Stringent and growth-rate-dependent control of the gua operon of Escherichia coli K-12

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The promoter of the gua operon has been located by transcript mapping using primer extension with reverse transcriptase. The surrounding nucleotide sequence has features characteristic of promoters under stringent and growth-rate-dependent regulation, namely a GC-rich discriminator next to the –10 hexamer, an upstream AT-rich sequence (the UP element) and potential FIS-binding sites. Transcriptional activity of the gua promoter was examined using transcriptional fusions to lacZ placed at a single chromosomal location. Expression from gua was reduced under stringent conditions in vivo, and varied with growth rate. Growth-rate control was independent of guanine-mediated repression. A fusion in which the CC-rich discriminator was mutated by insertion of an AT-rich oligonucleotide was used to demonstrate the importance of this region in control. Both stringent and growth-rate-dependent controls were abolished by the mutation. Other potential regulatory signals in the vicinity of the gua promoter are a pur operator (binding site for the PurR repressor), a gua operator, a DnaA-binding site and a CRP/FNR-binding sequence. The gua promoter lies back-to-back with the promoter for xseA (exonuclease VII), the two promoters being separated by only 20 bp.

Keywords: gua promoter, stringent control, growth-rate-dependent regulation, Escherichia coli

INTRODUCTION

In Escherichia coli, the flow of precursors into nucleic acids is a major metabolic commitment as RNA and DNA together comprise about one-quarter of the dry weight of the cell (Neidhardt, 1987). Stable RNA (rRNA and tRNA) constitutes the largest fraction of nucleic acids (83% of the total for cells in balanced growth at 37 °C in aerobic glucose minimal medium), DNA accounts for 13% of the total, and the remainder (approximately 4%) is mRNA, which has a rapid rate of turnover. We can expect efficient regulatory mechanisms for ensuring balanced production of nucleic acid precursors (nucleotides) for stable RNA and DNA synthesis to have evolved within E. coli, because provision of these precursors is highly demanding of resources and energy expenditure.

The purine nucleotide GMP is formed from IMP by the action of IMP dehydrogenase and GMP synthetase. These enzymes are coded by the two contiguous genes (guaB and guaA, respectively) of the gua operon. The nucleotide sequence of the guaBA operon has been determined (Teideman & Smith, 1985; Thomas & Drabble, 1985) but its regulatory features, including the promoter, have been only partially characterized (Thomas & Drabble, 1985) (Fig. 1). The operon needs to be responsive to changes in the metabolic state of the cell by sensing (a) the presence of externally available guanine (which is used in preference to synthesis de novo), (b) the need for precursors of stable RNA and (c) the need for precursors of DNA. The operon must also be expressed in a way that achieves balanced production of GMP with that of AMP and with the pyrimidine nucleotides. The combination and interaction of these various controls provides a system of multivalent regulation for the gua operon.

General control of the genes involved in purine and pyrimidine biosynthesis is mediated by the PurR repressor protein (He et al., 1990; Meng et al., 1990), the product of purR, which binds to a 16 bp palindromic sequence (the pur operator) in the promoter regions of these genes (Schumacher et al., 1994). All the genes involved in GMP biosynthesis from 5-phosphoribosyl 1-pyrophosphate are regulated by PurR as shown from measurement of enzyme activity and/or expression from pur gene–lacZ fusions in...
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PurR (Meng et al., 1990). Evidence for PurR binding at the pur operator in these genes (with the exception of\(guaB\)) has been provided by gel retardation and DNase I footprinting studies (He et al., 1990). The PurR corepressors have been identified as hypoxanthine and guanine (Choi & Zalkin, 1992; Schumacher et al., 1994).

The production of IMP dehydrogenase and GMP synthetase is coordinately repressed by guanine in the growth medium and induced by adenine (Mehra & Drabble, 1981; Spibey & Drabble, 1981; Thomas & Drabble, 1984). The range of\(gua\) operon expression from full repression to full derepression under conditions of guanine starvation is about 50-fold (Spibey & Drabble, 1981). This suggests an independent and specific system for controlling\(gua\) operon expression distinct from the PurR regulon.

We have reported that the\(gua\) operon is also regulated by DnaA protein (Tesfa-Selase & Drabble, 1992). High intracellular concentrations of DnaA (achieved by inducing a multi-copy plasmid carrying the\(dna\) gene fused to the\( tac\) promoter) reduce expression of the\(gua\) operon. Lowering the concentration of DnaA, either by sequestration with an\(oriC\) plasmid or by placing a temperature-sensitive\(dna\) mutant at the restrictive temperature, results in increased expression of\(gua\). Thus, transcriptional activity of the\(gua\) operon is sensitive to DnaA concentration and is thereby coupled to the DNA replication cycle.

As over 80% of the purine and pyrimidine nucleotides produced by\(E. coli\) are incorporated into stable RNA, the regulation of nucleotide biosynthesis would be expected to link into those mechanisms for regulating transcription of the genes for stable RNA. Control of stable RNA biosynthesis is through 'growth-rate-dependent' regulation and the associated 'stringent response' mediated by ppGpp (Baracchini & Bremer, 1988). These are devices for reducing stable RNA production when bacteria are placed under conditions requiring fewer ribosomes and tRNA for protein synthesis (e.g. amino acid starvation and 'shift-down' from enriched to minimal medium).

In this paper, we identify and locate the\(gua\) promoter by transcript mapping. This reveals several features within the surrounding sequence suggesting both transcriptional and translational control of\(gua\) operon expression. In particular, the\(gua\) promoter has features characteristic of promoters under stringent and growth-rate-dependent regulation and indeed responds to stringent conditions and to alterations in the growth rate. In addition, we identify a\(dna\) box (binding site for\(DNA\) protein), a potential\(pur\) operator, and a site with dyad symmetry which may be a candidate\(gua\) operator.

**METHODS**

**E. coli strains and bacteriophages.** ID1001 is derived from strain PL1087 (W3110\(guaB67\)) (Gilbert & Drabble, 1980) and carries a chromosomal\(gua\) point mutation and the plasmid pMT101 (Thomas & Drabble, 1984, 1985). pMT101 has a 2.1 kb insertion of DNA incorporating the entire\(xre\) gene and a portion of\(gua\) extending to position 880 as numbered in Fig. 1 (approximately one-third of the\(gua\) coding sequence). ID1001 was used for mRNA isolation. Strain LL309 (Gourse et al., 1986) was used to construct single-copy chromosomal fusions of the\(gua\) promoter to lacZ; ID1007 and ID1015 carry wild-type and mutant promoter–lacZ fusions, respectively (see below). Phage arms for the construction of these fusions were provided by ARS205-S7 and \(\phi\)G59 (Miura et al., 1981). HB101 (Thomas & Drabble, 1985) and JM103 (Yanisch-Perron et al., 1985) were the respective host strains for plasmids and phage M13mp18 (Yanisch-Perron et al., 1985).

**Growth media.** The minimal medium used is based on that of Vogel & Bonner (1956) with the addition of thiamin hydrochloride (5 \(\mu\)g ml\(^{-1}\)) and glucose (4 mg ml\(^{-1}\)) or glycerol (5 mg ml\(^{-1}\)) as carbon sources. The medium was enriched with Casamino acids (2 or 4 mg ml\(^{-1}\)) or by adding 18 l-amino acids (no valine or isoleucine) (40 \(\mu\)g ml\(^{-1}\) of each). M63 medium is described by Miller (1972). Bacteria were cultured routinely in L broth (Tesfa-Selase & Drabble, 1992).

**Preparation of RNA.** The method used is adapted from Summers (1970). Bacteria (ID1001) were centrifuged from exponential-phase cultures grown in minimal medium (80 ml) supplemented with glucose and Casamino acids, and washed with 20 ml ice-cold 0.1 M phosphate buffer (pH 7.0). The cells were resuspended in 20 ml ice-cold protoplast buffer (15 mM Tris/HCl, pH 8.0; 0.45 M sucrose; 8 mM EDTA; 80 \(\mu\)g lysozyme ml\(^{-1}\)) and incubated at 40 °C for 5 min. The cells were pelleted at 0 °C and resuspended in 2 ml lysis solution (10 mM Tris/HCl, pH 8.0; 10 mM NaCl; 1 mM sodium citrate; 1-5%, SDS) plus 60 \(\mu\)l diethylpyrocarbonate (DEPC), followed by incubation for 5 min at 37 °C. After cooling on ice, 1 ml ice-cold saturated NaCl was added and the mixture was centrifuged at 5000 \(g\) for 10 min at 0 °C. Ethanol (9 ml) at -20 °C was added to the supernatant and RNA was allowed to precipitate for 4 h at -20 °C. The RNA was collected by centrifugation and stored at -20 °C as a suspension in 70% (v/v) ethanol. Before use, the RNA suspension (1 ml) was centrifuged and the pellet was dissolved in 500 \(\mu\)l DEPC-treated water. The integrity of the RNA was checked by formaldehyde gel electrophoresis following heat denaturation (60 °C for 10 min). Contaminating DNA was removed with RNase-free DNase I, and protein by phenol extraction.

**Preparation and labelling of oligodeoxyribonucleotides.** Oligonucleotides were prepared by a solid-phase phosphoramidite method using an Applied Biosystems 381A DNA Synthesizer, and purified by reverse-phase HPLC. Primers (0.6 \(\mu\)g in 3 ml) were 5'-end-labelled by mixing with 5 \(\mu\)l 10× kinase buffer (0.5 mM Tris/HCl, pH 7.6; 0.1 mM MgCl\(_2\); 1 mM EDTA), 5 \(\mu\)l 100 \(\mu\)M spermidine, 2.5 \(\mu\)l 1 M DTT, 10 \(\mu\)l [\(\gamma\)-\(^{32}\)P]ATP (3-7 \(\times\) 10\(^{6}\) Bq; > 185 TBq mmol\(^{-1}\)), 21.5 \(\mu\)l sterile water and 3 \(\mu\)l (12 units) T4 polynucleotide kinase. The mixture was incubated at 37 °C for 30 min, then heated at 70 °C for 10 min to inactivate the enzyme. Gel filtration through Sephadex G-50 was used to remove unincorporated ATP.

**Transcript mapping by primer extension.** Transcription start points were mapped by a method based on that of Wilson et al. (1987). Total cellular RNA (50 \(\mu\)g), prepared as described above, was mixed with 5'-end-labelled oligodeoxyribonucleotide primer (minimum of 5 \(\times\) 10\(^{6}\) d.p.m.). The RNA mixture was precipitated from 2 vols ethanol at -20 °C by addition of 0.1 vol 4 M NaCl, collected by centrifugation, washed with 70% ethanol at -20 °C, dried at room temperature, and dissolved in 25 \(\mu\)l hybridization buffer (20 mM
Tris/HCl, pH 8.0; 0.1 M KCl; 0.1 mM EDTA). The solution was heated at 100 °C for 2 min and then placed at 37 °C for 4 h to allow hybridization. At this stage, E. coli single-strand binding protein (Pharmacia) (3.5 μg) was sometimes added to unravel any secondary structure in the RNA. The sample was cooled to room temperature and 25 μl of freshly prepared 2× buffer (0.2 M Tris/HCl, pH 8.3; 20 mM MgCl₂; 0.1 M KCl; 20 mM DTT) containing 1 nmol each of dATP, dGTP, dCTP and TTP was added, together with 12.5 units avian myeloblastosis virus (AMV) reverse transcriptase. The sample was incubated at 37 °C for 1 h then extracted with aqueous phenol. The primer extension products were precipitated by adding 2.5 vol 95% ethanol and placing the sample at ~70 °C for 20 min. The precipitate was collected by centrifugation, dissolved in 20 μl DEPC-treated water, mixed with dye-loading solution and applied to 7 M urea/8% polyacrylamide gels for electrophoresis alongside DNA sequencing reactions using the same primers.

Insertion mutagenesis of the gua promoter. An AT-rich 14-mer (5’AAAAAGTTA ACTTTGC) was synthesized and self-hybridized by incubating 0.2 nmol at 90 °C for 2 min and then cooling to room temperature over a 2 h period. This generated SstII-compatible ends to enable insertion of the 14-mer into the unique SstII restriction site of pMT104 (Thomas & Drabble, 1984, 1985). An internal Hpal site is also present in the sequence. The double-stranded oligomer (0.05 nmol) was ligated to 0.01 nmol SstII-cut pMT104 DNA overnight at 15 °C using 0.2 units T4 DNA ligase in a 10 μl reaction. The ligated DNA was incubated at 37 °C with SstII to digest any re-ligated pMT104 (vectors incorporating the mutagenic oligomer are SstII-resistant). The constructed plasmid DNA was transformed into HB101 with selection for ampicillin resistance. Plasmid DNA (pID101) isolated from transformants was resistant to SstII but, unlike pMT104, was sensitive to Hpal.

Construction of gua-lacZ transcriptional fusions. Plasmids pMT104 (Thomas & Drabble, 1984, 1985) and pID101 (see preceding section) were digested separately with AatI. The 310 bp AatI fragment E (Thomas & Drabble, 1984, 1985) from pMT104 and the corresponding 324 bp fragment E’ from pID101 (Fig. 1) were isolated by gel electrophoresis. These fragments were blunt end ligated into Smal-cut RF M13mp18 (20 ng cut M13mp18 RF DNA; 100 ng fragment E or E’; 0.4 units T4 DNA ligase; 20 mM Tris/HCl, pH 7.5; 1 mM MgCl₂; 10 mM DTT; 0.6 mM ATP; in a total of 10 μl) with incubation overnight at 15 °C. The ligated DNA was then used to transform JM103 and white plaques were picked from X-Gal plates. The orientation of the cloned DNA was determined by sequencing using Sequenase (US Biochemical). This revealed that: plasmid with fragments E and E’ in the required orientation for fusion to lacZ had been constructed, and confirmed that fragment E’ contained the additional 14 bp from the inserted oligonucleotide.

The construction of transcriptional fusions of the gua promoter to lacZ was achieved using two derivatives of phage λ (Miura et al., 1981). Digestion of DNA from λRS205-S7 (Bertrand et al., 1984) with HindIII releases a fragment carrying lacZ and trp ALV; this constituted the left arm of the fusion construction. The right arm was provided by digestion of λ59 DNA with EcoRI, M13mp18 RF DNA carrying either fragment E or fragment E’ (with the gua promoter orientated towards the HindIII site of the M13mp18 polynucleator) was treated in turn with HindIII and with EcoRI and fragments containing E and E’ were isolated by gel electrophoresis. Restriction enzymes were removed from these digests with aqueous phenol. The DNA fragments were mixed (13 μg of each λ digest with 13 μg fragment E or E’) to provide a molar excess of insert over the λ arms, and ligated using T4 DNA ligase at 15 °C overnight. The ligated DNA was packaged in vitro as described by Rosenberg et al. (1985). The constructed λ phage was used to infect the Lac- host LL309 (Gourse et al., 1986) and blue plaques were picked from X-Gal tryptone broth top agar plates. Lac- lysogens from within the plaques were purified on MacConkey agar. Single-copy lysogens were distinguished from multiple lysogens by β-galactosidase measurement using mid-exponential cultures in glucose minimal medium. Single lysogens (half the β-galactosidase activity of double lysogens) occurred at about fivefold greater frequency than double lysogens, as expected (Bertrand et al., 1984). It was noted that clones carrying the mutant (E’) gua-lacZ fusion had only approximately 5% of the enzyme activity of those containing the wild-type gua promoter (E). Two strains were selected as representative of single-copy wild-type (ID1007) and single-copy mutant (ID1015) gua-lacZ fusions.

β-Galactosidase assay. The enzyme was assayed in cells lysed with chloroform and SDS and with ONPG as substrate (Miller, 1972). One unit (U) of β-galactosidase hydrolyses 1 nmol ONPG min⁻¹ at 28 °C. The recorded β-galactosidase activities are means of assays from at least two independent cultures; the overall variation was not greater than ±10%.

Protein assay. The protein content of bacterial cells was determined by one of the following methods. When large numbers of measurements were to be made, samples (1 ml) of cultures were centrifuged, and the cells washed, resuspended in 1 ml 0.5 M NaOH, and placed at 100 °C for 5 min. After cooling to room temperature, protein concentration was determined by the Lowry method. The second procedure, used for smaller numbers of samples, involved sonication (two 30 s periods) of washed cells. Protein in the sonicates was measured by the bichoninic acid method of Smith et al. (1985).

RESULTS

Locating the gua promoter by transcript mapping

The guaB,A operon has been cloned and sequenced but two conflicting locations for the gua promoter and the guaB gene have been proposed. Using the S1 nuclease method for transcript mapping, Teideman & Smith (1985) placed the promoter (P2, Fig. 1) between positions 12 and 43 in the nucleotide sequence with the guaB coding region starting at A-242. Thomas & Drabble (1985), however, placed the gua promoter (P1, Fig. 1) between positions 239 and 267. Placement at this position was based on the identification of small overlapping restriction fragments carrying promoter activity and on the exact fit of the -10 element of P1 to the consensus TATAAT. The initiation codon for guaB at A-311 was deduced from N-terminal sequencing of IMP dehydrogenase, and from the presence of a potential Shine-Dalgarno sequence (Thomas & Drabble, 1985). The conflict between these two possible locations for the gua promoter has now been resolved by transcription mapping using primer extension by reverse transcriptase, with gua mRNA as template. For these experiments, the proportion of total mRNA which is gua mRNA was maximized by isolating RNA from strain ID1001 grown in guanine-free medium. This strain carries the chromosomal point mutation guaB67 and the plasmid pMT101. The plasmid has a 2.1 kb insertion of DNA incorporating the entire xreA gene and guaB to codon 190 (position 880 in the sequence as numbered in Fig. 1). A 26 kDa plasmid-derived polypeptide (assumed to be the
Fig. 1. Nucleotide sequence of the gua promoter region. The gua promoter (P1) and the divergent xseA promoter are indicated with −10 and −35 hexamers in bold-face type. P2 is another potential σ70 promoter. The GC-rich discriminator implicated in stringent control is shown together with the mutagenic sequence inserted at the SstI restriction site. Potential binding sites are identified for CRP and/or FNR, F15, PurR, and for a putative gua repressor (at the gua operator). End-points of cDNA identified by extension of primers complementary to sequences A and B (underlined) are indicated by asterisks. Regions with dyad symmetry are marked by diverging arrows. Coding sequences for guaB (IMP dehydrogenase) and xseA (exonuclease VII) are shown in lowercase letters. Fragments E and € used for constructing gua-lacZ fusions are between the two AluI restriction sites shown.

N-terminal portion of IMP dehydrogenase complements the defective chromosome-derived enzyme \textit{in vivo}, resulting in prototrophic growth. These bacteria are derepressed during growth in guanine-free medium (Thomas & Drabble, 1985), presumably to compensate for the low catalytic activity of the complemented enzyme.

The transcription start point for the gua operon was mapped by primer extension (see Methods). Two oligodeoxyribonucleotide 17-mers were synthesized with the sequences 5’-GAACCCTAGAGTGACG (primer A) and 5’-ATCTCTGGTCAATGTTA (primer B). Primer A is capable of hybridizing with mRNA transcribed through nt 364–380 of the gua sequence (Fig. 1) and will therefore map transcripts initiated from both P1 and P2 as the sequence is downstream of both promoters. The cDNA products of primer A extension using reverse transcriptase would be approximately 105 and 330 nt, respectively, for the two promoters. Primer B is capable of hybridizing with mRNA transcribed through nt 144–160, that is downstream of P2 but upstream of P1, and will therefore map transcripts originating at P2 only, as indicated by the production of an approximately 100 nt cDNA.

No cDNA was detected using primer B (Fig. 2a), so we conclude that no mRNA is transcribed upstream of and through the hybridization site of primer B and hence P2 is
not a functional promoter under the conditions assayed. However, the longest cDNA using primer A was only 67 nt. This does not correspond to a transcriptional start point close to promoter P1, but to position C-314 in a region containing no typical promoter sequence. AMV reverse transcriptase is known to terminate at certain stable secondary structures (Tuwerk et al., 1988) such as hairpins. The potential to form stable secondary structure in the leader sequence of gna mRNA has been noted previously (Thomas & Drabble, 1985) for a transcript starting at G-273 (Fig. 1). The stem-loop incorporates the first 37 nt of mRNA, so may cause reverse transcriptase to stop at C-314 (5 nt before the proposed hairpin). The primer extension reaction with primer A was repeated with the addition of E. coli single-strand binding protein to unravel secondary structure in the gna mRNA. Under these conditions cDNA transcripts 60, 67, 76, 93, 95, 97 and 107 nt long were detected (Fig. 2b). These correspond to termination of reverse transcription at the positions marked in Fig. 1 within the hairpin loop region. The longest cDNA detected corresponds to full-length transcripts originating at G-274 and thus identifies this nucleotide as the transcription start point for the gna promoter P1. The presence of a GC-rich 'discriminator' (Lindhal & Zengel, 1986) sequence between the -10 element and the transcription start point suggested stringent regulation at this promoter and led to the experiments described in the following section.

**Transcriptional regulation of the gna promoter**

Transcriptional fusions of the gna promoter to lacZ were constructed to facilitate investigation of transcriptional control of the gna operon by stringent and growth-rate-dependent regulation. The procedure involved cloning promoter-containing restriction fragments of DNA upstream of a promoter-less lacZ gene carried on λ phage (see Methods). Following phage DNA assembly, in vitro packaging and infection of Lac-c bacteria, single-copy Lac-c lysogens were isolated. These lysogens contain a single-copy lacZ gene, under control of the chosen promoter, integrated into the host genome at attλ. Two promoter-containing fragments were fused to lacZ; fragment E (Fig. 1) carrying the wild-type gna promoter and E' the corresponding fragment into which the sequence 5'-AAAGTTAACT'TGC had been inserted at the unique GC-rich Sir1 site adjacent to the -10 promoter hexamer. Two lysogens carrying these fusions (ID1007 and ID1015, respectively) were used in experiments to investigate the control mechanisms affecting gna expression.

The stringent response in bacteria occurs under conditions of amino acid starvation (Gallant et al., 1971). Using the lysogens ID1007 and ID1015, the effect of amino acid starvation on transcription initiated at the wild-type and mutant forms of the gna promoter was determined. Amino acid starvation was achieved by the addition of valine to cultures grown in the absence of isoleucine. Valine inhibits the first enzyme common to isoleucine and valine biosynthesis and thereby starves bacteria of isoleucine (Freundlich et al., 1962). The specific activity of β-galactosidase was measured with and without isoleucine starvation (Fig. 3). For bacteria growing normally (control culture), β-galactosidase formed a constant proportion of the cell protein (constant specific activity). However, after addition of valine the differential rate of synthesis of β-galactosidase fell by about 75% from that of the control culture. Hence expression of β-galactosidase is responding to stringent conditions in a manner that is predictable from the sequence determinants present at the promoter. Similar experiments with strain ID1015 indicated that isoleucine starvation had no effect on the specific activity of β-galactosidase (Fig. 3), indicating that mutation of the GC-rich discriminator region by insertion of an AT-rich sequence, while reducing the activity of the promoter, completely abolishes stringent regulation.

The two lysogens were grown in media that support a range of growth rates and the specific activity of β-galactosidase was determined during exponential growth (Fig. 4a). For the wild-type promoter fusion the specific activity of β-galactosidase increased with growth rate, showing that at higher growth rates the enzyme constitutes a larger fraction of the cell protein. β-Galactosidase specific activity for the culture growing at 1 doubling h⁻¹ was almost twice that for the culture growing at 0.43 doublings h⁻¹. This is characteristic of
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the products of genes that are under growth-rate-dependent control (Miura et al., 1981), and indicates that the gnu operon is under this form of regulation. The specific activity of β-galactosidase in the fusion strain carrying the insertion mutation showed no dependence on growth rate (Fig. 4a).

Guanine in the growth medium represses expression of the gnu operon (Mehra & Drabble, 1981). The effect of guanine on growth-rate-dependent control of the gnu-lacZ fusion was investigated (Fig. 4b). The presence of guanine led to a decrease of between 40 and 45% in the specific activity of β-galactosidase in each of the four media tested, but did not abolish the growth-rate-dependent response. This suggests that guanine repression is independent of the growth-rate response.

**DISCUSSION**

Primer extension with AMV reverse transcriptase was used to map the transcription initiation point of gnu mRNA and thus identify P1 as the gnu promoter (Fig. 1). On the basis of S1 nuclease mapping, it was proposed previously (Teideman & Smith, 1985) that the gnu promoter lies farther upstream at P2. This region of DNA does not, however, carry a functional promoter responding to guanine and adenine, as predicted for the gnu promoter (Thomas & Drabble, 1985). The region does contain several promoter-like sequences which may bind RNA polymerase in vivo and lead to the production of minor transcripts. These could protect DNA during S1 mapping, particularly at high RNA:DNA ratios. Furthermore, promoter P2 is within the xcuA coding sequence (Chase et al., 1986) and is more distant than P1 from the start of the gnuB coding region, which has been identified directly by N-terminal sequencing of purified IMP dehydrogenase (Thomas & Drabble, 1985). Promoter P1 has the preferred 17 bp separation of -35 and -10 hexamers, unlike P2 where the separation is 20 bp.

The gnu mRNA leader has the potential for forming a stable stem–loop secondary structure [ΔG° = -19.2 kcal mol⁻¹ (-80.6 kJ mol⁻¹) at 25 °C] incorporating the first 37 nt of the transcript (Thomas & Drabble, 1985). As the ribosome-binding site is sequenced in the stem–loop, this structure may be involved in regulating translation of gnu mRNA as has been proposed for mRNA transcribed from pyrC (Kelln & Neuhard, 1988). Formation of this stem–loop during the hybridization step in the transcript-mapping procedure explains the presence of truncated transcripts as AMV reverse transcriptase terminates prematurely at RNA hairpins (Tuerk et al., 1988), but some of the transcripts may have arisen from the copying of mRNA partially degraded at the 5′ end. Full-length cDNA was detected only when single-strand binding protein was added to unravel secondary structure in the mRNA.

Examination of the nucleotide sequence in the vicinity of the gnu promoter reveals features similar to those found in promoters for stable RNA. These promoters are subject to stringent control and growth-rate-dependent regu-

![Fig. 4. (a) Effect of growth rate on the expression of β-galactosidase in guaB-lacZ fusion strains ID1007 (○, wild-type gnu promoter) and ID1015 (△, mutated gnu promoter). Increasing growth rates were achieved by growing bacteria in defined minimal medium supplemented with: glycerol (5 mg ml⁻¹) and thiamin (1 µg ml⁻¹); glucose (4 mg ml⁻¹) and thiamin (1 µg ml⁻¹); glycerol (5 mg ml⁻¹), thiamin (1 µg ml⁻¹) and 18 L-amino acids (40 µg ml⁻¹ of each, excluding isoleucine and valine); glucose (4 mg ml⁻¹), thiamin and 18 amino acids as before; glucose (4 mg ml⁻¹) and Casamino acids (4 mg ml⁻¹). (b) Expression of β-galactosidase in ID1007 grown at different rates in the absence (●) and presence (▴) of guanine (40 µg ml⁻¹). Increasing growth rates were achieved by growing bacteria in defined minimal medium supplemented with: glycerol or glucose and thiamin [as in (a)]; glucose (2 mg ml⁻¹), thiamin (1 µg ml⁻¹) and Casamino acids (2 mg ml⁻¹); glucose (2 mg ml⁻¹), thiamin (1 µg ml⁻¹) and yeast extract (2 mg ml⁻¹) (yeast extract contains some guanine derivatives). All cultures were grown at 30 °C with aeration. At an OD₆₅₀ of 0.4, samples were removed for assay of protein and β-galactosidase.

![Graph showing the effect of growth rate on the expression of β-galactosidase.](image-url)
alterations to other promoters. Substitution of four the strain carrying a single-copy chromosomal promoter (Fig. 1); however, the consensus for the FIS-dependent regulation, have been observed following deletion of the discriminator by insertion of an AT-rich oligonucleotide resulted in a 90% reduction in promoter activity measured at a growth rate of μ = 0.6. This reduction in activity of the gna promoter may result partially from the effects of inserting a 14-mer on transcription initiation. The initiating nucleotide is altered from G to A, and the mRNA leader is increased by 14 nt.

During the stringent response there is a sharp fall in the GTP pool (which is then maintained at half its normal size) with a concomitant, almost equimolar, increase of ppGpp (Gallant et al., 1970; Gallant & Harada, 1969). Contraction of the GTP pool does not occur, however, if RNA synthesis is prevented by uracil starvation; in fact the GTP pool expands (Gallant & Harada, 1969). During isoleucine (but not uracil) starvation the conversion of IMP to GMP is blocked because ppGpp is a strong competitive inhibitor of IMP dehydrogenase (Gallant et al., 1970, 1971; Pao & Dyess, 1981). It is now apparent from our present work that contraction of the GTP pool during stringency may follow not only from enzyme inhibition by ppGpp but also from reduced transcription from the gna promoter.

As the primary function of purine and pyrimidine nucleotide biosynthesis is to provide precursors for stable RNA, stringent and growth-rate-dependent regulation of this synthesis is logical. In the absence of a need for stable RNA, it makes sense that synthesis of RNA precursors is curtailed. Apart from the gna promoter, the promoters of other genes involved in nucleotide biosynthesis show a close match to the GC-rich discriminator and may be stringently regulated. Indeed, experimental evidence has been presented for stringent regulation of purF (Makaroff & Zalkin, 1985), pyrBI (Turnbough, 1983) and carAB (Bouviex et al., 1984; Pette et al., 1984). Together with gnaBA, these genes encode enzymes that occupy pivotal points in purine and pyrimidine nucleotide biosynthesis.

Confirmation of the gnaBA promoter and +1 transcription initiation site justifies inspection of the surrounding sequence for features of potential regulatory significance. It is reported that the PurR repressor modulates gnaB expression by about threefold (Meng et al., 1990). A putative 16 bp pur operator, with 11/16 match to the consensus, can be identified. This is centred at C-249/G-250 within the gna promoter (Fig. 1). The full range of expression for the gna operon is, however, about 50-fold (Spibey & Drabble, 1981), so a regulatory mechanism must exist that is independent of PurR, stringent and growth-rate-dependent control, but is sensitive to changes in the cytoplasmic guanine and adenine nucleotide pools (Mehra & Drabble, 1981). The gna promoter region contains a sequence of imperfect dyad symmetry extending over 18–24 bp centred at G-271/C-272 (Fig. 1). This sequence overlaps the promoter and is therefore located where binding of a gna-specific repressor protein could interfere with transcription initiation. Work is now in progress to isolate and characterize a DNA-binding protein specific for this gna "operator". Expression of the operon is sensitive to changes in cellular DnaA protein concentration (Tesfa-Selase & Drabble, 1992). The gna promoter region has a DnaA box, the binding site for DnaA, with 8/9 match to the consensus, although a consensus sequence box within the gnaBA coding region is also implicated in this regulation (F. Tesfa-Selase & W. T. Drabble, unpublished results).
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promoter may have regulatory significance (Beck et al., 1986). Only 20 bp separate the —35 hexamers of these promoters with 161 bp between the initiation codons of IMP dehydrogenase and exo

uclease VII. Such close spacing of promoters may have regulatory significance (Beck & Warren, 1988) and will lead to stochastic hindrance as RNA polymerase molecules attempt to bind simultaneously, especially if the two promoters lie on the same face of DNA, as in this example. Inactivation of either promoter would be expected to increase the activity of the other. Several examples of divergent promoters regulated by the binding of CRP protein have been identified (Kolb et al., 1993). A sequence with 17/22 match with the consensus binding site for CRP (Kolb et al., 1993) lies at —117 with respect to initiation of gna transcription, but downstream of the divergent promoter for xreA. The core motif TGTGA of this site is conserved in the left half of the palindrome, but one difference in the right half confers the consensus core motif (TCACA) for binding of FNR. It has been demonstrated (Bell et al., 1989; Jennings & Beacham, 1993) that hybrid binding sites of this type respond to both CRP and FNR in vivo. The presence of CRP or FNR at the site may hinder binding of RNA polymerase to the xreA promoter and thus relieve competition at the gnaB-A promoter; the relevance, if any, of such a mechanism to the overall control of gnaB-A and xreA expression is being investigated.

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REFERENCES


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