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 GC-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

Abbreviation: FIS, factor for inversion stimulation.

purR mutant and *purR*⁺ host backgrounds. There is a threefold modulation of *guaB* expression mediated by PurR (Meng *et al.*, 1990). Evidence for PurR binding at the *pur* operator in these genes (with the exception of *guaB4*) 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 *guaB4* 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 *dnaA* 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 *dnaA* 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 *guaB4* 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 DnaA box (binding site for DnaA 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 *guaB* point mutation and the plasmid

pMT101 (Thomas & Drabble, 1984, 1985). pMT101 has a 2.1 kbp insertion of DNA incorporating the entire *xsxA* gene and a portion of *guaB* extending to position 880 as numbered in Fig. 1 (approximately one-third of the *guaB* 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 λ RS205-S7 and λ 459 (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 μ 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 μ 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 late-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 μ 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 μ 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 μ 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 phosphoramidate method using an Applied Biosystems 381A DNA Synthesizer, and purified by reverse-phase HPLC. Primers (0.6 μ g in 3 μ l) were 5'-end-labelled by mixing with 5 μ l 10 $\times$ kinase buffer (0.5 mM Tris/HCl, pH 7.6; 0.1 mM MgCl₂; 1 mM EDTA), 5 μ l 100 mM spermidine, 2.5 μ l 1 M DTT, 10 μ l [γ -³²P]ATP (3.7 $\times$ 10⁶ Bq; > 185 TBq mmol⁻¹), 21.5 μ l sterile water and 3 μ 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 μ g), prepared as described above, was mixed with 5'-end-labelled oligodeoxyribonucleotide primer (minimum of 5 $\times$ 10⁵ 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 μ 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 mM 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 vols 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'AAAGTTAACTTTGC) 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 *Sst*II-compatible ends to enable insertion of the 14-mer into the unique *Sst*II restriction site of pMT104 (Thomas & Drabble, 1984, 1985). An internal *Hpa*I site is also present in the sequence. The double-stranded oligomer (0.05 nmol) was ligated to 0.01 nmol *Sst*II-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 *Sst*II to digest any re-ligated pMT104 (vectors incorporating the mutagenic oligomer are *Sst*II-resistant). The constructed plasmid DNA was transformed into HB101 with selection for ampicillin resistance. Plasmid DNA (pID101) isolated from transformants was resistant to *Sst*II but, unlike pMT104, was sensitive to *Hpa*I.

Construction of *gua-lacZ* transcriptional fusions. Plasmids pMT104 (Thomas & Drabble, 1984, 1985) and pID101 (see preceding section) were digested separately with *A*luI. The 310 bp *A*luI 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 *Sma*I-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 transfect 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 phage 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 *Hind*III releases a fragment carrying *lacZ* and *trpAB*'; this constituted the left arm of the fusion construction. The right arm was provided by digestion of λ459 DNA with *Eco*RI. M13mp18 RF DNA carrying either fragment E or fragment E' (with the *gua* promoter orientated towards the *Hind*III site of the M13mp18 polylinker) was treated in turn with *Hind*III and with *Eco*RI 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 (1.3 µg of each λ digest with 1.3 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 µmol 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 bicinchoninic acid method of Smith *et al.* (1985).

RESULTS

Locating the *gua* promoter by transcript mapping

The *guaB* 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 kbp insertion of DNA incorporating the entire *xseA* 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

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 (Tuerk *et al.*, 1988) such as hairpins. The potential to form stable secondary structure in the leader sequence of *gua* 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 *gua* 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 *gua* 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 *gua* promoter

Transcriptional fusions of the *gua* promoter to *lacZ* were constructed to facilitate investigation of transcriptional control of the *gua* 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⁻ bacteria, single-copy Lac⁺ lysogens were isolated. These lysogens contain a single-copy *lacZ* gene, under control of the chosen promoter, integrated into the host genome at *att λ* . Two promoter-containing fragments were fused to *lacZ*; fragment E (Fig. 1) carrying the wild-type *gua* promoter and E' the corresponding fragment into which the sequence 5'-AAAGTTAACTTTGTC had been inserted at the unique GC-rich *Sst*II 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 *gua* 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 *gua* 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

Fig. 3. Effect of amino acid depletion on the expression of β -galactosidase in *guaB-lacZ* fusion strains. Bacteria were grown in M63 medium supplemented with glucose (2 mg ml⁻¹), MgSO₄ (1 mM) and 1 μ g ml⁻¹ each of 18 L-amino acids (excluding isoleucine and valine). Medium (100 ml vols) was inoculated with 10 ml overnight culture of ID1007 (●, ▲, wild-type *gua* promoter) or ID1015 (◆, ▼, mutated *gua* promoter) grown in the same medium. Cultures were grown at 30 °C with aeration (growth rates 0.95 and 0.90 doublings h⁻¹, respectively, for ID1007 and ID1015). Samples were removed periodically for β -galactosidase and protein assays. At mid-exponential growth, valine was added (▲, ▼) to a final concentration of 500 μ g ml⁻¹ to induce the stringent response (for these cultures growth rate gradually declined to zero over the course of 1 h following valine addition).

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

Fig. 4. (a) Effect of growth rate on the expression of β -galactosidase in *guaB-lacZ* fusion strains ID1007 (●, wild-type *gua* promoter) and ID1015 (▲, mutated *gua* promoter). Increasing growth rates were achieved by growing bacteria in defined minimal medium supplemented with: glycerol (5 mg ml⁻¹) and thiamin (1 μ g ml⁻¹); glucose (4 mg ml⁻¹) and thiamin (1 μ g ml⁻¹); glycerol (5 mg ml⁻¹), thiamin (1 μ g ml⁻¹) and 18 L-amino acids (40 μ g 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 μ g 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 μ g ml⁻¹) and Casamino acids (2 mg ml⁻¹); glucose (2 mg ml⁻¹), thiamin (1 μ g 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 OD₆₀₀ of 0.4, samples were removed for assay of protein and β -galactosidase.

the products of genes that are under growth-rate-dependent control (Miura *et al.*, 1981), and indicates that the *gua* 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 *gua* operon (Mehra & Drabble, 1981). The effect of guanine on growth-rate-dependent control of the *gua-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 *gua* mRNA and thus identify P1 as the *gua* promoter (Fig. 1). On the basis of S1 nuclease mapping, it was proposed previously (Teideman & Smith, 1985) that the *gua* 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 *gua* 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 *xseA* coding sequence (Chase *et al.*, 1986) and is more distant than P1 from the start of the *guaB* 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 *gua* mRNA leader has the potential for forming a stable stem-loop secondary structure [$\Delta G^0 = -19.2$ 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 sequestered in the stem-loop, this structure may be involved in regulating translation of *gua* mRNA as has been proposed for mRNA transcribed from *pyrC* (Kelln & Neuhaud, 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 *gua* promoter reveals features similar to those found in promoters for stable RNA. These promoters are subject to stringent control and growth-rate-dependent regu-

lation, but no single conserved feature has been shown to confer these properties (Zacharias *et al.*, 1990). Most highly conserved is a GC-rich sequence, known as the 'discriminator', downstream of and adjacent to the -10 hexamer. The consensus sequence for the discriminator is $\text{NCN}^{\text{G}}/\text{cC}^{\text{G}}/\text{cCC}$, where the first nucleotide is next to the last T of the -10 hexamer of the promoter (Lindhal & Zengel, 1986). The *gua* discriminator matches this consensus at seven of the eight positions. Also conserved in stringently regulated promoters is a *cis*-acting AT-rich sequence (the UP element) located between -60 and -40 with respect to the transcription start site. This has been ascribed a role in transcriptional activation (Ross *et al.*, 1993), improving RNA polymerase/promoter contact through DNA bending and compensating for the weak homology of these promoters with the -35 consensus (TTGACA). In addition, *trans*-activation by FIS has been recognized (Finkel & Johnson, 1992). FIS binds to sequences found between -71 and -60 of many rRNA and tRNA genes and activates transcription when there is a change to conditions that can support higher growth rates (Ball *et al.*, 1992). The AT-rich sequence and potential FIS sites are present upstream of the *gua* promoter (Fig. 1); however, the consensus for the FIS-binding site (Finkel & Johnson, 1992) is sufficiently redundant for the significance of the sites in *gua* to be considered only with caution pending further investigation.

In this study, we have presented experimental evidence both for stringent control and for growth-rate-dependent regulation of the *gua* promoter. Isoleucine starvation of the strain carrying a single-copy chromosomal *gua-lacZ* fusion caused a 75% fall in β -galactosidase synthesis (similar to the decrease reported for the P1 promoters of *rrn* operons; Condon *et al.*, 1992). Growth of the *gua-lacZ* fusion strain in defined media designed to support a range of growth rates showed that *gua* expression (measured as β -galactosidase specific activity) is subject to growth-rate-dependent regulation.

Disruption of the *gua* discriminator by insertion of an AT-rich linker removed stringent and growth-rate-dependent regulation. Similar effects, indicating the importance of the -1 to -10 region for stringent and growth-rate-dependent regulation, have been observed following alterations to other promoters. Substitution of four GC base pairs by AT base pairs in the discriminator of the *tyrT* promoter reduced stringent (Lamond & Travers, 1985) and growth-rate control (Travers *et al.*, 1986), as did alterations to the *rrnB* P1 discriminator sequence (Dickson *et al.*, 1989). Changes to the discriminator sequence appear to produce diverse effects on promoter strength. The substitutions in the *tyrT* promoter doubled its strength (Lamond & Travers, 1985) but single insertions and deletions in the discriminator sequence of *rrnB* P1 reduced promoter activity down to 20% of wild-type activity (Gaal *et al.*, 1989). Mutation of the *gua* discriminator with the AT-rich oligonucleotide resulted in a 90% reduction in promoter activity measured at a growth rate of $\mu = 0.6$. This reduction in activity of the *gua* 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 *gua* 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 *gua* 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* (Bouvier *et al.*, 1984; Piette *et al.*, 1984). Together with *guaBA*, these genes encode enzymes that occupy pivotal points in purine and pyrimidine nucleotide biosynthesis.

Confirmation of the *guaBA* 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 *guaB* 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 *gua* promoter (Fig. 1). The full range of expression for the *gua* 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 *gua* 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 *gua*-specific repressor protein could interfere with transcription initiation. Work is now in progress to isolate and characterize a DNA-binding protein specific for this *gua* 'operator'. Expression of the operon is sensitive to changes in cellular DnaA protein concentration (Tesfa-Selase & Drabble, 1992). The *gua* 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 *guaB* coding region is also implicated in this regulation (F. Tesfa-Selase & W. T. Drabble, unpublished results).

The *gua* promoter lies in the back-to-back arrangement with the promoter for *xseA* (encoding the mismatch repair enzyme exonuclease VII) (Chase *et al.*, 1986). Only 20 bp separate the -35 hexamers of these promoters with 161 bp between the initiation codons of IMP dehydrogenase and exonuclease VII. Such close spacing of promoters may have regulatory significance (Beck & Warren, 1988) and will lead to steric 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 *gua* transcription, but downstream of the divergent promoter for *xseA*. 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 (TCAAA) 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 *xseA* promoter and thus relieve competition at the *guaBA* promoter; the relevance, if any, of such a mechanism to the overall control of *guaBA* and *xseA* expression is being investigated.

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