Polarity Effects in the *hisG* Gene of Salmonella Require a Site Within the Coding Sequence

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ABSTRACT

A single site in the middle of the coding sequence of the *hisG* gene of Salmonella is required for most of the polar effect of mutations in this gene. Nonsense and insertion mutations mapping upstream of this point in the *hisG* gene all have strong polar effects on expression of downstream genes in the operon; mutations mapping promotor distal to this site have little or no polar effect. Two previously known *hisG* mutations, mapping in the region of the polarity site, abolish the polarity effect of insertion mutations mapping upstream of this region. New polarity site mutations have been selected which have lost the polar effect of upstream nonsense mutations. All mutations abolishing the function of the site are small deletions; three are identical, 28-bp deletions which have arisen independently. A fourth mutation is a deletion of 16 base pairs internal to the larger deletion. Several point mutations within this 16-bp region have no effect on the function of the polarity site. We believe that a small number of polarity sites of this type are responsible for polarity in all genes. The site in the *hisG* gene is more easily detected than most because it appears to be the only such site in the *hisG* gene and because it maps in the center of the coding sequence.

MOST nonsense and frameshift mutations in bacterial operons show a polar effect that reduces expression of promoter-distal genes in the same operon (FRANKLIN and LURIA 1961; JACOB and MONOD 1962; AMES and HARTMAN 1963). This effect has been shown to result from premature termination of mRNA synthesis at a point distal to a nonsense codon, but within the mutant gene (FRANKLIN and YANOFSKY 1976). This message termination has been shown to require the termination factor, *rho* (MORSE and PRIMAKOFF 1970; RICHARDSON, GRIMLEY and LOWERY 1975; ROBERTS 1969). The strength of a polarity effect is generally found to be function of the distance between the nonsense mutation and the next translation initiation site (NEWTON *et al.* 1965; FINK and MARTIN 1967).

A general model for the action of *rho* in polarity has been developed (DE CROMBRUGGE *et al.* 1973; SHIMIZU and HAYASHI 1974; VON HIPPEL *et al.* 1984). Specific models for the mechanism of *rho*-dependent message termination will be discussed later. The general model accounts for the basic features of polarity effects. According to this model, messenger RNA synthesis is coupled to translation and the ribosomes associated with a nascent message prevent binding of the *rho* factor to the message. When translation encounters a nonsense codon, ribosomes leave the message, but transcription continues. Thus, between a nonsense codon and RNA polymerase at the growing end of the message, no ribosomes are present until an effective translation initiation site is synthesized allowing ribosomes to reenter the message. This stretch of "naked" message allows binding of *rho* factor; *rho* then moves 5′-3′ along the message until it encounters a specific site that activates the NTPase activity of *rho* factor. The *rho* factor then proceeds until it encounters polymerase and triggers release of the growing RNA from the DNA being transcribed. According to the above model, the increase in polar effect as a function of distance to the next initiation site might be accounted for by suggesting that a longer "naked" message provides more sequences within which termination can occur at random, or by providing more specific sites which can serve to signal termination. Here we describe evidence for a specific site within coding sequence required for polarity in the *hisG* gene of *Salmonella typhimurium*.

The sites required for polarity effects within bacterial operons are not well known. Most work on *rho*-dependent message termination has involved termination at the ends of bacterial operons (WU, CHRISTIE and PLATT 1981; KUPPER *et al.* 1978) and the regulated terminators of phage lambda (ROSENBERG *et al.* 1978). However, *in vitro* transcription studies have revealed the existence of *rho*-dependent termination sites within several genes (DE CROMBRUGGE *et al.* 1973; SHIMIZU and HAYASHI 1974; LAU, ROBERTS and WU 1982; CALVA and BURGESS 1980). The exact nature of these internal sites is not clear and the precise role

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of such sites in the polarity effects seen for bacterial operons *in vivo* has not been demonstrated. It is likely, but not certain, that the sites within operons function similarly to the *rho*-dependent termination sites that have been intensively studied *in vitro*. In most bacterial operons, even those with gradients of polarity, some polar effect is shown by almost all nonsense mutations (even those at the downstream end of genes). Therefore, it seems likely that at least some sites essential for termination are located at the extreme distal end of each gene. Such sites may be common and widely scattered with at least some located at the end of each gene; such a situation could generate the polarity gradients seen for some genes.

In the course of work on transposon insertions in the histidine operon, we noted that the *hisG* gene of Salmonella appears to contain a single small region that is essential for the major polar effect of *hisG* mutations; this site is located well within the *hisG* coding sequence. Evidence supporting the existence of this site was reported previously by FINK and MARTIN (1967), but was discussed in terms of a gradient of polarity; in fact, their data fit better with the existence of a discontinuity, rather than a gradient of polar effects for *hisG* mutants with the break point located at the polarity site described here. We have analyzed this site genetically in hopes of learning more about the mechanism of mRNA termination and the nature and distribution of sites in bacterial operons that are essential for polarity. The results will be discussed in terms of known properties of *rho*-dependent terminators that have been revealed by *in vitro* transcription studies.

MATERIALS AND METHODS

Bacterial strains: The bacterial strains used in this study are listed in Table 1. They are all derived from *Salmonella typhimurium* strain LT2. The *rho-111*(ts) mutant was donated by H. WHITFIELD. This mutant was selected by its ability to suppress the polar effect of an IS2 insertion in the *gal* operon at 30°; the mutant is temperature sensitive for growth at 42° (HOUSLEY, LEAVITT and WHITFIELD 1981).

Media and growth conditions: The E medium of VOGEL and BONNER (1956) containing 0.2% glucose was used as minimal medium. Nutrient broth (Difco) containing 0.5% NaCl was used as rich medium. The media were prepared and supplemented, when necessary, as described by DAVIS, BOTSTEIN and ROTH (1980). Tetracycline was added at a concentration of 10 μg/ml in minimal medium and 15 μg/ml in nutrient broth. Kanamycin was added at a concentration of 125 μg/ml in minimal medium and 50 μg/ml in nutrient broth. Cells were grown on liquid and solid media at 37°. Strains containing the *rho-111*(ts) mutation were grown at 30° (HOUSLEY, LEAVITT and WHITFIELD 1981).

Transductional crosses: Phage P22 mutant HT105/1 int-201 was used in all transductions. This phage transduces at high frequency (SCHMIEGER 1972) and carries an *int* mutation isolated by GARY ROBERTS. Transductions were performed as described by JOHNSTON and ROTH (1979). Phage was grown according to HOPPE *et al.* (1979). When selecting for tetracycline resistance, phages and bacterial cells were mixed preincubated in drug-free rich medium for 20 min at 37° before plating on selective medium. When selecting for kanamycin resistance, the preincubation was carried out for 45 min.

TABLE 1

Bacterial strains

Strain	Genotype	Source
TR6215	*metE338 his01242 hisC2124 trpE49 amt-49 ara-9 rho-111*	H. J. WHITFIELD, JR.
TT2615	*hisG9647*::Tn*5*(A)	
TT2616	*hisG9648*::Tn*5*(A)	
TT7336	*hisG9425*::Tn*10*(B)	N. KLECKNER
TT7334	*hisG9424*::Tn*10*(A)	N. KLECKNER
	hisG618	P. E. HARTMAN
	hisG8649	P. E. HARTMAN
TT3897	*hisΔ644 zee-1*::Tn*10 leu-414 supE20*/ F'*pro*$^+$ *lac*$^+$/ M13Hol76*hisD6404*	L. BOSSI
TR6699	*his01242 hisC2148*/F'*hisB2405* (plasmid from *E. coli* K12)	J. SCOTT
TR5998	*his-3050* (deletion of entire *his* operon)	
TT10475	*hitG199*	This study
TT10476	*hitG200*	This study
TR5390	*hisGD9580*	I. HOPPE
TR5097	*hisG8659*	J. SCOTT

(A) or (B) after Tn*5* or Tn*10* insertions designates the orientation of the element in the chromosome. Unless otherwise noted, strains were obtained from the laboratory culture collection.

Isolation of mutants of the polarity site: Aminotriazole-resistant derivatives of strain TR6699(*his01242 hisG2148*) were isolated as described by SCOTT, ROTH and ARTZ (1975). The *his* operon of each AT-resistant clone was transduced into a haploid strain carrying the *his-3050* deletion (which removes the entire *his* operon) selecting for growth on histidinol. These HisD$^+$ transductants were screened as described in RESULTS, for those carrying a mutation within the *hisG* gene that relieves polarity and causes an increase in the levels of this *hisB* gene expression and thereby confers AT-resistance.

Separation of mutations relieving polarity from the *hisG2148* polar mutation: Polarity site mutations (*hit-199* and *hit-200*) were separated from the original polar mutation (*hisG2148*) by first introducing a Tn*10* insertion (*hisG9425*::Tn*20*), by transduction, into the AT^R derivatives of *hisG2148*. The Tet^{R}HisD$^+$ recombinants from this cross, which contained the *hisG9425*::Tn*10* insertion and the mutation relieving polarity, were then used as donors in transductional crosses with deletion mutant *hisGD9580* (TR5390) as recipient, selecting for HisD$^+$ (growth on histinol). Since the *hisGD* deletion carried by the recipient in this cross does not cover the site of the *hisG9425*::Tn*10* insertion, HisD$^+$Tet^S recombinants could be isolated. Among these, two types were expected, wild-type recombinants (in which the *hisGD* lesion of the recipient had been repaired without concomitant inheritance of the polarity site mutation) and recombinants in which the polarity site mutation had been inherited. These HisD$^+$Tet^S recombinants were tested for their His phenotype and both His$^+$ and His$^-$ types were found. The His$^-$ recombinant types carry the polarity site mutations (*hit-199* or *hit-200*).

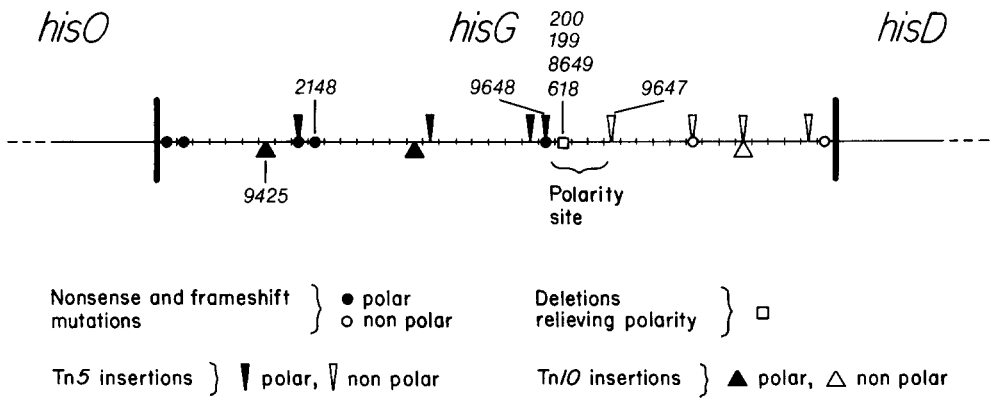

FIGURE 1.—Map distribution of polar and non polar *hisG* mutations. The position of Tn*5* insertions is a personal communication of D. BIEK. Additional sites for Tn*5* insertion, not shown in the figure, are present in the region of the polarity site (D. BIEK, personal communication). Behaviour of nonsense and frameshift mutations, with respect to polarity, is from FINK and MARTIN (1967) and their map positions are from HOPPE *et al.* (1979). Point mutations mapping to the left of the polarity site typically show 5% of normal expression of downstream genes. Point mutations mapping to the right of the polarity site designated "nonpolar" show 35% and 45% of downstream gene expression. The map position of polar Tn*10* insertions is from KLECKNER *et al.* (1979) and HOPPE *et al.* (1979); the site of the nonpolar Tn*10* insertion is a personal communication from AOQUAN WANG. Many additional nonpolar Tn*10* and IS10 insertions, other than the one shown in the figure, have been found (A. WANG and J. R. ROTH, manuscript in preparation); all map downstream of the polarity site.

Transfer of *hisG* mutations to the M13-*hisOGD* hybrid phage: The *hisG* mutations were sequenced by transferring them to the M13-*hisOGD* hybrid phage M13Ho176 (BARNES 1979) and using the phage DNA as a source for DNA sequencing. The M13Ho176 phage (BARNES 1979) carries the promoter region and the first two genes (*hisG* and *hisD*) of the histidine operon of Salmonella. The transfer of the mutations to the phage was carried out by using previously described genetic techniques (BOSSI and CIAMPI 1981; JOHNSTON and ROTH 1981). The technique takes advantage of the homology existing between the phage DNA and the bacterial chromosome in the region of the histidine operon. Recombination is allowed to occur between the phage DNA (*hisG*$^+$) and the chromosome of the strains carrying the *hisG* mutations, transferring the *hisG* mutations to the phage. The recombinant phage bearing *hisG* mutations was identified by its inability to transduce a His$^+$ phenotype into strains carrying the same *hisG* mutation that was expected to be on the phage. Those that failed to repair the mutation in question but could repair other nearby mutations were chosen. [(M13Ho176 can be transduced into new strains by P22-mediated transduction (JOHNSTON and ROTH 1981).]

Preparation of single-stranded template for DNA sequencing: Single-stranded DNA of the M13HOL76 derivatives carrying the *hisG* mutations to be sequenced was prepared by using a modification of the procedure of ZINDER and BOCKE (1982). After two polyethylene glycol precipitations, the phages were resuspended in DNA buffer and extracted four times with phenol. The aqueous layer was extracted four times with chloroform-isoamyl alcohol and the DNA precipitated in ethanol.

DNA sequencing: The choice of the primer to be used for sequencing the *hisG* mutations was facilitated by the knowledge of the genetic map position of the mutations (HOPPE, JOHNSTON, BIEK and ROTH 1979 and this work), the availability of the DNA sequence of the *hisG* gene (W. M. BARNES, personal communication) and the knowledge of the position of Tn*5* transposon insertion sites in the *hisG* sequence (BOSSI and CIAMPI 1981). Restriction fragment RH58 was used as primer in all sequencing experiments (BARNES 1978a, b). DNA sequencing experiments were carried out according to the procedure of SANGER, NICKLEN and COULSEN (1977) as modified by BARNES (1978b).

RESULTS

Initial evidence for a polarity site within the *hisG* gene: The transposons Tn*5* and Tn*10* usually cause an absolute polar effect when they insert into a bacterial operon (KLECKNER, CHAN and BOTSTEIN 1975; BERG, WEISS and CROSSLAND 1980). However, several exceptional insertions of these transposons have been noticed which do not eliminate expression of distal genes (BERG, WEISS and CROSSLAND 1980; CIAMPI, SCHMID and ROTH 1982; BLAZEY and BURNS 1982; BRASS, MANSON and LARSON 1984). At these sites, insertion of Tn*5* or Tn*10* blocks transcripts starting at the operon promoter but provides a promoter of its own which can direct transcription of downstream genes in the operon (CIAMPI, SCHMID and ROTH 1982; SIMONS *et al.* 1983). One such exceptional case is the *hisG* gene of Salmonella. Figure 1 presents a genetic map of the *hisG* gene with positions of several Tn*5* and Tn*10* insertion mutations. All insertions located promoter-proximal to a particular site in the gene are absolutely polar in the sense that they prevent expression of the distal *hisD* gene and leave cells unable to use histidinol as a source of histidine (the HisD$^-$ phenotype). These insertions also reduce expression of downstream genes since they cause a loss of *hisC* and *hisB* activity (data not shown). Insertions in the *hisG* gene, promoter-distal to this site express the *hisD* gene and show growth on histidinol. This distribution of HisD$^+$ and HisD$^-$ insertion mutations suggested the existence of a site within *hisG* that is required to terminate (presumably untranslated) transcripts emanating from upstream inserted elements. The mapping data on Tn*5* and evidence that transcription actually originates within Tn*5* will be presented in detail elsewhere (S. M. CIAMPI and D. BIEK, unpublished data; A.

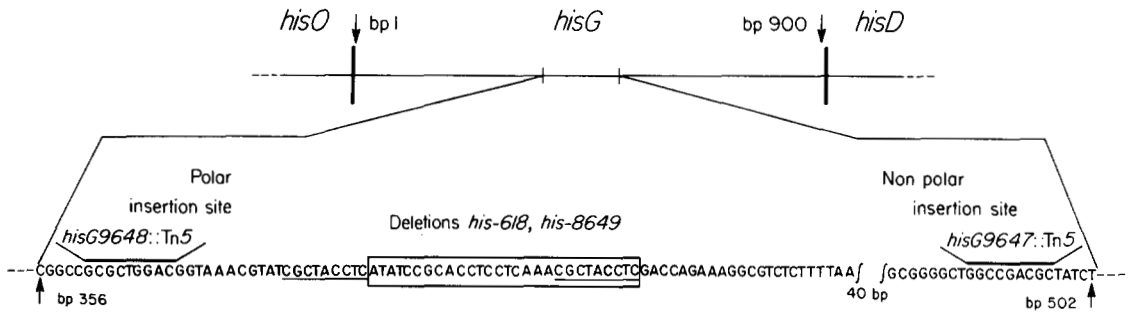

FIGURE 2.—Sequence alteration of two mutations relieving polarity. The region of DNA deleted in *his-618* and *his-8649* is boxed. Direct repeats of 9 bp likely to be involved in formation of the deletion are underlined. The sequences of the insertion sites of polar *hisG9648*::Tn*5* and of nonpolar *hisG9647*::Tn*5* are from BOSSI and CIAMPI (1981); the 9-base sequence that is duplicated to flank these inserts are indicated by "flying" overbars. Arrows point to base pairs numbered from the first base of this *hisG* gene.

WANG and J. R. ROTH, unpublished data). In essence, the evidence that transcription emanates from Tn*5* and Tn*10* inserts is that insertion of the element anywhere downstream of the polarity site activates the *hisD* gene even in strains lacking a *his* promoter. Insertion of either Tn*10* or Tn*5* upstream of the polarity site leaves *hisD* unexpressed, unless a *rho* mutation is present. SIMONS *et al.* (1983) have directly demonstrated the outward-directed promoter of Tn*10*.

The existence of the *hisG* polarity site is consistent with the polarity effects of nonsense mutations in the *hisG* gene. FINK and MARTIN (1967) measured the polarity effect of a series of *hisG* mutations whose map positions are known. While the polarity data were originally interpreted in terms of a smooth gradient of polarity, the distribution of data points is such that one could equally well argue for a discontinuity. All those mutations located promoter-proximal to an inferred polarity site show strong polar effects and those distal to this site show little or no polar effect (see Figure 1). Later we will show that mutations in the polarity site do, in fact, relieve polarity of an upstream polar point mutation.

Thus three promoters, of very different strengths are capable of expressing the *hisD* gene. The *his* promoter, when fully antiterminated is about 5 times the strength of the *lac* promoter (JOHNSTON and ROTH 1981); the Tn*10* (out) promoter has about 20% the strength of a *lac* promoter (CIAMPI, SSCHMID and ROTH 1982). The Tn*5* (out) promoter is very weak, approximately 2% of the *lac* promoter (D. STETLER and J. R. ROTH, unpublished results). In each case, expression of *hisD* can be blocked by the polar site; expression is restored by either a *rho* mutation or by mutations that damage the polarity site described here (see below).

Dependence of termination on *rho*-factor: Data on both Tn*5* and Tn*10* insertions suggest that message termination requiring this internal *hisG* site is dependent on the *rho* termination factor. Insertions of Tn*5* and Tn*10* at any point promoter-proximal to this site do not express *hisD* but become HisD$^+$ when a *rho* mutation is introduced into the strain. This has been shown previously for Tn*10* insertions (CIAMPI, SCHMID and ROTH 1982; BLAZEY and BURNS 1982). Data on Tn*5* insertions will be presented elsewhere.

Identification of *hisG* mutations that lack the polarity site: The general region of the *hisG* gene that includes the polarity site was identified as the sequence between the most promoter-distal Tn*5* insertion that fails to express *hisD* (*hisG9648*::Tn*5*) and the most promoter-proximal Tn*5* that has a HisD$^+$ phenotype (*hisG9647*::Tn*5*). These two insertion sites were identified following testing of many *hisG*::Tn*5* mutations (M. S. CIAMPI and D. BIEK, unpublished results) and are presented in Figure 2. These Tn*5* insertions have been genetically mapped by transduction crosses.

By comparing the map location of these insertions with the location of a variety of *hisG* point mutations on the detailed map of the *hisG* gene (HOPPE *et al.* 1979), it was possible to identify a small number of *hisG* mutations that map within the region between these inserts. These mutants were tested genetically to determine whether they have defects in their polarity site. To do this, each *hisG* mutant was crossed by transduction with a donor carrying a polar (HisD$^-$) *hisG*::Tn*10* insertion located upstream of the polarity site. Selection was made for tetracycline resistance (inheritance of Tn*10*) and the recombinants were scored for ability to grow on histidinol (HisD$^+$). If the recipient *hisG* mutant lacked a polarity site, the Tn*10* element should occasionally be inherited so as to generate a double mutant (*hisG*::Tn*10*, *hisG*$^-$) with a HisD$^+$ phenotype, since in the double mutants transcription could proceed across the defective polarity site into the *hisD* gene from the Tn*10* element. Of 13 mutations from this region tested, two (*his-618* and *his-8649*) gave rise to approximately 10% *hisD*$^+$ clones among their Tet^R transductants. By appropriate crosses we have demonstrated that these HisD$^+$ transductants do, in fact, carry both the donor Tn*10* insertion and the recipient *hisG* mutation

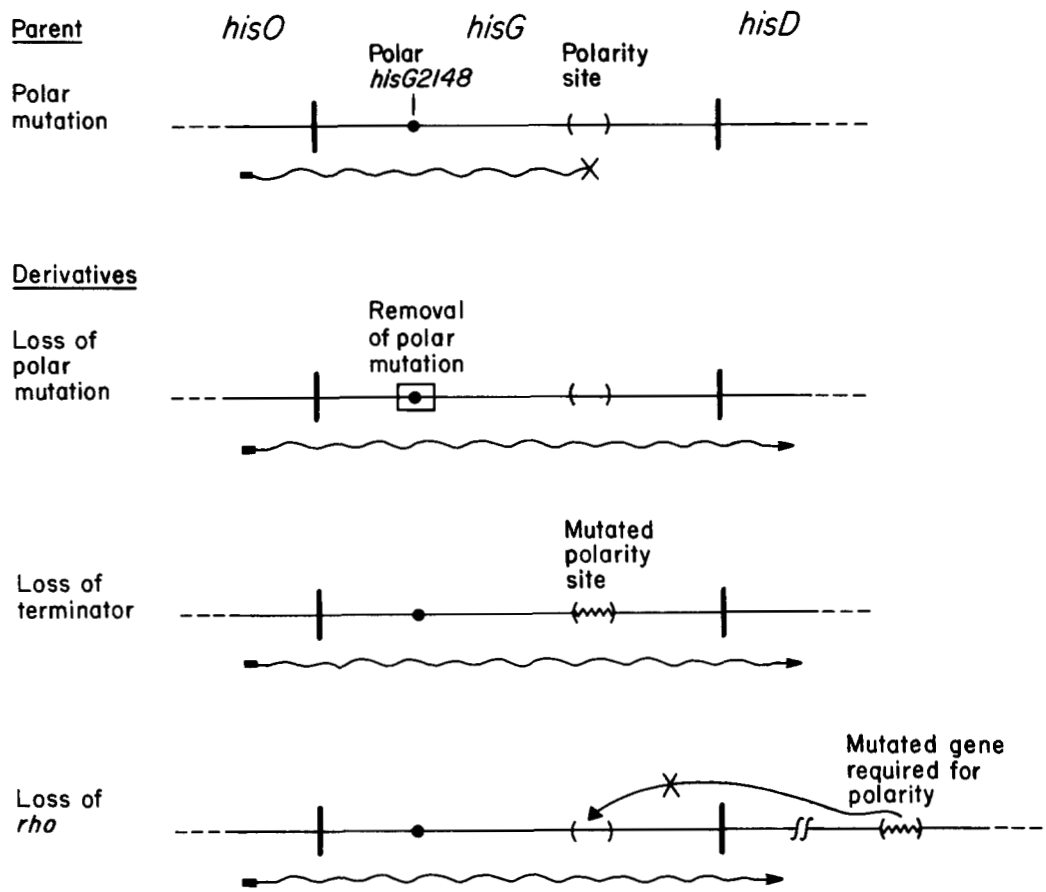

FIGURE 3.—Selection for mutants relieving polarity. Wavy lines indicate mRNA being synthesized. Zigzag lines indicate second site mutations relieving polarity.

(data not shown). The polarity site mutations do not express *hisD* by generating a new promoter; expression of *hisD* requires the presence of the transposon.

Since the genetic test that permitted identification of these two mutations is easy to perform, a large set of randomly chosen *hisG* mutations mapping outside the putative polarity site region were similarly tested. Of 60 additional point mutations tested, none shows a loss of the polarity function.

Genetic characteristics of two polarity site mutations: The two *hisG* mutations that appear to damage the polarity site, are stable (nonreverting) auxotrophic mutations mapping in the same deletion interval. They fail to show recombination when crossed with each other. Their ability to abolish the block to transcription was tested by introducing promoter-proximal Tn*10* insertions in both orientations (A and B). Both insertions tested, *hisG9425*::Tn*10* (B) and *hisG9424*::Tn*10* (A), gave approximately 10% HisD$^+$ recombinants when crossed with either *his-618* or *his-8649*. The ability of the two mutations to abolish the polarity site applies to both Tn*10* insertions. Thus, transcripts emanating from either end of Tn*10* reach the *hisD* gene only if the polarity site mutation is present. The same two mutations also permit transcription emanating from promoter-proximal Tn*5* insertions to reach the *hisD* gene.

DNA sequence alterations caused by *his-618* and *his-8649*: Both polarity site mutations were transferred by recombination to an M13 clone of the *hisOGD* region and the sequence of the critical region was determined by the dideoxynucleotide termination method of SANGER. The critical region was identified since the position of the flanking Tn*5* insertions has been previously determined (BOSSI and CIAMPI 1981). The sequence changes are presented in Figure 2. The two mutations are identical 28-bp deletions of a chromosomal segment located between repeated nine-base sequences. The affected wild-type sequence shows no unique features that suggest to us the exact structure of the terminator. Features of this sequence will be discussed later (see DISCUSSION).

Positive selection of mutants with an altered polarity site: To determine what features of the sequence are needed for polarity, we looked for additional mutations that affect the polarity site. This was done by selecting nonpolar mutants from a parent strain carrying a strongly polar point mutation at the promoter-proximal end of the *hisG* gene (*hisG2148*); the polarity effect of *hisG2148* is relieved by *rho*

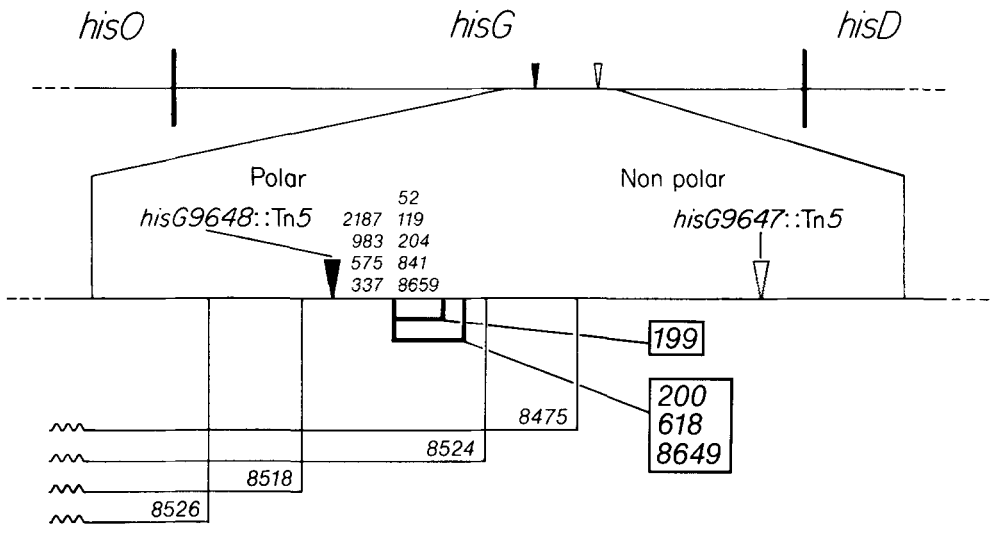

FIGURE 4.—Fine structure genetic map of terminator region. Deletions relieving polarity are indicated with thick lines and their allele numbers are boxed.

mutations. (See Figure 1 for the map position of *hisG2148*.) The selection method [that of SCOTT, ROTH and ARTZ (1975)] relies on a selection for increased function of the downstream *hisB* gene. One of the enzymatic activities of the *hisB* protein, imidazol glycerol phosphate dehydratase, is inhibited by the histidine analogue, aminotriazole (AT) (HILTON, KEARNEY and AMES 1965). Selection for resistance to AT demands increased levels of *hisB* gene expression. A strain carrying the polar *hisG* mutation and an F'*hisG*$^+$ *hisB*$^-$ plasmid was used. This prototrophic strain's only *hisB* gene is located *cis* to the strongly polar *hisG* mutation; therefore, the level of *hisB* gene expression is reduced. This strain cannot grow on minimal medium containing 15 mM AT. Mutants resistant to AT were selected and were encountered at a frequency of 2 per 10^7 cells plated.

The sorts of mutations expected from this selection were found and are described in Figure 3. These are: (1) deletions of the site of the parent polar mutation (2) mutations affecting the *rho* termination factor, (3) the desired mutations, affecting the polarity site or other sites within *hisG* essential to polarity.

Polarity site mutations were identified by the following procedure. First the AT^R transductants were crossed by transduction with a deletion mutant lacking the entire operon, in order to transfer their *his* region into a new genetic background; this was done using the AT^R strain as donor and selecting for HisD$^+$ transductants (able to grow on histidinol). Those mutants whose AT resistance was inherited in this cross must have a lesion in or near the *his* operon; *rho* mutations were eliminated. To identify polarity site mutants, each strain was transduced using phage grown on a Tn*10* insertion (*hisG9425*::Tn*10*) mapping promoter-proximal to the polarity site (see Figure 1). If transductants arise that still show a HisD$^+$ phenotype, then the recipient must have a mutation capable of preventing termination and allowing transcripts emerging from Tn*10* to reach *hisD*. Two new mutations met the above criteria, *hit-200* and *hit-199* (the *hit* designation is an extention of *his* allele number series). In principle, mutations generating new promoters capable of high level expression of *hisB* (and *hisD*) might have been expected in this class. Only one site in *hisG* is known to be mutable to generate a promoter (ST. PIERRE 1968). These promoters are weak and were not found.

The new candidates for polarity site mutations were separated from the other *his* mutation present in the original strain (as described in MATERIALS AND METHODS) and were found to have a His$^-$ phenotype. The new mutations map in the same deletion interval as the polarity site identified previously, far from the site of the original mutation, whose polar effect they correct. The new mutations fail to recombine with the original polarity site mutations (*hisG618* and *hisG8649*). When crossed with point mutations in the region of the polarity site, the new mutations behave identically to *hisG618* and *hisG8649*. These genetic results, summarized in Figure 4, suggest that the new mutations (*hit-199* and *hit-200*) affect the inferred polarity site.

DNA sequence changes of *hit-199* and *hit-200*: The two new polarity site mutations were transferred to the M13*his* clone and the mutant sequence was determined. The results are presented in Figure 5. Mutation *hit-200* is a recurrence of the same 28-bp deletion as mutations *his-618* and *his-8649*. Mutation *hit-199* removes an 18-bp sequence internal to the recurring 28-bp deletion. Repeated sequences are found near the ends of this shorter deletion that could account for the origin of a 16-bp deletion; these sequences may have been involved in generation of the 18-bp deletion actually found.

Mutations of the polarity site region that do not prevent polarity effects: The small region of the *hisG* map including the polarity site is well populated with

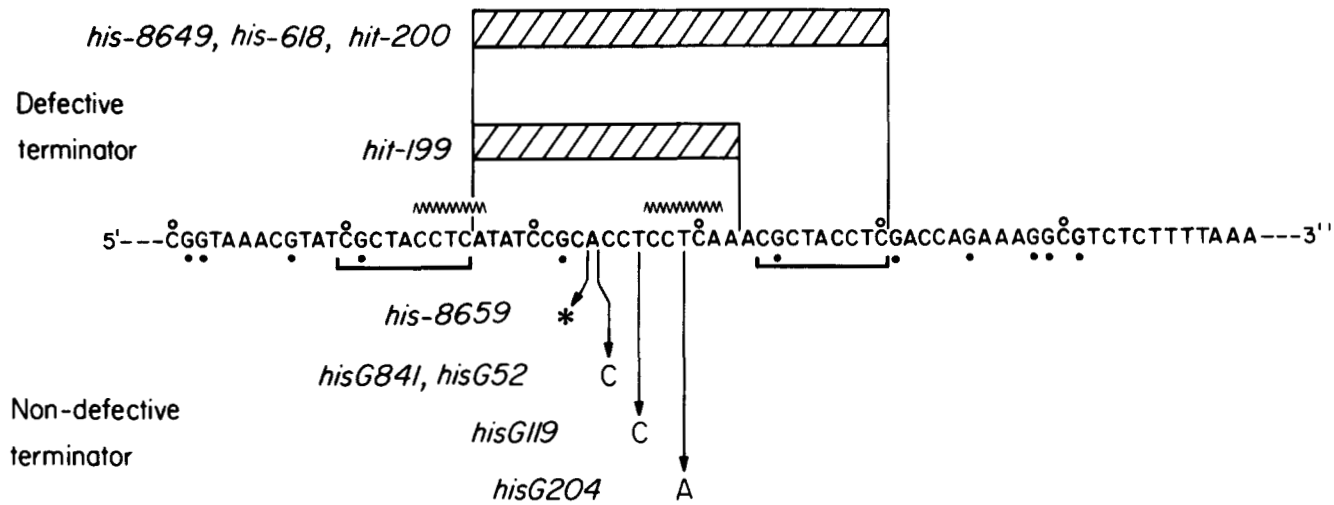

FIGURE 5.—Sequence alteration of mutations in the terminator region. The *hisG* sequence in the region of the terminator is shown (see also Figure 2). The rare G residues are indicated by dots just below the sequence. The spaced C residues suggested to be important by PLATT (1986) are indicated by small circles just above the sequence. Hatched boxes above the sequence indicate deletion mutations relieving polarity. Below the sequence, mutations that do not affect the terminator are shown. Repeated sequences that probably were involved in generation of the 28-bp deletion are underlined. Repeated sequences indicated by wavy lines are 16 bp apart but may have been involved in formation of the 18-bp deletion.

auxotrophic mutations that do not prevent polarity as judged by the crosses described earlier. The five point mutations which fail to recombine with both the 28-bp deletion and the 18-bp deletion were sequenced. The sequence alterations caused by these mutations are presented in Figure 5. As predicted by the genetic mapping, all changes do, in fact, fall within the shorter deletion (*hit-199*). Mutation *his-8659* is a 1-bp deletion; the other mutations are all base substitutions. None of these single base alterations prevents functioning of the polarity site. In an attempt to identify additional mutations impairing expression of polarity, revertants of the above four point mutations were selected and tested for ability to express polarity. All mutants except *his-8659* give rise to revertants, but none of the revertants has lost the ability to show polarity.

DISCUSSION

We have described a site within the coding region of the *hisG* gene of Salmonella that is responsible for most of the message termination within that gene. Termination of the outward directed transcripts of Tn*10* and Tn*5* requires this site. The site seems to be required for the polarity effect of nonsense and frameshift mutations in the *hisG* gene since the polar effect of such mutations is strong for those located upstream of this site and weak for mutations promoter-distal to this site. In addition, mutations in this site were isolated by selecting for removal of the polar effect of a point mutation *hisG2148* which maps far upstream of the polarity site. We have called this a "polarity site" but have not yet demonstrated what aspect of message termination requires this sequence. Since *rho*-dependent termination is involved, the site could, in principle, permit *rho* binding to mRNA; it could activate the *rho*-dependent ATPase (required for termination) or it could be the actual site at which termination occurs. It seems very likely that this site corresponds to the "upstream site" required for function of several *rho*-dependent terminators (LAU and ROBERTS 1985; SHARP and PLATT 1984; PLATT 1986; MORGAN, BEAR and VON HIPPEL 1983b; WU *et al.* 1980). These sites appear to activate the ATPase of the *rho*-factor and permit termination to occur at multiple points downstream from the site itself (WU, CHRISTIE and PLATT 1981). This possibility is supported by the recent observation of M. S. CIAMPI, M. S. BRUNI and C. B. CARLOMAGNO (personal communication) that *in vitro* transcription of *hisG* shows two major *rho*-dependent termination sites one of which corresponds closely with the polarity site described here; termination at this site does not occur in a polarity site deletion mutant. We will discuss the data presented here in terms of termination occurring at or near the site in question.

Sequence comparison for various *rho*-dependent terminators, including the *hisG* polarity site, has not revealed striking similarities, suggesting that no specific small sequence signals termination but rather that general characteristics of a larger region of the sequence might be responsible (discussed by PLATT 1986). The attempts to define these critical characteristics of a termination site have been based largely on the results of *in vitro* studies of *rho*-dependent termination. The following facts seem relevant:

1. *Rho*-dependent terminators require a site upstream of the point at which termination occurs (LAU and ROBERTS 1985; SHARP and PLATT 1984; MORGAN, BEAR and VON HIPPEL 1983b).

2. The function of *rho*-factor appears to require interaction with the mRNA, thus the critical aspects

of template recognition are likely to involve aspects of mRNA structure (SHARP and PLATT 1984; MORGAN, BEAR and VON HIPPEL 1983b; DARLIX 1973).

3. A region of message free of secondary structure appears to be necessary, but not sufficient, to define a termination site (MORGAN, BEAR and VON HIPPEL 1983a, b).

4. *Rho* factor possesses an ATPase activity that is stimulated by binding to poly-C, to specific sites in natural messages and to mixed polymers containing some C residues. The presence of G residues in the mRNA seems to inhibit activation of the ATPase (LOWERY-GOLDHAMMER and RICHARDSON 1974; RICHARDSON 1982; LOWERY and RICHARDSON 1977; SHARP and PLATT 1984).

5. This ATPase activity is required for message termination at multiple sites located downstream of the activating site (GALLUPPI and RICHARDSON 1980; HOWARD and DE CROMBRUGGE 1975).

These facts have been used to develop models for action of *rho* factor and for the function of the upstream site (MORGAN, BEAR and VON HIPPEL 1983b; PLATT 1986; RICHARDSON 1982). These models suggest that the site is an unstructured region of the message that is low in G content and contains C residues at appropriate spacing to activate the *rho* factor ATPase. A spacing of one C residue every 12 bases has been suggested by PLATT based on several theoretical considerations but relying mainly on comparing the sequences of several terminators including the unpublished *hisG* polarity site described here, the tryptophan operon terminator (trp t′) and a terminator upstream of the *rho* gene (PLATT 1986). This spacing of C residues can also be seen in a phage lambda terminator (tR1) (ROSENBERG *et al.* 1978; KUPPER *et al.* 1978) and in the terminator for the tyrosine tRNA transcript. Unfortunately, the existence of C residues with this spacing is likely to occur frequently on a random basis, since phase is not critical and slight deviations from 12 are likely to be permissible; this is especially true in a region poor in G residues and rich in C. This makes it difficult to assess whether the spacing seen in terminators is significant.

The data presented here for the *hisG* polarity site are consistent with the models for termination site structure including the suggestion of a 12-base spacing of C residues. The following points should be noted:

1. The sequence identified in *hisG* is poor in secondary structure and in G residues. (The position of G residues in the message is indicated by dots below the sequence in Figure 5.)

2. The approximate 12-base spacing of C residues is present. (These are indicated by small circles above the sequence in Figure 5.)

3. The two deletion types that destroy the polarity site remove much of the G-poor region and several of the C residues hypothesized to be critical. However, if one follows the sequence in either of the deletion types, C residues still occur every 12 residues in this region of the message. This suggests that perhaps removal of the G-poor region is the critical change caused by the deletions.

4. The point mutations that do not destroy terminator activity do not affect any of the critical C residues and they do not introduce new G residues into the critical region.

5. The one-base deletion mutation (*his-8659*), which does not destroy the site, also does not remove any critical C residues. However, the interval from which the base is removed is changed from 11 to 10 bases. Since this mutation does not destroy the polarity site, the new spacing in this interval is still assumed to be compatible with terminator activity.

While the interpretation above is consistent with the model developed by PLATT and others, the nature of the model is such that many sequences might fit. A more stringent test is clearly required to demonstrate definitively the sequence features that are critical to termination. The data presented here focus attention on a particular short sequence within coding sequence and restrict the models which might explain how termination occurs.

The *hisG* gene, with its apparent "step-gradient" of polarity, differs only slightly from genes showing a gradient of polarity (NEWTON *et al.* 1965; YANOFSKY and ITO 1966). The *hisC* gene of Salmonella is strikingly similar to *hisG* in that it too shows a polarity pattern suggesting a single point of discontinuity. All upstream *hisC* polar mutations show about 20% residual expression of distal genes in the operon; polar mutations near the downstream end of the *hisC* gene show only a 50% reduction in downstream gene expression (MARTIN *et al.* 1966). Unfortunately, the scatter in data points and the effects of reinitiation sites make it ultimately difficult, in all systems, to decide between a "smooth" gradient and a series of steps.

We suggest that the polarity patterns seen for all systems may reflect the placement and strength of a small number of polarity sites of the sort described here. Genes which include several weak, equivalent polarity sites may show a gradient of polarity. Genes like *hisC* and *hisG* may permit detection of the discontinuity because they have only one major termination site located within the gene. Genes with no apparent gradient may use polarity sites located near the end of that gene such that all mutations are subject to the effect of the same terminator. Alternatively, genes with no gradient may include no transcription terminator; their polar effects may reflect the efficiency of translational initiation of the subsequent gene as suggested by MARTIN *et al.* (1966).

Genes such as *hisG* and *hisC* which include single strong internal termination sites provide the opportunity of genetically analyzing the phenomenon of polarity.

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