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The sequence of the 6S RNA gene of *Pseudomonas aeruginosa*

Detlef W.Vogel, Roland K.Hartmann, Joachim C.R.Struck, Norbert Ulbrich and Volker A.Erdmann

Institut für Biochemie, Freie Universität Berlin, Thielallee 63, 1000 Berlin 33, FRG

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ABSTRACT

From the gram-negative eubacterium Pseudomonas aeruginosa we have isolated a stable 6S RNA, approximately 180 nucleotides in length. The RNA was partially sequenced and identified by comparison with the known Escherichia coli 6S RNA sequence. Southern hybridizations revealed a single copy gene coding for the 6S RNA. DNA from other prokaryotes, i.e. E. coli, Thermus thermophilus, Bacillus subtilis, Bacillus stearothermophilus and Halobacterium maris mortui , did not give detectable hybridization signals. The 6S RNA gene was cloned in E. coli and its complete primary structure was determined. Although the 6S RNA sequences from P. aeruginosa and E. coli share only a 60.4 % homology, we are able to propose a common secondary structural model.

INTRODUCTION

In E. coli several stable cytoplasmic RNAs have been identified (1), one of which is the 6S RNA (184 nucleotides), coded by one gene which is cotranscribed with a 22 kd protein gene (2). Its expression is not stringently controlled (3) and its concentration increases during the late log-phase of cell growth (4) to a maximal copy number of about 1000 molecules per cell (5). Although the 6S RNA was one of the first RNA-species to be sequenced (6), its function is still unknown. In E. coli the 6S RNA is part of an 11S ribonucleoprotein-complex (5) and it was supposed that this might be the eubacterial equivalent to the signal recognition particle (SRP), which mediates protein secretion in eukaryotes (7). Evidence for this hypothesis came from the observations (a) that the 6S RNA and one SRP-component, the 7SL RNA, share common structural features (7) and (b) that the 6S RNA could be coprecipitated with antibodies against an essential secretion factor, the secA-protein (8). On the other hand a mutant lacking the 6S RNA turned out to be viable and it had no deficiencies in secreting proteins (9). A heterologous reconstitution of the SRP using the 6S RNA instead of the 7SL RNA did not lead to active particles (7). Moreover an *in vitro* assay simulating

the protein secretion process of E. coli was shown to work without the 6S RNA (10).

What ever function the 6S RNA may have, it is of interest to learn more about the molecule itself. In order to define important structural features of the 6S RNA we have cloned and sequenced a 6S RNA gene of another gram-negative eubacterium, namely that of P. aeruginosa.

MATERIALS AND METHODS

Media and growth conditions for P. aeruginosa (ATCC 10145) were as described (11), except that cells were grown at 30°C. Agarose- and polyacrylamide gel electrophoresis, plasmid and genomic DNA isolation, electroelution and colony hybridization have been described in great detail and are not reiterated (11-15). Southern blotting was carried out as described (16) and the filters were washed at room temperature for 40 min. with 50% formamide/5 x SSC and for 20 min. with 2 x SSC. The 6S RNA was isolated from a crude 70S ribosomal fraction, by phenol extraction (17) and was separated by gelfiltration on Sephacryl S-200 (18). Fractions, which were shown by polyacrylamide gel electrophoresis to contain 6S RNA, were pooled, ethanol precipitated and radioactively labelled at their 3'-terminal end (19). Labelled 6S RNA was purified by electrophoresis and then partially sequenced using the enzymatic digestion method (20). To isolate the gene coding for the 6S RNA, genomic DNA was digested with Bam HI and Eco RI. The 6S DNA carrying DNA fragments were identified by hybridization utilizing the 3'-terminal labelled 6S RNA in the presence of unlabelled ribosomal RNA. In the Bam HI digest a signal at 3.0 kb could be detected and the respective DNA fragments were isolated by electroelution, ligated into a Bam HI linearized vector pBR 322 and E. coli X90 cells (21) were transformed. Postive clones were identified by colony hybridization (22). To determine the exact location of the 6S DNA carrying portion within the recombinant plasmid (pDV 52) it was digested with the restriction endonucleases Pvu II, Eco RI, Pst I, Ava I, Acc I, Sal I and Pvu I. To analyse the primary structure of the DNA, fragments were cloned into the double-standed replicative form of the M13 bacteriophages mp 8/9. The isolation of the recombinant phages was essentially as described (23).Phage DNA was collected by ethanol precipitation and dissolved in 14 µl TE buffer; 3 to 4 µl of this DNA solution were used for the sequencing reactions, which were performed according to the dideoxynulceotide chain termination method (24).

RESULTS

In search for an RNA species in P. aeruginosa, equivalent to the 6S RNA in E. coli, phenol extracted RNA from crude 70S ribosomes was separated by Sephacryl S-200 gel filtration and fractions were analysed by polyacrylamide gel electrophoresis (Fig. 1). Fractions 44-48, which exhibit several bands larger than 5S rRNA, were pooled, RNAs were labelled at their 3'-end and resolved by polyacrylamide gel electrophoresis. Two bands were eluted from the gel and analysed by enzymatic sequencing (data not shown). The most prominent band was identified to be a 23S rRNA fragment, comprising about

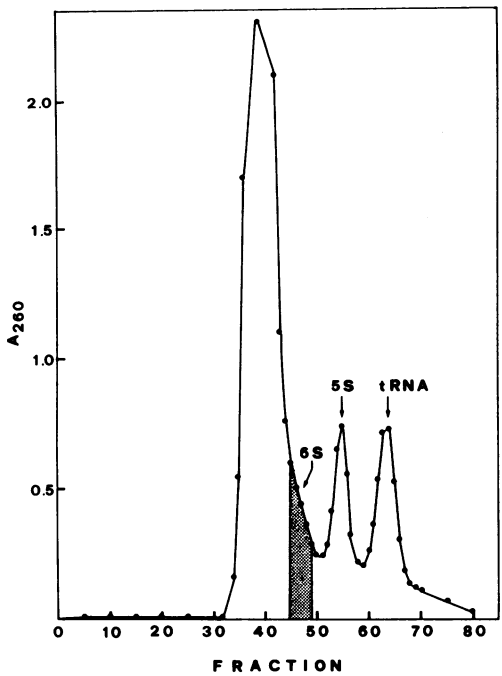

Figure 1. Gel filtration of P. aeruginosa RNA, extracted with phenol from a crude 70S ribosomal fraction. The sample (3000 A_{260} units), in buffer A (100mM Tris/HCl,pH 8.0; 500mM NaCl) was applied to a column of Sephacryl S-200 (2.6cm x 88cm) and eluted with buffer A. The flow rate was 20ml/hr and 4ml samples were collected. The elution profile was determined by measuring the absorbance at 260nm (1:100 dilutions). Fractions were analysed by employing 12% polyacrylamide gel electrophoresis in 7M urea/TBE (50mM Tris/borate pH 8.3, 1mM EDTA). 6S RNA containing fractions were pooled (dotted area). The arrow in the inset indicates the position of the 6S RNA.

230 nucleotides of the 3'-end. The band migrating slightly faster, approximately 180 nucleotides in length and indicated by an arrow in Figure 1, displayed significant homology to the E. coli 6S RNA, i.e. 11 consecutive nucleotides out of 60 nucleotides determined were identical. Additional interpretation of the sequencing gels was impaired by another contaminating rRNA fragment. Several attempts to label the molecule at its 5'-end remained unsuccessful.

Genomic P. aeruginosa DNA digested with BamH I and EcoR I, respectively, was analysed by Southern hybridization employing the 3'-end labelled 6S RNA in the presence of unlabelled rRNA. The addition of unlabelled rRNA was necessary due to the fact that the radioactively labelled 6S RNA probe -was

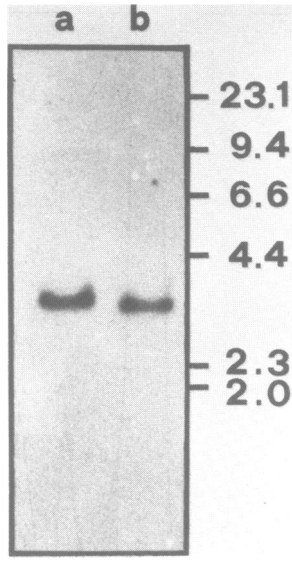

Figure 2. Autoradiograph of a Southern hybridization of restricted genomic DNA probed with 3'-labelled 6S RNA from P. aeruginosa. Lane a: Eco RI, b: Bam HI. As a size marker served a Hind III digest of lambda DNA.

contaminated with an rRNA fragment, as mentionend above. One signal , 3.0 kb in size, could be detected for both digests (Figure 2), indicating one 6S RNA gene copy per genome. Employing identical hybridization conditions no signals could be detected with other prokaryotic DNAs, i.e. E. coli, B. subtilis, B. stearothermophilus, T. thermophilus and H. maris mortui.

The 3.0 kb BamHI fragment was cloned into pBR 322 and subsequently into M13mp9, leading to the recombinat phage M13mp9DV52. The exact position of the 6S RNA gene could be determined by Southern hybridization of several restriction digests. The resulting restriction map is shown (Figure 3).

The 6S RNA coding portion localized on the SalI/PstI fragment, 0.34 kb in size, was subcloned into M13mp8 and M13mp9, respectively, and sequenced in

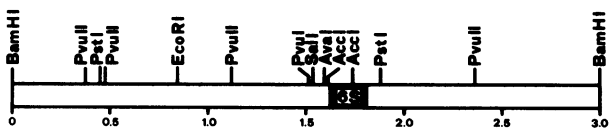

Figure 3. Restriction map of the Bam HI fragment, carrying the 6S RNA gene.

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                                                          + +++++
1     GTCGACGCGC CCTGGCGAAT CCGGCCGATG GCGCGAAGCC TGACCCGGGC GCGGCCAATT
                                                          -35

          ++++++          * * ***   ** ** ** **   **  *   ** **********
61    CGGGTATACT GGCCTCCGCT CCCTGGTGTG TTGGCCAGTC GGTGATGTCC CTGAGCCGAT
         -10           AUUU CUCUGAGAUG UUCGCAAGCG GGCCAGUCCC CUGAGCCGAU

      *  *   **  **  * * **  ** * *    *   **** * **** ** *   ** * ** *
121   AACTGCAACA AC--GGAGGT TGC-CAGTTG GACCGGTGTG CATG-TCCGC ACGACGGA-A
      AUUUCAUACC ACAAGAAUGU GGCGCUGCGC GGUUGGUGAG CAUGCUCGGU CCGUCCGAGA

      ****** **   *     *   **   ***** *****    * **********  **  * * *
176   AGCCTT-AAG GTCTACTGCA ACCGCCACCT TGAACTTTCG GGTTCAAGGG CTAACCCGAC
      AGCCUUAAAA CUGCGACGAC ACAUUCACCU UGAAC-CAAG GGUUCAAGGG UUA--CAGCC

       *****   *  *  **** *  **
235   AGCGGCACGA CCGGGGAGCT ACCTTTCCAC ATGATCGAAT CGCAAGACCT CAGCCGCCCG
      UGCGGCGGCA UCUCGGAGAU UCC

295   GCCCTGCGCC GCAAGCTCCG CCAGCGCGCC GCGCTCTCCC CCCTGCAG
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Figure 4. Sequence of the 6S RNA gene region of P. aeruginosa (upper row) compared with the 6S RNA of E. coli (6). To achieve maximum homology gaps were introduced in both sequences. Identical nucleotides are indicated by asterisks, the putative promoter region is marked by crosses. The nucleotide indicated by an arrow represents the arbitrary 5'-terminus in the secondary structural model (Figure 5, I).

both orientations. The primary structure determined was aligned with the 6S RNA sequence from E. coli (Figure 4). To achieve maximum homology gaps were introduced into both sequences. This alignment yields a homology of 60.4 %. A possible promoter region could be identified directly proximal to the 6S RNA gene. The -35 and -10 box show high homology to the E. coli promoter consensus sequence (25). In contrast to E. coli the -10 box is followed by a G/C rich sequence, a feature known from genes which are expressed under stringent control (26). A comparison of the 6S RNA coding sequences of E. coli and P. aeruginosa revealed a similar G/C content of 60% and 55% respectively, quite in contrast to the 76 base pairs of upstream sequence, including the putative promoter region, and the 88 bases of downstream sequence, where a significant higher G/C content can be observed in P. aeruginosa, i.e. 72% versus 37% for the upstream and 72% versus 48% for the downstream region. Within the downstream sequence we could not find a region of dyad symmetry which could act as an transcription termination signal.

Further analysis of the primary structure permitted the construction of a secondary structure model for P. aeruginosa 6S RNA (Figure 5), which is in parts identical to the structure previously proposed for E. coli 6S RNA (27).

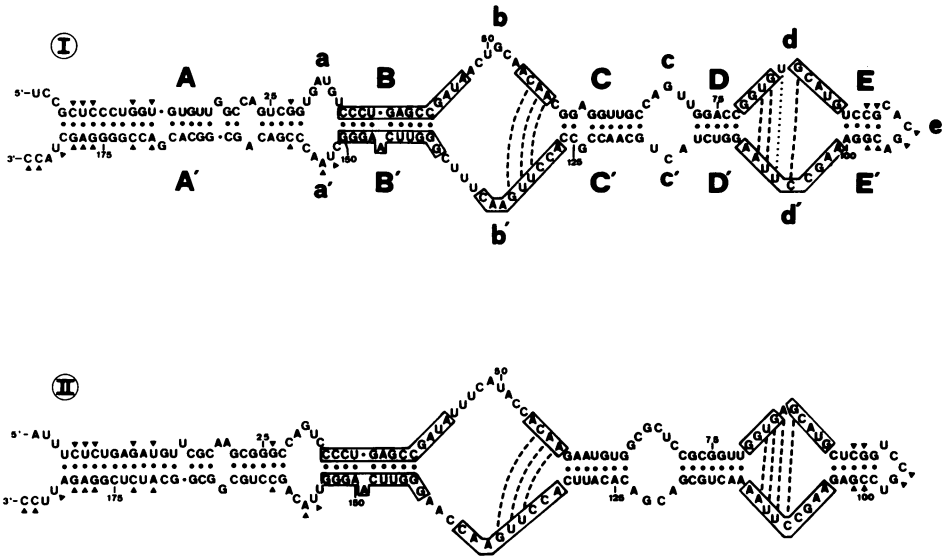

Figure 5. Secondary structural models of 6S RNAs from (I) P. aeruginosa and (II) E. coli (27). Extended regions of conserved nucleotides are boxed and single homologous bases are indicated by triangles. Possible base pairing in single stranded regions is marked by dashes. Looped regions are marked with letters, helical regions with capital letters.

DISCUSSION

We have been able to isolate a 6S RNA from the gram-negative eubacterium P. aeruginosa. In E. coli the 6S RNA was prepared from a cytoplasmic fraction (5). We isolated the P. aeruginosa 6S RNA from a crude 70S ribosomal fraction. The specificity of this association needs to be investigated.

Hybridization experiments revealed a single copy gene coding for the 6S RNA. This result clearly shows that the 6S RNA is not a fragment of a larger ribosomal RNA, since P. aeruginosa carries four rRNA gene sets (13).

In our hands the P. aeruginosa 6S RNA did not hybridize with E. coli DNA, reflecting the low sequence homology (60.4 %) between the 6S RNAs of both organisms. The longest section of consecutive identical sequence in both 6S RNAs comprises only 13 nucleotides. The homology of both 6S RNA sequences is much lower than that of the respective 5S ribosomal RNA sequences, where the homology is 80.0 % (28). A high divergence in primary structure is also known for another RNA-species, the RNA-component of RNase P. This enzyme processes primary t-RNA- and 4.5S RNA-transcripts at their 5'-end (29). The RNase P - RNA sequences from E. coli and B. subtilis are less than 50 %

homologous (30), but both molecules can cleave the same substrates (31), suggesting a common structural feature of higher order, which is responsible for their enzymatic activity.

It is of interest to note that hybridization signals could also not be detected between the P. aeruginosa 6S RNA and the DNAs of the extreme thermophilic gram-negative T. thermophilus, the gram-positive eubacteria B. subtilis and B. stearothermophilus and the halophilic archaebacterium H. maris mortui. It is obvious,that in these organisms the 6S RNA sequence, if at all existing, deviates significantly from that of P. aeruginosa.

The 6S RNA sequences from E. coli and P. aeruginosa fit very well in a consensus secondary structural model proposed in this communication (Figure 5). Both molecules display internal symmetry encompassing alternating elements of helical and looped structures. A striking feature of both 6S RNAs is the helical segment comprising nine base pairs located around positions 35 (B) and 150 (B'), respectively. All nucleotides in this helix and in addition some nucleotides in one proximal internal loop (b, b') are consecutively identical in both RNAs. The helix (B, B') displays a looped out adenine in both molecules. Such looped out bases are believed to be recognition signals for proteins (32). This central section described is the most conserved element, which might be important for function. The proximal domain (b, b'), comprising the positions 46-49 and 135-138, which recently has been proposed to be base paired in E. coli (27), cannot be formed in analogy with the P. aeruginosa sequence. Positions 81-84 of section d and 108-111 of section d' were suggested to be involved in base pairing in E. coli (27). An odd U-U base pair at position 82/106 in loop d/d' would be a prerequisite for constructing an identical secondary structure in this section of the P. aeruginosa 6S RNA. Such an odd base pair was shown to exist in 5S rRNA from Xenopus laevis (33).

A pentanucleotide sequence (5'-UCCGU-3') located in loop e of the E. coli structure (Figure 5) has been reported to be in a similar position in eukaryotic 7SL RNAs (27). This sequence is different in the P. aeruginosa 6S RNA (5'-CACGA-3'). On the basis of a secondary structural model it has been discussed, whether the bacterial 6S RNA is an equivalent to the eukaryotic 7SL RNA, a component of the signal recognition particle (SRP) (27). In our opinion the 6S RNA model presented here shows no significant structural homology to the central rod of the 7SL RNAs.

In E. coli the -10 promoter region was localized 15 nucleotides apart from the start of the 6S RNA coding region (2) and a precursor molecule has

been detected, which extends the mature 6S RNA by 8 nucleotides at the 5'-end (34). In P. aeruginosa the distance between the -10 promoter box (5'-TATACT-3'), which is in agreement with the E. coli consensus sequence (5'-TATAAT-3') (25), and the conjectured mature 5'-end comprises only 6 nucleotides in respect to the sequence alignment (Figure 4) and 4 nucleotides in order to achieve maximum homology of both secondary structures (Figure 5). This observation might reflect differences in the maturation of both 6S RNA species.

A -35 promoter box (CTGACC), which deviates in one strongly conserved position from the E. coli consensus sequence (TTGACA), could be identified 19 nucleotides upstream of the -10 box. A spacing of 15 to 21, optimally 17 base pairs between the two boxes was reported for functional promoters in E. coli (25). A transcription termination signal could not be detected within the 88 base pairs downstream the 6S RNA coding region, suggesting a situation as in E. coli, where the 6S RNA is the first part of a dual function transcription unit (2).

For the E. coli 6S RNA it has been demonstrated, that its expression is not under stringent response (3). Due to the presence of a G/C rich sequence (26) downstream the -10 box (Figure 4) in the P. aeruginosa 6S DNA it needs to be shown whether the expression of the gene is stringently controlled.

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REFERENCES

1. Ikemura, T. and Dahlberg, J.E. (1973) J. Biol. Chem. 248, 5024-5032.
2. Hsu, L.M., Zagorski, J., Wang, Z. and Fournier, M.J. (1985) J. Bacteriol. 161, 1162-1170.
3. Ikemura, T. and Dahlberg, J.E. (1973) J. Biol. Chem. 248, 5033-5041.
4. Griffin, B.E. (1975) J. Biol. Chem. 250, 5426-5437.
5. Lee, S.Y., Bailey, S.C. and Apirion, D. (1978) J. Bacteriol. 133, 1015-1023.
6. Brownlee, G.G. (1971) Nature 229, 147-149.
7. Walter, P. and Blobel, G. (1983) Cell 34, 525-533.
8. Robertson, M. (1984) Nature 307, 594-595.
9. Lee, C.A., Fournier, M.J. and Beckwith, J. (1985) J. Bacteriol. 161, 1156-1161.
10. Müller, M. and Blobel, G. (1984) Proc. Natl. Acad. Sci. USA 81, 7737-7741.

11. Timmis, K.N., Cabello, F. and Cohen, S.N. (1978) Molec. Gen. Genet. 162, 121-137.
12. Birnboim, H.C. and Doly, J. (1979), Nucl. Acids Res. 7, 1513-1523.
13. Hartmann, R.K., Toschka, H.Y., Ulbrich, N. and Erdmann, V.A. (1986) FEBS Lett. 195, 187-193.
14. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
15. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
16. Southern, E.M. (1974) J. Mol. Biol. 98, 503-517.
17. Horne, J.R. and Erdmann, V.A. (1972) Mol. Gen. Genet. 119, 337-344.
18. Ulbrich, N. and Wool, I.G. (1978) J. Biol. Chem. 253, 9049-9052.
19. England, T.E. and Uhlenbeck, O.C. (1978) Nature 275, 560-561.
20. Donnis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538.
21. Hanahan, D. (1983) J. Mol. Chem. 166, 557-580
22. Hartmann, R.K., Kröger, B., Vogel, D.W., Ulbrich, N. and Erdmann, V.A. (1987) Biochem. Intern. 14, 267-275.
23. Vogel, D.W., Hartmann, R.K., Kröger, B., Ulbrich, N. and Erdmann, V.A. (1987) Biochem. Intern. 14, 167-175.
24. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
25. Hawley, D.K. and McClure, W.R. (1983) Nucl. Acids Res. 11, 2237-2255.
26. Travers, A.A. (1984) Nucl. Acids Res. 12, 2605-2618.
27. Zwieb, C. (1986) Endocyt. C. Res. 3, 41-51.
28. Erdmann, V.A. and Wolters, J. (1986) Nucl. Acids Res. 14 suppl., r1-r59.
29. Altman, S., Guerrier-Takada, C., Frankfort, H. and Robertson, H.D. (1982) in Nucleases, pp. 243-274, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.
30. Reich, C., Gardiner, K.J., Olsen, G.J., Pace, B., Marsh, T.L. and Pace, N.R. (1986) J. Biol. Chem. 261, 7888-7893.
31. Gardiner, K. and Pace, N.R. (1980) J. Biol. Chem. 255, 7507-7509.
32. Christiansen, J., Douthwaite, S.R., Christensen, A. and Garrett, R.A. (1985) EMBO J. 4, 1019-1024.
33. Digweed, M., Pieler, T., Kluwe, D., Schuster, L., Walker, R. and Erdmann, V.A. (1986) Eur. J. Biochem. 154, 31-39.
34. Griffin, B.E. and Baillie, D.L. (1973) FEBS Lett. 34, 273-279.