ACTA ABP BIOCHIMICA POLONICA

Vol. 66, No 2/2019
139–146
https://doi.org/10.18388/abp.2018_2798

Review

When size matters – coordination of growth and cell cycle in bacteria

Joanna Morcinek-Orłowska, Justyna Galińska and Monika Glinkowska✉

Department of Bacterial Molecular Genetics, University of Gdansk, Gdańsk, Poland

Bacterial cells often inhabit environments where conditions can change rapidly. Therefore, a lot of bacterial species developed control strategies allowing them to grow and divide very fast during feast and slow down both parameters during famine. Under rich nutritional conditions, fast-growing bacteria can divide with time interval equal to half of the period required to synthesize their chromosomes. This is possible due to multifork replication which allows ancestor cells to start copying genetic material for their descendants. This reproduction scheme was most likely selected for, since it enables maximization of growth rate and hence – effective competition for resources, while ensuring that DNA replication will not become limiting for cell division. Even with this complexity of cell cycle, isogenic bacterial cells grown under defined conditions display remarkably narrow distribution of sizes. This may suggest that mechanisms exists to control cell size at division step. Alternative view, with great support in experimental data is that the only step coordinated with cell growth is the initiation of DNA replication. Despite decades of research we are still not sure what the driving forces in bacterial cell cycle are. In this work we review recent advances in understanding coordination of growth with DNA replication coming from single cell studies and systems biology approaches.

Key words: bacterial cell cycle, DNA replication, cell division, cell size control

Received: 30 March, 2019; revised: 07 April, 2019; accepted: 07 April, 2019; available on-line: 10 April, 2019

✉e-mail: monika.glinkowska@biol.ug.edu.pl
Acknowledgements of Financial Support: This work was supported by the National Science Center (Poland) No. UMO-2016/23/N/NZ2/02378 to J.M.O.
Abbreviations: SMK, Schaechter, Maaloe and Kjeldgaard; DAR, DnaA assembly region; DUE, DNA unwinding element; RIDA, regulatory inactivation of DnaA; DDAH, datA-dependent DnaA-ATP hydrolysis; DARS, DnaA reactivating sequences; NAPs, nucleoid associated proteins; SMC, structural maintenance of chromosome; SA, surface; V, volume; PG, peptidoglycan; PCC, progression control complex; SIS, sugar isomerase; S7P, sedoheptulose-7-phophasphate; M7P, D-glycero-D-manno-heptose-7-phosphate

EARLY MODELS OF CELL CYCLE COORDINATION IN BACTERIA – A BIT OF HISTORY

Bacterial cell cycle is defined as a set of consecutive events leading to production of two daughter cells. It is traditionally divided into three stages: a phase between cell birth and initiation of DNA replication (B period), genome duplication step (C period) and a phase between completion of replication and cell division (D period) (Skarstad *et al.*, 1983; Michelsen *et al.*, 2003). The two main processes of the cell cycle – DNA replication and cell division – have to be coordinated with cellular growth, so that reproduction is accompanied by the maintenance of characteristic cell size and shape. Such coordination is particularly important for microorganisms whose environment of can change very quickly and thus – rapid adaptation of bacterial cell cycle parameters to variable growth conditions is crucial for survival.

Over 60 years ago Schaechter, Maaloe and Kjeldgaard (SMK) formulated the principles of bacterial growth, also known as the growth law (Schechter *et al.*, 1958). They cultivated *Salmonella enterica* in different media and observed that cells grown under conditions supporting faster growth rate exhibit larger size and higher DNA, RNA and protein content per cell, irrespective of particular media composition. In other words, they proposed that the macromolecular composition of cells and their size is a function of growth rate, regardless of the specific nutrients present in the medium (Schaechter *et al.*, 1958). In their results, cell size scaled linearly with growth rate and was inversely correlated with doubling time. These observations linked also cell growth to cell cycle.

The C and D period in Schaechter's experiments were approximately constant and last for 40 and 20 minutes, respectively, whereas doubling time of fast growing bacteria is about 20 minutes. This paradox of bacterial cell cycle was solved in 1968 by Copper and Helmstetter's model, assuming that under fast growth conditions bacteria initiate the subsequent round of chromosome replication before the previous one is finished. Thus, the cell contains several replicating chromosomes, but replication is still initiated only once during the cell cycle (Cooper & Helmstetter, 1968). Cooper-Helmstetter's model of multifork replication together with the growth law led to the hypothesis that in the cell cycle replication is triggered by the achievement of a critical cell size (mass) per origin, which is constant regardless of growth conditions (Donachie, 1968). It was therefore assumed that there is a factor conveying the information about cell size growth directly to the replication machinery, however a perfect candidate for such a mechanism is still missing (for reasons why, see (Flåtten *et al.*, 2015; Barber *et al.*, 2017; Willis & Huang, 2017)).

Recently, development of single-cell techniques which enables testing the rules governing the cell cycle with respect to individual cells, renewed the interest in its principles. In this review we summarize the current knowledge about cell cycle coordination in bacteria, starting from brief description of the molecular mechanisms and regulation of main cell cycle stages followed by the newest insight into the role of cell size in proper cell cycle progression.

TOP-DOWN AND BACK AGAIN – MOLECULAR MECHANISMS UNDERLYING CONTROL OF THE KEY CELL CYCLE PROCESSES

Unless stated otherwise, mechanisms and protein terminology described below refer to the model Gram-negative bacterium *Escherichia coli*.

DNA replication

DNA replication has to be tightly controlled to ensure that bacterial chromosome is duplicated faithfully and only once during the cell cycle. This provides the stability and integrity of the next generations' genome.

The main regulatory mechanisms known so far, focus on the initiation of replication. The central player of that stage and the subject of control mechanisms is a highly conserved initiator protein DnaA, found in almost all eubacteria. DnaA binds to *oriC* and leads to the initial unwinding of the double DNA helix, permitting further formation of the replisome (reviewed in: Leonard & Grimwade, 2015; Jameson & Wilkinson, 2017; Katayama, 2017)

High degree of structural conservation pertains particularly to one of the four domains of DnaA, namely to domain III, responsible for ADP and ATP binding and hydrolysis of the latter (Erzberger *et al*., 2002; Nishida *et al*., 2002; Kawakamii *et al*., 2005). Decades of studies brought detailed understanding of the mechanism of DnaA action and its regulation. It was shown that DnaA binds to several types of sequences within the *oriC* ((Leonard & Grimwade, 2015) and references therein). *OriC* has a modular structure and consists of the DnaA Assembly Region (DAR), where DnaA binds initially, and DNA Unwinding Element (DUE) where DNA strands become separated during the initiation step. Three high affinity DnaA binding sites (R1, R2, R4) present in the *oriC* sequence are occupied throughout most of the cell cycle, and both DnaA- ADP and DnaA-ATP can interact with them. However, only DnaA-ATP can bind several low-affinity sites, forming two oppositely arrays between R1-R2 and R2-R4 DnaA-boxes (Hansen & Atlung, 2018).

Accumulation of a critical amount of DnaA-ATP bound to the oriC eventually results in the unwinding of the AT-rich DUE region (Kurokawa *et al*., 1999; Ozaki & Katayama, 2012; Sakiyama *et al*., 2017). Then further replication proteins are recruited to the so-called „open complex", namely DnaB helicase is loaded with the aid of DnaC, followed by DnaG primase and multi-subunit DNA polymerase III (Katayama, 2017).

DnaA concentration is stable during the cell cycle, but the ratio of the form associated with ATP to that bound to ADP changes – the level of DnaA-ATP peaks at the time of initiation, reaching 80% of the total cell pool of DnaA and shortly after initiation it falls up to around 20% (Kurokawa *et al*., 1999). There are several mechanisms responsible for the fluctuations of the levels of DnaA-ATP and DnaA-ADP and for the access of DnaA to oriC, which allow for precise control of the initiation timing and prevent premature reinitiation.

DnaA-ATP is converted to DnaA-ADP upon initiation via two independent mechanisms – RIDA (regulatory inactivation of DnaA) and DDAH (*datA*-dependent DnaA-ATP hydrolysis). In the first case, Hda protein, a homologue of DnaA, interacting with the β clamp of DNA polymerase III stimulates ATPase activity of DnaA during DNA synthesis (Kato & Katayama, 2001). In the second, chromosomal *datA* region with unusually high DnaA-binding capacity, stimulates ATP hydrolysis by DnaA in a manner dependent on IHF, one of the nucleoid associated, DNA-bending proteins (Kasho & Katayama, 2013; Kasho *et al*., 2017). DnaA-ADP can be also reactivated to DnaA-ATP by acidic phospholipids of the cell membrane (Saxena *et al*., 2013) and two chromosomal regions, called DARS (DnaA Reactivating Sequences) (Fujimitsu *et al*., 2009; Kasho *et al*., 2014) suggesting that cellular membrane synthesis and replication initiation may be related to each other. Moreover, DnaA autoregulates its cognate gene expression in an ATP-dependent manner (Speck *et al*., 1999; Grant *et al*., 2011). After the initiation, when Dna-ATP level is still high, unscheduled re-initiation is prevented by the SeqA protein, which sequesters both *oriC* region and the *dnaA* promoter for about one-third of the cell cycle (von Freiesleben *et al*., 2000; Hiraga *et al*., 2004; Guarné *et al*., 2005; Waldminghaus & Skarstad, 2009).

Another important regulatory protein that positively affects the initiator activity of DnaA is the DiaA (DnaA initiator-associating factor) protein. It directly interacts with DnaA, supporting its assembly to weak DnaA-affinity sites in the *oriC* (Ishida *et al*., 2004). Although DiaA is not an essential protein, its activity significantly promotes the initiation of replication and ensures that every origin in a cell is fired simultaneously (Ishida *et al*., 2004; Keyamura *et al*., 2007). Interestingly, DiaA also has a negative effect on the initiation of replication at its later stage. The protein binds to DnaA at the same site as DnaB helicase. Thus, as long as DiaA is bound to DnaA, it is impossible to recruit DnaB to the replisome and proceed with the initiation stage (Keyamura *et al*., 2009). Mild overexpression of DiaA does not affect the timing of replication in the cell cycle (Flåtten *et al*., 2015), so it is suggested that the inhibitory effect of DiaA on helicase loading does not result simply from binding competition, but a specific molecular mechanism allowing to release DiaA from the DnaA-DiaA complex. However, what cellular factor promotes replacement of DiaA with DnaB, is still unknown.

Chromosome segregation and cell division

Proper segregation of the replicated bacterial chromosome is essential for each of the daughter cells to inherit a full copy of the genome. However, this is the least understood process in bacteria, especially in *Escherichia coli* which lacks specific partition system. In contrast to eukaryotic organisms, chromosome segregation in bacteria occurs concomitantly with DNA replication, so the origin-close region is segregated first, followed by the rest of the chromosome with a certain delay (reviewed in (Badrinarayanan *et al*., 2015)).

It is assumed that the chromosome segregation occurs spontaneously and is driven by physical forces derived from the nucleoid topology, rather than by a biological mechanism (Jun & Wright, 2010; Le Chat & Espéli, 2012). Thus, proteins involved in the proper organization, compaction and maintenance of chromosome may also be involved in segregation process. These are nucleoid-associated proteins (NAPs) – key factors of proper chromosome organization – such as IHF, Fis, HU and H-NS, that are able to wrap, bend or bridge the DNA (Wang *et al*., 2013), as well as global chromosome organization factor – structural maintenance of chromosome (SMC) complex, in *E. coli* consisting of MukBEF proteins (Rybenkov *et al*., 2014).

It is also worth noting that during the replication, the emerging sister chromosomes are topologically in-

terwound – in order to be effectively segregated, they should be separated. Here, topoisomerase IV, which is essential in the segregation process, is involved in sister chromosomes decatenation (Zechiedrich *et al.*, 1997; Sand *et al.*, 2003; Joshi *et al.*, 2011; Zawadzki *et al.*, 2015).

Contrary to *E. coli*, up to 65% of bacterial species possess parABS partitioning system for the origin segregation (for more information see (Funnell, 2016)). It consists of parS sequences located near oriC and two protein components, ParA and ParB. ParB recognizes and binds to parS sequences, to which ParA protein is substequently recruited. The whole nucleoprotein complex is then pulled to the opposite poles of the cell in an ATP-dependent mechanism whose details have not been fully explained yet.

After completion of chromosomal DNA synthesis and segregation, the cell divides into two progeny cells of approximately equal size. This process is controlled by a protein complex called divisosome. Assembly of the division machinery is orchestrated by a tubulin homologue protein – FtsZ, which polymerizes in a GTP-dependent manner into a so-called Z-ring in the future septation region (Erickson *et al.*, 2010; Szwedziak *et al.*, 2014). In *E. coli*, *B. subtilis* and several other bacteria, the divisome structure has been proposed to assemble in two temporally distinct phases. During the first, the Z ring is formed and membrane-associated proteins (ZipA and FtsA in *E. coli*) tether it to the cell envelope, forming a complex called proto-ring. In the second stage, proto-ring recruits late divisome proteins responsible for peptidoglycan synthesis at the septum (FtsI in *E. coli*) and several proteins whose role relies most likely on coordinating the crosstalk between the proto ring and peptidoglycan synthetases (*E. coli* – FtsN, FtsLBQ) (for more detailed information see: (Rowlett & Margolin, 2015; Haeusser & Margolin, 2016). Constriction of the cytoplasmic membrane, cell wall synthesis at the septum and outer membrane invagination by the divisome finish the fission process.

Molecular regulation of cell division in bacteria concentrates basically on proper Z-ring positioning, coordinated in *E. coli* by at least two systems. The first, Min complex, ensures that FtsZ polymerization occurs exactly at midcell. Two components of this system, MinC and MinD oscillate between cell poles thanks to the help of a third protein, MinE. Min complex inhibits FtsZ polymerization, so the Z-ring cannot form at the poles, occupied with MinCD proteins (Rowlett & Margolin, 2013; Shih & Zheng, 2013). Second mechanism, named nucleoid occlusion, consists of SlmA – the protein that binds to the chromosome and represses Z ring formation over the nucleoid (for a recent review and references see: (Ortiz *et al.*, 2016)). The SlmA-specific sequences are dispersed throughout the genome with the exception of the replication terminus site. Thus, SlmA, directly interacting with FtsZ, restrains septum formation until the segregation process is almost completed. FtsZ is additionally linked to the ter region by such proteins as ZapA, ZapB and MatP, which contribute to the coupling between chromosome segregation and cell division (Espeli *et al.*, 2012). DNA translocase FtsK, which is an essential protein for *E. coli*, orchestrates chromosome segregation and cell division by interacting with chromosomal DNA, the divisome and topoisomerase IV (Crozat *et al.*, 2014).

Growth

Rapid growth is a guarantee of outgrowing competitors and producing progeny cells, so a maximization of growth rate and quick adaptation to changes in environmental conditions are the universal evolutionary strategy of most bacteria. However, bacterial growth rate depends directly on environmental conditions, i. e. on the availability of nutrients. Growth rate changes correspond to alterations in the scale of protein production, metabolism and synthesis of external cell layers – cell membranes and peptidoglycan.

The growth rate of bacterial cells is limited by the efficiency of cellular translation machinery, i.e. the amount and activity of rybosomes. Indeed, it has been confirmed that the fraction of ribosomal proteins increases linearly with growth rate (Dennis *et al.*, 2004; Jin *et al.*, 2012). However, apart from proper amount of ribosomes, growth is guaranteed also by a proper supply of aminoacids and tRNA and growth rate maximization is obtained when all rybosomes are saturated with their substrate. The translation rate is therefore regulated by both the amount of ribosomes and the diffusion of the complexes transporting aminoacyl-tRNA in the cytoplasm (Klumpp & Hwa, 2014; Scott *et al.*, 2014).

Systems biology research aiming to discover the complex interaction network within the cell has revealed that the cellular protein pool is partitioned into three fractions: a growth rate-independent fraction, ribosomal protein fraction and metabolic fraction including catabolic and anabolic enzymes (Scott & Hwa, 2011; Scott *et al.*, 2014). Two latter fractions always add up to a constant value as the total cellular protein concentration is fixed. However, the ratio between them changes depending on growth conditions. Namely, in the nutrient-rich environment the supply of nutrients such as amino acids is high and they do not need to be synthesized, so the metabolic fraction of proteins is reduced. As a result, production of ribosomal proteins increases, allowing for the cell to maximize the global translation rate (Scott *et al.*, 2014). Therefore, allocation of proteins between ribosomal and metabolic fraction is correlated with growth rate and – consequently - growth rate-dependent control of gene expression reflects that protein economy (Scott *et al.*, 2010, 2014; Scott & Hwa, 2011).

The genes encoding translational machinery are highly expressed and therefore, they need to be precisely regulated, because any deviation from the optimal level has a large impact on the proteome. Production of tRNA-affiliated and ribosomal proteins is co-regulated (Klumpp & Hwa, 2014). Ribosomes consist of rRNA and r-proteins and it is the rRNA component which is mainly subjected to precise, growth-rate dependent regulation ((Jin *et al.*, 2012) and references therein). rRNA genes are regulated by the alarmone of stringent response – ppGpp ((Potrykus & Cashel, 2008; Hauryliuk *et al.*, 2015) and references therein), and thus ppGpp is considered as a main source of growth rate control (Murphy *et al.*, 2010). In *E. coli* cells, ppGpp is produced by two proteins RelA and SpoT, the former is responsible for ppGpp synthesis during amino acid starvation. Appearance of an uncharged tRNA at the ribosomal A site activates RelA and induces ppGpp production. ppGpp in turn, inhibits rRNA promoters activity at the transcription initiation stage (Gralla, 2005; Brown *et al.*, 2016). As a result, a downshift in amino acids concentration will reduce tRNA charging and hence – increase ppGpp level, resulting in rRNA promoters inhibition. Consequently, a drop of ppGpp level upon an upshift of amino acids supply will have a contrary effect. Taken together, ppGpp-dependent regulation of rRNA synthesis contributes to the balance between amino acid supply and protein synthesis (Scott *et al.*, 2014) and supports the maintenance of

ribosomes saturated with their substrate under different growth conditions (Bosdriesz *et al.*, 2015).

Corse-grained model of growth-dependent protein allocation has also implications for the cell cycle control. Namely, it has been shown that *E. coli* cells during balanced growth under perturbations in various cellular processes, change their sizes and/or growth rate but maintain a remarkably stable initiation mass (mass per origin at the moment of initiation or a unit cell) (Si *et al.*, 2017). The authors proposed that this invariance of the unit cell under perturbations targeting transcription, translation, fatty acids or cell wall synthesis etc. can be explained assuming that cell size is the sum of all unit cells under any steady-state growth conditions. Furthermore, taking into account that cells attain a critical size prior to initiation and that the initiator concentration is constant under different steady-state growth conditions (as was shown for DnaA), the model of protein allocation predicts the existence of a specific protein sector that is constant despite changes of the ribosomal fraction of the proteome under different perturbations. Affiliation of replication initiators with that sector ensures invariance of initiation mass during balanced growth (Si *et al.*, 2017).

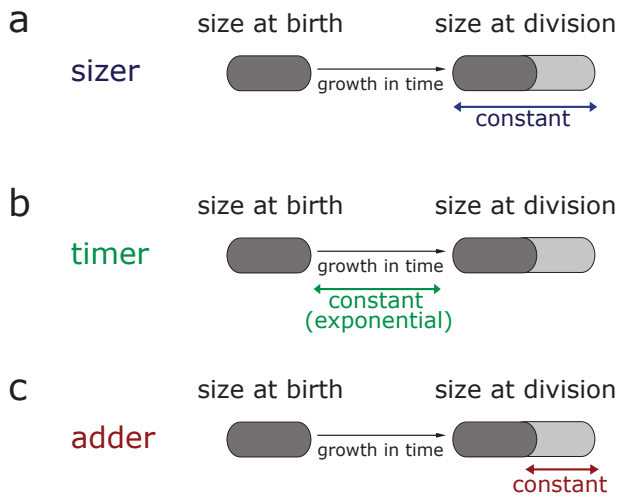

Figure 1. Alternative models of cell size control.
(**a**) "sizer" – bacteria cells divide after reaching defined size; (**b**) "timer" – cells divide after a certain period of time since birth; (**c**) "adder" – cells add a constant amount of volume between subsequent divisions. Adapted from (Willis & Huang, 2017).

BACTERIAL CELL CYCLE COORDINATION – NEW INSIGHTS FROM SINGLE CELL STUDIES AND MATHEMATICAL MODELING

The moments of replication initiation and division in the cell cycle need to be optimally chosen to meet certain criteria. Each initiation of DNA replication needs to be followed by division to maintain one genome duplication per cell cycle. Moreover, it has to be assured that each daughter cell receives a full copy of genetic material and maintains characteristic size after division. Despite availability of often detailed molecular description of mechanism underlying cell cycle progression, the big picture how key events are coordinated is still missing. In eukaryotic organisms it is achieved by a complex system of cell cycle checkpoints, however in bacteria no such cell cycle engine has been identified (Harashima *et al.*, 2013). A recent insight from single cell studies on cell size control and the chromosome cycle brings new scenarios and integrates previously described models, providing hints where to look for molecular control mechanisms. In this chapter, we review the conclusions of those studies and propose the role of metabolic cues in the integration of cell cycle events.

Cells of a defined type, under the same conditions display a narrow distribution of cell sizes (Amir, 2014). Early on, this fact resulted in the search of cell size control mechanisms and two models how such control can be achieved have been proposed. According to the first, cells divide upon reaching a certain size ('sizer') (Fig 1a). In the second, spending a certain amount of time in the cell cycle is the signal to trigger cell division ('timer') (Fig. 1b). The advent of single cell techniques enabled to revise these model by studying rules followed by individual cells. 'Sizer' or 'timer' models can be verified in such studies by analyzing the correlation of cells birth size and the amount of growth within the doubling time interval. Cells behaving like a perfect 'timer' would show no such correlation, whereas in the case of perfect 'sizer', the need to attain a defined size by cells in order to divide, would lead to the negative correlation between the birth size and the amount of growth prior to division (Willis & Huang, 2017).

Recently, high-throughput microfluidic techniques allowed to monitor the size and cell cycle of many individual bacterial cells simultaneously (reviewed in (Taheri-Araghi *et al.*, 2015a)). High amount of data collected from thousands of cells combined with mathematical modelling brought a third paradigm, dubbed 'adder' (Fig. 1c), stating that cells add a nearly constant volume between subsequent initiations or divisions irrespective of their birth size. 'Adder' principle turned out to be consistent with a large amount of data from evolutionarily distant bacterial species such as *Caulobacter crescentus*, *E. coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Deforet *et al.*, Osella *et al.*, 2014; Taheri-Araghi *et al.*, 2015b; Jun *et al.*, 2017). However, this coarse-grained model does not identify the mechanism by which the cells measure the added size or the point of the cell cycle where such regulation takes place. We also note here, that slow growing *E. coli* cells do not seem to follow "adder" growth mode, which will be described in more detail below.

The 'adder' model seems to finally disprove "sizer" based on feedback size-driven mechanisms. However, the question still remains what are the mechanisms underlying this constant size extension during the cell cycle. Later works based on single-cell data propose different schemes explaining which cell cycle stage is subjected to size-dependent control.

The prevalent view places cell size control at the initiation of DNA replication. The classical model of bacterial cell cycle control derived by Donachie from Cooper and Helmstetter and SMK data claimed that replication is initiated after reaching a critical size per origin at the moment of initiation regardless of growth conditions (Donachie, 1968; Donachie & Blakely, 2003). Cell division occurs after a fixed amount of time needed for completion of DNA replication and chromosome segregation (C+D period of cell cycle). A recent study by Wallden and coworkers (2016) based on monitoring of individual cell behavior supported this view and stated that initiation of DNA replication is set by a constant volume per origin ratio ("sizer" between initiations) and the respective division occurs C+D time after. The duration of C+D period is dependent on growth rate. In fast growing cells with overlapping replication rounds,

the initiation of DNA replication started in previous generations and the respective cell division will occur C+D time after, which is nearly constant under those conditions. Therefore the added size prior to division is uncorrelated to birth size, but depends on the individual cell's growth rate. In addition, Wallden and coworkers (2016) observed that *E. coli* cells grown under slow growth conditions do not follow "adder" behavior and instead behave like "sizers". The authors proposed that the observed negative correlation between birth size and the added volume in slow growing *E. coli* stems from size-dependent control of the initiation of DNA replication. Under conditions supporting only single replication round, cells small at birth add a greater volume before they can start DNA replication – and this results in a greater added volume, and hence – greater size prior to division.

Other works claimed that a threshold of size at any cell cycle stage, assuming nearly constant growth rate, would result in the loss of correlation between the birth volume and the division volume, in contrast to the situation seen in the data underlying "adder" principle. The authors proposed that cells add a constant volume per origin subsequent initiation rounds (behave like "adder" between initiations). This scenario preserves the "memory" of the previous cell cycle and predicts a strict relationship between cell size and the number of origin per cell (Haschemi *et al*., 2012; Ho & Amir, 2015).

In other words, in those chromosome-centric views – DNA replication and segregation are rate limiting steps for cell division. On the other hand, there are several opposite models, stating that the cell division, not initiation of replication is the rate-limiting process for the cell cycle progression. Along with the parallel discoveries of 'adder' phenomenon by several research group, some of them place control of the a constant volume preferentially at division step (Campos *et al*., 2014; Osella *et al*., 2014) or at a sub-period between initiation of replication and cell division (Adiciptaningrum *et al*). According to them, implementing cell size increment at division step ensures the compensation of the stochastic differences of cells' birth size and consequently, maintains cell size homeostasis (Campos *et al*., 2014).

Recently, another model standing in opposition to the chromosome-centric view has appeared. Namely, Harris and Theriot proposed that cells add a certain surface area between subsequent divisions (Harris & Theriot, 2016). They observed that although single cells may differ in individual sizes and shapes, they maintain surface (SA) to volume (V) ratio characteristic for given conditions (Harris & Theriot, 2016). They proposed that SA/V is the parameter controlled by cells and determined by the ratio of surface growth to volume growth. They based on the assumption that synthesis of cell surface precursors in the cytoplasm sets the rate of surface growth and leads to its scaling with volume. Harris and Theriot noted that as individual cell grows during the cell cycle SA/V drops due to larger proportion of low SA/V cylindrical body to high SA/V end-cap and the synthesis of the new cell polls restores characteristic SA/V, which implies that the rates of volume or surface growth must also display variation over the cell cycle. Indeed, the authors observed that cell volume growth rate is stable over the cell cycle, whereas SA growth speeds up at the end of the cycle which coincides with septation. They proposed that as the cell grows, cell surface material accumulates in the cell and is subsequently used up during the new cell pole synthesis. As artificial reduction of peptidoglycan (PG) synthesis leads to delay of cell division, Harris and Theriot concluded that accumulation of PG precursors may serve as a signal for cell division machinery to end septation process. This strategy ensures that cells accumulated enough material to finish this precursor-demanding step. The model based on accumulation of a threshold amount of cell wall building blocks reproduced the constant amount of volume added from birth to division, postulated by "adder" growth mode (Harris & Theriot, 2016, 2018).

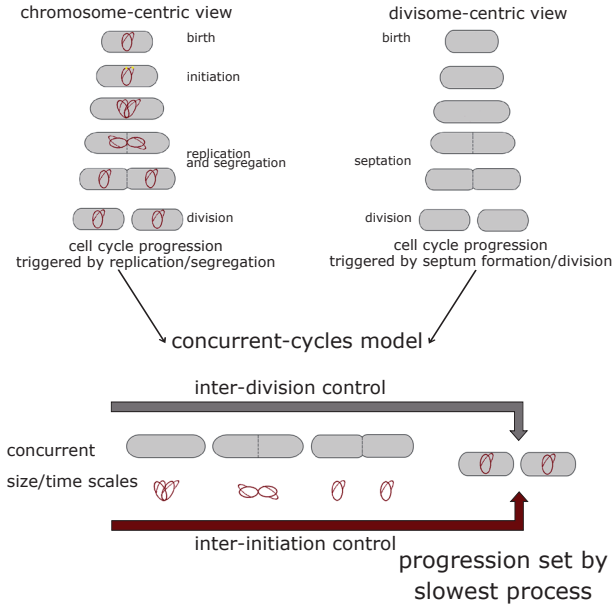

Figure 2. Competing models explaining timing of cell division during the cell cycle.
In the first scheme, DNA replication is always limiting for the cell cycle progression as proposed by Wallden and coworkers (Wallden *et al*., 2016) and Amir (Amir, 2014). In the second, chromosome replication is never limiting, i.e. division is set by accumulation of a certain amount of cell wall precursors, as proposed by Harris & Theriot (Harris & Theriot, 2016). Recently, Micali and coworkers (Micali *et al*., 2018a) have shown that neither of the two models recapitulates experimental data and proposed that both conditions have to be fulfilled – chromosome duplication and segregation has to be completed and divisome ready to finish off septation process. Division timing is set by the slowest of the two processes. Adapted from (Micali *et al*., 2018a).

Cell size control models, centralized either on either replication initiation or cell division seem to be incompatible and contrast one another. On the other hand, it has been shown recently that these models separately do not fit with all experimental observations from single-cell analysis. Namely, Micali et al. (Micali *et al*., 2018a, 2018b) reexamined previous single-cell data and concluded that neither the model, assuming DNA replication as the sole rate-liming process for cell division, nor models based on chromosome-independent interdivision cycles recapitulate the growth patterns observed in those studies. Instead the authors proposed that both mechanisms are at play and division is set by the slowest process, i.e. when chromosome replication and segregation is completed and when septum-synthesizing machinery is equipped with enough building-blocks (Fig. 2). This concurrent cycles view assumes that both chromosome replication-segregation and cell division are subjected to size-dependent control and these two size scales work in parallel. It results in a model where between subsequent initiations cells behave like 'adders' or 'sizers', depending on growth conditions, whereas interdivision process is

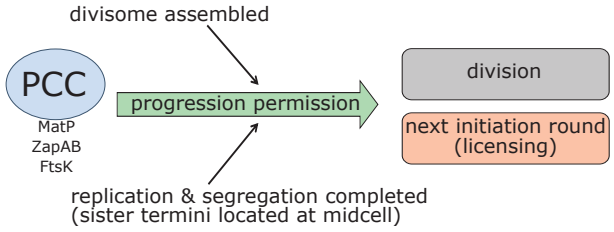

Figure 3. A model explaining cell cycle control by formation of Progression Control Complex (PCC).
Progression Control Complex is formed by physical interaction of the chromosome terminus with cell membrane in the forming septum after replicated sister chromosomes have moved to daughter cell compartments with termini situated on both sides of the septum. When other growth conditions are met and divisome maturates, progression permission is granted, allowing for subsequent cell division and initiation of DNA replication. Thus, progression permission is a licensing decision and it does not decide about timing of the downstream cell cycle events. Adapted from (Stouf *et al.*, 2018).

always an 'adder' (Micali *et al.*, 2018a). Thus, the integration of two processes sets the final decision about cell cycle progression.

The concept of integration of concurrent processes – DNA replication and cell division in setting the division time can be extended with a proposal of molecular progression control complex (PCC) (Stouf *et al.*, 2018). This system would constrain the onset of cell division as well as subsequent initiation of DNA replication until chromosome duplication and segregation is completed AND cells finished divisome maturation (Fig. 3). After both conditions are fulfilled progression permission is granted and cells complete division. At molecular level, this system is suggested to act as a link between chromosome segregation and division by direct physical interaction of the sister chromosomes terminus region with nascent septum, dependent on MatP, ZapAB and FtsK proteins. In support of this proposal, the authors observed that tethering of chromosomal DNA to cell membrane leads to inhibition of next replication rounds most likely due to loss of the negative supercoiling (Magnan & Bates, 2015; Magnan *et al.*, 2015). In addition, the authors argue that in conditional *ftsZ* and *ftsK* mutants chromosomal cell cycle progresses despite the lack of cell division and explain it by the absence of progression control complex in such cells. It is worth emphasizing that progression permission works as a licensing factor, not the mechanism controlling timing of replication initiation or cell division. Assumption that both replication and interdivision processes set division timing allows for existence of processes controlling both replication initiation and cell division. Such models account for the role of numerous proteins found in distinct bacterial species that regulate FtsZ assembly in response to the level of certain metabolites. For instance, moonlighting glucosyltransferases OpgH and UgtP link the UDP-glucose level to cell division in *E. coli* and *B. subtilis*, respectively (Weart *et al.*, 2007; Hill *et al.*, 2012; Westfall & Levin, 2017). For detailed molecular mechanisms and further examples of such proteins we refer the reader to recent excellent reviews on that subject (Chien *et al.*, 2012; Vadia & Levin, 2015; Jun *et al.*, 2017; Sperber & Herman, 2017; Westfall & Levin, 2017).

It is worth noting that eukaryotic cells also follow "adder" behavior leading to cell size homeostasis. To achieve this, they modulate both growth rate and cell cycle duration (Srivastava *et al.*, 2018). Therefore, one could say that genetically distinct organisms use different molecular strategies to achieve the same size control principle. This supports the view that cell size control is a result of coordination of many cellular functions like cell cycle, metabolism, protein synthesis, synthesis of the cell envelope. Indeed, mutation of many genes involved in very different cellular processes lead to changes in cell size (Cornet *et al.*, 2018). At molecular level, this coordination between cellular functions requires flow of information between the processes. Metabolic proteins engaged in the regulation of cell division, mentioned above, are an excellent example of such communication. In this light, it is also interesting that nucleotide sugars, used for cell envelope synthesis provide a link between replication precursors and cell surface expansion. Interestingly, also the level of S7P, a precursor of both nucleotides and outer cell membrane components seems to have a profound effect on the cell size, as shown by opposing effects of deletion of genes encoding enzymes that produce and consume it in the pentose-phosphate pathway (*tktA* and *talB*, respectively) (Westfall & Levin, 2018). Evidence has also accumulated suggesting a direct crosstalk between metabolism and regulation of DNA replication in *E. coli* and *B. subtilis* (Dalmais *et al.*, 2007; Węgrzyn *et al.*, 2011; Barańska *et al.*, 2013). In this light we also propose that DiaA protein could integrate the information on cell surface expansion and/or nucleotide synthesis capacity. DiaA contains a well conserved sugar isomerase domain (SIS) (Keyamura *et al.*, 2007). The role of the SIS domain in DiaA function is unknown. Notably, the SIS domain closely resembles that of another *E. coli* protein, sedoheptulose 7-phosphate isomerase GmhA (alternative name - LpcA) and its homologues in other bacterial species (Taylor *et al.*, 2008; Harmer, 2010; Do *et al.*, 2015). GmhA catalyzes isomerization of D-sedoheptulose-7-phosphate (S7P) into D-glycero-D-manno-heptose-7-phosphate (M7P), which constitutes a step in the pathway of lipopolysaccharide (LPS) synthesis – a part of the outer membrane of gram-negative bacteria (Kneidinger *et al.*, 2002; Taylor *et al.*, 2008). Interestingly, some bacteria encode only a single GmhA homologue, like for instance *P. aeruginosa* whose GmhA contains amino acid residues that were proven crucial for DiaA-DnaA interaction in *E. coli* (Keyamura *et al.*, 2007; Taylor *et al.*, 2008). This suggests that such proteins could join GmhA and DiaA functions. Binding of S7P or M7P by DiaA could regulate its activity with respect to DnaA and couple metabolism to DNA replication. Future effort in studies on cell cycle and size control should focus on clarification of a crosstalk between major processes: replication, cell envelope, metabolism and cell division.

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