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RNA editing of tRNA^{Phe} and tRNA^{Cys} in mitochondria of *Oenothera berteriana* is initiated in precursor molecules

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Abstract We have analyzed the role of RNA editing in the correction of mismatched base pairs in tRNA secondary structures in mitochondria of the flowering plant *Oenothera berteriana*. Comparison of genomic and cDNA sequences from unprocessed primary transcripts of the newly characterized genes for tRNA^{Cys}, tRNA^{Asn} and tRNA^{Ile} and the previously described gene for tRNA^{Phe} revealed single nucleotide discrepancies in the tRNA^{Cys} and tRNA^{Phe} sequences. While the change in the anticodon stem of tRNA^{Cys} alters a C-T to a T-T mismatch, the nucleotide transition in the tRNA^{Phe} restores a conventional T-A Watson-Crick base pair, replacing a C-A mismatch in the acceptor stem. Since both nucleotide alterations are conversions from genomic cytidines to thymidines in the cDNA (uridines in the tRNAs), they are attributed to RNA editing, which is observed in nearly all mRNAs from plant mitochondria.

Key words Plant mitochondria · Transfer RNAs RNA editing

Introduction

RNA editing constitutes a particular type of RNA processing, since it results in a posttranscriptional change in the primary sequence of RNA molecules. This phenomenon was first identified in kinetoplastids from *Trypanosoma brucei*, where uridines that are not genomically encoded are posttranscriptionally inserted into *cox*II mRNA molecules by RNA editing (Benne et al. 1986).

In recent years various types of RNA editing have been described in different organisms (Bonnard et al. 1992; Cattaneo 1991; Benne 1992). RNA editing is most frequently observed in the mitochondrial compartment, where it is predominantly involved in the maturation of mRNAs, although it is not restricted to this type of RNA molecule. In *Physarum polycephalum* primary sequences of mRNAs, rRNAs and tRNAs are altered by RNA editing (Miller et al. 1993). Pyrimidine transitions have been suggested to participate in the generation of the mature form of the mammalian cytosolic tRNA^{Asp} (Beier et al. 1992). A cytidine to uridine conversion changes the codon recognition loop of a marsupial mitochondrial tRNA (Janke and Pääbo 1993). RNA editing has also been shown to be required for maturation of tRNAs in mitochondria of the amoeboid protozoan *Acanthamoeba castellanii* (Lonergan and Gray 1993). In this organism, U to A, U to G and A to G conversions correct mismatched base pairs in the acceptor stems of four different tRNAs. These tRNA editing events occur within the first 3 bp of the acceptor stem, a region that normally provides major identity elements and specific contact points for the cognate aminoacyl-tRNA synthetase (Saks et al. 1994).

Almost all RNA editing events identified in higher plant mitochondria have been observed in mRNA molecules (Schuster et al. 1991a). Most of the cytidine to uridine (in rare cases uridine to cytidine) conversions are located in coding regions and alter the triplet identity, resulting in a change in the encoded amico acid (Schuster et al. 1991b). Some RNA editing events are observed in intron sequences, where the nucleotide conversions allow the formation of base pairs which are thought to be required for the adoption of a specific secondary structure by the intron (Wissinger et al. 1991; Binder et al. 1992). Several RNA editing sites have also been identified in 5' and 3' untranslated regions of various mRNAs; their function, however, is unclear at present (Schuster et al. 1991b). Only one cDNA clone derived from the 26S rRNA in *Oenothera berteriana* has been found with single C to U and U to C conversions. The significance of this putative rRNA editing remains unclear, since no RNA editing was found in any of

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the other rRNAs investigated in different plant species (Schuster et al. 1991a; Bonnard et al. 1992).

Recently Maréchal-Drouard et al. (1993) have identified an RNA editing event in the mitochondrial $tRNA^{Phe}$ of potato and bean. In both plant species a C_4-A_{69} mismatched base pair located in the tRNA acceptor stem is corrected to a regular U_4-A_{69} base pair.

The observation of several mismatched base pairings in secondary structure models deduced from tRNA genes encoded in mitochondria of several plant species led us to investigate a possible role of RNA editing in restoring correct base pairing in newly and previously described tRNAs from *Oenothera* mitochondria. Comparison of mitochondrial tRNA sequences from higher plants with the corresponding sequences from liverwort, where no RNA editing has been observed (Oda et al. 1992), reveals potential RNA editing sites in the tRNA sequences of higher plant mitochondria.

Materials and methods

Nucleic acids

Isolation and purification of mitochondrial nucleic acids have previously been described in detail (Schuster et al. 1988, Binder and Brennicke 1993a,b).

Southern blot analysis

Total mitochondrial DNA was digested with restriction enzymes, size fractionated on agarose gels and transferred to GeneScreen Plus nylon membranes (Dupont) by standard capillary techniques. Hybridization conditions followed the instructions given by the manufacturer. The oligonucleotide used for the identification of $tRNA^{Ile}$ was: 5'-CTCTAACCAACTGAGCTATTTCCCC-3'.

Northern blot analysis

Mitochondrial RNA was fractionated in 4% polyacrylamide gels. After electrophoresis the gels were soaked in transfer buffer (0.045 M TRIS-borate, 0.001 M EDTA, pH 8) for 10 min and subsequently electro-transferred for 16 h with 20 V to Duralon UV nylon filter (Stratagene) using a Biorad Trans-blot cell, which allows quantitative transfer of small and large RNA molecules.

cDNA synthesis and polymerase chain reaction (PCR) amplification

To remove possible DNA contamination from mitochondrial RNA preparations, nucleic acids were digested with RNase-free DNase I in a buffer containing 20 mM TRIS-HCl, pH 7.6 and 5 mM $MgCl_2$ for 15 min at room temperature. After phenol/chloroform extraction, 5 µg of mitochondrial RNA and 50 ng of the distal primer were incubated at 70°C for 10 min followed by another 10 min at room temperature. cDNA synthesis was carried out in a total volume of 20 µl using 200 U Superscript reverse transcriptase (BRL) in the presence of 20 mM TRIS-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 1 mg/ml bovine serum albinum (BSA), 10 mM dithiothreitol (DTT) and 500 nM dNTPs for 1 h. First strand cDNA was amplified on a Biomed thermocycler under the following conditions: 1 min at 94°C, 2 min at 42°C and 3 min at 72°C. This cycle was repeated 40 times with a final period of 10 min at 72°C. The amplification was performed in the presence of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM TRIS-HCl, pH 8.3, 50 nM dNTPs and 500 ng of each primer. Primers used in PCR amplifications were: $tRNA^{Asn}$, Asn2:5'-CAACTCGATCGGTGTATGTG-3' (nucleotides 2499–2518 in databank entry X76497), Asn3:5'-GATGTCCCTCGATTCTGC-3' (nucleotides 2748–2731 in databank entry X76497); $tRNA^{Cys}$, HC3:5'-CTTCTCTGGCAACCCGCC-3' (nucleotides 266–283 in databank entry X76497), HC1:5'-CCAGAATGTTGTCTGTGG-3' (nucleotides 539–522 in databank entry X76497); $tRNA^{Ile}$, Ile1:5'-TTCCGTCCGCTCTTCCCG-3' (nucleotides 163–180 in databank entry X76496), Ile2:5'-TTGAGATCTGCGGGGTGG-3' (nucleotides 346–329 in databank entry X76496); $tRNA^{Phe}$, P6:5'-CAAAAGACGGAAGACAAGAG-3' (nucleotides 313–332 in databank entry X74449), P1:5'-CTTGAATTTCCAAATCCGGC-3' (nucleotides 492–473 in databank entry X74449)

Miscellaneous methods

Mitochondrial DNA and PCR products were cloned into pKUN and pBluescript vectors following standard protocols (Sambrook et al. 1989). DNA sequencing was performed with a T7 sequencing kit (Pharmacia).

Results

Identification and genomic location of the tRNA genes

The genes for $tRNA^{Cys}$ and $tRNA^{Asn}$ were identified in the *Oenothera* mitochondrial genome during investigation of the region upstream of the $tRNA^{Tyr}$ gene and the distal part of the split *nad2* gene. The gene for $tRNA^{Asn}$ was detected on a 1 kb *Hin*dIII fragment that was mapped upstream of a 7 kb *Hin*dIII fragment containing the $tRNA^{Tyr}$ gene and the second segment of the *nad2* gene. Detailed mapping and sequence analysis of the region further upstream revealed a 5 kb *Hin*dIII fragment that contains the gene for $tRNA^{Cys}$. These 5 and 1 kb fragments are separated by a small 147 nucleotide *Hin*dIII DNA fragment (Binder et al. 1990, 1992). Southern blot hybridization confirmed the mitochondrial origin of the identified *Hin*dIII DNA fragments and the location of the genes for $tRNA^{Cys}$ and $tRNA^{Asn}$ on a common 2.85 kb *Bam*HI fragment (Fig. 1A,B). While $tRNA^{Cys}$ is encoded by a single locus, the gene for $tRNA^{Asn}$ is possibly present in two different environments in the *Oenothera* mitochondrial genome. Weak signals corresponding to a 6.5 kb *Bam*HI and a 3.1 kb *Hin*dIII fragment could either derive from such a second copy of this gene, which is present in substoichiometric amounts, or could result from a partial sequence duplication (Fig. 1A). The two tRNA genes for $tRNA^{Cys}$ and $tRNA^{Asn}$ occur together with the previously characterized gene for $tRNA^{Tyr}$ encoded upstream of *nad2* exon c. Sequence analysis showed the gene for $tRNA^{Cys}$ to be located 2145 bp upstream of the $tRNA^{Asn}$ coding region.

A 3 kb cloned fragment was isolated from an *Oenothera* mitochondrial DNA *Hin*dIII library by hybridization with an oligonucleotide that is complemen-

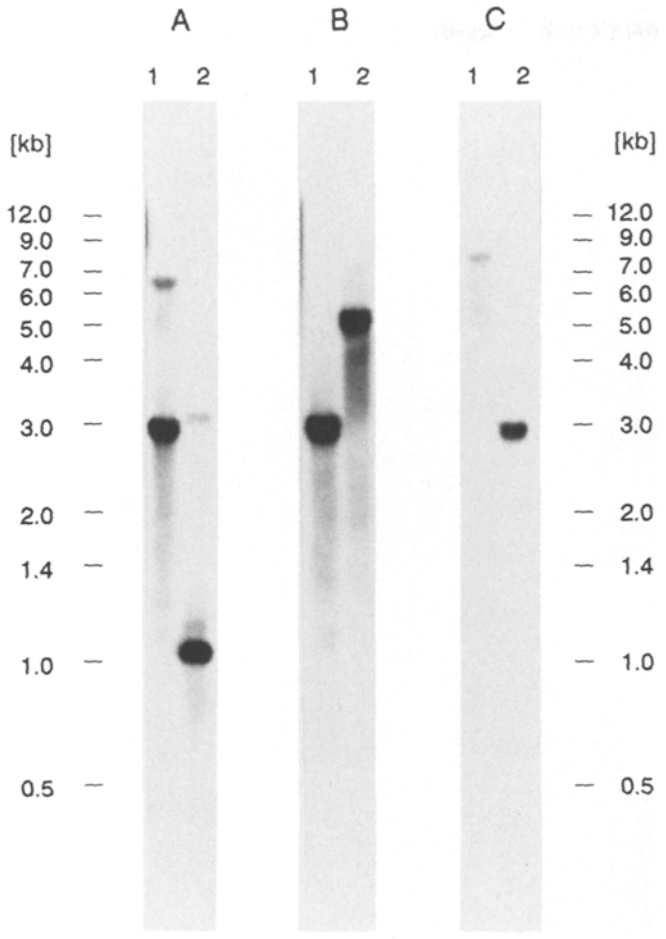


Fig. 1A-C Southern blot analysis of the genes for $tRNA^{Asn}$ (**A**), $tRNA^{Cys}$ (**B**) and $tRNA^{Ile}$ (**C**). Total mitochondrial DNA was digested with *Bam*HI (1) or *Hin*dIII (2) and probed with: **A** a DNA fragment containing the $tRNA^{Asn}$ gene with 558 bp of upstream and 167 bp of downstream region. This gene is present in two genomic environments in different stoichiometric amounts or only part of this region is duplicated in the mitochondrial DNA. **B** A 1.5 kb DNA fragment containing the $tRNA^{Cys}$ gene with 380 bp of upstream and 1030 bp of downstream sequence. Signals corresponding to a single DNA fragment in each digest indicate that $tRNA^{Cys}$ is encoded by a single-copy gene. **C** Hybridization of a 0.6 kb *Nru*I fragment containing the $tRNA^{Ile}$gene with 210 bp of upstream and 300 bp of downstream region detects a single DNA fragment in each lane, indicating that this tRNA is encoded by a single gene. Sizes of co-electrophoresed DNA length standards are given in kilobases

tary to the 5' part of maize $tRNA^{mMet}$ (Parks et al. 1984), which most probably is also modified in this plant species to decode an isoleucine codon (AUA), as has been shown for potato (Weber et al. 1990). Sequence analysis of a 0.58 kb *Nru*I DNA fragment revealed a tRNA gene that was identified as $tRNA^{Ile}$ by its homology to the respective gene from potato (Weber et al. 1990). Hybridization of the 0.58 kb *Nru*I fragment to total mtDNA indicates that this gene is present in a unique genomic environment in the *Oenothera* mitochondrial genome (Fig. 1C). So far no other coding region has been identified in the immediate vicinity of this gene.

The complete gene for $tRNA^{Phe}$ is present in four different genomic environments, while a fifth locus contains only a truncated $tRNA^{Phe}$ sequence in the mitochondrial genome of *Oenothera* (Binder and Brennicke 1993b).

Primary sequences and secondary structures

The tRNA sequences deduced from the genes for $tRNA^{Asn}$, $tRNA^{Cys}$ and $tRNA^{Ile}$ can be folded into the classical cloverleaf secondary structure (Fig. 2A-C). The sequences contain the typical nucleotides at invariant positions postulated by the generalized tRNA model (Singhal and Fallis 1979).

The gene for $tRNA^{Cys}$ (Fig. 2B), which is 71 nucleotides long shows 100% sequence identity with the corresponding gene from tomato (Izuchi and Sugita 1989). While the $tRNA^{Cys}$ gene is of "native" mitochondrial origin (Izuchi et al. 1990), the 72-nucleotide gene for $tRNA^{Asn}$ (Fig. 2A) shows 94.4%–100% identity to the chloroplast-like genes from tomato, wheat, and bean (Izuchi et al. 1990; Joyce and Gray 1989; Bird et al. 1989). Such chloroplast-like tRNA genes typically show very high sequence similarity to their chloroplast counterparts and are often located on plastid-derived DNA insertions in the mitochondrial DNA.

The primary sequence of the gene for $tRNA^{Ile}$ (Fig. 2C) is 98.6% homologous to the corresponding gene from potato. Both genes are 74 nucleotides long and contain a 5'-CAT-3' anticodon.

RNA editing in $tRNA^{Cys}$ and $tRNA^{Phe}$

In order to investigate RNA editing in tRNA molecules a cDNA library derived from *Oenothera* mitochondrial RNA was screened with specific probes for $tRNA^{Cys}$, $tRNA^{Asn}$ and $tRNA^{Phe}$ (Wissinger et al. 1991). Since only two tRNA-derived cDNA clones were isolated by this approach, we also analyzed PCR amplified cDNAs that were specifically synthesized from precursor molecules of $tRNA^{Cys}$, $tRNA^{Asn}$, $tRNA^{Ile}$ and $tRNA^{Phe}$. Comparison of genomic and cDNA sequences identified one cytidine to thymidine exchange each in the $tRNA^{Cys}$ and $tRNA^{Phe}$ coding regions (Figs. 3 and 4).

The secondary structure model (Fig. 2B) shows the nucleotide transition in $tRNA^{Cys}$ (Fig. 3A) to be located at the base of the anticodon stem. A C_{28}-T_{42} mismatch is changed to a T_{28}-T_{42} mismatch in 33.3% of the analyzed cDNA clones (Fig. 3B).

In the $tRNA^{Phe}$ gene a C to T exchange was identified by comparing the sequences of the four identical genomic copies with the respective cDNA sequences (Fig. 4A, Binder and Brennicke 1993b). This nucleotide transition converts a C_4-A_{69} mismatch in the acceptor stem to a conventional T_4-A_{69} Watson-Crick base pairing (Fig. 2D). The cDNA sequence analysis showed 46.7% of the 15 analyzed clones to be altered in this

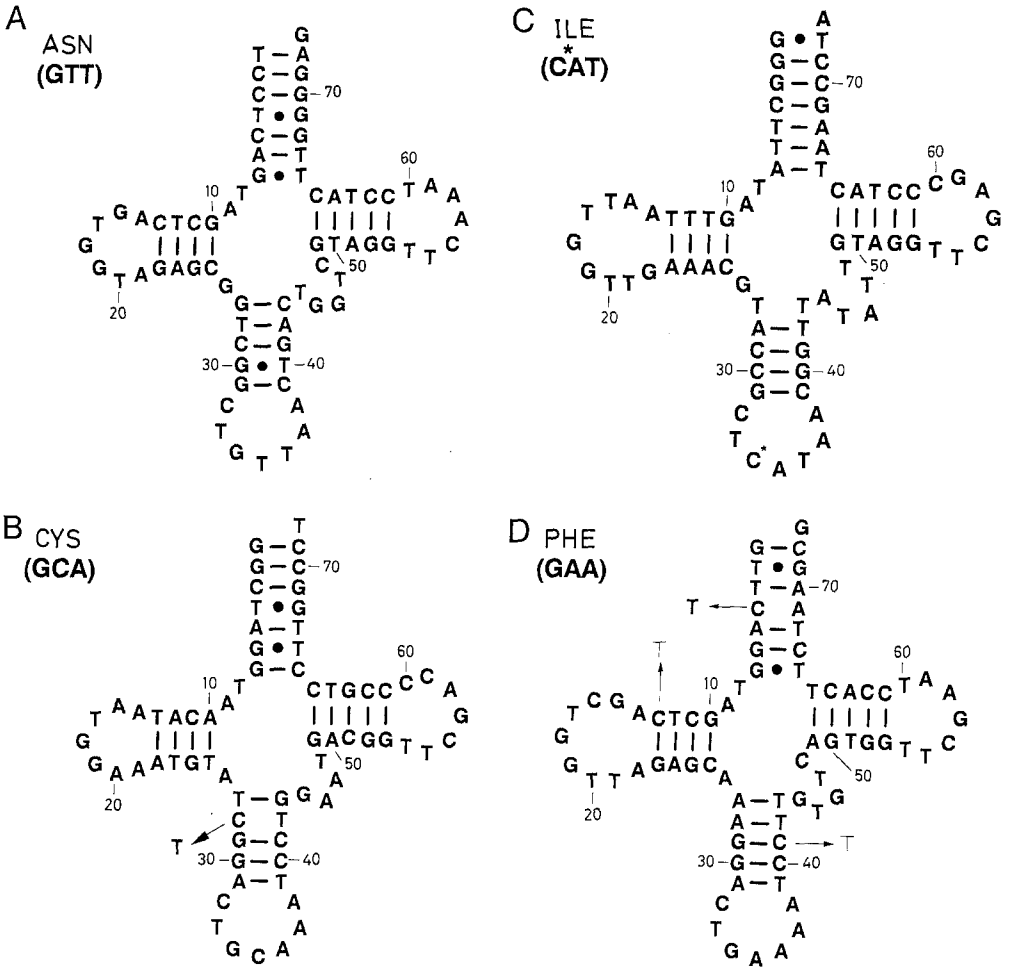


Fig. 2A-D Cloverleaf structures of the tRNAs deduced from the genes for $tRNA^{Asn}$ (**A**), $tRNA^{Cys}$ (B), $tRNA^{Ile}$ (**C**) and $tRNA^{Phe}$ (**D**) are shown as DNA sequences. Numbering of the nucleotides follows Steinberg et al. (1993). The C to T editing events are indicated by *arrows*. Major editing events are marked by *bold characters*, while the minor editing events observed in a single cDNA clone (no. 1182) are indicated by *thin letters*. The cytidine (C_{34}) in the first anticodon position of $tRNA^{Ile}$, which is hypermodified to a lysidine-like nucleotide in potato, is marked by an *asterisk*. The sequences of the newly characterized genes for $tRNA^{Cys}$, $tRNA^{Asn}$ and $tRNA^{Ile}$ with the respective upstream and downstream regions have been submitted to the Genbank/EMBL Data Bank with accession numbers X76496 and X76497

position. Sequence analysis of a cDNA clone derived from a pseudo-$tRNA^{Phe}$ showed that this nucleotide position (C_4) is also edited in a cDNA clone derived from a pseudo-copy of this gene (not shown), although the disruption in the 3' part of the gene does not allow correct formation of the acceptor stem of the pseudo-tRNA (Binder and Brennicke 1993b).

In the $tRNA^{Phe}$ two additional, unusual RNA editing events were observed in a single cDNA clone (no. 1182, Fig. 4B). Transition of C_{13} in the stem of the D-arm and C_{41} in the anticodon stem to thymidines converts two conventional C-G base pairings to less stable T-G base pairs (Fig. 2D). The significance of these two RNA editing events, which were detected only in a single cDNA clone, remains unclear. Since this clone is derived from PCR amplified cDNA, amplification artifacts cannot be excluded.

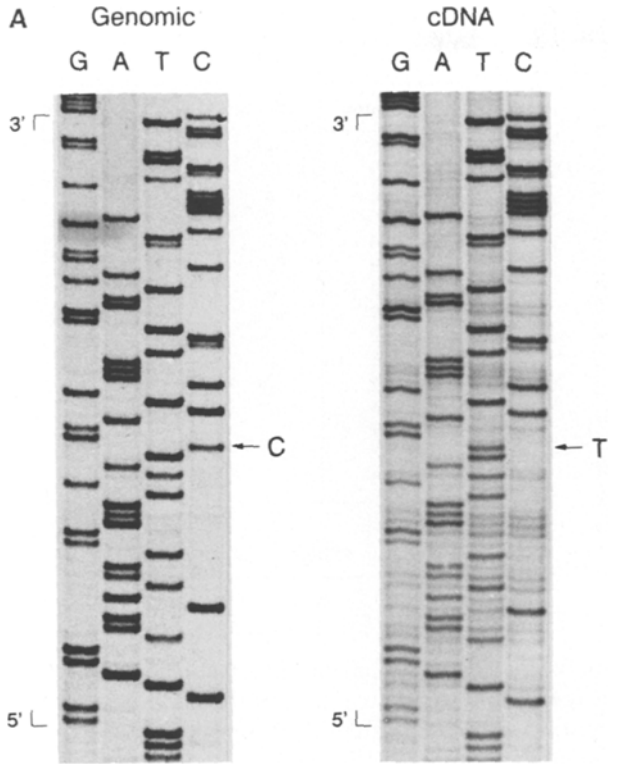
The seven cDNA clones derived from $tRNA^{Ile}$ precursor molecules show no differences from the genomic sequence. Only cytidines (C_{34}) were observed at the most probably hypermodified first anticodon position (Fig. 2C).

The analysis of genomic and cDNA sequences from $tRNA^{Asn}$ did not reveal any evidence for a nucleotide exchange. All 11 investigated cDNA clones are identical to the genomic sequence.

Since the observed nucleotide differences in the investigated tRNAs are alterations from genomic cytidines to thymidines in the cDNA (uridines in the tRNA), they are attributable to the RNA editing process observed in almost all plant mitochondrial mRNAs investigated so far.

The tRNA genes are transcribed into larger precursor RNAs

Transcription of the newly identified tRNA genes coding for $tRNA^{Cys}$, $tRNA^{Asn}$, and $tRNA^{Ile}$ was analyzed in detail by Northern blot experiments. Hybridization against total mitochondrial RNA was performed with



B

number of cDNA clones	edited	not edited	percentage of edited clones
12	4	8	33.3 %

Fig. 3A, B RNA editing in $tRNA^{Cys}$. **A** Comparison of genomic and cDNA sequences of $tRNA^{Cys}$. 5' and 3' ends of the tRNA gene are indicated and the editing site is identified by an *arrow*. **B** Statistical analysis of $tRNA^{Cys}$ editing events. In the precursor RNA population analyzed, 33.3% of the individual cDNA clones are found to be edited at position 28

probes containing the respective tRNA gene with upstream and downstream region sequences. Signals at about 75 nucleotides showed the mature tRNAs to be present in the steady state RNA of *Oenothera* mitochondria ($tRNA^{Asn}$, Fig. 5A; $tRNA^{Cys}$, Fig. 5B; $tRNA^{Ile}$, data not shown). Beside these mature tRNA-sized molecules a complex pattern of larger RNA species is detected by this approach. RNA molecules of sizes up to 2.5 kb for both $tRNA^{Cys}$ and $tRNA^{Asn}$ are most probably tRNA precursors. The various size classes of these precursor molecules may represent intermediates in the tRNA maturation process.

Discussion

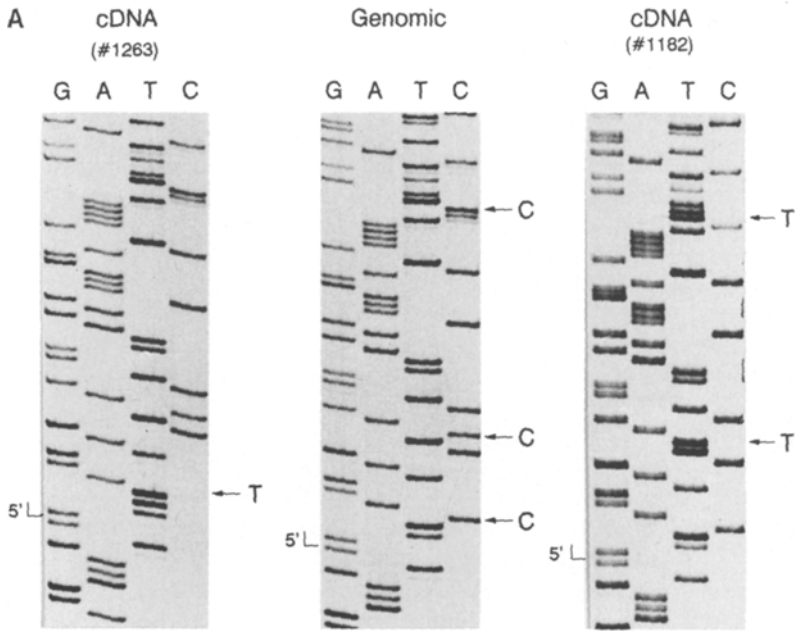
The genes for $tRNA^{Cys}$ and $tRNA^{Asn}$ have been newly identified in the *Oenothera* mitochondrial genome. Together with the previously identified gene for $tRNA^{Tyr}$ they are located upstream of exon c of subunit 2 of the NADH dehydrogenase. The organization of these three tRNA genes is identical in *Oenothera* and tomato, although the distances between the genes differ (Izuchi et al. 1990). The larger RNAs detected in the Northern blot analysis do indicate that these tRNA genes are transcribed into longer precursor RNAs (Fig. 5), but do not yet allow any conclusions to be drawn concerning possible cotranscription of the genes.

RNA editing or modification?

The sequence analysis of cDNAs derived from precursor tRNAs provided evidence for RNA editing in the mitochondrial $tRNA^{Cys}$ and $tRNA^{Phe}$ from the higher plant *O. berteriana*. One of these editing sites is located in the anticodon stem of $tRNA^{Cys}$. Another editing site is detected in the acceptor stem of $tRNA^{Phe}$. There are two arguments that support the idea that the transitions represent RNA editing rather than modification or hypermodification. First, in both cases genomic cytidines were altered to uridines (thymidines in the cDNA) as in the overwhelming majority of the editing events described in higher plant mitochondria. This notion is strongly supported by the analysis of the analogous $tRNA^{Phe}$ editing event in potato and bean, where the investigation showed a genuine C to U transition in the mature tRNAs (Maréchal-Drouard et al. 1993). Second, the mitochondrial $tRNA^{Ile}$ encoded in *Oenothera* most probably contains the same cytidine to lysidine-like nucleotide hypermodification as its counterpart in potato (Weber et al. 1990). A similar hypermodification in *E. coli* changes the codon recognition and amino acid specificity from methionine to isoleucine. The cDNA sequence analysis of the $tRNA^{Ile}$ precursor RNA in *Oenothera* mitochondria showed that this nucleotide is always identified as a cytidine residue. This implies that either the hypermodified nucleotide is transcribed by the reverse transcriptase as a normal guanosine or the precursor RNA is not yet modified at this nucleotide position. The clear detection of only cytidines at this position in the precursor RNA molecules indirectly supports the classification of the observed transitions in $tRNA^{Cys}$ and $tRNA^{Phe}$ as RNA editing events rather than nucleotide modifications, since they are already observed in precursor molecules.

Location of the RNA editing sites in the tRNA molecules

The tRNA editing sites identified in plant mitochondria are located in double-stranded regions of the tRNA secondary structures. As in the RNA editing of tRNAs in *A. castellanii* (Lonergan and Gray 1993) and in bean and potato $tRNA^{Phe}$ (Maréchal-Drouard et al. 1993) the C to T transition corrects a mismatch located in the



B

cDNA clone	editing	nucleotide position
#1175	+	4
#1179	-	-
#1182	+	13 and 41
#1233	-	-
#1246	-	-
#1249	-	-
#1259	+	4
#1260	+	4
#1262	-	-
#1263	+	4
#1264	-	-
#1265	+	4
#1266	+	4
#1267	+	4
#1268	-	-

number of cDNA clones	edited	not edited	percentage of edited clones
15	8	7	53.3 %

Fig. 4A, B RNA editing of $tRNA^{Phe}$. **A** Autoradiograms of $tRNA^{Phe}$ genomic and cDNA sequences are compared. Nucleotides changed by RNA editing are marked by *arrows*. The edited nucleotides are identified as C in the genomic and T in the cDNA sequences. Two rare and apparently "nonsense" editing events were detected in only a single cDNA clone (no. 1182). **B** Statistical analysis of the $tRNA^{Phe}$ editing events shows 53.3% of all clones to be edited with 46.7% altered at position 4 (Fig. 2D)

acceptor stem of *Oenothera* $tRNA^{Phe}$. As has been proposed for *A. castellanii*, the 3' part of the acceptor stem itself could provide the specificity for this editing event, assuming that the RNA editing mechanism is a form of directed mismatch repair. However, there are several arguments against a *cis*-guiding mechanism in higher plant mitochondria. Although the observed editing events are located in double-stranded regions, *cis*-guiding cannot be a prerequisite for editing, since editing at this position (C_4) also occurs in a cDNA derived from a pseudo-$tRNA^{Phe}$ gene, which shows a C_4-C_{69} mismatch in the non-functional acceptor stem of the potential secondary structure. In addition, *cis*-guiding is not possible at all in the RNA editing event in the $tRNA^{Cys}$, where a C-T mismatch is edited to a mismatched T-T pair. However, since all identified RNA editing events, including those observed in cDNA clone no. 1182, are located in base pairing regions, this structural feature might be somehow important for the RNA editing mechanism. These observations might suggest that formation of some kind of secondary structure is more generally involved in RNA editing in higher plant mitochondria.

RNA editing of tRNAs: functionally necessary for higher order structure?

Correct aminoacylation of tRNAs by their cognate aminoacyl-tRNA synthetases is directed by identity elements in the tRNA, which can be dispersed throughout the three-dimensional structure of the tRNA or may be concentrated in the anticodon loop and/or the acceptor stem (Schulman 1991). However, RNA editing of $tRNA^{Phe}$ in potato and bean, which is analogous to that observed in the corresponding tRNA from *Oenothera*, is proposed to have no influence on correct aminoacylation (Maréchal-Drouard et al. 1993). A function of RNA editing of the $tRNA^{Cys}$ in defining aminoacylation specificity is also rather unlikely, since the identity elements are usually not located in the stem of the anticodon arm. In *Marchantia* mitochondria, where no RNA editing has been observed so far, the mismatched T-T basepair, which is introduced into *Oenothera* $tRNA^{Cys}$ by RNA editing, is genomically encoded. It is therefore possible that the RNA editing of $tRNA^{Cys}$ is involved in the stabilization of the higher order structure. T-T mismatches are observed in several other mitochondrially encoded tRNAs from different plant species, either located in the acceptor stem as in $tRNA^{Ser}$ (GCU) (Binder et al. 1990, Maréchal-Drouard et al. 1993, Joyce et al. 1988) or in the stem of the anticodon arm as in $tRNA^{Ile}$ (Fig. 2C, Weber et al. 1990) or $tRNA^{Glu}$ (UUC) (Gualberto et al. 1989). This may indicate that mismatched T-T "basepairs" are less obstructive to formation of functional tRNA secondary structures than C-T mismatches. The significance of the RNA editing events observed in cDNA clone no. 1182 is questionable. Since these were detected only in a single cDNA clone, they might result from a kind of misediting as observed in *cox*I and *rps*3 mRNAs from *O. berteriana* (Schuster and Brennicke 1991) or alternatively result from PCR artifacts.

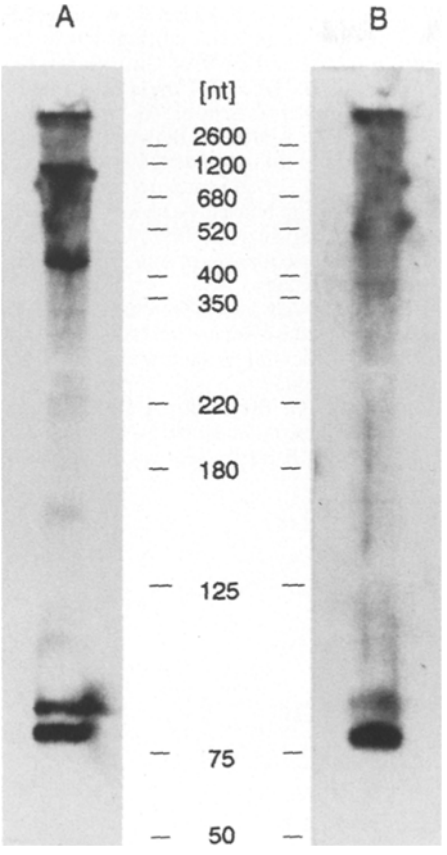


Fig. 5A, B Northern blot analysis of $tRNA^{Asn}$ (**A**) and $tRNA^{Cys}$ (**B**). For hybridization to total mitochondrial RNA from *Oenothera* the same probes as described for the Southern blot analysis were used. Strong signals from species of about 75 nucleotides correspond to the mature tRNA molecules. Signals corresponding to larger RNA species indicate the presence of tRNA precursor molecules in the steady state RNA of *Oenothera* mitochondria. Sizes of co-electrophoresed DNA standard fragments are given in nucleotides (*nt*)

RNA editing, a prerequisite for efficient processing of $tRNA^{Phe}$?

The T-A base pair introduced by RNA editing of the $tRNA^{Phe}$ repairs a mismatch in the acceptor stem and increases the stability of the secondary structure. The correctly paired stem might influence the processing steps required for maturation of the tRNA. While 46.6% of the investigated cDNA clones derived from precursor tRNA molecules from *Oenothera* are found to be edited at this position (Fig. 2D, position 4), no unedited forms of the mature tRNA from potato could be detected (Maréchal-Drouard et al. 1993). This suggests that the $tRNA^{Phe}$ editing is required for efficient 5' and 3' processing.

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