JOURNAL OF BACTERIOLOGY, Oct. 1989, p. 5523–5530
0021-9193/89/105523-08$02.00/0

Vol. 171, No. 10

New Mutations *fts-36*, *lts-33*, and *ftsW* Clustered in the *mra* Region of the *Escherichia coli* Chromosome Induce Thermosensitive Cell Growth and Division

FUMITOSHI ISHINO, HAI KWAN JUNG,† MASATO IKEDA, MASAKI DOI,‡ MASAAKI WACHI, AND MICHIO MATSUHASHI*

Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received 6 March 1989/Accepted 30 June 1989

Three new mutants of *Escherichia coli* showing thermosensitive cell growth and division were isolated, and the mutations were mapped to the *mra* region at 2 min on the *E. coli* chromosome map distal to *leuA*. Two mutations were mapped closely upstream of *ftsI* (also called *pbpB*), in a region of 600 bases; the *fts-36* mutant showed thermosensitive growth and formed filamentous cells at 42°C, whereas the *lts-33* mutant lysed at 42°C without forming filamentous cells. The mutation in the third new thermosensitive, filament-forming mutant, named *ftsW*, was mapped between *murF* and *murG*. By isolation of these three mutants, about 90% of the 17-kilobase region from *fts-36–lts-33* to *envA* could be filled with genes for cell division and growth, and the genes could be aligned.

Many genes are involved in the mechanism of cell growth and division of *Escherichia coli*. These genes are scattered along the entire length of the chromosome, but appreciable numbers have been found to be localized in a few restricted regions forming gene clusters. The region located at 2 min distal to *leuA*, which we called the *mra* (murein synthesis gene cluster a) region (17), contains several genes that code for enzymes synthesizing precursors of peptidoglycan: *murE*, *murF*, *murC*, and *ddl*. This cluster of genes for peptidoglycan synthesis is flanked by several genes involved in cell division: *ftsI* (filamentaous thermosensitive growth I [18]), coding for a septum peptidoglycan synthetase, penicillin-binding protein 3 (7) (also called *pbpB*), at the proximal end; and a series of *fts* genes, *ftsQ*, *ftsA*, and *ftsZ*, the precise functions of which are unknown, at the distal end. Of these genes, *ftsZ* is also involved in SOS control of cell division (13). The *mrb* (murein synthesis gene cluster b) area at 89 min (17) also contains several genes involved in cell growth, such as *mrbA* and *mrbB*. The area at 76 min is reported to contain four genes for cell division, *ftsE*, *ftsX*, *ftsY*, and *ftsS* (5). At 14.2 and 71 min there are areas called *mrd* (25) and *mre* (26) (murein synthesis gene clusters d and e, respectively), in which genes responsible for formation of the rod shape of the cells are located (4, 24–26).

The *mra* region, including the flanking *fts* regions, is one of the largest of these gene clusters for cell growth and division, extending 17 kilobases (kb) from *ftsI* to *envA*. The DNA sequences of a 5-kb portion from *ddl* to *envA* (1, 20, 21, 27) and a 3-kb portion encompassing *ftsI* (18) have been determined. However, the physical map of the remaining portions has not been precisely investigated, and some portions are still not covered by even putative genes. Therefore, we tried to investigate the *mra* region thoroughly by isolation of new mutants by localized mutagenesis. Our first aim was to fill in the open spaces in this region with new mutations and to construct a complete physical map of genes aligned in the total region. We expected by this method to obtain further information on the enzymatic and regulatory mechanisms of cell growth and division.

MATERIALS AND METHODS

Bacterial and bacteriophage strains and growth media. All *E. coli* strains used were derived from strain K-12. Their properties and sources are shown in Table 1. Phage strain λ6753 is a specialized transducing lambda phage carrying *leuA*. The original phage strain carrying the *leuA* region, from which the phages used in this work were derived, was isolated by Shimada et al. (23). Other specialized transducing lambda phages carrying various lengths of the *E. coli* chromosome encompassing *leuA* and its distal region were isolated from a mitomycin C (10 μg/ml) lysate of strain JE6198 that lacks the chromosomal *attλ* site and has lysogenized λ6753. The region of the *E. coli* chromosome on the phages was determined by complementation tests with *E. coli* strains carrying mutations in the *mra* region. Cells were grown in modified Lennox broth (9) (L′ broth) containing 1% Polypeptone (BBL Microbiology Systems), 0.5% yeast extract, 0.1% glucose, 20 mg of thymine per liter, and 0.5% NaCl, adjusted to pH 7.0; alternatively, cells were grown in L′ broth that did not contain NaCl (L′ΔNaCl broth). Synthetic M9 medium (15) with necessary supplements was used for determination of nutrient requirements and transduction of the *leu*$^+$ allele with linked markers. Media were solidified with 1.5% agar.

Isolation of mutants by localized mutagenesis. Phage P1 *vir* was mutagenized by treatment with hydroxylamine by the method of Hong and Ames (6). P1 *vir* was grown on wild-type *E. coli* W3110 at 37°C in L′ broth (titer of 10^{10}/ml). The phage was centrifuged at 100,000 × *g* for 2 h and suspended in 0.1 M sodium phosphate buffer (pH 6.0) containing 1 mM EDTA (PE buffer) to obtain a titer of 2×10^{11}/ml. To 2 ml of the concentrated phage suspension were added 10 ml of PE buffer and 8 ml of 1 M hydroxylamine hydrochloride containing 1 mM EDTA (adjusted to pH 6.0 with NaOH). The mixture was incubated for 13 h at 37°C. The mutagenized phage was collected by ultracentrifugation

* Corresponding author.
† Present address: National Institute of Safety Research, Nokbundong 5, Eunpyungku, Seoul, Korea.
‡ Present address: Yamanouchi Pharmaceutical Co., Ltd., Itabashi-ku, Tokyo 174, Japan.

TABLE 1. *E. coli* strains used

Strain	Relevant marker	Other markers	Source
JE1011	*leuB*	F⁻ *thr trp his thy ara lac gal xyl mtl rpsL tonA*	M. Ishibashi
JE1011R2		Same as JE1011 but *leuB*⁺, thermoresistant transductant (donor, JLA54)	This work
JE6198A	*leuA*	F⁻ Δ(*lac-pro*) *thi*	This work
JE6198		F⁻ *leuA* Δ(*lac-pro*) *thi* Δ(*gal-att-bio*)	S. Yasuda
W3110		F⁻	
TKL11	*murE*	F⁻ *thr-1 leuB codA pyrF101 his-108 thyA66 argG66 ilvA634 thi-1 deoC1 lacY1 tonA21 tsx-25 supE44*	E. Lugtenberg (12)
TKL46	*murF*	Same as TKL11 but *murF* instead of of *murE*	E. Lugtenberg (11)
OV58	*murG*(Am)	*ara*(Am) *lac*(Am) *gal*(Am) *galE trp*(Am)*leu ilv his thy suIIIA81*	W. D. Donachie (22)
ST222	*murC*	Same as JE1011	M. Matsuhashi (17)
JLB33	*lts-33*	Same as JE1011 but *leuB*⁺	This work
JLB36	*fts-36*	Same as JE1011 but *leuB*⁺, thermosensitive transductant (donor, JLA54)	This work
JLB17	*ftsW*	Same as JE1011 but *leuB*⁺	This work
JLA54	*fts-36*	Same as JE6198A but unknown mutation(s) and *leuA*⁺	This work
HB101		F⁻ *hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44*	D. Roulland-Dussoix (2)

at 80,000 × *g* for 2 h and washed twice with PE buffer. The survival rate of phages in the mutagenization procedure was about 10^{-2}. A *leuB* strain (JE1011) or a *leuA* strain (JE6198A) derived from strain JE6198 by P1 phage transduction (donor strain, W3110) was grown in L′ broth at 37°C to 5×10^8 cells per ml and transduced with hydroxylamine-treated P1 *vir* at a multiplicity of infection of 0.006. After incubation at 37°C for 20 min, the bacteria were washed twice with 0.85% NaCl solution and resuspended in the same solution. A 0.1-ml sample was then spread on M9 plates supplemented with all required amino acids except leucine, and the plates were incubated at 30°C for 48 h. Colonies of Leu⁺ transductants were purified, and the temperature sensitivity of their growth was tested on L′ΔNaCl plates at 42°C.

Complementation test with λ transducing phages. Complementation tests were carried out by the cross-streak method. Overnight cultures of each mutant were streaked on L′ΔNaCl plates, and lysates of λ transducing phages (more than 10^9/ml) that carried various lengths of chromosomal DNA encompassing *leuA* and the distal region were cross-streaked on the plates. The plates were then incubated overnight at 42°C. Complementation was judged positive when thermoresistant colonies were seen at the cross-section of streaks.

Subcloning of DNA fragments of the *mra* region into high-copy-number plasmids. Plasmid pLC26-6 (from the *E. coli* chromosome bank of Clarke and Carbon [3]), which carries the region of the chromosome from *leuA* to *murG*, and phage λJL149 (isolated in this laboratory), which carries the region from *leuA* to *ftsA*, were used as sources of subclones of the *mra* region. DNA fragments obtained from these chromosomal sources by digestion with various restriction enzymes were ligated with high-copy-number plasmids such as pBR322, pACYC184, and pHSG398. The procedure is shown in Fig. 1.

RESULTS

Isolation of mutants of the *mra* region by localized mutagenesis. About 100 thermosensitive mutants with mutations linked to *leuA* were isolated. The locations of the mutations were roughly mapped by complementation tests, using lambda transducing phages (Fig. 2). Fine mapping of the mutants was carried out by complementation with plasmids carrying subclones of the *mra* region (Fig. 2). Three mutants were selected for further studies. Strain JLA54, an *fts* mutant with the phenotype of thermosensitive growth and formation of filamentous cells, was obtained from strain JE6198A. Since this mutant strain contained multiple unidentified mutations, the *fts* phenotype was transduced into strain JE1011 by phage P1, and a putative single *fts* mutant, strain JLB36 (*fts-36*), was isolated from the transductants. An *lts* mutant with the phenotype of thermosensitive growth and cell lysis, strain JLB33 (*lts-33*), and another *fts* mutant, strain JLB17 (*ftsW*), were obtained from strain JE1011. The mutations *fts-36* and *lts-33* were mapped closely upstream of *ftsI*, which encodes penicillin-binding protein 3 (18), and *ftsW* was mapped between *murF* (11) and *murG* (22). Figure 2 shows only a 12-kb *Hin*dIII fragment encompassing *fts-36–lts-33*, *ftsI*, *murE* (12), *murF*, *ftsW*, *murG*, and part of *murC* (11). The three mutations could not be complemented by the transducing phage derived from λ16-2 (14) that complemented the more distal region encompassing *murC*, *ddl*, *ftsQ*, *ftsA*, *ftsZ*, and *envA*.

Other mutations were mapped in known genes of these regions. Some of the mutations showed complex phenotypes and will be described in detail elsewhere.

Properties of the mutants. Figure 3 shows the growth curves of the mutants *fts-36*, *lts-33*, and *ftsW* after the temperature shift-up in L′ΔNaCl broth; Fig. 4 shows the changes in cell shape after the temperature shift. A pair of strains isogenic to *fts-36*, JLB36 (*fts-36*) and JE1011R2 (*fts-36*⁺), which were prepared by transduction of the *leuB*⁺ *fts-36* allele into the *leuB fts-36*⁺ strain JE1011, showed no appreciable difference in growth at 30°C; at 42°C (cell concentration, 10^7/ml), however, growth of the *fts-36* strain was slower (Fig. 3A). Moreover, after the temperature shift-up at a lower cell concentration (10^6/ml), *fts-36* cells did not grow appreciably (Fig. 3A). In L′ broth in the presence of 0.5% NaCl, the *fts-36* mutant cells grew as long rods at 30°C but grew as filaments at 42°C (data not shown). In L′ΔNaCl broth, the *fts-36* mutant cells grew as short filamentous cells even at 30°C. At 42°C, these filaments were much longer than in the presence of NaCl and partially underwent lysis, whereas the *fts-36*⁺ cells grew as short rods at both temperatures (Fig. 4A).

The *lts-33* mutant showed cell lysis without formation of filamentous cells after the shift-up to 42°C (cell concentration, 2×10^7/ml) in L′ΔNaCl broth (Fig. 3B and 4B). In this experiment, the originally isolated *lts* mutant strain JLB33 was used.

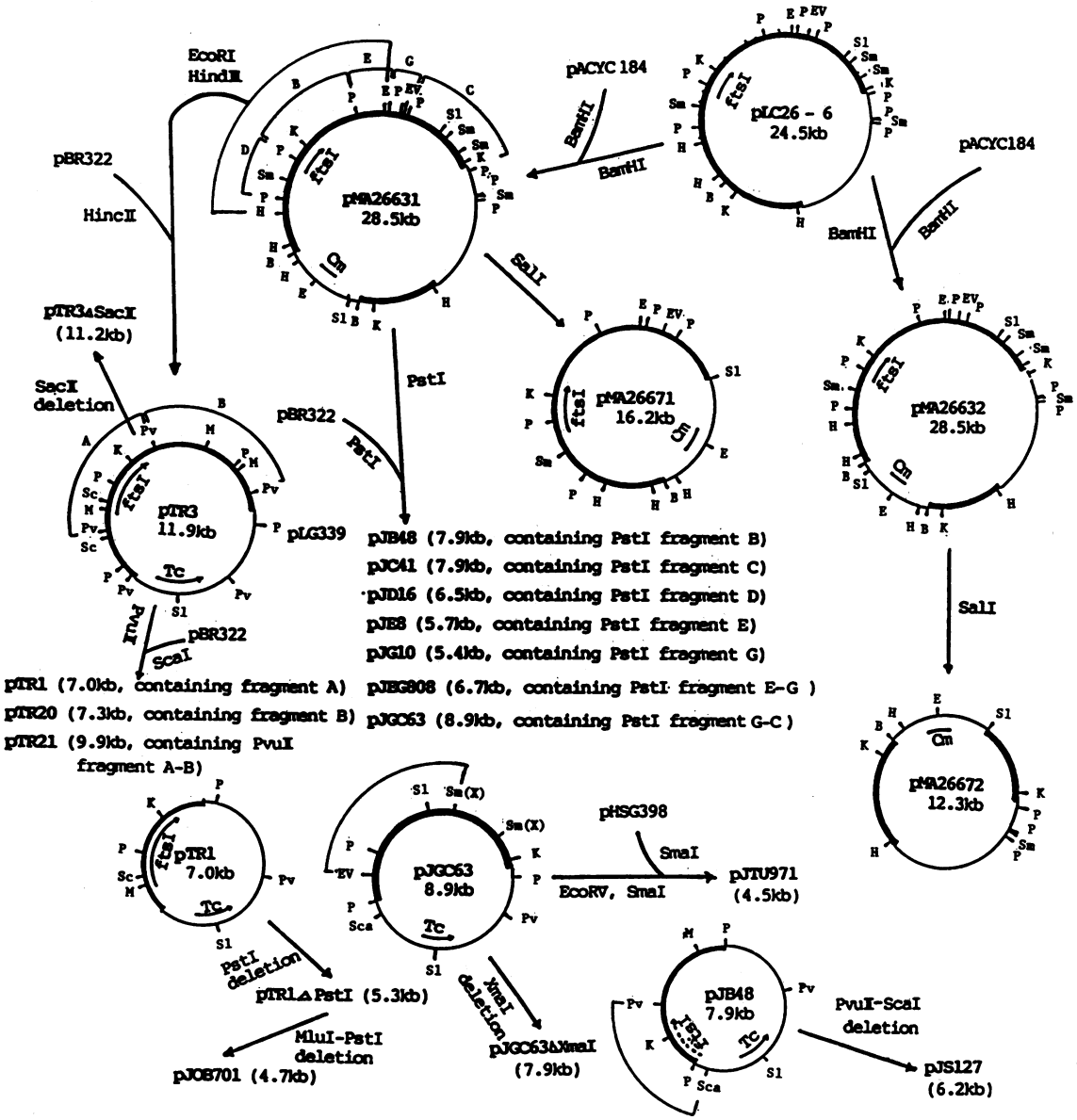


FIG. 1. Construction of plasmids. Abbreviations for restriction endonucleases: B, *Bam*HI; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; M, *Mlu*I; P, *Pst*I; Pv, *Pvu*II; Sc, *Sac*II; Sca, *Sca*I; Sl, *Sal*I; Sm, *Sma*I; X, *Xma*I. Plasmids constructed by ligation of the *Pst*I fragments of pMA26631 and pBR322, as well as plasmids pTR20 and pJTU971, contained chromosomal fragments downstream of the *bla* promoter of vector plasmids in the same direction as the *bla* promoter. Plasmid pTR21 contained chromosomal fragments in the reverse direction.

The *ftsW* mutant showed the typical phenotype of a temperature-sensitive cell division mutant (Fig. 3C and 4C): *ftsW* mutant JLB17 cells grew as rods at 30°C and as long filaments at 42°C (cell concentration, 10^7/ml). Upon the temperature shift-up at a lower cell concentration (10^6/ml), it showed no growth.

The *fts-36* and *ftsW* strains showed a more pronounced thermosensitive growth phenotype at lower cell density, which suggested the existence of some cell concentration-dependent factor(s).

These three mutations were recessive to the wild type, as shown by complementation by lambda transducing phage carrying the entire *mra* region of the chromosome.

Fine mapping of the *fts-36* and *lts-33* mutations by complementation with phage and plasmids. The *fts-36* mutation could be complemented by a transducing lambda phage, λJL148, that complemented *leuA* and *ftsI* and by those carrying longer chromosomal fragments, λJL195, λJL146, λJL16, and λ6752, but not by λ6753, which complemented only *leuA*. Cells carrying a complementing phage became thermoresistant, growing as rods at both 30 and 42°C in L′ΔNaCl broth. Complementations of the *lts-33* mutation by transducing lambda phages were similar to those of *fts-36*, causing prevention of cell lysis at 42°C in the same culture medium. Therefore, we concluded that both the *fts-36* and *lts-33* mutations were located in the region encompassing *leuA*, *ftsI*, and *murE*.

The results of fine mapping using plasmids are shown in Fig. 5. The *fts-36* and *lts-33* mutations could both be complemented by plasmid pTR3, which carries a 7.3-kb chromosomal fragment covering *ftsI*, *murE*, and *murF*, but not by pTR3Δ*Sac*II, which contains the same fragment except for

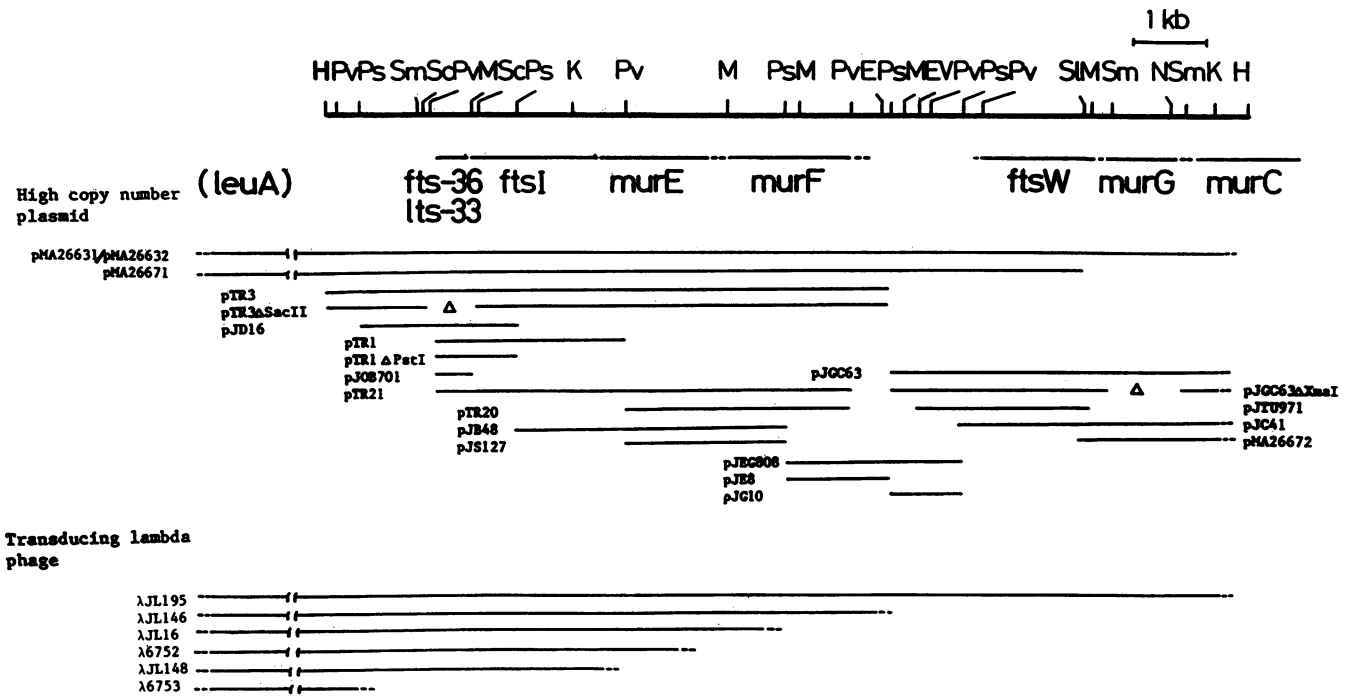


FIG. 2. Physical map of the 13-kb *Hin*dIII fragment of the *mra* region of the *E. coli* chromosome showing the extent of coverage by plasmids and transducing lambda phages. Δ, Deletion of a DNA fragment. Abbreviations for restriction endonucleases not given in the legend to Fig. 1: N, *Nru*I; C, *Cla*I.

deletion of a 0.7-kb *Sac*II fragment encompassing the 5′ end and upstream region of *ftsI*. Plasmid pTR1, carrying a 2.6-kb chromosomal fragment covering *ftsI* and part of the *murE* gene, was effective even after removal of a 1.45-kb *Pst*I-*Pvu*II fragment (pTR1Δ*Pst*I; Fig. 1) encompassing the major 3′ portion of *ftsI* and the 5′-terminal portion of *murE*. Plasmid pJOB701, which carried only a 600-base *Pvu*II-*Mlu*I fragment (Fig. 2), could also complement both the *fts-36* and *lts-33* mutations.

These results suggested that the *fts* and *lts* mutations were both located inside the small region directly upstream of the coding frame of *ftsI*.

Fine mapping of the *ftsW* mutation. The phenotype of the *ftsW* mutation, thermosensitive growth and filamentation, could be complemented by transducing phage λJL195, which carries a chromosomal fragment encompassing the region from *leuA* to *murG* (Fig. 2). Fine mapping with plasmids (Fig. 6) showed that plasmids pJGC63, pJGC63Δ*Xma*I, pJC41, and pJTU971 complemented the mutation but that plasmids pMA26671, pJG10, and pMA26672 did not. These results indicate that the most plausible location of the *ftsW* mutation is in a 2.2-kb *Pst*I-*Sma*I region between *murF* and *murG*, close to the *murG* gene.

DISCUSSION

By isolation of new mutants and physical mapping of their mutations on the chromosome by complementation experiments, the genes in the 12-kb *Hin*dIII fragment encompassing the *mra* region could mostly be filled, leaving only about

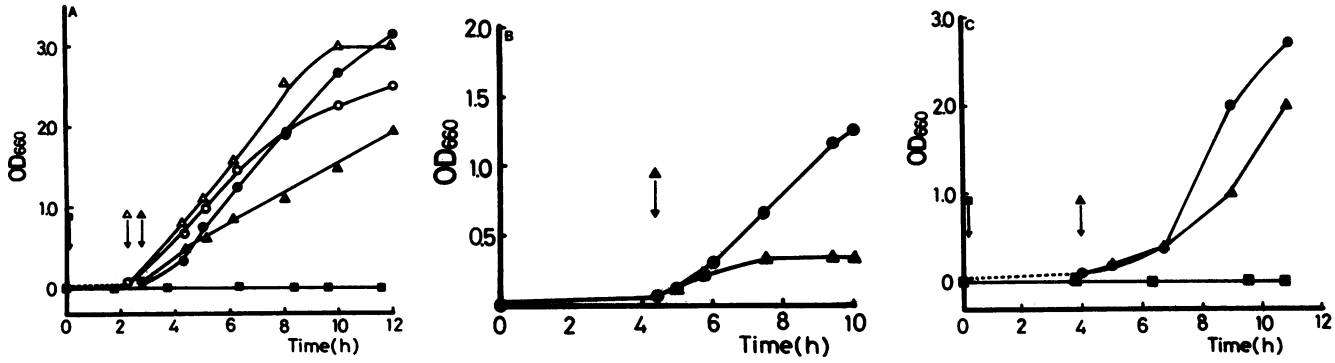


FIG. 3. Growth curves of *fts-36*, *lts-33*, and *ftsW* mutants. (A) Isogenic *fts-36* mutant strain JLB36 (closed symbols) and thermoresistant *fts-36*$^+$ strain JE1011R2 (open symbols) grown in L′ΔNaCl broth at 30°C (circles) or shifted to 42°C at 10^7 (triangles) or 10^6 (squares) cells per ml. (B) Mutant strain JLB33 containing the *lts-33* mutation grown in L′ΔNaCl broth at 30°C (circles) or shifted to 42°C at 2×10^7 cells per ml (triangles). (C) Mutant strain JLB17 containing the *ftsW* mutation grown in L′ΔNaCl broth at 30°C (circles) or shifted to 42°C at 10^7 (triangles) or 10^6 (squares) cells per ml. Arrows show the time of temperature shift.

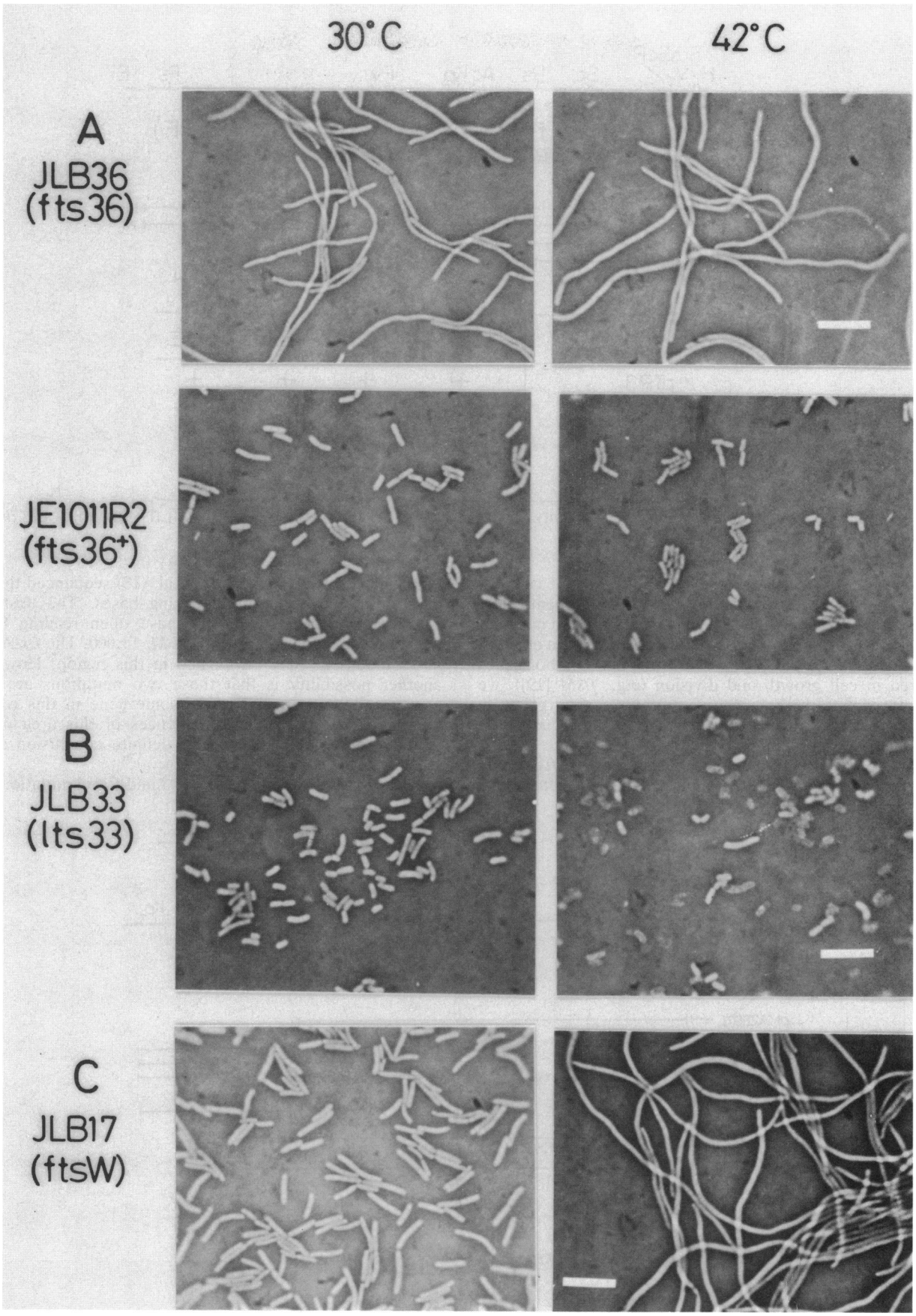
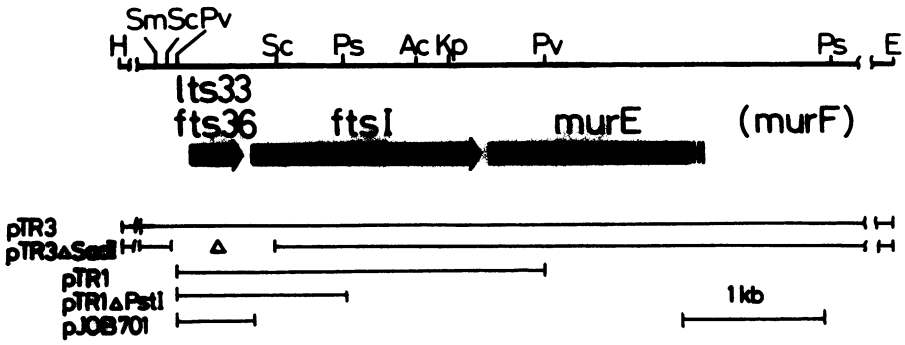


FIG. 4. Cell shapes of *fts-36*, *lts-33*, and *ftsW* mutants. Cells grown in L'ΔNaCl broth at 30 and 42°C were examined by dark-field phase-contrast microscopy. The temperature shift from 30 to 42°C was performed at a density of about 10^7 cells per ml. Bars, 10 μm.



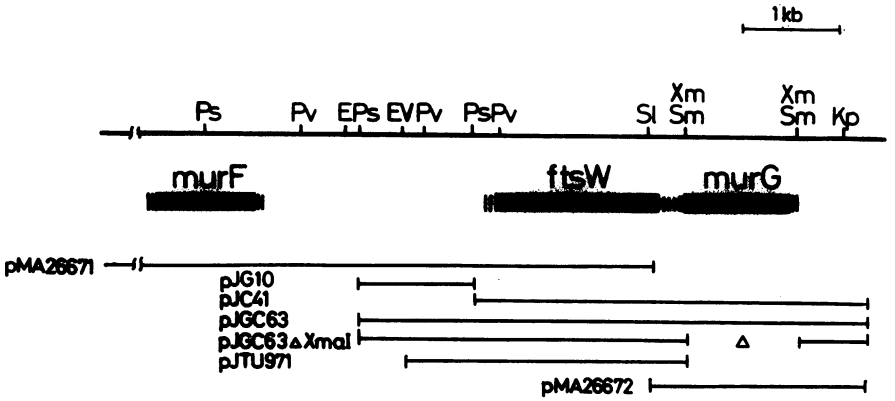
Plasmid \ Mutant	fts36	lts33	ftsI	murE
pTR3	+	+	+	+
pTR3ΔSacII	−	−	−	+
pTR1	+	+	+	−
pTR1Δ PstI	+	+	−	−
pJOB701	+	+	−	−

FIG. 5. Complementation of the temperature sensitivity of *fts-36* and *lts-33* mutants by plasmids. Strains JLB36 (*fts-36*), JLB33 (*lts-33*), JLB1 (*ftsI*), and TKL11 (*murE*) were used.

2 kb of open space between *murF* and *ftsW* that can still contain at least one coding frame (Fig. 2). Alignment of the *ftsI*, *murE*, and *murF* genes has also been reported independently by Maruyama et al. (16). Although the region of about 5 kb between *leuA* and *fts-36–lts-33* may also harbor genes involved in cell growth and division (e.g., *ftsM* [19]), we could not isolate thermosensitive mutants at this region by localized mutagenesis, even from among 100 independent mutants selected by linkage to *leuA*.

The two mutations with different phenotypes, *fts-36* and *lts-33*, are probably located in a 600-base chromosomal region. Previously, Nakamura et al. (18) sequenced the *ftsI* gene and several hundred flanking bases. The upstream flanking region contains a 330-base open reading frame capable of coding for a peptide of M_r 13,000. The *fts-36* and *lts-33* mutations may be located in this region. However, another possibility is that these two mutations are only suppressed by amplification of some gene in this region. Determination of the base sequences of this area of the mutants is required before any definite conclusion about these two mutations can be drawn.

The two phenotypes of the *lts-33* and *fts-36* mutations are



Plasmid \ Mutant	murF	ftsW	murG
pMA26671	+	−	−
pJG10	−	−	−
pJC41	−	+	+
pJGC63	−	+	+
pJGC63ΔXmaI	−	+	−
pJTU971	−	+	−
pMA26672	−	−	+

FIG. 6. Complementation of the temperature sensitivity of the *ftsW* mutant by plasmids. Strain TKL46 (*murF*), JLB17 (*ftsW*), and OV58 (*murG*) were used.

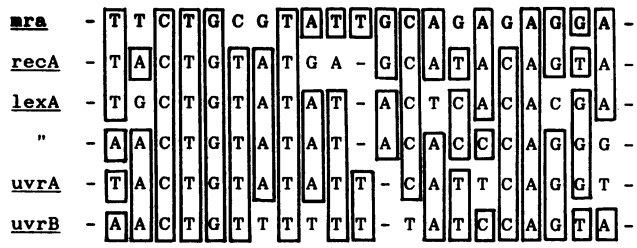


FIG. 7. Homology in the sequence of 21 bases (71 to 91 from the *Pvu*II site of the *mra* region) with the SOS box sequences reported. Identical bases are boxed.

thermosensitive cell lysis and filamentation. The former phenotype is the result of a defect in cell growth, such as formation of cell envelopes, or activation or overproduction of a lytic enzyme. The latter phenotype is certainly the result of inhibition of cell division. These two processes, cell growth and division, could be mutually competitive and may be strictly controlled in the cell. It is therefore of particular interest to determine whether a unique gene in the 600-base region can function in both cell growth and division. Such a gene may function in regulating these two processes. Cloning of the two mutant genes and determination of their base sequences are in progress.

The 330-base open reading frame in this 600-base chromosomal region has a start codon, ATG (bases 99 to 101 from the *Pvu*II site of plasmid pJOB701), and a Shine-Dalgarno-like sequence, GAGGACGA (87 to 94), 10 bases upstream. Furthermore, 21 and 43 bases upstream there are promoter-like sequences, TTCAGT (66 to 71) and CTGAGA (44–49). However, the most interesting sequence in this regulatory region is the 21-base sequence TTCTGCGTATTGCA GAGAGGA (71 to 91), which has high homology with the consensus sequence of the so-called SOS box (10; Fig. 7), a binding site of the *lexA* protein. Binding of the *lexA* protein to this sequence from the *mra* region has not, however, yet been shown. It is noteworthy that a putative *lexA*-controlled gene, *dinA* (8), has been reported to be located at 2 min on the *E. coli* chromosome map, close to *leuA*. The relationship between the *mra* region and the *dinA* gene needs investigation.

The *ftsW* gene is certainly involved in the process of cell division. The 12-kb *fts-36/lts-33–ddl* region, the *mra* region in a narrow sense, includes another gene for cell division, *ftsI*, and is flanked by a series of genes for cell division, *ftsQ*, *ftsA*, and *ftsZ*. Studies on the precise mechanism of function and base sequence of this new *fts* gene are also in progress.

ACKNOWLEDGMENTS

We thank Isao Shibuya and Akinori Ohta, Saitama University, for helpful experimental advice. We also thank Masao Yamada for providing lambda phage strains λ6752 and λ6753 and Y. Nakamura for phage P1 *vir*.

This work was partially supported by grants-in-aid for scientific research 61470157 and 63430020 to M.M. and 59580097 to F.I., grant-in-aid for scientific research on a special project 63615515 to M.M., and a grant in-aid for cooperative research from the Ministry of Education, Science and Culture of Japan.

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