Cell Growth and Size Homeostasis in Silico

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Abstract

With the recent technical advances in measuring cell size and sorting the newborn cells unperturbed, sophisticated experimental has been designed to study the cell growth and size homeostasis with high accuracy and time resolution. The result revealed that, the growth rate of some mammalian cells depends both on cell size and age and is under tight regulation [Tzur, A., et al. (2009) Science, 325, 167-171]. Here we propose a stochastic cell growth model with only two variables, namely, mRNA and ribosomes, that can explain the experimental results quantitatively. Consistent with the experiment, the model predicts three growth stages: An initial stage with suppressed growth rate, and an exponential growth stage with constant growth rate followed by a final sustaining stage with decaying growth rate. Abeit in an extremely simple form, the model can explain most of important features in cell growth and size homeostasis. Insights gained from the model may help us understand the design principles in cell size control.
Introduction

Controversial views regarding cell growth and size homeostasis have existed for a long time. There is evidence for a constant growth rate independent of cell size in some bacterias and Schwann cells [1]. Alternatively, evidence also exists for a size-dependent growth rate in budding yeast [2] and mammalian cells [3]. Early works modeling cell growth and cell cycle are usually based on assumptions that are difficult to be justified [4–6]. Recently there is a strong demanding in the development of more sophisticated models that explain the coordinance of cell growth and cell cycle and how size homeostasis is maintained in metazoan cells. However, the biggest limitation comes from insufficient measurement accuracy in cell size $s$ and age $a$ of a single cell unperturbed.

As an alternative, Collins-Richmond method [7] has been used to infer the cell growth rate $v = ds/dt$ as a function of size $s$. The rational behind this method is that, the proportion of cells of any given size is time-invariant in an asynchronous population at steady state, which means the ratio of cells with size smaller than $s$ is balanced by the growth of cells smaller than $s$ and the mitosis of cells larger than $s$. In order to apply this method to get $v(s)$, one needs to supply three cell size distributions for the asynchronous cells, the dividing cells and the newborns. The last two distributions are difficult to obtain and previous applications of this method need to reply on some unverified assumptions [3, 7]. Recently, in their seminal work [8], Tzur et al. developed a procedure that uses gentle flow to collect newborns almost nonperturbing. In addition, by measuring the size difference between two newborn sister cells, size distribution of the dividing population can be obtained by a convolution law. Combined with highly accurate cell size measurement, they are able to, for the first time, collect reliable data to apply the Collins-Richmond method.
As a major result, they found that the cell growth rate $v$ is in strong dependence with cell size $s$. In particular, when cell size is below a certain threshold the growth is exponential-like, i.e., the growth rate is linearly proportional to the cell size. Above this threshold, however, the growth rate begin to tacking down. The changing of growth pattern is a proof of size regulation but the underneath controlling mechanism is still a mystery. By tracking synchronous newborn cells, they also demonstrate that the growth rate is in dependence with cell age and cell cycle. After an initial growth suppression, there is a rapid increase in growth rate during the G1 phase, followed by a period of nearly constant exponential growth. Similar gear-shifting growth pattern is confirmed in a more recent work by the same group based on single cell measurement [9].

As revealed by the above experiments, cell growth is both size- and age-dependent, and is under tight control. The aim of the current work is to build a detailed cell growth model consistent with the experiments. Following the dynamics of mRNA and ribosomes, the two essential ingredients of protein synthesis, our stochastic model of cell growth predicts a gear-shifting growth pattern in which cells experience an initial transit stage with suppressed growth, followed by an exponential growth with constant growth rate and, if the cell has not divided yet, a sustaining stage with decaying growth rate. Abeit in a very simple form, the model captures most of the features observed in the experiments. In order to validate the model we repeat the same experiment procedure in silico and excellent quantitative agreement between the simulation results and the experimental results is obtained. Insights gained from the model may help us to understand the underlying mechanisms regulating cell growth and size homeostasis.
Model

Think of the cell as a container of organelles, then its size (denoted by $s$) is proportional to the amount of stuff it contains. Let ribosome be a representative organelle and assume the cell size passively expands as the number of ribosomes increases, so that the volume density of ribosomes in a cell will not exceed $\rho$. Let $n$ be the number of ribosomes within one cell, the above assumption establishes a relation between the cell size and ribosome number by $s = \max\{s, n/\rho\}$. Note that when the degradation rate of ribosomes is larger than the production rate, $n$ may decrease. However, since the constituting material still stays in the cells, the cell size is thus assumed to be non-decreasing during cell growth.

The production rate of ribosomes is assumed to be proportional to the protein synthesis capacity of its containing cell. mRNA and ribosomes are two essential component in protein synthesis. By rescaling the unit of mRNA so that each ribosome requires one unit of mRNA to work properly, the protein synthesis rate can be measured by the propensity of “loaded ribosomes”, i.e., ribosomes that can locate enough mRNA to work. Denote the available amount of mRNA in the cell to be $m$ (in the rescaled unit), the number of loaded ribosomes is then $\min\{m, n\}$.

The cell growth model is sketched in Fig. 1. mRNA and ribosomes levels are dynamically balanced by their production rates and degradation rates. Assume the degradation rates of mRNA ($\gamma_1$) and ribosomes ($\gamma_2$) are constants, and the production rate of ribosomes to be $\lambda_2 \min\{m, n\}$ (proportional to the protein synthesis capacity), the production rate of mRNA to be $\lambda_1 (\kappa a)^q/(1 + (\kappa a)^q)$. Here $a$ is the cell age and $q$ is the Hill’s coefficient. $\kappa$ is a rescaling coefficient. For newborns, the mRNA production rate is small as cells take time to unfold their chromosome and initiate transcription. After this the mRNA production rate saturates, either because the transcription process has hit its ca-
Figure 1. A stochastic cell growth model. The ribosome assembling rate are measured by the amount of “loaded ribosome”, which equals to the minimum of mRNA and ribosomes (with properly scaled units). The cell size expands passively so that the volume density of ribosomes will not exceed a certain threshold $\rho$. The amount of mRNA and ribosomes are dynamically balanced by their production rates and degradation rates.

Within each cell, the dynamics of $m$ and $n$ can be described by the following discrete chemical reaction system

\[
\begin{align*}
\text{DNA} & \quad \xrightarrow{\lambda_1(\kappa a)^q/(1+(\kappa a)^q)} \quad \text{mRNA}, \\
\text{mRNA} & \quad \xrightarrow{\gamma_1} \quad \emptyset, \\
\text{Amino acid} & \quad \xrightarrow{\lambda_2 \min\{m,n\}} \quad \text{Ribosome}, \\
\text{Ribosome} & \quad \xrightarrow{\gamma_2} \quad \emptyset.
\end{align*}
\]

Stochastic simulation algorithms such as Gillespie’s method can be used here to track $m$ and $n$ in each cell. However, since the number of $m$ and $n$ are usually very big, it is
more efficient to use the tau-leaping method to fire multiple reactions in one time-step [11], as will be used here. The stochastic model is more realistic especially when \( m \) or \( n \) is small, i.e., when cells are newly born. But it is still helpful for us to understand the general behavior of this system by considering the corresponding deterministic reaction rate equations (a continuous-variable ordinary differential equation system), given by

\[
\frac{dm}{da} = \lambda_1 \frac{(\kappa a)^q}{1 + (\kappa a)^q} - \gamma_1 m, \quad (1a)
\]
\[
\frac{dn}{da} = \lambda_2 \min\{m, n\} - \gamma_2 n. \quad (1b)
\]

Here we rescale the units of ribosome to get \( \rho = 1 \) so that the value of cell size, mRNA and ribosome are comparable. Note that different scaling factors only controls the magnitude of fluctuation in the stochastic model, and has no effect in the behavior of the above deterministic model. To determine the initial value of \( m \) and \( n \), it is assumed that, when a cell mitosis, all ribosomes are divided to the daughter cells proportional to their corresponding cell sizes (ribosome density remains the same). The mRNA level is set to zero for the newborns as mRNA usually degrades fast. Now solving the above equations with initial condition, say, \( m = 0 \) and \( n = 1000 \) gives trajectories shown in Fig. 2.

In consistent with [8], three different growth stages can be identified in Fig. 2. In the first stage, which last for a very short time, the newborn cells carries ribosomes inherited from its parent, but need to wait for mRNA supply to start synthesis of protein. As the chromosome unfolds and transcription initializes, the mRNA level quickly catches up. With enough mRNA, the cells first replenish ribosomes lost due to degradation during the first stage, then enter an exponential growth stage in which the synthesis of ribosome becomes the rate-limiting event. The growth rate is now a linear function of \( s \) with constant coefficient \( \lambda_2 \) (note that \( \min\{m, n\} = n \)). As the cell expands, however, limited
Figure 2. Solution of the reaction rate equations Eqs. (1a) and (1b). The ribosome density has been rescaled to $\rho = 1$ so that the amount of ribosome in the figure is comparable with the cell size (in fl). The loaded ribosome (dashed red) is the smaller one between $m$ (dashed black) and $n$ (solid blue). The cell size (solid green) overlaps with the ribosome number $n$ when the cell is expanding in size. Three growth stages can be identified (see main article). Parameters used here are: $\lambda_1 = 4000$, $\gamma_1 = 2$, $\lambda_2 = 0.25$, $\gamma_2 = 0.15$, $\kappa = 1$, $q = 4$. 
mRNA supplement can no longer support all ribosomes. This marks the third stage in
which the ribosomes still increase in number but the growth begins to slow down because
of larger degradation. Note that some cell may already undergo mitosis before entering
into stage III. Different growth stages reflect the internal change in the relative propensity
of mRNA and ribosomes which, in principle, could be adopted by cells as a mechanism
to regulate cell cycle. We will come back to this point in the Discussion section.

Next we want to repeat the cell culture experiment in silico, that is, to simulate a
large population of cells for a sufficient long time till the population become completely
asynchronous and their size and age distribution becomes stationary. To do so, we need to
specify how cells divide, which will be discussed below. In [8] it was found that, for cells
with the same size, cells with larger age tend to divide more. Meanwhile, for cells with
the same age, larger cells tend to divide more. They suspect there exist some form of size-
gate and age-gate for cell mitosis. Following their idea, we consider a phenomenological
mitosis rule in which a cell of size $s$ and age $a$ will divide with probability $p(s,a)dt$ during
an infinitesimal time $dt$. In particular, we choose

$$p(s, t) = \left( \alpha \frac{(s - s_c)^+}{s_c} \right)^2 + \left( \beta \frac{(a - a_c)^+}{a_c} \right)^2,$$

where $(x)_+$ means $\max\{0, x\}$. $s_c$ and $a_c$ correspond to the size-gate and age-gate, re-
spectively. The above cell division rule can generate simulation results quite close to the
experimental data. But one important feature is still missing, which is the long-tail be-

behavior found in the asynchronous and newborn cell size distribution. As a remedy, we let
cells with relative large size/age ratio, i. e., fast-growing cells, to live longer (by decrease
its $p(s, t)$). Among a lot of different choices of $p(s, t)$ that we tested, the above setting
best matches the experimental data. It is interesting to note here that, while the exact
shapes of cell size distributions are sensitive to the choice of $p(s, t)$, the shape of $v(s)$ is not, even though $v(s)$ is completely determined by those distributions (see Discussion).

When a cell divides, as measure in [8], the size difference between the two daughter cells is a Gaussian distribution with mean zero and standard deviation $68.8 \pm 10$ fl independent of cell size. In addition we assume the mitosis time is so small that can be neglected. During the simulation, without any constrain the total number of cells will grows exponentially in time, so we have to keep the population size without effecting the statistics. To do so we use the Moran population process [12] with fixed population number $N = 10,000$. In particular, when a cell mitosis, one of the daughter cells took the place of its mother cell, the other daughter cell will take the place of a cell that is randomly picked up from the total population (including its sister cell) with uniform probability weight. Doing so the total population remains constant and meanwhile the statistics remains the same.

**Results**

With the above cell growth model we repeat the experiment of [8] in silico. Initially all cells are assigned with cell size $s_0$ and age 0. After a sufficiently long time, an asynchronous population is reached with a steady cell size distribution which is independent with the initial condition.

First we study how growth rate is related with cell size. To be precise, we measure the growth rate of a cell with size $s$ and age $a$ defined by $ds/da = v(s, a)$. For example, $v(s, a) = v_0s$ corresponds to an exponential growth, and $v(s, a) = v_0$ corresponds to a linear growth. In the simulation, we obtain for each cell a time series of cell size $s(t)$ at discrete time point $\{0, \delta t, 2\delta t, \cdots \}$. So at one of the discrete time point $t$, the growth
rate of a single cell is approximated by \((s(t + \delta t) - s(t - \delta t))/2\delta t\). After the population reaches a steady state the simulation stops and growth rates of all cells are plotted in Fig. S1. Due to the stochastic nature of the problem, \(v(s,a)\) varies from cell to cell, and the growth rate is also age-dependent. So we divide the cell size into small intervals and then take the average of growth rate of cells whose size falls into each interval. The age-dependence in the growth rate \(v(s,a)\) is thus averaged out. It is straight forward to show that in this case the Collins-Richmond relation holds for this averaged \(v(s)\). In other words, the average growth rate we obtained above coincident with what has been measured in the experiment. Generally speaking, the simulation result (Fig. 3A) captures most of the features observed in the experimental result (Fig. 3B) quantitatively. The only disagreement is in the precise value of the slope between the range of \(s\) in \([1000, 2000]\). This is probably related with the long-tail behavior found in the actual distributions of the newborns (see blow).

The model in Eqs. (1a) and (1b) provides explanations to the shape of the growth rate curve: First of all, now we know that, at the turning point in the growth rate curve the cells are switching from phase II growth to phase III. The cell size at the transition is determined by the maximum availability of mRNA, denoted by \(m^*\). According to Eq. (1a), \(m^* = \lambda_1/\gamma_1\) (the Hill’s function is approximately 1 for large \(a\)), which is set to 2000 in order to match the experimental data. The growth rate at the transition is \((\lambda_2 - \gamma_2)m^*\) according to Eq. (1b) (now we have \(m = n = m^* = 2000\)), and is set to 200. Secondly, the slope of the curve on the right of the turning point is exactly \(\gamma_2\) (obtained by substituting \(dn/dt = v\) and \(n = s\) into \(dn/dt = \lambda_2m^* - \gamma_2n\)). Estimated from the experimental data \(\gamma_2\) is approximately 0.15. Lastly, the curve on the left hand side of the turning point is shifted away from the pure exponential growth curve (the red-dashed line in Fig. 3A). This is because, according to the model, a cell whose size is at this range can
Figure 3. Cell growth rate as a function of cell size. (A) Averaged growth rate from the simulation result. The dashed-red curve represents pure exponential growth \( v = \lambda_2 s \).

(B) Experimental result obtained using Collins-Richmond method in [8] (permission from AAAS to reuse this figure, different curves correspond to different detailed implementation). The parameters are: \( \rho = 100, \lambda_1 = 4000, \gamma_1 = 2, \lambda_2 = 0.25, \gamma_2 = 0.15, \kappa = 1, q = 4, s_c = 1100, a_c = 6, \alpha = 0.85, \beta = 0.8 \). Sample size is 10,000.

be found at both growth states I or II, which means it could either follow an exponential growth or have zero growth rate. The average growth curve is thus shifted down away from the exponential curve. The exact shape of this part of the growth curve, however, is collectively determined by the size distributions of the asynchronous cells, dividing cells and newborns.

Numerical result shows that a steady asynchronous cell size distribution can be reached. We mention by pass here that cell size homeostasis in this model is relatively easy to obtain. This is because the cell size is bounded by Eq. (1b) to be \( m^*\lambda^2/\gamma_2 \), so as long as the size gate \( s_c \) and age gate \( a_c \) in Eq. (2) are not too large (to prevent cells from dividing too early) the size distribution will converge to a steady state. However, the exact shape of the size distribution is affected by the particular choice of the model parameters. By fine tuning the parameters we obtain asynchronous, newborn and mitosis cell size distribution in Fig. 4 A, B and C, respectively (The same parameter setting is used to
generate Fig. 3. The newborn and mitosis cell size distributions are obtained by collect a sample of 10,000 newborns and dividing cells, respectively, after the population has reached equilibrium. The histograms as well as the fitted densities are plotted to compare with the corresponding experimental data in Fig. 4 D, E and F. Note that the mitosis size distribution (F) is not a directly measurement of experiment, but derived from the newborn cell distribution and cell size difference using a convolution law. These results show that the in silico results quantitatively matches with the experimental data.

Finally, we compare the in silico synchronous cell growth data with the experiment results. We collect newborns during the simulation and let them evolve synchronously. The heat-map in Fig. 5A shows how the cell size distribution change with time. The parameters are the same with those used to produce the previous results. Overall the simulation result is consistent with the experiment.

Together, the simulation results presented in this section demonstrate that the simple model captures most features of the complex process of cell growth and size homeostasis.

Discussions

Cell growth and division is an extremely complex process. It surprises us that a simple model as Eqs. (1a) and (1b) can capture most of the features of cell growth and size homeostasis of some mammalian cells quantitatively. Indeed, using only two variables (m and n) and four parameters (λ₁,₂ and γ₁,₂), the generally shape of the v(s) curve can be determined. By tuning other parameters the size distributions also fit the experimental quite well. Essentially the model describes the interplay between mRNA and ribosomes. The change in the relative propensity of these two components gives rise to three different growth stages: At stage I, the mRNA level quickly elevate from a low level to a high
Figure 4. Cell growth rate in comparison with experimental result. (A-C) Asynchronous, newborns and dividing cell size distributions obtained from simulation. (D-F) Asynchronous, newborns and dividing cell size distribution from experiment [8] (permission from AAAS to reuse these figures). Parameters are the same as those in Fig. 3.
level in the newborn cells. During this stage the production of mRNA is the rate-limiting process in protein synthesis. At stage II, the mRNA supplement over run the number of ribosomes, and now the production of new ribosomes become the rate-limiting process. At stage III, mRNA has already reaches its maximum production and become the rate-limiting process again if cells continue to grow in size. Generally speaking, the factors that newborns have a low level of mRNA (because DNA need to unfold) and the supplement of mRNA has a upper limit (because a cell has only one copy of DNA) is plausible in biological sense.

The model has its value not only in explaining the experimental results, but also in providing a possible mechanism for cell cycle regulation. The change in the relative propensity of mRNA and ribosome (or some other protein) could in potential be used by cells as a way to read their size information. On one hand, at the transition from growth stage I to stage II, the cells can receive two messages. One is “we need more ribosomes and a larger room”; The other is for chromatin, which says “mRNA is enough, time to replicate”. This seems to be the right time for a cell to enter the S phase in
the cell cycle. Interestingly, in a recent work [9], Son et al. measured the growth rate in dependence with cell size for single cells. They found a clear transition in which cell turn from a fast exponential-like growth into a relatively slower exponential-like growth. This is consistent with our model in that the change in growth rate is similar with dynamics of “loaded ribosomes” in our model (Fig. 3, red-dashed line). In addition, the experiment finding that significant correlation exists between the growth rate transition and the G1-S phase transition in cell cycle seems can also fit into our story. On the other hand, the transition from growth stage II to III may also be used as a size gauge. In yeast, there is evidence suggesting cell size is regulated in the G1 phase so that cells can reach a reasonable size before mitosis [13]. However, in our model, since a majority of cells have already gone mitosis before reaching the critical size 2000 fl, the size-dependent signal, if exists, must has already been released before the actual size hit 2000. Another possibility is that, besides passively control the cell size, the transition from stage II and III could be used as a cell differentiation or apoptosis signal.

In the experimental results, all size distributions exhibit a long-tail characteristic. In order to reproduce this feature in silico, in addition to the the mitosis probability (2), we add extra rules to let some cells with larger size/age ratio to live longer. Doing so leads to an excellent match in the asynchronous cell size distribution (Fig. 4A and D), but not as good for the newborns (one might need to modify the model itself to give the newborn cell distribution a long-tail because the maximum size a cell can grow is around 3000 ($\lambda_2 * m^* / \gamma_2$), so given the relative small variance in the size difference of the sister cells, it is not likely to found newborns with size larger than 1500). This tiny difference in the size distributions seems to be responsible for the mis-match in the growth rate curve around the size range [1500, 2000] in Fig. 3. Note that according to the Collins-Richmond relation, $v(s)$ is determined by distributions $f_a$ (asynchronous), $f_0$ (newborns) and $f_m$.
(mitosis) up to a constant, that is,

\[ v(s) \propto \frac{2F_0(s) - F_m(s) - F_a(s)}{f_a(s)}, \]

where \( F(s) \) represent the corresponding cumulative probability distributions. But in simulation we found that even though the cell size distributions are very different, the corresponding \( v(s) \) can be very similar. This may suggest the Collins-Richmond method is more sensitive to the shape of the tails of the distributions than the main bodies.

In the model we have not considered the cell-to-cell variation in the growth rate. Also, the mitosis probability we used is rather artificial. The effect on the growth rate v.s. cell size relation caused by these factors may not be significant. However, with more accurate experimental data in the future, these factors need to be investigated more carefully in order to build more realistic cell evolutionary models.

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Supplemental Information

Figure S1. Growth rate v.s. cell size. Each point represent the growth rate v.s. cell size of one cell at a fixed time. The averaged growth rate in Fig. 3 in the main article is obtained from the case of $\rho = 100$ by taking the average of growth rate within each small interval of cell size. Here $\rho$ is the maximum ribosome density. A small $\rho$ leads to large fluctuation in the stochastic simulation but does not effect the average growth rate and size distributions.