Biochimie 83 (2001) 121−124

S0300908400012190/FLA

Perpendicular planes of FtsZ arcs in spheroidal *Escherichia coli* cells

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(Received 10 October 2000; accepted 28 November 2000)

Abstract — Division planes in *Escherichia coli*, usually restricted to one dimension of the rod-shaped cell, were induced at all possible planes by transforming the cells to spheroids with mecillinam (inactivating PbpA). Such cells displayed many nucleoids and arcs of FtsZ, genetically tagged to green fluorescent protein, that developed to rings at constriction sites all around their surface. These observations are consistent with the view (Woldringh et al., J. Bacteriol. 176 (1994) 6030-6038) that nucleoids, forced during replication to segregate in the length axis of the cell by the rigid bacillary envelope, induce assembly of FtsZ to division rings in between them. © 2001 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS

mecillinam-induced spheroidal cells / nucleoid segregation / division planes / FtsZ-GFP rings and arcs / fluorescent microscopy / thymine limitation

1. Introduction

Bacillary bacteria grow by elongation only and divide in a perpendicular plane, while cocci extend and divide in all three dimensions. The question whether this stems from a fundamental difference is moot. Cooper [1] presumes that the peptidoglycan 'constrained-hoop' structure of rod-shaped bacteria such as *Escherichia coli* forces them to divide in one dimension, while Begg and Donachie [2] demonstrated that they could do so in two. We have argued [3] that the rigid peptidoglycan forces the two replicating nucleoids to segregate along cell length and that division ring is placed at the envelope adjacent to and between them, irrespective of the previous location upon termination of a chromosome replication cycle [4]. Our hypothesis implies three-dimensional randomization of division planes in spheroidal cells.

To test this working hypothesis, a physiological procedure has been devised to generate dividing spheroidal *E. coli* that initiate secondary constrictions before separation [5]. Spheroids were obtained by temporary mecillinam treatment [6] and division frequency was enhanced by thymine step-up of a thymine-limited *thyA* mutant [7]. These manipulations have indeed visualized two FtsZ arcs in tilted planes on spheroidal dividing cells [8] but the efficiency of the technique used (in situ immunofluorescence labeling [9]) was insufficient to resolve the controversy. By resorting to direct in vivo visualization using green fluorescent protein (GFP) tagged to FtsZ (FtsZ-Gfp), we confirmed our working hypothesis.

2. Materials and methods

2.1. Bacterial strain

To construct chimeric *ftsZ-gfp*, regulated by BAD promoter (Woldringh and Pas, to be published elsewhere), the improved GFP (from Stemmer and collaborators [10]) was used. It was inserted in pBAD (from Invitrogene) and introduced into the *thyA* strain CR34 of *E. coli* [3] by the *λInCh* system [11]. One of the clones (LMC1492) was found most suitable and displays FtsZ-Gfp fluorescent rings following addition of L-arabinose.

2.2. Experimental conditions

The following chemicals were simultaneously added to a culture of LMC1492, grown under 'balanced growth' conditions [12] in rich trypton-yeast medium containing low (though undefined) concentration of thymine: mecillinam (10 μg mL^{-1}), to affect rod-sphere transition; L-arabinose (0.2%), to induce *ftsZ-gfp*; thymine (20 μg mL^{-1}) and deoxyguanosine (100 μg mL^{-1}), to enhance the rate of chromosome replication hence, temporarily, the frequency of division signals. At 80–100 min, the cells were washed to release the division inhibition by mecillinam, and re-suspended in glucose salts medium without the required amino acids (but with the same concentrations of L-arabinose, thymine and deoxyguanosine) to

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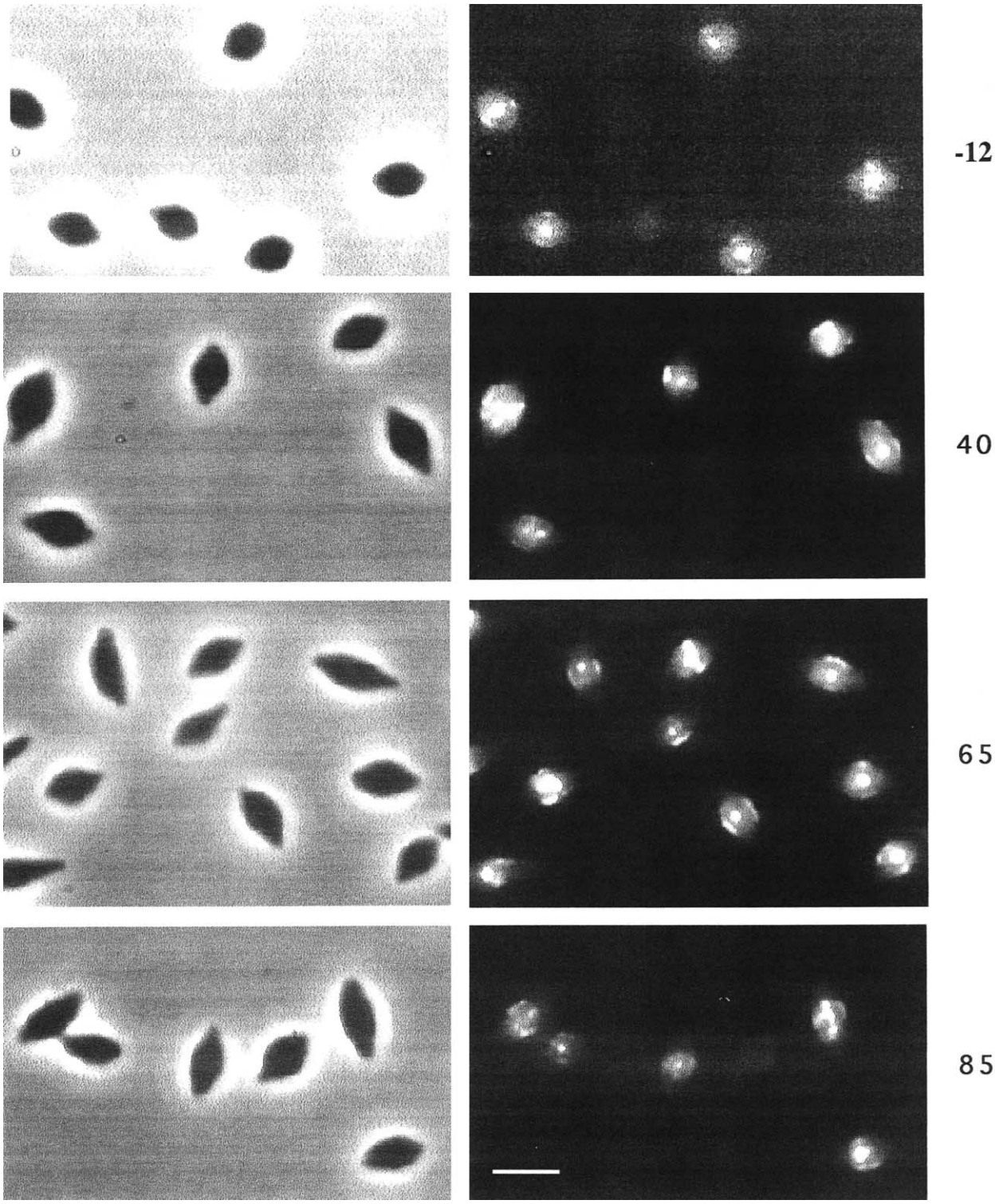

Figure 1. Phase contrast (left) and fluorescent (right) micrographs of *E. coli* K12 (CR34) cells before (–12 min) and various times (as indicated) after release from mecillinam treatment. Magnification bar, 5 μm.

block cell growth while allowing suspended divisions to terminate with fluorescent FtsZ-Gfp rings).

2.3. Microscopy

The cells were immobilized on agarose slides as described by Helvoort and Woldringh [13] and photographed with an air-cooled charged-coupled device (CCD) camera (Princeton Instruments; model RTE-1317-k-1), mounted on an Olympus BX –60 fluorescence microscope equipped with an oil immersion (× 100) lens. A computer (Quadra 840 AV, Apple) was used for image collection and exposure control via mechanical lamp and CCD camera shutters. Images were taken using the public domain program Object-Image 2.06 by N. Vischer (University of Amsterdam) [14], which is based on NIH Image by W. Rasband. The cells were photographed first in the phase-contrast mode, and then with a GFP fluorescence filter (U-MNG/narrow; excitation at 470 to 490 nm).

3. Results and discussion

Upon release from division-inhibition, the hybrid FtsZ-Gfp started to accumulate at various locations on the surface of the giant, multi-nucleated cells. The fluorescent spots (that looked at first like inclusion bodies) slowly developed into arcs in various planes (*figure 1*), many of which were positioned at constriction sites (visible by phase-contrast microscopy at later stages), some crossing each other (*figure 2*). A high proportion of the giant cells lysed during the recovery, and the rest recovered slowly (*figure 3*) because they were down-shifted into minimal glucose-supplemented medium to avoid elongation that masks the phenomenon of interest.

The slow recovery allowed easy visualization of 'butterfly-like' cells [5] with their two non-parallel FtsZ-Gfp rings representing a pair of secondary constrictions (indicated by arrows in *figure 3*).

The perpendicular arcs formed during the treatment (*figures 1, 2*) representing prospective division planes seem to be gradually forced during the recovery growth to eventually lie in parallel planes (*figure 3*) via acute angles (arrows) formed in the transition period. The molecular forces involve PbpA, but the mechanism is still an enigma.

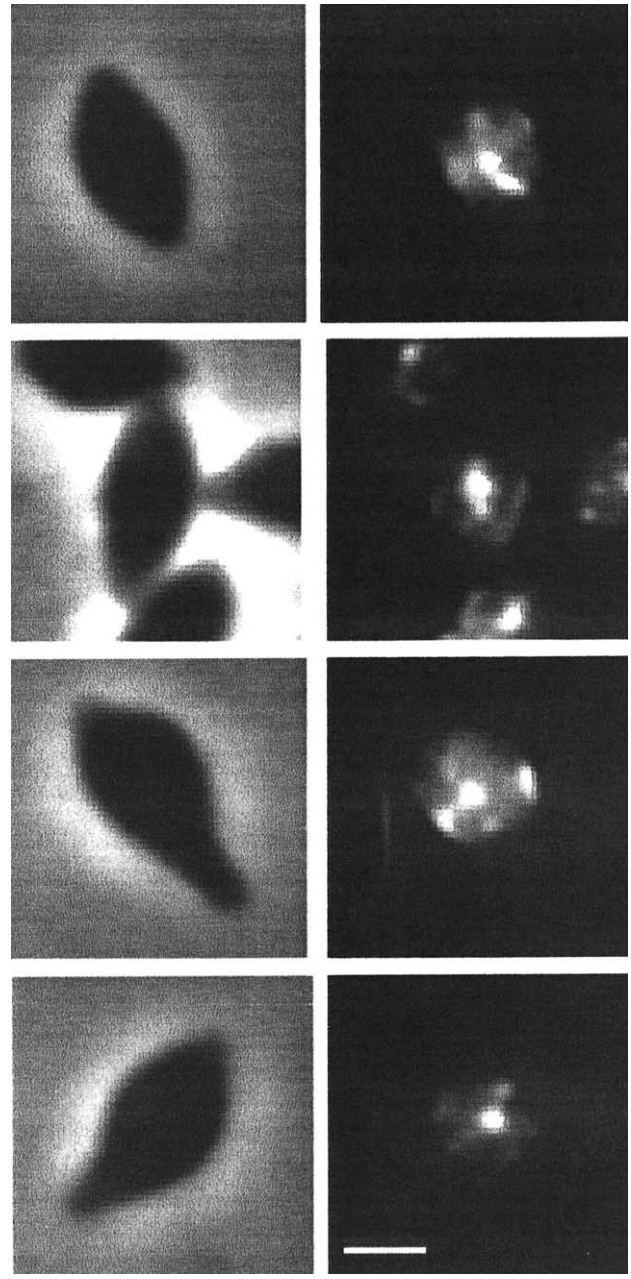

Figure 2. Phase contrast (left) and fluorescent (right) micrographs of a selection of cells with arcs and crosses developed 85 min after release of mecillinam treatment. Magnification bar, 2 μm

4. Conclusions

The results support our hypothesis that cell shape maintained by the rigid envelope restricts division planes in untreated bacillary *E. coli* to one dimension of the three possible in spheroids. The proposed function of the segregating nucleoids in positioning the constriction ring has also been supported by preliminary data (not shown), but these observations must be substantiated and quantitated. Three-dimensional virtual sectioning by confocal multi-channel microscopy is under way.

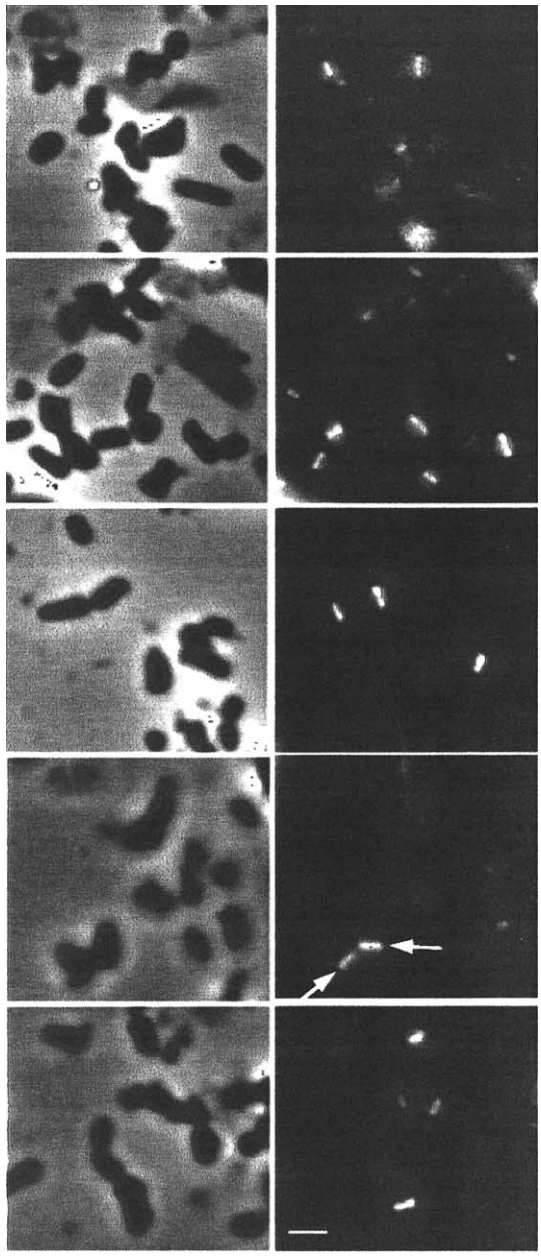

Figure 3. Phase contrast (left) and fluorescent (right) micrographs of cells partially recovered after overnight (12 h) incubation in minimal-glucose medium. Arrows indicate the non-parallel planes of cell division during recovery. Magnification bar, 2 μm.

The regime described is useful in general to study the division process; it can still be improved by modifying the time schedule of the manipulations involved.

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