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Growth and Division of Filamentous Forms of *Escherichia coli*

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ABSTRACT

ADLER, HOWARD I. (Oak Ridge National Laboratory, Oak Ridge, Tenn.), AND ALICE A. HARDIGREE. Growth and division of filamentous forms of *Escherichia coli*. J. Bacteriol. **90**:223–226. 1965.—Cells of certain mutant strains of *Escherichia coli* grow into long multinucleate filaments after exposure to radiation. Deoxyribonucleic acid, ribonucleic acid, and protein synthesis proceed, but cytokinesis does not occur. Cytokinesis (cross-septation) can be initiated by exposure of the filaments to pantoyl lactone or a temperature of 42 C. If growing filaments are treated with mitomycin C, nuclear division does not occur, and nuclear material is confined to the central region of the filament. Cytokinesis cannot be induced in mitomycin C-treated filaments by pantoyl lactone or treatment at 42 C.

The division of bacterial cells depends on a complex interaction of cell growth, nuclear synthesis, nuclear division, and cytokinesis (cross-septation). In some systems it is possible to isolate these factors for detailed study. We present here observations on a system in which cytokinesis can be isolated from other events in cell division and experimentally manipulated.

Certain mutant strains of *Escherichia coli* produce long nonseptate filaments when grown on nutrient media after exposure to ionizing or ultraviolet (2,537 A) radiation. This filament-forming property is under control of a single gene (Howard-Flanders, Simson, and Theriot, 1964; Adler and Hardigree, 1964*a*). Filaments grow for several hours after irradiation, synthesizing deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. Nuclear division occurs repeatedly during the growth period, and the filaments contain many nuclei distributed more or less uniformly throughout their length (Adler and Hardigree, 1965). Filaments may grow to 50 to 200 normal cell lengths before growth ceases and lysis occurs. If the growing filaments are exposed to pantoyl lactone or to a short period at 42 C, cytokinesis is initiated near one or both ends (Grula and Grula, 1962; Adler and Hardigree, 1965). The short cells produced by this process are capable of continued growth and division and will produce macrocolonies of normal-length cells. In this report we have particularly directed our attention to the question: Will the agents that stimulate cytokinesis be effective on filaments in which DNA synthesis and nuclear division have been inhibited?

MATERIALS AND METHODS

E. coli K-12 AB 1899 was obtained from Paul Howard-Flanders. The conditions for its growth and techniques for irradiation and phase microscopy have been published (Adler and Hardigree, 1964*a*). Mitomycin C (Kyowa-Hakko Kogyo Co., Ltd., Tokyo, Japan) and phenethyl alcohol (Eastman Organic Chemicals, Rochester, N.Y.) were added to autoclaved nutrient agar. Stationary-phase cultures of AB 1899 were diluted 20-fold in phosphate buffer, exposed to 10 to 20 kr of X rays, and spread on the surface of nutrient agar, nutrient agar plus mitomycin C, or nutrient agar plus phenethyl alcohol. Cover slip impressions were made after the samples had been incubated at 37 C for 2 to 5 hr. The cover slips were transferred to microscope slides coated with a layer containing 20% gelatin (Baker and Adamson, New York, N.Y.) and 1% agar in nutrient broth. The distribution of nuclear bodies could be clearly observed by use of this modification of the technique of Mason and Powelson (1956). Determination of rates of DNA, RNA, and protein synthesis were done on cultures of irradiated cells growing in nutrient broth at 37 C (Volkin and Cohn, 1954; Lowry et al., 1951).

RESULTS

If unirradiated, growing cells of *E. coli* K-12 AB1899 are observed by the phase microscope technique described, it can be seen that each cell contains approximately two nuclei. These appear as bright bodies in the cells of Fig. 1. When stationary-phase cells are exposed to ionizing radiation and then allowed to grow on the surface of nutrient agar, long nonseptate multinucleate filaments are formed (Fig. 2). The filaments grow

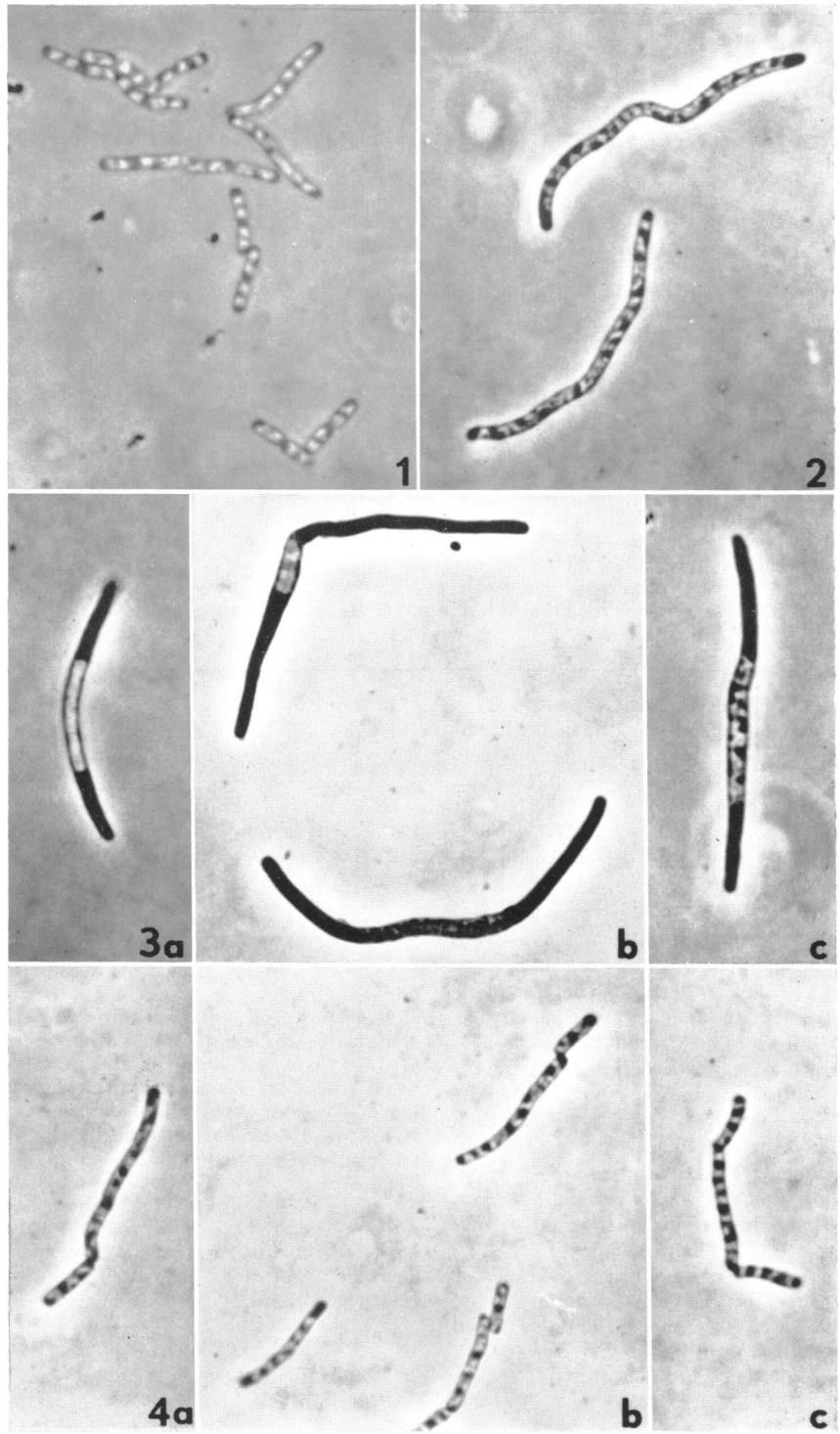

FIG. 1. *Unirradiated cells of Escherichia coli K-12 AB1899 dividing on nutrient agar. Ca. 2,000 ×. Nuclear material appears as bright bodies.*

FIG. 2. *Filament formed by the growth of an irradiated cell on nutrient agar for ca. 3 hr. Ca. 2,000 ×. Nuclear material appears as bright bodies.*

FIG. 3. *Examples of filaments formed by the growth of irradiated cells for ca. 3 hr on nutrient agar containing 10 μg of mitomycin C per ml. Ca. 2,000 ×. Nuclear material appears as bright bodies.*

FIG. 4. *Examples of division induced by exposure to pantoyl lactone. Ca 2,000 ×. Nuclear material appears as bright bodies.*

from both ends (Adler and Hardigree, 1964*b*). Nuclear material is distributed throughout the length of these filaments. It is usually present as discrete bodies, but sometimes several such bodies seem to be fused. In an occasional filament (less than 5%) nuclear material is confined to the central region. If, however, the growth of irradiated cells has taken place on nutrient agar containing 10 μg/ml of mitomycin C, the nuclear material is confined to the central region in more than 95% of the filaments (Fig. 3). At concentrations below 1 μg/ml, no effect of mitomycin C on distribution of nuclear material can be observed. At concentrations above 20 μg/ml, mitomycin C interferes with the growth of filaments.

Experiments in which phenethyl alcohol (Berrah and Konetzka, 1962) has been substituted for mitomycin C have not revealed any selective inhibition of DNA synthesis or nuclear replication. These experiments have been done with the following concentrations of phenethyl alcohol: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/ml. At the higher concentrations, inhibition of filament growth is observed, but nuclear division takes place and nuclei are well distributed.

If irradiated cells are grown on nutrient agar for 1 to 1.5 hr to initiate filament formation and then transferred to nutrient agar containing 0.12 M pantoyl lactone, cross septa begin to form in some filaments. This may take place at one or both ends (Fig. 4). The normal-length cells produced in this process contain at least one and usually two nuclear bodies. Similar results can be obtained by incubation of filaments at 42 C for 3 hr. Several hundred dividing filaments have been observed, and no examples of division resulting in the production of anucleate cells have been observed.

If filaments produced in the presence of mitomycin C are exposed to pantoyl lactone, no cross-septation is initiated. Many hundreds of filaments grown in the presence of mitomycin C and exposed to pantoyl lactone or a temperature of 42 C have been scanned to see if cross-septation does perhaps occur occasionally in a region of a filament lacking nuclei, but this has never been observed.

Two experiments have been done to follow the synthesis of DNA, RNA, and protein in filaments growing in nutrient broth containing 10 μg/ml of mitomycin C. Samples containing filaments were removed at intervals, washed once by centrifugation, and extracted with cold 5% trichloroacetic acid. Chemical determinations were performed on the acid-insoluble precipitate. In both experiments we observed an initial increase in diphenylamine-positive material per filament (ca. 40 to 50% in the first 1 hr of incubation) followed by an extended period (ca. 2.5 hr) during which no further increase occurs. RNA and protein synthesis rates are normal for the first 1 to 2 hr of incubation, but then fall below the rate observed for filaments not exposed to mitomycin C. Data from an experiment pertaining to DNA synthesis in growing filaments are given in Table 1. No evidence was obtained for the loss of diphenylamine-positive material from filaments growing in mitomycin C.

TABLE 1. *DNA synthesis in growing filaments**

Growth medium	Time (hr of growth at 37 C)				
	0	1	2	3	3.5
Nutrient broth.....	1	2.0	3.52	5.0	4.0
Nutrient broth + mitomycin C.....	1	1.52	1.5	1.54	1.50

* Amount of DNA per filament relative to amount at time zero.

DISCUSSION

The observations presented in this paper are a cytological manifestation of the action of a drug reacting specifically with DNA. Other antibiotics have been observed to affect nuclear architecture (Kellenberger and Ryter, 1955; Kaye and Chapman, 1963), but, from the chemical point of view, mitomycin C is probably the most specific of those that have been studied (Shiba et al., 1959). The concentration range producing the cytological effect in filaments is comparable to that which inhibits DNA synthesis (our experiments and Sekiguchi and Takagi, 1960).

Our observations on mitomycin C contrast sharply with those of Reich, Shatkin, and Tatum (1961). These authors presented phase microscope photographs of *E. coli* B filaments growing in the presence of mitomycin C, suggesting that, after an initial spreading of the nuclear material to all parts of the filament, it was lost from the cell completely. Their cytological observations were reinforced by biochemical experiments on *E. coli* 15T⁻ in which it was demonstrated that a substantial fraction of DNA was degraded and lost to the medium during growth in mitomycin C. Our observations suggest that there is only a limited spreading of nuclear material during the development of filaments (compare Fig. 1 with Fig. 3) and that the nuclear material is not lost from the cell prior to lysis. Determinations of the amount of acid-insoluble diphenylamine-positive material in filaments indicate that there is an increase (ca. 40 to 50%) during the first 1 hr of growth. The amount of this material per filament then remains constant for at least an additional 2.5 hr. These experiments on cultures grown in liquid media are not strictly comparable with our

cytological preparations on solid media, but do support the cytological observations.

There are several differences between the present experiments and those reported by Reich et al. (1961) that may account for the discrepancies. In addition to obvious factors such as choice of organisms, growth conditions, analytical techniques, and mitomycin C concentrations, it may be important to point out that, in our experiments, the mitomycin C treatment is imposed on X-irradiated cells. Reich and associates observed that conditions which permit growth are necessary for the demonstration of DNA breakdown and loss. It is conceivable that our irradiated cells, although still capable of growth, cannot perform certain metabolic activities that lead to the solubilization of DNA after mitomycin C treatment.

Perhaps the most useful of our observations relate to the initiation of cytokinesis by pantoyl lactone and the treatment at 42 C. If this phenomenon only involved an interaction between the initiating agent and the cell wall or membrane, we might expect it to occur in mitomycin C-treated filaments. The fact that no cytokinesis is observed in such filaments suggests that nuclear material plays an active role in the cross-septation process. Furthermore, it seems likely that nuclei must be located on both sides of the point at which cross-septation is to occur. We have never observed a case in which cross-septation, induced near the end of a filament, results in the formation of an anucleate cell. It may be that the division-promoting agents act directly on the nuclei, which in turn control the cross-septation process.

It is likely that the mechanism of cytokinesis in bacteria does involve an interaction between the nucleus and the cell membrane (Jacob, Brenner, and Cuzin, 1963; Ryter and Jacob, 1964; Ryter and Landman, 1964), and our observations support this concept.

The system we are using to study cell division is restricted by the resolution attainable with phase microscope technique, but it does have the advantages that no artifacts are introduced by fixatives or stains and observations are made on growing cells, in which nuclear replication and cytokinesis can be observed as they occur. We hope that it will be useful in further studies on cell division.

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