

Computational Analysis of the Mutual Constraints between Single-Cell Growth and Division Control Models

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Three models of division control are proposed to achieve cell size homeostasis: sizer, timer, and adder. However, few published studies of division control take into account the dynamics of single-cell growth and most assume that single-cell growth is exponential. Here, computational simulations considering exponential, linear, and bilinear growth models are performed. These simulations confirm that a timer division control model alone cannot lead to size homeostasis if the single-cell growth model is exponential. Furthermore, timer and adder division control models cannot be distinguished if the single-cell growth model is linear. Models of division control cannot be easily differentiated by analysis of average cell behavior because the birth sizes of the majority of cells are close to the population average. However, the differences between division control models are amplified in outlier cells whose birth size is far from the average. A method is introduced for vector field analysis of the speed of convergence of outlier lineages toward the steady-state birth size, which can help to distinguish between division control models and single-cell growth models.

1. Introduction

Cell size and shape are remarkably diverse. Bacterial cell size ranges from a few hundred nanometers for the smallest bacteria to a few hundred micrometers for the largest bacteria.^[1] Eukaryotic cells are typically larger than bacterial cells and can range from a few micrometers to even meters for cells of the nervous system. However, all cells have a limited size window where they function optimally and size control mechanisms may contribute to keeping them within their optimal range.^[2] Thus, the processes of cell growth and division may be coordinated in order to maintain cell size homeostasis over time.

Diverse models have been invoked to explain how individual cells grow and incorporate newly synthesized material. For example, some cells grow by extension of the lateral cell wall

(e.g., *Escherichia coli*) while others grow by elongation of the cell poles (e.g., *Mycobacterium* spp.). There have been many attempts to rationalize these growth patterns, such as Daniel and Errington's hypothesis that lateral cell wall extension is inherently exponential (because the growth zone expands continuously between birth and division) whereas, by similar reasoning, polar growth is inherently linear (because the size of the growth zone remains constant between birth and division).^[3] Other growth models are possible. For example, *Schizosaccharomyces pombe* is thought to grow by polar elongation in a bilinear manner, in part because the new cell pole initiates growth later than the old cell pole.^[4–7]

Many studies have converged on exponential growth as a model for cell growth based on pulse-labeling of cell populations, optical microscopy, spatial light

interference microscopy, and single-cell measurements of buoyant mass obtained with a suspended microchannel resonator.^[8–11] Earlier studies proposed linear growth as a general model for cell growth based on measurements made on single cells, synchronized cultures, and population distributions.^[12,13] In a few cases, bilinear growth has been invoked as a model to explain the pattern of single-cell growth observed using optical microscopy.^[4–7] However, it is difficult experimentally to distinguish between these growth models because the shapes of the growth curves generated by exponential, linear, and bilinear growth models are very similar and efforts to distinguish between them are confounded by experimental and biological sources of noise. To illustrate the lack of consensus in the literature, at different times using different methods, single-cell growth of a single species (*E. coli*) has variously been described as exponential, linear, or bilinear.^[11,12,14] The introduction of novel techniques for single-cell size measurements has not ended the debate. For example, although measurements of buoyant cell mass identified exponential growth as the “true” single-cell growth model in *Bacillus subtilis*, the “true” growth model for *E. coli* could not be identified with this technique.^[15]

Cell size homeostasis also requires that growth and division be coordinated. Otherwise, individual cells could become progressively bigger and bigger or smaller and smaller at every generation, eventually resulting in non-viability. When individual cells deviate significantly from the average birth size distribution—for example, following a transient block in cell cycle progression or due to an abnormally asymmetric cell division event—they do

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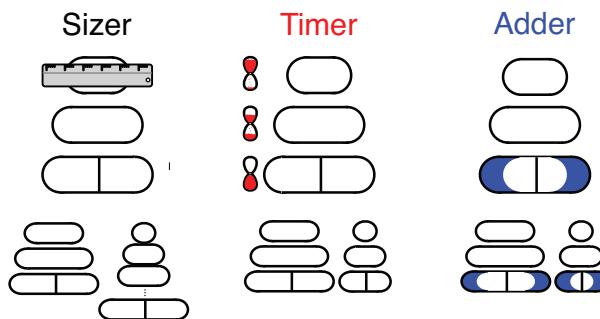


Figure 1. Schematic of three competing cell division control models. According to the sizer model, cells divide after reaching a predetermined size (ruler). In the timer model, cells divide after a certain amount of time has elapsed since their birth, following an internal clock (hourglass). In the adder model, cells must add a certain fixed amount of volume (blue area) before they divide, independent of their birth size. The contrasting behaviors of a large cell and a small cell are depicted for each scenario. No specific single-cell growth model is assumed.

eventually return to a “normal” size. This behavior indicates that there must be mechanisms responsible for cell size homeostasis.

In this context, three models for division control have been proposed.^[16] Originally referred to as sizer, clock, and incremental models, they are now known as sizer, timer, and adder models, respectively (Figure 1).^[17–19] According to the sizer model, cells divide after reaching a certain size. According to the timer model, cells divide after a certain amount of time has elapsed since their birth, following an internal clock. According to the adder model, cells must increase their size by a fixed amount before they divide, independent of their size at birth. This model seems to be supported by single-cell data for a large variety of organisms.^[20–25] These models for division control, however, are not mutually exclusive, as some studies have reported that combinations of the different mechanisms may operate in a single organism.^[26–32] It is also worth noting that these models (sizer, timer, adder) could be invoked to explain how other cell cycle transitions are controlled, for example, initiation of DNA replication.^[28]

Early studies considered the impact of different single-cell growth models on the control of cell division using a combination of experimental data and mathematical modeling.^[33–38] In more recent studies, the true single-cell growth model is usually assumed to be exponential and the potential consequences of other growth models on division control are seldom explored. Here, we assess the importance of determining the true dynamics of single-cell growth in order to distinguish between alternative division control models operating in the context of cell size homeostasis. We analyze the different methods that are used to identify the growth model based on single-cell growth curves and highlight how difficult it remains to assign the true growth model. We demonstrate that accurate knowledge of the single-cell growth model can, in some cases, eliminate a model of division control from further consideration. Keeping in mind the constraints imposed by the single-cell growth model, we demonstrate that vector field analysis of the time-dependent behavior of outliers in the size distribution is a useful method to distinguish between different models of division control.

2. Results

2.1. Boundary Conditions for Computational Simulations of Single-Cell Growth

We consider three possible growth models at the single-cell level: exponential, linear, and bilinear. In studies of single-cell growth it is common to distinguish only between exponential and linear growth. We have included the bilinear growth model in our analysis, in part because an important model organism, *S. pombe*, is thought to grow bilinearly.^[4–7] We implemented a model where cells grow bilinearly and the transition between slow growth and fast growth is size-dependent; under these conditions, linear and bilinear models give different results. The timing of the transition depends on the size at birth such that cells born small make the transition later, in terms of percentage of the interdivision time, than cells born large. Thus, cells following this model are situated somewhere between the exponential and linear models.

For the boundaries of the bilinear model, we define that cells born at an average size exhibit an increase in growth speed of 35% at 34% of their cell division cycle, as reported for *S. pombe*.^[5] Realistically, cells that are born very small or very large, compared to the average, may not reach the growth rate transition before dividing (small cells) or may not require a lag before making the transition (large cells). Therefore, as a lower boundary in our simulations, cells born smaller than 25% of the average birth size grow at their minimum speed during the entire cell cycle for birth until division (i.e., the transition to fast growth does not occur). This automatically sets the upper boundary to cells born 137.5% bigger than the average birth size. These large cells grow at their maximal speed during the entire cell cycle from birth until division (the transition to fast growth occurs at 0% of the interdivision time).

2.2. Single-Cell Growth Models Can Be Distinguished by Residual Analysis with Akaike and Bayesian Information Criteria

The growth model of single cells is usually deduced by fitting exponential, linear, or bilinear functions to the data obtained by time-lapse microscopy or other methods and evaluating the fittings using Pearson's correlation coefficient (r^2) or adjusted r^2 (Figure 2a–c). However, when we fitted each of these functions to simulated exponential, linear, or bilinear growth curves including small Gaussian noise, the adjusted r^2 values were very similar and close to 1, which indicates a very good fit for all of the models at this noise level. These fittings were made by adding Gaussian noise with a standard deviation of 2% of average cell size, which corresponds to the precision of measurement currently achievable with optical microscopy of small cells (e.g., a pixel size of 0.64 μm and an average cell size of 3 μm gives ~2% error).^[20] Since the adjusted r^2 is based on residuals, we performed an analysis of residuals to increase the discriminatory power (Figure 2d,e,g,i). In the case of adequate fit, residuals are normally distributed around zero; if the distribution falls into a pattern, this suggests the fit is inadequate.^[39] The distinction between different growth models

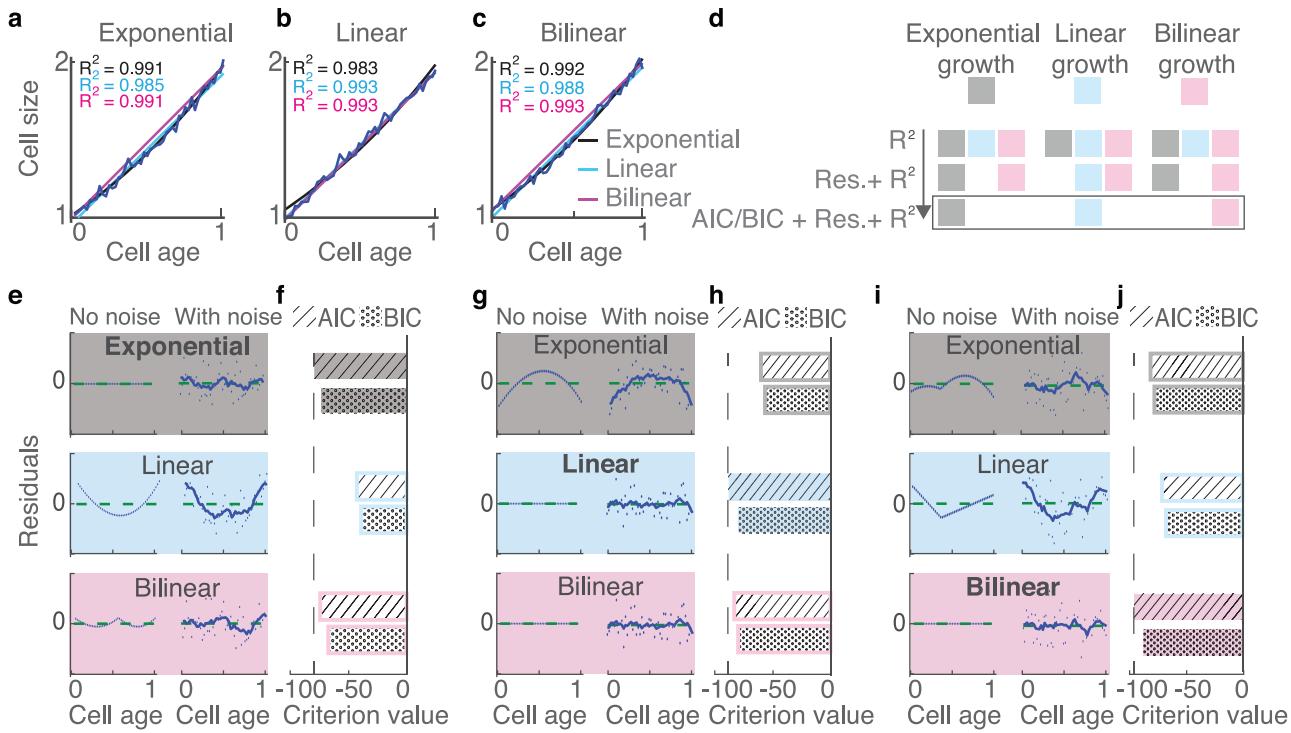


Figure 2. Single-cell growth models can be distinguished by analysis of residuals and AIC/BIC criteria. Fitting of single-cell growth curves at steady-state from a) a true exponential, b) true linear, or c) true bilinear model with added Gaussian noise (standard deviation of 2%). The adjusted R^2 values are very similar for the three fitted models. d) Summary of the model selection using different criteria. Only the combination of R^2 , residuals, and AIC/BIC accurately selects the true model. Residual analysis for all of the fittings of a) e) true exponential, g) true linear, i) or true bilinear growth curve fitted with exponential, linear, and bilinear models. The residuals obtained by comparing the true model and the fitted models are shown without (left) or with (right) added noise. The moving average considering sequences of seven values is shown. A good model shows no pattern in the distribution of residuals. f,h,i) AIC and BIC use the principle of parsimony to select the best model by discriminating against models that are too complex. The lowest value is associated with the best model.

can be further refined by applying the Akaike information criterion (AIC) or the Bayesian information criterion (BIC) to the curve fittings (Figure 2d,f,h,j).^[4,6] These criteria use the principle of parsimony and penalize models that are over-parameterized; BIC penalizes complex models more than AIC and is often preferred.^[40] For example, the bilinear fit is inferior to the exponential fit for a true exponential model (Figure 2f). This distinction is not clear using the adjusted R^2 (Figure 2a) or the residual analysis (Figure 2e) alone, demonstrating the additional discriminatory power of the AIC/BIC criteria. However, this method also has its limitations, and errors in model identification increase with increasing noise, especially for the bilinear model (Figure S1, Supporting Information).

2.3. Correct Identification of the Division Control Model Relies on Accurate Knowledge of the Single-Cell Growth Model

As discussed earlier, three main models for cell division control have been proposed: the sizer, timer, and adder models (Figure 1). Here, we show how these division control models are linked to the single-cell growth models in the context of cell size homeostasis, and how knowing the true growth model can help to distinguish between them. We investigate the exponen-

tial, linear, and bilinear models of single-cell growth to understand their stability and behavior in relation to the sizer, timer, and adder models of cell division control.

For all single-cell growth models, the cell size at birth after one generation can be described as

$$S_1 = (S_0 + \Delta S) \cdot k \quad (1)$$

where S_1 is the cell size at birth after one generation, S_0 is the initial ancestor cell size at birth, ΔS is the size increase between birth and division, and k is the asymmetry coefficient of division ($k = 0.5$ for symmetric division, $k = 0$ or 1 for totally asymmetric division).

If cells follow a timer model for cell division (the time elapsed between birth and division Δt is constant), the steady-state solution for birth sizes reached after many generations is found separately for exponential, linear, and bilinear single-cell growth models.

For exponential growth, ΔS in Equation (1) is replaced by $S_0 \cdot (e^{r\Delta t} - 1)$, where r is the growth rate (units in time^{-1}). The cell size at birth after one generation is thus given by

$$S_1 = S_0 \cdot e^{r\Delta t} \cdot k \quad (2)$$

The cell size at birth after n divisions is then given by

$$S_n = S_0 \cdot e^{r \cdot \Delta t \cdot n} \cdot k^n \quad (3)$$

Thus, over many divisions, the birth size of new generations of cells growing exponentially and following a timer reaches

$$\lim_{n \rightarrow \infty} S_n = \begin{cases} +\infty & \text{if } e^{r \cdot \Delta t} \cdot k > 1 \\ 0 & \text{if } e^{r \cdot \Delta t} \cdot k < 1 \\ S_0 & \text{if } e^{r \cdot \Delta t} \cdot k = 1 \end{cases} \quad (4)$$

Thus, cells either become larger and larger or smaller and smaller in subsequent generations, and the steady-state solution for birth size is reached only in the special case where

$$e^{r \cdot \Delta t} \cdot k = 1 \quad (5)$$

For linear growth, ΔS in Equation (1) is replaced by $\Delta t \cdot v$, where v is the growth speed (units in volume · time⁻¹). The cell size at birth after one generation is given by

$$S_1 = (S_0 + \Delta t \cdot v) \cdot k \quad (6)$$

The cell size at birth after n divisions is then given by

$$S_n = S_0 \cdot k^n + \Delta t \cdot v \cdot \sum_{i=1}^n k^i \quad (7)$$

Thus, over many divisions, the birth size of new generations of cells growing linearly and following a timer reaches a unique steady-state solution

$$\lim_{n \rightarrow \infty} S_n = \frac{\Delta t \cdot v \cdot k}{1 - k} \quad (8)$$

For bilinear growth, ΔS in Equation (1) is replaced by $\Delta t_1 \cdot v_1 + \Delta t_2 \cdot v_2$ where v_1 is the growth speed during the first period of the cell cycle Δt_1 , and v_2 is the growth speed during the second period of the cell cycle Δt_2 . Thus, the sum of Δt_1 and Δt_2 equals the full cell cycle duration Δt . The cell size at birth after one generation is given by

$$S_1 = (S_0 + \Delta t_1 \cdot v_1 + (\Delta t - \Delta t_1) \cdot v_2) \cdot k \quad (9)$$

The cell size at birth after n divisions is then given by

$$S_n = S_0 \cdot k^n + v_1 \cdot \sum_{i=1}^n k^i \delta_{1,i} + v_2 \cdot \sum_{i=1}^n k^i (\Delta t - \delta_{1,i}) \quad (10)$$

where $\delta_{1,i} = \Delta t_{1,n-i}$. Assuming that $0 < \delta_{1,i} < b_1$, and that $0 < \Delta t - \delta_{1,i} < \Delta t - b_1$ (where b_1 is the upper bound of $\delta_{1,i}$), the sequences $a_n = \sum_{i=1}^n k^i \delta_{1,i}$ and $b_n = \sum_{i=1}^n k^i (\Delta t - \delta_{1,i})$ are increasing and bounded, and thus converge. Therefore, over many divisions, the birth size of new generations of cells growing bilinearly and following a timer reaches a unique steady-state solution such as

$$\lim_{n \rightarrow \infty} S_n = v_1 \cdot \lim_{n \rightarrow \infty} a_n + v_2 \cdot \lim_{n \rightarrow \infty} b_n \quad (11)$$

If cells follow a sizer model of cell division control, the steady-state solution for birth sizes reached after many generations is common for exponential, linear, and bilinear single-cell growth and is given (by definition) by

$$\lim_{n \rightarrow \infty} S_n = S_{\text{sizer}} \quad (12)$$

with S_{sizer} , the size at birth as determined by the sizer.

If cells follow an adder model for cell division (ΔS is constant), the steady-state solution for birth sizes reached after many generations is common for exponential, linear, and bilinear single-cell growth models. The cell size at birth after one generation is given by

$$S_1 = (S_0 + \Delta S) \cdot k \quad (13)$$

The cell size at birth after n divisions is then given by

$$S_n = S_0 \cdot k^n + \Delta S \cdot \sum_{i=1}^n k^i \quad (14)$$

Thus, over many divisions, the birth size of new generations of cells following an adder control of cell division converges toward

$$\lim_{n \rightarrow \infty} S_n = \frac{\Delta S \cdot k}{1 - k} \quad (15)$$

Equation (15) is valid for all single-cell growth models.

In general, for all single-cell growth models, a sizer model or adder model will prevent cell size from diverging over generational time. In a sizer model because of an explicit restriction on size (Equation (12)), and in an adder model because cells reach a steady-state birth size no matter the growth model since the total added volume is kept constant (Equation (15)).

A timer model of cell division control results in an unstable birth size over successive generations if cells grow exponentially (Equation (4)). If an exponentially growing cell divides asymmetrically, or divides too soon or too late due to biological noise, cells may become smaller and smaller or larger and larger at each successive division and cell size homeostasis may not be maintained. These results are in good agreement with previous mathematical treatments of this problem.^[19,35–38] Under linear cell growth, cells following a timer model of division control reach a unique steady-state size at birth and can maintain size homeostasis (Equation (8)). The same is true for bilinear growth (Equation (11)).

To summarize, given that a timer model of cell division is unstable with exponential growth, these results underscore the importance of taking into account the model of single-cell growth before assigning models of division control.

2.4. The Speed at Which Outliers Return to a Steady-State Birth Size Is Characteristic of Each Combination of Single-Cell Growth and Division Models

Under steady-state growth, all three models of cell division control (sizer, timer, adder) are equivalent and not differentiable.

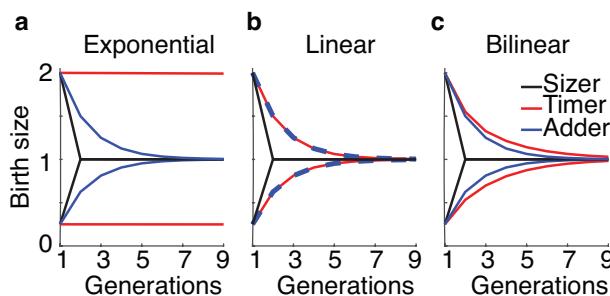


Figure 3. Outlier cells converge on the steady-state birth size at different speeds depending on the single-cell growth and division control models. The number of generations required for outlier cells to give rise to progeny cells that have a steady-state size at birth defined as “1” depends on the true division control model as well as the true single-cell growth model. a-c) The three division control models considered here are shown in black (sizer), red (timer), and blue (adder). Single-cell growth models were modeled for a) exponential growth, b) linear growth, and c) bilinear growth. Two outlier cells, one small (0.25 times the steady-state birth size of 1) and one large (2 times the steady-state birth size of 1), are shown in each case. See also Figure S2, Supporting Information.

Here, we consider a lineage of symmetrically dividing cells to be in steady-state growth if the birth size of all cells in the lineage remains constant over generations in a deterministic manner. Take, for example, a cell born at a volume v that will divide at $2v$, yielding two daughter cells of volume v that will also divide at $2v$. For this lineage, we cannot distinguish between a sizer model dictating that cells divide at exactly $2v$, an adder model dictating that cells add a volume v , or a timer model that allows cells to grow for a certain time, which happens to coincide with a volume of $2v$ at division. Under steady-state growth, all three models are possible and give the same result.

If we now consider outlier cells—for example, cells born at an abnormally small size of only 25% of the steady-state birth size or cells born at an abnormally large size of 200% of the steady-state birth size (Figure 3)—their descendants may eventually reach the steady-state birth size, depending on the single-cell growth model. Crucially, the rate of this convergence is determined by the model of division control (Figure 3), assuming that the descendants of the outlier cells return to a normal behavior.

If the true single-cell growth model is known, in most cases it is possible to distinguish between the models of division control when looking at the number of generations an outlier cell takes to reach the steady-state birth size. The timer and adder models of division control exhibit the slowest convergence of outlier cells toward steady-state. Cells growing exponentially under the control of a timer model remain at stable equilibria of large or small size (Figure 3a) because, under deterministic simulations, Equation (5) is satisfied. If Equation (5) were not satisfied, cells growing exponentially following a timer model would be born larger and larger or smaller and smaller in each generation, according to Equation (4). If cells grow linearly, the rate of convergence will not discriminate between a timer or an adder model: the two models are strictly equal (Figure 3b) because, during a fixed amount of time, a cell will add a fixed volume independently of its size at birth. Or, stating the case the other way around: adding a fixed volume will take the same amount of

time for all cells. Outlier cells growing bilinearly under a timer model will take slightly more time to reach cell size homeostasis than if they were governed by an adder model, because large cells grow faster and will add more volume than small cells in the same amount of time (Figure 3c). As large cells approach the steady-state birth size by becoming smaller and smaller in successive divisions, they will add a little less volume in each generation until the steady-state birth size is reached.

If we combine different models of division control during the cell cycle, the rate of convergence will adapt accordingly. For example, the more dominant the sizer model is, in terms of the fraction of the cell cycle it controls, the quicker the convergence will be. We note that this example of a “perfect sizer model” is unrealistic, as cells born at $2v$ would instantly divide to form two cells of v each, with insufficient time to complete DNA replication and segregation. Adding a more realistic “minimum interdivision time” constraint (effectively, a timer) reduces the rate of convergence, rendering a pattern somewhat more similar to the adder model (Figure 3). This effect is enhanced as the “minimum interdivision time” is increased. In theory, all intermediate convergence curves between the perfect sizer model and adder model could be found by combining models and adjusting their importance or duration.

Here, we assume that cell division is symmetric (the two daughter cells are equal in size at birth), but our conclusions would be similar for cells that divide asymmetrically. Indeed, in cases of asymmetric division, two steady-state birth sizes would be obtained when following the progeny of outlier cells: one for the smallest daughter cells, and one for the largest daughter cells. Every other cell lineage, between the smallest and largest outliers, will have a steady-state birth size intermediate between those two extremes.

Following a sizer model (Figure 4a) or adder model (Figure 4c), outlier cells following the three different single-cell growth models converge at the same rate in terms of generations. In the case of a timer model, the linear and bilinear models of single-cell growth converge to the steady state at different rates, whereas cells growing exponentially fail to converge (Figure 4b). Rather than looking at the number of generations that it takes for an outlier cell to reach the steady-state birth size, we can instead measure the absolute time. Because exponential growth is size-dependent, abnormally small cells take longer to converge to the steady-state birth size than abnormally large cells even though it takes them the same number of generations. This conclusion is also valid for bilinear growth, albeit to a lesser extent. Therefore, using absolute time as the measure of convergence speed, in theory, it should be possible to differentiate the single-cell growth models (Figure 4d-f). Experimentally, however, this distinction would be challenging due to measurement and biological noise, the latter including asymmetric division, which is common in some bacteria.

To summarize, although it is impossible to distinguish between the different models of cell division control when cells are in steady-state growth, analysis of the convergence rates of outlier cells can, in theory, distinguish between the models because the perturbed system is not in steady state. The only exception is that when single-cell growth follows a linear model, the adder model and timer model of division control cannot be differentiated.

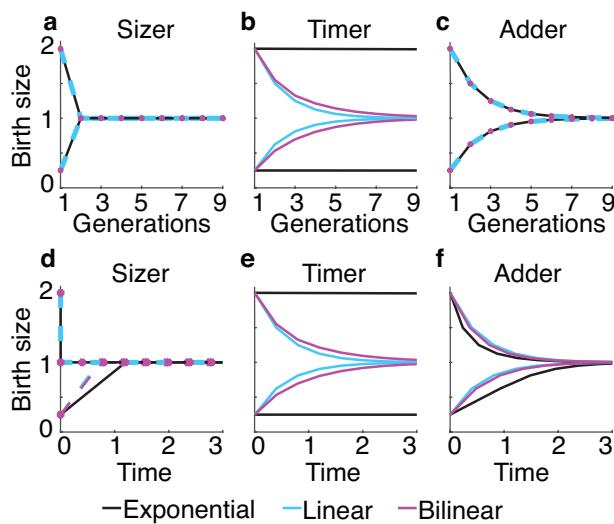


Figure 4. Single-cell growth models can be distinguished by the convergence of outlier cells on the steady-state birth size as a function of time. In the example shown here, two outlier cells, one small (0.25 times the steady-state birth size of 1) and one large (2 times the steady-state birth size of 1), were simulated. Convergence of these outliers on the steady-state birth size is plotted as a function of a–c) generations or d–f) time. Single-cell growth is exponential (black lines), linear (blue lines), or bilinear (pink lines). The timing of cell division was determined according to a,d) a sizer model, b,e) a timer model, or c,f) an adder model. When convergence is plotted as a function of generations (a–c), the speed of convergence is equivalent for all single-cell growth models when division is controlled by a sizer (a) or an adder (c), but the speed of convergence is different for each single-cell growth model under a timer (b). When convergence is plotted as a function of time (d–f), the speed of convergence can be differentiated for all combinations of single-cell growth models and division control models. These distinctions are blurred by addition of noise, and a “pure” sizer is unrealistic for large outlier cells, which divide immediately after birth with no time for DNA replication. See also Figure S3, Supporting Information.

2.5. The Correlation between Birth Size and Added Size Depends on the Division Control and Single-Cell Growth Models

Scatter plots of birth size against added size from birth to division (or variants thereof) have been widely used to deduce the true model of cell division control.^[17,18] In such plots, cells following a sizer model exhibit a slope of -1 (Figure 5b–d, black lines), while cells following an adder model exhibit a slope of 0 (Figure 5b–d, blue lines). Cells following these division control models exhibit the same slopes irrespective of the model of single-cell growth. However, the slope of a collection of cells following a timer model depends on the growth model: a slope of $s = 1$ if growth is exponential (Figure 5b, red line), $s = 0$ if growth is linear (Figure 5c, red line), or $0 < s < 1$ if growth is bilinear (Figure 5d, red line). In the last scenario, the slope depends on the parameters set for the bilinear model.

In the case of linear growth, the slope of cells following the timer model is equal to 0 because a small cell will add the same amount of volume as a large cell during the same interval of time. The timer and adder models therefore become undistinguishable and a constant added size can represent either model.

This point again underscores the importance of identifying the true model of single-cell growth.

2.6. Single-Cell Growth Models and Division Control Models Can Be Distinguished by the Speed of Convergence of Outlier Cells in Vector Fields of Birth Size Versus Added Size

Vector fields superimposed on a graph depicting birth size versus added size (Figure 5a) provide direct information about the rate of convergence of outlier cells toward the equilibrium point, defined here as the unique convergence point of the vector field. Thus, vector fields provide a better visualization of the underlying model of division control compared to conventional scatter plots. The vector field shows the direction followed by linear trajectories of cells and their descendants through multiple generations; where the trajectories pass through the equilibrium point, every cell has the same birth size and added size. The arrows indicate the direction of trajectories for successive birth size, under the assumption that the mechanisms responsible for cell growth and cell size homeostasis directly target the equilibrium point. In the “unstable” (cyan) areas the arrows point away from the equilibrium point and cells become progressively smaller or larger in each generation. Conversely, in the “stable” (magenta) areas the arrows point toward the equilibrium point and cells converge progressively toward this point in each generation. The darkness of the colors illustrates the speed at which the birth size of daughter cells becomes larger or smaller than the birth size of their mother cells in successive generations.

Under an exponential model of single-cell growth (Figure 5b), convergence toward the equilibrium point is reached the fastest with the sizer model of division control, as the trajectory lies within a dark magenta region. Under the timer model, cells lie within the extreme border of the “unstable” cyan regions. This region is completely white, indicating that daughter cells and mother cells have the same birth size, and convergence toward the equilibrium point will not occur. However, if noise is added, these cells will tend to fall into the “unstable” cyan region and move away from the equilibrium point. Under an adder model, cells that are far from the equilibrium point converge rapidly at the beginning and more slowly as they approach the equilibrium point, indicated by lightening of the magenta. Under a linear model of single-cell growth (Figure 5c), the sizer and adder models of division control behave exactly the same as for exponentially growing cells (cf. Figure 5b). Under a timer model, however, cells growing linearly behave the same as cells growing under an adder model. When the growth model is bilinear (Figure 5d), the sizer and adder models of division control behave exactly the same as for cells growing exponentially or linearly (cf. Figure 5b,c). Under a timer model, cells smaller than 25% of the average size and cells bigger than 137.5% of the average size grow linearly either at minimal or maximal speed, respectively, indicated by the dashed regions of the red line in Figure 5d. These cells are too small or too large to exhibit a change in growth speed and, therefore, they grow linearly and have another theoretical equilibrium point (not shown here).

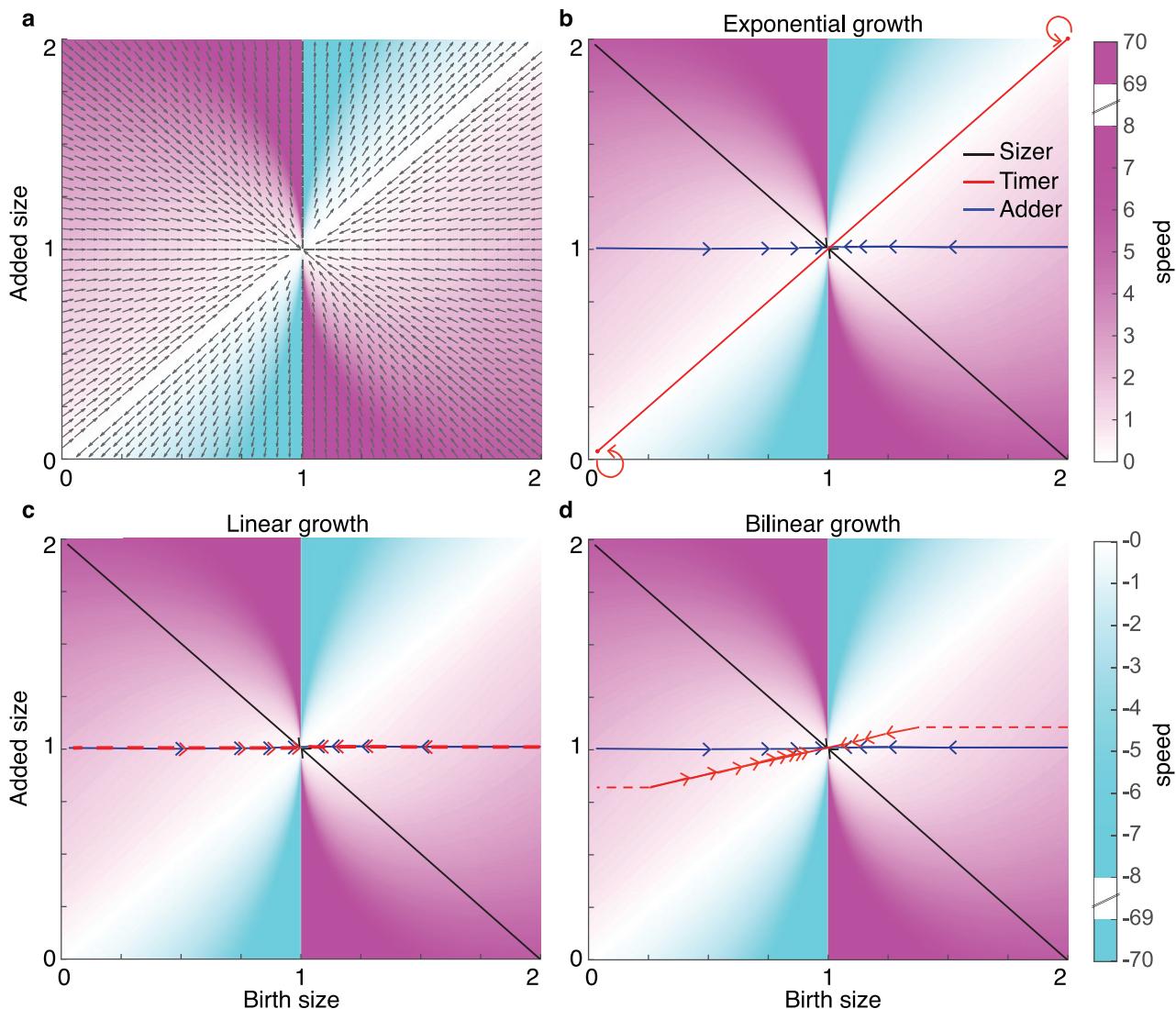


Figure 5. Vector fields of birth size versus added size show that the speed of convergence of outlier cells toward the equilibrium point depends on both the division control and single-cell growth models. a-d) Plots of birth size versus added size show that the correlation between them depends on the underlying single-cell growth and division control models. Superimposition of vector fields on these plots shows the speed of convergence (in arbitrary units per generation) of outlier cells toward the equilibrium point (1,1). a) Diverging regions of the vector field, where large cells grow larger and small cells grow smaller at every generation, are shown in cyan. Converging regions of the field are shown in magenta. The arrows representing the trajectory of birth size from generation to generation are pointing away from (diverging) or toward (converging) the equilibrium point. Increasing speed of divergence or convergence is indicated by darkening color. Simulated cells are born at specific points on the vector field and follow growth paths (direct trajectory toward the equilibrium point) before the next division following an adder, sizer, or timer model. b-d) Under an adder model (blue lines), a cell born at a size $2v$, twice bigger than the equilibrium size ($1v$), grows by $1v$. The cell then divides to generate two daughter cells of $1.5v$, which then grow by $1v$. Consequently, daughter cells in the next generation will be $1.25v$ at birth. Subsequent generations approach the equilibrium birth size of $1v$ following a horizontal trajectory, independent of the single-cell growth model. Under a sizer model (black lines), a cell born at $2v$ in size will not grow, thus giving rise to two daughter cells of $1v$ in size. In this case, the equilibrium birth size is reached within one generation, independent of the single-cell growth model. Under a timer model (red lines), the paths approaching the equilibrium point are different depending on whether a cell grows exponentially (b), linearly (c), or bilinearly (d). If cells grow exponentially and double their size during a cell cycle, all mother and daughter cells will retain the same size at birth. This scenario occurs in the white regions of the graphs, where the rate of convergence and divergence is zero. However, this situation becomes unstable if Equation (5) is not satisfied (due to asymmetric division, variable growth rate, or other sources of noise), in which case cells will fall into the unstable region and grow either larger or smaller in successive generations. With linear growth, the trajectory is the same as with an adder. When growth is bilinear, the slope is between 0 and 1 and depends on the point when the increase of growth speed occurs between birth and division.

As already mentioned for Figure 3, during asymmetric division, two extreme steady-state sizes would be obtained when following the lineage of outlier cells (the smallest and the

largest cells) through time. Thus, for asymmetrically dividing cells, two vector fields will be obtained, one for each extremum. All other cell lineages will reach a “birth size” and “added size”

situated somewhere on the interpolation curve of the two equilibrium points of the two vector fields.

3. Conclusion

Here, we highlight the importance of determining single-cell growth models, as the true growth model constrains the possible division control models. We show that conventional methods, such as r^2 analysis, are inadequate to determine the true growth model. Additional discriminating power can be achieved using analysis of residuals, especially when combined with Akaike or Bayesian information criteria.

In steady-state populations, the majority of cells provide little information about the model of division control because their birth sizes are near the population average. However, rare “outlier” cells with birth sizes significantly smaller or larger than the mean can be very informative. If scatter plots are used, we propose that the bulk population should be removed from the analysis, which should focus instead on outliers and their successive generations until the steady-state birth size is reached. We show that the speed (number of generations or absolute time) at which outliers return to a steady-state birth size is characteristic of each combination of single-cell growth model and division control model; only the timer and adder models are equivalent for cells growing linearly.

Experimentally, the speed of convergence of outliers to the steady-state birth size could also be studied by reversibly shifting the cell size distribution using mutations, antibiotics, or nutritional conditions that accelerate or delay division.^[41–43] Previously, mechanisms of nuclear division control based on the concentration or absolute number of stimulatory or inhibitory molecules have been proposed from studies with multi-nucleated syncytia of the slime mold *Physarum polycephalum*, which undergoes successive nuclear divisions without undergoing cell division.^[44–47] Experimentally, the authors attempted to distinguish between these mechanisms by perturbing the DNA-to-cytoplasm ratio and observing the convergence of intermitotic periods back to steady state in successive nuclear division cycles. A distinguishing feature of the sizer model is that it may return cells to steady state within a single division cycle after a perturbation, unlike the asymptotic approach to steady state that is characteristic of the timer and adder models. In *P. polycephalum*, if mitosis is delayed by UV irradiation or drug exposure, convergence of intermitotic periods back to steady state is achieved in a single nuclear division cycle following the perturbation.^[45–47] These results are consistent with a sizer model controlling nuclear division if we assume that both the intermitotic period and “size” (DNA-to-cytoplasm ratio) converge to steady state within one nuclear division cycle.

Although the sizer, timer, and adder models have been studied extensively in their abstract (mathematical) forms, the underlying and concrete mechanisms of size control are not well understood. In the case of a sizer model, it has been proposed that a metabolic sensor governs cell size at division in *E. coli* and *B. subtilis*, or that titration of intracellular molecules could serve as a proxy for cell size.^[48–50] This and other potential size-sensing mechanisms that could be used to control the timing of cell division have been reviewed recently.^[2,51–53] In the

case of a timer model, the time interval could be the result of the sum of multiple processes, each of which requires a specific and finite amount of time.^[16] In some organisms, a timer model could also be linked to the organism’s circadian rhythm, as cell size control has been shown to be driven in part by the circadian clock.^[54] In the case of an adder model, it was recently proposed that division is triggered by the accumulation of initiators and precursors of division proteins to a fixed threshold, combined with maintenance of their production in proportion to volume growth.^[55] As defined in our analytical analysis, $\Delta t \cdot v$ is fixed in an adder model under linear single-cell growth. Thus, mechanistically, the cell could achieve size homeostasis by modulating either Δt (the interdivision time) or v (the cell growth speed) or both, as proposed by Ginzberg et al.^[56] For bilinear growth, cells could modulate either Δt or v_1 and v_2 , or both. For exponential growth, cells could modulate either Δt or r , or both.

Conventionally, division control models are distinguished using scatter plots that correlate the birth size and added size of individual cells. For example, a constant added size irrespective of birth size is often taken to support the adder model of division control; however, this relationship also holds true for the timer model when single-cell growth is linear. As shown here, scatter plots can be overlaid with vector fields to capture additional parameters, such as cell lineages, speed of convergence to a steady-state birth size, and stability of cell size. Thus, vector fields contain both the information depicted in conventional scatter plots and convergence plots (Figure 3). Scatter plots depict the behavior of actual cells but they cannot predict the behavior of cells following an unstable combination of single-cell growth and division models, for example, cells growing exponentially under a timer model. In contrast, vector fields capture the predicted behavior of both stable and unstable combinations of models, which is a useful feature for ruling out unstable combinations without having to perform complex mathematical analyses.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

G.V.-T. and J.D.M. conceived the project. G.V.-T. created and implemented the models, performed the simulations, and interpreted

the results. G.V.-T. and A.R.V. generated the vector field plots. G.V.-T., N.D., and J.D.M. wrote the paper. N.D. and J.D.M. supervised the project. All authors commented on the manuscript and approved the paper.

Keywords

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