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.

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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).

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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.

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