RNA Editing of Cytochrome Oxidase Subunit III in Sunflower Mitochondria

Adolfo Saiardi and Carla Quagliariello

Dipartimento di Biologia Cellulare, Università della Calabria, 87030 Arcavacata di Rende, Italy

ABSTRACT

Direct sequencing of cytochrome oxidase subunit III (coxIII) mRNA with a specific primer confirms RNA editing in sunflower (Helianthus annuus) mitochondria. Six instances of mRNA editing could be verified, one of these specific to this species. All the editing events involve C to U transitions in the coxIII mRNA causing codon changes that lead to amino acids better conserved in evolution than those encoded in the genomic DNA. This observation confirms RNA editing to be widespread in higher plant mitochondria.

The term RNA editing is used to describe any process that results in the production of a messenger RNA with a nucleotide sequence different from its own DNA template. RNA editing seems to be operative in a wide range of biological systems likely as a specific control point of gene expression, though both the nature of the modification and the mechanisms proposed vary considerably.

The phenomenon was first discovered in the mitochondria of kinetoplastid trypanosomes (2, 18) where the mRNAs contain insertions or deletions of uridine residues relative to the sequence of their genes. Soon thereafter other examples of RNA editing were reported (reviewed in refs. 3 and 19): (a) a single precise, developmentally regulated cytosine to uridine change in mammalian apolipoprotein B mRNA; (b) insertion of one or more guanosine residues in paramyxovirus P mRNA; and (c) most recently, insertion of cytosine residues in Physarum polycephalum mitochondrial mRNAs (14).

RNA editing has also been described for several protein-coding genes in higher plant mitochondria (see ref. 20 for a review). All editing events described so far in the plant species investigated, primarily two monocots (wheat, maize) and two dicots (Oenothera, Petunia), involve a individual nucleotide alteration modifying the genome-encoded cytidines to uridines (4, 5, 8, 11) and in a few cases the genomic thymidines to cytidines (9, 17). Because most of the nucleotide conversions result in modifications of the codons which then specify amino acids that are conserved in the mitochondrial proteins of non-plant organisms, it appear that RNA editing in plant mitochondria plays a role in the conservation of protein sequences during evolution (8).

Moreover, as amino acid composition and peptide sequence analyses confirmed mRNA editing alterations at the protein level, this process seems to be essential for the correct expression of plant mitochondrial protein genes (1, 7). Recently, nucleotide and amino acid sequence comparisons of the positions shown to be edited in Oenothera (11) and wheat (9) coxIII cDNA led us to predict that editing is also required in sunflower (Helianthus annuus Gloriasol) mitochondria to synthesize an evolutionarily conserved, functional COXIII (16). To test these predictions we determined the sequence of a selected region in the coxIII mRNA from sunflower mitochondria by reverse transcriptase sequencing with a specific primer.

MATERIALS AND METHODS

Plant Material

Seeds of sunflower (Helianthus annuus) Gloriasol were provided by ISEA (Ancona, Italy). Gloriasol is a fertility-restored commercial hybrid seed line, based on CMS89, that carries the Helianthus petiolaris cytoplasm.

Isolation of Nucleic Acids

Mitochondrial DNA (mtDNA) and RNA (mtRNA) isolation from sunflower etiolated shoots has been described previously (16). The mtRNA was resuspended in sterile TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and an equal volume of 4 mM LiCl. After storing at 4°C for 12 h, the precipitated RNA was pelleted and washed with cold 2 mM LiCl, washed with 80% ethanol, and dried.

mRNA and mtDNA Sequencing

Forty nanograms of a coxIII-specific oligonucleotide primer (5' TGGGGCCAAATACCTCC 3', position 346 and 363 in Fig. 2 of ref. 16) was hybridized with 100 µg of total sunflower mtRNA in 15 µL of 50 mM KCl, 50 mM Tris-HCl, pH 8.3, and 8 mM MgCl2 and allowed to anneal for 1 h at 55°C. Sequencing was done by the dideoxy chain-termination method. Four microliters of 7.5 µM dNTP (minus dATP) (Sequenase Version 2.0, USB), 15 µCi of [α-32P]dATP (Amersham 3000 Ci/mmol; 1 Ci = 37 GBq), 15 units of avian myeloblastosis virus reverse transcriptase, 10 mM DTT, and 50 µg/mL actinomycin D were added to the annealing mixture and incubated at 37°C for 10 min. Subsequently 5 µL of

1 Supported by the Ministero della Ricerca Scientifica e Tecnologica (M.U.R.S.T., 60% and 40% Funds).

2 Abbreviations: coxIII, cytochrome oxidase subunit III; COXIII, coxIII polypeptide.
the labeling mix were distributed into each of four Eppendorf tubes containing 3 µL of termination mix (80 µM of each dNTP and 8 µM of ddNTP) (Sequenase Version 2.0, USB). The reactions were performed at 42.5°C for 45 min and chased by adding 1 µL of 0.2 mM dNTP to each Eppendorf tube and incubation at 42.5°C for 15 min. mtDNA sequencing is as given, or referred to, in Quagliariello et al. (16).

RESULTS AND DISCUSSION

In this communication we present the sequence of a selected region in the sunflower coxIII mRNA determined by reverse transcriptase sequencing using as primer a specific synthetic oligonucleotide. Comparing the mRNA sequence with the corresponding sequence from sunflower mitochondrial DNA (16), six cytidine to uridine modifications were identified clustered within a 72-nucleotide region of the sunflower coxIII mRNA. Representative mRNA and DNA sequence autoradiograms of the selected coxIII region are shown in Figure 1, as an example (others not shown). Tables I and II list sites of C to U editing within the investigated coxIII coding region and the effect of this editing on the predicted amino acid sequence of this protein. This compilation includes the two editing positions predicted on the basis of nucleotide and amino acid alignments (positions 97 and 102) with the corn coxIII mitochondrial genomic sequence (16). Amino acids at these positions in the COXIII polypeptide are highly conserved in nonplants while plants would show amino acid substitutions based on the genomic DNA sequence. These substitutions are eliminated by C to U editing occurring in the first position at these sites in the sunflower coxIII transcript.

Position 102 represents another example of the alteration that modifies a genomic arginine triplet CGG to UGG, where tryptophan is conserved in nonplant species polypeptides (Table II), as well as in monocot plants. A genomic TGG has been found in fact at the same position in the wheat (9), maize (15), rice (12) mitochondrial genomic sequences, whereas a genomic CGG codon is found at the same position in all the dicot coxIII genes analyzed so far (6, 10, 13). Editing at position 102, which does occur in sunflower, is probably also required in the other dicots coxIII mRNAs.

Four additional codons (Fig. 1 and/or Tables I and II) are found to be edited in the investigated coding region of coxIII (positions 82, 100, 104, and 105). At position 82 a CCT (Pro) codon is converted by a C to U transition in the second position to a CTT (Leu) codon. Incorporation of a leucine at

Table II. Amino Acid Comparison of the Protein Sequences Deduced from the Genomic Sequence of Sunflower (Su) with the Respectively Deduced Amino Acid Sequences from Oenothera (Oe) COXIII Polypeptide DNA-Encoded (10) and the Wheat COXIII Polypeptide Deduced from the cDNA Sequence (9)

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Table I. Comparison of Genomic and mRNA Sequences of coxIII in Sunflower Mitochondria

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**TABLES**

Table I. Comparison of Genomic and mRNA Sequences of coxIII in Sunflower Mitochondria

The amino acid specified after editing is given underneath the mRNA sequence.
position 82 improves conservation with nonplant COXIII polypeptides. Editing at this position also occurs in the wheat coxIII (9), whereas a genomic encoded CTT is present in Oenothera (10).

The nucleotide modification at position 100 of the sunflower coxIII mRNA, a CTT to TTT transition, seems not to be required in the other six known plant species (6, 9, 10, 12, 13, 15), where a genomic TTT codon was found at the same position and thus is unique to sunflower. The RNA editing events in the second positions of codons 104 and 105 also occur in the wheat coxIII mRNA and are probably also required in the other plant coxIII RNAs to maintain the functional conservation of the encoded polypeptide because they all share a genomic TCT codon at homologous sites. It would thus appear that those positions are evolutionarily conserved in plants.

These experimentally verified editing sites in the sunflower mitochondrial coxIII mRNA and the differences in the editing pattern found at homologous positions confirm the editing predictions based on nucleotide and amino acid sequence alignments (16) and reveal a number of additional nucleotide alterations at positions that are not highly conserved in non-plant COXIII polypeptides. Editing of the sunflower coxIII mRNA, therefore, improves the degree of similarity at the level of amino acid sequence for the COXIII polypeptide among higher plants as well as in comparisons between plants, fungi and animals. As final evidence for the uniformity of the COXIII loci-specified products in plant mitochondria direct protein sequencing will be necessary. These results confirm that RNA editing is a general feature of higher plant mitochondria.

ACKNOWLEDGMENTS

We thank Prof. Dr. A. Brennicke for helpful criticism of the manuscript. We are grateful to ISEA (Ancona, Italy) for providing seeds of Helianthus annuus Glorlasol.

LITERATURE CITED