JOURNAL OF BACTERIOLOGY, Apr. 1972, p. 117-121
Vol. 110, No. 1

Cell Division of *Escherichia coli* BUG-6: Effect of Varying the Length of Growth at the Nonpermissive Temperature

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Received for publication 6 December 1971

When *Escherichia coli* BUG-6 is grown at 42 C and then returned to 30 C, the division kinetics during the recovery at 30 C are dependent on the length of time the cells were grown at 42 C. If chloramphenicol is added when the cells are shifted from 42 to 30 C, no division occurs if the period at 42 C is less than 4 min or more than 110 min. Maximum division occurs when the period at 42 C is 50 min. A discussion of these results with reference to a previously proposed model is presented.

We recently described a reversible temperature-sensitive division mutant, *Escherichia coli* BUG-6, which divides normally at 30 C but fails to divide at 42 C and consequently grows into filaments at 42 C (8). Mutants of this type have been described elsewhere (1, 4, 6). We found that filaments of *E. coli* BUG-6 produced by a period of 45 min of growth at 42 C divide very rapidly on being replaced at 30 C. This division is not inhibited by the addition of chloramphenicol (CM) at the time of the 42 to 30 C shift. The present work describes the cell division kinetics at 30 C after varying periods of growth at 42 C.

MATERIALS AND METHODS

E. coli BUG-6 was derived from *E. coli* AB1157 *sm/r*, *gal*$^-$, *xyl*$^-$, and *mtl*$^-$ by a procedure described previously (8). Cultures in Erlenmeyer flasks were incubated in a shaking water bath. The growth medium consisted of 3 g of beef extract per liter, 5 g of pepticase per liter, and 5 g of NaCl per liter, adjusted to *p*H 7.3.

Cell numbers and cell size were measured with a modified Coulter counter coupled to a pulse height analyzer (2).

CM, obtained from Sigma Chemical Co., St. Louis, Mo., was used at a final concentration of 150 μg/ml.

RESULTS

Effect of different time periods at 42 C on recovery at 30 C.

E. coli BUG-6 was grown for several generations at 30 C, shifted to 42 C for different lengths of time, and then returned to 30 C. In Fig. 1, zero time is the time when the 42 C subcultures were replaced at 30 C. One minute of incubation at 42 C (Fig. 1a) has little effect on cell division, but a 2-min pulse at 42 C causes a lag before division resumes, and 34 min is required at 30 C before the control cell number is attained (Fig. 1b). The rate of cell division during the recovery period of the population pulsed for 2 min at 42 C is only slightly faster than the normal rate of division at 30 C. As the period of incubation at 42 C is increased (Fig. 1c-j), so the time required for the cell number of the culture incubated at 42 C to reach the 30 C control value decreases. The decrease results from an increasing rate of division during the recovery period as the length of the 42 C incubation period is increased. In all cases, no division occurs until 15 min have elapsed after the 42 to 30 C shift. Figure 2 is a summary of the information in Fig. 1.

The period required for the cell number of the 42 C-treated cultures to attain that of the control culture remains constant at 23 min after periods of growth at 42 C of between 15 and 35 min. (The optical density doubling at 42 C for *E. coli* BUG-6 is 35 min in the medium employed.) When the period of growth at 42 C exceeds 35 min, the time required for the cell number to attain the control value during the recovery period increases again. In Fig. 3 the cell division kinetics during the recovery

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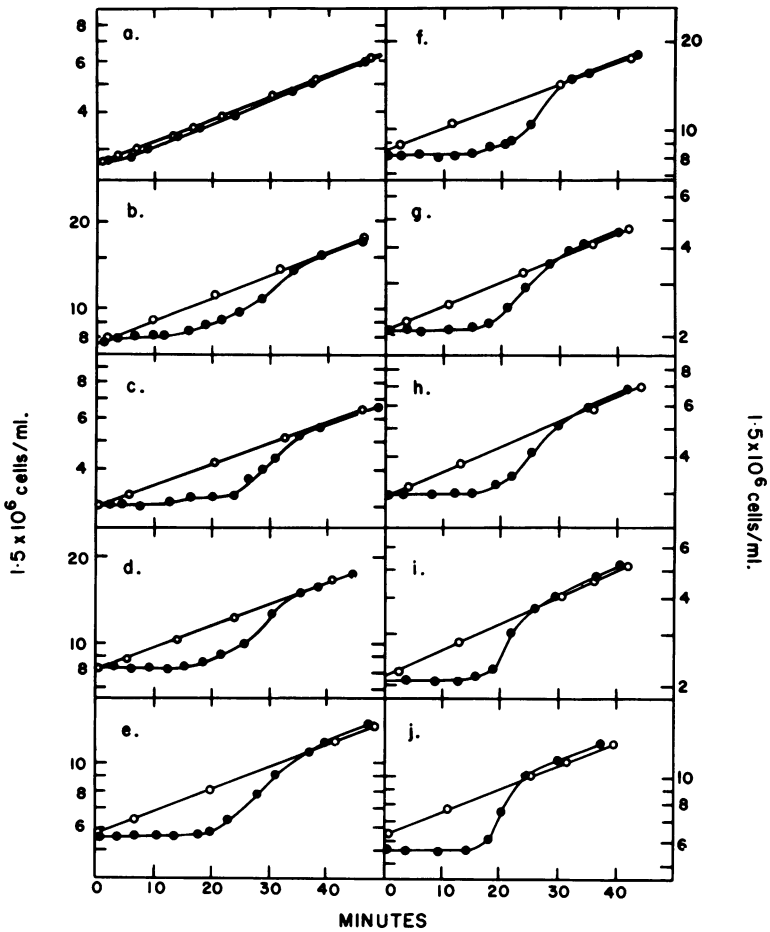

FIG. 1. *Effect on cell division of shifting Escherichia coli BUG-6 from 30 to 42 C for different time-intervals. E. coli BUG-6 was grown in broth for several generations at 30 C (○) and then shifted to 42 C (●) for (a) 1 min, (b) 2 min, (c) 3 min, (d) 4 min, (e) 5 min, (f) 6 min, (g) 7 min, (h) 8 min, (i) 10 min, and (j) 12 min before being replaced at 30 C. Time 0 min represents the time at which the 42 C cultures were replaced at 30 C.*

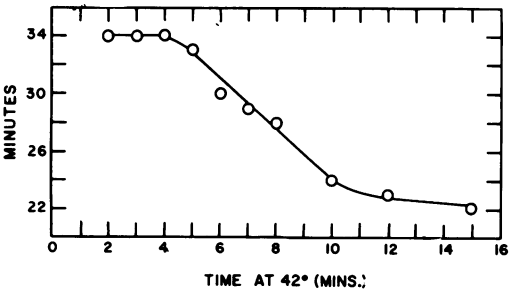

FIG. 2. *Variation in recovery time for cultures of Escherichia coli BUG-6 placed at 42 C for different time intervals. E. coli BUG-6 was grown for several generations at 30 C, and subcultures were placed at 42 C for different time intervals before being replaced at 30 C. The time required for the cell number of a subculture to attain the 30 C control value after being replaced at 30 C is plotted against the time that subculture was kept at 42 C.*

period at 30 C after periods of 45, 60, 75, 90, and 105 min at 42 C are shown. In each case, 15 min is required before cell division starts, and then one rapid doubling of cell number occurs, followed by a slower rate of division until the control rate of cell division at 30 C is obtained.

Effect of CM on cell division during the recovery period. Addition of CM at the time of the 42 to 30 C shift after 45 min at 42 C does not prevent cell division in the recovery period (8). In Fig. 4 CM is added at the time of the 42 to 30 C shift after periods of 35, 50, 65, 80, and 95 min of growth at 42 C. In each case cell division occurs in the presence of CM; however, the number of cell divisions is not directly proportional to the length of the growth period at 42 C. The number does initially increase as the 42 C growth period increases, but a maximum is reached and longer

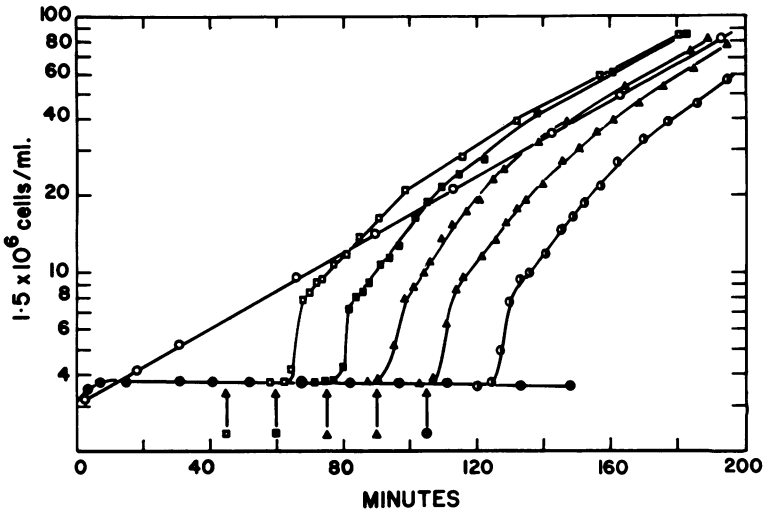

FIG. 3. *Effect on cell division of shifting Escherichia coli BUG-6 from 30 to 42 C for different time intervals. E. coli BUG-6 was grown in broth for several generations at 30 C (○), and at 0 min part of the culture was shifted to 42 C (●). Subcultures from 42 C were replaced at 30 C after 45 (□), 60 (■), 75 (△), 90 (▲), and 105 min (◑) at 42 C.*

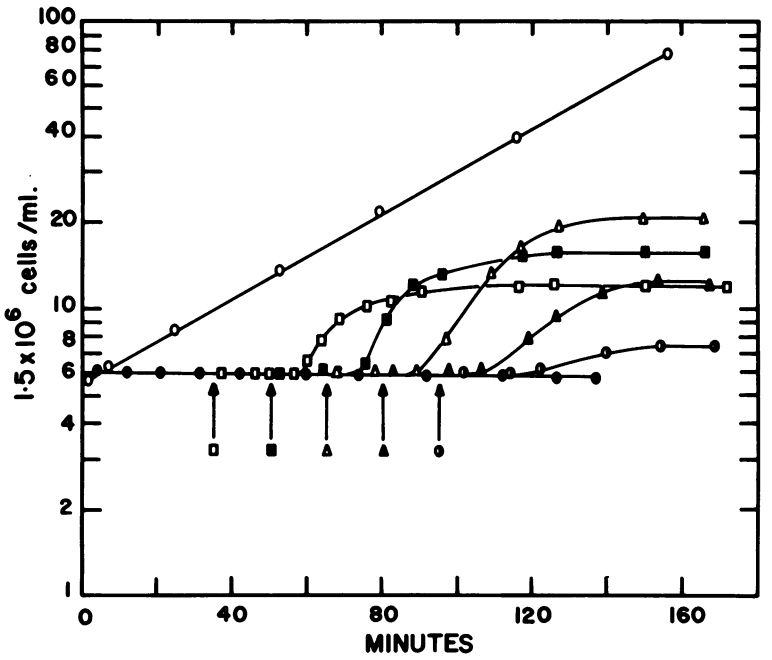

FIG. 4. *Effect on cell division of shifting Escherichia coli BUG-6 from 30 to 42 C for different time intervals and then replacing at 30 C in the presence of chloramphenicol. E. coli BUG-6 was grown in broth for several generations at 30 C (○), and at 0 min part of the culture was shifted to 42 C (●). Subcultures from 42 C were replaced at 30 C, and chloramphenicol was added (150 μg/ml final concentration) after 35 (□), 50 (■), 65 (△), 80 (▲), and 95 min (◑) at 42 C.*

periods of growth at 42 C result in increasingly less division during the recovery period at 30 C. During the recovery period the rates of division in the presence of CM are considerably less than in its absence (compare Fig. 3 and 4), and the fixed 15 min before the onset of cell division is lost. To determine exactly the amount of cell division in the presence of CM at 30 C following periods of growth at 42 C, a more detailed experiment was performed. A culture of *E. coli* BUG-6 was grown for several generations at 30 C and then shifted to 42 C. At frequent intervals, subcultures were removed and placed at 30 C in the presence of CM. In Fig. 5 the final cell number attained by each subculture is plotted against the length of time that the culture was at 42 C. Periods of less than 6 min or more than 110 min at 42 C allow little or no recovery division in the presence of CM at 30 C. A maximum number of divisions occurs after a period of approximately 50 min growth at 42 C. Plateau values occur for final cell number attained for cultures maintained at 42 C for between 18 to 22 min and 60 to 70 min. This experiment has been repeated many times, and, although slight variations occur in the timing of the plateaux and maximum, the shape of the curve is very reproducible.

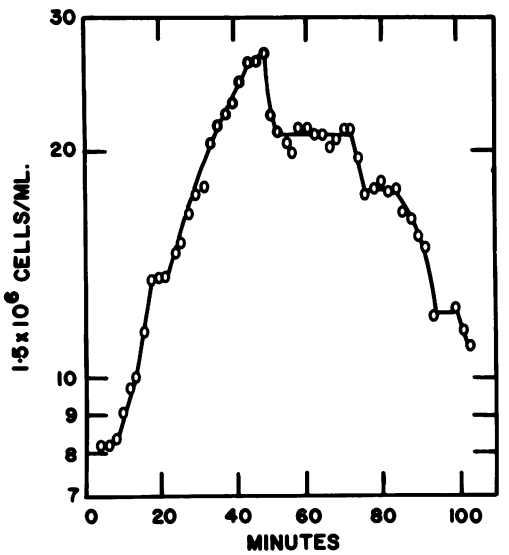

FIG. 5. *Effect of chloramphenicol on residual division of filaments during the recovery at 30 C after different time intervals at 42 C. Escherichia coli BUG-6 was grown for several generations in broth at 30 C and then placed at 42 C. Subcultures were removed and replaced at 30 C in the presence of chloramphenicol (150 μg/ml final concentration). The final cell number attained by each subculture is plotted against the time it was at 42 C.*

DISCUSSION

The division kinetics of *E. coli* BUG-6 upon transfer from restrictive to permissive temperature have been studied in detail. Filaments produced at 42 C resume division at 30 C, but the kinetics of this division are dependent upon the length of incubation at 42 C.

A 1-min incubation at 42 C is insufficient to inhibit cell division (Fig. 1a); 2-min incubation does stop cell division (Fig. 1b). Cell division during the recovery of cells after a 2-min incubation at 42 C is only slightly faster than the cell division rate of the 30 C control. Figures 1c through j indicate that longer periods at 42 C lead to more rapid cell division rates during the recovery period. In all cases there exists a 15-min period after the 42 to 30 C shift before cell division commences. This would indicate that at 42 C cells are blocked at a stage in cell division which occurs 15 min before actual cell separation or blocked in a process which takes 15 min to complete. As there is little or no residual division after a 30 to 42 C shift, the latter alternative seems more probable. It must be assumed in either case that a period at 42 C as short as 2 min requires the cells to return to a position in the cell division cycle of 15 min before cell separation. We have several cell division mutants of *E. coli* which differ from *E. coli* BUG-6 only in the length of the constant period after the 42 to 30 C shift and before the onset of cell division. Each mutant exhibits a characteristic constant period and these periods vary from as short as 12 min to as long as 70 min (*unpublished data*). A constant period of 60 to 70 min has been reported for a mutant of *Salmonella typhimurium* (1).

When the period of growth of *E. coli* BUG-6 at 42 C exceeds 35 min, the recovery cell division kinetics become more complicated although the constant 15-min period still exists. An initial burst of cell division produces one doubling of cell number. The rate of cell division then decreases slightly. The decreased rate is, however, still considerably faster than the control 30 C cell division rate. Eventually the control 30 C rate is obtained (Fig. 3). An initial division of filaments to produce a doubling of cell number has been observed when filaments produced by *lon*$^-$ mutants (5) or thymine starvation (3) divide and also when the temperature-sensitive cell division mutant *E. coli* PAT84 (Lazdunski and Shapiro, *personal communication*) is replaced at the permissive temperature. The initial completion of

one cell division regardless of filament length suggests that a preferential division site exists although size distribution analysis of the resulting cells indicates a random location of this site along the filament length (*unpublished data*). Paulton (7) has shown that, in *Bacillus subtilis*, control of cell division sites occurs and that, although several sites can coexist in the same cell, preferential completion of the earliest initiated division site occurs. It is tempting to suggest that the initial division utilizes a division site which was present at the time of the 30 to 42 C shift or that during growth at 42 C division sites are still produced, although not expressed, and that as in *B. subtilis* the sites are used sequentially when division is permitted at 30 C.

An alternative interpretation of the initial division would be that the filament at 42 C contains enough "division components" for one division at 30 C without any further synthesis being required. The experiment described in Fig. 4 was devised to test this proposal, assuming that, if CM were added at the time of the 42 to 30 C shift, then all cultures which had been at 42 C for longer than 35 min should divide just once. This result was not obtained. Division in the presence of CM is critically dependent on the length of the 42 C incubation period. Division does not occur if the culture is incubated at 42 C for less than 6 min or more than 110 min (Fig. 5). The rate of cell division in CM is decreased (compare Fig. 3 and 4), and the preferential completion of one cell division is lost (Fig. 4). It would therefore appear that cell division in the presence of CM is considerably different from cell division in the absence of CM. The results do indicate, however, that under certain conditions more than one cell division can occur in the absence of protein synthesis.

We previously proposed that *E. coli* BUG-6 normally produces a protein, division potential (D), which is required for cell division (Fig. 16 of reference 8). On shifting to 42 C this component is thought to change conformationally to an inactive form (X_1) thus blocking cell division. Furthermore, the model predicts that because of the conformational change, X_1 is not recognized by the metabolic controls governing the synthesis of D and the cell attempts to replenish its D by increasing the synthetic rate of D. As the cell is at 42 C, this rapidly synthesized D immediately becomes X_1, and a high level of X_1 results. On being replaced at 30 C, the X_1 reverts to D, which results in the observed burst in cell division.

This model adequately explains the division kinetics following a short period at 42 C. One minute at 42 C is insufficient to inactivate all the D, and division continues. Two minutes at 42 C inactivates all the D but is insufficient time for the development of a pool of X_1. As the period at 42 C increases, so the pool of X_1 can develop and be expressed on return of the cells to 30 C. Thus, as the period at 42 C increases, so the rate of recovery division increases, reducing the time required for the cell number to reach the control value (Fig. 1 and 2). A minimum recovery period is eventually attained (23 min; see Fig. 2) presumably due not to the level of X_1 but to the time required for its expression in division.

The division kinetics after long periods at 42 C cannot be explained solely by the proposed model without invoking additional controls, although the results do not contradict the basic proposal that at 42 C the filaments contain a high level of inactive division potential. The initial division following the shift from 42 to 30 C after extended periods at 42 C suggests that regardless of the amount of division potential in a filament, potential is used preferentially at one location. This preference is apparently lost if CM is added at the time of the 42 to 30 C shift.

ACKNOWLEDGMENTS

This work was supported by grant A4419 from the National Research Council of Canada.

We would like to thank N. H. Mendelson for helpful suggestions during preparation of the manuscript.

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