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Cell Growth and Division in *Escherichia coli:* a Common Genetic Control Involved in Cell Division and Minicell Formation

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Escherichia coli Div 124(ts) is a conditional-lethal cell division mutant formed from a cross between a mutant that produces polar anucleated minicells and a temperature-sensitive cell division mutant affected in a stage of cross-wall synthesis. Under permissive growth temperature (30 C), Div 124(ts) grows and produces normal progeny cells and anucleated minicells from its polar ends. When transferred to nonpermissive growth temperature (42 C), growth and macromolecular synthesis continue, but cell division and minicell formation are inhibited. Growth at 42 C results in formation of filamentous cells showing some constrictions along the length of the filaments. Return of the filaments from 42 to 30 C results in cell division and minicell formation in association with the constrictions and other areas along the length of the filaments. This gives rise to a "necklace-type" array of cells and minicells. Recovery of cell division is observed after a lag and is followed by a burst in cell division and finally by a return to the normal growth characteristic of 30 C cultures. Recovery of cell division takes place in the presence of chloramphenicol or nalidixic acid when these are added at the time of shift from 42 to 30 C, and indicates that a division potential for filament fragmentation is accumulated while the cells are at 42 C. This division potential is used for the production of both minicells and cells of normal length. The conditional-lethal temperature sensitive mutation controls a step(s) in cross-wall synthesis common to cell division and minicell formation.

The understanding of cell growth and division is fundamental to our knowledge of cell proliferation, differentiation, and cell-to-cell interaction. Much information on cell division has been gathered from both eukaryotic and prokaryotic cells (6). However, a detailed understanding of this process in bacteria remains far from complete (5, 7, 11).

Cell division in *Escherichia coli* is the end result of biochemical reactions and regulatory networks that begin at a specific time in the life cycle of the cell (G.G.K., unpublished results) and are coordinated with the chromosome replication cycle (G. G. Khachatourians, submitted for publication). These reactions normally proceed in an integrated manner and culminate in the physical separation of the two daughter cells. One way of beginning the analysis of these steps is to isolate and characterize, by morphological, genetic, and biochemical techniques, a collection of cell division mutants. These mutants, and hybrids made from them, should help us to sequence the steps required for cell division.

This approach has been used in our work, and in this paper some properties of a novel hybrid mutant are described. We will present evidence demonstrating the existence of a common step in normal cell division and minicell formation.

MATERIALS AND METHODS

Bacteria. From a conjugal cross between *E. coli* BUG-6 (10) and a rifampin-resistant derivative of *E. coli* P678-54 (1), several temperature-sensitive (ts), minicell-forming, hybrid mutants were obtained (Khachatourians and Clark, unpublished experiments). Mutant Div 124(ts) is one such mutant.

Growth of the cells. Cultures were grown in nutrient broth, as described by Reeve et al. (10), in 10-ml amounts in a shaking water-bath incubator at permissive (30 C) or nonpermissive temperatures

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(42 C). Absorbancy measurement at 620 nm (A_{620}) was performed with a Spectronic 20 (Bausch and Lomb, Inc., Rochester, N.Y.) spectrophotometer. The cell numbers and cell size distributions were monitored with a Coulter counter (model B) equipped with a 30-μm aperture probe and a model J automatic particle-size distribution analyzer-plotter (Coulter Electronics, Inc., Hialeah, Fla.).

Time-lapse microcinematography. Time-lapse cinematography was performed with a Zeiss phase-contrast microscope housed in a specially designed temperature-regulated chamber. Preparation of microcultures for cinematography has been described previously (3).

Chemicals. Chloramphenicol and nalidixic acid were purchased from Sigma Chemical Co. (St. Louis, Mo.), and were used at 150 and 10 μg/ml final concentrations, respectively.

RESULTS

E. coli BUG-6 is a conditional-lethal (ts) cell division mutant unable to continue cellular division when shifted from permissive (30 C) to nonpermissive (42 C) growth temperature (10). The *min* mutation(s) is responsible for the formation of small polar cells called minicells (2). *E. coli* Div 124(ts) is a hybrid cell division mutant that contains both mutations responsible for conditional-lethal cell division and the production of minicells. Div 124(ts) grows at 30 C, producing normal progeny cells and minicells. We have followed the effect of a temperature shift on Div 124(ts) by time-lapse cinematography. Figure 1 represents frames abstracted from one such sequence and shows the absence of additional cell division or minicell formation during growth at 42 C. The following features are noteworthy. (i) Elongation rates for individual cells parallel the growth rate (A_{620} at 42 C) of the exponential culture. (ii) The rate of cell elongation for one pole of the cell differs significantly from that of the opposite pole. Using newly produced minicells, which remain at rest on the slide, as markers, it could be shown that the end of the cell that has most recently

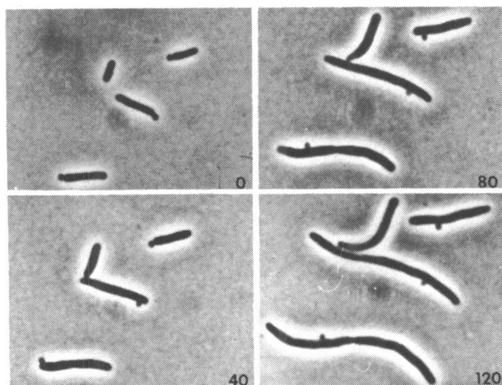

Fig. 1. *Growth of Div 124(ts) single cells at 42 C. Cell growth was abstracted from a 16-mm time-lapse film strip. Numbers at the bottom of each frame indicate growth time at 42 C. Phase-contrast approximate magnification. ×2,000.*

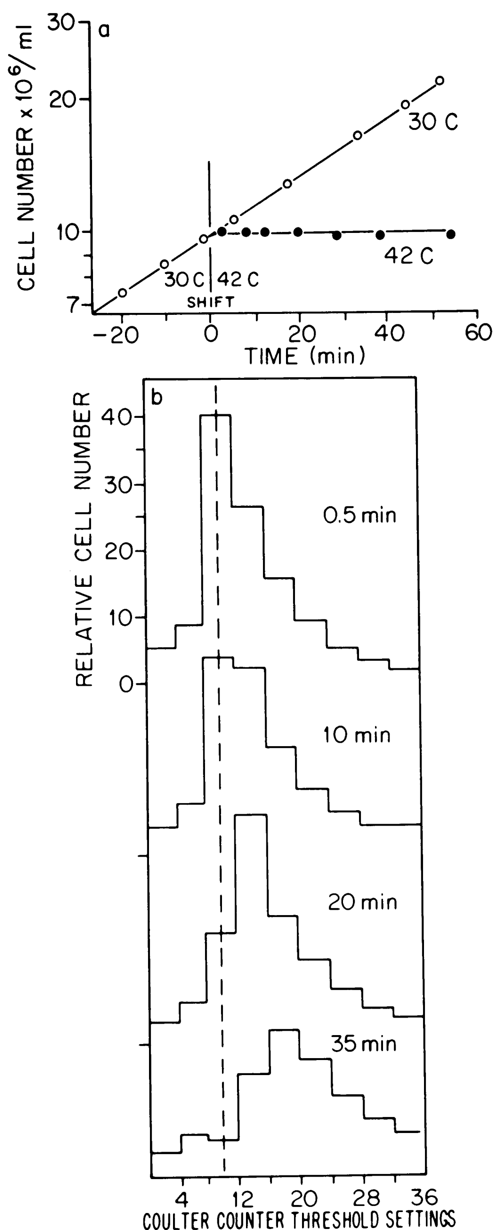

Fig. 2. *Effect of a shift from 30 to 42 C on Div 124(ts). Cell number (A) and cell volume distributions (B) are shown as a function of incubation time at 42 C. Vertical dashed line (in B) indicates the midpoint at the 30 C exponential size distributions.*

divided extends itself at one-half the rate of the opposite end. (iii) Regularly spaced constrictions appear along the walls of the Div 124(ts) filaments.

Analysis by Coulter counter technique of exponential cultures of Div 124(ts) grown at 30 C and shifted to 42 C agrees with Fig. 1. Coulter counter measurement of a shift-up condition is shown in Fig. 2. Increase in cell numbers ceases while cell growth continues, resulting in an increase in mean cell volume (filament formation). The mean cell volume for Div 124(ts) grown at 30 C is approximately twice that of *E. coli* BUG-6. We have found this to be a unique pleiotropic effect of the *min* mutation in *E. coli* strains (2) and in *Salmonella typhimurium* minicell-producing strains (Khachatourians and Hardigree, unpublished results). Aside from the mean cell volume, growth, and macromolecular synthesis examined by radioactive precursor incorporation in Div 124(ts), growth at 42 C (Khachatourians, unpublished results) is identical to that of *E. coli* BUG-6.

In contrast to the pattern of recovery of the BUG-6 parent, Div 124(ts) cells, when shifted from 30 to 42 C and returned to 30 C, never achieve a cell number equivalent to that of the control culture (Fig. 3; J. N. Reeve, personal communication). Under temperature shifts similar to that in Fig. 3, BUG-6 cells displayed a lag of 15 min followed by a rapid division phase which, within 23 min after shift-down (8, 9), result in a cell number equivalent to the 30 C control cultures. This was followed by a period of normal recovery of exponential division. Introduction of the *min* mutation clearly influences the BUG-6 recovery phenotype.

The underlying reasons for the reduced cell division during recovery were examined by studying the relationship between cell mass and nuclear equivalents to cell division capacity of filaments (Fig. 3). When nalidixic acid was used to inhibit further deoxyribonucleic acid synthesis, residual division at recovery was still observed. In this case the cell titer at the plateau extrapolates to the amount of cell division that could be obtained based on nuclear equivalents per mass of the filaments (from 30 C control culture). Inhibition of protein synthesis, on the other hand, revealed that at recovery there was enough division potential available for expression of cell division and minicell formation in the presence of the inhibitor. Analysis of cell size distribution profiles indicates a return to normal-sized cells at 30 C.

Abstracts similar to Fig. 1 were made from films of microcultures recovering at 30 C after 42 C incubation (Fig. 4). Results indicate that (i) filaments of Div 124(ts) can produce minicells and cells; (ii) at a given time, a filament will divide to yield a minicell or cell (that is, the division at recovery occurs by a sequential fragmentation of the filament); (iii) initially,

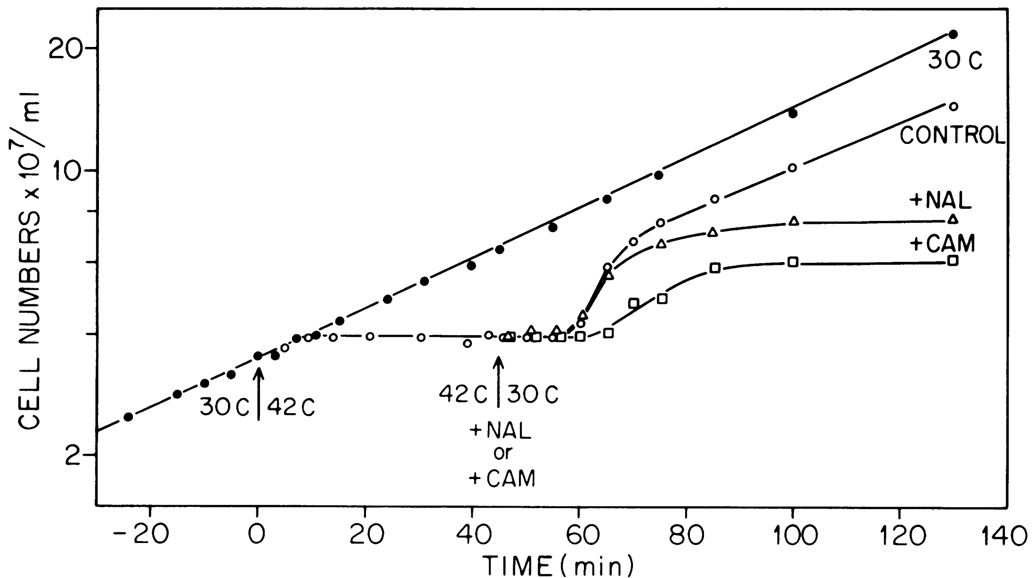

FIG. 3. *Recovery of Div 124(ts) from growth at 42 C. Part of a culture grown at 30 C (●) was shifted to 42 C (○) and was returned to 30 C at times indicated by the arrow. At the time of shift down chloramphenicol (□) or nalidixic acid (△) was added to the cultures.*

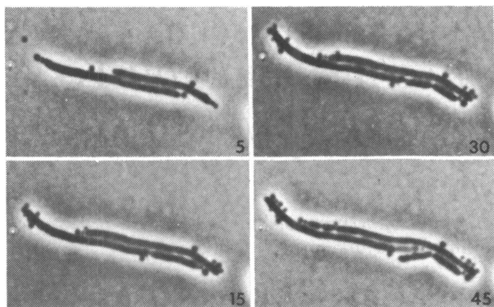

FIG. 4. *Cell division and minicell formation from filaments of Div 124 (ts) during recovery. Recovery division (42 C → 30 C shift) was abstracted as described in Fig. 1.*

minicells are produced throughout the length of the 42 C recovering filaments but become restricted to the polar ends when cells are grown further at 30 C; (iv) a given filament, on the average, produces more minicells than cells.

DISCUSSION

The observation that the mutant Div 124(ts) fails to form cross-walls leading to either normal cell division or minicell production at the nonpermissive temperature provides further support for the idea advanced earlier (4) that minicell production and normal cell division share at least one common biochemical step. Furthermore, the evidence presented in this and earlier publications suggests that the temperature-sensitive division protein known to accumulate in BUG-6 is at least one of the intermediates common to both normal and minicell-yielding divisions. When Div 124(ts) is returned to the permissive temperature, it begins to undergo normal and minicell-yielding divisions. The total of normal-sized cells formed is less than that of a control culture. However, if one adds together those divisions yielding minicells and those yielding normal cells, it can be demonstrated that the total division potential of the hybrid cell returned to permissive temperatures is equivalent to that of control cultures. From these observations we conclude that the division potential generated at the nonpermissive temperature is a common reservoir for all division events whether they yield cells or minicells. The manner in which the division potential is used, for example, at the catalytical or structural level in the control of cell division in minicell formation is still unknown.

Examination of Div 124(ts) should provide new information in understanding events involved in cell division. However, much more quantitative biochemical and genetic work is needed to elucidate its regulatory aspects. The mutant also directs our attention to questions regarding plasmid segregation, cell wall, and cell membrane growth and division. Some of these questions are currently under investigation.

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LITERATURE CITED

1. Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree. 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Nat. Acad. Sci. U.S.A. **57**:321-326.
2. Adler, H. I., W. D. Fisher, and A. A. Hardigree. 1969. Cell division in *Escherichia coli*. Trans. N.Y. Acad. Sci. (Ser. II) **31**:1059-1070.
3. Adler, H. I., and A. A. Hardigree. 1964. Analysis of a gene controlling cell division and sensitivity to radiation in *Escherichia coli*. J. Bacteriol. **87**:720-726.
4. Clark, D. J. 1968. Regulation of deoxyribonucleic acid replication and cell division in *Escherichia coli* B/r. J. Bacteriol. **96**:1214-1224.
5. Higgins, M. L., and G. D. Shockman. 1971. Procaryotic cell division with respect to wall and membranes. CRC Crit. Rev. Microbiol. **1**:29-72.
6. Mitchison, J. M. 1971. The biology of the cell cycle. Cambridge University Press, London.
7. Pato, M. L. 1972. Regulation of chromosome replication and the bacterial cell cycle. Annu. Rev. Microbiol. **26**:347-368.
8. Reeve, J. N., and D. J. Clark. 1972. Cell division of *Escherichia coli* BUG-6: effect of varying the length of growth at the nonpermissive temperature. J. Bacteriol. **110**:117-121.
9. Reeve, J. N., and D. J. Clark. 1972. Cell division of *Escherichia coli* BUG-6: effect of varying the temperature used as the non-permissive growth condition. J. Bacteriol. **110**:122-125.
10. Reeve, J. N., D. J. Groves, and D. J. Clark. 1970. Regulation of cell division in *Escherichia coli*: characterization of temperature-sensitive division mutants. J. Bacteriol. **104**:1052-1064.
11. Rowburry, R. J. 1972. Bacterial cell division: its regulation and relation to DNA synthesis. Sci. Progr. (Oxford) **60**:169-188.