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Quantifying gene expression variability arising from randomness in cell division times

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Abstract The level of a given mRNA or protein exhibits significant variations from cell-to-cell across a homogeneous population of living cells. Much work has focused on understanding the different sources of noise in the gene-expression process that drive this stochastic variability in gene-expression. Recent experiments tracking growth and division of individual cells reveal that cell division times have considerable inter-cellular heterogeneity. Here we investigate how randomness in the cell division times can create variability in population counts. We consider a model by which mRNA/protein levels in a given cell evolve according to a linear differential equation and cell divisions occur at times spaced by independent and identically distributed random intervals. Whenever the cell divides the levels of mRNA and protein are halved. For this model, we provide a method for computing any statistical moment (mean, variance, skewness, etcetera) of the mRNA and protein levels. The key to our approach is to establish that the time evolution of the mRNA and protein statistical moments is described by an upper triangular system of Volterra equations. Computation of the statistical moments for physiologically relevant parameter values shows that randomness in the cell division process can be a major factor in driving difference in protein levels across a population of cells.

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1 Introduction

Single-cell measurements reveal that the level of a protein or mRNA inside an individual cell can vary significantly across a genetically-identical population of cells exposed to the same environment (Raj and Oudenaarden 2008; Raj et al. 2006; Golding et al. 2005; Bar-Even et al. 2006; Newman et al. 2006; Kaern et al. 2005; Elowitz et al. 2002; Singh and Dennehy 2014). This stochastic variability has been shown to play a key role in cellular decision-making (Losick and Desplan 2008; Singh and Weinberger 2009; Weinberger et al. 2005; Arkin et al. 1998), information processing (Libby et al. 2007), and buffering populations from hostile changes in the environment (Eldar and Elowitz 2010; Veening et al. 2008; Kussell and Leibler 2005; Balaban et al. 2004). Much theoretical and experimental work has investigated how different sources of noise in the gene expression process drive intercellular variability in protein levels. More specifically, these sources include random promoter transitions between different transcriptional states and stochastic birth-death of individual mRNA transcripts and protein molecules (Singh et al. 2012; Shahrezaei and Swain 2008; Paulsson 2004; Friedman et al. 2006; Taniguchi et al. 2010; Munsky et al. 2009; Singh et al. 2010; Jia and Kulkarni 2011; Bokes et al. 2012; Kuang et al. 2013; Innocentini and Hornos 2007; Singh and Soltani 2013). Here we focus on an alternative mechanism for explaining gene-expression variability: randomness in cell division times. Living cells grow and divide, at which point the quantities of mRNAs and proteins are approximately divided equally between daughter cells. Since cell division times can vary from cell-to-cell (Roeder et al. 2010; Zilman et al. 2010; Hawkins et al. 2009; Stukalin et al. 2013), we investigate its role in driving variability in the level of a given mRNA or protein.

To quantify the contribution of random timing in the cell divisions to the observed variability in the level of a protein/mRNA, a deterministic gene expression model is considered where all other sources of noise are absent. In particular, the time evolution of cellular mRNA and protein levels are modeled by a standard set of linear differential equations. Cell divisions occur at times spaced by independent and identically distributed random intervals and when the cell divides both mRNA and protein levels are halved (assuming symmetric division). Our goal is to obtain explicit expressions for the statistical moments (mean, variance, skewness, etc.) of the mRNA/protein population counts in terms of model parameters (mRNA transcription rate, protein half-life, etc.) and the probability distribution of the intervals between cell divisions.

To this effect, we show that the statistical moments of the mRNA and protein levels can be explicitly obtained from the solution to an upper triangular system of Volterra equations. By studying the asymptotic behavior of these Volterra equations, one can provide expressions for the asymptotic moments of mRNA and protein levels, unveiling their dependence on model parameters. In the special case of Erlang distributed cell division intervals we show that the statistical moments can alternatively be described

by differential equations, leading to a simpler method to obtain equivalent expressions for the statistical moments.

We provide a numerical example with physiologically relevant parameter values and an experimentally obtained distribution for the cell division times. We use our results to study how cell-to-cell variability in protein levels varies with decreasing variability in the cell division times. Our results show that the randomness in the cell division process can be a major factor in driving intercellular difference in the level of a protein.

The remainder of the paper is organized as follows. The problem formulation including the stochastic model of gene-expression is presented in Sect. 2. In Sect. 3 we provide a method to compute the statistical moments for this model. In Sect. 4 we provide a numerical example and in Sect. 5 we discuss the results providing also possible directions for future work.

2 Stochastic model and problem formulation

In gene-expression, the mRNA count at time t in a cell, denoted by $m(t)$, and the protein count at time t in a cell, denoted by $p(t)$, can be described by the following linear system of differential equations

$$\begin{aligned} \dot{m}(t) &= k_m - \gamma_m m(t), & m(T) &= m_0, \\ \dot{p}(t) &= k_p m(t) - \gamma_p p(t), & p(T) &= p_0, \end{aligned} \tag{1}$$

for a time interval $[T, T + \epsilon)$, $\epsilon > 0$, in which there are no cell divisions (Alon 2006). The constant k_m is the mRNA transcription rate and γ_m is the mRNA degradation rate. Each mRNA produces proteins at a translation rate k_p and these molecules degrade at a constant rate γ_p . We assume that k_m, k_p, γ_m , and γ_p are positive.

Let the times at which the cell divides be denoted by $\{t_k\}_{k \in \mathbb{N}}$. Then (1) holds for $t \in \mathbb{R}_{\geq 0} \setminus \{t_k\}_{k \in \mathbb{N}}$ for an initial time $T = 0$. Let also $t_0 := 0$. At division times $\{t_k\}_{k \in \mathbb{N}}$ the mRNA and protein counts are halved, i.e.,

$$m(t_k) = \frac{1}{2} m(t_k^-), \quad p(t_k) = \frac{1}{2} p(t_k^-), \quad k \in \mathbb{N}, \tag{2}$$

where we use $u(t_k^-)$ to denote the limit from the left of a function u at t_k . The time intervals between these cell division times, $\{\tau_k := t_{k+1} - t_k\}_{k \in \mathbb{N}_0}$, are assumed to be independent and identically distributed and described by a given probability distribution, with finite moments. The mean of the cell division intervals $\{\tau_k\}_{k \in \mathbb{N}_0}$ is denoted by μ_τ and the covariance by σ_τ .

The goal of the present work is to obtain the statistical moments of the mRNA and protein levels, i.e.,

$$\mathbb{E}[m(t)], \mathbb{E}[p(t)], \mathbb{E}[m(t)^2], \mathbb{E}[p(t)^2], \mathbb{E}[m(t)^3], \dots \tag{3}$$

where $\mathbb{E}[\cdot]$ denotes the expected value, and the corresponding asymptotic values, as $t \rightarrow \infty$, provided that the limits exist. Computing (3) allows us to quantify variability

using the *coefficient of variation (CV)* of mRNA and protein levels. For a random variable a the coefficient of variation is defined as

$$CV_a := \frac{\sigma_a}{\mu_a},$$

where $\mu_a := \mathbb{E}[a]$ denotes the expected value and $\sigma_a := \sqrt{\mathbb{E}[(a - \mu_a)^2]} = \sqrt{\mathbb{E}[a^2] - \mu_a^2}$ denotes the standard deviation. The coefficient of variation of mRNA and protein levels are denoted by $CV_{m(t)}$ and $CV_{p(t)}$ respectively. Note that this measure is also considered in previous studies (Paulsson 2004). Our results shall allow us to assess how the variability in the inter-division times, measured by

$$CV_\tau := \frac{\sigma_\tau}{\mu_\tau},$$

influence the variability in mRNA and protein counts. Moreover, computing (3) enables us to provide further information on the distribution of the mRNA and protein by computing *skewness* and *kurtosis* defined as

$$SK_a := \frac{\mathbb{E}[(a - \mu_a)^3]}{\sigma_a^3}, \quad KU_a := \frac{\mathbb{E}[(a - \mu_a)^4]}{\sigma_a^4},$$

respectively, where a denotes a random variable. $SK_{m(t)}$ and $SK_{p(t)}$ denote skewness of mRNA and protein levels, respectively, and $KU_{m(t)}$ and $KU_{p(t)}$ denote kurtosis of mRNA and protein levels, respectively. Note that the third and fourth order centered moments in (3), can be computed via the uncentered moments, e.g., $\mathbb{E}[(a - \mu_a)^3] = \mathbb{E}[a^3] - 3\mathbb{E}[a^2]\mu_a + 2\mu_a^3$.

Note also that one can estimate the coefficient of variation, skewness, and kurtosis of mRNA and protein levels from experimental data. Hence such data can be compared with the expressions we shall provide by computing (3) to validate and estimate the parameters of the model (1), (2).

In order to guarantee that the limits as $t \rightarrow \infty$ exist for (3) we assume that the probability distribution of the intervals between divisions admits a probability density function, denoted by f , and that its support (the smallest closed set whose complement has probability zero) is an interval in the non-negative real line.

In fact we can establish the following result, proved in the appendix.

Proposition 1 *Suppose that the probability distribution of the intervals between divisions admits a density f and that $f(s) > 0$ in $s \in (\underline{\tau}, \bar{\tau})$, and $f(s) = 0$ for $s \in \mathbb{R}_{\geq 0} \setminus (\underline{\tau}, \bar{\tau})$ for $\underline{\tau} \in \mathbb{R}_{\geq 0}$, $\bar{\tau} \in \mathbb{R}_{> \underline{\tau}} \cup \{\infty\}$. Then $\lim_{t \rightarrow \infty} \mathbb{E}[m(t)^i]$, $\lim_{t \rightarrow \infty} \mathbb{E}[p(t)^j]$, and $\lim_{t \rightarrow \infty} \mathbb{E}[m(t)^i p(t)^j]$ exist for every $i, j \in \mathbb{N}$ and every non-negative initial conditions m_0, p_0 .*

A simple distribution for which the limits as $t \rightarrow \infty$ for (3) do not exist is a single point mass at a given point h , in which case the intervals between divisions are constant $t_{k+1} - t_k = h, \forall k \in \mathbb{N}$, and $\mu_\tau = h$. Then the evolution of (1), (2) is deterministic and the mRNA and protein levels oscillate indefinitely in a given bounded set. Note

however that most distributions of interest to model the cell division intervals (Erlang, log-normal, uniform, etc) comply with the assumptions of Proposition 1.

3 Main results

In Sect. 3.1 we show how to compute any statistical moment

$$\mathbb{E}[p(t)^i m(t)^j], \quad i, j \in \mathbb{N}_0, \tag{4}$$

for any time $t \in \mathbb{R}_{\geq 0}$. By considering $i = 0, j > 0$, and $i > 0, j = 0$, and taking the limit as t tends to infinity we will be able to obtain the desired asymptotic moments of mRNA and protein levels. In Sect. 3.2 we apply this method to provide explicit expressions for the two first asymptotic statistical moments of the mRNA level. In Sect. 3.3 we consider the special case of exponential and Erlang distributed intervals between divisions, providing a simpler method to compute statistical moments for these special cases.

3.1 Method for computing statistical moments

The key to our approach for computing (4) is to stack all the monomials $p(t)^i m(t)^j$, $i + j \leq n$ of order less or equal than a given arbitrary $n \in \mathbb{N}$ into a vector $x(t)$ ordered in a special way. To this effect, let $y^q \in \mathbb{R}^{q+1}$ contain the $q + 1$ monomials of a given order $q \in \mathbb{N}$ ordered in increasing exponent associated with $m(t)$, i.e.,

$$y^q(t) = \left[y_1^q(t) \dots y_{q+1}^q(t) \right]^T,$$

where

$$y_{i+1}^q(t) := p(t)^{q-i} m(t)^i, \quad i \in \{0, \dots, q\}.$$

Let also $y^0(t) := 1$ for every $t \in \mathbb{R}_{\geq 0}$. Then $x(t)$ is defined as

$$x(t) := \left[y^n(t)^T \ y^{n-1}(t)^T \dots \ y^1(t)^T \ y^0(t)^T \right]^T,$$

where we omit the dependence on n . Note that $x(0) = x_0$ where $x_0 := [p_0^n \ p_0^{n-1} m_0 \dots \ p_0 \ m_0 \ 1]^T$ and that $x(t) \in \mathbb{R}^r$, where

$$r := \frac{n(n+3)}{2} + 1.$$

The next lemma establishes that the time evolution of $x(t)$ can be obtained from the solution to a special impulsive system. We denote by I_j the identity matrix with dimensions $j \times j$, where the subscript indicating the dimension is dropped whenever clear from context.

Lemma 1 *The vector $x(t)$ satisfies the following impulsive system*

$$\begin{aligned} \dot{x}(t) &= Ax(t), & t \neq t_k, \quad t \geq 0, \quad k \in \mathbb{Z}_{>0}, \\ x(t_k) &= Jx(t_k^-), & t_0 = 0, \quad x(t_0) = x_0, \end{aligned} \tag{5}$$

where the intervals between consecutive transition times $\{t_{k+1} - t_k\}_{k \in \mathbb{N}_0}$ are distributed according to f , and the matrices A and J are described by

$$A := \begin{bmatrix} B_n & C_n & 0 & 0 & \dots & 0 \\ 0 & B_{n-1} & C_{n-1} & 0 & \dots & 0 \\ 0 & 0 & B_{n-2} & C_{n-2} & \ddots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & 0 & B_1 & C_1 \\ 0 & 0 & \dots & 0 & 0 & B_0 \end{bmatrix}, \quad J := \begin{bmatrix} \frac{1}{2^n} I_{n+1} & 0 & 0 & 0 & \dots & 0 \\ 0 & \frac{1}{2^{n-1}} I_n & 0 & 0 & \dots & 0 \\ 0 & 0 & \frac{1}{2^{n-2}} I_{n-1} & 0 & \ddots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & 0 & \frac{1}{2} I_2 & 0 \\ 0 & 0 & \dots & 0 & 0 & 1 \end{bmatrix},$$

where $B_q \in \mathbb{R}^{(q+1) \times (q+1)}$, $C_q \in \mathbb{R}^{(q+1) \times q}$, $q \in \{1, \dots, n\}$, are described by

$$B_q := \begin{bmatrix} -q\gamma_p & qk_p & 0 & 0 & \dots & 0 \\ 0 & -((q-1)\gamma_p + \gamma_m) & (q-1)k_p & 0 & \dots & 0 \\ 0 & 0 & -((q-2)\gamma_p + 2\gamma_m) & (q-2)k_p & \ddots & 0 \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & 0 & -(\gamma_p + (q-1)\gamma_m) & k_p \\ 0 & 0 & \dots & 0 & 0 & -q\gamma_m \end{bmatrix},$$

$$C_q := \begin{bmatrix} 0 & 0 & \dots & 0 & 0 \\ k_m & 0 & \ddots & 0 & 0 \\ 0 & 2k_m & \ddots & 0 & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & (q-1)k_m & 0 \\ 0 & 0 & \dots & 0 & qk_m \end{bmatrix},$$

and $B_0 := 0$.

Proof The proof follows from the definition of $x(t)$, and noticing that for non-negative integers i and j

$$\frac{d}{dt} p(t)^i m(t)^j = \begin{cases} -(i\gamma_p + j\gamma_m)p(t)^i m(t)^j + ik_p p(t)^{i-1} m(t)^{j+1} + jk_m p(t)^i m(t)^{j-1}, & \text{if } i > 0 \text{ and } j > 0, \\ -i\gamma_p p(t)^i + ik_p m(t) p(t)^{i-1}, & \text{if } i > 0 \text{ and } j = 0, \\ -j\gamma_m m(t)^j + jk_m m(t)^{j-1}, & \text{if } i = 0 \text{ and } j > 0, \end{cases}$$

and

$$p(t_k)^i m(t_k)^j = \frac{1}{2^{i+j}} p(t_k^-)^i m(t_k^-)^j.$$

□

The system (5) is an impulsive renewal system in the sense of (Antunes et al. 2012, 2013) with the key feature that A is an upper-triangular matrix and J is a diagonal matrix. The crucial aspect of (1), (2) that enables this feature is its cascade structure: mRNA generates protein, but the protein generation does not impact on the mRNA levels. The fact that we include the 0th moment $y^0(t) = 1$ in $x(t)$ allows us to describe the statistical moments $x(t)$ by a linear system of equations (5) instead of an affine one.

As in (Antunes et al. 2012, 2013) we can obtain $\mathbb{E}[x(t)]$ from the solution to a Volterra equation. In fact, the solution to (5) is described by

$$x(t) = S(t)x_0,$$

where

$$S(t) := e^{A(t-t_\ell)} J e^{A\tau_{\ell-1}} \dots J e^{A\tau_0},$$

for $\ell = \max\{k \in \mathbb{N}_0 : t_k \leq t\}$, and hence we have

$$\mathbb{E}[x(t)] = \Phi(t)x(0), \tag{6}$$

where

$$\Phi(t) := \mathbb{E}[S(t)]. \tag{7}$$

We show next that $\Phi(t)$ satisfies a Volterra equation.

Lemma 2 *The matrix $\Phi(t)$ satisfies the following Volterra equation*

$$\Phi(t) = \int_0^t \Phi(t-s) J e^{As} f(s) ds + e^{At} r(t), \tag{8}$$

where

$$r(t) := \int_t^\infty f(s) ds. \tag{9}$$

Proof Conditioning (7) on the time of the first jump t_1 , we obtain

$$\Phi(t) = \int_0^\infty \mathbb{E}[S(t)|t_1 = s] f(s) ds, \tag{10}$$

where

$$\mathbb{E}[S(t)|t_1 = s] = \begin{cases} e^{At}, & \text{if } s > t, \\ \mathbb{E}[S_1(t-s) J e^{As}], & \text{if } s \leq t, \end{cases} \tag{11}$$

and $S_1(t-s)$ is the transition matrix of (5) from $s = t_1$ to t , which depends on the intervals between divisions after the first division $\{\tau_k\}_{k \in \mathbb{N}_{\geq 1}}$. Due to the assumption that the intervals between transitions are independent and identically distributed we have $\mathbb{E}[S_1(t)] = \Phi(t)$. Thus, partitioning (10) using (11) we obtain (8). \square

Due to the fact that A is upper triangular and that J is diagonal, (8) is an upper triangular system of Volterra equations. Equation (8) can in some cases be solved analytically [cf. Gripenberg et al. (1990)] by recursively solving scalar Volterra equations. Instead, one can use a numerical method to solve (8) [see Linz (1985)]. A simple numerical method is to approximate the integral by a quadrature formula (e.g. a simple trapezoidal rule) at equally spaced points $jh \in [0, t]$, where h is the discretization step, i.e.,

$$\Phi(jh) = \sum_{\ell \in \mathbb{N}_0 \cap [0, j]} q_\ell \Phi(h(j - \ell)) J e^{A\ell h} f(\ell h) + e^{Ajh} r(jh), \quad j \in \mathbb{N}_0 \cap [0, t/h], \tag{12}$$

where the quadrature weights are denoted by q_ℓ . Then $\Phi(jh)$ can be obtained iteratively from (12). Note that this numerical method can easily incorporate distributions of the cell division intervals described from histograms, which is typically the case when these distributions are obtained experimentally [see e.g., Hawkins et al. (2009)].

An alternative analytical method to solve (8) is to use Laplace transforms. In fact, let

$$\begin{aligned} \hat{\Phi}(z) &:= \int_0^\infty \Phi(t) e^{-zt} dt, \\ \hat{H}(z) &:= \int_0^\infty e^{At} r(t) e^{-zt} dt, \\ \hat{K}(z) &:= \int_0^\infty J e^{At} f(t) e^{-zt} dt, \\ \hat{x}(z) &:= \int_0^\infty x(t) e^{-zt} dt, \end{aligned} \tag{13}$$

for $z \in \mathbb{C}$ for which the Laplace transforms exist. In the next result we provide a method to obtain the expressions for $\hat{K}(z)$, $\hat{H}(z)$ and $\hat{\Phi}(z)$ (and hence also $\hat{x}(z)$) in terms of

$$\hat{f}(z) := \int_0^\infty f(s) e^{-zs} ds.$$

Note that if z is real than $\hat{f}(z)$ is the moment generating function of f . Let $\alpha_j = A_{jj}$, $1 \leq j \leq r$ denote the diagonal elements of A and consider the following r partitions of A indexed by j : for $j = 1$,

$$A = \begin{bmatrix} \alpha_1 & h_1 \\ 0 & G_1 \end{bmatrix},$$

for $2 \leq j \leq r - 1$,

$$A = \begin{bmatrix} D_j & e_j & * \\ 0 & \alpha_j & h_j \\ 0 & 0 & G_j \end{bmatrix},$$

and for $j = r$,

$$A = \begin{bmatrix} D_r & e_r \\ 0 & \alpha_r \end{bmatrix},$$

where for $1 \leq j \leq r - 1$, $h_j \in \mathbb{R}^{1 \times (r-j)}$, $G_j \in \mathbb{R}^{(r-j) \times (r-j)}$ and for $2 \leq j \leq r$, $e_j \in \mathbb{R}^{(j-1) \times 1}$, $D_j \in \mathbb{R}^{(j-1) \times (j-1)}$.

Lemma 3 *The Laplace transforms $\hat{\Phi}(z)$, $\hat{H}(z)$, $\hat{K}(z)$, and $\hat{x}(z)$ exist in $\{z \in \mathbb{C} | \text{Re}(z) > 0\}$ and*

$$\hat{x}(z) = \hat{\Phi}(z)x_0, \tag{14}$$

where $\hat{\Phi}(z) = [\hat{\Phi}_{ij}(z)]$ is an upper-triangular matrix whose entries can be obtained recursively from the entries of $\hat{K}(z) = [\hat{K}_{ij}(z)]$ and $\hat{H}(z) = [\hat{H}_{ij}(z)]$ as

$$\begin{aligned} \hat{\Phi}_{ii}(z) &= \frac{\hat{H}_{ii}(z)}{1 - \hat{K}_{ii}(z)}, \quad 1 \leq i \leq r, \\ \hat{\Phi}_{ij}(z) &= \frac{\sum_{\ell=i}^{j-1} \hat{\Phi}_{i\ell}(z)\hat{K}_{\ell j}(z) + \hat{H}_{ij}(z)}{1 - \hat{K}_{jj}(z)}, \quad \text{for } i + 1 \leq j \leq r. \end{aligned} \tag{15}$$

Furthermore, if no two diagonal entries of A assume the same values, then

$$\begin{aligned} \hat{K}(z) &= \sum_{j=1}^r \hat{f}(z - \alpha_j) J Z_j, \\ \hat{H}(z) &= \sum_{j=1}^r \frac{1 - \hat{f}(z - \alpha_j)}{z - \alpha_j} Z_j, \end{aligned} \tag{16}$$

where

$$Z_j = \begin{cases} \begin{bmatrix} 1 & h_1(\alpha_1 I - G_1)^{-1} \\ 0 & 0 \end{bmatrix}, & \text{if } j = 1, \\ \begin{bmatrix} 0 & (\alpha_j I - D_j)^{-1} e_j & (\alpha_j I - D_j)^{-1} e_j h_j(\alpha_j I - G_j)^{-1} \\ 0 & 1 & h_j(\alpha_j I - G_j)^{-1} \\ 0 & 0 & 0 \end{bmatrix}, & \\ \text{if } 2 \leq j \leq r - 1, \\ \begin{bmatrix} 0 & (\alpha_r I - D_r)^{-1} e_r \\ 0 & 1 \end{bmatrix}, & \text{if } j = r. \end{cases}$$

The assumption in the last part of Lemma 3 that no two diagonal entries of A assume the same values is equivalent to assuming that there does not exist non-negative integers i, j , both less than n , such that $i\gamma_m = j\gamma_p$. This can be concluded by noticing that the diagonal entries of A take the form $-(k\gamma_m + \ell\gamma_p)$, for non-negative integers k, ℓ such that $k + \ell \leq n$. We assume this merely for the sake of simplicity since it guarantees that A is diagonalizable. If such assumption is not met we can obtain the desired Laplace transforms using the Jordan block decomposition of A , but the expressions are more intricate.

Proof Taking Laplace transforms on both sides of (6) and (8), and using well-known properties of Laplace transforms [see Gripenberg et al. (1990), Sect. 3.8], we obtain (14) and

$$\hat{\Phi}(z) = \hat{\Phi}(z)\hat{K}(z) + \hat{H}(z), \tag{17}$$

respectively.

Since A is an upper-triangular matrix and J is a diagonal matrix, $\hat{K}(z)$ and $\hat{H}(z)$ are upper-triangular and this implies that the solution to (17), $\hat{\Phi}(z)$, is also upper-triangular and can be obtained from (15). From (15) and it is clear that $\hat{\Phi}(z)$ exists when $\hat{K}(z)$ and $\hat{H}(z)$ exist and from (14) it is clear that $\hat{x}(z)$ exists when $\hat{\Phi}(z)$ exists. From (13) and the fact that the eigenvalues of A coincide with its diagonal entries, which are less or equal than zero, we see that each of the entries of $\hat{K}(z)$ and $\hat{H}(z)$ is the Laplace transform of linear combinations of functions taking the form $e^{\alpha t} f(t)$ for $\alpha \leq 0$ and $e^{\beta t} r(t)$ for $\beta \leq 0$, respectively. Hence $\hat{K}(z)$ and $\hat{H}(z)$ exist in $\{z \in \mathbb{C} | \text{Re}(z) > 0\}$.

To obtain (16) we note that the eigenvalues of A , α_j , $1 \leq j \leq r$, are simple under the assumption that the diagonal entries assume different values; the right eigenvector associated with the eigenvalue α_j is given by

$$v_j = \begin{cases} \begin{bmatrix} 1 \\ 0 \end{bmatrix}, & \text{if } j = 1, \\ \begin{bmatrix} (\alpha_j I - D_j)^{-1} e_j \\ 1 \\ 0 \end{bmatrix}, & \text{if } 2 \leq j \leq r - 1, \\ \begin{bmatrix} (\alpha_r I - D_r)^{-1} e_r \\ 1 \end{bmatrix}, & \text{if } j = r, \end{cases}$$

and the associated left eigenvector is given by

$$w_j^\top = \begin{cases} [1 \ h_1(\alpha_1 I - G_1)^{-1}], & \text{if } j = 1, \\ [0 \ 1 \ h_j(\alpha_j I - G_j)^{-1}], & \text{if } 2 \leq j \leq r - 1, \\ [0 \ 1], & \text{if } j = r. \end{cases}$$

Thus $A = \sum_{k=1}^r \alpha_j v_j w_j^\top$, which implies that

$$\begin{aligned} e^{As} &= \sum_{k=1}^r e^{\alpha_j s} v_j w_j^\top, \\ \hat{K}(z) &= \sum_{k=1}^r \int_0^\infty e^{\alpha_j s} e^{-zs} f(s) ds J v_j w_j^\top, \\ \hat{H}(z) &= \sum_{k=1}^r \int_0^\infty e^{\alpha_j s} e^{-zs} r(s) ds \alpha_j v_j w_j^\top. \end{aligned}$$

Then (16) follows by noticing that $Z_j = v_j w_j^\top$, $1 \leq j \leq r$, $\hat{f}(z - \alpha_j) = \int_0^\infty e^{\alpha_j s} e^{-zs} f(s) ds$ and

$$\int_0^\infty e^{\alpha_j s} e^{-zs} r(s) ds = \frac{1 - \hat{f}(z - \alpha_j)}{z - \alpha_j},$$

where the latter equality is obtained by replacing $r(s) = \int_s^\infty f(\delta) d\delta$ in the left-hand side and interchanging the integrals. □

This result can be used to obtain the time-evolution of the moments by taking Laplace inverse transforms, although this is useful only if the expression for the Laplace transforms have known inverse. This is the case, e.g., when f corresponds to an exponential or Erlang distribution [see Resnick (1992), p. 182].

More important to the present work is that Lemma 3 leads to the desired expressions for the asymptotic moments. In fact, having obtained $\hat{x}(z)$ we can make use of the final value theorem for Laplace transforms [see e.g., Chen et al. (2007)] to obtain expressions for the asymptotic value of x . The obtained expressions are summarized in the next result. Note that $\hat{K}(0)$ and $\hat{H}(0)$ can be decomposed as

$$\hat{K}(0) = \begin{bmatrix} M_K & n_K \\ 0 & 1 \end{bmatrix}, \quad \hat{H}(0) = \begin{bmatrix} M_H & n_H \\ 0 & \mu_\tau \end{bmatrix},$$

where $\mu_\tau = \int_0^\infty sf(s)ds$. Moreover, let

$$\hat{r}(z) := \int_0^\infty r(s)e^{-zs} ds, \tag{18}$$

where $r(s)$ is defined in (7).

Theorem 1 *Suppose that the probability distribution of the intervals between divisions satisfies the assumptions of Proposition 1. Then*

$$\lim_{t \rightarrow \infty} \mathbb{E}[x(t)] = \begin{bmatrix} \frac{1}{\mu_\tau} (M_H(I - M_K)^{-1}n_K + n_H) \\ 1 \end{bmatrix}. \tag{19}$$

Note that $(I - M_K)$ is an upper-triangular matrix and hence one can easily obtain an expression for $(I - M_K)^{-1}$.

Proof Note that

$$\hat{\Phi}(z) = \hat{H}(z)(I - \hat{K}(z))^{-1}$$

and consider the partitions

$$\hat{K}(z) = \begin{bmatrix} \hat{K}_1(z) & \hat{K}_2(z) \\ 0 & \hat{f}(z) \end{bmatrix}$$

and

$$\hat{H}(z) = \begin{bmatrix} \hat{H}_1(z) & \hat{H}_2(z) \\ 0 & \hat{r}(z) \end{bmatrix}.$$

Then

$$\hat{\Phi}(z) = \begin{bmatrix} \hat{H}_1(z)(I - \hat{K}_1(z))^{-1} & \frac{1}{1-\hat{f}(z)}\hat{H}_1(z)(I - \hat{K}_1(z))^{-1}\hat{K}_2(z) + \frac{1}{1-\hat{f}(z)}\hat{H}_2(z) \\ 0 & \frac{\hat{r}(z)}{1-\hat{f}(z)} \end{bmatrix}.$$

We can now use the final value theorem [see [Chen et al. \(2007\)](#), [Guest \(1991\)](#), p. 91] to compute

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[\Phi(t)] &= \lim_{z \rightarrow 0} z\hat{\Phi}(z) \\ &= \begin{bmatrix} 0 & \frac{1}{\mu_\tau} M_H(I - M_K)^{-1}n_K + \frac{1}{\mu_\tau}n_H \\ 0 & 1 \end{bmatrix}, \end{aligned} \tag{20}$$

where we used the fact that $\lim_{z \rightarrow 0} \frac{1-\hat{f}(z)}{z} = \lim_{z \rightarrow 0} \hat{r}(z) = \mu_\tau$, and the fact that $\hat{r}(z) = \frac{1-\hat{f}(z)}{z}$ (which can be concluded by interchanging the integral operations in

$\hat{r}(z) = \int_0^\infty \int_s^\infty f(\delta) d\delta e^{-zs} ds$). Note that the final value theorem requires the limits in the left and right-hand sides of (20) to exist [cf. Chen et al. (2007), Guest (1991), p. 91]. By inspection we see that $\lim_{z \rightarrow 0} z \hat{\Phi}(z)$ exists. From Proposition 1 we have that $\lim_{t \rightarrow \infty} \mathbb{E}[x(t)] = \lim_{t \rightarrow \infty} \mathbb{E}[\Phi(t)]x_0$ exists for every initial condition x_0 . This implies that $\lim_{t \rightarrow \infty} \mathbb{E}[\Phi(t)]$ exists. Since $x(t) = \Phi(t)x_0$ and the last component of $x(t)$ is equal to one for every time $t \geq 0$ we conclude (19). \square

Theorem 1 is one of the main results of the paper since it characterizes the asymptotic behaviour of the statistical moments (2). As we shall discuss in the sequel (see Remark 1) we could not have derived this theorem if we had considered the asymptotic behaviour of the embedded Markov chains $x(t_k)$ or $x(t_k^-)$, since in general

$$\lim_{k \rightarrow \infty} \mathbb{E}[x(t_k)] \neq \lim_{k \rightarrow \infty} \mathbb{E}[x(t_k^-)] \neq \lim_{t \rightarrow \infty} \mathbb{E}[x(t)]. \tag{21}$$

Furthermore, it has the following implication: since from (17) we conclude that $\hat{K}(0)$ and $\hat{H}(0)$ depend only on $\hat{f}(-\alpha_j)$, $1 \leq j \leq r$, the dependency of the statistical moments of order n of the state of (5) on the distribution between transitions is encapsulated in the moment generating function of the distribution evaluated only at the negative value of the diagonal entries of A . This can for example be seen in the explicit expression for the first two statistical moments of the mRNA level, which we give in the next section (see Theorem 2 below).

3.2 Expressions for the first two moments of the mRNA level

Lemma 3 and Theorem 1 allow us to obtain exact expressions for the moments of mRNA and protein levels. However, deriving these expressions analytically requires excessively long derivations and thus it is preferable to use symbolic computation. We illustrate the analytical procedure by deriving an expression for the mean mRNA level. In a supplementary file we derive expressions for the first and second moments of the protein level, and also present the procedure used for computing the second moment of the mRNA level.

Theorem 2 *The following holds*

$$\lim_{t \rightarrow \infty} \mathbb{E}[m(t)] = \frac{k_m}{\gamma_m} \left(1 - \frac{\hat{r}(\gamma_m)}{\mu_\tau(2 - \hat{f}(\gamma_m))} \right), \tag{22}$$

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)^2] = & \frac{k_m^2}{\gamma_m^2} \left(1 + \frac{\hat{r}(2\gamma_m) - 2\hat{r}(\gamma_m)}{\mu_\tau} + \frac{(\hat{r}(\gamma_m) - \hat{r}(2\gamma_m))(1 - \hat{f}(\gamma_m))}{\mu_\tau(1 - \frac{\hat{f}(\gamma_m)}{2})} \right. \\ & \left. + \frac{\hat{r}(2\gamma_m)}{4\mu_\tau} \left(\frac{(\hat{f}(2\gamma_m) - 2\hat{f}(\gamma_m) + 1)}{1 - \frac{\hat{f}(2\gamma_m)}{4}} + \frac{(1 - \hat{f}(\gamma_m))(\hat{f}(\gamma_m) - \hat{f}(2\gamma_m))}{(1 - \frac{\hat{f}(2\gamma_m)}{4})(1 - \frac{\hat{f}(\gamma_m)}{2})} \right) \right). \tag{23} \end{aligned}$$

Proof To prove (22) it suffices to consider $x(t) = [m(t) \ 1]^T$, which satisfies (5) for

$$A = \begin{bmatrix} -\gamma_m & k_m \\ 0 & 0 \end{bmatrix}, \quad J = \begin{bmatrix} \frac{1}{2} & 0 \\ 0 & 1 \end{bmatrix}.$$

Then from (17) we obtain

$$\hat{K}(0) = \begin{bmatrix} \frac{\hat{f}(\gamma_m)}{2} & \frac{k_m(1-\hat{f}(\gamma_m))}{2\gamma_m} \\ 0 & 1 \end{bmatrix}, \quad \hat{H}(0) = \begin{bmatrix} \frac{1-\hat{f}(\gamma_m)}{\gamma_m} & \left(\frac{k_m(\hat{f}(\gamma_m)-1)}{\gamma_m^2} + \mu_\tau \frac{k_m}{\gamma_m} \right) \\ 0 & \mu_\tau \end{bmatrix},$$

and from (19) we obtain

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)] &= \frac{1}{\mu_\tau} \left(\frac{\frac{(1-\hat{f}(\gamma_m)) k_m(1-\hat{f}(\gamma_m))}{\gamma_m 2\gamma_m}}{(1 - \frac{\hat{f}(\gamma_m)}{2})} + \frac{k_m(\hat{f}(\gamma_m) - 1)}{\gamma_m^2} + \mu_\tau \frac{k_m}{\gamma_m} \right) \\ &= \frac{k_m}{\gamma_m} \left(1 - \frac{1 - \hat{f}(\gamma_m)}{2\gamma_m \mu_\tau (1 - \frac{\hat{f}(\gamma_m)}{2})} \right), \end{aligned}$$

which is (22). □

We use the formulas presented in Theorem 2 to investigate variability in the level of a stable mRNA whose half-life is considerably longer than the mean cell division time (i.e., $\gamma_m \mu_\tau \ll 1$). Taking the limit as $\gamma_m \rightarrow 0$ for positive values of γ_m in (21) and (23) we obtain

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)] &= \frac{k_m \mu_\tau (3 + CV_\tau^2)}{2}, \\ \lim_{t \rightarrow \infty} CV^2_{m(t)} &= \frac{1}{27} + \frac{4(9\mathbb{E}[\tau^3]/\mu_\tau^3 - 9 - 6CV_\tau^2 - 7CV_\tau^4)}{27(3 + CV_\tau^2)^2}, \end{aligned} \tag{24}$$

where CV_τ^2 and $\mathbb{E}[\tau^3]$ denote the coefficient of variation squared and the third order moment of the cell division time, respectively. To understand how CV_τ^2 affects mRNA statistics we consider two extreme scenarios: exponential and deterministic cell division times ($CV_\tau \rightarrow 0$). For exponentially distributed cell division times ($CV_\tau = 1, \frac{\mathbb{E}[\tau^3]}{\mu_\tau^3} = 6$), (24) reduces to

$$\lim_{t \rightarrow \infty} \mathbb{E}[m(t)] = 2k_m \mu_\tau, \quad \lim_{t \rightarrow \infty} CV^2_{m(t)} = \frac{1}{3}. \tag{25}$$

In contrast, for deterministic cell division times ($CV_\tau = 0, \frac{\mathbb{E}[\tau^3]}{\mu_\tau^3} = 1$), (24) yields

$$\lim_{t \rightarrow \infty} \mathbb{E}[m(t)] = \frac{3k_m \mu_\tau}{2}, \quad \lim_{t \rightarrow \infty} CV^2_{m(t)} = \frac{1}{27}. \tag{26}$$

Comparison of (25) and (26) reveals that as cell division times become more and more precise ($CV_\tau^2 \rightarrow 0$), the steady-state mean mRNA level and mRNA CV^2 decrease and approach non-zero limits.

Next consider a more realistic scenario where cell division times are lognormally distributed with mean μ_τ and coefficient of variation squared CV_τ^2 . Recall that for a lognormal distribution $\mathbb{E}[\tau^3] = \mu_\tau^3(1 + CV_\tau^2)^3$. Assuming $CV_\tau^2 \ll 1$ and ignoring higher order terms in CV_τ^2 , we obtain from (24)

$$\lim_{t \rightarrow \infty} CV^2_{m(t)} = \frac{1}{27} + \frac{28}{81} CV_\tau^2. \tag{27}$$

Note that the first term on the right-hand-side of (27) represents variability in mRNA level when $CV_\tau^2 \rightarrow 0$, and the second term denotes additional variability arising due to randomness in cell division times.

Remark 1 The expressions provided in Theorem 2 can be used to confirm that (20) holds in general. We restrict ourselves to the expected value of the mRNA levels, one of the components of vector x . From (5) we conclude that the mRNA levels immediately after two consecutive divisions are related by

$$m(t_{k+1}) = \frac{1}{2} \left(e^{-\gamma_m \tau_k} m(t_k) + \frac{k_m(1 - e^{-\gamma_m \tau_k})}{\gamma_m} \right), \quad k \in \mathbb{N}_0.$$

Taking expected values on both sides of this equation we can conclude that

$$\mathbb{E}[m(t_{k+1})] = \frac{1}{2} \left(\hat{f}(\gamma_m) \mathbb{E}[m(t_k)] + \frac{k_m(1 - \hat{f}(\gamma_m))}{\gamma_m} \right). \tag{28}$$

We have that $\lim_{k \rightarrow \infty} \mathbb{E}[m(t_k)]$ exists under the conditions of Proposition 1, which is evident from the proof of this proposition given in the appendix. Assuming that it exists we conclude from (28) that it is given by

$$\lim_{k \rightarrow \infty} \mathbb{E}[m(t_k)] = \bar{m}_\infty, \quad \bar{m}_\infty := \frac{k_m(1 - \hat{f}(\gamma_m))}{2\gamma_m(1 - \frac{1}{2}(\hat{f}(\gamma_m)))},$$

which is different from (22). Since $m(t_k) = \frac{1}{2}m(t_k^-)$, $k \in \mathbb{N}$, we have

$$\lim_{k \rightarrow \infty} \mathbb{E}[m(t_k^-)] = 2\bar{m}_\infty,$$

which is also different from (22).

3.3 Exponential and Erlang distributions

In this section we show that when the distribution of the intervals between divisions is an exponential distribution with mean μ_τ and rate $\lambda = \frac{1}{\mu_\tau}$,

$$f(s) = \lambda e^{-\lambda s}, \quad s \in \mathbb{R}_{\geq 0},$$

or more generally an Erlang distribution with mean μ_τ and shape $\kappa \in \mathbb{N}$,

$$f(s) = \frac{(\kappa\lambda)^\kappa s^{\kappa-1}}{(\kappa - 1)!} e^{-\kappa\lambda s}, \quad s \in \mathbb{R}_{\geq 0},$$

where $\lambda = \frac{1}{\mu_\tau}$, the statistical moments can be obtained by solving linear differential equations. Note that this is simpler than solving the Volterra equation (8), which is required in the general case, and also facilitates the computation of asymptotic moments. While considering an exponential distribution is not biologically relevant, by properly choosing the shape parameter an Erlang distribution we will be able to accurately fit experimental data, as we shall see in Sect. 4. Still considering exponential distributions is relevant from a mathematical point of view and it allows us to compare the mRNA and protein counts obtained in this case with other biological relevant distributions.

The result for exponential distributions is given next.

Theorem 3 *When f corresponds to an exponential distribution with mean $\mu_\tau = \frac{1}{\lambda}$ the solution to (8) coincides with the solution to the linear differential equation*

$$\dot{\Phi}(t) = \Phi(t)(A + \lambda(J - I)), \quad \Phi(0) = I. \tag{29}$$

Hence

$$\lim_{t \rightarrow \infty} \Phi(t) = \begin{bmatrix} 0 & u \\ 0 & 1 \end{bmatrix}, \tag{30}$$

$$\lim_{t \rightarrow \infty} x(t) = \begin{bmatrix} u \\ 1 \end{bmatrix}, \tag{31}$$

where u is the unique vector such that

$$(A + \lambda(J - I)) \begin{bmatrix} u \\ 1 \end{bmatrix} = 0.$$

Proof When f is exponential (8) boils down to

$$\Phi(t) = \int_0^t \Phi(t - s) J e^{(A - \lambda I)s} \lambda ds + e^{(A - \lambda I)t}. \tag{32}$$

By inspection we conclude that $\Phi(0) = I$. The solution to this equation Φ is continuous [cf. Gripenberg et al. (1990), Th. 17, Ch. 3] and this implies that the right-hand side of (32) is differentiable, which in turn implies that Φ is differentiable. The derivative

with respect to t of the first term in the right-hand side of (32) is given by

$$\begin{aligned} \frac{d}{dt} \int_0^t \Phi(t-s) J e^{(A-\lambda I)s} \lambda ds &= J e^{(A-\lambda I)t} \lambda + \int_0^t -\frac{d}{ds} \Phi(t-s) J e^{(A-\lambda I)s} \lambda ds \\ &= \lambda \Phi(t) J + \int_0^t \Phi(t-s) J e^{(A-\lambda I)s} \lambda ds (A - \lambda I), \end{aligned} \tag{33}$$

where in the last equality we used integration by parts. Hence differentiating (32) we obtain

$$\begin{aligned} \frac{d}{dt} \Phi(t) &= \lambda \Phi(t) J + \int_0^t \Phi(t-s) J e^{(A-\lambda I)s} \lambda ds (A - \lambda I) + e^{(A-\lambda I)t} (A - \lambda I), \\ &= \lambda \Phi(t) J + \Phi(t) (A - \lambda I), \end{aligned}$$

which is (29). To prove (30) note that the solution to (29) is $\Phi(t) = e^{(A+\lambda(J-I))t}$ and that A has negative eigenvalues except for an eigenvalue at zero, whose corresponding left and right eigenvectors are $[0 \ 1]$ and $[u^T \ 1]^T$. Hence

$$\lim_{t \rightarrow \infty} \Phi(t) = \lim_{t \rightarrow \infty} e^{(A+\lambda(J-I))t} = \begin{bmatrix} u \\ 1 \end{bmatrix} [0 \ 1],$$

which is (30). Since the last component of $x(t)$ is equal to one for every positive time t we conclude (31). □

Theorem 3 provides a simple method to compute the asymptotic moments: it requires only to compute the kernel of the matrix $(A + \lambda(J - I))$. By doing this when $n = 2$ we can conclude that

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)] &= m_\infty, \quad m_\infty = \frac{k_m}{\frac{\lambda}{2} + \gamma_m}, \\ \lim_{t \rightarrow \infty} \mathbb{E}[m(t)^2] &= m_{2,\infty}, \quad m_{2,\infty} = \frac{k_m m_\infty}{\frac{3\lambda}{8} + \gamma_m}, \\ \lim_{t \rightarrow \infty} \mathbb{E}[p(t)] &= p_\infty, \quad p_\infty = \frac{k_m k_p}{(\frac{\lambda}{2} + \gamma_m)(\frac{\lambda}{2} + \gamma_p)}, \\ \lim_{t \rightarrow \infty} \mathbb{E}[p(t)m(t)] &= pm_\infty, \quad pm_\infty = \frac{k_m m_\infty}{\frac{3\kappa}{8\theta} + \gamma_m}, \\ \lim_{t \rightarrow \infty} \mathbb{E}[p(t)^2] &= p_{2,\infty}, \quad p_{2,\infty} = \frac{k_p (k_p m_{2,\infty} + k_m p_\infty)}{(\gamma_p + \frac{3\lambda}{8})(\gamma_m + \gamma_p + \frac{3\lambda}{4})}. \end{aligned}$$

In fact, for $n = 2$ one can check that

$$(A + \lambda(J - I)) [p_{2,\infty} \ pm_{\infty} \ m_{2,\infty} \ p_{\infty} \ m_{\infty} \ 1]^T = 0.$$

The asymptotic coefficients of variations squared are then given by

$$\begin{aligned} \lim_{t \rightarrow \infty} CV_{m(t)}^2 &= \frac{\frac{1}{2} + \gamma_m \mu_\tau}{\frac{3}{8} + \gamma_m \mu_\tau} - 1 \\ \lim_{t \rightarrow \infty} CV_{p(t)}^2 &= \frac{\left(\frac{1}{2} + \gamma_m \mu_\tau\right) \left(\frac{1}{2} + \gamma_p \mu_\tau\right) \left(\frac{7}{8} + \gamma_p \mu_\tau + \gamma_m \mu_\tau\right)}{\left(\frac{3}{8} + \gamma_m \mu_\tau\right) \left(\frac{3}{8} + \gamma_p \mu_\tau\right) \left(\frac{3}{4} + \gamma_m \mu_\tau + \gamma_p \mu_\tau\right)} - 1 \end{aligned}$$

where $\mu_\tau = \frac{1}{\lambda}$. We can then conclude that when the distribution of cell division intervals is exponential, we have

$$\begin{aligned} 0 < \lim_{t \rightarrow \infty} CV_{m(t)}^2 &< \frac{1}{3}, \\ 0 < \lim_{t \rightarrow \infty} CV_{p(t)}^2 &< \frac{29}{27}, \end{aligned}$$

where the upper bounds correspond to limit values as γ_m, γ_p become small with respect to the cell division rate λ , i.e., $\gamma_m \mu_\tau \rightarrow 0, \gamma_p \mu_\tau \rightarrow 0$, and the lower bounds corresponds to limit values as γ_m, γ_p become large with respect to the cell division rate.

For Erlang distributions we have the following result, analogous to Theorem 3. Let

$$\Phi^1(t) := \int_0^t \Phi(t - s) J e^{As} \kappa \lambda e^{-\kappa \lambda s} ds + e^{At} e^{-\kappa \lambda t}, \tag{34}$$

where $\Phi(t)$ is defined in (7) and for $2 \leq \ell \leq \kappa$ let $\Phi^\ell(t)$ be defined recursively as

$$\Phi^{\ell+1}(t) := \int_0^t \Phi^\ell(t - s) e^{As} \kappa \lambda e^{-\kappa \lambda s} ds + e^{At} e^{-\kappa \lambda t}, \quad 1 \leq \ell \leq \kappa - 1. \tag{35}$$

Theorem 4 *When f corresponds to an Erlang distribution with mean $\mu_\tau = \frac{1}{\lambda}$ and shape κ the solution to (8) coincides with $\Phi^\kappa(t)$ defined in (34), (35) and the $\Phi^\ell(t), 1 \leq \ell \leq \kappa$, can be obtained from the linear differential equation*

$$\frac{d}{dt} \bar{\Phi}(t) = \bar{\Phi}(t) P(A, J), \quad \bar{\Phi}(0) = [l_n \ l_n \ \dots \ l_n], \tag{36}$$

where

$$\bar{\Phi}(t) := [\Phi^1(t) \ \Phi^2(t) \ \dots \ \Phi^\kappa(t)], \tag{37}$$

and

$$P(A, J) := \begin{bmatrix} A - \kappa \lambda I_r & \kappa \lambda I_r & 0 & 0 & \dots & 0 \\ 0 & A - \kappa \lambda I_r & \kappa \lambda I_r & 0 & \ddots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \dots & 0 & A - \kappa \lambda I_r & \kappa \lambda I_r \\ \kappa \lambda J & 0 & 0 & \dots & 0 & A - \kappa \lambda I_r \end{bmatrix}.$$

□

Proof We can establish that

$$\Phi^\ell(t) = \int_0^t \Phi(t-s) J e^{As} \frac{(\kappa \lambda)^\ell s^{\ell-1}}{(\ell-1)!} e^{-\kappa \lambda s} ds + e^{At} \int_t^\infty \frac{(\kappa \lambda)^\ell s^{\ell-1}}{(\ell-1)!} e^{-\kappa \lambda s} ds \quad (38)$$

by induction. It holds for $\ell = 1$ and assuming it holds for a given $\ell - 1 < \kappa$, we can replace the right-hand side of (38) in the right-hand side of (35) obtaining (38) when ℓ is replaced by $\ell + 1$. Then, it is clear by uniqueness of solution to (8) that $\Phi^\kappa(t) = \Phi(t)$.

The differential equation (36) follows from differentiating (35) using similar arguments to the ones used in the proof of Theorem 3 to obtain (33) leading to

$$\frac{d}{dt} \Phi^\ell(t) = \begin{cases} \kappa \lambda \Phi(t) J + \Phi^\ell(t) (A - \kappa \lambda I), & \text{if } \ell = 1, \\ \kappa \lambda \Phi^{\ell+1}(t) + \Phi^\ell(t) (A - \kappa \lambda I), & \text{if } 2 \leq \ell \leq \kappa. \end{cases} \quad (39)$$

□

Similarly to Theorem 3 we can obtain the asymptotic behavior of the statistical moments of mRNA and protein levels by studying the asymptotic behavior of linear system (39).

4 Numerical results

For a particular cell type, recent experiments have approximated the distribution for the time intervals between cell divisions as a log-normal distribution with mean $\mu_\tau = 9.3$ h and standard deviation 2.54 h [Hawkins et al. (2009), Fig. 1d]. For the gene-expression model (1), (2) we consider the following parameters normalized by the mean cell division time $\mu_\tau = 9.3$ h:

$$\gamma_m = \frac{5}{\mu_\tau}, \quad \gamma_p = \frac{1}{\mu_\tau}, \quad \kappa_m = \frac{20}{\mu_\tau}, \quad \kappa_p = \frac{100}{\mu_\tau}.$$

These values imply a 10 h and a 2 h protein and mRNA half-life, respectively. We use the results of the present paper to compute the first two steady-state moments of

mRNA and protein levels, and the corresponding steady-state coefficients of variation. This gives

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)] &= 3.6027, & \lim_{t \rightarrow \infty} \mathbb{E}[p(t)] &= 218.7366, \\ \lim_{t \rightarrow \infty} \mathbb{E}[m(t)^2] &= 13.2270, & \lim_{t \rightarrow \infty} \mathbb{E}[p(t)^2] &= 5.0519 \times 10^4, \\ \lim_{t \rightarrow \infty} CV_{m(t)} &= 0.1381, & \lim_{t \rightarrow \infty} CV_{p(t)} &= 0.2364. \end{aligned} \tag{40}$$

A coefficient of variation of 0.2364 corresponds to a standard deviation that is about 24 % of the mean, showing significant heterogeneity in protein levels can be generated by randomness in the cell division process. Since our results also allow us to compute statistical moments of higher order we can also compute skewness and kurtosis. This gives

$$\begin{aligned} \lim_{t \rightarrow \infty} SK_{m(t)} &= -1.5168, & \lim_{t \rightarrow \infty} SK_{p(t)} &= 0.1779, \\ \lim_{t \rightarrow \infty} KU_{m(t)} &= 2.1554, & \lim_{t \rightarrow \infty} KU_{p(t)} &= 4.3484. \end{aligned}$$

A negative skewness for the mRNA level indicates that the corresponding probability distribution of mRNA leans to values smaller than the mean, and a positive skewness for the protein level indicates that the corresponding probability distribution leans to values larger than the mean.

Figure 1 depicts the transitory behavior of mRNA and protein levels starting from initial zero values. The plot for the mean values and corresponding standard deviations is obtained by using a numerical method (Linz 1985) to solve the Volterra equation (8) when $n = 2$. Note that the convergence to the steady state values is fast, i.e., the mean mRNA and protein values are already very close to the asymptotic values after a time corresponding to three times the mean inter-division interval. Two different realizations of the time evolution are also shown in Fig. 1.

Next, we investigate how the moments vary with the distribution. We assume that instead of the log-normal distribution considered above, the distribution of the intervals between divisions corresponds to an Erlang distribution with shape κ and we analyze how the shape of the distribution affects the coefficient of variation. We approximate the log-normal distribution above by an Erlang distribution. The coefficient of variation squared (CV_{τ}^2) of the log-normal distribution is given by $(2.54/9.3)^2 = 1/13.406$. Since the CV_{τ}^2 of the Erlang distribution is $1/\kappa$, an Erlang distribution with $\kappa \approx 13$ provides a good approximation. This approximation is illustrated in Fig. 3.

The fact that this is indeed a good approximation can be confirmed by computing the steady state second order moments and coefficients of variation leading to

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)] &= 3.6029, & \lim_{t \rightarrow \infty} \mathbb{E}[p(t)] &= 218.8723, \\ \lim_{t \rightarrow \infty} \mathbb{E}[m(t)^2] &= 13.2291, & \lim_{t \rightarrow \infty} \mathbb{E}[p(t)^2] &= 5.0601 \times 10^4, \\ \lim_{t \rightarrow \infty} CV_{m(t)} &= 0.1382, & \lim_{t \rightarrow \infty} CV_{p(t)} &= 0.2372. \end{aligned}$$

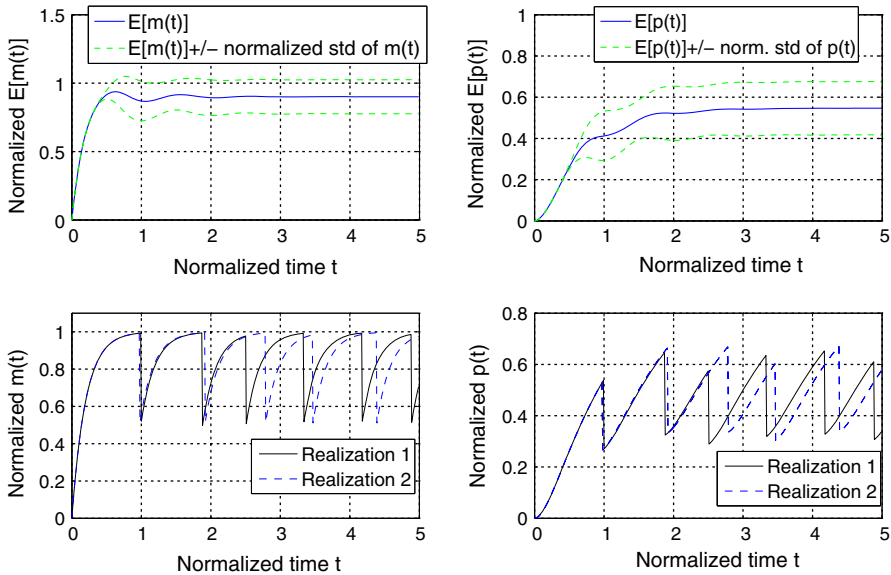


Fig. 1 Transitory behaviour of mRNA and protein levels for cell division intervals following a log normal inter-division distribution. Initial protein and mRNA counts are set to zero. Above Expected mRNA and protein counts. The dashed plots corresponds to mean plus/minus the standard deviation. Below Two realizations of mRNA and protein counts. The mRNA count $m(t)$ is normalized by the maximum value $\frac{k_m}{\gamma_m} = 4$ and the protein count $p(t)$ is normalized by the maximum value $\frac{k_m k_p}{\gamma_m \gamma_p} = 400$. Time t is normalized by the expected inter-division time length $\mu_\tau = 9.3$ h

The values are in fact very similar to (40). Also, one can plot the mean mRNA and protein levels starting at zero levels and show that they are almost coincident with those of Figure 3.

Figure 2 show analogous plots to Fig. 1 but for exponentially distributed division intervals with normalized rate $\lambda = 1$, which corresponds to an Erlang distribution with shape $\kappa = 1$. Note that the realizations are now very different from the case of log-normal distributed division intervals, and the plots shows significantly more variability. This can be confirmed by computing the steady-state second order moments

$$\begin{aligned} \lim_{t \rightarrow \infty} \mathbb{E}[m(t)] &= 3.6364, & \lim_{t \rightarrow \infty} \mathbb{E}[p(t)] &= 242.4242, \\ \lim_{t \rightarrow \infty} \mathbb{E}[m(t)^2] &= 13.5307, & \lim_{t \rightarrow \infty} \mathbb{E}[p(t)^2] &= 6.6818 \times 10^4, \\ \lim_{t \rightarrow \infty} CV_{m(t)} &= 0.1525, & \lim_{t \rightarrow \infty} CV_{p(t)} &= 0.3701. \end{aligned}$$

Note that the coefficients of variation are much larger from the previous case, considering log-normal distributed cell division intervals [see (40)].

In Fig. 4 we plot mRNA/protein means and CV for Erlang distributed cell division times with a unit mean and increasing shape parameter κ (which corresponds to decreasing variability of the Erlang distribution since $CV_\tau^2 = \frac{1}{\kappa}$). The moments first sharply decrease with increasing κ but then saturate to a lower limit for larger values

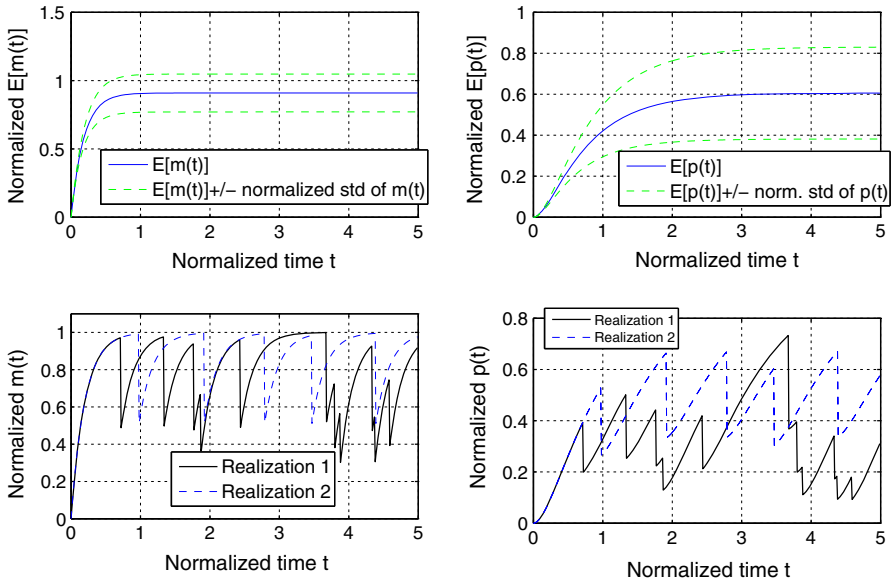
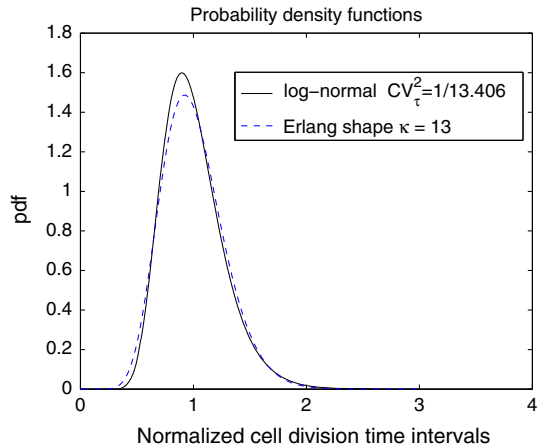


Fig. 2 Same as Fig. 1, but for inter-division following an exponential distribution with normalized rate parameter $\lambda = 1$

Fig. 3 Erlang distribution with shape parameter 13 approximating a log-normal distribution with $CV_{\tau}^2 = \frac{1}{13.406}$. Both distributions are normalized to have an unitary mean, i.e., the horizontal axis is normalized by μ_{τ}

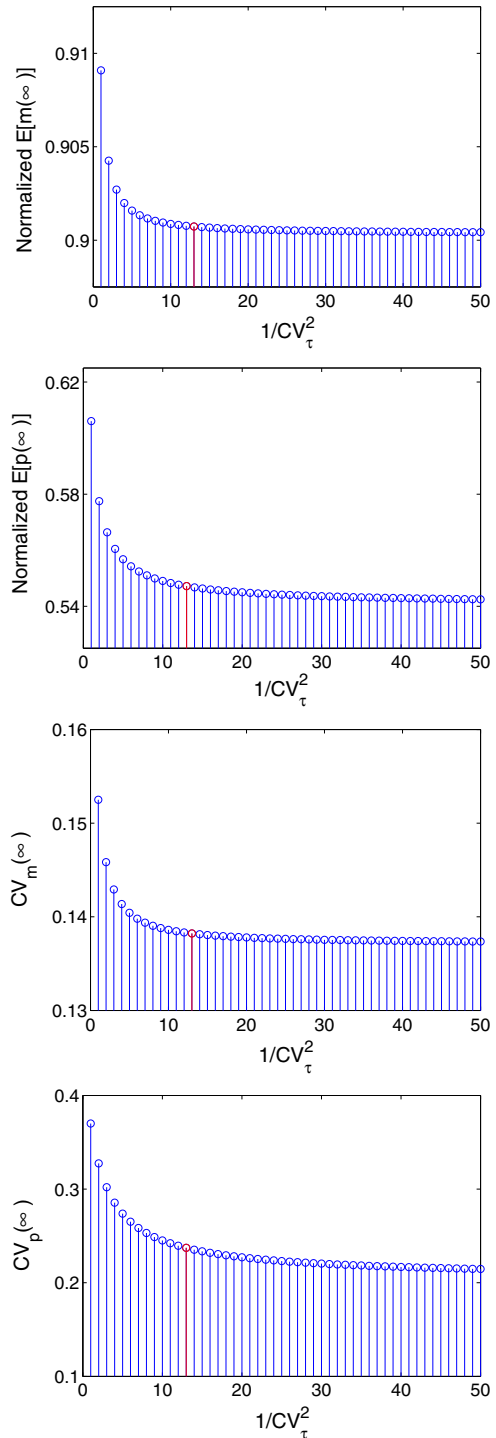


of κ . Note that such value is different from zero, although the probability that cell division intervals take values close to the mean approaches one as $\kappa \rightarrow \infty$. However, for each fixed κ even a small uncertainty on the inter-division intervals creates a large uncertainty on expected moments as $t \rightarrow \infty$.

5 Discussion and future work

Genome-wide quantification of protein half-lives has revealed that many gene encode stable proteins are not actively degraded but diluted through the process of cell divi-

Fig. 4 Steady-state mean and coefficient of variation of mRNA and protein population counts as a function of the shape of the Erlang inter-divisions distribution $\kappa = \frac{1}{CV_\tau^2}$ for an unitary mean. The shape of the Erlang distribution coincides with the squared inverse of the corresponding coefficient of variation for unitary mean. The mRNA count $m(t)$ is normalized by the maximum value $\frac{k_m}{\gamma_m} = 4$ and the protein count $p(t)$ is normalized by the maximum value $\frac{k_m k_p}{\gamma_m \gamma_p} = 400$. Increasing κ reduces the variance in the cell division time distributions and leads to lower variability in mRNA/protein levels



sion (Schwanhausser et al. 2011). Variations in cell division time between otherwise identical cells is bound to generate intercellular differences in the copy numbers of stable proteins. To quantify these intercellular differences we considered a renewal model of gene expression where mRNA/protein levels evolve deterministically, and reduce by half every time the cell divides into daughter cells. Stochasticity enters our model through the time interval between cell division, which is assumed to be an arbitrary random variable. We provided a method to obtain both the time-evolution and steady-state statistical moments of the mRNA/protein levels.

We have shown how to obtain explicit analytical expressions for the steady-state moments (see Theorems 1 and 2). Analytical expressions were useful in understanding how stochastic variability is connected to underlying model parameters. In particular, our results show that as the time interval between cell divisions becomes more deterministic, the moment dynamics becomes more oscillatory and both steady-state means/ CV converge to lower values. Formulas reported here would be useful in quantifying how much of the observed expression variability in a gene of interest can be attributed to random cell division versus the inherent stochasticity in the transcription/translation processes. Calculations using distributions obtained from experiments reveals that for a typical gene that encodes an unstable mRNA but stable protein (protein half-life = mean cell division time), randomness in the cell division process can be a significant factor in creating intercellular variability in protein levels (standard deviation in protein level was 25 % of the mean level). Note that this variability would be even higher for stable mRNAs or proteins with longer half-lives.

As many mRNAs/proteins are present at low-copy numbers inside cells two additional sources of noise come into play: (i) stochastic birth-death of individual mRNA/protein molecules and (ii) stochastic partitioning of molecules between daughter cell at the time of cell division, which could be modeled by a binomial distribution (Berg 1978). A recent study has quantified the extent of intercellular variability in protein levels arising from stochastic partitioning at cell division assuming a perfectly synchronized cell population, i.e., each cell is in the exact same cell cycle position (Huh and Paulsson 2011). In our work we considered an opposite scenario where both protein production/partitioning are modeled deterministically, and expression variability arises solely from desynchronization of cells due to random cell division times. An important direction of future work is to consider complex models of gene expression that incorporate stochastic synthesis with stochastic partitioning of molecules at arbitrary cell division times. Analysis of such models will enable a systematic understanding of how different source noise combine to drive non-genetic heterogeneity in mRNA/protein levels.

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6 Appendix

Proof (of Proposition 2) Let $z_k := [m(t_k^-) \ p(t_k^-)]^T, \forall k \in \mathbb{N}$, and note that

$$z_{k+1} = \mathcal{M}(z_k, \tau_k), \forall k \in \mathbb{N},$$

where

$$\mathcal{M}\left(\begin{bmatrix} a \\ b \end{bmatrix}, h\right) = \begin{bmatrix} e^{-\gamma_m h} \frac{a}{2} + \frac{k_m(1-e^{-\gamma_m h})}{\gamma_m} \\ e^{-\gamma_p h} \frac{b}{2} + \kappa_p \int_0^h e^{-\gamma_p(h-s)} \left(e^{-\gamma_m s} \frac{a}{2} + \frac{k_m(1-e^{-\gamma_m s})}{\gamma_m} \right) ds \end{bmatrix} \tag{41}$$

for positive a and b and $h \in (\underline{\tau}, \bar{\tau})$. We can assume without loss of generality that $z_1 \in \mathcal{I}_1$ where $\mathcal{I}_1 = [0, \frac{k_m}{\gamma_m}] \times [0, \frac{k_p}{\gamma_p}]$. In fact, one can conclude from (1), (2) that if the initial mRNA and protein counts do not belong to \mathcal{I}_1 then $(m(t_k), p(t_k)) \in \mathcal{I}_1$ after a sufficiently large time $k \in \mathbb{N}$ with probability one. Moreover if $(m(0), p(0)) \in \mathcal{I}_1$ then $(m(t), p(t)) \in \mathcal{I}_1$ for every time $t \in \mathbb{R}_{\geq 0}$. In particular $z_1 \in \mathcal{I}_1$. Now, if $z_1 \in \mathcal{I}_1$ then $z_k \in \mathcal{I}_k$ where the sets $\mathcal{I}_k, k \in \mathbb{N}$ are defined recursively

$$\mathcal{I}_{k+1} = \{\mathcal{M}(y, h) | y \in \mathcal{I}_k, h \in (\underline{\tau}, \bar{\tau})\}, \forall k \in \mathbb{N}.$$

From (41) we conclude that $\mathcal{I}_{k+1} \subseteq \mathcal{I}_k, \forall k \in \mathbb{N}$, and we can also conclude that $\bigcap_{k=0}^{\infty} \mathcal{I}_k$ has non-empty interior. Choose then an open set A in $\bigcap_{k=0}^{\infty} \mathcal{I}_k$. By construction the Markov chain z_k is irreducible [see Meyn and Tweedie (2009), Ch. 4] with respect to the indicator function of set A , which is equivalent to saying that one can reach any open set in A for any initial condition $z_1 \in \mathcal{I}_1$. One can also conclude that for any initial condition $z_1 \in \mathcal{I}_1$, the state $z_k, k \in \mathbb{N}$, visits the set A in infinite number of times and that the Markov chain is aperiodic meaning that it does not take values in disjoint sets visited periodically. This implies that the chain is Harris recurrent [see Meyn and Tweedie (2009), Ch. 9] and admits a unique invariant measure π_{MC} (which can be made a probability measure by properly scaling since the chain takes values in a bounded set) and, from the aperiodic ergodic theorem [cf. Meyn and Tweedie (2009), p. 309], we have

$$\lim_{n \rightarrow \infty} \mathbf{P}^n(z_1, B) = \pi_{MC}(B),$$

for any initial condition $z_1 \in \mathcal{I}_1$ and for any open set $B \subseteq \mathcal{I}_1$, where $\mathbf{P}^n(y, B) := \text{Prob}(z_{n+1} \in B | z_1 = y)$ for $n \in \mathbb{N}$. Using the results in (Antunes et al. 2013), one can prove that the process $w(t) := (m(t), p(t), \xi(t))$ where $\xi(t) := t - t_{N(t)}, N(t) := \max\{k \in \mathbb{N}_0 : t_k \leq t\}$ is the time since the last division, is a Piecewise deterministic process in the sense of (Davis 1993). Then z_k is the so called imbedded Markov chain of this process and using the connection between stationary distribution of the embedded chain and of the piecewise deterministic process (Davis 1993) one can conclude that the piecewise deterministic process also has an invariant distribution π_{PD} . Then for any function $g(m, p) = m^{n_1} p^{n_2}, n_1, n_2 \in \mathbb{N}$, we have

$$\lim_{t \rightarrow \infty} \mathbb{E}[g(m(t), p(t))] = \int g(m, p) \pi_{PD}(dw), \quad w = (m, p, \xi),$$

which concludes the proof. \square

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