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Thermus thermophilus 16S rRNA Is Transcribed from an Isolated Transcription Unit

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A cloned 16S rRNA gene from the extreme thermophilic eubacterium *Thermus thermophilus* HB8 was used to characterize the in vivo expression of the 16S rRNA genes in this organism by nuclease S1 mapping. The gene represents an isolated transcription unit encoding solely 16S rRNA. Under exponential growth conditions, transcription was initiated at a single promoter, which represents the structural equivalent of *Escherichia coli rrn* P2 promoters. The promoter-leader region was very similar to the *E. coli rrn* P2 promoter-leader segment that is responsible for antitermination. The *T. thermophilus* leader region was approximately 85 nucleotides shorter than its *E. coli* P2 counterpart. Potential processing intermediates were correlated with a proposed secondary structure of *T. thermophilus* pre-16S rRNA.

In the extreme thermophilic eubacterium *Thermus thermophilus*, each rRNA gene exists as two copies (10). Cloning of both 23S-5S rDNA gene sets, combined with genomic hybridization analyses, revealed the absence of 16S rDNA within 7.8 and 6.4 kilobases (kb), respectively, upstream of either 23S-5S rDNA gene copy. Accordingly, no 16S rRNA coding sequences were detected within 3.55 kb downstream of one cloned 23S-5S rDNA gene (10). In addition, our finding of a functional promoter immediately preceding one operon comprising a 23S rRNA, 5S rRNA, and $tRNA^{Gly}$ gene gave further evidence that the 16S rRNA genes of *T. thermophilus* are organized in separate transcription units, a situation so far unprecedented among procaryotes (12).

As a continuation of this work, we cloned a 16S rRNA gene from *T. thermophilus* HB8, located on a 2.3-kb *Bam*HI fragment. In this report we present 640 base pairs (bp) of upstream and 165 bp of downstream sequence surrounding the coding sequence for the mature 16S rRNA.

By using nuclease S1 protection, we were able to determine the in vivo start of transcription and the putative region of transcriptional termination. We characterized the in vivo maturation of the 16S rRNA, and in this report we present a stem-and-loop structure for pre-16S rRNA. We detected a segment of 14 bp, identical in the promoter-leader regions of the 16S and 23S-5S rRNA (12) operon from *T. thermophilus*, that is also present in the 23S-5S rRNA spacer (13). This sequence element includes a homology to the box A sequence that is part of *Escherichia coli rrn* promoter-leader (and spacer) regions responsible for antitermination (20).

In addition, the region upstream of the 16S rDNA promoter was probed by nuclease S1 mapping for further transcripts.

MATERIALS AND METHODS

Enzymes were purchased from Boehringer Mannheim Biochemicals; Pharmacia; New England BioLabs Inc.; and Bethesda Research Laboratories, Inc. Radioactive nucleotides were obtained from Amersham Corp.

Bacterial strains. *T. thermophilus* HB8 (ATCC 27634) cells were grown at 70 to 75°C in medium D, as described previously (4), supplemented with 5 g of tryptone (Difco Laboratories) per liter, 4 g of yeast extract per liter, 2 g of NaCl per liter, and 1 g of glucose per liter and harvested in the mid-log phase. *E. coli* BMH 71/18 (18) was used for M13 transformation.

Primer synthesis and DNA sequencing. Oligonucleotides used for M13 DNA sequencing were produced by the phosphoramidite method as described previously (12). DNA sequencing was performed by the M13-dideoxy method (29), with the use of a modified extended-DNA sequencing protocol provided by Bethesda Research Laboratories. For unequivocal sequence determination, both strands were completely sequenced by using consecutive primers (each 25 to 35 bases long) every 200 to 400 nucleotides.

Cloning of the 16S rRNA gene. DNA was isolated from *T. thermophilus* cells by standard procedures (24). One of the two 16S rRNA genes, located on a 2.3-kb *Bam*HI fragment (10), was cloned into pSP65 (25). *E. coli* X90 (9) competent cells were prepared and transformed as described previously (22). Colony hybridization was performed with 5′-end-labeled *T. thermophilus* 16S rRNA fragments (generated by limited RNase T_1 digestion) as the probe; modifications of the procedure reported (10) were as follows. After prehybridization, the hybridization was carried out in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 37°C for 48 h (11). One of several positive clones carrying the recombinant plasmid pTH16 was used for the analysis.

5′ and 3′ end labeling of DNA fragments used for nuclease S1 protection analysis. The 303-bp *Acc*I-*Bst*EII fragment derived from the recombinant plasmid pTH16 was dephosphorylated and labeled at its 5′ ends by using T4 polynucleotide kinase (23). The labeled fragment, comprising 125 nucleotides of 16S rDNA, was used for nuclease S1 protection analysis of the 16S rRNA 5′-flanking region.

For the analysis of the 3′-flanking region, a 321-bp *Asp*700 (*Xmn*I)-*Bam*HI fragment, including 160 nucleotides of 16S rRNA coding sequence, and a 283-bp *Nco*I-*Bam*HI fragment, including 122 nucleotides of 16S rRNA coding sequence, were labeled with [α-^{32}P]dCTP by using T4 DNA polymerase essentially as described previously (23). In detail, ca. 100 ng of the DNA fragment was incubated for 1 min at 37°C with 5 to 10 U of T4 DNA polymerase in a volume of 14.5 μl and in the absence of nucleotides. During this step, the 3′ exonuclease activity of the enzyme is unchained. The tube was placed on ice, and 1 μl of dATP-dGTP-dTTP (each

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2 mM) and 4 μl of [α-^{32}P]dCTP (10 to 40 μCi/μl) were added. During this step, the exonuclease activity is completely masked. After a 10-min incubation at 37°C, 1 μl of 2 mM dCTP was added, and the incubation was continued for 10 to 30 min. Compared with the mere filling-in reaction of recessed 3′ ends, the procedure yields thoroughly labeled probes of high specific activity.

To detect any transcripts originating from the region upstream of the 16S rDNA promoter, a 450-bp *Bam*HI-*Acc*I fragment was 5′ and 3′ end labeled as described above.

Nuclease S1 mapping. Preparation of total RNA from exponentially growing *T. thermophilus* cells and nuclease S1 protection analyses were performed as described previously (13). The hybridization temperature was 50 or 60°C. For controls, equal amounts (50 to 100 μg) of bulk tRNA from *E. coli* instead of total RNA from *T. thermophilus* were incubated with the labeled DNA probe. Size determinations of protected single-stranded DNA fragments (see Fig. 3) were verified by several independent gel electrophoresis steps in 5% polyacrylamide–7 M urea, with sequencing ladders of different M13 clones used as size markers.

RESULTS

Sequences surrounding the *T. thermophilus* 16S rRNA in the primary transcript (Fig. 1) could be formed into the secondary structure proposed in Fig. 2. The structure included a central section of extensive base pairing. Conspicuously, 15 consecutive purines of 5′-flanking sequences (Fig. 2, positions 34 to 48) and 15 successive pyrimidines of 3′-flanking sequences (Fig. 2, positions 1632 to 1646) were part of the helical region.

Nuclease S1 mapping. (i) 5′-Flanking region. S1 mapping studies were performed to identify the initiation site of transcription, the processing intermediates, and the region of transcriptional termination of the 16S rRNA gene. To analyze the 5′-flanking region, a 303-bp *Acc*I-*Bst*EII fragment comprising 125 nucleotides of 16S rDNA was 5′ end labeled (Fig. 1B), denatured, and hybridized to total RNA (50 or 100 μg) isolated from exponentially growing *T. thermophilus* HB8 cells. The lengths of protected single-stranded DNA fragments were determined by coelectrophoresis of M13 sequencing ladders (Fig. 3, lanes A, C, G, and T) and a *Cfo*I digest of the 5′-end-labeled 303-bp fragment (Fig. 3, lane 4) that yields a single strand which would correspond to protection by an RNA transcript initiated 11 nucleotides downstream of the −10 box (see Fig. 4A).

The DNA single strand protected by the primary transcript was four nucleotides longer than the *Cfo*I-generated single strand (211 bases) used as the size marker (Fig. 3, lanes 2 to 4). The difference of four nucleotides was verified by extended electrophoresis (data not shown). Therefore, transcription was initiated at the last of the four thymidines following the −10 box (see Fig. 4A).

As well as the signal corresponding to the primary transcript, bands of comparable intensities, 121 to 125 nucleotides in length (Fig. 3, lanes 2 and 3), could be assigned to cleavages of pre-16S rRNA at positions 90 to 94 (Fig. 2). These signals represent fragments protected by mature 16S rRNA molecules. The 5′ termini were at positions anticipated from an alignment with *E. coli* 16S rRNA. The presence of several bands of equal intensity implies a 5′-microheterogeneity.

After longer film exposures (Fig. 3, lane 11), a reproducible pattern of fainter bands was observed. We interpret these fragments to represent specific protection signals for the following reasons. Signals of comparable size were detected neither in the controls with bulk tRNA from *E. coli* (instead of total RNA from *T. thermophilus*) nor in the labeled DNA probe itself after extensive autoradiography (data not shown). The signals of protection correspond to cleavages sites at the 3′ boundary of box B (positions 19 to 22), in the long-range base-paired stem (positions 44, 48, and 50), and in the interior loop (positions 56 and 57) of pre-16S rRNA (Fig. 2).

(ii) 3′-Flanking region. For the analysis of the 16S rRNA 3′-flanking region, a 321-bp *Asp*700-*Bam*HI fragment, including 160 nucleotides of 16S rRNA coding sequence, and a 283-bp *Nco*I-*Bam*HI fragment, including 122 nucleotides of 16S rRNA coding sequence, were labeled with T4 DNA polymerase (Fig. 1B). By using DNA fragments which covered unequal portions of 3′ 16S rDNA at their 5′ termini but which were identical in their 3′ regions, we could unambiguously distinguish between protection by 3′ 16S rRNA precursor molecules and external processing products that do not include 3′ 16S rRNA sequences.

With both DNA fragments, we observed two pronounced signals or signal clusters (Fig. 3, lanes 6 and 7). With the 283-bp *Nco*I-*Bam*HI fragment (lane 7), bands of protection were shortened by exactly the number of missing 16S rDNA nucleotides relative to the *Asp*700-*Bam*HI fragment (used in lane 6). The larger signals in lanes 6 and 7 corresponded to RNA precursor molecules terminating around position 1670 (Fig. 2), i.e., within the stem of a potential hairpin containing seven consecutive G · C base pairs followed by a stretch of uridines, indicative of rho-independent terminators. Although the assignment to position 1670 was obtained with the *Asp*700-*Bam*HI as well as with the *Nco*I-*Bam*HI probe, the signal might correspond to transcripts terminating within the consecutive uridines, since the sequencing ladders used for size determination of protected fragments were not derived from the identical DNA used as the hybridization probe. Assuming that the assignment to position 1670 was correct, it is conceivable that transcripts terminating within the stretch of uridines were shortened by a nibbling activity of nuclease S1 owing to an instability of the RNA-DNA hybrids at the terminal U · A base pairs. Alternatively, the signal assigned to position 1670 (Fig. 2) might originate from an endonucleolytic cleavage within the hairpin structure. Conceivably, such a mechanism could ensure the production of uniform pre-16S rRNA molecules which do not display any 3′ heterogeneity caused by terminator readthrough. A strong endonucleolytic cleavage associated with a hairpin structure has also been detected in the *T. thermophilus* pre-23S-5S rRNA spacer (13).

The smaller signals, with estimated sizes of 155 to 161 nucleotides for the 321-bp *Asp*700-*Bam*HI fragment and 119 to 123 nucleotides for the 283-bp *Nco*I-*Bam*HI fragment (Fig. 3, lanes 6 and 7), corresponded to mature 3′ termini (Fig. 2, positions 1606 to 1610). The appearance of multiple bands of protection might reflect a 3′ microheterogeneity of mature 16S rRNA molecules. We cannot exclude, however, the possibility that these clustered signals originate from a slight degradation of the original DNA probes (Fig. 3, lanes 8 and 9).

The conjectural 3′ as well as the 5′ microheterogeneity of *T. thermophilus* 16S rRNA is less pronounced than in *T. thermophilus* pre-23S rRNA (12, 13). This might reflect differences in the maturation of 16S and 23S rRNA, as has been reported for *E. coli* (see Discussion).

Additional bands (approximately 115 nucleotides in length) were observed when the *Asp*700-*Bam*HI probe was

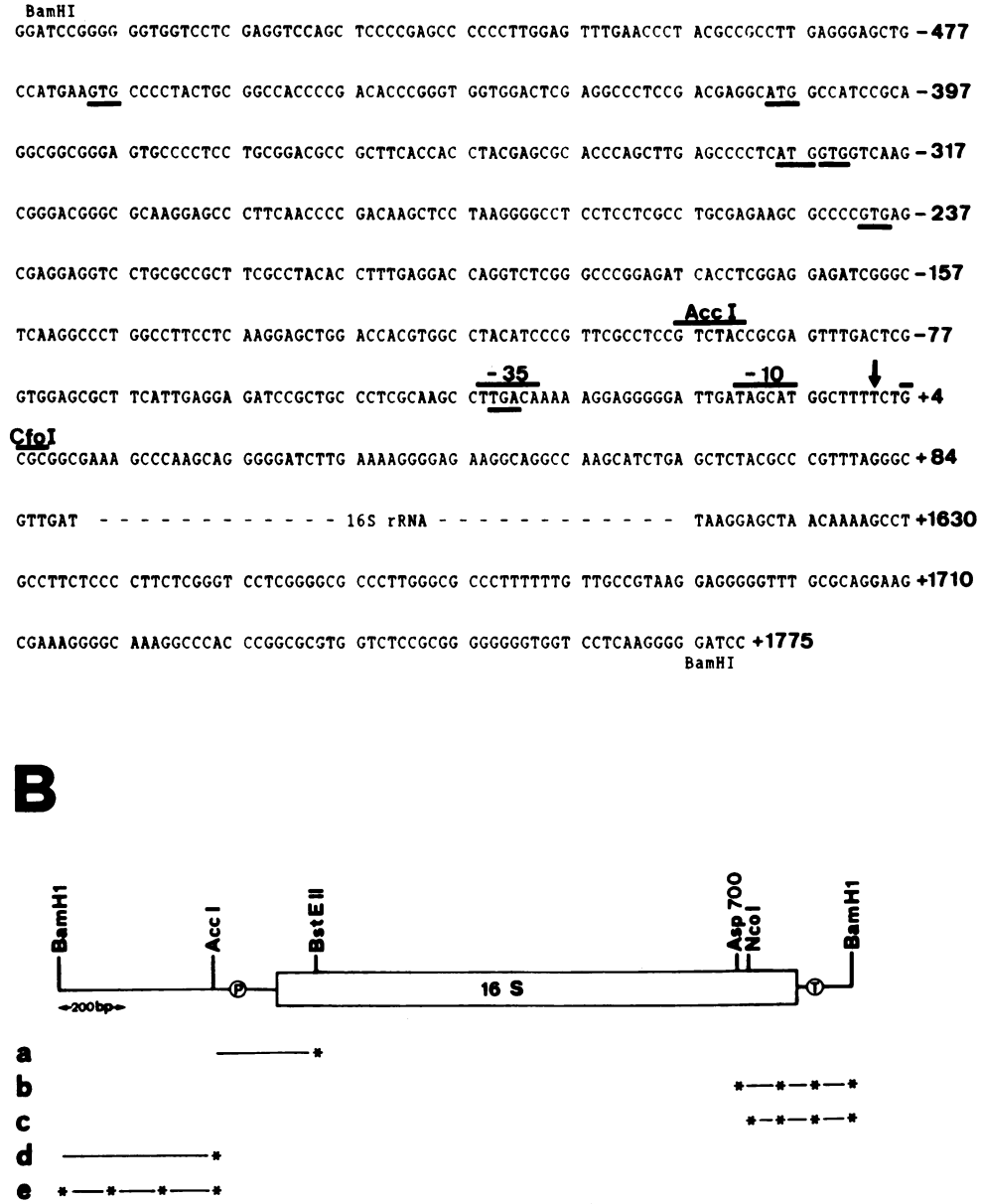

FIG. 1. (A) Nucleotide sequence of regions flanking the *T. thermophilus* 16S rRNA gene located on a 2.3-kb *Bam*HI fragment. The −35 and −10 regions (overlined), the start of transcription (vertical arrow, position +1), the *Acc*I and *Cfo*I sites (overlined), and potential start codons (underlined) of in-phase hypothetical open reading frames terminating within the −35 region are indicated. The conjectured 16S rRNA coding sequence (positions 90 to 1611) will be published separately (R. K. Hartmann, J. Wolters, B. Kröger, S. Schultze, T. Specht, and V. A. Erdmann, Syst. Appl. Microbiol., in press). Numbering of the sequence is based on the identified (Fig. 3) start of transcription (position +1). The *Bst*EII, *Asp*700, and *Nco*I recognition sites are located at positions +210 to 216, +1445 to 1454, and +1483 to 1488, respectively. (B) Schematic representation of the 16S rRNA gene region and DNA probes used for nuclease S1 mapping. The locations of the promoter and terminator are marked by P and T, respectively. Asterisks indicate ^{32}P-labeled nucleotides. Probes a and d were prepared by 5′ end labeling of the *Acc*I-*Bst*EII and *Bam*HI-*Acc*I fragments, respectively. Probes b (*Asp*700-*Bam*HI), c (*Nco*I-*Bam*HI), and e (*Bam*HI-*Acc*I) were thoroughly labeled by using T4 DNA polymerase, whose 3′ exonuclease activity is unleashed in the absence and masked in the presence of triphosphates. The latter method yields probes of high specific activity similar to those obtained by primer extension.

used (Fig. 3, lane 6); they were not detected at the identical position when the *Nco*I-*Bam*HI probe was used (lane 7), even after extended film exposure (lane 13). This means that the signals refer to protection within the 16S rRNA 3′ coding region, where the two DNA probes are heterogeneous. For the *Nco*I-*Bam*HI probe, equivalent bands approximately 75 nucleotides in length would have been expected; however, these were below the runoff limit of the gel. Therefore, the

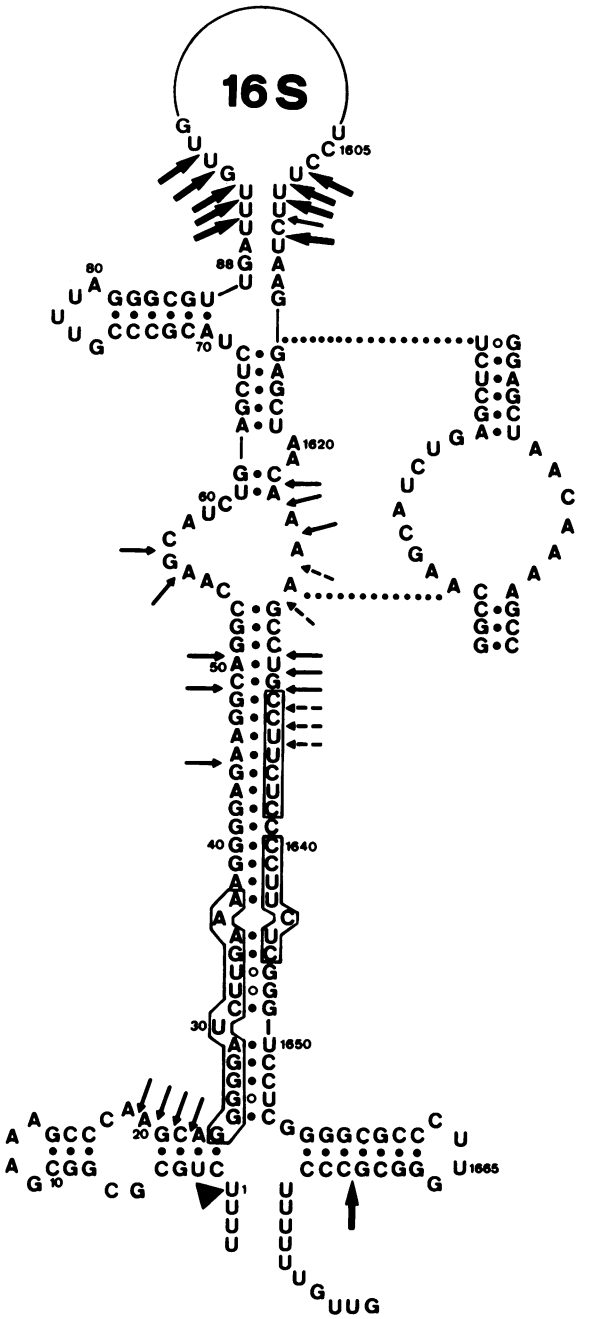

FIG. 2. Potential secondary structure of the *T. thermophilus* pre-16S rRNA transcript. Symbols: ▲, start of transcription (Fig. 3); →, potential cleavage sites of processing enzymes, deduced from data shown in Fig. 3, lanes 10 to 13; ➡, nuclease S1 protection signals of relatively high intensity. The thick arrow at the bottom, whose assignment to nucleotides 1670 to 1671 is an estimation (see text), might be positioned within the consecutive uridines (nucleotides 1674 to 1679), thus indicating a rho-independent release of the RNA transcript. Broken arrows indicate minor differences in size determination (Fig. 3, lanes 12 and 13) of signals using the *Asp*700/*Bam*HI fragment (broken arrows) or the shorter *Nco*I-*Bam*HI fragment (solid arrows adjacent to broken ones). Boxed nucleotides at positions 24 to 37 mark a homology to the *T. thermophilus* 23S rRNA promoter-leader and 23S-5S rRNA spacer (Fig. 5). Boxed nucleotides at positions 1632 to 1638 and 1640 to 1646 indicate a direct repeat. For part of the secondary structure, enclosed by dotted lines, an alternative structure can be conceived.

signals can be assigned to 16S rRNA transcripts lacking approximately 45 nucleotides at their 3′ ends. The discrete character of these protected DNA fragments (lane 6) is indicative of an endonucleolytic degradation pathway for 16S rRNA in *T. thermophilus*.

Fainter nuclease S1 protection signals (Fig. 3, lanes 12 and 13) can be assigned, as for the 5′ precursor region, to cleavages in the long-range base-paired stem (Fig. 2, positions 1629 to 1634), as well as to cleavages in the central interior loop (positions 1622 to 1626) of pre-16S rRNA. We should mention again that the observation of multiple signals does not necessarily reflect a clustered cleavage of pre-16S rRNA (instead of a single scission), as implied in Fig. 2, but could result from heterogeneities in the probe sizes (Fig. 3, lanes 8 and 9) or a terminal nibbling activity of nuclease S1.

Promoter region. The 16S and the 23S rDNA promoters display a striking homology to a −35 consensus sequence deduced from promoters functional in *E. coli* (14) (Fig. 4). A less stringent but still pronounced homology to a respective −10 consensus sequence can be observed. The promoter of the *T. thermophilus* 4.5S RNA gene (30) shows a reverse similarity, i.e., a less pronounced homology to the *E. coli* −35 consensus sequence but an almost perfect match to the respective −10 consensus sequence (Fig. 4C and D).

The regulatory mechanism termed stringent response can be induced in cells of *T. thermophilus* (21), and, in analogy to *E. coli*, a (p)ppGpp synthetase I could be extracted from a ribosomal fraction. As with the 23S rDNA promoter (12), we found a G+C-rich (discriminator) sequence located at the start of transcription of the 16S rRNA gene, which was similar to the region believed to be under stringent control in stable RNA and ribosomal protein genes of *E. coli* (31). The putative region, directly preceded by the −10 box in the reported *E. coli* promoters, was found to be separated by 6 to 9 nucleotides from the −10 box in the *T. thermophilus* promoter regions shown in Fig. 4, although the distance between the −10 region and start of transcription is comparable. Conspicuously, the 16S and 23S rDNA promoters of *T. thermophilus* displayed the common feature of four consecutive thymidines near the start of transcription (Fig. 4A and B).

Mapping of promoter upstream sequences. Analysis of sequences upstream of the 16S promoter revealed several in-phase reading frames all terminating within the −35 box (Fig. 1A). The longest reading frame would commence with the GTG codon at position −469 (Fig. 1A) and would code for a polypeptide of 145 amino acids. To detect any transcripts originating from this region, the 450-bp *Bam*HI-*Acc*I fragment was 5′ and 3′ end labeled (Fig. 1B, lines d and e). However, no specific bands of protection from nuclease S1 digestion could be observed when total RNA isolated from exponentially growing *T. thermophilus* cells was used (data not shown).

DISCUSSION

Antitermination elements of pre-16S rRNA. For the bacteriophage lambda antitermination system N, specific DNA segments, termed *nut* (or *nut*-like) loci, are essential. On the basis of a comparison of several of these loci, three conserved sequence elements have been reported (boxes A, B, and C [7]). In phage lambda, box B consists of a region of hyphenated dyad symmetry with a central purine-rich portion of the coding (RNA-equivalent) strand (20, 28). The stable association of phage lambda protein N with the transcription apparatus is promoted by the box B domain.

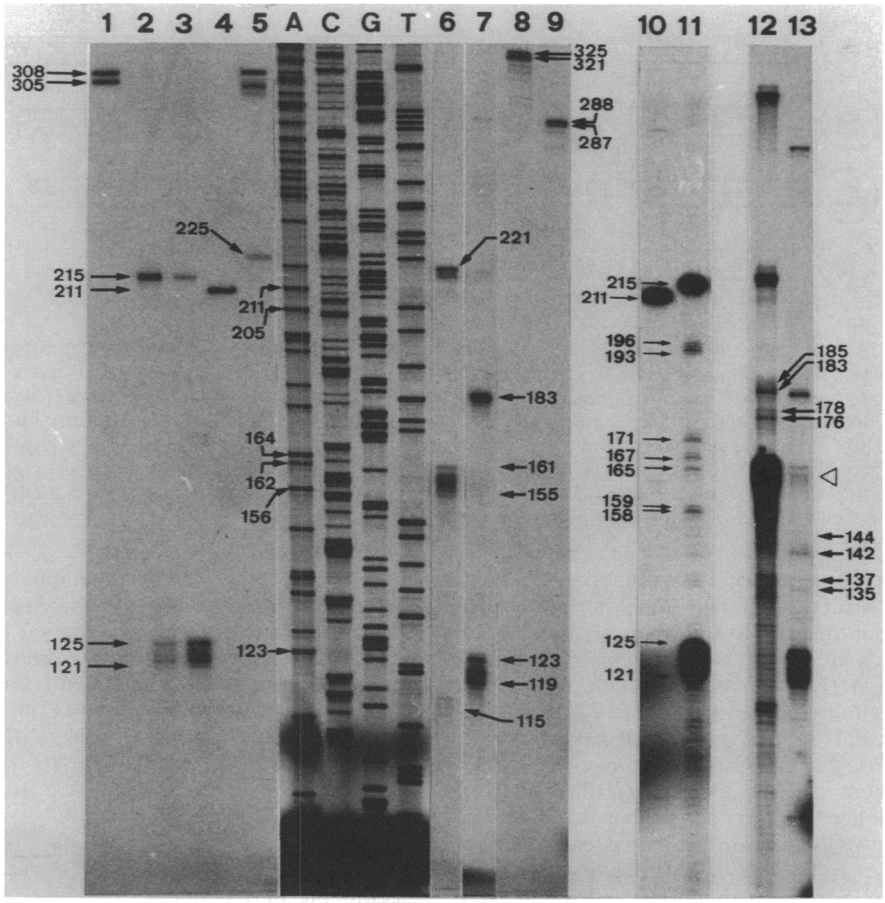

FIG. 3. Nuclease S1 mapping of the 5′ and 3′ regions of the 16S rRNA primary transcript from *T. thermophilus*. The size of single-stranded DNA fragments was determined by gel electrophoresis in 5% polyacrylamide–7 M urea. For the analysis of the pre-16S rRNA 5′-flanking region (lanes 1 to 5, 10, and 11), a 5′-end-labeled 303-bp *Acc*I-*Bst*EII fragment, comprising 125 nucleotides of 5′ 16S rDNA, was used as the probe. Lanes: 1, undigested probe; 2 and 3, probe hybridized to 50 μg of total RNA from *T. thermophilus* at 50°C (lane 2) or 60°C (lane 3) and digested with nuclease S1; 4 and 5, *Cfo*I and *Hph*I digests of the AccI-BstEII probe as size markers (the *Cfo*I digest yields a single-stranded fragment, 211 bases in length, that would correspond to protection by a transcript initiated 11 bases downstream of the −10 box [Fig. 4]); A to T, M13 sequencing ladders used as size markers, which lack, in contrast to the probe fragments protected from nuclease S1 digestion, the 5′-terminal phosphate. For the analysis of the 3′-flanking region, a 321-bp *Asp*700-*Bam*HI fragment (lanes 6 and 8), including 160 nucleotides of 16S rRNA coding sequence, and a 283-bp *Nco*I-*Bam*HI fragment (lanes 7 and 9), including 122 nucleotides of 16S rRNA coding sequence, were labeled with T4 DNA polymerase. Lanes: 6 and 7, probe hybridized to 50 μg of total RNA from *T. thermophilus* at 50°C and digested with nuclease S1; 8 and 9, undigested probes; 10 and 11, extended film exposure of lanes 4 and 3, respectively; 12 and 13, extended film exposure of lanes 6 and 7, respectively, but hybridized at 60°C. For the determined sizes (in bases) of potential processing intermediates, corresponding cleavage sites have been assigned in Fig. 2. For equivalent signals in lanes 12 and 13 (176 to 178 versus 135 to 137 bases, and 183 to 185 versus 142 to 144 bases), slightly different cleavage sites were assigned in Fig. 2 (indicated by broken adjacent to solid arrows in Fig. 2). Faint bands in lane 13 (◁) most probably originate from trace amounts of the *Asp*700-*Bam*HI fragment in the *Nco*I-*Bam*HI probe (data not shown), since the latter probe was derived from cleavage of the ^{32}P-labeled *Asp*700-*Bam*HI fragment. Size determinations in lanes 1 to 13 are based on several independent gel electrophoreses in 5% polyacrylamide–7 M urea, with sequencing ladders of different M13 clones as size markers; lanes A, C, G, and T represent one example. Uncertainties of length determinations were estimated not to exceed ±2 nucleotides. As controls for nuclease S1 protection analyses shown in lanes 2, 3, 6, 7, and 11 to 13, equal amounts of bulk tRNA from *E. coli* (instead of total RNA from *T. thermophilus*) were incubated with the corresponding DNA probe and subjected to the identical procedure. However, no unspecific bands of protection were detectable; as a consequence, corresponding lanes are omitted.

The event, that occurs when the polymerase has proceeded just beyond box B, induces the antitermination status of the transcription complex; i.e., during subsequent elongation, protein N traverses, along with the transcription complex, several defined terminators located downstream of *nut* (1).

Box A is probably involved in *nusA* protein binding (6), and box A mutations can cause antitermination activity to deteriorate (27). The function of box C is not clear yet.

In *E. coli rrn* promoter-leader and 16S-23S rRNA spacer regions, *nut*-like sequences have been identified (20, 26). The region essential for antitermination in *E. coli rrn* promoter-leader regions could be narrowed down to a 67-bp DNA fragment (Fig. 5, region I) immediately following the P2 promoter (20). Gourse et al. (8) have constructed plasmid encoded p_L-*rrnB* fusion operons that contained both box A and box B, only box A, or neither box A nor box B. Their data gave evidence that box A is essential, whereas box B is dispensable, for efficient rRNA transcription in *E. coli*.

The start of transcription of the 16S rRNA gene from *T. thermophilus* is followed by a hairpin structure, including an

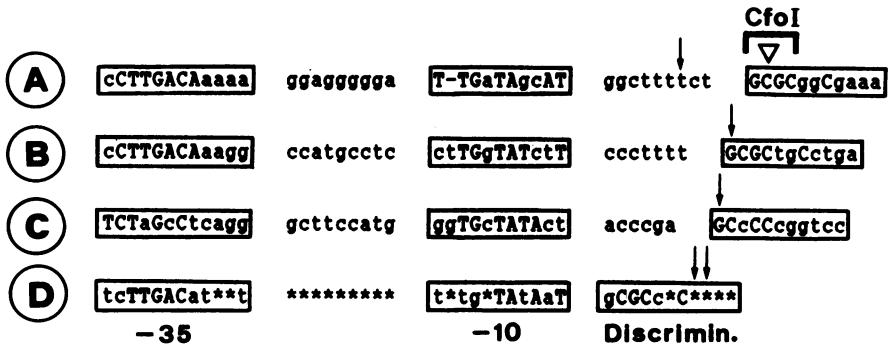

FIG. 4. Comparison of *T. thermophilus* 16S rDNA (A), 23S-5S rDNA (B) (12), and 4.5S RNA gene (C) (30) promoter regions with *E. coli* RNA polymerase promoter and discriminator consensus sequences (D) (14, 31). Vertical arrows indicate starts of transcription. Capital letters in panels A to C designate homology to the *E. coli* consensus sequences (panel D), whereas capital and lowercase letters in panel D mark highly conserved and semiconserved nucleotides, respectively, that are found in *E. coli* promoters. *, Arbitrary nucleotides. A gap has been introduced into the −10 box of the 16S rDNA promoter (panel A) to optimize the alignment. The *Cfo*I site was used to produce a restriction fragment of the 5′-end-labeled *Acc*I-*Bst*EII fragment (Fig 1B) as size marker to locate the starting point of transcription (Fig. 3, lanes 2 to 4); ▽, 3′ terminus of the coding strand of the *Cfo*I-*Bst*EII fragment. The coding strand was identical, apart from the four nucleotides lacking at the 3′ terminus, with the single-stranded fragment protected by the primary transcript.

internal loop that is reminiscent of a box B antitermination motif. Although this structure element does not allow formation of a stem of continuous base pairing as encountered in lambda *nut* regions or the *T. thermophilus* 23S rRNA promoter-leader region (Fig. 5, region III), it displays a pronounced similarity to its counterpart in *rrn* P2 promoter-leader regions of *E. coli* (Fig. 5, region I) and *Pseudomonas aeruginosa* (H. Y. Toschka, personal communication). The putative box B element of *T. thermophilus* 16S rRNA is followed by a sequence comprising 14 bases, which is identical in the *T. thermophilus* 23S promoter-leader region and is located there at an equivalent position (Fig. 2, positions 24 to 37; Fig. 5, regions II and III). The conserved sequence element harbors a box A antitermination motif, whose homology to the respective region in the *rrnG* P2 promoter-leader is depicted in Fig. 5. The box A sequence of the *rrnG* P2 promoter-leader (Fig. 5, region I) is identical with a consensus sequence deduced from an alignment of *nut*-like sequences of lambdoid phages and *E. coli rrn* leader and spacer regions (26). Interestingly, the conserved sequence element of the 16S and 23S rRNA leader harboring box A is also present in the 23S-5S RNA spacer region of the 23S-5S-tRNA^{Gly} operon from *T. thermophilus* (Fig. 5, region IV), where the homology spans 11 (23S rRNA leader [Fig. 5, region III]) and 12 (16 rRNA leader [Fig. 5, region II]) consecutive nucleotides, except for one C-to-U exchange in both cases.

Furthermore, we find a significant homology between the box C region of *E. coli rrn* promoter-leader sequences and the equivalent region of the *T. thermophilus* 16S rRNA promoter-leader sequence (Fig. 5, regions I and II). Conspicuously, a tandem box C element is present in the *T. thermophilus* 23S rRNA operon leader (Fig. 5, region III).

Just preceding the 5′ termini of mature 16S rRNA (Fig. 2 and 5, region II), the *T. thermophilus* pre-16S rRNA can form a hairpin ($\Delta G_{25°C}$ = −13.6 kcal [−56.9 kJ]). The structural element is reminiscent of the terminatorlike structure found in *E. coli rrn* leader regions 23 nucleotides upstream of mature 16S rRNA. The *E. coli* hairpin structure, which is similar to the *E. coli trp t′* terminator, is a pausing site for RNA polymerase in vitro (17). Conceivably, the terminatorlike structure in *T. thermophilus* pre-16S rRNA might also provide an additional regulatory signal affecting the efficiency of 16S rRNA transcription in the extreme thermophilic eubacterium. In summary, the *T. thermophilus* 16S rRNA promoter-leader comprised just those sequence elements which have been identified by Li et al. (20) to be essential for antitermination in *E. coli*. There is no upstream promoter equivalent to *E. coli* P1, and approximately 100 nucleotides located between box C and the 5′ terminus of *E. coli* 16S rRNA are absent in the *T. thermophilus* promoter-leader (Fig. 5, regions I and II). A very similar constellation was found for the 23S-5S rRNA operon of *T. thermophilus* (12) (Fig. 5, region III).

The extreme reduction of promoter-leader sequences preceded by only one promoter and the local and transcriptional decoupling of 16S and 23S-5S rRNA genes in *T. thermophilus* raise several questions, i.e., how synthesis of stoichiometric amounts of all rRNAs is accomplished, whether there is a growth-rate-dependent regulation of rRNA transcription, and, if so, whether there are common regulatory elements in 16S rRNA and 23S-5S rRNA operons. It is intriguing to speculate whether 16S and 23S-5S rRNA genes respond to different regulatory signals, thus providing a subunit-specific regulation of ribosome synthesis. In *E. coli*, sequences upstream of promoter P1 have been identified as target sites for ribosomal feedback inhibition and growth rate dependence of *rrn* transcription, and they have been found to be responsible for transcriptional activation (8). At P1 most transcripts initiate during exponential growth rates, whereas P2 maintains the basal level of *rrn* transcription at low growth rates. Since the structural P1 promoter equivalent is lacking in *T. thermophilus*, only sequences upstream of the single promoters preceding the 16S and 23S-5S rRNA operons or within the promoter-leader regions could harbor target sites for transcriptional activation, growth-rate-dependent regulation of transcription, and ribosomal feedback inhibition. So far, we have not detected any significant homologies between upstream sequences preceding the promoters for 16S and 23S-5S rRNA. Development of a cloning system for *Thermus* species is a prerequisite for addressing these questions.

Processing of pre-16S rRNA. In *E. coli*, cleavage of pre-16S rRNA by RNase III is not essential for 16S rRNA maturation. In RNase III-deficient strains, mature 16S rRNA termini form at the same rate as in wild-type cells (15), whereas 23S rRNA processing fails totally; i.e., all ribosomes are provided with unprocessed pre-23S rRNA, which are, how-

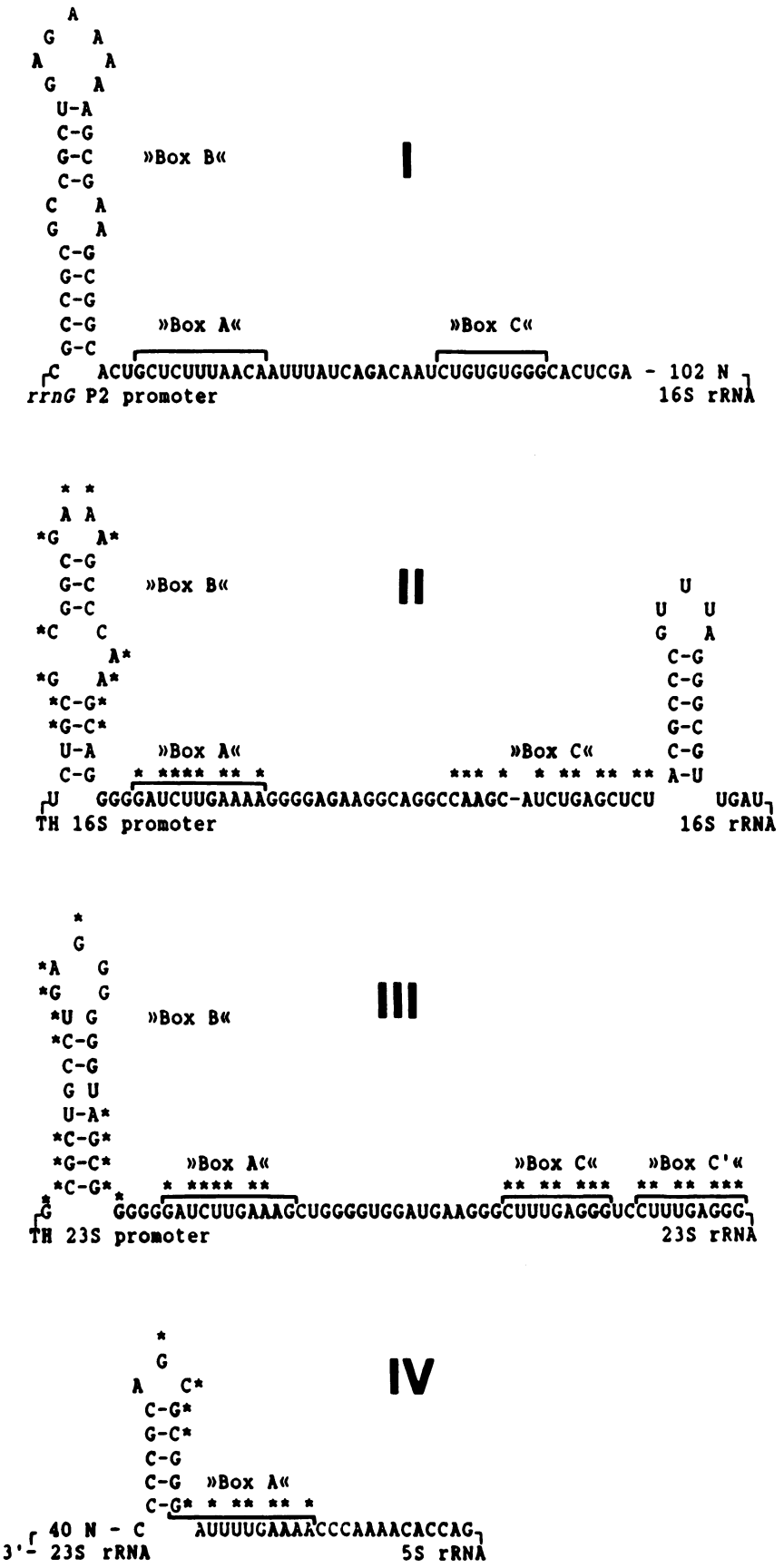

FIG. 5. Conserved sequence elements in promoter-leader regions of *E. coli rrnG* (region I) (20), *T. thermophilus* 16S (region II), and 23S-5S (region III) (12) rDNA and in the *T. thermophilus* 23S-5S rDNA spacer region (region IV) (13). The sequence shown in region I has been identified as the region responsible for antitermination in *E. coli rrn* transcription (20) harboring *nut*-like sequence elements (boxes A, B, and C). Asterisks in regions II to IV indicate homologies to boxes A, B, and C of the *E. coli rrnG* promoter-leader (region I). Box B denotes the entire hairpin. Abbreviation: N, nucleotides.

ever, functional in protein biosynthesis (15, 16). Recently, two-thirds of the normal promoter-leader of *E. coli rrnB* up to the RNase III cleavage site was replaced by the lambda p_L promoter and adjacent sequences (19). Only a few full-length transcripts originating from the hybrid operon could be detected after p_L promoter induction, but normal maturation of 5′ termini of 16S rRNA occurred. No transcripts at all were detected when the *rrnB* promoter-leader region was deleted entirely; i.e., the p_L promoter-leader region was fused directly to the 16S rRNA coding region. The latter results indicate the importance of 16S rRNA precursor sequences for the efficiency of rRNA transcription, transcript stability, and/or ribosome assembly.

For *T. thermophilus* 16S rRNA, signals which could be correlated to processing intermediates were detected by nuclease S1 anlyses (Fig. 3, lanes 11 to 13). The corresponding cleavage sites are located at an interior loop (around position 20 in Fig. 2), in the long base-paired stem, and in the interior loop (enclosed by dotted lines in Fig. 2) of the pre-16S rRNA. All these structural elements are reminiscent of *E. coli* RNase III cleavage sites. The enzyme cuts rRNA precursor molecules within long base-paired stem regions (3) and phage T7 mRNA precursor transcripts at interior loop structures (5).

In comparison with the signal obtained for the primary transcript, nuclease S1 protection signals for the putative processing intermediates of *T. thermophilus* pre-16S rRNA were relatively weak (Fig. 3). The result indicates a significant stability of the primary transcript but extremely short lives for intermediate cleavage products. It must be determined, however, whether the observed intermediate cleavage products represent authentic processing intermediates or by-products that do not reflect obligatory processing events. In the latter case, processing of pre-16S rRNA could be straightforward; i.e., maturation is achieved by a single step which yields the mature termini. This interpretation would be compatible with the findings, discussed above, that 16S rRNA maturation in *E. coli* can be accomplished in the absence of RNase III. In this context, it is interesting that transcription of *T. thermophilus* 4.5S RNA has been found to start at the mature 5′ terminus (30), whereas processing of pre-4.5S RNA by RNase P occurs in *E. coli* (2). The 4.5S rRNA gene is an example of a simplification of stable RNA synthesis in extreme thermophilic eubacteria.

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LITERATURE CITED

1. **Barik S., B. Ghosh, W. Whalen, D. Lazinski, and A. Das.** 1987. An antitermination protein engages the elongating transcription apparatus at a promoter-proximal recognition site. Cell **50**:885–899.
2. **Bothwell, A. L. M., R. L. Garber, and S. Altman.** 1976. Nucleotide sequencing and *in vitro* processing of a precursor molecule to *Escherichia coli* 4.5S RNA. J. Biol. Chem. **251**: 7709–7716.
3. **Bram, R. J., R. A. Young, and J. A. Steitz.** 1980. The ribonuclease III site flanking 23S sequences in the 30S ribosomal precursor RNA of *E. coli*. Cell **19**:393–401.
4. **Castenholz, R. W.** 1969. Thermophilic blue-green algae and the thermal environment. Bacteriol. Rev. **33**:476–504.
5. **Dunn, J. J., and F. W. Studier.** 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. **166**:477–535.
6. **Friedman, D., and E. Olson.** 1983. Evidence that a nucleotide sequence, "boxA," is involved in the action of the NusA protein. Cell **34**:143–149.
7. **Friedman, D. I., and M. Gottesman.** 1983. Lytic mode of lambda development, p. 21–52. *In* R. Hendrix, J. Roberts, F. Stahl, and R. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Gourse, R. L., H. A. De Boer, and M. Nomura.** 1986. DNA determinants of rRNA synthesis in *E. coli*: growth rate dependent regulation, feedback inhibition, upstream activation, antitermination. Cell **44**:197–205.
9. **Hanahan, D.** 1983. Studies of transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
10. **Hartmann, R. K., B. Kröger, D. W. Vogel, N. Ulbrich, and V. A. Erdmann.** 1987. Characterization of cloned rDNA from *Thermus thermophilus* HB8. Biochem. Int. **14**:267–275.
11. **Hartmann, R. K., H. Y. Toschka, N. Ulbrich, and V. A. Erdmann.** 1986. Genomic organization of rDNA in *Pseudomonas aeruginosa*. FEBS Lett. **195**:187–193.
12. **Hartmann, R. K., N. Ulbrich, and V. A. Erdmann.** 1987. An unusual rRNA operon constellation: in *Thermus thermophilus* HB8 the 23S/5S rRNA operon is a separate entity from the 16S rRNA operon. Biochimie **69**:1097–1104.
13. **Hartmann, R. K., N. Ulbrich, and V. A. Erdmann.** 1987. Sequences implicated in the processing of *Thermus thermophilus* HB8 23S rRNA. Nucleic Acids Res. **15**:7735–7747.
14. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
15. **King, T. C., and D. Schlessinger.** 1983. S1 nuclease mapping analysis of ribosomal RNA processing in wild type and processing deficient *Escherichia coli*. J. Biol. Chem. **258**:12034–12042.
16. **King, T. C., R. Sirdeshmukh, and D. Schlessinger.** 1984. RNase III cleavage is obligate for maturation but not for function of *Escherichia coli* pre-23S rRNA. Proc. Natl. Acad. Sci. USA **81**:185–188.
17. **Kingston, R. E., and M. J. Chamberlin.** 1981. Pausing and attenuation of *in vitro* transcription in the *rrnB* operon of *E. coli*. Cell **27**:523–531.
18. **Kramer, W., V. Drutsa, H.-W. Jansen, B. Kramer, M. Pflugfelder, and H.-J. Fritz.** 1984. The gapped duplex DNA approach to oligonucleotide-directed mutation construction. Nucleic Acids Res. **12**:9441–9456.
19. **Krych, M., R. Sirdeshmukh, R. Gourse, and D. Schlessinger.** 1987. Processing of *Escherichia coli* 16S rRNA with bacteriophage lambda leader sequences. J. Bacteriol. **169**:5523–5529.
20. **Li, S. C., C. L. Squires, and C. Squires.** 1984. Antitermination of *E. coli* rRNA transcription is caused by a control region segment containing lambda *nut*-like sequences. Cell **38**:851–860.
21. **Lienert, I., and D. Richter.** 1981. Stringent control in an extreme thermophilic bacterium. Preliminary characterization of a guanosine 3′, 5′-polyphosphate [(p)ppGpp] synthesizing enzyme from *Thermus thermophilus*. J. Gen. Microbiol. **123**:383–386.
22. **Mandel, M., and A. Higa.** 1970. Calcium dependent bacteriophage DNA infection. J. Mol. Biol. **53**:154–162.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 117–124. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organism. J. Mol. Biol. **3**:208–218.
25. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.

26. **Morgan, E. A.** 1986. Antitermination mechanisms in rRNA operons of *Escherichia coli*. J. Bacteriol. **168**:1–5.
27. **Olson, E., C.-S. Tomich, and D. Friedman.** 1984. The *nusA* recognition site. J. Mol. Biol. **180**:1053–1063.
28. **Salstrom, J., and W. Szybalski.** 1978. Coliphage *nutL*$^-$: a unique class of mutants defective in the site of gene N product utilization for antitermination of leftward transcription. J. Mol. Biol. **124**:195–221.
29. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
30. **Struck, J. C. R., H. Y. Toschka, and V. A. Erdmann.** 1988. Nucleotide sequence of the 4.5S RNA gene from *Thermus thermophilus* HB8. Nucleic Acids Res. **16**:9042.
31. **Travers, A. A.** 1984. Conserved features of coordinately regulated *E. coli* promoters. Nucleic Acids Res. **12**:2605–2618.
32. **Young, R., and J. Steitz.** 1978. Complementary sequences 1700 nucleotides apart from a ribonuclease III cleavage site in *Escherichia coli* ribosomal precursor RNA. Proc. Natl. Acad. Sci. USA **75**:3593–3597.