INTRODUCTION

The *Escherichia coli* guaB promoter (P_{guaB}) regulates transcription of two genes, guaB and guaA, which together constitute the *guaBA* operon. The *guaB* and *guaA* genes encode inosine 5'-monophosphate (IMP) dehydrogenase and guanosine 5'-monophosphate (GMP) synthetase, respectively, and are required for the biosynthesis of GMP from the common purine nucleotide precursor, IMP (Mehra & Drabble, 1981; Tiedeman & Smith, 1984). P_{guaB} is regulated by the cAMP receptor protein (CRP) and a putative CRP binding site is centred near position −117.5 relative to the *guaB* transcription start site (Hutchings & Drabble, 2000). Furthermore, transcription from P_{guaB} is strongly enhanced by an UP element (Husnain & Thomas, 2008). P_{guaB} also contains a putative binding site for PurR that overlaps the core promoter region, and PurR downregulates expression of *guaB* (Meng et al., 1990; Davies & Drabble, 1996). DNA binds to a sequence overlapping the core promoter region and also downregulates transcription from P_{guaB} (Tesfa-Selase & Drabble, 1996). It has also been shown that the rate of transcription from P_{guaB} per unit cell mass increases as a function of increasing cellular growth rate (Davies & Drabble, 1996; Husnain & Thomas, 2008). This phenomenon is commonly referred to as growth rate-dependent control (GRDC) (Gourse et al., 1996; Dennis et al., 2004). GRDC of P_{guaB} requires the UP element and sequences located upstream of the UP element (Husnain & Thomas, 2008).

The factor for inversion stimulation (FIS) regulates transcription by binding to highly degenerate 15 bp DNA sequences (Finkel & Johnson, 1992; Ross et al., 1999). At some *E. coli* promoters, FIS activates transcription by contacting the C-terminal domain of the RNA polymerase (RNAP) α subunit (αCTD) (Aiyar et al., 2002; McLeod et al., 2002). FIS can also promote transcription by decreasing the negative superhelicity of DNA (Travers et al., 2001). At other promoters, FIS downregulates promoter activity by binding to a site that overlaps or is located downstream from the RNAP binding site, or by forming a complex assembly with other nucleoid proteins (Gonzalez-Gil et al., 1998; Browning et al., 2000, 2004; Jackson et al., 2004). A previous study identified four putative binding sites for FIS that are located upstream of the P_{guaB} core promoter region (Hutchings & Drabble, 2000). The putative FIS binding sites are centred near positions −77, −92, −109 and −126 relative to the *guaB* transcription start, and were referred to as FIS sites I–IV, respectively (Fig. 1a). FIS contributes to GRDC of the thrU and *pdxA* promoters, and is required for growth rate-dependent synthesis of 4.5S RNA, tRNA<sup>ser</sup> and tRNA<sup>Thr</sup> (Emilsson & Nilsson, 1995; Dong et al., 1996). Moreover, transcription from the *fis* promoter is coupled to cellular growth rate (Mallik et al., 2006). Cellular levels of FIS and FIS mRNA also change with the growth phase, and they increase dramatically upon entry into the middle-exponential growth phase (Appleman et al., 1998; Ali Azam et al., 1999; Mallik et al., 2006).

In this work, we investigated whether FIS plays a role in the regulation of P_{guaB}. We show that the putative FIS binding sites located upstream of the P_{guaB} core elements do not recruit FIS (Hutchings & Drabble, 2000). Moreover, we demonstrate that FIS is recruited to three sites centred near −11, +8 and +29 relative to the *guaB* transcription start site, and all three sites are necessary for full FIS-mediated repression of P_{guaB} in vitro but not in vivo. Deletion of the *fis* gene results in increased P_{guaB} activity in vivo, but GRDC of P_{guaB} is maintained.

Abbreviations: CRP, cAMP receptor protein; EMSA, electromobility shift assay; FIS, factor for inversion stimulation; GRDC, growth rate-dependent control; RNAP, RNA polymerase.
repression. We also show that FIS is not required for GRDC of $P_{guaB}$.

**METHODS**

**Strains and plasmids.** All strains were derivatives of the *E. coli* K-12 strain VH1000. Each strain contained a chromosomally integrated transcriptional fusion of $lacZ$ to one of three $P_{guaB}$ derivatives: the full-length $guaB$ promoter (i.e. strain VH1000G-253), extending from positions $-253$ to $+36$ relative to the $guaB$ transcription start site [$P_{guaB}$ ($-253$ to $+36$)] (Husnain & Thomas, 2008), the $P_{guaB}$ ($-253$ to $+10$) promoter (i.e. strain VH1000G-25310, this work), which contains the same upstream end point as the full-length promoter but has a downstream end point at $+10$, and the $P_{guaB}$ ($-69$ to $+36$) promoter (i.e. strain VH1000G-69, this work), which has the same downstream end point as the full-length promoter but has an upstream end point at position $-69$ relative to the $guaB$ transcription start site. Fusions were carried on λ prophage and were constructed using a system based on simm21 (Simons et al., 1987; Rao et al., 1994). Strain VH1000G-253Afis was made by introducing the
**Fig. 1.** Identification of putative FIS sites at P_{guaB} (a) Sequence of the guaB promoter from positions -253 to +40 relative to the guaB transcription start site. Putative FIS sites I–IV, FIS sites 1–3, a putative CRP binding site centred at position -117.5 and binding sites for PurR and DnaA are indicated (Hutchings & Drabble, 2000). The core promoter elements (~35 and ~10 regions), UP element, and the initiating nucleotide are also shown, in bold. Apart from FIS sites I–IV, putative FIS sites that have been shown not to bind FIS in this work are not indicated. For clarity, FIS site 2 is not shown. (b) Candidate FIS binding sites were identified by comparing the consensus sequence for FIS (Ross et al., 1999; Shultzaberger et al., 2007) with P_{guaB} sequences located from positions –253 to +36. The consensus sequence employed is indicated accordingly; alternative bases at each position of the consensus sequence are shown in the second line. Conserved bases at the five positions considered to be critical for FIS binding (positions ~7, ~3, 0, +3 and +7) are emboldened, and the most frequently occurring base is shown in upper case. ‘n’ signifies any base. Candidate FIS sites are categorized according to their similarity to the consensus. Category 1 and 2 sites exhibit a 4/5 match at the critical positions, including positions +7 and −7, which are most strongly conserved among FIS sites. However, at category 2 sites, an infrequently occurring base is present at position ~3, 0 or +3 (in the case of P_{guaB} such mismatches only occur at position ~3). Category 3 sites also exhibit a 4/5 match at the critical positions but the mismatched base occurs at position +7 or −7. Other pertinent sites that do not fulfil the criteria for inclusion in categories 1–3 are shown as ‘other sites’. FIS sites that were protected by FIS in DNase I footprinting are as indicated.

**DNase I footprinting.** The EcoRI–Xhol DNA fragment in pBSG-253 was purified following electrophoresis in a 6% acrylamide gel (Husnain & Thomas, 2008). The promiscuous Xhol end was labeled at the downstream (Xhol) end with [α-32P]-dATP ([>7000 Ci (2.59 x 10^14 Bq) mmol^-1], MP Biomedicals) and subsequently purified according to a published procedure (Husnain & Thomas, 2008). The EcoRI–Xhol fragment in pBSG-133 was labelled similarly at the upstream end. Labelled DNA fragment (4 nM) was incubated at room temperature for 30 min in a volume of 20 µl containing 20 mM HEPES (pH 8.0), 5 mM MgCl2, 50 mM potassium glutamate, 1 mM DTT, 10% (v/v) glycerol and 20 µg ml^-1 sonicated calf thymus DNA (NE Healthcare), in the absence or presence of different concentrations of FIS. Samples were loaded (under tension) onto a 6% acrylamide gel (37.5 : 1 acrylamide : bis acrylamide) containing 7.5% (v/v) glycerol while running at ~15 V cm^-1, and gels were run for ~1 h at 4 °C. Radiolabelled DNA was visualized using a FujiFilm FLA3000 phosphorimager.

**Measurement of transcription in vitro.** Multiple-round transcription reactions were performed as described previously, using supercoiled pRLG770 derivatives containing P_{guaB} fragments (Husnain & Thomas, 2008). As a control, transcription was also measured from an rnbB P1 promoter derivative that did not contain any FIS sites (plasmid pRLG4238; Estrem et al., 1998). FIS (250 nM) was incubated with DNA in reaction buffer at room temperature for 30 min. Transcription was initiated at 30 °C with 10 nM E. coli RNAP holoenzyme (Epicentre), and reactions were allowed to proceed for 20 min.

**Measurement of transcription in vivo.** Strains containing a chromosomally integrated P_{guaB}-lacZ transcriptional fusion were employed in the measurement of promoter activity in vivo. Cells were inoculated from dense starter cultures into media that supported different cellular growth rates, as described previously (Husnain & Thomas, 2008). The β-galactosidase activity was determined following disruption of cells by sonication (Miller, 1972). To measure promoter activity at different stages of the growth cycle, cells were grown overnight in M9 minimal medium with 0.4% (w/v) glucose, 0.8% (w/v) Casamino acids and 5 µg thiamine ml^-1, and inoculated into fresh medium to an OD_{600} of ~0.01. Growth was monitored at OD_{600} and the β-galactosidase activity was measured at different points on the growth curve after cells were permeabilized with chloroform-SDS (Miller, 1972).

**RESULTS**

**Identification of putative FIS sites at P_{guaB}**

The 15 bp consensus sequence for FIS [5'-Gnn(c/t)(A/g)(a/t)(a/t)(T/A)(t/a)(t/a)(T/c)(g/a)nmC-3'] contains five highly conserved positions (underlined) that are the most significant for the recruitment of FIS (Finkel & Johnson,
positions. Five sequences were classified as category 3 sites—highly conserved bases that are located at the outermost positions. However, the mismatches occur at one of the additional sites fell into this category. Category 3 sites also contain bases that match the consensus at 4/5 critical positions, although they do include non-consensus but frequently occurring bases at the remaining two critical positions (positions −3 and +3) (Fig. 1b).

Analysis of FIS binding to P_{guaB}

EMSA was employed to determine whether FIS can bind to a DNA fragment containing the guaB promoter. As a comparison, binding of FIS to the rrnB P1 promoter, which is known to bind FIS under physiological conditions, was also analysed. The rrnB P1 promoter fragment employed contained the promoter-proximal FIS site, i.e. FIS site I (Ross et al., 1990; Bokal et al., 1995). The minimum concentration of FIS required to observe FIS–DNA interactions at either P_{guaB} (−253 to +36) or the rrnB P1 fragment by EMSA was 50 nM. Increasing the FIS concentration to 300 nM resulted in the formation of three different complexes between FIS and P_{guaB} (Fig. 2). At this concentration of FIS, a larger fraction of the rrnB P1 promoter fragment was bound by FIS, but there remained only a single FIS–DNA complex, and no FIS–DNA complexes were observed at an rrnB P1 promoter derivative that lacked a FIS site (Fig. 2). At a FIS concentration of 500 nM, an additional FIS–DNA complex was observed at both P_{guaB} and rrnB P1 harbouring FIS site I. As a complex was also observed with the promoter fragment that did not contain a FIS site, it is likely that the additional FIS–DNA interactions observed at 500 nM FIS are non-specific (Fig. 2). These results indicate that FIS binds to at least three sites at or near P_{guaB} under similar conditions to those in which FIS specifically binds to rrnB P1, and thereby suggest that FIS is likely to bind to these sites under physiological conditions.

Fig. 2. Analysis of FIS binding to P_{guaB} by EMSA. EMSA was employed to compare the relative binding affinity of FIS for a DNA fragment containing P_{guaB} (−253 to +36) with an rrnB P1 promoter derivative containing FIS site I (‘rrnB P1+ FIS site’) and rrnB P1 containing no FIS sites (‘rrnB P1–FIS site’). The concentration of FIS in each binding reaction is indicated above the corresponding gel lane.
Mapping the location of FIS binding sites at $P_{guaB}$ by DNase I footprinting

To determine the location of any FIS binding sites at $P_{guaB}$, a DNA fragment extending from positions $-253$ to $+36$ of $P_{guaB}$ (i.e. $P_{guaB} (-253$ to $+36)$) was radiolabelled at the downstream end, and DNase I footprinting was performed in the presence or absence of purified FIS. The results show that increasing the concentration of FIS up to 500 nM resulted in increased protection at two sites centred near positions $-11$ and $+8$ (FIS site 1 and FIS site 2, respectively) (Fig. 3). DNA fragments corresponding to $P_{guaB}$ sequences downstream of position $+16$ were not visible on this gel. To determine whether FIS bound to sequences downstream of position $+16$, a DNA fragment extending from positions $-133$ to $+36$ (i.e. $P_{guaB} (-133$ to $+36)$) was radiolabelled at the upstream end for DNase I footprinting. The results show that, in addition to the protection observed at FIS sites 1 and 2, a third site (FIS site 3) centred near position $+29$ was also bound by FIS. FIS sites 2 and 3 were identified by comparison with the consensus as being more likely to recruit FIS (i.e. FIS site 3 is a category 1 site, and FIS site 2 is a category 2 site) (Fig. 1b). However, protection of other category 1 and category 2 FIS sites that were identified by bioinformatic analysis was not observed. Interestingly, FIS site 1 contains mismatches to the consensus at two critical positions, and therefore was not identified by the bioinformatic analysis (Fig. 1b). FIS did not bind to any of the previously predicted FIS sites (FIS sites I–IV). It should be noted that another FIS site (site 2`) is located overlapping site 2, with its centre shifted by one base pair downstream of the centre of site 2 (Fig. 1). As sites that contain both an A at position $-4$ and a T at position $+4$ are bound by FIS much less efficiently than are sites that lack a G at position $-7$ (or a C at $+7$) (Shao et al., 2008), it is possible that site 2` may be preferred by FIS over site 2.

Role of FIS in the regulation of transcription from $P_{guaB}$ in vitro

To determine the role of FIS in the regulation of $P_{guaB}$ multiple-round transcription reactions were performed in the presence or absence of 250 nM FIS. Transcription was measured from $P_{guaB}$ $(-253$ to $+36)$ and shorter derivatives [$P_{guaB} (-133$ to $+36)$, $P_{guaB} (-59$ to $+36)$, $P_{guaB} (-37$ to $+36)$, $P_{guaB} (-133$ to $+21)$, $P_{guaB} (-133$ to $+1)$ and $P_{guaB} (-253$ to $+10)$] (end points as indicated). Addition of FIS to the transcription reaction resulted in $\sim$8–10-fold repression of transcription from $P_{guaB} (-253$ to $+36)$. Under the same conditions, there was no repressive effect of FIS on transcription from the rrrB promoter (Fig. 4a, b). This indicates that the repression of $P_{guaB}$ afforded by FIS at a concentration of 250 nM was due to a site-specific FIS–DNA interaction. Deletion of sequences upstream of the $P_{guaB}$ UP element (i.e. $P_{guaB} (-59$ to $+36)$) did not lead to any significant change in the degree of repression afforded by FIS, confirming that putative FIS sites I–IV do not play a role in the regulation of $P_{guaB}$ by FIS. Deletion of the $P_{guaB}$ UP element [$P_{guaB} (-37$ to $+36)$] gave rise to an undetectable level of transcripts from $P_{guaB}$ in the presence of FIS, which meant that the fold repression afforded by FIS could not be

![Figure 3. Mapping the location of FIS sites at $P_{guaB}$ by DNase I footprinting. A DNA fragment containing $P_{guaB} (-253$ to $+36)$ radiolabelled at the downstream end (relative to the $guaB$ transcription start site), and a DNA fragment containing $P_{guaB} (-133$ to $+36)$ labelled at the upstream end, were employed in DNase I footprinting in the presence of different concentrations of FIS (as shown). Nucleotide positions are shown relative to the $guaB$ transcription start site, and lanes containing the G+A ladder are indicated accordingly.](http://mic.sgmjournals.org)
Control of P\textsubscript{guaB} is not required for growth rate-dependent repression of P\textsubscript{guaB}. Deletion of FIS site 3 [i.e. P\textsubscript{guaB} (−133 to +1)] led to decreased repression by FIS (approximately sixfold repression), and deletion of both FIS site 2 and FIS site 3 [i.e. P\textsubscript{guaB} (−133 to +1)] gave rise to an approximately twofold repression by FIS (Fig. 4b). As P\textsubscript{guaB} (−133 to +1) retains FIS site 1 and is still subject to some degree of repression by FIS, these results indicate that FIS sites 1–3 each contribute to repression of P\textsubscript{guaB} and that FIS site 1 can function independently of the other FIS sites. A P\textsubscript{guaB} derivative harbouring a deletion of FIS site 3 and deletion of the downstream six bases of FIS site 2 [i.e. P\textsubscript{guaB} (−253 to +10)] was subject to an approximately fourfold repression by FIS, indicating that FIS site 2 had not been completely inactivated (Fig. 4b).

**FIS is not required for growth rate-dependent control of P\textsubscript{guaB}**

GRDC of P\textsubscript{guaB} (−253 to +36) was measured in a wild-type strain background and in a strain that harboured a deletion in the fis gene. To determine whether FIS site 3 is important for GRDC of P\textsubscript{guaB} activity of P\textsubscript{guaB} (−253 to +10) was also analysed. In exponentially growing wild-type E. coli cells, the activity of P\textsubscript{guaB} (−253 to +36) increased approximately twofold with every doubling of the growth rate as, shown previously (Fig. 5a, c; Davies & Drabble, 1996; Husnain & Thomas, 2008). In an otherwise isogenic fis strain, the activity of P\textsubscript{guaB} (−253 to +36) was higher than in the wild-type at all growth rates and was more pronounced at faster growth rates (i.e. an approximately twofold increase in activity was observed at the fastest growth rate) (Fig. 5a). However, the degree of GRDC was similar to that observed in a wild-type strain (i.e. in both cases, a doubling of the growth rate corresponded to an approximately twofold increase in promoter activity) (compare plots of relative activity versus growth rate, Fig. 5c). These results demonstrate that FIS is not required for GRDC of P\textsubscript{guaB}. Although FIS appears to downregulate transcription from P\textsubscript{guaB} in vivo, deletion of FIS site 3 and part of FIS site 2 did not lead to a significant change in P\textsubscript{guaB} activity in exponentially growing wild-type cells in comparison to P\textsubscript{guaB} containing the full complement of functional FIS sites [i.e. a 1.87-fold increase in P\textsubscript{guaB} (−253 to +10) activity occurred for every doubling of the growth rate in comparison to a 1.84-fold increase for P\textsubscript{guaB} (−253 to +36) (Fig. 5a, b)]. These results suggest either that FIS site 1 is able to effect full repression in vivo (contrasting with the results obtained in vitro) or that FIS sites 1–3 may not contribute to repression of P\textsubscript{guaB} in vivo under the conditions employed, and therefore the observed FIS-dependent repression is indirect.

**FIS is not required for growth phase-dependent regulation of P\textsubscript{guaB}**

Previous studies indicate that FIS levels are elevated during the mid-exponential growth phase, and they decrease sharply as cells enter stationary phase (Appleman et al.,...
This phenomenon is responsible for the known contribution of FIS to growth-phase-dependent regulation of some promoters (Nilsson et al., 1992; Appleman et al., 1998; Mallik et al., 2006; Bradley et al., 2007). To test whether FIS-dependent regulation of $P_{\text{guaB}}$ varies with the growth phase, transcription from a $P_{\text{guaB}}$ derivative with an upstream end point of $\sim 69$ that contained all three experimentally determined FIS sites [i.e. $P_{\text{guaB}}$ ($-69$ to $+36$)] was measured at different stages of growth in a wild-type strain, and in a strain that harboured a deletion in the fis gene. This promoter derivative was chosen as it lacks the putative CRP site centred near position $\sim 117.5$ (Hutchings & Drabble, 2000). It has previously been shown that FIS represses the crp1 promoter and this may alter cellular levels of CRP (Gonzalez-Gil et al., 1998).

In accordance with the results of the GRDC experiment, the activity of $P_{\text{guaB}}$ was higher in the fis background than in the wild-type strain throughout the course of the growth cycle. In the wild-type strain, $P_{\text{guaB}}$ activity increased by nearly 40% as cells entered the mid-exponential growth phase (i.e. the promoter activity at an $\text{OD}_{600}$ of $\sim 0.15$–$0.20$ was 40% higher than the activity at an $\text{OD}_{600}$ of $\sim 0.012$). The increase in activity in a fis strain over the corresponding part of the growth curve was less marked (i.e. there was a $\sim 16\%$ increase in promoter activity). Upon entry into stationary phase, there was a gradual decrease in the promoter activity in both strain backgrounds (Fig. 6). The results suggest that $P_{\text{guaB}}$ is subject to a degree of growth-phase-dependent regulation. However, there was no significant change in the transcription activity profile during the growth cycle when comparing the two strain backgrounds.

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**Fig. 5.** GRDC of $P_{\text{guaB}}$. (a, c) GRDC of a wild-type strain (○) or a strain containing the fis::aadA allele (●), harbouring a fusion of $P_{\text{guaB}}$ ($-253$ to $+36$) to lacZ, was analysed. (b) GRDC of a wild-type strain harbouring a $P_{\text{guaB}}$ ($-253$ to $+10$)-lacZ fusion was also measured. Strains were grown at different cellular growth rates to an $\text{OD}_{600}$ of 0.34–0.45, whereupon the β-galactosidase activity was determined. The promoter activity for $P_{\text{guaB}}$ ($-253$ to $+36$) in the presence and absence of functional fis is given both in Miller units (β-galactosidase activity) and expressed as a ratio to the activity at 1 doubling per hour (relative activity). The magnitude of the gradient in plots of relative promoter activity versus doublings per hour is proportional to the degree of GRDC. Each data point represents the mean promoter activity or mean growth rate. The mean was calculated using data obtained from three independent experiments.

**Fig. 6.** Growth-phase-dependent regulation of $P_{\text{guaB}}$. A wild-type strain or a strain containing the fis::aadA allele (Δfis), each harbouring a fusion of $P_{\text{guaB}}$ ($-69$ to $+36$) to lacZ, were inoculated from a dense culture into fresh growth medium [M9 minimal medium containing 0.4% (w/v) glucose, 0.8% (w/v) Casamino acids and 5 μg thiamine ml$^{-1}$] to an $\text{OD}_{600}$ of $\sim 0.01$. Samples were taken during different stages of growth and the β-galactosidase activity was determined. The promoter activity of the wild-type strain (○) and Δfis strain (●) is given in Miller units (β-galactosidase activity). Values presented are the mean ± SD, for three independent experiments. Cell density measurements ($\text{OD}_{600}$) for single cultures that are representative of the growth curve for the wild-type strain (○) and the Δfis strain (●), were plotted on a logarithmic axis.
backgrounds. Furthermore, $P_{guaB}$ activity peaks at the time that FIS levels are expected to be at their highest, and then falls off upon entry into stationary phase when FIS levels fall (Applemen et al., 1998; Ali Azam et al., 1999). These observations suggest that FIS does not significantly influence growth phase-dependent regulation of $P_{guaB}$ under the conditions employed.

**DISCUSSION**

A previous study identified four adjacent putative FIS binding sites in the FIS sites I–IV located upstream of the UP element at $P_{guaB}$. These sites were identified by comparison of the upstream $P_{guaB}$ sequence to a published consensus sequence for FIS (Finkel & Johnson, 1992, Hutchings & Drabble, 2000). By employing sequence alignment to compare $P_{guaB}$ sequences to a more representative consensus for FIS, we have identified four candidate FIS sites that bear a closer resemblance to this consensus than FIS sites I–IV (Ross et al., 1999). Two of these sites, sites 2 (or 2′) and 3 (centred at positions +8 and +29 relative to the $guaB$ transcription start site, respectively), are protected by FIS in DNase I footprinting experiments. One of the other two candidate FIS sites overlaps the UP element, and the remaining site is located at a distance upstream of the core promoter elements (centred at −213). Neither of these sites recruits FIS as judged by DNase I footprinting. Putative FIS sites I–IV also do not bind FIS. Interestingly, we show that a site centred at position −11, which was not identified as a likely FIS site, also recruits FIS. This suggests that although bioinformatic analyses are useful when searching for sites that are bound by FIS, they may not be useful in identifying some FIS binding sites that exhibit a weak match to the consensus.

At the tyrT promoter, FIS binding to sites II and III (centred at positions −91 and −122, respectively) cooperatively affects the binding of FIS to site I centred at −71 bp upstream of the transcription start site (Lazarus & Travers, 1993; Pemberton et al., 2002). As the B-form of DNA has a periodicity of 10.6 bp, this places these three sites on the same face of the DNA helix, with the centres of sites I and II separated by 21 bp. This suggests that cooperative interactions require adjacent FIS dimers to be positioned on the same face of the DNA, two turns of the DNA helix apart. At the rrnB P1 promoter, FIS sites I–III are centred at positions −71, −102 and −143, respectively, also placing them approximately on the same face of the DNA helix. However, FIS does not bind to rrnB P1 cooperatively in the absence of RNAP, and it is noteworthy that the central positions of these sites are separated by 32 or 42 bp (i.e. not 21 bp). Interestingly, at $P_{guaB}$, FIS sites 2 and 3 are positioned 22 bp apart (centre to centre) which will also place them on the same face of the DNA helix and a similar distance apart as FIS sites I and II at the tyrT promoter. However, the centres of FIS sites 1 and 2 at $P_{guaB}$ are 18 bp apart, making it unlikely that they are located on the same face of the DNA. Therefore, it is possible that FIS binds cooperatively to sites 2 and 3, but it appears less likely that occupancy of sites 2 and/or 3 stimulates binding of FIS to site 1.

Consistent with the location of functional FIS sites, we show that FIS represses transcription from $P_{guaB}$ ~8–10-fold in vitro. Deletion of FIS site 3 results in partial relief of repression, and deletion of FIS sites 2 and 3 together further relieves repression in vitro. The residual FIS-mediated repression of the $guaB$ promoter fragment containing the +1 downstream end point is likely to occur through interactions with site 1, and would suggest that binding of to site 1 does not require cooperative interactions with FIS dimers bound to adjacent sites. The binding of FIS to site 1 is likely to sterically hinder the recruitment of RNAP to $P_{guaB}$ as observed at the crp1 promoter (Gonzalez-Gil et al., 1998). The role of FIS sites 2 and 3, which together exert the most influence on transcription from $P_{guaB}$ in vitro, is less clear, although it is likely that the role of FIS site 3 is to stimulate binding of FIS to site 2, which in turn may play more of a direct role in repression. Our results suggest that the presence of FIS should decrease RNAP binding to $P_{guaB}$. However, DNase I footprinting experiments carried out in the presence of both FIS and RNAP were inconclusive (data not shown).

Although our results demonstrate that FIS represses transcription from $P_{guaB}$ in vitro, evidence for direct repression by FIS in vivo was not obtained (i.e. deletion of FIS sites 2 and 3 did not result in increased $P_{guaB}$ activity in wild-type exponentially growing cells). This is consistent with the results of a chromatin immunoprecipitation (ChIP)-chip analysis carried out under similar conditions, in which FIS binding at $P_{guaB}$ was not detected (see supplementary data in Grainger et al., 2006). However, in a *fis* strain we observed an increase in the activity of the $guaB$ promoter in derivatives containing all three FIS sites in the presence ($P_{guaB}$ (−253 to +36)) or absence [$P_{guaB}$ (−69 to +36)] of the putative CRP site centred at −117.5. This rules out the possibility that the effect of deleting *fis* on $guaB$ promoter activity is mediated by changes in CRP abundance [FIS has been shown to modulate transcription of *crp* (Gonzalez-Gil et al., 1998)]. However, it is possible that the change in transcription activity of $P_{guaB}$ in a *fis* background occurs as a result of altered regulation of $P_{guaB}$ by a transcription factor other than CRP, for example H-NS or HU (Claret & Rouviere-Yaniv, 1996; Falconi et al., 1996) or by changes in supercoiling (Schneider et al., 1997; Weinstein-Fischer et al., 2000). Another possible explanation is that the potential relief of $P_{guaB}$ repression that occurs upon deleting FIS sites 2 and 3 is masked in vivo through an alternative compensatory regulatory mechanism. A less likely explanation, in view of the poor match to the consensus FIS binding site, is that FIS binding to site 1 mediates full FIS-mediated repression in vivo.

A previous study has shown that the $P_{guaB}$ UP element, and sequences located further upstream, are required for GRDC of $P_{guaB}$ (Husnain & Thomas, 2008). Our results show that
FIS does not play a role in GRDC at this promoter, thereby implying that a different cellular factor is required for conferring GRDC on $P_{guaB}$ (Emilsson & Nilsson, 1995; Dennis et al., 2004; Paul et al., 2004). Experiments are under way to uncover the identity of this factor(s). Our results also suggest that $P_{guaB}$ is subject to growth phase-dependent control. However, although levels of FIS protein are also subject to growth phase-dependent control, it does not appear to play an important role in growth phase-dependent control at $P_{guaB}$. Thus, the physiological role of FIS at the $guaB$ promoter remains to be elucidated.

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**REFERENCES**


Jackson, L., Blake, T. & Green, J. (2004). Regulation of *ndh* expression in *Escherichia coli* by *Fis*. *Microbiology* 150, 407–413.


