TIME-LAPSE PHOTOMICROGRAPHY OF THE FORMATION OF A FREE SPHERICAL GRANULE IN AN *ESCHERICHIA COLI* CELL END

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ABSTRACT

HOFFMAN, HEINER (New York University, New York), AND MICHAEL E. FRANK. Time-lapse photomicrography of the formation of a free spherical granule in an *Escherichia coli* cell end. J. Bacteriol. **86**:1075–1078. 1963.—Only a single case of the formation of a free spherical granule at an *Escherichia coli* cell end was found among several thousand cells recorded by time-lapse photomicrography. The spherical end body apparently arose immediately upon or soon after binary fission, at the newly formed cell end. Several minutes elapsed between the appearance of the end body and its full separation, apparently by constriction, from the mother cell. The end body showed no cytological changes during a 51-min period of observation after its separation from the mother cell. A hypothesis concerning the nature of end bodies and the mechanisms underlying their production is presented.

A poorly studied and little-known form of irregular fission was described by Gardner (1930) among bacilli of the typhoid-coli and other groups, consisting of a constriction close to the extremity of the bacillus which resulted in the formation of a small spherical end segment. Since he never observed growth or change in form, Gardner believed that the coccal form represented either an abortive and lifeless cell or a cell fragment, rather than a phase of a life cycle. Califano, Falcone, and Pontieri (1956) were able to induce the liberation of apparently similar granules at the poles of *Escherichia coli* cells grown in a urethane medium. According to these investigators, the spherical bodies were expelled from the cell end; they then attached themselves laterally to the cell from which they originated, and later grew to coccobacilli and bacilli or divided into two coccoid units which then grew to bacilli. The end bodies did not seem to be connected with sexual phenomena or with the L cycle. In the same year, Pease (1956) observed the production of a small body from the end of *Spirillum serpens* cells, apparently by constriction. The fate of the small body was not determined, but Pease did point out that it resembled a gonidium.

The most elaborate study of end bodies which has come to our attention is that of Kvittingen (1949*a*, *b*) on "end piece" formation by *Proteus* Hauser. Kvittingen made serial photomicrographs of microcultures at irregular and long intervals, a procedure which precludes a detailed analysis of end-body formation. It appears, moreover, from an examination of his photomicrographs, that in some cases he may have confused end bodies with short cells fragmenting off the ends of filaments. Cell length measurements, not utilized by Kvittingen, are essential to avoid this difficulty. In Kvittingen's opinion, the "end pieces" contain half a polar body, which he interpreted as a haploid nucleus. Our recent time-lapse photomicrographic study (Hoffman and Frank, 1963) yielded a single instance of end-body segmentation among several thousand cells in a large series of recorded microcultures of *E. coli*, incubated at temperatures ranging between 37.5 and 43.5 C. Analysis of this unique record gave information bearing on previous conceptions of end bodies, and led us to formulate another possible mechanism in the genesis of these spherical structures.

MATERIALS AND METHODS

The clonal microcolony studied was labeled C61 in the previous report (Hoffman and Frank, 1963). This colony was grown in a Fleming covered smear preparation, and was photographed at 3-min intervals with Kodak high-contrast copy film (35 mm). The temperature of incubation was 43.5 C. Measurements of cell lengths were made with a sharply pointed draftsman's caliper from photographic prints at a magnification of 6000×.

and were read to the nearest 0.5 mm. The measurements were not reduced to microns. The genealogical scheme for naming the cells was described in an earlier publication (Hoffman and Frank, 1961).

RESULTS AND DISCUSSION

Several points are apparent from the photographic record of the early phases of the appearance of the coccal end body (Fig. 1 to 5), in spite of the loss of photographic frame 72. This frame is the

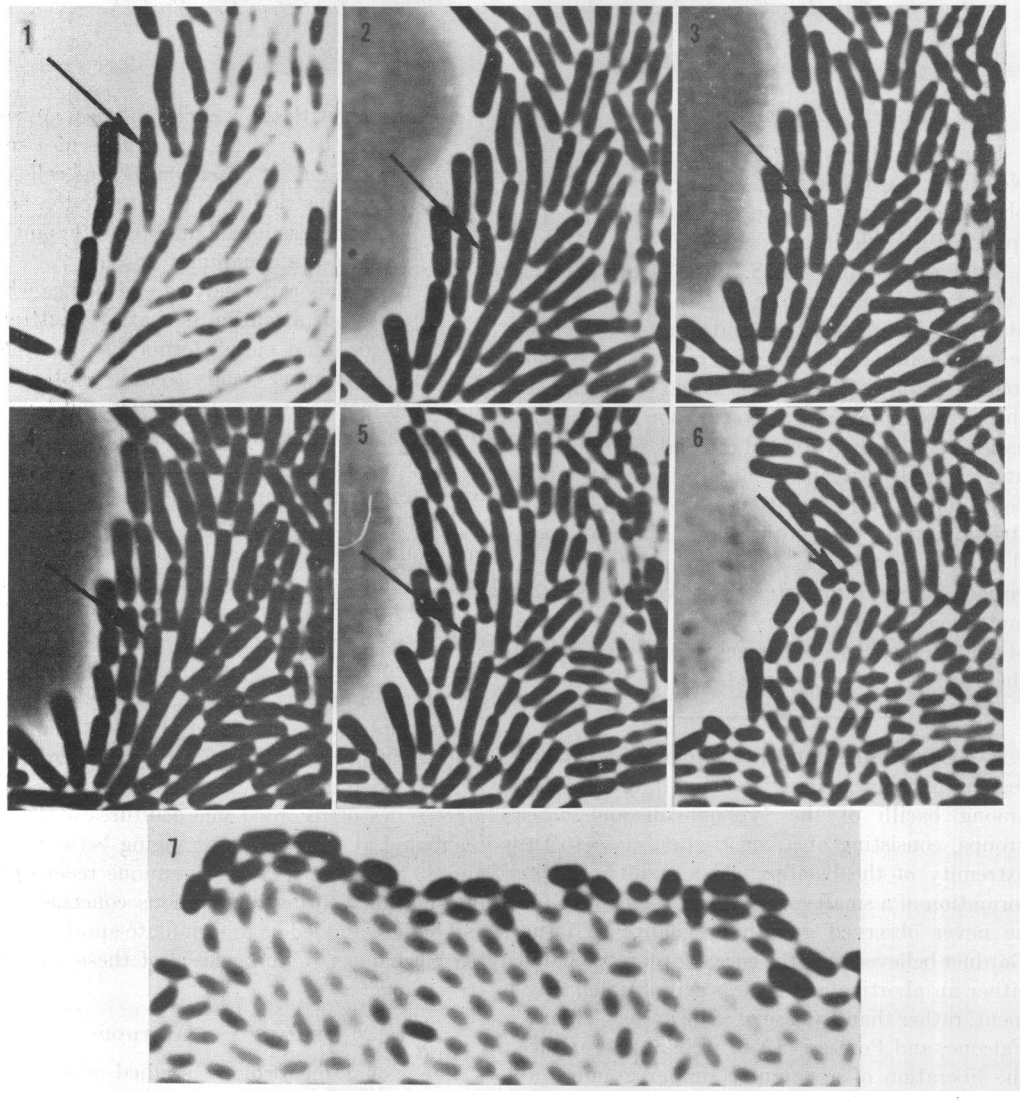

FIG. 1 to 7. *Microcultures of Escherichia coli. Phase microscopy, ×5000; arrow indicates cell named in the legend. (1) Frame 71, microcolony C61, cell* 7_{42} *just prior to division. (2) Frame 73, microcolony C61, cell* 8_{83}, *granule attached to cell end. (3) Frame 74, microcolony C61, cell* 8_{83}, *granule clearly outlined. (4) Frame 75, microcolony C61, cell* 8_{83}, *granule attached only tenuously. (5) Frame 77, microcolony C61, cell* 8_{83}, *granule fully separated from mother. (6) Frame 94, final photograph of microcolony C61, granule unchanged in diameter. (7) Overnight culture, microcolony B60, cell lengths at least double that of the granule diameter.*

TABLE 1. *Cell length measurements of cell 8_{83} (granule mother) and its sister cell 8_{84} during the period of granule detachment*

Frame no.	Cell no.	Cell length (mm)	Comment
73	8_{83}	12.5	Granule visible in mother; diameter 2.0 mm
	8_{84}	11.0	
74	8_{83}	12.5	Granule more clearly outlined; diameter 2.0 mm
	8_{84}	11.5	
75	8_{83}	11.0 + 2.0 (granule)	Granule attached to mother tenuously
	8_{84}	12.0	
76	8_{83}	12.0 + 2.0 (granule)	Granule attached to mother tenuously
	8_{84}	12.0	
77	8_{83}	12.0 + 2.0 (granule)	Granule fully separated from mother
	8_{84}	12.0	
78	8_{83}	12.5 + 2.0 (granule)	
	8_{84}	13.0	

midpoint of a 6-min interval in which cell no. 7_{42} divided to give rise to cell 8_{83}, the mother of the coccal end body. From Fig. 1, it may be seen that there were no constrictions in the middle of cell 7_{42} in frame 71 to indicate the formation of a spherical structure. The round granule first appeared in frame 73 (Fig. 2) at the cell end proximal to its sister cell (8_{84}), the cell end freshly formed by the division of cell 7_{42}. The coccus was clearly outlined in frames 73 and 74 (Fig. 3), but still attached to its cell of origin. In frame 75 (Fig. 4), only a tenuous strand of material maintained a connection between end body and mother cell. Thus, a period of at least 3 min elapsed before detachment of the spherical granule was well-established. Cell length measurements were made to determine whether detachment was due to extrusion or active movement of the coccal granule away from the mother cell, or to a constriction of the cell wall. It was found that the length occupied by the mother cell and the granule in frame 74 was the same as in frame 73 (Table 1). The data, therefore, indicate not only that the granule was not being extruded during this period, but that the growth of the mother cell itself had stopped during this same period. The accompanying sister cell (8_{84}), on the other hand, was gradually increasing in length (Table 1). Thus, the mother cell apparently cast off the spherical granule by constriction rather than expulsion or extrusion.

The lack of a photographic record at the exact time cell 7_{42} divided to give cells 8_{83} and 8_{84} leaves a serious gap in the evidence on the process by which the coccal form arises. It is possible, although unlikely, that the missing photographic frame may have provided this information, but to assure recording this process it would have been necessary to have made a time-lapse sequence at a much greater speed than that actually used. It is possible to obtain an approximation of the lengths of cells 8_{83} and 8_{84} in the missing frame 72 from their growth rates, and from the cell lengths at origin of their ancestors and their sisters (Table 2). The calculated cell lengths for cells 8_{83}

TABLE 2. *Cell length measurements at birth of the direct ancestors, with their sisters, and the descendants of cell 8_{83}*

Frame no.	Cell no.		Cell length (mm) at origin	
	Direct ancestor or descendant	Sister	Direct ancestor or descendant	Sister
9	1_1	1_2	9.0	7.5
21	2_2	2_1	10.5	10.5
31	3_3	3_4	10.5	10.5
39	4_6	4_5	10.0	10.5
47	5_{11}	5_{12}	10.5	10.5
56	6_{21}	6_{22}	11.0	11.0
63	7_{42}	7_{41}	10.5	10.5
72	8_{83}	8_{84}	10.5*	10.5†
79	9_{165}		6.5	
	9_{166}		6.5	
94	10_{329}		4.0	
	10_{330}		4.0	
	10_{331}		4.0	
	10_{332}		4.0	

* Calculated as the difference between the value for cell 8_{84} extrapolated to frame 72 and the value for cell 7_{42} also extrapolated to frame 72.

† Extrapolated from projection of growth rate of cell 8_{84} to frame 72.

and 8_{84} in the missing frame are 10.5 mm for each. This is practically identical to the cell lengths of the direct ancestors and their sisters for the previous six generations. The difference in lengths, therefore, between cells 8_{83} and 8_{84} in frames 73 and 74 (Table 1) most probably may be accounted for by evagination of the round granule from the newly formed cell end immediately after or shortly after division.

The large reduction in cell lengths at origin of cell 8_{83} descendants, after their direct ancestors had maintained a stable length for seven generations, suggested that the castoff granule may have had an influence upon this parameter. Cell length measurements of genealogical peer cells in various regions of the microcolony, however, failed to bear this out.

The diameter of the granule remained unchanged at 2.0 mm from its first appearance as a free body in frame 77 (Fig. 5) through to the end of cultivation, 51 min later (Fig. 6). On the other hand, the smallest size attained by normal cells was approximately 4.0 mm, and was first observed in the tenth generation (Table 2). Overnight incubation failed to result in any smaller cells (Fig. 7).

In our view, the spherical end body is a polar granule which has been pinched off after evagination from a newly formed cell end immediately after division. This implies a structural weakness in the transverse septum, and indicates a cellular lesion intermediate to that involved in filamentation, when transverse septa apparently are not formed (Hoffman and Frank, 1963). However, the phenomenon requires considerably more investigation before it will be understood clearly. An organism which produces the end bodies more readily than the strain of *E. coli* used by us will be required. The photographs of Kvittingen (1949*b*) indicate that *Proteus* may be a favorable subject, but it is also possible that under proper conditions larger numbers of end bodies may be induced by urethane or filament-evoking agents in low dilution.

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