RNA EDITING IN RAPESEED MITOCHONDRIAL TRANSCRIPTS: THE EXTENT OF RNA EDITING IN RAPESEED IS LOW COMPARED TO THOSE OF OTHER PLANTS

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ABSTRACT

RNA editing of gene transcripts seems to be a general phenomenon in higher plant mitochondria. However, the number of nucleotides altered differs between plant species and also between individual genes within a mitochondrion. RNA editing patterns of *atp6*, *atp9* and *orfB* transcripts in rapeseed were investigated by a method involving cDNA synthesis, using specific oligonucleotides as primers, followed by PCR amplification. It was shown that one, four and three RNA editing events occurred in the *atp6*, *atp9* and *orfB* coding regions, respectively. The extent of RNA editing in rapeseed is very low compared to those of other plant mitochondria. And in rapeseed, most of the amino acids which are specified only after editing in mitochondria of other plant species, are already encoded by the mitochondrial genomic sequence. Rapeseed already seems to have an "edited" sequence on its mitochondrial genome, while other species have a "pre-editing" sequence which needs extensive RNA editings to produce the mature mRNA. Such an extreme divergence of editing patterns between different higher plants is very interesting when considering the relationship between RNA editing and the evolution of plant mitochondrial genome.

INTRODUCTION

Recently the mRNAs of plant mitochondria have been shown to be modified; at specific locations, C residues on the genomic sequence are converted to U on its mRNA, resulting in polypeptide different from that deduced from its DNA sequence (reviewed in Bonnard *et al.*, 1992). This RNA editing event is essential for the correct expression of plant mitochondrial protein genes. The reasons for the existence of RNA editing in plant mitochondria is not clear, but it appears that RNA editing in plant mitochondria plays a role in the conservation of protein sequences during evolution. The number of nucleotides altered by editing differs, however, between plant species and also between individual genes within one mitochondrion.

To clarify that such a divergence of editing patterns is depended upon individual plant species, editing patterns of several genes in rapeseed mitochondria were examined. The author shows that the extent of RNA editing in rapeseed mitochondrial transcripts is low compared to those of other higher plants.

EXPERIMENTAL

The investigation of RNA editing in the mRNAs of three mitochondrial gene in

rapeseed shows that one, four and three editing events occur in the *atp6*, *atp9* and *orfB* coding regions, respectively. Comparative analysis of RNA editing patterns for these three gene transcripts among plant species indicates substantial variation in the extent of editing.

As for *atp6* transcripts, *Oenothera* and sorghum represent extreme examples, compared to rapeseed, the former two species having 21 and 19 editing events within the core polypeptide, while rapeseed having only one editing (Table 1) (Handa and Nakajima, 1992). In rapeseed, all of the amino acids except one which are specified only after editing in mitochondria of other two species, are already encoded by the mitochondrial genomic sequence. No variation of the extent and frequency of RNA editing between *atp6* transcripts from normal and cms cytoplasms can be observed, which suggests that RNA editing is not primary determinant for male-sterility induction in rapeseed.

Table 1. Editing characteristics of three mitochondrial gene transcripts.

Gene	Plant	Number of editing sites	Altered amino acids	Creation of stop codon by editing
	Rapeseed	1	1	no (genomic)
atp6	*Oenothera*	21	19	yes
	Sorghum	19	15	yes
	Rapeseed	4	4	no (genomic)
	Oenothera	4	3	yes
atp9	Petunia	10	7	yes
	Sorghum	8	6	yes
	Tobacco	10	6	yes
	Wheat	8	5	yes
	Rapeseed	3	1	no (genomic)
orfB	*Oenothera*	6	4	no (genomic)
	Wheat	4	3	no (genomic)

Such a divergence of editing patterns between different higher plant species is also found for the editing of *atp9* transcripts. Petunia and tobacco have 10 editing events within the core polypeptide, while rapeseed and *Oenothera* have only four editings (Table 1) (Handa, 1993).

Previous studies of RNA editing in *atp9* transcripts revealed that their termination codons were created by the editing of CAG and CAA codons. The creation of UGA was reported for the *atp9* mRNA of *Oenothera*, sorghum, and wheat and the creation of UAA for Petunia and tobacco. In the case of wheat, this modification was confirmed by protein sequencing. On the other hand, in rapeseed, termination codon has been already encoded by the genomic sequence (Table 1).

In the case of *orfB* transcripts, number of nucleotides altered don't differ so much among three species (Table 1) (Handa *et al.*, 1995). However, in rapeseed, all of the amino acids except one specified after editing in other species, are encoded by the genomic sequence.

As defined for these three genes, rapeseed seems to have an original sequence on its

mitochondrial genome among plant species. And rapeseed might be situated on the specific position for the RNA editing of mitochondrial transcripts. The specificity of rapeseed mtDNA is also supported by its genome size, because rapeseed has a smallest mitochondrial genome (about 221 kb) among higher plants.

Such an extreme divergence of editing patterns between different higher plant species is very interesting for considering the relationship between the RNA editing and the evolution of plant mitochondrial genome. Genomic sequence found in rapeseed mitochondria implies that genomic sequence of rapeseed remains in its original form, and does not show the mutations which can be seen in the other species. However, this hypothesis is not agreed with phylogenetical studies of higher plants. There is also another alternative, namely that during evolution, edited sequences have been integrated into the mitochondrial genome of rapeseed which no longer exhibit editing at these positions. The integration of edited sequences as RNA would require a reverse transcriptase-activity to be present in mitochondria. A reverse transcriptase-activity could be encoded in the nucleus and transported into the mitochondrion or could be synthesized form a mitochondrial gene within the organelle. The gene for mitochondrial reverse transcriptase has been found in *Oenothera* mitochondria, and in nuclear genome of *Brassica* species including rapeseed reverse transcriptase gene, which originated from copia-like retrotransposon, was also found.

Answers to these questions should be obtained through the continued analysis of RNA editing in rapeseed mitochondria, and through studying the evolution of plant mitochondrial genome and the molecular mechanism of RNA editing in plant mitochondria.

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