A DOMINANT CONSTITUTIVE phoR MUTATION IN
ESCHERICHIA COLI

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ABSTRACT

A dominant constitutive mutation of the phoR locus controlling alkaline phosphatase synthesis in Escherichia coli is described. Its phenotype can be explained by the production of a poisonous subunit of the phoR gene product. The phoR gene product is inferred to consist of at least 3 or 4 subunits.

GENETIC control over the rate of alkaline phosphatase synthesis in E. coli is exercised primarily by two loci, designated phoR and phoS. Both are located apart from the structural gene for alkaline phosphatase, phoA, and were originally defined by recessive constitutive mutations (ECHOLS et al. 1961; YAGIL, BRACHA and SILBERSTEIN 1970). In addition, recessive negative phoR mutations (phoR") have been described (GAREN and ECHOLS 1962). The existence of these types of mutations led GAREN and ECHOLS (1962) to propose a model for the regulation of alkaline phosphatase synthesis whereby the phoR gene specified an inducer. Under repressing conditions (excess phosphate) this inducer was converted to a repressor by the action of the phoS gene product. The repressor was assumed to act by binding to an operator for phoA. However, attempts to isolate phoA operator mutants which should be cis dominant constitutives have not been successful (ECHOLS et al. 1961). In the course of such an attempt a dominant constitutive phoR mutant was discovered. The mutant is described and its implication for the structure of the phoR gene product is discussed in this paper.

MATERIALS AND METHODS

Bacterial strains: The bacterial strains employed and their genotypes are listed in Table 1. All strains are Escherichia coli K12. Partially diploid strains were maintained in frozen culture to prevent loss of the F' factor.

Media: TXP minimal medium was routinely used. It contains per liter 12.1 g Trizma base (Sigma), 0.42 g Na3citrate·2H2O, 0.1 g MgSO4·7H2O, 1.0 g (NH4)2SO4, 0.97 g KH2PO4, 0.045 g FeCl3, and 2.0 g glucose or other carbon source. The pH of the Tris solution was adjusted to 7.4 before autoclaving. Sterile solutions of salts, carbon source, amino acids, pyrimidines, purines, vitamins, and antibiotics were added as required. Amino acids were added to a concentration of 20 μg/ml except for methionine (50 μg/ml) and threonine (100 μg/ml). Thymine was used at 20 μg/ml and adenine sulfate at 40 μg/ml. Vitamin B1 concentration was 1 μg/ml and streptomycin concentration 100 μg/ml. Casein hydrolysate at 0.02% was occasionally used to improve growth.

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2 This work was done to satisfy in part the requirements of the degree of Doctor of Philosophy at the University of Washington.

TABLE I

**Bacterial strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>lac phoR+ /F' lac phoR^d; P1 transductant of HD23 from HDP7</td>
</tr>
<tr>
<td>AB2345/F'</td>
<td>lac proC/F' lac phoA+ proC^- phoR^- sss; from E. Signer</td>
</tr>
<tr>
<td>B723</td>
<td>F^- lac phoR^- sss; P1 transductant of HD23 from HDP7</td>
</tr>
<tr>
<td>B723L</td>
<td>F^- lac phoR^- sss; UV mutant of B723</td>
</tr>
<tr>
<td>C3F2</td>
<td>F^- phoR^-; Echols et al. 1961</td>
</tr>
<tr>
<td>C8</td>
<td>HfrC phoR^-; Echols et al. 1961</td>
</tr>
<tr>
<td>G29</td>
<td>HfrC phoR^- (nonsense); Garen and Garen 1963a</td>
</tr>
<tr>
<td>DB98</td>
<td>F^- lac proC phoR^- sss (phoR C8 allele); from D. Berg</td>
</tr>
<tr>
<td>E15</td>
<td>HfrC phoA (deletion); Garen and Garen 1963b</td>
</tr>
<tr>
<td>HD23</td>
<td>F^- lac phoR^- purE thy sss; from Hfr 13 × DB98</td>
</tr>
<tr>
<td>HD234</td>
<td>lac phoR^- sss /F' lac phoR^- sss; F' recombinant from AB2345/F' × HD23</td>
</tr>
<tr>
<td>HDP7</td>
<td>HD234 phoR^-; by nitrosoguanidine mutagenesis</td>
</tr>
<tr>
<td>Hfr 13</td>
<td>Hfr 13 met sss phoR^-; Hirota and Sneath 1961</td>
</tr>
<tr>
<td>N3</td>
<td>lac phoR^- /F' lac phoR^- sss; F' recombinant from AB2345/F' × B723L</td>
</tr>
<tr>
<td>RC277</td>
<td>F^- proC phoR^-; from R. Curtis</td>
</tr>
<tr>
<td>U3</td>
<td>HfrC phoA; Garen 1960</td>
</tr>
</tbody>
</table>

TLP minimal medium, employed to derepress alkaline phosphatase synthesis, contained 0.00675 g KH₂PO₄ per liter. Solid minimal medium contained 1.7% Noble's special agar.

MacConkey's agar (Difco) was used to distinguish lactose fermenters from non-fermenters. L broth and L agar (Lennox 1955) were used for bacterial mating and to produce phage P1 stocks. Beef peptone broth, consisting of 10 g peptone, 10 g beef extract, 4 g NaCl per liter H₂O (pH 7.6) was used for acridine orange (50 μg/ml) curing of the F factor.

*Mutagenesis:* Mutants were induced by ultraviolet irradiation or by N-methyl-N'-nitro-N-nitrosoguanidine (Adelberg, Mandel and Chen 1965). Following exposure to nitrosoguanidine (100 μg/ml) for 30 min the mutagenized cells were washed, diluted 1:100 in minimal medium, and allowed to grow overnight before plating.

**Bacterial mating:** The cross streak technique for bacterial mating was employed (Berg and Curtis 1967).

**Transduction with phage P1:** The methods of Lennox (1955) were employed. To select transductants constitutive for alkaline phosphatase synthesis, plates containing β-glycerophosphate (0.2%) as the sole carbon source were used. For transduction mapping the bacteria were concentrated by centrifugation to approximately 2 × 10¹⁰ cells/ml and mixed with a suspension of 2 × 10¹⁰ P1/ml to give a multiplicity of infection about equal to one.

**Bacterial growth:** Bacterial growth was followed by the increase in optical density at 720 millimicrons. Protein synthesis in a culture is proportional to the increase in O.D._720μm (Gallant and Stapleton 1963). An O.D._720μm = 1 is equivalent to 220 ± 30 μg protein/ml.

**Assay of alkaline phosphatase:** Alkaline phosphatase was assayed by the method of Echols et al. (1961) as modified by Gallant and Stapleton (1963).

**Plate assays of alkaline phosphatase:** Colonies synthesizing alkaline phosphatase were distinguished from non-synthesizing colonies by one of two methods. In the method described by Echols et al. (1961) and by Gallant and Sportswood (1964) 10 mg p-nitrophenol phosphate/ml 1.0M Tris pH 8.0 (NPP) is sprayed on the plates. Synthesizing colonies turn yellow; non-synthesizing colonies remain white. In the second method, suggested by Dr. Ted Jones, about 6 ml of a solution of 2 mg fast red (TR salt, Sigma)/ml and 1 mg α-naphthyl acid phosphate/ml in a buffer consisting of 0.1 M Tris pH 8.0, 0.5 M NaF, and 2 × 10⁻⁴ M ZnCl₂ is layered over the Petri plate. A brown precipitate rapidly forms over synthesizing colonies.
RESULTS

Isolation of constitutive mutant HDP7: The partially diploid wild-type strain HD234 (phoS+phoA+phoR+/F’phoA+phoR+) was mutagenized with nitroso-guanidine as described in MATERIALS AND METHODS. The use of a partial diploid eliminates phoR− recessive constitutives from the screening procedure because this type of mutation is not expressed in the presence of the wild-type phoR+ allele. Moreover, a dominant constitutive mutation in the partially diploid region can readily be distinguished from a phoS constitutive by the segregation of wild-type clones. A constitutive phoS mutation would be stable because the phoS region of the chromosome is haploid in HD234.

Following mutagenesis approximately $10^4$ cells were plated on minimal lactose medium to ensure that only clones containing the lac+ F’ would grow. Fifteen constitutive colonies were detected by spraying with NPP. After purification by streaking, these constitutive mutants were tested for the segregation of repressed clones following stimulation of somatic recombination by ultraviolet irradiation (Berc 1969). Of the 15 mutants 14 did not segregate repressed clones. These 14 were presumed to have mutated at phoS and were not studied further. One mutant (HDP7) did segregate repressed clones. The mutation was designated hdp.

Curing of hdp by acridine orange: The segregation of repressed clones suggested that the hdp mutation could have occurred on the F’ or in the region of the chromosome covered by the F’. These two possibilities should have been distinguishable by testing strain HDP7 for the ability to transfer the hdp mutation to F− strains. However, HDP7 proved to be sterile. The presence of the F’ in HDP7 was indicated, however, by the segregation of lac− clones following ultraviolet irradiation.

An alternate means of determining whether the hdp mutation had occurred on the F’ was to attempt to cure it by growth of HDP7 in acridine orange (Hirot 1960). Growth in acridine orange resulted in loss of the episomal lac+ allele by 87% of the surviving cells. These lac− cells were considered to be cured of the F’ by acridine orange because there was almost no (<1%) loss of the lac+ allele in a control culture lacking acridine orange. Alkaline phosphatase synthesis was found to be repressed in all of the lac− clones. Because curing caused concomitant loss of the lac+ and hdp alleles, it was concluded that the hdp mutation had occurred on the F’ factor.

Mapping of hdp by P1 transduction: It seemed likely that hdp was a phoA operator constitutive or a phoR dominant constitutive. Because phoA and phoR are closely linked (Yagil, Bracha and Silberstein 1970), they should be cotransducible by phage P1 (Lennox 1955). Mapping of hdp was undertaken by determining its cotransduction frequency with the gene proC which lies between phoA and phoR (Yagil, Bracha and Silberstein 1970). This order, phoA−proC−phoR, makes possible an easy determination of whether hdp is in the phoR region or the phoA region.

A haploid hdp donor strain was required for mapping. To construct it the hdp mutation was transferred by P1 transduction from HDP7 to the F−lac phoA+
Table 2

Cotransduction frequencies of mutations affecting alkaline phosphatase synthesis with proC

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Genotype</th>
<th>proC+</th>
<th>Constutive or negative</th>
<th>Cotransduction frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>B723</td>
<td>hdp</td>
<td>1939</td>
<td>1490 constitutive</td>
<td>0.77</td>
</tr>
<tr>
<td>C3F2</td>
<td>phoR-</td>
<td>1411</td>
<td>1032 constitutive</td>
<td>0.73</td>
</tr>
<tr>
<td>C8</td>
<td>phoR-</td>
<td>1598</td>
<td>1139 constitutive</td>
<td>0.71</td>
</tr>
<tr>
<td>U3</td>
<td>phoA</td>
<td>480</td>
<td>432 negative</td>
<td>0.90</td>
</tr>
<tr>
<td>E15</td>
<td>phoA</td>
<td>480</td>
<td>450 negative</td>
<td>0.94</td>
</tr>
</tbody>
</table>

The P1 transduction recipient was RC277 which has the genotype phoR+ proC phoA+. Proline prototrophs were selected on TXP glucose supplemented with threonine, leucine, and B₁ in the B7/23, C3F2, and C8 crosses. TLP (limiting phosphate) with the same supplements was used in the U3 and E15 crosses to distinguish colonies capable of derepressing alkaline phosphatase (phoA+) from those incapable (phoA-). The colonies were sprayed with NPP to determine their phenotypes.

ProC+ phoR+ strain HD23. Of 18 lac+ transductants 8 were constitutive for alkaline phosphatase synthesis. They were purified and tested for UV-stimulated segregation of lac- and repressed clones. Both the lac+ and the hdp alleles were completely stable in the two strains. One of the strains, designated B723, was used as the P1 donor.

The first set of crosses, employing a phoA+ proC phoR+ recipient (RC277), was designed to determine the cotransduction frequency of hdp with proC+. ProC+ transductants of RC277 were selected and scored for constitutivity of alkaline phosphatase synthesis. The cotransduction frequency of hdp is compared to the cotransduction frequencies of other mutations known to occur in proA or phoR in Table 2.

The cotransduction frequency of hdp with proC+ is 0.77. This value is close to the cotransduction frequencies of the phoR- mutations C3 and C8 (ECHOLS et al. 1961), respectively 0.73 and 0.71, suggesting that hdp may be located in or near phoR. The cotransduction frequencies of the phoA mutations U3 and E15 (GAREN 1960; GAREN and GAREN 1963b), 0.90 and 0.94 respectively, differ sufficiently from the value 0.77 for hdp to make it unlikely that hdp is an operator mutation of phoA.

A more accurate measure of the proximity of the hdp mutation to phoR is the frequency of exchanges between hdp and a phoR mutation. In the second set of crosses B723 was used as the donor and DB98 which carries the phoR mutation C8 as the recipient. ProC+ transductants were selected. Those repressed for alkaline phosphatase synthesis could only have been produced by a crossover between the two mutations in a double or quadruple exchange depending upon the order as shown below.

transducing DNA: ---hdp--
chromosomal DNA: proC---C8

or

transducing DNA: ---hdp---
chromosomal DNA: proC---C8
TABLE 3

Frequency of repressed proC\textsuperscript{+} transductants in crosses between constitutive alkaline phosphatase mutants

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Genotype</th>
<th>Number of transductants proC\textsuperscript{+}</th>
<th>Repressed</th>
<th>Frequency of repressed transductants</th>
</tr>
</thead>
<tbody>
<tr>
<td>B723</td>
<td>hdp</td>
<td>17,312</td>
<td>12</td>
<td>0.0007</td>
</tr>
<tr>
<td>C29</td>
<td>phoR\textsuperscript{-}</td>
<td>15,108</td>
<td>100</td>
<td>0.0066</td>
</tr>
<tr>
<td>C8</td>
<td>phoR\textsuperscript{-}</td>
<td>27,491</td>
<td>1</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

The P1 transduction recipient was DB98 which carries the phoR\textsuperscript{-} mutation C8. Proline prototrophs were selected on TXP glucose agar supplemented with adenine and thymine. The colonies were sprayed with NPP to score alkaline phosphatase synthesis.

If hdp were located in or near phoR, this crossover should occur with a low frequency. For example, the frequency of trp\textsuperscript{+} recombinants in a P1 transduction cross between two mutants located at opposite ends of the trpA cistron is 2.5\% (Maling and Yanovsky 1961). By contrast, the data of Table 2 indicate that the frequency in a comparable cross between phoA and phoR would be greater than 30\%.

The frequency of repressed proC\textsuperscript{+} transductants in the cross B723 \times DB98 is compared with that in the cross C29 \times DB98 in Table 3. C29 is a phoR\textsuperscript{-} nonsense mutant (Garen and Garen 1963a). The frequency of 0.07\% observed in the cross B723 \times DB98 is less than 1/9 the frequency 0.66\% observed in the cross between the two phoR\textsuperscript{-} mutants. It may be inferred that hdp is closer to C8 than C29 is. Even if the formation of repressed recombinants required a quadruple exchange in the cross hdp \times C8 and only a double in the cross C29 \times C8, the distance between hdp and C8 would still be inferred to be 1/3 that between C29 and C8. This result strongly suggests that hdp is within the phoR locus and makes it plausible to designate hdp as a phoR\textsuperscript{d} mutation (constitutive dominant). This designation is made by analogy with the i\textsuperscript{d} mutations of the lac system which are dominant constitutive mutations in the gene specifying the lac repressor (Müller-Hill, Crapo and Gilbert 1968).

Phenotype of hdp: The expression of the phoR\textsuperscript{d} mutation hdp has been studied by measurement of the differential rates of alkaline phosphatase synthesis in strains carrying the mutation in various genetic configurations. The differential rate of enzyme synthesis is the increase in enzyme activity per unit increase in cell mass. Partially diploid heterozygous strains with phoR\textsuperscript{d} on the F\textsuperscript{'} episome or on the chromosome have been constructed. Measurement of the differential rates in these strains and in the wild-type partial diploid permits quantitation of the dominance of phoR\textsuperscript{d} over wild type. In addition, the expression of the phoR\textsuperscript{d} mutation is expected to be sensitive to the dosage of phoR\textsuperscript{+} because i\textsuperscript{d} mutations exhibit sensitivity to the amount of functional lac repressor in the cell (Müller-Hill, Crapo and Gilbert 1968). Hence, the differential rates of the two kinds of heterozygotes (phoR\textsuperscript{+}/F\textsuperscript{'}phoR\textsuperscript{d} and phoR\textsuperscript{d}/F\textsuperscript{'}phoR\textsuperscript{+}) are expected to differ because there are, on the average, about 1.5 copies of the F\textsuperscript{'} episome per copy of the chromosome during exponential growth (Sadler and Novick 1965). This
Dominant $phoR$ Mutation

Table 4

Summary of differential rates of alkaline phosphatase synthesis in $phoR^+$ and $phoR^-$ strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Differential rate (enzyme units/ml/ΔO.D._20 μΜ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In excess phosphate</td>
</tr>
<tr>
<td>HD23</td>
<td>F$^-$$phoR^+$</td>
<td>0.0008$^+$</td>
</tr>
<tr>
<td>HD234</td>
<td>$phoR^+/F'phoR^+$</td>
<td>0.025</td>
</tr>
<tr>
<td>73</td>
<td>$phoR^-/F'phoR^-$</td>
<td>0.71</td>
</tr>
<tr>
<td>N3</td>
<td>$phoR^-/F'phoR^+$</td>
<td>0.13</td>
</tr>
<tr>
<td>B723</td>
<td>F$^-$$phoR^-$</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Each value for an excess phosphate differential rate is the average from at least two experiments involving 12 measurements of alkaline phosphatase activity during exponential growth. Alkaline phosphatase was assayed as described. Increase in cell mass was measured by the change in O.D._20 μΜ_. In limiting phosphate experiments the culture was grown to early exponential phase in TXP, centrifuged, and resuspended in media containing $5 \times 10^{-5}$ M phosphate. The culture was allowed to exhaust the phosphate (Δ O.D._20 μΜ_ ≈ 0.2) to derepress alkaline phosphatase before samples were taken for assay.

Type of position effect should occur because the heterozygous strain with the $phoR^+$ allele on the episome should contain more functional $phoR$ gene product and synthesize less alkaline phosphatase than the heterozygote with the $phoR^+$ allele on the chromosome.

The origin of the haploid $phoR^-$ strain B723 has already been described. Strain N3 is an F$^-$ lac$^+$ recombinant from a cross between the progenitor wild-type partial diploid HD234 and a UV-induced lac$^-$ mutant of B723. It segregates lac$^-$ and $phoR^-$ clones but transfers only the $phoR^+$ allele with high frequency. Thus N3 is a partially diploid strain carrying $phoR^-$ on the chromosome. Strain 73 was selected as an alkaline phosphatase constitutive transductant on β-glycerol phosphate from a cross between HDP7 (P1 parent) and HD234. It is heterozygous at both lac and $phoR$ and synthesizes alkaline phosphatase at the same differential rate as HDP7. It transfers only the $phoR^-$ allele with high frequency indicating that it carries $phoR^-$ on the episome. Strain HD23 is the wild-type haploid progenitor of all the above strains. The genotypes and differential rates of alkaline phosphatase synthesis of these strains are summarized in Table 4.

The dominance of $phoR^-$ over $phoR^+$ is made clear by comparison of the differential rates of strains 73 and HD234. Alkaline phosphatase is synthesized by 73 ($phoR^-/F'phoR^+$) at a rate 28 fold that of the homozygous wild-type HD234 in excess phosphate. The dominance is not complete, however. The differential rate of synthesis by 73 in excess phosphate (0.71) is only 6.5% of the fully derepressed (limiting phosphate) rate of synthesis by HD234 (11.0). $PhoR^-$ is thus only partially dominant.

The dominance of $phoR^-$ is also evident from a comparison of the differential rates of N3 ($phoR^-/F'phoR^+$) and HD234 in excess phosphate. Here the difference is only 5 fold, however. The difference in differential rates (5.5 fold) between 73 and N3 indicates that the effect of dosage on the expression of $phoR^-$ is of the type expected. Strain N3 which should have 1.5 times as many copies of
the \( \textit{pho}R^+ \) allele as 73 and thus make more functional \( \textit{pho}R \) gene product, does indeed have a lower differential rate of alkaline phosphatase synthesis.

The differential rate values in Table 4 provide evidence to confirm the conclusion from the mapping results that \( \textit{hdp} \) is not a \( \textit{pho}A \) operator mutation. A heterozygote with a constitutive operator on the episome should have a differential rate only 1.5 times that of a heterozygote with the constitutive operator on the chromosome because of the episome to chromosome ratio of 1.5:1. The observed difference is 5.5. Additional confirmation comes from comparison of the haploid \( \textit{pho}R^- \) strain B723 with the partial diploid N3 \( (\textit{pho}R^-/\textsf{F'pho}R^+) \). The differential rate of the partial diploid in excess phosphate is 3-fold lower than that of the haploid implying that the introduction of a \( \textit{pho}A^+\textit{pho}R^+ \) episome into a cell with an \( \textit{hdp} \) chromosome produces a cytoplasmic interaction which cannot be explained in terms of an operator constitutive mutation. These negative results support the notion that \( \textit{hdp} \) is a \( \textit{pho}R \) mutation.

The fully derepressed rates of the \( \textit{pho}R^+ \) strains appear to be somewhat higher than those of the comparable wild-type strains. These results might imply that the \( \textit{pho}R^- \) mutation has enhanced inducing activity (that activity which is defective in \( \textit{pho}R^e \) mutants). However, variability in the limiting phosphate rates of these strains made this possibility difficult to assess.

An explanation for the 30-fold difference in repressed differential rate between the haploid \( \textit{pho}R^+ \) (0.00084) and the partial diploid \( \textit{pho}R^+/\textsf{F'pho}R^+ \) (0.025) is not provided by the data presented here. Two possible causes suggest themselves. Alkaline phosphatase is a dimer (Rothman and Byrne 1963) whose monomers associate spontaneously \textit{in vitro} to form active enzyme (Schlesinger and Levine-Thal 1963). This association reaction should be second order. Because the concentration of monomers in the partial diploid is 2.5-fold that in the haploid, the concentration of active enzyme (dimers) in the partial diploid should be \( (2.5)^2 = 6 \)-fold that in the haploid. Also, while the introduction of the \( \textsf{F'} \) episome carrying \( \textit{pho}A \) cisrons, it does reduce the 1:1 ratio of the \( \textit{pho}S \) cisrons to \( \textit{pho}A \) to 1:2.5. This relative deficiency of the \( \textit{pho}S \) gene products, which are required for repression, may be partially responsible for the increase in the repressed differential rate in the partial diploid.

**DISCUSSION**

The dominance and the position effect expressed by the \( \textit{pho}R^- \) mutation can be explained in terms of the poisonous subunit model advanced by Müller-Hill, Crafo and Gilbert (1968) for \( \textit{lac} i^- \) mutations. By this model a haploid \( \textit{pho}R^- \) cell would synthesize \( \textit{pho}R \) polypeptides which would aggregate to form a multimer partially defective in the ability to repress alkaline phosphatase. A heterozygous partially diploid cell would contain not only homologous \( \textit{pho}R^- \) and \( \textit{pho}R^+ \) multimers, but also a large proportion of heterologous multimers. These heterologous multimers would also be partially defective in repressor ability. The paucity of fully functional repressor multimers in the heterozygote would make it partially constitutive for alkaline phosphatase synthesis. Hence, \( \textit{pho}R^- \) would be dominant over \( \textit{pho}R^+ \). The position effect exerted on \( \textit{pho}R^- \)
expression in heterozygotes would simply be a consequence of the dosage of $phoR^+$ alleles. If the $phoR^+$ allele were on the episome, it would produce 1.5 times as many fully functional $phoR$ subunits as it would if it were on the chromosome. Because the concentration of fully functional multimers would be higher, the differential rate of alkaline phosphatase synthesis would be lower, as is actually the case.

The evidence that there are 1.5 copies of the F' episome per chromosome in exponentially growing cells is principally that the fully derepressed differential rate of β-galactosidase synthesis is about 2.5 times as great in an F' partial diploid as in a haploid (SADLER and NOVICK 1965). The factor of 2.5 implies that there are 1.5 copies of the episome per chromosome. GALLANT and SPOTTSWOOD (1964) have shown that this factor has a similar value when determined from alkaline phosphatase differential rates. They increased the dosage of $phoR^+$ by inserting an F'$phoR^+phoA^-$ into a haploid $phoR^+phoA^+$ strain. The differential rate in the F' strain was lower than that in the haploid by a factor of 2.3. Comparison of the fully derepressed differential rates in Table 4 shows that the F' HD234 has a differential rate 3.2-fold that of the F- HD23. The value of 3.2 is in approximate agreement with an episome to chromosome ratio of 1.5:1.

The poisonous subunit model may be used to estimate the number of subunits in the $phoR$ gene product from the dosage of $phoR^+$ in the partially diploid strains 73 and N3 and the differential rates of synthesis in these strains. It is necessary to assume, however, that the relationship between the concentration of the repressor, the gene for which has not been identified, and the differential rate also holds between the concentration of the $phoR$ gene product, which is required for repression, and the differential rate. The estimate is made from a comparison of the expected probabilities ($P$) of functional $phoR$ gene product formation calculated for $phoR$ gene products of various numbers of subunits from the episome to chromosome ratio of 1.5:1 with the actual probabilities ($p$) of repressor formation calculated from the differential rates of alkaline phosphatase synthesis.

The expected probabilities for $phoR$ gene products of 1 to 5 subunits are given in Table 5. Each of these probabilities is simply the fraction of $phoR$ gene product multimers expected to consist entirely of $phoR^+$ subunits in the heterozygotes 73 and N3.

### Table 5

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>73 $phoR^+/F'phoR^-$</th>
<th>73 $phoR^+/F'phoR^-$</th>
<th>N3 $phoR^-/F'phoR^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>$phoR^+/F'phoR^-$</td>
<td>0.40</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>$phoR^+/F'phoR^-$</td>
<td>0.16</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>$phoR^+/F'phoR^-$</td>
<td>0.064</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>$phoR^+/F'phoR^-$</td>
<td>0.026</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>$phoR^+/F'phoR^-$</td>
<td>0.010</td>
<td>0.078</td>
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</tbody>
</table>

The following assumptions are made in the calculation of $P$: (1) Only multimers consisting entirely of $phoR^+$ subunits have activity. (2) There are 1.5 copies of the episome per chromosome.
Actual probabilities \((p)\) of functional repressor formation in partial diploids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>HD234</th>
<th>73</th>
<th>N3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{pho}R^+/\text{F}'\text{pho}R^+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(G)</td>
<td>0.0027</td>
<td>0.0645</td>
<td>0.0118</td>
<td></td>
</tr>
<tr>
<td>(R)</td>
<td>439 (K_d)</td>
<td>14.5 (K_d)</td>
<td>83.6 (K_d)</td>
<td></td>
</tr>
<tr>
<td>(p)</td>
<td>1</td>
<td>0.033</td>
<td>0.19</td>
<td></td>
</tr>
</tbody>
</table>

Terms and calculations are explained in the text.

The actual probabilities of repressor formation may be calculated from the relationship between repressor concentration and differential rate. In the case of alkaline phosphatase synthesis this relationship has been inferred to be of the Michaelis type (Novick, Lennox and Jacob 1963; Gallant and Stapleton 1963). The repressor concentration in any partial diploid growing under any conditions can be calculated if we let \(G\) stand for its differential rate divided by the fully derepressed differential rate of the homozygous wild-type partial diploid, where \(R = 0\) (Sadler and Novick 1965).

\[
G = \frac{K_d}{(K_d + R)}
\]

Equation 1 can be solved for the repressor concentration \((R)\) which is given in units of the repressor-operator dissociation constant \((K_d)\).

The actual probability of repressor formation \((p)\) for any partial diploid is its value of \(R\) divided by the value of \(R\) for the fully repressed homozygous wild-type partial diploid (where \(p = 1\)). Values of \(G\), \(R\), and \(p\) for HD234, 73, and N3 are given in Table 6.

The actual probabilities \(p = 0.033\) for strain 73 \((\text{pho}R^+/\text{F}'\text{pho}R^-)\) and \(p = 0.19\) for strain N3 \((\text{pho}R^-/\text{F}'\text{pho}R^+)\) fall between the \(P\) values expected for \(\text{pho}R\) gene products of 3 and 4 subunits (Table 5). However, because \(\text{pho}R^-\) multimers would have partial repressing activity (Table 4), the \(p\) values are inflated with respect to the poisonous subunit model. Thus, 3 or 4 subunits is a minimum estimate for the number of subunits in the \(\text{pho}R\) gene product.

LITERATURE CITED


DOMINANT \textit{phoR} MUTATION


