Accelerated Evolution of Sites Undergoing mRNA Editing in Plant Mitochondria and Chloroplasts

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The selective constraints influencing mRNA editing in plant organelles are largely unknown. To investigate these, we compared patterns of editing between monocot and dicot mitochondrial mRNA. On average, 24% of sites that are edited from C to U in one species have been substituted during evolution by a genomic T in the other: this is four times the rate of evolution seen at nonedited synonymously variable C residues. A similar, but weaker trend (not statistically significant) is seen at sites edited in chloroplast mRNA. The elevated substitution rate does not appear to be a consequence of a higher mutability of the trinucleotide motif (T-C-purine) associated with editing, nor to be a result of reverse transcription from mature mRNA. Selection to replace the genomic C with a T may account for the accelerated evolution, either due to elimination of inefficient transcripts and protein products or as a consequence of the prior loss of components outside the edit site which are necessary for editing; the latter hypothesis is supported by the frequent loss of editing without genomic mutation at third codon positions. Whatever the cause, the rapid rate of evolution indicates that editing confers little selective advantage at most sites.

Introduction

Posttranscriptional modification of RNA to alter the coding specificity of the sequence is termed RNA editing, and may be caused by a variety of mechanisms in different organisms (Cattaneo 1991). A particular form of RNA editing, the replacement of cytidine (C) by uridine (U), is very common in the mitochondria of vascular plants (Hiesel, Combettes, and Brennicke 1994), and also occurs to a lesser extent in their chloroplasts (Gray and Covello 1993). This editing is mostly restricted to protein-coding sequences (Schuster et al. 1991), with a preference for second codon positions, and it appears to occur independently of transcription (Gualberto et al. 1991), of splicing (Sutton et al. 1991), and of translation (Zeltz et al. 1993). Editing occurs before and after splicing, and overediting may continue beyond the point necessary for a functional transcript, as there are cases of stop codons being introduced within coding regions (Schuster and Brennicke 1991*a*; Schuster et al. 1993). There are apparent preferences for certain bases near the edit site (Covello and Gray 1990; Maier et al. 1992), suggesting that the local RNA sequence may contribute in part to editing specificity. However, differences in editing patterns between species where the local RNA sequence is highly conserved suggest that the nearby sequence is not the only determinant of specificity (Zeltz et al. 1993).

Observed differences among plant species (Covello and Gray 1990; Freyer et al. 1995) in patterns of editing provide insights into the mechanism of editing. If editing was selectively advantageous, it would be expected to be conserved during evolution, and bases near the edited site which contribute to the editing process should also be conserved (Wolfe 1996). To test this hypothesis, we compiled a set of sequences homologous between monocots and dicots to compare the rates of evolution at edited and nonedited sites, and investigated the pattern of evolution in the bases near edited sites. We found that, far from being conserved, edited sites evolve at a very rapid rate.

Key words: RNA editing, mitochondrion, chloroplast, evolution, nucleotide substitution.

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Materials and Methods

A data set of mitochondrial genes was compiled for which there were sequences available, as well as experimental analysis of RNA editing, in both a monocot and a dicot. All mitochondrial monocot genes were from *Triticum aestivum,* except for *atp6* (*Sorghum bicolor*) and *mat-r* (*Zea mays*); all mitochondrial dicot genes are from *Oenothera berteriana,* except for *mat-r* (*Glycine max), nad9* (*Solanum tuberosum*), and *rps12* (*Petunia hybrida*). Edited sites are sites at which there is any evidence of C-to-U editing, taken from the cited literature (table 1). Residues were only included in the analysis if the protein sequence could be reliably aligned. This excluded parts of *coxII* (seven codons at dicot 5′ end) *matr* (monocot 3′ 11 codons), *orf589* (dicot 3′ seven codons) and *atp6* (dicot 5′ 29 codons and 3′ 35 codons). The analysis was restricted to comparing the frequency of C-to-T transition, as the analysis of the simple number of base differences could be biased by the unequal base frequencies at third codon positions, and by likely unequal rates of mutation between different bases. The frequency of transition from an edited C to a genomic T was calculated for the monocot-to-dicot direction, and vice versa. For comparison, the transition frequency was calculated at third codon position C's, excluding those which are edited in the first species. Transition frequencies were calculated as the total number of genomic T's in the second species, out of the total number of genomic C's in the first species, excluding any sites where the second species had a purine. This avoids underestimating the frequency of replacement of C by T at third codon positions, where replacements by a purine occur more often than at edited sites. An analysis of the frequency of mutation to other bases at each of the three codon positions was also performed for both edited sites and unedited C residues. No correction for

Table 1
Frequency of Transition from C to T at Edited Sites, and at Unedited Third Codon Positions

a. FREQUENCY OF GENOMIC C → T TRANSITIONS IN DICOT MITOCHONDRIAL GENES

Gene	At Sites Edited from C to U in Monocot	At Third Codon Position C in Monocot	GenBank; References (monocot)
atp6	2/18	4/34	X57100; Kempken et al. (1991)
atp9	4/8	1/12	X54621; Nowak and Kuck (1990)
coxII	5/19	6/40	X52867; Covello and Gray (1990)
coxIII	2/7	1/18	X52539; Gualberto, Weil, and Grienenberger (1990)
mat-r	4/15	9/205	U09987; Thompson et al. (1994)
nad1	5/17	2/37	X57965–8; Chapdelaine and Bonen (1991)
nad3	6/21	1/17	X59153; Gualberto et al. (1991)
nad9	5/12	0/30	X69720; Lamattina et al. (1993)
orfB	1/4	3/33	X59153; Gualberto et al. (1991)
orf589	8/33	5/110	X69205; Gonzalez, Bonnard, and Grienenberger (1993)
rps12	3/6	0/10	X59153; Gualberto et al. (1991)
Total	45/160	32/546	
Frequency	0.28[a] ± 0.04	0.06 ± 0.01	

b. FREQUENCY OF GENOMIC C → T TRANSITIONS IN MONOCOT MITOCHONDRIAL GENES

Gene	At Sites Edited from C to U in Dicot	At Third Codon Position C in Dicot	GenBank; Reference (dicot)
atp6	6/22	6/38	Y00465; Schuster and Brennicke (1991*a*)
atp9	1/4	0/12	X15765; Schuster and Brennicke (1991*b*)
coxII	4/20	4/35	X00212; Hiesel, Wissinger, and Brennicke (1990)
coxIII	2/8	3/19	X76275; Hiesel, Combettes, and Brennicke (1994)
mat-r	4/14	7/204	U09988; Thompson et al. (1994)
nad1	8/27	3/35	M63032–4; Wissinger, Schuster, and Brennicke (1991)
nad3	1/16	4/19	X52200; Schuster et al. (1990)
nad9	1/7	1/32	X79774; Grohmann et al. (1994)
orfB	2/7	5/34	X04764; Schuster et al. (1991)
orf589	6/43	5/107	X69555; Schuster et al. (1993)
rps12	2/5	1/11	U30458; GenBank 93
Total	37/173	39/546	
Frequency	0.21[a] ± 0.03	0.07 ± 0.01	

c. FREQUENCY OF GENOMIC C → T TRANSITIONS IN TOBACCO CHLOROPLAST GENES

	At Sites Edited from C to U in Maize	At Third Codon Position C in Maize	GenBank; Reference
Total	13/25	194/505	Z00044, X86563; Maier et al. (1995)
Frequency	0.52 ± 0.10	0.38 ± 0.02	

d. FREQUENCY OF GENOMIC C → T TRANSITIONS IN RICE CHLOROPLAST GENES

	At Sites Edited from C to U in Maize	At Third Codon Position C in Maize	GenBank; Reference
Total	5/25	59/610	X15901, X86563; Maier et al. (1995)
Frequency	0.20 ± 0.08	0.10 ± 0.01	

NOTE.—The transition rate excludes analysis of sites which have been substituted by a purine (see *Materials and Methods*), so that the total corresponds to the total in table 2, excluding transversions.

[a] Significantly different from third-codon-position frequencies, P values < 0.001, Fisher's exact test.

multiple hits was performed. For the calculation of chloroplast transition frequencies from monocot to dicot, the following 13 edited genes of *Zea mays* were used: *rpl2, ndhA, ndhB, petB, rpoB, ycf3, rpoC2, ndhD, ndhF, rps14, rpl20, atpA,* and *rps8* (Maier et al. 1995). For the calculation of chloroplast third-codon-position transition frequencies, the gene *rpoC2* was excluded because the alignment was unclear, while *ndhA* was excluded from the tobacco comparison because of an apparent frameshift.

The rate of evolution at bases flanking edited sites was investigated. To exclude biases arising from reading-frame constraints, only the contexts of second-codon-position edit sites were analyzed. Bases within eight residues of an edit site were analyzed (excluding those where the 17-base window included a gap in the alignment between dicot and monocot). The substitution frequencies (uncorrected for multiple hits) were calculated separately for the contexts of edit sites that are conserved between dicot and monocot and those that are

Table 2
Substitution Frequencies at Edited and Unedited Cytosines in Mitochondrial Genes

	Position in Codon			
	All	1	2	3
From edited C to edited C.....	0.58	0.53 (62)	0.71 (128)	0.20 (8)
From edited C to T.....	0.24	0.27 (32)	0.24 (44)	0.15 (6)
From edited C to unedited C.....	0.16	0.19 (22)	0.04 (8)	0.56 (23)
From edited C to A	0.02	0.02 (2)	0.01 (1)	0.07 (3)
From edited C to G	0.00	0.00 (0)	0.00 (0)	0.02 (1)
No. of sites	340	118	181	41
From unedited C to edited C	0.01	0.02 (22)	0.01 (8)	0.02 (23)
From unedited C to T	0.04	0.02 (26)	0.02 (27)	0.06 (71)
From unedited C to unedited C...	0.91	0.92 (1106)	0.94 (1,106)	0.86 (998)
From unedited C to A	0.03	0.03 (34)	0.02 (18)	0.04 (43)
From unedited C to G	0.01	0.01 (11)	0.01 (12)	0.02 (25)
No. of sites	3,530	1,199	1,171	1,160

Note.—Frequencies have been pooled from both the dicot/monocot and the monocot/dicot comparisons: actual numbers are given in parentheses.

diverged; for comparison, the substitution frequencies around conserved and diverged second-codon-position C residues were also calculated.

Results and Discussion

Rapid Evolutionary Turnover of Edited Sites

A site which is edited from a genomic C to a U in the mRNA may undergo a genomic mutation to a T without alteration of the amino acid sequence. All third-codon-position C residues can also synonymously substitute T. In the absence of selection constraints, it might be expected that these two groups of sequences would show similar rates of substitution. However, there is a higher rate of evolution at edited sites compared to synonymous nonedited sites both in mitochondria of dicots and monocots (table 1*a* and *b*) and in chloroplasts (table 1*c* and *d*). For the larger mitochondrial data set, this difference is highly significant (table 1*a* and *b*). In mitochondria, edited sites undergo four times more DNA replacements by T residues (28% of edited sites from monocot to dicot, and 21% of edited sites from dicot to monocot; table 1) compared to synonymously variable C residues (where only 6% of C residues are changed to T in both dicots and monocots). The elevated rate of evolution at mitochondrial edit sites is seen in almost all the genes investigated. Rates of C-to-T transition at third codon positions were almost identical when only two-fold degenerate sites were considered. A similar trend is seen at the 25 known chloroplast edited sites (table 1), although there are too few of these to assess its significance.

When the analysis is broken down according to codon position (table 2), it may be seen that there is a high rate of evolution for edited sites at both the first and second codon positions. The few sites edited at third codon positions show a higher frequency of loss of editing without mutation of the base (edited C transition to unedited C; table 2). This is not surprising, since the protein sequence is not altered by the loss of editing at the third base.

The high rate of evolution of edited sites seems likely to be a consequence of the editing; however, it is important to rule out the possibility that it reflects instead the higher mutability of a motif that is also coincidentally associated with editing. Covello and Gray (1990) showed that base frequencies around edited sites are biased. There is strong evidence for base preferences at the immediately upstream base, which is usually a T, and the downstream base, which is usually a purine R (Covello and Gray 1990). There are 77 unedited occurrences of the TCR motif in monocots where the C is at a third codon position, and is therefore unlikely to be subject to protein-coding constraints. Of these, only 6 (8%) have a T at the homologous site in the dicot. This is close to the frequency seen for all third-codon-position C's (table 2), indicating that the TCR motif shows no inherent propensity to be replaced by TTR in the absence of editing.

No Evidence of Sequence Conservation Around Conserved Edited Sites

While identification of a TCR edited motif might suggest that the contribution of neighboring RNA residues is limited to the flanking bases, other nearby residues may contribute in some way. They could possibly form particular motifs at a variable distance from the edit site (Gualberto, Weil, and Grienenberger 1990; Maier et al. 1992) or, by analogy with RNA editing in Trypanosomal mitochondria (Hajduk, Harris, and Pollard 1993), there may be a requirement for a "guide" RNA which is complementary to RNA around the edited site. However, there is no evidence for this as yet (Gualberto, Weil, and Grienenberger 1990; Bock and Maliga 1995). Covello and Gray (1990) found that differences in editing between plant species were associated with nearby base substitutions in the sequence of *coxII*. We investigated whether bases near a conserved edited site are conserved in 11 genes.

In order to remove the confounding influence of codon positional preferences, we only analyzed substitution rates around second-codon-position C residues.

Table 3
Frequencies of Base Substitution[a] Around Both Conserved and Diverged Second-Codon-Position Mitochondrial Editing Sites

Distance from Site	Conserved Sites[b]	Diverged Sites[b]
−8	0.06 (0.11)	0.10 (0.20)
−7	0.10 (0.07)	0.04 (0.13)
−6	0.02 (0.05)	0.02 (0.14)
−5	0.08 (0.10)	0.17 (0.24)
−4	0.02 (0.07)	0.02 (0.16)
−3	0.05 (0.05)	0.02 (0.12)
−2	0.05 (0.11)	0.08 (0.20)
−1	0.06 (0.06)	0.19 (0.22)
0	0 (0)	1 (1)
+1	0.11 (0.10)	0.12 (0.30)
+2	0.03 (0.07)	0.04 (0.15)
+3	0.02 (0.06)	0.04 (0.11)
+4	0.06 (0.11)	0.06 (0.20)
+5	0.10 (0.07)	0.02 (0.11)
+6	0.03 (0.05)	0.08 (0.11)
+7	0.23 (0.10)	0.17 (0.18)
+8	0.05 (0.07)	0.10 (0.15)

[a] Frequencies of substitution by any other base.

[b] Substitution rates in parentheses are for unedited second-codon-position C residues. Of the edited residues, 62 were conserved and 52 were found in only one species (compared with 2,530 conserved and 102 diverged unedited residues).

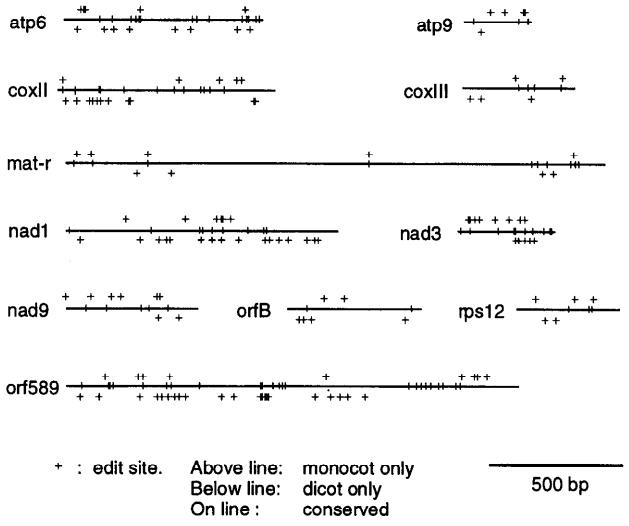

FIG. 1.—The distribution of conserved and diverged editing sites in monocot and dicot mitochondrial genes.

Initial inspection of the results suggests that there are differences in the levels of conservation between conserved and diverged edited sites: in particular, the upstream (−1) base is substituted more often at diverged sites (table 3). This would suggest that the substitution of the upstream base is associated with substitution of the editing site. However, the control group of unedited C residues showed a similar elevation in substitution rate when the residue was diverged (table 3). Therefore, the increased divergence may simply reflect a tendency for substitutions of dinucleotides to occur more often than expected by chance.

Substitution rates at unedited sites are highest at third codon positions (table 2), and are generally higher around diverged sites than around conserved sites. This may partly result from the clustering of amino acid replacements in unconstrained regions of the proteins. The pattern around edited sites is less regular, possibly because the sample sizes are smaller. However, substitutions occur at all distances from the edited sites, indicating that editing does not require the conservation of bases at any particular distance from the edited site. Thus, there is no strong sequence conservation around editing sites; neither is there significant sequence divergence around sites where editing differs between species.

Extensive Reverse Transcription Cannot Account for Rapid Evolution

It has been suggested that an apparent absence of editing sites in the last two-thirds of the monocot *coxI* gene (Covello and Gray 1990) may have arisen through a single gene conversion of the genomic sequence by an edited RNA via reverse transcription, prior to the divergence of maize, sorghum, rice, and wheat. This gene was not included in the present study because cDNA sequences were not available. A similar event is proposed to have converted the C-terminus of radish *atp6*, so that it does not require editing at about five sites (Gray and Covello 1993). The distribution of editing sites within the aligned coding sequences is largely random (fig. 1). The number of edited sites in all genes is similar in both dicot and monocot (table 1). Monocot edit sites which cluster within 20 bases of each other were placed into groups (41 in all, with an average of 3.0 sites in each). In order to test whether sites within a group tend to be conserved together or diverged together in dicots more often than expected at random, an analysis of variance was carried out. There was no strongly significant tendency for sites within a group to be more or less conserved in dicots ($P = 0.06$). It is certainly evident that many nonconserved edit sites are found in regions where editing is conserved (fig. 1), so that reverse transcription of DNA regions (of 20 bases or more) cannot account for the majority of editing differences, unless it predominantly uses RNA templates which are only very partially edited.

Role of Selection in Edit Site Evolution

The rapid evolution of editing sites cannot be explained by an increased rate of point mutation in the genomic DNA of the associated "TCR" motif, nor can it be explained by reverse transcription of tracts of DNA from a fully edited message. It is possible that selection may influence their gain and loss. As the numbers of edited sites in dicots and monocots are approximately similar, it is likely that similar selective constraints, if any, are acting in both lineages. There is no evident selective advantage for the acquisition of one set of editing sites in monocots and another in dicots. Therefore, if selection is indeed acting, the lack of conservation indicates that it is most likely to be selection against, rather than in favor of, editing sites. Editing is thus unlikely to have a significant regulatory role at the vast majority of edited sites. It is possible that the inefficien-

cies associated with editing, such as the production of a proportion of untranslatable messages and of nonfunctional proteins, represent a selective disadvantage.

A useful way of envisaging this situation is that at a given position there are three possible states: a uracil encoded by a genomic T, which is the most efficient, a uracil encoded by an edited C, which has an intermediate efficiency (i.e., an intermediate selection coefficient), and, finally, a cytidine which has not been edited and is strongly selectively disadvantageous. The frequency of editing sites is then determined by the selection coefficients associated with the three states. Such a simple three-state model is complicated by the fact that the edited state is dependent on editing factors outside the base itself. Thus, the strength of selection against an edited C and in favor of a genomic T may fluctuate as *trans*-acting editing factors evolve.

If editing is dependent on the existence of a long sequence motif or a large structure (either in *cis* within the neighboring RNA sequence or in *trans* such as that provided by a guide RNA), fixation of a mutation within the motif or structure which makes editing inefficient or nonexistent would create a strong selection pressure for the fixation of a C-to-T transition in the DNA. The high rate of loss of editing without genomic mutation of the C residue at third-codon-position sites (56%; table 2) supports this hypothesis, as it suggests that the rate of loss of editing is high in the absence of any influence on the protein-coding sequence of editing. While the number of third-codon-position edit sites is rather small, at first codon positions (whose mutations have less impact on amino acid properties compared to second-codon-position mutations) loss of editing without mutation has also occurred at a relatively high frequency (19%; table 2). However, there may be a tendency for synonymously variable sites to be only partially edited (e.g., Covello and Gray 1990; Hiesel, Combettes, and Brennicke 1990; Schuster et al. 1990; Gualberto et al. 1991), so that they may not be directly comparable with other edit sites: a larger and more uniformly characterized data set is required to establish that loss of editing is likely to precede replacement by a T in the DNA at sites which are constrained by protein conservation. An alternative model is that loss of editing factors outside the edited site itself follows mutation of the edited site (Bock and Maliga 1995), but this cannot account for the high rate of evolution of editing sites per se.

Bowe and dePamphilis (1996) reported that phylogenetic trees based on DNA are marginally more informative than those based on edited cDNAs; this property has been attributed to the increase in information resulting from the relaxation of constraint on nonsynonymous editable sites, which may be encoded by a T or a C. Our results indicate that edited sites may in fact be even more informative than synonymously variable sites over short evolutionary time spans, given their rapid turnover of pyrimidines. However, over greater evolutionary distances they could conceivably become saturated for change, so long as a given edited site which is substituted by a T retains a propensity for being edited, should it revert back to a C. Given that trees based on genomic DNA are at least as good as those based on cDNA sequences (Bowe and dePamphilis 1996), it is unlikely that saturation at editable sites seriously distorts the phylogenetic analyses of very divergent species.

In conclusion, this study has identified a high rate of evolution of edited sites. Regardless of whether this is driven by reverse transcription, by selection against inefficient protein production, or by frequent loss of editing motifs or structures, it is very clear that editing is not selectively advantageous at the bulk of edited sites. The continued maintenance of editing sites represents a balance between the chance mutation to sites that may be edited, and the processes of selection or gene conversion that rapidly remove them from the genome again. The low frequency of editing in chloroplasts compared to mitochondria could conceivably reflect higher selective constraints in the chloroplast, although the editing mechanisms may well differ in the two organelles. It is of interest that the TCR editing motif is more rigidly adhered to in the chloroplast editing sites (Maier et al. 1995), suggesting that these sites represent better substrates for the editing mechanism. The rarity of editing sites in certain species such as the club moss *Lycopodium squarrosum* (Hiesel, Combettes, and Brennicke 1994), and its apparent complete loss in the liverwort *Marchantia polymorpha* (Hiesel, Combettes, and Brennicke 1994; Gray 1996; Malek et al. 1996) is consistent with the hypothesis that selection pressures against editing may have been significantly large in certain evolutionary lineages. Differences in the numbers of edited sites in different species could reflect the intensity of selection against the mildly deleterious edited sites, which may be proportional to population size (i.e., the numbers of organelles within the cell, and/or the number of plants in the population). Given that edited sites are apparently not more advantageous than genomic T residues, editing may be viewed primarily as a historical accident that may have some beneficial effects, but whose continued existence is likely to reflect a dependency which is difficult to break (Gray and Covello 1993; Bock and Maliga 1995).

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LITERATURE CITED

BOCK, R., and P. MALIGA. 1995. In vivo testing of a tobacco plastid DNA segment for guide RNA function in *psbL* editing. Mol. Gen. Genet. **247**:439–443.

BOWE, L. M., and C. W. DEPAMPHILIS. 1996. Effects of RNA editing and gene processing on phylogenetic reconstruction. Mol. Biol. Evol. **13**:1159–1166.

CATTANEO, R. 1991. Different types of messenger RNA editing. Annu. Rev. Genet. **25**:71–88.

CHAPDELAINE, Y., and L. BONEN. 1991. The wheat mitochondrial gene of subunit I of the NADH dehydrogenase complex: a trans-splicing model for this gene-in-pieces. Cell **65**: 465–472.

COVELLO, P. S., and M. W. GRAY. 1990. Differences in editing at homologous sites in messenger RNAs from angiosperm mitochondria. Nucleic Acids Res. **18**:5189–5196.

FREYER, R., C. LOPEZ, R. M. MAIER, M. MARTIN, B. SABATER, and H. KOSSEL. 1995. Editing of the chloroplast *ndhB* encoded transcript shows dvergence between closely related members of the grass family (Poaceae). Plant Mol. Biol. **29**:679–684.

GONZALEZ, D. H., G. BONNARD, and J. M. GRIENENBERGER. 1993. A gene involved in the biogenesis of c-type cytochromes is co-transcribed with a ribosomal protein in wheat mitochondria. Curr. Genet. **24**:248–255.

GRAY, M. W. 1996. RNA editing in plant organelles: a fertile field. Proc. Natl. Acad. Sci. USA **93**:8157–8159.

GRAY, M. W., and P. S. COVELLO. 1993. RNA editing in plant mitochondria and chloroplasts. FASEB J. **7**:64–71.

GROHMANN, L., O. THIECK, U. HERZ, W. SCHROEDER, and A. BRENNICKE. 1994. Translation of *nad9* mRNAs in mitochondria from *Solanum tuberosum* is restricted to completely edited transcripts. Nucleic Acids Res. **22**:3304–3311.

GUALBERTO, J. J., J.-H. WEIL, and J. M. GRIENENBERGER. 1990. Editing of the wheat coxIII transcript: evidence for twelve C to U and one U to C conversions and for sequence similarities around editing sites. Nucleic Acids Res. **18**: 3771–3776.

GUALBERTO, J. M., G. BONNARD, L. LAMATTINA, and J. M. GRIENENBERGER. 1991. Expression of the wheat mitochondrial nad3-rps12 transcription unit: correlation between editing and mRNA maturation. Plant Cell **3**:1109–1120.

HAJDUK, S. L., M. E. HARRIS, and V. W. POLLARD. 1993. RNA editing in kinetoplastid mitochondria. FASEB J. **7**:54–63.

HIESEL, R., B. COMBETTES, and A. BRENNICKE. 1994. Evidence for RNA editing in mitochondria of all major groups of land plants except the Bryophyta. Proc. Natl. Acad. Sci. USA **91**:629–633.

HIESEL, R., R. WISSINGER, and A. BRENNICKE. 1990. Cytochrome oxidase subunit II mRNAs in *Oenothera* mitochondria are edited at 24 sites. Curr. Genet. **18**:371–375.

KEMPKEN, F., J. A. MULLEN, D. R. PRING, and H. V. TANG. 1991. RNA editing of sorghum mitochondrial *atp6* transcripts changes 15 amino acids and generates a carboxy-terminus identical to yeast. Curr. Genet. **20**:417–422.

LAMATTINA, L., D. GONZALEZ, J. GUALBERTO, and J. M. GRIENENBERGER. 1993. Higher plant mitochondria encode a homologue of the nuclear-encoded 30-kDa subunit of bovine mitochondrial complex I. Eur. J. Biochem **217**:831–838.

MAIER, R., K. NECKERMANN, B. HOCH, N. B. AKHMEDOV, and H. KOSSEL. 1992. Identification of editing positions in the *ndhB* transcript from maize chloroplasts reveals sequence similarities between editing sites of chloroplasts and plant mitochondria. Nucleic Acids Res. **20**:6189–6194.

MAIER, R. M., K. NECKERMANN, G. L. IGLOI, and H. KOSSEL. 1995. Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing. J. Mol. Biol. **251**: 614–628.

MALEK, O., K. LATTIG, R. HIESEL, A. BRENNICKE, V. KNOOP. 1996. RNA editing in bryophytes and a molecular phylogeny of land plants. EMBO J. **15**:1403–1411.

NOWAK, C., and U. KUCK. 1990. RNA editing of the mitochondrial atp9 transcript from wheat. Nucleic Acids Res. **18**:7164.

SCHUSTER, W., and A. BRENNICKE. 1991*a*. RNA editing in ATPase subunit 6 mRNAs in *Oenothera* mitochondria. FEBS Lett. **295**:97–101.

———. 1991*b*. RNA editing of ATPase subunit 9 transcripts in *Oenothera* mitochondria. FEBS Lett. **268**:252–256.

SCHUSTER, W., B. COMBETTES, K. FLIEGER, and A. BRENNICKE. 1993. A plant mitochondrial gene encodes a protein involved in cytochrome c biogenesis. Mol. Gen. Genet. **239**: 49–57.

SCHUSTER, W., R. TERNES, V. KNOOP, R. HIESEL, B. WISSINGER, and A. BRENNICKE. 1991. Distribution of RNA editing sites in *Oenothera* mitochondrial mRNAs and rRNAs. Curr. Genet. **20**:397–404.

SCHUSTER, W., B. WISSINGER, M. UNSELD, and A. BRENNICKE. 1990. Transcripts of the NADH-dehydrogenase subunit 3 gene are differentially edited in *Oenothera* mitochondria. EMBO J. **9**:263–269.

SUTTON, C. A., P. L. CONKLIN, K. D. PRUITT, and M. R. HANSON. 1991. Editing of pre-mRNAs can occur before cis- and trans-splicing in Petunia mitochondria. Mol. Cell. Biol. **11**:4274–4277.

THOMPSON, M. C., J. L. MACFARLANE, C. T. BEAGLEY, and D. R. WOLSTENHOLME. 1994. RNA editing of *mat-r* transcripts in maize and soybean increases similarity of the encoded protein to fungal and bryophyte group II intron maturases: evidence that *mat-r* encodes a functional protein. Nucleic Acids Res. **22**:5745–5752.

WISSINGER, B., W. SCHUSTER, and A. BRENNICKE. 1991. *Trans* splicing in Oenothera mitochondria: *nad1* mRNAs are edited in exon and *trans*-splicing group II intron sequences. Cell **65**:473–482.

WOLFE, K. H. 1996. Molecular evolution of plants: more genomes, fewer generalities. Pp. 45–57 *in* J. BARBER and B. ANDERSSON, eds. Molecular genetics of photosynthesis, *volume in* D. HAMES and D. GLOVER eds. Frontiers of molecular biology series. IRL Press, Oxford.

ZELTZ, P., W. R. HESS, K. NECKERMANN, T. BORNER, and H. KOSSEL. 1993. Editing of the chloroplast *rpoB* transcript is independent of chloroplast translation and shows different patterns in barley and maize. EMBO J. **12**:4291–4296.

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