Fig 4. Amino acid acceptance of *in vitro* synthesised tRNAs. $tRNA_1^{Tyr}$ (*a*), $tRNA_2^{Tyr}$ (*b*) and $tRNA^{Thr}$ (*c*) were synthesised *in vitro* in the presence (●) or absence (○) of 12 µg ρ factor in 200 µl reaction mixtures containing 0.05 M Tris-HCl (*p*H 7.9), 0.01 M $MgCl_2$, 0.05 M KCl, 1 mM dithiothreitol, 0.4 mM each of ATP, CTP, UTP and GTP, 20 µg $\varphi80psu_3^{+,-}$ DNA or 30 µg λh80T DNA, and 160 U RNA polymerase (1,600 U mg^{-1}). Synthesis was initiated after 5 min preincubation at 38 °C by addition of nucleoside triphosphates. After 30 min incubation at 38 °C the reaction was stopped by addition of 20 µg actinomycin D and 2 µg DNase I. tRNA-free S-100 extract (320 µg) was added and the incubation continued for 90 min. Synthesised tRNA was extracted with phenol, precipitated using ethanol and dissolved in 45 µl water. Aliquots of 3, 6, 9 and 12 µl were assayed for amino acid acceptance in 50 µl reaction mixtures containing 0.04 M Tris-HCl (*p*H 7.5), 0.01 M $MgCl_2$, 2 mM ATP, 3 mM glutathion, 1.5 µM L-3.5-^{3}H-tyrosine (41 Ci $mmol^{-1}$, Amersham UK) or 11 µM L-^{3}H-threonine 1.8 Ci $mmol^{-1}$ (New England Nuclear), 19 unlabelled amino acids (except tyrosine or except threonine respectively) and purified tyrosyl-tRNA synthetase[22] or 16 µg tRNA-free S-100 extract containing threonyl-tRNA synthetase. Incorporation of ^{3}H-labelled amino acids into acid-insoluble material was measured after 10 min incubation at 37 °C. tRNA-free S-100 extract was prepared by passing an *E. coli* S-100 extract adjusted to 0.4 M KCl through a DEAE-cellulose column.

during tRNA gene transcription on the amounts of amino acid-accepting tRNAs produced. Figure 4 shows that the presence of ρ increases the amount of $tRNA_1^{Tyr}$ transcribed on $\varphi80psu_3^{+,-}$ DNA by a factor of 3–4 and that of the $tRNA_2^{Tyr}$ and $tRNA^{Thr}$ transcribed on λh80T DNA by a factor of 1.5–2. These results confirm our previous observations concerning the effect of ρ factor on tRNA synthesis[11,12]. We have shown[13] that the presence of ρ during transcription of the $tRNA_1^{Tyr}$ gene increases the amounts of tRNA precursor produced without affecting its size. Thus, although it is generally assumed that ρ factor affects only the termination of an RNA molecule[14] our results do not indicate a termination effect for ρ in tRNA gene transcription. Note that transcription of tRNA genes by purified RNA polymerase also takes place in the absence of ρ and does not require any additional factor as suggested by Bikoff and Gefter[5,6].

The efficiency of production of amino acid chargeable tRNA molecules in the *in vitro* system is remarkable. From the data reported in the legend to Fig. 4 it can be calculated that the transcription of approximately 1.4 pmol $tRNA_1^{Tyr}$ gene DNA (20 µg $\varphi80psu_3^{+,-}$ DNA of molecular weight 30×10^6, containing 2 $tRNA_1^{Tyr}$ genes) resulted in the formation of 5.6 and 19.5 pmol $tRNA_1^{Tyr}$ when synthesised in the absence or presence of ρ respectively. Transcription *in vitro* of λh80T DNA was found to produce $tRNA_2^{Tyr}$ and $tRNA^{Thr}$ in equivalent amounts as determined by their amino acid acceptor activities (Fig. 4*b* and *c*). The glycine-accepting capacity of synthesised $tRNA^{Gly}$ was only 50% of that measured for $tRNA_2^{Tyr}$ or $tRNA^{Thr}$ (data not shown). This is probably due to the lower affinity of glycyl-tRNA synthetase for the $tRNA_2^{Gly}su^+A36$ species[15].

From the present *in vitro* studies we conclude that purified RNA polymerase alone can efficiently transcribe tRNA genes to produce primary tRNA percursors. The high fidelity of the transcription is illustrated by the fact that the synthesised RNA molecules are recognised as substrates by processing nucleases, by nucleoside modifying enzymes and by the aminoacyl-tRNA

synthetases. Note that tRNAs synthesised *in vitro*, when processed by dialysed S-100 extracts, are (with the exception of ψ formation) completely unmodified. The unmodified tRNA precursors can be correctly processed and the resulting unmodified tRNAs are capable of charging with the corresponding amino acid. These findings argue against a possible role of the modified bases in tRNA precursor processing or tRNA aminoacylation. The present study shows that the *in vitro* synthesised tRNA is a suitable substrate for the study of the enzymes involved in nucleoside modifications and may serve to elucidate further the role of the modified nucleosides in the biological functions of mature tRNA.

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MENACHEM ZEEVI
VIOLET DANIEL

Biochemistry Department,
Weizmann Institute of Science,
Rehovot, Israel

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Evolutionary origin of 5.8S ribosomal RNA

IN prokaryotes, the genes for 5S, 16S and 23S ribosomal RNA are linked in a polycistronic transcriptional unit[1,2]. In eukaryotes, on the other hand, the analogous unit contains the genes for 5.8S, 18S and 28S rRNAs, whereas the 5S RNA genes are found elsewhere in the genome. The analogous positions occupied by prokaryotic 5S RNA genes and eukaryotic 5.8S RNA in their respective genomes prompted Doolittle and Pace to speculate that these two RNA classes, both of which are components of the large ribosomal subunit, might have a common evolutionary origin[3]. If these RNAs are in fact homologous, one would expect their sequences to bear some relationship to each other. We report here our evaluation of possible structural relationships between known sequences of 5.8S and 5S rRNA, and conclude that they have no common evolutionary origin.

The comparison of RNA sequences is extremely difficult when insertions and deletions have to be considered. Recently, however, we have developed a test of similarity which can be applied to RNA sequences and which involves the establishment of the best alignment (most identities) of two sequences allowing a fixed number of insertions/deletions[4]. The number of positions in the alignment which are occupied by identical bases in the two sequences is counted. The process is repeated, increasing the allowed number of insertions/deletions by one. The number of identities are then compared with like values computed

Table 1 Comparison of 5S and 5.8S RNA sequences

Experiment	RNA	Insertions or deletions	Identities	Identities in random sequences
1	5.8S yeast 5.8S hepatoma	2	112	61
2	5.8S yeast 5S E. coli	8	67	66
3	5.8S yeast 5S KB cells	8	66	65
4	5.8S yeast 5S protoprokaryote	8	62	60
5	5.8S yeast 5S protoeukaryote	8	58	61
6	5.8S hepatoma 5S E. coli	8	67	69
7	5.8S hepatoma 5S KB cells	8	63	66
8	5.8S hepatoma 5S protoprokaryote	8	61	61
9	5.8S hepatoma 5S protoeukaryote	8	59	64

*The number of identities between random sequences is an average of five different experiments. Random sequences have the same composition and length as the two real sequences being compared in each experiment. We have chosen to give the number of identities found permitting eight insertions or deletions, since these are representative of values obtained permitting either more or less.

from random sequences. Related sequences show statistically significant increases in the number of identities found for each increase in the number of insertions/deletions permitted, up to a certain limit.

Previously, we have shown that 5S RNAs of eukaryotes and prokaryotes are homologous using this test. When applied to the comparison of the two known sequences of 5.8S RNA[5,6], an unequivocal relationship is apparent (see Table 1). It would seem, given the relatively large difference between the 5S sequences from yeast and humans[7,8], that 5.8S RNAs are more conservative to evolutionary change than eukaryotic 5S RNA. Such resistance to the fixation of natural mutations usually indicates an important structural and functional role. In fact, the sequences of all mammalian 5S RNA are apparently identical[8] and the same holds for 5.8S RNA[6]. The fact that both molecules are evolutionarily conservative increases the probability of finding a common origin, if in fact one exists.

As shown in Table 1, the test when applied to the comparison of either the eukaryotic or prokaryotic 5S RNA sequence with either 5.8S RNA sequence (yeast and hepatoma) shows them to be no more related than random sequences.

One might argue that in spite of the conservative evolution of these molecules, all similarities of sequences have been wiped out during their independant evolution from a hypothetical ancestor. We have, however, developed methods to infer hypothetical ancestral sequences using known 5S RNA sequences[10]. With a corpus of 15 known sequences we have estimated ancestral prokaryote and eukaryote sequences: no 5S RNA ancestral sequences show any relationship to known 5.8S RNA sequences.

These results indicate that 5.8S RNA does not share a common ancestor with 5S RNA and therefore did not arise by duplication of the 5S RNA gene followed by divergence. One interesting possibility is that the 5.8S RNA evolved from the intercistronic region of the bacterial ribosomal genome.

R. J. Cedergren
David Sankoff

Département de Biochimie et Centre de Recherches Mathématiques,
Université de Montréal,
Case Postale 6128, Montréal 101,
Québec, Canada

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Bean halo-blight toxin

Pseudomonas phaseolicola causes a disease of beans commonly called halo blight. The halo is a chlorotic zone which appears in the leaf round each infection site. It has been interpreted as the effect of a toxin produced at, and diffusing away from, the infection site. The zone characteristically contains a relatively large amount of the amino acid ornithine[1]. When *Ps. phaseolicola* was grown in a defined liquid culture medium at 18 °C the filter-sterilised medium, when applied to bean leaves, caused the chlorosis symptom of the disease[2]. From such media I have isolated a substance, 80–90% pure, which, when introduced to the leaf at a level of 100 ng per g fresh weight, causes both chlorosis and accumulation of ornithine. I report here the highly unusual structure of this compound. Its extreme lability in the presence of weak acids has made its isolation difficult, but has helped in the resolution of its structure.

The pure substance is water soluble, and hydrolysis with 6 M HCl (100 °C, 18 h) liberates three amino acids (ornithine, alanine and homoarginine). In spite of the presence of two basic amino acids (ornithine and homoarginine) the toxin itself behaves during electrophoresis at pH 2 like the acidic amino acids, suggesting that it also contains strong acid groups. More gentle hydrolysis of the toxin with dilute HCl (0.01 M, 40 °C, 2 h) yields a new compound which contains the same amino acids as the toxin but which shows no toxic properties. This new compound has, at pH 2, an electrophoretic mobility a little greater than that of ornithine, and now consistent with its content of basic amino acids. Sequential cleavage with carboxypeptidase and gas chromatography–mass spectrometry showed it to be the tripeptide ornithylalanylhomoarginine (structure 1, Fig. 1).

Because ornithine is the N-terminal amino acid of the tripeptide, both ornithine amino groups are unbonded. When, however, the intact toxin was reacted with 1-fluoro-2,4-dinitrobenzene and the product was hydrolysed, N^α-dinitrophenylornithine was the only amino acid derivative produced. The tripeptide, in contrast, gave N^α,N^δ-di-dinitrophenyl-ornithine. This shows that the δ-NH_2 of the toxin is bonded. There are two possible interpretations: either the toxin structure is cyclic (for example, cyclic-N^δ-ornithylalanylhomoarginyl) incorporating the ornithyl side chain in the ring (in which case dilute acid must open the ring at the ornithyl-N^δ-peptide bond), or the toxin structure is linear with a non-peptide group bonded to the ornithyl-N^δ (in which case dilute acid would yield peptide and non-peptide products; structure 2, Fig. 1).

The former was excluded when the action of carboxypeptidase enzymes on the toxin was studied. Homoarginine, then alanine, were sequentially cleaved, yielding a product which contained only one amino acid, ornithine. Because carboxypeptidase requires the presence of a free terminal carboxylic acid group to be able to act on the adjacent peptide bond (hence its sequential action), the cyclic structure would not have been attacked by it. I therefore conclude that the toxin is represented by structure 2, and that there is a further group present that is liberated from the toxin by the action of dilute acid.