FEMS Microbiology Letters 66 (1990) 1–4
Published by Elsevier

FEMSLE 03808

Hypothesis

Comparison of forward and backwards methods of cell cycle analysis

Stephen Cooper *

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, U S A

Received 12 July 1989
Revision received 14 August 1989
Accepted 17 August 1989

Key words: Cell cycle, Synchrony; Membrane-elution; DNA synthesis, Variability, Backwards methods

1. INTRODUCTION

We tend to look at life as a progression from youth to old age. This is reflected in our study of the bacterial division cycle, where newborn cells have an age of 0.0 and progress through the division cycle, finally dividing at age 1.0. There has been much effort expended in determining the rate and pattern of biosynthesis during the division cycle, and synchronization of cells has been the most common method for cell cycle analysis [1]. Synchronization may be called a forward method, as it directly reflects the increase in cell age in the usual manner; a synchronized population is one in which all cells are the same age during the division cycle. The rates of synthesis of various molecules during the division cycle may be obtained by taking samples from cells of all ages from newborn to dividing cells. Although there has been a great deal of work on synchronization, I am unaware of any generally accepted result analyzing the division cycle that was discovered using synchronized cells. Rather, the one aspect of the division cycle that is generally accepted, the pattern of DNA replication during the division cycle, was discovered using a non-synchrony method, Helmstetter's backwards-membrane-elution method ('the baby machine') [2]. The membrane-elution method is not a synchrony method. The cells eluting from the membrane are all newborn cells, and therefore the membrane-elution method produces a synchronized population. The membrane-elution method yields the best results, however, when prelabeled cells are placed on the membrane and newborn cells are eluted from the attached cells and analyzed without further growth. The pattern of incorporation during the division cycle is derived from the radioactivity eluted with the newborn cells. The newborn cells come off the membrane in a particular order; the first newborn cells eluted come from the oldest cells of the labeled population and with time the newborn cells descend from cells that were younger and younger at the time of labeling. Since this older-to-younger order is not usual way the division cycle is considered, the membrane-elution method is referred to as a backwards method. Growth of the newborn cells is not required; thus there is no synchronized culture to be analyzed.

Correspondence to Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620, U S A

0378-1097/89/$03 50 © 1989 Federation of European Microbiological Societies

Is there something about the backwards approach that may be better than the forward, synchrony, approach? I believe there is a difference, and I would like to present an explanation of why backwards methods may be inherently better, at least for the analysis of events that are more closely correlated with cell division rather than with cell birth

2. THE HYPOTHESIS

Assume that there is no perturbation of the cells by the technique used to produce the synchronously dividing population. Also assume that compared to the variability in cell interdivision times, the C period (the time for DNA to replicate from the origin to the terminus of the chromosome) and the D period (the time between termination and cell division) are relatively invariant This is related to, but not equivalent to, the observation that the time for the C and D periods are relatively invariant over a wide range of growth rates. In this discussion we are looking at a population of cells with variable interdivision times, and with relatively constant C and D periods. In Fig. 1 the characteristics of such a population are presented, with the cells aligned at division or at birth. When the cells are aligned at division (Fig 1, panel 1), DNA synthesis is shown to initiate and terminate at a constant time before cell division. When the cells are aligned at birth (Fig. 1 panel 2), the time periods between birth and the initiation of DNA synthesis are variable.

Consider a population of newborn cells that grow synchronously for one generation. The synchronized culture is produced by a physical separation of the smallest cells from the population, or by the selection of newborn cells from cells bound to, and growing on, a membrane. The rate of DNA synthesis during the division cycle can be determined by pulse-labeling this synchronized culture with radioactive thymidine at various times during the division cycle. The expected pattern of incorporation, and its interpretation in terms of C and D values, is shown in Fig. 1 (panel 3). Measuring the rate of DNA synthesis during the division cycle of a synchronized population with a

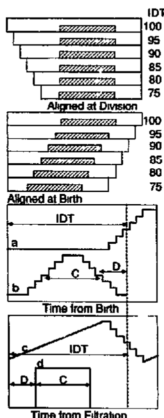

Fig 1 Graphic comparison of a synchrony experiment and a membrane-elution experiment The uppermost panel illustrates a culture with six representative cells with variable interdivision times (IDT) and invariant C and D periods (40 and 20 min respectively) In panel 1 the cells are aligned at division The cell with the shortest interdivision time also has the shortest time between birth and the initiation of DNA synthesis (The period of DNA synthesis is indicated by the shaded areas) Panel 2 shows the same cells aligned at birth in an ideal synchrony experiment The rate of DNA synthesis is determined during the growth of this synchronized culture and the results are seen in panel 3 There is a spread in the time at which cells divide, this gives the cell number curve (a). The observed pattern of thymidine incorporation, a measure of DNA synthesis, is shown in curve (b) This curve is obtained by looking at panel 2 and noting the number of cells in the original population that are synthesizing DNA at any particular time The C and D periods can now be obtained by measuring the times from the midpoints of the rises and falls in the DNA labeling curves to the midpoint of the rise in cell number In the fourth panel we see the result of a membrane-elution experiment The cell number curve (c) is not perfectly sharp due to the variability in cell interdivision times In this experiment the cells are pulse-labeled before being put on the membrane and the amount of label per cell eluted from the membrane is determined and plotted (d) Because there is no variability in the C and D periods, there is no slope in the curve of radioactivity per cell in the eluate from the membrane-elution experiment

variable time between birth and the start of DNA synthesis can yield C and D values. Unfortunately, one must estimate the mid-points of three sloping lines–the rise in the rate of DNA synthesis, the decrease in the rate of DNA synthesis, and the rise in the cell number—in order to get the C and D values.

In contrast to synchronized growth, the analysis of the division cycle using the prelabeling-membrane-elution technique gives a different result. The curves are sharper than in the synchrony experiment; this is because of the invariant C and D periods in the idealized example The rise and fall in the rate of DNA synthesis, measured by pulse-labeling the culture prior to placing the cells on the membrane, does not require estimates of the midpoints of the curves In this ideal example, one may measure the C and D periods in a backwards experiment (Fig. 1, panel 4) more easily than in a synchrony experiment (Fig. 1, panel 3). There would be a smoothing of the curves in subsequent generations of analysis, in either the synchronization or the membrane-elution experiment. Yet whatever increase in variation appeared in later generations, the membrane-elution experiment would always retain a sharper incorporation curve. In reality the membrane-elution curves would not be perfectly sharp as there is a finite labeling period as well as some variation in the C and D periods. To summarize the hypothesis events that are relatively constant in time prior to division will give sharper results when studied by a backwards method such as the membrane-elution technique

It should be pointed out that if the period of time from cell birth to the initiation of DNA synthesis were relatively constant, then forward synchronization methods, rather than backwards methods, would be preferred. The implicit point of the analysis made above is that that is not how the world is constructed. It is the time for DNA synthesis and the time between termination and cell division that are relatively constant, the remaining portions of the cell cycle are variable.

The DNA pattern shown in Fig. 1 is a relatively rare situation. A period devoid of DNA synthesis appears only in slow growing cells of some strains [2]. As the growth rate increases, the gap period decreases and finally disappears. With faster growth, initiation occurs prior to cell division. The same relationship of variability between cell birth and the next initiation is also found at these faster growth rates. The time between a particular cell division and the prior terminations or initiations of DNA synthesis is relatively constant. Thus, even at faster growth rates, this hypothesis holds

3. EXTRINSIC CONSIDERATIONS

It has been suggested that the membrane-elution method is prone to the same problems as synchronization. Some believe that the cells are perturbed by filtration and binding to a membrane, and some have argued that the order of cells completing division may be affected by the membrane-elution method. No matter how filtration may change the physiology of the cells, all that is required is that the bound cells divide in order and that the radioactivity, once incorporated, is not released by the cells. That the pattern of DNA synthesis has been determined by the membrane-elution method supports the hypothesis that backwards methods are superior. The cell elution curves [2] demonstrate that the order of elution of cells from the membrane is not perturbed

There is another advantage to the membrane-elution method In a synchronization experiment one must start and stop labeling a number of times during a synchronized cycle of growth. With the membrane-elution method, label is added only once and stopped simultaneously in all cells This single labeling period may also account for some of the succes of the backwards membrane-elution method

4 ADDITIONAL SUPPORT FOR THE HYPOTHESIS

Many laboratories find it possible to get a measurement of the C and D periods using the backwards membrane-elution experiment while no clear determinations of C and D periods could be obtained with the synchrony approach. This sup-

ports the hypothesis that the backwards membrane-elution approach is preferable to the forward synchrony method. One published example of this is the work of Helmstetter and Pierucci [3]. Another example is the apparent inability of synchronization method [4] to reveal slight deviations from exponentiality in cell wall synthesis that were obtained using the membrane-elution approach [5] In addition, the classic shift-up experiment of Kjeldgaard et al. [6] indicated a sharp break in the rate of cell increase following a shift. This sharp increase, subsequently confirmed using electronic cell counting and a number of different shifts [7], implies that C and D periods are relatively invariant in a population. The historical fact that a backwards method, the frequency-of-labeled-mitoses method, was successful in determining the phases of the eukaryotic cell cycle, again supports the hypothesis that backwards methods may be superior to forward synchrony methods.

ACKNOWLEDGEMENT

This work was supported by a Grant Number DMB 8417403 A01 from the National Science Foundation.

REFERENCES

[1] Evans, J E and Lennon, D E (1984) Synchronization of Microorganisms, In CRC Handbook of Microbiology Vol VI pp 163–208
[2] Helmstetter, C E (1967) J Mol Biol 24, 417–427
[3] Helmstetter, C.E. and Pierucci, O (1979) J Mol Biol 102, 477–486
[4] Wientjes, F B and Nanninga, N (1989) J Bacteriol 171, 3412–3419
[5] Cooper, S (1988) J Bacteriol 170, 422–430
[6] Kjeldgaard, N O Maaløe, O and Schaechter, M (1958) J Gen Microbiol 19, 607–616
[7] Cooper, S (1969) J Mol Biol 43, 1–11