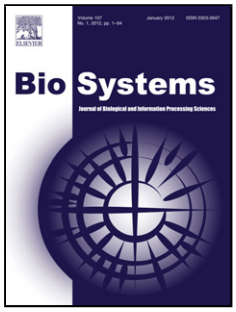

Title: Elucidating effects of reaction rates on dynamics of the lac circuit in Escherichia coli

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Abstract

Gene expression is regulated by a complex transcriptional network. It is of interest to quantify uncertainty of not knowing accurately reaction rates of underlying biochemical reactions, and to understand how they affect gene expression. Assuming a kinetic model of the lac circuit in *Escherichia coli*, regardless of how many reactions are involved in transcription regulation, transcription rate is shown to be the most important parameter affecting steady state production of mRNA and protein in the cell. In particular, doubling the transcription rate approximately doubles the number of mRNA synthesized at steady state for any rates of transcription inhibition and activation. On the other hand, increasing the rate of transcription inhibition by 10% reduces the average steady state count of mRNA by about 7%, whereas changes in the rate of transcription activation appear to have no such effect. Furthermore, for wide range of reaction rates in the kinetic model of the lac genetic switch considered, protein production was observed to always reach a maximum before the degradation reduces its count to zero, and this maximum was found to be always at least 27 protein molecules. Such value appears to be a fundamental structural property of genetic circuits making it very robust against changes in the internal and external conditions.

Keywords: *Escherichia coli*, lac genetic circuit, steady state synthesis, transcription.

1. Introduction

One of the main challenges in system biology is to elucidate design principles of gene regulation networks [1]. It is therefore of interest to study properties of regulatory structures or motifs which frequently compose regulatory networks [2]. Understanding the function of recurring regulatory motifs can shed light on the design of biological systems in which they appear. One such common motif implements a negative auto-regulation where transcription factors negatively regulate their own transcription [1]. This motif is present in over 40% of transcription factors known in *E. coli* [3]. In addition, understanding the relationship between the dynamics of mRNA and protein production and the rates of biochemical reactions comprising genetic circuits is fundamental in explaining the biological behavior of these circuits [4].

Synthesis of both mRNA and protein are outcomes of specific events, although their counts are only observed at selected (discrete) time instances giving rise to time series data. At time scales of interest, it is convenient to assume that mRNA and protein production is modeled by stochastic processes producing correlated molecule counts which are determined by the considered probability distributions and the functional model of gene expression. Moreover, for larger numbers of molecules, it is also convenient to approximate discrete molecule counts by continuous random variables in order not to exclude probability distributions for continuous random variables from the model. The probability distributions of molecule counts are obtained from single-molecule experiments which can be carried out at each stage of gene expression [5]. The frequency at which the bursts of mRNA and proteins are synthesized in the cell is more informative than the mean alone. General mechanisms of gene expressions can be inferred from mathematical models and the observed distributions. The models describing kinetic properties of genetic circuits can be either deterministic or stochastic [5-8], and they can assume continuous approximations, however, the kinetic mechanisms remain the same [9]. Stochasticity of gene expression emerges from random events in transcription and translation processes. The stochasticity can be vital for cell survival when the environmental conditions fluctuate [10]. Discovering protein interactions forming regulatory networks is important to understand dynamics of gene expression [1].

Transcription of a DNA strand is carried out by a multi-subunit DNA-dependent RNA polymerase (RNAP) [1] (Figure 1A). In the lac operon of *E. coli*, a separate regulatory gene (*lacI*) encodes the lac repressor which plays a pivotal role in operon control [11, 12] (Figure 1B). Switching between active and inactive state in gene circuits does not occur spontaneously [11]. In particular, repressor complexes bind to the operator to prevent RNAP to initiate transcription. Therefore, repressor-operator complex switches the lac circuit to inactive state whereas unbinding of the repressor from the operator switches the lac circuit back to active state [13]. Although regulatory events can affect every step of macromolecular synthesis within the cell, transcription initiation represents the most important control step which can be exploited to switch the gene and subsequent protein production on and off. In-vivo single-molecule measurements provided ample evidence that transcription initiation is a sequential process which plays a key role in mRNA dynamics subsequent protein synthesis [22]. It is also crucial to know how switching

production from a Poission process [17]. Transcription is one of the key steps governing dynamics of gene expression [18, 19]. In general, studying mRNA and protein synthesis in the cell and how it is affected by reaction rate values can (1) elucidate how the cell responds to internal and external stimuli, (2) motivate lab experiments to more precisely determine rates of the most essential reactions, and (3) inform techniques of synthetic biology how to effectively modify functions of gene circuits.

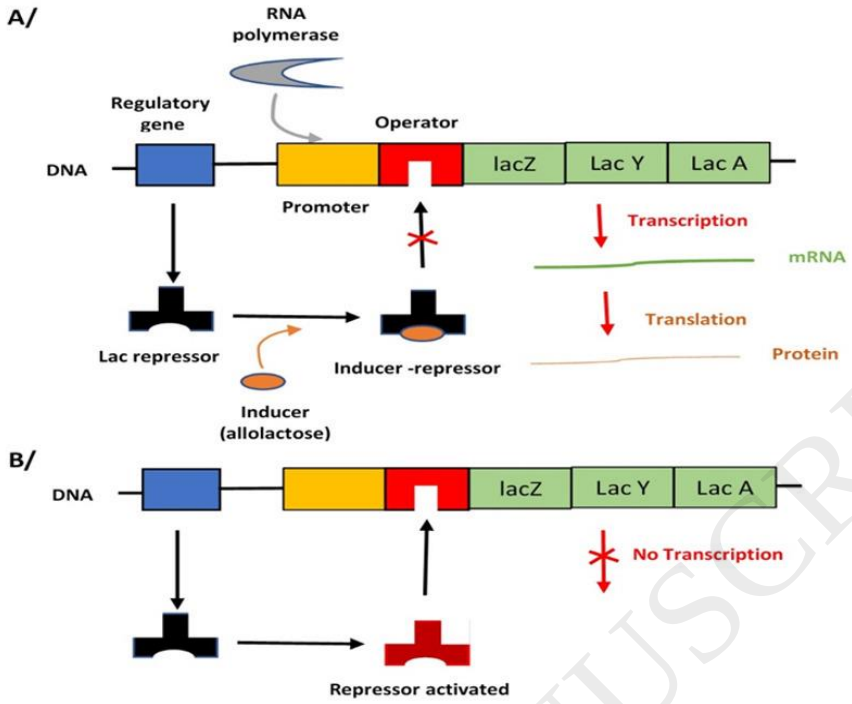

Figure 1. Model of operon regulation in the lac circuit of *E. coli*. (A) Transcription activation by binding of RNAP to the promoter. (B) Transcription inhibition by binding of activated repressor to the operator.

Our aim is to elucidate how reaction rates influence dynamics of the lac circuit by quantifying mRNA and protein synthesis. Ideally, insights from lab experiments, theory and computer simulations to study biological systems are aligned and can inform each other [23]. Stochastic kinetic models of genetic circuits are often assumed to provide more realistic insights than the models involving rate equations, even though the former are computationally and analytically expensive even for biochemical networks of moderate sizes [24]. Moreover, analytical solutions often require approximations, and they may become intractable when there are multiple or multi-level feedback control loops. The biochemical model of lac circuit considered in our work contains only a single feedback loop. However, the number of reaction rates in this model is relatively large, so we resort to stochastic simulations to study its steady-state dynamics. Specifically, we devise efficient sampling strategies of multi-dimensional space of reaction rates by utilizing the structure of lac circuit model. Specifically, we observed that regulatory reactions pertaining to gene activation and inactivation can be scaled jointly with no apparent loss of generality. This greatly reduces dimension of the parameter space of reaction rates to be explored. The values of reaction rates are uniformly sampled from specified intervals. In order to distinguish among different sampling sub-spaces, each sampling strategy of reaction rates is labeled by a model number. Productions of mRNA and protein are studied separately assuming different sampling strategies and numerical experiments. Biochemical reactions comprising the kinetic model of lac genetic switch are given in [13].

In this paper, we investigate how transcription and regulatory reaction rates affect mRNA and protein production in the lac circuit of *E. coli*. We explore how changes in transcription and translation rates modulate both mRNA and protein synthesis. We determine conditions when steady state production of mRNA and protein exist by measuring the mean molecule counts. This extends the results presented in [21, 26-28] on factors affecting mRNA production in *E. coli*. In general, it is challenging to rigorously define steady state of mRNA or protein production, especially for long-term dynamic processes or processes occurring at multiple time scales such as continuous proliferation of cells. However, it is well-known that mRNA levels at steady state determine steady-state protein levels [29].

2. Methods

2.1. Modeling and simulation framework

Kinetic model of the lac genetic switch considered in this paper is fully defined in [25] and [13], and,

values from which chosen reaction rates are uniformly sampled while all other reaction rates have their default values. We emphasize that all reaction rates in all simulations are kept constant. It is a different problem than, for example, investigating a genetic switch in [31] where some reaction rates are allowed to fluctuate during the cell lifetime. Moreover, we do not consider the problem how to estimate reaction rates from observed output molecule counts, nor we search for optimum rate values, for example, to maximize protein production under given conditions.

At steady state, time averages of species counts are equal to ensemble averages of random species counts observed over independent system time evolution traces [30]. In order to map reaction rates to steady state counts of mRNA and protein, we assume sampling strategies of reaction rates referred to as Model R1, R2 and R3 for analysis of steady state mRNA production, and rate sampling strategies denoted as Models P1, P2 and P3 for analysis of protein production. Models R1 and P1 represent baseline systems with unconstrained stochastic switching between active and inactive states and with all reaction rates having their default values. Model R2 explores the effects of gene inactivation rate on mRNA synthesis by assuming the default gene activation rate while the rate of transcription inhibition is sampled from a specified interval. Model R3 analyzes the effects of gene activation rate on mRNA synthesis by assuming the default gene inactivation rate while sampling the rate of transcription activation. Motivated by observation from [32] that the protein responsible for regulation of gene expression could be either locked in active or inactive state, we assume rate sampling Model P2 which has all reactions of unbinding repressor complexes from the operator removed, so the lac circuit is permanently locked in inactive state. Similarly, Model P3 has reactions of binding repressor complexes to the operator removed, so the lac circuit is always kept in active state. All other reactions in Models P2 and P3 otherwise remain unchanged, and have their default values.

Numerical experiments were performed in Lattice Microbe software version 2.3 which was downloaded and compiled with a GPU support on Fedora 25 Linux workstation. Time evolution traces of molecular species counts in a biochemical network of the lac circuit were generated independently using the Gillespie algorithm. The number of independent traces at each numerical experiment was set to be 100 unless stated otherwise. All simulations were performed over *E. coli* half lifetime which is $T = 1$ h [10]. All simulations also have the same initial species counts which are specified in [13].

In order to identify steady state of mRNA and protein production when evaluating observed molecule counts, we assume the definition provided in [33]. In particular, denote the observed time series of molecule counts as, $\{x_i\}_{i=1}^{T}$, and the associated weights, $\{w_i\}_{i=1}^{T}$. The steady state production can be then reliably detected by observing the weighted mean μ:

$$\mu = \frac{1}{\sum_{i=1}^{T} w_i} \sum_{i=1}^{T} w_i x_i \tag{1}$$

In our experiments, we observed that mRNA steady state occurs between 50 s and 1600 s from the initial simulation time for all reaction rate values considered, we assumed that 1600 s is sufficient time to consider production of both mRNA and protein to be in steady state.

In inactive state, the repressor can temporarily, for a short period of time, come off the operator before it re-binds again. During such event, already bound RNAP may initiate the transcription [34]. Such so-called basal synthesis is occurring at a rate of approximately one mRNA molecule over the cell half lifetime. Furthermore, there exists a maximum transcription rate which can be supported by the lac circuit in inactive state. Even for such maximum transcription rate, synthesized mRNA can be completely degraded or reach steady state while the lac circuit is still in inactive state. However, when the lac circuit switches to active state, transcription rate is significantly increased, and the minimum supported transcription rate required to reach steady state for mRNA in active state must be greater than the maximum supported transcription rate in inactive state [11, 35]. Moreover, previous experimental results such as those in [7] and [36] reported that, in active state, steady state mRNA production in the lac circuit can reach at most 50 mRNA molecules which can simultaneously exist in the cell.

2.2. Mapping regulatory reaction rates to mRNA synthesis

The mRNA steady state count is a function of kinetic rates K_{on} and K_{off} corresponding to repressor binding to the operator (the lac circuit turns to inactive state) and unbinding of repressor from the operator (the lac circuit switches to active state), respectively [37]. Thus, a regulatory state transition model of gene expression is simply:

$$\text{"On"} \underset{K_{off}}{\overset{K_{on}}{\rightleftarrows}} \text{"Off"}$$

In active state, transcription, translation and degradation rates are key parameters controlling mRNA and

jointly. In other words, we observed that, for arbitrary values of reaction rates within the same group, the same regulatory effect on steady state synthesis can be achieved by jointly scaling their default values. Hence, we consider the following two groups of regulatory reaction rates:

$$K_{on} = \{k_{ron}, k_{iron}, k_{i2ron}\} \text{ and } K_{off} = \{k_{roff}, k_{iroff}, k_{i2roff}\}.$$

The set K_{on} contains regulation reaction rates to switch the lac circuit to inactive state while the set K_{off} contains reaction rates to switch the lac circuit to active state. Parameters k_{ron}, k_{iron}, and k_{i2ron} in K_{on} are, respectively, the rates of binding inducer-repressor species R_2, IR_2 and I_2R_2 to the operator. Similarly, parameters k_{roff}, k_{iroff}, and k_{i2roff} in K_{off} represent rates of unbinding inducer-repressor species from the operator. Default values of these rates are given in [13], and also summarized in Table 1 where they are denoted as $K_{on\text{-}d}$ and $K_{off\text{-}d}$. The default rates $K_{on\text{-}d}$ and $K_{off\text{-}d}$ were obtained from single molecule in vivo experiments [13]. For convenience, all rates and variables considered in our work are summarized in Table S2 of Supporting Information.

Table 1. Regulation reactions in the lac circuit and their default rates [13]**.**

Transcription inactivation	**Rate set** $K_{on\text{-}d}$ ($M^{-1}s^{-1}$)
$R_2 + O \rightarrow R_2O$	$k_{ron} = 2.4e+06$
$IR_2 + O \rightarrow IR_2O$	$k_{iron} = 1.2e+06$
$I_2R_2 + O \rightarrow I_2R_2O$	$k_{i2ron} = 2.4e+04$
Transcription activation	**Rate set** $K_{off\text{-}d}$ (s^{-1})
$R_2O \rightarrow R_2 + O$	$k_{roff} = 6.3e-04$
$IR_2O \rightarrow IR_2 + O$	$ki_{roff} = 6.3e-04$
$I_2R_2O \rightarrow I_2R_2 + O$	$k_{i2roff} = 3.1e-01$

The grouping of parameters enables to scale them jointly as:

$$K_{on}(a) = a \times K_{on\text{-}d} = \{ak_{ron}, ak_{iron}, ak_{i2ron}\}, \text{ and}$$
$$K_{off}(a) = a \times K_{off\text{-}d} = \{ak_{roff}, ak_{iroff}, ak_{i2roff}\} \quad (2)$$

where $a>0$ is referred to as variation coefficient of the default sets $K_{on\text{-}d}$ and $K_{off\text{-}d}$. The parameters in these sets can be then sampled jointly by sampling the value of a. The regulatory rate samples are selected uniformly from a 3-dimensional hypercube:

$$K_{on} \subseteq (k_{on\text{-}min}, k_{on\text{-}max})^3 \text{ and } K_{off} \subseteq (k_{off\text{-}min}, k_{off\text{-}max})^3 \quad (3)$$

where $k_{on\text{-}min}$ and $k_{on\text{-}max}$ are the minimum and maximum rate values for transcription inhibition, and $k_{off\text{-}min}$ and $k_{off\text{-}max}$ are the minimum and maximum rate values for transcription activation, respectively. Hence, the same minimum and maximum values are assumed for all three repressor species:

$$k_{on\text{-}min} = \{k_{ron\text{-}min}, k_{iron\text{-}min}, k_{i2ron\text{-}min}\}$$
$$k_{on\text{-}max} = \{k_{ron\text{-}max}, k_{iron\text{-}max}, k_{i2ron\text{-}max}\}$$
$$k_{off\text{-}min} = \{k_{roff\text{-}min}, k_{iroff\text{-}min}, k_{i2roff\text{-}min}\}$$
$$k_{off\text{-}max} = \{k_{roff\text{-}max}, k_{iroff\text{-}max}, k_{i2roff\text{-}max}\}.$$

In order to evaluate the mRNA abundance dependence on regulatory rates, we consider sampling Models R1, R2 and R3 in Experiment 1 as defined in Table 2. Specifically, Experiment 1 evaluates Model R1 having default rates for gene inactivation and activation, Model R2 assumes 100 random samples of gene inactivation rate, and Model R3 assumes 100 random samples of gene activation rate. Gene activation and inactivation rates K_{off} and K_{on} were uniformly sampled assuming $k_{on\text{-}min} = 10^{-1}K_{on\text{-}d}$, $k_{on\text{-}max} = 10^{2}K_{on\text{-}d}$, $k_{off\text{-}min} = 10^{-1}K_{off\text{-}d}$, and $k_{off\text{-}max} = 10^{2}K_{off\text{-}d}$. For each set of sampled reaction rates, mRNA steady state count was determined by simulations. Samples of transcription rate k_{tr} were obtained within the range of supported transcription rates in active or inactive state, respectively.

Table 2. Sampling models to study steady state mRNA abundances.

	Rate	**Model R1**	**Model R2**	**Model R3**
Experiment 1	K_{off}	default	default	100 samples
	K_{on}	default	100 samples	default
	k_{tr}	1,000 samples	1,000 samples	1,000 samples
	# simulations	1,000	100,000	100,000

$$\frac{dm(t)}{dt} = \frac{K_{on}}{K_{on}+K_{off}} \cdot \frac{\delta_A}{V} + \frac{K_{off}}{K_{on}+K_{off}} \cdot \frac{\delta_R}{V} - \gamma_m m(t) \quad (4)$$

where V, δ_A, and δ_R represents, respectively, the cell volume, the average value synthesis for activated gene circuit, and the average synthesis for repressed gene circuit. At steady state, mRNA production does not vary, so that $dm_{ss}(t)/dt = 0$, and Eq. (4) becomes:

$$m_{ss}(t) = \frac{1}{\gamma_m}\left[\frac{K_{on}\delta_A + K_{off}\delta_R}{(K_{on}+K_{off})V}\right] \quad (5)$$

for

$$k_{tr} = \frac{K_{on}\delta_A + K_{off}\delta_R}{(K_{on}+K_{off})V}, \text{ and } \; m_{ss}(t) = k_{tr}/\gamma_m \quad (6)$$

Eq. (5) defines a deterministic linear relationship between regulatory kinetics rates and mRNA abundance at steady state. Eq. (6) presents a linear dependence between mRNA abundance at steady state and the transcription rate. Consequently, Eq. (5) and (6) predict that mRNA steady state abundances increase linearly with transcription rate.

2.4. Mapping rates of regulatory reactions to protein production

Understanding the relationship between regulatory reaction rates and protein production can reveal how much the lac circuit is robust against changes in regulation mechanisms, for example, due to genetic mutations, or due to changes in the intracellular and extracellular conditions. We designed numerical experiments to study this relationship similar to the experiments for investigating mRNA. The corresponding sampling strategies of reaction rates are denoted as Model P1, Model P2, and Model P3. These models are defined in Table 3. Again, unless stated otherwise, all other parameters have their default values given in [13]. Recall that the reactions considered and their rates are summarized in Table S3 in Supporting Information.

In Experiment 2, samples of transcription rates were generated from the range of transcription rates which are supported in inactive state (Model P2) or in active state (Model P3). These ranges of transcription rates are reported in Results. Transcription rates for Model P1 are assumed to be the same as for Model P3. Simulations were performed for each sample of transcription rate and all three sampling models considered. Experiment 3 is similar to Experiment 1, except now both K_{on} and K_{off} are sampled in given intervals. In particular, the sampling intervals defined in (3) assume $k_{on\text{-}min} = 10^{-1}k_{ond}$, $k_{on\text{-}max} = 10^{2}k_{ond}$, $k_{off\text{-}min} = 10^{-1}k_{offd}$, and $k_{off\text{-}max} = 10^{2}k_{offd}$. Experiment 4 explores the dependency of protein production on the protein degradation rate. The protein degradation rate was uniformly sampled from the interval $(2^4 k_{degpd},\ 10^3 k_{degpd})$ where k_{degpd} denotes the default value. In Experiment 5, we combined the samples of protein degradation rate generated for Experiment 4 with a new set of 100 samples of transcription rates as they were generated for Model P3 in Experiment 2. Finally, in order to assess the influence of translation rate on protein steady state abundance by Model P1 in Experiment 6, we first selected 100 representative transcription rate values while translation rate was uniformly sampled from the interval $(10^{-2}k_{tnd},\ 10^{2}k_{tnd})$ where k_{tnd} is the default translation rate.

Table 3. Sampling models to study steady state protein abundances.

	Rate	Model P1	Model P2	Model P3
Experiment 2	K_{off}	default	-	default
	K_{on}	default	default	-
	k_{tr}	1,000 samples	1,00 samples	1,000 samples
	# simulations	1,000	1,00	1,000
Experiment 3	K_{off}	100 samples	-	100 samples
	K_{on}	100 samples	100 samples	-
	# simulations	10,000	100	100
Experiment 4	k_{degp}	100 samples	100 samples	100 samples
	# simulations	100	100	100
Experiment 5	k_{tr}	-	-	100 samples
	k_{degp}	-	-	100 samples
	# simulations	-	-	10,000
Experiment 6	k_{tr}	100 samples	-	-
	k_{trans}	1000 samples	-	-

inactive state corresponds to the observed limit of at most 2 mRNA molecules generated by basal synthesis. Both these molecules are completely degraded during the cell half lifetime, $k_{tr\max}^{inac} = 0.021s^{-1}$. Therefore, in active state, the lac circuit must produce at least 2 mRNA molecules. We found that the measured transcription rate in active state is independent of particular inducer-repressor species present in the cell. Assuming that the maximum number of mRNA molecules synthesized in active state is about 50 [36], the corresponding maximum supported transcription rate is, $k_{tr\max}^{ac} = 0.504s^{-1}$. Figure 2 depicts basal mRNA synthesis in inactive state and mRNA synthesis in active state of the lac circuit. The corresponding measured ranges of transcription rates in both active and inactive states are also highlighted in Figure 2. Additional simulation results are presented in Figures S1 and S2.

3.2. Transcription rate controls mRNA abundance at steady state

The effects of transcription rate and gene inactivation rate on steady state mRNA synthesis were studied in Experiment 1. The 100 uniformly distributed samples of inactivation rates K_{on} were generated while activation rates K_{off} were kept at their default values. For each of 100 rate samples of K_{on}, the mRNA count synthesized at steady state was determined by simulation. For clarity of presentation, we chose the following 4 representative samples of K_{on} to illustrate the main results:

$$K_{on1}=K_{on}(10^{-1}),\ K_{on2}=K_{on}(1),\ K_{on3}=K_{on}(10),\ \text{and}\ K_{on4}=K_{on}(10^{2}).$$

Since $k_{tr}^{inac} \ll k_{tr}^{ac}$, 100 uniformly distributed samples of transcription rates for inactive state were generated from the interval $k_{tr}^{inac} = \left[k_{tr\min}^{inac}, k_{tr\max}^{inac}\right]$, and 1000 uniformly distributed samples of transcription rates for active state were generated from the interval $k_{tr}^{ac} = \left[k_{tr\min}^{ac}, k_{tr\max}^{ac}\right]$. For each sample of K_{on}, simulations were used to measure the mean mRNA count synthesized at steady state. Figure 3A shows the measured mRNA counts versus transcription rates for 4 selected values of transcription inactivation rates $K_{on}(a)$ defined in (1).

Considering deterministic model descripted in Eq. (5) and (6), we assume the *E. coli* cell volume $V = 1\mu m^3$ [40], and $\delta_A = 50$, and $\delta_R = 5$ [39]. Figure 3B shows the measured mRNA counts versus transcription rate for 4 selected values of transcription inactivation rates.

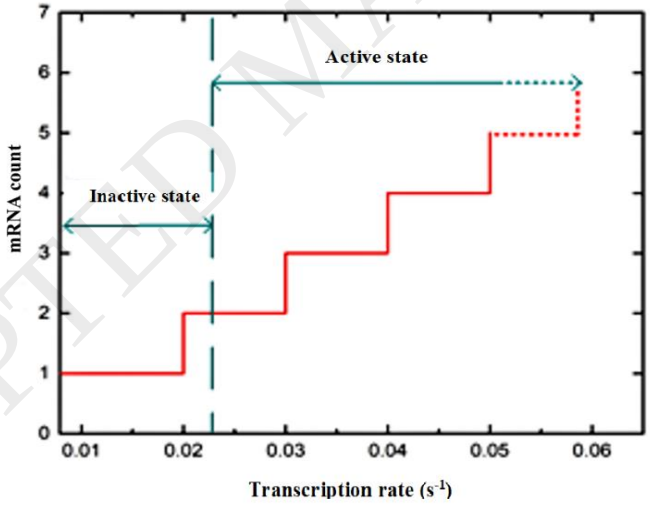

Figure 2: Transcription rates attainable in active and inactive state of the lac circuit. The red curve represents the number of mRNA molecules synthesized at given transcription rates (s^{-1}). The transcription rates in the range (0.00787, 0.021) (s^{-1}) defines basal synthesis of mRNA in inactive state. Active state experiences transcription rates at least 0.021 s^{-1} whist their maximum is unbounded. ***Graph origin:*** $7.87.10^{-3}$ on x-axis and 0 on y-axis

Due to very small number of mRNA molecules produced in inactive state (at most 2), the impact of transcription and gene regulation rates on mRNA synthesis in inactive state cannot be perceived easily. However, the results obtained in Experiment 1 can be summarized as follows. First, the number of mRNA molecules synthesized at steady state grows approximately linearly with transcription rate. Thus, provided that transcription rate k_{tr} produces N mRNA molecules at steady state, transcription rate $2\times k_{tr}$ produces about $2\times N$ mRNA molecules at steady state. This behavior was observed over a wide range of samples K_{on}. This result is somewhat intuitive (despite the presence of a positive feedback loop), and is also confirmed by the deterministic model in Eq. (5) and (6).

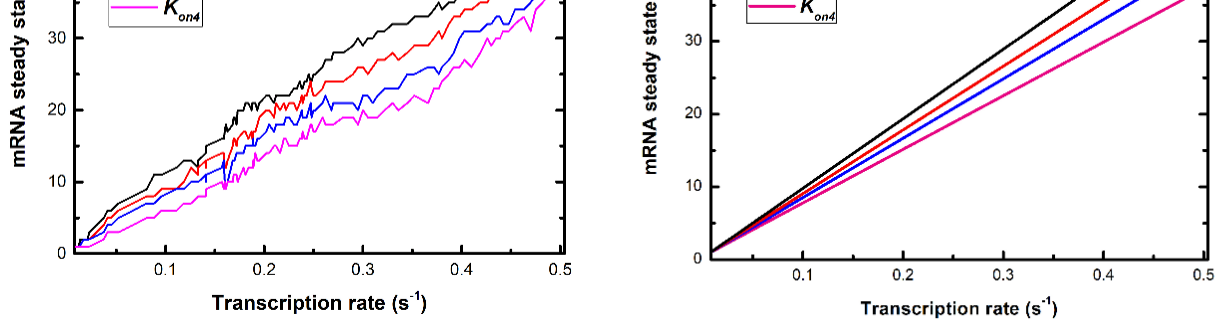

Figure 3. Steady state mRNA abundances for different transcription rates and gene inactivation rates. Transcription rates are modulated by selected inactivation rates K_{on1}, K_{on2}, K_{on3}, and K_{on4} shown as black, red, blue and purple lines, respectively. (A) Simulated stochastic model. (B) Deterministic model. ***Graph origin:*** $7.87.10^{-3}$ on x-axis and 0 on y-axis

We can conclude that there is a linear relationship between transcription rate and the steady state mRNA count. However, there is an inverse relationship between the number of mRNA synthesized at steady state and the scaling of reaction rates K_{on}. More specifically, the mRNA count at steady state slightly decreases when the rates K_{on} are scaled up by any factor greater than one. Additional simulation results to illustrate this behavior are provided in Figure S3.

3.3. Rates of transcription activation do not affect mRNA synthesis

We now investigate sampling Model R3 defined in Table 2, assuming that rates K_{on} have their default values while 100 random samples of rates K_{off} are uniformly generated from the interval ($k_{off\text{-}min}$, $k_{off\text{-}max}$) = (10^{-1},10^{2}). In addition, for each sample K_{off}, 1000 uniformly distributed samples of transcription rates were generated from the interval $k_{tr}^{ac} = \left[k_{tr\,\min}^{ac}, k_{tr\,\max}^{ac} \right]$ where $k_{tr\,\min}^{ac} = 0.021 s^{-1}$ and $k_{tr\,\max}^{ac} = 0.504 s^{-1}$. Overall, 1e5 simulations were performed in total in order to evaluate mRNA steady state abundances. For the sake of presentation clarity, we report the results for the following 4 representative samples of transcription activation rates:

$K_{off1} = K_{off}(10^{-1})$, $K_{off2} = K_{off}(1)$, $K_{off3} = K_{off}(10)$, and $K_{off4} = K_{off}(10^{2})$.

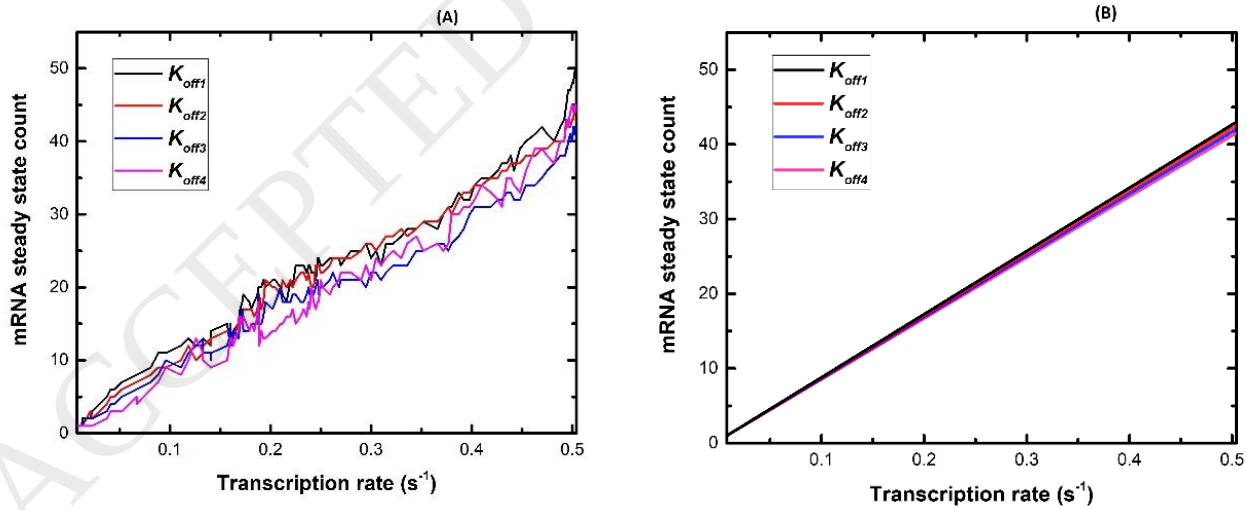

Figure 4. mRNA steady state abundances for varying transcription rates and 4 selected gene activation rates. The black, red, green and blue lines are mRNA steady state counts corresponding to rates K_{off1}, K_{off2}, K_{off3}, and K_{off4}, respectively, while rates K_{on} have their default values. (A) Simulated stochastic model. (B) Deterministic model. ***Graph origin:*** $7.87.10^{-3}$ on x-axis and 0 on y-axis

The mRNA steady state counts for different transcription rates and 4 selected rates K_{off} are shown in Figure 4A. We can make two observations from Figure 4A. First, we found that the number of mRNA molecules synthesized at steady state grows approximately linearly with transcription rate for all rate

3.4. Synthesized protein abundances in the lac circuit

Unlike Models R2 and R3 in Experiment 1, protein production is assessed while the lac circuit either freely switches between active and inactive state (Model P1), or while it is locked in inactive state (Model P2) or in active state (Model P3). Enforcing active or inactive state is achieved by removing corresponding reactions from the model, or by setting their reaction rates to zero. In particular, in order to keep the lac circuit in inactive state (Model P2), unbinding reactions of repressor complexes in Table 1 are removed. Similarly, the lac circuit is kept in active state (Model P3) by removing binding reactions of repressor complexes in Table 1. Assuming Experiments 2 and 3 with default reaction rates, Figure 5 presents the measured protein counts synthesized the cell half lifetime of 1 hour for all three Models P1, P2 and P3 considered. We found that, in full kinetic Model P1 as well as in Model P2, the protein counts did not reach steady state, but they attained a maximum before their gradual degradation which came into effect after some delay (Figure 5Aa and 5Ab). Model P3 shows similar behavior (Figure 5Ac). In order to investigate when steady state of protein synthesis occurs, we varied protein degradation rate between 2.1e-4 s^{-1} and 14.4e-3 s^{-1} while all other parameters had their default value. Even though the protein experiences a complete degradation in Model P1 (Figure 5Ba) and in Model P2 (Figure 5Bb), we found that steady state protein production only occurs when the lac circuit is locked in active state (Model P3, Figure 5Bc). Moreover, the maximum protein count produced in Model P3 is always larger than in Model P1. For example, Figure 5Aa and Figure 5Ac show maxima of 160 proteins in Model P1 versus 190 proteins in Model P3, respectively.

We now investigate how the maximum protein count synthesized in the lac circuit evolves when transcription rate is increased for all three models considered. Hence, we generated 1000 uniformly distributed samples of transcription rates for Models P1 and P3 but only 100 of such samples for Model P2 (Experiment 2 in Table 3). We found that whether the lac circuit is in active or inactive state, the maximum protein count synthesized before degradation increases with transcription rate for all three Models P1, P2 and P3. Additional simulation results are presented in Figure S5.

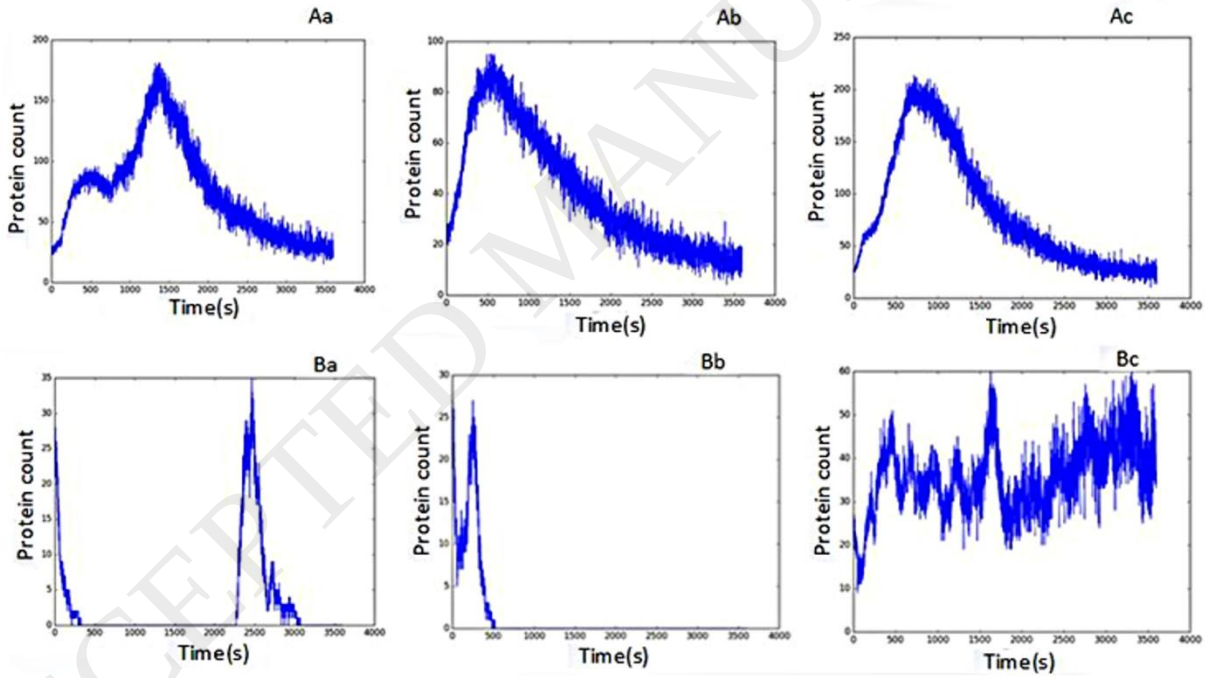

Figure 5. Protein counts synthesized in Models P1, P2 and P3 with default reaction rates. (a) Unconstrained Model P1, (b) Model P2 which remains in inactive state, and (c) Model P3 which is locked in active state. (A) The default protein degradation rate 2.1e-4 s^{-1}, and (B) the protein degradation rate is changed to 14.4e-3 s^{-1}. All other parameters have their default values. ***Graph origin:*** 0 on x-axis and 0 on y-axis

3.5. Steady state protein synthesis in active state

From the results presented so far, it is apparent that increasing protein degradation rate is an important factor for the emergence of steady state protein synthesis. Assuming Model P3 when the lac circuit is locked in active state, we investigate the relationship between protein degradation rate, transcription rate, and steady state protein counts (Experiment 5 in Table 3). Transcription rates k_{tr} were selected from the interval $[k_{tr0},\ 8k_{tr0}]$ where $k_{tr0} = k_{trd}/2^2$, and k_{trd} represents the default transcription rate, i.e., $k_{tr} \in$ (0.0315, 0.504) s^{-1}. The 100 samples of protein degradation rates were generated uniformly from the interval $(2^4 k_{degpd},\ 10^3 k_{degpd})$ where k_{degpd} is the default protein degradation rate. For all pairs of transcription and degradation rates, we found that steady state protein count in Model P3 varies between 1 and 70. Steady

Table 4. Protein counts synthesized at steady state in Model P3.

$k_{degp}(s^{-1})$	$k_{tr}(s^{-1})$				
	0.0315	**0.063**	**0.126**	**0.252**	**0.504**
3.36e-3	69				
6.72e-3	36	68			
13.44e-3	18	36	66		
26.88e-3	9	18	36	67	
53.76e-3	4	8	18	35	69
107.52e-3	2	4	8	17	37
215.04e-3	1	2	4	8	19
430.08e-3		1	2	4	9
860.16e-3			1	2	5
1720.32e-3				1	2
3440.64e-3					1

3.6. Maximum protein counts synthesized before their degradation

We further investigated how the maximum protein count which is produced before their degradation is affected by other reaction rates (Experiment 6 in Table 3). We assume full kinetic Model P1 to evaluate the relationship between protein production and translation rate. We generated 100 transcription rate samples from the interval $(k_{tr0}, 8k_{tr0})$. For each transcription rate, 1000 uniformly sampled translation rates were generated from the interval (k_{transd}/100, 10k_{transd}) with the default rate k_{transd} = 4.44e-2 s^{-1}. The simulation results for all 100 samples of transcription rates are similar; therefore, we chose 4 representative samples of transcription rates as, $k_{tr}(n) = 2^n \times k_{trd}$ where n={-2,-1,0,1}, so $n=0$ corresponds to the default value of 0.126 s^{-1}. We can make three important observations about protein production in the lac circuit from these results. First, increasing transcription rate increases the maximum number of proteins produced before their degradation (Figure 6A). Second, increasing translation rate increases slightly the maximum number of proteins produced before degradation (Figure 6A). Third, regardless of reduction in transcription and translation rates, the maximum number of proteins produced before the degradation is always at least 27 molecules (Figure 6B and 6C). Additional simulation results are provided in Figure S7.

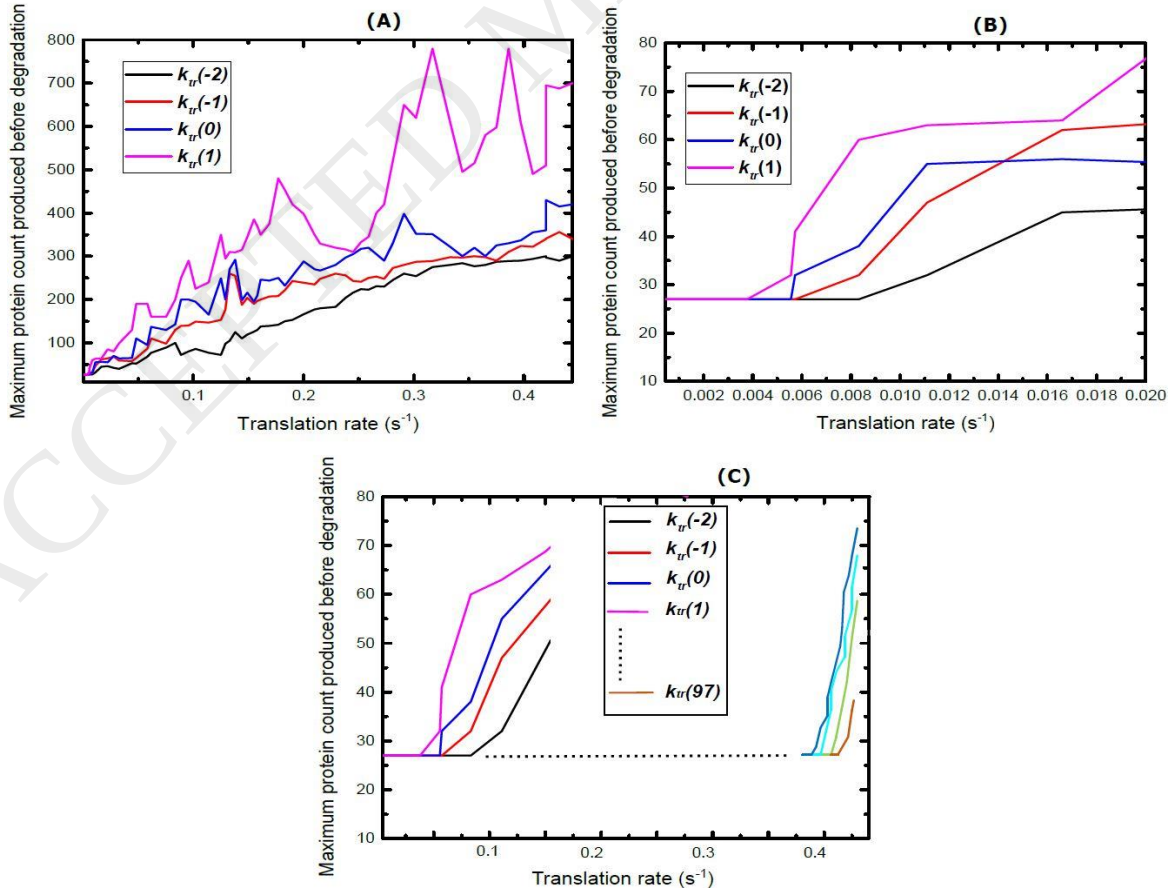

Figure 6. The maximum protein counts produced versus translation rate in Model P1. The 4 transcription rates $k_r(-2)$, $k_r(-1)$, $k_r(0)$ and $k_r(1)$ has value 0.31e^{-1} s^{-1}, 0.63e^{-1} s^{-1}, 1.26e^{-1} s^{-1}, and 2.52e^{-1} s^{-1}, respectively. The mRNA and the protein degradation rates have their default values. (A) The maximum

on y-axis, (C) $2.27 \cdot 10^{-3}$ on x-axis and 10 on y-axis

4. Discussion

Several recent studies point out that gene activity is normally bursty rather than Poissonian [41, 42]. The bursty nature of gene expression can be effectively modeled by a 2-stage or 3-stage model [42, 43]. Such models can be described by ordinary differential equations (Section 2.3) and solved analytically to obtain mean mRNA and protein production at steady state. Dependency of deterministic and stochastic models on reaction rates were compared in Figure 3 and Figure 4. Both models are showing good agreement in predicting linear dependency of the mean mRNA steady state production on transcription rate. The agreement between these two models is better when rates K_{on} are varied while rates K_{off} have their default values. Stochastic model can be also used to obtain probabilistic distributions of molecule counts in transition to and at steady state. The distribution of molecule counts is more informative than the mean counts in cases when the distribution is asymmetrical and highly skewed.

Our objective is to investigate how mRNA synthesis is modulated at steady state by rates controlling transcription activation and inhibition. Increasing rates K_{on} to improve the probability that the lac circuit is in inactive state showed a slight decrease of steady state mRNA counts. On the other hand, varying rates K_{off} affecting the probability of active state did not exhibit any noticeable effect on steady state mRNA synthesis. We then also considered steady state protein production. We found that protein production exhibits steady state only when the lac circuit is permanently locked in active state regardless whether the protein degradation is considered or not. Furthermore, for all reaction rates considered in Models P2 and P3, the protein counts produced at steady state are always at most 70 molecules. In full kinetic Model P1, time evolution of protein always exhibits a maximum before the degradation comes into effect. This maximum value appears to be never exceeded under any conditions or changes in reaction rate values.

Considering mRNA synthesis, we found that mRNA steady state abundances are strongly correlated with the rate of gene expression in inactive state for all rates assumed in Model R2. In particular, mRNA steady state abundance increases slightly when rates K_{on} are reduced. Provided that transcription is modeled as a Poisson process, it was shown in [44] that the probability of a single transcription event producing one mRNA molecule is proportional to the number of active genes. Thus, we can conclude that increasing rates K_{on} slightly reduces the probability of mRNA transcription. Our numerical results quantify how transcription regulation affects gene expression in the lac circuit. The rate at which the gene is transcribed is controlled by RNAP which binds to a specific DNA site known as the promoter (Figure 1) [45]. The affinity of RNAP binding to the promoter determines the gene transcription rate. Specific RNAP normally transcribes different genes. Binding affinity of RNAP to the promoter is crucially affected by the presence of inducer-repressor species at the operator [46]. Even in inactive state, the inducer-repressor may temporarily unbind from the operator which enables transcription to be initiated before the repressor again rebinds the operator. In active state, the rate of unbinding repressor complex from the operator does not affect transcription rate, as the rebinding of repressor complex to the operator rarely occurs, so the probability of temporarily blocking transcription is very small.

A number of previous studies assumed that transcription rate is one of the main factors determining mRNA abundance at steady state [21, 26-28]. We extended these studies by showing that, regardless of the rates assumed in the lac operon regulation, increasing transcription rate increases steady state mRNA synthesis. Specifically, doubling transcription rate approximately doubles steady state mRNA count over wide range of regulatory reaction rate values. Additional results are shown in Figures S3 and S4. Although there are multiple reactions involved in transcription regulation, a slight moderation of transcription rates have a linear effect on mRNA count produced at steady state. Therefore, we can claim that transcription rate represents the most important parameter affecting mRNA production at steady state.

Understanding why transcription rate is the most important parameter affecting gene expression is fundamental to elucidating design structure of genetic circuits. In particular, a popular coarse-grained 3-stage model of protein synthesis involves only these 6 reactions: switching of gene between active and inactive states, transcription of mRNA, translation of protein, and independent degradation of mRNA and protein. Such model is mainly motivated to mimic bursty production of mRNA and protein which has been observed in many living cells. More importantly, 3-stage model can be considered universally as a first order approximation of gene expression in all prokaryotic and eukaryotic cells. In other words, no matter how complex gene regulatory network there is, it can always be approximated by a simple 3-stage model. Typical modeling strategy in research papers appears to be to keep a given value of transcription rate determined from some experiment from fitting observed data, regardless whether more regulatory reactions are later added to the model. Our numerical results indicate that rate of transcription is more important for gene expression dynamics than gene switching between active and inactive states. This could be intuitively explained as follows. Occasional gene activation which is interrupted by relatively long periods of inactivity must be exploited efficiently in order to produce enough mRNA for protein synthesis. Since one mRNA molecule can translate several protein molecules, transcription affects gene

regardless whether the protein degradation is assumed. Previous studies have shown that there is a positive correlation between protein production rate and protein degradation rate [47-49]. We carried out numerical experiments to obtain protein steady state counts in active state (Model P3) while protein degradation rate varies and transcription rate remains constant. Selected results are presented in Table 4.

Measurements of mRNA and protein synthesized in the cell were performed in [26]. It was shown that, generally, in both bacteria and eukaryotes, the cellular concentration of protein is positively correlated with abundance of the corresponding mRNA, although not very strongly, and that molecular abundances are affected by interplay between the rates of production and degradation. Similar conclusions were reached in [50]. It was further shown that 40% of variations in protein production can be explained by varying mRNA counts in the cell [51, 52]. Moreover, many proteins show production changes during different cell growth phases, and these changes seem to be coordinated with the rate of cell growth rather than the environmental conditions or protein function [50]. Our numerical experiments again confirmed that transcription rate is one of the key factors affecting the maximum number of proteins synthesized before their degradation (Figure 4A).

More importantly, regardless of the internal and environmental conditions which may modulate reaction rates of the intracellular processes, we found that, under wide range of reaction rates, a certain minimum number of protein molecules is guaranteed to be always synthesized in the cell. It indicates the existence of a safety margin for the cell to be able to always rely on some minimum level of protein production. This is shown in Figures 5B and 5C for several values of transcription rates. In case of the lac switch model considered, we found that the minimum number of proteins produced is at least 27 irrespective of how much translation and transcription rates were reduced while the protein degradation rate remained constant. Such observation can be used to understand the minimal guaranteed functionality of essential systems in the cell during different phases of the cell cycle [53].

We need to also point out some limitations of our modeling methodology. The dependency of mRNA and corresponding protein production is certainly more complex than assumed in our model (Figure 1). For instance, the cellular resources (energy, molecular material) are shared by mRNA transcription and protein translation processes, so reaction rates in models of genetic expression cannot be varied independently [41]. Correlations between protein production statistics and mRNA statistics were obtained in [54]. In our numerical experiments, we assumed constant mRNA and protein degradation rates while sampling other reaction rates. We confirmed that a widely adopted two-state gene activity model [41, 55] affects both mRNA synthesis at steady state as well as the maximum number of proteins produced in active state before their degradation occur.

In some cases the rate sampling intervals are not symmetrical with respect to a default rate value. Our reasoning was to bias rate sampling towards larger values if the default value was already rather small. This does not affect accuracy or bias our analysis in any way. The number of reaction rates in the assumed lac circuit model to be sampled was reduced by grouping some of reaction rates, and then scaling them jointly. We verified that this does not bias the analysis, provided that grouping of reaction rates is done properly. In particular, we claim that for any values of reaction rates in the group, there exists a scaling factor of default reaction rate values which have the same effect on transcription and translation dynamics. In case of the lac circuit, these groups of reaction rates were naturally identified as regulatory reactions. However, for more complex biochemical models, how to group reaction rates, so that they can be scaled jointly without biasing the analysis can be much more difficult problem to consider.

5. Conclusion

Transcription rate appears to be one of the key factors affecting steady state mRNA and protein productions in the lac circuit of *E. coli*. In particular, we found that doubling transcription rate approximately doubles mRNA count synthesized at steady state. In addition, varying reaction rates of transcription inactivation in the lac circuit resulted in only small changes of steady state mRNA synthesis. Thus, increasing the rate of transcription inactivation by 10% led to a 7% decrease of mRNA count at steady state. On the other hand, varying the reaction rates controlling transcription activation has no effect on mRNA production at steady state.

For all values of reaction rates considered, a two-state kinetic model of lac circuit always exhibits time evolution of protein counts with a single maximum before the degradation comes into effect. Our analysis of the maximum protein count revealed that, over a wide range of environmental and cellular conditions, or equivalently, over a wide range of reaction rates, the lac circuit is guaranteed to always synthesize a certain minimum number of protein molecules. For the model of lac circuit in *E. coli* considered, we observed that no matter how much transcription and translation rates are reduced, the lac circuit always produces at least 27 protein molecules.

Authors' contributions. PL supervised the study. KA prepared and performed all numerical experiments including evaluation and visualization of the results, and drafted the initial manuscript. PR administered the project, validated the results, provided valuable suggestions, and participated in writing the manuscript. All authors gave final approval for publication.

Competing interests. The authors declare they have no competing interests.

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