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Escherichia coli 6S RNA Gene Is Part of a Dual-Function Transcription Unit

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The gene coding for the metabolically stable 6S RNA of *Escherichia coli* has been cloned, sequenced, and partially characterized in expression analyses. The DNA sequence results confirm the accuracy of the previously established RNA sequence and, with genomic hybridization data, reveal that there is only one copy of the 6S DNA in the chromosome. Consistent with its relaxed mode of expression, the promoter region of the 6S RNA gene was found to lack the hypothetical GC-rich discriminator domain common to other stable RNA genes under stringent control. The sequence results also revealed the occurrence of a 540-base-pair open reading frame immediately downstream from the 6S RNA coding region. Results from the expression analyses show that the protein and RNA coding regions are cotranscribed in vitro and that the open reading frame is translated in vivo.

The 6S RNA of *Escherichia coli* was one of the first RNAs to be sequenced (6), yet some years hence it remains only partially characterized and its function is still undefined. The 6S species contains 184 nucleotides, none of which are modified. A precursor form with six to eight additional bases at the 5′ terminus has been described, indicating that the mature RNA is derived from a larger primary transcript (12). Unlike the other small stable RNAs of *E. coli* characterized thus far, the 6S species is not subject to stringent control; that is, 6S RNA synthesis persists during amino acid starvation (20, 21). Results from attempts to assess its subcellular condition suggest that it is not associated with the ribosome, but it is part of a ribonucleoprotein complex with a sedimentation coefficient slightly larger than 10S (11, 23). To gain additional insight into the function of this unique RNA, we undertook the cloning of its gene. We demonstrate here that there is only one copy of the 6S DNA in the *E. coli* chromosome and present the nucleotide sequence for the 6S RNA gene region. We note the existence of an open reading frame (ORF) sequence downstream from the 6S RNA coding region. This ORF is expressed both in vitro and in vivo. Taken together, the results show that the 6S RNA gene and adjoining ORF occur in the same transcription unit.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. All *E. coli* strains used are K-12 derivatives. Strain FB105 (supplied by F. Blasi [7]) was used as the source of chromosomal DNA. *E. coli* JA221 (*hsdR ΔtrpE5 leuB6 recA1*; a derivative of strain C600) obtained from J. Carbon served as the host for transformation in the cloning work. The maxicell expression analyses were conducted with strain CSR603 (38). Cells used for chromosomal DNA and transformation were cultured in standard L broth (30). Plasmid DNA was isolated from cells grown in M9 salts medium supplemented with glucose (2 g/liter)–0.1 mM $CaCl_2$–1 mM $MgSO_4$–Casamino Acids (10 g/liter; Difco Laboratories, Detroit, Mich.)–D,L-tryptophan (80 mg/liter)–tetracycline (20 mg/liter) (30). The growth of transformants for hybridization screening was done in 10 ml of M9 medium containing tetracycline, with chloramphenicol (150 μg/ml) added at mid-log phase for plasmid amplification. In vivo labeling of RNA in *E. coli* JA221 was carried out in the defined morpholinepropanesulfonic acid medium of Neidhardt et al. (31) supplemented with 0.15 mCi of $^{32}P_i$ per ml (New England Nuclear Corp., Boston, Mass.)–L-leucine (100 μg/ml)–tryptophan (40 μg/ml) as described elsewhere (17). Culturing and labeling of *E. coli* CSR603 was essentially as previously described (36, 38). Modifications included the use of 10-ml cultures, washing and labeling of cells in a sulfur-free medium consisting of M9 medium with 1 mM $MgCl_2$ (in place of $MgSO_4$) and 10 μCi of [^{35}S]methionine (Amersham Corp., Arlington Heights, Ill.), and labeling for 1 h.

The vector plasmids pBR322 and pACYC184 have been previously described (5, 8). Plasmid pKK223-3, supplied by Jürgen Brosius, is a 4.6-kilobase (kb) derivative of pBR322 containing a part of the *tet* gene, the origin of replication, and an intact *bla* gene (with the *PstI* site inactivated). The insert includes a *trp-lac* fusion promoter (*ptac*) of 250 base pairs (3) followed by an M13mp8 polylinker and a 420-base-pair fragment from the termination region of the ribosomal RNA operon *rrnB*. The terminator segment includes sequences of 5S DNA and two transcriptional stop signals (5a).

Preparation of nucleic acids. High-molecular-weight chromosomal DNA was prepared as described by Marmur (27); plasmid DNA was prepared as described by Sidikaro and Nomura (40), except that the DNA was concentrated from a cleared lysate by polyethylene glycol precipitation (19).

Nonradioactive 6S RNA was isolated from preparations of crude *E. coli* tRNA by gel filtration chromatography and polyacrylamide gel electrophoresis as described previously (17). The RNA obtained migrated as a single band on electrophoretic analysis in a 10% polyacrylamide–7 M urea gel exhibiting an R_m value of 0.23 relative to bromophenol blue.

The preparation of in vivo-labeled ^{32}P small RNA was as described elsewhere (17). ^{32}P 6S RNA for hybridization assays was prepared by in vitro labeling of dephosphorylated RNA with [γ-^{32}P]ATP (1,000 to 3,000 Ci/mmol; New England Nuclear) and T4 phage polynucleotide kinase (New

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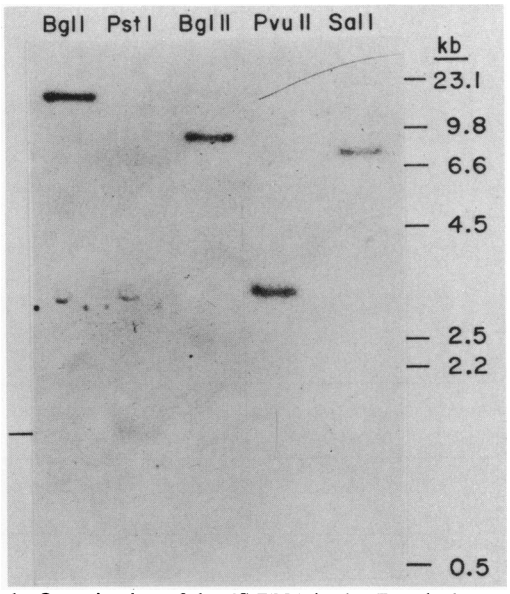


FIG. 1. Organization of the 6S DNA in the *E. coli* chromosome. Southern hybridization analyses were carried out with restriction nuclease digests of chromosomal DNA (7 μg each) by using 5′-labeled ^{32}P 6S RNA as the probe. Hybridization was carried out at 65°C in 6× SSC (1× SSC is 0.015 M sodium citrate [pH 7.0] plus 0.15 M sodium chloride)–0.2% (wt/vol) sodium dodecyl sulfate in the presence of 0.1 μg each of *E. coli* tRNA and 5S, 16S, and 23S ribosomal RNAs per ml. The autoradiographic results shown are for DNA digested with *Bgl*I, *Pst*I, *Bgl*II, *Pvu*II, and *Sal*I. The size markers are from a *Hin*dIII digest of λ DNA. The bar on the left border indicates the location of a faint band of hybridizing DNA present in the *Pst*I lane.

England Biolabs, Beverly, Mass.) as described by Simoncsits et al. (41). Specific activities of the labeled RNA fractions were of the order of 10^6 to 10^7 cpm/μg.

Recombinant DNA procedures. Restriction endonucleases, *Bal* 31 exonuclease, and DNA ligase were obtained from New England Biolabs and Bethesda Research Laboratories, Rockville, Md., and used as recommended by the suppliers. Restriction endonuclease digests were analyzed by electrophoresis in agarose gels (14), polyacrylamide gels (33), or agarose-acrylamide composite gels (2). The restriction nuclease map of pLH60-1 was constructed by using a blot hybridization procedure identified previously (17) and the partial digest procedure of Smith and Birnstiel (43).

Genomic 6S DNA was enriched ca. 150-fold before cloning by two cycles of restriction endonuclease digestion using *Sal*I and *Pst*I, respectively, and electrophoretic fractionation. One milligram of chromosomal DNA yielded 160 μg of *Sal*I DNA fragments of 6.6 kb. Subsequent enrichment of this material in the *Pst*I nuclease cycle generated 5 to 6 μg of 6S DNA with an average length of 1.3 kb. The *Pst*I-digested DNA fraction was ligated with *Pst*I-cut pBR322 DNA, and the resulting hybrid plasmids were used to transform *E. coli* JA221. Tetracycline-resistant, ampicillin-sensitive transformants were screened for cloned 6S DNA by the hybridization of ^{32}P-labeled 6S RNA to Southern blots (44) of uncut plasmid DNA. The DNA enrichment, ligation, transformation, and screening procedures have been described elsewhere (17, 26).

DNA sequencing. The method of Maxam and Gilbert (28) was used to determine the DNA sequence of the 6S RNA gene region. The results were derived from analyses of both strands which were carried out with overlapping sets of restriction nuclease fragments.

In vitro transcription. The in vitro transcription reactions contained (per 20 μl) the following: 0.25 μg of template DNA in 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol, 75 mM KCl, 100 μM each of ATP, GTP, and CTP, 10 μM UTP plus 10 μCi of [α-^{32}P]UTP (800 Ci/mmol; Amersham), 20% (vol/vol) glycerol, and 1 to 2 U of *E. coli* RNA polymerase (Pharmacia Fine Chemicals Inc., Piscataway, N.J.; P-L Biochemical, Inc., Milwaukee, Wis.). After a 30-min incubation at 37°C, the reactions were terminated and treated as described by Duester et al. (10). The RNA products were analyzed by electrophoresis in 5% polyacrylamide–7 M urea gels (33). The intact 6S DNA template was the *Pst*I DNA fragment recovered from agarose gels by electroelution in dialysis bags (42). Truncated templates were prepared by the cleavage of equivalent amounts of the *Pst*I fragment with appropriate restriction enzymes, with the reactions stopped by incubation at 65°C for 5 min. The resulting digests were used without further treatment.

RESULTS

The DNA coding for 6S RNA occurs at a single chromosomal locus. Southern hybridization analyses of genomic DNA were performed to determine the number of 6S RNA gene loci and to identify a restriction fractionation strategy that would allow the enrichment of 6S DNA for cloning. The results from this analysis, carried out with five restriction enzymes, are shown in Fig. 1. In each case, only a single band of hybridizable DNA was obtained, indicating that the 6S DNA is confined to a single chromosomal locus. Because of its unique small size, the 1.3-kb *Pst*I 6S DNA fragment was chosen for cloning.

Cloning of the 6S RNA gene. The absence of information about the 6S RNA function precluded the use of phenotypic screening to identify 6S DNA clones. Thus, screening was limited to either hybridization or RNA pattern analysis. Because it was operationally simpler and not dependent on expression, a hybridization assay was used. To avoid the background of chromosomal 6S DNA, screening was carried out with plasmid-enriched DNA. The screening problem was simplified further by preenrichment of the 6S DNA before cloning. The enrichment strategy consisted of two successive cycles of restriction nuclease digestion, fractionation, and recovery of the 6S DNA fraction. This scheme proved to be very successful earlier in our cloning of the *E. coli* 4.5S RNA gene (17).

Based on the results of the Southern hybridization analysis, two cycles of enrichment were used. The initial fragmentation of chromosomal DNA was by *Sal*I, yielding a 6S DNA fraction of 6.6 kb. *Pst*I was used in the second cycle and produced a mixture of 1.3-kb fragments. Overall purification of the 6S DNA was at least 150-fold, with enrichments of more than 6- and 23-fold for the two steps; this estimate represents a lower limit, as losses of material in recovery were not considered. The resulting *Pst*I-digested DNA was cloned into the *bla* gene of pBR322, and 6S DNA recombinants were identified by hybridization screening of plasmid DNA from tetracycline-resistant, ampicillin-sensitive transformants. About 20% of the 96 transformants tested were revealed to be 6S DNA clones, consistent with the yield anticipated from the enrichment estimate (9). One of these, designated pLH60-1, was used for the expression and sequence analyses described below.

The cloned 6S RNA gene is functional. An in vivo labeling analysis was next carried out to determine whether the cloned 6S DNA could be expressed. Figure 2 shows the electrophoretic patterns obtained for the small RNA species

from pLH60-1 transformants and control cells. Quantitation showed that transformants harboring pLH60-1 produce six- to eightfold more 6S RNA than do either the host cells or cells with vector DNA alone (latter result not shown). Normally, the cellular content of 6S RNA is about the same as that of a single tRNA: ca. 2% of the total small RNA, or 10^3 molecules per cell (21). In pLH60-1 transformants the relative abundance is elevated to 12 to 15% of the small RNA. Interestingly, the growth rate of the pLH60-1 transformants in L broth, M9, and morpholinepropanesulfonic acid media was similar to that of a control culture containing pBR322.

The 6S RNAs from the host and recombinant strain JA221(pLH60-1) were found to comigrate in high-resolution polyacrylamide gel analyses carried out under both denaturing and nondenaturing conditions (data not shown). The absence of detectable differences is taken as evidence that the plasmid and chromosomal 6S RNA genes are identical. The subsequent DNA sequence results confirmed this to be the case.

Restriction map of pLH60-1. A restriction map of pLH60-1 is shown in Fig. 3. Development of the map revealed the cloned insert to contain only one copy of the 6S RNA gene, located close to one end of the 1,330-bp *Pst*I insert. Evidence for the single-copy nature came initially from the finding that cutting the *Pst*I fragment with *Hin*fI yielded one hybridizable segment of 300 nucleotides which was large enough to encode only a single copy of the 184-base mature 6S RNA. This conclusion was later confirmed by DNA sequencing. The restriction map also enabled us to determine that the 6S RNA gene fragment in this case was inserted with a polarity opposite to that of the β-lactamase gene.

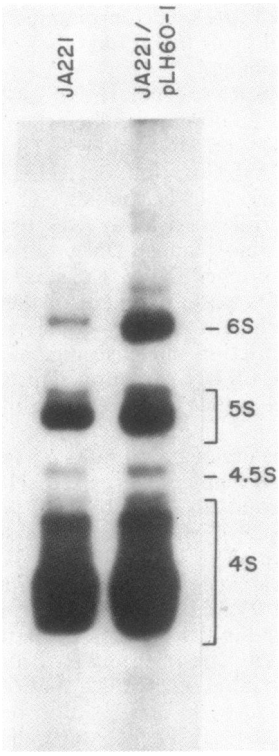
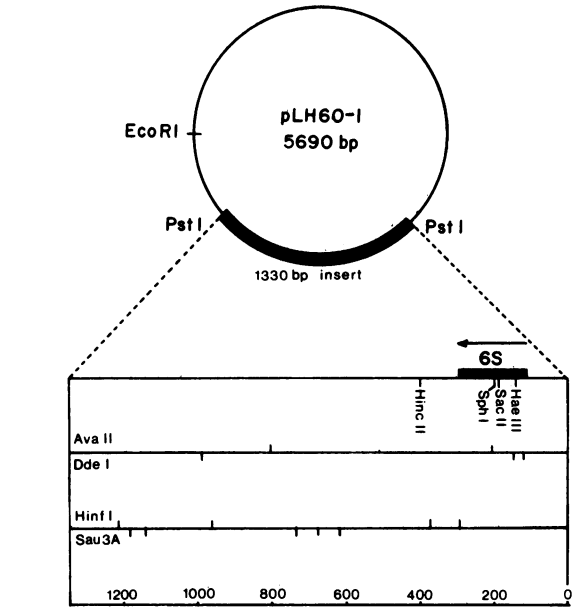


FIG. 2. In vivo expression of the cloned 6S DNA. The activity of the cloned 6S RNA gene was evaluated by determining the relative abundances of in vivo-labeled ^{32}P small RNA produced in transformants harboring pLH60-1. Cultures of *E. coli* JA221 with and without pLH60-1 and with the cloning vector pBR322 were labeled in a minimal salts medium with $^{32}P_i$. Cells were harvested at mid-log phase, and the small RNA was extracted and fractionated by electrophoresis through 10% polyacrylamide. Radiolabeled RNAs were detected by autoradiography and quantified by Cerenkov counting of excised gel slices.



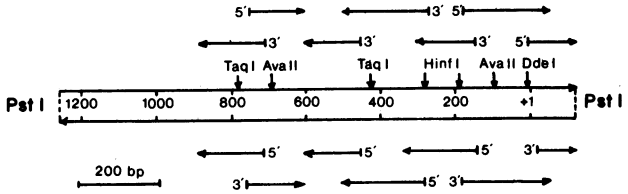


FIG. 3. Restriction map of pLH60-1 and sequencing strategy. The physical map of the 1.3-kb *Pst*I DNA insert was developed by single and multiple restriction enzyme analyses coupled with hybridization assays. The 6S DNA region was sequenced by the Maxam-Gilbert procedure by using the strategy indicated in the lower portion of the figure.

6S DNA sequence. The sequence of ca. 1,000 nucleotides starting from the *Pst*I site and extending beyond the 6S RNA coding region is presented in Fig. 4. The region encoding the mature 6S RNA corresponds to nucleotides 112 to 294 and is identical to the RNA sequence previously reported (6). The last nucleotide in the RNA sequence was reported as either C or A (6); however, it was found to be C in the DNA sequence. Inspection of the sequence preceding the 6S DNA revealed two candidate promoters with −10 domains centered three nucleotides apart at −19 and −22 bases from the nucleotide corresponding to the 5′ end of the mature 6S RNA. The −35 and −10 elements comprising these putative promoters are TTGAAT and TGTGGT for one candidate (spanning residues 63 to 91) and ATGACA and TAGAGT for the other (nucleotides 67 to 96). The two domains defining these promoters are separated by 17 and 18 nucleotides,

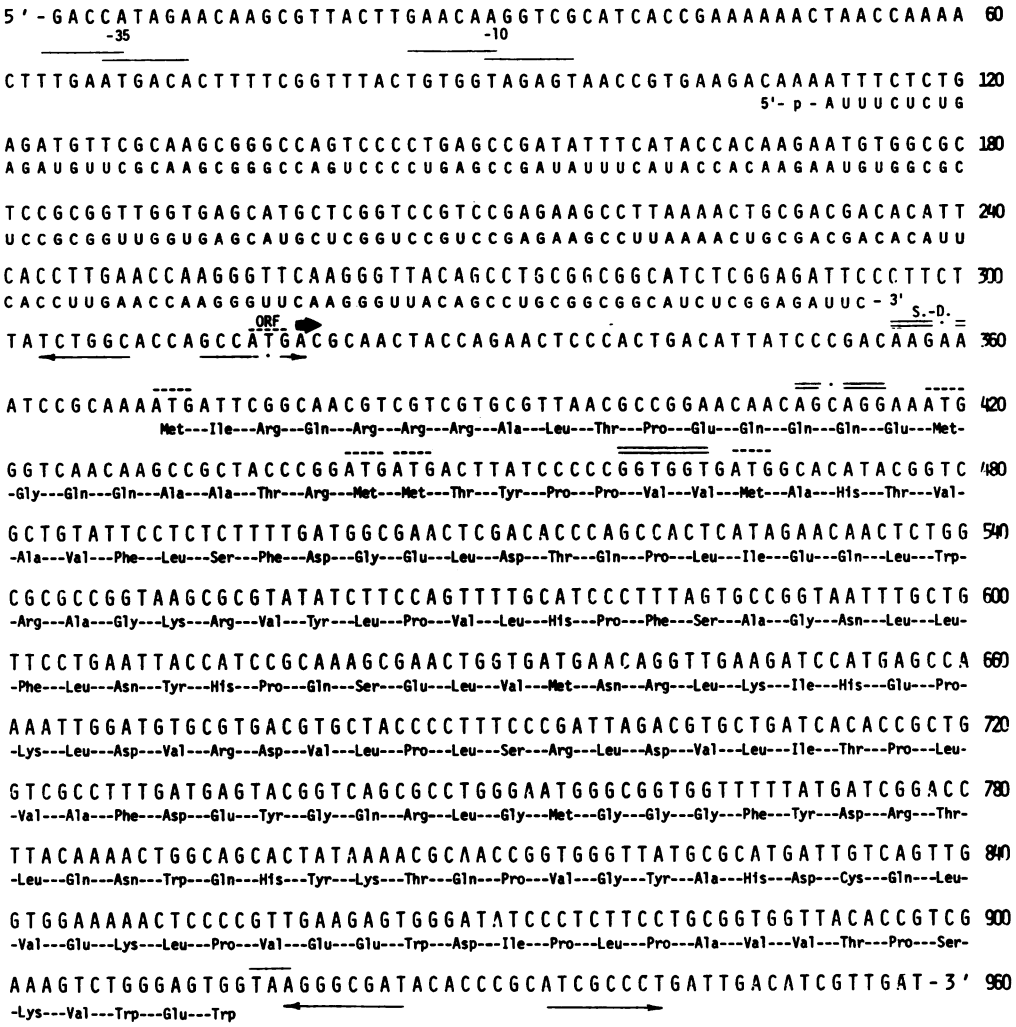


FIG. 4. Nucleotide sequence of the *E. coli* 6S RNA gene region. The sequence of the mature 6S RNA is given below the corresponding DNA sequence. Two possible promoter regions are identified with overlines above the candidate −35 and −10 domains, and two regions of dyad symmetry which could be potential terminator structures are underlined with arrows. An extended ORF distal to the 6S RNA coding region is identified, and the first six AUG codons are highlighted with a broken overline; a UAA stop codon is identified with an overline. The doubly overlined sequences at positions 356 to 360, 410 to 415, and 459 to 464 identify the best candidate Shine-Dalgarno (39) recognition sites in the ORF.

respectively. The second candidate is in better agreement with the consensus procaryotic promoter sequences TTGACA and TATAAT (13, 37). Consistent with the notion that the −10 element of this latter promoter is the one utilized is an earlier report describing the occurrence in preparations of in vivo-labeled RNA of a 6S RNA precursor with a 5′-terminal pppG (12). This starting nucleotide could correspond to G_{103} in the DNA sequence shown giving rise to a precursor with nine additional bases at the 5′ terminus. In contrast to stable RNA genes under stringent control, the promoter region of the 6S RNA gene lacks the GC-rich heptanucleotide sequence between the −10 domain and the transcription initiation site (46).

An examination of the DNA sequence immediately beyond the 3′ end of the 6S RNA coding region did not reveal a recognizable terminator structure (15, 37). A short stretch of nucleotides capable of forming a hairpin loop is evident (bases 303 to 319), but the trailing uridine residues characteristic of the rho-independent terminators are absent. Further scrutiny revealed an ORF downstream from the 6S RNA coding region, extending from base 316 to base 915 near the end of the sequenced portion. Of the eight AUG codons in the reading frame, three are preceded by plausible Shine-Dalgarno sequences (Fig. 4). Initiation at these codons would yield proteins of 21, 19, and 17 kilodaltons (kd). Immediately beyond the ORF is another region of dyad symmetry (residues 918 to 941) which could correspond to a transcriptional stop. As was the case with the inverted repeat following the 6S DNA, this element does not appear to correspond to a rho-independent terminator.

Is the ORF part of the 6S RNA transcription unit? The occurrence of the adjoining ORF and the lack of an identifiable transcription terminator or promoter between the 6S and ORF DNAs suggested the possibility that the 6S RNA gene may be part of a dual-function operon. In vitro and in vivo expression assays were carried out to identify the transcriptional stop point and to determine whether the ORF is actually transcribed and translated. The transcriptional analysis was done in vitro with the *Pst*I insert fragment, and various truncated derivatives of it as templates. The in vivo

assay involved the use of the maxicell expression system developed by Sancar et al. (38).

In sectioning the *Pst*I fragment for the transcription assays, we took advantage of conveniently located restriction sites within the ORF, the region between the 6S RNA sequence and the ORF, and the 6S DNA itself. Termination at the stem-loop domain immediately beyond the 6S RNA coding region would yield a transcript of ca. 200 nucleotides. However, transcription through the downstream ORF would produce a much larger transcript, presumably one in excess of 800 bases. The pattern of products obtained in the analysis are shown in Fig. 5.

Two major transcripts obtained with the intact *Pst*I fragment were estimated at ca. 800 and 120 nucleotides (Fig. 5, lane b). No product corresponding to a runoff transcript of 1,200 bases was detected. When the *Pst*I fragment was shortened stepwise from the 3′ end by *Sau*3A, *Hin*cII, and *Hin*fI, the larger product disappeared and was replaced with runoff transcripts of 550, 290, and 170 nucleotides, respectively (Fig. 5, lanes f, e, and d). Assuming that transcription initiates at G_{103}, the expected readthrough transcripts are 545, 293, and 188 nucleotides, which corresponded well with the pattern observed. The absence of a 6S RNA-like product from the largest template coupled with the pattern of runoff transcripts obtained indicate that the ORF is indeed cotranscribed with the 6S DNA, at least in vitro.

The 120-nucleotide product derived from the intact *Pst*I fragment was unexpected. Its persistence with the *Sau*3A and *Hin*cII fragments but its loss upon *Hin*fI treatment suggests that it is produced from an unidentified promoter near one of the *Hin*fI sites. The identity of this RNA species and its corresponding transcription unit are under further investigation.

Maxicell analyses were next undertaken to determine whether the ORF is translated in vivo. The plasmid DNA used included pLH60-1, the parent vector pBR322, and several new derivatives developed from the expression vector pKK223-3. This latter set of templates contain *bla* and the 6S RNA gene region with either an intact ORF or ORF sequence deleted of its 3′-distal half. The deletion fragment used lacks ca. 200 base pairs as a consequence of cleavage at an internal *Sau*3A site in the ORF and brief exposure to *Bal* 31 before recloning. The strategies used in the construction of the pKK223-3 derivatives are shown in Fig. 6. All four possible orientations were obtained for both the intact and shortened 6S DNA fragments. As constructed, the plasmids containing the intact ORF region, pZW21 and pZW41, were expected to direct the synthesis of both β-lactamase and the putative ORF protein; the deletion plasmids pZW45 and pZW63 should encode β-lactamase and perhaps a shortened ORF polypeptide of 10 to 12 kd. The expression results obtained with pLH60-1, the similarly oriented plasmid pair pZW21 and pZW45, and the relevant vector controls are shown in Fig. 7. The results for the templates representing the opposite orientation (pZW41 and pZW63) were similar to those for the pZW21 and pZW45 DNAs and are not shown.

Under conditions in which chromosome-mediated translation was barely detectable, one major protein of ca. 30 kd was observed for transformants harboring pBR322 or pKK223-3 DNA (Fig. 7, lanes 1 and 5). This protein is presumed to be the product of the *bla* gene (38, 45). Extract from the pLH60-1 transformant showed the presence of two labeled proteins with estimated sizes of 22 and 20 kd; as expected, the normal β-lactamase protein was absent. The presence of the unique protein species in pLH60-1 transformants suggests that the ORF at issue is translated, although the precise origin of the individual polypeptides is unclear. Without taking into account possible differences in the methionine content, it is apparent that the 20-kd product is markedly more abundant than the 22-kd species.

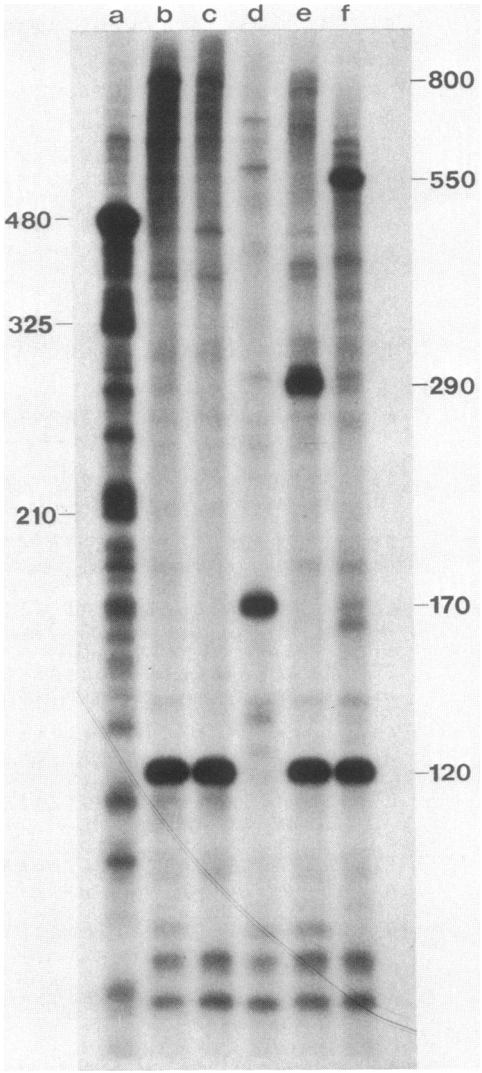


FIG. 5. In vitro transcription of the 6S RNA gene region. Restriction nuclease fragments containing various portions of the 6S RNA gene region were transcribed in vitro as described in the text. The ^{32}P-RNA products from the various reactions were deproteinized and fractionated by electrophoresis in gels of 10% polyacrylamide. Lane a, Size markers produced by in vitro transcription of a 1.9-kb restriction fragment containing the four tRNA genes of the *E. coli* $tRNA^{Arg}$ operon (16). Products were sized by using runoff transcripts generated from λDNA fragments cloned into the SP6 expression vector (Riboprobe-Promega, Inc.); the relative positions of the assorted transcripts and that of 5S ribosomal RNA are indicated. Lane b, Products resulting from the expression of the intact *Pst*I 6S DNA fragment. The remaining tracks contain the transcripts from templates produced by treatment of the *Pst*I fragment with *Hae*III (lane c), *Hin*fI (lane d), *Hin*cI (lane e), and *Sau*3A (lane f).

An inspection of the protein patterns for the pZW21 and pZW45 transformants (Fig. 7, lanes 3 and 4) provided stronger evidence that the ORF sequence indeed encodes an actual protein and that the product corresponds to the 22-kd species observed with the pLH60-1 DNA. Two proteins are

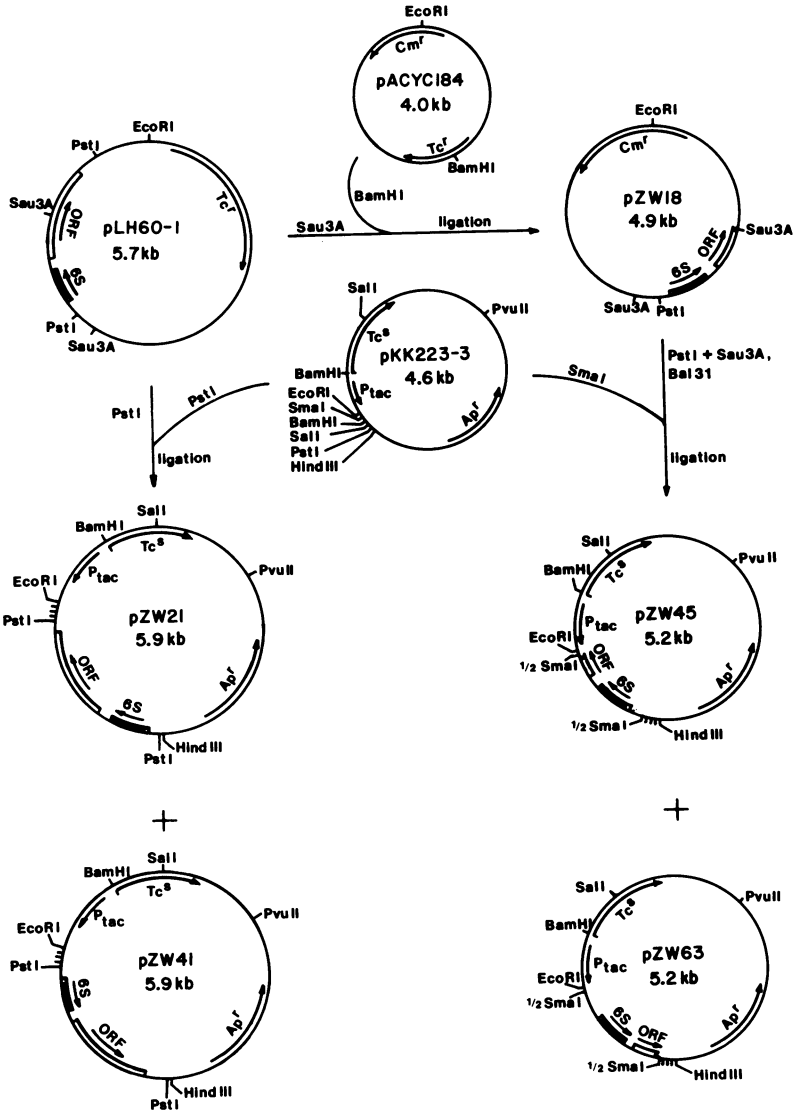


FIG. 6. Construction of the 6S DNA plasmids used in the in vivo expression analyses. The procedures are documented in the text.

expressed from the intact ORF template pZW21. The larger corresponds in size to the expected β-lactamase, and the smaller corresponds to the 22-kd product encoded by pLH60-1. The absence of the 22-kd product in transformants containing the ORF-deleted pZW45 DNA argues that this protein is derived from the ORF of the 6S RNA transcription unit. Of course, proof of this relationship will require more direct methods of analysis. The presence of the 20-kd protein in pLH60-1 transformants, but its absence in cells with pZW21 DNA, indicates that this protein does not originate from the ORF in question. Most likely, this product is a hybrid β-lactamase protein resulting from the fusion of vector and insert sequences at the original *Pst*I cloning site. Assuming that the 22-kd protein is derived from the ORF, its size is consistent with translation initiating at the second ATG codon in the designated ORF (residues 370 to 372). This potential start site is preceded by a good Shine-Dalgarno sequence, AAGAA (residues 356 to 360).

In this and other assays, we have not detected a recognizable truncated ORF protein. This product, anticipated to be ca. 10 to 12 kd, could be in low abundance because of instability. Another aspect of these results to be noted is the apparent difference in the expression of putative ORF-derived, 22-kd protein in various vectors. When carried in pLH60-1, the ORF protein appears to be made in consistently low levels as seen in independent labelings (data not shown). However, when encoded in the pKK223-3 derivatives, this product is as abundant as β-lactamase. The basis for the difference in expression is not known.

Additional evidence of cotranscription of the 6S RNA and adjoining ORF sequences has come from an analysis of these elements in a *galK* expression vector (29). The insertion of the 6S RNA transcription unit deleted of the distal ORF portion into a site upstream of *galK* DNA lacking its normal promoter alllowed *galK* expression (Z. Wang, unpublished data). This finding argues against the presence of a strong transcription terminator in the 6S RNA-ORF intercoding region.

DISCUSSION

The DNA sequence results revealed several important and interesting features of the 6S RNA gene region. Coupled

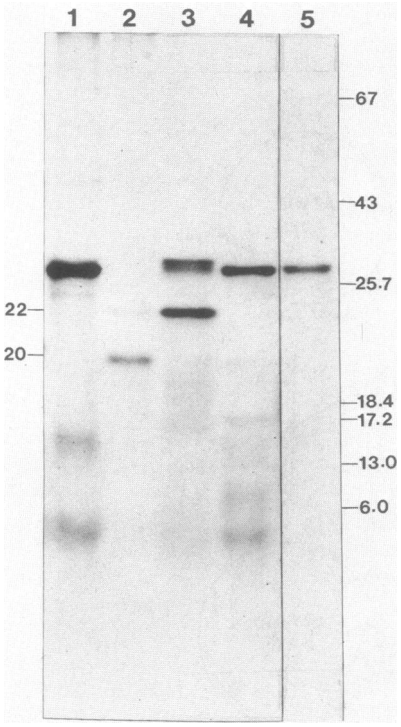


FIG. 7. In vivo translation of the cloned 6S DNA region. Protein synthesis directed by the 6S RNA gene region was evaluated by ^{35}S labeling of *E. coli* transformants by using the maxicell method (38). Log-phase cultures were irradiated with UV light and incubated in cycloserine overnight to eliminate chromosome-mediated expression. After incubation in medium containing 10 μCi of [^{35}S]methionine for 1 h, the labeled proteins were extracted and analyzed by gel electrophoresis in sodium dodecyl sulfate–12% polyacrylamide (32). The autoradiographic patterns shown are for *E. coli* CSR603 transformants with pBR322 (lane 1), pLH60-1 (lane 2), pZW21 (lane 3), pZW45 (lane 4), and pKK223-3 (lane 5). The positions of the various protein standards used are identified by molecular weight values (in kilodaltons). The standards were bovine serum albumin, ovalbumin, α-chymotrypsinogen, β-lactoglobulin, myoglobin, lysozyme, cytochrome *c*, and bovine trypsin inhibitor.

with the findings from the Southern analyses, the results clearly demonstrate that there is but one copy of the 6S DNA in the *E. coli* chromosome and that this sequence is in complete agreement with the previously determined RNA sequence (6). The discovery of the nearby ORF prompted the expression studies that showed it to be functional.

The sequence of the promoter region provides interesting insights, also. Thus far, the 6S RNA is the only metabolically stable small RNA in *E. coli* known not to be under stringent control. It follows that the comparison of promoter sequences for small RNA genes under stringent and relaxed controls has value. Although two candidate promoter sequences were detected for the 6S RNA gene, most likely only one is functional in vivo. Of the two possible promoters, that closest to the 6S RNA coding sequence is the more likely candidate. This view is based on its closer agreement with the procaryotic consensus sequence and the greater likelihood that transcription from this promoter will yield a precursor most like that detected in vivo—with eight additional nucleotides at the 5′ end and with a terminal pppG (12). Initiation at G_{103}, which is 7 nucleotides from the closer −10 domain versus 12 nucleotides for the upstream candidate, would yield a 5′-extended precursor with 9 extra nucleotides.

If the choice of candidate promoter is correct, then the sequence AACCGTG between −6 and +1 should be compared with the consensus stringency control discrimination element which occurs in this same relative position (46). For the stringently controlled stable RNA genes, the consensus sequence is gCGCc-C; upper- and lowercase letters correspond to strictly and moderately conserved nucleotides, respectively. Only the C nucleotide at position −3 in the 6S DNA sequence is found to agree; the agreement is no better for the equivalent domain of the alternate promoter. This lack of homology is consistent with the knowledge that the expression of the 6S RNA gene is under relaxed control.

Although unexpected, the occurrence of a dual-function transcript for the 6S RNA gene region is not unique among the *E. coli* small RNA genes. The operons encoding the *thrU tyrU glyT thrT tufB* gene cluster (4, 18), the *tyrT* su_3^+ tRNA gene (1), and the $tRNA_{f2}^{Met}$ *nusA* operon (22) are also mixed, coding for both tRNA and protein.

As noted above, the ORF identified in the sequence analysis possesses several in-frame AUG codons, three of which are preceded by candidate Shine-Dalgarno elements (Fig. 4). Initiation at the first of these would yield a protein of 21 kd, in closest agreement with the estimated 22-kd product observed in the expression analysis.

One of the goals of cloning the 6S RNA gene was to provide a means of assessing its essentiality and function. Nothing is known about its subcellular location, and little is known about its cellular condition. The 6S RNA does not sediment with the ribosome (6). This negative finding suggests but does not prove that the 6S species lacks a ribosome-related function. An analysis of cellular constituents fractionated by density gradient centrifugation has revealed that the 6S RNA occurs in a ribonucleoprotein complex with a sedimentation value that is somewhat larger than 10S (23). The detection of this particle coupled with the recent demonstration of a 7S RNA component in the mammalian signal recognition particle suggested the possibility that the bacterial ribonucleoprotein complex might be the functional equivalent of the animal cell signal recognition particle complex (35, 48, 49).

Consistent with the notion that the 6S and 7S RNAs might be related is the observation that the two RNAs do possess regions of homology. Two of these elements, consisting of stretches of 21 and 24 bases in the 6S RNA, show 70% homology with corresponding domains in the identical human (47) and rat (25) 7S RNAs. The related domains are defined by bases 133 to 153 and 157 to 180 in mature 6S RNA and bases 67 to 87 and 271 to 293 in the 303-base 7S species (the latter numbering is as described in reference 47). The second element of each pair occupies equivalent molecular positions at the 3′ end of the procaryotic and eucaryotic RNAs. (Both elements in the 7S RNA occur in regions observed to be homologous to the consensus sequence of the *Alu* family of repeated DNA [25, 47]).

Despite the observed homology, the case for functional relatedness of these RNAs is weak. Lee et al. (24) demonstrated that the 6S RNA gene is not essential for cell growth or maintenance of normal protein secretion function. This finding is in marked contrast with the pleiotropic nature of mutations affecting secretion and the results anticipated for a defective signal recognition particle involved in protein translocation.

A search for 6S RNA function must also consider the recent finding that the cellular level of 6S RNA is elevated some twofold in *E. coli* transformants harboring the gene for the so-called "spot 42" small RNA (34). The cloned frag-

ment encoding the spot 42 species was shown not to contain 6S DNA, and the two genes are now known to map at widely different sites (24, 34).

The organization of the 6S RNA gene in a dual-function transcription unit under relaxed control makes this RNA unique among the small stable RNAs of *E. coli*. The demonstration of nonessentiality does not necessarily mean that the 6S RNA lacks function, only that it is dispensable under the growth conditions evaluated. Taken together, the unique features of the 6S RNA and its gene system suggest that any biological role that can be identified will also be novel.

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