JOURNAL OF BACTERIOLOGY, May 1980, p. 615-620
0021-9193/80/05-0615/06$02.00/0

Vol. 142, No. 2

Organization of Genes in the *ftsA-envA* Region of the *Escherichia coli* Genetic Map and Identification of a New *fts* Locus *(ftsZ)*

J. F. LUTKENHAUS,[1]* HANS WOLF-WATZ,[2] AND W. D. DONACHIE[3]

Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06032[1]; Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden[2]; and Department of Molecular Biology, University of Edinburgh, Edinburgh, Scotland EH9 3JR[3]

Complementation tests have revealed that the mutation in the filamenting mutant PAT84 is distinct from *ftsA* and has been designated *ftsZ*. By isolating transducing phages carrying various amounts of the bacterial deoxyribonucleic acid in this region, it was possible to locate the *ftsZ* gene between *ftsA* and *envA*. It is concluded that these cell division genes are expressed independently of the neighboring murein genes.

The genes that map at approximately 2 min on the *Escherichia coli* K-12 genetic map affect the physiology of the cell wall. A mutation in *ftsA* leads to filament formation at the restrictive temperature (6, 15, 17), a mutation in *envA* results in altered permeability and the growth of cells in chains (13), and mutations in *murE,F,C* and *ddl* result in decreased murein biosynthesis and cell lysis (10–12, 17).

Several filament-forming mutants have been isolated independently, and the mutations have been found to map in this region. Three mutations, *fts-10, fts-12,* and *fts-15,* were isolated by van de Putte et al. (15) and were subsequently shown by Wijsman (17) to map at the *ftsA* locus between *murC* and *envA*. Hirota et al. (6) found that one of their filament-forming mutants, PAT84, carried a mutation which was linked to *leu* and was designated *ftsA*. Allen et al. (1) isolated a mutant temperature sensitive for septum formation, and this mutation (ts-1882) had the same P1 cotransduction frequency with *leu* as the mutation in PAT84 (5, 16). Recently, an amber mutation was isolated in the *ftsA* gene which allowed identification of the gene product (12). In addition, it was shown that this amber mutation was allelic to the *fts-12* mutation isolated by van de Putte et al. (15).

In this report it is shown that most of these *fts* mutations are allelic; however, the mutation in strain PAT84 defines a distinct locus. Because all of these mutants have been referred to as *ftsA,* the nomenclature is confusing. We propose that the earlier isolated mutants retain the *ftsA* designation and that the gene identified by the mutation in PAT84 be designated *ftsZ*. In addition, by analyzing newly isolated deletion derivatives of a previously isolated transducing phage, it has been possible to locate the *ftsZ* gene between *ftsA* and *envA*.

MATERIALS AND METHODS

Bacterial and phage strains. The *E. coli* K-12 strains used in this investigation are listed in Table 1. The parental transducing phage, λ16-2, was described previously (12), as was the derivative λ16-4. A lambda replacement vector for *Eco*RI fragments, stock number NM616, was provided by Noreen Murray.

Media. Bacteria were grown in Oxoid nutrient broth. Complementation tests were done on Oxoid nutrient agar plates containing thymine at 50 μg/ml, but no added NaCl. For tests involving *envA,* rifampin was added to plates at a final concentration of 5 μg/ml.

Complementation tests. Complementation tests involving a thermosensitivity marker were routinely performed by spot tests. About 10^7 to 10^8 cells of the mutant strain to be tested were mixed with 2.5 ml of soft nutrient agar and poured onto a nutrient agar plate. Then 10 μl of the phage lysate to be tested was spotted, and the plates were incubated at 43°C. When necessary 10 μl of helper phage was also added.

For transduction of the *envA* marker, phage were adsorbed to the recipient cells, and this suspension was diluted twofold into broth and grown for 2 h at 37°C to allow time for expression. Appropriate dilutions were then spread onto nutrient plates containing rifampin.

Isolation of phage deletion mutants and more transducing phage. (i) In vivo. The sensitivity of λ16-2, which contains 102% of wild-type λ DNA, to EDTA was determined by infection of a culture of W3110 followed by adding soft agar (containing no EDTA) and pouring the mixture on plates containing various concentrations of EDTA. On plates containing 1.1 mM EDTA, large plaques (but of variable size) appeared at a frequency of 10^{-4}. Experiments revealed that phage containing 95% of λ DNA would plaque with 100% efficiency on plates containing 1.1 mM EDTA, indicating that deletion mutants of λ16-2 which had lost as little as 7% of their DNA would plaque on these plates. Twenty such plaques were picked and purified, and the remainder were pooled and treated as described in the text.

(ii) In vitro. DNA (1 μg in 10 μl) from λ16-2 was

TABLE 1. *Bacterial strains*

Strain	Relevant marker	Other markers	Reference	Source
PAT84	*ftsZ84*(Ts)	*thr-1 leu-6 thi argH1 thy his trp rpsL lacY1 malA1 xyl-7 mtl-2 mel tonA2 supE44*	(6)	R. G. E. Murray
OV-2		F⁻ *ilv his leu thyA deo ara*(Am) *lac*-125(Am) *galU42*(Am) *tyrT* ($SupF_{tsA81}$)	(4)	
JFL100	*ftsZ84*(Ts)	*leu*⁺ *ftsZ84*(Ts) transductant of OV-2		
JFL101[a]	*ftsZ84*(Ts) *recA*	as JFL100 but *thy*⁺ *his*⁺		
OV-16	*ftsA16*(Am)	Derivative of OV-2, also *pro*	(12)	
TKF12	*ftsA12*(Ts)	*thr leu thi pyrF thyA ilvA his arg lac tonA tsx*	(15)	S. Normak
TKF10	*ftsA10*(Ts)	As TKF12	(15)	A. Rorsch
TKF15	*fts-15*(Ts)	As TKF12		A. Rorsch
GIA86	*envA*	*thr thi pyrF thyA ilvA his arg lac tonA tsx*	(17)	S. Normak
PC1358	*ddl*(Ts)	*thr leu trp his thyA thi lac gal xyl mH ara tonA phx rpsL ths*		E. J. J. Lugtenberg
PC1357	*murC*(Ts)	As PC1358		E. J. J. Lugtenberg
JFL110[a]	*ftsA12*(Ts) *recA*	As TKF12 but *thy*⁺ *his*⁺		
JFL111[a]	*ddl*(Ts) *recA*	As PC1358 but *thy*⁺ *his*⁺		
JFL112[a]	*murC*(Ts) *recA*	As PC1357 but *thy*⁺ *his*⁺		
JC5088	Hfr *recA*	*thr* prototroph *supE tonA*		N. Willets
W3110				N. Murray
C600				N. Murray
SA291 (λ *envA*⁺)		Δ*(uvrB-gal) his rpsL*	(12)	
NEM269				N. Murray
TKL22	*ftsA22*(Ts)	As TKF12	(17)	A. Rorsch

[a] The *recA* derivatives of the temperature-sensitive mutants were isolated among *his*⁺ *thy*⁺ exconjugants of a cross between the parent and JC5088.

digested with *Hin*dIII (Boehringer-Mannheim), and the reaction mixture was heated at 70°C to inactivate the enzyme. The resulting DNA fragments were then treated with T4 polynucleotide ligase, and plaques were recovered by transfection. Fifteen plaques were picked and purified, and the remainder were pooled and treated as described in the text.

(iii) Transducing phage. Transducing phages similar to λ16-2 were isolated by UV induction of the lysogen SA291(λ *envA*⁺). In this lysogen λ *envA*⁺ is integrated at the chromosomal *envA* gene. From the lysate, *ftsA*⁺ transducing phage (at a frequency of 10^{-5}) were selected by complementation of TKF12.

Preparation of phage and phage DNA. Phage were prepared after UV induction of a 500-ml culture of a lysogen, concentrated by polyethylene glycol precipitation (18) and a CsCl step gradient, and finally recovered by equilibrium centrifugation in 41.5% (wt/wt) cesium chloride solution (8). After dialysis, protein was extracted with phenol, and the DNA was dialyzed against a solution of 0.1 M NaCl, 10 mM Tris-hydrochloride (pH 7.5), and 1 mM EDTA.

Restriction endonuclease digestion and agarose gel electrophoresis. Samples containing 1 to 2 μg of phage DNA in 30 μl of 10 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 50 mM NaCl were incubated with 2 U of restriction endonuclease for 60 min at 37°C. The samples were then heated at 70°C for 10 min, cooled quickly in ice, and mixed with 5 μl of 50% glycerol containing 0.1% bromophenol blue. The samples were subjected to electrophoresis overnight in a horizontal slab agarose gel. The running buffer was 40 mM Tris-acetic acid (pH 8.2), 20 mM sodium acetate, 10 mM EDTA, and 0.2 μg of ethidium bromide per ml.

Ligation and transfection. Solutions of phage DNA which had been digested with endonucleases *Hin*dIII (Boehringer-Mannheim) or *Eco*RI (Sigma) were heated at 70°C for 10 min and then placed on ice. T4 polynucleotide ligase was added to them so that each reaction mixture contained, in a final volume of 0.2 ml, 10 μg of DNA, 66 mM Tris-hydrochloride (pH 7.5), 1 mM EDTA, 10 mM $MgCl_2$, 100 mM NaCl, 10 mM dithiothreitol, 0.1 mM ATP, 0.1 mg of bovine serum albumin per ml, and 1 U of T4 polynucleotide ligase (gift of Ken Murray). The reaction mixture was incubated at 10°C for 5 h and then at 4°C for 2 days before it was used for transfection. Transfection was carried out with strain NEM259 as described by Borck et al. (2).

RESULTS

Identification of a new cell division gene. The transducing phage, λ16-2, which was isolated previously, has been shown to carry the genes *envA, ftsA, ddl,* and *murC* (12). We found that this phage also complements the temperature-sensitive mutation *fts-84*(Ts) in strain PAT84. This result rules out the possibility that this temperature-sensitive mutation is dominant, one of the possibilities suggested previously (5, 16). Also, a derivative of λ16-2 that carried the *ftsA12*(Ts) allele was tested for its ability to complement *fts-84*(Ts). This phage, λ16-4, also complements this temperature-sensitive mutation. Thus, the *84*(Ts) mutation must represent a cell division locus distinct from *ftsA* [as defined by the *ftsA12*(Ts) allele]. We propose that this locus be designated *ftsZ.*

A number of other *fts* mutants were also isolated by van de Putte et al. (15). We found that *fts-10* and *fts-15* are alleles of *ftsA.* In addition, the mutation in strain TKL22 is also in the *ftsA* gene. This mutant was described as forming short filaments and then lysing at the restrictive temperature (17). However, we found that in several different media this mutant formed long filaments without any apparent lysis (data not shown).

Isolation of deletion mutants of λ16-2. To locate the *ftsZ* gene and to elucidate further the functional organization of the genes mapping in this region we set out to isolate deletion mutants of λ16-2. To do this two different approaches were used. In the first approach deletion mutants generated in vivo were selected by their increased resistance to EDTA. When λ16-2 was plated on nutrient agar plates containing 1.1 mM EDTA, plaques appeared at a frequency of 10^{-4}. Since this frequency is quite high, it was suspected that new deletions were occurring on the plate, and it was not just preexisting deletions that were being selected. (Similar observations have been made by Saito and Uchida [14].) Due to the presence of the *att* site in λ16-2, most of these deletions would be expected to have one endpoint at this site. Two deletion mutants, ΔBE1 and ΔLE3, which required phage for efficient transduction and thus were suspected to contain deletions originating at *att,* were analyzed in detail.

To select for deletions of λ16-2 that do not have an endpoint at *att,* approximately 5,000 plaques from the 1.1 mM EDTA plates were pooled. From this mixture phage were selected that complemented *envA* or *ftsZ,* one of which must be closer to the *att* site. Since mutants carrying deletions that originate from *att* can not lysogenize as efficiently as the wild type, they would be selected against. It was found that all deletions (16 tested) selected for by complementation of *ftsZ* also complemented *envA.* However, among the 15 deletions that were selected by their ability to complement *envA,* one, ΔJ, was found which no longer complemented *ftsZ,* suggesting that *envA* is nearer the *att* site. In addition to ΔJ two others, ΔB and ΔE, were analyzed further.

A second approach was to generate deletions of λ16-2 in vitro by deleting one or both of the bacterial DNA *Hin*dIII fragments. λ16-2 DNA was completely digested with *Hin*dIII and ligated, and plaques were recovered after transfection. Of 15 plaques that were screened (e.g., ΔR1), none complemented any of the mutations that are complemented by λ16-2. About 5,000 plaques were pooled and phage were selected by their ability to complement *ddl.* One such phage, ΔR2, was analyzed further.

In addition to these deletion derivatives, more transducing phage were generated in the same manner as λ16-2, i.e., by induction of λ *envA*$^+$ integrated at *envA.* Phage that had picked up additional DNA upon excision were selected by complementation of *ftsA12*(Ts). Of 30 phage isolated in this manner, one plaque-former, λ16-25, was found that did not complement *murC* or *ddl* and was studied further.

Physical characterization of λ16-2 and the deletion mutants. Figure 1 shows the restriction nuclease map of the bacterial DNA present in λ16-2. It confirms and extends the map presented by Irwin et al. (7) for this region. DNA from each of the deletion mutants was digested with *Hin*dIII and *Eco*RI to determine the extent of the deletions. These results are summarized in the diagram shown in Fig. 2.

Genetic characterization of the deletion phages. Each of the phages isolated in the previous section was tested for its ability to complement each of the mutations mapping in this region. The results are presented in Table 2. From these results, *ftsZ* is located between *ftsA* and *envA,* since ΔB complements *envA* and *ftsZ,* whereas ΔJ and λ *envA*$^+$ complement only *envA.* In addition, the five genes in this region have to be divided into a minimum of two functional units, one comprising *ddl* and *murC* and the other comprising *envA, ftsZ,* and *ftsA.*

In addition to the complementation tests, marker rescue experiments were carried out in a further attempt to locate the genes. As shown in Table 3, *ftsA12*(Ts) can be rescued by ΔB and ΔR2 but not λ *envA*$^+$. Also *ddl*(Ts) can be rescued by λ16-25 and ΔE but not by ΔR1, and *murC*(Ts) can be rescued by ΔR1 and ΔE but not by λ16-25. This was judged as marker rescue and not complementation due to the much lower

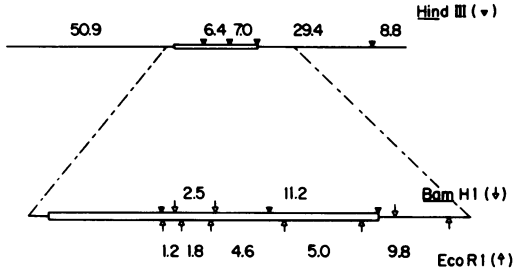

FIG. 1. *Restriction endonuclease map of the bacterial DNA present in λ16-2. The bacterial DNA present in the phage appears as an open rectangle. The sizes of the fragments are given as percentages of wild-type λ DNA. The ordering of the HindIII fragments was described previously (12). The ordering of the BamHI fragments was straightforward since endo R·BamHI only cuts the 6.4% HindIII fragment. The EcoRI fragments were ordered based on the following information. First, the 5.0% fragment was known to originate from within the 7.0% HindIII fragment based on restriction enzyme analysis of λ envA⁺ (data not shown). Second, since the 1.8% fragment is not cleaved by endo R·HindIII or endo R·BamHI, it must originate from within the 2.5% BamHI fragment. Third, the 4.6% fragment was cleaved by both endo R·BamHI and endo R·HindIII, yielding fragments that suggested it must be located between the 5.0 and 1.8% fragments. This left the 1.2% fragment to the left of the 1.8% fragment, where it should be cleaved by endo R·BamHI, and it is.*

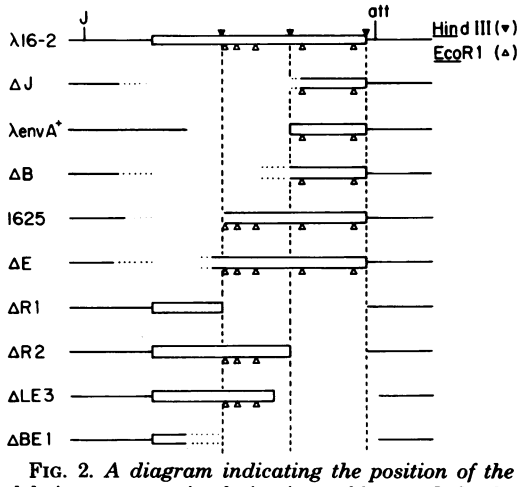

FIG. 2. *A diagram indicating the position of the deletions present in derivatives of λ16-2. Only the central regions of these phages are depicted here, and the bacterial DNA present in each phage is indicated by an open rectangle. The endpoints of the deletions were deduced from the restriction enzyme analyses of the phage DNAs. In some cases, the endpoints are uncertain, and this is indicated by dotted lines.*

frequency at which temperature-resistant clones appeared ($>10^{-5}$ for marker rescue versus $>10^{-1}$ for complementation). This was verified since there was no marker rescue if the recipients carried the *recA* allele (data not shown).

Cloning the *Eco*RI fragments from λ16-2. To elucidate further the functional organization of the cell division genes, the *Eco*RI fragments from λ16-2 were cloned into a λ *Eco*RI vector, λ616. This vector is useful since it is also integration proficient. Figure 3 shows the restriction nuclease analysis of λJFL40 and λJFL41 which contain the 5.0 and 4.6% *Eco*RI fragments, respectively. As shown in Table 4, λJFL40 complements *envA* and λJFL41 complements *ftsA*, but neither complements *ftsZ*. These results, along with those presented earlier, are summarized in the physical map of this region presented in Fig. 4.

DISCUSSION

Many temperature-sensitive, filament-forming mutants have been isolated, and their mutations have been mapped near *leu*. The isolation of transducing phages carrying the mutant and wild-type alleles has allowed a complementation analysis of these mutants to be carried out. As shown in this report, the mutation in strain PAT84 identifies a gene which is distinct from *ftsA* (as identified by the other *fts* mutations which are allelic). This new locus has been designated *ftsZ*. In addition, complementation analysis with newly isolated phage deletion mutants places the *ftsZ* gene between *ftsA* and *envA*.

Mutations in either *ftsA* or *ftsZ* result in the formation of multinucleated filaments at the restrictive temperature; however, the filaments are not identical. Mutations in *ftsA* result in filaments with indentations along the filaments which are presumably partially completed septa. This is true for both missense and amber mutations that have been isolated in *ftsA* (12, 16). In contrast, the *ftsZ84*(Ts) mutation results in filaments lacking any sign of constrictions. Thus, these mutations might be affecting different steps in septation, with the product of the *ftsZ* gene required at an earlier stage than the *ftsA* gene product. Such a view is supported by the behavior of these mutants in temperature shift experiments. Walker et al. (16) suggested from such experiments that the *ftsZ* mutant behaved as a septum initiation mutant, a conclusion in agreement with that of Burdett and Murray (3). The *ftsA* mutants behave differently in that the product of the *ftsA* gene is required throughout septation (15). Such results are consistent with the different morphologies of these mutants.

The genetic and physical characterization of the phages isolated in this study, which carry various lengths of the bacterial DNA, reveals

TABLE 2. *Complementation by the phage deletion mutants*[a]

Phage	Recipient				
	PC1357 *murC*(Ts)	PC1358 *ddl*(Ts)	TKF12 *ftsA12*(Ts)	JFL100 *ftsZ84*(Ts)	GIA86 *envA*
λ16-2	+	+	+	+	+
ΔJ	−	−	−	−	+
λ *envA*$^+$	−	−	−	−	+
ΔB	−	−	−	+	+
λ16-25	−	−	+	+	+
ΔE	−	−	+	+	+
ΔRI	−	−	−	−	−
ΔR2	+	+	−	−	−
ΔLE3[b]	+	+	−	−	−
ΔBE1[b]	−	−	−	−	−

[a] The same results were obtained with the thermosensitivity markers when *recA* derivatives of the recipients were used.

[b] The phage λ540 (2) was added as a helper.

TABLE 3. *Ability of the deletion phage to rescue various markers*

Phage	Recipient		
	PC1357 *murC*(Ts)	PC1358 *ddl*(Ts)	TKF12 *ftsA12*(Ts)
ΔR1	+	−	
ΔR2			+
λ16-25	−	+	
ΔB			+
ΔE	+	+	
λ *envA*$^+$			−

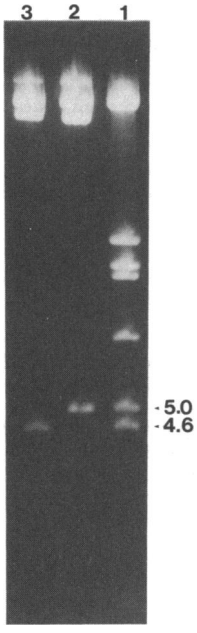

FIG. 3. *Restriction analysis of the phages containing the EcoRI fragments. The numbers to the right indicate the sizes of the EcoRI fragments in percentage of wild-type λ DNA. The phage DNAs were as follows: lane 1, λ16-2; lane 2, λJFL40; and lane 3, λJFL41.*

TABLE 4. *Complementation by phage carrying the EcoRI fragments*

Phage	Recipient			
	PC1358 *ddl*(Ts)	TKF12 *ftsA12*(Ts)	JFL100 *ftsZ84*(Ts)	GIA86 *envA*
λJFL40	−	−	−	+
λJFL41	−	+	−	−

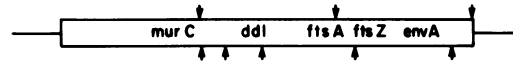

FIG. 4. *A diagram indicating the positions of the genes with respect to the HindIII and EcoRI sites. These results were deduced from the complementation tests in Tables 2 and 4 and the marker rescue experiments in Table 3.*

several aspects of the functional organization of the genes in this region. The *ftsA* gene is in a functional unit independent of the neighboring genes. First of all, the cloning of the 5.0 and 4.6% *Eco*RI fragments from λ16-2, each of which retains the ability to complement *envA* and *ftsA*, respectively, indicates that these two genes are in separate functional units. Also, *ftsA* can be expressed independently of *ftsZ*, since deletion phages (e.g., ΔB) were isolated which can complement *ftsZ* but not *ftsA* and since the 4.6% *Eco*RI fragment must contain the promoter and structural gene for *ftsA*. In addition, the expression of *ftsA* and *ddl*, the nearest gene on the other side, must also be independent, which can be reasoned in an analogous manner.

The two genes, *envA* and *ftsZ*, could be expressed independently or organized into one transcriptional unit. However, recent results from experiments in which the deletion phages were used to direct protein synthesis in UV-irradiated cells suggest that these two genes are expressed independently (manuscript in preparation).

The results suggest that *murC* and *ddl* constitute a single operon. These genes were located by the marker rescue experiments (Table 3) with *ddl* on the 6.4% *Hin*dIII fragment and *murC* on the bacterial DNA fragment closest to the phage *J* gene. Since the deletion phage ΔE can rescue both *murC* and *ddl*, it must contain the entire structural gene for *ddl*, although it does not complement. The simplest explanation is that the promoter is on the distal bacterial DNA fragment with *murC* transcribed before *ddl*. With this direction of transcription, the other murein genes in this region, *murE* and *murF* (17), must be in a separate operon.

Fletcher et al. (5) isolated λ-transducing phages that carried the bacterial DNA between *envA* and *leu*. Thus, their phage would carry the *ftsZ* gene, but they were unable to show complementation of the PAT84 mutant. Perhaps this difference from these results can be explained by the different λ transducing phages that were employed. Whereas an integration-proficient phage was used in this investigation, their transducing phage was integration defective, making it more difficult to achieve lysogeny. This combined with the high reversion rate of the PAT84 mutant would place their complementation frequencies at background levels. In addition, they reported that in complementation tests employing F prime plasmids they detected only partial complementation, whereas the transducing phage used in this study gave complete complementation (measured as colony-forming ability).

ACKNOWLEDGMENTS

We thank Douglas Ward and Noreen Murray for helpful discussion and for providing the phage vectors.

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. **Borck, K., J. D. Beggs, W. J. Brammar, A. S. Hopkins, and N. E. Murray.** 1976. The construction in vitro of transducing derivatives of phage lambda. Mol. Gen. Genet. **146**:199–207.
3. **Burdett, I. D. J., and R. G. E. Murray.** 1974. Septum formation in *Escherichia coli*: characterization of septal structure and the effects of antibiotics on cell division. J. Bacteriol. **119**:303–324.
4. **Donachie, W. D., K. Begg, and M. Vincente.** 1976. Cell length, cell growth and cell division. Nature (London) **264**:328–333.
5. **Fletcher, G., C. A. Irwin, J. M. Henson, C. Fillingham, M. M. Malone, and J. R. Walker.** 1978. 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. J. Bacteriol. **133**:91–100.
6. **Hirota, Y., A. Ryter, and F. Jacob.** 1968. Thermosensitive mutants of *E. coli* affected in the process of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677–693.
7. **Irwin, C. A., G. Fletcher, C. L. Sills, and J. R. Walker.** 1979. Expression of the *E. coli* cell division gene *sep* cloned in a charon phage. Science **206**:220.
8. **Kaiser, A. D., and D. S. Hogness.** 1960. The transformation of *E. coli* with deoxyribonucleic acid isolated from lambda dg. J. Mol. Biol. **2**:392–415.
9. **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.
10. **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.
11. **Lugtenberg, E. J. J., and A. van Schijndel-van Dam.** 1973. Temperature-sensitive mutant of *Escherichia coli* K-12 with an impaired D-alanyl-D-alanine ligase. J. Bacteriol. **113**:96–104.
12. **Lutkenhaus, J. F., and W. D. Donachie.** Identification of the *ftsA* gene product. J. Bacteriol. **137**:1088–1094.
13. **Normak, 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.
14. **Saito, H., and H. Uchida.** 1978. Organization and expression of the *dnaJ* and *dnaK* genes of *Escherichia coli* K12. Mol. Gen. Genet. **164**:1–8.
15. **van de Putte, P., J. van Dillewijn, and A. Rorsch.** 1964. The selection of mutants of *Escherichia coli* with impaired cell division at elevated temperatures. Mutat. Res. **1**:121–128.
16. **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.
17. **Wijsman, H. J. W.** 1972. A genetic map of several mutations affecting the mucopeptide layer of *Escherichia coli*. Genet. Res. **20**:65–74.
18. **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.