

Evolutionary fixation of RNA editing

SIR — Gene sequences encoded in the organellar genomes of many organisms are incompatible with function of the gene product. The ‘errors’ in the sequence are corrected after transcription of the gene by processes described by the term ‘RNA editing’¹. The persistence throughout evolution of organellar RNA editing is perplexing, because it might have been expected that back-mutations would have quickly removed the need for RNA editing systems.

In the mitochondrial genome of marsupials, the transfer (t)RNA^{Asp} gene carries the anticodon GCC, which potentially recognizes two glycine codons². The second position of the anticodon (position 35) is changed post-transcriptionally so that the canonical aspartate anticodon GUC is created in approximately 50% of the tRNA pool^{2,3}. Considering that this seems to be the only edited position in this genome⁴, and in view of the high rate of evolution of the mammalian mitochondrial genome⁵ that should allow for frequent back-mutations, it is particularly puzzling why this nucleotide has come to be corrected by an RNA editing event rather than by a simple substitution in the gene.

It is known that whereas the edited version of the tRNA^{Asp} (anticodon GUC) gets aminoacylated with aspartate, its

	32	38
opossum	UUUCAA	
kangaroo	U-----	
Platypus	C-----	
mouse	C-----	
cow	C-----	
human	C-----	
chicken	C-----	
frog	C-----	
Drosophila	C-----	

FIG. 1 Alignment of anticodon loop sequences (nucleotides 32–38) of the tRNA^{Gly} from two marsupials (ref. 4 and M. Dörner, personal communication) and some representative animals^{8,12}. The U at position 32 is green; identical bases are indicated by a dashed line.

unedited version (anticodon GCC) is aminoacylated with glycine⁶, suggesting that the unedited tRNA^{Asp}(GCC) functions as a glycyl-tRNA. At the same time, a functional tRNA^{Gly} with the expected anticodon UCC exists in marsupial mitochondria^{4,6}. Whereas the tRNA^{Gly}(UCC) will recognize all four glycine codons (GGN)⁷, the unedited version of tRNA^{Asp}(GCC) will recognize only two of these (GGY). Considering that the mitochondrial translation machinery of animals contains a limited set of only 22 tRNAs, it is surprising that two tRNAs recognizing an overlapping set of glycine codons should exist in marsupials.

A comparison of the marsupial

tRNA^{Gly}(UCC) gene sequences with those of other animals⁸ (Fig. 1) shows that at position 32, two nucleotides 5’ of the anticodon, marsupials carry a U residue whereas most other metazoans have a C residue. In an *Escherichia coli* translation system, it has been shown that a C-to-U substitution at that position restricts the decoding capacity of tRNA^{Gly}(UCC) to the codons GGA and GGG⁹. Thus, it is likely that the marsupial tRNA^{Gly}(UCC) decodes only the codons GGA and GGG, and that the remaining glycine codons GGC and GGU are read by the unedited tRNA^{Asp}(GCC). Consequently, the decoding capacities of the two glycyl-tRNAs would not overlap (Fig. 2).

These findings suggest the following evolutionary pathway for the fixation of RNA editing in marsupial mitochondria: the first mutation was a T-to-C transition in the anticodon of tRNA^{Asp}. Because this mutation would have been lethal for the organism, a ‘precursor’ editing activity probably existed. Such an activity could have been related to, for example, the de-amination of other biomolecules¹⁰. The adaptation of this activity to the editing of tRNA^{Asp} was presumably made possible by the multi-copy state of the mitochondrial genome, which can allow the selection of adaptive changes to take place. At first, the function of the unedited form of tRNA^{Asp} as a glycine isoacceptor would have been redundant. But it would have then created a situation where the substitution at position 32 of the tRNA^{Gly}(UCC) could occur, limiting its decoding capacity to the two glycine codons not decoded by the unedited form of tRNA^{Asp}(GCC).

Once fixed in the germ line, the secondary substitution in the tRNA^{Gly}(UCC) gene would have made an elimination of the tRNA^{Asp} substitution by a back-mutation lethal, as such a mutation would leave two out of four glycine codons without a decoding tRNA (Fig. 2). Thus, the elimination of the editing would require two back-mutations in the same mitochondrial lineage. In effect, the secondary mutation in the tRNA^{Gly} has therefore caused the editing of tRNA^{Asp} to become fixed in marsupial mitochondria.

We suggest that such combinations of mutations represent ‘genetic gridlocks’ that could be responsible for the occurrence of RNA editing in organellar

genomes. Because in other systems many positions have become dependent on RNA editing subsequent to the initial events, the initial mutations that have caused the evolutionary fixation of editing are probably indiscernible today.

An additional fascinating aspect of this system is that the primary transcript of the marsupial tRNA^{Asp} gene represents a naturally occurring tRNA that, depending on its state of editing, is assigned to one or the other codon

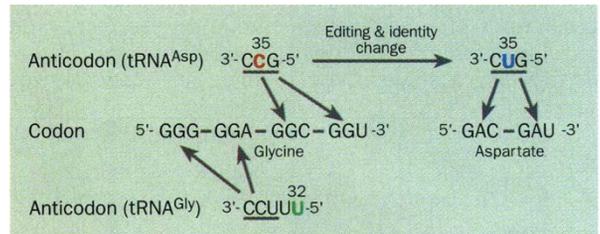


FIG. 2 Schematic illustration of the putative functions of tRNA^{Gly} and tRNA^{Asp} in marsupials. The tRNA^{Gly}(UCC) is restricted to two glycine codons owing to the substitution at position 32. The remaining two glycine codons can be recognized by the unedited version of tRNA^{Asp}(GCC). Subsequent to anticodon editing, this tRNA recognizes the two aspartate codons.

family. When such cases of RNA editing of anticodons are not accompanied by a change in tRNA identity, they may have been instrumental in the reassignments of codons to new amino acids in mitochondria¹¹.

**G. Valentin Börner
Svante Pääbo**

*Institute of Zoology,
University of Munich,
PO Box 202136,
D-80021 Munich,
Germany*

- Benne, R. *Curr. Opin. Genet. Dev.* **6**, 221–231 (1996).
- Janke, A. & Pääbo, S. *Nucleic Acids Res.* **21**, 1523–1525 (1993).
- Mörl, M., Dörner, M. & Pääbo, S. *Nucleic Acids Res.* **23**, 3380–3384 (1995).
- Janke, A., Feldmaier-Fuchs, G., Thomas, W. K., von Haeseler, A. & Pääbo, S. *Genetics* **137**, 243–256 (1994).
- Brown, W. M., George, M. & Wilson, A. C. *Proc. Natl Acad. Sci. USA* **76**, 1971–1976 (1979).
- Börner, G. V., Mörl, M., Janke, A. & Pääbo, S. *EMBO J.* (in the press).
- Barrell, B. G. et al. *Proc. Natl Acad. Sci. USA* **77**, 3164–3166 (1980).
- Steinberg, S., Misch, A. & Sprinzl, M. *Nucleic Acids Res.* **21**, 3011–3015 (1993).
- Lustig, F. et al. *Proc. Natl Acad. Sci. USA* **90**, 3343–3347 (1993).
- Covello, P. & Gray, M. W. *Trends Genet.* **9**, 265–268 (1993).
- Schultz, D. W. & Yarus, M. J. *Mol. Biol.* **235**, 1377–1380 (1994).
- Janke, A., Feldmaier-Fuchs, G., Gemmell, N., von Haeseler, A. & Pääbo, S. *J. Mol. Evol.* **42**, 153–159 (1996).

Correction

In the reply by D. Tilman, C. Lehman, R. May and M. Nowak to S. Budyanskiy in Scientific Correspondence of 18 July 1996 (*Nature* **382**, 215–216; 1996), the third full paragraph, line two, the number that appeared as ‘104’ should in fact have been ten raised to the fourth power, or 10,000. □