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DNA sequence of the 16S rRNA/23S rRNA intercistronic spacer of two rDNA operons of the archaebacterium *Methanococcus vannielii*

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ABSTRACT

The DNA sequence of the spacer (plus flanking) regions separating the 16S rRNA and 23S rRNA genes of two presumptive rDNA operons of the archaebacterium Methanococcus vannielii was determined. The spacers are 156 and 242 base pairs in size and they share a sequence homology of 49 base pairs following the 3' terminus of the 16S rRNA gene and of about 60 base pairs preceding the 5' end of the 23S rRNA gene. The 242 base pair spacer, in addition contains a sequence which can be transcribed into $tRNA^{Ala}$, whereas no tRNA-like secondary structure can be delineated from the 156 base pair spacer region. Almost complete sequence homology was detected between the end of the 16S rRNA gene and the 3' termini of either Escherichia coli or Halobacterium halobium 16S rRNA, whereas the putative 5' terminal 23S rRNA sequence shared partial homology with E. coli 23S rRNA and eukaryotic 5.8S rRNA.

INTRODUCTION

In eubacteria, genes for rRNA are invariably organized in rRNA operons and transcribed in the order 16S, 23S and 5S rRNA. Genes for tRNA may be situated in the spacer region between the 16S and 23S rRNA genes and co-transcribed with them (for review see ref. 1). In eukaryotes, on the other hand genes for cytoplasmic tRNA's have never been observed to be associated with those for ribosomal RNA.

Members of the third primary kingdom, the archaebacteria, exhibit a heterogeneous organization of genes for rRNA ranging from a typical eubacterial organization in Halobacterium (2) over a mixed type organization in Methanococcus (3) to a completely unlinked chromosomal location in certain thermoacidophiles (4). In Methanococcus, the existence of four apparent rDNA operons of the eubacterial type and of a single unlinked 5S rRNA gene has been reported. From their hybridization pattern with

bulk tRNA it was concluded that the 16S/23S intercistronic spacer contained (a) gene(s) for tRNA (3). To detail the hybridization data and to gain information on the type of tRNA encoded by the 16S/23S rRNA intercistronic spacer we have determined the DNA sequence of the spacers of two rRNA operons of this archaebacterium.

MATERIALS AND METHODS

Strains and Plasmids

Plasmids pMV1 and pMV15 with cloned M. vannielii rDNA were used for transformations. Vector plasmid pACYC184 (5) was a gift from A. Pecher, München.

DNA Preparation and Analysis

Plasmid DNA was prepared by the method of Clewell and Helinski (6) and restriction fragments were isolated from low-melting agarose gels as described by Maniatis et al. (7).

Restriction endonuclease digestion, ligation of DNA fragments and gel electrophoretic separations were carried out as described recently (3).

For transformation with plasmid DNA the method of Cohen et al. (8) was followed.

DNA Sequence Analysis

DNA fragments were labeled at the 3' end, using [α-^{32}P]dATP and DNA polymerase (large fragment according to Klenow) as described by Maniatis et al. (7). Labeling at the 5' end with [γ-^{32}P] ATP and polynucleotide kinase and sequencing reactions were carried out with the method of Maxam and Gilbert (9), exept for employing the modification of the A + G reaction described by Gray et al. (10). Electrophoresis was in 8 % and 20 % polyacrylamide gels containing 7M urea.

Materials

Restriction endonucleases, polynucleotide kinase, calf intestinal alkaline phosphatase and DNA polymerase I (large fragment according to Klenow) were supplied by Boehringer Mannheim GmbH.

[α-^{32}P]dATP and [γ-^{32}P]ATP were obtained from Amersham Buchler, Braunschweig.

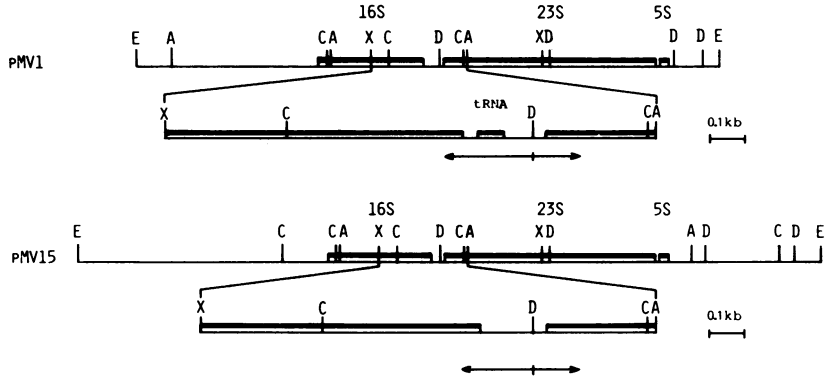

Figure 1. Physical maps of the EcoRI inserts of plasmids pMV1 and pMV15, each containing a complete rRNA gene cluster from M. vannielii (3). The subcloned XhoI-ClaI fragments with the 16S/23S spacers are drawn out enlarged. Coding regions for rRNA and tRNA are given as bars. Arrows indicate origin and extent of the sequenced regions (fragments were both 3' and 5' labeled in separate experiments). Sites for restriction endonucleases are designated as follows: E, EcoRI; A, ClaI; C, HincII; D, HindIII; X, XhoI.

RESULTS AND DISCUSSION

Subcloning and Sequencing

Fig. 1 gives the restriction map for the EcoRI inserts of plasmids pMV1 and pMV15, which each contain a complete M. vannielii rRNA operon (3). The XhoI-ClaI fragments of pMV1 and pMV15, which bear the 16S-23S spacer regions and adjacent parts of the 16S and 23S rRNA genes were subcloned into vector pACYC184 by replacing a SalI-ClaI fragment of the tet^R region.

For sequencing, these plasmids were linearized by digestion with restriction endonuclease HindIII, labeled either at the 3' or at the 5' end and recut with EcoRI at a restriction site located within the pACYC184 DNA. Fragments (labeled at only one end) were isolated from agarose gels and sequenced by the chemical method of Maxam and Gilbert (9). The origin and direction of each sequence analysis performed are indicated by arrows in Fig. 1; the sequences obtained from both rRNA operons, aligned for homologous regions, are given in Fig. 2.

The 3' Terminus of the 16S rRNA Gene

The M. vannielii 16S rRNA gene terminus was identified with

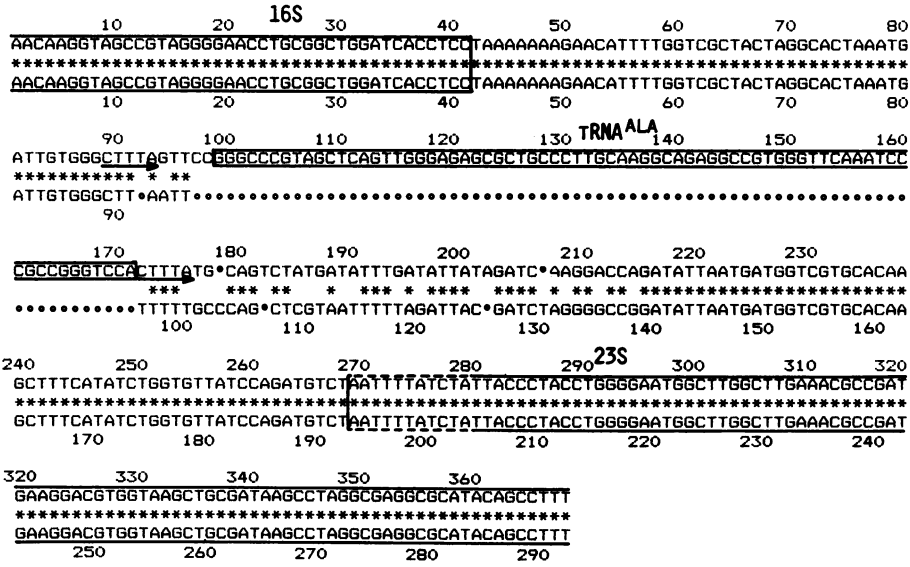

Figure 2. Nucleotide sequences of the 16S/23S spacer plus flanking parts of the 16S and 23S rRNA coding regions of pMV1 (upper line) and pMV15 (lower line). The non-coding RNA-like strands are shown; bases are numbered in the direction 5' to 3'. The sequences are aligned for optimal homology (indicated by asterisks) by introduction of gaps (•). Coding regions for $tRNA^{Ala}$, 16S and 23S rRNA are boxed. Broken lines give the putative start of the 23S rRNA gene (see also Fig. 4). Two pentanucleotide direct repeats flanking the $tRNA^{Ala}$ gene in the pMV1 sequence are marked by arrows.

the aid of the known 3' terminal oligonucleotide ($AUCACCUCC_{OH}$), (11) (Fig. 2). Fig. 3 shows a probable secondary structure model of the 3' end of the presumptive M. vannielii 16S rRNA and compares it with equivalent parts of the E. coli, H. halobium and Saccharomyces cerevisiae sequences (12).

The E. coli sequence has three additional bases at the 3' terminus and an exchange of two opposite bases in the stem of the 'dimethyladenine hairpin'. The H. halobium sequence differs in an additional U at the 3' terminus (which, however, also appears in the DNA sequence of M. vannielii but is obviously eliminated during rRNA processing) and one base exchange in the stem of the 'dimethyladenine hairpin', which converts a G·C to a G·U base pairing.

The main differences of the yeast sequence are the absence

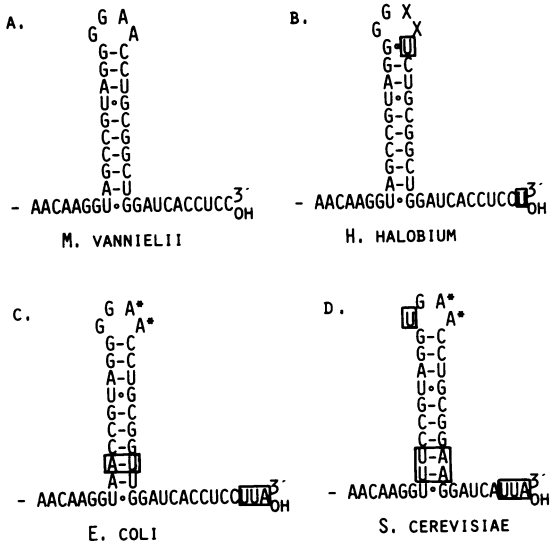

Figure 3. The primary and secondary structures of the 3' end of 16S rRNA's from M. vannielii (A.), H. halobium (B.), E. coli (C.) and S. cerevisiae (D.) (12). Altered bases with respect to the M. vannielii sequence are indicated by boxes. A* is N-6-dimethyladenine, X an unidentified modified base most likely N-6-dimethyladenine (12).

of the anti-Shine-Dalgarno sequence (CCUCC) and the presence of the sequence UGAA instead of GGAA in the loop of the dimethyladenine hairpin.

The 5' Terminus of the 23S rRNA Gene

As no sequence data for M. vannielii 23S rRNA were available an identification of the 5' terminus was attempted by alignment to optimal homology with E. coli 23S rRNA and with Xenopus laevis

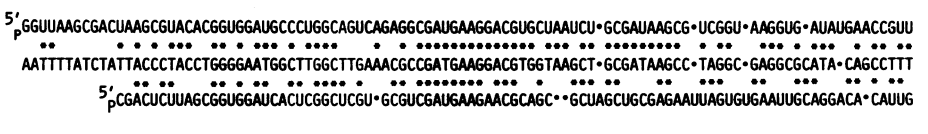

Figure 4. A comparison of the primary structure of the 5' end of the 23S rRNA gene from M. vannielii (middle line) with the 5' ends of E. coli 23S rRNA (upper line) and Xenopus laevis 5.8S rRNA (lower line) (13, 14). Identical bases are indicated by asterisks. Gaps (•) are introduced into the sequences to achieve optimal homology.

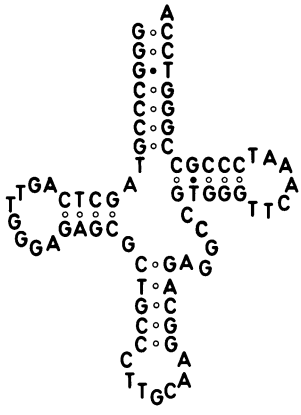

Figure 5. Cloverleaf structure formed by the non-coding, RNA-like strand of the putative $tRNA^{Ala}$ gene.

5.8S rRNA. On the basis of sequence homologies it had been suggested that the 5' end of prokaryotic 23S rRNA's and eukaryotic 5.8S rRNA's possess a common evolutionary origin (13, 14). This homology, as Fig. 4 shows, exists also between eukaryotic 5.8S rRNA and the 5' terminal sequence of archaebacterial 23S rRNA. There is also an extensive sequence homology between M. vannielii and E. coli 23S rRNA termini.

pMV1 but not pMV15 Contains a tRNA Gene

A comparison of the sequences of plasmid pMV1 and pMV15 reveals that the 16S rRNA coding regions and 49 base pairs downstream from the 3' terminus are completely identical. This is also found for the sequence starting at base No. 215 in pMV1 or base No. 139 in pMV15, respectively, and reaching into the 23S rRNA coding regions as far as determined.

In the non-homologous part of the pMV1 spacer sequence a tRNA-like structure was found. The transcript of the putative tRNA gene was identified as $tRNA^{Ala}$ (Fig. 5) by its anticodon and its sequence homologies with $tRNA^{Ala}_{1B}$ from E. coli (65 % identical bases) and $tRNA^{Ala}$ from yeast (67 %) and Halobacterium cutirubrum (78 %) (15). It should be emphasized that the M. vannielii $tRNA^{Ala}$ gene does not code for the 3' CCA end, unlike the situation in E. coli (16), Bacillus subtilis (17) and Anacystis nidulans (18) spacer $tRNA^{Ala}$ genes. The $tRNA^{Ala}$ gene is not interrupted by an intron, as in the case of Sulfolobus solfataricus $tRNA^{Ser}$ and $tRNA^{Leu}$ (19).

No tRNA-like structure could be detected in the spacer sequence carried by plasmid pMV15 although DNA fragments containing this spacer hybridized with bulk tRNA from Methanococcus (3). There are two possible explanations for this apparent discrepancy: (i) The 16S-23S intercistronic spacer may contain a partially deleted tRNA pseudogene, which still hybridizes to some tRNA, or (ii) an RNA fragment generated by RNA processing from the spacer region of the primary transcript of the presumptive operon or from the 3' end of the 16S rRNA may be stable enough to co-purify with tRNA and mimick tRNA homology. As a support to the second possibility, Alberty et al. (19) reported, that in the case of rat liver, tRNA preparations were contaminated by a small RNA fragment from the 3' end of 16S rRNA.

For any speculation on the relationship of the two spacer sequences it may be relevant that the $tRNA^{Ala}$ coding sequence of the rRNA operon carried by pMV1 is flanked by a direct repeat of 5 nucleotides. Apart from the possibility of being purely statistical, this may also be related to any excision/insertion of the tRNA sequence or to some RNA processing signal.

In conclusion: The two 16S/23S rRNA intercistronic spacers differ in length by 76 nucleotides and in the presence of a $tRNA^{Ala}$ coding sequence in the longer, 242 base pair spacer. No complete tRNA-like structure could be recognized in the short spacer. Features of the spacer plus flanking regions which are common to those from eubacteria are: the high homology of the 3' terminus of the 16S rRNA and the 5' end of the 23S rRNA genes with prokaryotic 16S rRNA and 23S rRNA (and eukaryotic 5.8S rRNA, respectively); the presence of the anti-Shine-Dalgarno consensus sequence at the 3' end of the 16S rRNA (see also ref. 11) and the GGAA sequence in the dimethyladenine loop; the presence of a $tRNA^{Ala}$ coding sequence. Unusual, however, is that the $tRNA^{Ala}$ gene is not associated with a coding sequence for $tRNA^{Ile}$ as in all other $tRNA^{Ala}$ containing spacers (16-18, 20-22).

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REFERENCES

1. Gray, M.W. and Doolittle, W.F. (1982) Microbiol. Rev. 46, 1-42.
2. Hofman, J.D., Lau, R.U., Doolittle, W.F. (1979) Nucl. Acids Res. 7, 1321-1333.
3. Jarsch, M., Altenbuchner, J., Böck, A. (1983) Mol. Gen. Genet. 189, 41-47.
4. Tu, J. and Zillig, W. (1982) Nucl. Acids Res. 10, 7231-7238.
5. Chang, A.C.Y. and Cohen, S.N. (1978) J. Bacteriol. 134, 1141-1156.
6. Clewell, D.B. and Helinski, D.R. (1969) Proc. Natl. Acad. Sci. USA 62, 1159-1166.
7. Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) in Molecular Cloning, pp. 113-116, p. 170, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
8. Cohen, S.N., Chang, A.C.Y., Hsu, L. (1977) Proc. Natl. Acad. Sci. USA 69, 2110-2114.
9. Maxam, A.M. and Gilbert, W. (1980) in Methods in Enzymology 65, Grossman, L. and Moldave, K., pp. 499-560, Academic Press, New York.
10. Gray, C.P., Sommer, R., Polke, C., Beck, E., Schaller, H. (1978) Proc. Natl. Acad. Sci. USA 75, 50-53.
11. Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., Wolfe, R. S. (1979) Microbiol. Rev. 43, 260-296.
12. Kagramanova, V.K., Mankin, A.S., Bogdanov, A.A. (1982) FEBS Lett. 144, 177-180.
13. Nazar, R.N. (1980) FEBS Lett. 119, 212-214.
14. Jacq, B. (1981) Nucl. Acids Res. 9, 2913-2932.
15. Gauss, D.H. and Sprinzl, M. (1983) Nucl. Acids Res. 11, r1-r53.
16. Young, R.A., Macklis, R., Steitz, J.A. (1979) J. Biol. Chem. 254, 3264-3271.
17. Loughney, K., Lund, E., Dahlberg, J.E. (1982) Nucl. Acids Res. 10, 1607-1624.
18. Williamson, S.E., Doolittle, W.F. (1983) Nucl. Acids Res.11, 225-235.
19. Kaine, B.P., Gupta, R., Woese, C.R. (1983) Proc. Natl. Acad. Sci. USA 69, 3309-3312.
20. Alberty, H., Raba, M., Gross, H.J. (1978) Nucl. Acids Res.5, 425-434.
21. Koch, W., Edwards, K., Kössel, H. (1981) Cell 25, 203-213.
22. Takaiwa, F. and Sugiura, M. (1982) Nucl. Acids Res. 10, 2665-2676.
23. Orozco, E.M.J., Rushow, K.E., Dodd, J.R., Hallick, R.B. (1980) J. Biol. Chem. 255, 10997-11003.
24. Graf, L., Kössel, H., Stutz, E. (1980) Nature 286, 908-910.