INTRODUCTION

In higher plants, mitochondrial transcripts undergo various posttranscriptional modifications: splicing, processing of the 5' and 3' ends, polyadenylation, and editing. An important step in posttranscriptional modification is site-specific cytidine deamination known as $U \rightarrow C$ editing. This process is widespread in plant mitochondria, affects almost all mitochondrial mRNAs, and is essential for gene expression, synthesis of functional polypeptides, stabilization of the secondary structures of introns and tRNA, and for 5'- and 3'-processing of tRNA [1, 2].

We have previously observed that mitochondrial rps13, atpA, cox1, and cox3 of wild perennial cereal *Elymus sibiricus* L. are highly homologous to their *Triticum aestivum* counterparts. Sequencing of the *E. sibiricus* genomic cox3 and its cDNA has shown that the differences between this and the *T. aestivum* genes are beyond the sites subject to editing and lead to substitutions in positions 53, 230, and 231 of the protein product [6, 7]. Here we demonstrate that the editing of the cox3 transcript proceeds similarly in *E. sibiricus* and *T. aestivum*, except that silent modification $U \rightarrow C$ takes place only in the latter. The editing involves 12 $C \rightarrow U$ conversions, which change 12 amino acid residues and the position of a hydrophobic amino acid cluster, thereby affecting the position and structure of the site of protein–protein interactions in Cox3.

EXPERIMENTAL

Isolation of mtDNA and total mtRNA, amplification of genomic cox3 and its cDNA. Mitochondria were isolated from 7-day-old etiolated seedlings as described previously [8]. DNA was obtained by lysis of isolated mitochondria with a detergent, extraction with phenol–chloroform, and precipitation with ethanol [9]. Total mtRNA was isolated with guanidine thiocyanate (Sigma, United States) in a lysis buffer and purified by phenol–chloroform extraction [10]. Isolated mtDNA was digested with RNase-free DNase (Sigma, 100 units of DNase per 0.5 mg RNA) at 37°C for 15 min [11]. The purity (a lack mtDNA admixture) was checked by amplification with proper primers without reverse transcription. The structures and sizes of PCR primers were based on the corresponding gene sequences available from the EMBL database for other plants. We used three primers: 5' (5'-ATGATTGAATTCCTCAGAGGCA), 3' (5'-TATACCTCCACCAATAGA), and internal (5'-TCCACGTGTG-
GAAGGGC). Genomic cox3 was amplified in 50 µl of the reaction mixture containing 50 mM Tris-HCl (pH 9.0), 50 mM NaCl, 10 mM MgCl2, 200 µM each dNTP, 20 pM each primer, and 2.5 units of Taq DNA polymerase (Sibenzyme). Amplification was run on a BIS-N 109 thermal cycler (Kol’tsovo) and included 35 cycles of 0.7 min at 94°C, 1.0 min at 50°C, and 1.5 min at 72°C, with first denaturation for 3 min and last synthesis for 10 min.

To be directly sequenced, the amplification product was purified on a Wizard column (Promega).

Cloning of genomic cox3 and its cDNA. After purification, amplified genomic and cDNA copies were cloned or directly sequenced. Cloning involved standard high-efficiency transformation of Escherichia coli TG2 [12]. The amplification products were treated with the Klenow fragment of E. coli DNA polymerase I to fill in the ends and cloned into the HindII site of pTZ19U.

Sequencing of genomic cox3 and its cDNA was performed with the above primers by Sanger’s method [13], without preliminary cloning. Cloned cDNA was sequenced with universal M13/pUC primers and the internal cox3 primer. Sequencing of both strands was carried out in three replicas. We examined at least three cDNAs obtained in independent experiments.

The sequencing product was resolved by denaturing PAGE in 5% gel at 1500 V. Gels were autoradiographed for 24 h at room temperature. In parallel, sequencing with an ABI PRISM kit was carried out in an ABI PRISM automated sequencer (Applied Biosystems). Sequence comparisons were done with the CLUSTALW program [14]. Amino acid sequence motifs were sought with the MOTIF: Sequence Motif Search program and the BLOCKS database of known motifs [15]. Conformation analysis employed the HyperChem 7 Evaluation program (ver. 2003, Hypercube).

RESULTS

Analysis of Amplified Mitochondrial cox3, Synthesis and Cloning of the cox3 cDNA

To study the editing of the transcript of cox3, which occurs in a single copy in the E. sibiricus mitochondrial genome [7], we analyzed the sequences of cox3 and the cDNA corresponding to its mature transcript. The product amplified from E. sibiricus mtDNA with cox3-specific primers was 795 bp and corresponded in size to homologous gene fragments of other plants.

Before cDNA synthesis, the inevitable mtDNA admixture was eliminated from total mtRNA preparations with RNase-free DNase, because mtDNA sequence could also be amplified. The first cDNA strand was synthesized with an oligonucleotide complementary to the 3’ end of the cox3 mRNA (see

Fig. 1. Electrophoresis of the product of cDNA synthesis with primers directed to E. sibiricus cox3 with (+) or without (-) initial reverse transcription (RT). M, molecular weight marker.