Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5011-5015, August 1982
Genetics

Transcriptional activity of the transposable element Tn*10* in the *Salmonella typhimurium ilvGEDA* operon

(polarity/gene activation/orientation-specific transcription/ribosome binding site)

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Communicated by Wolfgang K. Joklik, March 29, 1982

ABSTRACT Polarity of Tn*10* insertion mutations in the *Salmonella typhimurium ilvGEDA* operon depends on both the location and the orientation of the Tn*10* element. One orientation of Tn*10* insertions in *ilvG* and *ilvE* permits low-level expression of the downstream *ilvEDA* and *ilvDA* genes, respectively. Our analysis of *Salmonella ilv* recombinant plasmids shows that this residual *ilv* expression must result from Tn*10*-directed transcription and does not reflect the presence of internal promoters in the *ilvGEDA* operon, as was previously suggested. The opposite orientation of Tn*10* insertion in *ilvE* prevents *ilvDA* expression, indicating that only one end of Tn*10* is normally active in transcribing adjacent genes. Both orientations of Tn*10* insertion in *ilvD* exert absolute polarity on *ilvA* expression. Expression of *ilvA* is known to be dependent on effective translation of *ilvD*, perhaps reflecting the lack of a ribosome binding site proximal to the *ilvA* sequence. Therefore, recognition of the ability of Tn*10* to promote transcription of contiguous genes in the *ilvGEDA* operon apparently requires the presence of associated ribosome binding sites.

The *Salmonella typhimurium* LT2 *ilvGEDAYC* gene cluster (1, 2) encodes the enzymes responsible for isoleucine and valine biosynthesis and is located at 83 min on the standard linkage map (3). The *ilvGEDA* genes constitute a single transcriptional unit that is transcribed from *ilvG* to *ilvA* (1). Expression of the *ilvGEDA* operon is multivalently regulated by the branched chain amino acids isoleucine, leucine, and valine (4). DNA sequence analysis of the promoter region of this operon has shown the presence of an attenuator structure including a sequence that potentially encodes a 32-amino acid peptide rich in branched chain amino acid residues (5). Therefore, the mechanism of multivalent regulation of the *Salmonella ilvGEDA* operon probably involves translational control of transcription termination, as previously described for several amino acid biosynthetic systems including the *ilvGEDA* operon of *Escherichia coli* strain K-12 (6-10).

We have studied the effects of insertion of the tetracycline-resistance transposon Tn*10* into the *ilvGEDA* operon (1). Insertion of Tn*10* into *ilvG* or *ilvE* abolishes multivalently regulated transcription of the downstream genes, but low-level expression of the *ilvEDA* and *ilvDA* genes persists in *ilvG*::Tn*10* and *ilvE*::Tn*10* insertion mutants, respectively. Since the insertion of Tn*10* into other transcriptional units has been shown to be absolutely polar on downstream genes in the absence of intervening transcriptional start sites (11, 12), the partial polarity of *ilvG*::Tn*10* and *ilvE*::Tn*10* insertions was interpreted as evidence for the existence of internal promoters in the *ilvGEDA* operon, one located proximal to *ilvE* and one located before *ilvD*. The strong polarity of *ilvD*::Tn*10* insertions on *ilvA* expression indicated that no promoter exists between *ilvD* and *ilvA*.

The recent availability of *Salmonella ilvGEDA*$^+$ chimeric plasmids (2) has allowed us to examine further the structure of the *ilvGEDA* operon with particular interest in defining the nature of the putative internal promoters. Surprisingly, studies with various subclones of the *ilvGEDA* operon have failed to demonstrate the existence of internal promoters in the operon. We describe here these observations and present evidence that low-level expression of downstream *ilv* information in Tn*10* insertion mutants is dependent on transcription from the Tn*10* element itself; the productive use of Tn*10*-directed transcription of the *ilv* genes is ascribed to the presence of effective ribosome binding sites associated with these genes.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *S. typhimurium ilv*::Tn*10* insertion mutants have been described (1). Strains TT627 *strA1 pyrC7*/F'*ts114 lac*$^+$ *zzf-20*::Tn*10* and TT628 *strA1 pyrC7*/F' *ts114 lac*$^+$ *zzf-21*::Tn*10* were provided by John Roth. Recipient strains DW2 *pyrE cysE* and DU800 *metE338* were gifts from N. Kredich. Spontaneously occurring mutants of these strains resistant to nalidixic acid at 20 μg/ml were isolated in this laboratory. *Salmonella ilv*$^+$ recombinant plasmids have been characterized (2). *E. coli* K-12 strains N100 *galK recA*, N100 (pKO4), and N100 (pKO5) were provided by Keith McKenney (13), and *galK* plasmids pKO4 and pKO5 were prepared by standard techniques (2). Strain DU650 Δ*ilvEDAC leuB6 hsdR hsdM*$^+$ was the standard *E. coli* K-12 cloning recipient and was used for assay of *ilvEDA* expression from *ilv*$^+$ plasmids.

Recombinant DNA Studies. Restriction endonucleases and T4 DNA ligase were obtained from Bethesda Research Laboratories. Endonuclease digestions, DNA ligation, and subcloning of endonuclease-generated fragments of the *Salmonella ilvGEDA* operon into *galK* plasmids were carried out as described (2). BAL-31 nuclease was prepared from *Alteromonas espejiani* BAL-31 according to the method of Gray *et al.* (14). The *tet* promoter of pBR322 was deleted from the chimeric pDU5 by treatment with BAL-31 nuclease (15); 1 μg of *Eco*RI-cleaved pDU5 was digested with 0.2 unit of BAL-31 in 20 μl of 20 mM Tris·HCl, pH 8.1/200 mM NaCl/12 mM $CaCl_2$/12 mM $MgCl_2$/1 mM EDTA. The reaction mixture was incubated for 10 min at 30°C, and reaction was stopped by addition of 2.2 μl of 0.25 M EDTA. The DNA was extracted with phenol, subjected to blunt-end ligation, and used to transform strain DU650 to ampicillin resistance as described (2). Plasmid DNA was partially purified from a number of transformants and characterized by endonuclease digestion. One derivative, designated pDU51,

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was found that lacked the *Eco*RI and *Hin*dIII sites of pBR322 but retained the *Bam*HI site at 375 base pairs (16).

Media. Minimal lactose medium contained modified Davis-Mingioli salts (1), 0.5% lactose, L-isoleucine at 50 mg/liter, L-valine at 100 mg/liter, and L-leucine at 50 mg/liter; 1.3% agar (Difco) was added for preparation of solid medium. Solid minimal medium contained modified Davis-Mingioli salts, 0.5% glucose, and L-cysteine at 50 mg/liter; this medium was supplemented with filter-sterilized nalidixic acid at 20 μg/ml for purposes of counterselection. Cultures grown under conditions of excess isoleucine, valine, and leucine were prepared as described (1).

Determination of Tn*10* Orientation. The method of Chumley *et al.* (17) was used. Strain TT627/F′*ts114 lac*$^+$ *zzf-20*::Tn*10* was mated with each *ilv*::Tn*10* mutant, and Lac$^+$ transconjugants were selected at 30°C. The transconjugants were then streaked on the lactose selection medium and incubated at 42°C. Lac$^+$ colonies formed at high temperature were picked and grown to saturation at 42°C in liquid lactose medium. These Hfr derivatives were mated with DW2 *pyrE cysE* and DU800 *metE338*, selecting for Ura$^+$ or Met$^+$ transconjugants on minimal glucose agar at 37°C. Several Hfr colonies from each merodiploid were analyzed and, in each case, all Hfr derivatives exhibited the same direction of transfer. The orientation of each *ilv*::Tn*10* mutant was independently confirmed by repeating this procedure with TT628/F′*ts114 lac*$^+$ *zzf-21*::Tn*10*, which contains a Tn*10* insertion in the opposite orientation from F′*zzf-20*::Tn*10*. Accordingly, Hfr strains formed by integration of F′*zzf-21* showed the opposite direction of transfer from those containing F′*zzf-20*.

Enzyme Assays. The isoleucine-valine biosynthetic enzymes were measured by published methods (1). The galactokinase assay was according to McKenney *et al.* (13).

RESULTS

Effects of Tn*10* Insertion in the *ilvGEDA* Operon. A collection of *ilv*::Tn*10* insertion mutants was isolated by John Roth and generously provided to us for characterization. Each mutant was defined by standard genetic analysis and enzyme assay (1). Representative Tn*10* insertions in the *ilvGEDA* operon are shown in Fig. 1, which also compares *ilvGEDA* expression in the insertion mutants with that of wild-type *S. typhimurium* LT2. The *ilvEDA* genes in the *ilvG*::Tn*10* mutant (TT4) and the *ilvDA* genes in the *ilvE*::Tn*10* mutants (TT71, TT79, and TT379) are expressed at levels similar to those seen under repressing conditions in strain LT2. This low-level expression was not increased under conditions of branched chain amino acid limitation indicating that read-through transcription from the major *ilv* promoter, P_{GEDA}, did not occur (data not shown). Since Tn*10* insertion in a transcriptional unit has been shown to exert absolute polarity on downstream gene expression (11, 12), internal promoters, designated P_{EDA} and P_{DA}, were inferred to be present in the *ilvGEDA* operon. Absolute polarity of Tn*10* insertion is seen with the *ilvD*::Tn*10* insertion mutants, strains TT83 and TT87, in which no *ilvA* expression was detected. It was concluded, therefore, that no internal promoter exists between *ilvD* and *ilvA*.

Although most of the *ilvE*::Tn*10* insertion mutants characterized exhibited *ilvDA* expression, one unusual class of *ilvE*::Tn*10* insertions was found in which complete inactivation of *ilvDA* occurred. Such insertion mutations, as exemplified by strains TT81 and TT1260, are located in the *ilvE* gene, as shown by deletion mapping and reversion analysis. The phenotype of these strains, together with the pattern of expression of *ilvE* and *ilvD* from subclones of recombinant plasmids that lack portions of the *ilvE-ilvD* interface, led us to conclude that some overlap exists between the *ilvE* structural gene and the P_{DA} sequence

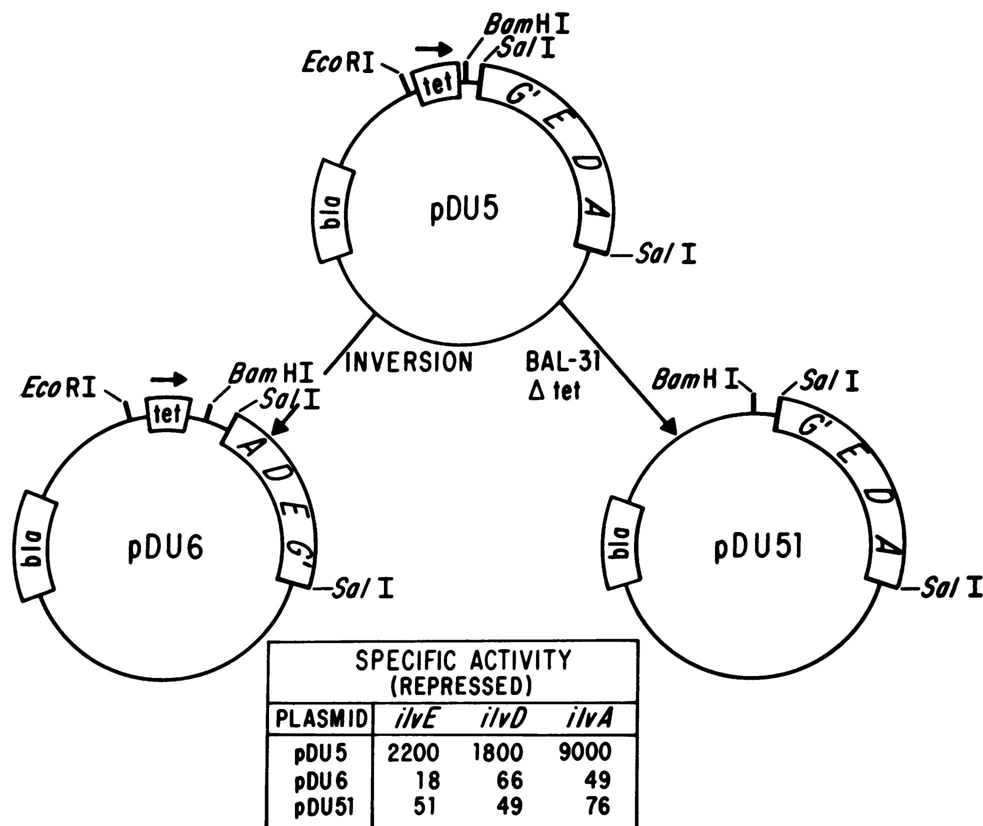
P_{GEDA}	G	E	D	A
LT2	3.9	62	22	85
TT4 *ilvG*::Tn10	→	49	31	70
TT71 *ilvE*::Tn10	4.6	→	65	190
TT79 *ilvE*::Tn10	4.9	→	69	230
TT379 *ilvE*::Tn10	3.5	→	55	200
TT81 *ilvE*::Tn10	3.0	←	N.D.	N.D.
TT1260 *ilvE*::Tn10	4.3	←	N.D.	N.D.
TT83 *ilvD*::Tn10	3.3	65	→	N.D.
TT87 *ilvD*::Tn10	4.0	79	←	N.D.

FIG. 1. The *S. typhimurium ilvGEDA* operon. The enzymes encoded by the operon are acetohydroxyacid synthase II (*ilvG*), transaminase B (*ilvE*), dihydroxyacid dehydrase (*ilvD*), and threonine deaminase (*ilvA*). The specific activities of the *ilvGEDA* gene products under repressed conditions are given in units of nanomoles of product formed per min per mg of protein. N.D., not detectable. The specific Tn*10* insertions are represented as boxes; Tn*10* causes inactivation of each gene in which it is inserted. Orientations of the Tn*10* elements are indicated by the enclosed arrows and were inferred from the experiments summarized in Table 1. Some of these enzyme measurements have been reported previously (1).

Table 1. Orientation of Tn*10* in the *ilvGEDA* operon

	Transconjugation frequency			
Hfr strain	× DW2 *pyrE cysE*	× DU800 *metE338*	Direction of transfer	Tn*10* orientation
TT4	120	1	O-*pyrE*-...-*metE*	+
TT71	>500	4	O-*pyrE*-...-*metE*	+
TT79	>500	0	O-*pyrE*-...-*metE*	+
TT379	>500	60	O-*pyrE*-...-*metE*	+
TT81	0	450	O-*metE*-...-*pyrE*	−
TT1260	5	450	O-*metE*-...-*pyrE*	−
TT83	113	2	O-*pyrE*-...-*metE*	+
TT87	2	>500	O-*metE*-...-*pyrE*	−

Hfr strains were generated by mating TT627 F′*ts114 lac*$^+$ *zzf-20*::Tn*10* with *ilv*::Tn*10* insertion mutants as described (17). The transconjugation frequency represents the number of Ura$^+$ (*pyrE*$^+$) or Met$^+$ (*metE*$^+$) colonies formed by plating a 1:100 dilution of the mating mixture on minimal glucose agar. Matings with Hfr-TT4 were carried out with derivatives of DW2 and DU800 resistant to nalidixic acid at 20 μg/ml in the selection medium.



SPECIFIC ACTIVITY (REPRESSED)			
PLASMID	*ilvE*	*ilvD*	*ilvA*
pDU5	2200	1800	9000
pDU6	18	66	49
pDU51	51	49	76

FIG. 2. Expression of *ilvEDA* from recombinant plasmids. Cloning of the 6-kilobase *Sal* I *ilvG'EDA* fragment into pBR322 has been described (2); one *Sal* I terminus lies toward the 3' end of *ilvG*, and the other is distal to *ilvY*, which is transcribed independently from the *ilvGEDA* operon. Inversion of the *Sal* I fragment was accomplished by digestion of pDU5 with *Sal* I endonuclease and religation under standard conditions (2). Removal of the *tet* promoter by BAL-31 nuclease resulted in a 130-base-pair deletion in the pBR322 vector that spans the *Eco*RI/*Hin*dIII region. This altered pBR322, when prepared without the *Sal* I insert, does not confer resistance to tetracycline. Measurements of *ilvEDA* activity from each plasmid were made in strain DU650 Δ*ilvEDAC* under repressed conditions. Specific activities are in nanomoles of product formed per min per mg of protein.

(18). The lack of *ilvDA* expression in strains TT81 and TT1260 assumes the insertion of Tn*10* into that overlap region, thus disrupting both the *ilvE* gene and the internal promoter needed for expression of *ilvDA* in the absence of read-through transcription from the *ilvE* gene. This hypothesis is shown to be incorrect by the results presented here, which indicate that the lack of *ilvDA* expression in TT81 and TT1260 is a consequence of the orientation of the Tn*10* element.

Expression of *ilvEDA* from Recombinant Plasmids. The *S. typhimurium ilvEDA* genes have been subcloned as a 6.0-kilobase *Sal* I fragment into pBR322 (2). One terminus of the fragment lies within the *ilvG* gene, so that the *ilvEDA* genes are dissociated from the major *ilv* promoter and expression of *ilvEDA* should be dependent solely on the internal promoters. Fig. 2 shows these plasmids containing the *Sal* I *ilvEDA*$^{+}$ fragment and indicates the level of *ilvEDA* expression from each plasmid. The *ilvEDA* genes in pDU5 are expressed at high levels, showing that these genes are intact on the *Sal* I fragment. The expression of *ilvEDA* from pDU5 does not result from promoters contained within this DNA but, rather, results from the plasmid *tet* promoter (16, 19). This was shown by inverting the *Sal* I fragment in orientation with respect to the *tet* promoter (pDU6), drastically curtailing *ilvEDA* expression. In addition, when the *tet* promoter was removed from pDU5 by digestion with nuclease BAL-31, a similar decrease in *ilvEDA* activity was observed (pDU51). The *ilvEDA* expression from pDU6 and pDU51 was comparable with the haploid gene activity in *ilvG*::Tn*10* and *ilvE*::Tn*10* insertion mutants (see TT4 and TT71 in Fig. 1). Since pBR322 exists in 20–30 copies per cell (20), the expression of *ilvEDA* as shown with pDU6 and pDU51 is much lower than expected, given the apparent strengths of the Tn*10*-defined internal promoters and the increased gene dosage with the multicopy recombinant plasmids.

To obtain additional evidence for the lack of internal promoters, the *S. typhimurium ilvGEDA* operon was fused with the *E. coli* K-12 *galK* gene by using the plasmid system of McKenney *et al.* (13). As shown in Fig. 3, the pDU30 and pDU40 chimeras contain *ilvGE* and *ilvGEDA'* fragments conjoined with *galK* so that *galK* is expressed from the *ilv* promoter P_{GEDA}; the levels of *galK* expression reflect the activity of P_{GEDA} under repressed conditions. When P_{GEDA} is removed by deleting a *Sal* I endonuclease fragment from these plasmids, *galK* expression from the derivatives pDU31 and pDU41 falls to $<1/10$th the repressed levels conferred by the parental plasmids. Furthermore, this low level expression is similar to that seen with the pKO4 and pKO5 vectors themselves, which is apparently the result of read-through transcription from plasmid promoters. Since the activity of the putative promoters suggested by *ilvEDA* expression in *ilvG*::Tn*10* and *ilvE*::Tn*10* insertion mutants is similar to the wild-type repressed levels of *ilvEDA* (see Fig. 1), the results obtained with the *galK* subclones indicate that there can be no significant transcription from internal sites in the *ilvGEDA* operon. In addition, this suggests that expression of *ilvEDA* and *ilvDA* in *ilvG*::Tn*10* and *ilvE*::Tn*10* mutants does not reflect the activity of autochthonous internal promoters but must be the result of transcription

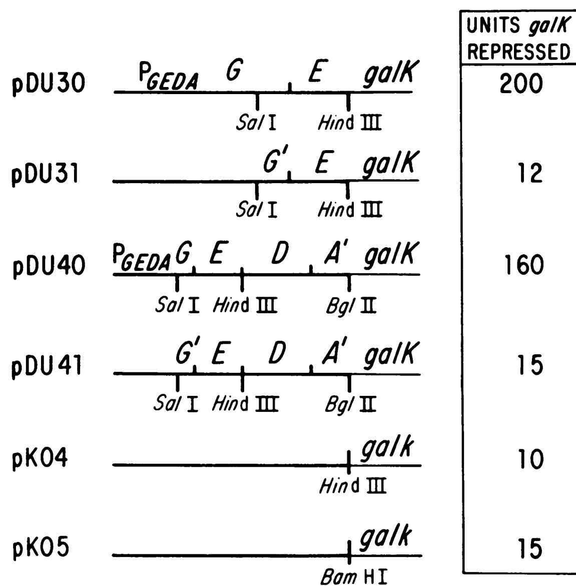


FIG. 3. Fusions of the *ilvGEDA* operon to *galK*. Specific endonuclease fragments of the *ilvGEDA* operon (2) were subcloned into pKO4 or pKO5. pDU30 consists of a 5.2-kilobase *Hin*dIII fragment inserted into pKO4; this *ilvGE*$^+$ fragment ends between the *ilvE* and *ilvD* structural genes. An 8-kilobase *Bgl* II fragment that is *ilvGED*$^+$ and terminates toward the 3' end of *ilvA* was cloned into the *Bam*HI site of pKO5, resulting in pDU40. A *Sal* I fragment present on both inserts and ending in *ilvG* was removed by *Sal* I digestion of pDU30 and pDU40 followed by intramolecular ligation. The resulting plasmids, pDU31 and pDU41, lack P_{GEDA} as well as most of the *ilvG* gene. *galK* expression from each plasmid was determined on strain N100 *galK recA* under repressed conditions by using the toluene-treated cell assay of McKenney *et al.* (13). Units of galactokinase activity are nanomoles of galactose 1-phosphate formed per min per A_{650} unit. Galactokinase expression from pKO4 and pKO5, which lack a promoter associated with *galK*, is also indicated and ranges from 10 to 15 units under these conditions.

from the inserted Tn*10* element.

We have attempted to demonstrate the transcriptional activity of Tn*10* by cloning the element from several *ilv*::Tn*10* insertion mutants. In no case has it been possible to obtain clones that contain intact *ilv*::Tn*10* insertions, suggesting that these mutations are susceptible to evolution during the course of molecular cloning. A similar observation concerning the instability of Tn*10* in multicopy plasmids has been made by Kleckner and co-workers (21) in their study of *his*::Tn*10* insertions.

Orientation of Tn*10* Determines the Degree of Polarity in the *ilvGEDA* Operon. Kleckner *et al.* (22) have recently shown that the DNA sequences of the inverted terminal repetitions of Tn*10* are not identical. In particular, a potential promoter sequence exists in the right-hand end of the element that could direct transcription outward from Tn*10* to the contiguous chromosome. If the expression of downstream genes in *ilv*::Tn*10* insertion mutants is dependent on transcription from Tn*10*, specifically from the potential promoter in the rightward sequence, then this expression should be correlated with the orientation of the Tn*10* element.

The orientation of *ilv*::Tn*10* insertions was determined by the method of Chumley *et al.* (17), which exploits the ability of Tn*10* to act as a region of homology by which an F'*lac*$^+$ Tn*10* plasmid can readily integrate into the chromosome. This integration depends on homologous recombination between the plasmid and chromosomal copies of Tn*10*. Therefore, all Hfr derivatives of a given merodiploid will exhibit the same direction of transfer of chromosomal markers as defined by the orientation of the chromosomal Tn*10* element.

Hfr strains were formed from each of the representative *ilv*::Tn*10* insertion mutants, and these strains were mated with either DW2 *pyrE cysE* or DU 800 *metE338*. The *pyrE* and *metE* loci are located in positions that flank *ilv* (3), so that Hfr strains generated by the insertion of F'*lac*$^+$ Tn*10* at the *ilv* gene cluster should transfer either *pyrE* or *metE* at high frequency, depending on the direction of transfer of a given Hfr. As shown in Table 1, the TT4-, TT71-, TT79-, and TT379-derived Hfr strains exhibit the same direction of transfer, implying that in each mutant the Tn*10* insertion is the same orientation. Since these mutants express the downstream *ilv* genes, the Tn*10* elements can be considered to be in the promoter-active or on orientation. The Hfr strains derived from TT81 and TT1260 transfer chromosomal markers in the opposite direction, showing that the Tn*10* element in TT81 and TT1260 is inverted with respect to Tn*10* in the other *ilvE* insertion strains. TT81 and TT1260 are Tn*10* insertion mutants that lack *ilvDA* expression, suggesting that the Tn*10* elements in these mutants are in the inactive or off orientation. We have studied other *ilv*::Tn*10* insertion mutants to obtain additional evidence for the orientation specificity of Tn*10*-mediated transcription. It should be noted that each of the five *ilvE*::Tn*10* insertion mutations depicted in Fig. 1 is distinct; that is, each is capable of recombining with the other *ilvE*::Tn*10* mutations to generate prototrophic transductants, indicating that each insertion site is different. During screening of the *ilv*::Tn*10* collection, a number of strains were shown to be identical. Three other *ilvE*::Tn*10* mutants, for example, were characterized that lacked *ilvEDA* expression but failed to recombine with TT81. In addition, these strains have the same Tn*10* orientation as that in TT81. This family of TT81-like mutants represents Tn*10* insertion in the same orientation at a specific site in the *ilvE* gene. Although Tn*10* can insert at many different sites on a target chromosome, its transposition does exhibit some preference for "hot spots," as shown for Tn*10* insertions in the *S. typhimurium his* operon (23). The number of distinct *ilv*::Tn*10* insertion mutants, therefore, appears to be limited by the presence of preferred sites for integration of Tn*10* in the *ilvGEDA* operon. Among the distinct *ilvG*::Tn*10* and *ilvE*::Tn*10* mutants characterized so far, we have discovered one exception to an orientation specificity for Tn*10* activation of downstream genes. Strain TT63 *ilvG*::Tn*10* was found to be in the off orientation yet expressed *ilvEDA* at levels similar to those seen with TT4. This exception could be explained, however, by the observation that the left-hand end of Tn*10* is susceptible to gene conversion, which appears to be promoted by high-frequency recombination in the P22::Tn*10* phage that serves as the transposition vector (N. Kleckner, personal communication). The left-hand end can therefore be converted to a fully functional right-hand end and should be capable of transcription of adjacent chromosomal sequences. These results are consistent with the hypothesis that only one end of the Tn*10* element—namely, the rightward end, which contains a potential promoter sequence—is active in transcribing adjacent chromosomal genes. The two *ilvD*::Tn*10* mutants, TT83 and TT87, have Tn*10* insertions in either orientation and yet both lack *ilvA* activity. This suggests that some genes cannot be expressed by transcription initiating at one end of Tn*10*.

Productive Transcription of Downstream *ilv* Genes by Tn*10* Requires Associated Ribosome Binding Sites. We have recently determined the amounts of each enzyme formed from the *ilvGEDA* operon when expressed from recombinant plasmids. Under the condition of valine limitation, acetohydroxyacid synthase II (*ilvG*), transaminase B (*ilvE*), dihydroxyacid dehydrase (*ilvD*), and threonine deaminase (*ilvA*) constitute 2.5%, 10%, 6%, and 3.5%, respectively, of the total cellular

protein; when corrected for the molecular weights of the polypeptide chains encoded by these genes the relative translation frequencies for *ilvGEDA*, respectively, are 1:7:2:1.5. Therefore, the sequences in the polycistronic *ilvGEDA* mRNA are translated with differential efficiency, perhaps reflecting ribosome binding sites of various strengths associated with *ilvG*, *ilvE*, and *ilvD*. The close correspondence of *ilvD* and *ilvA* expression suggests the possibility of translational coupling between these genes, as has been shown in the *trp* operon for the *trpED* genes (24). In fact, expression of *ilvA* does depend on effective translation of *ilvD*, as shown by deletions that remove the ribosome binding site and the sequence that encodes the amino-terminal portion of the *ilvD* gene and concomitantly prevent translation of downstream *ilvA* information (unpublished). This translational dependence of *ilvA* on *ilvD* implies the absence of a ribosome binding site associated with *ilvA*.

The *ilvE* and *ilvD* genes, both of which apparently have strong ribosome binding sites, can be expressed from Tn*10* when the element is inserted in the proper orientation as in TT4 and TT71. Translational coupling of *ilvD* with *ilvA* permits *ilvA* expression in both *ilvG*::Tn*10* and *ilvE*::Tn*10* mutants. However, in *ilvD*::Tn*10* insertion mutants no expression of *ilvA* occurs, even when the Tn*10* element is inserted in the on orientation for transcription of *ilvA* as in TT83 (Table 1). Transcription of the *ilvA* gene from Tn*10* does not allow for *ilvA* expression, presumably because of the lack of a ribosome binding site associated with *ilvA* and the resulting inability to translate the Tn*10*-derived *ilvA* transcript. Therefore, the only absolute polarity of Tn*10* insertion, regardless of orientation, that occurs in the *ilvGEDA* operon is apparently caused by perturbing the essential translational coupling of *ilvA* to *ilvD*.

DISCUSSION

The studies described above show that the partial polarity of certain *ilv*::Tn*10* insertion mutations must reflect the transcriptional activity of Tn*10* itself rather than the presence of internal promoters in the *ilvGEDA* operon. In addition, the ability of Tn*10* to promote downstream gene expression is associated with only one orientation of the Tn*10* element. This orientation dependence is explicable by the observations of Kleckner *et al.* (22) concerning the nonidentical nature of the 1,400-base-pair termini of Tn*10* and the potential promoter sequence encoded by the rightward IS*10*. At least one other group of transposable elements, the Tn3-related sequences, can also effect expression of contiguous genes by read-through transcription from the β-lactamase promoter and from the transposase (*tnpA*) promoter in the absence of repressor (*tnpR*) function (25, 26).

The proper orientation of Tn*10* is necessary but not sufficient for expression of downstream genes. As shown with the *ilvEDA* genes, both *ilvE* and *ilvD* are expressed from Tn*10*-directed transcripts because of associated ribosome binding sites. The expression of *ilvA* depends on translation of *ilvD*, presumably because of a lack of an effective *ilvA* ribosome binding site, so that Tn*10*-directed *ilvA* transcripts cannot be translated in the absence of *ilvD* expression. These observations indicate that the strong polarity effects that are noted on insertion of Tn*10* in a polygenic operon reflect the polarigenic orientation of the element or disruption of an essential translational coupling between genes or groups of genes. An additional impediment to Tn*10*-directed transcription of adjacent host sequences has been described by Ciampi *et al.* (27), who have shown that the ability of Tn*10* to promote transcription of adjacent genes in the *his* operon requires removal or inactivation of intervening *rho*-dependent termination sites. A similar impediment could be invoked to explain the inability of *ilvD*::Tn*10* insertions to cause expression of *ilvA*. We have shown, however, that, unlike the case of the *his* operon, the *rho*-111 mutation (28) does not cause *ilvA* expression in either strain TT83 or strain TT87.

We thank J. Roth for providing some of the *S. typhimurium* strains used in this study. Also, we thank J. Roth and N. Kleckner for helpful discussion and communication of unpublished results, H. J. Whitfield for strains carrying the *rho-111* mutation, and Dayle Wilkins for typing the manuscript. This work was supported by National Institutes of General Medical Sciences Grant GM12551. D.L.B. is a Trainee in the Medical Scientist Training Program and is funded by National Institutes of Health Grant GM07171.

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