Activation of *Escherichia coli* leuV Transcription by FIS

WILMA ROSS,1 JULIA SALOMON,1 WALTER M. HOLMES,2 AND RICHARD L. GOURSE1*

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706,1 and Department of Microbiology and The Massey Cancer Center, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 232982

Received 10 February 1999/Accepted 16 April 1999

The transcription factor FIS has been implicated in the regulation of several stable RNA promoters, including that for the major tRNA^{Leu} species in *Escherichia coli*, tRNA^{Leu}. However, no evidence for direct involvement of FIS in tRNA^{Leu} expression has been reported. We show here that FIS binds to a site upstream of the leuV promoter (centered at −71) and that it directly stimulates leuV transcription in vitro. A mutation in the FIS binding site reduces transcription from a leuV promoter in strains containing FIS but has no effect on transcription in strains lacking FIS, indicating that FIS contributes to leuV expression in vivo. We also find that RNA polymerase forms an unusual heparin-sensitive complex with the leuV promoter, having a downstream protection boundary of −7, and that the first two nucleotides of the transcript, GTP and UTP, are required for formation of a heparin-stable complex that extends downstream of the transcription start site. These studies have implications for the regulation of leuV transcription.

The leuV operon encodes three of the four genes for tRNA^{Leu}, one of the most abundant *Escherichia coli* tRNA species (12, 21). The promoter for leuV is strong, with activity similar to that of the rRNA promoter *rrnB* P1 (6, 7); like many other rRNA and tRNA promoters, it is regulated in response to growth rate and amino acid starvation (6, 37). The leuV promoter has several features similar to those of rRNA promoters, including near-consensus −10 and −35 hexamers spaced at the nonconsensus distance of 16 bp, a G+C-rich sequence (the discriminator region) between the −10 element and the transcription start site, and an upstream sequence that contributes to promoter activity (Fig. 1). However, the effect of upstream sequence at leuV is smaller than that at *rrnB* P1 (~10- to 40-fold versus ~300-fold) (6, 7, 34), and the mechanism(s) responsible for its effects has not been fully characterized.

The leuV upstream sequence has two components, and their contributions to promoter strength are similar (6, 7). The region just upstream of the −35 hexamer (−39 to −47) is likely to increase transcription by interacting with the C-terminal domain of the α subunit of RNA polymerase (RNAP), since it is similar to the promoter-proximal region of the UP element consensus (13) and since in vitro transcription of leuV in the absence of proteins other than RNAP is reduced by an RNAP α-subunit mutation that abolishes UP element recognition (αΔ235) (35). The second region, between −47 and −107, affects transcription by a previously uncharacterized mechanism. It was originally suggested that a T tract in this region (at −69 to −73) influences leuV promoter activity through its effects on DNA bending (7). However, a 2-bp substitution within this T tract (T−71G T−72G) abolished the upstream effect on transcription without affecting the anomalous electrophoretic mobility (bending) of the promoter fragment (7).

FIS is a 12-kDa DNA binding protein that directly activates transcription from a number of promoters by binding to sites upstream of the core promoter (e.g., *rrnB* P1, *thrU*/tufB, *tyrT*, *proP*, and *mar* [26, 29, 31, 32, 36, 40]). FIS also plays a role in other cellular processes, including repression of transcription (41), site-specific recombination (15), transposition (39), and DNA replication (14). It was suggested that FIS contributes to tRNA^{Leu} transcription, since at higher growth rates in *fis* mutant strains the concentration of tRNA^{Leu} (as well as of some other tRNAs) is reduced relative to that of 16S rRNA (30). However, it was not known whether this effect of *fis* was direct or indirect.

The concentration of FIS in the cell varies dramatically as a function of growth rate and growth phase (2, 3), and the extent of activation by FIS at some promoters varies as a function of growth rate (1, 11). However, regulation of *rrnB* P1 with growth rate appears to involve a different mechanism that involves sensing of the initiating nucleotide concentration (16). The extent of activation of *rrnB* P1 by FIS does not vary substantially with growth rate in wild-type strains (1), although FIS is responsible for growth rate-dependent regulation of *rrnB* P1 in strains with RNAP mutations that alter the nucleoside triphosphate (NTP)-sensing mechanism (4). Thus, the contribution of FIS to promoter activity and regulation can vary, depending on the specific kinetic properties of a promoter and other regulatory mechanisms that affect it.

In this work, we have identified a FIS binding site in the leuV promoter upstream region and we have examined the effects of FIS on leuV expression both in vivo and in vitro by using promoter derivatives with mutant or wild-type FIS binding sites. We have also identified an unusual heparin-sensitive RNAP complex with the leuV promoter. These studies support the proposal that multiple mechanisms, including activation by FIS and NTP sensing, contribute to the transcription and regulation of leuV.

**Identification of a FIS binding site upstream of the leuV promoter.** FIS binds to the upstream region in several stable RNA promoters (*rrnB* P1, *tyrT*, *tufB*, and *valU*) [10, 31, 36, 38], and putative FIS binding sites have been identified upstream of many others, including leuV (24, 25, 30). The proposed leuV FIS site contains a one-base mismatch from the consensus (Fig. 1) (15). However, the degeneracy of the FIS consensus sequence has limited its predictive value, and not all consensus sequences actually bind purified FIS (14, 15). We therefore...
FIG. 1. Sequence of the leuV promoter region. Positions protected by FIS in DNase I footprints and positions of enhanced DNase I cleavage within the FIS site (carets) are indicated. Enhanced DNase I cleavage at −38 and −48 in the presence of RNAP is indicated by vertical arrows. The 2-bp substitution mutant T−71G T−72G reduces FIS binding (Fig. 2). Boundaries of protection by RNAP in the absence (thin underline, −47 to −7) or the presence (thick underline, −47 to +20) of the initiating nucleotides GTP and UTP are indicated. Similarity of the FIS site to a consensus derived from information in references 15 and 20 is indicated by lines between the top and bottom strands. Dots between strands in the FIS site indicate poorly conserved positions in different FIS sites.

determined the location of FIS binding sites in the leuV promoter region experimentally.

In a DNase I footprinting experiment, FIS protected a site in the leuV promoter centered at −71 (Fig. 1 and 2A). The

FIG. 2. DNase I footprints of FIS bound to wild-type (A) or mutant (B) leuV promoter fragments. BglII-HindIII leuV promoter fragments were obtained from pRLG927 (wild-type leuV−109 to +33 [7]) or pHEB3 (leuV−109 to +11, T−71G T−72G [7]) and were 32P labeled in the bottom (template) strand at the BglII site, approximately 20 bp upstream from leuV position −109. Footprinting reactions were carried out at 22°C, essentially as described previously (35), in a solution of 10 mM Tris-Cl (pH 7.9), 10 mM MgCl2, 150 mM NaCl, 1 mM dithiothreitol, and 100 µg of bovine serum albumin per ml. FIS was present at the concentrations indicated. Sequence markers were prepared by the method of Maxam and Gilbert (27).
TABLE 1. Effects of FIS site substitution or deletion mutations on leuV promoter activity in wild-type fis and fis::kan strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Promoter</th>
<th>fis allele</th>
<th>Activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLG4043</td>
<td>leuV (−105 to +11)</td>
<td>Wild type</td>
<td>4,962</td>
<td>100</td>
</tr>
<tr>
<td>RLG4045</td>
<td>leuV (−105 to +11, T−71G T−72G)</td>
<td>Wild type</td>
<td>2,173</td>
<td>44</td>
</tr>
<tr>
<td>RLG4044</td>
<td>leuV (−47 to +11)</td>
<td>Wild type</td>
<td>1,393</td>
<td>28</td>
</tr>
<tr>
<td>RLG3274</td>
<td>leuV (−105 to +11, fis::kan-767)</td>
<td>Wild type</td>
<td>6,869</td>
<td>100</td>
</tr>
<tr>
<td>RLG3276</td>
<td>leuV (−105 to +11, fis::kan-767)</td>
<td>Wild type</td>
<td>6,895</td>
<td>100</td>
</tr>
<tr>
<td>RLG3285</td>
<td>leuV (−47 to +11)</td>
<td>fis::kan-767</td>
<td>5,065</td>
<td>74</td>
</tr>
</tbody>
</table>

<sup>a</sup> β-Galactosidase levels were determined in promoter-lacZ fusion-containing strains grown for several generations in Luria-Bertani medium (28) and are averages of duplicate determinations differing by less than 10%. Strains were constructed by P1 transduction from strain RJ1617 (23).

affinity for FIS of the wild-type and mutant promoters observed by DNase I footprinting (Fig. 2).

FIS activates transcription from the leuV promoter in vivo.

The effect of FIS on leuV transcription in vivo was determined by comparing the activity of the wild-type promoter with that of the promoter containing the FIS site mutation T−71G T−72G. Promoter activities were determined in strains containing single-copy chromosomal promoter-lacZ fusions. The FIS site mutation reduced promoter activity to about 44% of its wild-type activity in a strain containing FIS (Table 1 [see also Table 2]), a result consistent with previous observations with similar constructs (7). However, this mutation did not reduce leuV promoter activity in a strain lacking FIS (a fis::kan strain [Table 1]). This result indicates that the effect of the mutation in the wild-type strain is attributable to loss of FIS binding and activation.

Deletion of the entire FIS site (Δ−47) had a slightly larger effect on promoter activity than the 2-bp substitution, reducing it to 28% of wild-type activity (Table 1). This suggests either that the 2-bp substitution does not fully eliminate activation by FIS (consistent with the weak affinity of the 2-bp mutant DNA for FIS [Fig. 2B and 3]), that sequences upstream of −47 contribute slightly to the leuV UP element, or both. The latter possibility is consistent with the slight reduction in activity of the Δ−47 promoter (74% of the wild type) in a fis::kan strain (Table 1).

As observed previously for other promoters (26, 36), the activity of each of the leuV promoters was greater in fis::kan strains than in wild-type fis strains (Table 1). This increase may reflect contributions from at least two factors. First, some of the increase in leuV activity is likely to result from a compensating effect of the rRNA feedback system acting on core promoter function, as described previously for the rmb P1 and tufB promoters (32, 36). This feedback effect is thought to result from loss of activation of the rm operons by FIS and may operate through the recently described NTP-sensing mechanism for growth rate regulation of rm promoters (16). Transcription from growth rate-regulated rm P1 promoters lacking FIS sites is increased to a greater extent (approximately four- to fivefold) than transcription from control promoters (see below) in fis::kan strains (34b, 36). An effect of the feedback system on the leuV promoter is consistent with previous de-scribed effects of altered rm gene dosages on tRNA expression (19, 22).

In addition, some of the increase in leuV activity in fis::kan strains is likely to derive from a promoter-independent effect on the lacZ reporter system, since all promoter-lacZ fusions that we have tested (including non-growth-rate-regulated promoters such as lacUV5 and growth rate-defective mutant derivatives of rmb P1) show some degree of increase in activity in fis::kan strains (−1.5- to 2-fold) (34a). Since FIS has many roles in the cell and fis mutants have pleiotropic effects (14), this nonspecific effect is not surprising.

Although transcription of the leuV-lacZ fusion appears to be as active in fis::kan strains as in wild-type strains (Table 1), reduced levels of tRNA<sup>16S</sup> have been reported (relative to 16S rRNA) in fis mutant strains (30). These observations are consistent with the proposed contribution of a nonspecific increase in promoter-lacZ fusion activity in fis::kan strains, together with a feedback derepression of the leuV core promoter activity that may not be as severe as the derepression observed for rmb P1. This suggests that the leuV promoter may not be as responsive to the NTP-sensing mechanism as is rmb P1 (see also references 5 and 33). Alternatively, the apparent discrepancy between pleuV-lacZ fusion activity and reduced tRNA<sup>16S</sup> levels in fis::kan strains may reflect either an overestimate of tRNA<sup>16S</sup> production from the leuV operon (which encodes three tandem tRNA<sup>16S</sup> genes) with the promoter-lacZ fusion or reduced tRNA production from the argT operon, which encodes the fourth tRNA<sup>16S</sup> gene.

Properties of RNASP-leuV promoter complexes. Since RNAP forms an unstable, heparin-sensitive complex with the rmb P1 promoter, a feature responsible at least in part for its regulation by the NTP-sensing mechanism, we also characterized the properties of complexes formed between RNAP and the leuV promoter by using DNase I footprinting. RNAP formed a heparin-sensitive complex with the leuV promoter in the absence of NTPs (Fig. 4, lanes 4 and 5). The boundaries of this complex are somewhat unusual, extending from −47 to about −7, thus not including the transcription start site. At rmb P1, a closed, heparin-sensitive complex with protection approximately 20 bp upstream is observed under similar conditions (5, 8). These complexes differ from the open, heparin-stable complexes formed at most other promoters in the absence of nucleotides.

A heparin-stable leuV promoter-RNAP complex, in which protection extended downstream to −20, was formed in the
The presence of the initiating nucleotides GTP and UTP but not with GTP alone (Fig. 4, lanes 6 to 9). These results are similar to those obtained with rnbP1, where the initiating nucleotides ATP and CTP, generating a 5-mer slipped transcript (9, 18), were required for a heparin-stable complex. At ATP and CTP, generating a 5-mer slipped transcript (9, 18), complexes were formed with RNAP (10 nM) and the leuV promoter fragment (described in the legend to Fig. 2) in the presence or absence of the initiating nucleotides (500 μM GTP or 500 μM GTP and 50 μM UTP) at 22°C in buffer described in the legend to Fig. 2, except that it contained 30 mM KCl rather than 150 mM NaCl. Where indicated, heparin (10 μg/ml) was added prior to DNase I digestion.

The proximal UP element region of the leuV promoter (~40 to ~47) was protected by RNAP in both the heparin-sensitive (~47 to ~7) and heparin-stable (~47 to ~20) complexes, although the region upstream of ~47 was not protected. This protection pattern is consistent with stimulation of transcription by the sequence between ~39 and ~47 (7). Sites of enhanced DNase I cleavage occurred at positions ~38 and ~48 (Fig. 1 and 4), suggesting that RNAP may bend or distort the DNA at these sites. Similar enhanced cleavage was observed at position ~38 in the rnbP1 promoter (35).

Implications of these findings for the regulation of leuV promoter activity. The results presented here are consistent with the model that multiple mechanisms, including activation by FIS and an NTP concentration-sensing mechanism, may contribute to regulation of leuV transcription. We find that leuV transcription is directly activated by FIS and that, like rnb P1 promoters, it responds to a feedback regulation signal generated by mutation of the fis gene. However, the response of the leuV promoter to the feedback signal may not be as great as that observed for rnbP1, since tRNAγLeu levels are somewhat reduced in fis:kan strains (30). Consistent with this hypothesis, RNAP mutations that alter the NTP-sensing mechanism at rnbP1 also affect leuV transcription but to a lesser degree than rnbP1 (5). Other findings are also consistent with the possibility that the NTP-sensing mechanism described for rnbP1 affects leuV transcription. These include the formation of unusual heparin-sensitive complexes of the leuV promoter with RNAP (Fig. 4), the moderate level of growth rate-dependent regulation of leuV promoter derivatives lacking a FIS site (6, 33), and the dependence of leuV transcription in vitro on the concentration of the initiating nucleotides GTP and CTP (some transcripts were observed to initiate with CTP [33]). Thus, leuV transcription most likely reflects multiple regulatory inputs (33).

This work was supported by grant GM37408 from the National Institutes of Health to R.L.G. and by grant GM50747 to W.M.H.

We thank Yanira O’Neill-Morales and Mike Bartlett for construction of leuV promoter-lacZ fusions.

REFERENCES


34a. Ross, W. Unpublished observations.

34b. Ross, W., and V. Newburn. Unpublished observations.


