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Investigation of Dynamic Behaviour of Receptor Tyrosine Kinase and Protein Tyrosine Phospatase Reaction Network using Mathematical Model

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Summary: The dynamic and bifurcation behaviour of receptor tyrosin kinase (RTK) and protein tyrosine phospatase (PTP) reaction network model is investigated on the basis of Lyapunov-Andronov's theory. According to our qualitative and bifurcation analysis, propagation of phosphorylation is only possible in the unstable regime of the reaction network, i.e. when kinase activity of the receptor increases on phosphorylation. For some values of the model parameters first Lyapunov value can be positive or negative and bistability takes place.

Keywords: PTP-RTK Reaction Network, Mathematical Model, Bifurcation Analysis.

1. INTRODUCTION

In cells, biological responses are coordinated through signalling networks composed by interacting protein. The capacity of a cell to modulate its response in space and time is crucial for cell proliferation. Thus, the signals that control cell fate determination and cell differentiation and coordination in tissues and organs need to be exquisitely regulated in both time and space [5, 8]. The dynamical properties of a cell are determined by the topology of the protein-protein interaction networks that underlie cell physiology [11]. A major goal in the elucidation of biological networks is to develop mathematical models of the dynamics of different protein signalling pathways. However, in some cases mathematical

modelling is limited by the lack of appropriate, high-throughput experimental techniques to validate protein dynamics under different experimental conditions [7, 12].

Enzyme receptors are transmembrane receptors with intrinsic enzymatic activity. Good examples are the receptor tyrosine kinases (RTKs), high affinity cell surface receptors for many polypeptide growth factors, cytokines and hormones. These receptors can autophosphorylate their own tyrosine residues as well as the ones in growth factor receptors and the insulin receptor [4, 13]. The RTK family can be broadly divided into two groups depending on the covalent organization of the receptor. Most RTKs present a single polypeptide chain and are monomeric in the absence of ligand. RTK play significant roles in development, regulation of cell proliferation, differentiation and apoptosis, as well as in some diseases such as cancer and diabetes. Signalling through RTK is regulated by protein tyrosine phosphatases (PTPs) [3, 10]. While it is clear that PTPs are biologically important negative regulators for at least some RTKs [3, 9, 13 and references therein], little is known about the mechanisms and the specificity of these interactions [5]. To investigate the implications of a coupling between PTP and RTK activity in more detail, in [9] the authors formulated and analysed a minimal reaction model composed by two coupled ordinary nonlinear differential equations (scheme shown in Fig. 1). The model described the dynamics of the fraction of phosphorylated receptor tyrosine kinases (r) and the activated proteins tyrosine phsophatase (p) including the feedback-loop dephosphorylation process that couples the dynamics of both proteins. In the phosphorylation of the receptor tyrosine kinase, normal input signal-mediated process is considered as well as lateral propagation of the signal between activated and inactivated receptors.

$$\begin{aligned} \frac{dr}{dt} &= k_1^{'}\left(r_{tot}-r\right)^2 + k_1^{''}\left(r_{tot}-r\right)r - k_2^{'}rp, \\ \frac{dp}{dt} &= k_4\left(p_{tot}-p\right) - k_3^{'}rp, \end{aligned} \tag{1}$$

where

$$k_1^{'} = k_1\alpha_1, k_1^{''} = k_1\alpha_2, k_2^{'} = k_2\gamma, k_3^{'} = k_3\beta \quad (2)$$

Here k_1 is the basal kinase activity; α_1 represents the input signal-mediated contribution to the kinase activity and α_2 measures the enhancement in the kinase activity due to the lateral propagation-of the signal. β is rate constant associated to the deactivation of the phosphatase, while γ relates to the phosphatase-mediated deactivation of the tyrosine kinase. Finally, k_4 represents the activation of the phosphatase (p) via oxidation of the catalytic cysteine thiolate.

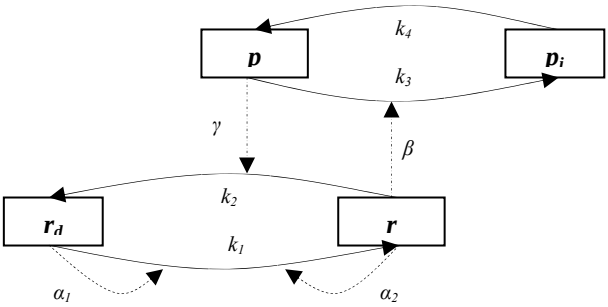

Fig. 1. Scheme showing the coupling between the dynamics of the receptor tyrosine kinase and phosphatase activation in the model proposed.
Legend: r, fraction of phosphorylated receptor tyrosine; r_d, dephosphorylated receptor tyrosine kinase; p, activated phosphatase; p_i, deactivated.

Given that uncatalysed reactions are negligible, they found that the steady states of the system are depended on three effective parameters: (i) the enhancement of kinase activity on receptor phosphorylation, α_2/α_1; (ii) the ratio of maximal phosphatase

activity to maximal kinase activity, $\frac{P}{K}=\frac{p_{tot}\gamma k_2}{r_{tot}\alpha_2 k_1}$; and (iii) the ratio of the maximal rate of phosphatase inhibition to the rate of phosphatase reactivation, $\frac{I}{R}=\frac{r_{tot}\beta k_3}{k_4}$. The authors also suggest that the system can operate in three possible conditions: 1) a unique stable steady state at low phosphorylation of receptor (system resting); 2) a unique stable steady state at high phosphorylation of receptor (system activated); 3) and a bistable state in which, depending on the initial conditions, the phosphorylation of the receptor can be either high ('activated') or low ('resting').

The plan of the paper is as follows: In Section 2 we qualitatively explore the model (1) using a specific bifurcation theory developed by Lyapunov-Andronov-Bautin. Afterwards, in Section 3 we discuss and unify results from previous section.

2. BIFURCATION ANALYSIS-ANALYTICAL STUDY

In this section, we consider the system (1), which present an autonomous dynamical model. All constants of this model are real and can be only positive. This system has three equilibrium (steady state) points:

$$\bar{r}^{3}+\frac{k_4\left(k_1^{'}-k_1^{''}\right)+r_{tot}k_3^{'}\left(k_1^{''}-2k_1^{'}\right)}{k_3^{'}\left(k_1^{'}-k_1^{''}\right)}\bar{r}^{2}+$$

$$+\frac{k_1^{'}k_3^{'}\left(r_{tot}\right)^2+k_4r_{tot}\left(k_1^{''}-2k_1^{'}\right)-k_2^{'}k_4p_{tot}}{k_3^{'}\left(k_1^{'}-k_1^{''}\right)}\bar{r}+\frac{k_1^{'}k_4\left(r_{tot}\right)^2}{k_3^{'}\left(k_1^{'}-k_1^{''}\right)}=0, \qquad (3)$$

$$\bar{p}=\frac{k_4p_{tot}}{k_4+k_3^{'}\bar{r}}$$

From the physiological point of view, $\bar{r}$ represents the concentration of a specie and must therefore be real positive. Thus, we further accomplish the equilibriums under consideration with positive

values. In the cubic case the equilibrium (steady state) values $\bar{r}$ of the system (1) are

$$X_1 = M_1 + M_2, \quad X_{2,3} = -\frac{M_1 + M_2}{2} \pm i\frac{M_1 - M_2}{2}\sqrt{3}, \tag{4}$$

where

$$\begin{aligned}
&\bar{r} = X - \frac{A}{3}, \quad A = \frac{k_4\left(k_1^{'} - k_1^{''}\right) + r_{tot}k_3^{'}\left(k_1^{''} - 2k_1^{'}\right)}{k_3^{'}\left(k_1^{'} - k_1^{''}\right)}, \quad C = \frac{k_4 k_1^{'}\left(r_{tot}\right)^2}{k_3^{'}\left(k_1^{'} - k_1^{''}\right)}, \\
&B = \frac{k_1^{'}k_3^{'}\left(r_{tot}\right)^2 + k_4 r_{tot}\left(k_1^{''} - 2k_1^{'}\right) - k_2^{'}k_4 p_{tot}}{k_3^{'}\left(k_1^{'} - k_1^{''}\right)}, \quad P = -\frac{A^2}{3} + B, \\
&N = 2\left(\frac{A}{3}\right)^2 + \frac{A}{3}B + C, \quad M_1 = \sqrt[3]{-\frac{N}{2} + \sqrt{Q}}, \\
&M_2 = \sqrt[3]{-\frac{N}{2} - \sqrt{Q}}, \quad Q = \left(\frac{P}{3}\right)^3 + \left(\frac{N}{2}\right)^2
\end{aligned} \tag{5}$$

It is well known [14] that if: (i) $Q > 0$, then the first equation in (3) has one real root and a pair of imaginary roots; (ii) $Q < 0$, then this equation has three different real roots and (iii) $Q = 0$, then for *P=N=0* the same equation has zero roots and for $P \neq 0, N \neq 0$ two real roots take place. Hence we obtain the initial condition for deviation of the model (1) parameters:

$$\left(\frac{P}{3}\right)^3 < -\left(\frac{N}{2}\right)^2 \tag{6}$$

Linearising around the steady states (3) by setting $r = \bar{r} + x_1$, $p = \bar{p} + x_2$ with x_1 and x_2 small perturbations, (1) becomes

$$\begin{aligned}
&\frac{dx_1}{dt} = ax_1 + bx_2 + \left(k_1^{'} - k_1^{''}\right)x_1^2 - k_2^{'}x_1x_2, \\
&\frac{dx_2}{dt} = cx_1 + dx_2 - k_3^{'}x_1x_2
\end{aligned} \tag{7}$$

where

$$a = -\left[k_2^{'} \bar{p} + r_{tot}\left(2k_1^{'} - k_1^{"}\right) + 2\bar{r}\left(k_1^{"} - k_1^{'}\right)\right], \quad b = -k_2^{'} \bar{r},$$
$$c = -k_3^{'} \bar{p}, \quad d = -\left(k_4 + k_3^{'} \bar{r}\right) \tag{8}$$

Following [2], the Routh-Hurwitz conditions for stability of (3) can be written in the form

$$R = -(a+d) = k_2^{'} \bar{p} + k_4 + r_{tot}\left(2k_1^{'} - k_1^{"}\right) + \bar{r}\left[k_3^{'} + 2\left(k_1^{"} - k_1^{'}\right)\right] =$$
$$= k_2 \gamma \bar{p} + k_4 + r_{tot} k_1 (2\alpha_1 - \alpha_2) + \bar{r}\left[k_3^{'} + 2k_1(\alpha_2 - 1)\right] > 0, \tag{9}$$

$$q = ad - bc = k_4\left[k_2^{'} \bar{p} + r_{tot}\left(2k_1^{'} - k_1^{"}\right) + 2\bar{r}\left(k_1^{"} - k_1\right)\right] +$$
$$+ k_3^{'} \bar{r}\left[r_{tot}\left(2k_1^{'} - k_1^{"}\right) + 2\bar{r}\left(k_1^{"} - k_1\right)\right] > 0. \tag{10}$$

Here the notations R and q are taken from [2]. Condition (9) can be positive or negative. When this condition is not valid (i.e. negative), the steady states (3) become unstable. In other words, if $2\alpha_1 < \alpha_2$ or $\alpha_2 < 1$ equilibrium points can be in the unstable zone of the system parametric space. In order to define whether the corresponding Andronov-Hopf bifurcation is sub (hard stability loss) or supercritical (soft stability loss) on the stability boundary of these equilibriums, it is necessary to calculate the so-called first Lyapunov value [1, 2, 6]. In the case of two first-order differential equations, this value can be determined analytically by the formula in [2].

$$L_1(\lambda_0) = -\frac{\pi}{4bq\sqrt{q}}\{[ac(a_{11}^2 + a_{11}b_{02} + a_{02}b_{11}) + \\ + ab(b_{11}^2 + a_{20} + a_{11}b_{20}) + c^2(a_{11}a_{02} + 2a_{02}b_{02}) - 2ac(b_{02}^2 - a_{20}a_{02}) - \\ - 2ab(a_{20}^2 - b_{20}b_{02}) - b^2(2a_{20}b_{20} + b_{11}b_{20}) + (bc - 2a^2)(b_{11}b_{02} - a_{11}a_{20})] - \\ - (a^2 + bc)[3(cb_{03} - ba_{30}) + 2a(a_{21} + b_{12}) + (ca_{12} - bb_{21})]\}, \tag{11}$$

where λ_0 is defined as a value of all system parameters of $\bar{r}$ and $\bar{p}$, for which the relation $R = 0$ takes place. The coefficients a_{ij} and b_{ij} $(i, j = 0, 1, 2, 3)$ are defined also in [2]. After accomplishing some transformations and algebraic operations, the first Lyapunov value $L_1(\lambda_0)$ for the states (4) is defined by the following equation

$$L_1(\lambda_0) = -\frac{a\pi}{4bq\sqrt{q}}\left[ca_{11}^2 + b(b_{11}^2 + a_{20}b_{11}) - 2ba_{20}^2 - a_{11}a_{20}\left(\frac{bc}{a} - 2a\right)\right] \tag{12}$$

Here, for the system (7) we have

$$a_{20} = k_1(1 - \alpha_2), \quad a_{11} = -k_2^{'} = -k_2\gamma, \quad b_{11} = -k_3^{'} = -k_3\beta, \\ a_{02} = b_{02} = b_{20} = a_{30} = b_{03} = a_{21} = a_{12} = b_{12} = b_{21} = 0. \tag{13}$$

It is seen that the first Lyapunov value (in (12)) can be negative or positive. If L_1 is negative, then in case of transition through the boundary $R = 0$ from positive values to negative ones, a stable limit cycle (self-oscillation) emerges. Inversely, in the case of transition from negative values to positive ones the stable limit cycle disappears, i.e. the self-oscillation ceases. In dynamic systems theory, this bifurcation behaviour near the boundary $R = 0$ is called *soft loss of stability*, and when the bifurcation parameter λ_0 changes, the system has reversible behaviour and the boundary $R = 0$ is called "safe".

If L_1 is positive, then in case of transition through the boundary $R = 0$ from positive values to negative ones, an unstable limit cycle emerges. Inversely, in case of transition from negative values to positive ones, the unstable limit cycle disappears. This type of bifurcation behaviour near the boundary $R = 0$ is called *hard loss of stability*, the system has an irreversible behaviour, and the boundary $R = 0$ is referred to as "dangerous" [2].

In other words, in case of safe boundaries, $L_1 < 0$, a slow drift of the parameters back into the stability region brings a system back into the original response, whereas in the dangerous case, $L_1 > 0$, this is generally impossible. Obviously, safe and dangerous boundaries are distinguished mainly by the stability or instability of the corresponding equilibrium state, or periodic trajectory, on the boundary [15].

3. CONCLUSIONS

According to our qualitative and bifurcation analysis, propagation of phosphorylation is only possible in the unstable regime of the reaction network, i.e. when kinase activity of the receptor increases on phosphorylation $2\alpha_1 < \alpha_2$. This theoretical finding is in good agreement with experimental studies in which activation of Ras was monitored by GFP-based sensors in cells [9, 16]. In addition, for some values of the model parameters first Lyapunov value can be positive or negative and bistability takes place. A stable steady state of the system with significant concentration of phosphorylated RTK kinase (system switched on) and a stable steady state with virtually no phosphorylated RTK (switched off) emerge in these cases, and certain external perturbations in the system can provoke a permanent transition between both states.

In conclusion, we shall note that this paper is a first step in the investigation of the RTK-PTP network. A number of questions still remain open for us. Indeed, we are interested on investigating how the change in the dynamic behaviour of the model relates to the pathological and the normal state of the system. Moreover, we plan to investigate how changes in particular parameters of the model affect its qualitative and quantitative behaviour.

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