Knowledge Representation Model for Systems-Level Analysis of Signal Transduction Networks

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Abstract

A Petri-net based model for knowledge representation has been developed to describe as explicitly and formally as possible the molecular mechanisms of cell signaling and their pathological implications. A conceptual framework has been established for reconstructing and analyzing signal transduction networks on the basis of the formal representation. Such a conceptual framework renders it possible to qualitatively understand the cell signaling behavior at systems-level. The mechanisms of the complex signaling network are explored by applying the established framework to the signal transduction induced by potent proinflammatory cytokines, IL-1β and TNF-α The corresponding expert-knowledge network is constructed to evaluate its mechanisms in detail. This strategy should be useful in drug target discovery and its validation.

Keywords: Petri nets, signal transduction networks, systems biology, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF- α)

1 Introduction

Biological signaling occurs at multiple levels including ligand-receptor interaction, formation of receptor signaling complexes, receptor signal transduction and transcription factor activation through consecutive phosphorylation and dephosphorylation steps whereby the phosphorylated (active) form of an intermediate protein acts as a catalyst (kinase) for the phosphorylation of the subsequent step. Generally, the first level of the signaling system derives from the interaction between receptor and ligand which function as sensor and stimuli, respectively. The large number of stimuli, e.g. extracellular matrix protein, cadherins, growth factors, hormones, cytokines, steroids, etc., are recognized by specific sensors, typically receptors embedded within the cell or plasma membrane to form a ligand-receptor complex and transduce the signal across the membrane. After further interactions of the ligand-receptor complex with adaptor proteins, a receptor signaling complex is formed and triggers specific intracellular signaling pathways. The final outcome of triggering signaling pathways is the activation of transcription factors, which, in turn, initiate transcription of specific genes in the cell nucleus [12, 20].

As pointed out elsewhere [8, 15, 35], the signaling system is indeed complex: multiple types of interactions among a large number of molecules as well as selective and nonlinear communication of different multifunctional elements with others lead to the system's complex and coherent behavior.

Moreover, a variety of signaling routes from the multiple stimuli are seamlessly interconnected for generating multiple cell functions through many cross-talks. Importantly, malfunction of the cellular signaling results in many diseases including cancer, diabetes, and inflammatory diseases. Thus, investigation of the relationships among the complex molecular mechanisms and biomolecular interactions in signaling pathways can provide a novel insight in identifying drug targets.

While some formal frameworks have already been proposed for defining the signaling components previously characterized [18, 30], much remains to be done to construct adequate models for representing, manipulating and analyzing biological signaling systems [1, 9]. Consequently, systems-level approaches are indeed required to understand the organization of the signaling system within the global context of the system [14, 17]. This challenge is addressed in the present study by developing a framework, methods and an appropriate tool for the reconstruction and qualitative analysis of signal transduction networks.

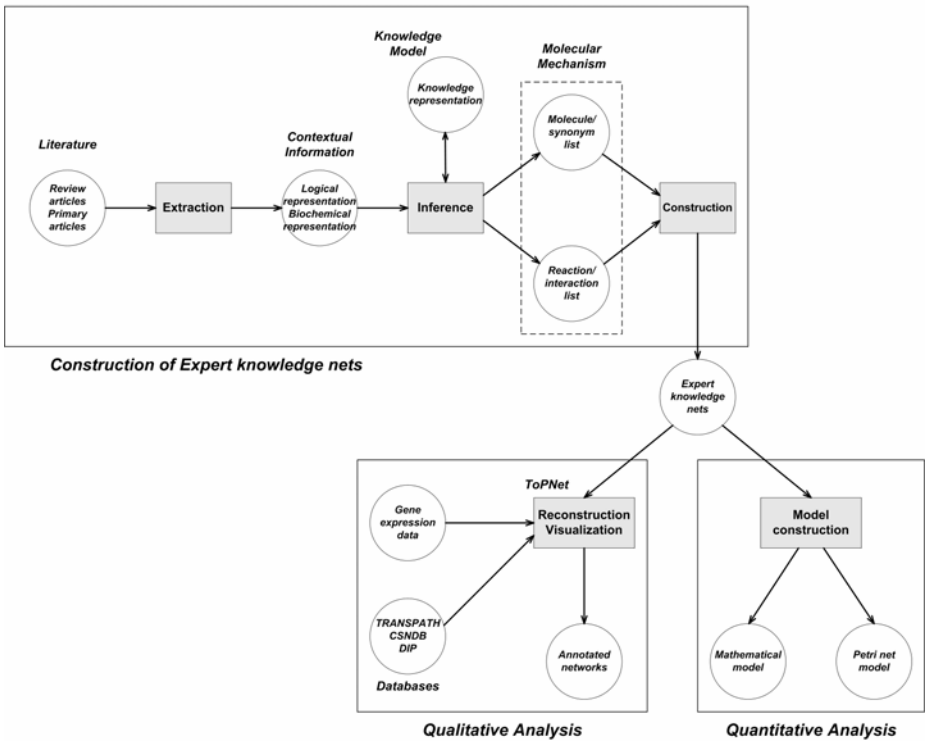


Figure 1: Framework for reconstructing and analyzing signal transduction networks. Initially, the available information on the mechanisms of signaling networks of interest is obtained and organized in a knowledge-based way, thus leading to the construction of expert knowledge networks comprising of signaling components and their interactions. Subsequently, the detailed mechanisms of the networks are investigated through qualitative and quantitative analyses.

2 Methods

2.1 Reconstruction and Analysis of Signal Transduction Networks

Figure 1 schematically outlines the conceptual framework for reconstructing and analyzing signal transduction pathways. First, the available information on the mechanisms of signal transduction networks of interest is obtained and organized according to a knowledge-based paradigm, thus giving rise to the current networks termed *expert knowledge networks* herein. These networks are composed of signaling components and a list of their interactions. The mechanisms of the networks are then explored through qualitative and quantitative analyses. The focus of the current study is on the reconstruction and qualitative analysis of the signal transduction systems on the basis of the formal representation.

To reconstruct the signal transduction networks of interest, a large number of original and review

articles were collected, from which the contextual information describing biological functions was extracted. Subsequently, this information was manually converted into a Petri net model, which describes the corresponding molecular mechanisms. In this study, the ToPNet (Toolbox for Protein Networks) program [11, 44] was used to reconstruct and visualize the networks.

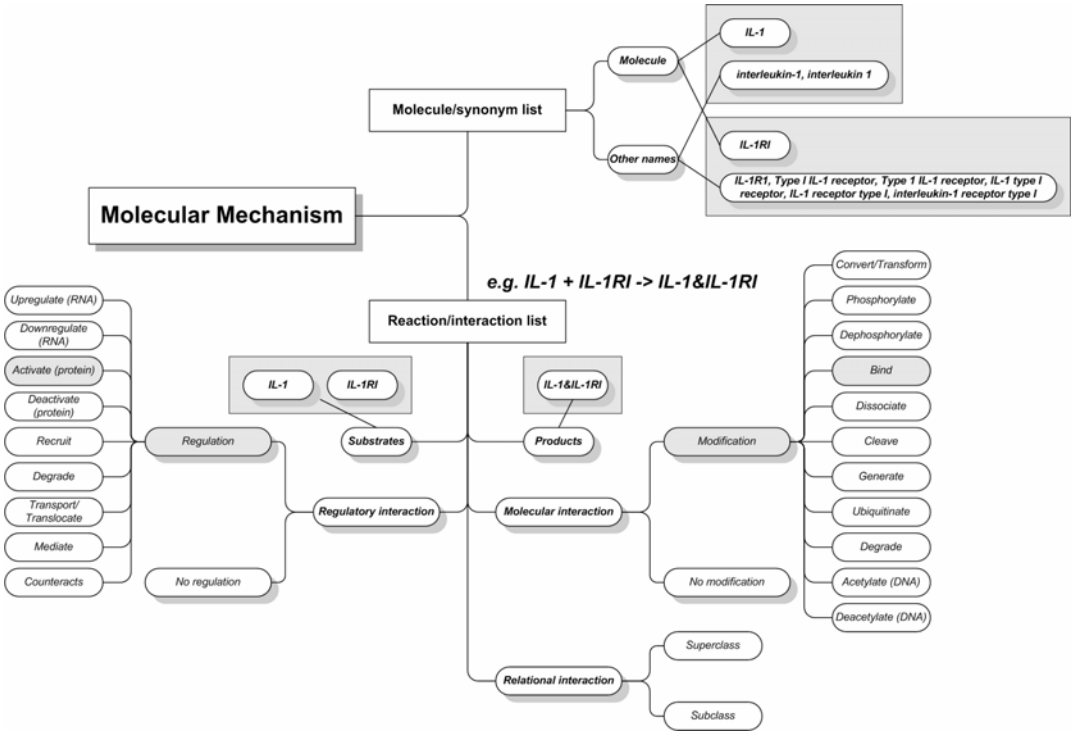


Figure 2: Representation of molecular mechanisms in the current knowledge model. Expert knowledge nets are specified according to this representation. For example, the sentence 'IL-1 binds IL-1RI to be complex' from the literature can be compiled into the knowledge model: Each molecule is listed in the Molecule/synonym class with its alternative names and synonyms; The 'activate' and 'bind' annotations for regulatory and molecular interactions, respectively, can be deduced from the sentence; to clarify the interaction, IL-1 and IL-1RI are considered to be substrates while their complex form IL-1&IL-1RI ('&' denotes a complex of the participating molecules) as the product of the complex building according to the reaction concept.

2.2 Knowledge Model for Representing Signal Transduction Pathways

The knowledge representation of the molecular mechanisms in signal transduction pathways is not easy because cell signaling is inherently diverse and incomplete [10]. Hence, a coherent knowledge representation is essential to describe as explicitly and formally as possible the available information on the molecular mechanisms of the biological system. A knowledge model is, therefore, developed in the current work, which is largely based on ontologies proposed in the previous works [10, 16, 27, 30, 45]. Figure 2 is the abbreviated illustration of the current knowledge model, in which the molecular mechanisms are represented by two classes. Each comprises several fields describing the biological information and regulation in the cell signaling as follows:

(1) Molecule/synonym: All molecules participating in the signaling pathways of interest are extracted, and alternative names of each molecule are added in the synonym list if available.

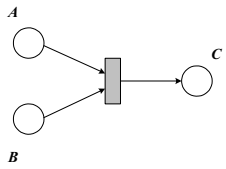
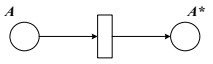
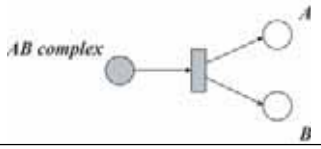
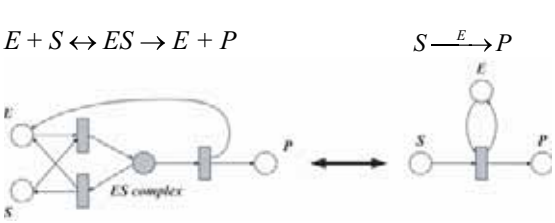
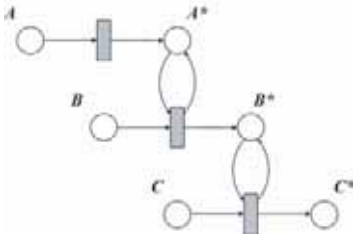
(2) Reaction/interaction: In the reaction class, any interaction type can be fully encoded with a list of substrates and that of products linked to the molecule class, thus explicitly specifying all molecular interactions. This enables the interactions to be mapped into Petri-net representation as described in detail later. In addition, to deal with an inherent duality of available descriptions, they are categorized as logical/semantic (e.g., 'activate', 'deactivate', 'upregulate',

'recruit', etc.) descriptions and biochemical/mechanistic (e.g., 'bind', 'convert', 'phosphorylate', 'dephosphorylate', etc.) descriptions. Subsequently, they are compiled as one of the predefined regulatory-interaction and molecular-interaction candidates given in Fig. 2. The relational interaction for the hierarchical relationship of molecules can also be described in this reaction class if available [27, 45].

2.3 Petri Net Representation for the Formal Description of Molecular Interactions

Various types of molecular interactions can be explicitly and clearly described by the Petri-net model [19, 27, 29, 45]. 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* [26]. In the current work, the places and transitions represent biomolecular species and molecular interactions (biochemical reactions), respectively. This basic graph representation suffices to describe the metabolic relationships, such as the chemical transformation and enzymatic reaction in metabolic pathways [29]. It can be extended to the regulatory relationships by simply adding regulatory transitions [19]. 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, whose Petri-net representations are identical. Hence, the interaction types that can be explicitly specified in two interaction categories of the knowledge model, i.e., logical and mechanistic descriptions, are represented as edge label annotations of Petri nets for the unambiguous representation of the molecular mechanisms.

Table 1: Petri-net representation of several reaction types in signal transduction pathways[a].

Chemical transformation	***Translocation***
$A + B \rightarrow C$ 	$A \rightarrow A^*$ 
Association	***Dissociation***
$A + B \rightarrow AB$ 	$AB \rightarrow A + B$ 
Enzymatic activation (Inhibition)	***Signaling cascade***
$E + S \leftrightarrow ES \rightarrow E + P$ $S \xrightarrow{E} P$ 	

[a]X* designates the active or different state of component, X

2.4 ToPNet (Toolbox for Protein Networks)

ToPNet (Toolbox for Protein Networks) was developed as a program for the integrated analysis of molecular-expression data in the context of biological networks [11, 44]. It allows easy reading, manipulation, and visualization of different biological networks by the users. ToPNet supports various

databases for molecular interactions via an appropriate Petri-net representation, for instance, the regulatory network database TRANSPATH [27, 45], CSNDB [28, 42] and the database of interacting proteins DIP [38, 43]. Furthermore, expert-knowledge networks have been specified in a simple tabular format manually by human experts. ToPNet also provides an integrated environment for the qualitative network analysis to allow the users to select regions of interest within these networks, and to build hulls around selected nodes or compute (shortest) paths through the network. Furthermore, gene-expression data can be loaded, mapped onto biological networks, and then inspected for the regions of interest with regard to such data.

Additionally, the match-program of the ToPNet-package takes the list of objects as input, where each object is specified by a list of names (synonyms). These names are parsed and tagged according to certain rules relevant to the topic of protein names. With these tagged names in hand, the objects in the first list are matched to those in the second list to unify the protein networks consisting of objects of the two lists. In particular, it is possible to effectively crosscheck the networks constructed with TRANSPATH and CSNDB. The detailed information on numerous mechanistic reactions and molecules retrieved from the literature is available in these databases. In this study, the ToPNet program was used as a supporting tool for validating, reconstructing, analyzing, and visualizing the networks constructed.

3 Results and discussion

3.1 Reconstruction and Analysis of IL-1β and TNF-α-Induced Signaling System

The approach presented in the preceding section is applied to the reconstruction and qualitative analysis of the signaling system stimulated by interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). IL-1β and TNF-α are potent proinflammatory cytokines that play critical roles in immunity and inflammation as well as in the control of cell proliferation, differentiation and apoptosis. In particular, they are significant in the pathogenesis of various inflammatory and autoimmune diseases, including rheumatoid arthritis, inflammatory bowel diseases, and asthma. Nevertheless, our understanding of inflammatory pathomechanisms associated with these diseases is far from sufficient although it has substantially increased during the past years. In fact, the two proinflammatory cytokines, IL-1β and TNF-α may activate every known signal transduction pathways [4]. Thus, understanding the mechanisms of these pathways through the reconstruction of the signal transduction network would generate information useful for identifying and validating new drug targets.

Initially, a signal transduction network is reconstructed by extracting the available information on the molecular mechanisms and interactions of IL-1β and TNF-α-induced signaling system from the literature. The detailed molecular mechanisms are then inferred from the extracted information, thus resulting in the possible pathways most likely associated with the three signaling mechanisms and various interactions among them (Fig. 3). The architecture of those pathways can be characterized by their hierarchical organization and many cross-talks which render the network complex and robust. All molecules and their mechanistic interactions involved in these three signaling pathways give rise to an expert knowledge network.

Each signal transduction system of IL-1β and TNF-α consists of four levels: ligand-receptor interaction, formation of receptor-signaling complex, signal transduction by the receptor-signaling complex, and transcription factor activation (Fig. 3). IL-1β signaling is initiated by the formation of a complex including IL-1β IL-1RI and IL-1RAcP. The formation of such a complex causes the intracellular adaptor molecule MyD88 to be recruited to the complex, which, in turn, facilitates the association of IRAK. This is followed by the interaction of IRAK with TRAF6, thereby leading to IL-1β-induced JNK and NF-κB activation [2]. Similar to IL-1β signaling, TNF-α signaling is initiated by the formation of a complex composed of TNF-α and TNFR1, which, in turn, recruits TRADD into the receptor signaling complex. Subsequently, this complex recruits RIP1 and TRAF2, thus triggering a cascade of signaling events, i.e., activation of JNK and NF-κB [3].

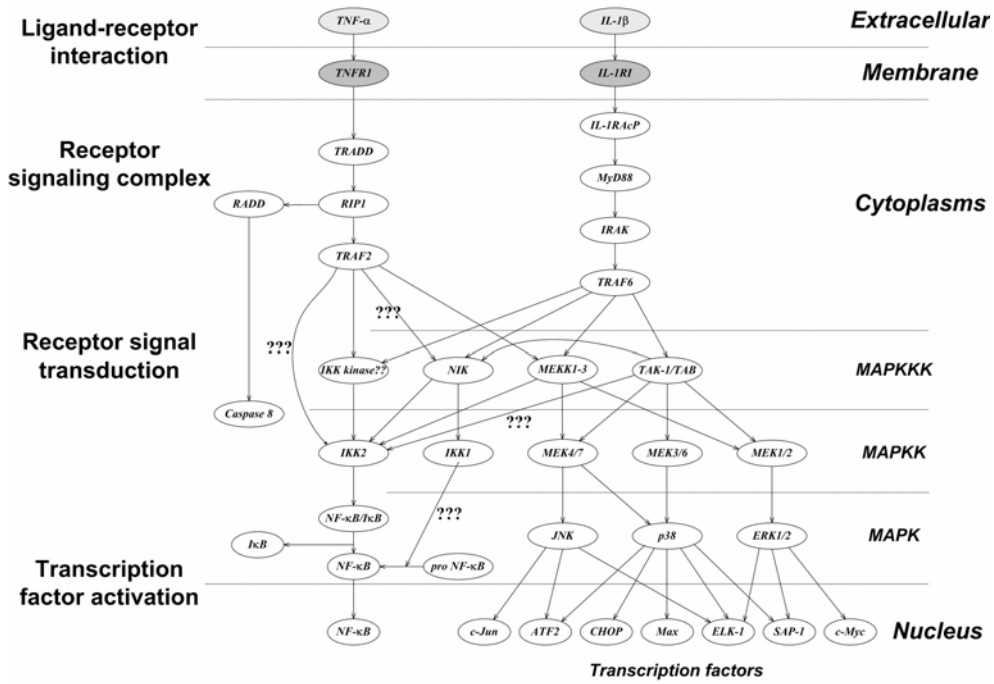


Figure 3: Signaling network associated with IL-1β and TNF-α Question marks (???) indicate unclear mechanisms and/or pathways.

The results have been validated using the match-program in the ToPNet package. According to the results for matching the molecule/synonym list involved in the IL-1β and TNF-α signaling pathways to the TRANSPATH and CSNDB databases, about 80% and 65% of the molecules of the list also appear in TRANSPATH and CSNDB, respectively, whereas 8 molecules (12.5%), including ProIL-1ra, IL-1ra, Ltbeta, LtbetaR, RIP1, TAB2, p38 and SAP-1, are founded in neither database. Among the 8 molecules, RIP1 is a well-known mediator participating in the TNFR1 signaling-complex system [3, 34]. This mismatch can be attributed to the listing of molecule RIP1, instead of RIP, in both databases.

Based on the given molecular mechanisms in the expert knowledge network, a signal transduction network can be reconstructed and visualized with ToPNet as a Petri-net representation (Fig. 4a). In this figure, the two receptors, TNFR1 and IL-1RI, are indicated by R, and the two ligands and transcription factors are L and TF, respectively. On the basis of the reconstructed network, the signal transduction mechanisms can be further explored. For example, the NF-κB activation triggered by the IL-1RI complex can be studied by setting modes manually (Fig. 4b). The resultant signaling pathways from IL-1β to NF-κB are visualized in the screen where information on the regulatory or molecular interaction is also shown according to the edge annotation mode.

The pathways can be reconstructed from the displayed result as the Petri-net representation depicted in Fig. 5. Mechanisms for the numbered molecular interactions are described in detail as follows: (1) After the IL-1β is bound to the IL-RI, (2) this ligand-receptor complex is associated with IL-1RAcP to form a complex containing IL-1β IL-1RI and IL-1RAcP [4]. The IL-1RAcP itself is unable to directly bind IL-1β ligand or transduce signals in the absence of IL-1RI. It serves as a functional partner to IL-1RI, generating an active receptor heterodimer in the presence of the ligand [36]. (3) Then, MyD88 is recruited to this complex, where it functions as an adaptor, supporting (4) the recruitment of IRAK and (5) adaptor protein TRAF6 in turn [5].

(4-6) It can be noted that TRAF6 is either recruited to receptor-bound IRAK or IRAK that is phosphorylated at the receptor complex prior to interaction with TRAF6 [6, 7]. The phosphorylation of IRAK is not necessary for JNK activation. However, in the case of NF-κB activation, IRAK is phosphorylated and (7) then interacts with TRAF6 [22]. Hence, IL-1β-induced NF-κB and JNK activation diverges at IRAK (Fig. 5). However, it has also been reported that phosphorylated IRAK interacts

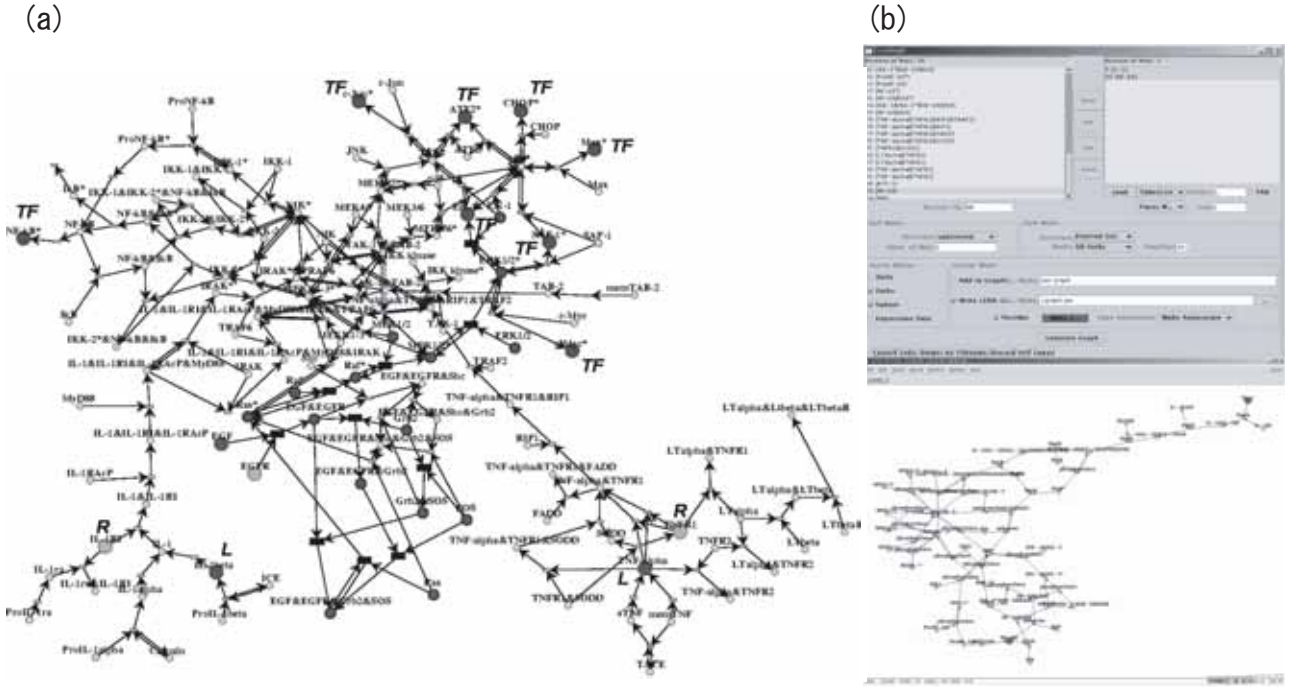


Figure 4: (a) Visualization of the annotated expert knowledge net induced by IL-1β and TNF-α as Petri-net representation using the ToPNet program. (b) Resultant screen shot of ToPNet for finding pathways and subnetworks from IL-1 to NF-κB.

with TRAF6 for both IL-1β-induced JNK and NF-κB activation. [7, 24]. Eventually, phosphorylated IRAK is ubiquitinated and degraded [40].

One of the major signaling pathways to be identified in IL-1β and TNF-α systems involves NF-κB activation. First, a MAP kinase kinase kinase (MAP3K) NIK is essential for IL-1β-mediated NF-κB activation [2]. (8-9) It is associated with TRAF6 induced by IL-1β [32]. However, NIK has no effect on IL-1β activation of MAPKs including JNK, p38 and ERK. Instead, TRAF6 can be directly linked to MAPKs activation.

(8-9) After activated by the receptor-bound IRAK-TRAF6 complex, (11) NIK interacts with the IKK complex consisting of IKK1 and IKK2 subunits [21, 37]. (10) IKK complexes containing homodimers of IKK1 and IKK2 as well as heterodimers may exist, each having somewhat different properties and therefore providing variations on the common theme of signal-regulated IκB phosphorylation. The existence of IKK homo- and heterodimers and the ability of NIK to interact with both IKK isoforms suggest two possible types of NIK-IKK complexes. In one scenario, monomeric IKK may interact with either another IKK subunit or with NIK. Alternatively, NIK may associate with dimeric forms of IKK, and thus all three kinases can exist in a single complex. All possible mechanisms to form complexes can be considered, and in Fig. 5 the heterodimeric form of IKK is shown as the Petri-net representation.

However, recent studies have demonstrated that IKK1 is not required for IKK activation by TNF-α [13]. As the activation of IKK2 invariably leads to IκB degradation, which results in NF-κB activation, they suggested that NIK is not linked to IKK2. Instead, NIK was reported recently to be an upstream kinase of the NF-κB precursor p100/NF-κB2 to the mature NF-κB p52 subunit [31, 39], which can be considered as a novel pathway for the NF-κB activation (not shown in Fig. 5). Note that in this work, all possible pathways regarding NIK were considered as putative pathways: the conflicting and contradictory results have been also reported in other studies [23, 25]. The invalid pathways are to be modified if additional detailed mechanisms are identified through further experimental studies. (12-15) The NF-κB activation occurs when IκB is degraded by IKK2 following its phosphorylation and ubiquitination. This releases NF-κB from IκB, and allows it to enter the nucleus [33].

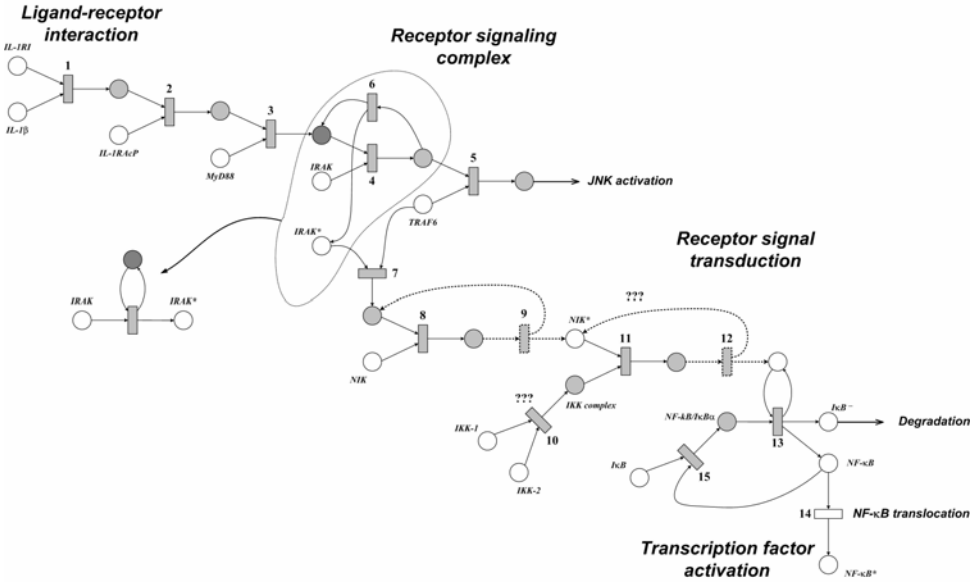


Figure 5: Reconstructed signal transduction pathways for NF-κB activation triggered by IL-1RI complex. Question marks (???) indicate unclear mechanisms which are considered as the putative ones. Detailed mechanisms for the numbered molecular interactions are described in the text.

3.2 Future Directions and Summary

A major future challenge is to explore drug effects on the pharmacological modulation within the pathways reconstructed in order to evaluate the efficacy, safety and cost-effectiveness of available drug candidates. To date, numerous anti-inflammatory drug targets have been identified at different levels in the signaling pathways [21, 41]. Currently being pursued by various investigators as potent anti-inflammatory drugs are the receptor antagonists, soluble receptors, compounds inhibiting protein kinase activity in intracellular signaling, and transcription factor blockers. Nevertheless, little has been done to investigate the pharmacodynamic and mechanistic effects of various drugs for signaling targets. This is attributable to the facts that the previous works have focused on the identification and characterization of drug targets, and that inflammatory pathomechanisms are yet to be well understood. Thus, the detailed molecular mechanisms of the reconstructed cellular signaling network can provide useful information for developing a rational approach to evaluate the mechanistic effects of drugs and for prioritizing drug candidates at the systems-level. We are currently investigating these possibilities.

In summary, the current study has established a conceptual framework for reconstructing signal transduction networks. This renders it possible to qualitatively analyze the network structure and the interactions among the network components on the basis of Petri-net representations. The detailed mechanisms of the network have been investigated through the application to the signal transduction system induced by IL-1β and TNF-α The results would be useful for the search of new targets for anti-inflammatory drugs. The main focus of the current study lies in the signal transduction system; nevertheless, some of the procedures adopted in the study can be extended to metabolic and gene regulation networks which can be characterized by 'material processing and energy transduction', and 'control theory and cellular programs', respectively. The methodologies and approaches presented in this paper should be also useful in expediting or facilitating drug discovery which would be the most important future undertaking.

Acknowledgments

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