Identification of the *Escherichia coli* Cell Division Gene *sep* and Organization of the Cell Division-Cell Envelope Genes in the *sep-mur-ftsA-envA* Cluster as Determined with Specialized Transducing Lambda Bacteriophages

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From a lysogen with λ integrated in the *leu* operon, specialized transducing phages that carry the cell division, murein biosynthesis, and envelope permeability genes located about 0.5 min to the right of *leu* were isolated. These phages were used to identify the previously undiscovered cell division gene *sep*. A genetic map proves that *sep* is located in the sequence *leuA* *sep* *murE* *murF* *murC* *ddl* *ftsA* *envA*. A physical map of this region was prepared by heteroduplex analysis of the phage DNAs. Overlapping segments of host DNA extended rightward for as much as 26.4 kilobase pairs from the prophage insertion point (thought to be in *leuA*) to include all the genes through *envA*.

The *Escherichia coli* chromosome region near min 2 contains several genes involved in cell division, murein biosynthesis, and envelope permeability. The cell division genes have been identified by temperature-sensitive (Ts) mutations that cause inhibition of septum formation at high temperature, with the result that long, nonseptate filaments form.

The *fts* gene was identified first by van de Putte et al. (23) and shown to map near *leu* by conjugation (23) and phage 363 transduction (26). Hirota et al. (8) later proposed that this locus be designated *ftsA* to distinguish it from other *fts* loci around the chromosome. A mutant that carries the cell division mutation 2158(Ts) was described by Allen et al. (1); the 2158(Ts) mutation was mapped 0.5 to 0.6 min to the right of *leu* by P1 transduction (25).

We now demonstrate that the 2158(Ts) mutation defines a cell division gene separate and distinct from *ftsA* and propose the designation *sep* for this gene. Specialized transducing λ phages, isolated from an unusual lysogen with λ1857 integrated in *leu* (18), have been used to prepare genetic and physical maps of the *sep* and *ftsA* genes, as well as the murein biosynthesis genes *murE,F,C* and *ddl* (26) and the envelope permeability gene *envA* (16). *envA* possibly is involved also in cell division because the *envA* mutant forms cells in chains (16). Wijesman (26) and Wijesman and Koopman (27) have previously shown by three- and four-factor crosses that the gene order is *leu muF* *murF* *murC* *ftsA* *envA* azi. The transducing λ phages prove that the order is *leuA* *sep* *murE* *murF* *murC* *ddl* *ftsA* *envA*, which confirms the sequence published by Wijesman (26) and Wijesman and Koopman (27) and extends it to include *sep* and *ddl*. These seven genes, *sep* through *envA*, and possibly others not yet identified, are located on a segment of chromosome not more than 20.8 kilobase pairs in length.

**MATERIALS AND METHODS**

Conventions. The transducing phages that carry a wild-type allele, as *sep*, will be designated λ*sep*+, in contrast to the usual convention, to permit the distinction in future publications from derivatives that carry defective *sep* genes, e.g., λ*sep*− (*am*). The λ*leuA* phages are thought to carry only a portion of *leuA* (6) and will not be designated λ*leuA*+. Phages are named after the selection that yielded them, although they might also carry other genes. λ*sep*46, for example, was obtained by transduction selecting for *sep*+; this phage also carries *murE*, *F*, *C*, *ddl*, *ftsA*, and *envA*.

Strains. The *E. coli* K-12 strains, their characteristics, and their sources are listed in Table 1. Phage λ*+* was from A. D. Kaiser; λ*leuA13Sam7* was from J. M. Calvo. Strain 2601c was the usual host for λ; strain RH288 was the permissive host for λ*leuA13Sam7*.

Media. Yeast extract-tryptone medium (YET) (10) was used. It was supplemented with 50 or 2 µg of thymine per ml, as required, or with 0.001 M MgSO4 for lysate preparation. The NaCl concentration was 5 mg/ml unless otherwise specified. Minimal medium (10) contained glucose (10 mg/ml), thiamine·HCl (5 µg/ml), and leucine (50 µg/ml) as required.

Culture conditions. Cultures were grown with shaking. For determination of cell number, cultures
were grown at 30°C for 12 generations before use. Cells were diluted in 0.9% NaCl-0.05% formaldehyde and counted in a model ZB Coulter Counter. Absorbance was measured at 540 nm in a Zeiss PMQII spectrophotometer, using a 10-mm light path.

Growth and purification of phages. λ was induced from a lysogen by UV irradiation. HfrH73 (λi857) was induced by heat. λimm444clts56Sam7 was grown as described previously (24). Presumed double lysogens of λ and λsep+ were induced by sequential UV and heat treatments or by sequential mitomycin C treatment and heat induction. The mitomycin C concentration was 10 μg/ml, and the period of treatment was 15 min. Phages were concentrated by precipitation with polyethylene glycol (28). λleuA13Sam7 was grown on E. coli B, and the cells were concentrated before lysis with CHCl3. Phages were purified in a step gradient of CaCl2 followed by an equilibrium gradient (22). Defective transducing phages were separated from λ helper in the second gradient. Phages collected from gradients were dialyzed either against 0.01 M tris(hydroxymethyl)aminomethane (Tris) (pH 7.5) containing 0.01 M MgSO4 or against that solution diluted 1:4 with water.

λ transduction in quantitative experiments. The λ transduction procedure was essentially that of Shimada et al. (19). Infected temperature-sensitive recipient were plated on YET plates, incubated at 30°C for 3.5 h, and then shifted to 42°C to select temperature-insensitive (TS+) transductants. For transduction of envA+, the envA(λ) recipient was infected, diluted in YET broth, incubated for 2 h at 30°C, and then plated on YET plates containing 3 μg of crystal violet per ml.

Spot tests for transduction. Sterile lysates were transferred (0.05 ml) to YET plates spread with about 2 × 105 temperature-sensitive λ+ recipient cells; the plates were incubated for 3.5 h at 30°C and then shifted to 42°C. (Exceptions are noted in Table 2.) λimm444clts56Sam7 was used to make transducing phage DNA from contour length measurements or from the density of the transducing phage. The absorbance at 260 nm of phage stocks was 0.18 to 1.0.

Electron microscopy. Electron microscopy of heteroduplexes was done essentially by the method of Davis et al. (7). The identity of single-strand DNA as λ or host was based on the known length of the transducing phage DNA from contour length measurements or from the density of the transducing phage.

Contour length measurements. Lengths of DNA molecules were measured after circularization as previously described (24), except that circularization was accomplished by heating to 45°C for 1 h and the measurements were made with a map measurer.

Heteroduplex preparations. DNAs were denatured directly from the phages. A final volume of 50 μl contained 0.02 M ethylenediaminetetraacetate (10 to 35 μl), 1 N NaOH (5 μl), λimm444clts56Sam7 phage stock (5 μl), and transducing phage stock (5 to 30 μl), added in that order. After 30 min at room temperature,
the mixture was neutralized by adding 15 μl of 1 M Tris (pH 8.0). Renaturation was achieved by annealing at 30°C for at least 6 h in the presence of 50% formamide.

For the formamide technique, the spreading solution contained 40% formamide, 0.1 M ammonium acetate, 0.01 M Tris (pH 8.0), 0.05 mg of cytochrome c per ml, and 0.1 volume of the annealed DNA. The hypophase was 10% formamide in 0.01 M Tris (pH 8.0)-0.001 M ethylenediaminetetraacetate.

φX174 single-strand and replicative-form-II DNAs were included as length standards for single-strand and duplex regions. φX174 single-strand DNA was prepared from φX174am3 by the method of Brown and Dowell (3). Replicative form I was prepared by the method of Zuccarelli et al. (29), except that E. coli C was the host and the lysate was poured directly onto a sucrose gradient. Conversion of replicative form I to form II was done by the method of Smith and Vinograd (20).

Lengths were expressed in percent λ+ DNA length and had a relative standard deviation of 5% or less of the length of the interval measured, except for λsep+69, λsep+27, and λsep+46, for which the greatest deviation was 10% for the single-strand regions.

RESULTS

Isolation of λsep+ transducing phages. The availability of a lysogen with λcl857 integrated in leu (18) prompted a search for transducing phages that could transduce a 2158(Ts) strain to temperature insensitivity. A lysate from strain 73 was used to transduce strain AX655 2158(Ts) to temperature insensitivity on YET plates. The plaque-forming phage titer of strain 73 lysates was 2 × 10^6 to 1 × 10^7/ml; the frequency of temperature-insensitive transduction was about 9 × 10^-1 per plaque-forming unit. The primary heterogenotes, presumed to be 2158(Ts) (λ+), were purified and induced to yield high-frequency-transducing (HFT) lysates. To determine whether the transducing phages were plaque-forming or defective, the HFT lysates were plated to yield individual plaques. Viable phages from individual plaques were resuspended in buffer and tested for the ability to transduce a 2158(Ts) (λ+) recipient to temperature insensitivity. None of the viable phages was capable of transduction, indicating that the transducing phages were defective.

To obtain phages that transduced the leuA, murF, and ddl markers, the selection procedure was repeated with leuA (λ), murF(Ts) (λ+) and ddl(Ts) (λ+) recipients, with similar results. The frequency of transduction ranged from 10^-4 per plaque-forming unit for ddl (with λddl+24) to 2.5 × 10^-3 for murF (with λsep+27) to 5 × 10^-4 for leuA (with λleuA13).

The transducing phages were purified genetically by infecting a 2158(Ts) (λ+) recipient at low multiplicity (10^-2 viable phage per cell) with HFT lysates and selecting temperature-insensitive transductants again. Defective λleuA

| Table 2. Transduction* of genes in the leuA-sep-ftsA-envA region |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Phage           | CV512 (leuA)    | AX655 (sep2158(Ts)) | PC1239 (murE)  | PC1242 (murF)  | PC1357 (murC)  | PC1358 (ddl)   | TKF10 (ftsA10(Ts)) | AX621 (ftsA1882(Ts)) | D22 (envA) |
| λleuA13         | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λsep+82'        | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λsep+69'        | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λsep+27'        | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λsep+36'        | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λddl+24         | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λmurF+121       | +               | +                | +               | +               | +               | +               | +               | +               | +               |
| λsep+46'        | +               | +                | +               | +               | +               | +               | +               | +               | +               |

* +, Positive transduction; -, no transduction.

† Recipient. All were λ lysogens.

Identical results obtained also with strain AX732(λ-), sep2158(Ts) derivative of strain UTH4113 prepared by P1 transduction (25).

Recipient lawn was 2 × 10^6 cells on YET plates with no added NaCl; phenotypic expression was for 1 h at 30°C.

Recipient lawn was obtained with the independently isolated murC(Ts) strain TKLA6(λ-). Recipient lawn was 2 × 10^6 cells on YET plates with no added NaCl; phenotypic expression was for 3 h at 30°C.

Recipient lawn consisted of 2 × 10^6 cells on YET plates with no added NaCl; phenotypic expression was for 2 h at 30°C.

Recipient lawn was 2 × 10^6 cells on YET plates with no added NaCl; phenotypic expression was for 1 h at 30°C.

Recipient lawn was obtained with the independently isolated murC(Ts) strain TKLA6(λ-) and PC1368 (λ+). Recipient lawn was 2 × 10^6 cells on YET plates with 0.5% NaCl; phenotypic expression was for 2 h at 30°C.

Results from quantitative experiments.

Nine other independent phages of this class were isolated.

Only one phage of this class isolated.

Eleven other independent phages of this class were isolated.

Eight other independent phages of this class were isolated.

Four other independent phages of this class were isolated.

Three other independent phages of this class were isolated.
phages were similarly purified with a leuA recipient. These transductants were used for all subsequent work.

Genetic map of the leuA-sep-ftsA-envA region. Transducing phages (as HFTs) were tested to determine which of the genes in the leu-envA region were present on each phage. The results of the spot tests (Table 2) are consistent with the models stating that, in strain 73, λcI857 is integrated in leuA (or perhaps leuB) (6) and that the transducing phages formed by excision of varying lengths of host DNA to the clockwise side of leuA. The transduction results permit the following conclusions. First, the septum formation gene defined by the mutation 2158(Ts) is located between leuA and murE; the ftsA gene, defined by the 10(Ts) allele (23, 26), is located between ddl and envA. Therefore, the 2158(Ts) mutation defines a previously undiscovered cell division gene, which we now designate sep. The sep product has previously been shown to be required continuously during septum formation (or perhaps in a late stage of septation) (25). Second, the previously described cell division mutation 1882(Ts) (1, 25) appears to be located in the ftsA gene. All five phages that carry the ftsA+ gene, but not envA+ (e.g., λmurF'121), also carry the wild-type allele defined by 1882(Ts). On the other hand, the phage with ddl+ as the distal marker (i.e., λddl'Ts24) fails to transduce ftsA10(Ts) and 1882(Ts) recipients to temperature insensitivity. The ftsA gene product, as studied in a mutant that carries 1882(Ts), has been demonstrated to be required continuously during (or perhaps in a late stage of) septation (25). A similar result was obtained with the ftsA10(Ts) mutant (see below). Third, the sequence of the genes in this region is: leuA sep murE murF' murC ddl ftsA envA (see Fig. 3). All of the phages isolated are consistent with this sequence (Table 2); that is, no unusual pattern of transduction was observed with any phage.

The frequency with which HFTs transduce lysogenic and nonlysogenic recipients was approximately the same with most of the phages (Table 3). This suggests that the initial formation of transductants by the λcI857 derivative at 30°C and subsequent stable maintenance of the λcI857 prophage at 42°C were not aided by the resident cI+ prophage. (Production of HFTs did, however, require λ+ helper.) How the λcI857 derivatives form transductants stable at 42°C in the absence of cI+ repressor has not been investigated.

Physical map of the leuA-sep-ftsA-envA region. A physical map of the leuA-envA region was provided by heteroduplex analysis of the transducing phage DNAs. Heteroduplexes were prepared between λimm434 and each transducing phage of Table 2, and contour lengths of all the duplex and single-strand regions were determined, using φX174 single-strand and duplex circles as standards. A representative heteroduplex molecule and a tracing of it are shown in Fig. 1. Diagrams of each heteroduplex are presented in Fig. 2.

The λcI857 prophage in strain 73, the unusual lysogen that yielded the transducing phages, must have been integrated at or very near POP'. The position of attachment as measured in Fig. 2 is 57.1% of the λ length (average of all determinations), which agrees well with the reported (5) position of 57.3%. Formation of these transducing phages involved the deletion of λ DNA from POP' (on the standard map) for varying distances. All the phages that carry sep, however, are deleted through at least the essential λ gene J, which explains their defectiveness. To form the λsep+ phages, the orientation of the prophage in strain 73 must be leuB λJ λA λR λN leuA sep, in agreement with the data of Davis and Calvo (6) and of Klingmüller et al. (11), obtained from studies of λleu phages. This orientation is opposite that of λ integrated at att BOB'.

Table 3. Transduction frequency with lysogenic and nonlysogenic AX555 sep2158(Ts) recipients

<table>
<thead>
<tr>
<th>Phagea</th>
<th>MOIB</th>
<th>Recipient</th>
<th>Transduc- tants/PFUc</th>
</tr>
</thead>
<tbody>
<tr>
<td>λsep*27</td>
<td>0.03</td>
<td>Nonlysogen</td>
<td>2 \times 10^{-5}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>λ lysogen</td>
<td>7 \times 10^{-5}</td>
</tr>
<tr>
<td>λmurF'121</td>
<td>0.02</td>
<td>Nonlysogen</td>
<td>5 \times 10^{-4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>λ lysogen</td>
<td>7 \times 10^{-3}</td>
</tr>
<tr>
<td>λleuA1894</td>
<td>0.14</td>
<td>Nonlysogen</td>
<td>1 \times 10^{-5}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>λ lysogen</td>
<td>1.5 \times 10^{-4}</td>
</tr>
<tr>
<td>λsep*46</td>
<td>0.33</td>
<td>Nonlysogen</td>
<td>1.8 \times 10^{-5}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>λ lysogen</td>
<td>1.6 \times 10^{-4}</td>
</tr>
</tbody>
</table>

* Supplied with λ+ helper in HFTs.

b Multiplicity of infection with viable phage.

c PFU, Plaque-forming unit.

d Genetically similar to λsep*46; the host insertion covers envA.
FIG. 1. (A) Electron micrograph of a heteroduplex molecule of λsep+82 and λimm48. (B) Trace of (A). The heavy and thin lines represent duplex and single-strand DNA, respectively.

000, would be about nine. In addition to the eight listed in Fig. 3, ilvH, ilvI, and polB are thought to be located between leuA and mureE (2), which means that the identity of most of the genes of this region might be known. It is possible that the transducing phages listed in Table 2 differ from each other in some cases by only one gene (Table 4); this would aid in the identification of gene products in subsequent work.

Properties of the ftsA10(Ts) mutant. The mutations 1882(Ts) and 10(Ts) apparently are alleles of one gene, ftsA (Table 2). Strains that carry 1882(Ts) behave, in multiple temperature shift experiments, as if the ftsA+ product is required continuously during septum formation (25). The ftsA mutant 10(Ts) also has the phenotype expected if the ftsA product participates continuously in septation (Fig. 4). After the cells were shifted from 30 to 42°C, cell division stopped abruptly (11% increase; average of seven experiments). If filamentous cells were shifted to the permissive temperature, division resumed after 12 to 15 min at a rate greater than the normal 30°C rate and rapidly converted the filaments into short cells (Fig. 4A). If rapidly dividing filaments were shifted a second time to 42°C, division was abruptly inhibited (Fig. 4D). If shifted to 42°C after 25 min at 30°C, the residual increase in cell number was 25%; if shifted after 35 min at 30°C, the cell number increased only 11% (Fig. 4D). If filaments were incubated at 30°C for brief intervals of 5 or 7 min (i.e., less time than was required for division to resume) and then shifted to 42°C, the division that occurred resulted in increases of 16 and 33%, respectively. If the ftsA+ product was required for initiating septation, a doubling or quadrupling in cell number might have occurred. The results of Fig. 4C are an exception to the pattern; in this case, the filaments had been incubated at 30°C until near the beginning of the most rapid division, 10 and 15 min. Under these conditions, the filaments divided at 42°C to the extent that the cell number increased by 80 and 120%. Perhaps the ftsA10(Ts) product was not immediately denatured when the temperature was increased, or perhaps septation was essentially complete in those filaments, but the daughter cells had not separated at the time of the shift.

Is 84(Ts) an allele of ftsA? Hirota et al. (8, 9) isolated a cell division mutant in which the mutation was designated ftsA84(Ts) because it mapped near leu. Transduction by P1 demonstrated that 84(Ts) and the sep2158(Ts) markers are cotransducible with leu to approximately the same extent—36 and 32%, respectively (25). However, map position cannot always be determined accurately by cotransduction frequency, because markers thought to reside within a single allele often cotransduce with an outside

VOL. 133, 1978

λsep+ PHAGES

95

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There are reasons to question whether $84(Ts)$ is in $ftsA$. F'101 apparently carries both $sep^+$ and $ftsA^+$. It transfers temperature insensitivity to both $sep^{2158}(Ts)$ and $ftsA^{1882}(Ts)$ mutants; both $sep^+$ and $ftsA^+$ are dominant over these temperature-sensitive alleles (25). However, F'101 does not transfer complete temperature insensitivity to $84(Ts)$ mutants. A partial diploid of genotype F'101/$84(Ts)$, when shifted to 42°C, undergoes three doublings in cell number and a 10-fold mass increase but then stops both division and growth (25). It was suggested, based on the assumption that $84(Ts)$ was an $ftsA$ allele, that the $84(Ts)$ allele was not recessive to the F'101 $ftsA^+$. In physiological experiments, a $84(Ts)$ mutant behaves as if the product of the gene defined by this mutation is required for initiation of septation (25). However, it is possible that the $84(Ts)$ product is denatured slowly at 42°C (25). Finally, none of the transducing phages transduced a $84(Ts)$ or $84(Ts)(\lambda^*)$ mutant to temperature insensitivity (Table 5). Although a $84(Ts)$ mutant reverted to temperature insensitivity with high frequency, transduction, had it occurred, should have been observed with HFTs (Table 4). Moreover, attempts to transduce a $84(Ts)(\lambda^*)$ mutant to temperature insensitivity with lysates from strain 73 failed.

Perhaps the correct explanation for all of these data is that the $84(Ts)$ mutation defines still another cell division gene, this one located on the clockwise side of $envA$. The explanation that $84(Ts)$ is a dominant $ftsA$ allele also is possible.

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Fig. 2. Schematic diagrams of heteroduplexes between $\lambda imm^{24}$ and $\lambda$ or the transducing phage DNAs. An exception was $\lambda dd+24$, which was heteroduplexed with $\lambda^*$ helper and $\lambda imm^{24}$ also. Two close parallel lines represent duplex DNA; separated solid lines represent single-strand DNA; and dotted lines show continuity. The single-strand host insertions are the bottom strand in all molecules. The numbers are percent $\lambda$ length. The numbers of molecules examined were: R, 9; $\lambda sep^{+}82$, 13; $\lambda sep^{+}69$, 12; $\lambda sep^{+}27$, 13; $\lambda sep^{+}24$, 6; $\lambda sep^{+}3$, 10; $\lambda dd+24$, 16. The map is from Davidson and Szymbalski (5).
**DISCUSSION**

From a lysogen with \( \lambda \) integrated in the \( \text{leu} \) operon, transducing phages that carry the genes within 26.4 kilobase pairs to the right of the site of prophage integration have been isolated. This segment of DNA is enough to code for about 22 genes, but perhaps as few as 11 or as many as 43. The different phages carry differing lengths of host DNA. Use of these phages made possible the identification of the previously undiscovered \( \text{sep} \) gene and the preparation of an unequivocal genetic map of the region. A physical map was prepared by analysis of heteroduplex molecules formed between single strands of transducing phage and \( \lambda \text{imm}\text{434} \) DNA. Of course, the genetic map might be incomplete because additional genes in this area might remain to be identified.
Also, the physical map might be defined more precisely by additional work, because the various host insertion end points (Fig. 3) are not expected to fall cleanly between genes, and fractions of genes are likely to exist on the various host insertion ends. Moreover, it is not certain that the genes presented as the rightmost genes on the various insertions actually are the most distal genes on that insertion (e.g., envA might not be the last gene on the end of the Asep+46 insertion [Fig. 3]). Some host insertions probably differ in length by only one gene.

The close proximity of sep, the mur genes, ddl, ftsA, and envA might be more than coincidence, as pointed out by Wijesman (26). These genes are located on a segment of DNA equal to or less than 20.8 kilobase pairs in length (26.4 to 5.6), which is at most 0.51 min on the chromosome map (2). sep and ftsA products are required for septum formation, which is presumed to be a function of the membrane. Whether sep+ and ftsA+ products act on or within the cytoplasmic membrane, or on or within the outer membrane, or within the cytoplasm is yet to be determined. The envA+ product is thought to function in or on the outer membrane because the envA mutants have increased permeability to antibiotics and a failure of septum separation (16, 17). The mur and ddl genes are involved in murein biosynthesis; temperature-sensitive mutations in these genes lead to lysis at 42°C (26). murE is thought to code for meso-diaminopimelic acid adding enzyme; murF, for D-alanyl-D-alanine adding enzyme; murC, for L-alanine adding enzyme; and ddl, for D-alanine:D-alanine ligase (12–15, 26). The fact that all of these genes are involved in cell membrane-cell wall synthesis or function raises questions as to whether they might be organized into one or a few functional units and whether the expression of these genes might be coordinately regulated. The host regulatory genes (or sites) are carried on the host segments of transducing phage DNA. This conclusion is based on the observation that sep and the other genes are

Table 5. Failure to transduce a 84(Ts) strain to temperature insensitivity

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Marker</th>
<th>Phage</th>
<th>MOI</th>
<th>Revertants/recipient</th>
<th>Transductants/recipient</th>
<th>Transductants/visible phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>AX655</td>
<td>sep2158(Ts)</td>
<td>λleuA169&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16</td>
<td>&lt;1 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>2.8 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>1.7 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>AX655(λ&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>sep2158(Ts)</td>
<td>λleuA169&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16</td>
<td>&lt;1 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>3.8 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>2.2 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>AX720</td>
<td>84(Ts)</td>
<td>λleuA169&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16</td>
<td>2 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>*</td>
<td>ND</td>
</tr>
<tr>
<td>AX720(λ&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>84(Ts)</td>
<td>λmurF&lt;sup&gt;F121&lt;/sup&gt;</td>
<td>0.5</td>
<td>2 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>*</td>
<td>ND</td>
</tr>
<tr>
<td>TKF10(λ&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>ftsA10(Ts)</td>
<td>λleuA169&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16</td>
<td>2 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>*</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Medium was VET with no added NaCl.

<sup>b</sup> Supplied with λ<sup>+</sup> helper in HFTs.

<sup>c</sup> Multiplicity of infection with viable phages.

<sup>d</sup> Genetically similar to Asep+46; the host insertion covers envA.

<sup>e</sup> Tests for transduction yielded survivors with a frequency of 2 ± 0.2 × 10<sup>-4</sup>.

<sup>f</sup> ND, None detected.
expressed from the transducing prophages even in the presence of a \( \lambda^+ \) prophage. It is known that the \( \lambda \) repressor represses all \( \lambda \) functions (except cl and rex) in a stable lysogen (21); therefore, the host genes on the transducing prophages must be controlled by host regulatory functions.

Two aspects of the physiology of the \( \lambda\text{sep}^+ \) prophages are surprising. First, HFT lysates, after infection at low multiplicity, transduce \( \text{sep}^+ \) recipients at approximately the same frequency whether they be \( \lambda^+ \) lysogens or nonlysogens. This suggests that the transductants do not require the wild-type \( \lambda^+ \) repressor to be dominant over the cI857 repressor coded for by the transducing phage chromosome. Second, the phage yield in HFT lysates from \( (\lambda^+) (\lambda\text{sep}^+) \) strains is considerably lower \((10^9 \text{ to } 10^8 \text{ plaque-forming units per ml; approximately equal numbers of plaque-forming and transducing particles}) \) than is usually observed after \( \lambda \) induction. Perhaps this lower yield is related to the peculiar pattern of lysogen formation.

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