Chromosomal Location of the mop (groE) Gene Necessary for Bacteriophage Morphogenesis in Escherichia coli

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The chromosomal location of a host gene, mop (groE), which is essential for the morphogenesis of several bacteriophages in Escherichia coli, was determined by two- and three-factor transductional crosses using phage P1. Cotransduction frequencies between mop and other markers were: aspA, 90%; ampA, 77%; frdA, 73%; mel, 24%. The sequence of markers in the corresponding segment (mel to purA; 91.5 to 93.5 min) of the E. coli linkage map was shown to be mel-aspA-mop(groE)-ampA-frdA-purA.

INTRODUCTION

The correct assembly of bacteriophage particles often requires the transient participation of host functions as well as phage-coded components (Casjens & King, 1975). Mutants of Escherichia coli defective in bacteriophage morphogenesis have been isolated and the corresponding loci have been designated mop (Takano & Kakefuda, 1972), tabB (Coppo et al., 1973) or groE (Sternberg, 1973; Georgopoulos et al., 1973). These mutants exhibit a range of different phenotypes including defective head morphogenesis (with phage λ, phage T4 or both phages), defective tail morphogenesis (with phage T5; Zweig & Cummings, 1975) and temperature-sensitive bacterial growth at 43 °C. Despite this diversity, the corresponding mutations are all located in the mel to purA (92 to 93 min) region of the linkage map of E. coli K12 (Bachmann et al., 1976) and only one gene is thought to be involved. This paper reports the mapping of a representative mop mutation (groE-I) relative to other markers (aspA, ampA and frdA) in the same region of the chromosome.

METHODS

Organisms. The sources and characteristics of the strains of Escherichia coli K12 are shown in Table 1. The groE-I (mop) mutation renders bacteria resistant to phage λ and temperature-sensitive for growth at 43 °C. Growth at 37 °C was unaffected but cultures of mop mutants were normally maintained at 30 °C and transductions with NS-1 as the recipient were also performed at this temperature. The ampAI mutation permits growth in the presence of sodium ampicillin (10 μg ml⁻¹; Penbritin, Beecham Research Laboratories, Brentford, Middx). Strains possessing the gltC8 mutation express the glutamate permease constitutively and as a consequence can use L-glutamate as sole carbon and energy source. However, their growth on glutamate is prevented by mutations in the transductionally unlinked L-aspartate gene (aspA). Mutations affecting the mel and frdA (fumarate reductase) genes prevent, respectively, growth on melibiose and anaerobic growth on glycerol medium with fumarate as the ultimate electron acceptor.

Media. The citrate-free basal minimal medium of Spencer et al. (1976) was used with substrates (mm): α-β-melibiose (5), monosodium L-glutamate (30) or glycerol (40) plus sodium fumarate (40). The glycerol plus fumarate medium also contained Difco vitamin-free Casamino acids (0.5 g l⁻¹). Media were supplemented with amino acids according to the requirements of particular strains and minimal media used in the primary selection of transductants were also supplemented with Difco nutrient broth (2 ml l⁻¹). Anaerobic incubation was in an atmosphere of H₂/CO₂ (95:5, v/v).

L broth and L agar (Lennox, 1955) was the complex medium used for maintaining and subculturing stocks. Media were solidified with Difco Bacto-agar (15 or 6 g l⁻¹) as required.
cultures had been washed in saline plus sodium citrate for 4 h to permit expression of the AmpR phenotype. The transductants were carefully purified by streaking.

JRG816 Hfr jrg823 F-

AmpA transductants were selected on L agar containing ampicillin (10 μg ml⁻¹) after transduced plating. For the 43 °C, and to plates spread with to single colonies on the selective medium before scoring the inheritance of unselected markers by replica-

el a/.

GI la1 Hfr (Frd'). Ampa transductants were selected on E. coli. The virulent derivative of phage A was kindly provided by Dr M. Gottesman and lysates of hvir and major substrates were prepared on E.

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Table 1. Strains of Escherichia coli k12

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Genotype</th>
<th>Source or derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-1</td>
<td>F⁻</td>
<td>gal, lac, groE-1 (mop), rpoB</td>
<td>N. Sternberg (Sternberg, 1973)</td>
</tr>
<tr>
<td>g11a1</td>
<td>Hfr</td>
<td>ilv, metB, ampA(λ)</td>
<td>K. G. Eriksson-Grennberg (Eriksson-Grennberg, 1968)</td>
</tr>
<tr>
<td>jrg807</td>
<td>Hfr</td>
<td>frdA(λ), ampA1, met, glutC(λ)(P1)</td>
<td>AmpA transductant from Gi11a1 into jrg810</td>
</tr>
<tr>
<td>jrg810</td>
<td>Hfr</td>
<td>aspA, met, glutC(λ)(P1)</td>
<td>R4frd/a of Spencer et al. (1976)</td>
</tr>
<tr>
<td>jrg816</td>
<td>Hfr</td>
<td>aspA, ampA1, met, glutC(λ)(P1)</td>
<td>R4/casp of Spencer et al. (1976)</td>
</tr>
<tr>
<td>jrg823</td>
<td>F⁻</td>
<td>mel-1, supE57, supF58, ampA1, glutC(λ)(P1)</td>
<td>Ymel/qa of Spencer et al. (1976)</td>
</tr>
</tbody>
</table>

Genetic methods. Transductions with the temperate phage P1 were performed by the method of Spencer et al. (1976) using a multiplicity of infection of 2. Transductants were selected on appropriate media with major substrates: melibiose (Mel⁺); glutamate (Asp⁺); glycerol plus fumarate and incubated anaerobically (Frd⁻). AmpA transductants were selected on L agar containing ampicillin (10 μg ml⁻¹) after transduced cultures had been washed in saline plus sodium citrate (5 mM), resuspended in L broth and then incubated for 4 h to permit expression of the AmpA phenotype. The transductants were carefully purified by streaking to single colonies on the selective medium before scoring the inheritance of unselected markers by replicating. For the mop marker, master plates were replicated to plates of L agar and incubated at 30 °C and 43 °C, and to plates spread with λvir and λvir. P1 (10⁶ plaque-forming units per plate) and incubated at 37 °C. The virulent derivative of phage λ was kindly provided by Dr M. Gottesman and lysates of λvir and λvir. P1 were prepared on E. coli strain w1485e and its P1-lysogen, respectively.

RESULTS AND DISCUSSION

Transduction studies with phage P1 have shown that the mop (groE, tab) locus is cotransducible with mel (22 to 32%) and purA (10 to 20%) and it has been placed between these genes at 92.6 min in the recalibrated linkage map of E. coli (Bachmann et al., 1976). This region also contains the aspartase gene, aspA, the penicillinase structural gene and its regulator, ampC and ampA respectively, and the fumarate reductase gene, frdA (Spencer et al., 1976; Normark & Burman, 1977). In addition, the gene order mel-aspAampC-ampAfrdA-purA has been established by two- and three-factor transductional crosses.

The linkage analysis has now been extended to determine the position of the mop locus relative to aspA, ampA and frdA. The results are presented in Table 2 and summarized above the linkage map shown in Fig. 1. In all the crosses mop was used as an unselected marker and inheritance of the mop mutation was detected in transductants by their resistance to λvir and P1-modified λvir and by their failure to grow at 43 °C. No segregation of these features of the Mop⁻ phenotype was ever observed. The three-factor crosses were designed so that mel (cross A), frdA (cross B) and aspA (cross C) could serve as selected markers whilst mop and ampA were unselected.

The position of mop relative to ampA was determined in cross A (Table 2) where a P1 lysate of NS-1, the mop donor, was used to transduce a mel ampA double mutant (jrg823) with mel as the outside marker for selection. The mel-mop linkage (23.7 %) is consistent with the values reported previously of 21.9 % (Takano & Kakefuda, 1972) and 32 % (Sternberg, 1973). The lower cotransduction frequency for ampA with mel (18.6 %) indicates that ampA is further than mop from mel. Also, by assuming that the least frequent class of Mel⁺ transductant (Amp⁺Mop⁺) arises by quadruple crossing-over, the gene order mel-mop-ampA is confirmed.

In cross B (Table 2) the same mop donor was used to transduce an ampA frdA double mutant (jrg807) with frdA as the selected marker. Analysis of the Frd⁺ transductants confirmed the very close linkage between ampA and frdA (95.5 %) previously reported by Spencer & Guest (1973). In addition, when the greater abundance of Amp⁺Mop⁺ transductants relative to the Amp⁺Mop⁻ class of transductants is considered in conjunction
Table 2. Transductional crosses involving mop, ampA, frdA, mel and aspA markers

<table>
<thead>
<tr>
<th>Cross</th>
<th>Donor</th>
<th>Recipient</th>
<th>Selection</th>
<th>Transductants per 10^8 P1</th>
<th>Number scored</th>
<th>Distribution of unselected markers</th>
<th>Cotransduction frequency (%)</th>
<th>Gene order indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NS-1</td>
<td>JRG823 (mel-ampAI)</td>
<td>Mel⁺</td>
<td>3.6</td>
<td>177</td>
<td>Amp⁺Mop⁻ 30</td>
<td>mel-mop (23.7)</td>
<td>mel-mop-ampA</td>
</tr>
<tr>
<td>B</td>
<td>NS-1</td>
<td>JRG807 (ampA1 frdA1)</td>
<td>Frd⁺</td>
<td>12.2</td>
<td>179</td>
<td>Amp⁺Mop⁻ 129</td>
<td>frd-mop (72.6)</td>
<td>frd-mop-ampA-frdA</td>
</tr>
<tr>
<td>C</td>
<td>NS-1</td>
<td>JRG816 (aspA ampAI)</td>
<td>Asp⁺</td>
<td>6.1</td>
<td>333</td>
<td>Amp⁺Mop⁻ 252</td>
<td>asp-mop (90.1)</td>
<td>aspA-mop-ampA</td>
</tr>
<tr>
<td>D</td>
<td>g11a1</td>
<td>NS-1</td>
<td>Amp⁻</td>
<td>38.0</td>
<td>218</td>
<td>Mop⁺ 168</td>
<td>amp-mop (77.1)</td>
<td></td>
</tr>
</tbody>
</table>

Gene order indicated: mel-mop-ampA-frdA, aspA-mop-ampA.
with the close linkage between mop and frdA (72.6%), the gene order mop–ampA–frdA is indicated. This in turn suggests that mop must be very close to aspA, because similar crosses involving aspA as the donor marker have shown that aspA is 67% linked to frdA on the same side as mop (Spencer et al., 1976).

In cross C (Table 2) mop was found to be closer to aspA than any of the other markers studied (cotransduction frequency, 90.1%). Moreover, the distribution of the unselected markers, ampA and mop, amongst the Asp+ transductants indicates the gene order aspA–mop–ampA. This confirms an earlier prediction, based solely on linkage data, that mop is further than aspA from mel (Spencer et al., 1976).

Cross D (Table 2) represents an independent determination of the mop–ampA cotransduction frequency (77.1%) based on the fraction of AmpR transductants of the mop mutant (N8-1) inheriting the mop+ marker from the ampA donor (G11a1). This cotransduction frequency agrees with indirect estimates of the mop–ampA linkage which can be calculated from the inheritance of the mop and ampA markers amongst appropriate classes of transductants in the foregoing crosses: 71.4% (cross A); 75.4% (cross B) and 84.0% (cross C).

All the information concerning the location of the mop gene is summarized in Fig. 1; relevant cotransduction frequencies determined previously are included below the map for comparison. The mop gene is clearly situated between aspA and ampA or, more precisely, between aspA and ampC because the penicillinase structural gene (ampC) has been placed next to its regulatory locus (ampA) on the side distal to purA (Normark & Burman, 1977).

Recently, the product of the mop (groE) gene has been identified as a protein of 65000 molecular weight by studies with artificially constructed λ transducing phages carrying the mop (groE) gene (Georgopoulos & Hohn, 1978; Hendrix & Tsui, 1978). The sizes of the

Fig. 1. Linkage map of Escherichia coli K12 in the 92 to 93 min region showing the relative positions of markers and frequencies of cotransduction for different pairs of markers. The cotransduction frequencies (%) are shown at the head of the arrow which points to the marker selected. The results of earlier studies are included below the map: a, Takano & Kakefuda (1972); b, Sternberg (1973); c, Coppo et al. (1973); d, Georgopoulos & Eisen (1974); e, Spencer & Guest (1973); f, Spencer et al. (1976).
**Location of the mop (groE) gene of E. coli**

cloned fragments were 7·7 and 8·4 kilobase pairs (kb). By assuming that the molecular length of phage P1 DNA is 97 kb the cotransducatonal linkages for pairs of markers can be converted into molecular lengths using the relationship of Wu (1966). This gives separations of 3·4 kb for aspA and mop and 8·0 kb for mop and ampA, indicating that the cloned mop fragments could contain just one or neither of these neighbouring genes. The latter seems more probable because no host functions other than mop were expressed by the transducing phages. By contrast, mutants hyperproducing penicillinase, due to multiple repetitions of the amp region, produce increased amounts of an unidentified protein having a molecular weight of 65000 (Normark et al., 1977). It seems highly probable that this protein is the mop (groE) gene product, particularly as the repeating units were estimated to be about 5 and 8 kb.

**REFERENCES**


 Также необходимо использовать семантический анализ для превью макроскопического анализа