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LETTER TO THE EDITOR

RNA editing missing in mitochondria

ANDREW T. ORR, JOHN C. RABETS, TAMARA L. HORTON, and LAURA F. LANDWEBER

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA

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Since the discovery of myriad forms of RNA editing in eukaryotes, there has been investigation into the origins and distribution of each type of editing (Benne, 1993; Landweber & Gilbert, 1994; Maslov et al., 1994; Yokobori & Pääbo, 1995). RNA editing is the insertion, deletion, or substitution of nucleotides in the primary sequence of transcripts. These events create initiation and termination codons, alter structural features of transcripts, and generate more than 90% of the amino acid codons in some mitochondrial genes (Benne, 1993; Landweber & Gilbert, 1993) (Fig. 1). These different types of RNA editing are identified by differences between RNA and genomic DNA sequences.

We investigated the mitochondrial genomes of the nematode *Caenorhabditis elegans* (Okimoto et al., 1992) and the ciliate *Paramecium tetraurelia* (Pritchard et al., 1990) for evidence of editing, because several unusual features of these fully sequenced mitochondrial genomes have been explained by editing in other organisms. We examined transcripts of 12 genes considered the most likely candidates for editing in *C. elegans* and *P. tetraurelia* (Fig. 1) based on the observation that: (1) all but one (ORF12) lack encoded AUG start codons; (2) many have extremely divergent predicted protein sequences, particularly at the N-terminus (*P. tetraurelia* CYb; ND2 in both species); (3) homologues are edited in other organisms; (4) one locus (ORF12/13) contains two overlapping reading frames that could be joined by an insertional editing event, similar to kinetoplastid editing of COII; and (5) *C. elegans* tRNAs (Okimoto et al., 1990) and SSU-rRNAs (Okimoto et al., 1994) are the smallest known for metazoa and lack many conventional features. Primer extension of the SSU-rRNA indicated single-base size heterogeneity in the first 70 nt (Okimoto et al., 1994), making this gene a candidate for insertional editing. In addition, *C. elegans* shares with Euglenozoa—including the kinetoplastids notorious for extensive RNA editing—another aberrant form of RNA processing: *trans* splicing, which has some similarities to kinetoplastid editing (Benne, 1993).

In all cases, the cDNA sequences matched the DNA sequences exactly, with the exception of single nucleotide differences among some clones. These were within RT-PCR error (Landweber et al., 1993), and there was no common pattern to suggest editing. The only change we observed in the *C. elegans* SSU-rRNA was a single nucleotide discrepancy at the 5′ terminus. Thus, we conclude that editing does not play a role in the maturation of these transcripts.

These data thus provide the strongest support for use of noncanonical initiation codons in these organisms (AUA, AUU, UUG assigned to *C. elegans* COIII, COII, ND2, respectively; AUC, UUG, GUG assigned to *P. tetraurelia* CYb, ND2, and COII [Pritchard et al., 1990]), including the first usage of UUG in metazoa (Okimoto et al., 1990) (Fig. 2). Editing of the $tRNA^{Met}$ anticodon, which could improve recognition of non-AUG initiation codons, was not found. These data suggest that the unusual features of these two mitochondrial genomes—including missing AUG start codons (Okimoto et al., 1990; Pritchard et al., 1990), and missing genes or 5′ portions of genes (Pritchard et al., 1990)—do not prevent the expression of functional genes. The possibility of finding RNA editing in other systems can only be tested by the examination of RNA sequences against DNA sequences in the expanding database.

Reprint requests to: Laura F. Landweber, Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA; e-mail: lfl@princeton.edu.

Abbreviations: CYb, cytochrome *b*; CO, cytochrome oxidase; ND, NADH dehydrogenase; ORF, open reading frame; SSU-rRNA, small subunit ribosomal RNA.

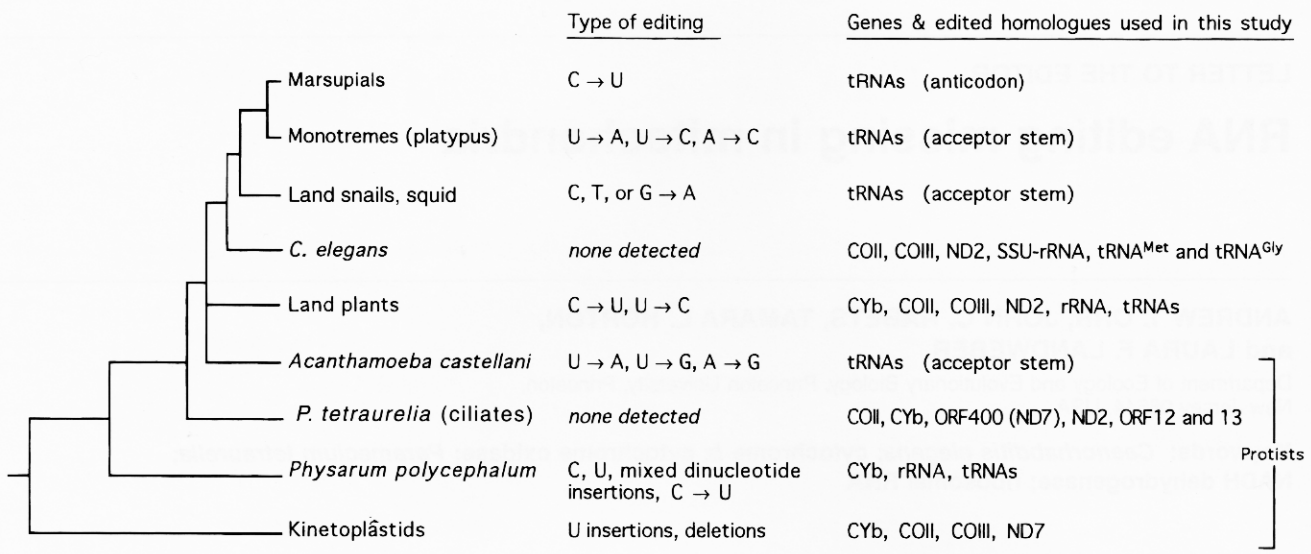

FIGURE 1. Distribution of RNA editing in mitochondria. Listed are taxa that contain mitochondrial editing systems and their edited homologues of genes investigated in this study. Branch lengths and order are schematic and based on published SSU-rRNA trees (Cavalier-Smith, 1993; Wainright et al., 1993; Winnepenninckx et al., 1995). cDNA sequences were obtained from *P. tetraurelia* (ATCC 30567) and *C. elegans* by 5′-RACE or RT-PCR (Landweber & Gilbert, 1993) using a Thermolyne Amplitron II thermocycler, followed by either direct sequencing or cloning and sequencing of several transcripts derived from total RNA. Primers were chosen from conserved regions to avoid bias against transcripts that might be edited in these regions. Sequences of primers and clones available upon request. The ORF12/13 DNA and cDNA sequences (GenBank accession no. U71284) differed from the published genomic sequence (Pritchard et al., 1990) at 14 nt, implying an error in the original sequence or polymorphism in this strain. One of these differences, a C insertion at position 27089, joined the two reading frames. 5′-RACE mapped the 5′ end of *P. tetraurelia* ND2 to position 13,478; CYb to 19,310; COII to 23,742 on X15917 (Pritchard et al., 1990), and the 5′ end of most *C. elegans* transcripts to the start codon.

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RNA  UUGAUUGUUUUUAUUUCUUUAUUU
DNA  TTGATTGTTTTTATTTCTTTATTT

RNA  UUUUUGAAUCUCCUUAUGAUAAAUCUU
DNA  TTTTTGAATCTCCTTATGATAAATCTT
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FIGURE 2. Alignment of 5′ ends of the ND2 transcript. Comparison of 5′ ends of representative RACE clones with the mtDNA for *C. elegans* (top) and *P. tetraurelia* (bottom). Lack of a 5′ untranslated region is typical for *C. elegans* mitochondrial genes. The UUG start codon (underlined) in the *Paramecium* sequence is located 69 nt downstream of the one assigned by Pritchard et al. (1990).

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REFERENCES

Benne R, ed. 1993. *RNA editing*. New York: Ellis Horwood.

Cavalier-Smith T. 1993. Kingdom Protozoa and its 18 phyla. *Microbiol Rev 57*:953–994.

Landweber LF, Fiks AG, Gilbert W. 1993. The boundaries of partially edited cytochrome *c* oxidase III transcripts are not conserved in kinetoplastids: Implications for the guide RNA model of editing. *Proc Natl Acad Sci USA 90*:9242–9246.

Landweber LF, Gilbert W. 1993. RNA editing as a source of genetic variation. *Nature 363*:179–182.

Landweber LF, Gilbert W. 1994. Phylogenetic analysis of RNA editing: A primitive genetic phenomenon. *Proc Natl Acad Sci USA 91*:918–921.

Maslov DA, Avila HA, Lake JA, Simpson L. 1994. Evolution of RNA editing in kinetoplastid protozoa. *Nature 368*:345–348.

Okimoto R, Macfarlane JL, Clary DO, Wolstenholme DR. 1992. The mitochondrial genomes of two nematodes, *Caenorhabditis elegans* and *Ascaris suum*. *Genetics 130*:471–498.

Okimoto R, Macfarlane JL, Wolstenholme DR. 1990. Evidence for the frequent use of TTG as the translation initiation codon of mitochondrial protein genes in the nematodes, *Ascaris suum* and *Caenorhabditis elegans*. *Nulceic Acids Res 18*:6113–6118.

Okimoto R, Macfarlane JL, Wolstenholme DR. 1994. The mitochondrial ribosomal RNA genes of the nematodes *Caenorhabditis elegans* and *Ascaris suum*: Consensus secondary-structure models and conserved nucleotide sets for phylogenetic analysis. *J Mol Evol 39*:598–613.

Pritchard AE, Seilhamer JJ, Mahalingam R, Sable CL, Venuti SE, Cummings DJ. 1990. Nucleotide sequence of the mitochondrial genome of *Paramecium*. *Nucleic Acids Res 18*:173–180.

Tomita K, Ueda T, Watanabe K. 1996. RNA editing in the acceptor stem of squid mitochondrial $tRNA^{Tyr}$. *Nucleic Acids Res 24*:4987–4991.

Wainright PO, Hinkle G, Sogin ML, Stickel SK. 1993. Monophyletic origins of the metazoa: An evolutionary link with fungi. *Science 260*:340–342.

Winnepenninckx B, Backeljau T, Mackey LY, Brooks JM, De Wachter R, Kumar S, Garey JR. 1995. 18S rRNA data indicate that aschelminthes are polyphyletic in origin and consist of at least 3 distinct clades. *Mol Biol Evol 12*:1132–1137.

Yokobori S, Pääbo S. 1995. tRNA editing in metozoans. *Nature 377*:490.