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Genetics

Transposon Tn*10* provides a promoter for transcription of adjacent sequences

(insertion elements/*Salmonella*/polarity/ρ factor/message termination)

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ABSTRACT **Promoters located within the Tn*10* insertion element cause transcription of "host" sequences adjacent to both ends of the inserted Tn*10* element. These promoters are usually not observed in genetic experiments because their transcripts are efficiently terminated at nearby ρ-dependent termination sites. The observations presented here provide an explanation for several confusing aspects of transposon behavior and suggest the possibility that many transposons possess promoters that have escaped detection for similar reasons.**

Insertion of the transposon Tn*10* within most bacterial operons has been found to cause an absolute polar block to transcription of downstream genes in the operon. This extremely strong polar effect has led to the assumptions that host transcription is terminated and that no transcripts emanate from Tn*10* into adjacent sequences. In contrast to Tn*10*'s absolutely polar effect on most operons, Tn*10* insertions in ribosomal RNA operons permit continued transcription of distal sequences (1). These conflicting observations can be reconciled if Tn*10* provides a promoter whose transcripts are efficiently terminated in some but not all situations. This paper presents evidence that Tn*10* provides a promoter for transcription of adjoining sequences. These transcripts have not been detected in the *his* operon because of the strong natural polarity effects that cause termination of the message. Based on independent results, Blazey and Burns (2) have inferred that the Tn*10* elements cause transcription of genes adjacent to insertion sites in the *ilv* operon. Possible implications of these results for other transposons will be discussed.

When Tn*10* insertion mutations were first characterized in operons (3–5) it was noted that most insertions were absolutely polar. In the *his* operon of *Salmonella typhimurium*, this conclusion was based on complementation tests. Insertions in the promoter-proximal gene *hisG* block expression of the immediately adjacent *hisD* and *hisC* genes (see Fig. 1); genes located promoter-distal to *hisC* still are expressed due to the secondary promoter P2 located between the *hisC* and *hisB* genes (3, 6, 7). Similarly, insertion mutations in the *hisB* gene (distal to the P2 promoter) prevent expression of the *hisH*, *-A*, and *-F* genes. The *hisI* and *hisE* genes are transcribed from the secondary promoter P3 located at the distal end of the *hisF* gene. Most complementation data fit this pattern and led to the conclusion that Tn*10* is an absolute block to transcription and provides no new promoter for distal sequences.

An exception was noted in the case of some *hisH*::Tn*10* insertions; several of these show weak expression of the downstream *hisA* and *hisF* genes (3). It was thought that some weak promoter site located within the *hisH* gene might be responsible for the observation. In the course of mapping internal promoters in the histidine operon, these *hisH*::Tn*10* insertions were reinvestigated. The weak expression of downstream genes in these insertion mutants was found to be due to a promoter associated with the Tn*10* element itself.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. All strains used are derivatives of *Salmonella typhimurium* strain LT2; full genotypes are listed in Table 1. Strains used for complementation tests have been described (8). Mutants carrying the *rho-111* mutation were isolated and kindly provided by Housley *et al.* (9). Media have been described elsewhere (8).

Complementation Tests. Expression of individual *his* genes was determined by complementation tests using F′ plasmids derived from *Escherichia coli*. Each plasmid carries a characterized *his* mutation (Table 1). Strains carrying these plasmids also carry auxotrophic mutations that permit their counter selection during plasmid transfer. Transfer was accomplished by spotting about 0.03 ml of each donor strain onto a lawn of recipient cells on medium selecting for histidine-independent growth. If complementation occurred, growth arose in the next 24–48 hr.

Histidinol Dehydrogenase Assays. The level of the *hisD* gene product was assayed according to the method of Ciesla *et al.* (10). Activities were normalized to cell number by determining turbidity of the cell suspension. In Table 4, activities are expressed relative to the level in LT2 which is defined as 1. For the assays in Table 4, cells were grown on minimal E medium containing 0.2% glucose and 0.1 mM histidine; no tetracycline was added.

RESULTS

Complementation Behavior of hisH::Tn*10* Insertions. The complementation data in Table 2 show the weak expression of distal genes seen in *hisH*::Tn*10* mutants. Lines 1 and 2 describe a set of *hisH* mutants; both showed weak but detectable expression of the *hisA* and *hisF* genes. Many other *hisH*::Tn*10* insertions showed identical complementation behavior. (The weakness of the complementation with the F′*hisB*$^-$ plasmid was unexpected but is characteristic of all *hisH*::Tn*10* mutations.)

There were a number of potential explanations for the lack of absolute polarity of *hisH*::Tn*10* insertion mutations. It seemed possible that the insertions in *hisH* might not completely block transcription from the *his* promoters (P1 and P2). A second explanation is that a promoter might be located within the *hisH* gene, either in the gene itself or associated with the Tn*10* element. In order to distinguish between these possibil-

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Table 1. Bacterial strains used

Strain	Genotype*
TT7159	*hisH8577*::Tn*10*(B) *his-57*
TT7160	*hisH8577*::Tn*10*(B)
TT7161	*hisH8541*::Tn*10*(A) *his-57*
TT7162	*hisH8541*::Tn*10*(A)
TT7184	*hisA8697*::Tn*10*(B) *his-57*
TT7185	*hisA8697*::Tn*10*(B)
TT7186	*hisB8686*::Tn*10*(A) *his-57*
TT7187	*hisB8686*::Tn*10*(A)
TT7188	*hisB8688*::Tn*10*(B) *his-57*
TT7189	*hisB8688*::Tn*10*(B)
TT7333	*hisG9424*::Tn*10*(A) *rho-111*
TT7334	*hisG9424*::Tn*10*(A) *rho*$^+$
TT7335	*hisG9425*::Tn*10*(B) *rho-111*
TT7336	*hisG9425*::Tn*10*(B) *rho*$^+$
TT7339	*hisG9424*::Tn*10*(A) *hisG8502 rho-111*
TT7340	*hisG9424*::Tn*10*(A) *hisG8502 rho*$^+$
TT7341	*hisG9425*::Tn*10*(B) *hisG8502 rho-111*
TT7342	*hisG9425*::Tn*10*(B) *hisG8502 rho*$^+$
TT7343	*his-9702 hisG1102*(feedback resistant) *rho-111*
TT7344	*his-9702 hisG1102*(feedback resistant) *rho*$^+$
TT7345	*his-9702 hisG9425*::Tn*10*(B) *rho-111*
TT7346	*his-9702 hisG9425*::Tn*10*(B) *rho*$^+$
TT7347	*his-9702 hisG9424*::Tn*10*(A) *rho-111*
TT7348	*his-9702 hisG9424*::Tn*10*(A) *rho*$^+$
TT7350	*his*$^+$ *rho*$^+$
TT7353	*his-9702 hisG9424*::Tn*10*(A) *hisG8502 rho-111*
TT7354	*his-9702 hisG9424*::Tn*10*(A) *hisG8502 rho*$^+$
TT7357	*his-9702 hisG9425*::Tn*10*(B) *hisG8502 rho-111*
TT7358	*his-9702 hisG9425*::Tn*10*(B) *hisG8502 rho*$^+$

* Letter A or B in parentheses after various Tn*10* insertions designates the orientation of the element in the chromosome.

ities, the host promoter regions P1 and P2 were removed by deletion.

Mutation *his-57* removes the promoter-proximal half of the operon, eliminating the *his* promoters P1 and P2 and extending into the *hisB* gene (Fig. 1). Removal of these promoters leaves the remaining *hisA* and *hisF* genes unexpressed as judged by complementation tests (Table 2, line 3). The failure of this deletion mutant to express downstream genes demonstrates that no promoter is present within the normal *hisH* gene. Deletion mutant *his-57* failed to express these genes even in the presence of a *rho* mutation (data not shown). However, when a *hisH*::Tn*10* insertion was added to the *his-57* deletion mutant, the resulting strain gained expression of the *hisA* and *hisF* genes (Table 2, lines 4 and 5). This expression was weak; histidine-independent growth of the diploid was just detectable in 24 hr; an additional 24 hr was required for a fully positive growth response. The weak expression provided by Tn*10* to deletion mutant *his-57* was indistinguishable from the weak expression seen for *hisH*::Tn*10* insertions in strains having functional *his* promoters. Thus, a *hisH*::Tn*10* insertion can provide expression of downstream genes whether or not the *his* promoters (P1 and P2) are present. This expression cannot be the result of transcription from *his* promoters reading across the inserted material nor can it be due to promoters located within the normal *hisH* gene.

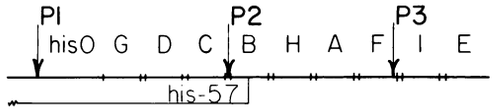

FIG. 1. Positions of genes and promoters in the *his* operon. Transcription proceeds from left to right. All promoters indicated can provide sufficient levels of distal gene products to permit growth on minimal medium. Deletion *his-57* removes both P1 and P2 and has no promoter capable of expressing the *hisH*, *hisA*, and *hisF* genes.

It seemed possible that expression of *hisA* and *hisF* genes might be due to a promoter generated fortuitously by juxtaposition of Tn*10* sequences and "host" sequences at a particular *hisH* insertion site. This possibility is unlikely because insertions at several sites showed expression of distal genes. The *hisH* insertion mutations tested are distributed between two sites in the gene. Insertions at six different sites in the *hisB* gene all provided weak expression of *hisA* and *hisF* genes; data for two of these *hisB* insertions are presented in Table 2, lines 6 and 7. In addition, an insertion in the *hisA* gene was able to provide weak expression of *hisF* (line 8). Apparently this low-level expression had been missed in previous complementation testing of *hisB* and *hisA* insertion mutations. The expression was more striking when the effect of Tn*10* was scored in strains that lack promoters P1 and P2; in these cases, the weak expression of downstream genes absolutely required the presence of Tn*10* (Table 2, compare line 3 to lines 4 and 5 and to 9–11). The observation of Tn*10*-generated promoters at several sites argues strongly against the possibility that promoters are formed at the junctions between Tn*10* and the neighboring sequences.

The behavior of the Tn*10* insertions described in Table 2 could be explained by a transcript emerging from the Tn*10* element. However, many other Tn*10* insertions do not show expression of distal genes. In order to account for the absolute polarity seen for some Tn*10* insertions, one must hypothesize that, in these cases, if transcripts emerge from Tn*10*, they must be terminated before the next gene in the operon. The possi-

Table 2. Complementation behavior of Tn*10* insertion mutations

Relevant chromosomal genotype	Growth of strain when various F′*his* plasmids were introduced								
	G$^-$	*D*$^-$	*C*$^-$	*B*$^-$	*A*$^-$	*F*$^-$	*I*$^-$	*IE*$^-$	*his*$^+$
1. *hisH8577*::Tn*10*(B)	+	+	+	+/−	+/−	+/−	+	+	+
2. *hisH8541*::Tn*10*(A)	+	+	+	+/−	+/−	+/−	+	+	+
3. *his-57*	−	−	−	−	−	−	+	+	+
4. *his-57 hisH8577*::Tn*10*	−	−	−	−	+/−	+/−	+	+	+
5. *his-57 hisH8541*::Tn*10*	−	−	−	−	+/−	+/−	+	+	+
6. *hisB8686*::Tn*10*(A)	+	+	+	−	+/−	+/−	+	+	+
7. *hisB8688*::Tn*10*(B)	+	+	+	−	+/−	+/−	+	+	+
8. *hisA8697*::Tn*10*(B)	+	+	+	+	−	+/−	+	+	+
9. *his-57 hisB8686*::Tn*10*	−	−	−	−	+/−	+/−	+	+	+
10. *his-57 hisB8688*::Tn*10*	−	−	−	−	+/−	+/−	+	+	+
11. *his-57 hisA8697*::Tn*10*	−	−	−	−	−	+/−	+	+	+

bility of such natural polarity effects on Tn*10*-promoted transcripts was investigated by using the "absolutely polar" insertion mutations in the *hisG* gene.

Effect of *rho* Mutations on the Tn*10* Outward Transcripts. If transcripts emanate from all Tn*10* elements, then those insertions that show no expression of distal genes must generate transcripts that terminate before they reach downstream genes. The message termination factor ρ is known to be involved in natural polarity in many operons (11, 12). Thus, termination of Tn*10* transcripts is likely to involve the ρ factor. In pursuing this, we checked the effect of *rho* mutations on the expression of genes located distal to absolutely polar Tn*10* insertions in the *hisG* gene.

Insertions of Tn*10* within the *hisG* gene have been studied extensively (3, 13, 14). These insertions permit no residual expression of the distal *hisD* or *hisC* genes as judged by sensitive complementation tests. Furthermore, strains carrying such *hisG* insertions show no growth on histidinol, an intermediate whose conversion to histidine is catalyzed by the adjacent *hisD* gene. Thus, by these two tests, the insertion of Tn*10* within the *hisG* gene blocks all transcripts emanating from the major *his* promoter P1 and provides no new transcripts capable of expressing the *hisD* and *hisC* genes.

A *rho* mutant of *S. typhimurium* has been isolated and extensively characterized by Housley *et al.* (9). When this *rho* mutation was introduced into strains carrying *hisG*::Tn*10* insertions, expression of the downstream *hisD* gene was restored (Table 3, compare lines 1 and 2 to lines 3 and 4). Strains carrying both a *hisG*::Tn*10* and the *rho* mutation acquired a $HisD^+$ phenotype (ability to utilize histidinol as a source of histidine).

The relief of Tn*10* polarity caused by *rho* is not dependent on a functional *his* promoter; expression is not due to transcripts from the *his* promoter being permitted to cross Tn*10* and reach downstream genes. This has been demonstrated by the use of deletion mutant *his-9702* which lacks the major *his* promoter P1 (Fig. 2). This deletion retains but cannot express the *hisD* gene (15) (see also Table 3, line 5). When a *rho* mutation occurred in this deletion mutant, no expression of the downstream genes resulted (Table 3, line 6). Thus, the deletion mutant alone possesses no promoter capable of expressing *hisD* even in the presence of a *rho* mutation. However, strains carrying the promoter deletion, a *hisG*::Tn*10* insertion, and the *rho* mutation expressed *hisD* function (Table 3, compare lines 7 and 8 to lines 9 and 10). This demonstrates that the presence of a Tn*10* element is essential for stimulation of *hisD* expression by a *rho* mutation. Furthermore, this expression is independent of the *his* promoter. In the absence of a *rho* mutation, the transcripts probably terminate within the *hisG* gene. If this is true, then it should be possible to demonstrate the existence of this termination site.

Table 3. Effect of *hisG*::Tn*10* mutation on expression of *hisD* in various genetic backgrounds

Relevant genotype	Strain	*hisD* expression (growth on histidinol)
1. *hisG9424*::Tn*10*(A)	TT7334	−
2. *hisG9425*::Tn*10*(B)	TT7336	−
3. *hisG9424*::Tn*10*, *rho-111*	TT7333	+
4. *hisG9425*::Tn*10*, *rho-111*	TT7335	+
5. *his-9702*	TT7344	−
6. *his-9702*, *rho-111*	TT7343	−
7. *his-9702*, *hisG9424*::Tn*10*	TT7348	−
8. *his-9702*, *hisG9425*::Tn*10*	TT7346	−
9. *his-9702*, *hisG9424*::Tn*10*, *rho-111*	TT7347	+
10. *his-9702*, *hisG9425*::Tn*10*, *rho-111*	TT7345	+
11. *hisG9424*::Tn*10*, *hisG8502*	TT7340	+
12. *hisG9425*::Tn*10*, *hisG8502*	TT7342	+
13. *hisG9424*::Tn*10*, *hisG8502*, *rho-111*	TT7339	+
14. *hisG9425*::Tn*10*, *hisG8502*, *rho-111*	TT7341	+
15. *his-9702*, *hisG9424*::Tn*10*, *hisG8502*	TT7354	+
16. *his-9702*, *hisG9425*::Tn*10*, *hisG8502*	TT7354	+
17. *his-9702*, *hisG9424*::Tn*10*, *hisG8502*, *rho-111*	TT7353	+
18. *his-9702*, *hisG9425*::Tn*10*, *hisG8502*, *rho-111*	TT7357	+

Transcription Termination Site Within the *HisG* Gene. Several lines of evidence suggest that a single site within the *hisG* gene may be responsible for the polarity associated with nonsense and frameshift mutations in that gene. Martin *et al.* (16) showed that *hisG* nonsense mutations mapping promoter-proximal to this site are strongly polar; nonsense mutations located within the *hisG* gene distal to this point show much weaker polarity or no polarity at all. In pursuing this we found that Tn*5* insertions promoter-proximal to this site are absolutely polar whereas Tn*5* insertions distal to this site retain expression of distal genes. We hypothesize that this site is the point at which ρ factor acts to terminate untranslated messages. Because both of the Tn*10* insertion sites in the *hisG* gene are located promoter-proximal to the hypothetical *rho* site, it is possible that the transcripts emanating from Tn*10* terminate there.

The deletion mutant *hisG8502* lacks the region of the *hisG* gene that contains this putative termination site. Double mutants were constructed that carry both a *hisG*::Tn*10* insertion and the *hisG8502* deletion. These strains are phenotypically $HisD^+$ (Table 3, compare lines 1 and 2 to lines 11 and 12). Activation of the *hisD* gene by the deletion *hisG8502* does not depend on the presence of a functional *his* promoter; activation was achieved even if the *his* promoter was removed by deletion *his-9702* (Table 3, compare lines 11 and 12 to lines 15 and 16). Furthermore, the size of deletion *hisG8502* is not critical to its effect on downstream gene expression because several *hisG* point mutations mapping within this deletion also abolished the polar effect of promoter-proximal Tn*10* insertions (unpublished data). The fact that mutations located nearby but outside Tn*10* permit detection of the Tn*10* transcript is strong evidence that the transcription revealed in the presence of a *rho* mutation is not due to escape of a Tn*10* transcript that normally terminates within the Tn*10* element; a mutation outside the Tn*10* element would not be expected to cause such escape transcription. It

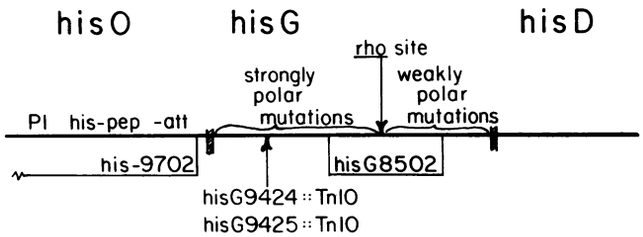

FIG. 2. Map of the *hisO*, *hisG*, and *hisD* genes. The Tn*10* insertions studied lie promoter-proximal to a point within the *hisG* gene inferred to be a site of ρ-dependent mRNA termination. Deletion *hisG8502* removes this termination site. Deletion *his-9702* removes the main *his* promoter P1 and the control region (*hisO*) including the leader peptide (*his–pep*) and attenuator (*his–att*) sites.

Table 4. Enzyme levels resulting from Tn*10* outward promoters

Relevant genotype	Strain	*hisD* level (relative specific activity)
1. *his*$^+$	TT7350	1.00
2. *hisG9424*::Tn*10*(A)	TT7334	0.05
3. *hisG9424*::Tn*10*, *rho-111*	TT7333	0.41
4. *his-9702*, *hisG9424*::Tn*10*	TT7348	0.03
5. *his-9702*, *hisG9424*::Tn*10*, *rho-111*	TT7347	0.31
6. *hisG9424*::Tn*10*, *hisG8502*	TT7340	1.31
7. *hisG9424*::Tn*10*, *hisG8502*, *rho-111*	TT7339	1.05
8. *hisG9425*::Tn*10*(B)	TT7336	0.04
9. *hisG9425*::Tn*10*, *rho-111*	TT7335	0.40
10. *his-9702*, *hisG9425*::Tn*10*	TT7346	0.03
11. *his-9702*, *hisG9425*::Tn*10*, *rho-111*	TT7345	0.32
12. *hisG9425*::Tn*10*, *hisG8502*	TT7342	1.13
13. *hisG9425*::Tn*10*, *hisG8502*, *rho-111*	TT7341	1.06
14. *his-9702*	TT7344	0.01
15. *his-9702*, *rho-111*	TT7343	0.03
16. *his-9702*, *hisG9424*::Tn*10*, *hisG8502*	TT7354	0.99
17. *his-9702*, *hisG9425*::Tn*10*, *hisG8502*	TT7358	1.17
18. *his-9702*, *hisG9424*::Tn*10*, *hisG8502*, *rho-111*	TT7353	0.89
19. *his-9702*, *hisG9425*::Tn*10*, *hisG8502*, *rho-111*	TT7357	0.92

seems more likely that these transcripts always emanate from Tn*10* but are normally terminated at sites in the adjacent "host" sequences by the action of *rho*.

Thus, it appears that the *hisG*::Tn*10* elements provide a promoter activity that can read the *hisD* gene if either (*i*) a *rho* mutation prevents transcription termination or (*ii*) mutations alter the site of *rho* action.

Strength of the Tn*10* Outward Promoter. The levels of the *hisD* product, the enzyme histidinol dehydrogenase, produced by the Tn*10* promoter under various genetic conditions have been assayed (Table 4). A series of isogeneic strains were compared; all strains were grown in the presence of excess histidine. Several points should be noted. (*i*) The *rho-111* mutation causes an 8- to 10-fold increase in the levels of the *hisD* enzyme. (*ii*) The *rho* effect is not dependent on the *his* promoter. (*iii*) The *rho* mutation does not affect *hisD* expression in the absence of Tn*10*. (*iv*) Deletion *hisG8502*, thought to remove the site of *rho* action, causes a 30-fold increase in the level of *hisD* expression. (*v*) The effect of deletion *hisG8502* on *hisD* expression does not require a functional *his* promoter.

The maximal *hisD* expression caused by Tn*10* was seen in strains lacking the termination site within the *hisG* gene. These levels are somewhat higher than those found in a maximally repressed operon (Table 4, line 1). Based on previous comparisons (17) the level seen would correspond to 10–15% of the transcription rate for a fully induced *lac* operon.

DISCUSSION

Evidence has been presented that transcripts initiated within the Tn*10* element extend into host sequences adjacent to the insertion site. The previous failure to detect these transcripts in most operons seems to be due to the efficiency of *rho*-dependent transcription termination at sites located within the operons studied. These sites have been hypothesized to account for the polarity effects shown by nonsense mutations on promoter-distal genes in an operon. The extensive work on this phenomenon has been reviewed by Franklin and Yanofsky (12). The widely accepted view of the polarity phenomenon is that transcription termination sites exist within coding sequences. These termination sites are not recognized when a translating ribosome follows the RNA polymerase closely. In the absence of such a ribosome, polymerase recognizes the termination site and ceases transcription.

Most of this termination activity seems to require the involvement of the protein ρ. The behavior of Tn*10* described here suggests that the transcripts emerging from Tn*10* are not translated and therefore are strongly subject to ρ-mediated termination. The efficiency of terminating these outward Tn*10* transcripts will probably vary widely depending on the sequences adjoining the insertion site. The insertions in the *hisB*, *-H*, and *-A* genes, which show weak expression of downstream genes, may generate transcripts that occasionally escape termination due to the weakness of the nearby termination sites or due to the presence of ribosome initiation sites that allow occasional ribosomes to enter the message and protect some messages from termination. In contrast, the insertions in the *hisG* gene show no expression of downstream genes, probably due to the strength of the message termination site within the *hisG* gene.

In ribosomal RNA operons, Tn*10* insertions appear to be nonpolar (1). We propose that this is due to transcripts emerging from the inserted Tn*10* element (and from other inserted elements that fail to show polarity in these operons). The ribosomal RNA cistrons present a special situation because they produce an untranslated RNA. Such operons must either be devoid of ρ-dependent termination sites or have a special mechanism for avoiding termination of the untranslated RNA. In either case, this operon would have no means of terminating transcripts emerging from inserted transposons; these transcripts would be detected and the insertions would appear nonpolar. It seems likely that this may account for the apparent nonpolar nature of Tn*10* insertions in the rRNA operons (1). Brewster and Morgan (18) have reported that transposons Tn*9* and IS*1* also show no polarity effects in ribosomal RNA operons. This suggests that these elements, like Tn*10*, provide outward promoters that have escaped detection in the nonribosomal operons tested because of early termination.

Reynolds *et al.* (19) have found that the *bgl* operon, which is normally unexpressed in *E. coli* K-12, can be activated by insertion of IS*1* or IS5. These insertion sequences are not thought to provide promoters for transcription of adjacent sequences. If these insertion sequences do possess promoters that have escaped detection because of the considerations described here, then the activation of *bgl* may be simply due to placement of a promoter near the *bgl* structural gene with no intervening *rho* sites.

The results reported here may also provide an explanation for the behavior of the transposon Tn5. Some, but not all, Tn5 insertions provide a promoter capable of expressing downstream genes (ref. 20; unpublished data). It has been suggested that this heterogeneity might be due to occasional creation of a promoter at the junction of Tn5 and sequence at the insertion site (20). Our unpublished results indicate that the heterogeneity of Tn5 insertions is due, at least in part, to the position of the insertion site *vis a vis* nearby *rho* sites. As is true for Tn*10*, the outside promoters of some Tn5 insertions lead to formation of transcripts whose detection is prevented by ρ-dependent termination.

R. W. Simons, B. C. Hoopes, W. R. McClure, and N. Kleckner (personal communication) have demonstrated the existence of a strong outward-directed promoter located near the outside end of IS*10*. Results discussed above suggest that IS*1* and Tn9 may also possess such transcripts. Transposons Tn5, Tn*A*, and IS2 have previously been shown to possess the same sort of promoter. The parallel behavior of these elements suggests that such outward transcripts may play an important role in the evolution or function of transposable elements.

Schmidt *et al.* (21) reported a short transcript produced at the outside ends of Tn*10* and Tn5. In the case of Tn*10* the transcript is induced by tetracycline. The relationship of the promoter described here to these inducible transcripts is not clear; the promoters described here do not depend on the presence of tetracycline for activity.

The presence of outward-directed promoters for bacterial transposons makes these transposons seem much less distinct from the transposable elements described in maize by McClintock (22). The maize elements affect regulation of gene expression but do not invariably lead to an abrupt and complete loss of gene activity. Recently, TY1 elements in yeast have been found to have regulatory effects similar to the maize element (23–25). In contrast, the bacterial insertion sequences and transposons have generally caused a complete loss of activity of the affected gene and a loss of expression of distal genes in the same operon by their absolute polar effects. The transcripts described here make it seem possible that Tn*10* and other transposons now thought to only block transcription could, in appropriate locations, serve to affect gene regulation and turn on silent genes.

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