Dimensional rearrangement of *Escherichia coli* B/r cells during a nutritional shift-down

ARIEH ZARITSKY,[1]* CONRAD L. WOLDRINGH,[2] CHARLES E. HELMSTETTER[3] and N. B. GROVER[4]

[1]*Department of Life Sciences, Ben-Gurion University of the Negev, PO Box 653, Be'er-Sheva 84105, Israel*
[2]*Department of Molecular Cell Biology, Section of Molecular Cytology, The University of Amsterdam, Plantage Muidergracht 14, 1018TV Amsterdam, The Netherlands*
[3]*Department of Biological Sciences (Cell Biology), Florida Institute of Technology, 150 W. University Boulevard, Melbourne, FL 32901, USA*
[4]*Hubert H. Humphrey Center for Experimental Medicine and Cancer Research, The Hebrew University Faculty of Medicine, PO Box 12272, Jerusalem 91120, Israel*

In a search for the mechanism underlying dimensional changes in bacteria, the glucose analogue methyl α-D-glucoside was used to effect a rapid reduction in the mass growth rate of *Escherichia coli* by competitively inhibiting glucose uptake, a so-called nutritional shift-down. The new steady-state cell mass and volume were reached after 1 h, during which the rate of cell division was maintained; rearrangement of the linear dimensions (cell length, diameter), however, required an additional 2 h and caused an undershoot in cell length, consistent with the view that *E. coli* is slow to modify its diameter. The results are compared with the overshoot in cell length that occurs following nutritional shift-up.

Introduction

Eubacteria are larger at rapid growth rates (Kjeldgaard *et al.*, 1958; Schaechter *et al.*, 1958). This is explained by the fact that two of the cell cycle parameters, the time (Helmstetter *et al.*, 1968) between initiation of chromosome replication and subsequent cell division $(C+D)$, and the cell mass per chromosome origin (Donachie, 1968; Pritchard *et al.*, 1969) at the time of initiation (M_i), are independent of the value of the doubling time (τ) when τ is less than 60 min. Thus, a fast-growing cell reaches a larger size (mass or volume) by the time it is ready to divide because it initiates replication at the same size (M_i) as a slow-growing cell but grows faster during the subsequent $C+D$.

Rod-shaped Gram-negative species such as *Escherichia coli* and *Salmonella typhimurium* change their cell size by adjusting both length and diameter (Schaechter *et al.*, 1958; Woldringh *et al.*, 1977; Zaritsky, 1975), despite the fact that at any particular growth rate $(1/\tau)$ they

extend in length only (Marr *et al.*, 1966). The mechanism by which cell diameter is modulated at different growth rates remains elusive. A crucial observation in this regard would seem to be the overshoot in cell length that takes place during the well-defined transition from slow to fast growth (Grover *et al.*, 1980; Woldringh *et al.*, 1980), the so-called nutritional shift-up (Kjeldgaard *et al.*, 1958): the process of dimensional rearrangement requires much more time than the $C+D$ needed for the cells to attain their final steady-state size (Zaritsky *et al.*, 1982). This has been explained in terms of linear extension of the cell envelope at a rate that depends on growth rate and doubles once during each cell cycle (Rosenberger *et al.*, 1978). However, recent analyses of peptidoglycan synthesis rate and its localization (Woldringh *et al.*, 1987; Cooper, 1988) are discordant with such a view.

The process of dimensional rearrangement during a nutritional shift-up is slow compared to $C+D$ because the cell is not able to adjust its diameter as rapidly as its mass or volume (Grover *et al.*, 1980; Woldringh *et al.*, 1980; Zaritsky *et al.*, 1982). Will it be easier, and thus quicker, for the cell to reduce its diameter upon a nutritional shift-down or will cell length again overcompensate, this time by undershooting its final steady-state value? Can we obtain an indication of the kinetics

* Author for correspondence. Tel. +972 57 278951 or +972 57 461712; fax 972 57 276201; e-mail ariehz@bgumail.bgu.ac.il.

Abbreviation: αMG, methyl α-D-glucoside.

of diameter accommodation during such a transition, with perhaps a hint as to the underlying mechanism? Such questions can only be addressed reliably under conditions in which the mass (and volume) growth-rate changes rapidly, a requirement that is satisfied (Kessler & Rickenberg, 1963) by the glucose analogue methyl α-D-glucoside (αMG), which has been used for studying the control of RNA and protein synthesis (Hansen *et al.*, 1975; Johnsen *et al.*, 1977; Molin *et al.*, 1977). This abrupt shift-down system has recently been exploited (Zaritsky & Helmstetter, 1992) to investigate the rate maintenance of cell division, enabling observation of the changes in the cell-cycle parameters $C + D$ and M_i with τ.

Here we present a detailed study of the dimensions of *E. coli* B/r during such a shift-down experiment and consider the implications for growth, division and shape formation in rod-shaped bacteria.

Methods

Strain, growth medium, shift-down conditions and growth measurements. E. coli B/r F26 (*thy his*) was cultured by vigorous shaking at 37 °C in minimal medium (Helmstetter, 1967) supplemented with glucose (0·1%), thymine (10 μg ml^{-1}) and histidine (20 μg ml^{-1}). To achieve a nutritional shift-down, αMG (Hansen *et al.*, 1975) was added as a powder to a final concentration of 1% (w/v). This ratio of 10:1 αMG:glucose was chosen because previous observations had indicated that rate-maintenance of cell division persisted under such conditions (Zaritsky & Helmstetter, 1992). Mass increase of the cultures was monitored at 450 nm with a Gilford micro-sample spectrophotometer and cell numbers were determined using an electronic particle counter developed in one of our laboratories (Huls *et al.*, 1992).

Preparation of cells for electron microscopy. Cells were fixed by the addition to the growth medium of 1% (w/v) OsO$_4$ to a final concentration of 0·1%, deposited on agar filters (Woldringh *et al.*, 1977) and photographed with a Philips EM300 electron microscope. Cellular dimensions were measured by touching, with an electronic pen, pictures projected on a transparent screen at a magnification of about × 10000. Distributions and means were calculated using the software described previously (Huls *et al.*, 1992).

Results and Discussion

Experiments described previously (Zaritsky & Helmstetter, 1992) showed that the addition of αMG (in a ratio to glucose of 10:1) to exponentially growing cells causes the mass doubling-time to increase abruptly from 42 to 100 min, then stabilize after about 70 min at a level of 80 min. In such shift-down experiments, the pre-shift rate of cell division (1·45 doublings h^{-1}) was maintained for about 65 min, after which it dropped to its new steady-state level (0·75 h^{-1}).

All cell dimensions were determined from a single, typical growth experiment. Cell length distributions at seven representative time points, and samples of agar-filtered cells at three time points, are displayed in Fig. 1.

The transition period was characterized by a sharp drop in mean cell length, from its pre-shift level of 2·71 μm to 1·77 μm at 60–80 min (Fig. 2a), due to division-rate maintenance (Zaritsky & Helmstetter, 1992), followed by a gradual rise of 6% to its final steady-state value of 1·87 μm. Mean cell diameter, on the other hand, remained approximately constant at 0·72 μm for about 2 h, then decreased by 10% over the next hour or so to its final steady-state value of 0·65 μm (Fig. 2b). This dissociation between length and diameter changes is emphasized by considering their ratio, the so-called (Zaritsky, 1975) shape factor (Fig. 2c). The ratio, 3·8 prior to the shift, undershot by around 35% (to 2·45 at 60–100 min) before reaching its new steady-state level of 2·8 about 3 h after the transition.

Adjustment of cell diameter during a transition between different growth rates is thus slow not only during nutritional shift-up, but under shift-down conditions as well. Qualitatively, two mechanisms can be conceived of through which the cell adjusts its diameter and establishes its shape. In one, it is the envelope alone that determines the response to the turgor pressure and the synthetic possibilities afforded by the metabolism of the cell. This is the idea underlying the constrained-hoop model (Cooper, 1989), which regards the cell as a pressurized balloon constrained by a tube of constant diameter, and ascribes its slow accommodation to a new radius to the molecular arrangement of the peptidoglycan chains in its surface.

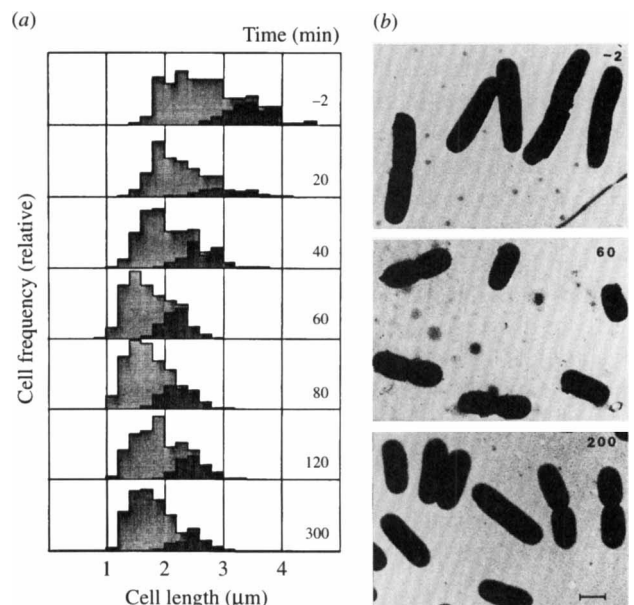The alternative view (Woldringh *et al.*, 1990) sees the

Fig. 1. (*a*) Relative frequencies of cell lengths and (*b*) agar-filtered *E. coli* F26 cells at selected time points before and after nutritional shift-down. Darker distributions (*a*) represent constricted cells. Bar (*b*), 1 μm.
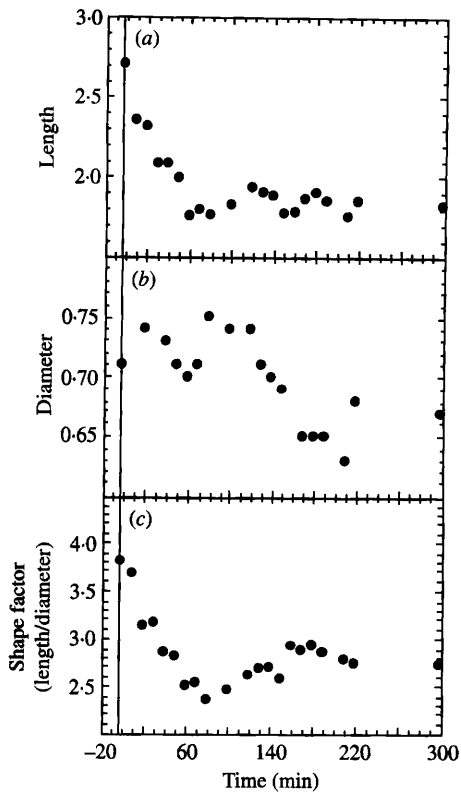
Fig. 2. (a) Mean cell length (μm), (b) cell diameter (μm), and (c) cell shape factor as functions of time before and after nutritional shift-down.

Fig. 3. Mean cell surface area (●, μm²), volume (○, μm³), and relative mass (▲, $OD_{450}/10^9$ cells) as functions of time before and after nutritional shift-down.

Fig. 4. Comparison between independent methods for cell volume determinations at selected time points before and after nutritional shift-down: electronic particle sizing (left; channel number), electron microscopy (right; μm³).

nucleoid influence as indirect (via the transcription/ translation activity in its vicinity, for instance), acting on the local rate of peptidoglycan synthesis through a process of reciprocal interactions: a change in the amount of replicating and segregating DNA forces the growing peptidoglycan to adjust its radius by a so-called nucleoid occlusion effect (Woldringh *et al.*, 1990). In turn, the peptidoglycan tube forces the extending nucleoid to segregate along the major axis of the cell. The data we have presented here are currently being utilized to test just such a model.

Volume and surface area were computed for each cell, from its measured length and diameter, by assuming an idealized geometry of a right circular cylinder with hemispherical polar caps, and the mean values are plotted in Fig. 3. Both dimensions appear to attain their new steady-state levels after about 60 min, followed by what appear to be slow oscillations that persist for several hours. Mean cell mass (relative values) was calculated from the ratio between OD_{450} and cell number (results not shown), and is included to demonstrate that cell density (mass/volume) remains constant during this transition, as in steady-state exponential growth (Kubitschek *et al.*, 1984). Cell volume distributions were also determined by a completely independent method, an
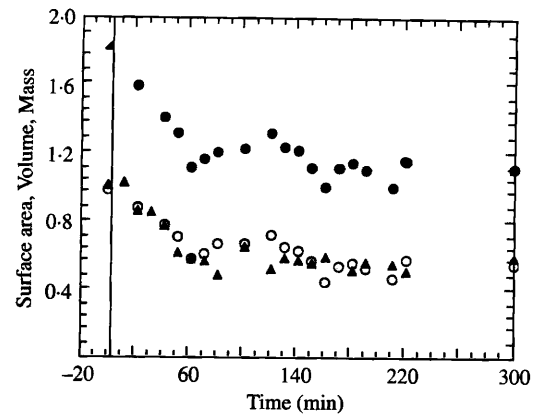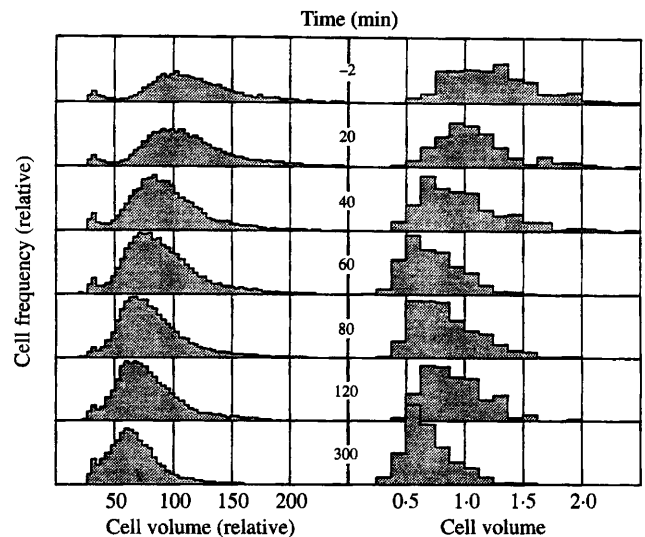
electronic particle counter (Kubitschek & Friske, 1986), and found to be comparable (Fig. 4); it is unlikely, therefore, that serious artifacts are present.

The very short cells (Fig. 2a) with small shape factors produced during the undershoot period (Fig. 2c) have an unusually high ratio of polar to cylindrical cell surface area (or volume) and so may be useful in testing the model (Helmstetter & Leonard, 1990) for nonrandom segregation of sister DNA strands to daughter cells at division.

References

COOPER, S. (1988). Rate and topography of cell wall synthesis during the division cycle of *Salmonella typhimurium. Journal of Bacteriology* **170**, 422–430.

COOPER, S. (1989). The constrained hoop: an explanation of the overshoot in cell length during a shift-up of *Escherichia coli. Journal of Bacteriology* **171**, 5239–5243.

DONACHIE, W. D. (1968). Relationship between cell size and time of initiation of DNA replication. *Nature, London* **219**, 100–104.

GROVER, N. B., ZARITSKY, A., WOLDRINGH, C. L. & ROSENBERGER, R. F. (1980). Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. I. Theory. *Journal of Theoretical Biology* **86**, 421–439.

HANSEN, M. T., PATO, M. L., MOLIN, S., FIIL, N. F. & VON MEYENBURG, K. (1975). Simple downshift and resulting lack of correlation between ppGpp pool size and ribonucleic acid accumulation. *Journal of Bacteriology* **122**, 585–591.

HELMSTETTER, C. E. (1967). Rate of DNA synthesis during the division cycle of *Escherichia coli. Journal of Molecular Biology* **24**, 417–427.

HELMSTETTER, C. E. & LEONARD, A. C. (1990). Involvement of cell shape in the replication and segregation of chromosomes in *Escherichia coli. Research in Microbiology* **141**, 30–39.

HELMSTETTER, C. E., COOPER, S., PIERUCCI, O. & REVELAS, L. (1968). The bacterial life sequence. *Cold Spring Harbor Symposia on Quantitative Biology* **33**, 809–822.

HULS, P. G., NANNINGA, N., VAN SPRONSEN, E. A., VALKENBURG, J. A. C., VISCHER, N. O. E. & WOLDRINGH, C. L. (1992). A computer-aided measuring system for the characterization of yeast populations combining 2D-image analysis, electronic particle counter, and flow cytometry. *Biotechnology and Bioengineering* **39**, 343–350.

JOHNSEN, K. S., MOLIN, S., KARLSTRÖM, O. & MAALØE, O. (1977). Control of protein synthesis in *Escherichia coli*: analysis of an energy source shift-down. *Journal of Bacteriology* **131**, 18–29.

KESSLER, D. P. & RICKENBERG, H. V. (1963). The competitive inhibition of α-methylglucoside uptake in *Escherichia coli. Biochemical and Biophysical Research Communications* **10**, 482–487.

KJELDGAARD, N. O., MAALØE, O. & SCHAECHTER, M. (1958). The transition between different physiological states during balanced growth of *Salmonella typhimurium. Journal of General Microbiology* **19**, 607–616.

KUBITSCHEK, H. E. & FRISKE, J. A. (1986). Determination of bacterial volume with the coulter counter. *Journal of Bacteriology* **168**, 1466–1467.

KUBITSCHEK, H. E., BALDWIN, W. W., SCHROETER, S. J. & GRAETZER, R. (1984). Independence of buoyant cell density and growth rate in *Escherichia coli. Journal of Bacteriology* **158**, 296–299.

MARR, A. G., HARVEY, R. J. & TRENTINI, W. C. (1966). Growth and division of *Escherichia coli. Journal of Bacteriology* **91**, 2388–2389.

MOLIN, S., VON MEYENBURG, K., MAALØE, O., HANSEN, M. T. & PATO, M. L. (1977). Control of ribosome synthesis in *Escherichia coli*: analysis of an energy source shift-down. *Journal of Bacteriology* **131**, 7–17.

PRITCHARD, R. H., BARTH, P. T. & COLLINS, J. (1969). Control of DNA synthesis in bacteria. *Symposia of the Society for General Microbiology* **19**, 263–297.

ROSENBERGER, R. F., GROVER, N. B., ZARITSKY, A. & WOLDRINGH, C. L. (1978). Surface growth in rod-shaped bacteria. *Journal of Theoretical Biology* **73**, 711–721.

SCHAECHTER, M., MAALØE, O. & KJELDGAARD, N. O. (1958). Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium. Journal of General Microbiology* **19**, 592–606.

WOLDRINGH, C. L., DE JONG, M. A., VAN DEN BERG, W. & KOPPES, L. (1977). Morphological analysis of the division cycle of two *Escherichia coli* B/r substrains during slow growth. *Journal of Bacteriology* **131**, 270–279.

WOLDRINGH, C. L., GROVER, N. B., ROSENBERGER, R. F. & ZARITSKY, A. (1980). Dimensional rearrangement of rod-shaped bacteria following nutritional shift-up. II. Experiments with *Escherichia coli* B/r. *Journal of Theoretical Biology* **86**, 441–454.

WOLDRINGH, C. L., HULS, P., PAS, E., BRAKENHOFF, G. J. & NANNINGA, N. (1987). Topography of peptidoglycan synthesis during elongation and polar cap formation in a cell division mutant of *Escherichia coli* MC4100. *Journal of General Microbiology* **133**, 575–586.

WOLDRINGH, C. L., MULDER, E., VALKENBURG, J. A. C., WIENTJES, F. B., ZARITSKY, A. & NANNINGA, N. (1990). Role of the nucleoid in the toporegulation of division. *Research in Microbiology* **141**, 39–49.

ZARITSKY, A. (1975). On dimensional determination of rod-shaped bacteria. *Journal of Theoretical Biology* **54**, 243–248.

ZARITSKY, A. & HELMSTETTER, C. E. (1992). Rate maintenance of cell division in *Escherichia coli* B/r: analysis of a simple nutritional shift-down. *Journal of Bacteriology* **174**, 8152–8155.

ZARITSKY, A., GROVER, N. B., NAAMAN, J., WOLDRINGH, C. L. & ROSENBERGER, R. F. (1982). Growth and form in bacteria. *Comments on Molecular and Cellular Biophysics* **1**, 237–260.