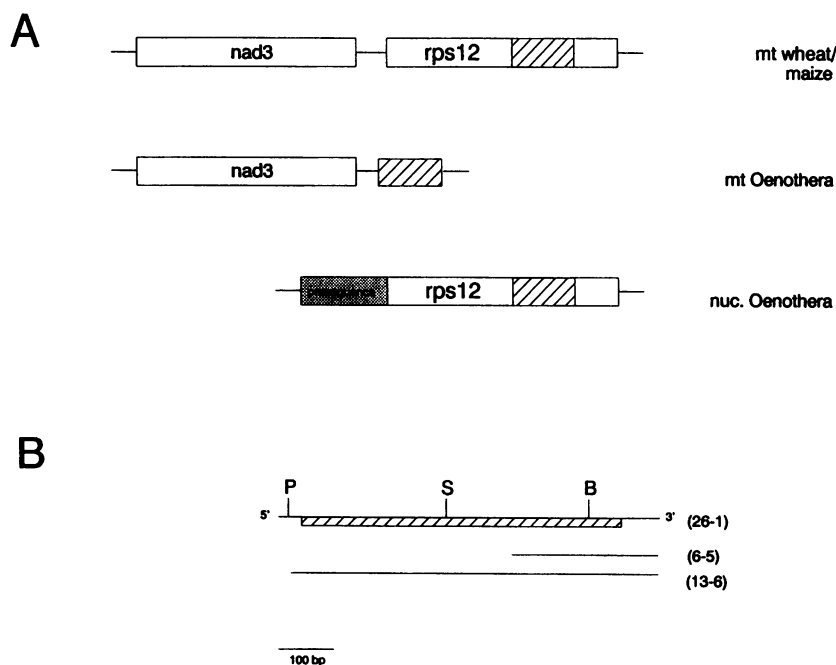


Figure 1. Organization of the *rps12* locus. (A) Comparison of the *rps12* genes in wheat and maize mitochondria (10; top part) with the gene fragment in *Oenothera* mitochondria (12; center part) and the nuclear gene of *Oenothera*. The partial *rps12* sequence present in the mitochondrial genome of *Oenothera* is highlighted by hatching. (B) Relative locations of three cDNA clones of the *Oenothera rps12* nuclear gene. The largest clone (26-1) is shown with the *rps12* open reading frame indicated by the box. Two smaller cDNAs (6-5 and 13-6) are indicated below. Restriction sites indicated are *Bam*HI (B), *Pst*I (P) and *Sac*I (S).

To analyse the gene structure particularly with respect to the occurrence of introns the corresponding genomic sequence was recovered from total *Oenothera* DNA by PCR amplification between primers derived from the terminal sequences of the

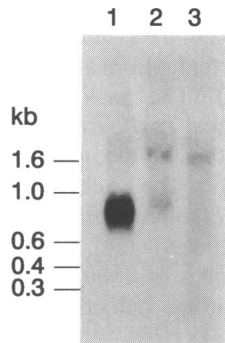


Figure 2. Northern blot analysis of *rps12* transcripts in *Oenothera*. Poly(A)⁺ RNA (lane 1), total cellular RNA depleted of the polyadenylated RNA (lane 2) and mitochondrial RNA (lane 3) were coelectrophoresed with the RNA length standards indicated on the left margin. The major mRNA species of 0.8 kb is detected in the polyadenylated mRNA fraction with some residual molecules still present in the depleted RNA. Amounts loaded were 10 μg polyadenylated RNA, 20 μg of the depleted RNA and 5 μg mitochondrial RNA. The weak hybridization signals of about 1.8 kb are probably due to unspecific hybridization to the 18S ribosomal RNAs.

```

CGCGAGGTGAGGACTGCACTTGCCTCCGAGAAGAAGCAAGACG ATG AGT GGT GGG AGG 57
W I S N L R Q V F G R S R A S I 5
TGG ATT TCC AAC CTG CGC CAA GTT TTT GGA AGA TCA AGA GCC TCA ATT 105
W I S N L R Q V F G R S R A S I 21
TCG TCG TCT CTT CTA CCT TTC CCT CCG ACT TCG ATT TCT AGT GGT TTC 153
S S S L L P F P P T S I S S G F 37
CAT GTC CCT CGC CTC CTT GAT GCC TGG AAG AAC CCT GTT GCA GCC GCC 201
H V P R L D A W K N P V A A A 53
GCC GCT TTC CGG CTT CCT CAA TCG TCC GGT TTC GCT ACA ATG AAT CAA 249
A A F R L P Q S S G F A T M N Q 69
CTG ATT CGT CAC GGT CGA GAA GAA AAG CAT CGC AGT GAT CGT AAA CGA 297
L I R H G R L D A W K N P V A A A 85
GCT CTA GGT AAA TGT CCG CAG AAG CAA GGA GTG TGC CTG CGT GTC TCA 345
A L G K C P Q K Q G V C L R V S 101
ACG AGA ACG CCG AAG AAA CCT AAT TCG GCT CTC GGG AAG ATT GCT AAA 393
T R T P K K K P K A L G K I A K 117
GTC CGA TTG AGC AAC CGA AAC GAT GTG TTT GCG TAC ATT CCA GGC GAA 441
V R L S N R N D V F A Y I P G E 133
GGT CAC AAT TTG CAG GAA CAT TCC ATG GTG CTG GTG CGA GGA GGT AGA 489
G H N L Q E H S M V L V R G G R 149
GTT AAA GAT TTG CCT GGT GTA AAG TTC CAT TGC ATT CGA GGA GTC AAG 537
V K D L P G V K F H C I R G G V K 165
GAT TTG CTG GGG ATC CCG GAT CCG AGA AGA GGC AGA TCC AAG TAC GGT 585
D L L G I P D R R R G R S K Y G 181
CGG GAG AAA CCG AAA TCG ACA TGA ATAGACTAGTAAGCTACGTTTCTCAGTTCC 640
A E K P K S T * 188
TTGAATTTGACAGCATCTATGGGGTCTGATTTGTTGTTGAATTCAGTTGCTTTTGGTTCTGT 702
TGCCGTTCAGCTGTTGTTTCGAAATTTGCGGTCTAGTACCTCTTGTTCACATTTTGGTT 765
ATAAAGAAGCTGTGGTTAGTAG 788
*****
    
```

Figure 3. Nucleotide sequence of the *Oenothera* nuclear encoded mitochondrial *rps12*. The entire sequence of the largest cDNA clone (26-1) is given. Amino acids are shown from the methionine codon closest to the beginning of the conserved *rps12* coding region (marked with a vertical arrow). Primers used in PCR analysis of the genomic DNA sequence are indicated by horizontal arrows above the sequence. The *Bam*HI, *Eco*RI, *Pst*I and *Sac*I restriction sites are underlined and the polyadenylation signal is marked by asterisks. The genomic DNA between the two primers is identical with the cDNA. The sequence is available in the EMBL and Genbank databases under accession no. X68040.

largest cDNA insert (Fig. 3). The sequences of the cDNA clones and the nuclear genomic DNA were identical and confirm their relationship. Southern hybridisation experiments suggest a single copy gene to code for the mitochondrial RPS12 polypeptide (Fig. 4). Single copy nuclear genes have also been found for all mitochondrial ribosomal proteins in animal and fungal species,

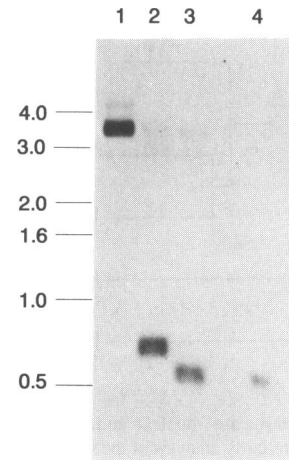


Figure 4. Southern blot analysis of the *rps12* gene in *Oenothera*. Genomic DNA was digested with *Bam*HI (lane 1), *Pst*I (lane 2) or *Bam*HI/*Pst*I (lane 3) and probed by hybridization with the ³²P-labelled *Eco*RI fragment of clone 26-1, containing the sequence between the 5'-linker and the internal *Eco*RI site in the 3'-non-coding region (Fig. 3). For comparison of the *rps12* genomic *Bam*HI/*Pst*I fragment a *Bam*HI/*Pst*I digest of clone 26-1 was coelectrophoresed (lane 4). Hybridization confirms the identity of the cloned fragment and shows the mitochondrial *RPS12* protein to be encoded by a single copy nuclear gene. The mitochondrial *rps12* fragment will not hybridize under these stringent conditions due to numerous nucleotide sequence differences mostly in silent positions.

```

Maize/Wheat mt. .... L 100
Oenothera mt. ....
Petunia mt. ....
Oenothera nuc. MSGGRWISNL RQVFGRSRAS ISSSLLPFPF TSISSGFHVP RLLDAWKNPV
E.coli .....

Maize/Wheat mt. .... MPTKNQL IRHGREEKWR TDRTRASDQC PQKQGVCLRV 100
Oenothera mt. ....
Petunia mt. .... MPSLNQL IRHGREEKWR TDRTRALDQC PQKQGVCPRV
Oenothera nuc. AAAAAFRLPQ SSGFATMNLQ IRHGREEKWR SDRKRALGKC PQKQGVCLRV
E.coli ..... ATVNQL VRKPRARKVA KSNVPALEAC PQKRGVCTRV

Maize/Wheat mt. .... Y L 150
Oenothera mt. ....
Petunia mt. ....
Oenothera nuc. STRTPKKPNS ALRKIAKVML SNRHDIFAH I PEGEHNLSQEH SIVLVRGGRV
E.coli ..... YTTTPKKPNS ALRKIVCRVRL TNGFEVTSYI GGEHNLQEH SMVLIRGGRV

Maize/Wheat mt. .... L K C 188
Oenothera mt. ....
Petunia mt. ....
Oenothera nuc. KDLPGVKVHC IRGVKDLLGI PDRRGRSKY GAERPCKS
E.coli .... KDLPGVRYHT VRGALDCSGV KDRKQARSKY GVKRPKA
    
```

Figure 5. Amino acid sequence alignment of the nuclear RPS12 with mitochondrial and bacterial proteins. The *Oenothera* nuclear (nuc.) encoded RPS12 sequence is shown from the methionine closest to the beginning of the mitochondrial and bacterial sequences. The maize, wheat and *Petunia* mitochondrial (mt.) protein sequences are given as deduced from genomic sequences with amino acids shown to be altered by RNA editing in maize and wheat indicated above the sequence (11, 26). Positions of editing in the two monocots are shaded in all species. Amino acids encoded by the *rps12* fragment in the mitochondrial genome of *Oenothera* are included in this alignment (12). The *E. coli* sequence is shown in the bottom line (35).

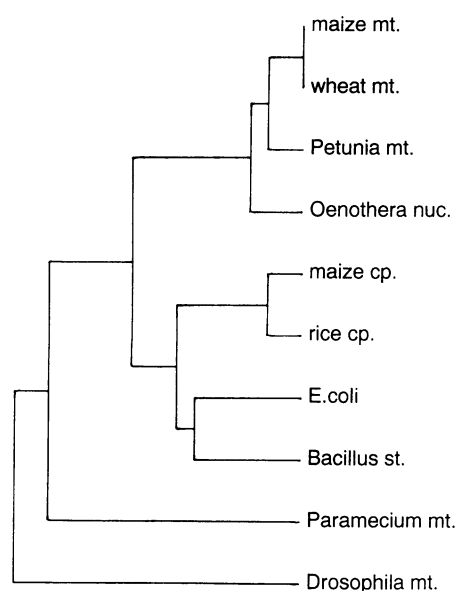


Figure 6. Sequence similarities of nuclear and mitochondrial encoded RPS12 polypeptides. Sequence similarity analysis of the the *rps12* proteins from different species shows the nuclear encoded protein of *Oenothera* to be most closely related to the mitochondrial encoded plant RPS12 polypeptides. The few differences may be a consequence of sequence alterations manifested during the transposition to the nucleus or due to the faster rate of nucleotide substitutions in the nuclear genome. Sequences for mitochondrial (mt.), chloroplast (cp.) and bacterial RPS12 polypeptides were taken from the databases.

like the *rps12* gene in *Drosophila* (20) and all thus far characterized yeast genes (21, 22).

NH₂-terminal mitochondrial transit sequence

The presequence from the first methionine to the phenylalanine codon corresponding to the start codon of the mitochondrial *rps12* genes in ginseng, wheat, maize and *Petunia* covers 63 amino acids (Figs. 3 and 5). Other transit peptides for plant mitochondria are of comparable size (23, 24). Several general features of transit peptides are conserved in this presequence, notably the abundant hydroxylated amino acids serine and threonine and frequent hydrophobic amino acids.

The putative *rps12* transit peptide, however, cannot be folded straightforward into an amphiphilic helix which has been proposed as a characteristic feature of mitochondrial target sequences (25). The scattered proline residues in the presequence are expected to introduce bends in the polypeptide chain and do not allow a helical structure. The small number of plant mitochondrial presequences known to date, however, precludes any clear consensus analysis of features common to such signal peptides in plants. No primary sequence similarity is observed between this sequence preceding the conserved *rps12* open reading frame in the *Oenothera* nuclear genome and any of the known mitochondrial presequences in yeast, plants or animals nor with any other published sequence.

The nuclear *rps12* gene corresponds to edited mitochondrial sequences

The protein sequence deduced from the *Oenothera* nuclear *rps12* gene (and the corresponding cDNAs) contains the amino acids specified by edited mitochondrial mRNAs in all instances of RNA

editing in mitochondrial transcripts of other plants (Fig. 5). In the *Oenothera* nuclear gene all six sites altered by RNA editing in wheat and maize mitochondria as well as the three sites edited in *Petunia* mitochondria are identical with the edited mitochondrial mRNAs.

RNA editing has been lost in the *Oenothera* mitochondrial gene fragment in at least one position. The phenylalanine codon at amino acid position 158 of the *Oenothera* nuclear encoded protein is generated by RNA editing from a serine triplet in maize, wheat and *Petunia* (Fig. 5). The analogous serine codon in the *Oenothera* mitochondrial *rps12* fragment was not found to be altered by RNA editing in the mitochondrial cDNA clones analysed previously (12). The leucine at amino acid position 153 is conserved even in *E.coli* and established by RNA editing in maize and wheat mitochondria (26). This codon is correctly found in the nuclear sequences of *Oenothera*, but has drifted to a phenylalanine triplet in the mitochondrial fragment.

DISCUSSION

Origin of the nuclear *rps12* gene in *Oenothera*

The mitochondrial ribosomal proteins are in animals exclusively encoded in the nucleus, whereas plants encode a number of ribosomal protein genes in their mitochondrial genomes. In the completely sequenced mitochondrial genome of the liverwort *Marchantia* 16 ribosomal protein genes have been identified (27). Several of these, including the *rps7-rps12* genes, are arranged in the same order as the respective bacterial gene clusters supporting their phylogenetic relationship. With an estimated total number of 70–80 proteins in mitochondrial ribosomes (28) most of their genes, however, must be nuclear encoded in the liverwort.

Protein S12 is one of the best conserved ribosomal proteins identified to date. The location of this polypeptide in the ribosome suggests its participation in binding translation initiation factors (29). It has also been implied in drug-induced misreading and certain alterations in the RPS12 sequence result in streptomycin resistance (30).

The mitochondrial RPS12 is encoded in the mitochondrion of *Marchantia* and all higher plants investigated to date with the sole exception of the flowering plant *Oenothera*. In this plant no complete *rps12* gene is located in the mitochondrial genome, only a small part of the reading frame is retained. In this investigation a complete copy of the coding sequence was identified as a nuclear encoded gene. Sequence comparison of this nuclear encoded protein from *Oenothera* with the respective polypeptides of other species shows the nuclear gene to be clearly related to the plant mitochondrial sequences (Fig. 6) and therefore to be derived from a former plant mitochondrial gene. In the deduced protein sequence 81.6% of the amino acids are identical with the maize and wheat mitochondrially encoded proteins. Similarity with the chloroplast polypeptides (56.4%) is slightly less than the number of amino acids identical with bacterial proteins, which amount to 59.3% and 64.5% with *E.coli* and *B.stearothermophilus* respectively (Fig. 6).

Transfer of the *rps12* gene must thus have occurred during speciation of higher plants in the branch leading to *Oenothera* species. The presence of a remnant of the original mitochondrial gene in *Oenothera* suggests the switch from active mitochondrial gene to the active nuclear gene to have occurred comparatively recently in the evolution of this lineage. Investigation of the

nuclear genomes of other plants for the presence or absence of mitochondrial *rps12* coding sequences will yield further information about when and how often this gene has been transferred.

Integration of transferred genes into the nucleus

The nuclear encoded open reading frame of *rps12* in *Oenothera* has like the nuclear *coxII* gene in cowpea (7) acquired an additional NH₂-terminal sequence that presumably functions as protein target sequence to the organelle. The cowpea presequence is separated in the genome from the actual coding region by an intron, whereas the *rps12* presequence is contiguous with the conserved *rps12* coding region. Exon shuffling as proposed for the *coxII* gene is thus unlikely to have been involved in adding the presequence to the *rps12* coding region in *Oenothera*. The nuclear *rpl22* gene encoding a chloroplast ribosomal protein in legumes (31), but none of the yeast nuclear encoded mitochondrial ribosomal proteins contain an intron at the presequence boundary (22, 28) suggesting both pathways of presequence addition to occur.

It is unclear how integration of the *rps12* mitochondrial sequences into the nuclear genome has occurred. No features indicative of the mode of integration are observed at the putative borders of the transferred *rps12* sequences. Only the open reading frame of *rps12* itself shows clear similarity with the respective mitochondrial sequences. Undirected insertion of the mitochondrial genes into the nucleus has to ensure correct addition of transcription and translation signals and of a mitochondrial target sequence. Such a situation could be provided if the mitochondrial gene inserts into an active protein coding gene that contains these signals and adds the presequence from its own reading frame. Although e.g. more than 2.7% of a bacterial chromosome have been found to potentially act as mitochondrial passage presequences (32) the probability of an active gene transfer into the nuclear genome arriving by chance in the correct reading frame in the right context is still low.

These requirements suggest that only a large number of undirected transfer and integration events will result in a single successful active gene transfer. The remnants of such transfers should be detectable in the nuclear genomes of all plants and eukaryotes if not rapidly eliminated. Several reports of mitochondrial sequences in the nuclear genomes of animals and plants support this consideration, but are far too limited to provide the necessary sequence pool (33 and refs in 8). A rapid loss of unused sequences in the nuclear genome is not observed for small repeated sequences and for the apparently non-transcribed *coxII* gene copy in the nuclear genome of pea for example (7). Such an undirected transfer and integration model could be operable only with a selection pressure for retaining potentially functional genes to explain the apparent absence of numerous other *rps12* or *coxII* sequence fragments in the nuclear genome.

An alternative model requires a (hypothetical) selection of the actual integration events for potential function. Only such integrations will occur that establish correct contexts for the mitochondrial reading frames in the nucleus. This theoretical model would thus exclude all random integrations and preclude a background of mitochondrial sequences in the nuclear DNA. A specific transfer and integration mechanism could operate via transposon-like shuttles that move between the different genomes. Retrotransposon derived sequences have been found in the mitochondrial genome of *Oenothera* (17) but their function remains as yet unclear.

Mode of the sequence transfer

Inferences about the sequence transfer mechanism can be made from analysis of the amino acids encoded by the nuclear *rps12* gene at the positions of mitochondrial RNA editing sites. The observation that all mitochondrial editing sites correspond in the nuclear *rps12* gene with the edited mitochondrial sequences suggests that the mitochondrial molecule from which the sequence transfer started was an edited mRNA rather than the genomic, unedited DNA. This conclusion is supported by the investigation of the other comparatively young sequence transfer of the *coxII* gene in legumes (7). The nuclear *coxII* gene in cowpea is colinear with the edited mRNA sequence of other species which was also interpreted as evidence supporting its derivation from mitochondrial mRNA. The lack of any mitochondrial sequences surrounding the integrated *rps12* nuclear gene can be accounted for by loss of these nucleotides through rapid drift in the nucleus or alternatively by preselection of the sequence either at the instance of integration or of reverse transcription.

The observation of these nuclear sequences corresponding to the edited RNA sequences in the mitochondrion of other plants furthermore suggests that analogous RNA editing does not commonly occur in the nucleus even in such transferred mitochondrial genes. This conclusion, however, does not preclude RNA editing of nuclear transcripts per se since specific factors may be required that are unlikely to cotransfer with the mitochondrial sequence if acting in trans.

How frequent are 'recent' gene transfers in plants ?

The pseudo-gene nature of the *rps12* fragment in the mitochondrial genome in *Oenothera* has been confirmed by the identification of the respective nuclear gene reported here. The former organellar gene has in the only other described instance of such a recent gene translocation, the *coxII* gene, been deleted from the mitochondrial genome of cowpea, but hybridization data suggest the gene transfer to have preceded the loss of the organellar gene in other legumes (7). Absence of complete or intact genes in mitochondria of one plant species but not others thus suggests further instances of such interorganellar gene transpositions.

The gene for ribosomal protein S13 (*rps13*) seems silent in wheat mitochondria although completely encoded in the organelle (34). The *rps13* gene appears to be absent from the mitochondrial genomes of pea and bean and may thus also be encoded by a nuclear gene in these three species (15). The only (in length) complete reading frame for ribosomal protein S19 is interrupted in genomic and cDNAs by a translational stop codon in *Oenothera* mitochondria and therefore is another candidate for a recent transfer to the nuclear compartment (13). Such sequence translocations from the mitochondrial genome to the nucleus may thus be comparatively recent in higher plants. In mammalia and yeast nearly all of the mitochondrial ribosomal protein genes reside in the nucleus and presumably have been transferred at some point early in evolution. The spurious similarity of the yeast nuclear genes with the corresponding bacterial sequences suggests these transfers to have occurred very early in evolution (21, 22, 28).

Sequence transfers between mitochondria, chloroplasts and the nucleus have also been found in most of the other potential directions, including integration of chloroplast sequences into the nuclear genome and chloroplast and nuclear sequences into the mitochondrial DNA (refs in 8). Nucleic acid movement between

the different organelles thus appears to be a common feature of the plant cell and suggests the compartmentation between the three genomes of plants to be more fluid than previously thought.

ACKNOWLEDGEMENTS

We thank Petra Härder for excellent technical assistance and Drs R.G.Herrmann and N.Wedel for their kind gift of the *Oenothera* nuclear cDNA library. We are grateful to Dr C.J.Leaver for his encouragement and advice. This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Bundesministerium für Forschung und Technologie.

REFERENCES

1. Leaver,C.J. and Gray,M.W. (1982) *Ann. Rev. Plant Physiol.*, **33**, 373–402.
2. Gray,M.W. 1989. *Trends Genet.*, **5**, 294–299.
3. Levings,C.S.III. and Brown,G.G. (1989) *Cell*, **56**, 171–179.
4. Covello,P.S. and Gray,M.W. (1989) *Nature*, **341**, 662–666.
5. Gualberto,J.M., Lamattina,L., Bonnard,G., Weil,J.-H. and Grienberger,J.M. (1989) *Nature*, **341**, 660–662.
6. Hiesel,R., Wissinger,B., Schuster,W. and Brennicke,A. (1989) *Science*, **246**, 1632–1634.
7. Nugent,J.M. and Palmer,J.D. (1991) *Cell*, **66**, 473–481.
8. Schuster,W. and Brennicke,A. (1988) *Plant Science*, **54**, 1–10.
9. Kim,K.-S., Schuster,W., Brennicke,A. and Choi,K.-T. (1991) *Plant Physiol.*, **9**, 1602–1603.
10. Gualberto,J.M., Wintz,H., Weil,J.-H. and Grienberger,J.M. (1988) *Mol. Gen. Genet.*, **215**, 118–127.
11. Rasmussen,J. and Hanson,M.R. (1989) *Mol. Gen. Genet.*, **215**, 332–336.
12. Schuster,W., Wissinger,B., Unseld,M. and Brennicke,A. (1990) *EMBO J.*, **9**, 263–269.
13. Schuster,W. and Brennicke,A. (1991) *Nucl. Acids Res.*, **19**, 6923–6928.
14. Wahleithner,J.A. and Wolstenholme,D.R. (1988) *Nucl. Acids Res.*, **14**, 6897–6913.
15. Bland,M.M., Matzinger,D.F., and Levings,C.S.III (1986) *Mol. Gen. Genet.*, **204**, 8–16.
16. Doyle,J.J. and Doyle,J.L. (1987) *Pytochem. Bull.*, **215**, 11–15.
17. Schuster,W. and Brennicke,A. (1987) *EMBO J.*, **6**, 2857–2863.
18. Chomczynski,P. and Sacchi,N. (1987) *Anal. Biochem.* **162**, 156–159.
19. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning* 2nd Edition. Cold Spring Harbor Press, New York.
20. Royden,C.S., Pirota,V. and Jan,L.Y. (1987) *Cell*, **51**, 165–173.
21. Grohmann,L., Graack,H.-R. and Kitakawa,M. (1989) *Eur. J. Biochem.*, **183**, 155–160.
22. Kitakawa,M., Grohmann,L., Graack,H.-R. and Isono,K. (1990) *Nucl. Acids Res.*, **18**, 1521–1529.
23. Boutry,M. and Chua,N.-H. (1985) *EMBO J.*, **4**, 2159–2165.
24. Braun,H.-P., Emmermann,M., Kruff,V. and Schmitz,U.K. (1991) *Mol. Gen. Genet.*, **231**, 217–225.
25. von Heijne,G., Steppuhn,J. and Herrmann,R.G. (1989) *Eur. J. Biochem.*, **180**, 535–545.
26. Gualberto,J.M., Bonnard,G., Lamattina,L. and Grienberger,J.M. (1991) *Plant Cell*, **3**, 1109–1120.
27. Takemura,M., Oda,K., Yamato,K., Ohta,E., Nakamura,Y., Nozato,N., Akashi,T. and Ohya,K. (1992) *Nucl. Acids Res.*, **20**, 3199–3205.
28. Grohmann,L. (1989) Dissertation, Freie Universität Berlin, Berlin.
29. Oakes,M., Henderson,E., Scheinman,A., Clark,M. and Lake,J.A. (1986) *In Hardesty,B. and Kramer,G. (eds.), Structure, Function and Genetics of Ribosomes*. Springer Verlag, Berlin, pp. 47–67.
30. Cundliffe,E. (1986) *In Hardesty,B. and Kramer,G. (eds.), Structure, Function and Genetics of Ribosomes*. Springer Verlag, Berlin, pp. 586–604.
31. Gantt,J.S., Baldauf,S.L., Calie,P.J., Weeden,N.F. and Palmer,J.D. (1991) *EMBO J.*, **10**, 3073–3078.
32. Baker,A. and Schatz,G. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 3117–3121.
33. Knoop,V. and Brennicke,A. (1991) *Curr. Genet.*, **20**, 423–425.
34. Bonen,L. (1987) *Nucl. Acids Res.*, **15**, 10393–10404.
35. Post,L.E. and Nomura,M. (1980) *J. Biol. Chem.*, **255**, 4660–4666.