Mutation Spectra and
the Neutrality of Mutations

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Abstract

The effect of amino acid replacements on enzyme function was studied in the β-galactosidase of
Escherichia coli. Mutants possessing 50% or less of normal enzyme activity were isolated and
examined. Of 733 amino acid substitutions calculated to have occurred, only 11 reduced β-galac-
tosidase activity below 50%. These mutations were expressed because they greatly impaired the
substrate affinity or catalytic efficiency of the enzyme. The inertness of the enzyme to amino acid
replacement was confirmed by immunological tests of β-galactosidase molecules changed in amino
acid sequence by suppression.

Most proteins are buffered against the effects of mutation by the structure of the genetic code and
the asymmetrical distribution of polar and non-polar amino acids. β-Galactosidase is additionally
protected by a potential capacity for increased synthesis to counteract partial inactivation.

A comparison of mutation spectra suggests that the apparent resistance of genes to mutation is
greatest in those specifying soluble enzymes, less in those whose products must fit into a structural
framework and least in those involved in protein–nucleic acid interactions.

It appears that most mutations in genes coding for enzymes are nearly neutral to natural selection
under customary conditions.

Introduction

It is commonly held that most mutations are harmful to the organism because it
is thought that efficient genetic functioning is dependent upon unique nucleotide and
amino acid sequences established by selection. Any sequence deviation introduced
by mutation would consequently be damaging. This view has been influential in
developing the current concept of evolution which relies on recombination and
segregation, rather than mutation, to provide adaptive genetic variation.

Recent comparisons of amino acid sequences in homologous proteins of related
organisms have shown that there is not a single best sequence corresponding to a
particular function, but that a wide variety is possible. This conclusion is supported
by the findings that amino acid changes altering the surface charge of proteins are
very common in natural populations as electrophoretic variants of enzymes. Many of
these amino acid differences seem to have little or no selective significance.

The conflicting evidence regarding the severity of mutations has led to a contro-
versy as to whether or not mutations occur that are neutral or nearly neutral to
natural selection. Most of the arguments for or against neutrality are either theoretical
or inferences drawn from selection experiments. With the hope of contributing some
direct experimental data to the question, an attempt has been made to recover as
many as possible of the mutations of a single gene and to assess their significance.
Mutations were studied in the gene for β-galactosidase in Escherichia coli because
of the favourable screening techniques for mutants. The results give a mutation spectrum for the gene, but because of limitations to mutant recovery the spectrum is incomplete.

Materials and Methods

The experimental organism was _E. coli_ K-12 Hfr Hayes. A strain constitutive for the proteins of the lactose operon was used, and certain mutants were derived from this strain. Methods of bacterial cultivation, mutagenesis, enzyme assay and identification of mutations have been described previously (Langridge 1968a, 1968b, 1968c; Langridge and Campbell 1969).

Galactose permease activity was measured by uptake of radioactive methyl β-thiogalactoside according to a modification of the method of Crandall and Koch (1971). Cultures for assay were first grown overnight at 37°C in mineral medium containing glycerol (0.2%) as the carbon source. They were then diluted 20-fold with fresh medium and grown to a density of about 5 x 10⁸ cells/ml. These cells were centrifuged at 4°C, resuspended in fresh mineral medium and kept on ice. A sample of 1·2 ml of bacterial suspension was added to 100 μl of solution containing 14C-labelled methyl β-thiogalactoside (2·5 μCi/ml) together with unlabelled methyl β-thiogalactoside (3·5 mg/ml) and incubated for 10 min at 37°C. The cells of 1-ml aliquots of the reaction mixture were collected on membrane filters and the radioactivity of the dried filters counted in a liquid scintillation counter. Controls for non-specific absorption were provided with a mutant with a deletion of most of the gene for galactoside permease. Levels of galactoside permease, which are the means of duplicate experiments, are expressed as percentages of the wild-type activity.

To determine the relationship between formazan formation by bacteria and the amount of β-galactosidase they contain, bacteria were incubated at 37°C overnight on agar plates containing mineral salts, yeast extract, lactose and triphenyltetrazolium chloride (0·01%). The bacteria of isolated colonies were removed from the plates and suspended in 1·5 ml of phosphate buffer. Samples (50 μl) of the suspensions were used for β-galactosidase assay after the addition of a drop each of tolune and sodium deoxycholate. The remainder of the sample was centrifuged and the bacteria extracted with 0·6 ml of isopropanol, which removes the formazan. The suspensions were again centrifuged and the absorption of the formazan measured at 482 nm.

To determine the enzyme activity per antibody unit, mutant extracts were diluted to roughly the same catalytic activities and incubated with enough β-galactosidase antibody to precipitate about 50% of the activity. The antiserum was diluted 300-fold in NaCl (0·85%) and 100 μl were added to 400 μl of enzyme extract. The mixture was left at room temperature for 90 min and then at 4°C for 12 h. The enzyme–antibody precipitate was removed by centrifugation and the supernatant was assayed for β-galactosidase activity.

Results

Detection of Mutants

To study the mutation spectrum, it is necessary to recover as many mutants as possible and not only those with greatly reduced enzyme levels. In the selection method bacteria are spread on plates containing lactose and triphenyltetrazolium chloride, where mutants deficient in β-galactosidase appear as colonies of varying degrees of redness according to the amount of enzyme they contain. Attempts to refine this method to give maximum sensitivity were made by varying the concentration of the dye in the medium and also the time and temperature of bacterial incubation.

To test the selection efficiency of each growth condition, a range of mutants possessing different levels of β-galactosidase was prepared. The indicator strains were made by suppressing amber mutations with a suppressor that inserts serine at the position of the mutation. Their enzyme levels varied from 6 to 46% of the fully induced wild-type strain; all suppressed mutants grew well on lactose. The tests
showed that a low concentration of triphenyltetrazolium chloride (0·01 %), a growth temperature of 37°C in the absence of light and an incubation period of 12 h gave best discrimination of the suppressed mutants. These cultural conditions were used in the isolation of mutants following mutagenic treatment.

There is a curvilinear relationship between the formazan concentration of mutant colonies grown under the conditions of selection and the β-galactosidase level of suppressed mutants (Fig. 1). The curve demonstrates that these modified culture conditions allow the detection of mutants with about 50% or less of normal β-galactosidase activity. This value sets the upper limit of mutant detection and means that only half of the activity range that mutation could theoretically produce can be examined. However, selection of β-galactosidase mutants on the basis of inability to grow on lactose only allows the detection of mutants with 0·6% or less of normal enzyme activity, unless the mutant enzymes have greatly reduced affinity for lactose.

**Mutants with Altered β-Galactosidase Activities**

Following mutagenic treatment with N-methyl-N'-nitro-N-nitrosoguanidine 448 mutants containing formazan, as judged by the red colour of colonies, were isolated. After purification the mutants were tested for their ability to grow on lactose and glucose as sole carbon sources. This test resulted in 144 mutants being discarded because they failed to grow on both lactose and glucose. They either had double mutations, one in the lactose operon and another in an essential biosynthetic pathway, or had single mutations affecting general sugar utilization. A further four mutants were discarded because the colonies were mucoid.

The remaining 300 mutants were assayed for specific β-galactosidase activity. Of these, 175 mutants had levels of enzyme not significantly different from wild type. They probably represent mutants carrying mutations affecting the galactose meta-
bolic pathway, the galactoside permease, or dehydrogenases acting on triphenyl-tetrazolium. Low \( \beta \)-galactosidase levels were found in the other 125 mutants, which must therefore carry mutations in the gene for \( \beta \)-galactosidase or in the genes controlling the synthesis of the enzyme.

The mutants with low enzyme levels comprised three classes. One class containing 64 mutants was devoid of enzyme activity and failed to recombine with mutations of the lactose operon. These mutations appear to be large deletions of the type previously found by Cook and Lederberg (1962). A second class of 47 mutants had low but measurable enzyme activity and failed to grow on lactose. Tests for suppressibility of the mutants showed that 14 were amber (UAG), 9 were ochre (UAA) and 13 were opal (UGA). Immunological assays for cross-reacting material established that three further mutants were missense; that is, the \( \beta \)-galactosidase had one amino acid replaced by another. The remaining eight mutants lacked cross-reacting material and, although they did not respond to suppressors, they were similar to chain-terminating mutants in their low enzyme activity and in their reversion patterns. The third class of 14 mutants grew on lactose, had intermediate levels of enzyme, and cross-reacted with antibody to normal \( \beta \)-galactosidase.

Although mutants having up to 50% of the normal level of \( \beta \)-galactosidase were detectable, 72% of all point mutations were shown or suspected to be of the chain-terminating type. It was found previously (Langridge and Campbell 1969) that chain-terminating mutations comprised 93% of the mutations of the \( \beta \)-galactosidase gene that prevented growth on lactose; i.e. those with 0·6% or less of normal enzyme activity. The more sensitive mutant detection method used in the present experiments has apparently allowed the isolation of more mutations leading to amino acid substitutions, but there is still a marked deficiency in this class. If all amino acid replacements produced a detectable mutant, about 16 missense mutants would be expected for each chain-terminating mutant (Langridge and Campbell 1969).

**Characteristics of Mutants with Intermediate Levels of \( \beta \)-Galactosidase**

The rarity of recoverable mutants with amino acid replacements implies that mutants of this class that are detected have enzymes altered in very unusual ways. Therefore, the 14 mutants with partial \( \beta \)-galactosidase activity were examined for their specific activities, temperature sensitivities, substrate-binding abilities and galactoside permease activities (Table 1).

\( \beta \)-Galactosidase that has been changed by mutation usually has increased heat sensitivity or decreased substrate binding or both. By these criteria 8 of the 14 mutants have an altered \( \beta \)-galactosidase. Six of these mutants were detectable because the amino acid replacement reduces the binding of lactose to the enzyme (mutants 833, 875, 936, 969, 1190, 1481). The other two heat-sensitive mutants (872 and 915) have apparently normal substrate binding properties. However, titration of the enzymes against antibody to normal \( \beta \)-galactosidase revealed that the catalytic efficiency per molecule is much less than in the wild-type enzyme. These mutants, therefore, seem to be ones in which the bond-rearranging ability of \( \beta \)-galactosidase is impaired.

In addition to reductions in \( \beta \)-galactosidase activity being caused by alteration of the enzyme, mutations in the promoter gene may decrease the amount of enzyme synthesized (Arditti *et al.* 1968). With a promoter mutation all three proteins of the
lactose operon (β-galactosidase, galactoside permease and galactoside acetyltransferase) will be reduced in amount but the protein molecules synthesized will be qualitatively identical with those of the wild type. In the six mutants (1008, 1084, 1304, 1354, 1472, 1488) that do not appear to have altered β-galactosidase, the permease activity was markedly reduced. These mutants therefore probably carry mutations of the promoter and are not part of the mutation spectrum of the β-galactosidase gene. In contrast, mutants that have changes in the gene for β-galactosidase did not have permease levels significantly different from the wild type, with the exception of mutants 872, 875 and 915. Since the latter mutants are known from kinetic and heat-sensitivity data to have enzyme alterations (Table 1), they are probably chain-terminating mutants that have been suppressed during the course of isolation. The amount of permease protein formed would then depend on the position of the mutation in the β-galactosidase gene (Newton et al. 1965).

Table 1. Characteristics of mutants having intermediate levels of β-galactosidase activity

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Growth on lactose</th>
<th>β-Galactosidase activity (% of wild type)</th>
<th>Half-life of β-galactosidase (min) (^A)</th>
<th>(10^4 \times K_m) (M) for ONPG (^B)</th>
<th>(10^9 \times K_i) (M) for lactose</th>
<th>Galactoside permease activity (% of wild type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3300 (wild type)</td>
<td>+</td>
<td>100</td>
<td>29</td>
<td>4-2</td>
<td>12-5</td>
<td>100</td>
</tr>
<tr>
<td>833</td>
<td>±</td>
<td>0-9</td>
<td>3-5</td>
<td>8-7</td>
<td>45</td>
<td>127</td>
</tr>
<tr>
<td>872</td>
<td>±</td>
<td>0-3</td>
<td>4</td>
<td>5-5</td>
<td>45</td>
<td>12</td>
</tr>
<tr>
<td>875</td>
<td>±</td>
<td>0-3</td>
<td>13</td>
<td>10-5</td>
<td>87</td>
<td>39</td>
</tr>
<tr>
<td>915</td>
<td>+</td>
<td>0-4</td>
<td>7</td>
<td>5-0</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>936</td>
<td>±</td>
<td>17-3</td>
<td>19</td>
<td>11-5</td>
<td>&gt;100</td>
<td>85</td>
</tr>
<tr>
<td>969</td>
<td>±</td>
<td>4-6</td>
<td>8-5</td>
<td>11-1</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>1008</td>
<td>+</td>
<td>5-4</td>
<td>25</td>
<td>5-0</td>
<td>12-5</td>
<td>13</td>
</tr>
<tr>
<td>1084</td>
<td>+</td>
<td>1-9</td>
<td>33</td>
<td>5-4</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>1190</td>
<td>±</td>
<td>1-3</td>
<td>7</td>
<td>10-5</td>
<td>&gt;100</td>
<td>102</td>
</tr>
<tr>
<td>1304</td>
<td>+</td>
<td>2-9</td>
<td>27</td>
<td>5-4</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>1354</td>
<td>+</td>
<td>0-5</td>
<td>34</td>
<td>4-6</td>
<td>7-5</td>
<td>46</td>
</tr>
<tr>
<td>1472</td>
<td>±</td>
<td>0-7</td>
<td>30</td>
<td>4-6</td>
<td>7-5</td>
<td>9</td>
</tr>
<tr>
<td>1481</td>
<td>±</td>
<td>2-2</td>
<td>13</td>
<td>25-0</td>
<td>95</td>
<td>129</td>
</tr>
<tr>
<td>1488</td>
<td>+</td>
<td>1-2</td>
<td>26</td>
<td>4-8</td>
<td>8-5</td>
<td>22</td>
</tr>
</tbody>
</table>

\(^A\) Calculated from \(t_{0.5} = -\log_e 2/k\), where \(k\) is the rate of enzyme inactivation per minute at 57°C.
\(^B\) o-Nitrophenyl β-D-galactoside.

Fig. 2 summarizes the types and frequencies of mutants detected in these experiments.

**Immunological Properties of Mutants**

A further test of the indication that amino acid replacement seldom affects β-galactosidase activity was made by measuring the catalytic efficiency of mutant molecules. A series of amino acid substitutions was prepared by suppressing 18 amber mutants of the β-galactosidase gene that had been isolated in earlier experiments. The enzyme molecules of the mutants are identical except that at a different position in each mutant the original amino acid is replaced by serine. The enzyme extracts of the suppressed mutants were diluted in buffer to give comparable catalytic activities and incubated with antibody to β-galactosidase as described in the Methods.
If the antigen–antibody binding remains the same for the wild type and the mutants, the antibody is expected to precipitate the same number of molecules in each. The amount of enzyme activity removed by the antibody will be unchanged if the catalytic efficiency per molecule is unaltered by amino acid replacement. The enzymatic activity removed from the wild type and each mutant extract by the same concentration of antibody is given in Table 2. The range of activities removed by antibody conforms to a normal distribution and none are significantly different from the mean with the exception of that for 564 sup D, which has a probability of occurrence of less than 0.01.

There is the possibility that the insertion of serine by the suppressor will restore the wild-type amino acid sequence, although only one of the six codons for serine is capable of being transformed by a single nucleotide change to the amber codon (UAG). Such wild-type sequences will have the same heat resistance as that of the parent (3300). Seven of the 18 suppressed mutants are of the resistant class (Table 2), but they do not have greater catalytic efficiency than the sensitive class, with the exception of 564 sup D. These data support the conclusion that β-galactosidase activity is usually unaffected by single amino acid replacements.

The exceptional enzyme of 564 sup D was examined to find the cause of the lowered catalytic efficiency. Kinetic studies showed that this enzyme was as efficient in binding lactose and o-nitrophenyl β-galactoside as that of the wild type. The enzyme was then titrated with antibody to find the equivalence point. As compared with the normal enzyme, the molecular catalytic efficiency of the 564 sup D enzyme was
about one-quarter (equivalence points: 3300, 0.19; 564 sup D, 0.05). As with two of the mutant enzymes listed in Table 1 (mutants 872 and 915), that of 564 appears to be deficient in catalysing the hydrolysis of the glycosidic bond.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>10^6 × Activity (m) removed by antibody</th>
<th>Heat sensitivity B</th>
<th>Mutant</th>
<th>10^6 × Activity (m) removed by antibody</th>
<th>Heat sensitivity B</th>
</tr>
</thead>
<tbody>
<tr>
<td>3300 (wild type)</td>
<td>16</td>
<td>Resistant</td>
<td>U366 sup D</td>
<td>16</td>
<td>Sensitive</td>
</tr>
<tr>
<td>13 sup D</td>
<td>12</td>
<td>Sensitive</td>
<td>U281 sup D</td>
<td>15</td>
<td>Sensitive</td>
</tr>
<tr>
<td>564 sup D</td>
<td>4</td>
<td>Sensitive</td>
<td>199 sup D</td>
<td>18</td>
<td>Resistant</td>
</tr>
<tr>
<td>2377 sup D</td>
<td>17</td>
<td>Resistant</td>
<td>19 sup D</td>
<td>17</td>
<td>Sensitive</td>
</tr>
<tr>
<td>2272 sup D</td>
<td>20</td>
<td>Resistant</td>
<td>653 sup D</td>
<td>19</td>
<td>Sensitive</td>
</tr>
<tr>
<td>1714 sup D</td>
<td>16</td>
<td>Sensitive</td>
<td>NG779 sup D</td>
<td>12</td>
<td>Sensitive</td>
</tr>
<tr>
<td>1519 sup D</td>
<td>15</td>
<td>Resistant</td>
<td>U367 sup D</td>
<td>21</td>
<td>Sensitive</td>
</tr>
<tr>
<td>U138 sup D</td>
<td>17</td>
<td>Resistant</td>
<td>Y14 sup D</td>
<td>16</td>
<td>Sensitive</td>
</tr>
<tr>
<td>1510 sup D</td>
<td>17</td>
<td>Resistant</td>
<td>200B sup D</td>
<td>16</td>
<td>Sensitive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1568 sup D</td>
<td>17</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

A Enzyme activity expressed as concentration of o-nitrophenol released per 5 min by the hydrolysis of 10^{-6}M o-nitrophenyl β-galactoside at 25°C and pH 7.0.

B Enzyme denoted resistant if mutant retained the same activity as the wild-type enzyme after being left at 57°C for 15 min, and sensitive if significantly less than wild-type activity remained after this treatment.

Derepression of the Lactose Operon

β-Galactosidase is an inducible enzyme capable of being synthesized in amounts up to 6.6% of the bacterial protein when fully induced (Cohn 1957). If less than this maximal amount of β-galactosidase is normally produced, buffering against mutation expression could be provided by further derepression. This aspect was examined by measuring the specific activity of β-galactosidase formed by an inducible strain at various lactose levels.

The bacteria were preadapted to lactose by overnight growth in mineral medium containing 0.1% lactose. They were then inoculated into tubes of mineral medium containing a range of lactose concentrations from 10^{-8} to 10^{-2}M. The fully induced β-galactosidase level was obtained by growing bacteria in mineral medium containing glycerol as a carbon source and isopropyl β-thiogalactoside (10^{-4}M) as inducer. Maximum β-galactosidase activity was formed in response to about 4 × 10^{-3}M lactose, but it did not exceed 33% of the fully induced level at higher lactose concentrations (Fig. 3). If a mutation decreases β-galactosidase quantitatively by impairing the promoter or qualitatively by reducing substrate binding or bond breakage, increased derepression could allow further enzyme synthesis to remove or reduce any effect on the growth rate.

Discussion

The experiments indicate that, for the range of mutation from 0 to 50% of normal enzyme activity, few amino acid substitutions have any effect on β-galactosidase function. Most mutations recovered (72%) produce their effects by causing termin-
ation of β-galactosidase synthesis. From the 44 probably chain-terminating mutants recovered it can be calculated that 733 amino acid substitutions occurred. However, only 11 were detected (Fig. 2) because they reduced the enzyme activity below the 50% level. The majority of missense mutations that cause drastic loss in enzymatic activity do so by reducing substrate binding (9 mutants of a total of 11). The remaining two mutations probably affect the bond-breaking mechanism.

![Graph showing β-Galactosidase activity vs. Lactose concentration](image)

**Fig. 3.** Amount of β-galactosidase synthesized by inducible *E. coli* grown on increasing levels of lactose.

These findings, together with other studies of mutations altering substrate binding and quaternary structure (Langridge 1968a, 1968b, 1968c, unpublished data), suggest that amino acid replacements have little effect unless they occur at or near amino acids that are in contact with substrate for binding or catalytic purposes or in regions concerned with subunit association. The tertiary structure of β-galactosidase formed by the majority of the 1000 amino acids of the enzyme appears to be little affected by amino acid change.

The reasons for inertness to amino acid alteration may be found in the buffering properties of the genetic code, in the protein structure and in the mode of enzyme synthesis. The genetic code is so arranged that amino acids with similar side-chain properties are coded for by triplets of related base composition (Volkenstein 1965). Thus, for hydrophilic amino acids, the greater the hydrophobic nature of the side chain the greater the tendency to mutate to an amino acid of the hydrophobic type. Similarly, hydrophobic amino acids, with the exception of tyrosine, always mutate by transition to another hydrophobic amino acid. In accordance with expectation, it is found that the most common naturally occurring amino acid replacements caused by single mutations in evolution involve rather small differences in side-chain character (Clarke 1970).

The importance of this dampening of the effects of mutational substitution is increased by the asymmetrical distribution of amino acids in soluble proteins. In proteins whose conformation is known nearly all polar and ionic side chains are in contact with water either at the surface of the molecule or in cavities. These amino acids may frequently be interchanged without affecting protein function. Changes that still give a functional molecule of haemoglobin (Perutz and Lehmann 1968) or cytochrome c (Dickerson 1971) are mostly changes of one type of hydrophilic side
chain for another hydrophilic one at the surface, but even certain changes to hydrophobic side chains are tolerated. On the other hand, large non-polar side chains lie either in the interior of the molecule, in crevices so designed as to minimize contact of these side chains with water, or on the surface where they form points of contact between subunits. The substitution of an interior hydrophobic amino acid for a hydrophilic one would destroy enzymatic activity, but the code structure minimizes such changes.

Even where an amino acid substitution decreases enzyme activity, increased synthesis of the enzyme may counteract the damage. The customary level of β-galactosidase appears to be such that further derepression could compensate for a mutational inactivation of two-thirds of the molecules or a reduction of molecular activity to one-third of normal.

Both calculation and experiment indicate that β-galactosidase is very resistant to inactivation by mutation. Most of the reasons for this resistance would be expected to apply to other enzymes. Whitfield et al. (1966) have found that about 66% of mutations affecting an amino transferase in Salmonella were chain-terminating, indicating that amino acid substitutions do not usually inactivate the enzyme. However, the reasons for enzymatic resistance to mutational damage do not necessarily apply to proteins that must fit into a precise structural organization or that have regulatory functions or to genes that are not translated.

In the gene for galactoside permease of E. coli, 63 mutations have been mapped and tested for suppressibility (Langridge, unpublished data). Only 16% of the mutants were chain-terminating and from this value it can be calculated that nearly one-third of the amino acid replacements have inactivated the permease. This sensitivity may reflect the necessity for exact protein conformation for fitting into membrane sites as well as for the still uncertain mechanism of sugar transport.

The data of Shineberg (1974) on lactose repressor mutants show that the function of the repressor protein is very susceptible to amino acid change. More than half of the mutants possessed intermediate repressor activities and no chain-terminating mutants were found in a sample of 37 tested. More extensive tests for suppression by Bourgeois et al. (1966) gave 3% of chain-terminating repressor mutants. This percentage is close to that expected if all nucleotide substitutions that are expressed in the protein are detected. It suggests that most amino acid substitutions of the repressor protein somewhat impair its function.

Many mutations of the operator gene of the lactose operon of E. coli have been isolated and studied by Smith and Sadler (1971). Unlike the other genes discussed, the operator is neither transcribed into RNA nor translated into protein. The mutations show an unusual localized distribution within the gene, probably because the interaction of the repressor with the operator may depend fairly directly upon the nucleotide sequence of the operator gene. As with the repressor mutants of Shineberg (1974), the operator mutants were isolated by selection for growth on phenyl β-galactoside. This substance is hydrolysed by β-galactosidase but is not an inducer of the enzyme. However, mutations that cause slight decreases in function are detected because enough enzyme is then produced to convert phenyl β-galactoside into an inducer and the effect is autocatalytic. The mutations of the repressor and operator therefore provide information on mutations with small effects and complement the data from the β-galactosidase gene.
In Fig. 4 the mutation spectra of the genes for \(\beta\)-galactosidase, \(\beta\)-galactoside permease, lactose operon repressor and lactose operon operator are drawn for comparison. All the spectra are partial, because there are limits to the detection of mutations in each gene, as indicated in the diagram.

![Mutation spectra diagram]

**Fig. 4.** Mutation spectra of two structural genes, \(\beta\)-galactosidase and galactoside permease (upper diagram) and two regulatory genes, lactose operon repressor and lactose operon operator (lower diagram). The frequencies of mutants in each functional class are plotted against the logarithm of the percentage of activity retained for \(\beta\)-galactosidase and permease or the logarithm of the percentage of activity lost for the repressor and operator. The frequencies of permease mutants are from Langridge (unpublished data). The number of operator mutants in each class, taken from the data of Smith and Sadler (1971), is only approximate. The frequencies of repressor mutants are from the data of Shineberg (1974). A, upper limit of \(\beta\)-galactosidase and permease mutant detection; B, wild-type level of \(\beta\)-galactosidase and permease; C, wild-type level of operator; D, wild-type level of repressor; E, lower limit of operator mutant detection; F, lower limit of repressor mutant detection.

It is evident that, in terms of altered function, some genes are far more sensitive to mutation than others. The few examples available suggest that sensitivity is greatest for genes involved in nucleic acid–protein interactions (operator, repressor), less so in genes whose products form part of a structural complex and least in genes specifying metabolic enzymes.

The concept of neutral mutations has received support mainly from amino acid sequence studies of enzymes and from the prevalence of electrophoretic variants of enzymes. The mutation spectrum for \(\beta\)-galactosidase provides evidence that the majority of amino acid replacements indeed have no detectable effect on enzyme
function. However, as shown previously (Langridge 1968a, 1968b, 1968c), many of these substitutions would be expressed under different conditions of substrate type or concentration, temperature, pH, etc. It would not be warranted to conclude that a mutation giving a particular amino acid substitution would be unaffected by selection under all conceivable conditions.

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References


