Volume 4 Number 3 March 1977 Nucleic Acids Research

Wheat embryo mitochondrial 18S ribosomal RNA: evidence for its prokaryotic nature

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Received 10 January 1977

ABSTRACT

We present a catalog of sequences of oligonucleotides produced by T1 ribonuclease digestion of ^{32}P-labeled small-ribosomal-subunit RNA ("18S" rRNA) isolated from purified wheat embryo mitochondria. This catalog is compared to catalogs published for prokaryotic and chloroplast 16S rRNAs and to preliminary results for wheat cytosol 18S rRNA. These comparisons indicate that: (1) wheat mitochondrial 18S rRNA is clearly prokaryotic in nature, showing significantly more sequence homology with 16S rRNAs than can be expected to arise by chance ($p<0.000001$); (2) shared oligonucleotide sequences include an especially high proportion of those identified as conserved in the evolution of prokaryotic rRNAs; and (3) wheat embryo mitochondrial and cytosol 18S rRNAs retain no more, and perhaps less, than the minimum sequence homology detectable by this sensitive method. These results argue in favor of an endosymbiotic origin for mitochondria.

INTRODUCTION

Small-ribosomal-subunit RNAs are known to be coded by the separate genomes of prokaryotes (16S rRNAs), eukaryotic nuclei (cytosol 18S rRNAs), and eukaryotic organelles (chloroplast 16S and mitochondrial 13S-18S rRNAs). The strong functional analogies and obvious indispensability of these molecules leave little doubt that they represent a family of evolutionary *homologs* suitable for molecular taxonomic and phylogenetic analyses. Indeed, Woese and collaborators[1-3] have shown the utility of partial sequence information in constructing bacterial phylogenies. More recently, we[4,5] and Zablen *et al.*[6] have concluded, from similarly-derived information, that the chloroplast 16S rRNAs of *Porphyridium* (a red alga) and *Euglena* are "prokaryotic" in nature, bearing strong (80-90%) homology to prokaryotic (and, in the case of *Porphyridium*, specifically blue-green algal) rRNAs, while sharing with cytosol 18S rRNAs less than the minimum homology detectable by these methods (60%). Such results can be most simply (although not exclusively) understood on the assumption that chloroplasts derive from photosynthetic prokaryotes incorporated as endosymbionts into the cytosol(s) of "protoeukaryotic" cells

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to which they were only remotely related phylogenetically[4-8].

Mitochondrial ribosomes show certain functionally "prokaryotic" properties (*e.g.*, antibiotic sensitivity and initiation with *N*-formylmethionyl-tRNA) which have been used as evidence that mitochondria, the organelles of eukaryotic respiration, also had an endosymbiotic origin (from aerobic bacteria). However, shared properties are certainly fewer and probably more superficial than those common to chloroplast and prokaryotic ribosomes. Mitochondrial ribosomes *do* differ substantially from their bacterial homologs (and among themselves) in size and protein content, and the rRNAs they contain show similarly "non-prokaryotic" (and variable) sizes, base compositions, and transcriptional organizations[9,10]. These and other considerations underlie recent suggestions that mitochondria, unlike chloroplasts, arose *de novo* ("autogenously") in relatively advanced aerobic protoeukaryotes[9,11,12].

In distinguishing autogenous from endosymbiotic origins, quantitative measurements of sequence homology between mitochondrial, cytosol and prokaryotic rRNAs can play a decisive role. We present here a catalog of sequences of the Gp-terminated oligonucleotides released by T1 ribonuclease digestion of purified wheat embryo mitochondrial small-subunit rRNA, and compare it to catalogs of (1) prokaryotic 16S rRNAs, (2) a chloroplast 16S rRNA, and (3) wheat embryo cytosol small-subunit rRNA. (Wheat mitochondrial and cytosol small-subunit rRNAs are of similar, although not identical, electrophoretic mobilities on polyacrylamide gels[13] and are both here designated as "18S" rRNAs.) These catalog comparisons show that wheat mitochondrial 18S rRNA, in spite of its "non-prokaryotic" size, bears strong homology to prokaryotic and chloroplast 16S rRNA molecules, and retains an especially high proportion of those sequences which can be defined (by their retention in a broad spectrum of bacterial and blue-green algal 16S rRNAs) as evolutionarily conservative and hence of particular importance to *prokaryotic* ribosomal function. In contrast, wheat mitochondrial and cytosol 18S rRNAs show little if any more homology than can be expected to arise by chance between evolutionarily-unrelated RNAs of similar size. These results argue in favor of an endosymbiotic origin for mitochondria.

MATERIALS AND METHODS

The procedure of Lau *et al.*[14] was used to produce ^{32}P-labeled RNA. Eight grams of viable wheat embryos (*Triticum vulgare*, var. Thatcher) were germinated (for each of two independent preparations) for 24 hr in the dark at room temperature in the presence of 100-120 mCi carrier-free ^{32}P-orthophosphate

(New England Nuclear). Total rRNA was isolated from purified mitochondria and from the post-mitochondrial supernatant (cytosol) fraction of embryo homogenates, as described in detail elsewhere[13]. The ^{32}P-labeled RNA fraction insoluble in 3 M NaCl at 0° was resolved on 5-25% continuous sucrose gradients. Purified cytosol and mitochondrial 18S rRNAs were collected from gradients and freed of salt. The labeled RNAs could not be attributed to contamination either by fungi (since none could be found growing with germinated embryos) or by bacteria (since (*i*) bacterial rRNAs migrate more rapidly than plant mitochondrial rRNAs on polyacrylamide gels, and (*ii*) bacterial RNA is not isolated with mitochondrial RNA by the procedures used[13]). Preliminary "fingerprint" analyses confirmed that mitochondrial and cytosol 18S rRNA molecules are distinct in sequence, as are mitochondrial and cytosol 26S and 5S rRNAs (see reference 15).

Purified mitochondrial and cytosol 18S rRNAs were digested with T1 ribonuclease (Calbiochem). The resultant Gp-terminated oligonucleotides were resolved by two-dimensional ionophoresis and sequenced through secondary and tertiary digestions with pancreatic, T1, and U_2 ribonucleases (Calbiochem), as described elsewhere[1,2,4,16]. Even with the high levels of ^{32}P-orthophosphate employed, specific activities of labeled mitochondrial rRNA preparations were lower than those normally employed in analyses of this type, and sequences of a fraction of the oligonucleotides derived from them consequently remain ambiguous. In all but a few such cases, information from secondary and tertiary analyses (indicated in Table 1) was nevertheless sufficient to allow scoring of coincidence or non-coincidence with oligonucleotide sequences (chain length ≥5) published for prokaryotic or chloroplast rRNAs. In comparing wheat mitochondrial and cytosol 18S rRNAs, simultaneous analyses of secondary and tertiary nuclease digestion products of similarly-migrating oligonucleotides from the two species allowed definitive conclusions in cases of non-coincidence and tentative conclusions in the (relatively few) cases of possible sequence identity.

RESULTS AND DISCUSSION

Table 1 presents the sequences of oligonucleotides of chain length (N) five or greater from wheat mitochondrial 18S rRNA and indicates the presence or absence of identical oligonucleotide sequences in (1) three bacterial (*Rhodopseudomonas spheroides*, *Escherichia coli*, and *Bacillus subtilis*) 16S rRNAs[2,16,17], (2) a blue-green algal (*Gloeocapsa alpicola*) 16S rRNA[5], (3) a red algal (*Porphyridium*) chloroplast 16S rRNA[4], and (4) wheat embryo cytosol

Table 1. Wheat mitochondrial 18S rRNA compared to prokaryotic, chloroplast and wheat cytosol rRNAs

Oligonucleotide sequence	*R. spherodes* 16S	*E. coli* 16S	*B. subtilis* 16S	*G. alpicola* 16S	*Porphyridium* chloroplast 16S	Wheat cytosol 18S
Pentamers						
CC*CCG	1	1	1	1	1	0
CCCCG	2	0	0	0	0	1
CCCAG	1	1	1	1	1	0
CCAAG (0-1)	0	0	0	0	0	1
ACACG	1	1-2	1	1	1	1
AACCG	0	1	0	0	0	0-1
AACAG	0	0	1	0	1	0
AAAAG	0	0	0	1	1	0
CUCCG	0	0	0	0	0	0
CCCUG	0	0	0	0	0	0
CUCAG	1	2	1	1	1	(0)
CUACG	0	0	0	0	0	0
CCAUG	1	1	0	0	0	(0)
CAUCG	1	0	0	1	1	1
ACUCG (1-2)	0	0	0	0	0	0
UCAAG (1-2)	1	1	0	1	(1)	0
UAACG	1-2	1	1	0	0	0
CAAUG	0	0	1	1	1	0
AUCAG	2	1	1	1	1	1
ACAUG	1	0	0	0	0	(0)
AAUCG	1	1	1	~2	1	0
AACUG	1	2-3	0	0	1	1
UAAAG (~2)	2	3-2	2	~2	2	0
AAUAG	1	0	1	0	1	0
CCUUG	1	1	1	0	1	1
CUCUG[1]	1	0	1	0	1	1
CUUAG	0	0	0	0	1	0
UUACG	0	0	0	0	0	0
UACUG	0	0	0	0	1	0
ACUUG[1]	0	1	0	0	0	0
UAUAG	0	0	0	2	0	0
AUUAG	1	2	1	2-3	2-3	0
AAUUG	2	1	1	1-2	1	1
CUUUG	0	1-2	0	(0)	0	0
UCUUG	0	2-1	1	2	0	0
UUCUG	0	0	0	0	1	1
UUUCG	0	0	0	0	0	0
UUUUG	0	0	0	0	0	0
unsequenced (0-1)	0	0	0	0	0	0
Hexamers						
CCACCG	0	0	0	0	0	0
CCCAAG	0	0	0	0	0	0
CACAAG	1	1	1	1	1	0
ACCAAG	0	0	0	0	0	0
AAAAAG	0	0	0	0	0	0
CCUCCG	0	0	0	0	0	0
CCUCAG	0	0	0	0	(1)	0
UACCCG	0	0	0	0	0	0
CCUAAG	0	0	0	0	(1)	0
UACCAG	0	0	0	0	0	0
CUAACG	0	1	1	0	1	0
CCAAUG[1]	0	0	0	0	0	0
AUCCAG	0	0	0	0	0	0
AAUCCG	0	0	0	1	0	0
AACCUG (0-1)	0	½	0	0	0	0
CUAAAG	0	0	0	0	0	0
UAAACG (0-1)	1	1	1	1	1	0
AUCAAG	0	0	0	0	0	0
ACAAUG	1	0	0	0	0	0
AACAUG	0	0	0	0	0	0
AAAUCG	0	0	0	0	0	0
AAAAUG	0	0	0	0	0	0
CUCUCG	0	0	0	0	0	0
CCUUCG	0	2	0	0	0	1
CCCUUG	0	1	0	0	0	0
UCUACG[2]	0	0	0	0	0	0
UCCAUG (½)	0	0	0	0	0	0
CCUAUG (½)	0	0	0	0	0	0
AUCCUG	1	0	1	1	1	(1)
UAAUCG	1	1	1	1	1	0
AUCAUG	0	1	0	0	0	0
AUACUG	1	1	0	0	0	0
AAUCUG	0	0	0	1	1	0
U,A,AUAG	0	0	0	0	0	0
AAUAUG	0	0	0	0	0	0
AAAUUG	0	0	0	0	0	0
UCUUCG	0	0	0	0	(1)	?
CCUUUG[1]	1	0	0	0	0	0
UUA,UA,G (~2)	0	0	0	0	0	(1)
UUUUCG	0	0	1	0	0	0
CUUUUG[1]	0	0	0	0	0	?
Heptamers						
ACCCACG	0	0	0	0	0	0
CCAAAAG[1]	0	0	0	0	0	0
AACAAAG	0	0	1	0	1	0
CAACUCG	1	1	1	(0)	0	0
ACCAAUG	0	0	0	0	0	0
AAAUCCG	0	0	0	0	0	0
CUCCUAG	0	0	0	0	0	0
AUCUACG	0	0	0	0	0	0
A(UA,CA)UG	0	0	0	0	0	0
CCUUUCG[2]	0	0	0	0	0	0
(A/C)UCUCUG[2]	0	0	0	0	0	0
CUUUCAG	0	0	0	0	0	0
AUUCCUG[2]	0	0	0	0	0	0
ACUUAUG[2] (0-1)	0	0	0	0	0	?
ACUAUUG[2] (0-1)	0	0	0	0	0	0
AAUUUCG[2]	0	0	0	0	0	0
AAUCUUG[2]	0	0	0	0	0	0
AUAUAUG[1]	0	0	0	0	0	(0)
$(CU_3)UCG$	0	0	0	0	0	0
$(CU_2)UCUG$	0	0	0	0	0	0

Table 1 (continued)

Oligonucleotide sequence	*R. spheroides* 16S	*E. coli* 16S	*B. subtilis* 16S	*G. alpicola* 16S	*Porphyridium* chloroplast 16S	Wheat cytosol 18S
Octamers						
CCA,CA,AAG	0	0	0	0	0	0
AAACAAAG	0	0	0	0	0	0
A_{0-1}(CCA,CA)CUG	?	?	?	?	?	0
C_{*1}(NN,AU)ACG[3]	0	0	0	0	0	0
C_{0-1},ACUCAA,CG[1]	0	0	0	0	0	0
AUACCCUG	1	1	1	0	0	0
A_{0-1}(CAA_{1-0},C_{0-1}UA)UCG	0	0	0	0	0	0
$C_{0-1}A_{0-1}$,C_{0-1}UA,CAA_{0-1},UG	?	?	?	0	?	0
CCCUUAUG	0	0	0	0	0	0
(C_2U)UUUCG	0	0	0	0	0	(0)
AAU,C_{1-2},U_3,G	0	0	0	0	0	(0)
UUCUA,UA,G	0	0	0	0	0	0
(C_3U_5)G[2]	0	0	0	0	0	0
(CU)UUAUUG	0	0	0	0	0	0
Nonamers						
$CCAAAAA_{1-3}$G	0	0	0	0	0	0
A(A/C)ACCUCG	0	0	0	0	0	0
A_{0-1},A(A/C)$(CA)_{0-1}$CUCCG	0	0	0	0	0	0
A_{0-1}UACA$(CA)_{1-0}$CCG[2]	?	?	?	?	?	?
C_{0-1}A,UA,CA,$(CA)_{1-0}$,CG[2]	0	0	0	0	0	0
UCCCUACCG[2]	0	0	0	0	0	(0)
CUAACUCCG[1]	1	1	0	1	1	0
UA(CUA,CCA)G[1]	0	0	0	0	0	0
UUUAAUUCG	1	1	1	1	0	0
unsequenced (1)	0	0	0	0	0	0
Decamers						
A_{0-1}(C_{3-2}U)AAAAG[2]	0	0	0	0	0	0
AAACUCAAAG	0	0	1	1	1	0
C_{0-2}A,C_{2-3}A,UCCUG	0	0	0	0	0	0
C_{0-2}A,C_{2-3}A,UCA,UG	?	0	0	0	0	0
CUUUCA,CA,CG[2]	0	0	0	0	0	0
CA,UCCUA,UA,G[1]	0	0	0	0	0	0
CA,(C_{0-1}A)$_{0-1}$((UC)$C_{0-1}$$UUA_{1-2}$)CG	0	0	0	0	0	0
CA,(C_{0-1}A)$_{0-1}$,$CUUA_{1-2}$,UG	0	0	0	?	?	0
UCAAAAUCUG	0	0	0	0	0	0
CUACUACUUG[1]	0	0	0	0	0	0
Undecamers and larger						
CCUA(CCUA,UA)G	0	0	0	0	0	0
CUUUCA,UA,CA,G	0	0	0	0	0	0
AACAACAACCUG[2]	0	0	0	0	0	0
(A_{0-2},UUUCA_{2-1}),CCA,UUG	0	0	0	0	0	0
CA,CUUCAAAA,CUA,G	0	0	0	0	0	0
unsequenced (2-3)	0	0	0	0	0	0

Table 1 lists all oligonucleotides larger than tetramers present in wheat mitochondrial 18S rRNA and indicates which of these are also present in (1) *R. spheroides* 16S[17], (2) *E. coli* 16S[16], (3) *B. subtilis* 16S[2], (4) *G. alpicola* (a unicellular blue-green alga) 16S[5], (5) *Porphyridium* (a eukaryotic red alga) chloroplast 16S[4], and (6) wheat cytosol 18S rRNA. Numerals indicate number of copies of coincident sequences. (1) and (0) indicate that coincidence is not certain but very probable, or very improbable, respectively. For those wheat mitochondrial 18S oligonucleotides which (because of low specific activity) cannot now be completely sequenced, results of secondary and tertiary analyses are indicated. It should be realized that comparisons of secondary and tertiary digestion products to those expected for (known) prokaryotic and chloroplast 16S rRNA-derived oligonucleotides of similar primary fingerprint position is usually sufficient to exclude sequence coincidence. Instances in which coincidence is possible are indicated by '?'. A/C denotes A *or* C. Asterisk indicates modified nucleoside. The 'unsequenced' pentamer *may* be modified.

[1]Sequence very probably, but not certainly, as indicated.

[2]Sequence uncertain. Secondary and tertiary digestion products most consistent with sequence as indicated. Decisions on coincidence made as described above.

[3]Secondary digestions produce unidentified modified dinucleotide.

18S rRNA. Table 2 summarizes, for each oligonucleotide size class (N≥5) and for all size classes taken together, (1) the number of oligonucleotide sequence coincidences observed between wheat mitochondrial 18S and each of the six RNAs to which it is compared, and (2) the (mean) number of sequence coincidences expected to arise by chance from any two RNAs of unrelated random sequence but similar size. (Mean expectations for N=5 and N=6 were determined by a computerized Monte Carlo simulation (1000 trials), while those for N≥7

Table 2. Summary of total number of coincident oligonucleotide sequences

Oligonucleotide Size Class	*R. spherodes* 16S	*E. coli* 16S	*B. subtilis* 16S	*G. alpicola* 16S	*Porphyridium* chloroplast 16S	Wheat cytosol 18S
Pentamers						
Observed	20	19	17	16	22	11
Expected	11	14	11	12	13	10
Hexamers						
Observed	7	9	6	6	9	3-5
Expected	4	3	3	3	3	4
Heptamers						
Observed	1	1	2	0	1	0-1
Expected	0.4	0.4	0.3	0.4	0.5	0.6
Octamers						
Observed	1-3	1-3	1-3	0-1	0-2	0
Expected	0.06	0.06	0.06	0.06	0.08	0.1
Nonamers						
Observed	2-3	2-3	1-2	2-3	1-2	0-1
Expected	0.02	0.02	0.02	0.02	0.02	0.02
Decamers						
Observed	0-1	0	1	1-2	1-2	0
Expected	0.003	0.002	0.003	0.005	0.004	0.006
All Size Classes						
Observed	31-35	32-35	28-31	25-28	34-38	14-18
Expected	15	17	13	15	16	15
Observed minus expected	16-20	15-18	15-18	10-13	18-22	(-1)-3

A summary of the number of sequence coincidences for oligonucleotides of size N=5 and greater between wheat mitochondrial 18S rRNA and each of the rRNAs for which data is presented in Table 1. Where ranges are indicated, lower values include only coincidences of sequences indicated (in Table 1) as certain or very probable; higher values include, in addition, those oligonucleotides for which sequences coincidence is considered possible (denoted '?' in Table 1).

were approximated as described previously[4].)

Wheat mitochondrial 18S rRNA is clearly prokaryotic in nature, sharing with each of the (bacterial, blue-green algal, and chloroplast) 16S rRNAs significantly more than the number of oligonucleotide sequences expected by chance. Even when scoring of coincidences is restricted to oligonucleotides with sequences indicated (in Table 1) as certain or very probable, the likelihood[4] that levels of coincidence observed arise at random (*i.e.*, do not reflect homology) is less than 0.000001. On the other hand, the number of sequence coincidences observed between mitochondrial 18S rRNA and cytosol 18S rRNA is little if any greater than that expected by chance, and base sequence homology between them is therefore at or below the limits detectable by these methods (60%)[5].

It is possible to show that sequences common to mitochondrial 18S and prokaryotic 16S rRNAs derive, as one might expect, largely from regions which show especially-strong evolutionary conservatism among prokaryotes. Such conservative regions have been identified by Woese *et al.*[3] in comparing catalogs of 16S rRNAs from 26 diverse bacterial species to that of *E. coli* 16S rRNA. These authors defined 26 oligonucleotides ($N \geq 5$) to be "universal" among bacteria (present in the 16S rRNAs of *E. coli* and at least 24 other species) and a further 27 sequences to be "conserved" (present in the 16S rRNAs of *E. coli* and at least 14 other species), and showed that these sequences together define nine relatively restricted regions of conservatism within the known primary sequence of *E. coli* 16S rRNA. Wheat mitochondrial 18S rRNA shares at most 35 oligonucleotide sequences ($N \geq 5$) with *E. coli* 16S rRNA. However, these comprise 17 (64%) of the universal sequences, ten (36%) of the conserved sequences, but only eight (13%) of the approximately 60 *E. coli* oligonucleotides defined as neither universal nor conserved. (Wheat mitochondrial 18S rRNA also retains the modified tetramer $G^{*}CCG$ found in all prokaryotic rRNAs catalogued by Woese *et al.*[3].) By contrast, wheat cytosol 18S rRNA contains at most four (15%) of the prokaryotic universal sequences[1 8] and only one of these is also found in preliminary catalogs of cytosol 18S rRNAs from *Porphyridium* and *Chlamydomonas* (unpublished).

Of the 28 universal or conserved sequences common to wheat mitochondrial 18S and *E. coli* 16S rRNAs, 22 can be assigned unambiguous (unique) positions in the primary sequence of the latter. Of these 22, more than two-thirds (15) appear in the 3'-terminal one-half of the molecule, and nearly one-third (7) are clustered in the most highly-conserved region identified by Woese *et al.*[3] (region 2, comprising only 7% of the total 16S rRNA length). A similar

clustering of conserved sequences (especially in region 2) has been reported for chloroplast rRNAs[4,6] and, together, these results suggest that at least some of the functional constraints on rRNA structure are similar in prokaryotes and organelles.

The present demonstration of strong homology between mitochondrial and prokaryotic rRNAs and lack of homology between mitochondrial and cytosol rRNAs clearly supports the endosymbiont hypothesis for the origin of mitochondria. It fails to prove it only insofar as it remains possible to argue that mitochondrial rRNAs are (in spite, somehow, of their demonstrable variability in size and base composition) evolutionarily much more conservative than their cytosol homologs, and thus more similar to a common protoeukaryotic ancestor of both cytosol and mitochondrial rRNAs. Such arguments[9,11,12] can be applied with equal force against any measure of homology between nuclear, mitochondrial, and prokaryotic gene products, and can only be overcome by the construction (with further data) of independent phylogenetic trees for mitochondria and nuclei which have *different* roots among the prokaryotes.

ACKNOWLEDGEMENTS

We thank the Medical Research and National Research Councils of Canada for support, and L. Weldon, L. Maclean and J. Oakley for help in statistical analysis. We also acknowledge the advice and criticism of C.R. Woese during the preparation of this manuscript.

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