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Supplemental Information

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Distribution of initiation times reveals mechanisms of transcriptional regulation in single cells

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Distribution of transcription initiation times for an arbitrary promoter architecture

In the following, we formulate a general theory of transcription initiation with an arbitrary promoter architecture to connect the distribution of times between transcription initiation events (or, equivalently, the distribution of distances between transcribing RNA polymerases along a gene) with mechanisms of transcription initiation. We assume that the promoter may exist in N different biochemical states (as defined by the different binding states of transcription factors, DNA conformations, etc.), and stochastically transitions between these states, causing fluctuations in the initiation rate. This stochastic initiation dynamics is followed by RNA elongation for which we assume that every RNA polymerase (RNAP) molecule moves along the gene at a constant speed v.

For this model we compute the inter-RNAP distance distribution along a gene of interest. In order to find the distances between adjacent RNAP molecules, we first compute the distribution of times between transcription initiation events $q_1(t)$ using a master equation approach (1–3). The rate of transitions from the m-th to the n-th state is $k_{m,n}$. The rate at which an RNAP molecules escape the m-th promoter state leading to transcription initiation is $k_{m,esc}$. To obtain a master equation for the general N-state case, we consider the probability P_m that the promoter is in the state m, at time t, given that no initiation event has taken place between 0 and t. The master equation for P_m is given by

$$\frac{dP_m}{dt} = \sum_{n=1}^{N}\left[k_{n,m}P_n - k_{m,n}P_m\right] - k_{m,esc}P_m. \qquad (1)$$

This accounts for all the ways by which the fraction of promoters in state m, which have not initiated transcription from 0 to time t, can change over the time interval (t, $t + dt$). Hence for all the promoter states the set of master equations are given by

$$\frac{d\boldsymbol{P}}{dt} = [\boldsymbol{K} - \boldsymbol{R}]\boldsymbol{P}. \qquad (2)$$

Where $\boldsymbol{P} = (P_1, P_2, \ldots, P_N)$, $\boldsymbol{K}$ and $\boldsymbol{R}$ are the transition and transcription initiation rate matrices respectively. We can solve the above set of master equations above to get,

$$\boldsymbol{P}(t) = e^{[\boldsymbol{K}-\boldsymbol{R}]t}\boldsymbol{P}(t=0). \qquad (3)$$

In order to compute the probability that two initiation events are separated by a time *t*, we compute the contribution of each promoter state to this quantity. For example, the probability that two initiation events are separated by time *t*, and the second initiation event happens while the promoter is in the state *m* is given by $k_{m,esc}P_m dt$. This is the product of the probability P_m that the promoter is in the *m*-th state at time *t* (and no other transcription events have happened before this time), and the probability $k_{m,esc}dt$ that an RNAP molecule escapes the promoter between *t* and *t* + *dt* while the promoter is in state *m*. The total probability that an initiation event happens in the time interval (*t*; *t* + *dt*) is simply the sum over all *N* states of the probability that this event occurs while the promoter is in any of these states:

$$q_1(t) = \sum_{m=1}^{N} k_{m,esc} P_m(t) \tag{4}$$

To find $P_m(t)$ from Eqn. 3 we need to specify the initial condition for $\boldsymbol{P}$ i.e. $\boldsymbol{P}(t=0)$. At time $t = 0$ there is an initiation event and that can take place from any promoter state. If we wait long enough, such that the system samples all the different promoter states, the probability that an initiation event occurs from a given promoter state should only depend on the steady state probability of being in that particular state and the rate of escape into elongation from that state. Hence it is given by

$$P_m(t=0) = \frac{k_{m,esc} P_m^{\,ss}}{\sum_{m=1}^{N} k_{m,esc} P_m^{\,ss}} \tag{5}$$

Where $P_m^{\,ss}$ is the steady state probability for the promoter to be in the *m*-th state. Steady state probabilities are given by

$$\boldsymbol{KP}=0 \tag{6}$$

Furthermore, there is the normalization condition, $\sum_{m=1}^{N} P_m^{\,ss} = 1$, and the average initiation rate is

$$I = \sum_{m=1}^{N} k_{m,esc} P_m^{\,ss} \tag{7}$$

We want to find the probability distribution $p_1(x)$ of inter-polymerase distances along the gene. Assuming a constant speed of elongation, $p_1(x)$ is essentially the imprint of the distribution of times between transcription initiation events. This allows for a very simple relationship between the inter-polymerase distance distributions and distribution of times between initiation events which is given by,

$$\begin{aligned} p_1(x)dx &= q_1(t)dt \\ p_1(x) &= q_1(t)\frac{1}{v} \end{aligned} \tag{8}$$

We use this equation to compare our theory with experiments which measure the inter-polymerase distance distributions along a gene.

Distribution of transcription initiation times for different models of initiation

We apply this general theoretical framework to obtain the probability distributions of initiation times and the inter-polymerase distance distributions (Eqns. 4 and 8), for different promoter architectures described below.

Two limiting steps model

The two limiting steps model is described in Fig. 2B. In this model, initiation happens in two sequential steps: the rate of RNAP loading on to the promoter occurs with rate k_{LOAD}, followed by RNA polymerase escaping the promoter leading to an initiation event at a rate k_{ESC} (4). The relevant master equations are in this case:

$$\begin{aligned}\frac{dP_1}{dt} &= -k_{LOAD}P_1 \\ \frac{dP_2}{dt} &= k_{LOAD}P_1 - k_{ESC}P_2.\end{aligned} \tag{9}$$

Using Eqn. 4,5 and 6, we can obtain the distribution of times between successive transcription initiation events, which is given by,

$$q_1(t) = \frac{k_{LOAD}k_{ESC}}{k_{LOAD} - k_{ESC}}\left[exp(-k_{ESC}t) - exp(-k_{LOAD}t)\right]. \tag{10}$$

Using Eqn. 8 and 10, we find the distribution of inter-RNAP initiation events to be

$$p_1(\mathrm{x}) = \frac{1}{v}\frac{k_{LOAD}k_{ESC}}{k_{LOAD} - k_{ESC}}\left[exp(-k_{ESC}(\frac{x}{v} - \frac{30}{v})) - exp(-k_{LOAD}(\frac{x}{v} - \frac{30}{v}))\right]. \tag{11}$$

where we have also taken into account the time delay $30/v$, which is the time it takes for the polymerase to clear the promoter, which is 30bp in length.

To find the initiation rate, we consider the different states of the promoter only and how they evolve in time. When an RNAP molecule initiates transcription by escaping the promoter, it goes back to state 1. Hence the master equations for the promoter states are given by

$$\begin{aligned}\frac{dP_1}{dt} &= -k_{LOAD}P_1 + k_{ESC}P_2 \\ \frac{dP_2}{dt} &= k_{LOAD}P_1 - k_{ESC}P_2.\end{aligned} \tag{12}$$

We get the steady state solution for P_1 and P_2 by setting the left sides of these two equations to zero. Then, from Eqn. 7 the transcription initiation rate is

$$\begin{aligned}I' &= P_2^{ss}k_{ESC} \\ &= \frac{k_{LOAD}k_{ESC}}{k_{LOAD} + k_{ESC}}.\end{aligned} \tag{13}$$

When we include the time to clear the promoter τ_{clear}, the effective initiation rate becomes

$$I = \frac{1}{1/I' + \tau_{CLEAR}} = \frac{k_{ESC}\, k_{LOAD}}{k_{ESC} + k_{LOAD} + k_{ESC}\, k_{LOAD}\tau_{CLEAR}}. \quad (14)$$

When all the rates are very slow τ_{CLEAR} becomes negligible.

Dead-end complex model

Dead-end complex model is described in Fig. 4A. This class of model considers the formation of a long-lived non-productive initiation complex at the promoter by RNAP (5, 6). After binding the promoter at a rate k_{LOAD}, each RNAP can initiate transcription at a rate k_{ESC} or make a dead-end complex at a rate k_{DEAD}. These dead-end complexes are unproductive and are removed at a rate k_{OFF}.

The master equations for this model are given by,

$$\begin{aligned} \frac{dP_1}{dt} &= -k_{LOAD}P_1 + k_{OFF}P_3 \\ \frac{dP_2}{dt} &= k_{LOAD}P_1 - (k_{ESC} + k_{DEAD})P_2 \\ \frac{dP_3}{dt} &= k_{DEAD}P_2 - k_{OFF}P_3. \end{aligned} \quad (15)$$

Using Eqn. 4, we find the inter-initiation time distribution, which is

$$q_1(t) = k_{ESC}P_2. \quad (16)$$

The initiation rate is then obtained using Eqn. 7 and 15

$$I' = \frac{k_{OFF}\, k_{ESC}\, k_{LOAD}}{k_{OFF}\, k_{ESC} + k_{OFF}\, k_{LOAD} + k_{OFF}\, k_{DEAD} + k_{DEAD}\, k_{LOAD}}. \quad (17)$$

When we include τ_{clear}, the average initiation rate using Eqn. 15, is given by

$$I = \frac{k_{OFF}\, k_{ESC}\, k_{LOAD}}{k_{OFF}\, k_{ESC} + k_{OFF}\, k_{LOAD} + k_{OFF}\, k_{DEAD} + k_{DEAD}\, k_{OPEN} + k_{OFF}\, k_{ESC}\, k_{LOAD}\tau_{CLEAR}}. \quad (18)$$

DNA supercoiling model

This model is described in Fig. 4B where RNAPs are recruited cooperatively in the supercoiled state of the promoter. RNAP molecules are loaded on to the promoter at a rate k_{LOAD}^{LOW}. After RNAP initiates transcription, at a rate k_{ESC}, it leaves the promoter DNA in a supercoiled state and subsequent loading of RNAP polymerases at the promoter occurs at a faster rate k_{LOAD}^{HIGH}. The rate of relaxation of the supercoiled state is k_{RELAX}. Master equations for this case are:

$$\begin{aligned}
\frac{dP_1}{dt} &= -k_{LOAD}^{LOW} P_1 + k_{RELAX} P_2 \\
\frac{dP_2}{dt} &= k_{LOAD}^{LOW} P_1 - k_{ESC} P_2 \\
\frac{dP_3}{dt} &= k_{ESC}(P_2 + P_4) - (k_{RELAX} + k_{LOAD}^{HIGH}) P_3 \\
\frac{dP_4}{dt} &= k_{LOAD}^{HIGH} P_3 - k_{ESC} P_4 .
\end{aligned} \tag{19}$$

As before, using equation Eqn. 4 we get the distribution of inter-initiation times,

$$q_1(\mathrm{t}) = k_{ESC}(P_2 + \mathrm{P}_4). \tag{20}$$

Using the same procedure as above, we find the average initiation rate to be,

$$I = \frac{k_{LOAD}^{HIGH} k_{LOAD}^{LOW} k_{ESC} + k_{RELAX} k_{LOAD}^{LOW} k_{ESC}}{k_{LOAD}^{LOW} k_{ESC} + k_{LOAD}^{HIGH} k_{LOAD}^{LOW} + k_{ESC} k_{RELAX} + k_{LOAD}^{LOW} k_{RELAX} + (k_{LOAD}^{HIGH} k_{LOAD}^{LOW} k_{ESC} + k_{RELAX} k_{LOAD}^{LOW} k_{ESC}) \tau_{CLEAR}}. \tag{21}$$

ppGpp model

The ppGpp model is described in Fig. 4C. As the number of ribosomal genes increases the rate of ribosomal RNA production goes up. This triggers the production of `control molecules' (e.g. ppGpp) which then reduce the initiation rate by modulating the promoter-RNAP interactions (7). ppGpp regulates the initiation process by converting the active promoter-RNAP complexes into inactive ones. It is described by the same kinetic scheme as the dead-end complex model but with a critical difference, namely in this case it is the rate of ppGpp binding to the RNAP-promoter complex (red arrow in Fig. 4C) and not the rate of RNAP loading on to the promoter is tuned as the copy number of ribosomal genes is changed. Here we call the rate of inactivation of the RNAP-DNA complex k_{ON}. To find the probability distribution of times between successive initiation events, as before we consider the following set of master equations,

$$\begin{aligned}
\frac{dP_1}{dt} &= -k_{LOAD} P_1 + k_{OFF} P_3 \\
\frac{dP_2}{dt} &= k_{LOAD} P_1 - (k_{ESC} + k_{ON}) P_2 \\
\frac{dP_3}{dt} &= k_{ON} P_2 - k_{OFF} P_3 .
\end{aligned} \tag{22}$$

Using Eqn. 4, we find the inter-initiation time distribution, which is given by,

$$q_1(t) = k_{ESC} P_2 . \tag{23}$$

When we include τ_{clear}, our average initiation rate, using Eqn. 7 and 22 , is given by

$$I = \frac{k_{OFF} k_{ESC} k_{LOAD}}{k_{OFF} k_{ESC} + k_{OFF} k_{LOAD} + k_{OFF} k_{ON} + k_{ON} k_{OPEN} + k_{OFF} k_{ESC} k_{LOAD} \tau_{CLEAR}}. \tag{24}$$

ON-OFF model

We consider the canonical model of transcription initiation, the ON-OFF model (1, 8). In this model, the promoter switches between two states: an ON state, from which transcription initiation can occur, and an OFF state from which initiation does not occur. The two states might correspond to a free promoter and one bound by a repressor protein. The rate of transitioning from the ON to OFF state is k_{OFF} and from OFF to ON is k_{ON}. The rate of initiation from the ON state is k_{ESC}. The probability distribution of times between successive initiation events, using Eqn. 4, is given by,

$$q_1(t) = A_1 k_1 exp(-k_1 t) + A_2 k_2 exp(-k_2 t). \tag{25}$$

where the constants k_1, k_2, A_1 and A_2 are given by

$$k_{1,2} = \frac{1}{2}\left[k_{ON} + k_{OFF} + k_{ESC} \pm \sqrt{\left(k_{ON} + k_{OFF} + k_{ESC}\right)^2 - 4k_{ESC}k_{ON}} \right], \tag{26}$$

$$A_1 = \frac{r - k_2}{k_1 - k_2}, \tag{27}$$

$$A_2 = 1 - A_1. \tag{28}$$

The mean and variance of the times between successive initiation events are given by

$$Mean = \frac{k_{ON} + k_{OFF}}{k_{ON}k_{ESC}} \tag{29}$$

$$Variance = \left[\frac{(k_{ON} + k_{OFF})^2 + 2k_{OFF}k_{ESC}}{(k_{ON}k_{ESC})^2} \right] \tag{30}$$

Effect of pausing on inter-polymerase distance distributions along the ribosomal genes

Ribosomal genes are very highly transcribed and hence even very short pauses can cause traffic jam of RNAPs along the path. This can dramatically reduce the rate of ribosomal RNA production. In order to keep the transcription rate high the anti-termination system suppresses most of the ubiquitous pauses that are observed in vitro (9). Depending on the efficiency of the anti-termination system pausing can play a vital role in the ribosomal RNA production process. Klumpp et al (9) did a systematic study of how RNAP pausing affects the process of transcription of ribosomal genes. We take their model and analyze the effect of RNAP pausing on the inter-polymerase distance distributions using the Gillespie algorithm (10, 11). This allows us to find the conditions under which the inter-polymerase distance distribution remains largely unaffected by transcriptional pausing and hence provides an imprint of the initiation dynamics.

Klumpp's model of RNAP pausing along the ribosomal genes

Klumpp et al (9) modeled (see Fig. S1A) the transcription of RNAPs in dense traffic, using a stochastic cellular automaton model. In this model the RNAP molecules are represented as extended objects moving along a one-dimensional lattice. Sites of this lattice represent the individual bases of a DNA template. Each RNAP can be in either the active or paused state, independent of the state of other RNAPs. An active RNAP transcribes along the DNA by making a single nucleotide step forward at a stepping or elongation rate k_{EL}. This can only happen when the next base is not blocked by the presence of another RNAP. During elongation, the active RNAP may switch stochastically to the paused state with a rate k_{P+}. Here the assumption is that each RNAP can pause at any site along the DNA. A paused RNAP remains at the same site and it can switch back to the active state with rate k_{P-}, so that the average duration of a pause is $1/ k_{P-}$. The rate at which the RNA polymerase molecules are loaded on the DNA template is k_{ESC}. The footprint of an RNAP molecule is L. Klumpp et al extended this model to include other effects of elongation such as backtracking of pauses or effects of rho dependent terminators. For our purposes though we don't take these into considerations as for a fully efficient anti-termination system the aforementioned factors do not matter (9).

Klumpp's model cannot explain the experimental observations when the operon number is increased

In order to explain the observed effect of Nus factor deletions on the distribution of RNAP distances, Klumpp et al. assumed that it was the pause duration along the ribosomal genes that increased (9). Similarly, to see whether the model can explain the observed features of inter-polymerase distance distributions when the operon number is increased, we change one of the parameters of the model at a time. We do this in such a way that the mean number of RNAPs along the gene, matches the number obtained in experiments (12) when the number of operons is increased. After tuning each parameter, we simulate the model to get the inter-polymerase distance distributions. Then we compare model predictions with the experimental results. In particular, we compare the intra-bunch mean distances and the average elongation rates of the RNAPs along the gene, both of which are measured to be constant in experiments (9). The parameter values are shown in Table.1. We did not change the pause frequency because no matter what value is chosen one cannot get the observed mean number of RNAPs along the gene when the operon number is increased. Our analysis shows that by changing one parameter at a time one cannot explain the experimental results as shown in Table.2. Also, we think it highly unlikely that multiple parameters of the pausing model change at the same time so as to keep both the elongation rate and intra-bunch mean fixed.

Table.1

Parameters	Seven genes	Ten genes
Elongation attempt rate (k_{EL})	100 bps/sec	160 bps/sec
Pause duration ($1/k_{P\text{-}}$)	0.23 sec	3 sec
Initiation rate (k_{ESC})	2 /sec	1.35 /sec

Effect of elongation on the inter-polymerase distance distributions

The distribution of inter-polymerase distances is determined by both the initiation and elongation dynamics. Depending on the timescales of the two processes, they affect the distribution differently. If the initiation timescales are much longer than the elongation timescales the distribution of distances will remain largely unaffected by the elongation dynamics, as in the case of most of the mRNA promoters in *E.coli*. As the ribosomal genes are very highly transcribed the initiation and elongation timescales become comparable and hence the effect of elongation on the RNAP distance distributions can be significant (9). We use the model proposed by Klumpp et al.(9) to see the extent of the effect of ubiquitous pauses on the elongating RNAPs. For a fully efficient anti-termination system most of the ubiquitous pauses are suppressed except few short lived pauses (9). Using the Gillespie algorithm (10, 11), we find the inter-polymerase distance distributions for two cases. In the first case we take the parameters proposed by Klumpp et al.(9) to find the distribution. In the second case we take the same parameters for the pausing dynamics but with lower initiation and elongation attempt rates so as to match the experimental observations (12). We compare both distributions with the ones we compute above and in the main text, which are derived only from the initiation dynamics. For the higher initiation rate the coefficient of variation (CV = 1.14±0.35) which is defined as the standard deviation divided by the mean of the distribution deviates significantly from the exponential distribution. When the initiation rate is lower the distribution obtained from the simulations matches well with the exponential distribution (CV = 1.03±0.042) as expected from the initiation dynamics. The distributions are shown in Fig. S2. We conclude that the inter-polymerase distribution as observed in the experiment (12) is a result of initiation dynamics and elongation has a very little effect on it.

Table. 2

Intra-bunch mean RNAP distance in bps	Gene copy number	Experimental results	Simulation results for different tuned parameters		
			Pause duration ($1/k_{P\text{-}}$)	Elongation attempt rate (k_{EL})	Initiation rate (k_{ESC})
	7	72±5	73.4	73.4	73.4
	10	72±4	52±3	87±4	83±3
			Simulation results for different tuned parameters		

RNAP elongation rate in bps/sec	**Gene copy number**	**Experimental results**	Pause duration ($1/k_{P\text{-}}$)	Elongation attempt rate (k_{EL})	Initiation rate (k_{ESC})
	7	78	78	78	78
	10	78	50±5	120±6	90±5

Estimating the number of ribosomal genes

It is experimentally difficult to measure the exact copy number for pBR322-based plasmids (13). Hence, we make an estimate in the following way. Voulgaris et al.(12) observed in their experiments that the total number of transcripts produced in the cell remains constant even when the number of genes is increased by inserting plasmids. The initiation rate per operon goes down to keep to the total initiation rate fixed. The number of RNAPs per gene was reduced by 34 percent. Hence the number of genes should be n =7/0.66 ~ 10. Using this number, we plot the data in Fig. 4.

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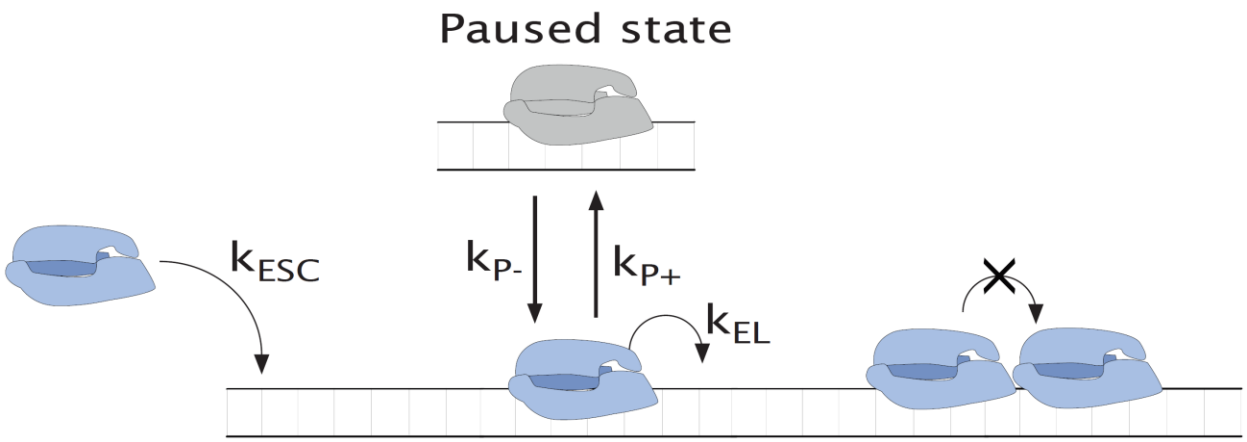

Figure S1: **Kinetic scheme of Klumpp's pausing model**: Active RNA polymerase molecules elongate along the ribosomal gene by making single-nucleotide forward steps along the DNA template. These elongation steps occur with rate k_{EL}, provided the site in front of the RNAP is not occupied by another RNAP. The rate at which RNAPs go to the paused state is k_{P+} and a paused RNAP returns to the active state with rate k_{P-}, the loading rate of RNAPs along the DNA template is k_{ESC}.

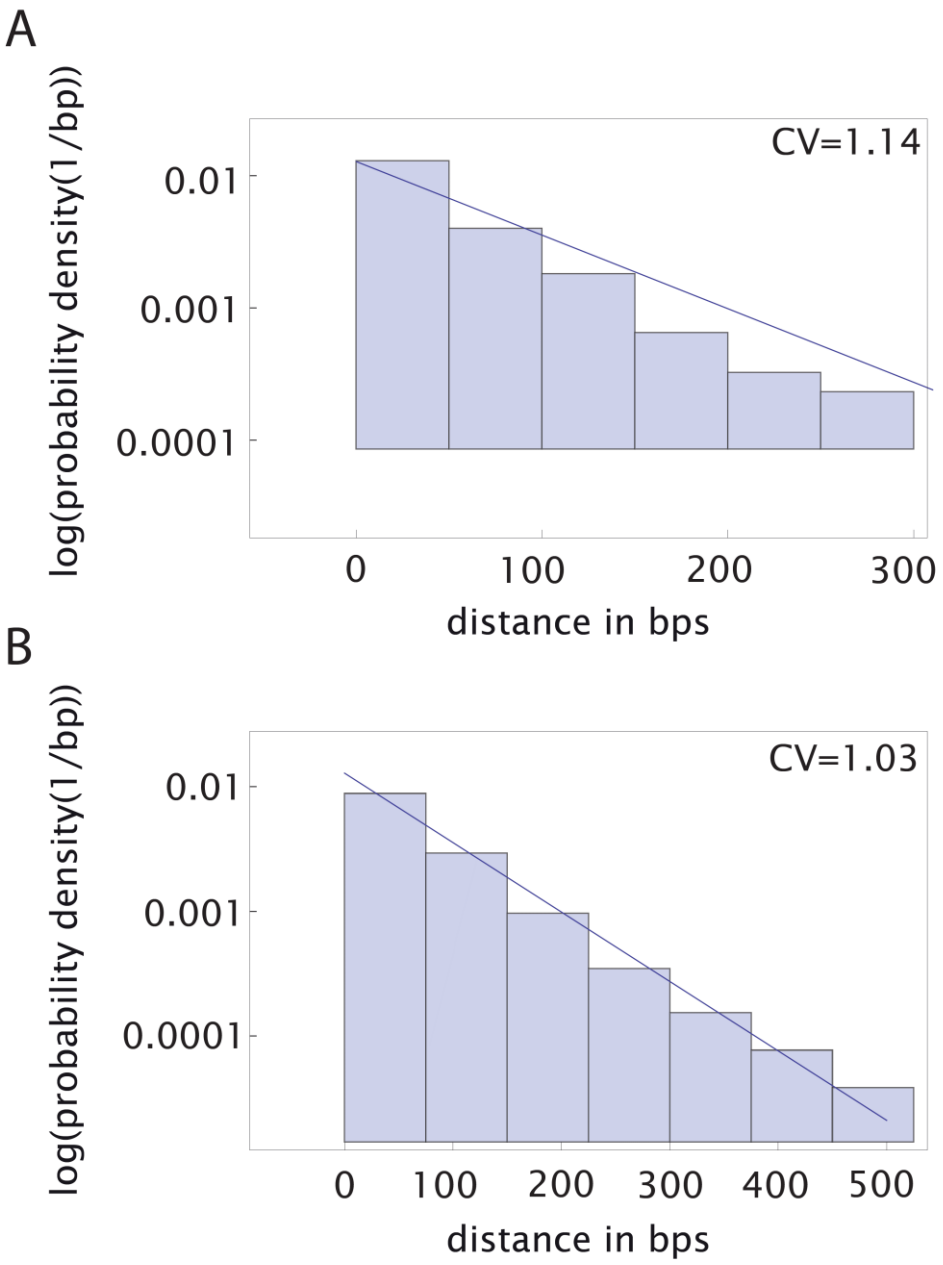

Figure S2: **Initiation versus elongation**: **A. Elongation limited regime:** Distance distribution of RNAPs along the gene gets affected substantially by the elongation process when the initiation time scale becomes comparable to elongation time scales. Using Klumpp et al.'s model (9) with the parameters they use i.e. k_{ESC} = 2/sec, k_{EL} = 100bps/sec, k_{P+} = 0.1/ sec, k_{P-}= 4.55/sec, and L = 50 bps we get the inter-polymerase distance distributions and compare it with an exponential distribution that one expects just from the initiation dynamics. It deviates quite a lot from an exponential distribution as suggested by the coefficient of variation. **B. Initiation limited regime:** With the same pausing dynamics but a lower initiation rate of k_{ESC} = 1/ sec and elongation attempt rate k_{EL} = 80/sec as observed in experiments [3], the distribution inter-polymerase distances

remains largely unaffected by the elongation dynamics. We compare it with an exponential distribution as expected from the initiation dynamics. Deviation of the distribution extracted from simulations from an exponential distribution is very small as suggested by the coefficient of variation.