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LETTERS TO THE EDITOR

Genetic Evidence that the Operator Locus is Distinct from the *z* gene in the *lac* Operon of *Escherichia coli*

The order of genes and controlling elements in the *lac* region of the *Escherichia coli* chromosome is *i-p-o-z-y-a* (Fig. 1), (Ippen, Miller, Scaife & Beckwith, 1968; Miller, Ippen, Scaife & Beckwith, 1968), where *i* is the structural gene for the repressor; *p*, the promoter region in which the initiation of transcription of the genes of the *lac* operon takes place; *o*, the operator site where repressor binds to prevent transcription, and *z*, *y*, and *a*, the genes of the *lac* operon which code for the structure of β-galactosidase, galactoside-permease (or M-protein) and thiogalactoside transacetylase, respectively. The location of mutants of the operator (O^c mutants) in the region of the N-terminal end of the *z*-structural gene raises the possibility that, in addition to acting as a binding site for the repressor, the operator also codes for an N-terminal portion of β-galactosidase. The main piece of evidence against this possibility comes from studies on an O^c mutant, O^c_{67} (Steers, Craven & Anfinsen, 1965). However, at

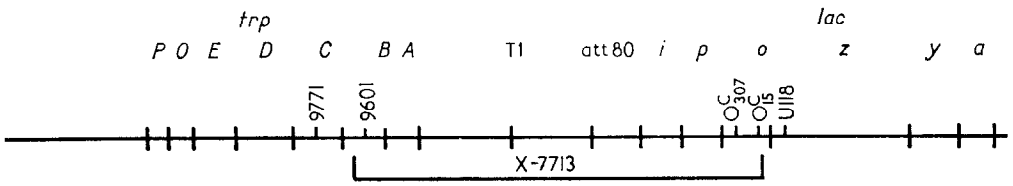

FIG. 1. A deletion which fuses the *lac* and *trp* operons

We have made the assumption in this Figure that the order of *p* and *o* is the same in the *trp* operon as it is in the *lac* operon. The mutants U118 (Newton, Beckwith, Zipser & Brenner, 1965; Schwartz & Beckwith, 1969), O^c_{307} and O^c_{15} were obtained from F. Jacob. The *trp*$^-$ mutants, *B9601* and *C9771* (Yanofsky & Ito, 1966) were obtained from C. Yanofsky. The techniques for mapping *trp*$^-$ point mutants will be described elsewhere (Reznikoff *et al.*, manuscript in preparation).

the time this mutant was analyzed, it was thought to belong to a class of operator deletions which extended into or beyond the *i* gene (Jacob, Ullman & Monod, 1964). Recent evidence indicates that these mutants are not deletions, but are either dominant i^- mutations or i^-O^c double mutants (Müller-Hill, Crapo & Gilbert, 1968; Davies & Jacob, 1968). The mutant, O^c_{67}, falls in the latter class (Beckwith, unpublished results). Therefore, since O^c_{67} may only be a point mutant, the finding that it has no observable effect on the activity and certain other properties of β-galactosidase is not convincing evidence of the distinction of operator and *z* gene. Furthermore, O^c_{67} was selected for exhibiting constitutive levels of active β-galactosidase.

In this paper, we describe the isolation of a deletion which removes the *i* gene, the promoter and a substantial portion of the *lac* operator. Despite the removal of the *lac* operator, this strain can produce high levels of β-galactosidase, suggesting that the operator is not a part of the structural gene.

We have isolated this deletion from a strain (X7700) in which the *lac* operon is transposed in a *ϕ80dlac* lysogen (Signer & Beckwith, 1966; Beckwith, Signer & Epstein, 1966) to the att_{80} site near the tryptophan operon (*trp*) on the *E. coli* chromosome (Fig. 1). Between *lac* and *trp* is a locus determining sensitivity to the bacteriophages T1 and *ϕ*80 and colicins V and B. Selection for resistance to these agents often yields deletions with ends in the *trp* or *lac* operons, or in both (Beckwith *et al.*, 1966; Miller *et al.*, 1968; Miller, Beckwith & Müller-Hill, 1969; Reznikoff, Miller, Scaife & Beckwith, manuscript in preparation). We have already described the isolation of deletions from this strain with ends in the *i*, *z* and *y* genes and in the *p* region. If the operator is not part of the *z* gene, from such a strain we should also be able to isolate a deletion which removes *i*, *p* and *o*, but leaves the *lac* structural genes intact.

Three hundred cultures of independent colonies of X7700 have been treated with a mixture of *ϕ*80 virulent and colicins V and B and plated on MacConkey agar (a *lac* indicator medium) to select for $T1^R$ type mutations (Miller, Reznikoff, Silverstone, Ippen, Signer & Beckwith, manuscript in preparation). From each plate, several lac^- colonies (when they occur) are purified and tested to determine whether the $T1^R$-lac^- deletion has one end within the *lac* operon. In this way, at least 73 independent deletions of this type have been characterized. In all but one case, the deletions could be shown to end within the *z* or *y* structural genes by mapping with point mutants in these genes. One deletion, X7713, recombined with all point mutants tested, including the earliest known z^- mutant, U118, an ochre mutation (Table 1). This mapping suggests that the *z* gene may still be intact. Further indication of this possibility is the finding that X7713 still makes a low but measureable amount of *β*-galactosidase (Table 2).

TABLE 1

Frequency of wild-type recombinants with X7713

U118	$1{\cdot}2\times10^{-3}$
O^c_{15}	$<3{\cdot}0\times10^{-7}$
O^c_{307}	$<2{\cdot}6\times10^{-7}$

Recombination studies were carried out by constructing diploids of X7713 with F-*lac-proA, B* episomes carrying one of the three markers. Recombination frequencies were measured as described elsewhere and are presented as recombinants per diploid bacterium (Miller *et al.*, 1968). One of the four independent cultures of the U118 diploid examined for lac^+ recombinants had a jackpot of recombinants which raises the recombination frequency about 7 times over the average of the other three. Four independent cultures of the O^c_{15} and O^c_{307} diploids were scored for O^+ recombinants. For frequencies with deletions known to recombine with these O^c mutants, see Ippen *et al.*, (1968) and Miller *et al.* (1968). The *lac* mutants used are described in Miller *et al.* (1968).

The deletion, X7713, was tested further and found not to recombine with two O^c mutants, O^c_{307} and O^c_{15}, which are supposed to map at opposite ends of the operator (Table 1) (Davies & Jacob, 1968).

In addition to being lac^-, the deletion X7713 is also trp^-. Crosses with various point mutants of the *trp* operon indicate that the deletion removes most of the *trp B* gene. This deletion, then, appears to fuse the *lac* operon to the *trp* operon. To verify this suggestion, we have introduced into X7713 a $trpR^-$ mutation which causes a 30 to 50

TABLE 2

β-Galactosidase activities

	−IPTG	+IPTG
X7713 *trpR*$^+$	9	—
X7713-*trpR*$^-$	151	—
X7713-*trpR*/F*i*$^+$*z*$^-$	169	159
X7700/F′*i*$^+$*z*$^-$	0·4	1192

Cultures were grown in PB minimal medium (Reznikoff *et al.*, manuscript in preparation) with or without 10^{-3} M-isopropyl-β-D-thio-galactoside (IPTG) to a density of about 3×10^8 cells/ml. The enzyme was assayed by the method of Pardee, Jacob & Monod (1959). Enzyme units are O.D. 420 mμ − 1·75 × O.D. 550 mμ/min divided by the O.D. at 600 mμ of the culture, multiplied by 1000. At least three independent assays were done with each strain, with less than 5% average error. X7700 is the *lac*$^+$ parent of X7713-*trpR*$^+$. We do not believe that we are observing completely efficient *trp*-initiated transcription into *z*, since we have isolated other somewhat different fusion strains with approximately 3 times higher levels of β-galactosidase (Reznikoff *et al.*, manuscript in preparation). The lower level in X7713 may be due either to inefficient re-initiation of translation at *z* because of polar effect created by the deletion, or to some interference with translation of *z* because of translation intiated at *trp B*, or to both.

times derepression of the *trpE* and *D* genes and an approximately 15 times derepression of the *C*, *B* and *A* genes (Imamoto, Ito & Yanofsky, 1966; Morse & Yanofsky, manuscript in preparation). If the *lac* and *trp* operons are fused and the *z* structural gene is intact, we should see an increase in the rate of β-galactosidase synthesis. In fact, the derepression of *trp* by the *trpR*$^-$ allele, results in a 17 times increase in the rate of β-galactosidase synthesis (Table 2).

We have described elsewhere the isolation of fusion strains similar to X7713, except that in these other strains, the *lac* operator is intact (Reznikoff *et al.*, manuscript in preparation). In these strains, the *lac* repressor markedly blocks the *trp*-initiated reading of the *lac* operon. However, in the *trpR*$^-$ derivative of strain X7713, there is absolutely no effect of *lac* repressor on β-galactosidase synthesis (Table 2). This result provides further indication of the deletion of the *lac* operator.

The deletion in the fusion strain X7713 removes a substantial portion of the *lac* operator and fuses it to the *trp B* gene. If the operator were part of the *z* structural gene, we would expect to see no β-galactosidase activity, unless the *o* region coded for an inessential portion of the enzyme. In fact, substantial levels of β-galactosidase are made in the *trpR*$^-$ derivative of X7713. It should be remembered that X7713 was isolated as a *lac*$^-$ derivative of X7700. Since there was no selection for a functioning β-galactosidase molecule, it cannot be argued that we have selected for a very special kind of deletion.

These results suggest that the *lac* operator is not part of the *z* structural gene. Further evidence in support of this suggestion is presented in the accompanying paper by Bhorjee, Fowler & Zabin, 1969. The location of the promoter between *i* and *o* indicates that the operator is probably transcribed (Ippen *et al.*, 1968). However, since the known function of the operator (repressor binding) (Gilbert & Müller-Hill, 1967; Riggs, Bourgeois, Newby & Cohn, 1968) does not require translation, and since *o* does not appear to be part of the *z* gene, there is no reason now to believe that *o* is translated at all.

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