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SUPPRESSION OF A *LAC O°* MUTATION IN *ESCHERICHIA COLI*

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ABSTRACT

SCHWARTZ, NORMAN M. (Yale University, New Haven, Conn.). Suppression of a *lac o°* mutation in *Escherichia coli*. J. Bacteriol. **88**:996-1001. 1964.—A class of *pro⁻* markers which can be cotransduced with *lac* map close to the *i* side of the *lac* region. The relative order of markers transferred by Hfr H is *thr leu proA proB lac proC ade*. Nine of ten slow-growing lactose-utilizing revertants of a *lac o°* mutant are suppressed mutants. The time of entry of *su-lac o-5* from an Hfr H derivative is 27 min. *Su-lac o-5* is separated from the *lac* region by a 10-min interval. *Su-lac o-5* is unstable, restores 7% of the wild-type level of β-galactosidase activity, suppresses a *try⁻* mutation, and has a deleterious effect upon growth rate. The primary *lac o°* mutation is interpreted as being a *z⁻* polarity mutation which constitutes nonsense. *Su-lac o-5* could act at the level of messenger ribonucleic acid translation converting nonsense to sense by promoting mistakes in protein synthesis.

Jacob and Monod (1961*a*) proposed that an operator was the initiation point for transcription of structural genes into messenger-ribonucleic acid (m-RNA) and the site of repressor action. However, it was also recognized (Jacob and Monod, 1961*b*) that the *o* region was inseparable from the adjacent extremity of the *z* gene. *Lac⁺* revertants of a *o°* mutant produced altered β-galactosidase and resulted from secondary mutations close to the original *o°* site. In addition, polarity mutations randomly distributed throughout the *z* gene were presumed to reduce transcription of the *y* and *x* genes (Jacob and Monod, 1961*b*).

Suppressors of *o°* (Beckwith, 1963) and of a *z⁻* polarity mutation (Schwartz, 1963) have been described. Beckwith (1964) further demonstrated that *o°* mutations neither map within the repressor-sensitive site nor define a site essential for m-RNA transcription. *O°* mutations, therefore, appear operationally indistinguishable from severe polarity mutations within a structural gene (*see also* Ames and Hartman, 1963).

The purpose of this study was to examine the characteristics of suppressed *lac o°* mutants.

MATERIALS AND METHODS

Conjugation experiments were performed as described by Adelberg and Burns (1960). Interrupted mating experiments performed to time the entry of various markers were carried out by the method of de Haan and Gross (1962). The media and culture methods employed were described by Adelberg and Burns (1960). Lactose-utilizing revertants of *lac⁻* strains were maintained on minimal-lactose agar. When employed in mating experiments or used to propagate transducing phage, these revertants were grown to stationary phase in minimal-lactose medium and then grown to exponential phase after 1:20 dilution in nutrient broth. Transducing phage P1kc, obtained from C. Yanofsky, was used as described by Lennox (1955). All of the strains used are derivatives of *Escherichia coli* K-12 (Table 1). *Lac⁻* strains were classified as *y⁻*, *z⁻*, or *o°* from the results of sexductional analyses with F-*z⁻*/*z⁻*, F-*y⁻*/*y⁻*, and F-*o°*/*o°* merodiploids (Jacob and Wollman, 1961). The inductive capacity of 10^{-3} M isopropyl-β,D-thiogalactopyranoside upon β-galactosidase in *y⁻* strains (Pardee, Jacob, and Monod, 1959) further served to distinguish *y⁻* from *z⁻* or *o°* mutations.

RESULTS

Characteristics and construction of strains. Four independently isolated *pro* mutations (*pro-13*, *pro K4*, *pro-156*, and *pro-158*) are cotransduced with *lac* (Schwartz, 1963). Figure 1 gives the results of an interrupted mating experiment timing the appearance of markers: *leu⁺*, *lac y⁺*, *pro-13⁺*, *ade⁺*, *and try⁺* from Hfr H donor AB259 into F⁻ recipient strain 13-6. *Pro-13* is separated from *lac* by about 1 min and maps on the side of *lac* adjacent to *ade*. Similar results for the time of entry of *pro-K4⁺*, *pro-156⁺*, and *pro-158⁺* (with

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respect to *lac* and *ade*) are obtained in crosses with AB259. In an interrupted mating between the Cavalli Hfr and strain 13-6, *pro-13*$^+$ is transferred prior to *lac*$^+$. These results demonstrate that the relative order of these markers is: *leu . . . lac pro-ade . . . . . try*.

Hfr H strain 3106, carrying the *lac o°* mutation of F$^-$ strain 2.320, was constructed by cotransduction of *pro-13*$^+$ *lac o°* in the following manner. P1kc prepared on *pro-13 z*$^+$ *i*$^-$ strain 13-6-Y2 was used as donor for transduction of a *z*$^-$ derivative of Hfr H AB259. Many of the *lac*$^+$ transductants selected on minimal-lactose agar containing proline also inherited *pro-13* and *i*$^-$. One such transductant strain (31) was treated with P1kc grown on strain 2.320 (*pro-13*$^+$ *lac o°*). About 20% of the proline-independent transductants obtained also received *lac o°*. Strain 3106 is a stable *pro-13*$^+$ *lac o°* P1kc sensitive derivative of Hfr H AB259.

F$^-$ strain Y201, *leu*$^-$ *lac o°* *ade*$^-$ *try*$^-$ *str*^{r} was prepared by transducing strain 13-6-Y2 (*pro-13 lac*$^+$) with P1kc derived from strain 2.320 (*pro-13*$^+$ *lac o°*). About 20% of the selected *pro*$^+$ transductants inherited *lac o°*.

Reversions of lac o°. Hfr strain 3106 (*lac o°*) was treated with 0.1 M ethyl methanesulfonate (EMS) and plated on minimal-lactose agar (Schwartz, 1963). Ten slow-growing revertant colonies (eight EMS-induced and two of spontaneous origin) were picked and purified by streaking on EMB-lactose-agar.

Each of the ten revertants was mated with F$^-$ strain 13-6-Y2 (*lac*$^+$ *pro-13*) to test for suppressors of *lac o°*. In a control cross, with the parent *o°* Hfr, 83% of the *pro-13*$^+$ selected recombinants inherited the closely linked *lac o°* mutation (Table 2, control cross). In similar crosses with nine of ten revertants (crosses 2 to 10, Table 2), 69 to 85% of the *pro*$^+$ recombinants were *lac o°*. Therefore, these nine strains are suppressed *lac o°* mutants. One of the suppressed mutants, *lac o° su-lac o-5* (revertant 5, Table 2), was chosen for further study.

Characteristics of lac o° su-lac o-5. Su-lac o-5 is unstable, since cultures of the suppressed mutant segregate lactose-negative types even after the strain has been purified from single-colony isolates. The suppressor restores 7% of the wild-type level of β-galactosidase activity and has a deleterious effect on growth rate. The generation time of strain *lac o° su-lac o-5* is 63 min in nutrient broth. Strains 31 *lac*$^+$, 3106 *lac o°* (from which *lac o° su-lac o-5* was derived), and three lactose-negative segregants obtained from the unstable suppressed mutant have a 35-min generation time. Lactose-negative segregants produced by the unstable suppressed mutant form larger colonies than does the lactose-utilizing suppressed strain on EMB-lactose-agar.

Ten *lac*$^-$ segregants obtained from *lac o° su-lac o-5* have the characteristics of *lac o°* in transduction crosses with eight *lac z*$^-$ strains (and *lac o°*). The *lac*$^+$ reversion pattern of these ten segregants is similar to *lac o°*, and it is likely that these *lac*$^-$ segregants carry the original *lac o°* point mutation.

TABLE 1. *List of strains of Escherichia coli**

Strain	Pertinent characteristics	Source and derivation
AB259	Hfr Hayes	3.000, Pardee
W1895	Hfr Cavalli	J. Lederberg
13-6	F$^-$ *str*^{r} *leu*$^-$ *lac y*$^-$ *pro-13 ade*$^-$ *try*$^-$	13 (Schwartz, 1963)
13-6-Y2	F$^-$ *str*^{r} *leu*$^-$ *lac*$^+$ *i*$^-$ *pro -13 ade*$^-$ *try*$^-$	13-6 × P1-3.300 (*lac*$^+$ *i*$^-$)
K4	F$^-$ *str*^{r} *thr*$^-$ *leu*$^-$ *lac z*$^-$ *pro-K4 ade*$^-$ *try*$^-$ *met*$^-$	Schwartz, 1963
156-5	F$^-$ *str*^{r} *leu*$^-$ *lac y*$^-$ *pro-156 ade*$^-$ *try*$^-$	156, R. Curtiss III
158-1	F$^-$ *str*^{r} *leu*$^-$ *lac y*$^-$ *pro-158 ade*$^-$ *try*$^-$	158, R. Curtiss III
2.320	F$^-$ *lac o°*	F. Jacob
31	Hfr Hayes, *lac*$^+$ *i*$^-$ *pro-13*	AB259 *lac z-6* × P1-13-6-Y2
3106	Hfr Hayes, *lac o°*	31 × P1-2.320
Y201	F$^-$ *str*^{r} *leu*$^-$ *lac o° ade*$^-$ *try*$^-$	13-6-Y2 × P1-2.320

* The following gene symbols are used in the table and the text: *thr*, threonine; *leu*, leucine; *lac*, lactose; *pro*, proline; *ade*, adenine; *try*, tryptophan; *met*, methionine; and *str*, streptomycin. Superscript − and + indicate mutant and wild-type alleles, respectively; superscript r and s designate resistance and sensitivity to streptomycin. Symbols for the genes of the lactose operon, *x*, *y*, *z*, *o*, and *i*, are used as suggested by Jacob and Monod (1961*b*). Different *pro*$^-$ mutations are distinguished by a strain number, identifying the strain in which that mutation was first isolated. For example, *pro-13* is the mutation resulting in the proline requirement of strain 13. P1 indicates transducing phage P1kc, and the notation P1-2.320 signifies P1kc propagated on bacterial strain 2.320; 31 × P1-2.320 symbolizes transduction of bacterial strain 31 by P1kc propagated on bacterial strain 2.320.

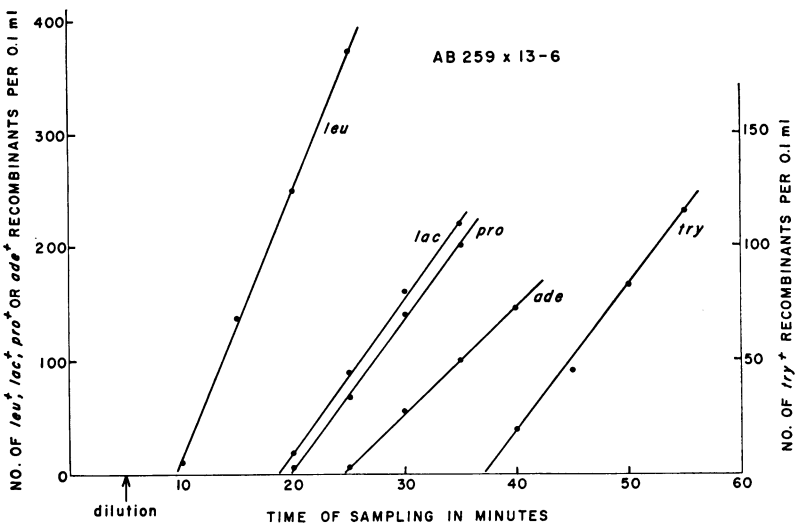

FIG. 1. *Transfer curves for leu$^+$, lac$^+$, pro$^+$, ade$^+$, and try$^+$ markers in a cross between prototrophic str^s AB259 (Hfr H 3.000) and F$^-$ 13-6: leu$^-$ lac$^-$ pro$^-$ ade$^-$ try$^-$ str^r. Exponential-phase broth-grown cultures of AB259 (10^8 cells per ml) and 13-6 (5 × 10^8 cells per ml) were mated by adding 0.5 ml of male strain to 4.5 ml of female strain in a 125-ml Erlenmeyer flask and standing at 37 C. After 5 min of incubation, the mating mixture was diluted 1:1,000 in prewarmed broth (100 ml total volume contained in a 2,800-ml Fernbach flask). Samples (2 ml) were removed at intervals, agitated on a Vortex Jr. mixer for 1 min, and 0.1-ml samples were plated on appropriately supplemented minimal media. (Note the difference in scale for try$^+$ str^r recombinants.)*

Mapping su-lac o-5. The time of entry of *su-lac o-5* was determined in an interrupted mating between Hfr H strain *lac o^o su-lac o-5* and F$^-$ strain Y201, *leu$^-$ lac o^o ade$^-$ try$^-$*. *Su-lac o-5* enters at about 27 min, 4 min after *ade$^+$* (Fig. 2). Since Hfr H transfers *lac$^+$* at about 18 min, 6 min prior to *ade$^+$* (Fig. 1), *su-lac o-5* is separated from *lac* by a 10-min interval. The kinetics obtained for the transfer to *leu$^+$*, *ade$^+$*, *try$^+$*, and *su-lac o-5* (Fig. 2) were repeated in three different experiments.

Try$^+$ maps very close to *su-lac o-5*, and appears to be injected by the suppressed strain 10 min earlier than in crosses with Hfr H AB259 (Fig. 1) or Hfr H 3106 *lac o^o* (results not shown). Either the *try* region of strain *lac o^o su-lac o-5* is transposed, or else *su-lac o-5* suppresses both *try$^-$* and *lac o^o*. The results of the following experiments support the latter conclusion.

When P1 derived from AB259 (*lac$^+$ try$^+$*) is used as donor in transduction of strain Y201 (*lac o^o try$^-$*), no lactose-utilizing tryptophan-independent recombinants are obtained (Table 3). On the other hand, *su-lac o-5* and the determinant conferring tryptophan independence are cotransduced at high frequency by P1 grown on *lac o^o su-lac o-5* (Table 3). If *su-lac o-5* suppresses *try$^-$*, 100% of the suppressed *lac o^o* recombinants selected from the latter transduction should be tryptophan-independent; this was found to be the case for 642 suppressed *lac o^o* transductants (Table 3). If tryptophan-independent transductants arise by receiving either *su-lac o-5* or *try$^+$*, only those transductants which inherited the suppressor would be able to utilize lactose. The data of Table 3 show that 65% of the selected tryptophan-independent transductants inherited *su-lac o-5*.

Instability. Su-lac o-5 is unstable, as are other suppressors of *lac$^-$* (Lederberg, 1952; Schwartz, 1963). After growth in minimal-lactose medium to stationary phase, about 0.5% of the colonies of *lac o^o su-lac o-5* screened on EMB-lactose-agar are *lac$^-$* or lactose-variegated. The genetic basis of the instability of a very unstable suppressed *lac$^-$* mutant will be the subject of another report.

DISCUSSION

Maps of the *E. coli* K-12 linkage group (Jacob and Wollman, 1961; Jacob and Monod, 1961*a*; Jacob and Monod, 1961*b*) place the *lac* region between a *pro* and an *ade* marker. The order of markers: *thr leu pro lac ade* was demonstrated by time of entry experiments with Hfr H (Jacob and Wollman, 1961). Recent experiments by R.

Curtiss III demonstrate that the *pro* region transferred by Hfr H (prior to *lac*) is composed of two *pro* loci, which we will refer to as *proA* and *proB*. These two *pro* loci are separated by about 2 min; *proB* is cotransduced with *lac* at high frequency, whereas *proA* is not cotransduced with *lac*. Hfr P4X-6 transfers $proA^+$ as an early marker and $proB^+$ as a terminal marker (Curtiss, *personal communication*). The *pro* markers reported in this paper map in the *proC* locus, are transferred by Hfr H after *lac*, are contransduced with *lac*, and are transferred as terminal markers by Hfr P4X-6. The relative order of markers transferred by Hfr H is: *thr leu proA proB lac proC ade.*

TABLE 2. *Genetic nature of slow-growing lactose-utilizing revertants of lac o° **

Cross no.	pro^+ donor	lac^-/pro^+ colonies tested	Per cent lac^-
1 (control)	3106 (*lac o°*)	230/276	83
2	Revertant 1 (EMS)	181/236	77
3	Revertant 2 (EMS)	192/245	78
4	Revertant 3 (EMS)	118/156	76
5	Revertant 4 (EMS)	93/123	76
6	Revertant 5 (EMS)	47/68	69
7	Revertant 6 (EMS)	103/142	73
8	Revertant 7 (EMS)	104/138	75
9	Revertant 9 (Sp)	106/124	85
10	Revertant 10 (Sp)	112/145	77
11	Revertant 8 (EMS)	0/182	0

* Revertant strains 1 to 8 were induced by ethyl methanesulfonate; revertants 9 and 10 are of spontaneous origin. Exponential-phase Hfr pro^+ str^r bacteria were mixed with exponential-phase pro^- lac^+ str^r F^- cells at a ratio of one male to ten females to give a final cell concentration of 5×10^8 bacteria per ml. After 90 min of mating by standing at 37 C, a sample of the mating mixture was agitated for 1 min on a Vortex Jr. mixer, diluted, and spread on agar selective for pro^+ str^r recombinants. The pro^+ recombinant frequency averaged 85%, expressed as the number of recombinants per number of input Hfr bacteria. The selected pro^+ recombinant colonies were replicated to minimal-lactose streptomycin agar (lacking proline) to determine the per cent capable of utilizing lactose. Pro^+ str^r recombinants from each cross were streaked on EMB-lactose-agar (containing streptomycin) to test fermentation reaction. (Each of the revertant strains gave a positive, but weaker than wild type, fermentation reaction after 24 hr of incubation at 37 C.)

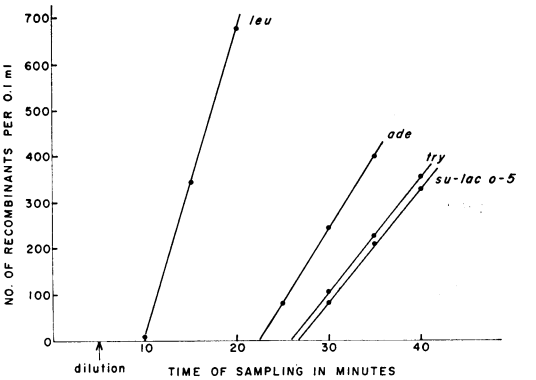

FIG. 2. *Transfer curves for leu^+, ade^+, try^+, and su-lac o-5 markers in a cross between Hfr H lac o° su-lac o-5 str^s and F^- Y201: leu^- lac o° ade^- try^- str^r. The experiment was performed as indicated in the legend to Fig. 1 with the following exception. The male donor strain was grown to stationary phase (10^9 cells per ml) in minimal-lactose medium and then grown to exponential phase (2×10^8 cells per ml) after 1:20 dilution in nutrient broth.*

A Hayes Hfr strain and a multiply marked F^- strain, each carrying a *lac o°* mutation, were constructed by cotransduction of $proC^+$ *lac o°*. Nine of ten revertants of *lac o°*, chosen because of their slow growth on minimal-lactose medium, still contain *lac o°* and therefore are suppressed *lac o°* mutants. One of these suppressors, *su-lac o-5*, is separated from *lac o°* by a 10-min interval. This suppressor also suppresses an independently isolated try^- mutation. Although suppressors of *lac o°* have been observed previously (Beckwith, 1963), time of entry results were not given. To our knowledge, *su-lac o-5* is the first reported *o°* reversion shown to be a suppressor from the results of a cross with wild type and mapped by determination of its time of entry.

Attardi et al. (1963) concluded that the *lac o°* mutation of strain 2.320 (which is used here) prevents transcription of deoxyribonucleic acid into m-RNA. If suppressors of *lac o°* (Beckwith, 1963) act at the level of m-RNA translation by producing mistakes in protein synthesis (Yanofsky and St. Lawrence, 1960; Yanofsky, Helinski, and Maling, 1961), then these suppressors must affect the transcription process indirectly (Attardi et al., 1963).

However, it is likely that the *o°* mutations actually map in the *z* gene, being identical to z^- mutants which have reduced permease and transacetylase activities, i.e., polarity mutants (Jacob

TABLE 3. *Transductional analysis of su-lac o-5**

Donor strain	Selected marker (or phenotype)	Unselected marker (or phenotype)	Per cent containing unselected marker (or phenotype)	No. of colonies tested
AB259	*lac*$^+$	*try*$^+$	0	436
(*lac*$^+$ *try*$^+$)	*try*$^+$	*lac*$^+$	0	440
lac o^o *su-lac o-5*	Tryptophan independence	*su-lac o-5*	65	879
	su-lac o-5	Tryptophan independence	100	642

* P1kc propagated on the indicated strains was used as donor in a cross with strain Y201 (*leu*$^-$ *lac o*^{o} *ade*$^-$ *try*$^-$). Lactose-utilizing transductants (either *lac*$^+$ or *lac o*^{o} *su-lac o-5*) were selected and purified from single colonies on minimal-lactose agar. The proportion of these recombinants which were independent of tryptophan was determined by replication onto minimal-lactose agar lacking tryptophan. Tryptophan-independent transductants were selected and purified on minimal-glucose agar lacking tryptophan. These recombinants were streaked onto EMB lactose agar to determine the proportion capable of utilizing lactose.

and Monod, 1961*b*; Franklin and Luria, 1961). Phenotypic *z*$^-$*y*$^+$ revertants of *lac o*^{o} (strain 2.320) can arise by deletion of the "*o*" region (Beckwith, 1964). These revertants are both sensitive to the repressor product of the *i* gene and produce almost wild-type levels of permease activity. Thus, the operator defined as the "site of action of repressor" and as the "initiation point for the cytoplasmic transcription of the structural genes" (Jacob and Monod, 1961*a*) cannot correspond to that region defined by the *o*^{o} mutations. Ames and Hartman (1963) found that *o*^{o} mutations in the histidine operon of *Salmonella* are indistinguishable from polarity mutations, and suggested that either reading frame mutations (Crick et al., 1961) or nonsense mutations (Benzer and Champe, 1962) produce polarity mutations.

Our results are most easily explained by considering the 2.320 *lac o*^{o} mutation as a polarity mutation within the *z* gene constituting "nonsense." *Su-lac o-5* is nonspecific, suppressing *lac o*^{o} and *try*$^-$, and could act by converting "nonsense" to "sense" at the level of m-RNA translation (Benzer and Champe, 1962; Garen and Siddiqi, 1962). The deleterious effect of *su-lac o-5* on growth and restoration of only 7% of the wild-type level of β-galactosidase is as anticipated from a class of suppressors proposed to act by promoting errors in protein synthesis (Brody and Yanofsky, 1963).

LITERATURE CITED

ADELBERG, E. A., AND S. BURNS. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**:321–330.

AMES, B. N., AND P. E. HARTMAN. 1963. The histidine operon. Cold Spring Harbor Symp. Quant. Biol. **28**:349–356.

ATTARDI, G., S. NAONO, J. ROUVIERE, F. JACOB, AND F. GROS. 1963. Production of messenger RNA and regulation of protein synthesis. Cold Spring Harbor Symp. Quant. Biol. **28**: 363–372.

BECKWITH, J. 1963. Restoration of operon activity by suppressors. Biochim. Biophys. Acta **76**: 162–164.

BECKWITH, J. 1964. A deletion analysis of the *lac* operator region in *Escherichia coli*. J. Mol. Biol. **8**:427–430.

BENZER, S., AND S. P. CHAMPE, 1962. A change from nonsense to sense in the genetic code. Proc. Natl. Acad. Sci. U.S. **48**:1114–1121.

BRODY, S., AND C. YANOFSKY. 1963. Suppressor gene alteration of protein primary structure. Proc. Natl. Acad. Sci. U.S. **50**:9–16.

CRICK, F. H. C., L. BARNETT, S. BRENNER, AND R. J. WATTS-TOBIN. 1961. General nature of the genetic code for proteins. Nature **192**:1227–1232.

DE HAAN, P. G., AND J. D. GROSS. 1962. Transfer delay and chromosome withdrawal during conjugation in *Escherichia coli*. Genet. Res. Cambridge **3**:251–272.

FRANKLIN, N. C., AND S. E. LURIA. 1961. Transduction by bacteriophage P1 and the proper-

ties of the *lac* genetic region in *E. coli* and *S. dysenteriae*. Virology **15**:299–311.

GAREN, A., AND O. SIDDIQI. 1962. Suppression of mutations in the alkaline phosphatase structural cistron of *E. coli*. Proc. Natl. Acad. Sci. U.S. **7**:1121–1127.

JACOB, F., AND J. MONOD. 1961*a*. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3**:318–356.

JACOB, F., AND J. MONOD. 1961*b*. On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**:193–211.

JACOB, F., AND E. L. WOLLMAN. 1961. Sexuality and the genetics of bacteria. Academic Press, Inc., New York.

LEDERBERG, E. M. 1952. Allelic relationships and reverse mutation in *E. coli* Genetics **37**:469–483.

LENNOX, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.

PARDEE, A. B., F. JACOB, AND J. MONOD. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**:165–178.

SCHWARTZ, N. M. 1963. Nature of ethyl methanesulfonate induced reversions of *lac*$^{-}$ mutants of *Escherichia coli*. Genetics **48**:1357–1375.

YANOFSKY, C., AND P. ST. LAWRENCE. 1960. Gene action. Ann. Rev. Microbiol. **14**:311–340.

YANOFSKY, C., D. R. HELINSKI, AND B. D. MALING. 1961. The effects of mutation on the composition and properties of the A protein of *Escherichia coli* tryptophan synthetase. Cold Spring Harbor Symp. Quant. Biol. **26**:11–24.