Regulation of rRNA Transcription Is Remarkably Robust: FIS Compensates for Altered Nucleoside Triphosphate Sensing by Mutant RNA Polymerases at Escherichia coli rrn P1 Promoters

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rRNA transcription is the rate-limiting step in ribosome synthesis and is subject to precise control by multiple regulatory systems (11, 19, 23). Since ribosome biosynthesis is an energetically expensive process, it is coupled to the cell’s nutritional status by being regulated in proportion to the cell’s growth rate (growth rate-dependent control).

Multiple mechanisms contribute to rRNA transcription initiation. The seven rRNA operons are transcribed from tandem promoters, P1 and P2, spaced about 120 bp apart (20). The P1 promoters are the targets of most of the known regulatory signals affecting rRNA transcription initiation and are responsible for growth rate-dependent regulation (17, 40). The best studied of the rrn P1 promoters, rrnB P1 (Fig. 1), consists of core promoter elements 10 and 35 bp upstream of the transcription start site, recognized by the sigma subunit of RNA polymerase (RNAP), and an UP element upstream of the −35 hexamer, recognized by the α subunit of RNAP (14, 35). In addition, there are three binding sites for the transcription factor FIS, centered at positions −71, −102, and −143 upstream of the rrnB P1 start site (37).

rrnB P1, rrnD P1, and perhaps all rrn P1 complexes with RNAP are unusually unstable. The stabilities of these promoter complexes are increased in vitro by the binding of the initiating nucleoside triphosphate (NTP) (GTP for rrnD P1 and ATP for the other six rrn P1 promoters), whose concentration increases with growth rate in vivo (15). We have suggested, therefore, that there is a kinetic competition between dissociation of the rrn P1 open complex and transcription initiation which is dependent on the concentration of the initiating NTP, leading to growth rate-dependent control of rRNA transcription (the NTP-sensing model) (7, 15). The core promoter (i.e., from about position −40 to the transcription start site) is sufficient for growth rate-dependent control of rrnB P1 and rrnD P1 transcription (6, 7).

We recently characterized two mutations, rpoCΔ215–220 and rpoBRH454 (in the genes for the β’ and β subunits of RNAP, respectively) that strongly reduce rrn P1 core promoter activity in vivo (7). The purified mutant RNAPs form less stable complexes with rrn P1 core promoters than are present in vivo. Nevertheless, the mutants grow almost as well as wild-type cells, suggesting that rRNA synthesis is not greatly perturbed. We report here that the rrn transcription factor FIS activates the mutant RNAPs more strongly than wild-type RNAP, thereby compensating for the altered properties of the mutant RNAPs. FIS activates the mutant RNAPs, at least in part, by reducing the apparent K_{m} for the initiating NTP. This and other results suggest that FIS affects a step in transcription initiation after closed-complex formation in addition to its stimulatory effect on initial RNAP binding. FIS and NTP levels increase with growth rate, suggesting that changing FIS concentrations, in conjunction with changing NTP concentrations, are responsible for growth rate-dependent regulation of rrn P1 transcription in the mutant strains. These results provide a dramatic demonstration of the interplay between regulatory mechanisms in rRNA transcription.

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RNA gene [12]), and FIS is responsible for a major component (but not all) of the growth rate-dependent regulation observed for the *leuV* promoter (32, 36).

In this study, we have investigated the effects of FIS on *rrnB* P1 transcription by the mutant RNAPs β’ Δ215–220 and β RH454 in vivo and in vitro. We conclude that the mutant strains grow nearly normally in spite of the altered properties of their transcription initiation complexes, because FIS provides almost wild-type activity and regulation to *rrn* P1 promoters. Our results suggest that *rrn* P1 promoters integrate multiple signals, including changing NTP levels and changing FIS levels, in order to regulate rRNA transcription initiation. Furthermore, our results illustrate how regulation of ribosome synthesis remains qualitatively unchanged in the face of substantial changes to the system components; i.e., the system is robust.

**FIG. 1.** The *rrnB* regulatory region, including the P1 and P2 promoters. FIS binding sites, UP elements, −10 and −35 elements, transcription start sites (+1), and the Nus factor binding site (BoxA) are indicated.

**TABLE 1.** Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference(s) or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG1655</td>
<td>Wild type</td>
<td>4</td>
</tr>
<tr>
<td>MGΔZB</td>
<td>MG1655 *lacZ145</td>
<td>42</td>
</tr>
<tr>
<td>CAG4000</td>
<td>MG1655 *lacX74</td>
<td>D. J. Jin and C. Gross</td>
</tr>
<tr>
<td>RJ1617</td>
<td>MC1000 *fix::kan767</td>
<td>22</td>
</tr>
<tr>
<td>CAG18500</td>
<td>MG1655 thi-39::Tn10</td>
<td>7</td>
</tr>
<tr>
<td>RLG3381</td>
<td>MG1655 <em>rrnB</em> P1 (−88 to +50) *lacZ</td>
<td>3, 17</td>
</tr>
<tr>
<td>RLG1829</td>
<td>MG1655 <em>rrnB</em> P1 (−154 to +50) *lacZ</td>
<td>8, 17</td>
</tr>
<tr>
<td>RLG1785</td>
<td>MGΔZB <em>rrnB</em> P1 (−88 to −37) <em>lac</em> (−36 to +57) *lacZ</td>
<td>3, 16</td>
</tr>
<tr>
<td>RLG1679</td>
<td>CAG4000 <em>rrnB</em> P1 (−88 to −37 Δ-72) <em>lac</em> (−36 to +57) *lacZ</td>
<td>16, 34</td>
</tr>
<tr>
<td>RLG1680</td>
<td>CAG18500 <em>rrnB</em> P1 (−61 to +50) *lacZ</td>
<td>7, 37</td>
</tr>
<tr>
<td>RLG3950</td>
<td>CAG18500 thi-39::Tn10</td>
<td>This work</td>
</tr>
<tr>
<td>RLG3993</td>
<td>CAG18500 <em>rrnB</em> P1 (−154 to +50) *lacZ</td>
<td>7, 37</td>
</tr>
<tr>
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<td>RLG1829 thi-39::Tn10</td>
<td>This work</td>
</tr>
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<td>CAG18500 thi-39::Tn10</td>
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<td>RLG1784 thi-39::Tn10 *rrnC Δ215–220</td>
<td>17; This work</td>
</tr>
<tr>
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<td>RLG1785 thi-39::Tn10 *rrnC Δ215–220</td>
<td>This work</td>
</tr>
<tr>
<td>RLG4397</td>
<td>RLG3398 *fix::kan767</td>
<td>This work</td>
</tr>
<tr>
<td>RLG4397</td>
<td>RLG3399 *fix::kan767</td>
<td>This work</td>
</tr>
<tr>
<td>RLG4397</td>
<td>RLG1680 thi-39::Tn10</td>
<td>This work</td>
</tr>
<tr>
<td>RLG4397</td>
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<td>This work</td>
</tr>
<tr>
<td>RLG4397</td>
<td>RLG1680 thi-39::Tn10 *rrnC Δ215–220</td>
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</tr>
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<td>RLG4397</td>
<td>RLG1679 thi-39::Tn10 *rrnC Δ215–220</td>
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<td>RLG4397</td>
<td>RLG1784 thi-39::Tn10 *rrnC Δ215–220</td>
<td>9, 17</td>
</tr>
<tr>
<td>RLG4397</td>
<td>RLG1678 <em>rrnB</em> P1 (−61 to +50) *lacZ</td>
<td>7</td>
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</tbody>
</table>

**MATERIALS AND METHODS**

**Bacterial strains and phages.** The strains used in this work are listed in Table 1. RNAP mutations and the *fus:kan* allele were moved between strains by bacteriophage P1 transduction (25). A phage monolysogens were constructed essentially as described previously (17).

**β-Galactosidase assays.** Cultures were grown at 30°C, and growth rates were varied using the media described previously (6, 7). For assays of *rrnB* P1 derivatives, strains were streaked from single colonies on plates containing media that supported growth rates lower than or equivalent to those supported by the media used in the experiment. Cells were scraped from the plate and diluted in the appropriate media, and after three to four generations of growth, mid-log-phase cultures were harvested, washed, sonicated, and assayed for β-galactosidase as described previously (7). All experiments were performed at least twice and on different days, and errors were less than 10% of the mean values.

**In vitro transcription.** Multiple-round transcription reactions were performed at 22°C as described previously (7), using a 0.2 mM concentration of a supercoiled plasmid (pRLG597) (37) containing an *rrnB* P1 promoter (positions −154 to +50), making a 220-nucleotide transcript terminated by *rrn* T1T2 terminators. The transcription buffer contained 115 mM NaCl (for Fig. 2), 100 mM NaCl (for
**RESULTS**

Upstream DNA sequences restore *rrnB* P1 transcription activity in *rrnB* mutant strains. Transcription of *rrnB* P1 promoter-*lacZ* fusions lacking FIS binding sites is severely reduced in *pcoDΔ215–220* and *pcoBRH454* cells relative to wild-type cells (7, 15). However, the mutant strains grow reasonably well (doubling times are 80% to 90% of that of the wild type), suggesting that rRNA synthesis is not strongly perturbed. Since *rrnB* P1 promoters in their natural context are activated by FIS (37), we tested whether the presence of their normal FIS sites would rescue the defects in transcription exhibited by *rrnB* P1 core promoter-*lacZ* fusions in the mutant strains.

We compared the activities of *rrnB* P1-*lacZ* fusions without FIS sites (positions −61 to +50), with only the proximal FIS site (−88 to +50), and with all three FIS sites (−154 to +50) in the wild-type and mutant strains (Table 2). The proximal FIS site increased transcription 3.8-fold, and three FIS sites increased transcription 14-fold, restoring almost full *rrnB* P1 promoter activity. Thus, the defect in transcription caused by the mutant RNAPs is much greater for *rrnB* P1 promoters lacking FIS sites than for *rrnB* P1 promoters containing FIS sites.

**FIS is responsible for the effect of upstream sequences on *rrnB* P1 promoter activity in the *pcoDΔ215–220* mutant.** In the previous section, we showed that upstream DNA sequences containing FIS sites compensate for the negative effect of the *pcoD* and *pcoP* mutations on *rrnB* P1 transcription. Consistent with the conclusion that activation by FIS was responsible for this effect of the upstream sequences in the *rrnB* mutant strains, transcription of an *rrnB* P1 promoter containing all three FIS sites dropped by about 60% in a *fis::kan pcoDΔ215–220* double mutant compared to the *pcoDΔ215–220* single mutant (Table 3). The requirement for FIS for the effect of the upstream sequence was further confirmed by measuring expression from an *rrnB* P1-*lacZ* fusion containing a single-base-pair deletion in FIS site I that eliminates FIS binding (−88 Δ−72 to +50) (37). This upstream sequence did not stimulate *rrnB* P1 transcription in the *rrnB* mutant strain (data not shown).

In contrast to the reduced transcription from *rrnB* P1 observed in the *fis::kan pcoDΔ215–220* double mutant strain, deletion of the *fis* gene did not reduce *rrnB* P1 transcription substantially in the wild-type strain (Table 3), consistent with our previous reports (34, 37). This apparent paradox results from an increase in *rrnB* P1 core promoter activity in the *fis::kan* mutant (34, 37). We have attributed this increase in *rrnB* P1 core promoter activity to the homeostatic nature of the regulatory system(s) controlling *rrnB* P1 transcription; i.e., rRNA transcription is feedback regulated such that disruptions that reduce ribosome synthesis increase *rrnB* P1 core promoter activity (19, 21). In the *rrnB* mutant strain, this feedback response was not able to increase transcription from the *rrnB* P1 core promoter enough to compensate fully for the loss of the *fis* gene (see Discussion).

Although *rrnB* P1 promoter activity decreases by about 60% in the *fis::kan pcoDΔ215–220* double mutant compared to the *pcoC* single mutant, the double mutant grows only about 10% slower than the *pcoDΔ215–220* strain; i.e., the small growth defect of the *pcoC* mutant strain is exacerbated only slightly by the *fis* mutation (Table 3). To account for the double mutant strain’s relative vigor, other mechanisms must increase rRNA transcription to compensate for the reduced activity of *rrnB* P1 promoters (see Discussion).

**Activation of β′ Δ215–220 RNAP by FIS in vitro.** The presence of FIS binding sites results in high *rrnB* P1 promoter activity in the *pcoDΔ215–220* strain. To further confirm that the increased activation by FIS in vivo was direct, we examined *rrnB* P1 transcription in vitro in the presence of purified RNAP.
and FIS. Under these conditions (see Materials and Methods), FIS increased transcription by the wild-type RNAP about 2.0-fold (Fig. 2, lanes 1 and 2), while it increased transcription by the β’ Δ215–220 RNAP about 12-fold (Fig. 2, lanes 3 and 4), resulting in similar overall promoter activity with the two enzymes (Fig. 2, lanes 2 and 4). Thus, as predicted from the results obtained in vivo, FIS directly compensates for the defect of the mutant RNAP by activating the mutant enzyme to a greater extent than the wild-type enzyme.

**Activation of transcription from the lac core promoter by FIS in the rpoCΔ215–220 mutant.** Although the mutant RNAPs formed less stable open complexes than wild-type RNAP at all promoters tested (7, 18; M. M. Barker, T. Gaal, and R. L. Gourse, unpublished data), they reduced transcription of only those promoters that formed intrinsically unstable open complexes with wild-type RNAP (e.g., *rrnB P1*). We predicted that the increased extent of activation by FIS observed in the mutant strains would be limited to promoters with kinetic properties similar to those of *rrnB P1*. To test this hypothesis, we measured transcription from a hybrid *rrnB-lac* promoter which was shown previously to be activated by FIS in a wild-type strain (3). We compared the activity of the hybrid promoter (which contains FIS site 1 and the UP element from *rrnB P1* fused to the lac core promoter) to that of an identical promoter with a Δ72 FIS site (which eliminates FIS binding) (37) in the rpoCΔ215–220 and wild-type strains. FIS activated the *rrnB-lac* hybrid promoter to approximately the same extent in both the wild-type and the mutant strains (3.9- versus 3.0-fold) (Table 4), in contrast to its differential effect on the *rrnB P1* promoter in the same two strains (Table 2). This result is consistent with the hypothesis that the differential effect of FIS in the wild-type strain versus the *rpoC* mutant strain is a function of a kinetic property of the promoter interaction with RNAP, presumably the intrinsic instability of the open complex.

**FIS reduces the concentration of the initiating NTP needed for *rrnB P1* transcription in vitro.** We next attempted to determine how FIS is able to activate *rrnB P1* transcription by the mutant RNAPs to a greater extent than by the wild-type RNAP (Table 2; Fig. 2). Transcription initiation at *rrnB P1* (and at other promoters) involves a series of steps in which RNAP first forms a closed complex that then isomerizes through a series of intermediates to an open complex capable of transcription (34). We showed previously that FIS increases closed-complex formation (8). However, closed-complex formation at *rrnB P1* is similar with mutant and wild-type RNAPs in the absence of FIS (7), suggesting that this might not be the step responsible for the differential effect of FIS on the mutant RNAPs. On the other hand, the mutant and wild-type RNAPs do differ in the stability of the open complexes they form at *rrnB P1*, which results in an increase in the required initiating NTP concentration for transcription with the mutant enzymes (7, 15). We therefore tested whether FIS might reduce the apparent **K<sub>ATP</sub>** for the initiating nucleotide in the mutants.

We measured the effect of FIS on transcription by β’ Δ215–220 RNAP at different ATP concentrations, using conditions that resulted in enough transcription in the absence of FIS for accurate measurement even at low ATP concentrations (Fig. 3A). FIS increased transcription by β’ Δ215–220 RNAP at all ATP concentrations under these conditions but did so the most at low ATP concentrations (Fig. 3A).We expressed transcription as a fraction of that obtained at a saturating ATP concentration (2 mM) in order to calculate apparent **K<sub>ATP</sub>** values for transcription by the mutant RNAP in the presence and absence of FIS (Fig. 3B). FIS greatly reduced the apparent **K<sub>ATP</sub>** needed for transcription initiation (from about 330 μM in the absence of FIS to about 60 μM in the presence of FIS). We conclude that FIS can affect both closed-complex formation (8) and a later step in transcription initiation. FIS thereby compensates for the decreased transcription exhibited by the mutant RNAP on *rrn P1* promoters in vitro in part by reducing the concentration requirement for the initiating NTP.

We also determined the effect of FIS on the apparent **K<sub>ATP</sub>** for transcription by the wild-type RNAP (Fig. 3C and D). FIS also greatly reduced the apparent **K<sub>ATP</sub>** for transcription by wild-type RNAP (from about 240 μM in the absence of FIS to about 30 μM in the presence of FIS). We conclude that FIS can facilitate transcription by both the wild-type and the mutant RNAPs in vitro by reducing the apparent **K<sub>ATP</sub>**.

**We emphasize that the salt concentrations in the buffers used for transcription with the two RNAPs were not identical in these experiments (see Materials and Methods), nor do we presume that these solution conditions are similar to those present in growing cells. Thus, the apparent **K<sub>ATP</sub>** for transcription by the two enzymes should not be compared directly, nor should they be considered the absolute **K<sub>ATP</sub>** for transcription initiation in vivo (see Discussion).**

**FIS is responsible for normal growth rate-dependent control of *rrnB P1* transcription in rpoCΔ215–220 and rpoB<sub>HR454</sub>**

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**TABLE 4. Effect of rpoCΔ215–220 on activation of the lac core promoter by fis**

<table>
<thead>
<tr>
<th>RNAP allele</th>
<th>Promoter</th>
<th>FIS site</th>
<th>β-Galactosidase activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Activation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td><em>rrnB (Δ-72-lac)</em></td>
<td>−</td>
<td>1.878</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>rrnB-lac</em></td>
<td>+</td>
<td>7,359</td>
<td>3.9</td>
</tr>
<tr>
<td>rpoCΔ215–220</td>
<td><em>rrnB (Δ-72-lac)</em></td>
<td>−</td>
<td>2,560</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>rrnB-lac</em></td>
<td>+</td>
<td>7,614</td>
<td>3.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results (in Miller Units) are means from two experiments; the variation was less than 5%.
mutants. We previously established that transcription of \( rnb \) P1 promoters lacking FIS sites is growth rate dependent in wild-type or in \( fis::kan \) strains (6, 15, 17, 37) but that growth rate-dependent regulation is substantially reduced in the \( rpoB \) and \( rpoC \) mutant strains (7, 15). We proposed that the mechanism responsible for this regulation involves, at least in part, \( rrn \) P1 sensing of the initiating NTP concentration in vivo, consistent with the altered NTP-sensing properties of complexes containing the mutant RNAPs observed in vitro (7, 15).

In the experiments shown in Fig. 4, we compared growth rate-dependent regulation of \( rnb \) P1 promoters lacking FIS sites with that of \( rnb \) P1 promoters containing FIS sites in the RNAP mutant strains. The presence of FIS sites not only increased \( rnb \) P1 promoter activity at high growth rates (as shown in Table 2) but also resulted in nearly normal growth rate-dependent regulation of \( rnb \) P1 (Fig. 4D to F). We conclude that FIS is an essential contributor to regulation of \( rnb \) P1 promoters in the RNAP mutant strains, although it is not essential for this purpose in wild-type cells.

Previous studies from our lab and others have demonstrated that the FIS concentration and the level of FIS-dependent activation of the \( rnb \) P1 promoter vary with growth conditions in wild-type cells (3, 5, 29, 33, 44). To determine whether changing FIS levels could contribute to growth rate-dependent regulation of \( rnb \) P1 transcription in the RNAP mutant strains, we examined FIS levels in cells grown in different media by using Western blot analysis with an anti-FIS antibody (Fig. 5). We found that FIS levels increased with growth rate similarly in the wild-type and \( rpoC \Delta 215-220 \) mutant strains; i.e., the \( rpoC \) mutation did not alter the expression of FIS. Thus, differential FIS-dependent activation with growth rate could contribute to the ability of FIS to restore growth rate-dependent regulation to \( rnb \) P1 promoters in the RNAP mutant strains.

We showed above that FIS activates \( rnb \) P1 transcription by...
the β’ mutant RNAP in vitro in part by reducing the apparent ATP concentration required for transcription initiation, and we suggested that this brings the initiating $K_{NTP}$ into a range sufficient for transcription by the mutant RNAP in vivo. Assuming that ATP and GTP concentrations change in the RNAP mutant strains as they do in the wild-type strain (15), we conclude that changing initiating NTP concentrations, in addition to changing FIS concentrations, likely contribute to growth rate-dependent regulation of $rrn$ transcription in the mutant strains.

**DISCUSSION**

**Rescue of mutant RNAP function by FIS.** We found previously that the intrinsic instability of $rrn$ core promoter complexes is responsible for their regulation with changing initiating NTP concentrations and for their sensitivity to the destabilizing effects of mutant RNAPs (7, 15). In the work presented here, we propose that this intrinsic instability, exacerbated by the mutant RNAPs, leads to the increased extent of activation by FIS in the mutant strains. The increased activation by FIS accounts for the almost normal $rrn$ transcription and growth rate-dependent regulation observed in the mutants.

In theory, FIS could increase $rrn$ transcription with a mutant RNAP by increasing open-complex formation ($K_{c}$), by decreasing open-complex dissociation, by decreasing the NTP concentration required for transcription initiation, or by some combination of these effects. We have shown previously that FIS activates transcription of $mmb$ P1 by wild-type RNAP in part by increasing the equilibrium constant for closed-complex formation (i.e., by increasing $K_{c}$) (8) through direct contacts with the C-terminal domain of the RNAP α subunit (9). However, since the mutant RNAPs and the wild-type RNAP had similar equilibrium binding constants for closed-complex formation in the absence of FIS (7) and since the mutations are unlikely to affect the interaction between FIS and the C-terminal domain of the RNAP α subunit directly, we suggest that the differential effect of FIS on the mutant versus wild-type RNAPs is not likely to occur at this initial RNAP binding step. Rather, the differential effect of FIS on the $rrn$ complex containing the mutant RNAP is most likely attributable to an effect on a step after initial closed-complex formation. Since FIS reduces the apparent $K_{NTP}$ for binding of the initiating ribonucleotide, which occurs in the open complex, we propose that FIS stabilizes an intermediate concurrent with or after strand opening, in addition to its effect on closed-complex formation described above. This proposal is consistent with the larger effect of FIS on the short-lived open complexes formed by the mutant RNAPs than on complexes containing the wild-type RNAP and with the fact that increasing the RNAP concentration did not alter the NTP concentration requirement for $rrn$ transcription in vitro (T. Gaal, W. Ross, and R. L. Gourse, unpublished data). A role for FIS in kinetic steps after closed complex formation has been proposed previously for other promoters ($mmd$ P1 [39] and $tyrT$ [26]). The effect of FIS on $mmb$ P1 that we have described here and that proposed for FIS on $mmd$ P1 and $tyrT$ could have a similar mechanistic basis. However, our results do not rule out the possibility that FIS could facilitate still other steps in the transcription mechanism. In addition, we note that some effects of FIS differ from those resulting from UP element-α interactions, since the latter did not alter the ATP concentration requirement for $mmb$ P1 transcription (Gaal et al., unpublished data).

**FIG. 4.** Effect of FIS on growth rate-dependent regulation of $mmb$ P1 transcription in wild-type (A and D), $rpoC$Δ215-220 (B and E), and $rpoBRH454$ (C and F) strains. (A to C) Transcription from the $mmb$ P1 promoter without FIS sites (−63 to +50). (D to F) Transcription from the $mmb$ P1 promoter containing three FIS sites (−154 to +50). Promoter activities were determined from β-galactosidase activities of promoter-lacZ fusions. Growth rates of cultures were varied as described previously (7).
In the mutant strains, but not in the wild type (6, 37) FIS is essential for efficient transcription of rmP1 promoters and for their regulation with growth rate. We ascribe this role in regulation in part to changing FIS concentrations with growth rate (Fig. 5) and thus to differential occupancy of the rmP1 FIS sites. In addition, since FIS brings the apparent K_eq of the mutant RNAP into the range where changes in NTP concentrations would most likely affect rmP1 transcription, FIS regulation in the mutant strains also would be accomplished in part through the changes in NTP concentrations that accompany changes in growth rate.

We do not propose that the apparent K_atp derived from the in vitro transcription experiments reported here are the absolute binding constants in vivo; the apparent K_atp derived from these experiments are far below the millimolar ATP concentrations present in vivo (reference 15 and references therein). However, the ATP concentration needed for rmP1 transcription initiation in vitro is extremely sensitive to cation concentration and to template supercoiling (15), both of which greatly affect the lifetime of the open complex, which is crucial in determining the K_eq (15). In fact, maximal rmP1 transcription in vitro requires initiating NTP concentrations that are in the millimolar range when the reactions are performed with high salt concentrations and/or on linear templates. The relevance of the relative K_atp determined for rmP1 transcription in vitro to transcription in vivo is supported strongly by the effects of artificial manipulation of ATP and GTP concentrations in vitro and in vivo (15) and by the correlation between the behaviors of the mutant RNAPs in vitro and in vivo (7, 15).

Role of FIS in growth rate-dependent control in wild-type strains. We have shown previously that deletion of the rmB P1 FIS sites or disruption of the fis gene has little effect on growth rate-dependent regulation of rmB P1 transcription in wild-type strains (6, 37). Furthermore, the high occupancy of rmP1 promoters with wild-type RNAP when NTP levels are maximal limits the potential for stimulation by FIS at high growth rates (Table 2) (J. A. Appleman, T. Gaal, M. S. Bartlett, W. Ross, and R. L. Gourse, unpublished data). Although seemingly paradoxical, the ability of FIS to rescue regulation of rmP1 promoters in the mutant strains and yet to be dispensable for this purpose in wild-type strains is consistent with the results described above: the kinetic characteristics of the rmB P1 complex are different in wild-type and RNAP mutant strains. FIS can thus affect the mutant RNAPs more than the wild-type RNAP and can confer growth rate-dependent regulation on rmP1 transcription in the mutant strains. Nevertheless, the changing FIS concentrations that occur with growth rate in both wild-type and mutant strains and the effect of FIS on the apparent K_atp of both the wild-type and mutant initiation complexes suggest that FIS could potentially contribute to the regulation of rmB P1 transcription under some conditions in wild-type strains, in conjunction with other regulatory mechanisms.

The data presented here also reinforce the previously recognized importance of FIS in growth rate-dependent control of other promoters. Some promoters are likely to owe their growth rate-dependent regulation almost entirely to changing FIS levels. Candidates for such promoters would be some tRNA promoters (13, 27) and the promoter for the 4.5S RNA (12), all of whose regulation is almost completely lost when FIS sites are deleted or in strains lacking the fis gene. Growth rate-dependent control of some other promoters may be attributable to the combined effects of changing FIS levels and changing NTP concentrations (or to the effects of other mechanisms). Candidates for such promoters would be those tRNAs whose growth rate-dependent regulation is aberrant, but not completely lost, in the absence of FIS sites or in strains lacking the fis gene. Growth rate-dependent control of some promoters is likely to be independent of FIS. Candidates for such promoters would be tRNAs whose regulation is completely unaffected in fis::kan strains or which contain no FIS binding sites (13, 27).

Growth rate-dependent control of FIS levels. The control of FIS expression has been studied extensively, and it appears to be complex. The promoter region of the fis operon contains binding sites for known regulatory proteins (integration host factor, FIS, and cyclic AMP receptor protein), and at least some of these clearly affect expression (5, 33, 44). In addition, the FIS promoter is sensitive to ppGpp levels; i.e., it displays a stringent response dependent on the relA gene product (30), and at least some of these clearly affect expression (5, 33, 44). In addition, the FIS promoter is sensitive to ppGpp levels; i.e., it displays a stringent response dependent on the relA gene product (30), and at least some of these clearly affect expression (5, 33, 44). In addition, the FIS promoter is sensitive to ppGpp levels; i.e., it displays a stringent response dependent on the relA gene product (30), and at least some of these clearly affect expression (5, 33, 44).
for growth rate-dependent regulation of the FIS promoter differs from that for rrn P1 promoters. Further studies will be required to understand whether growth rate-dependent regulation of FIS expression is determined primarily at the transcription level and, if so, whether transcription of the fis gene is affected by changing NTP concentrations.

**rRNA transcription regulation is robust.** Our results emphasize the resiliency of bacterial cells to the effects of mutation; i.e., like the bacteriophage lambda genetic switch (24), the rRNA transcription system is extremely robust. We have reported previously that although FIS activates rRNA transcription (as determined from the effects of FIS binding site mutations reported previously), deletion of the fis gene does not reduce rRNA synthesis (34, 37). This apparent contradiction can be explained by an observed increase in cis elements and interactions with the C-terminal domain of the RNA polymerase at the fis promoter (22). In the case of fis genes, the site mutations do not have pleiotropic effects on cell metabolism that complicate interpretation of transcriptional outputs.

We report here that not only does the cell compensate for the loss of FIS-dependent activation caused by fis gene mutations, but conversely FIS compensates for altered NTP sensing at rrn P1 core promoters caused by rpoB or rpoC mutations. Thus, FIS and NTPs can each regulate rrn P1 transcription independently, but changes in one can alter effects resulting from the other.

The mechanism(s) responsible for feedback regulation of rRNA transcription has not been identified. It is possible that multiple mechanisms could contribute to this homeostatic regulation, with different mechanisms responding to particular stimuli. We have proposed previously that adjustments in cellular NTP levels could provide one such mechanism for feedback control of rRNA transcription (15). We are currently investigating whether changes in cellular NTP levels could account for the increase in rrn P1 core promoter activity observed in the fis'kan strain. However, since FIS apparently plays a role (direct or indirect) in additional cell functions that affect rRNA transcription (e.g., see references 41 and 43), identifying the specific mechanism(s) responsible for the feedback response of rrn P1 promoters that results from the loss of the fis gene presents a complex challenge for the future.

Since double mutants containing deletions of the fis gene and rpoCΔ215–220 have rrn P1 promoter activity reduced by 60% yet display growth defects only slightly greater than cells mutated in either single gene (Table 3), another regulatory mechanism must prevent rRNA underproduction under these circumstances. Furthermore, although rrnB P1 transcription was reduced in the fis'kan rpoCΔ215–220 double mutant, we note that this 60% reduction in transcription did not result in transcription as low as that of an rrnB P1 promoter lacking FIS sites in the RNAP mutant strain. Thus, an unidentified mechanism may also be responsible for partial derepression of the rrnB P1 promoter in the double mutant. Our results illustrate how potential components of the rRNA transcription machinery can be unmasked when other mechanisms contributing to transcription are eliminated.

In summary, our present view is that regulation of rRNA transcription is affected by multiple trans-acting factors that regulate rrn P1 promoter activity, e.g., FIS, NTPs, and ppGpp (10, 15, 18). However, regulatory roles for additional cis- and trans-acting factors cannot be excluded. For example, the rrn P2 promoters likely play a crucial role in upshifts (40; Appleman et al., unpublished data). Nus factors are required to prevent premature rRNA transcription termination (11), and it has been reported that the histone-like protein H-NS affects rRNA promoter activity during the transition to stationary phase (1, 2). The results reported here provide a dramatic example of the interplay between some of these regulatory factors during rRNA transcription.

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**REFERENCES**


FIS COMPENSATES FOR ALTERED NTP SENSING

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