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Regulation of gene expression by Goodwin's loop with many genes

Regulacja ekspresji genów w pętli Goodwina z wieloma genami

ABSTRACT

The paper presents a simple analysis of a long Goodwin's loop containing many genes. The genes form a closed series. The rate of transcription of any gene is up or down regulated by the protein product of the preceding gene. We describe the loop with a system of ordinary differential equations of order s . Oscillatory solutions of the system are possible at the odd number of repressions and any number of inductions if the product of all Hill's coefficients, related to both repressions and inductions, is larger than:

$$\left(\cos^s \frac{\pi}{s} \right)^{-1}.$$

1. INTRODUCTION

We analyze loops of processes with negative or positive feedback. The most frequently considered realizations of such loops are some genes, whose expression is, directly or indirectly, regulated by their protein products. Since its invention by Goodwin in 1965 [1, 2], many papers have been devoted to this system (see [5] for review). S. Müller and co-authors [4] analyzed general properties of a long loop containing a number of genes repressed by products of the preceding gene. Their loop consisted of alternating transcription and translation reactions. They explored conditions for the existence of oscillatory solutions, multiple equilibriums and heteroclinic orbits. H. Smith [6] considered a loop containing a number of repressed genes, which he described by ordinary differential

equations with a delay. Invernizzi and Treu [3] have analyzed a long loop with a single repressed gene. In their system, a protein was produced as a result of the gene transcription and translation. After numerous transformations, this protein acquired the form capable of repression of the gene. All mentioned authors used more general derivations than we do. They arrived at the conclusion that oscillations are possible under condition, which is much like that mentioned in ABSTRACT. Our consideration is much simpler and includes the case with many genes with repressed or induced transcription. We hope that it will be more intelligible to undergraduate students.

2. THE SCHEME OF THE MODEL

We wish to analyze dynamical features of the loops containing both repressed and induced genes, whose products (proteins) undergo many changes before their interaction with the next gene. Let us assume that the loop contains a number of genes. Some of those genes are down regulated and some of them are up regulated by proteins encoded in the loop. The structure of the system is sketched in Figure 1. For the rate of transcription we accept the usual formulae of cooperative kinetics [1]. We assume that the rates of repressed and induced transcriptions are respectively proportional to $\frac{a}{b+x^n}$ or to $\frac{ax^n}{b+x^n}$; x is a concentration of the transcription factor (protein), a and b are constants and n is the Hill coefficient.

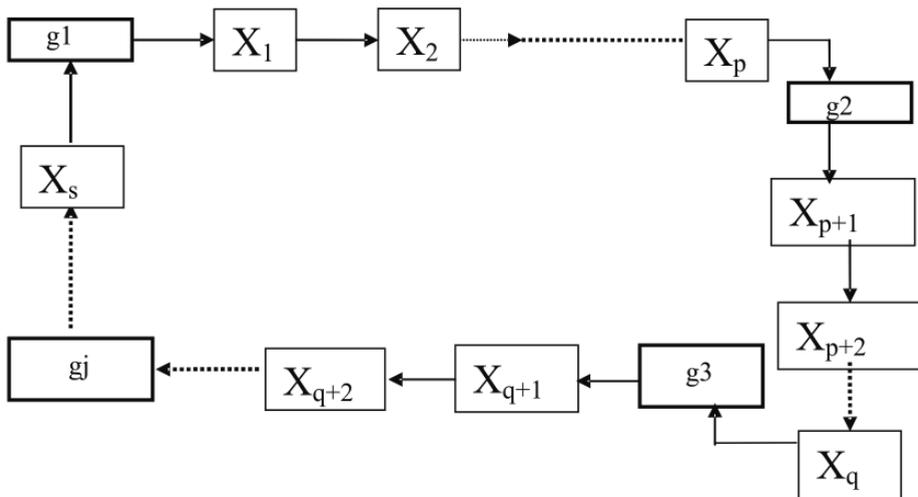


Fig. 1. Goodwin's loop with a few up- and down- regulated genes. Symbols X_1, X_{p+1}, X_{q+1} refer to mRNA. The rest of X_i denote proteins, g_j are regulated genes

3. GOODWIN'S LOOP CONTAINING SINGLE REPRESSED GENE

Goodwin's loop containing single repressed gene can be represented by a set of ordinary differential equations (1):

$$\begin{aligned}\dot{x}_1 &= \frac{a}{1+x_s^l} - x_1, \\ \dot{x}_i &= h_i x_{i-1} - k_i x_i, \quad i = 2, 3, \dots, s\end{aligned}\quad (1)$$

where dimensionless variables x_i are used with unit concentration adjusted to a concentration of x_s , which makes the rate of transcription equal to the half of its maximum value a . The unit time is equal to the mean lifetime of mRNA (x_1). In equations (1), l is the Hill coefficient of cooperativity. The symbols h_i and k_i refer to rate constants of synthesis (h_i) and decay (k_i) of the corresponding species.

The system (1) has a single equilibrium point determined by the relations:

$$\begin{aligned}\bar{x}_i &= \frac{\prod_{j=i+1}^s k_j}{\prod_{j=i+1}^s h_j} \bar{x}_s, \quad i = 1, \dots, s-1. \\ \frac{a}{1+\bar{x}_s^l} &= \alpha \bar{x}_s, \quad \alpha = \frac{\prod_{j=2}^s k_j}{\prod_{j=2}^s h_j},\end{aligned}\quad (2)$$

where \bar{x}_i denotes the values of the corresponding variable x_i in equilibrium points. The derivative of the nonlinear term in the first equation of (1) with respect to x_s is:

$$\frac{\partial f_1}{\partial x_s} = \frac{-a l x_s^{l-1}}{(1+x_s^l)^2}, \quad (3)$$

where f_1 replaces the function on the right of the first equation (1).

In the equilibrium point the value of this derivative (see (2)) can be expressed as:

$$g = \frac{-\alpha l \bar{x}_s^l}{1+\bar{x}_s^l}, \quad |g| < \alpha l. \quad (4)$$

The diagonal of the Jacobian matrix of the system (1) consists of the species decay rate constants (k_j). Positions ($j, j-1$) cover the rate constants for the

production of corresponding species (h_j). The position (l, s) is occupied by the derivative g . The structure of the Jacobian determines the following equation for its eigenvalues (λ):

$$(1 + \lambda) \prod_{j=2}^s (k_j + \lambda) - g \prod_{j=2}^s h_j = 0. \quad (5)$$

At what values of parameters does the equilibrium become unstable? Is it possible to have a Hopf bifurcation in this system? Sufficiently simple analytical solution of (5) can be found only for $s=1$ or $s=2$. One could find an answer to the second question, assuming for (5) pure imaginary λ and searching for conditions consistent with such an assumption. This way is effective for s not higher than 6. For the higher orders, equation (5) can be solved analytically only under severe simplification that the system has very high symmetry. If we assume that all $k_j = 1$, then (5) can be written as:

$$(1 + \lambda)^s = |g| \prod_{j=2}^s h_j (\cos \pi + i \sin \pi). \quad (6)$$

It follows from (6) that

$$\lambda_k = -1 + \left(|g| \prod_{j=2}^s h_j \right)^{\frac{1}{s}} \cos \frac{(2k+1)\pi}{s} + i \cdot \left(|g| \prod_{j=2}^s h_j \right)^{\frac{1}{s}} \sin \frac{(2k+1)\pi}{s}, \quad (7)$$

$k = 0, 1, \dots, s-1.$

The equilibrium will be unstable if the real part of any λ is positive. So far as we are looking for the minimum conditions of the equilibrium destabilization, we should consider those roots (7), which have the largest real parts. The two complex conjugate roots λ_0 and λ_{s-1} have the highest real parts. They will be positive and consistent with (4), if

$$\alpha l > |g| > \frac{1}{\prod_{j=2}^s h_j \cos^s \left(\frac{\pi}{s} \right)}. \quad (8)$$

Taking into account the definition of the α (2) and the assumption $k_j = 1$, one can conclude that double inequality (8) can be satisfied only under the condition

$$l > \frac{1}{\cos^s \left(\frac{\pi}{s} \right)}. \quad (9)$$

So, Hopf bifurcation in system (1) is possible, if the Hill coefficient for cooperative repression is high enough and $s \geq 3$. Let us note two features of the eigenvalues (7). All of them are placed in a complex plane on the circle with the radius equal to

$$R = \left(\left| g \prod_{j=2}^s h_j \right| \right)^{\frac{1}{s}} \quad (10)$$

centered at the point $(-1, 0)$. In the particular case

$$\lambda_0 = -1 + R \cos \frac{\pi}{s} + iR \sin \frac{\pi}{s} . \quad (10a)$$

At the bifurcation, when $Re(\lambda_0)=0$,

$$R = \cos^{-1} \frac{\pi}{s} \quad \text{and} \quad \text{Im}(\lambda_0) = \tan \frac{\pi}{s} . \quad (11)$$

The imaginary part of this eigenvalue, or the cyclic frequency of small oscillations around the equilibrium, is given by (11).

4. GOODWIN'S LOOP CONTAINING A SINGLE INDUCED GENE

The system of equations describing such a loop differs from the system (1) solely in the first equation referring to transcription.

$$\dot{x}_1 = \frac{\alpha x_s^I}{1 + x_s^I} - x_1 \quad (12)$$

$$\dot{x}_i = h_i x_{i-1} - k_i x_i, \quad i = 2, 3, \dots, s .$$

We can repeat calculations similarly to those done in the previous section and obtain corresponding relations determining the coordinates of equilibrium points:

$$\frac{\alpha \bar{x}_s^I}{1 + \bar{x}_s^I} = \alpha \bar{x}_s \quad (13)$$

where α is defined exactly in the same way as in (2). As it follows from (13), the equilibrium is found in the origin of coordinates. Other equilibrium points can be found at $x_s \neq 0$.

$$\frac{\bar{x}_s^{l-1}}{1 + \bar{x}_s^l} = \frac{\alpha}{a} . \quad (14)$$

The left hand side of (14) has a maximum at $\bar{x}_s^l = l - 1$. The additional points of equilibrium exist, when the maximum value of this function is higher than α/a . In the opposite case, graphs of the functions of x_s on the left and on the right sides of (14) will have no common points and equation (14) will have no real solutions:

$$\frac{(l-1)^{l-1}}{l} > \frac{\alpha}{a} . \quad (15)$$

The derivative of the nonlinear term in (12) is

$$\frac{\partial f_1}{\partial x_s} = \frac{alx_s^{l-1}}{(1+x_s^l)^2} . \quad (16)$$

The derivative (16) has the structure which is fully analogical to that of (3). They differ only by the sign. Let us note that the derivative (16) is equal to zero in the origin of coordinates. The value of this derivative in other equilibrium points can be obtained by inserting a from (14) into (16). This results in formula:

$$g = \frac{\alpha l}{1 + \bar{x}_s^l} \leq \alpha l . \quad (17)$$

The Jacobian structure of the system (12) is exactly the same as it was in (1). In consequence, characteristic equation of eigenvalues will also have the same structure:

$$(1 + \lambda) \prod_{j=2}^s (k_j + \lambda) = g \prod_{j=2}^s h_j . \quad (18)$$

This time, however, the right hand side of the equation is not negative. It follows immediately from (16) that equilibrium in the origin of coordinates is

stable for any physically meaningful values of the rate constants. For $g=0$ the solution of (18) becomes

$$\lambda_1 = -1, \quad \lambda_j = -k_j, \quad j = 2, \dots, s.$$

All of the eigenvalues are real and negative and the equilibrium is stable. Polynomial in λ on the left of (18) is a monotonously increasing function of its argument for positive λ . For $\lambda=0$, the polynomial has the value $\prod_{j=2}^s k_j$. Positive real eigenvalues appear only for

$$\prod_{j=2}^s k_j \leq g \prod_{j=2}^s h_j. \quad (19)$$

The case of both sides of (19) equal to each other corresponds to a saddle-node bifurcation. Taking into account that g satisfies at the same time (17), we arrive at the conclusion that bifurcation takes place for equilibrium value of x_s , when $\bar{x}_s^l = l - 1$. So, the condition for the existence of nonzero equilibrium points and the condition for unstable equilibrium are the same. Let us shortly consider the very symmetrical case with all $k_j=1$. In such a case characteristic equation (18) acquires the shape:

$$(1+\lambda)^s = g \prod_{j=2}^s h_j. \quad (20)$$

And eigenvalues are:

$$\lambda_k = -1 + \left(g \prod_{j=2}^s h_j \right)^{\frac{1}{s}} \cos \frac{2k\pi}{s} + i \left(g \prod_{j=2}^s h_j \right)^{\frac{1}{s}} \sin \frac{2k\pi}{s}$$

$$k = 0, 1, \dots, s-1. \quad (21)$$

There is at least one real eigenvalue (λ_0). It will be positive when the inequality (22) is satisfied.

$$1 \leq g \prod_{j=2}^s h_j. \quad (22)$$

In this case ($k_j=1$) inequalities (22) and (19) are equivalent. At a maximum transcription rate (a) which is high enough in comparison with the overall rate of proteins decay (15), the loop under consideration is a bistable trigger with one

of the stable equilibrium points in the origin of coordinates and the second at $\bar{x}_s^l > l - 1$. In the opposite case the system has only one equilibrium with all of the variables equal to zero.

5. GOODWIN'S LOOP CONTAINING MANY REGULATED GENES

We will not present in detail the systems with two regulated genes. It can be shown that a loop containing two repressed genes behaves as a bistable trigger at high enough maximum rate of transcription. Two stable equilibrium points correspond to high concentrations of one of the regulating proteins and low concentrations of the other. There is no equilibrium in the origin of coordinates. There are no oscillations, either.

At the low rate of transcription, a loop containing two induced genes behaves like the loop with a single induced gene. It can have an equilibrium point for all the variables equal to zero. Alternatively, it can have two additional points of equilibrium outside the origin of coordinates. One of these points is unstable and the second one is stable. The loop behaves then as a bistable trigger.

As an example of the loop with many regulated genes we are going to discuss the loop containing three regulated genes. One of these genes is repressed and two of them are induced. The system can be represented by a set of ordinary differential equations (23):

$$\begin{aligned}
 \frac{dx_1}{dt} &= \frac{C_1}{1+x_s^l} - x_1, \\
 \frac{dx_{q+1}}{dt} &= \frac{C_2 x_q^m}{1+x_q^m} - R_2 x_{q+1}, \\
 \frac{dx_{r+1}}{dt} &= \frac{C_3 x_r^n}{1+x_r^n} - R_3 x_{r+1}, \\
 \frac{dx_i}{dt} &= h_i x_{i-1} - k_i x_i, \\
 i &= 2, \dots, q, q+2, \dots, r, r+2, \dots, s. \\
 1 &< q < r < s.
 \end{aligned} \tag{23}$$

Nonlinear expressions in equations 1 , $q+1$ and $r+1$ refer to gene transcription with a repression or induction. Dynamical variables with the mentioned indices refer to mRNA concentration. The rest of variables denotes concentrations of proteins. Equations (23) have been written in a dimensionless form. The unit time

is equal to the mean lifetime of the mRNA, whose concentration is denoted by x_l in equations (23). Parameters R_2 and R_3 denote the ratio of the decay constants between x_{q+l} , x_{r+l} and x_l respectively. Concentrations units are not the same for all variables. It follows from equations 1, $q+1$ and $r+1$ in (23) that unit values of x_s , x_q and x_r make the rates of corresponding transcriptions equal to halves of their maximum values. The variables $x_1 \dots x_q$ are expressed in the same units as x_l . The variables $x_{q+1} \dots x_r$ have the same concentration units as x_q . Similarly, variables $x_{r+1} \dots x_s$ have the same concentration units as x_s . Equating the right sides of linear equations in (23), we can relate equilibrium values of all the variables to those of x_q , x_r and x_s .

$$\bar{x}_i = \frac{\prod_{j=i+1}^q k_j}{\prod_{j=i+1}^q h_j} \bar{x}_q, \quad i = 1, 2, \dots, q-1, \quad \text{and} \quad \bar{x}_1 = \frac{\prod_{j=2}^q k_j}{\prod_{j=2}^q h_j} \bar{x}_q = \alpha \bar{x}_q \quad (24)$$

$$\bar{x}_i = \frac{\prod_{j=i+1}^r k_j}{\prod_{j=i+1}^r h_j} \bar{x}_r, \quad i = q+1, q+2, \dots, r-1, \quad \bar{x}_{q+1} = \frac{\prod_{j=q+2}^r k_j}{\prod_{j=q+2}^r h_j} \bar{x}_r = \beta \bar{x}_r \quad (25)$$

$$\bar{x}_i = \frac{\prod_{j=i+1}^s k_j}{\prod_{j=i+1}^s h_j} \bar{x}_s, \quad i = r+1, r+2, \dots, s-1, \quad \bar{x}_{r+1} = \frac{\prod_{j=r+2}^s k_j}{\prod_{j=r+2}^s h_j} \bar{x}_s = \gamma \bar{x}_s \quad (26)$$

Equilibrium values of x_q , x_r and x_s can be obtained from equations (27):

$$\frac{C_1}{1 + \bar{x}_s^l} = \alpha \bar{x}_q, \quad \frac{C_2 \bar{x}_q^m}{1 + \bar{x}_q^m} = R_2 \beta \bar{x}_r, \quad \frac{C_3 \bar{x}_r^n}{1 + \bar{x}_r^n} = R_3 \gamma \bar{x}_s \quad (27)$$

To linearize the system (23), we will calculate derivatives of the nonlinear terms with respect to their arguments:

$$\frac{\partial f_1}{\partial x_s} = \frac{-C_1 l x_s^{l-1}}{(1 + x_s^l)^2}, \quad \frac{\partial f_{q+1}}{\partial x_q} = \frac{C_2 m x_q^{m-1}}{(1 + x_q^m)^2}, \quad \frac{\partial f_{r+1}}{\partial x_r} = \frac{C_3 n x_r^{n-1}}{(1 + x_r^n)^2} \quad (28)$$

The symbols f_l , f_{q+l} and f_{r+l} refer to functions in the right sides of the corresponding equations in (23). If we put the values of C_1 , C_2 and C_3 obtained from (27) into (28), we will arrive to expressions for the values of the derivatives in the equilibrium point:

$$\frac{\partial f_1}{\partial x_s} = \frac{-\alpha l y_q \bar{x}_s^{l-1}}{1 + \bar{x}_s^l} = g_1 \quad (29)$$

$$\frac{\partial f_{q+1}}{\partial x_q} = \frac{r_2 \beta m \bar{x}_r}{x_q (1 + \bar{x}_q^m)} = g_2 \quad (30)$$

$$\frac{\partial f_{r+1}}{\partial x_r} = \frac{r_3 \gamma n \bar{x}_s}{x_r (1 + \bar{x}_r^n)} = g_3 \quad (31)$$

Let us notice that the absolute value of the product

$$|g_1 g_2 g_3| = \frac{\alpha \beta \gamma l m n R_2 R_3 \bar{x}_s^l}{(1 + \bar{x}_s^l)(1 + \bar{x}_q^m)(1 + \bar{x}_r^n)} < \alpha \beta \gamma l m n R_2 R_3 \quad (32)$$

has an upper bound equal to the limit value at $\bar{x}_s \rightarrow \infty$ and $\bar{x}_q = \bar{x}_r = 0$ (32). Now, we ought to find eigenvalues of the Jacobian matrix of the system.

$$\begin{vmatrix} -(1+\lambda) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & g_1 \\ h_2 & -(k_2+\lambda) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & h_3 & -(k_3+\lambda) & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ * & * & * & * & * & * & * & * & * & * & * \\ 0 & 0 & 0 & h_q & -(k_q+\lambda) & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & g_2 & -(R_2+\lambda) & 0 & 0 & 0 & 0 & 0 \\ * & * & * & * & * & * & * & * & * & * & * \\ 0 & 0 & 0 & 0 & 0 & h_r & -(k_r+\lambda) & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & g_3 & -(R_3+\lambda) & 0 & 0 & 0 & 0 \\ * & * & * & * & * & * & * & * & * & * & * \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & h_s & -(k_s+\lambda) \end{vmatrix} = 0 \quad (33)$$

We have to transform the determinant (33) into polynomial and solve the resulting equation. The Jacobian contains two nonzero terms in each row. One of these terms is situated on the main diagonal, in the position (j, j) and is equal to the rate constant of decay of the respective substance. The other nonzero terms, the rate synthesis constants of the respective substances, have positions $(j, j-1)$.

In the rows l , $q+1$ and $r+1$, the values of derivatives of the terms describing transcription are placed in these positions instead of rate constants of synthesis. Derivatives are positive in the case of induction and they are negative in the case of repression. In the first row the derivative g_l is situated in the position (l, s) . Equation (33) is equivalent to polynomial equation (34).

$$(1+\lambda)(R_2+\lambda)(R_3+\lambda)\prod_{j=2, j \neq q+1, r+1}^s (k_j+\lambda) = g_1 g_2 g_3 \prod_{j=2, j \neq q+1, r+1}^s h_j \quad (34)$$

Because of the repression, there is no equilibrium in the origin of coordinates. Neither of derivatives g is equal to zero, but their product can be positive or negative. When the right hand side of (34) is positive, the system has a phase portrait of a trigger. When it is negative, the system evolves similar to that with one repressed gene. If parameters satisfy some additional condition, the system will be able to show auto-oscillations. These conditions consist in high enough maximum rates of transcription (C_1, C_2, C_3) and decay constants (R_2, R_3, k_j) not too much different from 1. In the particular case of the system (23) the product of g_i and the right side of (34) is negative.

Equation (34) has no simple solution in general case. But it is solvable under quite severe conditions that

$$R_2 = R_3 = k_j = 1, \quad j \neq 1, q+1, r+1. \quad (35)$$

The conditions (35) are equivalent to the assumption that all reagents of the system have the same mean rate constants of their decay. If it is so, then equation (34) can be written as

$$(1+\lambda)^s = g_1 g_2 g_3 \prod_{j=2, j \neq q+1, r+1}^s h_j \quad (36)$$

Let us to notice that under condition (35) the absolute value of $g_1 g_2 g_3$ (32)

$$|g_1 g_2 g_3| = \frac{\alpha \beta \gamma l m n \bar{x}_s^l}{(1+\bar{x}_s^l)(1+\bar{x}_q^m)(1+\bar{x}_r^n)} < \alpha \beta \gamma l m n \quad (37)$$

Taking into account that, according to (24–26 and 35)

$$\alpha \beta \gamma = \frac{\prod_{j=2, j \neq q+1, r+1}^s k_j}{\prod_{j=2, j \neq q+1, r+1}^s h_j} = \frac{1}{\prod_{j=2, j \neq q+1, r+1}^s h_j} \quad (38)$$

we obtain the relation (39):

$$|g_1 g_2 g_3| = \frac{1}{\prod_{j=2, j \neq q+1, r+1}^s h_j} \frac{lmn \bar{x}_s^l}{(1 + \bar{x}_s^l)(1 + \bar{x}_q^m)(1 + \bar{x}_r^n)} < \frac{1}{\prod_{j=2, j \neq q+1, r+1}^s h_j} lmn . \quad (39)$$

The insertion of (39) into (36) results in the equation for eigenvalues λ .

$$(1 + \lambda)^s = \text{sgn}(g_1 g_2 g_3) \frac{lmn \bar{x}_s^l}{(1 + \bar{x}_s^l)(1 + \bar{x}_q^m)(1 + \bar{x}_r^n)} . \quad (40)$$

It is evident that the absolute value of the right hand part of (40) satisfies the inequality:

$$R = \frac{lmn \bar{x}_s^l}{(1 + \bar{x}_s^l)(1 + \bar{x}_q^m)(1 + \bar{x}_r^n)} < lmn . \quad (41)$$

In the case of the negative product of derivatives, at odd number of representations, the equation (40) can be written as:

$$(1 + \lambda)^s = R(\cos \pi + i \sin \pi) . \quad (42)$$

and

$$\lambda_k = -1 + R^{\frac{1}{s}} \cos \frac{(2k+1)\pi}{s} + i R^{\frac{1}{s}} \sin \frac{(2k+1)\pi}{s}, \quad k = 0, \dots, s-1 . \quad (43)$$

All of the eigenvalues (43) are located on a circle with the radius $R^{\frac{1}{s}}$ and with the centre in the point $(-1, 0)$ on the complex plane.

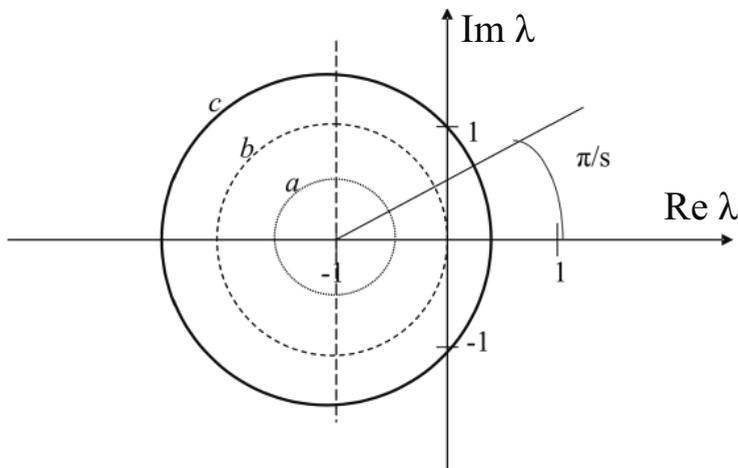


Fig. 2. Eigenvalues (21): a) $R < 1$, b) $R = 1$, c) $R > 1$

The roots λ_0 and λ_{s-1} have the same and the largest real part. Equilibrium of our system will be destabilized through Hopf bifurcation when the real part of these eigenvalues becomes positive. It happens when

$$R > \left(\cos^s \frac{\pi}{s} \right)^{-1} . \quad (44)$$

At the same time R should satisfy the inequality (41). It is possible, if

$$\left(\cos^s \frac{\pi}{s} \right)^{-1} < R < lmn . \quad (45)$$

Relation (45) and a negative product of nonlinear term derivatives in (23) constitute the necessary conditions of Hopf bifurcation in the system with a single repressed and two induced genes. This result can be generalized on a long loop with an odd number of repressed genes and with any number of induced genes. So, an oscillatory solution is possible in a Goodwin's loop when it contains an odd number of repressed genes and has the product of Hill coefficients higher than $\left(\cos^s \frac{\pi}{s} \right)^{-1}$, where s is the number of chemical species participating in the loop or the order of the system of ordinary differential equations describing the loop. The main requirement for the existence of oscillatory solutions in the loops under consideration is an odd number of repressed genes. However, the presence of cooperative induction makes the product of Hill coefficients higher. On the other hand, at the presence of many protein interconversions, even described by linear equations, s is higher and the extreme left side of (45) is lower. Both factors make oscillatory solution more probable.

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