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

By JOHN R. GUEST AND HELEN M. NICE

Department of Microbiology, University of Sheffield, Sheffield S10 2TN

(Received 29 June 1978)

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-1*) 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-1* (*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 *ampA1* mutation permits growth in the presence of sodium ampicillin (10 µg ml⁻¹; Penbritin, Beecham Research Laboratories, Brentford, Middx). Strains possessing the *gluC8* 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-aspartase 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 -D-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.

Table 1. *Strains of Escherichia coli* K12

Strain	Sex	Genotype	Source or derivation
NS-1	F ⁻	<i>gal, lac, groE-1 (mop), rpoB</i>	N. Sternberg (Sternberg, 1973)
G11a1	Hfr	<i>ilv, metB, ampA1(λ)</i>	K. G. Eriksson-Grennberg (Eriksson-Grennberg, 1968)
JRG807	Hfr	<i>jrdA1, ampA1, met, gltC8(λ)(P1)</i>	R4frd/ag of Spencer <i>et al.</i> (1976)
JRG810	Hfr	<i>aspA, met, gltC8(λ)(P1)</i>	R4/gasp of Spencer <i>et al.</i> (1976)
JRG816	Hfr	<i>aspA, ampA1, met, gltC8(λ)(P1)</i>	Amp ^B transductant from G11a1 into JRG810
JRG823	F ⁻	<i>mel-1, supE57, supF58, ampA1, gltC8(P1)</i>	Ymel/ga of Spencer <i>et al.</i> (1976)

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⁺). Amp^B 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 Amp^B phenotype. The transductants were carefully purified by streaking to single colonies on the selective medium before scoring the inheritance of unselected markers by replica-plating. 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 λ*vir* and λ*vir*. P1 (10⁸ 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 λ*vir* and λ*vir*. 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-aspA-ampC-ampA-frdA-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 λ*vir* and P1-modified λ*vir* 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^BMop⁺) 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^BMop⁺ transductants relative to the Amp^BMop⁻ class of transductants is considered in conjunction

Table 2. Transductional crosses involving *mop*, *ampA*, *frdA*, *mel* and *aspA* markers

Cross	Donor	Recipient	Selection	Trans- ductants per 10 ⁶ P1	Number scored	Distribution of unselected markers		Cotransduction frequency (%)	Gene order indicated
						Type	No.		
A	NS-1 (<i>mop</i>)	JRG823 (<i>mel-1 ampA1</i>)	Mel ⁺	3.6	177	Amp ^s Mop ⁻	30	<i>mel-mop</i> (23.7)	<i>mel-mop-ampA</i>
						Amp ^s Mop ⁺	3		
						Amp ^r Mop ⁻	12	<i>mel-amp</i> (18.6)	
						Amp ^r Mop ⁺	132		
B	NS-1 (<i>mop</i>)	JRG807 (<i>ampA1 frdA1</i>)	Frd ⁺	12.2	179	Amp ^s Mop ⁻	129	<i>frd-mop</i> (72.6)	<i>mop-ampA-frdA</i>
						Amp ^s Mop ⁺	42		
						Amp ^r Mop ⁻	1	<i>frd-amp</i> (95.5)	
						Amp ^r Mop ⁺	7		
C	NS-1 (<i>mop</i>)	JRG816 (<i>aspA ampA1</i>)	Asp ⁺	6.1	333	Amp ^s Mop ⁻	252	<i>asp-mop</i> (90.1)	<i>aspA-mop-ampA</i>
						Amp ^s Mop ⁺	2		
						Amp ^r Mop ⁻	48	<i>asp-amp</i> (76.3)	
						Amp ^r Mop ⁺	31		
D	G11a1 (<i>ampA</i>)	NS-1 (<i>mop</i>)	Amp ^r	38.0	218	Mop ⁺	168	<i>amp-mop</i> (77.1)	
						Mop ⁻	50

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).

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 *Amp*^R transductants of the *mop* mutant (NS-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

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 cotransductional 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

- BACHMANN, B. J., LOW, K. B. & TAYLOR, A. L. (1976). Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriological Reviews* **40**, 116-167.
- CASJENS, S. R. & KING, J. (1975). Virus assembly. *Annual Review of Biochemistry* **44**, 555-611.
- COPPO, A., MANZI, A., PULTIZER, J. F. & TAKAHASHI, H. (1973). Abortive bacteriophage T₄ head assembly in mutants of *Escherichia coli*. *Journal of Molecular Biology* **76**, 61-87.
- ERIKSSON-GRENBERG, K. G. (1968). Resistance of *Escherichia coli* to penicillins. II. An improved mapping of the *ampA* gene. *Genetical Research* **12**, 147-156.
- GEORGOPOULOS, C. P. & EISEN, H. (1974). Bacterial mutants which block phage assembly. *Journal of Supramolecular Structure* **2**, 349-359.
- GEORGOPOULOS, C. P. & HOHN, B. (1978). Identification of a host protein necessary for bacteriophage morphogenesis (the *groE* gene product). *Proceedings of the National Academy of Sciences of the United States of America* **75**, 131-135.
- GEORGOPOULOS, C. P., HENDRIX, R. W., CASJENS, S. R. & KAISER, A. D. (1973). Host participation in bacteriophage lambda head assembly. *Journal of Molecular Biology* **76**, 45-60.
- HENDRIX, R. W. & TSUI, L. (1978). Role of the host in virus assembly: cloning of the *Escherichia coli groE* gene and identification of its protein product. *Proceedings of the National Academy of Sciences of the United States of America* **75**, 135-139.
- LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**, 190-206.
- NORMARK, S. & BURMAN, L. G. (1977). Resistance of *Escherichia coli* to penicillins: fine-structure mapping and dominance of chromosomal beta-lactamase mutations. *Journal of Bacteriology* **132**, 1-7.
- NORMARK, S., EDLUND, T., GRUNDSTRÖM, T., BERGSTRÖM, S. & WOLF-WATZ, H. (1977). *Escherichia coli* K-12 mutants hyperproducing chromosomal beta-lactamase by gene repetitions. *Journal of Bacteriology* **132**, 912-922.
- SPENCER, M. E. & GUEST, J. R. (1973). Isolation and properties of fumarate reductase mutants of *Escherichia coli*. *Journal of Bacteriology* **114**, 563-570.
- SPENCER, M. E., LEBETER, V. M. & GUEST, J. R. (1976). Location of the aspartase gene (*aspA*) on the linkage map of *Escherichia coli* K12. *Journal of General Microbiology* **97**, 73-82.
- STERNBERG, N. (1973). Properties of a mutant of *Escherichia coli* defective in bacteriophage lambda head formation (*groE*). I. Initial characterization. *Journal of Molecular Biology* **76**, 1-23.
- TAKANO, T. & KAKEFUDA, T. (1972). Involvement of a bacterial factor in morphogenesis of bacteriophage capsid. *Nature New Biology* **239**, 34-37.
- WU, T. T. (1966). A model for three-point analysis of random general transduction. *Genetics* **54**, 405-410.
- ZWEIG, M. & CUMMINGS, D. J. (1975). Cleavage of head and tail proteins during bacteriophage T5 assembly: selective host involvement in the cleavage of a tail protein. *Journal of Molecular Biology* **80**, 505-518.