

## Paused in Translation: A Model for the Transcript Length-Dependent Impact of Ribosome-Targeting Antibiotics on Degrading mRNAs

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Ribosome-targeting antibiotics, such as chloramphenicol, stall elongating ribosomes during protein synthesis, disrupting mRNA translation. These antibiotic-induced pauses occur stochastically, alter collective ribosome dynamics, and transiently block protein production on the affected transcript. Existing models of ribosome traffic often rely on idealized assumptions, such as infinitely long and stable mRNAs and simplified pausing dynamics, overlooking key biological constraints. Here, we develop a totally asymmetric simple exclusion process that incorporates stochastic particle pausing and lattice degradation, using experimentally determined pausing and unpausing rates to model the effects of ribosome-targeting antibiotics. We introduce a single-cluster approximation, which is analytically treatable, tailored to capture the biologically relevant regime of rare and long antibiotic-induced pauses. This biologically constrained model reveals three key insights: (i) the inhibition of antibiotic-induced translation strongly depends on transcript length, with longer transcripts being disproportionately affected; (ii) reducing ribosome initiation rates significantly mitigates antibiotic vulnerability; and (iii) inhibition of translation is governed more by collective ribosome dynamics than by single-ribosome properties. Our analytical predictions match Gillespie simulations, align quantitatively with experimental observations, and yield testable hypotheses for future experiments. These findings may have broader implications for the mechanistic modeling of other biological transport processes (e.g., RNAP dynamics), and more generally for the community studying traffic models.

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### I. INTRODUCTION

The conversion of genetic information into functional proteins is a two-step process requiring precise coordination: the transcription of DNA into messenger RNA (mRNA) by RNA polymerases (RNAPs), followed by the translation of mRNA into proteins by ribosomes. However, because neither RNAPs nor ribosomes can overtake while moving along their respective templates, they are inherently prone to forming congested zones resembling traffic jams. Although such queuing phenomena might not be present in physiological conditions, it has been proposed that they can arise from a variety of causes, including random pausing of RNAPs along the DNA [1] as well as ribosome stalling induced by translation-inhibiting antibiotics [2].

To capture the collective dynamics of ribosomes and RNA polymerases moving along linear templates without overtaking, MacDonald and coworkers introduced the totally

asymmetric simple exclusion process (TASEP), which became the paradigmatic example of a driven lattice gas model [3]. In its basic formulation, the TASEP describes particles hopping unidirectionally along a one-dimensional lattice with simple exclusion: each site can be occupied by at most one particle. Although this model was initially proposed to describe ribosome traffic along mRNA during translation, it has since been adapted to transcription and other biological transport processes; see, e.g., Refs. [1,4–8]. Over time, extensions of the TASEP have been developed to better capture biological complexity, such as incorporating finite particle size [9], internal stepping cycles [5,6,10–12], site-dependent hopping [13–16], and obstacle-induced stochastic pausing dynamics [17,18]. In particular, TASEP models that include random pausing and unpausing events have been proposed to describe RNA polymerase behavior during transcription, where RNAPs can pause stochastically along the DNA [6,10] or be hindered by bound regulatory proteins [7,8,19]. However, it is important to recognize that the TASEP remains an idealized model: most theoretical studies assume infinite system sizes, rely on mean-field approximations, and focus on regimes where bulk properties dominate system behavior. In contrast, biological systems operate with finite lattice lengths, specific initiation and elongation rates, and well-defined pausing dynamics. It is not always clear a priori to what extent such modeling assumptions hold in biologically relevant parameter regimes.

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In this article, we focus on a framework that takes into account stochastic particle pausing to study the translation process under sub-lethal antibiotic stress, where ribosomes can transiently stall due to antibiotic binding. Despite extensive work on many TASEP variants, the question of how stochastic pausing shapes translation under biologically relevant conditions remains poorly understood. Here, we bridge this gap by a systematic study of a pausing TASEP model, constrained by measured biological parameters. We develop a stochastic TASEP model that explicitly incorporates ribosome pausing and unpausing dynamics driven by antibiotic binding and unbinding, using biologically relevant parameters. To render the complex dynamics analytically tractable while still preserving biological relevance, we introduce a Single-Cluster approximation that captures the system behavior across experimentally measured initiation, elongation, and pausing parameters. Within this framework, we derive analytical expressions for key observables, including the ribosome density, the ribosomal current (protein synthesis rate), as well as the fractions of *paused* (immobilized by antibiotic binding), *jammed* (blocked by exclusion), and actively translating ribosomes.

Our theoretical predictions show excellent agreement with extensive stochastic simulations. The model reveals how collective ribosome dynamics, strongly modulated by initiation rates and gene length, govern the response to translation-inhibiting antibiotics, an effect largely overlooked in previous theoretical approaches. Another key feature, often absent in previous models apart from a few rare exceptions (e.g., Ref. [20]), is that antibiotic unbinding times are comparable to mRNA degradation timescales, requiring explicit consideration of finite mRNA lifetimes when assessing translation dynamics under stress. To address this, we incorporate in our model transcript degradation, which allows us to capture the interplay between ribosome traffic, stochastic pausing, and mRNA stability. In particular, for the antibiotic studied here (chloramphenicol), the fate of the mRNA is effectively determined by the first antibiotic-binding event. Within this biologically constrained framework, our model predicts that, excluding other potential regulatory feedbacks, inhibition of translation by antibiotics becomes strongly dependent on gene length, with longer mRNAs more susceptible to disruption. We validate our predictions against experimental measurements of short and long gene expression under chloramphenicol treatment [21], finding that our analytical model quantitatively reproduces the observed trends and highlights the critical role of collective ribosome dynamics in mediating antibiotic action.

## II. BIOLOGICAL CONTEXT AND MODEL

Translation is one of the most common and critical cellular processes targeted by antibiotics. These compounds interfere with protein synthesis through a variety of mechanisms, including disrupting ribosome assembly, blocking initiation or recycling steps, interfering with the recruitment of charged tRNAs or translation factors, and inducing mistranslation [2,22]. Among these modes of action, a biologically significant class of antibiotics—such as chloramphenicol, tetracycline, and erythromycin—acts by binding to elongating

ribosomes thus causing them to transiently pause on the mRNA.

In this work, we focus specifically on this elongation-inhibiting mechanism. To develop a mathematical description of how ribosome-targeting antibiotics impact mRNA translation, we exploit the timescale separation between slow antibiotic-ribosome binding kinetics, rapid translation elongation, and mRNA degradation dynamics. Below, we introduce the model in its biological context, explain the relevant timescales, and present an approximation that enables analytical treatment of the system.

### A. Exclusion process in the context of ribosome-targeting antibiotics

In the presence of an intracellular concentration  $c$  of a ribosome-targeting antibiotic, such as chloramphenicol, antibiotic molecules can transiently interact with ribosomes at a rate  $k_p$ , resulting in temporary pauses of ribosomal movement along the mRNA strand. The rate at which ribosomes are hit by antibiotic molecules depends on the antibiotic concentration and the binding rate constant  $k_{\text{on}}$  through the relation

$$k_p = k_{\text{on}}c. \quad (1)$$

Paused ribosomes then resume elongation with an “unpausing” rate  $k_u$ , which depends on the antibiotic-ribosome unbinding. For chloramphenicol, one of the most widely used antibiotics in quantitative bacterial physiology studies [23], the rate constant  $k_{\text{on}}$  has been measured to be approximately  $5.6 \times 10^{-4} \mu\text{M}^{-1} \text{s}^{-1}$ , and the unpausing rate  $k_u$  to be  $1.4 \times 10^{-3} \text{s}^{-1}$  [24]. Even though measuring the precise binding and unbinding rates of antibiotics *in vivo* is challenging, *in vitro* studies reveal that other macrolides, such as erythromycin, exhibit unbinding times highly similar to chloramphenicol ( $k_u \approx 2.5 \times 10^{-3} \text{s}^{-1}$ ), even though their binding rate constants can be vastly different ( $k_{\text{on}} \approx 0.25 \mu\text{M}^{-1} \text{s}^{-1}$ ) [25]. Other classes of antibiotics, however, display markedly different kinetics; for instance, tetracycline exhibits much faster unbinding and binding rates, completely altering the timescale of the translation interruption.

To model this class of translation-inhibiting antibiotics, we develop an exclusion process in which particles (ribosomes) undergo stochastic pausing and unpausing as they progress along a one-dimensional lattice (the mRNA). This captures the essential features of ribosome dynamics under sub-lethal antibiotic stress. Beyond pausing dynamics, translation is governed by three primary stochastic rates of the standard TASEP: the initiation rate  $\alpha$ , at which ribosomes bind the mRNA entry site; the elongation rate  $\epsilon$  at which ribosomes move codon by codon, provided that the next codon is empty; and the termination rate  $\beta$  characterizing the detachment of the ribosomes from the last site. In the exclusion process, the mRNA strand is represented as a unidimensional discrete lattice, where each site stands for a codon. This is sketched in Fig. 1(a). In discussing the model, we will refer to a ribosome as a *particle*, to an mRNA strand as *lattice*, and to the initiation/termination rate as *entry* and *exit rate*.

Two quantities are important, both in terms of modeling and in terms of observation: density and current. The

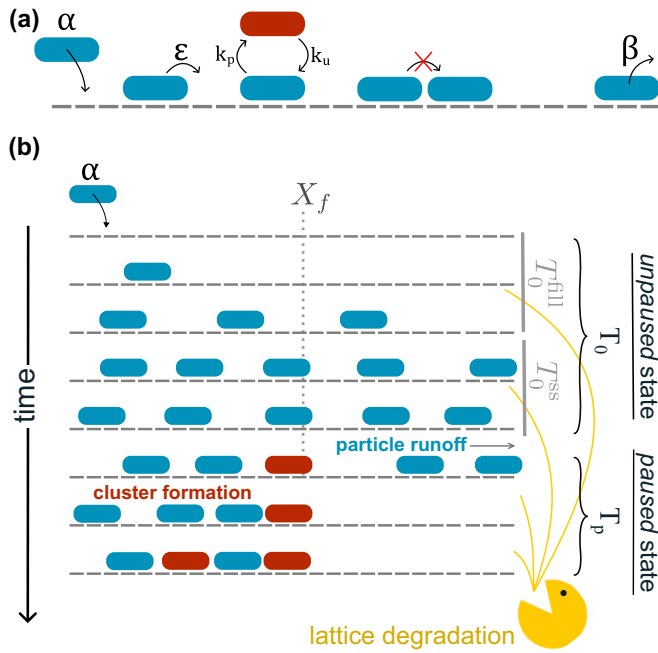


FIG. 1. (a) Schematic of the TASEP where each particle (ribosome) covers  $\ell$  sites (codons), here  $\ell = 3$ , for illustration. Particles enter (ribosomes initiate translation) at a rate  $\alpha$ , advance (elongate) at a rate  $\epsilon$  provided the next site (codon) is unoccupied, pause and unpause with rates  $k_p$  and  $k_u$ , respectively, and exit (terminate translation) at rate  $\beta$ . (b) Dynamics of pausing events and mRNA degradation. Initially, particles progressively fill the lattice (for a time  $T_0^{\text{fill}}$ ) until a TASEP steady state is established (lasting for a time  $T_0^{\text{ss}}$ ). A first pausing event occurs at a random position  $X_f$ . After that, a particle cluster forms upstream this position. Additional pausing events may subsequently occur at other positions (although this is unlikely in the regime we thoroughly study in the main text). The mRNA lattice has a finite lifetime (exponentially distributed with mean  $\tau$ ), represented by the Pac-Man symbol indicating stochastic degradation. Degradation can occur at any time during the process, including before any pausing events. The situation without lattice degradation, and thus allowing for the dissolution of the particle cluster, is investigated in the Supplemental Material [32].

(space-averaged) ribosome density  $\rho$  is the number of ribosomes on the lattice at a given time, divided by the number of lattice sites. The local variant of this quantity, for a single codon site, may be interpreted as the probability of finding a ribosome on this specific site. Finally, the local current is the number of ribosomes crossing any specific codon per unit of time. At the last site, the exit current therefore corresponds to the protein synthesis rate.

In *Escherichia coli*, the elongation rate typically lies between 10 and 20 codons per second, while the initiation rate is estimated between 0.1 and 1 ribosomes per second [26]. The termination process is generally considered fast relative to other translation events [27]. Ribosomes typically occupy a footprint of  $\ell = 10$  codons [28], and in *E. coli* the average mRNA length is 255 codons with a total range between  $L = 15$ –2368 codons [28,29]. Thus, a ribosome typically requires approximately 12–25 s to translate the entire transcript.

## B. Incorporating mRNA degradation dynamics

Bacterial mRNAs are short-lived, on the order of a few minutes, compared to their eukaryotic counterparts. In *E. coli*, recent genome-wide measurements reveal average mRNA lifetimes of approximately 1–5 min [29,30].

The finite lifetime of mRNAs fundamentally constrains the time available for translation and thus the total protein output from each transcript. In the following, we consider that mRNA degradation is instantaneous, meaning that translation stops immediately after a degradation event. Empirically, it has been shown that mRNA degradation in *E. coli* exhibits complex kinetics [30].

While the exact functional form of mRNA lifetime distributions has been debated, with evidence suggesting deviations from simple exponential decay, we model the distribution of mRNA degradation times as an exponential distribution for analytical tractability:

$$\varphi_{\text{mRNA}}(t) = \frac{1}{\tau} e^{-t/\tau}, \quad (2)$$

where  $\varphi_{\text{mRNA}}(t)$  denotes the probability density that an mRNA is degraded at time  $t$ , and  $\tau$  is the average mRNA lifetime.

This approximation, while simplified, captures the essential feature of stochastic mRNA degradation and enables closed-form analytical solutions for the model presented below. The exponential distribution represents a memoryless degradation process and provides a reasonable first-order description of mRNA turnover dynamics, particularly when considering ensemble-averaged behavior across many transcripts.

Given the timescales of chloramphenicol kinetics discussed earlier (corresponding to an unpausing time of  $\sim 12$  min), mRNA degradation with  $\tau$  on the order of 1–5 min implies that transcripts will typically degrade before all paused ribosomes have unpaused. This timescale separation between rapid translation elongation, slow antibiotic-ribosome binding/unbinding, and intermediate mRNA degradation fundamentally shapes the system dynamics.

In this regime, the first pausing ribosome is substantially determining the fate of the mRNA; because of the exclusion interactions, it blocks all upstream ribosomes, and a degradation event likely occurs before translation can be resumed (antibiotic unbinding). For antibiotics with faster unbinding kinetics, such as tetracycline (from Ref. [31] we estimate  $k_u \sim 0.14$ – $5.7$ /s), the interplay between degradation and unpausing may differ, a scenario we address in the Supplemental Material [32].

## C. Single-cluster picture

The full particle dynamics with pausing/unpausing due to the action of antibiotics is a very complex problem. However, a simplified picture emerges after observing that the unbinding rates of antibiotics such as chloramphenicol are typically three to four orders of magnitude lower than the ribosomal elongation rate (see values given above). Therefore, the antibiotic remains bound to a ribosome for a time equivalent to many elongation steps. The pronounced separation of timescales would therefore suggest a simplified representation in which a single paused ribosome initiates a traffic jam,

propagating backwards to rapidly reach a single, fully congested cluster reaching back to the initiation site, Fig. 1(b). We refer to this as the *single-cluster approximation*, which allows for a simplified yet tractable analytical description of the system's behavior in biologically relevant regimes.

As a simple back-of-the-envelope calculation supporting this picture, we take the reported rate binding constant  $k_{\text{on}}$  for chloramphenicol [24] at a concentration of 10  $\mu\text{M}$ . Under these conditions, the average time for a ribosome to get hit by the antibiotic is approximately 180 s. Given that a typical ribosome elongation step occurs at  $\sim 0.1$  s per codon (i.e., 10 aa/s [26]), a single ribosome would traverse  $\sim 1800$  codons before experiencing a binding event. Since the average protein length is around 255 codons [29], multiple binding events on the same ribosome are thus highly unlikely.

Additionally, the unbinding time for chloramphenicol is around  $\sim 700$  s [24]. With a time interval between initiation events of  $\sim 10$  s, there can be roughly 70 initiation events on an mRNA before the antibiotic unbinds from a ribosome paused on it. Assuming each ribosome footprint extends across 10 codons, this leads to a cluster size of  $\sim 700$  codons, which far exceeds the typical protein length [29]. These estimates support the conclusion that a *single-cluster approximation* is biologically reasonable under these conditions.

This simplified picture entails simplified dynamics, sketched in Fig. 1(b), which are as follows. During the first phase in the single cluster approximation, particles enter at the initiation site (with entry rate  $\alpha$ ), and these particles move across the lattice following the standard TASEP dynamics. As soon as a first pausing event occurs at some random position, particles downstream from this point evacuate and terminate. Simultaneously, further particles enter until the upstream section to the paused particle is fully filled. We are therefore looking at a system switching between two main states: a state with zero paused particle, obeying standard TASEP dynamics, which we refer to as the *unpaused state*, by opposition to its counterpart, the *paused state*, which has at least one paused particle on the lattice, entailing a jam of particles.

The lattice can be degraded independently of its state, as introduced in the previous subsection. Motivated by the separation of timescales between antibiotic unbinding and mRNA degradation, we may assume that once a cluster has formed, the mRNA is degraded before the cluster can dissolve by unblocking. The opposite situation with a nondegrading lattice is presented in the Supplemental Material [32].

### III. RESULTS: ANALYSIS OF THE MODEL

We next characterize the dynamics of these main states, which essentially amounts to understanding the entrance and duration of the paused state given the finite, stochastic lattice lifetime.

#### A. Unpaused state

To characterize the unpaused state (zero paused particles on the lattice, thus labeled with the suffix 0), we examine the evolution from an initially empty lattice toward a steady-state density profile, starting when particles enter with rate  $\alpha$  and move unidirectionally with an elongation rate  $\epsilon$ . In the

absence of pausing, the system follows the standard TASEP with extended particles [9]. In this article we focus on system parameters that set a low density phase ( $\alpha$  is limiting), as it is often assumed in mRNA translation [14]. As particles enter the empty lattice, a density front—often referred to as a *domain wall*—emerges, separating a region with positive particle density behind the wall from a zero-density region ahead of it [33–35]. The domain wall propagates through the lattice with velocity

$$v = \epsilon - \alpha. \quad (3)$$

The position of the domain wall or shock,  $x_s$ , at time  $t$  follows as

$$x_s(t) = vt. \quad (4)$$

The time required for the domain wall to traverse a lattice of length  $L$ , denoted as  $t_L$ , thus is

$$t_L = \frac{L}{\epsilon - \alpha}. \quad (5)$$

After this time, the lattice reaches a steady state characterized by a constant particle density and current [36]. Under standard TASEP assumptions for extended particles, these quantities are given by

$$J_{\text{ss}} = \frac{\alpha(\epsilon - \alpha)}{\epsilon + \alpha(\ell - 1)}, \quad \rho_{\text{ss}} = \frac{\alpha}{\epsilon + \alpha(\ell - 1)}, \quad (6)$$

where  $J_{\text{ss}}$  represents the steady-state particle current, and  $\rho_{\text{ss}}$  is the corresponding particle density, which follows directly from mean-field approximations commonly applied in exclusion processes [9]. Here,  $\ell$  is the particle footprint in terms of lattice sites.

#### 1. Lifetime distribution of the unpaused state

The random time  $T_0$  until the first pausing event can be analysed by viewing pauses as a *simple point process*, in which times at which pauses occur form a random set of points on the time axis. Particles pause independently following a stochastic Poisson process with rate  $k_p$ . However, the number of particles in the lattice grows linearly in time with the injection current  $J_{\text{ss}}$ , until the steady state time  $t_L$ , after which it remains constant:

$$N = \begin{cases} J_{\text{ss}}t, & t < t_L, \\ \rho_{\text{ss}}L, & t \geq t_L. \end{cases} \quad (7)$$

Therefore, we are dealing with an *inhomogeneous Poisson process* [37] (also called a nonstationary Poisson process) for the first phase,  $t < t_L$ . Here, the rate at which pausing occurs anywhere in the system is thus time-dependent, and given as  $\lambda(t) = k_p N(t)$ . Once the steady state has been reached, during the second phase ( $t \geq t_L$ ), this rate saturates as  $N$  saturates. All in all, we thus have the rate for the system to transition from an unpaused to a paused state as

$$\lambda(t) = \begin{cases} J_{\text{ss}}k_p t, & t < t_L, \\ \rho_{\text{ss}}Lk_p, & t \geq t_L. \end{cases} \quad (8)$$

The probability that no pausing has occurred up to time  $t$  is the survival function of the unpaused state, a standard result

for Poisson processes [38]:

$$S_p(t) = \exp[-\Lambda(t)]. \quad (9)$$

Here  $\Lambda(t)$  is the *cumulative* (or integrated) intensity, i.e., the expected number of pausing events up to time  $t$ , given by

$$\Lambda(t) = \int_0^t \lambda(s) ds = \begin{cases} at^2, & t < t_L, \\ at_L^2 + b(t - t_L), & t \geq t_L, \end{cases} \quad (10)$$

where we define  $a := J_{ss}k_p/2$  and  $b := \rho_{ss}Lk_p$ . Simultaneously, the lattice is subject to stochastic degradation. Assuming exponential lifetime with mean  $\tau$ , see Eq. (2), the survival function for degradation is

$$S_{\text{deg}}(t) = \exp\left[-\frac{t}{\tau}\right]. \quad (11)$$

For independent competing processes, the joint survival probability—representing the probability that neither event has occurred by time  $t$ —is the product of the individual survival probabilities:

$$S(t) = \exp\left[-\Lambda(t) - \frac{t}{\tau}\right]. \quad (12)$$

We now define the probability density  $F(t)$  for the first pausing event, anywhere in the system, as  $F(t) = \lambda(t)S(t)$ : this is the joint probability density that neither pausing nor degradation has occurred before  $t$ , and a pausing event takes place in the time interval  $dt$  is the product of the survival probability  $S(t)$ , and the instantaneous pausing rate  $\lambda(t)$ . Thus,

$$F(t) = \lambda(t)S(t) = \begin{cases} 2a e^{-at^2} e^{-t/\tau}, & t < t_L, \\ b e^{-at_L^2 - b(t-t_L)} e^{-t/\tau}, & t \geq t_L \end{cases} \quad (13)$$

from Eqs. (9) and (10).

## 2. Expected lifetime of the unpaused state

The expected duration  $T_0$  of the unpaused state, i.e., the expected time until either a pausing event or degradation occurs, can be computed directly by integrating the survival function over time:

$$T_0 = \int_0^\infty S(t) dt.$$

Splitting up the integral at  $t = t_L$  yields

$$T_0 = \int_0^{t_L} S(t) dt + \int_{t_L}^\infty S(t) dt, \quad (14)$$

that identifies two contributions, corresponding to whether the first pausing event arises before or after filling is reached.

The first integral corresponds to the expected duration of the unpaused state during the transient filling phase ( $t < t_L$ ), which we name  $T_0^{\text{fill}}$ . During this phase, from Eq. (10),  $\Lambda(t) = at^2$  with  $a = J_{ss}k_p/2$ , giving

$$T_0^{\text{fill}} := \int_0^{t_L} S(t) dt = \int_0^{t_L} \exp\left(-at^2 - \frac{t}{\tau}\right) dt. \quad (15)$$

By completing the square in the exponent, this integral can be solved analytically in terms of error functions. Defining the

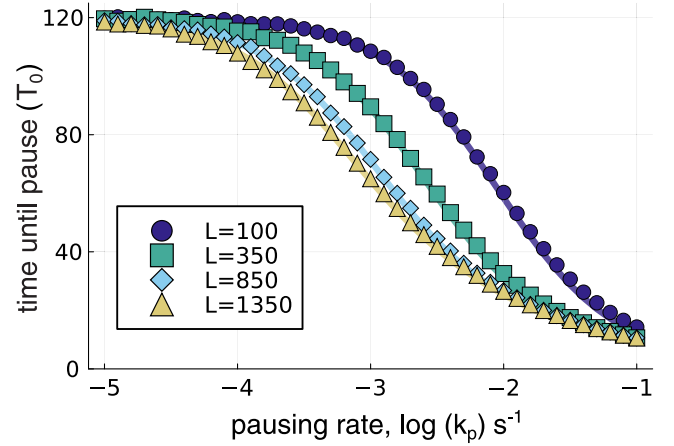


FIG. 2. Lifetime of the unpaused state, i.e., expected time until the first pausing event or degradation, as a function of the pausing rate, for different lattice lengths  $L$ . Analytical results (lines) closely match simulation data (markers). The simulation parameters here are  $\alpha = 0.2$ ,  $\epsilon = 20$ ,  $k_u = 1.4 \times 10^{-3}$ ,  $\tau = 120$  s.

prefactor  $\mathcal{A} = \frac{\sqrt{\pi}}{2\sqrt{a}} e^{\frac{1}{4a\tau^2}}$ , we obtain

$$T_0^{\text{fill}} = \mathcal{A} \left[ \text{erf}\left(\sqrt{a}t_L + \frac{1}{2\tau\sqrt{a}}\right) - \text{erf}\left(\frac{1}{2\tau\sqrt{a}}\right) \right], \quad (16)$$

with erf denoting the error function.

The second contribution to Eq. (14) arises from first unpaused events taking place after the system has reached steady state, i.e., after the domain wall has arrived at the exit site ( $t \geq t_L$ ). Here the cumulative pausing intensity grows linearly as  $\Lambda(t) = at_L^2 + b(t - t_L)$ , see Eq. (10), and we can compute the expected time  $T_0^{\text{ss}}$  of survival in the steady state as

$$\begin{aligned} T_0^{\text{ss}} &:= \int_{t_L}^\infty S(t) dt \\ &= \exp(-at_L^2 + bt_L) \int_{t_L}^\infty \exp\left(-t\left(b + \frac{1}{\tau}\right)\right) dt \\ &= \frac{\exp(-at_L^2 - t_L/\tau)}{b + 1/\tau}. \end{aligned} \quad (17)$$

Adding Eqs. (16) and (17) gives the total expected lifetime of the unpaused state:  $T_0 = T_0^{\text{fill}} + T_0^{\text{ss}}$ .

As illustrated in Fig. 2, this analytical result accurately captures the simulation data across different lattice lengths and pausing rates. Note that  $T_0$  represents the expected time spent in the unpaused state, including the scenario in which degradation occurs before any pausing event. Consequently, even in the limit of very fast pausing ( $k_p \rightarrow \infty$ ),  $T_0$  does not vanish: a fraction of mRNAs will always degrade before the first ribosome is loaded and paused, contributing their full lifetime to the unpaused average. This residual contribution is of order  $1/\alpha$ , the typical time to load the first ribosome, which remains small under physiological conditions.

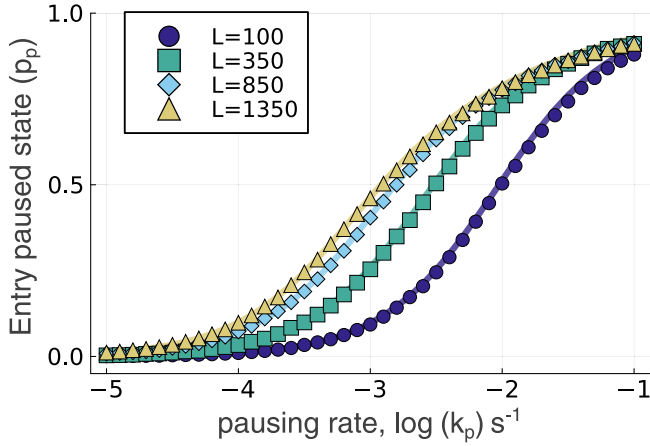


FIG. 3. Probability to enter the paused state before degradation occurs, as a function of the pausing rate, for different lattice lengths  $L$ . Analytical results (lines) closely match simulation data (markers). The residual  $T_0/\tau$  at high  $k_p$  reflects the probability that the mRNA degrades in the brief interval before the first ribosome even enters and gets hit. The parameters are the same as in Fig. 2.

## B. Paused state

### 1. Entering the paused state

The transition to the paused state is not guaranteed, as the lattice may degrade before any particle pauses. We define the probability  $p_p$  for actually entering the paused state as the likelihood that at least one pausing event occurs before lattice degradation. Since pausing and degradation are independent competing processes, this probability is obtained by integrating the pausing probability density  $F(t) = \lambda(t)S(t)$ , see Eq. (13), over all time:

$$\begin{aligned} p_p &= \int_0^\infty \lambda(t)S(t) dt \\ &= \int_0^{t_L} 2at S(t) dt + \int_{t_L}^\infty b S(t) dt. \end{aligned} \quad (18)$$

The first integral can be evaluated using integration by parts. Noting that  $2at \exp(-at^2) = -\frac{d}{dt}[\exp(-at^2)]$ , we obtain

$$\begin{aligned} \int_0^{t_L} 2at \exp\left(-at^2 - \frac{t}{\tau}\right) dt \\ = 1 - \exp\left(-at_L^2 - \frac{t_L}{\tau}\right) - \frac{1}{\tau} T_0^{\text{fill}}, \end{aligned} \quad (19)$$

where  $T_0^{\text{fill}}$  is given in Eq. (16). The second integral is simply  $bT_0^{\text{ss}}$  with  $T_0^{\text{ss}}$  from Eq. (17). Combining these results yields the compact form

$$p_p = 1 - \frac{T_0}{\tau}. \quad (20)$$

The probability  $p_p$  for the paused state to actually happen is close to unity when mRNA lifetimes are long compared to the time required for the first pausing event, but decreases significantly when degradation is fast relative to pausing, see Fig. 3.

We now characterize the paused state by the average length of the jam it entails, as well as its lifetime.

### 2. Expected first pausing position

To quantify the length of the jam in the paused state we must identify the position at which the first pausing event occurs. To this end we first consider the instantaneous position of the domain wall,  $x_s(t)$ , as defined in Eq. (4). Any particle behind this domain wall has an equal probability of pausing. Because the particle density upstream of the density front is homogeneous, pausing is equally likely to occur on each upstream site. Consequently, as long as the time of interest is  $t < t_L$ , the expected position of the first pausing event,  $x_f(t)$ , is at half the domain wall position,  $x_s(t)/2$ . However, once the domain wall reaches the exit site, at  $t \geq t_L$ , the density is uniform across the entire lattice, and thus the expected pausing position is simply the midpoint  $L/2$ . Therefore, the position  $x_f(t)$  at which, on average, the first pausing event is expected to occur can be summarized as

$$x_f(t) = \begin{cases} \frac{vt}{2}, & t < t_L, \\ \frac{L}{2}, & t \geq t_L. \end{cases} \quad (21)$$

To calculate the average position  $X_f$  where the paused state is initiated, by the first pausing event, we weigh the (time-dependent) position  $x_f$  where pausing is expected to occur by the probability density function  $F(t)$  from Eq. (13), and integrate over all possible times leading to

$$X_f = \frac{1}{p_p} \left[ \int_0^{t_L} \frac{vt}{2} F(t) dt + \int_{t_L}^\infty \frac{L}{2} F(t) dt \right]. \quad (22)$$

The factor  $1/p_p$  normalizes the average to those lattices that do undergo a pausing event, since the density  $F(t)$  integrates to  $p_p < 1$  rather than unity. The steady-state contribution ( $t \geq t_L$ ) to the expected pausing position is straightforward. From Eq. (17) we obtain that

$$\int_{t_L}^\infty \frac{L}{2} F(t) dt = \frac{L}{2} b T_0^{\text{ss}}. \quad (23)$$

The filling phase contribution requires computing the integral

$$\int_0^{t_L} \frac{vt}{2} F(t) dt = av \int_0^{t_L} t^2 e^{-at^2 - t/\tau} dt, \quad (24)$$

which can be solved exactly by completing the square in the exponent. Defining the shift parameter  $\Delta = \frac{1}{2a\tau}$  and transformed integration limits  $u_0 = \Delta\sqrt{a}$  and  $u_L = \sqrt{a}(t_L + \Delta)$ , the closed-form solution is

$$\begin{aligned} \int_0^{t_L} \frac{vt}{2} F(t) dt \\ = ve^{a\Delta^2} \left[ \left( \Delta^2 + \frac{1}{2a} \right) \frac{\sqrt{\pi}}{2\sqrt{a}} (\text{erf}(u_L) - \text{erf}(u_0)) \right. \\ \left. - \frac{t_L}{2} e^{-u_L^2} + \frac{\Delta}{\sqrt{a}} (e^{-u_0^2} - e^{-u_L^2}) \right]. \end{aligned} \quad (25)$$

Equation (25) can be simplified further by expressing the arguments of the last exponential function in terms of the

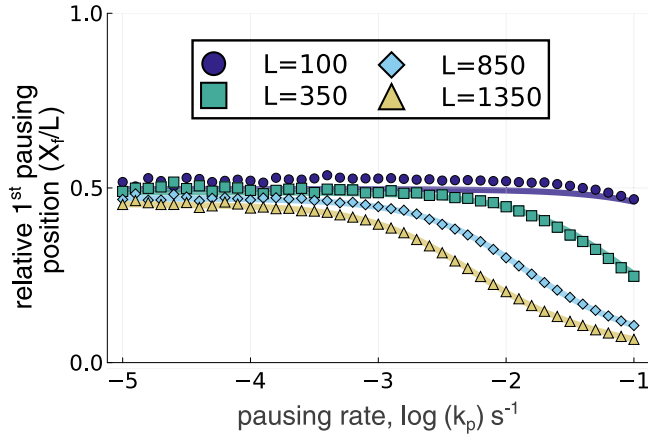


FIG. 4. Relative position  $X_f/L$  of the expected first pausing event for various lattice lengths as a function of the pausing rate  $k_p$ . The relative pausing position shifts toward the lattice midpoint 0.5 as lattice length decreases or pausing rate decreases. Analytical results (lines) closely match the simulation (markers). The parameters are the same as in Fig. 2.

previously defined quantities  $T_0^{\text{fill}}$  and  $T_0^{\text{ss}}$ . Using  $e^{a\Delta^2 - u_L^2} = (b + 1/\tau)T_0^{\text{ss}}$  and  $e^{a\Delta^2 - u_0^2} = 1$ , we obtain the more compact form:

$$\int_0^{t_L} \frac{v\tau}{2} F(t) dt = v \left[ \left( \Delta^2 + \frac{1}{2a} \right) T_0^{\text{fill}} + \left( \frac{t_L}{2} + \frac{\Delta}{\sqrt{a}} \right) (b + 1/\tau) T_0^{\text{ss}} - \frac{\Delta}{\sqrt{a}} \right], \quad (26)$$

where  $\Delta = \frac{1}{2a\tau}$  as before.

From Eqs. (23)–(26) it now becomes clear that the lattice size plays an important role: for a given pausing rate, as the lattice length increases the expected relative pausing position  $X_f/L$  shifts toward the entrance (i.e., becomes smaller). This is because the time required to reach the steady state increases with  $L$ , making it less likely that pausing will occur during the uniform-density steady state. Instead, pausing increasingly occurs during the filling phase, where the expected pausing position is  $< L/2$ . This is illustrated and validated by simulation results in Fig. 4.

### 3. Lifetime of the paused state

Given the mean lifetime of the lattice  $\tau$ , and the time  $T_0$  spent in the unpaused state, the expected time  $T_p$  spent in the paused state, averaged over all mRNAs (both those that pause and those that do not), is

$$T_p = \tau - T_0. \quad (27)$$

We can quantify the fractions of time that the system spends either in the paused state ( $P_p$ ) or in the unpaused state ( $P_0$ ) as it follows:

$$P_0 = \frac{T_0}{\tau}, \quad \text{and} \quad P_p = \frac{\tau - T_0}{\tau} = 1 - P_0. \quad (28)$$

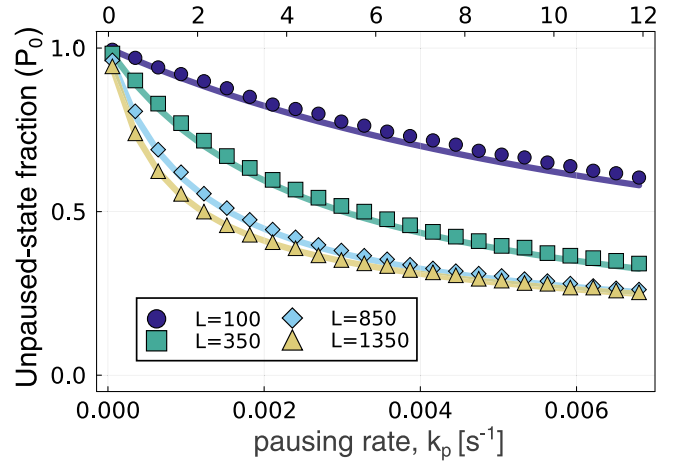


FIG. 5. Fraction of time spent in the unpaused state for different lattice lengths. The probability of being in the paused state can be obtained as  $1 - P_0$ . Symbols are simulation data, lines are model predictions from Eqs. (28).

These analytical predictions closely match simulation results, as illustrated in Fig. 5.

The lattice length influences these fractions primarily through its effect on the pausing rate. A larger lattice accommodates more particles, which increases the total pausing rate  $\lambda(t) = k_p N(t)$ , thereby shortening the expected duration of the unpaused state (see Fig. 2). Consequently, longer mRNAs spend a larger fraction of their lifetime in the paused state.

We note that, under the approximation that the cluster does not dissolve prior to lattice degradation (i.e., in the regime  $1/k_u \gg \tau$ ), one has  $P_p = p_p$ . In this limit, either the lattice enters the paused state with probability  $p_p$  and remains in that state until degradation, or it does not pause at all. Consequently, the expected fraction of time spent in the paused state ( $T_p/\tau$ ), is exactly equal to the probability of entering the paused state.

### C. Average observable quantities

Having established the fraction of time the system spends in the paused and unpaused states, respectively, we can now calculate the weighted averages of observable quantities.

#### 1. Particle density

The total particle density,  $\rho$ , is obtained as a weighted average of the paused and unpaused states,

$$\rho = P_0 \rho_0 + P_p \rho_p, \quad (29)$$

where the probabilities  $P_0$  and  $P_p$  are defined in Eq. (28); the average densities in the unpaused state  $\rho_0$  and in the paused state  $\rho_p$  are computed below.

The average density in the unpaused state is not simply the steady-state density  $\rho_{\text{ss}}$  because we must account for the (transitory) filling phase ( $t < t_L$ ), during which the number of particles on the lattice grows linearly from zero to its steady-state value. We define  $\rho_0$  as the time-averaged density during the unpaused state, weighted by the survival probability. This is computed as the total expected particle-time accumulated in the unpaused state, normalized by the lattice length  $L$  and the

expected duration  $T_0$ :

$$\rho_0 = \frac{1}{L T_0} \int_0^\infty N(t) S(t) dt. \quad (30)$$

Note that  $S(t)$  accounts for degradation, so the integral automatically restricts to times when the lattice is still present and in the unpaused state. This integral can be evaluated using the techniques used earlier (see Appendix B 1 for the full derivation), yielding the compact expression

$$\rho_0 = \frac{1}{L k_p} \left( \frac{1}{T_0} - \frac{1}{\tau} \right). \quad (31)$$

To derive the average density in the paused state  $\rho_p$ , we then need to account for (i) the formation and (ii) the persistence of the particle jam upstream of the first paused particle at position  $X_f$ , and for (iii) the run-off of particles downstream.

At the moment of pausing, the segment  $[0, X_f]$  contains  $\rho_{ss} X_f$  particles. New ones continue to enter at a rate set by the current  $J_{ss}$ , filling the lattice up to capacity  $X_f/\ell$  over a time  $t_{cf}$  given by

$$t_{cf} = \frac{X_f}{J_{ss}} \left( \frac{1}{\ell} - \rho_{ss} \right), \quad (32)$$

where the subscript “cf” denotes cluster formation. After this time, the cluster remains at maximum capacity until lattice degradation.

To compute the paused-state density, we introduce the time-averaged particle load  $\mathcal{L}$ , defined as the time integral of the number of particles on the lattice, weighted by the lattice survival probability. This quantity accumulates contributions from three phases: (i) cluster formation ( $\mathcal{L}_{cf}$ ), (ii) cluster persistence ( $\mathcal{L}_c$ ), (iii) run-off ( $\mathcal{L}_{ro}$ ). The average paused density is then obtained by normalizing the total load by the lattice length and the expected paused duration:

$$\rho_p = \frac{\mathcal{L}_{cf} + \mathcal{L}_c + \mathcal{L}_{ro}}{L T_p}, \quad (33)$$

the expression of which is given in Appendix B 2.

Having determined the density of both unpaused and paused states ( $\rho_0$  and  $\rho_p$ ) and their respective probabilities, Eq. (29) can now be used to fully characterize the system density as a function of the pausing rate  $k_p$ .

Figure 6(a) illustrates this dependency. Initially, increasing  $k_p$  results in a higher likelihood of entering the paused state, which leads to an increase in particle density. However, this trend saturates beyond a crossover value for  $k_p$ , as the system spends more and more time fully paused. Further increasing  $k_p$  then shifts the position of the initial paused particle closer to the initiation site, effectively reducing the initial cluster size and consequently lowering the overall density. This nonmonotonic behavior is observed in Fig. 6(a) for long lattices.

## 2. Current

Similarly to the density, we can also compute the average current,  $J$ , as a weighted sum:

$$J = P_0 J_0 + P_p J_p, \quad (34)$$

with  $J_0$  and  $J_p$  the contributions from the unpaused and paused state, respectively.

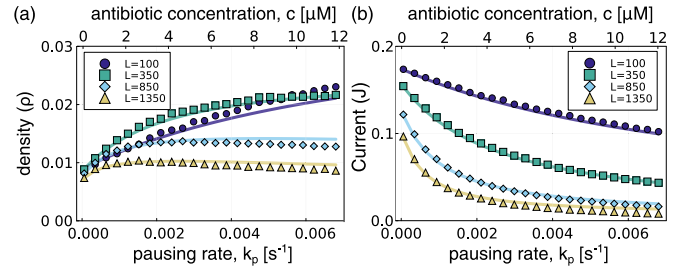


FIG. 6. (a) Particle density  $\rho$  vs pausing rate  $k_p$ . (b) The current  $J$  vs pausing rate. Parameters:  $\alpha = 0.2$ ,  $k_u = 0.0014$ ,  $\epsilon = 20$ ,  $\tau = 120$  s. Lines represent the theoretical predictions, symbols the simulation outcomes. The top  $x$  axis is expressed in antibiotic concentrations, using the relation  $k_p = k_{on}c$  and  $k_{on}$  estimated from chloramphenicol.

The unpaused current  $J_0$  is given by the total number of particles  $N_0$  leaving the lattice during the time  $T_0$ :  $J_0 = N_0/T_0$ . To calculate the total output  $N_0$ , we integrate the current  $J(t)$  weighted by the probability that the system is still in the unpaused state (neither paused nor degraded),  $S(t)$ . Since the flux is zero during the filling phase ( $t < t_L$ )—no particles exiting the lattice in this period—we compute the integral as

$$N_0 = \int_{t_L}^\infty J_{ss} S(t) dt = J_{ss} T_0^{ss}. \quad (35)$$

As a result,

$$J_0 = J_{ss} \frac{T_0^{ss}}{T_0}. \quad (36)$$

The paused current  $J_p$  is more complex to compute. After the first pausing event, the particles downstream (between  $X_f$  and  $L$ ) continue to elongate and exit, contributing to the current. We refer to this residual flux as the *run-off current*. If the unpausing rate is small (compared to degradation), as it is the case for chloramphenicol, those will be the only particles exiting the lattice. The opposite limiting case with fast antibiotic binding and unbinding is studied in the Supplemental Material [32]. Details on the computation of  $J_p$  are given in Appendix C.

Knowing  $J_0$  and  $J_p$ , we can then compute the total current  $J$  from Eq. (34), which reads

$$J = J_{ss} \left[ \frac{T_0^{ss}}{\tau} + (1 - e^{-t_{ro}/\tau}) \right], \quad (37)$$

with

$$t_{ro} = \begin{cases} \frac{L-X_f}{v}, & X_s \geq L, \\ \frac{X_f}{v}, & X_s < L. \end{cases} \quad (38)$$

Figure 6(b) shows that this analytical solution agrees well with simulations. In contrast to the density, the current is monotonic for all lattice sizes: increasing the pausing rate will always reduce the current.

We note that the expression for the runoff current is derived under the assumption that all downstream particles exit the lattice. However, for sufficiently large values of  $k_p$ , this assumption is no longer valid. In Appendix D we relax this

assumption and obtain an even closer agreement between the theoretical predictions and the simulation results.

### 3. Microscopic particle states

Having characterized the global properties of the system in terms of density and current, we now turn our attention to the microscopic dynamics by categorizing the states of individual particles.

To do that, we divide particles into three distinct categories: paused, mobile, and jammed (not paused, but blocked by exclusion by other particles downstream).

The *paused fraction*,  $f_p$ , represents the fraction of particles that are paused. As a function of time  $t$ , it approaches equilibrium according to

$$f_p(t) = \frac{k_p}{k_p + k_u} (1 - e^{-(k_p + k_u)t}). \quad (39)$$

With a finite mRNA lifetime  $\tau$ , the system may not reach the steady state before degradation. For instance, the estimates for chloramphenicol lead to  $k_p \sim k_u \sim 10^{-3} \text{ s}^{-1}$ ; since and  $\tau \sim 200 \text{ s}$ , one obtains  $\tau(k_p + k_u) \sim 0.2 \ll 1$ , indicating the system is far from pausing equilibrium before mRNA degradation. We compute the time-averaged paused fraction by weighting over the exponential lifetime distribution:

$$\langle f_p \rangle = \int_0^\infty f_p(t) \varphi_{\text{mRNA}}(t) dt, \quad (40)$$

which simplifies to

$$\langle f_p \rangle = \frac{k_p \tau}{1 + \tau(k_p + k_u)}. \quad (41)$$

For long mRNA lifetimes we obtain  $\langle f_p \rangle = k_p/(k_p + k_u)$ , which is the equilibrium value. For the sake of simplicity, in the following, we will use the symbol  $f_p$  for the time-averaged paused fraction.

Another relevant quantity is the *mobile fraction*,  $f_m$ , which accounts for particles that can move to the next site. Only this group of particles contributes to the overall current. The current can be expressed in terms of the number of mobile particles as  $J = \epsilon N_m/L$ , this leads to  $f_m = N_m/N = (JL/\epsilon)/N$ , and thus this fraction can be expressed as

$$f_m = \frac{J}{\epsilon \rho}. \quad (42)$$

Since every particle must belong to one of the three categories (paused, jammed, or mobile), their fractions sum to one. Thus, the *jammed fraction*,  $f_j$ —which represents particles that are not paused but are blocked due to steric hindrance—can be defined by

$$f_j = 1 - (f_m + f_p). \quad (43)$$

In the next section we will describe how neglecting the jammed fraction leads to overestimate the actively translating ribosomes under inhibition of translation by ribosome-targeting antibiotics.

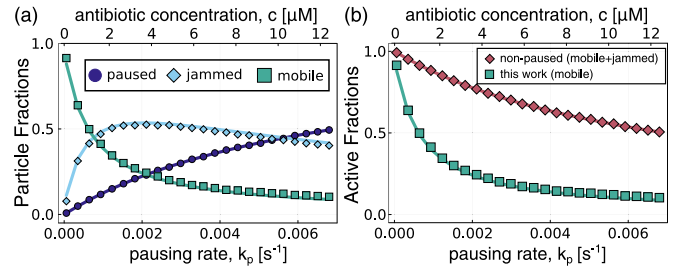


FIG. 7. Panel (a) shows how the fractions of jammed, mobile, and paused particles evolve with different pausing rates. Panel (b) compares various definitions of active particles (those contributing to the current) as a function of the pausing rate  $k_p$ . Steric interactions are often neglected when estimating the fraction of active ribosomes, so that jammed particles are still counted as contributing to the current (red circles). In contrast, our approach defines active particles only as those that are mobile (green squares). In this figure,  $\alpha = 0.02$ ,  $\epsilon = 20$ , and  $k_u = 1.4 \times 10^{-3}$ ,  $\tau = 180 \text{ s}$ .

## IV. RESULTS: BIOLOGICAL APPLICATIONS

In this section we discuss several biological situations which have motivated us to develop the model of a TASEP with stochastically pausing and unpausing particles.

### A. Model predicts antibiotics-induced ribosome jamming and sequestration

In models of quantitative physiology a key quantities is the biomass production rate (growth rate), which is often identified with protein synthesis made by ribosomes, i.e., the current  $J$  in this model multiplied by the entire set of mRNAs. Thus, in phenomenological models of quantitative physiology, “active” ribosomes at different environmental conditions determine biomass production and cellular growth [26,29]. In the literature, the definition of an active ribosome varies. Some studies define active ribosomes as those contributing to the current [26,29], while others classify any ribosome not in a paused state as active [6], regardless of whether they are temporarily blocked by downstream particles. Moreover, common models in quantitative physiology which link protein synthesis and mass growth neglect the role of ribosome interference (traffic). Here, we propose a more precise definition of active particles based on the previously introduced groups of paused, jammed, and mobile particles.

This allows us to compare the impact of elongation-targeting antibiotics (like chloramphenicol) on the population of translating ribosomes. Figure 7(a) shows the behavior of these fractions as functions of the pausing rate  $k_p$ , demonstrating excellent agreement between analytical predictions and simulation results. Intuitively, the fraction of ribosomes impacted by antibiotics (paused particles) steadily increases with antibiotic concentration. Instead, the mobile fraction abruptly decreases even at low antibiotic concentrations. This sharp decline is explained by the concurrent rise in jammed ribosomes, which, in the absence of ribosome-interference regulatory mechanisms (e.g., mRNA degradation), would sequester the majority of ribosomes away from active translation at low antibiotic levels.

Figure 7(b) compares active ribosomes as defined in our model (those contributing to total current, i.e., mobile particles) with the definition that neglects ribosome interference. The latter definition, typically used in the literature, considers active ribosomes as the total minus those paused, corresponding to the combined jammed and mobile fractions  $f_j + f_m$ .

The model thus predicts that antibiotics have an impact in lowering the fraction of active ribosomes, not only as a direct result of the antibiotics-ribosome binding, but also because they induce jamming by ribosome interference, as suggested in Ref. [2], but usually neglected in the literature. However, we highlight that this prediction does not account for potential traffic and ribosome biogenesis regulatory mechanisms [29,39].

### B. Collective ribosome dynamics affects hitting probability

The *hitting probability*—defined as the likelihood that a ribosome binds an antibiotic before completing translation—is often used to estimate the impact of elongation-inhibiting antibiotics on protein synthesis. An expression commonly used in the literature (see, e.g., Refs. [21,26]) is

$$P_{\text{hit}}^{\text{lit}} = 1 - e^{-k_p t_L}, \quad (44)$$

where  $k_p$  is the antibiotic binding rate and  $t_L$  is the time required for a ribosome to translate the full mRNA. While this expression is simple and analytically tractable, it is derived under the assumption that the mRNA is occupied by a single ribosome at a time.

However, under physiological conditions, ribosomes are frequently loaded onto mRNAs at initiation rates that allow multiple ribosomes to elongate simultaneously on the same transcript. In this setting, each ribosome can independently pause because it is targeted by the antibiotic, so the likelihood that at least one ribosome is interrupted during translation increases with the number of ribosomes on the mRNA. This introduces a collective effect that is not captured by the single-ribosome expression in Eq. (44).

When accounting for realistic ribosome traffic and stochastic mRNA degradation, the appropriate hitting probability must reflect the competition between three mutually exclusive first events: (i) a pausing event on any ribosome, (ii) completion of translation at time  $t_L$ , or (iii) degradation of the mRNA. In this setting, the hitting probability is the probability that a pausing event occurs before either translation finishes or the lattice degrades. This is given by the first-event density for pausing:

$$P_{\text{hit}} = \int_0^{t_L} F(t) dt, \quad (45)$$

where  $F(t)$ , defined in Eq. (13) is the product between  $\lambda(t)$ , the instantaneous total pausing rate, and  $S(t)$ , the joint survival probability that neither pausing nor degradation has occurred before time  $t$ . We already computed this integral in Eq. (19), which leads to

$$P_{\text{hit}} = 1 - e^{-at_L^2 - t_L/\tau} - \frac{1}{\tau} T_0^{\text{fill}}, \quad (46)$$

where  $T_0^{\text{fill}} = \int_0^{t_L} \exp(-at^2 - t/\tau) dt$  was defined in Eq. (16).

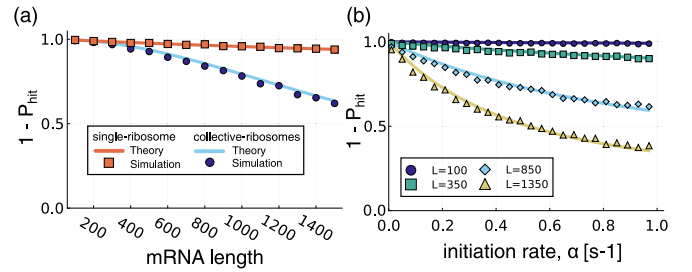


FIG. 8. (a) Probability of completing translation without antibiotic-induced pauses for varying lattice lengths, compared to the single-ribosome prediction [red line, Eq. (44)]. Parameters:  $\alpha = 0.25$ ,  $\epsilon = 20$ ,  $k_p = 10^{-3}$ ,  $k_u = 1.4 \times 10^{-3}$ . (b) Influence of the initiation rate  $\alpha$  on the probability of unimpeded translation for different lattice lengths. Decreasing  $\alpha$  makes a translation-halting event less likely. Lines are analytical predictions; symbols show simulation data. The parameters used here are  $k_p = 10^{-3}$ ,  $\epsilon = 20$ ,  $k_u = 1.4 \times 10^{-3}$ .

Figure 8(a) illustrates the contrast between the collective hitting probability obtained from Eq. (45) and the single-ribosome approximation from Eq. (44). For short lattices—corresponding to low ribosome numbers—the two expressions closely agree. However, for longer lattices, where more ribosomes can simultaneously occupy the mRNA, the collective hitting probability  $P_{\text{hit}}$  increases significantly, due to the increased likelihood that some ribosome will pause before translation completes. Figure 8(b) further demonstrates that the probability of successful translation (i.e., no pausing before completion) decreases not only with gene length but also with the initiation rate. Here too, having more ribosomes amplifies the probability that at least one will be hit during translation.

These results highlight a nontrivial collective effect: although the chance of any single ribosome pausing at a given moment is low, the probability that at least one ribosome pauses during the time from initiation to termination, given by  $t_L$ , increases sharply with the number of ribosomes translating the mRNA.

The generalized expression Eq. (45) shows that the effective hitting probability depends sensitively on ribosome traffic and on the interplay between pausing kinetics and mRNA degradation. Importantly, because mRNAs often degrade on a timescale comparable to or shorter than the antibiotic unbinding time, a pausing event almost always leads to loss of productive translation, making the timing of the first pause a key determinant of total protein yield.

### C. Gene-length-dependent expression differences emerge from collective dynamics

Building on the discussion of how antibiotic stress affects translation dynamics through pausing and collective effects, we now compare our model predictions with experimental data. One key prediction of our model is that, the longer the proteins, the more severe is the inhibition by ribosome-targeting antibiotic stress. This prediction qualitatively agrees with experimental data from Zhang *et al.* [21], who compared the expression under various antibiotic concentrations

of two genes, *gfpmut2* (238 codons) and *lacZ* (1024 codons), integrated at identical genomic locations. They observed an increased short-to-long protein expression ratio, consistent with our prediction of length-dependent translation inhibition.

In *E. coli*, the average lifetime of an mRNA is typically between 1 and 5 min [29], while the unbinding time of antibiotics like chloramphenicol is much longer—around 12 min [24]. This implies that once a ribosome is paused by an antibiotic, the mRNA is likely degraded before the ribosomes on the mRNA can resume any meaningful translation. Moreover, the pause of the ribosomes and the traffic jam generated may also trigger a cascade of signaling promoting the degradation of the affected transcripts. While ribosome drop-off may represent an alternative, though debated, fate for stalled ribosomes [40,41], there is evidence that the trailing ribosomes that collide with a stalled ribosome trigger dedicated surveillance pathways, including mRNA cleavage and ribosome rescue in *E. coli* [42,43].

Thus, we will assume that mRNA degradation lifetime follows an exponential distribution, as described in Eq. (2), but it occurs instantaneously upon ribosome collisions induced by the antibiotic binding to a ribosome. Consequently, for any given transcript, protein synthesis stops when the mRNA degrades or when a ribosome pauses.

To quantitatively describe the protein output per mRNA, we must first specify how protein numbers accumulate as a function of time. Given that the first protein is produced once the first ribosome crosses the lattice, the total number of proteins produced up to time  $t$  is

$$\mathcal{P}(t) = \begin{cases} 0, & t < t_L, \\ J_{ss}(t - t_L), & t \geq t_L, \end{cases} \quad (47)$$

where  $J_{ss}$  is the steady-state translation current, see Eq. (6), and  $t_L$  denotes the onset of steady-state translation. To determine the expected protein output per mRNA  $\mathcal{P} = \int \mathcal{P}(t) \varphi_{\text{mRNA}}(t) dt$ , we must consider both possible cases: mRNA degradation before pausing, or early ribosome pausing followed by transcript degradation before resumption of translation.

(i) *Degradation before pausing.* For those mRNAs that, by chance, degrade before the first pausing event at time  $T_0$ , translation proceeds until the degradation of the transcript occurs. The expected number of proteins produced by such mRNAs is given by

$$\int_{t_L}^{T_0} \mathcal{P}(t) \varphi_{\text{mRNA}}(t) dt, \quad (48)$$

where this integral represents the amount of protein produced up to time  $T_0$  (the onset of the paused state). It is obtained by weighting the protein output  $\mathcal{P}(t)$  by the probability distribution  $\varphi_{\text{mRNA}}(t)$ , which describes the likelihood of mRNA degradation occurring at time  $t$ .

(ii) *Pausing before degradation.* Conversely, consider the case in which an mRNAs has not yet degraded when the expected time of the first pausing event,  $T_0$ , is reached. In this case, translation is interrupted by antibiotic-induced pausing, and no further proteins are synthesized thereafter. All such mRNAs produce the same number of proteins,  $\mathcal{P}(T_0)$ , given by Eq. (47), evaluated at the first pausing time  $T_0$ . The total

contribution from these mRNAs is

$$\mathcal{P}(T_0) \int_{T_0}^{\infty} \varphi_{\text{mRNA}}(t) dt, \quad (49)$$

where the integral represents the weight of mRNAs that degrade after  $T_0$ .

Combining both terms, the total expected number of proteins produced per mRNA,  $\mathcal{P}$ , is given by

$$\mathcal{P} = \int_{t_L}^{T_0} \mathcal{P}(t) \varphi_{\text{mRNA}}(t) dt + \mathcal{P}(T_0) \int_{T_0}^{\infty} \varphi_{\text{mRNA}}(t) dt. \quad (50)$$

Evaluating Eq. (50) for the exponential mRNA lifetime distribution yields

$$\mathcal{P} = \begin{cases} J_{ss} \tau (e^{-t_L/\tau} - e^{-T_0/\tau}), & \text{if } T_0 > t_L, \\ 0, & \text{if } T_0 \leq t_L. \end{cases} \quad (51)$$

This expression reflects the effective time window between  $t_L$  and  $T_0$  during which protein synthesis occurs, weighted by the probability that the mRNA has not yet degraded. Note that the second case in Eq. (51) is justified since translation leads only to a negligible protein output if the pausing occurs before any ribosome completes. We note that this expression treats  $T_0$ —the expected time of the first pausing event—as a deterministic cutoff, thereby replacing the full distribution of first-pause times by its mean. This mean-field approximation is justified a posteriori by the close agreement with stochastic simulations we presented in the previous figures.

Because both reporters are driven by the same promoter at the same genomic locus, we assume any antibiotic-induced change in mRNA abundance is identical for GFP and *lacZ*. By normalizing each protein's expression under antibiotic treatment to its own untreated level, all effects of altered mRNA concentration cancel out—and only the relative change (fold-change) in protein output remains. Consequently, the ratio of fold-changes between long (*lacZ*) and the short (GFP) constructs,

$$\mathcal{R} = \frac{\mathcal{P}_\ell / \mathcal{P}_\ell^{(0)}}{\mathcal{P}_s / \mathcal{P}_s^{(0)}}, \quad (52)$$

reflects purely length-dependent translation inhibition, independent of transcript-level effects. Here, the indices  $s$  and  $\ell$  denote short and long proteins, respectively, and (0) indicates the reference conditions without antibiotics.

The predictions from our model quantitatively match the experimental data, as shown in Fig. 9. Without fitting separate parameters for each gene (such as different initiation or elongation rates), the model captures the observed decrease in the long-to-short expression ratio under increasing antibiotic concentrations. This agreement strongly supports the hypothesis that length-dependent inhibition naturally emerges from the interplay between collective ribosome dynamics and mRNA degradation, highlighting the model's predictive power. Furthermore, these results suggest that our framework can generalize to the expression of other genes under the same antibiotic, or to alternative inhibitory mechanisms affecting translation dynamics similarly to chloramphenicol.

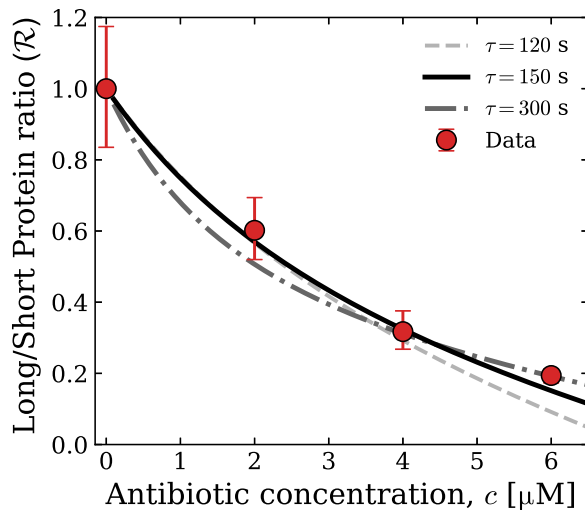


FIG. 9. Comparison between model predictions and experimental data from Zhang *et al.* [21]. The parameters used are  $k_{\text{on}} = 5.4 \times 10^{-4} \mu\text{M}^{-1}\text{s}^{-1}$ ,  $k_u = 1.4 \times 10^{-3} \text{s}^{-1}$  (giving  $K_D \approx 2.5 \mu\text{M}$ ), with a fixed initiation rate of  $0.1 \text{s}^{-1}$  and for different mean mRNA lifetime  $\tau$ .

## V. DISCUSSION

In this work, we developed a stochastic TASEP-based model to capture ribosome traffic under sub-lethal antibiotic stress, and that incorporates key biological constraints—finite mRNA length, transcript degradation, and experimentally measured antibiotic binding and unbinding rates. Although this model is motivated to study antibiotic-induced translation inhibition, in the limit  $k_p \rightarrow 0$  it provides a solid theory of translation on finite-lived mRNAs (TASEP on degrading lattices), which has only been tangentially studied in the exclusion process literature (e.g., Ref. [20]).

A strong timescale separation between translation dynamics and antibiotic binding/unbinding dynamics emerges from the experimental-derived parameters. This timescale separation enables us to develop a coarse-grained two-state description of translation consisting of an unpaused (translationally active mRNA) and a paused (translationally inhibited mRNA) state. This approach allows us to understand the interplay between ribosome-targeting antibiotic, protein synthesis, and mRNA features such as its lifetime, initiation rate, and its length.

After deriving the probability density function for the survival time of the unpaused state  $T_0$  and estimating the time  $T_p$  required to resolve the initial cluster and revert to a state without paused ribosomes, we calculated the fraction of time the system spends in each state, as illustrated in Fig. 5, where we highlight how the transcript length impacts the state of the mRNA (translating versus antibiotic-inhibited). The densities and currents are then calculated as weighted averages of the contributions from the unpaused (standard TASEP) and paused states. Both observables severely depend on the transcript length.

The dependence on system size arises as a collective effect, often not considered in previous studies. Such studies typically quantify the impact of translation-inhibiting antibiotics

by estimating the probability of a single ribosome becoming stalled during mRNA translation. In other words, they focus on the likelihood of antibiotic binding during the translation time  $t_L$  of a *single* ribosome [21,26]. In contrast, our model incorporates the collective nature of ribosome traffic, revealing a mechanism where the probability of translation disruption increases with the number of ribosomes actively translating the mRNA. As illustrated in Fig. 8(a), this leads to significantly higher hitting probabilities compared to predictions from single-particle models, especially for longer transcripts. Our model also predicts that longer mRNAs can reduce their vulnerability to ribosome-targeting antibiotics if they had lower initiation rates, thereby decreasing ribosome density and exposure to pausing events, Fig. 8(b). Interestingly, an anticorrelation between transcript lengths and initiation rates has been found in the literature, at least in yeast [44].

Moreover, as our theory explicitly addresses the impact of antibiotic binding and unbinding on overall ribosome dynamics and traffic, we introduce a formal definition of *active* ribosomes, commonly inferred through phenomenological frameworks [23,26]. Specifically, our model demonstrates that defining active ribosomes strictly as those contributing to the current (mobile particles) yields results different from estimates commonly found in the literature, where ribosome interference (jammed particles) is usually neglected, Fig. 7(a). However, we emphasize that, *in vivo*, cells may utilize various regulatory mechanisms to prevent ribosome clustering [45].

An important mechanism to prevent ribosome clustering and inefficient ribosome usage is mRNA degradation. The first pausing event significantly influences translational output under antibiotic stress since the mRNA lifetime is typically shorter than the chloramphenicol unbinding time. Thus, transcripts are often degraded before a paused ribosome can resume elongation, making protein production primarily occur before the first antibiotic-binding event. This insight also explains the observed length-dependent effects of ribosome-targeting antibiotics on gene expression. Our model quantitatively reproduces experimental differences in expression of *lacZ* and *gfp* (Fig. 9). We emphasize that this result is obtained without fitting the model to the data, but with only feeding biologically plausible parameter values obtained from the literature. As shown in the figure, the value of the mRNA lifetime (in the physiological range of 2–5 min) plays a minor role. Consequently, shorter transcripts—such as those encoding many ribosomal proteins—are predicted to be inherently more resistant to translation-inhibiting antibiotics compared to longer transcripts, such as those encoding RNA polymerase subunits.

While our model captures key features of ribosome dynamics under antibiotic stress, it also has important limitations. The most important one is the assumption of a single paused cluster, which holds for slow-binding antibiotics like chloramphenicol but breaks down at higher binding rates, for instance, in the case of fusidic acid [46]. Under such conditions, multiple paused clusters may form and interact through coagulation and decoagulation dynamics. Although the single-cluster approximation loses predictive reliability once multiple paused clusters emerge, length-dependent effects are still expected to matter even in a refined approach to resolve this more complex behavior.

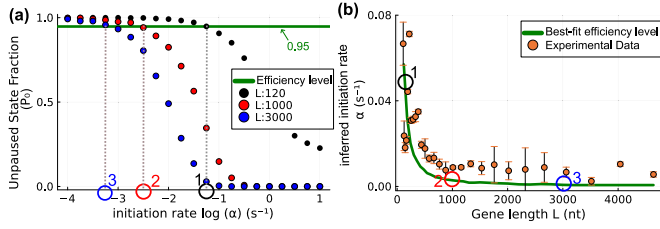


FIG. 10. The model suggests that promoter on-rates are set to minimize the clustering of RNAP (see Appendix E). (a) The simulated RNAP unpaused state fraction  $P_0$  as a function of initiation rates, for different gene lengths  $L$ . Here, we choose the efficiency level (green vertical line) so that the predicted initiation rate for a certain gene length matches the experimentally observed one, shown in panel (b). Experimental data from Ref. [29] (symbols) show a similar trend. Here, efficiency refers to the fraction of time spend in the unpaused state. The pausing and unpausing rate are taken from Ref. [1] and are here  $k_u = 0.5$ ,  $\varepsilon = 50$ ,  $\ell = 30$ , and  $k_p = 0.15$ .

Despite simplifying assumptions, our framework naturally extends to other biological systems characterized by stochastic pausing [19,47,48]. A key example is transcription, where RNAPs intermittently pause, causing local traffic jams [1]. These intrinsic pauses typically occur at rates significantly slower than elongation. Notably, shorter genes often exhibit higher transcription initiation rates in bacteria and yeast [29,49,50]. Our conclusion that the density would depend on both gene length and initiation rate allows us to speculate that initiation rates have been adapted to minimize the onset of RNAP clustering (see Fig. 10 and Appendix E). We further emphasize that, as shown in Ref. [51], our model of stochastically pausing particles can be mapped onto systems with dynamic obstacles [17,18] that temporarily impede particle movement. This analogy suggests broader applications, such as describing interactions between RNAPs and transcriptional regulatory elements or nucleosomes [7,8] or, beyond biology, systems such as vehicular traffic interrupted by traffic lights. Future work could extend this framework by connecting multiple mRNAs through a multi-TASEP approach [52], thereby linking antibiotic concentration to global bacterial outcomes such as growth rate and ribosome allocation.

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J.K. developed the theoretical framework and performed the simulations. L.C. developed the theoretical framework, conceptualized and supervised the work, supported by N.K. All authors discussed and interpreted the results and contributed to the final manuscript.

## DATA AVAILABILITY

The data that support the findings of this article are openly available [20,49].

## APPENDIX A: SIMULATIONS

We employ a standard Gillespie algorithm [53] for the simulations. The algorithm consists of two main phases. First, the system is allowed to run for a time  $t_{ss}$  to reach steady state. After achieving steady state, data is collected for a duration  $\Delta t$ .

In the Gillespie algorithm, updates occur as Poisson processes, which means they are independent of each other. To ensure that the system reaches steady state and that the data collection period  $\Delta t$  is sufficiently long, the total simulation time is made proportional to the lowest rate in the system. Unless otherwise stated, we set  $t_{ss} = 100 \times$  (lowest rate) and  $\Delta t = 1000 \times$  (lowest rate). Thus, the total simulation time  $t$  is equal to  $t = t_{ss} + \Delta t$ .

## APPENDIX B: DERIVATION OF THE DENSITY $\rho$

### 1. Derivation of the unpaused density $\rho_0$

The average density in the unpaused state is computed as the time-averaged density, weighted by the survival probability:

$$\rho_0 = \frac{1}{L T_0} \int_0^\infty N(t) S(t) dt, \quad (B1)$$

where  $N(t) = J_{ss}t$  for  $t < t_L$  and  $N(t) = \rho_{ss}L$  for  $t \geq t_L$ . This yields

$$\rho_0 = \frac{1}{L T_0} \left[ \underbrace{\int_0^{t_L} J_{ss}t S(t) dt}_{\text{Filling}} + \underbrace{\rho_{ss}L \int_{t_L}^\infty S(t) dt}_{\text{Steady state}} \right]. \quad (B2)$$

The steady-state phase contribution is straightforward:

$$\int_{t_L}^\infty S(t) dt = T_0^{ss}, \quad (B3)$$

as given in Eq. (17).

For the filling phase, we use the result derived for  $p_p$ :

$$\int_0^{t_L} 2at \exp\left(-at^2 - \frac{t}{\tau}\right) dt = 1 - \exp\left(-at_L^2 - \frac{t_L}{\tau}\right) - \frac{1}{\tau} T_0^{\text{fill}}. \quad (B4)$$

Dividing by  $2a$  gives

$$\int_0^{t_L} t S(t) dt = \frac{1}{2a} \left[ 1 - \exp\left(-at_L^2 - \frac{t_L}{\tau}\right) - \frac{1}{\tau} T_0^{\text{fill}} \right]. \quad (B5)$$

Recalling that  $\exp(-at_L^2 - t_L/\tau) = (b + 1/\tau)T_0^{ss}$ , this becomes

$$\int_0^{t_L} t S(t) dt = \frac{1}{2a} \left[ 1 - (b + 1/\tau)T_0^{ss} - \frac{1}{\tau} T_0^{\text{fill}} \right]. \quad (B6)$$

Substituting into the expression for  $\rho_0$ :

$$\rho_0 = \frac{1}{L T_0} \left[ \frac{J_{ss}}{2a} \left( 1 - (b + 1/\tau)T_0^{ss} - \frac{1}{\tau} T_0^{\text{fill}} \right) + \rho_{ss}L T_0^{ss} \right]. \quad (B7)$$

Since  $a = J_{ss}k_p/2$ , we have  $J_{ss}/(2a) = 1/k_p$ . Also,  $b = \rho_{ss}Lk_p$  and  $T_0 = T_0^{\text{fill}} + T_0^{\text{ss}}$ . Expanding:

$$\begin{aligned}\rho_0 &= \frac{1}{LT_0} \left[ \frac{1}{k_p} - \frac{bT_0^{\text{ss}}}{k_p} - \frac{T_0^{\text{ss}}}{k_p\tau} - \frac{T_0^{\text{fill}}}{k_p\tau} + \rho_{ss}LT_0^{\text{ss}} \right] \\ &= \frac{1}{LT_0} \left[ \frac{1}{k_p} - \frac{\rho_{ss}Lk_pT_0^{\text{ss}}}{k_p} - \frac{T_0^{\text{ss}}}{k_p\tau} - \frac{T_0^{\text{fill}}}{k_p\tau} + \rho_{ss}LT_0^{\text{ss}} \right] \\ &= \frac{1}{LT_0} \left[ \frac{1}{k_p} - \frac{T_0^{\text{ss}} + T_0^{\text{fill}}}{k_p\tau} \right] = \frac{1}{LT_0} \left[ \frac{1}{k_p} - \frac{T_0}{k_p\tau} \right] \\ &= \frac{1}{Lk_p} \left( \frac{1}{T_0} - \frac{1}{\tau} \right).\end{aligned}\quad (\text{B8})$$

Thus, the average density in the unpaused state is

$$\rho_0 = \frac{1}{Lk_p} \left( \frac{1}{T_0} - \frac{1}{\tau} \right). \quad (\text{B9})$$

## 2. Derivation of the paused density $\rho_p$

The average density in the paused state is computed as the time-averaged particle load, weighted by the lattice survival probability. We consider two phases: cluster formation and cluster persistence.

### a. Cluster formation phase

At the moment of pausing (defining this as  $t = 0$  in the paused state), the lattice segment  $[0, X_f]$  contains:  $\rho_{ss}X_f$  particles. New particles continue to enter at current  $J_{ss}$ , so the total number of particles in the cluster grows as

$$N_c(t) = \rho_{ss}X_f + J_{ss}t. \quad (\text{B10})$$

The cluster reaches maximum capacity when  $N_c(t_{cf}) = X_f/\ell$ . Solving for  $t_{cf}$ :

$$\rho_{ss}X_f + J_{ss}t_{cf} = \frac{X_f}{\ell} \quad (\text{B11})$$

$$t_{cf} = \frac{X_f}{J_{ss}} \left( \frac{1}{\ell} - \rho_{ss} \right). \quad (\text{B12})$$

The accumulated load during cluster formation, weighted by lattice survival, is

$$\mathcal{L}_{cf} = \int_0^{t_{cf}} (\rho_{ss}X_f + J_{ss}t) e^{-t/\tau_{\text{eff}}} dt, \quad (\text{B13})$$

where  $1/\tau_{\text{eff}} := 1/\tau + k_u$ . This effective rate accounts for the two independent processes that can reduce the cluster size: stochastic mRNA degradation (rate  $1/\tau$ ) and unpausing of the leading ribosome (rate  $k_u$ ). Although full cluster dissolution before mRNA degradation is unlikely in the chloramphenicol regime ( $1/k_u \gg \tau$ ), individual unpausing events at the cluster boundary do occur and reduce the effective time over which the cluster persists in its entirety. This phenomenological correction accounts for partial cluster dissolution, where a leading batch of particles becomes unblocked but the remainder of the cluster is still maintained intact by other blocked particles. It is necessary to obtain quantitative agreement with simulations; omitting the  $k_u$  term leads to a systematic overestimate of the paused-state density. Evaluating this

integral:

$$\begin{aligned}\mathcal{L}_{cf} &= \rho_{ss}X_f \int_0^{t_{cf}} e^{-t/\tau_{\text{eff}}} dt + J_{ss} \int_0^{t_{cf}} t e^{-t/\tau_{\text{eff}}} dt \\ &= \rho_{ss}X_f \tau_{\text{eff}} (1 - e^{-t_{cf}/\tau_{\text{eff}}}) \\ &\quad + J_{ss} [\tau_{\text{eff}}^2 (1 - e^{-t_{cf}/\tau_{\text{eff}}}) - \tau_{\text{eff}} t_{cf} e^{-t_{cf}/\tau_{\text{eff}}}] .\end{aligned}\quad (\text{B14})$$

### b. Cluster persistence phase

After reaching maximum capacity at  $t = t_{cf}$ , the cluster remains at size  $X_f/\ell$  until mRNA degradation. The accumulated load is

$$\mathcal{L}_c = \frac{X_f}{\ell} \int_{t_{cf}}^{\infty} e^{-t/\tau_{\text{eff}}} dt = \frac{X_f}{\ell} \tau_{\text{eff}} e^{-t_{cf}/\tau_{\text{eff}}}. \quad (\text{B15})$$

### c. Run-off contribution

The derivation above neglects particles downstream of the paused ribosome at position  $X_f$ . These particles continue to exit the lattice during the paused state, contributing to the density. Here we derive their contribution to the paused density.

At the moment of pausing, the spatial extent of downstream particles depends on whether the lattice has reached steady state.

If pausing occurs after the lattice has reached its steady-state density, the downstream segment  $[X_f, L]$  initially contains  $\rho_{ss}(L - X_f)$  particles. Because no inflow arrives from upstream once the pause is formed, this downstream population depletes linearly at the steady-state exit current  $J_{ss}$  up to when all run-off particles exit the system, at time  $t_{ro} = (L - X_f)/v$ . Writing the depletion in terms of the domain-wall velocity  $v = \epsilon - \alpha$  ( $J_{ss} = \rho_{ss}v$ ), the time-dependent downstream particle number for  $0 \leq t \leq t_{ro}$  is  $N_{ro}(t) = \rho_{ss}(L - X_f - vt)$ . Accounting for the stochastic lattice degradation, the *run-off load* (particle-time) is

$$\mathcal{L}_{ro} = \int_0^{t_{ro}} \rho_{ss}(L - X_f - vt) e^{-t/\tau} dt. \quad (\text{B16})$$

Finally, expressing the result with  $J_{ss} = \rho_{ss}v$  gives the compact form

$$\mathcal{L}_{ro} = \rho_{ss}(L - X_f) \tau - J_{ss} \tau^2 (1 - e^{-t_{ro}/\tau}). \quad (\text{B17})$$

If pausing occurs before steady state is reached, there is a gap between the domain wall at  $X_s = 2X_f$  and the exit at  $L$ . The downstream segment  $[X_f, X_s]$  initially contains  $\rho_{ss}(X_s - X_f) = \rho_{ss}X_f$  particles. The first particle reaches the exit after a delay  $t_{\text{start}} = (L - X_s)/v$ , after which particles exit at current  $J_{ss}$  until the last particle exits at  $t_{\text{exit}} = (L - X_f)/v$ . The run-off duration is  $t_{ro} = t_{\text{exit}} - t_{\text{start}} = X_f/v$ .

During the run-off window  $[0, t_{ro}]$  (measuring time from when particles start exiting), the number of particles decreases as  $N_{ro}(t) = \rho_{ss}(X_f - vt)$ .

The run-off load, accounting for the delay  $t_{\text{start}}$  before particles begin exiting and for lattice degradation, is

$$\mathcal{L}_{ro} = e^{-t_{\text{start}}/\tau} \int_0^{t_{ro}} \rho_{ss}(X_f - vt) e^{-t/\tau} dt. \quad (\text{B18})$$

This integral has the same form as Eq. (B17) with  $X_f$  replacing  $(L - X_f)$ , yielding

$$\mathcal{L}_{ro} = e^{-t_{start}/\tau} [\rho_{ss} X_f \tau - J_{ss} \tau^2 (1 - e^{-t_{ro}/\tau})], \quad (B19)$$

where  $t_{start} = (L - X_s)/v = (L - 2X_f)/v$  and  $t_{ro} = X_f/v$ .

#### d. Total paused density

Including cluster formation, persistence, and run-off contributions, the average paused density is

$$\rho_p = \frac{\mathcal{L}_{total}}{L T_p}, \quad (B20)$$

where the total load  $\mathcal{L}_{total} = \mathcal{L}_{cf} + \mathcal{L}_c + \mathcal{L}_{ro}$ . The last term depends on the status of the lattice at the pausing time. We thus distinguish two regimes:

*Steady-state case* ( $X_s \geq L$ ), when the initial density shock as already reached the end of the lattice:

$$\begin{aligned} \mathcal{L}_{total} = & \rho_{ss} X_f \tau_{eff} + J_{ss} \tau_{eff}^2 (1 - e^{-t_{cf}/\tau_{eff}}) \\ & + \rho_{ss} (L - X_f) \tau - J_{ss} \tau^2 (1 - e^{-t_{ro}/\tau}), \end{aligned} \quad (B21)$$

with  $t_{ro} = (L - X_f)/v$ .

*Transient case* ( $X_s < L$ ), when the initial density shock has not reached the end of the lattice:

$$\begin{aligned} \mathcal{L}_{total} = & \rho_{ss} X_f \tau_{eff} + J_{ss} \tau_{eff}^2 (1 - e^{-t_{cf}/\tau_{eff}}) \\ & + e^{-t_{start}/\tau} [\rho_{ss} X_f \tau - J_{ss} \tau^2 (1 - e^{-t_{ro}/\tau})], \end{aligned} \quad (B22)$$

with  $t_{ro} = X_f/v$  and  $t_{start} = (L - 2X_f)/v$ . In both cases,  $t_{cf} = \frac{X_f}{J_{ss}} (\frac{1}{\tau} - \rho_{ss})$  is the cluster formation time.

#### APPENDIX C: COMPUTING THE PAUSING CURRENT $J_p$

The paused current  $J_p$  is defined as the total expected number of run-off particles  $N_{ro}$  divided by the expected duration of the paused state:

$$J_p = \frac{N_{ro}}{T_p}. \quad (C1)$$

During run-off, particles exit at a rate  $J_{ss}$  for an interval of time  $t_{ro}$ , conditioned to the fact that the lattice has not been degraded. The number of particles exiting the lattice in the run-off period is thus

$$N_{ro} = J_{ss} \int_0^{t_{ro}} e^{-t/\tau} dt = J_{ss} \tau (1 - e^{-t_{ro}/\tau}). \quad (C2)$$

To evaluate  $t_{ro}$ , we count the number of particles downstream of the jam and calculate how long it takes before they all exit the lattice. We use the expected position of the density shock, or domain wall, at the time of the first pausing event  $X_s \approx 2X_f$  to distinguish between two regimes, based on whether the lattice was at steady state at the moment of pausing.

If the pause occurs when the lattice is at steady state (i.e., when  $X_s \geq L$ ), the segment from  $X_f$  to  $L$  contains  $\rho_{ss}(L - X_f)$  particles, and the duration of the runoff lasts  $t_{ro} = (L - X_f)/v$ .

If the pause occurs before steady state is reached (i.e., when  $X_s < L$ ), a gap exists between the domain wall at  $X_s$  and the exit at  $L$ . The downstream segment contains  $\rho_{ss}(X_s - X_f)$  particles. The first particle of this segment reaches the exit after a delay  $t_{start} \approx (L - X_s)/v$ , after which a steady current

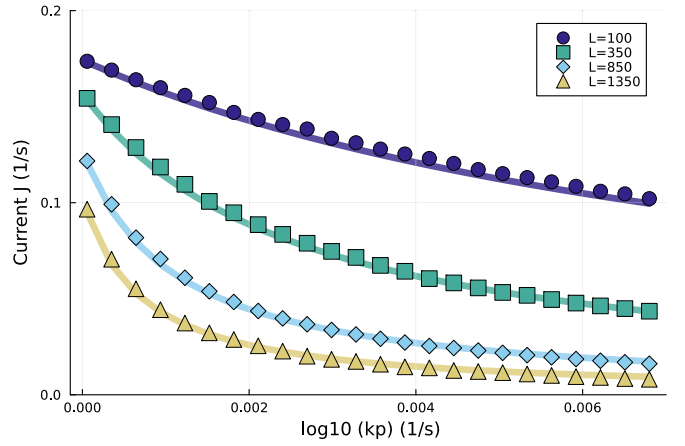


FIG. 11. Corrected paused current  $J$  vs pausing rate, similar to Fig. 6 but when using Eq. (D3).

$J_{ss}$  is established. The last particle exits at  $t_{exit} \approx (L - X_f)/v$ , giving a total run-off duration of  $t_{ro} = t_{exit} - t_{start} = X_f/v$ .

#### APPENDIX D: SECOND PAUSING IN RUNOFF CLUSTER

To account for the possibility of a second pausing event during run-off, we introduce a correction factor. The probability of at least one additional pause among the  $N_{ro}$  run-off particles during time  $t_{ro}$  is

$$p_{2nd} = 1 - e^{-k_p N_{ro} t_{ro}}, \quad (D1)$$

where

$$N_{ro} = \begin{cases} \rho_{ss}(L - X_f), & X_s \geq L, \\ \rho_{ss} X_f, & X_s < L. \end{cases} \quad (D2)$$

Since the second pause occurs at a random position within the run-off segment, it blocks on average half of the remaining current. The corrected paused current is

$$J_p^{corr} = J_p \left( 1 - \frac{p_{2nd}}{2} \right). \quad (D3)$$

The outcome is shown in Fig. 11.

#### APPENDIX E: RNAP PAUSING DYNAMICS AND GENE LENGTH-DEPENDENT INITIATION RATES

While our analysis has focused on translation, the TASEP framework with stochastic pausing is equally applicable to transcription. RNAPs elongating along DNA also undergo random pauses, a phenomenon observed across species [47,48]. These pauses arise from intrinsic mechanisms such as backtracking or proofreading [19], and typically occur on timescales two to three orders of magnitude slower than elongation [1,47]. Unlike pausing from external stress (e.g., antibiotics in translation), transcriptional pausing is an inherent feature of RNAP dynamics.

Notably, experiments have found that shorter genes tend to exhibit higher transcription initiation rates in both bacteria and yeast [29,49,50]. We hypothesize that this reflects an evolutionary adaptation to minimize RNAP congestion: longer genes, being more prone to queuing due to random

pausing, require lower initiation rates to maintain efficient transcription. For example, at the same initiation rate, longer genes spend far more time in the unpaused state compared to short ones, as seen in Fig. 10(a).

To test this idea, we define transcriptional efficiency as the fraction of time the system spends in the unpaused state. For each gene length, we use simulations to determine the initiation rate that best matches the experimental data shown in Fig. 10(b). Our analysis reveals that reproducing the observed initiation rates requires transcription to remain

in the unpaused state for over 90 percent of the time. This suggests that the initiation rates were optimized to reduce traffic and thus efficient RNAP usage, given their limited availability and high cellular cost. This analysis uncovers a clear trend: as gene length increases, the transcription process becomes more inefficient if the initiation rate does not change. This prediction aligns with experimental observations and supports the idea that stochastic pausing could impose a physical constraint on transcription, shaping gene-specific initiation rates.

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