Stochastic Delay Accelerates Signaling in Gene Networks

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Abstract

The creation of protein from DNA is a dynamic process consisting of numerous reactions, such as transcription, translation and protein folding. Each of these reactions is further comprised of hundreds or thousands of sub-steps that must be completed before a protein is fully mature. Consequently, the time it takes to create a single protein depends on the number of steps in the reaction chain and the nature of each step. One way to account for these reactions in models of gene regulatory networks is to incorporate dynamical delay. However, the stochastic nature of the reactions necessary to produce protein leads to a waiting time that is randomly distributed. Here, we use queueing theory to examine the effects of such distributed delay on the propagation of information through transcriptionally regulated genetic networks. In an analytically tractable model we find that increasing the randomness in protein production delay can increase signaling speed in transcriptional networks. The effect is confirmed in stochastic simulations, and we demonstrate its impact in several common transcriptional motifs. In particular, we show that in feedforward loops signaling time and magnitude are significantly affected by distributed delay. In addition, delay has previously been shown to cause stable oscillations in circuits with negative feedback. We show that the period and the amplitude of the oscillations monotonically decrease as the variability of the delay time increases.

Introduction

Gene regulation forms a basis for cellular decision-making processes and transcriptional signaling is one way in which cells can modulate gene expression patterns [1]. The intricate networks of transcription factors and their targets are of intense interest to theorists because it is hoped that topological similarities between networks will reveal functional parallels [2]. Models of gene regulatory networks have taken many forms, ranging from simplified Boolean networks [3,4], to full-scale, stochastic descriptions simulated using Gillespie's algorithm [5].

The majority of models, however, are systems of nonlinear ordinary differential equations (ODEs). Yet, because of the complexity of protein production, ODE models of transcriptional networks are at best heuristic reductions of the true system, and often fail to capture many aspects of network dynamics. Many ignored reactions, like oligomerization of transcription factors or enzyme-substrate binding, occur at much faster timescales than reactions such as transcription and degradation of proteins. Reduced models are frequently obtained by eliminating these fast reactions [6–9]. Unfortunately, even when such reductions are done correctly, problems might still exist. For instance, if within the reaction network there exists a linear (or approximately linear) sequence of reactions, the resulting dynamics can appear to be delayed. This type of behavior has long been known to exist in gene regulatory networks [10].

Delay differential equations (DDEs) have been used as an alternative to ODE models to address this problem. In protein production, one can think of delay as resulting from the sequential assembly of first mRNA and then protein [10–12]. Delay can qualitatively alter the local stability of genetic regulatory network models [13] as well as their dynamics, especially in those containing feedback. For instance, delay can lead to oscillations in models of transcriptional negative feedback [11,14–18], and experimental evidence suggests that robust oscillations in simple synthetic networks are due to transcriptional delay [19,20].

Protein production delay times are difficult to measure in live cells, though recent work has shown that the time it takes for transcription to occur in yeast can be on the order of minutes and is highly variable [21]. Still, transcriptional delay is thought to be important in a host of naturally occurring gene networks. For instance, mathematical models suggest that circadian oscillations are governed by delayed negative feedback systems [22,23], and this was experimentally shown to be true in mammalian cells [24]. Delay appears to play a role in cell cycle control [25,26], apoptosis induction by the p53 network [27], and the response of the NFκB network [15]. Delay can also affect the stochastic nature of gene expression, and the relation between the two can be subtle and complex [28–31].

Author Summary

Delay in gene regulatory networks often arises from the numerous sequential reactions necessary to create fully functional protein from DNA. While the molecular mechanisms behind protein production and maturation are known, it is still unknown to what extent the resulting delay affects signaling in transcriptional networks. In contrast to previous studies that have examined the consequences of fixed delay in gene networks, here we investigate how the variability of the delay time influences the resulting dynamics. The exact distribution of "transcriptional delay" is still unknown, and most likely greatly depends on both intrinsic and extrinsic factors. Nevertheless, we are able to deduce specific effects of distributed delay on transcriptional signaling that are independent of the underlying distribution. We find that the time it takes for a gene encoding a transcription factor to signal its downstream target decreases as the delay variability increases. We use queueing theory to derive a simple relationship describing this result, and use stochastic simulations to confirm it. The consequences of distributed delay for several common transcriptional motifs are also discussed.

In this study, we examine the consequences of randomly distributed delay on simple gene regulatory networks: We assume that the delay time for protein production, T, is not constant but instead a random variable. If f_T denotes the probability density function (PDF) of T, this situation can be described deterministically by an integro-delay differential equation [32] of the form

$$\dot{\mathbf{x}}(t) = \int \mathbf{g}[\mathbf{x}(t), \mathbf{x}(t-s)] f_T(s) \mathrm{d}s, \qquad (1)$$

where $\mathbf{x}$ is a positive definite state vector of protein concentrations, and $\mathbf{g}$ is a vector function representing the production and degradation rates of the proteins. Note that processes that do not require protein synthesis (like dilution and degradation) will depend on the instantaneous, rather than the delayed, state of the system. Therefore $\mathbf{g}$ is in general a function of both the present and past state of the system.

Equation (1) only holds in the limit of large protein numbers [32]. As protein numbers approach zero, the stochasticity associated with chemical interactions becomes non-negligible. Here, we address this issue by expanding on Eq. (1) using an exact stochastic algorithm that takes into account variability within the delay time [32]. We further use a queueing theory approach to examine how this variability affects timing in signaling cascades. We find that when the mean of the delay time is fixed, increased delay variability accelerates downstream signaling. Noise can thus increase signaling speed in gene networks. In addition, we find that in simple transcriptional networks containing feed-forward or feedback loops the variability in the delay time nontrivially affects network dynamics.

Queueing theory has recently been used to understand the behavior of genetic networks [33–35]. Here we are mainly interested in dynamical phenomena to which the theory of queues in equilibrium used in previous studies cannot be applied. As we explain below, gene networks can be modeled as thresholded queueing systems: Proteins exiting one queue do not enter another queue, as would be the case in typical queueing networks. Rather, they modulate the *rate* at which transcription is initiated, and thus affect the *rate* at which proteins enter other queues.

Results

Distributed delay in protein production

The transcription of genetic material into mRNA and its subsequent translation into protein involves potentially hundreds or thousands of biochemical reactions. Hence, detailed models of these processes are prohibitively complex. When simulating genetic circuits it is frequently assumed that gene expression instantaneously results in fully formed proteins. However, each step in the chain of reactions leading from transcription initiation to a folded protein takes time (Figure 1). Models that do not incorporate the resulting delay may not accurately capture the dynamical behavior of genetic circuits [17]. While earlier models have included either fixed or distributed delay [32,36,37], here we examine specifically the *effects* of delay variability on transcriptional signaling.

In one recent study, Bel *et al.* studied completion time distributions associated with Markov chains modeling linear chemical reaction pathways [38]. Using rigorous analysis and numerical simulations they show that, if the number of reactions is large, completion time distributions for an idealized class of models exhibit a sharp transition in the coefficient of variation (CV, defined as the standard deviation divided by the mean of the distribution), going from near 0 (indicating a nearly deterministic completion time) to near 1 (indicating an exponentially distributed completion time) as system bias moves from forward to reverse.

However, it is possible, and perhaps likely, that the limiting distributions described by Bel *et al.* do not provide good approximations for protein production. For instance, when the number of rate limiting reactions is small, but greater than one, the distribution of delay times can be more complex. Moreover, linear reaction pathways only represent one possible and necessarily simplified reaction scheme. Protein production involves many reaction types that are nonlinear and/or reversible, each of which is influenced by intrinsic and extrinsic noise [39], and these
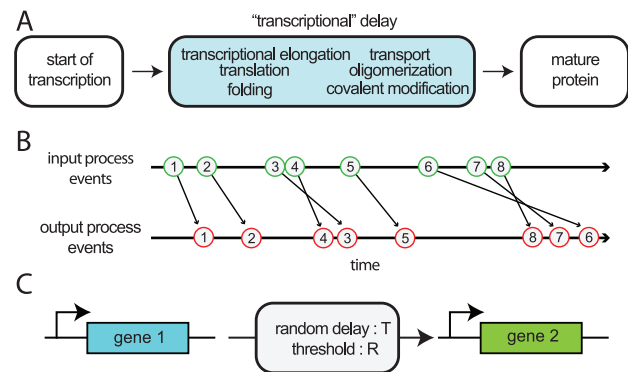

Figure 1. The origin of delay in transcriptional regulation. (A) Numerous reactions must occur between the time that transcription starts and when the resulting protein molecule is fully formed and mature. Though we call this phenomenon "transcriptional" delay, there are many reactions after transcription (such as translation) which contribute to the overall delay. (B) The creation of multiple proteins can be thought of as a queueing process. Nascent proteins enter the queue (an input event) and emerge fully matured (an output event) some time later depending on the distribution of delay times. Because the delay is random, it is possible that the order of proteins entering the queue is not preserved upon exit. (C) In a transcriptionally regulated signaling process the time it takes for changes in the expression of gene 1 to propagate to gene 2 depends on both the distribution of delay times, f_T, and the number of transcription factors needed to overcome the threshold of gene 2, R.
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reactions may impact the delay time distribution in complicated ways. Therefore, we do not try to derive the actual shape of f_T, but examine the effects its statistical properties have on transcriptional signaling. To do this, we represent protein production as a delayed reaction of the form

$$g \xrightarrow{\lambda(t,P),T} g + P, \tag{2}$$

where g is the gene, and transcription is initiated at rate $\lambda(t,P)$, which can depend explicitly on both time and protein number, P. After initiation, it takes a random time, T, for a protein to be formed. Note that the presence of time delay implies that scheme (2) defines a non-Markovian process. Such processes can be simulated exactly using an extension of the Gillespie algorithm (See Methods and [28,32]).

If the biochemical reaction pathway that leads to functional protein is known and relatively simple, direct stochastic simulation of every step in the network is preferable to simulation based on scheme (2). From the point of view of multi-scale modeling, however, paradigm (2) is useful when the biochemical reaction network is either extremely complex or poorly mapped, since one needs to know only the statistical properties of T.

## Protein formation as a queueing system

In the setting of scheme (2), first assume that $\lambda(t,P)$ does not depend on P, and protein formation is initiated according to a memoryless process with rate $\lambda(t)$. A fully formed protein enters the population a random time T after the initiation of protein formation. We assume that the molecules do not interact while forming; that is, the formation of one protein does not affect that of another. Each protein therefore emerges from an independent reaction channel after a random time. This process is equivalent to an $M/G/\infty$ queue [40], where M indicates a memoryless source (transcription initiation), G a general service time distribution (delay time distribution), and ∞ refers to the number of service channels.

In our model, the order in which initiation events enter a queue is not necessarily preserved. As Figure 1 (B) illustrates, it is possible for the initiation order to be permuted upon exit [32]. The assumption that proteins can "skip ahead" complicates the analysis of transient dynamics of such queues, and is essential in much of the following. While there are steps where such skipping can occur (such as protein folding), there are others for which it cannot. For instance, it is unlikely that one RNA polymerase can skip ahead of another – and similarly for ribosomes during translation off of the same transcript. Therefore, protein skipping may be more relevant in eukaryotes, where transcription and translation must occur separately, than prokaryotes, where they may occur simultaneously. However, if there is more than one copy of the gene (which is common for plasmid-based synthetic gene networks in *E. coli*), or more than one transcript, some skipping is likely occur. Therefore it is likely that the full results that follow are more relevant for genes of copy number greater than one.

Downstream transcriptional signaling

One purpose of transcription factors is to propagate signals to downstream target genes. Determining the dynamics and stochasticity of these signaling cascades is of both theoretical and experimental interest [41,42]. Therefore, we first examine the impact that distributed delay has on simple downstream signaling. Consider the situation depicted in Figure 1 (C), in which the product of the first gene regulates the transcription of a second gene. Using the same nomenclature as in scheme (2) we write

$$g_1 \xrightarrow{\lambda(t),T_1} g_1 + P_1 \tag{3a}$$

$$g_2 \xrightarrow{h(P_1),T_2} g_2 + P_2, \tag{3b}$$
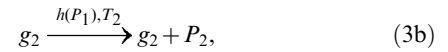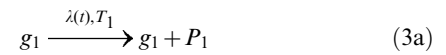
where g_1 and g_2 are the copy numbers of the upstream and downstream genes, P_1 and P_2 are the number of functional proteins of each type, and T_i is the random delay time of gene $i = 1,2$. The transcription rate of gene 2 depends on P_1 and is given by a Hill function $h(P_1)$. We consider the case in which P_1 activates g_2 (depicted in Figure 1) and the case in which P_1 represses g_2.

We now ask: If P_1 starts at zero and gene 1 is suddenly turned on, how long does it take until the signal is detected by gene 2? In other words, assume $\lambda(t) = \lambda_0\Theta(t)$, where $\Theta(t)$ is the Heaviside step function. At what time t does $P_1(t)$ reach a level that is detectable by gene 2? In order to make the problem tractable, we assume that the Hill function is steep and switch-like, so that we can make the approximation

$$h(P_1) = h_0\Theta(R - P_1) \qquad \text{(repressor case);} \tag{4a}$$

$$h(P_1) = h_0\Theta(P_1 - R)\text{(activator case).} \tag{4b}$$

Here h_0 is the maximum transcriptional initiation rate of g_2 and $R > 0$ is the threshold value of the Hill function, *i.e.* the number of molecules of P_1 needed for half repression (or half activation) of gene 2. The second gene therefore becomes repressed (or activated) at the time S_R at which R copies of protein P_1 have been fully formed.

We first examine reaction (3a). Assume that at time $t = 0$ there are no proteins in the system. Let $I_1(t)$ denote the number of transcription initiation events that have occurred by time t (the arrival process of the queueing system), $Q_1(t)$ the number of proteins being formed at time t (the size of the queue at time t), and $P_1(t)$ the number of functional proteins that have been completed by time t (the exit process of the queueing system). Since the arrival process is memoryless, $(I_1(t))_{t \geq 0}$ is a Poisson process with constant rate λ_0 for $t \geq 0$. Hence, the expected value of $I_1(t)$ is $E[I_1(t)] = \lambda_0 t\Theta(t)$.

The exit process, *i.e.* the number of fully functional proteins that have emerged from the queue, $P_1(t)$, is a nonhomogenous Poisson process with time-dependent rate $\lambda_{P_1}(t) = \lambda_0 F_{T_1}(t)$, where $F_{T_1}(t)$ is the cumulative distribution function (CDF) of the delay time T_1. It then follows that

$$\overline{P}_1(t) \equiv E[P_1(t)] = \lambda_0 \int_0^t F_{T_1}(s)\mathrm{d}s.$$

Inactivation (or activation) of gene 2 occurs when enough protein P_1 has accumulated to trigger a transcriptional change, according to Eq. (4a) or (4b). In other words, the random time it takes for the signal to propagate, S_R, is given by $S_R = \min\{t|P_1(t) = R\}$. Trivially, S_R changes by an amount identical to a change in the mean of the delay distribution. To examine the effects of randomness in delay on the signaling time, we therefore keep the mean of the delay distribution fixed, $E[T_1] = \tau$, and vary $\mathrm{Var}[T_1]$.

The probability density function of S_R is given by (See Methods)

$$f_{S_R}(t) = \frac{\overline{P}_1(t)^{R-1}}{(R-1)!} e^{-\overline{P}_1(t)} \frac{d\overline{P}_1}{dt}. \qquad (5)$$

Consequently, the mean and variance of the time it takes for the original signal to propagate to the downstream gene can be written as:

$$E[S_R] = \int_0^\infty \frac{s^{R-1}e^{-s}}{(R-1)!} \overline{P}_1^{-1}(s)\mathrm{d}s, \qquad (6)$$

$$\mathrm{Var}[S_R] = \int_0^\infty \frac{s^{R-1}e^{-s}}{(R-1)!} \left(\overline{P}_1^{-1}(s)\right)^2 \mathrm{d}s - E[S_R]^2. \qquad (7)$$

To gain insight into the behaviors of Eqs. (6) and (7), we first examine a representative, analytically tractable example. Assume that the delay time can take on 2 discrete values, $\tau+a$ and $\tau-a$ with equal probability. In this case,

$$E[S_R] = \tau - a + \frac{2R}{\lambda_0} + \frac{a\lambda_0\Gamma[R,a\lambda_0] - \Gamma[R+1,a\lambda_0]}{\lambda_0(R-1)!}, \qquad (8)$$

where $\Gamma(x,y)$ is the upper incomplete gamma function. Expanding for small a, we obtain (See Methods)

$$E[S_R] \approx \tau + \frac{R}{\lambda_0}, \qquad (9)$$

which is the deterministic limit. The first term is the mean delay time and the second is the average time to initiate R proteins at rate λ_0. A similar expansion for fixed R and large a gives (see panel (c) in Figure 2)

$$E[S_R] \approx \tau + \frac{R}{\lambda_0} - \left(a - \frac{R}{\lambda_0}\right). \qquad (10)$$

It follows that for larger delay variability, the mean signaling time *decreases* with delay variability (See Figure 2 (A)). Indeed, Eqs. (9) and (10) form the asymptotic boundaries for the mean signaling time. The intersection of the two asymptotes at $a = R/\lambda_0$, gives an estimate of when the behavior of the system changes from the deterministic limit (for $a < R/\lambda_0$) to a regime in which increasing the variability decreases the mean signaling time (for $a > R/\lambda_0$). It follows that the deterministic approximation given by Eq. (9) is valid in an increasing range, as R grows (See Figure 2 (C)). Indeed, an asymptotic analysis of Eq. (8) shows that the corrections to Eq. (9) are approximately of size $(a/R)^R$, and therefore rapidly decrease with R (See Methods).

The bottom row of Figure 2 shows that these observations hold more generally: When T_1 is gamma distributed the mean time to produce R proteins, $E[S_R]$, is very sensitive to randomness in delay time, but only when R is small to intermediate. As expected, the densities of the times to produce R proteins, f_{S_R}, are approximately normal and independent of the delay distribution when R is large (Middle panels of Figure 2).

We therefore expect that for each fixed threshold R, $E[S_R]$ is a decreasing function of the standard deviation σ_{T_1} of the delay. We

have proved this to be true for symmetric delay distributions (See Methods). Intuitively, this is due to the fact that the order in which proteins enter the queue is not the same as the order in which they exit. Proteins that enter the queue before the Rth protein, but exit after the Rth protein increase S_R, while the opposite is true for proteins that enter the queue after the Rth protein, and exit before it. Since only finitely many proteins enter the queue before the Rth protein, while infinitely many enter after it, the balance favors a decrease in the mean signaling time. Moreover, as delay variability increases, interchanges in exit order become more likely, and this effect becomes more pronounced. We outline the analytical argument: For each fixed time $t \geq 0$, $\overline{P}_1(t)$ is an increasing function of σ_{T_1}, hence $\overline{P}_1^{-1}(s)$ is decreasing function of σ_{T_1} for all $s \geq 0$. Referring to Eq. (6), this implies that $E[S_R]$ is a decreasing function of σ_{T_1} in the symmetric case.

In sum, mean signaling times decrease as delay variability increases (with fixed mean delay). This effect is most significant for small to moderate thresholds. We note that the decrease in mean signaling time phenomenon depends on a sufficient number of proteins entering the queue. If transcription is only active long enough for less than $2R-1$ proteins to be initiated, then mean signaling time will actually *increase* as delay variability increases. This phenomenon is explained in the subsection of the Methods section that analyzes repressor switches.

Example: Feedforward loops

Using the above results, we now examine more complicated transcriptional signaling networks. In particular, we turn to two common feedforward loops - the type 1 coherent and the type 1 incoherent feedforward loops (FFL) [43], shown in Figure 3. Each of these networks is a transcriptional cascade resulting in the specific response of the output, gene 3. The coherent FFL generally acts as a delayed response network, while the incoherent FFL has various possible responses, such as pulsatile response [43], response time acceleration [44], and fold-change detection [45].

To examine the effect of distributed delay on these networks we assume that at $t = 0$ gene g_1 starts transcription of protein P_1 at rate β_1, *i.e* $\lambda_1(t) = \beta_1\Theta(t)$. The second gene, g_2, starts transcription after $P_1(t)$ reaches the threshold R_{12}, so that $\lambda_2(t) = \beta_2\Theta(P_1(t) - R_{12})$. For the coherent FFL, we assume that the promoter of gene g_3 acts as an AND gate so that $\lambda_3(t) = \beta_3\Theta(P_1(t) - R_{13})\Theta(P_2(t) - R_{23})$. We further assume that the promoter of g_3 in the incoherent FFL is active only in the presence of P_1 and absence of P_2, so that we may write $\lambda_3(t) = \beta_3\Theta(P_1(t) - R_{13})\Theta(R_{23} - P_2(t))$.

The signaling time between any two nodes i and j within the network, *i.e.* the random time between the initiation of transcription of gene i to the formation of a total of R_{ij} proteins P_i is denoted S_{ij}. For each of the three pathways, the PDF of the signaling time is given by Eq. (5). In addition, because the random times S_{12} and S_{23} are additive (as are their variances), we can directly calculate the time at which P_2 reaches the threshold of gene 3 as $S_{123} = S_{12} + S_{23}$. Therefore, the random time at which the coherent FFL turns on is simply given by $t_{\mathrm{on}} = \max\{S_{13}, S_{123}\}$. Because $E[S_{13}]$ and $E[S_{123}]$ are decreasing functions of the delay variability, it can be expected that so is $E[t_{\mathrm{on}}]$.

In contrast to the coherent FFL, the dynamics of the pulse generating incoherent FFL are less trivial. Since the repressor (P_2) overrides the activator (P_1), assuming $S_{123} \geq S_{13}$ transcription of g_3 turns on at time S_{13} and turns off at time S_{123}, generating a pulse of duration $Z_{\mathrm{on}} = S_{123} - S_{13}$. Note that $E[Z_{\mathrm{on}}]$ can *increase or decrease* as a function of the standard deviation σ of the delay (see Figure 4 where σ was equal for all pathways).
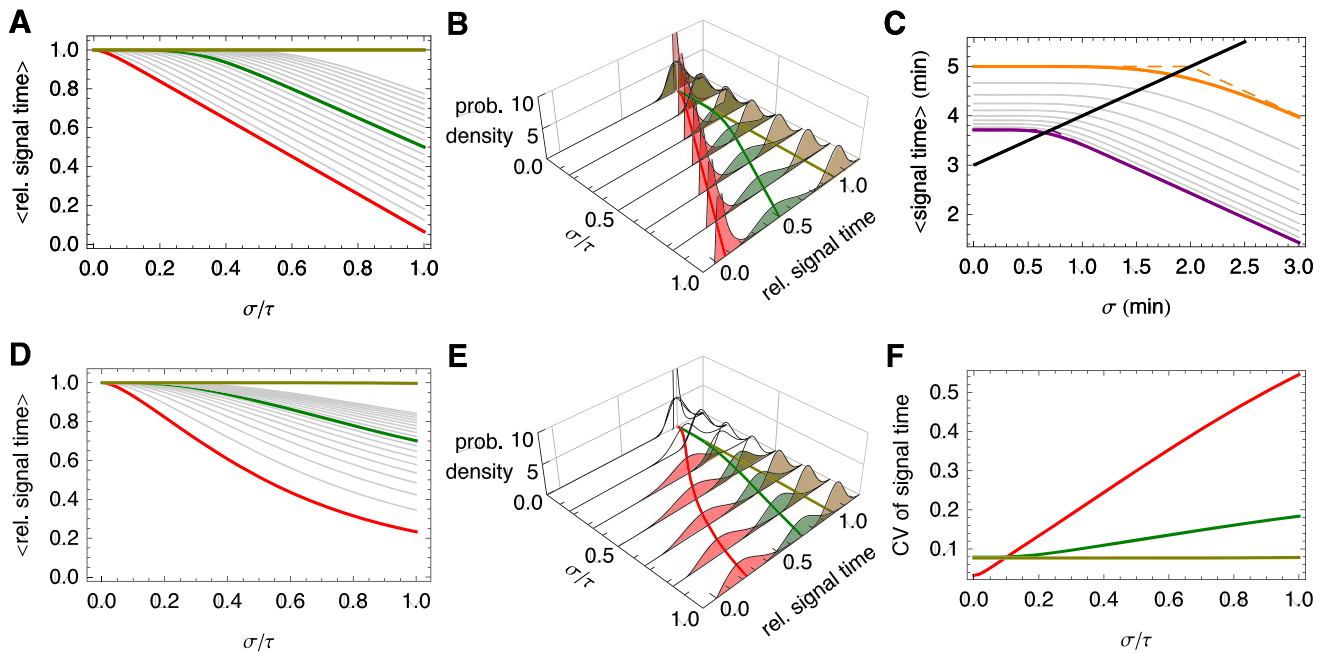
Figure 2. The effects of distributed delay on transcriptional signaling. (A) For the simplified symmetric distribution where the delay takes values $\tau + a$ and $\tau - a$ with equal probability, the mean signaling time decreases with increasing variability in delay time, Eq. (8). Shown are the signaling times (normalized by the time at $\sigma/\tau = 0$), versus CV of the delay time for signaling threshold values from $R = 1$ (red), through $R = 10$ (green) to $R = 19$ in steps of 1. Here $\tau = 3$ min and $\lambda = 10$ min^{-1}. When $R = 100$ (brown) increasing randomness in delay time has little effect on the mean. (B) Same as panel (A) but with the probability distribution, f_{S_R}, for different values of σ/τ. (C) The transition from the small σ regime to the large σ regime occurs when $\sigma \approx R/\lambda$. Here we fix $R = 10$ and between the different curves vary λ from 5 min^{-1} (magenta) to 14 min^{-1} (orange) in steps of 1. Dashed lines show the asymptotic approximations, Eqs. (9) and (10), which meet at the black line. Panels (D) and (E) are equivalent to panels (A) and (B), with T following a gamma distribution, $\tau = 3$ min, and $\lambda = 10$ min^{-1}. (F) The coefficient of variation of the signaling time, S_R, as a function of σ.
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To see this, write $E[Z_{\text{on}}]$ as follows:

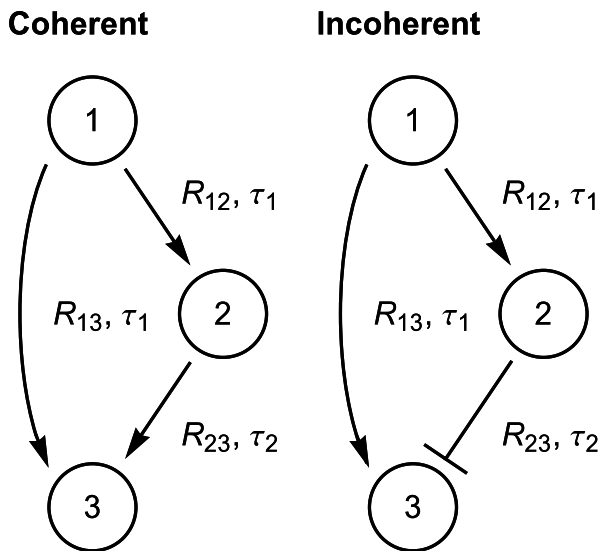$$E[Z_{\text{on}}] = E[S_{12}] + E[S_{23}] - E[S_{13}]. \tag{11}$$

Figure 3. Network schematics for the coherent and incoherent feedforward loops. Each pathway in the networks has an associated signaling threshold (R_{ij}) and mean delay time (τ_i). The random time between the initiation of transcription of gene i to the full formation of a total of R_{ij} proteins P_i is denoted S_{ij}, which is an implicit function of R_{ij}.
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Each of the terms on the right side of Eq. (11) is the expected signaling time of a single gene ($g_1 \rightarrow g_2$, $g_2 \rightarrow g_3$, and $g_1 \rightarrow g_3$, respectively). Consequently, $E[Z_{\text{on}}]$ depends on σ as a linear combination of 3 expected signaling time curves of the type pictured in Figure 2. The shapes of these signaling time curves determine the behavior of $E[Z_{\text{on}}]$ as a function of σ. Figure 4 shows that the behavior of the duration of the transcriptional pulse as a function of the delay variability depends on the values of each threshold within the network.

The delayed negative feedback oscillator

These observations can also be extended to networks with recurrent architectures. For instance, consider the transcriptional delayed negative feedback circuit [17], which can be described using an extension of scheme (2):

$$g \overset{h(P), T}{\rightarrow} g + P \tag{12a}$$

$$P \overset{\mu(P)}{\rightarrow} \varnothing, \tag{12b}$$

where $h(P)$ is a decreasing Hill function (i.e. P represses its own production) and $\mu(P)$ is the degradation rate due to dilution and proteolysis. Mather *et al.* examined the oscillations produced by systems of the type described by scheme (12) when the delay T is nonrandom (degrade and fire oscillators) [17]. Starting with no proteins, P is produced at a rate governed by the Hill function h. When the level of P exceeds the midpoint of the Hill function, gene g effectively shuts down. The proteins remaining in the queue exit,
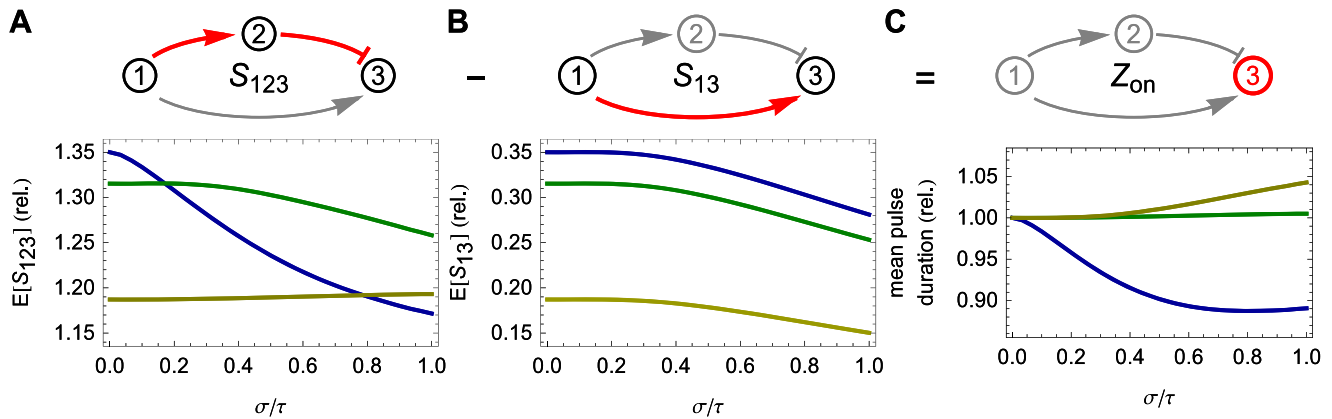
Figure 4. Distributed delay can either increase of decrease pulse duration in an incoherent feedforward loop. (A) *Top*: The longer pathway consists of the sum of two shorter pathways: $S_{123} = S_{12} + S_{23}$. (A) *Bottom*: The expected value of signaling time as a function of the relative standard deviation of the delay time. (B) *Top*: The shorter pathway is simply the signaling of the first gene to the third. (B) *Bottom*: Expected signaling time, S_{13}. (C) *Top*: The output pulse is determined by the amount of time gene 3 is actively transcribing. This time is simply the difference of the longer path duration (S_{123}) and the shorter path duration (S_{13}). (C) *Bottom*: Depending on the thresholds R_{12}, R_{13}, and R_{23}, the expected pulse duration can either increase or decrease as a function of the delay variability. In each of the three plots, the data on the vertical axis are presented relative to the mean pulse duration at $\sigma = 0$. Here, the colored lines correspond to $R_{23} = 1$ (blue), $R_{23} = 15$ (green), and $R_{23} = 100$ (brown), while $R_{12} = 100$, $R_{13} = 100$. In addition, the protein degradation rates are each $\beta = (\ln 2)/30\,\text{min}^{-1}$, all delays are gamma distributed with mean $\tau = 3\,\text{min}$.
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producing a spike, after which degradation diminishes P. When the protein level drops sufficiently, reaction (12a) reactivates and production of P resumes, commencing another oscillation cycle. Note that this circuit will not oscillate without delay.

As a result during each oscillation the gene is turned on until its own signal reaches itself, at which time the gene is turned off [17]. Therefore, the peak height of one oscillation is determined by the length of time the gene was in the "ON" state. Since that time is determined by the gene's signaling time, our theory predicts that the mean peak height of the oscillations will decrease as the variability in the delay time increases. Indeed, this is exactly what our stochastic simulations show in Figure 5. This is consistent with the fact that the negative feedback circuit is dynamically similar to the $g_2 - g_3$ sub-circuit within the incoherent FFL. Here we explicitly used a gamma-distributed delay time with mean $\tau = 3\,\text{min}$, $h(P) = \alpha C_0^n/(C_0 + P(t))^n$ and $\mu(P) = \beta P(t) + \gamma_R P(t)/(R_0 + P(t))$.

We can use our theory to predict the change in the peak height of the oscillator as a function of σ. For a delay that is gamma-distributed, the change in signaling time as a function of σ can be written as

$$f(\sigma) = E_{\sigma=0}[S_R] - E_{\sigma}[S_R], \qquad (13)$$

where $E_{\sigma}[S_R]$ is given by Eq. (6) and $f(\sigma)$ is the reduction in the expected signaling time. If we assume that the amount of time that protein is produced during a burst in the delayed negative feedback oscillator is also reduced by this amount, then it is possible to predict the change in the peak height accordingly. To a first approximation, if the promoter is in the "ON" state for a time that is $f(\sigma)$ less, then a total of $\alpha f(\sigma)$ less protein will be produced. Therefore we can write the expected peak height of the oscillator as

$$E_{\sigma}[PH] \approx E_{\sigma=0}[PH] - \alpha f(\sigma). \qquad (14)$$

However, due to degradation, Eq. (14) overestimates the correction to the peak height. Due to exponential degradation, only a fraction $\exp(-\beta f(\sigma))$ of the lost protein would have made it through to the peak. Also, the duration of enzymatic decay is

also reduced by a time $f(\sigma)$. Therefore, if we assume that the enzymatic decay reaction is saturated, we need to add an amount $f(\sigma)\gamma_R$ to Eq. (14). This gives us a more accurate prediction of the mean peak height as

$$E_{\sigma}[PH] \approx E_{\sigma=0}[PH] - \alpha f(\sigma)e^{-\beta f(\sigma)} + f(\sigma)\gamma_R. \qquad (15)$$

Figure 5 shows that this approximation works well, even for a Hill coefficient as low as 2.
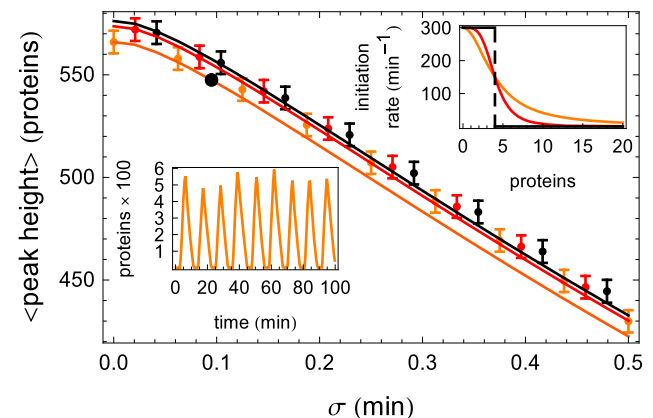

Figure 5. Distributed delay in the delayed negative feedback oscillator. Shown are the analytically predicted (solid lines) and numerically obtained (symbols with standard deviation error bars) mean peak heights of the negative feedback oscillator with Hill coefficients of $n = 2$ (orange), $n = 4$ (red), and $n = \infty$ (i.e. step function, black). The top inset shows the shape of the Hill function for the three values of n, with colors matching those in the main figure. The lower inset shows one realization of the oscillator at parameter values corresponding to the large black circle on the orange ($n = 2$) curve of the main figure. The average and the standard deviation of the peak heights were calculated from stochastic simulations of 10^5 oscillations. Here $\alpha = 300\,\text{min}^{-1}$, $\beta = 0.1\,\text{min}^{-1}$, $\gamma_R = 80\,\text{min}^{-1}$, $C_0 = 4$ and $R_0 = 1$.
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Discussion

The existence of delay in the production of protein has been known of for some time. For many systems its presence does not seriously impact performance. For example, the existence of fixed points in simple downstream regulatory networks without feedback is unaffected by delay. Delay is important if the timing of signal propagation impacts the function of the network. Delay can also change a network's dynamics. In networks with feedback, for instance, delay can result in bifurcations that are not present in the corresponding non-delayed system. The delayed negative feedback oscillator is a prime example [17]. Moreover, while the effect of delay in a single reaction may be small, it is cumulative and linearly additive in directed lines.

The intrinsic stochasticity of the reactions that create mature protein make some variation in delay time inevitable. However, we do not yet know the exact nature of this variability or the functional form of the probability density function f_T. To further complicate matters, there may exist a substantial amount of extrinsic variability in the delay time – the statistics of the PDF may vary from cell to cell.

We focused on the transient dynamics of $M/G/\infty$ queues in order to demonstrate the effects of distributed delay in a tractable setting. However, as mentioned earlier, $M/G/\infty$ queues may not always be a good model for protein production. For genes with low copy number or few available transcripts queues with $L < \infty$ service channels ($M/G/L$ queues) may provide a better description. For eukaryotic systems models in which transcription and translation are decoupled into separate queues may also be relevant. In addition, as protein production rates are often coupled with extrinsic factors such as growth rate and cell cycle phase, L may depend on time and on the state of the system.

The complexity of biochemical reaction networks suggests the use of networks of queues [46], and sources could be toggled on and off by other components of a reaction network. Even protein production from a single transcript may be more accurately described by a sequence of $M/G/1$ queues with each codon as one in a chain of service stations. In such a model ribosomes move from one codon station to the next, and are not able to skip ahead. Such models will be considered in future studies.

One further complication occurs if the burstiness of the promoter is large [47]. In the above analysis, we assumed that the initiation events of proteins were exponentially distributed in time. Since this is not necessarily the case due to the burstiness of promoters, some limits need to be put on the usefulness of the above results. Equations (9) and (10) suggest that the transition to accelerated behavior occurs when

$$\sigma > R/\lambda. \tag{16}$$

One can think of R/λ as the average time, $\overline{T}_R$, it takes to initiate R proteins, and rewrite the boundary as $\sigma > \overline{T}_R$. One can then assume that if the burstiness of the initiation events is not large, *i.e.* that the mean burst size is less than the signal threshold, then it does not matter what the distribution of initiation events is. In other words, as long as approximately R proteins are initiated in the time $\overline{T}_R$, and the variance of that number is not large, then Eq. (16) still holds.

Methods

Signaling time distributions associated with a single gene via queueing theory

Preliminary information. We first derive the signaling time distributions for a single gene that is modeled by an $M/G/\infty$ queue. An $M/G/\infty$ queue is a queueing system consisting of a memoryless arrival process (M) and infinitely many service channels (∞). The service time distribution is general (G) and there exists no maximal system size. Let

1. $(I(t))_{t \geq 0}$ denote the input (arrival) process,
2. $(Q(t))_{t \geq 0}$ denote the queue size process, and
3. $(P(t))_{t \geq 0}$ denote the departure (completion) process.

Thus $I(t)$, $Q(t)$, and $P(t)$ are the numbers of proteins that have entered the queue, are in the queue, and have departed the queue, respectively, at time t. Note that $I(t) = Q(t) + P(t)$ for all $t \geq 0$. Suppose that $(I(t))_{t \geq 0}$ is a nonhomogeneous Poisson process with rate function $\lambda(t)$. Let T denote the (random) service time and let F_T denote the cumulative distribution function (CDF) of T. This is the amount of time that a protein spends in the queue after entering. If the distribution of T is absolutely continuous, let f_T denote the probability density function (PDF) of T. For $t \geq 0$, define

$$\Lambda(t) = \int_0^t \lambda(s)\mathrm{d}s.$$

Notice that $E[I(t)] = \Lambda(t)$ for all $t \geq 0$.

Proposition. (transient distributions; see *e.g.* [40]) *Let* $t \geq 0$. *The random variables $Q(t)$ and $P(t)$ are Poisson with means*

$$E[Q(t)] = \Lambda(t) - \int_0^t F_T(s)\lambda(s)\mathrm{d}s,$$

$$E[P(t)] = \int_0^t F_T(s)\lambda(s)\mathrm{d}s.$$

Signaling time distributions. Let S_R denote the (random) first time at which $P(S_R) = R$, i.e. $S_R = \min\{t : P(t) = R\}$. We rescale time so that the rescaled completion process is a homogeneous Poisson process with rate 1. For $t \geq 0$ define

$$\bar{P}(t) := E[P(t)] = \int_0^t F_T(s)\lambda(s)\mathrm{d}s.$$

Define the rescaled departure process $(\tilde{P}(\zeta))$ by $\tilde{P}(\zeta) = P(\bar{P}^{-1}(\zeta))$. Let ξ_R denote the (random) time at which $\tilde{P}(\xi_R) = R$. The random time ξ_R has a gamma distribution with PDF

$$g_{\xi_R}(\zeta) = \frac{\zeta^{R-1}}{(R-1)!}e^{-\zeta}, \tag{17}$$

so S_R has PDF

$$f_{S_R}(t) = \frac{(\bar{P}(t))^{R-1}}{(R-1)!}e^{-\bar{P}(t)}\bar{P}'(t). \tag{18}$$

Computing the expectation of S_R, we have

$$E[S_R] = \int_0^\infty t\left(\frac{(\bar{P}(t))^{R-1}}{(R-1)!}e^{-\bar{P}(t)}\bar{P}'(t)\right)\mathrm{d}t \tag{19a}$$

$$= \int_0^{\bar{P}(\infty)} \bar{P}^{-1}(\zeta) \left(\frac{\zeta^{R-1}}{(R-1)!} e^{-\zeta} \right) d\zeta. \qquad (19b)$$

We now show that if T is symmetrically distributed about its mean and λ is a constant function, then for every fixed value of R, increasing the standard deviation σ_T of T *decreases* the expected signaling time.

Proposition. *Assume that T is symmetrically distributed about its mean and that λ is a constant function. Let $R \in \mathbb{N}$. The function $E[S_R]$ is a decreasing function of σ_T.*

Proof. Suppose that $\lambda \equiv \lambda_0$. In light of (19b), it suffices to show that for every fixed $t \geq 0$, $\bar{P}(t)$ is an increasing function of σ_T. We write $\bar{P}(t,\sigma_T)$ and $F_T(t,\sigma_T)$ to explicitly indicate the dependence of $\bar{P}$ and F_T on σ_T as well as t. Fix $t \geq 0$ and let $\gamma_2 > \gamma_1$. Define $\tau = E[T]$. For every $0 < z \leq \tau$, we have

$$F_T(\tau - z, \gamma_2) - F_T(\tau - z, \gamma_1) = F_T(\tau + z, \gamma_1) - F_T(\tau + z, \gamma_2) \geq 0.$$

Therefore, if $t < \tau$, we have

$$\bar{P}(t,\gamma_2) = \lambda_0 \int_0^t F_T(s,\gamma_2) ds \geq \lambda_0 \int_0^t F_T(s,\gamma_1) ds = \bar{P}(t,\gamma_1).$$

If $\tau < t < 2\tau$, we have

$$\begin{aligned}
\bar{P}(t,\gamma_2) &= \lambda_0 \int_0^{2\tau-t} F_T(s,\gamma_2) ds + \lambda_0 \int_{2\tau-t}^t F_T(s,\gamma_2) ds \\
&\geq \lambda_0 \int_0^{2\tau-t} F_T(s,\gamma_1) ds + \lambda_0 \int_{2\tau-t}^t F_T(s,\gamma_2) ds \qquad (20) \\
&= \lambda_0 \int_0^{2\tau-t} F_T(s,\gamma_1) ds + \lambda_0 \int_{2\tau-t}^t F_T(s,\gamma_1) ds \\
&= \bar{P}(t,\gamma_1).
\end{aligned}$$

Finally, if $t > 2\tau$, then the inequality $\bar{P}(t,\gamma_2) \geq \bar{P}(t,\gamma_1)$ follows from computation (20) and the fact that for $s > 2\tau$, $F_T(s,\sigma_T) = 1$ for all relevant values of σ_T.

Expected value of $Q(S_R)$. Computing the expectation of $Q(S_R)$, we have

$$\begin{aligned}
E[Q(S_R)] &= E[E[Q(S_R)|S_R = t]] = \\
&\int_0^\infty \left(\int_0^t \lambda(s)(1 - F_T(s)) ds \right) \frac{\bar{P}(t)^{R-1}}{(R-1)!} e^{-\bar{P}(t)} \bar{P}'(t) dt = \\
&\int_0^{\bar{P}(\infty)} \left(\int_0^{\bar{P}^{-1}(\zeta)} \lambda(s)(1 - F_T(s)) ds \right) \frac{\zeta^{R-1}}{(R-1)!} e^{-\zeta} d\zeta.
\end{aligned}$$

Example - Bernoulli delay distributions. Suppose that the rate function of the input process is constant and equal to λ_0, and T is a Bernoulli random variable described by the probability measure

$$\beta \delta_{\tau - 2a(1-\beta)} + (1-\beta) \delta_{\tau + 2a\beta},$$

where $0 < \beta < 1$. We begin by computing $E[S_R]$.

Let $x_1 = \tau - 2a(1-\beta)$ and $x_2 = \tau + 2a\beta$. The CDF of T is given by

$$F_T(t) = \begin{cases} 0, & t < x_1; \\ \beta, & x_1 \leq t < x_2; \\ 1, & t \geq x_2. \end{cases}$$

For $t \geq 0$ we have

$$\bar{P}(t) = \int_0^t \lambda_0 F_T(s) ds = \begin{cases} 0, & t < x_1; \\ \lambda_0 \beta(t - x_1), & x_1 \leq t < x_2; \\ \lambda_0(t - \tau), & t \geq x_2. \end{cases}$$

The signaling time S_R has PDF

$$f_{S_R}(t) = \frac{(\bar{P}(t))^{R-1}}{(R-1)!} e^{-\bar{P}(t)} \bar{P}'(t) =$$

$$\begin{cases} 0, & t < x_1; \\ \dfrac{(\lambda_0 \beta(t-x_1))^{R-1}}{(R-1)!} e^{-\lambda_0 \beta(t-x_1)} \lambda_0 \beta, & x_1 \leq t < x_2; \\ \dfrac{(\lambda_0(t-\tau))^{R-1}}{(R-1)!} e^{-\lambda_0(t-\tau)} \lambda_0, & t \geq x_2. \end{cases}$$

We compute $E[S_R]$ using (19b). The inverse of $\bar{P}$ is defined only for $t \geq x_1$, thus

$$\bar{P}^{-1}(\zeta) = \begin{cases} \dfrac{\zeta}{\lambda_0 \beta} + x_1, & 0 \leq \zeta < \lambda_0 \beta(x_2 - x_1); \\ \dfrac{\zeta}{\lambda_0} + \tau, & \zeta \geq \lambda_0 \beta(x_2 - x_1). \end{cases}$$

Substituting for x_1 and x_2 yields

$$\bar{P}^{-1}(\zeta) = \begin{cases} \dfrac{\zeta}{\lambda_0 \beta} + \tau - 2a(1-\beta), & 0 \leq \zeta < 2\lambda_0 a\beta; \\ \dfrac{\zeta}{\lambda_0} + \tau, & \zeta \geq 2\lambda_0 a\beta. \end{cases}$$

Using (17) and (19b), we have

$$\begin{aligned}
E[S_R] &= \int_0^{2\lambda_0 a\beta} \left(\frac{\zeta}{\lambda_0 \beta} + \tau - 2a(1-\beta) \right) g_{\xi_R}(\zeta) d\zeta + \\
&\quad \int_{2\lambda_0 a\beta}^\infty \left(\frac{\zeta}{\lambda_0} + \tau \right) g_{\xi_R}(\zeta) d\zeta \\
&= \int_0^{2\lambda_0 a\beta} \left(\frac{\zeta}{\lambda_0 \beta} - 2a(1-\beta) \right) g_{\xi_R}(\zeta) d\zeta + \\
&\quad \int_{2\lambda_0 a\beta}^\infty \frac{\zeta}{\lambda_0} g_{\xi_R}(\zeta) d\zeta + \tau \int_0^\infty g_{\xi_R}(\zeta) d\zeta.
\end{aligned}$$

Adding and subtracting $\int_0^{2\lambda_0 a\beta} (\zeta/\lambda_0) g_{\xi_R}(\zeta) \mathrm{d}\zeta$ gives

$$E[S_R] = \int_0^{2\lambda_0 a\beta} \left(\frac{\zeta}{\lambda_0 \beta} - 2a(1-\beta) \right) g_{\xi_R}(\zeta) \mathrm{d}\zeta - \int_0^{2\lambda_0 a\beta} \frac{\zeta}{\lambda_0} g_{\xi_R}(\zeta) \mathrm{d}\zeta$$

$$+ \int_0^{2\lambda_0 a\beta} \frac{\zeta}{\lambda_0} g_{\xi_R}(\zeta) \mathrm{d}\zeta + \int_{2\lambda_0 a\beta}^{\infty} \frac{\zeta}{\lambda_0} g_{\xi_R}(\zeta) \mathrm{d}\zeta + \tau$$

$$= \int_0^{2\lambda_0 a\beta} \left(\frac{\zeta}{\lambda_0 \beta} - \frac{\zeta}{\lambda_0} - 2a(1-\beta) \right) g_{\xi_R}(\zeta) \mathrm{d}\zeta + \int_0^{\infty} \frac{\zeta}{\lambda_0} g_{\xi_R}(\zeta) \mathrm{d}\zeta + \tau.$$

Using $(\zeta/\lambda_0) g_{\xi_R}(\zeta) = (R/\lambda_0) g_{\xi_{R+1}}$, we have

$$E[S_R] = \tau + \frac{R}{\lambda_0} + \int_0^{2\lambda_0 a\beta} \tag{21}$$
$$\left(\left(\frac{\zeta}{\lambda_0} \right) \left(\frac{1-\beta}{\beta} \right) - 2a(1-\beta) \right) \frac{\zeta^{R-1}}{(R-1)!} e^{-\zeta} \mathrm{d}\zeta.$$

Finally, we express (21) using gamma functions:

$$E[S_R] = \frac{(\beta-1)(\Gamma(R+1, 2a\beta\lambda_0) - 2a\beta\lambda_0 \Gamma(R, 2a\beta\lambda_0))}{\beta\lambda_0 \Gamma(R)} +$$
$$2a(\beta-1) + \frac{R}{\beta\lambda_0} + \tau, \tag{22}$$

where $\Gamma(z) = \int_0^{\infty} t^{z-1} e^{-t} \mathrm{d}t$ and $\Gamma(z, a) = \int_a^{\infty} t^{z-1} e^{-t} \mathrm{d}t$.

We now examine the asymptotics of $E[S_R]$ in the $a \to 0$ limit. The first and second partial derivatives of $E[S_R]$ with respect to a are given by

$$\partial_a E[S_R] = \frac{2(\beta-1)(\Gamma(R) - \Gamma(R, 2a\beta\lambda_0))}{\Gamma(R)},$$

$$\partial_a^2 E[S_R] = \frac{2^{R+1}(\beta-1)e^{-2a\beta\lambda_0}(a\beta\lambda_0)^R}{a\Gamma(R)}.$$

Expanding $E[S_R]$ for small values of a gives

$$E[S_R] \sim \tau + \frac{R}{\lambda_0} + \frac{2^{R+1}(\beta-1)(\beta\lambda_0)^R a^{R+1}}{\Gamma(R+2)}.$$

Using the Stirling approximation $n! \sim n^n e^{-n} \sqrt{2\pi n}$ we therefore obtain

$$\frac{2a(\beta-1)(2\lambda_0 a\beta)^R}{(R+1)R!} \sim \frac{2a(\beta-1)}{(R+1)\sqrt{2\pi R}} \left(\frac{2\lambda_0 a\beta e}{R} \right)^R,$$

and therefore

$$E[S_R] \sim \tau + \frac{R}{\lambda_0} + \frac{2a(\beta-1)}{(R+1)\sqrt{2\pi R}} \left(\frac{2\lambda_0 a\beta e}{R} \right)^R.$$

In particular, for $\beta = 1/2$ we have

$$E[S_R] \sim \tau + \frac{R}{\lambda_0} - \frac{a}{(R+1)\sqrt{2\pi R}} \left(\frac{\lambda_0 a e}{R} \right)^R.$$

In this case the correction to the deterministic limit is of order $a^{R+1}/R^{R+3/2}$.

We obtain linear large a asymptotics by noting that the first term on the right side of (22) vanishes in the $a \to \infty$ limit:

$$E[S_R] \sim 2a(\beta-1) + \frac{R}{\beta\lambda_0} + \tau.$$

Figure 6 shows a comparison between these analytical results and stochastic simulations.

Example 2- Normal delay distributions. Suppose that the rate function of the input process is constant and equal to λ_0. Suppose that T is a normal random variable with mean τ and standard deviation σ.

The CDF of T is given by

$$F_T(t) = \frac{1}{2} \left(\mathrm{erf}\left(\frac{t-\tau}{\sqrt{2}\sigma} \right) + 1 \right)$$

where erf is the error function. For $t \geq 0$ we have

$$\bar{P}(t) = \frac{1}{2}\lambda_0 \left((t-\tau)\mathrm{erf}\left(\frac{t-\tau}{\sqrt{2}\sigma} \right) - \tau \cdot \mathrm{erf}\left(\frac{\tau}{\sqrt{2}\sigma} \right) + \right.$$
$$\left. \sqrt{\frac{2}{\pi}}\sigma \left(\exp\left(-\frac{(t-\tau)^2}{2\sigma^2} \right) - \exp\left(-\frac{\tau^2}{2\sigma^2} \right) \right) + t \right).$$

Expanding $\bar{P}(t)$ about $\sigma = 0$ we obtain

$$\bar{P}(t) =$$
$$\begin{cases} 0 + \mathcal{O}(\sigma^3) \left(\exp\left(-\frac{(t-\tau)^2}{2\sigma^2} \right) + \exp\left(-\frac{\tau^2}{2\sigma^2} \right) \right), & t < \tau; \\ \lambda_0(t-\tau) + \mathcal{O}(\sigma^3) \left(\exp\left(-\frac{(t-\tau)^2}{2\sigma^2} \right) + \exp\left(-\frac{\tau^2}{2\sigma^2} \right) \right), & t \geq \tau. \end{cases}$$

Note that the corrections to the first terms in the expansions are exponentially small in σ^2 in both regimes. We denote by P^* the approximation for $\bar{P}$ which omits terms exponentially small in σ^2. The signaling time PDF of S_R can then be approximated by

$$f_{S_R}(t) \approx \frac{(P^*(t))^{R-1}}{(R-1)!} e^{-P^*(t)} \frac{\mathrm{d}}{\mathrm{d}t} P^*(t).$$

Using (19a), we have

$$E[S_R] \approx \int_0^{\infty} t \left(\frac{(P^*(t))^{R-1}}{(R-1)!} e^{-P^*(t)} \frac{\mathrm{d}}{\mathrm{d}t} P^*(t) \right) \mathrm{d}t =$$
$$\int_{\tau}^{\infty} t \left(\frac{\lambda_0 e^{-\lambda_0(t-\tau)}(\lambda_0(t-\tau))^{R-1}}{(R-1)!} \right) \mathrm{d}t = \frac{R}{\lambda_0} + \tau,$$

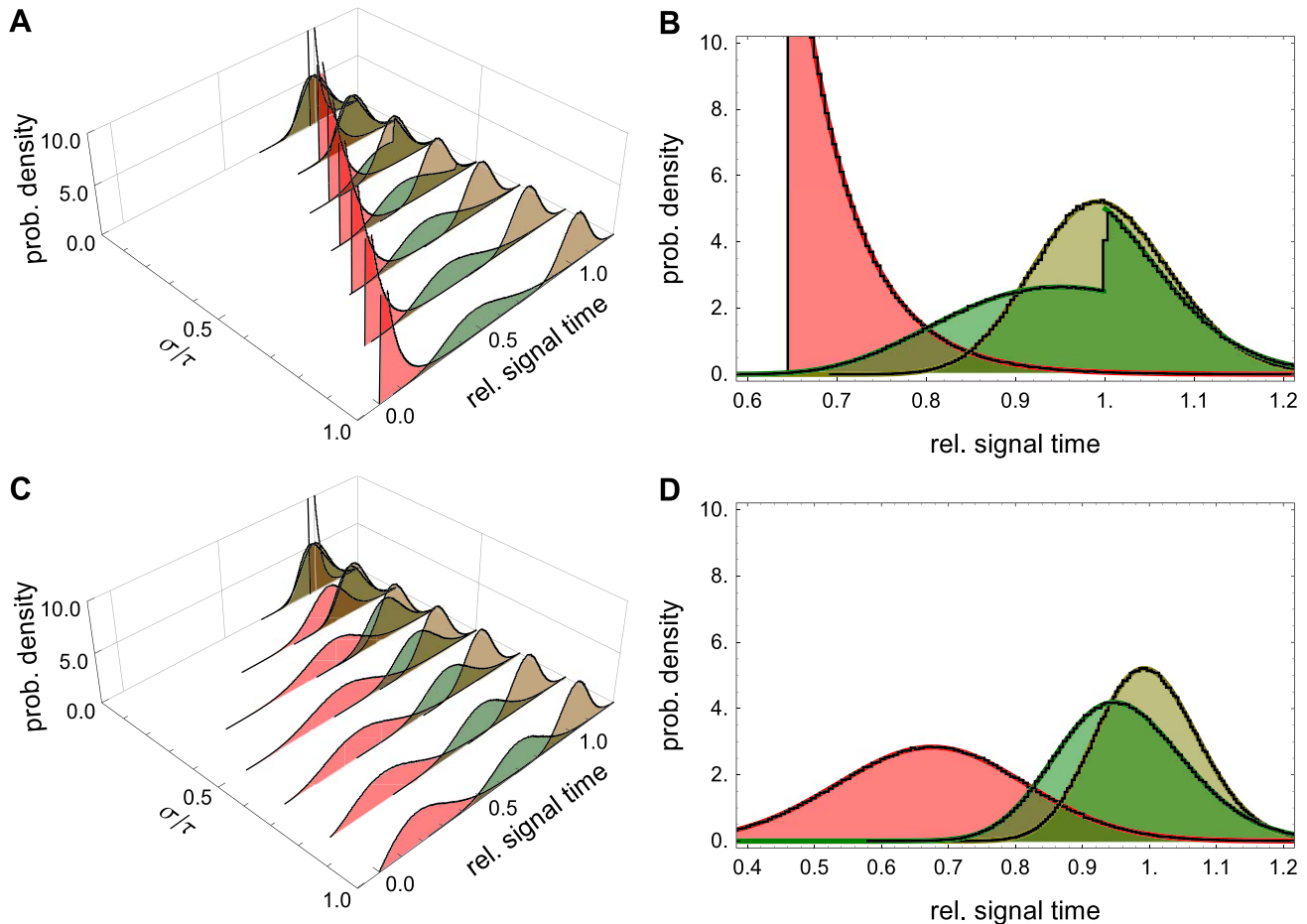which is again correct up to terms exponentially small in σ^2.

Figure 6. The effects of distributed delay on transcriptional signaling. (A) PDFs for the signaling time using the delay distribution T from Example with $\beta = 1/2$. The PDFs in red correspond to signal threshold value $R = 1$, green to $R = 10$ and brown to $R = 100$. Here $\tau = 3\,\mathrm{min}$ and $\lambda_0 = 10\,\mathrm{min}^{-1}$. (B) A 2D view of panel (A) with $\sigma = 1\,\mathrm{min}$. Solid lines show analytical results which are nearly indistinguishable from those obtained through stochastic simulation (black lines). Note that the discontinuity in the green curve is due to the discrete nature of the Bernoulli delay distribution. The CDF, F_T, has jump discontinuities that, in light of Eq. (18), produce jump discontinuities in the signaling time PDF. The discontinuity is apparent in both the theoretical prediction (green line) and the stochastic simulations (black line). Panels (C) and (D) are equivalent to panels (A) and (B) with T following a gamma distribution. The PDFs were discretized over 200 bins using 10^6 trials.
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Feed-forward network architectures

Feed-forward switches. Consider a network of two $M/G/\infty$ queues with input processes $(I_1(t))_{t\geq 0}$ and $(I_2(t))_{t\geq 0}$, queue size processes $(Q_1(t))_{t\geq 0}$ and $(Q_2(t))_{t\geq 0}$, and departure processes $(P_1(t))_{t\geq 0}$ and $(P_2(t))_{t\geq 0}$. Let $\lambda_1(\cdot)$ and $\lambda_2(\cdot)$ denote the input rate functions of queues 1 and 2, respectively. Queueing system 1 evolves independently of queueing system 2 and acts as a switch: at a time which depends on the exit process of the first system, the input process I_2 switches on (activator switch) or off (repressor switch).

Activator switches. Variances of signaling times propagate additively through linear chains of genes in which each gene up-regulates the next. Let $R_{12}, R_{23} \in \mathbb{N}$ be threshold values for protein 1 acting on promoter 2 and protein 2 acting on promoter 3, respectively. We assume that gene 2 is switched on at time

$$S_{12} := \min\{t \geq 0 : P_1(t) = R_{12}\}.$$

Analogously, let S_{23} denote the length of time between S_{12} and the time at which the P_2 process first reaches level R_{23}. The

distributions of S_{12} and S_{23} have PDFs of the form given in (18). Since S_{12} and S_{23} are independent, we have

$$\mathrm{Var}[S_{12} + S_{23}] = \mathrm{Var}[S_{12}] + \mathrm{Var}[S_{23}].$$

This argument extends inductively to directed pathways in which the product of each gene activates the subsequent gene in the sequence.

Repressor switches. Suppose that I_2 is on until time S_{12}, at which point I_2 switches off. Queueing system 2 now has modified input rate function $\lambda_2 \mathbf{1}_{[0,S_{12}]}$, where $\mathbf{1}_J$ is the characteristic function of the interval J. We compute $E[P_2(t)]$ for $t \geq 0$ by conditioning on S_{12}. Let $t \geq 0$. We have

$$E[P_2(t)|S_{12} = t^*] = \begin{cases} \int_0^t \lambda_2(s) F_{T_2}(s)\mathrm{d}s, & \text{if } t \leq t^*; \\ \int_0^{t^*} \lambda_2(s) F_{T_2}(t - t^* + s)\mathrm{d}s, & \text{if } t > t^*. \end{cases} \quad (23)$$

Therefore

$$E[P_2(t)] = E[E[P_2(t)|S_{12} = t^*]]$$

$$= \int_0^\infty E[P_2(t)|S_{12} = t^*] f_{S_{12}}(t^*) dt^*$$

$$= \int_0^t \left(\int_0^{t^*} \lambda_2(s) F_{T_2}(t - t^* + s) ds \right) f_{S_{12}}(t^*) dt^*$$

$$+ \int_t^\infty \left(\int_0^t \lambda_2(s) F_{T_2}(s) ds \right) f_{S_{12}}(t^*) dt^*.$$

Higher moments may be obtained in a similar manner.

For a repressor switch, the P_2 process and therefore the ability of gene 2 to signal downstream components depend in complex ways on the statistical properties of T_2. We examine these complex relationships by conditioning first on S_{12} and then on I_2. Suppose that $S_{12} = t^*$. The key observation is this: for fixed $t > t^*$, $E[P_2(t)|S_{12} = t^*]$ can *increase or decrease* with the standard deviation

σ_{T_2} of T_2. We verify this assuming T_2 is symmetrically distributed about its mean and assuming λ_2 is a constant function.

If the midpoint $t - t^*/2$ of the time interval $[t - t^*, t]$ satisfies $t - t^*/2 < E[T_2]$, then

$$\int_0^{t^*} F_{T_2}(t - t^* + s) ds \tag{24}$$

is an increasing function of σ_{T_2} and therefore $E[P_2(t)|S_{12} = t^*]$ *increases* as σ_{T_2} increases. By contrast, if $t - t^*/2 > E[T_2]$, then the integral in (24) is a decreasing function of σ_{T_2} and therefore $E[P_2(t)|S_{12} = t^*]$ *decreases* as σ_{T_2} increases. Repressive signaling can therefore qualitatively affect the response of P_2 production to changes in the variability of T_2.

We now examine the ability of gene 2 to signal downstream components by conditioning on I_2. Let $R_{2*} \in \mathbb{N}$. Let S_{2*} denote the time at which P_2 first reaches level R_{2*}. The key observation is
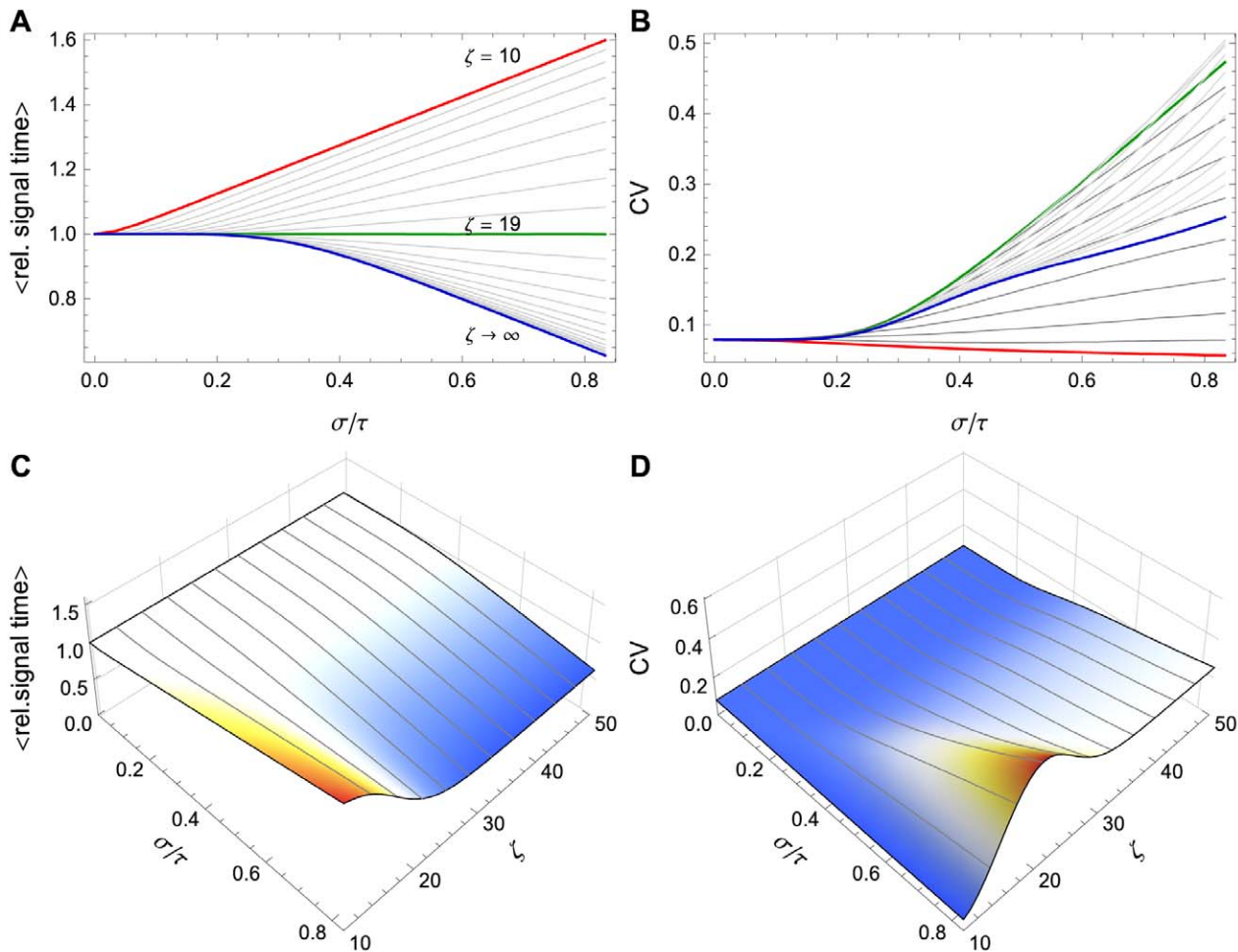

Figure 7. Signaling time depends on the number of initiation events. $E[S_{2*}|\zeta]$ can *increase or decrease* as a function of σ_{T_2} depending on the value of ζ. Here $R_{2*} = \lambda_2 = 10$. (A) $E[S_{2*}|\zeta]$ vs. CV of T_2 for ζ varying from $\zeta = 10$ (red) to $\zeta = 19$ (green) to $\zeta = \infty$ (blue) using the Bernoulli delay distribution T_2 in Example with $\beta = 1/2$. Note the transition that occurs at $\zeta = 19$. (B) Equivalent to (A), but plotting CV of the signaling time instead of conditional expectation. (C) and (D) Contour plots corresponding to (A) and (B), respectively. Notice that for fixed σ/τ, signaling time CV can change non-monotonically with ζ. For instance, at $\sigma/\tau = 0.6$, signaling time CV starts low (red), increases to ~ 0.3 (green) and then decreases thereafter. Plots were obtained through stochastic simulation with 10^6 trials.
doi:10.1371/journal.pcbi.1002264.g007

this: If we assume that gene 2 shuts off after exactly ζ transcription initiation events, then $E[S_{2*}|\zeta]$ can *increase or decrease* as a function of σ_{T_2}. Figure 7 demonstrates this numerically for a case in which λ_2 is a constant function and T_2 is symmetrically distributed. In this case, we find that $E[S_{2*}|\zeta]$

1. increases as σ_{T_2} increases if $\zeta < 2R_{2*} - 1$,
2. does not depend on σ_{T_2} if $\zeta = 2R_{2*} - 1$, and
3. decreases as σ_{T_2} increases if $\zeta > 2R_{2*} - 1$.

Intuitively, this is due to the fact that the order in which proteins enter the queue is not necessarily the same as the order in which they exit. Consider again Figure 7. When the total number of transcription initiation events, ζ, is smaller than 19, then more proteins enter the queue before the 10th protein than after it. It is therefore more likely that a protein entering before protein 10 will exit ahead of it than that a protein entering after protein 10 will exit before it. As a result, the expected time $E[S_{2*}|\zeta]$ increases with σ_{T_2}. When the balance favors proteins that enter the queue after protein 10, the opposite is true, and $E[S_{2*}|\zeta]$ decreases with σ_{T_2}.

We conjecture that this trichotomy holds in general if λ_2 is a constant function and T_2 is symmetrically distributed about its mean.

Stochastic simulations

Gillespie's stochastic simulation algorithm generates an exact stochastic realization for a system of N species interacting through M reactions. The state of the system is stored in the vector X, and each reaction j is characterized by a state change vector Z_j and its propensity function $a_j : \mathbb{R}^N \to \mathbb{R}$. If the system is in state X and reaction j occurs then the system state changes to $X + Z_j$ [5].

The idea behind extending Gillespie's SSA to model distributed delay is that if a reaction is to be delayed by some amount of time then we temporarily store this reaction along with the time at which the event will occur and we only apply this reaction at the given time. We used a version of the algorithm equivalent to those described in [32,48]. Note that [48] also describes a more efficient version of the algorithm.

References

1. Jacob F, Monod J (1961) Genetic regulatory mechanisms in synthesis of proteins. J Mol Biol 3: 318–356.
2. Alon U (2007) Network motifs: theory and experimental approaches. Nat Rev Genet 8: 450–461.
3. De Jong H (2002) Modeling and simulation of genetic regulatory systems: a literature review. J Comp Biol 9: 67–103.
4. Kærn M, Blake WJ, Collins JJ (2003) The engineering of gene regulatory networks. Annu Rev Biomed Eng 5: 179–206.
5. Gillespie DT (1977) Exact stochastic simulation of coupled chemical reactions. J Phys Chem 81: 2340–2361.
6. Kepler TB, Elston TC (2001) Stochasticity in transcriptional regulation: origins consequences, and mathematical representations. Biophys J 81: 3116–3136.
7. Bundschuh R, Hayot F, Jayaprakash C (2003) Fluctuations and slow variables in genetic networks. Biophys J 84: 1606–1615.
8. Bennett MR, Volfson D, Tsimring L, Hasty J (2007) Transient dynamics of genetic regulatory networks. Biophys J 92: 3501–3512.
9. Lan YH, Elston TC, Papoian GA (2008) Elimination of fast variables in chemical Langevin equations. J Chem Phys 129: 214115.
10. McAdams HH, Shapiro L (1995) Circuit simulation of genetic networks. Science 269: 650–656.
11. Goodwin BC (1965) Oscillatory behavior in enzymatic control processes. Adv Enzyme Regul 3: 425–438.
12. Mahaffy JM, Pao CV (1984) Models of genetic control by repression with time delays and spatial effects. J Math Biol 20: 39–57.
13. Chen L, Aihara K (2002) Stability of genetic regulatory networks with time delay. IEEE Trans Circ Syst 49: 602–608.
14. Smolen P, Baxter DA, Byrne JH (1999) Effects of macromolecular transport and stochastic uctuations on dynamics of genetic regulatory systems. Am J Physiol Cell Physiol 277: C777–C790.
15. Monk NA (2003) Oscillatory expression of Hes1, p53, and NF–κB driven by transcriptional time delays. Curr Biol 13: 1409–1413.
16. Lewis J (2003) Autoinhibition with transcriptional delay: A simple mechanism for the zebrafish somitogeneis oscillator. Curr Biol 13: 1398–1408.
17. Mather W, Bennett MR, Hasty J, Tsimring LS (2009) Delay-induced degrade-and-fire oscillations in small genetic circuits. Phys Rev Lett 102: 068105.
18. Amir A, Meshner S, Beatus T, Stavans J (2010) Damped oscillations in the adaptive response of the iron homeostasis network in *E. coli*. Molec Microbiol 76: 428–436.
19. Stricker J, Cookson S, Bennett MR, Mather WH, Tsimring LS, et al. (2008) A fast, robust and tunable synthetic gene oscillator. Nature 456: 516–519.
20. Tigges M, Marquez-Lago TT, Stelling J, Fussenegger M (2009) A tunable synthetic mammalian oscillator. Nature 457: 309–12.
21. Larson DR, Zenklusen D, Wu B, Chao JA, Singer RH (2011) Real-time observation of transcription initiation and elongation on an endogenous yeast gene. Science 332: 475–478.
22. Smolen P, Baxter DA, Byrne JH (2002) A reduced model clarifies the role of feedback loops and time delays in the drosophila circadian oscillator. Biophys J 83: 2349–2359.
23. Sriram K, Gopinathan MS (2004) A two variable delay model for the circadian rhythm of neurospora crassa. J Theor Biol 231: 23–38.
24. Ukai-Tadenuma M, Yamada RG, Xu H, Ripperger JA, Liu AC, et al. (2011) Delay in feedback repression by Cryptochrome 1 is required for circadian clock function. Cell 144: 268–281.
25. Chen KC, Csikasz-Nagy A, Gyorffy B, Val J, Novak B, et al. (2000) Kinetic analysis of a molecular model of the budding yeast cell cycle. Molec Biol Cell 11: 369–391.
26. Sevim V, Gong XW, Socolar JES (2010) Reliability of transcriptional cycles and the yeast cell-cycle oscillator. PLoS Comp Biol 6: e1000842.
27. Tiana G, Jensen MH, Sneppen K (2002) Time delay as a key to apoptosis induction in the p53 network. Eur Phys J 29: 135–140.
28. Bratsun D, Volfson D, Tsimring LS, Hasty J (2005) Delay-induced stochastic oscillations in gene regulation. Proc Natl Acad Sci U S A 102: 14593–14598.
29. Maithreye R, Sarkar RR, Parnaik V, Sinha S (2008) Delay-induced transient increase and heterogeneity in gene expression in negatively auto-regulated gene circuits. PLoS One 3: e2972.
30. Scott M (2009) Long delay times in reaction rates increase intrinsic uctuations. Phys Rev E 80: 031129.
31. Grönlund A, Lötstedt P, Elf J (2010) Costs and constraints from time-delayed feedback in small regulatory motifs. Proc Natl Acad Sci U S A 107: 8171–8176.
32. Schlicht R, Winkler G (2008) A delay stochastic process with applications in molecular biology. J Math Biol 57: 613–648.
33. Arazi A, Ben-Jacob E, Yechiali U (2004) Bridging genetic networks and queueing theory. Physica A 332: 585–616.
34. Levine E, Hwa T (2007) Stochastic uctuations in metabolic pathways. Proc Natl Acad Sci USA 104: 9224–9229.
35. Mather WH, Cookson NA, Hasty J, Tsimring LS, Williams RJ (2010) Correlation resonance by coupled enzymatic processing. Biophys J 99: 3172–3181.
36. Zhu R, Ribeiro AS, Salahub D, Kauffman SA (2007) Studying genetic regulatory networks at the molecular level: Delayed reaction stochastic models. J Theor Biol 246: 725–745.
37. Zhu R, Salahub D (2008) Delay stochastic simulation of single-gene expression reveals detailed relationship between protein noise and mean abundance. FEBS Lett 582: 2905–2910.
38. Bel G, Munsky M, Nemenman I (2010) The simplicity of completion time distributions for common complex biochemical processes. Phys Biol 7: 016003.
39. Elowitz MB, Levine AJ, Siggia ED, Swain PS (2002) Stochastic gene expression in a single cell. Science 297: 1183–1186.
40. Gross D, Shortle J, Thompson J, Harris C (2008) Fundamentals of queueing theory. 4th edition. Hoboken, New Jersey: John Wiley & Sons Inc.
41. Thattai M, van Oudenaarden A (2002) Attenuation of noise in ultrasensitive signaling cascades. Biophys J 82: 2943–2950.
42. Hooshangi S, Thiberge S, Weiss R (2005) Ultrasensitivity and noise propogation in a synthetic transcriptional cascade. Proc Natl Acad Sci U S A 102: 3581–3586.
43. Mangan S, Alon U (2003) Structure and function of the feed-forward loop network motif. Proc Natl Acad Sci U S A 100: 11980–11985.
44. Mangan S, Itzkovitz S, Zaslaver A, Alon U (2006) The incoherent feed-forward loop accelerates the response-time of the *gal* system in *escherichia coli*. J Mol Biol 356: 1073–1081.

45. Goentoro L, Shoval O, Kirschner MW, Alon U (2009) The incoherent feedforward loop can provide fold-change detection in gene regulation. Mol Cell 36: 894–899.
46. Amir A, Kobiler O, Rokney A, Oppenheim AB, Stavans J (2007) Noise in timing and precision of gene activities in a genetic cascade. Mol Syst Biol 3: 71.
47. Golding I, Paulsson J, Zawilski SM, Cox EC (2005) Real-time kinetics of gene activity in individual bacteria. Cell 123: 1025–1036.
48. Cai X (2007) Exact stochastic simulation of coupled chemical reactions with delays. J Chem Phys 126: 124108.