One is Nothing without the Other: Theoretical and Empirical Analysis of Cell Growth and Cell Cycle Progression☆

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Abstract

Small, fast-growing bacteria make ideal subjects for genetic and quantitative analysis alike. Long the darling of theoreticians, efforts to understand the relationship between cell growth and cell cycle progression in bacterial systems have been propelled by modelers and empiricist in equal measure. Taking a historical approach, here we break down early work in this area, the impact it had on how the bacterial cell cycle is understood and interrogated, and changes brought by the advent of high-throughput techniques for the analysis of individual bacterial cells in culture.

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Introduction

Bacterial model systems are notable for their ability to generate as many as 10^9 isogenic cells per milliliter from a single parent in a matter of hours. Given these large numbers, it is no coincidence that quantitative analysis and modeling are the bread and butter of both classical and modern bacteriology. Models derived from the observation of cells growing in batch culture provide the foundation for molecular and genetic studies examining a wide range of cellular behaviors, from as general as the random nature of mutation [1] to as specialized as the bistability of a developmental decision in *Bacillus subtilis* [2].

An area that has benefited disproportionately from quantitative analysis and modeling is investigations into the relationship between cell growth and cell cycle. In a groundbreaking study in 1958, Schaechter, Maaløe, and Kjeldgaard (SMK) determined that the size of *Salmonella* cells scales exponentially with nutrient-dependent changes in growth rate [3]. Subsequent work by Helmstetter and Cooper [4–6] established a framework for understanding the bacterial cell cycle. Combining data from these earlier studies, Donachie [7] identified a strong correlation between one aspect of cell cycle progression, replication initiation, and achievement of a specific cell size. Quantitative models have both inspired and shaped genetic, molecular, and cytological analysis of cell growth and cell cycle progression for over 50 years.

While these models stimulated efforts to understand the control mechanisms governing DNA replication, division, and cell size, their law like aura and focus on two closely related model systems (*Salmonella* and *Escherichia coli*), together with limitations inherent in genetic analysis of essential processes, significantly restricted the nature and scope of subsequent work.

Here we discuss the genesis of these seminal models, their role as valuable foundations for empirical analysis of growth and cell cycle progression, and their limitations for the same. Special attention is given to recent advances in single-cell analysis that have altered our understanding of the relationship between growth and division, requiring reassessment of old models, and opening new and exciting areas of inquiry.

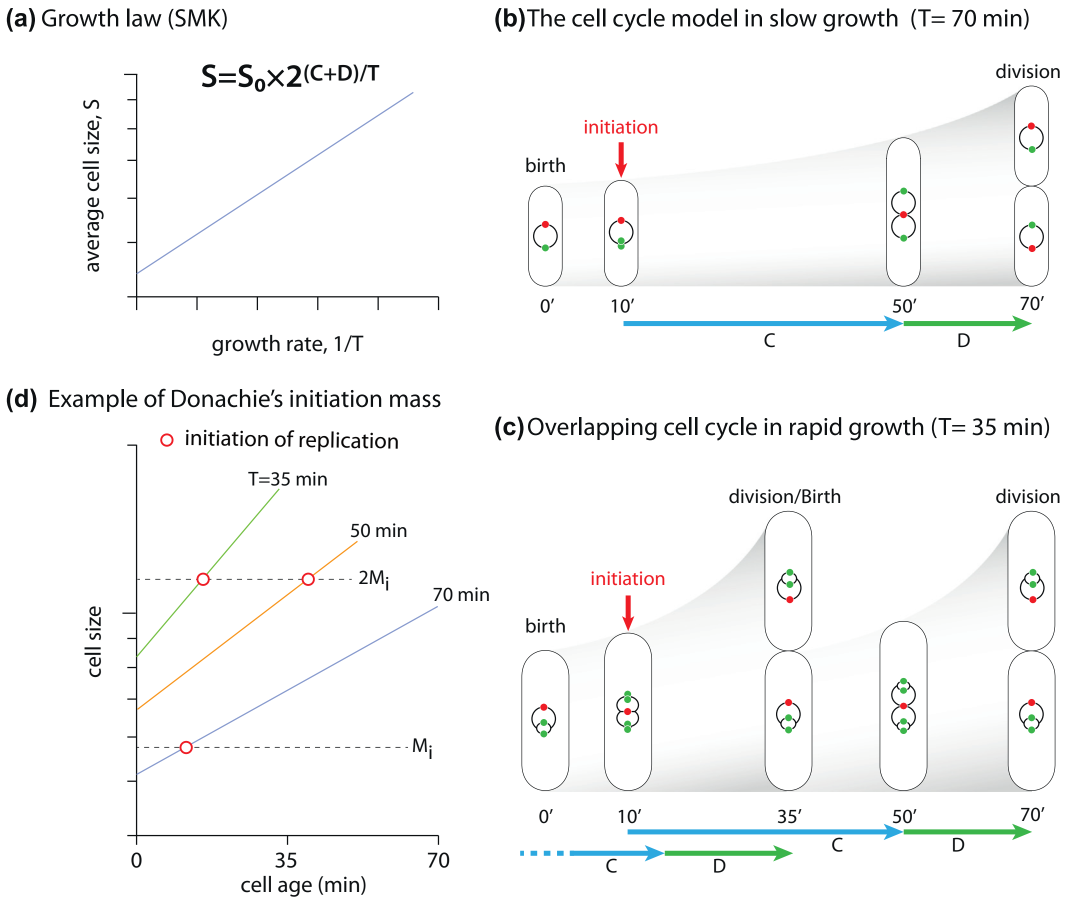


Fig. 1. (a) "The Growth Law": average cell size scales exponentially with nutrient-imposed growth rate, independent of the chemical details of the growth media. The exponent was later determined to be (C + D), the time interval between an initiation of chromosome replication and a cell division. (b) Cooper and Helmstetter model of *E. coli* cell cycle (green dots denote origin of replication, and red dots denote terminus of replication). The time required to complete a round of replication is ~40 min (C-period) followed by a ~20-min gap (D-period) before cells divide. Shown are slow-growing cells that have sufficient time to start and complete a single round of replication within their lifetime. (c) Rapidly dividing cells maintain multiple replication cycles. The time interval between consecutive initiations is shorter than (C + D), but there is a one-to-one correspondence between initiation and division events. (d) Donachie's critical mass model. Combining the growth law (a) with the cell cycle model (b and c), Donachie deduced that the cell size at the initiation of chromosome replication is a multiple of a fixed mass, M_i, and the number of origins of replication.

The Growth Law and Its Corollaries: Three Seminal Papers

Schaechter, Maaløe, and Kjeldgaard (1958): dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*

To gain insight into the relationship between nutrient availability and cellular physiology, SMK assessed the size and composition of *Salmonella* cells during steady-state growth in upward of 30 different nutrient conditions. Using this deceptively simple approach, they determined that DNA and RNA content, as well as average cell size scale exponentially with nutrient-dependent changes in growth rate (Fig. 1a). Dubbed "The Growth Law", this relationship is remarkably independent of the chemical details of the growth media, suggesting that cells balance metabolic flux to maximize growth rate even when individual components are limiting. An exponential increase in size is accompanied by an exponential increase in DNA and RNA synthesis, thereby ensuring that concentrations of both remain constant across growth conditions. Large cells cultured in nutrient-rich medium thus have proportionally more DNA and RNA than smaller, isogenic organisms cultured under nutrient limiting conditions.

Caveats. As SMK point out, it is only nutrient-dependent increases in growth rate that result in increases in size. Experiments in which growth rate was varied by reducing ambient temperature had no impact on cell size or composition. In addition, while the growth law applies to other bacteria including the gram-positive model *B. subtilis* [8,9], not all bacteria are capable of growing in such a wide range of nutrient conditions, making it unclear how applicable the “law" is to other species, like *Caulobacter crescentus*.

Cooper and Helmstetter (1968): chromosome replication and the division cycle of *E. coli* B/r

Cooper and Helmstetter set out to solve a conundrum. *E. coli* is capable of sustaining mass doubling times as short as 20 min. The period required to initiate and complete a round of replication plateaus at ~40 min in nutrient-rich medium supporting mass doubling times <60 min [4]. Given these parameters, how can cells preserve viability if the time required to complete a round of DNA replication is greater than that required to generate new daughter cells? Building on SMK's data indicating that DNA content increases with growth rate, Cooper and Helmstetter deduced that rapidly dividing cells must maintain multiple ongoing DNA replication cycles in order to remain viable. While the time interval between consecutive initiation events can be shorter than that required to replicate the chromosome, a one-to-one correspondence between an initiation event and a cell division event is retained. In this manner, overlapping replication cycles ensure that DNA is fully replicated and segregated prior to each cell division even at the fastest growth rates.

Caveats. The Cooper–Helmstetter model of the bacterial cell cycle is derived from fast-growing (mass doubling time <60 min) *E. coli* cells in which the time required to replicate the chromosome is constant. However, in less auspicious conditions (mass doubling time>60 min), C-period varies with growth rate and the bacterial cell cycle resembles that of eukaryotic cells with a gap before and after chromosome replication. In addition, the model is based entirely on observation of wild-type cells at steady state. Whether or not replication initiation and division are coupled—as the authors would like to suggest—or merely appear to be based on averaged data from thousands of cells thus remained an open question.

Donachie (1968): relationship between cell size and time of initiation of DNA replication

Combining SMK's observation that size increases predictably with growth rate in *Salmonella* with Cooper and Helmstetter's model suggesting the interval between initiation and division is constant (~60 min) in fast-growing *E. coli*, Donachie discovered that cell size at initiation (M_i) is a multiple of a fixed mass (Fig. 1d). At doubling times >60 min, M_i is equivalent to a fixed constant, while at doubling times between 60 and 30 min, it is closer to twice this (Fig. 1d). Based on this observation, he suggested that chromosome replication initiates once cell size per the origin of replication reaches a critical value. Donachie proposed the presence of an initiator protein that accumulates in a growth-dependent manner until it reaches threshold levels and triggers replication. Threshold number is achieved only when cells reach “critical mass.” This idea had implications not only for the regulation of DNA replication but also for the regulation of cell size: if initiation and division are coupled, as suggested by Cooper and Helmstetter, linking initiation to size means that division will be similarly dependent.

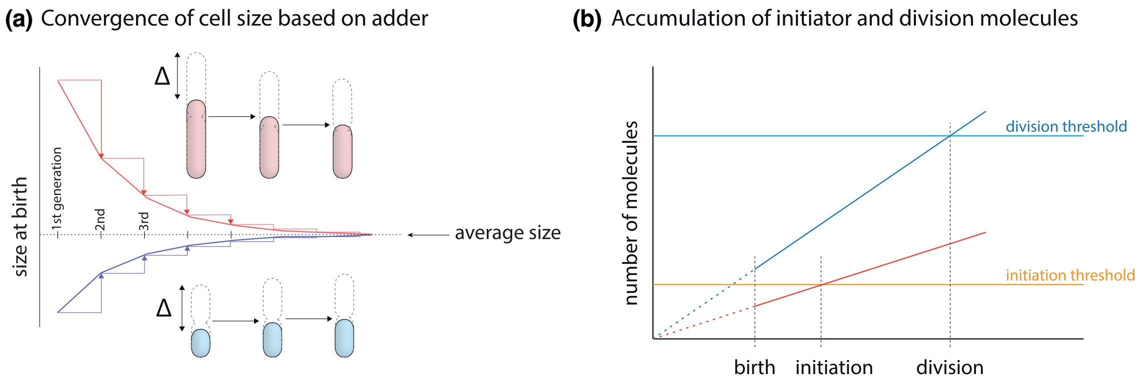
Caveats. Based on data from SMK and Cooper and Helmstetter, Donachie's findings are subject to the same caveats as the earlier studies. In addition, because he combined data from two different studies and two different organisms—*Salmonella* and *E. coli*—Donachie's findings are a meta-analysis and need to be viewed as such. Finally, as with Cooper–Helmstetter, while there is clearly a strong correlation between replication initiation and achievement of a specific size, Donachie does not present data supporting a causal relationship between the two phenomena.

Models Inspire, Models Define: Attempts to Understand the Molecular Basis of the Growth Law and Its Corollaries

The work of SMK, Cooper–Helmstetter, and Donachie have served as the intellectual foundation for empirical analysis of cell growth and cell cycle progression in bacteria for over 50 years. While much of this work supports the conclusions of these seminal studies, significant anomalies exist, particularly with regard to the *determinative* models of Cooper–Helmstetter and Donachie that suggest the need for reassessment.

A single, growth-dependent initiator? Or not? Donachie's meta-analysis and his model were immediately and broadly appealing. From a physiologist's point of view, Donachie's model predicted the traits expected of an initiator protein. Any putative initiator should be essential and ideally highly conserved. Assuming the initiator functioned to activate replication in a threshold-dependent manner, as Donachie proposed, depletion should delay replication initiation and increase average cell size, while overproduction should lead to premature initiation and a reduction in cell size.

Approximately 20 years after Donachie first observed a link between cell size and replication initiation, work by Løbner-Olesen and colleagues [10] suggested that the elusive size-dependent initiator was the highly conserved AAA+ ATPase DnaA. Isolated by Nobel Prize winner Arthur Kornberg [11], DnaA assembles


(a) Convergence of cell size based on adder

(b) Accumulation of initiator and division molecules

Fig. 2. (a) The Adder principle of size control. Cells add a constant volume—independent of their size at birth—before dividing to form two daughter cells. Large cells (red) grow the same amount as small (blue) cells. Over generations, the cell size approaches an average. (Adopted with permission from Ref. [18].) (b) Accumulation of initiators involved in chromosome replication and cell division parallels increases in cell size. Replication and division are triggered when their respective ignition factors achieve critical numbers.

upstream on DNA proximal to the origin of replication and drives a conformational change that permits assembly of the replication machinery and the start of the elongation phase of replication. Supporting its role as a size-dependent initiation factor, depletion of DnaA delays replication initiation and increases initiation mass, while overexpression leads to over initiation and reduces cell size. Consistent with a growth-dependent accumulation mechanism, DnaA concentration is constant across different media, while the number of DnaA molecules per cell scales with cell size.

It was clear from the outset, however, that the story was incomplete. Although 5-fold overproduction of DnaA results in over-initiation, initiation mass is only reduced ~20%, suggesting that additional initiation factors need to reach threshold levels to support productive rounds of replication. In fact, depletion of any other protein required for initiation and even several required for elongation increases the size of both *E. coli* and *B. subtilis* [12,13]. The universality of DnaA as a size-dependent initiation factor is also questionable. Mutations that reduce size, delay replication initiation in *E. coli*, consistent with DnaA levels needing to reach threshold. However, in *B. subtilis*, the timing of initiation is unaffected in diminutive mutants [14].

Coupled? Or not? Despite appearances [4,7], data supporting a model in which initiation and division are coupled are at best mixed. The disconnect between DnaA's strong impact on replication initiation and modest impact on cell size argues against a direct link between the two processes. This disconnect is further enhanced by the discovery that while initiation is required for proper positioning of the division machinery, the cytokinetic ring forms regardless of whether initiation occurs (reviewed in Ref. [15]), as well as data indicating that cells can divide through a partially replicated chromosome [16]. Perhaps the best evidence for a link between initiation and division comes from *B. subtilis*, whereas extended block in division inhibits new rounds of DNA replication [17]. The mechanism underlying this connection remains unknown.

Single-Cell Analysis Reveals New Insights Requiring Revisitation of Classic Models

These early studies were all conducted with the assumption that bacteria double in size before division. Whether division was under the control of its own sizer (division occurs once the cell reaches a specific size) or dependent on the one controlling replication initiation, or under the control of a timer (division occurs at a specific cell age) was unknown. The concept of mass doubling is intuitive to anyone who has watched bacterial cells grow and divide. In the absence of precision measurements and with a limited sample size, it appears that a new born cell grows until it is roughly twice its birth size and then divides in the middle producing two equivalent daughter cells. This impression is reinforced by analysis of cells in batch culture in which mass and cell number increases logarithmically under ideal growth conditions.

The advent of robust techniques for the analysis of thousands of individual cells has not only shattered this view but also led to a reassessment of the mechanisms coordinating cell growth and cell cycle progression and, in some cases, even reinterpretation of the meaning of “old" data. These techniques include microfluidic devices engineered for microscopic applications and specialized software that facilitates analysis of thousands of cells at steady state. Microfluidic devices include the “mother machine” and the “turbidostat” [18–21], both of which hold cells in one or two dimensions, permitting analysis of the bacterial growth and cell size across multiple generations and lineages.

A desire to understand how bacteria deal with fluctuations in size that are passed along from generation to generation led to a major shift in our understanding of the bacterial cell cycle. Single-cell analysis revealed individual cell size to be a dynamic and stochastic variable, rather than the deterministic variable. Variability is a natural outcome of the fluctuations in gene expression, cell division, and other variables, arguing against the presence of a "size sensor" that guards entry into the division phase of the cell cycle.

Tracking these natural fluctuations over generations, investigators were able to discern that *E. coli*, *B. subtilis*, and *C. crescentus* cells grow by adding a constant volume of material, independent of their size at birth [18,20,22]. Stochastically small cells add the same amount of material as stochastically large ones. While this "Adder" mechanism does not immediately restore "normal" size, over generations, the addition of an equivalent volume ensures that cells in lineage derived from even the most (size) deviant mother cell approach the idealized "average" as shown in Fig. 2a. Adder is thus not a strict cell-size control executing in one generation, but a "passive" control mechanism that results in gradual control of cell size over multiple generations. Follow-up studies revealed Adder to be present in other bacteria, and even in some eukaryotes, a phenomenological means of ensuring cell-size fluctuations are controlled during steady-state growth [23–25].

The Search for the Mechanistic Origin of Adder

The discovery of Adder necessitated a new look at classic models for cell growth and cell cycle progression. Unlike a sizer that ensures cells divide only when they reach a specific volume or mass, Adder is not a strict size control mechanism. Adder is instead the product of coordination between the biosynthetic processes that generate new cellular material and the cell cycle. Because Adder requires concurrent regulation of growth, cell size, and the cell cycle, it may appear to be less intuitive than a sizer. The existence of Adder highlights the importance of the homeostatic control mechanisms that ensure growth and cell cycle progression are in lock-step.

How should we view the Growth Law in the context of Adder? The volume added per generation must increase under nutrient-rich conditions, but how much? And how quickly is this increase achieved (one generation after the nutrient shift? many?). Nutrient-dependent increases in growth rate are accompanied by an increase in the rate of cell cycle progression. Does the threshold model for initiation mass put forward by Donachie explain Adder? What is the relationship between initiation and division in the context of Adder?

Significantly, both Adder and cell size homeostasis in general are robust to a wide range of genetic and chemical perturbations [26,27]. While some perturbations altered the volume added prior to division, Adder remains intact at steady state. In one of the first studies to systematically interrogate Adder, Wallden and colleagues [28] employed a microfluidic turbidostat to determine that the initiation of chromosome replication is triggered at a fixed volume per chromosome origin, independent of a cell's birth volume and growth condition. Consistent with Donachie's model as well as experimental data from diminutive mutants [7,29], stochastically short *E. coli* increased the period between birth and replication initiation. However, although suggestive of coupling between size and cell cycle progression, this study failed to illuminate the mechanistic basis for this correlation.

Illuminating the mechanisms underlying Adder ultimately required a new approach, one that enabled investigators to overcome the homeostatic control mechanisms that dominate at steady state. Realizing that it would be prohibitive to test every possible parameter for its ability to perturb Adder, Si and colleagues [30] ran computational simulations to vary the autocorrelation between mother and daughter cell size. This effort suggested that Adder was a product of homeostatic control. Factors required for different cell cycle events would be produced at rates concomitant with cell growth, as reported by SMK [3]. These factors accumulate as cells grow, reaching threshold numbers, only when cells reach a critical size, as proposed by Donachie [7], and then collapse allowing the accumulation start from zero for the next cell cycle. Breaking Adder would thus require levels of these proteins to be varied independent of cell growth. To test this model, Si and colleagues modulated synthesis of DnaA (a representative initiation protein) in *E. coli* and FtsZ (a tubulin-like component of the division machinery and representative cell division protein) in both *E. coli* and *B. subtilis*. This unorthodox approach led to a number of important discoveries. First, *E. coli* and *B. subtilis* both appear to possess two Adders, one functioning at replication initiation and one functioning at division. Oscillating the concentration of DnaA and FtsZ in otherwise steady-state cells is sufficient to break the initiation and division Adders, respectively. Second, the division adder appears to be paramount with regard to cell size homeostasis: the initiation Adder by varying DnaA concentration has no impact on impact the division Adder. This latter finding, that the division adder is independent of initiation, provides further evidence that initiation and division—although clearly coordinated—are not coupled.

What Does This All Mean for Experimental Bacterial Physiologists

The identification of Adder and recent refinements have important implications for how we understand and discuss the bacterial cell cycle. For us, as experimentalists, these advances clarify how we think about cell cycle regulation in general. Until recently, it has been conceptually challenging to provide a coherent, cell cycle explanation for cell size phenotypes observed in steady-state mutant populations. While the strong impulse is to say that such mutants divide "earlier" (if short) or "later" (if long), in reality this is problematic. Dividing repeatedly too early would reduce daughter cell size in each generation. Dividing repeatedly too late would have the opposite effect, increasing size in subsequent generations. Instead, incorporating both Adder and the idea that threshold numbers of specific proteins are required for division to proceed, shorter cells are almost certainly the result of a reduction in this number, either directly or by relieving an inhibitory mechanism that increases the threshold.

Outstanding questions include the nature of the threshold underlying the replication and division Adders. Obviously neither DnaA nor FtsZ is sufficient for replication initiation and division, respectively. What other proteins meet the standards for "threshold" initiation and division factors? Are more threshold factors better? Or do too many "cooks" make the burden for these essential processes too high? Equally interesting is whether the division Adder is paramount in bacteria other than *E. coli* and *B. subtilis*, particularly organisms that exhibit different morphologies and growth patterns. Such organisms may rely on the initiation Adder or use an entirely different mechanism to maintain average cell size. High-throughput single-cell analysis has led to a major revision in our understanding of the mechanisms coordinating cell growth and cell cycle progression. What the next technical advance will be, and how it will impact our understanding of Adder and its role in coordinating growth and cell cycle progression remains to be seen. In the meantime, those of us in the field should remember to keep our eyes and our minds open.

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References

[1] S.E. Luria, M. Delbrück, Mutations of bacteria from virus sensitivity to virus resistance, Genetics 28 (1943) 491–511.
[2] T.M. Norman, N.D. Lord, J. Paulsson, R. Losick, Memory and modularity in cell-fate decision making, Nature 503 (2013) 481–486.
[3] M. Schaechter, O. Maaløe, N.O. Kjeldgaard, Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*, Microbiology 19 (1958) 592–606.
[4] S. Cooper, C.E. Helmstetter, Chromosome replication and the division cycle of *Escherichia coli* B/r, J. Mol. Biol. 31 (1968) 519–540.
[5] C.E. Helmstetter, S. Cooper, DNA synthesis during the division cycle of rapidly growing *Escherichia coli*, J. Mol. Biol. 31 (1968) 507–518.
[6] C.E. Helmstetter, Rate of DNA synthesis during the division cycle of *Escherichia coli* B/r, J. Mol. Biol. 24 (1967) 417–427.
[7] W.D. Donachie, Relationship between cell size and time of initiation of DNA replication, Nature 219 (1968) 1077–1079.
[8] M.G. Sargent, Control of cell length in *Bacillus subtilis*, J. Bacteriol. 123 (1975) 7–19.
[9] M.E. Sharpe, P.M. Hauser, R.G. Sharpe, J. Errington, *Bacillus subtilis* cell cycle as studied by fluorescence microscopy: constancy of cell length at initiation of DNA replication and evidence for active nucleoid partitioning, J. Bacteriol. 180 (1998) 547–555.
[10] A. Løbner-Olesen, K. Skarstad, F.G. Hansen, K. von Meyenburg, E. Boye, The DnaA protein determines the initiation mass of *Escherichia coli* K-12, Cell 57 (1989) 881–889.
[11] R.S. Fuller, B.E. Funnell, A. Kornberg, The dnaA protein complex with the *E. coli* chromosomal replication origin (oriC) and other DNA sites, Cell 38 (1984) 889–900.
[12] E.J. Harry, J. Rodwell, R.G. Wake, Co-ordinating DNA replication with cell division in bacteria: a link between the early stages of a round of replication and mid-cell Z ring assembly, Mol. Microbiol. 33 (1999) 33–40.
[13] Y. Hirota, A. Ryter, F. Jacob, Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division, Cold Spring Harb. Symp. Quant. Biol. 33 (1968) 677–693.
[14] N.S. Hill, P.J. Buske, Y. Shi, P.A. Levin, A moonlighting enzyme links *Escherichia coli* cell size with central metabolism, PLoS Genet. 9 (2013), e1003663.
[15] I.V. Hajduk, C.D.A. Rodrigues, E.J. Harry, Connecting the dots of the bacterial cell cycle: coordinating chromosome replication and segregation with cell division, Semin. Cell Dev. Biol. 53 (2016) 2–9.
[16] L.J. Wu, A.H. Franks, R.G. Wake, Replication through the terminus region of the *Bacillus subtilis* chromosome is not essential for the formation of a division septum that partitions the DNA, J. Bacteriol. 177 (1995) 5711–5715.

[17] H.A. Arjes, A. Kriel, N.A. Sorto, J.T. Shaw, J.D. Wang, P.A. Levin, Failsafe mechanisms couple division and DNA replication in bacteria, Curr. Biol. 24 (2014) 2149–2155.

[18] S. Taheri-Araghi, S. Bradde, J.T. Sauls, N.S. Hill, P.A. Levin, J. Paulsson, M. Vergassola, S. Jun, Cell-size control and homeostasis in bacteria, Curr. Biol. 25 (2015) 385–391.

[19] P. Wang, L. Robert, J. Pelletier, W.L. Dang, F. Taddei, A. Wright, S. Jun, Robust growth of *Escherichia coli*, Curr. Biol. 20 (2010) 1099–1103.

[20] M. Campos, I.V. Surovtsev, S. Kato, A. Paintdakhi, B. Beltran, S.E. Ebmeier, C. Jacobs-Wagner, A constant size extension drives bacterial cell size homeostasis, Cell 159 (2014) 1433–1446.

[21] G. Ullman, M. Wallden, E.G. Marklund, A. Mahmutovic, I. Razinkov, J. Elf, High-throughput gene expression analysis at the level of single proteins using a microfluidic turbidostat and automated cell tracking, Philos. Trans. R. Soc. B Biol. Sci. 368 (2013) 20120025.

[22] A. Amir, Cell size regulation in bacteria, Phys. Rev. Lett. 112 (2014).

[23] M. Deforet, D. van Ditmarsch, J.B. Xavier, Cell-size homeostasis and the incremental rule in a bacterial pathogen, Biophys. J. 109 (2015) 521–528.

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

[25] I. Santi, N. Dhar, D. Bousbaine, Y. Wakamoto, J.D. McKinney, Single-cell dynamics of the chromosome replication and cell division cycles in mycobacteria, Nat. Commun. 4 (2013).

[26] F. Si, D. Li, S.E. Cox, J.T. Sauls, O. Azizi, C. Sou, A.B. Schwartz, M.J. Erickstad, Y. Jun, X. Li, S. Jun, Invariance of initiation mass and predictability of cell size in *Escherichia coli*, Curr. Biol. 27 (2017) 1278–1287.

[27] M. Campos, S.K. Govers, I. Irnov, G.S. Dobihal, F. Cornet, C. J. Wagner, Genomewide phenotypic analysis of growth, cell morphogenesis, and cell cycle events in *Escherichia coli*, Mol. Syst. Biol. 14 (2018), e7573.

[28] M. Wallden, D. Fange, E.G. Lundius, Ö. Baltekin, J. Elf, The synchronization of replication and division cycles in individual *E. coli* cells, Cell 166 (2016) 729–739.

[29] N.S. Hill, R. Kadoya, D.K. Chattoraj, P.A. Levin, Cell size and the initiation of DNA replication in bacteria, PLoS Genet. 8 (2012), e1002549.

[30] F. Si, G. Le Treut, J.T. Sauls, S. Vadia, P.A. Levin, S. Jun, Mechanistic origin of cell-size control and homeostasis in bacteria, bioRxiv (2018) https://doi.org/10.1101/478818.