

Cellular Properties and Population Asymptotics in the Population Balance Equation

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Proliferating cell populations at steady-state growth often exhibit broad protein distributions with exponential tails. The sources of this variation and its universality are of much theoretical interest. Here we address the problem by asymptotic analysis of the population balance equation. We show that the steady-state distribution tail is determined by a combination of protein production and cell division and is insensitive to other model details. Under general conditions this tail is exponential with a dependence on parameters consistent with experiment. We discuss the conditions for this effect to be dominant over other sources of variation and the relation to experiments.

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Biological cell populations are diverse in their physiological properties, even if genetically identical. Since physiology rather than genetics ultimately carries biological function, there is much interest in understanding this aspect of biological variation. A good model system for this problem is a microorganism population that is genetically uniform and grows under uniform conditions; these systems have been studied for many years, and have recently received renewed attention following developments in experiment design and technique of single-cell measurements (reviewed by [1,2]). Experiments using fluorescence tagging combined with microscopy and cytometry have focused on variation in particular proteins inside cells, while theoretical studies have provided models of specific circuits and noise sources. Under steady-state growth conditions, several experiments have shown that even for regulated proteins, distribution shapes are insensitive to many details and are often observed to be broad with exponential tails [3,4]. This calls for a more physical

perspective of the problem, raising questions such as the universality of the resulting distributions. We here show that an exponential tailed distribution with the correct dependence on system parameters follows from a description involving a balance between deterministic protein production and dilution at cell division if these processes satisfy reasonable conditions. Such tails, reflecting variation in division time, are thus expected even if stochastic fluctuations in gene expression are negligible. The conditions for this effect to be dominant relative to noise in protein production are discussed.

A general theoretical framework for describing population distributions of quantities that obey a balance of growth and division, such as cell size or protein content, is the population balance equation (PBE) [5,6]. In its most general form it can incorporate many details and multiple internal cellular properties. We here focus on the case where the relevant physiological property of each cell can be described by a single variable x [7,8]:

$$\frac{\partial}{\partial t} f(t, x) + \frac{\partial}{\partial x} [g(x)f(t, x)] = -b(x)f(t, x) + 2 \int_0^1 \frac{d(p)}{p} b\left(\frac{x}{p}\right) f\left(t, \frac{x}{p}\right) dp - f(t, x) \int_0^\infty b(\xi) f(t, \xi) d\xi. \quad (1)$$

Here $f(t, x)$ is the probability density for the quantity x at time t in the population, and $g(x)$ is the individual growth rate of x . Cell division is assumed to follow a “sloppy control” mechanism [9]: $b(x)$ is the probability per unit time for a cell of quantity x to divide. Once division occurs, $d(p)$ is the probability for dividing into two daughter cells with fractions p and $1 - p$ of the mother cell. To obey mass conservation $d(p) = d(1 - p)$. The last term in the equation accounts for normalization. Underlying this model is the assumption that the growth process occurs gradually and with small fluctuations throughout the cell cycle, whereas division abruptly induces a large change in x .

A large body of previous work on this model is dedicated to theorems regarding the existence and uniqueness of solutions [10], numerical algorithms ([11] and referen-

ces there) and special case solutions [12]. Traditionally the coordinate x was interpreted as related to cell size (mass, linear dimension, etc.), and the dependence of the probability per unit time to divide on cell size reflects the combination of deterministic size-dependent and random aspects of cell division [9]. However, for our purpose of analyzing the asymptotic properties of the steady-state distributions, x can also be interpreted as the amount of a particular protein or molecule in the cell, any quantity which is produced and preserved at cell division. This follows because the probability per unit time to divide generally saturates for large values of cell size, age, or protein content, reflecting the inherent probabilistic component of the cell cycle [13]. This point, as well as the effect of an additional stochastic component in $g(x)$, will be further discussed below.

Our analysis begins by considering the steady-state solution of Eq. (1). Assuming such a solution exists, $f(t, x) = f(x)$ and the last integral becomes a constant, $\int_0^\infty b(x)f(t, x)dx = R$. This constant is the specific growth rate of the number of cells in balanced exponential growth, and can be viewed as a parameter in the equation. Therefore at steady state,

$$\frac{d}{dx}[g(x)f(x)] = -b(x)f(x) + 2 \int_0^1 \frac{d(p)}{p} b\left(\frac{x}{p}\right) f\left(\frac{x}{p}\right) dp - Rf(x). \quad (2)$$

Now consider the incoming flow contributing to the probability density at large x , where $f(x)$ is a decreasing function. It comes from two processes: growth, bringing cells of low x to a higher one; and division, breaking high- x cells into pairs of smaller x . If the probability density decreases rapidly enough, then for large x the first of these incoming flows is dominant over the second. We shall assume that this is the case for now, neglect the integral term representing the second flow in Eq. (2), and return to examine the consistency of this assumption later. One then obtains the following ordinary differential equation:

$$\frac{d}{dx}[g(x)f(x)] = -(b(x) + R)f(x) \quad (3)$$

with the solution

$$f(x) = C \exp\left(-\int^x \frac{b(\xi) + R + g'(\xi)}{g(\xi)} d\xi\right). \quad (4)$$

A related integral was found for the case of exactly symmetric division and a finite ranged variable [7]. Here we argue that in general under the assumption of a rapidly decreasing $f(x)$ the ratio between two points at the tail of the distribution is given by Eq. (4) with the limits of integration at the two points.

If x represents cell size, $g(x)$ is the growth function of the individual cell. Experiments directly measuring this function are not straightforward [14]; theoretical works have mostly assumed either linear or constant functions for simplicity. If x is interpreted as the amount of a protein, then a constant g represents a mean rate of protein production that is independent of the protein level. Assuming $g(x) = \gamma$ and a saturating probability per unit time to divide $b(x) \rightarrow b_0$ for large x ,

$$f(x) \sim e^{-\kappa x}, \quad \kappa = (b_0 + R)/\gamma \quad (5)$$

Returning now to the question of the validity of the naive approximation Eq. (4), a resulting exponential tail hints to consistency of the approximation since the function decreases rapidly. More precisely, we assumed that

$$2 \int_0^1 \frac{d(p)}{p} b\left(\frac{x}{p}\right) f\left(\frac{x}{p}\right) dp \ll -\frac{d}{dx}[g(x)f(x)]. \quad (6)$$

Substituting the above exponential one finds that this re-

quirement is satisfied by $x \gg d_{\max} \rho / \kappa^2$, where $d_{\max} = \max_p \{d(p)\}$ and $\rho = 2b_0/\gamma$; this defines the regions of consistency of the approximation.

The population balance equation Eq. (1) can be solved numerically ([11] and references there). We have developed a numerical procedure to solve the time-dependent equation on a semi-infinite range based on the method of time-evolution operators [7]. Figure 1 shows the steady-state solution with functions g, b that saturate at large x . As predicted by the argument above, the distributions exhibit exponential tails. Starting the dynamics from various initial conditions always relaxed to the same steady-state distribution. An exponential tail was found for all division functions $d(p)$, consistent with Eq. (5).

Using this observation, we proceed without much loss of generality to a more accurate asymptotic approximation for the case $d(p) = 1$. Assuming once again $g(x) = \gamma$ and $b(x) \rightarrow b_0$ for large x , Eq. (2) is equivalent, by a change of variables and an additional differentiation, to

$$\frac{d^2 f}{dx^2} + \kappa \frac{df}{dx} + \rho \frac{1}{x} f = 0. \quad (7)$$

$x = \infty$ is an irregular singular point of this equation [17]. Trying a solution $f(x) = \exp(\mu x) x^\lambda \eta(x)$ with $\lambda \in \mathcal{R}$ and $\eta(x)$ analytical at $x = \infty$, we obtain to leading order

$$f(x)_{x \rightarrow \infty} \sim C_1 x^{\rho/\kappa} e^{-\kappa x} + C_2 x^{-\rho/\kappa}. \quad (8)$$

Since Eq. (7) is of second order we have two independent solutions; however, as $0 < R \leq b_0$ it follows that $1 \leq \rho/\kappa < 2$ and hence the mean of the second solution diverges. This observation, while obviously not a proof of uniqueness, supports the numerical result of relaxation to a

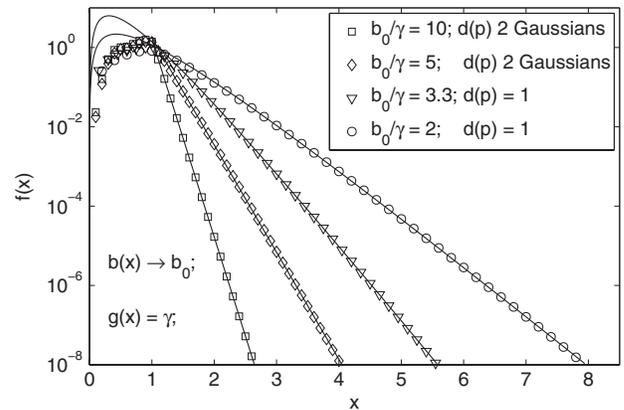


FIG. 1. Steady state population distributions with exponential tails. Numerical solution for constant growth $g(x) = \gamma$ and saturating probability of division per unit time. (\square, \diamond): $b(x) = b_0 H(x - \theta)$; $d(p)$ sum of two Gaussians at $p = 0.3$ and $p = 0.7$. This function describes asymmetric division, such as that observed for budding yeast cells. (∇, \circ): $b(x) = \frac{b_0}{2} [\tanh(k(x - \theta)) + 1]$ with $k = 5$, $\theta = 1$ and $d(p) = 1$. Asymptotic approximations [Eq. (8)] are shown by solid lines.

unique steady-state distribution from many initial conditions.

An exactly solvable case occurs when $b(x) = b_0$, then $f(x) = \kappa^2 x e^{-\kappa x}$. Here $R = b_0$, then $\kappa = \rho$ so the first asymptotic function in Eq. (8) is an exact solution; the second, $f(x) \sim x^{-1}$, is non-normalizable. The PBE here reduces to a model studied in [18], where protein is produced at a constant rate and cells divide with constant probability per unit time.

We thus establish that under general conditions the steady-state distribution exhibits an exponential tail, as has been observed in several experiments [3,4]. The exponential tail is obtained neglecting variation in the source g , and stems from a balance between the first-order kinetics of cell division and a constant or saturating deterministic source. The dependence of the exponent on parameters is such that upon increase of production, represented by g , the exponential tail broadens. This is consistent with experimental observations on protein production at steady state in populations of yeast cells [4], and inconsistent with most models that account for population variation by production noise.

Formally Eq. (4) indicates that the distribution tail is determined by the ratio of the growth and division functions, not by each of them separately. Thus, if for large x these functions do not saturate but have the same x dependence, an exponential tail will also arise. Figure 2 shows the numerical solution for linearly increasing $g(x)$, $b(x)$, supporting this prediction. While not immediately relevant to protein production, this result illustrates how exponential tails can arise by different growth and division functions maintaining constant ratio. It thus supports our analytic conclusion about how the combination of these functions shapes the distribution tails.

A growth, or production, function $g(x)$ that increases with x is relevant for several biological contexts. For example, if food uptake is related to the surface area of

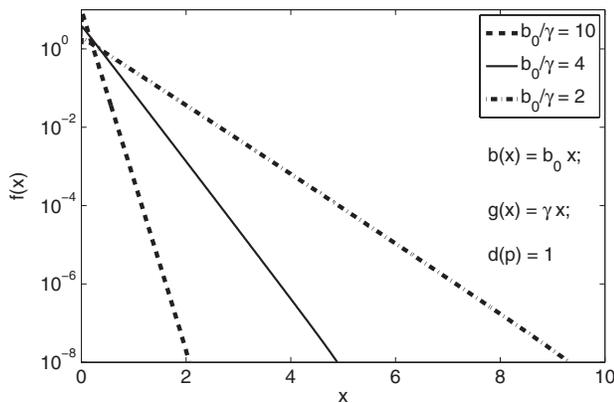


FIG. 2. Steady state distributions for $g(x) = \gamma x$, $b(x) = b_0 x$ and $d(p) = 1$. This example shows that the distribution tail is determined by the ratio between $b(x)$ to $g(x)$ and not by each function separately.

the organism and x is a linear dimension, then growth is an increasing function of x [7]. For $g(x) = \gamma x$ one can show that $R = \gamma$ and therefore $\kappa = \rho/2 + 1$. Using the same procedure as before to write an equivalent ordinary differential equation for $b(x)$ saturating to b_0 at large x and $d(p) = 1$, we find

$$\frac{d^2 f}{dx^2} + [\rho/2 + 3] \frac{1}{x} \frac{df}{dx} + \rho \frac{1}{x^2} f = 0. \quad (9)$$

This is the Euler equation [17] with power-law solutions $f(x) = Cx^\alpha$ where $\alpha = -\rho/2, -2$. Of the two independent solutions to the asymptotic equation, only $\alpha = -\rho/2$ with $\rho > 4$ is consistent with $f(x)$ being a probability density with a finite mean. Indeed, numerical simulations in this parameter regime always relax to a steady state with a tail $f(x) \sim x^{-\rho/2} = x^{-b_0/\gamma}$; see Fig. 3 for a comparison between the numerical solution and the asymptotic tail.

The special case of $b(x) = b_0 H(x - \theta)$, where H is the Heaviside function with threshold θ , is exactly solvable. Here the Euler equation (9) holds exactly in the region $x > \theta$. By continuity and normalization requirements one can show that the coefficient of the solution with $\alpha = -2$ vanishes, and the unique solution is

$$f(x) = \begin{cases} (1 - 2/\rho) \frac{1}{\theta} & x \leq \theta \\ (1 - 2/\rho) \theta^{\rho/2 - 1} x^{-\rho/2} & x > \theta \end{cases}. \quad (10)$$

Once again, this solution is valid for $\rho > 4$ ($b_0 > 2\gamma$). Note that the naive argument leading to Eq. (4) is self-consistent in this case only for a more severely limited region of parameters ($b_0 \gg 3\gamma$).

In summary, we used the population balance equation to study the interplay between intracellular and population processes in shaping the steady-state distribution in a dividing cell population. The novel component in our approach is to consider the variable x describing the cell state as unbounded and to focus on the asymptotic prop-

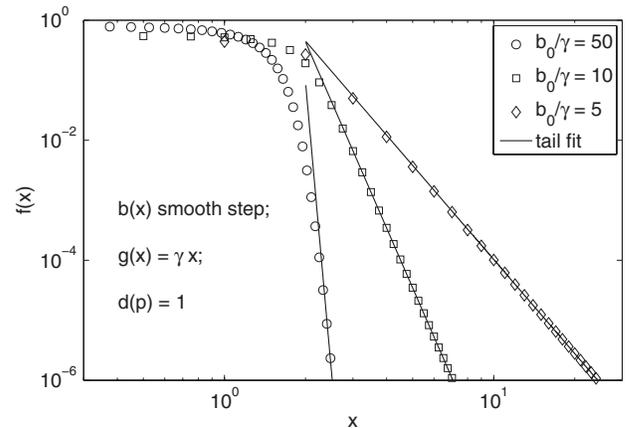


FIG. 3. Steady state distributions for $g(x) = \gamma x$, $b(x) = \frac{b_0}{2} \times [\tanh(k(x - \theta)) + 1]$ with $k = 2$, $\theta = 2$ and $d(p) = 1$. Numerical solutions (symbols) are shown with asymptotic approximation to the tail (lines).

erties of its distribution. This enables us to extend the interpretation of x as a particular protein or molecule in the cell, since asymptotically the probability per unit time to divide becomes independent of the variable, $b(x) \rightarrow b_0$ for large x . This probabilistic component of the cell cycle is a well-established property for many cell types [9,13].

We have shown that generally the functional forms of mean growth or production $g(x)$ and probability per unit time to divide $b(x)$ determine the tail of the distribution through a particular combination, Eq. (4). Because the PBE takes into account the kinetics of cell division as a discrete process, randomness in the timing of cell division is sufficient to yield an exponentially tailed distribution at steady state. In reality, the single-cell function $g(x)$ itself has a stochastic component, and this can be added to the model using the diffusion approximation. Such an extension will be a good approximation if $\frac{\delta g^2}{\langle g \rangle^2} \ll \frac{1}{b_0 + R}$.

At the other extreme, if internal stochasticity is dominant, it should be modeled in detail. For example, previous work has shown that bursts in mRNA production cause an exponential distribution of protein produced in each cell, which in turn is reflected as exponential tails in the population distribution [19–21]. Division can then be assumed synchronous with symmetric binomial distribution [19,20], or it can be altogether neglected and described as a continuous dissipative process [21], without changing the result. The validity of each regime depends on the relative variation of the two processes, production and division, and on their relative time scales. One way to identify the regime in experiment is the dependence of the exponential tail on parameters: if the tail results from microscopic effects, then a larger mean production results in relatively narrower distributions and the slope of the tail remains intact. However, if the exponent results from a combination of sloppy division and deterministic production as suggested here, then larger mean production results in a broader exponential tail. Experiments on yeast populations have shown that increasing the mean protein production, either by an increase in the number of promoters or by adding inducing agents, increases the mean and at the same time broadens the exponential tail [4]. This dependence suggests that it is the population effects, rather than microscopic noise, which govern the distribution tails in these experiments.

In any interpretation of x , our results predict that the distribution tails will be insensitive to the division function $d(p)$. This is supported by the universality of protein distribution tails in yeast cells grown under various steady-state conditions [4]. Yeast cells divide asymmetrically, with the degree of asymmetry depending on growth rate and environment [9]. The observation that under all growth conditions the protein distribution exhibited exponential tails is consistent with our prediction. Moreover, unpublished results on bacteria populations grown at

steady state [22] show that even this symmetrically dividing organism exhibits similar exponential tails.

Taken together, our results suggest that exponential tails in the distribution of an abundant protein in a dividing population may be a much more universal feature than previously thought, since they reflect fundamental properties of randomness in cell division times and not necessarily the particular microscopic details of protein production circuits.

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