PLEIOTROPIC EFFECTS OF SUPPRESSORS OF A LAC-"OPERATOR NEGATIVE" MUTATION IN ESCHERICHIA COLI*

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A suppressor mutation is a genetic change which removes the mutant phenotype associated with another mutation, and which is separable from the first mutation by recombination. Of particular interest are suppressor mutations which act at the level of translation of the gene modified by the first mutation. In this context, it is useful to consider if the lesion occasioned by the original mutation leads to the formation of a mis-sense or nonsense triplet. A mis-sense triplet results in an amino acid substitution, while a nonsense triplet results in an untranslatable codon. Extragenic suppression of a mis-sense triplet has been demonstrated for an allele of the tryptophan synthetase system of Escherichia coli. In the same organism, extragenic suppression of a nonsense triplet has been demonstrated for certain ambivalent alleles of the A cistron of the rII system of phage T4, for certain amber mutants of the cistron which codes for the head protein of T4, for a mutant (O2) allele of the β-galactosidase structural gene, and for a mutant allele of the alcaline phosphatase structural gene.

The mechanism(s) of suppression of mis-sense or nonsense triplets has not been demonstrated experimentally. It has been speculated that alterations of either an activating enzyme or a transfer-RNA could account for suppression. More recently, ribosomes have been implicated as another component whose alteration can result in suppression. Needless to say, alteration of any component of the translation system which does or can interact with messenger-RNA during the translation process might result in suppression. Whatever the mechanism, it might be expected that alteration of the translation machinery of a cell would, in certain instances, affect the translation of genetic information other than the original mutant triplet. This type of alteration of the translation machinery could be indicated by an unusual physiological behavior of a strain harboring the responsible suppressor mutation.

In this paper, we report the properties of certain mutants capable of suppressing the lac phenotype determined by the "operator negative" mutation in strain 2320(λ) of E. coli. The suppressors were selected for further study because of their concomitant acquisition of (a) the ability to suppress a set of rII mutants of phage T4 and certain mutants of T7, and (b) a variety of new physiological properties not obviously related to suppression of the lac phenotype. Suppression of the lac phenotype is thought to be accomplished by the acquisition of the ability to translate the nonsense triplet in the O2 allele in strain 2320(λ). Pleiotropic ef-
fects associated with a suppressor of the Oq° allele in a different genetic background have been reported recently.\textsuperscript{13} Our evidence indicates that the pleiotropic effects are not the result of the simultaneous alteration of the genetic information of several cistrons (such as a deletion, for example\textsuperscript{14}). Rather, the variety of pleiotropic effects appear to stem from hereditary changes primarily affecting the translation of genetic information other than the nonsense triplet in the lac operon. This evidence also indicates that the pleiotropic effects of any one suppressor can be quite limited, such as the acquisition of a simple nutritional requirement completely repairable by an exogenous supply of a growth factor. Conjugation studies have revealed the serious limitations inherent in the use of nonisogenic strains in attempting to map extragenic suppressors of the type described.

\textit{Materials and Methods}.—Tryptone B\textsubscript{1} medium contains per liter, 10 gm of Difco Bacto tryptone, 5 gm of NaCl, and 1 mg of vitamin B\textsubscript{1}. Solid tryptone B\textsubscript{1} medium contains, in addition, 1\% agar. The minimal medium contains the salt composition of M63\textsuperscript{15} and was supplemented with either 0.2\% glycerol, 0.1\% D-xylose, or 0.1\% β-lactose as the carbon source. Solid minimal medium contains 1.5\% agar in addition. Difco Bacto yeast extract at 10 mg/liter or vitamin B\textsubscript{1} at 1 mg/liter were used to supplement the minimal medium, where indicated.

The source of strain 2230(\textsuperscript{16}) of \textit{E. coli} and of the transducing phage P1K, was reported previously.\textsuperscript{6} Strain AB313\textsuperscript{16} was obtained originally from Dr. E. Adelberg. Strain C600(\textsuperscript{17}) was prepared by lysogenizing strain C600 with phage \textlambda{} from a lysate prepared by UV induction (method of Weigle\textsuperscript{18}) of strain 3110(\textsuperscript{16}); both bacterial strains were originally obtained from Dr. J. Weigle. Mutants of the phage T4 suppressed by a suppressor of the Oq° allele in 2230(\textsuperscript{16}) were obtained from Dr. S. P. Champe, and have been previously described.\textsuperscript{4} Mutants of T7 with similar properties were isolated on the basis of their ability to form plaques on at least one of the suppressor strains and not on 2320(\textsuperscript{16}). These mutants, designated with the prefix \textit{oss}, will be described in more detail in a subsequent paper.\textsuperscript{11} The wild-type strain of T7, from which these mutants were derived, was obtained originally from Dr. R. S. Edgar.

Suppressor mutants of 2230(\textsuperscript{16}) were selected on solid lactose minimal medium supplemented with yeast extract. Prior to plating for selection, cells were grown to the stationary phase in tryptone B\textsubscript{1} medium with aeration. Mutant selection was done by the following methods to increase the variety of mutants obtained.

\textit{Method 1}:
A 0.1-ml sample of a stationary phase culture, grown at 37°C, was spread on each of a number of selective plates, and the plates were incubated at 37°C.

\textit{Method 2}:
Ten independent cultures, each started from a small inoculum, were incubated at 25°C; after they reached stationary phase, a 0.1-ml sample of each culture was spread on a separate selective plate. After incubation of the plates at 42°C for 7 days, colonies were picked (method 2a). The plates were then shifted to 25°C for further incubation. Mutants obtained after the shift to 25°C are said to result from method 2b.

\textit{Method 3}:
Sixteen independent cultures were grown at 30°C; a 0.1-ml sample of each final culture was spread on a separate selective plate and incubated at 15°C for approximately 2 weeks. At this time, the resultant colonies were picked (method 3a) and the plates were then shifted to 37°C for further incubation. Mutants obtained after this shift are said to result from method 3b.

In all cases, the mutant colonies were purified and tested for their phage suppressor pattern by the method described under Table 1.

Growth rate data were obtained from exponentially growing cultures. Whenever possible, the cells were pregrown in the test medium and then shifted to fresh medium for growth studies. Mixing and aeration of the cultures was accomplished by forcing filtered air through "bubbler tubes" immersed in the medium.

The methods used in the transduction studies were described previously.\textsuperscript{6} Interrupted conjugation studies were done in tryptone B\textsubscript{1} medium using a slight modification of the method of Hayes.\textsuperscript{18} Mating was interrupted by the addition of phage T6.

\textit{Results}.—\textit{Physiological properties of suppressor mutants}:
The isolation of strain
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Unlike 2320(X), the strain from which it was derived, R8 ceases to grow exponentially in glycerol minimal medium as a consequence of the absence of an exogenous supply of vitamin B₁. However, R8 grows exponentially at the same rate as 2320(X) in tryptone B₁ or in glycerol B₁ minimal medium at 37°C (Fig. 1). The B₁ requirement is cotransducible (using phage P1K₀) with the suppressor of both the O₁₁ allele and the phage mutants. Thus, the B₁ requirement is a pleiotropic effect caused by the suppressor in R8, known not to map in the lac operon. In this case, as in all other cases to be described below, the pleiotropic effects are expressed even under conditions which do not require translation of the lac operon message.

Mutant QTS31, obtained by method 2b, manifests a B₁ requirement identical to that of R8; however, the two strains have a different phage suppressor pattern (Table 1). Conversely, mutants have also been isolated which have the same phage suppressor pattern as either R8 or QTS31, but which are free of the B₁ requirement. Thus, there is no strict correlation between phage suppressor pattern and a B₁ requirement.

### Table 1

<table>
<thead>
<tr>
<th>Phage mutant</th>
<th>R8</th>
<th>QTS31</th>
<th>Q5</th>
<th>Y14</th>
<th>15B</th>
<th>2320(λ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oss 11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Oss 14</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Oss 15</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Oss 20</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T7 oss⁺</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

A sample containing about 10⁶ particles was spotted on the bacterial lawn, incubated at 42°C, and scored after at least 4 hr; +, confluent lysis; and −, no lysis of the bacterial lawn, respectively.

Method 2a resulted in the selection of mutants such as Q3 which, unlike 2320(λ), are able to grow exponentially at 42°C in glycerol minimal medium supplemented with yeast extract. The phage suppressor pattern of Q3 is also unique in the set of bacterial mutants reported here (Table 1).

### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>2320(λ)</th>
<th>Y14</th>
<th>Y14-1</th>
<th>15B</th>
<th>15B-L4</th>
<th>14B-L4⁺</th>
<th>SM</th>
<th>2320(λ)⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubling time</td>
<td>38</td>
<td>60</td>
<td>37</td>
<td>80</td>
<td>36</td>
<td>60</td>
<td>57</td>
<td></td>
</tr>
</tbody>
</table>

The doubling time in minutes was determined for cells growing exponentially with aeration in tryptone B₁ medium (in the presence of 500 μg/ml of SM, where indicated).
Mutant Y14, possessing a still different phage suppressor pattern (Table 1), was isolated by method 1. The doubling time of this mutant in tryptone B₁ medium is 50 per cent longer than that of 2320(λ) under the same conditions (Table 2). Also, in contrast to 2320(λ), Y14 cannot sustain exponential growth in tryptone B₁ at 18°C.

Mutant 15B, possessing another unique phage suppressor pattern (Table 1), was isolated by method 3b. The doubling time of this mutant is twice that of 2320(λ) in tryptone B₁ medium at 37°C. Furthermore, 15B is much less resistant to streptomycin (SM) than the parental strain. In tryptone B₁ medium containing 200 μg/ml of SM, 15B grows exponentially but at a lesser rate than in the absence of SM. With 500 μg/ml of SM, the growth of 15B eventually almost ceases, even though the doubling time of 2320(λ) under the same conditions is only increased by 50 per cent (Fig. 2). (The decreased resistance of 15B to SM is different from classical SM sensitivity; this is illustrated in Figure 2 by the behavior of strain C600(λ): this strain ceases growth immediately after exposure to as little as 100 μg/ml of SM.) Thus, the pleiotropic effects of the suppressor in 15B can be detected in two ways: a significantly decreased growth rate in complex medium at 37°C and an increased sensitivity to SM, as compared with its parental strain.

Many of the lac+ isolates of 2320(λ) were capable of suppressing a variety of phage mutants but did not reveal an unusual physiological behavior as a consequence of harboring an active suppressor gene. These mutants were not studied in greater detail.

Repair of pleiotropic effects by subsequent mutation: The pleiotropic effects were found to be subject to repair by subsequent mutation in all cases where selective pressure could be applied.

Strain 15B-L₄, a revertant of 15B, was isolated on the basis of large colony formation at 37°C on tryptone B₁ plates. This mutant is indistinguishable from 2320(λ) by the following criteria: (a) doubling time at 37°C in tryptone B₁ medium with and without SM (Table 2); (b) inability to form colonies on solid lactose minimal medium; and (c) inability to suppress any of the phage mutants tested. The possibility that a second mutation suppresses the pleiotropic effects of 15B is excluded by the following experiment. Phage P1K₄ grown on 15B-L₄ was used to attempt to transduce the lac+ phenotype associated with the 15B mutation into 2320(λ). No lac+ transductants were obtained, even though the same phage lysate was able
to transduce freedom from a tryptophan requirement to a tryptophan synthetase deletion mutant (with an efficiency of $0.5 \times 10^{-4}$ transductants per P1K, plaque-former). Thus, by all testable criteria, 15B-L4 acts genetically and physiologically as a true revertant.

Mutant R8-1 was isolated as a replacement population of R8 growing in glycerol minimal medium with limiting $B_1$ at $37^\circ C$. R8-1 has the same phage suppressor pattern as R8 and is still lac+. This mutant grows in glycerol minimal medium at nearly the same rate as 2320(λ) (Fig. 1).

Mutant Y14-1 is a revertant of Y14, isolated on the basis of large colony formation at $37^\circ C$ on solid lactose minimal medium supplemented with yeast extract. In addition to the lac+ phenotype, it has the same phage suppressor pattern as Y14 and grows at approximately the same rate as 2320(λ) in tryptone $B_1$ medium at $37^\circ C$ (Table 2).

**Mapping experiments:** An attempt to determine the location of the suppressor mutation in 15B was made by using strain AB313 as a donor and 15B as a recipient in mating experiments. AB313 does not suppress any of the T7 mutants tested. Selection for recombinants which had lost the su46B allele was made on the basis of large colony formation on solid tryptone $B_1$ medium at $37^\circ C$. An independent control for gene transfer was made by selecting recombinants on xylose minimal medium at $37^\circ C$, taking advantage of the fact that 15B, as well as 2320(λ), are xyl-.

Further analysis of the results was discontinued when at least two phage suppressor patterns different from those of either parent turned up among 10 xyl+ recombinants tested. In view of these results, it is difficult to interpret data obtained from conjugation studies designed to map suppressors of the type described here by using nonisogenic strains.

**Discussion.**—All of the suppressors described above share the following properties, which most likely result from a single mutational event: (a) they allow translation of the nonsense triplet in the Oγ allele of strain 2320(λ); (b) they suppress a variety of mutants of phages T4 and T7; and (c) they show pleiotropic effects which are expressed even under conditions where suppression of the lac+ phenotype is not required for growth. It is clear, however, that the various suppressors described are genetically different from one another, as judged by their differences in phage suppressor pattern and (with the exception of R8 and QTS31) in physiological behavior. The possibility that all of the suppressor mutations produced different alleles of the same cistron remains to be excluded, however, pending further genetic analysis.

Concerning the basis of the pleiotropic effects, physiologically unrelated functions could be simultaneously affected if the suppressor was, in fact, an extended alteration affecting several adjacent cistrons, as demonstrated for a similar system. This possibility appears very unlikely in view of the ability of subsequent mutations to repair the pleiotropic effects, and can be virtually excluded by the more rigorous analysis described for 15B-LA. It seems more likely that, in all cases, the suppressor mutation involves a base pair substitution rather than an extended alteration.

With this possibility excluded, and considering that (a) selection of the mutants was based on the ability to suppress a nonsense triplet, and (b) all the suppressors affect the expression of a variety of phage mutants, the pleiotropic effects are most simply explained as the result of errors in the translation of other genetic information.
The number of triplets which is subject to faulty translation as a consequence of the action of the suppressor gene seems quite limited in some, if not all, of the mutants described. For example, R8 and QTS31 are phenotypically normal if grown in minimal medium supplemented with B1. Hence, it is particularly significant that the growth of both mutants ceases completely in the absence of B1. Thus, the translation error in these two strains must not only eliminate the activity of the affected enzyme(s), but must also occur in nearly every molecule of the enzyme(s) synthesized. If it is assumed that the error is made with the same high frequency in every affected triplet, it seems very unlikely that a normal physiology would be regained upon the addition of B1 if many triplets (other than those in the enzyme(s) required for B1 synthesis) were affected. For the same reason, the increased temperature range of Q8 cannot be explained by errors affecting many triplets with high frequency.

In contrast, it is more difficult to estimate the amount of genetic information affected at 37°C by the suppressors in Y14 and 15B, respectively. In particular, the evidence does not allow a distinction between a high frequency of errors at a few triplets and a low frequency of errors at many triplets. In either case, each of the errors must have only mildly deleterious effects and they must not be limited to enzymes involved in the synthesis of the kinds of small molecules supplied by the complex growth medium.

Concerning the nature of the translation errors of genetic information (other than the O° allele) in a given suppressor strain, two possibilities can be considered: the affected triplet(s) is converted either to mis-sense or nonsense. The latter possibility appears less likely, but one means of converting a triplet from sense to nonsense might be the alteration (caused by the suppressor mutation) of a previously functional suppressor gene. Thus, the nature and extent of translation errors in a given suppressor strain may depend upon (a) the new properties of the translation system resulting from the changed allele, and (b) the amount and distribution of the genetic information whose translation required the participation of the component which is now altered. Herein may reside the basis of the differences between the various suppressor strains described.

Summary.—Some physiological properties of certain lac+ revertants of a lac- "operator negative" strain of E. coli are described. The revertants were selected for study because they suppress a set of T4 and T7 mutants and differ physiologically from the parental strain in a number of ways not obviously related to the acquisition of the lac+ phenotype. These pleiotropic effects of the suppressor mutations do not result from an extensive alteration of a number of cistrons, and can be explained in terms of errors primarily affecting the translation of other genetic information. The amount of genetic information affected by these errors seems quite limited in some, if not all, of the mutants described. The type(s) and extent of the translation errors caused by the various suppressor genes remain a matter of speculation.

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1 Benzer, S., and S. P. Champe, these PROCEEDINGS, 48, 1114 (1962).
CORRELATION OF GENETIC ACTIVITY, HETEROCHROMATIZATION, AND RNA METABOLISM*

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Recent experimentation has pointed to differential RNA synthesis as a manifestation of differential gene action during development. Independently, the process of facultative heterochromatization has been shown to result in differential genetic inactivity. Because an entire haploid set of chromosomes undergoes facultative heterochromatization in male mealy bugs (Coccoidea, Homoptera), these species provide ideal material for correlating heterochromatization, gene action, and RNA metabolism.

In the giant chromosomes of Chironomus spp. the high RNA content of both puffs and some bands can be revealed by metachromatic staining, while their RNA metabolism can be shown with tritiated uridine labeling. The differential appearance of puffs in various tissues, in both normal and hormone-influenced development, provides strong circumstantial evidence that development is controlled through differential synthesis of RNA. On the other hand, relatively little correlation has been made between the action of known genetic loci and RNA synthesis. Through crossing-over experiments, one gene has been localized in a chromosome segment containing a puff; this puff appears as such only in the few cells which show the phenotype. However, the gene has not been placed precisely in the puff; nor have comparative or developmental studies of the RNA synthesis of this important segment been made available.