Step by step, cell by cell: quantification of the bacterial cell cycle

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Abstract

The *Escherichia coli* cell cycle is a classic, but we are still missing some of its essential aspects. The reason is that our knowledge is mostly based on population data, and our grasp of the behavior of single cells is still very limited. Today, new dynamic single-cell data promise to overcome this barrier. Existing data from single cells has already led to findings and hypotheses that challenge standard views, and raised new questions. Here, we review these recent developments and propose that a systematic exploration of the correlation patterns between "cell cycle intervals" defined by key molecular events measured in many single cells could lead to a quantitative characterization of the cell cycle in terms of inherent stochasticity and homeostatic controls.

Keywords: Cell-cycle, Division Homeostasis, E. coli, Stochasticity, Key intervals, Replication initiation

1. The cell cycle is a feature of single cells.

Based on all the research on the cell cycle of *E. coli*, We might think that we know a great deal about this problem. And to some extent, this is true. The discovery of a key role of **replication initiation** (see Glossary) dates back to work from the late 1960s [1, 2], and by the early 1990s most of the molecular players that we consider relevant today had been identified [3].

However, it is not difficult to convince ourselves that we are missing some essential aspects of the problem. The reason is that most of the information in our possession comes from bulk **population measurements** and indirect inference. In this Opinion piece, we argue that today it is the right moment to revisit the *E. coli* cell cycle exploiting dynamic **single-cell measurements**. Such experiments are challenging, as they require efficient imaging and cell segmentation-tracking methods, but are now possible at high throughput, and with microfluidic control of nutrient exchange. Used in combination with reporters of molecular events and new theoretical models, they they provide a unique opportunity to build a new basis for understanding the cell cycle.

This data will be more and more accessible in the coming years, but the recent literature leaves us with a wealth of conclusions and models that seem incompatible and incoherent. The main hypotheses that we put forward in this opinion article is that (i) this apparent incoherence can be overcome and is not due to intrinsic limitations of the approach, and (ii) it is a smoking gun for the need of more comprehensive and precise quantitative methods in both data analysis and theoretical descriptions. Such tools are necessary to properly handle the large amount of data and, most importantly, to formulate

and test falsifiable models of cell cycle control. This approach can be applied broadly, including eukaryotes [4, 5, 6].

2. The average cell is not the typical single cell

From the pioneers of quantitative bacterial physiology of the "Copenhagen School" [7], empirical observations in terms of quantitative relations between physiology-related variables averaged over large populations have been used to infer specific control mechanisms of the cell cycle [8, 7]. The problem is that the average cell behaviour does not correspond necessarily to the typical behaviour of single cells. Therefore, models based on population averages have limitations, and ought to be revisited and tested with single-cell data. The classic example is the model proposed by Donachie [2] in the 1960s, stating that DNA replication is initiated at a critical mass per replication origin. As we will discuss, although appealing and describing properly the measured cell-cycle response to changes in growth media, this model may be insufficient to describe recent single-cell measurements. In other words, there are unique specific behaviors of single live cells that are obscured if we average everything.

For the cell cycle to progress, events related to DNA replication and **segregation**, metabolism, growth and cell division must occur in a specific time order for each cell (across many divisions along lineages [9, 10]), despite considerable molecular noise and variability of parameters. This hierarchy has major impact on single cells, but may be missed drawing data from population averages. For example, a missed **septation** due to late segregation may lead to failure of cell division, formation of a filamentous cell and subse-

quent rescue, which can be accompanied by a non-symmetric division, with consequences on the balance of cell size, DNA amounts and cell-cycle regulators observable over several generations. However, these events typically affect a small fraction of the population, and their consequences would not be observable in population averages, unless most of the population becomes filamentous. Therefore, dissecting such a cascade of errors and controls would be impossible without a single-cell view, since the complex temporal interplay of several concurrent processes and the important role of **stochasticity** are hidden by population averages

3. From phenomenological to mechanistic models

A clear sign that the application of high-throughput single-cell techniques is effective comes from recent work. These measurements have already unveiled the stochastic nature of metabolism and resulting growth [11], intriguing universal properties of the joint distribution of cell size and interdivision times [12, 13], as well as an effective principle where cells add, on average, a constant volume to their initial one every cell division (sometimes called the **adder mechanism**) [14, 15, 12, 16], which is consistent with long-term **homeostasis** of the cell-size distribution in a population.

However, the molecular basis of this observation remains unknown. Indeed, several phenomenological models can in principle reproduce the empirical observation of a constant average added size. For example, a "concerted" control of cell division based on cell size and on time [17, 13, 18, 19] as well as a completely different mechanism based on the ratio between cell surface and volume [20] can both reproduce this behaviour. Thus, the recent papers

leave us with a rather obscure picture. Clearly, part of such differences may be due to sensitivity of measurements to differences in growth conditions, genetic background, experimental and analysis methods, and so on. However, we argue that insufficient integration of theoretical approaches and data also plays a large role, and the lack of a general theoretical framework to interpret the data (and compare and falsify models) also limits the experiments. Therefore, it is necessary to link more closely theoretical models to data on one side and to molecular mechanisms on the other.

4. Cell-cycle intervals

To move our mathematical descriptions towards more specific biological mechanisms, a first step is to focus the analysis on specific cell cycle events that have been directly linked to molecular controls. Indeed, a common way to describe qualitatively the progression of the cell cycle [1, 22, 23] is to define **cell-cycle intervals** (Fig. 1), by key landmark events (e.g., replication, formation of the Z-ring, septation, etc.), and establishing their relative timing and connection with global observables such as cell growth rate, size, total protein concentration, as well as with concentration of selected metabolic or cell-cycle proteins (e.g., a reliable reporter of the initiator protein DnaA [24, 25]).

Note that cell-cycle intervals may span multiple consecutive generations, and are not necessarily defined within two consecutive divisions. For example, it might make sense to consider the events of completion of a successful segregation and onset of septation in one cycle, and link them to the event of replication initiation in the following one, or consider the period between ter-

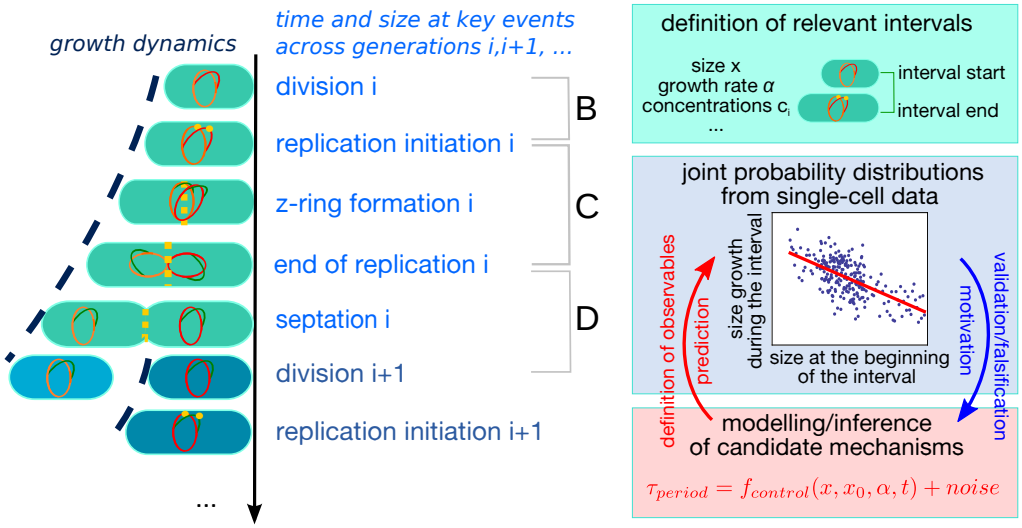

Figure 1: Key events in the *E. coli* cell cycle define cell-cycle intervals subject to stochastic variation and exerting homeostatic control. The left panel illustrates some of the key cellular events (replication cycle, Z ring formation, septation, etc.) which may be used to define intervals across generations (indexed as $i, i+1, ...$). Cell-cycle intervals may not span just the time between two cell divisions, but can be defined across consecutive generations. The right panels illustrate the outlook for future analyses supported in this opinion paper. First (top panel), cell-cycle intervals are defined, and values of key variables (including size x) are collected at the beginning of each interval. Second, joint distributions of measured variables are used to motivate a mathematical model. The example plot is the joint distribution of size growth and initial size in an interval, conventionally used to detect size homeostasis [21]. Third (bottom panel) mathematical models define key observables and falsifiable predictions to test in the data. The formula in the example relates the duration of an interval τ to stochasticity ("noise") due to intrinsic cell-to-cell variability of cellular parameters, and homeostatic control based on measurable variables. Reciprocal feedback between falsifiable models and data helps identifying mechanisms and molecular players of cell-cycle progression.

mination and initiation (typically in the next generation), where ATP-DnaA is supposed to increase [26, 27]. More trivially, it is well known that the timing of replication, the "C period", can be longer than the interdivision time [1, 28], since fast-growing *E. coli* cells support multiple DNA replication rounds at the same time, and thus the replication initiation in one cell cycle will lead to a complete chromosome in a subsequent cycle of a daughter or grand-daughter cell. Reporters of at least some of the key players of the cell-cycle are at hand, thanks to many previous studies characterizing several aspects of the E. coli cell cycle [29, 30, 31].

The cellular process or event defining cell-cycle intervals can both cause an increase of cell-to-cell variability in size (by an intrinsic stochasticity due to biochemical noise, e.g., the timing of the completion of replication for the C period varies from cell to cell) and correct for this increase in variability by enacting decisions on the cell cycle (for instance by compensatory effects that suppress variability, e.g., B period duration may be shorter on average for cells that are born larger.) Cells adjust the cell-cycle to respond to specific needs (e.g. conditioning division to successful nucleoid segregation), creating a structure of correlations between interval durations and measurable parameters. While we still have to fully learn how to decipher such trends, we know that they enable the production of testable (and falsifiable) quantitative mathematical descriptions of the cell cycle.

Note that organizing the cell cycle into discrete events may be difficult, and this simplification has its limitations. For example intervals might be hard to define for chromosome segregation, which is a multi-step process [32]. Equally, reporters of expression of cell-cycle proteins do not define intervals

per se, but they rather exhibit temporal oscillations or specific spatial organizations. More in general, protein expression and spatial distributions can be correlated with cell cycle processes and events.

5. Studying single cells can challenge long-standing hypotheses.

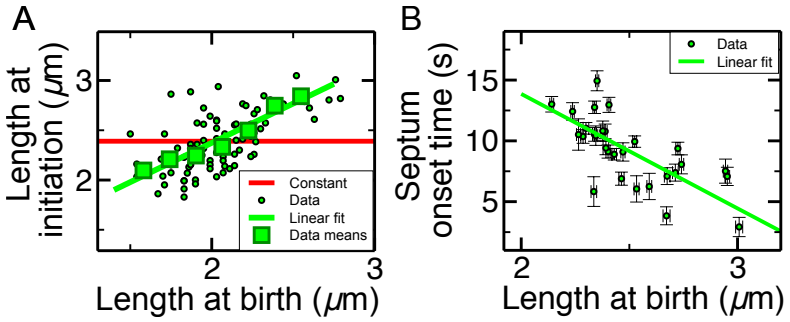

Figure 2: Examples of insights from correlation analysis of cell-cycle intervals. A: Available data for replication initiation in single cells challenge the hypothesis of initiation at a critical cell size (mass). The plot (data from ref. [18]) shows the cell size at replication initiation (estimated by the appearance of SeqA-GFP foci) versus the initial cell size (estimated by cell length, cell width being constant across cells). Each circle corresponds to a different cell cycle. A critical mass model (red line) would predict that the initiation size is the same regardless of size at birth. Instead, while there is some control on size, cells tend to be larger at initiation if they are born larger (green, squares are binned averages of data, solid line is a linear fit). B: Septum onset time enforces size homeostasis. The plot (data from ref. [33]) shows the septum onset time (measured by cell shape segmentation [34]) versus the initial cell size. A pure characteristic septation time would show no correlation with length at birth, but the existence of a correlation suggests a correction mechanism. A recent study on perturbations of volume and surface growth rates supports this observation [20].

A number of recent studies have produced data that are already challenging existing models, and highlight the importance of further investiga-

tions [33, 23, 18]. One example is the "licensing hypothesis" for replication initiation [23], which, based on observations on single cells, proposes that septation or occurrence of cell division may license (by activating the origin or releasing an inhibitory signal) the chromosomes for the next round of replication initiation (and unlicensed origins cannot initiate).

Concerning the timing of replication initiation and cell division, Donachie [2], based on population data [35, 1], proposed that DNA replication is initiated at a critical mass per replication origin. Notably, this author was very aware that the critical mass hypothesis applies *on average*, in response to changes in the external conditions, and need not be valid for single cells in a fixed condition. Additionally, theoretical descriptions that do not comply with the critical mass hypothesis are present in the literature. For example, a recent modeling study [36] argues that initiation may occur after a constant size has been added between consecutive initiations.

Two recent studies used fluorescent labeling of replication forks to measure replication initiation in single cells [18, 19]. Both studies show size compensation in the B period, i.e., cells that are born larger than average initiate earlier. This supports a link between replication initiation and size homeostasis, by initiation following cell size, by initiation controlling cell division, or both.

A similar correlation with size was observed in one study for the D-period between termination and division [18]. Whether size compensation at initiation is governed by a critical size remains incompletely resolved. Both data sets show that initiation size increases with initial size in slower growth conditions (Fig. 2). The Wallden *et al.* data at faster growth rate suggest that

initiation size is independent on initial size, leading them to argue in favor of a constant initiation volume model also for the slower growth rates [19], and attributing the correlation to missed initiation events before birth, in the mother cell. Initiations could occur before cell division without being detectable by SeqA foci, or be inhibited by a licensing-like phenomenon (see above and ref. [23]), visible. However, these events of initiation before division are not clearly characterized in current studies. In comparing different studies, one must also consider possible effects of growth conditions, strains used, and labelling schemes: one can for instance label all SeqA proteins [19], or only a fraction of them [18], or label different proteins involved in the replication process, such as DnaQ [19].

Thus, new tests and measurements are necessary, together with theoretical frameworks to clearly test or falsify alternative models. Importantly, the interpretation differences between the two studies is also reflected in differences in the mathematical models that were used and shown to be consistent with the data. While one model assumes a control without a precise critical mass for the B period and a size-coupled D period [18], the other assumes a critical mass at initiation and a duration of the sum of C and D period that is coupled to single-cell growth rate and not cell size [19]. A third model, mentioned above, suggests a constant added size per origin between initiations [36]. Of course, not all predictions of these models are expected to coincide and and it would be fruitful to compare them in further detail.

A second example of useful information from cell-cycle intervals at the single-cell level is the hypothesis of a role of septum formation in size homeostasis. Single-cell analysis (Fig. 2) indicates that the cell-cycle interval from

cell birth to onset of septation (measured by cell segmentation) may be size-dependent [33]. More recent results [20] have led to speculation that septum formation may be the main ("rate limiting") checkpoint in deciding cell division in most conditions. A size-coupled septation mechanism corresponds to the hypothesis that replication might not be the driver, or the only driver of the coupling between cell division and size (as assumed in the models of refs [36, 19, 18], whereas the model by Harris and Theriot [20] takes the complementary assumption that septation is the only driver of cell division). Conversely, the cell-cycle interval defined by the timing between onset of septation and cell division fluctuates around a constant value, independent of the total interdivision time, much like the C period [33].

In conclusion, these studies illustrate the gaps of knowledge regarding the cell cycle at the single-cell level, provide preliminary answers, and show the potential of correlating events and processes in single cells.

6. How do cell-cycle intervals add up to produce size homeostasis and cell-cycle control?

Deeper knowledge of the most relevant cell-cycle intervals, reflecting key processes such as replication cycle and Z-ring contraction, will help in answering how different controls contribute to size homeostasis, a constant added size, and the observed scaling of cell sizes and interdivision times [13]. From the biological viewpoint, characterizing the cell cycle is a broader aim than mere characterization of cell-size homeostasis, but understanding the link between cell-size distribution, metabolism and key molecular determinants may have important implications. Taking the example of the observed con-

stant added size [12, 15], one may link this behavior to a classic "initiator" model [37] where the key step (replication initiation) is triggered by the accumulation of an initiator protein to a constant copy number, and not a constant concentration, which is compatible with the observation that the total amount of active DnaA appears to be relevant for initiation timing in *E. coli* [29]. However, several processes may contribute to the decision to divide [38]. Besides the process of replication initiation by DnaA [26, 27], the division triggering of the Z-ring [22, 39], conditioned on successful segregation [40], as well as metabolic cues [30, 41] and septum synthesis [20] have all been linked to cell division.

Analysis of the concerted action of these control mechanisms should show whether the decision to divide is captured by a single "rate limiting" principle [20], whether different controls may be rate limiting in different conditions [19], or whether parallel controls are active on overlapping time scales. Incidentally, none of the intervals defined by DNA replication (B,C,D periods) appear to obey constant added size in slow-growing cells, but a model incorporating the observed couplings of net elongation during B,C,D, with size at the beginning of each period [21] does reproduce the overall constant added size behavior [18].

Cells might want to try to add a constant size for many reasons, such as (i) an architectural constraint (due, e.g., to cell-wall growth [20]), (ii) a consequence of how the control mechanism is effected at the molecular level [37, 12] or (iii) by a circuit that measures asolute size, but happens to show this behavior [19]. A complementary possibility is that observations such as the constant added size or the scaling of size and doubling time

fluctuations could be the result of selection, such as optimization for colony growth or lineage expansion or ecosystem-level interactions [42], or avoidance of detrimental effects such as waste accumulation.

7. Concluding remarks and future perspectives.

Perhaps the most important feature of the future direction we propose is that it is fully quantitative, and benefits from integration and feedback between theoretical predictions and experiments that is not yet achieved in the field. We see this area as a major challenge for the applications of statistical physics to biology. Precise quantitative measurement of the key players with sufficient statistics (see Outstanding Questions) can be achieved, with the ultimate goal of producing mathematical equations capturing coherently all observed behaviors and able to predict phenotypes at the single-cell level. New theoretical work should compare alternative mechanisms, identify which observatbles are central to distinguish, and determine the precision/statistics needed in the experiments for optimal measurements [43].

A quantification of cell-cycle intervals in single cells can be complemented by their change in response to the external conditions, mutations, and other perturbations such as arresting replication, depleting DnaA, overexpressing both functional and non-functional proteins, etc. [20, 44, 45, 46]. For example, nutrient shifts were used classically to look at cell division dynamics [47, 48], but they can potentially give a wealth of further information in single cells. A further challenge will be to understand adaptation behavior in non-steady conditions and linking this dynamic behavior to the homeostatis observed in fixed environments.

Finally, focusing on cell-cycle intervals that are closely linked to molecular mechanisms would give us a minimal but fully mechanistic description of the available data. Important limitations are apparaent as well. First, the tools for linking conditional dependencies between experimental observables to mathematical models still need to be fully developed. Second, correlations do not necessarily reveal causality. Third, correlations may be sensitive to external conditions and genetic background. However, we believe that such a development, combined with molecular biology and biochemistry, will bring us closer to a mechanistic description of the cell cycle at the single-cell level.

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Outstanding Questions Box

What are the key molecular events in the cell cycle of single *E. coli* cells and how do they compromise or promote homeostasis of basic parameters such as cell size, protein concentration, and DNA copy number?

How are metabolic signals and housekeeping events (replication, segregation, etc.) integrated to decide when to divide?

Does the observed constant added size mechanism emerge from the integration of multiple decisions or is it the result of a single process?

TRENDS BOX

[900 characters / 3-5 bullet points]

The cell cycle is stochastic due to intrinsic cellular noise, affecting decision-making related to key steps (i.e., initiation of replication, chromosome segregation, Z-ring contraction and septation)

Recent high-throughput single-cell measurements of growing *E. coli* show a constant average added size between consecutive cell divisions.

Similar measurements allowing the full stochastic unraveling of the *E. coli* cell cycle will likely become available in the coming years.

These data will open new perspectives and challenge classic views, starting from the long-standing hypothesis that a critical mass per origin triggers replication initiation.

GLOSSARY

Adder mechanism: The hypothesized mechanism by which *E. coli* cells tend to add a constant volume or mass to the initial size to decide the moment of cell division. This mechanism enforces size homeostasis [15, 12]

Cell-cycle interval: Defined here as the period of time between two key events in the cell cycle (Fig. 1). For example, three cell-cycle intervals are classically defined with respect to DNA replication: the B,C,D sperated by replication initiation and the end of replication.

DnaA: ATP-ase protein that accumulates in its active ATP-bound form to a threshold value during the cell cycle inducing DNA melting by binding cooperatively to the origin(s) and thus triggering initiation of DNA replication [26].

Homeostasis: The process through which single cells control key variables (such as size, concentrations) in order to ensure their stability along lineages. There is, in general, a difference between homeostasis in fixed conditions and the average response to a perturbation.

Population measurements: Measurements of average quantities over large cell populations. Most of growth-related laws in bacterial physiology are based on such measurements [8], typically for exponentially growing populations. For example, the typical population estimate of the average cell size consists in a measurement of optical density divided by a cell count [7].

Replication initiation: The start of DNA replication, defining the

end of the B period in bacteria, and corresponding to the G1/S transition in mammalian cells.

Segregation: The process of disentanglement and separation of duplicated chromosomes necessary to ensure a chromosome copy to each daughter cell.

Septation: Formation of a cell wall that constricts the cell (approximately in the middle for symmetrically dividing bacteria like *E. coli*) and leads to new cell poles.

Single-cell measurements: Experiments following dynamically many cells with single-cell resolution, monitoring size, shape and fluorescent probes, and allowing to quantify the cell-to-cell variability and correlations.

Stochasticity: In the context of cell cycle events, represents the tendency of cell-cycle progression to be different in each individual cell, due to values of internal variables (e.g. key protein amounts or concentrations) and molecular noise. As a consequence, mathematical models have to describe the cell-cycle progression as a stochastic process, typically representing the interplay of cell-to-cell variability and homeostatic control.

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[1] S. Cooper, C. E. Helmstetter, Chromosome replication and the division cycle of *Escherichia coli* B/r, J. Mol. Biol. 31 (3) (1968) 519–40.

[2] W. D. Donachie, Relationship between Cell Size and Time of Initiation of DNA Replication, Nature 219 (1968) 1077–9.

[3] W. D. Donachie, The cell cycle of Escherichia coli., Annu Rev Microbiol 47 (1993) 199–230. doi:10.1146/annurev.mi.47.100193.001215.

[4] M. B. Ginzberg, R. Kafri, M. Kirschner, Cell biology. on being the right (cell) size., Science 348 (6236) (2015) 1245075. doi:10.1126/science.1245075.
URL http://dx.doi.org/10.1126/science.1245075

[5] K. M. Schmoller, J. J. Turner, M. Kõivomägi, J. M. Skotheim, Dilution of the cell cycle inhibitor Whi5 controls budding-yeast cell size., Nature 526 (7572) (2015) 268–272. doi:10.1038/nature14908.
URL http://dx.doi.org/10.1038/nature14908

[6] S. Son, A. Tzur, Y. Weng, P. Jorgensen, J. Kim, M. W. Kirschner, S. R. Manalis, Direct observation of mammalian cell growth and size regulation., Nat Methods 9 (9) (2012) 910–2. doi:10.1038/nmeth.2133.

[7] M. Schaechter, A brief history of bacterial growth physiology., Front Microbiol 6 (2015) 289. doi:10.3389/fmicb.2015.00289.
URL http://dx.doi.org/10.3389/fmicb.2015.00289

[8] H. Bremer, P. P. Dennis, Modulation of chemical composition and other parameters of the cell at different exponential growth rates., EcoSal Plus 3 (1). doi:10.1128/ecosal.5.2.3.
URL http://dx.doi.org/10.1128/ecosal.5.2.3

[9] M. Hashimoto, T. Nozoe, H. Nakaoka, R. Okura, S. Akiyoshi, K. Kaneko, E. Kussell, Y. Wakamoto, Noise-driven growth rate gain in clonal cellular populations., Proc Natl Acad Sci U S A 113 (12) (2016)

384 3251–3256. doi:10.1073/pnas.1519412113.
385 URL http://dx.doi.org/10.1073/pnas.1519412113

386 [10] G. Lambert, E. Kussell, Quantifying selective pressures driving
387 bacterial evolution using lineage analysis., Phys Rev X 5 (1).
388 doi:10.1103/PhysRevX.5.011016.
389 URL http://dx.doi.org/10.1103/PhysRevX.5.011016

390 [11] D. J. Kiviet, P. Nghe, N. Walker, S. Boulineau, V. Sunderlikova, S. J.
391 Tans, Stochasticity of metabolism and growth at the single-cell level.,
392 Nature 514 (7522) (2014) 376–379. doi:10.1038/nature13582.
393 URL http://dx.doi.org/10.1038/nature13582

394 [12] S. Taheri-Araghi, S. Bradde, J. T. Sauls, N. S. Hill, P. A. Levin, J. Pauls-
395 son, M. Vergassola, S. Jun, Cell-size control and homeostasis in bacteria.,
396 Curr Biol 25 (3) (2014) 385–91. doi:10.1016/j.cub.2014.12.009.

397 [13] A. S. Kennard, M. Osella, A. Javer, J. Grilli, P. Nghe, S. J. Tans,
398 P. Cicuta, M. Cosentino Lagomarsino, Individuality and universality in
399 the growth-division laws of single E. coli cells., Phys Rev E 93 (1) (2016)
400 012408. doi:10.1103/PhysRevE.93.012408.

401 [14] A. Amir, Cell size regulation in bacteria, Phys. Rev. Lett. 112 (2014)
402 208102. doi:10.1103/PhysRevLett.112.208102.
403 URL http://link.aps.org/doi/10.1103/PhysRevLett.112.208102

404 [15] M. Campos, I. V. Surovtsev, S. Kato, A. Paintdakhi, B. Bel-
405 tran, S. E. Ebmeier, C. Jacobs-Wagner, A constant size extension

406 drives bacterial cell size homeostasis., Cell 159 (6) (2014) 1433–46.
407 doi:10.1016/j.cell.2014.11.022.

408 [16] I. Soifer, L. Robert, A. Amir, Single-cell analysis of growth in budding
409 yeast and bacteria reveals a common size regulation strategy., Curr Biol
410 26 (3) (2016) 356–361. doi:10.1016/j.cub.2015.11.067.

411 [17] M. Osella, E. Nugent, M. Cosentino Lagomarsino, Concerted control of
412 *Escherichia coli* cell division, Proc. Natl. Acad. Sci. (U.S.A.) 111 (9)
413 (2014) 3431–5.

414 [18] A. Adiciptaningrum, M. Osella, M. C. Moolman, M. Cosentino Lago-
415 marsino, S. J. Tans, Stochasticity and homeostasis in the E. coli replica-
416 tion and division cycle., Sci Rep 5 (2015) 18261. doi:10.1038/srep18261.

417 [19] M. Wallden, D. Fange, E. G. Lundius, Ö. Baltekin, J. Elf, The synchro-
418 nization of replication and division cycles in individual E. coli cells., Cell
419 166 (3) (2016) 729–739. doi:10.1016/j.cell.2016.06.052.
420 URL `http://dx.doi.org/10.1016/j.cell.2016.06.052`

421 [20] L. K. Harris, J. A. Theriot, Relative rates of surface and vol-
422 ume synthesis set bacterial cell size., Cell 165 (6) (2016) 1479–1492.
423 doi:10.1016/j.cell.2016.05.045.
424 URL `http://dx.doi.org/10.1016/j.cell.2016.05.045`

425 [21] S. Di Talia, J. M. Skotheim, J. M. Bean, E. D. Siggia, F. R.
426 Cross, The effects of molecular noise and size control on variability
427 in the budding yeast cell cycle., Nature 448 (7156) (2007) 947–951.

doi:10.1038/nature06072.
URL http://dx.doi.org/10.1038/nature06072

[22] T. Den Blaauwen, N. Buddelmeijer, M. E. Aarsman, C. M. Hameete, N. Nanninga, Timing of FtsZ assembly in Escherichia coli., J Bacteriol 181 (17) (1999) 5167–5175.

[23] D. Bates, N. Kleckner, Chromosome and replisome dynamics in E. coli: loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation., Cell 121 (6) (2005) 899–911.

[24] C. Saggioro, A. Olliver, B. Sclavi, Temperature-dependence of the DnaA-DNA interaction and its effect on the autoregulation of dnaA expression., Biochem J 449 (2) (2013) 333–341. doi:10.1042/BJ20120876.
URL http://dx.doi.org/10.1042/BJ20120876

[25] A. Olliver, C. Saggioro, J. Herrick, B. Sclavi, DnaA-ATP acts as a molecular switch to control levels of ribonucleotide reductase expression in Escherichia coli., Mol Microbiol 76 (6) (2010) 1555–1571. doi:10.1111/j.1365-2958.2010.07185.x.
URL http://dx.doi.org/10.1111/j.1365-2958.2010.07185.x

[26] W. D. Donachie, G. W. Blakely, Coupling the initiation of chromosome replication to cell size in *Escherichia coli*, Current Opinion in Microbiology 6 (2) (2003) 146–50.

[27] M. A. A. Grant, C. Saggioro, U. Ferrari, B. Bassetti, B. Sclavi, M. Cosentino Lagomarsino, DnaA and the timing of chromosome repli-

cation in *Escherichia coli* as a function of growth rate., BMC Syst Biol 5 (2011) 201. doi:10.1186/1752-0509-5-201.

[28] O. Michelsen, M. J. Teixeira de Mattos, P. R. Jensen, F. G. Hansen, Precise determinations of C and D periods by flow cytometry in *Escherichia coli* K-12 and B/r, Microbiology 149 (4) (2003) 1001–10. doi:10.1099/mic.0.26058-0.

[29] N. S. Hill, R. Kadoya, D. K. Chattoraj, P. A. Levin, Cell size and the initiation of DNA replication in bacteria., PLoS genetics 8 (3) (2012) e1002549. doi:10.1371/journal.pgen.1002549.

[30] L. G. Monahan, E. J. Harry, You are what you eat: Metabolic control of bacterial division., Trends Microbiol 24 (3) (2016) 181–189. doi:10.1016/j.tim.2015.11.007.

[31] A.-C. Chien, N. S. Hill, P. A. Levin, Cell size control in bacteria., Curr Biol 22 (9) (2012) R340–R349. doi:10.1016/j.cub.2012.02.032.

[32] N. Kleckner, J. K. Fisher, M. Stouf, M. A. White, D. Bates, G. Witz, The bacterial nucleoid: nature, dynamics and sister segregation., Curr Opin Microbiol 22 (2014) 127–137. doi:10.1016/j.mib.2014.10.001.
URL `http://dx.doi.org/10.1016/j.mib.2014.10.001`

[33] R. Tsukanov, G. Reshes, G. Carmon, E. Fischer-Friedrich, N. S. Gov, I. Fishov, M. Feingold, Timing of Z-ring localization in Escherichia coli., Phys Biol 8 (6) (2011) 066003. doi:10.1088/1478-3975/8/6/066003.

[34] G. Reshes, S. Vanounou, I. Fishov, M. Feingold, Cell shape dynamics in *Escherichia coli.*, Biophysical journal 94 (1) (2008) 251–64. doi:10.1529/biophysj.107.104398.

[35] M. Schaechter, O. Maaløe, N. O. Kjeldgaard, Dependency on medium and temperature of cell size and chemical composition during balanced grown of salmonella typhimurium., J. Gen. Microbiol. 19 (3) (1958) 592–606.

[36] P.-Y. Ho, A. Amir, Simultaneous regulation of cell size and chromosome replication in bacteria., Front Microbiol 6 (2015) 662. doi:10.3389/fmicb.2015.00662.

[37] L. Sompayrac, O. Maaløe, Autorepressor Model for Control of DNA Replication, Nature 241 (1973) 133–5.

[38] L. Robert, Size sensors in bacteria, cell cycle control, and size control., Front Microbiol 6 (2015) 515. doi:10.3389/fmicb.2015.00515.
URL `http://dx.doi.org/10.3389/fmicb.2015.00515`

[39] A. J. F. Egan, W. Vollmer, The physiology of bacterial cell division., Ann N Y Acad Sci 1277 (2013) 8–28. doi:10.1111/j.1749-6632.2012.06818.x.

[40] H. Cho, H. R. McManus, S. L. Dove, T. G. Bernhardt, Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist., Proc Natl Acad Sci U S A 108 (9) (2011) 3773–3778. doi:10.1073/pnas.1018674108.

[41] N. S. Hill, P. J. Buske, Y. Shi, P. A. Levin, A moonlighting enzyme links

Escherichia coli cell size with central metabolism., PLoS Genetics 9 (7) (2013) e1003663. doi:10.1371/journal.pgen.1003663.

[42] A. Giometto, F. Altermatt, F. Carrara, A. Maritan, A. Rinaldo, Scaling body size fluctuations., Proc. Natl. Acad. Sci. (U.S.A.) 110 (12) (2013) 4646–50.

[43] J. Grilli, M. Osella, A. S. Kennard, M. Cosentino Lagomarsino, Relevant parameters in models of cell division controlarXiv:1606.09284.

[44] A. Zaritsky, Cell-shape homeostasis in Escherichia coli is driven by growth, division, and nucleoid complexity., Biophys J 109 (2) (2015) 178–181. doi:10.1016/j.bpj.2015.06.026.
URL `http://dx.doi.org/10.1016/j.bpj.2015.06.026`

[45] H. Zheng, P.-Y. Ho, M. Jiang, B. Tang, W. Liu, D. Li, X. Yu, N. Kleckner, A. Amir, C. Liu, Interrogating the e. coli cell cycle by cell dimension perturbations, PNAS, in press.

[46] F. Si, D. Li, S. E. Cox, J. T. Sauls, O. Azizi, A. B. Schwartz, M. J. Erickstad, Y. Jun, X. Li, S. Jun, Deconstructing cell size control into physiological modules in escherichia coli, bioRxivarXiv:http://biorxiv.org/content/early/2016/10/17/081422.full.pdf, doi:10.1101/081422.
URL `http://biorxiv.org/content/early/2016/10/17/081422`

[47] A. Zaritsky, S. Zabrovitz, DNA synthesis in Escherichia coli during a nutritional shift-up., Mol Gen Genet 181 (4) (1981) 564–566.

[48] A. Zaritsky, C. L. Woldringh, C. E. Helmstetter, N. B. Grover, Dimensional rearrangement of Escherichia coli B/r cells during a nutritional shift-down., J Gen Microbiol 139 (11) (1993) 2711–2714. doi:10.1099/00221287-139-11-2711.
URL http://dx.doi.org/10.1099/00221287-139-11-2711