The author is a Postdoctoral Fellow of the United States Public Health Service. All *lac*⁻ mutants were kindly supplied by Dr. F. Jacob.

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## Restoration of operon activity by suppressors

The genes clustered in the *lac* region of the *Escherichia coli* chromosome are under the control of a single operator region adjacent to the gene for β-galactosidase (EC 3.2.1.2). $o^0$ mutants, located in this region, shut off the activities of all three genes in the operon, β- galactosidase (*z*), permease (*y*), and galactoside transacetylase[1]. It has been shown that these mutants, including the one described below, do not produce any detectable messenger-RNA from the operon[2]. Since according to this result there are no cytoplasmic gene products from the operon in mutants of this type, the ways in which reversion to the $lac^+$ state can occur should be limited. It is the purpose of this note to describe experiments which test this expectation by determining the nature of revertants of such a mutant.

We have previously reported the isolation of phenotypically $z^+y^+$ revertants of an $o^0$ mutant for the lactose operon[3]. The isolation and characterisation of a new type of revertant of this same mutant is reported here. By the use of melibiose, an α-galactoside which requires permease but not β-galactosidase for its metabolism[4], it was possible to select for phenotypically $z^-y^+$ revertants. The $o^0$ mutant used, 2320, was an $i^-$ strain (produces no repressor) obtained from Dr. F. JACOB. More than 50 % of the revertants isolated using melibiose produced little or no β-galactosidase. These phenotypically $z^-y^+$ revertants are not the result of a mutation at another locus producing a new type of permease, since mutants deleted in the *lac* region do not revert in this manner. After purification, the revertants were assayed for permease by a new method involving the measurement of *o*-nitrophenyl-β-D-galactoside uptake. This method will be described in detail elsewhere[5]. In Table I are listed some of the properties of the "$z^-y^+$" revertants which were isolated. The very occurrence of this class of revertant indicates that they are not true back-mutants and that suppressors are involved.

Genetic studies were carried out to determine whether any of the suppressors in these revertants were located outside the *lac* region. In interrupted mating experiments, various wild-type Hfr donors were mated with these $F^-$ revertants. The

TABLE I

CHARACTERISTICS OF $z^-y^+$ REVERTANTS OF 2320

All activities and the levels of CRM production are in terms of % of the amount produced by a fully constitutive strain, 33co.

| *Revertant number* | *β-Galactosidase* | *Permease* | *CRM* production* | *Generation time** (min)* |
|---|---|---|---|---|
| M12 | 0 | 72 | 0 | 80 |
| M15 | 0 | 91 | 10 | 80 |
| M22 | $3 \cdot 10^{-3}$ | 2 | 0 | 85 |
| M31 | $3 \cdot 10^{-3}$ | 18 | 0 | 95 |
| M32 | $3 \cdot 10^{-3}$ | 5 | — | 80 |
| M35 | $3 \cdot 10^{-3}$ | 0–1 | — | — |
| M41 | 0 | 26 | — | 80 |
| M42 | $3 \cdot 10^{-3}$ | 12 | 0 | 110 |
| M43 | $3 \cdot 10^{-3}$ | 7 | 0 | 105 |
| 2320 | $3 \cdot 10^{-3}$ | <1 | 0 | 80 |

* CRM, material cross-reacting with β-galactosidase antibody, was determined according to the method of M. Cohn and D. Perrin as described by MASTERS AND PARDEE[6].

** Generation times are in minutes on glycerol–minimal medium.

time of entry of the wild-type allele of the suppressor locus was determined by scoring the appearance of $su^0o^0$ recombinants (the original $o^0$ mutant). These melibiose$^-$ recombinants could be distinguished from the revertants themselves on tetrazolium plates. In addition to the "$z^-y^+$" revertants, three "$z^+y^+$" revertants[3] (28, 1.0 %; 26, 1.5 % and 16, 60 % β-galactosidase), were mapped by this technique.

The 10 revertants which were examined fell into 4 classes according to suppressor location (Fig. 1). 1, two of the "$z^+y^+$" strains, 26 and 28, carry suppressors near the

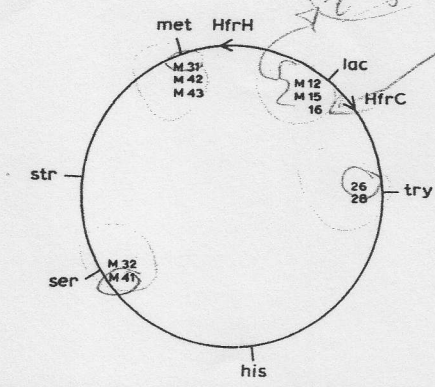


Fig. 1. Location of suppressors on *E. coli* chromosomal map. The map positions of the suppressors are rough estimates except for the three internal suppressors in the *lac* region.

tryptophan marker. 2, the suppressors in M32 and M41 map near the serine marker. 3, the wild-type suppressor loci for M31, M42 and M43 enter close to the methionine marker. 4, three suppressors, in M12, M15 and 16, are located within the *lac* region. None of these gave $su^0o^0$ recombinants with the Hfr donors used.

Two of this last group, M12 and M15, were studied further. Several Hfr strains carrying mutations either in the *z* or *y* cistrons were mated with M12 and M15, and

the frequency of $z^+y^+$ recombinants from the "$z^-y^+$" revertants was determined. By this method it was determined that the reversion in M15 is at the same site or very close to the $o^0$ mutation. In contrast, the reversion in M12 involves a deletion extending over a major part of the *z* region.

AMES *et al.*[7] have suggested two mechanisms for restoration of operon activity in suppressed $o^0$ mutants for the histidine pathway. They propose that in one revertant a deletion connects the inactivated histidine operon to an operator for another system, thus restoring its activity. In the second case, the defective operon appears to have been duplicated on an extrachromosomal fragment, again possibly coming under the control of a new operator. The revertants reported on in this note do not belong to either of these classes. M12 does not appear to carry a deletion extending out of the *lac* operon. It was found that when the heterogenote, $F^-i^+z^+y^-/i^-z^-y^+$, was formed by introducing an *F-lac* factor into M12, repression of the previously constitutive permease activity resulted. The repressor-sensitive region of the operon is thus probably still intact. It does not seem plausible that the $i^+$ repressor could act on the new operator for the *lac* region as would have to be supposed if the model of AMES *et al.* is valid for this system. Secondly, when an Hfr carrying a deletion of the *lac* region was mated with representatives of the three external suppressors, 26, M31 and M41, it was found that melibiose$^-$ (or *lac*$^-$) recombinants appeared immediately the lac region entered the recipient. If these suppressor *loci* were actually translocated and reactivated operons, such recombinants should not appear until after the wild-type "suppressor loci" have entered the cell. Thus, neither extended deletions nor translocations can be invoked to account for the various suppressors described here.

With these mechanisms excluded for restoration of operon activity, it becomes difficult to interpret the many suppressors in terms of the accepted model of gene control. Either the factors involved in action at the operator site are more complex than previously supposed, or new types of suppressors which affect the gene transcription process are involved.

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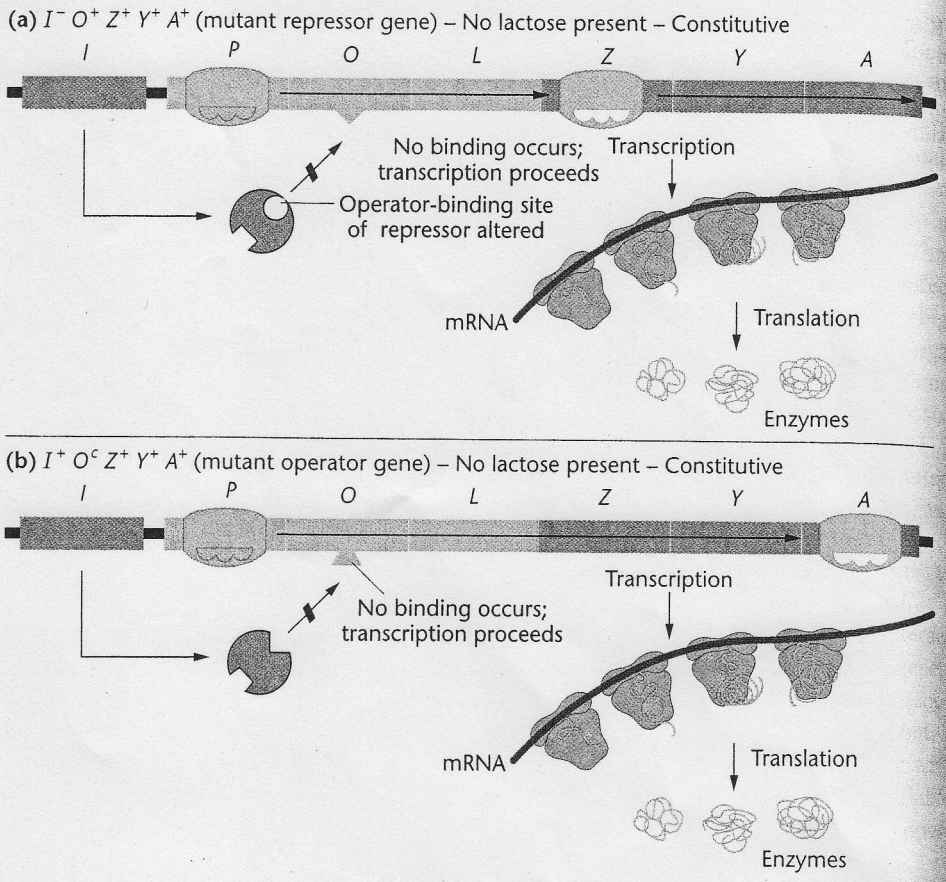


**FIGURE 17–6** The response of the *lac* operon in the absence of lactose when a cell bears either the $I^-$ or the $O^C$ mutation.

molecule, no direct chemical evidence was immediately forthcoming. A single *E. coli* cell contains no more than 10 or so copies of the *lac* repressor; direct chemical identification of 10 molecules in a population of millions of proteins and RNAs in a single cell presented a tremendous challenge.

**TABLE 17–1** A Comparison of Gene Activity (+ or −) in the Presence or Absence of Lactose for Various *E. coli* Genotypes

| Genotype | Presence of β-Galactosidase Activity: Lactose Present | Lactose Absent |
|---|---|---|
| $I^+O^+Z^+$ | + | − |
| A. $I^+O^+Z^-$ | − | − |
| $I^-O^+Z^+$ | + | + |
| $I^+O^cZ^+$ | + | + |
| B. $I^-O^+Z^+/F'I^+$ | + | − |
| $I^+O^cZ^+/F'O^+$ | + | + |
| C. $I^+O^+Z^+/F'I^-$ | + | − |
| $I^+O^+Z^+/F'O^c$ | + | − |
| D. $I^sO^+Z^+$ | − | − |
| $I^sO^+Z^+/F'I^+$ | − | − |

*Note*: In parts B to D, most genotypes are partially diploid, containing an F factor plus attached genes (F′).

In 1966, Walter Gilbert and Benno Müller-Hill reported the isolation of the *lac* repressor in partially purified form. To achieve the isolation, they used a *regulator quantity* ($I^q$) mutant strain that contains about 10 times as much repressor as do wild-type *E. coli* cells. Also instrumental in their success was the use of the gratuitous inducer, IPTG, which binds to the repressor, and the technique of **equilibrium dialysis**. In this technique, extracts of $I^q$ cells were placed in a dialysis bag and allowed to attain equilibrium with an external solution of radioactive IPTG, which is small enough to diffuse freely in and out of the bag. At equilibrium, the concentration of IPTG was higher inside the bag than in the external solution, indicating that an IPTG-binding material was present in the cell extract and that this material was too large to diffuse across the wall of the bag.

Ultimately, the IPTG-binding material was purified and shown to have various characteristics of a protein. In contrast, extracts of $I^-$ constitutive cells having no *lac* repressor activity did not exhibit IPTG-binding activity, strongly suggesting that the isolated protein was the repressor molecule.

To confirm this thinking, Gilbert and Müller-Hill grew *E. coli* cells in a medium containing radioactive sulfur and then isolated the IPTG-binding protein, which was labeled in its sulfur-containing amino acids. This protein was mixed with DNA from a strain of phage lambda (λ), which carries the $lacO^+$ gene. The DNA sediments at 40*S*, while the IPTG-binding protein sediments at 7*S*. The DNA and protein were mixed and sedimented in a gradient, using ultracentrifugation. The radioactive protein sediments at the same rate as