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Comparing negative feedback mechanisms in gene expression: From single cells to cell populations

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Abstract— **Negative feedback regulation is a well-known motif for suppressing deleterious fluctuations in gene product levels. We systematically compare two scenarios where negative feedback is either implemented in the protein production rate (regulated synthesis) or in the protein degradation rate (regulated degradation). Our results show that while in low-noise regimes both schemes are identical, they begin to show remarkable differences in high-noise regimes. Analytically solving for the probability distributions of the protein levels reveals that regulated synthesis is a better strategy to suppress random fluctuations while also minimizing protein levels dipping below a threshold. In contrast, regulated degradation is preferred if the goal is to minimize protein levels going beyond a threshold. Finally, we compare and contrast these distributions not only in a single cell over time but also in an expanding cell population where these effects can be buffered or exacerbated due to the coupling between expression and cell growth.**

I. Introduction

Gene expression is the process by which cells synthesize proteins based on information stored in DNA. Due to the small number of molecules involved in this process and their short lifetimes, random fluctuations or noise in protein levels are an inherent feature of gene expression. Single-cell studies have shown that noise in protein levels is physiologically relevant and affects biological function often negatively and, in other cases, positively [1]–[4]. For example, while random fluctuations can enhance population adaptation to uncertain environments [5], they can be disadvantageous when homeostasis is required around a set protein level [6]. Consequently, cells may evolve different mechanisms to suppress gene expression noise when it is detrimental [7]. In this article, we are interested in negative feedback mechanisms in which gene expression is controlled based on the intracellular protein concentration [8]–[11].

To understand how cells implement feedback, we should consider that, as a dynamical process, the gene expression process can be simplified as a result of two mechanisms: protein synthesis and protein degradation (or dilution). When these processes reach equilibrium, the protein concentration fluctuates randomly around a set point. Negative feedback can be implemented mainly in two different ways: The first occurs when an increase in protein levels results in a decrease in the synthesis rate. Another, when increasing protein levels yields a speeding up of degradation rate (Fig. 1A). These different strategies can exhibit distinct properties in terms of response time, efficiency, and stochastic behavior.

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Among the applications of negative feedback implemented through the self-regulation of protein synthesis, we can include a fast kinetic response [12], suppressing random fluctuations in protein concentrations [13], stabilizing gene expression against mutations [14], and protein synthesis on demand [15]. On the other hand, negative feedback through self-regulation of the degradation rate has found applications in sharp triggering and adaptation of the response to stress [16], and in improving molecular sensing [10].

Both negative feedback mechanisms have been mainly compared at the level of small noise approximation [17]. At this level, both mechanisms show equivalence in terms of protein statistics. However, in a regime of higher random fluctuations in protein concentration, we expect appreciable differences in the noise suppression dynamics [18]. To understand this divergence, we explore regimes beyond the small noise approximation using a combination of analytical approaches and stochastic simulations of gene expression in both single cells and in proliferating cell populations.

We start by introducing a simple one-dimensional linear system to capture the dynamics of protein levels and transform it into a nonlinear system by having either the synthesis rate, or the degradation rate be protein-regulated. Next, we consider a stochastic formulation of this system, where protein synthesis occurs in random bursts of activity, and feedback is implemented in either the frequency with which bursts occur or the rate of protein degradation in between two consecutive bursts. The simplicity of this system results in an exact analytical solution for the steady-state distributions, which we contrast between the two feedback strategies in both small and high-noise regimes. Finally, we explore, through simulations, the effects of the implementation of these feedback strategies in proliferating populations where the dilution rate is equivalent to the cell proliferation rate.

II. Deterministic formulation of self-regulation mechanisms

To get a basic understanding of the gene expression process, let us simplify it as a deterministic system following the first-order differential equation:

$$\frac{dx}{dt} = k - \gamma x, \tag{1}$$

where the positively-valued scalar $x(t)$ is the protein concentration at time t with steady-state

$$\bar{x} := \lim_{t\to\infty} x(t) = \frac{k}{\gamma}. \tag{2}$$

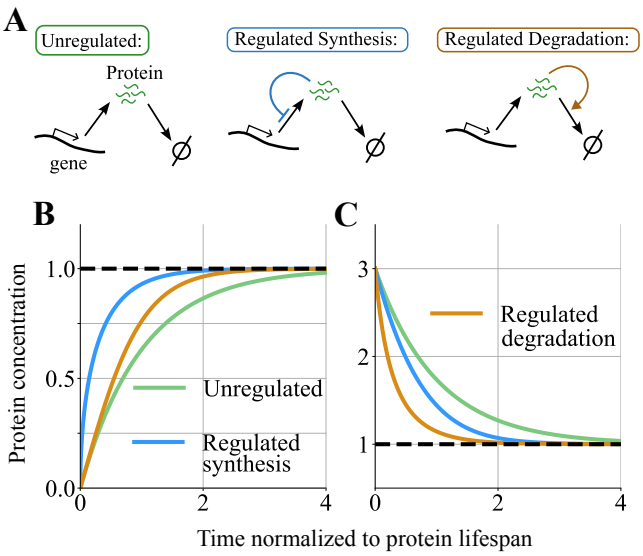

Fig. 1. **Negative feedback mechanisms in gene expression and their differential responses to deviations from equilibrium.** (**A**) In the unregulated system, production and degradation rates are constant. For regulated synthesis, the production rate decreases with the protein level and, for the regulated degradation, the degradation increases with the protein level. The mean protein level is set to 1. The solution of the nonlinear ordinary differential equations (3)-(4) are plotted over time by assuming $k=\gamma=1$ and $g(x)=x$ for a starting initial condition of $x(0)=0$ (**B**) and $x(0)=3$ (**C**).

Here k is the synthesis rate that combines both transcription/translation processes and γ is the rate at which proteins are degraded. A key assumption underlying this one-dimensional model is that the corresponding mRNAs are short-lived and are degraded rapidly compared to γ [19].

Let $g(x)$ be a positively-valued continuously-differentiable function that monotonically increases with x. Then, negative feedback control can be implemented independently in two alternative and orthogonal ways - increasing protein concentration either decreases the synthesis rate

$$\textit{Feedback in Synthesis}: \qquad \frac{dx}{dt} = k/g(x) - \gamma x, \tag{3}$$

or enhances the degradation rate

$$\textit{Feedback in Degradation}: \qquad \frac{dx}{dt} = k - \gamma g(x)x. \tag{4}$$

We further assume that $g(\bar{x})=1$, which ensures the exact same equilibrium point $\bar{x}=k/\gamma$ for both feedback scenarios.

To obtain analytical expressions, it is common to linearize the system considering small perturbations $e=x-\bar{x}$ around the equilibrium point. Hence, feedback models (3) and (4) yields the same linear system:

$$\frac{de}{dt} = -\gamma(1+s)e,\ s := \frac{\bar{x}}{g(\bar{x})}\frac{dg(x)}{dx}|_{x=\bar{x}} \geq 0. \tag{5}$$

s is the dimensionless log-sensitivity of $g(x)$ with respect to x evaluated at the equilibrium point.

Although the dynamics of feedback-regulated synthesis and degradation are identical for small perturbations, when we consider non-linearities, that is, solving (3) and (4), we observe different responses for large perturbations: While negative feedback in synthesis provides a faster response when starting from a low concentration (Fig. 1B), degradation feedback is faster in reverting the system back to equilibrium when starting from a high initial concentration (Fig. 1C). These response kinetics to large perturbations are critical in determining the extent and shape of concentration fluctuations in the stochastic model, where noise constantly "kicks" the system out of equilibrium.

III. Stochastic Formulation of Self-Regulation Mechanisms at Low-Noise Regime

In this section, we explore how protein noise is affected by different feedbacks using the Linear Noise Approximation (LNA), where $g(x)$ is linearized around the equilibrium. From this section onward, the concentration $x(t)$ will be a random process, and the angular bracket notation $\langle\ \rangle$ will denote the expected value. Therefore, noise in protein levels is measured using the squared coefficient of variation CV_x^2 and we redefine $\bar{x}$ as the mean concentration in steady state. These metrics satisfy:

$$\bar{x} := \lim_{t\to\infty}\langle x(t)\rangle; \quad CV_x^2 := \lim_{t\to\infty}\frac{\langle x^2\rangle - \langle x\rangle^2}{\langle x\rangle^2}. \tag{6}$$

A. Unregulated gene expression

Given its analytical tractability, we model protein synthesis as random bursts. The burst size B_x is related to an abrupt increase in protein concentration during translation. B_x is assumed to be an independent, identically distributed random variable with a positive-valued probability distribution and mean $\langle B_x\rangle$. Without regulation, bursts follow a Poisson process at a rate k_x (burst frequency), each increasing protein concentration by B_x. Assuming a relatively high protein count, we approximate protein dynamics as a continuous decay between bursts, following first-order kinetics [20].

These processes can be gathered in a Stochastic Hybrid System (SHS) that is conveniently represented by

$$x \xrightarrow{k_x} x + B_x, \quad \frac{dx}{dt} = -\gamma x. \tag{7}$$

To quantify the extent of fluctuation in $x(t)$ we use the tools of moment dynamics – for the SHS (7) the time evolution of uncentered moments is given by [21],

$$\frac{d\langle x^m\rangle}{dt} = \left\langle k_x\left((x+B_x)^m - x^m\right) + \frac{dx^m}{dx}(-\gamma x)\right\rangle \tag{8}$$

Simplifying (8) for $m=1$ and $m=2$ yields

$$\frac{d\langle x\rangle}{dt} = k_x\langle B_x\rangle - \gamma\langle x\rangle, \tag{9a}$$

$$\frac{d\langle x^2\rangle}{dt} = k_x\langle B_x^2\rangle + 2k_x\langle B_x\rangle\langle x\rangle - 2\gamma\langle x^2\rangle, \tag{9b}$$

where for the equivalence of (9a) to the deterministic model (1) we need the net synthesis rate $k=k_x\langle B_x\rangle$.

Solving this system of differential equations at steady-state, we obtain

$$\bar{x} = \frac{k_x \langle B_x \rangle}{\gamma} = \frac{k}{\gamma}, \quad CV_x^2 = \frac{\langle B_x^2 \rangle}{2\langle B_x \rangle \bar{x}}. \tag{10}$$

We can highlight that, if the bursts follow an exponential distribution, $\langle B_x^2 \rangle = 2\langle B_x \rangle^2$, therefore CV_x^2 is proportional to $\langle B_x \rangle$ as can be seen in Fig. 2.

B. Self-regulated synthesis

Within this framework of bursty expression, negative feedback control of protein synthesis can occur both in the burst frequency or burst size [22]. Here, we consider feedback on the arrival of bursts, and alter the burst frequency to $k_x/g(x)$. Recall that as $g(x)$ is a monotonically-increasing function, the arrival of bursts slows down with increasing protein concentrations. The moment dynamics for this case is similar to (8) with k_x now replaced by $k_x/g(x)$,

$$\frac{d\langle x^m \rangle}{dt} = \left\langle \frac{k_x}{g(x)} \left((x+B_x)^m - x^m \right) + \frac{dx^m}{dx}(-\gamma x) \right\rangle \tag{11}$$

and these moment equations cannot be solved for arbitrary nonlinear functions $g(x)$ due to issues with moment closure [23]–[25]. Towards that end, we consider small fluctuations of $x(t)$ around the mean $\bar{x}$ [26], [27], one can linearize

$$\frac{k_x}{g(x)} \approx k_x \left(1 - s \left(\frac{x-\bar{x}}{\bar{x}} \right) \right), \quad s := \frac{\bar{x}}{g(\bar{x})} \frac{dg(x)}{dx}|_{x=\bar{x}} \geq 0 \tag{12}$$

using the fact that $g(\bar{x}) = 1$. Now using this linear approximation in place of $k_x/g(x)$ in (11), and again performing the steady-state moment computations results in

$$\bar{x} = \frac{k_x \langle B_x \rangle}{\gamma}, \quad CV_x^2 = \frac{\langle B_x^2 \rangle}{2\langle B_x \rangle \bar{x}(1+s)}. \tag{13}$$

Note that the noise levels with feedback are lower by a factor of $1+s$ compared to the case with no regulation (10).

C. Self-regulated degradation

Negative feedback in degradation can be implemented by having a constant burst frequency and modifying the decay kinetics to

$$\frac{dx}{dt} = -\gamma g(x) x. \tag{14}$$

It is important to point out that if one considers a protein that is not actively degraded but its concentration is diluted from an increase in cell size (as is the case of most proteins in *E. coli*), this feedback implies a coupling between expression and cell growth. More specifically, lower expression of this protein will result in a lower exponential growth rate for cell size, resulting in longer cell-cycle times. We discuss some implications of this coupling in more detail in Section 5. With the altered nonlinear degradation, the moment dynamics is given by

$$\frac{d\langle x^m \rangle}{dt} = \left\langle k_x \left((x+B_x)^m - x^m \right) + \frac{dx^m}{dx}(-\gamma g(x) x) \right\rangle. \tag{15}$$

It turns out that linearizing $g(x)x$ in (15) for small concentration fluctuations around the mean results in the exact same mean and noise levels as computed in (13) for feedback in synthesis. Thus consistent with previous observations [8], [28], *both feedback are identical in terms of noise attenuation for small fluctuations*.

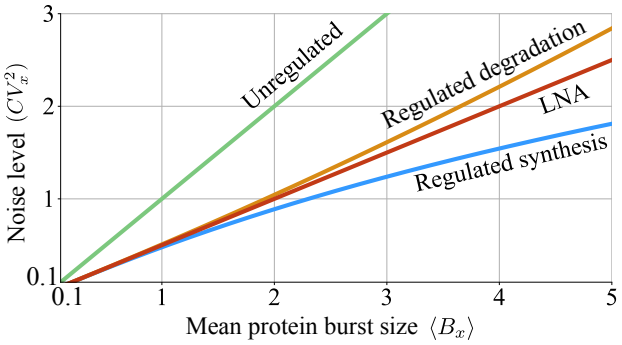

Fig. 2. **Self-regulated synthesis outperforms self-regulated degradation in attenuating noise at high protein burst sizes**. The noise levels (19) and (22) obtained for self-regulated synthesis and degradation, respectively, are plotted with increasing mean protein burst size $\langle B_x \rangle$. For comparison, noise level (10) when there is no feedback, and LNA-predicted noise level (13) are also plotted. For this plot, B_x is drawn from an exponential distribution with mean $\langle B_x \rangle$, $k_x = 1/\langle B_x \rangle$, $\gamma = 1$ and $g(x) = x$.

IV. Feedback Comparison in High-Noise Regime

We now explore the stochastic feedback systems in the high-noise regime that correspond to large protein burst sizes. Throughout this section and later, we consider the physiological case of B_x drawn from an exponential distribution with mean $\langle B_x \rangle$ [29]. Our approach relies on analytically solving the steady-state distribution for the protein concentration and contrasting their shapes between feedback strategies as a function of $\langle B_x \rangle$. As we increase $\langle B_x \rangle$, we also correspondingly decrease k_x to maintain the same $\bar{x}$ in the small noise regime (13). As we shall later see, given the nonlinearities of the system, the mean protein concentration in the stochastic model deviates considerably from its deterministic counterpart in the high-noise regime.

A. Self-regulated synthesis

Recall that in this case, bursts arrive with a rate $k_x/g(x)$. Let $p(x,t)$ denote the probability density function (pdf) of the protein concentration at time t. Then based on the SHS formalism introduced earlier, the time evolution of $p(x,t)$ is described by the Chapman-Kolmogorov equation [30]

$$\begin{aligned} &\frac{\partial p(x,t)}{\partial t} + \frac{\partial J}{\partial x} = 0, \\ &J = -\gamma x p(x,t) + k_x \int_0^x e^{-(x-x')/\langle B_x \rangle} \frac{p(x')}{g(x')} dx'. \end{aligned} \tag{16}$$

Assuming $k_x = 1/\langle B_x \rangle$, $\gamma = 1$ and $g(x) = x$ that ensures a steady-state mean protein concentration of 1 units in the small-noise regime for both regulated and unregulated

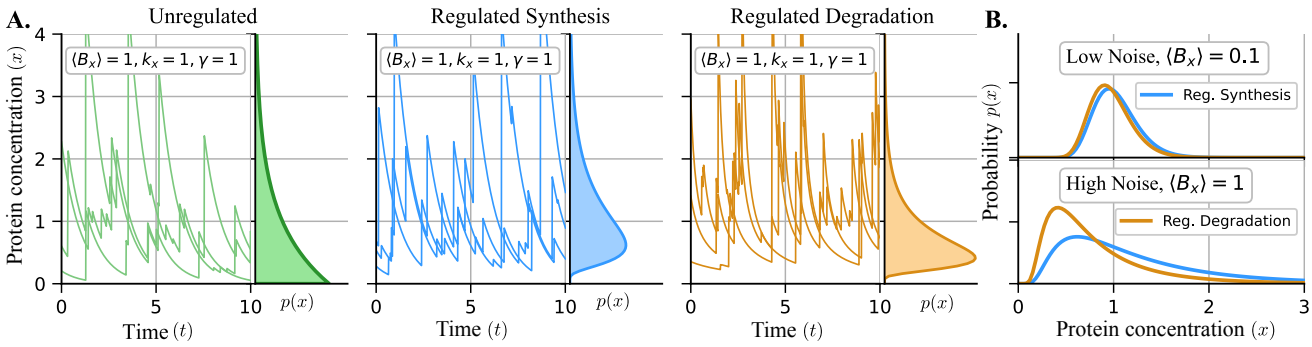

Fig. 3. **Self-regulated degradation leads to thinner-tailed steady-state protein concentration distributions compared to self-regulated synthesis.** (**A**) Sample trajectories for protein concentrations for unregulated and regulated gene expression with $g(x) = x$. B_x is drawn from an exponential distribution with mean $\langle B_x \rangle$. (**B**) The solutions of the steady-state protein distributions given by (17) and (21) demonstrate a close similarity in the low noise regime (top), where $\langle B_x \rangle = 0.1$, $k_x = 10$, and $\gamma = 1$. A noticeable deviation in the distribution of the two systems is observed in the high noise regime (bottom), where $\langle B_x \rangle = k_x = \gamma = 1$.

systems, (16) has an exact analytical solution for the steady-state pdf [28]

$$p(x) := \lim_{t\to\infty} p(x,t) = \frac{e^{-k_x(1+x^2)/x}}{2xY_0(2k_x)}. \tag{17}$$

Here $Y_0(2k_x)$ is the modified Bessel function of the second kind that can be computed in *Wolfram Mathematica* using

$$Y_i(2k_x) := BesselK[i, 2k_x]. \tag{18}$$

The steady-state pdf (17) leads to the following mean and the noise levels

$$\bar{x} = \frac{Y_1(2k_x)}{Y_0(2k_x)}, \quad CV_x^2 = \frac{Y_2(2k_x)Y_0(2k_x)}{Y_1(2k_x)^2} - 1. \tag{19}$$

B. Self-regulated degradation

The complementary case of feedback in degradation has exponentially-distributed bursts arrive with constant rate $k_x = 1/\langle B_x \rangle$, and protein concentrations decay nonlinearly between consecutive bursts as

$$\frac{dx}{dt} = -g(x)x = -x^2. \tag{20}$$

Formulating an analogous Chapman-Kolmogorov equation to (16) yields the following steady-state pdf

$$p(x) = \frac{e^{-k_x(1+x^2)/x}}{2x^2Y_1(2k_x)} \tag{21}$$

with the mean and the noise levels

$$\bar{x} = \frac{Y_0(2k_x)}{Y_1(2k_x)}, \quad CV_x^2 = \left(\frac{Y_1(2k_x)}{Y_0(2k_x)}\right)^2 - 1. \tag{22}$$

V. Different perspectives for population statistics: Single-cell vs. population snapshots

Fig. 2 presents a comparison of noise levels (19) and (22) with increasing mean burst size, together with unregulated noise levels (10), and the LNA-predicted noise levels (13). For an exponentially-distributed burst size B_x, $k_x = 1/\langle B_x \rangle$, $\gamma = 1$ and $g(x) = x$, the unregulated and LNA-predicted noise levels are simply $\langle B_x \rangle$ and $\langle B_x \rangle/2$, respectively. It is quite evident in Fig. 2 that feedback regulation strategies have a considerable impact on noise suppression. As predicted earlier, noise levels for both feedbacks converge together with the LNA-predicted level in the low-noise regime. Conversely, in the high-noise regime, we see a divergence among them, with feedback in synthesis providing the lowest noise.

Fig. 3 shows sample trajectories of protein concentration evolving over time as well as the steady-state pdfs for different regulation strategies. For $\langle B_x \rangle = 0.1$, in the low noise regime, the protein pdfs of both systems exhibit notable similarities. Meanwhile, Fig. 3B (bottom) presents the distributions of the two systems for $\langle B_x \rangle = 1$, the high-noise regime showing important differences. The distribution for the regulated degradation system demonstrates a faster tail decay (also evident in the $1/x^2$ scaling in (21) as compared to $1/x$ in (17)). The faster decay of the tail can be intuitively understood from the perturbation responses in Fig. 1 with quicker mean reversion from a high concentration seen in degradation feedback as compared to synthesis feedback. Therefore, to minimize concentration fluctuations above a high critical threshold, the regulated degradation approach is preferred. Using similar logic, it can be seen that in scenarios where minimization of protein fluctuations below a low threshold is desired, the regulated synthesis strategy proves to be more effective.

This article has focused on gene expression statistics

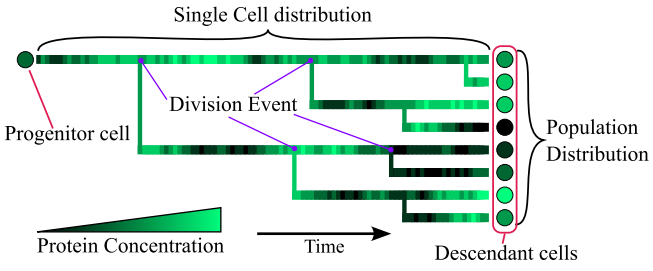

Fig. 4. **Comparing the statistics of protein concentration between single-cell and population perspectives**. The schematic shows the expansion of the colony starting from an individual cell that proliferates to give birth to descendant cells.

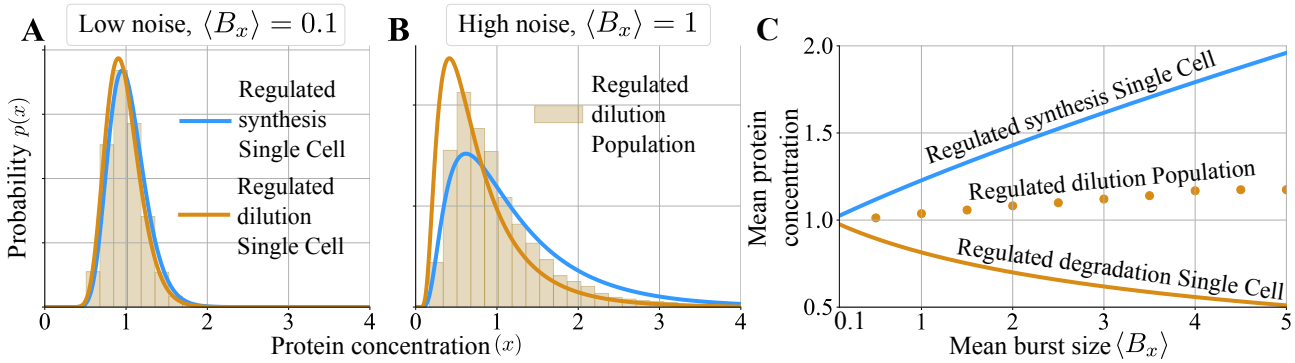

Fig. 5. **Negative feedback coupling between protein concentration and dilution drive differences in single-cell and population-level concentration pdfs**. The single-cell pdf for the self-regulated dilution system (21) is compared to its population counterpart in the low noise regime (**A**), characterized by $\langle B_x \rangle = 0.1$, and a high noise (**B**), where $\langle B_x \rangle = 1$. For comparison purposes, the concentration pdf for self-regulated synthesis is also shown here. (**C**) The mean steady-state protein concentration calculated from the perspective of the single cell (19) and (22), and at the population level is shown as a function of the mean burst size $\langle B_x \rangle$. *Parameters:* $\gamma = 1$, $k_x \langle B_x \rangle = 1$. The burst size follows an exponential distribution with mean $\langle B_x \rangle$ depicted on each figure.

within single-cell trajectories. However, experimentally not always a single cell is tracked, but the statistics is done over a population of lineage-related cells [31]. As depicted in Fig. 4, in a population-based approach, we consider all progeny of a cell, computing the pdf across all cells at a specific time snapshot [32], [33].

To model the protein level distribution at a population perspective, we employ an agent-based model for a growing cell colony[1]. When a cell dilutes its protein at a rate $\gamma g(x)$, division becomes a stochastic process with probability $\mathbb{P}(\text{division during}(t, t+dt)|x) = \gamma g(x) dt$. After division, a new cell inherits the mother's protein concentration. For self-regulated synthesis, our result shows that all cells share the same division rate (γ), resulting in no difference between analytically-obtained protein concentration pdf (17) and the population-level histogram in both low- and high-noise conditions (see our preprint version for more details [34]).

With self-regulated dilution, cells with higher protein concentrations replicate faster. Therefore, from the population perspective, the pdf of the protein level is shifted to higher values compared to the single-cell counterpart (Fig. 5B). To quantify this effect, Fig. 5C shows the mean protein concentration against the burst size, with both feedback mechanisms converging to the same mean concentration in the absence of intrinsic noise. We observe how, in the high-noise regime, the mean protein level for the regulated dilution in the population approach exceeds its single-cell perspective. This shift approaches the mean protein level from the population perspective to the single-cell mean protein level with feedback in protein synthesis.

VI. Discussion

In this contribution, we investigated stochastic dynamical systems motivated by the central question of noise attenuation in gene expression. By comparing two different feedback strategies for self-regulating protein synthesis versus degradation, we show that their perturbative linear dynamics around the set point are identical. This leads to the same noise level for both feedbacks when stochasticity is added through bursty synthesis.

As perturbations around the equilibrium begin to increase, these self-regulation mechanisms begin to diverge. At large protein burst sizes, regulated synthesis produces a lower CV_x as compared to feedback in degradation (Fig. 2). These differences are exemplified in the steady-state pdfs (Fig. 3) - *regulation in degradation results in a faster decay of the distribution tail*. Thus, regulated degradation would be a better strategy if protein levels that cross a high critical threshold are detrimental to cell fitness. In contrast, for an essential protein whose levels need to be maintained above a critical low threshold, negatively regulating protein synthesis is a better option. Similar observations have been made via stochastic simulation of genetic circuits [17], and here we quantify them analytically with exact solutions of the underlying Chapman–Kolmogorov equations.

Another key contribution of this work explores the steady-state protein level distribution across a cell population at a given time snapshot. In this population perspective, the protein distribution can differ from the distribution obtained by observing an individual cell over time. This is particularly relevant for stable proteins with no active degradation, where the protein dilution rate is essentially the cellular growth rate. *Our results show that both single-cell and population-level distributions are identical for self-regulated synthesis*, where cellular growth rates are constant [34]. However, when high protein levels increase the dilution and cell growth rate, cells with higher protein concentrations proliferate faster and become a larger part of the population. This results in a right shift of the population-level distribution compared to its single-cell counterpart (Fig. 5). Based on these results, we expect a left-shifted population-level distribution for the case of positive feedback between expression level and cell growth rate. Positive feedback is implemented when a high intracellular protein concentration slows the cellular growth

[1]The algorithm and associated code have been provided on https://doi.org/10.5281/zenodo.8343055.

rate, leading to a lower dilution rate, which further increases the concentration [35], [36].

It is interesting to note that the population-level distributions of both feedback strategies seem closer to each other, as compared to their single-cell distributions (Fig. 5). This leads to an interesting conclusion that negative feedback may suppress distribution differences at the population level, which in turn could be exacerbated by positive feedback where cellular growth is inversely related to expression levels. In summary, we have dissected the role of feedback in shaping the protein level distribution in single cells and in a proliferating population. Future work will consider mixtures of feedback in burst size, frequency, and degradation in combination with sequestration-based feedbacks [37]–[39] in determining the magnitude, time-scale, and exact shape of fluctuations in gene product levels.

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