0021-9193/78/0133-0091$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 91-100
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Vol. 133, No. 1
Printed in U.S.A.

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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Received for publication 20 July 1977

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 λcI857 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). Wijsman (26) and Wijsman and Koopman (27) have previously shown by three- and four-factor crosses that the gene order is *leu murE 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 Wijsman (26) and Wijsman 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 *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*leuA*13*Sam*7 was from J. M. Calvo. Strain 2e01c was the usual host for λ; strain RH288 was the permissive host for λ*leuA*13*Sam*7.

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 $MgSO_4$ 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

TABLE 1. *Principal strains*[a]

Strain	Relevant marker	Other characteristics	Source or reference
AX621(λ⁺)	*ftsA1882*(Ts)	F⁻ *thr leu thi arg proA his gal xyl ara mtl lac rpsL*	1, 25
AX655	*sep2158*(Ts)	F⁻ *thr leu thi arg proA his gal xyl ara mtl lac rpsL*	1, 25
AX655(λ⁺)	*sep2158*(Ts)		
AX710(λ⁺)	*ftsA1882*(Ts)	F⁻ *thr nadC rpsL*	25
AX720	*84*(Ts)	F⁻ *thr nadC rpsL*	25
AX720(λ⁺)	*84*(Ts)		
AX732(λ⁺)	*sep2158*(Ts)	F⁻ *thr nadC rpsL*	25
CV512(λ)	*leuA371*	F⁺	B. Bachmann
PC1239(λ⁺)	*murE*(Ts)	F⁻ *thr leu thi pyrF codA thyA argG ilvA his lacY tonA tsx phx supE ths dra vtr uvrB sus*	E. Lugtenberg
PC1242(λ⁺)	*murF*(Ts)	As PC1239 but *xyl*	E. Lugtenberg
TKL46(λ⁺)	*murF*(Ts)	F⁻ *thr leu thi pyrF thyA lac tonA his ilvA arg tsx*	A. Rörsch
PC1357(λ⁺)	*murC*(Ts)	F⁻ *thr leu trp his thyA thi lac gal xyl mtl ara tonA phx rpsL*	E. Lugtenberg
PC1358(λ⁺)	*ddl*(Ts)	As PC1357	E. Lugtenberg
TKF10(λ⁺)	*ftsA10*(Ts)	F⁻ *thr leu thi pyrF thyA lac tonA*	H. Wijsman
D22(λ)	*envA*	F⁻ *ampA1 proA trp his rpsL*	S. Normark
D21(λ)	*envA*⁺	Parental strain of D22	S. Normark
2e01c		F⁻ *thr leu thi lac rpsL*	M. Malamy
RH288		*supF*	R. Gayda
73(λcI857)	*leu*	HfrH (*gal-uvrB*)Δ *thi*	R. Weisberg
W3101(λ*imm*⁴³⁴*cIts56-Sam7*)		*sup*⁺	L. Reichardt

[a] Strains CV512(λ), D21(λ), and D22(λ) were received as lysogens; the origins of the prophages are not certain, but they have λ immunity.

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 Z_B 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 (λcI857) was induced by heat. λ*imm*⁴³⁴*cIts56Sam7* was grown as described previously (24). Presumed double lysogens of λ⁺ and λ*sep*⁺ 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). λ*leuA13Sam7* was grown on 2e01c, and the cells were concentrated before lysis with $CHCl_3$. Phages were purified in a step gradient of CsCl 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 $MgSO_4$ 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 recipients 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 2×10^7 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.) *leu*⁺ transduction was observed by transferring 0.05 ml of lysate to about 10^7 cells of strain CV512(λ) on minimal medium lacking leucine and incubating the plate at 37°C.

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. The absorbance at 260 nm of phage stocks was 0.18 to 1.0.

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), λ*imm*⁴³⁴*cIts56Sam7* 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 φX174*am3* 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 λ*sep*+69, λ*sep*+27, and λ*sep*+46, for which the greatest deviation was 10% for the single-strand regions.

RESULTS

Isolation of λ*sep*+ transducing phages. The availability of a lysogen with λ*c*I857 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)(λ^+) to temperature insensitivity on YET plates. The plaque-forming phage titer of strain 73 lysates was 2×10^4 to 1×10^5/ml; the frequency of temperature-insensitive transduction was about 9×10^{-4} per plaque-forming unit. The primary heterogenotes, presumed to be *2158*(Ts) (λ^+) (λ*c*I857TS+), 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^{-2} per plaque-forming unit for *ddl*+ (with λ*ddl*+24) to 2.5×10^{-3} for *murF*+ (with λ*sep*+27) to 5×10^{-4} for *leuA*+ (with λ*leuA*13).

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 λ*leuA*

TABLE 2. *Transduction*[a] *of genes in the leuA-sep-ftsA-envA region*

Phage	Recipient[b]								
	CV512 *leuA*	AX655[c] *sep2158*(Ts)	PC1239[d] *murE*	PC1242[d] *murF*	PC1357[e] *murC*	PC1358[f] *ddl*	TKF10[g] *ftsA10*(Ts)	AX621[h] *ftsA1882*(Ts)	D22[i] *envA*
λ*leuA*13	+	−	−	−	−	−	−	−	−
λ*sep*+82[j]	+	+	−	−	−	−	−	−	−
λ*sep*+69[k]	+	+	+	−	−	−	−	−	−
λ*sep*+27[l]	+	+	+	+	−	−	−	−	−
λ*sep*+3[m]	+	+	+	+	+	−	−	−	−
λ*ddl*+24[k]	+	+	+	+	+	+	−	−	−
λ*murF*+121[n]	+	+	+	+	+	+	+	+	−
λ*sep*+46[o]	+	+	+	+	+	+	+	+	+

[a] +, Positive transduction; −, no transduction.

[b] Recipient. All were were λ lysogens.

[c] Identical results obtained also with strain AX732(λ^+), a *sep2158*(Ts) derivative of strain UTH4113 prepared by P1 transduction (25).

[d] Recipient lawn was 2×10^8 cells on YET plates with no added NaCl; phenotypic expression was for 1 h at 30°C.

[e] Identical results were obtained also with the independently isolated *murC*(Ts) strain TKL46(λ^+). Recipient lawn was 2×10^7 cells on YET plates with no added NaCl; phenotypic expression was for 3 h at 30°C.

[f] Recipient lawn consisted of 2×10^8 cells on YET plates with no added NaCl; phenotypic expression was for 2 h at 30°C.

[g] Recipient lawn was 2×10^7 cells on YET plates with no added NaCl; phenotypic expression was for 1 h at 30°C.

[h] Identical results were obtained also with strain AX710(λ^+), an *ftsA1882*(Ts) derivative of strain UTH4113 prepared by P1 transduction (25). Recipient lawn was 5×10^7 cells on YET plates with 0.5% NaCl; phenotypic expression was for 2 h at 30°C.

[i] Results from quantitative experiments.

[j] Nine other independent phages of this class were isolated.

[k] Only one phage of this class isolated.

[l] Eleven other independent phages of this class were isolated.

[m] Eight other independent phages of this class were isolated.

[n] Four other independent phages of this class were isolated.

[o] 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., λ*murF*$^+$121), also carry the wild-type allele defined by *1882*(Ts). On the other hand, the phage with *ddl*$^+$ as the distal marker (i.e., λ*ddl*$^+$24) 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 *c*I$^+$ prophage. (Production of HFTs did, however, require λ$^+$ helper.) How the λcI857 derivatives form transductants stable at 42°C in the absence of *c*I$^+$ 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 λ*imm*^{434} 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.

TABLE 3. *Transduction frequency with lysogenic and nonlysogenic AX655 sep2158(Ts) recipients*

Phage[a]	MOI[b]	Recipient	Transductants/PFU[c]
λ*sep*$^+$27	0.03	Nonlysogen	2×10^{-5}
		λ lysogen	7×10^{-5}
λ*murF*$^+$121	0.02	Nonlysogen	5×10^{-4}
		λ lysogen	7×10^{-3}
λ*leuA*169[d]	0.14	Nonlysogen	1×10^{-5}
		λ lysogen	1.5×10^{-5}
λ*sep*$^+$46	0.33	Nonlysogen	1.8×10^{-5}
		λ lysogen	1.6×10^{-5}

[a] Supplied with λ$^+$ helper in HFTs.
[b] Multiplicity of infection with viable phage.
[c] PFU, Plaque-forming unit.
[d] Genetically similar to λ*sep*$^+$46; the host insertion covers *envA*.

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' leftward (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 λ*sep*$^+$ phages, the orientation of the prophage in strain 73 must be *leuB* λ*J* λ*A* λ*R* λ*N* *leuA sep*, in agreement with the data of Davis and Calvo (6) and of Klingmüller et al. (11), obtained from studies of λ*leu* phages. This orientation is opposite that of λ integrated at *att* BOB'.

Measurement of the size of the host DNA segment in each transducing phage permits the construction of a physical map of the *leuA-sep-ftsA-envA* region (Fig. 3; Table 4). The segment of chromosome that extends from the prophage integration site in strain 73 (thought to be in *leuA* [6]) to cover *envA*, the rightmost known gene of this cluster, is about 26,400 base pairs. This is enough DNA to code for 22 genes, assuming that the protein products have an average molecular weight of 40,000. The minimum number of proteins that this region could code for, assuming an average molecular weight of 100,-

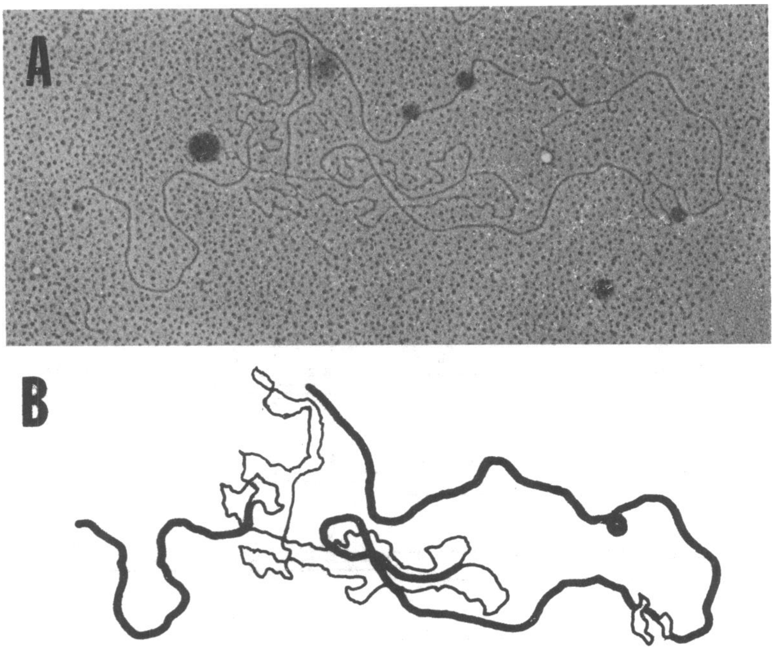

FIG. 1. *(A) Electron micrograph of a heteroduplex molecule of λsep^+82 and λimm^{434}. (B) Trace of (A). The heavy and thin lines represent duplex and single-strand DNA, respectively.*

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 *murE* (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 were 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

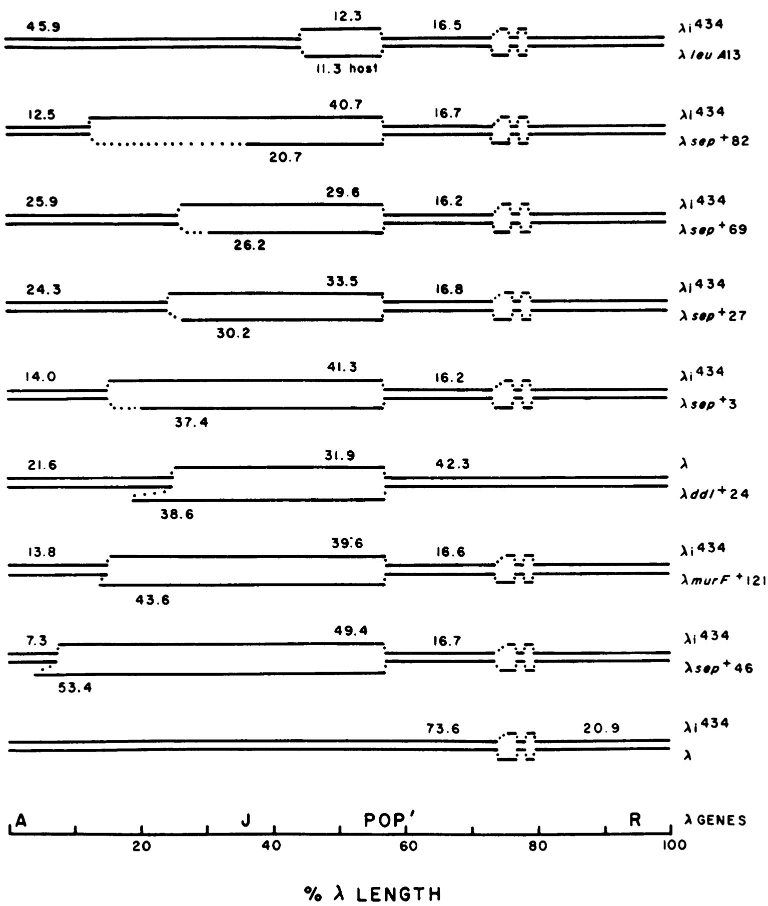

FIG. 2. *Schematic diagrams of heteroduplexes between λimm*[434] *and λ or the transducing phage DNAs. An exception was λddl*+*24, which was heteroduplexed with λ*+ *helper and λimm*[434] *also. Two close parallel lines represent duplex DNA; separated solid lines represent single-strand DNA; and dotted lines show continuity. The single-strand host insertions are the bottom strand in all molecules. The numbers are percent λ length. The numbers of molecules examined were: λleuA13/λimm*[434]*, 10; λsep*+*82/λimm*[434]*, 25; λsep*+*69/λimm*[434]*, 13; λsep*+*27/λimm*[434]*, 12; λsep*+*3/λimm*[434]*, 11; λddl*+*24/λ or λimm*[434]*, 11; λmurF*+*121/λimm*[434]*, 13; λsep*+*46/λimm*[434]*, 6. The map is from Davidson and Szybalski (5).*

marker with widely varying frequencies (e.g., 4).

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 *sep2158*(Ts) and *ftsA1882*(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)(λ+) 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)(λ+) 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.

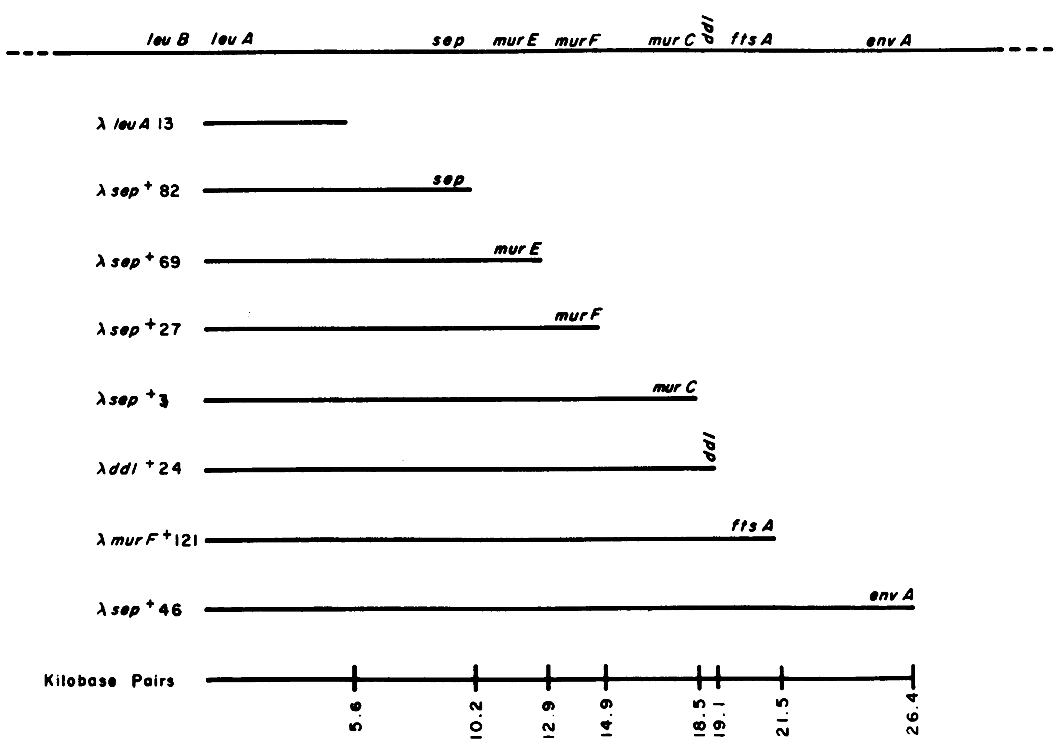

FIG. 3. *Physical map of the leuA-sep-ftsA-envA region. The host insertion of each transducing phage of Fig. 2 is diagrammed to correspond in orientation from left to right to the usual E. coli map. The E. coli chromosome segment is shown on top.*

TABLE 4. *Physical structure of the leuA-sep-ftsA-envA region*

Phage	Host insertion			Coding capacity			
	% λ Length	Kilobase pairs	Daltons ($\times 10^6$)	Protein (daltons $\times 10^5$)	Genes[a]	Genes[b]	Additional genes[c]
λ*leuA*13	11.3	5.6	3.7	1.88	5	2–9	
λ*sep*+82	20.7	10.2	6.7	3.37	8	3–17	1–8
λ*sep*+69	26.2	12.9	8.5	4.26	10	4–21	1–4
λ*sep*+27	30.2	14.9	9.8	4.92	12	5–25	1–4
λ*sep*+3	37.4	18.5	12.2	6.10	15	6–30	1–4
λ*ddl*+24	38.6	19.1	12.6	6.30	16	6–31	1
λ*murF*+121	43.6	21.5	14.2	7.10	18	7–35	1–4
λ*sep*+46	53.4	26.4	17.4	8.71	22	9–43	2–8

[a] Assuming the protein molecular weight average is 40,000.

[b] Assuming the range of protein molecular weights is 20,000 to 100,000.

[c] Increment in the number of genes carried on each phage; range of molecular weights assumed to be 20,000 to 100,000.

DISCUSSION

From a lysogen with λ integrated in the *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 *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 λ*imm*^{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 λ*sep*+46 insertion [Fig. 3]). Some host insertions probably differ in length by only one gene.

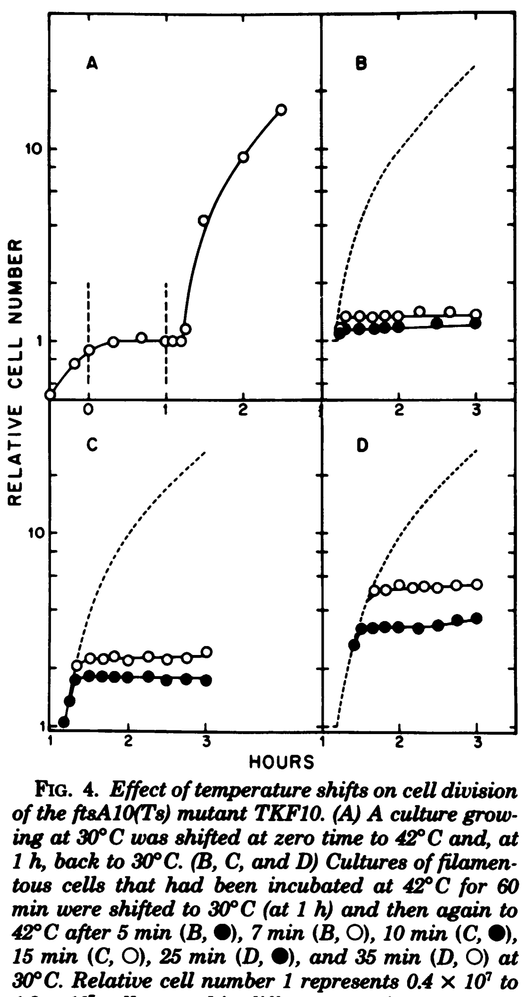

FIG. 4. *Effect of temperature shifts on cell division of the ftsA10(Ts) mutant TKF10. (A) A culture growing at 30°C was shifted at zero time to 42°C and, at 1 h, back to 30°C. (B, C, and D) Cultures of filamentous cells that had been incubated at 42°C for 60 min were shifted to 30°C (at 1 h) and then again to 42°C after 5 min (B, ●), 7 min (B, ○), 10 min (C, ●), 15 min (C, ○), 25 min (D, ●), and 35 min (D, ○) at 30°C. Relative cell number 1 represents 0.4 × 10⁷ to 1.2 × 10⁷ cells per ml in different experiments.*

The close proximity of *sep*, the *mur* genes, *ddl*, *ftsA*, and *envA* might be more than coincidence, as pointed out by Wijsman (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, 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] a 84(Ts) strain to temperature insensitivity*

Recipient	Marker	Phage[b]	MOI[c]	Revertants/recipient	Transductants/recipient	Transductants/viable phage
AX655	*sep2158*(Ts)	λ*leuA*169[d]	16	$<1 \times 10^{-7}$	2.8×10^{-4}	1.7×10^{-5}
AX655(λ+)	*sep2158*(Ts)	λ*leuA*169[d]	16	$<1 \times 10^{-7}$	3.8×10^{-4}	2.2 10^{-5}
AX720	*84*(Ts)	λ*leuA*169[d]	16	2×10^{-5}	[e]	ND[f]
		λ*murF*+121	0.5	2×10^{-5}	[e]	ND
AX720(λ+)	*84*(Ts)	λ*leuA*169[d]	16	2×10^{-5}	[e]	ND
		λ*murF*+121	0.5	2×10^{-5}	[e]	ND
TKF10(λ+)	*ftsA10*(Ts)	λ*murF*+121	0.5	5×10^{-7}	1.8×10^{-4}	4.4×10^{-4}

[a] Medium was YET with no added NaCl.
[b] Supplied with λ+ helper in HFTs.
[c] Multiplicity of infection with viable phages.
[d] Genetically similar to λ*sep*+46; the host insertion covers *envA*.
[e] Tests for transduction yielded survivors with a frequency of $2 \pm 0.2 \times 10^{-5}$.
[f] ND, None detected.

expressed from the transducing prophages even in the presence of a λ^+ prophage. It is known that the λ repressor represses all λ functions (except *c*I 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 λ*sep*⁺ phages are surprising. First, HFT lysates, after infection at low multiplicity, transduce *sep*(Ts) recipients at approximately the same frequency whether they be λ^+ lysogens or nonlysogens. This suggests that the transductants do not require the wild-type λ^+ repressor to be dominant over the *c*I857 repressor coded for by the transducing phage chromosome. Second, the phage yield in HFT lysates from $(\lambda^+)(\lambda sep^+)$ strains is considerably lower (10^8 to 10^9 plaque-forming units per ml; approximately equal numbers of plaque-forming and transducing particles) than is usually observed after λ induction. Perhaps this lower yield is related to the peculiar pattern of lysogen formation.

ACKNOWLEDGMENTS

We thank C. S. Lee and Leodocia Pope for advice and help with the electron microscopy and S. Normark, A. Rörsch, E. Lugtenberg, H. Wijsman, R. Weisberg, J. Calvo, and B. Bachmann for strains.

This work was supported by Public Health Service grant AI08286 from the National Institute of Allergy and Infectious Diseases and by American Cancer Society grant NP169D. C.A.I. is a National Science Foundation Predoctoral Fellow; J.M.H. is supported by Public Health Service training grant 1 T32 GM07126 from the National Institute of General Medical Sciences; J.R.W. was supported during a portion of the work by Public Health Service research career development award GM29413 from the same institute.

LITERATURE CITED

1. **Allen, J. S., C. C. Filip, R. A. Gustafson, R. G. Allen, and J. R. Walker.** 1974. Regulation of bacterial cell division: genetic and phenotypic analysis of temperature-sensitive, multinucleate, filament-forming mutants of *Escherichia coli*. J. Bacteriol. 117:978–986.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116–167.
3. **Brown, L. R., and C. E. Dowell.** 1968. Replication of coliphage M13. II. Intracellar deoxyribonucleic acid forms associated with M13 infection of mitomycin C-treated cells. J. Virol. 2:1296–1307.
4. **Cronan, J. E., Jr., and G. N. Godson.** 1972. Mutants of *Escherichia coli* with temperature-sensitive lesions in membrane phospholipid synthesis: genetic analysis of glycerol-3-phosphate acyltransferase mutants. Mol. Gen. Genet. 116:199–210.
5. **Davidson, N., and W. Szybalski.** 1971. Physical and chemical characteristics of lambda DNA, p. 45–82. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Davis, M. G., and J. M. Calvo.** 1977. Isolation and characterization of λ*pleu* bacteriophages. J. Bacteriol. 129:1078–1090.
7. **Davis, R. W., M. Simon, and N. Davidson.** 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. Methods Enzymol. 21D:413–438.
8. **Hirota, Y., M. Ricard, and B. Shapiro.** 1971. The use of thermosensitive mutants of *E. coli* in the analysis of cell division, p. 13–31. *In* L. A. Manson (ed.), Biomembranes, vol. 2. Plenum Publishing Co., New York.
9. **Hirota, Y., A. Ryter, and F. Jacob.** 1968. Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. 33:677–693.
10. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics 49:237–246.
11. **Klingmüller, W., K. Krell, and K. Shimada.** 1973. Genetic analysis of bacteriophage lambda strains which transduce the genes for leucine biosynthesis. Mol. Gen. Genet. 126:1–6.
12. **Lugtenberg, E. J. J., and A. van Schijndel-van Dam.** 1972. Temperature-sensitive mutants of *Escherichia coli* K-12 with low activities of the L-alanine adding enzyme and the D-alanyl-D-alanine adding enzyme. J. Bacteriol. 110:35–40.
13. **Lugtenberg, E. J. J., and A. van Schijndel-van Dam.** 1972. Temperature-sensitive mutants of *Escherichia coli* K-12 with low activity of the diaminopimelic acid adding enzyme. J. Bacteriol. 110:41–46.
14. **Lugtenberg, E. J. J., and A. van Schijndel-van Dam.** 1973. Temperature-sensitive mutant of *Escherichia coli* K-12 with an impaired D-alanine:D-alanine ligase. J. Bacteriol. 113:96–104.
15. **Miyakawa, T., H. Matsuzawa, M. Matsuhashi, and Y. Sugino.** 1972. Cell wall peptidoglycan mutants of *Escherichia coli* K-12: existence of two clusters of genes, *mra* and *mrb*, for cell wall peptidoglycan biosynthesis. J. Bacteriol. 112:950–958.
16. **Normark, S.** 1970. Genetics of a chain forming mutant of *Escherichia coli*. Transduction and dominance of the *envA* gene mediating increased penetration to some antibacterial agents. Genet. Res. 16:63–78.
17. **Normark, S., H. G. Boman, and G. D. Bloom.** 1971. Cell division in a chain-forming *envA* mutant of *Escherichia coli* K-12. Fine structure of division sites and effects of EDTA, lysozyme and ampicillin. Acta Pathol. Microbiol. Scand. Sect. B 79:651–664.
18. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. 63:483–503.
19. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1973. Prophage lambda at unusual chromosomal locations. II. Mutations induced by bacteriophage lambda in *Escherichia coli* K-12. J. Mol. Biol. 80:297–314.
20. **Smith, C. A., and J. Vinograd.** 1972. Small polydisperse circular DNA of HeLa cells. J. Mol. Biol. 69:163–178.
21. **Taylor, K., Z. Hradecna, and W. Szybalski.** 1967. Asymmetric distribution of the transducing regions on the complementary strands of coliphage λ DNA. Proc. Natl. Acad. Sci. U.S.A. 57:1618–1625.
22. **Thomas, M., and R. W. Davis.** 1975. Studies on the cleavage of bacteriophage lambda DNA with *Eco*RI restriction endonuclease. J. Mol. Biol. 91:315–328.
23. **van de Putte, P., J. van Dillewijn, and A. Rörsch.** 1964. The selection of mutants of *Escherichia coli* with impaired cell division at elevated temperature. Mutat. Res. 1:121–128.
24. **Walker, J. R., J. M. Henson, and C. S. Lee.** 1977. Isolation and characterization of plaque-forming λ*dnaZ*⁺ transducing bacteriophages. J. Bacteriol. 130:354–365.
25. **Walker, J. R., A. Kovarik, J. S. Allen, and R. A.**

Gustafson. 1975. Regulation of bacterial cell division: temperature-sensitive mutants of *Escherichia coli* that are defective in septum formation. J. Bacteriol. **123**:693–703.
26. **Wijsman, H. J. W.** 1972. A genetic map of several mutations affecting the mucopeptide layer of *Escherichia coli*. Genet. Res. **20**:65–74.
27. **Wijsman, H. J. W., and C. R. M. Koopman.** 1976. The relation of the genes *envA* and *ftsA* in *Escherichia coli*. Mol. Gen. Genet. **147**:99–102.
28. **Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber.** 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology **40**:734–744.
29. **Zuccarelli, A. L., R. M. Benbow, and R. L. Sinsheimer.** 1976. Formation of the parental replicative form of bacteriophage ϕX174. J. Mol. Biol. **106**:375–402.