Time-Lapse Photomicrography of Cell Growth and Division in Escherichia coli

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ABSTRACT

Hoffman, Heiner (New York University, New York, N.Y.), and Michael E. Frank. Time-lapse photomicrography of cell growth and division in Escherichia coli. J. Bacteriol. 89:212-216. 1965.—Photomicrographs at 15-sec intervals of cells growing at 37 C disclosed that in a cell with a generation time of 21.0 min the processes of furrowing, cross-wall formation, and cell separation are completed within 2.5 min after the division furrow first becomes clearly visible. Among a large number of cultivations examined, only a few cells late in one microculture at 43.5 C failed to separate once the cross wall was completed. Measurements of cell lengths during a 5-min period, extending from just before to just after division, showed that elongation of the cell is a discontinuous process, although the growth rate over the 5-min period is exponential. At the time of cell division, it appears that the synthesis of cell-wall material is diverted entirely into formation of the cross wall.

The introduction of phase-contrast microscopy greatly improved the possibilities for the direct cytological investigation of single-cell growth of living bacteria in relation to cytokinesis. Nevertheless, very few studies of this problem have been carried out. Among the better known earlier attempts is that of Mason and Powelson (1956), who were primarily interested in nuclear division. They took photomicrographs at 2-min intervals, with bright phase-contrast optics, of microcultures on a gelatin medium with a refractive index designed to disclose greater internal detail than seemed possible with agar. Although well-defined "nuclear figures" were found in Escherichia coli cells, the cell wall could not be seen, and Mason and Powelson were unable to establish when the transverse septum completely separated the sister cells. More recently, Schaechter et al. (1962) employed a modified version of the technique in a study which timed the period required for division of bacterial nuclei (nuclear interdivision time). Photographs were taken at intervals ranging between 1 and 3 min. From measurements of cell lengths, these authors concluded that E. coli cells increased in length at all times during the division cycle without noticeable discontinuities.

In the present investigation, we have re-examined the problem of cell growth in relation to cytokinesis in E. coli by means of dark phase optics rather than bright. The study was carried out primarily on the basis of one microcolony which was photographed at 15-sec intervals and a second which was photographed at 3-min intervals. Analysis of the photomicrographs revealed some previously undescribed aspects of the time required for certain stages in the division process, but failed to confirm some of the observations of Schaechter et al. (1962) in regard to cell growth.

MATERIALS AND METHODS

The organism used was rough-phase E. coli ATCC 8077. The Fleming microculture technique, in which a dry film of smeared broth culture on a cover slip is overlaid with agar, was used for growing the microcolonies. A 37 C "rapid-sequence" cultivation was photographed at 15-sec intervals; the record of this cultivation has been used in an earlier paper (Hoffman and Frank, 1964b), which describes some details of the techniques used (see also Hoffman and Frank, 1961, 1963a). The second cultivation, photographed at 3-min intervals, was incubated at 43.5 C in a Zeiss heating stage rather than in the microscope incubator box used for the first culture. Measurements of cell length were made with a draftsman's caliper to the closest 0.5 mm from photographic prints at 7,000X for the rapid-sequence cultivation and at 3,780X for the 43.5 C cultivation.

RESULTS

Rapid-sequence cultivation at 37 C. The center cell in Fig. 1 is in the fourth generation, and had a generation time of 21.0 min. Twenty cells were present in the microcolony at this time, and the center cell in Fig. 1 was well in the middle of them. The first visual indication that the cell was under-
going division was the presence of a well-defined dark body in the center of the cell. The origin of this central body could not be traced with certainty in the earlier photomicrographs. The initial phase of division itself was marked by the appearance of a slight division furrow (Fig. 1), which gradually developed. Within 1.5 min (Fig. 2), it was so fully developed that the cell was noticeably constricted at the center. Within the next minute (Fig. 6), the dark central body apparently split into two parts separated by a narrow transverse band which was not as dark as the adjoining central bodies. The time at which this band appeared was the point which we arbitrarily took as the completion of division. Thus, division was completed for the described cell within 2.5 min after the slight indication of furrowing in Fig. 1. The proximal ends of the newly formed daughters then gradually rounded off and became more clearly defined (Fig. 7 and 8). The two granules resulting from the splitting of the central body in the mother remained as polar bodies in the daughters. The daughter cells then began to move apart, and were well separated (Fig. 10) within 2.75 min after the transverse wall was formed. However, a strand of what may be capsular material still extended between the proximal ends of the daughters.

Caliper measurements from time-lapse photomicrographs (Fig. 1 to 10) of the lengths of the cell and its 2 daughters at 15-sec intervals during the period from shortly before to shortly after division indicated that there are intervals during this period when growth in length does not occur. The longest period of such apparent growth suspension in the cell was found in an interval extending from 0.75 min before completion of division to 0.75 min after. Similar observations were obtained from measurements of an additional dozen cells in this microcolony. Nevertheless, plotting the length of the central cell in each 15-sec photographic frame over a 5-min period, including the time required for cell division, indicated that growth in the direction of the longitudinal axis of the cell was exponential for the 5-min period as a whole (Fig. 11).

Microculture at 43.5°C photographed at 3-min intervals. This microcolony was described as C61 in an earlier report (Hoffman and Frank, 1963a). With the exception of heat-induced filaments, the cells in this microcolony appeared quite normal in their growth and division behavior through the greater part of the cultivation. In the ninth generation, however, cell growth slowed

Fig. 1 to 10. Microculture at 37°C, dark phase-contrast microscopy, photographed at 15-sec intervals, 7,000X. Selected representative frames over a 5-min period to show division of the centrally located cell (fourth generation).
down considerably or stopped completely, although division continued. In most cases, the daughter cells in a sibling pair easily separated from each other after division (Fig. 12 to 21), the process apparently taking about as much time as in the early phases of cultivation. A few cells were found, however, which developed a cross septum (Fig. 14), but the resulting daughters did not separate from each other during the following 21-min period to the end of the photomicrographic record (Fig. 15 to 21).

**DISCUSSION**

The quality of the photomicrographs obtained is satisfying for the purposes of the present study, but the amount of detail of the intracytoplasmic structures visible in the photomicrographs was not as great as had been hoped for in view of the high resolution of Kodak high-contrast copy film and the very fine quality of the optical lenses used. One of the reasons for this result is the probable occurrence of some slight movement of

**Fig. 11.** Growth of the centrally located cell and its daughters over the 5-min period shown in Fig. 1 to 10. Cell lengths are plotted in millimeters as read directly from the enlarged prints of the photomicrographs.

**Fig. 12 to 21.** Microculture at 43.5°C, dark phase-contrast microscopy, photographed at 3-min intervals. 3,750X. Cells are in the ninth generation; encircled cell divided in Fig. 14, but the daughters are still closely attached 21 min later (Fig. 21) at the end of the photomicrographic record; cell enclosed by square exhibits normal separation following division.
the cellular organelles during the relatively long film exposure time of 5 to 6 sec. An additional and more significant reason involves the difference in refractive index between the cytoplasm and the enclosed structures. No attempt was made in the present study to use the technique of Mason and Powelson (1956) to increase contrast by adding gelatin to the mounting medium, since Mason and Powelson had found that the cell wall in *E. coli* was not revealed in this way, and since they also had been unable to establish the time of appearance of the transverse cell wall. Difficulties with the technique have been reported by others also (Adams, 1963; Brieger, 1963).

The cytological details of division visible in the light microscope have interested a number of recent investigators concerned with bacterial generation times. Powell (1958) distinguished three cell generation times, the intervals between successive divisions of nuclei, cytoplasm, and cell wall. He utilized cell-wall division as the end point in determining generation times. Questnel (1963) took the time at which light appeared between formative sister pairs as the extremity of the period required for completion of cell-wall division. Our own observations indicate that this point can be determined with greater certainty than any other aspect of bacterial cell division visible in the light microscope.

The photomicrographic record of cell division which was obtained from the rapid-sequence cultivation is apparently the first time-lapse study to be presented with short intervals subjected to frame-by-frame analysis. From an extensive search of the literature, we have found only a single report (Bisset, 1939) on the length of time required by a rod form to pass through the division process itself. Bisset (1939), observing *Bacillus myceoides* with an oil immersion lens, found that the time required for the completion of division after the appearance of an extremely fine line across the organism was 5 to 15 min. The generation times of these cells were not given.

The central body, which precedes the optical appearance of the transverse cell wall, may well be the intracytoplasmic membranous organelle which Imaeda and Ogura (1963) had associated with the formation of cell wall during division of mycobacteria. Up to the present, however, it must be acknowledged that attempts to demonstrate these organelles in *E. coli* through electron microscopy have not been entirely successful. Studies by Conti and Gettner (1962) and Edwards and Stevens (1963) failed to demonstrate them, although Kaye and Chapman (1963) found some rare examples in control cells and more prominent and numerous examples in cells treated with colistin sulfate.

Only a single microcolony was uncovered, the culture at 43.5°C, in which we found that the appearance of the transverse cell wall was not promptly followed by a separation of the daughter cells. Even in this case, only a few cells failed to separate, and this was late in the cultivation when the colony was obviously exhibiting very little growth. No cases were found, in this carefully examined microcolony of three- or four-member chains or filaments with cross walls, which would have indicated further divisions without separation. These points are emphasized since, on the basis of cell-wall staining, it has been claimed (Bisset, 1955) that rough-phase eubacteria are normally four-celled.

Particularly interesting were the findings on fluctuations of growth in cell length, especially in relation to cell division, since the observations and discussions by previous investigators of these problems did not encourage the experimental approach taken. Schaechter et al. (1962) had found from their measurements of individual *E. coli* cells, made from projections of photomicrographs taken at intervals ranging between 1 and 3 min during growth, that *E. coli* cells increased in length at all times during the division cycle, without noticeable discontinuities. The discrepancy with our results may perhaps be due to the longer interval between photomicrographs in the study by Schaechter et al. Furthermore, Mitchison (1961) rejected rod-shaped bacteria in his study of cell growth of single bacteria on the grounds that, since bacterial rods are usually multinucleate, there is opportunity for variation depending upon the degree to which the cellular units are out of phase with each other in their nuclear or cell cycles. Our data do not seem to have reflected this possibility. In addition to the consistent agreement of the described central cell with the measurements of the other dozen cells in the rapid-sequence recorded microcolony, we have also established (Hoffman and Frank, 1964a) that microcolonies cultivated by the techniques developed in our laboratory exhibit a high degree of synchrony of division throughout the course of the cultivation.

Fluctuations in cell length very similar to those noted in the present study may be found in Table 1 of an earlier report (Hoffman and Frank, 1963b), which indicates that cell elongation is suspended during the pinching off of an end granule. Here also, the cell quickly catches up to the length of its sister after the granule has been separated from the cell.

The observation that the cell length does not increase during the period that the cross wall apparently is being formed indicates that the
resources for synthesizing cell-wall material are diverted entirely into formation of the cross wall.

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Literature Cited


