Robustness Analysis of the PI3K/AKT Cell Signaling Module

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***Abstract*—Cell responses are actuated by tightly controlled signal transduction pathways. Due to the complex nature of molecular processes inside the cell, mathematical models recently have been used as a powerful tool to analyze the dynamic properties of cell signaling pathways. Although the concept of an integrated signaling network replete with inter-pathway crosstalk and feedback regulation is broadly appreciated, kinetic data of the type needed to characterize such interactions in conjunction with mathematical models are lacking. In recent years a number of additional criteria such as robustness property have been proposed to validate the proposed mathematical models that should maintain certain important properties of biological systems. In this work we analyze the robustness property of the PI3K/Akt module using the method of perturbation to model rate constants. Simulation results suggested that the perturbation to the activation of Akt and PI3K kinases has more influence on the signal output of the module than other processes in the system.**

***Index Terms*—Cell signaling pathway, PI3K/Akt module, mathematical model, robustness analysis, perturbation.**

I. Introduction

Cell signal transduction pathways convey information from the cell exterior to nuclear targets. A canonical signalling pathway is epidermal growth factor (EGF) regulation of MAP kinase activity. This pathway was among the first to be characterized at a molecular level using a combination of genetics and biochemistry [1]. An important subsystem of this pathway is the PI3K/Akt module. Since its discovery in the 1980s, the family of lipid kinases termed PI3Ks has been found to have key regulatory roles in many cellular processes, including cell survival, proliferation and differentiation [2]. As major effectors downstream of receptor tyrosine kinases and G protein-coupled receptors, PI3Ks transduce signals from various growth factors and cytokines into intracellular messages by generating phospholipids, which activate the serine–threonine protein kinase Akt and other downstream effector pathways. Recent human cancer genomic studies have revealed that many components of the PI3K pathway are frequently targeted by germline mutations and somatic mutations in a broad range of human cancers. These findings, and the fact that PI3K and other kinases in the PI3K pathway are amenable to pharmacological intervention, make this pathway one of the most attractive targets for therapeutic intervention in cancer [3], [4].

EGF-induced MAP kinase activation is an ideal model system for mathematical modelling because of the availability of a large amount of experimental data and a relatively comprehensive understanding of the regulatory mechanisms in operation. Over the last decade, EGF signalling has been used repeatedly as a testable paradigm for pioneering computational systems biology. By focusing on Ras-dependent activation of the MAP kinase module, Huang and Ferrell developed the first mathematical model that predicted highly ultra-sensitive responses of the MAP kinase cascade, which were then confirmed by experimentation [5]. The success of this work stimulated a great deal of interests in designing kinetic models that provided testable predictions and novel insights into signaling events. For example, Bhalla et al. combined experiments and modeling to support MAP kinase involvement in a bistable feedback loop [6]; Schoeberl et al. developed the mathematical model for the EGF-regulated MAP kinase pathway [7]; we have demonstrated that the critical function of Ras nanoclusters in generating high-fidelity signal transduction [8]; and recent research works investigated the cross-talk between the MAP kinase pathway and other parallel signaling pathways [9], [10], [11]. Nevertheless, the molecular mechanisms that allow for precise yet robust control of MAP kinase signal intensity with a range of activation kinetics and diverse biological outcomes still remain poorly understood [11], [12].

Compared with the widely studied Ras-activated ERK kinase module,properties of the PI3K/Akt module are much less investigated. In this work we will study the robustness property of the PI3K/Akt module. The rest of this paper is organized as follows. Section II introduces a mathematical model of the PI3K module that is activated by growth factor receptors. Section III discusses the robustness property of four important activation processes of this module. Section IV studies the functions of kinase inhibitors in decreasing the signal output and their impact on robustness property of this important signaling module.

II. Mathematical Model

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In this study, we examined the dynamic property of the PI3K-Akt pathway that is involved in Heregulin (HRG)-stimulated ErbB4 receptor signaling. HRG-stimulated ErbB4 receptor has been known to interact with Src-homology and collagen domain protein (Shc) adaptor protein and p85 subunit of PI3K. The membrane receptor association has been observed to activate PI3K, and the activated PI3K catalyses phosphatidylinositol (PI), resulting in the release of second messengers to activate protein kinase B/Akt in response to external growth hormones in several types of cell lines [10].

In this work we discuss the following mathematical model for the PI3K/Akt pathway. This pathway is a sub-system of the proposed mathematical model in [10] that includes the MAP kinase pathway, PI3K pathway as well as the cross-talk between these two pathways. We used the same notations in the original model in this work. The dynamics of the kinase concentrations and activities is described as follows.

$$
\begin{aligned}
&d[R]/dt = -v_1 \\
&d[HRG]dt = -v_1 \\
&d[R-HRG]/dt = v_1 - 2v_2 \\
&d[R-HRG2]/dt = v_2 - v_3 + v_4 \\
&d[RP]/dt = v_3 - v_4 - v_{23} + v_{25} - v_{34} \\
&d[\text{internalization}]/dt = v_{34} \\
&d[PI3K]/dt = -v_{23} + v_{26} \\
&d[R-PI3K]/dt = v_{23} - v_{24} \\
&d[R-PI3K^*]/dt = v_{24} - v_{25} \\
&d[PI3K^*]/dt = v_{25} - v_{26} \\
&d[PI]/dt = -v_{27} + v_{28} \\
&d[PIP_3]/dt = v_{27} - v_{28} - v_{29} \\
&d[Akt]/dt = -v_{29} \\
&d[Akt-PIP_3]/dt = v_{29} - v_{30} + v_{31} \\
&d[Akt-PIP]/dt = v_{30} - v_{31} - v_{32} + v_{33} \\
&d[Akt-PIPP]/dt = v_{32} - v_{33}
\end{aligned}
\tag{1}
$$

where the reaction rates in this model are given below:

$$
\begin{aligned}
&v_1 = k_1[R][HRG] - k_{-1}[R-HRG] \\
&v_2 = k_2[R-HRG]^2 - k_{-2}[R-HGR2] \\
&v_3 = k_3[R-HRG2] - k_{-3}[RP] \\
&v_4 = V_4[RP]/(K_4 + [RP]) \\
&v_{23} = k_{23}[RP][PI3K] - k_{-23}[R-PI3K] \\
&v_{24} = k_{24}[R-PI3K] - k_{-24}[R-PI3K^*] \\
&v_{25} = k_{25}[R-PI3K^*] - k_{-25}[RP][PI3K^*] \\
&v_{26} = V_{26}[PI3K^*]/(K_{26} + [PI3K^*]) \\
&v_{27} = k_{27}[PI3K^*][PI]/(K_{27} + [PI]) \\
&v_{28} = V_{28}[PIP_3]/(K_{28} + [PIP_3]) \\
&v_{29} = k_{29}[PIP_3][Akt] - k_{-29}[Akt-PIP_3] \\
&v_{30} = V_{30}[Akt-PIP_3]/\left[K_{30}\left(1+\frac{[Akt-PIP]}{K_{32}}\right)+[Akt-PIP_3]\right] \\
&v_{31} = k_{31}[PP2A][Akt-PIP]/\left[K_{31}\left(1+\frac{[Akt-PIPP]}{K_{33}}\right)+[Akt-PIP]\right] \\
&v_{32} = V_{32}[Akt-PIP]/\left[K_{32}\left(1+\frac{[Akt-PIP_3]}{K_{30}}\right)+[Akt-PIP]\right] \\
&v_{33} = k_{33}[PP2A][Akt-PIPP]/\left[K_{33}\left(1+\frac{[Akt-PIP]}{K_{31}}\right)+[Akt-PIP]\right] \\
&v_{34} = k_{34}[RP] - k_{-34}[\text{Internalization}]
\end{aligned}
$$

The rate constants in the above reaction rates are: $k_1 = 0.001$, $k_{-1} = 0.00076$, $k_2 = 0.01$, $k_{-2} = 0.1$, $k_3 = 1.0$, $k_{-3} = 0.01$, $V_4 = 62.5$, $K_4 = 50$, $k_{23} = 0.1$, $k_{-23} = 2$, $k_{24} = 9.85$, $k_{-24} = 0.0985$, $k_{25} = 45.8$, $k_{-25} = 0.047$, $V_{26} = 2620$, $K_{26} = 3680$, $k_{27} = 16.9$, $K_{27} = 16.9$, $V_{28} = 17000$, $K_{28} = 9.02$, $k_{29} = 507$, $k_{-29} = 234$, $V_{30} = 20000$, $K_{30} = 80000$, $k_{31} = 0.107$, $K_{31} = 4.35$, $V_{32} = 20000$, $K_{32} = 80000$, $k_{33} = 0.211$, $K_{33} = 12$, $k_{34} = 0.003$, $k_{-34} = 0$.

We used this mathematical model to simulate the system dynamics. The initial condition is (Receptor ([R])=80nM, [HGR]=10nM, [PI3K]=10nM, [PI]=800nM, [Akt]=10nM). In the original model in [10], phosphatase [PP2A] deactivates both activated MEK kinase in the MAP kinase pathway and activated Akt kinase in the PI3K/Akt module, we thus assumed the concentration of PP2A is 2.85nM, which is a quarter of the concentration of PP2A in [10]. All initial conditions of other variables are zero.

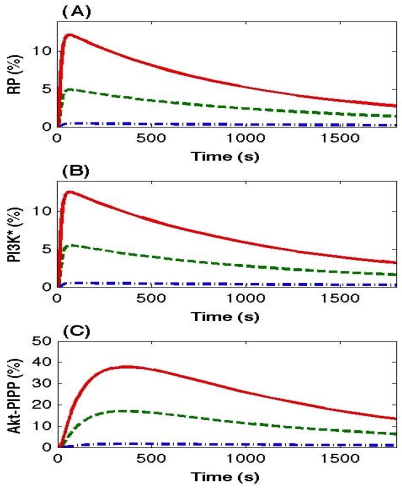

Figure 1. Simulated system dynamics of the PI3K/Akt module. (A) Related PR activity. (B) Related PI3K activity. (C) Related Akt activity. (Solid-line: HRG=10nM, dash-line: HRG=5nM, dash-dot-line: HRG=1nM).

Fig. 1 shows the simulated kinase activities based on different concentrations of HRG. Due to the process of internalization, the activity of RP was peaked at ~60s and then decreased gradually over time. Similar dynamics was also observed for the activity of kinase PI3K. However, kinase Akt shows more sustained activities. In addition, based on different input signal strengths, the activities of all kinases in the network are proportional to the signal input, namely the HRG concentrations

III. Robustness Analysis

Robustness, in both biological and engineering systems, can be defined as the ability of a system to function correctly in the presence of both internal and external uncertainty [13]. This theory has been extensively studied by Kitano and co-workers [14]. Since robustness is a ubiquitously observed property of biological systems, this property has been widely used recently as an important measure to select the optimal network structure or model rate constants from estimated candidates, including the MAP kinase pathway. A formal and abstract definition of the robustness property, given by Kitano [14], is well consistent with the general principle of the robustness property of complex systems, and has been widely used in analyzing robustness properties of biological systems. Recently more detailed definitions have been proposed to calculate the robustness property of biological systems and there is accumulated literature for studying the robustness property of various biological systems.

We used the concept defined by Kitano [14] to measure the robustness property of the proposed model. The robustness property of a mathematical model with respect to a set of perturbations P is defined as the average of an evaluation function $D^s_{a,P}$ of the system over all perturbations $p \in P$, weighted by the perturbation probabilities $prob(p)$, given by

$$R^s_{a,P} = \int_{p\in P} prob(p) D^s_{a,P} dp$$

Here we use the following measure [15] to evaluate the average behavior

$$R^M_{a,P} = \sum_{i,j} \left[\int_{p\in P} prob(p) x_{ij}(p) dp \right]$$

which is the mean of kinase activities that should be close to the simulated kinase activity obtained from the unperturbed rate constants. In addition, the impact of perturbations on nominal behaviour is defined by

$$R^N_{a,P} = \sum_{i,j} \left[\int_{p\in P} prob(p) \left(x_{ij} - x_{ij}(p) \right)^2 dp \right]$$

where $x_{ij}(p)$ and x_{ij} are the simulated activities of kinase x_i at time point t_j with perturbed and unperturbed rate constants, respectively. For each rate constant k_i, the perturbation is set to

$$\overline{k_i} = \max\{k_i(1+\mu N), 0\} \qquad (2)$$

with the standard Gaussian random variable *N(0,1)*. Here μ represents the perturbation strength.

To study the robustness property, we perturbed the value of each parameter by using the generated random number with various values of the perturbation strength μ. New simulations were obtained by using the perturbed rate constants, and we compared the new simulations with the standard simulation derived from the unperturbed model rate constants. The system is more stable regarding a particular perturbation condition if the difference between the new simulations and standard simulation is smaller. For each perturbed condition, we generated 2,000 sets of perturbed rate constants by using the standard Gaussian random variable and $\mu = 0.01 \sim 0.2$ in equation (2). To make a fair comparison, the same random numbers in the Gaussian random variable were used in each set of rate estimate.

According to the structure of the PI3K pathway, we considered four activation modules, namely the activation processes for receptors, PI3K, PI and Akt. To examine the robustness of each activation module, we perturbed all the rate constants in each module in a single test. For example, when testing the robustness property of the receptor activation module, we perturbed the values of parameters $k_1, k_{-1}, k_2, k_{-2}, k_3, k_{-3}$ and kept the rate constants in all other modules unchanged. Here we are interested in the perturbation of the activities of kinase PI3K and in particular Akt. Except for the Akt activation module, the perturbation of each module will not influence the activation of the up-stream activation modules.

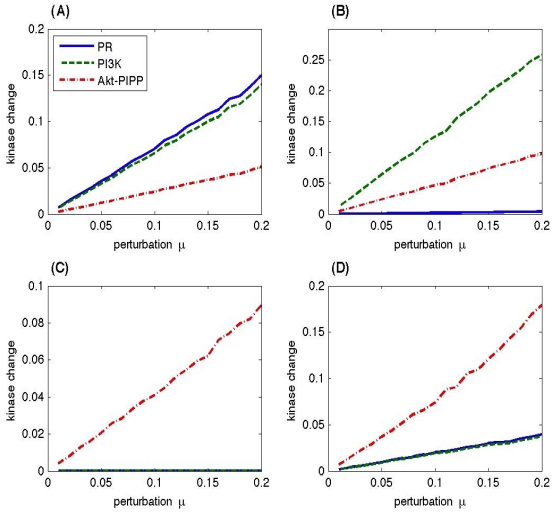

Figure 2. Robustness analysis of the PI3K/Akt module. The averaged changes of kinase activities when perturbing all kinetic rates related to activation of receptor (A), PI3K activation (B), PI activation (C), and Akt activation (D) (Solid-line: receptor activity, dash-line: PI3K activity, dash-dot-line: Akt activity).

In Fig. 2A, the perturbation to the receptor activation has changed the activities of receptor, PI3K and Akt. However, when the perturbation was made to the PI3K activation, the activity of receptors was barely changed. Similar observation was found in Fig. 2C where only the activity of Akt was changed when the PI module was perturbed. However, the perturbation to the Akt activation module will influence the activity of receptor and PI3K. When comparing the variation of Akt activity in these four figures, we found that the perturbation to the Akt module caused the largest change in the Akt activity.

The second important module to Akt activation is the PI3K activation module. However, the variation in the receptor activation module has the least impact of the Akt activation module.

IV. Effect of Kinase Inhibitor

Kinase PI3K activates an important cell survival signaling pathway, and constitutive activation is seen in ovarian, head and neck, urinary tract, cervical and small cell lung cancer. Targeting the PI3K Pathway offers opportunities to inhibit a major cell survival signaling pathway for therapeutic developments, in a large number of human diseases, such as cancer, metabolic diseases and inflammation, to name a few. In recent year a variety of PI3K inhibitors have been tested in pre-clinical studies and several have now entered clinical trials [3], [4].

Next we simulate the effect of the kinase inhibitors and study the robustness property of the inhibitor functions. In this work we tested two important inhibitors, namely Wortmannin and PP2A, for the two important kinases PI3K and Akt, respectively. Wortmannin is a well-known and first-generation PI3K inhibitor. It is a natural product isolated from *Penicillium wortmannin* that binds irreversibly to PI3K enzymes by covalent modification of a lysine residue that is necessary for catalytic activity [3]. In this work the function of Wortmannin was realized by reduced kinase binding rate constant and phosphorylation rate, given by

$$k_9 = k_{9,0}\frac{1}{1+[Wort]}, \qquad k_{11} = k_{11,0}\frac{1}{1+[Wort]}, \tag{3}$$

where [Wort] is the concentration of Wortmannin, $k_{9,0}$ and $k_{11,0}$ are rate constant when the amount of Wortmannin is zero in the system.

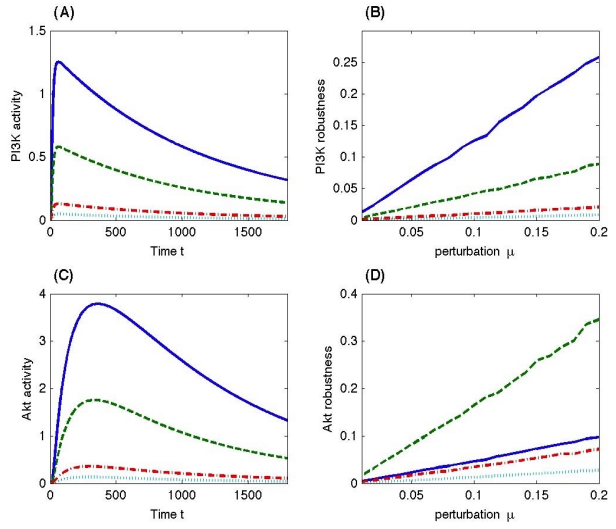

Figure 3. System dynamics and robustness property based on different concentrations of Wortmannin. (A) Activities of kinase PI3K. (B) Robustness property of kinase PI3K. (C) Activities of kinase Akt. (D) Robustness property of kinase Akt. (Solid line: [Wort]=0; dash-line: [Wort]=1nM; dash-dot line: [Wort]=5nM; dot-line: [Wort]=10nM).

Fig. 3 gives simulated system dynamics of kinase PI3K and Akt activities as well as the robustness property of the system when different amount of Wortmannin is added into the system. The kinase activities in Fig. 3A and 3C are reduced proportionally when more amount of Wortmannin was added into the system. Regarding the robustness property of kinase PI3K, it shows in Fig. 3B that the averaged perturbation of kinase activity is proportional to the corresponding kinase activity. However, the robustness property of kinase Akt in Figure 3D is different from that of other kinases in the system. When the amount of Wortmannin is in the range of ~1nM, the perturbation of rate constants to the Akt activation module has more influence than that when the system has larger signal output because of no amount of wortmannin in the system. This observation is consistent with that in Fig. 2D showing that the robustness of the Akt activation module has profound influence on the system dynamics.

Kinase Akt1 is involved in cellular survival pathways, by inhibiting apoptotic processes. Akt1 is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. Since it can block apoptosis, and thereby promote cell survival, Akt1 has been implicated as a major factor in many types of cancer [3]. In this work we tested the function of overexpressed PP2A that was realized by a large amount of phosphatase PP2A in the system. Since the concentration of PP2A is an initial condition of the mathematical model, no rate constant in the model was changed.

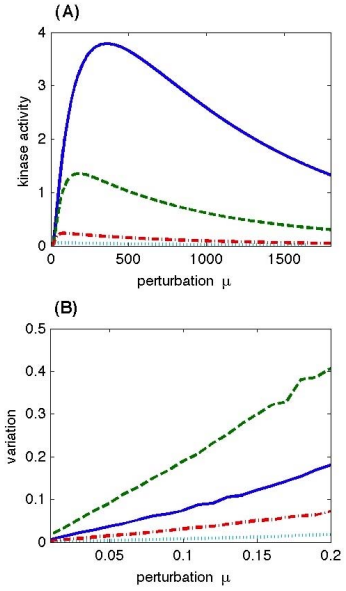

Figure 4. Signal output of the PI3K/Akt module regulated by different concentrations of phosphatase PP2A. (A) Simulated Akt activity. (B) Robustness property of signal output (Akt). (Solid-line: [PP2A]=2.85nM, dash-line: [PP2A]=5.7nM, dash-dot-line: [PP2A]=14.25nM, dash-line: [PP2A]=28.5nM).

Fig. 4A gives the simulated kinase Akt activities when using different concentrations of phosphatase PP2A. When the amount of PP2A is larger, it is reasonable to expect that the corresponding signal output in terms of Akt activities is smaller. However, when we test the robustness property of the PI3K/Akt module with different concentrations of PP2A, Fig. 4B suggested that slightly increased PP2A concentrations has much more influence on the robustness property of the PI3K/Akt

module. Again, this interesting observation is consistent with the robustness property of kinase Akt showing in Fig. 2 and 3.

V. Discussion

In this work we studied the robustness property of the PI3K/Akt module that was activated by the external cell signal. We perturbed the model parameters using the Gaussian random variables. In particular, we tested the influence of different modules in the network, namely the receptor activation module, PI3K activation module, PI activation module, and Akt activation module. Except for the Akt activation module, all the perturbation in one module will influence the activity of itself and down-stream kinase. In addition, we tested the influence of inhibitor on the kinase activity and also system robustness property. This analysis provided important information for the robustness of the PI3K/Akt module that is a key subsystem of the growth factor activated MAP kinase pathway.

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