Colored Petri net modeling and simulation of signal transduction pathways

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Abstract

Presented herein is a methodology for quantitatively analyzing the complex signaling network by resorting to colored Petri nets (CPN). The mathematical as well as Petri net models for two basic reaction types were established, followed by the extension to a large signal transduction system stimulated by epidermal growth factor (EGF) in an application study. The CPN models based on the Petri net representation and the conservation and kinetic equations were used to examine the dynamic behavior of the EGF signaling pathway. The usefulness of Petri nets is demonstrated for the quantitative analysis of the signal transduction pathway. Moreover, the trade-offs between modeling capability and simulation efficiency of this pathway are explored, suggesting that the Petri net model can be invaluable in the initial stage of building a dynamic model.

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1. Introduction

Petri-net-based models have been widely adopted for studying biological systems, especially metabolic and genetic networks. Petri net models allow formal and clear representation of the biological systems based on their firm mathematical foundation for the analysis of performance measure and properties, such as liveness, boundedness and reachability, and their high-level extensibility for modeling and dynamic simulation (Reddy et al., 1996; Mounts and Liebman, 1997; Goss and Peccoud, 1998; Hofestäd and Thelen, 1998; Küffner et al., 2000; Matsuno et al., 2000; Genrich et al., 2001; Heiner et al., 2001; Oliveira et al., 2001; Schacherer, 2001; Peleg et al., 2002; Pinney et al., 2003). However, only a handful of attempts have been made to model and simulate signal transduction pathways by resorting to Petri nets (Matsuno et al., 2003; Heiner et al., 2004).

Conventionally, the dynamics of the signal transduction system has been investigated by simulations using the continuous, mass-action differential equations (Huang and Ferrell, 1996; Bhalla and Iyengar, 1999; El-Masri and Portier, 1999; Levchenko et al., 1999; Levenson et al., 2000; Asthagiri and Lauffenburger, 2001). Although such simulation is highly efficient computationally, it is restricted to a well-defined system because appropriate kinetic parameters should be provided for successful simulations. On the other hand, the discrete-event model with timing consideration by high-level Petri nets, e.g., colored Petri nets (CPN) and timed Petri nets, provides a substantial flexibility in modeling the
systems; the designer can observe in detail a variety of the
dynamic behaviors during the simulation with increased
modeling power since the system can be easily modified by
changing the kinetic parameters interactively (Matsuno
et al., 2000; Genrich et al., 2001). Note that this is almost
equivalent to the program execution and subsequent
debugging process in case of convergence failure. In this
study, we adopted CPN for the simulation of signal
transduction networks on the basis of Petri net representa-
tion and continuous differential equations, thereby explor-
ing the usefulness of Petri nets for the quantitative analysis
of the system.

2. Methodology

Both the Petri net model and mathematical-modeling
approach comprising the conservation (mass balance) and
kinetic equations were employed to explore the dynamic
behavior of the signal transduction system. We first
describe Petri net representation of molecular interactions,
and then the mathematical as well as Petri net models for
two basic reaction types in detail.

2.1. Petri net representation for molecular interactions

Various types of molecular interactions can be explicitly
and clearly described by the Petri-net model (Reddy et al.,
1996; Goss and Pecquod, 1998; Hofestädt and Thelen,
1998; Küffner et al., 2000; Oliveira et al., 2001). Petri nets
are bipartite directed graphs $G = (V, E)$ composed of two
kinds of vertices, $V_1$ and $V_2$; naturally $V_1 \cup V_2 = V$. The
former is termed places ($V_1 = P$), and the latter is termed
transitions ($V_2 = T$), where edges $e \in E \subseteq (V_1 \times V_2) \cup
(V_2 \times V_1)$ link the places with transitions, and vice versa
(Murata, 1989). In the current work, the places and
transitions represent biomolecular species and molecular
interactions or reactions, respectively. This basic graph
representation suffices to describe the metabolic relation-
ships, such as the chemical transformation and enzymatic
reaction in metabolic pathways (Reddy et al., 1996). It can be
extended to the regulatory relationships by simply
adding regulatory transitions (Küffner et al., 2000). For
signal transduction systems, however, supplementary
information on the regulatory/molecular interactions
should be specified to distinguish among different types
of molecular interactions, e.g., chemical transformation
and association (Lee et al., 2004). Nevertheless, from the
mechanistic point of view, Petri net representations of both
cases are identical.

2.2. Mathematical model and simulation for basic reaction
types

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

$$\sum_{i=1}^{m} a_i R_i \xleftrightarrow{\mu_{i}} \sum_{j=1}^{n} b_j P_j, \quad (1)$$

where $a_i$ and $b_j$ denote the stoichiometric coefficients of the
$i$th reactant and the $j$th product, respectively; $R_i$ and $P_j$, the
$i$th reactant and the $j$th product, respectively; and $\mu_f$ and $\mu_b$,
the forward and backward rate constants, respectively.

Then, the reaction rate, which is dependent on the
concentrations of reactants and products, can be expressed
as follows:

$$v = \frac{d[R_i]}{dt} = \frac{d[P_j]}{dt} = k_b \prod_{j=1}^{n} [P_j]^{\mu_b} - k_f \prod_{i=1}^{m} [R_i]^{\mu_f}, \quad (2)$$

where $[R_i]$ and $[P_j]$ are the concentrations of the $i$th reactant
and the $j$th product, respectively.

For example, the association reaction or complex
formation can be represented by

$$A + B \xleftrightarrow{\mu_a, \mu_d} AB, \quad (3)$$

where $\mu_a$ and $\mu_d$ denote the association and dissociation
rate constants, respectively. The dynamic behavior of this
system can be observed by the following rate expressions

![Fig. 1. Simulation of the mathematical models for (A) complex formation
and (B) enzymatic reaction.](image-url)
upon pre-specification of the initial concentrations of $A$, $B$, and $AB$:

$$
\frac{dA}{dt} = k_d [AB] - k_a [A][B],
$$

(4)

$$
\frac{dB}{dt} = k_d [AB] - k_a [A][B],
$$

(5)

$$
\frac{d[AB]}{dt} = k_a [A][B] - k_d [AB].
$$

(6)

In addition, two conservation equations can be considered as follows:

$$
\frac{dA}{C_{138}} = \frac{k_d [AB]}{C_0} - \frac{k_a [A]}{C_{138}},
$$

(7)

$$
\frac{dB}{C_{138}} = \frac{k_d [AB]}{C_0} - \frac{k_a [B]}{C_{138}},
$$

(8)

where $[A]_0$ and $[B]_0$ are the initial concentrations of $A$ and $B$, respectively. Upon eliminating $[A]$ and $[B]$ via Eqs. (7) and (8), Eq. (6) reduces to a kinetic model comprising a single variable of $[AB]$. In other words, Eqs. (6)–(8) collectively constitute a system of differential-algebraic equations.

In implementing the simple reaction model presented above, we assume that $k_a = 0.007; k_d = 0.25$; and the initial concentrations of species, $[A]_0 = 100, [B]_0 = 166,$ and $[AB]_0 = 0$. Then, Eqs. (4)–(6) with the given kinetic parameters and initial concentrations can be solved. Fig. 1A shows the resultant time profiles of the concentrations of $A, B,$ and $AB$.

The second type of reaction is an enzyme reaction represented by the combination of the reversible association and irreversible dissociation steps involving one substrate and one product (uni–uni mechanism), as illustrated below:

$$
E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P,
$$

(9)

where $k_1$ is the association rate constant between substrate ($S$) and enzyme ($E$); $k_2$, the dissociation constant of $ES$; and $k_3$, the rate constant for the formation of a product ($P$) from $ES$. Similar to the first type reaction, the mass balance and kinetic equations can be derived, yielding the following four rate equations and two mass balance equations.

$$
\frac{dE}{dt} = (k_2 + k_3)[ES] - k_1[E][S],
$$

(10)

$$
\frac{dS}{dt} = k_2[ES] - k_1[E][S],
$$

(11)

$$
\frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES],
$$

(12)

$$
\frac{d[P]}{dt} = k_3[ES],
$$

(13)

$$
[E] = [E]_0 - [ES],
$$

(14)

$$
[S] = [S]_0 - [ES] - [P],
$$

(15)

The results of simulation by assuming that $k_1 = 5, k_2 = 4, k_3 = 1, [E]_0 = 20, [S]_0 = 100, [ES]_0 = 0$, and $[P]_0 = 0$ are shown in Fig. 1B. Initially, the active sites of $E$ are

![Fig. 2. Petri net model for the complex formation. (A) The model can be depicted by association and dissociation reactions as Petri net representation. For the simulation, we can construct various executable Petri net models, (B) without timing consideration, (C) with timing consideration, and (D) with one transition under the design/CPN environment.](image-url)
rapidly saturated with $S$, thereby forming $ES$. The increased concentration of $ES$ causes the steady increase of product $P$, eventually reaching a plateau. Finally, all substrate is transformed into the product, resulting in the termination of reaction. This approach can be extended in a straightforward manner to an enzymatic reaction with multiple reactants and products (Bish and Mavrovouniotis, 1998).

2.3. Petri net model and simulation for basic reaction types

A high-level Petri net model (or executable Petri net model) based on the Petri net representation and CPN can also be constructed for the dynamic simulation of the cell signaling system as has been attempted for the analysis of metabolic pathways (Genrich et al., 2001). In the current study, the software package Design/CPN (http://www.daimi.au.dk/Cpnets/) and its Window version, CPN tools (http://www.daimi.au.dk/CPNtools/) were employed for the implementation of the Petri net model. 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 derived from the mathematical model, in code regions of transitions. Then, the concentration of each substrate is updated whenever the reaction transition specified by the function is fired according to its occurrence rule.

For example, the complex formation model described in the preceding subsection can be depicted as the Petri net representation shown in Fig. 2A. The model consists of three places ($A$, $B$, and $AB$) represented by circles and two transitions (forward reaction $R1$ and backward reaction $R2$) by rectangular boxes. Fig. 2B shows the corresponding executable Petri net model for this example in the Design/CPN environment where two transitions denote the reactions, $R1$ and $R2$, and every substrate is represented by a place connected through an outgoing arc and an ingoing arc to the transitions.

To calculate the reaction rate, we have defined the kinetic functions, $R1$ and $R2$, in the code regions of the reaction transitions, whereby the concentration of each substrate is updated whenever the transition is fired (i.e., occurs). For example, when the transition, $R1$, is fired, the function, $R1$, is called with the current concentrations of $A$, $B$ and $AB$ (denoted by $cA$, $cB$, and $cAB$, respectively) as the input values to its code region. Then, their concentration changes within a unit time, $\Delta[A]/\Delta t = k_d[A][B] - k_{[A][B]}$, $\Delta[B]/\Delta t = k_d[AB] - k_{[A][B]}$, and $\Delta[AB]/\Delta t = k_d[A][B] - k_{[A][B]}$, which are derived from Eqs. (4)–(6), are computed in the global declaration node that is not explicitly shown. Finally, the updated concentrations are returned as the output values to each connected place through the outgoing arc from the transition which is fired. This procedure continues during the simulation. Note that in this discrete-event model, the reaction rate is considered as the extent of the concentration change within unit interval, $\Delta c / \Delta t$, as described above. If accurate results are required, a time interval must be small. Thus, the efficiency and accuracy of simulation for a large system depend highly on the time interval predefined, the appropriate value of which should also be selected to prevent excessive extrapolation invoking a negative concentration (Genrich et al., 2001). In the current work, the primitive I/O commands of Standard ML (called CPN ML) language supported in Design/CPN are adopted to store the data, whereby the post-updated values of $cA$, $cB$ and $cAB$, i.e., input or output values of the transition, $R1$, are added in a flat-file whenever the $R1$ is fired.

Fig. 3A illustrates the time profiles of the concentrations of $A$, $B$ and $AB$ obtained by Petri net simulation. Note that each concentration increases or decreases with some fluctuations, which were not observed when using the mathematical model as in Fig. 1A. This is a consequence of the fact that timing is not considered in this discrete-event model; two existing transitions are randomly fired, thus resulting in the different occurrence distribution between them in every run. To take into account the timing, it should be assumed that the simulation time is increased
only after all the reactions are run first; at the same step, the already fired transitions await until all the remaining transitions are fired. This timed Petri net can be achieved by using timed colorset and delay expression in arcs connected to the place state of Fig. 2C.

When the two reactions are considered to occur in a single transition of Fig. 2D, the same result as the case of the mathematical model is obtained as shown in Fig. 3B. In terms of simulation time, this model is more efficient than the two-transitions model described above: one occurrence

---

**Fig. 4.** Various executable Petri net models (A) without timing consideration, (B) with timing consideration, and (C) with one transition under the CPN tools environment.

---

**Fig. 5.** Petri net model for the enzymatic reaction. The reaction model with three transitions and four places (A) can be transformed into the simpler one with one transition and three places, (B) known as the Michaelis–Menten model according to reduction rules. For the simulation, corresponding executable Petri net models in the design/CPN (C) and CPN tools (D) environments can be represented as one transition.
in the former is identical to the two occurrences in the latter. In a large system with many reactions, however, the consideration of one transition connected to many places loses the modeling capability; as such, it is meaningless to construct such Petri net model. Strictly speaking, this resembles more closely to the simulation by the mathematical model with a fixed step rather than Petri net simulation. Note that Fig. 5 shows the executable Petri net models for the basic reaction type in the CPN tools environment. Figs. 4A–C are equivalent to Figs. 2B–D, respectively.

Figs. 5A and B are two Petri net representations of the enzymatic-reaction type. The reaction model with three transitions and four places can be transformed into a simpler one with one transition and three places according to reduction rules (Reddy et al., 1996; Murata, 1989). This simple reaction model corresponds to the Michaelis–Menten model in which it is assumed that enzyme concentration is constant. In various simulation studies on biochemical reaction systems (Bhalla and Iyengar, 1999; Tomita et al., 1999), this model has been used due to its simplicity in form and its efficiency in simulation. However, the model is not adopted in this study. Our executable Petri net models can be represented as one transition, preserving the system properties (see Figs. 5C and D). In this study, therefore, one-transition model can be defined for each elementary reaction and enzymatic reaction, and all the reactions are incorporated into the timed Petri net model. Figs. 6A and B show the simulation results for the models without and with timing consideration, respectively. The time profiles of the concentrations of the reaction compounds are similar to those predicted by the mathematical model.

Based on the aforementioned continuous, mass-action differential equations and the Petri net representation, we can construct mathematical models and executable Petri net models for dynamically investigating molecular mechanisms comprising various reaction types in signal transduction pathways. In the following section, the dynamics of the complex signaling system, the epidermal growth factor (EGF)-induced signal transduction pathway, is explored by applying this strategy.

3. Results and discussion

The strategy of employing the executable Petri net model is applied to the analysis of the signaling system stimulated by EGF. EGF receptor (EGFR), belonging to the family of tyrosine kinase receptors, plays important roles in cell growth, survival, proliferation, and differentiation. Since EGFR is associated with tumorigenesis in particular, it has been recognized as a drug target in the development of potent anti-cancer agents (Fry et al., 1995; Kim and Muller, 1999). In addition, EGFR signaling network has been relatively well studied, and thus is a good model system for examining the dynamics of signal transduction systems. Indeed, much effort has been made to develop mathematical models of this signal transduction pathway (Kholodenko et al., 1999; Brightman and Fell, 2000; Asthagiri and Lauffenburger, 2001; Moehren et al., 2002; Schoeberl et al., 2002). This is because understanding the mechanisms and dynamics of the pathway through the reconstruction of the signal transduction network would generate information useful for finding and verifying new drug targets. The current simulation study follows the modeling scheme presented by Asthagiri and Lauffenburger (2001) and adopts the necessary kinetic parameters from the literature (Kholodenko et al., 1999; Levchenko et al., 2000).

3.1. Petri net representation of EGF-induced signal transduction pathways

A schematic representation of the signal transduction system induced by EGF is depicted in Fig. 7, and the corresponding Petri net representation in Fig. 8. First, EGF binds to EGFR, forming the EGF.EGFR complex (Step 1). These receptor–ligand complexes then interact with each other to form a dimer, EGF.EGFR2 (Step 2), which becomes phosphorylated to yield an activated form of a receptor complex, EGF.EGFR2* (Step 3). Then, the
first adaptor protein, Grb2, is recruited into the activated receptor complex (Step 4). This complex, EGFR2Grb2, then associates itself with the second adaptor protein, SOS, to form a larger complex, EGFR2Grb2.SOS (Step 5). Both adaptors, Grb2 and SOS, may precouple within the cytosol in the absence of any stimulus to form the cytosolic heteroadaptor complex, Grb2.SOS (Step 6). This complex may also directly interact with the activated receptor complex, EGF.EGFR2 to form EGF.EGFR2Grb2.SOS (Step 7).

The formation of the receptor-signaling complex, EGF.EGFR2Grb2.SOS, regulates a cascade of signaling events. First, it activates Ras; as a result, Ras is phosphorylated from its inactive state. Once Ras is converted to its active state, Ras* (Step 8-1), it initiates a cascade of enzyme activations through a sequence of phosphorylation steps whereby the phosphorylated (active) form of an intermediate protein acts as a catalyst for the phosphorylation of the subsequent step. These steps form a Raf-MEK-ERK-Elk cascade (Steps 9-1, 10-1, 11-1, and 12-1). In addition, dephosphorylation of each active substrate, including Ras*, Raf*, MEK*, ERK* and Elk*, by phosphatases, such as GAP, PP2-A, and MKP-1 (Steps 8-2, 9-2, 10-2, 11-2, and 12-2), also represents one of the major regulatory mechanisms in the EGF-induced signaling system.

It is assumed that two modes of negative feedback exist in the current system (Buday et al., 1995; Langlois et al., 1995; Wartmann et al., 1997). One is the dissociation of SOS from the EGF.EGFR2Grb2.SOS complex (Step 13). In this mode, the active enzyme ERK* directly catalyzes the covalent modification of SOS in the complex EGF.EGFR2Grb2.SOS (Buday et al., 1995; Langlois et al., 1995). Presumably, this modification converts SOS into its inactive form, SOS−, which is no longer capable of associating with Grb2. Therefore, the existing EGF.EGFR2Grb2.SOS complex is disassembled into its constituents, i.e., EGF, EGFR2Grb2 and SOS−. The other mode of negative feedback is Raf hyperphosphorylation by ERK* (Wartmann et al., 1997). In this mode, the active enzyme Raf* is converted into Raf− by ERK* (Step 14).

### 3.2. Modeling and simulation of EGF-induced signal transduction pathways

The continuous, mass-action differential-equation model comprised of the conservation and kinetic equations was developed to investigate the dynamic behavior of the EGF signaling pathways. Each elementary reaction with letter abbreviations (Fig. 7) and kinetic parameters for the concomitant rate equation is given in Table 1, where the step designating a reaction represents the corresponding
reaction number indicated in Fig. 7. In addition, the rate constants for each reaction were included in the corresponding rate equation derived from the model for the basic reaction types discussed in the previous section. First the model without two feedback modes (Steps 13 and 14 in Fig. 7 and Table 1) was considered as follows:

Kinetic equations (without feedback):

\[
\begin{align*}
\frac{d[C^* A_1]}{dt} &= k_1^1[C^*][A_1] - k_1^2[C^* A_1] - k_1^2[C^* A_1][A_2] + k_2^1[E_0^*], \\
\frac{d[E_0^*]}{dt} &= k_2^1[C^* A_1][A_2] - k_2^2[E_0^*] + k_2^3[C^* A_1][A_2] - k_2^4[E_0^*] \quad \text{(19)}, \\
\frac{d[A_1 A_2]}{dt} &= -k_1^2[C^* A_1][A_2] + k_1^3[E_0^*] \\
&\quad + k_2^4[C^* A_1][A_2] - k_2^5[E_0^*], \quad \text{(20)}, \\
\frac{d[E_i E_{i-1}]}{dt} &= k_1^5[E_i E_{i-1}] - (k_1^6 + k_2^6)[E_i E_{i-1}], \quad \text{for all } i, \\
\frac{d[E_i P_i]}{dt} &= k_1^7[E_i][P_i] - (k_2^7 + k_3^7)[E_i P_i], \\
\frac{d[E_i]}{dt} &= k_1^8[E_i][E^*_{i-1}] + k_1^9[E_i][E^*_{i+1}] + k_2^8[E_i][E^*_{i+1}] \\
&\quad - (k_2^9 + k_3^9)[E_i E_{i-1}] + k_2^{10}[E_i P_i]. \quad \text{(24)}
\end{align*}
\]

Mass balance equations (without feedback):

\[
\begin{align*}
[R]_{\text{total}} &= [R] + [C] + 2([C_2] + [C^*]) + [C^* A_1] + [E_0^*] + [E_1 E_0^*], \quad \text{(25)} \\
[L]_{\text{total}} &= [L] + [R]_{\text{total}} - [R], \quad \text{(26)} \\
[A_1]_{\text{total}} &= [A_1] + [C^* A_1] + [A_1 A_2] + [E_0^*] + [E_1 E_0^*], \quad \text{(27)} \\
[A_2]_{\text{total}} &= [A_2] + [A_1 A_2] + [E_0^*] + [E_1 E_0^*], \quad \text{(28)} \\
[E_i]_{\text{total}} &= [E_i] + [E_i E_{i-1}] + [E_i^*] + [E_i^* E_{i+1}] + [E_i^* P_i], \quad \text{(29)} \\
[P_i]_{\text{total}} &= [P_i] + [E_i^* P_i]. \quad \text{(30)}
\end{align*}
\]

In the case of the model with two feedback modes, the reactions, R13 and R14 (steps 13 and 14 in Table 1) are additionally considered. Thus, among Eqs. (16)-(30), those related to two reactions are modified, resulting in Eqs. (31)-(36), and two rate Equations (37) and (38) are additionally considered.

Kinetic equations (with feedback):

\[
\begin{align*}
\frac{d[C^* A_1]}{dt} &= k_1^1[C^*][A_1] - k_1^2[C^* A_1] - k_1^2[C^* A_1][A_2] + k_2^1[E_0^*] + k_{\text{cat},1}[E_0^* E_4^*], \quad \text{(31)} \\
\frac{d[E_0^*]}{dt} &= k_2^1[C^* A_1][A_2] - k_2^2[E_0^*] + k_2^3[C^* A_1][A_2] - k_2^4[E_0^*] \\
&- k_1^3[E_0^*] - k_1^4[E_0^*][E_1] + (k_1^5 + k_{\text{cat},1,3}[E_1 E_0^*] - k_2^5[E_0^*][E_4^*] + k_2^6[E_0^* E_4^*] + k_2^7[E_0^* E_4^*], \quad \text{(32)} \\
\frac{d[E_2^* E_4^*]}{dt} &= k_2^*E_2^*[E_4^*] - (k_2^* + k_{\text{cat},2})[E_2^* E_4^*], \quad \text{(33)} \\
\frac{d[E_0^* E_4^*]}{dt} &= k_2^*E_0^*[E_4^*] - (k_2^* + k_{\text{cat},3})[E_0^* E_4^*], \quad \text{(34)}
\end{align*}
\]
Table 1
Biochemical reactions and kinetic parameters of the EGF-induced signal transduction system

<table>
<thead>
<tr>
<th>Reaction step</th>
<th>Reactions</th>
<th>Kinetic constants</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>$L + R \rightarrow C$</td>
<td>$k_1 = 0.003 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_i = 0.06 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 2</td>
<td>$2C + C_2 \rightarrow 2C$</td>
<td>$k_a = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_i = 0.1 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 3</td>
<td>$C + C \rightarrow C^*$</td>
<td>$k_1^+ = 1.0 \text{s}^{-1}$</td>
<td>$k_0^+ = 0.01 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 4</td>
<td>$C + A_1 \rightarrow C^*A_1$</td>
<td>$k_1^+ = 0.003 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_i^+ = 0.05 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 5</td>
<td>$C^*A_1 + A_1 \rightarrow E_0$</td>
<td>$k_2 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_i^+ = 0.06 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 6</td>
<td>$A_1 + A_2 \rightarrow A_2$</td>
<td>$k_{12} = 0.0001 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{12} = 0.0015 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 7</td>
<td>$C^* + A_1 \rightarrow E_0$</td>
<td>$k_{12} = 0.0045 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{12} = 0.03 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 8-1</td>
<td>$E_0 + E_0 \rightarrow E_0E_0 \rightarrow E_0 + E_1^*$</td>
<td>$k_1 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_1 = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 8-2</td>
<td>$E_1 + P_1 \rightarrow E_1P_1 \rightarrow P_1 + E_1$</td>
<td>$k_{p_1} = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{p_1} = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 9-1</td>
<td>$E_0 + E_0 \rightarrow E_0E_0 \rightarrow E_0 + E_2^*$</td>
<td>$k_1 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_2 = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 9-2</td>
<td>$E_2^* + P_2 \rightarrow E_2P_2 \rightarrow P_2 + E_2$</td>
<td>$k_{p_2} = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{p_2} = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 10-1</td>
<td>$E_1 + E_2 \rightarrow E_1E_2 \rightarrow E_1 + E_3^*$</td>
<td>$k_1 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_3 = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 10-2</td>
<td>$E_2^* + P_2 \rightarrow E_2P_2 \rightarrow P_2 + E_2$</td>
<td>$k_{p_1} = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{p_2} = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 11-1</td>
<td>$E_0 + E_0 + E_1 \rightarrow E_0E_1 \rightarrow E_0 + E_2^*$</td>
<td>$k_1 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_4 = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 11-2</td>
<td>$E_2^* + P_2 \rightarrow E_2P_2 \rightarrow P_2 + E_2$</td>
<td>$k_{p_1} = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{p_2} = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 12-1</td>
<td>$E_0 + E_0 + E_1 \rightarrow E_0E_1 \rightarrow E_0 + E_3^*$</td>
<td>$k_1 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_5 = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 12-2</td>
<td>$E_2^* + P_2 \rightarrow E_2P_2 \rightarrow P_2 + E_2$</td>
<td>$k_{p_1} = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{p_2} = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 13</td>
<td>$E_0 + E_0 + E_1 \rightarrow E_0E_1 \rightarrow E_0 + E_3^*$</td>
<td>$k_1 = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_5 = 0.5 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Step 14</td>
<td>$E_2^* + P_2 \rightarrow E_2P_2 \rightarrow P_2 + E_2$</td>
<td>$k_{p_1} = 0.01 \text{mM}^{-1} \text{s}^{-1}$</td>
<td>$k_{p_2} = 0.5 \text{s}^{-1}$</td>
</tr>
</tbody>
</table>

$\text{[E]}_{\text{total}} = \text{[E]}_1 + \text{[E]}_2 + \text{[E]}_3 + \text{[E]}_4 + \text{[E]}_5 + \text{[E]}_6 + \text{[E]}_7 + \text{[E]}_8 + \text{[E]}_9 + \text{[E]}_{10}$

$\text{[A]}_{\text{total}} = \text{[A]}_1 + \text{[A]}_2 + \text{[A]}_3 + \text{[A]}_4 + \text{[A]}_5 + \text{[A]}_6 + \text{[A]}_7 + \text{[A]}_8 + \text{[A]}_9 + \text{[A]}_{10}$

$\text{[E]}_{4,\text{total}} = \text{[E]}_4 + \text{[E]}_5 + \text{[E]}_6 + \text{[E]}_7 + \text{[E]}_8 + \text{[E]}_9 + \text{[E]}_{10}$

$\text{[P]}_{\text{total}} = \text{[P]}_1 + \text{[P]}_2 + \text{[P]}_3 + \text{[P]}_4 + \text{[P]}_5 + \text{[P]}_6 + \text{[P]}_7 + \text{[P]}_8 + \text{[P]}_9 + \text{[P]}_{10}$

Based on the kinetic and mass balance equations of the EGF-induced signal transduction system and the corresponding Petri net representation depicted in Fig. 8A, the executable Petri net model was constructed to explore the dynamic behavior of the system using design/CPN and CPN tools (Fig. 8B). Fig. 9 exhibits the results of simulation of the executable Petri net model. Herein, the concentrations of all enzymes, except $E_0$, are expressed in terms of the fractions of the initial concentrations in their inactive states ($E_i$'s), which are assumed to be identical. In the absence of feedback, an overall amplification of signal is observed across the enzyme cascade. Specifically, the steady-state concentration of each enzyme in its active state ($E_i^*$) increases as the signal progresses down the Ras($E_1$)– Raf($E_2$)– MEK($E_3$)– ERK($E_4$)–Elk($E_5$) cascade (Fig. 9A). In this case, negative regulatory mechanisms, such as dissociation of adaptor protein-containing signaling complexes (Step 13) and deactivator-mediated shut-down of signaling enzymes (Step 14), are unable to induce signal adaptation. When the two feedback modes were considered, complete adaptation is achieved for signals downstream of the feedback target as observed in Fig. 9B. The implication of the feedback effects in the MAPK pathway has extensively been analyzed and discussed by Asthagiri and Lauffenburger (2001).

Once the structure and dynamic properties of the EGF signaling pathways are well characterized and understood...
through the presented approach, the kinetic model in conjunction with the drug models, e.g., pharmacokinetic and pharmacodynamic models, can be exploited to quantitatively analyze drug targets in the development of potent anti-cancer agents. To date, numerous drug targets for cancer therapies have been identified at different levels in the signaling pathways (Adjei, 2000). Currently being pursued by various investigators as potent anti-cancer agents include the receptor antagonists (e.g., antibodies and soluble receptors), compounds inhibiting protein kinase activity in intracellular signaling, and transcription factor blockers (Chang et al., 2003; García-Echeverría, 2004). A major future challenge is to explore such identified drug effects on the pharmacological modulation within the pathways and to investigate the pharmacodynamic and mechanistic effects of various drugs for signaling targets, thereby evaluating the efficacy, safety and cost-effectiveness of drug targets available (Lee et al., 2004; Rao et al., 2005). In this regard, the detailed molecular mechanisms and kinetic models of the cell-signaling network could provide useful information for developing a rational approach to the quantification of the mechanistic effects of drugs and prioritizing drug candidates at the system level; this awaits our future efforts.

The executable Petri net model is based on the discrete-event model with timing consideration. Events occurring at the same moment are introduced randomly; each event changes the system state and may entail a future event to occur. Since the signaling system is simulated stepwise according to the occurrence rule, the detailed behavior of each node, or substrate, can be observed during the simulation. This renders it possible to intuitively observe the regulatory mechanisms in the biologically meaningful sense, e.g., how signaling molecules interact with each other and behave in response to environmental changes via feedback loops and oscillators (Oliveira et al., 2001). In addition, the system can be easily modified by altering the kinetic parameters specified in code regions of transitions, thus providing the designer with modeling capability. Furthermore, the changes in the concentrations of all molecules can be displayed in the corresponding places whenever the reaction transition is fired according to the occurrence rule during the simulation, thus allowing direct observation of the effects of the concentration changes on various steps in the pathways. These characteristics will be useful for the system with uncertain kinetic parameters, which need to be estimated for the simulation. The speed of simulation, however, remains a significant drawback of the Petri net model when compared to the mathematical model in which the kinetic and mass balance equations written in differential equations are numerically solved. In the mathematical model, the solution simultaneously satisfying the constraints under the specified tolerances is updated after each small time increment, which is either fixed or varied depending on the solver. Consequently, the approach based on the Petri net model should be useful for the interactive simulation of signaling pathways in the initial stage of constructing a dynamic model.

In summary, a methodology has been established for investigating the dynamic behavior of the signal transduction system by means of CPN. To our knowledge, this work is the first application of CPN to the simulation of signal transduction pathways. The results of this study provide a proof of the principle for the efficient dynamic simulation of highly complex signaling networks by using executable Petri net models. Such models are consistent with the conventional models in the form of differential equations. The main focus of the current study was on the signal transduction system, but the procedures similar to those adopted for quantifying the interactions among the network components can be extended to metabolic and gene regulatory networks.

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References


