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Regulation of Bacterial Cell Division: Genetic and Phenotypic Analysis of Temperature-Sensitive, Multinucleate, Filament-Forming Mutants of *Escherichia coli*

JANE SMITH ALLEN, CAMILLE C. FILIP, RALPH A. GUSTAFSON, ROBERT G. ALLEN, AND JAMES R. WALKER

Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712

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Three mutants of *Escherichia coli* K-12 which form filaments during 42 C incubation have been characterized. The mutant strains AX621, AX629, and AX655 continued to grow and to synthesize deoxyribonucleic acid at 42 C for 150 to 180 min, after which time growth ceased. When cultures of the mutants were transferred from 42 to 28 C, septation of the filaments began after a 25- to 30-min period and continued at a greater than normal rate until no filaments remained. Addition of chloramphenicol at the time of transfer from 42 to 28 C prevented cell division in strain AX655 and caused lysis of strains AX621 and AX629. The temperature sensitivity mutation in each strain mapped near *leu*. For strain AX621, the mutation was specifically located between *leu* and *nadC* by P1 transduction. Properties of these strains are compared with those of other cell division mutants.

Bacterial cell division is a complex process which involves chromosome replication, nuclear segregation, septation, and physical separation of daughter cells. Attempts to understand how the individual steps involved in cell division are accomplished and how these steps are related to each other have led to the isolation and study of various mutants. In particular, mutants defective in septation have been discovered which either construct septa at inappropriate sites (1, 8, 14, 19, 24, 28) or fail to form septa under nonpermissive conditions (2, 3, 5, 7, 11, 12, 16, 23, 28, 29, 34).

A class of *Escherichia coli* K-12 mutants which fail to form septa at the nonpermissive temperature, but which septate when returned to the permissive temperature, includes PAT84 (11), BUG-6 (23), ts-20 (17), and AX629 (3). In each of these mutants, cell division ceases abruptly upon shifting to the nonpermissive temperature. Deoxyribonucleic acid (DNA) synthesis and total mass increase continue, and the nuclei are evenly distributed along the filaments which form during incubation at nonpermissive temperature. When a culture is shifted back to the permissive temperature, cell division continues to be inhibited for a short period and then resumes at a greater than normal rate.

In this paper, we will further characterize AX629 and two other closely related mutants, AX621 and AX655. The three mutants were isolated on separate occasions after selecting for thermosensitivity and then screening with a microscope for filament formation (3). Although some differences were apparent, such as in rate of killing at 42 C and in the degree of lysis after chloramphenicol (CM) treatment, the three strains in general had similar physiological characteristics. The mutation in all three strains mapped near *leu*, and one, $ts_{18\text{-}82}$, was located between *leu* and *nadC* by P1 transduction.

MATERIALS AND METHODS

Strains. The *E. coli* K-12 strains, their characteristics, and their source or derivation are listed in Table 1. The mutants AX655 ($ts_{21\text{-}55}$), AX621 ($ts_{18\text{-}82}$), and AX629 (ts_{424}) were isolated from AB1157 after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (3). Mutagen survivors were screened for inability to form colonies at 42 C, and the temperature-sensitive (ts) mutants then were screened for filament formation at the nonpermissive temperature.

Bacteriophage P1*vir* was a gift of J. L. Rosner.

Media. Enriched tryptone-yeast extract (YET) medium and defined medium base (12) supplemented with glucose (10 mg/ml), thiamine hydrochloride (5 μg/ml), and L-amino acids (50 μg/ml) were used. Medium for testing azide resistance was that used by Yura and Wada (33). Media for phage growth and transduction were those of Rosner (25) for liquid media and Caro and Berg (6) for solid media.

Cell number and mass determinations. After

TABLE 1. *Principal E. coli strains*

Strain	Characteristics	Source
AB1157	F⁻ *thi thr leu arg proA his gal xyl ara mtl lac str*	P. Howard-Flanders
AX655	ts mutant of AB1157 (ts_{21-58})	
AX621	ts mutant of AB1157 (ts_{18-82})	
AX629	ts mutant of AB1157 (ts_{424})[a]	
AX700	*leu*⁺ revertant of AX621	
UTH4113	F⁻ *thr leu nadC22*	T. Matney
S9080	F⁻ *lac thi purE str*	M. Malamy
HfrH	Hfr *str*⁺	E. Moody
Hfr6	Hfr *str*⁺ *met*	E. Moody
KL14	Hfr *str*⁺	E. Moody
KL16	Hfr *str*⁺	E. Moody
KL25	Hfr *str*⁺	E. Moody
KL96	Hfr *str*⁺	E. Moody
KL99	Hfr *str*⁺	E. Moody
KL226	Hfr *str*⁺	E. Moody
PK191	Hfr *str*⁺ *pro*	E. Moody

[a] Complete nutritional requirements for this strain have not been determined.

dilution in 0.9% NaCl-0.05% formaldehyde, cells were counted with a model Z_B Coulter counter, with an amplitude of 2 and a current setting of 1. Absorbance was measured at 450 nm in a Zeiss PMQ II Spectrophotometer using a 10-mm light path.

Growth conditions. Cultures were inoculated into 2 ml of YET broth and grown for 24 h with shaking at 28 C, diluted 10^{-6} into fresh YET broth, and grown again at 28 C with shaking until an absorbance of 0.08 to 0.09 was reached. Each culture was then divided into two portions; one portion was incubated at 28 C, the other at 42 C.

Measurement of DNA synthesis. Cultures were grown for 24 h at 28 C in 2 ml of YET broth supplemented with 250 μg of deoxyadenosine per ml, and then diluted 10^{-6} into YET broth supplemented with 250 μg of deoxyadenosine per ml and [*methyl*-^{3}H]-thymidine (0.33 μCi per 0.004 μg per ml) (4, 30). Growth conditions described above were then maintained.

Radioactive isotope counting. Samples were mixed with cold trichloroacetic acid (final concentration 5%), chilled, and filtered onto glass-fiber filters. The filters were washed, dried, and counted in a scintillation fluid of toluene plus 0.5% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(4-methyl-5-diphenyloxazolyl)]-benzene.

Staining and microscopy of cells. Cells for light microscopy were prepared by the method of Piéchaud (21). The stained cells were examined with a Leitz Ortholux microscope equipped with Phaco NPL achromatic objectives.

Cells for electron microscopy were prepared by the method of Peters and Wigand (20) as modified by Zusman et al. (34). Grids were examined with a Hitachi H-7 electron microscope.

Chemicals. Deoxyadenosine and thymidine were obtained from Calbiochem, La Jolla, Calif. CM was purchased from Sigma Chemical Co., St. Louis, Mo., and [*methyl*-^{3}H]thymidine (20 Ci/mmol) was purchased from New England Nuclear, Boston, Mass.

Mating conditions. The procedure for matings used was that of Moody and Lukin (15), except that when F′ and F⁻ cells were mated the F′:F⁻ ratio was 10:1.

Interrupted mating. Samples were removed with prewarmed pipettes, diluted, violently agitated on a saber saw (13), and plated on prewarmed agar plates.

Preparation and titering of P1vir lysates. The procedure of Rosner (25) was used for preparing P1*vir* lysates. Lysates were grown on the donor host twice before being used for transduction.

P1vir transduction. Transduction was performed by the procedure of Willetts et al. (31).

RESULTS

Growth at 28 and 42 C. The thermosensitive mutants AX621, AX629, and AX655 were isolated as filament-forming strains found among mutagen survivors which could not form colonies at 42 C (3). In all three independently isolated mutants, septum formation was specifically inhibited at 42 C, but when filaments were transferred to 28 C cell division occurred at a rate greater than the usual 28 C rate. In addition, all three mutations map approximately 1 min from *leu* on the standard genetic map.

The properties of strain AX655 will be presented in detail; variations observed in the other two strains will be noted.

When a culture of strain AX655 which had been growing at 28 C was shifted to 42 C, mass continued to increase but total cell number remained constant, indicating that filaments formed during 42 C incubation (Fig. 1). The filaments remained viable (capable of forming colonies at 28 C) for 90 min at 42 C, but then the number of viable cells decreased exponentially with a half-life of 10 to 15 min. Growth (as measured by absorbancy at 450 nm) did not continue indefinitely at 42 C, but ceased after 150 min and the absorbance remained constant. This constancy of absorbance reflected primarily cessation of growth rather than lysis of some filaments and continued growth of other filaments because (i) the total number of the (nondividing) cells remained constant, (ii) the filaments increased in length only 50% from 120 to 150 min at which time their maximal length was established, and (iii) lysed cell envelopes accounted for less than 1% of all the filaments observed with a microscope in the cultures.

Strain AX629 showed a similar growth pattern. At 42 C, absorbance increased 20-fold

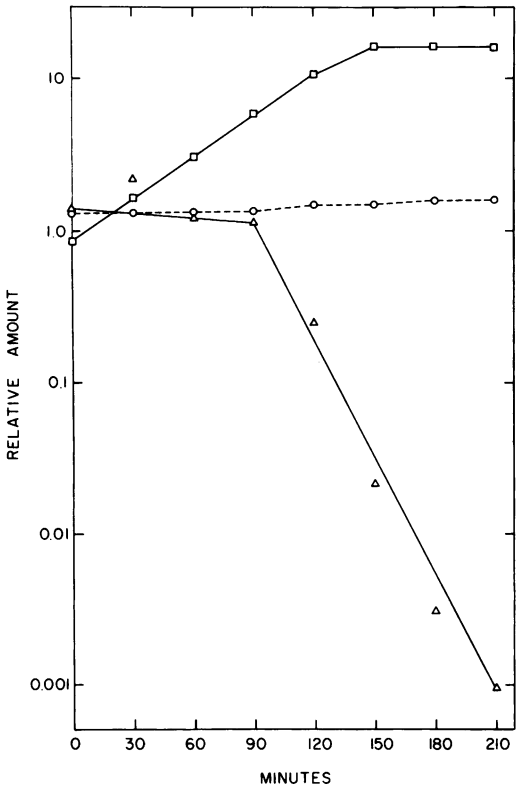

FIG. 1. *Effects of 42 C incubation on strain AX655. Relative amount 1 represents an absorbance (□) of 0.1 and 10^7 cells/ml as determined by viable cell (△) or Coulter (○) counts.*

during the 180-min incubation period and then remained constant; viability remained constant for about 100 min and then decreased with a half-life of 15 min.

Strain AX621 increased 12-fold in mass during 180 min of incubation at 42 C. Viability, as determined by plating, decreased immediately at 42 C, with a half-life of 30 min. It is possible that the physical process of spreading the AX621 filaments on agar plates to measure viability contributed to killing; AX621 filaments divided rapidly in liquid medium when the temperature was reduced to 28 C after being held at 42 C for 60 min, indicating that this strain remained viable at 42 C in liquid medium.

In all three mutants, the mass increase at 42 C represented predominantly an increase in length, for the diameter of the growing filaments was relatively constant.

Growth pattern after temperature reduction. When a culture of AX655 was returned to 28 C after a 60-min period at 42 C, cell number remained constant for 25 to 30 min and then rapidly increased at four to five times the 28 C pre-shift rate (Fig. 2). This rapid increase gradually slowed until filaments were eliminated from the culture and the cells were normal in length at 135 min after temperature reduction. After this time, the original 28 C rate of cell number increase was resumed.

Absorbance and cell number changes very similar to those obtained with strain AX655 were observed when strain AX629 was incubated at 42 C.

Strain AX621 showed a similar pattern during the 42 C incubation, except that limited lysis of filaments occurred when the culture was returned to 28 C after 60 min at 42 C. When strain AX621 was shifted to 28 C after being held at 42 C for 120 min, the absorbance increased 10% in 70 min and then remained constant for at least 180 min. Examination of the cells revealed that some lysis occurred. Growth did not resume for at least 180 min.

Microscope observation of dividing filaments. The division pattern of filaments formed during incubation at 42 C was studied

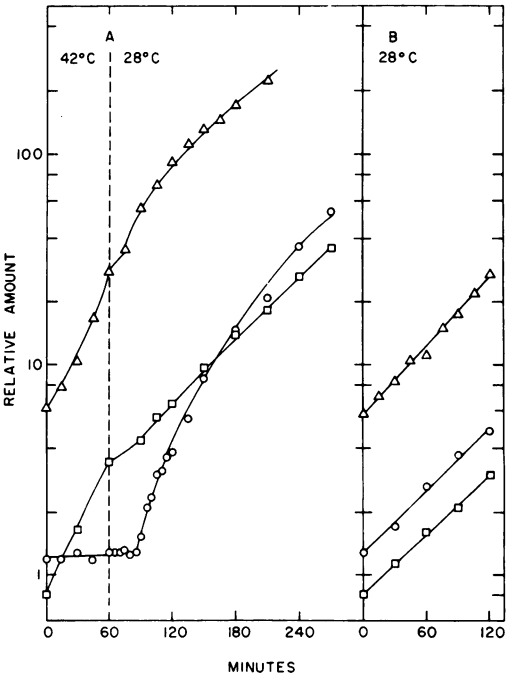

FIG. 2. *Growth and DNA synthesis in strain AX655. The culture was incubated at 42 C for 60 min and then at 28 C. Relative amount 1 refers to an initial absorbance (□) of 0.10, 1,000 counts/min of trichloroacetic acid-insoluble [methyl-^3H]thymidine (△), and 10^7 cells/ml (○) as determined by the Coulter counter.*

by placing cells which had been incubated for 60 min at 42 C on a glass slide covered with YET agar. The slide was then incubated at 28 or 25 C. Similar patterns of new cell formation were observed at each temperature.

Figure 3A shows photographs of three typical filaments of strain AX655 during incubation at 25 C. During the 120-min observation period after being shifted from 42 C, each filament (which was initially about four times the size of 28 C grown cells) divided both at the center of the filament and at each end, although not in a fixed sequence. Daughter cells, which sometimes were shorter filaments about twice the size of 28 C grown cells, subsequently divided until a microcolony resulted (Fig. 3B). Similar results were obtained with strains AX621 and AX629.

DNA synthesis. DNA synthesis in the three mutant strains continued apparently normally at 42 C. When AX655 was incubated for 60 min at 42 C and then returned to 28 C, DNA synthesis continued with no lag and, after about 45 min, paralleled the rate of increase in absorbance (Fig. 2). Similar results were found during incubation at 42 C for 60 min or 120 min for strain AX629 and for 120 min with strain AX621.

Filaments which formed during incubation at 42 C were stained by the Piéchaud technique (21). The nuclear regions in all three mutant strains were evenly distributed along the length of the elongating filaments. Both the length of the filaments and the number of nuclear bodies doubled approximately every 30 min (Fig. 4). Electron micrographs confirmed that DNA was distributed evenly throughout the length of the filaments.

Treatment with sodium deoxycholate. Sensitivity to deoxycholate has been interpreted as evidence of alteration in the structure of bacterial membranes (18); in the presence of deoxycholate concentrations which do not affect wild-type cells, cells with altered membrane properties either lyse (9) or lack normal membrane structure when examined by electron microscopy (10). However, all of the three of the strains AX655, AX629, and AX621 continued mass increase at 28 and 42 C when the medium was supplemented with 0.5% sodium deoxycholate.

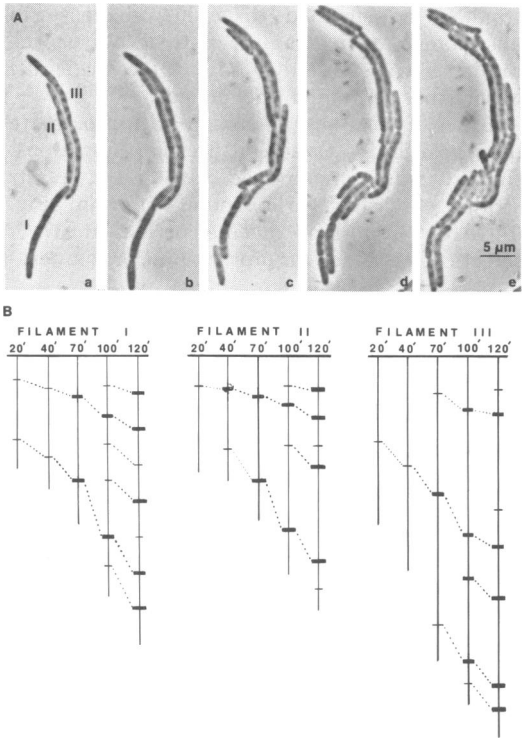

FIG. 3. *Division of filaments formed by strain AX655. (A) Photographs of unstained cells on agar slides at 25 C at 20 (a), 40 (b), 70 (c), 100 (d), and 120 (e) min after shifting the culture from 42 C to 25 C. The bar represents 5 µm. (B) Diagram showing location of septa (|) and physical separation (■) of daughter cells for the individual filaments shown in A. After 20 min at 25 C, filaments I, II, and III were 15.4, 15.9, 23.1 µm long, respectively.*

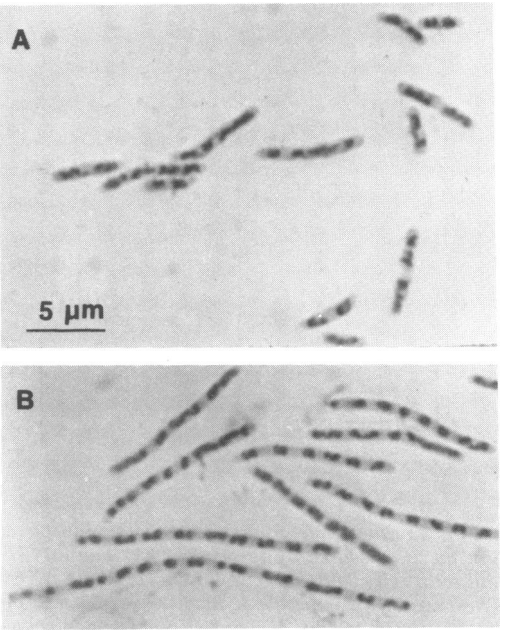

FIG. 4. *Piéchaud nuclear region stains of AX655. Cells were collected after 0 (A) or 60 (B) min of incubation at 42 C. Nuclear regions appear dark.*

Effect of culture density on filament formation. Cultures of each of the three mutant strains were shifted from 28 to 42 C when the cell density per ml reached 2×10^7 or 2×10^8. Subsequent patterns of absorbance and cell number changes revealed that filament formation in these strains was independent of culture density.

Effect of CM on division of filaments. A culture which had been growing at 28 C was incubated at 42 C for 60 min and then divided into several fractions, each of which was returned to 28 C for further incubation. CM was added to the fractions at various intervals thereafter to give a final concentration of 150 μg/ml. A control culture received no CM. Samples were removed at intervals from each fraction to measure absorbance and the number of cells.

When CM was added to strain AX655 at 0, 15 or 30 min after the shift to 28 C, absorbance increased 25 to 40% during 120 min after CM addition (Fig. 5A). The number of viable cells remained constant for about 90 min during incubation with CM, if the CM was added at 0 or 15 min after temperature reduction, and then decreased; the viable cells of the control culture remained constant for 30 min after the shift to 28 C and then increased (Fig. 5B). The total number of cells also remained constant if CM was added at the time the temperature was reduced (Fig. 5C). Thus, filaments of AX655 were inhibited from dividing by the addition of CM at the time of shifting to the permissive temperature.

The effect of CM on filaments of AX629 and AX621 was more drastic; extensive lysis occurred. Fractions of AX629 cultures which received CM at 15 min before reducing the temperature to 28 C or at 0 or 15 min after the shift exhibited a decrease in absorbance soon after introduction of CM. This decrease was exponential and resulted in a 75% reduction in absorbance over a 3-h period. The number of viable cells began to decrease immediately after addition of CM.

Addition of CM to AX621 filaments 15 min prior to transfer of the culture to 28 C or at the time of transfer led to a 50% decrease in absorbance during a 3-h period. When the inhibitor was added 15 min after the shift, absorbance remained unchanged.

Hfr × F⁻ crosses. To assign an approximate map position to these cell division mutations, the mutants were crossed with a set of Hfr strains which had various points of origin (Fig. 6). After a 30-min mating period at 37 C, selection was made for prototrophic or tempera-

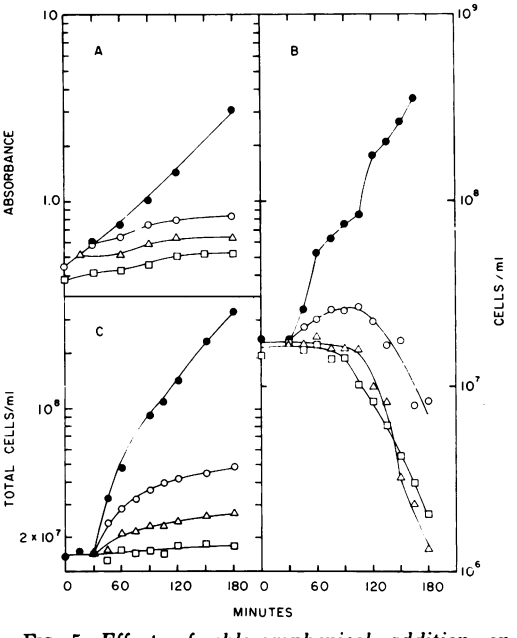

FIG. 5. *Effect of chloramphenicol addition on growth, as measured by absorbance (A), viability (B), and total cell counts (C) of filaments of strain AX655. The culture was incubated at 42 C for 60 min and then returned to 28 C at 0 min. Chloramphenicol was added 0 (□), 15 (Δ), or 30 (○) min after return to 28 C. The control culture (●) received no chloramphenicol.*

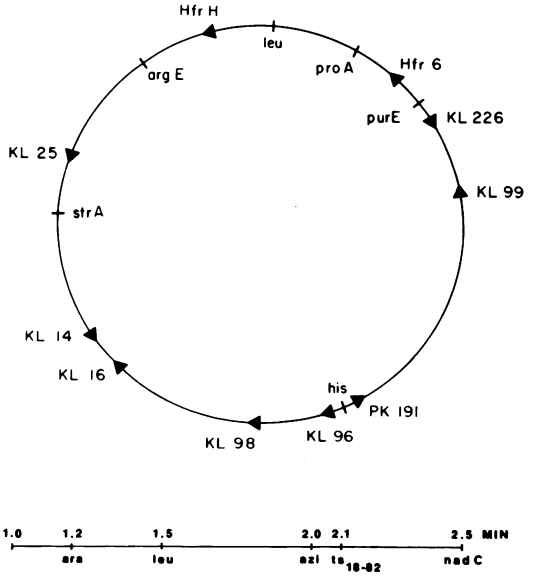

FIG. 6. *Map of the E. coli chromosome showing origins of Hfr strains and an expanded map covering the leu-nadC region.*

ture-insensitive recombinants; counter selection against the Hfr strains was accomplished by the use of streptomycin.

When AX655 was mated with the Hfr strains of Fig. 6, temperature insensitivity was donated by strains HfrH and KL226 (Table 2). To map the temperature-sensitivity mutation more accurately, selected recombinants were scored for other markers. The thermosensitive defect appears to map near *leu* and *proA* and to be more closely linked to *leu* than to *proA*. For the HfrH mating, 91% (89 to 98) of the temperature-insensitive (ts^+) colonies were also leu^+, and only 72% (71 of 98) were $proA^+$; when KL226 was used, 91% (95 of 104) of the ts^+ recombinants were leu^+, and only 79% (82 of 104) were $proA^+$; when KL14 was used, 82% (9 of 11) of the ts^+ recombinants were leu^+ and only 18% (2 of 11) were $proA^+$.

Similarly, ability to form colonies at 42 C was donated early and with high frequency to strain AX629 and strain AX621 only by HfrH and KL226.

When recombinants from matings with AX621 were scored for unselected markers, $ts_{18\text{-}82}$ was determined to be closely linked to *leu*. When KL14 was mated with AX621 for 60 min and leu^+ recombinants were selected, 99% (98 of 99) were ts^+ and 64% (64 of 100) were $proA^+$; when KL96 was used, the leu^+ recombinants were 100% (16 of 16) ts^+ and 44% (7 of 15) $proA^+$. Strain AX629 has an as yet unidentified auxotrophic requirement(s) in addition to the requirements of the parent strain, AB1157. Consequently, experiments involving selection and scoring for auxotrophic markers were not performed with this mutant.

TABLE 2. *Mating of AX655 with Hfr strains*[a]

Hfr strain	Selected marker	Recombinants (%)
HfrH	ts^+	2.4
	leu^+	7.9
	$proA^+$	5.6
KL226	ts^+	0.14
	$proA^+$	1.2
	leu^+	0.34
Hfr6	ts^+	<0.002
	his^+	<0.002
KL14	ts^+	0.03
	arg^+	0.58
KL16	ts^+	<0.003
	his^+	4.5
KL96	ts^+	<0.001
	his^+	1.2
KL99	ts^+	<0.002
	his^+	0.002
PK191	ts^+	0.05
	his^+	5.5

[a] Reversion frequency of AX655 to temperature insensitivity was 10^{-6}. Hfr strains mutated to streptomycin resistance at a frequency of $<10^{-7}$. Mating period was 30 min.

Interrupted matings. HfrH, which had been shown in previous experiments to donate the ability to form colonies at 42 C with high efficiency to all three mutants, was used for interrupted mating studies. Samples were removed from the mating flask at 5-min intervals, subjected to treatment with a modified saber saw, and plated on selective media.

Mating with mutant AX655 indicated that leu^+ entered the recipient approximately 9 min after mixing of the donor and recipient cells (Fig. 7). The ts^+ marker ($ts_{21\text{-}58}$) entered at about 9 or 10 min, and $proA^+$ entered between 14 and 17 min after mixing. The time interval between entrance of leu^+ and $proA^+$ is in

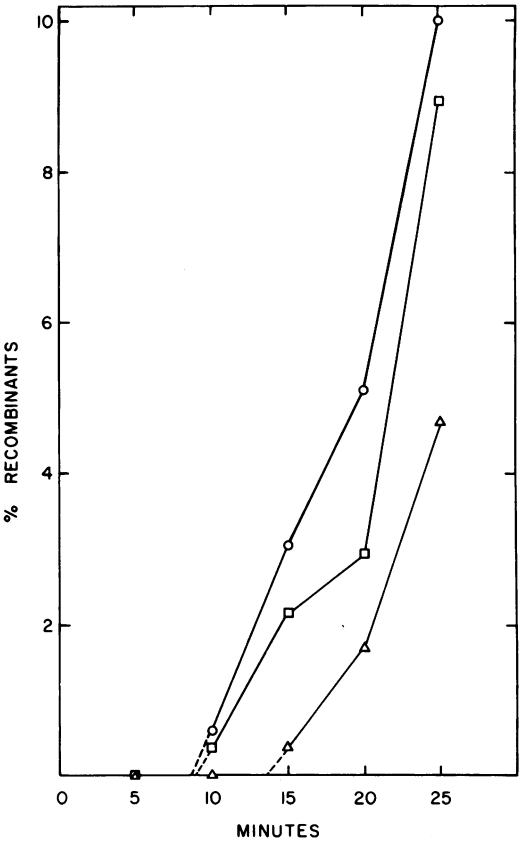

FIG. 7. *Interrupted mating of AX655 with HfrH. Samples were removed from the mating vessel at 5-min intervals and plated to determine the percentage of leu^+ (○), temperature-insensitive (□), and $proA^+$ (△) recombinants.*

agreement with the known map distance of 6 min (27) between them. Thus the ts gene maps within one min clockwise from *leu*.

Interrupted mating experiments with HfrH and mutants AX621 and AX629 indicated that the thermosensitive mutations in these strains ($ts_{18\text{-}82}$ and ts_{424}, respectively) also mapped within 1 min clockwise of *leu*.

Orientation of ts relative to ara, leu, and nadC. To determine the orientation of ts relative to *ara* and *leu*, *leu*$^+$ and ts$^+$ were transduced into AX621 from S9080. Unselected markers were scored after purification (Table 3).

If the gene order were *ara leu* ts, transductants of the genotype *ara*$^+$ *leu*$^-$ ts$^+$ would require four crossover events and would be found least often when the selection was for ts$^+$. In fact, this class was not found. To clarify the position of ts relative to *nadC*, *leu*$^+$ was transduced into UTH4113 from AX700, a *leu*$^+$ revertant of AX621. *leu*$^+$ transductants were purified and scored for ts and *nadC*. Analysis of the linkage data revealed that the gene order must be *leu* ts *nadC*, because the class of transductants found least often was *leu*$^+$ ts$^+$ *nadC*$^+$ (Table 3), the class which would require four crossover events. (The sequence *leu nadC* ts would require that the class *leu*$^+$ ts$^-$ *nadC*$^-$ be most infrequent.)

Specifically, the $ts_{18\text{-}82}$ mutation is located at min 2.1 on the standard chromosome map (Fig. 6). With data from the transductants selected for *leu*$^+$ (Table 3), the Wu formula (32), and the known map position of *leu* at min 1.5 (27), $ts_{18\text{-}82}$ was determined to be 0.6 min to the right of *leu* (Fig. 6).

TABLE 3. *P1vir transduction analysis of the orientation of* $ts_{18\text{-}82}$

Donor	Recipient	Selected marker	Scored markers	Transductants having scored markers
S9080	AX621	*leu*$^+$	*ara*$^+$ ts$^-$	53
			ara$^+$ ts$^+$	20
			ara$^-$ ts$^+$	22
			ara$^-$ ts$^-$	14
S9080	AX621	ts$^+$	*ara*$^-$ *leu*$^-$	10
			ara$^-$ *leu*$^+$	10
			ara$^+$ *leu*$^-$	0
			ara$^+$ *leu*$^+$	21
AX700	UTH4113	*leu*$^+$	ts$^+$ *nadC*$^+$	10
			ts$^+$ *nadC*$^-$	280
			ts$^-$ *nadC*$^+$	42
			ts$^-$ *nadC*$^-$	125

Azide sensitivity. It has been reported that *azi*^{r} mutants, which are resistant to sodium azide and which form filaments during incubation at 42 C, map at min 2 of the *E. coli* chromosome, very near to the *leu* locus (27, 33). Consequently, thermosensitive mutants were plated on medium containing 0.0034 M sodium azide at 30 C. Neither AX621, AX629, AX655, nor the parent AB1157 was able to form colonies, and thus none was azide resistant.

DISCUSSION

In liquid medium at 42 C, all three mutants grew to a maximum of 12- to 20-fold increase in mass and then growth stopped. The defect which prevented septum formation at 42 C caused the eventual cessation of growth for recombinants selected for ability to form colonies at 42 C and also regained the ability to form septa at 42 C. Also, ts$^-$ transductants obtained with P1*vir* grown on the ts$^-$ mutant donor had lost the ability to form septa and divide at 42 C. It is possible that the ts mutation causes defective functioning or assembly of membrane components. The defective membrane components would prevent septum formation and eventually fail even to support growth. This model is supported to some extent by the finding that CM, when added to filaments at the time of shifting to 28 C, caused extensive lysis of two of the mutants. CM prevented division of the third mutant when filaments were shifted to 28 C.

The specific reason why growth stopped at 42 C is unknown, but the stopping of growth was not unexpected because of the nature of the isolation procedure. There were no detectable infectious phage particles released after growth stopped.

Many laboratories have now described bacterial mutants which fail to divide at elevated temperatures but do not show impaired DNA synthesis. Some mutants show residual division at the restrictive temperature (5, 34), whereas others cease cell division immediately. Examples of the latter type include 4a (2) and TK159 and TK463 (6) in *Salmonella typhimurium*, and PAT84 (11), BUG-6 (23), ts-20 (17), AX629 (3), and AX621 and AX655 (this paper) in *E. coli*.

Each of these strains forms filaments at the restrictive temperature, although mass continues to increase and DNA synthesis appears normal. When the strains are shifted to the permissive temperature, cell division resumes at a rapid rate after a lag period. However, even the *E. coli* mutants differ among themselves in basic characteristics. Filaments of mutants PAT84 and BUG-6, when shifted from 42 to

28 C, completed one division in the presence of chloramphenicol (17, 22). When filaments of AX655, AX629, and AX621 were shifted from 42 to 28 C, CM not only prevented division but also caused extensive lysis of AX629 and AX621. Mutant ts-20 was unique in that, when the temperature was reduced, filaments divided synchronously, but only near one end (17).

Mutations $ts_{18\text{-}82}$, ts_{424}, and $ts_{21\text{-}58}$ (carried by strains AX621, AX629, and AX655, respectively) all map in the same region of the *E. coli* chromosome, about 1 min clockwise of *leu*. Specifically, $ts_{18\text{-}82}$ is located at min 2.1, 0.6 min to the right of *leu*.

The *ftsA* locus also maps in this region (26), although details of the FTSA phenotype are not clear. Van de Putte et al. (29) placed fts_2^-, fts_7^-, and fts_8^- slightly clockwise of *leu*. Their strains formed filaments at the restrictive temperature and apparently had normal DNA synthesis; no information was given about behavior of the strains after shifting back to the permissive temperature. Subsequently, Taylor (26) referred to this locus as *ftsA* and indicated that it is identical to *azi*, presumably because Yura and Wada (33) had shown that some (although not all) azi^r strains are temperature sensitive for growth and form filaments at 42 C. Hirota et al. (10, 11) placed the defect in strain PAT84 at *ftsA*, although no mapping data were presented. It has not been reported whether PAT84 is azide resistant.

In *S. typhimurium*, the cell division mutation *divC* has also been mapped near *leu* (6). Thus, it appears that a number of cell division mutants which form filaments during incubation at the restrictive temperature, which synthesize DNA apparently normally, and which form septa at a faster than normal rate after return to the permissive temperature all map near *leu*. It is possible that the various phenotypes seen in strains AX621, AX629, AX655, and PAT84 result from different mutations in the same operon or cistron. Map positions of the mutations in BUG-6 and ts-20 have not been published; *divA* of *S. typhimurium* maps near *metB* (6).

Complementation studies will determine the relationship between mutations ts_{424}, $ts_{18\text{-}82}$, $ts_{21\text{-}58}$, and the *ftsA* in PAT84.

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