MODELING AND CONSTRUCTION OF MOLECULAR TRIGGER
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Summary

Motivation: Design of genetically engineered constructs that would allow for controlling operation
of certain genes is among the most topical problems in the modern biology. We are developing the
theory of modeling hypothetical and actual gene networks, which provides prediction of the main
modes of gene network operation. The goal of this work was to integrate theoretical and experimental
approaches to analysis of the operation patterns of relatively simple vector system with trigger
properties.

Results: Behavior of simplest gene networks with trigger and cyclic operation modes was studied
qualitatively and quantitatively using mathematical models (portrait and regulatory circuits).
Functioning of trigger plasmids under the effect of inducers in E. coli cells was verified
experimentally. Optimal conditions for studying the dependence of fluorescent signal intensity on
the presence/absence of inducer and duration of its action on repressor were selected.

Introduction

Earlier, we developed a method for modeling GNs (Likhoshvai et al., 2000; Likhoshvai et al., 2001a)
and several computer models (Bazhan et al., 1995; Ratushny et al., 2003; etc.) as well as the
fundamentals of the theory of hypothetical gene networks (HGNs), which are actually the models
Mathematical tools for studying HGNs were proposed. It was demonstrated that HGNs could have
stable stationary and cyclic operation modes as well as attractors of a more intricate nature.
Numerical calculations showed that only the structure–function relations determine the limit
properties of canonical HGNs at sufficiently large values of synthesis and repression parameters.
A number of empirical criteria connecting the limit properties of HGNs with the properties of the
corresponding structural graphs were formulated. With reference to the real gene networks, this
means that a certain minimal complexity (nonlinearity) of the processes regulating the activities of
GN elements is the necessary condition for existence of the necessary number of stationary and/
or cyclic GN operation modes. The necessary complexity may be reached by multimerization of
repressor proteins and/or occurrence of a sufficiently large number of intermediate stages between
the gene and the regulatory protein.

Research into HGNs opens the possibility for synthesizing gene networks with any prespecified
number of stationary and/or oscillating modes as well as for realizing many other, possibly, more
intricate systems with operation modes oriented to solving practical problems.
Results and Discussion

Models. We constructed and numerically studied models of self-regulating operon (SRO), molecular trigger (MT), and molecular oscillator (MO) composed of three operons successively repressing one another. The models were constructed using generalized chemical method of modeling (Likhoshvai et al., 2000). It was demonstrated that a region of at least 2000 bp between promoter and protein coding part is necessary for the onset of self-oscillations in SRO. MT and MO function according to experimental data (Elowitz, Leibier, 2000; Fig. 1).

For SRO and MO, properties of periodicity were studied. The minimal period for SRO at physiological parameters amounted to ~15 min; for MO, at least 100 min. Numerical experiments with MO demonstrated that the length of the period depended essentially on the gene copy number (Fig. 2). This suggests fluctuations in copy numbers of plasmids underlies a large variation of the periods observed (Elowitz, Leibier, 2000). In the model of MT, the mean time of changeover amounted to

Fig. 1. Self regulation of operon expression efficiency: 1, insertion with a length of 10 000 nucleotides (the period T = 46 min); 2, insertion with a length of 5000 nucleotides (the period T = 26 min); 3, insertion with a length of 3000 nucleotides (the period T = 15 min); 4, insertion with a length of 2000 nucleotides (stationary); the ordinate, concentration of the reporter protein; the abscissa, time in min.

Fig. 2. A three operon oscillator: 1, the plasmid copy number is 1/100 plasmids/cell (the period T = 110 min; 2, the copy number is 10/100 plasmids/cell (the period T = 156 min; and 3, the copy number is 1/10 plasmids/cell (the period T = 104 min).
approximately 25–30 min. To verify the adequacy of the conclusions obtained using the model, we commenced experimental construction of the trigger system.

**Experiment.** Earlier, trigger plasmids containing two repressors and two constitutive promoters were constructed (Elowitz, Leibier, 2000; Gardner et al., 2000; Tropynina et al., 2002). The plasmids pTAK and pIKE were kindly provided by J.J. Collins (Fig. 1). Both plasmids contain lacI gene. Lac repressor (R2) binds to promoter Ptrc-2 (P2) to form the first pair promoter–repressor. As the second pair (P1 and R1), the promoter Pls1con and thermosensitive repressor cIts of λ phage are used in plasmids pTAK; tetR gene, encoding Tet repressor, binding to the promoter P1 tetO-1, in plasmids pIKE. Changeover in plasmids pTAK occurs when IPTG, which interacts with LacI repressor, is added or when temperature is increased. Changeover in plasmids pIKE occurs when IPTG or tetracycline (Tc) is introduced. The state of triggers was monitored according to change in the expression level of GFP protein, which was under the control of Pτc-2 promoter.

Individual colonies of JM2.300 cells, carrying pTAK or pIKE plasmids were transferred into selective medium supplemented (if necessary) with 2 mM IPTG to grow a night culture at 37 °C (pIKE) or 32 °C (pTAK). Then, the cells were grown in either the presence or absence of the corresponding inducer. Cells were transferred into minimal medium to record the intensity of GFP fluorescence at an excitation wavelength of 480 nm in a SFM 25 (KONTRON INSTRUMENTS, Italy) fluorimeter. All the measurements were made during the logarithmic growth phase. If necessary, the medium was supplemented with tetracycline as the second inducer (pIKE) or cells were incubated at 42 °C (pTAK). Upon a 3–6-h induction, cells were transferred into the fresh medium free of inducer. The cells retained the state when GFP was expressed in the absence of the inducer. Upon the effect of the second inducer, the triggers switched to the functional state when GFP expression was absent.

![Fig. 3](image_url)  
**Fig. 3.** Dependence of GFP fluorescence on time and presence of inducer.

This state was stably inherited by the next cell generations. Fig. 3 shows the dependence of GFP expression level on time and presence/absence of inducers.

As control experiments, cell cultures were divided into two parts. The first was grown in the presence of IPTG; the second, in the absence. Upon 6 h of growth in the presence of inducer, the cells were transferred into an inducer-free fresh medium. Both cultures retained the corresponding states (with a high or low GFP expression level) for 1 day. The ability of trigger plasmids to preserve two stable functional states in a succession of cell divisions is shown in Fig. 4.
Thus, it is demonstrated that the trigger plasmids under certain conditions are able to preserve stably one of the two alternative functional states in the succession of E. coli cell divisions. Further, we plan to construct a system with cyclic operation mode as well as more complex systems combining both stationary points and cycles.

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References