Physica D 318–319 (2016) 116–123

Contents lists available at ScienceDirect

Physica D

journal homepage: www.elsevier.com/locate/physd

Spatiotemporal dynamics of distributed synthetic genetic circuits

Oleg Kanakov [a,*], Tetyana Laptyeva [a], Lev Tsimring [b], Mikhail Ivanchenko [a]

[a] Lobachevsky State University of Nizhniy Novgorod, Prospekt Gagarina 23, 603950 Nizhniy Novgorod, Russia
[b] BioCircuits Institute, University of California – San Diego, La Jolla, CA 92093-0328, USA

HIGHLIGHTS

- We propose and study distributed gene networks, a toggle-switch and an oscillator.
- The networks consist of two interacting subunits shared between two cell strains.
- The toggle-switch cell culture is controllable with external stimuli.
- The oscillatory cell culture gets synchronized by intercellular signaling.
- We discuss potential biosensing properties of the proposed circuits.

ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form
24 October 2015
Accepted 26 October 2015
Available online 3 November 2015

Keywords:
Synthetic gene networks
Reaction–diffusion systems
Biosensing

ABSTRACT

We propose and study models of two distributed synthetic gene circuits, toggle-switch and oscillator, each split between two cell strains and coupled via quorum-sensing signals. The distributed toggle switch relies on mutual repression of the two strains, and oscillator is comprised of two strains, one of which acts as an activator for another that in turn acts as a repressor. Distributed toggle switch can exhibit mobile fronts, switching the system from the weaker to the stronger spatially homogeneous state. The circuit can also act as a biosensor, with the switching front dynamics determined by the properties of an external signal. Distributed oscillator system displays another biosensor functionality: oscillations emerge once a small amount of one cell strain appears amid the other, present in abundance. Distribution of synthetic gene circuits among multiple strains allows one to reduce crosstalk among different parts of the overall system and also decrease the energetic burden of the synthetic circuit per cell, which may allow for enhanced functionality and viability of engineered cells.

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1. Introduction

Over the last fifteen years we witnessed an outstanding progress in engineering of synthetic gene networks and understanding their complex dynamics. Following the development of the toggle switch [1] and the repressilator [2], there appeared a lineage of transcriptional or metabolic oscillators [3–5], further synchronized by quorum sensing [6,7], event counters [8], pattern forming cultures [9], learning systems [10], optogenetic devices [11], memory circuits and logic gates [12–16].

Currently, much attention is paid to designing cells which synthetic gene circuits would interact with specific genes in the other cells, with promising applications in medicine and, in particular, oncology, from recognition, to targeted drug delivery and killing of cancer cells [17–19]. Accordingly, there arises a general problem of engineering and studying multi-cell distributed synthetic gene circuits, and a considerable progress has already been achieved. The intercell communication has proved to be conveniently realized by (though, not limited to) acyl homoserine lactone (AHL) quorum-sensing signals [20]. Versatile examples of collective functioning were demonstrated, to name pattern formation in a sender–receiver cell strains mixture [9], manipulated biofilm dispersal by a population-driven switch [21], complex logic networks from basic and compatible blocks placed in different cells [13,14], and the dynamics of two cell species cooperation [22,23] or predator–prey interaction [24] and other regimes of microbial ecosystems under the action of environment [25].

The latter examples of collective dynamics relied on the interplay between gene circuit and population dynamics, that is, up or down regulation of synthetic genes would induce cell death or survival. This approach is obviously not very efficient when the engineered cells are the carriers of a function of interest, since it implies

* Corresponding author.
E-mail address: okanakov@rf.unn.ru (O. Kanakov).

http://dx.doi.org/10.1016/j.physd.2015.10.016
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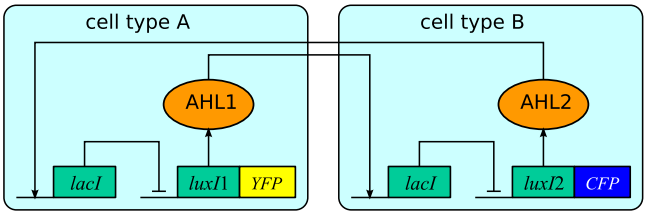

Fig. 1. Scheme of a two-component genetic toggle switch, where mutual repression is realized via diffusion of the quorum-sensing molecules between the cells, and activation of the built in LacI repressors; the activity of a cell can be observed due to fluorescent reporters.

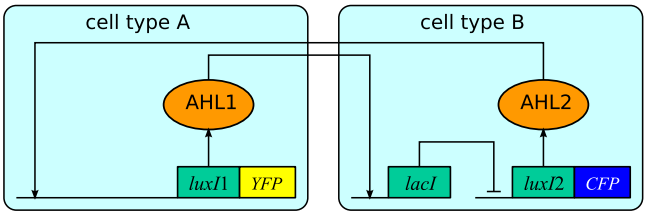

Fig. 2. Scheme of a two-component genetic oscillator, where communication between cell strains is realized via diffusion of the quorum-sensing molecules between the cells. A quorum-sensing signal from the strain B activates the circuit in the strain A, while its peer represses activity in the strain B, due to the built in LacI repressor; the activity of a cell can be observed due to fluorescent reporters.

elimination of at least a part of them. We aim to demonstrate that the complex distributed gene dynamics can be realized without affecting cell survival, and exemplify it in two fundamental dynamical regimes: toggle-switch and oscillator. The potential biosensing properties are discussed.

2. Mathematical model

We introduce model synthetic gene circuits whose parts are distributed between two cell populations (A and B) and communicate by own AHL-family quorum-sensing mediators, which penetrate the other cells and bind with the constitutively produced Lux-R family regulators to form transcription activating complexes [20].

To implement the toggle-switch regime the subcircuits interact competitively, repressing production of AHL in opposing cells by the intermediary lacI repressor gene (see Fig. 1). Self-sustained oscillations can develop if only one subcircuit is repressing its counterpart, while the latter acts as an activator (see Fig. 2). Routine genetically encoded fluorescent reporters (here, yellow and cyan fluorescent proteins, YFP and CFP) can visualize the arising dynamics in biological experiments.

In the first case, a mathematical model under the assumption of a homogeneous mixture of both cell types follows from Michaelis–Menten enzyme kinetics equations [26] and can be written in dimensionless form as a system of partial differential equations:

$$\begin{aligned}
&\partial_t x = \frac{1}{1+(l_1/L)^m} - x \qquad \partial_t y = \frac{1}{1+(l_2/L)^m} - y \\
&\frac{1}{\gamma_2}\partial_t l_1 = l_0\frac{\mu + r_\tau}{1+r_\tau} - l_1 \qquad \frac{1}{\gamma_2}\partial_t l_2 = l_0\frac{\mu + a_\tau}{1+a_\tau} - l_2 \\
&\partial_t a = b_a x - \gamma_3 a + D\Delta a \qquad \partial_t r = b_r y - \gamma_3 r + D\Delta r,
\end{aligned} \tag{1}$$

where the dimensionless state variables are normalized concentrations: x and y—of LuxI1 and LuxI2 (proteins which mediate synthesis of AHL1,2), l_1 and l_2—of LacI in cells of type A and type B, a and r—of AHL1 and AHL2. Parameter l_0 is the relative strength of lacI gene expression, b_a and b_r determine the synthesis rates of AHL1 and AHL2 per unit of volume of the medium (by implication, they are directly proportional to the corresponding cell strain concentrations). L is an inverse sensitivity parameter of luxI to its inhibitor LacI (due to possible renormalization $L = 1$ taken unless stated otherwise), m is a cooperativity parameter for intermediate repressor (in particular, $m = 4$ for LacI), $\mu \ll 1$ determines the background (leakage) expression of luxI1 and luxI2 in the absence of activator, γ_2 and γ_3 are relative degradation rates of lacI and AHL (normalized by that of luxI1 and luxI2 which are assumed equal), D is AHL diffusion coefficient (assumed equal for both AHL types) and Δ is the Laplacian operator. The intracellular diffusion and the time to AHL synthesis are incorporated in the time delay τ.

In case of $b_a = b_r$ model (1) becomes invariant to mutual permutation of triplets (x, l_1, a) and (y, l_2, r). Thus, the only asymmetry between the network components A and B, which is taken into account in the model, is the difference in AHL1 and AHL2 synthesis rates, which are determined by parameters b_a and b_r. For the sake of analysis simplicity we neglect all other possible kinds of asymmetry.

Similarly, a mathematical model for the second circuit reads:

$$\begin{aligned}
&\partial_t x = l_0\frac{\mu + r_\tau}{1+r_\tau} - \frac{x}{1+fx} \\
&\partial_t y = \frac{1}{1+(l_2/L)^m} - \frac{y}{1+f(y+l_2)} \\
&\frac{1}{\gamma_2}\partial_t l_2 = l_0\frac{\mu + a_\tau}{1+a_\tau} - \frac{l_2}{1+f(y+l_2)} \\
&\partial_t a = b_a x - \gamma_3 a + D\Delta a \\
&\partial_t r = b_r y - \gamma_3 r + D\Delta r,
\end{aligned} \tag{2}$$

where we additionally take into account the saturation coefficient of enzymatic degradation f, essential for oscillation dynamics [3,27], let $\gamma_2 = 1$ without the loss of generality, and explicitly write cell densities $n_{1,2}$ in AHL production coefficients $b_a = bn_1$, $b_r = bn_2$.

Numerical simulations of the model are performed in one and two spatial dimensions, for (1) and (2), respectively, using the forward finite-difference method. The Laplacian operator (which in 1D reduces to second derivative in the spatial coordinate) is approximated by second-order central finite difference. Evolution in time is modeled by explicit fourth-order Runge–Kutta scheme. The size of 1D spatial grid used to produce the left panel of Fig. 4 is 800 nodes, and in all other 1D simulations—400 nodes. The coefficient of the discretized Laplacian $D_d = D/\Delta z^2$ (Δz being the spatial grid spacing) in toggle switch medium simulations (Sections 3.2, 3.3) is $D_d = 40$. The spatial coordinate z is normalized by the characteristic scale $z_0 = \sqrt{D/\gamma_3}$, so that the spatial grid step size amounts to $\Delta z \approx 0.16 z_0$. In synchronization simulations (Section 4.2) coefficient D_d is varied, and grid size in 2D simulations is 10×10 nodes with lattice spacing assumed to be unity (physically, it implies that distance is measured in the units of typical correlation length of spatial inhomogeneity). The time step is $\Delta t = 0.01$. Fulfilling the von Neumann condition $D_d \Delta t \leq 1/2$ ensures the stability of forward explicit finite-difference scheme for parabolic equations. Boundary conditions are zero flux conditions.

3. Distributed genetic switch

3.1. Local dynamics

A model describing local dynamics of a physically small volume (which contains a sufficient number of cells, but is small enough to neglect the spatial variation of all variables within the volume) is a system of ordinary differential equations (ODEs) obtained from (1) by omitting the diffusion term (setting $D = 0$). Additionally, we neglect the time-delay, $\tau = 0$. To get an insight into the dynamics

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