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Petri Net Based Descriptions for Systematic Understanding of Biological Pathways

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SUMMARY Petri nets have recently become widely accepted as a description method for biological pathways by researchers in computer science as well as those in biology. This paper gives an overview of Petri net formalisms to describe biological pathways and discusses their use in modelings and simulations for the systematic understandings of biological pathways. After reviewing the use of various types of Petri nets for the biological pathway modelings, we showed the examples that analyze fundamental properties of biological pathways using T-invariant, P-invariant, siphon, and trap. Applications of hybrid Petri nets for producing new biological hypotheses through simulations are also illustrated.

key words: *Petri net, systems biology, biological pathways, modeling, simulation*

1. Introduction

Systems biology [20] is a new field that aims to integrate different levels of information to understand how biological processes function in a cell. The experimentally covered biological facts are usually summarized in a picture of network composed of figures of various shapes and several types of arrows reflecting the underlying biological images (See Fig. 3). Such pictures are called *biological pathways*. Many researches on biological pathway modelings using formal description methods have been made [19], and the systematic behaviors of the biological pathways have been observed by means of computer simulations.

Petri net is a formal description for modeling concurrent systems mainly applied to the artificial systems such as manufacturing systems [42] and communication protocols [53]. Petri nets have recently become widely accepted as a description method for biological pathways by researchers in computer science as well as those in biochemistry [40].

This paper gives an overview of Petri net formalisms to describe biological pathways and discusses their use in modelings and simulations for the systematic understandings of biological pathways. Section 2 reviews the use of various Petri nets in modeling biological pathways. This section also discusses about the existing simulation tools used for biological pathway simulations with referring the literature comparing these simulation tools.

Biological pathways can be classified into three categories; gene regulatory networks, metabolic pathways, and signaling pathways. Section 3 focuses on the formalization and analysis of metabolic pathways and signaling pathways using Petri nets. This section shows that structural properties of both of the metabolic and signaling pathways can be formulated by T-invariant, P-invariant, siphon, and trap which exhibit the fundamental behavioral properties of Petri nets.

In Sect. 4, we illustrate several practical examples using hybrid Petri nets and its extensions to produce biological hypotheses by means of simulations. Section 5 concludes this paper with introducing our new website available for the analyzes of biological pathways with Petri nets.

2. Petri Net Based Formalism of Biological Pathways and Simulation Tools

2.1 Hybrid Functional Petri Net with Extension

Biological pathways are reaction-networks of biological processes in a cell, which can be classified into three categories: metabolic pathways, gene regulatory networks, and signaling pathways. Due to the nature of concurrency of biological pathways, many researches using Petri nets on modeling biological pathways has been made. Metabolic pathways are the first biological pathways modeled by ordinary Petri nets [16], [43].

After these works, several kinds of high-level Petri nets have been employed to model biological pathways: colored Petri net [7], [26], [52], stochastic Petri net [10], [39], and hybrid Petri net [4]–[6], [23], [28]–[33], [35], [49]. Differences in modeling biological pathways between colored Petri net, stochastic Petri net, and hybrid Petri net are well discussed in [11] based on characteristics of these Petri nets. For other mathematical formalisms of biological pathways such as Bayesian networks, Boolean Networks, differential equations, and rule-based formalisms, the review [19] will be worth to read. This review focused on gene regulatory systems, but these formalisms have been also applied to modeling of metabolic pathways and signaling pathways.

These many attempts using high-level Petri nets prove that there exist a variety of requirements in biological pathway modeling. Based on this observation, Nagasaki et al. [35] proposed a new powerful Petri net architecture *hybrid functional Petri net with extension* (HFPNe) which involves all the functions of existing high-level Petri nets. In other words, each of ordinary Petri net, stochastic Petri net,

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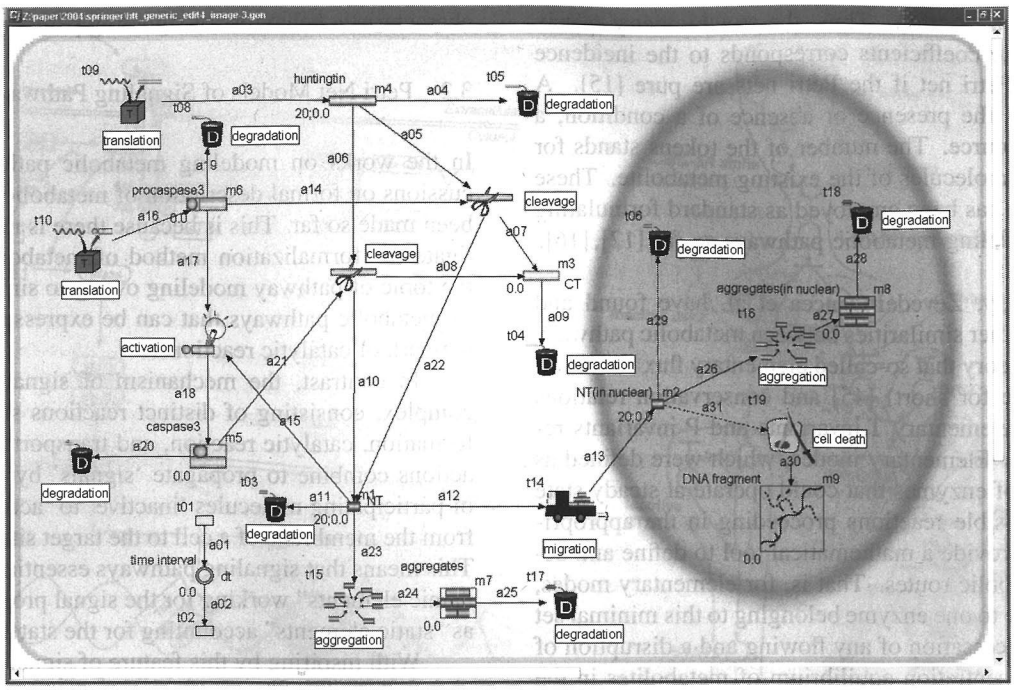

Fig. 1 Huntington's disease HFPNe model in the screen shot of Cell Illustrator [37].

colored Petri net, and hybrid Petri net can be treated as a subset of the HFPNe. Precise definition of the HFPNe as well as relationships with other Petri nets are described in [35]. Another aspect on this topic was presented in Peleg [39], suggesting that the best high-level Petri net tool must be selected depending on the requirements for the modeling goals or multiple tools must be used.

2.2 Biological Pathway Simulation Tools

Cell Illustrator [59] is a pathway simulation tool which employs the HFPNe as a basic architecture. Figure 1 shows a screen shot of Cell Illustrator which displays Huntington's disease model with HFPNe. In this figure, Petri net elements of places and transitions have been changed to pictures of biological images which reflect the roles of these elements. These changes make pathway models with HFPNe more familiar to biologists. Please refer to [35] for the details of this pathway.

Peleg et al. [39] surveyed Petri net formalisms and tools and compared them based on their mathematical capabilities as well as by their appropriateness to represent typical biological processes. They compared 5 tools, Mobius [3], TimeNET [8], Design/CPN [18], Genomic Object Net (GON) [4], [36]†, and Woflan, evaluating the ability of these tools to model specific features of biological pathways by answering a set of biological questions that they originally defined in the paper. Another suggestion had been made by them [38] prior to this paper, proposing to combine Workflow/Petri Net and a biological concept model in order to represent and simulate biological processes, under the assessment of eleven diverse models that were developed in the field of software engineering, business, and biology.

For the comparisons of simulations tools with other formalisms, Chen and Hofestädt [1] evaluate four tools Gepasi [34], Jarnac [58], DBsolve [55], and E-Cell [48] in terms of 11 criteria including core algorithms, user interface, and programming language. In addition, they mentioned about advantages of Petri net based modeling method with respect to graphical modeling representation and sound mathematical background. Nagasaki et al. [37] compared six software packages including GON, E-Cell, and Gepasi on 16 criteria especially with the view of enrichment of GUI and performance on XML descriptions for their software packages.

3. Qualitative Modeling of Biological Pathways with Petri Nets

3.1 Analysis of Metabolic Pathways with Petri Nets

Metabolic pathways have such intrinsic characteristic that are series of chemical reactions catalyzed by enzymes, resulting in either the formation of a metabolic product or the initiation of other metabolic pathways. The idea to use Petri nets for modeling and analyzing metabolic pathways have been popularly developed owing to the unique and explicit modeling foundation of metabolic pathways from the first paper by Reddy et al. [43]. In the paper, they have used two kinds of nodes: places and transitions of Petri net to denote metabolites and reactions respectively. Further in metabolic pathways, the stoichiometric coefficients described as arc-weights indicate the amounts of substances and products

†Former software name of Cell Illustrator.

participated in a reaction. Thus, the stoichiometry matrix containing these coefficients corresponds to the incidence matrix of the Petri net if the Petri nets are pure [15]. A token indicates the presence or absence of a condition, a signal, or a resource. The number of the tokens stands for the number of molecules of the existing metabolite. These modeling rules has been employed as standard formulating patterns in modeling metabolic pathways so far [12], [16], [24], [52], [54].

Interestingly, Zevedei-Oancea et al. have found and discussed the other similarities between metabolic pathways and Petri net theory that so-called elementary flux mode (elementary mode for short) [45] and conservation relations correspond to elementary T-invariants and P-invariants respectively [54]. Elementary modes, which were defined as a minimal set of enzymes that could operate at steady state with all irreversible reactions proceeding in the appropriate direction, provide a mathematical tool to define and describe all metabolic routes. That is, for elementary modes, any disturbance to one enzyme belonging to this minimal set will result in a cessation of any flowing and a disruption of a dynamic concentration equilibrium of metabolites in the system. In [52], elementary modes have been discussed to show the correspondence of elementary T-invariants to Petri nets. Further, the authors have calculated elementary T-invariant from Petri nets that model glycolytic pathway and pentose phosphate pathway with showing the process to discover elementary modes. Similarly, elementary P-invariants have been calculated to represent the preservation law for the amounts of all metabolites in the system, i.e. a positive linear combination of all concentrations (token numbers in Petri nets) is constant in time.

Moreover, the concepts of traps, siphon, deadlock and liveness have been considered to reveal some properties of metabolic pathways by Zevedei-Oancea et al. They have revealed that (1) a trap is such a set of places corresponding to storage metabolites steadily increased during growth of an organism; (2) a siphon is such a set of places corresponding to storage metabolites gradually decreased during starvation; (3) the check for deadlock helps to determine whether a biochemical pathway can reach a false equilibrium where it is blocked; (4) the liveness of a system indicates that all transitions are able to fire infinitely. They have also presented an example of *Trypanosoma brucei* metabolism to detect siphons and traps for validation of their consideration.

The authors of M. Heiner, I. Koch and K. Voss have proposed a method for applying higher-level Petri net to design and qualitatively analyze metabolic steady state models [52]. They have used colored Petri net with individual tokens which offers the possibility to distinguish the metabolites to detect and further give the interpretation of invariants.

As for intracellular interaction pathways, there also exist signaling pathways besides metabolic pathways. As we have stated above, the metabolic pathways can be described by elementary modes, in the following, we will inquire the characteristic behaviors of signaling pathways.

3.2 Petri Net Models of Signaling Pathways

In the works on modeling metabolic pathways, few discussions on formal description of metabolic pathways have been made so far. This is because there is no need to investigate the formalization method of metabolic pathways as the topic of pathway modeling owing to simple mechanism of metabolic pathways that can be expressed by a uniform network of catalytic reactions.

In contrast, the mechanism of signaling pathway is complex, consisting of distinct reactions such as complex formation, catalytic reaction, and transportation. These reactions combine to propagate 'signals' by changing states of participating molecules 'inactive' to 'active' in sequence from the membrane of a cell to the target site in the nucleus. This means that signaling pathways essentially involve "dynamic elements" working for the signal propagation as well as "static elements" accounting for the states of molecules.

With inspiring by this feature of signaling pathways of the combination of dynamic and static elements, a few researchers have tried to formalize signaling pathways with Petri nets. Lee et al. [25] modeled a signaling pathway of NF-κB activation process triggered by IL-1RI complex with the Petri net components presented in their paper. They have recently simulated a signaling pathway stimulated by EGF with Design/CPN [26]. Choi et al. [2] presented a bipartite directed graph[†] of the TGF-β pathway from ligand-receptor interaction to activation of Smad transcription factor heterodimer.

The attempts to model and analyze signaling pathways have been made by Heiner et al. in 2004. The paper explains how to model and validate the apoptosis pathways (cell death) by using qualitative Petri nets. In [13], they have given the modeling methods that (1) each biochemical compound or receptor is assigned to a place; (2) the relations between any biochemical substances are represented by transitions with corresponding arcs (3) the enzyme reactions are modeled by test arc. Further they have performed the model validation by changing all the test arcs to unidirectional ones and calculating elementary T-invariants that can give the description of biological pathways.

Li et al. [27] have proposed methodology to model and analyze signaling pathways. Their modeling methods based on Petri net have focused on the molecular interactions and mechanisms and presented 12 reaction types (e.g. autophosphorylation, chemical reaction, gathering action and so on) and corresponding Petri net models as long as the biological facts have been known. Figure 2 illustrates the signaling pathways mediated by cytokine Interleukin-3 (IL-3) in which a cytokine promotes the proliferation and differentiation of bone marrow-derived hematopoietic cells. IL-3 signaling regulates the cell through several major pathways in-

[†]They do not use the term "Petri net," while the signaling pathway models by their bipartite directed graph consist in Petri nets.

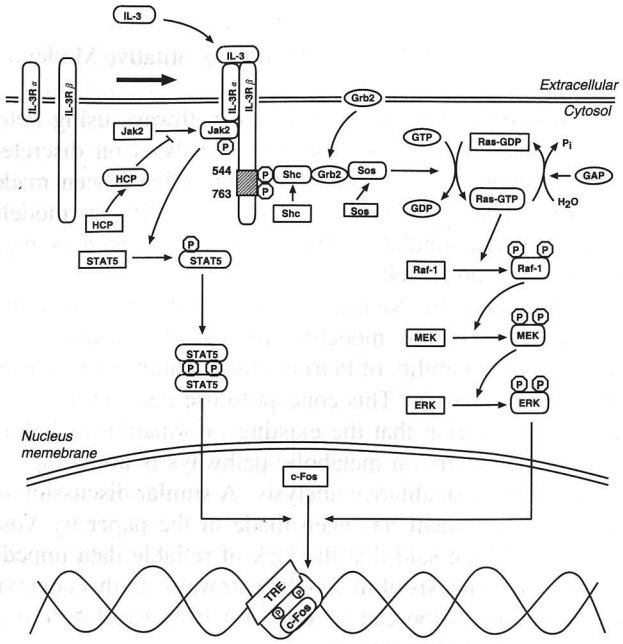

Fig. 2 IL-3 signaling pathway.

cluding JAK/Stat and Ras-MAPK. Based on their modeling methods, IL-3-induced signaling pathways can be modeled naturally and explicitly to a Petri net model.

Furthermore, they have introduced a new notion *activation transduction components* (ATC for short) in order to describe an enzymic activation process of reactions in signaling pathways and show its correspondence to elementary T-invariant in the Petri net models. The purpose of their paper is to inquire into the behaviors of sequential enzymic activation processes of signaling pathways. And the features of signaling pathways have been given that the signal propagates itself through a series of sequential enzymic activation processes where a certain enzyme changes from "inactivate" state to "activate" state depending on the functions of upstream enzymes. In this way, they have introduced the notion of ATC to represent a set of reactions and related substances that make an enzyme active.

They also have revealed that such an ATC corresponds to the subnet generated by the elementary T-invariant representing a fundamental periodic behavior. This is because, the subnet has such features that, (i) before and after any firing sequence corresponding to an elementary T-invariant, the tokens on each place in the subnet are kept constant; and (ii) all the transitions in subnet take part in the firing sequence.

Last, they have also proposed an algorithm to give relations among ATCs in signaling pathways in order to clarify how enzymic activation processes occur in their paper. Figure 3 shows all the subnets of the Petri net model of IL-3-induced signaling pathways. Then they have used the result of algorithm, to schematize the connection relations between the subnets that correspond to ATCs in signaling pathways. So far, this paper gives us a new insight into the

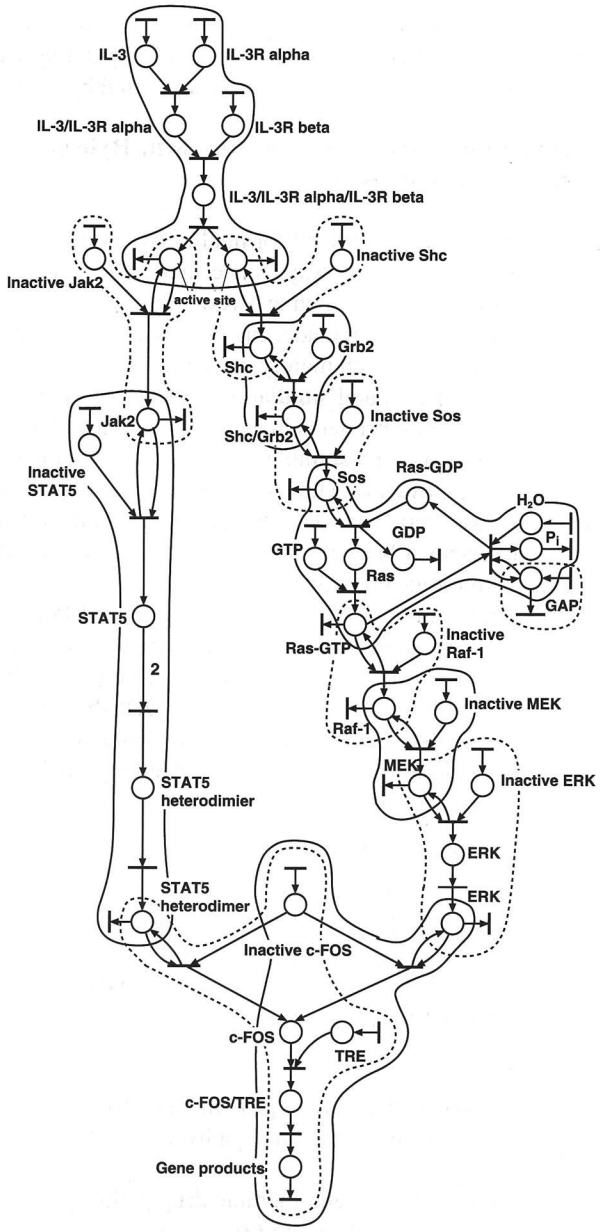

Fig. 3 Petri net model of IL-3 signaling pathway. Each subnet surrounded by a line corresponds to the activation transduction component.

architecture of signaling pathways to grasp structural and behavioral properties based on the novel consistent modeling methods.

While agreeing with the usefulness of Petri nets in modeling and analyzing large complicated signaling pathways, Takai-Igarashi [47] pointed out the inconsistencies of existing Petri net representations of signaling pathways, which may diminish the further utilization of the accumulated Petri net models such as in reusing, sharing and scaling up them. She has proposed a set of standard units of Petri net based on Cell Signaling Network Ontology (CSNO) [46], which allows us to construct signaling pathway models with no inconsistency. CSNO is a clear structured framework for systematic, consistent, and shareable description of signal-

ing pathways. The study aims at reconstructing comprehensive knowledge of cellular functions into a computer system on the basis of Petri net representation supported by CSNO.

4. Quantitative Pathway Modeling with Hybrid Petri Net and Its Extension

Petri nets have been employed for qualitative modeling and analysis of biological pathways since many theoretical investigations on Petri nets such as structural analysis of systems in the past have been made. In contrast, ordinary differential equations have been mainly used as the techniques for quantitative modeling and simulations. It has likely been a common sense that Petri net is in good agreement with qualitative evaluation but not suitable for quantitative evaluation of biological pathways. However, there have been some attempts to use Petri net for quantitative evaluation. Section 4.1 describes a brief history using Petri nets to model and analyze biological pathways.

Matsuno et al. [29] demonstrated that hybrid Petri net (HPN) has high potential to model and simulate biological pathways through the example of λ-phage genetic switch mechanism; for this pathway model, many ODE-based qualitative evaluations have been conducted. After this work, many biological pathways have been created with the technique of HPN or its extension as listed below.

- Gene Regulatory Networks
  - λ phage genetic switch [29]
  - circadian rhythms of fruit fly [30]
  - circadian rhythms of mouse [33]
  - cancer gene regulation (p53, MDM2, and p19ARF) [5]
- Signaling Pathways
  - apoptosis induced by protein Fas [30]
  - Notch-Delta signaling pathway in *Drosophila* [32]
  - gemcitabine chemotherapic drug pathway [39]
  - Huntington's disease [37]
  - role of interleukin-6 in the fate of haematopoietic stem cells [49]
  - Raf-1 kinase inhibitor protein on the extracelluar signal regulated kinase [14]
- Metabolic Pathways
  - urea cycle and its regulation [1]
  - *lac* operon and glycolytic pathway [31]
- Protein Networks (cell cycle)
  - cell division process of *Xeopus* [28]
  - fission yeast cell cycle [6]

In Sect. 4.2, the prominent feature of HPN-based pathway modeling in finding a new biological hypothesis is illustrated using circadian genetic control mechanism [33]. Sections 4.3 and 4.4 are dedicated to introduce other practical works using the HPN to find new biological facts.

4.1 Petri Net Based Formalism for Quantitative Modeling

The structural analysis of biological pathways using Petri net in Sect. 3 consists in qualitative analysis on discrete-event system. On the other hand, there have been made quantitative analysis on the systems of continuous models such as kinetic simulations with differential equations, e.g., Gepasi [34] and E-Cell [48].

The paper by Reddy et al. [43], which have firstly applied Petri net to modeling biological pathways, describes the availability of Petri net for qualitative analysis of metabolic pathways. This concept to use Petri net is based on the observation that the existing information including kinetic parameters on metabolic pathways is not complete for conducting qualitative analysis. A similar discussion to the above argument has been made in the paper by Voss et al. [52] which said that the lack of reliable data impede quantitative analysis of metabolic pathways. In this context, the same discussion can be done for gene regulatory networks and signaling pathways.

It can be considered that discrete nature of Petri net does not promote the use of Petri net for quantitative analysis of biological pathways. However, a few attempts aiming at quantitative analysis by discrete-based Petri net are found in the papers [7], [17], [41]. Hofestädt and Thelen [17] have introduced "functional Petri net" which allows to calculate dynamic biocatalytic process by using functions for specifying the arrow weight. With the functional Petri net, they illustrated the method to simulate biochemical networks. Interesting application of the functional Petri net is found in [21] which tries to infer the structure and the set of parameters of functional Petri net representing a metabolic pathway from observed data with the evolutionary algorithm. Genrich et al. [7] used Design/CPN to produce continuous behaviors of substances in metabolic processes of glycolysis. Popova-Zeugmann et al. [41] have tried to bridge the gap between qualitative and quantitative models in steady state, showing a procedure to transform a qualitative model into a quantitative one by exploiting T-invariants.

4.2 Process of Hypothesis Production Using HFPN Model in Circadian Genetic Control Mechanism

Figure 4 shows the interaction map of mammalian circadian regulatory system. *Per* and *Cry* genes are transcribed by the CLOCK/BMAL protein complex, and translated into proteins that form heterodimers before returning to the nucleus. Products of the *Clock* and *Bmal* genes heterodimerize to form the positive transcription factor for *Per*, *Cry*, and *Rev-Erb* genes; their effects are counteracted by the PER/CRY protein complex. The REV-ERB protein represses the transcription of the *Bmal* gene.

The bold dotted arc in Fig. 4 is a hypothetical interaction "PER/CRY activates *Bmal*," which has not been identified by biological experiments yet. This hypothetical interaction is also included in the corresponding HFPN model of

Fig. 5 as the bold dotted arc. In the following, we describe the process finding this hypothetical interaction.

First, using the HFPN model without the bold dotted arc, we simulated the behavior of the five genes above. Figure 6(a) is the result of the simulation. In this figure, the *Bmal* expression peaks at almost the same time as the *Cry* and *Per* expressions. However, as per the biological observations [44], the peak of *Bmal* expression has to be located approximately at the mid point of two successive *Per* or *Cry* expression.

Then, we use the following known fact for addressing this inconsistency between the two results from the simulation and from the biological experiment; in circadian genetic control mechanism of fruit fly, the interaction corresponding to the interaction "PER/CRY activates *Bmal*" of mouse has been already found [9]. Accordingly, we conducted simulations with the HFPN model incorporating this interaction, i.e., the HFPN model of Fig. 5.

Figure 6(b) shows the result of simulation on this hypothetical-interaction-incorporated HFPN model. After some parameter tuning, the *Bmal* expression peak can successfully be located almost at the mid point of two successive peaks of *Per* or *Cry* expressions. This result implies that the interaction "PER/CRY activates *Bmal*" exists in mammal circadian genetic control mechanism, while this interaction has not been biologically identified. In addition, we have shown the reason that *Bmal* expression peak can not locate at the mid point of *Per* (*Cry*) expressions. Please refer to the paper [33] for the details.

4.3 New Roles of Cell Cycle Proteins Suggested by Simulations

A cell is divided into two cells. This dividing process is basically the process of DNA replication in the nucleus followed by physical separation of the two complete genomes to daughter nuclei. While a cell is in proliferation, this dividing process are repeated, thus making a "cycle" of DNA

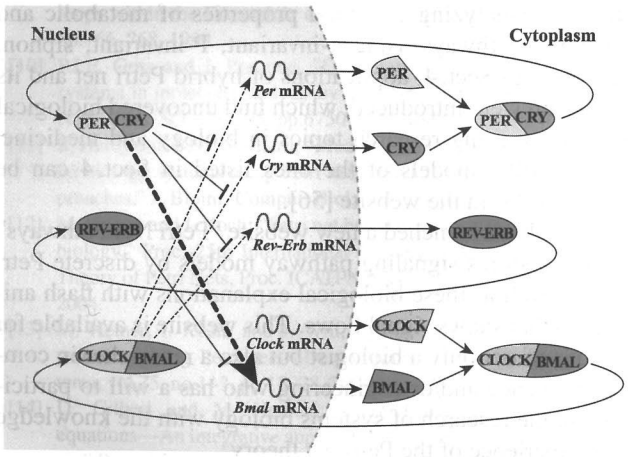

Fig. 4 Mammalian circadian gene regulatory mechanism [33]. The bold dotted arc indicates the hypothetical interaction which has not been biologically confirmed.

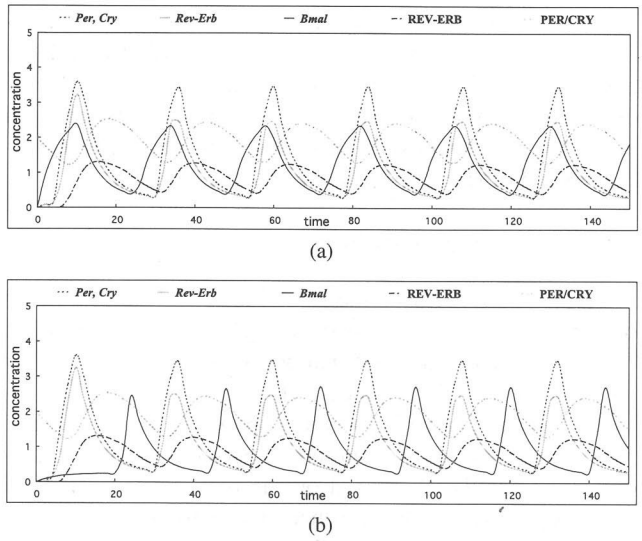

Fig. 6 Expressions of *Bmal*, *Cry*, *Per*, *Rev-Erb*, and *Clock* genes [33]. (a) Without the hypothetical interaction. The *Bmal* expression peaks at almost the same time as the *Cry* and *Per* expressions. (b) Hypothetical interaction is introduced. The peak of *Bmal* expression is located almost at the mid point of two successive peaks of *Per* and *Cry* expressions.

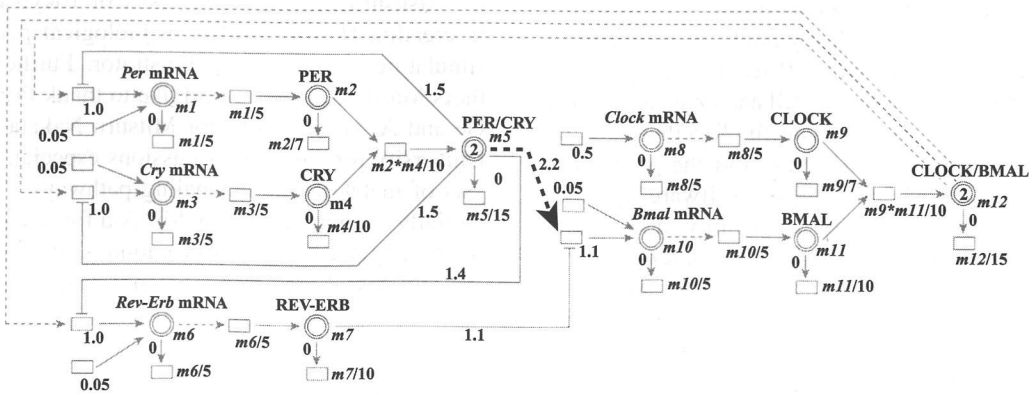

Fig. 5 HFPN model of mammalian circadian gene regulatory mechanism [33]. The bold dotted arc corresponds to the hypothetical interaction in Fig. 4.

replication and genome separation. This cycle is called "cell cycle." Cell cycle is driven by many proteins; each protein has its individual role, expressing at the stage when it should work in cell cycle. Details of the protein regulation in cell cycle is explained in the review by Tyson et al. [50].

MPF, which is known as one of the important protein complex of cell cycle, works when a cell goes into the stage of genome separation. Kotani et al. [23] examined the role of the protein Wee1 of human, which has been known to work as an inhibitor of the MPF, showing that Wee1 essentially works to regulate the rate of cell division, by means of computer simulations with the hybrid Petri net. In addition, their simulation results suggest that the possible range of Wee1 expression for cell division is very narrow, and in the narrow range, the number of cell division increases with less amount of expression of Wee1 protein.

Another suggestion on the cell cycle protein regulation is found in the paper by Fujita et al. [6]. Rum1 is a protein which suppresses the activation of MPF during the phase after cell division, thus over-expression of Rum1 causes the arrest of the MPF activation. With the created HFPN model of fission yeast cell cycle, they gave a suggestion for the Rum1 expression that the period of cell cycle becomes longer if Rum1 expresses weakly. This suggestion should be examined by a biological experiment in the near future.

4.4 Applications in Medicine

Troncale et al. [49] used the HFPN to model the regulation of haematopoiesis and investigated the role of Interleukin-6 (IL-6) in human early haematopoiesis by simulations with the constructed HFPN model. IL-6 activation can be considered as an epigenetic switch, responsible for bi-stability; self-renewal and differentiation of permissive haematopoietic stem cell (HSC). Based on the biological observation [51], they suggested the presence of an autocrine loop which functions as the positive feedback circuit for activating IL-6. This suggested positive feedback loop was examined with the HFPN model. Simulation result showed that this positive feedback loop is not functional and that production of sIL-6 (substance produced by this feedback loop) does not constitute an epigenetic modification determining the fate of HSCs, in the actual experimental conditions.

Peleg et al. [39] gave a pathway model of gemcitabine chemotherapeutic drug with hybrid Petri net. The pathway starts from the drug's intake by the cell and ends in its effect on cancerous cells resulting in cell death. Together with the hybrid Petri net model, a Mobius model of the pathway is presented in the paper. Mobius [60] is a software for modeling and studying the reliability, availability, and performance of complex systems, which supports multiple high-level modeling formalisms, including stochastic extensions to Petri nets, Markov chains and extensions, and stochastic process algebras. They performed simulations on these pathway models and presented the results from Mobius tool which evaluates the effect of drug by examining the occurrence of cell death as a function of drug dose.

5. Conclusions

Along with the progress of techniques to measure substances in a cell, a whole sketch of biological pathways in a cell will become uncovered. For the systematic understanding of biological pathways, computer simulation is indispensable, since it is too hard to grasp dynamic behaviors in a cell even for a small network consisting of 10 substances.

Petri net is a promising method for biological pathway modeling and simulation because it has the following characteristics;

- "firm mathematical foundation" enabling formal and clear representation of biological pathways as well as their structural analyses, and
- "visual representation of networks" which provides intuitive understanding of biological pathways without any mathematical descriptions which are basically difficult for ordinary biologists.

The first and second characteristics were mainly discussed in Sects. 3 and 4 with reviewing the published literature, respectively. Section 3 described the contribution of Petri net theory in analyzing structural properties of metabolic and signaling pathways using T-invariant, P-invariant, siphon, and trap. In Sect. 4, applications of hybrid Petri net and its extension were introduced, which find uncovered biological facts of ongoing research topics in biology and medicine. Some HFPN models of the ones listed in Sect. 4 can be download from the website [56].

We have launched a new website "Petri Net Pathways" [57] that stores signaling pathway models by discrete Petri nets as well as these biological explanations with flash animation that shows signal flows. This website is available for the use of not only a biologist but also a researcher in computer science and/or engineering who has a will to participate in the research of systems biology with the knowledge and experience of the Petri net theory.

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