Genetic Mapping of the phoR Regulator Gene of Alkaline Phosphatase in Escherichia coli

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SUMMARY

Genetic mapping of the structural phoA and the linked regulatory phoR genes for alkaline phosphatase synthesis in Escherichia coli was carried out by conjugation. Distal markers were selected and the segregation of proximal markers was determined. The gene order lac-phoA-phoR-tsx is proposed.

INTRODUCTION

The synthesis of alkaline phosphatase in Escherichia coli is repressed by inorganic orthophosphate (Torriani, 1960). This enzyme is coded by the structural gene phoA and its synthesis is controlled by two regulatory genes, phoR and phoS. A mutation in either of the regulatory genes results in a phenotype constitutive for alkaline phosphatase synthesis. The structural gene and the phoR regulatory gene are linked and are located close to the lacI gene of the lactose operon. The phoS regulatory gene is located in a distant region, between the isoleucine-valine (ilv) and tryptophanase (tnaA) loci (Garen, 1960; Echols, Garen, Garen & Torriani, 1961; Aono & Otsuji, 1968). The order of the linked phoA and phoR genes has not yet been determined. This communication describes a series of crosses determining the order and the position of these two loci relative to the linked lac and resistance to bacteriophage T6 (tsx) markers.

METHODS

Bacterial strains. Table 1 lists the strains used; all are derivatives of Escherichia coli K12.

Media. Tris buffered minimal media were used as described by Echols et al. (1961. See also Torriani, 1968). These were supplemented with 0.2% of the appropriate sugar, 2% Difco Noble agar and, where necessary, 20 μg./ml. L-amino acid, 1 μg./ml. thiamin and 100 μg./ml. streptomycin. The concentration of KH2PO4 in these plates (10⁻³ M) was in excess for the repression of alkaline phosphatase synthesis. For derepression of enzyme synthesis, plates with low concentration (3.3 × 10⁻³ M) of KH2PO4 were used. Tryptone broth contained, per litre, 10 g. Difco Bacto-Tryptone, 5 g. yeast extract, 5 g. NaCl and 40 mg. NaOH. For plating, 2% agar was added and 0.7% for tryptone soft agar. EMBlac medium as described by Lederberg (1950) was used.

Determination of alkaline phosphatase phenotype. A modification of the method of Levinthal, Signer & Fetherolf (1962) was used. The plates were sprayed with a 1+1 (v/v) mixture of α-naphthyl phosphate (Calbiochem; 2 mg./ml. in 1 M Tris-chloride buffer, pH 8-0) and tetrazotized o-dianisidine (Sigma; 10 mg./ml. in water). These
reagents were dissolved and mixed in an ice bath just before spraying. Colonies with a purple coloration indicated enzyme activity. Constitutive colonies stained on media containing KH$_2$PO$_4$ at excess or low concentrations; wild type repressible colonies were stained at low phosphate concentration only and phoA mutants were unstained on both media.

### Table 1. Characteristics of the Escherichia coli k-12 strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Genotype*</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>K10</td>
<td>Hfr C</td>
<td>wild type</td>
<td>Echols et al. (1961)</td>
</tr>
<tr>
<td>C600</td>
<td>F-</td>
<td>thi thr leu lac tsx-r str-r</td>
<td>F. Jacob</td>
</tr>
<tr>
<td>AB1899</td>
<td>F-</td>
<td>thi thr leu proA lac tsx-r str-r</td>
<td>P. Howard-Flanders</td>
</tr>
<tr>
<td>NA1</td>
<td>Hfr C</td>
<td>phoR</td>
<td>K10 by NG</td>
</tr>
<tr>
<td>P21-NA1</td>
<td>Hfr C</td>
<td>phoA phoR tsx-r</td>
<td>NA1 by NG</td>
</tr>
<tr>
<td>RE2</td>
<td>F-</td>
<td>thr leu lac phoA phoR tsx-r str-r</td>
<td>P21-NA1 × C600 by selection for tsx-r</td>
</tr>
</tbody>
</table>

*Non-relevant markers are given in parentheses.

Abbreviations used: thi = thiamin, thr = threonine, leu = leucine, pro = proline, arg = arginine, his = histidine, lac = lactose, xyl = xylose. mil = manitol, ara = arabinose, gal = galactose. tsx-r = resistance to bacteriophage T6, str-r = resistance to streptomycin. pho = phosphatase, lon = long form. NG = N-methyl-N'-nitro-N-nitrosoguanidine.

**Mutant isolation**

(a) phoR constitutive mutant. Approximately $4 \times 10^7$ cells of K10 were spread on plates containing β-glycerol phosphate as the sole carbon source (Torriani & Rothman, 1961). Pieces of filter paper soaked in a solution of the mutagen N-methyl-N'-nitro-N-nitrosoguanidine at 5 mg./ml. were placed in the centre of the plates. Bacteria from three of the constitutive colonies which grew after 70 hr of incubation at 37° were crossed with strain C600, and the fraction of thr$^+$leu$^+$ and lac$^+$ recombinant colonies synthesizing alkaline phosphatase constitutively was determined. In one of the crosses (NA1 × C600) 50% of the thr$^+$leu$^+$ recombinants and 90% of the lac$^+$ recombinants were constitutive. Strain NA1 was therefore designated phoR (Echols et al. 1961).

(b) phoAphoR double mutant. Strain NA1 was treated with N-methyl-N'-nitro-N-nitrosoguanidine by the method of Adelberg, Mandel & Chen (1965). After 4 hr of growth to allow segregation, surviving bacteria were plated on minimal medium containing excess KH$_2$PO$_4$. Colonies that did not synthesize alkaline phosphatase were picked, purified and spotted on minimal medium plates containing KH$_2$PO$_4$ at a low concentration. Of the 22 colonies tested, two failed to synthesize the enzyme on both media and were designated phoAphoR double mutants. The remaining colonies were revertants to the repressible wild type phenotype.

(c) Resistance to bacteriophage T6. Approximately $10^8$ bacteria were mixed with $2 \times 10^8$ phage particles in melted soft tryptone agar (at 43°) and plated on top of tryptone agar. To confirm the phage resistance of bacteria in colonies that grew after incubation, they were cross streaked, after purification, against the phage at $2 \times 10^{10}$ particles/ml. either on tryptone agar or on EMBlac agar.

**Conjugation.** The parental strains were grown in tryptone broth. Exponentially growing F$^-$ cells (approximately $5 \times 10^8$ organisms/ml.) were centrifuged and re-suspended in one-fifth of the original volume. Exponentially growing Hfr cells were
Mapping of the phoR gene in E. coli

added to give a final ratio of 1Hfr:10F− cells. The conjugants were gently shaken in 37° for 1 hr and were then plated on supplemented selective minimal agar. When tsx-r recombinants were selected, the conjugants were first incubated with shaking in tryptone broth supplemented with streptomycin, to allow segregation. After three generations of growth they were treated for 25 min. with T6 particles at a multiplicity of 10 before plating for survivors.

RESULTS AND DISCUSSION

Table 2 shows the results of a cross between strains NA1 and AB1899. The recombinant colonies on the selective media were sprayed to determine their phoR genotype. The fewest exchanges occurred between the lac and the phoR markers, indicating the closest linkage between them. Since the majority of phoR+ in either lac+ or pro+ recombinants were tsx-r (Table 2), it is likely (though not necessary) that the phoR marker is located between lac and tsx. This, however, becomes evident from results of the cross between strains K10 and RE2. Three kinds of recombinants were obtained and the order of phoA and phoR loci relative to the linked lac and tsx markers was determined (Fig. 1, Table 3):

(A) phoA+phoR constitutive recombinants. These recombinants, requiring a cross-over in region c (Fig. 1), were obtained by plating the conjugants on minimal medium containing β-glycerol phosphate as carbon source (Torriani & Rothman, 1961). After two cycles of single colony isolation their lac and tsx genotype was determined. The results are shown in Table 3A. Since the majority of the phoA+phoR recombinants possessed the lac− marker from the Hfr strain and the tsx-r marker from the F− strain, the gene order lac-phoA-phoR-lac− is most likely.

(B) lac+ recombinants. These were obtained on medium with lactose as the carbon source and were sprayed for the determination of their phoR phenotype. The constitutive (phoR) recombinants must have resulted from cross-overs in regions a and c, and of 731 lac+ recombinants only 11 (1.5%) were constitutive. (These results agree
with the results obtained by Echols et al. 1961, who reported that in the cross between Hfr thr+leu+phoA+phoR and F- thr leu phoA phoR+, of the thr+leu+ recombinants 1.8% resulted from a cross-over between the phoA and phoR loci. These lac+phoR recombinants were isolated and after two cycles of single colony purification their tsx genotype was determined (Table 3B). The majority (82.2%) were tsx-r, and this is also compatible with the gene order lac-phoA-phoR-tsx.

Table 3. The analysis of recombinants from the cross Hfr k10 lac+ phoA+ phoR+ tsx-s str-s x F- R62 lac phoA phoR tsx-r str-r

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of colonies scored</th>
<th>Unselected markers</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A phoA+phoR</td>
<td>100</td>
<td>lac+tsx-r</td>
<td>83.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lac tsx-r</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lac+tsx-s</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lac tsx-s</td>
<td>0</td>
</tr>
<tr>
<td>B lac+phoR</td>
<td>28</td>
<td>tsx-r</td>
<td>82.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tsx-s</td>
<td>17.8</td>
</tr>
<tr>
<td>C lac+tsx-r</td>
<td>96</td>
<td>phoA+phoR+</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phoA+phoR*</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phoA (phoR)*</td>
<td>48.3</td>
</tr>
</tbody>
</table>

* Due to the mutation of the phoA structural gene, the phoR genotype cannot be determined, but it is most likely to be mutant rather than wild type.

(C) lac+tsx-r recombinants. This class of recombinants was selected in order to determine the relative distances between the genes involved. They could result from cross-overs between lac and tsx in either regions b, c or d (Fig. 1), and the genotype of such recombinants was determined by their ability to synthesize alkaline phosphatase on media with either excess or low phosphate concentrations. The frequency of each recombinant genotype is shown in Table 3C. The fact that all three types of segregants (phoA+phoR+, phoA+phoR, phoA phoR) were obtained conclusively proves the gene order proposed. In addition, assuming that the observed frequency of cross-overs is proportional to the distance between the markers, it is calculated that the relative lengths of regions b, c and d are 2:17:1:1:33, respectively. The ratio of the distances c/(b+c) can be calculated either from selection (C) (22.2/(48.3 + 22.2) = 0.32) or by dividing the 1.5% lac+phoA+phoR recombinants obtained in selection (B) (above) by the 5.4% lac+phoR+ recombinants obtained in Table 2 (1.5/5.4 = 0.28). These two values are in good agreement. However, the assumption concerning the strict proportionality between distance and frequency of crossing-over should be made with caution, since negative interference may have resulted in a higher incidence of cross-overs proximal to the lac marker (Maccacaro & Hayes, 1961). The observation that the frequency of lac phoA+phoR constitutive recombinants (7%) was lower than that of phoA+ phoR tsx-s recombinants (10% — Table 3A) is probably due to spontaneous chromosomal breakage during transfer, since lac is a distal marker (Hayes, 1968).

The orientation on the genetic map of the phoR regulatory gene is the same as in almost all other operons known in E. coli in which the operator or one of the regulator sites are linked to the structural genes (cf. Taylor & Trotter, 1967; Epstein & Beckwith, 1968).
Mapping of the phoR gene in E. coli

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REFERENCES


