Transcriptional Organization of the *rfaGBIJ* Locus of *Salmonella typhimurium*

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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-rfaI*) 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-antigenic 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* Δ*lac*) and M1174 (*rfaH*⁺ Δ*lac*) are isogenic *E. coli* K-12 strains provided by M. Achtman. *S. typhimurium* AF21 (*rfaI*::Mudlac *rfaH*⁺) was constructed as described below. Strain AF21-106 (*rfaH487 rfaI*::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 transductional 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*⁺ *rfaI*::Mudlac *metE*::Tn10) was obtained from the same transduction and was identified as a tetracycline-resistant recombinant that had retained the P1-sensitive phenotype of AF21. Strain LE392 (*hsdR supE44 supF58 lacY1 galK2 galT22 metB1 trpR55*) was obtained from W. Nunn.

Plasmids. (i) **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).

(ii) **pRB18 to pRB85.** The *lacZ* minitransposon from λ1048

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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 present study (Table 1). Coordinates are given in kilobases from the *HindIII* site in the *rfaG* end of the insert. Abbreviations: C, *ClaI*; H, *HindIII*; E, *EcoRI*; B, *BamHI*; Hp, *HpaI*; P, *PstI*; Pv, *PvuI*; N, *NdeI*.

was inserted at random locations within pKZ26 by IPTG induction of LE392(λ 1048)/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::lacZ*18).

(iii) **pSL plasmids.** In the pSL plasmids (provided by C. Squires), the *cat* gene serves as a transcriptional reporter for transcripts exiting from a polycloning 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 polycloning 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 polycloning 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 polycloning site (Fig. 3). pSL140 is similar to pSL102, except that the UV5 P_{lac} promoter is present instead of the P2 promoter of pSL102 (Fig. 3).

(iv) **pRB101 to pRB188.** The *rfaGBIJ* fragments shown in Fig. 2 and 3 were cloned into the polycloning 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

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 the resistance (R) or sensitivity (S) to chloramphenicol are shown on the right of the figure. In the diagram for the pSL100 vector, the solid line indicates the polycloning site. The arrows indicate the location and orientation of the chromosomal insertions inserted into the polycloning 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 *Bal* 31 deletion mutant plasmids that were examined in this study, as described in the text. Bg, *BglI*; nd, not determined. For other abbreviations, see the legend to Fig. 1.

FIG. 3. Effects of promoter-distal *rfa* fragments on transcription from exogenous promoters. The indicated plasmids were introduced into *rfaH*⁺ and *rfaH*⁻ strains. Asterisks indicate that the cells were grown in the presence of IPTG. The individual chromosomal inserts are shown below their locations in the physical map of the *rfa* locus (Fig. 1). In the diagram for the pSL102 and pSL140 vectors, the solid line indicates the polycloning site. Bg, *Bgl*I. For other details, see Fig. 1 and 2.

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 *Bgl*II and then treated with *Bal* 31 exonuclease for approximately 15 min at 30°C. *Bam*HI linkers were ligated to the ends of the partially digested linear fragments. The preparation was then treated with *Bam*HI to generate *Bam*HI-*Bam*HI 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 *Bam*HI site of pSL100 to yield a series of plasmids (including pRBD14 and pRBD19). 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 (*rfaI*), and SL3750 (*rfaJ*). Correction of the mutant phenotypes was indicated by correction of the Fo-resistant phage resistance pattern of the test strains (11).

Isolation of chromosomal *rfa::Mudlac* insertions. Random insertions of Mud1(Ap^r *lac*) into the chromosome of *S. typhimurium* TV119 (*rfaB*) were generated by coinfection with phages Mud1(Ap^r *lac*) and Mucts62hP1-1 as described by Csonka et al. (6). Ampicillin-resistant clones in which Mud1(Ap^r *lac*) was inserted into *rfa* genes were selected on the basis of their resistance to the *rfa*-specific bacteriophage 6SR. The insertion mutants isolated in this way were further characterized and assigned to specific *rfa* genes on the basis of their phage 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_{lac}.

Enzyme measurements in *rfaH*⁺ and *rfaH*⁻ strains. The *rfaH*⁺ and *rfaH*⁻ strains used were M1174 and M1170, respectively. When β -galactosidase was assayed, the strains also contained F'481 (*lacI*^r *lacZ*::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 μ mol 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

TABLE 1. *lacZ* expression from *rfa::lacZ* gene fusions^a

Fusion	Location (kb) ^b	Orientation ^c	Gene ^d	β-Galactosidase activity for ^e :		
				<i>rfaH</i> ⁺	<i>rfaH</i>	<i>rfaH</i> ⁺ / <i>rfaH</i>
85	2.1	CW	<i>rfaG</i>	951	345	2.8
18	3.5	CW		1,217	454	2.7
56	3.9	CW		785	155	5.1
67	4.4	CW	<i>rfaB</i>	652	130	5.0
53	4.8	CW	<i>rfaB</i>	679	159	4.3
71	5.5	CW	<i>rfaI</i>	753	237	3.2
73	5.7	CW	<i>rfaI</i>	804	191	4.2
66	6.17	CW	<i>rfaI</i>	639	146	4.4
55	0.2	CCW		380	531	0.72
81	2.2	CCW	<i>rfaG</i>	130	254	0.51
44	3.5	CCW		85	178	0.5
64	4.8	CCW	<i>rfaB</i>	111	215	0.51
59	5.5	CCW	<i>rfaI</i>	96	156	0.61
61	5.7	CCW	<i>rfaI</i>	72	110	0.65
78	7.35	CCW		403	582	0.09

^a Plasmids pRB18 to pRB85, containing the indicated *rfa::lacZ* transcriptional fusions, were introduced into *rfaH*⁺ and *rfaH* strains, and activities of β-galactosidase were determined.

^b The locations of the *lacZ* insertions are given from the left end of the chromosomal insert in pKZ26 (Fig. 1).

^c CW (clockwise) and CCW (counterclockwise) refer to the orientation of *lacZ* relative to the genetic map of pBR322 (the parent of pKZ26).

^d 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.

^e 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 *lacZ*55 and *lacZ*78, 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 *cat* 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 pRBD19). In contrast, cells containing plasmids with *rfa* sequences that lay downstream of this region (pRB187, pRB157, pRB101, pRB103, and pRBD14) 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 pRBD19 in Fig. 2), as monitored by chloramphenicol resistance and by CAT assay. Fragments that began at or after 0.7 map unit (illustrated by pRBD14 in Fig. 2) lacked significant promoter activity by these criteria. These results indicate that the major promoter(s) associated with the *rfaGBIJ* locus (*P*_{rfa} 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 into the insert from the vector are 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 pRBD19) (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 pRBD19) 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 (*P*_{rfa}) 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

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

Strain	Relevant genotype	β -Galactosidase activity (units) ^a
<i>S. typhimurium</i> AF21-106	<i>rfaI::Mudlac rfaH</i>	1.9
<i>S. typhimurium</i> AF21-107	<i>rfaI::Mudlac rfaH</i> ⁺	25.9
<i>S. typhimurium</i> AF21	<i>rfaI::Mudlac rfaH</i> ⁺	30.3
<i>E. coli</i> JK268 (+IPTG)		97.0
<i>E. coli</i> JK268 (–IPTG)		1.2

^a β -galactosidase units are as defined by Miller (15).

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 P_{rfa} into pSL140, thereby placing it downstream of P_{lac} (pRB179 in Fig. 3). When pRB179 was studied under conditions where the level of *cat* expression in *rfaH*⁺ cells was similar to expression from P_{rfa}, 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 P_{rfa}.

Effect of *rfaH* on transcription of chromosomal *rfaI* 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 *rfaI* gene by lysogenization with phage Mud1(Ap^r *lac*). β -Galactosidase activity from the chromosomal *rfaI::MudlacZ* insertion was approximately 5% of the activity from the plasmid-borne *rfaI::lacZ* probes. The difference presumably reflects the increased number of cellular copies of *rfaI::lacZ* with the plasmid-borne probes. As shown in Table 2, *lacZ* expression from the chromosomal *rfaI::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 *rfaI::lacZ* constructs (*rfaI::lacZ71*, *rfaI::lacZ73*, and *rfaI::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 *rfaI*, appears to be driven by one or more promoters located upstream of *rfaG* (P_{rfa} 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 *rfaGBI* 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

FIG. 4. Diagram of the general organization of the *rfaGBIJ* region. Only the major promoter activities (P) are indicated. Terminators (t) are shown within the region in which their presence was suggested from the experiments shown in Fig. 3; the question mark indicates that their presence is speculative.

gene cluster forms an operon that is transcribed in the order *rfaG-rfaB-rfaI(-rfaJ)*.

Although *rfaJ* is likely to be part of the same unit, the possibility remains that *rfaJ* is transcribed from another promoter oriented in the counterclockwise direction, since none of the available transcriptional probes monitored clockwise 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 λ N and λ Q 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 λ Q protein, in which the *qut* site responsible for interaction with Q overlaps the relevant promoter (9, 22).

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