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REVIEW ARTICLE

Bacterial cell proliferation: from molecules to cells

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One sentence summary: Newly characterized simple principles that describe how bacterial cells grow and divide at the cellular scale need to be related with the underlying complex and interwoven molecular mechanisms.

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ABSTRACT

Bacterial cell proliferation is highly efficient, both because bacteria grow fast and multiply with a low failure rate. This efficiency is underpinned by the robustness of the cell cycle and its synchronization with cell growth and cytokinesis. Recent advances in bacterial cell biology brought about by single-cell physiology in microfluidic chambers suggest a series of simple phenomenological models at the cellular scale, coupling cell size and growth with the cell cycle. We contrast the apparent simplicity of these mechanisms based on the addition of a constant size between cell cycle events (e.g. two consecutive initiation of DNA replication or cell division) with the complexity of the underlying regulatory networks. Beyond the paradigm of cell cycle checkpoints, the coordination between the DNA and division cycles and cell growth is largely mediated by a wealth of other mechanisms. We propose our perspective on these mechanisms, through the prism of the known crosstalk between DNA replication and segregation, cell division and cell growth or size. We argue that the precise knowledge of these molecular mechanisms is critical to integrate the diverse layers of controls at different time and space scales into synthetic and verifiable models.

Keywords: bacterial cell proliferation; cell cycle regulation; adder; spatio-temporal coordination; resource allocation

BACTERIA ARE VERY EFFICIENT AT PROLIFERATING

The mechanisms underlying the capacity of a cell to self-replicate are primordial for life. The core mechanisms are highly conserved throughout evolution. Bacteria have evolved over billions of years into very efficient and highly integrated proliferative agents. As single-cell organisms, they rely on cell duplication to proliferate. The population growth rate is often argued to be the parameter under selective pressure through evolution. The efficiency of proliferation may be just as important. Bacteria typically multiply by binary fission and cannot increase the number of offspring per generation beyond two. However, they have acquired mechanisms to maintain an average number of descendants close to the maximal value of two, even under non-optimal growth conditions. Maintaining cell growth during the repair of DNA damage allows buffering a temporary delay in the completion of DNA replication, and sustaining the same population growth rate as unstressed

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cells (Darmon *et al.* 2014). Cultivation of clonal populations of millions of cells in the laboratory highlights the faithfulness and the efficiency of the cell duplication process. An estimation of the death rate of *Escherichia coli* cells under constant growth conditions shows that *E. coli* cells are able to proceed through all the cellular duplication process and give birth to two viable daughter cells with >99.95% efficiency (1 death in 2000 exponentially growing cells) (Stewart *et al.* 2005). The bacterial cell cycle is thus highly efficient and robust, requiring an effective coordination of the different cell cycle events, between each other and with cell growth.

EARLY EVIDENCE OF COUPLING BETWEEN THE CELL CYCLE AND GROWTH

In bacteria, as in every living cell, two main cycles have to be coordinated: the DNA cycle—DNA replication and segregation—and the division cycle. Both cycles must also be coupled to cell growth. These connections are fundamental because they define the time and size scales relevant to a cell. Cytological observations in the first half of the 20th century already pointed to such coupling. Gerhard Piekarski and Bern Stille successfully used Feulgen's staining method to describe the dynamics of what was then called 'nucleoid' (term coined by G. Piekarski; Piekarski 1937) along the life cycle of a bacterial cell. The stained 'thymonic material', or DNA, in multiple Gram-positive and Gram-negative bacteria formed nucleus-like structures, hence the term nucleoid. The nucleoid was shown to be duplicated and segregated to future daughter cells, or spores (Piekarski 1937; Stille 1937; Moore 1941). Somehow, the nucleoid dynamics was coupled to cell growth and division to maintain a stable number of nucleoids per cell.

One of the most influential results in bacterial cell physiology came two decades later with the discovery by Schaechter and colleagues that the mass (or size) of individual cells in a growing population is tightly coupled to the growth rate of the population and can be described by an exponential relationship (Schaechter, Maaloe and Kjeldgaard 1958). This simple law (i.e. an equation describing empirical data) has been a central element in bacterial cell biology over the next 60 years. Importantly, the composition of the medium is irrelevant to predict cell mass, as long as we know which growth rate is achieved. Moreover, in a given growth medium, a change of growth rate by a temperature shift does not alter cell mass. Growth rate is simply a measure of the 'pattern of biochemical activities imposed by the medium' that sets the average cell size (Schaechter, Maaloe and Kjeldgaard 1958). This strong correlation between cell mass/size and growth rate reflects (i) a sharp coupling between growth rate and division rate and (ii) a predictable change in this coupling according to the growth rate.

Cell size results from the balance between growth and division rates, and is widely used as a proxy for cell mass since cell density does not change significantly along the cell cycle under steady state growth (Martínez-Salas, Martín and Vicente 1981). The tight coupling between cell size and cell growth has therefore strong implications on cell cycle progression. Within the next decade, measurement of the timing of the replication and post-replication periods (C and D periods, respectively, in the bacterial nomenclature) showed that, for fast growth conditions (>0.8–1 doubling per hour), these periods were nearly constant, irrespective of the doubling time, at $C = 40$ min and $D = 20$ min (Cooper and Helmstetter 1968). The definition of the C and D periods, as well as the B period, extending from cell birth to the initiation of DNA replication, formalized the current bacterial cell cycle model, which is therefore centrally defined by the DNA replication period C. Note that the BCD model is not fully equivalent to the eukaryotic G1/S/G2/M cell cycle model. In bacteria, DNA segregation occurs concomitantly with DNA replication and each locus segregates shortly after its replication (on average 10–20 min; Wang, Reyes-Lamothe and Sherratt 2008; Lesterlin *et al.* 2012), while in eukaryotic cells, DNA synthesis (S phase) is independent from chromosome segregation, which occurs during mitosis (M phase).

The first prominent model of the coupling between cell growth and the cell cycle

Combining population average data from Schaechter and colleagues on *Salmonella typhimurium* (Schaechter, Maaloe and Kjeldgaard 1958) and Cooper and Helmstetter on *E. coli* B/r (Cooper and Helmstetter 1968), William Donachie proposed that the mass versus growth rate relationship discovered by Schaechter Maaløe and Kjeldgaard (the SMK law) results from initiation of DNA replication at multiples of a critical cell mass (Donachie 1968). This model assumes that the initiation of DNA replication, occurring at multiples of a fixed cell mass, is the molecular event determining the timing of all other cell cycle events, including cytokinesis.

William Donachie's insight was that the correlation between cell size and growth rate—or maybe more appropriately growth medium richness—finds a quantitative interpretation in the way cells progress through the cell cycle based on the SMK law. He proposed that for fast growth conditions, the population average cell size can be expressed as

$$S = \frac{S_i}{N_i} 2^{(C+D)/T} = \frac{S_i}{N_i} e^{\lambda(C+D)}, \quad (1)$$

where S_i/N_i is the ratio of population averages of size over the number of origin of replication at the time of initiation of DNA replication, C and D are the durations of the C and D periods, T the population doubling time and λ the growth rate. The major assumptions of this model are that the C + D period and the ratio S_i/N_i, later dubbed 'unit cell', are constant across growth conditions. Stated differently, 'If cells have a constant C and D, and if the initiation mass is a constant, the mass per cell will be an exponential function when plotted against growth rate' (Cooper 1997).

This interpretation of the coupling between mass (or size), growth and the cell cycle based on population averages suggests a cell size homeostasis model where a critical size triggers the initiation of DNA replication (sizer model), which is followed by cell division after a constant period C + D. This is the sizer model applied to cell mass at the initiation of DNA replication, instead of cell size at division as it was initially envisioned (Koch and Schaechter 1962). However, both the SMK growth law and the assumptions of the sizer models have been challenged.

Debated assumptions

The interpretation of the relationship between cell growth and cell cycle encapsulated in Equation (1) does not capture the increase in C and D period durations in slow growth conditions. In fact, the C and D periods have been shown to not be as constant as initially proposed and they tend to increase with the generation time (Woldringh 1976; Skarstad, Steen and Boye 1983; Allman, Schjerven and Boye 1991; Bipatnath, Dennis and Bremer

1998; Michelsen *et al.* 2003; Stokke, Flåtten and Skarstad 2012). Moreover, the fundamental hypothesis of invariance of the unit cell S_i/N_i has been repeatedly challenged. On the one hand, some studies substantiated the model by showing a narrower variation of cell size at the onset of DNA replication as compared with cell age (Koppes *et al.* 1980; Boye *et al.* 1996), thereby identifying the initiation of DNA replication as the most likely cell cycle event coupled to cell mass or size. The constancy of the initiation mass also received strong experimental support (Herrick *et al.* 1996; Hill *et al.* 2012; Wallden *et al.* 2016; Si *et al.* 2017) under the assumption that the C + D period is constant (see also Cooper 1997, 2006 for a review of the arguments in favor of this model). On the other hand, some studies challenged the invariance of the initiation mass (Churchward, Estiva and Bremer 1981; Wold *et al.* 1994), and other results are difficult to reconcile with the simplest versions of the model of constant mass at initiation. For instance, the number of origin of replication in the cell cannot play a role in size sensing as *E. coli* cells can maintain multiple copies of mini-chromosomes (plasmids with the chromosomal origin of replication as only origin of replication) (Messer *et al.* 1978; Leonard and Helmstetter 1986), or even live with multiple copies of the origin of replication on the chromosome (Løbner-Olesen and von Freiesleben 1996; Wang *et al.* 2011).

Cell size results from the balance between growth and cell division rates

Zheng and colleagues proposed a more comprehensive interpretation of the empirical relationship between cell growth and the cell cycle. This growth law, unlike the SMK law, also describes the dependence of cell size/mass on growth rate and the duration of C and D periods under slow growth conditions (Zheng *et al.* 2020). The growth law becomes

$$S = S_0 \lambda\ (C + D) = S_0 \log(2)\,(C + D)/T, \qquad (2)$$

where symbols have the same meaning as in Equation (1) and S_0 is a 'fundamental' cell size (or mass) unit without explicit meaning so far. The model from Zheng and colleagues (Equation 2) is incompatible with the one presented in Equation (1). In this framework, the initiation mass is dependent on growth rate. Despite the small degree of variation (20–50%; Wold *et al.* 1994; Zheng *et al.* 2020) and the experimental difficulty of measuring the initiation mass, this result is probably the best circumstantial evidence in favor of the model defined in Equation (2). Importantly, while Donachie's model assumes constant C + D period, this model is based on the observation that the duration of the C + D period is inversely proportional to the growth rate, with $C + D = 0.3\lambda^{-1} + 0.99$ (Zheng *et al.* 2020). This expectation is in line with the proportional relationship identified between the C period at slow growth rates and the generation time T (Kubitschek and Newman 1978), hence a reciprocal relationship between the C period and the growth rate λ. The precise nature of the dependence of the C + D period on the growth rate may require further consensus as others have identified a power–law relationship $C + D = 0.3\lambda^{-0.84} + 0.7$ (Wallden *et al.* 2016). Note that we modified the time unit in the latter expression, from minutes to hours, to match the former relationship from Zheng and colleagues. The exponent of −0.84 is slightly different from the value of −1 implied in the reciprocal relationship, but both expressions are in broad agreement. The exact value of the exponent changes the respective impacts of the growth rate and the C + D period on cell size or mass, but does not alter the model presented in Equation (2). Importantly, the relationship between the duration of the C + D period and the growth rate predicts a small change in C + D period (∼15% decrease) as the growth rate increases from 1 to 3 doublings per hour. This limited increase, together with the experimental difficulties to measuring C and D periods, may explain why the C + D period has been considered to be invariant at fast growth rates.

CELL BIOLOGY AT THE SINGLE-CELL LEVEL: NEW PERSPECTIVES ON THE CORRELATION BETWEEN CELL SIZE AND GROWTH RATE

How cells know and control how big they are? The answer to this cell size homeostasis question is inherently linked to the coordination of cell growth with the cell cycle. This old question remains unanswered, but has been intensely investigated over decades. And although we do not have definitive answers yet, tremendous progress has been made over the last decade. In the following paragraphs, we review the different models of cell size homeostasis, how they emerged and why they may not be fully satisfactory.

It was clear from the beginning that the growth laws, derived from population averages, do not necessarily describe the behavior of single cells and do not constrain the list of possible cell size homeostasis mechanisms. Under *steady state conditions*, every cell cycle event will indeed occur on *average* at the same average cell size or age, and will be followed *on average* by other cell cycle events after a constant time period, irrespective of the underlying cell size homeostasis mechanism (see Boye and Nordström 2003). Therefore, it is of paramount importance to take advantage of the noisy nature of cellular physiology to explore how cells respond to the small perturbations they experience at each generation.

Clues from cell size distributions and correlations

The underlying mechanisms coupling cell growth with cell size and the cell cycle induce specific patterns in the distribution or correlation between cell size, growth or cell cycle parameters. In the following paragraphs, we present examples illustrating how statistical features, i.e. (i) the degree of variability of cell size at the initiation of DNA replication, (ii) the correlation between cell lengths at birth and division and (iii) the skewness of the inter-division time distribution can help falsify or support a specific family of model of cell size homeostasis and coupling between cell size and the cell cycle.

Single-cell information was collected in many studies, often by quantitative analyses of light or electronic microscopy images. Identifying the cell cycle stage associated with the smallest cell size variability would strongly suggest that cell size or growth is coordinated with the cell cycle at this specific stage. As a corollary, the relatively high variability of cell size at the time of initiation of DNA replication questioned the sizer model from Cooper and Helmstetter (1968) and Donachie (1968) (see Koch 1977 for a comprehensive discussion on the topic).

Correlations between cell size or age distributions at various cell cycle stages are highly predictive of the mode of size control at work. Simple snapshots of synchronized or even asynchronous populations provide us with cell size distributions. Cell

size distributions allow for the estimation of the degree of correlation between the inception and termination of cell cycle periods, respective to cell age or size. For instance, the correlation between cell length at birth and division was estimated to be as high as 0.55 (Koppes *et al.* 1980). This result is incompatible with the absence of correlation predicted by a sizer model as in this family of models cell division is triggered irrespectively of cell size at birth (Koch and Schaechter 1962; Cooper and Helmstetter 1968; Donachie 1968).

Quantitative single-cell tracking was achieved as early as 1932 (Rahn 1932), unveiling the variability in generation times of bacterial cells growing in the same growth conditions. Complementary studies confirmed this variability and revealed the continuous nature of the single-cell growth rate (i.e. no cell cycle arrest) (Powell 1956; Schaechter *et al.* 1962). Voorn and Koppes realized that the different cell size control mechanisms implied very different levels of skewness of the interdivision time distribution and used the experimental estimation of the skewness to falsify the sizer model as proposed by Schaechter *et al.* (1962); Cooper and Helmstetter (1968); and Donachie (1968), and favor an incremental, or adder model where cells would grow on average by the same amount, irrespectively of cell size at birth (Voorn and Koppes 1997).

More recently, Ariel Amir developed a mathematical framework able to capture the mode of size control in one parameter (Amir 2014). This versatile model allowed for an objective comparison between modes of control. Both correlations (length at birth versus division and length at birth versus interdivision time) and the skewness of the interdivision time distribution were used to estimate that the most likely range of value for this control parameter corresponds to the incremental model.

Direct experimental observation of the incremental/adder phenomenon

Inspection of the relation between cell cycle, cell size and the individual growth rate of the cells using microfluidic devices revealed that cells grow indeed on average by a constant amount before dividing, irrespective of their size at birth. As a result, cells shorter than average will tend to be relatively longer, while cells longer than average tend to be relatively shorter, and cell size converges toward the average added size between divisions (Campos *et al.* 2014; Taheri-Araghi *et al.* 2015). The name of 'adder' designing the incremental model was coined (Taheri-Araghi *et al.* 2015) to match other generic names for cell size control mechanisms sizer and timer. The adder behavior seems to be conserved and was characterized in a diverse set of organisms (Campos *et al.* 2014; Deforet, van Ditmarsch and Xavier 2015; Fievet *et al.* 2015; Taheri-Araghi *et al.* 2015; Willis and Huang 2017), including yeast cells (Soifer, Robert and Amir 2016), and even in mammalian cells (Cadart *et al.* 2018). Given the diversity of concerned organisms, this degree of conservation suggests that the adder phenomenon is an emergent cellular property rather than based on a molecular mechanism sensing added size.

The adder phenomenon does not specify a family of conserved mechanisms and could arise from a diversity of control mechanisms. In fact, an adder between division events was rapidly shown to be compatible with a replication centric model reminiscent of the sizer model at the initiation of DNA replication: an adder between replication initiation events defines cell size and is coupled to the division cycle through a constant C + D period (Ho and Amir 2015; Taheri-Araghi 2015). The added size per division cycle is proportional to the number of origins of replication in the cell. Others proposed that the very same old sizer model could recapitulate the adder behavior between divisions if the C + D period is not constant and depends on growth rate (Wallden *et al.* 2016). The interdivision adder was shown to be also compatible with a division centric model where cell division is the limiting process through the accumulation of a cell envelope precursor necessary to build the new poles, provided that the rates of cell surface area and cell volume are proportional (Harris and Theriot 2016).

Ojkic and colleagues observed a remarkable scaling of cell surface area and volume (Ojkic, Serbanescu and Banerjee 2019):

$$S = \gamma V^{\frac{2}{3}}, \tag{3}$$

where S represents the cell surface area, V cell volume and γ a constant pre-factor dependent on cell shape. Equation (3) captures the tight control of the cell aspect ratio (cell length over width) (Zaritsky and Pritchard 1973; Zaritsky 2015). This scaling relationship is proposed to emerge from cell shape homeostasis at the single-cell level. Regardless of the growth conditions and the achieved growth rate, cells tend to add on average the same added length when normalized by cell width (constant $\Delta L/width$) (Ojkic, Serbanescu and Banerjee 2019). This generalization of the adder phenomenon is consistent with the work from Harris and Theriot and with the cell division being the rate limiting process.

From a single limiting process to a concerted control

All the models presented in the previous section were based on a *single* rate limiting process that was size dependent (i.e. the division or replication process is sensitive to cell size, not age). However, the division rate of *E. coli* cells was shown to be both size- and age dependent: the division rate of a young cell remains lower than an older cell with the same size. These results called for the notion of concerted control (Osella, Nugent and Lagomarsino 2014) with two interdependent but different controlling elements, or triggers.

Exploring cell size homeostasis beyond the interdivision time, by segmenting the cell cycle into multiple periods, further substantiated this notion of concerted control. In *E. coli*, cell size compensation occurs during the B and D, but not C period of the cell cycle under relatively slow growth conditions (no overlapping cycles) (Adiciptaningrum *et al.* 2015). This study echoes earlier findings showing that restricting DNA replication through thymine limitations resulted in longer C period and shorter D period (Meacock and Pritchard 1975), and highlights at least two important points: (i) the durations of the B and D periods can be modulated by both size and growth rate and (ii) the D period can be modulated, suggesting that cell division can be licensed by an event other than DNA replication initiation.

Concurrent and parallel processes: a matter of correlations and variances

The problems of the coupling between the division and DNA cycles and of the varying C + D (or D only) period called for more complex models than a single adder driving a rate limiting process. Single-cell tracking of the replication process revealed that the adder phenomenon could be observed between consecutive replication initiation in *Mycobacterium smegmatis* and *E. coli* (Logsdon *et al.* 2017; Witz, van Nimwegen and Julou 2019). In both cases, the comparison between observed and simulated

variances and correlations between variables led the authors to propose a second adder running from the initiation of DNA replication to the following cell division to couple division and DNA cycles in order to recapitulate the experimental observations (Fig. 1A). Both models posit a size-dependent trigger for the initiation of DNA replication, while DNA replication triggers cell division through an independent process (see Koch 1977 for early support for this family of models).

A fundamentally different perspective came from Lagomarsino's group who proposed that any of the two cycles could be rate limiting for triggering cell division at each division cycle (Micali *et al.* 2018a,b) (Fig. 1B). In this model, the division and the replication cycles are coupled to cell size via near-adders running between consecutive replication initiation or cell division events. The two cycles are coupled through the duration of the C + D period. In this AND gate, the slowest process between divisome assembly and DNA replication plus segregation period sets division size (Fig. 1B). As a result, the added size during the C + D period negatively correlates with cell size at the initiation of DNA replication, but to a milder degree than if DNA replication was never limiting. In addition, the C + D period should depend on growth rate: if the division process sets the division size though an adder, cells growing faster will grow by the added size sooner, thereby shortening the D period whenever the division process is limiting. This property of the model describes the relationship between C + D duration and growth rate measured at the single-cell level (Adiciptaningrum *et al.* 2015; Wallden *et al.* 2016). Note that this concurrent processes model may be parametrized to avoid any correlation between the size at replication initiation and the added size during the C + D period and match the prediction of the double adder proposed by Witz, van Nimwegen and Julou (2019).

Interestingly, Si and coworkers experimentally altered independently the duration of the division and replication processes, effectively making each of the two processes the limiting process in independent experiments (Si *et al.* 2019). They observed that altering the adder behavior between division events had no impact on the adder between interdivision events. They also observed that altering the inter-initiation adder did not affect the interdivision adder. These results led the author to conclude that the DNA and division cycles are not coupled to cell size through the same cell cycle event (Fig. 1C). In fact, they propose that cell size is set by the replication process (added size proportional to the number of origin of replication), while the control of the division process ensures cell size homeostasis. In light of the concurrent processes model (Micali *et al.* 2018a), one would expect a strong negative correlation between the added size during the C + D period and the size at the initiation of DNA replication, indicative of cell division being always the limiting process. Note that the double adder model proposed in Si *et al.* (2019) is compatible with earlier data (Cooper and Helmstetter 1968) to the extent that the C + D period is not constant and is predicted to negatively correlate with growth rate. This negative correlation explained by Micali's and Si's models is consistent with the observed relationship between these two parameters at the single-cell level (Adiciptaningrum *et al.* 2015; Wallden *et al.* 2016) and links the single-cell behavior to the population average data described by the model presented in Equation (2). The clear difference of dependence of C and D periods on growth rate (Adiciptaningrum *et al.* 2015) suggests that the two periods must be separated to establish a meaningful relationship with growth rate.

About correlations and variances

The differences between the models lie in their ability to capture correlations between parameters across the population or over generations. The characterization of cell to cell variability in single-cell studies enables the estimation of these correlations and tests or falsifies the different models. Early studies focused on specific correlation coefficients or degree of variation to build and choose the model best describing the data, or falsify other models (e.g. the correlation between size at birth and division or the skewness of the interdivision time distribution; Voorn and Koppes 1997; Amir 2014). Logsdon and colleagues used stochastic simulations of different models to estimate 13 parameters (coefficients of correlation and variation). Witz *et al.* proposed a more systematic method based on the generalized variance to identify the set of most independent variables to identify the model that describes best the data (Witz, van Nimwegen and Julou 2019). In fact, all studies dealing with single-cell variability were built on the premise that the minimal set of independent variables providing maximal information about the system would define the natural variables for cell size homeostasis.

Grilli and colleagues proposed a more formal and general approach, based on the linear response theory framework, and built a general and versatile model capturing all considered models through a limited set of parameters (Grilli *et al.* 2017, 2018). This approach also allows for the exploration of the importance of the coupling parameters and the sensitivity to noise under different parametrization of the general model corresponding to the different modes of control envisioned. This formalism is largely inaccessible to biologists, but opens potential fruitful collaboration between theorists and experimentalists.

It remains to be seen how all these models are sensitive to the segmentation of the cell cycle in specific steps. The end of DNA replication, or late DNA segregation steps have the potential to be major cell cycle transitions as mentioned earlier (Meacock and Pritchard 1975; Adiciptaningrum *et al.* 2015) (see also Huls, Vischer and Woldringh 2018). How would models evolve if new cell cycle periods were included in the measurements (e.g. the time from DNA replication initiation to the inception of cell constriction, or U period)? Would we identify new natural variables? The present models suggest that it might be useful to further investigate the coupling of the late cell cycle periods with cell size and growth.

Experimental considerations

On a technical note, the relative democratization of microfluidic devices led to a substantial increase in the number of cells tracked over generations and provided us with the necessary statistics to evaluate first order correlations and estimate the degree of variability of the different variables (e.g. generation time). The distinction between the different families of models proposed may require the estimation of higher order statistics and greater numbers of cell cycles (Grilli *et al.* 2017).

In addition, the field may gain from establishing a common standard experimental setup, from the imaging procedures to the analysis pipeline, to gain in reproducibility. This standard setup should take into consideration the limitations reported for the PDMS-based fluidic devices (friction forces can limit cell expansion) (Yang *et al.* 2018). It should also take into account the fact that the distribution of cell ages of cells at any given time during an experiment has consequences on the interpretation we can make of time-averaged variables. The uniformity

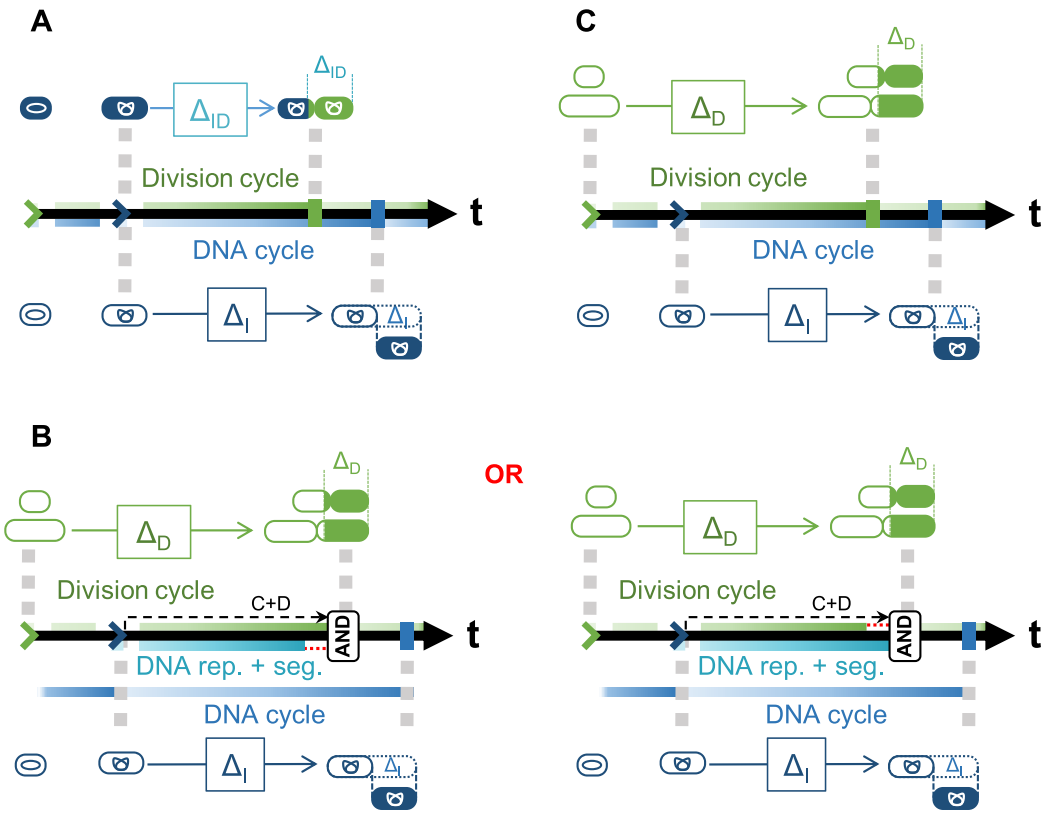

Figure 1. Models of parallel and concurrent processes driving cell size control. To ensure cell size homeostasis, cells must coordinate cell growth with the DNA and division cycles. At least two connections are therefore necessary to coordinate these three processes together. Double adder models seem to be able to recapitulate cell-scale observations by connecting the division (in green) and replication cycles (in blue) to themselves and to each other. Note that the period covering DNA replication and chromosome segregation can be different from what the C + D period has indicated in panel **(B)**, where they are represented with the turquoise color and a black dotted arrow. **(A)** A replication centric model with one adder is running between two consecutive initiation of DNA replication (Δ_I) and the other interlinking the event of initiation of DNA replication with the following cell division event (Δ_{ID}) (Logsdon *et al.* 2017; Witz *et al.* 2019). (B) The 'AND gate' model where adders are running between two consecutive events of (i) initiation of DNA replication (Δ_I) and (ii) cell division (Δ_D), but in which the competition between the division process and the C + D period sets the timing of division. On the left panel, the division cycle is rate limiting, while on the right panel the segregation and replication processes are the slowest. Note that by definition the D period does not necessarily end with the end of DNA segregation, hence the difference between the C + D period and the time required for DNA replication and segregation processes on the right panel (Micali *et al.* 2018a,b). **(C)** Two-adder model where independent adders are running between two consecutive events of (i) initiation of DNA replication (Δ_I) and (ii) cell division (Δ_D) (Si *et al.* 2019).

of cell age distribution allows for a better match with ergodicity assumptions (Rochman, Popescu and Sun 2018).

Toward molecular bases of the coupling between growth and the cell cycle

Microfluidic experiments provided us with the dynamics of single cell progressing through the cell cycle (Santi *et al.* 2013; Adiciptaningrum *et al.* 2015; Wallden *et al.* 2016; Logsdon *et al.* 2017; Si *et al.* 2019; Witz, van Nimwegen and Julou 2019). The segmentation of interdivision period in B, C and D periods consistently led the authors to decouple the inception of the C period from cell division. The DNA cycle is coupled to size by a size-dependent mechanism triggering the initiation of DNA replication after the addition of a constant size (or mass) per origin of replication. The coupling between the DNA cycle with the division cycle and growth is more problematic as it requires specific correlations such as a negative correlation between single-cell growth rate and their C + D period. Note that the concurrent processes model proposed by Micali *et al.* (2018a) predicts this correlation instead of assuming it (Wallden *et al.* 2016). Since the C period was shown to be quite insensitive to size and growth rate (Adiciptaningrum *et al.* 2015), it suggests a role for late DNA cycle events (end of DNA replication or segregation) in triggering cell division. As we will see below, the existence of a crosstalk between cell division and DNA segregation has been established (Kennedy, Chevalier and Barre 2008; Lesterlin *et al.* 2008), and a few molecular mechanisms may provide a molecular basis to this peculiar role of the D period. We may gain insights by separating the D period in sub-periods in the future, to capture the adaptation of the D period due to the effects linked to DNA segregation requirements from those related to cell constriction.

The requirement for two independent triggers to couple cell size with the cell cycle (two adders or potentially one adder and a timer in some bacterial species; Santi *et al.* 2013) reflects the relative independence of the processes driving the DNA and the division cycles (i.e. the fact that they are not strictly interdependent, see below) (Nordstrom, Bernander and Dasgupta 1991; Boye and Nordström 2003). To account for the added volume, the accumulation of a single protein (DnaA for DNA replication and FtsZ for cell division) (Ojkic, Serbanescu and Banerjee 2019; Si *et al.* 2019), or of a cell pole precursor (Harris and Theriot 2016), is proposed to trigger the commitment to the next cell cycle event in a growth-dependent manner. This factor is synthesized at a rate proportional to the cellular growth rate and its activity

depends on a given number of molecules rather than its concentration. These hypotheses are analogous to the autorepressor model presented by Sompayrac and Maaløe (1973), one of the simplest circuit recapitulating the adder behavior. These models are strongly supported by the invariance of initiation size in *E. coli* and *Bacillus subtilis* (Si *et al.* 2017; Sauls *et al.* 2019). However, as we have seen earlier, this invariance may not hold true, and as we shall see below, the regulatory network of the cell cycle is far more complex than monitoring the level of a single protein or metabolite. Understanding how a 'simple' cellular behavior such as the adder phenomenon can emerge from a high molecular complexity constitutes a unique opportunity to close the gap between the observed dynamics of cellular proliferation and its mechanistic bases. A wealth of cell biology and molecular data describing the mechanistic bases of many aspects of cellular proliferation has been reported. In the following, we attempt to review these mechanisms through the lens of the required coordination between DNA and division cycles and cell growth.

THE CELL CYCLE PACEMAKERS AND THE CHECKPOINT PARADIGM

The cell cycle is defined by periods and by the molecular events triggering the transitions between them. Most importantly, many bacteria can manage overlapping cell cycles and a cell can be at the same time at different cell cycle stages. Under fast growth conditions a cell can be replicating its chromosomes (C period of cycle n+1) and dividing (during the D period of cycle n, and more precisely the cytokinetic or T period). As such, the cell cycle is better viewed as a set of recurrent cellular processes that are synchronized via a heap of molecular mechanisms, rather than a linear succession of events (Nordstrom, Bernander and Dasgupta 1991; Boye and Nordström 2003). Supporting this view, cell division can be genetically impaired without affecting DNA replication, segregation and cell growth, leading to the formation of filamentous cells (e.g. the *fts* mutants, for *f*ilamentous *t*hermosensitive, reviewed in Donachie and Robinson 1987). Conversely, DNA replication and/or segregation can be stopped without temporarily inhibiting cell division. Even the inhibition of DNA segregation does not prevent further rounds of DNA replication in *E. coli* (Wang, Reyes-Lamothe and Sherratt 2008).

The concept of cell cycle checkpoint encapsulates the idea of control mechanisms enforcing dependency between cell cycle events (e.g. licensing mechanisms), as opposed to intrinsically coupled events. The former type of dependency may be bypassed with loss of function mutations and the possibility to genetically disrupt the normal succession of cell cycle events remains a gold standard to define what is a cell cycle checkpoint or not (Hartwell and Weinert 1989). Checkpoints typically integrate information from surveillance systems monitoring the level of completion of the previous phase and the capacity of the cell to complete the next step (e.g. completion of the assembly of the division machinery, or the spindle in Eukaryotic cells, or the absence of DNA damage).

In the BCD bacterial cell cycle, the B period extends from cell birth to the initiation of DNA replication while the C and D periods correpond to the replicative phase and the time elapsed between the end of DNA replication and cell division, respectively. More periods were defined to include observables related to cell division and the dynamics of the nucleoid (e.g. T- or U-period; Helmstetter 1987). Two classical checkpoints control the onset of the DNA cycle, and the activation of the division machinery (divisome). These two molecular switches integrate a multiplicity of information and are believed to set the pace of the cell cycle. These two switches crystalized thus far virtually all efforts to develop mechanistic models for cell size homeostasis.

Initiation of DNA replication

The control of the initiation of DNA replication has been extensively studied and is well described in recent and excellent reviews (Katayama *et al.* 2010; O'Donnell, Langston and Stillman 2013; Kaur *et al.* 2014; Katayama, Kasho and Kawakami 2017). Briefly, the mechanistic switch in the regulation of the initiation of DNA replication at the unique replication origin, *oriC*, lies on two essential elements—the nucleotide-bound state of the DnaA protein and the topology of the *oriC* DNA region. DnaA binds to multiple sites (DnaA box motifs) within *oriC* in an ordered manner according to the affinity of each motif to DnaA in its ATP- or ADP-bound forms. The interplay between DnaA and *oriC* culminates in the formation of a structured orisome, promoting the opening of the replication bubble (Leonard *et al.* 2019).

It is often argued that a critical threshold of the initiator protein (DnaA) necessary for building the orisome must be attained to trigger the initiation of DNA replication. A large collection of studies highlights the preeminence of the control of the amount, not concentration (Boye *et al.* 1996), of ATP-bound DnaA protein in the cell as the central regulatory mechanism (Hansen, Christensen and Atlung 1991a; Hansen and Atlung 2018). DnaA concentration has been reported to be nearly constant over a wide range of growth rate (Hansen *et al.* 1991b; Herrick *et al.* 1996). The amount of DnaA protein is therefore proportional to cell mass or size. A critical threshold of DnaA amounts would trigger initiation of DNA replication at a specific cell size, while an integral threshold of DnaA (amount of DnaA produced since the last replication initiation event) would result in an adder phenomenon between initiation events. This latter mechanism implies that the DnaA protein is the limiting factor and that the DnaA molecules used for the previous initiation event cannot be involved in the next. However, increasing the levels of DnaA per cell by up to 50% did not alter the timing of DNA replication, arguing that the DnaA protein is not limiting in the cell (Flåtten *et al.* 2015).

Beyond this simplistic view centered on a single protein, it appears that the regulation of the initiation of DNA replication is complex, and that multiple signals and modulators interfere with this minimal view (Ryan *et al.* 2004; Riber *et al.* 2016). In fact, beyond protein amount and concentration, the balance between ATP- and ADP-loaded DnaA seems to be the relevant molecular cue that integrates regulatory signals controlling the initiation of DNA replication. The nucleotide-bound state of DnaA (ATP versus ADP) is highly regulated along the cell cycle, through multiple protein–protein, protein–DNA (reviewed in Katayama, Kasho and Kawakami 2017) and protein–phospholipids interactions (Sekimizu and Kornberg 1988; Xia and Dowhan 1995). Moreover, the topology of the *oriC* DNA region is influenced both locally and globally, for example through the control of the transcription of neighboring genes (*gidA* and *mioC*) (Theisen *et al.* 1993), and more globally by the structure of the chromosome in the cell (Magnan and Bates 2015).

Our understanding of the large regulatory network controlling the initiation of DNA replication offers good insights on how a single round of DNA replication occurs for each *oriC*

opening event or on the basis of the synchrony between initiation events in a single cell. However, it is still unclear how growth information is integrated in this checkpoint, leading to the coupling between cell mass/size and DNA replication presented in 1968 (Cooper and Helmstetter 1968; Donachie 1968). In *B. subtilis*, replacing *oriC* and *dnaA* by a plasmidic origin of replication, in the presence or not of a functional *dnaA* gene, unveiled multiple and independent connections between *oriC*, DnaA and respiration, central carbon metabolism, fatty acid synthesis, phospholipid synthesis and protein synthesis (Murray and Koh 2014). Furthermore, in *B. subtilis* and *E. coli*, suppressors of thermosensitive alleles of essential DNA cycle genes have been repeatedly mapped to genes related to the central carbon metabolism, pointing to a possible integration via yet-to-be described mechanisms (Jannière *et al*. 2007; Maciąg *et al*. 2011; Maciąg-Dorszyńska *et al*. 2012; Tymecka-Mulik *et al*. 2017; Nouri *et al*. 2018).

In a real tour de force, Camsund and colleagues combined single-cell tracking and cell cycle dynamic analysis from video microscopy in a microfluidic device with CRISPR-Cas9 RNAi (Camsund *et al*. 2020). This technology allows for the characterization of the alteration in cell cycle dynamics associated with the inhibition of expression of tens of genes in a single experiment. Following a fluorescent reporter for DNA replication, they clustered lineages according to their cell cycle and growth dynamics (e.g. small/large size at birth or at the time of initiation of DNA replication). Focusing on cells with an altered average initiation size, they identified multiple genes known to be involved in the processes that are directly or indirectly regulating the balance between ATP- and ADP-loaded DnaA. These results highlight the central role of the balance between ADP- and ATP-loaded DnaA in integrating regulatory information, but also illustrate the multiplicity of mechanisms and sources of regulatory information that feed into the initiation of DNA replication. The effects of these multiple regulatory mechanisms are difficult to reconcile with models of replication initiation through the accumulation of DnaA protein up to a threshold. At the population level, the ATP- and ADP-loaded DnaA balance may be a good descriptor for the average timing of initiation of DNA replication, but the multiplicity of regulatory signals suggests that the DnaA protein is not the limiting factor for DNA replication; a finer description may be required at the single-cell level.

The control of the initiation of DNA replication remains an active field of research, which has been influenced by the concept of invariance of the initiation mass. It will be crucial to reconsider the massive amount of molecular data in light of the results from Zheng *et al*. (2020).

Divisome assembly and activation of cytokinesis

The field of bacterial cell division has gained tremendous molecular insights on how cell division works and how it is controlled (for reviews, see for example Du and Lutkenhaus 2017; Mahone and Goley 2020). Our purpose here is to highlight a few elements that are relevant to our understanding of the coordination between cell size, cell growth and the cell cycle. The same models proposed for DnaA and the initiation of DNA replication were proposed to be applicable for the activation of cell constriction through the accumulation of FtsZ protein up to a critical or integral threshold. However, here again, the multiplicity of the regulation layers suggests that these models are too simplistic.

The divisome (i.e. the multiprotein complex mediating cytokinesis) assembles progressively through a cascade of recruitments of proteins. The chain of recruitment ensures the maturation of the divisome so that cell constriction occurs in a timely manner, in concert at all three layers of the cell envelope (inner and outer membranes and the peptidoglycan layer) and in between the two copies of the genetic material. The highly conserved tubulin-like protein FtsZ assembles at midcell into short polymers that are anchored to the cytoplasmic membrane by ZipA (γ-proteobacteria specific protein) and FtsA proteins. The 'Z-ring' results from the treadmilling dynamics of FtsZ short polymers around the circumference of the cell (Bisson-Filho *et al*. 2017; Yang *et al*. 2017).

FtsA is also able to polymerize. The polymeric state of FtsA may be a first control point in the assembly and dynamics of the divisome (Pichoff *et al*. 2012), although the signal remains unclear. FtsA promotes the recruitment of intermediate proteins that connect the division machinery to the cell envelope and the chromosome, recruit functional modules and/or maintain the synthetic activity of the divisome in an 'off' state until activation. Among them, the sub-complex FtsEX was shown to establish a physical link between the cytoplasmic FtsZ polymers at the membrane and the peptidoglycan. FtsEX mediates the recruitment of amidases (through the intermediate activator protein EnvC in *E. coli*) that denude the glycan strains of the peptidoglycan and promote the recruitment of late cell division protein (Sham *et al*. 2011; Yang *et al*. 2011; Pichoff, Du and Lutkenhaus 2019). The next protein to be recruited, the essential division protein FtsK, is also involved in chromosome segregation (Bigot *et al*. 2007). FtsK recruits the FtsQBL sub-complex by interacting at least with FtsQ (Di Lallo *et al*. 2003; Dubarry, Possoz and Barre 2010). The role of FtsQBL is to hold in an 'off'-state the synthetic activity of the FtsWI complex (transglycosylase and transpeptidase, respectively) (Boes *et al*. 2019). The impact on cell division of FtsK variants unable to fulfill chromosome-related functions suggests a defect in the constriction process (Lesterlin *et al*. 2008; Stouf, Meile and Cornet 2013). These results naturally bring to mind the possibility of a checkpoint for the activation of the divisome dependent on the segregation status of the chromosome (Dubarry, Possoz and Barre 2010; Grainge 2010).

Once the cell division machinery is activated through an as-yet-unknown mechanism, FtsZ seems to play the role of conductor by constantly redistributing the active sites of peptidoglycan synthesis around the division site (Bisson-Filho *et al*. 2017; Yang *et al*. 2017). The GTPase activity of the FtsZ protein promotes the treadmilling dynamics of short FtsZ filaments around the constriction site. FtsZ proteins may remain static in filaments, but the affinity of components of the peptidoglycan synthesis machinery for FtsZ filaments may allow FtsZ dynamic structures

to displace the sites of peptidoglycan synthesis away from the most constricted regions of the constriction ring. In *E. coli*, the rate of FtsZ treadmilling does not dictate cell constriction rate (Yang *et al.* 2017), while it does in *B. subtilis* (Bisson-Filho *et al.* 2017). This difference may be related to the constraints associated with the synthesis of a septum in *B. subtilis* instead of two new poles 'on the fly' in *E. coli*. Regardless, the short and dynamic nature of FtsZ structures and their distributive function call for a revision of simple integral threshold models based on the accumulation of FtsZ up to a critical added amount. These models have the didactic advantage of relating the adder phenomenon at the cellular level to molecular elements, but they require that the FtsZ molecules used in one septum should not be used in another one. Otherwise, the added amount of FtsZ protein could not be linked to the initiation of cell division. A critical experiment would be to test whether FtsZ proteins can be used at multiple constriction sites, within the same cell or over generations.

The divisome appears more than ever as a dynamic machinery that is assembled in a complex manner, and its activation can depend on external information (e.g. from the cell envelope, the chromosome). To further illustrate the diversity of the sources of signals integrated by the divisome, let us just mention a few other examples. In a couple of seminal studies, Levin and co-workers identified a molecular link between enzymes related to UDP-glucose and cell division: in *B. subtilis* and *E. coli*, distinct enzymes (UgtP and OpgH, respectively) appear to inhibit FtsZ polymerization under nutrient-rich conditions, thus leading to an increase in cell size (Weart *et al.* 2007; Hill *et al.* 2013). In *E. coli*, the production of the cell division protein YmgF (Karimova, Robichon and Ladant 2009) depends on the cell cycle-dependent expression of the two genes flanking *oriC*, *gidA* and *mioC* (Lies *et al.* 2015). Moreover, the diguanylate cyclase YfiN acts as a cell division inhibitor in response to reductive and cell envelope stresses (Kim and Harshey 2016). Lastly, mutations in amino acid metabolism genes suggest that diverting the carbon flux from glycine, threonine and methionine biosynthetic pathways rescues some cell division defects associated with thermosensitive alleles of *ftsK*, *ftsQ* and to a lesser extent, *ftsI* (Vega and Margolin 2018).

The complexity of the regulation of the divisome is unlikely to be determined by the accumulation of a single component as in the conceptually elegant integral threshold model developed around FtsZ (Ojkic, Serbanescu and Banerjee 2019; Si *et al.* 2019). An assumption of these models requires that the amount of newly synthesized FtsZ constitutes the limiting factor for the activation of the division machinery, in spite of the large bundle of regulatory mechanisms that are necessary under a wide range of growth conditions. Making a parallel with the ATP- and ADP-loaded DnaA balance, the focalization of FtsZ treadmilling at midcell remains the major hub integrating regulatory information for the division process. However, FtsZ is clearly not the only relevant molecular player and probably not the rate limiting factor at each division cycle (Coltharp *et al.* 2016).

Driving the cell cycle via hierarchical transcription and protein degradation networks

A complex regulatory network driving the cell cycle in *Caulobacter crescentus* (reviewed in Lasker, Mann and Shapiro 2016) sets the pace of both DNA replication and cell division. Owing to their short half-life, RNA turnover is most often tuned by *de novo* transcription, while protein turnover is largely controlled by their degradation. The cell cycle transcriptional network of the bacterium *C. crescentus* is a prominent example of cell cycle control through cyclic expression of cell cycle master regulators (Lasker, Mann and Shapiro 2016). In parallel, the targeted degradation of the proteins is mediated by adaptor proteins so as to modulate protein amounts along the cell cycle (Joshi *et al.* 2015; Joshi and Chien 2016; Lasker, Mann and Shapiro 2016).

The control of the oscillations of cell cycle proteins is a knob that allows for the integration of nutritional and environmental cues. In *C. crescentus*, DnaA activity drives the timing of the initiation of DNA replication, while the oscillation in the amounts of the master regulator CtrA regulates the asymmetry of replication in the two asymmetric swarmer and stalked daughter cells (Jonas, Chen and Laub 2011). A nutritional stress reduces the rate of translation of the DnaA, which quickly reduces the amounts of DnaA protein in the cell because of the constitutive Lon-dependent degradation of DnaA. It also stabilizes the master regulator CtrA, which is a negative regulator of DNA replication. As a result, strong nutritional limitations quickly lead to cell cycle arrest in G1 phase (Leslie *et al.* 2015). In addition, stresses such as exposure to ethanol or high salt concentrations lead to the inactivation of the master regulator CtrA via its dephosphorylation and subsequent degradation. In the absence of CtrA, DNA replication is positively regulated while cell division is blocked. Stressed cells therefore become filamentous, with multiple copies of their genome. This stress response allows for the maintenance of growth in mass during the stress period in presence of nutrients, which provides a clear growth advantage (Heinrich, Sobetzko and Jonas 2016).

The existence of a gene expression network, with a defined temporal cycling, may be obscured in many bacterial species by the lack of synchronization method necessary to characterize systematically the temporal dynamics of gene expression along the cell cycle. However, such a regulatory network controlling both classical cell cycle checkpoints (initiation of DNA replication and cytokinesis) is difficult to envision in *E. coli* or *B. subtilis* because of their ability to manage overlapping cell cycles.

ORGANIZING CENTERS COORDINATE A MULTIPLICITY OF MECHANISMS TO SYNCHRONIZE CELL CYCLE EVENTS

The mechanisms interlinking cell cycle events are variable from bacterium to bacterium. However, a common theme emerges where the DNA and division cycles are synchronized by a coherent cross-regulation network (see graphical abstract). At early stages of the DNA cycle, the assembly of the divisome is inhibited, while at late stages DNA segregation and cell division are involved in a crosstalk that precipitates the completion of both processes. It appears that the chromosome architecture at the cellular level plays a pivotal role in this interplay between DNA and division cycles (Haeusser and Levin 2019).

Getting organized to coordinate cell cycle events

Bacteria are highly organized unicellular organisms (Hoppert and Mayer 1999). This high degree of cellular organization is vital for the proper coordination of all cellular processes. Every aspect of DNA and division cycles and cell growth takes on an organizational dimension: from chromosomes, secondary replicons and their physical assembly as nucleoids, to protein patterning across the cytoplasm and the cell envelope. Cell cycle

progression is fundamentally based on dynamic spatial patterning of cell cycle regulators, and the cellular organization of the chromosome plays a central role in their localization as much as the regulators define the choreography of the chromosome along the cell cycle. In the model organism *C. crescentus*, the cell cycle has historically been studied through the dynamic patterning of cell cycle regulating proteins, while in *E. coli* and *B. subtilis* the cellular architecture of the chromosome has often taken the center stage. The latter chromosome centric view has the advantage of grouping bacteria in two major classes—chromosome dominantly organized around their (i) origin of replication region or (ii) terminally replicated region—and we will use this point of view to briefly describe how cellular organization promotes the coordination of multiple cellular processes.

Bacterial chromosomes are typically circular DNA molecules defined by three major features: (i) the origin of replication *oriC*, (ii) the recombination site *dif* on the opposite side of *oriC* (halfway through the circular DNA molecular starting from *oriC*) and (iii) *oriC*-*dif* oriented motifs such as KOPS (Bigot *et al.* 2005) and Chi sites (El Karoui *et al.* 1999), and base composition biases as the GC skew. Chromosomes are thus bipolarized from *oriC* to *dif*. These two sites are also part of large chromosomal domains (or macrodomains) displaying homogeneous subcellular localization and dynamics (Niki, Yamaichi and Hiraga 2000; Valens *et al.* 2004). The *oriC* and *dif* sites together with their surrounding sequences thus occupy specific subcellular locations. These locations may vary between bacteria. Strikingly, each chromosome locus also occupies a typical location in the cell that follows its linear arrangement along the DNA molecule (Viollier *et al.* 2004; Espeli, Mercier and Boccard 2008). Therefore, the bulk of chromosomal DNA, as the specific *oriC* and *dif*-carrying regions, carries both genetic and spatial information. It follows that proteins binding to specific chromosomal loci are spatially patterned in the cell.

The 3D organization of one part of the bacterial chromosome seems to be sufficient to direct the global conformation of the chromosome in the cell. Most bacteria rely on a ParABS system to guide DNA segregation (Livny, Yamaichi and Waldor 2007). Briefly, the ParB protein binds specifically the centromeric sequence(s) *parS* to nucleate the formation of a large complex containing other ParB protein copies bound dynamically and non-specifically to the DNA around *parS* over several kilobases (Breier and Grossman 2007). The subcellular positioning of this partition complex and/or its bi-polar migration leading to DNA segregation depends on the cognate ParA ATPase protein (Lim *et al.* 2014; Sanchez *et al.* 2015). In organisms with a chromosome-borne ParABS system, *parS* sites are most often concentrated near the origin of replication, leading to long range organization of the *oriC* regions as macrodomains and to ordered chromosome positioning and segregation following the *oriC* to *dif* axis. In addition, an interplay between the ParB/*parS* and the SMC (structural maintenance of chromosomes) complexes resulting in ordered pairing of the two *oriC* to *dif* chromosome halves (or replichores; Blattner *et al.* 1997) has been revealed in different organisms (*C. crescentus*, *B. subtilis*, *Pseudomonas aeruginosa*; Le *et al.* 2013; Vallet-Gely and Boccard 2013; Wang *et al.* 2017). It thus seems that the *oriC*-*parS* region contains the required information for global chromosome arrangement in these organisms.

Enterobacteriaceae are a noticeable example where SMC and the chromosomal ParABS systems seem to have been lost through evolution. Although it is still unclear how the organization and bipolar migration of the *oriC* regions is achieved, the whole organization of the chromosome seems to have switched from an *ori*-centric to a *dif*-centric mode into which the terminal part of the chromosome (*ter* domain) containing *dif* seems to hold the information necessary for chromosome structuration. These bacteria contain a specific cluster of genes including genes encoding the condensin-like complex MukBEF and the MatP protein (Brézellec *et al.* 2006). MatP specifically binds *matS* sites scattered along a large *ter* region (800 kb in *E. coli* K12) (Mercier *et al.* 2008). It interacts with divisome-borne proteins (see below), keeping *ter* regions at midcell during the D period and with the MukBEF complex, acting in the global cellular positioning of the chromosome (Nolivos *et al.* 2016). Importantly, recent work identified functional homologs of the *E. coli* components outside *Enterobacteriaceae*, promoting the necessary integration of the late events of chromosome segregation with cytokinesis (Woldemeskel *et al.* 2017; Ozaki, Jenal and Katayama 2020) (see below).

Mechanistic coupling between DNA replication and segregation

A common feature in bacteria is that DNA replication and segregation are two largely overlapping events. These two processes forming the DNA cycle are not intrinsically coupled, but are made interdependent by two types of mechanisms. The initiation of DNA replication integrates the capacity of the cell to perform DNA segregation as a signal (i.e. sensing the presence of a complete partition complex). In addition, the completion of DNA replication can be facilitated by a proper segregation of newly synthesized sister chromatids.

The DNA replication initiation factor DnaA and segregation system ParA/ParB-*parS* (similar to Soj/Spo0J-*parS* in *Bacillus subtilis*, RctA/RctB-*parS* in *Vibrio cholerae*) interlink DNA replication and segregation. Deletion of *spo0J*/*parB* in *B. subtilis* or of *parA1*, *parB1* or *parS1* in *V. cholerae* led to abnormal localizations of *oriC* and a dysregulation of DNA replication (Lee *et al.* 2003; Yamaichi *et al.* 2011). Scholefield *et al.* showed in *B. subtilis* that the partition mechanism seems to regulate DNA replication through the dimerization of Soj (ParA) and its fixation to Spo0J (ParB): the monomeric form of Soj is able to depolymerize oligomers of DnaA both *in vitro* and *in vivo*, thus imposing a delay in DNA replication initiation. The physical interaction of Spo0J with Soj limits the regulatory activity of Soj on DnaA (Scholefield *et al.* 2011; Scholefield, Errington and Murray 2012). In *V. cholerae*, the two chromosomes regulate differently their replication. The replication initiation factor RctB of chromosome II is able to bind specifically to *parS*, leading to the titration of RctB and a delay in the initiation of DNA replication (Gerding *et al.* 2015). A similar behavior has been described in *C. crescentus*, where DnaA also exhibits the capacity to bind *parS*. However, in *C. crescentus*, recent evidence suggested that DnaA might promote DNA segregation (Mera, Kalogeraki and Shapiro 2014), while in *B. subtilis* and *V. cholerae*, the partition system modulates the activity of the initiator protein. It is tempting to speculate on the role of this regulatory inversion (DnaA acts on ParA). The initiator protein can induce the accumulation of ParA at the new pole as replication is initiated and may therefore drive the assembly of a new polar hub as ParA will help forming a second PopZ matrix at the new pole (Laloux and Jacobs-Wagner 2013).

DNA replication provokes topological constraints on the DNA with two consequences: accumulation of positive supercoiling ahead of the replication forks provokes their arrest and the transmission of the constraints behind the forks leads to interwoven sister chromatids. The release of these topological

constraints by the type II topoisomerases is thus essential for the completion of DNA replication as well as for subsequent segregation of sister chromatids. This release is tightly controlled, suggesting its timing is important. This is the case in the two following examples. In *C. crescentus*, the high-fitness cost (nearly essential) gene *gapR* encodes a nucleoid-associated DNA-binding protein with peculiar DNA-binding dynamics (i.e. a very low dissociation constant). GapR accumulates in front of the replication forks (Arias-Cartin *et al.* 2017) and drives the activity of gyrase, the type II topoisomerase with prominent swivel activity in front of the forks (Guo *et al.* 2018). This release of topological constraints ahead of the forks controlled by GapR is essential for the completion of DNA replication under fast growth conditions. In *E. coli*, the resolution of pre-catenanes (interwoven nascent chromatids) by TopoIV appears controlled by an orchestrated delay in methylation of newly synthetized DNA (Wang, Reyes-Lamothe and Sherratt 2008; Lesterlin *et al.* 2012; Joshi *et al.* 2013). This is thought to promote a 5–8 min (~400 kb) period of post-replicative cohesion behind progressing forks and shortening this time provokes global segregation defects. Note that in *Enterobacteriaceae* this does not apply to the *ter* region, into which catenane resolution is primarily controlled by MatP and cohesion times are longer (Nolivos *et al.* 2016).

DNA segregation regulates negatively cell division at early stages and positively at final stages

Cell division is synchronized with DNA segregation in multiple ways. Yet, the combination of these mechanisms generates a coherent coordination of DNA and division cycles. DNA replication and early DNA segregation stages inhibit cell division, while late segregation steps are positively coordinated with cell constriction (Fig.2).

The dynamics of the cell cycle at the single-cell level, in agreement with earlier results, revealed that the D period plays a peculiar role in cell size homeostasis (see above). The mechanisms described below could play a pivotal role in coupling the cell cycle with growth by modulating the duration of the D period. Most mechanisms are involved with focalizing FtsZ treadmilling at midcell. One mechanism involving the large essential division protein FtsK stands out and seems to mediate a crosstalk between cell division and DNA segregation.

Cellular patterning of cell division inhibitors prevents premature cell division

Multiple mechanisms grouped under the term nucleoid occlusion (NO) are known to prevent the assembly and activation of the division machinery over unsegregated chromosomes (Fig. 2A and B). The DNA-binding protein SlmA (*E. coli*) and Noc (*B. subtilis*) exemplify the first NO mechanism discovered, based on the cellular patterning of FtsZ polymerization inhibitors. The global organization and dynamics of the chromosomes in these organisms establish an inhibitor free zone at midcell as soon as the bulk of DNA is segregated toward each daughter cell and cleared away from midcell (Wu and Errington 2004; Bernhardt and de Boer 2005; Wu *et al.* 2009; Cho *et al.* 2011; Tonthat *et al.* 2011). Beyond SlmA in *E. coli* and Noc in *B. subtilis*, the Nucleoid Occlusion phenomenon is not fully understood and may be achieved by diverse mechanisms. The controlled activity of the RocS protein in time and space in *Streptococcus pneumoniae* cells seems to accomplish a very similar function, termed nucleoid protection (Mercy *et al.* 2019). The position of the division site is selected very early on (in the preceding division cycle) in *S. pneumoniae* cells. Taking advantage of the spatial information carried by the global structure of the chromosome, RocS interacts with the centromeric region of the chromosome and the protein ParB and localizes at the future division sites at the $\frac{1}{4}$ and $\frac{3}{4}$ positions of *S. pneumoniae* cells (Mercy *et al.* 2019). Thus, RocS localizes to the future division site to prevent septum closure over the chromosome instead of being distributed over the chromosome (Fig. 2C). The regulatory activity of RocS on the division machinery remains elusive.

The MinC/D/E proteins form a tri-partite system controlling the positioning of the division site through the pole-to-pole oscillation of MinC, which inhibits off-center cell division and FtsZ polymerization (Adler *et al.* 1967; Lutkenhaus 2007) (Fig. 2A and B). Interestingly, the Min systems have also been shown in *B. subtilis* (Kloosterman *et al.* 2016), and proposed in *E. coli* (Di Ventura *et al.* 2013), to participate to the efficient segregation of the chromosome. In this hypothesis, the pole-to-pole oscillation of the MinD protein, which also binds DNA, may help the segregation of chromosomes by dynamically tethering DNA to the membrane in a biased manner toward the poles. Different proteins of the same family fulfill related functions in different organisms. In *C. crescentus*, the MinC functional homolog protein MipZ interacts with the partitioning protein ParB (Mohl, Easter and Gober 2001). The tethering of the ParB-*parS* partition complex at the poles drives the formation of the bipolar gradient of MipZ once segregation is completed, allowing cytokinesis at midcell (Thanbichler and Shapiro 2006). In *Myxococcus xanthus*, the Pom system (PomX/Y/Z) performs a similar function by localizing PomZ, a positive regulator of FtsZ localization, at midcell through oscillation over the nucleoid (Treuner-Lange *et al.* 2013; Schumacher and Søgaard-Andersen 2017; Schumacher *et al.* 2017). In *S. pneumoniae*, division plane and site selection is mediated by the protein MapZ and the segregation of the origins of replication *via* its ParABS system (Fleurie *et al.* 2014; Raaphorst, van Kjos and Veening 2017).

ParB proteins provide yet another link between DNA segregation and the control of cell division. As mentioned in the previous paragraphs, in *S. pneumoniae*, ParB is involved in the division site selection and in the recruitment of the nucleoid protection factor RocS. Moreover, the segregation-related ParB homolog Spo0J from *B. subtilis* has also been shown to act synergistically with the Min system and nucleoid occlusion (Kloosterman *et al.* 2016; Haeusser and Levin 2019; Hajduk *et al.* 2019) to couple DNA segregation with division site selection and divisome dynamics (Raaphorst, van Kjos and Veening 2017; Haeusser and Levin 2019; Hajduk *et al.* 2019).

In *E. coli*, DNA segregation also depends on cell size. The duration of the D period positively correlates with cell size when cell length increases upon *ftsZ* depletion, or cell width increases upon *mreB* depletion (Zheng *et al.* 2016). In addition, the segregation of bulk DNA in the cell, a step referred to as nucleoid splitting and corresponding to the apparition of bilobed nucleoids (Bates and Kleckner 2005), is dependent on cell size: the larger the cell, the earlier the nucleoid splits (Campos *et al.* 2018). Furthermore, the distribution among bacteria of the mechanisms linking the DNA and division cycle is remarkably dependent on cell morphology. For example, cocci generally do not possess homologs of the Min and Noc/SlmA proteins (Pinho, Kjos and Veening 2013). Without poles, the Min system is unlikely to provide the correct information about the position of the division site.

The early inhibition of cell division by DNA segregation can take many forms and is also cell shape dependent. Importantly, the relation between cell size and the DNA cycle is not limited to the D period. In some *E. coli* mutants with reduced size (by ~30%)

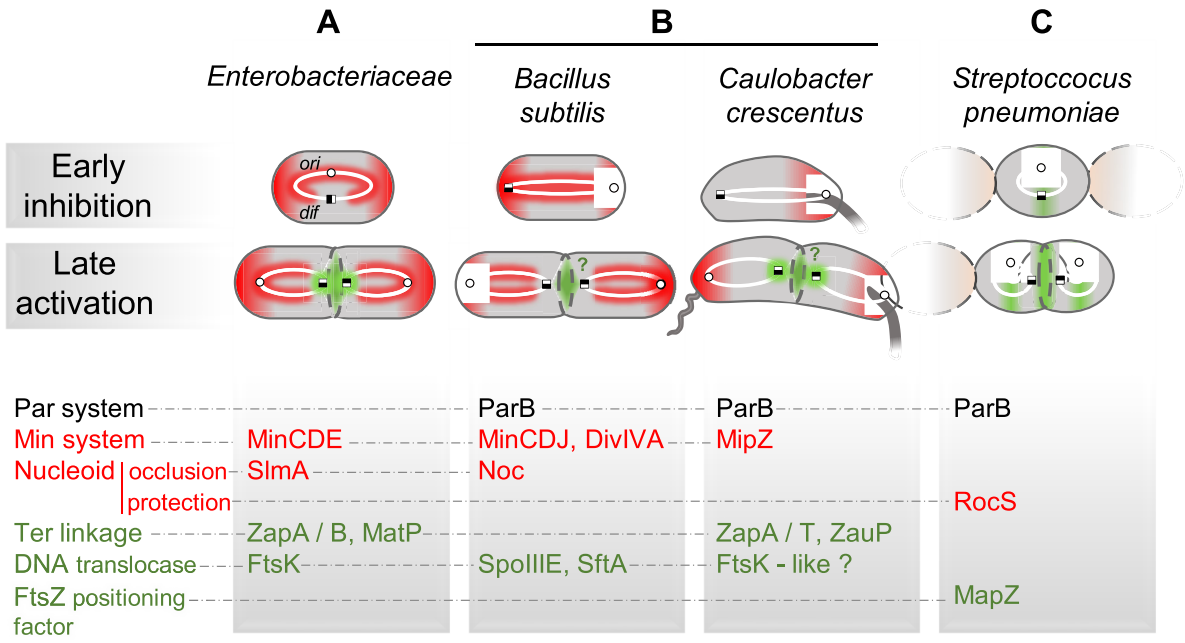

Figure 2. Phases of early inhibition and late activation drive the coordination between cell division and DNA segregation. The choreography of the chromosome along the cell cycle drives the temporal switch between inhibition and by spatially reorganizing key regulatory factors. On panels **(A)**, **(B)** and **(C)** are represented the positive (green) or negative (red) effects of key factors on the coordination between DNA segregation and cell division. Chromosomes are represented in white with their origin of replication, *ori* (white circle) and *dif* site (black and white square). The names of the major positive (green) and negative (red) regulators described in the text for each model organism are listed below each schematic. The black color for ParB indicates a dual role. (A) In *Enterobacteriaceae*, the early inhibition of divisome assembly is mediated by the oscillatory Min system and the nucleoid occlusion factor SlmA. At later stages, SlmA has cleared away from midcell and multiple activators drive the localization of the *ter* macrodomain, centered around *dif*, at the division site and coordinate late segregation events with cytokinesis. (B) In *B. subtilis* and *C. crescentus*, ParB, Noc and Min (ParB and MipZ in *C. crescentus*) organized at the pole(s) and around *ori* cooperate to inhibit Z-ring formation at midcell. At later stages, the relocation of the two *ori* copies to both poles lifts the early inhibition. The crosstalk between late segregation steps and the division machinery via FtsK homologs (SpoIIIE and SftA in *B. subtilis*) remains to be established. (C) In *S. pneumoniae*, the Min system is absent and poles do not serve as organizing centers. Instead, the segregation of the two copies of *ori* lifts the inhibition of the division machinery activity at midcell and drives the assembly of new division machineries at $\frac{1}{4}$–$\frac{3}{4}$ positions in coordination with MapZ. The role the DNA translocase FtsK has not been investigated in this organism. These new division sites are maintained inactive by the presence of RocS (functionally homologous to nucleoid occlusion). In *S. pneumoniae*, ParB plays a dual role by helping localize FtsZ and the nucleoid protection factor RocS. The position of the *dif* site schematized in *S. pneumoniae* cells does not reflect experimental observations and has been postulated for illustration purposes.

growing at the same rate as the wild type strain, it is not the D period but the C period that is shortened, although the mutated genes (*ftsA* and *pgm*) are unlikely to have a direct effect on the speed up of ~25% of DNA replication (Hill *et al.* 2012).

Late segregation events promote divisome assembly and activate cell division

Multiple molecular mechanisms are at play to coordinate the late stages of DNA segregation with cell division (Fig. 2). The late stages of segregation occur during the D period. They include the resolution of two major types of physical links between sister chromosomes. (i) The bidirectional replication of circular DNA molecules results in covalently interlocked chromosomes. This molecular architecture of replicated chromosomes, called catenanes, is resolved by TopoIV. (ii) Chromosome dimers (the joining of the two daughter chromosomes into a single circular DNA molecule), frequently formed by recombinational repair, are resolved by XerCD-mediated site-specific recombination at the *dif* site. Catenanes and dimer resolution appear to be linked in time and space and both are controlled by an interplay between MatP and the FtsK proteins (Stouf, Meile and Cornet 2013; El Sayyed *et al.* 2016). Two direct connections between late segregation and cytokinesis have been reported (Mannik and Bailey 2015), both of them involving global chromosome structure and dynamics.

MatP interacts with ZapB, an abundant small protein that interacts indirectly with FtsZ *via* ZapA (Espeli *et al.* 2012). ZapA and ZapB are thought to form a large highly dynamic structure localizing both at the *ter* region of the chromosome and at the divisome (Buss *et al.* 2017). They help focalize FtsZ at midcell and induce the co-localization of the *ter* region with the divisome (Bailey *et al.* 2014). Since MatP is also required for normal cell division, it has been proposed that it helps localizing ZapB and ZapA at midcell depending on the positioning of the *ter* region, mediating a positive control on divisome assembly referred to as the *ter*-linkage (Bailey *et al.* 2014). Note that the *ter* region is devoid of SlmA-binding sites (Cho and Bernhardt 2013), preventing contradictory signals between positive and negative signals mediated by DNA-bound MatP-ZapAB and SlmA, respectively. In addition, without MatP, cells constrict faster (Coltharp *et al.* 2016). Taken together, these results suggest that the *ter*-linkage can both promote the assembly of the divisome and slow down its activity once activated, if MatP remains at midcell with unsegregated *ter* regions at the time of cell constriction. As mentioned earlier, functional homologs of the components of the *ter*-linkage have been found outside of the *Vibionaceae* and *Enterobacteriaceae* (Woldemeskel *et al.* 2017; Ozaki, Jenal and Katayama 2020)—ZapA was initially identified in *B. subtilis* (Gueiros-Filho and Losick 2002). The functional homolog of MatP in *C. crescentus*, ZapT, was found to preferentially bind DNA around the *dif* site and to help localize the *ter* domain of the chromosome with

the division machinery (Ozaki, Jenal and Katayama 2020). The same study reports the presence of ZapT homologs in a diverse set of proteobacteria, highlighting the importance of connecting late segregation steps with division, even when the chromosome adopts an *ori*-centric organization.

The FtsK protein is an obvious link between late segregation and cell division since it is physically involved in both processes (Fig. 2A and B). This highly conserved protein is large, multifunctional, multidomain and broadly organized in three spatial domains (Bigot *et al.* 2004, 2007; Crozat *et al.* 2014). The N-terminal side of the protein anchors FtsK to the inner membrane specifically at the divisome. In *E. coli*, this domain is essential to cell division (Begg, Dewar and Donachie 1995; Dubarry, Possoz and Barre 2010). The highly conserved C-terminal region is organized in three subdomains: α and β form an ATP-fueled DNA translocation motor (Massey *et al.* 2006; Sivanathan *et al.* 2006), while γ controls translocation (Bigot *et al.* 2005; Ptacin *et al.* 2006; Sivanathan *et al.* 2006; Yates *et al.* 2006). The central portion separating the N- and C-terminal domains is a highly variable linker containing interaction interfaces with proteins of the divisome (Di Lallo *et al.* 2003; Dubarry, Possoz and Barre 2010). The DNA-translocation activity of FtsK is oriented by recognition of the KOPS DNA motifs, which orientation most preferentially follows the *oriC* to *dif* axis of the chromosome, by the γ subdomain (Bigot *et al.* 2005; Levy *et al.* 2005). Although KOPS motifs are present and their orientation biased on the whole chromosome, *E. coli* FtsK most preferentially acts in a restricted region around *dif*, roughly corresponding to the *matS*-containing region (Deghorain *et al.* 2011). This region displays an ordered segregation pattern, *dif* being segregated last, which depends on MatP and the KOPS-reading activity of FtsK (Stouf, Meile and Cornet 2013). It has been proposed that MatP, by keeping the *ter* region at midcell, creates a substrate for FtsK that in turn removes MatP while translocating (Graham *et al.* 2010), allowing segregation to complete (Stouf, Meile and Cornet 2013). Translocation stops at *dif* upon interaction of the γ domain with XerCD, which also induces recombination to resolve dimers (Graham *et al.* 2010). Both FtsK and XerCD positively control the activity of TopoIV (El Sayyed *et al.* 2016). All events of late chromosome segregation thus appear coupled in time and space: ordered segregation by FtsK and final chromosome untangling, including resolution of dimers and removal of catenanes.

In *E. coli*, late segregation events occur concomitantly with cell constriction or slightly before, i.e. at late steps of divisome assembly (Steiner and Kuempel 1998; Kennedy, Chevalier and Barre 2008; Stouf, Meile and Cornet 2013; Galli *et al.* 2017). The current model posits that this is due to activation of FtsK-mediated DNA translocation at the time of division. It has been proposed that this activation relies on the hexamerization of the N-terminal domain in the septum (Bisicchia *et al.* 2013). This concomitance of events appears under selection pressure during evolution. Indeed, in bacteria with multiple chromosomes, replication of the individual chromosomes is tuned so that each termination of replication events is coupled with cell division in the same manner (Du *et al.* 2016; Frage *et al.* 2016).

Several observations suggest that a reciprocal control, i.e. exerted by FtsK on cell division depending on the progression of segregation, also exists. Indeed, FtsK catalytic mutants unable to translocate (FtsK ATPase mutants) display strong cell shape defects and a lysis phenotype suggesting a defect in the control of cell envelope synthesis (Lesterlin *et al.* 2008; Stouf, Meile and Cornet 2013). Strains with large chromosome inversion altering the *oriC* to *dif* symmetry show a cell division delay phenotype that turns lethal, involving massive cell lysis, when FtsK translocation is impaired (Lesterlin *et al.* 2008). In addition, an FtsK variant unable to recognizes KOPS provokes both late *ter* segregation and a delay in cell division (our unpublished results). Taken together, these data support the idea that FtsK activities on the chromosome modulate the divisome synthetic activity. It thus appears that the dynamics of the *ter* region, primarily controlled by MatP, and FtsK are at the core of a positive feedback loop leading to the concomitant closing of the septum with the translocation of the terminal region of the chromosome out of the division site. Interestingly, FtsK is genetically linked to PBP5 (*dacA*) (Begg, Dewar and Donachie 1995) and interacts physically with PBP3 (Di Lallo *et al.* 2003), two enzymes involved in peptidoglycan synthesis at the division septum.

All the mechanisms presented above could potentially play a role in the control of the duration of the D period to achieve the adequate coupling between cell size and the DNA and division cycles. These mechanisms are expected to influence the age dependence of the division process (Osella, Nugent and Lagomarsino 2014), although the tight correlation between nucleoid and cell size (Campos *et al.* 2018; Gray *et al.* 2019; Wu *et al.* 2019) may introduce another size-dependent sensing element to couple the DNA and division cycles to cell size. In any case, the dynamics of the 3D organization of bacterial genomes is proposed to be of paramount importance for the coordination of the cell cycle with cell size and growth (i.e. for cell proliferation).

External signals: modulation and control of cell proliferation

Beyond the metabolic information integrated at both the initiation of DNA replication and divisome maturation, growth and metabolism deeply influence cell cycle progression through multiple molecular mechanisms operating at different time and space scales.

Constant survey by secondary messengers

Small molecules derived from nucleotides are widely distributed secondary messengers involved in cell morphogenesis and cell differentiation (Jenal, Reinders and Lori 2017). Despite high regulatory potential, those small molecules received little interest so far as potential instantaneous coupling signals between cellular processes driving cell growth and the DNA and division cycles. Some of them have been shown to alter cell cycle progression and their role in the control of cell proliferation may have been underestimated.

Cyclic-di-GMP has been recognized as a major effector involved in cell differentiation in multiple bacterial species (*C. crescentus*, *Pseudomonas aeruginosa*, *Myxococcus xanthus*, *Bdellovibrio bacteriovorus*—reviewed in Jenal, Reinders and Lori 2017). It is also involved in the control of cell cycle progression, at the very least in the α–proteobacterium *C. crescentus*, via interactions with ATPase domains of cell cycle regulators (Lori *et al.* 2015; Jenal, Reinders and Lori 2017).

The stringent response mediated by the synthesis of ppGpp (guanosine tetraphosphate) by the RelA and SpoT proteins, 'adapts' the protein and lipid biosynthetic flux to the corresponding biosynthetic capacities. Low synthetic fluxes compared with synthetic capacities signal an impoverishment of growth conditions and the stringent response, mediated by an increase in ppGpp level in the cell, induces a global change in gene expression, eventually resulting in cell growth and cell cycle arrest (Ferullo and Lovett 2008). The ppGpp molecule

has been found to inhibit DNA replication by directly interacting with the DNA primase DnaG in *E. coli* and *B. subtilis* (Wang, Sanders and Grossman 2007; Maciąg *et al.* 2010; Maciąg-Dorszyńska, Szalewska-Pałasz and Węgrzyn 2013). Basal ppGpp levels have also been found to be critical for relationship between growth rate and the DNA cycle. In the absence of ppGpp, the initiation mass and the positive correlation of the rate of initiation of DNA replication with growth rate are changed, and DNA segregation seems impaired (Fernández-Coll *et al.* 2020). This impact of the cellular concentration of ppGpp may be largely explained by the ppGpp-dependent regulation of the expression of the DNA gyrase. In the absence of ppGpp, at least one of the gyrase genes (*gyrA*) is overexpressed (Fernández-Coll *et al.* 2020). The overproduction of the DNA gyrase is expected to induce a high degree of negative supercoiling that may inhibit the initiation of DNA replication (see above) as well as DNA segregation.

The secondary messenger Ap4A (di-adenosyl tetraphosphate) has been shown to modulate the timing of cell division in *E. coli* (Nishimura *et al.* 1997). Oxidative stress has been shown to induce the synthesis of Ap4A (among other di-nucleotidyl polyphosphate molecules) in *Salmonella typhimurium* (Bochner *et al.* 1984). It is tempting to speculate on the existence of a redox sensing mechanism modulating cell cycle progression in γ-proteobacteria.

In the α-proteobacterium *C. crescentus*, the oxidoreductive state of the cell constitutes a regulatory signal monitored by the proteins KidO and GdhZ and that modulates the cell cycle progression both at an early stage, by controlling the decatenation of DNA by Topo IV (KidO) (Narayanan *et al.* 2015), and at later stages, by modulating the FtsZ polymerization at midcell (KidO and GdhZ) (Radhakrishnan, Pritchard and Viollier 2010; Beaufay *et al.* 2015).

Small molecules allow for controls with short time-scales. Their role in the control of cell growth and the cell cycle is well documented. It is tempting to envision a more general role, beyond the specific examples reported here. Secondary messengers may provide a constant coupling between cellular processes by tuning up or down all physiological processes in response to general signals (e.g. metabolic capacity, oxidative state).

Do finite resource effects enslave cell cycle progression to cell growth?

At the cellular scale, an intrinsic feedback between all cellular processes is imposed by a finite resource effect. This finite resource effect may be yet another possible mechanism at the origin of the proportional coupling between the rates of passage through the D period and the growth rate.

The notion of finite resources is perhaps best understood through the ribosome autocatalytic synthesis paradigm. The number of ribosomes per cell can be optimized by balancing translation capacity with the associated flux of amino acids (Scott and Hwa 2011; Scott *et al.* 2014; Kafri *et al.* 2016). Too few ribosomes would reduce the cellular growth rate while too many ribosomes would consume too much amino acids for autocatalytic ribosome synthesis and reduce growth rate as well. Resource allocation models recapitulate the correlation observed between the ribosomal content of a cell and growth rate (Scott *et al.* 2010; Scott and Hwa 2011). Proteomics data support this proteome allocation model (Hui *et al.* 2015). Assuming proportionality to enzyme production rates, metabolic fluxes can be used to optimize energy allocation to proteome sectors in a genome-scale metabolic model, thereby interlinking metabolism with the SMK growth law through protein costs (Mori, Marinari and Martino 2019). Reframing the resource allocation as a self-replicating machine explicitly introduces a cell duplication program in the model (Pugatch 2015; Jun *et al.* 2018) and will offer a versatile framework to explore, support or disprove cell cycle and growth control models in a more holistic manner, at the cellular scale (see Groot *et al.* 2020; Pandey, Singh and Jain 2020).

Finite resources effectively impose correlations between all cellular processes, including the ones underlying cell growth and the DNA and division cycles. As a result, all processes display homeostatic behaviors, even if they are not the controlling ones. As an illustration, protein amounts per cell follow the cell size homeostatic behavior, without being the controlling element (Susman *et al.* 2018). As a consequence, we cannot take the homeostatic behavior of a phenotype (e.g. cell size, DnaA/FtsZ amounts) under steady state conditions as a proof that it is homeostatically controlled (Amir 2017).

Continuous monitoring of cell physiology and the finite resource effects open the intriguing possibility for an as-yet-poorly explored type of cell proliferation control. Time and size scales at the cellular level would be set by the continuously adjusted balance of the cell cycle progression and growth rates. This type of control is coherent with the remarkable number of genetic evidences linking the cell cycle and cellular metabolism (Jannière *et al.* 2007; Maciąg *et al.* 2011; Maciąg-Dorszyńska *et al.* 2012; Tymecka-Mulik *et al.* 2017; Nouri *et al.* 2018; Vega and Margolin 2018). The complexity of such a connection between metabolism and cell proliferation may seem a little dizzying. However, similarly to the growth laws describing the dependence of cell size with growth [see Equations (1) and (2)], the connection between cell growth and metabolism can be described with a very low number of variables. Up to a growth rate of $\sim$0.7 doubling per hour, growth rate is linearly related with carbon intake (Groot *et al.* 2019). These results suggest that a limited number of constraints shape the resource allocation strategy, at least at relatively slow growth rates. It is therefore tempting to ask how resources are allocated to the cell cycle and cell growth and whether this allocation can explain the nearly reciprocal relationship observed between growth and cell cycle progression rates, both at the population and single-cell level (Wallden *et al.* 2016; Zheng *et al.* 2020).

CONCLUSIONS

Maybe not so surprisingly, many molecular mechanisms feed into cell cycle progression and its coordination with cytokinesis and cell growth. These mechanisms can be globally classified and ordered in a logical set of rules underpinning cell proliferation. The cell cycle checkpoints are the pacemakers. In *C. crescentus*, a transcriptional regulatory network drives the activity of DnaA and FtsZ. In *E. coli*, it is believed that cell growth dictates the rate of accumulation of these two proteins up to a threshold that triggers the transition toward the next step. Following the rhythm pulsed by the checkpoints, cells commit to DNA replication, segregation and cytokinesis and make use of organizing centers to coordinate them. Polar hubs (*C. crescentus* and *V. cholerae*) or chromosomal domains (*ori* in *B. subtilis* and S. pneumoniae, *ter* in *E. coli*) concentrate key regulators interactions to coordinate the different cell cycle phases. DNA replication and segregation are coupled through DnaA-ParA interactions and the resolution of topological structures. Then, cell division and DNA segregation are coupled through multiple mechanisms (depending on the organism) that, in essence, prevent

FtsZ polymerization at midcell at early stages of DNA segregation and activate cell constriction at final stages (Fig. 2). Finally, a number of mechanisms signal metabolic information, or other external signal to the different machineries driving the cell cycle and cytokinesis. Among them, cell growth may play a special role. The necessary resource allocation to the different cellular processes prescribes a growth rate. This growth rate enslaves the rates of metabolite, RNA and protein productions, and may thereby set the same tempo to all cellular processes (provided that the resource allocation strategy is optimal).

At the cellular scale, the tracking of single cells progressing through their cycle strongly suggests that more than one cell cycle event must be coupled to cell size. Typically, at least one cell division event and one DNA cycle event are linked to cell size via an adder between consecutive events to couple the cell cycle with cell growth. The coupling between the division and DNA cycles remains unclear (Fig. 1). The molecular mechanisms outlined in this review would rather support a model where the D period may be variable because of a coupling between DNA segregation and cell constriction.

The complexity and the multiplicity of mechanisms involved in the coordination of cell proliferation events preclude any chance of a comprehensive understanding without the help of models. Intriguingly, models derived from cell size homeostasis studies elegantly couple the DNA and division cycles to growth with simple rules. It is an exciting prospect to explore how the complexity of the regulatory network vanishes out at the cellular scale. In this pursuit, the profound knowledge of the molecular mechanisms underpinning cell proliferation is necessary to construct meaningful coarse-grain models of the cell cycle and cell proliferation. Knowing how *E. coli* cells grow, replicate and segregate their DNA or assemble their division machinery allowed for the interrogation of the SMK growth law by introducing independent perturbations in each variable of the model through manipulations of the different molecular mechanisms at play (Zheng *et al.* 2016; Si *et al.* 2017). In this respect, it appears that introducing more molecular complexity in cellular scale models of cell size homeostasis will be necessary. Neither DnaA nor FtsZ protein amounts per cell can account for the molecular complexity of the regulation of the initiation of DNA replication and the inception of cell constriction. At this time, more caution should be taken when naming the possible controlling factors responsible for sensing growth and added size and include other factors such as ZapA or FtsK and the related mechanisms (Kleckner *et al.* 2018; Zheng *et al.* 2020).

Our conception of the coupling between cell growth and the cell cycle has profoundly changed over the last decade, from a pure size threshold to a couple of independent triggers that depend on added size, rather than cell size/mass/volume itself. A number of different models have been proposed where either the DNA cycle or the division cycle is limiting, or both. The differences between these models reside in their ability to describe all the variability and coupling parameters between cell cycle events that we can measure. We anticipate that these differences will also emerge from their ability to describe the behavior of cells progressing through other cell cycle periods such as the inception of cell constriction, nucleoid splitting, or the initiation of synthesis of pre-septal peptidoglycan. Accordingly, it appears to us that a better temporal definition of the bacterial cell cycle, beyond the initiation and termination of DNA replication, will be required. Exploring the role of late steps of the DNA cycle in coupling DNA and division cycles seems to be the next frontier and may lead to unexpected parallels with the coupling of cytokinesis with chromosome segregation during the anaphase and telophase stages of mitosis.

We also anticipate that the exploration of the law describing the dependency of the C + D period duration on growth rate will constitute one of the next goals in the field. The nearly reciprocal relationship between these two variables has strong implications on the coupling between cell growth and the cell cycle. A remarkable feature of this relationship is that it seems to hold at the population level (Michelsen *et al.* 2003; Zheng *et al.* 2020) and at the single-cell level (Wallden *et al.* 2016). This tight relationship between the growth rate and the rate of progression through DNA replication and segregation reminds us that, beyond the coupling of one or more cell cycle stages with cell size, the relative control of the rates (growth and cell cycle progression) is likely another crucial element underpinning cell proliferation efficiency. Cell size, growth and the cell cycle may be coupled not only via isolated cell cycle events but also through a controlled balance of the rates of growth and progression through cell cycle periods.

It appears that a 'core' cell cycle and cell division machinery drives the basic cell duplication process. For instance, DnaA and FtsZ as well as ParA and ParB are widely conserved proteins in the bacterial kingdom. This core machinery is then modulated by a substantial number (on the order of 10 in *E. coli*) of mechanisms that may vary among bacterial organisms (Pinho, Kjos and Veening 2013). These differences reflect, at least in part, functional and evolutionary constraints (e.g. cell shape) and that these different organisms have adopted different growth strategies. For instance, *C. crescentus* rely on a proliferation program that would be difficult to adapt to overlapping cycles as seen in *E. coli* or *B. subtilis*. Making sense of the diversity of proliferation strategies among bacterial strains and species will undoubtedly offer new perspectives on the evolutionary relevant mechanisms driving and controlling cell proliferation.

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REFERENCES

Adiciptaningrum A, Osella M, Moolman MC *et al.* Stochasticity and homeostasis in the *E. coli* replication and division cycle. *Sci Rep* 2015;**5**:1–8.

Adler HI, Fisher WD, Cohen A *et al.* Miniature *Escherichia coli* cells deficient in DNA. *Proc Natl Acad Sci USA* 1967;**57**:321–6.

Allman R, Schjerven T, Boye E. Cell cycle parameters of *Escherichia coli* K-12. *J Bacteriol* 1991;**173**:7970–4.

Amir A. Cell size regulation in bacteria. *Phys Rev Lett* 2014;**112**:208102.

Amir A. Is cell size a spandrel? *eLife* 2017;**6**:e22186.

Arias-Cartin R, Dobihal GS, Campos M *et al*. Replication fork passage drives asymmetric dynamics of a critical nucleoid-associated protein in *Caulobacter*. *EMBO J* 2017;**36**: 301–18.

Bailey MW, Bisicchia P, Warren BT *et al*. Evidence for divisome localization mechanisms independent of the Min system and SlmA in *Escherichia coli*. *PLoS Genet* 2014;**10**:e1004504.

Bates D, Kleckner N. Chromosome and replisome dynamics in *E. coli*: loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. *Cell* 2005;**121**:899–911.

Beaufay F, Coppine J, Mayard A *et al*. A NAD-dependent glutamate dehydrogenase coordinates metabolism with cell division in *Caulobacter crescentus*. *EMBO J* 2015;**34**:1786–800.

Begg KJ, Dewar SJ, Donachie WD. A new *Escherichia coli* cell division gene, *ftsK*. *J Bacteriol* 1995;**177**:6211–22.

Bernhardt TG, de Boer PAJ. SlmA, a nucleoid-associated, FtsZ-binding protein required for blocking septal ring assembly over Chromosomes in *E. coli*. *Mol Cell* 2005;**18**:555–64.

Bigot S, Corre J, Louarn J-M *et al*. FtsK activities in Xer recombination, DNA mobilization and cell division involve overlapping and separate domains of the protein. *Mol Microbiol* 2004;**54**:876–86.

Bigot S, Saleh OA, Lesterlin C *et al*. KOPS: DNA motifs that control *E. coli* chromosome segregation by orienting the FtsK translocase. *EMBO J* 2005;**24**:3770–80.

Bigot S, Sivanathan V, Possoz C *et al*. FtsK, a literate chromosome segregation machine. *Mol Microbiol* 2007;**64**:1434–41.

Bipatnath M, Dennis PP, Bremer H. Initiation and velocity of chromosome replication in *Escherichia coli* B/r and K-12. *J Bacteriol* 1998;**180**:265–73.

Bisicchia P, Steel B, Mariam Debela MH *et al*. The N-terminal membrane-spanning domain of the *Escherichia coli* DNA translocase FtsK hexamerizes at midcell. *mBio* 2013;**4**:e00800–13.

Bisson-Filho AW, Hsu Y-P, Squyres GR *et al*. Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. *Science* 2017;**355**:739–43.

Blattner FR, Plunkett G, Bloch CA *et al*. The complete genome sequence of *Escherichia coli* K-12. *Science* 1997;**277**:1453–62.

Bochner BR, Lee PC, Wilson SW *et al*. AppppA and related adenylylated nucleotides are synthesized as a consequence of oxidation stress. *Cell* 1984;**37**:225–32.

Boes A, Olatunji S, Breukink E *et al*. Regulation of the peptidoglycan polymerase activity of PBP1b by antagonist actions of the core divisome proteins FtsBLQ and FtsN. *mBio* 2019;**10**:e01912–18.

Boye E, Nordström K. Coupling the cell cycle to cell growth. *EMBO Rep* 2003;**4**:757–60.

Boye E, Stokke T, Kleckner N *et al*. Coordinating DNA replication initiation with cell growth: differential roles for DnaA and SeqA proteins. *Proc Natl Acad Sci USA* 1996;**93**:12206–11.

Breier AM, Grossman AD Whole-genome analysis of the chromosome partitioning and sporulation protein Spo0J (ParB) reveals spreading and origin-distal sites on the *Bacillus subtilis* chromosome. *Mol Microbiol* 2007;**64**:703–18.

Brézellec P, Hoebeke M, Hiet MS *et al*. DomainSieve: a protein domain-based screen that led to the identification of *dam*-associated genes with potential link to DNA maintenance. *Bioinformatics* 2006;**22**:1935–41.

Buss JA, Peters NT, Xiao J *et al*. ZapA and ZapB form an FtsZ-independent structure at midcell. *Mol Microbiol* 2017;**104**: 652–63.

Cadart C, Monnier S, Grilli J *et al*. Size control in mammalian cells involves modulation of both growth rate and cell cycle duration. *Nat Commun* 2018;**9**:1–15.

Campos M, Govers SK, Irnov I *et al*. Genomewide phenotypic analysis of growth, cell morphogenesis, and cell cycle events in *Escherichia coli*. *Mol Syst Biol* 2018;**14**:e7573.

Campos M, Surovtsev IV, Kato S *et al*. A constant size extension drives bacterial cell size homeostasis. *Cell* 2014;**159**:1433–46.

Camsund D, Lawson MJ, Larsson J *et al*. Time-resolved imaging-based CRISPRi screening. *Nat Methods* 2020;**17**:86–92.

Cho H, Bernhardt TG. Identification of the SlmA active site responsible for blocking bacterial cytokinetic ring assembly over the chromosome. *PLoS Genet* 2013;**9**:e1003304.

Cho H, McManus HR, Dove SL *et al*. Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist. *Proc Natl Acad Sci USA* 2011;**108**:3773–8.

Churchward G, Estiva E, Bremer H. Growth rate-dependent control of chromosome replication initiation in *Escherichia coli*. *J Bacteriol* 1981;**145**:1232–8.

Coltharp C, Buss J, Plumer TM *et al*. Defining the rate-limiting processes of bacterial cytokinesis. *Proc Natl Acad Sci USA* 2016;**113**:E1044–53.

Cooper S, Helmstetter CE. Chromosome replication and the division cycle of *Escherichia coli* B/r. *J Mol Biol* 1968;**31**:519–40.

Cooper S. MicroCorrespondence: does the initiation mass for DNA replication in *Escherichia coli* vary with growth rate? *Mol Microbiol* 1997;**26**:1137–43.

Cooper S. Regulation of DNA synthesis in bacteria: analysis of the Bates/Kleckner licensing/initiation-mass model for cell cycle control. *Mol Microbiol* 2006;**62**:303–7.

Crozat E, Rousseau P, Fournes F *et al*.,, The FtsK family of DNA translocases finds the ends of circles. *J Mol Microbiol Biotechnol* 2014;**24**:396–408.

Darmon E, Eykelenboom JK, Lopez-Vernaza MA *et al*. Repair on the Go: *E. coli* maintains a high proliferation rate while repairing a chronic dna double-strand break. *PLoS One* 2014;**9**:e110784.

Deforet M, van Ditmarsch D, Xavier JB. Cell-size homeostasis and the incremental rule in a bacterial pathogen. *Biophys J* 2015;**109**:521–8.

Deghorain M, Pagès C, Meile J-C *et al*. A defined terminal region of the *E. coli* chromosome shows late segregation and high FtsK activity. *PLoS One* 2011;**6**:e22164.

Di Lallo G, Fagioli M, Barionovi D *et al*. Use of a two-hybrid assay to study the assembly of a complex multicomponent protein machinery: bacterial septosome differentiation. *Microbiology* 2003;**149**:3353–9.

Di Ventura B, Knecht B, Andreas H *et al*. Chromosome segregation by the *Escherichia coli* Min system. *Mol Syst Biol* 2013;**9**:686.

Donachie WD, Robinson AC,. Cell division: parameter values and the process. In: *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*. Washington, DC: ASM PRess, 1987, 1578–93.

Donachie WD. Relationship between cell size and time of initiation of DNA replication. *Nature* 1968;**219**:1077–9.

Dubarry N, Possoz C, Barre F-X. Multiple regions along the Escherichia coli FtsK protein are implicated in cell division. *Mol Microbiol* 2010;**78**:1088–100.

Du S, Lutkenhaus J. Assembly and activation of the *Escherichia coli* divisome. *Mol Microbiol* 2017;**105**:177–87.

Du W-L, Dubarry N, Passot FM *et al*. Orderly replication and segregation of the four replicons of Burkholderia cenocepacia J2315. *PLoS Genet* 2016;**12**:e1006172.

El Karoui M, Biaudet V, Schbath S *et al*. Characteristics of Chi distribution on different bacterial genomes. *Res Microbiol* 1999;**150**:579–87.

El Sayyed H, Le Chat L, Lebailly E *et al*. Mapping topoisomerase IV binding and activity sites on the E. coli genome. *PLoS Genet* 2016;**12**:e1006025.

Espeli O, Borne R, Dupaigne P *et al*. A MatP-divisome interaction coordinates chromosome segregation with cell division in E. coli. *EMBO J* 2012;**31**:3198–211.

Espeli O, Mercier R, Boccard F. DNA dynamics vary according to macrodomain topography in the E. coli chromosome. *Mol Microbiol* 2008;**68**:1418–27.

Fernández-Coll L, Maciag-Dorszynska M, Tailor K *et al*. The absence of (p)ppGpp renders initiation of Escherichia coli chromosomal DNA synthesis independent of growth rates. *mBio* 2020;**11**:e03223–19.

Ferullo DJ, Lovett ST. The stringent response and cell cycle arrest in Escherichia coli. *PLoS Genet* 2008;**4**:e1000300.

Fievet A, Ducret A, Mignot T *et al*. Single-cell analysis of growth and cell division of the anaerobe Desulfovibrio vulgaris Hildenborough. *Front Microbiol* 2015;**6**:1378.

Fleurie A, Lesterlin C, Manuse S *et al*. MapZ marks the division sites and positions FtsZ rings in Streptococcus pneumoniae. *Nature* 2014;**516**:259–62.

Flåtten I, Fossum-Raunehaug S, Taipale R *et al*. The DnaA protein is not the limiting factor for initiation of replication in Escherichia coli. *PLoS Genet* 2015;**11**:e1005276.

Frage B, Döhlemann J, Robledo M *et al*. Spatiotemporal choreography of chromosome and megaplasmids in the Sinorhizobium meliloti cell cycle. *Mol Microbiol* 2016;**100**:808–23.

Galli E, Midonet C, Paly E *et al*. Fast growth conditions uncouple the final stages of chromosome segregation and cell division in Escherichia coli. *PLoS Genet* 2017;**13**:e1006702.

Gerding MA, Chao MC, Davis BM *et al*. Molecular dissection of the essential features of the origin of replication of the second Vibrio cholerae chromosome. *mBio* 2015;**6**:e00973.

Graham JE, Sivanathan V, Sherratt DJ *et al*. FtsK translocation on DNA stops at XerCD-dif. *Nucleic Acids Res* 2010;**38**:72–81.

Grainge I. FtsK – a bacterial cell division checkpoint? *Mol Microbiol* 2010;**78**:1055–7.

Gray WT, Govers SK, Xiang Y *et al*. Nucleoid size scaling and intracellular organization of translation across bacteria. *Cell* 2019;**177**:1632–48.e20.

Grilli J, Cadart C, Micali G *et al*. The empirical fluctuation pattern of E. coli division control. *Front Microbiol* 2018;**9**:1541.

Grilli J, Osella M, Kennard AS *et al*. Relevant parameters in models of cell division control. *Phys Rev E* 2017;**95**:032411.

Groot DH, Boxtel C, Planqué R *et al*. The number of active metabolic pathways is bounded by the number of cellular constraints at maximal metabolic rates. *PLoS Comput Biol* 2019;**15**:e1006858.

Groot DH, Hulshof J, Teusink B *et al*. Elementary growth modes provide a molecular description of cellular self-fabrication. *PLoS Comput Biol* 2020;**16**:e1007559.

Gueiros-Filho FJ, Losick R. A widely conserved bacterial cell division protein that promotes assembly of the tubulin-like protein FtsZ. *Genes Dev* 2002;**16**:2544–56.

Guo MS, Haakonsen DL, Zeng W *et al*. A bacterial chromosome structuring protein binds overtwisted DNA to stimulate type II topoisomerases and enable DNA replication. *Cell* 2018;**175**:583–97.e23.

Haeusser DP, Levin PA. Keeping replication on par with division in Bacillus. *Mol Microbiol* 2019;**112**:747–50.

Hajduk IV, Mann R, Rodrigues CDA *et al*. The ParB homologs, Spo0J and Noc, together prevent premature midcell Z ring assembly when the early stages of replication are blocked in Bacillus subtilis. *Mol Microbiol* 2019;**112**:766–84.

Hansen FG, Atlung T, Braun RE *et al*. Initiator (DnaA) protein concentration as a function of growth rate in Escherichia coli and Salmonella typhimurium. *J Bacteriol* 1991b;**173**:5194–9.

Hansen FG, Atlung T. The DnaA Tale. *Front Microbiol* 2018;**9**:319.

Hansen FG, Christensen BB, Atlung T. The initiator titration model: computer simulation of chromosome and minichromosome control. *Res Microbiol* 1991a;**142**:161–7.

Harris LK, Theriot JA. Relative rates of surface and volume synthesis set bacterial cell size. *Cell* 2016;**165**:1479–92.

Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. *Science* 1989;**246**:629–34.

Heinrich K, Sobetzko P, Jonas K. A kinase-phosphatase switch transduces environmental information into a bacterial cell cycle circuit. *PLoS Genet* 2016;**12**:e1006522.

Helmstetter CE. Timing of synthetic activities in the cell cycle. In: *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*. Washington, DC: ASM Press, 1987, 1594–605.

Herrick J, Kohiyama M, Atlung T *et al*. The initiation mess? *Mol Microbiol* 1996;**19**:659–66.

Hill NS, Buske PJ, Shi Y *et al*. A moonlighting enzyme links Escherichia coli cell size with central metabolism. *PLoS Genet* 2013;**9**:e1003663.

Hill NS, Kadoya R, Chattoraj DK *et al*. Cell size and the initiation of DNA replication in bacteria. *PLoS Genet* 2012;**8**:e1002549.

Hoppert M, Mayer F. Prokaryotes: even without membrane-bounded compartments, prokaryotes display a high degree of subcellular organization. *Am Sci* 1999;**87**:518–25.

Ho PY, Amir A. Simultaneous regulation of cell size and chromosome replication in bacteria. *Front Microbiol* 2015;**6**:662.

Hui S, Silverman JM, Chen SS *et al*. Quantitative proteomic analysis reveals a simple strategy of global resource allocation in bacteria. *Mol Syst Biol* 2015;**11**:784.

Huls PG, Vischer NOE, Woldringh CL. Different amounts of DNA in newborn cells of Escherichia coli preclude a role for the chromosome in size control according to the "adder" model. *Front Microbiol* 2018;**9**:664.

Jannière L, Canceill D, Suski C *et al*. Genetic evidence for a link between glycolysis and dna replication. *PLoS One* 2007;**2**:e447.

Jenal U, Reinders A, Lori C. Cyclic di-GMP: second messenger extraordinaire. *Nat Rev Microbiol* 2017;**15**:271–84.

Jonas K, Chen YE, Laub MT. Modularity of the bacterial cell cycle enables independent spatial and temporal control of DNA replication. *Curr Biol* 2011;**21**:1092–101.

Joshi KK, Bergé M, Radhakrishnan SK *et al*. An adaptor hierarchy regulates proteolysis during a bacterial cell cycle. *Cell* 2015;**163**:419–31.

Joshi KK, Chien P Regulated proteolysis in bacteria: *Caulobacter*. *Annu Rev Genet* 2016;**50**:423–45.

Joshi MC, Magnan D, Montminy TP *et al*. Regulation of sister chromosome cohesion by the replication fork tracking protein SeqA. *PLoS Genet* 2013;**9**:e1003673.

Jun S, Si F, Pugatch R *et al*. Fundamental principles in bacterial physiology—history, recent progress, and the future with focus on cell size control: a review. *Rep Prog Phys* 2018;**81**:056601.

Kafri M, Metzl-Raz E, Jonas F *et al*. Rethinking cell growth models. *FEMS Yeast Res* 2016;**16**:fow081.

Karimova G, Robichon C, Ladant D Characterization of YmgF, a 72-residue inner membrane protein that associates with

the Escherichia coli cell division machinery. *J Bacteriol*. 2009;**191**:333–46.
Katayama T, Kasho K, Kawakami H. The DnaA cycle in Escherichia coli: activation, function and inactivation of the initiator protein. *Front Microbiol* 2017;**8**:2496.
Katayama T, Ozaki S, Keyamura K *et al*. Regulation of the replication cycle: conserved and diverse regulatory systems for DnaA and oriC. *Nat Rev Microbiol* 2010;**8**:163–70.
Kaur G, Vora MP, Czerwonka CA *et al*. Building the bacterial orisome: high-affinity DnaA recognition plays a role in setting the conformation of oriC DNA. *Mol Microbiol* 2014;**91**:1148–63.
Kennedy SP, Chevalier F, Barre FX. Delayed activation of Xer recombination at dif by FtsK during septum assembly in Escherichia coli. *Mol Microbiol* 2008;**68**:1018–28.
Kim HK, Harshey RM. A diguanylate cyclase acts as a cell division inhibitor in a two-step response to reductive and envelope stresses. *mBio* 2016;**7**:e00822–16.
Kleckner NE, Chatzi K, White MA *et al*. Coordination of growth, chromosome replication/segregation, and cell division in E. coli. *Front Microbiol* 2018;**9**:1469.
Kloosterman TG, Lenarcic R, Willis CR *et al*. Complex polar machinery required for proper chromosome segregation in vegetative and sporulating cells of Bacillus subtilis. *Mol Microbiol* 2016;**101**:333–50.
Koch AL, Schaechter M. A model for statistics of the cell division process. *Microbiology* 1962;**29**:435–54.
Koch AL. Does the initiation of chromosome replication regulate cell division?In: Rose AH, Tempest DW (eds). *Advances in Microbial Physiology*. Academic Press, 1977, 49–98.
Koppes LJ, Meyer M, Oonk HB *et al*. Correlation between size and age at different events in the cell division cycle of Escherichia coli. *J Bacteriol* 1980;**143**:1241–52.
Kubitschek HE, Newman CN. Chromosome replication during the division cycle in slowly growing, steady-state cultures of three Escherichia coli B/r strains. *J Bacteriol* 1978;**136**:179–90.
Laloux G, Jacobs-Wagner C. Spatiotemporal control of PopZ localization through cell cycle–coupled multimerization. *J Cell Biol* 2013;**201**:827–41.
Lasker K, Mann TH, Shapiro L. An intracellular compass spatially coordinates cell cycle modules in *Caulobacter crescentus*. *Curr Opin Microbiol* 2016;**33**:131–9.
Lee PS, Lin DC-H, Moriya S *et al*. Effects of the chromosome partitioning protein Spo0J (ParB) on oriC positioning and replication initiation in Bacillus subtilis. *J Bacteriol* 2003;**185**:1326–37.
Leonard AC, Helmstetter CE. Cell cycle-specific replication of Escherichia coli minichromosomes. *Proc Natl Acad Sci* 1986;**83**:5101–5.
Leonard AC, Rao P, Kadam RP *et al*. Changing perspectives on the role of DnaA-ATP in orisome function and timing regulation. *Front Microbiol* 2019;**10**:2009.
Leslie DJ, Heinen C, Schramm FD *et al*. Nutritional control of DNA replication initiation through the proteolysis and regulated translation of DnaA. *PLoS Genet* 2015;**11**:e1005342.
Lesterlin C, Gigant E, Boccard F *et al*. Sister chromatid interactions in bacteria revealed by a site-specific recombination assay. *EMBO J* 2012;**31**:3468–79.
Lesterlin C, Pages C, Dubarry N *et al*. Asymmetry of chromosome replichores renders the DNA translocase activity of FtsK essential for cell division and cell shape maintenance in Escherichia coli. *PLoS Genet* 2008;**4**:e1000288.
Le TBK, Imakaev MV, Mirny LA *et al*. High-resolution mapping of the spatial organization of a bacterial chromosome. *Science* 2013;**342**:731–4.
Levy O, Ptacin JL, Pease PJ *et al*. Identification of oligonucleotide sequences that direct the movement of the Escherichia coli FtsK translocase. *Proc Natl Acad Sci USA* 2005;**102**:17618–23.
Lies M, Visser BJ, Joshi, MC *et al*. MioC and GidA proteins promote cell division in E. coli. *Front Microbiol* 2015;**6**:516.
Lim HC, Surovtsev IV, Beltran BG *et al*. Evidence for a DNA-relay mechanism in ParABS-mediated chromosome segregation. *eLife* 2014;**3**:e02758.
Livny J, Yamaichi Y, Waldor MK Distribution of centromere-like parS sites in bacteria: insights from comparative genomics. *J Bacteriol* 2007;**189**:8693–703.
Logsdon MM, Ho P-Y, Papavinasasundaram K *et al*. A parallel adder coordinates mycobacterial cell-cycle progression and cell-size homeostasis in the context of asymmetric growth and organization. *Curr Biol* 2017;**27**:3367–74.e7.
Lori C, Ozaki S, Steiner S *et al*. Cyclic di-GMP acts as a cell cycle oscillator to drive chromosome replication. *Nature* 2015;**523**:236–9.
Lutkenhaus J. Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu Rev Biochem* 2007;**76**:539–62.
Løbner-Olesen A, von Freiesleben U. Chromosomal replication incompatibility in Dam methyltransferase deficient Escherichia coli cells. *EMBO J* 1996;**15**:5999–6008.
Maciąg-Dorszyńska M, Ignatowska M, Jannière L *et al*. Mutations in central carbon metabolism genes suppress defects in nucleoid position and cell division of replication mutants in Escherichia coli. *Gene* 2012;**503**:31–35.
Maciąg-Dorszyńska M, Szalewska-Pałasz A, Węgrzyn G. Different effects of ppGpp on Escherichia coli DNA replication in vivo and in vitro. *FEBS Open Bio* 2013;**3**:161–4.
Maciąg M, Kochanowska M, Lyżeń R *et al*. ppGpp inhibits the activity of Escherichia coli DnaG primase. *Plasmid* 2010;**63**:61–7.
Maciąg M, Nowicki D, Janniere L *et al*. Genetic response to metabolic fluctuations: correlation between central carbon metabolism and DNA replication in Escherichia coli. *Microb Cell Fact* 2011;**10**:19.
Magnan D, Bates D. Regulation of DNA Replication Initiation by chromosome structure. *J Bacteriol* 2015;**197**:3370–7.
Mahone CR, Goley ED. Bacterial cell division at a glance. *J Cell Sci* 2020;**133**:jcs237057.
Mannik J, Bailey MW. Spatial coordination between chromosomes and cell division proteins in Escherichia coli. *Front Microbiol* 2015;**6**:306.
Martínez-Salas E, Martín JA, Vicente M. Relationship of Escherichia coli density to growth rate and cell age. *J Bacteriol* 1981;**147**:97–100.
Massey TH, Mercogliano CP, Yates J *et al*. Double-stranded DNA Translocation: structure and mechanism of hexameric FtsK. *Mol Cell* 2006;**23**:457–69.
Meacock PA, Pritchard RH. Relationship between chromosome replication and cell division in a thymineless mutant of Escherichia coli B/r. *J Bacteriol* 1975;**122**:931–42.
Mera PE, Kalogeraki VS, Shapiro L. Replication initiator DnaA binds at the Caulobacter centromere and enables chromosome segregation. *Proc Natl Acad Sci USA* 2014;**111**:16100–5.
Mercier R, Petit MA, Schbath S *et al*. The MatP/matS site-specific system organizes the terminus region of the E. coli chromosome into a macrodomain. *Cell* 2008;**135**:475–85.
Mercy C, Ducret A, Slager J *et al*. RocS drives chromosome segregation and nucleoid protection in Streptococcus pneumoniae. *Nat Microbiol* 2019;**4**:1661–70.

Messer W, Bergmans HEN, Meijer M *et al*. Mini-chromosomes: plasmids which carry the E. coli replication origin. *Molec Gen Genet* 1978;**162**:269–75.

Micali G, Grilli J, Marchi J *et al*. Dissecting the control mechanisms for DNA replication and cell division in E. coli. *Cell Rep* 2018b;**25**:761–71.e4.

Micali G, Grilli J, Osella M *et al*. Concurrent processes set E. coli cell division. *Sci Adv* 2018a;**4**:eaau3324.

Michelsen O, Teixeira de Mattos MJ, Jensen PR *et al*. Precise determinations of C and D periods by flow cytometry in *Escherichia coli* K-12 and B/r. *Microbiology* 2003;**149**:1001–10.

Mohl DA, Easter J, Gober JW. The chromosome partitioning protein, ParB, is required for cytokinesis in Caulobacter crescentus. *Mol Microbiol* 2001;**42**:741–55.

Moore MM. The cytology of bacteria. The nuclear apparatus as shown by Feulgen's "Nuclealfarbung" reaction. *P Roy Irish Acad B* 1941;**47**:21–52.

Mori M, Marinari E, Martino AD. A yield-cost tradeoff governs *Escherichia coli*'s decision between fermentation and respiration in carbon-limited growth. *npj Syst Biol Appl* 2019;**5**:1–9.

Murray H, Koh A. Multiple regulatory systems coordinate DNA replication with cell growth in Bacillus subtilis. *Plos Genet* 2014;**10**:e1004731.

Narayanan S, Janakiraman B, Kumar L *et al*. A cell cycle-controlled redox switch regulates the topoisomerase IV activity. *Genes Dev* 2015;**29**:1175–87.

Niki H, Yamaichi Y, Hiraga S. Dynamic organization of chromosomal DNA in Escherichia coli. *Genes Dev* 2000;**14**:212–23.

Nishimura A, Moriya S, Ukai H *et al*. Diadenosine 5′,5‴-P1,P4-tetraphosphate (Ap4A) controls the timing of cell division in Escherichia coli. *Genes Cells* 1997;**2**:401–13.

Nolivos S, Upton AL, Badrinarayanan A *et al*. MatP regulates the coordinated action of topoisomerase IV and MukBEF in chromosome segregation. *Nat Commun* 2016;**7**:1–12.

Nordstrom K, Bernander R, Dasgupta S. The Escherichia coli cell cycle: one cycle or multiple independent processes that are co-ordinated? *Mol Microbiol* 1991;**5**:769–74.

Nouri H, Monnier A-F, Fossum-Raunehaug S *et al*. Multiple links connect central carbon metabolism to DNA replication initiation and elongation in Bacillus subtilis. *DNA Res* 2018;**25**:641–53.

O'Donnell M, Langston L, Stillman B Principles and concepts of DNA replication in bacteria, archaea, and eukarya. *Cold Spring Harb Perspect Biol* 2013;**5**:a010108.

Ojkic N, Serbanescu D, Banerjee S Surface-to-volume scaling and aspect ratio preservation in rod-shaped bacteria. *eLife* 2019;**8**:e47033.

Osella M, Nugent E, Lagomarsino MC Concerted control of Escherichia coli cell division. *Proc Natl Acad Sci USA* 2014;**111**:3431–5.

Ozaki S, Jenal U, Katayama T Novel divisome-associated protein spatially coupling the Z-ring with the chromosomal replication terminus in Caulobacter crescentus. *mBio* 2020;**11**:e00487–20.

Pandey PP, Singh H, Jain S. Exponential trajectories, cell size fluctuations, and the adder property in bacteria follow from simple chemical dynamics and division control. *Phys Rev E* 2020;**101**:062406.

Pichoff S, Du S, Lutkenhaus J. Roles of FtsEX in cell division. *Res Microbiol* 2019;**170**:374–80.

Pichoff S, Shen B, Sullivan B *et al*. FtsA mutants impaired for self-interaction bypass ZipA suggesting a model in which FtsA's self-interaction competes with its ability to recruit downstream division proteins. *Mol Microbiol* 2012;**83**:151–67.

Piekarski G. Cytologische Untersuchungen an Paratyphus-und Colibakterien. *Archiv Mikrobiol* 1937;**8**:428–39.

Pinho MG, Kjos M, Veening J-W. How to get (a)round: mechanisms controlling growth and division of coccoid bacteria. *Nat Rev Microbiol* 2013;**11**:601–14.

Powell EO. Growth rate and generation time of bacteria, with special reference to continuous culture. *J Gen Microbiol* 1956;**15**:492–511.

Ptacin JL, Nöllmann M, Bustamante C *et al*. Identification of the FtsK sequence-recognition domain. *Nat Struct Mol Biol* 2006;**13**:1023–5.

Pugatch R. Greedy scheduling of cellular self-replication leads to optimal doubling times with a log-Frechet distribution. *Proc Natl Acad Sci USA* 2015;**112**:2611–6.

Raaphorst R, Kjos M, Veening J-W. Chromosome segregation drives division site selection in Streptococcus pneumoniae. *Proc Natl Acad Sci USA* 2017;**114**:E5959–68.

Radhakrishnan SK, Pritchard S, Viollier PH. Coupling prokaryotic cell fate and division control with a bifunctional and oscillating oxidoreductase homolog. *Dev Cell* 2010;**18**:90–101.

Rahn O. A chemical explanation of the variability of the growth rate. *J Gen Physiol* 1932;**15**:257–77.

Riber L, Frimodt-Møller J, Charbon G *et al*. Multiple DNA-binding proteins contribute to timing of chromosome replication in E. coli. *Front Mol Biosci* 2016;**3**:29.

Rochman ND, Popescu DM, Sun SX. Ergodicity, hidden bias and the growth rate gain. *Phys Biol* 2018;**15**:036006.

Ryan VT, Grimwade JE, Camara JE *et al*. Escherichia coli prereplication complex assembly is regulated by dynamic interplay among Fis, IHF and DnaA. *Mol Microbiol* 2004;**51**:1347–59.

Sanchez A, Cattoni DI, Walter J-C *et al*. Stochastic self-assembly of ParB proteins builds the bacterial DNA segregation apparatus. *Cell Syst* 2015;**1**:163–73.

Santi I, Dhar N, Bousbaine D *et al*. Single-cell dynamics of the chromosome replication and cell division cycles in mycobacteria. *Nat Commun* 2013;**4**:1–11.

Sauls JT, Cox SE, Do Q *et al*. Control of Bacillus subtilis replication initiation during physiological transitions and perturbations. *mBio* 2019;**10**:e02205–19.

Schaechter M, Maaloe O, Kjeldgaard NO. Dependency on medium and temperature of cell size and chemical composition during balanced grown of Salmonella typhimurium. *J Gen Microbiol* 1958;**19**:592–606.

Schaechter M, Williamson JP, Hood JR *et al*. Growth, cell and nuclear divisions in some bacteria. *Microbiology* 1962;**29**:421–34.

Scholefield G, Errington J, Murray H. Soj/ParA stalls DNA replication by inhibiting helix formation of the initiator protein DnaA. *EMBO J* 2012;**31**:1542–55.

Scholefield G, Whiting R, Errington J *et al*. Spo0J regulates the oligomeric state of Soj to trigger its switch from an activator to an inhibitor of DNA replication initiation. *Mol Microbiol* 2011;**79**:1089–100.

Schumacher D, Bergeler S, Harms A *et al*. The PomXYZ proteins self-organize on the bacterial nucleoid to stimulate cell division. *Dev Cell* 2017;**41**:299–314.e13.

Schumacher D, Søgaard-Andersen L. Regulation of cell polarity in motility and cell division in Myxococcus xanthus. *Annu Rev Microbiol* 2017;**71**:61–78.

Scott M, Gunderson CW, Mateescu EM *et al*. Interdependence of cell growth and gene expression: origins and consequences. *Science* 2010;**330**:1099–102.

Scott M, Hwa T. Bacterial growth laws and their applications. *Curr Opin Biotechnol* 2011;**22**:559–65.

Scott M, Klumpp S, Mateescu EM *et al*. Emergence of robust growth laws from optimal regulation of ribosome synthesis. *Mol Sys Biol* 2014;**10**:747.

Sekimizu K, Kornberg A. Cardiolipin activation of dnaA protein, the initiation protein of replication in Escherichia coli. *J Biol Chem* 1988;**263**:7131–5.

Sham L-T, Barendt SM, Kopecky KE *et al*. Essential PcsB putative peptidoglycan hydrolase interacts with the essential FtsXSpn cell division protein in Streptococcus pneumoniae D39. *Proc Natl Acad Sci USA* 2011;**108**:E1061–9.

Si F, Le Treut G, Sauls JT *et al*. Mechanistic origin of cell-size control and homeostasis in bacteria. *Curr Biol* 2019;**29**: 1760–70.e7.

Si F, Li D, Cox SE *et al*. Invariance of initiation mass and predictability of cell size in *Escherichia coli*. *Curr Biol* 2017;**27**: 1278–87.

Sivanathan V, Allen MD, de Bekker C *et al*. The FtsK γ domain directs oriented DNA translocation by interacting with KOPS. *Nat Struct Mol Biol* 2006;**13**:965–72.

Skarstad K, Steen HB, Boye E. Cell cycle parameters of slowly growing *Escherichia coli* B/r studied by flow cytometry. *J Bacteriol* 1983;**154**:656–62.

Soifer I, Robert L, Amir A. Single-cell analysis of growth in budding yeast and bacteria reveals a common size regulation strategy. *Curr Biol* 2016;**26**:356–61.

Sompayrac L, Maaløe O. Autorepressor model for control of DNA replication. *Nat New Biol* 1973;**241**:133–5.

Steiner WW, Kuempel PL. Sister chromatid exchange frequencies in *Escherichia coli* analyzed by recombination at the dif resolvase site. *J Bacteriol* 1998;**180**:6269–75.

Stewart EJ, Madden R, Paul G *et al*. Aging and death in an organism that reproduces by morphologically symmetric division. *PLoS Biol* 2005;**3**:e45.

Stille B. Zytologische Untersuchungen an Bakterien mit Hilfe der Feulgenschen Nuclealreaktion. *Archiv Mikrobiol* 1937;**8**: 125–48.

Stokke C, Flåtten I, Skarstad K. An easy-to-use simulation program demonstrates variations in bacterial cell cycle parameters depending on medium and temperature. *PLoS One* 2012;**7**:e30981.

Stouf M, Meile J-C, Cornet F. FtsK actively segregates sister chromosomes in *Escherichia coli*. *Proc Natl Acad Sci USA* 2013;**110**:11157–62.

Susman L, Kohram M, Vashistha H *et al*. Individuality and slow dynamics in bacterial growth homeostasis. *Proc Natl Acad Sci USA* 2018;**115**:E5679–87.

Taheri-Araghi S, Bradde S, Sauls JT *et al*. Cell-size control and homeostasis in bacteria. *Curr Biol* 2015;**25**:385–91.

Taheri-Araghi S. Self-consistent examination of Donachie's constant initiation size at the single-cell level. *Front Microbiol* 2015;**6**:1349.

Thanbichler M, Shapiro L. MipZ, a spatial regulator coordinating chromosome segregation with cell division in Caulobacter. *Cell* 2006;**126**:147–62.

Theisen PW, Grimwade JE, Leonard AC *et al*. Correlation of gene transcription with the time of initiation of chromosome replication in *Escherichia coli*. *Mol Microbiol* 1993;**10**: 575–84.

Tonthat NK, Arold ST, Pickering BF *et al*. Molecular mechanism by which the nucleoid occlusion factor, SlmA, keeps cytokinesis in check. *EMBO J* 2011;**30**:154–64.

Treuner-Lange A, Aguiluz K, Does C *et al*. PomZ, a ParA-like protein, regulates Z-ring formation and cell division in Myxococcus xanthus. *Mol Microbiol* 2013;**87**:235–53.

Tymecka-Mulik J, Boss L, Maciąg-Dorszyńska M *et al*. Suppression of the *Escherichia coli* dnaA46 mutation by changes in the activities of the pyruvate-acetate node links DNA replication regulation to central carbon metabolism. *PLoS One* 2017;**12**:e0176050.

Valens M, Penaud S, Rossignol M *et al*. Macrodomain organization of the *Escherichia coli* chromosome. *EMBO J* 2004;**23**: 4330–41.

Vallet-Gely I, Boccard F. Chromosomal organization and segregation in *Pseudomonas aeruginosa*. *PLoS Genet* 2013;**9**:e1003492.

Vega DE, Margolin W. Suppression of a thermosensitive zipA cell division mutant by altering amino acid metabolism. *J Bacteriol* 2018;**200**:e00535–17.

Viollier PH, Thanbichler M, McGrath PT *et al*. Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc Natl Acad Sci USA* 2004;**101**:9257–62.

Voorn WJ, Koppes LJH. Skew or third moment of bacterial generation times. *Arch Microbiol* 1997;**169**:43–51.

Wallden M, Fange D, Lundius EG *et al*. The synchronization of replication and division cycles in individual *E. coli* cells. *Cell* 2016;**166**:729–39.

Wang JD, Sanders GM, Grossman AD. Nutritional control of elongation of DNA replication by (p)ppGpp. *Cell* 2007;**128**:865–75.

Wang X, Brandão HB, Le TBK *et al*. Bacillus subtilis SMC complexes juxtapose chromosome arms as they travel from origin to terminus. *Science* 2017;**355**:524–7.

Wang X, Lesterlin C, Reyes-Lamothe R *et al*. Replication and segregation of an *Escherichia coli* chromosome with two replication origins. *Proc Natl Acad Sci USA* 2011;**108**:E243–50.

Wang X, Reyes-Lamothe R, Sherratt DJ. Modulation of *Escherichia coli* sister chromosome cohesion by topoisomerase IV. *Genes Dev* 2008;**22**:2426–33.

Weart RB, Lee AH, Chien AC *et al*. A metabolic sensor governing cell size in bacteria. *Cell* 2007;**130**:335–47.

Willis L, Huang KC. Sizing up the bacterial cell cycle. *Nat Rev Microbiol* 2017;**15**:606–20.

Witz G, van Nimwegen E, Julou T. Initiation of chromosome replication controls both division and replication cycles in *E. coli* through a double-adder mechanism. *eLife* 2019;**8**:e48063.

Woldemeskel SA, McQuillen R, Hessel AM *et al*. A conserved coiled-coil protein pair focuses the cytokinetic Z-ring in *Caulobacter crescentus*. *Mol Microbiol* 2017;**105**:721–40.

Woldringh CL. Morphological analysis of nuclear separation and cell division during the life cycle of *Escherichia coli*. *J Bacteriol* 1976;**125**:248–57.

Wold S, Skarstad K, Steen HB *et al*. The initiation mass for DNA replication in *Escherichia coli* K-12 is dependent on growth rate. *EMBO J* 1994;**13**:2097–102.

Wu F, Swain P, Kuijpers L *et al*. Cell boundary confinement sets the size and position of the *E. coli* chromosome. *Curr Biol* 2019;**29**:2131–2144.e4.

Wu LJ, Errington J. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in Bacillus subtilis. *Cell* 2004;**117**:915–25.

Wu LJ, Ishikawa S, Kawai Y *et al*. Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation. *EMBO J* 2009;**28**:1940–52.

Xia W, Dowhan W. In vivo evidence for the involvement of anionic phospholipids in initiation of DNA replication in *Escherichia coli*. *Proc Natl Acad Sci USA* 1995;**92**:783–7.

Yamaichi Y, Gerding MA, Davis BM *et al*. Regulatory cross-talk links Vibrio cholerae chromosome II replication and segregation. *PLoS Genet* 2011;**7**:e1002189.

Yang D, Jennings AD, Borrego E *et al*. Analysis of factors limiting bacterial growth in PDMS mother machine devices. *Front. Microbiol* 2018;**9**.

Yang DC, Peters NT, Parzych KR *et al*. An ATP-binding cassette transporter-like complex governs cell-wall hydrolysis at the bacterial cytokinetic ring. *Proc Natl Acad Sci* 2011;**108**: E1052–60.

Yang X, Lyu Z, Miguel A *et al*. GTPase activity–coupled treadmilling of the bacterial tubulin FtsZ organizes septal cell wall synthesis. *Science* 2017;**355**:744–7.

Yates J, Zhekov I, Baker R *et al*. Dissection of a functional interaction between the DNA translocase, FtsK, and the XerD recombinase. *Mol Microbiol* 2006;**59**:1754–66.

Zaritsky A, Pritchard RH. Changes in cell size and shape associated with changes in the replication time of the chromosome of Escherichia coli. *J Bacteriol* 1973;**114**: 824–37.

Zaritsky A. Cell-shape homeostasis in *Escherichia coli* is driven by growth, division, and nucleoid complexity. *Biophys J* 2015;**109**:178–81.

Zheng H, Bai Y, Jiang M *et al*. General quantitative relations linking cell growth and the cell cycle in *Escherichia coli*. *Nat Microbiol* 2020;1–7.

Zheng H, Ho PY, Jiang M *et al*. Interrogating the *Escherichia coli* cell cycle by cell dimension perturbations. *Proc Natl Acad Sci USA* 2016;**113**:15000–5.