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POSITIVE AND NEGATIVE FEEDBACK LOOPS COUPLED BY COMMON TRANSCRIPTION ACTIVATOR AND REPRESSOR

Jan Sielewiesiuk, Agata Łopaciuk

Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, Lublin, Poland, e-mail sielewiesiuk@poczta.umcs.lublin.pl

ABSTRACT

Dynamical systems consisting of two interlocked loops with negative and positive feedback have been studied using the linear analysis of stability and numerical solutions. Conditions for saddle-node bifurcation were formulated in a general form. Conditions for Hopf bifurcations were found in a few symmetrical cases. Auto-oscillations, when they exist, are generated by the negative feedback repressive loop. This loop determines the frequency and amplitude of oscillations. The positive feedback loop of activation slightly modifies the oscillations. Oscillations are possible when the difference between Hilll's coefficients of the repression and activation is sufficiently high. The highly cooperative activation loop with a fast turnover slows down or even makes the oscillations impossible. The system under consideration can constitute a component of epigenetic or enzymatic regulation network.

Keywords: circadian rhythms, mathematical model, coupled loops, oscillations

1. INTRODUCTION

Circadian rhythms in eukaryotic organisms are generated by special motifs in transcription regulation networks. The most important of these motifs are closed negative feedback loops. Transcription of a gene in such a loop gives mRNA, whose translation produces a protein. The protein undergoes a number of transformations and becomes the repressor of its own gene. The scheme of this kind can also work in a chain of enzymatic reactions, if the end product of the chain inhibits an enzyme catalyzing one of the first reactions. As a matter of fact, this system, known as Goodwin's oscillator, was originally applied to enzymatic reactions (Goodwin 1966). In the simplest variant, the system consists of one cooperative process (transcription, allosteric enzyme, membrane receptor) and several reactions with the linear monomolecular kinetics. The systems which generate oscillations *in vivo* have more complex structure (Jolma et al. 2010, Feng and Lazar 2012, Dunlap 1999, Alon 2007). Circadian clocks, as a rule, contain interlocked negative and positive feedback loops (Saithong et al. 2010). The existence of oscillations and their period are determined by the negative feedback loop. On the other hand, the positive feedback loop can to some degree modify the period, amplitude and robustness of the oscillations. It can even fully damp the oscillations in some special cases.

We consider a hypothetical system of two genes whose transcription is governed by the same transcription factors. One of these genes encodes a protein which can become a repressor. The other gene encodes a precursor protein of the activator. Transcription of both genes is possible provided that the concentration of the repressor is sufficiently low and the concentration of the activator is sufficiently high. From the viewpoint of mathematical modeling, the essential feature of the system is logic of the coupling between the repressor and activator feedback loops. The logical conjunction (~repression $\cap$ activation) ought to be satisfied to start transcription.

Simultaneous transcription of many genes, induced by common factors, occurs in prokaryotic and eukaryotic cells. In lactose operon of *Escherichia coli* the same promoter and operator control transcription of polycistronic genes encoding three proteins (Hogg 2005). Polycistronic genes and mRNA are quite usual phenomena in bacterial operons. In eukaryotes, as a rule, the synexpression of proteins fuctioning in the same process is coordinated by trans-acting factors (Niehrs and Pollet 1999). The alternative splicing of pre-mRNA makes it possible to obtain two different proteins on the basis of the same transcript. In such a case, expression of these proteins is induced in the same promoter (Majercak, Wen-Feng Chen and Edery 2004). Both mechanisms occur in circadin clocks (Ripperger and Brown 2010, Steiger and Köster 2011).

The system is represented in our model by a set of ordinary differential equations. Linear stability analysis (Nayfeh and Balachandram 1995) and numerical solutions were used as tools in our search. We tried to determine how the phase portrait of the system depends on parameters. We have found general relations between parameter values when the number of equilibrium points was changed in a saddle-node or transcritical bifurcation. The conditions of the Hopf bifurcation have been found in a less general form in a few special, symmetrical, cases.

2. STRUCTURE OF THE SYSTEM AND ITS MATHEMATICAL MODEL

We discuss a specific hypothetic system which could regulate expression of genes. The system contains two genes which are transcribed simultaneously. Their transcription is regulated by two transcription factors, repressor and activator. The transcription is going on when concentration of the repressor is low and concentration of the activator is sufficiently high. Such relations between trancriptions of the two genes can be realized by a common promoter in an extremaly simplified model, as in Fig. 2.1. Qualitatively, the same situation is formed in circadian systems by E-boxes, which are activated by an activator. In such a case, a repressor interacting with the activator prevents its interaction with E-box. Two proteins encoded in the regulated genes undergo many transformations with the transcription factors as end products. The system is represented by a scheme in Fig. 2.1 and a set of q ordinary differential equations (1). Our model is consistent with highly simplified schemes of the mammalian circadian clock presented by Jolma et al. (2010), Fig.1 and by Oster (2010), Fig. 5.3.

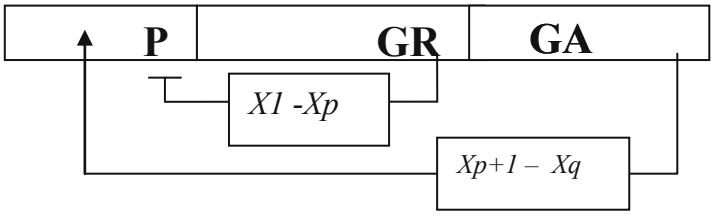

FIG. 2.1. Scheme of the regulatory system. P – promoter, GR – gene of the repressor, GA – gene of the activator.

$$\begin{aligned}
\frac{dx_1}{dt} &= \frac{ax_q^n}{\left(1+x_p^m\right)\left(1+x_q^n\right)} - k_1 x_1, \\
\frac{dx_i}{dt} &= h_{i-1}x_{i-1} - k_i x_i, \quad i = 2,\ldots,p, \\
\frac{dx_{p+1}}{dt} &= \frac{bx_q^n}{\left(1+x_p^m\right)\left(1+x_q^n\right)} - k_{p+1}x_{p+1}, \\
\frac{dx_j}{dt} &= h_{j-1}x_{j-1} - k_j x_j, \quad j = p+2,\ldots,q.
\end{aligned} \tag{1}$$

In equations (1), x_p and x_q are concentrations of the repressor and activator respectively. The variables x_1 and x_{p+1} are concentrations of mRNA encoding repressor's and activator's precursors. The rest of variables refer to concentrations of transient forms of the proteins. Equations with indices *1* and *p+1* correspond to mRNA synthesis and decay. We use dimensionless variables. The unit value for the first p variables (1) is the repressor concentration which reduces the rate of transcription to the half of its value in the repressor absence. The unit value of the variables x_{p+1} ...x_q corresponds to such activator concentration at which the rate of transcription becomes equal to the half of its maximum value. Hill's coefficients of cooperativity for the repressor and activator are respectively m and n. The k_i and h_i are rate constants. The constants a and b are maximum rates of transcription at the most favorable conditions ($x_p \to 0$, $x_q \to \infty$).

The system (1) is positively invariant at positive values of the rate constants. If all the variables have non-negative initial values then they will rest non-negative during the evolution of the system. In searching equilibrium points, all the variables except for x_p and x_q can be eliminated. Conditions for equilibrium can be given the following shape (2):

$$\frac{ay^n}{\left(1+x^m\right)\left(1+y^n\right)} = \alpha x, \quad \frac{by^n}{\left(1+x^m\right)\left(1+y^n\right)} = \beta y, \tag{2}$$

where x and y are equilibrium values of x_p and x_q respectively.

$$\alpha = \frac{\prod_{i=1}^{p} k_i}{\prod_{i=1}^{p-1} h_i}, \qquad \beta = \frac{\prod_{i=p+1}^{q} k_i}{\prod_{i=p+1}^{q-1} h_i}.$$

Equilibrium values of the remaining variables are

$$\overline{x}_j = \frac{\prod_{i=j+1}^{p} k_i}{\prod_{i=j}^{p-1} h_i} x, \quad j = 1, \ldots, p-1,$$

$$\overline{x}_j = \frac{\prod_{i=j+1}^{q} k_i}{\prod_{i=j}^{q-1} h_i} y, \quad j = p+1, \ldots, q-1. \tag{3}$$

Equations (2) have one obvious solution $x = y = 0$. It means, in view of (3), that the origin of phase coordinates is an equilibrium point of the system (1). It follows from (2) that the number of equilibrium points and their coordinates depends on two parameters $A = a/\alpha$ and $B = b/\beta$, which are simple functions of the $2q$ rate constants. Possible equilibrium points beyond the origin ($y \neq 0$) can be found from equations (2) in the following form (4):

$$\frac{By^{n-1}}{\left(1+x^m\right)\left(1+y^n\right)} = 1, \quad y = \frac{B}{A}x. \tag{4}$$

Equations (4) can be reduced to the equivalent polynomial equation

$$B^n x^{m+n} + A^n x^m + B^n x^n - AB^n x^{n-1} + A^n = 0. \tag{4'}$$

In the case of $n=1$, the equation (4') takes the shape:

$$Bx^{m+1} + Ax^m + Bx + A\left(1-B\right) = 0.$$

According to Descartes rule, it has then one ($B>1$) or none ($B<1$) real and positive root. So, at $n=1$, the system (1) has one or two equilibrium points. The number of equilibrium points with non-negative phase coordinates is changed

through transcritical bifurcation at $B=1$. In the case of $n\geq 2$, there are two sign changes in the polynomial (4') and it can have two or none positive roots. The system can have one, two or three equilibrium points. The number of equilibrium points changes from one to three in a saddle-node bifurcation. Bifurcational values of the parameters we will find using characteristic equation of the system.

3. CHARACTERISTIC EQUATIONS OF THE SYSTEM AND THE NUMBER OF EQUILIBRIUM POINTS

Two equations with indices 1 and $p+1$ of the system (1) have nonlinear functions in their right hand parts. Let us call these functions f_1 and f_{p+1}:

$$\begin{aligned} f_1(x_1, x_p, x_q) &= \frac{ax_q^n}{\left(1+x_p^m\right)\left(1+x_q^n\right)} - k_1 x_1, \\ f_{p+1}(x_p, x_{p+1}, x_q) &= \frac{bx_q^n}{\left(1+x_p^m\right)\left(1+x_q^n\right)} - k_{p+1} x_{p+1} \end{aligned} \tag{5}$$

and write their partial derivatives (6).

$$\begin{aligned} g_{1,p} &= \frac{\partial f_1}{\partial x_p} = \frac{-amx_p^{m-1}x_q^n}{\left(1+x_p^m\right)^2\left(1+x_q^n\right)}, \\ g_{1,q} &= \frac{\partial f_1}{\partial x_q} = \frac{anx_q^{n-1}}{\left(1+x_p^m\right)\left(1+x_q^n\right)^2}, \\ g_{p+1,p} &= \frac{\partial f_{p+1}}{\partial x_p} = \frac{-bmx_p^{m-1}x_q^n}{\left(1+x_p^m\right)^2\left(1+x_q^n\right)}, \\ g_{p+1,q} &= \frac{\partial f_{p+1}}{\partial x_q} = \frac{bnx_q^{n-1}}{\left(1+x_p^m\right)\left(1+x_q^n\right)^2}. \end{aligned} \tag{6}$$

Indices in the left part of (6) refer to positions of particular derivatives in the Jacobian of the system (6').

$$J=\begin{bmatrix} -k_1 & 0 & 0 & g_{1,p} & 0 & 0 & g_{1,q} \\ h_1 & -k_2 & 0 & 0 & 0 & 0 & 0 \\ 0 & h_{i-1} & -k_i & 0 & 0 & 0 & 0 \\ 0 & 0 & h_{p-1} & -k_p & 0 & 0 & 0 \\ 0 & 0 & 0 & g_{p+1,p} & -k_{p+1} & 0 & g_{p+1,q} \\ 0 & 0 & 0 & 0 & h_{j-1} & -k_j & 0 \\ 0 & 0 & 0 & 0 & 0 & h_{q-1} & -k_q \end{bmatrix} \tag{6'}$$

$$i=3,...,p-1$$
$$j=p+2,...,q-1.$$

In a general case, the characteristic equation of the system (1) has the shape (7):

$$\begin{aligned} &\prod_{i=1}^{q} u_i + (-1)^{q-p-1} g_{p+1,q} \prod_{i=p+1}^{q-1} h_i \prod_{i=1}^{p} u_i + \\ &+(-1)^{p-1} g_{1,p} \prod_{i=1}^{p-1} h_i \prod_{i=p+1}^{q} u_i + \\ &+(-1)^{q} \left(g_{p+1,q} g_{1,p} - g_{1,q} g_{p+1,p} \right) \prod_{i=1, i\neq p}^{q-1} h_i = 0 \\ &u_i = -(k_i + \lambda) \end{aligned} \tag{7}$$

where λ is an eigenvalue of the matrix J (6'). As it follows from (6), the term $\left(g_{p+1,q} g_{1,p} - g_{1,q} g_{p+1,p} \right) \prod_{i=1, i\neq p}^{q-1} h_i$ in the equation (7) vanishes.

With any $n>1$ all the derivatives (6) are equal to zero in the origin, and characteristic equation appears to be quite simple and solvable in this equilibrium point (8).

$$(-1)^q \prod_{i=1}^{q} (k_i + \lambda) = 0, \quad \lambda_i = -k_i, \quad i = 1,...,q. \tag{8}$$

All eigenvalues for this point of equilibrium are real and negative. The equilibrium at origin is stable at any meaningful values of parameters.

In the case of $n=1$, $g_{p+1,q}=b$, $g_{1,q}=a$. We can use definitions of parameters β and B and give to the characteristic equation for an equilibrium at origin the shape of (9).

$$\prod_{i=1}^{p}\left(k_i+\lambda\right)\left[\prod_{i=p+1}^{q}\left(k_i+\lambda\right)-B\prod_{i=p+1}^{q}k_i\right]=0. \tag{9}$$

There are p negative eigenvalues $\lambda_i=-k_i$, $i=1,...,p$. All of the remaining eigenvalues have their real parts negative at $B<1$, one of them becomes equal to zero at $B=1$ and at least one of them is positive at $B>1$. Now ($n=1$), the equilibrium at origin is stable at $B<1$ and unstable at $B>1$. There is a transcritical bifurcation at $B=1$. An additional point of equilibrium with positive coordinates appears and equilibrium at origin becomes unstable.

In order to examine stability of the equilibrium points beyond the origin, one should solve equations (2) or (4), substitute obtained x and y for x_p and x_q in (6) and find eigenvalues of the resulting Jacobian. But, it is rather impossible to obtain a clear symbolic solution of the mentioned equations. Instead, we use equations (2) to express parameters a and b as functions of x and y and insert obtained expressions into (6). Next, we use resulting expressions as respective entries of the Jacobian. Such a procedure results in the following characteristic equation (10):

$$\prod_{i=1}^{q}\left(k_i+\lambda\right)-\frac{n}{1+y^n}\prod_{i=p+1}^{q}k_i\prod_{i=1}^{p}\left(k_i+\lambda\right)+\frac{mx^m}{1+x^m}\prod_{i=1}^{p}k_i\prod_{i=p+1}^{q}\left(k_i+\lambda\right)=0. \tag{10}$$

The equation is valid in the points of equilibrium which are situated beyond the origin of coordinates, $\left(x\neq 0\right)\wedge\left(y\neq 0\right)$, including $n=1$. We have not used any additional assumption in derivation of the equation (10).

It is easy to find conditions for saddle-node bifurcation using equation (10). In the point of such bifurcation, at least one of eigenvalues is equal to zero. With $\lambda=0$, equation (10) is reduced to the relation (11):

$$1-\frac{n}{1+y^n}+\frac{mx^m}{1+x^m}=0. \tag{11}$$

This relation constitutes the condition which is to be satisfied by coordinates of an equilibrium point at a saddle-node bifurcation. It can be written as a function $y(x)$ (12):

$$y^n = \frac{n-1-(m+1-n)x^m}{1+(m+1)x^m}. \tag{12}$$

Formula (12) allows us to calculate y for an arbitrary chosen value of x. Next, both x and y can be used to calculate the parameters A and B according to equations (4). In such a way, we can find at what values of A and B the saddle-node bifurcation takes place.

$$\begin{aligned} A &= \frac{nx\left(1+x^m\right)^2}{n-1+(n-1-m)x^m}, \\ B &= \frac{n\left(1+x^m\right)^2}{\left[n-1+(n-1-m)x^m\right]^{1-\frac{1}{n}}\left[1+(m+1)x^m\right]^{\frac{1}{n}}}. \end{aligned} \tag{13}$$

In order to get physically meaningful, positive, values of y, A and B from (12) and (13) , we should confine our choice of m, n and x values to:

$$0 < x^m < \frac{n-1}{m+1-n}, \qquad m > n-1 \geq 1. \tag{14}$$

We can now draw a parametric plot *B(x)[A(x)]* according to (13) with the parameter x and any particular pair of Hill coefficients. The plot would divide the plane *(A,B)* into areas with one or three equilibrium points. It follows from (14) that there is no bifurcation with λ=0 at n=1 and nonzero values of variables.

It will be useful to introduce more complex parameters

$$\gamma = \frac{n}{1+y^n}, \quad \mu = \frac{mx^m}{1+x^m}. \tag{15}$$

In terms of the parameters γ and μ, characteristic equation (10) and condition for saddle-node bifurcation (11) obtain somewhat simpler and more general forms of (16) and (17):

$$\prod_{i=1}^{q}\left(k_i+\lambda\right) - \gamma\prod_{i=p+1}^{q}k_i\prod_{i=1}^{p}\left(k_i+\lambda\right) + \mu\prod_{i=1}^{p}k_i\prod_{i=p+1}^{q}\left(k_i+\lambda\right) = 0 \tag{16}$$

$$\gamma = \mu + 1. \tag{17}$$

However, the new parameters have a small disadvantage. A pair of values γ and μ characterizes one specific point of equilibrium, not the whole system. Each particular system is represented in the plane (μ, γ) by a number of points equal to the number of equilibrium points. In any case there is a point (0, n) corresponding to the point of equilibrium in the origin of the phase coordinates. An unstable saddle point can be found at $\gamma > \mu + 1$. The third point of equilibrium can be found at $\gamma < \mu + 1$. Autooscillations around this point can be generated after a possible Hopf bifurcation.

4. HOPF BIFURCATION AND OSCILLATIONS IN THE SYSTEM

4.1. THE CASE OF THE HIGHEST SYMMETRY WITH ALL K_I=1 AND Q=2P

We could find conditions of Hopf bifurcation in the form of analytical relations only in some special cases. Let us consider the system satisfying two essential limitations. Rate constants of decay (k_i) for all the variables in the system are equal to each other. The repressor's loop consists of the same number of substances as the activator's loop ($q=2p$). The assumption that all decay constants are equal to 1 will make our calculations more simple but it will not make them less general. Under such assumptions the characteristic equation (16) adopts the simple and solvable form (18).

$$(1+\lambda)^p \left[(1+\lambda)^p - \gamma + \mu \right] = 0. \tag{18}$$

There are p roots λ=-1. The remaining p eigenvalues satisfy the equation (19)

$$(1+\lambda)^p = R, \quad R = \gamma - \mu, \quad -m < R < n. \tag{19}$$

The inequality in (19) follows from definitions of R, γ and μ. In the case of $|R| < 1$ all roots of the equation (19)

$$\lambda = R^{\frac{1}{p}} - 1 \tag{20}$$

have negative real parts and respective point of equilibrium is stable. At R=1, one real eigenvalue vanishes and a saddle- node bifurcation takes place. With R=1, the second part of (19), leads to the relation, which we have already obtained in the fully general case (17).

In the case of negative *R,* equation (19) can be given the form of (21)

$$(1+\lambda)^p = |R|(\cos\pi + i\sin\pi), \quad |R| = \mu - \gamma < m. \tag{21}$$

The equation has roots:

$$\lambda_j = |R|^{\frac{1}{p}} \cos\frac{(2j+1)\pi}{p} - 1 + i|R|^{\frac{1}{p}} \sin\frac{(2j+1)\pi}{p}, \quad j = 0,1,...,p-1. \tag{22}$$

Eigenvalues with j=0 or j=p-1 have the highest real parts. The equilibrium will be destabilized when the real part of these two eigenvalues becomes positive. It takes place at

$$|R| = \mu - \gamma \geq \left(\cos^p\frac{\pi}{p}\right)^{-1}. \tag{23}$$

The case of equality in (23) gives a relation between the coordinates of equilibrium points at Hopf bifurcation.

$$y^n = \frac{n\cos^p\frac{\pi}{p} + 1 - \left[(m-n)\cos^p\frac{\pi}{p} - 1\right]x^m}{\left(m\cos^p\frac{\pi}{p} - 1\right)x^m - 1}. \tag{24}$$

In order to obtain meaningful values of y, parameters of the system and the coordinate x should satisfy the following limitations:

$$m - n > (\cos^p\frac{\pi}{p})^{-1}, \quad \frac{1}{m\cos^p\frac{\pi}{p} - 1} < x^m < \frac{n\cos^p\frac{\pi}{p} + 1}{(m-n)\cos^p\frac{\pi}{p} - 1}. \tag{25}$$

Let us note that the Hopf bifurcation is possible when difference between Hill coefficients of repression and activation exceeds a certain value shown in the first part of (25). The same value bounded Hill coefficient at Hopf bifurcation in a loop with one repressed gen (Invernizzi and Treu 1991), as well as a product of Hill coefficients of all cooperative process in a single loop containing many repressed or activated genes (Sielewiesiuk and Łopaciuk 2012).

The relation (24) is satisfied in equilibrium points undergoing a Hopf bifurcation. Using this relation and conditions of equilibrium (2) we can express parameters $A=a/\alpha$ and $B=b/\beta$ as functions of x - repressor's concentration in equilibrium (26).

$$A=\frac{nx\left(1+x^m\right)^2\cos^p\frac{\pi}{p}}{n\cos^p\frac{\pi}{p}+1-\left[\left(m-n\right)\cos^p\frac{\pi}{p}-1\right]x^m},$$
$$B=\frac{n\left(1+x^m\right)^2\cos^p\frac{\pi}{p}}{\left\{n\cos^p\frac{\pi}{p}+1-\left[\left(m-n\right)\cos^p\frac{\pi}{p}-1\right]x^m\right\}^{1-\frac{1}{n}}\left[\left(m\cos^p\frac{\pi}{p}-1\right)x^m-1\right]^{\frac{1}{n}}}. \tag{26}$$

Equations (26) describe a parametric curve *B(x)[A(x)]* which separates in the plane *(A,B)* the area with possible oscillatory solutions from the area where oscillation are impossible.

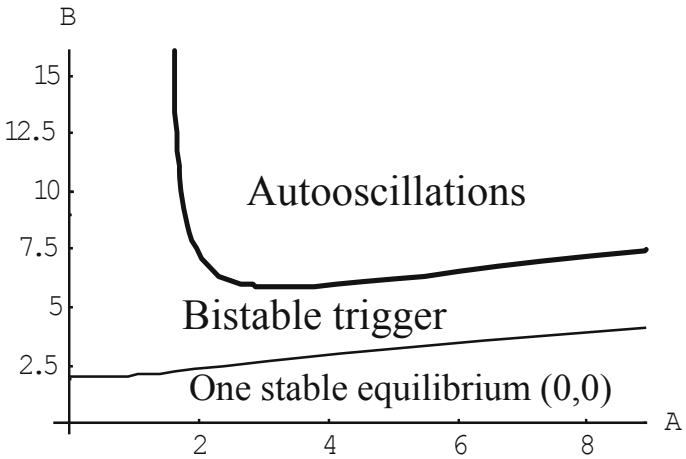

FIG. 1. Parameter plane of the system with {*p, q, m, n*}={10, 20, 4, 2}. Lower curve corresponds to the saddle-node bifurcation, the upper one – to Hopf bifurcation.

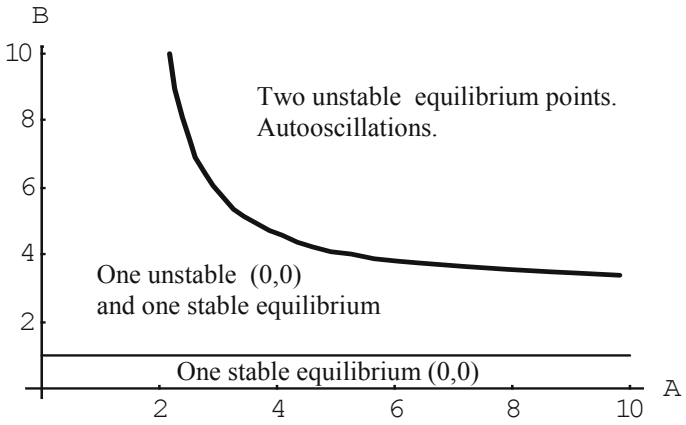

FIG. 2. Parameter plane of the system with {*p, q, m, n*}={10, 20, 4, 1}. Lower curve (*B=1)* corresponds to the transcritical bifurcation, the upper one – to Hopf bifurcation.

Systems with a cooperative ($n>1$, Fig. 1) and non-cooperative ($n=1$, Fig. 2) activation have some qualitative similarities and differences. Systems of both the kinds have a point of equilibrium at the origin of coordinates at any values of A and B. Two other, nonzero, points of equilibrium appear through saddle-node bifurcation in systems with $n>1$, and one additional equilibrium appears through transcritical bifurcation when $n=1$. The equilibrium at the origin is stable at all values of parameters with $n>1$, but it becomes unstable for $B>1$ with $n=1$. Hopf bifurcation takes place in both the kinds of activation at sufficiently high values of the parameters A and B.

4.2. REPRESSION AND ACTIVATION LOOPS WITH DIFFERENT NUMBERS OF ELEMENTS

Let us consider some systems with slightly lower symmetry, where the two loops consist of different numbers of protein transformations. As in section 4.1, we continue to use all the decay rate constants equal to one and the characteristic equation (16) in the form:

$$(1+\lambda)^q - \gamma(1+\lambda)^p + \mu(1+\lambda)^{q-p} = 0. \qquad (27)$$

In order to find values of the parameters γ and μ at Hopf bifurcation, we supposed a purely imaginary eigenvalue $\lambda = i\omega$ and introduced a new complex variable

$$z = 1+\lambda = 1+i\omega = |z|(\cos\varphi + i\sin\varphi) \qquad (28)$$

with $\tan\varphi = \omega, \quad |z| = \sqrt{1+\omega^2} = \dfrac{1}{|\cos\varphi|}.$

The substitution of the variable z for $1+\lambda$ in (27) results in equations (29):

$$\begin{aligned} \gamma\,|\cos\varphi|^{q-p}\cos p\varphi - \mu\,|\cos\varphi|^{p}\cos(q-p)\varphi &= \cos q\varphi \\ \gamma\,|\cos\varphi|^{q-p}\sin p\varphi - \mu\,|\cos\varphi|^{p}\sin(q-p)\varphi &= \sin q\varphi \end{aligned} \tag{29}$$

and bifurcational values of the parameters γ and μ:

$$\gamma = \frac{\sin p\varphi}{|\cos\varphi|^{q-p}\sin(2p-q)\varphi}, \quad \mu = \frac{\sin(q-p)\varphi}{|\cos\varphi|^{p}\sin(2p-q)\varphi}. \tag{30}$$

These relations allowed us to construct parametric plots $\gamma(\mu)$ using φ as a parameter. Sets of such plots are shown in Fig. 3 ($q<2p$) and in Fig. 4 ($q>2p$). In both the figures, the straight line $\gamma = \mu + 1$ corresponds to points of the saddle node bifurcation. At least one eigenvalue of these points of equilibrium is equal to zero in all systems under consideration. There is at least one real and positive eigenvalue at $\gamma > \mu + 1$. The remaining curves correspond to equilibrium points having a pair of complex eigenvalues with zero real part.

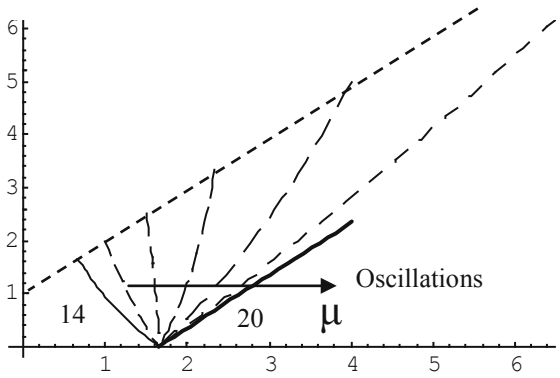

FIG. 3. Curves $\gamma(\mu)$ for Hopf bifurcation in systems having repression loop consisting of p=10 reagents. Activation loops contain q-*10* reagents with q=14, 15, 16, 17, 18, 19, 20, in the order from the left to the right. Dashed line $\gamma = \mu + 1$ corresponds to saddle-node bifurcation.

Numerical calculations were done for the number of reagents in the repression loop p=10 and that in the activation loop is q-p. In the cases of q-p= 4, 5, 6, 7, 8 and 9, the curves end on the straight line $\gamma = \mu + 1$ with φ=0. As it was shown earlier (23), the straight line $\gamma = \mu - 1.65172$ corresponds to Hopf bifurcation in the most symmetrical system with both loops of equal sizes (p=10 and q-p=10). Oscillations are possible around the equilibrium points with $\gamma < \mu + 1$ belonging to the area below saddle-node line and to the right from the curves of Hopf bifurcation. Let us remind that any particular system has in the plane (γ, μ) an accessible area with $\mu \le m$ and $\gamma \le n$. It is possible, at high n, that the curve of Hopf bifurcation does not divide the accessible rectangle m x n into two separate parts. An example of such situation can be seen in Fig. 3 for q=14 or 15 and n=2, where there are no stable oscillations in spite of existing complex eigenvalues with positive real part. The system goes to the stable equilibrium with zero values of all variables. It appears that oscillations are impossible, when inductive loop is too short.

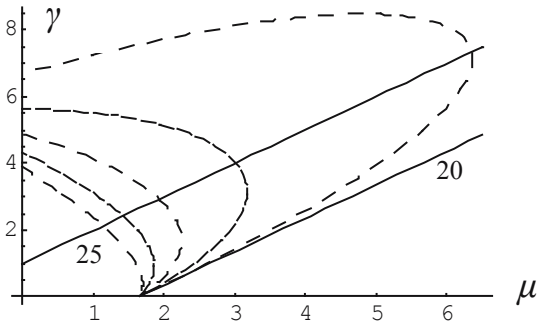

FIG. 4. Curves $\gamma(\mu)$ for Hopf bifurcation in systems having repression loop consisting of p=10 reagents. Activation loops contain q-p reagents with q=25, 24, 23, 22, 21, 20. Again, the straight line $\gamma = \mu + 1$ corresponds to saddle-node bifurcation.

On the contrary, long loops of activation do not suppress oscillations. We present in Fig. 5 values of the period of oscillations obtained from numerical solutions. The oscillations have the highest frequency when the loop of activation is slightly longer than the loop of repression (p=10, q-p=12). The period approaches a constant value with increasing number of elements in the loop of activation. At $q<p$, the shorter loop of activation slows down the oscillations. In the point with $\mu = \left|\cos\frac{\pi}{p}\right|^{-p}$ and γ=0, pure imaginary eigenvalue is equal to $\tan(\pi/p)$ and implies the period of 19.34 for p=10 (confront formulas

(22) and (23)). As can be seen in Fig. 5, the period of oscillations has only slightly higher value with long activation loops.

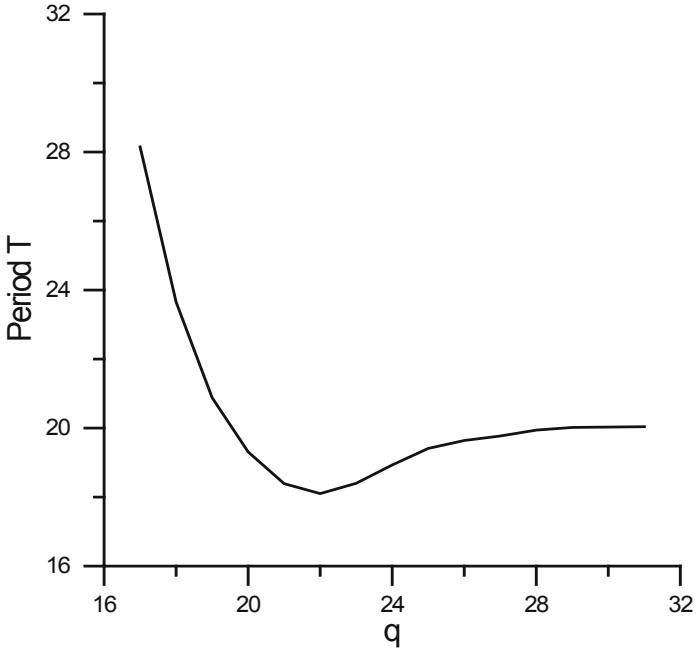

FIG. 5. Dependence of oscillations period on the number of elements in the loop of activation (*q-10*). The unit of time is the reciprocity of decay rate constants, or mean life time of mRNA (x_1), *m=4, n=2.*

Let us consider equations (29) and relations (30) in a special case of $\phi = \pi / p$. Solutions (30) give then $\gamma=0$ and $\mu = \left|\cos\frac{\pi}{p}\right|^{-p}$ in a general case of any $q>p$. These coordinates (μ,γ) refer to the point which is common for all of the bifurcational curves in Figs 3 and 4. In cases of long activation loops, when q is an integer multiple of p:

$$q = jp, \quad j = 2,3,... \tag{31}$$

the second one of the two equations (29) is satisfied with $\varphi=\pi/p$ for any value of μ and γ. Substitution of these values of q and φ into the first equation of (29) gives relation $\gamma(\mu)$ which should be satisfied at Hopf bifurcation

$$\gamma = (-1)^j \left|\cos\frac{\pi}{p}\right|^{(2-j)p} \left(\mu - \left|\cos\frac{\pi}{p}\right|^{-p}\right). \qquad (32)$$

Relation (32) is illustrated in Fig. 6. The straight line with *j=2* reproduces exactly the condition of Hopf bifurcation in the most symmetrical systems (23). The biggest part of

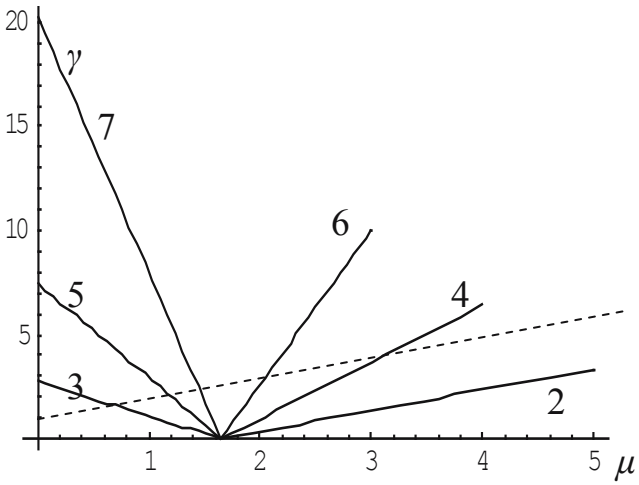

FIG. 6. Hopf bifurcation curves in systems where q is an integer multiple of p *(q=jp).* The values of j are shown at respective straight tlines. Dotted line corresponds to the saddle-node bifurcation ($\gamma=\mu+1$).

the plane (μ, γ), corresponding to autooscillations, have the systems with $q=3$ when the activation loop is twice as long as the loop of repression. In the limit of very high values of j (at very long loops of activation) the function (32) goes to the straight line with an infinite slope

$$\mu = \left|\cos\frac{\pi}{p}\right|^{-p} \qquad (33)$$

and the existence of oscillatory solutions does not more depend on the parameter γ.

Relations obtained in the section 4.2. enable us to find equilibrium points with a pair of pure imaginary eigenvalues. In Hopf bifurcation, the real part of this pair of complex eigenvalues should change its sign. We used numerical calculations and checked in several cases that the change of sign does really take place. Unfortunately, we have not found any clear proof of this change in

general case. In the case of $q=2p$, relations (22) and (23) imply an analytical expression for the bifurcating pair of eigenvalues:

$$\lambda = (\mu-\gamma)^{\frac{1}{p}} \cos\frac{\pi}{p} - 1 \pm i(\mu-\gamma)^{\frac{1}{p}} \sin\frac{\pi}{p}. \tag{34}$$

In this case, the change of the sign of $Re(\lambda)$ at crossing bifurcational value of μ-γ (curve 2 in Fig. 6) is evident. The characteristic equation (27) has a relatively simple analytical solution also in the case of $q=3p$. The bifurcating pair of eigenvalues is then given by (35):

$$\lambda = \left(\frac{\mu+\sqrt{\mu^2+4\gamma}}{2}\right)^{\frac{1}{p}} \cos\frac{\pi}{p} - 1 \pm i\left(\frac{\mu+\sqrt{\mu^2+4\gamma}}{2}\right)^{\frac{1}{p}} \sin\frac{\pi}{p}. \tag{35}$$

The real part of (35) is an icreasing function of μ and γ and vanishes at μ and γ satisfying relation (32) with $j=3$ (curve 3 in Fig. 6). So, it must change its sign by crossing this curve.

4.3. REPRESSION AND ACTIVATION LOOPS WITH DIFFERENT RATE CONSTANTS OF DECAY

Let us now consider the system whose both loops consist of the same number of elements ($q=2p$), but they differ in the rate of decay of their reagents. We assume that the constants k_i in the repression loop ($i=1,..,p$) have the unit value and those in the activation loop are equal to k. In such a case the characteristic equation (16) can be written as:

$$(1+\lambda)^p (k+\lambda)^p - \gamma k^p (1+\lambda)^p + \mu(k+\lambda)^p = 0. \tag{36}$$

Introducing $p=10$ and $\lambda=i\omega$ into equation (36) allows us to express parameters γ and μ as functions of ω^2. Parametric plots $\gamma(\omega^2)[\mu(\omega^2)]$ show the values of γ and μ, which correspond to Hopf bifurcation. The plots are presented in Figs 7 and 8. Oscillatory solutions are possible in areas below the straight line $\gamma=\mu+1$ and values of μ higher than those in bifurcation curves.

All of the considered asymmetrical systems have the loop of repression consisted of 10 reagents decaying with the rate constant equal to 1. Their activation loops differ one from another by numbers of elements in the loops of activation or by the rate constants of decay. There is obvious qualitative similarity between the systems with elongated activation loops and the systems with slower decay of reagents in these loops (Figs 4 and 7). Finite imaginary

eigenvalues appear on both sides of the straight line $\gamma=\mu+1$. In spite of this fact, oscillations around equilibrium points with $\gamma>\mu+1$ are impossible. All equilibrium points from this part of the (μ,γ) plane have one real positive eigenvalue. Evolution of the system takes it away from the vicinity of such an equilibrium. Let us note that in both cases the turnover of activator is slower than that of the repressor.

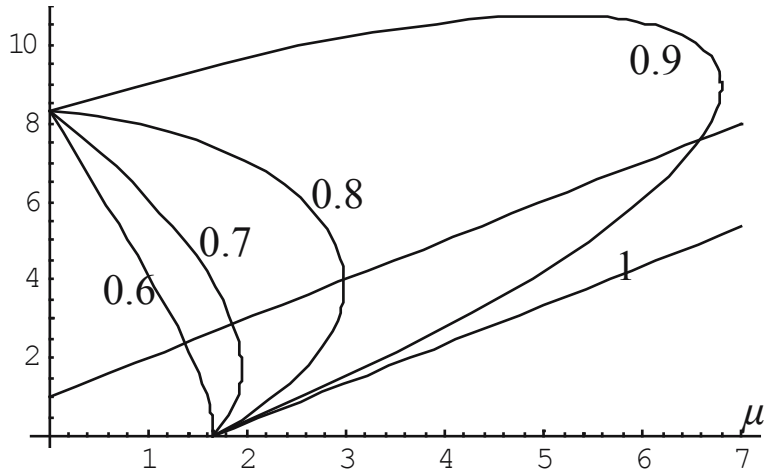

FIG. 7. Curves $\gamma(\mu)$ for Hopf bifurcation in the systems with equal number of elements in both loops. Rates of decay constants are equal to unity in the loop of repression. The values of decay rate constants in the loop of activation (k) are shown at curves.

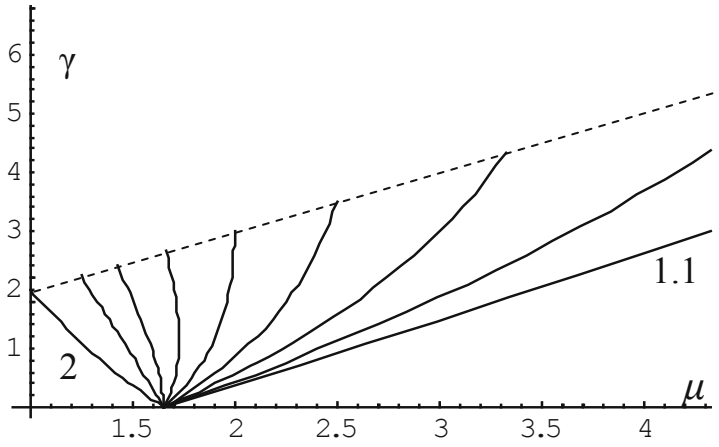

FIG. 8. Curves $\gamma(\mu)$ for Hopf bifurcation in the systems with equal number of elements in both loops. Rate of decay constants are equal to unity in the loop of repression. The curves corresponds to decay rate constants in the loop of activation k=2, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, and 1.1.

On the other hand, bifurcational curves of the systems with shortened loops of activation are much similar to those of the systems with higher rate constants of decay in these loops (Figs 3 and 8). The latter systems have pure imaginary eigenvalues only at $\gamma<\mu+1$. At $\gamma=\mu+1$, imaginary eigenvalue attains zero. Possible oscillations around the equilibrium points with γ slightly lower than $\mu+1$ should have extremely low frequency. In this pair of asymmetrical cases, activator's loop has shorter time of turnover than the repressor's loop.

All bifurcation curves in Figs 3, 4, 7 and 8 represent equilibrium points with a pure imaginary eigenvalue They have one common point with $\mu = \left(\cos\frac{\pi}{p}\right)^{-p}$, $\mu\approx1.65172$ at $p=10$, and $\gamma=0$. It can be inferred from relations (22) and (23) that in this bifurcation point imaginary eigenvalue $\omega = \tan\frac{\pi}{p}$ and we can expect oscillations with a period $T = \frac{2\pi}{\tan(\pi / p)} \approx 2p$. Numerical solutions suggest that this evaluation of the period is better than those based on the actual imaginary part of complex eigenvalue. The turnover time of the repression loop is the main factor determining the period of oscillations. The activation loop with short turnover time excludes oscillations or makes them slower. The slower activation loop has no significant influence on the period of oscillations. These rules are illustrated in Figs 5 and 9.

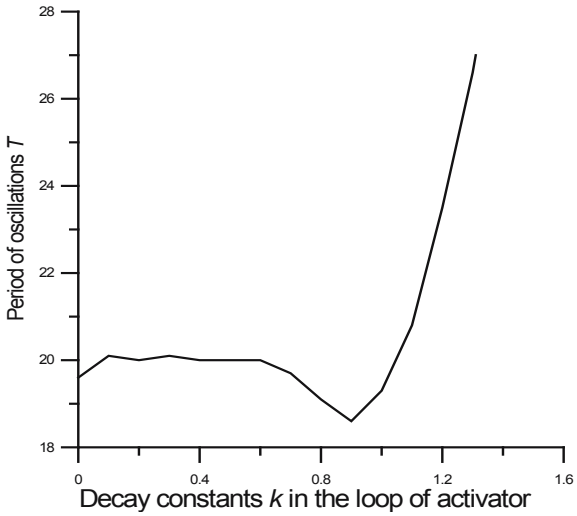

FIG. 9. Period of oscillations obtained from numerical solutions in systems with 10 elements in both loops, $k_i=1$ in the loop of repression, $k_i=k$ in the loop of activation, $m=4$, $n=2$.

5. CONCLUSIONS

We considered a model system of gene expression regulated by two transcription factors, repressor and activator. We assumed that transcription of the gene takes place provided that the concentration of the repressor is low and, at the same time, the concentration of the activator is high. Repression and activation are both cooperative processes with respective Hill coefficients m and n.

High values of m promotes oscillations and high values of n make them less probable. Oscillatory solutions are possible when difference m-n is sufficiently high, see relation (25). We introduced parameters γ and μ, functions of coordinates of equilibrium points, which enabled us to derive the characteristic equation and the saddle-node bifurcation in a quite general way (16,17). The Hopf bifurcation was analyzed in a few special cases. Oscillations are generated in our system by a negative feedback loop of the Goodwin's type. A coupled loop with positive feedback does not disturb oscillations when its turnover time is longer than the turnover time of the negative feedback loop. Oscillations are slowed down or even fully damped, when the loop of activation has the turnover time shorter than that of the repression loop.

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