# INITIAL CHARACTERIZATION OF TEMPERATURE-SENSITIVE CELL DIVISION MUTANTS OF *ESCHERICHIA COLI*

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SUMMARY: Thermosensitive mutants of *E. coli* which form filaments at 42 C were isolated. These mutants were divided into 3 classes according to optical density and cell number patterns during growth at 42 C and after being shifted from 42 C to 30 C. Nuclear regions in 2 classes were positioned along the filament with the same number per unit length of cell as were those of wild-type cells. However, one class appeared to be defective in nuclear region positioning in that the nuclear bodies of its filaments were separated by large areas of cytoplasm.

In an attempt to elucidate the biochemistry of cell division, various thermosensitive mutants of *E. coli* have been isolated by several laboratories. One group of mutants is conditionally deficient in septum formation and the cells elongate into filaments during incubation at the restrictive temperature. A class of these mutants which is indefinitely inhibited from forming septa at 40 C, but which, on reducing the temperature to 30 C, fragments completely into short cells by the formation of septa along the length of the filaments, has been described in detail (Hirota *et al.*, 1968; Reeve *et al.*, 1970; and Nagai *et al.*, 1971). Fragmentation occurs rapidly and is completed within about a 40 min period. This class of mutants is readily selected by the differential filtration procedure of Van de Putte *et al.* (1964).

In this paper, we report the initial characterization of three additional classes of mutants conditionally defective in septum formation

which were isolated by direct screening of temperature-sensitive mutants.

## METHODS AND MATERIALS

Strain and medium: All mutants were derived from *E. coli* AB1157 ($F^-$ *thi*$^-$ *thr*$^-$ *leu*$^-$ *arg*$^-$ *pro*$^-$ *his*$^-$ *ara*$^-$ *lac*$^-$ *gal*$^-$ *xyl*$^-$ *mtl*$^-$ *str*$^r$) obtained from P. Howard-Flanders. Culture were grown in tryptone-yeast extract medium.

Mutant isolation: Thermosensitive mutants were obtained using the procedure of Kohiyama *et al.* (1966), except that 100 µg/ml of N-methyl-N'-nitro-N-nitrosoguanidine was used. These strains were screened microscopically for filament formation at 30 C and at 42 C.

Staining: The method of Piéchaud (1954) was used

Cell number and mass determination: Cells were counted with a Model B Coulter Counter. Optical density was measured at 450 nm in a Zeiss PMQ II spectrophotometer.

## RESULTS

Approximately 12% of the mutagen survivors incubated at 30 C were defective in colony-forming ability at 42 C. Of 191 such mutants which were screened microscopically, 42 formed filaments when incubated for 3 hr in liquid medium at 42 C.

Optical density and cell number changes of 21 filament formers were examined after temperature shifts. Cells were grown at 30 C until balanced growth was achieved, diluted into prewarmed broth at 42 C for various times, and diluted again for 30 C incubation. Three classes of mutants will be described.

In Class A, represented by AX629, mass continued to increase at 42 C; cell number remained constant for 1 hr and then increased (Fig. 1). When the culture was shifted to 30 C after 120 min at 42 C, cell mass increased 50% during 60 min and then remained constant for an additional 60 min. Cell number increased at about 1.4 times the 30 C rate for 120 min, at which time the proper mass:cell number ratio was

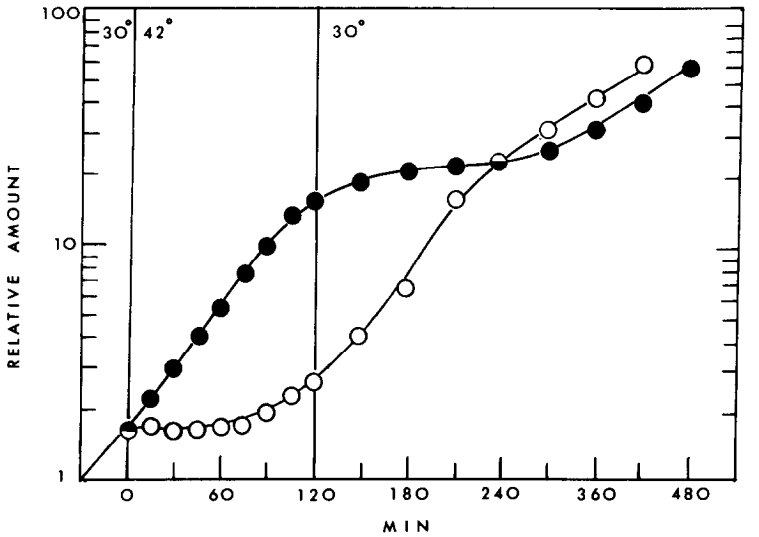


Fig. 1. Growth and cell division of strain AX629 at 42 C and after shifting from 42 C to 30 C. Relative amount 1 represents 1 x $10^7$ cells/ml (O) and an absorbance of 0.10 (●).

reestablished. Then mass and cell number increased at approximately the pre-shift rate.

When the culture was shifted to 30 C after 90 min at 42 C, mass increased at 0.7 times the pre-shift 30 C rate, and cell number increased at 1.5 times the pre-shift 30 C rate until the proper mass:cell number ratio was reestablished.

The nuclear regions of AX629 filaments were regularly distributed along the entire length of the filament (Plate 1A).

Class B (AX612) mutant cell number remained constant throughout the 42 C incubation period whereas mass continued to increase (Fig. 2). After shifting from 42 C to 30 C both mass and cell number increased at a rate of one doubling per 100 min for approximately 100 min. This parallel increase was not a permanent alternative state, however. During the next 180 min, cell division occurred at a rate greater than the rate of mass increase until the average cell size was approximately equal to that in the 30 C control culture.

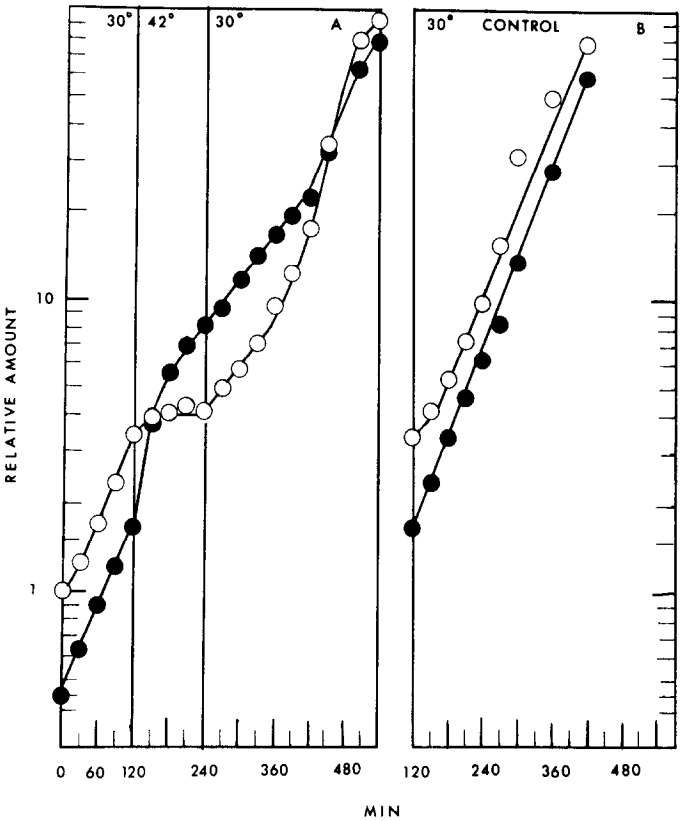


Fig. 2. (A) Growth and cell division of strain AX612 at 42 C and after shifting from 42 C to 30 C. Relative amount 1 represents 1 x $10^7$ cells/ml (○) and an absorbance of 0.04 (●). (B) Growth and cell division of strain AX612 grown continuously at 30 C. 120 min represents the time at which a part of the initial 30 C culture was shifted to 42 C.

When the 42 C incubation period was 60 min, subsequent 30 C incubation resulted in mass and cell number increases at 1.3 times the pre-shift 30 C rate for at least 180 min.

Nuclear regions of AX612 were distributed in a manner similar to that of Class A (Plate 1B). The significance of the distinctive shape of the nuclear regions is unknown at present.

Mutants of Class C (AX633) showed little or no increase in cell number at 30 C following a 60 or 120 min incubation period at 42 C (Fig. 3). Incubation at the non-permissive temperature was lethal for AX633. During 120 min incubation at 42 C, colony-forming ability

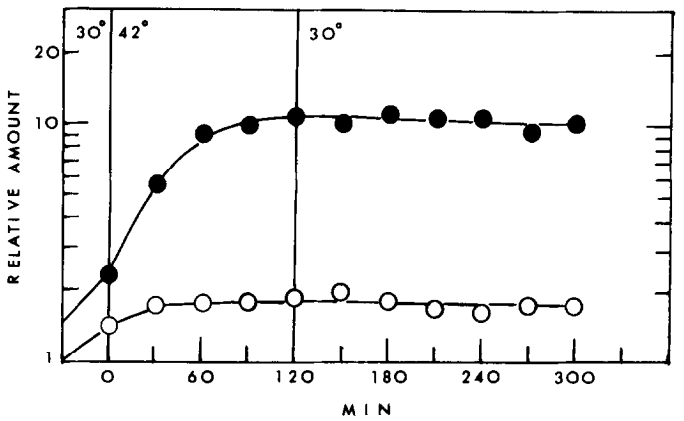


Fig. 3. Growth and cell division of strain AX633 at 42 C and after shifting from 42 C to 30 C. Relative amount 1 represents $1 \times 10^7$ cells/ml (O) and an absorbance of 0.16 (●).

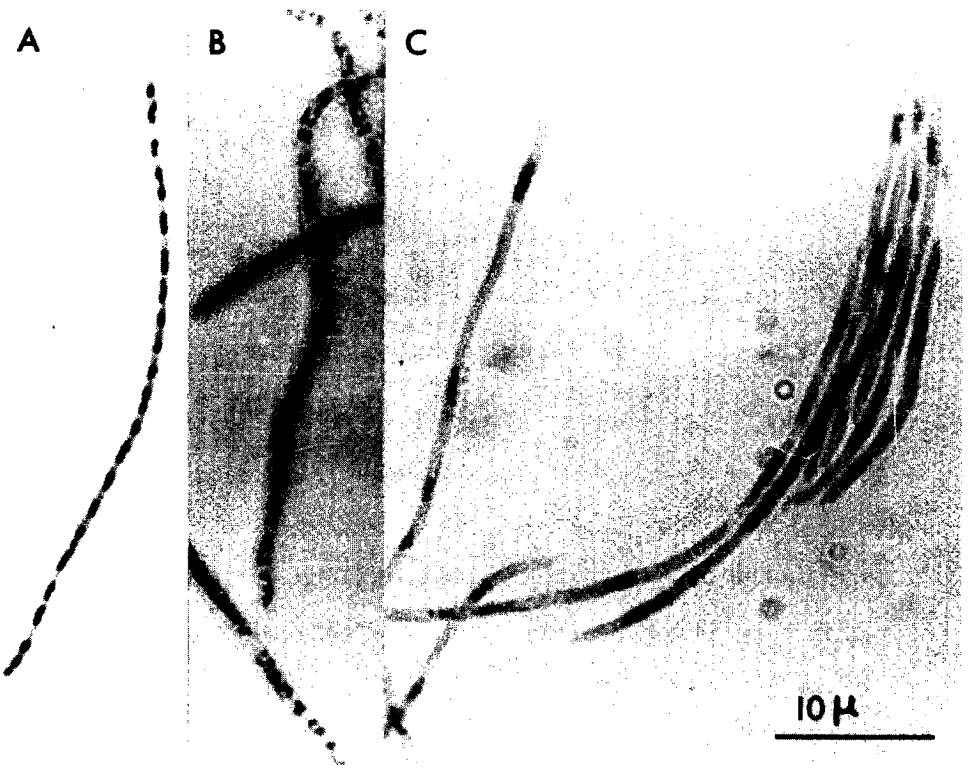


Plate I. Nuclear stained filaments formed during 90 min of 42 C incubation. A, AX629; B, AX612; C, AX633. Nuclear regions appear dark.

decreased exponentially and 3% of the initial number of colony-forming cells survived after 120 min. This fraction remained approximately constant during the subsequent incubation at 30 C for 180 min. A shift to 30 C after 30 min at 42 C resulted in only a 30% increase in cell number and only a 100% increase in absorbance over a 120 min period.

The nuclear regions of AX633 filaments were separated by large areas of cytoplasm which apparently contained no nuclear material, so that fewer nuclear regions existed per unit of cell length than in wild-type cells (Plate 1C). This type of nuclear positioning was observed also with AX634, a strain which increased 2.5 fold in cell number and 50% in cell mass during 120 min of 30 C incubation after 120 min at 42 C. The possible relationship between aberrant nuclear distribution and septum formation inhibition is under investigation.

Detailed physiological and genetic characterization of these and other classes of mutants will be used to analyze the process of septum formation.

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