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Spatiotemporal dynamics of distributed synthetic genetic circuits

Oleg Kanakov^{a,*}, Tetyana Lapyteva^a, Lev Tsimring^b, and Mikhail Ivanchenko^a

^aLobachevsky State University of Nizhniy Novgorod, Prospekt Gagarina 23, 603950 Nizhniy Novgorod, Russia

^bBioCircuits Institute, University of California – San Diego, La Jolla, CA 92093-0328, USA

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.

Keywords

synthetic gene networks; reaction-diffusion systems; biosensing

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, 4, 5], further synchronised by quorum sensing [6, 7], event counters [8], pattern forming cultures [9], learning systems [10], optogenetic devices [11], memory circuits and logic gates [12, 13, 14, 15, 16].

*Corresponding author okanakov@rf.unn.ru (Oleg Kanakov).

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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, 18, 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 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, that 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:

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

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), $\mu < 1$ 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 is the Laplacian operator. The intracellular diffusion and the time to AHL synthesis is 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:

$$\begin{aligned} \partial_t x &= l_0 \frac{\mu + r\tau}{1 + r\tau} - \frac{x}{1 + fx} \\ \partial_t y &= \frac{1}{1 + (l_2/L)^m} - \frac{y}{1 + f(y + l_2)} \\ \frac{1}{\gamma_2} \partial_t l_2 &= l_0 \frac{\mu + a\tau}{1 + a\tau} - \frac{l_2}{1 + f(y + l_1)} \\ \partial_t a &= b_a x - \gamma_3 a + D \Delta a \\ \partial_t r &= b_r y - \gamma_3 r + D \Delta r, \end{aligned} \quad (2)$$

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 $D_d = D / z^2$ (z 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 $z \approx 0.16z_0$. In synchronization simulations (Sec. 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 of this local model, we start with analyzing the symmetric case $b_a = b_r = b$. In this case the system has an invariant manifold $x = y$, $l_1 = l_2$, $a = r$. A straightforward algebra allows for deriving an approximate expression for an equilibrium point on this manifold

$$x=y \approx l_{1,2}^{-m}, \quad l_{1,2} \approx l_0 r, \quad r^{m+1} \approx \frac{b}{\gamma_3} \frac{1}{l_0^m} \quad (3)$$

under simplifying assumptions

$$\mu \ll \left(\frac{b}{\gamma_3} \frac{1}{l_0^m} \right)^{\frac{1}{m+1}}, \quad (4a)$$

$$\frac{1}{l_0} \ll \frac{b}{\gamma_3} \ll l_0^m. \quad (4b)$$

These requirements can be fulfilled by choosing sufficiently large value of l_0 (which corresponds to a relatively strong expression of lacI genes and can be achieved, for example, by increasing the copy number for this gene), and small enough value of μ (which implies weak background expression of luxI1 and luxI2 genes in the absence of activator; for real promoters values of the order $\mu \approx 0.01$ are typical). Condition (4b) then defines a range of allowed production to degradation ratio of AHL1 and AHL2. In particular, its left-hand part imposes a lower bound on cell density: if it is too small, then AHL concentration is insufficient for mutual suppression, and both cell strains are simultaneously active without any bistability.

The linearized dynamics about the equilibrium point is split into two non-interacting parts, describing the independent evolution of small perturbations, tangent and transversal to the symmetric invariant manifold. Under the assumptions (4a,b), the transversal part exhibits instability, as soon as the additional requirement

$$m > 1 \quad (5)$$

is fulfilled. This is actually the case for the lacI gene, which exhibits cooperativity $m = 4$. Tangential instability is also formally possible within the same assumptions (4a,b), but is found to require much higher cooperativity $m > 8$, which does not hold for the lacI repressor and is not typical in general.

Conditions (4a,b) and (5) together ensure the transversal (symmetry-breaking) instability of the symmetric equilibrium point. Generally speaking, this kind of (local) instability does not imply bistability in the global phase space, since the analysis above does not exclude the existence of a globally stable attractor on the symmetric manifold. Furthermore, this analysis does not apply to the asymmetric case. That said, the set of conditions (4a,b), (5) yields a good starting hint to search for bistability in the local dynamics, and, accordingly, for wave fronts in the spatially extended model.

3.2. Wave fronts in the extended model

Equilibrium states of local dynamics correspond to spatially homogeneous states of the extended model (1), which is a multi-component reaction-diffusion system. In case of local bistability, reaction-diffusion systems commonly admit solutions in the form of fronts which separate quasi-homogeneous domains corresponding to either stable state. Emergence and propagation of such fronts is almost completely characterized in case of scalar (one-component) reaction-diffusion systems (see [28, 29] and references therein). Namely, in single spatial dimension immobile fronts are non-generic (exist only in a codimension-1 set of parameters); generically, fronts propagate in the direction which is uniquely determined by relative “strengths” of the two asymptotic stable states on the sides of the front: the “stronger” state domain always expands at the expense of the “weaker” one. In higher space dimensions, curvature of a front also affects front velocity [29]. In multi-component bistable reaction-diffusion systems, even in single spatial dimension, front velocity (and even direction of propagation), generally speaking, is no longer uniquely determined by front asymptotics (at fixed system parameters) and may depend upon initial conditions [30, 31]. In this case front propagation cannot be explained merely by ascribing relative “strengths” to the stable states and assuming expansion of the stronger one.

Assuming bistability in the local version of (1), in case of $b_a = b_r$ we expect zero-velocity front solutions to (1) due to symmetry. Since immobile fronts of (1) are still codimension-1 in the parameter space (as results from the fact that seeking for a time-independent solution to (1) leads to a system of two equations both having spatial derivatives, see details in [32]), asymmetry $b_a \neq b_r$ leads to front propagation. We did not prove existence of these front solutions rigorously, neither did not we study their uniqueness and stability. However, our numerical results below in this section support the conjecture that at the chosen parameter values fronts exist and are stable, and their velocity (in particular, direction of propagation) is uniquely determined by the system parameters. This justifies the use of “state strengths” terminology, with the asymptotic state ahead of (in back of) the propagating front designated as the weaker (the stronger) one at given parameter values.

In order to obtain stationary front profiles in the symmetric and asymmetric cases we used parameter values $\gamma_2 = \gamma_3 = 1.0$, $l_0 = 3.0$, $\mu = 0.01$, $m = 4$, with $b_a = b_r = 5.0$ for the symmetric case, and $b_r = 5.0$, $b_a = 5.2$ for the asymmetric one. These parameter values satisfy conditions (4a,b) and (5). Initial conditions are specified in the form of a step-shaped front: $x = y = l_1 = l_2 = 0.5$ in the whole system, $a = 0.1$ and $r = 1.0$ to the left of the initial position of the front, while $a = 1.0$ and $r = 0.1$ to the right. The stationary front is obtained by modeling the evolution of the system on the time interval $T_{\text{relax}} = 100$, which is sufficient

for the stationary profile to establish. In Fig. 3 we plot stationary profiles of $a(z)$ and $r(z)$ for stable fronts in the symmetric (solid lines) and asymmetric (dashed lines) cases. The origin of the spatial coordinate $z = 0$ is placed at the center of the front which is defined as the point where $a(z, t) = r(z, t)$, and calculated by cubic spline interpolation between grid nodes. Note, that two curves for $r(z)$ visually coincide, which implies almost identical profiles of $r(z)$ in both cases. The profile of $a(z)$ in the asymmetric case (blue dashed line) is noticeably shifted upwards from the symmetric one (blue solid line), which makes the state to the right of the front center “stronger” than the one to the left, and leads to front propagation in the leftward direction.

We measured the dependence of the stationary front speed upon parameter b_a at constant $b_r = 5.0$ by letting the front propagate for time interval $T_{\text{propag}} = 1500$ (after initial relaxation on interval $T_{\text{relax}} = 100$) and dividing the observed shift of the front center by T_{propag} . The result is plotted in Fig. 4 (left panel), confirming that any small asymmetry leads to front propagation.

3.3. Switching dynamics in response to external perturbations

Since, in our case, no immobile fronts or structures are found in an asymmetric bistable system, only homogeneous states persist. This makes any finite-sized (bounded) extended system also essentially bistable, with the whole medium ultimately relaxing to one of the two stable states. A bounded bistable medium can thus be used as a single robust multi-cellular bistable unit.

The distributed toggle switch can be used as a robust biosensing system that transitions to one of the two stable states depending on the structure of an external stimulus. To implement it one can add extra luxI genes with promoters sensitive to a certain external signal (e.g. light, chemical concentration, etc.). This sensitivity may be either direct, or mediated by a signaling pathway of the cell. In our mathematical model it can be taken into account by modifying the equations describing the evolution of LuxI concentration (first line in (1)) with additional terms which are generally time- and space-dependent:

$$\partial_t x = \frac{1}{1+l_1^m} - x + E_x(t, z), \quad \partial_t y = \frac{1}{1+l_2^m} - y + E_y(t, z), \quad (6)$$

where $E_x(t, z)$, $E_y(t, z)$ are relative expression rates of additional luxI1 and luxI2 genes controlled by external stimuli.

Depending on the magnitude of the stimulus, the system either remains bistable (at weaker stimuli), or loses bistability and only one stable state remains (at greater ones). Weaker stimuli can be used to control the speed and direction of wave front propagation. In Fig. 4 (right panel) we plot the measured dependence of front speed versus stimulus magnitude E_y (constant in time and applied uniformly in space) in the asymmetric case $b_a = 5.2$, $b_r = 5.0$ (other parameters same as in previous simulations). We observe, that tuning E_y allows to immobilize the front by effectively canceling out the asymmetry, and even to reverse the front propagation by swapping the roles of the stronger and the weaker states.

Stronger stimuli destroy bistability, which can be used to switch the system. Due to asymmetry of states important differences occur for switching in opposite directions. When the whole system resides in the stronger state, it can be switched to the weaker one only by a global stimulus. Otherwise, a part of the system remains in the stronger state, which after the stimulus is removed will propagate in the wake of the front and occupy the medium. On the contrary, if the system initially resides in the weaker state, then switching a part of the system may eventually switch the whole medium due to front propagation.

We confirm this reasoning by numerical simulations. The asymmetry is achieved by letting $b_r = 5.0$, $b_a = 5.2$, with all other parameters same as in the previous simulations. We start with switching from the stronger to the weaker state. To prepare the medium in the stronger state, we specify initial conditions $a = 1.0$, $r = 0.1$, all other dynamic variables at 0.5 in the whole system, and let the system equilibrate for time interval $T_{\text{relax}} = 100$. The origin of time in the plots which are presented below is the end of this relaxation interval. To record the pre-stimulus stationary state, we simulate the system for another $T_{\text{prestim}} = 100$ time units. After that we apply the stimulus $E_y(t, z)$ of constant amplitude 0.1 in the whole system for time duration $T_{\text{stim}} = 400$. After the end of the stimulus we simulate free evolution of the system on time interval $T_{\text{free}} = 700$. In this case the whole system changes the state after stimulus. Since the whole setting is uniform in space, the solution does not depend on the spatial coordinate. Plots of $a(t)$ and $r(t)$ taken at the middle point of the system versus time are presented in Fig. 5.

When a stimulus of the same magnitude is applied locally on a section of size W , defined by $|z| \leq W/2$ (the origin $z = 0$ is placed in the middle of the system), with $W/z_0 \approx 15$, then the local state is switched by the stimulus, but due to front propagation the system eventually switches itself back to the stronger state after the stimulus is removed, as expected. This is shown in Fig. 6, where the left and the middle panels present the evolution of variables $a(z, t)$ and $r(z, t)$ in the whole system in color code, and the right panel contains plots of $a(0, t)$ and $r(0, t)$ measured at the middle point of the system ($z = 0$) versus time.

Next, we consider switching to the stronger state at the same parameter values. We put the system into the weaker state by using initial conditions $a = 0.1$, $r = 1.0$, other dynamic variables at 0.5 in the whole system, and letting the system equilibrate on interval $T_{\text{relax}} = 100$. Then we follow the same protocol as in the previous simulation with same timings $T_{\text{prestim}} = 100$, $T_{\text{stim}} = 400$, $T_{\text{free}} = 700$, but now applying stimulus $E_x(t, z)$. The magnitude of the stimulus is $E = 0.1$, and the spatial width W of the stimulus took on three values: $W/z_0 \approx 15$ (top row in Fig. 7), $W/z_0 \approx 6$ (middle row) and $W/z_0 \approx 3$ (bottom row). We observe, that in cases when the stimulus is sufficient to switch the system locally (top and middle rows in Fig. 7), the area occupied by the stronger state expands in the wake of the propagating front, which implies the ultimate switching of the whole system of any finite size. At the same time, when the stimulus is not sufficient to switch the system even locally, then no fronts are triggered, and the system returns to the initial (weaker) state after the stimulus is removed (bottom row in Fig. 7).

The result of the previous simulation suggests that the capacity of the stimulus to switch the medium and trigger the front propagation depends not only on its magnitude, but as well on

the spatial scale of its application (the size of the medium section on which the stimulus is applied). We performed a series of simulations applying local stimuli $E_x(t, z)$ of constant magnitudes ranging from 0.03 to 0.5 on spatial scales W/z_0 ranging from 0.95 to 15.0 (approximately), with duration $T_{\text{stim}} = 1000$. After the stimulus is removed, free evolution of the system is simulated on the time interval $T_{\text{free}} = 500$. In Fig. 8 we plot the value of variable $a(z, t)$ found in the center of the system ($z = 0$) at the end of the simulation. We observe, that even strong stimuli are not capable of switching the medium, unless they are applied in a wide enough region. This property may be employed to construct a controllable multicellular bistable unit with increased robustness to noise, since uncorrelated spatial patterns of noise are not expected to lead to erroneous switching of the system state.

4. Distributed oscillator

4.1. Spatially homogeneous regimes

Let us analyze stationary and spatially homogeneous solutions to (2), considering its ODE counterpart, as obtained by letting $D = 0$:

$$\begin{aligned}\partial_t x &= l_0 \frac{\mu + r_\tau}{1 + r_\tau} - \frac{x}{1 + f x}, \\ \partial_t y &= \frac{1}{1 + (l_2/L)^m} - \frac{y}{1 + f(y + l_2)}, \\ \frac{1}{\gamma_2} \partial_t l_2 &= l_0 \frac{\mu + a_\tau}{1 + a_\tau} - \frac{l_2}{1 + f(y + l_2)}, \\ \partial_t a &= b n_1 x - \gamma_3 a, \\ \partial_t r &= b n_2 y - \gamma_3 r.\end{aligned}\quad (7)$$

Neglecting enzymatic degradation, $f = 0$, one arrives at the $(m + 1)$ -th order polynomial in l_2 , the LacI concentration, whose non-negative roots yield coordinates of equilibria of (7). For $m = 4$, which is the actual cooperativity of LacI, we get

$$\frac{\beta_2 (\beta_1 + l_0 \mu)}{L^4} l_2^5 - \frac{l_0 \beta_2 \mu}{L^4} (l_0 + \beta_1) l_2^4 + (l_0 + \beta_1 \beta_2 + \beta_1 + \mu l_0 \beta_2) l_2 - l_0 (l_0 + \mu (\beta_1 + \beta_1 \beta_2 + l_0 \beta_2)) = 0, \quad (8)$$

where $\beta_{1,2} = \gamma_3 / (b n_{1,2})$.

It can be further demonstrated that the polynomial (8) always possesses a single positive root, and, correspondingly, the original system (2) always has a single stationary spatially homogeneous solution. The stability of this solution against spatially homogeneous perturbations can be studied analytically, as the linear stability of the equilibrium of (7). Tedious algebra, which we do not provide here for the sake of brevity, shows its persistent stability at least for $\mu > 0$, $\tau = 0$, $f = 0$.

The main results are obtained numerically. First, we study the stability of the equilibrium of (7) and the onset of oscillations due to Andronov-Hopf bifurcation (cf. Fig. 9) in dependence of the enzymatic degradation, f , inverse sensitivity of luxR repression by LacI, L (varied in experiment, e.g. by modulating concentration of the IPTG inducer), Fig. 10, and density of activator cells, n_2 , Fig. 11 (left panel). Similarly, we confirmed that non-zero time delay, $\tau > 0$, leads to development of self-sustained oscillations in a wide range of parameters, even if saturation of enzymatic degradation is neglected, $f = 0$.

4.2. Spatially inhomogeneous and biosensing dynamics

Cells may naturally have non-uniform distribution in space. In this case the medium will have different local properties: oscillating or non-oscillating, and in the former case, local frequencies may differ. Thus, to obtain a common frequency output, necessary when periodic functionality is required, synchronization has to be established.

Studying the potential sources of oscillation frequency variance we calculate the frequency ω of the limit cycle in (7) to find its dependence on the cell densities (see Fig.11) pronounced in presence of both enzymatic degradation and non-zero time delay (in this case oscillations are more robust which allows for tuning their frequency in a broader range). Instructively, higher densities correspond to lower frequencies.

Synchronization of oscillations across the space can be achieved due to diffusion of AHL. We demonstrate it by simulating a discretized version of (2) on a 10×10 lattice with unity spacing of sites, with cell densities $n_{1,2} \in [0, 1]$ randomly chosen for each lattice site, and with varied coefficient of discretized Laplacian D_d (“discrete diffusion coefficient”). The other parameters were set to the typical $\mu = 0.01$, $l_0 = 2$, $b = 2$, $\gamma_3 = 1$, $L = 0.5$, $\tau = 2$, $f = 0.5$. The results shown in Fig.12 (a)-(c) confirm that already at moderate values of the diffusion coefficient synchronization sets in and the whole system oscillates at the same frequency.

Remarkably, oscillations emerge even when the relative density of the two cell strains is highly imbalanced, as the right panel of Fig.11 shows. For example, about 5% of activator cells is enough to destabilize equilibrium. This result not only additionally confirms the robustness of the proposed distributed oscillator, but suggests its potential application as a biosensor, when the cells of one strain can indicate the appearance of another by developing an oscillatory component in the fluorescent reporter signal, see illustration in Fig.12 (d). Note that as variation in the density of cells changes oscillation frequency, it can also report the population dynamics of the culture.

Conclusions

We proposed and studied distributed synthetic genetic toggle-switch and oscillator, the parts of both circuits distributed between two cell strains and coupled via quorum sensing signals. The distributed toggle switch relies on mutual repression of the parts in each strain, and oscillator comprises activating and repressing units. In contrast to the previously studied distributed synthetic gene circuits, the requested dynamics does not involve population dynamics. Minimization of exogenous constructs in a single cell decreases the energetic burden and allows for introducing additional synthetic genes of a useful functionality under control of the distributed dynamics.

Distributed bistable system can exhibit mobile fronts (or immobile, in case of symmetry), switching the system from the weaker to the stronger state. The circuit can also behave as a biosensor, with the switching front induced by external signal (transcriptional regulator, chemical inducer, optical signal). This response can be implemented for switching on and off the genetically encoded treatment or killing of tumor cells [33, 34, 35, 36, 37, 17, 38, 39, 40].

Distributed oscillator is shown to persist in a wide region of system parameters. Despite the dependence of its frequency on the local properties of population (e.g. cell density), the quorum sensing signaling can synchronize local oscillations on a common frequency, making it possible to implement a periodic modulation of a therapy. Importantly, the emergence of oscillations itself, which requires the mutual presence of both cell strains, is manifested already at small densities of one or another strain. Thus, a single strain culture can play a role of a biosensor, with the oscillations launched in response to the appearance of the second strain. This effect can also be implemented for initiating and modulating the above mentioned tumor therapies by synthetically engineered cells.

These results call for further studies of complex dynamical regimes of synthetic gene networks that can be realized in interacting multi-strain cell cultures.

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Highlights (for review)

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.

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Figure 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.

Figure 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.

Figure 3.

Profiles $a(z)$ (blue) and $r(z)$ (red) in stationary wave fronts in the symmetric (solid lines) and asymmetric (dashed lines) cases. Note, that two curves for $r(z)$ visually are not discriminated and fuse into one. Asymmetric front propagates to the left.

Figure 4.

Dependence of the kink velocity upon b_a at fixed $b_r = 5.0$, $E_y = 0$ (a), upon E_y at fixed $b_a = 5.2$, $b_r = 5.0$ (b).

Figure 5.

Switching the system from the stronger state to the weaker one by globally applied stimulus: plots of $a(t)$ (blue solid line) and $r(t)$ (red dashed line) taken at the middle point of the system versus time.

Figure 6.

Failure to switch the system from the stronger state to the weaker one by locally applied stimulus. Panel (a): $a(z, t)$; panel (b): $r(z, t)$; panel (c): middle-point plots $a(0, t)$ (blue solid line) and $r(0, t)$ (red dotted line).

Figure 7.

Switching the system by external stimulus from the weaker state to the stronger one. Left column: $a(z, t)$; middle column: $r(z, t)$; right column: plots of $a(0, t)$ (blue solid line) and $r(0, t)$ (red dashed line). Spatial scale of stimulus application W/z_0 is approximately 15 (top row), 6 (middle row), 3 (bottom row).

Figure 8. Switching the medium from weaker to stronger state: the established value of variable a in the center of the system versus strength E and width W of the applied stimulus.

Figure 9.

From stable equilibrium to self-sustained oscillations in (7) as the inverse sensitivity to LacI transcriptional repression, L , increases. Here $\mu = 0.01$, $l_0 = 2$, $b = 2$, $\gamma_3 = 1$, $n_1 = n_2 = 1$, $f = 1$ and $\tau = 0$.

Figure 10.

Maximal real part of characteristic values (top panel) and corresponding imaginary part (bottom panel) of the equilibrium of (7) in dependence on the inverse sensitivity to LacI repressor L for a set of enzymatic degradation saturation coefficient values f . Here $\mu = 0.01$, $l_0 = 2$, $b = 2$, $\gamma_3 = 1$, $n_1 = n_2 = 1$, $f = 1$, and $\tau = 0$.

Figure 11.

Oscillation frequency of the limit cycle in (7) in dependence on the density of activator cells n_2 for a set of enzymatic degradation saturation coefficient values f (a) and on density of both repressors and activators, n_1 and n_2 (b). Here $\mu = 0.01$, $l_0 = 2$, $b = 2$, $\gamma_3 = 1$, $L = 0.5$, $\tau = 2$ and $n_1 = 1$ (a) and $f = 0.5$ (b).

Figure 12.

(a) Observed oscillation frequencies in a discretized version of (2), a lattice 10×10 , converge to a synchronization frequency as the discrete diffusion coefficient D_d is increased. Here $\mu = 0.01$, $l_0 = 2$, $b = 2$, $\gamma_3 = 1$, $L = 0.5$, $f = 0.5$, $\tau = 2$, and random $n_{1,2} \in [0, 1]$. (b) and (c) Spatio-temporal patterns for a lattice cross-section (column) in the asynchronous ($D_d = 0.01$) and synchronous ($D_d = 0.1$) regimes. (d) Reporting appearance of activator cells: $n_2 = 0$ for times $t < 250$ and $n_2 = 0.1$ at the center of the area for $t > 250$. Emerging periodic oscillations indicate the presence of second cell strain. Here $\mu = 0.01$, $l_0 = 2$, $b = 2$, $\gamma_3 = 1$, $L = 0.5$, $f = 1.0$, $\tau = 2$, $D_d = 0.1$.