Cell Size Regulation in Bacteria

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Various bacteria such as the canonical gram negative *Escherichia coli* or the well-studied gram positive *Bacillus subtilis* divide symmetrically after they approximately double their volume. Their size at division is not constant, but is typically distributed over a narrow range. Here, we propose an analytically tractable model for cell size control, and calculate the cell size and interdivision time distributions, as well as the correlations between these variables. We suggest ways of extracting the model parameters from experimental data, and show that existing data for *E. coli* supports partial size control, and a particular explanation: a cell attempts to add a constant volume from the time of initiation of DNA replication to the next initiation event. This hypothesis accounts for the experimentally observed correlations between mother and daughter cells as well as the exponential dependence of size on growth rate.

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Microorganisms such as bacteria come in a diverse set of shapes and sizes. Nonetheless, individual strains have remarkably reproducible shapes, and a narrow distribution of sizes [1–4]. Many bacteria, such as *E. coli*, are rod shaped, and during their exponential growth phase they elongate while maintaining a constant diameter. After approximately doubling their length (as well as mass and volume), and completing DNA replication for their offspring, they divide symmetrically into two approximately identical daughter cells. In spite of decades of research, we still do not have a good understanding of how cells regulate their shape, both mechanically (i.e., what is the biophysical feedback necessary to achieve a rod-shaped cell? [5]) and dimensionally: the coefficient of variation (standard deviation:mean, CV) can be as low as 0.1 for bacteria [2]. Bacteria are also remarkable in their ability to have a generation time that is shorter than the time it takes them to replicate DNA: doubling time τ_d for *E. coli* in rich media at 37 °C is about 20 mins, while $T_r \approx 60$ mins are needed from initiation of DNA replication to cell division. This apparent paradox is explained by the existence of multiple replication forks: in these situations, a cell will already start replicating DNA for its four granddaughters (or eight great granddaughters) in order for the replication to complete in time.

Many models for cell size regulation exist in the literature [1,2,6–14]. Different strategies will yield particular cell size and interdivision time distributions, as well as distinct correlations. Hence, it is important to understand the connection between different regulation models and the resulting distributions and correlations. Moreover, there are two seemingly contradictory results in the literature: the first is the model by Donachie [15], which shows that the measured exponential dependence of bacterial size on growth rate [16] is consistent with initiation of DNA replication at a constant, growth-rate-independent volume per replication fork—suggesting a mechanistic picture in which a cell "knows" of its size and initiates replication when reaching a critical one. This model would imply that size at birth and division would not be correlated: since the time from initiation to division is constant [17], the size at division will be independent of the size at birth. However, experiments show that there are strong correlations between the two [18].

We will show here how these two results can be reconciled within a minimal model, which will be analytically tractable. We will suggest a mathematical framework which is able to capture and extend several existing models, and will use it to analyze the correlations and cell size distributions. We shall show that the aforementioned experimental data for *E. coli* support a mechanism of cell size regulation in which the cell attempts to add a constant volume from the event of initiation of DNA replication to the next initiation event [19]. This model will be consistent with the results discussed in Ref. [15], will predict an exponential dependence of cell size on growth rate, and will also quantitatively account for the positive correlations between size at birth and division [18] and negative correlations between size at birth and interdivision time [14]. We will show that for size-additive noise the size distribution is Gaussian, while for time-additive (i.e., size-multiplicative) noise the resulting size distribution is log-normal—and hence right skewed. As discussed in the Supplemental Material [20], experimentally measured distributions are indeed skewed, and for this reason we focus on the analysis of time-additive noise in the main text and defer the size-additive case to the Supplemental Material [20]. Within the model, the standard deviations of both size and interdivision time distributions are controlled by a single parameter.

The tools which we shall use will parallel those used when solving problems in statistical mechanics, in particular those

involving Langevin equations [21]. Multiplicative noise and the log-normal distributions which emerge from our model also occur in other problems in physics, such as relaxations in glasses [22] and the modeling of financial markets [23,24]. However, in contrast to most physical systems, negative feedback (i.e., control) is a necessary feature of biological systems, including the problem studied here.

Exponential growth of a single cell and regulation models.—The question of the mode of growth of a single bacterium has been a long standing problem, with linear and exponential growth the most common models considered [1,2,25]. Recent experiments show that individual cell volume grows exponentially for various bacterial strains [3,26–28]. In fact, if cells grow at a rate that is proportional to the amount of protein they contain [29,30], as long as the protein concentration is constant, the cells will grow exponentially in mass and volume. We shall assume exponential growth of volume throughout this paper, $v(t) \propto 2^{t/\tau_d}$, and neglect fluctuations in the growth rate. Furthermore, we will assume that cells divide precisely in half since experimental results [31,4] show that division occurs at the midcell to an excellent approximation.

Cells need a feedback mechanism that will control their size distribution. If cells grew for a constant time $t = \tau_d$, random fluctuations in the timing would make the size of the cells at division v_d perform a random walk on the volume axis, and thus this mechanism does not control size. Another regulatory strategy is that of division at a critical size, or of initiation of DNA replication at a critical size. These ideas are prevalent in the literature [1,2], but we will show that existing experimental data for *E. coli* argues against them. We shall consider the following class of models: upon being born at a size v_b, the cell would attempt to grow for a time $\tau(v_b)$ such that its final volume at division is $v_d = f(v_b)$. If the function $f(v_b) = \text{const}$, we are back to the critical size model. The constant time model can also be cast in this language: since the growth is exponential, attempting to grow for a prescribed, constant time τ_d is the same as having $f(v_b) = 2v_b$. Another important model that has been suggested is the so-called "incremental model," in which the cell attempts to add a constant volume v_0 to its newborn size [32]. In this case

$$f(v_b) = v_b + \Delta. \tag{1}$$

In the following, we suggest a method through which an arbitrary regulatory model described by a function $f(v_b)$ can be approximately solved; i.e., we can find all the involved distributions and correlation coefficients analytically, finding excellent agreement with the numerically exact solutions. We also provide methods to extract the model parameters from the experimental data.

The model.—We assume that the cell attempts to divide at a volume $v_d = f(v_b)$, as previously explained, by attempting to grow for the appropriate amount of time t_a which is a function of v_d. We assume that to this time is added a random noise t_n, which we assume to be Gaussian. The magnitude of this noise will dictate the width of the resulting size and interdivision time distributions. Thus we have

$$t_{\text{growth}} = t_a + t_n = \tau_d \log_2[f(v_b)/v_b] + t_n, \tag{2}$$

with t_n assumed to be a random variable with $P(t_n) = (1/\sqrt{2\pi\sigma_T^2}) e^{-(t_n^2/2\sigma_T^2)}$. The model is similar to that discussed in Ref. [33], where the molecular mechanisms leading to the noise in budding yeast are studied.

We will calculate the interdivision time and volume distributions. The key insight is that for noise that is not too large (equivalent to size distributions which are not too broad, i.e., with a small CV), it is the behavior of $f(v_b)$ around the average newborn size $\bar{v}$ that is the most important. Therefore, we can Taylor expand $f(v_b)$ around $\bar{v}$:

$$f(v_b) \approx f(\bar{v}) + f'(\bar{v})(v_b - \bar{v}). \tag{3}$$

As an example, the incremental model has $f'(\bar{v}) = 1$ and $\bar{v} = \Delta$, while the critical size model has $f'(\bar{v}) = 0$.

Any two models that agree to lowest order will result in similar distributions—provided the noise is not too large. We therefore choose to solve an equivalent model that will be amenable to analytic treatment, and that can be viewed as an interpolation between the critical size model and the constant doubling time model. We choose

$$t_a = \tau_d[1 + \alpha \log_2(v_0/v_b)], \tag{4}$$

which corresponds to the regulatory function: $f(v_b) = v_0 2^{t_a} = 2v_b^{1-\alpha} v_0^{\alpha}$. The case $\alpha = 0$ corresponds to a constant doubling time model [$f'(v_0) = 2$], while $\alpha = 1$ corresponds to the critical size model [$f'(v_0) = 0$]. Importantly, for $\alpha = 1/2$ we have $f'(v_0) = 1$, as does the incremental model; hence, using a target function like this gives results close to a perfect realization of the incremental mode. We shall show that the parameter v_0 in Eq. (4) will be very close to the average newborn cell size $\bar{v}$.

Solution of size and interdivision time distributions.—We shall consider the case of symmetric division, relevant for many bacteria. For a newborn size v_b, we have for the next newborn volume $v_b^{\text{new}} = v_0^{\alpha} v_b^{1-\alpha} 2^{t_n/\tau_d}$.

Therefore,

$$\log_2(v_b^{\text{new}}/v_0) = (1-\alpha)\log_2(v_b/v_0) + t_n/\tau_d. \tag{5}$$

From stationarity of the stochastic process we know that $P(v_b^{\text{new}}) = P(v_b)$. Since t_n is a Gaussian variable, we find that $\log_2(v_b)$ is also a Gaussian variable, and hence $P(v_b)$ would be a log-normal distribution. If we denote the variance of $\log_2(v_b/v_0)$ by σ_v^2, we have

$\sigma_v^2 = \sigma_v^2(1-\alpha)^2 + ({\sigma_T}^2/{\tau_d}^2)$; therefore, the newborn size distribution is

$$P(v_b) = \frac{1}{\sqrt{2\pi}\ln(2)\sigma_v}\frac{e^{-([\log_2(v_b/v_0)]^2/2\sigma_v^2)}}{v_b}, \quad (6)$$

with

$$\sigma_v^2 = \frac{{\sigma_T}^2}{\tau_d^2\alpha(2-\alpha)}. \quad (7)$$

Note that the average cell size is $\bar{v} = v_0 e^{\ln^2(2)\sigma_v^2/2}$; for realistic values of σ_v it will only be a few percent larger than v_0. Similarly, the standard deviation of the size distribution will be approximately $\sigma_s \approx \ln(2)\sigma_v v_0$, and the coefficient of variation is, thus, $v_{\mathrm{CV}} \approx \ln(2)\sigma_v$. The skewness of the distribution is positive, $\gamma_1 \approx 3\ln(2)\sigma_v$, and provides a useful test of the assumption of a time-additive rather than size-additive noise, as we elaborate on in the Supplemental Material [20].

We can now find the distribution of division times using $t_d = t_a + t_n$. Since v_b depends only on the noise of previous generations, t_a is independent of t_n, and since $\log_2(v_b/v_0)$ and t_n are Gaussian variables, the resulting interdivision time distribution is also Gaussian, and has a variance given by

$$\mathrm{Var}[t_d] = \tau_d^2\alpha^2\sigma_v^2 + {\sigma_T}^2 = {\sigma_T}^2\frac{2}{2-\alpha}. \quad (8)$$

In the case $\alpha \to 0$, we find that σ_v diverges (an extremely broad distribution of newborn sizes), but the interdivision time distribution is narrow: $\mathrm{Var}[t_d] \to {\sigma_T}^2$, as should clearly be the case since there is no size feedback mechanism in this case. Note that a stationary distribution exists for $0 < \alpha < 2$, and that the size distribution is narrowest for $\alpha = 1$.

From Eq. (8) we find that the CV of the distribution of interdivision times is given by $t_{\mathrm{CV}} = (\sigma_T/\tau_d)\sqrt{(2/2-\alpha)}$. It is instructive to consider the dimensionless quantity:

$$\gamma \equiv v_{\mathrm{CV}}/t_{\mathrm{CV}} \approx \frac{\ln(2)}{\sqrt{2\alpha}}. \quad (9)$$

By constructing γ from the experimental distributions we can extract the value of α and find the form of the size regulation utilized by the organism, if the division is symmetric. Later we shall show an additional, independent way of extracting α, which will rely on correlation coefficients.

Figure 1 compares the numerically obtained size distribution for various values of α and the incremental model, with the result of Eq. (6), finding excellent agreement. In the Supplemental Material [20] we extend this comparison to various noise magnitudes. Our model captures the numerically exact solution very well and Eq. (4) provides a useful tool to capture a generic division strategy characterized by an arbitrary function $f(v_b)$.

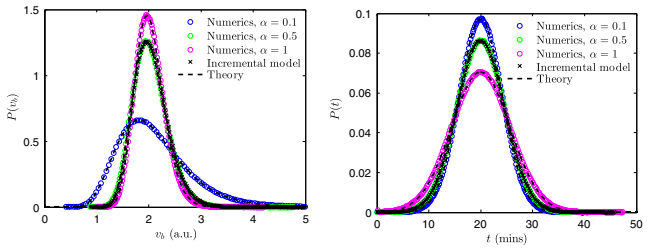

FIG. 1 (color online). Comparison between the analytical results of the model for varying values of α [Eqs. (6)–(8)] and numerics. Choosing $\alpha = 1/2$ provides an excellent approximation for the incremental model, as the effective size regulation of the two models agrees to lowest order. The parameters of the model are chosen according to their realistic values for *E. coli* growing at 37°: doubling time is $\tau_d = 20$ mins and $\sigma_T/\tau_d = 0.2$ [2]. For each case, the numerical distribution is extracted from a sequence of 10^7 divisions.

Extracting the parameters from experiments.—Within the class of models proposed here, the value of α can be obtained by considering the correlations between size at birth and size at division. In the Supplemental Material [20] we show that the Pearson correlation coefficient between size at birth and division (equal to the correlation coefficient between mother and daughter size at birth) equals

$$C \approx 1 - \alpha. \quad (10)$$

In particular, for the incremental model the correlation coefficient between mother and daughter cells should be 0.5.

Upon fixing the value of α, a single parameter σ_T will determine the distributions of both size and division time, and the calculations performed here would allow one to scale both distributions using this single parameter. For the time-additive noise analyzed here, the model predicts an approximately log-normal newborn size distribution, given by Eq. (6), and a Gaussian interdivision time distribution, given by Eq. (8), whose standard deviation is larger than σ_T. In the Supplemental Material [20] we show that for size-additive noise, one obtains a skewed time distribution but a Gaussian size distribution—in contrast to what is observed experimentally for *E. coli* [13]. Therefore, observing the distribution shape provides useful information regarding the source of the noise. Further experiments are needed to elucidate the molecular source of this multiplicative noise.

Cell size control in E. coli.—Experimentally, various correlation coefficients were measured for *E. coli* at slow growth conditions in Ref. [18], using the membrane elution technique. The correlation coefficient between mother and daughter size at birth was found to be $C = 0.55$, close to the theoretical $1/2$ value expected for the incremental model.

There was a strong correlation (0.8) between size at initiation of DNA replication and size at division, as we would expect from the assumption of exponential growth and that the time from initiation to division is constant [17]. Yet these observations appear to be in direct contradiction to the idea that initiation occurs at a critical size [15]. The key point is that Donachie's analysis [15] shows that there is a critical size for initiation of DNA replication (independent of growth rate), *on average*. It is only from the fluctuations (i.e., correlations) that one can understand whether the underlying regulatory mechanism utilizes a critical size or integrates volume—as we shall propose is the case. Reference [19] gives a simple biophysical implementation of the incremental model, which will reconcile these seemingly contradictory results and will realize a particular case of the class of model we proposed here: in this model, a protein A is forced to have a growth-rate-independent concentration throughout the cell using a negative feedback in its regulation, and a second protein B is produced whenever A is. In this way, when cell volume grows (and only then), more A and B proteins are generated in an amount proportional to the change in volume. The hypothesis is that B proteins localize at their potential initiation site (i.e., one of the origins of replication), and only when their total number at each origin reaches a critical value does initiation of DNA replication occur, after which B is degraded. Note that two types of proteins are necessary, since in order to measure volume differences A must be spread throughout the cell, while B has to localize to measure an absolute number (rather than concentration). See Ref. [19] for further details.

In the Supplemental Material [20] we show that this model reduces to the incremental model, albeit with an effective Δ [see Eq. (1)] which strongly depends on the doubling time τ_d and the time from initiation to division T_r:

$$\hat{\Delta} \equiv \Delta 2^{T_r/\tau_d}. \tag{11}$$

According to our results the average cell size will be $\hat{\Delta}$—in agreement with the experimental results seeing precisely this exponential dependence of bacterial size on growth rate, with T_r the exponent [16]. This model naturally accounts for the "quantization" of the cell critical size at initiation at different growth rates [15], without necessitating the measurement of an absolute mass or volume. Moreover, it is plausible that the source of noise in adding the incremental volume will be due to "molecular noise" (number fluctuations of protein B), and would therefore be weakly dependent on growth rate. The same calculation which leads to Eq. (11) would suggest that σ_T (the noise standard deviation) should depend on the growth rate in the same exponential way as $\hat{\Delta}$. This implies that the CV of size distributions should be weakly dependent on growth rate [see Eq. (7)], an expectation supported by Ref. [34].

Thus, we have shown that using our calculations and the interpretation in terms of the incremental model we can elucidate various experimental results. In fact, the model also makes precise predictions with regards to additional correlations: for example, it is possible to show that for the incremental model the size correlation coefficient between cells N generations apart is 2^{-N}. Similarly, the model predicts a negative correlation of $-1/2$ between the size at birth and the interdivision time; see the Supplemental Material [20] for further details. This correlation coefficient was recently analyzed by Robert *et al.* [14] using data from two different experimental systems, finding a correlation coefficient of -0.5 in both cases, exactly as predicted by our model. This gives a particularly simple and transparent interpretation to their analysis, and provide additional, strong support for the incremental model. References [13,35] find similar negative correlations between newborn size and interdivision size, supporting our conclusion.

All of these provide additional support for the relevance of this model to cell size control in *E. coli*, and most likely to other organisms as well. It is possible, however, that alternative biophysical mechanisms may lead to the same correlations and size dependencies calculated here, and for this reason finding the details of the underlying biological mechanism is important. In recent years, DnaA has been shown to have properties reminiscent of the biophysical model described here [9], where its active and inactive forms correspond to the roles of proteins A and B above—see the Supplemental Material [20] for further details, which includes Refs. [36–41].

Discussion.—In this work we suggested a phenomenological model which is able to describe partial size control within a broad class of control strategies, and interpolate between the case of constant time to division and division at a critical size, for both size-additive and time-additive noise. We are able to analytically calculate the size and interdivision time distributions for the case of symmetric division relevant to various bacteria. For *E. coli*, we have shown that a simple biophysical model in which a constant volume is added from consequent events of initiation of DNA replication predicts the following. (1) Cell size depends exponentially on growth rate. (2) Cell size distributions are approximately log-normal. (3) The correlation coefficient between size at birth and division is approximately $1/2$. (4) The correlation coefficient between size at birth and time to division is approximately $-1/2$. (5) The ratio of the CV of size and interdivision time distributions is approximately $\ln(2)$. The simplicity of a biophysical model which implements this idea [19] suggests that this may be a robust way of regulating cell size and coupling DNA replication and growth.

This interpretation in terms of the incremental model suggests an outstanding puzzle: can we underpin the precise molecular mechanism responsible for volume integration? Can the source of the noise in interdivision times be

elucidated? Testing this model further in other microorganisms may yield important insights into cell size regulation, and, in particular, it is intriguing to see if the same ideas are applicable to cell size control in higher organisms. Recently, size distributions in other microorganisms were shown to obey simple scaling laws [42], suggesting this to be a promising direction, and that the model discussed here may have a broader range of applicability.

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