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Gene *ilvY* of *Salmonella typhimurium*

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Evidence is presented for the existence in *Salmonella typhimurium* LT2 of the regulatory gene *ilvY*. The *Escherichia coli* K-12 *ilvY* gene product is shown to complement a *S. typhimurium ilvY* mutation in vivo.

The *ilvC* gene product, α-acetohydroxy acid isomeroreductase, is one of the four enzymes common to isoleucine and valine biosynthesis in a number of enteric bacteria including *Escherichia coli* K-12 and *Salmonella typhimurium* LT2 (14). The synthesis of the isomeroreductase is induced by its substrates, α-acetolactate or α-aceto-α-hydroxybutyrate (2). The synthesis of the remaining enzymes of the pathway is repressed by the branched-chain amino acids (6, 7).

The analysis of *ilvC* regulation in *E. coli* K-12 has been facilitated by the isolation of a λ phage with the *lacZ* gene fused to the *ilvC* regulatory elements (12), and by the development of an in vitro protein-synthesizing system using the phage DNA as a template (16). The synthesis of β-galactosidase in this in vitro system is dependent upon the acetohydroxy acids and guanosine tetraphosphate. In addition, a positive regulatory protein, designated upsilon, has been identified which is absolutely required for *ilvC*-dependent β-galactosidase synthesis (15). Mapping with recombinant plasmids demonstrates that the *E. coli* K-12 *ilvY* gene codes for upsilon and is located between *ilvA* and *ilvC*. The upsilon protein presumably interacts with the acetohydroxy acids in vivo to form an induction complex that is essential for *ilvC* expression. Therefore, an *ilvY* mutation that destroys the *ilvC* regulatory activity of upsilon should prevent adequate *ilvC* expression and confer a *trans*-recessive Ilv^- phenotype.

We have recently employed the translocatable drug resistance element Tn*10* to study the genetic organization of the *S. typhimurium ilvGEDAC* gene cluster (3); during this investigation we obtained genetic evidence for the presence of a regulatory locus analogous to the *E. coli* K-12 *ilvY* gene, probably situated between *ilvA* and *ilvC*. The present report describes the characterization of a *Salmonella ilvY* mutant and the results of *cis-trans* analysis, which demonstrate the positive *trans* effect of the *ilvY* gene product on in vivo expression of *ilvC*.

The spontaneous excision of Tn*10* to generate tetracycline-sensitive variants is often associated with irreversible alterations of the contiguous chromosome extending from either one side or the other of the inserted element (10). These alterations include both deletions and inversions. We selected *Salmonella* Tet^s Ilv^- derivatives from an *ilvA*::Tn*10* insertion strain, TT58 (Table 1). The derivatives were characterized as either deletions or inversions by P22 transductions with standard *ilv* mutants. The Ilv^- phenotype of these mutants could arise by Tn*10*-generated alterations from the left end of the *ilvA*::Tn*10* insertion that involve *ilvD*, encoding the dihydroxyacid dehydrase, or by alterations extending rightward into *ilvC*, encoding the isomeroreductase. In addition, if the *ilvY* locus exists in *Salmonella* between *ilvA* and *ilvC*, then rightward-extending Tn*10* inversions or deletions could also mutate the *ilvY* gene. If the *ilvY* gene product is essential for *ilvC* expression in vivo, this would confer an Ilv^- phenotype.

The direction of the Tn*10*-generated lesion in a set of 30 Tet^s Ilv^- variants of TT58 was determined by transducing each with P22 phage grown on either strain DU601 Δ*ilvDA* or DU603 Δ*ilvAC* (Table 1). Since these deletions were derived from TT58, all TT58 Ilv^- variants resulting from Tn*10*-generated chromosomal alterations extending from the right end of the *ilvA*::Tn*10* insertion can be transduced to Ile^- by phage grown on DU601 but not by phage grown on DU603. The transductants remain Ile^- presumably because of a residual Tn*10*-derived insertion in the *ilvA* gene, as has been shown for both Tn*10*-promoted deletions and inversions in the *rex* gene of λ phage (10). Using this transductional screening, 6 of 30 TT58 Ilv^- variants were judged to involve alterations extending to the right of *ilvA*::Tn*10*.

The six derivatives were used as donors in P22 transductions with strain DU201 *ilvC8*. Two of the six failed to transduce DU201 to prototrophy, indicating that these are Tn*10*-generated deletions that extend into *ilvC* at least as far as *ilvC8*. Each of the remaining four clones recombined with DU201 to generate prototrophic

TABLE 1. *Bacterial strains used in this study*[a]

Strain designation	Pertinent genotype	Source
F⁻		
TT58	*ilvA*::Tn*10*	J. Roth
TT521	*srl*::Tn*10 recA*	J. Roth
DU601	Δ*ilvDA*	From TT58 (3)
DU6011	Δ*ilvDA srl*::Tn*10 recA*	From DU601[b]
DU603	Δ*ilvAC*	From TT58 (3)
DU6031	Δ*ilvAC srl*::Tn*10 recA*	From DU603
DU201	*ilvC8*	F. Armstrong
DU202	*ilvA*::Tn*10 ilvC8*	From DU201[c]
409	*leuA409*	(4)
DU2	*ilvD18 ilvA2280 leuA409*	From 409[d]
DU3	*ilvD18 ilvA2280 leuA409 srl*::Tn*10 recA*	From DU2[b]
DU701	*ilvA ilvY*	This study
DU7011	*ilvA ilvY srl*::Tn*10 recA*	From DU701[b]
DU976(pMD7)	*ilvA454* Δ(*proAB-lac*)(pMD7) *ilvA*⁺ *ilvY*⁺ *ilvC-lacZ*	H. E. Umbarger (15)
Hfr		
SA722	*serA15 pur-268*	K. E. Sanderson (11)
DU203	*serA15 pur-268 ilvA*::Tn*10 ilvC8*	From SA722[e]
F′		
DU3(F′*ilvC8*)	*ilvD18 ilvA2280 leuA409 srl*::Tn*10 recA*(F′*ilvGED*⁺)*ilvA*::Tn*10 ilvC8*	DU203 × DU3[f]

[a] All strains are *S. typhimurium* LT2 except for CU976(pMD7), which is *E. coli* K-12.

[b] The *recA* allele was introduced by P22 transduction from strain TT521 according to Anderson and Roth (1). The *srl*::Tn*10* insertion is 50% linked to *recA* so that approximately 50% of Tet^r transductants with TT521 as the donor also receive the *recA* allele. Coinheritance of *recA* is scored by enhanced sensitivity to UV light radiation.

[c] The *ilvA*::Tn*10* insertion is about 60% linked to *ilvC8* (data not shown). A single Tet^r Ilv$^-$ transductant was cloned. Enzyme assay was performed on the transductant and showed no threonine deaminase or isomeroreductase activities. This clone was designated DU202.

[d] The *ilvA2280* allele encodes a threonine deaminase that is insensitive to feedback inhibition by isoleucine. The feedback insensitivity allows growth on threonine as the sole nitrogen source, and this phenotype can be readily scored as a transductional marker. The catalytically inactive *ilvD18* mutation is approximately 90% linked to *ilvA2280*. The configuration *ilvD18 ilvA2280* was introduced into strain 409 by P22 transduction selecting for growth on threonine as the sole nitrogen source. Coinheritance of *ilvD18* was confirmed by enzyme assay. The details of this procedure will be published elsewhere.

[e] The *ilvA*::Tn*10 ilvC8* markers were transduced from DU202 into SA722 by selecting for Tet^r. A single Tet^r Ilv$^-$ transductant was cloned, and enzyme assay was performed to confirm the inherited genotype.

[f] A 30-min mating between DU203 and DU3 was performed essentially as described (9), selecting for Ilv$^+$ transconjugants. Six of the transconjugants were characterized in detail. In each case the Ilv$^+$ phenotype was sensitive to curing by acridine orange and could be transferred at high frequency to appropriate recipients. The F′*ilv* clones also transfer *metE* but not *rbsP*. Since the parental Hfr transfers chromosomal markers in the order O-*ilv-rbsP*-pyrE-. . .-*metE-cya* (11), these F′ factors must be the result of type II excisions. A single clone was designated DU3(F′*ilvC8*) and used for the complementation analysis.

transductants. Enzyme assay of these four variants showed that each lacks both threonine deaminase (*ilvA*) and isomeroreductase (*ilvC*) activities. Therefore, these mutants could arise by Tn*10*-generated alterations involving *ilvC* but retaining the *ilvC8* site, or they could represent mutations affecting the putative *Salmonella ilvY* locus, which is essential for *ilvC* expression. This reasoning is based upon the assumption that the lack of isomeroreductase in strain DU201 is, in fact, caused by a mutation in the structural gene (*ilvC*) rather than in the regulatory gene (*ilvY*); the results of the following *cis-trans* test show the assumption to be valid in that the functions affected by the *ilvC8* mutation and the Tn*10*-generated mutation are complementary for isomeroreductase activity. Strain DU3(F′*ilvC8*) was mated with each mutant under standard conditions (9). No transconjugants are expected on isoleucine minimal medium if the endogenote possesses a Tn*10* alteration involving *ilvC*, because the F′ factor carries the *ilvC8* mutation. However, if the recipient is Ilv$^-$ because of an *ilvY* mutation, then the F′ plasmid will provide the *ilvY* gene product in *trans* to induce the expression of the endogenote *ilvC*

gene, and the transconjugants should grow on isoleucine medium. Only one of the clones, designated DU701, was complemented in the mating with DU3(F'*ilvC8*). To rule out the possibility of intragenic recombination in the *ilvC* gene, DU701 was made *recA* according to the method of Anderson and Roth (1), and the mating with DU3(F'*ilvC8*) was repeated. The transconjugation frequency in the mating between DU3(F'*ilvC8*) and DU7011 was 1.0×10^{-4} Ilv$^+$ transconjugants per F' donor cell. This compares well with the frequency of 1.5×10^{-4} Ilv$^+$ transconjugants per F' donor cell obtained in the mating of DU3(F'*ilvC8*) with DU6011 Δ*ilvDA recA*. Therefore, the ability to complement the DU7011 mutation is transferred by the F' factor at the same frequency as an *ilv* structural gene, *ilvD*. No transconjugation occurred in a mating between DU3(F'*ilvC8*) and DU6031 Δ*ilvAC recA*, confirming that the F'*ilvC8* plasmid does not complement an *ilvC* mutation in *trans*.

Enzyme assay was performed on DU7011 and DU7011(F'*ilvC8*) under conditions of excess branched-chain amino acids or isoleucine limitation. In both *E. coli* K-12 and *S. typhimurium*, the level of *ilvC* expression is correlated with the activity of the acetohydroxy acid synthases because the products of the synthase reactions, α-acetolactate and α-aceto-α-hydroxybutyrate, are the natural inducers of *ilvC* expression (2). This correlation depends upon the presence of functional apo-inducer, the *ilvY* gene product (15). As shown in Table 2, DU7011 lacks detectable *ilvC* activity even under the condition of isoleucine limitation with a large derepression of the synthase activities. However, the introduction of the F'*ilvC8* plasmid allows for a normal repressed level of *ilvC* as well as the essentially normal induction of *ilvC* upon isoleucine limitation.

We conclude from these results that the Tn*10*-generated lesion in strain DU7011 affects a *trans*-active regulatory element, essential for *ilvC* expression, which we believe represents the *Salmonella ilvY* gene. Since Tn*10*-generated chromosomal alterations begin at one end of the inserted element (10), the *ilvY* locus is clearly located to the right of *ilvA*. We have not been able to characterize the DU7011 mutation as either a deletion or inversion because of the lack of useful mapping mutations in this region. If the lesion is a deletion, then *ilvY* must map between *ilvA* and *ilvC*, since the *ilvC* gene is unaffected. If the lesion is an inversion, it is not now possible to map *ilvY* unambiguously between *ilvA* and *ilvC*, since an inversion could conceivably involve a gene that is distal to *ilvC* without affecting the *ilvC* structural gene. However, in view of the *E. coli* K-12 mapping results, we favor the interpretation that the *Salmonella ilvY* is situated between *ilvA* and *ilvC*.

TABLE 2. *Enzyme assay of strains DU7011 and DU7011(F'ilvC8)*

Strain	Medium[a]	Sp act[b]	
		Acetohydroxy acid synthase[c]	Acetohydroxy acid isomereroductase[d]
DU7011	Repressing	17	ND[e]
	Limiting isoleucine	150	ND
DU7011 (F'*ilvC8*)	Repressing	19	57
	Limiting isoleucine	160	660

[a] Cells were grown as batch cultures in the minimal medium of Davis and Mingioli (5) with the modifications described previously (3). Repressing medium contained 0.76 mM leucine, 0.85 mM valine, and 0.38 mM isoleucine. Limitation for isoleucine was achieved by decreasing the isoleucine concentration to 0.038 mM. Cells were harvested in late log phase and disrupted by sonication as described previously (3).

[b] Specific activities are expressed as nanomoles per minute per milligram of protein.

[c] Assayed according to Størmer and Umbarger (13).

[d] Assayed according to Arfin et al. (2) with α-aceto-α-hydroxybutyrate as the substrate.

[e] ND, Not detectable.

The close similarity between the *E. coli* K-12 and *S. typhimurium ilv* gene clusters suggests the possibility that the *E. coli ilvY* gene could complement the *S. typhimurium ilvY* mutation. A fragment of the *E. coli* K-12 *ilv* gene cluster containing the *ilvA*, *ilvY*, and *ilvC-lacZ* fusion genes has been inserted on the conjugally active plasmid RP4 by in vitro recombination techniques (15). We mated this plasmid, designated pMD7, into DU701 and selected for prototrophic transconjugants. These were obtained at a high frequency, indicating that the *E. coli* K-12 *ilvY* gene product does complement the *Salmonella ilvY* mutation (in addition, the *ilvA* gene contained on pMD7 is adequately expressed to allow for isoleucine biosynthesis). Enzyme assay was performed on a purified DU701(pMD7) merodiploid grown either in excess branched-chain amino acids or in minimal medium (Table 3). The adequate levels of *ilvC* formed under either of these conditions indicate that the *E. coli* K-12 *ilvY* gene product does effectively recognize *cis*-active *Salmonella ilvC* regulatory elements. The induction of β-galactosidase is the result of the action of the *E. coli* K-12 *ilvY* gene product at the *E. coli ilvC* regulatory elements controlling β-galactosidase expression. The basis for the

TABLE 3. *Enzyme assay of strain DU701(pMD7)*

Medium[a]	Sp act[b]		
	Acetohydroxy acid synthase	Acetohydroxy acid isomereroreductase	β-galactosidase
Repressing	41	10	93
Minimal	90	230	3100

[a] Cells were grown and harvested in the same manner as described in Table 2, footnote *a*. Minimal medium contained no amino acid supplements.

[b] Specific activities are expressed as nanomoles per minute per milligram of protein. The acetohydroxy acid synthase and the isomeroreductase were assayed as described in Table 2, footnote *a*. The β-galactosidase was assayed according to Miller (9).

fact that the induction ratio of β-galactosidase (33-fold) is greater than that of isomeroreductase (23-fold) is unknown, but it may be explained by preferential interaction of the *E. coli* K-12 *ilvY* gene product with the homologous *ilvC* regulatory elements.

The genetic evidence presented here identifies the *S. typhimurium ilvY* gene and establishes the essential role of the *ilvY* gene product in regulation of *ilvC* expression in vivo. An alternate explanation of our results, invoking the presence of two contiguous *ilvC* genes that specify different subunits of the isomeroreductase, has been ruled out by the demonstration that the *Salmonella* isomeroreductase is composed of four identical subunits (8). Our genetic analysis demonstrates that the *Salmonella ilvY* gene maps to the right of *ilvA*, and in view of the evidence from the closely related *E. coli* K-12 system, we favor the gene order *ilvA-ilvY-ilvC*. The *E. coli* K-12 and *S. typhimurium ilvC* regulatory systems are similar, and the *E. coli* K-12 *ilvY* gene product can function in vivo to promote *Salmonella ilvC* expression.

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