ДИНАМИКА СВЯЗАННЫХ СИНТЕТИЧЕСКИХ ГЕНЕТИЧЕСКИХ ОСЦИЛЛЯТОРОВ

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Как их связать:

- quorum sensing

Связанные Репрессилаторы:

- Inhomogeneous steady states,
- Inhomogeneous limit cycles,
- Oscillatory regimes

Генетический триггер

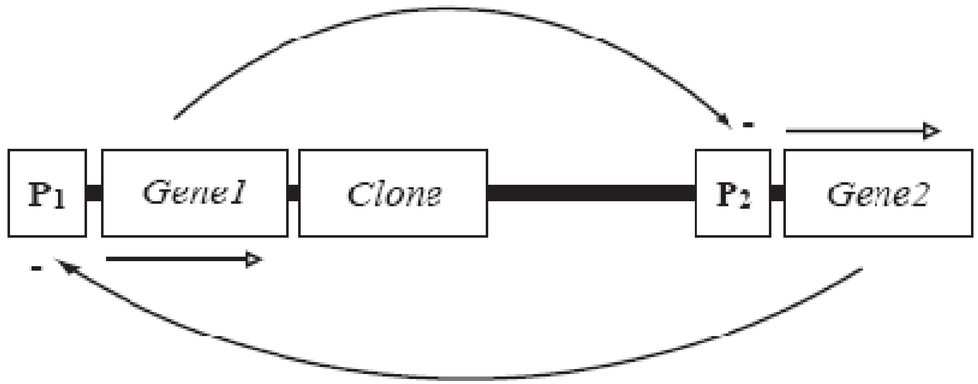

Figure 3: Schematic of toggle switch. Promoter 1, P_1, efficiently transcribes gene 1 unless inhibited by the repressor protein encoded by gene 2. Promoter 2, P_2, efficiently transcribes gene 2 unless inhibited by the repressor protein encoded by gene 1. Open arrows indicate direction of transcription. Clone: an additional gene or genes can be placed under the control of P_1 or P_2.

Two mutual repressors: Nature 2000

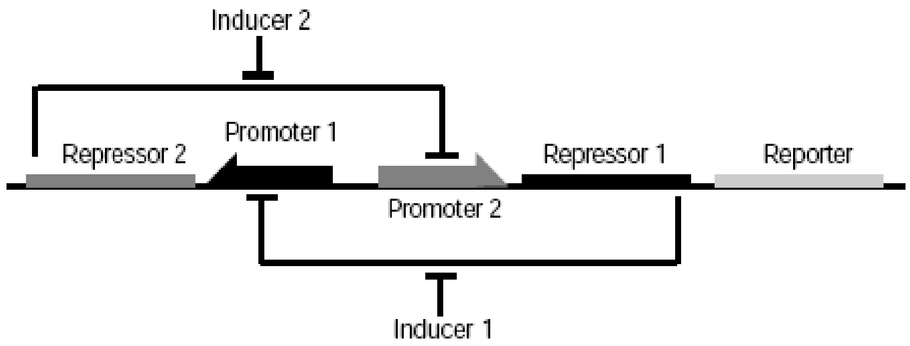

Figure 1 Toggle switch desi NATURE | VOL 403 | 20 JANUARY 2000 tion from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2.

Construction of a genetic toggle switch in *Escherichia coli*

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Фазовый портрет триггера – Nature 2000

$$\frac{du}{dt} = \frac{\alpha_1}{1 + v^{\beta}} - u$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1 + u^{\gamma}} - v$$

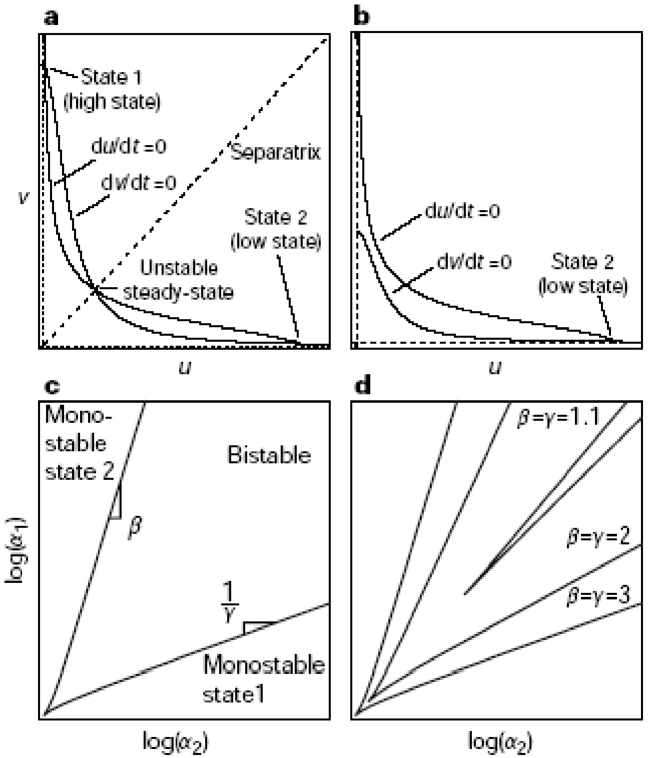
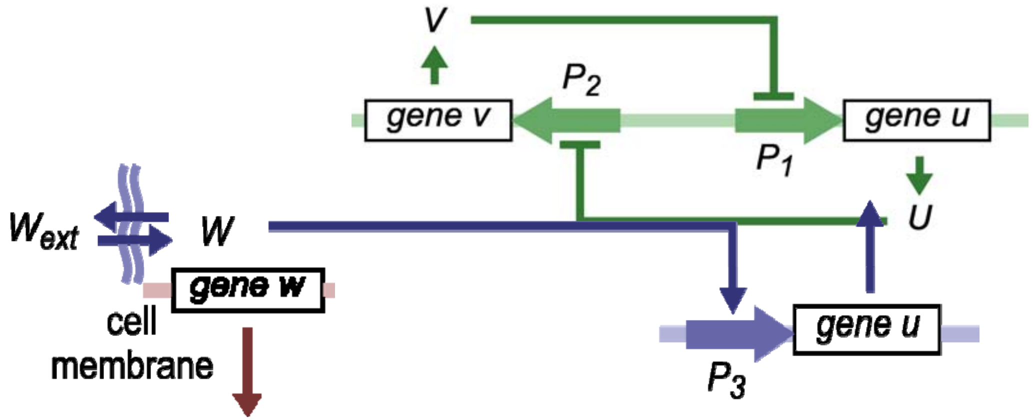


Figure 2 Geometric structure of the toggle equations. **a**, A bistable toggle network with balanced promoter strengths. **b**, A monostable toggle network with imbalanced promoter strengths. **c**, The bistable region. The lines mark the transition (bifurcation) between bistability and monostability. The slopes of the bifurcation lines are determined by the exponents β and γ for large α_1 and α_2. **d**, Reducing the cooperativity of repression (β and γ) reduces the size of the bistable region. Bifurcation lines are illustrated for three different values of β and γ. The bistable region lies inside of each pair of curves.

- Генетический релаксационный осциллятор – Kuznetsov A. et al, SIAM, 2004



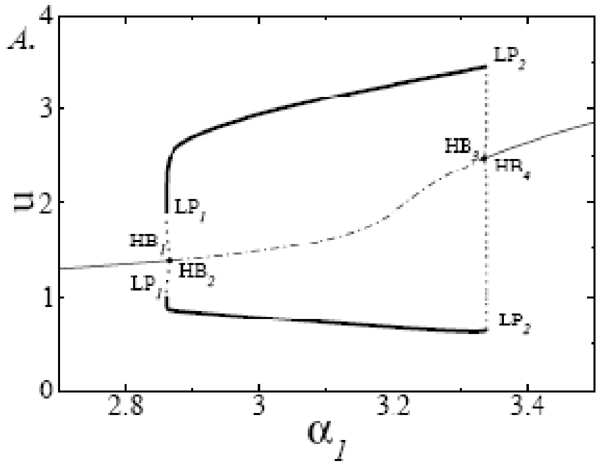
$$\frac{du_i}{dt} = \alpha_1 f(v_i) - u_i + \alpha_3 h(\omega_i) \qquad \frac{dv_i}{dt} = \alpha_2 g(u_i) - v_i$$

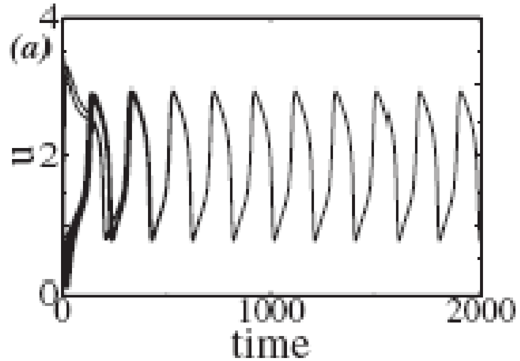
$$\frac{d\omega_i}{dt} = \varepsilon(\alpha_4 g(u_i) - \omega_i) + 2d(\omega_e - \omega_i) \qquad f(v) = \frac{1}{1+v^\beta},\ g(u) = \frac{1}{1+u^\gamma},\ h(w) = \frac{w^\eta}{1+w^\eta},$$

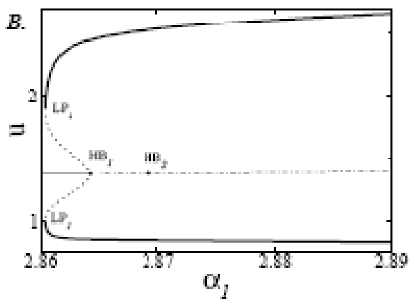
$$\frac{d\omega_e}{dt} = \frac{d_e}{N}\sum_{i=1}^{N}(\omega_i - \omega_e).$$

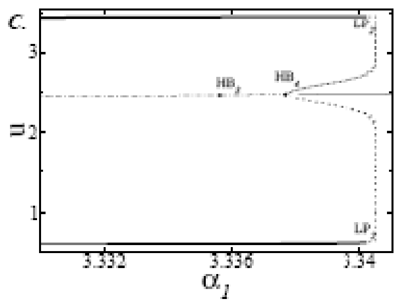
Связь обеспечивается диффузией медленной переменной

Изолированный генетический Релаксатор

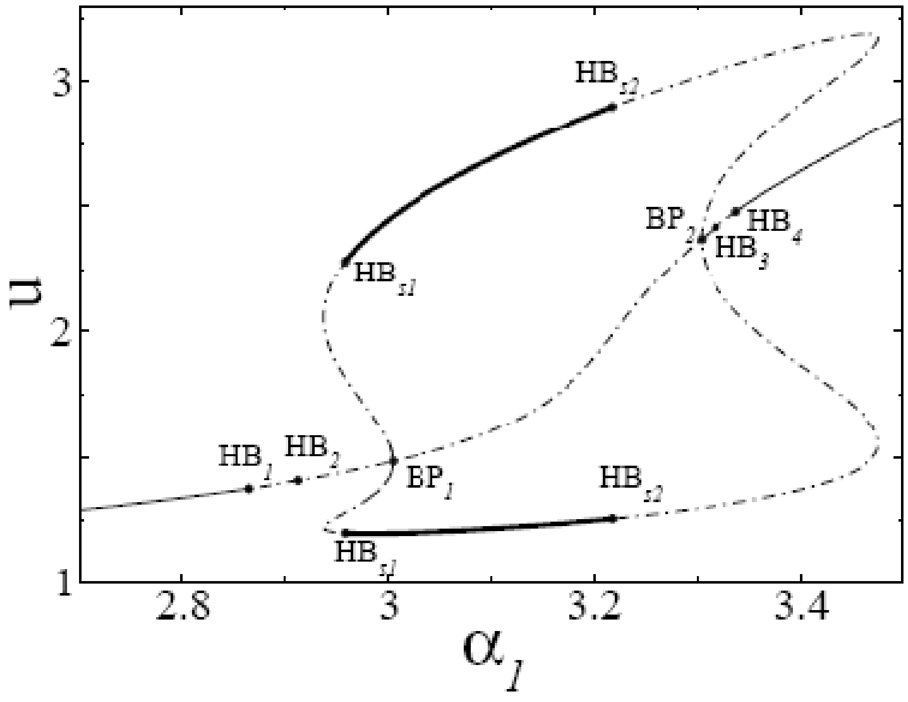








Oscillator Death (2 Релаксатора)



Oscillator Death (2 Relaxators)

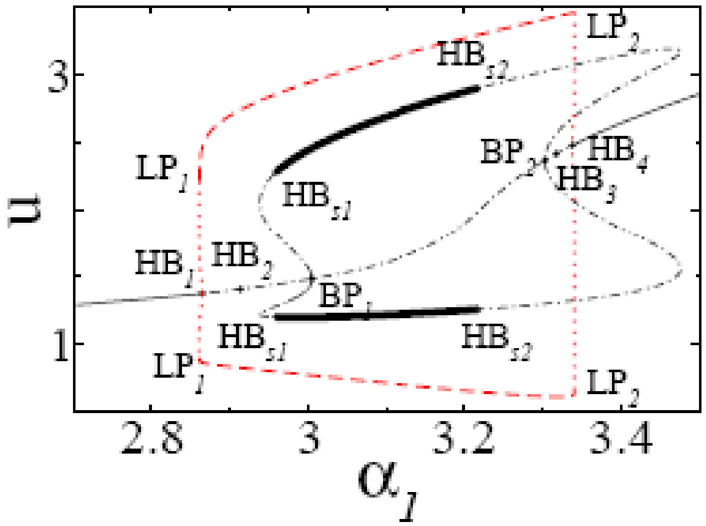


FIG. 4: Bifurcation diagram obtained by variation of α_1, to illustrate the OD. The coupling strength is $d = 0.01$. Other parameters are the same as in Fig. 2. Thin solid lines denote stable steady state, thick solid lines - a stable OD regime, dash - dotted lines denote unstable steady state, dotted-lines - unstable limit cycle and dashed lines - stable limit cycle.

Many oscillators - OD

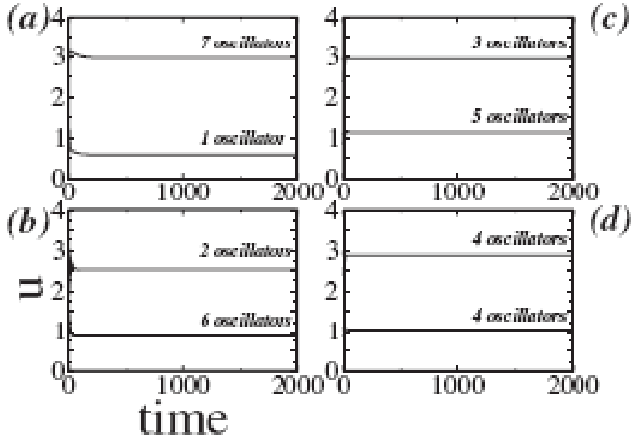


FIG. 2. Examples of different distributions in the steady state clusters for the system (1)–(4) with $N=8$ oscillators. Parameters: $\varepsilon=0.01$, $\alpha_1=3$, $\alpha_2=5$, $\alpha_3=1$, $\alpha_4=4$, $\beta=\eta=\gamma=2$, $d=0.3$, and $d_e=1$. Distribution (a) 1:7, where seven oscillators occupy the higher level; (b) 2:6; (c) 3:5; and (d) 4:4. Note the different protein levels for different oscillator distributions.

OD + α-Detuning = ODD

Detuning=0.96, coupling d = 0.005, 0.006, 0.008

tem is governed by the dimensionless Eqs. (see [15] for details):

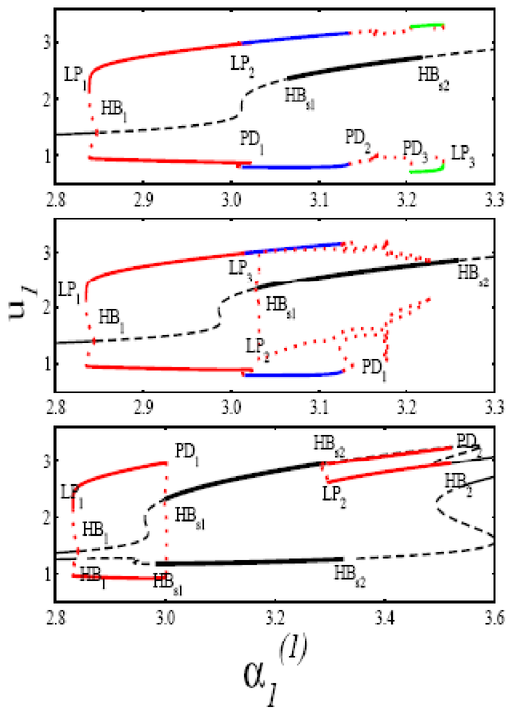
$$\frac{du_i}{dt} = \alpha_1^{(i)} f(v_i) - u_i + \alpha_3 h(w_i) \quad (1)$$

$$\frac{dv_i}{dt} = \alpha_2 g(u_i) - v_i \quad (2)$$

$$\frac{dw_i}{dt} = \varepsilon(\alpha_4 g(u_i) - w_i) + 2d(w_e - w_i) \quad (3)$$

$$\frac{dw_e}{dt} = \frac{d_e}{N}\sum_{i=1}^{N}(w_i - w_e). \quad (4)$$

where N is the total number of oscillators, u_i and v_i represent the proteins of which the toggle switch is made in the i-th cell, and w_i and w_e are the intra- and extra-cellular AI concentrations, respectively. The dimensionless parameters α_{1i} and α_2 regulate the oper-



Реконструкция разверток двух Релаксаторов при изменении бифуркационного параметра

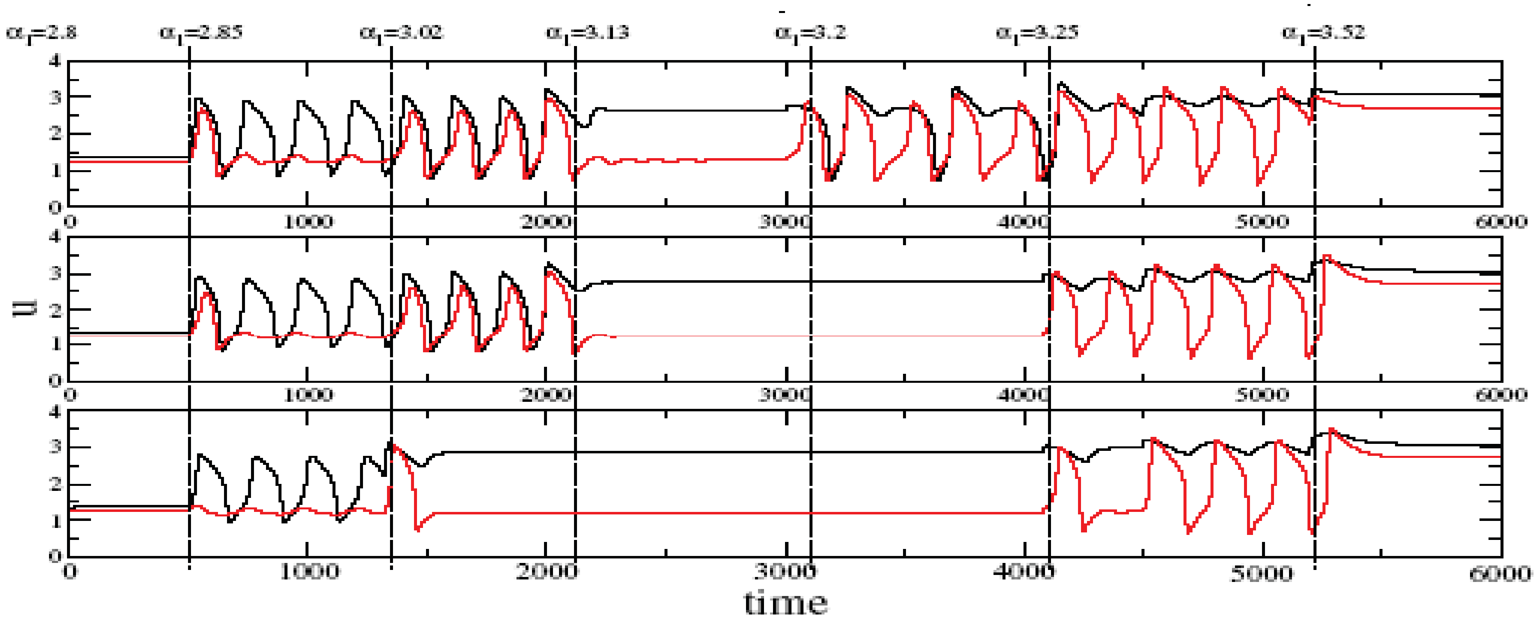


Fig. 1: (Colour on-line) Conjugation lines representing time series (ts) for different interval of α_1 values, which correspond to the qualitative changes of the dynamic of the system (1–4), for top: $d = 0.005$, middle: $d = 0.006$, bottom: $d = 0.008$ and $d_{ij} = 0.96$. These α_1 intervals (the vertical dashed lines) correspond to stable regimes for $d = 0.005$. We continue them below to show the dynamical changes in the system. Other parameters are: $N = 2$, $\varepsilon = 0.01$, $\alpha_2 = 5$, $\alpha_3 = 1$, $\alpha_4 = 4$, $\beta = \eta = \gamma = 2$, and $d_e = 1$.

5 coupled genetic relaxators

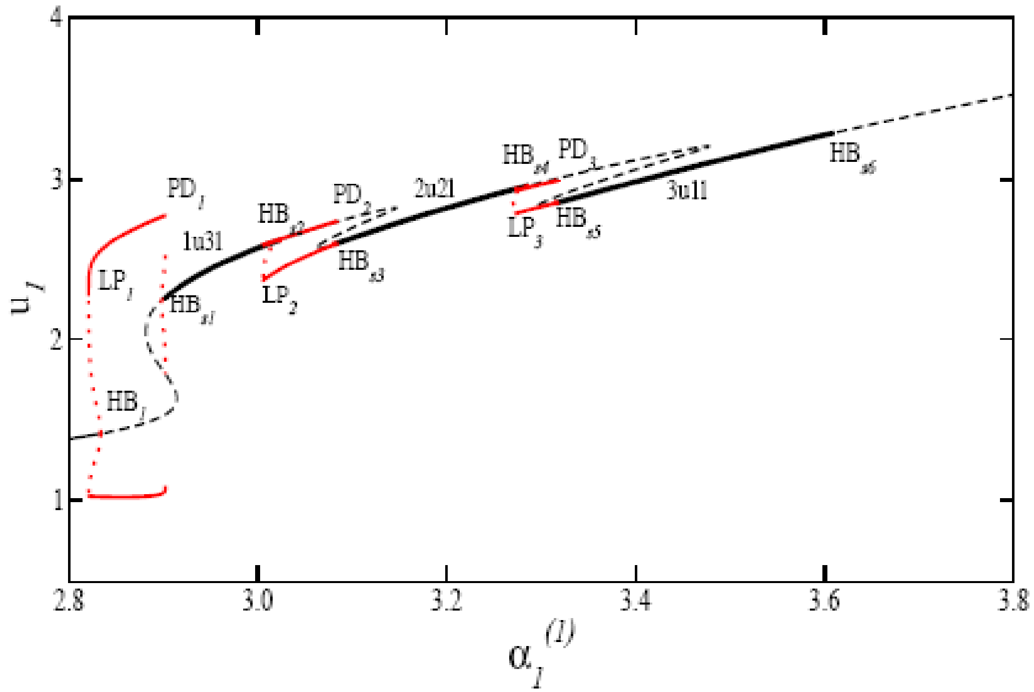


FIG. 7: Different stable cluster distributions for $N = 5$ coupled oscillators (the bifurcation branch for one oscillator is plotted). From left to right: 1 oscillators located in the '(**u**)pper' OD cluster, 4 in the '(**l**)ower' one - $1u|4l$ distribution, $2u|3l$, $3u|2l$ and $4u|1l$ distribution. The oscillatory solutions (asymmetric oscillations) are 'pushed' between the stable distributions, establishing OD dominance.

Many Relaxators - Clustering

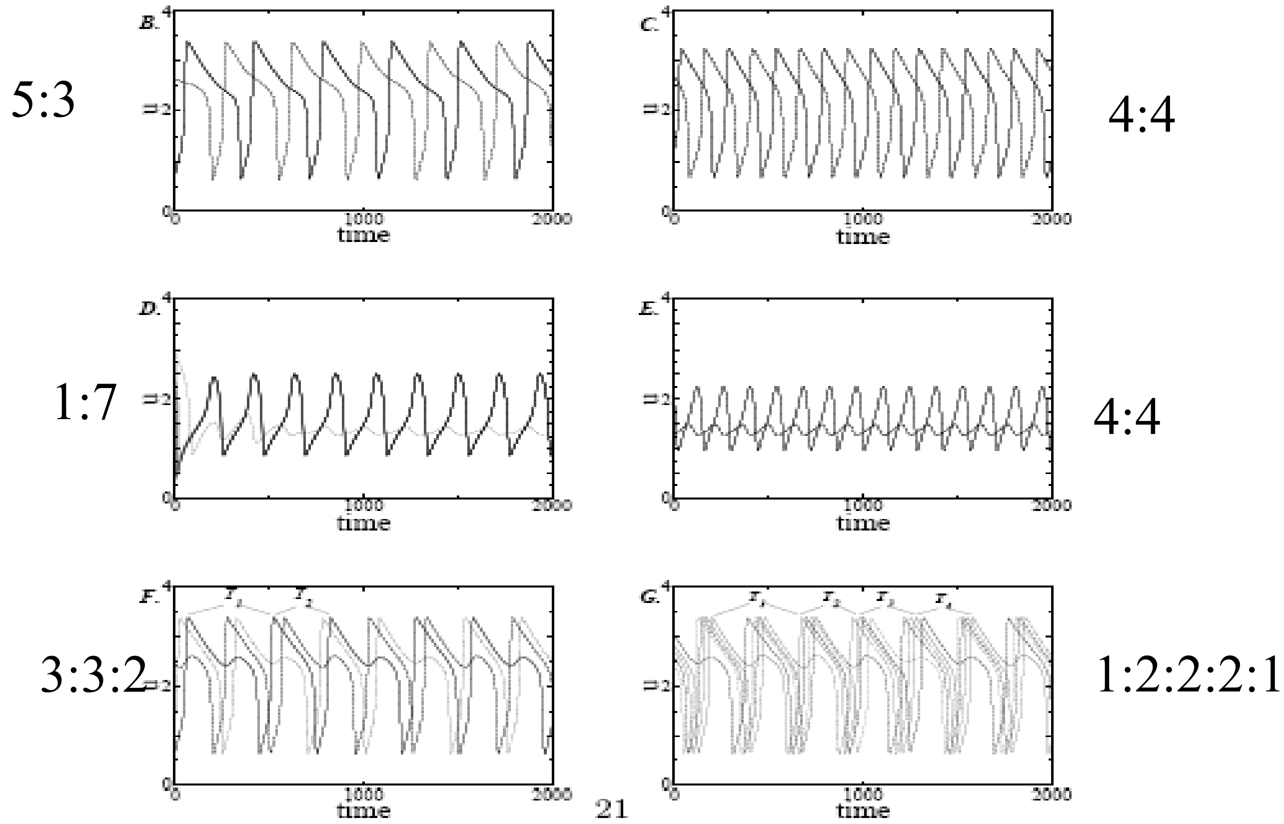
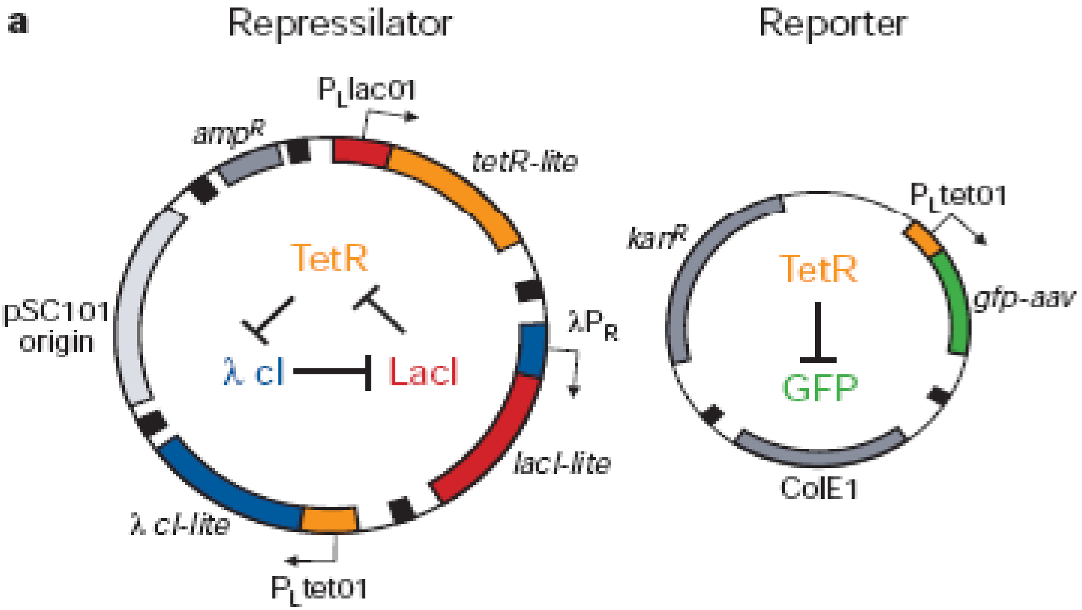


FIG. 3: Different oscillatory clusters for system of $N = 8$ oscillators. **(a)**: in-phase oscillations: $\alpha_1 = 3, d = 0.005$; **(b)**,**(c)**: anti-phase oscillations with different distributions of the oscillators between clusters: $\alpha_1 = 3.3, d = 0.001$; **(d)**,**(e)**: asymmetric solution with different distribution of the oscillators: $\alpha_1 = 2.868, d = 0.001$; **(f)**: three oscillatory clusters: $\alpha_1 = 3.3, d = 0.00105$ and **(g)**: five oscillatory clusters: $\alpha_1 = 3.3, d = 0.001$. For other parameters see Fig.2.

Nature, 403, 2000

A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler



Repressilator

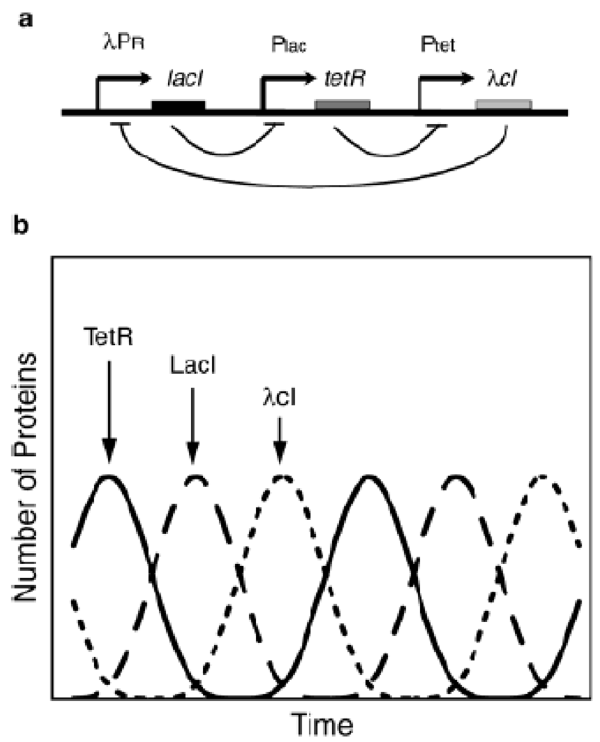


Figure 2. a: The repressilator. The promoters and repressors used were slightly modified versions of the wildtype. **b:** In oscillatory cells the three repressor populations oscillate with lagged phases. Coupling of the TetR repressor to the reporter plasmid expressing a short-lived GFP molecule permits observation of the oscillations.

Repressilator works – Elowitz, Leibler: Nature, 2000

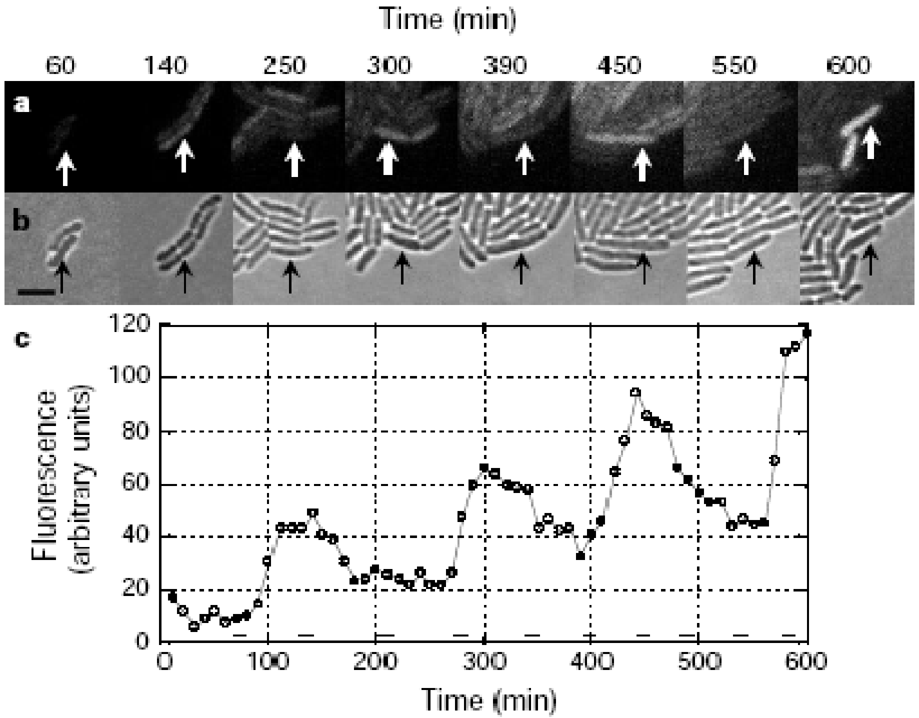


Figure 2 Repressilation in living bacteria. **a**, **b**, The growth and timecourse of GFP expression for a single cell of *E. coli* host strain MC4100 containing the repressilator plasmids (Fig. 1a). Snapshots of a growing microcolony were taken periodically both in fluorescence (**a**) and bright-field (**b**). **c**, The pictures in **a** and **b** correspond to peaks and troughs in the timecourse of GFP fluorescence density of the selected cell. Scale bar, 4 μm. Bars at the bottom of **c** indicate the timing of septation events, as estimated from bright-field images.

Модификации Репрессилаторов с разными типами связи

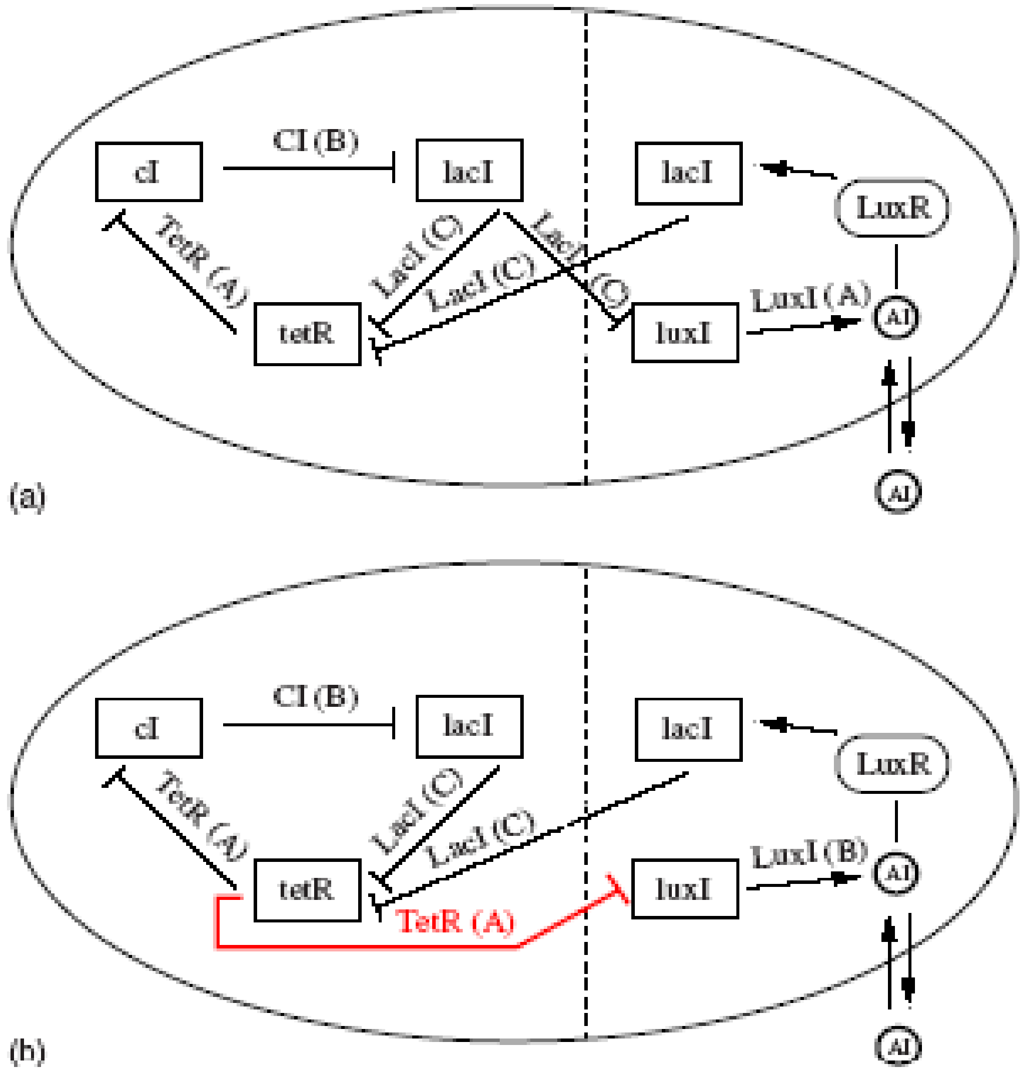
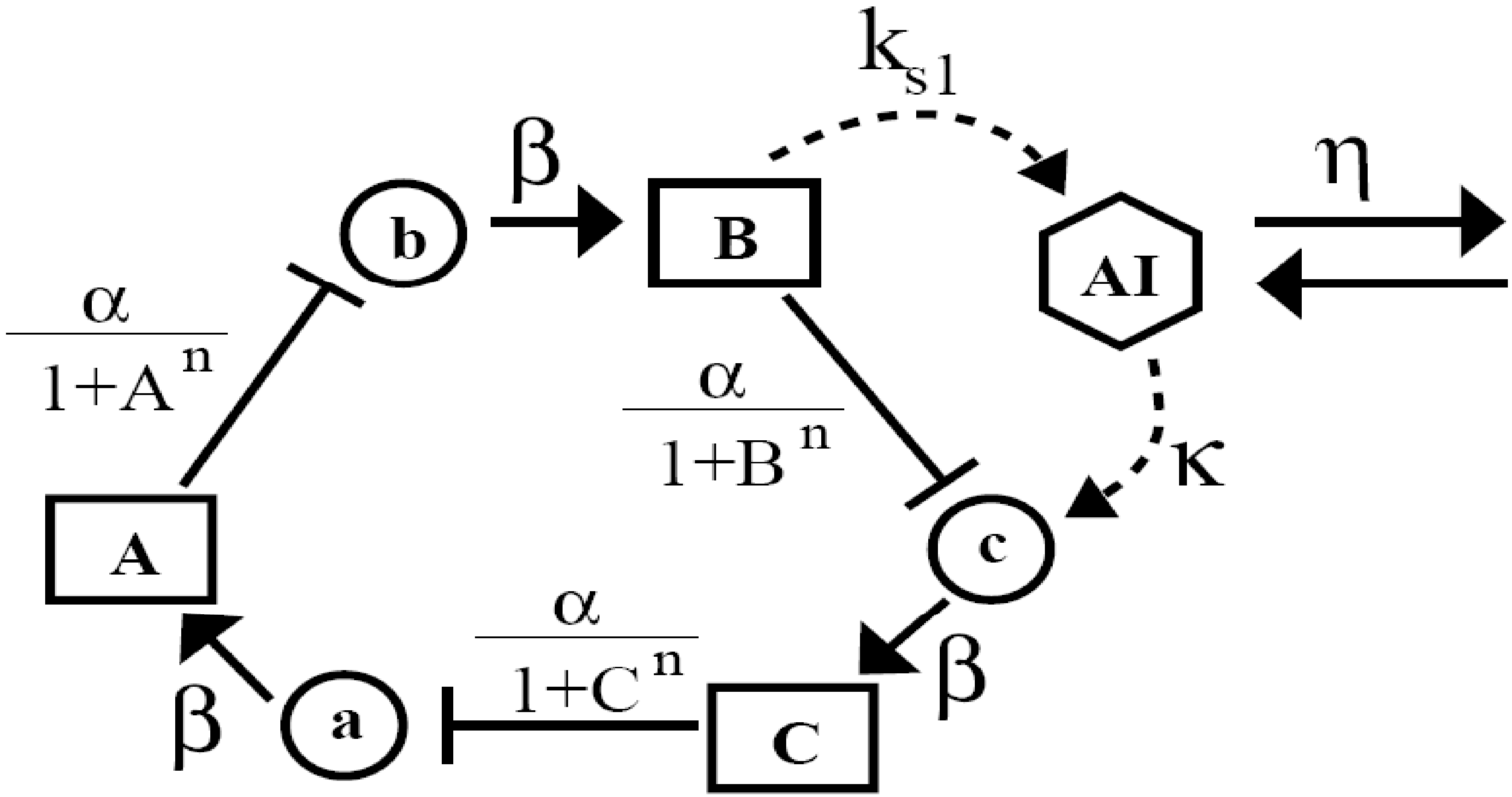


FIG. 1. (Color online) Scheme of the repressilator with quorum sensing cell-to-cell communication: with reinforcing coupling [5] (top) and repressive coupling (bottom).

Схема Репрессилатора с разбиением по стадиям



Безразмерные уравнения глобально связанных репрессилаторов

RNA

$$\dot{a}_i = -a_i + \frac{\alpha}{1 + C_i^n}$$

$$\dot{b}_i = -b_i + \frac{\alpha}{1 + A_i^n}$$

$$\dot{c}_i = -c_i + \frac{\alpha}{1 + B_i^n} + \kappa \frac{S_i}{1 + S_i}$$

proteins

$$\dot{A}_i = \beta_a (a_i - A_i)$$

$$\dot{B}_i = \beta_b (b_i - B_i)$$

$$\dot{C}_i = \beta_c (c_i - C_i)$$

Производство аутоиндусера и связь

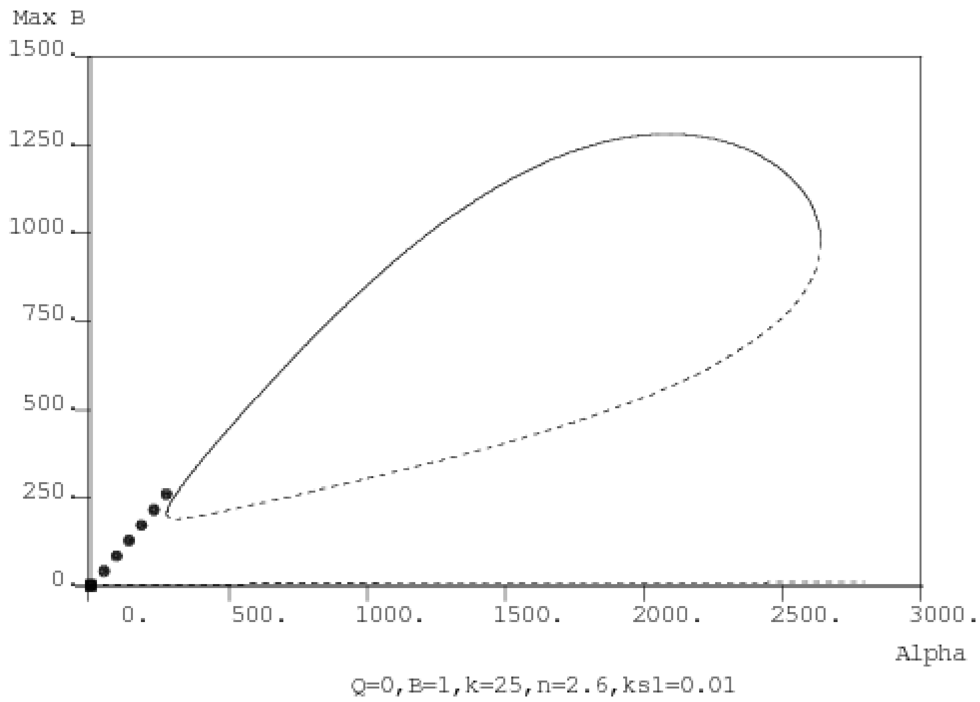
$$\dot{S}_i = -k_{s0} S_i + k_{s1} B_i - \eta (S_i - S_e)$$

$$S_e = Q \bar{S}$$

$$\bar{S} = \frac{1}{N} \sum_{i=1}^{N} S_i$$

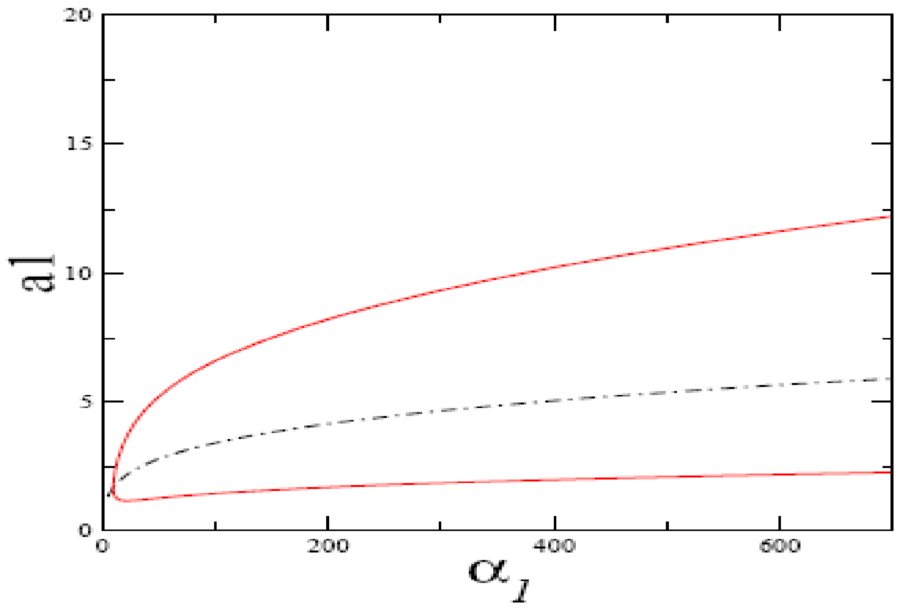
$Q = \frac{\delta N / V_{ext}}{k_{se} + \delta N / V_{ext}}$ определяет силу связи

Динамика изолированного Репрессилатора с ростом скорости транскрипции: переход от автоколебаний к устойчивому стационарному состоянию.

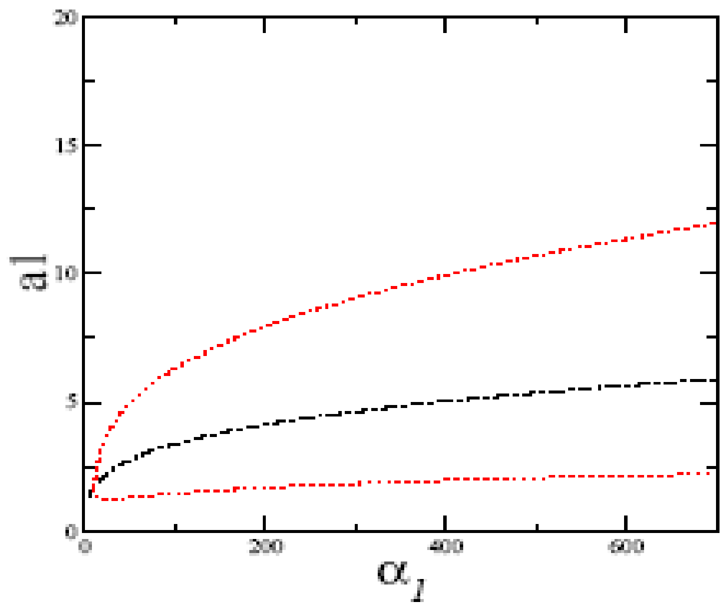


Каков тип связи?

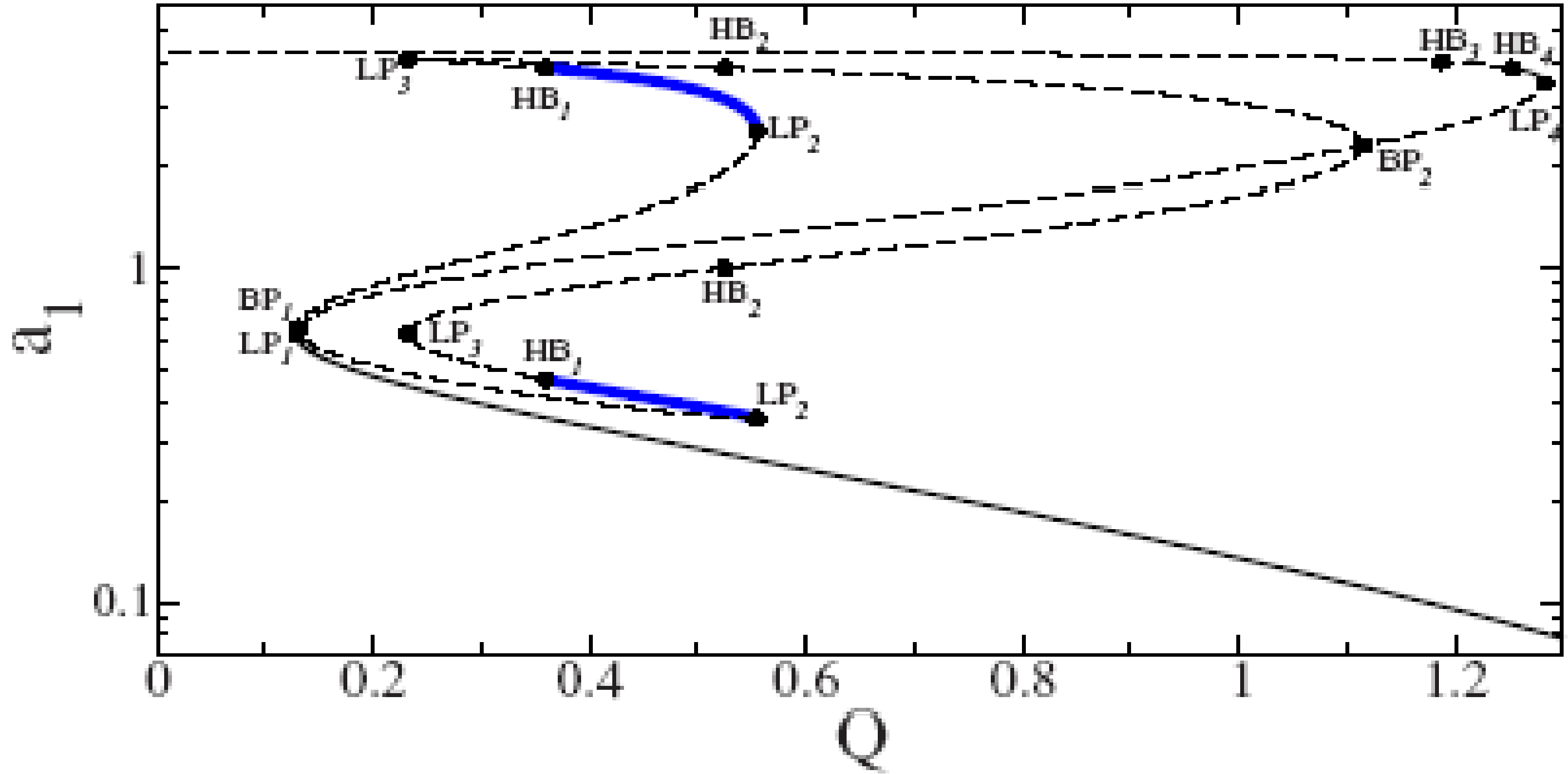
stable anti-phase limit cycle



unstable in-phase LC

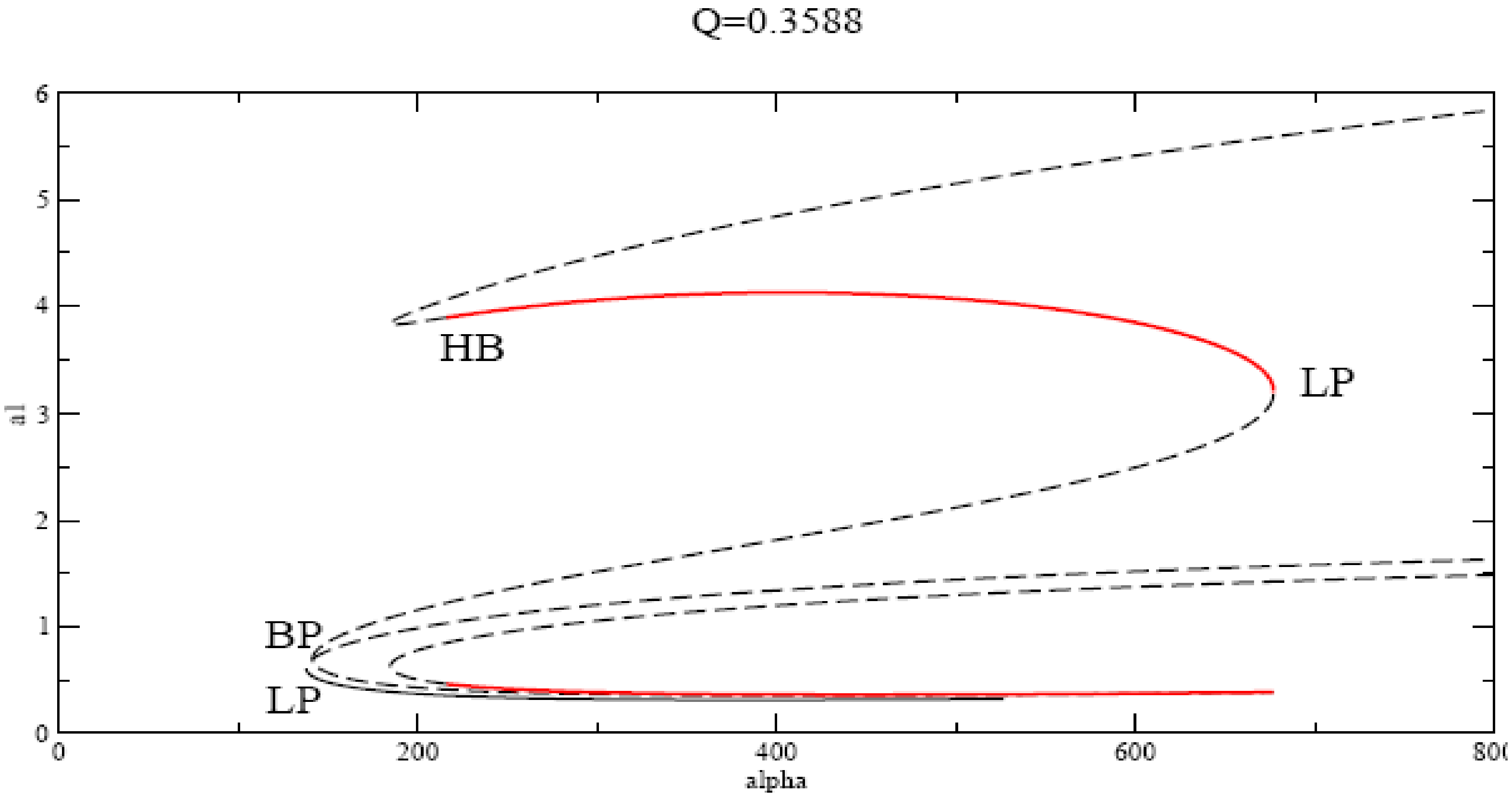


Bifurcation analysis for two coupled Repressilators: the steady states: α is fixed



Q regulates how much AI is returned to cells from the medium

Bifurcation analysis for two coupled Repressilators: steady states - Q is fixed



Bifurcation analysis for two coupled Repressilators: inhomogeneous oscillations

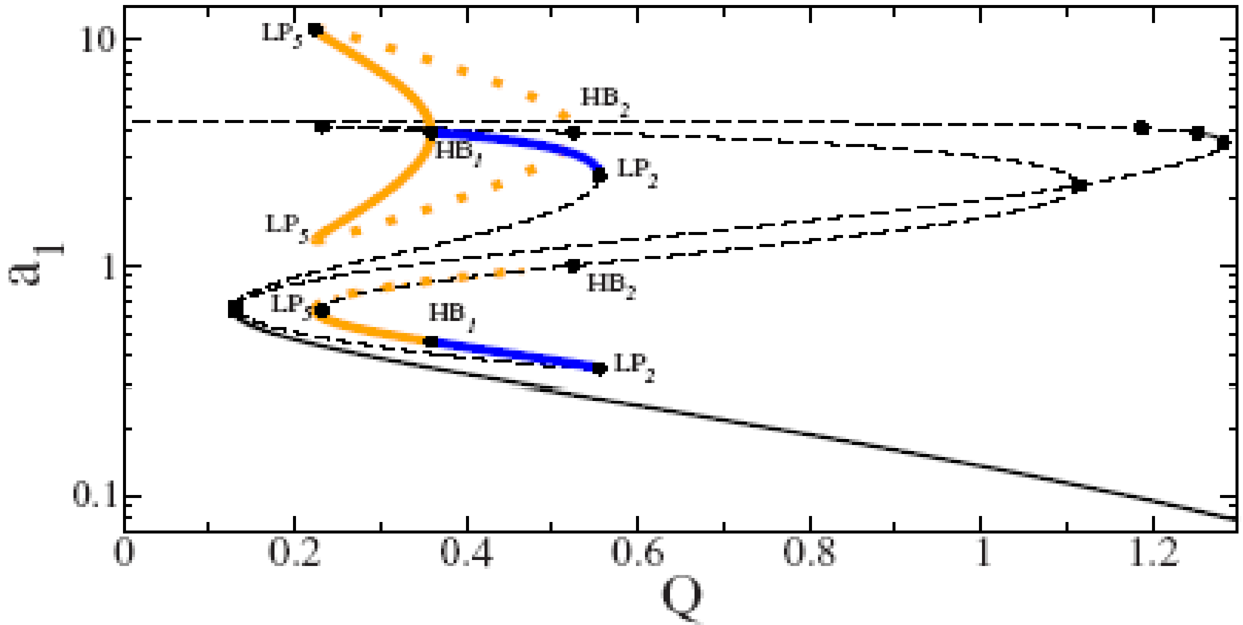
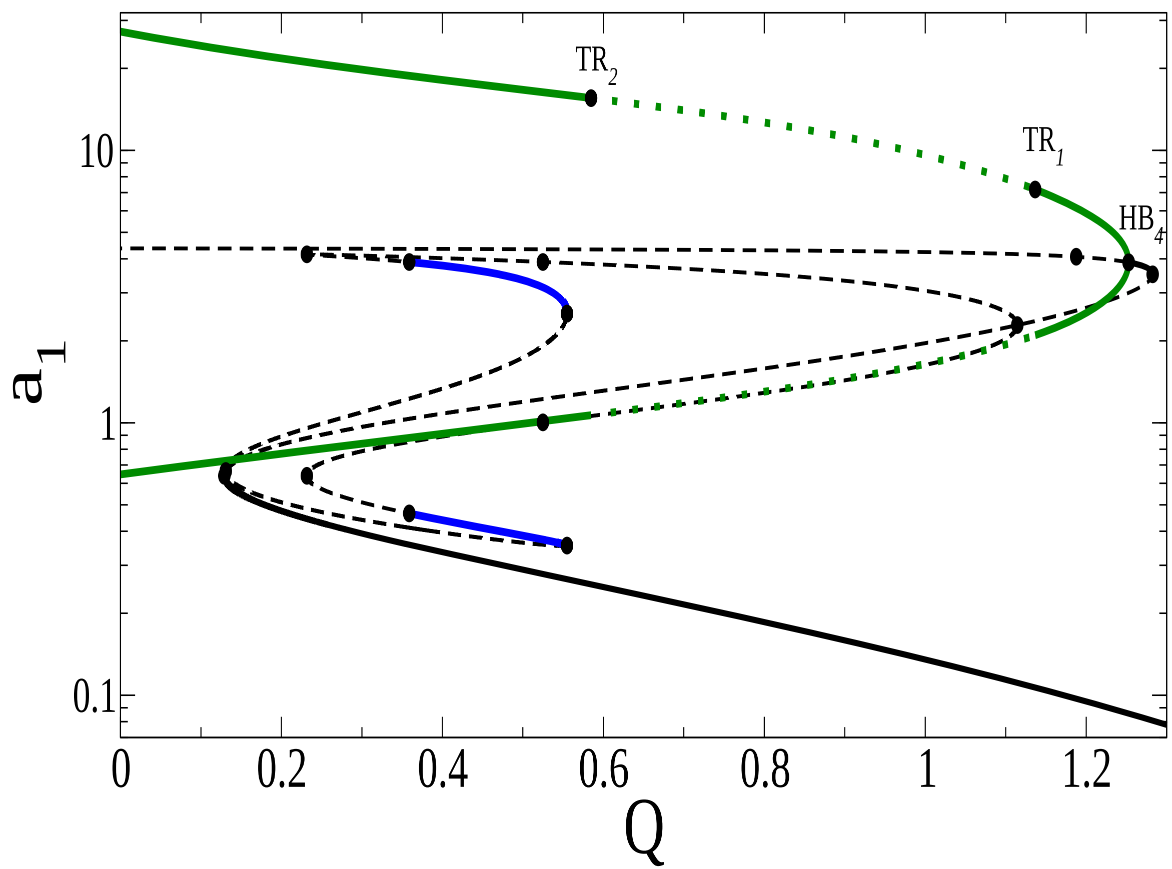


FIG. 4. (Color online) Bifurcation diagram versus coupling Q, with a focus on the IHLC and the IHSS. The stable IHSS is represented by a thick blue line, the stable IHLC with a thick orange line, and the unstable IHLC is represented with a dashed yellow line. Parameters are those of Fig. 2.

Bifurcation analysis for two coupled Repressilators: oscillations



Bifurcation analysis for two coupled Repressilators: regular oscillations

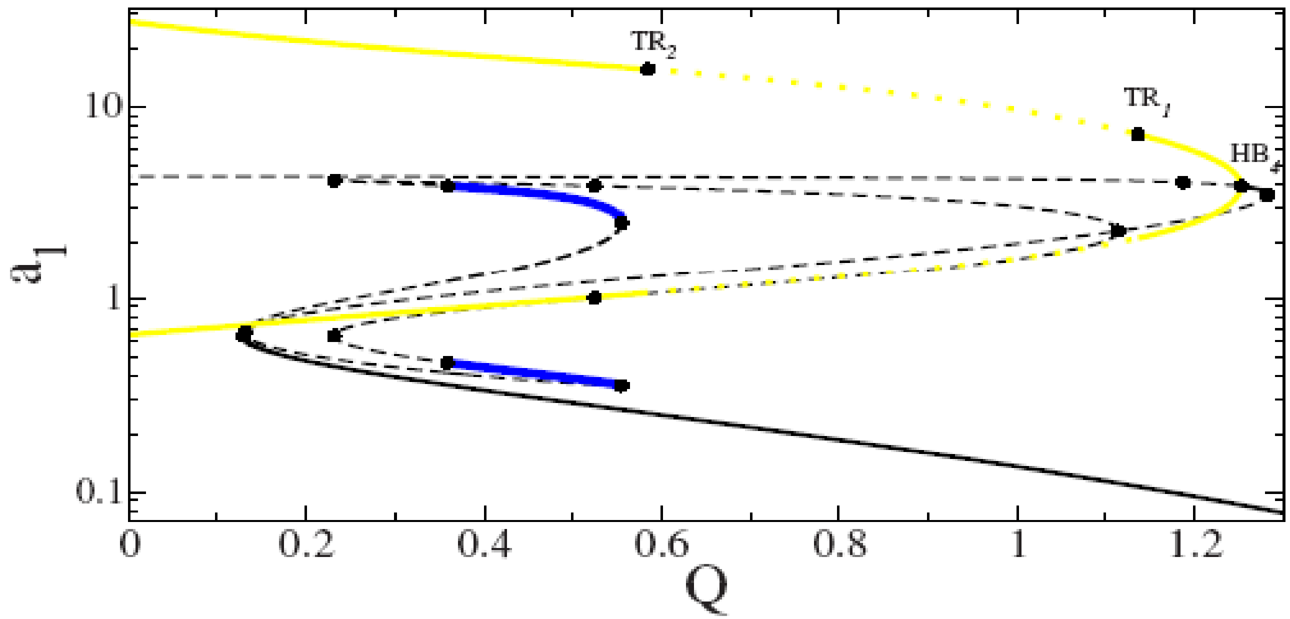
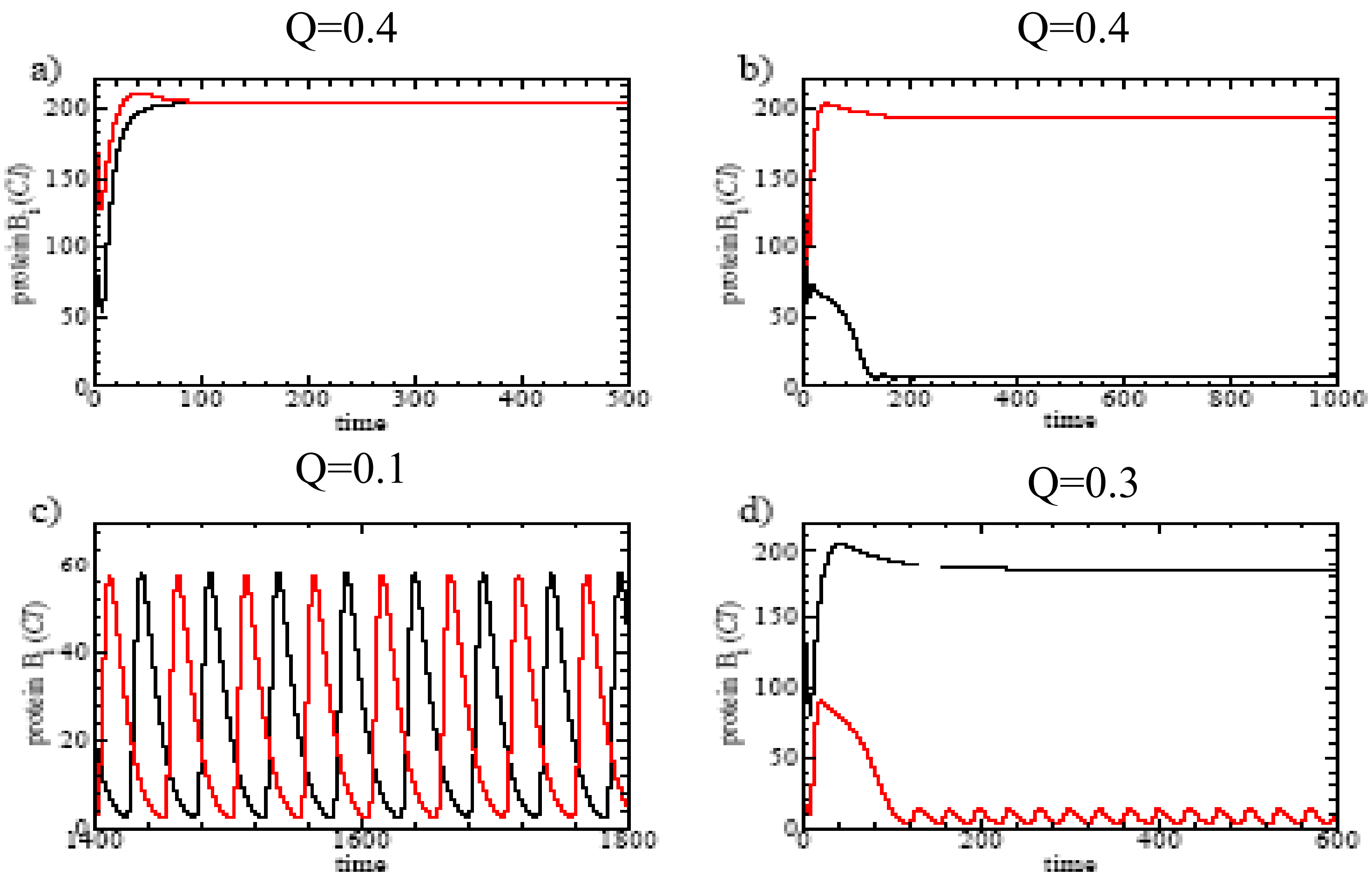


FIG. 5. (Color online) Bifurcation diagram versus coupling Q, focusing on the stable antiphase oscillations (thick yellow line). Parameters are those of Fig. 2.

Режимы в паре Репрессилаторов



Выводы

1. Основная мотивация изучения синтетических генетических систем – построить простые экспериментальные модели, на которых можно проверить принципы регуляции генетических систем.
2. Показано, что даже простое взаимодействие простых генетических осцилляторов может приводить к богатому наборы динамических режимов, которые могут рассматриваться как основа «динамической дифференцировки» в популяции синтетических клеток. А может и в реальных популяциях.
3. Развитые модели связанных Триггеров, Релаксаторов, Репрессилаторов порождают новые задачи для нелинейной физики и формулируют требования к эксперименту.