Conditional Cooperativity of Toxin - Antitoxin Regulation Can Mediate Bistability between Growth and Dormancy

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Abstract

Many toxin-antitoxin operons are regulated by the toxin/antitoxin ratio by mechanisms collectively coined “conditional cooperativity”. Toxin and antitoxin form heteromers with different stoichiometric ratios, and the complex with the intermediate ratio works best as a transcription repressor. This allows transcription at low toxin level, strong repression at intermediate toxin level, and then again transcription at high toxin level. Such regulation has two interesting features; firstly, it provides a non-monotonous response to the concentration of one of the proteins, and secondly, it opens for ultra-sensitivity mediated by the sequestration of the functioning heteromers. We explore possible functions of conditional regulation in simple feedback motifs, and show that it can provide bistability for a wide range of parameters. We then demonstrate that the conditional cooperativity in toxin-antitoxin systems combined with the growth-inhibition activity of free toxin can mediate bistability between a growing state and a dormant state.

Introduction

Many bacteria and archaea have multiple Toxin-Antitoxin (TA) loci [1], where the toxin normally inhibits cell growth, while the antitoxin neutralizes the activity of the toxin by forming a tight TA complex. One of the known functions of TA loci is to respond to nutritional stress [2], namely, toxins are activated upon nutritional starvation and slow down the rate of translation. When cells are under normal fast growth conditions, on the other hand, the majority of the cells will be in the antitoxin-dominated state, such that toxin activity is fully inhibited.

It has been found that many bacterial TA loci are auto-regulated at the transcriptional level by a mechanism called “Conditional Cooperativity” (CC) [3], where the transcription factor can bind cooperatively to the operator only if the concentrations of two different proteins satisfy a certain stoichiometric ratio. CC was quantitatively studied in one of the known functions of TA loci to be responding to nutritional stress [2], namely, toxins are activated upon nutritional starvation and slow down the rate of translation. When cells are under normal fast growth conditions, on the other hand, the majority of the cells will be in the antitoxin-dominated state, such that toxin activity is fully inhibited.

Interestingly, all plasmid and chromosome-encoded TA loci investigated are found to be regulated by CC so far, including relBE of E. coli [3,4], vapBC of Salmonella enterica [7], phd/doc of plasmid P1 [8,9] and cadA/cadB of plasmid F [10]. This suggests that CC is a common feature for TA loci.

In our previous work, we have explored the function of CC in the starvation response of the RelBE system, and showed that CC prevents random toxin activation and promotes fast translational recovery when starvation conditions terminate. However, to reproduce the full dynamics of the starvation response, we took into account details of the RelBE system, which made the model rather specific to it. The primary purpose of this paper is to construct a simple mathematical model that demonstrates the functions of CC in a more general perspective.

TA loci have been suggested to be involved in persister formation [11–16]. When an antibiotic is applied to a growing bacterial population, the majority of the bacteria are killed. However, a very small fraction of them survives and re-grows after the antibiotic is removed. If the progeny of the bacteria is again sensitive to the same antibiotic, they are called persisters, in contrast to the resistant bacteria that have acquired resistance to antibiotic by mutation. Persisters are genetically identical to the sensitive cells, but believed to be in a non- or slow-growing, dormant state. Since the majority of antibiotics interfere with the cell growth and division process, cells can survive if they grow slowly or not at all.

The exact molecular mechanism underlying persistence is not fully understood. However, it has been found that mutations in hkiAB genes severely increase the level of of persisters formation. Interestingly hkiAB is one of the TA loci in E. coli [11,13,14]. In
**Author Summary**

The effectiveness of antibiotics on many pathogenic bacteria is compromised by multidrug tolerance. This is caused by a small sub-population of bacteria that happen to be in a dormant, non-dividing state when antibiotics are applied and thus are protected from being killed. These bacteria are called persisters. Unraveling the basic mechanism underlying this phenomenon is a necessary first step to overcome persistent and recurring infections. Experiments have shown a connection between persister formation and the battle between a toxin and its antitoxin inside an *E. coli* cell. Toxin inhibits the cell growth but is neutralized by the antitoxin by forming a complex. The proteins also regulate their own production through this complex, thereby forming a feedback system that controls the growth of the bacterium. In this work we provide mathematical modeling of the feedback module and explore its abilities. We find that the auto-regulation with reduced growth associated with free toxins allows the cell to be bistable between two states: an antitoxin-dominated, normal growing one, or a dormant one caused by the activity of the toxin. The latter can be the simplest description of persister state. The toxin-antitoxin system presents a powerful example of mixed feedback design, which can support epigenetics.

In addition, recent experiments [15] showed that removal of 10 mRNA-encoding TA loci reduced the persister fraction significantly. These observations strongly suggest that TA loci are important factors for persister formation.

One of the possible explanations is that stochastic activation of the toxin will slow down cell growth, resulting in a dormant state. This will be possible if the TA locus dynamics exhibits bistability, where a cell can be either in the antitoxin-dominated state that ensures the growth or in the toxin-dominated state that inhibits the growth. This viewpoint is also consistent with the observation that the persister state can be described as a metastable state with a constant stochastic switching rate to and from normal growing state [12].

This idea was theoretically pursued by Lou et al. [17] with a simple mathematical model that did not take CC into account. They concluded that, for bistability to be achieved, high cooperativity (Hill-coefficients \(\sim 4\)) is necessary, both in transcriptional auto-regulation of the TA operon and in the free toxin activity.

In this paper, we explore the basic features of CC as a regulation mechanism mediated by heteromer formation. We demonstrate that CC provides bistability in a simple feedback motif in a wide range of the parameters. We then construct a simplified model of TA system regulation and demonstrate that CC with growth rate-mediated feedback via toxin activity can provide the bistable alternatives between the antitoxin-dominated and the toxin-dominated states.

**Results**

**Conditional regulation**

**Complex regulation.** We examine a simplified system, where protein A and T can form two kinds of heteromers, AT and ATT (Fig.1A):

\[
A + T + T \rightarrow AT + T \rightarrow ATT.
\]

Here, we assume that AT is the active molecule that act as a transcriptional repressor, whereas free A, free T, and ATT are not active in transcriptional control. This is a simplification of the transcriptional regulation by RelBE, where RelB\(_2\) corresponds to one A, while RelE corresponds to one T.

The amount of active molecule \([AT]\) shown in Fig. 1 is determined from total A and T distributed among complexes \([AT]\) and \([ATT]\) according to

\[
[AT] = \frac{[A]_r[T]}{K_T},
\]

\[
[ATT] = \frac{[T][AT]}{K_{TT}}.
\]

Here \(K_T\) and \(K_{TT}\) are the dissociation constants for AT and ATT, respectively, whereas the concentration of free A (T) is denoted \([A]_r\) (\([T]_r\)).
Fig. 1B shows $[AT]$ as a function of $T$ for fixed $A$, pinpointing that when $T<A$, $T$ is limiting the amount of AT, while $T>A$ implies that a substantial fraction of $A$ is sequestered in the AT complex. For $T>2A$, ATT formation sequesters nearly all AT and $[AT]$ drops sharply to a value close to zero. This last transition can be ultrasensitive, provided that the binding between AT and ATT is strong, $K_{T}T \ll A$. For RelB-E system the binding is indeed very strong, with a measured $K_{T}T$ in the nanomolar regime \[18–21\] as well as in transcription factors \[22–25\]. In the present case, just a factor two difference in $T$ around $T \approx 2A$ can change $[AT]$ dramatically.

This ultra-sensitivity is reflected in the promoter activity behavior, that shows a sharp de-repression occurring at $T \approx 2A$ (Fig. 1C), where $[AT]$ drops. Another unique feature of CC is its non-monotonicity, and an associated derepression for small $T$ because $[AT]$ is small, see Fig. 1B,C.

Note that Fig. 1C does not include possible cooperativity in AT-DNA binding. The unique characteristics of CC, ultra-sensitivity by sequestration and non-monotonicity, do not require this cooperativity. For simplicity, therefore, we focus on regulation by AT without cooperativity, and we call it “conditional regulation” (CR), rather than CC. Of course, adding cooperativity will make the response even sharper, and the following results hold for the cooperative case, too.

**Bistability in a simple feedback motif.** We now study production of $T$ repressed by AT, while $A$ is fixed. The regulatory circuit is described by

$$\frac{dT}{dt} = \frac{\sigma}{1 + \frac{[AT]}{K_{O}}} - T, \quad (4)$$

where $\sigma$ is the maximum production rate of $T$, and $K_{O}$ is the dissociation constant of AT molecule to DNA. We assume that total $A$ can be controlled and maintained at a steady state by a AT independent promoter. In this subsection, we take the lifetime of $T$ to be the time unit and set $K_{T} = K_{T}T = 1$ for the dissociation constants, thus measuring concentrations of AT and ATT in units of their mutual binding strength. Further, focusing on CR, we assume that there is no cooperativity in binding of AT to promoter.

Fig. 2(A) shows the production term of eq. (4) as a function of $T$ for three different values of $A$ with each of them two different values of $K_{O}$. The repression is always strongest at $T = A$, and sharp de-repression happens at $T > 2A$ for all the cases. The higher $A$, the more $[AT]$ will present when $A = T$, resulting in stronger repression at $A = T$ for larger $A$. The AT-DNA dissociation constant $K_{O}$ also contribute to the repression strength.

The thick black line represents the degradation term in eq. (4), and the intersection between this and the production gives the steady state values of $T$. For small $A$ ($=20$) with $K_{O} = 1$, there is only one crossing, happening at a relatively high value of $T$ ($\approx 900$). At intermediate $A (=100)$, there are two stable fixed points and one unstable fixed point in between ($T \approx 200$), reflecting a bistable system. At high $A$ ($=400$), the high $T$ fixed point vanishes and the system settles at a monostable state with low $T$. We have also analyzed the systems systematically for weaker repression, i.e. higher values of $K_{O}$, and again found bistability provided that $A$ (and thus $T$) is increased accordingly.

In addition, the non-monotonicity of the CR has a striking implication in regulation at low $T$ values: It guarantees that the low (uninduced) $T$ steady state value has finite amount of $T$ that is maintained at a level nearly independent of $A$ (Fig. 2A, compare $A = 100$ and 400 with $K_{O} = 1$). This is an important feature for TA system in terms of the starvation response, as discussed later.

Remarkably, the system exhibits bistability without cooperative binding to DNA. In the TA system the cooperativity is instead provided by the ultrasensitive de-repression at $T = 2A$ that is facilitated by a very strong protein-protein binding \[22–25\]. This bistability is seen in a wide range of $A$ and $\sigma$ values as shown in Fig. 2(B). The larger $\sigma$ and $A$, the high-$T$ steady state value increase proportionally, while the low-$T$ steady state value remains practically unchanged. Thus, as externally imposed $A$ is increased, the model predict a larger contrast between the two steady states. If the binding to DNA is cooperative, the de-repression at ATT formation becomes even sharper, thereby favouring bistability.

We have also studied other possible motifs, where either $T$ or $A$ is repressed or activated by AT complex (data not shown). For example we found that if AT activate $A$ while $T$ is kept constant, one can obtain bistability between a high $A$ state and a low $A$ state in a wide range of parameters. This bistability is again supported by the ultrasensitivity of AT sequestration, as $[AT]$ increase sharply with increasing $A$ around $T/2$.\[doi:10.1371/journal.pcbi.1003174.g002\]
Simple model of persister formation

In this section, we construct a simple model of TA activity control with CR, a model aimed at capturing the central features of persister formation. We use the RelBE system as a reference because the molecular interactions and parameters are best known here. The reference parameters are listed in Materials and Methods.

In RelBE [6], the antitoxin RelB and the toxin RelE are encoded by the same operon, and transcriptionally auto-regulated by CC. RelE is metabolically stable, and its concentration decreases only by dilution due to cell division (generation time ~30 min in log phase growth in rich medium). On the other hand, RelB is actively degraded by protease Lon, resulting in its very short half-life of ~3 min. In spite of this, the RelB concentration in a normally growing cell is about 10 times of that of RelE [4], suggesting that the RelB mRNA is translated about 100 times more often than RelE mRNA [6].

This situation is depicted in Fig. 3A1. Since both toxin T and antitoxin A are regulated by the same promoter, the corresponding equations apply:

\[ \frac{dT}{dt} = \frac{\sigma_T}{1 + \left(\frac{AT}{K_O}\right)}T - \Gamma_A A \]
\[ \frac{dA}{dt} = \frac{\sigma_A}{1 + \left(\frac{AT}{K_O}\right)} - \Gamma_A A. \quad (5) \]

where \( \sigma_T \) and \( \sigma_A \) are the maximal production rate for \( T \) and for \( A \), respectively. The dilution rate of \( T \) is given by cell division, and is taken as a unit rate, while \( \Gamma_A \) is the active degradation rate of \( A \).

This motif, however, cannot exhibit bistability. Fig. 3A2 shows example null-clines, which have only one stable fixed point at the antitoxin dominated state. We performed parameter scan spanning from 1/8 to 8 fold relative to the values used for Fig. 3A2, but did not find any combination of parameters that gives bistability, even if we allow cooperative binding of AT to DNA with Hill coefficient 2 (data not shown). This absence of bistability is due to \( A \) being regulated identically to \( T \). Accordingly, the de-repression of the promoter around \( T \approx 2A \) increases not only the toxin production but also the antitoxin production, and the latter is so large that the system remains in the antitoxin-dominated state.

When we include the activity of free toxin on cell growth, however, the model system can show bistability. This is because the toxin-induced arrest of cell growth prolong lifetime of \( T \), while leaving \( A \) being degraded by Lon at a high rate. The mathematical formulation of this extended model is

\[ \frac{dT}{dt} = \frac{\sigma_T}{1 + \left(\frac{AT}{K_O}\right)}T - \frac{1}{1 + \beta_M [T_f]} \frac{\sigma_A}{1 + \left(\frac{AT}{K_O}\right)} - \Gamma_A A. \quad (6) \]
\[ \frac{dA}{dt} = \frac{\sigma_A}{1 + \left(\frac{AT}{K_O}\right)} - \Gamma_A A. \quad (7) \]

Figure 3. TA system with CR without and with feedback through free toxin activity. (A.1) Schematic representation of the genetic circuit described by eq. (5) for TA system with CR, without considering toxic activity of free T. (A.2) Null-clines for eq. (5). Blue line represents \( \frac{dT}{dt} = 0 \), and red line represents \( \frac{dA}{dt} = 0 \). For comparable values of \( A \) and \( T \) the two null clines become parallel and does not cross, as shown in the area highlighted in grey, i.e. the system does not show bistability. The parameters used are listed in Table 1 in Materials and Methods. Dashed lines with arrows show the flow to the fixed point. (B.1) Schematic representation of the genetic circuit described by the model (6) and (7). (B.2) Null-clines for the system of eqs. (6) and (7) with \( \beta_M = \beta_C \approx 11 \). Blue line \( \frac{dT}{dt} = 0 \), Red line \( \frac{dA}{dt} = 0 \). Dashed lines with arrows show the flow to the stable fixed points. doi:10.1371/journal.pcbi.1003174.g003
expressing that $[T_f]$ reduces all protein production, and accordingly also decreases the dilution by cell growth. $\beta_M$ represents the reduction of protein expression per free toxin ($T_f$) molecule, and $\beta_C$ represents the growth inhibition per free toxin molecule. Notice that $[T_f]$ does not influence degradation of A, because it is anyway so unstable that cell division hardly affects its concentration.

These terms correspond to the growth-rate dependent feedback [17,26,27]. The reduction of the protein production ($\beta_M$ term) can account for both direct activity of free toxin to TA locus and the global slowdown of the transcription rate due to change of physiological conditions [26]. Comparison of the present model with the steady state growth data in Ref.[26] is given in Text S1. We expect $\beta_M \approx \beta_C$ because the slowing down of the growth rate is due to the global slowing down of the protein production. At the same time, there can be some quantitative difference because $\beta_M$ may include the effect specific to the TA locus.

The growth-rate mediation mediated by T constitutes a positive feedback [17,26,27] on $T$ accumulation, which is essential for bistability and persistor formation. The term with $\beta_M$ reduces the production of both antitoxin and toxin, and thus overall weaken the ability to maintain the bistability. Note that $\beta_M$ primarily influences the transition state from A to T dominated state, because the reduction of production targets the short lived A protein first.

Fig. 3B1 examines eqs. (6)–(7) with parameters extracted from the RelBE system [6] (see the figure caption of Fig. 3). The nullclines in Fig. 3B2 are from the $\beta_M = \beta_C \approx 11$ case, exhibiting two stable fixed point, one at the antitoxin-dominated state (the low-$T$ state, $A \approx 10$, $T \approx 1$) and another at the toxin dominated state (the high-$T$ state, $A \approx 1$, $T \approx 100$). Note that the antitoxin dominated state has almost the same concentrations as the stable fixed point in Fig. 3A2 with $\beta_M = \beta_C = 0$. The antitoxin dominated state scarpsely depends on $\beta_M$ and $\beta_C$, since there is almost no free toxin ($[T_f] \approx 0$) in the antitoxin dominated state.

Figure 4A shows the ratio between the $T$ dilution rates at the low and high $T$ steady state, $[1+\beta_M T_f(\text{high})]/[1+\beta_M T_f(\text{low})]$. The figure illustrates that our model predicts bistability for a wide range of parameters, and further that this bistability is indeed governed by the increase in cell generation parameterized by the $\beta_C$ term. For too large $\beta_M$ the bistability is counteracted because the toxin production is reduced too much by free toxin to accumulate enough for the stable high toxin state. Remarkably, for proportional reduction of protein production and increased cell generation, $\beta_M = \beta_C$, the model predicts bistability for all $\beta_M = \beta_C > 1$.

We further performed scanning of other parameters. We fixed one parameter at a time and sampled the rest of the parameters randomly to test 1000 samples in logarithmic scale within the range between 1/8 to 8 fold of the reference values. We then systematically changed the fixed parameters between 1/8 to 8 fold and repeated the procedure, to see the effect of the parameter. We found that 20% to 80% of the samples showed bistability. The detailed results are given in Text S2. We also explored the effect of the dissociation constant $K_T$ and $K_{TT}$ more intensively, by changing $K_T = K_{TT}$ from the reference value to 64 fold, since they describe the sharpness of the CR and this is expected to influence the bistability. We find that the number of bistability parameter sets decreases gradually with the fold change of $K_T$ and $K_{TT}$. Details are given in Fig. S4.
Using known parameters for the RelBE system in *E. coli*, we constructed a minimal model for TA activity, combining conditional regulation with a feedback from free toxin to the cell growth. It was demonstrated that this model shows bistability for a wide range of parameters, with a stable state corresponding to the antitoxin-dominated, normal growing state, and another metastable state corresponding to the toxin-dominated state, potentially corresponding to the persister state.

Noticeably, the model eqs. (6)–(7) did not rely on details of the molecular mechanisms of how the toxin works, and therefore the model is not limited to the RelBE system. The important assumptions are: (i) The TA system is conditionally regulated, (ii) toxins are stable and diluted mainly by cell division, while antitoxins are metabolically unstable, and (iii) free toxins reduce the productions of proteins and hence cell growth. All the

**Figure 5. Schematic summary of the role of conditional regulation in persister formation.** The red curves show the toxin production rate and the blue lines give the degradation rate, both from eq. (6). Both terms depend on \( A \), and here we make approximation that \( A \) is always in steady state (eq. 7 with \( dA/dt = 0 \)) for given \( T \), because dynamics of \( A \) is much faster than \( T \) due to high production and degradation rate. Since production term of \( A \) and \( T \) are proportional to each other and \( A \) is degraded at a constant rate, resulting \( A \) concentration is proportional to the production term of \( T \) (red curves). The scales of curves are modified from actual functional forms so that the characteristic behaviours can be grasped easily. The ultra-sensitivity mediated by protein-protein binding combined with feedback from free toxin activity is reflected in the peak of the production rate and drop of the degradation rate, resulting in bistability of the system. This accounts for the type II persister where a cell can spontaneously switch to and out of the persister state. The non-monotonicity of the conditional regulation secures that some toxins are stored in antitoxin dominated state, helping the transition to the stress-induced activation of toxin [6], which becomes the base for type I persister formation.

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**Discussion**

Using known parameters for the RelBE system in *E. coli*, we constructed a minimal model for TA activity, combining conditional regulation with a feedback from free toxin to the cell growth. It was demonstrated that this model shows bistability for a wide range of parameters, with a stable state corresponding to the antitoxin-dominated, normal growing state, and another metastable state corresponding to the toxin-dominated state, potentially corresponding to the persister state.

Noticeably, the model eqs. (6)–(7) did not rely on details of the molecular mechanisms of how the toxin works, and therefore the model is not limited to the RelBE system. The important assumptions are: (i) The TA system is conditionally regulated, (ii) toxins are stable and diluted mainly by cell division, while antitoxins are metabolically unstable, and (iii) free toxins reduce the productions of proteins and hence cell growth. All the
Conditional Cooperativity Mediates Bistability

Table 1. Reference parameter values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_T$</td>
<td>166.28 nM min$^{-1}$</td>
<td></td>
<td>Growth rate</td>
</tr>
<tr>
<td>$K_0$</td>
<td>1 nM</td>
<td></td>
<td>Activation</td>
</tr>
<tr>
<td>$K_T$</td>
<td>0.3 nM</td>
<td></td>
<td>Degradation</td>
</tr>
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<td>$k_{a/TT}$</td>
<td>0.3 nM</td>
<td></td>
<td>Association</td>
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<tr>
<td>$\Gamma_T$</td>
<td>0.2 min$^{-1}$</td>
<td></td>
<td>Degradation</td>
</tr>
<tr>
<td>$\beta_C$</td>
<td>0.16 nM$^{-1}$</td>
<td></td>
<td>Antitoxin</td>
</tr>
<tr>
<td>$\beta_M$</td>
<td>0.16 nM$^{-1}$</td>
<td></td>
<td>Antitoxin</td>
</tr>
</tbody>
</table>

All the numerical analyses are done using C++ codes developed by the authors. When necessary, $[AT]$ was calculated by solving algebraic equations (2) and (3) with conservation of mass for a given amount of $(A,T)$ by Newton’s method [32]. The bistable solutions in Fig. 2 B (Fig. 4) were obtained by finding the fixed points for $dT/dt = 0$ with eq. (4) $dT/dt = 0$ and $dA/dt = 0$ with
eqs. 6 and 7) by Newton’s method and then evaluating the stability based on the Jacobian. The trajectories that constitute the flux in Figs. 3A2 and 3B2 were calculated by the 4th-order Runge-Kutta method [32].

Reference parameters

The values of the parameters used in the ODEs correspond to a conversion to dimensionless numbers of the parameters relative to the RelBE system we studied in [6].

In particular we used the lifetime of RelE in exponential growth conditions (\( \tau_s \)) as time-unit (\( \tau_a \)) and the maximal amount of A proteins produced in the unit time as concentration unit (\( C_a \)). In the RelBE system \( \frac{\sigma_a}{\sigma_0} \approx 715000 \) nM thus fixing \( \sigma_a = 10000 \) we obtain \( C_a = 71.5 \) nM, while \( \tau_a = \frac{\sigma_a}{\sigma_0} = 43 \) min. The value of \( \beta_M \) in the starved condition [6] was evaluated to be around 1000 in this units. However, it is expected to be smaller in the normal condition, since RelE cleaves mRNA at the ribosomal A-cite, units. However, it is expected to be smaller in the normal condition, since RelE cleaves mRNA at the ribosomal A-cite, which is expected to be more accessible at the starvation. Therefore, we mostly explore \( \beta_M \) values smaller than 1000.

The reference parameters are shown in table 1.

Supporting Information

Figure S1 Fit of the free toxin activity parameters to the grow-rate dependent global transcription rate. **Left:** Red points: Global transcription rate \( \dot{z}_M(\Gamma) \) from Klumpp et al. [26]. Green Line: normalized production rate \( \tilde{z}(\Gamma) \) from our model with \( \beta = 0.4 \). Right: Red points: Normalized global transcription rate multiplied by gene copy number, \( \tilde{z}_M(\Gamma)(g(\Gamma))/g(1) \) from Klumpp et al. [26]. Green Line: normalized production rate \( \tilde{z}(\Gamma) \) from our model with \( \beta = 1.2 \).

Figure S2 \( \beta_M/\beta_C \) fitted to the global transcription rate lies in the bistable region. Each green dot in the plot represents a combination of \( \beta_M \) and \( \beta_C \) that give bistable results. The red line represents \( \beta_M/\beta_C = 0.4 \), and the black line \( \beta_M/\beta_C = 1.2 \).

Figure S3 The robustness of the bistability against parameter change. We fix \( \sigma_a = 100 \) and \( \Gamma_0 = 1 \), and vary rest of the parameters. In (a) \( \beta_M \) is changed systematically between \( \frac{1}{8} \) and 8 fold of the value used in the main text \( \beta_M^0 = 11.4475 \); we change it between \( \frac{1}{8} \beta_M = 1.4309 \) and 8 \( \beta_M^0 = 91.58 \) with a pace given by 2\( n \) with an integer \( n \in [-3,3] \). For each value of \( \beta_M \), we sample rest of the parameters randomly and independently of each other, and they can take any values from the set \( 2^{10} \) (the reference value) with \( n \in [-3,3] \). The reference values are given in Table 1. We collect a sample of 1000 points in the parameter space. The bars in the histogram represent the fraction of this sample of points in the parameter space that still shows bistable behavior. The same procedure is then carried out for \( \beta_C \), \( \Gamma_B \), \( \tilde{K}_T \), \( \tilde{K}_{TT} \), \( \tilde{K}_D \), and \( \tilde{\sigma}_a \).

Figure S4 The robustness of the bistability against the change of the dissociation constants \( \tilde{K}_T \) and \( \tilde{K}_{TT} \). We set \( \tilde{K}_T = \tilde{K}_{TT} \), and increase them systematically from the reference value (0.004) to 64 fold of the reference value. Since the dissociation constants set the concentration of A and T at which \( AT \) and \( ATT \) formation is significant, we fix \( \tilde{\sigma}_a = 10000 \) and \( \Gamma_0 = 10 \) in addition to fixing \( \tilde{\sigma}_T = 100 \) and \( \Gamma_0 = 1 \). We then sample the rest of the parameters randomly in the base 2 logarithmic scale, within 1/8 to 8 fold of the reference value. We tried 1000 parameter sets for each values of \( \tilde{K}_T = \tilde{K}_{TT} \). The plot shows the fraction of the parameter set that shows the bistability. We see that the number of bistability parameter sets decrease gradually with fold increase of the dissociation constants.

Text S1 Correspondence of parameters with the growth rate dependence data of protein production rate in the steady state growth.

Text S2 Parameter scan by Monte Carlo sampling to test the robustness of bistability.

Author Contributions

Wrote the paper: IC KS KG NM. Model building and model analysis: IC KS KG NM.

References