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Inhibition of Growth of *ftsQ*, *ftsA*, and *ftsZ* Mutant Cells of *Escherichia coli* by Amplification of a Chromosomal Region Encompassing Closely Aligned Cell Division and Cell Growth Genes

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Amplification of a 2.6-kilobase chromosomal fragment of the *mra* region of *Escherichia coli* encompassing the *ftsI*(*pbpB*) gene and an open reading frame upstream was lethal to *E. coli* strains with mutations of the flanking cell division genes *ftsQ*, *ftsA*, and *ftsZ*. A shortened fragment in which the major portion of *ftsI* was deleted also had lethal effects on *ftsQ* and *ftsZ* mutants.

Several genes responsible for cell growth and division are located on the *Escherichia coli* chromosome in gene clusters. The largest of these clusters known is located at about 2 min on the *E. coli* chromosome map; since this region involves several genes of peptidoglycan (murein) synthesis, we called it the *mra* (murein cluster a) region (8). Detailed investigations (4, 5, 14) have shown that at least 12 genes involved in cell growth or division are aligned in this region: *fts*-36/*lts-33*, *ftsI* (also called *pbpB* [11]), *murE*, *murF* (followed by an unidentified region of about 2 kilobases [kb]), *ftsW*, *murG*, *murC*, *ddl*, *ftsQ*, *ftsA*, *ftsZ*, and *envA* (5). The functions of the protein products of only five of these genes are known: *ftsI* codes for penicillin-binding protein 3 (9, 11), a septum peptidoglycan synthetase (6); and *murC*, *murE*, *murF*, and *ddl* code for enzymes involved in the synthesis of nucleotide precursors of peptidoglycan (8, 14). Four *fts* genes, *ftsW* (5), *ftsQ*, *ftsA*, and *ftsZ* (7, 10), and a small chromosomal region in which the *fts-36*/*lts-33* mutation is located (5) are involved in cell division, but their precise functions have not been clarified. Cell division is a complex phenomenon in which many genes functioning in septum formation are expressed sequentially, or probably sometimes in concert. Cell division is also dependent on the cell growth: cell growth is required before septum formation, but it may compete with division of cells, in which lateral growth of cell wall peptidoglycan causing elongation of the cells and its vertical growth causing formation of a septum are supposed to be well controlled (1a, 13).

Previously, two mutations, *fts-36*, and *lts-33*, mapped in a 0.4-kb region upstream of *ftsI*, were found to cause different phenotypes: a defect of cell division (*fts-36*) and a defect of cell growth (*lts-33*) (5). Amplification of a chromosomal fragment encompassing the area that complemented these two novel cell division-cell growth mutations had remarkable effects on several cell division mutants, as reported here. These observations may provide a clue to the interaction of cell division-growth genes or their products.

The *E. coli* mutant strains used are listed in Table 1.

TABLE 1. *E. coli* K-12 strains used

Strain	Genetic markers	Source
JE1011	F⁻ *thr leu trp his thy ara lac gal xyl mtl rspL tonA*	M. Ishibashi
JLB1	Same as JE1011 but *leu*⁺ and *ftsI*	This laboratory
JLB17	Same as JE1011 but *leu*⁺ and *ftsW*	This laboratory
TOEL1	*thr pro his arg thi thy ftsQ*	*leu*⁺ transductant of TOE1 (1)
TOEL13	*thr pro his arg thi thy ftsA*	*leu*⁺ transductant of TOE13 (1)
KEN90	*ilv his trp thy ftsZ tyr ara lac gal supF*	K. J. Begg (1)
JEFQ1	Same as JE1011 but *leu*⁺ and *ftsQ*	This work, by *leu*⁺ transduction to JE1011 (donor: TOEL1)
JEFA1	Same as JE1011 but *leu*⁺ and *ftsA*	This work, by *leu*⁺ transduction to JE1011 (donor: TOEL13)
JEFZ1	Same as JE1011 but *leu*⁺ and *ftsZ*	This work, by *leu*⁺ transduction to JE1011 (donor: KEN90)

Plasmids that carried *E. coli* chromosomal fragments of different lengths were isolated previously in this laboratory by cloning in high-copy-number plasmids (5). The extent of coverage of the chromosome by these plasmids is shown in Fig. 1. These plasmids were introduced into several *fts* mutants of *E. coli* by transformation, and the cells were cultured on agar plates of modified Lennox broth (12) containing 1% (wt/vol) polypeptone, 0.5% (wt/vol) yeast extract, 0.1% (wt/vol) glucose, and 20 mg of thymine per liter, adjusted to pH 7.0, in the presence or absence of 0.5%

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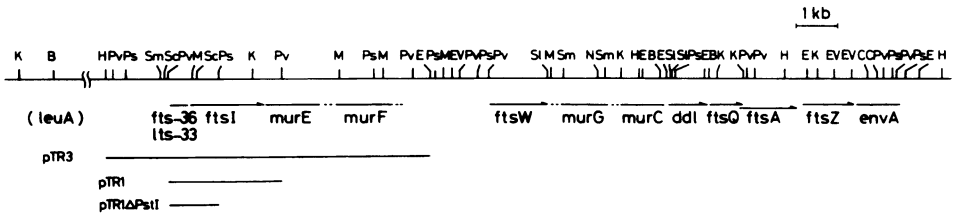

FIG. 1. Alignment of genes of *mra* and its flanking regions and its coverage by the plasmids used. All plasmids contained the driving unit and tetracycline resistance from PBR322. Chromosomal fragments were inserted downstream of the *bla* promoter. The directions of the *bla* promoter, the 330-base open reading frame, and *ftsI* are the same. Abbreviations for restriction endonucleases: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; M, *Mlu*I; N, *Nru*I; Ps, *Pst*I; Pv, *Pvu*II; Sc, *Sca*I; Sl, *Sal*I; Sm, *Sma*I.

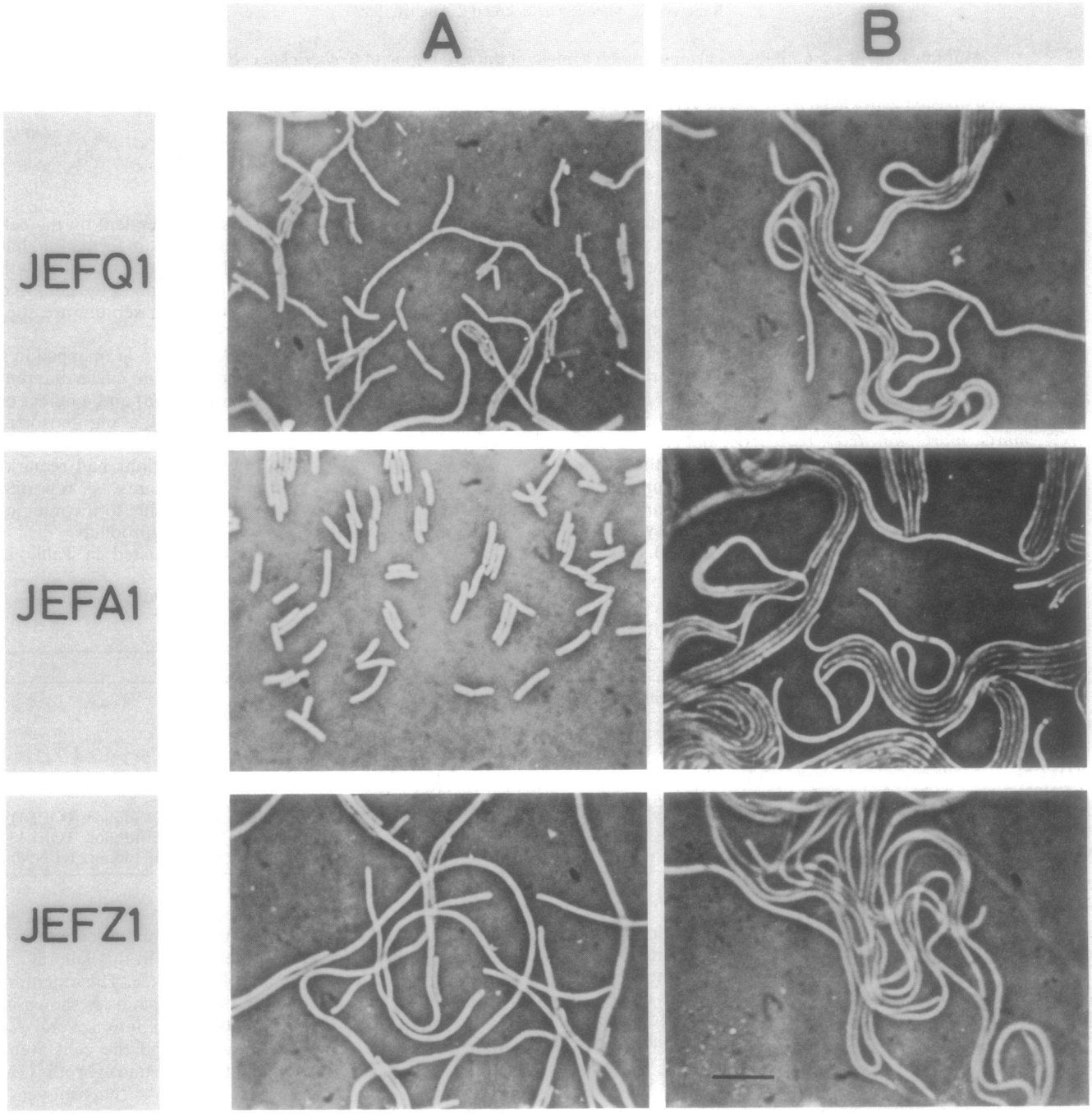

FIG. 2. Morphological changes of *ftsQ*, *ftsA*, and *ftsZ* mutants induced by introduction of plasmid pTR1: (A) no plasmid; (B) with plasmid pTR1. Freshly prepared single colonies of the transformants in L′ agar plates at 30°C were streaked on L′ΔNaCl agar plates and cultured at 30°C for 8 h. Bar, 10 μm.

TABLE 2. Comparison of colony-forming abilities of *fts* mutants containing plasmids containing *fts-36/lts-33-ftsI* regions in the presence and absence of NaCl[a]

Strain	Genotype	Colony-forming ability of mutants containing the following plasmids:											
		pTR1			pTR1ΔPstI			pBR322			None		
		ΔNaCl	NaCl	ΔNaCl/NaCl	ΔNaCl	NaCl	ΔNaCl/NaCl	ΔNaCl	NaCl	ΔNaCl/NaCl	ΔNaCl	NaCl	ΔNaCl/NaCl
JLB1	*ftsI*	542	530	1.0		ND[b]		909	980	0.9	1,055	1,032	1.0
JLB17	*ftsW*	156	164	1.0	225	289	0.8	219	225	1.0	90	73	1.2
JEFQ1	*ftsQ*	3	483	0.006	5	524	0.009	191	185	1.0	260	257	1.0
JEFA1	*ftsA*	4	426	0.009	404	563	0.7	519	571	0.9	206	225	0.9
JFFZ1	*ftsZ*	3	345	0.009	24	455	0.05	288	281	1.0	75	59	1.3
JE1011		305	337	0.9	287	284	1.0	758	744	1.0	977	951	1.0

[a] Values are numbers of colonies formed at 30°C on L′ agar plates in the absence and presence of 0.5% NaCl and their ratios. Inoculum sizes were arbitrary.
[b] ND, Exact measurement was not possible due to the low plating efficiencies (10^{-2}) even in the presence of NaCl.

(wt/vol) NaCl (referred to as L′ broth and L′ΔNaCl broth, respectively) at 30°C, a permissive temperature for growth of the mutant cells. In L′ΔNaCl broth at this temperature, the *ftsZ* mutant strain JEFZ1 grew poorly, forming small colonies of elongated cells, whereas the *ftsQ* and *ftsA* mutant strains JEFQ1 and JEFA1 grew normally, forming shorter rods (Fig. 2). When the plasmid pTR1, carrying a 2.6-kb chromosomal fragment encompassing the entire region of *ftsI* gene and its 0.5-kb proximal and 0.45-kb distal flanking regions, was introduced into the mutants, they all formed long filamentous cells (Fig. 2) and died at 30°C on plates of L′ΔNaCl broth solidified with 0.5% (wt/vol) agar. The plasmid pTR3, which carried a larger, 7.6-kb chromosomal fragment encompassing the entire region covered by pTR1 and the *murE* and *murF* genes, also had similar effects (data not shown). The vector plasmid pBR322 alone had no such effects. At 42°C, cells of the three *fts* mutants *ftsQ*, *ftsA*, and *ftsZ* died, irrespective of the presence of a plasmid. In these experiments cells were used very soon after transformation of the mutant cells with plasmids. Transformant colonies were picked up from the selection plates, and single colonies were isolated directly from the transformant colonies and applied immediately on the plates for the morphology and viability assays. This precaution was important because, on aging, transformed cells may change to suppress unfavorable effects of introduced plasmids, thus obscuring results.

The lethal effect of amplification of the 2.6-kb chromosomal region is shown more quantitatively in Table 2 by comparing the colony-forming abilities of the mutant strain carrying the plasmid on L′ΔNaCl agar and L′ agar plates at 30°C.

Amplification of the 2.6-kb chromosomal region (pTR1) had lowered the colony-forming abilities of *ftsQ*, *ftsA*, and *ftsZ* cells to the extent of 10^{-2} in the absence of NaCl compared with that in the presence of NaCl. However, it showed no effect on *ftsI* or *ftsW* at 30°C. At 42°C, it recovered the rod shape and normal growth of *ftsI* cells but not of *ftsW* cells. When the 2.6-kb chromosomal fragment of pTR1 was shortened to 1.2 kb (plasmid pTR1Δ*Pst*I), encompassing only the 480-base proximal and the 719-base N-terminal regions of the *ftsI* gene, growth inhibition of the mutants *ftsQ* and *ftsZ* on L′ΔNaCl agar plates at 30°C was still observed, but inhibition of the *ftsA* mutant was not. Growth of the *ftsI* mutant at 30°C was also inhibited by pTR1Δ*Pst*I even in the presence of NaCl, probably because the product of the incomplete *ftsI* gene on this short fragment may compete with the function of the mutant *ftsI* gene or its product, mutant-type penicillin-binding protein 3, which may be sparingly active at 30°C.

The effects of the 2.6-kb fragment encompassing *fts-36/lts-33* and *ftsI* on *ftsQ*, *ftsA*, and *ftsZ* mutants and those of the shortened 1.2-kb fragment on *ftsQ* and *ftsZ* mutants are of special interest, because they suggest that some balance in formation of genes or gene products involved in cell division and cell growth is required for their normal functioning in the cell cycle process and that cell death occurs when one of these genes is overexpressed while another is sparingly functional. It is not yet known which portion of the 2.6- or 1.2-kb fragment actually functions in this lethal effect: a strong candidate is the 360-base sequence containing an SOS box-like sequence (5) and a 330-base open reading frame upstream of *ftsI* (9), but experiments with shorter subclones containing the entire 360-base sequence have so far not been successful.

Some investigators have reported a similar interaction between *ftsQ*, *ftsA*, and *ftsZ* (2), whereas others consider that there is no interaction between *ftsI* and *ftsQ*, *ftsA*, and *ftsZ* (3). More extensive studies on the interactions of the genes for cell growth and division should contribute to the understanding of the mechanisms of cell growth and division in *E. coli*.

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