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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-monotonic response to the concentration of one of the proteins, and secondly, it opens for ultrasensitivity 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 Escherichia coli TA loci, relBE [3–6]. Here the two proteins, the toxin (mRNase) RelE and the antitoxin RelB, are encoded by the same operon, which is negatively auto-regulated. The tight dimer RelB2 is a weak transcriptional auto-repressor, but this repression is strongly enhanced by the presence of RelE and becomes strongest at RelB2/RelE ratio 1:1. Over-expression of RelE above twice of RelB2, though, will result in an abrupt de-repression of the promoter. This unique behavior is a consequence of formation of alternative hetero-complexes of RelB and RelE, RelB2RelE and RelB3RelE2. Two RelB2RelE bind to the promoter site cooperatively to repress the promoter strongly, while RelB3RelE2 does not bind to the promoter.

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], vpaBC of Salmonella enterica [7], phd/doc of plasmid P1 [8,9] and cdaA/cdaB of plasmid F [10]. This suggest 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 exposed to the antibiotic, a very small fraction of them survives and re-grows again. This behavior is one of the reasons why the bacteria are resistant to the antibiotic. The persister bacteria function is maintained by the persister loci that encode for the persister proteins.

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

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):

$$A + T + T \rightarrow AT + T \leftrightarrow ATT.$$  

(1)

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 RelB2 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

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

(2)

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

(3)

Here $K_T$ and $K_{TT}$ are the dissociation constants for AT and ATT, respectively, whereas the concentration of free A (T) is denoted $[A]/([T])$. 

Figure 1. Heterocomplex formation in a TA system. (A) Reaction scheme of the heterocomplex formations, implying that the active complex [AT] is constrained by through $A = [A] + [AT] + [ATT]$ and $T = [T] + [AT] + 2[ATT]$ with complex concentrations expressed by eq. (2), (B) Concentration of AT heteromers for a fixed value of $A = 100$ as a function of $T$ with $K_T = K_{TT} = 1$. Note that it has a peak at $A = T$. In the strong binding limit of $K_T \rightarrow \infty$ with $K_T = rK_T$ (r kept constant), $[AT]$ for $T < 2A$ is given by

$$\frac{1}{1 + \frac{[AT]}{K_T}} = \frac{1}{1 + \frac{[AT]}{K_T}},$$

for $r \neq 4$ and $T(2A - T)/(2A)$ for $r = 4$, where $[AT]$ always has a peak at $A = T$. In this limit, $[AT] = 0$ for $T \geq 2A$. (C) The behavior of $[AT]$ shown in (B) is reflected in the behavior of the repression factor $1/(1 + [AT]/K_T)$ as a function of $T$, calculated for fixed $A = 100$, and dissociation constant for AT-DNA binding $K_D = 1$.

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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 ATT 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 ultra-sensitive, provided that the binding between AT and ATT is strong, \( K_{AT} < A \). For RelB-E system the binding is indeed very strong, with a measured \( K_{AT} \) 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

\[
\frac{dT}{dt} = \frac{\sigma}{1 + \frac{[AT]}{K_O}} - T, \tag{4}
\]

where \( \sigma \) is the maximum production rate of T, and \( K_O \) is the dissociation constant for the binding of AT molecule to DNA. We assume that total A can be controlled and maintained at a steady state by an AT independent promotor. In this subsection, we take the lifetime of T to be the time unit and set \( K_T = K_{TT} = 1 \) 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 promotor.

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 \( T \approx 2A \).
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 \(\approx 30\) 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 \(\approx 3\) 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. 3A. Since both toxin T and antitoxin A are regulated by the same promoter, the corresponding equations apply:

\[
\frac{dT}{dt} = \frac{\sigma_T}{1 + \frac{[AT]}{K_T}} - T \quad \text{and} \quad \frac{dA}{dt} = \frac{\sigma_A}{1 + \frac{[AT]}{K_A}} - \Gamma_A \cdot A, \quad (5)
\]

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 prolongs lifetime of T, while leaving A being degraded by Lon at a high rate. The mathematical formulation of this extended model is

\[
\frac{dT}{dt} = \frac{\sigma_T}{1 + \frac{[AT]}{K_T}}(1 + \beta_M[T_f]) - \frac{1}{1 + \beta_C[T_f]} \cdot T \quad (6)
\]

\[
\frac{dA}{dt} = \frac{\sigma_A}{1 + \frac{[AT]}{K_A}}(1 + \beta_M[T_f]) - \Gamma_A \cdot A. \quad (7)
\]

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 \(\frac{A}{dt} = 0\). 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. (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 \(\beta_C = \beta_M \approx 11\). Blue line \(\frac{dT}{dt} = 0\), Red line \(\frac{dA}{dt} = 0\). Dashed lines with arrows show the flow to the fixed point. doi:10.1371/journal.pcbi.1003174.g003
expressing that $[T_f]$ reduces all protein production, and accordingly also decreases the dilution by cell growth. $b_M$ represents the reduction of protein expression per free toxin ($T_f$) molecule, and $b_C$ 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 ($b_M$ 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 $b_M \approx b_C$ 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 $b_M$ may include the effect specific to the TA locus.

The growth-rate reduction mediated by T constitutes a positive feedback [17,26,27] on T accumulation, which is essential for bistability and persister formation. The term with $b_M$ reduces the production of both antitoxin and toxin, and thus overall weaken the ability to maintain the bistability. Note that $b_M$ 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 $b_M=b_C\approx 11$ 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 $b_M=b_C=0$. The antitoxin dominated state scarcely depends on $b_M$ and $b_C$, 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, $[1+b_T T_f(\text{high})]/[1+b_T T_f(\text{low})]$. 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 $b_C$ term. For too large $b_M$ 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, $b_M=b_C$, the model predicts bistability for all $b_M=b_C>1$.

We also studied the robustness of the bistability against parameter change. One of the most crucial parameters for the bistability is the ratio $\sigma_A/\sigma_T$, because this determines the difference of the concentration of $A$ and $T$. We therefore varied $\sigma_A/\sigma_T$ with keeping $\sigma_T$ constant, and searched for the bistable regime in $(b_M,b_C)$ space. The rest of the parameters are kept same as those used in Fig. 4A. Only when $\sigma_A/\sigma_T\approx 10$ is considered, because lower ratios prevent antitoxin domination due to its 10 times higher degradation rate. For rather small $\sigma_A/\sigma_T\approx 20$, too large $b_C$ makes the anti-toxin dominated state unstable, because very small amount of free toxin is enough to activate the positive feedback to toxin via the growth rate. With even larger $\sigma_A/\sigma_T$, stronger feedback is needed to stabilize toxin-dominated state, reflected in larger values of $b_C$ and $b_M$.

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 $K_F$ and $K_{FT}$ more intensively, by changing $K_T=K_{FT}$ 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 $K_{FT}$. Details are given in Fig. S4.
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
Table 1. Reference parameter values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{\alpha} )</td>
<td>166.28 nM min^{-1}</td>
</tr>
<tr>
<td>( k_{\beta} )</td>
<td>0.3 nM</td>
</tr>
<tr>
<td>( k_{T} )</td>
<td>0.3 nM</td>
</tr>
<tr>
<td>( k_{C} )</td>
<td>0.2 min^{-1}</td>
</tr>
<tr>
<td>( \beta_{C} )</td>
<td>0.16 nM^{-1}</td>
</tr>
<tr>
<td>( \beta_{M} )</td>
<td>0.16 nM^{-1}</td>
</tr>
</tbody>
</table>

Reference parameter values.

Predicted: 0.004 min^{-1}.

Materials and Methods

Numerical solutions of the model equations

All the numerical analyses are done using C++ codes developed by the authors. When necessary, [4T] 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. 2B (Fig. 4) were obtained by finding the fixed points for \( dT/dt = 0 \) and \( dA/dt = 0 \) with

\( \sigma_{T} = \frac{166.28 \text{nM min}^{-1}}{C_{0}} \) and \( \sigma_{C} = \frac{166.28 \text{nM min}^{-1}}{43 \text{min}} \).
Conditional Cooperativity Mediates Bistability

References


