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E.coli Fis protein activates ribosomal RNA transcription *in vitro* and *in vivo*

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An upstream activation region (UAR) contributes to the extremely high activity of the *Escherichia coli* ribosomal RNA promoter, *rrnB* P1, increasing its activity 20- to 30-fold over that of the same promoter lacking the UAR. We have used DNase footprinting to define three specific sites in the *rrnB* P1 UAR that bind Fis, a protein identified previously by its role in recombinational enhancer function in other systems. We find that purified Fis activates transcription from promoters containing these sites 10- to 20-fold *in vitro* at concentrations correlating with the filling of these sites. Three approaches indicate that Fis contributes to the function of the UAR *in vivo*. First, there is a progressive loss in the activity of *rrnB* P1–*lacZ* fusions as Fis binding sites are deleted. Second, an *rrnB* P1 promoter with a mutation in a Fis binding site has 5-fold reduced transcription activity *in vivo*, dramatically reduced Fis binding *in vitro*, and shows no Fis dependent transcription activation *in vitro*. Third, upstream activation is reduced 5-fold in a *fis*$^-$ strain. We show that rRNA promoters derepress in response to the loss of Fis *in vivo* in accord with the predictions of the negative feedback model for rRNA regulation. We find that *fis* is not essential for the function of two control systems known to regulate rRNA, growth rate dependent control and stringent control. On the basis of these results, we propose roles for Fis and the upstream activation system in rRNA synthesis.

Key words: Fis/rRNA promoter/transcriptional regulation/ upstream activation

Introduction

Ribosomal RNA synthesis is the rate-limiting step in ribosome synthesis in *Escherichia coli*. Most rRNA synthesis at higher growth rates derives from the first of two promoters (P1) in each of the seven rRNA operons (Lund and Dahlberg, 1979; deBoer and Nomura, 1979). *rrnB* P1 promoters respond to at least two control systems, growth rate dependent control, in which promoter activity varies roughly with the square of the growth rate, and stringent control, in which amino acid starvation results in severe repression of stable RNA operons (Nomura *et al.*, 1984; Lindahl and Zengel, 1986; Jinks-Robertson and Nomura, 1987; Cashel and Rudd, 1987; Gaal and Gourse, 1990). *rrnB* P1 promoters are among the strongest known in *E.coli*, with rRNA synthesis from the seven operons representing >50% of the total transcriptional activity of cells at high growth rates (Bremer and Dennis, 1987).

The *rrnB* P1 promoter has been studied extensively and can be divided into two functional domains (Gourse *et al.*, 1986). *rrnB* P1 sequences between −48 and the transcription initiation site (+1) contain the RNA polymerase binding site and are sufficient to confer growth rate dependent control, believed to be mediated by a negative feedback system (Jinks-Robertson *et al.*, 1983; Gourse *et al.*, 1985, 1986; Dickson *et al.*, 1989). Sequences upstream of −48 and extending to −154 increase the level of promoter activity ~20 to 30-fold *in vivo* and are called the upstream activation region (UAR; Gourse *et al.*, 1986; Gaal *et al.*, 1989). *rrnB* P1 promoter mutations affecting the response of the promoter to growth rate control or upstream activation have been identified (Gaal *et al.*, 1989; Dickson *et al.*, 1989). The upstream activation system is a major contributor to the exceptional strength of rRNA promoters and is the subject of this paper.

Restriction fragments containing the UAR of *rrnB*, or from the tRNA operons *tufB* or *hisR* which also have upstream activation regions, display anomalous electrophoretic mobility, suggesting the presence of bent DNA (Bossi and Smith, 1984; Gourse *et al.*, 1986; Vijgenboom *et al.*, 1988). The correlation of an unusual DNA structure with the region required for increased *rrnB* P1 transcription led us to investigate a possible role in upstream function for proteins known to bend DNA sequences to which they bind, such as Integration Host Factor (reviewed in Friedman, 1988) and Fis (Johnson *et al.*, 1987; Thompson and Landy, 1988), which might affect the curvature of *rrnB* P1 *in vivo*.

Fis is a 12 kd protein, first identified biochemically as a host factor required *in vitro* for the phage Mu *gin* and *Salmonella hin* site specific DNA inversion reactions (Kahmann *et al.*, 1985; Johnson and Simon, 1985; Koch and Kahmann, 1986; Johnson *et al.*, 1986). Fis was also found to participate in a related recombination system, *cin*, from phage P1 (Haffter and Bickle, 1987) and to stimulate the excision reaction of the λ site specific recombination system (Thompson *et al.*, 1987). Purified Fis is a specific DNA binding protein (Bruist *et al.*, 1987; Haffter and Bickle, 1987; Thompson *et al.*, 1987; Betermier *et al.*, 1989), and a consensus recognition sequence has been defined (Hubner and Arber, 1989). The *fis* gene has been sequenced and is located at 71.5 min on the *E.coli* chromosome (Johnson *et al.*, 1988; Koch *et al.*, 1988). Intracellular concentrations of Fis are significantly higher in exponentially growing cells than in stationary phase cells (Thompson *et al.*, 1987; R.Johnson, personal communication), but under the conditions tested *fis* is not an essential gene (Johnson *et al.*, 1988; Koch *et al.*, 1988).

We present biochemical and genetic evidence here that Fis is required for maximal upstream activation of the rRNA

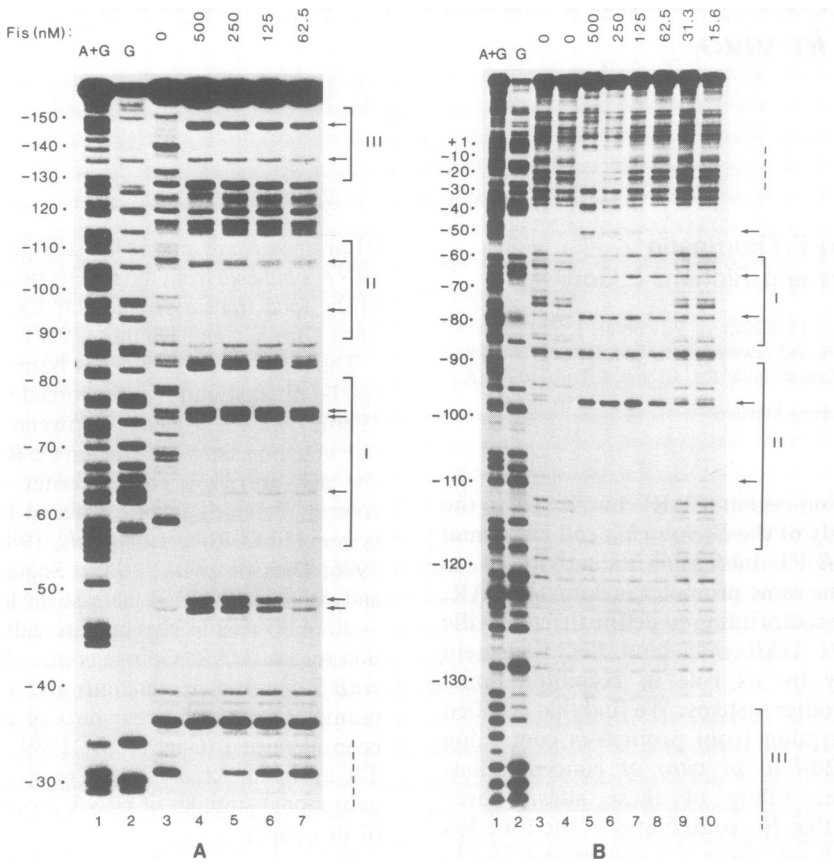

Fig. 1. DNase I footprints of Fis on a +UAR *rrnB* P1 promoter fragment. The −154 to +50 *Eco*RI−*Hin*dIII DNA fragment was 3′-end-labeled at either the *Hin*dIII linker (+50; top strand; **panel A**), or the *Eco*RI linker (−154; bottom strand; **panel B**). DNA was incubated with the indicated concentrations of purified Fis in buffer containing 150 mM NaCl, then partially digested with DNase I and electrophoresed as described (Materials and methods). Lanes 1 and 2 contain sequence markers (A+G and G) generated by the method of Maxam and Gilbert (1980). Three higher affinity Fis binding sites (I−III) are indicated by brackets, and positions of enhanced DNase I cleavage within them are indicated with arrows. Additional Fis binding sites seen at higher Fis concentrations (panel A, lane 4; panel B, lanes 5 and 6) are indicated with dashed lines. Positions of enhanced cleavage in the −50 region are also indicated.

promoter *rrnB* P1. We use DNase I footprints to show that Fis recognizes specific sites in the *rrnB* P1 UAR, and we show that purified Fis activates transcription from *rrnB* P1 *in vitro*. We define the Fis binding sites in the UAR as the targets of Fis action by correlating the DNA requirements required for transcription activation by Fis *in vitro* with the DNA limits required for maximal activity *in vivo*. In addition, we show that an *rrnB* P1 promoter with a Fis binding site mutation is defective in Fis binding and transcription *in vitro* and has reduced activity *in vivo*. Furthermore, we find that upstream activation of rRNA promoters is substantially reduced *in vivo* in *fis*$^-$ strains, but that growth rate regulation and stringent control are unaffected. Finally, we suggest possible regulatory functions for the upstream activation system. These studies, along with a concurrent publication from Nilsson *et al.* (1990) which also suggests that the *fis* plays a role in stable RNA transcription, illustrate how stable RNA operons utilize both positive and negative control elements to maintain the appropriate amount of rRNA synthesis during growth.

Results

Fis protein binds specifically to the rrnB P1 promoter

The unusual conformation of DNA containing the *rrnB* P1 upstream activating region (UAR; Gourse *et al.*, 1986) suggested that protein(s) influencing such a conformation might play a role in UAR function *in vivo*. To evaluate this possibility, we asked whether two proteins known to bend DNA with which they interact, Integration Host Factor (IHF; reviewed in Friedman, 1988) and Fis, could bind specifically to *rrnB* P1 upstream sequences. We found no evidence for specific interaction of IHF with this region (data not shown). However, we found three relatively high affinity Fis binding sites in the *rrnB* P1 UAR using DNase I footprint experiments (sites I−III; Figures 1 and 2). Sites I and III are completely protected against DNase I cleavage at 62.5 nM Fis, and protection of site II is nearly complete at this Fis concentration. Sites I and III, centered at −71 and −143, contain the degenerate consensus sequence for Fis defined by Hubner and Arber (1989; G/T--YR--A/T--YR--C/A), while Site II, centered at −102, has a one base mismatch from this consensus (Figure 2).

Approximately 25 bp are protected in each site. Two symmetrically positioned sites of enhanced DNase I cleavage within each site are separated by 11−12 bp and offset on the two strands by 2−3 bp (Figures 1 and 2). The positions of these enhancements with respect to the consensus sequence are approximately the same as those observed previously for Fis interactions with other sites (Bruist *et al.*, 1987; Betermier *et al.*, 1989). At concentrations of Fis 4- to 8-fold higher (250−500 nM), additional lower affinity sites are

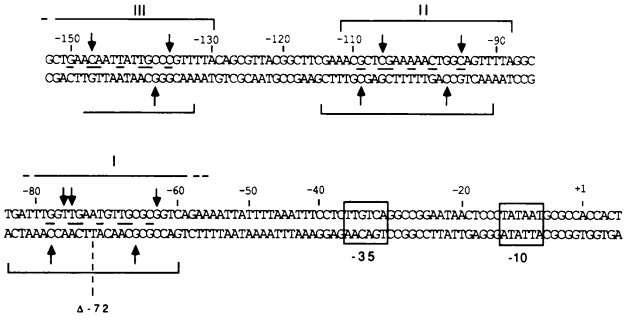

Fig. 2. Fis-protected sites within the UAR of the *rrnB* P1 promoter. The extent of protection against DNase I cleavage in three high affinity binding sites (I–III) on both top and bottom strands (Figure 1 and data not shown), is indicated by brackets above and below the sequence. Identification of some boundaries of protection is imprecise due to inefficient cutting by DNase I in these regions. Sites of enhanced DNase I cleavage are indicated with vertical arrows. (The region containing the predicted enhancement at −149 on the bottom strand was not observed on the gels due to proximity of this region to the labeled end. The degree of enhancement at different positions is variable; see Figure 1.) Positions of homology with the Fis consensus sequence (G/T--YR--A/T--YR--C/A; Hubner and Arber, 1989) in sites I–III are indicated by underlining between the two strands. The −10 and −35 promoter hexamers are boxed. The position of deletion mutation AΔ-72 (Gaal *et al.*, 1989) is indicated. Numbering of the sequence is with respect to the start of transcription (+1).

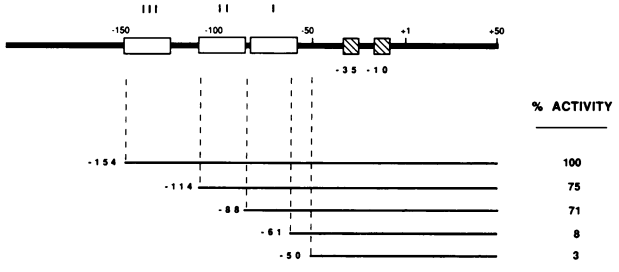

Fig. 3. Correlation of *rrnB* P1 promoter activity *in vivo* with the presence of Fis binding sites. *rrnB* P1 promoter sequence, numbered with respect to the transcription start site, +1, is represented by a thick solid line. The −10 and −35 promoter hexamers are indicated by hatched boxes, and the three high affinity Fis binding sites (I–III) by open boxes. *rrnB* sequences present in different promoter–*lacZ* fusions are represented by thin solid lines. The downstream boundary of *rrnB* P1 sequence in the fusions is +50. β-galactosidase activities of single copy fusions present on λ phage in the chromosome were determined in the NK5031 strain background (Table III) and were normalized to the activity of the −154 to +50 promoter fusion. Percentage activities indicated are the mean of two or more determinations, and standard deviations are within 10% of the indicated percentage values.

partially protected by Fis, including two apparently overlapping sites spanning the −30 to +1 region of the promoter, and a site in the A/T rich −50 region, defined only by enhanced cleavage sites at −48 to −50 (Figure 1).

Fis binding sites correlate with transcriptional activity in vivo

A role for Fis in activating transcription from the *rrnB* P1 promoter is suggested by the behavior of a series of *rrnB* P1–*lacZ* fusions containing different amounts of *rrnB* DNA upstream of the transcription start site (Figure 3 and Gourse *et al.*, 1986). Promoter fusions in which either Fis binding site III, or both sites II and III have been deleted (−114 to +50; and −88 to +50) have somewhat reduced activities (75% or 71%) when compared with a longer promoter fusion (−154 to +50). Promoters lacking all three Fis sites (−61 to +50 and −50 to +50) have only 8% and 3%, respectively, of the activity of the fusion containing the three sites (−154 to +50) (Figure 3).

Fis activates transcription in vitro from rrnB P1 promoters containing upstream Fis binding site(s)

To test whether Fis plays a direct role in transcription activation of *rrnB* P1, *in vitro* transcription reactions were carried out in the presence of purified Fis, using DNA templates containing *rrnB* P1 promoters with different amounts of upstream sequence (to −154, −88, −61 or −50) (Figure 4). At Fis concentrations in which only the high affinity sites (I–III) were occupied in footprinting experiments (62.5 and 125 nM Fis; Figure 1), 10- to 20-fold increases in transcription were observed with *rrnB* P1 promoters containing either Fis sites I–III (−154 template) or only Fis site I (−88 template). No activation of transcription was found at any Fis concentration tested (31.25 nM–500 nM) from *rrnB* P1 promoters lacking UAR Fis site(s) (−61 or −50 templates). These results correlate with the high promoter activities of the −88 and −154 promoter–*lacZ* fusions observed *in vivo*, compared with the −61 and −50 promoter fusions (Figure 3). Fis dependent transcriptional activation was not observed for other promoters tested *in vitro*, including *lacUV5* and *rrnB* P2 (data not shown) or for a transcript from the plasmid vector, thought to be RNA-1 (Morita and Oka, 1979; see Figure 4).

At higher Fis concentrations (250 and 500 nM) there was either no increase, or a significant decrease in the amount of *in vitro* transcript made from the *rrnB* P1 promoter (Figure 4 and data not shown). At these higher Fis concentrations, low affinity sites in the *rrnB* P1 promoter region, as well as the high affinity sites in the UAR, are occupied in footprinting experiments (Figure 1). The inhibition observed was independent of the presence of the upstream Fis sites (I–III), demonstrating that it is not mediated by these sites. Inhibition at high Fis concentrations was also observed for other promoters tested (*rrnB* P2, *lacUV5*, and RNA-1). We note that inhibition of λ site specific recombination *in vitro* was also observed with Fis concentrations above optimal levels (Thompson *et al.*, 1987).

Fis dependent transcriptional activation of *rrnB* P1 was greatest at the highest salt concentration tested (150 mM NaCl, Figure 4), and was also observed at 100 and 125 mM NaCl or 150 mM KCl, but not at a condition found previously to be optimal for *rrnB* P1 activity *in vitro* in the absence of Fis (30 mM KCl; data not shown). The *rrnB* P1 promoter has been shown previously to be unusually salt sensitive *in vitro* (Gourse, 1988; Leirmo, 1989; Petho *et al.*, 1986). Fis appears to alleviate this extreme salt sensitivity.

Templates containing sequences to −61, −88 or −154 are more active in the absence of Fis than the template containing sequences only to −50 (Figure 4). This factor independent effect of the upstream region on transcription is described in detail in a separate communication (S.Leirmo and R.Gourse, in preparation).

Promoters with mutations in the Fis Site I consensus sequence have reduced activities in vivo

The mutant *rrnB P1* promoter collection reported previously (Gaal *et al.*, 1989; Josaitis *et al.*, 1990) includes several mutations in the region containing Fis site I. In Table I, we

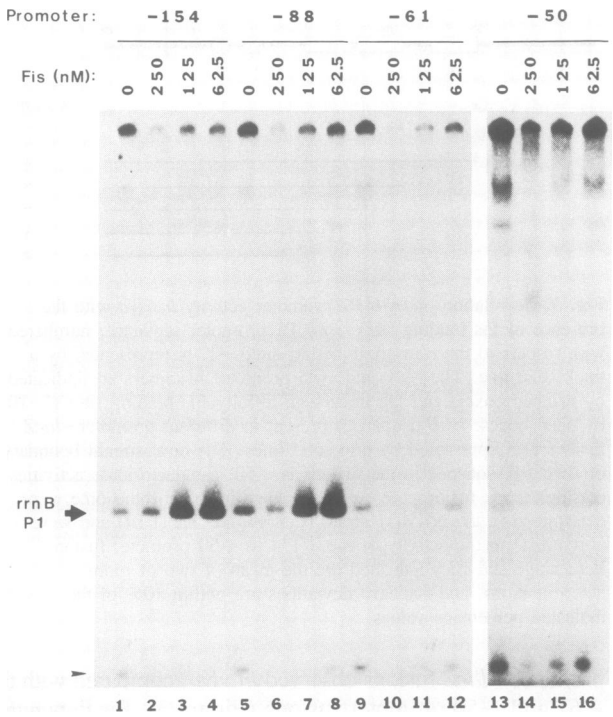

Fig. 4. The effect of purified Fis on *in vitro* transcription of *rrnB* P1 promoters with different upstream endpoints. Supercoiled plasmid DNA templates contained *rrnB* P1 promoters with the indicated upstream endpoints (−154, −88, −61 or −50) and downstream endpoints of +50, inserted upstream of an *rrnB* terminator region. The DNA templates were transcribed *in vitro* in buffer containing 150 mM NaCl and the indicated concentrations of purified Fis as described (Materials and methods). The ~220 base *rrnB* P1 specific transcript (large arrow) is absent in reactions carried out with the plasmid lacking a promoter insert (pRLG770, data not shown). The smaller arrow indicates a vector derived transcript believed to be RNA-1 (Morita and Oka, 1979). Lanes 13−16 were autoradiographed approximately 6-fold longer than lanes 1−12 to permit visualization of the less abundant −50 promoter transcript (see text).

list mutations from our collection upstream of position −61, the endpoint of the promoter fusion that failed to be activated by Fis (see above). There is a reasonable correlation between the effects of the mutations on promoter activity *in vivo* and their effects on the Fis consensus sequence. In general, single substitutions away from the consensus (G-67C, G-74C) have a smaller effect than single deletions which alter the spacing between the consensus elements (GΔ-65, GΔ-67, AΔ-72, GΔ-74). Similar mutations in the Fis sites of the phage P1 *cin* enhancer reduce or abolish recombinational enhancer function (Hubner and Arber, 1989). Single base deletions or sustitutions just upstream of Fis site I have little or no effect. We speculate that the relatively small effects of some mutations just downstream of Fis site I might be attributable to effects on factor independent activation (see above and Discussion).

A promoter with a Fis site mutation is defective in Fis binding and Fis dependent transcription activation in vitro

While the correlation between Fis consensus sequence and mutant promoter activity is suggestive of a role for Fis *in vivo*, we wished to test directly the Fis binding capacity of a representative member of the group of mutant *rrnB* P1 promoters. A single base deletion at −72 [AΔ-72, 18% of wild-type activity *in vivo* (Gaal *et al.*, 1989)] was compared with a wild-type promoter in a DNA restriction fragment binding experiment with purified Fis. The electrophoretic mobility of a very short *Eco*RI−*Dra*I restriction fragment containing Fis Site I (*rrnB* P1 sequence coordinates −88 to −46) is partially retarded at 3.9 nM Fis, and quantitatively retarded at 62.5 nM Fis and higher (Figure 5). In contrast, the mutant *Eco*RI−*Dra*I fragment shows partial retardation only at 64-fold higher Fis concentration (250 nM). At this Fis concentration, interaction of Fis with the much lower affinity sites in the −46 to +1 fragment also occurs.

Fis also alters the mobility of the two labeled M13 restriction fragments present in the DNAs analyzed in this experiment. A 500 bp M13 fragment (M13mpl8 sequence coordinates 6284−6784) interacts with Fis at concentrations 1.0 nM and higher, while a 1608 bp M13 fragment (4622−6230) interacts with Fis only at higher Fis concentrations. Binding to the 500 bp M13 fragment may reflect interaction with a site previously noted in a region of pUC18 DNA containing some of the same sequences (Haffter and Bickle, 1987).

As predicted from the fact that the AΔ-72 mutant is defective in Fis binding, transcription from a template containing this mutant *rrnB* P1 promoter is not activated by Fis *in vitro*, and the residual transcription is inhibited at high Fis concentrations (Figure 6). The amount of transcript obtained from the mutant and wild-type promoters in the absence of Fis is comparable. These findings confirm that binding of Fis to Site I confers the ability of Fis to activate transcription from this promoter. Together with the

Table I. Effect of mutations within Fis site I in the *rrnB* P1 promoter on the Fis consensus sequence and on promoter activity

Mutation[a]	Effect on Fis consensus (−64 to −78)[b]	*In vivo* activity (% of wild-type activity)[c]
A. Single base deletions		
GΔ-62	No effect	43
GΔ-65	Alters	22
GΔ-67	Alters	24
AΔ-72	Alters	18
GΔ-74	Alters	30
GΔ-83	No effect	94
B. Single base substitutions		
G-67C	Alters	44
A-72T	No effect	96
G-74C	Alters	49
T-75C	No effect	108
G-77T	No effect[d]	56
T-84C	No effect	95
C-85T	No effect	85
G-87C	No effect	87

[a]Mutations were reported either in Gaal *et al.* (1989) or for GΔ-67 in Josaitis *et al.* (1990).
[b]The Fis consensus sequence, G/T--YR--A/T--YR--C/A (Hubner and Arber, 1989), is found between −64 and −78 in Fis Site I in the *rrnB* P1 UAR (Figure 1 and 2).
[c]*In vivo* β-galactosidase activities of the mutant promoter−*lacZ* fusions are from Gaal *et al.* (1989) or Josaitis *et al.* (1990) and are expressed as a percentage of the activity of the wild-type promoter of the same length (−88 to +1).
[d]Although not a consensus position, a G at this relative position within the proximal domain Fis binding site in the *hin* enhancer was partially protected against methylation by DMS (Bruist *et al.*, 1987), suggesting a role in Fis interaction.

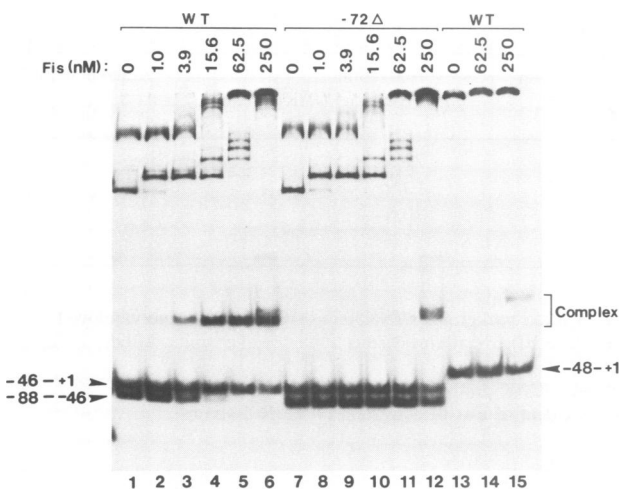

Fig. 5. Gel electrophoresis of complexes formed with purified Fis and either wild-type or mutant *rrnB* P1 promoter-containing restriction fragments. Restriction digested and ^{32}P-end-labeled M13RF DNAs containing either the +UAR wild-type promoter (lanes 1–6), the +UAR AΔ-72 mutant promoter (lanes 7–12), or the −UAR wild-type promoter (lanes 13–15) were incubated with indicated concentrations of purified Fis prior to electrophoresis on a 7% acrylamide gel. Free promoter region DNA fragments are labeled with their *rrnB* sequence coordinates, and the Fis–DNA fragment complex is indicated. The −88 to −46 fragment contains Fis binding site I (see Figure 2). The −48 to +1 −UAR fragment is larger because of a linker at −48.

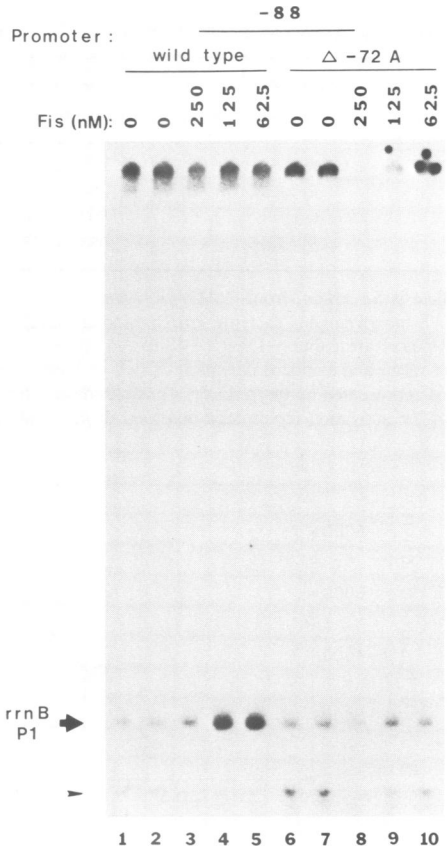

Fig. 6. *In vitro* transcription of supercoiled DNA containing the wild-type −88 to +1 *rrnB* P1 promoter (lanes 1–5) or the −88 to +1 promoter containing the AΔ-72 mutation (lanes 6–10). Transcription reactions were carried out in the presence of the indicated concentrations of purified Fis in buffer containing 150 mM NaCl as described (Materials and methods). The small arrow indicates RNA-1 transcription.

observation that this mutant promoter has 5-fold reduced activity *in vivo*, these findings indicate that Fis action affects *rrnB* P1 transcription *in vivo*.

Fis is required for maximal activation in vivo

The *in vivo* activities of *rrnB* P1 promoters with different deletion endpoints or with mutations within Fis site I correlate strongly with their ability to be activated by purified Fis *in vitro*, supporting a role for Fis in determining the strength of this promoter *in vivo*. The role of Fis in the upstream activation system was evaluated further by comparing the activities of *rrnB* P1 promoter–*lacZ* fusions containing (+UAR; −88 to +1) or lacking (−UAR; −48 to +1) the upstream region in *fis*$^+$ and *fis*$^-$ strains. The extent of activation, expressed as the ratio of β-galactosidase activities of the +UAR and −UAR *lacZ* fusions, is 22.3-fold or 10.6-fold in two different *fis*$^+$ strains (Table II, column 5), as reported previously (Gourse, 1986; Gaal *et al.*, 1989; Dickson *et al.*, 1989). However, in *fis*$^-$ derivatives of these two strains the UAR region exerts only a 3.6- or 2.3-fold activating effect (Table II, column 5). Thus, in each strain background there is an ~5-fold loss of function of the UAR region in the *fis*$^-$ strain (Table II, column 6). The *fis*$^-$ allele in these experiments contains a kanamycin resistance cassette cloned into the *fis* gene (Koch *et al.*, 1988). A comparable loss of activation was observed with a second *fis*$^-$ allele (*fis*::*767*) containing a partial deletion of the *fis* gene and insertion of a kanamycin resistance cassette (Johnson *et al.*, 1988; data not shown).

Although there is a loss of activation by the UAR in a *fis*$^-$ strain, β-galactosidase activity from the +UAR promoter–*lacZ* fusion in a *fis*$^-$ strain is nearly the same as that observed in a wild-type (*fis*$^+$) strain (Table II, column 4). The reduction in the +UAR/−UAR activity ratio observed in *fis*$^-$ strains occurs largely because of a 4–5-fold increase in the activity of the −UAR promoter (Table II, column 3). These findings are in accord with predictions of the feedback regulation system shown previously to control rRNA and tRNA synthesis (Jinks-Robertson *et al.*, 1983; Nomura *et al.*, 1984; Gourse and Nomura, 1984; Gourse *et al.*, 1985, 1986; Cole *et al.*, 1987; Yamagishi *et al.*, 1987). We interpret the increase in −UAR activity as a reflection, at least in part, of compensation by the growth rate control (feedback) system for the loss of upstream activation function from all or most host *rrn* operons (see Discussion).

Fis is not essential for growth rate dependent control or stringent control

We have argued previously that the feedback system is the mechanism responsible for growth rate dependent control of rRNA transcription (Jinks-Robertson *et al.*, 1983; Gourse *et al.*, 1985, 1986; Gaal and Gourse, 1990). If feedback derepression is responsible for the increase in −UAR–*lacZ* fusion activity in the *fis*$^-$ strain (see above), then the growth rate control system should be intact. Furthermore, Fis activates *rrnB* P1 transcription by interacting with sites in the UAR, a region not required for growth rate control (Gourse *et al.*, 1986; Dickson *et al.*, 1989). However, since low affinity Fis binding sites are present in the region required for growth rate control, we tested expression of an *rrnB* P1 promoter–*lacZ* fusion at a series of different

Table II. β-galactosidase activities of *rrnB* P1 promoters and the extent of upstream activation *in vivo* in *fis*$^+$ and *fis*$^-$ strains

Strain background	*fis* allele[a]	β-galactosidase activity[b]		Activation (+UAR/−UAR)[c]	Activation Fis$^+$ / Activation Fis$^-$[d]
		−UAR	+UAR		
NK5031	w.t.	249 ± 33	5553 ± 802	22.3	5.7
NK5031	*fis*::*kan*	1254 ± 218	4926 ± 592	3.9	
CAG4000	w.t.	507 ± 66	5378 ± 337	10.6	4.6
CAG4000	*fis*::*kan*	2054 ± 78	4690 ± 50	2.3	

[a]The *fis*$^-$ allele is *fis* :: *kan*, from CSH50*fis* :: *kan*.
[b]*fis*$^+$ or *fis*$^-$ λ monolysogens carrying *rrnB* promoter−*lacZ* fusions in either of two strain backgrounds (NK5031 or CAG4000) were employed. *rrnB* P1 promoters are either −UAR (−48 to +1) or +UAR (−88 to +1) (see Table III). β-galactosidase activities (Miller units; Miller, 1972) represent the mean and standard deivation of two or more separate determinations.
[c]Activation is represented as the ratio of β-galactosidase activities of the +UAR and −UAR promoter−*lacZ* fusions in each strain.
[d]The ratio of the activation (column 5) observed in *fis*$^+$ and *fis*$^-$ strains reflects the extent of loss of activation in the *fis*$^-$ strains.

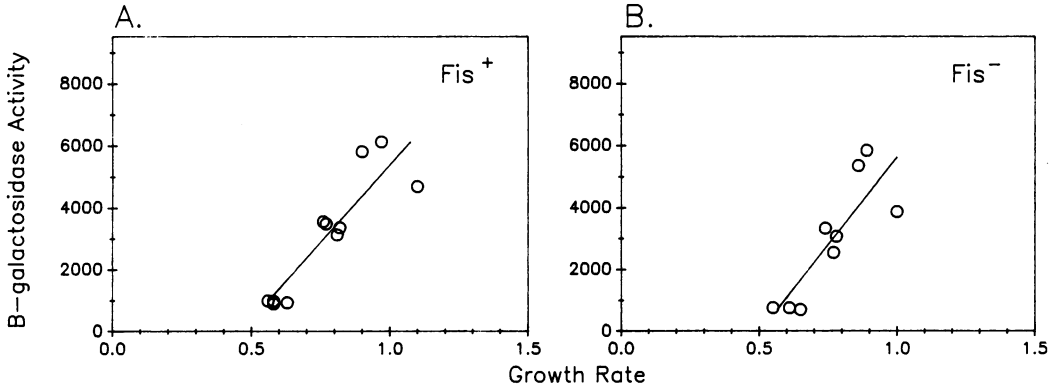

Fig. 7. Growth rate dependent control of the wild-type +UAR *rrnB* P1 promoter (−88 to +1) in isogenic *fis*$^+$ and *fis*$^-$ strains. β-galactosidase activities (Miller units; Miller, 1972) were measured in cells grown at several different steady state growth rates (cell doublings/h) using a single copy promoter−*lacZ* fusion carried on phage λ in the chromosome of either a *fis*$^+$ strain (**panel A**) or an isogenic *fis*$^-$ strain (**panel B**). Lysogens used were those in the NK5031 strain background (RLG1100, RLG1303). Determinations were from two separate experiments.

steady state growth conditions in isogenic *fis*$^+$ and *fis*$^-$ strains. Comparable growth rate dependent control of this promoter fusion was observed in the two strains (Figure 7A and B), indicating that *fis* is not essential for regulation of rRNA transcription during steady state growth. A control promoter−*lacZ* fusion (*lacUV5*), shown previously not to be growth rate regulated (Gourse *et al.*, 1986), was also found not to be growth rate regulated in the *fis*$^-$ strain (data not shown), indicating that our conditions distinguished between growth rate regulated and non-regulated promoters. The growth rates of *fis*$^-$ strains were comparable with or only slightly (5−10%) slower than those of the isogenic *fis*$^+$ strains in both strain backgrounds and with each of the different growth media used.

The *fis* gene is also not essential for function of the stringent control system. Incorporation of $^{32}PO_4$ into RNA (the majority of which is rRNA; Bremer and Dennis, 1987), was monitored in isogenic *fis*$^+$ and *fis*$^-$ strains following induction of the stringent response by isoleucine starvation. In both strains incorporation is dramatically reduced following induction, as expected for a stringent response (Figure 8A and B). An isogenic *fis*$^+$ *relA*$^-$ strain showed the expected relaxed response (Figure 8C), indicating our ability to detect relaxation of the control system. In the absence of isoleucine starvation (closed symbols, Figure 8), comparable incorporation of $^{32}PO_4$ into RNA was observed in *fis*$^+$ and *fis*$^-$ strains, further indicating that wild-type rRNA promoter activity is virtually the same when Fis is absent during steady state growth.

Discussion

Fis plays a direct role in upstream activation of rRNA transcription in vivo

Several lines of evidence indicate that Fis plays a direct role in the upstream activation of rRNA promoters. Purified Fis binds *in vitro* to three high affinity sites in the *rrnB* P1 promoter, all located within the UAR, and activates transcription *in vitro* 10- to 20-fold in a reaction requiring the presence of the UAR. Three different experimental approaches indicate that Fis contributes to UAR function *in vivo*. First, the extent of *rrnB* P1 promoter activity correlates with the presence of the Fis binding sites. Second, a mutation in Fis site I (AΔ-72) reduces Fis binding at least 64-fold, eliminates transcription activation by Fis *in vitro* (Figures 5 and 6), and decreases promoter activity 5-fold *in vivo* (Gaal *et al.*, 1989). The *in vivo* activities of promoters with other mutations in Fis site I also correlate with effects on the Fis consensus sequence (Table I). Third, upstream activation of *rrnB* P1 is reduced 5-fold in *fis*$^-$ strains (Table II).

The 5-fold effect of the loss of Fis *in vivo* (Table II) was observed using constructs containing only Fis site I. It is probable that the magnitude of the Fis effect *in vivo* would be slightly greater if measured using fusions containing sequences extending to −154, since these sequences contain Fis binding sites (Figures 1 and 2) and contribute slightly to upstream function (Figure 3).

The loss of *rrnB* P1 upstream activation function *in vivo* when the *fis* gene is inactivated or when there is a Fis binding

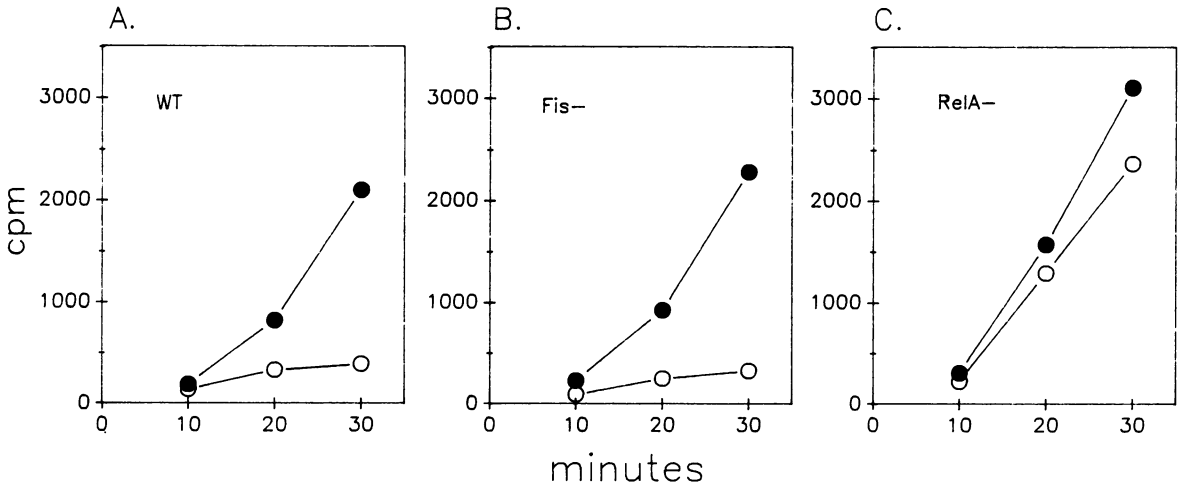

Fig. 8. Stringent control measured in isogenic wild-type, *fis*$^-$ and *relA*$^-$ strains. Cultures of CAG4000, RLG1347 and RLG1292 were grown for several generations at 30°C. Valine was added to a portion of the cultures at $t = 0$ to induce isoleucine starvation. $^{32}PO_4$ was added at $t = 5$ min and incorporation into TCA precipitable material (the majority of which is rRNA) was determined at indicated times for cultures starved (open circles) or unstarved (filled circles).

site mutation in the UAR is 5-fold. However, comparing the same promoter−*lacZ* constructs (*rrnB* P1 −88 to +1 and *rrnB* P1 −48 to +1), the UAR exerts a 10- or 20-fold effect *in vivo* in different *fis*$^+$ strains (Table II). Thus, there is 2- to 4-fold residual upstream activation in *fis*$^-$ strains. This residual activation might be attributable to an effect of the upstream DNA sequences alone, since we have found that the upstream sequences of *rrnB* P1 activate transcription *in vitro* in the absence of Fis by increasing the bimolecular step in the RNA polymerase−promoter interaction (factor independent activation; S.Leirmo and R.Gourse, in preparation). However, we cannot rule out the possibility that other cellular proteins could play a role in the activation observed in *fis*$^-$ strains.

Intracellular concentrations of Fis vary with the cell growth cycle, and are maximal in early log phase, declining as cells approach stationary phase (Thompson *et al.*, 1987; R.Johnson, personal communication). Our measurements of *rrnB* P1 promoter activity *in vivo* (Figure 3 and Tables I and II), which indicate an effect of Fis on *rrnB* P1 promoters containing an intact Fis binding site in the UAR, were made using mid-log phase cells growing under steady state conditions. Although it is likely that the β-galactosidase activities derived from promoter fusions reflect synthesis rates in steady state conditions, it is conceivable that differences in *accumulation* of β-galactosidase observed after several generations of growth still reflect differences in β-galactosidase *synthesis rates* from a time following upshift when the effects of Fis on rRNA synthesis rates might be more dramatic. Nilsson *et al.* (1990) have indicated that a UAR and Fis dependent increase in the accumulation of galactokinase from a *thrU(tufB'):galK* operon fusion might occur following dilution from stationary phase. However, it has been suggested that the rapidity of the rRNA synthesis increase upon upshift precludes a role for auxiliary proteins in this response (Gausing, 1980). Direct experiments correlating synthesis rates from *rrnB* P1 with UAR Fis site occupancy and intracellular Fis concentration are required to test roles for Fis and upstream activation during growth transitions, steady state growth and other physiological conditions.

Redundancy of systems affecting rRNA expression

A gene product affecting as critical a cell function as rRNA synthesis might be expected to be essential for cell viability, yet the construction and properties of the *fis*::*kan* alleles (Johnson *et al.*, 1988; Koch *et al.*, 1988) indicate that at least under standard growth conditions *fis* is not essential. This finding can be understood in light of our predictions and observations that the well documented feedback control system affecting rRNA synthesis can compensate for the loss of upstream function.

There is a precedent for derepression of the feedback system to compensate for defects that occur in rRNA synthesis (Sharrock *et al.*, 1985). In this case, a mutation (*nusB5*) reducing anti-termination of rRNA transcripts results in derepression of rRNA and tRNA promoters to keep cellular rRNA levels normal. If the loss of upstream activation of *rrnB* P1−*lacZ* fusions observed in a *fis*$^-$ strain (Table II) also occurs for all or most of the seven host *rrn* operons (see also below), a compensating derepression of the feedback regulation system is predicted to occur to restore rRNA synthesis to appropriate levels. Our data are consistent with the interpretation that compensating derepression is occurring: the β-galactosidase activity of an *rrnB* P1 promoter−*lacZ* fusion containing the UAR is nearly the same in *fis*$^+$ and *fis*$^-$ strains, while the −UAR promoter, since it lacks the UAR Fis sites, is subject only to the effects of derepression of the feedback system. The −UAR promoter therefore increases in the *fis*$^-$ strain (Table II). We also find that rRNA synthesis during steady state growth is normal in a *fis*$^-$ strain (Figure 8), and that growth rate control (which is proposed to be mediated by the feedback mechanism) is functional in a *fis*$^-$ strain (Figure 7). It is also conceivable that variation in Fis levels contributes to growth rate regulation, but that the feedback system is sufficient to accomplish the same result in the absence of *fis*.

Compensating rRNA synthesis in *fis*$^-$ strains raises the question as to why the upstream activation system is maintained *in vivo*. Redundancy is a common feature in bacterial gene expression, and it is not surprising that a system as central to cell survival as rRNA transcription has a means of ensuring production should one system become

dysfunctional. In fact, it was shown previously (Ellwood and Nomura, 1980) that deletion of one rRNA operon has no discernable effect on cell physiology. The data reported here indicate further the capacity for compensation of the rRNA transcription machinery. It seems likely that the cell could survive the loss of several rRNA operons, unless the tRNAs encoded by individual rRNA operons become limiting. On the other hand, there may be conditions in which maximum rRNA promoter activity requires both derepression of the feedback mechanism and upstream activation, for example, at high steady state growth rates (faster than attained in the experiments reported here) or during nutrient upshifts. In order to reach new steady state rRNA levels quickly, rRNA synthesis rates are stimulated transiently following nutrient upshifts, before reaching an increased steady state rate (Gausing, 1980). Since Fis concentrations increase dramatically early in logarithmic growth (Thompson *et al.*, 1987; R.Johnson, personal communicaton), it is reasonable that Fis could play a role in the rapid increase in rRNA synthesis rates that occurs when cells come out of stationary phase or are presented with a medium supporting faster growth rates.

We observed *rrnB* P1 inhibition at relatively high Fis concentrations *in vitro*. However, we have no evidence that Fis plays a negative role in rRNA expression *in vivo*. The negative control systems known to affect rRNA synthesis, growth rate dependent control and stringent control, both function in *fis*$^-$ strains. At this time, a negative role for Fis in ribosome regulation cannot be excluded, however.

Role of Fis in transcription of other operons

The increased activity of the −UAR *rrnB* P1−*lacZ* fusion in the *fis*$^-$ strain implies that many or most host rRNA operons are affected by the loss of Fis, resulting in compensating derepression of the feedback system. An upstream activating region has also been demonstrated for *rrnA*, and a protein factor found only in exponential phase cells was shown by band shift experiments to interact with the upstream region (Nachaliel *et al.*, 1989). This factor may well be Fis. Upstream activation regions have been demonstrated in several tRNA operons (Lamond and Travers, 1983; Bossi and Smith, 1984; van Delft *et al.*, 1987; Bauer *et al.*, 1988). These tRNA operons, as well as other rRNA operons, have a Fis consensus sequence or a single mismatch from consensus centered at −71 (+/− 1 bp) (Josaitis *et al.*, 1990). While the presence of the degenerate Fis consensus sequence is probably of little value in assessing function since it is predicted to occur rather frequently, the precise positioning of the consensus at −71 in these upstream regions is more suggestive of a functional role. In addition, there is evidence to suggest that precise positioning of the upstream region of *rrnB* P1 may be important to its function (Josaitis *et al.*, 1990).

Concurrent with completion of this work, Nilsson *et al.* (1990) reported that purified Fis protein binds to the UAR of the *tufB* operon, and that a partially purified EFTu preparation probably containing Fis binds to the UAR regions of *tyrT* and *rrnB* P1 as well. The authors concluded that Fis activates rRNA promoters on the basis that these three templates compete for binding of a common factor. Our biochemical and genetic data strongly support these interpretations.

Mechanism of rrnB P1 transcriptional activation by Fis

Fis site I in the *rrnB* P1 UAR is capable of mediating a strong upstream effect *in vivo* in the absence of sites II and III (Figure 3 and Table I). Site III, or possibly both sites II and III, may contribute to the 25−30% increase in activity of *lacZ* fusions containing these sites over that of fusions containing only site I. Activities of promoter−*lacZ* fusions containing sequences further upstream than −154 (Gourse *et al.*, 1986) indicate that additional Fis sites further upstream, if present, play little if any role in *rrnB* P1 activation.

The unusual salt sensitivity of the *rrnB* P1 promoter *in vitro* (Petho *et al.*, 1986; Gourse, 1988; Leirmo *et al.*, 1989) is to a large extent overcome by Fis. The relevance of salt sensitivity to the mechanism of this promoter and its activation is not yet understood, but we note that salt sensitivity is also found for other stable RNA promoters (Kupper *et al.*, 1976; Hamming *et al.*, 1979; Lamond, 1985).

Future experiments will address whether Fis dependent activation utilizes and potentiates the factor independent activation mechanism, whether DNA bending is involved, and whether Fis−RNA polymerase interactions play a role in Fis dependent activation. We note that direct Fis interactions with the Hin recombinase in a synaptic complex have been proposed for Fis mediated enhancement of site specific inversion (Bruist *et al.*, 1987; Johnson *et al.*, 1987; Heichman and Johnson, 1990).

Materials and methods

Bacterial strains

Bacterial strains are listed in Table III. Strains monolysogenic for λ carrying promoter−*lacZ* fusions were described previously or constructed as described (Gourse *et al.*, 1986; Gaal *et al.*, 1989). *Fis*:: *kan* derivatives of monolysogenic strains were constructed by P1 transduction (Ross *et al.*, 1986) using P1 *vir* grown on CSH50*fis*:: *kan* or on RJ1617, with selection on LB agar containing 50 μg/ml kanamycin. Transductants were obtained at a frequency (10^{-6}) comparable with that observed by Johnson *et al.* (1988). β-galactosidase activities of six independent *fis*$^-$ transductants of strains RLG1348 and RLG1350, and at least two *fis*$^-$ derivatives of other strains were determined, with good agreement in values obtained. RLG1292 was constructed by transduction of CAG4000 with P1 *vir* grown on CF1651.

Phage and plasmids

P1 *vir* was from this laboratory's collection. M13mp18 or mp19 clones carrying *rrnB* P1 promoter inserts were previously described (Gaal *et al.*, 1989). Construction of phage λ carrying promoter−*lacZ* fusions was described previously (Gourse *et al.*, 1986; Gaal *et al.*, 1989). The fusions contain promoters inserted as *Eco*RI−*Hin*dIII fragments upstream of the W205 *trp*−*lac* fusion. Plasmid pRLG770 was constructed by R.A.Sharrock by insertion of a 1 kb *Hin*dIII fragment from pPS1 (Sarmientos *et al.*, 1983), containing the *rrnB* terminator region, into the *Hin*dIII site of pKM2 (deBoer, 1984). The resultant plasmid was linearized by partial digestion with *Hin*dIII, the ends filled in with Klenow enzyme and ligated. An isolate in which the downstream *Hin*dIII site was inactivated was chosen for further work (pRLG770). Plasmid derivatives of pRLG770 contain the following promoter inserts (*rrnB* P1 unless otherwise noted) as *Eco*RI−*Hin*dIII fragments: pRLG597 (−154 to +50); pRLG589 (−88 to +50); pRLG591 (−61 to +50); pRLG592 (−50 to +50); pRLG862 (−88 to +1); pWR52 (−88 to +1 with the AΔ-72 mutation); pRLG593 (lacUV5); pWR51 (*rrnB* P2).

General methods

Plasmid DNAs were purified by standard CsCl methods, or using Qiagen columns (Qiagen, Inc.). Qiagen purified DNA was twice phenol extracted and ethanol precipitated following column purification. Restriction enzymes were obtained from New England Biolabs. *Eco*RI and *Hin*dIII restriction fragment ends were 3′-labeled by filling in with Sequenase (US

Table III. Bacterial strains

Strain	Genotype	Source or reference
NK5031	*ΔlacM5262 supF Nal*^{R}	Gaal *et al.*, 1989
CAG4000	MG1655 *ΔlacX74*	D.-J.Jin, C.Gross
CF1651	MG1655 *relA251*[a]	M.Cashel
RLG 1292	CAG4000 relA251	this work
CSH50*fis* :: *kan*	*ara Δpro-lac thi rpsL fis* :: *kan*	Koch *et al.*, 1988
RLG 1347	CAG4000 *fis* :*kan*	this work
λ Lysogens[b] in NK5031:		
RLG 731	NK5031/λ*rrnB* P1 (−154-+50)−*lacZ*	this work
RLG 732	NK5031/λ*rrnB* P1 (−50-+50)−*lacZ*	this work
RLG 733	NK5031/λ*rrnB* P1 (−88-+50)−*lacZ*	this work
RLG 954	NK5031/λ*rrnB* P1 (−114-+50)−*lacZ*	this work
RLG 957	NK5031/λ*rrnB* P1 (−61-+50)−*lacZ*	this work
RLG 1065	NK5031/λ*rrnB* P1 (−48-+1)−*lacZ*	Gaal *et al.*, 1989
RLG 1305	RLG 1065 *fis* :: *kan*	this work
RLG 1100	NK5031/λ*rrnB* P1 (−88-+1)−*lacZ*	Gaal *et al.*, 1989
RLG 1303	RLG 1100 *fis* :: *kan*	this work
λ Lysogens[b] in CAG4000:		
RLG 1348	CAG4000/λ*rrnB* P1 (−48-+1)−*lacZ*	this work
RLG 1349	RLG 1348 *fis* :: *kan*	this work
RLG 1350	CAG4000/λ*rrnB* P1 (−88-+1)−*lacZ*	this work
RLG 1351	RLG 1350 *fis* :: *kan*	this work

[a]The *relA251* allele is described in Metzger *et al.* (1989). Strain CF1651 was from M.Cashel.
[b]λ monolysogens carry promoter−*trp*−*lacZ* fusions on the phage chromosome.
Sequence coordinates of *rrnB* P1 promoters (Gourse *et al.*, 1986; Gaal *et al.*, 1989) are indicated.

Biochemicals), [α-^{32}P]dATP, (DuPont/NEN) and either 0.5 mM ddGTP (for *Hin*dIII ends) or ddTTP (for *Eco*RI ends). Fragments were purified by acrylamide gel electrophoresis (Gourse, 1988). Acrylamide gels contained TBE buffer: 90 mM Tris−borate pH 8.3, 2.5 mM EDTA.

Purified proteins were kindly provided to us: highly purified Fis protein was obtained from Regina Kahmann and Christian Koch, and from Reid Johnson. The two preparations gave similar results. RNA polymerase was from Dayle Hager and Richard Burgess, and was 40% active (+/− 10%) as determined by S.Leirmo (Gourse, 1988). IHF was from Marcin Filutowicz.

DNase I footprinting

pNO1353 DNA, containing the −154 to +50 *rrnB* P1 promoter flanked by *Eco*RI and *Hin*dIII linkers (Gourse *et al.*, 1986), was digested with either *Eco*RI or *Hin*dIII, ^{32}P-end-labeled (see above), then digested with *Hin*dIII or *Eco*RI and the promoter fragments purified. Labeled DNA (0.2 nM) was incubated for 10 min at 22°C, with varying concentrations of purified Fis in 25 μl of 10 mM Tris−Cl pH 8.0, 10 mM $MgCl_2$, either 100 mM or 150 mM NaCl, 1 mM DTT and 100 μg/ml BSA. Freshly prepared serial dilutions of Fis were made in 25 mM Tris−Cl pH 7.5, 10% glycerol and 50 mM NaCl. DNase I (Worthington) was added to a final concentration of 4 μg/ml, incubated for 30 s at 22°C, and the reaction terminated by rapid phenol extraction in the presence of 10 mM EDTA. 2.5 μg sonicated calf thymus DNA was added as carrier, the samples were ethanol precipitated, dried, resuspended in 4 μl of 0.5× TBE buffer containing 8 M urea and bromophenol blue and xylene cyanole indicator dyes, and electrophoresed on 8% acrylamide−7 M urea gels. Gels were dried and autoradiographed at −80°C using Kodak XAR-5 film and Dupont Cronex Lightning Plus intensifying screens.

In vitro transcription

25 μl reactions containing 0.2 nM supercoiled plasmid DNA were pre-incubated for 10 min at 22°C with varying concentrations of purified Fis in 10 mM Tris−Cl pH 8.0, 10 mM $MgCl_2$, either NaCl (100 mM, 125 mM or 150 mM) or KCl (150 mM), 1 mM DTT, 100 μg/ml BSA, 500 μM ATP, 50 μM CTP, 10 μM CTP, 10 μM GTP and [α-^{32}P]UTP (DuPont/NEN) at a specific activity of ~30 Ci/mmol. Reactions containing 30 mM KCl contained 40 mM Tris−acetate pH 7.9 rather than 10 mM Tris−Cl pH 8.0 (conditions used for RNA polymerase footprinting with this promoter; Gourse, 1988) and were otherwise identical to those described above. Transcription was initiated by addition of purified RNA polymerase (0.1−0.2 nM), and reactions were terminated after 15 min at 22°C by addition of 25 μl of 7 M urea, 10 mM EDTA, 1% SDS, 2× TBE, 0.05% bromophenol blue and 0.025% xylene cyanole. Equivalent aliquots of these samples were electrophoresed on 5% or 6% acrylamide−7 M urea gels containing 1× TBE for 2 h at 250 V. The relative yield of the 220 base *rrnB* P1 transcript was determined by scanning dried gels using an Ambis Radioanalytic Imaging System (Ambis Systems, San Diego, CA), and/or by densitometry of autoradiographs using a Hoefer Scanning Densitometer (Hoefer Scientific Instruments, San Fransisco).

β-Galactosidase determinations and growth rate control experiments

β-galactosidase activities (Table II) were determined as described (Miller, 1972) in cells grown in LB for approximately five generations at 30°C to an OD_{600} of 0.35−0.50. For growth rate control experiments (see Dickson *et al.*, 1989), overnight cultures grown in Medium 2 (below), were used to inoculate cultures of several different media and grown as above. The media used were: (1) minimal AB (Clark and Maaløe, 1967) with 0.4% glycerol, 20 μg/ml tryptophan, 2.5 μg/ml thiamine; (2) minimal AB or minimal A (Miller, 1972) with 0.2% or 0.4% glucose, 20 μg/ml tryptophan and 2.5 μg/ml thiamine; (3) Medium 2 with 0.1% Casamino acids; (4) LB (Miller, 1972); (5) 20% LB plus 80% medium 3. Chilled cells were combined with Z buffer (Miller, 1972), sonicated for 40 s using a Branson Sonifier, and β-galactosidase activities then determined as described (Miller, 1972).

Stringent control experiments

CAG4000, RLG1347 and RLG1292 cultures were grown in MOPS medium (Neidhardt *et al.*, 1974) containing 4 mM K_2HPO_4, 0.4% glucose, 40 μg/ml of 18 amino acids (no valine or isoleucine), 40 μg/ml uracil, and 10 μg/ml thiamine for several generations at 30°C. At an OD_{600} of ~0.15, two 0.5 ml aliquots were removed from each culture to prewarmed tubes, and either water or 400 μg/ml valine (to induce isoleucine starvation) was added to each aliquot. After 5 min, 10 μCi $^{32}PO_4$ was added to each aliquot with continued shaking. 150 μl samples were removed to 2 ml 10% cold trichloroacetic acid (TCA) at t = 10, 20 and 30 min. After at least 20 min

on ice, samples were passed through glass fiber filters, washed extensively with 5% TCA, dried and counted. The resulting values were corrected for small differences in cell number between strains.

Fragment retardation

M13 RF DNA from M13mp18 or mp19 clones carrying *rrnB* P1 promoters as *Eco*RI−*Hin*dIII inserts was extracted using a modification of the alkaline lysis procedure of Birnboim and Doly (1979) and treated with RNase A. DNAs were digested with *Eco*RI and *Hin*dIII, and ^{32}P-end-labeled as described above. +UAR promoter DNAs were then digested with *Dra*I (at *rrnB* position −46) to generate separate fragments containing the UAR (−88 to −46) and the immediate promoter region (−46 to +1). Identification of the resultant two *rrnB* fragments was confirmed by demonstrating the presence of a predicted *Hae*III site (−28) in the −46 to +1 fragment (data not shown). Digested and labeled DNAs were incubated for 10 min at 22°C with varying concentrations of purified Fis in 15 μl containing 10 mM Tris−Cl pH 8.0, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM DTT and 5 μg/ml sonicated calf thymus DNA (Sigma). Gel loading solution was added to generate a final concentration of 1× TBE, 10% glycerol with bromophenol blue, and samples were electrophoresed for 2 h at 200 V on 7% acrylamide gels with 0.5× TBE, which had been prerun for 2 h. Autoradiography was as for sequencing gels (above).

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References

Bauer,B.F., Kar,E.G., Elford,R.M. and Holmes,W.M. (1988) *Gene*, **63**, 123−134.

Betermier,M., Lefrere,V., Koch,C., Alazard,R. and Chandler,M. (1989) *Mol. Microbiol.*, **3**, 459−468.

Birnboim,H.C. and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513−1523.

Bossi,L. and Smith,D.M. (1984) *Cell*, **39**, 643−652.

Bremer,H. and Dennis,P.P. (1987) In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 1527−1542.

Bruist,M.F., Glasgow,A.C., Johnson,R.C. and Simon,M.I. (1987) *Genes Dev.*, **1**, 762−772.

Cashel,M. and Rudd,K.E. (1987) In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 1410−1438.

Clark,D.J. and Maaløe,O. (1967) *J. Mol. Biol.*, **23**, 99−112.

Cole,J.R., Olsson,C.L., Hershey,J.W.B., Grunberg-Manago,M. and Nomura,M. (1987) *J. Mol. Biol.*, **198**, 383−392.

deBoer,H.A. (1984) *Gene*, **30**, 251−255.

deBoer,H.A. and Nomura,M. (1979) *J. Biol. Chem.*, **254**, 5609−5612.

Dickson,R.R., Gaal,T., deBoer,H.A., deHaseth,P.L. and Gourse,R.L. (1989) *J. Bacteriol.*, **171**, 4862−4870.

Ellwood,M. and Nomura,M. (1980) *J. Bacteriol.*, **143**, 1077−1080.

Friedman,D.I. (1988) *Cell*, **55**, 545−554.

Gaal,T. and Gourse,R.L. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 5533−5537.

Gaal,T., Barkei,J., Dickson,R.R., deBoer,H.A., deHaseth,P.L., Alavi,H. and Gourse,R.L. (1989) *J. Bacteriol.*, **171**, 4852−4861.

Gausing,K. (1980) In Chambliss,G., Craven,G.R., Davies,J., Davis,K., Kahan,L. and Nomura,M. (eds). *Ribosomes: Structure, Function, and Genetics*. University Park Press, Baltimore, pp. 693−718.

Gourse,R.L. (1988) *Nucleic Acids Res.*, **16**, 9789−9809.

Gourse,R.L. and Nomura,M. (1984) *J. Bacteriol.*, **160**, 1022−1026.

Gourse,R.L., Takebe,Y., Sharrock,R.A. and Nomura,M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1069−1073.

Gourse,R.L., deBoer,H. and Nomura,M. (1986) *Cell*, **44**, 197−205.

Haffter,P. and Bickle,T.A. (1987) *J. Mol. Biol.*, **198**, 579−587.

Hamming,J., Gruber,M. and AB,G. (1979) *Nucleic Acids Res.*, **7**, 1019−1033.

Heichman,K.A. and Johnson,R.C. (1990) *Science*, **249**, 511−517.

Hubner,P. and Arber,W. (1989) *EMBO J.*, **8**, 577−585.

Jinks-Robertson,S. and Nomura,M. (1987) In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 1358−1385.

Jinks-Robertson,S., Gourse,R.L. and Nomura,M. (1983) *Cell*, **33**, 865−876.

Johnson,R. and Simon,M.I. (1985) *Cell*, **41**, 781−791.

Johnson,R., Bruist,M.F. and Simon,M.I. (1986) *Cell*, **46**, 531−539.

Johnson,R.C., Glasgow,A.C. and Simon,M.I. (1987) *Nature*, **329**, 462−465.

Johnson,R.C., Ball,C.A., Pfeffer,D. and Simon,M.I. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 3484−3488.

Josaitis,C., Gaal,T., Ross,W. and Gourse,R.L. (1990) *Biochim. Biophys. Acta*, in press.

Kahmann,R., Rudt,F., Koch,C. and Merlens,G. (1985) *Cell*, **41**, 771−780.

Koch,C. and Kahmann,R. (1986) *J. Biol. Chem.*, **261**, 15673−15678.

Koch,C., Vandederckhove,J. and Kahmann,R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4237−4241.

Kupper,H., Contreras,R., Khorana,H.G. and Landy,A. (1976) In Losick,R. and Chamberlin,M. (eds), *RNA Polymerase*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 473−484.

Lamond,A.I. (1985) *EMBO J.*, **4**, 501−507.

Lamond,A.I. and Travers,A.A. (1983) *Nature*, **305**, 248−250.

Leirmo,S. (1989) The mechanism of interaction of *E.coli* RNA polymerases with bacteriophage, and bacterial, promoters. Ph.D Thesis. University of Wisconsin, Madison.

Lindahl,L. and Zengel,J.M. (1986) *Annu. Rev. Genet.*, **20**, 297−326.

Lund,E. and Dahlberg,J.E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5480−5484.

Maxam,A. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499−560.

Metzger,S., Schreiber,G., Aizenman,E., Cashel,M. and Glaser,G. (1989) *J. Biol. Chem.*, **264**, 21146−21152.

Miller,J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Morita,M. and Oka,A. (1979) *Eur. J. Biochem.*, **97**, 435−443.

Nachaliel,N., Melnick,J., Gafny,R. and Glaser,G. (1989) *Nucleic Acids Res.*, **17**, 9811−9822.

Neidhardt,F.C., Block,P.L. and Smith,D.F. (1974) *J. Bacteriol.*, **119**, 736−747.

Nilsson,L., Vanet,A., Vijgenboom,E. and Bosch,L. (1990) *EMBO J.*, **9**, 727−734.

Nomura,M., Gourse,R. and Baughman,G. (1984) *Annu. Rev. Biochem.*, **53**, 75−117.

Petho,A., Belter,J., Boros,I. and Venetianer,P. (1986) *Biochim. Biophys. Acta*, **866**, 37−43.

Ross,W., Shore,S.H. and Howe,M.M. (1986) *J. Bacteriol.*, **167**, 905−919.

Sarmientos,P., Sylvester,J.E., Contente,S. and Cashel,M. (1983) *Cell*, **32**, 1337−1346.

Sharrock,R.A., Gourse,R.L. and Nomura,M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 5275−5279.

Thompson,J.F. and Landy,A. (1988) *Nucleic Acids Res.*, **16**, 9687−9705.

Thompson,J.F., Moitosa de Vargas,L., Koch,C., Kahmann,R. and Landy,A. (1987) *Cell*, **50**, 901−908.

van Delft,J.H.M., Marinon,B., Schmidt,D.S. and Bosch,L. (1987) *Nucleic Acids Res.*, **15**, 9515−9530.

Vijgenboom,E., Nilsson,L. and Bosch,L. (1988) *Nucleic Acids Res.*, **16**, 10183−10197.

Yamagishi,M., de Boer,H.A. and Nomura,M. (1987) *J. Mol. Biol.*, **198**, 547−550.

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