Stability analysis of a time delay model for the JAK-STAT signaling pathway

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Abstract

The paper presents a stability analysis of a time delay model for the JAK-STAT signaling pathway by using an equivalent system of ordinary differential equations. This is realized on the basis of an analytical formula for the Lyapunov function. The following conclusions are made based on the results found: when the import rate of STAT5 into the nucleus is larger than (or equal to) the export rate: (i) the equivalent system has many fixed points different from zero or it is in a trivial state where all variables are zero; and (ii) at the beginning and end of signal transduction (i.e. when the concentration of the hormone Erythropoietin is low) the system is in a stable regime.

Keywords: Signaling pathway, time delay model, stability analysis, Lyapunov function

1. Introduction

It is generally accepted that signaling and cell functions are dynamic processes. Stability analysis of such systems is primarily a matter of finding the number of steady states, their nature (stable/unstable) and characterizing transitions between them (structural stability analysis). Following a dynamic systems approach to cell signaling [1, 2] the present paper introduces an advanced analysis of an established experimental system. Pathways, understood as biochemical reaction networks, are complex systems. Complexity arises from the presence of feedback loops in the cell [1], the relatively large number of molecules involved and the nonlinear nature of interactions between molecules [2].

With advances in technologies and the design of experiments that generate quantitative data sets, there is an increasing demand for mathematical tools to elucidate the dynamic behavior of cells. For example, the time delays, that are a consequence of transport processes especially between the nucleus and cytoplasm in a cell, are particularly relevant to cyclic changes observed in experiments [15, 19]. Oscillations have long been known in metabolic pathways [20, 21] and are now also considered as an important aspect of cell signaling [26].

STATs (signal transducers and activators of transcription) are a family of latent cytoplasmatic proteins that are activated to participate in gene expression [3]. Seven different mammalian STAT genes have been identified in three chromosomal clusters. Phosphorylation on a single tyrosine located around residue 700 in each protein is obligatory for STAT activation. Ligand-activated receptors, which catalyze this phosphorylation include receptors with intrinsic tyrosine kinase activity as well as receptors that lack intrinsic tyrosine kinase activity, but which Janus kinases (JAKs) are noncovalently associated to [3]. Phosphorylation of the kinase is the first of three tyrosine phosphorylations culminating in STAT

activation. The activated JAKs phosphorylate tyrosine sites on the cytoplasmic rail of the receptor that serve as docking sites for the SH2 domains that occur in all the STATs. It is important that the activated JAK protein kinases do not seem to have specificity for a particular STAT substrate [3].

Functioning cells originate from undifferentiated progenitor cells. Differentiation of progenitor cells is triggered by hormones. Erythropoietin ($EpoR_A$) is the hormone that promotes the development of progenitor cells into red blood cells [3, 5, 13].

The JAK/STAT pathway was originally identified in mammals. This pathway is involved in signaling through multiple cell surface receptors, including receptor tyrosine kinases, G- protein-coupled receptors, and hemotopoietic cytokine receptors such as the erythropoietin receptor ($EpoR_A$). Signal transduction through the $EpoR_A$ is essential for the proliferation and differentiation of erythroid progenitor cells [2, 5 and references therein]. Studies of this pathway in mice cell lines have revealed that JAK/STAT signaling plays a central role during hematopoeisis and other developmental processes [4]. In particular, this pathway is a global factor in the control of cell movement. Information storage in DNA and translation of that information into proteins is central to thermodynamic stability maintenance, through increased order that results from synthesis of specific macromolecules from monomeric precursors while heat and other reaction products are exported into the environment [24, 25]. In view of the general considerations for homeostasis of living cell, the dynamical stability of the pathway's steady states is of particular interest. Extending previously published models and analyses [2, 5, 14] we expand the analysis of the dynamic properties by translating a delay differential equation model into an equivalent system of ordinary differential equations.

The paper is organized as follows: In Section 2 we investigate how a change of the fixed point type of the equivalent system of ordinary differential equations influences over the time delay model of the JAK-STAT signaling pathway (obtained in [5]). Section 3 provides a discussion and conclusions of our study.

2. Time delay model.

In this section, the general form of the time delay model of the JAK-STAT signaling pathway is investigated. This model was originally developed by Timmer *et. al.* [5]. It considers mass-action kinetics and denotes the amount of activated Epo-receptors by $EpoR_A$, unphosphorylated monomeric STAT-5 by x_1, phosphorylated monomeric STAT-5 by x_2, phosphorylated dimeric STAT-5 in the cytoplasm by x_3 and phosphorylated dimeric STAT-5 in the nucleus by x_4. Hence we can write

$$\begin{aligned}
\dot{x}_1 &= -k_1 c x_1 + 2k_4 x_3(t-\tau),\\
\dot{x}_2 &= k_1 c x_1 + 2k_3' x_3 - k_2 x_2^2,\\
\dot{x}_3 &= -(k_3 + k_3')x_3 + 0.5 k_2 x_2^2,\\
\dot{x}_4 &= k_3 x_3 - k_4 x_3(t-\tau),
\end{aligned} \tag{1}$$

where $c = EpoR_A = const.$, and k_1, k_2, k_3, k_3', k_4 are constants with dimension [min^{-1}]. According to [2, 5], the initial values of x_2, x_3, x_4 are zero, and the initial value of x_1 is a free parameter which is estimated from experimental data.

In the following sections we at first consider the general properties of the approximation of delay systems by ordinary differential equations and then apply a so called 'linear chain trick' for the delay system (1).

2.1. Approximation of the system with delay. Linear chain trick

We follow [6-8] and consider a differential equation with delay

$$\frac{dx}{dt} = f\left(t, x(t), x(t-\tau)\right), \tag{2}$$

where $t \in R$, $x \in R^n$, and function $f(t, u, v)$ is continuous in t and satisfies the Lipschitz condition with respect to u and v, as well as the following condition

$$f(t, 0, 0) = 0. \tag{3}$$

After substitution of the delay elements by a sequence of m aperiodic segments (linear chain trick) [6-8], we can turn Eq. (2) into an equivalent system of ordinary differential equations (ODEs)

$$\begin{aligned} &\frac{dx}{dt} = f\left(t, x, z_m\right), \\ &\frac{dz_i}{dt} = \frac{m}{\tau}\left(z_{i-1} - z_i\right), \quad i = 1, ..., m, \quad m \in N. \end{aligned} \tag{4}$$

Note that $z_0(t) = x(t) = \tau \dot{z}_1 + z_1$ at $m = 1$. In the case where the trivial solution of Eq.(2) is asymptotically stable, the accuracy of approximation can be considered on the infinite interval $[0, \infty]$. In this situation, the following statement applies [6]:

Theorem 1.
If the trivial solution of system (2) is uniformly asymptotically stable, then, for sufficiently large m, the trivial solution of system (4) is uniformly asymptotically stable. If the trivial solution of system (4) is uniformly asymptotically stable, then the trivial solution of (2) is uniformly asymptotically stable for sufficiently large m.

By virtue of Theorem 1, there exists a number $m_0 > 0$ such that, for $m \geq m_0$, the asymptotic stability of trivial solution of the system with delay (2) is equivalent to the asymptotic stability of the system of ordinary differential equations (4). Number m_0 must be estimated in each case, which is why a general form of *Theorem 1* has been was proved later in [9], i.e.:

Theorem 2.
If the trivial solution of Eq.(2) is exponentially stable (unstable), then there exists $m_0>0$ such that, for $m \geq m_0$, the trivial solution of system (4) is exponentially stable (unstable). If for all $m \geq m_0$, the trivial solution of system (4) is exponentially stable (unstable), then the trivial solution of Eq. (2) is also exponentially stable (unstable).

2.2. Linear chain trick for system (1)

Introduce a family of z_i's in (1), where i=1, 2. Hence, system (1) with z defined in Section 2.1 can be written equivalently as a set of ODEs:

$$\dot{x}_1 = -k_1 c x_1 + 2k_4 z_2, \tag{5}$$

$$\dot{x}_2 = k_1 c x_1 + 2k_3' x_3 - k_2 x_2^2, \tag{6}$$

$$\dot{x}_3 = -\left(k_3 + k_3'\right) x_3 + 0.5 k_2 x_2^2, \tag{7}$$

$$\dot{x}_4 = k_3 x_3 - k_4 z_2, \tag{8}$$

$$\dot{z}_1 = \frac{1}{\tau}\left(x_3 - z_1\right), \tag{9}$$

$$\dot{z}_2 = \frac{2}{\tau}\left(z_1 - z_2\right). \tag{10}$$

We take the approximate values for the parameters of the model (5)-(10) from the literature [2, 5]:

$$\begin{aligned} &k_1 = 0.021\left[\min^{-1}\right], \quad k_2 = 2.46\left[\min^{-1} mol^{-1}\right] / x_1(0), \\ &k_3 = 0.1066\left[\min^{-1}\right], \; k_4 = 0.10658\left[\min^{-1}\right], \; \tau = 6.4[\min]. \end{aligned} \tag{11}$$

Since data for the parameter k_3' lack, we assume $k_3' = 0.001\left[\min^{-1}\right]$ while all numerical realizations of delay system (1) and system (5)-(10) are accomplished with the following initial conditions

$$\begin{aligned} &x_1(0) = 5.5,\; x_2(0) = x_3(0) = 0.001, \\ &x_4 = 0,\; x_5(0) = 0.5,\; x_6(0) = 1.9 \end{aligned} \tag{12}$$

Solutions of system (1) (fine lines) and system (5)-(10) (thick lines) are shown in Fig.1. Note that a solution for x_4 is not shown because it does not affect the qualitative behavior of both the time delay and the ODE system. Comparing numerical results for these two systems we see that the solution of (5)-(10) is very close to the delay model throughout the interval (0, 60).

Thus, we conclude that the approximation of system (1) with delays by system (5)-(10) with ordinary differential equations is correct and all conclusions for the ODEs system are valid for the time delay system (1). Here we note the following fact.

According to experimental results found for Erythropoietin ($EpoR_A$=c) in [2, 5] we assume that:

$$\begin{aligned} &c = 10 \text{ for } t \in [0, 2);\; c = 15 \text{ for } t \in [2, 3);\; c = 20 \text{ for } t \in [3, 5);\; c = 45 \text{ for } t \in [5, 12); \\ &c = 25 \text{ for } t \in [12, 19);\; c = 10 \text{ for } t \in [19, 30);\; c = 5 \text{ for } t \in [30, 60]. \end{aligned} \tag{13}$$

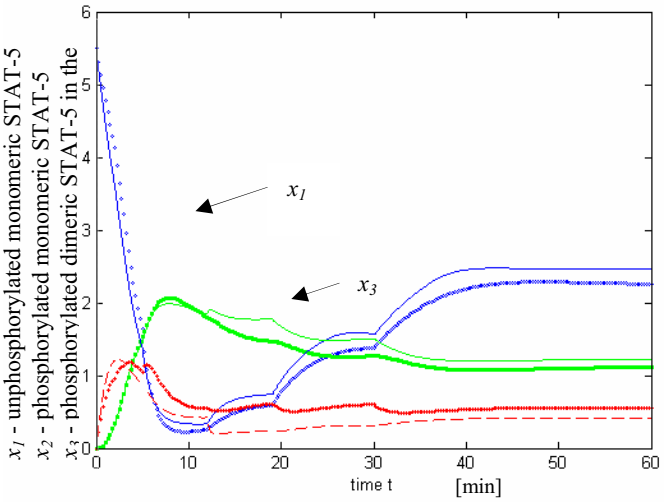

Fig. 1. The unphosphorylated monomeric STAT-5-x_1 (blue), the phosphorylated monomeric STAT-5-x_2 (red) and the phosphorylated dimeric STAT-5 in the cytoplasm-x_3 (green) as functions of time. For further explanations see text.

We investigate the behavior of system (5)-(10) in the section that follows.

2.3. Qualitative analysis of the system (5)-(10)

Equations (5)-(10), together with initial conditions (12) represent an equivalent system for the delay model of the JAK-STAT signaling pathway published in [2]. To facilitate the analysis, we first non-dimensionalize the system, and then carry out a steady-state analysis on the scaled system. We also

discuss the biological (physiological) relevance of our results. We note that only the fourth equation (Eq. (8)) contains the variable x_4. Using this fact, we obtain the following reduced form for system (5)-(10)

$$\begin{aligned}
\dot{x}_1 &= -k_1 c x_1 + 2k_4 z_2, \\
\dot{x}_2 &= k_1 c x_1 + 2k_3' x_3 - k_2 x_2^2, \\
\dot{x}_3 &= -\left(k_3 + k_3'\right) x_3 + 0.5 k_2 x_2^2, \\
\dot{z}_1 &= \frac{1}{\tau}\left(x_3 - z_1\right), \\
\dot{z}_2 &= \frac{2}{\tau}\left(z_1 - z_2\right).
\end{aligned} \tag{14}$$

Next, we non-dimensionalize the model (14) using the following relations

$$\begin{aligned} &x_1 = x_1^0 X_1,\ x_2 = x_2^0 X_2,\ x_3 = x_3^0 X_3, \\ &z_1 = z_1^0 Z_1,\ z_2 = z_2^0 Z_2,\ t = T\tau_1 \end{aligned}, \tag{15}$$

where T is the expected period of JAK-STAT signaling. Constants $x_1^0 \div x_3^0$, z_1^0 and z_2^0 are initial conditions in different intervals of change of parameter c (see (13)), respectively. After substitution of (15) into (14) and accomplishing some transformations the system (14) takes the form

$$\begin{aligned} \dot{X}_1 &= -a_1 X_1 + a_2 Z_2, \\ \dot{X}_2 &= a_3 X_1 + a_4 X_3 - a_5 X_2^2, \\ \dot{X}_3 &= -a_6 X_3 + a_7 X_2^2, \\ \dot{Z}_1 &= a_8 X_3 - a_9 Z_1, \\ \dot{Z}_2 &= 2a_9 Z_1 - a_{10} Z_2, \end{aligned} \tag{16}$$

where

$$\begin{aligned} &a_1 = k_1 cT,\ a_2 = \frac{2k_4 z_2^0 T}{x_1^0},\ a_3 = \frac{k_1 c x_1^0 T}{x_2^0},\ a_4 = \frac{2k_3^{'} x_3^0 T}{x_2^0}, \\ &a_5 = k_2 x_2^0 T,\ a_6 = \left(k_3 + k_3^{'}\right)T,\ a_7 = \frac{0.5k_2 \left(x_2^0\right)^2 T}{x_3^0}, \\ &a_8 = \frac{x_3^0 T}{\tau},\ a_9 = \frac{z_1^0 T}{\tau},\ a_{10} = \frac{2z_2^0 T}{\tau} \end{aligned} \tag{17}$$

Here we note that the constant coefficients $a_1 \div a_{10}$ are dimensionless, too. The stationary points of system (16) are

$$\begin{aligned} &\bar{X}_1 = \frac{2a_2 a_7 a_8}{a_1 a_6 a_{10}} \bar{X}_2^2,\quad \bar{X}_3 = \frac{a_7}{a_6} \bar{X}_2^2,\quad \bar{Z}_1 = \frac{a_8}{a_9} \bar{X}_3, \\ &\bar{Z}_2 = \frac{2a_9}{a_{10}} \bar{Z}_1,\ \bar{X}_2^2 \left(\frac{2a_2 a_3 a_7 a_8}{a_1 a_6 a_{10}} + \frac{a_4 a_7}{a_6} - a_5 \right) = 0, \end{aligned} \tag{18}$$

It follows from Eq. (18) that system (16) has two fixed points. When the relation

$$a_5 = \frac{2a_2 a_3 a_7 a_8}{a_1 a_6 a_{10}} + \frac{a_4 a_7}{a_6}, \text{ i.e. } k_3 = k_4, \tag{19}$$

is valid, system (16) has many fixed points different from zero. According to [2], in this case ($k_3 = k_4$) the import rate k_3 is equal to the export rate k_4. To preserve mass conservation, the condition $k_3 \geq k_4$ has to hold. If this condition is not valid, STAT5 accumulates in the nucleus. Thus, we take $\bar{X}_2 = 1.3$ from Fig.1. Therefore, according to (18) we obtain for the first fixed point

$$\bar{X}_1 = 3.38\frac{a_2 a_7 a_8}{a_1 a_6 a_{10}},\ \bar{X}_2 = 1.3,\ \bar{X}_3 = 1.69\frac{a_7}{a_6},$$
$$\bar{Z}_1 = \frac{a_8}{a_9}\bar{X}_3,\ \bar{Z}_2 = \frac{2a_9}{a_{10}}\bar{Z}_1 \tag{20}$$

In order to investigate the character of the fixed point (Eq. (20)), we make the following substitutions in the model (16)

$$X_1 = \bar{X}_1 + y_1,\ X_2 = \bar{X}_2 + y_2,$$
$$X_3 = \bar{X}_3 + y_3,\ Z_1 = \bar{Z}_1 + y_4, \tag{21}$$
$$Z_2 = \bar{Z}_2 + y_5$$

Hence, after some transformations system (16) gets the following form in local coordinates

$$\dot{y}_1 = -a_1 y_1 + a_2 y_5,$$
$$\dot{y}_2 = a_3 y_1 - A_1 y_2 + a_4 y_3 - a_5 y_2^2,$$
$$\dot{y}_3 = A_2 y_2 - a_6 y_3 + a_7 y_2^2, \tag{22}$$
$$\dot{y}_4 = a_8 y_3 - a_9 y_4,$$
$$\dot{y}_5 = 2a_9 y_4 - a_{10} y_5,$$

where

$$A_1 = 2a_5 \bar{X}_2,\ A_2 = 2a_7 \bar{X}_2\ . \tag{23}$$

The divergence of flow (22) is

$$D_5 = \sum_{i=1}^{5} \frac{\partial \dot{y}_i}{\partial y_i} = -\left(a_1 + a_6 + a_9 + a_{10} + A_1 + 2a_5 y_2\right) \tag{24}$$

It is seen that D_5 is always negative and therefore system (22) is always dissipative. On the other hand, if relation (19) is not valid, it is easy to see that the equilibrium (the steady state) is the trivial state where all variables are zero, i.e.:

$$\bar{X}_1 = \bar{X}_2 = \bar{X}_3 = \bar{Z}_1 = \bar{Z}_2 = 0. \tag{25}$$

The basis of stability theory for systems with structurally unstable equilibrium states was developed by Lyapunov. Critical equilibrium states have been the subject of a number of studies. Here we mention only the two most common and simple cases, where the characteristic equation

$$\chi^n + a_1\chi^{n-1} + ... + a_{n-1}\chi + a_n = 0 \tag{26}$$

(i) has one zero root; or
(ii) has a pair of complex-conjugate roots on the imaginary axis.

The first case is determined by the condition

$$a_n = 0, \Delta_k > 0, k = 1,...,n-1, \tag{27}$$

where Δ_k is the Routh-Hurwitz determinant. Recall that $a_n = (-1)^n \det A$, where A is the matrix of the linearized system at the equilibrium state. Therefore, according to [10, 11, 12], from a practical point of view for high-dimensional systems ($n \geq 4$) the basic tools for studying critical cases include the so called method of the Lyapunov functions. Typically, a proof concerning stability consist of either designing a Lyapunov function (LF), or proving its existence. Its applicability is not limited to critical equilibriums. What follows is a definition for a Lyapunov functions.

Definition.
A continuous function $V(x)$ defined in a neighborhood D of equilibrium state O and smooth in D\O, is called a Lyapunov function for systems (16) and (22) if it satisfies the following conditions (d.1) $V(0) = 0$; *(d.2)* $V(x) > 0$ *if* $x \neq 0$; *(d.3)* $\dot{V}(x) \leq 0$ *at* $x \neq 0$.

The use of Lyapunov functions to guarantee stability is based upon the following Theorem.

Theorem 3.
If there exists a function $V(x)$ satisfying conditions (d.1)-(d.3), then the equilibrium state O is Lyapunov-stable. Furthermore, if the inequality (d.3) is strict for all $x \neq 0$, then all trajectories in D tend to the point O as $t \to +\infty$, i.e. the equilibrium state O is asymptotically stable.

We omit here the proof because it has been discussed at length elsewhere [12, 16, 17]. To prove that an equilibrium state is unstable one can use analogies to Lyapunov functions. If there exists a function $V(x)$ satisfying conditions (d.1) and (d.2) but (d.4) $\dot{V}(x) > 0$ at $x \neq 0$, then the corresponding equilibrium state *O* is unstable. Therefore, by virtue of *Theorem 3* all trajectories tend to *O* as $t \to -\infty$, i.e. such an equilibrium state is repelling, or completely unstable.

However, it is possible to have an unstable equilibrium state *O* such that some trajectories converge to *O* as $t \to +\infty$ [12]. The simplest example is a rough saddle. To prove instability of a saddle in critical cases one can use Chetaev's function where conditions (d.1), (d.2) and (d.4) hold only within some sector adjoining the point *O* [12, 20].

From the practical point of view, stability in the sense of Lyapunov is less important than asymptotic stability. In particular, it follows from simple continuity arguments that if a critical equilibrium state is asymptotically stable, then the trajectories of any nearby system will also converge to a small neighborhood of the origin where they will stay forever. The behavior of trajectories in this small neighborhood may be nontrivial. Nevertheless, any deviations from zero of trajectories of a nearby system must remain small because the equilibrium state is asymptotically stable at the critical parameter value [10, 12, 18].

Following the definition for a Lyapunov function, we can write for V and $\dot{V}$ that

(i) for the first fixed point (20),. the import rate k_3 is equal to the export rate k_4
(ii)

$$V_1 = y_1^2 + y_2^2 + y_3^2 + y_4^3 + y_5^2 \tag{28}$$

$$\frac{dV_1}{dt} = \dot{V}_1 = \frac{\partial V_1}{\partial y_1}\dot{y}_1 + \frac{\partial V_1}{\partial y_2}\dot{y}_2 + \frac{\partial V_1}{\partial y_3}\dot{y}_3 + \frac{\partial V_1}{\partial y_4}\dot{y}_4 + \frac{\partial V_1}{\partial y_5}\dot{y}_5 . \tag{29}$$

After substituting (22) and (28) into (29) and performing some transformations, Eq. (29) takes the form

$$\begin{aligned}\dot{V}_1 = 2\big[&y_1\left(-a_1 y_1 + a_2 y_5\right) + y_2\left(a_3 y_1 - A_1 y_2 + a_4 y_3 - a_5 y_2^2\right) + \\ &+ y_3\left(A_2 y_2 - a_6 y_3 + a_7 y_2^2\right) + y_4\left(a_8 y_3 - a_9 y_4\right) + \\ &+ y_5\left(2a_9 y_4 - a_{10} y_5\right)\big]\end{aligned} \tag{30}$$

(ii) for the second fixed point (25)

$$V_2 = X_1^2 + X_2^2 + X_3^2 + Z_1^2 + Z_2^2 , \tag{31}$$

$$\frac{dV_2}{dt} = \dot{V}_2 = \frac{\partial V_2}{\partial X_1}\dot{X}_1 + \frac{\partial V_2}{\partial X_2}\dot{X}_2 + \frac{\partial V_2}{\partial X_3}\dot{X}_3 + \frac{\partial V_2}{\partial Z_1}\dot{Z}_1 + \frac{\partial V_2}{\partial Z_2}\dot{Z}_2 . \tag{32}$$

After substituting (16) and (31) into (32) and performing some calculations for (32) we obtain

$$\begin{aligned}\dot{V}_2 = 2\big[&X_1\left(-a_1 X_1 + a_2 Z_2\right) + X_2\left(a_3 X_1 + a_4 X_3 - a_5 X_2^2\right) + \\ &+ X_3\left(-a_6 X_3 + a_7 X_2^2\right) + \\ &+ Z_1\left(a_8 X_3 - a_9 Z_1\right) + Z_2\left(2a_9 Z_1 - a_{10} Z_2\right)\big].\end{aligned} \tag{33}$$

Using result for $\dot{V}_1$ and $\dot{V}_2$ (obtained in (30) and (33)), $\dot{V}_1$ and $\dot{V}_2$ are shown in Figure 2 for different values of time *t*, i.e. when $t \in [0, 60]$. It is seen that $\dot{V}_1$ and $\dot{V}_2$ pass through regions where they are negative or positive. A more detailed investigation of $\dot{V}_1$ and $\dot{V}_2$ in these regions will be shown in the following Figures 3 and 4.

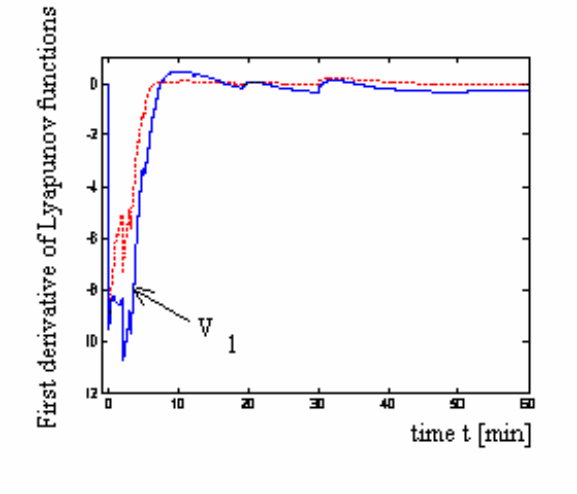

Fig. 2. Current first derivatives $\dot{V}_1$ (blue) and $\dot{V}_2$ (red) of Lyapunov functions (30) and (33) at $t \in [0, 60]$.

The dependence of $\dot{V}_1$ on t is shown in Figs. 3a, 3b and 3c, for $t \in [0, 7.4]$, $t \in [7.4, 19]$ and $t \in [19, 60]$. Initially, $\dot{V}_1$ is negative and for $t \approx 7.8$ it passes through zero while for $t \approx 7.9$ it is positive (see Figs. 3a and 3b) and remains positive to the end of the interval $t \in [7.4, 16.8]$. After that, for $t \in [16.9, 20]$ and $t \in [39, 60]$ $\dot{V}_1$ is negative (see Fig.3c). It is interesting to note that for $t \in [20, 23]$ and $t \in [31, 36]$ $\dot{V}_1$ is positive. Therefore, following [9, 10, 11] as well as the above definition and *Theorem 3* for LF, we have for $t \in [0, 7.4]$, $t \in [16.9, 20]$ and $t \in [39, 60]$ that the equilibrium state (20) is stable. On the other hand, for $t \in [7.4, 16.8]$, $t \in [20, 23]$ and $t \in [31, 36]$ the equilibrium (20) is unstable. Hence, following the theorems introduced in Section 2.1, we conclude that in those intervals for time t the time delay system (1) has also stable/unstable solutions, i.e. JAK-STAT signaling pathway is stable for $t \in [0, 7.4]$, $t \in [16.9, 20]$ and $t \in [39, 60]$, and for $t \in [7.4, 16.8]$, $t \in [20, 23]$ and $t \in [31, 36]$ is unstable, respectively.

From a biological point of view, the stability of cell signaling pathways could be connected with homeostasis, i.e., the process of keeping an internal environment stable by making adjustments to changes in the external environment. This is achieved by a system of feedback control loops. In other words, for the stability of cell signaling processes it could be useful to define a condition where the cell maintains a stable condition where in fact a constant flux of molecules occurs. In a more narrow sense homeostasis could be refered to the maintenance of water and salt concentrations in the cell. Instability can be connected respectively with: (i) building new spatial structures; (ii) transitional processes (bifurcations) from one to other structure; (iii) appearance of self-oscillations after Turing bifurcation and building new structures as a result of transport being a stabilization element. In [22], Turing proved that it is possible for a homogeneous attracting equilibrium to lose stability due to the interaction with diffusion processes [23].

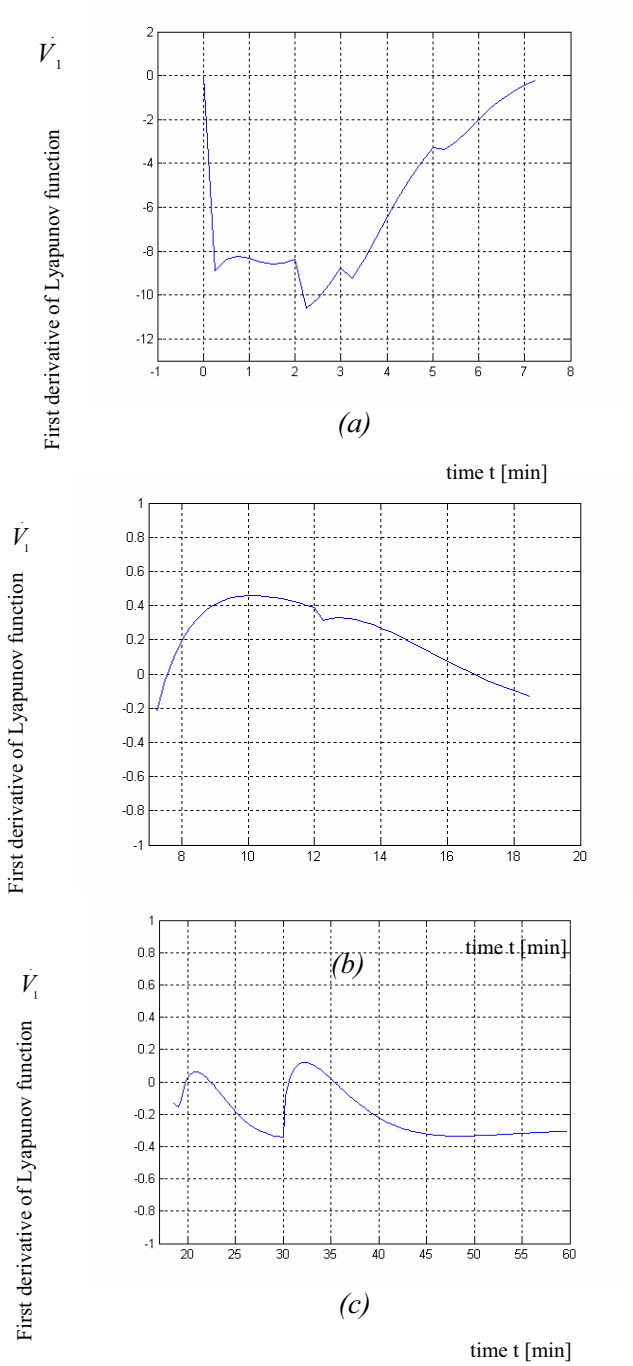

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Fig. 3. The first derivative $\dot{V}_1$ of Lyapunov function (30) at $t \in [0, 7.4]$ is shown in subfigure (**a**). (b) illustrates the behavior of $\dot{V}_1$ for $t \in [7.4, 19]$ and (c**)** illustrates the behavior of $\dot{V}_1$ for $t \in [19, 60]$, respectively. For more details see the text.

The change of $\dot{V}_2$ for $t \in [0, 4]$ and $t \in [4, 14]$ are shown in Figs. 4a and 4b. It can be seen that $\dot{V}_2$ passes through regions for which it is negative or positive. More precisely, $\dot{V}_2$ becomes positive after $t \approx 6.9$, and remains positive till the end of the interval $t \in [6.9, 19]$ (see Fig. 4c) . It is interesting to note here that $\dot{V}_2$ is positive also in the intervals $t \in [20, 25]$ and $t \in [30, 45]$. In other words, as a result of evidence shown in Figs. 2, 4a, 4b and 4c we may conclude that the behavior of the equilibrium state (25) for $t \in [0, 6.9]$, $t \in [19, 20]$, $t \in [25, 30]$ and $t \in [45, 60]$ is stable, i.e. here we have homeostasis. And vice versa- for $t \in [6.9, 19]$, $t \in [20, 25]$ and $t \in [30, 45]$ the second fixed point (25) is unstable. According to Theorems 1, 2 and 3 we conclude that in those cases the JAK-STAT signaling pathway is unstable.

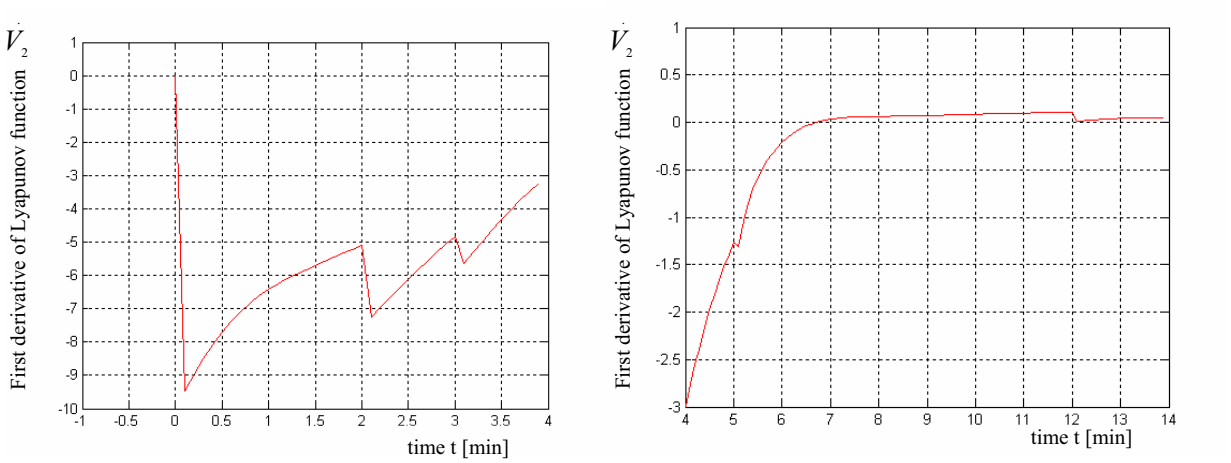

Fig. 4. First derivative $\dot{V}_2$ of Lyapunov function (30) at $t \in [0, 4]$ is shown in (a). (b) illustrating the behavior of $\dot{V}_2$ for $t \in [4, 14]$ and subfigure (c) illustrating the behavior of $\dot{V}_2$ for $t \in [19, 60]$, respectively.

Finally, we note by comparing results in Fig.3 and Fig.4 that the JAK-STAT pathway (for $k_3 \geq k_4$) is always stable in the beginning and at the end of the signaling process (in response to activation of the Epo-receptor).

3. Summary and conclusions

This paper studies changes in the dynamics of a time delay model for the JAK-STAT signaling pathway using dynamic systems stability analysis. The basic approach is to approximate a time delay model by means of equivalent system of ordinary differential equations using the linear chain trick. From numerical results presented in Section 2.1 it is shown that solutions of the original and the approximating system are very close. Note that governing equations of the model, presented by Eqs (1) and (5)-(10), are solved numerically using MATLAB. We subsequently analyze the qualitative behavior of solutions of the approximated system using the Lyapunov function approach to stability analysis. According Theorems 1 and 2 the qualitative behavior of these two systems is one and the same. On the basis of this fact, the following conclusions are made:

1. In JAK-STAT signaling pathway dynamics, a key role is played by parameters k_3 and k_4:

(i) for $k_3 = k_4$ (the import rate k_3 is equal to the export rate k_4), the equivalent system has many fixed points different from zero.

(ii) for $k_3 > k_4$, the fixed point is only one, equal to zero.

2. JAK-STAT pathway is always stable from 0 to 7 minute and from 45 to 60 minute, i.e. when the concentration of $EpoR_A$ is low we have homeostasis.

3. When the condition $k_3 > k_4$ holds, then

(i) at $t \in [0, 6.9]$, $t \in [19, 20]$, $t \in [25, 30]$ and $t \in [45, 60]$ the JAK-STAT pathway is in a stable regime;

(ii) at $t \in [6.9, 19]$, $t \in [20, 25]$ and $t \in [30, 45]$ JAK-STAT pathway is in an unstable regime.

4. When the condition $k_3 = k_4$ holds, then

(i) at $t \in [0, 7.4]$, $t \in [16.9, 20]$ and $t \in [39, 60]$ JAK-STAT pathway is stable.

(ii) at $t \in [6.9, 19]$, $t \in [20, 25]$ and $t \in [30, 45]$ this signaling pathway is unstable.

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