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Fusions of the *lac* and *trp* Regions of the *Escherichia coli* Chromosome[1]

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Two classes of strains were studied in which the *lac* operon is transposed to a chromosomal site close to the *tonB* and *trp* loci. The two classes differ in the orientation of the *lac* region on the chromosome. In both types of strains, *tonB* mutants were selected in which deletions removing the *tonB* locus also caused a fusion of the *lac* and *trp* regions. The study of the properties of such fusion strains provides information on the control of both the *lac* and *trp* operons.

The *lac* and *trp* region in *Escherichia coli* are normally separated by approximately 12% of the bacterial chromosome (29). However, it is possible to isolate strains in which the *lac* region is transposed to a site very close to the *trp* operon (3). In certain of these strains, the *lac* genes are inserted in the chromosome as part of the defective φ80d*lac* transducing phages (26). In φ80d*lac* type I (Fig. 1), the *lac* region replaces late genes of φ80; when this phage is inserted at the φ80 attachment region (att80), its *lac* operon is oriented in the same direction as the *trp* operon. In φ80d*lac* type II, (Fig. 2), the *lac* region replaces early genes of φ80; when this phage is inserted at *att*80, its *lac* operon is oriented in the opposite direction for the *trp* operon. The close proximity of the *lac* and *trp* operons in these strains has, in the following way, allowed isolation of deletion mutations which fuse the two operons. A locus (*tonB*) determining the sensitivity of *E. coli* to bacteriophage T1 lies between *att*80 and *trp* (27), and selection for T1^r mutations in φ80 lysogens often results in deletions that enter prophage φ80 on one side or the *trp* operon on the other, or both (6). In lysogens carrying either type of φ80d*lac*, *tonB* deletions often enter the *lac* operon of the transducing phage on one side and the *trp* operon on the other, thereby fusing the two operons (4). In this paper we present detailed studies on the isolation and properties of such fused operons.

MATERIALS AND METHODS

Strains. X-5097 is an F$^-$Sm^r strain which carries the *lac-proA,B* deletion, X111. X-5131 is a φ80d*lac* type I derivative (Fig. 1) of this strain and X-5144, a φ80d*lac* type II derivative (15, Table 9, Fig. 2). X-7700 is an *ara*$^-$ derivative of X-5131. *Lac z15* is described by Beckwith (2); *lac y1092* was obtained from M. Malamy (15); and the *lac z* mutations, U118, U131, X90 (19), and *lac i694*(i^s), were obtained from F. Jacob. The *trp*$^-$ mutations and the *trpR*$^-$ mutation were obtained from C. Yanofsky (9, 30). Other strains are described by Reznikoff et al. (24).

Media. Media are described elsewhere (8, 24).

Chemicals. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside(XG) was obtained from Cyclo Chemical Corp. This compound, which is a substrate but not an inducer of β-galactosidase at the concentration used, is colorless. When it is hydrolyzed, two indolyl moieties associate spontaneously to produce the insoluble colored dye, indigo. Amino acids were obtained from Calbiochem, acetyl coenzyme (CoA) from Pabst Laboratories, dithiobisnitrophenol and 3-indole propionic acid from Eastman Organic Chemicals, and isopropyl-β-D-thiogalactoside (IPTG) lysozyme and deoxyribonucleic acid (DNA) from Mann Research. Chorismic acid was prepared by the method of Gibson (7).

Lysate preparation and selection for T1^r mutations. These procedures are described in great detail by Gottesman and Beckwith (8).

Mapping. For mapping deletion ends within the

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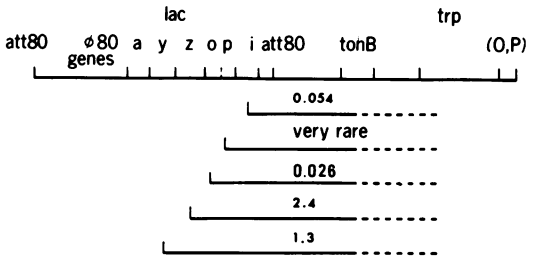

FIG. 1. *TonB-lac deletions in φ80dlac lysogen, type I. The numbers for each deletion class represent the average per cent of tonB mutations which were deletions of this type in the cultures analyzed. This per cent was calculated by multiplying the fraction of the lac⁻ colonies examined which belonged to the particular class by the average per cent of lac⁻ colonies (16%) in those cultures which were quantitated. There are two independent deletions which were assumed to end within the z gene, despite failure to recombine with lac z mutations, because of their ability to use melibiose as sole carbon source at 42 C (22). These are included in the 93 z deletions. The order of the trp promoter and operator has not yet been determined. The following abbreviations are used: lac—genes determining utilization of lactose; trp—genes determining the biosynthesis of tryptophan; tonB—locus governing sensitivity to bacteriophage T1; att80—chromosomal attachment site for bacteriophage φ80; col—colicinogenic factor.*

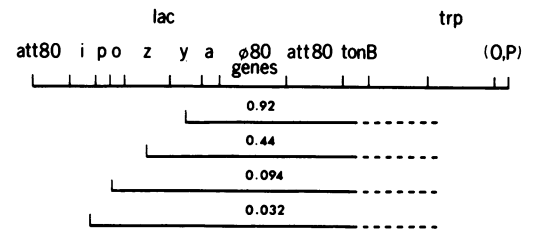

FIG. 2. *TonB-lac⁻ deletions in φ80dlac lysogen, type II. The average per cent of lac⁻ colonies in the cultures quantitated was 24%.*

lac operon, a drop of a donor culture (carrying a reference mutation on an F-*lac* episome) is mixed with a drop of the F⁻ *tonB-lac* derivative to be tested on lactose-minimal agar, containing citrate [required for growth of T1ʳ colonies on minimal agar (24)] and proline. The plates are scored after 2 to 3 days for *lac*⁺ recombinants. *Lac*⁻ derivatives of X-7700 were mapped with the *z*⁻ mutants described above.

The F-*trp* episomes carrying the various *trp*⁻ point mutations were obtained and used for mapping *tonB-lac*⁻-*trp*⁻ deletions of X7700 as previously described (24). The *trp* ends of the *tonB-i*⁻-*trp*⁻ deletions from X7700 and the *tonB-lac*⁻-*trp*⁻ deletions from X-5144 were determined by complementation with φ80 transducing phages carrying different portions of the *trp* operon.

The *tonB-i*⁻ deletions were tested for recombination with the *i* mutant, 694(iˢ), in the following way. Diploids of the type F-*lac*⁻(i694)*proA,B*⁺/Δ (*lac-pro*)X111⁻ *tonB-i*⁻ were first constructed. Cultures of these *lac* diploids were spread on lactose minimal agar, and after 2 days the plates were screened for *lac*⁺ recombinants. Since the *i*ˢ mutation reverts to *lac*⁺ character (*i*⁻ or *o*ᶜ) at an appreciable rate (24), a number of potential *lac*⁺ recombinants were tested on XG glucose-minimal agar to insure that they were not constitutive revertants. Constitutive revertants show up as deep-blue colonies on this medium.

Construction of trpR⁻ derivatives of fusion strains. First, an Hfr Cavalli carrying the *trpR*⁻ mutation was constructed as follows. Hfr Cavalli *met*⁻*trpR*⁺*str*ˢ was mated with F⁻*trpR*⁻*cysB*⁻*str*ˢ for 3 hr, and the mixture was plated out on glucose minimal agar. The only cells which will grow on glucose minimal plates after this cross are recipients which have received the *cys*⁺ allele from the Hfr. Since the *cysB* marker is donated very late by Hfr Cavalli, some of the *cys*⁺ recombinants should also have received the Hfr origin. Fifteen per cent of the *cys*⁺,*met*⁺ colonies were shown to be Hfr strains by their ability to donate the marker *lac*⁺ to a *lac*⁻ recipient. About half of these were still 5-methyltryptophan resistant, indicating they still had the *trpR*⁻ character. One of these (D-7011) was used in crosses to introduce the *trpR* mutation into fusion strains.

Since the fusion strains derived from X-7700 carried an *ara*⁻ mutation, and since the *ara* locus and the *trpR* gene are closely linked, the *trpR*⁻ mutation could be easily crossed into these fusion strains with D-7011. Bacterial matings were done between D-7011 and these fusion strains; *ara*⁺ recombinants were selected, counterselecting with streptomycin; and *trpR*⁻ derivatives were detected by screening for the 5-methyltryptophan-resistant character. To use the same technique with X-8511 (*see* Fig. 4), this strain was first crossed with an *ara*⁻, Hfr Hayes, and an *ara*⁻ derivative of X-8511 was isolated by screening on arabinose MacConkey Agar.

Assays: extract preparation. A 100-ml amount of exponentially growing cells (in phosphate buffer minimal media, containing 5 μg of thiamine per ml and 0.2% glycerol) was spun down in a centrifuge. The cells were washed with 10 ml of tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.5, 0.05 M), centrifuged, and concentrated to a 1-ml volume in the same buffer. Lysozyme (250 μg/ml) was added to the cells; the mixture was maintained for a few minutes at room temperature. Deoxyribonuclease (50 μg/ml) in 0.005 M $MgCl_2$ was added; the cells were then frozen in a methanol-dry ice bath. They were quickly thawed at 37 C, and the freeze-thaw procedure was repeated. Cell debris was centrifuged at 12,000 × *g* for ca. 45 min. For assays done under more carefully controlled conditions (Table 1), the strains were grown in 1% glucose with excess tryptophan (100 μg/ml). The bacteria were concentrated as previously described and sonically oscillated.

lac Enzymes. β-Galactosidase was assayed by the method of Pardee et al. (21). Thiogalactoside transacetylase was assayed by a procedure developed by M. Malamy (*personal communication*). To 25 μliters of

TABLE 1. *Control of transacetylase in lac-trp fusions, type I*[a].

Strain	Anthranilate synthetase		Transacetylase		Genes in which deletions end	
	trpR$^+$	*trpR*$^-$	*trpR*$^+$	*trpR*$^-$	*trp*	*lac*
X-7700	0.003	0.47	0.72	0.76	Unfused[b]	Unfused[b]
593	Low	High	Low	High	A	Y
5	Low	High	Low	Low	B	Y
23	Low	High	Low	High	B	Z
106	Low	High	Low	High	B	Z
330	Low	High	Low	High	B	Z
471	Low	High	Low	High	B	O
248	Low	High	Low	High	B	Z
484	Low	High	Low	Low	C	Z
321	Low	High	Low	High	D	Y
549	Low	High	Low	High	D	Y
205	Low	High	Low	High	D	Y
345	Low	High	Low	High	D	Y
28	0.01	1.00	0.0007	0.0031	E	Y
287	0.02	0.97	0.001	0.040	E	Z
291	0.004	1.02	0.002	0.41	E	Z
293	0.006	0.49	0.001	0.13	E	Z
425	0.013	1.12	0.009	0.93	E	Z
461	0.011	0.58	0.002	0.07	E	Z
594	0.007	1.24	0.001	0.20	E9851$^-$	Z
650	Low	High	Low	High	E9851$^-$	Z
677	Low	High	Low	High	E9851$^-$	Z

[a] All strains with intact *trpD* and *trpE* genes were assayed for anthranilate synthetase under repressed and derepressed conditions to insure that the method of derepression of the *trp* operon was working. In the case of mutants carrying deletions which extend into *trpD* or beyond, an F *trp* (*colV, B*) episome (24) was introduced into the strain, and the effects of derepression were assayed on the episomally controlled anthranilate synthetase. In all cases except strains 549, 650, and 677, *trpR*$^+$ and *trpR*$^-$ derivatives were assayed. For these three strains, derepression with indoleproprionic acid was used. The strains for which values are presented were assayed under more carefully controlled conditions than the others. These values represent the average of two assays with less than 10% error. The levels of the *trp* enzymes are normalized to a control strain carrying a *trpE-laci* deletion, W3 (24), with a *trp* episome. The levels of the *lac* enzymes are normalized to 3.300 (an *i*$^-$ wild-type strain), grown in glycerol-minimal medium. The low-high comparison indicates a derepression ratio of at least 20. In the cases of strains 5 and 484, the derepression ratio was at least 10, but levels in the *trpR*$^+$ derivatives were difficult to measure accurately.

[b] With isopropyl-β-thiogalactoside.

reaction mixture A (1 M IPTG; 0.01 M acetyl-CoA; 0.01 M ethylenediaminetetraacetic acid; in 0.1 M potassium phosphate buffer, *p*H 7.0) on a parafilm square is added 10 μliters of extract which has been heated for 5 min at 70 C, clarified by centrifugation, and diluted appropriately. This 35-μliter volume is then taken off the parafilm in a 50-μliter microcap, one end is plugged with plasticene, and the weighted capillary is dropped into a bath at 37 C and allowed to incubate for at least 20 min. After incubation, the capillary is removed from the bath, and the contents are blown into a 2-ml volume of dithiobisnitrophenol, 0.0001 M in 0.005 M Tris (*p*H 7.8). The yellow color is allowed to develop for 15 min and is read within 2 hr before fading begins. A blank extract from a *lac* deletion strain is used as a reference. Units are expressed as change in absorbancy at 412 nm per minute per mg of protein.

Trp enzymes. Anthranilate synthetase was assayed by measuring the appearance of anthranilic acid in a Turner model 111 recording spectrofluorometer. Procedure is as reported by Bauerle and Margolin (1) for assaying component 1. Units are expressed as increase in Turner units per minute per milligram of protein. The procedure for assaying phosphoribosyl transferase is also that of Bauerle and Margolin (1), and the same units are used. Indoleglycerol phosphate synthetase was assayed by the method of Ito and Crawford (11). Units are expressed as the change in absorbancy at 290 nm per minute per milligram of protein × 10^4. Tryptophan synthetase, B subunit was assayed by the method of Smith and Yanofsky (28). Units are expressed as the change in absorbancy at 540 nm per minute per milligram of protein × 10^4.

Protein. Protein was measured by the Folin method (14).

RESULTS

Deletions from type I strains: deletions with ends in the lac operon. The strain (X-7700) in which these deletions were selected is *ara*$^-$, *lac*$^+$

(ϕ80d*lac* lysogen, type I), *trp*$^+$; it carries a single deletion mutation which covers the *lac*, *proA* and *proB* genes at the normal *lac* region. This deletion eliminates all of the homology between this region and the chromosomal region carried by the ϕ80d*lac* (26). For selection of deletions into the *lac* region, strain X-7700 was treated with a combination of lysates of colicin V and ϕ80 virulent and spread on *lac* MacConkey Agar; all colonies which appear after this treatment carry mutations in the *trp*-linked *tonB* locus.

One-hundred-ninety-four cultures from independent clones of X-7700 were treated in this way to select T1^r mutants. For each culture, up to 4 *lac*$^-$ colonies were picked and purified. The frequency of *lac*$^-$ colonies measured in 52 of these cultures varied from 0 to 95% of the total T1^r colonies with a mean at about 4%. A very small fraction of *tonB-lac*$^-$ colonies might have resulted from *tonB* mutations in cells which had already been spontaneously cured of the prophage. The average fraction of cured colonies from cultures of both X-7700 and X-5144 is about $\frac{1}{5,000}$.

Lac end point. Of the 621 *lac*$^-$ isolates tested, 147 recombined with the *y*$^-$ mutation furthest from the operator, 1092, and were studied further. Crosses with *z*$^-$ point mutants showed that 93 of these isolates were deletions ending in the *z* gene, and 53 were deletions ending beyond the last *z*$^-$ mutant tested (i.e., probably in the *y* gene; Fig. 1). One deletion, 471 (X-7713), recombined with all of the *z*$^-$ mutants tested; it also had a low constitutive level of β-galactosidase activity (about 3% of a fully induced wild-type strain). This deletion, which appears to end between the *lac* operator and the beginning of the *z* gene, is described in detail elsewhere (5, 23). The average distribution of deletion end points is shown in Fig. 1.

The 147 deletions that end before *lac y1092* come from 70 different cultures. In three cases, two demonstrably different deletions came from the same culture. Thus, at least 73 of the *tonB* deletions that end within the *lac* operon are independent.

The deletions that do not recombine with *lac y1092* either stop at a point further in the *lac* operon or cover the entire locus.

Trp-end point. The orientation of *trp* genes is shown in Fig. 3. Of the 73 *lac*$^-$ deletions known to be independent, 30 are *trp*$^-$ and therefore extend either into or beyond the *trp* operon. Only 1 of the 70 cultures tested gave 2 deletions ending in *lac*, one of which was *trp*$^+$ and one *trp*$^-$. (This culture is one of the three mentioned in the preceding paragraph.)

The *trp* end point of these deletions was determined by complementation tests with F-*trp* episomes carrying point mutants in the different *trp* structural genes. One deletion ends in the *trpA* gene, six in the *trpB* gene, one in the *trpC* gene, and four in the *trpD* gene. Of the remaining 18 which do not have an active *trpE* gene as measured by complementation, 6 recombine with the early *trpE* point mutant 9851; 3 others were shown by physiological tests described below to retain intact at least the *trp* controlling elements, *p* and *o*, if not a portion of the *trpE* gene. Thus, of 73 *tonB* deletions which end within the *lac* operon, 21 have their other end within the *trp* operon.

Deletions with ends in the lac i gene. We can screen for *tonB* deletions which have one end in the *i* gene in strain X-7700 by using the sensitive *lac* indicator dye XG. On minimal agar plates containing XG and a carbon source such as glucose, colonies of *i*$^-$ bacteria are deep blue, whereas colonies of *i*$^+$ bacteria are very pale blue. Two-hundred-sixty-five cultures from independent clones of strain X-7700, treated to select T1^r cells, were spread on minimal glucose XG agar. An average of 0.054% of the colonies from these cultures were deep blue. Forty-four *tonB-i*$^-$ isolates, each from an independent culture, were purified and assayed for their levels of β-galactosidase. All but 1 of the 44 had the same levels of activity as wild type. The exception (strain X-8605) which had 0.4% of wild-type activity removes the entire *i* gene and at least part of the *lac* promoter (18).

Twelve of the 44 *tonB-i*$^-$ deletions were crossed with an *i* gene mutation, *i694*. Six of the 12 recombined with this mutation.

Of the 44 *tonB-i*$^-$ deletions, 19 were *trp*$^-$. Of these 19, 7 were shown by complementation tests to end within the *trp* operon.

Properties of type I fusions. All of the deletions from strain X-7700 which are *lac*$^-$ remove the controlling elements *p* and *o*. We shall consider the properties of those *lac*$^-$ deletions which have one end within the *lac* operon and the other end within the *trp* operon. Since the *lac* controlling

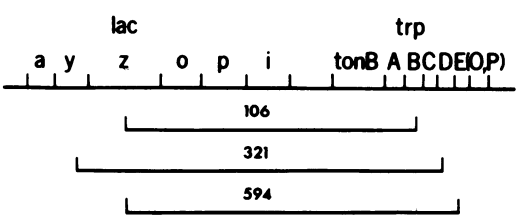

FIG. 3. *Fusions of the lac and trp operons from ϕ80dlac lysogens, type I. Representative deletions have been selected for this figure. For properties of the deletion strains, see Table 1.*

elements are removed and the *lac* structural genes effectively fused to the *trp* operon (Fig. 3), the remaining *lac* enzymes should come under the control of the *trp* regulatory gene, *trpR*. To test this prediction, we have derepressed the *trp* operon in these strains either by adding to growth media the *trp* operon inducer, indolepropionic acid (IPA; 19), or by introducing into the strains by recombination a constitutive allele of the *trpR* gene (*trpR*$^-$). Derepression of the *trp* operon always resulted in an increase in activity of the *lac* enzyme thiogalactoside transacetylase. The quantitative results for certain of the *trpE-lac* fusions are presented in Table 1. In addition, derepression of the *trp* operon increased the β-galactosidase activity of deletion strain 471, which, as noted earlier, has low levels of this enzyme (5, 23).

In some of the 12 *trp*$^-$-*lac*$^-$ deletions which do not have an active E gene and do not recombine with the *trpE 9851* mutation, the deletion might have cut out part of the *trpE* gene but not the *trp* operon controlling elements. Such strains should also exhibit tryptophan control of the remaining *lac* enzymes. Addition of IPA to the growth medium of 2 of the 12 strains resulted in a considerable increase in the level of thiogalactosidase transacetylase activity. In a third strain, the introduction of the *trpR*$^-$ mutation had the same effect (Table 1). The transacetylase derepression ratios in these *trpE*$^-$-*lac*$^-$ deletion strains were comparable to the anthranilate synthetase derepression ratios in these same strains. (The anthranilate synthetase was coded for by a *trp* episome carried in these strains.) Thus, these three strains must still carry intact *trp* controlling elements.

Deletion mutants which fuse the *i* gene to the *trp* operon leave the *lac* controlling elements intact. As might be expected, the levels of the *lac* enzymes are affected little or not at all by derepression of the *trp* operon (17, 24).

Deletions from type II strains. These deletions were isolated in a strain (X-5144) which is *ara*$^+$, *lac*$^+$, φ80d*lac* lysogen (type II), and is otherwise identical to X-7700. Up to 8 *tonB-lac*$^-$ colonies from each of 195 independent cultures were picked and purified. The frequency of *lac*$^-$ mutants among *tonB* mutants in 140 cultures varied from 0 to 100% of the total *tonB* mutants, with a mean of 12.5%.

Lac end point. In strain X-5144, the *i* gene is the furthest *lac* gene from the *tonB* locus (Fig. 2). To test for the presence of a remaining portion of the *lac* region in these deletions, each *lac*$^-$ was crossed by spot test with the mutation *lac i694* (which is in the *i* gene but gives a *lac*$^-$ phenotype), and *lac*$^+$ recombinants were scored. Of the 765 *lac*$^-$ colonies tested, in this way, 46 gave *lac*$^+$ recombinants. These were isolated from 16 of the 195 cultures treated. These deletions were mapped more extensively by crossing with a series of mutations in the *p* and *o* region and the *z* gene (see also 10, 17). By these tests, 28 deletions end in the *y* gene, 14 in the *z* gene, 3 in the *o-p* region and one in the *i* gene.

Trp end point. Thirty one of the 46 *tonB-lac*$^-$ deletions were also *trp*$^-$. However, of these, only 10 could be shown by complementation tests to end within rather than beyond the *trp* operon. Eight of the ten are isolates from one culture and appear to be repeats of the same deletion (X-8511); the other two (X-8504 and X-8508) come from independent cultures (Fig. 4). Deletion X-8511 has one end in the *trpA* gene, deletion X-8504 in the *trpD* gene, and deletion X-8508 in either *trpB* or *trpC*.

Properties of type II fusions. Deletions X-8511 and X-8504 remove the operator-distal ends of both the *lac* and *trp* operons but leave the controlling elements of both operons intact. In strain X-8511, derepression of the *trp* operon has very little or no effect on the basal or induced level of β-D-galactosidase, whereas induction of the *lac* operon has no effect on the basal or derepressed levels of the *trp* enzymes (Table 2).

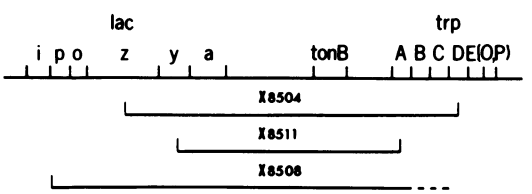

FIG. 4. *Fusions of the lac and trp operons from φ80dlac lysogens, type II. Detailed mapping of X-8504 and X-8508 is presented elsewhere (10, 18).*

TABLE 2. *Properties of type II fusions*[a]

Strain	AS-ase	PRT-ase	IGPS-ase	TS-ase B	β-Galac-tosidase
X8511	1.39	ND[b]	2.2	9.8	23
X8511 + IPTG[c]	1.25	ND	1.3	7.54	17,600
X8511 *trpR*$^-$	120	48.4	57.7	202	14
X8511 *trpR*$^-$ + IPTG	139	53.2	66.6	217	15,300

[a] Abbreviations: ASase, anthranilate synthetase; PRTase, phosphoribosyl transferase; IGPS, indoleglycerol phosphate synthetase; and TSase B, tryptophan synthetase, B subunit. Values are averages of 4 assays for X8511 and 10 assays for X8511 *trpR*$^-$. The *trp* gene specifying each enzyme is as follows: E, ASase; D, PRTase; C, IGPSase; B, TSase B.

[b] Not done.

[c] Isopropyl-β-thiogalactoside.

DISCUSSION

The transposition of *lac* genes to the *trp* region of the chromosome provides a powerful tool for analyzing the structure of operons. By selecting for mutations in the *tonB* locus, we have isolated deletions which have one end in both the normally oriented and inverted *lac* operons. In this paper, the deletions were selected in strains which are $trpR^+$, repressed for the *trp* operon. Elsewhere, we described selection of deletions in strains which are $trpR^-$ and carry *lac* promoter mutants (24). The combination of the two selections has allowed us to select deletions which fuse the *lac* and *trp* operons in many ways. Studies of these deletions have led to the following information on the *lac* region. (i) The *lac* promoter is located between the *i* gene and the operator (10, 18); (ii) the *i* gene is transcribed in the same direction as the *lac* operon (17); (iii) the *lac* operator is not part of the structural gene for β-galactosidase (5, 23); (iv) a messenger ribonucleic acid stop signal may exist at the end of the *i* gene nearest the *lac* promoter (24).

As expected, all deletion strains isolated from type I strains which fuse the *z* or *y* genes to the tryptophan operon result in control of the remaining *lac* genes by the *trp* repressor. The amount of thiogalactoside transacetylase produced in these strains under conditions of *trp* depression varies widely. In three exceptional cases, there is only very little transacetylase made. This effect might be due in part to polarity caused by out-of-phase deletions. Jacob et al. (12) have described similar fusions of the *lac* operon to the *purE* operon. Such fusions result in the control of the remaining *lac* genes by the concentration of purines in the cell.

Three deletions (594, 650, 677) which remove all of the *trpE* sites tested and end in the *lac z* gene still show normal control of transacetylase by the *trp* repressor. Since the *trp* controlling elements must, therefore, be still intact in these strains, this finding indicates that the *trp* operator is either close to the right end of the *trpE* gene or lies outside of it. Such fusions thus provide a means of analyzing the *trp* controlling elements.

Deletions isolated from type II strains (*lac* and *trp* antiparallel) remove the terminal portions of both the *trp* and *lac* operons. In these strains the two operons retain their controlling elements intact and are transcribed from opposite strands of the DNA. Transcription initiated at one operator might continue on the antisense strand into the other operon and conceivably interfere with expression of the latter. However, we fail to see any such interference in one such strain, X-8511. It is possible that transcription and translation from the antisense strand results in an early termination of one or both processes. In a different system involving a similar fusion of the histidine and rough B operons, effects were observed on expression of one operon by derepression of the other (13).

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