Downregulation of the *Escherichia coli* guaB promoter by FIS

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**INTRODUCTION**

The *Escherichia coli* guaB promoter (P\textsubscript{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\textsubscript{guaB} is regulated by the CRP 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\textsubscript{guaB} is strongly enhanced by an UP element (Husnain \& Thomas, 2008). P\textsubscript{guaB} also contains a putative binding site for PurR that overlaps the core promoter region, and PurR downregulates expression of guaB (Meng \textit{et al.}, 1990; Davies \& Drabble, 1996). DnaA binds to a sequence overlapping the core promoter region and also downregulates transcription from P\textsubscript{guaB} (Tesfa-Selase \& Drabble, 1996). It has also been shown that the rate of transcription from P\textsubscript{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 \textit{et al.}, 1996; Dennis \textit{et al.}, 2004). GRDC of P\textsubscript{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 \textit{et al.}, 1999). At some *E. coli* promoters, FIS activates transcription by contacting the C-terminal domain of the RNA polymerase (RNAP) \( \alpha \) subunit (\( \alpha \)CTD) (Aiyar \textit{et al.}, 2002; McLeod \textit{et al.}, 2002). FIS can also promote transcription by decreasing the negative superhelicity of DNA (Travers \textit{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 \textit{et al.}, 1998; Browning \textit{et al.}, 2000, 2004; Jackson \textit{et al.}, 2004). A previous study identified four putative binding sites for FIS that are located upstream of the P\textsubscript{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\textsubscript{Ser} and tRNA\textsubscript{Thr} (Emilsson \& Nilsson, 1995; Dong \textit{et al.}, 1996). Moreover, transcription from the fis promoter is coupled to cellular growth rate (Mallik \textit{et al.}, 2006). Cellular levels of FIS and FIS mRNA also change with the growth phase, and they increase dramatically upon entry into the mid-exponential growth phase (Appleman \textit{et al.}, 1998; Ali Azam \textit{et al.}, 1999; Mallik \textit{et al.}, 2006). In this work, we investigated whether FIS plays a role in the regulation of P\textsubscript{guaB}. We show that the putative FIS binding sites located upstream of the P\textsubscript{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. We also show that FIS is not required for GRDC of $\text{P}_{\text{guaB}}$.

**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 $\text{lacZ}$ to one of three $\text{P}_{\text{guaB}}$ derivatives: the full-length $\text{guaB}$ promoter (i.e. strain VH1000G-253), extending from positions $-253$ to $+36$ relative to the $\text{guaB}$ transcription start site [$\text{P}_{\text{guaB}}$ ($-253$ to $+36$)] (Husnain & Thomas, 2008), the $\text{P}_{\text{guaB}}$ ($-253$ 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 $\text{P}_{\text{guaB}}$ ($-69$ 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 $\text{guaB}$ transcription start site. Fusions were carried on $\lambda$ 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
**RESULTS**

**Identification of putative FIS sites at \(P_{guaB}\)**

The 15 bp consensus sequence for FIS \([5'-\text{Gnn}(c/t)(A/g)(a/t)(a/t)(T/A)(t/a)/(T/c)/(g/a))\text{mnC-3'}\) contains five highly conserved positions (underlined) that are the most significant for the recruitment of FIS (Finkel & Johnson, 1972).
positions. Five sequences were classified as category 3 sites containing bases that match the consensus at 4/5 critical positions. However, mismatches occur at one of the outermost positions. Additional sites fell into this category. Category 3 sites also contain bases that match the consensus at 4/5 critical positions, including the outer bases (positions 2 and 7) and the conserved T residue at position +3. These sequences were subdivided into three categories. Category 1 sites contain bases that match the consensus at 4/5 critical positions, including the outer bases (positions -7 and +7) that are most strongly conserved among FIS sites and which are presumed to be bound by the D helices of FIS (Shultzaberger et al., 2007). The remaining critical position (position -3) contained the alternative purine base G that occurred less frequently at that position. Two candidate FIS sites were identified that fell into this category (Fig. 1b). Category 2 sites differ from category 1 sites in having a less frequently occurring C or T residue at position -3. Two additional sites fell into this category. Category 3 sites also contain bases that match the consensus at 4/5 critical positions. However, the mismatches occur at one of the highly conserved bases that are located at the outermost positions. Five sequences were classified as category 3 sites (Fig. 1b). Two of them correspond to the previously identified putative FIS sites III and IV located upstream of P_{guaB} (Hutchings & Drabble, 2000). Putative FIS sites I and II were not identified by this analysis as they harbour bases that match the consensus at only 3/5 of the 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.](image-url)
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 ~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 $rrnB$ P1 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

![Fig. 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)
determined. Deletion of FIS site 3 [i.e. P_{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_{guaB} (-133 to +1)] gave rise to an approximately twofold repression by FIS (Fig. 4b). As P_{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_{guaB} and that FIS site 1 can function independently of the other FIS sites. A P_{guaB} derivative harbouring a deletion of FIS site 3 and deletion of the downstream six bases of FIS site 2 [i.e. P_{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_{guaB}**

GRDC of P_{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_{guaB} activity of P_{guaB} (-253 to +10) was also analysed. In exponentially growing wild-type E. coli cells, the activity of P_{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_{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_{guaB}. Although FIS appears to downregulate transcription from P_{guaB} in vivo, deletion of FIS site 3 and part of FIS site 2 did not lead to a significant change in P_{guaB} activity in exponentially growing wild-type cells in comparison to P_{guaB} containing the full complement of functional FIS sites [i.e. a 1.87-fold increase in P_{guaB} (-253 to +10) activity occurred for every doubling of the growth rate in comparison to a 1.84-fold increase for P_{guaB} (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_{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_{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\textsubscript{guaB} varies with the growth phase, transcription from a P\textsubscript{guaB} derivative with an upstream end point of −69 that contained all three experimentally determined FIS sites [i.e. P\textsubscript{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 −117.5 (Hutchings & Drabble, 2000). It has previously been shown that FIS represses the crp\textsubscript{I} 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\textsubscript{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\textsubscript{guaB} activity increased by nearly 40% as cells entered the mid-exponential growth phase (i.e. the promoter activity at an OD\textsubscript{600} of −0.15~0.20 was 40% higher than the activity at an OD\textsubscript{600} of ~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 ~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\textsubscript{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\textsubscript{guaB}. (a, c) GRDC of a wild-type strain (●) or a strain containing the fis::aad\textsubscript{A} allele (○), harbouring a fusion of P\textsubscript{guaB} (−253 to +36) to lac\textsubscript{Z}, was analysed. (b) GRDC of a wild-type strain harbouring a P\textsubscript{guaB} (−253 to +10)-lac\textsubscript{Z} fusion was also measured. Strains were grown at different cellular growth rates to an OD\textsubscript{600} of 0.34~0.45, whereupon the β-galactosidase activity was determined. The promoter activity for P\textsubscript{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.

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**Fig. 6.** Growth-phase-dependent regulation of P\textsubscript{guaB}. A wild-type strain or a strain containing the fis::aad\textsubscript{A} allele (Δfis), each harbouring a fusion of P\textsubscript{guaB} (−69 to +36) to lac\textsubscript{Z}, 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\textsuperscript{-1}] to an OD\textsubscript{600} of ~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 (OD\textsubscript{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.
DNA helix apart. At the positioned on the same face of the DNA, two turns of the sites I and II separated by 21 bp. This suggests that DNA has a periodicity of 10.6 bp, this places these three cooperatively affects the binding of FIS to site I centred DNA helix. However, FIS does not bind to also placing them approximately on the same face of the 2

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.

ACKNOWLEDGEMENTS

This work was supported by a family PhD sponsorship awarded to S.I.H., kindly provided by S. M. Husnain, and a research project grant awarded to M. S. T. by the Wellcome Trust (grant ref. 073917). We thank W. Ross, T. Gaal, H. Murray and R. L. Gourse (University of Wisconsin–Madison) for strains, plasmids and purified FIS protein. We are grateful to T. Belyaeva (University of Leeds) for advice on EMSA, and also to S. J. W. Busby and D. Browning (University of Birmingham) for strains and advice on DNase I footprinting.

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Edited by: L. S. Frost