

Rapid Transcription Factor Fluctuations Drive Nonequilibrium Gene Regulatory Dynamics in Bacteria

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(Received 20 December 2024; accepted 28 May 2025; published 18 July 2025)

In bacteria, fluctuations in transcription factor (TF) concentrations and stochastic binding to promoters drive gene expression fluctuations in their target genes. However, how this “noise propagation” from TFs to their targets determines the expression fluctuations in target genes has so far not been quantitatively characterized. Here, we use fluorescence time-lapse microscopy in combination with microfluidics and automated image analysis to quantitatively track the single-cell expression dynamics of target promoters of the SOS response regulator LexA in *Escherichia coli* under mild DNA damage using the antibiotic Ciprofloxacin (Cipro). We find that all target promoters exhibit short stochastic expression bursts which become more frequent as the Cipro concentration increases, but whose durations are identical for all promoters, with exponentially distributed heights that vary across promoters, but that are independent of Cipro concentration. We show that these observations are inconsistent with popular “equilibrium” models of bacterial gene regulation that assume that binding and unbinding of TFs to promoters is fast relative to TF concentration fluctuations. Instead, all observations are quantitatively fit by a model in which DNA damage events lead to rapid transient dips in LexA concentration, and an expression burst occurs when LexA unbinds from the target promoter during such a short dip. Crucially, because these LexA concentration fluctuations occur on the same timescale as individual TF unbinding events, the responses of different target promoters to individual DNA damage events are complex stochastic and nonequilibrium functions of the strengths of their LexA binding sites and the durations of the events. Our results show that when induction events cause rapid TF concentration changes, i.e., on the timescale of individual TF binding and unbinding events, different target promoters exhibit distinct stochastic responses that read out different kinetics of the induction events. Since many bacterial TFs are activated through phosphorylation or binding to small signaling molecules, such nonequilibrium gene regulation is likely pervasive in bacteria, and may have been exploited by natural selection to encode distinct responses by different targets of the same TF.

DOI: [10.1103/nvk3-jsvm](https://doi.org/10.1103/nvk3-jsvm)

I. INTRODUCTION

Bacterial cells can adapt their gene expression to a large variety of environments including many combinations of nutrients and stresses. Much of this regulation occurs through the sequence-specific binding of transcription factors (TFs) to sites near the transcription start of genes, altering the rate at which transcription is initiated [1–3]. Since TF binding and transcription initiation are single molecule events at promoters, they are subject to inherent thermal molecular noise, causing intrinsic stochasticity in gene expression. Indeed, early studies of gene expression at the single-cell level not only showed that gene expression varies between identical promoters in the same cell, but also that the actions of TFs can propagate noise so as to increase the cell-to-cell variation in gene expression of their targets [4,5].

Recent work from our laboratory has shown that noise propagation from regulators to their targets is a key determinant of gene expression noise on a genome-wide scale in *Escherichia coli* [6,7], and this raises the question of how noise is propagated from regulators to their targets, i.e., how the actions of TFs determine the gene expression dynamics of their targets in single cells.

A popular modeling approach is to assume that the dynamics TF binding and unbinding is fast relative to fluctuations in TF concentrations [8–10] so that the fractions of time that different TF binding configurations occur at a promoter equal the frequencies given by assuming “thermodynamic equilibrium” in the TF binding dynamics. Under this assumption, the effective transcription rate from a promoter is then simply the average of the transcription rates over different binding configurations, weighted with their equilibrium frequencies. Notably, in this “equilibrium” model, noise propagation from regulators to their targets is mainly due to cell-to-cell fluctuations in TF concentrations. However, it is currently unclear to what extent these assumptions actually hold for bacterial regulatory circuitry.

To investigate how regulators affect the expression dynamics of their targets in single cells we here focus on the LexA regulon in *E. coli*, which is activated in response to DNA damage [11,12]. This regulon is attractive for a study

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of noise propagation for several reasons. The system has been intensively studied, its induction mechanism has been dissected in substantial detail, its target genes have been well characterized, including the sequence specificity of LexA and its binding sites in target promoters, and many of the kinetic parameters of this system are known. Together, this strongly constrains possible models of the regulatory dynamics of this system. In addition, the LexA regulon can be easily induced in the laboratory by damaging DNA either through UV light or using particular antibiotics.

Briefly, in the absence of DNA damage, the repressor LexA is bound to the promoters of its target genes, keeping them repressed. When DNA damage occurs, the protein RecA forms filaments on single-stranded DNA and LexA molecules auto-cleave on these RecA filaments, leading to a decrease in active LexA concentration, which in turn induces its target genes. Once the DNA damage is repaired, the RecA filaments disassemble, and the LexA expression recovers [11–15].

The LexA regulon was initially mostly studied in response to UV radiation at the bulk population level, leading to several models for the regulatory dynamics, e.g., Refs. [15–18]. Subsequent single-cell studies of the induction dynamics in response to UV radiation revealed that the population measurements mask a more complex single-cell dynamics with multiple roughly synchronized peaks in the activity of LexA target promoters [19]. However, because UV radiation induces a complex cascade of downstream effects including major physiological changes such as filamentation and growth arrest, it has been challenging to disentangle how the various processes downstream of UV radiation cause the observed peaks in LexA target expression. Indeed, a host of different models has been proposed in the literature to explain these complex temporal modulations in LexA activity [17,20–22].

We reasoned that expression regulation of LexA targets in single cells might be easier to interpret in a regime with only mild induction of this regulon. Recent time-lapse microscopy studies with a fluorescent transcriptional reporter of the LexA target promoter RecA have shown that, even in the absence of stressors that induce DNA damage, there are stochastic expression bursts in single cells [23,24], suggesting that stochastic induction of LexA targets in single cells can be readily observed even at low levels of DNA damage.

We selected a set of LexA target genes that are currently believed not to be regulated by any other TF. Using fluorescent transcriptional reporters of the promoters of these target genes [25] we tracked growth and gene expression in lineages of single cells using an integrated setup that combines fluorescence time-lapse microscopy with microfluidics and automated image analysis [26,27]. Using the DNA damaging antibiotic Ciprofloxacin (Cipro) [28] we exposed the cells to increasing levels of mild DNA damage, inducing the LexA targets without substantially affecting either the growth rates or the physiology of the cells, i.e., avoiding filamentation. Using this setup, we quantified the time-dependent stochastic gene expression for different target promoters and Cipro concentrations.

Below we first show that different promoters show different levels of induction and that the induction is driven by short stochastic bursts in expression. We then show that the frequencies, heights and durations of these gene expression bursts have very specific quantitative characteristics and

that ‘equilibrium’ models of gene regulation are unable to reproduce this behavior. Instead, we show that all our data can be accurately fit by a model in which stochastic DNA damage events cause short transient dips in LexA levels and that expression bursts occur whenever LexA unbinds from the promoter during such a transient dip. Importantly, we show that because the dips in LexA concentration occur on the same timescale as the binding/unbinding of individual LexA molecules to their binding sites, the response from each promoter is a complex stochastic function of the frequency and durations of DNA damage events in the recent history of the cell. That is, because of the nonequilibrium nature of the regulatory response, different target promoters can show highly distinct responses to the same induction of a single TF, effectively reading out different characteristics of the induction dynamics.

While we here demonstrated this nonequilibrium dynamics for the LexA regulon, we note that many TFs in bacteria are activated through phosphorylation or binding to small signaling molecules, so that rapid transient changes in active TF levels likely occur in many regulatory circuits, and the type of nonequilibrium regulatory responses that we observe for the LexA regulon might well be pervasive in bacterial gene regulation.

II. RESULTS

A. LexA target genes show different levels of induction at the population level

To study the induction of the LexA regulon we selected eight promoters that are known to be regulated by LexA and are currently believed not to be regulated by any other TF (apart from the sigma factor Sigma70) [29]. In addition, we included two synthetic promoters that we previously obtained by screening a library of completely random short sequence fragments for fragments that drive expression [6]. These promoters, which we denote by “synthetic high” and “synthetic medium,” exhibit average expression levels at the 97.5th and 50th percentile of all native *E. coli* promoters, respectively. In addition, these synthetic promoters do not appear to contain TF binding sites other than a Sigma70 site [6] and were observed to be constitutively expressed across a range of growth conditions [7]. We thus use these synthetic promoters as constitutively expressed controls.

We induced the LexA target promoters by inducing DNA damage using low levels of Cipro. Our microfluidic setup enables controlled variation of the Cipro concentration over time, allowing direct observation of gene regulatory responses to different induction levels in single cells [26]. In particular, we monitored the cells over a period of 30 h, starting with no antibiotic in the first 6 h, switching to 1 ng/ml in the following 12 h and finally to 2 ng/ml in the final 12 h. Notably, these concentrations are all far below the MIC of Cipro and do not cause major physiological changes to the cells such as filamentation. Indeed, at these concentrations the growth rates are not affected by the presence of the antibiotic (see Fig. 18 of Appendix F).

In Fig. 1(c), we show snapshot images across time of cells and their fluorescence levels from one growth lane containing cells that express GFP from the synthetic high promoter (bot-

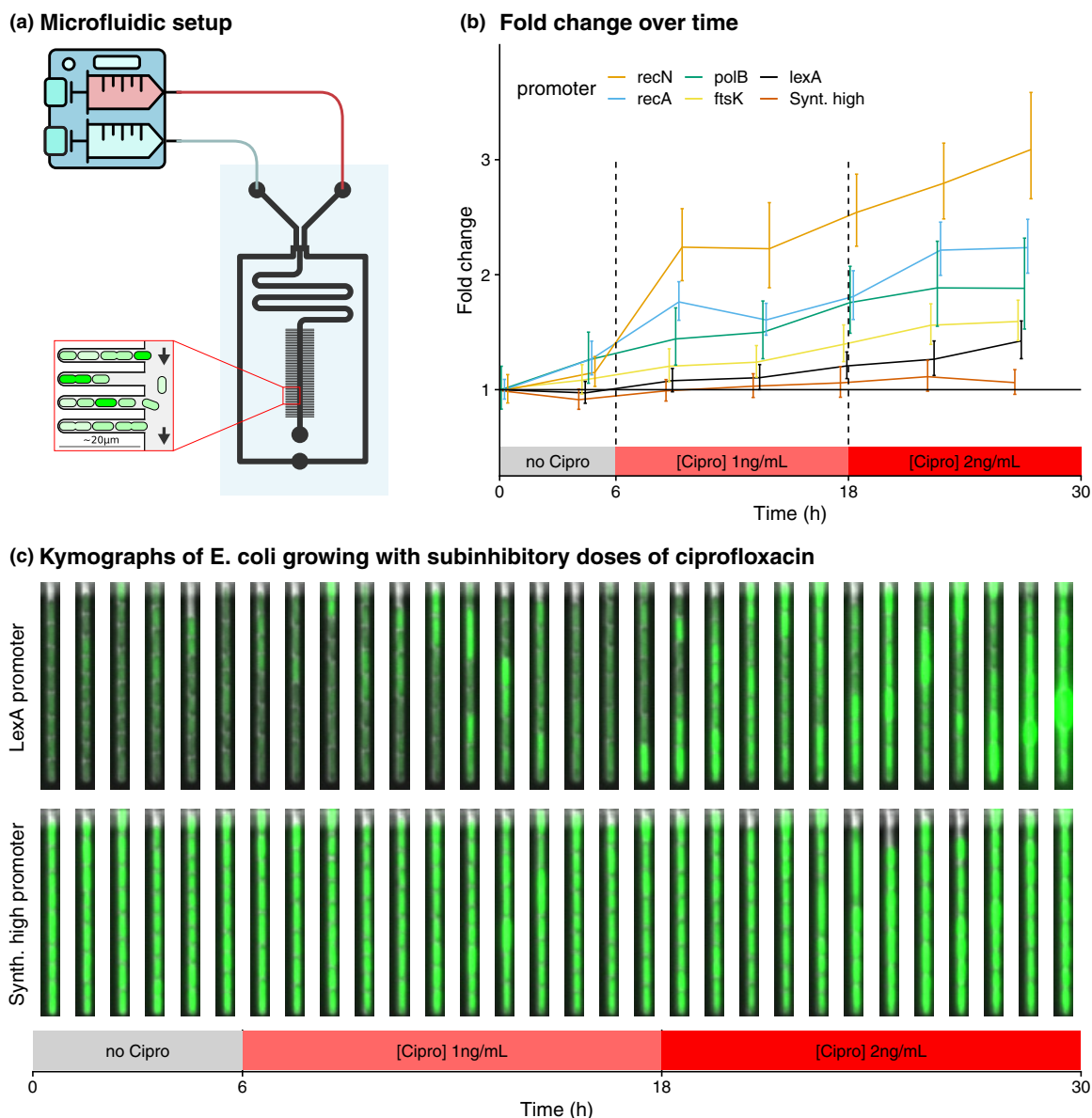


FIG. 1. Induction of promoters under control of LexA. (a) Schematic of the dual input mother machine microfluidic setup used in the experiments. Programmable pressure pumps allow arbitrary mixtures of two input media which first flow through a mixing junction and then pass by many independent growth channels where bacteria grow in single file. (b) Fold changes in average expression as a function of time for five different LexA target promoters, and the unregulated synthetic high promoter (mean $\pm$ one standard-error). (c) Example kymographs of *E. coli* growing under increasing subinhibitory concentrations of Cipro. The upper strip shows cells expressing GFP from the *recA* promoter, while the bottom strip shows cells expressing GFP from the synthetic high promoter. Note that Cipro is known to induce DNA strand breaks.

tom) and one growth lane containing cells expressing GFP from the *recA* promoter (top). We can see that, while the synthetic promoters exhibit approximately constant expression across the different Cipro concentrations, *recA* promoters exhibit much more variable expression which on average increases as the concentration of Cipro increases. Figure 1(b) shows the fold change of the mean fluorescence level (averaged over all cells) as a function of time for the synthetic high promotes and the 5 LexA target promoters that were induced in our setup. Note that we did not observe a detectable induction for three additional promoters (*ruvA*, *uvrD*, and *umuD*) that are reported to be regulated by LexA (see Fig. 19 of Appendix F).

Importantly, Fig. 1(b) shows that the expression of different promoters is upregulated to different extents in response to the same change in conditions. In addition, Fig. 19 of Appendix F shows the basal GFP concentrations (in the absence of Cipro) and the fold changes for all the LexA regulated promoters.

B. GFP production from LexA regulated promoters occurs in short bursts

To better understand how different target promoters respond to the same induction of the LexA TF, we monitored the expression dynamics in single cell lineages. For each cell, the microfluidic setup enables us to track the total GFP intensity

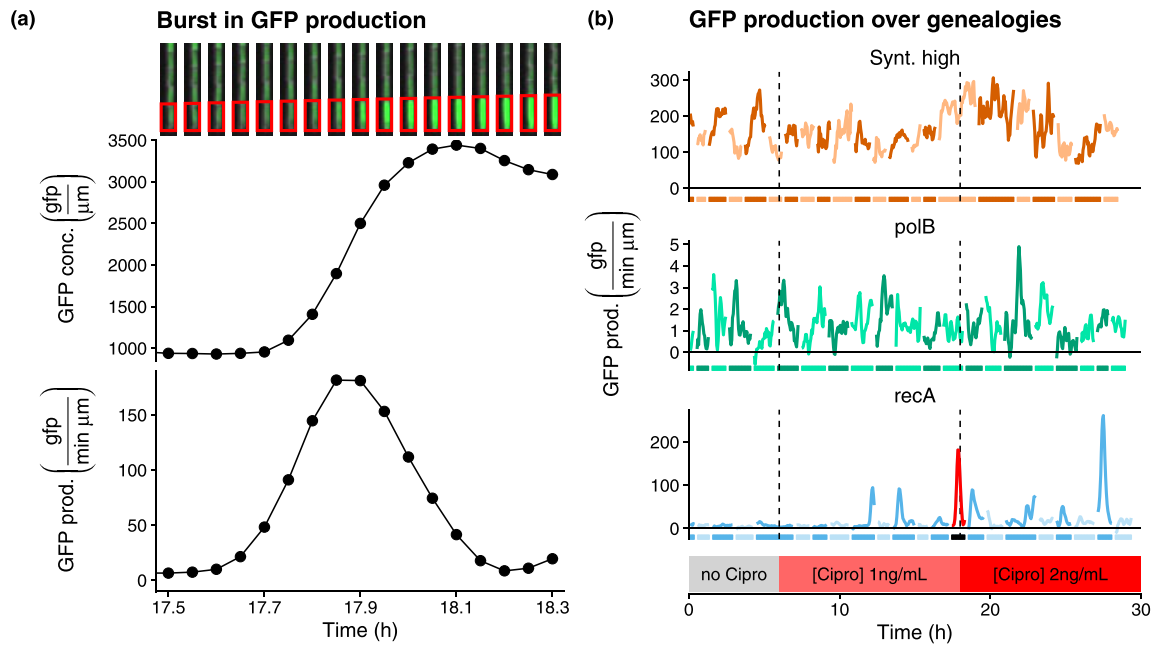


FIG. 2. GFP production over time for single cells. (a) Time-lapse images highlighting a sudden increase in GFP fluorescence for a cell expressing GFP from the *recA* promoter (top, red rectangles), the corresponding GFP concentration (middle) and inferred volumic GFP production $q(t)$ (bottom). The production shows a transient burst from 17.7 to 18.1 h (approximately 20 min). (b) Example of volumic GFP production $q(t)$ along the entire duration of the experiment for three promoters. For each promoter, the production $q(t)$ of only one representative cell genealogy is displayed, and the boundaries between the cells of the genealogy are shown by the rectangles below each trace. The dotted vertical lines separate the three different Cipro concentrations. The red cell in the *recA* trace corresponds to the cell shown in panel (a).

over time, as shown in the top panel of Fig. 2(a). To quantify gene expression from each promoter we define the volumic GFP production rate $q(t)$ by modeling the dynamics of the cells' total GFP fluorescence $G(t)$ as a function of time t using the following equation:

$$\frac{dG}{dt} = q(t)V(t) - \delta_g G(t), \quad (1)$$

where $q(t)$ is the volumic production rate, $V(t)$ the volume of the cell, and the last term represents the loss in fluorescence due to bleaching and GFP protein decay, which occurs at rate δ_g .

Although our analysis does not depend on a specific biophysical interpretation of the volumic production rate $q(t)$, note that if the concentration of active ribosomes and the average translation rate of all mRNAs in the cell are both roughly constant across the cell cycle, then $q(t)$ is proportional to the fraction of GFP mRNAs among all mRNAs in the cell at time t . Since mRNAs turn over on a timescale of just a few minutes [30], the production rate $q(t)$ thus corresponds to the recent transcriptional activity of the promoter. As detailed in the Appendix B, we estimate $q(t)$ from measurements of total fluorescence in short sliding windows, assuming that $q(t)$ changes smoothly within these windows.

We observed that the traces of volumic production $q(t)$ across time for LexA target promoters exhibit relatively short bursts. As an example, Fig. 2(a) shows the raw fluorescence images, GFP concentration, and volumic production in a single cell carrying the GFP reporter of the *recA* promoter during a 45 min time segment, illustrating the occurrence

of a production burst. Examples of time traces of GFP volumic production $q(t)$ over the entire experiment are shown in Fig. 2(b) for three representative lineages of cells expressing GFP from different promoters. The stochasticity of gene expression is underscored by the substantial fluctuations in volumic production, i.e., even the constitutively expressed promoter [top row of Fig. 2(b)] exhibits close to threefold variation in volumic production $q(t)$. However, because GFP concentration is determined by the integral of production on the timescale of dilution, fluctuations in GFP concentration are much smaller than those of the production rate. Most importantly, however, there is a clear difference between the time traces of $q(t)$ for the LexA targets and that of the constitutively expressed promoter. Whereas the production of the synthetic high promoter fluctuates in a manner resembling a random walk around a mean value, the LexA target promoters *recA* and *polB* are characterized by a low background production interspersed by production bursts, which are especially clear for the *recA* promoter.

C. Characterization of the expression bursts

Although the expression bursts are very clear for some promoters, e.g., *recA*, for others such as *polB*, it is more challenging to distinguish the bursts in production from the “background expression.” To more rigorously characterize and quantify the production bursts, we calculated the distribution of volumic production rate q across all cell lineages for each promoter and observed that, in contrast to the constitutive promoters, all LexA targets exhibit exponential tails in the

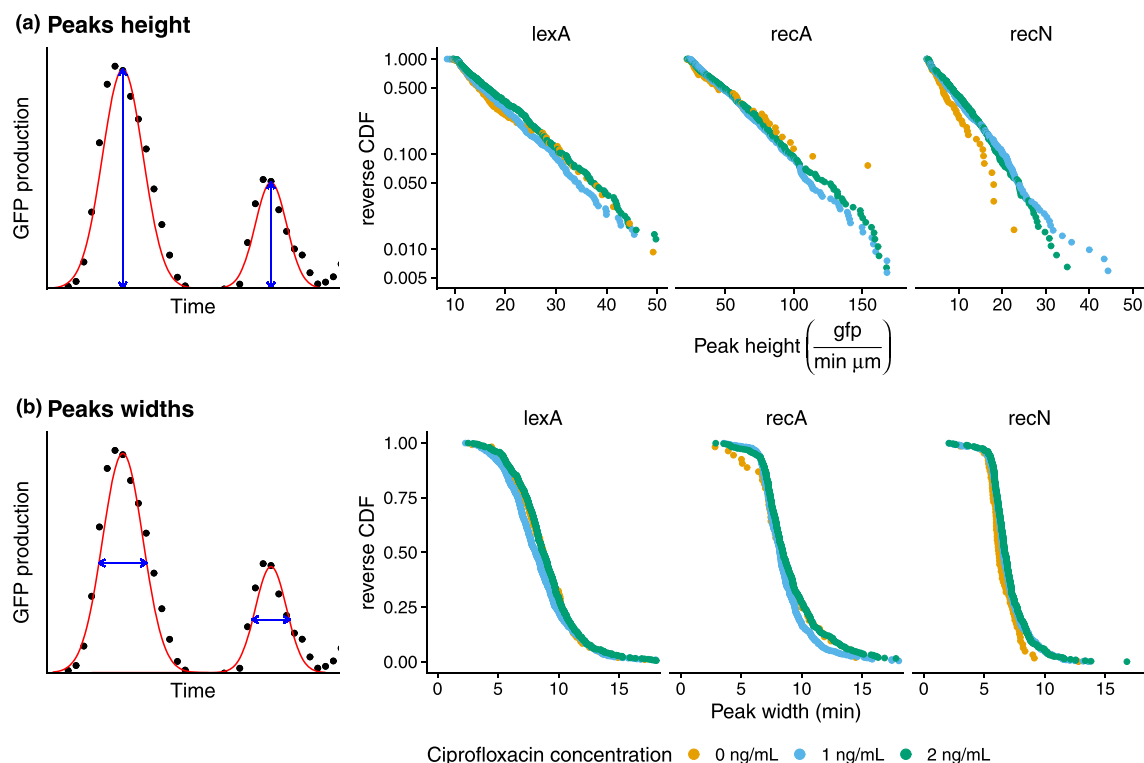


FIG. 3. Quantitative characteristics of the bursts in volumic GFP production. (a) Bursts in volumic production are fitted to Gaussian shaped peaks to estimate their heights (top left panel). The other panels show reverse cumulative distributions of the peak heights for three target promoters (different panels) and for the three Cipro concentrations (colors, see legend at the bottom). Note that the vertical axes are shown on logarithmic scales. (b) Reverse cumulative distributions of peak widths for the same three promoters and Cipro concentrations. Note that peak heights are exponentially distributed and independent of the Cipro concentration, but with a different mean for each promoter. Peak durations have the same distribution across all promoters and Cipro concentrations.

distribution of q (see Fig. 12 of Appendix B). In addition, we further verified that q values in these tails correspond to peaks in the time traces of volumic production. As detailed in the Appendix C, we define bursts in production as local maxima in the time traces of volumic production $q(t)$, whose heights lie within the exponential tail of the distribution of q . In addition, we estimate the height and width of each production peak by fitting the trace $q(t)$ around the local maximum to a Gaussian function. In particular, we define the width of the peak as the standard-deviation of the fitted Gaussian.

Figure 3 shows the distributions of the heights and widths of production peaks that we find for different promoters and at different levels of Cipro. For clarity, we here only show the distributions for *lexA*, *recA*, and *recN* and show analogous results for *polB* and *ftsK* in Figs. 14 and 15 of Appendix C. These figures additionally show that the peak statistics are insensitive to the cutoff used to define the peaks.

First, we observed that the heights of the production peaks are almost perfectly exponentially distributed and independent of Cipro concentration for each promoter [Fig. 3(a)]. That is, each promoter has its own exponential distribution of peak heights, with a given average peak height that is unchanged by changes in the Cipro concentration. Second, we observed that the distributions of peak durations are not only independent of Cipro concentration, but also virtually identical for all three promoters, with a median duration of around 8 min [Fig. 3(b)].

Note that, since the peak duration is defined as the standard-deviation of the fitted Gaussian, the entire duration from the start to the end of the production burst is about 20–30 min.

Finally, to estimate the relative frequencies of bursts across promoters and Cipro concentrations, we extend the exponential tails of the peak frequency as a function of the height cutoff back to zero, and in this way estimate an effective “pseudofrequency” of each promoter at each Cipro concentration (see Appendix C 4). We find that these pseudofrequencies increase with the concentration of Cipro in a similar although not strictly identical manner across promoters (Fig. 4).

In summary, we find that all target promoters exhibit production bursts which become more frequent as the Cipro concentration increases, but whose durations are identical for all promoters, and whose heights depend on the promoter, but are independent of Cipro concentration. These quantitative observations put strong constraints on the possible biophysical mechanisms underlying these production bursts, as we explore in the next section.

D. A simple model of stochastic single-cell gene expression

Our observations clearly show that the gene expression dynamics are stochastic, i.e., with different cells exhibiting bursts at different points in time and with varying frequencies across cell lineages. To explore what single cell regulatory

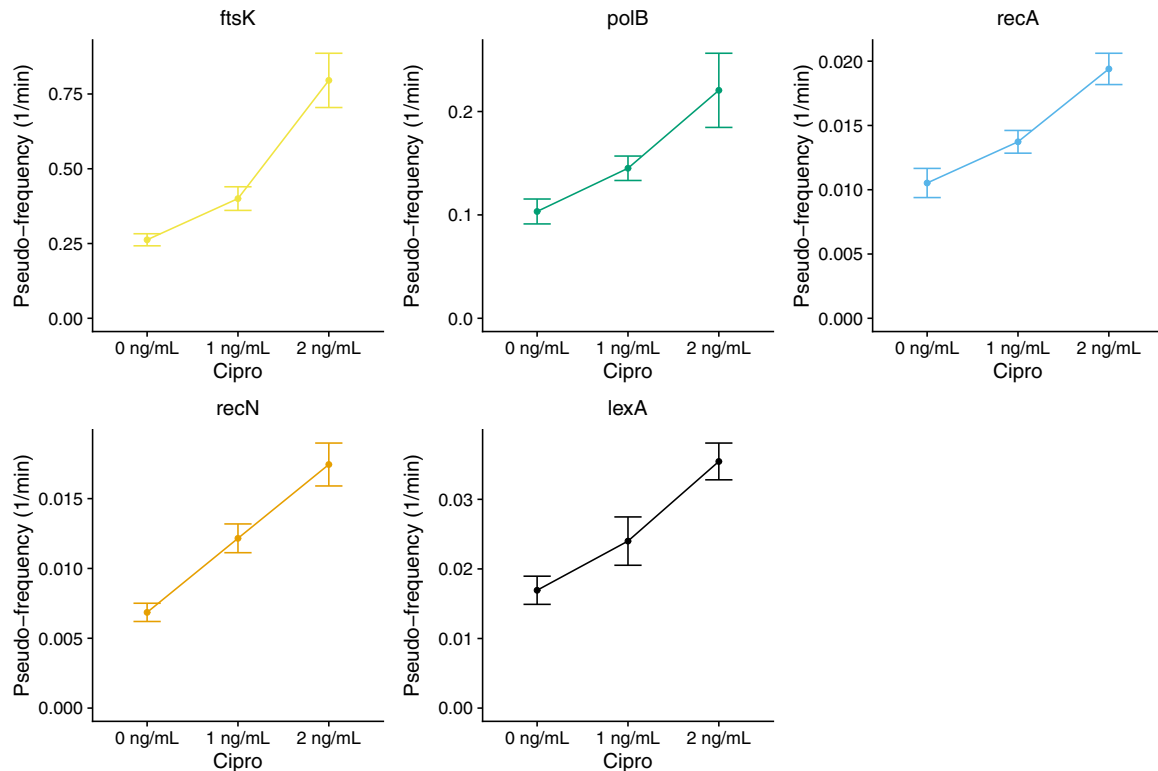


FIG. 4. Relative frequencies of the bursts in GFP production as a function of Cipro concentration across LexA target promoters. Each panel shows the estimated pseudofrequency of production bursts (see text) as a function of Cipro concentration for one LexA target promoter (indicated at the top). The error bars represent standard-errors of the pseudo frequencies across independent growth channels. The burst frequency increases monotonically with Cipro concentration for all promoters but rises more steeply for some promoters (i.e., recN) than for others (i.e., polB).

mechanisms can explain our observations we will thus use a stochastic modeling framework that explicitly models the expression dynamics at promoters in single cells, including individual binding and unbinding events of LexA to the promoter, transcription events, translation events, and so on. In particular, our model follows a single cell lineage through time including the exponential growth of the cell and division events.

We model the cell growth and division as deterministic. Following the observation that added length between birth and division is approximately independent of growth-rate and cell size at birth [31,32] we model the cell cycle by, after each division, drawing a random added length ΔL from a Gaussian distribution with mean and variance matching those observed in the experimental data. We then let the cell grow exponentially until a length ΔL has been added, upon which it divides again. For simplicity, we ignore growth-rate fluctuations and let all cells grow at the same rate, given by the median observed growth rate in the data (doubling roughly once every 80 min). Our model also ignores small cell size asymmetries at cell division, assuming the mother cells divide exactly in half at each cell division. Notably, since the growth-rate and cell division fluctuations are small relative to the large stochastic fluctuations in cell-to-cell burst frequency and burst heights, these approximations do not meaningfully affect the predictions of the model. Similarly, we also ignore that growth rates can systematically vary across the cell cycle [33] because these variations are very minor, i.e., con-

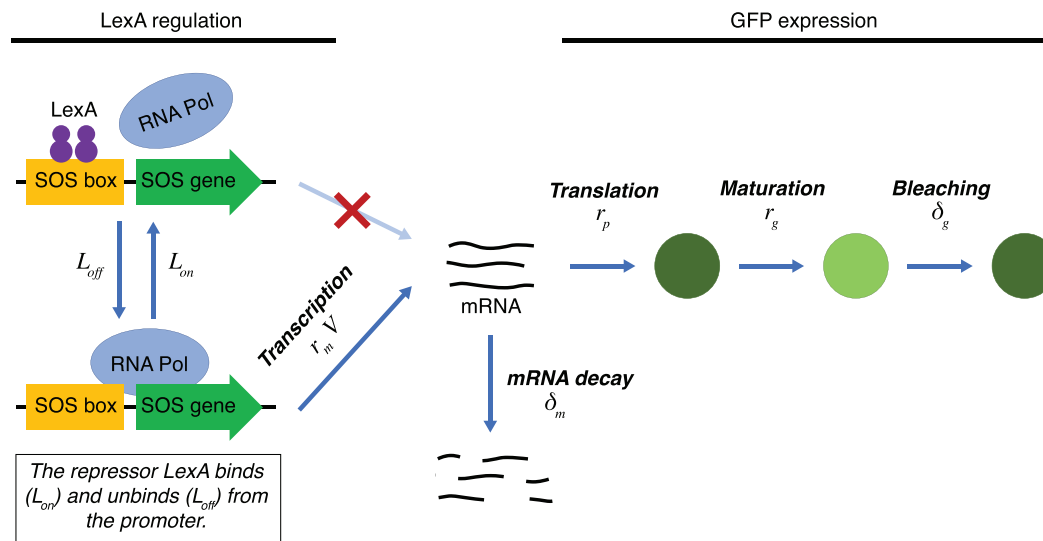
siderably smaller than the cell-to-cell fluctuations in growth rate.

Our model of the gene expression dynamics of a LexA target promoter in a given cell consists of the stochastic reactions shown in Fig. 5(a) (see Appendix E and Table III for a list of the reactions). Notably, most of these reactions involve single molecule events such as the binding of a single TF molecule to the promoter, the transcription of a single mRNA from the promoter, and so on. These reactions are therefore intrinsically stochastic and our model thus calculates the *rates* of these reactions based on the state of the cell (i.e., the probability per unit time for these reactions to occur) and simulates the stochastic dynamics using the standard Gillespie algorithm.

We assume that the promoter can exist in either a LexA-bound or LexA-free state and switches stochastically between these two states. LexA unbinds at a rate L_{off} , which depends on the affinity of the binding site(s) in the promoter, and LexA binds at a rate L_{on} which is proportional to the concentration of free LexA in the cytoplasm.

In general, LexA target promoters will transcribe at a lower rate when bound by LexA than when unbound. We make the idealization that promoters only transcribe when unbound. In the unbound state, transcription events occur at a rate $r_m V$, i.e., proportional to the volume V of the cell. This assumption is in line with the simplest model of gene expression along *E. coli*'s cell cycle, i.e., with molecule numbers of all types, e.g., RNA polymerases, ribosomes, TFs, etcetera, all increasing

(a) Gene expression model



(b) Separation of timescales

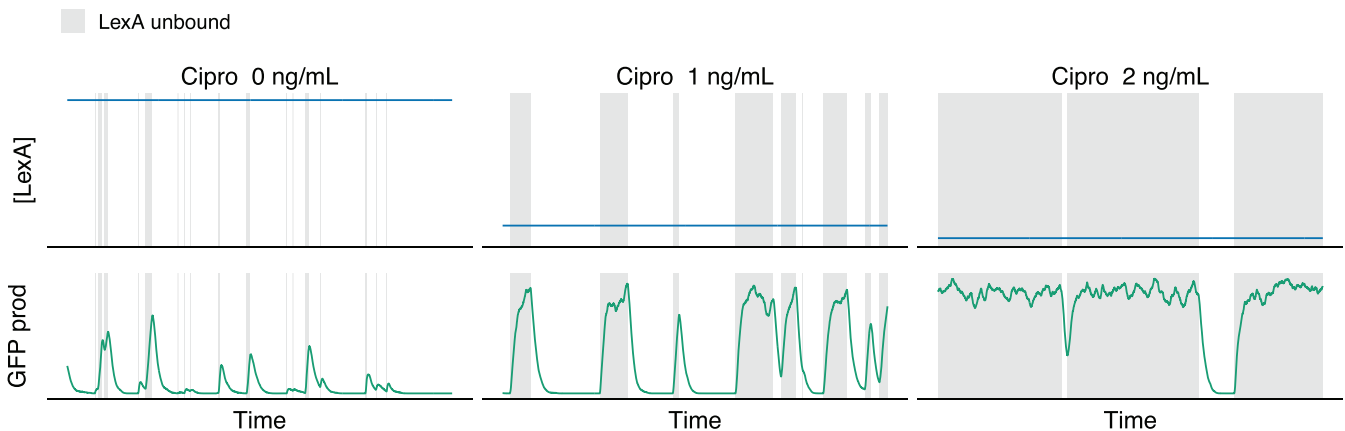


FIG. 5. Gene expression and equilibrium regulation models for the LexA regulatory network. (a) Our gene expression model for a LexA target promoter includes stochastic switches between a transcribing state (unbound) and a repressed state (bound by LexA) as well as stochastic transcription events, translation events, mRNA decay events, GFP maturation, and bleaching/decay, each occurring at a given rate per molecule. (b) Example simulation of the ‘equilibrium’ model where the concentration of LexA is assumed to be effectively constant over time and decreasing with the Cipro concentration (top row). In this model, expression bursts correspond to periods where the promoter is unbound (gray intervals), leading to peaks in volumic production (bottom row). Note that, as the average concentration of LexA decreases, LexA takes longer to rebind, causing production bursts to increase in duration.

in proportion to cell volume such that concentrations remain constant on average [34].

Each mRNA has a probability δ_m per unit time to decay and a probability r_p per unit time to be translated into a GFP molecule. Notably, for realistic parameter settings there are typically only a few mRNA copies per cell, so that translation and decay events are also intrinsically stochastic and cannot be modeled by deterministic mass-action kinetics.

To correctly capture the timescales of the bursts, we take into account the maturation of the GFP molecules. Each GFP produced is initially immature (i.e., nonfluorescent) and has a probability r_g per unit time to mature into a fluorescent protein. Finally, each GFP has a probability δ_g per unit time to decay, which represents the combined effects of photo-

bleaching and protein decay [26]. Note that many of the parameters of this model, such as the mRNA decay rate, the translation rate of the GFP reporter, the GFP maturation rate, and the rate of LexA unbinding from the RecA and consensus binding site are known from the literature, at least approximately. We used this knowledge to set many of the parameter values of the model (see Table I).

The final quantity to specify in this model is the dynamics of LexA concentration in the cell and its dependence on the concentration of Cipro. Below we will first explore a model which follows the popular ‘equilibrium’ assumption that TF binding configurations at promoters can be described by their thermodynamic equilibrium frequencies and show that this leads to predictions that are clearly at odds with our observations.

E. Observed burst characteristics disagree with predictions of “equilibrium” models

As mentioned in the Introduction, one of the assumptions often adopted in modeling gene expression in bacteria is that the binding configurations at promoters are in statistical equilibrium given the TF concentrations in the cell, e.g., Ref. [35]. Indeed, starting from the seminal work of Ackers *et al.* [36], this strategy has been applied in a large number of studies, including systems with relatively complex regulation, e.g., Refs. [8,37–44].

The key assumption of these “equilibrium” models is that the production from each promoter is an average of transcription rates in different binding configurations, which assumes that the timescales of TF binding and unbinding from the promoters, as well as the timescale of transcription initiation events, are fast relative to the timescale of TF concentration changes [10,35].

To investigate the burst characteristics predicted by this equilibrium assumption, we considered the idealized scenario in which, during each time segment at a given Cipro concentration, the promoters are experiencing an effectively constant LexA concentration, which decreases with Cipro levels [Fig. 5(b), top row]. We used our gene expression model to investigate this parameter regime and representative results of simulations of the model are shown in Fig. 5(b). In this regime, the bursts in production correspond to periods of time when LexA is unbound from the promoter. As the concentration of Cipro increases, and the average concentration of LexA decreases, the rate at which LexA rebinds to unbound promoters is lowered, causing the time intervals in which the promoter is free from LexA to increase in duration [Fig. 5(b), top row]. This in turn causes higher production bursts of longer durations [Fig. 5(b), bottom row].

These predictions of the model clearly contradict the experimental observations of Figs. 3 and 4, which show peak heights and durations are independent of Cipro concentration, whereas their frequency increases with Cipro concentration.

One could argue that our assumption of constant LexA levels is too restrictive, and that the equilibrium model allows LexA levels to vary in time and across cells, as long as these variations are slow relative to the rates of LexA binding and unbinding to the promoters. However, unbinding rates of LexA from its binding sites have been measured and can be as low as once every several minutes [18]. Therefore, for the equilibrium assumption to hold, LexA concentration changes must be slow relative to this timescale, and since production bursts last on the order of 10–20 min, this in turn implies LexA concentration must be roughly constant on the timescale of the production bursts. Furthermore, since bursts correspond to periods in which the promoter is unbound, and the rebounding rate is set by the LexA concentration, in the equilibrium model the distribution of burst heights and durations must reflect the distribution of LexA concentrations within a time segment. Therefore, as the LexA levels decrease with increasing Cipro concentration, the equilibrium model necessarily predicts longer and higher bursts, which contradicts the experimental observations. Therefore, it is clear that explaining the experimental observations requires a model in which LexA concentration fluctuations must occur on the same timescale as the observed bursts in production.

F. Production bursts correspond to stochastic transient dips in LexA concentration

A successful model needs to explain why the durations of production bursts are independent of promoter and Cipro concentration, and why peaks heights are exponentially distributed and also independent of Cipro concentration. We start by considering what is known about LexA concentrations in the cell, and the timescales for its binding and unbinding from target sites. The timescale for LexA to unbind from one of its sites ranges from just seconds for weak binding sites to almost 10 min for the consensus site [18]. This already implies that not every LexA unbinding event can generate a production burst, because production bursts are observed much less frequently.

Next is the LexA binding rate in conditions without DNA damage, i.e., when LexA concentration is highest. This can be estimated as follows. Previous studies have reported that it takes a single TF molecule on the order of 3–5 min to find a single binding site on the genome [45,46]. Therefore, the waiting time for an unbound promoter to become bound by LexA is roughly 3–5 min, divided by the number of active LexA repressors. The number of LexA molecules per cell (in absence of DNA damage) has been estimated as 1100–1300 [13,47], resulting in 550–650 dimers [23]. Since the total number of genomic binding sites of LexA is about an order of magnitude less than this [29], the effects of titration are small and the number of free LexA dimers in the cytoplasm is on the same order of magnitude. Therefore, when LexA is at its steady-state level, the timescale for a single unbound binding site to become rebound is predicted to be less than a second. This suggests that in the absence of DNA damage, unbound promoters are rebound so quickly by LexA that it is unlikely for multiple transcription initiation events to occur in between. That is, these previous measurements suggest that production bursts require LexA concentration to decrease substantially.

This raises the question as to what is known about LexA concentration dynamics. It is known that, upon DNA damage events that produce single-stranded DNA, RecA forms filaments on the single-stranded DNA and LexA auto-cleaves itself on these filaments, leading to a decrease in LexA concentration [48,49]. The decay rate of LexA has been extensively studied under UV-induced damage. However, it is little studied for continuous antibiotics exposure [50]. Moreover, it is challenging to interpret the LexA decay observed in bulk population studies, since this likely represents a heterogeneous mixture of LexA decay and recovery across single cells. Notably, *in vitro* studies reported decay rates on the order of minutes [51–53], and these results agree with several *in vivo* studies that also found LexA concentrations decay within minutes [13,54]. Most relevant for our current study, a recent single-cell study in cells subjected to low levels of Cipro (i.e., 0.5 ng/ml) found exponential decay of LexA concentration with a half-life of less than a minute [50]. The available data thus suggest that, upon formation of RecA filaments, LexA levels decay 10–100 fold within a few minutes.

These considerations suggest the following picture of the concentration dynamics within single cells [Fig. 6(a)]. First, the fact that cells show bursts at different times and at

TABLE I. Parameters of the gene expression model. For most parameters an approximate value is known from the literature. Only for the last three parameters in *italic*, a range of possible values needs to be fixed by the requirements of fitting to our observations (see Appendix F).

Rate	Description	Value/Constraint
δ_g	GFP bleaching+decay rate	0.0032 min^{-1} [26]
r_g	GFP maturation rate	$1/8\text{--}1/6 \text{ min}^{-1}$ [55,56]
r_p	Translation initiation rate	20 min^{-1} [57–60]
α	LexA decay rate during DNA damage	1.5 min^{-1} [50]
β	LexA recovery rate after repair	1.5 min^{-1}
L_{ss}	Steady-state LexA binding rate	200 min^{-1}
L_{off}	LexA unbinding rate	0.1 min^{-1} (consensus sequence),
δ_m	mRNA decay rate	3 min^{-1} (recA site) [18,61,62] Between $1/2\text{--}1/6 \text{ min}^{-1}$ [63]
r_r	<i>DNA damage repair rate</i>	$0.5\text{--}1.0 \text{ min}^{-1}$
r_m	<i>Transcription initiation rate per unit volume for the unbound promoter</i>	Fixed by r_r and observed average peak height
r_b	<i>Frequency of DNA damage events</i>	Fixed by observed peak frequency

fluctuating intervals and frequencies strongly suggests that DNA damage events occur stochastically. We thus assume that damage events occur at a probability r_b per unit time, which will depend on Cipro concentration. Whenever a damage event occurs, the LexA concentration starts to decay exponentially at a rate α (which is larger than 1 per minute, see Table I). After the DNA damage is repaired, RecA filaments will disappear, and the concentration of LexA recovers at a rate β (which, to reduce the number of free parameters, we assume equal to α).

We don't know how long DNA damage events take to repair, but repair times are very likely stochastic and fluctuate from event to event. For the mild damage resulting from the low levels of Cipro, we will assume that damage is repaired at a constant rate r_r , leading to an exponential distribution of repair times, which is arguably the simplest assumption that can be made for the distribution of repair times. As we will see below, this simple assumption results in an exponential distribution of peak heights, in agreement with our experimental observations.

Together, these considerations define a dynamic nonequilibrium model of the stochastic gene expression dynamics (detailed in Appendix E) with parameters that are listed in Table I. Notably, this model is “nonequilibrium” not in the sense that detailed balance is broken, but in the sense that transient fluctuations in the concentration of LexA are so fast that promoters do not have time to sample the equilibrium distribution of binding configurations. Therefore, the key assumption of so called “equilibrium” models, i.e., that a promoter's output is the weighted average of transcription rates in each TF binding configuration weighted with the corresponding equilibrium frequency of that binding configuration, fundamentally breaks down. As we will see below, this leads to a much richer variety of responses to the induction of the regulon.

Figure 6(b) shows a representative example of the DNA damage events, LexA concentration, and gene expression dynamics of a LexA target promoter from a simulation of this

dynamic model. Note that because LexA copy numbers per cell are relatively large, we model the LexA dynamics as effectively deterministic, i.e., decreasing exponentially during a damage event, and recovering exponentially afterwards.

Most of the kinetic parameters of this model are known (at least approximately) from previous work (Table I), leaving only three unknown parameters (*italic* parameters in Table I): the rate of DNA damage repair r_r , the volumic rate r_m at which a given target promoter transcribes when unbound, and the frequency of DNA damage events at each Cipro concentration r_b .

We next investigated whether these three remaining parameters of the model can be set to reproduce the observed burst durations, the exponential distributions of burst heights, and the burst frequencies. We find that this is indeed the case, but that this requires that the repair rate r_r lies in a narrow range. First, given that mRNA decay times are in the range 2–6 min and that GFP maturation time is 6–8 min, even an instantaneous burst of mRNA production results in a peak of volumic production q with a duration only a bit less than the observed average peak duration. This implies that the bursts in mRNA production must be very short. Indeed, simulations of our dynamic model show that the distribution of peak durations matches those observed experimentally when DNA damage events are shortlived [Fig. 6(c) and Fig. 20 of Appendix F]. In addition, the observed exponential distribution of peak heights is only reproduced in the simulations for repair rates in the range $0.5 \leq r_r \leq 1.0$ per minute [Fig. 6(d), Fig. 21 of Appendix F]. In Appendix F we provide analytical derivations that explain why peak heights are exponentially distributed in this regime, which we briefly summarize here.

Outside of DNA damage events promoters are almost always bound by LexA. Depending on the strength of the LexA site(s), LexA will stochastically unbind from the promoter up to a few times each minute, but the rate of rebinding of LexA to its site(s) will be so much higher than the transcription rate that no burst in mRNA production can occur.

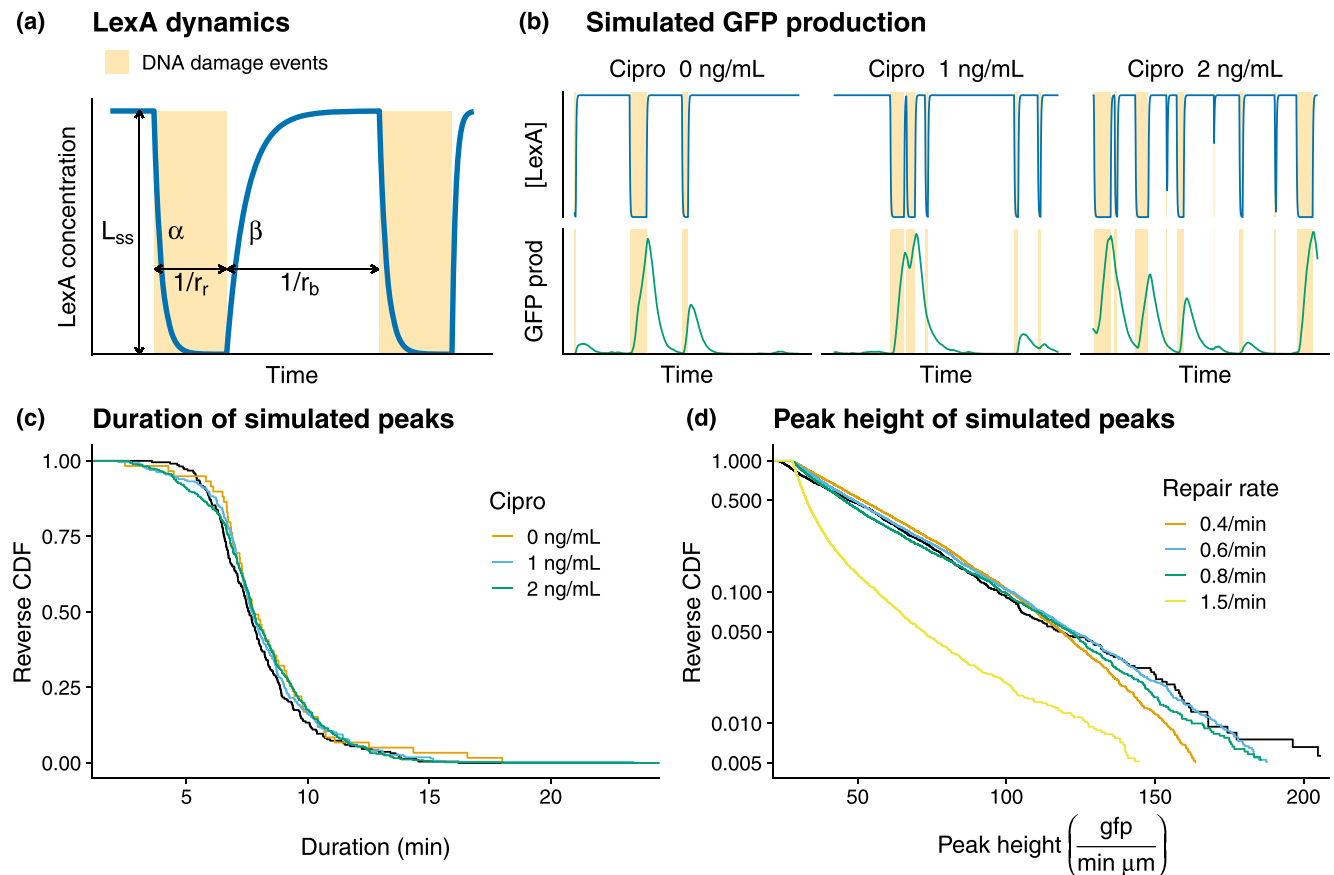


FIG. 6. Dynamic model for the induction of the LexA regulatory network. (a) LexA dynamics in the presence of DNA damage events that leads to the formation of RecA filaments. Without damage (white areas), LexA has a steady-state concentration with binding rate L_{ss} . Upon a DNA damage event, LexA concentration decays exponentially at rate α (yellow areas) until the damage is repaired. We assume damage is repaired at a constant rate r_r , and that upon repair, the LexA concentration recovers at a rate β . Finally, we assume the DNA damage events occur at a rate r_b , which increases with Cipro concentration. (b) Example simulation results of the dynamic model, where the LexA concentration dynamics of panel A is explicitly taken into account (top row). Production bursts occur when LexA unbinds from a promoter during a DNA damage period when the LexA concentration is low (bottom row). We refer to the Appendix E for a detailed description of the simulation algorithm. (c) Reverse cumulative distributions of peak durations for the simulations of the dynamic model (black) and for the experimental data from the *recA* promoter in different conditions (colored lines) show that peak durations match provided damage events are short-lived. In this simulation mRNA decay time was 4 min, GFP maturation time was 7 min and repair rate again $r_r = 0.8 \text{ min}^{-1}$. (d) Reverse cumulative distributions of peak heights of the *recA* promoter for the experimental data (black) and simulations of our model with DNA damage repair rates in the range 0.4–1.5 min^{-1} . Note that when the repair rate is too low (orange) or too high (yellow), the peak height distribution starts deviating from an exponential.

Thus, a transcription burst only occurs when LexA unbinds from the promoter during a time interval when the LexA concentration is so low that the rate of LexA rebinding is smaller than the transcription rate. Because DNA damage is repaired quickly on average, damage events that last long enough for LexA to decay sufficiently may be relatively rare. However, in the subset of damage events that last long enough such that LexA has decayed sufficiently and, in addition, LexA has unbound from the promoter, there is now an exponentially distributed additional amount of time before the damage is repaired. Consequently, the number of mRNAs produced during this time interval will be geometrically distributed, and the fact that these time intervals are generally short (because $r_r \geq 0.5$ per minute) also ensures that the resulting heights of the peaks in q are simply proportional to the number mRNAs produced (Appendix F).

This model thus not only explains why peak durations are independent of the promoter and Cipro concentration, it also explains why peak heights are exponentially distributed, and controlled by the ratio of the transcription initiation rate r_m and the damage repair rate r_r , which is naturally promoter-dependent, but not dependent on Cipro concentrations.

The burst frequency of each promoter will depend on how often DNA damage lasts long enough such that LexA rebinding rates fall below the promoter's transcription rate, and additionally the LexA repressor unbinds from the promoter. This thus depends nontrivially both on the volumic transcription rate of the promoter r_m and on the strength of its LexA binding site(s). Thus, while the frequency generally increases with Cipro concentration, it does so in a promoter-dependent manner. Although we don't know how the rate of DNA damage events increases with Cipro concentration, it is

straightforward to set the rate of DNA damage events r_b such that our dynamic model reproduces the observed distribution of peak frequencies as a function of their cutoff (see Fig. 22 of Appendix F).

Finally, although our simple model ignores several features of the stochastic gene expression dynamics in real cells such as the fluctuations in growth rate and the unequal distribution of cell volume at division, Fig. 23 of Appendix F shows that our simple model also reproduces the observed distributions of GFP concentration across cells and Cipro concentrations for the *recA* promoter with reasonable accuracy.

G. Promoter response functions as a function of LexA binding site strength and DNA damage durations

As explained above, in our nonequilibrium model the response of different LexA target promoters to DNA damage events depends in a nontrivial manner on the strengths of the LexA target sites in the promoters, their volumic production rates when unbound, as well as on the number and durations of the DNA damage events. Here we use simulations of our model to explore how this “promoter response function” depends on these parameters.

Although in our experiments with low doses of Cipro, DNA damage events were short-lived, more generally the durations of the DNA damage events will highly depend on the type of damage, and may well last much longer for some types of damage. We thus first investigated how the promoter output resulting from a single DNA damage event depends on the duration of the event and the strength of the LexA site in the promoter. We set all parameters of our model as in Table I and set the transcription initiation rate to $r_m = 5 \text{ min}^{-1} \mu\text{m}^{-1}$, which approximately corresponds to the inferred r_m for the *RecA* promoter. We then performed simulations of the model under many repetitions of a single DNA damage event of different durations [see Fig. 7(a)], and measured the distributions of the total number of mRNA molecules produced during the damage event as a function of both the duration of the damage event and the strength of LexA binding sites, as quantified by the promoter’s unbinding rate L_{off} . Figure 7(b) shows box-whisker plots of the distribution of promoter output for different L_{off} and damage event durations, normalized to the total output that would be observed if the promoter had been unbound throughout the entire DNA damage event.

The strongest possible LexA binding site has been reported to have an unbinding rate of 0.1 per minute [18] and because it takes on average 10 min for LexA to unbind, promoters with such a LexA site only respond to damage events that last 10 min or more [Fig. 7(b), top left panel]. In addition, for damage events that last 10–20 min, the response is highly variable, i.e., for promoters with such a strong LexA site the gene expression response is very noisy.

Many native LexA target promoters have binding sites with unbinding rates in the range of 1–10 per minute. For such promoters the output increases monotonically, and with less variability, over the entire range of damage durations from 1 to 20 min [Fig. 7(b), top right and bottom left panels]. The weaker the binding site, the more sensitive the promoter becomes to short damage events. However, for weaker sites the output also saturates more quickly as a function of the

damage duration. Thus, the strength of a promoter’s LexA binding site determines over which range of DNA damage durations the promoter goes from not responding to a saturating response. In addition, we also see that the stronger the LexA site, the more variable the responses are across damage events. Finally, when the binding site becomes very weak, the promoter starts to become active even in the absence of any damage events, and the range of the response to DNA damage becomes strongly attenuated [Fig. 7(b), bottom right panel].

We note that, if the promoter response could be described by an equilibrium model, its output should only depend on the total amount of time that LexA concentration is low. Therefore, the nonequilibrium behavior of the LexA regulon can be illustrated by comparing promoter responses to one long DNA damage event versus multiple short events of the same total duration. We performed simulations of the dynamic model for a period of 35 min in which n equally spaced DNA damage events occurred that together have a total duration of 17.5 min [Fig. 8(a)] and measured the distribution of the total number of mRNA molecules produced as a function of the number of events n and the strength of the LexA binding site [Fig. 8(b)].

We see that the total output not only depends on the total duration of DNA damage, but is highly dependent on whether the damage is spread over multiple short or fewer longer events, confirming the intrinsically nonequilibrium nature of the promoter response. The promoter response generally decreases with the number of events. For the promoter with the strongest LexA site, there is only a (highly variable) response for the longest events [Fig. 8(b), top left panel]. For unbinding rates in the regime of the sites in many LexA target promoters, i.e., from 1–10 per minute, the response decreases systematically with the number of damage events and disappears when the events become short relative to the average time for LexA to unbind [Fig. 8(b), top right and bottom left panels]. That is, the unbinding rate of the LexA site sets a lower bound on the duration of the DNA damage events that are detected by the promoter.

The results also illustrate that the dynamic range in promoter response as the DNA damage varies from $n = 1$ to $n = 13$ events depends strongly on the unbinding rate of the promoter. In particular, the dynamic range is largest for the promoter with unbinding rate 10 per minute because it is able to detect even the shortest damage events, while still not being saturated by the longest event. This illustrates that the unbinding rate of the promoter sets the range of damage event durations over which its response varies. Finally, when the unbinding rate becomes so high that it is on the order of the LexA binding rate, the variation in response becomes highly attenuated because the promoter is active even in the absence of DNA damage [Fig. 8(b), bottom right panel]. This illustrates that the LexA concentration in the absence of DNA damage sets a lower bound on the durations of damage events that can be detected.

In summary, the output from a LexA target promoter depends in a nonlinear manner on the strength of its LexA binding site(s) and the durations and numbers of DNA damage events. Consequently, the expression levels of different LexA target promoters effectively “read out” different statistical properties of the DNA damage events in the recent history of the cell.

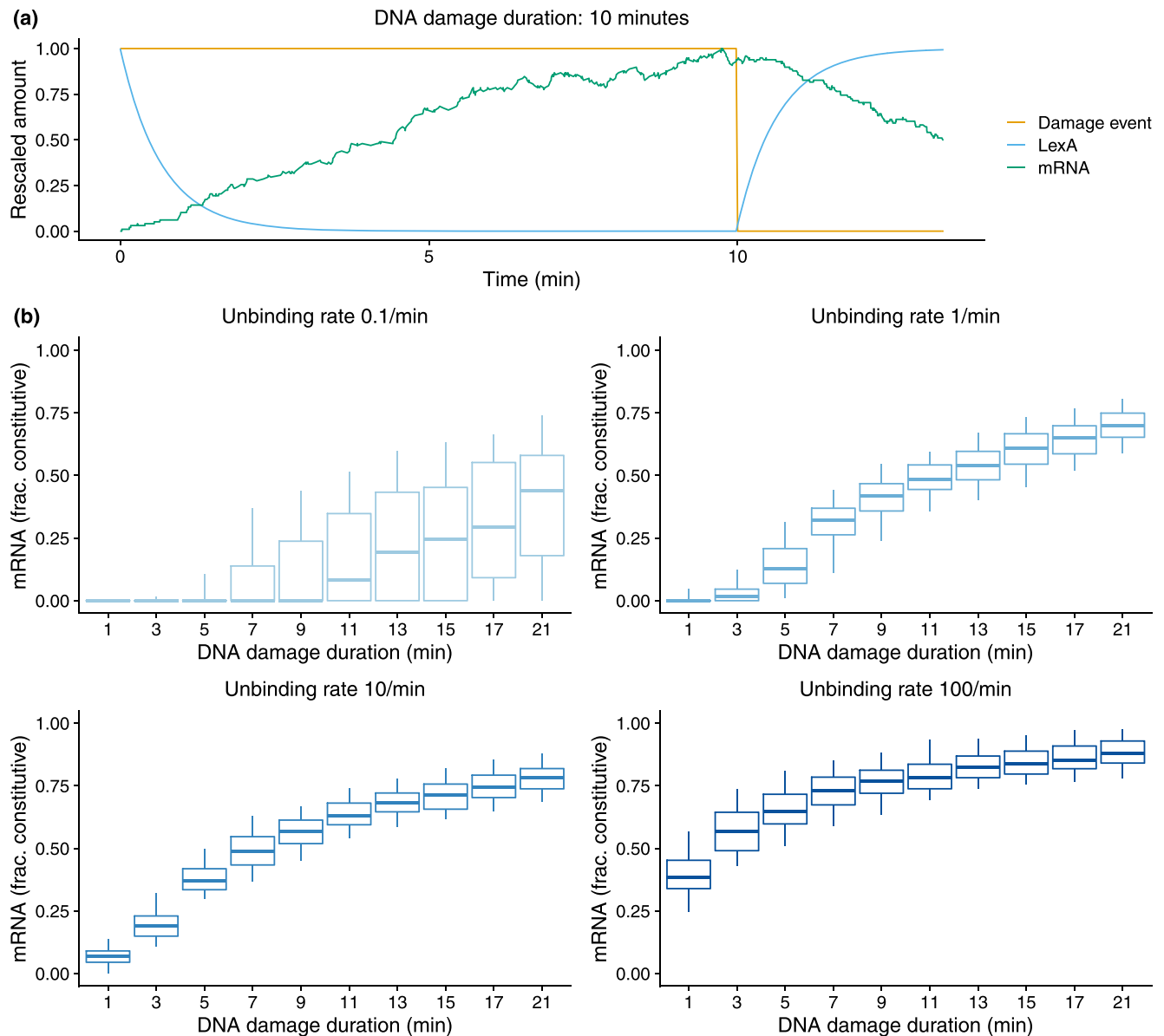


FIG. 7. Response of a LexA target promoter to single DNA damage events of different durations. (a) Example dynamics of DNA damage (red), LexA concentration (green), and mRNA level (blue) for a DNA damage event that lasts 10 min. All quantities are rescaled between 0 and 1 for ease of visualization. (b) Distributions of the amount of mRNA produced (as fraction of the mRNA produced by the fully unbound promoter) as a function of the duration of the DNA damage event (horizontal axis) for LexA target promoters that have different rates of LexA unbinding from 0.1 to 100 per minute (panels). The box-whisker symbols show the 5th, 25th, 50th, 75th, and 95th percentile of the amount of mRNA produced across replicate simulations of DNA damage events of the same duration. We see that unbinding rates in the range 1–10 per minute, which correspond to those observed for native LexA sites, give the most sensitive and reproducible response for damage events that last 1–20 min.

III. DISCUSSION

Due to unavoidable thermal noise, gene expression is inherently stochastic at the single-cell level [5] and also the effects of TFs on the expression of their target genes are inherently stochastic, leading to propagation of noise from regulators to their targets [4]. Indeed, recent genome-wide studies have shown that, in *E. coli*, noise propagation drives highly condition-dependent patterns of gene expression noise [6,7]. However, there is currently little quantitative understanding of how the actions of TFs determine expression responses of different target promoters in single cells.

To study how the induction of a single TF affects the expression dynamics of its target genes in single cells, we here made use of recent technical advances that use fluorescence time-lapse microscopy in combination with microfluidics to allow tracking of cell growth and gene expression in many lineages of single cells with high resolution and quantitative accuracy [26,27]. Furthermore, we chose the LexA regulon in *E. coli* as object of our study because it combines several attractive features that facilitate the development of realistic quantitative models of its stochastic single-cell dynamics, i.e., (1) it can easily be induced to different levels in the laboratory

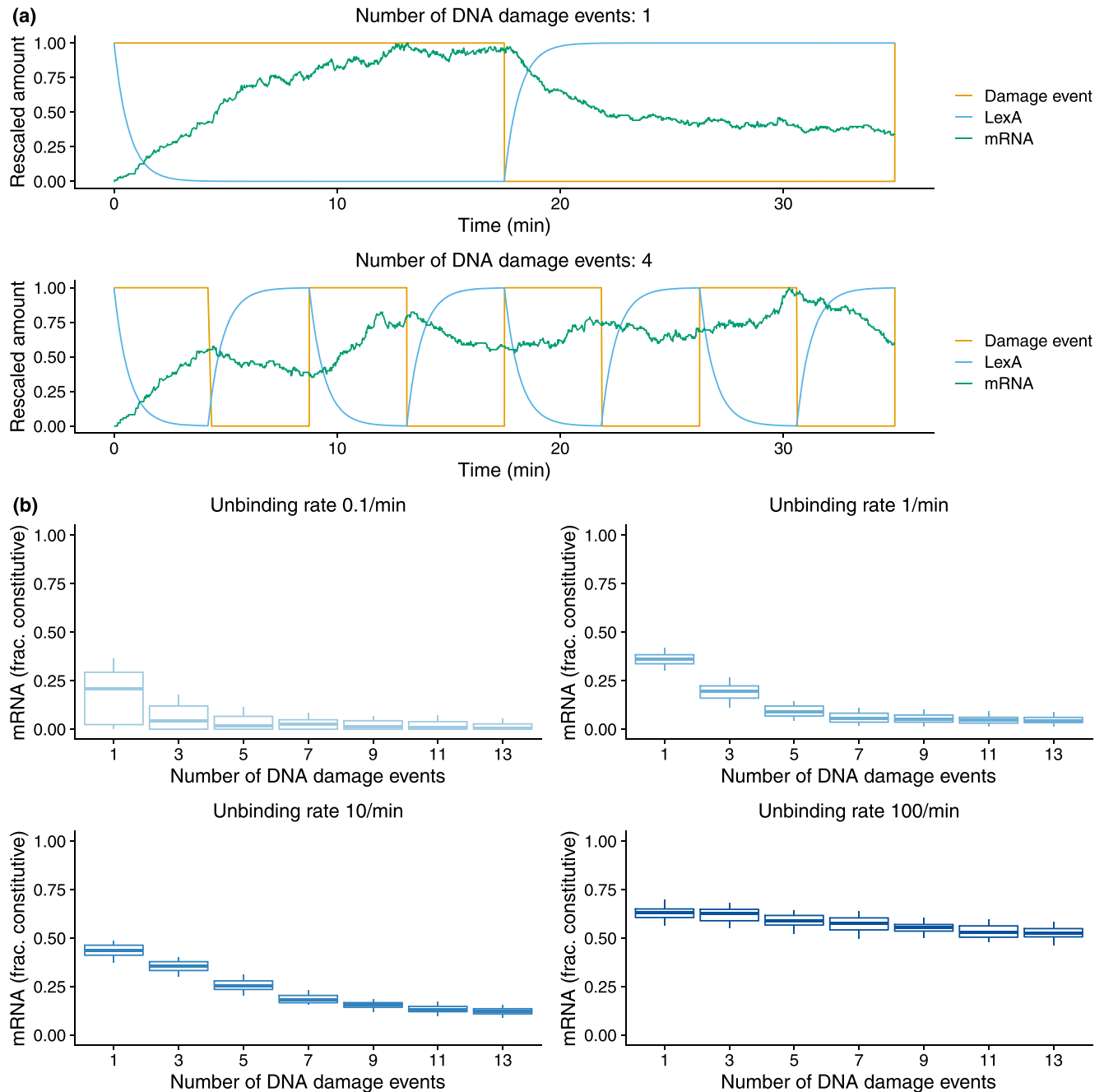


FIG. 8. Response of LexA target promoters to different numbers of DNA damage events with the same total duration. (a) Example dynamics of DNA damage (red), LexA concentration (green), and mRNA number (blue) for either a single damage event lasting 17.5 min (top), or four damage events lasting $17.5/4 = 4.375$ min each. The quantities are rescaled between 0 and 1 for representation purposes. (b) Total amount of mRNA produced (as fraction of the mRNA produced by the fully unbound promoter) as a function of the number of damage events (horizontal axes) for different LexA unbinding rates ranging from 0.1–100 per minute (different panels). The box-whisker symbols shows the 5th, 25th, 50 (line), 75, and 95th percentile of the mRNA produced across replicate simulations. Note that, since the sum of the duration of the DNA damage events is kept constant, the dependence on the number of events shows that the output from the promoter depends nonlinearly on the duration of DNA damage events.

using low doses of antibiotics, (2) it has several different target promoters that are all known to be only regulated by LexA, and (3) much is already known about the induction mechanism of the LexA regulon as well as many of its kinetic parameters. In particular, the LexA regulon responds to DNA damage and we monitored the single-cell responses

of a collection of target promoters to mild DNA damage using different levels of Cipro.

We found that LexA target promoters express in bursts that exhibit very particular quantitative characteristics. While the frequencies of expression bursts systematically increase with Cipro level, their heights and their durations are

independent of Cipro level. In addition, the heights of expression peaks are exponentially distributed for all promoters and peak durations have narrow distributions that are identical for all promoters. The observation that induction of the *lexA* regulon occurs through increasing frequency of expression bursts is reminiscent of previous observations, mostly in eukaryotes, where induction of gene regulatory circuits was found to occur through modulation of the frequency of pulses in gene expression [64]. However, the mechanism that we identified here is arguably more basic than those causing pulsatile behavior in the eukaryotic systems described in Ref. [64].

In particular, we showed that our observations are explained by a simple model in which stochastic DNA damage events cause short transient dips in the concentration of LexA and that expression bursts occur when LexA unbinds from target promoters during these concentration dips. Crucially, because unbinding of LexA from its target sites (and its subsequent rebinding) occurs on the same timescale as the duration of the LexA concentration dips, the output from each promoter is a complex function of the strength of its *lexA* binding site(s), its transcription rates when the promoter is either bound or unbound by LexA, and the statistics of the durations of DNA damage events (Figs. 7 and 8).

This is in contrast to popular “equilibrium” models which assume that TF binding and unbinding dynamics is sufficiently fast so that the transcriptional output from a promoter can be calculated by averaging the transcription rate over all possible TF binding configurations, each weighted with their thermodynamic equilibrium probability of occurrence [8–10]. Notably, in such equilibrium models the output of a target promoter is a relatively simple logistic function of TF concentration that is characterized by the maximum fold change and the TF concentration at half-maximum. Consequently, under an equilibrium model there is relatively little variation in the possible responses that different target promoters can give to a change in the concentration of the TF that regulates them.

In contrast, we find that our observations are explained by transient dips in LexA concentration that take place on the same timescale as individual TF binding and unbinding events. Consequently, promoters do not sample the equilibrium distribution of TF binding configurations and this “nonequilibrium” behavior causes different target promoters to effectively read out different statistical properties of the DNA damage events in the recent history of the cell.

In this regard it is important to note that our model makes various simplifications. For example, the model assumes that each LexA target promoter can only take on two states, i.e., bound and unbound, and that it only transcribes in the unbound state. However, many native LexA target promoters in *E. coli* have multiple LexA binding sites, so that there are multiple binding states that may all have different rates of transcription. Therefore, in reality the response of each promoter is an even more complex function of the durations and numbers of DNA damage events than in our simple model.

Our data suggest that, in our conditions with only low levels of DNA damaging agent, there are only isolated DNA damage events and LexA levels recover in between. In contrast, when damage events become more frequent or multiple events occur at once, as may occur under UV radiation, the depletion of LexA protein will start feeding back positively

on itself and the concentration dynamics of LexA will become much more complex. Although modeling such scenarios are beyond the scope of this work, we believe that the bursting behavior that we observe here, and that is explained by our dynamic model, may also help explain previous observations that under UV damage different LexA target promoters appear to respond at different times [14,18,19,65–67]. This is an important avenue for future study.

Another interesting question is to what extent natural selection might have shaped the configurations and strengths of LexA binding sites to fine-tune the response characteristics of different promoters, i.e., how much induction each promoter should exhibit in response to different types of DNA damage, and whether there are any evolutionary trade-offs in this respect. Our results show that the strength of the LexA binding site sets a lower bound on the length of DNA damage events that can be detected, because LexA is unlikely to unbind during events that are short relative to the unbinding rate. Consequently, weaker sites are much more sensitive to short events. However, this sensitivity requires that, in the absence of DNA damage, the promoters with weak sites are still kept sufficiently repressed, and this in turn requires high expression levels of LexA. Thus, cells ultimately “pay” for the sensitivity in promoter responses by requiring high levels of LexA expression. This suggests that the LexA expression level may reflect a trade-off between cost of expression, and the advantage of allowing different promoters to have distinct responses to different types of DNA damage.

Finally, a key question is whether the nonequilibrium behavior of the LexA regulon is an exception among bacterial regulons, or whether it is to be expected that many regulons exhibit such nonequilibrium behavior. Notably, the nonequilibrium dynamics arises when concentrations of active TFs vary on the same timescale as individual TF binding and unbinding events. In the case of LexA, this occurred because of its fast degradation during DNA damage events and this may be specific to this particular TF. However, many other TFs change between active and inactive configurations either by binding small signaling molecules, e.g., LacI binding allolactose, or by phosphorylation, e.g., as in two-component systems. Since both the concentrations of small signaling molecules and the activities of kinases can easily change on very short timescales, it is actually very likely that many bacterial regulatory systems will exhibit fluctuations in active TF levels on fast timescales. That is, the nonequilibrium behavior that we identified here for the LexA regulon likely applies to many regulatory systems in bacteria and may have been exploited by natural selection to tune the regulatory responses of different targets of the same TF across many regulatory circuits.

IV. MATERIALS AND METHODS

A. Strains and transcriptional reporters

We measured fluorescence in single cells for a set of *E. coli* MG1655 strains that each carry a transcriptional reporter inserted upstream of a gene for the fluorescent protein GFP-mut2 and expressed from a low copy-number plasmid [25]. All the sequences were verified by Sanger sequencing. We chose reporters corresponding to promoters that are known

to be regulated by LexA and currently believed to not be targeted by any other TF [29]: *ftsK*, *lexA*, *polB*, *recA*, *recN*, *ruvA*, *umuD*, and *uvrD*. In addition, as a control we also measured fluorescence from analogous reporters of two synthetic promoters that were obtained by screening a large library of random sequence fragments for driving expression at either the median or at 97.5th percentile of all native *E. coli* promoters [6]. Throughout the paper, we refer to these two synthetic promoters as synthetic medium and synthetic high.

B. Time-lapse microscopy

Mother machine experiments were performed over 30 h as described in Ref. [26], using M9 + 0.4% glucose, and a pressure controller for flow control (OB1 mk3, Elveflow). One phase contrast and one GFP fluorescence image (Lumencor Spectra X, Cyan LED at 17% with ND4 filter, 2000 ms exposure) were acquired over multiple positions every 3 min. To study three increasing concentrations of Ciprofloxacin (Sigma Aldrich) consecutively, we used two media inputs, without Cipro and with 2ng/ml respectively, and set the flow rates to expose the bacteria to the following ratio of those inputs: 100/0 (first 6 h, no Cipro), 50/50 (from 6 to 18 h, 1 ng/ml), 0/100 (from 18 to 30 h: 2 ng/ml). We refer to Ref. [26] for a detailed description of the dual input mother machine design that allows accurate dynamic control of the growth media that flow to the growth channels. To avoid perturbing the response to Cipro during the experiment, the selection antibiotic for the reporter plasmid (50 µg/ml of kanamycin) was supplemented during the overnight preculture only. We note that plasmid loss during the experiment would lead to a gradual decrease of the fluorescence level in the corresponding cell due to cytoplasm dilution, eventually producing nonfluorescent cells. Remarkably, we do not observe any cell becoming non fluorescent during the 30 h experiments, which indicates a very robust partitioning mechanism for the pSC101 origin. All experiments were performed at 37 °C.

C. Data preprocessing

To measure cell sizes and their gene expression, we analyzed the images using the mother machine analyzer (MoMA) software as described in Ref. [26], which provides accurate segmentation and tracking of the cells.

From the phase contrast imaging data we obtain estimates of the cell length at each time point and from the fluorescence data we obtain estimates of the total fluorescence using a method described previously [26]. Briefly, the fluorescence signal across the channel shows a Cauchy-shaped peak that decays to background fluorescence between the channels, and the amplitude of this peak is proportional to the total number of GFP molecules in the cell. We previously estimated the conversion factor between the fluorescence peak amplitude and GFP molecule number from the fluctuations in the distribution of fluorescence at division [26] and we use this to convert fluorescence intensities into estimated GFP molecule numbers.

D. Normalization of GFP expression

We find that the estimated number of GFP molecules generally correlates strongly with the size of the cell (see Fig. 9

of Appendix A), especially for the constitutive synthetic promoters. We thus decided to measure gene expression as GFP concentration, which we define as estimated total number of GFP molecules divided by estimated cell length (which is proportional to cell volume since cells generally keep their width constant in these growth conditions).

Since GFP concentration changes smoothly in time, measurements of GFP concentration from consecutive time points of the same cell are highly correlated. Therefore, to calculate distributions of GFP concentrations across the “population” of cells, we use averages over time segments of length roughly equal to the correlation time of GFP concentration fluctuations.

As explained in Sec. A 2 of the Appendix, we observed some day-to-day variability in the absolute fluorescence levels of the same constitutive synthetic promoter, which are likely due to subtle uncontrolled variations in the growth-conditions across days (see Fig. 10 of Appendix A). In contrast, we find that relative expression levels are highly reproducible across days. To compare results from different days we thus normalized expression levels from different days by the average expression of the synthetic high promoter measured on that day (see Appendix A 2 and Fig. 10 of Appendix A).

Finally, we noticed that due to the effects of the illumination, cell behavior transiently adapts during the first 1–2 h of the experiment, and we therefore discarded all measurements taken during the first 2 h (Fig. 11 of Appendix A and Appendix A 3).

A detailed overview of the number of cells observed per promoter and medium is reported in Table II.

E. Estimating volumic GFP production rate

We observed that, in general, the rate of GFP production is proportional to cell volume V , which is consistent with an approximately constant concentration of ribosomes in the cells leading to an overall production of proteins proportional to cell volume. As in previous works from other labs, e.g., Refs. [19,24], we thus write the time-dependent total production rate of GFP as a product of cell size $V(t)$ and a volumic production rate $q(t)$, which we use to quantify the activity of the promoter. Further, the total GFP fluorescence of a cell decays at a constant rate β due to the combined effects of bleaching and dilution. We thus obtain the following differential equation for the total GFP fluorescence $G(t)$ of an exponentially growing cell,

$$\frac{dG}{dt} = q(t)V(t) - \beta G(t) = q(t)V_0 e^{\lambda t} - \beta G(t), \quad (2)$$

where in the second equation we have assumed that the volume $V(t)$ grows exponentially at a rate λ starting from a volume V_0 at the beginning of the cell cycle. We have previously used control experiments to measure β [26] and, as detailed in Appendix B, we combine estimates of the instantaneous growth-rate λ from the phase contrast measurements with measurements of the total fluorescence $G(t)$ to estimate the instantaneous production rate $q(t)$ during short time windows using the above equation. In particular, to obtain a local estimate for the volumic production rate $q(t)$ at time t , we solve the above differential equation for a time window

TABLE II. Overview of the time-lapse microscopy data in this study. The table lists, for each promoter and separate experiment performed with that promoter, the number of independent full cell cycles that were observed during the three time segments with different Cipro concentrations.

Promoter	Experiment	Cipro 0 ng/ml	Cipro 1 ng/ml	Cipro 2 ng/ml
Synt. high	Experiment 1	195	410	331
Synt. high	Experiment 2	395	871	492
Synt. high	Experiment 3	587	1304	995
Synt. high	Experiment 4	536	1118	870
Synt. high	Experiment 5	261	600	499
Synt. high	Experiment 6	101	274	216
Synt. medium	Experiment 1	81	219	149
ftsK	Experiment 3	527	1197	1007
lexA	Experiment 5	725	1708	1448
polB	Experiment 3	156	349	285
polB	Experiment 5	350	743	655
recA	Experiment 1	421	994	766
recA	Experiment 4	836	1942	1541
recN	Experiment 4	778	1811	1473
ruvA	Experiment 6	217	488	448
umuD	Experiment 2	205	378	285
uvrD	Experiment 6	101	235	203

centered on t spanning five acquisition time points (that is, covering 15 min) and estimate the local growth rate using a window of 9 time points centered on the same time point (since, empirically, we find that growth rate varies relatively little over this 27 min timescale). We checked that reducing the width of these windows has no significant effect on the statistics of peaks in $q(t)$, apart from slightly reducing the peak widths.

F. Peak identification

The GFP production traces of several LexA target promoters show clear bursts, i.e., relatively short-lived peaks in volumic production $q(t)$. These peaks in volumic production are characterized by exponential tails in the distributions of q (see Fig. 12 of Appendix B). To characterize these bursts, we defined production peaks as any contiguous segment of the volumic GFP production $q(t)$ above a given cutoff q_c , and we fit $q(t)$ in each such segment to a Gaussian curve from which we measure the height and duration of the peak. In Appendix C we give a detailed description of our peak-annotation algorithm, and show that the statistics of the peaks are insensitive to the precise value of the cutoff used (Figs. 13–16 in Appendices B and C).

ACKNOWLEDGMENTS

We thank members of the van Nimwegen group for useful discussions and feedback on this work. This work was supported by Grants No. 31003A_159673 and No. 310030_184937 of the Swiss National Science foundation to E.v.N.

DATA AVAILABILITY

The data that support the findings of this article are openly available [68], embargo periods may apply.

APPENDIX A: DATA ANALYSIS

1. Number of GFP molecules versus GFP concentration

As cells grow exponentially to approximately double their size over a cell cycle, the total number of GFP molecules in the cell (as estimated from the fluorescence signal) typically grows in proportion to cell size, while GFP concentration remains roughly constant (see Fig. 9 of Appendix A). In our analyses, we thus decided to focus on the dynamics of GFP concentration (GFP molecules divided by estimated cell length) and how this concentration changes due to fluctuations in the amount of GFP produced per unit time and volume of the cell.

2. Day-to-day variation in absolute expression levels

We noticed that there were clearly measurable differences in GFP levels from experiments done on different days, even for the reporters with constitutive synthetic promoters. To illustrate this, Fig. 10(a) of Appendix A we show the average GFP concentration of the synthetic high promoter across six different experiments (horizontal axis) and stratified into seven different time segments of the experiments (panels). It is clear that there is substantial variation in GFP concentration across experiments, but the fact that the patterns in each panel look similar suggests that, within each experiment, GFP concentrations are relatively constant across the 4 h time segments.

We normalized GFP concentrations by dividing by the average GFP concentration across all cells and time points within a given experiment, and confirmed that this indeed results in constant expression across time for each experiment [see Fig. 10(b) of Appendix A]. In particular, the expression variation is now within the estimated error bars across days and time segments. Thus, it appears that subtle variations in

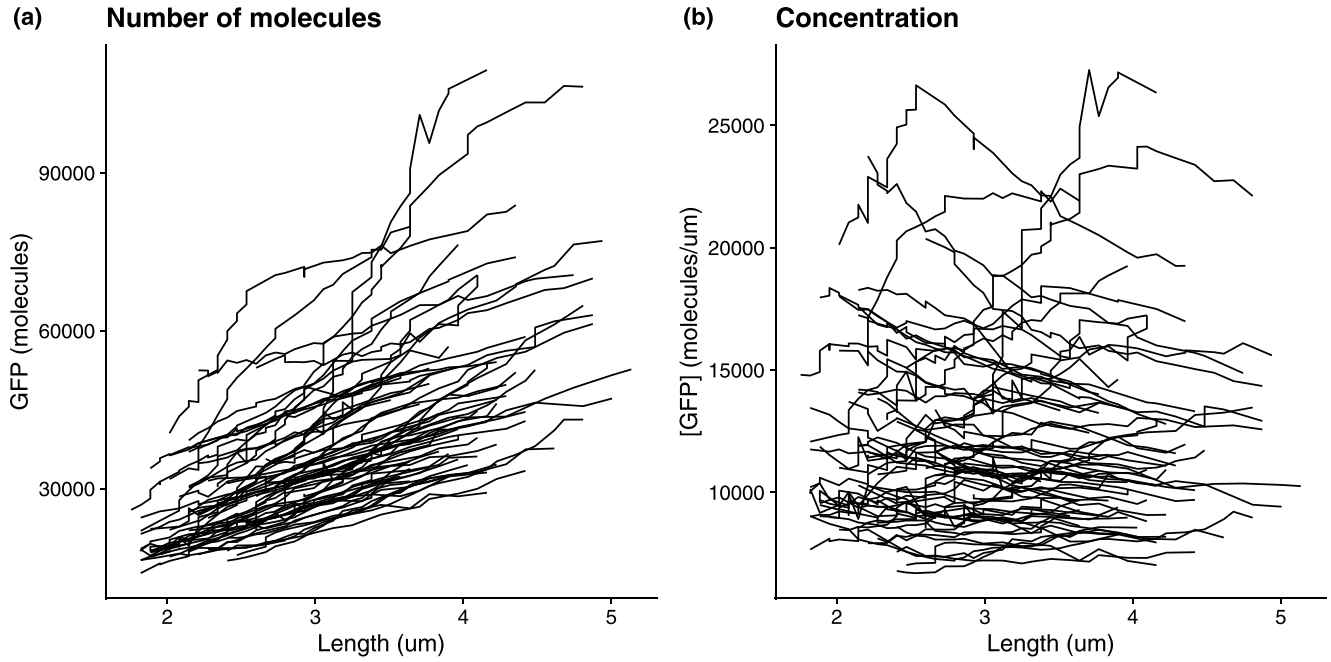


FIG. 9. Total GFP molecule numbers are proportional to cell size. (a) Total GFP number as function of cell length for a selection of cells expressing GFP from the synthetic high promoter. Each line corresponds to one cell cycle. (b) The same data but now showing GFP concentration (GFP molecule number per unit cell length) versus cell length. Notice there is no systematic correlation between GFP concentration and cell length.

the growth conditions on different days lead to measurable changes in absolute GFP levels, but that the relative concentrations across time within an experiment are highly reproducible.

As a further confirmation, Fig. 19 of Appendix F shows the average GFP concentration in glucose and the fold changes upon treatment with Cipro for all the promoters, and shows that for *recA* and *polB*, the normalized concentrations also agree across different measurement days.

3. Adaptation to the mother machine

Increasing the illumination of the cells allows for increased accuracy and sensitivity in the GFP measurements. However, when illumination levels become too high, they become toxic to the cells. Indeed, we suspect that the stochastic induction of the *recA* promoter seen in recent mother machine experiments may be the result of illumination leading to low levels of DNA damage [23,24]. Compromising between accuracy and phototoxicity we chose an illumination where the cells grow only marginally slower than without illumination.

Measuring the distribution of growth rates as a function of time (obtained by local linear fits of log cell length versus time) we observed that the median growth rate of cells drops during the first 2 h of the experiment, presumably due to the cells adapting to the illumination (see Fig. 11 of Appendix A). To exclude effects from this transient adaptation phase, all measurements from cells born in the first 2 h were discarded for the analyses presented in this work.

APPENDIX B: INFERRING THE VOLUMIC GFP PRODUCTION

Let $G(t)$ be the amount of GFP inside a cell at a time t . We denote by $q(t)$ the amount of GFP that is created per unit volume and per unit time at time t . We assume that GFP fluorescence decays at a rate β , which represents the combined effects of bleaching and protein decay. We thus have for the dynamics of $G(t)$:

$$\frac{dG}{dt} = q(t)V(t) - \beta G(t) = q(t)V_0 e^{\lambda t} - \beta G(t), \quad (B1)$$

where in the second equation we have assumed that cell volume grows exponentially at a rate λ starting from volume V_0 at time $t = 0$. We can solve this equation by rewriting it as

$$\frac{d}{dt}[Ge^{\beta t}] = q(t)V_0 e^{(\lambda+\beta)t}. \quad (B2)$$

Integrating and setting the initial condition $G(t_0) = G_0$ we find

$$G(t) = G_0 e^{-\beta(t-t_0)} + V_0 e^{-\beta t} \int_{t_0}^t d\tau q(\tau) e^{(\lambda+\beta)\tau}. \quad (B3)$$

To estimate a volumic production rate q_i at each measurement time point t_i we will make the approximation that $q(t)$ is approximately constant over a time window of $T = 5$ time points centered on t_i and estimate this q_i from the measurements of total fluorescence in this time window.

If we set the volumic production rate $q(t)$ to a constant q for a short time window, then we find the following for the

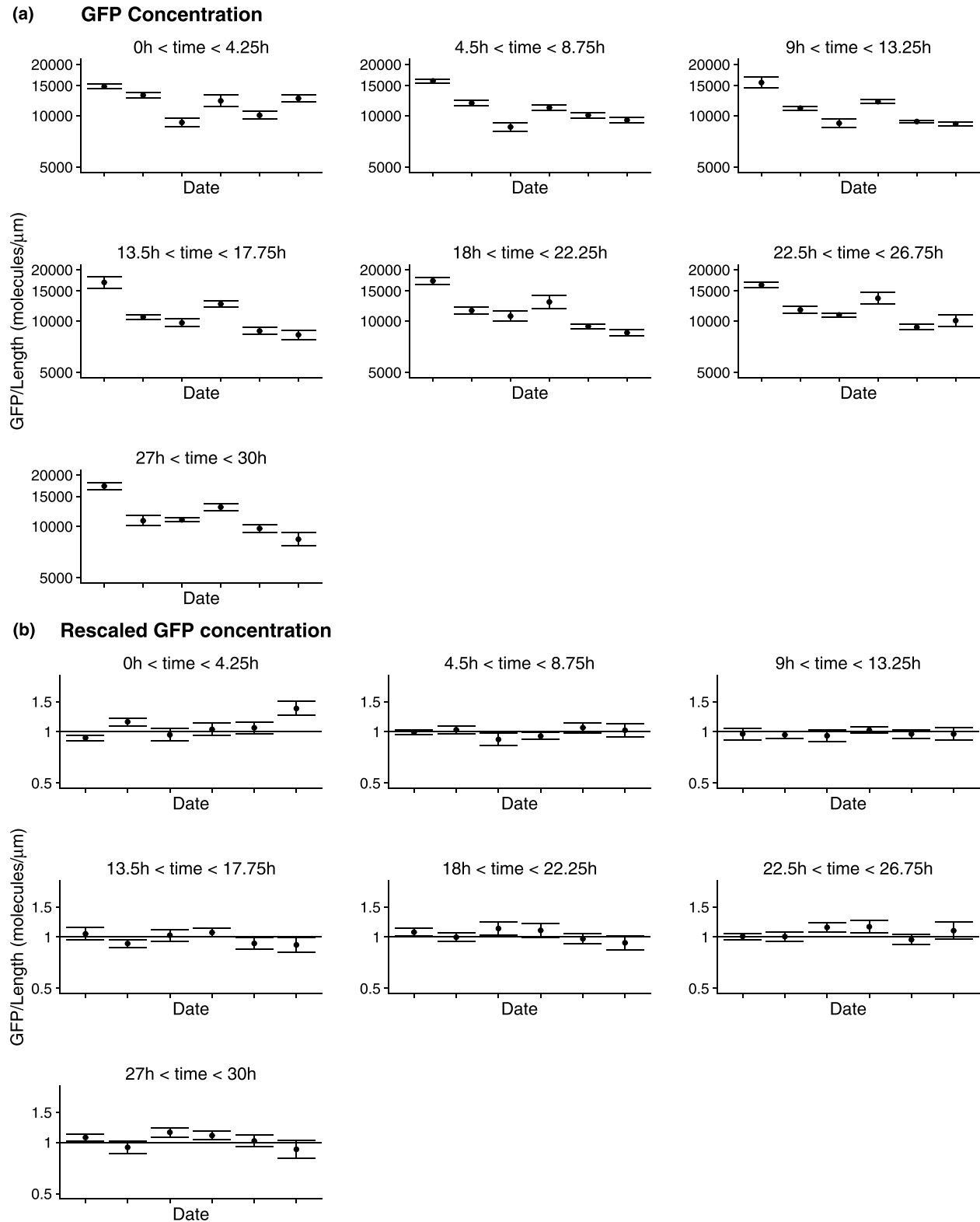


FIG. 10. Average GFP concentration. (a) Average GFP concentration as driven by the constitutive Synthetic High promoter across a population of cells from experiments performed on different days (horizontal axis), and stratified by time bins of 4.5 h segments of each experiment (one panel per time segment). Note that clear day-to-day variation in average GFP levels is evident, and that expression levels are relatively constant across different time segments. (b) Same as panel (a), but we divided each concentration by the average GFP concentration over the entire day. The results show that, after this normalization, expression levels are approximately constant across time on all days. As a guide for the eye, the horizontal lines show the value 1.

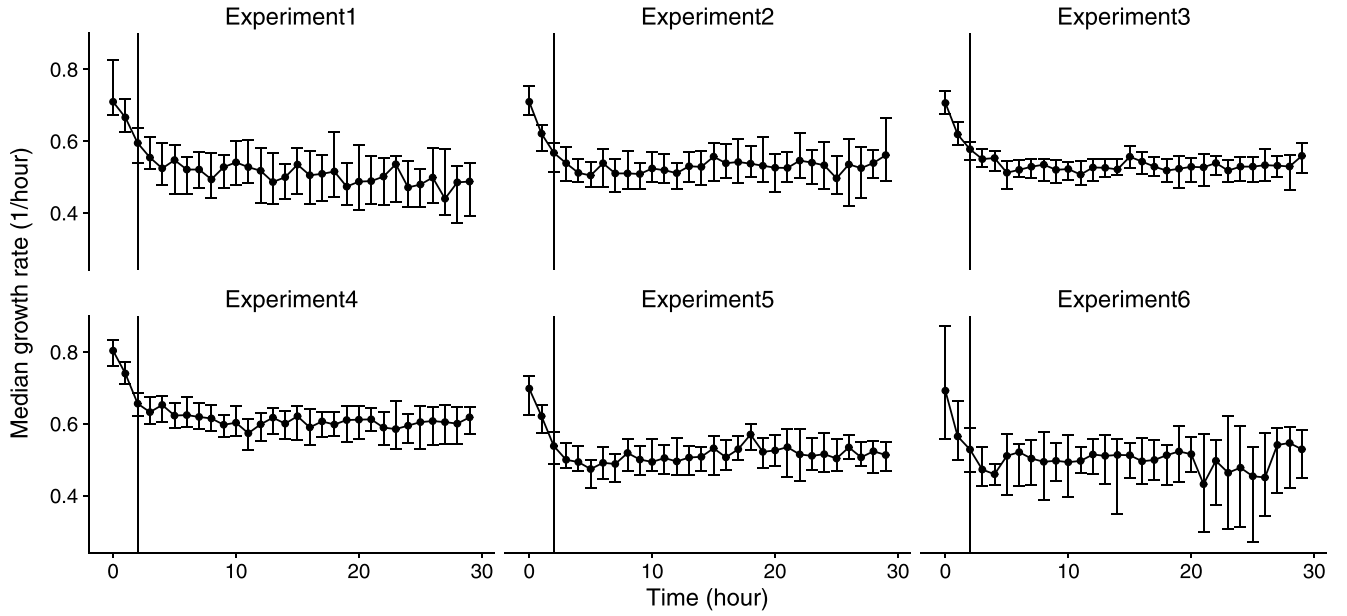
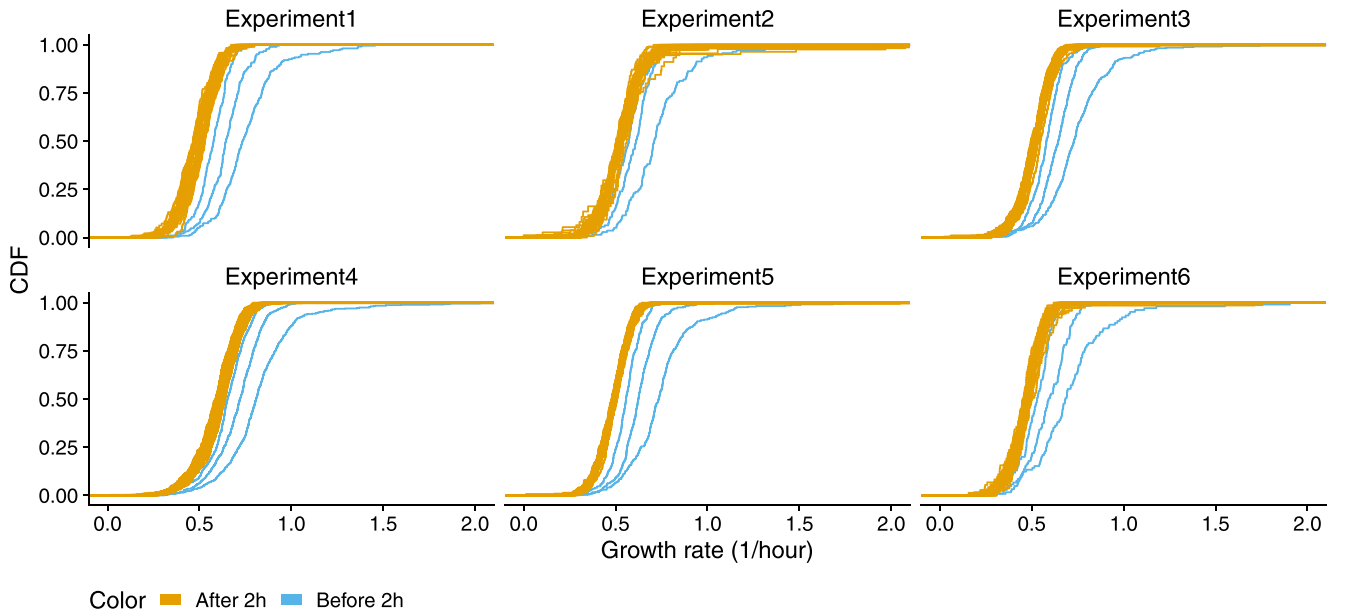
(a) Adaptation to the Mother Machine**(b) Distribution of growth rates**

FIG. 11. Cells adapt to illumination during the first 2 h. (a) Median growth rate of the cells during different periods of the experiment (horizontal axis) as measured on different days (different panels). After the illumination commences at time 0 h, the growth rate first decreases and then stabilizes after approximately 2 h. Error-bars correspond to two standard deviations of the posterior of the median. (b) Cumulative distributions of single-cell growth rates stratified by time bin. The blue lines show the distributions coming from time bins earlier than 2 h and they are clearly higher than the red distributions coming from time bins after 2 h. Each panel corresponds to a different biological experiment.

total GFP content during this time window:

$$G(t) = \alpha(t)q + G_0 e^{-\beta t}, \quad (\text{B4})$$

where we have defined

$$\alpha(t) = \frac{V_0}{\lambda + \beta} [e^{\lambda t} - e^{-\beta t}]. \quad (\text{B5})$$

Let G_i^m correspond to the measured total GFP fluorescence at each time point t_i in the short time window and assume that each measurement G_i^m corresponds to the true value G_i plus Gaussian measurement noise of (unknown) standard deviation σ . That is, we assume

$$G_i^m = \alpha_i q + G_0 e^{-\beta t_i} + \mathcal{N}(0, \sigma^2), \quad (\text{B6})$$

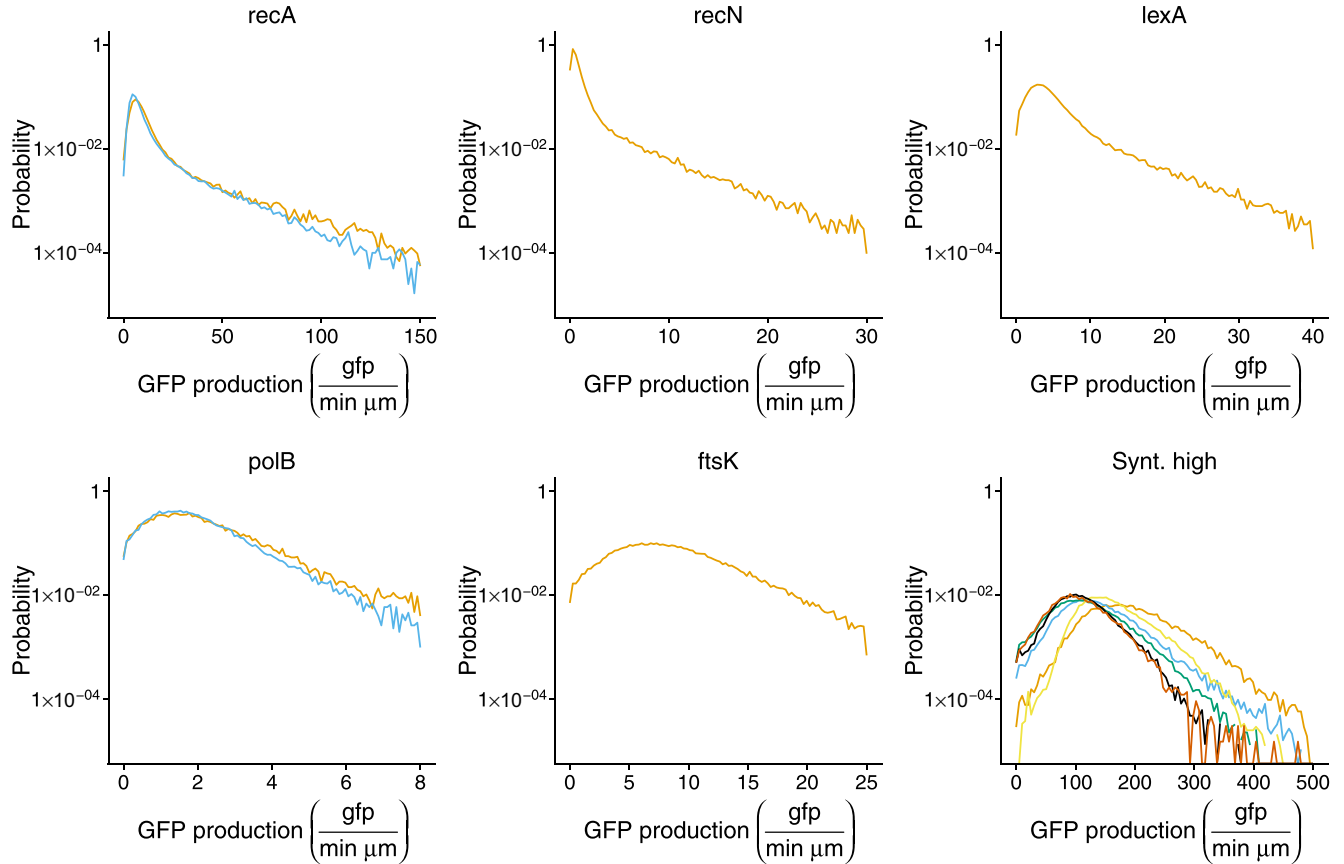


FIG. 12. Distribution of volumic GFP production q for the LexA target promoters and the Synthetic high promoter. Probability densities of the estimated volumic production rates q for each promoter. The y-axes are shown on logarithmic scales so that exponential tails appear as straight lines. The production rates q were pooled from the entire experiment, regardless of the Cipro concentration. Note that for LexA targets all distributions exhibit an initial peak at low q followed by an exponential tail of large q values, which inspection suggests corresponds to short-lived peaks in production. In contrast, the synthetic high promoter does not exhibit a similar exponential tail in q .

where

$$\alpha_i = \frac{V_0}{\lambda + \beta} [e^{\lambda t_i} - e^{-\beta t_i}]. \quad (\text{B7})$$

To estimate the α_i for our time window of $T = 5$ time points, we use a slightly larger window of nine time points centered on the same time point and perform a linear regression of log cell-length versus time to estimate the growth rate λ and the cell length V_0 at the start of the time window. We further define $b_i = e^{-\beta t_i}$, using the total decay plus bleaching rate β that we estimated previously [26].

Given the estimated values of the α_i and b_i , the probability of the data is given by

$$\begin{aligned} P(\{G_i^m\} | q, \{\alpha_i\}, \beta, \sigma, G_0) \\ = \frac{1}{(2\pi\sigma^2)^{\frac{T}{2}}} \exp \left[-\frac{1}{2\sigma^2} \sum_{i=1}^T (G_i^m - q\alpha_i - G_0 b_i)^2 \right]. \end{aligned} \quad (\text{B8})$$

To remove the dependence on the unknown starting GFP level G_0 and the unknown size of measurement errors σ , we marginalize over these variables with a uniform prior for G_0

and a scale prior $d\sigma/\sigma$ for σ . Finally, assuming a uniform prior for q as well we find that the posterior for q is given by the Student t distribution

$$P(q | \{G_i^m\}, \{\alpha_i\}, \beta) \propto \left[1 + \frac{\text{var}(\alpha) \left(q - \frac{\text{Cov}(G^m, \alpha)}{\text{var}(\alpha)} \right)^2}{\text{var}(G^m)(1 - \rho^2)} \right]^{-\frac{T-1}{2}}, \quad (\text{B9})$$

where we defined the variances, covariance, and correlation

$$\text{var}(\alpha) = \frac{1}{T} \sum_{i=1}^T (\alpha_i)^2 - \frac{(\sum_{i=1}^T \alpha_i b_i / T)^2}{\sum_{i=1}^T b_i^2 / T}, \quad (\text{B10})$$

$$\text{var}(G^m) = \frac{1}{T} \sum_{i=1}^T (G_i^m)^2 - \frac{(\sum_{i=1}^T G_i^m b_i / T)^2}{\sum_{i=1}^T b_i^2 / T}, \quad (\text{B11})$$

$$\text{cov}(G^m, \alpha) = \frac{1}{T} \sum_{i=1}^T G_i^m \alpha_i - \frac{(\sum_{i=1}^T G_i^m b_i / T)(\sum_{i=1}^T \alpha_i b_i / T)}{\sum_{i=1}^T b_i^2 / T}, \quad (\text{B12})$$

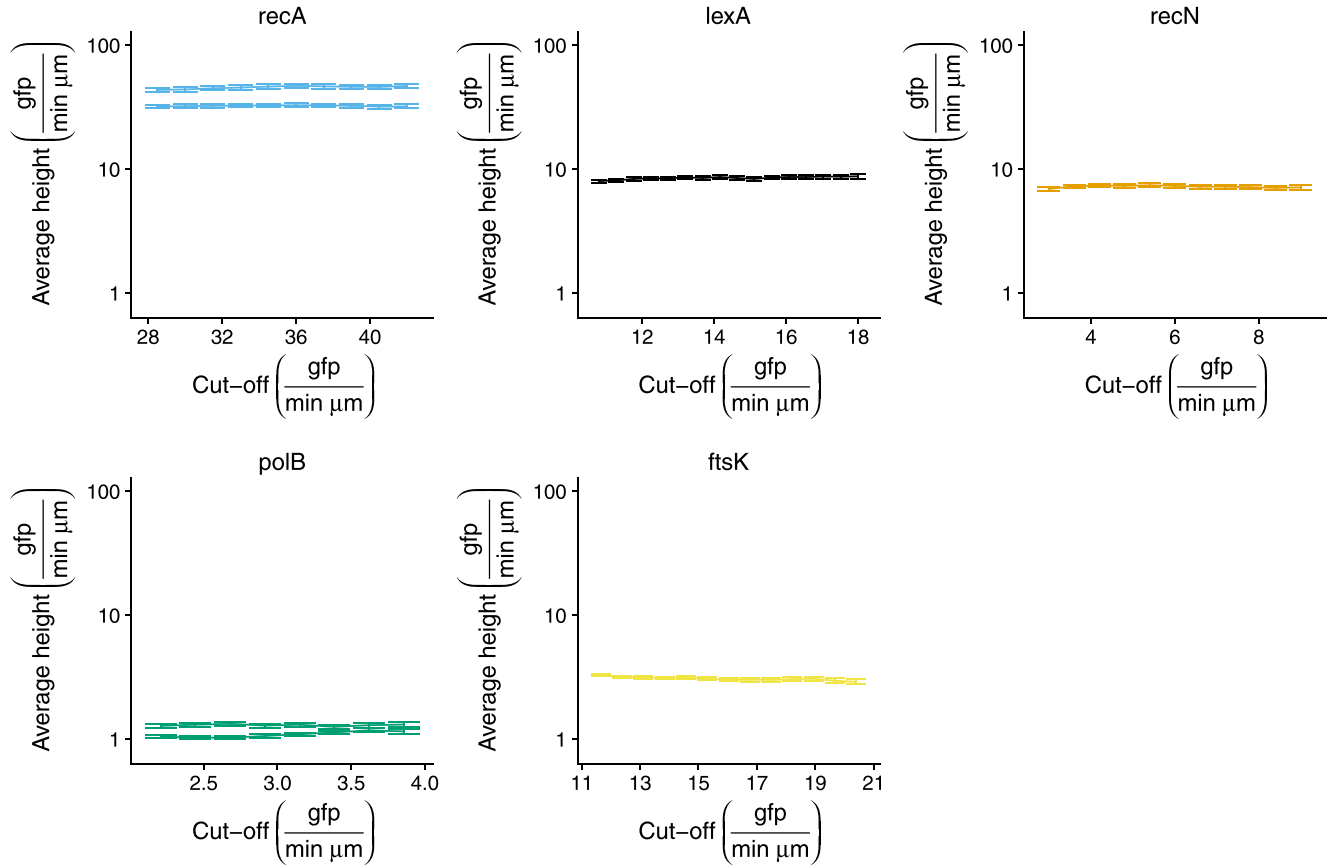


FIG. 13. Average peak height $h(q_c)$ as function of the cutoff q_c . Estimated average height $h(q_c)$ as a function of the cutoff on peak height q_c for each of the target promoters. The error bars show estimated height $h(q_c)$ plus and minus one standard deviation $\sigma_h(q_c)$. The cutoff q_c for each promoter is chosen to lie in the exponential tail of Fig. 12 of Appendix B. Note that the estimate of h is insensitive to the cutoff q_c . For recA and polB estimates from experiments on two separate days are shown.

and the correlation coefficient

$$\rho = \frac{\text{cov}(G^m, \alpha)}{\sqrt{\text{var}(\alpha)\text{var}(G^m)}}. \quad (\text{B13})$$

Note that, because the effects of bleaching are incorporated through the dependence on the b_i , these definitions of the variances and covariances are slightly different from the usual expressions.

From this we have the maximal posterior estimate for q :

$$q^* = \frac{\text{cov}(G^m, \alpha)}{\text{var}(\alpha)}, \quad (\text{B14})$$

and approximate error-bar

$$\sigma_q = \sqrt{\frac{(1 - \rho^2)\text{var}(G^m)}{(T - 1)\text{var}(\alpha)}}. \quad (\text{B15})$$

Note that these results are easy to interpret. The best estimate for q is simply the usual best estimate for a linear model. The error goes to zero as the correlation ρ goes to one, which occurs when the measurements fall on a perfect straight line. The error-bar is further proportional to the standard deviation of the measured GFP fluorescences G^m and inversely proportional to the standard deviation of the α_i , which means that

if the measurements span a larger interval in α , the estimate becomes more accurate. Finally, the error depends as usual on the inverse square-root of the number of independent measurements $(T - 1)$ (since one independent measurement was lost when marginalizing over G_0).

We thus divide the entire dataset into sliding windows of T time points each and for each window estimate a local value of q . We checked that reducing the width of the windows below $T = 5$ had no significant effect on the peak statistics, apart from slightly reducing the peaks width.

APPENDIX C: IDENTIFICATION OF PEAKS IN VOLUMIC PRODUCTION

1. Peak finding method

We observed that for all target promoters that show an induction by LexA (i.e., recA , lexA , recN , polB , and ftsK), the distribution of volumic production rate q has an exponentially distributed tail, as shown in see Fig. 12 of Appendix B. Visual inspection of the time traces of q confirmed that values out in these tails correspond to bursts in GFP production. In contrast, the distribution of volumic production of the synthetic high promoter does not show such an exponential tail (see Fig. 12 of Appendix B).

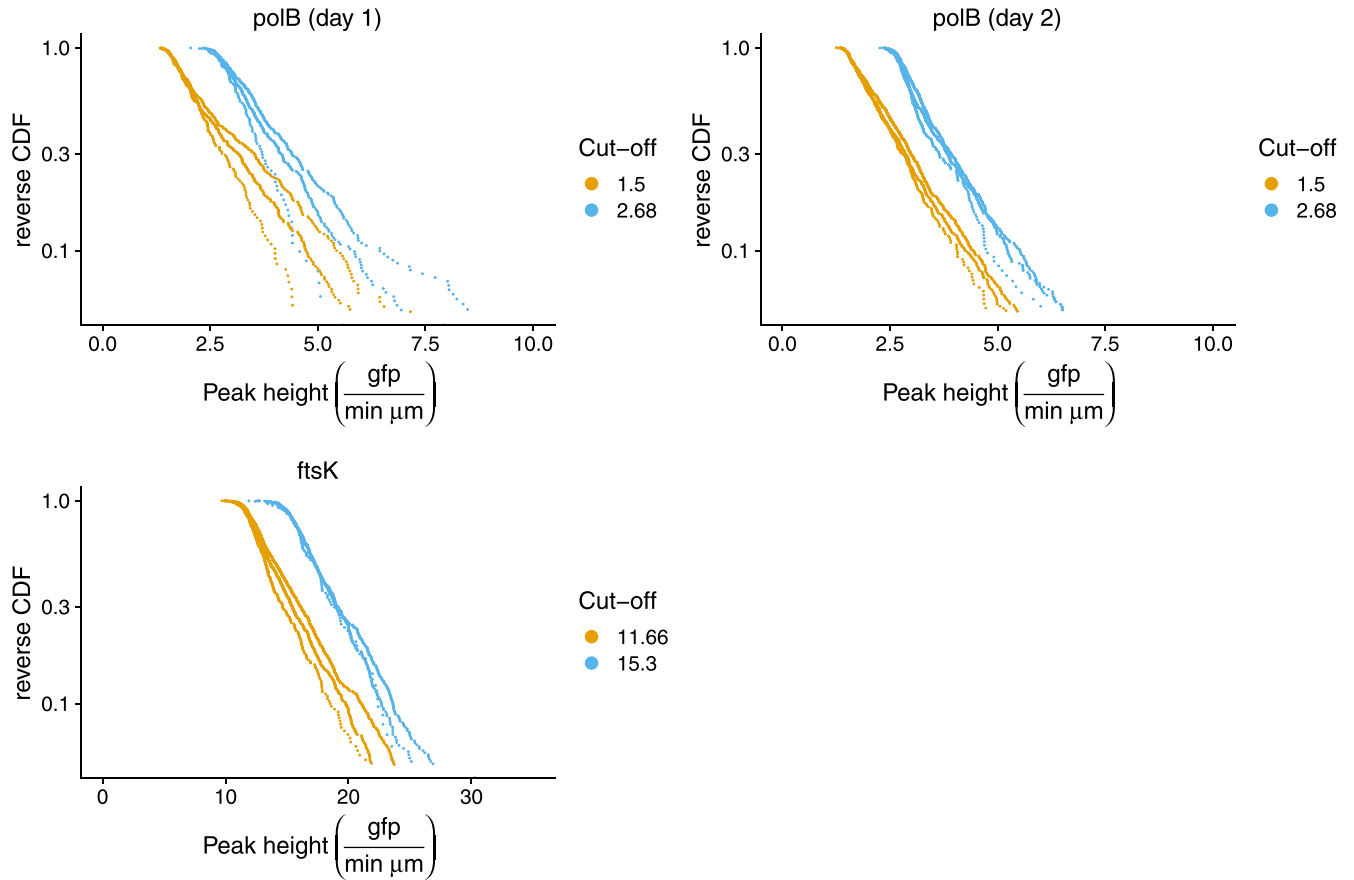


FIG. 14. The peak height distribution is exponential and independent of the cutoff q_c . The peak heights for polB and ftsK are exponentially distributed for two different values of the cutoff q_c in the range of the exponential tail (see Fig. 12 of Appendix B). Each color represents a different cutoff. Curves of the same color represent the distributions of q during the three experimental segments with different antibiotic concentrations. This figure complements Fig. 3 of the main text.

For each LexA target promoter we identified peaks in the time traces of the volumic GFP production rate $q(t)$ by finding all segments of consecutive time points for which $q(t)$ is over a cutoff value q_c , where q_c is taken to lie in the exponential tail for the corresponding promoter.

We then used the following algorithm:

(1) Find all contiguous time segments for which $q(t) > q_c$.

(2) For each such segment, find all local maxima of $q(t)$ within the segment.

(3) For each local maximum, we extract a segment of data points around the maximum to fit it to a Gaussian shaped peak. To meaningfully fit a Gaussian-shaped peak, the segment needs to capture the curvature around the peak. We create a segment by including contiguous data points to the left and right of the local maximum for as long as their q values keep decreasing, and as long as the q values are at least a fraction $f = 0.25$ of the maximum. For each maximum we thus include a segment of falling q values to the left and right, until q drops to a quarter of its maximum value.

(4) Before we fit the q values in the segment to a Gaussian, we first perform two checks: The maximum must not lie at the edge of the segment, and the segment must have at least 3 data points in it. Otherwise the maximum is discarded.

(5) For each segment of consecutive $q(t)$ measurements $[q(t_1), q(t_2), \dots, q(t_n)]$ around a maximum, we fit a Gaussian by minimizing the sum of squared deviations

$$\Delta^2 = \sum_{i=1}^n \left[q(t_i) - A \exp \left(-\frac{(t_i - \mu)^2}{2\sigma^2} \right) \right]^2, \quad (C1)$$

with respect to A (height of the peak), μ (time of the peak maximum), and σ (peak width). In this way we obtain a set of extracted peaks for each mother machine experiment, together with a time μ of each peak's occurrence, its height A , and its duration σ .

2. Peak height distributions have exponential tails

We next set out to estimate the average peak height h of the exponential tail of the distribution for each promoter. In particular, we assume that for peaks with height A larger than a cutoff q_c , the distribution is exponential, that is

$$P(A | A \geq q_c, h) = e^{-(A-q_c)/h} / h, \quad (C2)$$

with h the average peak height. Imagine that we have n peaks with $A \geq q_c$ and that $\langle A \rangle_{q_c}$ is the average height of these n

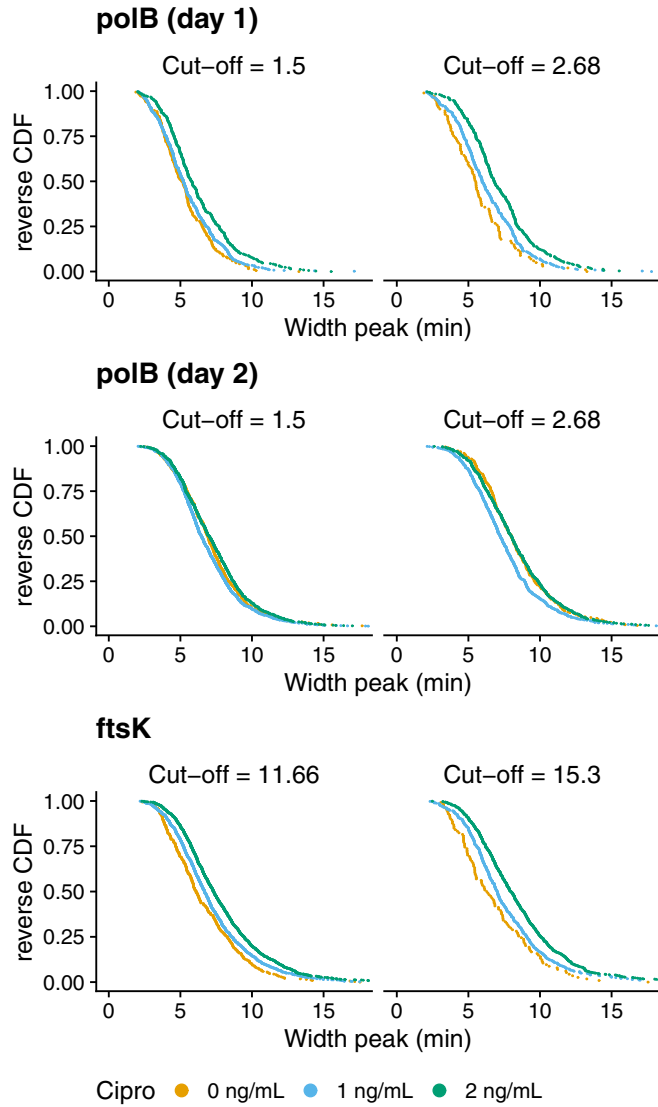


FIG. 15. Peak durations do not depend on the promoter, Cipro concentration, or the cutoff q_c . Reverse cumulative distributions of the peak durations for polB and ftsK for two different values of the cutoff q_c (different panels) in the range of the exponential tail (see Fig. 12 of Appendix B), separately for each Cipro concentration (different colors). This figure complements Fig. 3 of the main text. Note that for polB results from experiments on two different days are shown.

peaks. It is easy to show that the maximum likelihood fit of average peak height h to the distribution (C2) is given by setting h equal to

$$h(q_c) = \langle A \rangle_{q_c} - q_c, \quad (\text{C3})$$

with an error-bar of

$$\sigma_h(q_c) = \frac{h(q_c)}{\sqrt{n}}, \quad (\text{C4})$$

i.e., standard deviation of the posterior distribution.

To the extent that the tail of the distribution of q is really exponential, we should find that the average heights $h(q_c)$ are relatively insensitive to q_c , when the cutoff lies

in the exponential tail of the distribution. Figure 13 of Appendix B shows that this is indeed the case. Also, the distribution of heights above q_c , as shown in Fig. 3 of the main text, clearly confirms that the peak heights are exponentially distributed.

3. Peak height and width distributions for polB and ftsK

In Figs. 14 and 15 of Appendix C, we show that the distributions of heights and durations of the peaks for the polB and ftsK promoters also exhibit the same features we observed in the main text for recA, recN, and lexA. That is, the heights are exponentially distributed and depend only on the promoter, while the durations are independent of both the promoter and the Cipro concentration. The figures also show the distributions for two different values of the cutoff q_c in the range where the GFP production is exponential (see Fig. 12 of Appendix B).

4. Peak frequencies increase with antibiotic concentration

As explained in Appendix C 1, we identified peaks in the time traces of the estimated volumic GFP production rate $q(t)$ by finding all segments of consecutive time points for which $q(t)$ is above a cutoff value q_c . The frequencies of the peaks, defined as number of peaks divided by total time, of course depends on the chosen cutoff q_c . However, since the heights are exponentially distributed, we expect that the number of identified peaks, and hence the frequencies, are also exponentially decreasing with the cutoff. This behavior is indeed verified in Fig. 16 of Appendix C, where the cutoff used for each promoter runs over the exponential tail of $q(t)$ (see Fig. 12 of Appendix B). To define an effective peak frequency that is independent of the cutoff q_c , we extrapolate the exponential curve to $q_c = 0$ for each promoter. Note that, by effectively assuming that peaks can be arbitrarily shallow this effective frequency overestimates the frequency of true expression bursts. The estimate is solely intended to compare frequencies of different promoters and different time segments in a manner that removes the dependency on the cutoff q_c .

This extrapolation is done by simply fitting the observed log-frequencies to linear functions of q_c and inferring the intercepts. Notice that for each promoter there are three lines, corresponding to the log-frequencies of the peaks in the three different Cipro concentrations. However, the slope for each Cipro concentration is expected to be the same, since the height distribution is independent of the Cipro concentration. Therefore, we fit each promoter using a maximum likelihood model where the slope is constrained to be the same for the three different concentrations of Cipro. In particular, let us suppose we have a set of points from N linear models indexed by an index i , with the same slope m , but different intercepts q_i . Then

$$m = \frac{\sum_i \sigma_{xy_i}}{N\sigma_x^2}, \quad (\text{C5})$$

$$q_i = \langle y_i \rangle - m\langle x \rangle, \quad (\text{C6})$$

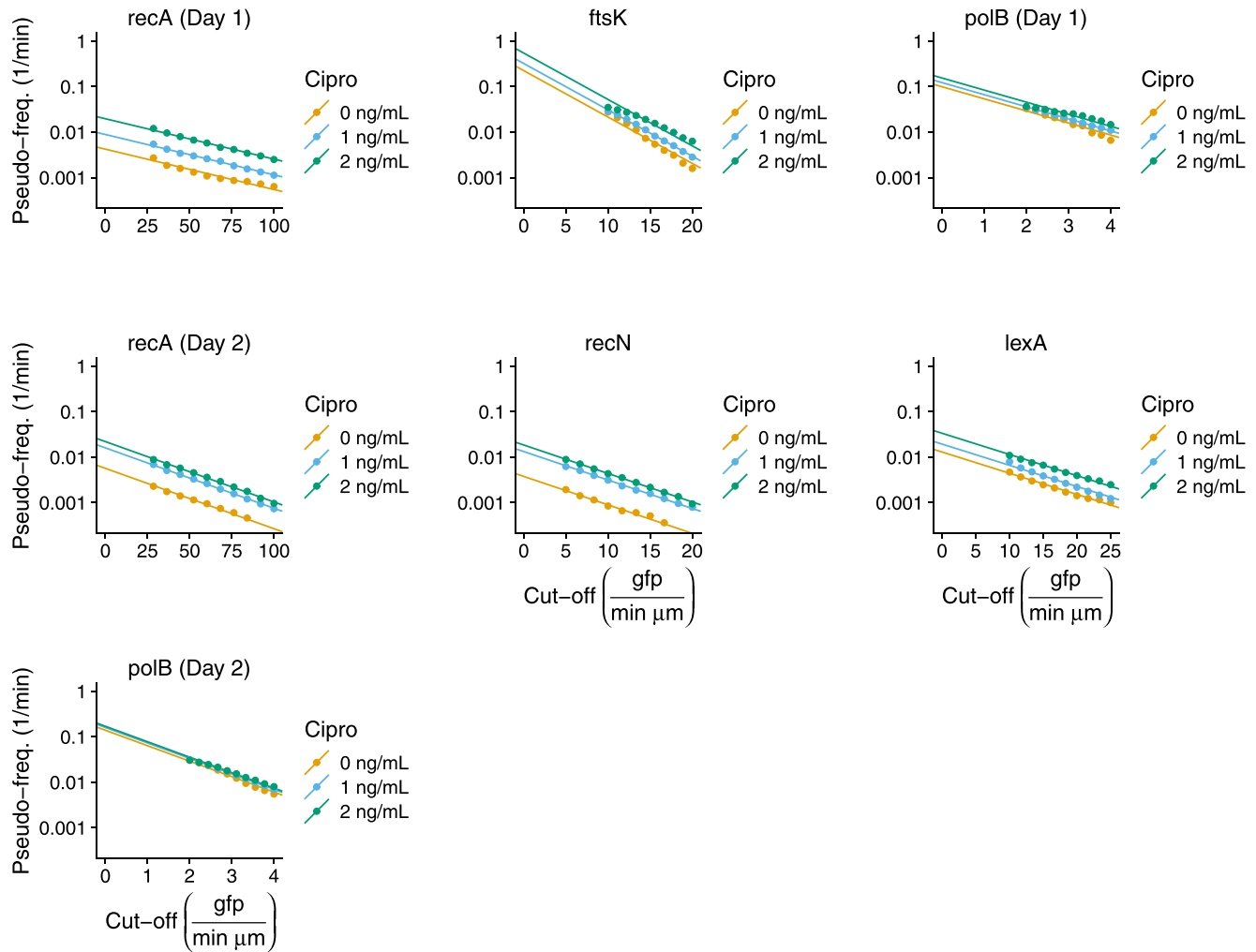


FIG. 16. Peak frequency as function of the cutoff q_c . Each panel shows the experimentally observed frequencies of production peaks as a function of cutoff q_c for different Cipro concentrations (colors) for a given promoter and replicate experiment (indicated at the top). Peak frequencies decay exponentially with cutoff q_c for each promoter and increase with Cipro concentration. To define an effective peak frequency that is independent of the cutoff q_c we fit exponential functions (solid lines) and extend the curves to $q_c = 0$. All vertical axes are shown on a logarithmic scale. Note that *recA* and *polB* have been measured in duplicate on separate days. Note also that in the fitting we impose that a given promoter has the same exponential dependence on cutoff in all Cipro concentrations, i.e., equal slopes in the log-linear plot.

where i runs over the N lines and we assumed that the independent variables x (the Ciprofloxacin concentrations) are the same for all the lines.

The results of the fits are shown in Fig. 16 of Appendix C, while the inferred peak frequencies are reported in the main text in Fig. 4. Figure 17 of Appendix C shows that the results are consistent between different days.

APPENDIX D: EFFECTS OF CIPROFLOXACIN ON CELL GROWTH AND GENE EXPRESSION

1. Single cell growth rates are not affected by Ciprofloxacin

The concentrations of Ciprofloxacin used in our experiments are low enough not to significantly affect the physiology of the cells. Indeed, the distribution of single-cell growth

rates is not meaningfully affected by the Ciprofloxacin, as shown in Fig. 18 of Appendix D.

2. Different LexA target promoters exhibit different levels of induction

For each promoter studied in this work, we measured both the average GFP concentration when growing without Ciprofloxacin [see Fig. 19(a) of Appendix D] as well as its average fold change as a function of time along the experiment [see Fig. 19(b) of Appendix D]. Although *ruvA*, *uvrD*, and *umuD* are reported to be regulated by LexA, they did not show any induction in their gene expression in our experiments. It is possible that these promoters are only induced at more severe levels of DNA damage, which could cause more prolonged

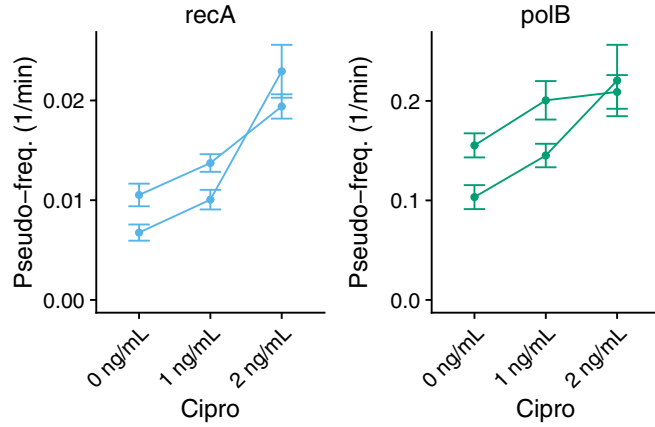


FIG. 17. Comparison of estimated peak pseudofrequencies from experiments on different days. Each panel shows the estimated pseudofrequencies of production bursts from experiments performed on two different days. The results show that, although there are clear day-to-day variations in the behavior, the pseudofrequencies are similar on different days.

periods of decrease in LexA expression than in our experiments.

APPENDIX E: SIMULATION OF GENE EXPRESSION DYNAMICS IN GROWING CELLS

To rigorously test the predictions of different models for the stochastic gene regulation of the LexA targets we developed a simulation framework that tracks a lineage of cells as they grow, divide, and stochastically express from a LexA target promoter. The algorithm, which we implemented in C++, implements the steps depicted in Fig. 5(a) of the main text:

(1) The simulation is initialized from a very small cell length (2 nm) whose precise value is not important as the dynamics reaches a stationary cell length distribution relatively quickly, and we discard data from the initial transient phase.

(2) Cell cycles follow the adder model [31,32] and at the beginning of each cell cycle, an added length ΔL is drawn from a Gaussian distribution with mean 1.86 and standard deviation 0.35 micrometer, which correspond to the mean and standard deviation of cell length observed in our experiments. The cell then grows exponentially with a constant growth rate of 0.009/min (matching the median doubling time of 76 min we observed in the experiments), until it the added length ΔL is reached.

(3) After adding a length ΔL , the cell divides into two daughters with each exactly half the volume of the mother. Each mRNA, immature GFP, and mature GFP in the mother cell is assigned to one of the daughters with equal probability. The simulation subsequently tracks only one of the daughters (which is randomly chosen).

(4) During the growth of each cell, the following stochastic events are simulated using the Gillespie algorithm [69]:

(a) A new instance of DNA damage is created at rate r_b .

TABLE III. Reactions in the Gillespie simulation. A list of all the reactions considered in the Gillespie simulation, together with their rates. [LexA] is the LexA concentration and V is the volume of the cell.

Reaction	Rate
DNA damage	r_b if a damage is not already there. Otherwise 0
DNA damage repair	r_r if the DNA is damaged Otherwise 0
LexA binding	[LexA] if LexA is unbound Otherwise 0
LexA unbinding	L_{off} if LexA is bound Otherwise 0
mRNA transcription	$r_m V$
mRNA decay	(number mRNA) δ_m
Protein translation	(number mRNA) r_p
Protein maturation	(number immature proteins) r_g
Protein bleaching	(number folded proteins) δ_g

(b) Each existing DNA damage instance is repaired at rate r_r .

(c) If the promoter is not bound by LexA, then LexA binds to the promoter at rate proportional to the current concentration of LexA. Note that, when LexA is at its equilibrium concentration in the absence of DNA damage, this rate is L_{SS} .

(d) If the promoter is bound by LexA, then LexA unbinds at a fixed rate L_{off} , which is determined by the strength(s) of the LexA binding site(s) in the promoter.

(e) If the promoter is not bound by LexA, then a new mRNA molecule is created (i.e., transcribed) at rate $r_m V$. Note that we assume that the transcription rate is proportional to cell volume V because plasmid copy number is approximately proportional to cell volume and we assume that the concentration of mRNA polymerase is approximately constant across the cell cycle. That is, we assign larger cells a larger transcription rate, but do not explicitly model the transcription dynamics at the individual plasmids.

(f) Each molecule of mRNA decays at a rate δ_m .

(g) Each molecule of mRNA is translated into immature GFP at rate r_p .

(h) Each immature GFP matures into fluorescing GFP at rate r_g .

(i) Each mature GFP disappears to due bleaching (or decay) at a total rate δ_g .

Note that, in the above simulation algorithm, growth and division of the cells are deterministic whereas all gene expression processes are stochastic. Table III provides a list of all reactions considered in the Gillespie simulations, together with their rates.

The concentration dynamics of LexA follows one of the following two scenarios. In the equilibrium model, the concentration of free LexA in the cell is assumed to be constant and dependent on the amount of Ciprofloxacin (i.e., there is a fixed LexA concentration at each Ciprofloxacin level). In the dynamic model, the concentration of LexA in the absence

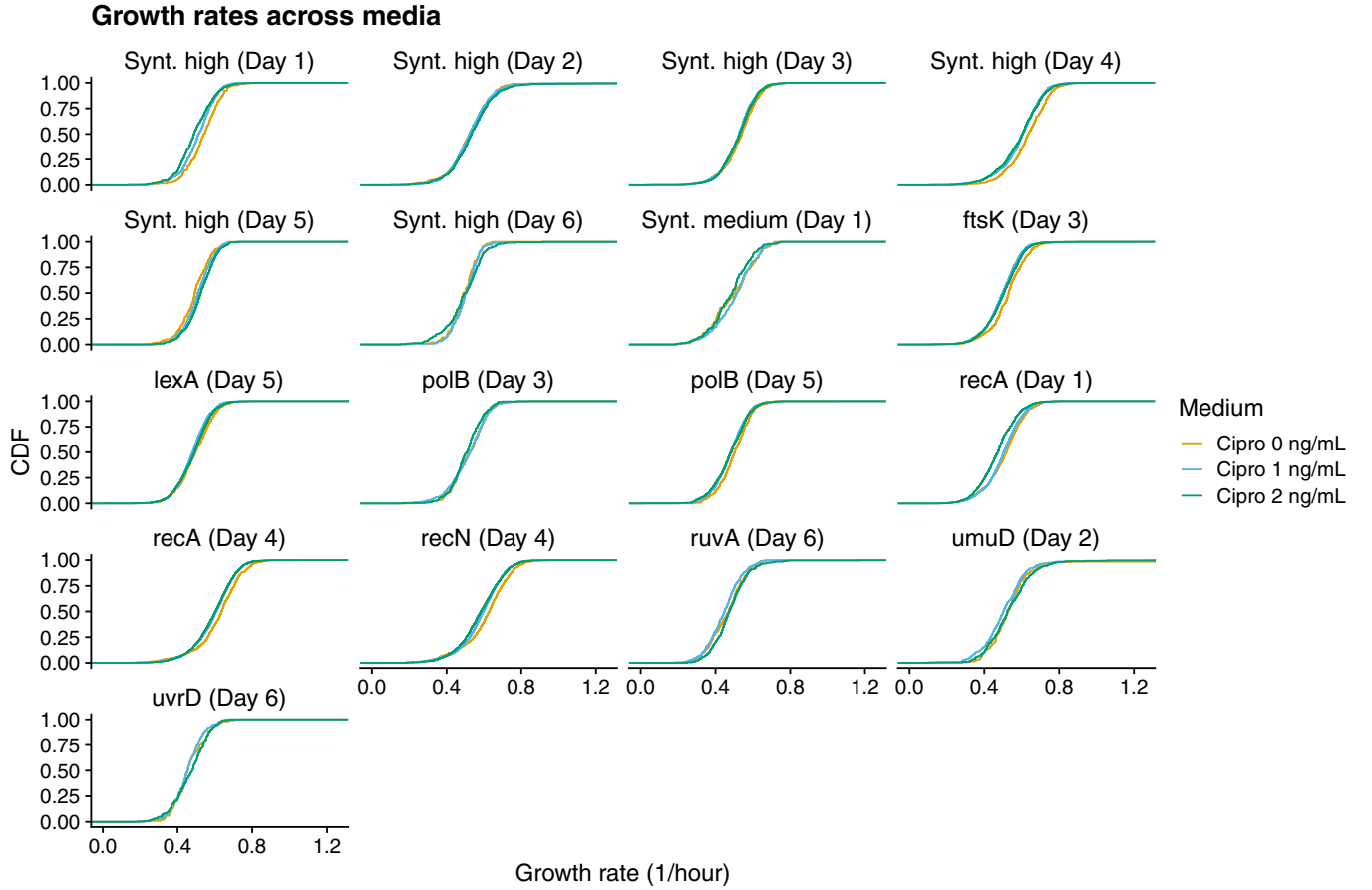


FIG. 18. Single-cell growth rates are not affected by Ciprofloxacin. For each observed single cell cycle in each experiment, a growth-rate was estimated by a linear fit of log cell length against time. Each panel shows the cumulative distribution of single-cell growth rates for a given promoter measured on a given day. The different colors represent the three different concentrations of Ciprofloxacin used during each experiment. Note that the growth rates are virtually independent of the Cipro concentration and promoter.

of DNA damage is assumed constant and at a level corresponding to a binding rate L_{SS} . Whenever DNA damage is present, the concentration is assumed to decay exponentially at a rate α . After the damage is repaired, we assume that the concentration of LexA recovers at a rate β until it saturates back to the steady-state rate L_{SS} . We generally assume for simplicity that α and β are equal, i.e., that it takes roughly as much time for LexA concentration to decrease as it takes to recover after the DNA damage is repaired. Note that, while the introduction and repair of instances of DNA damage is stochastic, the LexA concentration in responses to these DNA damage instances is deterministic.

APPENDIX F: THEORETICAL ANALYSIS OF THE DYNAMIC MODEL

In this section we present a number of analytical derivations to characterize how peak durations, heights and frequencies are related to the parameters of the dynamic model (Table I in the main text). In particular, we will show that, to fit the experimental data, instances of DNA damage must be repaired relatively quickly, that in this regime peak heights are exponentially distributed, and that the ratio of the

promoter's transcription initiation rate r_m and the rate of DNA damage repair r_r sets the average peak height.

1. DNA damage events must be short-lived

The duration of the peaks is determined by several rates, including the DNA damage repair rate r_r , the decay rate δ_m of the mRNA, and the maturation rate r_g of the GFP. Here we first show that, because the GFP maturation time is known to be 6–8 min [55,56] and the mRNA decay times are typically in the range of 2–10 min [63], even an instantaneous burst of mRNA production would lead to a peak in volumic production $q(t)$ of a duration that is only a little bit less than the observed durations of the peaks (which have an approximately Gaussian distributed duration with mean around 8 min).

Let's assume that at time $t = 0$ an instantaneous burst of m_0 mRNA molecules is produced, which then decay at a rate δ_m and are translated into immature GFP proteins at a rate r_p . In addition, immature GFP proteins mature into fluorescing GFP at a rate r_g . The volumic production rate q is defined as the production of mature GFP per unit time and volume.

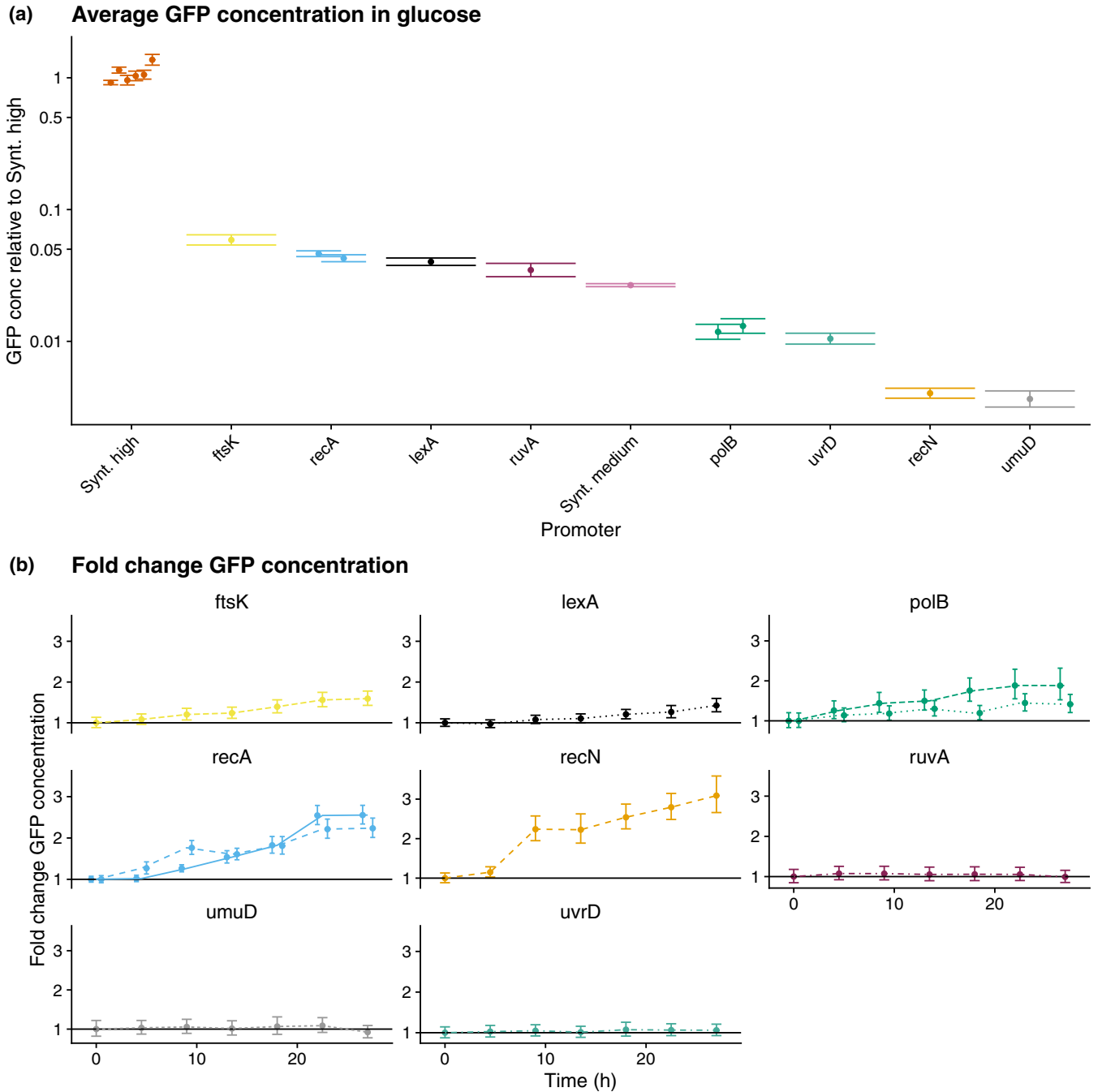


FIG. 19. Population average expression levels under treatment with Ciprofloxacin. (a) Average GFP concentration for the different promoters studied in this work in conditions without Ciprofloxacin. Note that, to take small day-to-day fluctuations in absolute fluorescence levels into account, the basal expression is reported relative to the average GFP concentration of the synthetic high promoter on the same day. Note also that *polB* and *recA* have been measured on two different days and different colors represent different experimental dates. Error bars correspond to mean $\pm$ one standard-error. The vertical axis is on a logarithmic scale. (b) Fold change in population average GFP concentration as a function of time during the experiment, with no Cipro for 0–6 h, 1 ng/ml for 6–18 h, and 2 ng/ml for 18–30 h. Error bars correspond to mean $\pm$ one standard-error. Note that for *ruvA*, *uvrD*, and *umuD* we did not detect any induction.

For this simple analytical argument, we will ignore stochastic fluctuations and treat mRNA number as a continuous quantity that simply decays exponentially with time

$$m(t) = m_0 e^{-\delta_m t}. \quad (\text{F1})$$

Similarly, for the total amount of immature protein P that has been produced we write

$$\frac{dP}{dt} = r_p m(t) - r_g P(t). \quad (\text{F2})$$

Notably, P corresponds to the number of proteins produced by this burst of m_0 mRNAs, not the total number of proteins in the cell.

The volumic production associated with this burst is related to $P(t)$ through

$$q(t) = \frac{r_g}{V} P(t), \quad (\text{F3})$$

where V is the volume of the cell. Note that since the peak durations are short compared to the cell cycle, we will ignore the growth of the volume V during the peak. Solving for $q(t)$ we find

$$q(t) = \frac{r_p m_0}{V} \frac{e^{-r_g t} - e^{-\delta_m t}}{x - 1}, \quad (\text{F4})$$

where $x = \frac{\delta_m}{r_g}$ is the ratio of the mRNA decay to the protein maturation rate.

Let t^* denote the time at which the maximum of the peak in volumic GFP production $q(t)$ occurs, q^* the height of this maximum, and dt the time for which the peak is above half of its maximum. First, the maximum production occurs at

$$t^* = \frac{1}{r_g} \frac{\log(x)}{x - 1}. \quad (\text{F5})$$

Using this, we find for the height of the peak

$$q^* = \frac{r_p m_0}{V} x^{-\frac{x}{x-1}}, \quad (\text{F6})$$

which is proportional to the mRNA burst size m_0 and inversely proportional to the volume V .

That is, when a burst of m_0 mRNAs occurs at a cell volume of size V , this leads to a burst of production of size q^* . Below we combine this with a calculation of the distribution of mRNA burst sizes m_0 at different volumes V to show that the distribution of peak heights q^* is exponential. In particular, we will see below that the dependence on volume will cancel out since the mRNA burst sizes m_0 are themselves proportional to volume V .

Finally, there is no simple analytical expression for the time dt that $q(t)$ is larger than half its maximum $q^*/2$. However, from equation (F4) we numerically find that with a GFP maturation rate of $r_g = 1/7$ min, dt runs from $dt \approx 11$ min at a $\delta_m = 1/2$ per minute mRNA decay rate to $dt \approx 18$ min at $\delta_m = 1/6$ per minute. The latter is already longer than the width of the peaks we observe in the data (full width half maximum: $2\sqrt{2\log(2)}\sigma = 17$ min). That, using the known GFP maturation time of about 7 min, we have to assume mRNA lifetime is less than 6 min for the peak duration from an instantaneous burst of mRNA production to remain below the experimentally observed peak durations. Consequently, the mRNA production bursts must be short, i.e., at most a few minutes long.

The parameter that controls the duration of transcriptional bursts is the DNA damage repair rate r_r , i.e., the average duration of DNA damage events is $1/r_r$ minutes. For a relatively high repair rate of $r_r = 0.8$ per minute, only one in fifty DNA damage events take longer than 5 min to repair, and we used our simulation framework to compare the peak durations at this repair rate with those observed experimentally as we

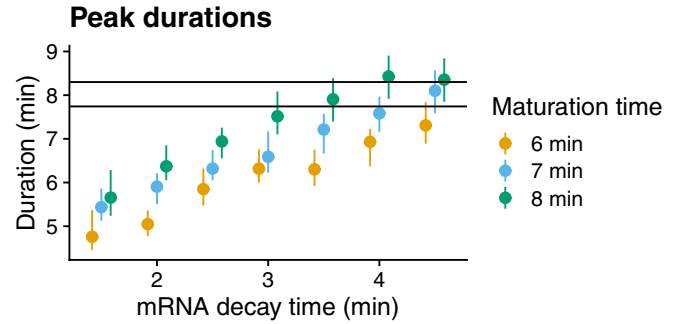


FIG. 20. Duration of peaks in volumic GFP production from bursts of mRNA production. Median peak durations (mean $\pm$ standard-error of the standard deviations of the Gaussians fitted to peaks in volumic production) in simulations of the dynamic model using different values for the mRNA decay rate (horizontal axis) and the GFP maturation time (symbol colors, see legend) and a DNA damage repair rate of $r_r = 0.8 \text{ min}^{-1}$. The black horizontal lines correspond to the average durations of the peaks in the two biological replicate experiments with recA.

varied mRNA lifetime from 1.5–4.5 min and GFP maturation time ranging from 6–8 min.

Figure 20 of Appendix F shows that, with this repair rate, the mean duration of the peaks matches the experimentally observed mean duration for maturation times of 7–8 min when mRNA lifetime is 3.5–4.5 min. Moreover, at an mRNA decay time of 4 min and a maturation time of 7 min, the distribution of durations of the simulated peaks exactly matches those observed in the experiments when the repair rate is $r_r = 0.8$ per minute [Fig. 6(c) in the main text].

2. When damage events are short-lived, peak heights are exponentially distributed

As we have seen in the last section, the short durations of the observed peaks in volumic production require that instances of DNA damage are repaired relatively quickly. We also saw that the observed distribution of peak durations can be well fit by the model when the repair rate is $r_r = 0.8$ per minute. We first used our simulation framework to investigate whether the distribution of peak heights also matches the exponential distribution that is observed experimentally. We find that, for a relatively narrow range of repair rates from $r_r = 0.5$ to $r_r = 1.0$ per minute, the distribution of peak heights in the simulations indeed matches the experimental distribution observed experimentally (see Fig. 21 of Appendix F). However, from repair rates of $r_r = 0.4$ per minute or below, and from $r_r = 1.5$ per minute or above, the distributions of peak heights already start deviating from what is observed experimentally (see Fig. 21 of Appendix F).

Here we provide a simple analytical argument to explain why, when DNA damage events are short-lived, the distribution of peak heights has an exponential tail.

Equation (F6) shows that, when mRNAs are produced in a short burst, the height of the resulting peak in volumic production is proportional to the number of mRNAs m produced in the burst, and inversely proportional to the volume V at which

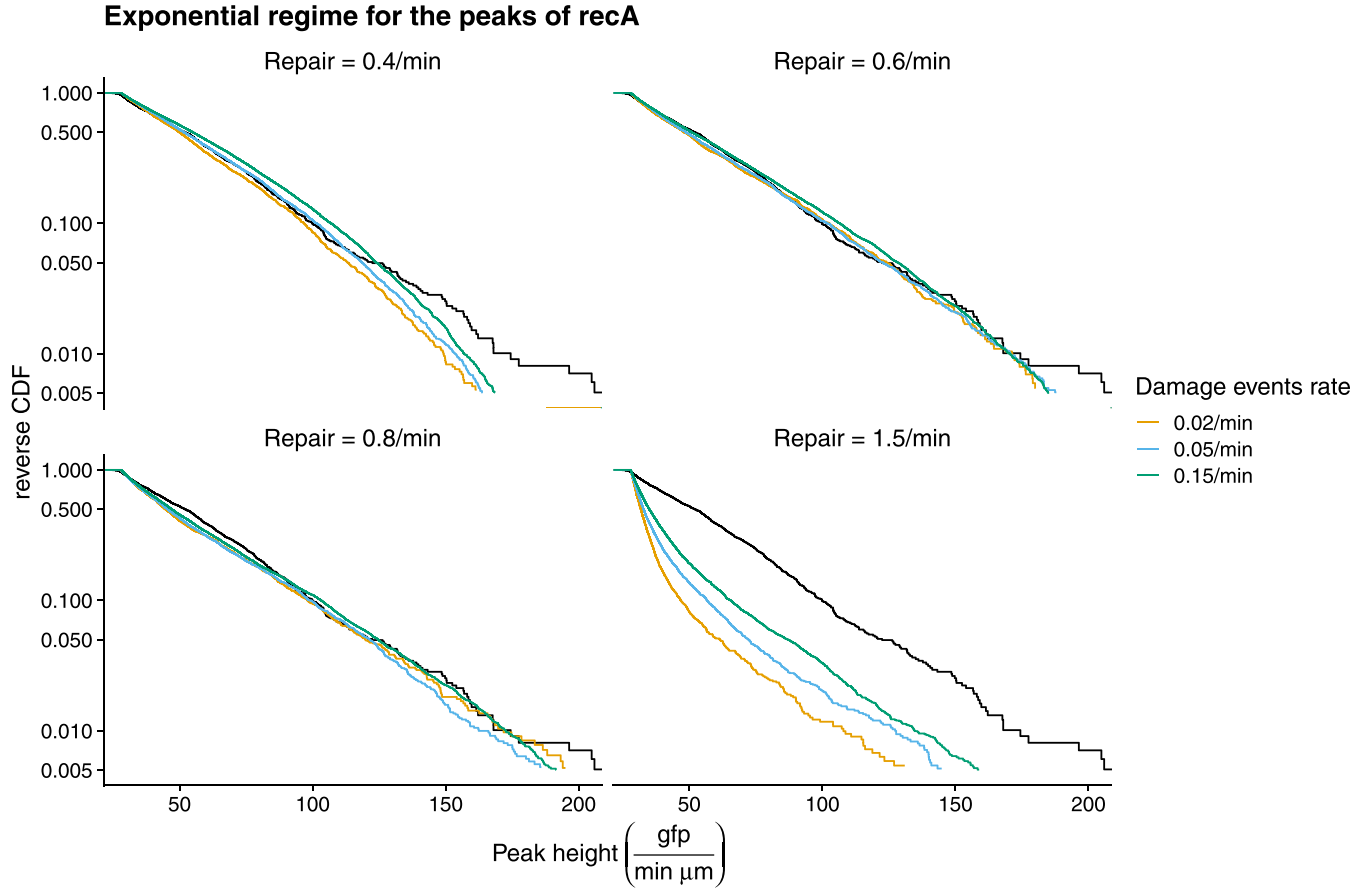


FIG. 21. Reverse cumulative distributions of peak heights from simulations of the dynamic model. Each panel shows the reverse-cumulative distributions of peak heights obtained using simulations of the dynamic model with the *recA* promoter for a given repair rate (shown at the top of the panel) and for different frequencies of DNA damage events (different colored lines, see legend). The transcription rate r_m has been set to match the average of the exponential distributions observed experimentally (black lines). Vertical axes are shown on a logarithmic scale. Note that at the lowest and highest of these repair rates the distributions of peak heights start deviating from an exponential.

the burst occurred. For a transcriptional burst to occur, two conditions need to be satisfied. First, LexA needs to unbind from the promoter and second, the LexA concentration needs to be low enough such that the transcription rate $r_m V$ is at least on the order of the LexA rebinding rate, which only occurs during DNA damage events. Note that the longer the DNA damage lasts, the lower the LexA concentration and the lower the rate of LexA rebinding. Thus, the larger bursts of mRNA production correspond to DNA damage events that last longer. The exponential tail of the distribution of peak heights thus corresponds to relatively long DNA damage events where the LexA concentration has become low.

For these longer DNA damage events, we will approximate the LexA concentration dynamics by ignoring the transient phase during which the concentration drops from L_{ss} to a new low steady state at L_{low} , as well as the transient recovery phase during which it grows from L_{low} back to L_{ss} . That is, we model such DNA damage events as if the LexA concentration is L_{low} during the entire DNA damage event.

During such DNA damage events the following events can occur stochastically: LexA-bound promoters switch to unbound at rate L_{off} , unbound promoters switch to LexA-bound at rate L_{low} , new transcripts are initiated from unbound promoters at rate $r_m V$, and the damage event ends at a rate

r_r . Because these rates are constant, it is straight-forward to calculate the probability P_m that m mRNAs have been created at the time that the damage event ends.

At any time, the promoter can be either in the LexA-bound state b or unbound state u . If the promoter is in state b , then only two events can occur next. Either the promoter switches to unbound, or the DNA damage event ends. The probability $P(u|b)$ that the next event is a switch to the unbound state is

$$P(u|b) = \frac{L_{off}}{L_{off} + r_r}, \quad (F7)$$

and the probability $P(e|b)$ that the next event is the end of the damage event is

$$P(e|b) = 1 - P(u|b) = \frac{r_r}{L_{off} + r_r}. \quad (F8)$$

If the promoter is in the unbound state u , then the possible next events are a switch to the bound state b , the end of the damage event, or the generation of a new transcript. The probability that the next event is a switch to the bound state is

$$P(b|u) = \frac{L_{low}}{L_{low} + r_r + r_m V}, \quad (F9)$$

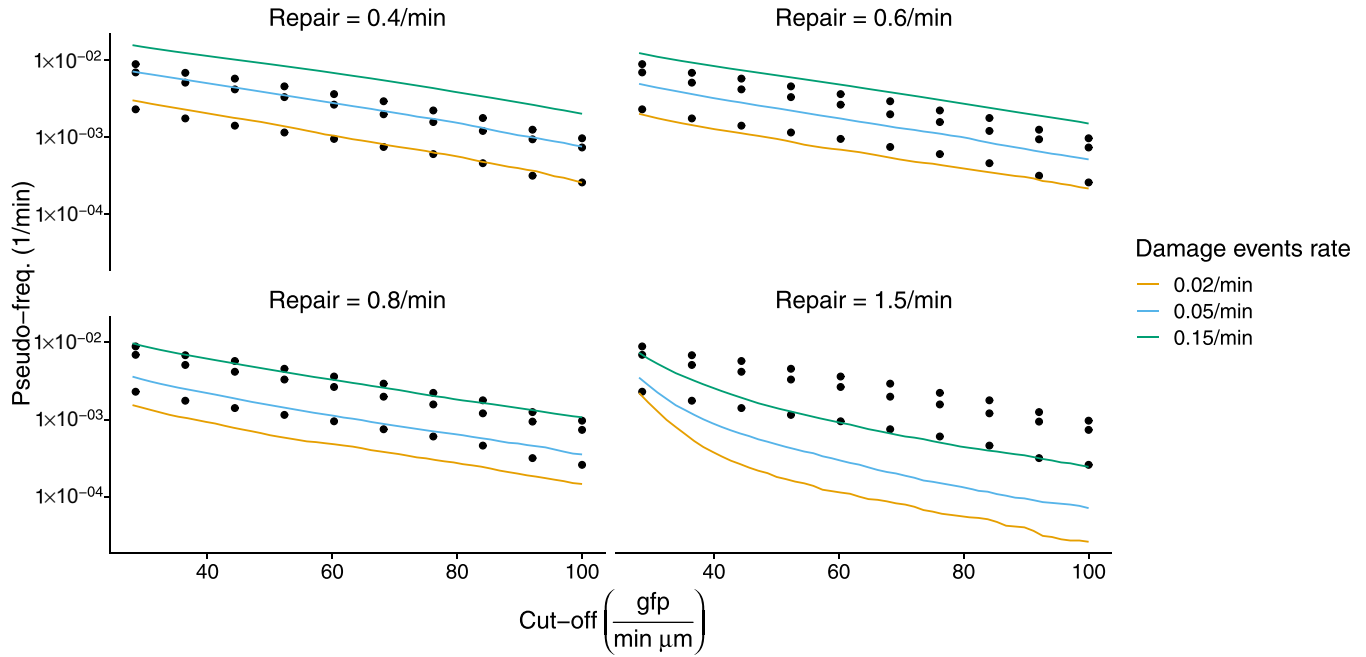


FIG. 22. Peak frequencies in simulations of the dynamical model with the *recA* promoter. Each panel shows the observed peak frequencies as a function of cutoff in peak height (horizontal axis) from simulations of the dynamical model with the *recA* promoter for different frequencies of DNA damage events (colored lines, see legend) and for a given rate of damage repair (indicated at the top). For reference, the black dots show the frequencies observed experimentally at different Cipro concentrations (i.e., the same data as in Fig. 16). The vertical axes are shown on a logarithmic scale. The results show that, by appropriately setting the frequency of DNA damage events in the model, the peak frequencies as a function of cutoff can be matched between simulations and experiments, provided that the repair rate lies in a relatively narrow range of 0.5–1.0 per minute.

the probability that the next event is the end of the damage event is

$$P(e|u) = \frac{r_r}{L_{\text{low}} + r_r + r_m V}, \quad (\text{F10})$$

and the probability $P(t|u)$ that the next event is the initiation of a transcript is

$$P(t|u) = \frac{r_m V}{L_{\text{low}} + r_r + r_m V}. \quad (\text{F11})$$

Imagine that the promoter is in state u and that m mRNAs have already been generated. We now want to calculate the probability ρ that, before the damage is repaired, another transcript is generated. This can happen either by directly generating a transcript with probability $P(t|u)$, or switching back and forth from bound to unbound and back k times with probability $[P(b|u)P(u|b)]^k$ before creating the transcript. We thus have

$$\rho = P(t|u) \sum_{k=0}^{\infty} [P(b|u)P(u|b)]^k = \frac{P(t|u)}{1 - P(b|u)P(u|b)}. \quad (\text{F12})$$

Thus, given that the promoter starts out in the unbound state, with probability ρ another transcript is created, and with probability $1 - \rho$ the damage event ends.

Thus, assuming that at the start of the damage event the promoter is bound, the probability that 0 transcripts are gen-

erated at the time the damage is repaired is

$$P_0 = P(e|b) + P(u|b)(1 - \rho), \quad (\text{F13})$$

and the probability that $m \geq 1$ transcripts are generated is

$$P_m = P(u|b)\rho^m(1 - \rho). \quad (\text{F14})$$

Note that this is a geometric distribution which is the discrete equivalent of the exponential distribution. The average number of mRNAs produced in the burst is given by

$$\langle m \rangle = P(u|b) \frac{\rho}{1 - \rho} = \frac{r_m V}{r_r} \frac{L_{\text{off}}}{L_{\text{low}} + L_{\text{off}} + r_r}. \quad (\text{F15})$$

Note that equation has an intuitive interpretation. The first factor $r_m V / r_r$ is the expected number of transcripts that would be produced if the promoter were unbound during the entire event. The second factor is the expected fraction of the time that the promoter is unbound during the event, which depends on the strength of the binding site.

Therefore, using equation (F6), the distribution of peak heights q^* resulting from bursts that occurred at volume V is approximately exponential with mean

$$\langle q^* \rangle = \frac{r_p \langle m \rangle}{V} x^{-\frac{x}{x-1}} = \frac{r_p r_m}{r_r} \frac{L_{\text{off}}}{L_{\text{low}} + L_{\text{off}} + r_r} x^{-\frac{x}{x-1}}, \quad (\text{F16})$$

which, notably, is independent of V , so that one exponential describes peaks resulting from bursts occurring at different cell volumes V .

In summary, the tail of the distribution of peak heights is approximately exponentially distributed with a mean that is proportional to transcription rate r_m of the promoter. Thus,

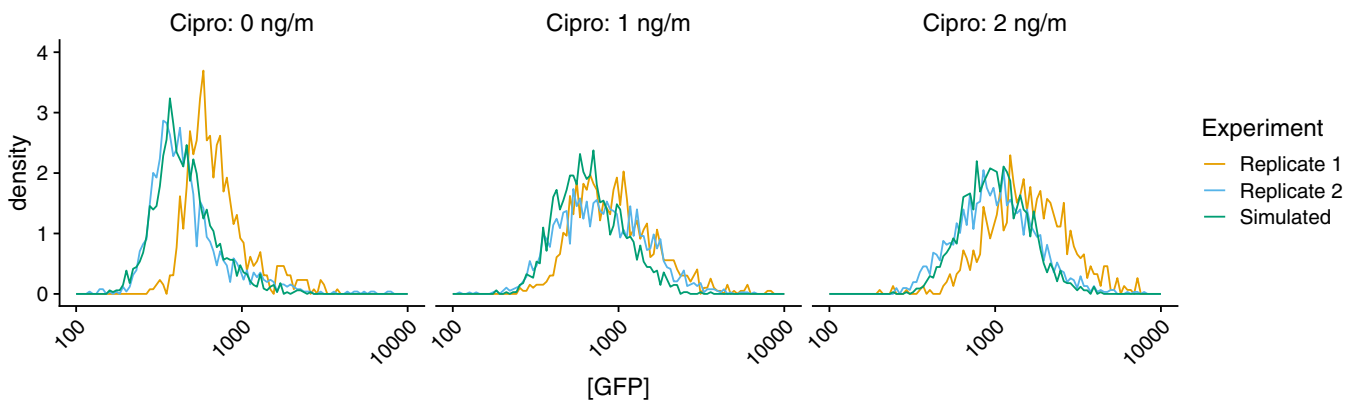
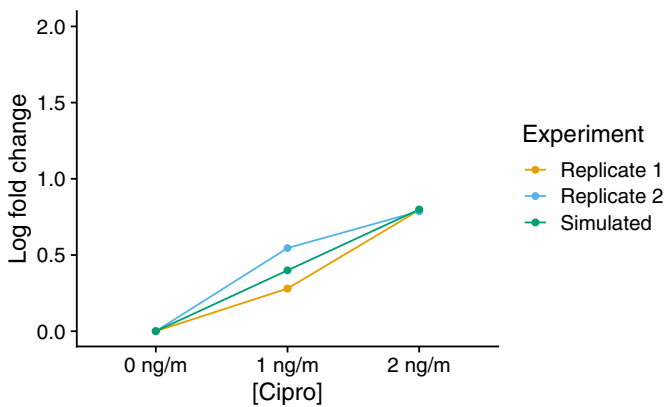
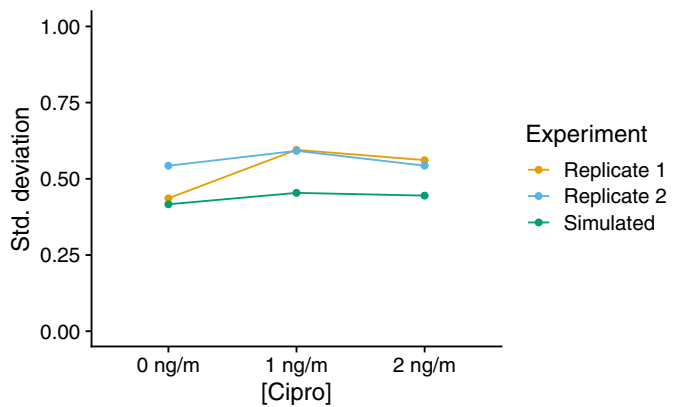
(a) GFP concentration**(b) Log fold change GFP conc.****(c) Std. deviation log(GFP conc.)**

FIG. 23. Distribution of simulated vs observed GFP concentrations. (a) Comparison between the GFP concentration distributions for the *recA* promoter as observed in the two replicates and in the simulations of our simple dynamical model (colors) for each of the Cipro concentrations (panels). (b) Log-fold changes in the average expression of the *recA* promoter as a function of Cipro concentration for the two replicates and the simulations. (c) Standard deviation in expression of the *recA* promoters as a function of Cipro concentration for the two replicates and the simulations. Note that the model fairly accurate captures the GFP concentration distributions and fold changes observed in the experiments although it somewhat underestimates the standard-deviation across cells.

the our dynamical model can be made to fit the exponential height distributions of each promoter by simply setting the transcription rate r_m to fit the average peak heights.

However, it should be noted that this derivation relied on the assumptions that damage events are short-lived. As Fig. 21 of Appendix F shows, at a repair rate of 0.4/min the DNA damage events start to be sufficiently long that the assumption that transcriptional bursts are short starts breaking down, and we notice that the height distribution starts to bend away from a pure exponential tail. We also see that at very high repair rates (i.e., $r_r = 1.5$ per minute), longer damage events become so rare that the exponential tail no longer holds over the range of peak heights that we observed in experiments. That is, apart from the burst durations, also the

fact that the observed distribution of peak heights is exponential restricts the repair rate to lie in a relatively narrow range.

In Fig. 22 of Appendix F we show that for repair rates in this range, we can also recapitulate the observed absolute frequencies of peaks as a function of cutoff q_c for *recA*, provided that the repair rate lies within the relatively narrow range of 0.5–1.0 per minute.

Finally, although our simple model ignores relevant fluctuations such as fluctuations in growth rate and the unequal sizes of daughters at division, Fig. 23 of Appendix F shows that it is able to capture the distributions of GFP concentrations across cells and Cipro concentrations for the *recA* promoter with reasonable accuracy.

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