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The nucleotide sequence of the 16S ribosomal RNA gene of the archaebacterium *Halococcus morrhua*

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The sequence of the 16S rRNA gene from the archaebacterium *Halococcus morrhua* was determined by the dideoxynucleotide sequencing method. It is 1475 nucleotides long. This is the second archaebacterial sequence to be determined and it provides sequence comparison evidence for the secondary structural elements confined to the RNAs of this kingdom and, also, support for controversial or additional base pairing in the eubacterial RNAs. Six structural features are localized that have varied during the evolution of the archaebacteria, eubacteria and eukaryotes. Moreover, although the secondary structures of both sequenced archaebacterial RNAs strongly resemble those of eubacteria, they contain sufficient eukaryotic-like structural characteristics to reinforce the view that they belong to a separate line of evolutionary descent.

Key words: archaebacteria/dideoxynucleotide sequencing/ RNA secondary structure

Introduction

Comparative studies of catalogues of oligonucleotides deriving from ribonuclease T1 digestion of the small subunit RNAs first led to the concept that archaebacteria constitute a third kingdom with an evolutionary heritage distinct from those of eubacteria and eukaryotes (Woese *et al.*, 1978; Magnum *et al.*, 1978; Woese and Gupta, 1981). This concept was reinforced by other comparative studies (reviewed by Woese, 1981) on cell membranes (De Rosa *et al.*, 1976), cell envelopes (Kandler, 1979) and DNA-dependent RNA polymerase subunits (Huet *et al.*, 1983), in addition to other components of the protein synthesizing machinery including ribosomal proteins (Matheson *et al.*, 1980), elongation factors (Kessel and Klink, 1980) and aminoacyl-tRNA synthetases (Kwok and Wong, 1980).

To define the evolutionary status of archaebacteria more precisely, it is important to extend the comparative sequence approach from oligonucleotides to whole sequences. Moreover, now that secondary structural models of the eubacterial RNAs have reached a reasonable level of refinement (reviewed by Woese *et al.*, 1983; Maly and Brimacombe, 1983) we can consider possible changes in secondary structure that have occurred during evolution with some confidence. Recently, the first nucleotide sequence of the 16S RNA gene of an archaebacterium was completed for *Halobacterium volcanii* (Gupta *et al.*, 1983). Here we present the sequence of a second 16S RNA from *Halococcus morrhua* which has a separate line of evolutionary descent from the halobacterium (Woese, 1981). The dideoxynucleotide sequencing method (Sanger *et al.*, 1977) was employed using phage M13 vectors (Messing *et al.*, 1981; Messing and Vieira, 1982); a rapid liquid hybridization technique was used to determine the orientations of the cloned DNA fragments.

Results

An 8.5-kb fragment was prepared from the *H. morrhua* chromosomal DNA using the restriction enzyme *Eco*RI. It contained the gene for 16S RNA, the spacer region between 16S and 23S RNA, and the first 1800 bp of the 23S RNA gene. It was cloned into phage λgtWES.λB and mapped as illustrated in Figure 1. Two DNA fragments were prepared that strongly hybridized to the 16S RNA, one with restriction enzyme *Sau*3A and the other with *Hind*III. The former contained almost the whole 16S RNA gene except for nine nucleotides at both ends, whereas the latter contained the 3′ half of the 16S RNA gene, the spacer region and the first 280 nucleotides of the 23S RNA gene. These fragments were further digested with restriction enzymes that were specific for four base pairs. The products are illustrated for the *Sau*3A fragment in Figure 1; each of these subfragments was cloned into phage M13mp7, mp8 or mp9 vectors.

The nine nucleotides at the 5′ end of the 16S RNA gene were cloned by the following procedure. The *Sau*3A restriction fragment was digested with *Alu*I and the fragment near the 5′ end (base pairs 9–87; see Figure 1) was isolated and cloned into the M13mp8 vector. The complementary strand was synthesized by DNA polymerase in the presence of a primer and [α-^{32}P]ATP (Amersham). The radioactive insert and the flanking vector sequences (98 bp) were excised by *Eco*RI/*Hind*III digestion and used as a probe (Hu and Messing, 1982). The 8.5-kb fragment of chromosomal DNA was then digested with *Alu*I, and the fragments were cloned into the M13mp7 vector. Clones extending downstream from the *Alu*I site at nucleotide 87 (Figure 1) were detected by the plaque hybridization technique using the radioactively labelled strand of the *Eco*RI/*Hind*III fragment described above.

The orientations of the cloned inserts, depicted in Figure 1, were determined using a liquid hybridization technique which was simpler and faster than the standard Southern blotting procedure (Southern, 1975). The method is illustrated in Figure 2 for a *Hae*III fragment cloned in both directions into M13mp7. It exploits the large difference in size between the labelled RNA probe and the single-stranded DNA of the M13 DNA vector. A large number of clones were screened within 24 h. The procedure is also suitable for detecting hybrid formation between small labelled DNA fragments and phage DNA.

The nucleotide sequences of the DNA fragments cloned into the M13 vectors were determined by the dideoxynucleotide method (Sanger *et al.*, 1977). The sequence of the 16S RNA gene is delineated in Figure 3; 97% was obtained by sequencing both DNA strands at least twice (Figure 1) and we sequenced through each of the restriction sites. The extremities of the 16S RNA were defined by comparison with sequenced

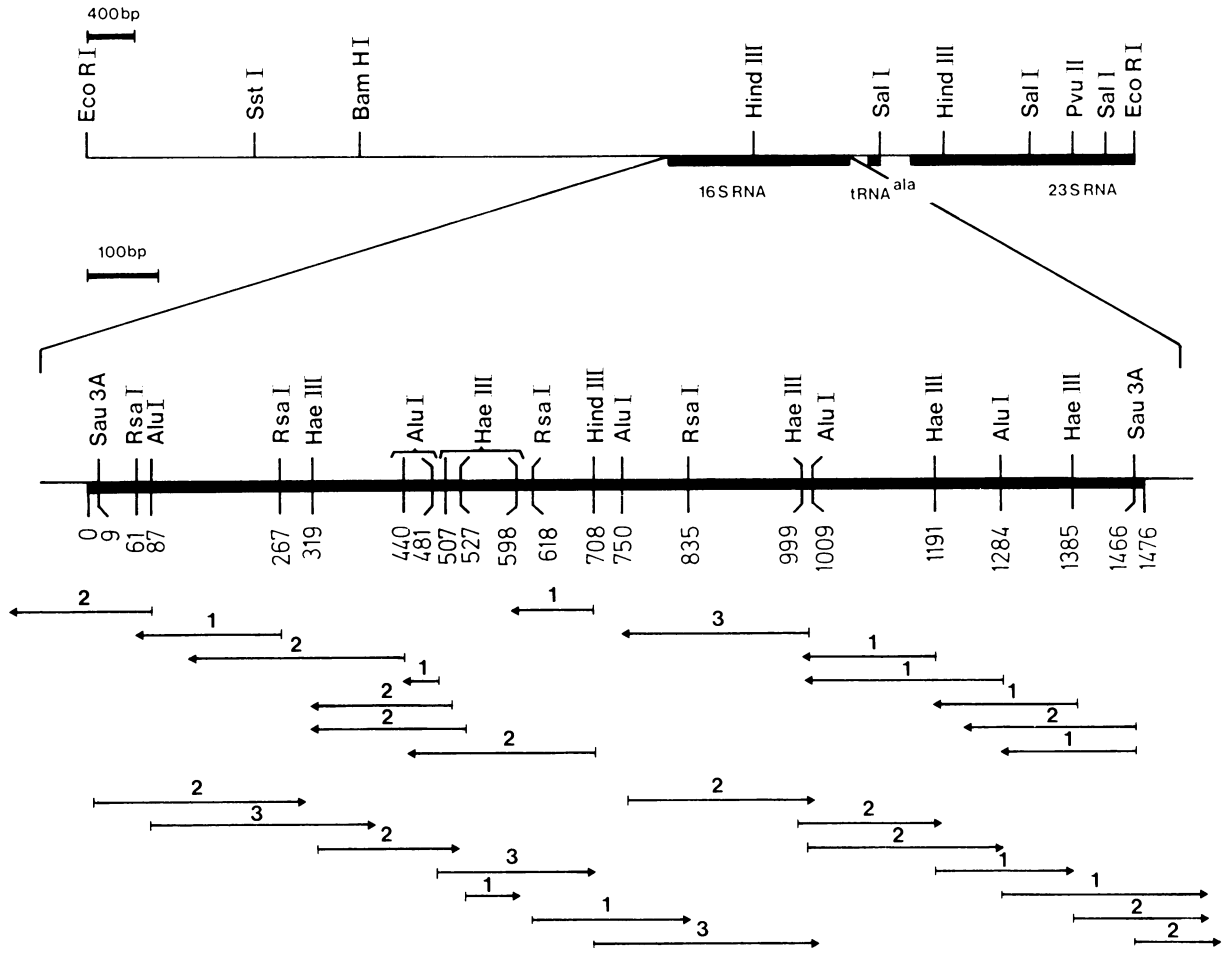

Fig. 1. Restriction endonuclease map of the *H. morrhua* gene showing the cloned fragments that were sequenced. The upper portion of the figure shows the 16S RNA gene, the spacer region, and part of the 23S RNA gene cloned into phage λgtWES.λB. Most of the 16S RNA gene was excised with the *Sau*3A enzyme and the lower part of the figure shows the DNA fragments that it yielded on further restriction nuclease digestion. These were subcloned into M13 vectors. The clones that were sequenced, and the direction of the sequencing, are indicated by arrows; the numbers on the arrows signify how many times each clone was sequenced.

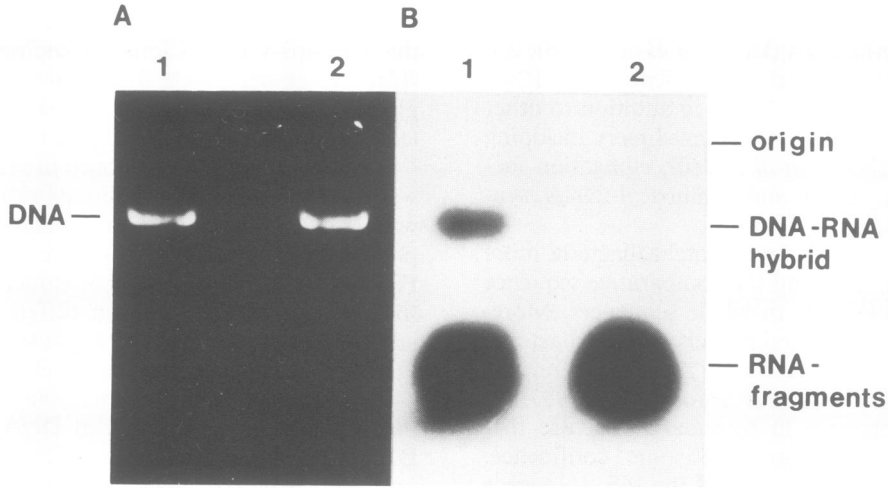

Fig. 2. Agarose gel showing the result of liquid hybridization of 16S RNA fragments, prepared by digestion with RNase S1 and end-labelled with [5′-^{32}P]pCp, to a 187-bp *Hae*III restriction fragment that had been cloned in both directions into phage M13mp7 vector. Hybrids formed with both clones were electrophoresed in an agarose gel as described in Materials and methods. **(A)** Agarose gel stained with ethidium bromide. **(B)** Autoradiograph of the gel. **Samples 1** and **2** contain the single-stranded DNA fragment oriented the same as the transcribing and non-transcribing strands of the DNA fragment, respectively.

AUUCCGGUUG	AUCCUGCCGG	AGGCUAUUGC	UAUCGGGGUC	CGAUUCAGCC	50
AUGCUAGUUG	UACGGGUUCA	GACCCGUAGC	AAAUAGCUCC	GUAACACGUG	100
GUCAAACUAC	CCUCUGGACC	GGGAUAUCCU	CGGGAAACUG	AGGUCAAUCC	150
CAGAUACUGC	UUUCAUGUUG	GAAUACAGAA	AGUCGGAAAC	GGUCCGCCGC	200
CGGAGGACGU	GACUGCGGCC	GAUUAGGUAG	ACGGUGGGGU	AACGGCCCAC	250
CGUGCCGAUA	AUCGGUACGG	GUUGUGAGAG	CAAGAACCCG	GAGACGGUAU	300
CUGAGACAAG	AUACCGGGCC	CUACGGGGCG	CAGCAGGCGC	GAAACCUUUA	350
CACUGCACGC	CAGUGCGAUA	AGGGGACCCC	GAGUGCGAGG	GCAUACAGUC	400
CUCGCUUUUC	GUGACCGUAA	GAAGGUCUCA	GAAUAAGAGC	UGGGCAAGAC	450
CGGUGCCAGC	CGCCGCGGUA	AUACCGGCAG	CUCGAGUGAU	AGCCACUAUU	500
AUUGGGCCUA	AAGCGUCCGU	AGCCGGCCGA	ACAGGUCCGU	CGGGAAAUCC	550
ACCCGCUCAA	CGGGUGGGAC	GUCCGGCGGA	AACCAGUCGG	CUUGGGGCCG	600
GGAGACCAGA	GAGGUACGUC	CGGGGUAGGA	GUGAAAUCCU	GUAAUCCUGG	650
ACGGACCACC	GGUAGCGAAA	GCGUCUCUGG	AGAACGGACC	CGACGGUGAG	700
GGACGAAAGC	UUGGGUCUCG	AACCGGAUUA	GAUACCCGGG	UAGUCCAAGC	750
UGUAAACGAU	GCUCGCUAGG	UGUGGCGUUG	GCUACGAGCC	AGCGCUGUGC	800
CGUAGGGAAG	CCGAGAAGCG	AGCCGCCUGG	GAAGUACGUC	CGCAAGGAUG	850
AAACUUAAAG	GAAUUGGCGG	GGGAGCACUA	CAACCGGAGG	AGCCUGCGGU	900
UUAAUUGGAC	UCAACGCCGG	ACAUCUCACC	GGCACCGACA	GUGUGCAGUG	950
ACAGUCAGUC	CGAUGGGCUU	ACUUGAGCCA	CUGAGAGGAG	GUGCAUGGCC	1000
GCCGUCAGCU	CGUACCGUGA	GGCGUCCUGU	UAAGUCAGGC	AACGAGCGAG	1050
ACCCGCGUCC	CUAAUUGCCA	GCAGCAGCCU	UGUGCUGGCU	GGGUACAUUA	1100
GGGAGACUGC	CGUCGCUAAG	ACGGAGGAAG	GAACGGGCAA	CGGUAGGUCA	1150
GUAUGCCCCG	AAUGUGCCGG	GCGACACGCG	GGCUACAAUG	GCCGAGACAG	1200
UGGGACGCUA	CCCCGAGAGG	GGACGCUAAU	CUCCUAACCU	CGGUCGUAGU	1250
UCGGAUUGCG	GGUUGAAACC	CACCCGCAUG	AAGCUGGAUU	CGGUAGUAAU	1300
CGCAUUUCAG	AAGAGUGCGG	UGAAUACGUC	CCUGCUCCUU	GCACACACCG	1350
CCCGUCAAAU	CACCCGAGUG	AGGUCCGGAU	GAGGCCGGCG	CAACGCCGGU	1400
CGAAUCUGGG	CUUCGCAAGG	GGGAUUAAGU	CGUAACAAGG	UAGCCGUAGG	1450
GGAAUCUGCG	GCUGGAUCAC	CUCCU			1475

Fig. 3. The nucleotide sequence of the 16S RNA gene from *H. morrhua*.

RNA molecules (Kagramanova *et al.*, 1982). The gene contains 1475 nucleotides.

Discussion

Comparison of the RNA sequence with those of H. volcanii, eubacteria and eukaryotes

The *H. morrhua* RNA is 1475 nucleotides long; three nucleotides longer than the *H. volcanii* RNA but 67 nucleotides shorter than that of *Escherichia coli* and up to 300 nucleotides shorter than the largest small subunit RNA of eukaryotes (Chan *et al.*, 1984). No insertions were detected comparable with that found in the *H. morrhua* 5S RNA sequence (Luehrsen *et al.*, 1981). Most of the insertions in the larger RNAs occur at a few points; they are considered below with respect to the secondary structural model. The degree of homology between the *H. morrhua* and *E. coli* sequences is 60%; it is 50% in the putative double helices compared with 81% in the loop regions. In contrast, the archaebacteria and

Table I. Exceptional aspects of conserved sequences in the *H. morrhua* 16S RNA

	H. morrhua	*H. volcanii*	Eubacteria	Eukaryotes	Loop-helix	
1.	G-A-U-A-U_{127}	G-A-U-A-A	G-A-U-A-A_{152}	G-A-U-A-A_{150}	9	–
2.	U-C-A-A-U-C_{148}	C-U-A-A-U-A	C-U-A-A-U-A_{174}	C-U-A-A-U-A_{173}	9	9
3.	G-A-C-G_{209}	G-A-U-G	G-A-U-G_{230}	absent	8	–
4.	G-C-C-A_{362}	G-C-A-A	G-C-A-A_{383}	variable	16	–
5.	G-C-G_{1173}	G-C-U	G-C-U_{1224}	G-C-U_{1523}	33/31	–
6.	U_{1263}-A_{1272}	C-G	C_{1314}-G_{1323}	non-conserved	–	43

The numbering system for the eubacteria derives from the *E. coli* 16S RNA sequence (Brosius *et al.*, 1978) and for eukaryotes it originates from the rat sequence (Chan *et al.*, 1984).

eukaryotes share little common sequence except at their 3′ ends. The homology with the sequence of *H. volcanii*, which has a separate line of descent from *H. morrhua* (Woese, 1981), is 89%; it is 85% in the double helices and 98% in the loop regions. This high latter value reflects that the loop regions tend to contain conserved sequences of functional importance (Noller, 1980). However, there are a few changes in such sequences in *H. morrhua* that are otherwise highly conserved in the RNAs of eubacteria and *H. volcanii*. They are listed in Table I.

Secondary structure of the RNA

Secondary structural models have been derived for the small subunit RNAs mainly on the basis of phylogenetic sequence comparisons, where evidence for the presence of a double helix is provided by base pairing in one organism being replaced by alternative base pairing in another; the phenomenon is termed compensating base changes. The latest versions of the eubacterial RNA models from Strasbourg (Stiegler *et al.*, 1981), Berlin (Maly and Brimacombe, 1983) and Santa Cruz/Urbana (Woese *et al.*, 1983) show a large measure of agreement. Moreover, the extensive phylogenetic evidence based upon 16 completed sequences and nearly 200 catalogues of ribonuclease T1 oligonucleotide sequences from small subunit RNAs has been summarized recently by Woese *et al.* (1983). Recent models have also appeared for the larger eukaryotic 18S RNAs from *Dictyostelium discoideum* (Olsen *et al.*, 1983) and rat (Chan *et al.*, 1984) which show extensive structural homology with eubacterial RNAs, especially in the central and 3′ domains.

In Figure 4, we present a secondary structural model for the *H. morrhua* 16S RNA. Below we consider two applications of the model. (1) Compensating base changes with the *H. volcanii* structure (Gupta *et al.*, 1983) were localized and they provide the first phylogenetic evidence for those structural elements that are confined to the archaebacteria (Table II). (2). Owing to their wide evolutionary divergence, many compensating base changes can be found between the common double helices of the archaebacteria and eubacteria which provide evidence for uncertain or additional helices in both structures. These inferences are considered with respect to the individual helices which are numbered in Figure 4.

Helix 9a. Our results provide further phylogenetic support for this helix which is present only in the Santa Cruz/Urbana model. It is more stable in *H. morrhua* than in most eubacteria and eukaryotes.

Helix 12. The relatively unstable region at the centre of this helix was only base paired in the Santa Cruz/Urbana model. Both archaebacterial RNAs are more stable in this region and exhibit a compensating change.

Helix 13. While the base of this helix is common to all models the remainder can be drawn either as a continuous helix (Berlin/Strasbourg) or by looping out G-A-A and generating four new base pairs (Santa Cruz/Urbana – see Figure 3); our data provide further phylogenetic evidence for the latter.

Helix 23b. This has been extended into the internal loop by 4 bp (G-G-U-A_{616}/G-A-C-C_{657}); this regular base pairing is a eukaryotic feature (see below).

Helix 27. The additional 2 bp drawn at the base of the helix (Sant Cruz/Urbana) are supported by both archaebacterial sequences.

Helix 29. This has been extended by 2 bp with a looped out guanosine; the extension is compatible with the phylogenetic data for eubacteria and eukaryotes.

Helix 40b. An extra 8 bp are drawn between 1065 – 1074/1087 – 1094. This region is more stable in both archaebacteria than in either eubacteria or eukaryotes. The base pairing is compatible with the phylogenetic evidence.

Helix 41. This was extended at the base of the helix by 3 bp that are supported phylogenetically.

Helix 43a. We have closed up this region, as in the Berlin model, although the additional base pairing is only weakly supported phylogenetically.

Helix 45. The cytidine bulged from the helix of *H. volcanii* at position 1376 (Gupta *et al.*, 1983) is paired with guanosine in *H. morrhua*.

Several sites occur where weak structuring in the eubacterial RNAs becomes more stable in the halophile RNAs; at present we cannot infer whether this is a general feature of the archaebacterial RNAs or whether it reflects the adaptation of the halophile RNAs to their high salt microenvironments.

The secondary structural model is valuable for detecting structural regions that have remained unstable during the evolution of the three kingdoms, and for directly comparing the archaebacterial and eukaryotic RNA structures; these points are considered separately below.

Labile regions of the RNA structure during evolution

Six regions of secondary structure are listed in Table II which vary in each of the three primary kingdoms. Five lie in the 5′ domain which, significantly, has been accredited with a mainly structural role in the ribosome structure (Noller, 1980). The sixth occurs within the 3′-terminal subdomain and although this has been implicated in both mRNA and tRNA binding the variable helix, itself, has not.

Eukaryotic-like characteristics of the RNA

When aligning the RNA sequences of *H. volcanii* with those

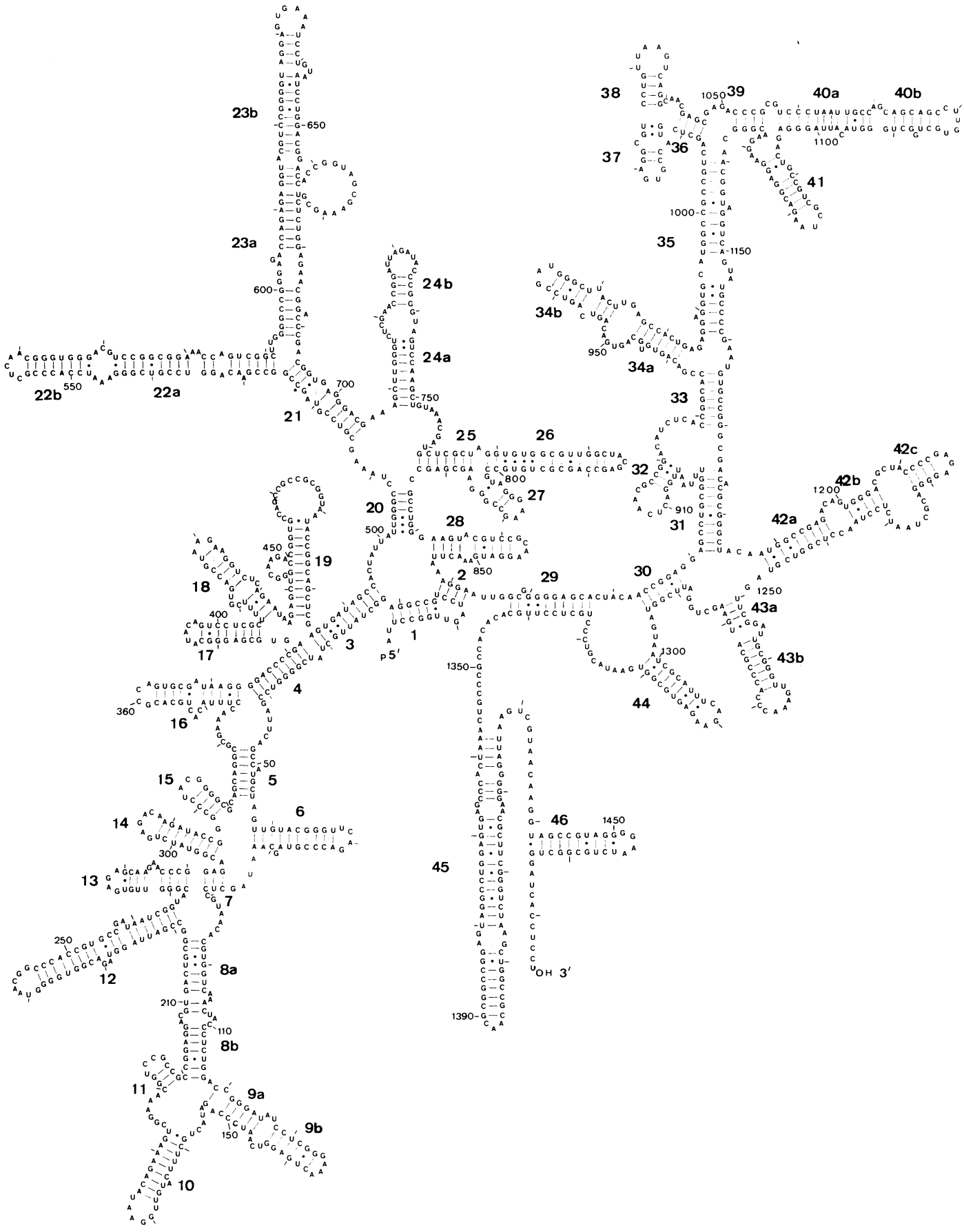

Fig. 4. Proposed secondary structural model for the 16S RNA of *H. morrhua*. The structure derives from phylogenetic sequence and oligonucleotide comparisons for 16S RNA (reviewed by Woese *et al.*, 1983). The helices are numbered from the 5′ end. Some differences from the latest versions of the generalized models (Stiegler *et al.*, 1981; Maly and Brimacombe, 1983; Woese *et al.*, 1983) are considered in the text.

Table II. Labile features of the RNA structure during the evolution of the eubacteria, archaebacteria and eukaryotes

Helix/ loop	Eubacteria	Archaebacteria	Eukaryotes
6	large loop at base of helix	loop partially deleted	partial deletion of helix 6 and loop
10	short helix (3 bp)	long helix (9 bp)	80 n insert
11	long helix (8 bp)	short helix (3 bp)	80 n insert
17	helix (8 bp) with large internal loop	continuous helix (8 bp)	helix deleted – only a loop present
18	long helix (18 bp) – large internal loop	smaller helix (10 bp)	25 n insert
45	intermediate helix	shortened helix	elongated helix

bp = base pair; n = nucleotide.

Table III. Structural similarities between the archaebacterial and eukaryotic small subunit RNAs

Helix/	Eubacteria	Archaebacteria	Eukaryotes
7	G-U-G_{115}/C-A-C_{314}	C-U-C_{89}/G-A-G_{293}	C-U-C_{98}/G-A-G_{337}
16	internal loop C-A-$\underline{A}_{374}$	internal loop C-A-$\underline{C}_{353}$	internal loop C-A-$\underline{C}_{497}$
19	internal loop in centre of helix	loop contains extra nucleotide and displaced 1 bp	loop contains extra nucleotide and displaced 1 bp
23b	mainly purine juxtapositions	G-G-U-A_{616}/G-A-C-C_{657}	U-U-C-G-U-A_{948}/ G-A-C-G-A-A_{991}
29	G-C-A-C-A_{937}	G-C-A-C-$\underline{U}$-A_{880}	G-C-A-C-$\underline{U}$/$\underline{C}$-A_{1221}
34	–	U·C 'pairing'	U·C 'pairing'

The numbering systems for eubacteria, archaebacteria and eukaryotes derive from *E. coli* (Brosius *et al.*, 1978), *H. morrhua* and rat (Chan *et al.*, 1984).

of eubacteria and eukaryotes, Gupta *et al.* (1983) noted that the invariant nucleotides C_{47}, C_{912}, G_{966}, U_{1381} and C_{1384} in eubacteria (*E. coli* numbers) became A_{43}, U_{856}, U_{911}, C_{1330} and U_{1333} in both the archaebacterium and eukaryotes (*H. morrhua* numbers); the results from *H. morrhua* reinforce this conclusion. In addition, other features which are common to the sequences of the two archaebacterial and eukaryotic RNAs are listed in Table III. Only one example was noted of the eubacterial and eukaryotic RNAs being closer to one another than to those of archaebacteria. This was in the conserved sequence G-C-$\underline{C}$-G-C_{462} in *H. morrhua* and *H. volcanii* which became G-C-$\underline{A}$-G-C in the eubacteria and eukaryotes. The results serve to reinforce both the concept that the archaebacteria are closer to the other two primary kingdoms than the latter are to one another, and the conclusion that therefore they must each have a distinct evolutionary heritage.

Materials and methods

Preparation of bacteria and labelled rRNA fragments

H. morrhua strain ATCC 17082 was a gift of Dr. G.E.Fox. It was cultured in high salt medium (Bayley, 1971) and harvested by centrifugation (15 min at 8000 *g*). Ribosomes were extracted by grinding the cells with alumina and the RNAs were fractionated on a sucrose gradient containing lithium dodecylsulphate (Fellner, 1969).

The RNA was prepared for hybridizing by dissolving 50 μg of 16S or 23S RNA in 0.12 ml digestion buffer (100 mM Na acetate, 10 mM Mg acetate, 1 mM Zn acetate, 100 mM KCl, pH 4.8). It was treated with 20 units RNase S1 for 15 min at 20°C and the enzyme was removed by two phenol and two chloroform extractions. The RNA was precipitated by 2.5 volumes of ethanol, washed with 1 ml ethanol, dried in a vacuum centrifuge and 3′ end-labelled with [^{32}P]pCp (Amersham) using the T4 RNA ligase (P.L. Biochemicals) as described by Bruce and Uhlenbeck (1978).

Preparation of H. morrhua and phage λ DNA

H. morrhua cells were lysed by suspending 1 g cells in 10 ml, 30 mM Tris-HCl, 1 mM EDTA, pH 7.4, 2% Triton X-100. The cleared lysate was extracted with phenol, and precipitated with ethanol and centrifuged to equilibrium in a caesium chloride gradient (48 h at 42 000 r.p.m. and 15°C in a Beckmann Ti 80 rotor). The DNA band was removed and the ethidium bromide was extracted five times with butanol. The DNA was dialysed against 20 mM Tris-HCl, 1 mM EDTA, pH 8 overnight and stored at 4°C.

DNA from λgtWES.λB phage, and the *E. coli* strains BHB 2688, BHB 2690 and LE 392 were kindly provided by E.Ø.Jensen. λ phages, λ phage DNA and the *in vitro* packaging mixture were prepared essentially as described by Maniatis *et al.* (1982).

λ Cloning and screening of the constructed genomic library

Total DNA from *H. morrhua* was digested with *Eco*RI and mixed with DNA from phage λgtWES.λB that had been digested with *Eco*RI/*Sst*I. The mixture was ligated with T4 DNA ligase at a DNA concentration of 0.8 mg/ml. 1–2 μg ligated DNA was mixed with the *in vitro* packaging mixture of phage λ and aliquots yielding 10 000–15 000 plaques were spread on agar plates. The plaques were transferred to nitrocellulose filters (Gene screen, NEN) and hybridized overnight with the 3′ end-labelled fragments of 16S RNA in the presence of 50% formamide and 30 mM Na citrate, 300 mM Na chloride essentially as described by Benton and Davis (1977). Plaques containing hybrids were purified by replating and screening twice. The phage λ DNA was prepared on a large scale (Maniatis *et al.*, 1982). A clone containing a 8.5-kb fragment was digested with various restriction endonucleases (Amersham and Biolabs) and electrophoresed on 0.8% agarose gels. DNA was transferred to the gene screen filters and hybridized with 16S or 23S RNA fragments. Those DNA fragments that hybridized with the 16S RNA were further digested and separated on 4.5% polyacrylamide gels. The resulting fragments were extracted and cloned into M13mp7, mp8 and mp9 vectors (P.L. Biochemicals: Messing *et al.*, 1981; Messing and Vieira, 1982).

The orientations of the cloned inserts were ascertained either by 'figure-eight' mapping or by liquid hybridization with 3′ end-labelled 16S RNA fragments. Liquid hybridization was performed by mixing 0.5 μg single-stranded DNA from the M13 vector with the 3′ end-labelled RNA fragments in 20 μl buffer (40 mM Pipes, pH 6.4, 1 mM EDTA, 0.2 M Na chloride, 80% deionized formamide). The mixture was incubated at 50°C for 30 min and slowly cooled to 35°C before loading onto a 0.8% agarose gel containing 50 mM Tris-borate, 1 mM EDTA, pH 8.3 and electrophoresed for 1–2 h at 80 V. The gels were autoradiographed overnight using an intensifying screen. The cloned fragments were sequenced by the procedure of Sanger *et al.* (1977); DNA polymerase and dideoxynucleotides were obtained from P.L. Biochemicals.

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References

Bayley,S.T. (1971) in Last,J.A. and Laskin,A.I. (eds.), *Protein Synthesis in Bacterial Systems*, M. Dekker Inc., NY, pp. 89-111.

Benton,W.D. and Davis,R.W. (1977) *Science (Wash.)*, **196**, 180-184.

Brosius,J., Palmer,M.L., Kennedy,P.J. and Noller,H.F. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 4801-4806.

Bruce,A.G. and Uhlenbeck,O.C. (1978) *Nucleic Acids Res.*, **5**, 3665-3677.

Chan,Y.L., Gutell,R., Noller,H.F. and Wool,I.G. (1984) *J. Biol. Chem.*, **259**, 224-232.

De Rosa,M., Gabacorta,A. and Bullock,J.D. (1976) *Phytochemistry*, **15**, 143-145.

Fellner,P. (1969) *Eur. J. Biochem.*, **11**, 12-27.

Gupta,R., Lanter,J.M. and Woese,C.R. (1983) *Science (Wash.)*, **221**, 656-659.

Hu.Y. and Messing,J. (1982) *Gene*, **17**, 271-277.

Huet,J., Schnabel,R., Sentenac,A. and Zillig,W. (1983) *EMBO J.*, **2**, 1291-1294.

Kagramanova,V.K., Mankin,A.S., Baratora,L.A. and Bogdanov,A.A. (1982) *FEBS Lett.*, **144**, 177-180.

Kandler,O. (1979) *Naturwissenschaften,* **66**, 95-105.
Kessel,M. and Klink,F. (1980) *Nature,* **287**, 250-251.
Kwok,Y. and Wong,J.T.F. (1980) *Can. J. Biochem.,* **58**, 213-222.
Luehrsen,K.R., Nicholson,D.E., Eubanks,D.C. and Fox,G.E. (1981) *Nature,* **293**, 755-756.
Magnum,L.J., Luehrsen,K.R. and Woese,C.R. (1978) *J. Mol. Evol.,* **11**, 1-8.
Maly,P. and Brimacombe,R. (1983) *Nucleic Acids Res.,* **11**, 7263-7286.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual,* published by Cold Spring Harbor Laboratory Press, NY.
Matheson,A.T., Yaguchi,M., Balch,W.E. and Wolfe,R.S. (1980) *Biochim. Biophys. Acta,* **626**, 162-177.
Messing,J. and Vieira,J. (1982) *Gene,* **19**, 269-276.
Messing,J., Crea,R. and Seeburg,P.H. (1981) *Nucleic Acids Res.,* **9**, 309-321.
Noller,H.F. (1980) Chambliss,G. *et al.* (eds.), *Ribosomes: Structure, Function and Genetics,* University Park Press, Baltimore, pp. 3-22.
Olsen,G., McCarroll,R. and Sogin,M.L. (1983) *Nucleic Acids Res.,* **11**, 8037-8049.
Sanger,F., Nicklen,S. and Coulsen,A.R. (1977) *Proc. Natl. Acad. Sci. USA,* **74**, 5463-5467.
Southern,E.M. (1975) *J. Mol. Biol.,* **98**, 503-517.
Stiegler,P., Carbon,P., Ebel,J.P. and Ehresmann,C. (1981) *Eur. J. Biochem.,* **120**, 487-495.
Woese,C.R. (1981) *Sci. Am.,* **7**, 94-106.
Woese,C.R. and Gupta,R. (1981) *Nature,* **289**, 95-96.
Woese,C.R., Magnum,L.J. and Fox,G.E. (1978) *J. Mol. Evol.,* **11**, 245-252.
Woese,C.R., Gutell,R., Gupta,R. and Noller,H.F. (1983) *Microbiol. Rev.,* **47**, 621-669.
Zwieb,C., Glotz,C. and Brimacombe,R. (1981) *Nucleic Acids Res.,* **9**, 3621-3640.

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