Volume 9 Number 14 1981 Nucleic Acids Research

Is wheat mitochondrial 5S ribosomal RNA prokaryotic in nature?

M.W.Gray and D.F.Spencer

Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

Received 12 May 1981

ABSTRACT

Küntzel *et al.* (1981) (*Nucleic Acids Res. 9*, 1451-1461) recently concluded that the sequence of wheat mitochondrial 5S rRNA is significantly more related to prokaryotic than to eukaryotic 5S rRNA sequences, and displays an especially high affinity to that of the thermophilic Gram-negative bacterium, *Thermus aquaticus*. However, the sequence on which this conclusion was based, although attributed to us, differs in several places from the one determined by us. We show here that the correct sequence (Spencer, D.F., Bonen, L. and Gray, M.W. (1981) *Biochemistry*, in press) does not support the conclusions of Küntzel *et al.* about potential secondary structure in wheat mitochondrial 5S rRNA and its phlyogenetic significance. We further show that when the wheat mitochondrial 5S rRNA sequence is matched against published alignments for *E. coli*, *T. aquaticus*, and wheat cytosol 5S rRNAs, the mitochondrial sequence shows no greater homology to the *T. aquaticus* sequence than to the *E. coli* sequence, and only slightly more homology to these two sequences than to wheat cytosol 5S rRNA. This analysis confirms our original view (*Biochemistry*, in press) that wheat mitochondrial 5S rRNA is neither obviously prokaryotic nor eukaryotic in nature, but shows characteristics of both classes of 5S rRNA, as well as some unique features.

INTRODUCTION

Plant mitochondria contain 5S rRNA [1], while those of other eukaryotes apparently do not. Wheat mitochondrial 5S rRNA is distinguished from its cytosol counterpart by its unique T_1 oligonucleotide catalogue [2] and by its specific hybridization to wheat mitochondrial DNA [3]. More recently, determination of the complete nucleotide sequence of wheat mitochondrial 5S rRNA proved that it is indeed a structural homologue of other 5S rRNAs [4], and also showed that it possesses a mix of eukaryotic and prokaryotic characteristics, as well as some unique features. Sequence homology between wheat mitochondrial and *E. coli* 5S rRNAs was found to be only slightly greater than between wheat mitochondrial and cytosol 5S rRNAs, leading us to conclude that the mitochondrial sequence is neither obviously prokaryotic nor eukaryotic in nature.

Prior to publication of the details of our sequence determination [4],

Küntzel *et al.* [5] published a paper in which they claimed that wheat mitochondrial 5S rRNA is significantly more related to prokaryotic than to eukaryotic 5S rRNA sequences, and displays an especially high affinity to the 5S rRNA of the thermophilic Gram-negative bacterium, *Thermus aquaticus*. Our intent here is to discuss why we and Küntzel's group come to quite different conclusions about the phylogenetic status of wheat mitochondrial 5S rRNA. We also comment briefly on the difficulties in aligning sequences that are obviously homologous but widely divergent, which is at the crux of all attempts to derive phylogenetic relationships from sequence comparisons.

DISCUSSION

Küntzel *et al.* [5] cite as their source of the wheat mitochondrial 5S rRNA sequence an abstract of a poster presented by us at the 12th International Bari Conference on Mitochondria (Martina Franca, Italy, June 23-28, 1980). However, the sequence of wheat mitochondrial 5S rRNA did not actually appear in the abstract cited by Küntzel's group, and the sequence they attribute to us differs in several places (Fig. 1) from the one presented by us at the Bari Conference and documented in full in [4].

In the sequence used by Küntzel *et al.*, one of the inaccuracies occurs in region L5, which they suggest is involved in complementary base pairing with region L3. The putative duplex is $\begin{matrix} 5'...CCG...3' \text{ (L3)} \\ 3'...GGU...5' \text{ (L5)} \end{matrix}$. The potential presence of this structure was considered by Küntzel *et al.* to be a prokaryotic property of wheat mitochondrial 5S rRNA, since an analogous duplex can be postulated for

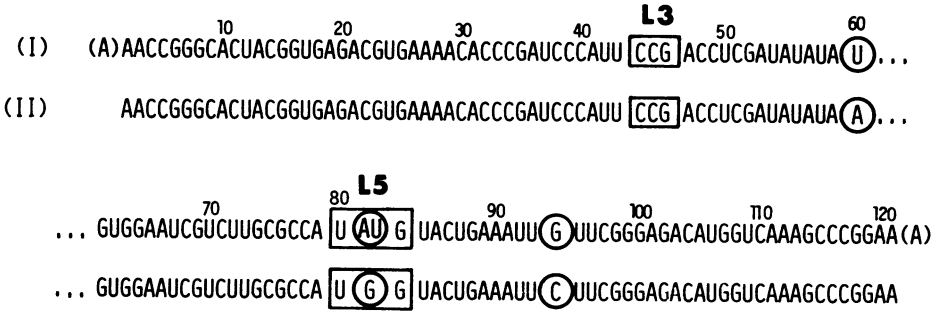

FIGURE 1. Primary sequence of wheat mitochondrial 5S rRNA. (I) as determined by Spencer *et al.* [4] (correct sequence); (II) as listed by Küntzel *et al.* [5] (incorrect sequence). The circled residues denote positions which differ in the two renditions. The boxes enclose the two regions, L3 and L5, which Küntzel *et al.* suggest could interact by forming three adjacent base pairs.

E. coli 5S rRNA. However, it is evident (Fig. 1) that when the correct version of the wheat mitochondrial 5S sequence is used, base pairing between L5 and L3 cannot exist.

Potential Secondary Structure

Küntzel *et al.* further assert that the "eukaryotic stem" (S4/S4' in their terminology) cannot be formed in wheat mitochondrial 5S rRNA. However, as shown in Fig. 2 and documented more fully in [4], moderately-stable duplexes (EE' and FF'; Fig. 2) corresponding in position to those which can be written for wheat cytosol and other eukaryotic 5S rRNA sequences [6-8] are potentially present in wheat mitochondrial but not in *E. coli* or *T. aquaticus* 5S rRNA.

The potential mitochondrial helix EE' shares with the analogous eukaryotic helix (see [9]) the following characteristics: (i) a length of at least five consecutive base pairs; (ii) a conserved G·U base pair in the penultimate position; and (iii) a low thermodynamic stability. However, as previously noted [4], the mitochondrial helix EE' is closed by a small (4 nucleotide) prokaryotic-like loop rather than the large (9-12 nucleotide), well-conserved loop that closes helix EE' in eukaryotic cytosol 5S rRNA.

Prokaryotic 5S rRNAs, including those of *E. coli* and *T. aquaticus*, contain a different potential helix (DD'; Fig. 2), one having considerable thermodynamic stability. However, no helix corresponding in position to eukaryotic helix FF' can be formed in prokaryotic 5S rRNA.

In a recently proposed generalized model of secondary structure in eukaryotic cytosol 5S rRNA [10], helix AA' (the base-paired region comprising the 3' and 5' ends) is made continuous with helix FF' by the addition of further base

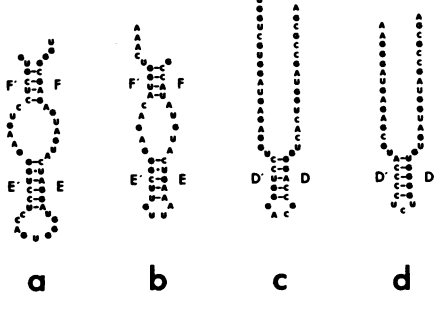

FIGURE 2. Potential secondary structure in the highly variable [9,11,12] region of (a) wheat cytosol [16], (b) wheat mitochondrial [4], (c) *T. aquaticus* [17] and (d) *E. coli* [18] 5S rRNAs. This is the so-called "prokaryotic loop" region of eubacterial 5S rRNAs [11,12]. The sequences listed encompass the region between sections B' and A' in the alignment of Hori and Osawa [9]. Potentially helical regions are designated as in [4], following published conventions [9,11, 12]. Note that in both *E. coli* and *T. aquaticus* 5S rRNAs, helix DD' could be three base pairs longer than shown in the figure and indicated in [9]. Helix FF' corresponds in S4/S4' in [5].

pairs between these two duplexes. It is noteworthy that the wheat cytosol 5S rRNA sequence fits this model [10], but the wheat mitochondrial 5S rRNA sequence does not.

The region of 5S rRNA that contains helices EE' and FF' (eukaryotic cytosol) or DD' (prokaryotes) is the region most variable in sequence between the two classes of 5S rRNA, and the one that distinguishes most clearly between them [9-12]. The additional observations made above reinforce our previous conclusion [4] that in this region, wheat mitochondrial 5S rRNA is as distinct from both prokaryotic and eukaryotic 5S rRNAs as these are from each other, although the mitochondrial RNA does possess certain features of both classes.

Alignment of Sequences

In evaluating sequence homologies [4], we aligned the wheat mitochondrial 5S rRNA sequence with representatives of eukaryotic cytosol (wheat) and prokaryotic (*E. coli*) 5S sequences, these two being matched against one another according to the scheme of Hori and Osawa [9]. Since the latter investigators included the sequence of *T. aquaticus* in their analysis, its alignment against the wheat mitochondrial 5S rRNA sequence follows automatically from the data presented in [4] and [9]. Fig. 3 presents a matrix of the resulting homologies calculated from pairwise comparisons. It is evident that the wheat mitochondrial 5S rRNA shows a similar degree of homology with *E. coli*, *T. aquaticus*, and wheat cytosol 5S rRNAs, whereas the two prokaryotic 5S sequences are significantly more homologous to each other than either is to the wheat cytosol or mitochondrial 5S sequences. It should be noted that the alignment employed by Küntzel *et al.* differs substantially from ours. When the two alignments for the wheat mitochondrial 5S rRNA are matched against the *E. coli* sequence, only about one-half of the positions correspond. Moreover, Küntzel's alignment between *E. coli* and *T. aquaticus* 5S rRNAs differs from that of Hori and Osawa [9] at about 20% of the positions.

	wheat cytosol	*E. coli*	*T. aquaticus*
wheat mitochondria	55	61	58
wheat cytosol		63	53
E. coli			73

FIGURE 3. Numbers of identical residues at equivalent positions in pairwise comparisons among 5S rRNA sequences from wheat mitochondria, wheat cytosol, *T. aquaticus*, and *E. coli*.

For assessment of homologies, our approach [4] was to fit the wheat mitochondrial 5S rRNA sequence into the scheme of Hori and Osawa [9] by following general principles [9,11,12] which emphasize the juxtaposition of helical regions (particularly within the highly-variable region, residues 76-113, of the mitochondrial sequence). In doing so, we made the following assumptions (helical regions are designated as in Fig. 5-7, ref. [4]):

(i) Positions 55-60 (AUAUAU) constitute an insertion. The unusual sequence AUAUAUAU, which is unique to wheat mitochondrial 5S rRNA, is located between helices CC' and GG' [4], in the same position as an AA sequence in *E. coli*, *T. aquaticus*, many other prokaryotic 5S rRNAs [9].

(ii) U67 is an insertion. This aligns C68 with the universal C residue which corresponds to the 5'-terminal residue of B' in all (except higher plant cytosol) sequenced 5S rRNAs, including that of wheat mitochondria.

(iii) Single nucleotide deletions occur between C51 and G52 and between U73 and G74. *E. coli* and wheat cytosol 5S rRNAs have different nucleotides at the place of each assumed deletion, whereas residues on either side are identical in these two RNAs.

(iv) U24 and G25 are additions. This provides a better register of sections A and B with the corresponding regions in *E. coli* and wheat cytosol 5S rRNAs.

(v) Deletions occur between U82 and G83, U92 and U93, and G94 and U95. In this region, wheat mitochondrial 5S rRNA aligns better with wheat cytosol than with *E. coli* 5S rRNA; in particular, such an alignment produces an excellent match of sequences corresponding to helices EE' and FF' [4]. Note that 8 of the assumed deletions (3 between U92 and U93, 5 between G94 and U95) fall within the 4-nucleotide loop which encloses helix FF' and which is 9-12 nucleotides long in eukaryotic cytosol 5S rRNA. The remaining 4 deletions (between U82 and G83) are placed where 1-2 deletions have been assumed for eukaryotic 5S rRNAs.

CONCLUSIONS

Obviously, the phylogenetic conclusions that one draws from primary sequence comparisons are crucially dependent on the alignment chosen [9]. The availability of reasonable numbers of eukaryotic cytosol and eubacterial 5S rRNA sequences lends some credibility to additions and deletions assumed in aligning these sequences against one another, and in certain cases deletions in widely separated parts of the sequence are seen to be concerted, leading to the elimination of individual base pairs in helical regions [9]. The difficulty in the case of wheat mitochondrial 5S rRNA is that its primary sequence is very different from all other 5S sequences, both prokaryotic and eukaryotic. We

know of no totally objective method for validating the alignment of such a divergent sequence, and therefore the additions and deletions we have assumed, although reasonable and soundly based, are nevertheless somewhat arbitrary.

For this reason, we feel it is premature to draw firm phylogenetic conclusions from a single very divergent sequence. Rather, it is important first to broaden the data base by determining additional plant mitochondrial 5S rRNA sequences, as well as further eukaryotic and prokaryotic 5S sequences. This will not only provide a much more convincing basis for the alignment ultimately chosen for plant mitochondrial 5S rRNA, but may also produce more suitable 5S rRNA sequences for comparison. In this regard, it will be particularly important to determine the sequences of 5S rRNA from those organisms (such as *Paracoccus denitrificans* and *Rhodopseudomonas spheroides*) for which a specific phylogenetic affinity with mitochondria has been proposed on other grounds [13].

In summary, we do not agree with Küntzel *et al.* [5] that the sequence of wheat mitochondrial 5S rRNA "strongly supports the idea of an endosymbiotic origin of plant mitochondria". We would point out that much more convincing data in favor of such a proposal already exist in the literature [14,15]. Indeed, these data, based on the T_1 oligonucleotide catalogue of wheat mitochondrial 18S rRNA, still constitute the strongest available evidence for the specifically prokaryotic nature of any mtDNA-encoded macromolecule.

REFERENCES

1. Leaver, C.J. and Harmey, M.A. (1976) *Biochem. J. 157*, 275-277.
2. Cunningham, R.S., Bonen, L., Doolittle, W.F. and Gray, M.W. (1976) *FEBS Lett. 69*, 116-122.
3. Bonen, L. and Gray, M.W. (1980) *Nucleic Acids Res. 8*, 319-335.
4. Spencer, D.F., Bonen, L. and Gray, M.W. (1981) *Biochemistry*, in press.
5. Küntzel, H., Heidrich, M. and Piechulla, B. (1981) *Nucleic Acids Res. 9*, 1451-1461.
6. Hori, H. (1976) *Mol. Gen. Genet. 145*, 119-123.
7. Nishikawa, K. and Takemura, S. (1974) *J. Biochem. (Tokyo) 76*, 935-947.
8. Vigne, R. and Jordan, B.R. (1977) *J. Mol. Evol. 10*, 77-86.
9. Hori, H. and Osawa, S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 381-385.
10. Luehrsen, K.R. and Fox, G.E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2150-2154.
11. Fox, G.E. and Woese, C.R. (1975) *Nature 256*, 505-507.
12. Fox, G.E. and Woese, C.R. (1975) *J. Mol. Evol. 6*, 61-76.
13. Whatley, F.R. (1981) *Ann. N.Y. Acad. Sci. 361*, 330-340.
14. Bonen, L., Cunningham, R.S., Gray, M.W. and Doolittle, W.F. (1977) *Nucleic Acids Res. 4*, 663-671.
15. Cunningham, R.S., Gray, M.W., Doolittle, W.F. and Bonen, L. (1977) in *Acides Nucléiques et Synthèse des Protéines Chez les Végétaux* (Bogorad, L. and Weil, J.H., eds.), pp. 243-248, Éditions du Centre National de la Recherche Scientifique, Paris.

16. MacKay, R.M., Spencer, D.F., Doolittle, W.F. and Gray, M.W. (1980) *Eur. J. Biochem. 112*, 561-576.
17. Nazar, R.N. and Matheson, A.T. (1977) *J. Biol. Chem. 252*, 4256-4261.
18. Brownlee, G.G., Sanger, F. and Barrell, B.G. (1967) *Nature 215*, 735-736.