Control of Repressilators' oscillatory behavior by transcription cooperativity and RNA/protein time scales

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1 Introduction

Regulatory molecular networks are collections of interacting molecules in a cell. One particular kind, oscillatory networks, has been discovered in many pathways. Well-known examples are the circadian clock [1] and the cell cycle [2], where the oscillatory nature of the process plays a central role. Abnormalities of these processes lead to various diseases, from sleep disorders to cancer [3, 4]. For this reason, the regulatory oscillators attract significant attention among biologists and biophysicists.

These natural regulatory networks are very complex and include many types of molecules, from genes to small messengers. It is necessary to study the regulatory mechanisms by means of highly simplified models. These models are particularly valuable because *artificial* regulatory networks can be engineered experimentally [5, 6, 7, 8, 9, 10, 11]. The qualitative agreement between models and experiments is remarkable and validates the mathematical approach to the analysis of regulatory networks. Our goal is revealing general principles of cellular regulation by studying various artificial networks.

We study an artificial oscillatory network called the repressilator [7], which borrows the idea of a ring oscillator coming from engineering. The oscillatory mechanism of the repressilator is based on connecting an odd number of inverters (negative control elements) in a ring. Its genetic implementation uses three proteins that cyclically repress the synthesis of one another by inhibition of corresponding mRNA production.

A challenging area of the research is communication among cells in a population or organism. It has been proposed theoretically to design artificial interaction among cellular oscillators using quorum sensing [12, 13]. Artificial communication among cells containing regulatory oscillators can lead to various effects from synchronization to suppression of oscillations [12, 13, 14, 15]. These collective dynamical effects further contrasted artificial regulatory oscillators different by the design. A homogeneous population composed of repressilators, along with some other networks, was shown to display robust in-phase synchronization [13, 12, 16]. The property was regarded as a characteristic of the regulatory structure that they have in common. Accordingly, the coupling structure was called phase-attractive as opposed to the phase-repulsive that leads to the anti-phase synchronization [15]. In this paper, we question that the in-phase synchrony is the only option in phase-attractive systems. We show that changing timescales and transcription cooperativity may dramatically alter synchronization properties and lead to other interesting dynamical effects in the network.

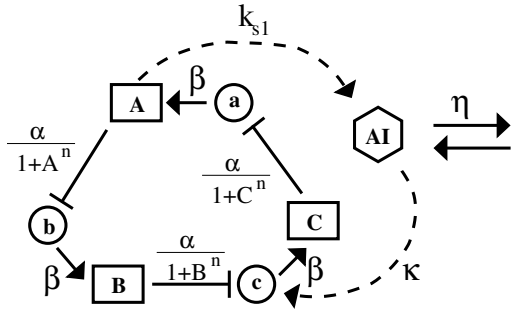


Figure 1: The minimal scheme of repressilator with autoinducer production [13].

2 Model

The idea for the oscillatory mechanism of the repressilator is based on connecting an odd number of inverters (negative control elements) in a ring. Its genetic implementation uses three proteins that cyclically repress the synthesis of one another by inhibition of corresponding mRNA production (Fig. 1). A small molecule, autoinducer (AI), carries out the coupling function which is based on quorum sensing [12, 13]. The following system of dimensionless equations describes the behavior of coupled repressilators [13]:

$$\begin{aligned}
\frac{\mathrm{da_i}}{\mathrm{d}t} &= -a_i + \frac{\alpha}{1+C_i^n}; & \frac{\mathrm{dA_i}}{\mathrm{d}t} &= -\beta(A_i - a_i) \\
\frac{\mathrm{db_i}}{\mathrm{d}t} &= -b_i + \frac{\alpha}{1+A_i^n}; & \frac{\mathrm{dB_i}}{\mathrm{d}t} &= -\beta(B_i - b_i) \\
\frac{\mathrm{dc_i}}{\mathrm{d}t} &= -c_i + \frac{\alpha}{1+B_i^n} + \kappa\frac{S_i}{1+S_i}; & \frac{\mathrm{dC_i}}{\mathrm{d}t} &= -\beta(C_i - c_i) \\
\frac{\mathrm{dS_i}}{\mathrm{d}t} &= -k_{s0}S_i + k_{s1}A_i - \eta(S_i - Q\bar{S})
\end{aligned}$$

The uppercase letters A_i, B_i and C_i denote protein concentrations, while lowercase a_i, b_i and c_i are proportional to the concentrations of mRNA corresponding to those proteins, S_i denotes AI concentration, where i is a cell index. $\bar{S} = \frac{1}{N}\sum_{i=1}^{N} S_i$, where N is the total number of cells; $N = 2$ in this work. All negative terms in 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. Parameter Q reflects degree of the AI dilution in the medium. It is proportional to population density $\frac{V_{\mathrm{cell}}}{V_{\mathrm{medium}}}$ and can be varied from 0 (AI is strongly diluted) up to 1 (dense cell packing) [13]. 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.

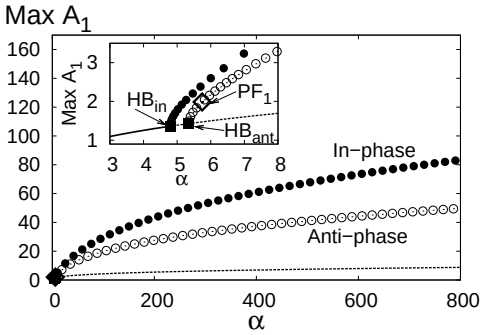
The system and the scheme on Fig. 1 present a highly simplified model of the oscillatory network. In particular, intermediate reaction steps such as binding of an effector to a promoter are assumed to be very fast and, therefore, are not explicitly shown in the model. The system has been shown to oscillate both in experiments and in simulations for big enough α [7, 17, 18].

3 Results

We study an example of two interacting repressilators. We show that increasing the cooperativity of transcription repression (Hill coefficient) and changing the reaction time-scales dramatically alter synchronization properties. The network demonstrates in- and anti-phase oscillatory regimes and can be birhythmic, choosing between those two types of synchronization, in a wide range of parameters. In some region of parametric space there are whole cascades of complex anti-phase oscillatory solutions, which coexist with in-phase regime. Thus, the type of synchronization is not characteristic for the network structure. However, we conclude that the specific scenario of emergence and stabilization of synchronous solutions is much more characteristic.

In particular, anti-phase oscillations emerge at elevated cooperativity values. We choose the maximal synthesis rate for the mRNA as the main control parameter for our analysis. We calculate bifurcation diagrams with respect to this

(a)



(b)

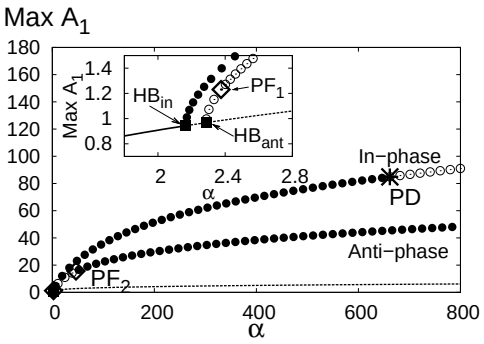


Figure 2: The network switches from in- to anti-phase synchrony when the transcription cooperativity n is elevated. (a) $n = 2$, the in-phase limit cycle is stable; (b) $n = 2.6$, the in-phase cycle loses stability at higher values of α, the anti-phase one becomes stable as α increases. Solid (dashed) lines and solid (empty) circles denote stable (unstable) steady state and periodic solution, respectively. HB — Hopf, PF — pitchfork and PD — period doubling bifurcations. Parameters are: $\beta = 1.0$, $\kappa = 25.0$, $k_{s0} = 1.0$, $k_{s1} = 0.01$, $\eta = 2.0$, $Q = 1.0$ [13].

parameter and study how regimes found in these diagrams depend on other parameters. At the initial cooperativity value of 2.0, the in-phase synchronization remains stable and anti-phase remains unstable at any synthesis rate (Fig. 2(a)). When the cooperativity is elevated only to 2.6, the anti-phase solution becomes stable at a sufficiently high synthesis rate. In contrast, the in-phase solution loses its stability at these elevated cooperativity and high synthesis rate (Fig. 2(b)).

Additionally, fast mRNA kinetics provides birhythmicity in a wide range of the synthesis rate (Fig. 3). Initially, the time-scales of the protein and mRNA kinetics were identical ($\beta = 1.0$). We make mRNA kinetics much faster than protein, which is a more natural case ($\beta = 0.1$). The sequence in which the oscillatory solutions emerge from Hopf bifurcations changes — the anti-phase emerges first. As a result, the anti-phase solution emerges stable, and the in-phase emerges unstable. In the birhythmic parameter regime, both solutions must be stable. Three bifurcations always precede the birhythmic parameter regime when the synthesis rate increases. The in-phase solution becomes stable as a result of a repelling invariant torus emanating from the limit cycle. The

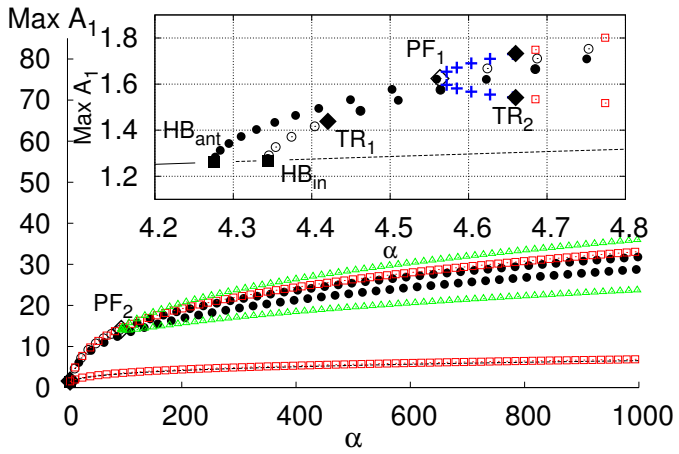


Figure 3: A reduction in the timescale β provides birhythmicity in a wide range of α. At $\beta = 0.1$ and $n = 2.6$, in-phase and anti-phase rhythms are stable at both moderate and high α. PF_1 gives rise to inhomogeneous anti-phase solutions — stable (blue crosses) and unstable (red squares), which are separated by a torus bifurcation (TR_2). PF_2 gives rise only to an unstable inhomogeneous anti-phase solution (green triangles). Other parameters and notations are the same as in Fig. 2.

other two bifurcations are unexpected: The anti-phase limit cycle first loses its stability, and then regains it. Both transitions are pitchfork bifurcations of limit cycles. The second bifurcation cancels the effect of the first one on the stability of the anti-phase solution. Thus, both in-phase and anti-phase solutions are stable in a very wide range of the synthesis rate (Fig. 3).

Our work presents a novel scenario of emerging birhythmicity and switching between the in- and anti-phase solutions in regulatory oscillators. Since the types of synchronization coexist in one network, they are not characteristic for the network structure. However, the bifurcation scenario may be much more characteristic. This may help to address a central question in the analysis of regulatory networks — how to connect structural characteristics to dynamical and functional properties of a network.

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