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Plant Gene Register

The *cox1* Initiation Codon Is Created by RNA Editing in Potato Mitochondria[1]

Verónica Quiñones, Silvana Zanlungo, Loreto Holuigue, Simón Litvak, and Xavier Jordana*

Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago, Chile (V.Q., S.Z., L.H., X.J.); and Institut de Biochimie et Génétique Cellulaires, Centre National de la Recherche Scientifique, 1, rue Camille Saint Saëns, 33077 Bordeaux, France (S.L.)

Cyt *c* oxidase is one of the multisubunit complexes located in the inner mt membrane of eukaryotes. This enzyme catalyzes the transfer of electrons from Cyt *c* to molecular oxygen. In most organisms, subunits I, II, and III are encoded in the mt genome, whereas the other subunits are encoded in the nuclear genome and imported into the mitochondria (Bonen, 1991). We report here the nt sequence of the potato (*Solanum tuberosum*) mt gene *cox1*, encoding subunit I of Cyt oxidase (Table I). The potato single-copy *cox1* gene was found to be present on a previously isolated 5.1-kb *Eco*RI restriction fragment, downstream of a truncated *cob* pseudogene and a split gene coding for ribosomal protein S10 (Zanlungo et al., 1994, 1995). A similar *rps10-cox1* arrangement was also found in the pea mt genome (Knoop et al., 1995).

We subcloned 2.2-kb *Bam*HI, 1.4-kb *Sal*I/*Bam*HI, and 1.5-kb *Bam*HI/*Eco*RI DNA fragments into Bluescribe M13+, M13mp18, and/or M13mp19 vectors (for restriction map, see Zanlungo et al., 1995). Both strands of the *cox1* locus were completely sequenced by the dideoxy chain termination method. The potato *cox1* open reading frame is located 193 nts downstream of the *rps10* UGA stop codon, which is created by RNA editing of a CGA codon (Zanlungo et al., 1995). The alignment of *cox1* nt sequences from plant species shows strong conservation (93.8–96.9% similarity), which is indicative of a functional gene. However, the *cox1* gene of potato mitochondria contains an ACG codon at the position where *cox1* from both monocotyledonous and dicotyledonous plant species have an ATG initiation codon (Isaac et al., 1985; Bailey-Serres et al., 1986; Grabau, 1986; Hiesel et al., 1987; Kemmerer et al., 1989).

We consider here whether this ACG codon is converted to a functional AUG initiation codon by C to U RNA editing. RNA editing is required for maturation of mRNAs in plant organelles and occurs almost exclusively by C to U conversions. AUG initiation codons have been shown to be created by editing of ACG codons in chloroplast *rpl2* and *psbL* transcripts of maize and tobacco, respectively, and in the mt *nad1* mRNAs of wheat (Gray and Covello, 1993). To address this issue, cDNAs containing 117 nts upstream and 748 nts downstream of the ACG codon were obtained by reverse transcription of total mtRNA and PCR amplification between appropriate oligonucleotide primers. In eight of nine cloned and sequenced PCR-amplified cDNAs, an ATG Met codon was found instead of the Thr ACG codon, showing that a C to U editing creates the AUG initiation codon for the potato *cox1* mRNA. This RNA-editing event can be assumed to be essential for protein synthesis to proceed. The creation of the *cox1* initiation codon may be indicative of a regulatory role for the editing process whereby conversion of a nonfunctional to a translatable mRNA is achieved.

Four additional C to U RNA-editing events were detected in the first 90 codons of the *cox1* coding region that were analyzed by cDNA sequencing. One of these modifications, detected in seven of nine cDNA clones, gives no change in the predicted protein sequence (silent editing): at amino acid position 5, a GTC Val codon is converted to GTT. The other three editing events change the specified amino acid. At amino acid position 4, a CCG Pro codon is converted to a CTG Leu codon in nine cDNA clones analyzed, and at positions 81 and 85, the TCT Ser codons are converted to TTT Phe codons in three cDNA clones analyzed. These editing events restore the codons found in mtDNA from maize and sorghum (Isaac et al., 1985; Bailey-Serres et al., 1986). It appears likely, therefore, that the CCG Pro and TCT Ser codons found at the same positions in pea (Kemmerer et al., 1989), *Oenothera* (Hiesel et al., 1987), and soybean (Grabau, 1986) *cox1* genes are also converted to CTG Leu and TTT Phe codons.

The amino acid sequence, deduced from the genomic sequence, predicts that potato COX1 is a very hydrophobic polypeptide, consisting of 527 amino acids, with a molecular mass of 57,592 D. Comparison of the deduced amino acid sequence with those from other higher plants shows that the percentage of sequence similarity ranges from 96.6% (soybean; Grabau, 1986) to 94.9% (*Oenothera*; Hiesel et al., 1987). Examination of codon usage in the potato *cox1* gene reveals that, as in other plant mt genes, there is a strong bias toward the use of T (38.2%) in the third position of the codons.

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* Corresponding author; fax 56-2-2225515.

Abbreviations: mt, mitochondrial; nt, nucleotide.

Table I. *Characteristics of the potato mt cox1 gene*

Organism:
Solanum tuberosum cv Bintje.
Location:
mt genome.
Gene Product:
Subunit one of the mt Cyt *c* oxidase (complex IV).
Cloning Techniques:
The *cox1* gene is located on a 5.1-kb *Eco*RI restriction fragment, downstream of a truncated *cob* pseudogene and an *rps10* split gene (Zanlungo et al., 1995). We cloned the 5.1-kb fragment (pE5.1; Zanlungo et al., 1994), and fragments obtained from pE5.1 (2.2 kb *Bam*HI, 1.4 kb *Sal*I/*Bam*HI, and 1.5 kb *Bam*HI/*Eco*RI) were subcloned into Bluescribe, M13mp18, and/or M13mp19 vectors. The sequence of the *cox1* locus was determined by primer walking, using the dideoxy chain termination method. Both strands of the genomic DNA were completely sequenced. cDNAs were obtained by reverse transcription of potato total mtRNA and PCR amplification between oligonucleotide primers that carried *Bam*HI sites. These cDNAs were digested with *Bam*HI, cloned into Bluescribe, and sequenced by the dideoxy chain termination method.
Sequence Identification:
DNA and deduced amino acid sequence comparisons to the other plant mt *cox1* genes (Isaac et al., 1985; Bailey-Serres et al., 1986; Grabau, 1986; Hiesel et al., 1987; Kemmerer et al., 1989).
Features of Gene Sequence:
Sequenced DNA is 2030 bp long. Open reading frame from nts 171 to 1754. The potato *cox1* gene has an ACG codon at the position where *cox1* of other plants have their AUG initiation codon. Instead of this ACG codon, an ATG codon was found in eight of nine cDNA clones analyzed by sequence analysis, showing that a C to U editing creates the potato *cox1* AUG initiation codon.
Gene Copy Number:
One copy is present in the potato mtDNA.
Features of the Predicted Amino Acid Sequence:
The open reading frame consists of 527 amino acids, with a molecular mass of 57,592 D. Four of the five detected RNA-editing events change the specified amino acid: Met instead of Thr (amino acid 1), Leu instead of Pro (amino acid 4), and Phe instead of Ser (amino acids 81 and 85). Only 90 codons from the N-terminal region were analyzed for RNA editing. Sequence similarity with other known plant mt *cox1* genes on the amino acid level ranges from 96.6% (soybean; Grabau, 1986) to 94.9% (*Oenothera*; Hiesel et al., 1987).
Codon Bias:
Third codon position biased for T (38.2%).
Antibodies:
None available.

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