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Suppression of the Abnormal Phenotype of *Salmonella typhimurium rfaH* Mutants by Mutations in the Gene for Transcription Termination Factor Rho

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Mutations in the *rfaH* gene have previously been shown to cause premature termination of transcription of the *traYZ* operon of the F factor and also to prevent expression of the *rfaGBIJ* gene cluster of *Salmonella typhimurium*. In the present study, mutants were selected for their ability to restore the normal pattern of *rfaGBIJ* function. On the basis of this initial selection, several classes of extragenic suppressor mutants were isolated that completely or partially corrected the Tra$^-$ and Rfa$^-$ phenotypes of the prototype *rfaH* mutant. The suppressor mutations included mutations in *rho* and mutations that mapped in or close to *rpoBC*. Other suppressor mutations were located elsewhere on the chromosome, presumably identifying other genes that play a role in the RfaH-mediated transcriptional regulation.

Mutations in the *rfaH* gene of *Salmonella typhimurium* and *Escherichia coli* (the *rfaH* gene of *E. coli* was previously called *sfrB* [2]) are associated with loss of expression of several genes that code for membrane proteins. The affected genes include *traT*, a part of the *traYZ* operon of the F factor (3, 9), and genes of the *rfaGBIJ* gene cluster that are thought to code for membrane glycosyltransferases involved in lipopolysaccharide biosynthesis (6).

The isolation of amber mutations of the *rfaH* gene implies that *rfaH* function is mediated by a protein product (6).

Studies of transcription of the *traYZ* operon in *rfaH*$^+$ and *rfaH* mutant cells have shown that transcription of the operon is prematurely terminated in *rfaH* mutant cells (3, 9). This led Beutin et al. (3) to suggest that the *rfaH* gene product is a transcriptional antiterminator required for transcription through intracistronic terminators that otherwise prevent complete expression of the regulated genes. This view was supported by the demonstration that the *tra* defect was partially suppressed in a *rho*(Ts) mutant grown at nonpermissive temperature (3).

Regulation at the level of premature termination of transcription has been well studied in phages such as λ, in which the *N* and *Q* proteins are required for operon-specific antitermination events (16). In the case of *N*, several additional proteins are involved in the antitermination event (8). Antitermination mechanisms also play a role in the regulation of chromosomal genes, such as the genes of the *rrnG* operon, where transcription in vivo continues past several strong transcription terminators (1, 14). Thus far the only chromosomal antitermination systems in which proteins required for the antitermination activities have been identified are the *rfaH* and *bglG* systems (3, 12).

We have attempted to identify other genes or gene products that may be involved in the positive regulatory function of the RfaH protein by identifying extragenic mutations that suppress the abnormal phenotype of *rfaH* mutants. In this paper, we report the isolation of a number of such extragenic suppressor mutants (hereafter called *sup*^{rfaH}). The group of *sup*^{rfaH} mutations includes mutations in *rho*, encoding transcriptional termination factor rho. The fact that loss of rho activity restores *rfa* gene expression in *rfaH* mutant cells supports the view that the *rfaH* gene product acts at the level of transcription termination. In addition, other *sup*^{rfaH} mutations lie in genes whose functions have not yet been identified, suggesting that additional protein factors may be involved in RfaH-associated antitermination activity.

MATERIALS AND METHODS

Strains and growth conditions. Strains used in this study are listed in Table 1. Unless otherwise noted, bacterial cultures were grown at 37°C in either proteose peptone beef extract (PPBE; Difco) or glucose-containing minimal medium containing the required nutritional supplements (13). When histidinol was used, it was present at 1.5 mg/ml. When *pmi* strains were tested for phage sensitivity or were used as recipients for P22-mediated transduction, mannose was present in PPBE at 10 μg/ml. When *galE* strains were tested for phage sensitivity or were used as recipients for P22-mediated transduction, the cells were exposed to 0.1% galactose for 30 min prior to the addition of phage. Galactose was also present in the medium used for phage sensitivity testing but was omitted when *galE* strains were used as recipients for P1-mediated transduction. In Hfr mating and transduction experiments, transconjugants and transductants were purified several times by restreaking on selective medium before doing phenotypic characterization. Phage sensitivities were determined by cross-streaking and were confirmed by spotting the phage on a lawn of the test organism. Bacteriophages have been previously described (18). In strain constructions, transductants were selected for the presence of Tn*10* or Tn*5* markers on the basis of tetracycline or kanamycin resistance, respectively.

Plasmid pEG25 (constructed by Elio Gulletta), which

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TABLE 1. Strains of *S. typhimurium* used

Strain	Relevant characteristics	Source or reference
SL1060	*rfaH1060 pmi trp met* Str^r	18
SL1060IH	SL1060 *ilvC*::Tn*5 hisG*::Tn*10*	This study
SL1030	*rfaH*$^+$ *pmi trp met*	B. A. D. Stocker
SL1032	*rfaG pmi trp met*	5
SL3750	*rfaJ417*	B. A. D. Stocker
SL1297	*metE*::Tn*10 rfaH*$^+$	B. A. D. Stocker
SL4213	*hsdL6 hsdSA29 galE*	7
SL4213H	SL4213 *hisG*::Tn*10*	This study
TT4697E	*ilvC*::Tn*5 galE bio*::Tn*10*	This study
SL5014	*cysE his pyrE* P22sie	10
TV148	*rfaI432*	19
TV119	*rfb*	B. A. D. Stocker
NK1531	F′ *zzf-20*::Tn*10 lac*$^+$ *pro*$^+$ Amp^r/r$^-$ m$^+$ *cys* Nal^r Rif^r	N. Kleckner
KP1494T	*rpo thiA*::Tn*10*	This study
TT7349	*rho-111*	J. Roth
TT7349E	*rho-111 galE bio*::Tn*10*	This study
TT7349H	TT7349 *hisG*::Tn*10*	This study
TT7336	*hisG9425*::Tn*10*	J. Roth
TT8	*ilvA2174*::Tn*10*	J. Roth
JM3	*rfaH sup*^{rfaH}3	This study
JM4	*rfaH sup*^{rfaH}4(Ts)[a]	This study
JM15	*rfaH sup*^{rfaH}15	This study
JM15E	JM15 *galE bio*::Tn*10*	This study
JM41	*rfaH sup*^{rfaH}41(Ts)	This study
JM42	*rfaH sup*^{rfaH}42	This study
JM58	*rfaH sup*^{rfaH}58	This study
JM58E	JM58 *galE bio*::Tn*10*	This study
JM75	*rfaH sup*^{rfaH}75	This study
JM206	*rfaH sup*^{rfaH}206(Ts)	This study
JM245	*rfaH sup*^{rfaH}245	This study
JM245E	JM245 *galE bio*::Tn*10*	This study
JM247	*rfaH sup*^{rfaH}247(Ts)	This study
AF100	*rfaH*(Am) *galE metE*::Tn*10*	This study
AF102	as AF100 but *rfaH*$^+$	This study
AF108	*rfaH cysE pyrE*	This study

[a] Ts, no growth on PPBE plates after 24 h of incubation at 42°C.

contains the *E. coli rho* gene, was obtained from A. Das and was transferred from its original *E. coli* host to *S. typhimurium* SL4213 (*hsdL6 hsdSA29*) before use in the experiments described here.

Strain constructions. Unless otherwise noted, introduction of *galE* into *galE*$^+$ strains was accomplished by P22- or P1-mediated cotransduction with *bio*::Tn*10* from strain G30T3 (*galE bio*::Tn*10*). The presence of the *galE* mutation was established on the basis of the galactose-negative phenotype on indicator plates and the sensitivity to galactose toxicity that characterize *galE* strains.

SL1060IH. *ilvC*::Tn*5* and *hisG*::Tn*10* were introduced into SL1060 by successive P1-mediated transductions from strains TT4697E and SL4213H, selecting for kanamycin resistance and tetracycline resistance, respectively.

KP1494T. *thiA*::Tn*10* was introduced into KP1494 (11) by P22-mediated transduction from TT501 (*thiA*::Tn*10*).

AF100. *rfaH*(Am) was introduced into strain G30 by P1-mediated cotransduction with *metE*::Tn*10* from SL1297. Tetracycline-resistant transductants were screened for the P22^r Ffm^s phenotype of RfaH$^-$ cells to yield AF100. AF102 was obtained from the same experiment as a tetracycline-resistant P22^s Ffm^r transductant.

AF108. *rfaH* was introduced into strain SL5014 by P1-mediated transduction from SL1297. Tetracycline-resistant transductants were screened for the P22^r Ffm^s phenotype of RfaH$^-$ cells to yield AF108.

TT7349H. *hisG*::Tn*10* was introduced into strain TT7349 by P22-mediated transduction from TT7336.

F′ transfer. F′ transfer was performed by standard methods (13), using a donor-to-recipient ratio of 1:3. The F′ in all donor strains originated from strain NK1531. Transconjugants were selected on the basis of their ability to grow in the presence of tetracycline. The donor was selected against by plating in the presence of streptomycin (for NK1531) or in the absence of tryptophan. Transfer efficiency was expressed as number of transconjugants per recipient cell, normalized in each case to the yield of transconjugants when the donor was the isogenic *rfaH*$^+$ strain (approximately 3×10^{-3}).

Isolation of *sup*^{rfaH} mutants. Cells from an actively growing culture of strain SL1060 were suspended at a density of 5×10^8 cells per ml and exposed to nitrosoguanidine (1 mg/ml) at 37°C for 60 min, essentially as described by Miller (13); this resulted in approximately 50% killing. Survivors were spread on PPBE-mannose plates which were then overlaid with PPBE soft agar containing approximately 10^9 PFU of bacteriophage Ffm. After overnight incubation at 37°C, Ffm-resistant colonies were picked, purified, and retested for phage sensitivity pattern and genetic markers. About 2×10^{-5} of the mutagenized cells had become resistant to Ffm (Ffm^r) and sensitive to P22 (P22^s). To isolate spontaneous mutants, the cells were treated identically except that nitrosoguanidine was omitted. The yield of spontaneous Ffm^r P22^s mutants was approximately 10^{-7}. The Ffm^r P22^s isolates from the two selections formed the original pool of *sup*^{rfaH} mutants.

Other microbiological procedures. To measure the frequency of cotransduction of *thiA*::Tn*10* and *rpoBC*, strain KP1494T was used as donor in P22-mediated transduction into the wild-type strain SL1030. Tetracycline-resistant transductants were selected, purified, and scored for transfer of the temperature-sensitive *rpoBC* mutation by testing for their ability to grow on PPBE plates at 42°C and their resistance to rifampin (0.1 mg/ml). In all cases, the appearance of temperature sensitivity was accompanied by resistance to 0.1 mg of rifampin per ml. A similar procedure was used to measure the frequency of cotransduction of *thiA*::Tn*10* and *sup*^{rfaH}247(Ts) into SL1030. The donor was strain JM247T (*sup*^{rfaH}247 *thiA*::Tn*10*). Transductants were scored for temperature sensitivity as an indicator of transfer of the temperature-sensitive *sup*^{rfaH}247 mutation.

The reversion frequency of *sup*^{rfaH}247 was determined by selecting for colonies of JM247 that appeared on PPBE plates after incubation for 24 h at 42°C. The frequency of reversion to temperature resistance was 2×10^{-7}. The temperature-resistant colonies were repurified twice at 42°C and then tested for their phage resistance pattern.

RESULTS

Isolation of suppressor mutants. To identify mutations that suppress the abnormal phenotype of *rfaH* mutants of *S. typhimurium*, we took advantage of the fact that RfaH$^-$ and RfaH$^+$ cells can be distinguished on the basis of their altered sensitivity to bacteriophages that utilize lipopolysaccharide as their cellular receptors. RfaH$^+$ cells synthesize the complete core lipopolysaccharide. This permits the subsequent addition of O antigen, thereby conferring sensitivity to bacteriophage P22 and resistance to bacteriophage Ffm (Fig. 1). Conversely, the incomplete lipopolysaccharide synthesized by *rfaH* mutant cells confers P22 resistance and Ffm sensitivity.

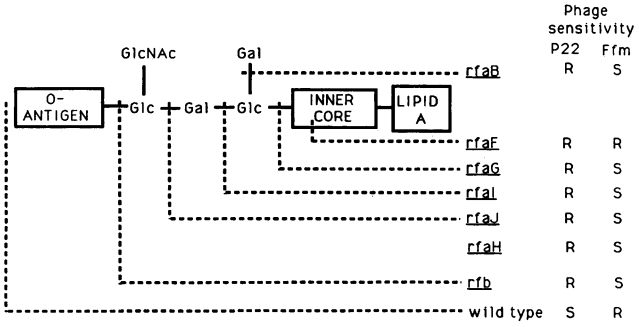

FIG. 1. Lipopolysaccharide structure and phage sensitivities of wild-type and mutant strains of *S. typhimurium*. Interrupted lines indicate the points at which elongation of the polysaccharide core is blocked in *rfaG*, *rfaB*, *rfaJ*, *rfaI*, *rfaF*, and *rfb* mutants, resulting in formation of truncated lipopolysaccharides. *rfaF* mutants fail to incorporate the distal heptose residue of the inner core. *rfb* genes are responsible for synthesis and addition of the O antigen and are not part of the *rfa* cluster. Sensitivity (S) or resistance (R) to phages P22 and Ffm are indicated on the right for the wild type and for each of the mutant classes. For further structural details, see reference 15.

We therefore selected Ffm-resistant colonies from a culture of strain SL1060 (*rfaH*) to identify *rfaH* mutant cells that had become phenotypically RfaH$^+$. This imposed a requirement for relatively high-level suppression since the residual presence of a relatively small number of "RfaH$^-$" phage receptors would be expected to make the cells sensitive to Ffm. Conversion of the phenotype of RfaH$^-$ strains from Ffm^s to Ffm^r indicates that either (i) a mutation has led to loss of a glycosyltransferase required for formation of the inner core, leading to synthesis of a truncated lipopolysaccharide that has lost the Ffm receptor site (illustrated for *rfaF* in Fig. 1), or (ii) the defect in *rfaGBIJ* expression that results from the *rfaH* mutation has been corrected by mutation in a regulatory site within the *rfaGBIJ* cluster or by mutation elsewhere in the chromosome.

To eliminate mutations in genes required for synthesis of the inner core, the Ffm-resistant mutants were screened to identify those that had also acquired sensitivity to bacteriophage P22. Since the complete wild-type lipopolysaccharide is required for P22 adsorption, suppressor strains that have become P22 sensitive cannot represent mutants in inner-core biosynthesis.

Three types of suppressor mutation can be expected from such a selection: (i) mutations within *rfaH* that correct the original *rfaH* defect and restore normal function to the RfaH protein, (ii) mutations in the presumed RfaH-responsive regulatory site(s) within the *rfaGBIJ* cluster that either eliminate the need for the RfaH protein or that generate altered regulatory sites that can interact productively with the mutant *rfaH* gene product, and (iii) mutations in other genes whose gene products may be involved in the RfaH regulatory system.

Ten independently isolated Ffm^r P22^s mutants were selected for further study. Seven were spontaneous mutants (JM3, JM4, JM15, JM41, JM42, JM58, and JM75), and three were isolated after treatment with nitrosoguanidine (JM206, JM245, and JM247). Four of the mutants (JM4, JM41, JM206, and JM247) were temperature sensitive for growth at 42°C on nutrient agar or PPBE plates.

Mutations within the *rfaH* gene were excluded by the following transductional mapping results. (i) The suppressor mutations were not cotransducible with *metE*$^+$, which was 30 to 50% linked to *rfaH* in parallel transductions. The criterion for transfer of the suppressor mutation was conversion of the bacteriophage resistance pattern of an *rfaH* mutant recipient from Ffm^s P22^r to Ffm^r P22^s (Table 2). (ii) The *rfaH* mutant allele was still present in the suppressor strains as shown by transductional transfer into AF102 (*rfaH*$^+$) (Table 2). Transfer of the *rfaH* mutant allele from the suppressor strains into AF102 (*rfaH*$^+$ *metE*::Tn*10*) by cotransduction with *metE*$^+$ was indicated by conversion of the bacteriophage resistance pattern of the recipient from Ffm^r P22^s to Ffm^s P22^r. These results show that the *sup*^{rfaH} mutations were not revertants or intragenic suppressors of the original *rfaH1060* mutation.

TABLE 2. ES18-mediated transductional analysis of *rfaH* and *rfaGBIJ* regions of *sup*^{rfaH} mutants

Donor[a]	No. of transductants		
	Ffm^s Met$^+$[b]	Met$^+$ Ffm^r[c] (10^6)	Ffm^s Pyr$^+$ Cys$^+$[d]
rfaH$^+$	0.0	1.0	ND[e]
rfaH	0.33	0.002	ND
rfaG	ND	ND	0.92[f]
rfaI	ND	ND	0.92[f]
rfaJ	ND	ND	0.98[f]
None	ND	ND	0.0[f]
JM3	0.22	0.002	1.0[g]
JM4	0.5	0.003	1.0[g]
JM15	0.22	0.002	1.0[g]
JM58	0.33	0.008	ND
JM41	0.25	0.006	1.0[g]
JM42	0.56	0.002	1.0[g]
JM75	0.33	0.002	1.0[g]
JM206	0.3	0.003	1.0[g]
JM245	0.17	0.002	1.0[g]
JM247	0.1	0.002	1.0[g]

[a] Donor strains were as follows: *rfaH*$^+$, SL1030; *rfaH*, SL1060; *rfaG*, SL1032; *rfaI*, TV148; *rfaJ*, SL3750.

[b] The recipient was AF102 (*metE*::Tn*10* *rfaH*$^+$). Met$^+$ transductants were selected and tested for sensitivity to Ffm. The numbers indicate the fraction of total Met$^+$ transductants that were Ffm^s.

[c] The recipient was AF100 [*rfaH*(Am) *metE*::Tn*10*]. Met$^+$ Ffm^r transductants were selected. The numbers indicate Met$^+$ Ffm^r transductants per recipient cell.

[d] Cys$^+$ Pyr$^+$ transductants were selected and tested for sensitivity to Ffm. The numbers indicate the fraction of Cys$^+$ Pyr$^+$ transductants that were Ffm^s.

[e] ND, not determined.

[f] The recipient was AF109 (*cysE pyrE rfaH*$^+$).

[g] The recipient was AF108 (*cysE pyrE rfaH*).

Suppressor mutations within the *rfa* target(s) were excluded by demonstrating that none of the *sup*^{rfaH} strains mapped within the *rfaGBIJ* cluster. This was shown by using each of the *sup*^{rfaH} strains as transductional donors for transfer into AF108 (*rfaH cysE pyrE*). Transductants that had received the *rfaGBIJ* region were identified by selecting for Cys$^+$ Pyr$^+$ recombinants since *rfaGBIJ* lies between *pyrE* and *cysE* on the genetic map of *S. typhimurium* (17). None of the Cys$^+$ Pyr$^+$ transductants showed the Ffm^r phenotype of the *sup*^{rfaH} *rfaH* donors (Table 2). In parallel experiments, the cotransductional linkage of genes of the *rfaGBIJ* cluster with *cysE*$^+$ *pyrE*$^+$ was 92 to 98%. Therefore, none of the 12 *sup*^{rfaH} mutations represented mutations within or close to the regulated *rfaGBIJ* genes.

Since the suppressor mutations were located neither in *rfaH* nor in the *rfaGBIJ* cluster, we conclude that they represent mutations in other genes involved in the RfaH-mediated positive regulation of *rfaGBIJ* gene expression.

Effects of *sup*^{rfaH} on *tra* function. As noted above, loss of

TABLE 3. Phage sensitivity patterns and conjugal efficiencies of sup^{rfaH} mutants

Strain	Phage sensitivity (P22/Ffm)[a]	F′ transfer
SL1030 (*rfaH*$^+$)	S/R	1.0
SL1060 (*rfaH*)	R/S	<0.004
JM206 [sup^{rfaH}206(Ts) *rfaH*]	S/R	1.0
JM4 [sup^{rfaH}4(Ts) *rfaH*]	S/R	0.5
JM245 (sup^{rfaH}245 *rfaH*)	S/R	0.16
JM75 (sup^{rfaH}75 *rfaH*)	S/R	0.12
JM247 [sup^{rfaH}247(Ts) *rfaH*]	S/R	0.1
JM58 (sup^{rfaH}58 *rfaH*)	S/R	0.08
JM15 (sup^{rfaH}15 *rfaH*)	S/R	0.04
JM42 (sup^{rfaH}42 *rfaH*)	S/R	0.006
JM41 [sup^{rfaH}41(Ts) *rfaH*]	S/R	<0.004
JM3 (sup^{rfaH}3 *rfaH*)	S/R	[b]

[a] Resistance (R) or sensitivity (S) to phage P22/resistance (R) or sensitivity (S) to phage Ffm.
[b] We were unable to obtain a stable F′ derivative of JM3.

rfaH function is associated with a defect in expression of the *traYZ* operon of the F factor, which is required for the host cell to act as a conjugal donor. Therefore, we asked whether the effects of the sup^{rfaH} mutations were limited to the *rfaGBIJ* genes or whether the sup^{rfaH} mutations also suppressed the effect of the *rfaH* mutation on *tra* gene expression. As shown in Table 3, the ability to act as donors in conjugal transfer was partially or completely restored by 8 of the 10 sup^{rfaH} mutations. The increase in transfer efficiency varied between 10- and 250-fold among the different sup^{rfaH} strains.

Some sup^{rfaH} mutations are mutations in rho. Preliminary mapping by Hfr-mediated conjugation defined a subset of sup^{rfaH} mutations that mapped between 78 and 83.3 U on the *S. typhimurium* genetic map. When these strains were used as recipients in interrupted matings with Hfr strain SA722, the *sup*$^+$ allele was transferred with high frequency as shown by conversion of the bacteriophage resistance pattern of the recipient from Ffm^s P22^r to Ffm^r P22^s. This placed the mutations counterclockwise and close to the origin of transfer of SA722, which is located between *cya* and *ilv* at approximately 83.3 U on the *E. coli* genetic map.

Because the *rho* gene, coding for transcriptional termination factor rho, maps in this region, we asked whether the sup^{rfaH} mutations in these strains represented *rho* mutations. In these experiments, rho function was monitored by measuring the ability of strains containing *hisG*::Tn*10* to grow on histidinol in the absence of histidine. The presence of downstream rho-dependent terminator(s) prevents the progression of transcripts that originate within Tn*10*, thereby preventing transcription of downstream genes. As a result, the *hisG*::Tn*10* strains are unable to utilize histidinol as a source of histidine (4). Replacement of the chromosomal *rho*$^+$ gene by a *rho* mutant allele permits transcription through the terminator(s), restoring the ability to grow on histidinol. As expected, when the chromosomal *rho*$^+$ gene of the *hisG*::Tn*10* indicator strain was replaced by the *rho-111* mutant allele (in strain TT7349H), the strain was able to grow on histidinol in the absence of histidine (see Table 5).

The following evidence indicated that the sup^{rfaH} mutations in three of the suppressor strains (JM15, JM58, and JM245) represented *rho* mutations.

(i) The sup^{rfaH} mutations in JM15, JM58, and JM245 were cotransducible with *ilv*, which lies at approximately 83 map units, within 0.5 min of *rho* (17). The linkage of each of the sup^{rfaH} alleles to *ilv* was similar to that of a known *rho* mutation (*rho-111*) (Table 4).

TABLE 4. Transductional linkage of sup^{rfaH} and *rho* to *ilvA*

Donor genotype[a]	Recipient	Fraction of transductants
		Hol$^+$ Ilv^{+b}
rho-111	SL060IH	0.71
		RfaH$^+$ Ilv^{+b}
sup-15	SL1060IH	0.63
sup-58	SL1060IH	0.69
sup-245	SL1060IH	0.64
		RfaH$^-$ Tet^{rc}
ilv::Tn*10*	JM15	0.25
ilv::Tn*10*	JM58	0.34
ilv::Tn*10*	JM245	0.21
ilv::Tn*10*	JM3	<0.009
ilv::Tn*10*	JM41	0.025
ilv::Tn*10*	JM42	<0.008
ilv::Tn*10*	JM75	0.008
ilv::Tn*10*	JM206	0.63

[a] Donor strains were as follows: *rho-111*, TT7349E; *sup-15*, JM15E; *sup-58*, JM58E; *sup-245*, JM245E; *ilv*::Tn*10*, TT8.
[b] The *ilv*$^+$ allele from each of the indicated donors was transferred by P1-mediated transduction into SL1060IH (*rfaH ilvC*::Tn*5 hisG*::Tn*10*). The numbers indicate the fraction of Ilv$^+$ transductants that were able to grow on histidinol in place of histidine (Hol$^+$) or were Pss^s Ffm^r (RfaH$^+$).
[c] The *ilv*::Tn*10* allele from strain TT8 was transferred by P22-mediated transduction into each of the indicated sup^{rfaH} *rfaH* recipients. The numbers indicate the fraction of Ilv$^+$ transductants that were P22^r Ffm^s (RfaH$^-$).

(ii) The sup^{rfaH} mutations in JM15, JM58, and JM245 were associated with alteration of rho function. This was shown by introducing *hisG*::Tn*10* into each of the three strains. All of the resulting *hisG*::Tn*10* sup^{rfaH} transductants were able to grow in the presence of histidinol and absence of histidine (Table 5). Therefore, each of the three sup^{rfaH} strains had also become Rho$^-$. In contrast, when *hisG*::Tn*10* was introduced into the parental *rfaH* mutant strain SL1060 or into

TABLE 5. Effect of sup^{rfaH} mutations on readthrough past a rho-dependent terminator

Recipient	Transductants[a]	
	Tet^r Hol$^+$/Tet^r	Relevant genotype
SL1030	0/14	*rho*$^+$ *hisG*::Tn*10*
TT7349H	39/39	*rho-111 hisG*::Tn*10*
JM15	22/22	*sup-15 hisG*::Tn*10*
JM58	8/8	*sup-58 hisG*::Tn*10*
JM245	8/8	*sup-245 hisG*::Tn*10*
JM3	0/42	*sup-3 hisG*::Tn*10*
JM4	0/8	*sup-4 hisG*::Tn*10*
JM41	0/8	*sup-41 hisG*::Tn*10*
JM42	0/8	*sup-42 hisG*::Tn*10*
JM75	0/8	*sup-75 hisG*::Tn*10*
JM206	0/8	*sup-206 hisG*::Tn*10*
JM247	0/8	*sup-247 hisG*::Tn*10*

[a] The *hisG*::Tn*10* allele was transferred from strain TT7336 into the indicated recipient strains by P22-mediated transduction. Tet^r recombinants were selected and tested for their ability to grow on histidinol (Hol$^+$) in the absence of histidine. Tet^r Hol$^+$/Tet^r, fraction of Tet^r transductants that were Hol$^+$. None of the Tet^r transductants were able to grow in the absence of both histidine and histidinol.

TABLE 6. Correction of the phage sensitivity pattern of *sup*^{rfaH} mutants by a *rho*$^+$ plasmid

Recipient	Phenotype of transformant[a]		
	No plasmid	pBR322	pEG25 (*rho*$^+$)
SL1030 (*rfaH*$^+$)	RfaH$^+$	RfaH$^+$	ND
SL1060 (*rfaH*)	RfaH$^-$	RfaH$^-$	RfaH$^-$
JM15 (*sup-15 rfaH*)	RfaH$^+$	RfaH$^+$	RfaH$^-$
JM58 (*sup-58 rfaH*)	RfaH$^+$	ND	RfaH$^-$
JM245 (*sup-245 rfaH*)	RfaH$^+$	ND	RfaH$^-$
JM4 (*sup-4 rfaH*)	RfaH$^+$	RfaH$^+$	RfaH$^+$
JM41 (*sup-41 rfaH*)	RfaH$^+$	ND	RfaH$^+$
JM42 (*sup-42 rfaH*)	RfaH$^+$	RfaH$^+$	RfaH^{+b}
JM75 (*sup-75 rfaH*)	RfaH$^+$	ND	RfaH$^+$
JM206 (*sup-206 rfaH*)	RfaH$^+$	ND	RfaH^{+b}
JM247 (*sup-247 rfaH*)	RfaH$^+$	RfaH$^+$	RfaH$^+$

[a] The indicated plasmids were transformed into the recipient strains, and transformants were selected on the basis of resistance to ampicillin. The purified transformants were tested for sensitivity to P22 and Ffm. We were unable to obtain stable transformants of strain JM3. RfaH$^+$, P22^s Ffm^r; RfaH$^-$, P22^r Ffm^s. ND, not determined.

[b] Ambiguous phage sensitivity patterns in approximately 20% of transformants.

the other seven *sup*^{rfaH} strains, the *hisG*::Tn*10* transductants were unable to grow when histidine was replaced by histidinol, confirming the presence of *rho*$^+$.

(iii) When the *sup*^{rfaH} mutations in JM15, JM58, and JM245 were transduced into a *hisG*::Tn*10* *rfaH* recipient together with *ilvA*, all transductants that had acquired the RfaH$^+$ phenotype had also acquired the ability to grow on histidinol. Therefore, there was 100% transductional linkage of *sup*^{rfaH} with the *rho* mutations of the three suppressor strains.

(iv) The phenotypes of JM15, JM58, and JM245 were converted from RfaH$^+$ to RfaH$^-$ by introduction of the *rho*$^+$ plasmid pEG25, as shown by the conversion of their phage sensitivity patterns from Ffm^r resistant to Ffm^s (Table 6).

We conclude from these experiments that the *sup*^{rfaH} mutations in JM15, JM58 and JM245 represented *rho* mutations.

Allele specificity of *sup*^{rfaH} (Rho$^-$) mutations. To determine whether other *rho* mutations were also capable of suppressing the RfaH$^-$ phenotype of *rfaH1060*, another *S. typhimurium rho* mutant allele [*rho-111*(Ts)] was introduced into strain SL1060 (*rfaH*). In contrast to the *sup*^{rfaH} (Rho$^-$) alleles described above, *rho-111* was unable to correct the RfaH$^-$ phage sensitivity pattern of SL1060 at either the permissive or nonpermissive temperature. However, the *rho-111* allele did partially suppress the defect in conjugal transfer of isogenic *rho*$^+$ *rfaH1060* strains, as shown by a 15-fold increase in the efficiency of F′ transfer. This compares with a 10- to 40-fold increase in transfer efficiency resulting from the presence of the three *sup*^{rfaH} (Rho$^-$) alleles (Table 3).

Other *sup*^{rfaH} mutations. Of the *sup*^{rfaH} mutants that were not *rho* mutants, one temperature-sensitive *sup*^{rfaH} allele (JM247) mapped close to *rpoBC*, which codes for the β and β′ subunits of RNA polymerase. The frequency of cotransduction of *thiA*::Tn*10* with *rpoBC* was 0.13 to 0.21; the frequency of transduction of *thiA*::Tn*10* with the temperature-sensitive phenotype of *sup*^{rfaH}247 was 0.12 to 0.33. Reversion analysis of JM247 indicated that the temperature-sensitive defect and the effects on *rfa* function were probably due to the same mutation, since 10 of 10 spontaneous temperature-resistant revertants had also acquired increased resistance to P22.

The other *sup*^{rfaH} mutations mapped elsewhere on the chromosome and therefore were not located in *rho* or in the genes for the two RNA polymerase subunits. None were cotransducible with *argG*, which in *E. coli* maps close to *nusA*.

DISCUSSION

The mutations identified in the present study were selected on the basis of their ability to suppress the effects of an *rfaH* mutation on expression of the *rfaGBIJ* locus. The resulting *sup*^{rfaH} mutants fell into several groups.

Group 1 consisted of *rho* mutants. This was most clearly shown by the observation that replacement of the chromosomal *rho*$^+$ gene by the *sup*^{rfaH} (Rho$^-$) alleles permitted transcription to proceed through a rho-dependent terminator in *hisG*::Tn*10* strains. It seems reasonable to conclude that the *sup*^{rfaH} (Rho$^-$) suppressor mutations restore *rfa* function by permitting transcription past rho-dependent terminator(s) within the *rfaGBIJ* locus, thereby bypassing the need for the RfaH protein.

This result, in conjunction with the prior demonstration that *rfaH* mutations are associated with premature termination of transcription of the *traYZ* operon (3, 9), supports the view that the RfaH protein acts directly or indirectly as an operon-specific transcriptional antiterminator as originally suggested by Beutin et al. (3). It should be noted that the relevant rho-dependent sites may lie within the ultimate targets (*rfaGBIJ* and *traYZ*) but also could be located elsewhere, for example within transcriptional units whose gene products are actually responsible for the transcriptional effects on the target genes.

Although the ultimate target site(s) must be located within the *rfaGBIJ* cluster, none of the 10 *sup*^{rfaH} mutations were located in this region. This may reflect the fact that the mutant selection technique only identifies mutations that restore activity of all of the affected glycosyltransferases since correction of the RfaH$^-$ bacteriophage resistance pattern requires that complete wild-type lipopolysaccharide be synthesized. Therefore, suppressor mutations within the *rfaGBIJ* cluster would only have been found if a single regulatory site were responsible for the positive regulation of all of the *rfa* structural genes. Although the failure to find *sup*^{rfaH} mutations that were located within the *rfaGBIJ* cluster could mean that multiple targets are present within the cluster, the relatively small number of *sup*^{rfaH} mutants that were examined makes this conclusion quite speculative.

Several of the *sup*^{rfaH} mutations were located in genes that code for known components of the transcriptional machinery. In addition, however, six of the original *sup*^{rfaH} mutations were not located in *rho* or *rpoBC*. Since these mutations reversed the effects of *rfaH* mutations on lipopolysaccharide structure and on Tra function, they are likely to identify genes that code for other proteins that play a role in the RfaH-mediated regulatory system. This implies that the regulatory system may involve several additional proteins in addition to RfaH. Study of the genes and gene products of the remaining group of *sup*^{rfaH} mutations therefore can be expected to provide further information about the mechanism responsible for the RfaH-mediated transcriptional regulation.

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