Control of the dynamical behavior of the Repressilator by Quorum Sensing

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1 Introduction

With advance achieved in gene engineering it has become possible to construct genetic circuits with programmed behavior. Genetic toggle switch might be considered as good example [1, 2], which can be used as a simple switching device responding to any external stimulus or as a bit-wise memory unit in biocomputing. Other prominent example of synthetic gene circuits is genetic oscillator [2, 3, 4, 5]. The first synthetic genetic oscillatory circuit was repressilator [3].

The ideas behind synthetic circuits are simple. The toggle switch consists of two elements and each of them inhibits the other. Such a scheme is characterized with two possible stable steady states: full expression of the one of the elements and no expression of the other one and vice versa [1]. In turn, the repressilator consists of at least three elements inhibiting each other in a cyclic way.

Oscillatory processes are discovered in many biological contexts, e.g. circadian rhythms [6] and cell cycle [7]. Synthetic gene oscillators are simpler in their organization and have potential to increase our knowledge of natural gene networks with oscillatory behavior.

A challenging area of the research is communication among cells in a population or organism. It has been proposed theoretically to use quorum sensing mechanism [8] to introduce a coupling between oscillators [9, 10]. The quorum sensing is based on diffusion of a small molecule (autoinducer). There are two genes to introduce to the system expressing: protein synthesizing autoinducer and protein which is autoinducer receptor. Active complex of receptor and autoinducer activates gene expression from target gene(s). Such a coupling is sensitive to population density.

In [10] it has been proposed that protein A synthesizes autoinducer, which activates target gene C. Such topology provides phase-attractive type of coupling — autoinducer operates by inhibiting own production in the cells. System constructed in this way demonstrates in-phase synchronization over a population of cells. This model also exhibits some other important dynamical regimes (accepted for publication).

In [11] it has been proposed that protein B synthesizes autoinducer, which activates target gene C. Such topology provides phase-repulsive coupling — autoinducer stimulates its own synthesis by the intermediate steps along the repressilator core (Fig. 1). It has been shown in the system that in broad parameter areas the main regime is anti-phase oscillations [12].

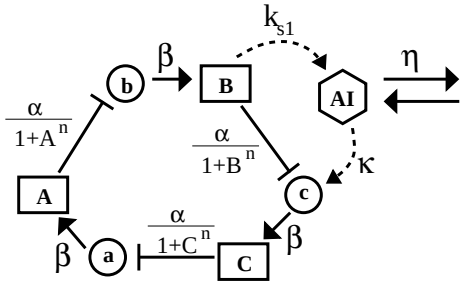

Figure 1: The minimal scheme of the repressilator with autoinducer production [11].

In present work we investigate dynamical behavior of single repressilator with phase-repulsive coupling mechanism depending on maximal transcription rate from a single promoter (α), taking into account difference in protein and mRNA timescales.

We have shown that single oscillator with coupling module added exhibits a special steady state limited in parametric space. This steady state is shown to coexist with limit cycle and the size of hysteresis area varies with ratio between protein and mRNA degradation rate constants.

2 Model

The following system of dimensionless equations describes the dynamical behavior of the single repressilator with phase-repulsive coupling mechanism [11]:

$$\begin{aligned}
\tfrac{\mathrm{da}}{\mathrm{d}t} &= -a + \tfrac{\alpha}{1+C^n}; & \tfrac{\mathrm{dA}}{\mathrm{d}t} &= -\beta(A-a) \\
\tfrac{\mathrm{db}}{\mathrm{d}t} &= -b + \tfrac{\alpha}{1+A^n}; & \tfrac{\mathrm{dB}}{\mathrm{d}t} &= -\beta(B-b) \\
\tfrac{\mathrm{dc}}{\mathrm{d}t} &= -c + \tfrac{\alpha}{1+B^n} + \kappa\tfrac{S}{1+S}; & \tfrac{\mathrm{dC}}{\mathrm{d}t} &= -\beta(C-c) \\
\tfrac{\mathrm{dS}}{\mathrm{d}t} &= -k_{s0}S + k_{s1}B - \eta S
\end{aligned}$$

The uppercase letters A, B and C denote protein concentrations, while lowercase a, b and c are proportional to the concentrations of mRNA corresponding to those proteins, S denotes autoinducer concentration. All negative terms in

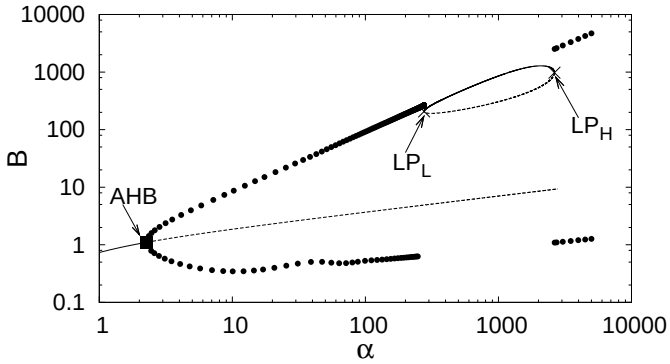

Figure 2: Emergence of the limit cycle in the single repressilator cell through Hopf bifurcation (AHB, black square) by varying maximal transcription rate α. Solid (dashed) lines denote stable (unstable) steady state; solid circles denote stable periodic solution. $\mathrm{LP_L}$, $\mathrm{LP_H}$ — saddle-node bifurcations of the steady state. Parameters used: $n = 2.6$, $\kappa = 25.0$, $\beta_a = \beta_b = \beta_c \equiv 1.0$, $k_{s0} = 1.0$, $k_{s1} = 0.01$, $\eta = 2.0$.

the right-hand side represent degradation of the molecules. The nonlinear function $f(x) = \frac{\alpha}{1+x^n}$ reflects synthesis of the mRNAs from the DNA controlled by regulatory elements called promoters. α defines transcription rate in the absence of the repressor (x). α indirectly depends on several factors, such as the abundance of the RNA polymerase and that of the repressilator plasmid in the cell. Therefore, this parameter may take very different values and we choose α as a bifurcation parameter, i.e. one to be varied. n is called Hill coefficient or cooperativity and reflects multimerization of the protein required to affect the promoter. The parameter β is a ratio between the decay rates of proteins and mRNAs. The three proteins are assumed to have identical kinetics, making the model symmetric. η is a diffusion rate constant which, in case of the single repressilator, is an additional degradation term.

3 Results

Introducing the new molecule to the system may affect dynamics of the single repressilator. We have shown that in the system of one oscillator with autoinducer production module there is a steady state at high values of transcription rate (Fig. 2). The steady state is not related to Hopf bifurcation from which the limit cycle emerges.

The steady state is provided by the high concentration of the autoinducer which, in turn, is based on high concentration of the protein B. Thus, protein B plays two roles: on the one hand, it inhibits synthesis of mRNA C, and this is required for oscillations in the core repressilator; on the other hand, it activates synthesis of the same mRNA C through intermediate autoinducer production step (Fig. 1). This is how phase-repulsive coupling works.

At some high values of transcription rate α the concentration of the protein B might be so much that a lot of autoinducer molecules appear and can compensate the inhibition role of the protein B by its activation role, and the steady state

appears. This steady state makes the limit cycle undergo the infinite period bifurcation (IPB). The changes of other parameters, which increase the role of the autoinducer, can also result in steady state appearance.

Thus, one might need to understand behavior of the system around the steady state in phase space in order to see whether stationary point changes oscillatory dynamics.

The limit cycle emerged at Hopf bifurcation AHB is stable in a wide range of transcription rate α and undergoes IPB near saddle-node bifurcation points $\mathrm{LP_L}$ and $\mathrm{LP_H}$ of the steady state at high values of α (Fig. 2). The position of the IPB might be dependent on some other parameters. We have found that the ratio between mRNA and protein life times (β) is crucial for determining the transcription rate at which IPB occurs.

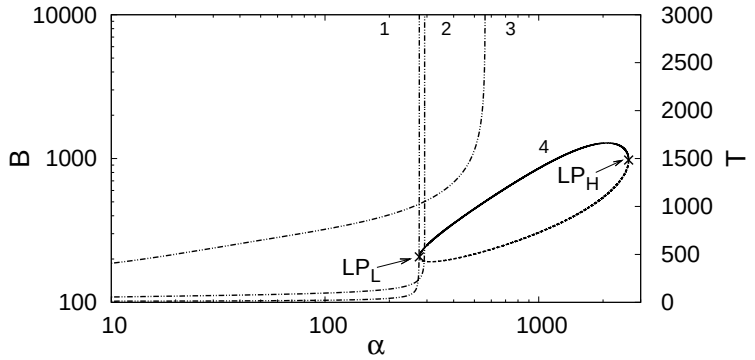

Figure 3: Coexistence (hysteresis) of oscillatory and stationary solutions. Lines 1, 2 and 3 show how the period (T) of the oscillations depends on transcription rate α. Line 4 — the steady state (protein B vs. α). Line 1: $\beta_a = \beta_b = \beta_c \equiv \beta = 1.0$. Line 2: $\beta = 0.1$. Line 3: $\beta = 0.01$. Line 4 does not depend on β_i (see equations). Other parameters as in Fig. 2.

Line 1 in Figure 3 shows how period of the limit cycle depends on α in case of equal life times of mRNA and protein ($\beta = 1.0$) which corresponds with diagram shown in Fig. 2. We make mRNA kinetics much faster than protein which is a more natural case ($\beta = 0.1$ and $\beta = 0.01$). The limit cycle persists with reasonable period in the region of the steady state (lines 2 and 3 in Fig. 3). Thus, the lower values of β provide the bigger region of the hysteresis between steady state and limit cycle.

We have shown an interesting effect of appearance of the stable steady state not related to the Hopf bifurcation in the system of single repressilator with signal molecule included such that in the population this molecule would provide phase-repulsive coupling. The faster mRNA kinetics provide hysteresis (coexistence) between limit cycle and stable steady state solution. Thus, the single cell, while not in population, with quorum sensing mechanism can control self dynamics by switching between oscillations in time and stationary behavior.

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