Cell Growth and Size Homeostasis in Silico

Yucheng Hu[†*] and Tianqi Zhu[‡]
[†]Zhou Pei-yuan Center for Applied Mathematics, Tsinghua University, Beijing, China; and [‡]Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China

ABSTRACT Cell growth in size is a complex process coordinated by intrinsic and environmental signals. In a research work performed by a different group, size distributions of an exponentially growing population of mammalian cells were used to infer cell-growth rate in size. The results suggested that cell growth was neither linear nor exponential, but subject to size-dependent regulation. To explain the observed growth pattern, we built a mathematical model in which growth rate was regulated by the relative amount of mRNA and ribosomes in a cell. Under the growth model and a stochastic division rule, we simulated the evolution of a population of cells. Both the sampled growth rate and size distribution from this in silico population agreed well with experimental data. To explore the model space, alternative growth models and division rules were studied. This work may serve as a starting point to understand the mechanisms behind cell growth and size regulation using predictive models.

INTRODUCTION

How cells grow in size between divisions has been a classic problem in biology. Despite extensive research over the decades, much about the topic still remains unknown (1–5). To measure cell-growth rate, two approaches are usually taken. The first is to directly monitor the size of single cells (early attempts to measure cell size at single-cell level suffered from technical limitations (6–8), but much progress has been made to allow accurate measurement of the size of a single cell (9,10)). The other approach is based on collective measurement of large populations of cells in a synchronized or asynchronized state (8,11,12). Together, these two types of measurements provide complementary data, shedding light on the mechanisms that regulate cell growth.

In 2009, Tzur et al. (13) estimated the mean growth rate in size of a mouse lymphoblast cell line (L1210) using a population level approach. In particular, measurements of size distributions of the asynchronized, newborn, and dividing cell populations were conducted. With these three size distributions as input, the averaged cell-growth rate as a function of cell size was computed from the Collins-Richmond equation (11). This equation is built on the observation that the balance of flux among subpopulations allows the size distribution of asynchronous populations of cells to remain at dynamic equilibrium. Similar methods have been used to study the growth rate of bacteria and animal cells (11,14,15).

The estimated growth rate as a function of cell size obtained by Tzur et al. (13) is replotted here in Fig. 1 *A* (see Fig. 2A of Tzur et al. (13)). On average, cell-growth rate first increases with cell size, then decreases after reaching a critical size. This Λ-shaped growth pattern is consistent with results previously reported in Collins and Richmond (11) and Anderson et al. (15) and is confirmed in a more recent work in Kafri et al. (12). As proposed in Tzur et al. (13), the reduction in growth rate seen in very large cells could be related to some size-dependent regulation of cell growth. Little is known about the mechanism behind the regulation. It is also not clear how such regulation would affect cell growth and division.

By mathematical modeling and stochastic simulation, we investigate what growth models could give rise to the observed growth pattern, and how they affect the size homeostasis in a cell population. In particular, we propose a simple cell-growth model to explain the experimental data in Tzur et al. (13). The model assumes that a cell's growth rate is determined by both its ribosome number and mRNA level. During a cell-cycle, the relative abundance of ribosome and mRNA undergoes change, coordinating cell growth. With a probabilistic division rule that tells a cell when and how to divide, the evolution of an in silico cell population can be simulated. With fitted parameters, the in silico population reproduces the Λ-shaped growth curve and cell size distributions as observed in experiment. We further explore alternative cell-growth models and division rules to study how they affect cell size distributions in the population. This work provides a phenomenological explanation of the complex experimental data in Tzur et al. (13) and reveals the intricate connections among growth regulation, division control, and size homeostasis.

CELL-GROWTH MODEL

As sketched in Fig. 2 *A*, we build our model based on some intuitive assumptions:

Assumptions

The size (volume) of a cell, denoted by s, is assumed to be proportional to its protein mass, which in turn is considered

Submitted July 2, 2013, and accepted for publication January 29, 2014.

*Correspondence: huyc@tsinghua.edu.cn

Yucheng Hu and Tianqi Zhu contributed equally to this work.

Editor: Rong Li.

0006-3495/14/03/0991/7 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.01.038

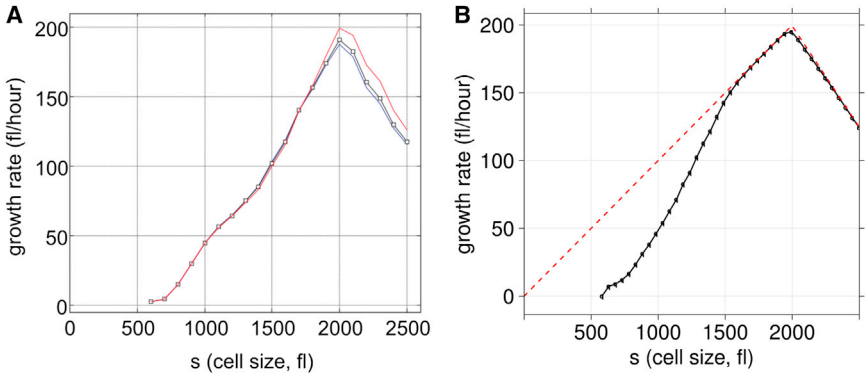

FIGURE 1 Cell-growth rate as a function of cell size. (*A*) Experimental result obtained using the Collins-Richmond method in Tzur et al. (13). Permission was obtained from the American Association for the Advancement of Science (AAAA, Washington, DC, www.aaas.org) to reuse this figure. Note that different curves correspond to different detailed implementations. (*B*) Averaged growth rate obtained from the in silico population simulated using our cell-growth model (*black curve*). Pure exponential growth ($v(s) = (\lambda_2 - \gamma_2)s$) for $0 \le s \le 2000$ and linear decay in growth rate ($v(s) = 500 - \gamma_2 s$) for $s \ge 2000$ (*Dashed-red curve*). To see this figure in color, go online.

to be proportional to the total number of ribosomes in the cell (ribosome is considered to be a representative of proteome). These notions allow us to rescale the units of protein mass and ribosome quantity so that 1fL of cell volume contains one unit of protein and one unit of ribosome. Here γ_2 denotes protein degradation rate per unit of protein mass.

The protein synthesis rate is assumed to be proportional to the total number of the cell's working ribosomes, which are specifically those that can allocate mRNA to carry out translation. In case there is a shortage of mRNA, some ribosomes become idle.

The unit of m, the mRNA level in a cell, is rescaled so that one unit of ribosome needs one unit of mRNA. In the rescaled units, the amount of working ribosomes in a cell equals to $\min\{m,s\}$ (m and s are treated as continuous variables). Thus, the protein synthesis rate is $\lambda_2 \min\{m,s\}$.

The mRNA has a degradation rate of γ_1 and an age-dependent production rate of

$$\lambda_1 (\kappa t)^\eta / (1 + (\kappa t)^\eta),$$

where t is the cell age, and η and κ are two parameters controlling the transcription rate of mRNAs.

Mathematically, the dynamics of s and m is given by

$$\frac{dm}{dt} = \frac{\lambda_1 (\kappa t)^\eta}{1 + (\kappa t)^\eta} - \gamma_1 m, \tag{1a}$$

$$\frac{ds}{dt} = [\lambda_2 \min\{m, s\} - \gamma_2 s]^+. \tag{1b}$$

The condition $[x]^+ = \max\{0,x\}$ keeps ds/dt nonnegative, due to the fact that the cell size does not shrink because constituting amino acids remain in the cell even if protein degradation occurs faster than synthesis. The Hill's function term allows mRNA level to saturate quickly after the cell is born.

A typical trajectory of the above system is shown in Fig. 2 *B* (see Methods for the parameter values). mRNA level is initially low in the newborn cell, because old mRNA degraded during mitosis and chromosomes need time to unfold before new mRNA can be transcribed. This phase is called Growth Stage I, in which insufficient mRNA supply limits protein synthesis. In Growth Stage II, mRNA level builds up quickly, allowing all ribosomes in the cell to work full time. Meanwhile, new ribosomes are produced at a rate proportional to the total amount of ribosomes currently available in the cell. As a result, cell size grows exponentially. Nevertheless, cells can only have limited mRNA supply due to limited DNA copy number. In Growth Stage III, if a cell keeps growing beyond a critical size, mRNA will become rate-limiting again and its growth rate will decrease as a result of increased protein decay due to the growing cell mass.

In addition to a model that specifies how each cell grows, we also need to know how and when a cell divides to simulate an evolving population of cells. Following Tzur et al. (13), we assume the size difference between two sibling daughter cells

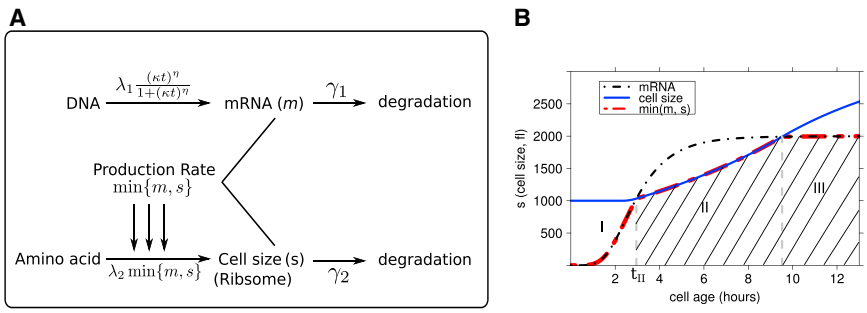

FIGURE 2 (*A*) A two-variable cell-growth model. Cell size is proportional to the number of ribosomes it contains. The decay rate per cell volume is γ_2 and the production rate is proportional to the amount of working ribosomes, $\lambda_2 \min\{m,s\}$. (*B*) Trajectory of mRNA and cell size simulated using Eqs. 1a and 1b. Initially, the mRNA level is set to zero. According to the relative abundance of mRNA and ribosomes, three growth stages can be identified in which mRNA and ribosomes play different roles in regulating cell growth (see main article). To see this figure in color, go online.