Effects of the promoter open complex formation on gene expression dynamics

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Little is known about the biological mechanisms that shape the distribution of intervals between the completion of RNA molecules (T_p^{RNA}), and thus transcriptional noise. We characterize numerically and analytically how the promoter open complex delay (τ_P) and the transcription initiation rate (k_t) shape T_p^{RNA}. From this, we assess the noise and mean of transcript levels and show that these can be tuned both independently and simultaneously by τ_P and k_t. Finally, we characterize how τ_P affects bursting in RNA production and show that the τ_P measured for a lac promoter best fits independent measurements of the burst distribution of the same promoter. Since τ_P affects noise in gene expression, and given that it is sequence dependent, it is likely to be evolvable.

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I. INTRODUCTION

The stochastic nature of the multiple sequential chemical reactions involved in transcription and translation cause fluctuations in genes' expression levels, which are further enhanced by the low number of transcription events, transcription factors, and transcription factor binding sites [1,2]. This noise affects cellular functioning [3,4], cell differentiation pathway selection [5,6], and adaptability of organisms to the environment [7], besides having implications in pathological processes [2,8].

The first stochastic models of gene expression [5] assumed this process as instantaneous, and can match mean expression of genes and of some small gene regulatory networks (GRNs) at quasiequilibrium [9]. Subsequent studies showed that the time between transcription initiation and the production of an active protein affects the dynamics of GRNs [9–13].

Transcription and translation comprise multiple steps. In prokaryotes, transcription starts with the binding of an RNA polymerase (Rp) to a promoter (P) and the formation of the open complex [14,15], during which time no other Rp can bind to the promoter (ranging from seconds to minutes [14]). This process delays the production of the RNA molecule after transcription initiation, and is here referred to as the "promoter delay" (τ_P).

This delay varies between transcription events [16]. For example, τ_P of the lac promoter follows a Gaussian-like distribution with a mean of 40 s and a standard deviation of 4 s [17]. τ_P also varies widely from gene to gene [18].

Simulations of a multidelayed stochastic toggle switch [19] provided evidence that the effects of τ_P on GRN dynamics is not accountable by having only a delay on protein completion, as τ_P makes the promoter unavailable for reactions for a time period.

Given that the delay on protein production alone does not capture effects of other delays on GRN dynamics, multidelayed stochastic models of prokaryotic transcription and translation were proposed [20] and, consequently, multidelayed stochastic models of prokaryote GRNs [21]. Subsequent studies using these models assessed by inspection how several parameters, including τ_P, affect protein fluctuations [10,13].

Pedraza and Paulsson [22] characterized how the distribution of time intervals between the production of consecutive RNA molecules (T_p^{RNA}) and proteins affects noise in RNA and proteins levels, using previous theoretical results [23]. Their study did not focus on what determines the shape of this distribution.

Using Monte Carlo (MC) and analytical methods, we study how in prokaryote gene expression the promoter delay, τ_P, and the transcription initiation rate, k_t, shape the distribution T_p^{RNA}. Our analysis is made for all values of τ_P and k_t within realistic intervals according to measurements, and we further extend previous works [10,13] by accounting for the fact that τ_P varies between transcription events, in agreement with measurements [14,17].

While we focus on the role of τ_P and k_t, events in elongation such as stochastic stepwise movement in the DNA template [15], collisions between Rp [20], backtracking [24–26] and pauses [26,27], also affect T_p^{RNA} and transcriptional noise [24,25]. Their effects are here accounted for in the distribution of intervals between completion of RNA molecules. Relevantly, these events do not affect the distribution of intervals between completion of ribosome binding site region of the RNA (RBS), which allows initiating translation in prokaryotes.

We first present the multidelayed stochastic model of gene expression, followed by the analytical deduction of the distribution of time intervals between completions of consecutive RNA molecules as a function of τ_P and k_t. We then estimate numerically and analytically the noise and mean level of transcripts and show that these can be tuned independently and simultaneously, by varying τ_P and k_t. Finally, we assess numerically and analytically how τ_P directly affects transcriptional bursting and confront our calculations with measurements.

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II. DELAYED STOCHASTIC MODEL OF GENE EXPRESSION

We model gene expression by reactions with multiple delays [21] and simulate the dynamics according to the delayed stochastic simulation algorithm (SSA) [20], which innovates relative to previous ones [9,11] in that it can handle more than one delayed event per reaction.

Transcription has three main phases: initiation, elongation, and termination. In *E. coli*, for transcription to start, the Rp has to find a start site, form a closed complex, isomerize into an open complex, and clear the promoter region [17]. The duration of this process is sequence dependent [17], varies from one transcription event to the next [16] and differs between different genes. After, the Rp moves along the DNA strand (elongation) while forming the RNA molecule. In the end, a single-stranded RNA molecule is released (termination) [14].

Reaction 1 models transcription [21] as a single-step multidelayed reaction. An Rp can bind to P and initiate transcription with a stochastic rate constant k_t. This reaction has three products, all delayed. First, P is released for new reactions after τ_P elapses and, at approximately the same time, a ribosome binding site molecule is produced [13] (not modeled explicitly). This is followed by the release of the Rp for new reactions, and of a complete RNA molecule

$$P + \mathrm{Rp} \xrightarrow{k_t} P(\tau_P) + \mathrm{Rp}(\tau_{\mathrm{Rp}}) + \mathrm{RNA}(\tau_{\mathrm{RNA}}). \quad (1)$$

If product X has a delay τ, represented by $X(\tau)$, it takes τ seconds for X to be produced after the reaction occurs. τ can be randomly drawn from a distribution each time the reaction occurs [28]. If all delays were set to zero, when an Rp bound to P, it would instantaneously produce an RNA and release Rp and P for new reactions.

Each of these delays affects the dynamics differently. τ_P imposes a minimum time between transcription events [14]. τ_{Rp} can cause the number of available Rp to vary significantly if these exist in low amounts, affecting the propensity of transcription. τ_{RNA}, which can vary significantly from one transcription event to the next [26], causes a temporal translation in the amount of RNA, and consequently, of proteins in the cell [22]. In [13], it was numerically assessed that if $[(|\mathrm{Rp}| \times k_t)^{-1} + \tau_P] \gg \tau_{\mathrm{Rp}}$, then τ_{Rp} does not affect the dynamics significant ($|\cdot|$ denotes the amount of a substance).

Note that under normal conditions in prokaryotic transcription, τ_{Rp} and τ_{RNA} are very similar but not identical. I.e., the RNA is usually released slightly before the Rp [29]. The difference between these two delays might vary from gene to gene (sequence dependence), thus we opted for defining each delay by a unique variable, although in our simulations τ_{Rp} and τ_{RNA} are set to the same value.

In E. coli, in normal conditions, there are $\approx$28 Rp's available for transcription at any moment [30]. Stress can cause overexpression of many genes, decreasing the number of available Rp's, consequently limiting transcription. Since we do not address such stress situations, we maintain the number of *available* Rp's constant and disregard the explicit presence of Rp's in analytical estimations.

Reaction 2 models RNA degradation at the rate d_{RNA}

$$\mathrm{RNA} \xrightarrow{d_{\mathrm{RNA}}} \varnothing . \quad (2)$$

Values of delays, rate constants, and initial quantities of each substance are described in the results section. Note that the model used here is applicable to prokaryotes, but not to eukaryotes, which would require at least one extra step to account for chromatin remodeling [31].

TABLE I. Probability distribution of T_p^M, where M is either RBS or RNA, and its mean, variance, and coefficient of variation. X_t is a random variable drawn from the distribution $\mathrm{Exp}(\lambda_t)$, where $\lambda_t = k_t|\mathrm{Rp}|$. All τ's are also random variables.

		Delayed	Nondelayed
T_p^M	=	$X_t + \tau_P + \tau_M - \tau_M^{\mathrm{prev}}$	X_t
$\mu(T_p^M)$	=	$\lambda_t^{-1} + \mu(\tau_P)$	λ_t^{-1}
$\sigma^2(T_p^M)$	=	$\lambda_t^{-2} + \sigma^2(\tau_P) + 2\sigma^2(\tau_M)$	λ_t^{-2}
$c_v(T_p^M)$	=	$\sqrt{\lambda_t^{-2} + \sigma^2(\tau_P) + 2\sigma^2(\tau_M)} / \lambda_t^{-1} + \mu(\tau_P)$	1

III. RESULTS

A. Distribution of time intervals between consecutive completions of RNA molecules

We determined analytically the distribution of intervals between the production of consecutive RBS, T_p^{RBS} (released at the same time as the promoter [13,21]), and between the production of consecutive RNA, T_p^{RNA}, accounting for the delay τ_P and elongation time. For comparison, we also deduce expressions assuming no delays.

The model being stochastic, we study the probability distribution of T_p^{RBS} and T_p^{RNA}: their mean μ, variance σ^2 and coefficient of variation c_v (defined as σ/μ). These quantities can be analytically deduced from properties of the delayed SSA [20]. $\mathrm{Exp}(\lambda)$ denotes the exponential distribution with a mean of λ^{-1}, while $\lambda_t = k_t|\mathrm{Rp}|$ is the propensity of transcription [32]. Let $X_t \sim \mathrm{Exp}(\lambda_t)$ denote the time for a free promoter to initiate transcription. The time between the production of two consecutive RBS molecules equals $X_t + \tau_P + \tau_{\mathrm{RBS}} - \tau_{\mathrm{RBS}}^{\mathrm{prev}}$, where τ_{RBS} is the time it takes to produce the RBS under consideration and $\tau_{\mathrm{RBS}}^{\mathrm{prev}}$ is the time it took to produce the previous one. Both τ_{RBS} and $\tau_{\mathrm{RBS}}^{\mathrm{prev}}$ need to be considered as transcription initiation can occur again before the preceding one is fully complete. The expression for the time between the creations of two complete RNA molecules is obtained similarly. The expressions in Table I describe how the mean, the variance, and the c_v of the distributions explicitly depend on the delays τ_P, τ_{RBS}, and τ_{RNA}.

Importantly, for constant delays or a distribution with small variance in comparison to the mean (as in the lac promoter in E. coli, which has a mean of 40 s and a standard deviation of 4 s [17]), the time between transcription events varies less as τ_P increases, with consequences on the noise levels of transcripts.

We verified the expressions in Table I by MC simulations. As an example, we set $k_t = 10^{-2}$ s^{-1} [13], $\tau_P \sim \mathrm{N}(40, 4^2)$s [17], and $\tau_{\mathrm{RBS}} \sim \mathrm{N}(45, 4^2)$s [10]. Thus, from Table I,

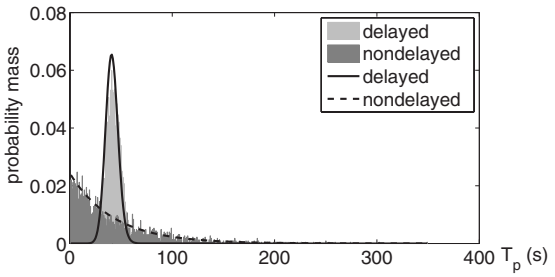

FIG. 1. Normalized MC and analytical T_p^{RBS} distributions for $\tau_P \sim \mathrm{N}(40,4^2)$ and $\tau_P=0$. Lines correspond to the analytical predictions.

$\mu(T_p^{\mathrm{RBS}})=42.5$ s. To ensure the same $\mu(T_p^{\mathrm{RBS}})$ in the nondelayed case, we set $k_t=5.9\times10^{-4}$ s^{-1}. The two scenarios are simulated for 10^5 s. Figure 1 shows the T_p^{RBS} distributions determined numerically and analytically for these parameter values. The means of the distributions are 42.4 and 42.6 s, while the standard deviations are 7.2 and 41.5 for the delayed and nondelayed case, respectively, as analytically predicted.

By affecting T_p^{RBS} and T_p^{RNA}, τ_P is expected to affect the distribution of bursts in transcription, and thus the noise in transcript levels, protein levels, and, consequently, GRNs' dynamics.

B. Effects of τ_P and k_t on the transcript levels

In [22], it was shown that the noise in RNA and protein levels depends on $\mu(T_p^{\mathrm{RNA}})$ and $\sigma(T_p^{\mathrm{RNA}})$. For the RNA, this noise is [22]

$$c_v(\mathrm{RNA}) = \sqrt{d_{\mathrm{RNA}} \times \mu(T_p^{\mathrm{RNA}}) \frac{c_v^2(T_p^{\mathrm{RNA}})+1}{2}}. \qquad (3)$$

As mentioned in [22], many physical parameters affect this distribution, e.g., elongation times, which are affected by events such as pauses, arrests, etc [24]. Formulas in Table I show how $\mu(T_p^{\mathrm{RNA}})$ and $\sigma(T_p^{\mathrm{RNA}})$ can be varied as a function of two parameters, namely, τ_P and k_t. Also, importantly, the formulas show that $\mu(T_p^{\mathrm{RNA}})$ and $\sigma(T_p^{\mathrm{RNA}})$ can be varied independently of one another.

By identifying and characterizing how these two physical parameters of genes' promoters, namely, the distribution of the durations of the open complex formation and the affinity between Rp and promoter region, shape T_p^{RNA} distribution, one could predict, e.g., the effect of point mutations in the promoter sequence on T_p^{RNA}, as these two parameters are sequence dependent [16,17].

We assessed numerically how well the formulas predict RNA noise and mean level as a function of τ_P and k_t. We simulated gene expression and RNA degradation (reactions 1 and 2) for various values of τ_P and k_t. d_{RNA} is set to 1.389×10^{-3} s^{-1} (within the interval of known RNA half-lives in E. coli, i.e., 0.5–30 min [33]). To maintain the number of Rp molecules constant, its delay is set to zero. Finally, τ_{RNA} is set to zero so as to better assess the effects of τ_P alone on transcripts' mean and noise level.

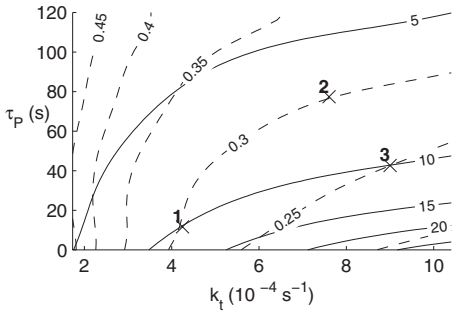

FIG. 2. Contour plot for the mean and coefficient of variation (c_v) of the RNA level as a function of τ_P and k_t. The mean stays constant when moving along the solid lines and the c_v stays constant when moving along the dashed lines.

Figure 2 illustrates how the mean and the coefficient of variation in the RNA level can be varied independently or simultaneously. Point (1) located at $k_t=4.25\times10^{-4}$ s^{-1} and $\tau_P=11.61$ s has a mean of 10.1 and $c_v=0.30$. Point (2) located at $k_t=7.6\times10^{-4}$ s^{-1} and $\tau_P=77.42$ has the same c_v as point (1) but different mean of 6.41. Point (3) at $k_t=9\times10^{-4}$ s^{-1} and $\tau_P=42.58$ s has the same mean as point (1) but a c_v of 0.25. One example of a change in both mean and noise by tuning k_t and τ_P is moving from point (2) to (3).

Further, for smaller values of k_t and not too large values of τ_P, the c_v (but not the mean) is roughly independent of τ_P (c_v contours are nearly vertical). In this regime, the intervals between transcription initiation events (determined mostly by k_t), are much longer than τ_P. Thereby, τ_P plays almost no role (if smaller than 50 s) as a limiting factor of transcription initiation, as mentioned when describing the effects of each delay in reaction 1 and as expected given the expression for $\mu(T_p^{\mathrm{RNA}})$ (Table I). On the other hand, the mean RNA level in this regime varies almost linearly with k_t, as it would in the nondelayed case, again because the delay is not affecting noise and rate of transcription.

One could argue that in this regime there is no particular evolutionary constrain on the value of τ_P, up to a certain extent (increasing beyond a certain duration would affect the dynamics of transcription, thus be under evolutionary pressure).

C. Bursts in transcriptional dynamics

To assert if τ_P can directly affect the noise in gene expression, one needs to confront the transcriptional dynamics of the model with measurements of the dynamical patterns of RNA levels [22,34] for varying values of τ_P.

T_p^{RNA} determines the distribution of bursts in RNA levels, defined as rapid increases in RNA levels from one measurement to the next, thus RNA noise level [22]. We focus on the effect of τ_P on T_p^{RNA}, thus, on RNA noise level. The relevance of characterizing τ_P effects on noise is that τ_P is sequence dependent [17], thus evolvable.

We measure the distribution of changes in RNA amounts from one measurement to the next in the simulation, for various values of τ_P. From this, we obtain the burst size distribution and compare it to the one obtained from measure-

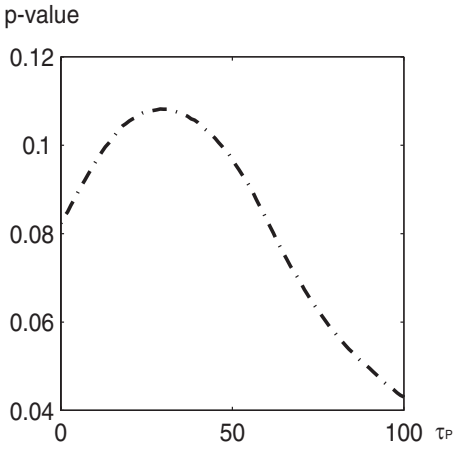

FIG. 3. p-values from the two-sampled K-S test between the distribution of variations in RNA amounts in experiments and model for various values of τ_P (from 0 to 100, step size of 1).

ments [35]. Note that other measurements, in eukaryotes, as, e.g., in [36], are not comparable to our model, given the differences in transcriptional regulatory mechanisms between eukaryotes and prokaryotes.

Fortunately, the measurements with which we confront our model were made in E. coli [35], on a gene driven by the lac promoter, for which the promoter delay length is well known from independent measurements [17] ($\approx$40 s). We thus expect the best match between model and experiments [35] to occur for $\tau_P \approx 40$ s [17].

In the experiment described in [35], E. coli cells were grown and induced for MS2-GFP (fluorescent RNA binding protein). Measurements of fluorescence taken every minute over a 2 h period were reported, from which the number of RNA molecules can be determined [35].

To confront model and experiments, we measured the distribution of bursts in RNA amounts, from measurements of number of RNA molecules taken in intervals of 60 s in individual model cells, for mean τ_P ranging from 0 to 100 s. The number of samples taken (10^7) for each value of $\mu(\tau_P)$ is sufficient to obtain a good estimation of the real distribution. These distributions were then confronted to the one obtained from the measurements (120 data points [35]) by the two-sample Kolmogorov-Smirnov (K-S) test, to assess their goodness of fit.

We set k_t to 0.4 min^{-1} [35] and a decay rate of 0.44 min^{-1} so as to match the mean RNA levels ($\approx$9 molecules) reported in [35]. RNA decay is modeled by reaction 2.

We disregard noise sources in elongation [13,14,24,25]. These can be significant if, e.g., the gene has sequences prone to long pauses [15,24,27], which is not the case for the gene sequence used in these experiments. Also, for the value of k_t used, collisions between Rp's in the strand are rare [20], not contributing to noise significantly. To assess which τ_P best fits the experiments, we computed the p-values of the K-S test between experiments and simulations for values of τ_P from 0 to 100 s (Fig. 3). The values of τ_P between 30 and 50 s have the highest p-values, i.e., these are the values that best match the experimental data. While the p-values are not very high, note that the experimental data consists of 120 data points and is affected by measurement noise [35].

These results imply that τ_P has a significant effect on the transcriptional dynamics of a gene. Importantly, the bursting dynamics of a gene driven by a lac promoter [35] is best matched by setting τ_P to the duration measured for that promoter's open complex formation in an independent experiment [17]. This demonstrates the accuracy of this model of prokaryotic gene expression.

Given the effects on the bursts distribution, it is then expected that τ_P affects the noise level of transcripts.

It is known that transcriptional and, consequently, translational noise vary if mean and standard deviation of T_p^{RNA} vary [22,23]. Our results allow characterizing how τ_P and k_t affect T_p^{RNA}, and thus, RNA and protein noise levels. Given the dependency of the dynamics of GRNs on the noise level of its constituent genes [1,5,13,10,19,21] one can then conclude that the τ_P of each gene affects the dynamics of GRNs.

IV. CONCLUSIONS

The effects of k_t on the noise in gene expression are well known [5,2]. Less is known about how the various delays during transcription and translation affect this noise.

We studied, analytically and numerically, the effects of the delay associated to the open complex formation step in the dynamics of transcription and translation. This delay strongly affects the distribution of intervals between transcription completions, and thus the bursting distribution of RNA levels. Importantly, the best fit of our model to measurements of a lac promoter's bursting dynamics [35] is attained by assuming a delay following a distribution identical to the one assessed experimentally in an independent measurement [17].

Our main results are the numerical and analytical characterization of T_p^{RNA} dependency on k_t and τ_P, and from there, the observation that the noise and mean of transcript and protein levels can be varied independently, or simultaneously, by varying these two parameters. Notably, k_t and τ_P are sequence dependent, implying that mutations in the promoter sequence are likely to allow the tuning of genes' noise and mean expression level, simultaneously or independently.

In the study of noise in gene expression, results regarding its regulation on a general level exist [22], i.e., how different T_p^{RNA} distributions cause different noise levels. However, little is known about the biological mechanisms that shape T_p^{RNA} and thus transcriptional noise. The formula for T_p^{RNA} as a function of τ_P and k_t provides a partial answer to this question in prokaryotic gene expression, namely, how the initiation step of transcription contributes to noise.

Regarding general modeling strategies of stochastic gene expression and genetic networks, our results suggest that these need to represent explicitly the delay on the promoter release and thus, the promoter itself, besides the delays associated to the completion of RNA and, in translation, of proteins [9,11], given that for experimentally measured time lengths, the promoter open complex formation delay [16,17] affects transcriptional dynamics.

Our results also suggest that, in translation, the delay associated to the RBS release [13] needs to be explicitly modeled as well. Further studies are needed to characterize the effects on T_p^{RNA} of events in elongation, such as pauses [24], and consequently on transcriptional noise.

Since τ_P affects noise in gene expression and thus the dynamics of GRNs, it is likely to be subject to selection. Given that it is sequence dependent [14,15], it is likely to be evolvable.

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