Identification of editing positions in the *ndh*B transcript from maize chloroplasts reveals sequence similarities between editing sites of chloroplasts and plant mitochondria

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ABSTRACT

A comparison of the nucleotide sequences from genomic DNA and cDNA of the *ndh*B gene from maize chloroplasts shows that the *ndh*B transcript is edited by C-to-U transitions at six positions which appear to exist as editing sites also in the chloroplast *ndh*B genes from rice and tobacco but not from liverwort. In order to identify possible sequence determinants necessary for editing, the sequences surrounding the newly identified *ndh*B and previously identified *ndh*A editing sites were compared with each other and with editing sites observed in plant mitochondrial transcripts. Among the chloroplast editing sites two closely positioned *ndh*B sites show similarity by sharing a common octanucleotide. The existence of the identical octanucleotide in the *ndh*J gene whose transcript is not edited at the respective position, shows, however, that this octanucleotide is not sufficient to elicit the editing process. On the other hand, several of the chloroplast editing sites show sequence similarities with certain sets of consensus sequences reported earlier for editing sites of plant mitochondria. This supports the view that the editing processes of both plant organelles share common components and/or mechanistic steps and that the consensus sequences are part of the determinants necessary for editing.

INTRODUCTION

The nucleotide sequences of mRNAs can, as a result of editing processes, deviate from their respective gene sequences. This observation was originally reported for kinetoplast DNA encoded transcripts of trypanosomes, in which U residues are inserted or deleted with the aid of guide RNAs (1,2).

Subsequently, editing was also observed for a small number of nuclear mRNAs (3,4,5), for mitochondrial transcripts of the slime mold *Physarum* (6) and for plant mitochondrial mRNAs (7,8,9), which are particularly abundant in editing sites (10). In the latter case editing causes mainly C-to-U and to a lesser extent U-to-C

transitions. Editing in the chloroplast genetic system was detected only recently in the transcript of the maize *rpl2* gene in which the AUG initiator codon is created by a C-to-U transition from an ACG codon (11). This was followed by a similar observation for the initiator codon of the *psb*L gene from tobacco chloroplasts (12). That chloroplast editing is not restricted to the rare cases where nonfunctional initiator codons are encoded by the respective genes was then shown for the transcript of the maize *ndh*A gene which contains four internal editing positions (13).

Nothing is, however, known about the components and mechanistic steps involved in the chloroplast editing process. A straightforward assumption would imply that the sequences surrounding the editing positions function as determinants for eliciting the editing process by interacting either directly with the editing machinery or indirectly by attracting guide RNAs as is already known for the editing process in trypanosomes (1,2,14). The possibility that certain sets of consensus sequences act as editing determinants has been suggested for several plant mitochondrial editing sites (15, 16) and should also be taken into account for chloroplast editing. As a first step in this direction, identification of additional chloroplast editing sites appeared necessary. Here we report the existence of six internal editing positions in the transcript of the *ndh*B gene from maize chloroplasts, which together with the previously identified editing sites of the *ndh*A transcript allows a comparison of ten internal chloroplast editing sites. Similarity among the chloroplast editing sites could be detected between two of the *ndh*B encoded sites, which share a common octanucleotide. This might be part of the sequence determinants necessary for editing of the two sites. The fortuitous existence of the same octanucleotide in the *ndh*J transcript where no editing is observed, excludes its function as a complete determinant. Surprisingly, however, several of the chloroplast editing sites show sequence similarities with certain sets of consensus sequences reported for editing sites of plant mitochondrial transcripts (15, 16). This, together with other features of the chloroplast editing system, provides suggestive evidence that the editing processes of both plant organelles share common components and/or mechanistic steps.

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MATERIALS AND METHODS

Isolation of organelles

Intact chloroplasts were isolated from 10-day-old green maize seedlings by Percoll gradient centrifugation as described (17). Mitochondria were isolated from 6-day-old etiolated maize coleoptiles by differential centrifugation and sucrose gradient centrifugation (18).

Nucleic acids preparation

Nucleic acids from purified chloroplasts and mitochondria were prepared by guanidinium hydrochloride and CsCl gradient centrifugation (19) as described previously (13).

cDNA synthesis and amplification

RNA was reverse transcribed with a random primer mixture using avian myeloblastosis reverse transcriptase as described (13). DNA and cDNA were amplified in the presence of 1.5 mM $MgCl_2$ according to a standard protocol with 32 cycles of 93°C (for 30 sec), 55°C (for 1 min) and 72°C (for 1 min) with a 2-min extension at 93°C of the first cycle and a 7-min extension at 72°C of the final cycle. Amplification products were separated on 1.5% agarose gels, transferred onto a DEAE membrane, eluted with NET buffer (1.0 M NaCl, 0.1 mM EDTA, 20 mM Tris/HCl pH 8.0) at 65°C for 1 hour, extracted twice with phenol/chloroform saturated with TE buffer (10mM Tris/HCl pH 8.0, 1 mM EDTA) and precipitated with 2.5 volumes of ethanol.

Direct sequencing of amplification products

Gel purified amplification products were sequenced directly by a modified chain termination method (20).

List of oligonucleotides

Oligonucleotides used for PCR and sequencing were synthesized on a DNA synthesizer (modell No. 394; Applied Biosystems). The positions of primers nb1 to nb9 are shown in Figure 2. The sequences and positions of the coxII specific primers A and B were described previously (11).

nb1: 5'-CGAAACAATCGGGACTTTTCGG-3'
nb2: 5'-GGCGGAACAGATCTACTAATTC-3'
nb3: 5'-GCTCGTAAGGAGTCCTATTG-3'
nb4: 5'-ATCCTGCATAATCTCGAATG-3'
nb5: 5'-CAAGGAGATTCCCCAATATC-3'
nb6: 5'-CAATTGAAATTCCTGGGGAG-3'
nb7: 5'-AGACCGTAGATCTCTCTTGG-3'
nb8: 5'-ATGTACTCTACGGATAGAGG-3'
nb9: 5'-GGGGGAGATCGAGCTTCAAG-3'

RESULTS AND DISCUSSION

The ndhB genes of the maize plastome

Identical copies of the ndhB gene are located on each of the inverted repeat regions, IR_A and IR_B, of the plastomes of higher plants (Figure 1A). This situation, which had been known for the plastomes of tobacco (21), liverwort (22) and rice (23), has been confirmed by the present work for the maize plastome. The peptides encoded by the ndhB gene and other chloroplast ndh genes are homologous to subunits of mitochondrial NADH-ubiquinone reductase (24,25) which are encoded by mitochondrial or nuclear nad genes. The existence of the chloroplast ndh genes lends strong support to the postulation of a respiratory chain located in chloroplasts (i.e., chlororespiration, for review see reference 26). As shown in Figure 1B, the ndhB gene is preceded
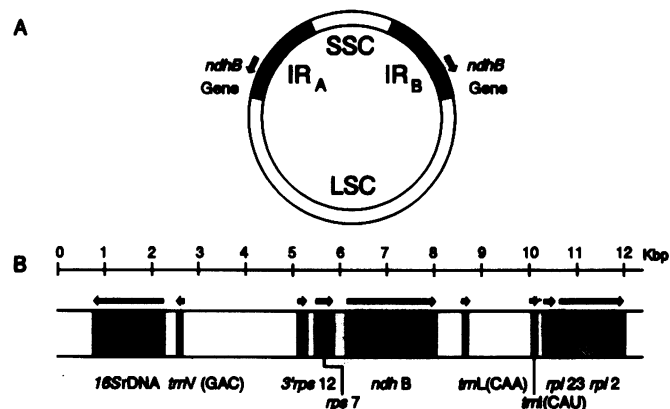

Figure 1. Position of the ndhB gene of the maize plastome. In (A) the location and copy number of the ndhB gene in relation to the inverted repeat regions IR_A and IR_B and to the small and large single copy regions (SSC and LSC), respectively, are shown. In (B) the position of the ndhB gene within the inverted repeat region is depicted. Introns are marked by striations. The direction of transcription is indicated by arrows.

in the same orientation and in close proximity by the rps7 gene and the 3'exon of the rps12 gene. This suggests that the three units are cotranscribed in the form of a larger transcript as is common for other gene clusters occurring in the plastomes of higher plants (27). In Figure 2 the nucleotide sequence of the two exons of the ndhB gene together with the deduced amino acid sequence of the encoded peptide is depicted. The intron/exon borders which originally had been inferred from an alignment with the rice ndhB sequence (23) have been confirmed experimentally during the course of this work by sequence analysis of the cDNA (data not shown).

The ndhB transcript is edited at six positions

Figure 2 shows the position of the primer pair nb1/nb2 which was used for amplification of a ndhB cDNA obtained by random priming of total chloroplast RNA with avian myeloblastosis reverse transcriptase. As depicted in Figure 3, the mobility of the amplification product derived from the chloroplast cDNA (lane b) is in accordance with the expected size of 1650 bp which, due to the absence of the intron, is 703 bp shorter than the genomic amplification product of 2353 bp (lane a). No products are formed in controls without nucleic acids (lane c and f).

A primer pair specific for amplification of the mitochondrial coxII gene (11, 13) leads to the expected 514 bp product with mitochondrial cDNA (lane e), but not with chloroplast cDNA (lane d). This control shows that the chloroplast cDNA is free of mitochondrial cDNA contaminants. The possibility can therefore be excluded that the cDNA observed in lane b is derived from ndhB sequences transferred from the chloroplast genome to the mitochondrial genome, as has been observed for this and other chloroplast genes (28,29). The editing sites identified in the chloroplast cDNA sequence cannot originate from mitochondrial editing.

Sequencing of the amplification products derived from either genomic DNA or cDNA was performed directly with the products of lanes a and b of Figure 3, using the primers nb1 to nb9 as shown in Figure 2. This allowed identification of six positions I to VI shown in Figure 4 where the cDNA deviates from the genomic DNA by C-to-T transitions (site IV) or by G-to-A transitions (sites I, II, III, V and VI, due to the polarities
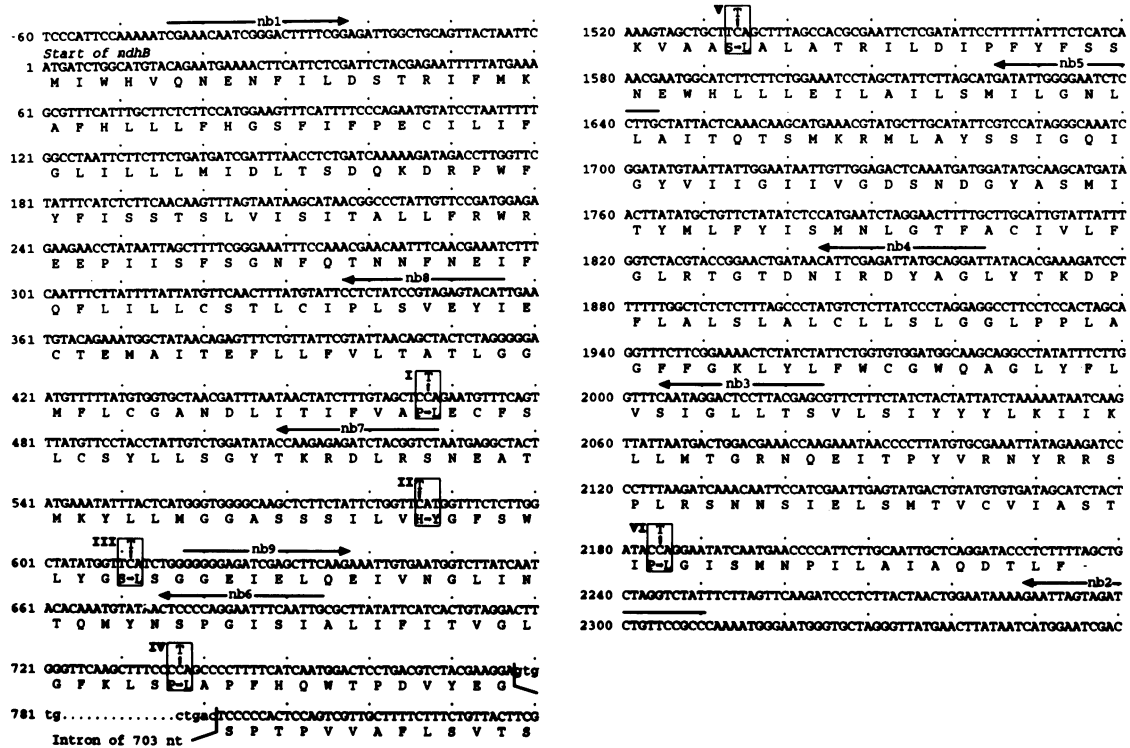
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                 ────nb1────▶
-60 TCCCATTCCAAAAATCGAAACAATCGGGACTTTTCGGAGATTGGCTGCAGTTACTAATTC
Start of ndhB
  1 ATGATCTGGCATGTACAGAATGAAAACTTCATTCTCGATTCTACGAGAATTTTTATGAAA
    M  I  W  H  V  Q  N  E  N  F  I  L  D  S  T  R  I  F  M  K

 61 GCGTTTCATTTGCTTCTCTTCCATGGAAGTTTCATTTTCCCAGAATGTATCCTAATTTTT
    A  F  H  L  L  L  F  H  G  S  P  I  F  P  E  C  I  L  I  F

121 GGCCTAATTCTTCTTCTGATGATCGATTTAACCTCTGATCAAAAAGATAGACCTTGGTTC
    G  L  I  L  L  L  M  I  D  L  T  S  D  Q  K  D  R  P  W  F

181 TATTTCATCTCTTCAACAAGTTTAGTAATAAGCATAACGGCCCTATTGTTCCGATGGAGA
    Y  F  I  S  S  T  S  L  V  I  S  I  T  A  L  L  F  R  W  R

241 GAAGAACCTATAATTAGCTTTTCGGGAAATTTCCAAACGAACAATTTCAACGAAATCTTT
    E  E  P  I  I  S  F  S  G  N  F  Q  T  N  N  F  N  E  I  F
                                          ◀────nb8────
301 CAATTTCTTATTTTATTATGTTCAACTTTATGTATTCCTCTATCCGTAGAGTACATTGAA
    Q  F  L  I  L  L  C  S  T  L  C  I  P  L  S  V  E  Y  I  E

361 TGTACAGAAATGGCTATAACAGAGTTTCTGTTATTCGTATTAACAGCTACTCTAGGGGGA
    C  T  E  M  A  I  T  E  F  L  L  F  V  L  T  A  T  L  G  G
                                          I□
421 ATGTTTTTATGTGGTGCTAACGATTTAATAACTATCTTTGTAGCTCCAAATGTTTCAGT
    M  F  L  C  G  A  N  D  L  I  T  I  F  V  A [P-L] E  C  F  S
                                        ◀────nb7────
481 TTATGTTCCTACCTATTGTCTGGATATACCAAGAGAGATCTACGGTCTAATGAGGCTACT
    L  C  S  Y  L  L  S  G  Y  T  K  R  D  L  R  S  N  E  A  T
                                              II□
541 ATGAAATATTTACTCATGGGTGGGGCAAGCTCTTCTATTCTGGTTCATGGTTTCTCTTGG
    M  K  Y  L  L  M  G  G  A  S  S  S  I  L  V [H-Y] G  F  S  W
       III□                  ────nb9────
601 CTATATGGTCATCTGGGGGGGAGATCGAGCTTCAAGAAATTGTGAATGGTCTTATCAAT
    L  Y  G [E-S] S  G  G  E  I  E  L  Q  E  I  V  N  G  L  I  N
                                    ◀────nb6────
661 ACACAAATGTATAACTCCCCAGGAATTTCAATTGCGCTTATATTCATCACTGTAGGACTT
    T  Q  M  Y  N  S  P  G  I  S  I  A  L  I  F  I  T  V  G  L
                    IV□
721 GGGTTCAAGCTTTCCCCAGCCCCTTTTCATCAATGGACTCCTGACGTCTACGAAGGAgtg
    G  F  K  L  S [P-L] A  P  F  H  Q  W  T  P  D  V  Y  E  G  L

781 tg............ctgagtCCCCCACTCCAGTCGTTGCTTTTCTTTCTGTTACTTCG
Intron of 703 nt        S  P  T  P  V  V  A  F  L  S  V  T  S
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                    V□
1520 AAAGTAGCTGCTTCAGCTTTAGCCACGCGAATTCTCGATATTCCTTTTTATTTCTCATCA
     K  V  A  A [S-L] A  L  A  T  R  I  L  D  I  P  F  Y  F  S  S
                                            ◀────nb5────
1580 AACGAATGGCATCTTCTTCTGGAAATCCTAGCTATTCTTAGCATGATATTGGGGAATCTC
     N  E  W  H  L  L  L  E  I  L  A  I  L  S  M  I  L  G  N  L

1640 CTTGCTATTACTCAAACAAGCATGAAACGTATGCTTGCATATTCGTCCATAGGGCAAATC
     L  A  I  T  Q  T  S  M  K  R  M  L  A  Y  S  S  I  G  Q  I

1700 GGATATGTAATTATTGGAATAATTGTTGGAGACTCAAATGATGGATATGCAAGCATGATA
     G  Y  V  I  I  G  I  I  V  G  D  S  N  D  G  Y  A  S  M  I

1760 ACTTATATGCTGTTCTATATCTCCATGAATCTAGGAACTTTTGCTTGCATTGTATTATTT
     T  Y  M  L  F  Y  I  S  M  N  L  G  T  F  A  C  I  V  L  F
                         ────nb4────
1820 GGTCTACGTACCGGAACTGATAACATTCGAGATTATGCAGGATTATACACGAAAGATCCT
     G  L  R  T  G  T  D  N  I  R  D  Y  A  G  L  Y  T  K  D  P

1880 TTTTTGGCTCTCTCTTTAGCCCTATGTGTCTCTTATCCCTAGGAGGCCTTCCTCCACTAGCA
     F  L  A  L  S  L  A  L  C  L  L  S  L  G  G  L  P  P  L  A

1940 GGTTTCTTCGGAAAACTCTATCTATTCTGGTGTGGATGGCAAGCAGGCCTATATTTCTTG
     G  F  F  G  K  L  Y  L  F  W  C  G  W  Q  A  G  L  Y  F  L
     ────nb3────
2000 GTTTCAATAGGACTCCTTACGAGCGTTCTTTCTATCTACTATTATCTAAAAATAATCAAG
     V  S  I  G  L  L  T  S  V  L  S  I  Y  Y  Y  L  K  I  I  K

2060 TTATTAATGACTGGACGAAACCAAGAAATAACCCCTTATGTGCGAAATTATAGAAGATCC
     L  L  M  T  G  R  N  Q  E  I  T  P  Y  V  R  N  Y  R  R  S

2120 CCTTTAAGATCAAACAATTCCATCGAATTGAGTATGACTGTATGTGTGATAGCATCTACT
     P  L  R  S  N  N  S  I  E  L  S  M  T  V  C  V  I  A  S  T
     VI□
2180 ATACCAGGAATATCAATGAACCCCATTCTTGCAATTGCTCAGGATACCCTCTTTTAGCTG
     I [P-L] G  I  S  M  N  P  I  L  A  I  A  Q  D  T  L  F  -
                                            ◀────nb2────
2240 CTAGGTCTATTTCTTAGTTCAAGATCCCTCTTACTAACTGGAATAAAAGAATTAGTAGAT

2300 CTGTTCCGCCCAAAAATGGGAATGGGTGCTAGGGTTATGAACTTATAATCATGGAATCGAC
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Figure 2. Nucleotide sequence and deduced amino acid sequence of the two *ndh*B exons including the intron boundery sites. The positions and orientations of the primers nb1 (5′primer) and nb2 (3′primer) used for PCR and sequencing and of the additional sequencing primers nb3 to nb9 are indicated by horizontal arrows. The six editing positions I to VI where C-to-U transitions alter the amino acid sequence are boxed.

of the used sequencing primers). The positions of the six editing sites within the sequence of the *ndh*B coding strand and with respect to the encoded amino acids are indicated in Figure 2. Five of the affected *ndh*B triplets are edited at the second codon position as was observed for all chloroplast editing sites identified previously (11, 12, 13). A first codon position is involved for the CAT (His) to TAT (Tyr) transition of editing site II. This excludes a frameshift and/or translation dependent mechanism of editing which would have been an attractive possibility if always the same of the three codon positions was to be the subject of editing.

Editing restores codons for conserved amino acids

An alignment of amino acid sequences encoded by the mRNA sequences surrounding the *ndh*B editing sites with homologous sequences from rice (23), tobacco (21) and liverwort (22) chloroplasts but also with the homologues from the cyanobacterium Synechococcus 7942 (30) and from three plant mitochondrial *nad*2 encoded peptides is depicted in Figure 5. The amino acids resulting from editing in maize are in all cases conserved in liverwort chloroplast by the respective codons existing already at the gene level. On the other hand, the rice and tobacco sequences in all cases encode the amino acids encoded by the unedited transcript in maize, which suggests that all the editing sites exist also in the *ndh*B transcripts of rice and tobacco. The alignment with the cyanobacterium homologue indicates conservation of the edited amino acid residues from maize (and of the genomically encoded residues from liverwort chloroplast) for sites I, II and III. Alignment of the regions around the editing positions of the *ndh*B encoded peptides with the homologous plant mitochondrial *nad*2 encoded peptides shows
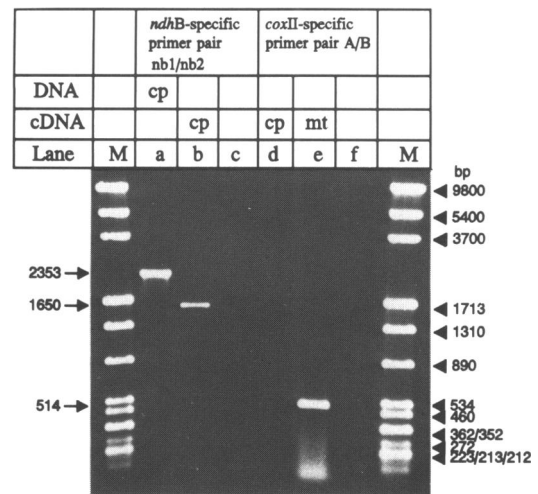
similar or even identical amino acid residues as compared to the edited maize residues. The transcripts for the mitochondrial peptide from *Oenothera* contain 36 editing positions (31), of which two coincide with chloroplast editing sites. These are

	*ndh*B-specific primer pair nb1/nb2			*cox*II-specific primer pair A/B			
DNA	cp						
cDNA		cp		cp	mt		
Lane	M a	b	c	d	e	f	M

bp markers (right): ◀ 9800, ◀ 5400, ◀ 3700, ◀ 1713, ◀ 1310, ◀ 890, ◀ 534/460, ◀ 362/352, ◀ 277, ◀ 223/213/212

markers (left): 2353→, 1650→, 514→

Figure 3. Amplification products obtained by PCR with the primer pair nb1/nb2 specific for the *ndh*B gene (lanes a, b and c) and primer pair A/B specific for the mitochondrial *cox*II gene (lanes d, e and f). Amplifications were carried out either with DNA from maize chloroplasts (lane a) or with cDNA obtained by reverse transcription of RNA from maize chloroplasts (lanes b and d) or from maize mitochondria (lane e). The positions and expected lengths in base pairs of the amplification products are marked by horizontal arrows at the left. The positions of the length markers (lanes M) are indicated by arrowheads at the right. Controls using buffer instead of DNA were run in lanes c and f.

editing site V and VI, existing also in the homologous positions of the wheat (32) (and as expected not in liverwort (33)) mitochondrial *nad2* transcript. However, in contrast to the UCA (Ser) to UUA (Leu) transition caused in editing site V of maize
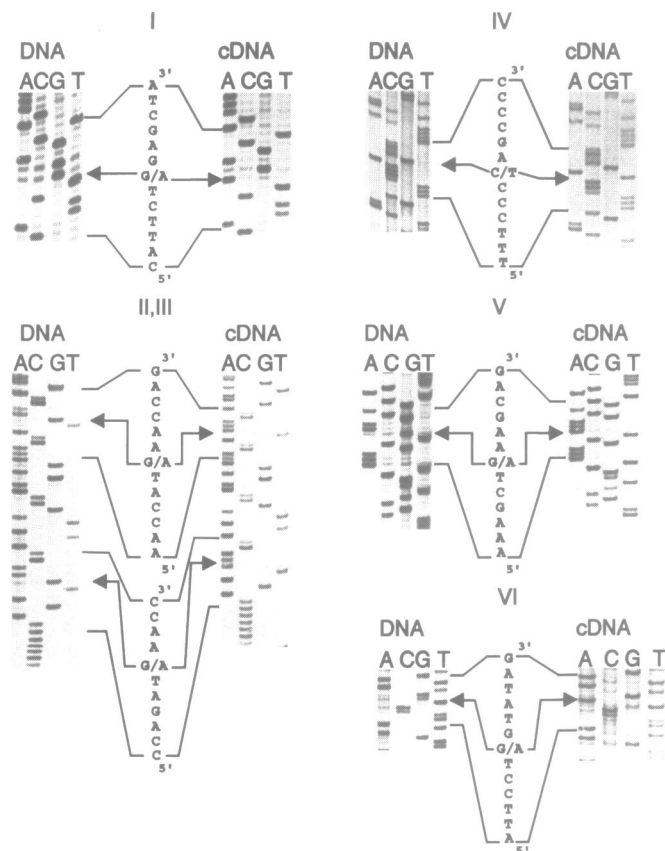

Figure 4. Identification of editing positions by comparison of the *ndh*B nucleotide sequences obtained either with amplified cDNA or amplified plastome DNA as template. The 2353- and 1650 bp amplification products (Figure 3, lanes a and b) were isolated and sequenced directly by a modified chain termination method using primers nb7 for editing position I, nb6 for editing positions II and III, nb9 for editing position IV, nb5 for editing position V and nb2 for editing position VI. The editing positions where chloroplast DNA and cDNA differ are marked by arrows. Because of the polarity of the primers nb2, nb5, nb6 and nb7 the corresponding autoradiograms show sequences complementary to the *ndh*B transcript.

chloroplast (Figure 2) a UCU (Ser) to UUU (Phe) transition results from the mitochondrial editing. A similar situation exists for editing site VI where in maize chloroplasts a CCA (Pro) to CUA (Leu) transition occurs whereas the homologous *Oenothera* and wheat mitochondrial editing sites show an UCC (Ser) to UUC (Phe) transition (see also following section and Figure 7C and E). Thus, inspite of the homology of these editing sites between chloroplasts and mitochondria, codons for different amino acids result from the editing events.

Editing at homologous positions has already been observed previously for the maize chloroplast *ndh*A transcript and the plant mitochondrial *nad1* transcripts (13). The present finding of two other editing sites common to the two plant organelles adds further support to the suggestion that the editing processes in both plant organelles share common components and/or mechanistic steps. This suggestion gains even further support from the observation that no transition from alanine codons (GCN) to valine codons (GUN) have been observed so far in both plant organellar editing systems. The existence of several positions in the *ndh*A and *ndh*B transcripts where valine codons exist in the liverwort sequence and alanine codons in the sequences from maize and other chloroplast species (see for instance the position directly adjacent to editing site IV of Figure 5) was highly suggestive for these transitions to occur; however, the cDNA sequences of all these potential editing sites obtained so far clearly disproved this suggestion (see for example the sequence pattern of editing site IV depicted in Figure 4). This apparent absence of the alanine to valine transition in the so far identified editing positions of chloroplasts and in plant mitochondria where almost 300 editing sites have been reported (10) suggests that the GCN-to-GUN transition is probably 'forbidden' as editing event in both plant organelles for mechanistic reasons.

Two *ndh*B editing sites share common sequences

As outlined in Figure 6 the closely positioned editing sites II and III are contained in a common octanucleotide sequence which is preceded—though with a different spacing of two and one nucleotides respectively—by a common tetranucleotide. The latter is preceded directly or separated by two G residues by a common pentanucleotide. This situation is conserved in rice and tobacco (also depicted in Figure 6) but not in the liverwort chloroplast sequence. The common sequences are not in the same reading frame which, as already mentioned above, is a result of the fact that editing position II involves a first codon position and editing position III a second codon position.
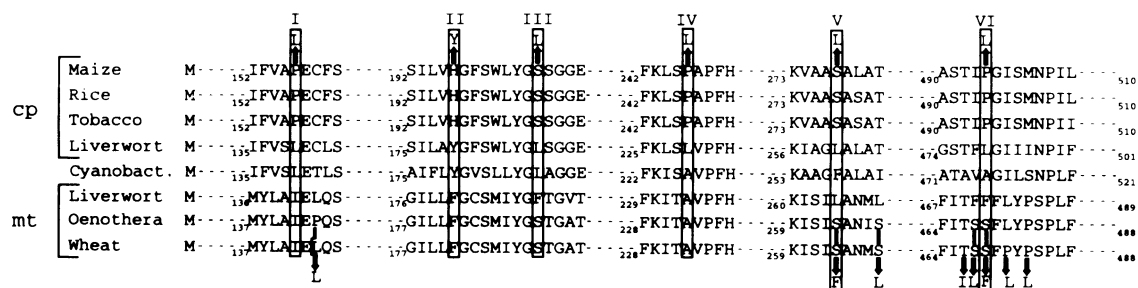

Figure 5. Alignment of the amino acid sequences encoded by the maize *ndh*B gene with homologous sequences from rice, tobacco, and liverwort chloroplasts, from a cyanobacterium and with the peptides encoded by the mitochondrial *nad2* genes from liverwort, *Oenothera* and wheat. Amino acid substitutions resulting from editing are marked by small vertical arrows.

It is tempting to speculate that the common sequences shared by the two editing sites function as determinants necessary to elicit the editing process similar to a proposal made for plant mitochondrial editing sites (15, 16). In order to test this possibility a computer aided screening for the octanucleotide sequence in the maize plastome was carried out which led to the detection of the identical octanucleotide sequence in the *ndh*J gene. Analysis of the *ndh*J cDNA sequence however, showed that no editing occurs in the *ndh*J transcript at this position. This observation excludes a function of the octanucleotide as an absolute determinant but leaves the possibility that it functions in conjunction with additional sequences such as the preceding common tetranucleotide and pentanucleotide, which are not present in the *ndh*J transcript. This possibility remains to be tested
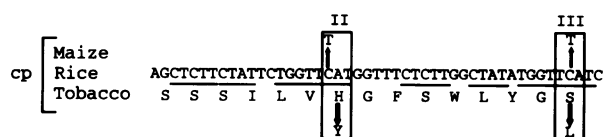
Figure 6. Identical sequences surrounding the editing sites II and III of the maize *ndh*B gene. The positions where editing leads to C-to-U transitions are marked by arrows. The sequence identities between the two sites are marked by underlining. The identical sequence is also found at the homologous position in rice and tobacco chloroplast *ndh*B genes.
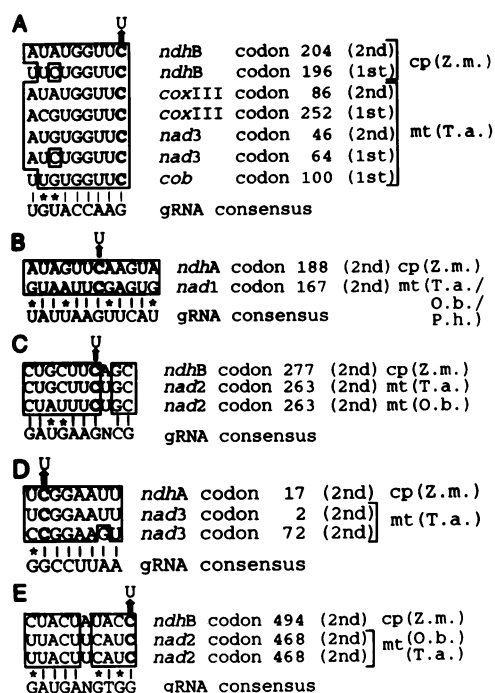
Figure 7. Sequence similarities around editing sites of maize chloroplast (Z.m.), wheat (T.a.), *Oenothera* (O.b.) and *Petunia* (P.h.) mitochondrial transcripts. The positions where C residues are edited to U residues are indicated by arrows. The position of the edited nucleotide (1st or 2nd) within the modified codon is also given. Nucleotide sequences which could be involved in base pairing with putative guide RNAs are boxed. The occurence of G-U base pairs is indicated by asterisks. The codons 188 and 357 of the maize *ndh*A transcript coincides with the editing positions reported previously (13). Likewise the codons 196, 204, 277 and 494 of the maize *ndh*B transcript correspond to the editing positions II, III, V and VI (see Figures 2 and 5).

experimentally in an in vitro editing system, which needs to be developed.

Several chloroplast editing sites show similarities with sequences common to plant mitochondrial editing sites

The observation reported in the previous section encouraged us to extend the sequence comparison of chloroplast editing sites to the consensus sequences reported for plant mitochondrial editing sites (15,16), which have been suggested as possibly reflecting the existence of guide RNAs (15). As outlined in Figure 7 six of the chloroplast *ndh*A and *ndh*B editing sites share common sequences with mitochondrial editing sites. Interestingly, these sequence similarities are not restricted to homologous sequences such as the three pairs of common editing sites between the *ndh*A/*nad*1 (13) and *ndh*B/*nad*2 (this work; see section above) depicted in Figures 7B, C and E, respectively. As demonstrated in Figures 7A and D, sequence similarities occur also between the editing sites of nonhomologous chloroplast and mitochondrial transcripts such as from chloroplast *ndh*A/B and the mitochondrial *nad*3/*cox*III/*cob* genes. While most of the similarities are observed within the monocots (maize chloroplast versus wheat mitochondria (15,16,32,34)) three *Oenothera* (31,35) (Figure 7B, C and E) and one *Petunia* (36) (Figure 7B) mitochondrial editing sites are also included.

As also outlined in Figure 7, the sequence similarities allow the deduction of putative guide RNA sequences which would be complementary to the respective editing sites. The existence of A/G and U/C substitutions observed at several positions of the shared sequences, which would imply UG base pairing between the editing sites and the hypothetical guide RNAs provides additional support to this suggestion as pointed out earlier for the mitochondrial sequences (15). However, attempts to isolate and characterize guide RNAs from plant mitochondria have not as yet met with success (10). In view of our present observations the existence of chloroplast guide RNAs remains an option seriously to be considered for experimental exploration. However, a functioning of the common sequences as determinants for direct interaction with the editing apparatus should be regarded as an attractive alternative.

In conclusion, the following observations strongly suggest that the editing processes of both plant organelles share common components and/or mechanistic steps:

1. C-to-U transitions are found in chloroplast transcripts and the same transition predominates in plant mitochondria.

2. Common editing sites exist at homologous positions of the chloroplast *ndh*A and B and the plant mitochondrial *nad*1 and 2 transcripts.

3. Common sequences are shared between editing sites of homologous and even nonhomologous transcripts of the two organelles.

4. The apparent absence of chloroplast editing in liverwort is paralleled by the absence of editing in liverwort mitochondria.

5. The frequency of codon transitions corresponds to the situation observed for plant mitochondria:

Absence of Ala→Val and Thr(ACC)→Ile(AUC) transitions.
High frequency of Ser→Phe, Ser→Leu and Pro→Leu transitions.

ACKNOWLEDGEMENTS

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