The Isolation of *fumB* Mutants of *Escherichia coli*

By JOAN M. HENSON,* NANCY K. BLAKE AND LYNN E. MAREK

Department of Microbiology, Montana State University, Bozeman, Montana 59715, USA

(Received 13 February 1987; revised 18 April 1987)

Escherichia coli strains lacking the terminus region of the chromosome (min 29–36) due to an IS10-promoted deletion did not grow well in rich medium; they also did not grow on fumarate minimal medium because *fumAC* (min 35·7) is deleted. Strains with secondary mutations that partially suppress the deletion phenotype displayed healthier growth on rich medium and grew on minimal fumarate medium. These suppressor mutants had an IS10 insertion just upstream of the *fumB* structural gene (min 93·4). A strain with a Tn10 insertion at this location was constructed and used to delete nonessential *fumB*; *fumB* deletion mutants grew well on both rich and minimal fumarate media.

INTRODUCTION

In a previous study, *Escherichia coli* strains with large terminus region deletions were isolated (Henson & Kuempel, 1985). One mutant, strain PLK1608, lacked 7 min of terminus region DNA (min 29–36). The deletion event was promoted by IS10 and the deletion strain had at least one remaining IS10 in its genome. This strain had a number of unusual properties; it formed many filamentous cells and lacked DNA termination blocks that are present in wild-type strains. In addition, the strain grew poorly on complex medium and not at all on minimal fumarate medium. These growth deficiencies were at least partially attributed to deletion of *fumAC* (min 35·7), because Guest & Roberts (1983) found that *fumA* point mutants also grow poorly on rich medium and do not grow aerobically on fumarate minimal medium. It was also reported (Guest & Roberts, 1983; Guest *et al.*, 1985) that *fumA* point mutations could be complemented by *fumB* (min 93·7) if *fumB* were present in high copy, such as on a multicopy vector.

We describe here the isolation of strains with insertion mutations that suppress the Fum[−] phenotype of large terminus region deletion strains.

METHODS

Strains and media. *Escherichia coli* K12 strains used in this study are listed in Table 1. Bacteria were grown on LB (complex) or M9 (minimal) media (Miller, 1972) at 37 °C. M9 medium supplemented with 1·0% (w/v) fumarate instead of glucose was used to score strains for *fumA* and *fumB*. Tetracycline resistant strains (Tc^r) were selected and scored on LB medium containing 20 µg tetracycline ml^{−1}, whereas tetracycline sensitive (Tc^s) strains were isolated from fusaric acid medium (Bochner *et al.*, 1980). MacConkey medium (Miller, 1972) with 1% (w/v) mannose was used to score *manA*; M9 medium containing 1% (w/v) melibiose instead of glucose was used to score *melA*; *purA* was scored on minimal medium lacking adenine, and *arg*⁺, *leuB*⁺ and *his*⁺ recombinants were selected on minimal media lacking arginine, leucine and histidine, respectively. 'Dark' and 'light' colony morphology phenotypes were scored on LB plates that were incubated for approximately 1 week.

Genetic manipulations. Bacteriophage P1 transductions and Hfr matings were done as described by Miller (1972) and purified recombinants were scored for other markers on appropriate media. Tc^s *fumB*–*mel* deletion mutants were isolated from fusaric acid medium and then scored for *mel*.

Plasmid pJH69 was constructed by subcloning *Pst*I restriction fragments from pLC4-5, which carries *fumB*⁺ (Guest *et al.*, 1985) (Fig. 1), into the *Pst*I site of pACYC177 (Chang & Cohen, 1978). Plasmids pLC4-5 and pACYC177 were digested with *Pst*I, mixed and ligated with T4 DNA ligase (New England Biolabs).

Table 1. *E. coli* K12 strains

Strain	Relevant genotype of phenotype	Reference or source
PLK1608	F ⁻ <i>trpR trpA9605 his-29 ilv pro-2 arg-427 thyA deoB</i> (or <i>deoC</i>) <i>tsx</i> λ ⁻ Δ5	Henson & Kuempel (1985)
JH122	F ⁻ <i>thr-1 leuB6 thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL31 tsx-33</i> λ ⁻ <i>supE44 zdg-232::Tn10</i> Δ5	This study
JH123	Hfr PO 2A of Cavalli Hfr between <i>purE</i> and <i>fep</i> , transferring <i>purE</i> first. <i>relA1 tonA22</i> λ ⁻ <i>zdg-232::Tn10</i> Δ5 <i>zjd::IS10</i>	This study
JH125	Hfr PO 45 between <i>serA</i> and <i>lysA</i> , transferring <i>lysA</i> first. <i>thi-1 relA1</i> λ ⁻ <i>zdg-232::Tn10</i> Δ5 <i>zjd::IS10</i>	This study
JH148	Hfr PO 1 between <i>pyrB</i> and <i>pil</i> , transferring <i>pil</i> first. <i>thi-1 relA spoT1</i> λ ⁻ <i>supQ8 zdg-232::Tn10</i> Δ5 <i>zjd::IS10</i>	This study
JH162	Hfr PO 13 between <i>rbs</i> and <i>ilvE</i> , transferring <i>rbs</i> first. <i>thi-1 leu-6 gal-6 lacY1</i> (or <i>lacZ4</i>) λ ⁻ <i>supE44 zdg-232::Tn10</i> Δ5 <i>zjd::IS10</i>	This study
JH163	Hfr PO 18 between <i>argE</i> and <i>purA</i> , transferring <i>argE</i> first. <i>malB</i> λ ⁻ <i>supE44 zdg-232::Tn10</i> Δ5 <i>zjd::IS10</i>	This study
JH207	Hfr PO 46 between <i>pyrE</i> and <i>ilvE</i> , transferring <i>ilvE</i> first. <i>supE44</i> λ ⁻ <i>zdg-232::Tn10</i> Δ5	This study
JH208	JH207 with <i>zjd::IS10</i>	This study
JH246	JH207 with <i>zjd-593::Tn10</i>	This study
M2508	Hfr PO2A between <i>purE</i> and <i>fep</i> transferring <i>purE</i> first. <i>relA1 spoT1 metB1 melA7</i>	<i>E. coli</i> *
ES4	F ⁻ <i>tonA2 lacY</i> (or <i>lacZ</i>) <i>tsx-1</i> (or 70) <i>supE44 gal-6</i> λ ⁻ <i>mtl-2 purA45</i>	<i>E. coli</i> *
JH272	M2508 with <i>zdg-232::Tn10</i> and Δ5	This study
JH290	M2508 with <i>zjd-593::Tn10</i> and <i>melA</i> ⁺ by phage P1 transduction	This study
JH374	JH290 with a <i>Tn10</i> -promoted deletion from <i>zjd-593::Tn10-mel</i>	This study
JH377	JH290 with a <i>Tn10</i> -promoted deletion from <i>zjd-593::Tn10-mel</i>	This study
JH378	JH290 with a <i>Tn10</i> -promoted deletion from <i>zjd-593::Tn10-mel</i>	This study
JH400	JH374 with <i>zdg-232::Tn10</i> and a terminus region deletion extending from <i>manA-dgsA</i> by P1 transduction	This study
pLC4-5 /JA200	pLC4-5 is from the hybrid plasmid collection of Clarke & Carbon (1976) and carries <i>fumB</i> ⁺ , <i>lysU</i> ⁺ and <i>cadA</i> ⁺ . JA200: F ⁺ <i>thr-1 leuB6 trpE5 thi-1 recA56</i>	<i>E. coli</i> *

* Genetic Stock Center, Yale University, Conn., USA.

Transformation was done as described by Dagert & Ehrlich (1979) and recombinant plasmids were purified from kanamycin-resistant but ampicillin-sensitive clones. Purified plasmids were subjected to further restriction enzyme analysis, and a comparison of their restriction sites with the restriction map of the *fumB* region devised by Guest *et al.* (1985) allowed identification of subcloned pLC4-5 *Pst*I fragments.

Plasmid pJH25 was constructed by digesting pLC4-5 and pACYC177 with *Hind*III restriction enzyme, mixing the digests, and ligating the fragments with T4 DNA ligase. Since both plasmids had only one *Hind*III site, pJH25 was a composite of pACYC177 and pLC4-5. Recombinant plasmid was isolated from ampicillin-resistant but kanamycin-sensitive transformants and subjected to further restriction enzyme analysis to confirm its identity.

DNA preparations. Chromosomal DNA was extracted from 100 ml cultures by the method of Deonier & Mirels (1977) except that the DNA was not deliberately sheared. Plasmid DNA was prepared essentially according to the Sarkosyl lysate procedure of Clewell (1972) but Triton X-100 (0.2% final concentration) was added instead of Sarkosyl.

Restriction enzymes were purchased from New England Biolabs and used as directed. Electrophoresis of DNA was done in 0.6% submerged, horizontal agarose gels in a buffer of 80 mM-Tris/borate (pH 8.3)/1 mM-EDTA. Restriction fragments of λc1857Sam7 served as *M*_r standards (Daniels & Blattner, 1982). Gels were run at 50 mA for 4–6 h, stained with ethidium bromide and photographed under UV light. Gels were dried on Whatman paper at room temperature and stored until their use for DNA–DNA hybridization.

Nick translation and hybridization. Thymidine [α -³²P] triphosphate [approximately 3200 Ci mmol⁻¹ (1.184 × 10¹⁴ Bq mmol⁻¹), New England Nuclear] was used to nick translate plasmid DNA according to the procedure of Rigby *et al.* (1977). Ethanol-precipitated, labelled plasmid DNA was resuspended in 1 ml buffer (10 mM-Tris, pH 7.6) and boiled for 10 min to denature the DNA; the probe contained approximately 10⁸ c.p.m. (μg DNA)⁻¹.

Fig. 1. Map of the *fumB* region of the chromosome (Guest *et al.*, 1985) indicating positions of suppressor insertions upstream of the *fumB* structural gene. pLC4-5 is from the Clarke-Carbon collection of plasmids (Clarke & Carbon, 1976). $\uparrow$ are *Hind*III sites; $\top$ are *Pst*I sites.

Probe DNA was added to 50 ml 5 $\times$ SSC (0.75 M-NaCl/0.075 M-sodium citrate) with 50 mM-sodium phosphate buffer (pH 7.0) and nonfat dry milk (2.5 mg ml⁻¹) (Carnation). In DNA-DNA hybridizations on nitrocellulose filters, nonfat dry milk (BLOTTO) is a good substitute for Denhardt's ingredients (Ficoll, polyvinylpyrrolidone and bovine serum albumin) and salmon testes DNA (Johnson *et al.*, 1984). We report here and in a preliminary communication (Makara & Henson, 1986) that BLOTTO could also replace Denhardt's ingredients when DNA-DNA hybridizations were done in dried gels.

DNA in dried gels was hybridized with ³²P-labelled plasmid DNA according to Tsao *et al.* (1983) with several modifications. A gel dried on Whatman paper was soaked in 0.5 M-NaOH/1.5 M-NaCl in a Pyrex tray to denature the DNA in the gel. The Whatman paper was peeled from the gel when it became wet. After 15–30 min, the gel was folded and placed in a 50 ml disposable centrifuge tube (Falcon 2098). It was briefly rinsed with 50 ml 20 $\times$ TES (1.0 M-Trizma Base/20 mM-EDTA/3.0 M-NaCl, brought to pH 8.0 with HCl) and then allowed to soak in another 50 ml of this solution for 15–30 min. The TES was removed and the probe solution was added. Hybridization was done in the Falcon tube at 68 °C in a shaking water bath (100 r.p.m.). After 24 h the gel was placed in a Pyrex pan, washed twice with 5 $\times$ SSC for 30 min at room temperature and washed three times (1–2 h for each wash) in 2 $\times$ SSC containing 0.1% SDS at 42 °C. The gel was then air dried on a 'seal-a-meal' bag, wrapped and placed on Kodak XAR-5 film with an intensifying screen at –70 °C for an appropriate period after which the film was developed.

RESULTS AND DISCUSSION

Mutant isolation

An unhealthy *E. coli* terminus region deletion mutant (strain PLK1608, containing $\Delta 5$) formed flat, transparent colonies which we called 'light' morphology (Henson & Kuempel, 1985). After several days 'dark' papillae grew in the centre of these colonies and appeared to be growing more vigorously than the colonies from which they arose. It was possible that these

Table 2. *Interrupted matings with various Hfr strains and recipient strain JH122*

Donor Hfr and direction of transfer	Marker selected	Percentage recombinants with 'dark' phenotype
JH125 (min 63→62)	<i>his</i> ⁺	0 (0/100)
JH148 (min 97→98)	<i>leuB</i> ⁺	0 (0/100)
JH162 (min 85→84)	<i>his</i> ⁺	0 (0/74)
JH163 (min 91.5→90)	<i>argE</i> ⁺	0 (0/100)
JH208 (min 81→82)	<i>argE</i> ⁺	26 (9/34)
JH123 (min 13→12)	<i>leuB</i> ⁺	8 (8/100)

healthier derivatives of strain PLK1608 had suppressor mutations that resulted from transposition of an *IS10* because strain PLK1608 was known to have at least one remaining *IS10* in its genome (Henson & Kuempel, 1985), and because the number of papillae which arose in each colony was consistent with the frequency of *IS10* transposition (Kleckner, 1983). Also, since *Tn10* and *IS10* are capable of inactivating genes, or altering gene expression, upon transposition (reviewed by Kleckner, 1983) we speculated that the 'dark' colony phenotype could be the result of either gene activation or inactivation by *IS10*.

To test whether cells of the 'dark' colonies had a transposed *IS10*, chromosomal DNA was extracted from purified 'light' and 'dark' strains, digested with *Pst*I, and hybridized with nick-translated, ³²P-labelled pRT16, a plasmid that carries *Tn10* (Jorgensen *et al.*, 1979). Both 'light' and 'dark' strains had at least one *Pst*I fragment that contained *IS10*, as was expected since the parental strain PLK1608 still contained an *IS10*. However, 'dark' strains contained an additional fragment that hybridized with the *Tn10* plasmid (G. P. Magnant and J. M. Henson, unpublished). The size (8.0 kb) of this new *Pst*I fragment was identical in seven 'dark' strains that were analysed, suggesting that the 'dark' colony phenotype was the result of transposition of *IS10*, which is 1.4 kb in size and lacks *Pst*I restriction sites, into a particular 6.6 kb *Pst*I fragment.

Preliminary mapping of the IS10 suppressor mutation

Mutations that suppressed any defects caused by a terminus region deletion were of particular interest since there was a possibility that they defined new genes or sites that are involved in DNA replication. The general location of the suppressor mutation was determined by interrupted mating experiments. Deletion 5 was introduced into various Hfr strains by phage P1 transduction and 'dark' derivatives of these deletion-mutant Hfr strains were isolated (Table 1). 'Dark' Hfr strains were then mated with light $\Delta 5$ recipients and the number of recombinants that displayed the 'dark' colony phenotype was scored (Table 2). Only two Hfr strains transferred the suppressor mutation relatively early. One of these, strain JH208, had a transfer point of origin at min 81 and transferred min 82 early, whereas the other Hfr (strain JH123) had a transfer point of origin at min 13 and transferred min 12 early. These two mating experiments suggested that the suppressor mutation was located between min 82 and min 13 on the *E. coli* map (Bachmann, 1983). Because Hfr strains JH163 and JH148 did not transfer the suppressor mutation, its location was further assigned to the region between min 92 and 97.

Isolation of a Tn10 suppressor mutation

Unlike the dark phenotype, Tc^r is a selectable and easily scored phenotype. Therefore, we next looked for a 'dark' derivative of Hfr JH207 which had a complete *Tn10*, encoding Tc^r, inserted at the suppressor site. Strain JH207 contained *zdg-232::Tn10* (min 36.6), as well as an *IS10* associated with $\Delta 5$, and we expected that this *Tn10* could also occasionally transpose to the

suppressor site between min 92 and 97 to generate 'dark' derivatives of strain JH207. Of 76 'dark' derivatives of strain JH207, two transferred Tc^r early to a wild-type recipient and one of these (strain JH246) was chosen for further study. Strain JH246 contained a Tn10 at the suppressor site, because when it was used as the donor in a bacteriophage P1 transduction to transduce Tc^r into strain PLK1608 ('light', $\Delta 5$), 100% of the Tc^r transductants also had the 'dark' colony phenotype.

Mapping the Tn10 suppressor mutation

Although the suppressor Tn10 did not cotransduce with *pyrB* (min 96.5), it did cotransduce at a high frequency with *melA* (min 93.4); when P1 was grown on strain JH246 (*melA*⁺ Tc^r) and used to transduce Tc^r into strain M2508 (*melA* Tc^s), 68% (272/400) of the Tc^r transductants were *melA*⁺. The suppressor Tn10 also cotransduced at a detectable rate with *purA* (min 95). Again P1 was grown on strain JH246 (*purA*⁺ Tc^r); 21.5% (86/400) of the Tc^r transductants of recipient strain ES4 (*purA* Tc^s) were also *purA*⁺. Some transductants were probably Tc^r because of transduction of *zdg-232::Tn10* instead of the suppressor Tn10 from the donor, although *zdg-232::Tn10* transduces at low frequency because of its close proximity to $\Delta 5$ in strain JH246. Hence, cotransduction of the suppressor Tn10 with *melA* and *purA* was probably higher than 68% and 21.5%, respectively. Because these transduction data placed the suppressor Tn10 very near *melA*, between min 93 and 94, it was designated *zjd-593::Tn10*.

Guest and co-workers (Guest & Roberts, 1983; Guest *et al.*, 1985) recently identified and mapped *fumB*, a gene that allows growth of a *fumA1* point mutant on fumarate minimal medium when present in high copy. They also found that *fumB* was closely linked to *melA*, constructed a restriction map of several plasmids which carry the *melA-fumB* region of the chromosome and determined the direction of transcription of the *fumB* gene. *fumA1* mutants are unable to grow aerobically on fumarate or acetate, but *fumB*⁺, if present in high copy, such as on a ColEI-*fumB*⁺ plasmid, allows growth of *fumA1* mutants on fumarate minimal medium.

The *fumA* gene is located in the terminus region of the chromosome and is deleted from strain PLK1608. Because of the close proximity of *fumB* to *melA*, it seemed possible that *zjd-593::Tn10* was located upstream of the *fumB* structural gene and caused an increased expression of this gene. A finding that supported this possibility was that 'dark' strains grew on minimal fumarate medium, whereas 'light' strains did not. A relatively strong promoter (p-OUT) is present on IS10 and Tn10 and capable of directing transcription of adjacent chromosomal DNA (Kleckner, 1983).

To determine if *zjd-593::Tn10* was located near the promoter region of the *fumB* gene, DNA-DNA hybridization experiments were conducted with nick-translated pLC4-5 (*fumB*⁺) and chromosomal restriction fragments from several insertion mutants and a wild-type strain (Fig. 2). Parental strain M2508 (Fig. 2*a*, lane 1) had two *Pst*I fragments, approximately 4.6 and 6.6 kb, that hybridized with pLC4-5. Smaller *Pst*I fragments that hybridized with pLC4-5 were cut from the photograph of the autoradiogram in Fig. 2. In 'dark' strains that had *zjd-593::Tn10* (Fig. 2*a*, lanes 2-4), the 6.6 kb *Pst*I fragment was missing and a 16 kb fragment appeared, as would be expected if Tn10 (which is 9.6 kb and contains no *Pst*I sites) inserted into the 6.6 kb *Pst*I fragment. In strains that had an IS10 insertion (Fig. 2*a*, lanes 6 and 7) the 6.6 kb fragment is missing and an 8.0 kb band appears, as would also be expected if IS10 inserted into the 6.6 kb fragment. Of 16 insertion mutants tested by hybridization, all 16 had an insertion in the 6.6 kb *Pst*I fragment just upstream of the *fumB* structural gene (kb 11-17 on Guest's map of the *fumB* region) (Fig. 1). Chromosomal DNA from strain M2508 and various insertion strains was also digested with *Hind*III and hybridized with pLC4-5 (Fig. 2*b*). M2508 had a high-*M_r* fragment and an 8.0 kb *Hind*III fragment that hybridized with labelled probe (Fig. 2*b*, lane 1). Most insertion strains had a *Hind*III pattern that looked identical to the parental strain (Fig. 2*b*, lanes 2 and 3). This would be expected if their insertions were in the large *Hind*III fragment; high-*M_r* fragments with IS10 insertions would not be resolved from the high-*M_r* wild-type fragment with electrophoresis conditions used in this study. An occasional IS10 insertion strain, however, was missing the 8.0 kb *Hind*III fragment and a 9.5 kb band appeared instead (Fig. 2*b*, lane 4). These results indicated that although all insertions occurred in the 11-17 kb *Pst*I fragment, they could

Fig. 2. Autoradiogram of a 0.6% agarose gel of DNA that was hybridized with ^{32}P -labelled pLC4-5. (a) Chromosomal DNA from various strains digested with *Pst*I: lane 1, DNA from strain M2508; lanes 2-4, DNA from several strains with *zjd-593*; lane 5, DNA from strain JH272 (M2508 with *zdg-232::Tn10* and $\Delta 5$); lanes 6 and 7, DNA from two strains that have *zjd::IS10*. (b) Chromosomal DNA digested with *Hind*III: lane 1, chromosomal DNA from M2508; lanes 2 and 3, chromosomal DNA from strains with *zjd-595::IS10*; lane 4, chromosomal DNA from a strain with *zjd-594::IS10*. (c) Chromosomal DNA from two *zjd-593::Tn10-mel* deletion strains (JH377, JH378) and a wild-type strain digested with *Hind*III and hybridized with pLC4-5.

occur on either side of the *Hind*III site located at kb 12.5 on Guest's map of the *fumB* region. One *IS10*, located to the left of the *Hind*III site at kb 12.5, was designated *zjd-594::IS10*, whereas another *IS10* on the right of the *Hind*III site was called *zjd-595::IS10*. Because *zjd-594::IS10* was in the 11-17 kb *Pst*I fragment and the 4-12 kb *Hind*III fragment, its location could be narrowed to within kb 11-12. Guest *et al.* (1985) (J. R. Guest, personal communication) predicted that the *fumB* structural gene starts at kb 11.5 ± 0.2 kb. This would place *zjd-594::IS10* immediately upstream of *fumB*. Since *zjd-595::IS10* was to the right of the *Hind*III site at 12.5 kb but still within the 11-17 kb *Pst* fragment, it was situated between kb 12.5 and 17 kb on the *fumB* region map.

The exact location of *zjd-593::Tn10* could not be discerned from our hybridization experiments. Strain JH290 had *Hind*III fragments of the same size as its parental strain M2508; however, because *Tn10* has two *Hind*III sites, the *Tn10* could have been located on either side of the kb 12.5 *Hind*III site and given this result. The *Tn10* was within 2 kb of the *Hind*III site; if it was at a greater distance, new *Hind*III fragments of sufficient size to be detected by hybridization would have been generated.

Construction of *fumB* deletion mutants

Since there are no known *fumB* mutants, we used *zjd-593::Tn10* to construct *fumB-mel* deletions. *Tn10* is known to promote deletions of adjacent DNA (Kleckner, 1983) and Tc^S deletion mutants can be isolated on fusaric acid medium (Bochner *et al.*, 1980). Tc^S derivatives of strain JH290 were screened for Mel^- ; since *fumB* lies between the *mel* operon and *zdg-593::Tn10*, it was assumed to be deleted in these strains. Subsequent hybridization experiments with pLC4-5 demonstrated that all of these deletions were missing the *fumB* *Pst*I restriction fragment and all of the DNA on the *Hind*III fragment from kb 4-12.5 that shares homology with pLC4-5 (Fig. 2c). Since *IS10* is also notorious for causing inversions, as well as deletions of adjacent DNA (Kleckner, 1983), we also did a hybridization experiment with pLC4-5 and deletion strain chromosomal DNA doubly digested with *Pst*I and *Eco*RI. All eight

deletion strains were missing the *Pst*I/*Eco*RI fragment from kb 9–11 (data not shown). This fragment would have been present in any strains that had an inversion of DNA from *zjd-593::Tn10-mel* instead of a deletion of that DNA.

All eight deletion strains grew well aerobically in fumarate minimal or rich media, which demonstrated that the *fumB* gene is not essential for growth under these conditions.

Complementation studies with pLC4-5 subcloned fragments

Although it seemed likely that *Tn10* was providing a relatively strong promoter for increased expression of *fumB*, it was also possible that *zjd-593::Tn10* was inactivating a gene whose product represses *fumB*. Therefore, we subcloned the *Pst*I fragment from kb 11–17 into the *Pst*I site of pACYC177 (Chang & Cohen, 1978) and transformed 'dark' strains with the recombinant plasmid pJH69. If the subcloned fragment encoded a repressor of *fumB*, one might expect that the phenotype of transformed 'dark' strains would turn to 'light' and they would be unable to grow on fumarate. However, 'dark' strains remained 'dark' and grew on fumarate minimal medium. We also did complementation tests with plasmid pJH25 which is a composite plasmid of pLC4-5 and pACYC177. If pLC4-5 has a *fumB* repressor gene, it must span the *Hind*III site in the plasmid because it can be inactivated on either side of this site. We reasoned that if pLC4-5 was cut with *Hind*III and subcloned into the *Hind*III site of pACYC177, then the putative repressor gene would be inactivated and Δ *fumAC* Δ *fumB* strains transformed with this hybrid plasmid would grow faster on minimal fumarate medium than Δ *fumAC* Δ *fumB* mutants that were transformed with pLC4-5 (with an intact putative *fumB* repressor gene). A mutant deleted for *fumAC* and *fumB*, strain JH400, was constructed by transducing Δ 5 into strain JH374 (Δ *fumB*). The mutant transformed with pJH25 did not grow faster on fumarate than the mutant transformed with pLC4-5 (data not shown).

Guest *et al.* (1985) suggested that *fumA* and *fumB*, which share homology, may act as positive regulatory elements for *fumC*, which in turn would encode a fumarase that catalyses the interconversion of fumarate and L-malate in the TCA cycle. However, growth can occur on minimal fumarate in the absence of *fumC* and *fumA* which suggests that *fumB* encodes a fumarase instead of a positively regulating factor acting on *fumC*. Recent experiments done by S. A. Woods and J. R. Guest demonstrated that *fumA* and *fumC* encode independent fumarases (J. R. Guest, personal communication). Because *fumB* and *fumA* share homology, *fumB* would also presumably encode a fumarase. Perhaps *fumB* is a poorly expressed vestigial *fumA* gene, as suggested by Guest *et al.* (1985).

The Fum⁻ phenotype of smaller *fumAC* deletions was also suppressed by *zjd::IS10* or *Tn10* insertions. However, the insertions did not suppress other abnormal phenotypic properties of large terminus region deletion strains; for example, 'dark' derivatives of strain PLK1608 still formed many filamentous cells. Although other possibilities exist, it was likely that *Tn10* or *IS10* insertions 1–3 kb upstream of the *fumB* structural gene suppressed the Fum⁻ phenotype of terminus region deletion strains by providing a stronger promoter for greater expression of *fumB*.

We thank Drs B. Bachmann, J. R. Guest and P. K. Kuempel for strains and helpful discussion. We especially thank Dr Guest for communicating his results prior to publication. This work was funded by MONTS, and grant DMB-8507753 from the National Science Foundation.

REFERENCES

- BACHMANN, B. J. (1983). Linkage map of *Escherichia coli* K-12, edition 7. *Microbiological Reviews* **47**, 180–230.
- BOCHNER, B. R., HUANG, H. C., SCHIEVEN, G. L. & AMES, B. N. (1980). Positive selection for loss of tetracycline resistance. *Journal of Bacteriology* **143**, 926–933.
- CHANG, A. C. Y. & COHEN, S. N. (1978). Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *Journal of Bacteriology* **134**, 1141–1150.
- CLARKE, L. & CARBON, J. (1976). A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *Escherichia coli* genome. *Cell* **9**, 91–99.

- CLEWELL, D. B. (1972). Nature of ColE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. *Journal of Bacteriology* **110**, 667-676.
- DAGERT, M. & EHRLICH, S. D. (1979). Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. *Gene* **6**, 23-28.
- DANIELS, D. & BLATTNER, F. (1982). Nucleotide sequence of the Q gene and Q to S intergenic region of bacteriophage λ . *Virology* **117**, 81-92.
- DEONIER, R. C. & MIRELS, L. (1977). Excision of F plasmid sequences by recombination of directly repeated insertion sequence 2 elements. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 3965-3969.
- GUEST, J. R. & ROBERTS, R. E. (1983). Cloning, mapping and expression of the fumarase gene of *Escherichia coli* K12. *Journal of Bacteriology* **153**, 588-596.
- GUEST, J. R., MILES, J. S., ROBERTS, R. E. & WOODS, S. A. (1985). The fumarase genes of *Escherichia coli*: location of the *fumB* gene and discovery of a new gene (*fumC*). *Journal of General Microbiology* **131**, 2971-2984.
- HENSON, J. M. & KUEMPEL, P. L. (1985). Deletion of the terminus region (340 kilobase pairs of DNA) from the chromosome of *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 3766-3770.
- JOHNSON, D. A., GAUTSCH, J. W., SPORTSMAN, J. R. & ELDER, J. H. (1984). Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. *Gene Analytical Techniques* **1**, 3-8.
- JORGENSEN, R. A., BERG, D., ALLET, B. & REZNIKOFF, W. S. (1979). Restriction enzyme cleavage map of Tn10, a transposon which encodes tetracycline resistance. *Journal of Bacteriology* **237**, 681-685.
- KLECKNER, N. (1983). Transposon Tn10. In *Mobile Genetic Elements*, pp. 261-298. Edited by J. A. Shapiro. Orlando, Florida: Academic Press.
- MAKARA, M. P. & HENSON, J. M. (1986). BLOTTO for dried agarose gels. In *Focus*, vol. 8, pp. 14-15. Bethesda, Maryland, USA: Bethesda Research Laboratories.
- MILLER, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- TSAO, S., BRUNK, C. & PEARLMAN, R. (1983). Hybridization of nucleic acids directly in agarose gels. *Analytical Biochemistry* **131**, 365-372.