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The Generation of Promoter-Mediated Transcriptional Noise in Bacteria

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Abstract

Noise in the expression of a gene produces fluctuations in the concentration of the gene product. These fluctuations can interfere with optimal function or can be exploited to generate beneficial diversity between cells; gene expression noise is therefore expected to be subject to evolutionary pressure. Shifts between modes of high and low rates of transcription initiation at a promoter appear to contribute to this noise both in eukaryotes and prokaryotes. However, models invoked for eukaryotic promoter noise such as stable activation scaffolds or persistent nucleosome alterations seem unlikely to apply to prokaryotic promoters. We consider the relative importance of the steps required for transcription initiation. The 3-step transcription initiation model of McClure is extended into a mathematical model that can be used to predict consequences of additional promoter properties. We show in principle that the transcriptional bursting observed at an *E. coli* promoter by Golding et al. (2005) can be explained by stimulation of initiation by the negative supercoiling behind a transcribing RNA polymerase (RNAP) or by the formation of moribund or dead-end RNAP-promoter complexes. Both mechanisms are tunable by the alteration of promoter kinetics and therefore allow the optimization of promoter mediated noise.

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Introduction

Cellular processes involve stochastic reactions between limited numbers of molecules, and therefore are subject to random noise. The existence of noise in the intracellular concentration of various species has been highlighted in a number of natural and engineered genetic circuits [1–6], which has been coupled with an increasing focus on the theory of how noise might be controlled or exploited by the cell.

Gene expression is perhaps the most important stochastic process in the cell. Transcription involves the production of small numbers of mRNAs, which are then translated multiple times, creating and amplifying noise in protein concentrations. Therefore, the probability distribution underlying the timing of transcription initiation is important for understanding cellular dynamics. A distribution where initiations are evenly spaced will result in less noise and a more uniform cell population. In contrast, a highly variable rate of initiation will produce large fluctuations that can lead to heterogeneous behavior across populations of genetically identical cells. This variability is important to allow populations of unicellular organisms to cope with variable environments [1,5]. Another example is the spontaneous induction of ‘non-inducible’ prophages such as P2 [7], where stochastic flipping of a genetic switch allows a low rate of transition from lysogeny into lytic development. Noise in transcriptional initiation also has implications for transcriptional interference between convergent promoters [8].

Bertrand [9] and colleagues have developed a system where an mRNA containing multiple MS2 binding sites can be visualized by the binding of MS2-GFP fusion proteins to the mRNA. Golding and colleagues [10] placed such an mRNA under the control of the $P_{lac/ara}$ promoter in *E. coli* and could thereby detect production of individual mRNAs. When the promoter was induced, transcription was observed to occur in an unexpectedly irregular fashion, with bursts of transcription separated by long periods of inactivity. This phenomenon was called transcriptional bursting. The bursts of activity (on-periods) lasted an exponentially distributed amount of time, with a mean of 6 minutes at 22°C. During an on period a geometrically distributed number of transcripts are produced in rapid succession, with a mean of 2.2 transcripts per on-period. The long periods without transcription (off-periods) were also exponentially distributed, with a mean of 37 minutes. Golding et al. also report that similar behavior is seen with the P_{RM} promoter of phage lambda.

Golding et al. [10] showed that this behavior was inconsistent with transcription occurring as a Poisson process. Here we consider the McClure model of transcription initiation [11–13], a more general model of transcription initiation, and show that it is still unable to reproduce the transcriptional bursting observed by Golding et al. We then consider current hypotheses for the mechanism of transcriptional bursting and find them wanting. Finally we propose two novel hypotheses for the mechanism behind transcriptional bursting, demonstrating that they are able to explain the results of Golding et al.

Author Summary

Noise in gene expression is important for phenotypic variation among genetically identical cells. The gene expression will be particularly sensitive to noise in transcription initiation. Transcription initiation from a given promoter involves multiple steps, each of which could be rate limiting. In this paper we discuss how transcription initiation could come in bursts, separated by long periods where the promoter is inactive. Our results are compared to recent data of Golding *et al.* (2005), which suggest that transcriptions from some prokaryotic promoters occur in a highly irregular burst-like fashion. We show that the observed bursting could be caused by one of two alternate mechanisms. One possibility is that changes in supercoiling induced by previous RNA polymerase can help a subsequent RNAP to enter directly into open complex. Another possibility is that an RNAP at the promoter sometimes forms a dead-end complex, and thereby occludes the promoter for a sizeable amount of time.

Results

The Standard Promoter Model Does Not Produce Bursting

Golding *et al.* showed that their results were not consistent with transcription initiation being a single Poisson process. By considering the McClure model of transcription initiation (Figure 1A) we show that initiation as a single Poisson process is a special case where only one step is rate limiting, and that while the more general case is not a single Poisson process it is still unable to fit the results of Golding *et al.*

In prokaryotes, the initiation of transcription requires the binding of an RNAP to the promoter, the isomerisation of the RNAP through several intermediate forms, rounds of abortive initiation and then finally release from the promoter. Here we consider the McClure model of transcription [11–13] (Figure 1A), where transcription initiation requires three steps: RNA polymerase (RNAP) binding to the promoter to form a closed complex, followed by isomerisation of the closed complex to an open complex in which the DNA at the promoter is melted, and the escape of the open complex to form an RNAP complex engaged in elongation of the transcript. The closed complex is assumed to be in rapid equilibrium with free RNAP, while isomerisation and escape are treated as being slower and irreversible. This model is a simplified but useful version of the full kinetics of initiation.

The kinetics of each elementary reaction in initiation determines the final distribution of transcription initiation. Transcription is often treated as a Poisson process, i.e. the probability of initiation at a given moment is a constant, which results in an exponential distribution of times between transcripts. Golding *et al.* were able to show through several methods that the distribution of transcription initiation was non-Poisson. However, the exponential distribution is a special case where there is only one rate limiting step in the initiation of transcription.

For the analytical analysis of the McClure model, we make the assumption that the rates of binding k_b and unbinding k_u of the closed complex are relatively fast, and therefore that there are only two kinetically significant steps, isomerisation of the closed complex to an open complex, and promoter escape by the open complex. We assume that each step is elementary, i.e. that it can be approximated as a single chemical reaction. We also ignore the effect of self-occlusion, where an RNAP prevents further initiation

at the promoter until it has transcribed far enough to no longer occlude the promoter (50 bp), as the time needed to transcribe this distance (1–4 seconds) is negligible compared to the time between initiations in the Golding *et al.* experiments. The average time needed to complete the first step, t_o , is therefore $t_o = (1+K)/O$, where $K = k_u/k_b$ is the equilibrium constant of dissociation for the closed complex and O is the rate of transition from closed to open complex. The inverse of the rate of the open to elongating transition (E) gives the average time needed for the second kinetically important step, t_E (Figure 1A). The average time taken for initiation (and therefore the time gap between initiations, $\langle \Delta t \rangle$, with $\langle \dots \rangle$ indicating the average) is the sum of two exponentially distributed random variables, $\langle \Delta t \rangle = \langle \tau_o + \tau_E \rangle$. The probability distribution of time gaps between initiations is given by

$$P(\Delta t) = \frac{\left[\exp\left(-\frac{\Delta t}{\tau_o}\right) - \exp\left(-\frac{\Delta t}{\tau_E}\right) \right]}{\tau_o - \tau_E} \quad (1)$$

for $t_o \neq t_E$. For $t_o = t_E = t$, we get

$$P(\Delta t) = \frac{1}{\tau^2} \Delta t \exp\left(-\frac{\Delta t}{\tau}\right). \quad (2)$$

In the case where one step is much slower than the other (Class I), there is only one rate-limiting step in initiation and the distribution of Dt approaches a single exponential with mean $t_L = \max(t_o, t_E)$ (Equation 1; Figure 1B), i.e. it approaches a single Poisson process. Here, the data points in Figure 1B) have been obtained by simulating the model of the promoter in Figure 1A) using the Gillespie algorithm [14], which stochastically determines the next reaction to occur and the time interval between reactions based on the given rates. The other extreme, where $t_o = t_E$ (Class II), is shown in Figure 1C. In Class II, the chance of rapid successive firings faster than the average ($Dt \ll t_o + t_E$) is smaller than for a Class I promoter, as for a Class II promoter a low Dt requires both the isomerisation and the escape to productive transcription to occur in rapid succession, whereas for a Class I promoter a low Dt requires the rapid occurrence of the rate limiting step only. As a consequence the distribution in Class II shows a peak at non-zero Dt . Promoter models that specify more kinetically significant reaction intermediates produce more extreme versions of the Class II distribution, with a larger peak centered around $\langle \Delta t \rangle$, resulting in more regular firing intervals.

The Class I type promoter shows the most fluctuation in Dt , and the effect of adding more kinetically significant intermediate steps is to reduce the amount of variability in Dt . Therefore neither the standard model nor models that take into account more intermediates can reproduce the bunched activity observed by Golding *et al.* [10], which show greater fluctuations in Dt than a Poisson process. In order to reproduce the bunched activity, it is necessary to consider a model with a branched pathway, where the system can go into either an active state or an inactive state with a switching mechanism between them.

Previously Proposed Mechanisms for Bunched Activity

Here we consider several hypotheses for the mechanism of transcriptional bursting and argue that they are unlikely to be correct. The promoter used by Golding *et al.* [10], $P_{lac/ara}$, can be repressed about 70-fold by the lac repressor and activated about 30 fold by AraC [15]. Therefore, a simple hypothesis put forward by Golding *et al.* is that the silent periods are periods where the lac

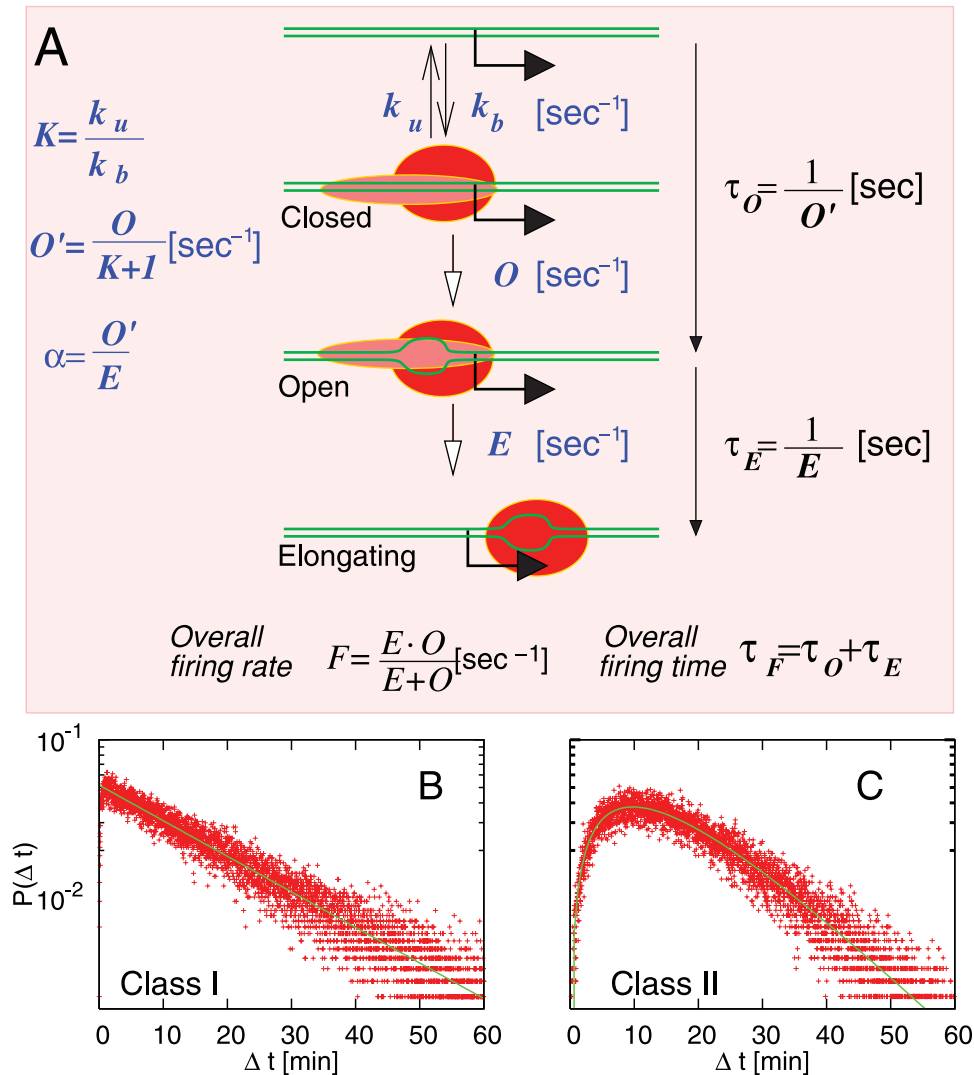


Figure 1. The Hawley-McClure 3-step model of transcription initiation. (A) First an RNAP forms a closed complex at the promoter with some on (k_b) and off rates (k_u), and subsequently forms an open complex with rate O . This process is a directed non-equilibrium transition. Finally, the open complex escapes the promoter into productive transcription with the one way rate E . (B–C) The distribution of intervals between transcription events for the standard 3-step model. For binding and unbinding rates of the closed complex we use $k_b = k_u = 60$ [1/min]. The average total strength is $F = 1/20$ [min], whereas $l = 35$ [bp] and $v = 25$ [bp/sec]. (B) Class I: Probability distribution for time between transcripts Δt for a promoter with a single rate limiting step. Here, it is isomerisation with $O = 1/9.7$ [min]. The escape rate from the open complex is $E = 1/0.19$ [min]. Red dots: stochastic simulation results. Solid line: predicted distribution, $(1/\tau)\exp[-\Delta t/\tau]$ with $\tau = 19.4$ [min]. (C) Class II; Δt probability distribution for a promoter with two rate limiting steps because the isomerisation and escape rates are similar ($O = 1/4.9$ [min], $E = 1/9.7$ [min]). Solid line shows the predicted distribution, $(1/\tau)^2 \Delta t \exp[-\Delta t/\tau]$ with $\tau = 9.7$ [min].
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repressor is bound to the promoter, and the bursts are periods of activity when the promoter is free. However, the mean duration of off-periods is 37 min while on periods are only 6 min in duration, despite the fact that the promoter has been fully induced by 1 mM IPTG. It seems impossible for the lac repressor to remain bound to the DNA for 37 minutes under these conditions; especially considering that 1 mM IPTG derepresses the lac promoter in less than 5 sec [16].

A similar idea is that the off-periods represent periods where AraC is not bound to the promoter [10]. To make this feasible the on rate for AraC in an *E. coli* cell would have to be exceedingly small given the large off periods. This is unreasonable in view of the high association rate for AraC to other operators [17].

Presumably association rate is diffusion limited, meaning that it would take one AraC molecule less than a minute to bind to the operator [18]. In conclusion we find it unlikely that binding AraC is sufficient to produce bunched activity.

Another hypothesis put forward by Golding *et al.* is that RNAP might be able to re-initiate after termination, aided by the retention of sigma factor during transcription [19]. Presumably the RNAP would have to be positioned to rebind to the same promoter after termination for re-initiation to occur with any reliability, and it is not clear how this would be caused. One possibility is that a transcription factor might remain in contact with both the RNAP and the promoter via a DNA loop. This would render the promoter unavailable during transcription,

which has some support from the data in that the lengths of the observed on-periods were approximately equal to the number of initiations multiplied by the time taken to transcribe the reporter mRNA for both $P_{lac/ara}$ and P_{RM} (Golding, private communication), which would be expected if transcription does not occur simultaneously. However, this data is somewhat anecdotal, and stands in contradiction to the simultaneous transcription observed with electron microscopy [20]. Also, this mechanism requires binding of a closed complex to the DNA to be the rate limiting step that causes the 37 minute long off-period, and we consider it unlikely that simple recognition of the promoter by RNAP would take this long, especially given that closed complex formation is often thought to be a rapid equilibrium process.

Multiple RNAP can cooperate to overcome pause sites [21]. It might therefore be possible that the burst is due to multiple RNAP building up at a pause site and overcoming it together. However, this would require the RNAP to pause for a length of time on the same scale as the off-period; such an extreme pause is unlikely given that even the strongest pauses measured *in vitro* only last for around one minute.

Bursting could also result if there were distinct regions of high and low transcriptional activity within bacteria, akin to the idea of transcription factories in eukaryotes, and the promoter moved in and out of these regions on a slow time scale [22,23]. Although this is an interesting possibility, not enough is known to evaluate such a mechanism in bacteria in much detail.

Fluctuations in the availability of free RNAP within the cell could contribute to variable initiation rates but it is difficult to see how such severe and long-lasting fluctuations capable of producing extended periods of complete inactivity could occur in cells where ~ 3000 RNAPs [24] produce $>10^5$ RNAs per generation.

Supercoiling-Mediated Recruitment

There is both theoretical [25] and experimental evidence [26,27] that an elongating RNAP can increase the negative supercoiling of the DNA behind it.

Promoters can be very sensitive to supercoiling; for example, *in vitro* the activity of the LacP promoter increases by more than a factor of 10 when the super-coilings is changed from zero to -0.065 (which is the average supercoiling of DNA in *E. coli*) [26]. We therefore consider it a possibility that the bursts of transcription might be caused by a transcribing RNAP assisting the recruitment of further RNAP via the wake of supercoiling left behind it. In principle one could argue that perturbed supercoil states could relax quickly in a plasmid [25] like the one used by Golding *et al.*, but it has been demonstrated that a promoter can induce huge changes in supercoiling of a plasmid [28].

Consider a promoter where open complex formation is a rate limiting step that is assisted by negative supercoiling. To model this, we assume that the negative supercoiling assists this step to the extent that it is no longer rate limiting. We parameterize this effect of supercoiling into a single number q , the probability that supercoiling left in the wake of a prior RNAP allows a subsequent RNAP to rapidly form an open complex before the supercoiling is relaxed (Figure 2A). This then creates two possible behaviors at the promoter. If the promoter is in the supercoiled state, open complex formation is enhanced to the point where it is not rate limiting, and transcription events occur at rate E and are exponentially distributed. If the promoter is not in the supercoiled state, then open complex formation is very much slower and now rate limiting; transcriptional events are still exponentially distributed but now with the much lower rate O . This creates the long periods of inactivity associated with off periods (Figure 3A) and

holds when $O \ll E$, and gives a distribution

$$P(\Delta t) = \frac{q}{\tau_E} \exp\left(-\frac{\Delta t}{\tau_E}\right) + \frac{(1-q)}{\tau_O} \exp\left(-\frac{\Delta t}{\tau_O}\right) \quad (3)$$

(shown in Figure 3B).

The supercoiling need not persist for the full length of the on-period, or for the length of time between two initiations. In the scheme we present here, it is only required that the supercoiling persists long enough to allow an open complex to form rapidly. The final escape step is assumed to be neutral with respect to supercoiling and hence as soon as an open complex has formed at the promoter the supercoiling can be relaxed without interrupting the on-period. This assumption can be varied without changing the general behavior of the model.

If the supercoiling is relaxed before an open complex is formed, the promoter has switched to an off-period where initiation occurs at a much slower rate. The parameter q determines the size of the on-periods, as after each initiation there is a probability q that another open complex will be recruited and the on-period will continue, or a probability $1-q$ that an off-period will start. Therefore, the probability of getting a burst of $\langle \Delta n \rangle$ initiations is proportional to q^{Dn-1} . In this model a promoter is in the on-state when it is in the supercoiled state or when it has an open complex. Table 1 gives equations relating model parameters to the average $\langle \Delta n \rangle$, $\langle t_{on} \rangle$, and $\langle t_{off} \rangle$ (Derivations are given in Text S1).

This mechanism can reproduce the observations of Golding *et al.* [10] with the parameters $t_O = 37$ [min], $t_E = 29$ [min] and $q = 0.545$. We simulated the recruitment model using the Gillespie algorithm [14]. It gives the expected shape for the $P(Dt)$ distribution (Figure 3B) and matches the distribution of Dn measured by Golding *et al.* (3C) and also the distributions of on and off-periods measured by Golding *et al.* (3D). In these plots the on-periods are defined as being the time intervals when there is rapid successive initiation (Figure 3A), following the procedure in Golding *et al.* [10]; the detailed definition is given in the Materials and Methods section.

Formation of a Dead-End Complex

Another possibility is that the off periods are due to the formation of long-lived non-productive initiation complexes at the promoter [29–31]. These non-productive complexes have been observed *in vitro* and may be arrested backtracked complexes or complexes that cannot exit the abortive initiation state into productive elongation. In both cases initiation can be made more efficient by the GreA/B RNAP-binding factors [29,30]. The random formation of such ‘dead-end’ complexes could block the promoter for extended periods of time, causing productive transcription to be confined to those times when the promoter is free. For the promoter IPR the lifetime of these complexes was found to be in the order of 10–20 minutes under *in-vitro* conditions, thus dead-end complexes can last long enough to cause the observed off-periods [31].

For the analytical treatment of this model we call the probability that a promoter bound complex will undergo a productive initiation Q and the probability that the promoter bound complex enters a moribund state is therefore $1-Q$. We assume that removal of the moribund complexes is a Poisson process with a rate d , which gives $\langle t_{off} \rangle = \tau_{dead}/Q$ with $t_{dead} = 1/d$, which allows for the fact that a single off-period can be caused by multiple subsequent moribund complexes (Table 1). Here we consider a promoter to be in the off-period if it is occupied by dead-end complexes; otherwise it is on. The derivations of on- and off-times are given in Text S1. The dead-end complex mechanism is also capable of causing the behavior observed by Golding *et al.* The data of Golding *et al.* are reproduced with $Q = 0.545$, $t_{dead} = 20$ [min], and $t_O + t_E = 2.9$ [min].

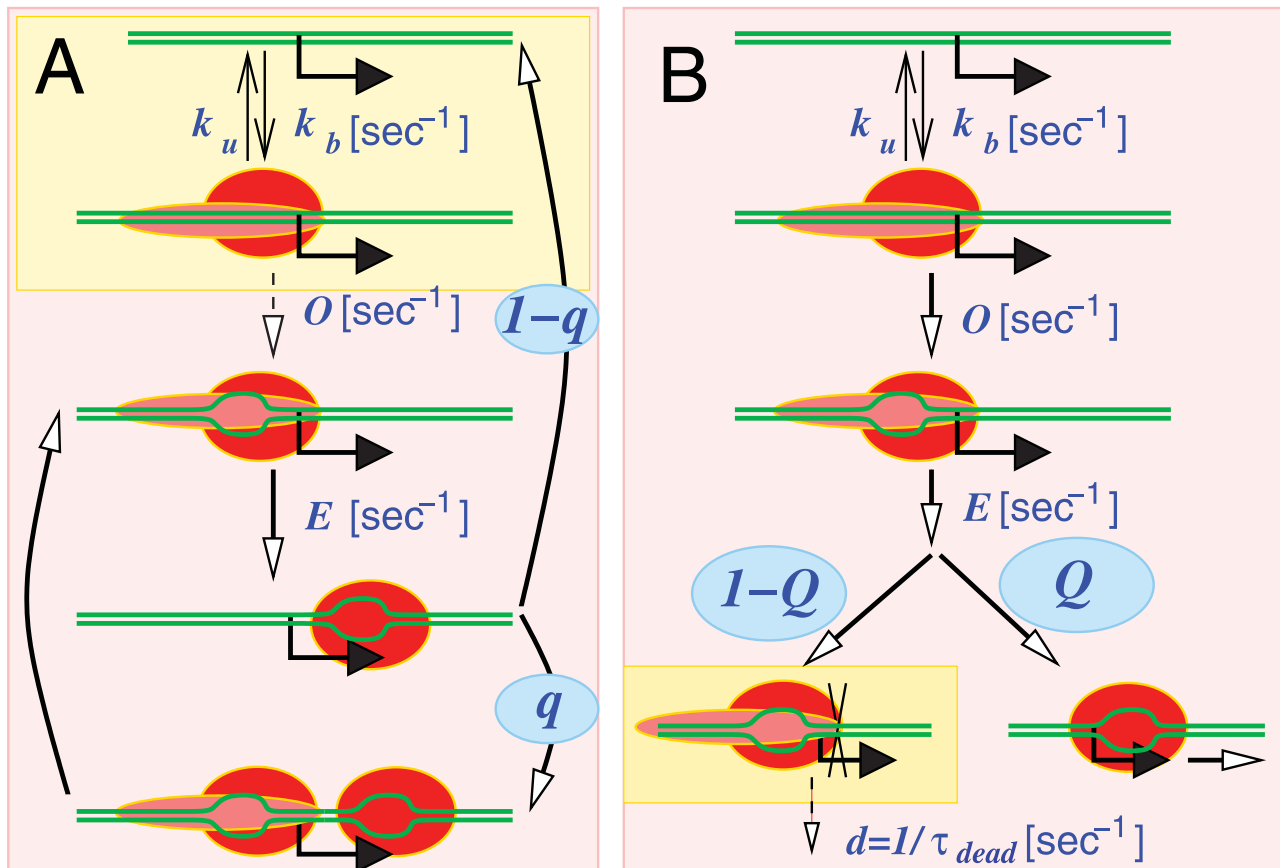


Figure 2. Revisiting mechanisms for bunched firing. We propose (A) supercoiling assisted open complex formation, and (B) possible stalling of an RNAP into a “moribund” complex. The yellow background indicates the state where most of the time is spent in the off period. (A) An elongating RNAP might recruit a subsequent RNAP into an open complex with probability q , thus by-passing the time needed to recruit an RNAP and form open complex. In the limit of large k_b , the firing rate is given by $F = \frac{E \cdot O'}{O' + (1-q)E} = \frac{O'}{\alpha + (1-q)}$ with $\alpha = \frac{O'}{E}$. (B) Two alternative open complexes, of which one is productive and the other is a dead end complex that is removed with rate d . In this case Q denotes the probability that a closed complex enters into the productive open complex. In the limit of large k_b , the firing rate is given by $F = \frac{QEO'}{E + O' + (1-Q)OE/d} = \frac{Qd}{\beta + (1-Q)}$ with $\beta = \frac{\tau_O + \tau_E}{\tau_{dead}} = \frac{d(E + O')}{EO'}$. The detailed calculations and equations for limiting k_b are given in Text S1. Both the dead-end and the recruitment model can be simulated on-line using the java applet on <http://www.cmol.nbi.dk/models/transcription/RNAPInitiation.html>. doi:10.1371/journal.pcbi.1000109.g002

Figure 3E shows the distribution $P(Dt)$ with these parameters obtained by the simulation using the Gillespie algorithm [14]. It has been confirmed that the distributions of Dn , t_{on} and t_{off} are reproduced as well as the recruitment model (data not shown).

The formation of dead-end complexes is favored by low temperatures at the lac UV5 promoter [32]. If this were also the case for the $P_{lac/ara}$ promoter, it could be part of the explanation for why the $P_{lac/ara}$ promoter is so weak in the conditions used by Golding *et al.* (22°C) when it is reported to be a strong promoter elsewhere [15]. However, the activity of the promoter observed by Golding *et al.* at 37°C is still rather low compared to the previously reported estimate [15]. This could be associated with the fact that there is almost no activation of the promoter caused by AraC/arabinose under their experimental conditions (see Figure 1E in Golding *et al.*). Another possibility could be the presence of an unknown terminator, which would imply that the number of complete transcripts represents only a fraction of the transcription initiation events.

Control of Transcriptional Noise

One of observations made by Golding *et al.* that was used as evidence for transcriptional bursting was that the Fano factor for

the distribution of number of transcripts \mathcal{N} , $v = \langle (\mathcal{N} - \langle \mathcal{N} \rangle)^2 \rangle / \langle \mathcal{N} \rangle$, was approximately 4 for the $P_{lac/ara}$ promoter at 37°C, rather than 1 predicted for Poisson transcription. The Fano factor is a measure of noise; higher values indicating a more noisy process. When the on-periods are much shorter than the off-periods, the Fano factor n is linked to the burst size Dn as $v \approx \langle \Delta n \rangle$. If the on-time is sizable, on the other hand, $\langle \Delta n \rangle$ needs to be much larger to give the same n .

By considering a population of cells where transcripts are degraded with rate g , we can relate n to model parameters. Figure 4 shows how n varies with model parameters for each model while keeping $\langle \mathcal{N} \rangle = 10$ obtained by analytical calculations (The detailed calculations are in the Text S1.). In the recruitment case the Fano factor is larger for smaller a and larger q , i.e., when the open complex formation is the rate limiting step and once a firing has occurred further recruitment occurs successively. In the dead-end model the Fano factor is larger for smaller $b = (t_O + t_E)/t_{dead}$ and larger Q which occurs when moribund persist for long periods of time, but transcription during the on periods is rapid and occurs many times before another off period occurs. One should note that the Fano factor can be changed depending on parameters for a given $\langle \mathcal{N} \rangle$; This means that the noise can be tuned for a given promoter strength under either model, which can allow the

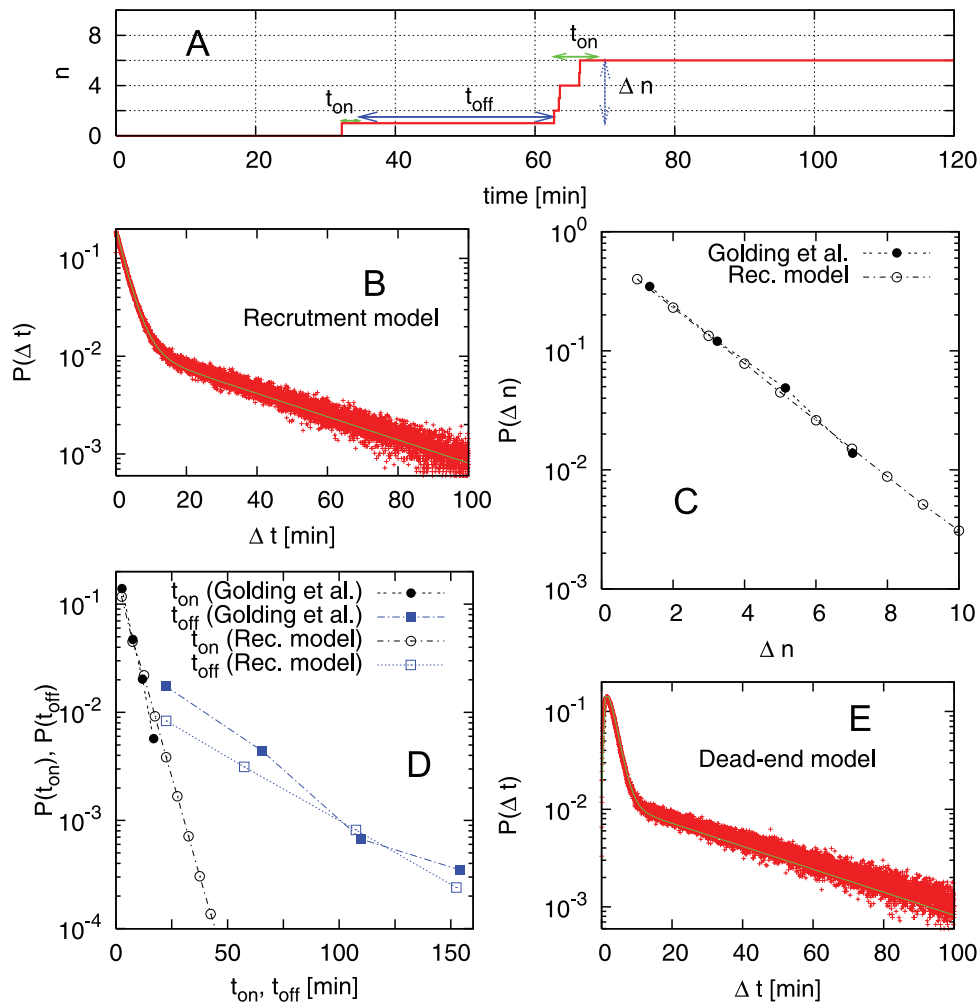


Figure 3. Transcriptional bursting from three step model with supercoiling assisted recruitment and the dead-end complex model. (A–D) show the results from the recruitment model with the probability of recruitment $q=0.545$. The average firing rate is $1/20$ [1/min]. $k_b=k_u=60$ [1/min], $E=1/2.9$ [1/min], $O=1/18.3$ [1/min], $l=35$ [bp], $v=25$ [bp/sec]. (A) Accumulated number of mRNAs, showing on periods and off-periods. Transcriptional bursting can be seen around 62 [min] to 70 [min], where 5 firings occur in rapid succession. See Materials and Methods for the choice of parameters and definitions of t_{on} and t_{off} . (B) The distribution of the durations between firing, $P(\Delta t)$. The solid line shows Equation 3. (C) The distribution of the number of firings per on-period, $P(\Delta n)$. The filled circles show experimental data from Golding *et al* [10], and the open circles are Δn from the recruitment model. (D) The distribution of the “on-times” t_{on} (open circles) and the “off-times” (open boxes) t_{off} . The experimental data from Golding *et al.* for the on-time (filled circles) and off-time (filled boxes) are also shown. (E) The distribution of intervals between initiations for the dead-end complex model (Figure 2B). The probability of productive elongation is $Q=0.545$, and the rate of removal of dead-end complexes is $d=1/\tau_{dead}=1/(20$ [min]). The average firing rate is $1/22$ [1/min]. $k_b=k_u=60$ [1/min], $E=1/1.5$ [1/min], $O=1/0.7$ [1/min], $l=35$ [bp], $v=25$ [bp/sec]. This parameter choice corresponds to a Class 2 promoter in the on periods, which gives a round curve for short timescales (around 3 [min]). The solid line shows $Q\Delta t \exp[-\Delta t/\tau]/\tau^2+(1-Q) \exp[-\Delta t/(\tau_{dead}/Q)]/(\tau_{dead}/Q)$ with $\tau=\tau_O=\tau_E$.
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Table 1. Relations between model parameters and the average $\langle \Delta n \rangle$, $\langle t_{on} \rangle$, and $\langle t_{off} \rangle$.

Parameters	$\langle \Delta n \rangle$	$\langle t_{on} \rangle$	$\langle t_{off} \rangle$
<i>P. laclara</i>	2.2	6 [min]	37 [min]
Recruitment	$1/(1-q)$	$(\langle \Delta n \rangle - 1)\tau_E + \Delta$	τ_O
Dead-end	$1/(1-Q)$	$(\langle \Delta n \rangle - 1)(\tau_O + \tau_E) + \Delta$	τ_{dead}/Q

Here, the duration Δ for a RNAP to transcribe one mRNA after it has been fired from the promoter is added, because in Golding’s experiment the mRNA is already visible when it is being made.
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promoter noise to evolve to reflect a level that provides the best fitness for the cell.

Discussion

We have analyzed possible mechanisms of transcriptional bursting in terms of a simple recruitment/isomerisation/escape model. A model where supercoiling created by an RNAP engaged in transcription assists in the recruitment of subsequent RNAPs is able to reproduce all the features of the experiments, without resorting to very large timescales for on-off equilibrium rates, or unknown pause sites or localization effects. Alternatively, the data of Golding *et al.* could also be reproduced if the investigated promoters spent a sizable fraction of their time by being occupied by an RNAP in a non-productive state.

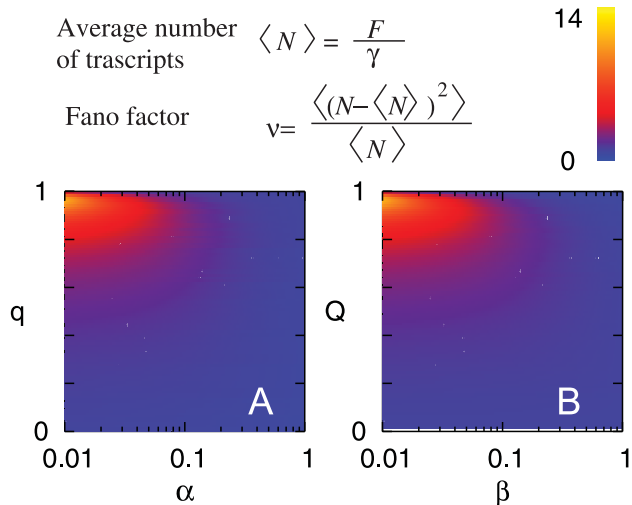


Figure 4. Parameter dependence of Fano factor. (A) Fano factor

$v = 1 + \frac{(1-q)(q-x)}{(x+1-q)(x+1-q+x/(\langle N \rangle(1-q-x)))}$ in the recruitment model for $\langle N \rangle = F/\gamma = 10$. The horizontal axis shows the aspect ratio $\alpha = O'/E$, and the vertical axis shows the recruitment probability q . The fluctuations are larger for smaller α , where the formation of the closed complex is the rate-limiting step. (B) Fano factor for the dead-end model,

$$v = 1 + Q \left[(1-Q) - \frac{(\gamma/d+1)\alpha\beta^2}{(\alpha+1)^2} \right] \left[(\beta+1-Q) \left(\beta+1-Q + \beta \frac{\gamma}{d} + \frac{(1+d/\gamma)(\gamma/d)^2\alpha\beta^2}{(\alpha+1)^2} \right) \right]^{-1}$$

with $\frac{\gamma}{d} = \frac{Q}{\langle N \rangle(\beta+1-Q)}$, for $\langle N \rangle = 10$ and $\alpha = 1$. The horizontal axis is $\beta = (\tau_O + \tau_E)/\tau_{dead}$, the ratio of the average time required for successful firing to the average time taken to remove a dead-end complex, and the vertical axis is the probability of successful firing, Q . Small β and large Q gives large fluctuations, which enables burst like firing through successive normal firings (from large Q) and long silent periods until the dead-end complex is removed (from small β). The detailed calculations are given in Text S1.

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Transcription bursts have been reported in eukaryotic systems [33,34] and have also been proposed to facilitate cell to cell variability. These eukaryotic model systems both included transcription factors and in addition they may be influenced by chromatin remodeling. The bunched expression of nearby genes is correlated [34], a feature that fits with extended states of chromatin. The dead end complex cannot give such spatial correlations, whereas supercoiling mediated recruitment in principle could correlate expression from two promoters if they are close to each other.

In one mammalian system, the reported pulse duration and silenced periods are similar to the ones modeled in this paper [33]. However, in that system subsequent bursts of transcription are correlated, with one transcription burst priming the system for another one [33], which has not been reported in Golding *et al.* This is again consistent with the larger scale genomic silencing associated with, for example, chromatin states or the genes repositioning relative to transcriptional factories [22]. The recruitment model cannot account for correlations between subsequent bursts, whereas the dead end model could give such time correlations between bursts if the dead end complexes come in different categories, each with their characteristic lifetime.

Overall we stress that our current modeling demonstrates two plausible mechanisms for generating bursts of transcription at an isolated promoter. Additional mechanisms come into play when

the promoter is regulated by a transcription factor with a low on-rate, or when large scale reorganization of the chromosome takes place on a slow timescale.

Both the dead-end and the recruitment model can be simulated on-line using the java applet on <http://www.cmol.nbi.dk/models/transcription/RNAPInitiation.html>.

Testing the Recruitment Model

The recruitment model implies a number of predictions that can be tested. In particular, promoters with bunched transcription initiation will be highly sensitive to negative supercoiling of the DNA. And conversely, promoters that are insensitive to supercoiling will have transcription events which are separated by more regular time intervals.

For promoters that are sensitive to supercoiling, one could selectively shorten the long off periods by introducing a second nearby promoter. One option is to add a divergent promoter that might be able to donate its negative supercoil wake. Such a construct was investigated by Opel *et al.* [27], who reported that a second promoter could indeed increase the activity of a supercoiling sensitive promoter in the *ilvYC* operon. This predicts that if a similar experiment was done with the $P_{lac/ara}$ promoter, then reduced off periods would be observed.

Another prediction is that for promoters with bunched activity the isomerisation step is rate limiting. Thus the fraction of time spent in open complex is small compared to the time between transcription initiations. One might be able to show an inverse correlation between the noisiness of a promoter and the occupancy of the promoter by open complexes using potassium permanganate DNA footprinting [35].

Testing the Dead-End Model

The dead-end mechanism implies that the promoter is mostly occluded by an RNAP with an open transcription bubble. This could be identified permanganate footprinting [35].

The availability of GreA/B could affect the rate of removal of the dead-end complex, d [29,30]. Overexpression of GreA/B could increase d and reduce off-periods, while longer off-periods, due to lower d , could be observed in greA/B mutants.

It is possible that the dead-end complexes could be removed by a collision with an RNAP transcribing from a second promoter in a fashion similar to the removal of an open complex by transcriptional interference [36]. The off-times of a promoter could therefore in principle be shortened by using other RNAP's initiated from another promoter that transcribes across the promoter in question. If a promoter spent a substantial fraction of the time occupied by a dead-end complex, it could be strongly activated by tandem or even convergent promoters, which would be a novel twist on the usually repressive effect of transcriptional interference. If d is reduced in Table 1, the "off-times" could be reduced by a factor set by the ratio of the strength of the two promoters, and the promoter activity could increase. Thus, if $P_{lac/ara}$ activity is affected by dead-end complex formation, then placing a weak divergent promoter upstream should not increase $P_{lac/ara}$ activity but placing this promoter in a convergent orientation may activate $P_{lac/ara}$.

Perspectives for the Regulation of Transcriptional Noise

The sensitivity of a promoter to supercoiling mediated recruitment or dead-end complex formation provides additional avenues for control of overall promoter strength, either by evolution or by regulatory factors.

DNA supercoiling can increase or decrease promoter activity both *in vitro* [26] and *in vivo* [37] in a promoter specific manner.

Supercoiling can affect RNAP binding to the promoter and open complex formation *in vitro* and presumably can affect other steps as well. RNAP recruitment induced by the supercoiling created by an elongating transcription complex may contribute significantly to the activity of certain promoters. We expect that, except for very active promoters, rapid dissipation of the supercoil would make inhibition of a supercoiling-repressed promoter by this mechanism unlikely. Stimulation by the departing elongating complex should similarly only apply to the early steps in initiation. Thus only promoters whose early steps are rate-limiting and can be enhanced by supercoiling should be stimulated by this mechanism.

The reduction of promoter activity by the formation of dead-end complexes is potentially very strong. The effect increases with the probability of forming such a complex ($1-Q$) and with the lifetime of the complex ($1/d$), parameters which could be determined both by the promoter sequence and by the availability of factors such as GreA/B that may remove the complex [29,30]. This mechanism would seem to be an inefficient way to set the strength of a promoter, as it would sequester an RNAP. However, it would allow regulation by transcription factors that change the fraction of RNAPs that enter into dead-end complexes or that stabilized the dead-end complex. As a consequence, genes which are silenced through this mechanism will have relatively high fluctuations in expression level, and thereby some cells can explore advantages afforded by relatively high expressions, even when most cells are kept at near zero expression. Bunched activity for a near silenced promoter could, for example, be important in the pathway for the spontaneous induction of lysogeny for some temperate phages, like P2.

High noise in protein levels can also be obtained at the translation level. If a single mRNA molecule is rapidly translated many times the result is a burst of protein production. Therefore transcriptional bursting is not strictly required for protein production to occur in bursts. However, transcriptional bursting might allow for additional modes of regulation by transcription factors or other proteins that influence the state of the DNA around the promoter site. It may also complement bursts of protein production produced by rapid translation by removing constraints placed on burst size by the upper limits of mRNA translation rate.

Dynamics and the interplay between timescales presents an open, and until recently, quite unexplored part of molecular biology. The present analysis suggests a new mechanism for *in vivo* regulation, where long silent timescales emerge as the result of some particularly large rate limiting step in the promoter. These steps are open for new levels of regulation by transcription factors, which naturally will be most effective when they influence the rate limiting step of transcription initiation [38].

Materials and Methods

Calculation Methods

To calculate the activity of a promoter we first calculate the probability that the promoter will be occupied by closed (h) and open (q) complexes using steady state conditions. The total activity of the promoter is given by $F = Eq$ for the standard model and the recruitment model, and $F = QEq$ for the dead-end model. Details of the calculation are found in the Text S1.

The time between subsequent initiations is calculated by considering the time needed for each step as described in the Text S1. For class I there is only one step and the distribution is a

simple exponential. For class II there is two steps. If these steps take an average time of t_o and t_E , the total waiting time between events is distributed with

$$P(\Delta t) = \int_0^{\Delta t} \frac{1}{\tau_o} \exp\left(-\frac{s}{\tau_o}\right) \times \frac{1}{\tau_E} \exp\left(-\frac{\Delta t - s}{\tau_E}\right) ds, \quad (4)$$

giving eq. (1) in the main text for $t_o \neq t_E$. For one t much greater than the other, this distribution degenerates into a simple exponential. For $t_o = t_E$, eq. (4) gives eq. (2) in the main text.

For the recruitment model, the intervals between initiations are partitioned between the supercoiling assisted or unassisted outcomes, with a partitioning ratio given by q . Details are in the Text S1. For the dead-end model the distribution is similarly partitioned between the two distributions with a partition ratio given by Q . Details are in the Text S1. In the Text S1 we also show how to calculate the distribution of “on” and “off” times from q or Q . Finally, we calculate the Fano factor $v = \langle (N - \langle N \rangle)^2 \rangle / \langle N \rangle$ by using generating functions as described in the Text S1.

Protocol to Determine On-Periods and Off-Periods

We distinguish “on-periods” and “off-periods” in the simulation data following the procedure used by Golding *et al.* [10]. They analyzed the experimentally obtained time series of fluorescent signal manually. The system is considered to be in “off-period” when the signal does not change for a while, and otherwise it is in “on-period”. The specific time resolution to detect an “off-period” was not given, but the shortest off-time measured was around 6 [min] (Golding, private communication); in other words, transcription events separated by less than 6 [min] were considered to be in the same “on-period”.

During an on-period, the number of messages transcribed, $Dn \geq 1$, and the duration t_{on} were recorded; the time to transcribe one message D was 2.5 [min] [10], which corresponds to the on-time for $Dn = 1$ case.

Considering this protocol used by Golding *et al.* [10], we defined Δn , t_{on} , and the duration of the off-time t_{off} out of the time series of firings from our model (Figure 3A) as follows: (i) When firings are separated by more than $\tau_c = 6$ [min] + $\Delta = 8.5$ [min], the promoter is in an off period. (iii) Otherwise, if successive firings are separated by an interval less than τ_c , the gene is considered to be on until we observe an interval greater than τ_c . This defines the on-time t_{on} , and we count the number of transcripts per on-time Δn .

Supporting Information

Text S1 Supplemental materials

Found at: doi:10.1371/journal.pcbi.1000109.s001 (0.13 MB PDF)

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Author Contributions

Conceived and designed the experiments: NM ID MC KS. Performed the experiments: NM ID MC KS. Analyzed the data: NM ID MC KS. Contributed reagents/materials/analysis tools: NM ID MC KS. Wrote the paper: NM ID MC KS.

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