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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.

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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

on the purpose of modern tRNA introns. Most introns of archaeal tRNAs are too short to have a different function other than to disrupt their tRNA genes. Therefore, our article (Randau & Söll, 2008) addresses the question as to what the evolutionary advantage of these disruptions would be and why they would be maintained in the genome.

Di Giulio offers predictions that do not follow logically from our hypothesis. These predictions include that *Nanoarchaeum equitans* should contain more disrupted tRNA genes than it does and that, in general, intron-containing tRNA genes should outnumber continuous genes. It is not correct to assume that, if disrupted tRNA genes can be advantageous, all (or most) genes would be expected to be split. We believe that the occurrence of tRNA introns can only be evaluated in consideration of (i) the entirety of integrative elements and (ii) the degree of the negative impact that the integration of these elements has on the host. Integrative elements harmful to the host might target only specific tRNA isoacceptors. Some integration events are beneficial to the host and should be tolerated. Therefore we disagree that a 'the more introns—the better' scenario would be favourable for the cell as evolution must strike a balance between these opposing selection pressures. We believe that the process of intron acquisition and integration resistance is a rather dynamic process in the ongoing co-evolution between integrative elements and their hosts. This is in agreement with the observation that even closely related species—for example, the four genome sequences from *Pyrobaculum* species—display a fairly divergent set of tRNA introns and intron placements. Di Giulio states that the ancient character of introns is evidenced by the similarity of archaeal and eukaryal introns (Haugen *et al.*, 2005). Here, one should distinguish that although the splicing machinery is

expected to be ancient, this is not necessarily true for the introns found in modern tRNA. Clearly the tRNA splicing endonucleases co-evolved with their substrates' splicing motifs (Randau *et al.*, 2005; Tocchini-Valentini *et al.*, 2005) and would be able to process the same intron type in different tRNA genes as introns are acquired and lost during evolution.

We would also like to state that we do not believe that the split tRNA genes of *N. equitans* would provide a different or even better protection against integrative elements than introns. We consider that split tRNA genes are the result of the same genomic rearrangements that led (i) to an unusually high number of genes split between functional domains of the encoded proteins and (ii) to the dispersion of conserved gene clusters such as the ribosomal protein cluster (Waters *et al.*, 2003). These unique rearrangements are a possible consequence of the fast evolutionary pace and the parasitic lifestyle of *N. equitans* (Brochier *et al.*, 2005).

In the final paragraph, Di Giulio comments on our evidence for the acquisition of introns exemplified by the flexible insertion sites of crenarchaeal introns and the presence of multiple introns. We do not see why it can be agreed that these unusual introns are acquired, whereas an intron located in the anticodon has to be considered as the ancestral 'main intron'. All tRNA introns within one organism are uniformly processed by the same splicing machinery. The increased flexibility of the intron insertion site in Crenarchaeota can merely be seen in conjunction with the relaxed splicing motif recognition of their tRNA endonuclease family (Calvin *et al.*, 2005; Randau *et al.*, 2005).

In conclusion, we think that our presented hypothesis on the dynamics of intron evolution conforms to investigations of the

ancient splicing machinery and encourages not only a stimulating discussion but also an analysis of the relationship between viruses and other integrative elements and their hosts' tRNA gene structure.

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