CONSTANCY OF THE RATIO OF DNA TO CELL VOLUME IN STEADY-STATE CULTURES OF *ESCHERICHIA COLI* B/r

H. E. KUBITSCHEK

From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

Bacterial dry mass and content of DNA, RNA, and protein increase with growth rate in steady-state bacterial cultures (1, 2). While dry mass and RNA content increase more rapidly than DNA or protein, the ratio of DNA to protein is often virtually independent of the rate of growth (3), although some exceptions have been noted (4). I report here on the relationship between DNA content and another parameter, cell volume, which may also be critically related to control of cell growth.

Earlier data for DNA per cell (5) in steady-state cultures of *Escherichia coli* B/r growing at 37°C were averaged over growth rate intervals of 0.2 divisions/h for convenience in graphical presentation, Fig. 1 A (52 cultures). Mean cell volumes were determined for these and additional cultures of *E. coli* B/r with a modified Coulter electronic cell size analyzer calibrated with polystyrene latex microspheres of known diameters, Fig. 1 B (105 cultures). These techniques, the resolution of the instrument, and the similarity in the shape of bacterial size distributions at all growth rates were described earlier (6). Simple exponentials were fitted to each data set by (unweighted) least mean squares, giving the empirical relationships

$$\overline{\mathrm{DNA}} = 4.17 \times 10^{-15} \exp (0.56\, q),$$

and

$$\bar{V} = 3.81 \times 10^{-12} \exp (0.51\, q),$$

where q is the cell division rate (per hour), $\overline{\mathrm{DNA}}$ is the average amount of DNA in grams per cell, and $\bar{V}$ is the average cell volume in cubic centimeters. The coefficients of q in the two equations are not significantly different (standard errors of 0.017 and 0.019, respectively). The average value of the coefficient of q is 0.54.

To show the constancy of DNA per unit cell volume more directly, values of DNA per cell were divided by corresponding values of $\bar{V}$. As shown in Fig. 1 C, individual values for these ratios do not differ significantly from the mean value

of 1.20 mg/cm^3 (standard deviation, 0.11; standard error of the mean, 0.036). That is, within experimental errors, values of DNA per unit cell volume are independent of growth rate, and consequently, of mean cell size.

The constancy of DNA to cell volume in Fig. 1 C reveals the incompatibility of some earlier proposals for the timing and control of initiation of DNA synthesis. It has been suggested that initiation occurs when the ratio of DNA to cell mass or the cell mass per chromosomal origin reaches a critical value, (7–10) and that DNA synthesis begins soon after division in slowly growing cultures (11). Examination of DNA synthesis and cell growth in slowly growing cultures, at rates less than 0.8 divisions/h, shows that the two suggestions cannot hold simultaneously. At these slow growth rates the amount of DNA per cell at initiation is constant because replication is initiated on single unbranched chromosomes (5, 11). However, volumes of newly divided cells increase with division rate in essentially the same manner as does mean cell volume of the culture, as is evident from the fact that distributions of cell volume remain nearly invariant at different growth rates (6). Thus, if initiation of DNA synthesis occurs very early in the cell cycle, the DNA to cell volume ratio at initiation cannot remain constant, but must decrease as division rates increase from 0 to 0.8 divisions/h.

In order to determine the effect of growth rate upon the ratio of DNA to cell mass at initiation of DNA synthesis, mean cell densities were determined by equilibrium centrifugation of cells in linear Ficoll (Pharmacia Fine Chemicals, Inc., Picataway, N. J.) gradients (15–30% or 20–40%). Band positions were measured after centrifuging for 30 min at 10,000 rpm in a swinging bucket rotor (Sorvall centrifuge, RC2-B; rotor, HB-4; Ivan Sorvall, Inc., Newtown, Conn.). A chlorobenzine droplet was added to each gradient for a secondary density standard. In control experiments, the position of the band was independent of time of centrifugation after about 15 min, and did not change when sodium azide or dinitrophenol was added to poison the cells. The experimental density values in grams per cubic centimeters and their standard errors are the following: in broth (3.0 divisions/h), 1.114 $\pm$ 0.005; in glucose minimal medium (1.2 divisions/h), 1.108 $\pm$ 0.002; in acetate minimal medium (0.3 divisions/h, 1.104 $\pm$ 0.003; and in chemostat cultures (0.2—0.3 divisions/h), 1.103 $\pm$ 0.003.

These results support the conclusion that mean cell densities in these steady-state cultures do not vary by more than about 1% at different growth rates in these cultures, and therefore, that the DNA to cell mass ratio is proportional to the DNA to cell volume ratio. Thus, if initiation occurs shortly after division the DNA to cell mass ratio also cannot remain constant in slowly growing cells.

Alternatively, if DNA synthesis is initiated in these slowly growing cells at a constant interval before division (5) then the present data do not rule out the possible constancy of DNA to mass at initiation. Although mean cell mass at initiation increases with growth rate, initiation might occur earlier in the cell cycle in more rapidly growing cultures, and the two effects could be compensatory.

The results in Fig. 1 do, however, rule out Donachie's simple graphical proof (Fig. 1 in ref. 12) for the constancy of DNA to mass at initiation. This proof implicitly assumes that mean cell mass at the beginning of the cell cycle is proportional to 2^q, where q is the cell division rate, as before. But because distributions of cell volume are independent of growth rate, mean cell volumes both at the beginning of the cycle and throughout the entire cycle increase with cell division rate in the same manner. For *E. coli* B/r, therefore, the data in Fig. 1 are consistent with an increase in initial cell mass that is proportional to $e^{0.54q} = 2^{0.78q}$, rather than 2^q. Thus, when considered over a growth rate range of 2 cell divisions/h, initial cell mass does not increase by the assumed factor of 4, but in fact by a factor of slightly less than 3 for *E. coli* B/r. Consequently, the values given (12) for relative cell mass at initiation of DNA synthesis no longer fall on horizontal lines, and these values are no longer the same or multiples of the same cell mass. Thus, there is no compelling

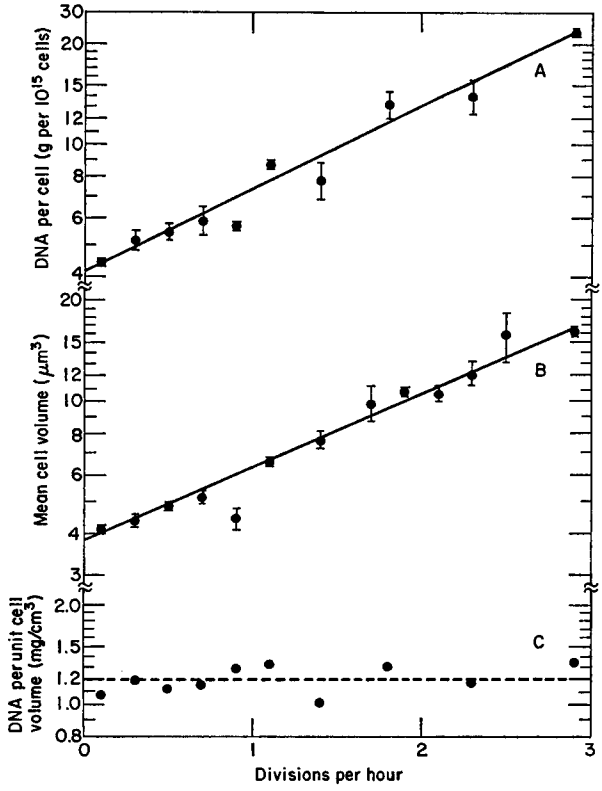

FIGURE 1 Average DNA content and mean cell volume in steady-state cultures of *E. coli* B/r. The dashed horizontal line is the average of all observed values. Each data point is the average of two or more observations. The vertical bars above and below the points represent observed standard errors of the mean. (A) DNA per cell, in units of 10^{-15} g. (B) Mean cell volume, in units of 10^{-12} cm^3 (cubic micrometers). (C) DNA per unit cell volume, in units of mg/cm^3.

theoretical reason to suppose that the ratio of DNA to mass need remain constant at the instant of initiation of DNA replication.

Nevertheless, the essential constancy of DNA to cell volume over a 4- to 5-fold range of DNA and cell volumes and an approximately 30-fold range in growth rate indicates the presence of control mechanisms to maintain this constancy. These may be weak control mechanisms, in the sense that they do not operate continuously throughout individual cell cycles, as is evident from the fact that slowly growing bacteria have gaps in DNA synthesis (5, 12–15) but not in cell volume increase (16). An attempt to explain the similar constancy of the DNA to protein ratio led Leick (3) to suggest that messenger RNA is the growth-limiting factor and that the synthesis of messenger RNA is in turn limited by the amount of template DNA or that it is regulated in some way by ribosome density.

All such purely chemical explanations overlook two important physical factors that may ultimately limit cell growth. The first is the surface area of the cell which may ultimately limit transport of raw materials into the cell. By itself, however, surface area would seem to be incapable of accounting for the rather sharp limit to division rate commonly observed for bacteria. These are known to be able to double every 20 min under the most favorable conditions, but few or no cases of more rapid sustained bacterial growth in steady-state cultures are known. I suggest that the major factor limiting rapidity of growth rate is the increasing accumulation of ribosomes. Cytoplasms of cells in rapidly growing bacteria are uniformly and tightly packed with ribosomes (17) which should interfere not only with the transport of nutrients into the cell, but also with the free diffusion of products of synthesis within the cell.

This work was supported by the U. S. Atomic Energy Commission.

Received for publication 15 June 1973 and in revised form 26 September 1973.

REFERENCES

1. SCHAECHTER, J., O. MAALØE, and N. O. KJELDGAARD. 1958. *J. Gen. Microbiol.* **19**:952.
2. HERBERT, D. 1959. *In* Recent Progress in Microbiology. G. Tunevall, editor. Charles C. Thomas, Publisher, Springfield, Ill. 381.
3. LEICK, V. 1968. *Nature (Lond.)*. **217**:1153.
4. KUBITSCHEK, H. E. 1970. Introduction to Research with Continuous Cultures. Prentice-Hall, Inc., Englewood Cliffs, N. J.
5. KUBITSCHEK, H. E., and M. L. FREEDMAN, 1971. *J. Bacteriol.* **107**:95.
6. KUBITSCHEK, H. E. 1969. *Biophys. J.* **9**:792.
7. LARK, K. G., and C. LARK. 1960. *Biochim. Biophys. Acta.* **43**:520.
8. MAALØE, O., and P. C. HANAWALT. 1961. *J. Mol. Biol.* **3**:144.
9. MAALØE, O., and N. O. KJELDGAARD. 1966. Control of Macromolecular Synthesis. W. A. Benjamin, Inc., Menlo Park, Calif.
10. DONACHIE, W. E. 1969. *J. Bacteriol.* **100**:260.
11. COOPER, S., and C. E. HELMSTETTER. 1968. *J. Mol. Biol.* **31**:519.
12. DONACHIE, W. D. 1968. *Nature (Lond.)*. **219**:1077.

13. LARK, C. 1969. *Biochim. Biophys. Acta.* **119**:517.
14. HELMSTETTER, C. E. 1967. *J. Mol. Biol.* **24**:417.
15. KUBITSCHEK, H. E., H. E. BENDIGKEIT, and M. R. LOKEN. 1967. *Proc. Natl. Acad. Sci. U. S. A.* **57**:1611.
16. KUBITSCHEK, H. E. 1968. *Biophys. J.* **8**:1401.
17. KJELDGAARD, N. O. 1967. *Adv. Microb. Physiol.* **1**:39.