American Journal of Plant Sciences, **2011, 2, 535-538**
doi:10.4236/ajps.2011.24063 Published Online October 2011 (http://www.SciRP.org/journal/ajps)

The Post-Transcriptional mRNA Editing Analysis of cox3 Mitochondrial Gene in Fern *Asplenium nidus* Reveals Important Features

Sebastian Panarese, Guglielmo Rainaldi

Department of Biochemistry and Molecular Biology, University of BARI "Aldo Moro", Bari, Italy.
Email: g.rainaldi@biologia.uniba.it

Received July 18th, 2011; revised August 20th, 2011; accepted September 5th, 2011.

ABSTRACT

In the mitochondria and chloroplasts of flowering plants (*angiosperms*), *transcripts of protein-coding genes are altered after synthesis so that their final primary nucleotide sequence differs from that of the corresponding DNA sequence. This posttranscriptional mRNA editing consists almost exclusively of C-to-U substitutions* (*direct*) *and less frequently of U-to-C substitution* (*reverse*). *Editing occurs predominantly within coding regions, mostly at isolated C residues, and usually at first or second positions of codons, thereby almost always changing the amino acid from that specified by the unedited codon. Editing may also create initiation and termination codons. The effect of C-to-U RNA editing in plants is to make proteins encoded by plant organelles more similar in sequence to their non plant homologs, then specific C-to-U editing events are essential for the production of functional plant mitochondrial proteins. Our attention has been devoted to the study of the mRNA editing in cox3 mitochondrial gene of fern Asplenium nidus. This study reveals the extreme importance of both C-to-U and U-to-C substitutions for protein expression.*

Keywords*: *mtDNA, Plant, Monilophytes, Asplenium nidus

1. Introduction

Mitochondrial genomes of land plants have been fully sequenced and characterized in several species belonging to the Briophytes (*Marchantia polymorpha* [1] and *Physcomitrella patens* [2]) and Spermatophytes (*Arabidopsis thaliana* [3], *Beta vulgaris* [4], *Oryza sativa* [5], *Brassica napus* [6], *Zea mays* [7], *Nicotiana tabacum* [8] and *Triticum aestivum* [9]).

The comparison of organization, structure and expression between Spermatophyte mitochondrial genomes reveals several homogeneous features which can be summarized as follows: i) the presence of repeated sequences, ii) a heterogeneous structure, iii) the presence of DNA segments of extra mitochondrial origin (mainly chloroplastic) carrying in some cases active genes (usually for tRNAs) [10-12], iv) the editing of transcription products of structural genes, v) an incomplete set of tRNA genes.

To gain more knowledge on the mitochondrial biogenesis of Monilophytes, in particular respect to editing process, we chose plants of a filicales family, the fern *Asplenium nidus*, available at the Botanical Garden of the University of Bari.

In our previous investigations [13] we verified high level of editing process in filicales with to respect Spermatophytes plants. In our system, the editing analysis of *cox*3 mitochondrial gene confirms this hypothesis.

2. Materials and Methods

2.1. Sources of Mitochondrial DNA

Two alternative procedures for the isolation of organelles have been developed depending on the tissue used as starting material: roots or leaves. In the former the soil contained in the thick network of roots was removed by hand and washed in distilled water. This step was followed by drying the roots on filter paper and weighing and wrapping them with a double layer of sterile gauze. After washing several times with sterile water, the roots were suspended in sterile buffer (Mannitol 0,4 M, Mops 25 mM, EGTA 1 mM, PVP 1% pH 7,8) and homogenized in a blender with five hits every five seconds at medium speed.

Fractionation, lysis of organelles and DNA extraction were as reported by Hanson [14].

The isolation of organelles and DNA from green

leaves (leaf-procedure) was carried out using the same protocol with few modifications [14].

The mitochondrial DNA isolated by the two alternative procedures showed significant different levels of chloroplast contamination, as judged from the PCR amplification of highly conserved chloroplast regions [15].

Using as a template the mtDNA prepared from the roots, where the copy number of plastid DNA are reduced [16], no amplified products could be detected. The results of these experiments are reported in **Figure 1**.

The leaf procedure was used mainly for the isolation of total RNA employed for reverse transcription and cDNA synthesis and for the investigation concerning editing of *cox*3 gene transcripts.

2.2. Total RNA Extraction from *A. Nidus*

RNeasy Plant Mini Kit (Qiagen) was used for the purification of total RNA from fresh or frozen plant tissues. An accurate photometric detection of starting material was essential in order to obtain optimal RNA yield and purity. A maximum amount of 100 mg plant material was generally processed.

2.3. RT-PCR

RT-PCR experiments were carried out in a single step by means of SuperScript One-Step RT-PCR with Platinum *Taq*. Components for both cDNA synthesis and PCR were combined in a single tube, using gene-specific primers and target RNAs from total RNA. Reverse transcription automatically followed PCR cycling without additional steps. The system consisted of two major components: RT/Platinum *Taq* Mix and 2X Reaction Mix where the RT/Platinum *Taq* Mix contained a mixture of SuperScript II Reverse Transcriptase and Platinum *Taq* DNA Polymerase for optimal cDNA synthesis and PCR amplification respectively.

The following cycling conditions were established: cDNA synthesis pre-denaturation (1X) at 50˚C for 15-30'; 94˚C for 2'; PCR amplification (40X) at 94˚C for 20"; 50˚C - 60˚C for 30"; 68˚C - 72˚C for 1'/kb; final extension (1X) at 72˚C for 7'.

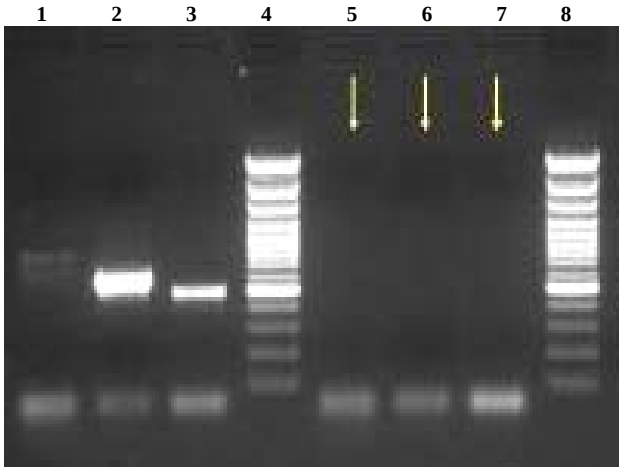

Figure 1. Multiple test of amplification performed on nucleic acids extracted with the two different procedures. The panels show two groups of three amplifications carried out with the same pairs of universal primers (BA48557-A49291, BA49317-A49855, BA49873-A50272, [14] for the detection of cpDNA in the nucleic acid fractions isolated by the two procedures. Lanes 1-3: template isolated by the leaf procedure. 5-7: template (arrows) isolated by the root procedure. 4 and 8: 1 Kb DNA ladder (3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp).

2.4. DNA Cloning in Ta Vectors

The cloning of dsDNA fragments was carried out using pGEM-T Easy Vector Systems and following the manufacturer instructions.

2.5. DNA Sequencing and Determination of Editing Rates

Sequencing of individual cDNA of 23 clones was performed automatically with the Big Dye1 Terminator Cycle Sequencing Kit (Applied Biosystems).

ClustalW program was used to determine editing sites, aligning DNA and cDNA sequences.

Sequence of *cox*3 gene were deposited in GeneBank database (accession number: FR669448).

3. Results and Discussion

3.1. Analysis of Editing Sites on Cox3 Transcripts

The **Figure 2** shows the comparison of mitochondrial sequence of *cox*3 gene with the corresponding cDNA. **Table 1** shows editing data about some *cox*3 mitochondrial genes of different plant species.

The analysis of results obtained in this investigation shows some specific features for the *A. nidus* mitochon-

Table 1. Comparison of editing value in cox3 mitochondrial transcripts between some land plants.

	Editing C-U	Editing U-C	Site Tot	%	Gene bp
P. patens	1		1	0.1	798
B. vulgaris	4		4	0.5	798
B. napus	7		7	0.9	798
A. thaliana	8		8	1.0	798
T. aestivum	12	1	13	1.6	798
O. sativa	1		1	0.1	843
A. nidus	**19**	**13**	**32**	**4.0**	**798**

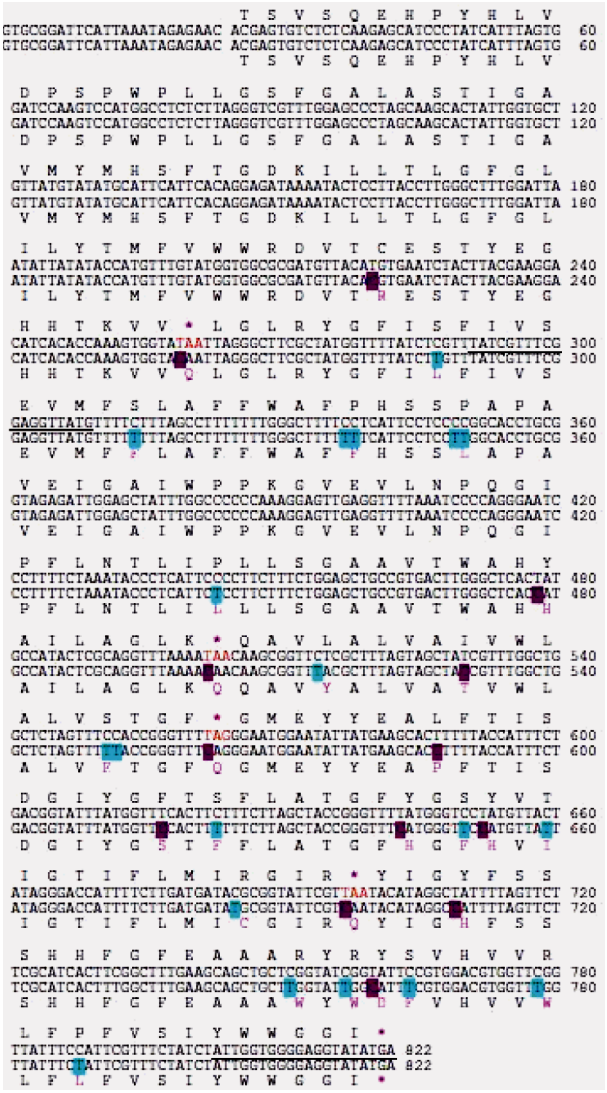

Figure 2. Alignment of genome sequence (upper) with cDNA counterpart of *cox*3 mitochondrial gene of *A. nidus*. Nucleotides underlined correspond to sequencing primers.

drial *cox*3 gene:

1) for the transcript of the gene, direct editing events (19 C-U; 2,3%) and reverse editing events (13 U-C; 1,7%) are almost equivalent;

2) the editing events have a high relevance: they suppress four stop codons within the *cox*3 gene;

3) 25 amino acids changes and 4 suppressions of stop codons (3 TAA-1 TAG) are found. These stop codons are corrected by U to C editing to sense codons of the amino acid Q (**Figure 3**);

4) in particularly critical positions, editing events of both types generate transcripts which can be translated as functional proteins;

5) the initial codon of *cox*3 is an ACG codon which is not changed to AUG by a direct editing event.

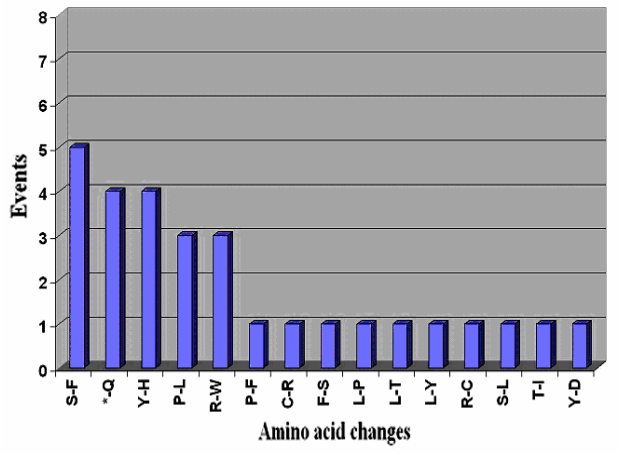

Figure 3. Amino acids changes after editing events in *A. nidus* cox3 mitochondrial transcript.

This observation can be considered in agreement with the finding of Dong F. G. [17] who found that the radish (*Raphanus sativus* L.) mitochondrial *cox*2 gene contains an ACG at the predicted translation initiation site which is not converted to AUG codon in the mRNA, although 15 other RNA editing sites were identified. So our finding confirms that in plant mitochondria ACG codon can be utilized as initiation codon;

6) *cox*3 editing events have a higher frequency on *A. nidus* transcripts compared with the same genes studied on other angiosperm genomes (**Table 1**);

7) reverse editing events (U-C) are considerably more frequent in cox3 transcripts of *A. nidus* than in other plant species, where they are nearly absent (**Table 1**);

8) as shown in **Figure 4**, for 17 codons changes occur in first position, for 13 codons in second position and for only one codon in third position.

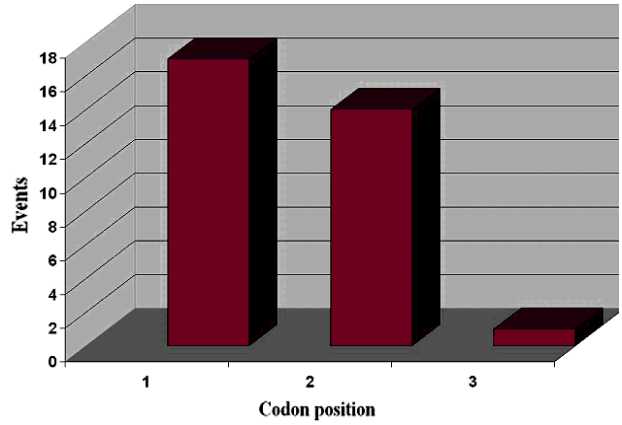

Figure 4. Distribution of editing events across codon positions.

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