Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioelectrochemistry 63 (2004) 87–90
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Feedback loops in the signal paths controlling gene expression

F. Šrobár*

Institute of Radio Engineering and Electronics, Academy of Sciences of the Czech Republic, Chaberská 57, CZ 182 51, Prague 8, Czech Republic

Received 23 June 2003; received in revised form 10 November 2003; accepted 20 November 2003

Abstract

Theoretical and empirical studies support the concepts suggesting emission of electromagnetic fields by living cells. Experiments performed in our laboratory indicate, in addition, tight links between attributes of these fields and the phase of cell division cycle, i.e., processes governed by gene transcription. This work supplements the now quite extensive literature concering gene networks in that it identifies the well-known feedback mechanisms in exact topological and quantitative terms. Special diagrammatic apparatus is employed for this purpose. The simple system comprising one gene and one transcription factor (binding as a dimer to the gene regulatory region), controlled via phosphorylation process by external signal, is analyzed to demonstrate the approach. The diagram representative of the system's dynamics contains a feedback loop; the so-called transmission function of this feature of diagram topology is shown to control the dependences of gene product concentration on the stimulus intensity.

Keywords: Feedback loops; Signal paths; Gene expression

1. Introduction

In the late 1960s, H. Fröhlich [1] put forward a hypothesis of the existence of endogenous electromagnetic fields generated by ordinary biological cells, not specially dedicated to this purpose. The idea has since been elaborated both theoretically and experimentally, even though the progress has been hampered by the prohibitively low power levels (10^{-15} W or less) and low spatial reach (below 100 nm) of the putative fields. Work performed in our laboratory [2,3] suggests that electromagnetic emission of the synchronized yeast cell populations in the 9-MHz frequency region is strongly linked with the actual phase of the cell division cycle, i.e., with processes involving gene transcription and related phenomena.

The theme of genomic regulation is now a well established subject which not only found its place in the curricula of courses in biology and medicine, but also spilled over to disciplines traditionally considered to be quite distant from this. (The November 2002 issue of Proceedings of the IEEE—Institute of Electrical and Electronics Engineers—was devoted to this subject; see also Ref. [4].) The immediate background for the present contribution is contained in the work of Smolen, Baxter, and Byrne [5] and Wolf and Eeckman [6]. The two hallmarks of the gene regulatory dynamics are nonlinearity and feedback. Both are addressed here in a peculiar way afforded by the use of a specific mathematical tool—the causal diagrams (a version of the signal flow graphs, see Ref. [7]).

2. The model and its diagrammatical analysis

To demonstrate the approach, we take the simple case of one gene whose transcription is activated by the regulatory protein transcription factor, TF, encoded by it. The protein must be bound to operator site (promoter region) adjacent to the gene as a phosphorylated dimer in order to activate transcription. Examples of such mechanism are the Lac9 protein (the Ga14 homologue from *Kluyveromyces lactis*), which binds as a homodimer or the proteins jun and fos that jointly form a transcriptional activator [8]. Simplifying slightly the notation introduced in Ref. [5], let us denote c as the monomer concentration [TF]; the homodimer concentration is to a good approximation proportional to c^2. The transcription rate of the gene is supposed to saturate

* Tel.: +420-266-77-3473; fax: +420-284-68-0222.
E-mail address: srobar@ure.cas.cz (F. Šrobár).

1567-5394/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2003.11.003

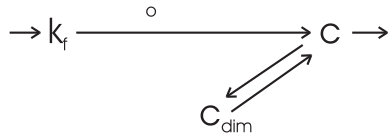

Fig. 1. Diagram representing causal relationships in the dimer-controlled gene regulation system, Eqs. (1) and (2), with variables k_f, c, and c_{dim} selected to be assigned vertices. Changes δk_f are considered as external input at vertex k_f, the response δc is monitored at vertex c.

with TF dimer concentration so as to be proportional to what we will call the effective dimer concentration

$$c_{\mathrm{dim}} = \frac{c^2}{c^2 + K_{\mathrm{d}}} \tag{1}$$

with K_d the dissociation constant of the TF dimer from the promoter. The proportionality coefficient k_f is the maximal (saturated) value of the transcription rate. Assuming that TF is synthesized also in the absence of dimer at a low basal rate r and that its concentration decays with a rate k_d, one gets the following rate equation for c:

$$\frac{\mathrm{d}c}{\mathrm{d}t} = K_{\mathrm{f}} c_{\mathrm{dim}} - k_{\mathrm{d}} c + r. \tag{2}$$

The quantity k_f is supposed to be proportional to the degree of TF phosphorylation effected by the agency of kinases and phosphatases. As such, it can be regarded as a variable representing external influences. In keeping with [5], c and K_d are construed as dimensionless entities.

Diagrammatical analysis is commenced with differentiating the model equations, namely Eq. (1) and the stationary ($\mathrm{d}c/\mathrm{d}t = 0$) version of Eq. (2), with respect to variables c, c_{dim}, and k_f:

$$\begin{aligned} \delta c_{\mathrm{dim}} &= 2K_{\mathrm{d}}(c_{\mathrm{dim}}^2/c)\delta c \\ c_{\mathrm{dim}}\delta k_{\mathrm{f}} &+ k_{\mathrm{f}}\delta c_{\mathrm{dim}} - k_{\mathrm{d}}\delta c = 0 \end{aligned} \tag{3}$$

After some algebra and diagram building [7], we get the diagram shown in Fig. 1 with the transmission function between vertices k_f and c

$$t(k_{\mathrm{f}}c) \equiv \frac{\mathrm{d}c}{\mathrm{d}k_{\mathrm{f}}} = \frac{k_{\mathrm{d}}^{-1}}{1 - t_{\mathrm{loop}}}. \tag{4}$$

Quantity k_d^{-1} in the numerator is the transmission function of the diagram line $^{o}(k_f c)$; t_{loop}, expressed as a function of c by the expression

$$t_{\mathrm{loop}}(c) = K_{\mathrm{d}}\left(\frac{1}{c^2} - \frac{2r}{k_{\mathrm{d}}c^3}\right), \tag{5}$$

is the transmission function of the feedback loop $cc_{dim}c$ seen in the diagram. Diagram corresponds to situation in which the system is excited by an externally controlled change δk_f at the input vertex k_f; the reaction δc is monitored at the diagram output c.

3. Numerical results and discussion

Eq. (4) in conjunction with Eq. (5) was integrated which afforded to plot TF concentration c, effective dimer concentration c_{dim}, and transmission function of the feedback loop t_{loop} against what is considered to be an externally controlled variable, namely the maximal gene transcription rate k_f. As is often the case in modelling phenomena in microscopic realm, one is faced with the problem of the choice of realistic values of parameters entering the equations. In the absence of ab initio theoretical results or experimental data fitting concrete genetic regulatory system, one can be guided by the generally observed time scales of crucial component processes, such as transient phosphorylation of TFs [5]. These considerations point to values of k_d on the order of 1 min^{-1}; values of r can be expected to lie about one order of magnitude below this. (The rationale for choice of the value K_d and for the dynamic interval of k_f variations is given below.) To compensate for the lack of more precise knowledge, the dependences that follow were evaluated each for three values of the fixed parameter, covering a one-decade interval.

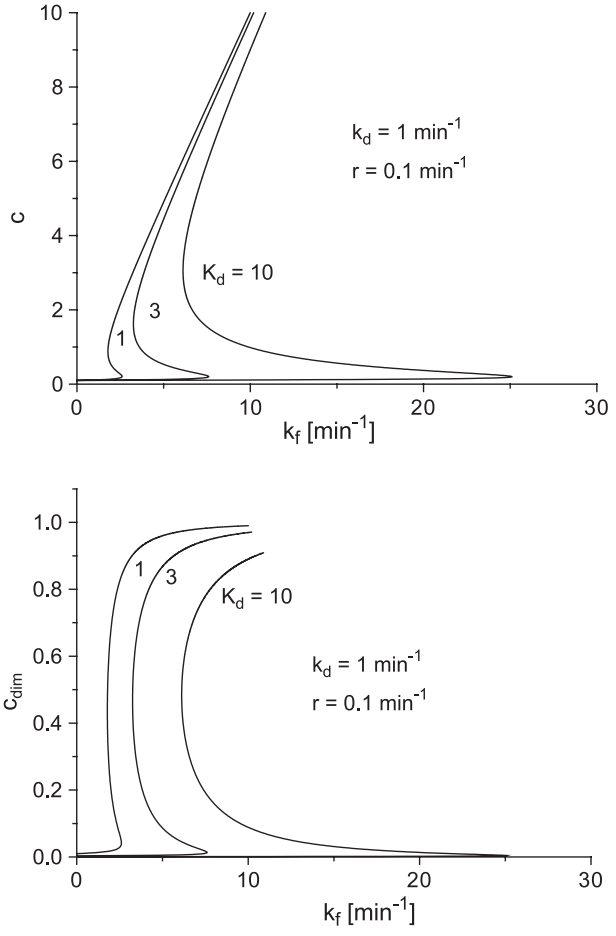

Fig. 2. Dependences of the TF protein concentration c (upper panel) and the effective dimer conentration c_{dim} (lower panel) on the maximal transcription rate k_f for selected values of the dissociation constant K_d.

Fig. 2 presents correlated dependences of c and c_{dim} vs. k_f for fixed values of the dissociation constant of the TF dimer from the promoter region, K_d. All curves of the two families possess sigmoidal character which is most pronounced for higher values of K_d. While for the c vs. k_f curves we see a general, for large c values asymptotically almost linearly increasing, tendency, interrupted in the low c region by an interval of thermodynamically unstable ($dc/dk_f<0$) states, the c_{dim} vs. k_f dependences in the lower panel exhibit an almost step-like shape. Dependences shown in Fig. 3, with k_d instead of K_d as the labelling parameter, exhibit similar character. It should be noted that both the dissociation constant K_d and the degradation rate k_d are, by their definitions by Eqs. (1) and (2), factors whose increase disrupts the gene replication process. On the other hand, the whole model becomes invalid for $k_d=0$ (see Eq. (4)) and feedback loop ceases to exist for $K_d=0$ (Eq. (5)).

Fig. 4 contains the t_{loop} vs. k_f dependences for selected values of K_d (the upper panel, complementary to Fig. 2) or k_d (the lower panel, to be considered in conjunction with Fig. 3). These curves control the course of the c vs. k_f dependences in that the points for which $t_{loop}=1$ correspond to singular points of dc/dk_f and the interval $I=\{k_f;\ t_{loop}>1\}$

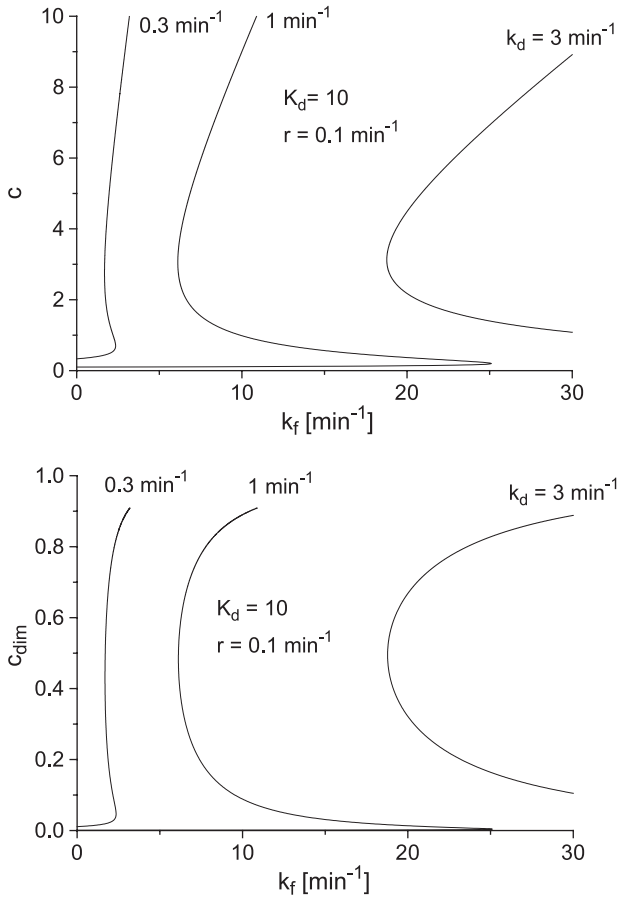

Fig. 3. Dependences of the TF protein concentration c (upper panel) and the effective dimer conentration c_{dim} (lower panel) on the maximal transcription rate k_f for selected values of the TF degradation rate k_d.

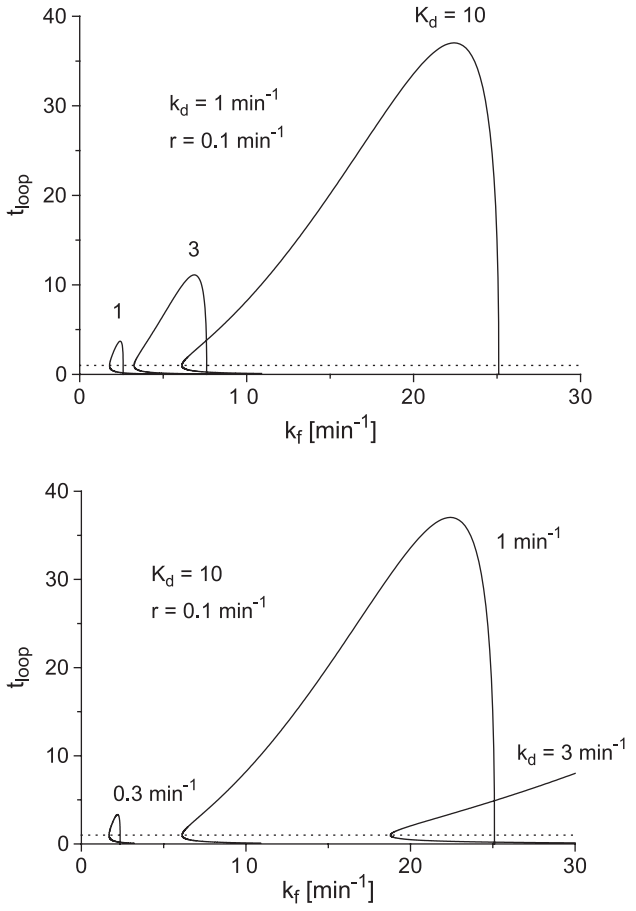

Fig. 4. Dependences of the transmission function of the feedback loop $cc_{dim}c$ in the diagram in Fig. 1 on the maximal transcription rate k_f. Upper panel: Selected values of K_d (complement to Fig. 2). Lower panel: Selected values of k_d (complement to Fig. 3). The dotted horizontal lines indicate the critical level $t_{loop}=1$.

corresponds to the unstable states marked by $dc/dk_f<0$. In this interval, there are two stable protein concentration values lying on the two branches of the c vs. k_f curves. Interval I is quite narrow when the replication process can proceed with little hindrances (small K_d and/or k_d values). In that case, small fluctuations in the externally controlled paramter k_f would cause large perturbations of the gene transcription system; a feature that would be eliminated in the course of evolution as potentially threatening integrity of the organism. For high K_d and k_d values, this interval is quite large, the feedback loop influence for $k_f \in I$ becomes dominant ($t_{loop} \gg 1$ in the denominator of Eq. (4)). In particular, for $K_d>10$ and/or $k_d>3$ min^{-1}, the change in k_f required for switching event to occur would be too large to be encountered in customary physiological environment. These considerations substantiate the choice of K_d and k_d values as indicated in the figures.

The analyzed model, according to the results of Wolf and Eeckman [6], is one of the two simplest cases of a gene regulation system capable of acting as a switch. (The other is a monomer-controlled system with one gene and two

operator sites.) Our findings afford referring to the notion of feedback phenomena in this context in more rigorous terms: They have a certain topology and can be quantified by means of the transmisssion function of the feedback loop. Criterion of bistability is that the t_{loop} dependence on external variable k_f crosses the critical level of unity at two distinct points.

Feedback mechanisms operate also on the level of intracellular generators of electromagnetic radiation [9,10]. This activity is supported by the polar molecular structures within the cell (like microtubule filaments) or the cell membrane and is driven by the cell's energy sources (GTP or ATP hydrolysis). These processes are closely connected with the events of the cell household involving gene replication. It might be of interest to study mutual relationships between these two feedback-controlled systems. As an example, time dependence of the electromagnetic activity of the tub-401 mutant of the synchronized yeast cells that were induced to start the cell division cycle by abruptly increasing the temperature from restrictive (14 °C) to permissive (28 °C) values exhibited four peaks correlated, respectively, with formation of mitotic spindle, binding of chromatids to kinetochore microtubules, elongation of the spindle in anaphase A, and elongation of the spindle in anaphase B [3]. Given the fact that duration of this whole series of events is on the order of tens of minutes, the values of rate constants used in the present computations (on the order of 0.1 and 1 min^{-1}) are quite congruent with these findings.

The approach suggested here affords also study of more complex gene regulation systems exhibiting both positive and negative feedback influences. According to Ref. [11], the negative feedback guarantees homeostasis in levels of gene products while the positive feedback creates conditions favourable for occurrence of multiple stable states of these levels. Synergy of both positive and negative feedback is necessary for a gene regulation system to support oscillations.

References

[1] H. Fröhlich, in: M. Maurois (Ed.), Quantum Mechanical Concepts in Biology, Theoretical Physics and Biology, North-Holland, Amsterdam, 1969, pp. 13–22.
[2] F. Jelínek, J. Pokorný, J. Šaroch, V. Trkal, J. Hašek, B. Palán, Microelectronic sensors for measurement of electromagnetic fields of living cells and experimental results, Bioelectrochem. Bioenerg. 48 (1999) 261–266.
[3] J. Pokorný, J. Hašek, F. Jelínek, J. Šaroch, B. Palán, Electromagnetic activity of yeast cells in the M phase, Electro- Magnetobiol. 20 (2001) 371–396.
[4] L. Chen, K. Aihara, A model of periodic oscillation for genetic regulatory systems, IEEE Trans. Circuits Syst. 1, Fundam. Theory Appl. 49 (2002) 1429–1436.
[5] P. Smolen, D.A. Baxter, J.H. Byrne, Frequency selectivity, multistability, and oscillations emerge from models of genetic regulatory systems, Am. J. Physiol. 274 (1998) C531–C542.
[6] D.M. Wolf, F.H. Eeckman, On the relationship between genomic regulatory element organization and gene regulatory dynamics, J. Theor. Biol. 195 (1998) 167–186.
[7] F. Šrobár, Feedback relationships—a neglected theme in physics, Eur. J. Phys. 13 (1992) 1–8.
[8] A.D. Keller, Model genetic circuits encoding autoregulatory transcription factors, J. Theor. Biol. 172 (1995) 169–185.
[9] F. Šrobár, J. Pokorný, Topology of mutual relationships implicit in the Fröhlich model, Bioelectrochem. Bioenerg. 41 (1996) 31–33.
[10] F. Šrobár, J. Pokorný, Causal structure of the Fröhlich model of cellular electromagnetic activity, Electro- Magnetobiol. 18 (1999) 257–268.
[11] R. Thomas, D. Thieffry, M. Kaufman, Dynamical behavior of biological regulatory networks, Bull. Math. Biol. 57 (1995) 247–276.