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NOTES

Leucine Uptake and Protein Synthesis Are Exponential during the Division Cycle of *Escherichia coli* B/r

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The rate of leucine uptake, which is a measure of protein synthesis, was measured during the division cycle of *Escherichia coli* B/r by the membrane elution technique. The rate of leucine uptake was exponential, indicating that protein synthesis is exponential, and not linear, during the division cycle. These results, coupled with the results of other work on the exponential rate of RNA synthesis during the division cycle, indicate that the accumulation of mass in *E. coli* and other gram-negative organisms is exponential during the division cycle.

It has recently been proposed that the accumulation of mass during the bacterial division cycle is linear (15). Earlier work had demonstrated that the uptake of leucine is constant during the division cycle (12). These two results are consistent with a general model proposing that cell growth is linear for a wide range of cells (13).

I now present a simple experiment demonstrating that the rate of uptake of leucine is not linear, as had been proposed (12), and that the rate of protein synthesis during the division cycle is not linear (15) but exponential. These results indicate that accumulation of mass is exponential during the division cycle.

The membrane elution method has been described previously (1, 4–6, 10, 11). Briefly, bacteria are grown in minimal medium, and a radioactive label is added to the exponentially growing, unperturbed cells. After a short period of radiolabel incorporation, the cells are filtered through a nitrocellulose membrane, the unincorporated radioactivity is removed from the cells by washing them with warm medium, and the nitrocellulose membrane is inverted and fresh medium is pumped through it. A fraction of the cells bind to the membrane, and after an initial period of release of unbound cells the only cells released from the membrane are newborn cells released by division. A fraction of each sample was taken for a cell count with a Coulter Counter (Coulter Electronics, Inc.). A larger fraction (4 to 5 ml) was taken for the determination of the amount of radioactive leucine in the cells. The radioactivity in the first cells released from the membrane by division reflects the incorporation of radioactivity into the oldest cells in the labeled culture, and over time the newborn cells are descendants of progressively younger cells in the labeled culture. Therefore, the rate of uptake of a labeled substance during the division cycle can be determined. Since the measured pools of label after washing of cells are low (as determined by comparing total incorporation with that remaining in the cell after trichloroacetic acid precipitation of the cells [4]), the rate of leucine incorporation is a measure of the rate of protein synthesis. The major point of interpretation that should be noted is that if the datum points representing incorporation of radioactivity per cell as a function of time yield a straight line when plotted on semilogarithmic graph paper (solid line, Fig. 1), then uptake is exponential (10). Discontinuities would be observed if synthesis were linear or bilinear during the division cycle (dashed line, Fig. 1). The plateaus indicated by the dashed line in Fig. 1 indicate that the rate of incorporation of the molecule of interest is constant during the division cycle. This is what a linear increase in mass (or a constant rate of uptake) during the division cycle means. The dashed line is predicted by the model of Kubitschek (12–15).

Uptake of leucine and synthesis of protein during the division cycle. The results of an experiment on the uptake of leucine into *Escherichia coli* B/r during the division cycle are presented in Fig. 2. As the datum points representing radioactivity incorporated per cell fall on a straight line, one can conclude that the uptake is exponential. No indication of a constant rate of uptake during the division cycle (dashed line, Fig. 2) was observed. Other experiments (data not shown) performed over the past two decades have consistently shown that the uptake of many different compounds is not linear (1, 4–8, 10, 11), and in particular the uptake of compounds reflecting protein and RNA synthesis during the division cycle is exponential. No variations or discontinuities indicating linear or bilinear synthesis have been observed. The uptake of leucine and the synthesis of protein are also exponential during the division cycle of *Salmonella typhimurium* (4). Since the protein and RNA of the cell compose a large fraction of the cell mass (>75%) and since uracil uptake and RNA synthesis are also exponential during the division cycle (7, 8), I suggest that accumulation of mass during the division cycle is also indistinguishable from an exponential pattern.

Kubitschek (15) has recently proposed that the accumulation of mass during the division cycle of *E. coli* is linear. This proposal was made on the basis of size measurements of cells that were synchronized by using sucrose gradients to select the smallest cells from an exponential culture. Cell sizes were determined with an electronic cell size analyzer. I have recalculated the data of Kubitschek (15), and as shown in Fig. 3 the results of Kubitschek cannot be used to distinguish between linear and exponential growth. Kubitschek (15) plotted the measured sizes of cells of different ages on a rectangular graph (Fig. 3a), drawing the best straight line through the datum points. He then drew a line for

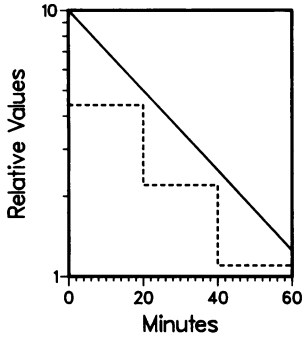

FIG. 1. Comparison of results (radioactivity per cell) expected from the membrane elution method for exponential and linear accumulation of mass during the division cycle. ——, Expected result for exponential accumulation of mass during the division cycle; -----, expected result for linear increase in mass during the division cycle.

exponential growth which deviated visibly from these datum points. His statistical analysis of this type of graph indicated that the data were consistent with the proposal of linear growth and excluded exponential growth. The exponential line tested was not the best fit to the data but was determined by only two datum points. A reanalysis of the published data of Kubitschek on a semilogarithmic plot is shown in Fig. 3b. This replotting was performed as follows. (i) The location of each of the datum points on the published graph was measured by using a ruler. (ii) Each datum point was converted to the proper numerical value by adjusting each measured value to the size of the original graph. (iii) The original datum points were replotted on a graph with linear coordinates to yield the original graph of Kubitschek (graph a in reference 15). (iv) The datum points were transformed to an exponential function by the method of Kubitschek (15), $y = \ln(\text{mass})/\ln 2 + 0.8979$, and these transformed datum points were plotted on a rectangular graph. (The use of 0.8979 rather than of 1.0 allowed the line to pass through the zero intercept.) (v) A linear regression analysis was performed to test whether each of the two lines were straight lines, and the statistically determined best fit lines were drawn with the data of each graph by using the University of Michigan statistical program MIDAS. The relevant statistical parameter, R^2, was determined for each regression line and was found to be 0.99251 for the linear plot (Fig. 3a) and 0.98559 for the transformed plot (Fig. 3b). A value of 1.0 indicates a perfect correlation with a straight line. The values obtained are so close to 1.0 that it can be concluded that there is no statistical difference in the data that would allow one to say that the data fit a linear model rather than an exponential model. It may be noted that there is an apparent curvature of the line when it is plotted by using the logarithmic transformation (e.g., for cell ages up to about 0.2 the first 7 points all lie below the line, for cell ages in the range between 0.35 and 0.8 34 out of 46 points are above the line, and of the final 18 points 16 are below the line). This curvature however, does not allow a statistical elimination of the exponential model because of the insensitivity of size analysis and its inability to distinguish the difference of 6% between the models. Thus, the size measurements of Kubitschek (15) are compatible with an exponential rate of synthesis during the division cycle. Any deviations as noted in Fig. 3b are extremely slight in terms of the differences in cell size measured with a Coulter Counter.

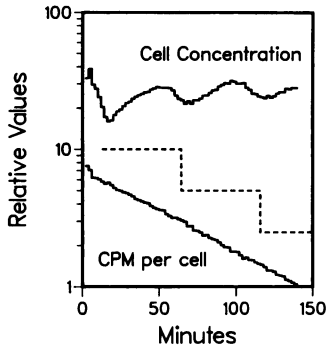

FIG. 2. Cell cycle analysis of leucine uptake (and protein synthesis) during the division cycle. A100-ml amount of *E. coli* B/r *lys* mutant cells in culture medium (10^8 cells per ml growing in minimal medium with glycerol and lysine) was labeled for 2 min with 2 μCi of [^{14}C]leucine (450 mCi/mmol; New England Nuclear Corp.). The cells were then filtered, washed, and analyzed as described in the text. The dashed line is the expected pattern for a constant rate of leucine uptake and protein synthesis during the division cycle. This constant rate is predicted by a model of linear rate of increase in mass during the division cycle. The upper cell elution curve has oscillations that are due to the initial cell age distribution of the cells at the time they were filtered. The decrease in the dashed line is placed at the end of the first division cycle as indicated by the cell elution curve.

The experiment described here demonstrates that protein synthesis, and presumably a correlate of protein synthesis, accumulation of mass, is exponential during the division cycle. Dennis (7, 8) has shown that stable RNA is also synthesized exponentially during the division cycle. Since these two components compose a large fraction of the cell mass, I suggest that mass is accumulated exponentially during the division cycle. Ecker and Kokaisl (9) have presented evidence, by using autoradiographic analysis of individual cells, that the rate of protein synthesis is exponential. In addition, the evidence presented in the present study does not support the fundamental data on which the linear model for increase in mass was derived, that is, the constant uptake of molecules during the division cycle of bacteria (12). The uptake of molecules is exponential for precursors of protein (data of the present study), stepwise for precursors of DNA (1, 5, 6, 10, 11), exponential for precursors of RNA (7, 8), and complex but almost exponential for precursors of peptidoglycan (4).

The experiment presented here uses a technique, membrane elution, that has been used to determine the rates of both thymidine uptake and DNA synthesis during the divi-

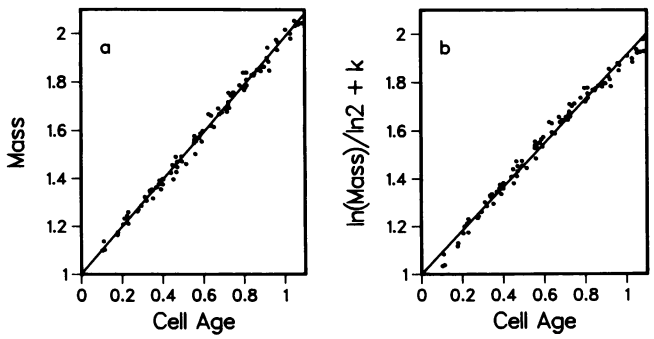

FIG. 3. Reanalysis of the data of Kubitschek (15). See the text for details.

sion cycle; these experimental results (1, 5, 10, 11) have been confirmed by a completely independent method (16). To my knowledge, no other cell cycle analysis method has received such support. In addition, the membrane elution method is both a differential method and a method that measures the rate of uptake of a compound. The membrane elution technique is not a synchrony method. Although newborn cells that are eluted from the membrane can be considered to be, and are, a synchronized culture, the method as applied here does not use these synchronized cells. Any perturbation that may occur on the membrane occurs after the period of labeling and therefore does not affect the experimental measurements. In the experiment presented above, the uptake of leucine is measured as a function of the division cycle, and it is shown that the uptake is exponential.

The model of Kubitschek implies the presence of a global system that regulates accumulation of mass and results in linear increase in mass. If macromolecular synthesis is essentially exponential (14), this global control system adjusts the uptake of molecules during the division cycle so that the total increase in mass is linear. The passive continuum model (2, 3), in contrast, does not propose any global control system. Each of the components of the cell—protein, DNA, peptidoglycan, lipid, RNA—is synthesized on the basis of signals transmitted by the state of the cell, and there is no mechanism that adjusts accumulation of mass to yield a linear increase in mass during the division cycle. The increase in mass of the cell is the simple sum of the masses of its individual components, and although it is essentially exponential, in detail it is a complex function of the sum of the rates of synthesis of the different macromolecular and micromolecular components of the cell.

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LITERATURE CITED

1. **Cooper, S.** 1969. Cell division and DNA replication following a shift to a richer medium. J. Mol. Biol. **43**:1–11.
2. **Cooper, S.** 1979. A unifying model for the G1 period of prokaryotes and eukaryotes. Nature (London) **280**:17–19.
3. **Cooper, S.** 1982. The continuum model: application to G1-arrest and G(0), p. 315–336. *In* C. Nicolini (ed.), Cell growth. Plenum Publishing Corp., New York.
4. **Cooper, S.** 1987. Rate and topography of cell wall synthesis during the division cycle of *Salmonella typhimurium*. J. Bacteriol. **170**:422–430.
5. **Cooper, S., and C. E. Helmstetter.** 1968. Chromosome replication and the division cycle of *Escherichia coli*. J. Mol. Biol. **31**: 519–540.
6. **Cooper, S., and T. Ruettinger.** 1973. Replication of deoxyribonucleic acid during the division cycle of *Salmonella typhimurium*. J. Bacteriol. **111**:966–973.
7. **Dennis, P. P.** 1971. Regulation of stable RNA synthesis in *Escherichia coli*. Nature (London) New Biol. **232**:43–47.
8. **Dennis, P. P.** 1972. Stable ribonucleic acid synthesis during the cell division cycle in slowly growing *Escherichia coli* B/r. J. Biol. Chem. **247**:204–208.
9. **Ecker, R. E., and G. Kokaisl.** 1969. Synthesis of protein, ribonucleic acid, and ribosomes by individual bacterial cells in balanced growth. J. Bacteriol. **98**:1219–1226.
10. **Helmstetter, C. E.** 1967. Rate of DNA synthesis during the division cycle of *E. coli* B/r. J. Mol. Biol. **24**:417–427.
11. **Helmstetter, C. E., and S. Cooper.** 1968. DNA synthesis during the division cycle of rapidly growing *E. coli* B/r. J. Mol. Biol. **31**:507–518.
12. **Kubitschek, H. E.** 1968. Linear cell growth in *Escherichia coli*. Biophys. J. **8**:792–804.
13. **Kubitschek, H. E.** 1970. Evidence for the generality of linear cell growth. J. Theor. Biol. **28**:15–29.
14. **Kubitschek, H. E.** 1981. Bilinear cell growth of *Escherichia coli*. J. Bacteriol. **148**:730–733.
15. **Kubitschek, H. E.** 1986. Increase in cell mass during the division cycle of *Escherichia coli* B/rA. J. Bacteriol. **168**:613–618.
16. **Skarstad, K., H. B. Steen, and E. Boye.** 1985. *Escherichia coli* DNA distributions measured by flow cytometry and compared with theoretical computer simulations. J. Bacteriol. **163**: 661–668.