Evgenii Volkov
Department of Theoretical Physics, Lebedev Physical Inst., Leninskii 53, Moscow, Russia
e-mail: volkov@td.lpi.ru
Ilya Potapov
Department of Signal Processing, Tampere University of Technology, Korkeakoulunkatu 10, Tampere, Finland and Biophysics Department, Lomonosov Moscow State University, GSP-1, Leninskie Gory, Moscow, Russia
e-mail: ilya.potapov@tut.fi
Alexey Kuznetsov
Department of Mathematical Sciences and Center for Mathematical Biosciences, IUPUI, 402 N. Blackford St., Indianapolis, IN 46202, USA.
e-mail: alexey@math.iupui.edu

Dynamics of coupled repressilators: the role of mRNA kinetics and transcription cooperativity

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.

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 [3, 4, 5]. Our computational study [6] suggests that the oscillatory mechanisms implemented in regulatory oscillators are qualitatively different. Comparing various artificial networks helps revealing general principles of cellular regulation.

We study an artificial oscillatory network called the repressilator [4], 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 [7, 8]. A small molecule, autoinducer (AI), carries out the coupling function. Synchronization is only one and simplest outcome of such interaction. It is suggested that the outcome depends on the structure of the network. A phase-attractive (synchronizing) and phase-repulsive coupling structures were distinguished for regulatory oscillators. In this paper, we question this separation.

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 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. 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.

Additionally, fast mRNA kinetics provides birhythmicity in a wide range of the synthesis rate. Initially, the time-scales of the protein and mRNA kinetics were identical. We make mRNA kinetics much faster than protein, which is a more natural case. 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 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.

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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