Localizing cell division in spherical *Escherichia coli* by nucleoid occlusion

Arieh Zaritsky [a,*], Conrad L. Woldringh [b]

[a] *Department of Life Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Be'er-Sheva 84105, Israel*
[b] *Swammerdam Institute for Life Sciences, BioCentrum, University of Amsterdam, Kruislaan 316, 1098 SM Amsterdam, The Netherlands*

Abstract

The spatial relationship between FtsZ localization and nucleoid segregation was followed in *Escherichia coli thyA* cells, made spheroidal by brief exposure to mecillinam and after manipulating chromosome replication time using changes ('steps') in thymine concentration [Zaritsky et al., Microbiology 145 (1999) 1015–1022]. In such cells, fluorescent FtsZ-GFP arcs did not overlap the DAPI-stained nucleoids. It is concluded that FtsZ rings are deposited between segregating nucleoids, consistent with the nucleoid occlusion model [Woldringh et al., J. Bacteriol. 176 (1994) 6030–6038].
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1. Introduction

Bacterial cell division is related to chromosome segregation in such a way that the DNA is replicated and partitioned between the daughters before division [1]. While segregation is ongoing during the replication process itself [2], central positioning of the division site between two daughter nucleoids occurs towards termination [1–4]. Two complementary mechanisms have been proposed for localizing cell division in rod-shaped *Escherichia coli* cells: nucleoid occlusion [5] prevents FtsZ ring assembly on membrane regions immediately surrounding nucleoids that are actively involved in transertion (see for review [2]), and the Min system [6] inhibits FtsZ ring assembly at cell poles, while allowing ring formation at midcell.

In mutants lacking the oscillating Min proteins many divisions occur at the nucleoid-free regions of the cell poles producing minicells [7]. At the same time, however,

FtsZ rings are also properly localized between daughter nucleoids resulting in normal central divisions [8]. Understanding the mode of localizing cell division between two fully duplicated nucleoids is likely to yield new insights into an essential life process at a very small scale. Here, we study the effect of nucleoid occlusion on the assembly of the FtsZ ring in spherical *E. coli* cells that possess multiple chromosomes and divide in alternating planes [9,10]. It has been suggested that "the larger diameter of most cocci may require them to find an alternative mode to regulate their division machine" [11]. We have proposed [12] that the same mechanism, based on a structural signal, is responsible, namely, the relative positions of the fully replicated daughter nucleoids at termination. According to our 'segregation-dependent division' model, division pattern is determined by cell shape [13]. It is based on the observations that the nucleoids move apart during replication [2] and that peptidoglycan synthesis is slowed down at the surface surrounding them [14]. In bacilli, the rigid wall forces the replicating/segregating nucleoids to extend in one dimension only. The model predicts that in cocci, the nucleoids would be free to move away in three dimensions, still leaving nucleoid-free regions for FtsZ assembly. This prediction is confirmed here by visualizing the mutually exclusive positions of nucleoids and FtsZ rings in spherical and irregularly shaped cells.

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* Corresponding author. Tel.: +972 (8) 6461 712;
Fax: +972 (8) 6278 951.
E-mail address: ariehz@bgumail.bgu.ac.il (A. Zaritsky).

2. Materials and methods

2.1. Bacterial strain and growth

Strain LMC1492 was used in this study [9]. It had previously been derived from *E. coli* K12 (CR34; *thr-1 leuB6 thyA* [15]) to include a chimeric *ftsZ-gfp* using the lambda-in-chromosome (λ*InCh*) system [9], regulated by the BAD promoter hence displaying FtsZ-GFP (green fluorescent protein) rings following addition of L-arabinose.

Growth at 37°C was followed by OD_{450} (measured with a spectrophotometer) and cell numbers (with an electronic particle counter, 30 μm orifice). Balanced cultures were grown 'normally' [16] for at least 10 doublings by successive dilutions ($OD_{450} < 0.4$).

2.2. Cells with secondary constrictions

Large cells with multi-forked chromosomes were obtained during balanced growth in rich tryptone-yeast medium containing a low (though undefined) concentration of thymine [17]. Treatment with mecillinam resulted in spheroids [15]. To obtain secondarily constricted cells, 2′-deoxyguanosine (100 μg ml^{-1}) and thymine (to 20 μg ml^{-1}) were added.

2.3. Experimental design

The following chemicals were simultaneously added to a balanced culture of LMC1492, as previously described [9]: mecillinam (1 μg ml^{-1}), to affect rod–sphere transition; L-arabinose (0.2%), to induce *ftsZ-gfp*; DAPI (4′,6-diamidino-2-phenylindole dihydrochloride hydrate; 250 ng ml^{-1}) to visualize DNA; thymine and 2′-deoxyguanosine (as above) to enhance the rate of chromosome replication and hence, temporarily, the frequency of division signals. At 80–100 min, the cells were washed to release the division inhibition by mecillinam, and resuspended in glucose salts medium without the required amino acids (but with the same concentrations of L-arabinose, thymine and deoxyguanosine) to block cell growth while allowing suspended divisions to terminate with fluorescent FtsZ-Gfp rings. To inhibit protein synthesis without wash, chloramphenicol (CAM) was added to 200 μg ml^{-1}.

2.4. Microscopy

The cells were immobilized on agarose slides as described previously [18] and photographed with a 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 840AV, 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) [19], which is based on NIH Image by W. Rasband. The cells were photographed first in the phase contrast mode, second with a DAPI fluorescence filter (U-MWU: excitation at 330–385 nm, transmission > 420 nm) and then with a GFP fluorescence filter (U-MNB: excitation at 470–490 nm, transmission > 515 nm).

3. Results

Rod–sphere transformation by inactivating Penicillin-Binding-Protein-2 postpones division, so that secondary constrictions only appear when the primary is already advanced; hence the two dividing daughters are not clearly seen in relation to each other ([20], and unpublished observations). To visualize consecutive constrictions in a single cell early enough, the time *D* between replication termination and cell separation must be extended or division conditions enhanced, at least temporarily. This is obtained by manipulating the chromosome replication time *C* in *thyA* strains using thymine 'steps' [21]. Slower replication/segregation delays termination and hence the subsequent division. That is why thymine auxotrophs growing with a low thymine concentration form larger cells with more complex nucleoids and more DNA without any change in mass growth rate [17]. The larger cell size is accommodated by a gradual increase in diameter [21] resulting in extended time to finalize division. Stepping up the concentration of thymine (thus replication rate) increases temporarily the frequency of terminations. The large cells with more segregating nucleoids now have more space on their surface to localize FtsZ assembly [10]. In these wider, thymine-limited cells, DAPI-stained nucleoids are tilted at acute angles to the long cell axis (fig. 3a in [15]). Relieving the cylindrical constraint of the rigid peptidoglycan further by mecillinam indeed releases them from moving in a single dimension; sphere-like cells develop, with nucleoids extending in all directions (fig. 3b in [15]).

When FtsZ is tagged to GFP fluorescent arcs and rings are formed, in many cells crossing each other perpendicularly [9]. The impression that the developing arcs are placed on surface between segregating nucleoids was clarified here during mecillinam treatment. Some FtsZ-GFP nucleation centers were formed between segregated nucleoids as early as 30 min after mecillinam addition (Fig. 1A). This effect was emphasized at 80 min (Fig. 1B). Of the 93 cells scored, it appeared that FtsZ arcs or rings did not overlap the nucleoids in 72% of the cases. To make this point clearer, the DAPI-stained nucleoids were false colored in red and superimposed on GFP, displayed in green (Fig. 1D). Outlined nucleoids (Fig. 1E) confirmed the mutually exclusive spatial relationship between the two crucial cellular structures. In 13% of the 93 cells no FtsZ-GFP fluorescence was present. In the rest (15%) an overlap of
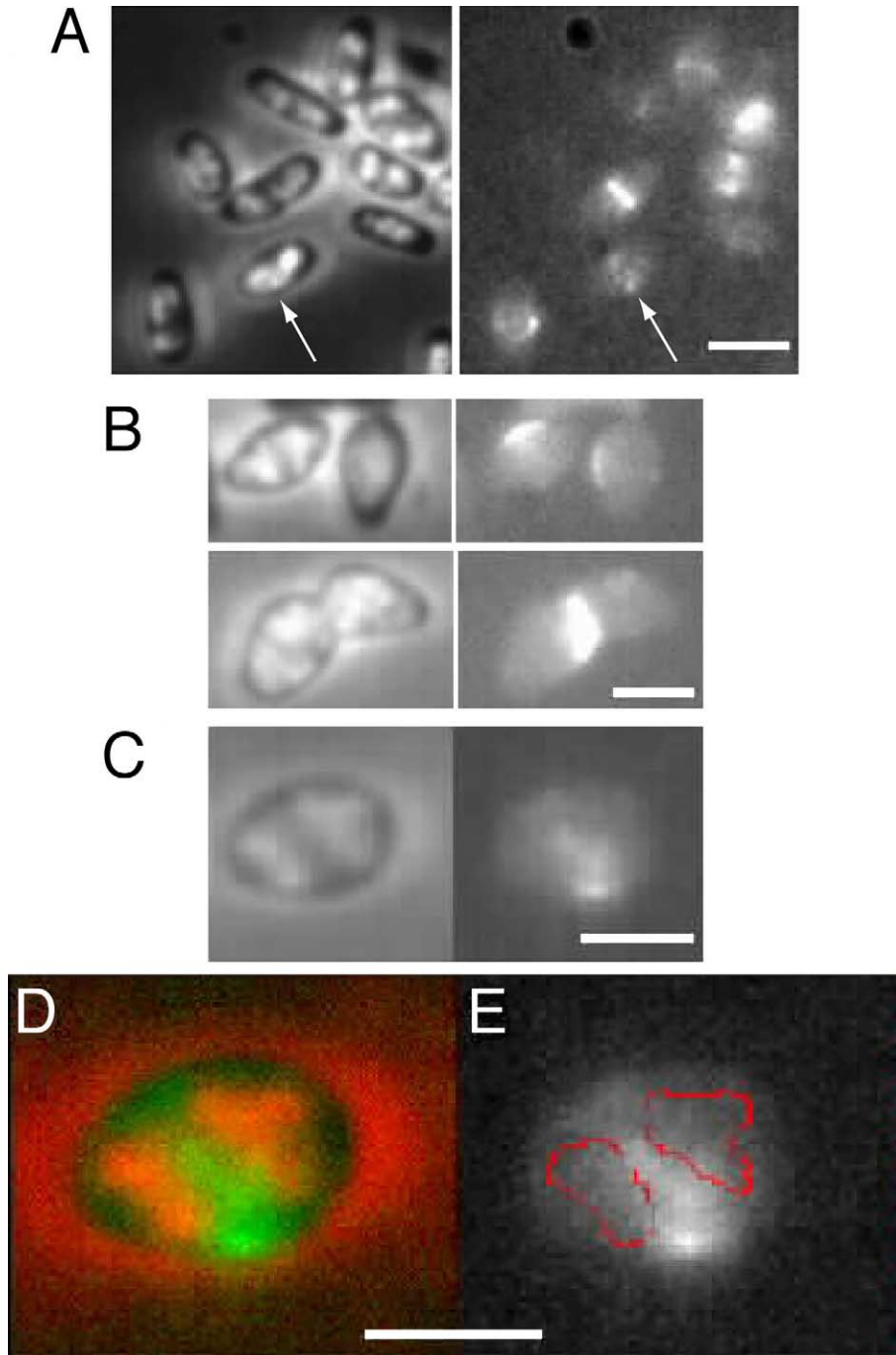
Fig. 1. Assembling FtsZ rings (right hand side in A–C) are excluded from surface surrounding nucleoids (left hand side in A–C). FtsZ-GFP nucleation centers formed between segregated nucleoids at 30 min (A) and this was emphasized at 80 min after mecillinam addition (B). The arrow in A points to an overlap between DAPI and GFP fluorescence; this could be due to the structures being in different focal planes. Enlarged picture (C) of such a typical cell demonstrates lack of overlap between the crossing arcs and the four segregated nucleoids. D: DAPI-stained nucleoids false colored in red and superimposed on GFP-tagged FtsZ, displayed in green. E: Nucleoids, outlined in red, 'avoiding' FtsZ-GFP cross-like arcs. All magnification bars represent 2 μm.

GFP and DAPI fluorescence occurred (e.g. arrow in Fig. 1A), but this can be ascribed to the structures occurring in different focal planes.

To see whether localization of FtsZ rings between segregating nucleoids depends on active cell growth, translation was inhibited with CAM. CAM-treated cells run out chromosome replication and complete constrictions that are ongoing upon CAM addition [22]. In the large spheroidal cells, however, not all FtsZ arcs and rings completed division and many remained visible (Fig. 2). As segregated nucleoids are known to compact and coalesce or fuse following CAM treatment [12,18], GFP and DAPI
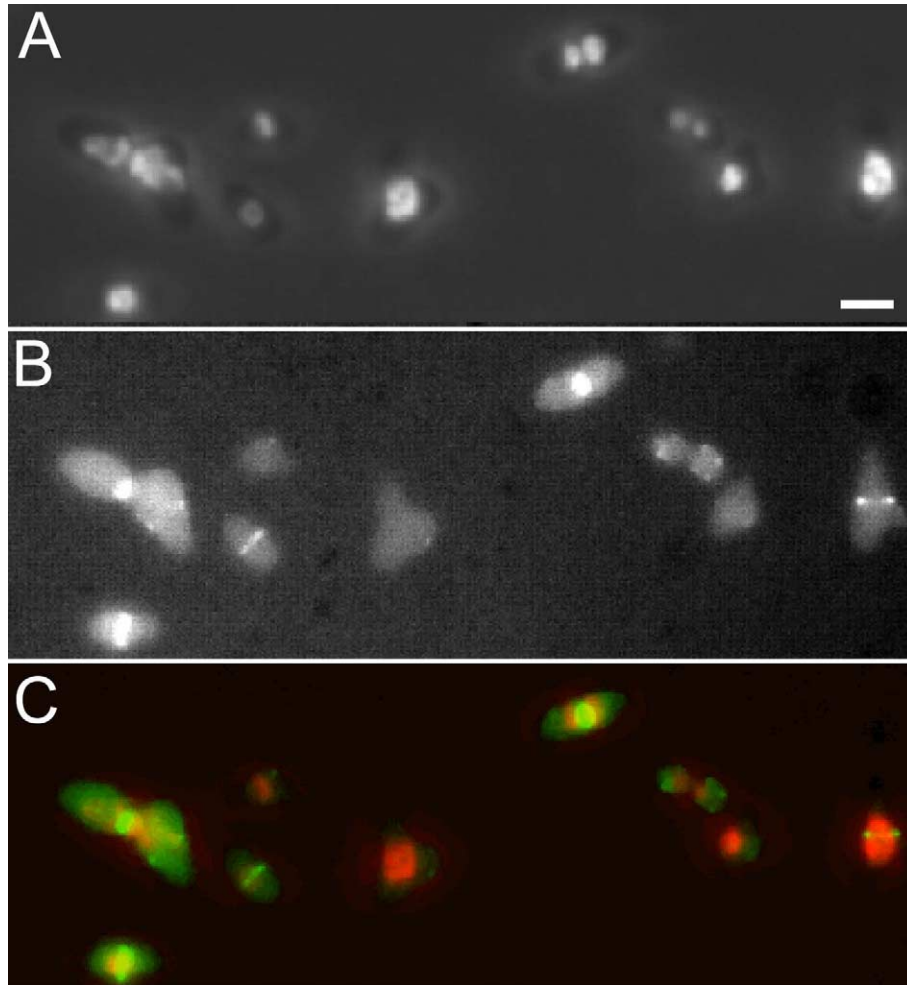
Fig. 2. DAPI-stained nucleoids (A) and GFP-tagged FtsZ arcs and rings (B) in 40 min CAM-treated cells prepared as in Fig. 1. Note that in these non-growing cells FtsZ rings often overlap nucleoid structures. This is demonstrated in C using false colors as in Fig. 1D. Magnification bar represents 2 μm.

fluorescence could now be seen to overlap in most cells (Fig. 2C).

4. Discussion

In this study we demonstrated, in vivo and simultaneously, that the FtsZ ring is deposited on cell surface that is free from DAPI-stained material in spherical cells. We conclude that nucleoid material (DNA) forbids placing FtsZ rings around it, an observation that supports the so-called nucleoid occlusion model [5,12]. The fully replicated nucleoid thus seems to transmit a positive signal or relieve an inhibitory one (or both). Deciphering this mechanism is expected to shed light on a fundamental series of processes leading to bacterial cell division.

It can be argued that FtsZ is excluded from cytoplasmic nucleoid regions thus concentrating it in other cell parts, leading to assembly at the neighboring membrane. Our favorable explanation is that the replicating and segregating nucleoid prevents assembly of FtsZ proteins on nearby

surface regions through the so-called transertion process [2], i.e. coupled transcription, translation and translocation of membrane proteins. This process establishes a crowded condition in the vicinity of the nucleoid which hinders any FtsZ self-assembly thus resulting in nucleoid occlusion. Having a decreased number of expressed genes towards the chromosomal terminus [23], the transertion-exerted hindrance diminishes when the replication forks progress towards it and the bulk of daughter nucleoids have segregated apart. This may be the reason why a slower replication rate (longer C) results in a shorter period D between termination and division [21]; in contrast to the situation in a spherical cell, such a rod-shaped cell continues to elongate thus creating enough surface membrane to fully segregate the daughter nucleoids at termination. FtsZ ring assembly can thus begin at or before termination leading to a short D period.

It is thus the diminished crowding by transertion rather than a 'positive signal' evoked by an event at termination or completed segregation that triggers FtsZ assembly. This view of cell division localization does not need (pre)orga-
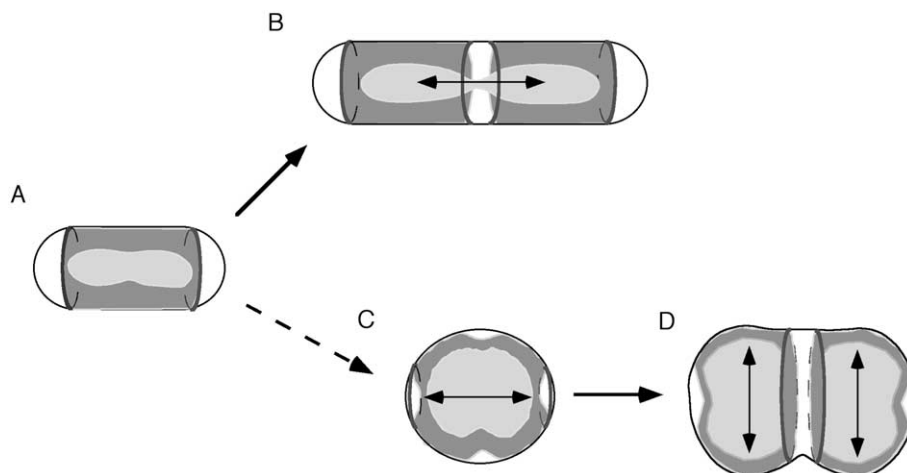
Fig. 3. Schematic representation of nucleoid occlusion. Dark gray areas represent membrane surface surrounding the segregating nucleoid (light gray bodies). White regions represent surface areas where FtsZ rings are free to assemble. Elongation of a rod-shaped cell (A) allows nucleoid segregation along the length axis (double-headed arrow). At termination, when most of the daughter nucleoids are separated, a transertion-free area is generated perpendicular to the segregation axis, where FtsZ can assemble (B). In the transition to a spherical cell (dashed arrow) with nucleoids containing more than two chromosomes (C), the cell division period is extended. Sufficient membrane surface is obtained to generate DNA-free regions for FtsZ assembly (D) when secondary nucleoids are segregated due to faster replication rate at the high thymine concentration. (Compare with fig. 6 in [15] and with fig. 8 in [24].)

nization of cytoskeleton-like proteins to serve as ruler or track for site specificity or motor-based DNA movement. This explanation does not contradict the observations [24] that MinD oscillation in parallel to the longest axis of coccoid cells helps to localize cell division; it may rather complement them as follows. Nucleoids seem to create surface domains [25], in the vicinity of which the peptidoglycan synthetic activity is slowed down, and segregate towards the most actively growing membrane regions as transertion sites develop more easily where new membrane space is available [2]. In the case of several (bidirectionally) segregating nucleoids in a sphere-like cell, each nucleoid creates its own long axis (double arrows in Fig. 3 and in fig. 6 in [15]). If MinD oscillations [24] follow this axis in such cells (which is still to be determined), this may be due to their faster diffusion through the nucleoid than through the denser cytoplasm. In other words, the Min system and the nucleoids support each other.

The images displayed here are of live cells in which DNA is expanded and occurs in a single or in two nucleoid masses. This may explain why there is usually only one FtsZ arc between these masses, even in cells with four or eight chromosomes. In these cases, there are no 'multiple nucleoid-free spaces' [24]; such spaces, however, become visible by OsO$_4$ or formaldehyde fixation [15]. They also appear in vivo upon CAM treatment, but in this case no new FtsZ rings seem to form because de novo protein synthesis is blocked. Under these conditions, the physical hindrance is abolished and the rings occur 'over' the location of the nucleoids (Fig. 2) due to coalescence ('fusion') of the (partially or fully) segregated nucleoids that move together to the division site [18]. FtsZ rings and subsequent recruited division proteins have indeed been demonstrated to enhance the last stage of nucleoid segregation [26].

Note added in proof

Rabinovitch et al. ('DNA-membrane interactions can localize bacterial cell center', J. Theoret. Biol., in press) have recently proposed another means by which the transertion process can signal FtsZ ring formation when nucleoids separate. Analysis of the unevenness in stress that DNA exerts on cell membrane through so-called 'transertion strings' concludes that the maximum rate of transition from a minimum to zero stress predicted in cell's midpoint during nucleoid separation may activate FtsZ ring formation.

Acknowledgements

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