Role of editing in plant mitochondrial transfer RNAs

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Abstract

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\textsubscript{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\textsubscript{His} (GUG). For both these mt tRNA\textsubscript{Phe} and tRNA\textsubscript{His}, editing of the precursors is a prerequisite for their processing into mature tRNAs. In potato mt tRNA\textsubscript{Cys} (GCA), editing converts a C\textsubscript{28}:U\textsubscript{42} mismatch in the anticodon stem into a U\textsubscript{28}:U\textsubscript{42} non-canonical base pair, and reverse transcriptase minisequencing has shown that the mature mt tRNA\textsubscript{Cys} is fully edited. In the bryophyte \textit{Marchantia polymorpha} this U residue is encoded in the mt genome and evolutionary studies suggest that restoration of a U\textsubscript{28} 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\textsubscript{Cys}, requires C to U editing at this position. But sequencing of the purified mt tRNA\textsubscript{Cys} has shown that C is present at position 28, indicating that C to U editing is a prerequisite for the subsequent isomerization of U into C at position 28. © 2002 Published by Elsevier Science B.V.

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1. Introduction

1.1. 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 chloroplast (reviewed by Smith et al., 1997). This was again recently illustrated when editing of an RNA from human immunodeficiency virus 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 either nucleotide deletions and insertions or nucleotide conversions occur? What is the function of RNA editing?

1.2. RNA editing in plant mitochondria

In plants, a similar kind of RNA editing, consisting of 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 are present on a genome. This was recently confirmed by Giegé and Brennicke, 1999 who found 441 editing sites on transcripts covering the whole mitochondrial genome of \textit{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 allows to maintain sequence conservation at the protein level.

The question of how RNA editing occurs was only partially answered. Yu and 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 has been unsuccessful (Faiivre-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, the 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.

2. tRNA editing in plant mitochondria

2.1. 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., 1996a). In the mitochondrial 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(4th) (GUG) (Maréchal-Drouard et al., 1996b). The third example is the native tRNA(5th) (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.

2.2. 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 the 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.

2.3. Function of tRNA editing

In the case of both tRNA(Phe) and tRNA(4th), editing of precursor transcripts was shown to be a prerequisite for 5’ and 3’ processing to generate a mature tRNA (Maréchal-Drouard et al., 1996a,b; Marchfelder et al., 1996; Kunzmann et al., 1998). These experiments were conducted by incubating an 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 a C42:A69 mispairing into a normal U42: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 C42−G69. Although less efficiently, a mature tRNA-size product was also obtained when the C42−G69 in vivo transcript of this mutated gene was incubated in the presence of the mitochondrial lysate, suggesting that proper folding of the tRNA precursor is probably required for recognition by RNase P and/or other processing enzymes.

More recently we have studied the editing of potato mitochondrial tRNA(5th) (Fey et al., 2000). In that case, in vitro processing experiments showed no evidence that editing was required for tRNA maturation. Other in vitro assays were developed, but again editing seemed to affect neither the aminocacylation of this tRNA, nor the addition of the 3’-CCA sequence. We then decided to study the evolution of this tRNA sequence. Having noticed that this C to U editing at position 28 restores the ancestral sequence found in the mitochondrial trnC gene of the Bryophyte Marchantia polymorpha, we first sequenced this gene from several plant species. While the ancestral U coding sequence was also found in a fern and in the Prespermaphyte Ginkgo biloba, two Cycads (also Prespermaphytes) and Magnolia grandiflora (belonging to the earliest dicot sub-class) exhibited a C in the same gene at position 28. If this tRNA was not edited in the two Cycads, it would have suggested that this editing site is not essential. On the contrary, we found that the tRNA(5th) is also edited in the two Cycads. As this study clearly indicated that editing has allowed to maintain a U residue at position 28 during evolution, we reconsidered the importance of this tRNA editing. The identification of editing sites is usually based on reverse transcription of tRNA and sequencing of the resulting cDNA. To check the in vivo sequence of tRNA(5th), 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 a Ψ could still be considered as RNA editing. Only one similar case has been previously described before in Escherichia coli, where tRNA(Ser) (GGA) undergoes a conversion of the C20.
residue into a 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 and 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 Ψ_{28} can be generated in potato mt tRNA^Cys (Fig. 3). The necessity for this editing event could be related to the function of this Ψ_{28}. 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 and Poulter, 1991) and human tRNA^{Lys-3} (Durant and 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 N1 atom in the Ψ pyrimidine ring (Arnez and Steitz, 1994; Westhof and Moras, 1988).

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<th>tRNAs</th>
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<td>C4</td>
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<tr>
<td>His</td>
<td>Larch</td>
<td>C7(*)</td>
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<td>Cys</td>
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Fig. 1. Editing sites in plant mitochondrial tRNAs and their functions. (*) In this larch mt tRNA^His, there is an additional G at position −1 (which is post-transcriptionally added), so that C_7 becomes C_6 if this extra G is not taken into account.
3. Conclusion

We have come to a point were 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 RNase Z will probably explain the importance of these editing events. In the case of tRNA^{A^0}, more experimental evidence for the importance of $\Psi$ 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.

References


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