The EMBO Journal vol.9 no.1 pp.263 – 269, 1990

Transcripts of the NADH-dehydrogenase subunit 3 gene are differentially edited in *Oenothera* mitochondria

Wolfgang Schuster, Bernd Wissinger, Michael Unseld and Axel Brennicke

Institut für Genbiologische Forschung, Ihnestrasse 63, D-1000 Berlin, FRG

Communicated by C.J.Leaver

A number of cytosines are altered to be recognized as uridines in transcripts of the *nad*3 locus in mitochondria of the higher plant *Oenothera*. Such nucleotide modifications can be found at 16 different sites within the *nad*3 coding region. Most of these alterations in the mRNA sequence change codon identities to specify amino acids better conserved in evolution. Individual cDNA clones differ in their degree of editing at five nucleotide positions, three of which are silent, while two lead to codon alterations specifying different amino acids. None of the cDNA clones analysed is maximally edited at all possible sites, suggesting slow processing or lowered stringency of editing at these nucleotides. Differentially edited transcripts could be editing intermediates or could code for differing polypeptides. Two edited nucleotides in an open reading frame located upstream of *nad*3 change two amino acids in the deduced polypeptide. Part of the well-conserved ribosomal protein gene *rps*12 also encoded downstream of *nad*3 in other plants, is lost in *Oenothera* mitochondria by recombination events. The functional *rps*12 protein must be imported from the cytoplasm since the deleted sequences of this gene are not found in the *Oenothera* mitochondrial genome. The pseudogene sequence is not edited at any nucleotide position.

Key words: *nad*3 gene/plant mitochondria/RNA editing/ RNA editing differences

Introduction

The recognition of RNA editing in trypanosome mitochondria has expanded our understanding of the dynamic control and manifestation of the basic genetic programme specified by DNA in biological systems (Benne, 1989; Borst *et al.*, 1989; Stuart, 1989; Simpson and Shaw, 1989). Previous attention on post-transcriptional nucleotide modifications had been focused on the chemical alterations in structural RNAs, notably the ribosomal and transfer RNAs. RNA processing in mRNAs had been restricted to the excision of introns and 5′- and 3′-terminal modifications from trimming to capping and poly(A) addition. With the observation of extensive post-transcriptional RNA sequence alterations in trypanosomes it became clear that not all polypeptide sequences are directly encoded in the linear genomic DNA sequence. Since then RNA editing—defined as the modification of the primary DNA-derived sequence to potentially specify a different protein sequence—has been observed with a RNA unwinding/modifying activity in *Xenopus*, converting adenosine to inosine residues (Bass and Weintraub, 1988); the insertion of regularly spaced C residues in mitochondria of the acellular slime mould *Physarum polycephalum* (Benne, 1989; Simpson and Shaw, 1989); and a single C to U conversion in the apolipoprotein B pre-mRNA in mammalia (Driscoll *et al.*, 1989).

Alteration of the DNA-derived mRNA sequence in plant mitochondria has so far been found in every gene investigated in this respect in *Oenothera* and mostly involves C to U modifications. These RNA sequence alterations occur in coding and non-coding regions at irregular sites without any apparent primary sequence motifs potentially conferring specificity to the editing process. It is an open question whether the RNA editing activity in plant mitochondria recognizes internal signals or moves along the RNA in a progressive way co- or post-transcriptionally 5′ to 3′ or post-transcriptionally 3′ to 5′.

The identification of partially edited transcripts in trypanosome mitochondria suggests a 3′ to 5′ progressive editing process for these organisms (Feagin *et al.*, 1987; Simpson and Shaw, 1989). These potential editing intermediates isolated as individual cDNA clones have inserted and/or deleted U residues in their 3′ sequences and terminate at different 5′ positions with oligo(U) stretches that are presumably inserted in excess, only to be trimmed in a second step to the correct final sequence in the mature transcript.

We have now investigated a number of independent cDNA clones of the NADH-dehydrogenase subunit 3 locus (*nad*3) in the higher plant *Oenothera* to determine the extent and potential variation of RNA editing in the transcript population. The state of editing in individual molecules varies at specific sites, suggesting a post-transcriptional internal editing process and a hierarchy of site affinities, or alternatively a differentiated stringency at individual nucleotide identities leading to potentially mixed polypeptide populations.

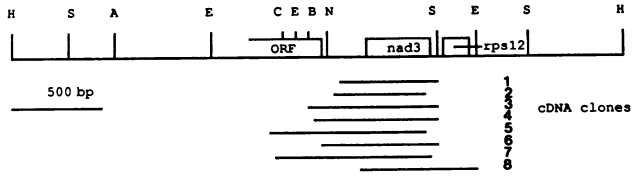


Fig. 1. Organization of the *nad*3 locus in *Oenothera* mitochondria. The *nad*3 gene in *Oenothera* mitochondria is encoded downstream of an unidentified reading frame (ORF) and upstream of a truncated *rps*12 gene. The sequence regions covered by cDNA clones discussed in this paper (1 – 8) are indicated below the genomic arrangement. Restriction sites are indicated for *Hin*dIII (H), *Spe*I (S), *Sal*I (A), *Eco*RI (E), *Sca*I (C), *Bgl*II (B) and *Nsi*I (N). The 3.4 kb *Hin*dIII restriction fragment is orientated with the 5′ termini of the ORF and *nad*3 on the left-hand side.

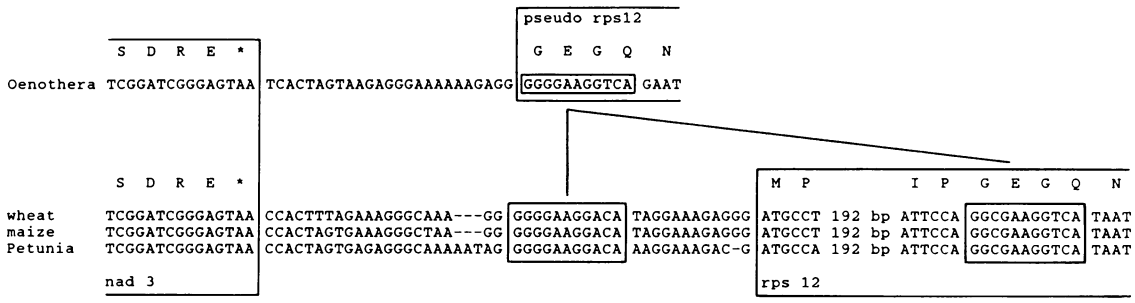


Fig. 2. Recombination via an 11 nucleotide imperfect repeat has deleted part of the *rps*12 gene. Left-hand and right-hand nucleotides of the imperfect repeat in the wheat, maize and *Petunia* spacer region between *nad*3 and *rps*12 and within the *rps*12 open reading frame are recombined in *Oenothera* (indicated by boxes and connecting lines). This event has deleted spacer sequences and part of the *rps*12 coding region, creating a pseudogene of *rps*12. The wheat and maize sequences are taken from Gualberto *et al.* (1988), the *Petunia* sequences from Rasmussen and Hanson (1989).

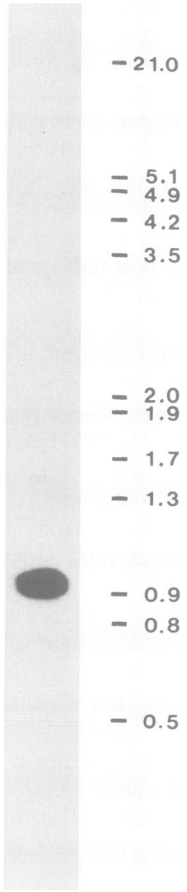


Fig. 3. Southern analysis shows no intact copy of the *rps*12 gene in *Oenothera* mitochondria. *Eco*RI restricted mitochondrial DNA probed with *nad*3 and/or the *rps*12 region deleted in *Oenothera* shows hybridization only to the fragment found in the genomic arrangement described here (Figure 1). Incompletely digested molecules containing this and the adjacent *Eco*RI fragment appear at ~1.5 kb in the extremely long exposure shown here to detect also low copy number molecules. Sizes of the DNA length standards are indicated in kb. This result suggests that no intact copy of the *rps*12 gene is encoded elsewhere in the mitochondrial genome of *Oenothera* besides the pseudogene.

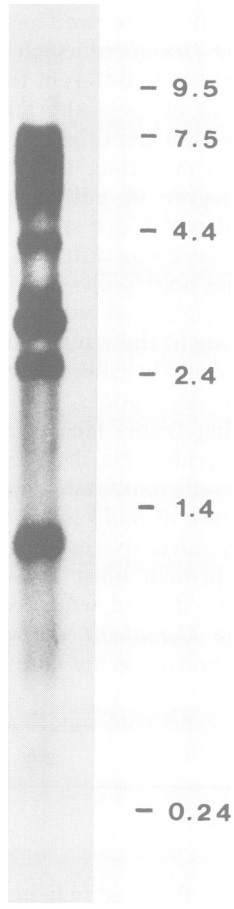


Fig. 4. Northern analysis of transcripts from the *nad*3 locus in *Oenothera* mitochondria. A number of transcripts with different sizes are detected with a subcloned probe covering the *rps*12 coding region in size-fractionated mitochondrial RNA of *Oenothera*. Abundant transcripts range from 1.2 to 7.5 kb with still larger RNAs occurring infrequently. Size markers (indicated in kb) are RNA length standards co-fractionated in the agarose–formaldehyde gel.

Results

Organization of the nad3 locus in Oenothera

The *nad*3 gene in *Oenothera* mitochondria was identified with a heterologous probe from the respective wheat locus. The *nad*3 gene in the wheat and maize mitochondrial genomes is located downstreawm from a $tRNA^{Ser}$ and a tRNA pseudogene and upstream of ribosomal protein rps12 that is co-transcribed with *nad*3 (Gualberto *et al.*, 1988).

The *nad*3 gene is located on a 3.4 kb *Hin*dIII fragment in the mitochondrial genome of *Oenothera* (Figure 1). This fragment was isolated from a *Hin*dIII genomic DNA library and sequenced at the *nad*3 locus. The *Oenothera* mitochondrial DNA sequence of this region diverges from the wheat and maize sequences upstream of the *nad*3 coding

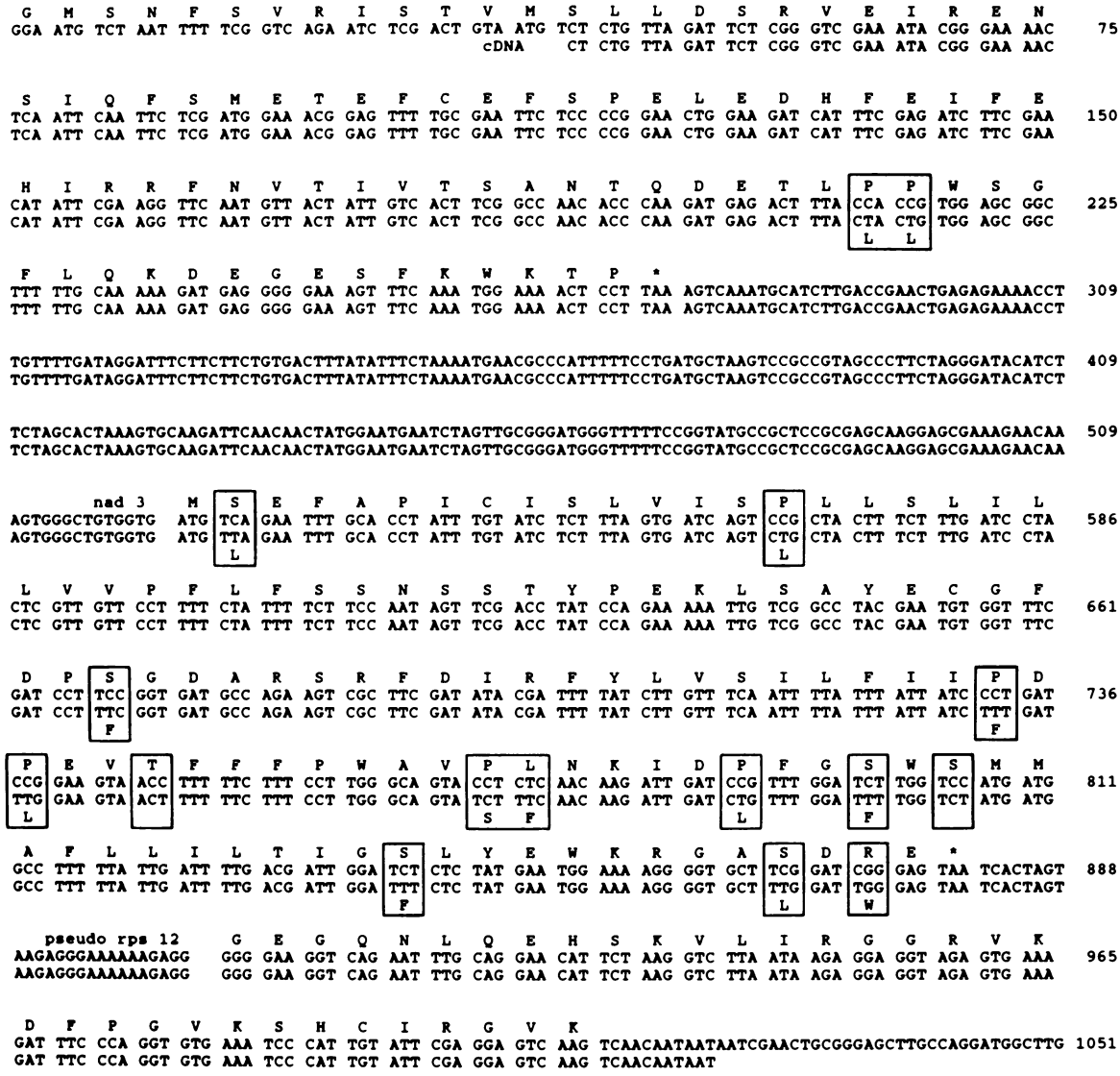


Fig. 5. Comparison of genomic and cDNA sequences around the *nad*3 gene. The genomic sequence (top line of nucleotides) begins within the open reading frame upstream of *nad*3 and continues through the co-transcribed spacers, the *nad*3 coding region and the *rps*12 pseudogene. The cDNA sequence in the lower nucleotide line has been derived from the eight independent cDNA clones shown in Figure 1. All nucleotides edited in at least one cDNA clone are incorporated and highlighted by boxes. Amino acids specified in the cDNA differing from the genomic encoded amino acid are given underneath the cDNA nucleotide sequence.

region (Figure 1). The $tRNA^{Ser}$ has been lost from this region and an open reading frame is now found upstream of the *nad*3 gene in *Oenothera* in the same orientation. This ORF has no identifiable similarity to any sequence in the data banks (not shown). The high percentage of T in third codon positions commonly found in plant mitochondrial genes, however, suggests this ORF to be a genuine plant mitochondrial gene.

Mitochondrial rps12 is not encoded in Oenothera mitochondria

A sequence recombination event has occurred downstream of *nad*3 and deleted part of the intervening sequence between *nad*3 and *rps*12, together with a portion of the actual coding region of *rps*12 in the *Oenothera* mitochondrial genome. An imperfect, direct repeat of 11 nucleotides in wheat, maize and *Petunia* at the borders of the sequence deleted in *Oenothera* suggests that recombination involved alignment and excision via this repeat (Figure 2).

Since part of the *rps*12 coding region is missing at this locus, we searched the mitochondrial genome of *Oenothera* for another, intact copy of the *rps*12 gene. Southern hybridization experiments indicate, however, this truncated pseudogene in connection with the *nad*3 gene to be the only genomic location containing *rps*12 sequences (Figure 3).

The functional mitochondrial *rps*12 polypeptide therefore appears to be nuclear encoded in *Oenothera* and imported into the mitochondrion post-transcriptionally.

nad3, the upstream ORF and the rps12 fragment are co-transcribed

Northern blot analysis of mitochondrial RNA probed with the *nad*3 and/or the *rps*12 fragment sequences shows that both sequences are contained in a complex pattern of mRNA species (Figure 4 and data not shown). The *nad*3 and *rps*12 genes in wheat and maize are similarly co-transcribed in several transcripts of different sizes (Gualberto *et al.*, 1988). The large transcripts and some of the cDNA clones in

Oenothera mitochondria cover the coding regions of both the upstream unknown ORF and *nad*3, indicating co-transcription of this ORF with *nad*3 from further upstream promoter(s) (Figure 1).

Analysis of independently derived cDNA clones suggests a potential 3′ terminus between the *nad*3 coding region and the *rps*12 fragment. Although these cDNA clones were obtained from randomly primed cDNA synthesis and are not expected to reflect *in vivo* 3′ termini some of them have identical (or nearly identical, ±1 nucleotide) 3′ termini with different 5′ extensions (cDNA clones 1, 3, 4, 6 in Figure 1 and data not shown).

The genomic sequence is altered in the mRNA-derived cDNAs

Comparison of genomic and cDNA-derived sequences of this region shows a number of divergences in nucleotide identities in the sequences encoding *nad*3 and the upstream ORF (Figure 5). The cDNA sequence derived from the most extensively edited sequences shows 16 nucleotide modifications from genomic cytosines to uridines (or analogues) within the *nad*3 sequence and two such transitions in the upstream ORF, but none in the *rps*12 pseudogene in the cDNA clones analysed. Only two of the edited nucleotides in *nad*3 are silent, third position alterations in codons specifying the same amino acid in cDNA and genomic sequences.

Amino acid sequence alignment of genomic and cDNA-derived polypeptides with the respective proteins encoded in the mitochondrial and chloroplast genomes of other species show RNA editing to maintain a better degree of conservation for the *Oenothera* nad3 protein in evolution (Figure 6).

The only genomic CGG codon near the carboxy terminus of the *nad*3 polypeptide is edited in the cDNA sequence to a UGG codon specifying the evolutionarily conserved tryptophane according to the universal genetic code (discussion in Hiesel, *et al.*, 1989).

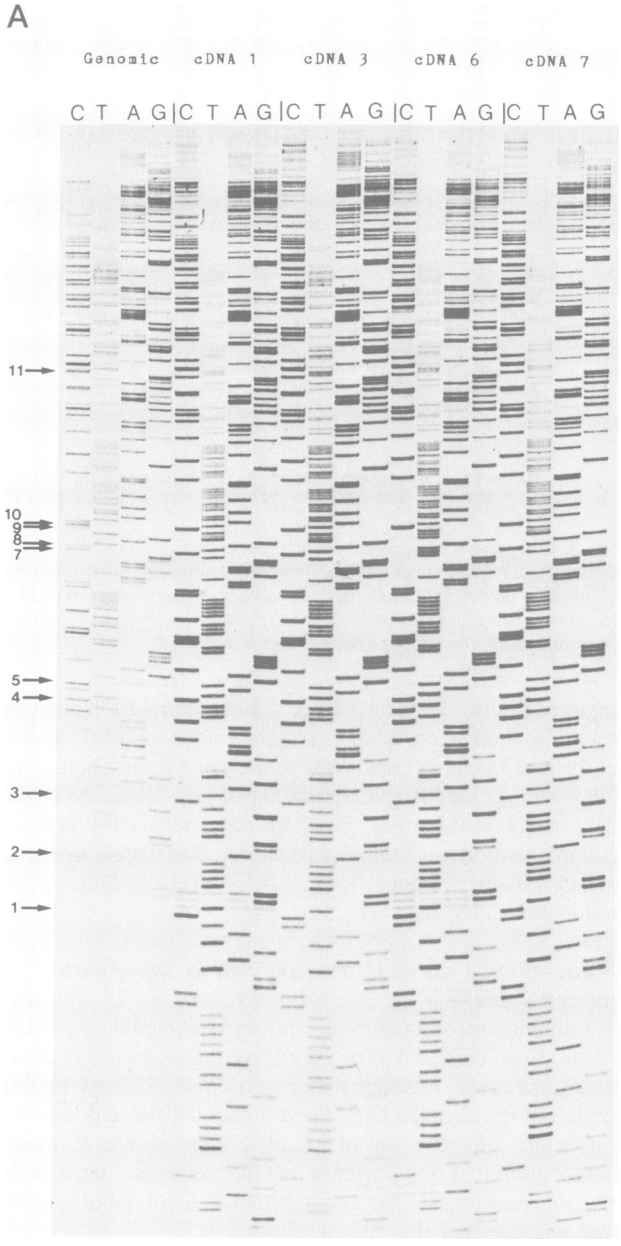
```
Oenothera cDNA   L          L                              F
                 ◆          ◆                              ◆
  Oenothera mt  MSEFAPICISLVISPLLSLILLVVPFLFSSNS  STYPEKLSAYECGFDPSGDAR
      Human mt     MNFALILMINTLLALLIMIITFWLPQLNG    YMEKSTPYECGFDPMSPAR
      Mouse mt     INLYTVIFINILLSLTLILVAFWLPQMNL    YSE ANPYECGFDPTSSAR
     Bovine mt     MNLMLALLTNFTLATLLVIIAFWLPQLNV    YSEKTSPYECGFDPMGSAR
  X. laevis mt      MTATILMIAMTLSTILAILSFWLPQMTP    DMEKLSPYECGFDPLGSMR
    Tobacco cp  MFLLYEYDFFWAFLIISILVPILAFLISGVLAPISKGPEKLSTYESGIEPMGDAW
    Liverwort cp MFLLEKYDYFFVFLLIISFFSILIFSLSKWIAPINKGPEKFTSYESGIEPMGEAC
                        10         20         30         40         50

                  F L          SF    L F            F          L W
                  ◆◆           ◆◆    ◆◆             ◆          ◆◆
  SRFDIRFYLVSILFIIPDPEVTFFFPWAVPLNKIDPFGSWSMMAFLLILTIGSL YEWKRGASDRE
  VPFSMKFFLVAITFLLFDLEIALLLPLPWALQTTNLPLMVMSSLLLIIILALSLAYEWLQKGLDWTE
  LPFSMKFFLVAITFLLFDLEIALLLPLPWAIQTIKTSTMMIMAFILVTILSLGLAYEWTQKGLEWTE
  LPFSMKFFLVAITFLLFDLEIALLLPLPWASQTANLNTMLTMALFLIILLAVSLAYEWTQKGLEWTE
  LPFSMRFFLIAILFLLFDLEIALLLPFWAAQLNTPSIVILWAALILTLLTLGLI YEWLQGGLEWAE
  LQFRIRYYMFALVFVVFDVETVFLYPWAMSFDVLGVSVFIEAFIFVLILIIGLV YAWRKGALEWS
  IQFQIRYYMFALVFVIFDVETVFLYPWAMSFYNFGISSFIEALIFILILIIGLV YAWRKGALEWS
       60         70         80         90        100        110      118
```

Fig. 6. Comparison of the amino acid sequences deduced from the genomic and cDNA nucleotide sequences. Amino acid sequences deduced from the *nad*3 genomic sequences encoded in mitochondria (mt) and chloroplasts (cp) of different species are aligned with the respective sequence from *Oenothera* mitochondria. Amino acids differently specified by the edited cDNA sequences given in Figure 5 are indicated by arrows in the top line labelled cDNA. Most of the amino acids specified differently by the cDNA sequence are better conserved in evolution than the genomic encoded residues when compared to the *nad*3 sequences from the human (Anderson *et al.*, 1981), mouse (Bibb *et al.*, 1981), bovine (Anderson *et al.*, 1982), *Xenopus* (Roe *et al.*, 1985) mitochondrial and the liverwort (Ohyama *et al.*, 1986) and tobacco (Shinozaki *et al.*, 1986) chloroplast genomes.

Partially edited cDNAs

Individual cDNA clones differ in the degree of editing at specific nucleotide positions (Figure 7A). Figure 7(B) shows a sequence alignment of the *nad*3 coding region in eight exemplary cDNA clones, reflecting different states of editing. These clones were randomly selected for detailed analysis from >100 cDNA clones of this region identified in the mitochondrial cDNA library.

None of the cDNA clones analysed so far for the *nad*3 region could be identified as a 'completely' edited sequence containing all observed possible nucleotide modifications. Each of the clones lacks at least two nucleotide alterations with no apparent hierarchy in their distribution between five different nucleotide positions. Two of these variably edited nucleotides are in silent third-codon positions, another one is without effect on the specified amino acid in the first position of a leucine codon. The other two of these only in some cDNAs edited cytosines are in first-codon positions in adjacent triplets, altering proline to serine and leucine to phenylalanine codons respectively when modified to uridine



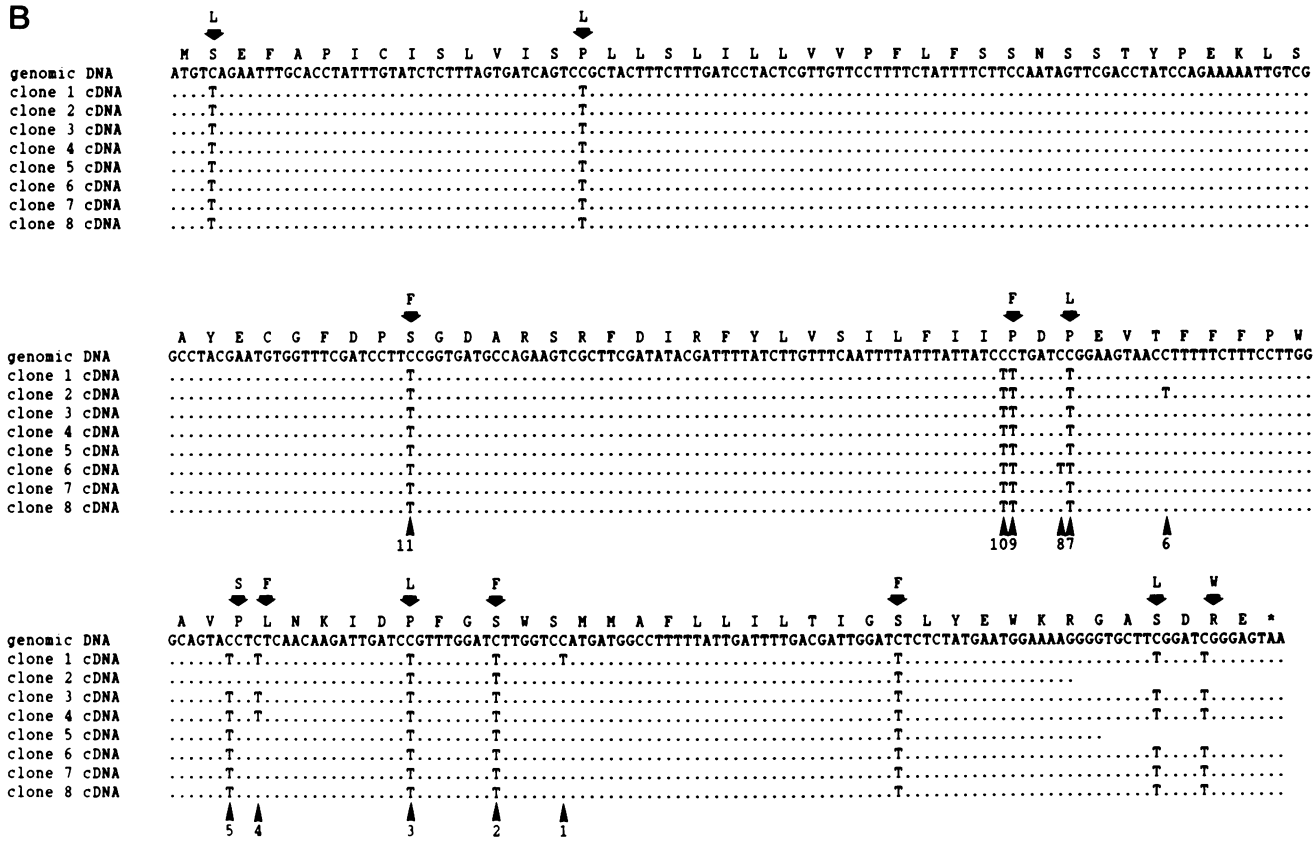


Fig. 7. Individual cDNA clones show different patterns of editing. (**A**) The sequence of a region where several cytosines are differently edited in the cDNA clones 1, 3, 6 and 7 (Figure 1) is compared comparison with the sequence of the genomic DNA. G, A, T, C indicate the four ladders obtained from a synthetic primer with the sequence 5′-TTTTTTCCCTCTTACTAGTG-3′ located 3′ of the *nad*3 coding region. This primer lies outside the sequence covered by cDNA clone 2, the only cDNA with nucleotide position 6 altered. Nucleotides altered in at least one of the cDNA clones shown are indicated by arrows and numbered as in (B). (**B**) Alignment of eight individual cDNA sequences with the genomic nucleotide sequence of the *nad*3 coding region. Only the altered nucleotides are shown in the cDNA sequences. Amino acids divergent in the protein sequence altered by editing of the mRNA from the genomic encoded polypeptide sequence are given in the top lane and indicated by arrows. Nucleotides altered between genomic and cDNA clones in the sequence region covered by the gel analysis shown in (B) are identified by numbers and arrowheads.

(Figures 5–7). The genomic proline codon is modified in seven of eight cDNA clones, whereas the neighbouring genomic leucine codon is edited in only three out of the eight shown (Figure 7).

The observation that none of the investigated cDNA clones is maximally edited in the *nad*3 coding region suggests that such transcripts are of very low abundance in the steady-state mRNA population. The randomly primed cDNA library has been found to reflect correctly relative abundances of different mRNA species in the isolated mitochondrial RNA by comparison of the number of cDNA clones with Northern blot mRNA hybridization intensities. Similarly, processed and unprocessed intron–exon junctions are present in the cDNA library in relative amounts representative of the splicing efficiencies of individual introns in different genes in *Oenothera* mitochondria (B.Wissinger, unpublished).

Discussion

In the work reported here we analysed the extent of RNA editing in plant mitochondria in the locus encoding subunit 3 of the NADH-dehydrogenase in *Oenothera*. The investigated cDNA clones suggest a mixture of partially edited transcripts from this gene in mitochondria of *Oenothera*.

Previous analysis of RNA editing in higher plant mitochondrial transcripts specifying the cytochrome b (cyt*b*), subunit 1 of the NADH-dehydrogenase (*nad*1) and cytochrome oxidase subunits II and III (*cox*II and *cox*III) had revealed homogeneously edited cDNAs for these loci (Hiesel, *et al.*, 1989). RNA editing in these genes and the *nad*3 locus investigated here predominantly involves modification of cytidines to be recognized as uridines by the reverse transcriptase and presumably the ribosome. One reverse alteration has been observed in the cytochrome b locus modifying a genomic encoded U to C in the cDNA sequence (Hiesel, *et al.*, 1989).

The restriction of RNA editing in plant mitochondria to altering nucleotide identities between C and U is most easily explained by an enzymatic activity modified from enzymes of nucleotide metabolism such as cytidine deaminase (O'Donovan and Neuhard, 1970) or CTP synthetase (O'Donovan and Neuhard, 1970; Zalkin, 1985). The hypothetical editing activity, however, requires additional specificity (factors) for the recognition of polynucleotide chains and therein for specific editing sites.

Post-transcriptional modification changing C to U has also been found in a tissue-specific single nucleotide conversion, creating a new termination codon in the apolipoprotein B gene in mammalia (Chen *et al.*, 1987; Powell *et al.*, 1987).

This alteration of the mRNA sequence has been included in the many different RNA processing events while narrowing the term RNA editing to the mRNA sequence alteration by addition of non-genomically encoded nucleotides in trypanosome mitochondria (Borst *et al.*, 1989). The extensive sequence alterations in the mRNAs in plant mitochondria by many specific C to U conversions in each gene investigated in this respect in *Oenothera*, however, suggest the term RNA editing also for this process in higher plants, since the polypeptides specified by the newly edited sequences differ substantially from the proteins deduced from the genomic DNA.

The enzymatic properties and specificities of this RNA editing process in higher plant mitochondria now need to be investigated in detail. The cDNA analysis presented in this study allows some tentative deductions on stringency and efficiency of the editing process in plant mitochondria—and raises more questions.

Efficiency of the editing process appears to vary between different sites in the mRNA sequence. Partially or differently edited nucleotides in the cDNA clone population derived from the *nad*3 locus are restricted to five of the 16 positions found modified (Figure 7), with the other 11 positions effectively edited in all cDNA clones investigated. Although efficiency clearly differs between the partially and fully edited sites, the sequence of editing events at the partially processed sites does not appear to proceed in any strict order or hierarchy. Distribution of modified and not-altered nucleotides appears to be more or less random between individual cDNAs without any coherent pattern at the five differently edited sites.

The mixture of partially edited cDNA clones can be taken to reflect the steady-state distribution of different transcripts but does not allow one to differentiate between RNA editing intermediates and terminally edited mRNAs. Partially edited mRNAs would be end-products if the editing process occurs co-transcriptionally and does not change the mRNA sequence any further once released from such a hypothetical RNA polymerase editing complex. Alternatively editing could scan fully synthesized mRNAs post-transcriptionally for sites to be modified. The latter pathway could further process partially edited transcripts by going through multiple rounds of attachment, site recognition and modification for individual mRNA molecules and sites.

Whether or not the partially edited transcripts will be further processed has further implications as to the identity of the final, mature mRNA and the actual polypeptide that will be made from this gene. So far no 'completely' edited cDNA clone with all potential sites actually modified has been found for *nad*3 in *Oenothera*. If such molecules do exist in the plant mitochondrion they must be of much lower abundance than the different, partially edited mRNAs, as reflected by the cDNA population, unless they are selectively lost during the mRNA extraction procedure.

The high percentage of partially edited transcripts in trypanosome mitochondria from the *cox*III and in some species also the *cob* and *MURF3* genes does not pose a comparable problem since they are inaccessible to translation until completely processed. As RNA editing in trypanosome mitochondria appears to proceed progressively in the 3′ to 5′ direction, functional AUG codons and long open reading frames will only be generated after the entire transcript has been edited (Benne, 1989). Variability of editing between individual mRNA molecules has been found in the AU tails in trypanosome mitochondria, suggesting a less strictly controlled RNA editing process in these particular sequences without, however, any consequences on the translation products.

Equal affinity and accessibility of all transcripts from the *nad*3 gene in *Oenothera* mitochondria to the ribosome would consequently lead to a mixture of different proteins. The polypeptides specified by the analysed, partially edited transcripts would of course not differ at the three observed silent, potentially altered positions. Alternatively incorporated proline and serine would significantly change the possible secondary structure in this region of the protein. Since the genomic proline at this position (amino acid 83 in Figure 6) is maintained in only one cDNA clone, the predominant protein fraction should contain serine, which is also specified in the respective chloroplast proteins in *Marchantia* and tobacco (Figure 6). The adjacent amino acid 84 is encoded by five cDNA clones as the genomic leucine and by three edited cDNAs as phenylalanine. The latter amino acid is found in the chloroplast deduced proteins, but leucine is integrated in the human *nad*3 subunit. This observation may suggest that different polypeptides derived from one or the other partially edited transcript might potentially be functional in plant mitochondria. Only direct protein sequence data, however, will be able to decide whether indeed a mixture of different proteins is being synthesized in *Oenothera* mitochondria or whether only specific mRNAs are selected for translation of the functional *nad*3 subunit.

Materials and methods

Tissue culture

Oenothera tissue culture cells were grown as callus cultures on solid agar medium in the dark as previously described and were harvested after 3 weeks of subculture (Brennicke, 1980).

Isolation of mitochondrial nucleic acids

Mitochondrial DNAs were purified from mitochondrial fractions obtained after differential centrifugation as described (Brennicke, 1980; Hiesel and Brennicke, 1983). Genomic clone libraries of *Oenothera* mitochondrial DNA have been established with different restriction fragments in pBR and pUC vectors (Hiesel and Brennicke, 1983; Schuster, 1988). Mitochondrial RNA was isolated from mitochondria additionally enriched by sucrose gradient centrifugation and was purified by several rounds of phenol extractions and LiCl precipitations (Hiesel and Brennicke, 1987; Schuster *et al.*, 1988).

cDNA cloning

A random oligonucleotide mixture was used as primers for cDNA synthesis in addition to the previously described first-strand synthesis for a 3′ initiated cDNA library of *Oenothera* mitochondrial transcripts (Hiesel and Brennicke, 1987). Double-stranded cDNAs were ligated to synthetic adaptors containing a *Bam*HI recognition site overlap and ligated into pUC vectors (B.Wissinger, unpublished procedures). The final randomly primed cDNA library contains ~500 000 independent clones.

Gel electrophoresis, hybridization and sequencing

Selection of genomic and cDNA clones was done by several rounds of filter lifts from the bacterial colonies plated at lowered densities in each round until individual colonies could be identified. These were analysed by standard gel and hybridization procedures and sequenced by combinations of controlled chemical modification and chain termination procedures.

Acknowledgements

We thank Drs J.M.Grienenberger and J.H.Weil for the wheat genomic probe for the *nad*3 and *rps*12 locus. We also thank Dr C.J.Leaver FRS for many fruitful discussions. This work was supported by generous grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

References

Anderson,S., Bankier,A.T., Barrell,B.G., De Bruijn,M.H.L., Coulson, A.R., Drouin,J., Eperon,I.C., Nierlich,D.P., Roe,B.A., Sanger,F., Schreier,P.H., Smith,A.J.H., Staden,R. and Young,I.G. (1981) *Nature*, **290**, 457–465.

Anderson,S., De Bruijn,M.H.L., Coulson,A.R., Eperon,I.C., Sanger,F. and Young,I.G. (1982) *J. Mol. Biol.*, **156**, 683–717.

Bass,B.L. and Weintraub,H. (1988) *Cell*, **55**, 1089–1098.

Benne,R. (1989) *Biochim. Biophys. Acta*, **1007**, 131–139.

Bibb,M.J., Van Etten,R.A., Wright,C.T., Walberg,M.W. and Clayton,D.A. (1981) *Cell*, **26**, 167–180.

Borst,P., Benne,R. and Tabak,H.F. (1989) *Cell*, **58**, 421–422.

Brennicke,A. (1980) *Plant Physiol.*, **65**, 1207–1210.

Chen,S.-H., Habib,G., Yang,C.-Y., Gu,Z.-W., Lee,B.R., Weng,S.-A., Silberman,S.R., Cai,S.-J., Deslypere,J.P., Rosseneu,M., Gotto,A.M., Li,W.-H. and Chan,L. (1987) *Science*, **238**, 363–366.

Driscoll,D.M., Wynne,J.K., Wallis,S.C. and Scott,J. (1989) *Cell*, **58**, 519–525.

Feagin,J.E., Jasmer,D.J. and Stuart,K. (1987) *Cell*, **49**, 337–345.

Gualberto,J.M., Wintz,H., Weil,J.H. and Grienenberger,J.M. (1988) *Mol. Gen. Genet.*, **215**, 118–127.

Hiesel,R. and Brennicke,A. (1983) *EMBO J.*, **2**, 2173–2178.

Hiesel,R. and Brennicke,A. (1987) *Plant Sci.*, **51**, 225–230.

Hiesel,R., Wissinger,B., Schuster,W. and Brennicke,A. (1989) *Science*, in press.

O'Donovan,G.A. and Neuhard,J. (1970) *Bacteriol. Rev.*, **34**, 278–343.

Ohyama,K., Fukuzawa,H., Kohchi,T., Shirai,H., Sano,T., Sano,S., Umesono,K., Shiki,Y., Takeuchi,M., Chang,Z., Aota,S., Inokuchi,H. and Ozeki,H. (1986) *Nature*, **322**, 572–574.

Powell,L.M., Wallis,S.C., Pease,R.J., Edwards,Y.H., Knott,T.J. and Scott,J. (1987) *Cell*, **50**, 831–840.

Rasmussen,J. and Hanson,M.R. (1989) *Mol. Gen. Genet.*, **215**, 332–336.

Roe,B.A., Ma,D.P., Wilson,R.K. and Wong,J.F.H. (1985) *J. Biol. Chem.*, **260**, 9759–9774.

Schuster,W. (1988) Dissertation, Universität Tübingen.

Schuster,W., Hiesel,R., Wissinger,B., Schobel,W. and Brennicke,A. (1988) *In Shaw,C. (ed.), Plant Molecular Biology—A Practical Approach.* IRL Press, Oxford, pp. 79–102.

Shinozaki,K., Ohme,M., Tanaka,M., Wakasugi,T., Hayashida,N., Matsubayashi,T., Zaita,N., Chungwongse,J., Obokata,J., Yamaguchi-Shonozaki,K., Ohto,C., Torazawa,K., Meng,B.Y., Sugita,M., Deno,H., Kamogashira,T., Yamada,K., Kusuda,J., Takaiwa,F., Kato,A., Tohdoh,N., Shimada,H. and Sugiura,M. (1986) *EMBO J.*, **5**, 2043–2049.

Simpson,L. and Shaw,J. (1989) *Cell*, **57**, 355–366.

Stuart,K. (1989) *Parasitol. Today*, **5**, 5–8.

Wisdom,G.B. and Orsi,B.A. (1969) *Eur. J. Biochem.*, **7**, 223–230.

Zalkin,H. (1985) *Methods Enzymol.*, **113**, 282–287.

Received on October 19, 1989