RNA Editing in the Cytochrome b Locus of the Higher Plant Oenothera berteriana Includes a U-to-C Transition

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RNA editing in the cytochrome b locus of *Oenothera berteriana* mitochondria modified a number of cytidine nucleotides to uridines, mostly altering codon identities. One nucleotide alteration involved a reverse modification changing a genomic thymidine to a cytidine in the cDNA sequence. The enzymatic editing activity in higher-plant mitochondria thus appears to be able to catalyze the interconversion of pyrimidines in both directions at specific nucleotides in the mRNA template.

Mitochondrial transcripts in the higher plant Oenothera berteriana are edited by individual nucleotide alterations modifying the genomically encoded amino acid sequences to proteins better conserved in evolution (12). The loci investigated to date include the mRNAs for cytochrome oxidase subunits II (coxII) and III (coxIII), NADH dehydrogenase subunits 1 (nad1) and 3 (nad3), and ribosomal protein S14 (rps14; R. Hiesel, W. Schuster, B. Wissinger, and A. Brennicke, unpublished observations). The nucleotide alterations previously observed exclusively involve transitions from cytidines encoded by the genomic sequences to uridines. This report generalizes the process of RNA editing in higher-plant mitochondria to include both types of transitions between pyrimidines and identifies a reverse editing event in the cytochrome b mRNA of O. berteriana.

To analyze the extent of RNA editing in the cytochrome b locus of O. berteriana, we compared the sequences of genomic and cDNA clones. Figure 1 shows sample gels of the comparison of part of the investigated coding region with regions of several edited nucleotides. Five of the six editing events involve cytidine-to-uridine alterations in the mRNA, similar to the editing events observed for other Oenothera gene loci. Editing event 3 in Fig. 1 involves the reverse alteration, with a change from a genomic T to a C in the cDNA sequence. Like most editing events in O. berteriana mitochondria, this modification altered the encoded amino acid, at this site from isoleucine to threonine (Fig. 2). In several instances, the arginine codon CGG has been found in plant mitochondrial genes in which the amino acid tryptophan is conserved in the corresponding protein sequences of other species and has appropriately been discussed as potentially coding tryptophan instead of arginine (7, 9, 11, 14). The RNA editing process now appears to change the cytidine residue in these codons to a uridine, thereby altering the codon and obliterating the need for a deviant genetic code in plant mitochondria. An example of a CGG-to-TGG alteration in the cDNA sequence was found in the analyzed region of the cytochrome b locus (Fig. 1 and 2). Furthermore, this and most other alterations in the cytochrome bcDNA sequence improved the degree of conservation between the deduced plant protein sequence and the homologous polypeptide sequences from other organisms (Fig. 3). However, not all edited nucleotides changed the encoded amino acid, as evidenced by a third-position change in a The six nucleotide modifications shown in Fig. 1 occurred clustered within a region of 86 nucleotides in the cytochrome b cDNA sequence, including the silent alteration and the T-to-C transition (Fig. 1 and 2). In the further downstream part of the cytochrome b locus, nucleotide alterations were spaced much further (up to 285 nucleotides) apart (Fig. 2). The overall frequency of nucleotide exchanges in the analyzed part of the cytochrome b locus in O. berteriana amounted to 1.3%, with 12 nucleotides edited of the 873 investigated. The percentage of altered cytidines calculates to 6.9%, equivalent to one nucleotide edition every 14.5 cytidines.

The editing process thus appears to select specific nucleotides for alteration that are determined not in a random process of evenly spaced modifications based solely on physical distances but rather in a process that must be determined by other specificities. These do not appear to be simple sequence motifs, since altered codons and the surrounding sequences differ completely between the individual editing events. The parameters determining an individual C (or U) to be edited in *O. berteriana* mitochondria are just as unclear as the specificity of the U insertion-excision process of RNA editing in trypanosome mitochondria, where uridines are inserted into or excised from the mRNA in specific sites and numbers (2, 3, 15, 16).

An enzyme-specific activity similar to that required for the editing process in *O. berteriana* mitochondria has been observed in mammalian cells and is responsible for altering tissue specifically one single cytidine to create a new termination codon. This modifying activity could be prepared from cellular extracts in an active state, maintaining in vitro the specificity for both site and reaction (8).

The activity involved in plant mitochondrial editing, however, must fulfill additional requirements, including the specific selection of many highly divergent sites and, with the observation of a U-to-C transition, the ability to catalyze both directions of the modification reaction. This latter requirement could be met either by two different moieties or by a single enzymatic complex. Such single enzymatic activities capable of performing both directions of pyrimidine transitions, albeit with highly asymmetric efficiencies, are found among enzymes involved in the metabolism of nucleotides such as cytidine deaminase and CTP synthetase (13, 17, 18). These enzymes catalyze both forward and backward reactions in establishing specific equilibria and

leucine CTC codon to a CTT codon that specifies the same amino acid (Fig. 1 and 2).

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FIG. 1. Comparison of genomic and cDNA sequences of the cytochrome b locus in O. berteriana mitochondria. Six nucleotide differences between the genome and mRNA-derived clones were found clustered within the region between nucleotides 286 and 372 (Fig. 2; 14). The altered nucleotides are indicated by numbered arrows in two gel loadings of the genomic sequence determined by controlled chemical modification and a corresponding cDNA sequencing gel derived by the chain termination method. The cDNA gel is shown upside down in the mirrored orientation for easier sequence alignment. Sequences and gels read 5' to 3' from top to bottom.

GG GGC TGC TTC CCT TAT ATC CAT GCT AAT GGG GCA AGT ATC TTT GTG GTT CAC GGG GGC TGG TTG CTC CGT TAT ATC CAT GCT AAT GGG GCA AGT ATG TTT TTC ATT GTG GTT TAC genomic cDNA L H T F R G L Y H A S Y S S P R E F V R C L G V V CTT CAT ATT TTT CGT GGT CTA TAT CAT GCG AGT TAT AGC AGT CCT AGG GAA TTT GTT CGG TGT CTC GGA GTT GTA CTT CAT ACT TTT CGT GGT CTA TAT TAT TAT CGG AGT TAT AGC AGT CCT AGG GAA TTT GTT TGG TGT CTT GGA GTT GTA I F L L M I V T A F T GG Y V L P W G Q M S F W G A ATC TTC CTA TTA ATG ATT GTG ACA GCT TTT ACA GGA TAC GTA CTA CTT CTG GGT CAG ATG AGC TTT TGG GGA GCT ATC TTC CTA TTA ATG ATT GTG ACA GCT TTT TTT GGA TAC GTA CTA CTT CGT GGT CAG ATG AGC TTT TGG GGA GCT T V I T S L A S A I P V V G D T I V T W L W G G F ACA GTA ATT ACA AGC TTA GCT AGC GCC ATA CCA GTA GTA GGA GAT ACC ATA GTG ACT TGG GTT TGG GGT GGT TTC ACA GTA ATT ACA AGC TTA GCT AGC GCC ATA CCA GTA GTA GGA GAT ACC ATA GTG ACT TGG GTT GG GGT GCT TTC S V D N A T L N R F F S L H H L L P F I L V G A S TCC GTG GAC AAT GCC ACC TTA AAT CGT TTT TTT AGT CTT CAT CAT TAT CTC CCC TTT ATT CTA GTA GGC GCC AGT TCC GTG GAC AAT GCC ACC TTA AAT CGT TTT TTT AGT CTT CAT CAT TAT TTA CTC CCC TTT ATT CTA GTA GGC GCC AGT L L H L A A L H Q Y G S S N P L G V H S E M D K I CTT CTT CAT CTG GCC GCA TTG CAT CAA TAT GGA TCT TCT AAT CCA TTG GGT GTA CAT TCA GAG ATG GAT AAA ATT CTT CTT CAT CTG GCC GCA TTG CAT CAA TAT GGA TCT TCT AAT CCA TTG GGT GTA CAT TCA GAG ATG GAT AAA ATT 753 S F Y P Y F Y V K D L V C W V A F A I F F S I W I TCT TTT TAC CCT TAT TTT TAT GTA AAG GAT CTA GTA GGT TGG GTA GCT TTT GCT ATC TTT TTT TCC ATT TGG ATT TCT TTT TAC CCT TAT TTT TAT GTA AAG GAT CTA GTA GGT TGG GTA GCT TTT GCT ATC TTT TTT TCC ATT TGG ATT
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FIG. 2. Comparison of cytochrome *b*-encoding genomic and cDNA nucleotide sequences for the region analyzed. Numbering starts from the first coding nucleotide (14). Edited nucleotides are boxed, with the cDNA-specified amino acids given underneath. The first six nucleotide differences are those shown in Fig. 1 and include a T-to-C modification, a silent C-to-T change, and a CGG codon alteration.

0e	c DNA		F	Y 1	r P	Y 🖶	W	I I	1	167
0e Hs Sc Nc	genomic	GGWLLRYMHANGASI YGWIIRYLHANGASI NGYILRYLHANGASI NGWLVRYLHSNTASI	AFLIVV AFFICL FFFMVM AFFFLV	/HLHI .FLHI IFMHM YLHI	EFRGLI EGRGLI AAKGLI EGRGMI	YHASYSSPREF YYGSFLYSE YYGSYRSPRVT YYGSYRAPRTL	VRCLGVVIFLLMIV TWNIGIILLLATMA LWNVGVIIFILTIA VWAIGTVILILMMA	FAFTGYVLPWGQMSFWGATV FAFMGYVLPWGQMSFWGATV FAFLGYCCVYGQMSHWGATV FAFLGYVLPYGQMSLWGATV	'ITSLASAIPVVC 'ITNLLSAIPYIC 'ITNLFSAIPFVC 'ITNLISAIPWIC	GDT GTD GND GQD
0e	c DNA								:	254
0e Hs Sc Nc	geno s ic	IVTWLWGGFSVDNA LVQWIWGGYSVDSP IVSWLWGGFSVSNP IVELHLGGFSVNNA	LNRFF LTRFF LIQRFF LNRFF	FSLHH FIFHH FALHY FALYH	HLLPFI FILPFI YLVPFI FVLLFI	ILVGASLLHLA IIAALATLHLL IIAAMVIMLHM ILVVLVLMYLI	ALHQY-GSSNPLGVI FLHET-GSNNPLGI ALHIH-GSSNPLGI VLYDIVGLSNPLGA	HSEMDKISFYPYFYVKDLV(TSHSDKITFHPYYTIKDAL(TGNLGRIPMHSYFIFKDLV1 LGNYDRIIFAPYYLFKDL11	;WVAFAIFFSIW] ;LLLFLLSLMTL [VFLFMLILALF [IFIFIYVLSSF]	IFY TLF VFY VFF
0e	c DNA					Y			Y	341
0e Hs Sc Nc	genomic	APNVLGHPDNYIPA SPDLLGDPDNYTLA SPNTLGHPDNYIPG MPNVLGDSENYIMA	IPMSTP IPLNTP IPLVTP IPMQTP	PPHIN PPHIN PASIN PPAIN	VPEWYI Kpewyi Vpewyi Vpewyi	FLPIHAILRSH FLFAYTILRSV LLPFYAILRSI LLPFDAILRSI	PDKAGGVAAIAPVF PNKLGGVLALLLSI PDKLLGVITMFAAI PNKLLGVIAMFSAI	ICLLALPFFKDMYVRSSSFI LILAMIPILHMSKQQSMMFI LVLLVLPFTDRSVVRGNTFI LAIMLLPITDLGRSKGLQFI	<pre>{PIHQGIFWLLL, {PLSQSLYWLLA, KVLSKFFFFIFV RPLSKFAFWAFV</pre>	ADC ADL FNF VNF
0e	c DNA			S	. :	372				
0e Hs Sc Nc	genomic	LLLGWIGCQPVEAP LILTWIGGQPVSYP VLLGQIGACHVEVP LILMKLGACHVESP	VTIGO TIIGO VLMGO TIELGO	QISPI QVASV QIATH QFSTI	IVFFLI VLYFT FIYFA IFYLS	FFA TIL YFL YFI				

FIG. 3. Demonstration that RNA editing in the cytochrome b mRNA in O. berteriana mitochondria alters the polypeptide encoded by the gene, compensating frequently for the genomic nucleotide sequence drift. The mRNA-specified protein is thus better conserved in evolution than is the genome-encoded polypeptide. Amino acid sequences deduced from *Oenothera* (Oe) genomic and cDNA nucleotides are aligned with the respective sequences from other organisms: humans (Hs; 1), *Saccharomyces cerevisiae* (Sc; 5), and *Neurospora crassa* (Nc; 4). The cDNA-encoded amino acids divergent from the genome-specified sequence are indicated by arrows.

could therefore carry out an activity similar to the bidirectional editing in plant mitochondria that alters most frequently C to U and occasionally U to C. Further investigation of the involved enzymatic activities and the necessary specificity factors will allow us to determine more conclusively the biochemical reactions and requirements of RNA editing in higher plant mitochondria.

After this report was completed, two papers reporting RNA editing in wheat mitochondria were published (6, 10). These observations of RNA editing in a monocot plant strengthen the suggestion that RNA editing occurs in many higher plants.

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