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Cell Cycle Regulation in Escherichia coli. I

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Biofisica. — *Cell Cycle Regulation in* Escherichia coli. I (*). Nota di MARIO AGENO, CARLA BACCI e OLGA MARIA QUARANTA, presentata (**) dal Corrisp. M. AGENO.

RIASSUNTO. — Bloccando per un certo tempo la sintesi del DNA e/o quella delle proteine in una coltura di *E. coli* in crescita esponenziale, si ottengono dopo il blocco curve di crescita il cui andamento dipende in modo drammatico dalla durata del blocco.

Si osserva come una interpretazione particolareggiata di tali curve e un accurato confronto tra esse, tenendo conto delle durate dei relativi blocchi, costituiscano un semplice valido metodo per ottenere informazioni sulla organizzazione interna della cellula e sulle relazioni temporali tra i vari processi che portano alla divisione cellulare.

Si presentano poi a titolo di esempio le curve di crescita seguenti blocco (mediante acido nalidixico) della sintesi del DNA per 22, 27 e 45 minuti, e se ne trae un semplice modello per spiegare la fenomenologia, che accompagna il blocco della sintesi del DNA mentre la sintesi proteica continua. Tale modello fa prevedere che in alcuni casi si debbano avere dopo il blocco divisioni cellulari in cui una delle due cellule figlie è non vitale. Tale previsione risulta confermata dall'esperimento.

As is well-known, the duplication of a prokaryotic cell can be described in terms of a model proposed in 1968 by Helmstetter *et al.* [1]. According to this model the process, which ends in the physical separation of two newborn cells, can be divided into three successive stages, three relevant time intervals being defined.

1) In the first stage the cell prepares the biosynthetic and structural components needed for the *initiation* of chromosome replication. The relevant interval of time last I minutes.

2) In the second stage *chromosome replication* initiates and goes to completion. The time required is C minutes.

3) The third stage lasts from the completion of chromosome replication to the subsequent *cell division*. Its duration is D minutes.

Subsequent cycles of cell duplication are interconnected, a new cycle beginning as soon as a round of chromosome replication starts. Obviously, the interdivision time τ (or: the cell number duplication time in an exponential growing culture) equals I. Realistic values for *Escherichia coli* are:

$$I = \tau = 35 \text{ min.} \quad , \quad C = 40 \text{ min} \quad , \quad D = 20 \text{ min.},$$

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All the cellular processes can be placed and described in the frame of this (I + C + D)-model. However, it is not clear whether a system of intracellular signals exists, coordinating all the events necessary for the cell to divide.

Available results on the internal timing of the cell are scanty and sometimes in disagreement, so that several different models for the control of cell division were proposed in the past and no really decisive test exists to date [2].

As far as the spatial organization is concerned, the prokaryotic cell does not possess any system of inner membranes, so that it is significant for cellular components which in a new-born cell are present in only one or very few copies. This is the case of the chromosome, which in the interdivision time must not only bring a round of duplication to completion but must do it in such a way that each of the cells eventually possesses one of the two copies. At the centre in the equatorial plane of the cell it must give way to the septum-cross-wall formation.

A simple and interesting model for chromosome segregation was proposed by Jacob, Brenner and Cuzin in 1963 [3]. Their suggestion was that each parental DNA strand is anchored to the cell envelope and, while the synthesis of the new complementary strands goes on, the two copies of the chromosome are passively separated into the daughter cells by the growth of the envelope between the attachment sites. There is no final confirmation of this model. However, a lot of indirect evidence favours it [4] and we can conclude that it is very likely correct.

Furthermore, we now know much more on the chromosome of *E. coli*.

Its duplication is bidirectional [5] and (if $\tau \leq C$) the DNA synthesis is continuing for the entire interdivision time, so that we can maintain that, very likely, the *origin* of the chromosome is permanently attached to the cell envelope [4]. The *E. coli* chromosome is a very compact structure forming about fifty highly supercoiled loops [6]. Taking into account the cell size, one sees that the greatest distance between the two attachment sites on the cell membrane is of the order of 1 μm. In order that the segregation be effective, one must therefore assume that the chromosome unwinds at the replication forks only (perhaps a loop at a time) and resumes its compactness just at the back of the forks, so that the entire structure is always kept in some tension by the two moving sites on the membrane.

Helmstetter's and Jacob's models offer a starting point for the study of the overall space-time organization of the cell cycle in prokaryotes. However some difficulties remain as far as suitable experimental technique are concerned.

Usually one cannot follow a particular process in a single cell. The ideal method is considered to be that of making use of synchronized cultures, whose logarithmic growth-curves look like a flight of steps. Samples to be tested are taken from such a culture at intervals, assuming that in each of them all the cells are in the same physiological state.

In practice this method generally fails and the results obtained seem difficult to explain and even conflicting. In fact, a set of stairs in the growth-curve only shows that some cell divisions are synchronized. One cannot assume that all the other processes in the cells are also synchronized, and it may well be that all the cells of the some age in the culture *are not* in the same physiological state.

Furthermore, the method can be questioned also from a more general point of view, It assumes gratuitously that a well-defined normal physiological state of the cell exists (that in an exponentially growing culture), in which all the intracellular processes are coordinated in a well-defined way. However, one cannot say that the overall organization of the cell must be strictly determined. Haploid prokaryotic organisms do not possess a stock of latent mutations, securing population survival in variable environmental conditions. They face the challenge of the environment, reducing the length of the reproductive cycle as much as possible. On the other hand, growth conditions similar to those in our experimental cultures are hardly possible in nature. Usually, times of plenty will be short, a few duplication cycles at most, with long periods of famine in between. Therefore, in the case of bacteria such as *E. coli*, which do not form spores, a fairly rigid internal organization would be a serious drawback from the evolutionary point of view. We may surmise that, within the limits of bacterial physiology, the timing of cell processes is really a loose one, and that phase relations of cellular events in the cell cycle are not strictly determined, but depend also on the environmental conditions and past history of the bacterial population. In that case, the above-mentioned use of synchronized cultures in order to detect time relations in the reproductive cycle could not give consistent and significant results.

This does not mean that cultures with synchronized cell divisions are useless: it is only a question of using them suitably, in order to decipher the overall organization of the cell.

With this purpose in mind, we have planned experiments in which, in an exponentially growing culture, one of the fundamental biosynthetic processes is selectively and reversibly blocked for a given interval of time, usually DNA- or protein-synthesis.

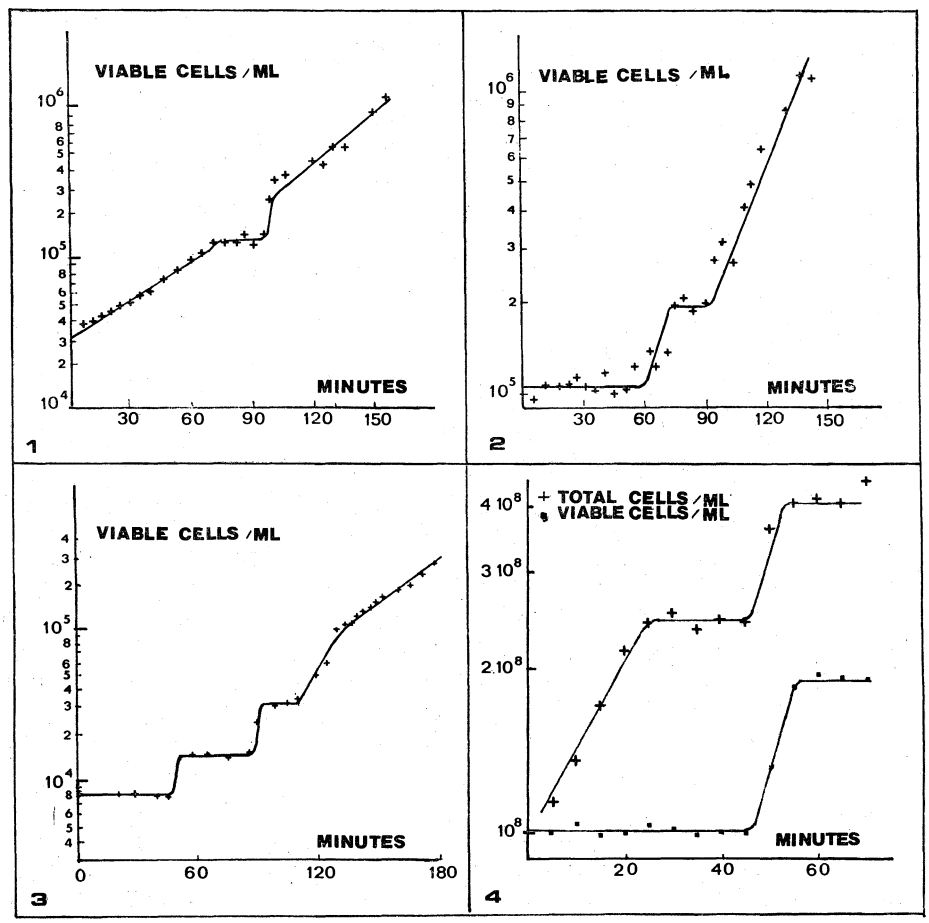
What matters mostly is not that a number of synchronized cell divisions usually follow when the block is suppressed, but that the general trend of the growth-curve after the block *depends dramatically on the length of the block.*

As an example, the growth-curves obtained by treating an exponential culture ($\tau = 45$ min.) of *Escherichia coli B* with nalidixic acid (7,5 mg/ml) for 22, 27 and 45 minutes respectively, are shown in Figures 1–3.

A detailed interpretation of such growth-curves and an accurate comparison of these can provide a lot of information on the coordination of the various cellular events and on the physiological variation limits of their phase relations in the course of the reproductive cell cycle. As a starting point for such an interpretation one can use the previously reported models of Helmstetter

et al. and of Jacob *et al.*. However, the growth-curves themselves must offer a conclusive test of the models, or the elements to correct them.

Without entering into interpretative deails, it is evident that the growth-curves following a 22 min. block of DNA synthesis have a normal trend in the interval $0 \leq t < 2/3\ \tau$, the effect of the block appearing only afterwards,



Figures 1-4.

as an alternation of plateaus and steps. The block causes phase displacement between chromosome replication and cell division. However, the old replicating forks, arrested by the drug, start again as soon as the block is suppressed and the interrupted round of replication goes to completion normally.

On the contrary, let us examine from this point of view the two growth-curves following longer blocks of DNA synthesis. Here, the first cell divisions after the suppression of the block are synchronous divisions relative to a round of chromosome replication starting in every cell as soon as the block is suppressed. There is no trace of the old replicating forks: only five minutes

more of the action of the drug are sufficient to eliminate them. Obviously, they cannot be all destroyed by the drug in five minutes, after 22 minutes of ineffective traetment.

A possible explanation of this effect is suggested by the model of Jacob, Brenner and Cuzin. We have seen that the segregation mechanism in this model can be effective only if the entire chromosomal structure is always kept under some tension by the motion of the two attachment sites on the cell membrane. Whereas DNA synthesis is blocked by nalidixic acid, protein synthesis is not, and cell elongation goes on. However, the chromosomal structure is now rigid and the distance between its two origins cannot increase. We must assume that the tension of the structure increases and it is conceivable that, after a while (if the block lasts more than 22 minutes) it causes the detachment of the chromosome at one of the two attachment sites on the membrane: perhaps that of the parental strand more recently synthetized, which seems to be the weakest point in the structure [7].

We can assume that also in this case the old replicating forks start again as soon as the block is suppressed, the interrupted round of chromosome replication goes to completion and the cell eventually divides. However, one of the two daughter cells now has its chromosome detached from the membrane, and can no longer reproduce itself.

The initial plateaus of the two growth-curves following a 27 and 45 minute block of DNA synthesis, may not be real. Perhaps in their course the cells divide and the cell number does increase, but if one titrates the culture by counting on agar plates as usual, a constant number is found.

It may be that this mechanistic explanation it too simple, and the correct one is different and more complex. However, we have tested it by determining the total cell number with a microscope, using a standard Petroff-Hauser chamber.

Total and viable cell numbers in the first hour of the growth-curve following a 45 minute block of DNA synthesis are compared in Fig. 4. As is evident, all the cells divide three times. In each of the first two divisions one of the two daughter cells is non-viable, while the third one is the synchronous division, relating to the extraordinary round of chromosome replication beginning at the end of the block. The total cell number at the end of the first hour is just four times the initial number. As far as this forecast is concerned, our model is confirmed.

This instance show show one can determine the physiological limits of phase displacement between chromosome replication and cell envelope elongation from the length of the block during which non-viable cells first occur. Similarly the deviations from the exponential trend of the first growth-curve allows us to determine the limits of phase displacement between stage C and stage D of the cell cycle.

A detailed analysis of the previous growth-curves will be published shortly.

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