

# PETRI NET BASED MODEL OF THE T CELL RECEPTOR SIGNALING PATHWAY

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## Abstract

*Intracellular signaling pathways as well as the interactions and coordination that exist among them are complex and difficult to visualize and understand. Computer-based models of such pathways can aid in understanding the mechanics of their operation. Such models allow the display and manipulation of the network of interacting components. Simulations that run on the constructed model emulate the dynamics of the modeled process. This allows the simultaneous interplay of the represented variables to be observed and analyzed, thus facilitating an understanding of the modeled process. The modeling of such pathways is the focus of this work. In particular, we have focused on creating an executable Petri net model of the T Cell Receptor (TCR) mediated signaling pathway. This pathway is a critical part of the body's defense mechanism against infectious agents. The model depicts the pathway in terms of the elements involved and events that occur.*

**Keywords:** Pathways, Signaling, Modeling, T-Cell, Petri Nets

## 1. Introduction

Intracellular signaling pathways are chains of sequential and concurrent biochemical reactions triggered inside cells in response to events that occur at the cell's surface. Cell surface receptors can recognize and bind specific molecules or "signals" from their environment, setting off a chain of *intracellular signaling pathways*. Events that occur at the cell surface are transmitted or signaled to the cell's interior through these signaling pathways. Notification to the cell's interior is necessary to shape the cell's response to the external event.

T cells are a special class of lymphocytes (White Blood Cells). Their surface is lined with receptors (T Cell Receptors) that can recognize and bind infectious agents (foreign antigens). They are therefore crucial components of the immune system with a major role in the body's immune response against infectious agents. Their absence results in impaired immunity and death from infections. Their strategy of containing and eliminating infectious agents has been well studied and continues to be the target of active research to help combat diseases. The TCR mediated signaling pathway includes the set of events that 'signal' or relay to the T cell's interior the binding of TCRs to foreign antigens and lead to the activation of an IL-2 gene to produce an IL-2 protein. The IL-2 protein promotes the division and proliferation of T cells. The proliferation of T cells is an important part of the T cell mediated combat strategy. It helps create an army of T cells to launch a successful attack on the invading foreign antigen.

Signaling through immune system receptors is therefore an important area of biological research. A clear and precise understanding of the regulatory control that leads to the activation, differentiation and proliferation of foreign antigen specific T cells is necessary to be able to manipulate the process with a therapeutic aim.

Computer based models of the highly integrated networks of intracellular signaling pathways provide a quick, cheap and convenient means of reproducing some semblance of the real process. Simulations capture the dynamics of the modeled system. Formulated hypotheses can be refined on the basis of the constructed model and tested in-vivo.

Constructive guidance on the design of new experiments can be gained to help identify all the parameters involved in the signaling pathway under study.

Petri nets have been used to model biological systems [1, 2, 3]. Our Petri net model is created from a biological description of the signaling process. Specifically, starting from a textual description of the pathway, we first identify the elements, events, relationships and dependencies that exist in that pathway and create a Petri net model based on these factors. We then use a Petri net-based tool to simulate and validate the series of events that lead to the generation of the IL-2 protein. Our model serves as a reference or qualitative base model for further extensions with the eventual aim of aiding the understanding of associated gene regulation. It provides a basic understanding of the modeled process, allows analysis on the basis of the biochemical reactions that result from a given concentration of reactants and products and can be used for intermediate validation. It is only intended to capture functional dependencies involved in signal propagation within a pathway.

The next section of this paper talks about the significance of T cells and their signaling mechanism. Section 3 contains the definition and details of Petri nets. Section 4 details our modeling approach, the resulting Petri net model and tools to validate it. Our conclusions are presented in Section 5.

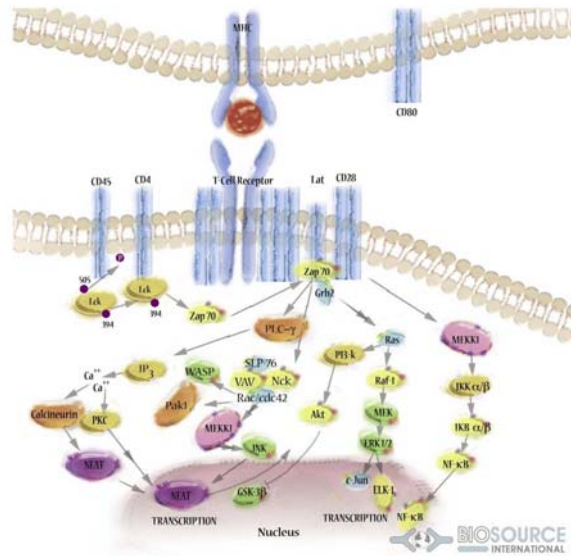
## **2. T Cell and Signaling**

We chose to study T cells and their signaling mechanism since T cells are regarded as the ‘epicenter’ of the immune system. They mature in the thymus and are responsible for cell-mediated immunity. There are 2 types of T cells – CD4 or the helper and CD8 or the killer T cells. The former enhance the response of other white blood cells while the latter directly kill infected cells. The number of helper T cells is seen to drop dramatically when infected with AIDS. Hence T cells that recognize HIV are being isolated and multiplied to inject into the bloodstream to target HIV. This is called T cell immunotherapy. T cells that attack certain herpes viruses have also been identified. T cells are being genetically engineered to improve the immune response. This paper focuses on CD4 helper T cells and their mode of action.

Signaling is initiated at the T Cell Receptors (TCRs) when they detect an infection within the body and launch an attack to remove it. The first step of the TCR mediated defense involves the interaction of TCRs with a foreign antigen presented on the surface of an Antigen Presenting Cell (APC). An APC is a cell that has engulfed and digested the foreign antigen. The digested antigen’s peptide fragments are displayed on the surface of the APC along with Major Histocompatibility Complex (MHC) class II proteins. TCRs that recognize the peptide fragment bind to the peptide:MHC ligand and initiate the process of annihilating the antigen. Another molecule (CD28) present on the T cell is also involved in the signaling process. It binds to CD80. These bindings trigger the T cell receptor mediated signal transduction pathway. Presence and engagement of TCRs and CD28 are critical for the initiation of the signal.

The binding event is signaled from the cell surface via the cytoplasm to the nucleus to effect the transcription of Interleukin-2 gene (IL-2). This is a growth factor for T cells. It is translated into IL-2 protein and secreted outside the T cell, where it is bound by IL-2 receptors present on the T cell surface. It induces T cells to divide, increase in number and produces an army of T cells to successfully attack and eliminate the infecting agent.

Chapter 6 of the text “Signaling Through Immune System Receptors” by C. A. Janeway et al. provides an excellent description of the role of T cells and their signaling mechanism [4]. A pictorial depiction of the T cell signaling pathway is given in Figure 1.



**Figure 1:** A pictorial depiction of the T Cell Signaling Pathway [5]

### 3. Petri Nets

Petri Nets are a formal graphical modeling and analysis language (notation). They can be executed to study the dynamics of the modeled system. Mathematical aspects of Petri Nets are closely related to automata theory [6, 7]. The static structure of a Petri net is a graph. Formally, a Petri net is a 4-tuple  $(S, T, F, M)$  where

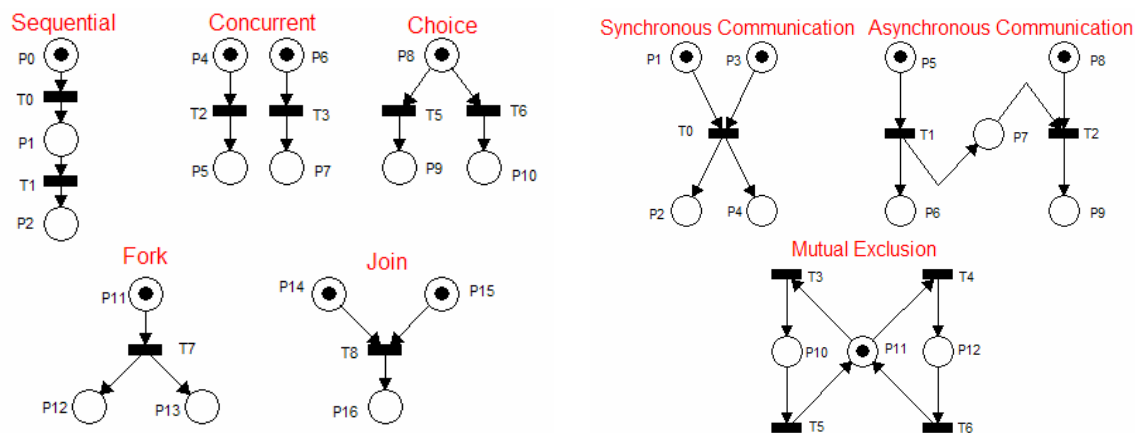
- $S$  is a finite set of *places*
- $T$  is a finite set of *transitions*
- $F$  is a subset of  $(S \times T) \cup (T \times S)$  and defines the *flow relation* or the *dependency relation*
- $S$  and  $T$  are disjoint

To understand the definition of a Petri net, it is helpful to have a very abstract level view of the workings of a system. Such a view stipulates existence of “state-like” objects ( $S$ ) and “event-like” objects ( $T$ ) and dependencies between these objects ( $F$ ). The basic idea is that “any” phenomena or system can be described in terms of “cause and effect”. The state-like objects become the cause for the event-like objects to “occur” and the resulting effect is “another” state-like object. There is an elegant graphical representation of nets.

In this representation, it is customary to denote places by a circle ( $\circ$ ) and transitions by a box/bar ( $\text{—}$ ). The flow relation is depicted by directed arcs ( $\rightarrow$ ) joining places and transitions and vice versa. The disjointness of  $S$  and  $T$  makes a Petri net a bipartite graph.

A *marked* Petri net is a Petri net together with a mapping  $M: S \rightarrow \mathcal{N}$ , where  $\mathcal{N}$  is the set of natural numbers. The graphical notation represents markings as bullets ( $\bullet$ ) inside circles that represent places on the net. In Petri net parlance, these bullets are called *tokens*. The distribution of tokens represents the combined current state of the underlying system being modeled. A transition in a marked Petri net is *enabled* if all its input places contain at least one token. An enabled transition may *fire* by removing one token from each of its input places and depositing a token in each of its output places. Multiple transitions may be enabled and fired to capture concurrent behavior. The firings of various transitions capture the dynamic aspect of a Petri net and hence the behavior of the underlying system. Through transition firings, a Petri net evolves from one marking to a new marking. A marking  $M'$  is *directly reachable* from a marking  $M$  if there is a transition that is enabled in  $M$  and whose firing yields  $M'$ . A marking  $M'$  is *reachable* from a marking  $M$  if there is a sequence of transitions whose firing begins at  $M$  and yields  $M'$ . The *state-space* of a Petri net is the set of all markings reachable from an *initial marking*.

Systems modeled as Petri Nets are analyzed to derive emergent properties of the modeled system. They are ideal for modeling sequential, concurrent, synchronous and asynchronous behavior. Sequential execution depicts causal relationship in a Petri net structure whereas lack of causality captures concurrency. Petri nets also model scenarios where there is a conflict for shared resources. The following configurations (Figure 2 below) are helpful when modeling a system using Petri nets. These configurations depict some common situations that one may encounter during the modeling phase and are often helpful in debugging and analyzing a Petri net.



**Figure 2:** Petri net configuration examples

## 4. Approach and Results

Our model is intended to only capture the functional dependencies involved in signal propagation within a pathway. Specifically, starting from a textual description of the pathway, we first identify the key elements, events, causal relationships and dependencies that exist in that pathway. Absence of a causal relationship indicated concurrency. The key steps of the pathway were translated to elements of a Petri net model and validated. We give details of our translation of the natural language description of the T cell signaling pathway into a Petri net.

### 4.1 Key Steps in the Creation of the Petri net Model

- Use compounds or set of molecular activity values to represent places.
- Use an activity or response (e.g., phosphorylation, activation, binding, cleavage, release of ions etc) to denote a transition.
- Dependencies become causal relationships and get translated to a sequence of transition firings.
- Concurrent activities are captured naturally by having no causal relationship.

Applying these steps to the entire description of the T cell signaling pathway, we obtain the resulting Petri net shown in Figure 3.



**Figure 3: The resulting Petri net**

## 4.2 Observations

The resulting Petri net looks very similar to a flow diagram of the signal transduction process in appearance. It is hence very easy to follow and understand. Our net is of course more than just a static structural representation. It is a dynamic model with the added power of simulating the signal transduction pathway.

$\text{Ca}^{2+}$  represent a shared resource in this process. They are required for two of the intracellular signaling pathways leading to the production of the NFAT and NF $\kappa$ B transcription factors. The first version of our Petri net modeled this situation as a conflict scenario leading to the production of either NFAT or NF $\kappa$ B but not both. We rectified this behavior to simulate the in-vivo behavior of producing both NFAT and NF $\kappa$ B. We represented  $\text{Ca}^{2+}$  twice as two separate places on the Petri net. One provided input to the NFAT pathway while the other triggered the NF $\kappa$ B production path. This removed the conflict and allowed all tokens to pass through to the end in the simulation.

Recall that the three intracellular signaling pathways leading to the production of the NFAT, NF $\kappa$ B and AP-1 transcription factors are concurrent pathways and show up as causally independent sequences of transitions in the model.

All the three transcription factors, namely NFAT, NF $\kappa$ B and AP-1 must be available to proceed further. Absence of any one of them will not allow the pathway to proceed. In terms of Petri net, this is synchronization. It symbolizes the “waiting” state for the three transcription factors to become available before they can merge and act together (confluence of information). Synchronizations like this are also seen in other instances such as the following:

- LAT and SLP-76 together activate GEFs and Tec kinases
- Mek1 and Mek2 together activate Erk1 and Erk2
- Erk1 and Erk2 together phosphorylate and activate the transcription factor Elk

The following are instances of forking scenarios in our Petri net:

- Phosphorylation of LAT and SLP-76 by ZAP-70 produces phosphorylated LAT and phosphorylated SLP-76
- Production of activated Mek1 and Mek2 by Raf
- Production of activated Erk1 and Erk2 by Mek1 and Mek2

## 4.3 Tool and Validation

A free software tool called HPSim was used to create and execute the Petri net model. HPSim provides a simple graphical user interface to create, edit and simulate Petri nets. See [8] for more information.

We used the HPSim tool to:

- Create a simulated network of the T cell signaling pathway,
- Verify all causal relationships against biological descriptions,
- Check absence of deadlock states during execution, and
- Ensure completion of the Petri net’s execution.

## 5. Conclusions

The key steps of the TCR mediated signaling pathway were identified in terms of the bio-molecular complexes involved, their properties and the relationships among them. The compounds and events were translated to elements of a Petri net to depict and simulate the signal transduction pathway. Our Petri net specifically models and simulates the series of events triggered by TCRs that lead to the generation of the IL-2 protein. It was validated against biological textual descriptions as well as pictorial depictions.

Our Petri net can be viewed as a ‘base’ model of the T cell signaling pathway, providing a basis for an extended model using hierarchical colored Petri nets or hybrid Petri nets.

Data mining approaches can be applied to the extraction and (semi) automatic creation of such models since descriptions of the various aspects of biological pathways are seldom available in their entirety in a single source.

Our Petri net is a step in the direction of gaining a clear and precise understanding of the regulatory control that leads to the activation, differentiation and proliferation of antigen specific T cells. Such an understanding is critical to manipulate the process with a therapeutic aim. It will facilitate the discovery and design of new drugs.

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