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Regulation of Bacterial Cell Division: Temperature-Sensitive Mutants of *Escherichia coli* That Are Defective in Septum Formation

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Mutations ts2158 and ts1882, which confer temperature sensitivity of septum formation, map near *leu* in the region of min 2.0 to 2.1 on the *Escherichia coli* chromosome. These mutants stop division abruptly and grow as filaments at 42 C; when returned to 28 C, division resumes after about 30 min to produce short cells. The product of the gene defined by these mutations probably is required during all stages of septum formation rather than specifically for initiation of septation. Filaments that formed at 42 C contained incomplete constrictions (septa). When actively dividing filaments (i.e., those incubated at 28 C until division resumed) were shifted to 42 C a second time, division again stopped abruptly and incomplete constrictions persisted during the incubation at 42 C. Filaments that were subjected to 28 C incubation for a brief time (10, 20, or 30 min) before being shifted again to 42 C did not resume division as would be expected of a strain defective in initiating septation. Mutations ts1882 and ts2158 are recessive to the ts$^+$ allele, which is consistent with the interpretation that these mutations cause the loss of a function. They did not complement each other and presumably represent one cistron. Mutants carrying ts1882 and ts2158 mutations were compared with a mutant defective in the *ftsA* allele, also known to map near *leu*.

Some temperature-sensitive (ts) cell division mutants of *Escherichia coli* (2, 8, 14, 17, 23, 28), of *Salmonella typhimurium* (1, 6), and of *Bacillus subtilis* (3, 13, 22) continue deoxyribonucleic acid synthesis and growth at elevated temperature but do not form septa. These mutants presumably are defective in the initiation or in the process of septum formation. In *E. coli*, at least seven (18), and probably more, alleles are involved in septum formation.

Two mutations that confer temperature sensitivity in septum formation, ts1882 and ts2158, map at approximately min 2.0 to 2.1 near *leu* (2). Mutants carrying these mutations stop division abruptly when shifted to 42 C and continue growth as filaments; after reduction of temperature to 28 C they resume division at a rate greater than normal until the filaments have divided to form short cells (2). The *ftsA* gene, defective in strain PAT84, also maps near *leu; ftsA* mutants stop division at 41 C and resume division after the temperature is reduced to 30 C (18). The *ftsA*, ts1882, and ts2158 mutants all were identified as clones which, at 42 C, grew as filaments but could not form colonies (2, 8).

In this paper, we present evidence that the ts1882 and ts2158 mutations define an allele that codes for a product required during the process of septum assembly, rather than for initiation of septation. Filaments forming during incubation at 42 C retain visible, incomplete septa. The ts$^+$ allele is dominant over ts1882 and ts2158; also, these mutations probably represent one cistron. Properties of the mutants that contain ts1882 and ts2158 are compared with those of an *ftsA* mutant.

MATERIALS AND METHODS

Strains. The *E. coli* K-12 strains, their characteristics, and their sources are listed in Table 1. Bacteriophage P1*vira* was obtained from E. Moody.

Media. Enriched tryptone-yeast extract (YET) medium and defined medium base (9) supplemented with glucose (10 mg/ml), thiamine hydrochloride and nicotinic acid (5 μg/ml), L-amino acids (50 μg/ml), and streptomycin (200 μg/ml) as needed, were used. The YET broth contained 0.5% NaCl for use with all strains except *ftsA*84 derivatives, for which the NaCl concentration was 0.4%. The *ftsA*84 phenotype is suppressed by concentrations of NaCl of 0.5% or

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TABLE 1. *Principal strains*

Strain	Characteristics	Source
AX655	ts2158 derivative of AB1157 F⁻ *thr elu thi arg proA his gal xyl ara mtl lac str*	(2)
AX621	As AX655 but ts1882	(2)
P4X8	Hfr *leu ftsA*84 (PO3)	B. Shapiro
UTH4113	F⁻ *thr leu str nadC*22	T. Matney
AX710	*leu*⁺ ts1882 transductant of UTH4113	
AX720	*leu*⁺ *ftsA*84 transductant of UTH4113	
AX732	*leu*⁺ ts2158 transductant of UTH4113	
F'_{101}/C600	F'_{101} *thr*⁺ *leu*⁺ ts⁺ *nadC*⁺/*thr leu str*⁺	
F'_{101}ts2158/AX655	Isolated from F'_{101}/AX655	

higher (J. R. Walker, unpublished data; according to Ricard and Hirota [18], the minimum NaCl concentration required for suppression is 0.65%). The ts1882 and ts2158 mutants are not affected by variations in NaCl concentration from 0.2 to 1%. Media for phage growth and transduction were those of Rosner (19) for liquid media and of Caro and Berg (5) for solid media.

Cell number and mass determinations. Cells were diluted in 0.9% NaCl-0.05% formaldehyde and counted in a model Z_B Coulter counter. Absorbance was measured at 450 nm in a Zeiss PMQ II spectrophotometer, using a 10-mm light path.

Growth conditions. Cultures were grown in YET broth at 28 C with shaking for 12 generations before use. Changes in temperature were made without dilution by pouring cultures into prewarmed flasks.

Staining of cells for light microscopy. The cell wall stain procedure of Breakefield and Landman (3) was used.

P1vir transduction. The procedure of Rosner (19) was used for preparation of lysates. Lysates were grown on the donor host twice before use in the transduction procedure of Willetts et al. (25).

RESULTS

Resumption of division after 42 C incubation. In strain AX655 ts2158, division ceased abruptly at 42 C but growth, as measured by absorbance, continued for 2.5 mass doublings during 60 min (Fig. 1). The residual division at 42 C was limited to an increase of 6% in the total number of cells (average of nine experiments, range of 1 to 11%). Division resumed 28 to 30 min after reducing the temperature to 28 C at a rate greater than the normal rate at 28 C. As the filaments divided into shorter cells, the division rate gradually decreased over 180 min until the rate slowed to the usual 28 C rate.

In the *ftsA* strain, division also stopped at 42 C after a residual increase of 15% over 60 min (average of 12 experiments, range of 8 to 23%); growth continued for three mass doublings over 90 min, after which growth ceased (Fig. 2). When the temperature was reduced to 28 C after 60 min at 42 C, division resumed at 14 min (average of eight experiments, range 12 to 15 min) with a burst of division that yielded approximately four short cells per filament during a 15-min period. After these initial rapid divisions, the division rate gradually returned to the normal 28 C rate.

Although both mutants AX655 ts2158 and P4X8 *ftsA* stopped dividing abruptly at 42 C, they differed in that AX655 filaments retained incomplete septa (Fig. 3). There were no apparent septa in filaments of P4X8 when viewed by light (Fig. 4) or, as sections, by electron microscopy (data not shown).

Effects of multiple temperature shifts on cell division. When cultures were incubated at 42 C for 60 min, then for a limited period at 28 C, and finally again at 42 C, AX655 ts2158 and P4X8 *ftsA* differed in ability to divide during the second 42 C incubation period. When the 28 C incubation period was shorter than the time required for division to resume at this temperature, P4X8 *ftsA*, but not AX655

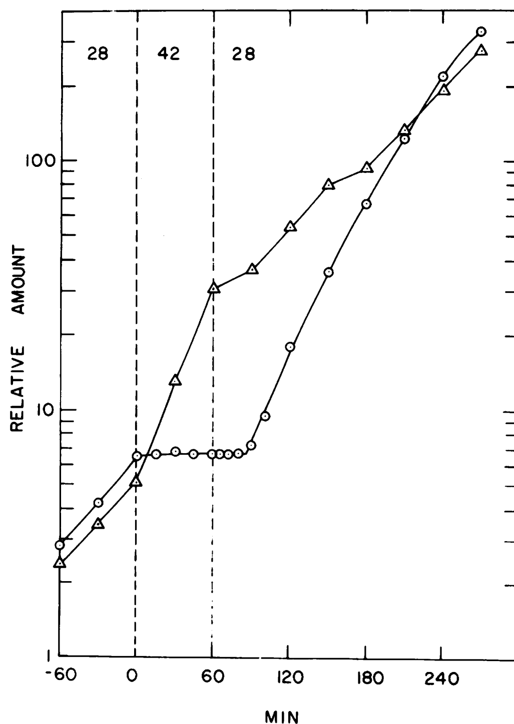

FIG. 1. *Growth and cell division of AX655 ts2158 at 28 and 42 C. Relative amount 1 represents 10⁶ cells/ml* (O) *and an absorbance* (Δ) *of 0.01.*

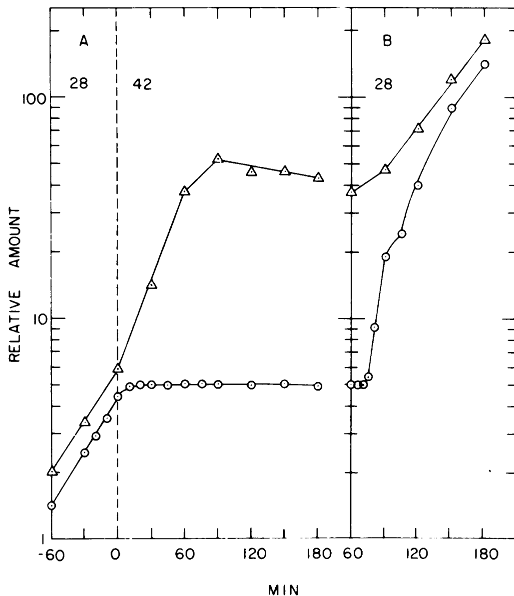

FIG. 2. *Growth and division of P4X8 ftsA at 28 and 42 C. A culture was grown at 28 C and shifted at 0 min to 42 C(A); after 60 min at 42 C, a portion of the culture was shifted to 28 C (B). Relative amount 1 represents 10^6 cells/ml* (O) *and an absorbance* (Δ) *of 0.01.*

ts2158, was able to divide during the second period at 42 C. Filaments of strain AX655 ts2158 required 28 to 30 min (Fig. 1) to resume division at 28 C; if only 10 or 20 min at 28 C was permitted, a second shift to 42 C prevented the resumption of division (Fig. 5). The percentage increase in cell number was limited to 0 and 10, respectively, under these conditions. If the second shift to 42 C occurred after 30 min of 28 C incubation, i.e., as filament division resumed, the residual division that occurred over the ensuing 30 min resulted only in a 15% increase in cell number (Fig. 5).

In contrast, a brief period of 28 C incubation after 60 min of 42 C incubation was adequate to permit division of filaments of strain P4X8 *ftsA* during a second period of incubation at 42 C (Fig. 6A, B). A 5-min and a 10-min incubation period at 28 C permitted subsequent increases in cell number at 42 C of 35 and 110%, respectively. This division occurred at 42 C immediately upon the temperature increase and earlier than division resumed in a control culture maintained at 28 C (Fig. 6A, B).

When filaments that were actively dividing were shifted to 42 C, AX655 ts2158 stopped division abruptly but P4X8 *ftsA* continued division. When filaments of AX655 were shifted a second time to 42 C after 40 or 50 min at 28 C, the residual division was limited to increases in cell number of 5 and 17%, respectively (Fig. 7). Thus, although filaments were forming septa (Fig. 3d), the divisions were not completed at 42 C. Filaments observed during the second period of incubation at 42 C contained incomplete septa (Fig. 3e), as did filaments observed during the initial period of incubation at 42 C. In contrast, actively dividing filaments of P4X8 *ftsA* continued division when shifted a second time to 42 C (Fig. 6C). A 127% increase in cell number followed a shift to 42 C made after 17 min of incubation at 28 C (Fig. 6C); no incomplete septa were found in filaments of the *ftsA* mutant under these conditions (Fig. 4e).

Effect of length of period at 42 C on resumption of division. Strain AX655 ts2158, when shifted to 42 C for periods of 5 to 10 min, slowed the division rate for an ensuing 30 min (Fig. 8), but division then resumed at a rate greater than the normal 28 C rate for about 55 min, after which division occurred at the normal rate. Incubation at 42 C for 15 or 30 min resulted in almost complete inhibition of division for a period of 30 min during subsequent incubation at 28 C (Fig. 9; cf. Fig. 1); when division resumed, rapid division occurred for periods of 70 and 90 min, respectively, before the normal division rate was reestablished. In mutant P4X8, subsequent division at 28 C was slowed even by 3 min of 42 C incubation; periods longer than 3 min (i.e., 5 to 45 min) had the effect of stopping subsequent division at 28 C for approximately 15 min in all cases tested (Fig. 10).

After resumption of division, P4X8 *ftsA* divided with a rate proportional to the duration of 42 C incubation (Table 2), until a minimum of 7 min for septum formation and cell separation was reached. In AX655 ts2158, the rate of division of filaments was much less affected by the length of time of 42 C incubation (Table 2).

Genetic mapping of ftsA and ts2158. We (2) reported that ts1882, which confers a ts phenotype similar to that conferred by ts2158, maps at min 2.1 between *leu* (at 1.5 min) and *nadC* (at 2.5 min). The *ftsA* allele was reported to map near min 1 to 1.5 (18). The orientation of *ftsA* and of ts2158 relative to *leu* and *nadC* and their map positions have been determined by P1 transduction. P1 was grown on leu^+ revertants of P4X8 *ftsA* and of AX655 ts2158. The leu^+ marker was transduced into the *leu* ts^+ *nadC* strain UTH4113, and the transductants were scored for ts and *nadC* (Table 3). The class of transductants found least often (or not at all) was the leu^+ ts^+ $nadC^+$ group; formation of this

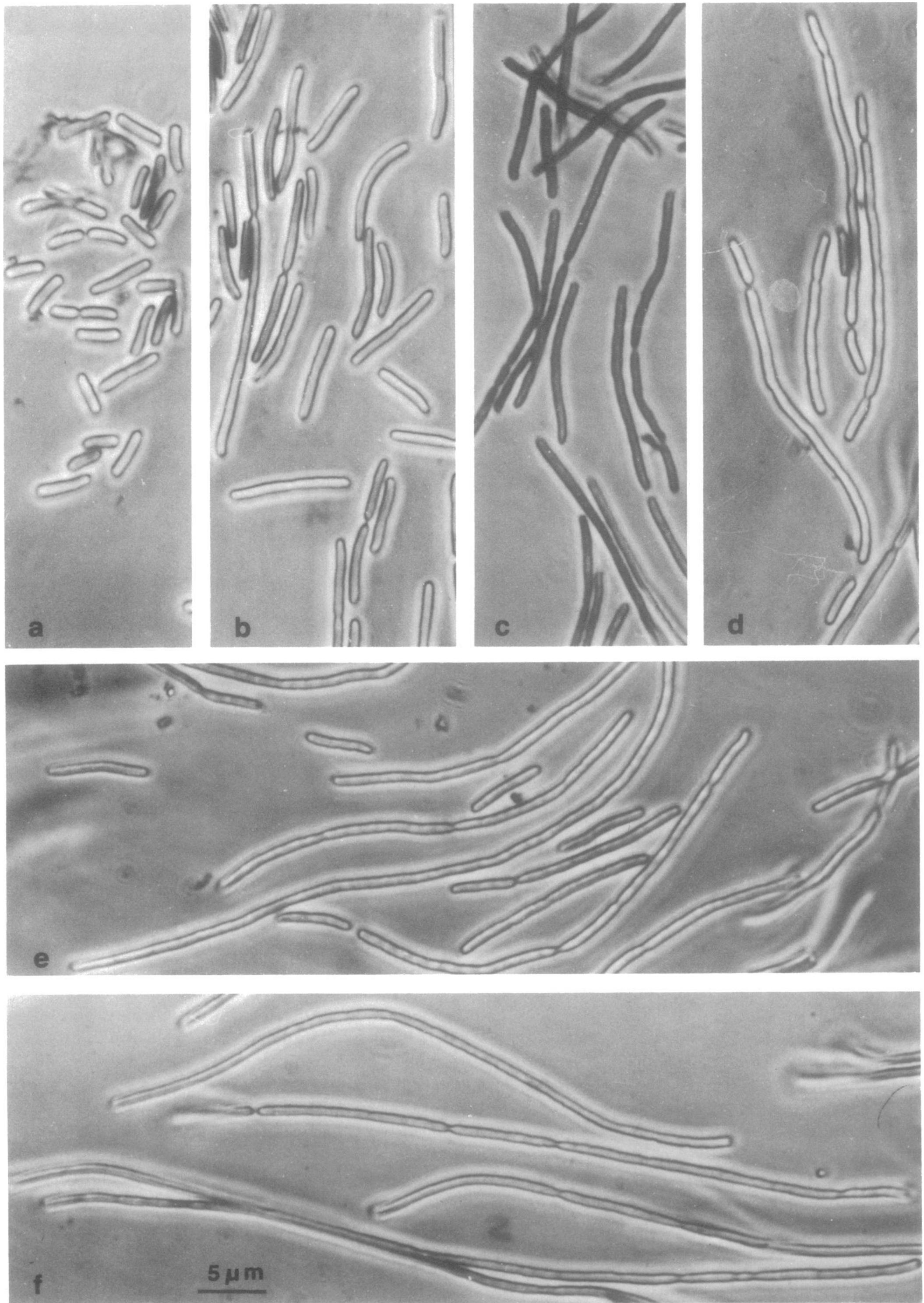

FIG. 3. *Incomplete septa of AX655 ts2158 filaments. A culture in YET broth was shifted in the sequence 28 C, 42 C for 60 min, 28 C for 40 min, 42 C. (a) Cells grown at 28 C; (b) nondividing filaments after 30 min at 42 C; (c) nondividing filaments after 60 min at 42 C; (d) dividing filaments 40 min after temperature reduction; (e) nondividing filaments 30 min after second shift to 42 C; (f) nondividing filaments 60 min after second shift to 42 C. The magnification was the same for (a) through (f).*

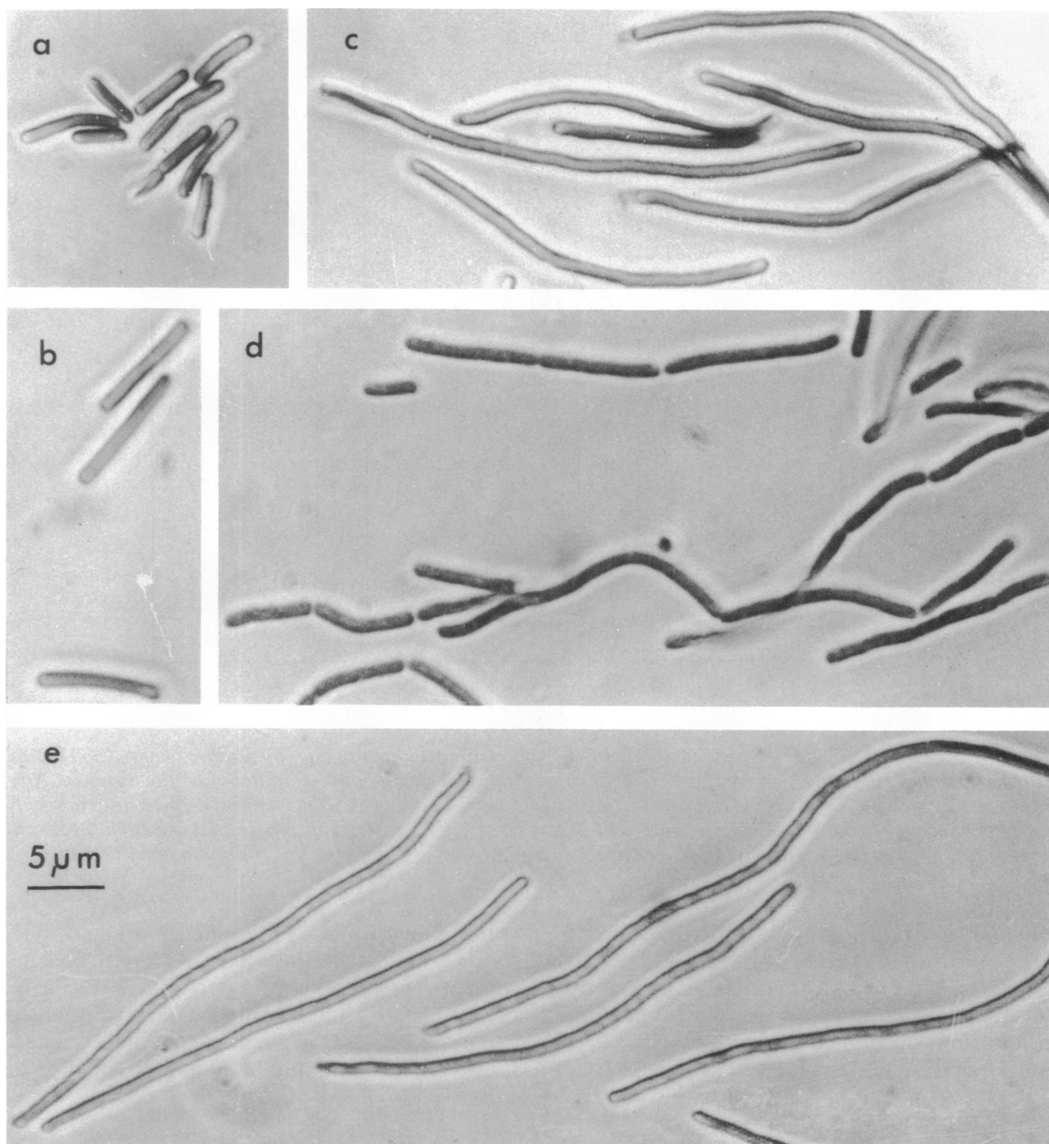

FIG. 4. *Filaments of P4X8 ftsA. Cells in broth culture were shifted in the sequence 28 C, 42 C for 60 min, 28 C for 20 min, and 42 C. (a) Cells grown at 28 C; (b) filaments after 20 min at 42 C; (c) filaments after 60 min at 42 C; (d) dividing filaments 20 min after reduction of temperature to 28 C; (e) filaments 60 min after increasing the temperature to 42 C the second time. Magnification was the same for (a) through (e).*

class would require four cross-over events, and would be rare, only if the sequence of markers were *leu ftsA* (or ts2158) *nadC*. From cotransduction frequency data (ref. 2 and Table 3) and the Wu formula (26), *ftsA*84, ts2158, and ts1882 are all located 0.5 to 0.6 min to the right of *leu; nadC* is 0.9 min to the right of *leu*. *ftsA*84 was cotransducible with *leu* at the level of 36%, ts2158 at the level of 32.5%, and ts1882 at the level of 38% (2); thus it is not possible to determined the sequence of these three mutations from these transduction data alone.

Dominance and complementation analyses. The episome F'_{101} covers *thr* and *leu* (10) and must extend to the right of *nadC* because it donates $nadC^+$ when introduced into the *nadC* strain UTH4113. F'_{101} thus covers the area in which *ftsA*, ts2158, and ts1882 are located. Partial diploid strains with the genotypes $F'_{101}ts^+$/ts2158 were insensitive to 42 C incuba-

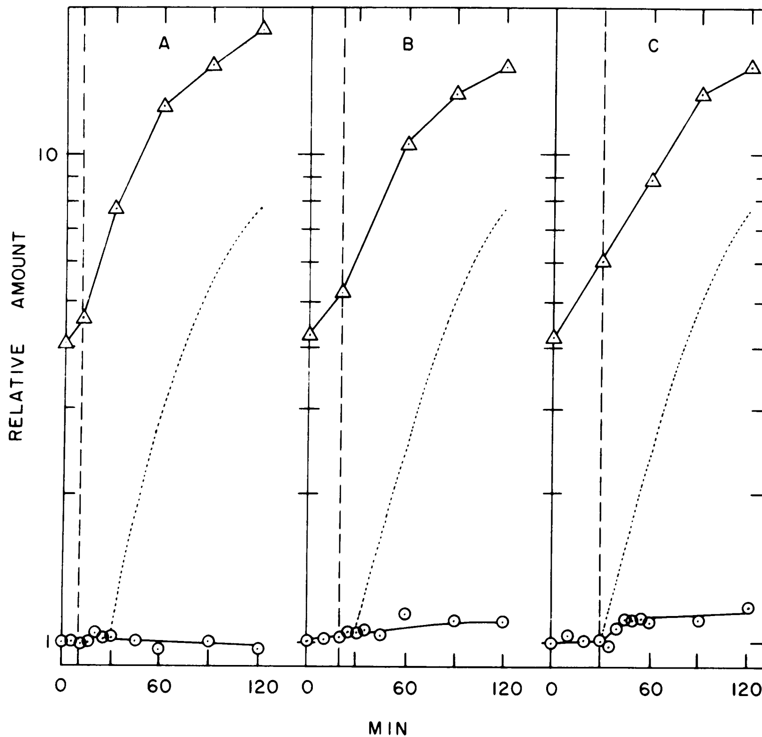

FIG. 5. *Effect of a second temperature shift to 42 C on resumption of division by filaments of AX655 ts2158. A culture was grown at 28 C, shifted to 42 C for 60 min, and then shifted to 28 C at 0 min on the abscissa. After 10 (A), 20 (B), and 30 (C) min, the temperature was again increased to 42 C. The vertical dashed lines mark the time of the second increase to 42 C; the dotted lines represent cell number in an experiment in which the second increase to 42 C did not occur. Relative amount 1 represents 4.8 × 10^6 cells/ml (○) and an absorbance (Δ) of 0.04.*

tion and continued growth and division (Fig. 11A). Similar results were obtained with $F'_{101}ts^+$/ts1882. Thus, the ts2158 and ts1882 mutations are recessive to the wild-type allele. In addition, a partial diploid strain of the genotype F'_{101}ts2158/ts1882 was temperature sensitive (Fig. 11C), indicating that both ts mutations are located within one cistron or that one mutation is polar. The latter possibility is less likely because the ts mutations are presumed to be missense. Partial diploid strains of the genotype F'_{101}*ftsA*$^+$/*ftsA* did not have the wild-type phenotype (Fig. 11B): growth continued for 4 h but then stopped. Division was initially inhibited and then resumed, but with a decreasing rate, for about 4 h. This lack of dominance of *ftsA*$^+$ over *ftsA*84 prevents complementation analysis of the relationship between *ftsA* (as defined by mutation *ftsA*84) and ts2158 or ts1882. It is possible that *ftsA*$^+$ is not dominant over any *ftsA* allele. Alternatively, the product of *ftsA*$^+$ might function as an oligomeric structure; mixing of *ftsA*$^+$ and *ftsA*84 subunits within one functional unit could inactivate that structure. The residual division suggests the presence of a partially active product, at least for 4 h. Partial diploid strains of the genotype F'_{101}ts2158/*ftsA*84 resembled $F'_{101}ts^+$/*ftsA*84 in growth and division at 42 C (Fig. 11D).

DISCUSSION

The allele defined by ts2158 and ts1882 provides a product that is probably required continuously during septum formation. This interpretation is based on several lines of evidence obtained with AX655 ts2158, AX621 ts1882, and ts transductants prepared from then. (i) When shifted to 42 C, these ts mutants ceased division abruptly; the residual division resulted in an increase in cell number of only about 6% over a 60-min period. (ii) After growth for 60 min at 42 C, incubation at 28 C for a brief period (5 or 10 min) was not adequate to initiate divisions that could be completed during a second period at 42 C. (iii) When rapidly dividing filaments

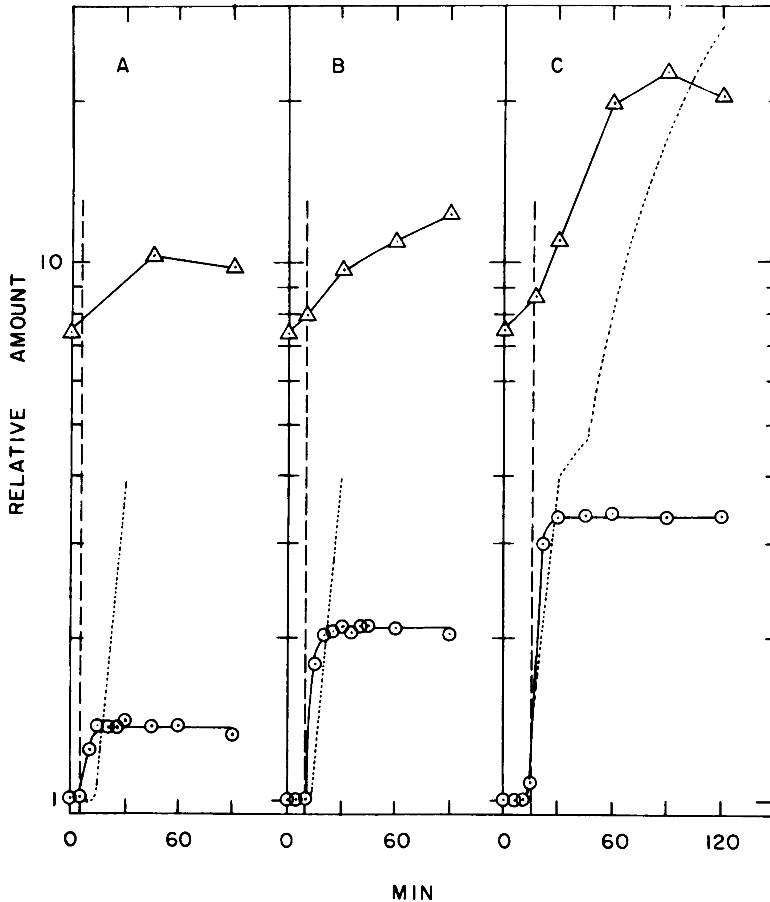

FIG. 6. *Effect of shifting temperature to 42 C a second time on resumption of division by filaments of P4X8 ftsA. A culture was grown at 28 C, shifted to 42 C for 60 min, and then shifted to 28 C at min 0 on the abscissa. After 5 (A), 10 (B), and 17 (C) min, the temperature was again increased to 42 C. The vertical dashed lines mark the time of the second increase to 42 C; the dotted lines represent cell number in an experiment in which the second increase to 42 C did not occur. Relative amount 1 represents 5 × 10⁶ cells/ml (O) and an absorbance (Δ) of 0.07.*

were shifted to 42 C a second time, division stopped abruptly. (iv) The filaments that formed during incubation at 42 C retained incomplete septa, apparently representing many stages of septum formation (Fig. 3). (v) When shifted in the sequence 28 C, 42 C, 28 C until rapid division resumed and then 42 C again, incomplete septa also persisted during the second period of 42 C incubation. These data indicate that septation requires the function of at least one specific gene product during septum formation, although the possibility that the gene product functions only during a final step in septum assembly cannot be excluded. Other ts mutants that stop division abruptly at 42 C have been described (e.g., references 1, 6, 8, 14, 17). It might be assumed that such strains are defective in a late stage of septum formation; however, incomplete septa have not been demonstrated in them. If such mutants are blocked in a late step in septation, it must be concluded that septation is completed in a small fraction of one generation or that incipient septa are resorbed or obscured during growth of the filament, as suggested by Slater and Schaechter (20). Even in the ts1882 and ts2158 mutants, not all the septa present in cells at the time of shifting to 42 C persisted throughout the 42 C incubation. After 60 min at 42 C, there were about 25 to 35% as many incomplete septa in filaments of these mutants as there were in dividing cells at the time of the shift.

The phenotype of the *ftsA* strain P4X8 and ts transductants prepared from it can be ex-

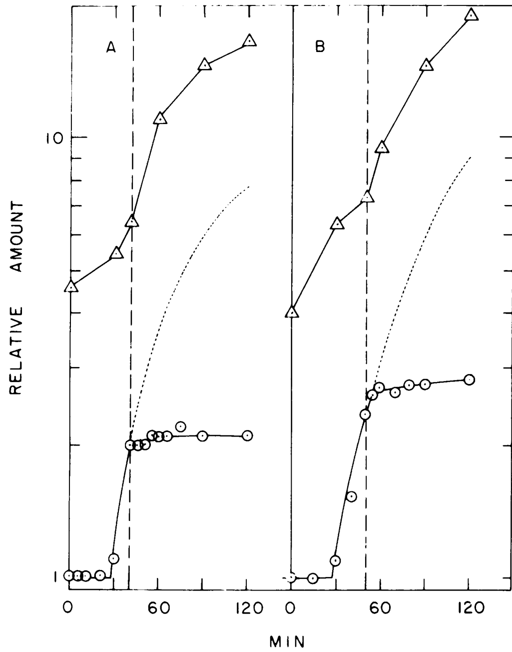

FIG. 7. *Effect of shifting temperature to 42 C a second time on actively dividing filaments of AX655 ts2158. A culture was grown at 28 C, shifted to 42 C for 60 min, and then shifted back to 28 C (at 0 min on the abscissa). After 40 (A) and 50 (B) min, the culture was again shifted to 42 C. The vertical dashed lines mark the time of the second increase to 42 C; the dotted lines represent cell number in a control culture which was not shifted to 42 C the second time. Relative amount 1 represents 4.8 × 10^6 cells/ml (○) and an absorbance (Δ) of 0.04.*

plained by at least two models. The first assumes these mutants to be defective in initiating septum formation. The evidence includes the following: (i) division stops at 42 C with a residual increase of 15% over a 60-min period; (ii) after growth at 42 C, a brief period of 5 to 10 min at 28 C permits the initiation of divisions that can be completed during a second period at 42 C; (iii) when rapidly dividing filaments were shifted to 42 C for a second time, division continued, permitting an increase of over 100%, as if divisions initiated at 28 C were continued at 42 C; and (iv) filaments of these mutants did not contain visible incomplete septa. Burdett and Murray (4) have concluded that the *ftsA* mutant PAT84 is defective in an early stage of division involving wall modification before assembly of the septum. A second model is that the *ftsA* allele codes for a product that functions in initiation or completion of septa (or in both processes) and is denatured slowly at 42 C. This model is correct only if incipient septa are resorbed or obscured during filament elongation. The fact that even 3 min of 42 C incubation delayed subsequent division at 28 C for 30 min (Fig. 10) suggests that the *ftsA*84 product is

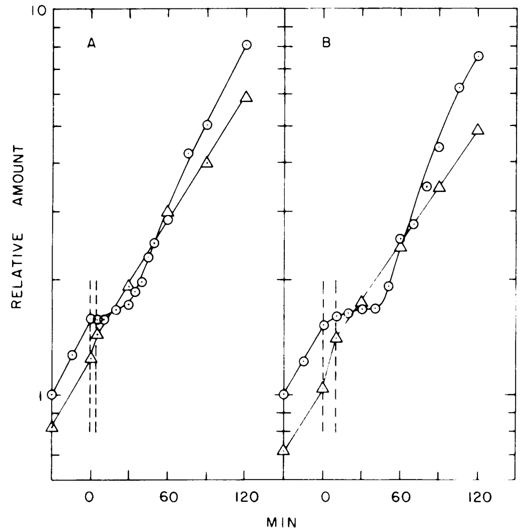

FIG. 8. *Effect of 5- (A) and 10- (B) min periods of incubation at 42 C on subsequent division by strain AX655 ts2158 at 28 C. Cells were grown at 28 C, shifted to 42 C at time 0 on the abscissa, and then shifted to 28 C again. Relative amount 1 represents 10^6 cells/ml (○) and an absorbance (Δ) of 0.01. Dashed lines represent times of temperature shifts.*

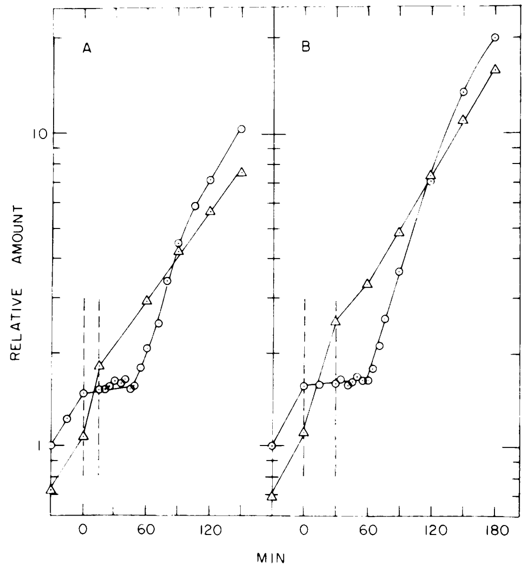

FIG. 9. *Effect of 15- (A) and 30- (B) min periods of incubation at 42 C on subsequent division at 28 C by strain AX655 ts2158. Conditions and symbols are as defined in Fig. 8 legend.*

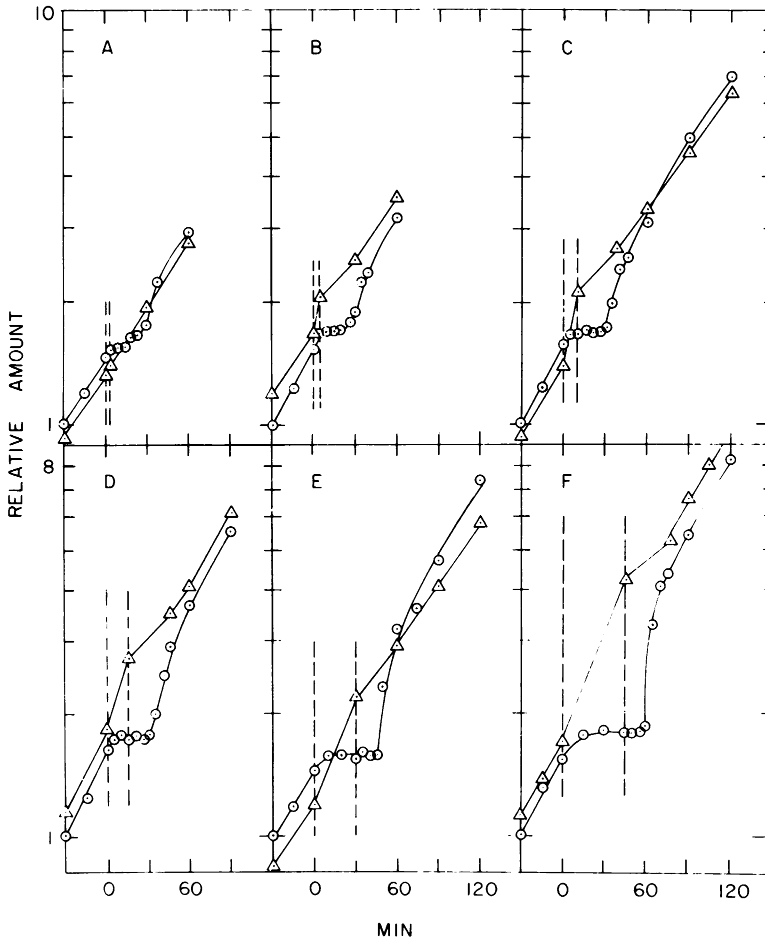

FIG. 10. *Resumption of division by strain P4X8 ftsA after periods of 3 (A), 5 (B), 10 (C), 15 (D), 30 (E), and 45 (F) min at 42 C during subsequent incubation at 28 C. Conditions and symbols are as defined in the Fig. 8 legend.*

TABLE 2. *Effect of duration of 42 C incubation on subsequent rate of division at 28 C*

Incubation period at 42 C (min)	Time required to complete first division (min)[a]	
	AX655 ts2158	P4X8 *ftsA*
5	—[b]	44
10	37	31
15	30	27
30	27	18
45	NT[c]	7
60	25	7

[a] After resumption of division at 28 C.

[b] Subsequent division was not stopped by a 5-min period at 42 C.

[c] NT, Not tested.

TABLE 3. *Analysis of map position of ftsA84 and ts2158 by P1 transduction*

Donor	Recipient	Selected marker	Unselected markers	Transductants with unselected markers
leu$^+$ P4X8	UTH4113	*leu*$^+$	ts$^+$*nadC*$^+$	0
			ts$^+$*nadC*$^-$	159
			ts$^-$*nadC*$^+$	52
			ts$^-$*nadC*$^-$	38
leu$^+$ AX655	UTH4113	*leu*$^+$	ts$^+$*nadC*$^+$	3
			ts$^+$*nadC*$^-$	111
			ts$^-$*nadC*$^+$	27
			ts$^-$*nadC*$^-$	28

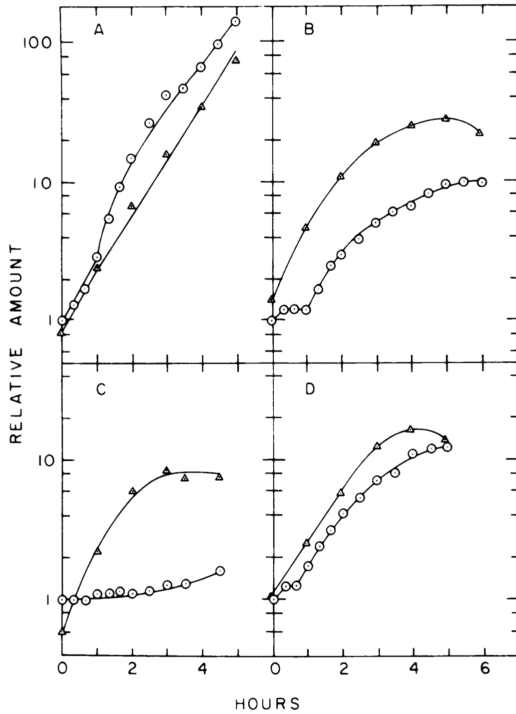

FIG. 11. *Growth and division of partial diploid strains at 42 C. For complementation and dominance analysis, the ts alleles were transferred into strain UTH4113. leu⁺ transductants (Table 3 and reference 2) that were ts and nadC were prepared from P1 grown on ts donors. Strains AX710 ts1882, AX720 ftsA84, and AX732 ts2158 were prepared by P1 transduction from donors that were leu⁺ revertants of strains AX621 ts1882, P4X8 ftsA, and AX655 ts2158, respectively. Partial diploid derivatives of AX710 ts1882, AX720 ftsA84, and AX732 ts2158 were prepared by mating each with F'_{101}/C600 and selecting thr⁺ nadC⁺ str merodiploids. An F'_{101} episome carrying the ts2158 mutation was isolated by screening clones of F'_{101}ts⁺/AX655 ts2158 for temperature sensitivity until an F'_{101}ts2158/AX655 ts2158 clone was identified. F'_{101}ts2158 was transferred from F'_{101}ts2158/AX655 ts2158 to AX710 and to an AX720 by mating and selecting thr⁺ nadC⁺ his⁺ merodiploids. Episome-containing strains were confirmed as males by their sensitivity to M13 or f_2 phage. (A) F'_{101}ts⁺/AX732 ts2158; (B) F'_{101}ts⁺/AX720 ftsA84; (C) F'_{101}ts2158/AX710 ts1882; (D) F'_{101}ts2158/AX720 ftsA84. Relative amount 1 represents 10^6 cells/ml (○) and an absorbance (△) of 0.01.*

not denatured slowly. Because both P4X8 *ftsA*84 and *ftsA*84 transductants of another K-12 strain form filaments without incomplete septa, this property depends on the *ftsA*84 mutation rather than on some physiological property of the cell.

The observation that the rate of division of filaments of P4X8 at 28 C, after temporary incubation at 42 C, was proportional to the period of 42 C incubation (Table 2) suggests that synthesis of an inactive *ftsA*84 product continues at 42 C but that the product is renaturable at 28 C. A similar interpretation has been proposed by Reeve and Clark (16) to account for a similar phenomenon observed with the ts strain BUG-6. However, the data can be explained also by more complex models such as one assuming excessive synthesis of a division activator at 42 C while the synthesis of *ftsA* product, normally synthesized and depleted during each cycle, is thermosensitive. The rate of division of filaments of AX655 ts2158 was much less dependent on the period of 42 C incubation (Table 2). Thus, models involving thermosensitive synthesis or activity of the product of the gene defined by this mutation are equally plausible, although the rapid inhibition of division after a shift to 42 C (Fig. 1 and 9) supports the thermolabile, non-renaturable product model.

The relation between *ftsA*84 and the allele defined by mutations ts2158 and ts1882 is not clear. All three map within the region of 2.0 to 2.1 min on the chromosome. Both ts1882 and ts2158 are presumably in one cistron because both are recessive to the ts⁺ allele, and complementation could not be demonstrated between the two mutants. The *ftsA*⁺ allele was not dominant over *ftsA*84, which means that complementation analysis of the relation between ts2158 and *ftsA*84 is not presently possible. Although *ftsA*⁺ was not dominant, neither was it completely recessive. Residual, slow division of the F'_{101}*ftsA*⁺/*ftsA*84 strain suggests that the *ftsA* product functions as an oligomer of several subunits. Mixing of wild-type and mutant subunits could lead to slow residual division. (Somewhat similar interpretations have been suggested for other systems, e.g., reference 11.) Growth, as measured by mass, stopped in the diploid after 4 h at 42 C. The fact that the F'_{101}ts2158/*ftsA*84 partial diploid at 42 C resembled the division and growth pattern of the F'_{101}ts⁺/*ftsA*84 strain, rather than the F'_{101} ts2158/ts1882 strain, suggests that the *ftsA*84 and ts2158 mutations might be in different cistrons.

Several mutations that affect cell division have been mapped near *leu*. Originally, van de Putte et al. (23) placed *fts*-2, *fts*-7, and *fts*-8 slightly clockwise of *leu;* these mutations conferred ts filament formation and were selected by filtration. Hirota et al. (7, 18) referred to the allele defined by mutation T84 as *ftsA* and reported its map position as min 1.0 to 1.5. Wijsman (24) referred to mutations *fts*-10,

fts-12, and *fts*-15, selected by the procedure of van de Putte et al. (23), as *ftsA;* the map sequence was determined to be *leu mur ftsA azi* (24). The P1 transduction analysis of *ftsA*84, ts1882, and ts2158 places these mutations at min 2.0 to 2.1, in the sequence *leu* ts *nadC*. (The term *ftsA* was used also by Taylor [21] to refer to azide-resistant mutants because some of them formed filaments at high temperature [27]. Azide resistance and *ftsA* should not be used interchangeably; the cell division mutants studied appear to be distinct genetically from *azi* [24].) In *S. typhimurium*, the *divC* locus, which also affects cell division, was mapped near *leu* (1).

It might be significant that genes coding for enzymes involved in murein precursor biosynthesis (*murE, F,* and *C* and *ddl*) map very close to *ftsA* (12, 24). Mutants defective in these genes lyse at 42 C (12, 24), presumably because of inhibition of murein synthesis. *ftsA* mutants were not defective in the murein biosynthetic enzymes assayed (24). The *envA* mutation, which leads to chain formation and increased sensitivity to ampicillin and several other antibiotics, also has been mapped by P1 transduction in the sequence *leu envA azi,* with *envA* and *azi* very close to each other (15).

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