Activation of Silent Genes by Transposons Tn*5* and Tn*10*

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ABSTRACT

The presence of transposons Tn*10* or Tn*5* in the genome increases the frequency with which a silent (promoter-less) gene (*hisD*) is mutationally activated. The activation frequency is increased 5–25-fold by Tn*10* and 30–90-fold by Tn*5*. Activation of the *hisD* gene is achieved by transposition of the entire transposon or one of its flanking insertion sequences to a region just upstream of the silent gene, between a Rho-dependent termination site in the adjacent *hisG* gene and the *hisD* gene. For both Tn*5* and Tn*10* the component insertion sequences were found to transpose much more frequently than the entire composite element. Transposons Tn*5* and Tn*10* have previously been shown to carry promoters which direct transcripts into sequences adjacent to their insertion sites.

SEVERAL insertion sequences (IS) are able to activate a silent (promoter-less) gene by inserting near the unexpressed coding sequence (SAEDLER *et al.* 1974; PILACINSKI *et al.* 1977; BOYEN *et al.* 1978; GLANSDORFF, CHARLIER and ZAFARULLAH 1978; WATZ, RATZKIN and CARBON 1978; BRENNAN and STRUHL 1980; BERG, WEISS and CROSSLAND 1980; REYNOLDS, FENTON and WRIGHT 1981). In each case, the insertion appears to provide a promoter for the expressed gene, but the general phenomenon is difficult to interpret, since not all insertions of a given element serve to activate distal genes (SAEDLER *et al.* 1974). As exemplified by IS*2*, several factors complicate the situation. Rearrangements within the element can generate a promoter activity directed out of the element (GHOSAL and SAEDLER 1978). Second, it is possible that the cell harbors several varieties of IS*2*, only some of which are capable of providing a promoter. In at least one case, the new promoter was generated by juxtaposition of IS and host sequences (JAURIN and NORMARK 1983). All of these complications could, in principle, explain why only some insertions activate expression of genes near the insertion site.

Transposons Tn*5* and Tn*10* cause an absolute polar effect when inserted at most sites in polycistronic operons. That is, at most insertion sites, they appear to cause termination of transcripts initiated by the operon's promoter and do not themselves provide a promoter capable of expressing distal genes. However insertions of Tn*5* and Tn*10* at certain sites do show expression of genes promoter-distal to the insertion site; this expression is independent of the main operon promoter and appears to be due to a promoter provided by the insertion itself (BERG, WEISS and CROSSLAND 1980; BLAZEY and BURNS 1982; CIAMPI, SCHMID and ROTH 1982; CIAMPI and ROTH 1988). Evidence that transposon Tn*10* possesses an outward promoter has been presented by SIMONS *et al.* (1983); gene activation by the IS*50*L element of Tn*5* has been observed in *Escherichia coli by* KENDRICK and REZNIKOFF (1988).

When classifying auxotrophic Tn*5* insertions in the *hisG* gene of *Salmonella typhimurium*, we noted that Tn*5* insertions fall into two approximately equal classes, one which could and one which could not provide a promoter capable of activating the immediately adjacent *hisD* gene. The ability of *hisG*::Tn*5* insertion mutants to express the adjacent gene shows a clear dependence on map position within the *hisG* gene (D. BIEK, M. S. CIAMPI and J. R. ROTH, unpublished results). Those insertions mapping upstream of a particular point in the *hisG* gene (the polarity site) are unable to activate *hisD*; those mapping distal to the polarity site do activate *hisD*. Furthermore Tn*10* insertions in *hisG* all fail to express the *hisD* gene, and all are located at two sites upstream of the polarity site.

The polarity site within the *hisG* has been characterized genetically and physically. The site, located near the middle of the *hisG* gene, is responsible for most of the polarity effect of point mutations in the *hisG* gene and appears to be required for Rho-dependent termination of untranslated transcripts (CIAMPI and ROTH 1988). The map distribution of His^+ and His^- insertions with respect to this site suggested that the variability in gene activation seen previously for Tn*5* and Tn*10* and for other transposable elements might be due to location of the insertion sites *vis-á-vis* Rho-dependent termination sites.

We propose that these elements at all insertion sites

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block the standard transcripts of the target region, but provide transcripts which start within the element and extend outward across downstream bacterial sequences. Up to the point of the first translation initiation site, these transcripts are untranslated and are therefore subject to the action of Rho-dependent transcription termination at polarity sites. Most insertions are absolutely polar; they block normal transcription of the target region and their own transcripts are terminated before reaching the next downstream gene. This suggests that polarity sites are common and are usually present within the untranslated region downstream of the element. Insertions activate the adjacent gene only if no polarity site is located between the inserted material and the first translation start site. In a wild-type gene (no insertion) polarity sites would not interfere with transcription since translation would prevent the action of the Rho termination factor.

To test aspects of this proposal we made use of an observation made by ANDERSON and ROTH (1978) in the course of their work on duplications which activate a silent (promoter-less) *hisD* gene. ANDERSON noted that hisD$^+$ revertants of the promoter deletion mutant *his-203* are much more frequent in strains carrying transposon Tn*10*. We have pursued this observation and report here that most HisD$^+$ revertants isolated in such strains have acquired an insertion of transposon material near the *hisD* gene. We describe here the stimulatory effect of both Tn*5* and Tn*10* on the frequency of mutations activating the silent *hisD* gene and the nature and positions of the activating insertions. The results support the idea that position of the insertion *vis-á-vis* a transcription termination site is critical.

MATERIALS AND METHODS

Bacterial strains: Bacterial strains are listed in Table 1; all strains are derivatives of *S. typhimurium* strain LT2. Plasmids with various derivatives of Tn*10* were kindly provided by N. KLECKNER and co-workers (HALLING *et al.* 1982; WAY and KLECKNER 1984).

Growth conditions and general methods: Culture media and conditions for growth of bacteria and transducing phage were as described by DAVIS, BOTSTEIN and ROTH (1980). Plasmids were introduced into Salmonella by transformation and subsequently were moved from one Salmonella strain to another by P22-mediated transduction.

Identification of extended deletions that activate the *hisD* gene: Previously it has been shown that revertants of *his-203* able to grow on histidinol (Hol$^+$) can occur by deletions that serve to fuse the *hisD* gene to a foreign promoter located to the left of the *his* operon (AMES, HARTMAN and JACOB 1963). We screened all Hol$^+$ revertants for possession of such deletions by crossing with phage grown on *hisG* point mutations (*hisG*3025, *hisG*6608, *hisG*337) mapping just promoter-distal to deletion *his-203*. Of the 150 spontaneous revertants tested here only three failed to recombine with these point mutations and appear to carry extended deletion mutations. Thus the previously described deletion class is rare.

Identification of *hisG* internal promoter mutations: The *hisD* gene can be activated by mutations that generate a new promoter within the *hisG* gene. All such mutations, when separated from *his-203*, lead to production of a cold sensitive *hisG* enzyme and auxotrophy at 25° (ST. PIERRE 1968). We sought this characteristic phenotype among the mutations causing activation of the *hisD* gene. Each of the Hol$^+$ revertants was used as a recipient in crosses with *hisG* point mutations located near the right end of the *his-203* deletion (see previous section). These His$^+$ recombinants, selected at 37°, have acquired material to repair the recipient *his-203* deletion, but retain with high probability the recipient region of *hisG* gene between deletion *his-203* and the *hisD* gene. If any Hol$^+$ revertant carries a promoter internal to *hisG*, a high percentage of these His$^+$ transductants show the characteristic cold sensitive *his* phenotype and (histidine dependent growth at 30°). Among the 150 Hol$^+$ revertants tested, 8 were due to formation of such a promoter within the *hisG* gene.

Identification of tandem duplications that activate the *hisD* gene: One class of the original Hol$^+$ revertants of *his-203* was found to be unstable, giving rise to Hol$^-$ segregants at high frequency (AMES, HARTMAN and JACOB 1963). These unstable revertants were found to be due to duplications with one endpoint between the *hisG* and *hisD* genes; the duplications fuse the *hisD* gene to a foreign promoter at the duplication join point (ANDERSON and ROTH 1978). In search of duplication types, we screened all revertants for instability by plating cells of the revertant strains for single colonies on medium containing histidinol (2 mM) and a low concentration of histidine (0.005 mM). On this medium the original Hol$^+$ revertants form large colonies, but any segregant that has lost the ability to utilize histidinol forms a tiny colony before exhausting the supply of histidine. Four such duplication types were found among 150 revertants screened.

Identification of inversions that activate the *hisD* gene: Inversion mutations can survive this selection if they place the *hisD* gene near a foreign promoter at the rearrangement break point (SCHMID and ROTH 1983b). Such inversion mutations, if large, can be identified by disruption of linkage at the *his* locus. This is checked by transduction crosses, using a Hol$^+$ revertant strain as a donor and a deletion of the entire *his* operon (*his-3050*) as recipient. If the donor's Hol$^+$ phenotype is due to an inversion larger that a transduced fragment, it will be impossible to transduce the Hol$^+$ determinant into the *his* deletion recipient, because the donor inversion places unrelated sequences on one side of the *his* region, preventing transductional repair of the recipient deletion. This method has been described in detail (SCHMID and ROTH 1983a). This test revealed no inversions among the set of 150 Hol$^+$ revertants tested.

Demonstrating linkage of the activating mutation to the histidine operon: The transduction test for inversions (described above) demonstrated that all the mutations activating the *hisD* gene map in or near the *his* operon. That is, all Hol$^+$ revertants of deletion *his-203*, when used as donors, were able to generate Hol$^+$ recombinants when crossed with the recipient deletion mutant *his-3050*. Since recipient deletion *his-3050* removes the entire *his* operon and overlaps deletion *his-203*, all Hol$^+$ transductants must inherit the donors *his* region, including deletion *his-203* and any mutation that can serves to activate the donor *hisD* gene. All Hol$^+$ revertants yielded transductants in this cross, demonstrating transductional linkage between *his-203* and the mutation responsible for activation of the *hisD* gene.

TABLE 1

Strain list

Strain	Genotype	Source
TR6936	*his-203*	Lab collection
TT7500	*his-203 trp-2451*::Tn*10*	Lab collection
TT7501	*his-203 hisC8579*::Tn*10*	Lab collection
TT7499	*his-203 guaB544*::Tn*10*	Lab collection
TT7498	*his-203 ileA597*::Tn*10*	Lab collection
TT8372	*his-203*/F'*lac*$^+$*zzf-20*::Tn*10*	Lab collection
TT7579	*his-203 thr-450*::Tn*5*	Lab collection
TT7582	*his-203 trp-2475*::Tn*5*	Lab collection
TT9044	*his-203 hisC9642*::Tn*5*	Lab collection
TT7581	*his-203 purG2140*::Tn*5*	Lab collection
TT9045	*his-203 srl-211*::Tn*5*	Lab collection
TT12230	*his-203 ilvD2171*::Tn*5*	Lab collection
TT9046	*his-203 metE2092*::Tn*5*	Lab collection
TT8376	*his-203*/F'lacI^Q L8 *lacZ*::Tn*10*(del-4)	N. KLECKNER
TR7520	*his-203*/pNK378	N. KLECKNER
TT8378	*his-203*/F'lacI^Q L8 *lacZ*::Tn*10*(del-4; HH104)	N. KLECKNER
TT7521	*his-203*/pNK579 Tn*10*(del-4; HH104)	N. KLECKNER
TT8381	*his-203*/pNK373	N. KLECKNER
TT8382	*his-203*/pNK474	N. KLECKNER
TT8798	*his-203 zzz-1081*::Tn*5*(*tnf-55*. amber)	Lab collection
TT8799	*his-203 zzz-1082*::Tn*5*(wild type)	Lab collection
TT9421	*his09702 hsiG316*::Tn*5*(*neo-116*)	Lab collection
TT9423	*his09702 hsiG316*::Tn*5*(*neo-111*)	Lab collection
TR5998	*his-3050* (deletion of entire operon)	P. E. HARTMAN
TT8389-8471	*his-203. hsiG*::IS*10. ileA597*::Tn*10* (original isolates)	
TT8472-8473	*his-203. hsiG*::Tn*10. ileA597*::Tn*10* (original isolates)	
TT8474-TT8556	*his-203. hsiG*::IS*10* (in *his-3050* background)	
TT8557-TT8558	*his-203. hsiG*::Tn*10* (in *his-3050* background)	
TT8559-TT8593	*his-203. hsiG*::IS*50 trp-2475*::Tn*5* (original isolates)	
TT8594-TT8604	*his-203, hsiG*::Tn*50 trp-2475*::Tn*5* (original isolates)	
TT8605-TT8639	*his-203. hsiG*::IS*50* (in *his-3050* background)	
TT8640-TT8650	*his-203. hsiG*::Tn*50* (in *his-3050* background)	

Testing the presence of a drug resistance element near the activated *hisD* gene: Since the cross described above moves the activating mutation into a new genetic background, it is possible to determine if that mutation was caused by insertion of an element encoding drug resistance. Each Hol$^+$ transductant of deletion mutant *his-3050* was scored for inheritance of resistance to kanamycin (for revertants arising in the presence of a Tn*5* element) or tetracycline (for those arising in the presence of Tn*10*). This permitted identification of the 11 revertants caused by insertion of complete Tn*5* elements and the 2 revertants caused by insertion of Tn*10*.

Genetic mapping of mutations that activate the *hisD* gene: Each insertion mutation was mapped by P22-mediated transduction crosses using as recipients a series of deletion mutations that enter the *hisG* gene from the right side (see Figure 2). Some of these deletions have been described previously (HOPPE, JOHNSTON and ROTH 1979); others were characterized by I. HOPPE, S. LAM, S. CIAMPI and D. BIEK (unpublished results). The donors carry both deletion *his-203* (entering *hisG* from the left) and the mutation activating the *hisD* gene. The parent *his-203* mutation recombines at high frequency (500–1000 recombinants per plate under conditions used) with all of the deletions presented in Figure 1. Tests scored as negative gave no recombinants on two full plate tests; thus these crosses could detect at least a 1000-fold decrease in recombination frequency.

Southern transfers and dot blots: Southern transfers were performed as described by DAVIS, BOTSTEIN and ROTH (1980). The probe used for detecting histidine operon sequences is the replicative form of clone M13Hol68 constructed by BARNES (1979); the inserted sequence includes the *hisO* region and the *hisG* and *hisD* genes. Probe for detecting Tn*5* sequences was plasmid pRZ102 (ColE1::Tn*5*) provided by W. REZNIKOFF. The probe for detecting Tn*10* sequences was plasmid pNK579 supplied by N. KLECKNER. Hybridization was carried out at 65° for 12 hr in 5× SSPE, 0.3% sodium dodecyl sulfate (SDS) and 100 μg/ml salmon sperm DNA. Membranes were washed at 45° with 2× SSPE with 0.2% SDS.

RESULTS

Test system for gene activation: The last enzyme in the histidine biosynthetic pathway, histidinol dehydrogenase, is encoded by the second gene (*hisD*) in the histidine operon. The *hisD* gene can be silenced by deletions (such as *his-203*) which do not affect the *hisD* gene itself but remove the *his* promoter and part of the first gene in the operon, *hisG* (see Figure 2, below). Thus deletion mutant *his-203* possesses an intact but unexpressed copy of the *hisD* gene. Cells with no active *hisD* gene cannot use the last intermediate in the *his* pathway, histidinol, as a source of

TABLE 2

Effect of transposons on reversion of *his-203* to HisD$^+$

Strain	Transposon carried[a]	Frequency of HisD$^+$ revertants ($\times 10^8$)	Increase in frequency	Map position of Tn *5* or Tn*10*
TR6936	None	1.4		None
TT7500	*trp-2451*::Tn*10*	17	13	34 min
TT7501	*hisC8579*::Tn*10*	10	7	44 min
TT7499	*guaB544*::Tn*10*	11	8	54 min
TT7498	*ileA597*::Tn*10*	35	26	83 min
TT8372	/F'*lac*$^+$ *zzf-20*::Tn*10*	8	6	F plasmid
TT7579	*thr-450*::Tn*5*	70	50	0 min
TT7582	*trp-2475*::Tn*5*	102	73	34 min
TT9044	*hisC9642*::Tn*5*	7	5	44 min
TT7581	*purG2140*::Tn*5*	105	75	57 min
TT9045	*srl-211*::Tn*5*	163	116	59 min
TT12230	*ilvD2171*::Tn*5*	179	128	83 min
TT9046	*metE2092*::Tn*5*	182	130	84 min

[a] All strains listed include promoter deletion mutation *his-203* and are phenotypically HisD$^-$.

histidine. By selecting for derivatives of deletion mutant *his-203* that can use histidinol as a source of histidine, one selects for acquisition of a new promoter that can serve to express the previously silent *hisD* gene. This selection was initially used to select extended deletions (AMES, HARTMAN and JACOB 1963) and has since been used to select promoters formed by point mutation (ST. PIERRE 1968), chromosome duplications (ANDERSON and ROTH 1978), and inversions (SCHMID and ROTH 1983b). In this paper, the selection will be applied to the study of gene activation by transposons Tn*5* and Tn*10*.

Presence of Tn*5* or Tn*10* increases the frequency of spontaneous mutations that activate the silent *hisD* gene: Strains with only the *his-203* deletion mutation are phenotypically HisD$^-$ and show HisD$^+$ revertants (able to grow on histidinol) at a frequency of about 1 per 10^8 cells. Isogenic strains containing Tn*5* or Tn*10* show a substantial increase in this revertant frequency. This increase in gene activation frequency is seen for strains having transposons at widely separated sites in the chromosome and on an F' plasmid (Table 2).

Activation of the *hisD* gene depends on ability of the element to transpose: Mutants of Tn*5* and Tn*10* that lack the transposase gene (carried by one of the flanking IS) are unable to transpose. Such mutants are also unable to stimulate the frequency of activation of the *hisD* gene (Table 3). Plasmids carrying altered Tn*10* elements were constructed and made available by N. KLECKNER and co-workers. Line 1 presents a strain lacking Tn*10* and line 2 presents a strain with a shortened, but functional Tn*10* element. As seen in line 3, a transposition-defective transposon causes no increase in reversion frequency over that seen for strains lacking Tn*10*. Conversely transposons with elevated transposition frequency show an increased frequency of *hisD* gene activation. The strain in line 4 has a Tn*10*(HH104) element (increased transposase) on an F' plasmid. The most frequent *hisD* activation (line 5) was seen in a strain carrying the hyperactive element Tn*10*(HH104) on a high copy plasmid.

A mutant of Tn*5* with an amber mutation in its transposase gene was made by D. BIEK (BIEK and ROTH 1980). This mutant element has lost the ability to transpose and does not increase the frequency of the spontaneous frequency of *hisD* gene activation (Table 3; line 7). An isogenic strain carrying a wild-type Tn*5* element (line 8) shows the typical increase in reversion of *his-203*. Because of strain construction problems, the strains presented in lines 7 and 8 are independent transposition mutants; the insertions are in a common genetic background (*his-203*) but are not at the same chromosomal position. We have shown that chromosomal position of the parent insertion is not critical to ability to stimulate reversion of *his-203*.

The above results are consistent with two alternative models for gene activation. First, the transposase itself could serve as an agent for DNA alteration (*e.g.*, making endonucleolytic cuts and stimulating error-prone repair); this mutagenic activity could serve to create a promoter near the *hisD* gene or stimulate rearrangement events that place the *hisD* gene near a foreign promoter. A second possibility is that the complete act of transposition is required and insertion of material near *hisD* serves to activate expression. A derivative of Tn*10* which has one end of the element damaged but still produces high levels of transposase was constructed by N. KLECKNER and co-workers. This element is unable to transpose despite the presence of high levels of transposase. This element does not stimulate activation of the silent *hisD* gene, suggesting that transposition and not just transposase is essential to the activation process (see Table 3, line 6).

Characterization of mutations that activate the *hisD* gene: In order to study the events causing gene activation, a series of HisD$^+$ revertants were generated and analyzed. One hundred revertants were selected in strain TT7598, which carries deletion *his-203* and the mutation *ileA597*::Tn*10*. Fifty revertants were selected in strain TT7582 which carries deletion *his-203* and the mutation *trp-2474*::Tn*5*. These (150) revertants were characterized genetically to test the following possibilities:

1. Are the revertants due to a base substitution mutation that creates a new promoter? ST. PIERRE (1968) showed the *hisD* gene can be activated by a new promoter, generated by a point mutation at one specific site in the *hisG* gene. Such point mutations all generate a *hisG*-cold sensitive phenotype

TABLE 3

Effect of transposon alterations on ability to activate *hisD*

Line	Strain	Relevant genotype[a]	Hol$^+$ revertant frequency ($\times 10^8$)
1	TR6936	No Tn*10*	1.0
2	TT8376	/F'*lacZ*::Tn*10* (del-4) (shortened but functional Tn*10*)	8
3	TT7520	/pNK378 (Tn*10* defective for transposase)	0.8
4	TT8378	/F' *lacZ*::Tn*10*(del-4; HH104) (shortened Tn*10* with increased expression of transposase)	229
5	TT7521	/pNK579 Tn*10* (del-4; HH104) (shortened Tn*10* with high transposition frequency on a high copy number plasmid)	3100
6	TT8381	/pNK474 (high transposase expression; no functional Tn*10* or IS*10*	1
7	TT8798	*his-203 zzz-1081*::Tn*5* (*tnf-55*, amber) (Tn*5* defective for transposition)	2
8	TT8799	*his-203 zzz-1082*::Tn*5* (wild type)	90

[a] All strains listed include promoter deletion mutation *his-203* and are phenotypically HisD$^+$.

when separated from the *his-203* deletion mutation.

2. Are the revertants due to deletions which fuse the *hisD* gene to a foreign promoter near the *his* operon? Such a means of *hisD* activation was described by AMES, HARTMAN and JACOB (1963). Such deletions can be identified by their inability to recombine with point mutations within the *hisG* gene.
3. Are the revertants due to formation of tandem duplications which place *hisD* under the control of a foreign promoter at the duplication join point? (ANDERSON and ROTH 1978). Revertants of this type would have an unstable HisD$^+$ phenotype due to frequent recombination events that cause loss of the duplication.
4. Are the revertants due to occurrances of inversions which rearrange the chromosome so as to juxtapose a *hisD* gene and a foreign promoter? Although such events are rare, several have previously been isolated using this selection method and can be identified by linkage disruption at their endpoints (SCHMID and ROTH 1983b).
5. Did any new auxotrophic lesion in the *hisG* gene occur in the course of *hisD* activation? This would be expected if a transposable element inserted within the *hisG* gene.

The genetic tests used to answer these questions have been described previously in the references cited above and are outlined in MATERIALS AND METHODS. Table 4 summarizes the results. Of 50 *hisD* activation mutations isolated in the Tn*5* carrying parent strain, only three are due to previously described events; 47 have become HisD$^+$ due to a new sort of mutation in the immediate vicinity of the *hisD* gene and are candidates for being transposition mutations. Similarly 85 of 100 Tn*10*-stimulated *HisD*$^+$ revertants show novel mutation types near *hisD* which could be insertions. We presume that the novel types were caused by the transposon present in the parental genetic background and that the few revertants of previously described types are due to the background of spontaneous events unrelated to Tn*5* or Tn*10*.

The candidates for insertions were tested for possession of a drug resistance gene associated with the *his* region. Phage P22 grown on the *HisD*$^+$ revertant strains was used to transduce the *his* region of the revertant (HisD$^+$) into deletion mutant *his-3050*. The transductants were scored for drug resistance (tetracycline or kanamycin depending on whether the revertant was Tn*10*- or Tn*5*-stimulated). As indicated in Table 4, two revertants have acquired a *his*-associated Tet^R and 11 have acquired a *his*-associated Kan^R. This suggests that some, but not all of the revertants are due to transposition of a complete Tn*5* or Tn*10*. We will present evidence that the mutations activating *hisD* without providing drug resistance are due to insertion of one of the component IS elements, which are known to transpose independently of the drug resistance determinant (FOSTER *et al.* 1981; BERG *et al.* 1985).

Table 4 summarizes the map position of the mutations within the *hisG* gene. (This mapping will be described in more detail below.) Of the 47 HisD$^+$ revertants due to insertion of Tn*5* material, 29 have acquired an auxotrophic lesion within the *hisG* gene (three due to Tn*5*, 26 to IS*50*). Of the 85 HisD$^+$ mutants due to Tn*10*, 77 acquired a *hisG* mutation (two due to Tn*10*, 75 to IS*10*). This HisD$^+$ insertion mutants that do not show a new auxotrophic *hisG* mutation will be shown to carry insertions in the intercistronic region between the *hisG* and *hisD* gene (see below and Figure 2).

TABLE 4

Mutations that activate the *hisD* gene

	Parent strain with Tn*5* (TT7582)	Parent strain with Tn*10* (TT7498)
Insertions in hisG		
IS	26	75
Tn	3	2
Insertions in hisG-D border		
IS	10	8
Tn	8	0
Noninsertion types		
Point mutations (new promoters)	2	6
Extended deletions	1	3
Duplications	0	4
Inversions	0	0
Uncharacterized	0	2
Total tested	50	100

Analysis of Tn*5*-generated HisD$^+$ revertants by DNA hybridization: Genetic tests suggested that 47 of 50 HisD$^+$ revertants might have acquired, in or near the *his* operon, an insertion of material derived from Tn*5*. Eleven of these revertants carry a Kan^R determinant within the *his* region and were inferred to have acquired an entire Tn*5* element; the others were presumed to have acquired only IS*50*. These conclusions were verified by Southern hybridizations.

Figure 1 presents hybridization results for two Hol$^+$ revertants presumed to be due to insertion of IS*50*, and one presumed to be due to Tn*5* insertion. Lane P presents the original HisD$^-$ parent strain which has insertion *trp* ::Tn*5* and deletion *his-203*. The original Hol$^+$ revertant strains are shown in lanes 1, 3 and 5. In addition, for each of these revertants, a transductant was tested which carries the activated *hisD* gene separated from the *trp*::Tn*5* insertion by transduction into deletion mutant *his-3050* (lanes 2, 4 and 6). DNA from each strain was cut with restriction enzyme *Eco*RI, which has no site within the Tn*5* element. Probes were Tn*5* DNA and *his* DNA hybridized sequentially to the same array of fragments to visualize the relevant bands.

Using the Tn*5* probe, it can be seen that HisD$^+$ revertants have acquired an extra band homologous to Tn*5* in addition to the parental band due to the *trp*::Tn*5* insertion (compare the lane P to lanes 1, 3 and 5). When the *his* region of these revertants is transduced into a new genetic background the resulting transductants (lanes 4 and 6) show only this extra band and lack the parental *trp*::Tn*5* band. The transductant in lane 2 shows a new band in addition to the band associated with *his* material; this new band is probably due to a transposition event occurring during transduction of the *his*-associated IS*50* insertion into strain *his-3050*. It is known that introduction of Tn*5* into a new genetic background induces transposition; presumably this induction also applies to IS*50*.

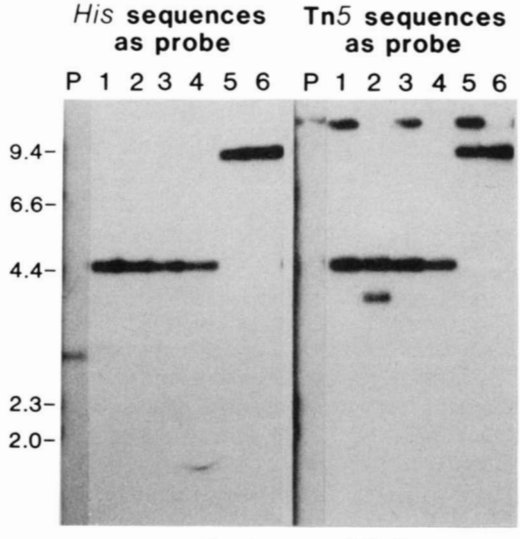

FIGURE 1.—DNA hybridization tests of Hol$^+$-revertants arising in a strain with Tn*5*. DNA from each of the strains tested was cut with enzyme *Eco*RI; the fragments were distributed by electrophoresis and blotted to a nitrocellulose membrane. The same array of fragments was probed first with labeled *his* sequences, then washed and with labeled Tn*5* sequences. Lane P contains DNA from the parent strain (TT7582). Lanes 1 and 3 contain DNA from Hol$^+$ revertants inferred to have acquired on IS*50* insertion in the *his* region (TT8559 and TT8560). Lanes 2 and 4 contain DNA from transductants of deletion mutant *his-3050* (TT8605 and TT8506) which have inherited the *his* region of the revertants presented in lanes 1 and 3. Lane 5 contains DNA from a Hol$^+$ revertant which was inferred to carry a complete Tn*5* element in the *his* region (TT8595). Lane 6 contains DNA from a transductant of deletion mutant *his-3050* (TT8641) which has inherited the *his* region of the strain presented in lane 5.

When the same array of restriction fragments is hybridized to the *his* probe, the HisD$^+$ revertants show an increase in the size of the *his Eco*RI fragment which includes the entire *hisG* and *hisD* genes. The size increase is characteristic of IS*50* (~1.5 kb) for both of the two putative IS*50* insertion mutants tested (lanes

1, 2, 3 and 4). The HisD$^+$ revertant that acquired a *his*-linked kanamycin resistance determinant shows an increase in the size of its *his* fragment (~5 kb) that approximates the size of an entire Tn*5* element (lanes 5 and 6).

The HisD$^+$ revertants show bands of the same size hybridizing with both the Tn*5* and *his* probes, consistent with insertion of the Tn*5* sequences within the *his Eco*RI fragment. Several strains were tested in this way and gave identical results. All putative Tn*5*/IS*50* insertion mutations were tested using DNA from transductants carrying the activated *hisD* gene in an otherwise Tn*5*-free strain. Their DNA was checked by "dot blots" for the presence of Tn*5* homology. All transductants which received the activated *hisD* gene, also received DNA homologous to Tn*5* (data not shown). Thus all of the putative insertion mutations selected in a Tn*5*-containing parent strain (listed in Table 4) acquired Tn*5* material adjacent to the *hisD* gene.

Analysis of Tn*10*-generated HisD$^+$ revertants by restriction fragment hybridization: All of the 85 HisD$^+$ revertants inferred to be caused by insertion of Tn*10*-derived material have similarly acquired Tn*10* sequences associated with the *his* region (data not shown). This was demonstrated by experiments similar to those described above for the Tn*5* insertion mutations. Several of the original *hisD*$^+$ mutants (carrying insertion *ileA*::Tn*10*) were used as a DNA source; transductants which received the activated *hisD* gene without the original *ileA*::Tn*10* insertion were also tested. DNA was cut with *Eco*RI which does not cut within IS*10*, but has one site in the central region of Tn*10*. Fragments were visualized by hybridization to both Tn*10* and *his* probe sequences. Four strains were checked, three putative IS*10* insertion mutants and one putative Tn*10* insertion mutant.

The parent strain (*his-203 trp*::Tn*10*) shows one band homologous to the *his* probe. In all strains thought to have acquired an IS*10*, this size of this band has increased about 1500 bp (the approximate size of IS*10*). Transductants carrying the activated *his* region in a new genetic background also show this larger fragment. When the Tn*10* sequence is used as probe, the original *HisD*$^+$ revertant shows one new band homologous to Tn*10*. This band is the same size as the band which showed homology to the *his* probe. When the reactivated *his* region is transduced into a new strain, the transductants acquire this same band having homology to Tn*10* sequences.

In strains thought to have acquired a complete Tn*10* element, the *his* probe hybridizes with two new bands, while the band containing *his* sequences characteristic of the parent strain is not seen. This is consistent with introduction of the Tn*10* element with its internal *Eco*RI site within the *his Eco*RI fragment.

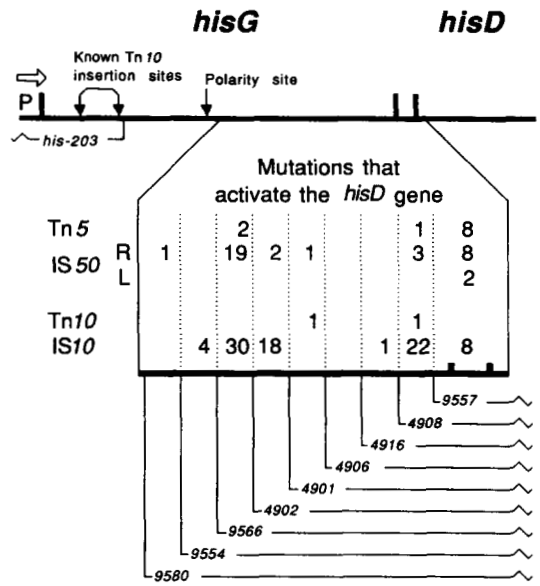

FIGURE 2.—Map distribution of *hisG* insertion mutations that activate the *hisD* gene. Mutations were mapped by P22-mediated transductional crosses using Hol$^+$ revertants of *his-203* as donors and the deletions indicated above as recipients. The parental *his-203* deletion mutation recombines with all of the recipient deletions. The intergene spacer region is 100 bp in length; the polarity site is in the middle of the *hisG* gene, 500 bp from each end. The polarity site is genetically marked by mutation *hisG618*, a 20-bp deletion which destroys the polarity site. The resolution of the map is such that intervals scored as positive show recombinants at a frequency reduced 1000-fold or less from the frequency seen with a *his*$^+$ donor.

This interpretation is strengthened by the fact that the sum of the size of the two new fragments equals the size of the parental *his* fragment plus the size of the complete Tn*10* element (10 kb). Transduction of the activated *his* region from the original strain into a new genetic background yields a transductant which shows these same two new bands of material homologous to the *his* probe. When the same array of fragments is exposed to a Tn*10* probe, it can be seen that revertants show two fragments complementary to Tn*10* sequences; these fragments have the same mobility as fragments complementary to the *his* probe.

All 85 strains inferred to have acquired IS*10* were tested by "dot blots." The activated *his* regions of all of these strains were transduced into a genetic background free of Tn*10* and DNA from these transductants was tested for homology to Tn*10* DNA by "dot blots." All of the transductants except two had acquired Tn*10* sequences. The two strains which failed to show homology are listed as "uncharacterized" in Table 4.

Genetic mapping of insertion mutations: All of the insertion mutations that activate the silent *hisD* gene have been mapped by P22-mediated transduction crosses using a set of deletions that enter the *hisG* gene from the right. The map positions of these inserts are presented in Figure 2. It should be noted that the

inserts that activate *hisD* all lie to the right of the known Rho-dependent transcription termination site within the *hisG* gene. The inserts fall in several deletion intervals. None of the previously known *his*::Tn*10* insertions, isolated as *hisG* auxotrophs, lies in this region of the map.

Mapping of insertion mutations located within the *hisG* gene was straighforward since these mutations have a His^- phenotype and can be crossed with deletion mutants selecting for prototrophy. Mapping of insertions in the spacer region between the *hisG* and *hisD* genes was more difficult since these insertions do not cause a His^- phenotype. Mapping was made possible by the fact that these inserts block transcription from the *his* promoter and provide a weaker promoter to transcribe downstream genes in the *his* operon. Thus strains carrying these insertions are prototrophic, but express distal *his* genes (including *hisB*) at a low level. This renders the mutants sensitive to an inhibitor of *hisB* enzyme, aminotriazole (HILTON, KEARNEY and AMES 1965) and makes it possible to counterselect the insertion mutation. The $HisD^+$ revertants were used as donors in crosses with recipient deletion mutants selecting for His^+ recombinants able to grow on minimal medium containing aminotriazole. Thus one can select for recombination between the donor insertion mutation and recipient deletion mutation. These crosses demonstrate that the prototrophic insertions map between the *hisG* and *hisD* genes. Sequence data of W. BARNES (personal communication) demonstrates that this spacer region is approximately 100 bp in length; this region includes several examples of the repeated sequence element REP (HIGGINS *et al.* 1982; STERN *et al.* 1984; GILSON *et al.* 1984).

Identification of insertions of IS*50*L and IS*50*R: Transposon Tn*5* includes two flanking IS*50* elements known as IS*50*L and IS*50*R (left or right side of the element as it is usually presented). The IS*50*R element encodes both a transposase and an inhibitor of transposition; IS*50*L encodes neither function (JOHNSON, YIN and REZNIKOFF 1982; ISBERG, LAZAAR and SYVANEN 1982). We have genetically classified the IS*50* insertions that activate the *hisD* gene by making use of the fact that only IS*50*R encodes an inhibitor of Tn*5* transposition. An isogenic series of strains carrying the IS*50* insertions, but no other Tn*5* material was used as recipient for Tn*5* in a cross that selects for transposition of the introduced Tn*5* element. We scored the effect of the recipient IS element on Tn*5* transposition.

Each strain was identically infected with P22 phage grown on a donor strain that carries Tn*5* in an *E. coli* F′*lac* plasmid. Inheritance of Tn*5* can not occur by recombination due to lack of homology between the chromosomes of Salmonella and *E. coli*. To generate a Kan^R transductant, the transduced Tn*5* element must transpose to a site in the recipient chromosome. This cross has been described in detail (BIEK and ROTH 1980, 1981).

Results are presented in Table 5. The control strain, deletion mutant *his-3050*, shows a frequency of Kan^R transposition mutants that has been normalized to 1. Most of the IS*50* insertion mutants show a 5–10-fold reduction in this frequency; this is typical of strains carrying IS*50*R. Two exceptional Hol^+ strains show very little inhibition of Tn*5* transposition. These are inferred to carry IS*50*L, which does not inhibit transposition (BIEK and ROTH 1980; ROTHSTEIN and REZNIKOFF 1981). Table 5 also includes control strains which are known to contain IS*50*R or IS*50*L alone; these are deletion mutants of Tn*5* that lack one or the other of the flanking IS*50* elements (D. BIEK and J. ROTH, unpublished results). In summary, the vast majority of the IS*50* inserts that activate *hisD* are inferred to be due to transposition of IS*50*R, which encodes both the transposase and the inhibitor of transposition.

DISCUSSION

A silent gene in the Salmonella chromosome can be activated by spontaneous mutations. The frequency of activation is greatly stimulated in strains carrying transposons Tn*5* and Tn*10*. The frequency increase is due to transposition of these elements to sites in the immediate vicinity of the activated gene. The nature and map distribution of the insertion mutations causing activation permit several conclusions regarding the behavior of transposable elements and the nature of transcription termination in the *his* operon.

Transposition can represent a major mechanism for activation of silent genes. In strains harboring Tn*5* or Tn*10*, 132 of 150 mutants with activated *hisD* genes are due to transposition. These $hisD^+$ revertants were found at a frequency of 10^{-6} (Tn*5*) or 10^{-7} (Tn*10*). This frequency is extremely high when one considers that the transposition target is only about 500 bp (the distance between the polarity site within the *hisG* gene and the adjacent *hisD* gene).

In Salmonella strains lacking Tn*10* or Tn*5*, activation of the *hisD* gene has never been seen to occur by insertion of endogenous elements such as IS*200*, the only insertion sequence known to be present in *S. typhimurium* (LAM and ROTH 1983). While the two uncharacterized mutations in Table 4 could be insertions, they are not examples of IS*200* (J. CASADESUS, unpublished results). While this could be explained if IS*200* lacked an outward promoter, the low frequency of IS*200* transposition is probably sufficient to account for our failure to observe gene activation by IS*200*. Only one insertion mutation (caused by IS*200*) has been documented in Salmonella (LAM and ROTH

TABLE 5

Inhibition of Tn*5* transposition by various *hisG*:IS *50* insertions

Recipient	Relevant genotype	Relative transposition frequency of Tn*5*
Parental strain (*his-3050*)	(no Tn*5* or IS*50*)	=1
Typical IS*50* insertions (34 mutations that activate *hisD*) (*hsiG1120–hsiG1154*)	*hisG*::IS*50*R (inferred)	0.05–0.26 (average 0.14)
Exceptional insertions (2 mutations that activate *hisD*)		
(*hsiG1129*)	*hisG*::IS*50*L (inferred)	0.89
(*hsiG1140*)	*hisG*::IS*50*L (inferred)	0.90
Control strains		
TT9421	Tn*5*(*neo-116*) retains IS*50*L	1.0
TT9423	Tn*5*(*neo-111*) retains IS*50*R	0.17

1983), despite an extensive search for such mutants (J. CASADESUS and J. R. ROTH, unpublished results).

Both Tn*5* and Tn*10* are able to activate expression of genes located near their insertion site. In the case of Tn*10*, the promoters responsible have been inferred previously (CIAMPI, SCHMID and ROTH 1982; BLAZEY and BURNS 1982) and characterized in detail biochemically (SIMONS *et al.* 1983). In the case of Tn*5* these promoters have been inferred from the lack of absolute polarity of some Tn*5* inserts (BERG, WEISS and CROSSLAND 1980; CIAMPI, SCHMID and ROTH 1982; D. BIEK, M. S. CIAMPI and J. R. ROTH, unpublished results). Activation of silent genes by insertion of IS*50*-left has recently been described in *E. coli*, with results that differ slightly from those reported here (see below). It seems likely that these promoters are within the Tn*5* element. All Tn*5* insertions in the *hisG* gene distal to the internal termination site cause expression of the *hisD* gene; all Tn*5* insertions upstream of the terminator fail to express *hisD*. Many of the insertions on which this conclusion is based were isolated as *hisG* mutations, without regard for *hisD* gene expression (D. BIEK, M. S. CIAMPI and J. R. ROTH, unpublished data). Insertions of Tn*10* or Tn*5* upstream of the polarity site can express the *hisD* gene if a *rho* mutation is introduced; this expression requires the presence of the transposon and does not require a *his* promoter (D. BIEK, M. S. CIAMPI, and J. R. ROTH, unpublished data).

For both Tn*5* and Tn*10*, the flanking IS sequences transpose substantially more frequently than the entire element. Of 47 insertions of Tn*5* material, 36 were IS*50* and only 11 were Tn*5*; of 85 insertions generated by Tn*10*, 83 were IS*10* and only two were Tn*10*. This confirms previous observations on these elements (BERG *et al.* 1985; SHEN, RALEIGH and KLECKNER 1987).

The IS*50* element at the right side of Tn*5* appears to transpose much more frequently than the left IS*50*. Of 36 examples of IS*50* transposition, 34 appear to be IS*50*R and only two are IS*50*L. This is consistent with the preferential action of transposases at sites immediately *cis* to the transposase gene, which is encoded by IS*50*R (ROTHSTEIN and REZNIKOFF 1981; ISBERG and SYVANEN 1981; MORISATO *et al.* 1983). In *E. coli*, selective activation of a silent gene by Tn*5* was seen to occur primarily by insertion of IS*50*L; this expression was attributed to the *neo* promoter which is located at the inside end of IS*50*L; only minimal gene activation by IS*50*R or by the complete Tn*5* element was seen. This contrasts with our conclusions that both IS*50* right and Tn*5* were frequent causes of *hisD* gene activation in Salmonella. We suggest two explanations for these differences. First, the selection system used here may be more sensitive in that very little gene expression is required to generate a Hol$^+$ (HisD$^+$) phenotype. Second, it is possible that outward-directed promoters exist in Tn*5* that are more highly expressed in Salmonella.

Transposon Tn*10* inserts at specific target sites described by a consensus sequence (HALLING and KLECKNER 1982). Previously isolated *hisG*::Tn*10* insertions are all at the two sites indicated in Figure 2 (KLECKNER *et al.* 1979). The selection system described here demands insertion at sites to the right (promoter distal) of the polarity site and thus does not permit use of either of the preferred sites. We describe here many new Tn*10*/IS*10* insertion sites within the *hisG* gene. These new insertions demonstrate that Tn*10* must recognize, possibly at lower frequency, additional sites within *hisG*; these new sites may deviate more widely from the consensus sequence identified previously.

Only insertions distal to the internal polarity site in the *hisG* gene can serve to activate the *hisD* gene; all insertions that activate *hisD* lie to the right of this site (Figure 2). This termination site lies near the middle of the *hisG* gene (CIAMPI and ROTH 1988). Apparently

no message termination sites are present between this point and the *hisD* gene. Thus a 500-bp sequence is the target site for transposons that activate the *hisD* gene.

Activation of silent genes by IS elements has been seen repeatedly in *E. coli*, however substantial questions remain as to how activation occurs and why all insertions of a particular element do not activate downstream genes. Results presented here suggest that one requirement may be that no polarity site intervene between the site of insertion and the gene to be expressed.

In interpreting the behavior of natural IS elements, one faces the problem of heterogeneity; in strains with multiple IS sequences, one does not know the source of the particular element causing gene activation. Heterogeneity of donors has been eliminated in the experiments presented here since only one donor transposon capable of gene activation is present. The method used here allows positive selection for transposition of elements present within a single genome, even if those elements do not encode drug resistance.

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