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LONG - LASTING SYNCHRONY OF THE DIVISION OF ENTERIC BACTERIA

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SUMMARY

Escherichia coli and Enterobacter cloacae were synchronized by periodically repeated doublings in a phosphate limited medium. The synchronized populations were incubated as batch cultures during 11 cell generations. The quality of synchrony, monitored by cell counts and size distribution, decayed slowly but a relative synchrony was still obvious after 10 cycles of free growth. No gross asymmetry was found in the interdivision time distribution of E.coli. Its coefficient of variation was found to be significantly lower than most figures reported in the literature. Since these experiments imply thousandfold dilutions of the initial stable cell constituents as well as of the original medium, the hypothesis of a diffusible molecule responsible for the maintenance of synchrony gained no support.

INTRODUCTION

Considerable variability of intervals between successive cell divisions of bacteria has been reported on the basis of observations on single cells and their progeny (1-9), as well as on a statistical evaluation of the time course of cell divisions in synchronized populations (10 - 13). Individual bacteria were shown to differ also with respect to the cell size at birth or at division (8, 14 - 19), to the time elapsing between two successive homologous events of the DNA cycle (8, 20, 21) or to the size or age at initiation of chromosome replication (22, 17, 19, 23, 13).

Many curves of synchronous growth reported in the literature show a strong tendency toward randomization at the second or third synchronous cell division and few reports if any describe four or more synchronous cycles.

As pointed out by Maaløe (24), the very possibility of long-lasting synchrony under conditions of free growth (e.g., 25, 26) appears incompatible

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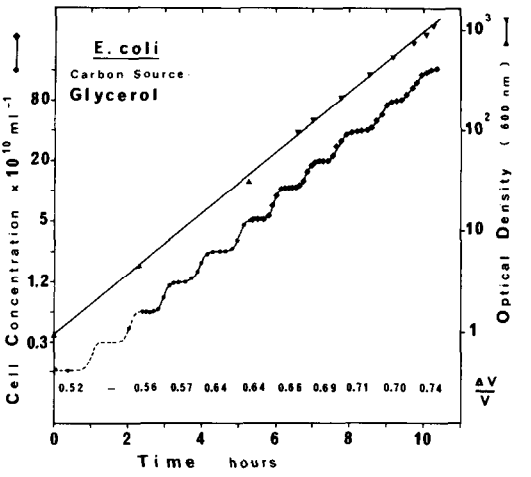

Figure 1 - Cell concentration, optical density and population heterogeneity in a non-limited synchronized culture of Escherichia coli.

After 16 phasing cycles on glycerol as sole carbon source, the culture of E.coli was diluted 22-fold in the same medium, supplemented with a non-limiting concentration of phosphate, 1 mM. Samples from this culture were counted (full dots). This culture was further diluted 39-fold after 15 minutes and sampled after the fifth hour (full squares). Cell counts and optical density were multiplied by the dilution factors. Mean values of population heterogeneity between divisions $\Delta V/V$ are indicated under the corresponding cell cycles. For cycle number 6, the given heterogeneity is a mean between those obtained from the two cultures. $\Delta V/V$ was found to be around 0.80 for a random population under identical conditions of culture.

with the reported variability of most of the cell cycle parameters. Since we recently reported synchrony lasting up to six cell generations in a population of Escherichia coli growing in batch culture after synchronization by automatic phasing (27), we extend these observations further in the present report in order to try to understand the apparent contradiction.

RESULTS

The measurements performed during the course of a synchronous growth of E.coli K 12, strain 3300, are represented on figure 1.

The bacteria were grown in the synchronization medium limited by inorganic phosphate (0.25 mM), with glycerol as the carbon source. After 5 hours of phosphate starvation, the culture was submitted to automatic phasing (27) by twofold dilutions every 90 minutes, during 24 hours (16 cycles). The

doubling time before phosphate starvation was around 65 minutes. After harvest the culture was diluted 22-fold in the same medium supplemented with 1 mM phosphate. This was time zero of the experiment. 15 minutes later an additional dilution brought the bacterial density to $1/850^{th}$ of the density at harvest. Batch cultures were maintained in conical flasks in a shaking water bath at 37°C. Cell concentrations were measured with a Coulter counter (Model ZB, Coultronics France) on samples withdrawn from the 22-fold dilution during cycles number 3 to 6, and from the 850-fold dilution during cycles number 6 to 11. Data of cell counts and of optical density were multiplied by the dilution ratio so as to display a continuous curve.

Another prolonged experiment, performed with a strain of Enterobacter cloacae, which was phased on glucose as the carbon source, is represented on figure 2. The number of observed cell cycles was about the same as in figure 1, but here the first part of the time course was sampled from a 30 - fold dilution of the harvest, and a second 30 - fold dilution, made after five generation times, served for the following cell cycles. Five generations later, a further 16 - fold dilution proved necessary in order to maintain the conditions of free growth. The cell cycle which followed the second dilution exhibited a premature cell division, which was later compensated for by a longer interdivision interval.

In both experiments the quality of synchrony was significantly deteriorated during the 2 or 4 last observed cell cycles but the randomization of the culture was far from complete. The remaining synchrony was made even more obvious by the observation of the distribution of cell sizes between divisions (figures 1 and 2). Cell volumes were estimated by using a size distribution analyzer (model P 64, Coulter Electronics Inc., USA). Population heterogeneity $\Delta V/V$ is expressed as the ratio of the width of the size distribution curve at half maximal frequency, ΔV, over the modal size, V (27). This parameter is progressively increasing but does not reach 0.8, its usual

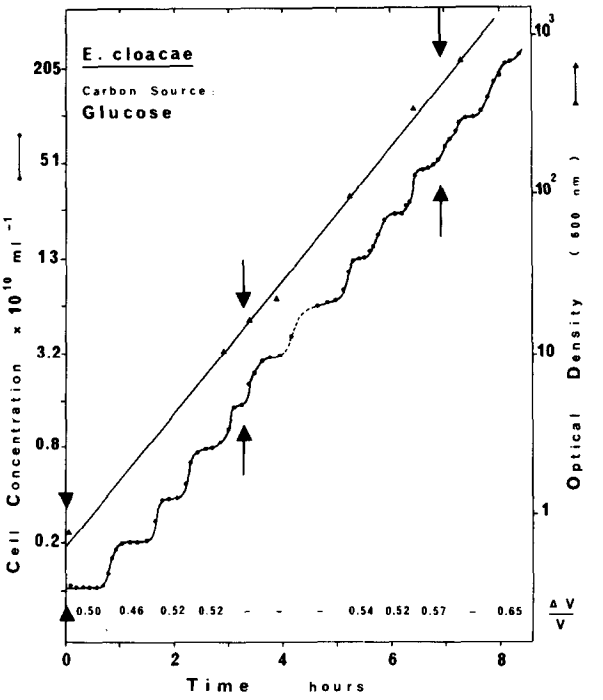

Figure 2 - Cell concentration, optical density and population heterogeneity in a non-limited synchronized culture of Enterobacter cloacae.

After 13 phasing cycles on glucose as the carbon source, the culture of E.cloacae was diluted 30-fold in the same medium, supplemented with 3 mM phosphate. 200 minutes later, it was diluted again by the same factor, and after another 215 minutes it was once more diluted 16-fold. Cell counts and optical density were multiplied by the total dilution ratios. Mean values of population heterogeneity between divisions $\Delta V/V$ are indicated under the corresponding cycle. $\Delta V/V$ was around 0.76 for a similar but random culture.

value in a log phase culture. The maximal resolution on $\Delta V/V$ is for instrumental reasons around 7%.

The dispersion of E.coli cell divisions in time was estimated by a graphical method and it was found to increase slowly from one cell cycle to the next. No significant skewness was found in the distribution of inter-division times, which was thereafter assumed to be Gaussian.

DISCUSSION

From the long-lasting synchrony in batch cultures it can be safely concluded that bacteria have a stable timing device able to control

cell divisions. This control however is not a rigid one, since cell parameters subject to fluctuations (13) can displace cell division away from synchrony. This can be visualized as due to a secondary timer of lesser stability toward environmental influences. The primary timing device tends to counteract randomization by means of a fluctuation in the opposite direction during the next cell cycle. This is the meaning of the coefficient of variation of the interdivision time, which is 0.125 ± 0.019 in the experiment of figure 1, and of the mother-daughter interdivision time correlation coefficient, which was estimated to be -0.37 ± 0.08. As a result, the overall trend toward randomization is about 2 % of the doubling time per cycle in this experiment.

Coefficients of variation of the interdivision time range in the literature from about 0.12 to 0.33 (13, 16) for E.coli. On the other hand, many studies support the existence of correlations between the interdivision times of related individuals in a given family tree (3, 4, 6, 8, 28, 29, 11, 12). There seems to be positive correlation between relatives in parallel lines, and negative correlation between relatives in a same line as observed here.

Environmental factors such as those utilized to induce synchrony can greatly influence the next or the next few cell division times, but it can be assumed that they have a more limited effect on the primary timing device, which subsequently tends to compensate for the deviations toward synchrony, and thereby causes the early randomization usually observed after synchronization procedures using one or a few inductive stimuli. The premature cell division which followed the second dilution in the experiment of figure 2 might be an example of the effect of environmental factors. The second cell cycle which followed was longer than average, so that the steady-state values of cell parameters were reestablished and the rate of exponential growth was undisturbed. In summary, the event which disturbed the regular rhythm of cell divisions had little or no effect on the primary timer.

If in a random population, not only the cell division, but also the setting of the primary timer is random, it can be assumed that during a synchronization process the synchronous setting of the primary timers of individual cells is lagging behind the observed synchronization of cell division. During the repeated phasing cycles utilized in our method, we can assume that the setting of the primary timer is finally thoroughly adjusted and that this is the reason of the long-lasting synchrony observed.

An alternative explanation, namely that a cyclic variation of some diffusible message might greatly contribute to establish and/or to maintain synchrony might have been suggested by the previous results encompassing 6 synchronous cell cycles (27). Since the source of the hypothetical diffusible substance is in the cells, its efficient range of cyclic variations should be linked to a limited range of cell concentrations, which was maintained in the previous experiments by successive dilutions at short intervals. The observation here reported that thirtyfold and nearly thousandfold dilutions did not significantly disturb cell synchrony leaves very little chance to substantiate this hypothesis.

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