On the Distribution of State Values of Reproducing Cells

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Abstract. Characterizing cell state by measuring the degree of gene expression as well as its noise has gathered much attention. The distribution of such state values (e.g., abundances of some proteins) over cells has been is measured, which is not only a result of intracellular process, but is also influenced by the growth in cell number that depends on the state. By incorporating the growth-death process into the standard Fokker–Planck equation, a nonlinear temporal evolution equation of distribution is derived and then solved by means of eigenfunction expansions. This general formalism is applied to the linear relaxation case. First, when the growth rate of a cell increases linearly with the state value x, the shift of the average x due to the growth effect is shown to be proportional to the variance of x and the relaxation time, similarly with the biological fluctuation- response relationship. Second, when there is a threshold value of x for growth, existence of critical growth rate, represented again by the variance and the relaxation time, is demonstrated. The relevance of the results to the analysis of biological data on the distribution of cell states, as obtained for example by flow cytometry, is discussed.

1. Introduction

Biological systems suffer fluctuations. No intracellular biochemical process can avoid fluctuations, because they arise from the motion and reaction of molecules. For example, abundance of mRNAs and proteins in a cell fluctuate in time or by cells, even if they are measured at the same time after a cell division, for cells with identical genes (clones). Indeed, Elowitz has explicitly measured the numeric fluctuations of proteins in Escherichia coli by distinguishing intrinsic and extrinsic fluctuations [1]. Such intracellular fluctuations have attracted both theoretical and experimental attention [2, 3, 4, 5, 6, 7], while the significance of the phenotypic fluctuations for adaptation [8] and evolution [9, 10] has also been investigated.

In general, let us consider the fluctuation of some quantity x characterizing the state of a cell, such as the number of proteins. Now, as a result of intra-cellular dynamics, x fluctuates among cells or in time. Let us denote the single-cell distribution of x by $P_{single}(x)$. In principle, it can be obtained by repeating a single-cell measurement over an ensemble of cells.

Here, however, we must be careful about the choice of the initial ensemble itself for such distributions. The initial distribution of cells chosen for an experiment depends on whether the cell can proliferate or not and the speed of cell replication, which may depend on the cell state x. Consider, for example, taking an ensemble of cells from a culture. Then the probability of choosing cells that have higher replication speeds will be larger, and the initial distribution of x will be biased accordingly.

This problem is prominent in the measurement of cells from continuous cultures using flow cytometry or some other means [11]. In flow cytometry, the characteristics of each cell (e.g., the magnitude of fluorescence when a fluorescent protein gene is introduced) are measured over a huge number of cells. It is now established as a standard, powerful tool to measure the distribution of states of cells. Here, if the growth rate of a cell is independent of the quantity x, the choice of cell ensemble is not biased by the value x, and thus the observed distribution $P(x)$ by flow cytometry is simply that given by the distribution $P_{single}(x)$. On the other hand, if the growth rate depends on x, the distribution $P(x)$ may be altered from the distribution from single-cell dynamics.

As an illustration, consider the case in which $P_{single}(x)$ is a Gaussian distribution around $x = x_0$, while the replication rate of a cell increases strongly with x for $x > x_0$, assuming that x represents the abundance of some chemical that mediates the growth of the cell. In this case, it is naturally expected that the observed distribution $P(x)$ should be biased towards $x > x_0$.

In general, the distribution $P_{single}(x, t)$ has been studied with the use of stochastic processes to characterize the intra-cellular dynamics of the state x. Established mathematical tools such as Master's equation, Langeving's equation, and the Fokker–Planck equation [12, 13] are applied for such studies. On the other hand, as a biological unit (cell) replicates, the number of cells increases accordingly. This effect of replication must be incorporated with these stochastic processes to include both the single-cell

fluctuations and the growth dynamics of the cells.

Recently, there has been growing interest in exploring the relationship between the fluctuations of the intracellular state and the response of the state to the change in external conditions, both theoretically and experimentally [1, 6, 14, 15, 16]. For example, a change in the concentration of some protein against the change in the external condition (e.g., concentration of some chemical in the medium) may be measured experimentally, from which the response of such an intracellular state to the environmental change may be discerned. Here, however, the growth speed of a cell generally depends on the intracellular state, e.g., the abundance of such protein, because the protein is important to the function of the cell. Hence, the measured change of the protein concentration in response to external change involves both the internal change of the intracellular state and the change in the cell number distribution caused by the state-dependent growth rate. Thus, we should develop a theoretical tool to distinguish the two effects, based on the measurable quantities. In the present paper, by setting up an equation for $P(x, t)$ that takes into account both the intra-cellular stochastic process and the state-dependent cell reproduction rate, we address this issue.

We first derive the evolution equation of the distribution $P(x, t)$ by extending the Fokker–Planck equation to incorporate state-dependent growth. (In the present paper, 'growth' means the replication of a cell, and the replication rate in time is called the growth rate). The derived equation includes a term for state-dependent growth, from which is subtracted the average growth rate over all cells, leading to a source/sink term that corresponds to the growth/death process of a cell. The average growth rate gives a self-consistent term that is nonlinear in distribution $P(x, t)$, but we can formally solve the equation through the eigenvalue properties of a Sturm–Liouville-type operator. After giving a general formulation of the equation, we present two simple examples of this formulation, by assuming the linear Langevin equation for the single-cell dynamics of the state variable. First, by considering the linear dependence of the growth speed on x, we obtain a formula for the shift of the average value of the state x. The shift is proportional to the product of the variance of the state, the relaxation time, and the proportion coefficient of the growth speed with x. For our second example, we study the case in which there is a threshold value of the state x for growth, and derive a formula for the change of $P(x, t)$ to 'feel' the state-dependent growth. Cautious remarks are made on the interpretation of the distribution obtained from flow cytometry, while the relevance of our theory to evolution is also briefly discussed.

Note that we do not discuss specific mechanisms for the cell growth here. Rather, we introduce a function characterizing state-dependence growth generally and derive the distribution function.

2. Derivation of the equation for the distribution of cell state with reproduction

Let us first introduce a variable, x, which represents a state value of a cell, for example, a concentration of some chemical (or its deviation from the mean value). We assume that the temporal evolution of variable x in a single cell obeys some Markovian dynamics, that is, the value of x at time t is determined only by the value of x at some previous time. (Although biological systems may often retain some memory, this assumption can be acceptable as a first-step approximation, and indeed is adopted for most models.) Based on this assumption, we consider the following Langevin equation,

$$\frac{dx(t)}{dt} = -f(x(t)) + \sqrt{g(x(t))}\,\eta(t),$$ (1)

where the function f represents the force acting on the value toward its mean value and $g(\geq 0)$ represents the strength of the diffusion at the value, and $\eta(t)$ is a Gaussian white noise term having the statistical properties: $\langle \eta(t) \rangle = 0$ for any t and $\langle \eta(t_1)\eta(t_2) \rangle = 2\delta(t_1 - t_2)$ for any t_1 and t_2. The distribution function $P_{single}(x,t)$ indeed obeys the Fokker–Planck equation‡ derived from the Langevin equation (1) [12, 13, 26]

$$\frac{\partial P_{single}(x,t)}{\partial t} = \frac{\partial}{\partial x}\left[f(x) + \frac{\partial}{\partial x}g(x)\right]P_{single}(x,t).$$ (2)

We now introduce the growth (replication) of the cell, whose rate μ is dependent on the state value of x in the cell, and is a function of $x(t)$, denoted by $\mu(x(t))$. To derive the equation for the distribution $P(x,t)$ for this growth rate of the cell, we first recall that the distribution function at time $t + \Delta t$ is expanded with the rate $(1 + \mu(x)\Delta t)$ due to the cell growth, while the distribution thus obtained (at $t + \Delta t$) is not normalized by itself, so that we need to normalize it. After straightforward calculation§, we obtain

$$\frac{\partial P(x,t)}{\partial t} = (\mu(x) - \bar{\mu}(t))P(x,t) + \frac{\partial}{\partial x}\left[f(x) + \frac{\partial}{\partial x}g(x)\right]P(x,t).$$ (3)

Here $\bar{\mu}$ is defined by

$$\bar{\mu}(t) = \int_{x_1}^{x_2} \mu(x)P(x,t)dx,$$ (4)

which gives the mean growth rate of the cells at time t.

If $\mu(x) = $ constant, i.e., for x-independent cell growth, the first term in equation (3), $(\mu(x(t)) - \bar{\mu}(t))P(x,t)$, vanishes and accordingly equation (3) is reduced to just the usual Fokker–Planck equation (2); the influence of the state-dependent cell-growth

‡ Here we have adopted Ito calculus; for Stratonovich calculus, one can simply replace f by $f - \frac{1}{2}\frac{dg}{dx}$ in equation (3). If g is constant, there is no difference.

§ By introducing $W(x, x', \Delta t)$ as the transition probability that the system changes from the state with x' to that with x during the time interval Δt, the distribution function at $t + \Delta t$ is written as $P(x, t + \Delta t) = \frac{\int_{x_1}^{x_2} dx'\, W(x,x',\Delta t)P(x',t)(1+\mu(x')\Delta t)}{\int_{x_1}^{x_2} dx \int_{x_1}^{x_2} dx'\, W(x,x',\Delta t)P(x',t)(1+\mu(x')\Delta t)} \simeq (\mu(x) - \bar{\mu}(t))P(x,t)\Delta t + \int_{x_1}^{x_2} W(x,x',\Delta t)P(x',t)dx'$, where we have used the property of the transition probability, $\int_{x_1}^{x_2} W(x,x',\Delta t)dx = 1$ for any x' and any Δt.

appears only in the term $(\mu(x(t)) - \bar{\mu}(t))P(x,t)$, which plays the role of source (sink) in the distribution density, if the growth rate at some point x is greater (smaller) than the mean growth rate, $\bar{\mu}$. This term is essentially a continuous-state version in the evolution equation by Eigen et al [17].

As in the standard Fokker–Planck equation for the probability, we take the no-flux boundary condition as:

$$\left[f(x) + \frac{\partial}{\partial x} g(x) \right] P(x,t) \Bigg|_{x=x_1, x_2} = 0. \tag{5}$$

3. Analysis of the evolution equation of the distribution with growth

Equation (3) obtained in the last section is nonlinear in P because the term $\mu(t)$ involves P itself, so that it first looks rather difficult to analyze. Fortunately, however, the analysis turns out not to be so difficult, with the aid of linear operators and eigenvalues. We first introduce a linear operator

$$L = \mu(x) + \frac{\partial}{\partial x} \left[f(x) + \frac{\partial}{\partial x} g(x) \right] \tag{6}$$

and rewrite equation (3) as

$$\frac{\partial P(x,t)}{\partial t} = -\bar{\mu}(t)P(x,t) + L(x)P(x,t). \tag{7}$$

As the operator L is of the Sturm–Liouville type, we can, in principle, find all of its eigenvalues and corresponding eigenfunctions, and all the eigenvalues are real [18]. We denote the eigenvalues and the corresponding eigenfunctions by λ_i and $\phi_i(x)$, where the index i runs over the non-negative integers, $i = 0, 1, 2, ..$, and the eigenvalues are ordered so that $\lambda_i \geq \lambda_j$ for $i < j$. From the definition, λ_i and $\phi_i(x)$ satisfy the relation

$$L(x)\phi_i(x) = \lambda_i \phi_i(x). \tag{8}$$

By expanding $P(x,t)$ in terms of these eigenfunctions as

$$P(x,t) = \sum_{j=0}^{\infty} a_j(t)\phi_j(x), \tag{9}$$

we obtain (see Appendix A)

$$\frac{da_i(t)}{dt} = \left(\lambda_i - \sum_{j=0}^{\infty} \lambda_j a_j(t) \int_{x_1}^{x_2} \phi_i(x)dx \right) a_i(t) \tag{10}$$

Hence, the partial differential equation (3) for P is reduced to a set of ordinary differential equations for $\{a_i\}$, while the initial conditions of a_i are given from $P(x, t_0)$. By further fixing a normalization factor for $\phi_i(x)$ properly (see Appendix A), equation (10) is simplified as

$$\frac{da_i(t)}{dt} = \left(\lambda_i - \sum_{j=0}^{\infty} {}' \lambda_j a_j(t) \right) a_i(t), \tag{11}$$

where the prime over the summation symbol indicates that the summation is taken over all eigenfunctions except non-contributing ones.

Equation (11) tells us that any eigenfunction $\phi_i(x)$ of the linear operator L, except for the non-contributing ones, gives a stationary solution of equation (3), because any set $\{a_i(t) = 1 \text{ and } a_j(t) = 0 \text{ for } j \neq i\}$ is a stationary solution of (10). Among those stationary solutions, however, only the solution with $a_j(t) = \delta_{j,0}$, is stable, as shown in Appendix A. Indeed, by recalling that the eigenvalues are ordered so that $\lambda_i > \lambda_j$ for $i < j$, all the stationary solutions for $k > 0$ are shown to be unstable, while if $\lambda_0 > 0$ the solution with $k = 0$ (i.e., with $a_i = \delta_{i0}$) is stable. In other words, only the mode with the largest growth rate remains as a stationary solution, as is expected.

The requirement $\lambda_0 > 0$ for the stability of the system is quite reasonable. Otherwise, all λ_j are negative, which means there is no growth at any state, and all the cells would become extinct with time (recall that λ_i is equal to the growth rate of the mode represented by the ith eigenfunction). To have a positive growth rate for the stationary distribution, $\lambda_0 > 0$ is therefore necessary. The condition $\lambda_0 > 0$ simply means that the cells (or units) continue reproduction without extinction.

Now, the stationary solution of equation (3) is given by $\phi_0(x)$, the eigenfunction of the operator L corresponding to the maximal eigenvalue λ_0. Similarly to the case of the standard Fokker–Planck equation, the eigenvalue problem of the operator L can be transformed into that for the Schrödinger-type equation whose "potential" is given by the functions $f(x)$, $g(x)$, and $\mu(x)$ (see Appendix B). Hence we can use the methods and solutions developed in quantum mechanics.

4. Two simple examples of the evolution of the distribution

In this section we study two simple examples of equation (3) by linear or threshold-type dependence of the growth rate on x. We choose $f(x) = kx$ and $g(x) = D$ in equation (3) with k and D positive constants. The reasons for this choice are: (i) that the Gaussian distribution is often observed to be the stationary distribution of a biological state, while this linear Langevin equation is the simplest to realize the Gaussian distribution (the log-normal distribution is sometimes observed in cells [5, 9, 19], but in this case we can simply use the logarithm of the quantity as the variable x that concerns us), and (ii) that this linear Langevin equation has been thoroughly investigated in physics and mathematics; it models the motion of a Brownian particle in a harmonic potential, so that we can easily see the effect of the state-dependent growth introduced here.

4.1. $\mu(x)$ linearly dependent on x

We study the case $\mu(x) = ax + b$ for x to $[-\infty, \infty]$ in equation (3), where a and b are constants. It is natural to study the linear case as the simplest non-trivial example. Indeed, as long as the range of x in concern is small, gradual change in $\mu(x)$ can be approximated by linear change.

In this case, we can obtain all eigenvalues and their corresponding eigenfunctions of L as $\lambda_n = \frac{Da^2}{k^2} + b - kn$ and $\phi_n(x) = N_n H_n(\sqrt{\frac{k}{2D}}(x - \frac{2Da}{k^2})) \exp[-\frac{k}{4D}(x - \frac{2Da}{k^2})^2 - \frac{k}{4D}x^2]$, where $H_n(x)$ is the nth Hermite polynomial in x and N_n is the normalization constant determined by the normalization condition (A.2). In particular, the stationary distribution is obtained directly as

$$\phi_0(x) = N_0 \exp[-\frac{k}{2D}(x - \frac{Da}{k^2})^2], \tag{12}$$

while the temporal evolution of the distribution is obtained with these eigenvalues and eigenfunctions and with the reduced equations (11) for $\{a_i\}$.

Fortunately, however, in this case there is a more convenient way to obtain the dynamics of the system: if the system starts with a Gaussian distribution at some initial time, the temporal evolution of the distribution preserves the Gaussian form. By taking a Gaussian distribution $P(x, t) = \frac{1}{\sqrt{2\pi\beta(t)}}e^{-\frac{(x-\alpha(t))^2}{2\beta(t)}}$ with α and β as the mean value and the variance, it can be shown (see Appendix C), that the temporal evolution preserves the Gaussian form when the time evolution equations for α and β are given by $\frac{d\alpha(t)}{dt} = a\beta(t) - k\alpha(t)$ and $\frac{d\beta(t)}{dt} = -2k\beta(t) + 2D$.

These equations indicate that while the temporal evolution of the variance is completely the same as the case for a constant μ, the evolution of the mean value is influenced by the state-dependent growth; the mean value is shifted in the direction of larger μ, driven by its variance. In the stationary state, as is also given in equation (12), the mean value (peak position) shifts with the degree aD/k^2 compared with the case without the growth term (or, from the case with constant μ (i.e., $a = 0$)). Note that this change in the mean value in the stationary state is proportional to the variance of the original distribution, which is given by D/k, i.e.,

$$\Delta x = \frac{aD}{k^2} = \frac{a}{k}\left\langle (\delta x)^2 \right\rangle, \tag{13}$$

where $\langle ... \rangle$ is the average of the stationary distribution $P(x)$, and $\delta x = x - \langle x \rangle$.

In other words, the larger the variance of the distribution is, the more the mean value shifts. Correspondence with the fluctuation–response relationship [20, 9] is interesting, because the shift in the growth is proportional to the original fluctuation. In addition, response to a higher growth state is possible only under the fluctuation of the state, which demonstrates the relevance of phenotypic fluctuation to adaptation. With this shift of Δx, the average growth rate of a cell changes with

$$\Delta\overline{\mu} = a\Delta x, \tag{14}$$

which is an experimentally measurable quantity. Hence, the right hand side of equation (13) is represented by measurable quantities, because k is simply the relaxation time, a is estimated from equation (14) and the variance $\langle (\delta x)^2 \rangle$ is measurable.

4.2. A threshold for growth: the step function $\mu(x)$

We consider equation (3) with $\mu(x) = a, \Theta(x - x_0) + b$, where a, b, and x_0 are constants, and Θ is the so-called Heaviside step function; $\Theta(x) = 0$ for $x < 0$ and $\Theta(x) = 1$ for

$x \geq 0$. We study this case because, in biological systems, a threshold for reproduction sometimes exists.

In this case, the eigenfunctions are written analytically with the use of confluent geometric series and the corresponding eigenvalues are obtained by transforming the equation to the Schrödinger equation (see Appendix B). Because the complete analytic form is rather complicated, we discuss only the results of numerical calculations here.

First, we consider the stationary distribution of equation (3). When the position x_0 of the step of $\mu(x)$ is within the standard deviation of $P_{single}(x)$, i.e., $0 \leq x_0 < \sqrt{\frac{D}{k}}$ (we consider only the case of non-negative x_0), the stationary distribution gradually moves toward the position x_0, as the parameter a increases. On the other hand, when the position x_0 is outside the standard deviation of $P_{single}(x)$, i.e., $x_0 > \sqrt{\frac{D}{k}}$, the stationary distribution does not change much until the parameter a reaches some critical value a_c. As a increases beyond that value, the distribution shifts smoothly to larger x. The existence of the critical value a_c is demonstrated in figure (1), which is a plot of the total amount of the distribution in region $x > x_0$ against the relative growth rate a (see figure (2)).

The critical value of a_c is estimated to be $a_c \simeq k x_0 \sqrt{\frac{k}{D}}$, as is confirmed numerically (see inset of Fig (1)). Indeed, this value of a_c coincides with the inverse of some characteristic time that is the average time required for a cell in a higher-growth state $(x > x_0)$ to change to the lower-growth state $(x < x_0)$. This numerical result is reasonable: if the relative growth rate a is smaller than a_c, cells change to the state $x < x_0$ before they grow sufficiently in the higher-growth region $x > x_0$. The cells cannot 'feel' the higher-growth region, so that the difference in growth rates does not influence the cell population distribution.

Next, we briefly explain the dynamic behavior of the distribution when the relative growth rate is greater than a_c and the distribution is initially localized at $x < x_0$. To be specific, we set $P(x, t_0) = \delta(x)$, i.e., localized at $x = 0$. The temporal evolution of the distribution is given in figure (3). Here: (i) first, the distribution behaves as if it does not 'feel' the state-dependence of $\mu(x)$ until its tail touches x_0, the edge of the step function. (ii) After the tail of the distribution reaches the edge of the step function, the distribution in this tail region starts to grow faster (see figure (3)); at this stage, the distribution has two peaks. (iii) Finally, the distribution converges to a single peak at the mean value, around x_0, the position of the step of $\mu(x)$. This temporal evolution to a higher growth state is in contrast to the linear case, where a single-peak distribution is preserved and only the peak position is shifted.

In the present example, the stationary distribution has a single peak. For some forms of $f(x)$, however, the stationary distribution has two peaks, even though the single cell distribution (without the x dependence of $\mu(x)$) has a single peak. For example, for $f(x) = 2\text{sgn}(x)$ with the present form of $\mu(x)$, two peaks coexist (see figure (4)). Here, for large x $(x > x_0(= 4))$, the growth rate is high and the distribution is confined within some range, so that the distribution has one peak in that region, while for small x $(x < x_0)$, not all cells grow, so that the distribution of the cells tends to

decrease. However, many cells that have grown in the higher-growth region flow into the lower-growth region because of the effect of the force of f, so that the distribution has another peak there.

5. Conclusion and Outlook

In the present paper we have posed the question of how the distribution of an intracellular state variable (say the abundances of some chemical or degree of gene expression) is altered due to the state dependence of the replication rate of a cell. To discuss the temporal evolution of the distribution of the internal state x of such replication units, we have incorporated the state-dependent growth rate into the standard Fokker–Planck equation. By considering the population distribution of replication units with Langevin equation dynamics, we have derived a general equation for the temporal evolution of the distribution of states $P(x, t)$. The derived equation includes a self-consistent term arising from the growth rate. In spite of this non-linear term, we can formally solve the equation as an eigenvalue problem of the Sturm–Liouville type. Note that the formalism presented here is rather general, as is the Fokker–Planck equation.

After giving a general analysis of the equation, we have studied two simple examples, assuming the linear Langevin equation for single-cellular dynamics. First, when the growth rate increases linearly with the state value x, the average of x over cells increases in proportion to its variance, which reminds us of the fluctuation-response relationship in physics, while the proportion coefficient is estimated by the increase of the growth rate and the relaxation time. Note that the shift of population distribution to a higher growth state is possible only with the fluctuation of the internal state. Our result implies that the response of x to environmental change is proportional to its variance. In other words, fluctuations in chemical concentration, which have been studied extensively, are relevant to biological adaptation.

Now let us return to the question raised in the introduction. We measure an intracellular state variable $\langle x \rangle$ from an ensemble of cells, and study its change against the change in external conditions. Here we change the environmental condition (e.g., nutrient concentration) and the cell state value x (e.g., the concentration of some enzyme) is changed accordingly. After the cell distribution becomes stationary, we can measure this change of the average x denoted by $\langle \Delta x \rangle_{total}$ that is caused by the change in the environmental condition. Now, from this measurement, we are often interested in detecting the change in the stationary state of x, to explore intracellular dynamics. However, such an intracellular state variable x is often also related to the ability for cell growth. Hence $\langle \Delta x \rangle_{total}$ is also influenced by the change in cell growth speed, and this may deviate from the change caused by the intracellular dynamics $\langle \Delta x \rangle_{single}$. Then, can we estimate the change of the internal state $\langle \Delta x \rangle_{single}$ from the measurement of $\langle \Delta x \rangle_{total}$? If we confine our discussion only to the linear regime, we find

$$\langle \Delta x \rangle_{total} = \langle \Delta x \rangle_{single} + \frac{a}{k} \left\langle (\delta x)^2 \right\rangle . \tag{15}$$

from equation (13). Here the latter term can be estimated from the standard measurements. First, through equation (14), a can be estimated from the change in the average growth rate of cells. Second, k is simply the relaxation time. Hence, by measuring the temporal change of $\langle x(t) \rangle$, and by fitting the approach to its stationary value by an exponential form, one can estimate k. Finally, from the variance of the state value x at a stationary state (by flow cytometry or by other means), we can obtain $\langle (\delta x)^2 \rangle$. Accordingly, we can estimate the term $\frac{a}{k} \langle (\delta x)^2 \rangle$, so that the intracellular change of x is estimated from the observable quantity $\langle \Delta x \rangle_{tot}$.

In our second example, we studied the case with a threshold-type dependence of the growth rate on the state x. When the position x_0 of the step of $\mu(x)$ is outside the standard deviation of $P_{single}(x)$, i.e., when $x_0 > \sqrt{\frac{D}{k}}$, the distribution does not change significantly until the relative growth rate a reaches a critical value a_c, beyond which the distribution starts to shift to the higher-growth region. From the biophysical viewpoint, the value a_c corresponds to the inverse of the average time required for a cell to change from the higher-growth state $(x > x_0)$ to the lower-growth state$(x < x_0)$.

Here we have found that the distribution of the state variable often exhibits double peaks over a long transient time. For some form of $f(x)$ and $\mu(x)$, a double-peak stationary distribution is also obtained, even if $P_{single}(x)$ has only a single peak. This raises a cautious remark on the interpretation of the distribution observed in flow cytometry. Even if double peaks are observed, this does not necessarily mean that the internal cell dynamics (e.g., gene expression network dynamics or metabolic dynamics) have bistable states. One of the peaks may be associated with the flow of population due to the difference in reproduction speeds.

In general, the growth rate of a cell depends on the degree of some gene expression, while there are fluctuations in it. Hence our theory will be relevant to in the study of distribution of gene expression in relationship with the growth rate (see e.g., Banerjee et al.[21]). For example, consider the growth of E.coli, in the medium lacking glutamine but having glutamate[22]. In this case E. coli are able to synthesize glutamine from glutamate with the aid of glutamine synthetase (abbreviated as "GS" hereafter). As the glutamine is essential to the growth, the growth rate of the E. coli depends on the degree of gene expression of GS, denoted by x. Indeed, Suzuki et al.[22] measured the distribution of x, with the aid of fluorescent protein and flow-cytometry, to examine how it changes with the environmental condition. The change of average x and the variance $\langle (\delta x)^2 \rangle$ are thus obtained. As the growth rate and relaxation time scale are also measured, it will be possible for us to examine the relationship between fluctuation and response.

Dependence of bacterial growth rate on its phenotypic state has also been studied in "bacterial persistence"[24, 23], where the bacterium switches its state between a usual state and a dormant state stochastically. Although the population distribution of these phenotype states is analyzed by a discrete-state model[25], it will also be important to study a continuous-state version, as given in our formulation, since the degree of gene expression is generally continuous.

Several extensions of the present formulation are straightforward. Although we mainly discussed the case with a single state variable, extension to a higher-dimensional case is straightforward. Inclusion of a memory term to go beyond Markovian dynamics will be possible, although we expect that most of the results on the linear and step-function cases above are still valid in the non-Markovian case.

Although we have given our formulation here for a reproducing cell with an internal state (e.g., chemical concentration), the present formulation can be applied generally to any reproducing system with a growth rate dependent on its internal state. For example, it can be applied to an artificial cell or a replicating biochemical system with a growth rate that depends on its internal catalytic activity. Furthermore, application to continuous evolution is possible. By taking x as a Hamming distance from a typical gene, the evolution process to change x to a given phenotype having some function can be considered. Here, the reproduction rate depends on x, which gives $\mu(x)$, while the diffusion process in x is simply the mutation, with D as the mutation rate. As non-functional mutants are more common, the mutation in the change of function (or activity) has a drift to a smaller regime, leading to a 'force' term towards $x = 0$ as in equation(1). The temporal evolution of the distribution of gene x is thus analyzed by using our equation (3), while in some examples, the steady state with positive growth rate collapses [10], with the increase of the mutation rate, as the largest growth speed λ_0 becomes negative, which leads to error catastrophe.

A biological unit reproduces at a rate that depends on its state. The present Fokker–Planck equation with growth and death provides a basic equation for such problems in general.

Acknowledgments

Appendix A. Eigenfunction analysis

Corresponding to equation(8), we can generally introduce the adjoint operator of L, denoted by $L^\dagger$, and introduce the "left" eigenfunctions of $L^\dagger$ denoted by $\psi_i(x)$, for the eigenvalue λ_i. As is well known, left and right eigenfunctions for different eigenvalues are orthogonal and can be normalized as $\int_{x_1}^{x_2} \psi_i(x)\phi_j(x)dx = \delta_{ij}$, where δ_{ij} is the Kronecker delta ($\delta_{ij} = 0$ for $i \neq j$ and $\delta_{ij} = 1$ for $i = j$). With these relationships, we can expand $P(x,t)$ in terms of the eigenfunctions, as given by equation (9), while the initial condition for $a_i(t)$ is given by $a_i(t_0) = \int_{x_1}^{x_2} \psi_i(x)P(x,t_0)dx$ for the initial time t_0.

Next, we will express $\bar{\mu}(t)$ in terms of $\{a_i\}$ and $\{\lambda_i\}$. From the definition (4) of $\bar{\mu}$,

$$\bar{\mu}(t) = \int_{x_1}^{x_2} \mu(x)P(x,t)dx = \sum_{i=0}^{\infty} \lambda_i a_i(t) \int_{x_1}^{x_2} \phi_i(x)dx, \qquad (A.1)$$

where we have used the relation (6), the boundary conditions (5), and the relations (8) and (9), successively. Then, the time evolution equation for $a_i(t)$ is straightforwardly

obtained by inserting (9) into (7), multiplying by $\psi_i(x)$ and integrating it over x, which leads to equation (10) in the text.

Note that there remains a freedom in the choice of $\phi_i(x)$ and $\psi_i(x)$, because the normalization condition is still satisfied under the change of $\phi_i(x) \to c_i\phi_i(x)$ and $\psi_i(x) \to (1/c_i)\psi_i(x)$ with any constant $c_i \neq 0$. By taking advantage of this freedom, we can introduce, for convenience, another normalization condition:

$$\int_{x_1}^{x_2} \phi_i(x)dx = 1 \tag{A.2}$$

for all the right eigenfunctions whose integral over x does not vanish. Indeed, this normalization (A.2) is easily achieved by re-scaling the eigenfunctions $\psi_i(x) \to \psi_i(x) \int_{x_1}^{x_2} \phi_i(x')dx'$ and $\phi_i(x) \to \phi_i(x)/ \int_{x_1}^{x_2} \phi_i(x')dx'$. If $\int_{x_1}^{x_2} \phi_i(x')dx'$ vanishes, we simply leave the original eigenfunctions, and we call eigenfunctions with $\int_{x_1}^{x_2} \phi_i(x')dx' = 0$ "non-contributing eigenfunctions". Note that for the 0th right eigenfunction, ϕ_0, this normalization is always possible, because the 0th right eigenfunction does not take $\phi_0(x) = 0$ for any x [18]. With this choice of normalization, equation (10) is reduced to equation (11), i.e., $\frac{da_i(t)}{dt} = (\lambda_i - \sum_{j=0}^{\infty}{}' \lambda_j a_j(t))a_i(t)$.

Any set { $a_i(t) = 1$ and $a_j(t) = 0$ for $j \neq i$ } is a stationary solution of the above equation. Now we make a linear stability analysis of these solutions. Consider the solution $a_i(t) = \delta_{ik}$ for given k, and introduce a perturbation $\delta a_i(t)$ as $a_i(t) = \delta_{ik} + \delta a_i(t)$ $(i = 0, 1, ...,)$. Then, inserting this into (11) and retaining only the terms of first order in δa, we obtain

$$\frac{d\delta a_i(t)}{dt} = (\lambda_i - \lambda_k)\delta a_i(t) - \delta_{ik}\sum_{j=0}^{\infty}{}' \lambda_j \delta a_j(t) \equiv \sum_{j=0}^{\infty}{}' \Lambda_{ij}\delta a_j(t).$$

The eigenvalues of the matrix $\{\Lambda_{ij}\}$ are easily shown to be $(\lambda_0 - \lambda_k)$, ... , $-\lambda_k$, $(\lambda_{k+1} - \lambda_k)$, ... Recalling that $\lambda_i > \lambda_j$ for $i < j$, we can easily show that all the stationary solutions for $k > 0$ are unstable. On the other hand, as long as $\lambda_0 > 0$ the solution with $k = 0$ is stable.

Appendix B. Transformation of the linear operator L to an Hermite operator

In this section we transform equation (3) to a type of Schrödinger equation, to show explicitly that the operator L defined by (6) is transformed to an Hermite operator. Here we follow the standard transformation from the Fokker–Planck equation to the Schrödinger equation [26], except for the existence of the terms concerning $\mu(x)$.

We first introduce a new variable y defined as $y(x) = \int_{x_0}^{x} \sqrt{\frac{D}{g(x')}}dx'$, where x_0 is some number on $[x_1, x_2]$. According to this transformation, the distribution can change to $\hat{P}(y, t) = \frac{1}{dy/dx}P(x, t) = \sqrt{\frac{g(x)}{D}}P(x, t)$. With these new variables, we can write equation (3) as:

$$\dot{\hat{P}}(y, t) = -\bar{\mu}(t)\hat{P}(y, t) + \left[\hat{\mu}(y) + \frac{\partial}{\partial y}\left[\hat{f}(y) + D\frac{\partial}{\partial y}\right]\right]\hat{P}(y, t), \tag{B.1}$$

where $\hat{f}(y) = \sqrt{\frac{D}{g(x)}}(f(x) + \frac{1}{2}g'(x))$ and $\hat{\mu}(y) = \mu(x(y))$. $g'(x)$ is the derivative of g with respect to x and $x(y)$ is the inverse of the function $y(x)$. Note that $\bar{\mu}$ does not change by this transformation.

By further introducing two new quantities $\Phi(y) = \int_{y_0}^{y} \frac{\hat{f}(y')}{D}dy'$ and $\Psi(y,t) = e^{\frac{\Phi(y)}{2}}\hat{P}(y,t)$, equation (B.1) is rewritten as

$$\frac{\partial \Psi(y,t)}{\partial t} = -\bar{\mu}(t)\Psi(y,t) + \left[V(y) + D\frac{\partial^2}{\partial y^2}\right]\Psi(y,t) \tag{B.2}$$

$$= -\bar{\mu}(t)\Psi(y,t) + H(y)\Psi(y,t) \tag{B.3}$$

where $V(y) = \hat{\mu}(y) - \frac{\hat{f}(y)^2}{4D} + \frac{\hat{f}'(y)}{2}$ and $H(y) = \left[V(y) + D\frac{\partial^2}{\partial y^2}\right]$. The operator H obtained above is evidently a Hermite operator, and indeed the eigenvalue problem of $H\Psi$ is simply a type of Schrödinger equation. Accordingly, the exact solutions or techniques developed for Schrödinger equations can be applied to our problem.

Appendix C. Temporal evolution preserving a Gaussian distribution for the linear $\mu(x)$ case

When $f(x) = kx$, $g(x) = D$, and $\mu(x) = ax + b$, equation (3) becomes

$$\frac{\partial P(x,t)}{\partial t} = a(x - \langle x \rangle_t)P(x,t) + \frac{\partial}{\partial x}\left[kx + D\frac{\partial}{\partial x}\right]P(x,t), \tag{C.1}$$

where we have used the normalization condition $\int_{x_1}^{x_2} P(x,t)dx = 1$, and have adopted the notation $\langle ... \rangle_t \equiv \int_{x_1}^{x_2} ...P(x,t)dx$. Multiplying both sides of equation (C.1) by x and x^2 and integrating each case over x, we obtain

$$\frac{d\langle x \rangle_t}{dt} = a(\langle x^2 \rangle_t - \langle x \rangle_t^2) - k\langle x \rangle_t \tag{C.2}$$

$$\frac{d\langle x^2 \rangle_t}{dt} = a(\langle x^3 \rangle_t - \langle x \rangle_t\langle x^2 \rangle_t) - 2k\langle x^2 \rangle_t + 2D. \tag{C.3}$$

Suppose now that the solution of equation (C.1) is a Gaussian distribution, i.e.,

$$P(x,t) = \frac{1}{\sqrt{2\pi\beta(t)}}e^{-\frac{(x-\alpha(t))^2}{2\beta(t)}}, \tag{C.4}$$

where α and β correspond to the mean value of x and its variance, respectively, which are related to $\langle x \rangle_t$ and $\langle x^2 \rangle_t$ as $\alpha(t) = \langle x \rangle_t$ and $\beta(t) = \langle x^2 \rangle_t - \langle x \rangle_t^2$. Using equations (C.2) and (C.3) and the property of the Gaussian distribution $\langle x^3 \rangle_t = 3\alpha(t)\beta(t)^2 + 3\alpha(t)^3$, we can derive the time evolution equation of α and β as follows:

$$\frac{d\alpha(t)}{dt} = a\beta(t) - k\alpha(t) \tag{C.5}$$

$$\frac{d\beta(t)}{dt} = \frac{d\langle x^2 \rangle_t}{dt} - 2\langle x \rangle_t\frac{d\langle x \rangle_t}{dt}$$

$$= -2k\beta(t) + 2D. \tag{C.6}$$

On the other hand, inserting the form of (C.4) into equation (C.1) and simplifying the equation, we obtain the equation

$$2(x - \alpha(t))\beta(t)(k\alpha(t) - a\beta(t) + \frac{d\alpha(t)}{dt}) + ((x - \alpha(t))^2 - \beta(t))(-2D + 2k\beta(t) + \frac{d\beta(t)}{dt}) = 0.$$

The time evolution equations of α and β satisfy the above equations (C.5) and (C.6), and the Gaussian distribution is the solution of equation (C.1) (as the solution with temporal evolution is unique).

Glossary

Flow cytometry. A method for measuring biophysical characteristics (such as size and fluorescence) of single cells primarily by optical means. Although the cells are measured one by one by making them flow, it can process thousands of cells per second.

Growth rate. Although generally it refers to the speed of growth of an organism, here it means only the "replication speed" of a cell.

Phenotypic fluctuation. Even for clone cells (i.e., having identical genes), the intracellular state, such as the copy number of some m-RNA or protein, can vary. Hence the state values are distributed by cells, giving rise to fluctuation around its mean value.

Cell state. A cell consists of a huge number of chemicals. Depending on the composition of these chemicals, the cell changes its state. Hence the cell state is characterized by abundances of the chemicals, which are adopted as state values here.

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Figures
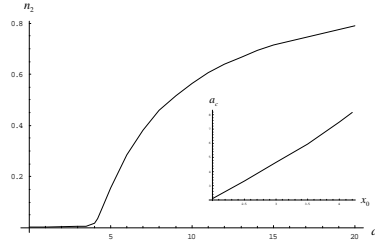
Figure 1. The total amount n_2 of the distribution in the higher-growth region $(x > x_0 = 3)$, calculated as $n_2 = \int_{x_0}^{x_2} P(x)dx$, against the relative growth rate a. This plot clearly indicates the existence of the critical value of a, a_c, which is defined here as the value where $n_2 = 1/10$. The inset, a plot of the critical value of a_c against the position x_0 of the step of $\mu(x)$, shows the dependence of a_c on x_0, which is fitted well by: $a_c \simeq kx_0\sqrt{\frac{k}{D}}$. These calculations were carried out for $f(x) = x$, $g(x) = 1$, $\mu(x) = a\Theta(x-3)$, for the range of x, $[-6, 6]$.
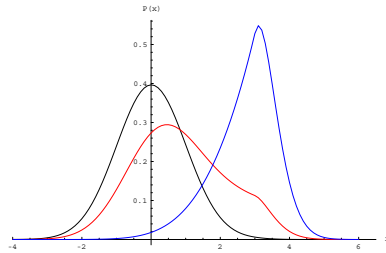

Figure 2. Some profiles of the stationary distributions for different relative growth rates $a = 2$(black), 5(red), and 8(blue). We can see that the distribution for $a = 2$ is hardly influenced by $\mu(x)$. We choose the same equation as for Figure 1, i.e., $f(x) = x$, $g(x) = 1$, $\mu(x) = a\Theta(x-3)$, with the range of x, $[-6, 6]$.
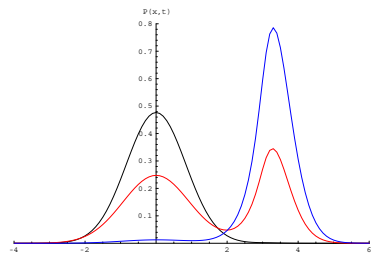
Figure 3. Temporal evolution of the distribution for equation (3) for $\mu(x) = 20\ \Theta(x-3)$, $g(x) = 1$, $f(x) = x$, with the range of x, $[-6, 6]$. The initial condition is given by $P(x, t_0 = 0) = \delta(x)$. The black, red, and blue curves show the distributions at $t = 0.6$, 1.04, and 1.6, respectively. The double-peak distribution is observed during an intermediate period.
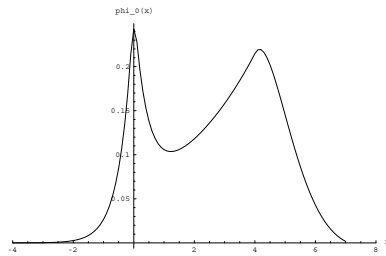
Figure 4. An example of the stationary distribution of equation (3) having double peaks, for $f(x) = 2 \, \text{sgn}(x)$, $g(x) = 1$, $\mu(x) = 2.4 \, \Theta(x - 4)$, with the range of x, $[-7, 7]$.