Transcriptional Organization of the rfaGBIJ Locus of Salmonella typhimurium

R. BRAZAS,† E. DAVIE, A. FAREWELL,‡ AND L. I. ROTHFIELD*
Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06032

Received 22 April 1991/accepted 19 July 1991

The transcriptional organization of the rfaGBIJ gene cluster of Salmonella typhimurium was studied by using lacZ and cat transcriptional probes. The results indicated that the leftward end of the gene cluster (rfaG-rfaB-rfaIJ) is an operon that is transcribed from one or more promoters that lie upstream of rfaG. The results further indicated that the product of the rfaH (sfrB) gene acts as a positive regulator of transcription of the entire rfaGBIJ cluster. At least one site required for the RfaH-mediated transcriptional regulation lies within or very close to the upstream promoter.

The lipopolysaccharide of gram-negative bacteria contains a core region that is similar in all gram-negative bacteria (16). The lipopolysaccharide core provides the attachment site for the O-antigen polysaccharide that is a major determinant of virulence in gram-negative organisms. Loss of the ability to synthesize the complete core lipopolysaccharide is associated with a significant decrease or loss of virulence.

The core is synthesized by a series of glycosyltransferases that catalyze the stepwise transfer of sugars from their nucleotide-linked precursors (16). The genes coding for the glycosyltransferases are located in the rfaGBIJ gene cluster at approximately 79.5 units on the Salmonella typhimurium and Escherichia coli genetic maps (2, 19). The relative positions of several of the genes within the cluster (Fig. 1) have been established for S. typhimurium by using genetic methods (4, 19) and by studying the ability of cloned chromosomal fragments to complement known rfaG, rfaB, rfaI, and rfaJ mutations (12).

Expression of the genes of the rfaGBIJ cluster is thought to be positively regulated by the product of the rfaH gene (formerly the sfrB gene in E. coli), which is located at 84 units on the genetic map (19). The evidence for the positive regulation of the rfa genes by RfaH is indirect. It is based on the loss of specific glycosyltransferase activities and a marked decrease in the amount of one of the transferase proteins in an rfaH amber mutant (5). The existence of the rfaH amber mutant suggests that rfaH acts via its protein product.

RfaH is also a positive regulator of expression of the traY-Z operon of the F factor. Beutin et al. (3) showed that truncated traY-Z transcripts accumulate in rfaH mutant cells; this led them to suggest that the RfaH protein acts to prevent premature termination of transcription within the traY-Z transcriptional unit. Support for this view has also come from studies involving lacZ transcriptional probes (9). It is not known whether rfaGBIJ gene expression is transcriptionally regulated by a similar antitermination mechanism.

In this paper we describe studies of the transcriptional organization of the rfaGBIJ gene cluster and the effects of RfaH on the rfaGBIJ transcriptional pattern, by using lacZ and cat probes as transcriptional monitors.

MATERIALS AND METHODS

Bacteriological procedures. Culture conditions and microbiological procedures were as described by Miller (15). Cells were grown at 37°C in L-broth (LB) and harvested in the midexponential growth phase. Resistance to chloramphenicol was defined as the ability to form colonies after overnight growth at 37°C on LB plates containing 100 μg of chloramphenicol per ml. When isopropyl-β-D-thiogalactoside (IPTG) was used, it was present at a concentration of 1 mM for three generations before the cells were harvested.

Strains. E. coli M1170 (rfaH Δlac) and M1174 (rfaH+ Δlac) were isogenic E. coli K-12 strains provided by M. Achters. S. typhimurium AF21 (rfaH::Mudlac ΔrfaH+) was constructed as described below. Strain AF21-106 (rfaH487 ΔrfaH::Mudlac metE::Tn10) was constructed by ES18-mediated cotransduction of rfaH487 and metE::Tn10 from AF106 (rfaH487 metE::Tn10) into AF21, making use of the close transcriptional linkage of the rfaH and metE genes (19). Recombinants that had received metE::Tn10 were selected on the basis of their tetracycline resistance. Within this group, transductants that had acquired the rfaH487 allele were identified on the basis of their resistance to bacteriophage P1 (22). Strain AF21-107 (rfaH+ rfaH::Mudlac metE::Tn10) was obtained from W. Nunn.

Plasmids. (1) pKZ26. pKZ26 (provided by K. Sanderson) is a pBR322 derivative that contains 7.5 kb of chromosomal DNA from S. typhimurium (12). The genes within the insert (Fig. 1) were identified on the basis of genetic complementation of known rfa mutants. The rfaGBIJ gene order in pKZ26 was deduced from the gene order of the chromosomal genes as obtained by standard genetic transduction and conjugation analysis (19). The gene order was partially confirmed by complementation studies with plasmids containing fragments of the chromosomal insert of pKZ26 (12) and by sequencing of the rfaI and rfaJ genes (4).

(i) pRB18 to pRB85. The lacZ mini-transposon from λ1048
1. The lacZ insert in each of the derivative plasmids carries the indicated insertion (e.g., 85 represents the lacZ insertion in pRB85). The approximate locations of the rfa genes is taken from Kadam et al. (12). In the case of rfaG, rfaB, and rfaI, the positions also correspond to the genes inactivated by specific lacZ insertions in the rfaG end of the insert. Abbreviations: C, ClaI; H, HindIII; E, EcoRI; B, BamHI; HpaI; P, PstI; Pv, PvuI; N, NdeI.

was inserted at random locations within pKZ26 by IPTG induction of LE392(10488)/pKZ26, essentially as described by Way et al. (20). The location and orientation of the lacZ sequences in each of the derivative plasmids are shown in Fig. 1. The 5.4-kb lacZ insert in each case contains a BamHI site at either end of the inserted sequences. The numbers assigned to the inserts (Fig. 1) are also used to identify the plasmids. Thus, pRB18 contains the 7.5-kb chromosomal rfa segment containing insert 18 (rfa::lacZ18).

(iii) pSL plasmids. In the pSL plasmids (provided by C. Squires), the cat gene serves as a transcriptional reporter for transcripts exiting from a polycoding site (13). Transcriptional terminators prevent transcription from the plasmid into the reporter gene, and translational terminators prevent formation of a fusion protein between the cat gene product and the protein product of genes cloned into the polycoding site. Transcription into cat was monitored either by measurement of chloramphenicol acetyltransferase (CAT) enzyme activity or by the appearance of resistance to chloramphenicol. In pSL100 there is no promoter between the transcriptional terminators and the polycoding site (Fig. 2). Therefore, cat expression requires that the inserted fragment contain a promoter oriented toward the cat gene. In pSL102, a ribosomal P2 promoter is present that provides an exogenous promoter for sequences inserted into the polycoding site (Fig. 3). pSL140 is similar to pSL102, except that the UV5 P2 promoter is present instead of the P2 promoter of pSL102 (Fig. 3).

(iv) pRB101 to pRB188. The rfaGBIJJ fragments shown in Fig. 2 and 3 were cloned into the polycoding sites of pSL100, pSL102, or pSL140 by standard methods. The donor fragments were obtained from pRB56, pRB66, or pRB78 by using the BamHI site at the left end of the lacZ insert (Fig. 1; Table 1) as the downstream end of the subcloned fragment, except for pRB179, for which the donor fragment was obtained from pRB178 (Fig. 3). Other restriction sites used for the cloning are indicated in Fig. 2 and 3.

(v) pRBD14 and pRBD19. Plasmids pRBD14 and pRBD19 contain partial deletions of the left end of the chromosomal

![Diagram of rfaGBIJJ loci](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3693903/bin/fig1.jpg)

**Fig. 1.** Locations and orientations of lacZ insertions. The locations and orientations of 15 rfa::lacZ insertions are shown under the physical map of the chromosomal insert in pKZ26. Each number corresponds to the plasmid that carries the indicated insertion (e.g., 85 represents the lacZ insertion in pRB85). The approximate locations of the rfa genes is taken from Kadam et al. (12). In the case of rfaG, rfaB, and rfaI, the positions also correspond to the genes inactivated by specific lacZ insertions in the rfaG end of the insert. Abbreviations: C, ClaI; H, HindIII; E, EcoRI; B, BamHI; HpaI; P, PstI; Pv, PvuI; N, NdeI.

![Diagram of promoter activities](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3693903/bin/fig2.jpg)

**Fig. 2.** Promoter activities of rfa fragments in pSL100-derived plasmids. The indicated plasmids, in which cat expression requires the presence of a promoter in the chromosomal insert, were introduced into rfaH* and rfaH strains. Activities of β-galactosidase (LacZ) and CAT and the resistance (R) or sensitivity (S) of each of the plasmid-containing strains to chloramphenicol are shown on the right of the figure. In the diagram for the plasmid vector, the solid line indicates the polycoding site. The arrows indicate the location and orientation of the chromosomal insertions inserted into the polycoding site upstream of the cat reporter gene, relative to the physical map of the rfa locus shown in Fig. 1. pRB19 and pRB14 are 2 of the 22 different Bal31 deletion mutant plasmids that were examined in this study, as described in the text. Bg, BglII; nd, not determined. For other abbreviations, see the legend to Fig. 1.
insert of pRB161 (Fig. 2) and were prepared as described below.

Construction of partial deletions of rfaGBIJ sequences. pRB161 (Fig. 2) was linearized by treatment with BgII and then treated with Bal 31 exonuclease for approximately 15 min at 30°C. BamHI linkers were ligated to the ends of the partially digested linear fragments. The preparation was then treated with BamHI to generate BamHI-BamHI fragments from which a variable amount of DNA had been removed from the upstream end of the pRB161 chromosomal insert. Fragments of approximately 3 to 3.5 kb were eluted from an agarose gel and ligated into the BamHI site of pSL100 to yield a series of plasmids (including pRB14 and pRB19). The structure, orientation, and length of the chromosomal fragments in the resulting plasmids were determined by restriction analysis.

Complementation analysis. Plasmids pRB18 to pRB85 (Table 1) and pKZ26 were transformed into the following rfa indicator strains: S. typhimurium SL1032 (rfaG), SL4805 (rfaB), TV148 (rfaH), and SL3750 (rfaI). Correction of the mutant phenotypes was indicated by correction of the F-resistant phage resistance pattern of the test strains (11).

Isolation of chromosomal rfa::Mudlac insertions. Random insertions of Mud1(Ap' lac) into the chromosome of S. typhimurium TV119 (rfb) were generated by coinfection with phages Mud1(Ap' lac) and MuG562hP1-1 as described by Csonka et al. (6). Ampicillin-resistant colonies in which Mud1(Ap' lac) was inserted into rfa genes were selected on the basis of their resistance to the rfb-specific bacteriophage 6SR. The insertion mutants isolated in this way were further characterized and assigned to specific rfa genes on the basis of their phase resistance patterns (11) and the characteristic electrophoretic patterns of lipopolysaccharides isolated from the mutant strains (10). About 50% of the rfa::Mudlac mutants were Lac+ on indicator plates. This result is consistent with the expectation that half of the insertions would be in the correct orientation to permit transcription to proceed from the mutated gene into the lacZ probe. One of the Lac- mutants (AF21 [rfaI::Mudlac]) was used in the studies described here. β-Galactosidase activity from the chromosomal rfaI::MudlacZ insertion was approximately 10% of the activity of a fully induced chromosomal copy of lacZ under control of P\textsubscript{lac}.

Enzyme measurements in rfaH\textsuperscript{+} and rfaH strains. The rfaH\textsuperscript{+} and rfaH strains used were M1174 and M1170, respectively. When β-galactosidase was assayed, the strains also contained F\textsuperscript{481} (lac\textsuperscript{I} lac::Tn5). Assays were performed on parallel samples of cells harvested in mid-exponential growth. β-Galactosidase, β-lactamase, and CAT assays were performed as previously described (13, 15, 18). Units are defined as originally described except for CAT, in which 1 unit is defined as 1 μmol of chloramphenicol acetylated in 10 min. The results are expressed as the ratio of the activity of β-galactosidase or CAT to the activity of β-lactamase, thereby normalizing for possible differences in plasmid copy number in the different samples.

Other procedures. Unless otherwise described, procedures were performed as described by Miller (15) or Maniatis et al. (14). Physical maps and orientations of the plasmids described in Fig. 1 to 3 were determined by restriction analysis.

RESULTS

Organization of the rfa cluster. To monitor transcription from different regions of the rfaGBIJ gene cluster, a lacZ transcriptional probe was inserted at random locations within plasmid pKZ26, whose chromosomal insert includes

![Diagram](http://jb.asm.org/Downloadedfromhttp://jb.asm.org/)}
TABLE 1. lacZ expression from rfa::lacZ gene fusion

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Location (kb)*</th>
<th>Orientation</th>
<th>Gene†</th>
<th>β-Galactosidase activity for†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rfaH⁺</td>
</tr>
<tr>
<td>85</td>
<td>2.1</td>
<td>CW</td>
<td>rfaG</td>
<td>951</td>
</tr>
<tr>
<td>18</td>
<td>3.5</td>
<td>CW</td>
<td>rfaG</td>
<td>1,217</td>
</tr>
<tr>
<td>56</td>
<td>3.9</td>
<td>CW</td>
<td>rfaG</td>
<td>785</td>
</tr>
<tr>
<td>67</td>
<td>4.4</td>
<td>CW</td>
<td>rfaB</td>
<td>652</td>
</tr>
<tr>
<td>53</td>
<td>4.8</td>
<td>CW</td>
<td>rfaB</td>
<td>679</td>
</tr>
<tr>
<td>71</td>
<td>5.5</td>
<td>CW</td>
<td>rfaI</td>
<td>753</td>
</tr>
<tr>
<td>73</td>
<td>5.7</td>
<td>CW</td>
<td>rfaI</td>
<td>804</td>
</tr>
<tr>
<td>66</td>
<td>6.17</td>
<td>CW</td>
<td>rfaI</td>
<td>639</td>
</tr>
<tr>
<td>55</td>
<td>0.2</td>
<td>CCW</td>
<td>rfaG</td>
<td>380</td>
</tr>
<tr>
<td>81</td>
<td>2.2</td>
<td>CCW</td>
<td>rfaG</td>
<td>130</td>
</tr>
<tr>
<td>44</td>
<td>3.5</td>
<td>CCW</td>
<td>rfaG</td>
<td>85</td>
</tr>
<tr>
<td>64</td>
<td>4.8</td>
<td>CCW</td>
<td>rfaB</td>
<td>111</td>
</tr>
<tr>
<td>59</td>
<td>5.5</td>
<td>CCW</td>
<td>rfaI</td>
<td>96</td>
</tr>
<tr>
<td>61</td>
<td>5.7</td>
<td>CCW</td>
<td>rfaI</td>
<td>72</td>
</tr>
<tr>
<td>78</td>
<td>7.35</td>
<td>CCW</td>
<td>rfaI</td>
<td>403</td>
</tr>
</tbody>
</table>

* Plasmids pRB18 to pRB5, containing the indicated rfa::lacZ transcriptional fusions, were introduced into rfaH⁺ and rfaH strains, and activities of β-galactosidase were determined.

† The locations of the lacZ insertions are given from the left end of the chromosomal insert in pKZ26 (Fig. 1). CW (clockwise) and CCW (counterclockwise) refer to the orientation of lacZ relative to the genetic map of pBR322 (the parent of pKZ26).

‡ The indicated plasmid-encoded gene was inactivated by the lacZ insertion. Where no gene is indicated, the rfa::lacZ plasmid corrected all of the chromosomal mutations tested.

Values are expressed relative to β-lactamase (see Materials and Methods).

The rfaG, rfaB, rfaI, and rfaJ genes. Fifteen independent insertions that were distributed throughout the 7.5-kb rfaGBIJ insert were selected for further study (Fig. 1).

Several of the lacZ insertions resulted in inactivation of the rfaG, rfaB, or rfaI genes of the parental plasmid, as shown by loss of the ability of the rfa::lacZ plasmids to complement chromosomal rfaG, rfaB, or rfaI mutations (Table 1). These presumably were located within coding sequences or essential upstream regions of the affected genes. None of the lacZ insertions affected the ability of the other known rfa genes of pKZ26 to complement their corresponding chromosomal mutant alleles.

All of the probes that were oriented in the clockwise direction (relative to the pBR322 genetic map) showed significant levels of lacZ expression in rfaH⁺ cells, as monitored by β-galactosidase activity (Table 1). In contrast, β-galactosidase activity from most of the counterclockwise inserts was very low, ranging from 8 to 15% of the average activity from the clockwise inserts. The only exceptions were inserts lacZ53 and lacZ78, which are located at the extreme ends of the chromosomal fragment.

Evidence that the rfaH gene product affects transcription of the genes of the rfaGBIJ cluster was obtained by comparing lacZ expression from the rfa::lacZ plasmids in isogenic rfaH⁺ and rfaH mutant cells. In the case of the clockwise-oriented lacZ insertions, lacZ expression was significantly higher in rfaH⁺ than in rfaH cells (Table 1). In contrast, for the counterclockwise probes, β-galactosidase activities in rfaH⁺ cells were actually lower than the activities in rfaH⁻ cells. These results indicate that all of the genes within the locus are transcribed in the same direction. The results also show that RfaH positively regulates rfaGBIJ expression at the transcriptional level.

Promoter organization. Evidence that transcription of the rfaGBIJ cluster was driven by a promoter that lies upstream of rfaG was obtained by inserting fragments of the cloned rfa region into pSL100, which lacks a promoter capable of driving transcription of the lacZ gene (Fig. 2). Expression of the downstream cat gene therefore requires an intrinsic promoter within the cloned insert.

Insertion into pSL100 of fragments that included the leftward 1.1 kb of the chromosomal insert resulted in significant cat expression, as shown by resistance to chloramphenicol killing and/or by increased activity of CAT (Fig. 2, pRB161, pRB160, and pRB160). In contrast, cells containing plasmids with rfa sequences that lay downstream of this region (pRB187, pRB157, pRB101, and pRB103) and pRB14) remained sensitive to chloramphenicol and showed only 5 to 15% of the CAT activity induced by plasmids containing the more leftward region of the locus. These fragments therefore lacked significant intrinsic promoter activity.

The upstream promoter was more precisely mapped by constructing a series of deletions that extended into the chromosomal sequences from the left. These revealed significant promoter activity from all of the several fragments whose left ends were located at or before 0.65 units on the map of the chromosomal insert (illustrated by pRB19 in Fig. 2), as monitored by chloramphenicol resistance and by CAT assay. Fragments that began at or after 0.7 map unit (illustrated by pRB14 in Fig. 2) lacked significant promoter activity by these criteria. These results indicate that the major promoter(s) associated with the rfaGBIJ locus (P₁ in Fig. 4) lies upstream of rfaG, between 0.65 and 0.7 kb from the left end of the original chromosomal insert.

When overlapping promoter-distal fragments that covered the 0.7- to 3.9-kb region were interposed between the ribosomal P2 promoter and the cat transcriptional monitor of pSL102 (pRB188, pRB107, pRB165, and pRB178), there was a 78 to 91% decrease in CAT expression as compared with that for the unsubstituted pSL102 vector. A significant decrease in CAT expression was also seen with the nonoverlapping fragments present in pRB188 and pRB109. This suggests that one or more transcriptional terminators may be located within this 3.2-kb region of the rfaGBIJ locus.

Effect of rfaH on transcription of cloned rfa genes. To further localize the sites responsible for the RfaH-mediated transcriptional regulation, we studied rfaGBIJ fragments in the pSL100 vector, where possible artifacts due to transcription in the insert from vector sequences were avoided. This confirmed that all elements required for the rfaH-mediated transcriptional regulation were present in the leftward end of the rfa locus. This was shown by the four- to fivefold-higher expression of the lacZ (pRB158) and cat (pRB161) reporter genes and by the chloramphenicol resistance associated with the presence of the plasmids in rfaH⁺ but not rfaH cells (pRB161, pRB160, and pRB160) (Fig. 2). As expected, when the 3.9-kb chromosomal fragment of pRB161 was inserted into pSL100 in the opposite orientation (in pRB162), there was no significant difference in cat expression between rfaH⁺ and rfaH⁻ cells. These results established that at least one site that was required for the rfaH-mediated transcriptional regulation was located between 0.65 unit (the start of the insert in pRB19) and 1.05 units (the downstream end of the insert in pRB160) on the rfaGBIJ map.

We then attempted to separate the RfaH-responsive site from the rfaGBIJ promoter region (P₁) by examining the effect of rfaH⁺ and rfaH alleles on expression from promoterless rfa fragments that were transcribed from the ribosomal P2 promoter of pSL102 (Fig. 3). In all cases, transcripts that originated from the exogenous promoter were...
insensitive to RfaH-mediated regulation, as shown by the unchanged levels of CAT in rfaH+ and rfaH hosts (pRB188, pRB165, and pRB178 in Fig. 3).

Because of the possibility that the high level of transcription from the strong P2 promoter might have masked the presence of RfaH-responsive sites in the promoterless inserts, we inserted the promoterless 3.2-kb fragment that lies immediately distal to P2 into pSL140, thereby placing it downstream of P1ac (pRB179 in Fig. 3). When pRB179 was studied under conditions where the level of cat expression in rfaH+ cells was similar to expression from P2ac, there was no significant difference in the levels of CAT between rfaH+ and rfaH cells (Fig. 3).

The inability to obtain promoterless fragments that retained their sensitivity to RfaH-mediated regulation indicates that at least one site needed for the transcriptional regulation was located in or very close to P2ac.

**Effect of rfaH on transcription of chromosomal rfa gene.** To confirm that the effect of rfaH expression on transcription of the plasmid-borne rfa genes was a valid representation of the behavior of the chromosomal genes, we inserted a lacZ transcriptional probe into the chromosomal rfaL gene by lysogenization with phage Mudl(Ap' lac). β-Galactosidase activity from the chromosomal rfaL::MudlacZ insertion was approximately 5% of the activity from the plasmid-borne rfaL::lacZ probes. The difference presumably reflects the increased number of cellular copies of rfaL::lacZ with the plasmid-borne probes. As shown in Table 2, lacZ expression from the chromosomal rfaL::lacZ probe was 14-fold higher in rfaH+ than in rfaH cells. This increase was significantly greater than the approximately fourfold stimulation of the corresponding plasmid-borne rfaL::lacZ constructs (rfaL::lacZ71, rfaL::lacZ73, and rfaL::lacZ66 [Table 1]). This may indicate that the protein responsible for the RfaH-mediated transcriptional regulation is present in amounts that are insufficient to activate all of the plasmid-borne targets.

**DISCUSSION**

The present results suggest the model of transcriptional organization of the rfaGBIJ locus of S. typhimurium that is shown in Fig. 4. The entire leftward portion of the locus, including rfaG, rfaB, and rfaL, appears to be driven by one or more promoters located upstream of rfaG (Prf in Fig. 4). This conclusion is based on the absence of significant transcription into the lacZ and cat transcriptional probes from fragments that included the rfaGBIJ region but lacked the upstream sequences. The conclusion is supported by the observation that significant expression was seen only with clockwise-oriented probes and that these were the only probes that showed the expected response to the presence or absence of the wild-type rfaH allele. This suggests that the gene cluster forms an operon that is transcribed in the order rfaG-rfaB-rfaL(-rfaJ).

Although rfaL is likely to be part of the same unit, the possibility remains that rfaL is transcribed from another promoter oriented in the counterclockwise direction, since none of the available transcriptional probes monitored clock-wise transcripts within the rfaJ structural gene. This is made less likely by the failure of lacZ78 to respond to the presence or absence of the rfaH+ allele (Table 1), but it still cannot be excluded.

It has been suggested that in E. coli the rfa gene cluster is also transcribed unidirectionally, with promoters located both upstream and within the locus (1).

The present results also demonstrate that the RfaH-mediated regulation of rfaGBIJ operates at the transcriptional level, as has previously been shown for tra genes. The results of the tra studies also suggested that the RfaH-mediated positive regulation occurred by an antitermination mechanism, in which the rfaH gene product permitted transcription to proceed past terminators located within the transcriptional unit (3, 8). A suggestion that a similar mechanism may be responsible for the RfaH-mediated regulation of rfaGBIJ in S. typhimurium has come from the demonstration that certain mutations in the gene for transcriptional termination protein rho can suppress the abnormal phenotype of rfaH mutants (7).

Although consistent with the idea that RfaH acts as a transcriptional antiterminator, the present study does not establish that such a mechanism is responsible for the RfaH-mediated regulation of the rfaGBIJ cluster since it was not possible to separate the site(s) required for RfaH regulation from the upstream promoter(s). Therefore, the possibility that the rfaH system regulates rfaGBIJ expression at the level of initiation of transcription rather than at the level of premature termination cannot be excluded. If antitermination is involved, at least one essential site must be located very close to the upstream promoter(s).

In the well-studied antitermination systems mediated by the λN and λQ proteins, the site at which the antitermination protein engages the transcriptional complex is distinct from and lies upstream of the termination sites at which the antitermination effect is manifested (17). If the RfaH-mediated transcriptional regulation occurred by such a mechanism, the inability to separate an upstream RfaH-responsive site from the promoter would resemble the situation with the λQ protein, in which the qut site responsible for interaction with Q overlaps the relevant promoter (9, 22).

**TABLE 2. Effect of rfaH gene expression on chromosomal rfaL::lacZ expression**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>β-Galactosidase activity (units)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium AF21-106 rfaL::Mudlac rfaH</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium AF21-107 rfaL::Mudlac rfaH+</td>
<td>25.9</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium AF21 rfaL::Mudlac rfaH+</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>E. coli JK268 (+IPTG)</td>
<td>97.0</td>
<td></td>
</tr>
<tr>
<td>E. coli JK268 (-IPTG)</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

*β-galactosidase units are as defined by Miller (15).
ACKNOWLEDGMENTS

We thank Asis Das for helpful discussions and advice. This work was supported by NIH grant AM13407.

REFERENCES


