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Minireview

Editing of plant mitochondrial transfer RNAs

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Editing in plant mitochondria consists in C to U changes and mainly affects messenger RNAs, thus providing the correct genetic information for the biosynthesis of mitochondrial (mt) proteins. But editing can also affect some of the plant mt tRNAs encoded by the mt genome. In dicots, a C to U editing event corrects a C : A mismatch into a U : A base-pair in the acceptor stem of mt tRNA^{Phe} (GAA). In larch mitochondria, three C to U editing events restore U : A base-pairs in the acceptor stem, D stem and anticodon stem, respectively, of mt tRNA^{His} (GUG). For both these mt tRNAs editing of the precursors is a prerequisite for their processing into mature tRNAs.

In potato mt tRNA^{Cys} (GCA), editing converts a C28 : U42 mismatch in the anticodon stem into a U28 : U42 non-canonical base-pair, and reverse transcriptase minisequencing has shown that the mature mt tRNA^{Cys} is fully edited. In the bryophyte *Marchantia polymorpha* this U residue is encoded in the mt genome and evolutionary studies suggest that restoration of the U28 residue is necessary when it is not encoded in the gene. However, *in vitro* studies have shown that neither processing of the precursor nor aminoacylation of tRNA^{Cys} requires C to U editing at this position. But sequencing of the purified mt tRNA^{Cys} has shown that Ψ is present at position 28, indicating that C to U editing is a prerequisite for the subsequent isomerization of U into Ψ at position 28.

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Abbreviation: mt, mitochondrial.

RNA editing

RNA editing is usually defined as a modification of the RNA primary sequence, thus causing a divergence from the coding DNA sequence. Rather than an unusual phenomenon restricted to a few genetic systems, RNA editing appears more and more to be an essential step in gene expression in a wide variety of organisms such as protists, animals and plants, and in different compartments such as mitochondria and chloroplasts (reviewed by Smith *et al.,* 1997). This was again recently illustrated when editing of an RNA from HIV was described by Bourara *et al.* (2000).

For each type of RNA editing the same questions have to be considered: How did RNA editing appear and evolve? How do nucleotide deletions and insertions or nucleotide conversions occur? What is the function of RNA editing?

RNA editing in plant mitochondria

In plants, a similar kind of RNA editing, consisting in C to U conversions, has been identified in both chloroplasts and mitochondria (reviewed by Maier *et al.*, 1996). One of the striking differences, however, is the number of editing sites per genome. In a chloroplast genome only a few editing sites can be identified. In plant mitochondria, studies based on single transcript led to the prediction that several hundreds of editing sites could be present in a genome. This was recently confirmed by Giegé & Brennicke (1999) who found 441 editing sites in transcripts covering the whole mitochondrial genome of *Arabidopsis thaliana*. Editing has been observed in all the major groups of land plants, but not in algae (Hiesel *et al.,* 1994; Malek *et al.,* 1996).

A hypothesis to explain how RNA editing appeared and was maintained in plant organelles is to consider that the factor(s) responsible for this nucleotide conversion first derived from another RNA processing activity. Following this event, mutations were conserved in the genome while being compensated at the RNA level. This idea originates essentially from the fact that editing often makes it possible to maintain sequence conservation at the protein level.

The question of how RNA editing occurs has only partially been answered. Yu & Schuster (1995) were able to demonstrate that editing results from the deamination of a C residue into U, rather than nucleotide replacement, but until now attempts to identify a nuclear gene coding for the cytidine deaminase involved have been unsuccessful (Faivre-Nitschke *et al.,* 2000). Another major aspect of the editing mechanism remains completely unknown in plant mitochondria: the deamination reaction has to be restricted to specific C residues and the *cis*- and/or *trans*-acting factors involved in the selection of these editing sites still have to be identified.

The function of editing can vary. Most of the editing sites are in the coding sequences of messenger RNAs, so that editing results in a modification at the protein level and often increases the conservation of protein sequences between different species. However, the question of their necessity should be addressed for each protein. The role of editing in non-coding sequences is even more difficult to identify, as the importance of 5′ and 3′ untranslated regions of mitochondrial mRNAs in mechanisms such as RNA stability, processing or degradation and translation initiation remains poorly documented. However, a study of an editing event in an intron showed that it was a prerequisite to splicing (Börner *et al.*, 1995).

Although editing mainly affects mRNAs, editing sites have also been identified in three different plant mitochondrial tRNAs. In this report, we summarize the results obtained on the editing of these three tRNAs.

tRNA EDITING IN PLANT MITOCHONDRIA

The three edited tRNAs

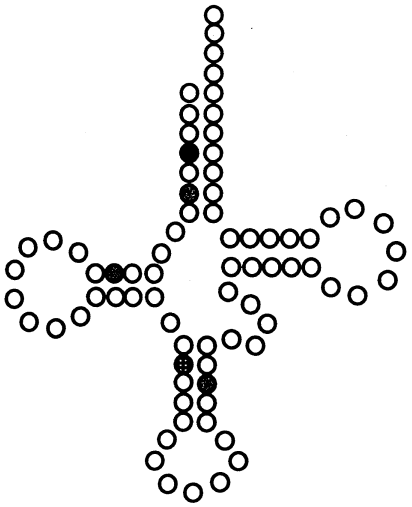
In dicot mitochondria a C to U editing event corrects a C:A mismatch into a U:A base-pair in the acceptor stem of $tRNA^{Phe}$ (GAA) (Binder *et al.,* 1994; Maréchal-Drouard *et al.*, 1993). In the mitochondria of the gymnosperm *Larix leptoeuropaea*, three C to U conversions restore U:A base-pairs in the acceptor stem, D stem and anticodon stem, respectively, of $tRNA^{His}$ (GUG) (Maréchal-Drouard *et al.*, 1996b). The third example is the native $tRNA^{Cys}$ (GCA) expressed in dicot mitochondria where a C28:U42 mismatch is converted into a U28:U42 non canonical base-pair (Binder *et al.*, 1994; Fey *et al.*, 2000). These editing sites are indicated in Fig. 1.

About the mechanism conferring specificity

As editing occurs in double-stranded regions of these tRNAs and restores base-pairing, it could be hypothesized that tRNA editing is directed by the presence of a mismatch in a tRNA arm. However, similar mismatches are present in $tRNA^{Gly}$ (GCC) and $tRNA^{Ser}$ (GCA) of pea and potato mitochondria, but no editing event could be observed (Schock *et al.*, 1998) Thus, a mismatch alone in a tRNA arm is not sufficient to lead to a specific editing event.

Function of tRNA editing

In the case of both $tRNA^{Phe}$ and $tRNA^{His}$, editing of precursor transcripts was shown to be a prerequisite for 5′ and 3′ processing to generate mature tRNA (Maréchal-Drouard *et al.*, 1996a, 1996b; Marchfelder *et al.*, 1996, Kunzmann *et al.,* 1998). These experiments were conducted by incubating *in vitro* synthesized RNA (corresponding to a tRNA precursor transcript) in the presence of partially purified mitochondrial proteins containing both RNase P and RNase Z processing activities (Fig. 2). In the case of mt $tRNA^{Phe}$, since editing of the C4-A69 mispairing into the normal U4-A69 base pair appeared to promote efficient processing of the corresponding precursor RNA *in vitro*, we wondered whether the same effect would be obtained when this mismatch is changed into another base pair C4-G69. Although less efficiently, a mature tRNA-size product was also obtained when the C4-G69 *in vitro* transcript of this mutated gene was incubated in the presence of the mi-



tRNAs	Plants	Positions	Functions
Phe ●	Dicots	C4	Maturation
His ●	Larch	C7 C12 C41	Maturation
Cys ●	Dicots	C28	Pseudouridylation

Figure 1. Editing sites in plant mitochondrial tRNAs and their functions.

tochondrial lysate, suggesting that proper fold ing of the tRNA pre cur sor is prob a bly re quired for recognition by RNase P and/or other processing enzymes.

More re cently we have stud ied the ed it ing of potato mitochondrial $tRNA^{Cys}$ (Fey *et al.*, 2000). In that case, *in vi tro* pro cess ing ex per i ments showed no ev i dence that ed it ing was re quired for tRNA mat u ra tion. Other *in vi tro* as says were developed, but again editing seemed to affect neither the aminoacylation of this tRNA, nor the addition of the 3′-CCA se quence. We then de cided to study the evo lu tion of this tRNA sequence. Having noticed that the C to U ed it ing at po si tion 28 re stores the an ces tral se quence found in the mi to chon drial *trnC* gene of the Bryophyte *Marchantia polymorpha*, we first se quenced this gene from several plant species. While the ancestral U cod ing se quence was also found in a fern and in the Prespermaphyte *Gingko biloba*, two Cycads (also Prespermaphytes) and *Magnolia grandiflora* (belonging to the earliest dicot sub-class) ex hib ited a C in the same gene at position 28. If this tRNA was not edited in the two Cy cads, it would have sug gested that this ed it ing site is not es sen tial. On the con trary, we found that $tRNA^{Cys}$ is also edited in the two Cycads. As this study clearly indicated that ed it ing has al lowed the con ser va tion of a U res i due at po si tion 28 dur ing evo lu tion, we re con sid ered the im por tance of this tRNA ed it ing. The iden ti fi ca tion of ed it ing sites is usually based on reverse transcription of tRNA

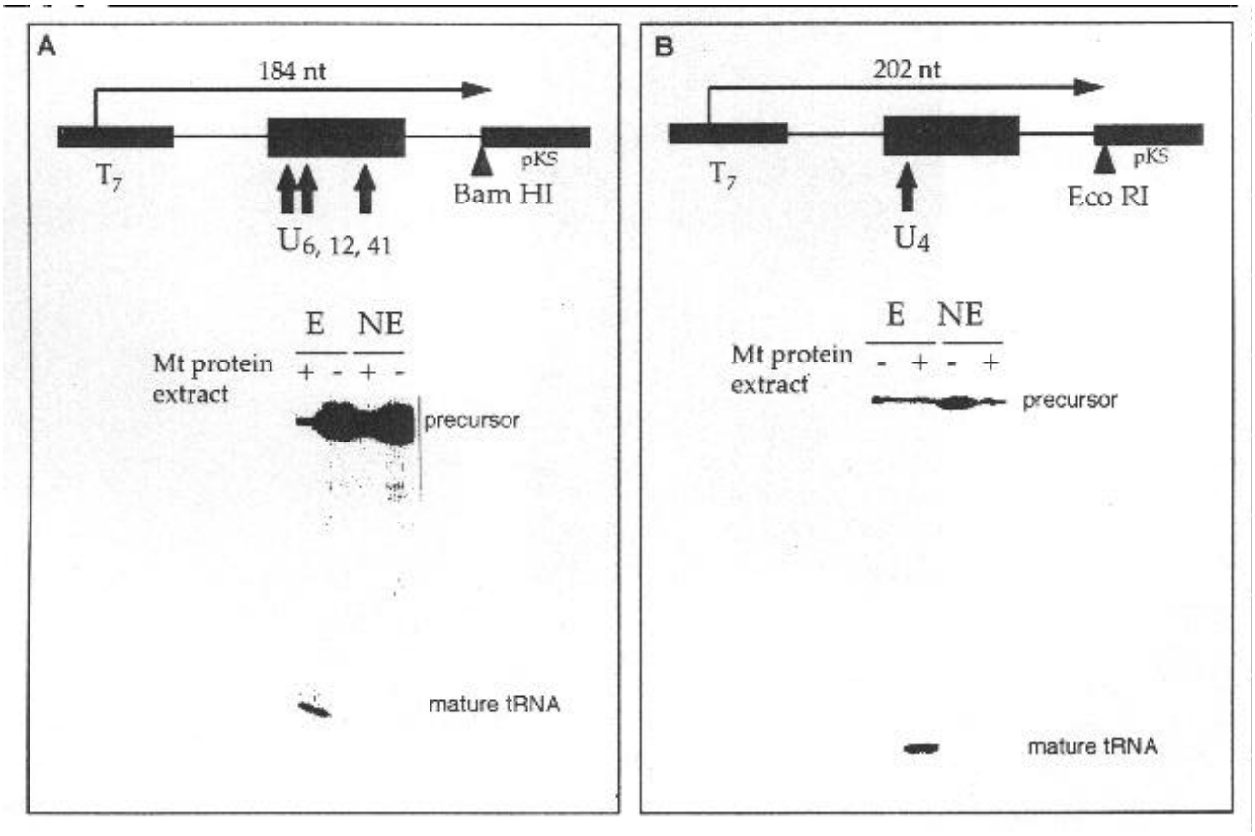


Figure 2. Editing is a pre req ui site for the pro cess ing of $tRNA^{His}$ and $tRNA^{Phe}$ precursors.

The up per part of the fig ure shows sche matic rep re sen ta tions of the gene con structs used as tem plates for the synthe sis of A) $tRNA^{His}$ and B) $tRNA^{Phe}$ pre cur sors. Lower part: *in vi tro*-syn the sized la beled pre cur sor RNAs cor re spond ing to the nonedited (NE) or ed ited (E) forms of A) $tRNA^{His}$ and B) $tRNA^{Phe}$ were in cu bated in the pres ence (+) or ab sence (–) of a po tato mi to chon drial pro tein ex tract. Pro cessing prod ucts were an a lyzed on 15% polyacrylamide gels.

and sequencing of the resulting cDNA. To check the *in vivo* sequence of $tRNA^{Cys}$, we purified it from potato mitochondria and found that the residue at position 28 was not a uridine but rather a pseudouridine (Ψ). This result raises the question whether the mechanism allowing the conversion of a C residue into Ψ could still be considered as RNA editing. Only one similar case has been previously described in *Escherichia coli*, where $tRNA^{Ser}$ (GGA) undergoes a conversion of the C20 residue into dihydrouridine (Motorin *et al*., 1996), probably due to a two-step mechanism involving first a C to U deamination (personal communication of H. Grosjean in Price & Gray, 1998). As C to U deamination and U to Ψ isomerization (by a Ψ synthase) affect different atoms in the pyrimidine ring, we propose a two step model to explain how Ψ28 can be generated in potato mt $tRNA^{Cys}$ (Fig. 3). The necessity for this editing event could be related to the function of this Ψ28. It is generally assumed that Ψ residues provide a fine improvement of the tRNA structure. For instance, it has been shown that Ψ residues can stabilize RNA by improving RNA stacking (Davis, 1995).

Stabilization of tRNA structure by a Ψ residue at position 39 was also shown in the case of *E. coli* $tRNA^{Phe}$ (Davis & Poulter, 1991) and human $tRNA^{Lys-3}$ (Durant & Davis, 1999). More generally, these residues could represent favorable sites for tRNA hydration, where a water molecule can be involved in hydrogen bonds with the phosphate backbone and with the NI atom in the Ψ pyrimidine ring (Arnez & Steitz, 1994; Westhof & Moras, 1988).

CONCLUSION

We have come to a point where a more detailed understanding of the function of editing in these three tRNAs is related to other biological processes. In the case of $tRNA^{Phe}$ and $tRNA^{His}$, the identification of the sequence and structural features required for a tRNA precursor to be recognized as a substrate by RNase P and/or RNase Z will probably explain the importance of these editing events.

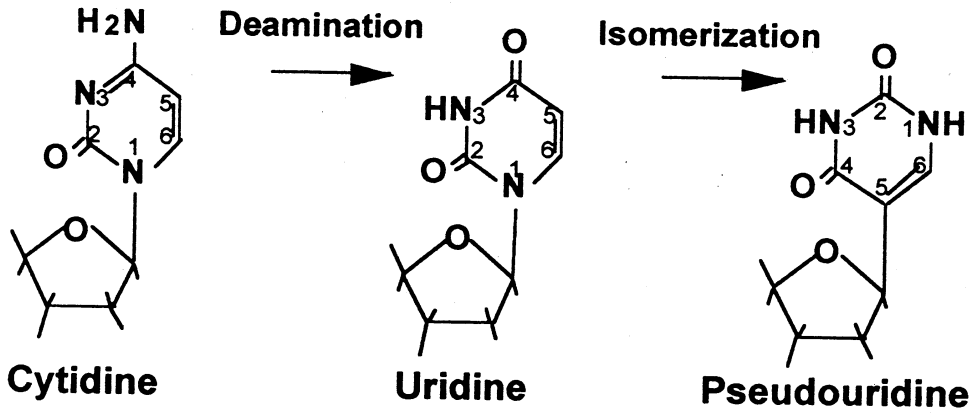


Figure 3. A two-step model proposed to generate Ψ at position 28 in potato mt $tRNA^{Cys}$.

In the case of $tRNA^{Cys}$, more experimental evidence for the importance of Ψ residues in tRNAs will be required before one can conclude on the necessity of this editing site to stabilize the structure of this tRNA *in vivo*.

Other editing sites in mt tRNAs might be identified in the future, but our studies have already provided evidence that the importance of RNA editing is not restricted to messenger RNAs in plant mitochondrial gene expression, but also to structural RNAs.

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