Probing Noise in Gene Expression and Protein Production

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We derive exact solutions of simplified models for the temporal evolution of the protein concentration within a cell population arbitrarily far from the stationary state. We show that monitoring the dynamics can assist in modeling and understanding the nature of the noise and its role in gene expression and protein production. We introduce a new measure, the cell turnover distribution, which can be used to probe the phase of transcription of DNA into messenger RNA.

Advances in experimental techniques, that enable the direct observation of gene expression in individual cells, have demonstrated the importance of stochasticity in gene expression, the translation into proteins of the information encoded within DNA.1–5 Such variability can lead to deleterious effects in cell function and cause diseases.6 On the positive side, stochasticity in gene expression confers on cells the ability to be responsive to unexpected stresses and may augment growth rates of bacterial cells compared to homogeneous populations.7 Disentangling the various contributions to production fluctuations is further complicated by the recent finding that different stochastic processes
yield the same response in the variance in protein abundance at stationarity.\textsuperscript{8} A population of isogenic cells growing under the same environmental conditions can exhibit protein abundances that vary greatly from cell to cell. The sources of variability have been identified at multiple levels,\textsuperscript{9–13} with transcription and translation playing a major role under certain circumstances.\textsuperscript{14–16}

The low concentration of reactants potentially has two important consequences: the first is that fluctuations around the mean can be large; the second is that the nature of the stochastic noise should be taken into account in some detail because one may not simply invoke the central limit theorem\textsuperscript{17} which leads to the universal and ubiquitous Gaussian noise. Thus, two genes expressed at the same average abundance can produce populations with different phenotypic noise strengths, defined as the ratio of the variance over the mean value of the number of proteins.\textsuperscript{18} We show here that two distinct models, one taking into account the detailed nature of the noise and the other following from an application of the central limit theorem, yield exactly the same stationary solution for the distribution of proteins in isogenic cells under the same environmental conditions. The exact dynamical solution of these two simplified models demonstrate the value of monitoring the dynamics for understanding the nature of the noise in a cell.

We make the simplified assumption that the kinetics of gene expression can be described approximately by four rate constants: $k_1$ and $k_2$ are the transcription and translation rates, respectively and $\gamma_1$ and $\gamma_2$ are the degradation rates for mRNA and proteins, respectively. It has been found experimentally that proteins are produced in bursts\textsuperscript{5, 18–20} with an exponential distribution of the number of proteins produced in a given event. Following Paulsson \textit{et al.}\textsuperscript{21} and Friedman \textit{et al.}\textsuperscript{22} we will assume that transcription pulses are Poisson events and that the probability distribution that in a single event $I > 0$ proteins are produced, $w(I)$, is approximated by $w(I) = \frac{\gamma_1}{k_2} e^{-\frac{\gamma_1}{k_2} I}$, where $k_2/\gamma_1$ is the translation efficiency, i.e. the mean number of proteins produced in a given burst. Here we consider a simple model for the production of proteins without memory and aging of molecules. Using the specific burst distribution given above allows one to obtain the shape of the protein distribution even far from stationarity. Under these assumptions, the stochastic equation that governs the single-variable dynamics of gene expression, can be written as

$$\dot{x}(t) = \delta - \gamma_2 x(t) + \Lambda(t).$$
Figure 1: The stationary distribution of proteins in a prokaryotic cell population taken from Ref. 18 fitted to Eq. (3) with $\delta = 0$ or $\delta / \gamma_2 = 60.3$ (dashed). The best fit parameters are $\gamma_1/k_2 = 0.038$, $k_1/\gamma_2 = 12.88$ ($\chi^2 \simeq 6100$) and $\gamma_1/k_2 = 0.030$, $k_1/\gamma_2 = 8.33$ ($\chi^2 \simeq 8700$), respectively. From the experimental data it is hard to distinguish between the steady state distributions predicted by Eq. (3) with $\delta = 0$ and $\delta > 0$.

This pseudo-equation describes the real-time stochastic evolution of gene expression through a deterministic part and a stochastic term $\Lambda(t)$, which will be defined later on. Here $x$ is a continuous variable that represents the number of proteins within a cell. $\delta$ is a term added for generality which can be incorporated in the average noise.

In order to understand the nature of the noise for the gene expression case, let us consider the random variable, $I_k$, that is a measure of the number of proteins in the $k^{th}$ transcription event, where $k = 1, 2, \ldots, n$. A key quantity of interest is $\sum_{k=1}^{n(t)} I_k \equiv \Lambda(t) \Delta t$ where $n(t)$, the number of events in the time interval $(t, t + \Delta t)$, is a random variable independent of both $x$ and the $I_k$s. As in the experiment, let us postulate that: i) the $I_k$s are independent and identically distributed with exponential distribution; and ii) the probability of $n$ events occurring during the time interval $\Delta t$ is given by the Poisson distribution $q_n(\Delta t) = (k_1 \Delta t)^n \exp(-k_1 \Delta t)/n!$. The distribution of $\Lambda(t)$ that we use in Eq. (1) can be explicitly calculated and leads to the following expression for the cumulants: $\langle \Lambda(t_1) \cdots \Lambda(t_n) \rangle = n! k_1 (k_2/\gamma_1)^n \prod_{i=2}^{n} \delta(t_i - t_1)$ for $n \geq 2$ and $\langle \Lambda(t) \rangle = k_1 k_2/\gamma_1$, independent of time. Because the cumulants
are delta functions, the noise is still white (events are uncorrelated if they occur at different times); however the noise is no longer Gaussian because cumulants with \( n \) greater than two are non-zero.

The master equation that describes this burst-like process is\(^\text{17}\)

\[
\frac{\partial p(x,t)}{\partial t} = -\frac{\partial}{\partial x}[(\delta - \gamma_2 x)p(x,t)] + k_1 \int_0^x w(x-y)p(y,t)\,dy - k_1 p(x,t),
\]

where \( p(x,t) \equiv p(x,t|x_0,0) \) is the conditional probability that the protein concentration has a value \( x \) at time \( t \) given that it has a value \( x_0 \) at time 0; and \( w(x) = \frac{\gamma_1}{\gamma_2} e^{-\frac{\delta}{\gamma_2} x} \). The stationary solution of this model (with \( \delta = 0 \)) was first obtained by Paulsson et al.\(^\text{21}\) and subsequently re-derived by Friedman et al.\(^\text{22}\) For arbitrary \( \delta > 0 \), we find the stationary solution is

\[
p_s(x) = \frac{\gamma_1}{k_2} \frac{k_1}{\Gamma(k_1/\gamma_2)} \left(x - \frac{\delta}{\gamma_2}\right)^{k_1/\gamma_2 - 1} e^{-\frac{\gamma_1}{\gamma_2} (x - \frac{\delta}{\gamma_2})}
\]

where \( \Theta(x) \) is the step function equal to 1 when \( x > 0 \) and zero otherwise. This distinctive feature is a sharp signature of the nature of the noise even in the stationary solution but is present only when \( \delta \neq 0 \). However, as shown in the fit to the stationary solution in Fig. (1), the singularity, if it exists, is easily masked by other noise effects leading to a rounding effect.

Although experiments on gene expression\(^\text{5,18}\) are consistent with a burst-like protein production, steady-state distributions of protein abundances are equally compatible with alternative explanations. In fact, because mRNA is unstable compared to protein lifetime (\( \gamma_1 \gg \gamma_2 \)), one can assume that transcripts give rise to a constant flux of proteins \( f \) and subsequently any protein degrades at a constant rate \( \gamma_2 \). Because of the great amount of available molecules, one can apply the central limit theorem and suppose that the amplitude of fluctuations is simply proportional to \( \sqrt{x} \). Within this framework there is no burst-like production, nevertheless the stationary solutions that one obtains for a burst-like process, including that of the extended autoregulation model,\(^\text{24,25}\) are also obtained in models with appropriately chosen random multiplicative Gaussian noise.\(^\text{23}\) Within this scenario the stochastic evolution of the protein concentration \( x(t) \) is governed by the equation

\[
\dot{x}(t) = f - \gamma_2 x(t) + \sqrt{Dx(t)} \eta(t),
\]
Figure 2: Protein distribution dynamics for different types of noise and with the same initial conditions, i.e. $x_0 = 0$ proteins at $t = 0$. The dashed curve is for the multiplicative Gaussian noise, i.e. Eq. (4) with $f \equiv k_1 k_2 / \gamma_1$ and $D \equiv \gamma_2 k_2 / \gamma_1$; whereas the other curve is for the non-Gaussian noise, i.e. for Eq. (5). In both cases the parameters are $\delta = 0$, $\gamma_2 / D = \gamma_1 / k_2 = 0.038$, $f / D = k_1 / \gamma_2 = 12.88$ and we have set $\gamma_2^{-1} = 40$ min, $\gamma_1^{-1} = 2$ min.

where $\eta(t)$ is a Gaussian white noise with autocorrelation $\langle \eta(t) \eta(t') \rangle = 2\delta(t - t')$. Note that the same equation could be obtained on setting $\langle \Lambda(t) \rangle = f$ and $\langle \langle \Lambda(t) \Lambda(t') \rangle \rangle \equiv \langle \Lambda(t) \Lambda(t') \rangle - \langle \Lambda(t) \rangle \langle \Lambda(t') \rangle = 2Dx(t)\delta(t - t')$ in Eq. (1) with all higher order cumulants being identically zero. We point out that in ecology Eq. (1) is useful for studying the evolution of tropical forests, where the detailed nature of the stochastic noise is not important because of the relatively large numbers of trees of a given species. In the field of finance, Eq. (1) has been used to study the evolution of interest rates (the Cox-Ingersoll-Ross model), where analogous considerations on fluctuations can be made. On defining $f \equiv k_1 k_2 / \gamma_1$ and $D \equiv \gamma_2 k_2 / \gamma_1$, Eq. (1) yields the same stationary state as in Eq. (2) with $\delta = 0$, i.e. Eq. (3). The mean number of proteins at stationarity is $k_1 k_2 / \gamma_1 \gamma_2$ and the phenotypic noise strength at stationarity is
In order to take into account the effects of feedback in a system undergoing auto-regulation, one can introduce the physically transparent modification $f \rightarrow Dc(x)$, where $c$ is a response function which can be modeled as having two distinct limiting values at zero and at infinity with the latter being smaller than the former. Even in this situation, we obtain the same stationary distribution with bistability as Friedman et al. Despite this much more realistic analysis, the final stationary protein distribution is experimentally indistinguishable from Eq. (3) with $\delta = 0$. Thus, a theoretical modeling of the stationary state of protein production provides little insight into the microscopic nature of the noise that leads to stationarity. According to our approach, stochasticity in gene expression ensues from the large number of available components which entangle a lot of different mechanisms within a cell. Interestingly, this calls for effective mechanisms which can dampen the deleterious effects of protein noise. Such efficient noise-reducing mechanisms could be a combination of gestation and senescence, because of their ability to prevent fluctuations rather than correcting.

These results raise the question whether the agreement between the stationary solutions of the theoretical models and experiments are in fact a direct probe of the nature of the microscopic noise and whether the asymmetric stationary solutions derive from a careful consideration of the bursty nature of the noise. In order to circumvent the indistinguishability of steady-states, one can look into empirical protein abundances far from stationarity, for which we provide analytical formulas. Thus we turn now to a study of the dynamics of Eq. (2) which is a powerful probe of the noise effects. We have derived the solution at arbitrary time,

\[ p(x, t) = e^{-k_1 t} \left[ \delta(x - \xi_t) + \Theta(x - \xi_t) \frac{k_1 \gamma_1}{k_2 \gamma_2} (e^{\gamma_2 t} - 1) e^{-k_1 t} \times \exp \left[ -\frac{\gamma_1}{k_2} e^{\gamma_2 t} (x - \xi_t) \right] {\text{1}}_F^1 \left( \frac{k_1 \gamma_2}{\gamma_2} + 1, 2; \frac{\gamma_1}{k_2} (e^{\gamma_2 t} - 1) (x - \xi_t) \right) \right), \tag{5} \]

where $\text{1}_F^1 (a, b; x)$ is the confluent hypergeometric function and $\xi(t) \equiv x_0 e^{-\gamma_2 t} + \frac{\delta}{\gamma_2} (1 - e^{-\gamma_2 t})$ is the solution of the deterministic part of the equation, i.e. without the noise. On using Eq. (5), one can calculate the phenotypic noise at any time, arbitrarily far from stationarity, and furthermore one can study its behavior starting from an arbitrary initial amount of proteins. Note that $\delta$
enters only through $\xi(t)$ and the temporal evolution scales are determined by the transcription rate and the degradation rate of proteins. Interestingly, one obtains a distribution of proteins with a cut off along the interval $[0, \xi_t]$ at any time whenever $\delta > 0$. By exploiting the exact dynamical solution one can study the evolution of the phenotypic noise strength in finer detail and probe the experimental consequences of the burst process hypothesis (Fig. 2).

A measurable quantity that directly probes the protein distribution and its temporal evolution is the cell-turnover distribution (CTD) denoted by $P(\rho, t)$ and defined as the probability that at time $t$ the ratio $x(t)/x(0)$ is equal to $\rho$, where $x(t)$ and $x(0)$ are the number of proteins within an isogenic cell population at time $t > 0$ and $t = 0$, respectively. This quantity can be defined both close to and far from stationarity: if the initial distribution of the proteins within the cell population is in the steady state given by Eq. (3) with $\delta = 0$, then at a subsequent time $t > 0$ the CTD is

$$P_{CTD}(\rho, t) = e^{-k_1 t} \delta \left( \rho - e^{-\gamma_2 t} \right) + \left( \frac{k_1}{\gamma_2} \right)^{2} \frac{e^{-k_1 t} (e^{\gamma_2 t} - 1) \Theta (\rho - e^{-\gamma_2 t})}{\left[ 1 + e^{\gamma_2 t} (\rho - e^{-\gamma_2 t}) \right]^{\frac{1}{\gamma_2} + 1}} \times \left( k_1 \gamma_2 + 1, \frac{k_1}{\gamma_2} + 1, 2; \frac{(e^{\gamma_2 t} - 1)(\rho - e^{-\gamma_2 t})}{1 + e^{\gamma_2 t} (\rho - e^{-\gamma_2 t})} \right).$$

(6)

where $_2F_1(a, b, c; x)$ is the standard hypergeometric function. Thus, according to Eq. (6), under the burst process hypothesis we predict that i) the CTD vanishes between 0 and $e^{-\gamma_2 t}$ even though the system is at stationarity, an effect which ought to be detectable for time scales less than or of the order of $1/\gamma_2$, ii) the CTD depends only on $k_1$ and $\gamma_2$ but not on translational efficiency and other rates, iii) at very large time separation there is only one free parameter, the ratio $k_1/\gamma_2$, and the CTDs predicted by the Gaussian and non-Gaussian noises become the same (fig.3).

The analogous time dependent solutions for the Gaussian white noise can be compared with Eqs. (5) and (6). The closer the system is to its steady-state, the more difficult it is to distinguish among the effects of gestation, senescence and burst-like production. Thus an experimental protocol capable of analyzing the cell population and its time evolution with different initial conditions would be helpful to disentangle the nature of stochastic noise. At early times, the evolution of the distribution is strongly affected by the specific mechanisms involved in the dynamics. At this stage, different distributions of waiting times
Figure 3: Cell Turnover Distribution (CTD). The dashed curve is for the Gaussian noise case, i.e. the CTD is calculated assuming that the governing equation is Eq. (1) with \( f \equiv k_1k_2/\gamma_1 \) and \( D \equiv \gamma_2k_2/\gamma_1 \); whereas the other curve is for the non-Gaussian noise case, i.e. for Eq. (6). In this case the arrow indicates the cut-off point. Note, however, that extrinsic noise could tend to smooth out the discontinuity. In both cases \( k_1/\gamma_2 = 12.88 \) and we have set \( \gamma_2^{-1} = 40 \) min.

between events or burst sizes produce non-stationary distributions that are very different, and the distinctive effects of noise, deterministic driving forces or coupling of degrees of freedom can be elucidated. Different conditions at initial times propagate into the early temporal evolution in strongly different ways according to the different effects of involved mechanisms, but inexorably lead to the same distribution for large time separation.

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References

[1] Kaern, M. et al., *Nature Genet.* 6, 451-464 (2005).

[2] Raser, J. M. & O’Shea, E. K., *Science* 309, 2010-2013 (2005).

[3] Paulsson, J., *Nature* 427, 415-418 (2004).

[4] Golding, I., Paulsson, J., Zawilski S. M. & Cox, E. C., *Cell* 123, 1025-1036 (2005).

[5] Cai, L., Friedman, N. & Xie, X. S., *Nature* 440, 358-362 (2006).

[6] Magee, J. A., Abdulkadir, S. A. & Milbrandt, J., *Cancer Cell* 3, 273-283 (2003).

[7] Thattai, M. & van Oudenaarden, A., *Genetics* 167, 523-530 (2004).

[8] Pedraza, J. M. & Paulsson, J., *Science* 319, 339-343 (2008).

[9] Elowitz, M. B., Levine, A. J., Siggia, E. D. & Swain, P. S., *Science* 297, 1183-1186 (2002).

[10] Raser, J. M. & O’Shea, E. K., *Science* 304, 1811-1814 (2004).

[11] Becskei, A., Kaufmann, B. B. & van Oudenaarden, A., *Nature Genet.* 37, 937-944 (2005).

[12] Rosenfeld, N. et al., *Science* 307, 1962-1965 (2005).

[13] Pedraza, J. M. & van Oudenaarden, A., *Science* 307, 1965-1969 (2005).

[14] Newman, J. R. S. et al., *Nature* 441, 840-846 (2006).

[15] Bar-Even, A. et al., *Nature Genet.* 38, 636-643 (2006).

[16] McAdams, H. H. & Arkin, A., *Proc. Natl Acad. Sci. USA* 94, 814-819 (1997).

[17] van Kampen, N. G. *Stochastic Processes in Physics and Chemistry* (Elsevier, 2004).

[18] Ozbudak, E. M., Thattai, M., Kurtser, I., Grossman, A. D. & van Oudenaarden, A., *Nature Genet.* 31, 69-73 (2002).
[19] Thattai, M. & van Oudenaarden, A., Proc. Natl Acad. Sci. USA 98, 8614-8619 (2001).

[20] Kierzek, A. M., Zaim, J. & Zielenkiewicz, P., J. Biol. Chem. 276, 8165-8172 (2001).

[21] Paulsson, J. & Ehrenberg, M., Phys. Rev. Lett. 84, 5447-5450 (2000).

[22] Friedman, N., Cai, L. & Xie, X. S., Phys. Rev. Lett. 97, 168302 (2006).

[23] See Supplementary Information for further details.

[24] Becskei, A. & Serrano, L., Nature 405, 590-593 (2000).

[25] Isaacs, F. J., Hasty, J., Cantor, C. R. & Collins, J. J., Proc. Natl Acad. Sci. USA 100, 7714-7719 (2003).

[26] Azaele, S., Pigolotti, S., Banavar, J. R. & Maritan, A., Nature 444, 926-928 (2006).

[27] Cox, J., Ingersoll, J. & Ross, S., Econometrica 53, 385-407 (1985).

[28] Lebedev, N. N. Special Functions and their Applications (Dover, 1972).