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Synchrony of Division in Clonal Microcolonies of *Escherichia coli*

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ABSTRACT

HOFFMAN, HEINER (New York University, New York, N.Y.), AND MICHAEL E. FRANK. Synchrony of division in clonal microcolonies of *Escherichia coli*. J. Bacteriol. **89**:513–517. 1965.—A common pattern of synchrony of division was found in 18 clonal microcolonies studied by means of time-lapse photomicrography. One of these is described in detail. Cell counts at 1-min intervals were carried into the 11th generation of a microcolony photographed for 5.25 hr. The counts indicated that an early synchrony of division occurred which apparently was gradually dissipated during the course of cultivation. However, when times of cell division for each generation were plotted, it became apparent that each generation conformed to a distribution curve which gradually changed in its characteristics from one generation to the next. The range of time during which the divisions occurred increased from 16 min in the 6th generation to at least 74 min in the 11th. Overlap between distribution curves increased from 3 min between the 7th and 8th generations, where it initially occurred, to at least 20 min between the 10th and 11th. Skewness of the distribution curve progressed from a negative value (−0.943) in the 5th generation to a positive value (+0.339) in the 10th. Genealogical identification of the first and last quarters of the cells to divide within a generation revealed, early in the course of cultivation, a cell subline which was dividing in a pattern of synchrony independent of the colony as a whole. The characteristics of this synchronous pattern, however, gradually progressed with each new generation toward those of the microcolony as a whole.

Our studies with time-lapse photomicrography of developing microcolonies indicate that there are some interesting aspects of synchrony of division which can be detected only in clonal microcolonies. Since only brief attention was paid to this phenomenon previously (Hoffman and Frank, 1963; Hoffman and Frank, 1964*a*), we carried out in the present study a more thorough analysis based primarily upon one microcolony, although a large number of cultivations were examined.

MATERIALS AND METHODS

The techniques for cultivation, microscopy, photography, and film analysis have been described (Hoffman and Frank, 1964*b*). The genealogical records utilized for this study were based on time-lapse photomicrography of rough-phase clonal microcolonies of *Escherichia coli* ATCC 8677. We examined 18 microcolonies incubated at temperatures ranging from 37 to 43.5 C; 10 were relatively short cultivations extending over four or five generations, and 8 were relatively long, extending over six or more generations.

RESULTS

Although variations in details occurred from one clonal microcolony to another, a common pattern seemed to be dominant among them all, and, therefore, only microcolony 63-3 (Fig. 1 to 4) was subjected to close analysis. This microcolony was incubated at 37 C and photographed at 1-min intervals. It was especially suited for analysis, since it had been photographed over a relatively long period of 5.25 hr, at the end of which time members of the 14th generation were beginning to appear. Moreover, comparatively little piling of cells took place so that a large proportion of the cells in each generation could be identified (Table 1). All the cells within a generation could be accounted for through the 6th generation. By the 10th generation, approximately 25% of the cells were lost; with few exceptions, this was due to piling. In some rare instances, a cell failed to grow or failed to divide. All available cells through the 10th generation were traced, as well as a substantial number of cells in the 11th generation. In all, the genealogical identification and the time

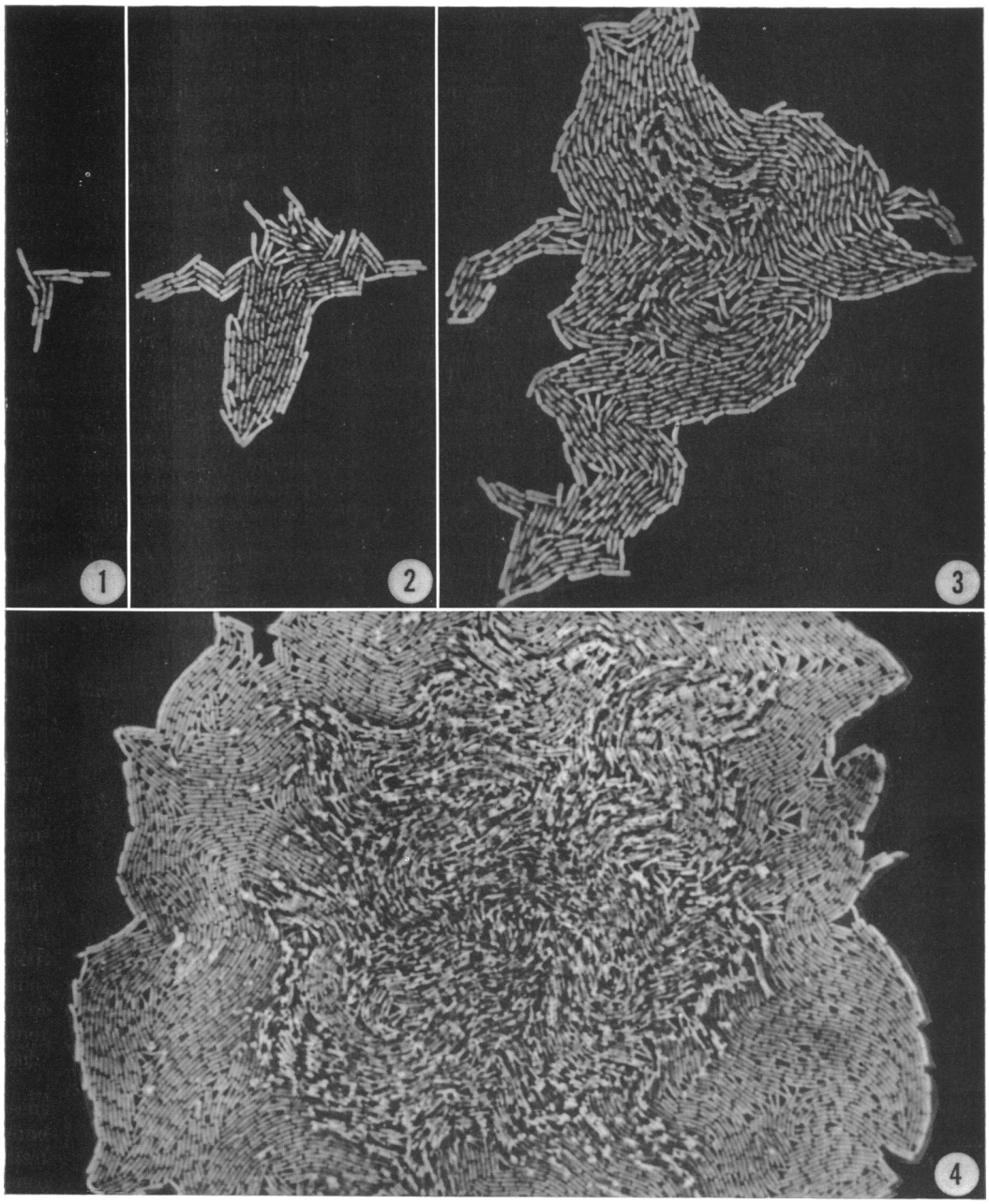

FIG. 1 to 4. *Microcolony 63-3, dark phase contrast microscopy, photographic image reversed, 850×.* (1) *Microcolony after 80-min incubation period, 3rd and 4th generation cells present.* (2) *Microcolony after 155-min incubation period, at the beginning of the 7th generation.* (3) *Microcolony in the 9th generation, when 15% of the cells could not be found.* (4) *Microcolony after 5.25-hr incubation period; some 14th generation cells are present. Note piling in center of colony.*

TABLE 1. *Times of division of genealogically identified cells (microcolony 63-3)*

Generation	No. of cells identified	Range of times of division	Range overlap	Range with base zero	Median of times of division	Mean ($\bar{X}$) of times of division	SD of mean	*P* of difference between consecutive means	Skewness* of entire colony	Skewness* of 2_3 cell subline	Scherbaum's (1959) synchronization index
		min	*min*	*min*	*min*	*min*					
1	2	33–39		6	3.00	3.00					
			0								
2	4	54–60		6	3.50	3.50					
			0								
3	8	72–87		15	7.50	7.75					
			0								
4	16	91–109		18	7.00	7.19					
			0								
5	32	111–125		14	9.25	8.09	±3.69		−0.943		0.43
			0					0.6			
6	64	132–148		16	9.14	8.55	±4.03		−0.439		0.37
			0					0.01			
7	123	151–174		23	12.50	11.94	±4.46		−0.377	+0.58	0.35
			3					0.01			
8	231	172–204		32	14.21	14.33	±6.32		+0.057	+0.59	0.29
			12					0.3			
9	451	193–226		33	14.38	14.89	±5.69		+0.269	+0.25	0.26
			15					0.01			
10	772	212–255		44	17.84	18.71	±7.69		+0.339	+0.29	0.16
			20					0.01			
11†	277	236–310		74	24.69	24.12	±10.15				

* Skewness = 3 ($\bar{X}$ − median)/SD of mean.
† The 11th generation was not completed.

at which each cell divided was determined for 1,980 cells in this microcolony.

Total cell counts of the colony were determined at each 1-min interval, and the values were plotted to obtain a distribution curve. The resulting picture (Fig. 5) suggests the conventional growth curve for a bacterial culture. The periodic leveling off, or steplike inflections, are periods during which no cells or very few cells divided. These inflections became progressively damped with each new generation, starting with the 7th. The reason for the damping became clear when the times at which the cell divisions occurred were plotted for the cells within each generation (Fig. 6).

The times at which cell divisions took place within a generation occurred in curved distributions with markedly high and narrow central portions and tails which drop off sharply (Fig. 6). Skewness of the distribution curves (Table 1) gradually shifted from a negative value (−0.943) in the 5th generation to a positive value (+0.339) in the 10th, with the smallest degree of skewness in the 8th generation. This pattern of variation in skewness was unique for microcolony 63-3, and no consistent pattern was apparent from an examination of the data from the other cultivations.

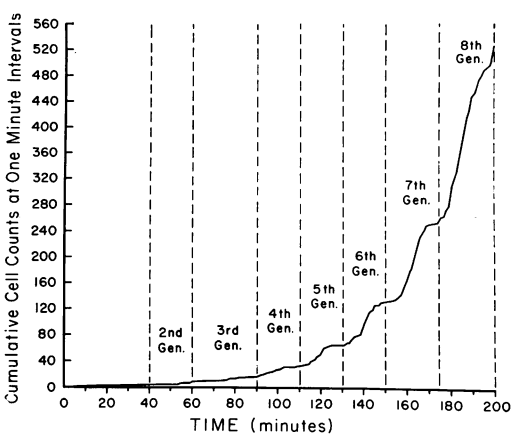

FIG. 5. *Total number of countable cells in microcolony 63-3 at 1-min intervals, with the time range for each generation marked approximately by dashed lines.*

No overlap between generations was found through the 7th generation. Overlap between generations 7 and 8 was 3 min, but it gradually in-

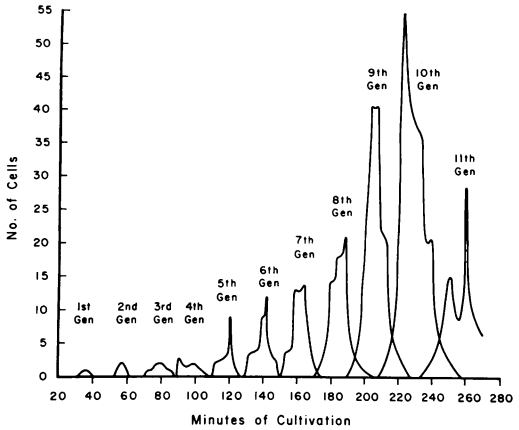

FIG. 6. *Times of cell divisions for cells identified by generation in microcolony 63-3.*

TABLE 2. *Percentage of cells in the first and last quarters of a times-of-division distribution within a generation whose mothers occupied that respective quarter in the previous generation (microcolony 63-3)*

Generation	First quarter	Last quarter
	%	%
5	75	50
6	81	68
7	79	67
8	57	51
9	74	65
10	64	63

creased with each new generation. Between the 10th and 11th generations, the overlap was 20 min (Table 1). Remarkably few cells, however, were involved in the overlapping ends of the distributions.

With Scherbaum's (1959) synchronization index of cell division, a progressive loss of synchronization was indicated, coursing from a high of 0.43 in the 5th generation to a low value of 0.16 in the 10th generation (Table 1). The drop in the index appears to be primarily a reflection of an increase in spread of the times at which the cells of a generation divided. This change in spread (Table 1) resulted in the gradual and generally significant increase, from generation to generation, of the mean time at which the cell divisions occurred. (To facilitate this comparison, the times of division of cells within each generation were referred back to a zero baseline, the time at which the first cell division of that generation occurred.)

The percentage was determined of the first and last quarters of the cells to divide in a generation, whose mothers occupied that same quarter in the previous generation. A large proportion of the cells in the first and last quarters of one generation were derived from mothers occupying the same position in the previous generation (Table 2). Only in the first quarter, however, was there a persistent cell line (subline 2_3) which could be found through the length of the cultivation. Nevertheless, there was a continuous decrease in the number of 2_3 subline cells from generation to generation which appeared in the first quarter. Thus, in the 5th generation, 88% of the 2_3 subline cells appeared in the first quarter, but in the 10th generation only 36% appeared in the first quarter

TABLE 3. *Percentage of the descendants of cell 2_3 that appear in the first quarter of the cells in each generation (microcolony 63-3)*

Generation	Theoretical no. of 2_3 descendants	2_3 Descendants found	2_3 Descendants found in first quarter
		%	%
5	8	100	88
6	16	100	81
7	32	100	69
8	64	94	50
9	128	90	43
10	256	82	36

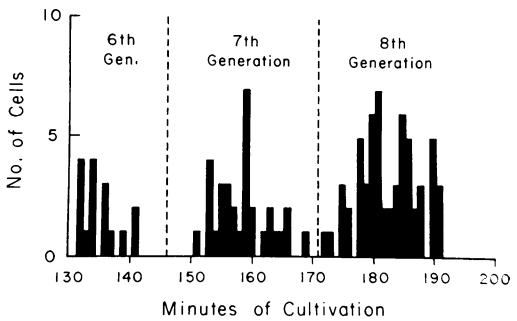

FIG. 7. *Times of cell divisions for several generations of the 2_3 cell subline in microcolony 63-3.*

TABLE 4. *Mean generation times of 2_3 cell line and of entire clone (microcolony 63-3)*

Generation	2_3 Cell line	Entire clone
	min	*min*
3	15.5	21.5
4	17.8	18.2
5	20.9	20.7
6	21.1	21.1
7	22.8	22.2

(Table 3). In the case of the quarter containing the late-dividing cells, there was no cell line comparable in prominence to that of 2_3 for the first quarter.

Three changes in the characteristics of the distribution curves appeared to be involved in the gradual loss of prominence of the 2_3 cell line in the first quarter of the cell division time distribution: (i) the progressive shift in skewness of the distribution curves for the entire colony from a negative toward a positive value with each generation (Fig. 3; Table 1), which had the effect of narrowing the time range covered by the first-quarter cells; (ii) the time range required by the 2_3 subline to complete its divisions increased with each generation (Fig. 7); and (iii) the progressive shift of skewness in the distribution curves for 2_3 descendants (Fig. 7; Table 1) in the direction opposite to that occurring for the colony as a whole.

A comparison of the generation times of the 2_3 subline with those for the rest of the microcolony (Table 4) shows that there are no appreciable differences, with the single but significant exception of the 3rd generation, where the daughters of 2_3 subline have a much shorter generation time than the mean of all the cells in that generation. The short generation time in the 3rd generation only accounts nicely for the early phasing and for the numerical prominence of the 2_3 subline in the early part of the times of division distribution.

DISCUSSION

The evidence indicates that cell sublines within a clonal microcolony may form independently phased and differently shaped distribution curves of synchronous division which, however, gradually shift toward the characteristics of the colony as a whole. Moreover, this independent phasing in the synchrony of division does not appear to be a result of spatial relationships within the colony, since cell sublines traverse the entire colony (Hoffman and Frank, 1961). It seems, therefore, that the distribution curve of times of cell division for a single generation may not be based upon a homogeneous population, and may show a greater deviation from a strictly gaussian form than we had originally believed (Hoffman and Frank, 1964*a*) from a preliminary study. This is a surprising conclusion, since a clonal microcolony is the product of a single cell and presumably has a common genetic constitution. Possibly, an isolated and transitory event involving the division mechanisms of the two daughters of cell 2_3 in the 3rd generation speeded up division enough to establish the distinctive phasing of division synchrony in this cell line. The subsequent drift toward the curve characteristics of the entire colony may have occurred as a result of equilibration within the colonial milieu of diffusible products of metabolism involved in cell division. It is planned to subject to close analysis the generation times of all the available cells in each generation of microcolony 63-3 with the hope of uncovering additional aspects of the generation times which may influence the patterns of division synchrony described here.

In spite of the findings on heterogeneity, the present observations indicate that the general outlines of the curves describing synchrony of division in a clonal microcolony are very similar under a variety of cultural conditions. This suggests that the early loss of synchrony of division in treated broth cultures may be more apparent than real. In view of the characteristics of the overlap in times of division in consecutive generations of a microcolony, the possibility exists that essentially uncontaminated populations of single generations may be obtained from broth cultivations over a longer period of time than had seemed possible on the basis of cell counts alone.

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