MOLECULAR AND CELLULAR BIOLOGY, May 1990, p. 2428–2431
0270-7306/90/052428-04$02.00/0

Vol. 10, No. 5

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

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Received 23 October 1989/Accepted 10 January 1990

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1 (*nad1*) and 3 (*nad3*), and ribosomal protein S14 (*rps14*; R. Hiesel, W. Schuster, B. Wissinger, and A. Brennicke, unpublished observations).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 *b* cDNA 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 leucine CTC codon to a CTT codon that specifies the same amino acid (Fig. 1 and 2).

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

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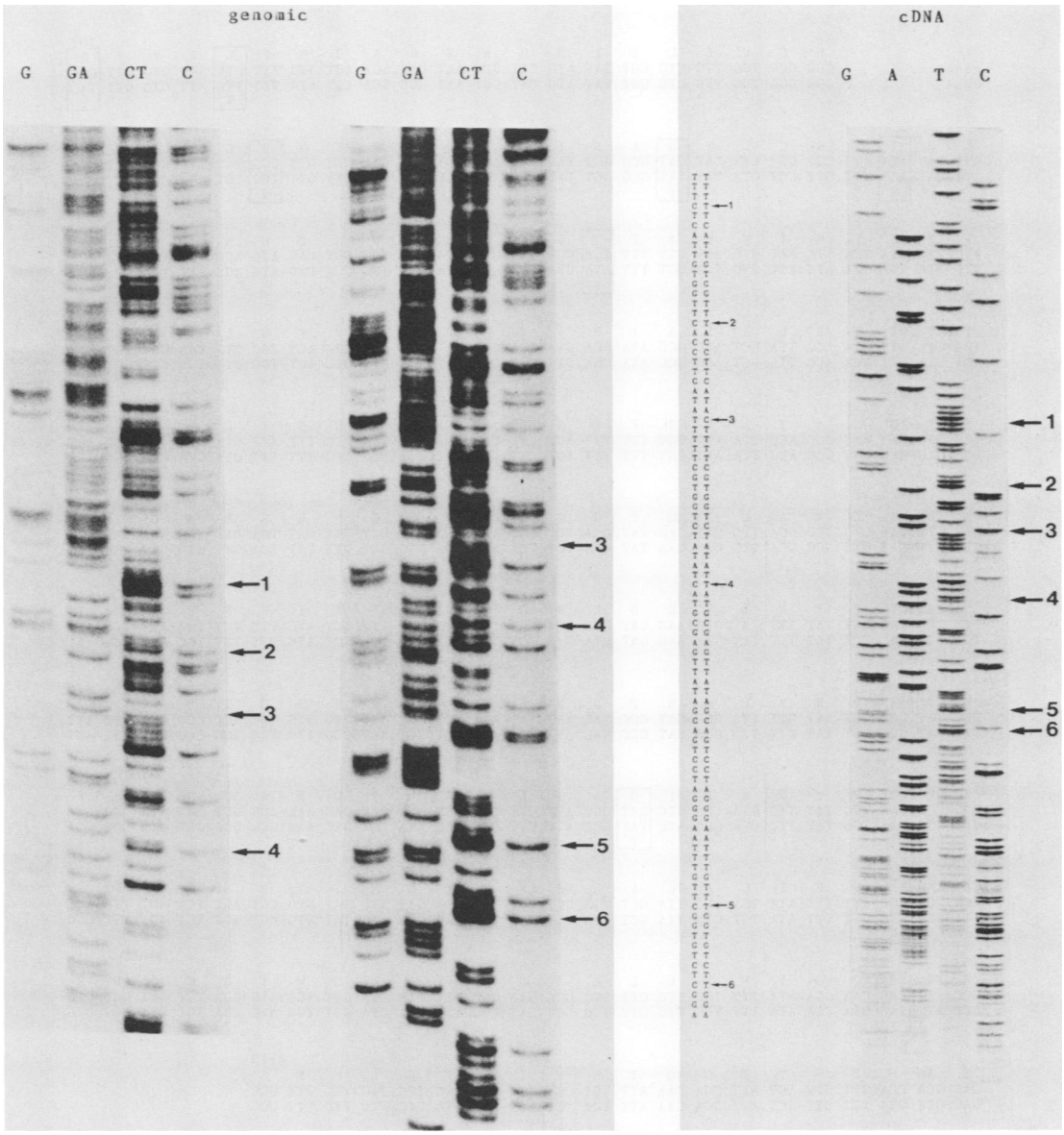

FIG. 1. Comparison of genomic and cDNA sequences of the cytochrome *b* locus in *O. berteriana* mitochondria. Six nucleotide differences between the genome and mRNA-derived clones were found clustered within the region between nucleotides 286 and 372 (Fig. 2; 14). The altered nucleotides are indicated 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.

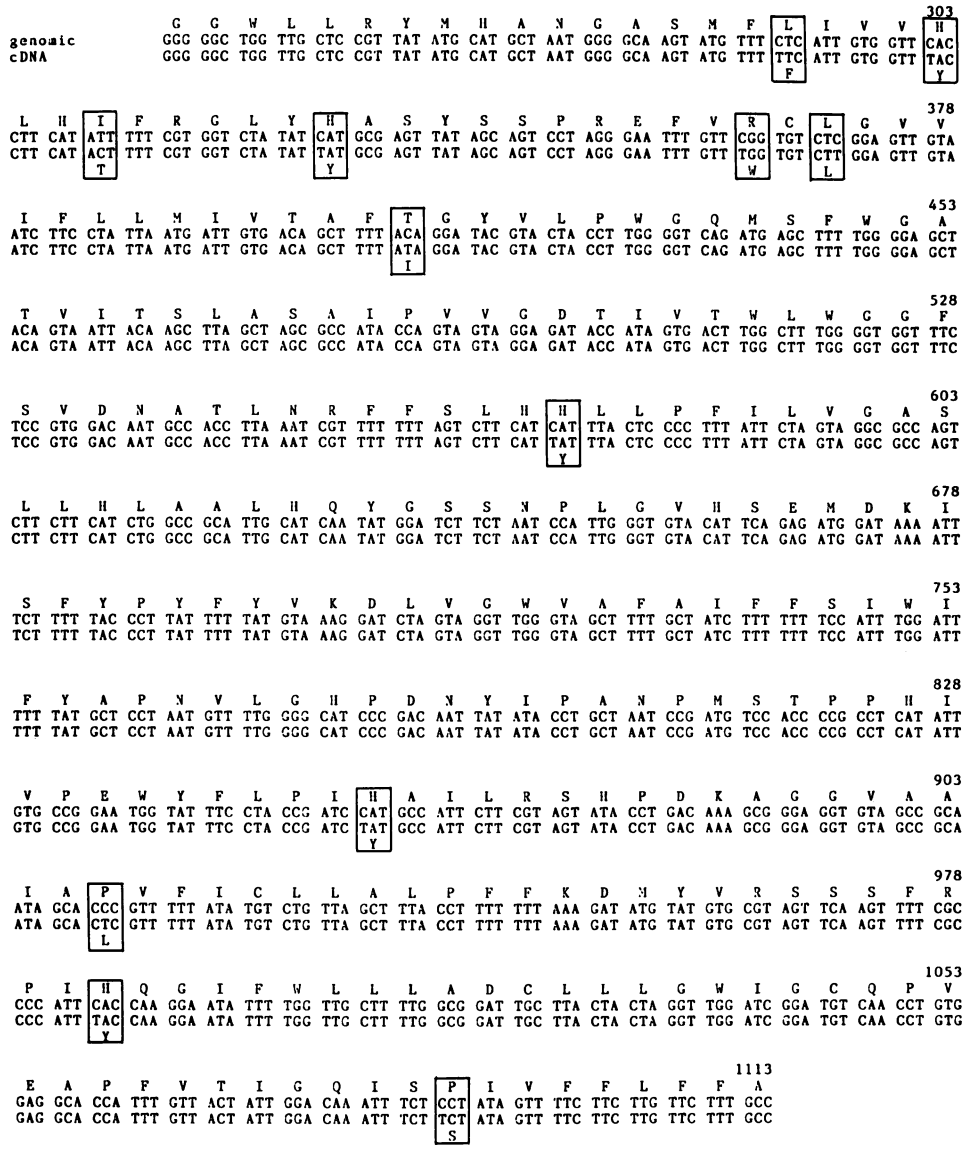

FIG. 2. Comparison of cytochrome *b*-encoding genomic and cDNA nucleotide sequences for the region analyzed. Numbering starts from the first coding nucleotide (14). Edited nucleotides are boxed, with the cDNA-specified amino acids given underneath. The first six nucleotide differences are those shown in Fig. 1 and include a T-to-C modification, a silent C-to-T change, and a CGG codon alteration.

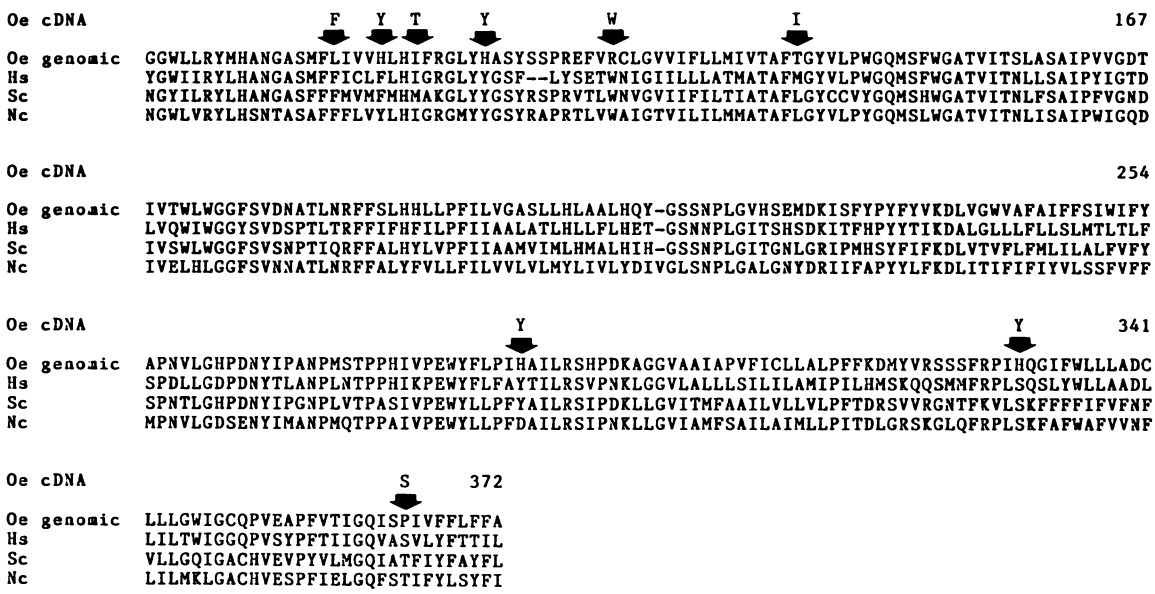

FIG. 3. Demonstration that RNA editing in the cytochrome *b* mRNA in *O. berteriana* mitochondria alters the polypeptide encoded by the gene, compensating frequently for the genomic nucleotide sequence drift. The mRNA-specified protein is thus better conserved in evolution than is the genome-encoded 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.

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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