페트리 네트를 이용한 신호전달체계의 표현방법 및 모사

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Petri Net Representation and Simulation of Signal Transduction Network

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Introduction

Our knowledge of cell signal transduction has advanced over the last two decades, via the identification of increasingly larger numbers of cell signaling components such as growth factors, cytokines, and their receptors. Moreover, the information on the interaction of this molecules, i.e., molecular interaction or biochemical reaction, has become more and more available. Nevertheless, much effort should be done to understand the cell signaling behavior qualitatively but also quantitatively. In this study, for both purposes - qualitative and quantitative modeling of signaling networks - a conceptual framework for the representation and simulation of large signal transduction networks based on Petri nets will be presented, followed by a case study application to a signaling system induced by Interleukin-1 (IL-1) and Tumor necrosis factor (TNF) known as potent proinflammatory cytokines.

Methodology

Framework for the reconstruction of signal transduction networks

Figure 1 describes the overall procedure for the reconstruction of signal transduction networks.

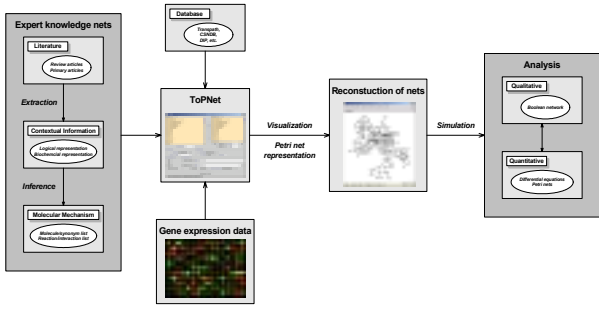

Figure 1. Framework for the reconstruction of signal transduction network

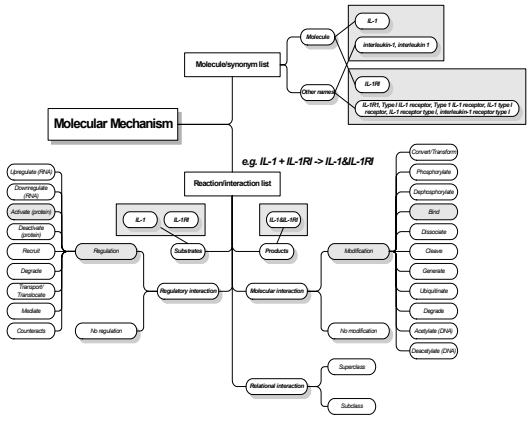

Figure 2. Data format of molecular mechanism stored in Expert knowledge nets

As shown in Figure 1, to construct *Expert knowledge nets*, first the contextual information on the system of interest is extracted from a large number of review and primary articles, which consists of *logical* and *biochemical* representation (Rzhetsky *et al.*, 2000). Then, molecular mechanisms are inferred by converting of the contextual information extracted into the data format in Figure 2. For the clear representation of molecular mechanisms, we make use of Petri net models, which are also required for the subsequent quantitative analysis in the simulation study. In this study, *ToPNet* (Toolbox for Protein Networks) program developed in FhG-SCAI/LMU München (Daniel Hanisch, personal communication/unpublished) is employed to reconstruct

and visualize the network of interest. In the analysis part, both an executable Petri net model and a mathematical modeling approach are adopted to investigate the dynamic behavior of signal transduction pathway.

Modeling and simulation of signal transduction system

Signal transduction pathways mainly consist of two kinds of reaction type (Bhalla and Iyengar, 1999). One is an elementary reaction step such as chemical transformation, association and dissociation. General chemical reactions can be expressed as follows:

$$\sum_{i=1}^{m} a_i R_i \xleftrightarrow{k_f, k_b} \sum_{j=1}^{n} b_j P_j \tag{1}$$

where a_i and b_j are the stoichiometric coefficients of the ith reactant and jth product, respectively, R_i and P_j are the ith reactant and jth product, respectively, and k_f and k_b are the forward and backward rate constants, respectively. Then, the reaction rate directly depends on the concentrations of reactants and products and can be presented as follows:

$$v = \frac{d[R_i]}{a_i dt} = -\frac{d[P_j]}{b_j dt} = k_b \prod_{j=1}^{n} [P_j]^{b_j} - k_f \prod_{i=1}^{m} [R_i]^{a_i} \tag{2}$$

where $[R_i]$ and $[P_j]$ are the concentrations of ith reactant and jth product, respectively.

The other type is an enzymatic reaction represented by the combination of reversible association step and irreversible dissociation step with one substrate and one product (uni-uni mechanism), as follows:

$$E + S \xleftrightarrow{k_1, k_2} ES \xrightarrow{k_3} E + P \tag{3}$$

where k_1 and k_2 are the association and dissociation rate constants, respectively, and k_3 is the rate constant for the formation of a product (P) from an enzyme-reactant (ES) complex. First, a substrate (S) diffuses towards an enzyme (E) and binds to its active site, forming the enzyme-reactant (ES) complex. Then, the ES complex transforms into the enzyme (E) and the product (P) for the next reaction.

In this study, a high-level Petri net model (or executable Petri net model) is also constructed for the dynamic simulation of the cell signaling system as done for the analysis of metabolic pathways (Genrich *et al.*, 2001). For the implementation of the Petri net model, the software package Design/CPN (http://www.daimi.au.dk/Cpnets/) is used. In the Design/CPN environment transitions denote reactions and every substrate is represented as a place connected through an outgoing and an ingoing arc to the transitions. For the calculation of the reaction rate, we can define kinetic functions, in "code regions" of transitions. Then, the concentration of each substrate is updated to new value whenever the reaction transition specified by the functions is fired according to its occurrence. In addition, primitive I/O commands of Standard ML (called CPN ML) language supported in Design/CPN are used to store simulation results.

Based on the above described continuous, mass-action differential equations and the Petri net representation, we can constitute the mathematical model and the executable Petri net model.

Application

We apply the methods sketched above for the reconstruction and analysis of signal transduction networks to the signaling system induced by Interleukin-1 (IL-1) and Tumor necrosis factor (TNF). IL-1 and TNF-α are potent proinflammatory cytokines that play a critical role in immunity and inflammation, and in the control of cell proliferation, differentiation and apoptosis. Especially, they are important in the pathogenesis of various inflammatory and autoimmune diseases, including rheumatoid arthritis, inflammatory bowel diseases, and asthma. However, little is known about inflammatory pathomechanisms associated with those diseases although our understanding has increased dramatically. Nearly every known signal transduction pathway may be activated by these two proinflammatory cytokines (Bian *et al.*, 2001). Thus, understanding their mechanisms and dynamics through the reconstruction of the signal transduction network will provide useful information to find new drug targets.

Reconstruction of a signal transduction network

Following the procedure for the reconstruction of signal transduction networks, current information on the molecular mechanism and interaction of IL-1β and TNF-α signaling systems is extracted from scientific and

review articles through literature survey, and then detailed molecular mechanism are inferred from the extracted information. As a result, the likely and possible pathways associated with IL-1β, TNF-α, and EGF signal transduction and many cross-talks between them were investigated as shown in Figures 3 and 4.

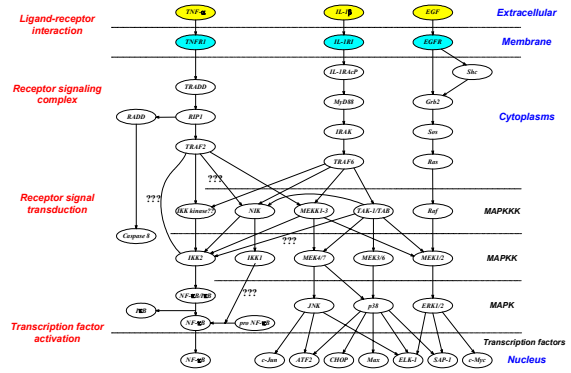

Figure 3. IL-1β and TNF-α signaling pathways

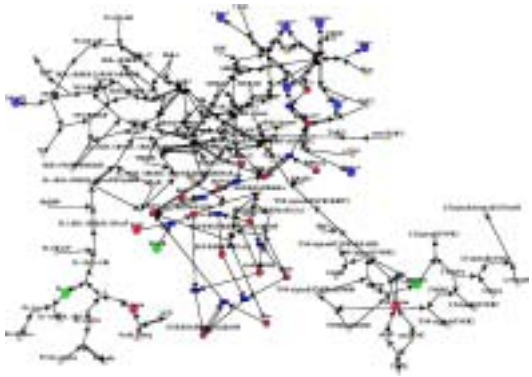

Figure 4. Petri net representation of IL-1β and TNF-α signaling pathways

The signal transduction systems of EGF and the two cytokines, IL-1 and TNF-α consist of four levels, including the ligand-receptor interaction, the formation of receptor signaling complex, signal transduction by receptor signaling complex and transcription factor activation (Figure 3). IL-1 signaling is initiated by the formation of a complex including IL-1, IL-1RI, and IL-1RAcP. Formation of this complex causes the intracellular adaptor molecule MyD88 to be recruited to the complex, which in turn facilitates the association of IRAK. Next, IRAK interacts with TRAF6, which leads to IL-1-induced JNK and NF-κB activation (Auron, 1998). As with the similar way in IL-1 signaling, TNF-α signaling is initiated by the formation of a complex composed of TNF-α and TNFR1. Then, TRADD is recruited into the receptor signaling complex. Next, TRADD recruits RIP1 and TRAF2, which triggers a cascade of signaling events, including activation of JNK and NF-κB (Baud and Karin, 2001). Using the ToPNet, signal transduction network with all the molecules and reactions involved in these three signaling pathways can be constructed and visualized as a Petri net representation as shown in Figure 4.

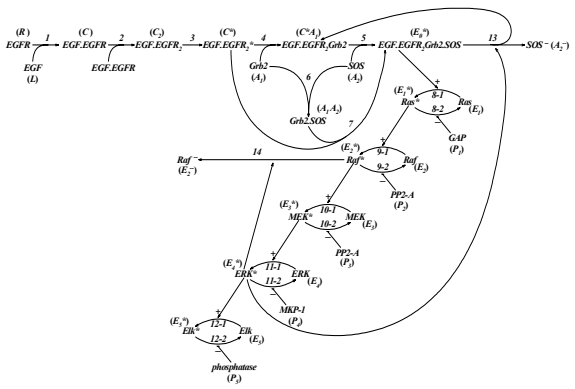

Figure 5. Schematic view of the signal transduction system induced by EGF

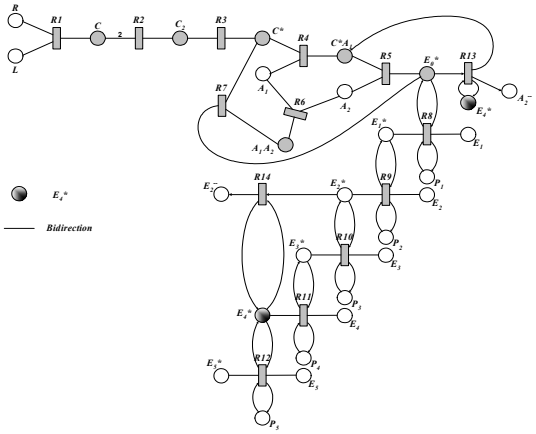

Figure 6. Petri net representation of EGF signaling pathway

Modeling and simulation of signal transduction system

Once the signaling network is reconstructed, both the executable Petri net model and the mathematical modeling approach are employed to understand the dynamic behavior of signal transduction pathway. Based on the basic reaction types for two modeling approaches, we extend the EGF signaling pathway to a slightly more complex system (Asthagiri and Lauffenburger, 2001; Kholodenko *et al.*, 1999) which has been investigated as one of pathways related to the signal transduction network induced by IL-1 and TNF-α. A schematic representation of the signal transduction system induced by EGF is shown in Figure 5 and the corresponding executable Petri net

model in Figure 6.

Figures 7 and 8 show the simulation results using the two models. In the absence of feedback, an overall amplification of signal is observed across the enzyme cascade, namely, the steady-state concentration of each enzyme in its active state (*Ei**) increases as the signal progresses down the *Ras*(*E*1)-*Raf*(*E*2)-*MEK*(*E*3)-*ERK*(*E*4)-*Elk*(*E*5) cascade (Figure 7). When two feedback modes are emanated from the enzyme *ERK** (*E*4*), complete adaptation is achieved for signals down-stream of feedback target as can be observed in Figure 8. For a more detailed investigation and analysis of the feedback effects in MAPK pathway, see the reference (Asthagiri and Lauffenburger, 2001).

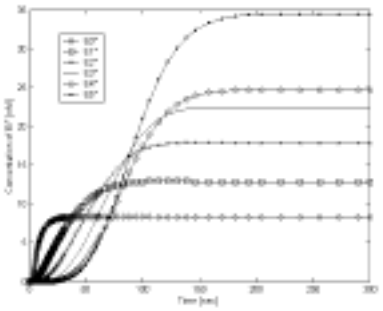

Figure 7. Signal amplification across the enzyme cascade in EGF signaling pathway

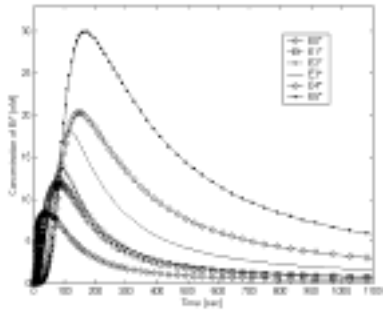

Figure 8. Complete signal adaptation of the enzyme cascade with two negative feedback modes in EGF signaling pathway

Conclusion

In this study, we have developed a conceptual framework for the Petri net representation and simulation of signal transduction networks, to implement the quantitative and qualitative analysis of the network structure and the interaction between network components. As a result of the application to the signal transduction system induced by IL-1 and TNF-α, and EGF, mechanisms and dynamics of the network have been investigated, which could be useful for the search of new targets for anti-inflammatory drugs. The application study provides a proof of principle for the feasibility of modeling quite complex signaling networks both qualitatively and quantitatively by executable Petri net models consistently with conventional differential equation models.

Acknowledgement

This work was partially supported by BK21 project and Center for Ultramicrochemical Process Systems sponsored by KOSEF.

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