JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5759–5764
0021-9193/88/125759-06$02.00/0

Vol. 170, No. 12

Role of the 5′ Upstream Sequence and Tandem Promoters in Regulation of the *rpsU-dnaG-rpoD* Macromolecular Synthesis Operon

MIRJANA NESIN,† JAMES R. LUPSKI,‡ AND G. NIGEL GODSON*

Biochemistry Department, New York University Medical Center, 550 First Avenue, New York, New York 10016

Received 3 May 1988/Accepted 19 September 1988

***Bal*31 exonuclease deletion analysis and transposon Tn*5* mutagenesis of the 5′ regulatory region of the *rpsU-dnaG-rpoD* macromolecular synthesis operon fused to the chloramphenicol acetyltransferase gene (pGLR301) demonstrated that sequences 5′ to the operon promoters were not involved in operon transcriptional regulation and that the three tandem promoters P_1, P_2, and P_3 were functionally independent. P_2 was the strongest promoter, and P_3 was the weakest. P_1, P_2, and P_3 acting in combination appeared to be stronger than the individual promoters.**

The *rpsU-dnaG-rpoD* operon is one of the most complex operons described for *Escherichia coli*. It contains three external promoters (P_1, P_2, and P_3), three internal promoters (P_a, P_b, and the heat shock promoter P_{hs}, all located in the coding region of *dnaG*), an internal terminator (T_1), a potential LexA-binding site on P_3, and a potential antitermination *nut* equivalent site (7, 20, 22, 24). The operon is clearly under complex regulation, and its structure appears to allow discoordinate gene expression, producing in the steady state S21 protein, primase, and sigma protein in a ratio of 50,000:50:5,000 copies per cell. The operon also appears to be able to modulate the relative amount of each gene product synthesized during stress. In the case of heat shock (22, 36), stringent response (14), and lambda phage infection (28, 29), relative increases in sigma have been observed. Also, NusA has been shown to affect *rpoD* gene expression (32). The presence of the RNase III cleavage site following the *dnaG* coding region (7) and the *nut* equivalent site suggest that they can also be used to modulate the relative gene expression within the operon.

There are several other operons in *E. coli* that are similar to the *rpsU-dnaG-rpoD* operon in that they have ribosomal genes adjacent to genes coding for transcription and translational proteins, with the gene products being synthesized in discoordinate amounts. These are the *rpsL10-rpsL7-12-rpoB-rpoC* operon (31, 39), the *rpsL13-rpsL11-rpsL4-rpoA-rpsL17* operon (17), the *rpsL12-rpsL7*-EFG-EF-Tu operon (9), the *rpsO pnp* operon (34), the *rpsB tsf* operon (1), and the *rpsL16-trmD-rpsL19* operon (8). Most of these operons have multiple tandem promoters, and several have internal terminators or attenuators. However, the *rpsU-dnaG-rpoD* macromolecular synthesis operon is the only one identified to date containing genes whose products are involved in transcription, translation, and DNA replication.

The question arises whether these complex operons are regulated by upstream sequences other than promoters and whether the tandem promoters can operate independently and represent a flexible system of changing the rate of initiation of operon transcripts. This paper examines the role of the 5′ upstream sequence of the *rpsU-dnaG-rpoD* operon by using *Bal*31 deletion and Tn*5* insertional mutagenesis and examines the ability of the three operon promoters to act independently.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. *Escherichia coli* K-12 HB101 (F^- *hsdS20* r_B^- m_B^- *recA13 ara-14 proA2 lacY1 galK2 rpsL20* Sm^r *xyl-5 mtl-1 supE44* λ^-) (3) was used for all the experiments to assay plasmid-encoded chloramphenicol acetyltransferase (CAT) and β-lactamase activity. Phage lambda 467 (λ *b221 rex*::Tn*5* *c*I857 *O*am29 *P*am80) (10) propagated in LE392 (F^- *hsdR514* r_k^- m_k^- *supE44 supF58 lacY galK2 metB1 trpR55* λ^-) (10) was used as a source of transposon Tn*5*. LB broth (25) and minimal 2xYT medium (25) were used to grow *E. coli* cells.

Plasmids and constructions. The recombinant plasmids pRLM47 (38), pGLR301, pGLR306 (22), and pBS105 (23) were used as sources of the operon regulatory regions. Plasmid pKK232-8, the CAT promoter probe plasmid (5, 6), was used for gene fusion experiments. pGLN312, -314, and -316 were derived as single-restricted enzyme fragment deletions from pGLR301. pGLN312 has a 539-bp *Bgl*II fragment deleted; pGLN314 has a 1,080-bp *Cla*I DNA fragment deleted; and pGLN316 has a 1,788-bp *Eco*RI DNA fragment deleted. The structure of the constructs was checked by using appropriate restriction enzyme cleavages. pGLN313 and pGLN306B were made by inserting DNA fragments derived from pGLR301 into *Sma*I-cut pKK232-8. pGLN313 contains a *Pst*I-*Hin*dIII fragment, and pGLN306B contains a *Cla*I-*Hin*dIII fragment (see Fig. 1).

Three independent sets of deletion clones generated by *Bal*31 exonuclease were made from pGLR301, pGLN312, and pGLN314. pGLR301 was cleaved with *Bam*HI, pGLN312 was cleaved with *Bgl*II, and pGLN314 was cleaved with *Cla*I prior to *Bal*31 treatment. All of these enzymes cleaved the DNA only once. The conditions of *Bal*31 cleavage are those of Legerski et al. (19).

Transposon mutagenesis. The transposon Tn*5* was randomly inserted into pGLR301 by using the methods described by de Bruijn and Lupski (10). Approximately 500 individual Ap^r Km^r Cm^r (50 μg/ml, 50 μg/ml, and 5 μg/ml,

* Corresponding author.
† Present address: Department of Pediatrics, Lenox Hill Hospital, New York, NY 10021.
‡ Present address: Department of Pediatrics and Institute for Molecular Genetics, Baylor College of Medicine, Texas Medical Center, Houston, TX 77030.

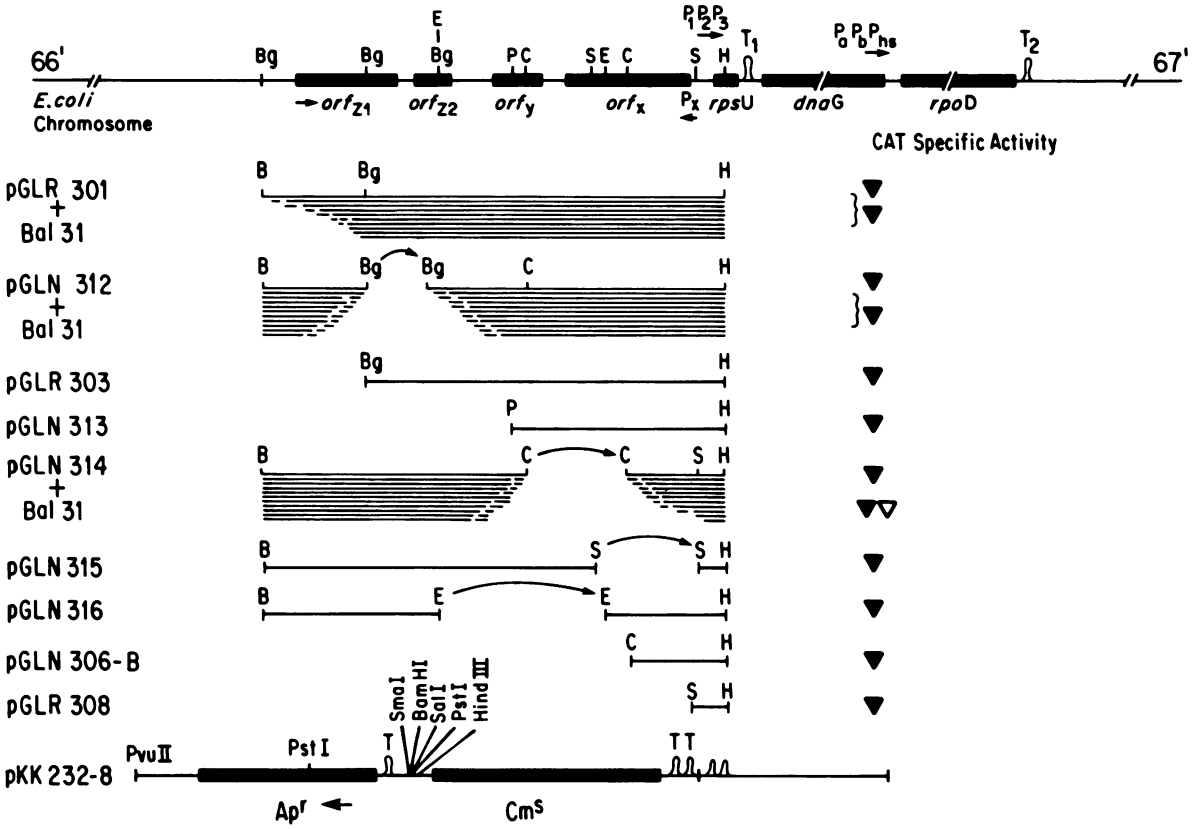

FIG. 1. Construction of the serial deletion clones of the 5-kb sequence from the *E. coli* chromosome preceding the *rpsU-dnaG-rpoD* operon and their effect on CAT expression in plasmid constructs. The genetic map of the region of the *E. coli* chromosome containing the *rpsU-dnaG-rpoD* operon and open reading frame sequences orf_x, orf_y orf_{z1}, and orf_{z2} is shown above. Restriction sites used in the cloning are marked as follows: B, *Bam*HI; Bg, *Bgl*II (1.272 kb and 1.811 kb from the *Bam*HI site); E, *Eco*RV (1.917 kb and 3,705 kb from the *Bam*HI site); P, *Pst*I (2.70 kb from the *Bam*HI site); C, *Cla*I (2.85 kb and 3.93 kb from the *Bam*HI site); S, *Sac*II (3.54 kb and 4.07 kb from the *Bam*HI site); and H, *Hin*dIII (5 kb from the *Bam*HI site). DNA fragments shown on the lower part of the figure are cloned into the polylinker region of pKK232-8, a CAT promoter probe vector whose structure is shown elsewhere (5, 6). Below pGLR301, pGLN312, and pGLN314 are drawn the *Bal*31 deletion clones that were generated from them. Plasmids pGLR301, pGLR303, and pGLR308 are described in Lupski et al. (22). The CAT activity of the constructs was assayed as outlined in the Materials and Methods section. All of the constructs gave a specific activity almost identical to that of pGLR301 (1.7 to 2.0 U/mg of protein) except for the *Bal*31 deletion clones of pGLN314 in which the deletion passed the *Sac*II cleavage site (<0.10 U/mg of protein). The high activities are marked with a solid arrow, and the lower activity with an open arrow. For interpretation of the results, see the text.

respectively) transformants were isolated and shown to contain pGLR301::Tn*5* plasmid DNA. The position of the individual Tn*5* insertions within the independent pGLR301 Tn*5* clones was determined by restriction endonuclease analysis.

CAT assay. A 6-ml amount of freshly grown HB101 cells containing the Cm^r gene fusion plasmids were grown in LB medium supplemented with ampicillin (50 μg/ml) to an A_{600} of 0.6. The cells were harvested, lysed, and assayed for CAT either spectrophotometrically as described in detail in Lupski et al. (22) or with [^{14}C]chloramphenicol acetylation as described before (13).

RESULTS

Deletion analysis of upstream regions of the *rpsU-dnaG-rpoD* operon. Wold and McMacken (38) and Lupski et al. (22) independently postulated that sequences 5′ upstream from the *rpsU-dnaG-rpoD* operon are involved in the control of its expression. In an attempt to localize and further characterize these regulatory sequences, a series of deletion clones were constructed in pGRL301 (Fig. 1). Plasmid pGLR301 (22) contains a 5-kb *Bam*HI-*Hin*dIII fragment 5′ upstream from the operon coding sequences fused to the Cm^r gene in the promoter probe plasmid pKK232-8 (5). This construction was digested with *Bgl*II, *Cla*I, *Pst*I, *Eco*RV, *Sac*II, and *Bam*HI and *Cla*I and religated by using DNA concentrations that favor intramolecular closure (11). Deletion clones pGLN312-316 and pGLN306B were obtained from this procedure and shown by restriction enzyme digestion to have the expected structure (Fig. 1). pGLN312 has a 5,396-bp *Bgl*II DNA fragment missing, deleting part of orf_{z1} and orf_{z2} (31): pGLN314 has a 1,080-bp *Cla*I DNA fragment missing, deleting orf_y and part of orf_x; pGLN315 has a 530-bp *Sac*II DNA fragment missing, deleting most of orf_x; pGLN316 has a 1,788-bp *Eco*RI fragment missing, deleting part of orf_{z2}, all of orf_y, and part of orf_x. pGLN313 and pGLN306B were constructed directly by using the appropriate DNA fragments (Fig. 1). pGLR303 and pGLR308 have been described previously (22).

Three independent groups of deletion clones were also obtained by cleaving pGLR301, pGLN312, and pGLN314 to

linear molecules with *Bam*HI, *Bgl*II, and *Cla*I, respectively, and digesting with *Bal*31 (Fig. 1).

To examine the effect of these deletions on transcription from the operon promoters, CAT specific activities were determined for all clones. The results are shown in Fig. 1.

It was found that the CAT activity of the restriction enzyme fragment deletion clones (pGLN15, pGLN16, pGLN306B, and pGLR308) were all high and similar to that of pGLR301. It was also found that *Bal*31 deletion of the upstream region in pGLR301 and pGLN312 also did not affect CAT activity. However, in pGLN314 *Bal*31 deletion clones, the CAT activity dropped at least 10-fold as soon as the deletion crossed the *Sac*II site (4,072 bp for the *Bam*HI upstream site).

As the *Sac*II site is close to the operon promoter, the data suggest that sequences upstream of the promoter do not affect its expression.

Insertion mutations in upstream regions. To independently determine the sequences upstream from the *rpsU-dnaG-rpoD* operon that affect its expression, Tn*5* insertional mutagenesis was performed. The use of a second method of selectively inactivating upstream sequences is important, because both methods generate novel joints which may influence transcription. The *E. coli* HB101 cells harboring pGLR301 were infected with a λ::Tn*5* transducing phage. Plasmid DNA with potential Tn*5* insertions was isolated from all Km^r colonies and used to transform competent *E. coli* HB101 cells. Transformants were screened on plates containing ampicillin, kanamycin, and chloramphenicol for the insertional mutations in the sequences upstream from the operon. Plasmid DNA from Ap^r Km^r Cm^r colonies was digested with *Bam*HI plus *Hin*dIII restriction enzymes to determine whether the insertion was within the desired 5-kb *Bam*HI-*Hin*dIII fragment of pGLR301; if so, the unique *Hin*dIII site located in inverted repeats of Tn*5* (18) was used to measure its approximate location to ±50 bp. The map position of approximately 100 independent, random insertional mutations is shown in Fig. 2. To determine whether any of these Tn*5* mutants influenced transcription initiation from the operon promoters, their CAT specific activities were measured in extracts of cells harboring these Tn*5*-mutated plasmids. As shown in Fig. 2, the only mutations causing a significant (10-fold) drop in CAT specific activity were located close to the operon *Hin*dIII site.

Exact locations of insertions which affect operon expression. The Tn*5* and *Bal*31 mutations that caused a significant drop in CAT activity all mapped within a 250-bp *Cla*I-*Hin*dIII DNA fragment. This contains part of the *rpsU* gene, all of the operon promoters, and part of orf_x. The exact location of most of these insertions was determined by sequencing an *Hpa*I-*Hin*dIII fragment excised from the Tn*5* mutant plasmids. This fragment encompasses the last 185 bp of the Tn*5* inverted terminal repeat (2) plus part of the operon up to the *Hin*dIII site. The sequence was determined after subcloning into *Acc*I-*Hin*dIII-cleaved M13mp9. The first 185 bases were therefore Tn*5*, and the junction with the *E. coli* DNA defined the point of transposon insertion.

Surprisingly, many of the insertions were within promoters. The exact sites of insertion within P_1, P_2, P_3, and P_x plus those in other parts of the *Cla*I-*Hin*dIII DNA fragment are shown in Fig. 3, and the relative CAT activity of the sequenced mutants is given in Table 1.

When the insertion mutation occurred within promoter P_1, P_2, or P_3, expression of the *cat* gene fusions decreased substantially. Comparison of mutations in all three promoters demonstrated that all were functional but had different

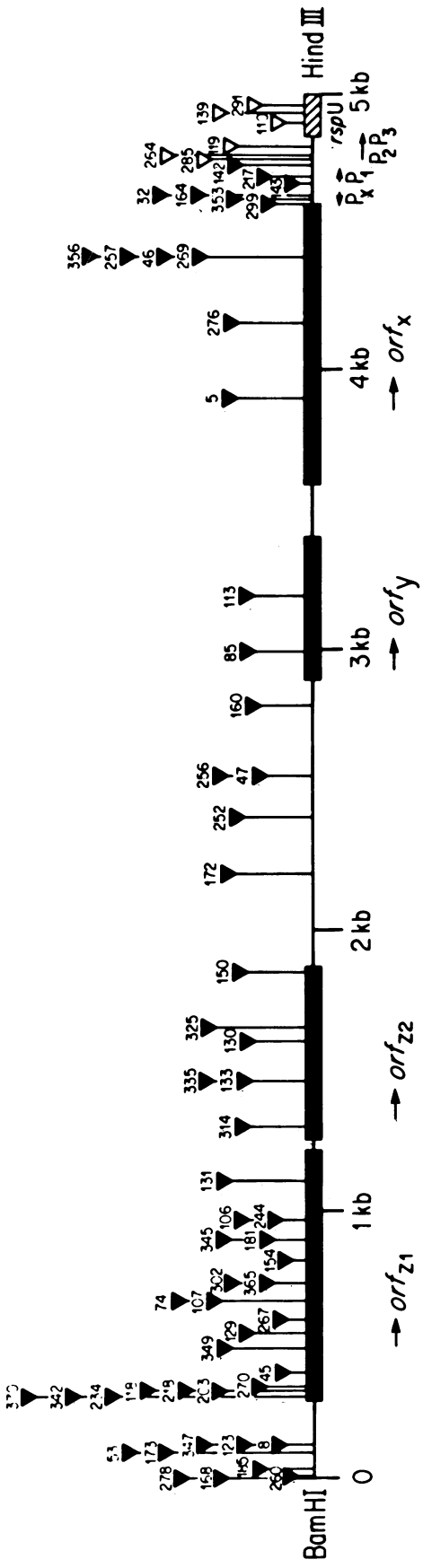

FIG. 2. Position of 62 independent Tn*5* insertions in the *Bam*HI-*Hin*dIII DNA fragment, 5′ to the *rpsU-dnaG-rpoD* operon, cloned in pKK232-8, and their effect on CAT activity. Plasmid pGLR301 (22) was subjected to Tn*5* mutagenesis (see Materials and Methods), and the positions of Tn*5* insertions within the individual pGLR301::Tn*5* clones were measured by restriction endonuclease cleavage (to within ±50 bp). The triangles indicate the sites of Tn*5* insertion and a relative value for CAT specific activity. Solid triangles (as in Fig. 1 and 3) represent approximately 10-times-higher values than open triangles. Promoters P_1, P_2, P_3, and P_x, orf_x (22), orf_y, orf_{Z1}, and orf_{Z2} (30) and the direction of transcription are indicated. Note the only Tn*5* insertions which affect transcription from the promoters are located within 250 bp of the *Hin*dIII site.

TABLE 1. CAT activity

Plasmid or Tn*5* insertion	Mutation site	Promoter(s) operating	CAT sp act (A_{412}/0.036/mg of protein)
pGLR301	None	P_1, P_2, P_3	1.77
pGLN306B	None	P_1, P_2, P_3	1.53
Tn*5*::301			
-164	P_x −10	(P_1), P_2, P_3	0.75
-217	P_1 −10	P_2, P_3	0.60
-142	Between P_1 + P_2 P_3	P_2, P_3	0.90
-264	P_2 −35	P_3	0.08
-139	*rpsU*		0.007
-291	*rpsU*		0.03
-110	*rpsU*		0.03
pKK232-8	None		0.00

strengths. The fact that the mutation in P_x promoter caused decreased CAT activity may indicate some influence of *orf*$_x$ on *rpsU-dnaG-rpoD* operon transcription or may be due to proximity to P_1.

The low level of CAT activity of mutants with an insertion within the coding region of *rpsU* may be explained by the low-level transcription initiation occurring from transposon Tn*5* itself. Tn*5* appears to have an outward-directed promoter activity (21).

To test the accuracy and reproducibility of the results, the CAT activity was measured by both the spectrophotometric and [^{14}C]chloramphenicol chromatographic assays. Both assays were sensitive enough to detect small differences in promoter strength, and both gave comparable results.

DISCUSSION

The presence of multiple tandem promoters in the *rpsU-dnaG-rpoD* operon raises the possibility that all three promoters can act independently and be independently regulated. The presence of the LexA-binding site on P_3 supports this idea. The published data from this laboratory (22) have shown that P_1 alone and P_2 plus P_3 when tested in the promoter probe plasmid pKK232-8 (pGLR305 and pGLR308) can function independently. No data for P_3 alone could be generated from these constructs, however. Experiments described in this paper with Tn*5* insertional mutagenesis demonstrate that all three promoters are functional in vivo and that their contribution to overall transcriptional initiation is not the same. It was found that inactivation of P_1 by Tn*5* insertion (Tn*5*::301-217 and 142) reduces expression of the *cat* reporter gene to approximately half of its activity when all three promoters are present (Table 1). Insertion of Tn*5* into P_2 (Tn*5*::301-264), which removes the influence of both P_1 and P_2, showed that P_3 alone is active in vivo. The strength of P_3 can be deduced by comparing the CAT activity of mutant Tn*5*::301-264 with the CAT activities of mutants Tn*5*::301-139, -291, and -110, in which Tn*5* insertions are in the *rpsU* gene. The hierarchy of activity appears

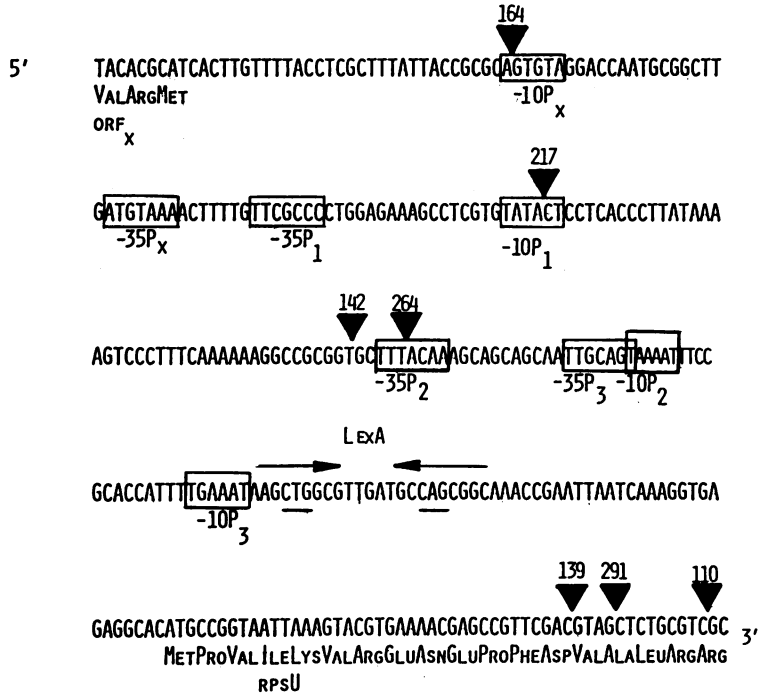

FIG. 3. Exact location of Tn*5* insertions into the promoter regions that affect CAT activity. The nucleotide sequence of the Tn*5* insertion sites within the *Bam*HI-*Hin*dIII fragment of pGLR301 was determined by cloning the *Hpa*II-*Hin*dIII fragments from pGLR301::Tn*5* mutants of interest into M13mp9 (*Sma*I-*Hin*dIII-cleaved) and using the dideoxy chain termination method (35). *Cla*I-*Hin*dIII fragments from pGLR306 and pBS105 were cloned into *Sma*I-cleaved M13mp9 to determine their DNA sequences. Plasmid DNA was also directly sequenced by using the method of Messing and Vieira (27). The intergenic region is demarcated by the start points of *rpsU* and *orf*$_x$ represented by their amino acid sequence transcribed on opposite DNA strands. For reasons of clarity, only the sequence of the transcribed DNA strand for the *rpsU-dnaG-rpoD* operon is presented; *orf*$_x$ is encoded by a complementary DNA strand. The three operon promoters P_1, P_2, and P_3, and P_x for *orf*$_x$, were determined by either S1 mapping (7) or gene fusion experiments (22, 24) and their −10 and −35 sequences are boxed. The putative LexA-binding site is shown (22). The sites of Tn*5* insertional mutations are marked by solid triangles. The CAT values for the clones depicted on this figure are given in Table 1.

to be P_1, P_2, P_3 (1.5 to 1.7) > P_2, P_3 (0.6 to 0.9) > P_3 (0.08) > no promoter (0.007 to 0.03) (Table 1).

The degree of similarity of the nucleotide sequence of P_1, P_2, and P_3 with the consensus sequence that Hawley and McClure (16) and McClure (26) deduced from a comparison of 168 natural and mutated *E. coli* promoters fits well with the observed CAT expression data and predicts that all three promoters should be functional in vivo and that P_2 will be the strongest.

The S1 mapping data of Burton et al. (7) also support the hypothesis that P_1, P_2, and P_3 are independent. They found that 80% of the operon transcription originates from P_2 and 20% from P_1; none was detected from P_3. However, if the promoter can be independently modulated, then it might be expected that the 5′ origin of the transcripts would vary with the physiological condition of the cell (growth rate, age, etc.) and that under the conditions of these experiments, P_3 transcripts were not made.

If the tandem promoters can be independently regulated, the question arises as to what regulates them. Experiments of Wold and McMacken (38) suggested that sequences 5′ upstream of the operon enhance its expression and stability. They found that plasmid clones containing only the operon were unstable and appeared to kill the cells by unregulated overexpression of *dnaG* primase. However, by adding 5 kb of upstream sequences, the plasmids were stabilized. Similar results were found in this laboratory (22, 23) when the operon promoters and upstream regions were joined to reporter genes or cloned with *dnaG* intact. However, the results of *Bal*31 deletion analysis and Tn*5* insertional mutagenesis experiments (Fig. 1 and 2) suggest that transcription from the external promoters (P_1, P_2, and P_3) is not enhanced by 5′ sequences in *cis*. Also, the experiments were performed under physiological conditions, and it is known that gene expression of the activators is not always present or active in exponentially growing cells but is produced in response to stress (13, 14, 37).

ACKNOWLEDGMENTS

We thank Steven J. Projan for suggestions and discussions.

This work was funded by Public Health Service grant GM-38292 to G.N.G.

LITERATURE CITED

1. **An, G., D. S. Bendiak, L. A. Mamelar, and J. D. Friesen.** 1981. Organization and nucleotide sequence of a new ribosomal operon in *Escherichia coli* containing the genes for ribosomal protein S2 and elongation factor Ts. Nucleic Acids Res. **9**:4163–4172.
2. **Auerswald, A. E., G. Ludwig, and H. Schaller.** 1981. Structural analysis of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45**:107–113.
3. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–471.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Brosius, J.** 1984. Plasmid vectors for the selection of promoters. Gene **27**:151–160.
6. **Brosius, J., and J. R. Lupski.** 1987. Plasmids for the selection and analysis of prokaryotic promoter. Methods Enzymol. **153**:54–68.
7. **Burton, Z. F., C. A. Gross, K. K. Watanabe, and R. R. Burgess.** 1983. The operon that encodes the sigma subunit of RNA polymerase also encodes ribosomal protein S21 and DNA primase in *E. coli* K-12. Cell **32**:335–349.
8. **Bystrom, A. S., J. K. Hjalmarsson, P. M. Wikstrom, and G. R. Bjork.** 1983. The nucleotide sequence of an *Escherichia coli* operon containing genes for tRNA in G methyltransferase, the ribosomal proteins S16 and L19 and a 22k polypeptide. EMBO J. **2**:899–905.
9. **Dean, D., J. L. Yates, and M. Nomura.** 1981. Identification of ribosomal protein S7 as a repressor of translation within the *stR* operon of *E. coli* Cell **24**:413–419.
10. **de Bruijn, F. J., and J. R. Lupski.** 1984. The use of transposon Tn*5* mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids—a review. Gene **27**:131–149.
11. **Dugaicyck, A., H. W. Boyer, and H. M. Goodman.** 1975. Ligation of *EcoRI* endonuclease generated DNA fragments into linear and circular structures. J. Mol. Biol. **96**: 171–184.
12. **Godson, G. N.** 1983. Sequencing DNA by the Sanger chain termination method, p. 69–111. *In* S. M. Weissman (ed.), Methods of DNA and RNA sequencing. Praeger Publishers, New York.
13. **Gorman, C. M., F. L. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1047.
14. **Gottesman, S.** 1984. Bacterial regulation: global regulatory networks. Annu. Rev. Genet. **18**:415–441.
15. **Grossman, A. D., W. E. Taylor, Z. F. Burton, R. R. Burgess, and C. A. Gross.** 1985. Stringent response in *Escherichia coli* induces expression of heat shock proteins. J. Mol. Biol. **186**:357–365.
16. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *E. coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
17. **Jaskunas, S. R., R. R. Burgess, and M. Nomura.** 1975. Identification of a gene for the α subunit of RNA polymerase at the *str-spc* region of the *E. coli* chromosome. Proc. Natl. Acad. Sci. USA **72**:5036–5040.
18. **Jorgensen, R. A., S. J. Rothstein, and W. S. Reznikoff.** 1979. A restriction enzyme cleavage map of Tn*5* and location of a region encoding neomycin resistance. Mol. Gen. Genet. **177**:65–73.
19. **Legerski, R. J., J. L. Hodnett, and H. B. Gray, Jr.** 1978. Extracellular nucleases of pseudomonas *Bal*31. III. Use of the double-strand deoxyriboexonuclease activity as the basis of a convenient method for the mapping of fragments of DNA produced by cleavage with restriction enzymes. Nucleic Acids Res. **5**:1445.
20. **Lupski, J. R., and G. N. Godson.** 1984. The *rpsU-dnaG-rpoD* macromolecular synthesis operon of *E. coli*. Cell **39**:251–252.
21. **Lupski, J. R., S. J. Projan, L. S. Ozaki, and G. N. Godson.** 1986. A pBR322 plasmid copy number mutant due to a Tn*5* position effect. Proc. Natl. Acad. Sci. USA **83**:7381–7385.
22. **Lupski, J. R., A. Ruiz, and G. N. Godson.** 1984. Promotion, termination, and anti-termination in the *rpsU-dnaG-rpoD* macromolecular synthesis operon. Mol. Gen. Genet. **195**:391–401.
23. **Lupski, J. R., B. L. Smiley, F. R. Blattner, and G. N. Godson.** 1982. Cloning and characterization of the *Escherichia coli* chromosomal region surrounding the *dnaG* gene, with a correlated physical and genetic map of *dnaG* generated via transposon Tn*5* mutagenesis. Mol. Gen. Genet. **185**:120–128.
24. **Lupski, J. R., B. L. Smiley, and G. N. Godson.** 1983. Regulation of the *rpsU-dnaG-rpoD* macromolecular synthesis operon and the initiation of DNA replication in *Escherichia coli* K-12. Mol. Gen. Genet. **189**:48–57.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **McClure, W. R.** 1985. Mechanism and control of transcription initiation in prokaryotes. Annu. Rev. Biochem. **54**:171–204.
27. **Messing, J., and J. Vieira.** 1982. The pUC plasmids, an M13 mp7-derived system for mutagenesis and sequencing with synthetic antisense primers. Gene **19**:259–268.
28. **Nakamura, Y., and T. Yura.** 1976. Induction of sigma factor synthesis in *Escherichia coli* by the *N* gene product of bacteriophage lambda. Proc. Natl. Acad. Sci. USA **73**:4405–4409.
29. **Nakamura, Y.** 1984. Amber *dnaG* mutation exerting a polar effect on the synthesis of RNA polymerase sigma factor in *E.*

coli. Mol. Gen. Genet. **196**:179–182.
30. **Nesin, M., J. R. Lupski, P. Svec, and G. N. Godson.** 1987. Possible new genes as revealed by molecular analysis of 5.0 kb *E. coli* chromosomal region 5′ to the macromolecular synthesis operon. Gene **55**:149–161.
31. **Nomura, M., R. Gourse, and G. Baughman.** 1984. Regulation of the synthesis of ribosomes and ribosomal components. Annu. Rev. Biochem. **53**:75–118.
32. **Peacock, S., J. R. Lupski, G. N. Godson, and H. Weissbach.** 1985. In vitro stimulation of *E. coli* RNA polymerase sigma subunit synthesis by NusA protein. Gene **33**:227–234.
33. **Raiband, O., and M. Schwartz.** 1984. Positive control of transcription initiation in bacteria. Annu. Rev. Genet. **18**:173–206.
34. **Regnier, P., and C. Portier.** 1986. Initiation, attentuation and RNase III processing of transcripts from the *E. coli* operon encoding ribosomal protein S15 and polynucleotide phosphorylase. J. Mol. Biol. **187**:23–32.
35. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
36. **Taylor, W. E., B. D. Straus, A. D. Grossman, Z. F. Burton, C. A. Gross, and R. R. Burgess.** 1984. Transcription from a heat-inducible promoter causes heat shock regulation of the sigma subunit of *E. coli* RNA polymerase. Cell **38**:371–381.
37. **von Hippel, P. H., D. G. Beor, W. D. Morgan, and J. A. McSwiggen.** 1984. Protein nucleic acid interactions in transcription. Annu. Rev. Biochem. **53**:389–446.
38. **Wold, M. S., and R. McMacken.** 1982. Regulation of expression of the *Escherichia coli dnaG* gene and amplification of the *dnaG* primase. Proc. Natl. Acad. Sci. USA **79**:4907–4911.
39. **Yamamoto, M., and M. Nomura.** 1978. Cotranscription of genes for RNA polymerase subunits n and n′ with genes for ribosomal proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **75**:3891–3895.