Journal of Bacteriology, Mar. 1987, p. 995–1002
0021-9193/87/030995-08$02.00/0

Vol. 169, No. 3

# *Bacillus subtilis* rRNA Promoters Are Growth Rate Regulated in *Escherichia coli*

HARRY G. DENEER† AND GEORGE B. SPIEGELMAN*

*Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5*

Received 9 June 1986/Accepted 31 October 1986

**rRNA promoters from the *rrnB* locus of *Bacillus subtilis* and from the *rrnB* locus of *Escherichia coli* were fused to the gene for chloramphenicol acetyltransferase (CAT). The level of expression of CAT in *E. coli* showed growth rate dependence when the CAT gene was linked to either *E. coli* or *B. subtilis* tandem promoters. The downstream promoter of the tandem *Bacillus* pair showed growth rate regulation, while the upstream promoter did not, whereas for the *E. coli* tandem promoters, only the upstream promoter was growth rate regulated.**

In enteric bacteria, the rate of rRNA synthesis is under growth rate-dependent control, increasing as the environment supports faster overall growth (15, 16, 21). The mechanisms which modulate the growth rate-dependent synthesis of rRNA and other stable RNAs are unknown, but there is strong evidence to suggest that for rRNA operons, the promoter regions are involved. All *Escherichia coli* rRNA operons thus far sequenced have tandemly arranged promoters, consisting of two distinct −35 and −10 regions separated by about 110 to 120 base pairs (bp) (15, 16). Results of studies with operon fusions and studies in which stable RNA promoters have been linked to transcription terminators have shown that the promoter region alone is sufficient to confer growth rate-dependent control (9, 20, 22, 26).

Recently Gourse et al. (10) have studied a series of deleted promoters from the *E. coli rrnB* and *rrnE* operons and concluded that the region between −51 and −20 with respect to the upstream promoter ($p_1$) transcription initiation site contains the sequences necessary for growth rate-dependent control. The downstream promoter ($p_2$) is transcriptionally much less active and not growth rate regulated, but it may be important during the recovery from the stationary phase of growth (25). Finally, the region between −51 and −88 of $p_1$ appears to stimulate transcription as much as 15-fold, while a region downstream of the $p_2$ promoter is required for efficient elongation of rRNA transcripts (10, 14).

We have been investigating the control of rRNA synthesis in *Bacillus subtilis* and have established that the overall control of rRNA synthesis appears to be the same as that in *E. coli* (13, 33). In this study we used a gene fusion system to show that *B. subtilis rrnB* promoters are functional in *E. coli* and are expressed under growth rate-dependent control. In contrast to *E. coli* promoters, however, the *B. subtilis* $p_2$ promoter is growth rate regulated and is the more transcriptionally active promoter of the $p_1$-$p_2$ pair.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *E. coli* HB101 was used as the host for all recombinant plasmids. The sources of plasmid were as follows: pKK232-8 and pKK10-2, J. Brosius; pGS227, K. Bott; pBR328, T. Warren.

**Cloning of *B. subtilis* and *E. coli* rRNA promoters.** Plasmid pHD1.8 was constructed by cloning a 1.8-kilobase (kb) fragment from pGS227 (25) into the *Eco*RI site of pKM-1, which has been described previously (7). Plasmid pGS227 contained two contigous *Eco*RI fragments (1.8 and 1.1 kb) derived from the 5′ terminus of the *B. subtilis rrnB* operon. Following *Eco*RI treatment of pGS227, the 1.8-kb fragment was separated by agarose gel electrophoresis and recovered by electroelution. The 1.8-kb fragment was cloned into pKM-1. The recombinants were screened for size, and a plasmid carrying the 1.8 kb fragment was identified. The presence of the 1.8-kb fragment was confirmed by hybridization to *Eco*RI-cut pGS227, which was electrophoresed through 0.7% agarose and transferred to nitrocellulose, and by DNA sequencing.

The smaller DNA fragments carrying *Bacillus* rRNA promoters were subcloned from the 1.8-kb insert on pHD1.8. Restriction fragments were isolated, blunt ended with the Klenow fragment of DNA polymerase, and ligated to *Sma*I-digested pKK232-8 under standard conditions (18). Recombinant clones were selected on LB media (18) containing 50 μg of chloramphenicol per ml. Only clones carrying a functional promoter in the proper orientation are able to grow under these selective conditions (2). In Fig. 1A the various rRNA promoter subclones used here are illustrated. pKK427B carries the tandem $p_1$-$p_2$ promoters on a 427-bp *Dde*I-*Sau*96I fragment pKK211B also carries the $p_1$-$p_2$ pair as a 211-bp *Dra*I fragment. To separate the promoters, the 427-bp fragment from pKK427B was digested with *Hin*cII, and a 282-bp fragment with a 3′ terminus that ended in the −35 region of the $p_2$ promoter was subcloned into pKK232-8. The resulting plasmid (pKK282B) contained only the $p_1$ promoter intact. To yield a plasmid with only the $p_2$ promoter, pKK427B was cut with *Eco*RI and treated with BAL 31 exonuclease as described by Maniatis et al. (18), and deleted fragments were subsequently liberated with *Bam*HI. DNA fragments were fractionated on polyacrylamide gels, isolated, and cloned into pKK232-8. Two of these deletions were used further. pKK285B contained an intact $p_1$-$p_2$ pair with the 5′ end 46 bp upstream of the −35 region of $p_1$ (see Fig. 1B for deletion endpoints). pKK220B was found to be deleted up to position 907 (Fig. 1B), which lies between the −10 and −35 regions of $p_1$, thus inactivating this promoter.

* Corresponding author.
† Present address: Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan, Canada S7N 0W0.

In addition to the *B. subtilis* rRNA promoters, analogous promoters from the *E. coli rrnB* operon (on pKK10-2 [3]) were also cloned into pKK232-8 to give pKK292Ec. pKK351Ec carries the $p_1$ promoter alone as a 351-bp *Hin*fI fragment, while pKK128Ec carries the single $p_2$ promoter as a 128-bp *Hha*I fragment (4). Finally, a nonribosomal promoter from *E. coli* was also used. The 377-bp *Eco*RI-*Bam*HI fragment from pBR322, carrying the promoter for the tetracycline resistance gene, was cloned to give pKKTet.

**Growth conditions.** The basal media for all growth rate experiments was AB media (20) supplemented with 20 μg of thiamine per ml, 50 μg of proline and leucine per ml, and 50 μg chloramphenicol per ml. To achieve different growth rates, this was further supplemented with (i) 0.4% glucose–0.4% yeast extract–0.4% Casamino Acids (Difco Laboratories, Detroit, Mich.); (ii) 0.4% glucose–0.01% yeast extract; (iii) 0.5% sodium succinate–0.4% yeast extract; (iv) 0.5% sodium succinate–0.01% yeast extract; or (v) 0.5% sodium acetate–0.01% yeast extract. Logarithmically growing cultures of *E. coli* HB101 carrying the various clones were diluted 1 in 400 with fresh, prewarmed media of the same type and then shaken at 37°C in 250-ml sidearm flasks. Growth was followed with a Klett-Summerson spectrophotometer equipped with a green filter. When a Klett reading of 20 to 25 was reached, the entire 20-ml culture was chilled on ice. The cells were harvested by centrifugation, washed once with EB buffer (50 mM Tris hydrochloride [pH 7.8], 30 μM dithiothreitol), pelleted, and stored at −20°C until assayed for chloramphenicol acetyltransferase (CAT) activity.

**CAT assay.** Frozen cells were thawed, suspended in 3.0 ml of EB buffer (27), and sonicated for 1 min. Cellular debris was removed by centrifugation and 10- to 50-μl fractions of the extract were assayed for CAT activity by the spectrophotometric method described by Shaw (27). Separate fractions were assayed for total protein by a modified Lowry's assay (24), and CAT-specific activities were calculated. One unit of activity is defined as a change of $10^{-3}$ optical density units at 412 nm per minute per milligram of total protein at 37°C.

**CAT mRNA determination. (i) Isolation of [$^3$H]RNA.** Cultures of *E. coli* HB101 carrying the recombinant plasmids were grown as described above and labeled for 1 min by the addition of 10 μCi of [5,6-$^3$H]uridine per ml. Incorporation of label was stopped, mRNA was isolated, and the amount of incorporation was determined as described by Daniels and Bertrand (6).

**(ii) Construction of M13-CAT hybridization probe.** A 773-bp *Taq*I fragment containing the entire CAT structural gene (5) was isolated from pBR328 and cloned into the *Sma*I site of M13 mp18 by the method described by Messing (19). Both orientations of the 773-bp CAT fragment were obtained, and the sense clone was used as the blank in the hybridization assay. Large quantities of single-stranded M13-CAT DNA were prepared by scaling up the mini-prep procedure described previously (19). M13-CAT DNA was bound to nitrocellulose filters (diameter, 115 mm; BA85; Schleicher & Schuell, Inc., Keene, N.H.) as described by Daniels and Bertrand (6). Individual filters (diameter, 6 mm) were subsequently punched out with a cork borer. Enough DNA was added to the large filter such that each 6-mm-diameter filter contained 4 μg of DNA.

**(iii) [$^3$H]RNA-DNA hybridization.** Relative rates of CAT mRNA synthesis were determined by hybridizing portions of the [$^3$H]RNA with an excess of filter-bound M13-CAT DNA. Reaction conditions, washing, and counting of filters were as described by Daniels and Bertrand (6). Levels of CAT mRNA were calculated by subtracting the radioactivity bound to blank filters from that bound to M13-CAT filters and expressing the result as a percentage of the input trichloroacetic acid-precipitable radioactivity.

**Determination of CAT mRNA half-life.** The chemical half-life of CAT mRNA was determined by monitoring the decay of hybridizable CAT mRNA following inhibition of transcription by rifampin. Plasmid-carrying cultures grown at different rates were labeled with [$^3$H]uridine as described above, and further incorporation of label into RNA was stopped by the addition of 200 μg of rifampin per ml and 0.8 μg of uridine per ml. Samples (10 ml) of the cultures were removed immediately before, and 30, 60, and 90 s after rifampin addition. Labeled RNA was extracted, and levels of CAT mRNA were determined by hybridization to M13-CAT filters as described above.

**Determination of plasmid copy number.** Plasmid copy numbers in cells growing at different rates were determined by the hybridization method described by Adams and Hatfield (1), as described by Daniels and Bertrand (6). Nick-translated pKK232-8 DNA was used as the hybridization probe. Numbers of plasmid copies are expressed as nanograms of plasmid DNA per microgram of total protein in the sonicated cell extract.

**S1 nuclease mapping of transcripts from *rrnB*-CAT fusions.** RNA was isolated from *E. coli* carrying pKK427B, which was grown in LB media, as described by Daniels and Bertrand (6). To generate a DNA probe, pKK211B was treated with *Hin*dIII and dephosphorylated with calf intestinal alkaline phosphatase (1 U/μg of DNA; Boehringer/Mannheim, Canada, Dorval, Quebec, Canada). The DNA was labelled with [γ-$^{32}$P]ATP and polynucleotide kinase as described previously (8). The kinase reaction was terminated with heat, and the DNA was treated with *Eco*RI to liberate the radioactive fragments that were isolated by electrophoresis through an 8% polyacrylamide gel followed by electroelution.

Hybridizations were carried out in the buffer described by Maniatis et al. (18), with 20 μg of RNA per reaction and a hybridization temperature (40°C) that has been shown to give maximum hybrid formation. After hybridization hybrids were treated with 75 U of S1 nuclease (Sigma Chemical Co., St. Louis, Mo.) in 0.3 ml of S1 nuclease buffer (18) for 1.5 h at 30°C and then precipitated. DNA sequencing was done by the chemical method (8, 18), and a fraction of the same DNA was used in the mapping experiment.

## RESULTS

**Cloning of rRNA promoters.** The vector pKK232-8 (2) carries a CAT gene that lacks a functional promoter but that retains the necessary translation signals. The presence of promoters on DNA fragments inserted upstream of the CAT gene can be detected by production of CAT and, hence, growth of recombinant cells in the presence of chloramphenicol. In Fig. 1A the various *B. subtilis rrnB* promoter fragments cloned into pKK232-8 are shown. The tandem $p_1$-$p_2$ promoter pair was represented on three clones. pKK427B included 220 bp DNA upstream of the $p_1$ transcription start site, while pKK285B retained only 83 bp of the upstream flanking sequences. The third clone, pKK211B, had 45 bp upstream of $p_1$ remaining and 77 bp downstream of the $p_2$ promoter remaining. In Fig. 1B the precise endpoints of all cloned promoters are illustrated. In addition, we separated and individually cloned the $p_1$ and $p_2$ promoters (pKK282B and pKK220B, respectively). Finally,

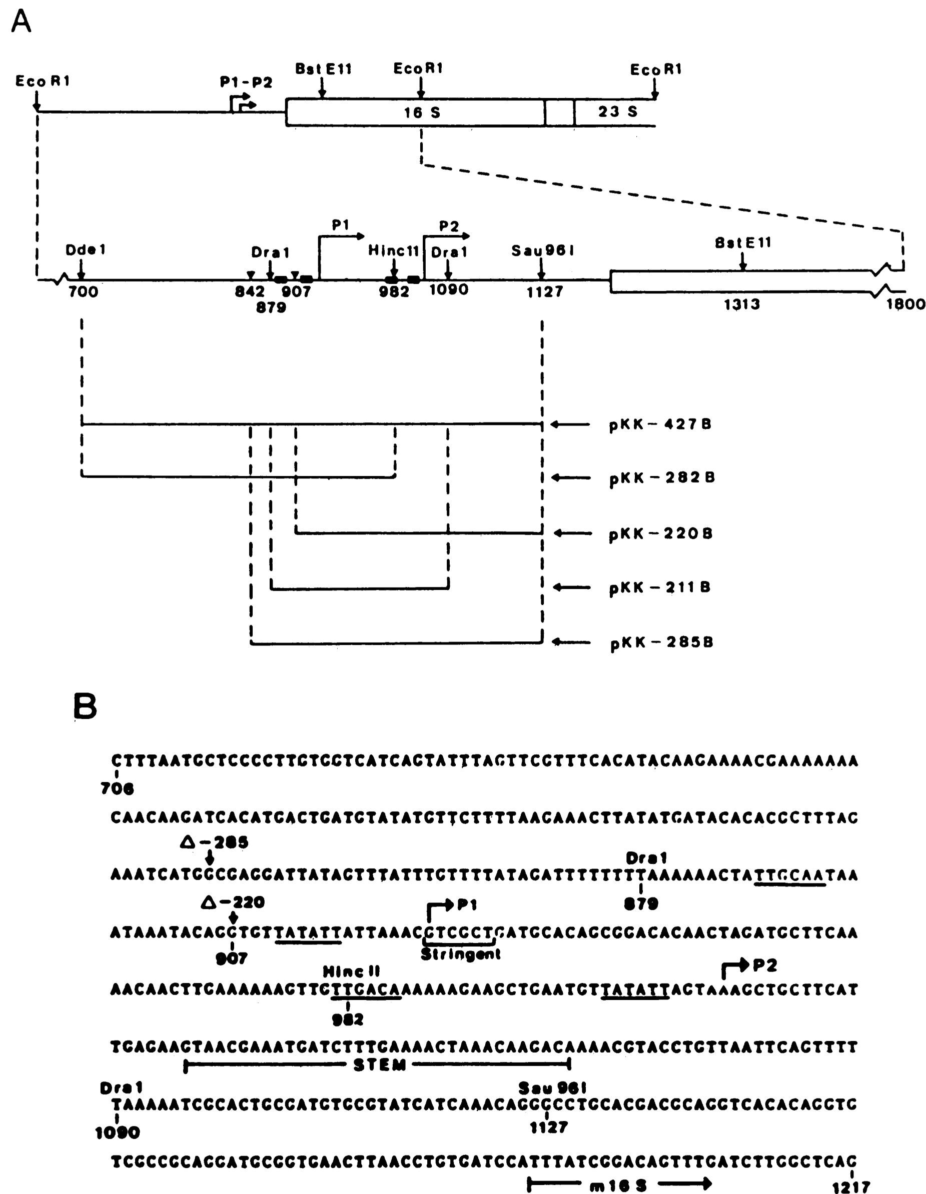


FIG. 1. (A) Schematic diagram of the origin of the *B. subtilis* rRNA promoter fragments. The top line represents the 2.9-kb *Eco*RI fragment from the *rrnB* operon cloned in pKM-1 to give pGS227 (28). The middle line shows the 1.8-kb *Eco*RI fragment subcloned in pKM-1 to give pHD1.8 (7). The −35 and −10 regions of the tandem promoters are indicated by small filled boxes. The start point and direction of transcription from $p_1$ and $p_2$ are indicated by arrows. The double lines represent the 16S RNA coding region. Numbering corresponds to that given by Stewart and Bott (28), in which 1 is the first base of the 5′ *Eco*RI site. The lower part of panel A represents the promoter fragments cloned into pKK232-8, as described in the text. (B) DNA sequence of the promoter region of the *B. subtilis rrnB* operon, as determined by Stewart and Bott (28). The −35 and −10 regions of $p_1$ and $p_2$ are underlined, and the start points of transcription indicated previously (28) are shown by arrows. The *Hin*cII site at position 982 was used to isolate the single $p_1$ promoter on pKK282B, and the endpoints of the pKK285B and pKK220B deletion fragments are indicated at positions 842 and 907, respectively. The sequence around $p_1$ marked "Stringent" has homology to the region that may be involved in stringent control (11, 12).

we cloned the analogous regions from the *E. coli rrnB* operon, including the tandem $p_1$-$p_2$ pair (pKK292Ec) and the separated $p_1$ and $p_2$ promoters (pKK351Ec and pKK128Ec, respectively). All promoters cloned into pKK232-8 were stable as long as cells were grown in the presence of chloramphenicol.

**Expression of promoters at different growth rates.** Host *E. coli* cells carrying the various promoter-CAT fusions were grown in different media to achieve a range of growth rates and were assayed for CAT activity. The results for the three clones carrying the *E. coli* rRNA promoters are shown in Fig. 2A and B. In agreement with others (10, 25) we found that only the upstream $p_1$ promoter (pKK351Ec; Fig. 2B) gave the steep positive slope of activity with increasing growth rate which is characteristic of growth rate regulation. Transcription from the $p_2$ promoter was lower overall and did not increase with increasing growth rates. A clone carrying the pBR322 tetracycline resistance gene promoter

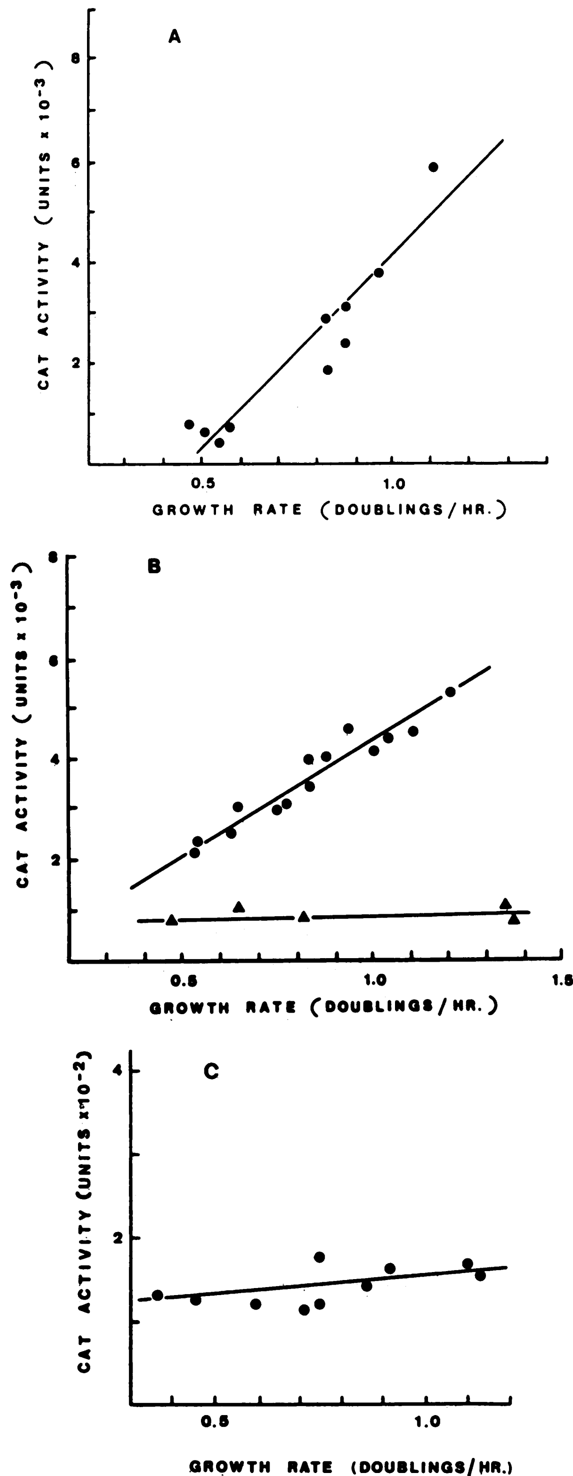


FIG. 2. Promoter activity as a function of growth rate. CAT specific activity was measured as a function of cellular growth rate, as described in the text. The slopes of lines were determined by linear regression. (A) *E. coli rrnB* tandem $p_1$-$p_2$ promoters on pKK292Ec. (B) *E. coli rrnB* single upstream ($p_1$) promoter on pKK351Ec (●); *E. coli rrnB* downstream ($p_2$) promoter on pKK128Ec (▲). (C) pBR322 tetracycline resistance promoter on pKKTet.

also failed to show a growth rate-dependent change in CAT activity, and the overall level of expression was similar to that of the $p_2$ rRNA promoter (Fig. 2C).

The results for the *B. subtilis* rRNA promoter-CAT fusions, expressed in the heterologous *E. coli* host grown at different rates, are shown in Fig. 3A to D. The tandem $p_1$-$p_2$ promoter pair (pKK427B; Fig. 3A) showed the steep positive slope that is indicative of a classical growth rate-dependent response. This same trend was seen for two other clones that differed in the amount of 5'- and 3'-flanking DNA remaining (pKK285B and pKK211B, respectively; Fig. 3B and C). Thus, for the tandem promoter, sequences beyond position −83 relative to the $p_1$ transcription initiation site and beyond position +77 relative to $p_2$ were not required to elicit the growth rate-dependent response. Interestingly, the level of activity from the *Bacillus* rRNA promoters was at least as high, if not greater than, that seen for the analogous *E. coli* promoters (compare pKK427B and pKK292Ec). Finally, Fig. 3D illustrates the response obtained for the separated promoters of the $p_1$-$p_2$ pair. Only the downstream $p_2$ promoter showed growth rate-dependent activity, and when compared with the $p_1$ promoter, $p_2$ was much more active at all growth rates.

The overall response of the *E. coli rrnB* promoters to growth rate (Fig. 2) was similar to the response seen with fusions that have been integrated into the chromosome (10) and to fusions that have been created with truncated genes (25, 26). Thus, the presence of the *rrnB*-CAT fusions on multicopy plasmids did not appear to substantially alter the cell regulatory mechanisms. The presence of the fusions used in this study appeared to impose a metabolic load because cells carrying plasmids grew more slowly than cells without plasmids (the maximum doubling times achieved by strains with CAT fusions was about 1.2 $h^{-1}$), although the slower growth may have been due to the presence of chloramphenicol. The constructs did not appear to differ significantly in their effect on overall growth rate.

**Measurement of CAT mRNA, mRNA half-life, and plasmid copy number.** To ensure that the growth rate response seen for rRNA promoters cloned into pKK232-8 accurately reflected the transcriptional activity of the promoters, the level of CAT mRNA at different growth rates was directly determined by hybridization of RNA labeled in a 1-min pulse to filter bound M13 DNA carrying a fragment complementary to the CAT mRNA. The level of CAT mRNA in cells carrying either pKK427B or pKK211B increased in a growth rate-dependent manner (Fig. 4). The ratio of mRNA to CAT protein was constant over the growth range tested, indicating that there were no differential translation effects with growth rate. However, the ratio of CAT activity to mRNA was greater for pKK211B than for pKK427B, suggesting some difference in translation efficiency between mRNA from these clones.

It has been shown that the half-lives of some mRNAs can vary greatly at different cellular growth rates (32). To eliminate the possibility that an increase in mRNA stability at high growth rates was responsible for the increase in the CAT message, the chemical half-life of CAT mRNA was measured at high and low growth rates. CAT mRNA transcribed from pKK427B had a half-life of approximately 43 s in slow-growing cells and 30 s in faster growing cells (Fig. 5). Thus, CAT mRNA was slightly more labile under faster growth conditions, suggesting that the data in Fig. 3A underestimate the growth rate-dependent response of the *Bacillus rrnB* promoters.

Finally, because pKK232-8 is a multicopy plasmid, we

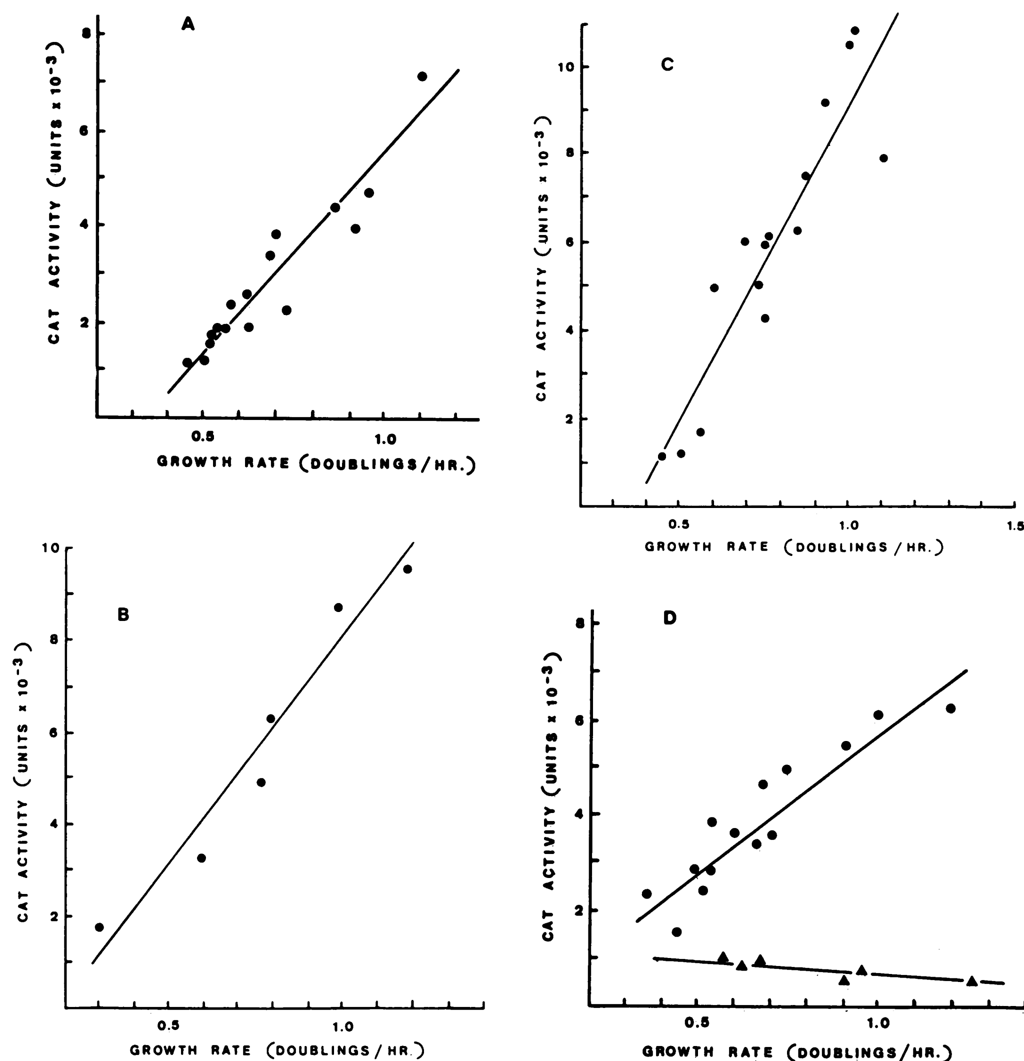


FIG. 3. Promoter activity as a function of growth rate. CAT activity was measured as described in the text. Slopes were calculated by linear regression. All promoters used here were derived from the *B. subtilis rrnB* operon. (A) pKK427B; $p_1$-$p_2$ tandem promoters. (B) pKK285B; $p_1$-$p_2$ tandem promoters with 5'-flanking deletion. (C) pKK211B; $p_1$-$p_2$ tandem promoters with 5'- and 3'-flanking deletion. (D) pKK220B (isolated $p_2$ promoter; ●); pKK282B (isolated $p_1$ promoter; ▲).

considered the possibility that extreme fluctuations in plasmid copy number with varying growth rate could mimic a growth rate-dependent response. Others have found that growth conditions and strengths of inserted promoters can significantly affect the copy number (1, 29). Therefore, an estimate of the amount of plasmid DNA per unit of total cellular protein was obtained for several clones. The results for pKK427B (Fig. 6) indicate that while there was a slight overall increase in the amount of plasmid DNA as the growth rate increased, the increase was not large enough to account for the increase seen in CAT mRNA or CAT-specific activity. Measurements for other clones gave similar results (data not shown).

**S1 nuclease map of the RNA produced from the *Bacillus rrnB* promoters.** While the acquisition of chloramphenicol resistance and production of CAT mRNA demonstrated that the fragments inserted into pKK232-8 contained functional promoters, S1 nuclease mapping was carried out to support the claim that both *Bacillus rrnB* promoters function in *E. coli*. In Fig. 7 are shown the results of an S1 nuclease mapping experiment, along with a DNA sequence of the *rrnB* promoter region. The RNA used was isolated from cells carrying pKK427B, while the DNA probe was the *Eco*RI to *Hin*dIII promoter-containing fragment from pKK211B. Two sizes of protected fragments were seen. By using the numbering shown in Fig. 1B, there was a major start at position A-1014. This is the same site observed in vivo for $p_2$ in *B. subtilis* by Stewart and Bott (28). Minor bands on either side of position 1014 could be seen on the autoradiograph but were not visible in Fig 1B. At $p_1$ a doublet was seen corresponding to positions A-922 and C-923. Such doublets may arise from the S1 nuclease treatment. These positions were slightly closer to the −10 region than the position determined by Stewart and Bott which was G-924; the cause of the variation is not known. The signal obtained was very weak, which is not surprising in light of the short chemical half-life of the mRNA (Fig. 5). Furthermore, in agreement with Fig. 3, it appears that significantly more RNA was transcribed from $p_2$ than from $p_1$ in vivo, although the levels were not quantitated.

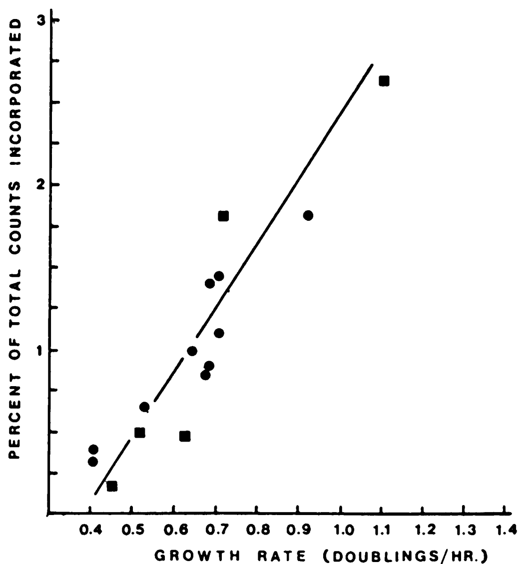


FIG. 4. Amount of CAT-specific mRNA versus growth rate. The amount of CAT mRNA was determined by hybridization to filter-bound M13-CAT DNA and is expressed as a percentage of the total input radioactivity that bound to the CAT DNA filter. Symbols: ●, values obtained for pKK427B; ■, values for pKK211B. The slope of the line was obtained by linear regression.

## DISCUSSION

We wish to point out that the analysis of promoter activity using a gene fusion is in some ways artificial. However, we have endeavored to control for any potential artifacts within this system that could mimic a growth rate-dependent response by directly measuring CAT mRNA, CAT mRNA half-life, and plasmid copy number at different growth rates. These measurements were all consistent with the conclusion

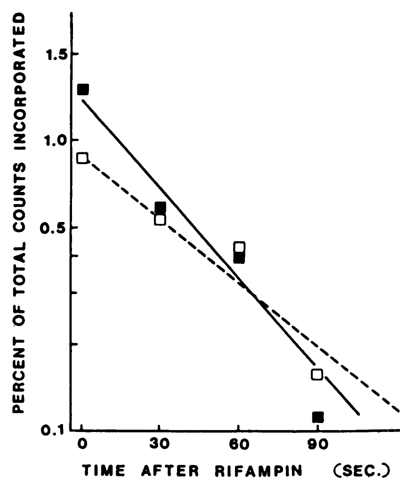


FIG. 5. Chemical half-life of CAT mRNA versus growth rate. RNA in cells carrying pKK427B was pulse-labeled and hybridized to filter-bound M13-CAT DNA. The percentage of total input radioactivity which bound to CAT DNA filters is shown for cells labeled at different times after inhibition of transcription with rifampin. Symbols: ■, cells grown at 0.97 doubling per h; □, cells growing at 0.55 doubling per h. The calculated mRNA half-lives are 30 and 43 s, respectively.

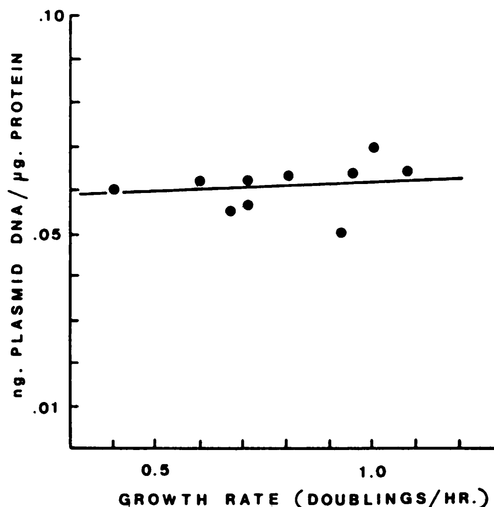


FIG. 6. Amount of pKK427B plasmid DNA versus growth rate. Sonicated cell extracts used for CAT assays were also assayed for the amount of plasmid DNA using a dot-blot hybridization procedure as described in Methods. Results are expressed as the amount of plasmid DNA per microgram of total protein in the cell extract.

that the gene fusion system was a valid means of assessing the growth rate-dependent regulation of cloned promoters.

When the *B. subtilis* rRNA ($p_1$-$p_2$)-CAT fusions were tested in *E. coli* HB101 as a function of increasing growth rate, a steep increase in CAT-specific activity was seen, which is indicative of classical growth rate-dependent expression. The response of the *Bacillus* rRNA promoters supports the suggestion that the overall control of rRNA synthesis in *B. subtilis* is similar to that in *E. coli* (13). Furthermore, the features that govern growth rate regulation, either in the DNA sequence or in ancillary factors, appear to be compatible in both bacteria. Thus, basic regulatory mechanisms governing the synthesis of key components of the cellular translational apparatus seem to be highly conserved from an evolutionary standpoint.

While the response of the tandem *B. subtilis* rRNA promoters to changing growth rate was the same in *E. coli* as the response of the native *E. coli* promoters, some important differences were obvious when the individual $p_1$ and $p_2$ promoter elements were examined. In contrast to the *E. coli* promoters, the *B. subtilis* upstream $p_1$ promoter was not growth rate regulated and was only weakly expressed (Fig. 3D). The *B. subtilis* downstream promoter was the more active and regulated promoter of this pair. The $p_2$ promoter fragment used here still retained the −10 region of the $p_1$ promoter; however, examination of the DNA sequence upstream of the 220-bp insert did not reveal any potential −35 sequence (data not shown). Therefore, the critical region for growth rate control of the *B. subtilis rrnB* promoters would appear to lie closer to the $p_2$ promoter than position 907 (Fig. 1B).

*B. subtilis* $p_1$ and $p_2$ promoters provide a means of testing aspects of the mechanisms that affect promoters subject to growth rate-dependent control in *E. coli*. For example, the AT-rich region immediately upstream of many rRNA and tRNA promoters, including the *Bacillus* $p_1$ promoter used here, is thought to play a role in modulating downstream transcription (30). In pKK211B, however, this region was largely deleted, with no change in overall transcriptional activity or in growth rate regulation of the tandem $p_1$-$p_2$

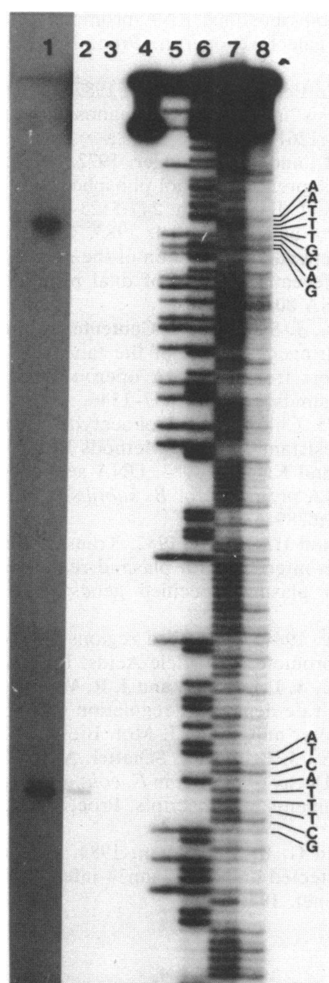


FIG. 7. S1 nuclease map of the CAT fusion mRNA. RNA isolated from cells carrying pKK427B was hybridized to the promoter containing the *Eco*RI to *Hin*dIII fragment from pKK211B which was labeled with polynucleotide kinase at the *Hin*dIII end. After hybridization and treatment with S1 nuclease, the protected DNA was collected and electrophoresed along with fractions of the same DNA subjected to chemical sequencing reactions. The DNAs were electrophoresed on an 8% 7 M urea–polyacrylamide gel. Lanes 1–4, S1 nuclease mapping reactions; lane 4, DNA incubated under hybridization conditions but not treated with S1 nuclease; lane 3, DNA incubated under hybridization conditions without RNA and treated with S1 nuclease; lane 2, DNA incubated under hybridization in the presence of RNA and treated with S1 nuclease; lane 1 shows a longer exposure of lane 2; lanes 5 to 8, DNA sequencing reactions of G-, G+A-, T+C-, and C-specific reactions, respectively. The protected fragments correspond to positions 922 and 923 for $p_1$ and position 1014 for $p_2$.

promoters. In fact, the pKK220B construction, which was still growth rate regulated, lacked this region entirely, although there was an AT bias in the region immediately preceding the $p_2$ site at position −35 (Fig. 1B). Gourse et al. (10) have recently reported that for the *E. coli rrnB* operon the sequences that endow growth rate-dependent control are between −20 and −51 of the $p_1$ promoter. The intervening sequences between the *B. subtilis* $p_1$ and $p_2$ promoters must similarly encode growth rate regulation of transcription from $p_2$. The exact nucleotides which are significant must be assessed by the analysis of further deletions.

A large body of evidence has shown that the synthesis of rRNA is regulated primarily at the initiation of synthesis (15, 16, 21). Two models of the mechanism of growth rate regulation of rRNA synthesis have received recent attention. In one model, the intracellular concentration of guanosine 5′-diphosphate, 3′-diphosphate (ppGpp), which varies with growth conditions, affects the distribution of RNA polymerase between forms with different affinities to rRNA promoters (17, 23). In the second model, free, nontranslating ribosomes regulate rRNA synthesis directly through feedback inhibition (10, 21). These models are compatible if a means to mediate between ppGpp and nontranslating ribosomes is invoked (16). Of significance to this study is that in both models the DNA sequences involved in the target for growth rate regulation are in, or close to, the rRNA promoters (9, 15, 31).

We have demonstrated that a *rrnB* promoter from *B. subtilis* is recognized as a target for growth rate regulation in *E. coli*. The sequences surrounding the seven *E. coli* rRNA $p_1$ promoters are not identical to each other but share considerable homology (for a review, see reference 16). Two of the *E. coli* $p_1$ *rrn* promoters that have been analyzed (*rrnA* and *rrnB*) (10, 20, 25) are identical in the −51 to −20 region, which has been shown to be important (10). The sequence of a third *rrn* $p_1$ promoter (*rrnE*) diverges somewhat, although it is still growth rate regulated (10). In contrast, apart from the −10 and −35 consensus regions, the *B. subtilis rrnB* promoter shows little sequence homology to any of the *E. coli* $p_1$ promoters. It is important to remember that the precise nucleotides that affect growth rate regulation have not been identified for any *rrn* promoter. However, the lack of sequence homology between the *B. subtilis rrnB* $p_2$ and *E. coli rrn* $p_1$ promoters suggests that the control points for regulation may reflect structural or topological characteristics of the rRNA promoters.

## ACKNOWLEDGMENTS

This study was supported by grant 67-6783 from the National Sciences and Engineering Research Council, Ottawa, Ontario, Canada, and grant 68-9558 from the Medical Research Council, Canada.

We thank K. Bott and J. Brosius for generously providing plasmids and P. Gontier and C. Hirsch for preparing the manuscript.

## LITERATURE CITED

1. **Adams, C., and G. Hatfield.** 1984. Effects of promoter strengths and growth conditions on copy number of transcription-fusion vectors. J. Biol. Chem. **259**:7399–7403.
2. **Brosius, J.** 1984. Plasmid vector for the selection of promoters. Gene **27**:151–160.
3. **Brosius, J.** 1984. Toxicity of an overproduced foreign gene product in *E. coli* and its use in plasmid vectors for the selection of transcription terminators. Gene **27**:161–172.
4. **Brosius, J., T. Dull, D. Sleeter, and H. Noller.** 1981. Gene organization and primary structure of a ribosomal RNA operon from *E. coli*. J. Mol. Biol. **148**:107–127.
5. **Close, T., and R. Rodriguez.** 1982. Construction and characterization of a chloramphenicol resistance gene cartridge: a new approach to the transcriptional mapping of extrachromosomal elements. Gene **20**:305–316.
6. **Daniels, D., and K. Bertrand.** 1985. Promoter mutations affecting divergent transcription in the Tn10 tetracycline resistance determinant. J. Mol. Biol. **184**:599–610.
7. **DeBoer, H. A., L. J. Comstock, and M. Vasser.** 1983. The *tac* promoter: a functional hybrid derived from the *trp* and *lac* promoters. Proc. Natl. Acad. Sci. USA **80**:21–25.
8. **Dobinson, K. D., and G. B. Spiegelman.** 1985. Nucleotide sequence and transcription of a bacteriophage φ29 early promoter. J. Biol. Chem. **260**:2950–5955.
9. **Duester, G., R. M. Elford, and W. M. Holmes.** 1982. Fusion of

the *E. coli* tRNA$^{Leu}$ promoter to the *gal*K gene: analysis of sequences necessary for growth rate dependent regulation. Cell **30**:855–864.

10. **Gourse, R., H. A. DeBoer, 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.
11. **Lamond, A. I., and A. A. Travers.** 1985. Genetically separable functional elements mediate the optimal expression and stringent regulation of a tRNA gene. Cell **40**:319–326.
12. **Lamond, A. I., and A. A. Travers.** 1985. Stringent control of bacterial transcription. Cell **41**:6–8.
13. **Leduc, E., M. Hoekstra, and G. B. Spiegelman.** 1982. Relationship between synthesis of ribosomes and RNA polymerase in *Bacillus subtilis*. Can. J. Microbiol. **28**:1280–1288.
14. **Li, S., 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.
15. **Lindahl, L., and J. Zengel.** 1982. Expression of ribosomal genes in bacteria. Adv. Genet. **21**:53–121.
16. **Lindahl, L., and J. M. Zengel.** 1986. Ribosomal genes in *Escherichia coli*. Annu. Rev. Genet. **20**:297–329.
17. **Little, R., and H. Bremer.** 1984. Transcription of ribosomal component genes and *lac* in a *relA*$^+$/*relA*$^-$ pair of *E. coli* strains. J. Bacteriol. **159**:863–869.
18. **Maniatis, T., G. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–52.
20. **Muira, A., J. Krueger, S. Itoh, H. de Boer, and M. Nomura.** 1981. Growth rate dependent regulation of ribosome synthesis in *E. coli*: expression of the *lac*Z and *gal*K genes fused to ribosomal promoters. Cell **25**:773–782.
21. **Nomura, M., R. Gourse, and G. Baughman.** 1984. Regulation of the synthesis of ribosomes and ribosomal components. Annu. Rev. Biochem. **53**:75–117.
22. **Ota, Y., A. Kikuchi, and M. Cashel.** 1979. Gene expression of an *Escherichia coli* ribosomal RNA promoter fused to structural genes of the galactose operon. Proc. Natl. Acad. Sci. USA **76**:5799–5803.
23. **Ryals, J., R. Little, and H. Bremer.** 1982. Control of rRNA and tRNA synthesis in *E. coli* by guanosine tetraphosphate. J. Bacteriol. **151**:1261–1268.
24. **Sanderham, H., and J. Strominger.** 1972. Purification and properties of $C_{55}$ isoprenoid alcohol phosphokinase from *Staphylococcus aureus*. J. Biol. Chem. **247**:5123–5131.
25. **Sarmientos, P., and M. Cashel.** 1983. Carbon starvation and growth rate dependent regulation of the *E. coli* ribosomal RNA promoter: differential control of dual promoters. Proc. Natl. Acad. Sci. USA **80**:7010–7013.
26. **Sarmientos, P., J. Sylvester, S. Contente, and M. Cashel.** 1983. Differential stringent control of the tandem *E. coli* ribosomal RNA promoters from the *rrn*A operon expressed *in vivo* on multicopy plasmids. Cell **32**:1337–1346.
27. **Shaw, W.** 1975. Chloramphenicol acetyltransferase from chloramphenicol-resistant bacteria. Methods Enzymol. **43**:737–755.
28. **Stewart, G., and K. Bott.** 1983. DNA sequence of the tandem ribosomal RNA promoter for *B. subtilis* operon *rrn*B. Nucleic Acids Res. **11**:6289–6300.
29. **Stueber, D., and H. Bujard.** 1982. Transcription from efficient promoters can interfere with plasmid replication and diminish expression of plasmid specified genes. EMBO J. **11**:1399–1404.
30. **Travers, A. A.** 1984. Conserved regions of coordinately regulated *E. coli* promoters. Nucleic Acids. Res. **12**:2605–2618.
31. **Travers, A. A., A. I. Lamond, and J. R. Weeks.** 1986. Alteration of the growth rate dependent regulation of *E. coli tyrT* expression by promoter mutations. J. Mol. Biol. **189**:251–255.
32. **von Gabain, A., J. Belasco, J. Schattel, A. C. Y. Chang, and S. Cohen.** 1983. Decay of mRNA in *E. coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80**:653–657.
33. **Webb, V., and G. B. Spiegelman.** 1984. Ribosomal RNA synthesis in uninfected and SPO1 am34 infected *Bacillus subtilis*. Mol. Gen. Genet. **194**:98–104.