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.

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 Plac/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 Pks 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 initiation (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, $t_{c_1}$, is therefore $t_{c_1} = (1 + K_0) / o_0$, where $K_0 = k_b / k_u$ is the equilibrium constant of dissociation for the closed complex and $O_0$ 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, $\Delta t$), with $\langle .. \rangle$ indicating the average, is the sum of two exponentially distributed random variables, $\langle \Delta t \rangle = (t_{c_1} + t_E)$. The probability distribution of time gaps between initiations is given by

$$P(\Delta t) = \frac{\exp(-\Delta t / t_{c_1}) - \exp(-\Delta t / t_E)}{t_{c_1} / t_E}$$

(1)

for $t_{c_1} \neq t_E$. For $t_{c_1} = t_E$, we get

$$P(\Delta t) = \frac{1}{t_E} \Delta t \exp\left(-\frac{\Delta t}{t_E}\right).$$

(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 $\Delta t$ approaches a single exponential with mean $t_L = \max(t_{c_1}, t_E)$ (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 $t_{c_1} = t_E$ (Class II), is shown in Figure 1C. In Class II, the chance of rapid successive firings faster than the average $\langle \Delta t \rangle$ is smaller than for a Class I promoter, as for a Class II promoter a low $\Delta t$ requires both the isomerisation and the escape to productive transcription to occur in rapid succession, whereas for a Class I promoter a low $\Delta t$ requires the rapid occurrence of the rate limiting step only. As a consequence the distribution in Class II shows a peak at non-zero $\Delta t$. 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 $\Delta t$, and the effect of adding more kinetically significant intermediate steps is to reduce the amount of variability in $\Delta t$. 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 $\Delta t$ 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], $\lambda_{lac/ara}$, 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
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_ara and P_M (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 >10^7 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 10 when the supercoils are 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

\[
P(\Delta t) = \frac{q}{\tau_E} \exp\left(-\frac{\Delta t}{\tau_E}\right) + \frac{1-q}{\tau_O} \exp\left(-\frac{\Delta t}{\tau_O}\right)
\]

(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 \(\Delta\) initiations is proportional to \(q^{\Delta-1}\). 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 \(\langle \Delta\rangle\), \(\langle t_O\rangle\), and \(\langle t_d\rangle\) (Derivations are given in Text S1).

This mechanism can reproduce the observations of Golding et al. [10] with the parameters \(t_O = 37\) [min], \(t_d = 29\) [min] and \(q = 0.545\). We simulated the recruitment model using the Gillespie algorithm [14]. It gives the expected shape for the PDF distribution (Figure 3B) and matches the distribution of \(D_n\) 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 \(\langle t_d\rangle = \frac{1}{d}\) 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 \(t_d+t_E = 2.9\) [min].
calculations and equations for limiting the productive open complex. In the limit of large 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 $F = \frac{Q \cdot k_b}{E + (1-Q) \cdot k_u}$, with $x = \frac{k_u}{k_b}$.

(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 $F = \frac{Q \cdot k_b}{E + (1-Q) \cdot k_u}$, with $x = \frac{k_u}{k_b}$. 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.

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Figure 3E shows the distribution $P(D|n)$ with these parameters obtained by the simulation using the Gillespie algorithm [14]. It has been confirmed that the distributions of $D_n$, $t_{on}$, 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. [37°C] is still rather low compared 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 $N$, $v = \langle N^2 \rangle / \langle N \rangle$, was approximately 4 for the $P_{sec/ara}$ 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 is larger for smaller $Q_b$ model the Fano factor is larger for smaller $Q$, 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 $Q$; This means that the noise can be tuned for a given promoter strength under either model, which can allow the...
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 being occupied by an RNAP in a non-productive state.
Perspectives for the Regulation of Transcriptional Noise

The sensitivity of a promoter to 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 wake 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/τ), 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 activity of factors such as GreA/B that may remove the complex [29,30].

Calculation Methods

To calculate the activity of a promoter we first calculate the probability that the promoter will be occupied by closed (|) 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 are two steps. If these steps take an average time of τs and τE, the total waiting time between events is distributed with

\[ P(\Delta t) = \int_{0}^{\Delta t} \frac{1}{\tau_{O}} \exp \left( -\frac{s}{\tau_{O}} \right) ds \]

\[ \times \frac{1}{\tau_{E}} \exp \left( -\frac{\Delta t - s}{\tau_{E}} \right) ds, \]

giving eq. (1) in the main text for τs≠τE. For one τ much greater than the other, this distribution degenerates into a simple exponential. For τs=τE, eq. (4) gives eq. (2) in the main text.

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≠1, and the duration τm 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, τm, and the duration of the off-time τg out of the time series of firings from our model (Figure 3A) as follows: (i) When firings are separated by more than τg = 6 [min]+Δ=8.5 [min], the promoter is in an off period. (ii) Otherwise, if successive firings are separated by an interval less than τg, the gene is considered to be on until we observe an interval greater than τg. This defines the on-time τo and we count the number of transcripts per on-time Δn.

Materials and Methods

Calculation Methods

Text S1  Supplemental materials

Acknowledgments

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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