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Supplementary Material for Beyond initiation-limited translational bursting: the effects of burst size distributions on the stability of gene expression

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S1 Probability density of V

Following the main text, T_1 represents a random variable (r.v.) for the reaction time of mRNA degradation, while T_2 represents a r.v. for the reaction time of translation. Let $V(t)$ be a r.v. representing the number of translation reaction events from a single transcript within time t. Then,

$$p_V(v) = \int_0^\infty Prob\{V(t) = v\}\, f_{T1}(t)dt$$

where $f_{T1}(t)$ is the density function of T_1. Let $T(v)$ be a r.v. representing a time to complete v translation reaction events and $f_T(t; v)$ be its density function. Then,

$$\begin{aligned} Prob\{V(t) = v\} &= Prob\{V(t) \geq v\} - Prob\{V(t) \geq v + 1\} \\ &= Prob\{T(v) < t\} - Prob\{T(v + 1) < t\} \\ &= \int_0^t f_T(t'; v)dt' - \int_0^t f_T(t'; v + 1)dt' \end{aligned}$$

where $f_T(t; v)$ can be given by

$$f_T(t; v) = \int_0^t \cdots \int_0^t \delta(t - \sum_{i=1}^{v} t_i)\, f_{T2}(t_1) \cdots f_{T2}(t_v)\, dt_1 \cdots dt_v$$

where $\delta(\cdot)$ is the Dirac delta function and $f_{T2}(t)$ is the density function of T_2.

S2 Derivation of Fokker-Planck equation

For each copy of mRNA molecule synthesized via transcription, the probability that this copy of mRNA leads to the production of v copies of X is $p_V(v)$. The propensity function of the protein synthesis reaction to produce exactly v molecules of the protein following a single transcription event becomes $q(s)\, p_V(v)$. Thus, our gene expression model describes the kinetics of the synthesis reaction of v protein molecules from a single mRNA molecule by $q(s)\, p_V(v)$ and the protein degradation by $k_{deg}\, x$. Let $p(x, t \mid s)$ be the probability that $X(t) = x$ given that signal level (i.e., transcription factor level) is s. Then, the chemical master equation [1] is given by

$$\begin{aligned} \frac{\partial p(x, t \mid s)}{\partial t} = &\sum_{v=0}^{\infty} p_V(v)\, [\, q(s)\, p(x - v, t \mid s) - q(s)\, p(x, t \mid s)] \\ &+ k_{deg}\, (x + 1)\, p(x + 1, t \mid s) - k_{deg}\, x\, p(x, t \mid s). \end{aligned} \tag{S1}$$

By making continuous approximation and resorting to the second-order Taylor expansion [2, 3], we have

$$\begin{aligned}\frac{\partial p(x,t \mid s)}{\partial t} = &- \sum_{v\in\mathbb{Z}^*} p_V(v)\, v\, \frac{\partial}{\partial x}\left[q(x,s)\, p(x,t \mid s)\right] \\ &+ \sum_{v\in\mathbb{Z}^*} p_V(v)\, v^2\, \frac{1}{2}\frac{\partial^2}{\partial x^2}\left[q(x,s)\, p(x,t \mid s)\right] \\ &+ \frac{\partial}{\partial x}\left[k_{deg}\, x p(x,t \mid s)\right] + \frac{1}{2}\frac{\partial^2}{\partial x^2}\left[k_{deg}\, x p(x,t \mid s)\right],\end{aligned}$$

which, as shown in the main text, can be simplified to the following Fokker-Planck equation:

$$\frac{\partial p(x,t \mid s)}{\partial t} = -\frac{\partial}{\partial x}\left[A(x \mid s)\, p(x,t \mid s)\right] + \frac{1}{2}\frac{\partial^2}{\partial x^2}\left[D(x \mid s)\, p(x,t \mid s)\right] \tag{S2}$$

where the drift function $A(x \mid s) = \mu_1\, q(s) - kx$ and the diffusion function $D(x \mid s) = \mu_2\, q(s) + kx$.

S3 Simulation procedure

To test the accuracy of our analytical model, we performed simulations of the underlying discrete-stochastic model governed by the chemical master equation (Eq. S1). The stochastic simulation was based on the Gillespie stochastic simulation algorithm (SSA) [4], which was implemented in R specifically for analysis of our models. To model the dynamic of the protein burst size, an extended version of the standard SSA was used so that the value of V is sampled from a given distribution (e.g., a truncated geometric distribution and a negavite binominal distribution) each time a reaction event fires. To obtain the distribution of X_∞, 10,000 runs of simulation were performed for 5,000 minutes and the copy number of protein X at the final time point was recorded for each simulation run. In the simulation of the gene expression model with the regulated promoter, the dynamic of the transcription factor S was modeled by sampling a value from a specific distribution (i.e., a negavite binominal distribution for the results in Fig. 4) each time a reaction event fires. Statistical analysis was made for each model setting based on the 10,000 samples of X_∞ from the simulation.

S4 Conditions for stable states and unstable states

In the stationary regime, we have

$$\frac{\partial}{\partial x}\{A(x \mid s)p_s(x \mid s) - \frac{1}{2}\frac{\partial}{\partial x}[D(x \mid s)p_s(x \mid s)]\} = 0.$$

From the boundary condition $p_s(\infty \mid s) = 0$ and $p'_s(\infty \mid s) = 0$, we have

$$A(x \mid s)p_s(x \mid s) - \frac{1}{2}\frac{\partial}{\partial x}[D(x \mid s)p_s(x \mid s)] = 0,$$

yielding

$$p'_s(x \mid s) = \frac{\alpha(x \mid s)}{D(x \mid s)}p_s(x \mid s),$$

where $\alpha(x \mid s) = 2\,A(x \mid s) - D'(x \mid s)$. At the local extrema positions of $p'_s(x \mid s)$, then, it is clear that $\alpha(x \mid s) = 0$. At the stable state positions, $p_s(x \mid s)$ is a peak, so we have $p'_s(x_{ss} \mid s) = 0$ and $p''_s(x_{ss} \mid s) < 0$. At the unstable state positions, $p_s(x \mid s)$ is the bottom of a valley, so we have $p'_s(x_{us} \mid s) = 0$ and $p''_s(x_{us} \mid s) > 0$. By taking the derivative of $p'_s(x \mid s)$, we can express the second derivative of $p_s(x \mid s)$ as

$$p''_s(x \mid s) = p_s(x \mid s)\frac{d}{dx}\frac{\alpha(x \mid s)}{D(x \mid s)} + p'_s(x \mid s)\frac{\alpha(x \mid s)}{D(x \mid s)}.$$

At the local extrema points, we have $\alpha(x \mid s) = 0$, so at these points, the expression of $p''_s(x \mid s)$ is simplified to

$$\begin{aligned} p''_s(x \mid s) &= p_s(x \mid s)\left[\frac{\alpha'(x \mid s)\,D(x \mid s) - \alpha(x \mid s)\,D'(x \mid s)}{D^2(x \mid s)}\right] \\ &= p_s(x \mid s)\frac{\alpha'(x \mid s)}{D(x \mid s)}. \end{aligned}$$

Here, since $p_s(x \mid s)$ and $D(x \mid s)$ are non-negative, the sign of $p''_s(x \mid s)$ must agree with the sign of $\alpha'(x \mid s)$. That is, in a stable state, we have $\alpha'(x_{ss} \mid s) < 0$, while, in an unstable state, we have $\alpha'(x_{ss} \mid s) > 0$ Therefore, a stable state, x_{ss}, satisfies $\alpha(x_{ss} \mid s) = 0$ and $\alpha'(x_{ss} \mid s) < 0$, while an unstable state, x_{us}, satisfies $\alpha(x_{us} \mid s) = 0$ and $\alpha'(x_{us} \mid s) > 0$.

S5 Analysis of stability in a constitutive promoter system

We examine $\alpha(x) = 2\,A(x) - D'(x)$ where the drift function $A(x) = \mu_1\,k_{prod} - k_{deg}\,x$ and the first derivative of the diffusion function $D'(x) = k_{deg}$. Since $2\,A(x)$ is a monotonically decreasing function and $D'(x)$ is a constant, they have only one intersection point if $2\,A(0) \geq D'(0)$ and no intersection point, otherwise. In addition, the first derivative of $\alpha(x)$ with respect to x is always negative, implying that this intersection point is a stable state. This means that, in order for the stable protein level to be positive, we need to have $2\,A(0) \geq D'(0)$, that is, $2\,\mu_1\,k_{prod} > k_{deg}$. Solving $\alpha(x_{ss}) = 0$ for x_{ss} yields

$$x_{ss} = \mu_1\,\frac{k_{prod}}{k_{deg}} - \frac{1}{2}.$$

To derive the expression of stability width w, we approximate $p_s(x)$ around x_{ss} by the following Gaussian function

$$f(x) = p_s(x_{ss})\, \exp\left[-(x - x_{ss})^2/2\, w^2\right].$$

From this setup, it is clear that $p_s(x_{ss}) = f(x_{ss})$ and $p_s'(x_{ss}) = f'(x_{ss}) = 0$. To find the expression of w, we impose another condition: $p_s''(x_{ss}) = f''(x_{ss})$. The second derivative of $p_s(x)$ at a stable state position is $p_s''(x_{ss}) = p_s(x_{ss})\alpha'(x_{ss})/D(x_{ss})$, The second derivative of $f(x)$ with respect to x is $f''(x) = -f(x)/w^2 - f'(x)\,(x - x_{ss})/w^2$. Since $f(x_{ss}) = 0$, we have $f''(x_{ss}) = -f(x_{ss})/w^2$. From these, we obtain $w = \sqrt{-D(x_{ss})/\alpha'(x_{ss})}$. For the constitutive promoter setting, thus, we can express the width w as

$$w = \sqrt{\frac{1}{2}\frac{k_{prod}}{k_{deg}}\,(\mu_2 + \mu_1) - \frac{1}{4}}.$$

S6 Analysis of stability in feedback systems

S6.1 Shape of 2A(x) and D'(x)

For the feedback-based gene expression process, we have

$$\begin{aligned} 2A(x) &= 2\,\mu_1\, q(x) - 2\, k_{deg}\, x, \\ D'(x) &= \mu_2\, q'(x) + k_{deg}. \end{aligned}$$

Since $A(0^+) = \mu_1\, k_b$ and $A(\infty) = -\infty$, in the positive domain, $2\,A(x)$ must have an odd number of zero-crossings (i.e., in the deterministic context). The first and the second derivatives of $2\,A(x)$ are given by $2\,A'(x) = 2\mu_1\, q'(x) - 2\, k_{deg}$ and $2\,A''(x) = 2\mu_1\, q''(x)$, while the first and second derivatives of $D'(x)$ are $D''(x) = \mu_2\, q''(x)$ and $D'''(x) = \mu_2\, q'''(x)$, respectively. The first three derivatives of $q(x)$ are

$$\begin{aligned} q'(x) &= \frac{\nu\, x^{n-1}}{{K_d}^n\left[(x/K_d)^n + 1\right]^2}, \\ q''(x) &= \nu\frac{(n-1)\,(x/K_d)^n - (n+1)\,(x/K_d)^{2\,n}}{x^2\,\left[(x/K_d)^n + 1\right]^3}, \\ q'''(x) &= \nu\frac{\sum_{i=1}^{3} f_i(n)(x/K_d)^{i\,n}}{x^3\left[(x/K_d)^n + 1\right]^4} \end{aligned}$$

where $\nu = n\,(k_a - k_b)$, $f_1(n) = (n-2)(n-1)$, $f_2(n) = 4\,(1 - n^2)$, and $f_3(n) = (n+2)(n+1)$. If $n > 1$, then $q''(x)$ has a single positive root at $x = x_{in}$ where $x_{in} = K_d\, \sqrt[n]{(n-1)/(n+1)}$. Thus, $2\,A(x)$ has an inflection point and $D'(x)$ has a local extremum at $x = x_{in}$ when $n > 1$.

The expression of $q'(0^+)$ and $q''(0^+)$ depends on the value of n and they are given by

$$\lim_{x\to 0^+} q'(x) = \begin{cases} \infty & \text{if } n < 1 \\ (k_a - k_b)/K_d & \text{if } n = 1 \\ 0 & \text{if } n > 1, \end{cases} \tag{S3}$$

$$\lim_{x\to 0^+} q''(x) = \begin{cases} -\infty & \text{if } n < 1 \\ -2\,(k_a - k_b)/{K_d}^2 & \text{if } n = 1 \\ 0 & \text{if } n > 1. \end{cases} \tag{S4}$$

S6.2 Positive feedback systems

From these results, given that $k_a > k_b$ (i.e., positive feedback systems), we can obtain characteristics of the shape of $2\,A(x)$ and $D'(x)$ as follows. In this setting, we have $q'(x) \geq 0$ for all $x > 0$. If $n \leq 1$, then $q''(x) < 0$ for all $x > 0$, indicating that $2\,A(x)$ is a concave function, which approaches $-\infty$ as $x \to \infty$. On the other hand, if $n > 1$, then $q''(x)$ has a single positive root at $x = x_{in}$, and the curve of $2\,A(x)$ changes from convex to concave at $x = x_{in}$.

If $n \leq 1$, then $q''(x) < 0$ and $q'''(x) > 0$ for all $x > 0$, making $D'(x)$ a monotonically decreasing, convex function, that starts from ∞ if $n < 1$ and $\mu_2(k_a - k_b)/K_d + k_{deg}$ if $n = 1$ and converges to k_{deg} as $x \to \infty$. On the other hand, if $n > 1$, then $D'(x)$ starts from k_{deg} at $x = 0$ and has a local maximum at $x = x_{in}$, which approaches k_{deg} as $x \to \infty$.

S6.3 Negative cooperative positive feedback case

In this setting, we have $\alpha(0) < 0$ and $\alpha(\infty) < 0$. We also know that $2A(x)$ is a concave function and $D'(x)$ is a monotonically decreasing convex function (see Sections S5.1 and S5.2). From these, we have two cases for the number of sign-changes in $\alpha(x)$ in the $x > 0$ domain:

Case (i): $\alpha(x)$ has two sign-changes. In this case, $p_s(x)$ has two local extrema. The lower zero-crossing point is an unstable state, while the higher one is a stable state.

Case (ii): $\alpha(x)$ has no sign change. This implies that for all $x > 0$, $D'(x) \geq 2\,A(x)$. In this case, $p_s(x)$ has no local extremum, indicating that $p_s(x)$ is a monotonically decreasing function.

These cases correspond to the cases presented in the main text.

By taking the variance of the burst size as small as possible (i.e., $\mu_2 \approx {\mu_1}^2$), we may be able to control $D'(x)$ so that it transitions from infinity to k_{deg} rapidly in a wide range of parameter conditions. This guarantees the condition for Case (i), but the position of the unstable state becomes very close to zero since the slopes of $2\,A(x)$ and $D'(x)$ are

initially ∞ and $-\infty$, respectively, making it unnoticeable (Fig. 5a). Thus, even though there is a point of attraction at $x = 0$, this attractor is not noticeable since the threshold is very close to 0 under this condition. Another analytical observation is that, if $D'(x)$ with the lowest possible value of μ_2 (i.e., ${\mu_1}^2$) results in Case (ii), then any changes in μ_2 would result in Case (ii). Suppose we observe Case (i) (i.e., the points of attraction at 0 and a high protein expression level separated by the threshold) in a positive feedback gene expression system with a transcription factor of negative cooperativity. Then, if we are to increase the variance of the burst size by increasing μ_2, the distance between the threshold and the higher expression state state becomes shorter because an increase in μ_2 results in increase the threshold point and decrease the level of the higher expression state state (Fig. 5a). As we further increase the variance of the burst size, eventually, $D'(x)$ becomes so large that it can no longer intersect with $2A(x)$, leaving the point of attraction only at 0.

S6.4 Non-cooperative positive feedback case

In this setting, $2A(x)$ is a concave function, and $D'(x)$ is a monotonically decreasing convex function (see Sections S5.1 and S5.2). The sign of $\alpha(0)$, however, now becomes dependent on μ_2, implying that the number of stable states depends on the variance of the burst size. Since $\alpha(\infty) < 0$, $\alpha(x)$ has a single zero-crossing only if $\alpha(0) > 0$, otherwise, it has an even number of zero-crossings (Fig. 5b). $D'(0)$ is a linear function of μ_2 when $n = 1$ (see Section S5.1). From this, the sufficient condition for $\alpha(x)$ to have a single zero-crossing based on the range of the second moment of the burst size becomes

$$\mu_2 < K_d \left(2\mu_1 \, k_b - k_{deg}\right) / \left(k_a - k_b\right). \tag{S5}$$

When this condition is satisfied, the sign changes from positive to negative, indicating that the protein level has a positive stable state. Condition S5 also implies that $2\mu_1 \, k_b > k_{deg}$ is a necessary condition for the single zero-crossing. Since the variance of the burst size must be non-negative, we can also constrain the validity range of μ_1 to have a stable protein level to be

$$\frac{k_{deg}}{2\,k_b} < \mu_1 < \frac{\sqrt{{k_b}^2 \, {K_d}^2 - \left(k_a - k_b\right) \, k_{deg} \, K_d} + k_b \, K_d}{k_a - k_b} \tag{S6}$$

In contrast, if $\alpha(0) \leq 0$, similar to the negatively cooperative setting, $\alpha(x)$ has two or zero sign-changes, implying that $p_s(x)$ has either two or zero local extrema. This also shows that, if the value of μ_1 does not satisfy Condition S6, $p_s(x)$ has again two local extrema, that is, the protein copy number has two points of attraction at 0 and higher protein expression level separated by a threshold.

S6.5 Positive cooperative positive feedback case

In this setting, $A''(x)$ is zero when $x = x_{in}$ where $x_{in} = K_d \sqrt[n]{(n-1)/(n+1)}$ (see Section S6.1). Thus, $2A(x)$ is locally convex up to x_{in}, while it is locally concave from x_{in} (see Sections S6.1 and S6.2). This means that $2A(x)$ has two local extrema: a local minimum at $x = x_1$ and a local maximum at $x = x_2$, which are separated by x_{in}. In the deterministic context, the process is bistable, only if $2A(x_1) < 0$ and $2A(x_2) > 0$, that is, only if the value of the drift function at the local minimum point and the local maximum point is negative and positive, respectively. In the stochastic context, however, the stability of the system can be widely different because of nonlinear $D(x)$. Indeed, given that $2\mu_1 k_b < k_{deg}$, $\alpha(x)$ guarantees to have an even number of zero-crossings whereas $2A(x)$ has an odd number of them. The manipulation of the burst size variance can control the number of positive stable states. By tuning μ_2, we can alter the amplitude of the local maximum of $D'(x)$ proportionally, which can change the number of intersection between $2A(x)$ and $D'(x)$. This predicts that changes in the burst size distribution can give rise to a multimodal protein distribution. This point is illustrated in Fig. 5c, in which the system exhibits bistability when μ_2 has the lowest possible value (i.e., $\mu_2 = 400$ when $\mu_1 = 20$). As the value of μ_2 is increased to the level close to that of the geometric random variable (i.e., $\mu_2 = 400$), the amplitude of the peak of $D'(x)$ becomes so high that two intersection points disappear, leading to a monostable state with the lower expression stable state.

S6.6 Negative feedback systems

Given that $k_a = 0$ (i.e., negative feedback systems), we can obtain characteristics of the shape of $2\,A(x)$ and $D'(x)$ as follows. In this setting, we have $q'(x) \leq 0$ for all $x > 0$. If $n \leq 1$, then $q''(x) > 0$ for all $x > 0$, indicating that $2\,A(x)$ is a monotonically decreasing convex function, which approaches $-\infty$ as $x \to \infty$. If $n \leq 1$, then $D'(x)$ is a monotonically increasing function with $D'(0) < 2\,A(0)$, implying that there is only one intersection point between $2\,A(x)$ and $D'(x)$. Thus, there is only one intersection point between $2\,A(x)$ and $D'(x)$, indicating that the protein expression process has one positive stable state.

On the other hand, if $n > 1$, then $q''(x)$ has a single positive root at $x = x_{in}$, and $2\,A(x)$ is a monotonically decreasing function that changes from concave to convex at $x = x_{in}$. Thus, $D'(x)$ starts from k_{deg} at $x = 0$ and has a local minimum at $x = x_{in}$, which approaches k_{deg} as $x \to \infty$. In any case, thus, $2\,A(x)$ is a monotonically decreasing convex function and $D'(x)$ is a monotonically increasing function in the range $x \in [x_{in}, \infty)$ for all n. In this setting, $2\,A(x)$ has an inflection point and $D'(x)$ has a local minimum when $x = x_{in}$ where $x_{in} = K_d \sqrt[n]{(n-1)/(n+1)}$. Since the maximum value of $D'(x)$ is k_{deg}, if the value of $2A(x)$ at $x = x_{in}$ is found to be greater than the protein degradation rate constant, then we can guarantee that this gene expression process has a high protein expression level with any setting of the protein burst size distribution.

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