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Identification of the *ftsA* Gene Product

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A nonsense mutation was identified in the essential cell division gene *ftsA* of *Escherichia coli*. A λ-transducing phage was isolated which complemented this mutation. This phage programmed the synthesis of four bacterial proteins in UV-irradiated cells. By substituting the nonsense mutation for the *ftsA*$^+$ allele in this transducing phage and comparing the proteins programmed by it in UV-treated Su$^+$ and Su$^-$ cells, the product of the *ftsA* gene was identified as a protein with a molecular weight of 50,000.

The study of the cell cycle in *Escherichia coli* is essentially an investigation into the two major periodic events of the cell cycle, i.e., DNA replication and cell division. The investigation into DNA replication has proceeded more rapidly than that into cell division on both a genetic and a biochemical level. Recent results (19) suggest that most of the genes involved in DNA replication may be identified. In addition, many of the gene products have been identified and their functions determined (10).

Many genes involved in cell division have also been identified. Hirota et al. (7, 8, 18) made a preliminary classification of division mutants, but this list is still increasing (6, 13). However, on a biochemical level, the investigation of cell division lags behind that of DNA replication. This is chiefly due to the lack of an assay for the gene products involved. To circumvent this problem we have isolated a unique collection of conditional mutants which have nonsense mutations in genes involved in cell division (M. Vincente, N. Otsuji, J. F. Lutkenhaus, K. Begg, G. Salmond, and W. D. Donachie, manuscript in preparation). Such mutants will allow the identification of the corresponding gene products.

In this article we report the first successful use of this method. We have identified the primary gene product of a cell division gene. This gene was shown by complementation to be identical to a gene previously designated as *ftsA* (7, 8, 21, 24).

MATERIALS AND METHODS

Bacterial and phage strains. The *E. coli* K-12 strains used in this study are summarized in Table 1. The phage strains used are listed in Table 2.

Growth media. Oxoid nutrient broth was used for liquid cultures and added to agar for plates. The NaCl concentration was 5 mg/ml. To test for the *envA* phenotype, rifampin was added to plates at a final concentration of 2 to 10 μg/ml, depending on the strain.

Minimal medium was Vogel-Bonner salts (22) supplemented with 0.2% maltose and vitamin B_1 (10 μg/ml).

Growth of phage and preparation of DNA. High-titer stocks of phage were prepared in the following way. Phage were plated for single plaques on W3110. Bacteria from the center of the turbid plaque were streaked, and single colonies were picked and tested for lysogeny by cross-streaking against the λ*imm*^{21}cI mutants (Table 2). Lysogens were grown in liquid and induced by UV irradiation. Phage were concentrated by polyethylene glycol precipitation (26). The phage were further purified and concentrated by a CsCl step gradient, and in some cases this was followed by a CsCl equilibrium gradient.

To prepare DNA, the phage were dialyzed to remove the CsCl and the protein was extracted with phenol. The DNA was then dialyzed against a solution of 0.1 M NaCl, 10 mM Tris-hydrochloride (pH 7.5), and 1 mM EDTA to remove the phenol.

λ transduction. To test for transduction of a thermosensitivity (Ts) marker, 10^8 cells of the temperature-sensitive recipient were mixed with 0.1 ml of the appropriate sterile lysate. After incubation to allow adsorption, appropriate dilutions of the mixture were spread on nutrient agar plates and incubated directly at 42°C. For transduction of OV16 to temperature resistance with the low-frequency transducing lysate, all resultant clones were screened for the Trp phenotype at 42°C. This was employed as a screen for reversion of *tyrT*(SupF_{tsA81}) to temperature resistance, which occurs at a high frequency. All clones that required tryptophan at 42°C and were resistant to the λ*imm*^{21}cI mutants (Table 2) were presumed to be transductants. These were then induced to obtain high-frequency transducing lysates. In the case of a defective phage, λ540 was added as a helper after UV induction.

For transduction involving *envA*, the recipient cells, after time was allowed for adsorption of the phage, were diluted into nutrient broth and grown for 2 h to allow time for expression. Appropriate dilutions were

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TABLE 1. *Bacterial strains*

Bacterial strain	Genotype	Source or reference
OV-2	F⁻ *ilv his leu thyA deo ara*(Am) *lac-125*(Am) *galU42*(Am) *galE trp*(Am) *tyrT*($SupF_{tsA81}$)	5
OV-16	*pro ftsA16*(Am) derivative of OV-2	Vincente et al., manuscript in preparation
SA291	Δ(*gal att*$^{\lambda}$ *bio uvrB*) *his rpsL*	Noreen Murray
JFL74	spontaneous *leu* derivative of SA291	This work
JFL75	*leu*$^{+}$ *envA* transductant of JFL74	This work
TKF12	*ftsA*(Ts) *thr leu thi pyrF thyA ilvA his arg lac tonA tsx*	25
ATK131	*envA ftsA*(Ts) *leu*$^{+}$ derivative of TKF12	25
159	*uvr gal rpsL*	Tom Linn
159(λ*imm*^{434}*supF*)	λ*imm*^{434}*supF* lysogen of 159	This work
W3110	Prototroph	Laboratory stock
D_{22}(λ)	*envA his pro trp rpsL*	14
PC1358	*ddl thr leu trp his thyA thi lac gal xyl mtl ara tonA phx rpsL ths*	E. J. Lugtenberg; 24
PC1242	*murF thr leu thi pyrE codA thyA argG ilvA his lacY xyl tonA tsx phx supE ths dra uvrB vtr*	E. J. Lugtenberg; 24
PC1357	*murC thr leu trp his thyA thi gal xyl mtl ara tonA phx rpsL*	E. J. Lugtenberg; 24
KLF4/AB2463	F'104 *thi thr leu arg his pro recA mtl xyl ara galK2 lacY rpsL tsx supE44*	B. Bachmann
KLF1/AB2463	F'101 *thi thr leu arg his pro recA mtl xyl ara galK2 lacY*	B. Bachmann

TABLE 2. *Phage strains*

Phage strain	Bacterial genes on phage	Source or reference
λ540*imm*^{21}		2, 12
λ*envA*$^{+}$	*envA*$^{+}$	Wolf-Watz and Lutkenhaus (unpublished data)
λ16-2[a]	*envA*$^{+}$ *ftsA*$^{+}$	This work
λ16-3[a]	*envA*$^{-}$ *ftsA*$^{+}$	This work
λ16-4[a]	*envA*$^{+}$ *ftsA*(Ts)	This work
λ16-5[a]	*envA*$^{+}$ *ftsA-16*(Am)	This work
λ*imm*^{434}*supF*	*supF*	Noreen Murray
λ*imm*^{21}*supF*	*supF*	2
λcI857		Noreen Murray
λ*imm*^{21}*clh80*		Noreen Murray
λ*imm*^{21}*clh*λ		Noreen Murray
λ*b2*		Bill Brammar

[a] These phage also carry genes *ddl*$^{+}$ and *murC*$^{+}$.

then spread onto nutrient plates containing rifampin.

Electron microscopy. The procedure followed was essentially that of Davis et al. (4). Denatured DNA was prepared directly from intact phage by treatment with alkali in a final volume of 500 μl, which contained 1.5 μg of DNA of each of the two phages in 0.25 M NaOH and 20 mM EDTA. After 20 min at 27°C the solution was neutralized by the addition of 50 μl of 2.0 M Tris-hydrochloride (pH 8.5). Renaturation was achieved by allowing the solution to stand at 27°C for 2 to 3 h in the presence of 50% formamide. The spreading solution contained 0.2 volume of the DNA solution in 0.1 mM Tris-hydrochloride (pH 8.5), 10 mM EDTA, 50% formamide, and 0.1 mg of cytochrome *c*. The hypophase was 15% formamide in 13 mM Tris-hydrochloride (pH 8.5).

The immunity bubble (*imm*$^{\lambda}$/*imm*^{21}), 13.7% of λ (3), was used as a standard for referring single-strand lengths. The right arm of λ was used as a standard for double-strand lengths.

Protein synthesis in UV-irradiated bacteria. The procedure was essentially that of T. Linn, M. Goman, and J. Scaife (J. Mol. Biol., in press). The cells were grown in minimal medium supplemented with 0.2% maltose. At an optical density at 540 nm of 0.2 the cells were collected by centrifugation and resuspended in the same medium containing 0.02 M $MgCl_2$ at a density of 10^9 cells per ml. After a UV dose of 6000 ergs/mm^2, the culture was split into 100-μl aliquots and infected with phage at a multiplicity of infection of 10. The samples were incubated for 20 min at 37°C to allow adsorption and then diluted by the addition of 4 volumes of prewarmed minimal medium. After a further 20-min incubation, 20 μCi of [^{35}S]methionine was added to each sample. After 5 min, the samples were centrifuged, and the cell pellet was resuspended in 50 μl of sodium dodecyl sulfate sample buffer, which contained 2% sodium dodecyl sulfate, 20% glycerol, 5% 2-mercaptoethanol, and 125 mM Tris-hydrochloride (pH 6.8).

Electrophoresis and autoradiography were performed as described previously (11) except that the polyacrylamide gels were 10 to 17% gradient gels.

Agarose gel electrophoresis. Samples containing 1 to 2 μg of phage DNA in 30 μl were incubated with 2 units of restriction endonuclease *Hin*dIII (Boehringer-Mannheim) at 37°C for 1 h. After heating at 70°C for 10 min, 5 μl of 0.1% bromophenol blue in 50% glycerol was added, and the samples were loaded onto a 0.8% agarose gel. Electrophoresis was at 30 V/cm for 15 h. 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.

RESULTS

Physiology and genetic mapping of OV16. OV16 was isolated as a temperature-sensitive mutant after nitrosoguanidine mutagenesis of a strain carrying a temperature-sensitive suppressor, *tyrT*($SupF_{tsA81}$) (M. Vincente, J. F. Lutkenhaus, K. Begg, N. Otsuji, G. Salmond, and W. D. Donachie, submitted for publication). Lysogenization of this mutant with an integration-proficient $\lambda imm^{21} supF$ (a non-temperature-sensitive suppressor) conferred temperature resistance, thus confirming that the temperature sensitivity was due to the presence of an amber mutation. Following curing of $\lambda imm^{21} supF$ by $\lambda imm^{\lambda} b2$ the mutant again became temperature sensitive. After a shift to 42°C, asynchronous populations of this mutant stop dividing after about 20 min (an interval equivalent to the D period). Figure 1 shows photographs of this mutant growing at 30°C and 150 min after a shift to 42°C.

The mutation in OV16 was located near *leu* by F′ complementation. It was complemented by F′104 but not by F′101. The mutation also cotransduced (by P1 transduction) with *leu*, but it was difficult to quantitate due to the poor viability of OV16 on minimal plates. It therefore appeared possible that the mutation might fall within the cluster of division-related genes (including *ftsA*) that lie near *leu* (Fig. 2). In addition, the filaments formed at 42°C have a very characteristic shape, with indentations at sites that would presumably have been septa (see Fig. 1B). Similar filaments are formed by temperature-sensitive *ftsA* missense mutants (23).

Isolation of a transducing phage for OV16. The *envA* mutation maps within this cluster of genes, but a $\lambda envA^{+}$ transducing phage (selected from in vitro-constructed transducing derivatives of a plaque-forming, integration-proficient phage λimm^{21}; ref. 2; Wolf-Watz and Lutkenhaus, unpublished data) failed to complement both the mutation of interest and a known *ftsA* mutation. This phage was extended to include additional chromosomal loci near *envA*. To do this, SA291, an *E. coli* K-12 strain with an att^{λ} deletion, was lysogenized with $\lambda envA^{+}$. Then a lysate made by UV induction of this lysogen was screened for its ability to transduce OV16 to temperature resistance. One difficulty in this procedure is the high reversion rate of the suppressor to temperature resistance (95% of all revertants). However, OV16 also carries a *trp*(Am) mutation, and therefore all clones could be checked for the Trp phenotype at 42°C. Twelve clones that required tryptophan at 42°C were tested for phage release. Two of these yielded high-frequency transducing lysates for OV16 upon induction. The remaining 10 contained defective transducing phage, since they yielded high-frequency transducing lysates if λ540 was added as a helper following induction. One of the plaque formers, λ16-2, was chosen for the subsequent work. This phage also complemented *ftsA* (TKF12), *ddl* (PC1358), and *murC*

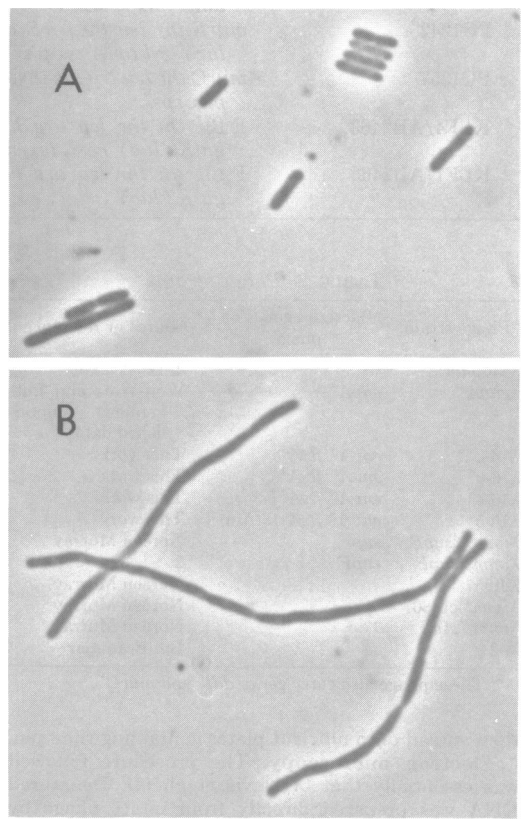

FIG. 1. *(A) OV16 grown in nutrient broth at 30°C. (B) OV16 shifted to 42°C for 150 min.*

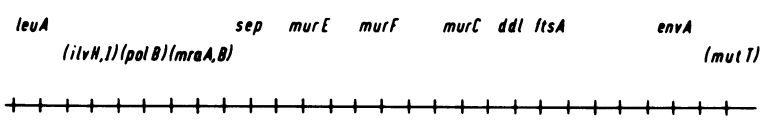

FIG. 2. *The genetic and physical map of the region between leu and envA. Each segment corresponds to 1 kilobase. The positions of leuA, sep, murE, murF, murC, ddl, ftsA, and envA are taken from Fletcher et al. (6). The positions of the remaining genes are taken from Bachmann et al. (1), but are not as well characterized.*

(PC1357), but did not complement *murF* (PC1242).

To determine the size of the bacterial DNA insert, λ16-2 was heteroduplexed with λ*c*I857. A typical molecule is shown in Fig. 3. There are two bubbles, one corresponding to the different immunity regions of the two phages and the other, larger bubble resulting from the bacterial DNA insertion. By comparing this heteroduplex with the λ*envA*$^+$/λ*c*I857 heteroduplex (not shown) it was calculated that the bacterial DNA insertion had been increased from 7% of λ in λ*envA*$^+$ to 21% in λ16-2. In addition, 3% of the phage DNA from the *b*2 region (adjacent to gene J) was lost during the formation of λ16-2.

Analysis of the *Hin*dIII digests of these two phage DNAs (Fig. 4, columns 3 and 4) confirmed the size of the bacterial DNA insert as 7%. Also, the additional bacterial DNA in λ16-2 contains a *Hin*dIII site resulting in a 6.4% fragment (Fig. 5).

Isolation of a phage carrying the amber mutation. To identify the product of the gene mutated in OV16, it was necessary to obtain the amber allele on the phage. To do this the *envA* allele (from strain D22) was first put onto λ16-2. This was done by using phage P1 to cotransduce *envA* with *leu*$^+$ into a strain lacking the *att*$^\lambda$ site (JFL74). This strain was then lysogenized with λ16-2 (the phage was expected to integrate in the vicinity of *envA* by homologous recombination). This lysogen was induced, and the phage obtained were used to make lysogens of a strain carrying the *envA* allele. These lysogens were then checked for sensitivity to rifampin to determine which allele was carried by the phage. Five percent of the phage were found to carry *envA*, and the remainder carried *envA*$^+$. One of the phage carrying *envA*, designated λ16-3, was chosen for subsequent manipulations.

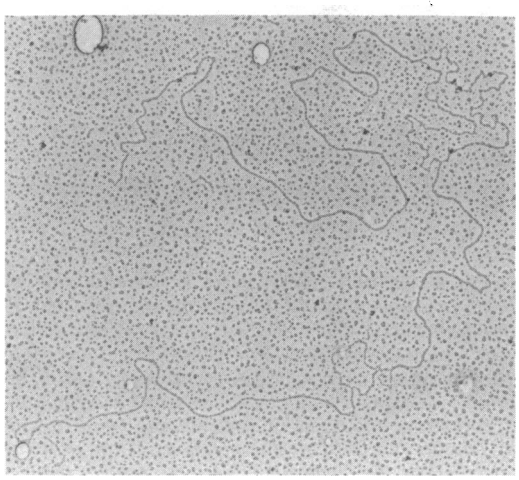

FIG. 3. *An electron micrograph of a heteroduplex molecule of λ16-2 and λcI857. The smaller bubble is due to the two different immunity regions, and the larger bubble is due to the bacterial DNA present in λ16-2.*

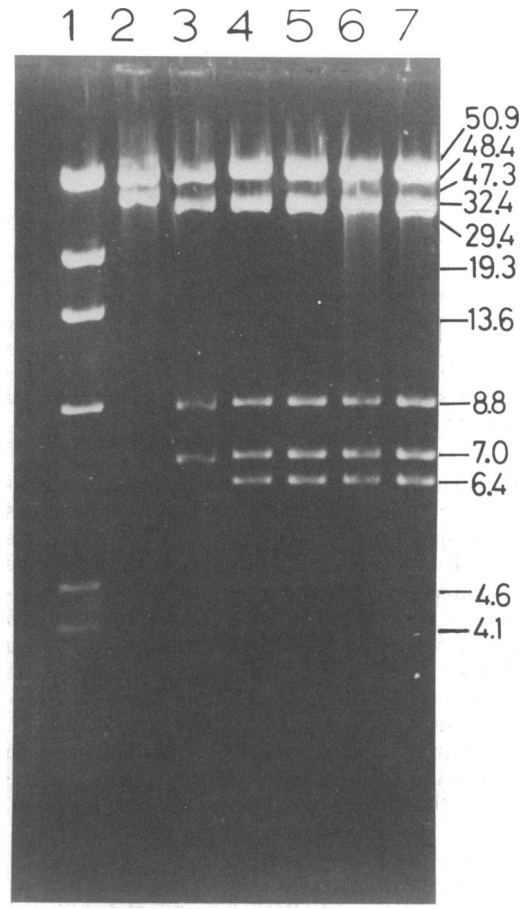

FIG. 4. *Agarose gel electrophoresis of phage DNA digested with HindIII: column 1, λcI857; column 2, λ540; column 3, λenvA*$^+$*; column 4, λ16-2; column 5, λ16-3; column 6, λ16-4; and column 7, λ16-5. λ540 contains a single HindIII site. λenvA*$^+$*, the transducing phage constructed from λ540, contains three HindIII sites, one at each end of the bacterial DNA insertion (7.0% fragment) and another near the right end generating an 8.8% fragment. This latter site was picked up when λenvA*$^+$ *recombined with λ*$^+$ *during its isolation (suspected from the heteroduplex analysis since this phage no longer has the nin deletion). The remaining phages yield identical fragments. In addition to those fragments contained in λenvA*$^+$ *there is a 6.6% fragment generated from the extra bacterial DNA contained in these phage. Also, the extra bacterial DNA results in an increase in the large fragment from the left end.*

Although we wanted the amber mutation from OV16 on the phage, it was easier to put the

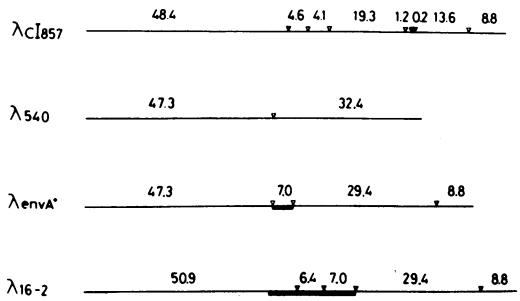

FIG. 5. *A schematic diagram of the various phages used in this investigation. The triangles indicate the sites cleaved by HindIII, deduced from Fig. 4. The numbers to the right refer to the percentage of total lambda DNA.*

ftsA(Ts) allele onto the phage first because suitable bacterial strains were available. This was done by plating λ16-3 on strain TKF12, which is *ftsA*(Ts) and *envA*$^+$. We then selected for phage that had regained the *envA*$^+$ allele by recombination with the bacterial chromosome. Such phage would have a high probability of also picking up the *ftsA*(Ts) allele because of its proximity. All λ*envA*$^+$ were isolated by transduction of ATK131 [*envA ftsA*(Ts)] to *envA*$^+$. Each transductant was then also screened to determine the *ftsA* allele on the phage. Of the phage that were *envA*$^+$, 37% were also *ftsA*(Ts). One of these, λ16-4, was used further.

If *ftsA* and the mutation in OV16 are in the same gene, then λ16-4 should not complement OV16. Lysogens of OV16 were constructed and found to be still temperature sensitive. Thus, the amber mutation in OV16 is in the *ftsA* gene.

This same procedure could also be used to put the amber mutation from OV16 onto the phage. Therefore, λ16-3 was plated on OV16, and λ*envA*$^+$ recombinants were again selected by complementation of strain ATK131 [*envA ftsA*(Ts)]. Of the phage that became *envA*$^+$, 32% were found to no longer complement the *ftsA*(Ts) mutation. These had presumably picked up the amber mutation, confirming that this mutation is in the *ftsA* gene. One of these phage, λ16-5, was used further.

These derivatives of λ16-2 were assumed to carry the mutated alleles since they no longer complemented the corresponding mutants. It is unlikely that the respective gene was deleted, because in each case the phage were generated by an event that occurred at high frequency. This is confirmed, since the *Hin*dIII profiles of these phage DNAs are identical to λ16-2 (Fig. 4, columns 3–6).

Identification of the *ftsA* gene product. The proteins coded for by the inserted bacterial DNA in these phages were determined by infection of UV-irradiated cells followed by pulse-labeling with [^{35}S]methionine. Comparison (Fig. 6, columns 2 and 3) of the patterns obtained with the transducing phage, λ16-2, and the original vector, λ540, revealed the presence of four additional proteins (molecular weights of 40,000, 50,000, 52,000, and 60,000) which must be expressed from the bacterial DNA. Infection with the phage carrying the *ftsA*(Am) mutation, λ16-5 (Fig. 6, column 4), resulted in the synthesis of only three of these proteins. One protein with a

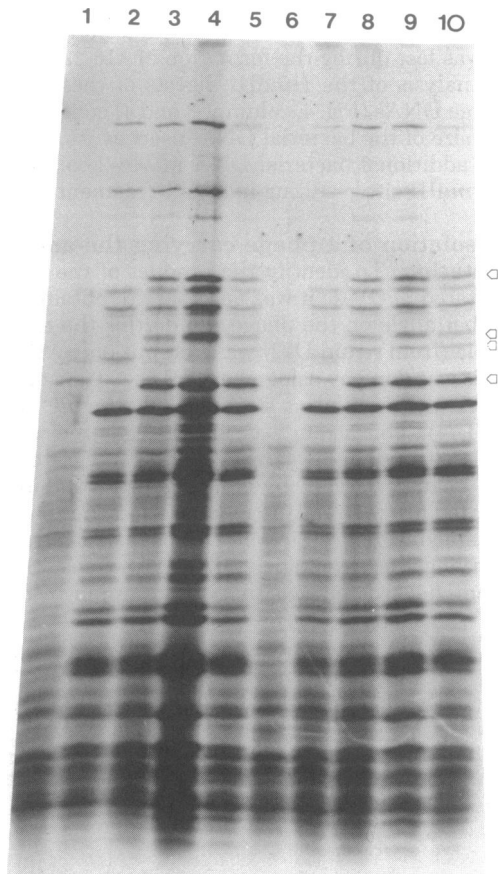

FIG. 6. *An autoradiogram of [^{35}S]methionine-labeled proteins obtained after phage infection of UV-irradiated cells. The cells were strain 159 in columns 1 to 5 and strain 159 (λimm^{434}supF) in columns 6 to 10. The phage were as follows: columns 1 and 6, no phage; columns 2 and 7, λ540; columns 3 and 8, λ16-2; columns 4 and 9, λ16-5; and columns 5 and 10, λ16-4. The arrows indicate the positions of the proteins specified by the bacterial DNA. One protein (molecular weight of 48,000) present in the λ540-infected cells is not present in the cells infected with the various transducing phages. The gene for this protein lies within the b2 region (17) and was deleted during formation of λ16-2.*

molecular weight of 50,000 was not synthesized. However, in the presence of a suppressor the synthesis of this protein was restored (Fig. 6, column 8). Thus, this protein must be the product of the *ftsA* gene.

Infection with the phage carrying the *ftsA*(Ts) allele (Fig. 6, columns 5 and 10) resulted in a pattern of protein synthesis that was identical with that obtained with λ16-2. In addition, these samples were run in the two-dimensional gel system of O'Farrell (15) (not shown) to determine whether the *ftsA*(Ts) mutation had resulted in a change in charge of the 50,000-dalton protein, but there was no change.

DISCUSSION

Many temperature-sensitive missense mutants that affect cell division have been isolated in *E. coli*, but few of these have led to the identification of the corresponding gene products. The exceptions are those in which the mutation also leads to a loss in the ability of the protein to bind penicillin, which can then be used as an assay for their identification (20). This assay is, of course, limited to very few of the proteins actually involved in cell division (based on the larger number of genes that have been implicated in cell division).

As a first step in identifying the gene products involved in cell division, we have isolated a collection of mutants with nonsense mutations in various genes that affect this process. In the second stage we have begun to isolate specialized λ-transducing phages that complement these mutants, the first of which is described here. Others have also been isolated and will be described in later publications.

Complementation tests show that the nonsense mutation in OV16 is in the gene that has been previously designated *ftsA*. With the use of the λ-transducing phage carrying the nonsense mutation we have been able to identify the product of the *ftsA* gene as a protein with a molecular weight of 50,000. We have also run the protein coded for by the original *ftsA*(Ts) allele in the two-dimensional gel system of O'Farrell (not shown). It has the same charge and molecular weight as the wild-type protein, thus confirming that *ftsA*(Ts) is a missense mutant.

In addition to the product of the *ftsA* gene, three other proteins are coded for by this transducing phage. They have approximate molecular weights of 40,000, 52,000, and 60,000. These could be the gene products of *envA*, *ddl*, or *murC* or of some other as yet unidentified genes that might be in this region. At present work is progressing in trying to identify them. The bacterial DNA insertion in this transducing phage is 21% the length of lambda, or about 10.5 kilobases. The four proteins we have found occupy 50% of the coding capacity of this DNA. It is possible that other smaller proteins are present which are not seen due to the large number of phage proteins in this region of the gel (although no new proteins were seen in the two-dimensional gels). Therefore, it is possible that at least some of this DNA is not expressed under these conditions, suggesting that it is noncoding DNA.

Wijsman (24) has pointed out that the genes in this region of the map are related in that they all affect the cell envelope. Fletcher et al. (6) raised the possibility that these genes might even be in one functional unit. However, this possibility can now be ruled out. The family of λ-transducing phages isolated by Fletcher et al. (6) start from a point in the *leu* operon and extend in the direction of *envA*. Our transducing phage starts to the right of *envA* and extends back towards *leu* until *murC*. In addition, we have a transducing phage that complements just *envA* (Wolf-Watz and Lutkenhaus, unpublished data). Since all of these phage can complement the appropriate mutant in the prophage state, we can assume that the promoter(s) for these genes are intact. Therefore, we can conclude that the genes in this region must have at least three separate promoters.

In the experiment presented in Fig. 6 either a nonlysogen or a heteroimmune lysogen was used for the analysis of the proteins synthesized by the various transducing phages. As a result the synthesis of the bacterial proteins coded for by the phage is increased due either to expression from the phage promoters or to gene dosage as a result of phage DNA synthesis. The level of expression of the *ftsA* gene in a homoimmune lysogen under these conditions (not shown) is the same order of magnitude as the expression of *polA* from λ*polA* (A. Newman, T. Linn, and R. Hayward, Mol. Gen. Genet., in press). This would suggest a level of approximately 400 molecules of the *ftsA* gene product per cell (9).

The role of the *ftsA* gene product in division is not yet clear, although the available evidence suggests that it plays an essential role. Investigation of a missense mutant in this gene reveals that the gene product is required throughout septation (23). The kinetics of division in the mutant containing the amber mutation show that the gene product cannot be reutilized and is presumably used up in the process of septation. In addition, the product appears to reach a critical level just as the cells become committed to divide (Vincente et al., submitted for publication).

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ADDENDUM IN PROOF

Recent results from this laboratory indicate that the *ftsA* locus can be divided into two cistrons. One cistron is identified by the *ftsA12*(Ts) mutation (21, 24), and the other is identified by the *ftsA84*(Ts) mutation (7, 8). This will be the subject of a subsequent publication.

LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116-167.
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. **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.
4. **Davis, R., 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.
5. **Donachie, W. D., K. Begg, and M. Vincente.** 1976. Cell length, cell growth and cell division. Nature (London) **264**:328-333.
6. **Fletcher, G., C. A. Irwin, J. M. Henson, C. Fillingim, 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.
7. **Hirota, Y., M. Richard, 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.
8. **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.
9. **Kornberg, A.** 1974. DNA synthesis. W. H. Freeman and Co., San Francisco.
10. **Kornberg, A.** 1978. Enzymatic replication of DNA in *E. coli* probed by small phages, p. 705-728. *In* I. Molineux and M. Kohiyama (ed.), DNA synthesis, present and future. Plenum Publishing Co., New York.
11. **Lutkenhaus, J. F.** 1977. The role of a major outer membrane protein in *Escherichia coli*. J. Bacteriol. **131**: 631-637.
12. **Murray, K., and N. E. Murray.** 1975. Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease III of *Haemophilus influenzae* and restriction endonuclease I of *E. coli*. J. Mol. Biol. **98**:551-564.
13. **Nishimura, Y., Y. Takeda, A. Nishimura, H. Suzuki, M. Inouye, and Y. Hirota.** 1977. Synthetic colE1 plasmids carrying genes for cell division in *Escherichia coli*. Plasmid **1**:67-77.
14. **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.
15. **O'Farrell, P. H.** 1975. High-resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007-4021.
16. **Ptashne, M.** 1967. Isolation of the λ phage repressor. Proc. Natl. Acad. Sci. U.S.A. **57**:306-313.
17. **Ray, P., and H. Murialdo.** 1975. The role of gene Nu3 in bacteriophage lambda head morphogenesis. Virology **64**:247-263.
18. **Ricard, M., and Y. Hirota.** 1973. Process of cellular division in *Escherichia coli*: physiological study on thermosensitive mutants defective in cell division. J. Bacteriol. **116**:314-322.
19. **Sevastopoulos, C. G., C. T. Wehr, and D. A. Glazer.** 1977. Large-scale automated isolation of *Escherichia coli* mutants with thermosensitive DNA replication. Proc. Natl. Acad. Sci. U.S.A. **74**:3485-3489.
20. **Spratt, B. G.** 1977. Temperature-sensitive cell division mutants of *Escherichia coli* with thermolabile penicillin-binding proteins. J. Bacteriol. **131**:293-305.
21. **van de Putte, P., J. van Dillewijn, and A. Rorsch.** 1964. The selection of mutants of *Escherichia coli* with impaired cell division at elevated temperature. Mutat. Res. **1**:121-128.
22. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
23. **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.
24. **Wijsman, H. J. W.** 1972. A genetic map of several mutations affecting the mucopeptide layer of *Escherichia coli*. Genet. Res. **20**:65-74.
25. **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.
26. **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.