SUPPORTING MATERIAL

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

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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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