On Modeling and Analyzing Signaling Pathways with Inhibitory Interactions Based on Petri Net

Chen Li[1] Shunichi Suzuki[1] Qi-Wei Ge[2] Mitsuru Nakata[2]
Hiroshi Matsuno[3] Satoru Miyano[4]

[1] *Graduate School of Science and Engineering,* [2] *Faculty of Education,* [3] *Faculty of Science, Yamaguchi University, Japan*

[4] *Human Genome Center, Institute of Medical Science, University of Tokyo, Japan*

Email: {li, toshikazu}@ib.sci.yamaguchi-u.ac.jp, {gqw, nakata}@inf.edu.yamaguchi-u.ac.jp, matsuno@sci.yamaguchi-u.ac.jp, miyano@ims.u-tokyo.ac.jp

ABSTRACT: In this paper we discuss the formulation and the analysis of a signaling pathway by Petri nets. In order to explicitly and formally describe the molecular mechanisms and pathological characteristics of signaling pathways, we propose a new modeling method to construct signaling pathways on the basis of formal representation of Petri net. Our proposed extended algorithm effectively finds basic enzymic components of signaling pathways by employing T-invariants of Petri nets with considering the origination leading to an occurrence of inhibition functions than existing methods. An application of the proposed algorithm is given with the example of Interleukin-1 and Interleukin-6 signaling pathways.

1 INTRODUCTION

Signaling pathways have been widely studied in cell biology. They are information cascades of enzyme reactions from transmembrane receptors to the nucleus DNA, which ultimately regulate intracellular responses such as programmed cellular proliferation, gene expression, differentiation, secretion and apoptosis. Up to now, the formulation and analysis of biological networks have been investigated from quantitative and qualitative aspects [1, 2, 3, 4] by using various types of Petri nets such as stochastic Petri nets [5, 6], hybrid Petri nets [7, 8] and coloured Petri nets [9]. By using qualitative method, the researchers could gain immense important insights into the behaviors of the models, even in the absence of quantitative data, at a relatively low cost in terms of effort and high degree computational time. The analysis of even large scale and complex networks can be handled with the same set of simple structural and behavioral properties defined by Petri nets.

Today much research of modeling and analyzing metabolic pathways in qualitative term has been developed from the first paper by Reddy *et al.* [2] in 1993. In the meantime, since the signaling pathways are actually extremely complex as we know, a bit of investigation of relationships among complex molecular mechanisms and interactions in signaling pathways have been provided. Heiner *et al.* [3] have proposed a method for developing and analyzing models of biological pathways in systematic manner by calculating the T-invariants to obtain all paths in signaling pathways. However, their method is not sufficient to discuss the general systematic behavior, since they did not take the effect of enzymes into account. Therefore, we have proposed a new method of formulating and analyzing a signaling pathway with a focus on enzymes by applying Petri nets, which provide all the chains consisting of enzymic reaction elements in signaling pathways [10].

Inhibition functions are essential for biological systems, and there are numerous recent reports on signaling pathways in which inhibition functions play important roles [11]. Currently it is still hard to model and analyze signaling pathways including inhibition functions with the existing modeling methods. Therefore, in this paper we are to improve our proposed method to handle inhibition functions in signaling pathways. Our proposed method would give a fresh insight into medical treatment and support any drug therapies.

2 BASIC STRUCTURAL PATTERNS

Petri nets are powerful tools in modeling various concurrent systems and the theories have been widely applied to inquiring systems' behaviors [12]. In this section, we give the necessary definitions used in this paper as follows:

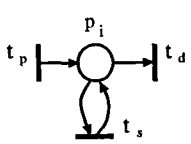

Figure 1: An enzyme place in Petri net model.

[Definition 1] A Petri net is denoted as $PN=(T,P,E,\alpha,\beta)$ which is a bipartite graph [12], where $E=E^{+}\cup E^{-}$ and

T: a set of transitions $\{t_1,t_2,\cdots,t_{|T|}\}$

P: a set of place $\{p_1,p_2,\cdots,p_{|P|}\}$

E^{+}: a set of edges from transitions to places $e=(t,p)$

E^{-}: a set of edges from places to transitions $e=(p,t)$

α: $\alpha(e)$ is the weight of edge $e=(p,t)$

β: $\beta(e)$ is the weight of edge $e=(t,p)$. □

[Definition 2]

(1) $^{\circ}t$ (or t°) is a set of the input (or output) places of t and called the *pre-set* (or *post-set*) of transition t.

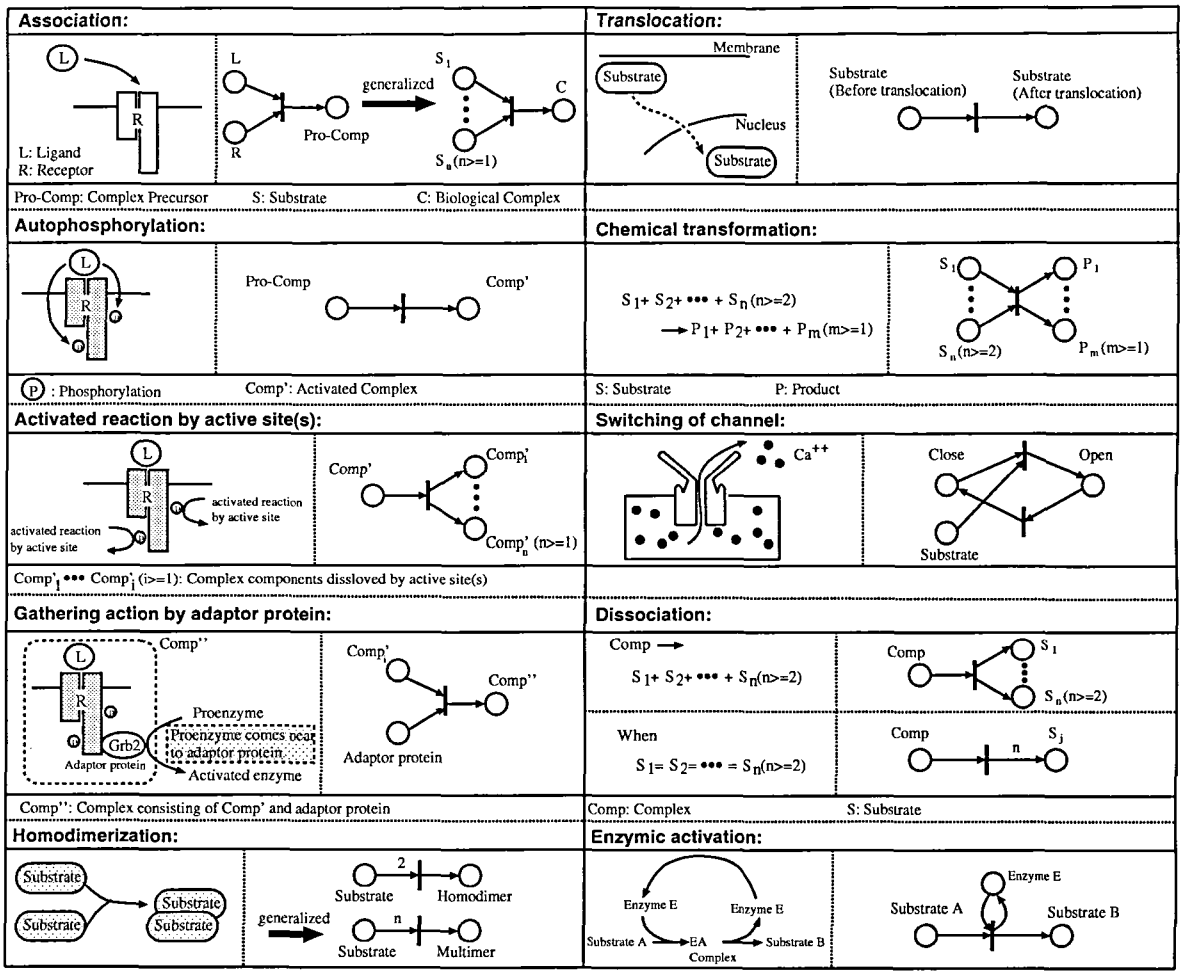

Figure 2: Petri net models of various reaction types in signaling pathways.

(2) The structure of a Petri net PN can be represented by a matrix, called *place–transition incidence matrix* (*incidence matrix* for short) $C=C^+-C^-$, where:

$$C^+(i,j)=\begin{cases}\beta_e & \text{if } e=(t_j,p_i)\in E^+\\ 0 & \text{otherwise}\end{cases}$$

$$C^-(i,j)=\begin{cases}\alpha_e & \text{if } e=(p_i,t_j)\in E^-\\ 0 & \text{otherwise}\end{cases}$$

(3) Token distribution to places is called *marking* and expressed by $M=(m_1,m_2,\cdots,m_{|P|})^t$, where, m_i is the number of tokens at p_i.

(4) A transition sequence $\sigma=t_1t_2\cdots t_k$ is called *firing sequence* from M_I to M_F, if the firing simulation of σ on M_I can be carried out all the way to the last element of σ, which leads to the marking M_F. The marking transition is expressed by $M_I[\sigma>M_F$ and the firing numbers of all the transitions are expressed by a firing count vector $J=(j_1,j_2,\cdots,j_{|T|})^t$. The relationship among C, J, M_I and M_F can be expressed by $M_F=M_I+CJ$. □

[Definition 3]

(1) A non-negative integer vector J satisfying $CJ=0$ is called *T-invariant* and the set of transitions $T_J=\{t_i\in T|j_i\neq 0\}$ is called the *support* of J.

(2) For a T-invariant J with the support T_J, if there exists no such T-invariant J' whose support $T_{J'}$ satisfies $T_{J'}\subset T_J$, then T_J is called *minimum support*. Further for a T-invariant J with minimum support T_J, if all the values $\{j_i|t_i\in T_J\}$ have no common divisor then J is called *elementary T-invariant*.

(3) A subnet N is called "generated by a set of transition T_J" if N is such a subnet that N is composed of all the transitions t included in T_J and all the places included in the pre-set and post-set of any $t\in T_J$.

(4) An inhibitor arc which is depicted as a line with a hollow circle at the end where the arrowhead normally appears, represents the function of inhibition. An inhibitor arc disables a transition to fire if the upstream place is occupied by a token, but does not consume the token. □

We give a new modeling method for formulating signaling pathways to Petri net models that can be naturally modeled according to the following rules:

(1) Places denote static elements including chemical compounds, conditions, states and substance participating in the biological systems. Tokens indicate the presence of these elements.

(2) Transitions denote active elements including chemical

reactions, events, actions, conversions and catalyzed reactions. Arc weight is omitted when its weight is 1.

(3) Since an enzyme itself plays a role of catalyzer in biological system and there occurs no consumption in biochemical reactions, an enzyme is modeled by an enzyme place as to be defined in **Definition 4** in the following.

(4) An inhibition function existing in biological system is modeled by an inhibition arc directly affecting transition sequence σ.

In this paper, an enzyme place is defined as follows:

[Definition 4]

(i) An enzyme in biological system is modeled by a place (called an *enzyme place* hereafter) with such a self-loop (refer to Fig.1) that once an enzyme place is occupied by a token, the token will return to the same place again, keeping the firable state.

(ii) Let p_i is an enzyme place, t_s denotes a transition in a self-loop of p_i, t_d denotes a sink output transition of p_i implying an extremely small natural degradation in biological system. The sets of p_i, t_s and t_d in PN are denoted by P_e, T_s and T_d, respectively. Here we give a formal definition of the set P_e;

$P_e=\{p_i|\exists t,t_d,s.t.\ p_i\in{}^\circ t\cap t^\circ, t_d^\circ=\phi, p_i={}^\circ t_d,\ |e(t_p,p_i)|=1,$
$p_i={}^\circ t_s=t_s^\circ,\ |t_s|=1, t\in T, t_p\in T_p, t_d\in T_d, \alpha(p_i,t_d)\ll 1\}$. □

Numerous types of molecular interactions can be clearly described by Petri net model [13], which suffices to give the description of the metabolic pathway presently [2]. For signaling pathways, as is pointed out in [14], the additional information among the molecular interactions also should be extraordinarily distinguished according to different types of interactions. As to explicitly understand the structural complicated signaling pathways, the formulation of each essential molecular interaction in a signaling pathway by using Petri net is the first step in modeling the network of signaling pathways as a qualitative event system. The molecular mechanisms and interactions existing in signaling pathways (left side of dot-line) and corresponding Petri net graph (right side of dot-line) are shown in Fig.2. From all the models illustrated in Fig.2, we extracted just four basic structural patterns with which any reaction types existing in signaling pathways can be represented (see Fig.3). On the basis of this interpretation, it provides us a basic platform to describe and analyze the properties and behaviors of signaling pathways.

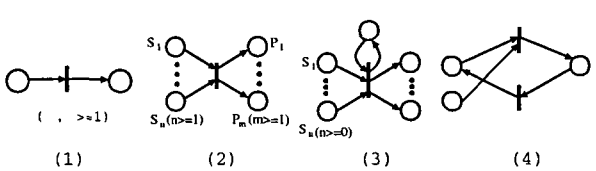

Figure 3: Four basic structural patterns extracted from Fig.2 for modeling signaling pathways.

In the next section, we give an extended general algorithm including inhibitory interactions, and analyze the behavioral properties of the Petri net model to obtain functional qualities of the biological system.

3 MODELING SIGNALING PATHWAYS

3.1 Enzymic reaction and T-invariant

Signaling pathway is the chain of intracellular signaling events which starts by attaching ligands at receptors and ends by altering target proteins, which are responsible for modifying the behaviors of a cell. These signaling events are mediated by intracellular signaling proteins (enzymes for short) that relay the signal into the cell by activating the next enzyme from inactive state to active state on receipt of signal in the chain. Many of the enzymes controlled by reactions such as phosphorylation are themselves enzymes. In the *enzymic cascades*, an enzyme activated by phosphorylation phosphorylates the next enzyme in sequence, that is to say, an *enzymic reaction* is sequentially connected by an enzyme activation reaction (called *an enzymic reaction element* hereafter). Therefore, for this fundamental mechanism of signaling pathways, the understanding of the behaviors of each enzyme seems extremely important.

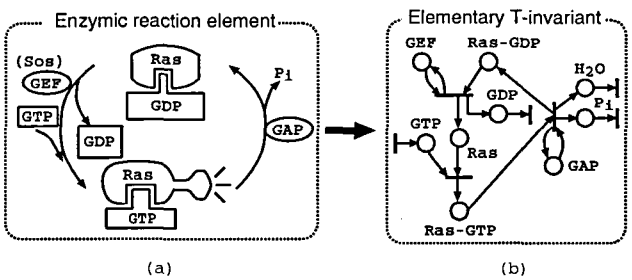

Figure 4: The association between enzymic reaction elements and elementary T-invariants mapping between the models and the pathways with the example. (a) The mechanism of the regulation of Ras activity. (b) The Petri net model of (a).

After modeling an enzymic reaction element according to the method of representation explained above, it is easy to interpret that an elementary T-invariant is exactly equivalent to an enzymic reaction element mapping between the Petri net models and the pathways (see Fig.4). The token's presence of the precedent enzyme place is importantly associated with the firing possibility of successive enzyme place. In every elementary T-invariant the precedent enzyme place acts as controllers, reflecting the properties of enzymic reactions transmitting the signals from the precedent steps to the next one of signaling pathways. That is, elementary T-invariants will give more precise understanding of structural properties of signaling pathways.

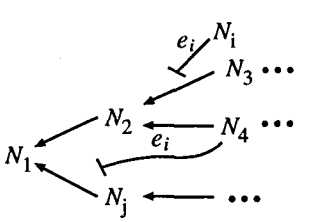

Figure 5: The chains obtained by applying proposed new extended algorithm with highlighting inhibition functions.

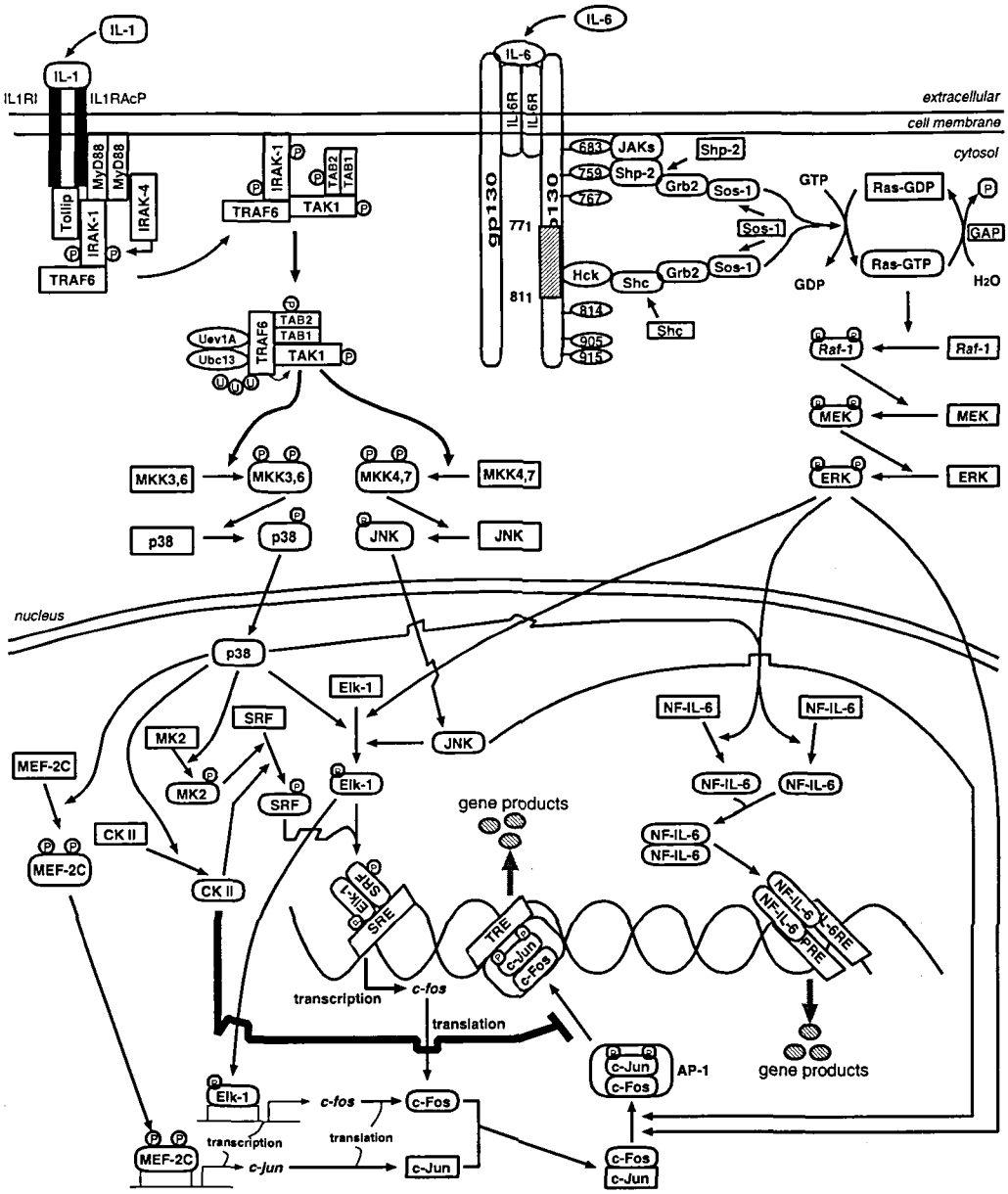

Figure 6: Petri net models of various reaction types in signaling pathways.

3.2 New extended algorithm

Since elementary T-invariants express minimum periodic behaviors of Petri nets, an enzymic reaction element of signaling pathways can be expressed by a subnet corresponding to an elementary T-invariant. A chain consisting of enzymic reaction elements can be identified by finding a series of subnets from sink transition(s) as our consideration [10]. Still, there is a problem that we have simply deleted inhibitor arcs to omit the inhibition functions due to the difficulty of dealing with enzymic reactions whose modeled nets include inhibitor arcs. Therefore, we expand the proposed algorithm in order to handle the inhibition functions and give a way of clarifying the interactions of enzymic reaction elements in signaling pathways.

In the following, we are to give only the outline of our new extended algorithm due to the space limitation of this paper:

step 1° To delete each inhibitor arc e_i from Petri net model and add one output transition t_i to the place from which the inhibitor arc was connected.

step 2° To rewrite the incidence matrix C for the transformed model obtained in step 1°.

step 3° To apply the previous proposed algorithm [10] to get elementary T-invariants and chains of the subnets N_j (j=1,2,$\cdots$), which correspond to the calculated elementary T-invariants, in the form of "$N_1 \leftarrow N_2 \leftarrow N_3 \leftarrow \cdots$", "$N_1 \leftarrow N_j \leftarrow \cdots$" and so on as shown in Fig.5.

step 4° To calculate the elementary T-invariant J_i to get the inhibition function subnet N_i by solving linear equation: CJ_i=0 under the condition of $J_i(t_i)>0$.

step 5° To add the inhibition function subnet N_i to the chains obtained in step 3° by connecting subnet N_i to the corresponding transition whose firing status can be restrained by inhibitor arc e_i as shown in Fig.5.

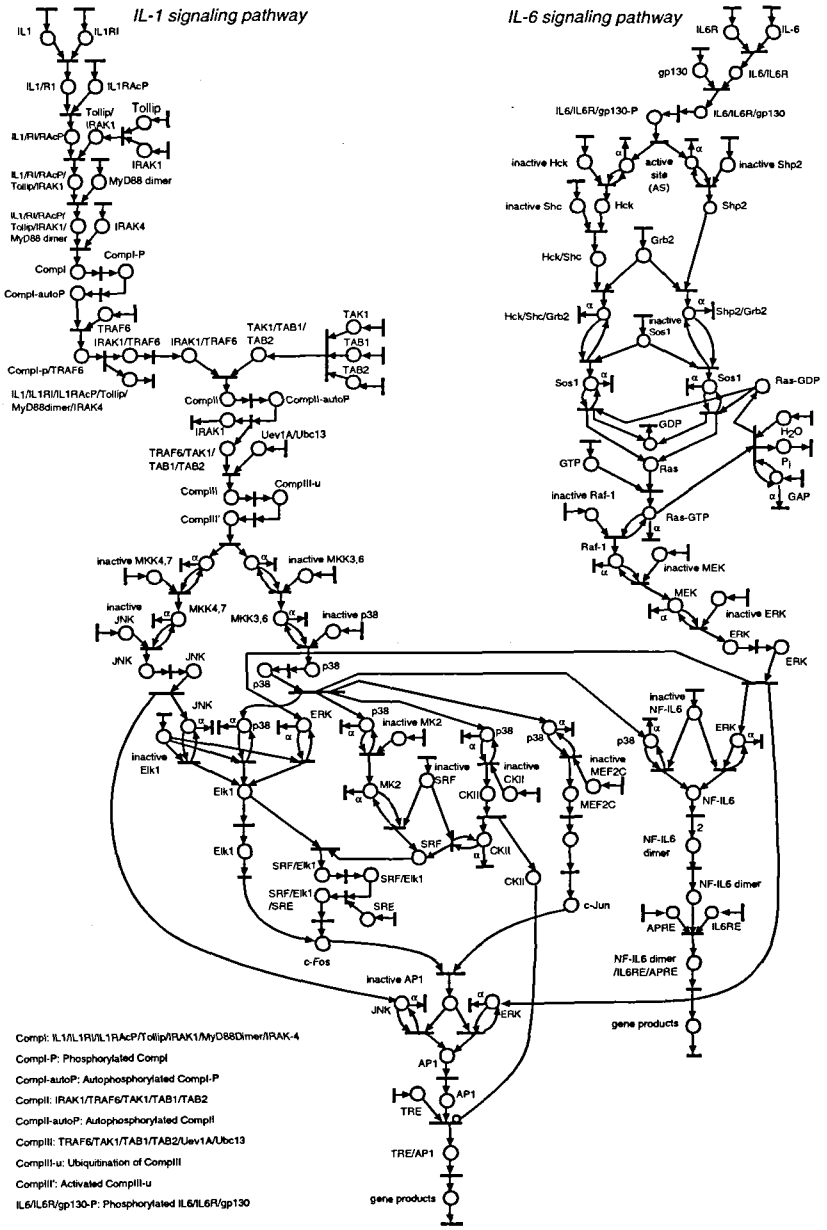

Figure 7: Petri net models of various reaction types in signaling pathways.

In this way, all the enzymic reaction element chains existing in signaling pathways can be naturally found out easily on account of the chains based on a series of subnets by calculating all the elementary T-invariants [15] in Petri net models while emphasizing the properties of each enzymic reaction element with considering the origination leading to an occurrence of inhibition functions.

3.3 An application to IL-1 and IL-6 pathways

This subsection gives an example possessing cross-talks with suppressive effect between Interleukin-1 and Interleukin-6 (IL-1 and IL-6 for short) signaling pathways for demonstrating the effectiveness of our improved method.

(1) IL-1 is a cytokine that primarily regulate inflammatory and immune responses. Via its type I receptor it activates specific protein kinases, including the NFκB inducing kinase (NIK) and the mitogen-activated protein (MAP) kinase cascade. These modulate a number of transcription factors including NFκB, SRF and AP-1 that respond to different signals in this pathways and regulate a variety of complex promoters and enhancers to the inflammatory response [16, 17, 18].

(2) IL-6 is also a cytokine that provokes a broad range of cellular and physiological responses playing a role in inflammation and hematopoiesis. An extracellular ligand activates IL-6 signaling pathway by binding to a receptor composed of an α subunit and gp130 shared in common with other cytokines in the IL-6 family, and results in cellular events including activation of JAK-STAT pathway and activation of Ras-MAPK pathway [19].

There exist extremely complicated mechanisms of cross-

talks in the example signaling pathway of IL-1 and IL-6 as shown in Fig.6. The upstream signal of either p38 from IL-1 pathway or ERK from IL-6 pathway activates transcription factors Elk-1 and NF-IL-6 that can act through its own genome in the DNA [20, 21, 22]. There is only one inhibitory interaction in example pathway that CKII activated by MAP kinase cascade of IL-1 pathway can inhibit the activity of transcription factor AP-1 through phosphorylation of c-Jun which is a component of AP-1, as well as activate the SRF stated in (1) [23, 24].

We remodel the signaling pathway illustrated in Fig.6 to Petri net model by our proposed new modeling method based on the formal representation rules in section 2 (see Fig.7). By using our proposed algorithm in section 3.2, we can get all subnet chains in the formation like Fig.5 based on calculating elementary T-invariants from sink transitions as well as finding the the pathway leading to an occurrence of inhibition functions (the inhibition function to the activation function of AP-1 by CKII in this example). Finally, we can check and rewrite the signaling pathway of biological system against these subnet chains in Petri net model. Details are omitted since the limitation of space.

Acknowledgments

This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research on Priority Areas, 17014067 and 17017007, 2005.

REFERENCES

[1] V.N. Reddy, M.N. Liebman, and M.L. Mavrovouniotis. Qualitative analysis of biochemical reaction systems. Comput. Biol. Med, 26(1):9–24, 1996.

[2] V.N. Reddy, M.L. Mavrovouniotis, and M.N. Liebman. Petri net representations in metabolic pathways. In Proc. Int. Conf. Intell. Syst. Mol. Biol. 1, 328–336, 1993.

[3] M. Heiner, I., Koch, and J., Will. Model validation of biological pathways using Petri nets, demonstrated for apoptosis. Biosystems, 75(1–3):15–28, 2004.

[4] L. Popova-Zeugmann and M. Heiner. Worst-case analysis of concurrent systems with duration interval Petri nets. Entwurf komplexer Automatisierungssysteme, pages 162–179, 1997.

[5] Y. Narahari, K. Suryanarayanan, and N.V.S. Reddy. Discrete event simulation of distributed systems using stochastic Petri nets. Energy, Electronics, Computers, Communications, pages 622–625, 1989.

[6] J. Peccoud. Stochastic Petri nets for genetic networks. Medicine Sciences, 14(8–9):991–993, 1998.

[7] H. Matsuno, S. Fujita, A. Doi, M. Nagasaki, and S. Miyano. Towards biopathway modeling and simulation. In Proc. 24th ICATPN, Lecture Notes in Computer Science, 2679:3–22, 2003.

[8] H. Matsuno, Y. Tanaka, H. Aoshima, A. Doi, M. Matsui, and S. Miyano. Biopathways representation and simulation on hybrid functional Petri net. In Silico Biol., 3(3):389–404, 2003.

[9] H. Genrich, R. Küffner, and K. Voss. Executable Petri net models for the analysis of metabolic pathways. International Journal on Software Tools for Technology Transfer, 3(4):394–404, 2001.

[10] C. Li, S. Suzuki, Q.W. Ge, M. Nakata, H. Matsuno, and S. Miyano. A new proposal of Petri net based formulation for analyzing signaling pathways. Technical report of IEICE,104(593):1–6, 2005.

[11] M. Garcia-Calvo, E.P. Peterson, B.Leiting, R.Ruel, D.W. Nicholson, N.A. Thornberry. Inhibition of human caspases by peptide-based and macromolecular inhibitors. J Biol Chem, 273(49):32608–32613, 1998.

[12] J. Peterson. Petri net theory and the modeling of systems. Englewood Cliffs, NJ, Prectice-Hall, 1981.

[13] R. Hofestädt. A Petri net application to model metabolic processes. Syst. Anal. Mod. Simul., 16:113–122, 1994.

[14] T. Takai-Igarashi and R. Mizoguchi. Cell signaling networks ontology. In Silico Biol., 4(1):81–87, 2004.

[15] T. Fukunaga, Q.W. Ge and M. Nakata. On obtaining all the elementary T-invariants using linear programming. Technical report of IEICE, 104(402):59–64, 2004.

[16] A.K. Roshak, J.F. Callahan and S.M. Blake. Small-molecule inhibitors of NF-kappaB for the treatment of inflammatory joint disease. Curr Opin Pharmacol., 2(3):316–321, 2002.

[17] E. Stylianou, and J. Saklatvala. Molecules in focus interleukin-1. The International Journal of Biochemistry & Cell Biology, 30:1075–1079, 1998.

[18] S. Janssens and R. Beyaert. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. Molecular Cell, 11:293–302, 2003.

[19] P. C. Heinrich, I. Behrmann, S. Haan, H. M. Hermanns, G. Müller-newen and F. Schaper. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem. J., 374:1–20, 2003.

[20] Y. Shi and M. Gaestel. In the cellular garden of forking paths: how p38 MAPKs signal for downstream assistance. Biol. Chem., 383:1519–1536, 2002.

[21] E. Herlaar and Z. Brown. p38 MAPK signalling cascades in inflammatory disease. Molecular Medicine Today, 5(10):439–447, 1999.

[22] A.B. Carter, M.M. Monick and G. W. Hunninghake. Both erk and p38 kinases are necessary for cytokine gene transcription. Cell Mol. Biol., 20:751–758, 1999.

[23] J. A. McElhinny, S.A. Trushin, G.D. Bren, N. Chester and C.V. Paya. Casein kinase II phosphorylates IκBα at S-283, S-289, S-293 and T-291 and is required for its degradation. Molecular and cellular biology, 16:899–906, 1996.

[24] A. Lin, J. Frost, T. Deng, T. Smeal, N. AL-Alawi, U. Kikkawa, T. Hunter, D. Brenner and M. Karin. Casein kinase II is a negative regulator of c-Jun DNA binding and AP-1 activity. Cell, 70(5):777-789, 1992.