Biophysical Letter

Cell-Shape Homeostasis in *Escherichia coli* Is Driven by Growth, Division, and Nucleoid Complexity

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ABSTRACT Analysis of recently published high-throughput measurements of wild-type *Escherichia coli* cells growing at a wide range of rates demonstrates that cell width W, which is constant at any particular growth rate, is related (with a $CV = 2.4\%$) to the level of nucleoid complexity, expressed as the amount of DNA in genome equivalents that is associated with chromosome terminus ($G/terC$). The relatively constant ($CV = 7.3\%$) aspect ratio of newborn cells (L_b/W) in populations growing at different rates indicates existence of cell-shape homeostasis. Enlarged W of thymine-limited *thyA* mutants growing at identical rates support the hypothesis that nucleoid complexity actively affects W. Nucleoid dynamics is proposed to transmit a primary signal to the peptidoglycan-synthesizing system through the transertion mechanism, i.e., coupled transcription/translation of genes encoding membrane proteins and inserting these proteins into the membrane.

Received for publication 20 February 2015 and in final form 8 June 2015.

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Two essential, unique macromolecules (structures) exist in a bacterial cell: DNA (nucleoid), which stores the genetic information; and the shape-maintaining peptidoglycan (sacculus), which protects the cell from rupture by its osmotic pressure (turgor). For species survival, division must occur after the genome doubles and between the two emerging sets, hence duplications of the two are coupled, temporally and spatially. A mechanism responsible for the link is still puzzling.

Temporal aspects of the bacterial cell division cycle

The time C taken to replicate the circular chromosome of wild-type *Escherichia coli*, bidirectionally from *oriC* to *terC*, is ~40 min at 37°C irrespective of the nutrition-modulated doubling time τ (1). The cell splits to two morphologically identical daughters (2) at a nearly constant time $D \approx$ 20 min after termination of replication. Fast-growing or slow-replicating cells, where $\tau < C$, initiate a replication cycle before termination of the preceding one, thus forming multiforked chromosomes containing more DNA (3,4). Under such conditions, an initiation event can occur in the mother or grandmother cell (1). This model, valid for cells growing at a wide range of rates μ (reciprocal of τ), has survived a half a century, with only minor changes of parameter values (e.g., Bipatnath et al. (5) and Michelsen et al. (6)). This model's conclusions have also been confirmed in other bacteria (e.g., Helmstetter (7)). A cell cycle is divided into three periods by two major events between two successive fissions—initiation and termination of replication—that can also occur in reverse order, depending on the values of C, D, and τ (8). A cell grows exponentially and divides on average ca $(C+D)$ min after initiating replication at a nearly constant volume V_i (or 2^n-multiples thereof (1)), simultaneous at all τ-values, where $n = C/\tau$ is the mean number of replication positions (9), at size $V_d = V_i\, 2^{(C+D)/\tau}$.

Applying the age-distribution function $f(a) = \ln 2 \times 2^{(1-a)}$ (10), where $0 < a < 1$, to both cells and replication positions in a steady-state culture (11), the volume V and DNA content G of an average cell are given, respectively, by $V = (V_i \ln 2) 2^{(C+D)/\tau}$ and (12,13) $G = \tau[2^{(C+D)/\tau} - 2^{D/\tau}]/(C \ln 2)$. (See https://sils.fnwi.uva.nl/bcb/ for the CELL CYCLE SIMULATION program, annotated in Zaritsky et al. (4).)

Cell dimensions and aspect ratio

Bacillary bacteria grow by elongation only (2), but faster growing cells are also wider, resulting in a nearly constant average aspect ratio (length/width) $A = L/W$ (14). Approximating such cells to cylinders, $V = \pi(W/2)^2 L$, yields $A = (4/\pi)(V/W^3)$. The changes in cell width during nutritional shifts occur slowly during the division process around the deepening constriction sites, thus forming temporarily tapered cells (15,16). As of this writing, the mechanism governing W changes is unknown; however, it must involve some signal transduced to the peptidoglycan biosynthetic system (17).

Editor: Zemer Gitai.
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http://dx.doi.org/10.1016/j.bpj.2015.06.026

As is common in biological systems, the variance of cell-length distribution at later stages is anticipated to be larger, but the variation at division is smaller than those at earlier events in the cycle (18). Koppes et al. (19) proposed that cells initiate constriction after a constant length increment after initiation of DNA replication or between two successive divisions (20). A recent surge of articles (21–24) resurrects this old question; analyses of high throughput results suggest that a steady-state growing culture maintains a stable size distribution by adding a constant, growth-rate-dependent, mean incremental length ΔL, which is equal to the mean length of a newborn cell L_b, each generation irrespective of its real size at birth. This mode of division, observed in live cells of various symmetrically dividing species (21–24), seems to result in size homeostasis.

Does nucleoid complexity determine cell dimensions?

Just as cell size is fixed by V_i and the periods C, D, and τ, the cell-width W has been proposed to be determined by nucleoid structure by means of a still-unknown mechanism (25). The average amount of DNA (in genome equivalents G) that is associated with a *terC* (termed nucleoid complexity (NC), or $NC = G/terC = [\tau/(C\ln 2)]\ [2^{(C+D)/\tau} - 2^{D/\tau}]/2^{D/\tau} = [\tau/(C\ln 2)]\ (2^{C/\tau}-1) = (2^n-1)/(n\ln 2)$, has been implicated in determining cell-width W (25,26), but the supporting data have been weak and scarce.

Analysis (Table 1) of the results reported recently by Taheri-Araghi et al. (24) conclude that both ΔL and W are correlated with NC, thus generating a constant aspect ratio L_b/W, termed here "cell -shape homeostasis". The practically identical ratio W/NC, 0.404 μm (SD = 0.01; coefficient of variation (CV) = ~2.5%), over a threefold range of doubling times studied, $17.1 < \tau < 51.3$ min, reinforces the idea that nucleoid structure, expressed as NC, affects W. It is furthermore supported by the observation (4,26) that *thyA* mutant cells in which chromosome replication time C is prolonged by lowering the thymine concentration without changing τ are also wider (26,27), presumably for the same cause (namely, a higher weighted-mean NC [$= (2^n-1)/(n\ln 2)$]). Width of stationary cells with a single non-replicating nucleoid after slow growth in poor media is only slightly larger (Fig. 3 in Woldringh et al. (25), and C.L. Woldringh, Amsterdam University, personal communication, 2015) than the predicted 0.404 μm, also consistent with the hypothesis.

The ratio between the length-increment ΔL and NC, $\Delta L_{avg}/(G/terC)$, remains surprisingly constant at 1.485 ± 0.09 (CV = 6.1%). The constant cell aspect ratio A ($= L_b/W$), 3.722 ± 0.271 (CV = 7.3%), implies a larger relative increment in cell volume than in length ($\Delta V/V > \Delta L/L$) as μ rises because a cylinder's volume is a function of the radius squared $(W/2)^2$. The constant A implies that the growth-rate dependent ΔV is accommodated equally in three dimensions (x, y, and z axes), two of which are perpendicular to the length axis L. Thus, describing cell size by its length is only valid under steady state at a certain growth rate. The small (2.4%) coefficient of variation in the ratio W/NC suggests that NC directly affects W, but a mechanism, to date, is sorely lacking.

Inconsistencies

The relation between cell width and nucleoid complexity is consistent with the previously published learned guess (4,25,26). The analysis here is based on the following geometrical and physiological considerations: a cell is regarded as a cylinder. It divides at a constant time after

TABLE 1 Dimensions of *E. coli* cells growing at varying rates with different nucleoid complexities

τ (min)[a]	μ (h^{-1})[b]	L_b (μm)[a]	ΔL (μm)[a]	$(\Delta L + L_b)/2$ (μm)[a]	W (μm)[a]	$n = C/\tau$ (C = 44)[c]	$NC = G/terC$[d]	$\Delta L_{avg}/NC$[e]	W/NC[e]	$\Delta L_{avg}/W$[e]
51.35	1.17	2.08	2.03	2.055	0.55	0.857	1.366	1.5044	0.4026	3.7364
50.85	1.18	2.27	2.13	2.200	0.56	0.865	1.370	1.6058	0.4088	3.9286
37.70	1.60	2.11	2.17	2.140	0.64	1.167	1.540	1.3896	0.4156	3.3438
30.15	2.00	2.36	2.40	2.380	0.71	1.459	1.730	1.3757	0.4108	3.3521
26.65	2.25	2.88	2.90	2.890	0.72	1.651	1.870	1.5455	0.3851	4.0139
22.50	2.67	3.34	3.27	3.305	0.85	1.956	2.124	1.5560	0.4002	3.8882
17.10	3.51	3.98	3.91	3.945	1.04	2.573	2.776	1.4211	0.4042	3.7933
Mean								1.4854	0.4039	3.7223
SD								0.090	0.0098	0.2710
CV (= SD/Mean)								0.061	0.0243	0.0728

L_b, mean cell length at birth; ΔL, mean incremental cell length from birth to division; $\Delta L_{avg} = (\Delta L + L_b)/2$, i.e., average, normalized cell length at birth. (Note that ΔL and L_b must be identical. They were measured separately. The small differences between the determinations were averaged/normalized.) W, Cell width (diameter).

[a]Measured data points were taken from Taheri-Araghi et al. (24).

[b]Mean growth rate μ (in h^{-1}) is reciprocal of doubling time τ (=60/μ; in min).

[c]Mean number per nucleoid of replication positions (9) n (= C/τ), where C (= 44 min) is the time (1,24) to replicate the chromosome, bidirectionally from *oriC* to *terC*.

[d]$G/terC$ (= nucleoid complexity), the amount of DNA in genome equivalent units (G) covalently attached to the chromosome terminus (*terC*), calculated from $(2^n-1)/(n\ln 2)$, with C = 44 min in all cases.

[e]Calculated ratios from previous columns.

initiating a cycle of chromosome replication with doubling of volume. Cell volume grows exponentially and DNA replicates linearly. Thus, a cell's aspect ratio A is proportional to V/W^3, and because both dimensions are related to NC [$= (2^n-1)/(n\ln 2)$], L in the first power and W in the second, both V and W^3 are scaled to NC^3, which results in a constant A irrespective of τ, as reported here (see last column in Table 1). However, cell-volume V has repeatedly been calculated (e.g., Amir (21)) to conform to $V_i 2^{(C+D)/\tau}$, hence A should be related to $2^n 2^{D/\tau} n^3/(2^n-1)^3$, which is inconsistent with our finding (data not shown). The only parameter that has not been determined in Taheri-Araghi et al. (24) is D, hence one wonders whether it really does not change with τ (1,13), at least in this studied strain. Mutant cells with longer D period are indeed larger (28), and the degree of diameter W flexibility varies among strains (mentioned and discussed in Zaritsky et al. (26); and see Begg and Donachie (29)). This apparent contradiction hints to at least one other factor involved in determining cell shape, which may be discovered by resolving the seeming paradox.

Conclusions

Constant ΔL may be overridden by a type of fail-safe mechanism envisioned to ensure proper DNA segregation (23). Is it related to nucleoid occlusion (30,31), or to the nucleoid acting as a "molecular ruler" (23)? The answer needs not be mutually exclusive, and hence the observed correlations among ΔL, W, and NC restore the classical view (32) that cell-size control and cell-cycle control are coupled.

Finally, it is envisaged that just as M_i aligns all existing *oriC*s to initiate synchronously when cell size reaches a constant $V_i/oriC$, so does NC ($= G/terC$) for cell dimensions through width. The mechanisms for both rules are still to be deciphered in biophysical and biochemical terms. (See the Supporting Material for a proposed mechanism.)

SUPPORTING MATERIAL

Supporting Material is available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(15)00609-8.

ACKNOWLEDGMENTS

This Letter is dedicated to the memory of my Ph.D. mentor (1969–1971), the late Robert H. (aka Bob) Pritchard (1930–2015). I am indebted to Conrad L. Woldringh for initiating the idea and four decades of cooperation, and Charles E. Helmstetter for 45 years of inspiration and encouragement. Waldemar Vollmer, Alfonso Jiménez Sánchez, and Assaf Zaritsky are gratefully acknowledged for enlightening remarks, the latter for statistical help as well. Ariel Amir's suggestions and remarks significantly improved this Letter.

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SUPPORTING MATERIAL

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Implications, predictions and proposed mechanism

The idea that nucleoid complexity plays a major role in determining cell dimensions in bacteria predicts that the distance between external nucleoid border(s) and cell pole(s) is (are) larger in large cells than in small cells, consistent with published analysis (S1).

Nucleoid complexity *NC* (26), expressed as *G*/*terC* (25), changes continuously during the cell cycle, with a single jump at replication-termination to half its value an instant earlier when the replicating chromosome turns into two (1,8). If the critical value of the presumed *NC* signal affecting cell width *W* is sensed at termination *D* min before daughter cells separation, implying continuous signal-sensing, *W* will correspondingly vary during the cell cycle. The constant *W* in a steady-state culture (2) and its relation to *NC* under varying growth rates (Table 1) hint that the signal is age-weighted during the whole cycle but relayed to the forming divisome around the time of replication-termination. For this reason, and because the two possibilities are linearly related, which one is chosen does not make a conceptual difference; for simplicity we remain with scaling to the average value $(2^n-1)/(n\ln 2)$.

A possible answer to the immediately-arising question how a cell 'averages' during its life cycle a dynamic feature such as *NC* lies in the refractive nature of peptidoglycan structure: cell width can only be modulated during a limited time – the division process (15,16). The data describing the kinetics of dimensional rearrangements during such transitions are qualitatively consistent with an average: the new steady-state cell dimensions are reached after several division cycles whereas it takes *C* and (*C*+*D*) min to achieve the new steady-state, post-transition values of *NC* and cell volume respectively (https://sils.fnwi.uva.nl/bcb/ ; *eg*, 4). The observed variability of ΔL (24) may partially reflect suspected variations in *C* – another testable, quantitative prediction of the model presented here.

The signal is thus presumed to be relayed continuously and its effect averaged until nucleoids segregate and constriction is initiated. Such an explanation, which is consistent with larger width of stationary cells after growing faster in richer media (unpublished observations by CL Woldringh), can be handled analytically for the long periods required to reach new steady-state cell width during nutritional shifts (15,16) or thymine steps (26,27). This concept directs attention to sorely lacking signals transmitted from the replicating chromosome to the elongating and constricting sacculus during growth and division. Such instruction, if experimentally confirmed, will add a function to the many already attributed to DNA.

Several questions arise: are the discovered correlations between cell dimensions and *NC* fortuitous or do they indicate a yet-unresolved mechanism? If the latter, what does it involve? What causes a cell growing by elongation to widen upon a nutritional shift to a richer medium and how is this widening triggered by the nucleoid structure?

The *primary* signal conveyed from the nucleoid to the peptidoglycan synthetic machinery was envisaged (S2) to be of a physical nature, namely transertion (30): coupled transcription / translation of genes encoding membrane proteins and insertion of these proteins into the membrane. It may be related to a presumed crucial role played by DNA dynamics (replication, transcription, segregation, partition) affecting the biosynthetic activities of the peptidoglycan (elongation, constriction, division; see *eg* (17)), two singular molecules in a bacterial cell, the duplications of which must be precise and coordinately regulated. This interaction is one of the last remaining fundamental questions in basic bacteriology; the correlations found (Table 1) may hint to the direction our attention should be attracted.

The alternative mechanism proposed recently (24), that a Proteome sector is involved in determining ΔL, is doubtful: (a) identical growth rates with similar cell dimensions can be reached in different media compositions that necessarily result in different protein profiles (S3,S4); (b) precise segregation of daughter nucleoids between daughter cells is highly unlikely to depend on a sloppy process such as the partition of numerous proteins, the precise partition of which is not crucial; (c) constant ΔL was observed even in nucleate Δ*minC* mutant cells displaying a highly distorted division but proper nucleoid segregation (23); (d) if "the average P-sector proteins per cell is constant with respect to nutrient-imposed growth rate" (24), it is unable to explain the change of ΔL with growth rate μ (Table 1). The undetailed and vague Proteome model is theoretical, indefinite and hence untestable quantitatively.

The suggestion presented here is simpler: both cell dimensions, length *L* and width *W* depend on a single factor, *NC* that is constant in any particular growth rate μ $(= \tau^{-1})$ and proportional to μ, as observed (Table 1; (24)). It may serve as 'a measuring stick' to which both are related: newborn cells contain each a nucleoid with similar amounts of DNA, and the length-increment ΔL added during an inter-division time τ enables proper segregation and partition of the daughter nucleoids and cell division perpendicularly in between.

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