correspondence

Transfer RNA genes in pieces are an ancestral character

Randau & Söll (2008) suggest that the anticodon loop region of transfer RNA (tRNA) genes is the target site of a huge variety of mobile genetic elements and that this resulted in the evolution of tRNA genes in pieces. They therefore suggest that the presence of introns in the anticodon loop might not be a plesiomorphic trait that is important in tRNA origin (Di Giulio, 2006), but an acquired trait, as it endowed tRNA genes with a precious protection mechanism against the integration of viruses and other autonomous genetic elements, in that the intron removed the integration site of these mobile genetic elements. Similarly, Randau & Söll maintain that split and permuted tRNA genes might also have been able to prevent the integration of mobile genetic elements, as their attachment sites were evidently disrupted and hence restricted their propagation within the genome, thus conferring a selective advantage. This evolution should not have been possible as Randau & Söll consider split tRNA genes to be derived from tRNA genes containing the intron, which, once inserted into the anticodon loop region of tRNA genes, would have disrupted the attachment site for mobile genetic elements. After this disruption there would have been no selective pressure to continue the evolutionary process—that is, to actually fragment the tRNA genes. More generally, it is expected that the ease with which introns might have been inserted into tRNA genes compared with the complexity of evolving tRNA genes in pieces (Di Giulio, 2006, 2008) would lead to the conclusion that the insertion of introns would always have been preferred to the evolution of tRNA genes in pieces as a mechanism to remove the attachment sites of mobile genetic elements.

Furthermore, if the intron in the anticodon loop of tRNA were truly an acquired trait, as Randau & Söll maintain, this would lead to the prediction that an organism such

as *Nanoarchaeum equitans* should have numerous tRNA genes with introns, as this organism must have been subjected to a considerable selective pressure from mobile genetic elements, as they hypothesize, in that this is the only organism in which split tRNA genes have been observed. This prediction is not supported by the facts: in *N. equitans* there are only four tRNA genes with introns and six tRNA split genes, whereas all the others are genes continuously coding for tRNA (Lowe & Eddy, 1997). The hypothesis of Randau & Söll would also predict—if the intron in the anticodon loop of tRNA genes had truly bestowed a strong selective advantage such as to make tRNA genes no longer vulnerable to the attack of mobile genetic elements—that the frequency of the class of tRNA genes with introns should be generally higher than that of the class of uninterrupted tRNA genes. However, this prediction is not supported by the observations: the most frequent tRNA genes are the ones that continuously code for tRNA (Lowe & Eddy, 1997). Nevertheless, in several archaeobacteria and in some eukaryotes, it can be observed that the number of tRNA genes with an intron is higher than that of uninterrupted tRNA genes (Lowe & Eddy, 1997). However, although the hypothesis of Randau & Söll suggests that the selective pressure to remove the integration site in the anticodon loop of tRNA genes must have been particularly high, split tRNA genes, for example, have not been produced or other types of tRNA genes in pieces been observed in these organisms. In short, the facts contradict the predictions of Randau & Söll's hypothesis.

Randau & Söll maintain that the most convincing evidence for a late acquisition of introns originates from the identification of highly variable insertion sites of introns in the crenarchaeal tRNA genes. In their examples, introns of the type bulge-helix-bulge are inserted into other domains of the tRNA molecule and also disconnect the two halves of tRNA as multiple introns. Although these examples show that these introns were acquired, this does not prove that the main intron in the anticodon loop is not a plesiomorphic trait. In

other words, the intron present in the anticodon loop of tRNA genes might well be a plesiomorphic trait, whereas the same type of intron present in other parts of the molecule might be an apomorphy. This is in perfect agreement with the observation that the introns present in the anticodon loop of tRNA genes of Archaea and Eukarya are of the same type (Haugen *et al*, 2005) and are therefore probably extremely ancient.

REFERENCES

- Di Giulio M (2006) The non-monophyletic origin of the tRNA molecule and the origin of genes only after the evolutionary stage of the Last Universal Common Ancestor (LUCA). *J Theor Biol* **240**: 343–352
- Di Giulio M (2008) Permuted tRNA genes of *Cyanidioschyzon merolae*, the origin of the tRNA molecule and the root of the Eukarya domain. *J Theor Biol* **253**: 587–592
- Haugen P, Simon DM, Bhattacharya D (2005) The natural history of group I introns. *Trends Genet* **21**: 111–119
- Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improving detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* **25**: 955–964 (<http://lowelab.ucsc.edu/GtRNAdb/>)
- Randau L, Söll D (2008) Transfer RNA genes in pieces. *EMBO Rep* **9**: 623–628

Massimo Di Giulio is at the Laboratory for Molecular Evolution, Institute of Genetics and Biophysics 'Adriano Buzzati Traverso', CNR, Via P. Castellino 111, 80131 Naples, Italy. E-mail: digiulio@igb.cnr.it

doi:10.1038/embor.2008.153

Response by Lennart Randau & Dieter Söll

We appreciate the comments of Massimo Di Giulio on the ancestral character of intron-containing transfer RNAs (tRNAs), which reinforce his previously published views on the origin of the tRNA molecule (Di Giulio, 1999, 2006).

Our main motivation to describe a scenario of tRNA introns as acquired resistance against integrative elements was not to contribute to the long-fought intron-late/intron-early discussion, but to present a view