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(RESEARCH ARTICLE)

Did transfer RNA evolve from a ribozyme? An *in-silico* study

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Abstract

Transfer RNA (tRNA) is widely believed to be one of the oldest, if not *the* oldest nucleic acid on Earth. Concurrently, ribozymes, RNA-only catalysts that perform many of the same functions as present-day protein enzymes, are also thought to be just as ancient. While the position has been posited that tRNA, nature's chief aminoacylator of amino acids with the assistance of aminoacyl-tRNA synthetase (aaRS) protein enzymes, evolved from a self-aminoacylating ribozyme, no studies have been performed, to the best of this author's knowledge, searching for nucleotide sequence correlation between the two; such correlation would indicate the conservation of part or all of such a ribozyme in modern-day tRNA. To that end, an *in-silico* study utilizing several databases was performed to search for a high percentage of highly conserved nucleotide sequences in archaea, believed to be the most ancient of organisms, with very successful results and their implications discussed here.

Keywords: tRNA; Ribozyme; Nucleic Acid; Evolution; aaRS; *In-silico*

1. Introduction

Transfer RNA (tRNA) is widely believed to be one of the oldest, if not *the* oldest nucleic acid on Earth [1]. Concurrently, ribozymes, RNA-only catalysts that perform many of the same functions as present-day protein enzymes, are also thought to be just as ancient [2]. While the position has been posited that tRNA, nature's chief aminoacylator of amino acids with the assistance of aminoacyl-tRNA synthetase (aaRS) protein enzymes, evolved from a self-aminoacylating ribozyme, no studies have been performed, to the best of this author's knowledge, searching for nucleotide (nt) sequence correlation between the two; such correlation would indicate the conservation of part or all of such a ribozyme in modern-day tRNA [3]. To that end, an *in-silico* study utilizing several databases was performed to search for a high percentage of highly conserved nt sequences among archaea, believed to be the most ancient of organisms [1], with very successful results and their implications discussed here.

2. Methodology

The search for conserved aminoacylating ribozyme nts in archaea was based on Burton's tRNA model (Figure 1) [1]. This model posits that primordial tRNA ($tRNA^{Pri}$) was composed of three 31-nucleotide (nt) minihelices (the D-Loop, anticodon (Ac) loop, and T-Loop), all formed from prebiotic condensation/dehydration reactions. These were then ligated together to form the 93-nt $tRNA^{Pri}$, with the nearly universally conserved -ACCA acceptor stem sequence added for a total of 97-nts. Two subsequent 9-nt deletions formed first the longer type II tRNAs (88-nts) and second the shorter type I tRNAs (79-nts) [1], To facilitate the search, the middle Ac loop was replaced with a 31-nt minihelix containing the sequence of a 26-nt truncated C3 RNA derived from Hepatitis D Virus/viroid (HDV); this RNA is that from which Yarus' five-nucleotide self-aminoacylating ribozyme is derived [4]. The resulting 97-nt primordial prime sequence ($tRNA^{Pri'}$, Figure 2) was then entered into both the tRNAscan-SE (http://trna.ucsc.edu/tRNAscan-SE/) and the NCBI Nucleotide

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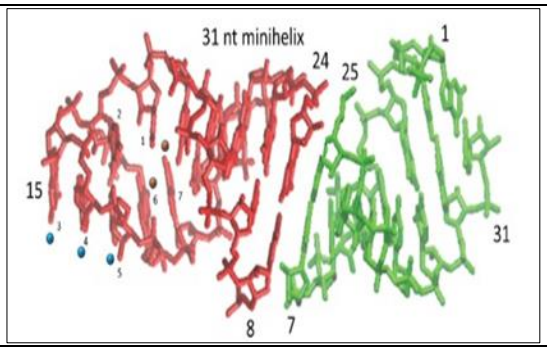
Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) databases and the results compared. While modern archaea employ protein endonuclease enzymes for scission of introns and subsequent ligation, in a prebiotic environment. these actions were most likely performed by ribozymes, and it should be noted here that, in addition to self-aminoacylation ribozymes, HDV RNA also possesses both lyase and ligase ribozymes as well [5]

Figure 1 Models for the evolution of type-I and type-II tRNAs. 5′ and 3′ acceptor stems are shaded green. The D loop 17-nt microhelix is shaded magenta. U-turn stem-loop-stems are shaded yellow (stems) and red (7-nt U-turn loop).

GCGGCGGUAGCCUAGCCUGGCCUACCGCCGC 31-nt D loop minihelix GGACUUCGGUCCUGUGGCGAAAGCCUAACCC 31-nt minihelix containing truncated C3 aminoacylating ribozyme GGCCGCCCCGGGUUCAAAUCCCGGCCGCCGCACCA 31-nt T loop minihelix + 3′-ACCA GCGGCGGUAGCCUAGCCUGGCCUACCGCCGCGGACUUCGGUCCUGUGGCGAAAGCCUAACCC GGCCGCCCCGGGUUCAAAUCCCGGCCGCCGCACCA $tRNA^{Pro}$ 93 + 4 Cloverleaf precursor	Diagrammatic Schema for ribozyme-mediated ligation
	31-nt D Loop minihelix +

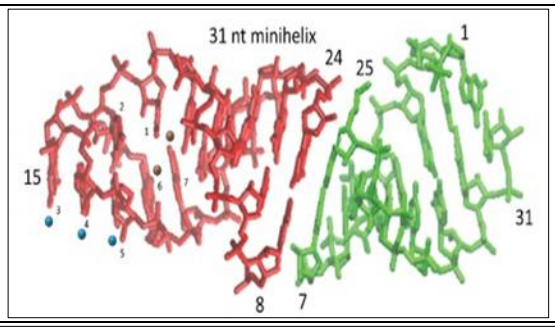
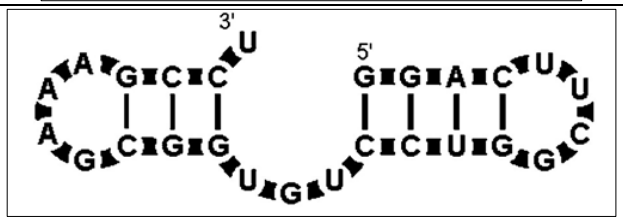
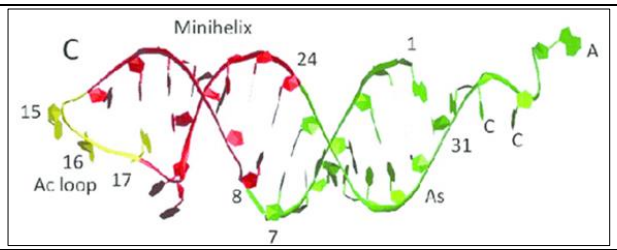
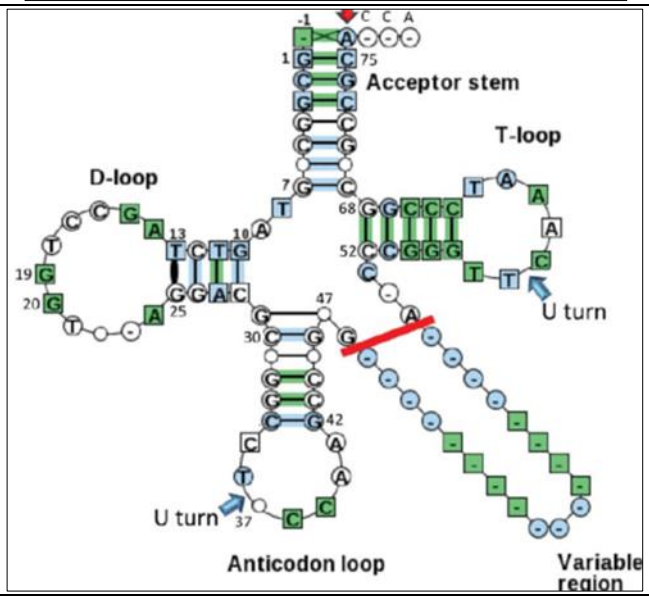
	31-nt minihelix containing
	26-nt Truncated C3 aminoacylating ribozyme +
	31-nt T-Loop and Acceptor stem
	97-nt tRNA$^{Pri'}$ resembling L-leucine tRNA (tRNA^{Leu})and L-serine tRNA (tRNA^{Ser}), type II tRNAs, or pre-intron glycine tRNA (tRNA^{Gly}), a type I tRNA (post-intron), widely believed to be the first amino acid coded for in the genetic code [1]. Note red line indicating intron.

Figure 2 Nucleotide sequences for the three 31-nt minihelices (D-Loop, 31-nt minihelix containing the 26-nt truncated C3 aminoacylating ribozyme, and T-Loop); nucleotide sequence for the 97-nt tRNAPri'; and diagrammatic schema for their ribozyme-mediated ligation leading to the 97-nt tRNAPri', which resembles L-leucine tRNA (tRNALeu) and L-serine tRNA (tRNASer), type II tRNAs, or pre-intron glycine tRNA (tRNAGly), a type I tRNA, widely believed to be the first amino acid coded for in the genetic code [1].

3. Results and Discussion

The tRNAscan-SE entry results concurred with the finding of the tRNA$^{Pri'}$ resemblance to tRNA^{Leu} and tRNA^{Ser} or pre-intron tRNA^{Gly} and revealed the presence of a possible non-canonical intron from nts 41–58, consistent with Yoshihisa's 2014 review of non-canonical archaeal tRNA introns [6] (Figure 3).

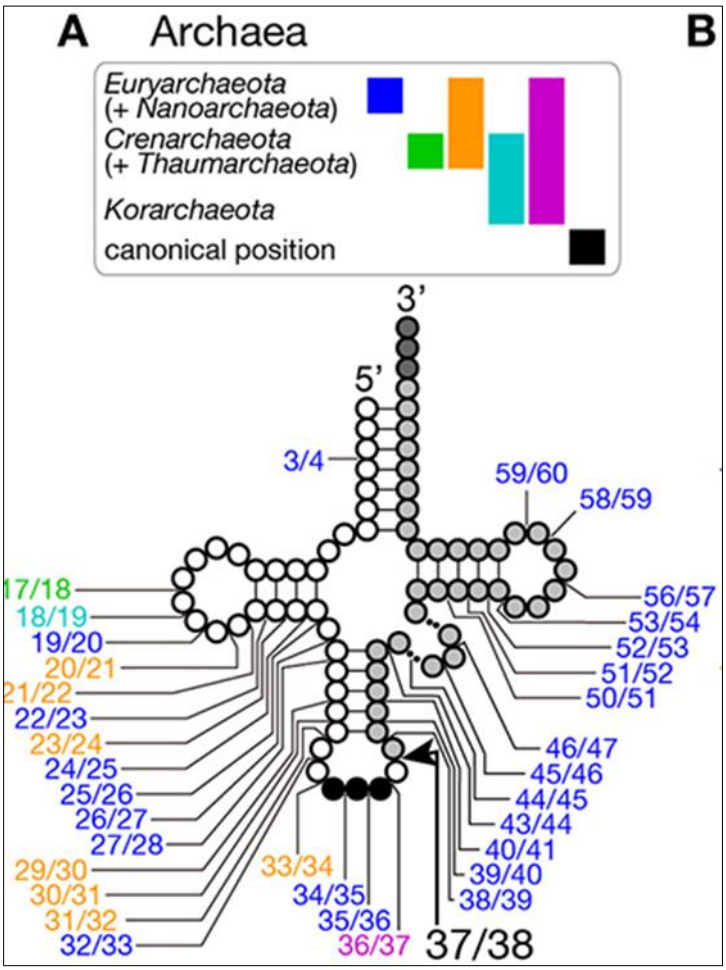


Figure 3 Insertion points of non-canonical introns in tRNA genes**.** Insertion points of non-canonical introns in various tRNA genes from archaea. Positions of intron insertion found in certain groups of organisms are color-coded as shown on top of the tRNA models. Intron insertion points are summarized from data in Sugahara et al. (2008), Sugahara et al. (2009), and Chan et al. (2011)**.**

Possible intron: 41-58 (41-58)

Seq: GCGGCGGTAGCCTAGCCtGGCcTAGGCgGCCGGACTTCGGACCCGGCCGcCCCGGGTTCAAATCCCGGCCGCCGCACCA

Pre:
GCGGCGGTAGCCTAGCCTGGCCTAGGCGGCCGGACTTCGG[TCCTGTGGCGAAAGCCTA]ACCCGGCCGCCCCGGGTTCAAATCCC
GGCCGCCGCACCA

tRNA$^{Pri'}$ nt positions of possible intron [in brackets]. Abbreviations: Seq: post-intron nt sequence; Pre: pre-intron nt sequence (generated from tRNAscan-SE data).

A blastn query of the 97-nt tRNA$^{Pri'}$ sequence, pre-intron removal, in the NCBI Nucleotide Blast database revealed similar results to the tRNAscan-SE results, i.e., the highest percentage matches to the tRNA^{Leu} type II tRNA of the archaeon *Methanopyrus kandleri*, with an overall match of 75% (66/88) nts, 69% (18/26) of which were conserved aminoacylating ribozyme nts and an Expect (E) value of 8e-05. The next highest percentage match was to the pre-intron tRNA^{Gly} type I tRNA of the archaeon *Pyrobaculum aerophilum*, with an overall match of 74% (70/94), 54% (14/26) of

which were conserved aminoacylating ribozyme nts and an E value of 2e-05. It should be noted that *Methanopyrus kandleri* is widely believed to be the microorganism closest in the phylogenic tree to the Last Universal Common Ancestor (LUCA) [7], effectively suggesting pre-intron $tRNA^{Pri'}$ to be a representation of the precursor to both type I and type II tRNAs, as well as resembling what LUCA pre-intron-processed tRNA may have possibly looked like (Figures 4 and 5).

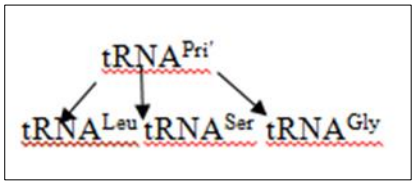


Figure 4 $tRNA^{Pri'}$ serves as a precursor to both type II ($tRNA^{Ser}$ and $tRNA^{Leu}$) as well as type I ($tRNA^{Gly}$) tRNAs

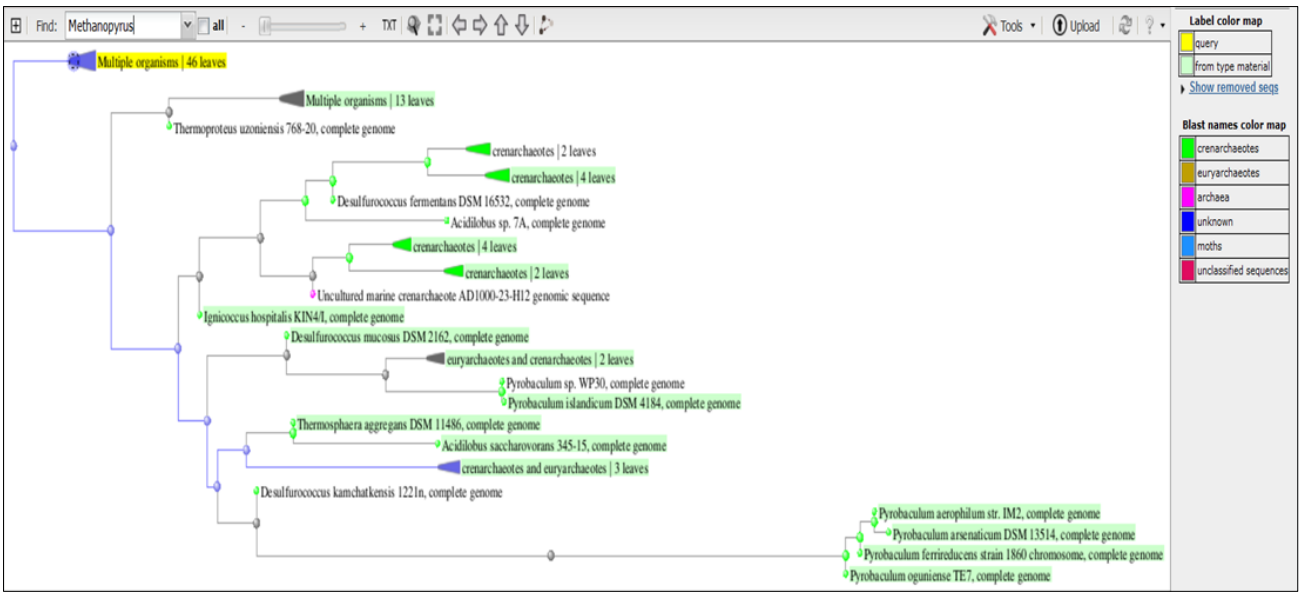


Figure 5 Screenshot of the archaeal phylogenetic tree generated from NCBI Nucleotide Blast data with LUCA on the far left. Note the proximity to LUCA of the pre-intron $tRNA^{Pri'}$ sequence (yellow). Blue represents the pathway to *Methanopyrus kandleri*. Green-shaded organisms' sequences match ≥ 50% of overall nt sequences, unshaded < 50%

Next, the 79-nt sequence representing $tRNA^{Pri'}$ after removal (processing) of its 13-nt intron (Figure 5) was queried in the NCBI Nucleotide Blast database. Results revealed high-percentage matches with several *Pyrococci*; for example, type I *Pyrococcus yayanosii* tRNAs, included:

Post-intron-processed glycine $tRNA^{Gly}$ with an overall match of 84% and 62% (8 of 13) conserved ribozyme nts; L-tyrosine $tRNA^{Tyr}$ with an overall match of 79% and 69% (9 of 13) conserved ribozyme nts; and L-proline $tRNA^{Pro}$ with an overall match of 80% and 85% (11 of 13) conserved ribozyme nts. While *Methanopyrus kandleri* did not appear in the top 100 results, a comparison of its type I glycine $tRNA^{Gly}$ with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database revealed an overall match of 66% and 62% (8 of 13) conserved ribozyme nts. In all cases, for both type I and type II tRNAs, the percentage of overall matched nts was above 65%, ranging from 66% to 84% and averaging 75% while the percentage of conserved self-aminoacylating ribozyme nts was above 50%, ranging from 54% to 85% and averaging 70%. Again, these results very effectively suggest the post-intron $tRNA^{Pri'}$ to also be a strong representation of the precursor to type I tRNAs, as well as resembling what LUCA post-intron-processed tRNA may have looked like (Figures 6 and 7).

Possible intron: 41-53 (41-53)

Seq: GCGGCGGTAGCCTAGCCtGGCcTAGGCGGCCgGACTTCGGGCCtGGCAGTCCCGGGTTCAAATCCCGGCCGCCGCACCA

Pre:
GCGGCGGTAGCCTAGCCTGGCCTAGGCGGCCGGACTTCGG[TCCTGTGGCGAAA]GCCTGGCAGTCCCGGGTTCAAATCCCGGCCGCCGCACCA

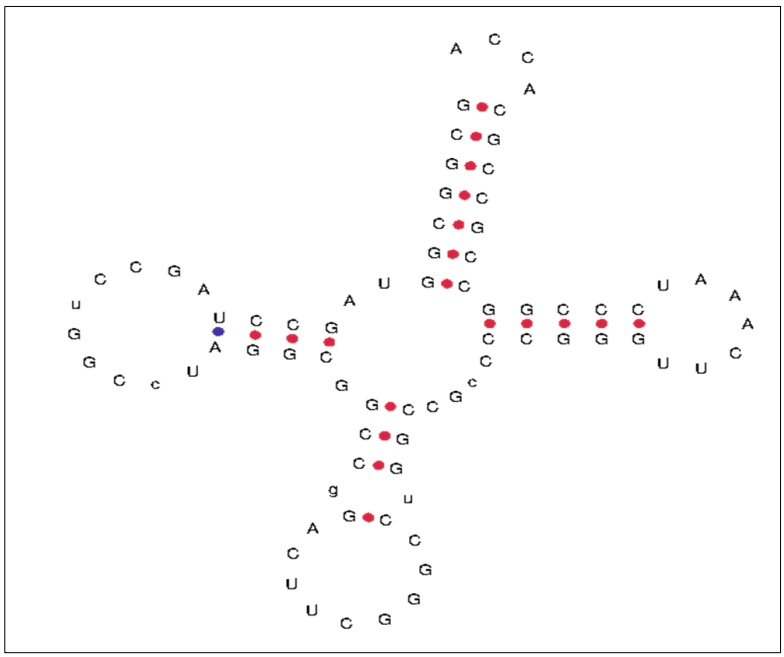


Figure 6 tRNA$^{Pri'}$ nt positions of possible intron [in brackets] and sequence and structure of the type I 79-nt post-intron tRNA$^{Pri'}$ cloverleaf. Abbreviations: Seq: post-intron nt sequence; Pre: pre-intron nt sequence (all generated from tRNAscan-SE data)

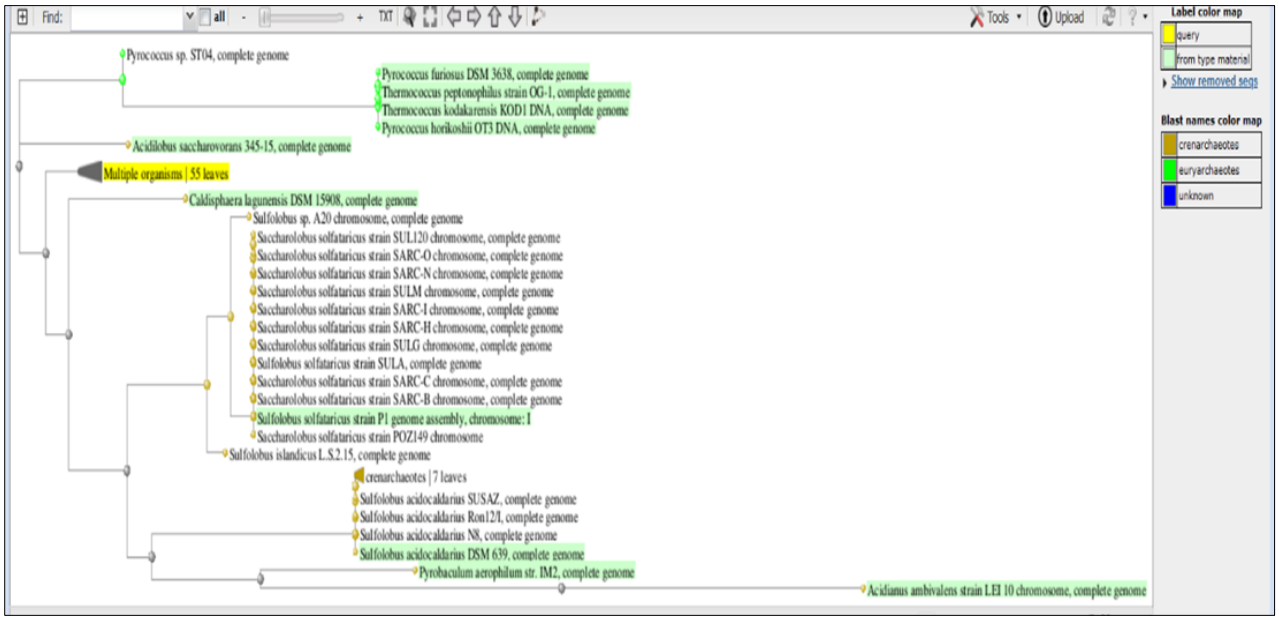


Figure 7 Screenshot of the archaeal phylogenic tree generated from NCBI Nucleotide Blast data with LUCA on the far left. Note the proximity to LUCA of the post-intron tRNAPri' sequence (yellow). Also note the proximity of Pyrococcus. Green-shaded organisms' sequences match ≥ 50%, unshaded < 50%

4. Conclusion

In conclusion, the high percentage of highly conserved sequence results in this *in-silico* study strongly suggest that both modern type I and type II archaeal tRNAs evolved from a self-aminoacylating ribozyme, with the aid of other lyase and ligase ribozymes, all resembling those derived from present-day HDV RNA. Such a conclusion may also help to delineate the ambiguity surrounding the phylogenic origin and position of viroids and viruses [10], affirming their place, along with both the pre- and post-intron $tRNA^{Pri'}$ proposed here, very close to LUCA and therefore the root of the tree of life.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest is declared by the author in the writing of this paper.

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