International Journal of Pharma and Bio Sciences V1(2)2010

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

SHRUTI JAIN*[1], PRADEEP K. NAIK[2], SUNIL V. BHOOSHAN[1]

[1, 3] Department of Electronics and Communication Engineering, Jaypee University of Information Technology, Solan-173215, India

[2]Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Solan-173215, India

***Corresponding Author** jain.shruti15@gmail.com

ABSTRACT

This paper demonstrates the steps of a new integrating methodology to develop and analyze models of biological pathways in a systematic manner using well established Petri net technologies. The whole approach comprises stepwise modeling, animation, model validation as well as qualitative and quantitative analysis for behavior prediction. After a short introduction into systems biology we demonstrate how to develop and validate qualitative models of biological pathways in a systematic manner using the well-established Petri net analysis technique of place and transition invariants. This work examines signaling networks that control the survival decision treated with combinations of three primary signals, *tumor necrosis factor-α* (TNF), *epidermal growth factor* (EGF) and insulin. The example used in this paper is devoted to apoptosis, the genetically programmed cell death. Apoptosis is an essential part of normal physiology for most metazoan species. Disturbances in the apoptotic process could lead to several diseases. The signal transduction pathway of apoptosis includes highly complex mechanisms to control and execute programmed cell death.

KEYWORDS

TNF, EGF, Insulin, Places, Transitions

1. INTRODUCTION

Systems biology is an approach in which the digital information of the genome, acted upon by environmental cues, generates the many molecular signatures of gene and protein expression, as well as other, more phenomenological experimental observations. These data may be integrated together to form a testable hypothesis of how a biological organism functions as a system. The central components of systems biology are genetically programmed networks (circuits) within cells and networks of cells. These components establish the organization and function of individual cells and tissues in response to environmental signals such as cell-to-cell communication within organ systems and whole organisms. The emerging area of systems biology is a whole-istic approach to understanding biology. It aims at system-level understanding of biology, and to understand biological systems as a system. Systems Biology takes a different approach and tries to integrate the biological knowledge and to understand how the molecules act together within the

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

network of interaction that makes up life. Again, model building promises to be the key in advancing understanding. Modeling biological problems, as modeling in general, is always subject to simplifications, required to make modeling tractable from statistical and computational point of view, to make the models understandable to humans, or both. The essential properties of the system, given the desired level of abstraction and time-scale, should be captured by the model. Apoptosis is a process in which cells play an active role in their own death (which is why apoptosis is often referred to as cell suicide)[1, 2, 3, 4]. It is an organized self destruction of redundant and incorrigibly damaged living cells and is different from the usual or necrotic cell death [5, 6, 7]. The word, apoptosis, is derived from Greek roots meaning, "Dropping off" e.g. falling of leaves. It plays an important role in the development and maintenance of tissue homeostasis but also represents an effective mechanism by which abnormal cells, such as tumor cells, can be eliminated. Abnormalities in apoptotic function or resistance to apoptosis have been identified as important events in the pathogenesis of colorectal cancer and its resistance to chemotherapeutic drugs and radiotherapy.

The ability of cells to die by apoptosis is a fundamental property of animal cells [8, 9, 10, 11], it is an invariable part of animal development, and it often continues into adulthood. During development, the role of apoptosis is often very clear. For example, the cells that are needed only for the formation, not the final function of a certain organ or part of a body, can be carefully cleaned away. In particular in the nervous system, the cells that are "in the wrong place at the wrong time" are terminated before they cost too much, for example, in terms of energy consumption. Although the apoptotic destruction itself is an "expensive" process that consumes much energy and building materials to no constructive purpose, it is a sound investment in terms of the organism as an entity. When compared to the life of the whole organism, cells are apparently cheap and expendable.

Petri nets, or place-transition nets, are classical models of concurrency, non-determinism, and control flow, first proposed by Carl Adam Petri in 1962. Petri nets are bipartite graphs and provide an elegant and mathematically rigorous modeling framework for discrete event dynamically systems. Petri nets are directed, bipartite, attributed graphs. Bipartite means that they consist of two types of nodes (vertices), which are called in our context places $P = (p_1, p_2, p_3,...,\ldots p_m)$ and transitions $T = (t_1, t_2, t_3,...,\ldots t_n)$, and directed arcs (edges), which connect only nodes of different typ. Instead of the classical graph theoretic terms vertex and edges we use here the terms nodes and arcs, which are in the given context more popular.

Definition: A Petri net is a quadruple $\mathcal{N} = (P, T, f, m_o)$ where

1. P and T are finite, non-empty, and disjoint sets. P is the set of places. T is the set of Transitions
2. $f:((P\times T)\cup(T\times P))\rightarrow \mathrm{N}_0$ defines the set of directed arcs, weighted by non- negative integers.
3. $m_0 : P \rightarrow \mathrm{N}_0$ gives the initial marking.

Graphically places are represented by circles and transitions represented by square boxes. Petri net is a description method for modeling concurrent systems which has been mainly used to model artificial systems such as manufacturing system and communication protocols. From the first attempt by, several types of Petri nets including stochastic Petri net and colored Petri net have been employed to model biological pathways.

The Petri nets [12, 13, 14] can be used to describe logical relations among the states in a discrete event dynamic system, such as manufacturing system and product development process. The hybrid Petri nets is an integration of discrete Petri nets and continuous Petri nets, in which the discrete places and transitions

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

or the continuous places and transitions can describe the discrete event dynamics and the continuous variable dynamics respectively, and the interactions between them are described by the arcs between places and transitions and the functions defined on the transitions or arcs. To describe the behaviors of networked manufacturing systems in a more detail and quantitative way, the mathematical formulation of the dynamics is included in the hybrid Petri nets model.

The use of Petri nets for modeling quantitative (kinetic) properties of biochemical networks, especially for genetic and cell communication processes, was discussed in[13, 14, 15]. Other contributions followed, using various types of Petri nets like stochastic nets and hybrid nets.

2. BIOLOGY OF APOPTOSIS

Almost all cells, regardless of their phylogenetic origin[16, 17] or physiological specialization, ultimately senesce and die. There are several ways of dying, depending on the nature and severity of the death stimulus, type of the cell affected, and homeostatic conditions of the cell and its surroundings. The two major forms of cell death recognized today are apoptosis and necrosis[18, 19, 20, 21]. The main difference is thought to be the requirement for energy: apoptosis is an active process consuming energy and requiring macromolecular synthesis, while necrosis occurs passively[22, 23, 24, 25]. In spite the fact that there are several features that can distinguish these processes from each other, also common and overlapping characteristics appear, and there are cases where no clear cut distinction between apoptosis and necrosis can be found. While the characterization of differences between apoptotic and necrotic cell death remains incomplete, recent findings suggest that apoptotic cell death differs even from cell to cell, and each induction strategy is likely to involve a unique set of genes shown in Table 1.

Table 1
Programmed Cell death pathways

APOPTOSIS	NECROSIS
Caspase activation inhibition of mRNA translation	Pro-inflammatory signaling and cytokine production
Condensation of cell and organelles	Swelling of the cell and organelles
Chromatin condensation DNA fragmentation	Mottled chromatin condensation
Loss of membrane asymmetry	Loss of membrane asymmetry
Membrane remains impermeable	Rapid loss of membrane permeability
Cell falls apart into apoptotic bodies	Cell membrane explodes. Remains stay together

Apoptosis is a highly regulated process, being controlled by various ligands and signaling pathways. However, some pathways and events of the apoptotic program have been conserved among species and are considered as mediators of fundamental events in apoptotic signaling and cell death process itself, and

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

therefore, they appear to be of particular significance. These include for example, the action and regulation of the Bcl-family of proteins, cytochrome c release from mitochondria which is accompanied with mitochondrial dysfunction, and activation of caspases. The molecular genetic studies in the nematode Caernorhabditis elegans (C.elegans)[24, 25] have revealed the existence of conserved genes and gene families, such as ced-3 (homologs of mammalian caspases), ced-4 (Apaf-1), and ced-9 (the Bcl-family of proteins), which are involved in apoptosis.

3. SYSTEM APPROACH

During apoptosis, a complex death program is initiated that ultimately leads to the fragmentation of the cell. The death program can be either initiated by the cell itself or by certain external stimuli. These external stimuli may induce apoptosis by targeting one of two pathways. The 'extrinsic' pathway is initiated by triggering cell death receptors on the cell surface, leading to activation of the intracellular apoptotic machinery (*death signal-induced, death receptor-mediated pathway*). The 'intrinsic' pathway of apoptosis is initiated via the mitochondria by cellular stress, such as chemotherapeutic drugs and radiation (*the stress-induced, mitochondrion-mediated pathway*) (i.e. a caspase-9-dependent pathway) shown in Figure 1. The elucidation of the molecular mechanisms regulating these processes is of primary interest.

Caspases constitute one of most specific cysteine proteases with an absolute requirement for cleavage site after aspartic acid (hence the name *caspase*). At least four amino acids to the NH_2 terminal side of aspartate serve as the recognition site for cleavage and functional catalysis. The tetra peptide recognition motif differs significantly among caspases, explaining the diversity of their biological functions. The cleavage of protein by caspases is very specific and efficient. Caspases are specific cysteine-rich proteases. The involvement of caspases has been proved by a discovery of CED-3, a cell death gene product of a nematode (*Caenorhabditis elegun*). Caspases are requested for terminal differentiation of specific cell types, whether this differentiation process leads to enucleation or not. These enzymes also play a role in T and B lymphocyte proliferation and, in some circumstances, appear to be cytoprotective rather than cytotoxic. These pleiotropic functions implicate caspases in the control of life and death but the fine regulation of their dual effect remains poorly understood. Deregulation of the pathways in which caspases exert these non apoptotic functions is suspected to play a role in the pathophysiology of several human diseases. A total of 12 cysteine proteases known as caspases have been identified in mammals: caspase-1 to -10, caspase-12 and caspase-14. The protein initially named caspase-13 was later found to represent a bovine homolog of caspase-4, and caspase-11 is most likely the murine homolog of human caspase-4 and -5. They are divided into three groups: apoptosis activators like caspase 2, 8, 9, 10 that contain a long prodomain at the N-terminus; apoptosis executioners such as caspase 3, 6, 7 that have a short prodomain and inflammatory mediator with caspase 1, 4, 5, 11, 12, 13 and 14 .

Generally, there are two pathways through which the caspase family proteases can be activated: one is the *death signal-induced, death receptor-mediated pathway*; the other is the *stress-induced, mitochondrion-mediated pathway* (i.e. a caspase-9-dependent pathway)

3.1 Death receptor-mediated procaspase-activation pathway

3.1.1 Death receptor-dependent procaspase-activation pathway of caspase-8/caspase-10:

Cell death signals, such as Fas ligand (FasL) and tumor necrosis factor (TNF)-2, can be specifically

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

recognized by their corresponding death receptors, such as Fas or TNF receptor (TNFR)-1, in the plasma membrane. Their binding will in turn activate the death receptors. Fas can bind to the Fas-associated death domain (FADD) (or TNFR-associated death domain, TRADD) and cause FADD aggregation and the emergence of DEDs[26]. These exposed DEDs interact with the DEDs in the pro domain of procaspase-8, which will induce the oligomerization of procaspase-8 localized on the cytosolic side of the plasma membrane. Then a massive molecule complex known as the death-inducing signal complex (DISC) is formed. In DISC, two linear subunits of procaspase-8 compact to each other followed by procaspase-8 auto activation to caspase-8. The activation of the downstream pathways of caspase-8 varies with different cell types (Figure 1). In Type I cells (cells of some lymphoid cell lines), caspase-8 is vigorously activated and can directly activate the downstream procaspases (e.g. procaspase-3). In Type II cells (other than Type I cells), caspase-8 is only mildly activated and unable to activate procaspase-3 directly. However, it can activate the mitochondrion-mediated pathway by truncating Bid (a pro-apoptotic Bcl-2 family member), a kind of proapoptotic protein in the cytosol, into its active form, tBid. tBid will trigger the activation of the mitochondrion pathway: cytochrome c, apoptosis-inducing factor (AIF) and other molecules are released from mitochondria, and apoptosis will be induced.

The activation pathway mediated by procaspase-10, with a DED-containing prodomain, is similar to that mediated by procaspase-8. Caspase-10 functions mainly in the apoptosis of lymphoid cells [28]. It can function independently of caspase-8 in initiating Fas- and TNF-related apoptosis. Moreover, Fas crosslinking in primary human T cells leads to the recruitment and activation of procaspase-10. Although caspase-8 and caspase-10 both interact with the DED of FADD in death receptor signaling, they may have different apoptosis substrates and therefore potentially function distinctly in death receptor signaling or other cellular processes.

3.1.2 Death receptor-dependent pro caspase-activation pathway of caspase-2: Once death signals bind to their corresponding death receptors on the plasma membrane, death receptors will be activated. The activated receptors recruit procaspase-2 by adaptors, such as receptor-interacting protein (RIP), RIP associated ICH-1/CED-3 homologous protein with a death domain and TRADD, by means of the prodomain of procaspase-2. Procaspase-2 is activated after the recruitment. Very little has been understood so far concerning the downstream substrates of caspase-2.

3.2 Mitochondrion-mediated procaspase-activation pathway

3.2.1 Mitochondrion-mediated procaspase-activation pathway of caspase-8 : Apart from being recruited to form a DISC complex after autoactivation, procaspase-8 could also be activated through a cytochrome c-dependent pathway. After cytochrome c is released from mitochondria to the cytosol, caspase-6 is the only cytosolic caspase with the ability to activate procaspase-8, which depends solely on procaspase-6 activation by prodomain cleaving. It means that, in the cytochrome c-dependent pathway, the activation of procaspase-8 requires neither the interaction with FADD nor the formation of a DISC complex[26].

3.2.2 Mitochondrion-mediated procaspase-activation pathway of caspase-9 : When cellular stress (e.g. DNA damage) occurs, proapoptotic proteins in the cytosol will be activated, which will in turn induce the opening of mitochondrion permeability transition pores (MPTPs). As a result, cytochrome c localized in mitochondria will be released to the cytosol. With the

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

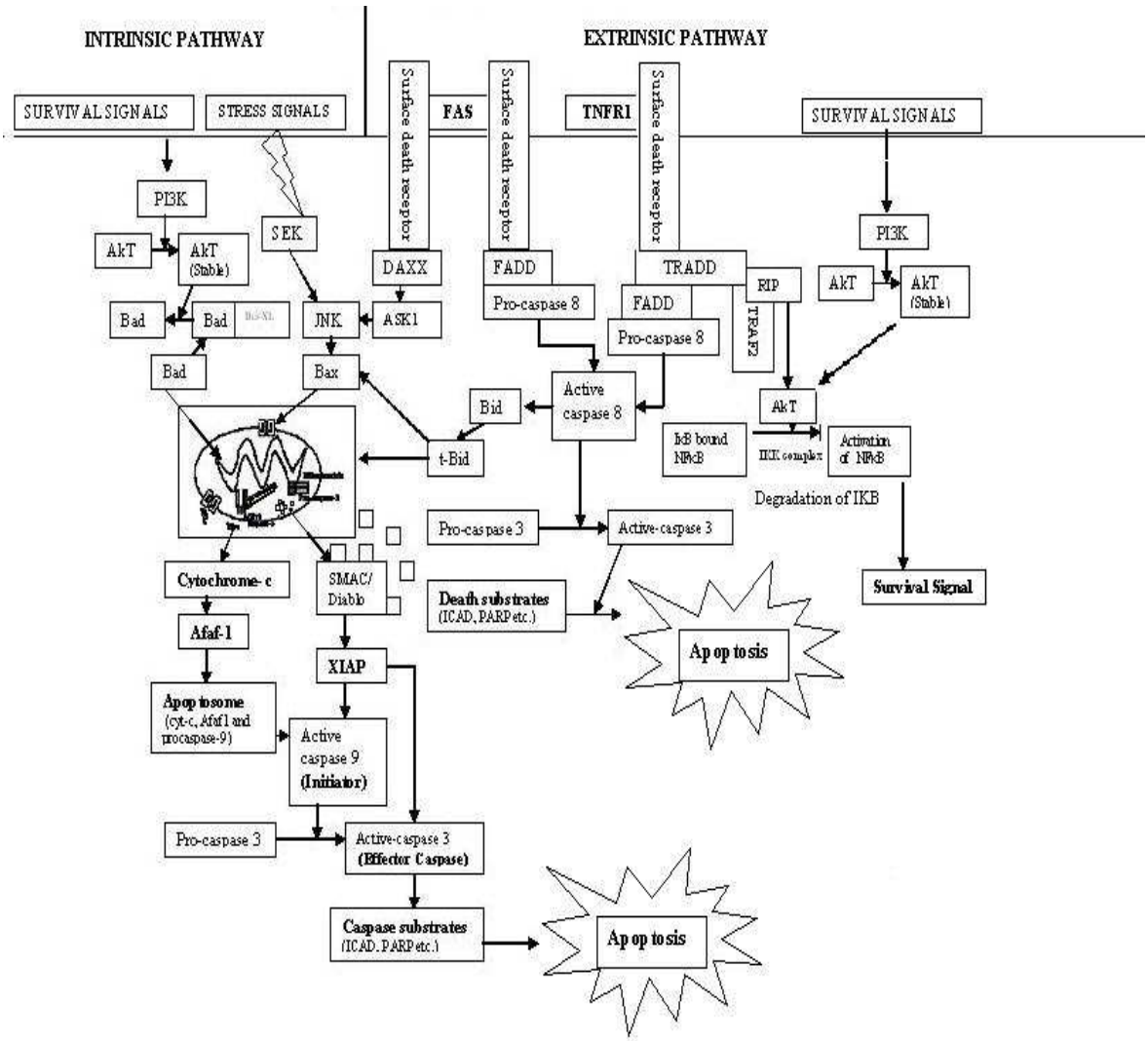
presence of cytosolic dATP (deoxyadenosine triphosphate) or ATP, apoptotic protease activation factor-1 (Apaf-1) oligomerizes. Together with cytosolic procaspase-9, dATP and cytochrome c, oligomerized Apaf-1 can result in the formation of a massive complex known as apoptosome. The N-terminal of Apaf-1 and the prodomain of procaspase-9 both have CARDs, with complementary shapes and opposite charges. They interact with each other by CARDs and form a complex in the proportion of 1:1. Activated caspase-9 can in turn activate procaspase-3 and procaspase-7. The activated caspase-3 will then activate procaspase-9 and form a positive feedback activation pathway (Figure 1).

In the mitochondrion-mediated activation pathway, Apaf-1 is a central component of the apoptosome. Apaf-1 has three distinct domains: an N-terminal CARD, a nucleotide-binding domain and 12–13 repeats of WD40 near its C-terminal. At least four different isoforms of Apaf-1 have been found, all of which contain the three domains resulted from the alternative splicing of Apaf-1 pre-mRNA. CARD is responsible for binding the prodomain of procaspase-9, thus it is important in procaspase-9 recruitment and activation. The sequence of the nucleotide-binding domain is very similar to CED-4 in *C. elegans*. For this reason, the domain is also referred to as the CED-4-homologous domain. This domain is responsible for Apaf-1 oligomerization in the presence of cytochrome c and dATP. The dATP-binding ability of Apaf-1 alone is poor, but with cytochrome c it can be greatly enhanced. Procaspase-9 also has a synergic promotion to the binding. WD40 repeats are involved in the interaction of Apaf-1 and cytochrome c. Recently, there have been many reports concerning the activation of caspase-9, which have challenged traditional ideas. Under normal physiological conditions, inactive caspase-9 exists in the form of a monomer. When caspase-9 is artificially crystallized or is recruited by Apaf-1 *in vivo*, the formation of a caspase-9 dimer results in the activation of caspase-9 [28]. According to these new results, alternative ideas have been brought forward about how procaspase-9 is activated and what molecules are required during the activation. One view generally held is that, although the prodomain of procaspase-9 is cleaved, the formation of the caspase-9 (or procaspase-9) dimer, rather than the cleavage, is essential to the activation of caspase-9. However, under some circumstances, the activation of procaspase-9 may be independent of mitochondrial factors, such as cytochrome c.

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

Figure 1 .

Schematic representing the core components of apoptosis pathways.



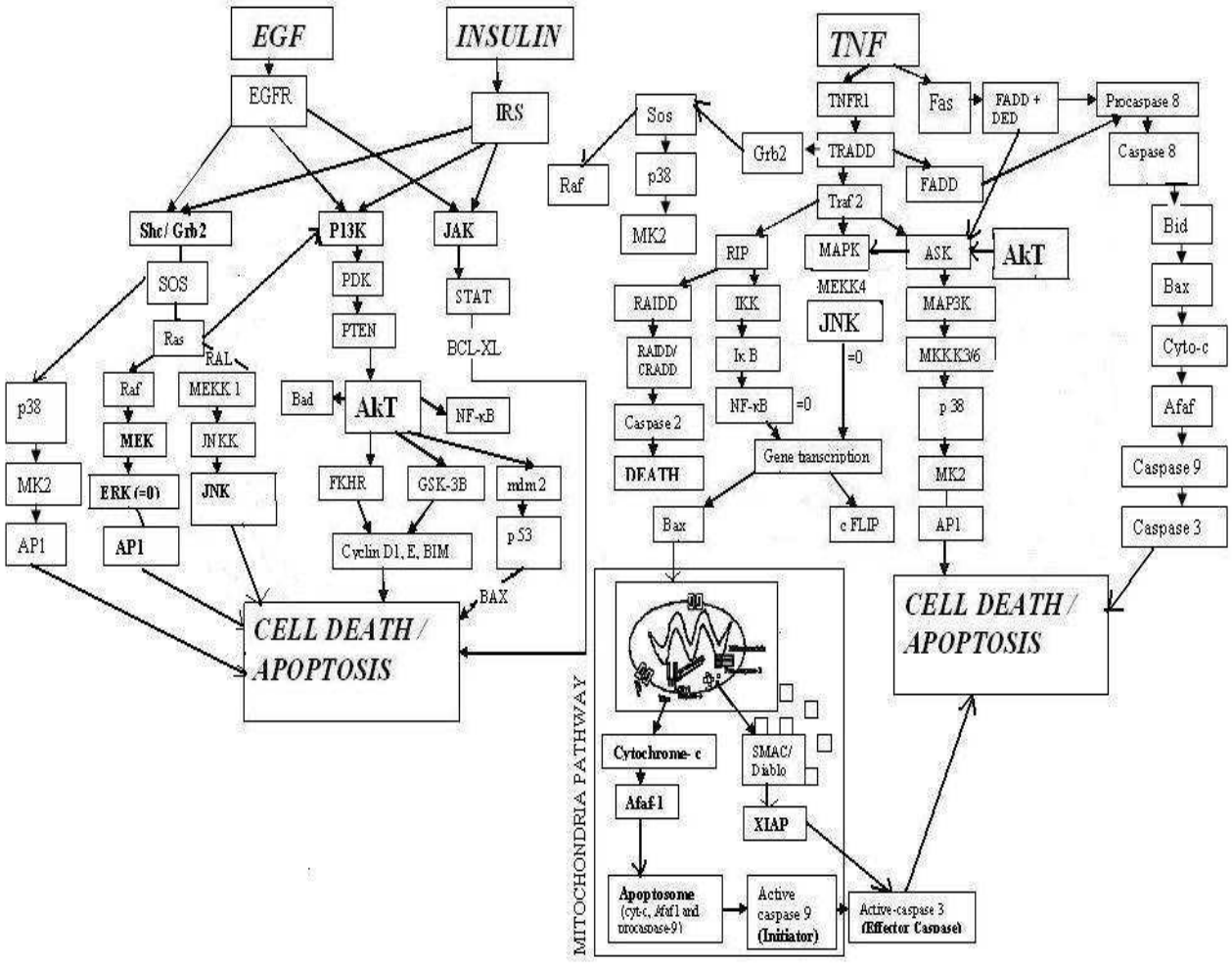
In the extrinsic pathway, TNF super family members including Fas Ligands binding to a death receptor and forming a death inducing signalling complex (DISC), which activate caspase-8. In the intrinsic pathway, cytochrome c released from mitochondria causes apoptosome formation and caspase-9 activation. Both caspase-8 and caspase-9 activate down stream caspases like caspase-3 and leading to apoptosis.

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

4. RESULTS AND DISCUSSIONS

We have implemented the signaling system heading by three input signals such as TNF, EGF and insulin. The block diagram of the signaling system that was modeled is shown in Figure 2.

Figure.2
Model of Cell Death



TNF induces apoptosis, although receptor ligation is rarely enough on its own to initiate apoptosis as is the case with Fas ligand binding. Binding of TNF alpha to TNFR1 results in receptor trimerisation and clustering

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

of intracellular death domains. This allows binding of an intracellular adapter molecule called TRADD (TNFR-associated death domain) via interactions between death domains. TRADD has the ability to recruit a number of different proteins to the activated receptor. Recruitment of TNF-associated factor 2 (TRAF2) can lead to activation of NF-kB and the JNK pathway. Other viral proteins prevent apoptosis by blocking stimulation of death receptors by antagonizing their activation or stimulating their turnover or inducing NF-κB stimulation. Inhibition of NF-κB activation produces a corresponding increase in apoptosis, indicating that the balance of cell viability vs cell death is maintained by the degree of NF-κB activation. The activation and subsequent activity of caspases are targeted by a variety of antiapoptotic proteins. The mammalian protein cFLIP (Flice (caspase-8) inhibitor protein; with homologs encoded by a variety of viruses) contains DED protein domains and an inactive caspase domain that prevents both the binding of caspase-8 to various death receptors and its activation. cFLIP does not, however, prevent apoptosis induced by granzyme B or by chemotherapeutic drugs and irradiation, which are both activated by a caspase-9 dependent mechanism. A different class of viral proteins that inhibited caspase activation is the inhibitor of apoptosis proteins (IAPs) originally identified in baculovirus where they prevented insect cell apoptosis. Mammalian counterparts to the IAPs have been found associated with the activated TNF receptors where they block the activation of caspase- 8, an association and activity which is upregulated by NF-κB activation. They also act downstream of mitochondrial release of cytochrome C to prevent activation of caspase-9. While the IAP proteins generally have less ability to block apoptosis by proactivated caspases, one member, XIAP, has been shown to block caspase-3 and caspase-7 activity directly via binding to one of its BIR regions. A third class of antiapoptotic proteins are direct inhibitors of caspase activity. These include the baculoviral inhibitor p35, a potent specific inhibitor of activated caspases that acts as a caspase pseudosubstrate. TRADD can also associate with FADD, which leads to the induction of apoptosis via the recruitment and cleavage of pro-caspase 8. The ligand for Fas (FasL or CD95L) activated apoptosis in a similar way to the TNF receptor. Binding of the ligand promotes receptor clustering, DISC formation and the activation of the caspase cascade. However, signalling through the Fas receptor is slightly simpler than through the TNF receptor. The adapter protein FADD can be recruited directly to the death domain on the Fas receptor, without requiring the prior recruitment of TRADD. When over expressed, Apoptosis signal-regulating kinase 1 (ASK1) induces apoptosis via the mitochondrial-dependent caspase pathway and, based on studies in mice deleted for ASK1, it is essential for TNFα and oxidant stress induced, but not Fas-induced, apoptosis. Because TNF α can cause apoptosis via mitochondrial independent mechanisms, ASK1 must act via additional mechanisms to induce apoptosis in response to TNFα. It was found that a small molecule antagonists of X-chromosome-linked inhibitor of apoptosis protein (XIAP) that overcome inhibition of caspase-3 directly induced cell death in tumor cells while having little toxicity on normal cells, which indicates a critical role of caspase-3 in cancer cell apoptosis. Bcl-2, an anti-apoptotic protein, also exists abundantly in the mitochondrial membrane. In a cell-free system, mitochondrial presence is necessary to induce the nuclear condensation and DNA fragmentation, which is considered as a signature of apoptosis. Induction of caspase, that actually executes the cell death in presence of ATP, also requires cytochrome-c (cyto-c) released from mitochondria. Upon ligand-binding receptors homo-dimerise or hetero-dimerise triggering tyrosine trans-phosphorylation of the receptor sub-units.

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

Intracellular tyrosine kinases of the Src family and Abl family are also able to tyrosine phosphorylate ErbB receptors. These tyrosine phosphorylated sites allow proteins to bind through their Src homology 2 (SH2) domains leading to the activation of downstream signaling cascades including the RAS/extracellular signal regulated kinase (ERK) pathway, the phosphatidylinositol 3 kinase(PI3K) pathway and the Janus kinase/Signal transducer and activator of transcription (JAK/ STAT) pathway. Differences in the C-terminal domains of the ErbB receptors govern the exact second messenger cascades that are elicited conferring signaling specificity. The EGF signal is terminated primarily through endocytosis of the receptor-ligand complex. The contents of the endosomes are then either degraded or recycled to the cell surface. A number of signal transduction pathways branch out from the receptor signalling complex as shown in Figure. 2. Activation of the EGFR occurs through receptor dimerization, conformational change, and auto phosphorylation. Phosphorylated receptors recruit adaptor proteins, and these then activate multiple signaling proteins including extracellular-regulated kinase (ERK) via Ras and the Akt kinase via phosphatidylinositol 3-kinase (PI3K) if PI3K is active. But if PI3K is inactive than there is cell death. The binding of *Insulin* to the insulin receptor also activates extracellular signal-regulated kinases (ERK) and Akt, but in contrast to EGFR, the insulin receptor is constitutively dimerized, and most insulininduced signaling involves modification of insulin receptor substrate 1 (IRS1), a multidomain adaptor protein. Insulin is the major hormone controlling critical energy functions such as glucose and lipid metabolism. Insulin activates the insulin receptor tyrosine kinase (IR), which phosphorylates and recruits different substrate adaptors such as the IRS family of proteins. Insulin receptor (IRS-1) substrate1 plays important biological function for both metabolic and mitogenic (growth promoting) pathways. IRS-1 plays a key role in transmitting signals from the insulin and insulin-like growth factor-1 (IGF-1) receptors to intracellular pathways PI3K / Akt and ERK MAP kinase pathways. Tyrosine phosphorylated IRS then displays binding sites for numerous signaling partners. Among them, PI3K has a major role in insulin function, mainly via the activation of the Akt/PKB. Activated Akt induces glycogen synthesis through inhibition of GSK-3; protein synthesis via mTOR and downstream elements; and cell survival through inhibition of several pro-apoptotic agents, including Bad, Forkhead family transcription factors and GSK-3. Insulin signaling also has growth and mitogenic effects, which are mostly mediated by the Akt cascade as well as by activation of the Ras/MAPK pathway.

Based on the block diagram we have made Petrinet model for each input i.e. TNF, EGF and Insulin.

4.1 For TNF : Figure 3 shows the petrinet model for TNF. Based on that model we have made the following equations :

- **FAS induced** :
    1. s2, s31, s26, s27, s28, s29, s30, s12 – FAS/ FADD/ ASK/ MAP3K/ p38/ MK2/ AP1.
    2. s2, s31, s26, s25, s20, s21, s38, s41, s42, s43, s34, s44 – FAS/ FADD/ ASK/ MAPK/ JNK/ BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
    3. s2, s31, s26, s25, s20, s21, s38, s39, s40, s35, s44 – FAS/ FADD/ ASK/ MAPK/ JNK/ BAX / Mitochondria/ SMAC/ XIAP/ Caspase 3.
    4. s2, s31, s26, s25, s20, s22, s24 – FAS/ FADD/ ASK/ MAPK/ JNK/c-FLIP
    5. s2, s31, s17, s32, s33, s44 – FAS/ FADD/ Caspase 8 / Caspase 3

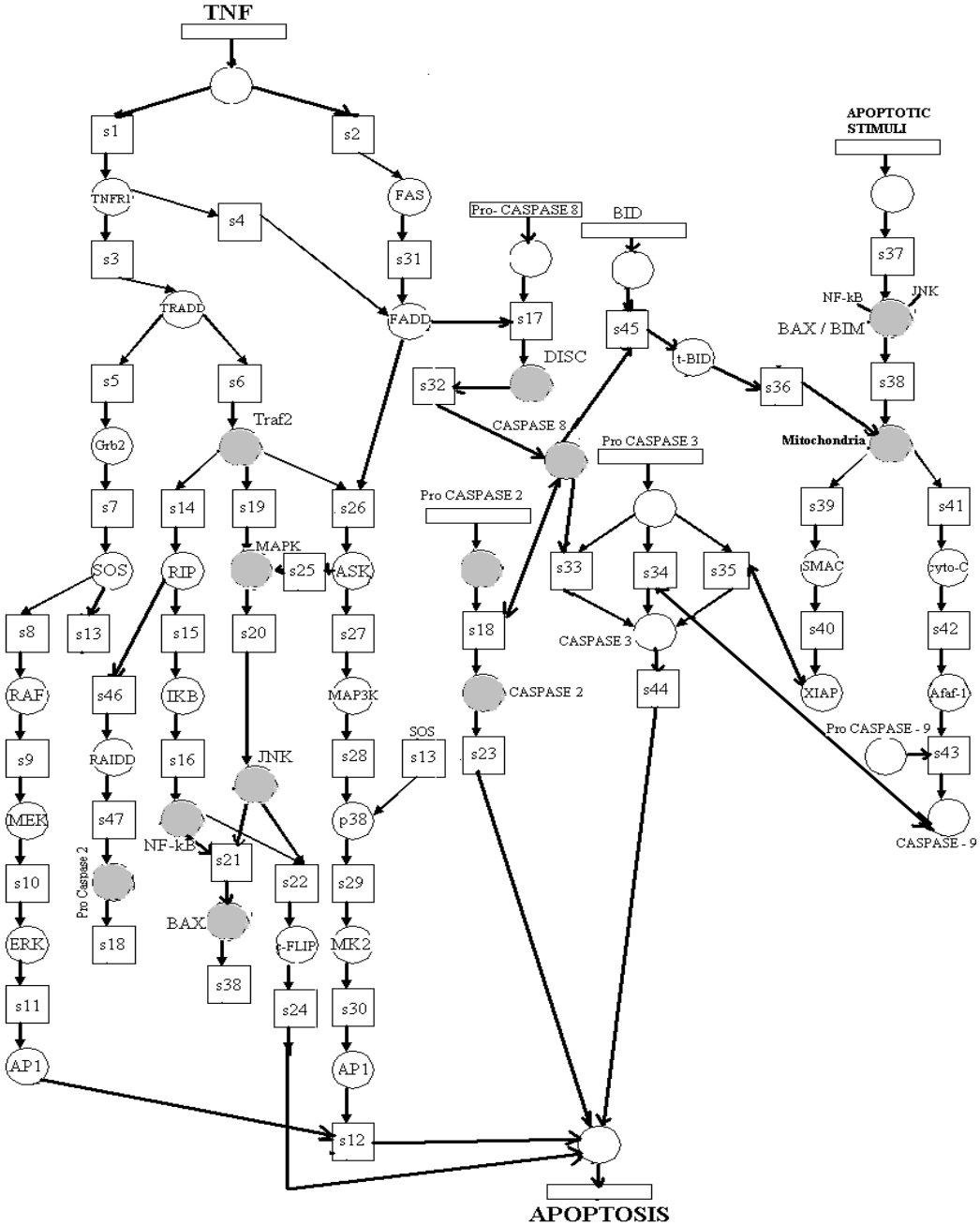
PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

6. s2, s31, s17, s32, s18, s23 – FAS/ FADD/ Caspase 8 / Caspase 2
7. s2, s31, s17, s32, s45, s36, s41, s42, s43, s34, s44 – FAS/ FADD/ Caspase 8/ Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
8. s2, s31, s17, s32, s45, s36, s39, s40, s35, s44 – FAS/ FADD/ Caspase 8/ Mitochondria/ SMAC/ XIAP/ Caspase 3.

- **Apoptotic stimuli induced** :
  1. s37, s38, s41, s42, s43, s34, s44 - apoptotic stimuli/ Bax, Bid, Bim/ Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  2. s37, s38, s39, s40, s35, s44 - apoptotic stimuli/ Bax, Bid, Bim/ Mitochondria/ SMAC/ XIAP/ Caspase 3.
- **TNFR1- induced** :
  1. s1, s3, s5, s7, s8, s9, s10, s11, s12, - TNFR1/ TRADD/ Grb2/ SOS/ RAF/ MEK/ ERK/ AP1
  2. s1, s3, s5, s7, s13, s29, s30, s12 - TNFR1/ TRADD/ Grb2/ SOS/ p38/ MK2/ AP1.
  3. s1, s3, s6, s14, s15, s16, s22, s24- TNFR1/ TRADD/ traf2/ RIP/ IκB/ NF-κB/ c-FLIP
  4. s1, s3, s6, s14, s15, s16, s17, s38, s41, s42, s43, s34, s44 - TNFR1/ TRADD/ Traf2/ RIP/ IκB/ NF-κB/ BAX/ Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  5. s1, s3, s6, s14, s15, s16, s17, s38, s39, s40, s35, s44 - TNFR1/ TRADD/ Traf2/ RIP/ IκB/ NF-κB/ BAX/ Mitochondria/ SMAC/ XIAP/ Caspase 3.
  6. s1, s3, s6, s14, s46, s47, s18, s23 - TNFR1/ TRADD/ Traf2/ RIP/ RAIDD/ Pro caspase 2/ Caspase 2.
  7. s1, s3, s6, s14, s46, s47, s18, s33, s44 - TNFR1/ TRADD/ Traf2/ RIP/ RAIDD/ Pro caspase 2/ Caspase 8 / Pro- Caspase 3 / Caspase 3.
  8. s1, s3, s6, s19, s20, s21, s38, s41, s42, s43, s34, s44 - TNFR1/ TRADD/ Traf2/ MAPK/ JNK/ BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  9. s1, s3, s6, s19, s20, s21, s38, s39, s40, s35, s44 - TNFR1/ TRADD/ Traf2/ MAPK/ JNK/ BAX /Mitochondria/ Mitochondria/ SMAC/ XIAP/ Caspase 3.
  10. s1, s3, s6, s19, s20, s22, s24- TNFR1/ TRADD/ Traf2/ MAPK/ JNK/ c-FLIP
  11. s1, s3, s6, s26, s27, s28, s29, s30, s12 - TNFR1/ TRADD/ Traf2/ ASK/ MAP3K/ p38/ MK2/ AP1.
  12. s1, s4, s26, s27, s28, s29, s30, s12- TNFR1/ FADD/ ASK/ MAP3K/ p38/ MK2/ AP1.
  13. s1, s4, s17, s32, s33, s44 – TNFR1/ FADD/ Caspase 8/ Caspase 3
  14. s1, s4, s17, s32, s18/ s23 – TNFR1/ FADD/ Caspase 8/ Caspase 2.
  15. s1, s4, s17, s32, s45, s36, s41, s42, s43, s34, s44 – TNFR1/ FADD/ Caspase 8/ Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  16. s1, s4, s17, s32, s45, s36, s39, s40, s35, s44 – TNFR1/ FADD/ Caspase 8/ Mitochondria/ SMAC/ XIAP/ Caspase 3.

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

Figure 3
Petri Net Model of TNF



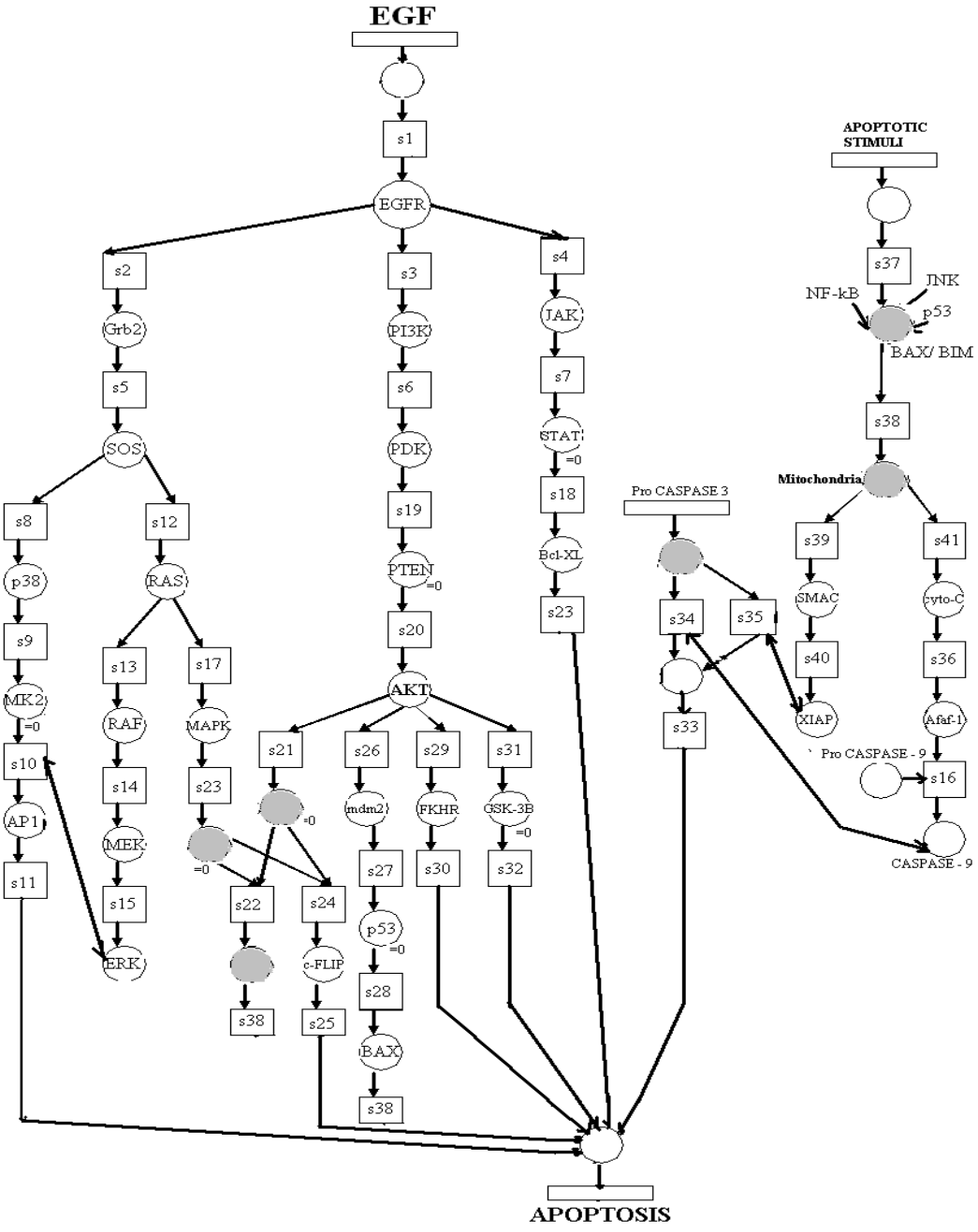
PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

4. 2 For EGF : Figure 4 shows the Petri net model for EGF. Based on that model we have made the following equations :

- **EGFR induced** :
  1. s1, s2, s5, s12, s13, s14, s15, s10, s11 - EGFR/ Grb2/ SOS/ RAS/ RAF/ MEK/ ERK/ AP1
  2. s1, s2, s5, s8, s9, s10, s11 - EGFR/ Grb2/ SOS/ p38/ MK2/ AP1.
  3. s1, s2, s5, s12, s17, s23, s22, s38, s41, s36, s16, s34, s33 – EGFR / Grb2/ SOS/ RAS/ MAPK/ JNK/ BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  4. s1, s2, s5, s12, s17, s23, s22, s38, s39, s40, s35, s33 - EGFR / Grb2/ SOS/ RAS/ MAPK/ JNK/ BAX /Mitochondria/ SMAC/ XIAP/ Caspase 3.
  5. s1, s2, s5, s12, s17, s23, s24, s25- EGFR / Grb2/ SOS/ RAS/ MAPK/ JNK/ c-FLIP
  6. s1, s3, s6, s19, s20, s21, s22, s38, s41, s36, s16, s34, s33 – EGFR / PI3K/ PDK/ PTEN/ AkT/ NF-κB / BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  7. s1, s3, s6, s19, s20, s21, s22, s38, s39, s40, s35, s33 – EGFR / PI3K/ PDK/ PTEN/ AkT/ NF-κB / BAX /Mitochondria/ SMAC/ XIAP/ Caspase 3.
  8. s1, s3, s6, s19, s20, s21, s24, s25 – EGFR / PI3K/ PDK/ PTEN/ AkT/ NF-κB / c-FLIP.
  9. s1, s3, s6, s19, s20, s26, s27, s28, s38 s41, s36, s16, s34, s33 – EGFR / PI3K/ PDK/ PTEN/ AkT/ mdm2, p53/ BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  10. s1, s3, s6, s19, s20, s26, s27, s28, s38 s39, s40, s35, s33 – EGFR / PI3K/ PDK/ PTEN/ AkT/ mdm2, p53/ BAX / Mitochondria/ SMAC/ XIAP/ Caspase 3.
  11. s1, s3, s6, s19, s20, s29, s30 - EGFR / PI3K/ PDK/ PTEN/ AkT/ FKHR.
  12. s1, s3, s6, s19, s20, s31, s32 - EGFR / PI3K/ PDK/ PTEN/ AkT/ GSK-3B.
  13. s1, s4, s7, s18, s23 – EGFR/ JAK/ STAT/ Bcl-XL.
- **Apoptotic stimuli induced** :
  1. s37, s38, s41, s42, s43, s34, s44 - apoptotic stimuli/ Bax, Bid, Bim/ Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
  2. s37, s38, s39, s40, s35, s44 - apoptotic stimuli/ Bax, Bid, Bim/ Mitochondria/ SMAC/ XIAP/ Caspase 3.

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

Figure 4
Petri Net Model of EGF for cell death



PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

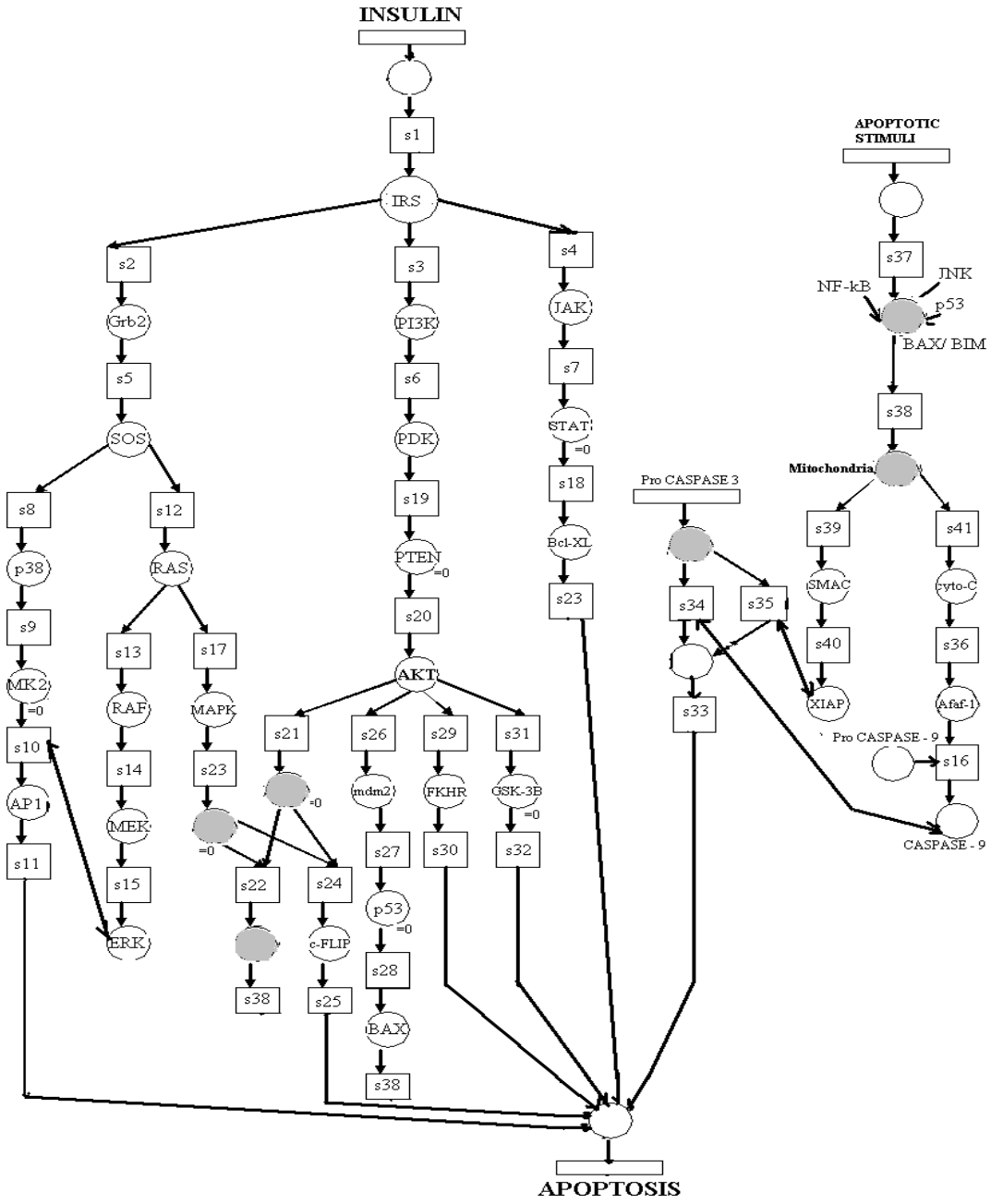
4. 3 For Insulin : Figure 5 shows the Petri net model for INSULIN. Based on that model we have made the following equations :

- **IRS induced** :
    1. s1, s2, s5, s12, s13, s14, s15, s10, s11 - IRS/ Grb2/ SOS/ RAS/ RAF/ MEK/ ERK/ AP1
    2. s1, s2, s5, s8, s9, s10, s11 - IRS / Grb2/ SOS/ p38/ MK2/ AP1.
    3. s1, s2, s5, s12, s17, s23, s22, s38, s41, s36, s16, s34, s33 – IRS / Grb2/ SOS/ RAS/ MAPK/ JNK/ BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
    4. s1, s2, s5, s12, s17, s23, s22, s38, s39, s40, s35, s33 - IRS / Grb2/ SOS/ RAS/ MAPK/ JNK/ BAX /Mitochondria/ SMAC/ XIAP/ Caspase 3.
    5. s1, s2, s5, s12, s17, s23, s24, s25- IRS / Grb2/ SOS/ RAS/ MAPK/ JNK/ c-FLIP
    6. s1, s3, s6, s19, s20, s21, s22, s38, s41, s36, s16, s34, s33 – IRS / PI3K/ PDK/ PTEN/ AkT/ NF-κB / BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
    7. s1, s3, s6, s19, s20, s21, s22, s38, s39, s40, s35, s33 – IRS / PI3K/ PDK/ PTEN/ AkT/ NF-κB / BAX /Mitochondria/ SMAC/ XIAP/ Caspase 3.
    8. s1, s3, s6, s19, s20, s21, s24, s25– IRS / PI3K/ PDK/ PTEN/ AkT/ NF-κB / c-FLIP.
    9. s1, s3, s6, s19, s20, s26, s27, s28, s38, s41, s36, s16, s34, s33 – IRS / PI3K/ PDK/ PTEN/ AkT/ mdm2, p53/ BAX /Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
    10. s1, s3, s6, s19, s20, s26, s27, s28, s38 s39, s40, s35, s33 – IRS / PI3K/ PDK/ PTEN/ AkT/ mdm2, p53/ BAX / Mitochondria/ SMAC/ XIAP/ Caspase 3.
    11. s1, s3, s6, s19, s20, s29, s30 - IRS / PI3K/ PDK/ PTEN/ AkT/ FKHR.
    12. s1, s3, s6, s19, s20, s31, s32 - IRS / PI3K/ PDK/ PTEN/ AkT/ GSK-3B.
    13. s1, s4, s7, s18, s23 – IRS / JAK/ STAT/ Bcl-XL.
- **Apoptotic stimuli induced** :
    1. s37, s38, s41, s42, s43, s34, s44 - apoptotic stimuli/ Bax, Bid, Bim/ Mitochondria/ ctyo-c/ Afaf 1/ Caspase 9/ Caspase 3.
    2. s37, s38, s39, s40, s35, s44 - apoptotic stimuli/ Bax, Bid, Bim/ Mitochondria/ SMAC/ XIAP/ Caspase 3.

Altogether we get 44 places and 48 transitions for TNF, 39 places and 41 transitions for EGF, and 39 places and 41 transitions for Insulin.

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

Figure 5
Petri Net Model of Insulin for cell death



PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

5. CONCLUSION

We had successfully made computational model for cell death using three inputs such as TNF, EGF and insulin With that model we had made Petri Net Model for each input.

REFERNCES

1. Belta C, Schug J, Thao D, Kumar V, Pappas G. J, Rubin, H.,& Dunlap, P. Stability and reachability analysis of a hybrid model of luminescence in the marine bacterium Vibrio fischeri. 40th IEEE Conference on Decision and Control, 1(4), 869–874, 2001.
2. Weiss R. Cellular computation and communications using engineered genetic regulatory networks. PhD Thesis, MIT, September 2001.
3. Nand K. Sah, Tarvinder K Taneja, and Seyed E Hasnain, Mitochondria can power cells to life and death, Resonance pp. 74-84, April 2001.
4. Aravind L, Dixit V M and Koonin E V, The domains of death: evolution of the apoptosis machinery. Trends Biochem Sci;24:47-53, 1999.
5. Lü C X, Fan T J, Hu G B, Cong R S, Apoptosis-inducing factor and apoptosis. Acta Biochim Biophys Sin 35: 881−885, 2003.
6. Vermeulen Katrien, Van Bockstaele DR, Berneman ZN, Apoptosis: mechanisms and relevance in cancer Ann Hematol 84: 627-639, 2005.
7. Kerr J F R, Wyllie A H, Currie A R., Apoptosis: A basic biologic phenomenon with wide ranging implications in tissue kinetics. Br J Cancer; 26: 239–257, 1972.
8. Launay S, Hermine O, Fontenay M, Kromer G, Solary E, Garrido C, Vital functions for lethal caspases Oncogene , 24: 5137−5148, 2005.
9. Kidd V J. Proteolytic activities that mediate apoptosis. Annu Rev Physiol, 60: 533–573, 1998.
10. Thompson C. Apoptosis in the pathogenesis and treatment of disease. Science, 267: 1456–1462, 1995.
11. Lieberthal W, TriacaV, Levine J., Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: Apoptosis vs. necrosis. Am J Physiol, 270: F700–708, 1996.
12. Reddy, V.N., Mavrovouniotis, M.L. and Liebman, M.N., Petri net representations in metabolic pathways. Proc. First ISMB, 328-336, 1993.
13. Hofestädt, R., A Petri net application to model metabolic processes. SAMS 16, 113-122, 1994.
14. Genrich, H., Küffner, R. and Voss, K., Executable Petri net models for the analysis of metabolic pathways. International Journal on Software Tools for Technology Transfer, 3(4), 394-404, 2001.
15. Alla, H, David, R, Continuous and hybrid Petri nets. J Circuits Syst Comput, 8, 159-188, 1998.
16. Lotem, J, Sachs L., Cytokines as suppressors of apoptosis, Apoptosis, 4: 187–196, 1999.
17. Wolf B B, Green D R.,Suicidal tendencies: Apoptotic cell death by caspase family proteases. J Biol Chem, 274: 20048–20052, 1999.
18. Majno G, Joris I.,Apoptosis, oncosis, and necrosis: An overview of cell death. Am J Pathol, 146: 3–15. 98 *Apoptosis of renal tubular cells,* 1995.
19. Wyllie A, Morris R, Smith A, Dunlop D.,Chromatin cleavage in apoptosis: Association with condensed chromatin morphology and dependence on

PETRI NET IMPLEMENTATION OF CELL SIGNALING FOR CELL DEATH

macromolecular sythnesis. J Pathol, 142: 67–77, 1984.
20. Enari M, Sakahira H, Yokayama H, Okawa K, Iwamatsu A, Nagata S., A caspase-activated endonuclease that degrades DNA during apoptosis, and its inhibitor ICAD. Nature, 391: 43–50, 1998.
21. Lieberthal W, Menza S A, Levine J S, Graded ATP depletion can induce apoptosis or necrosis of cultured mouse proximal tubular cells. Am J Phyiol, 274: F315–F327, 1998.
22. Earnshaw W, Nuclear changes in apoptosis. Current Opinion in Cell Biology ,7: 337–343, 1995.
23. Lieberthal W, Koh J S, Levine J S, Necrosis and apoptosis in acute renal failure. Semin Nephrol, 18: 505–518, 1998.
24. Fan Ting-Jun., Han LH, Cong RS, Liang J, Caspase Family Proteases and Apoptosis Acta Biochimica et Biophysics Sinica 2005, 37 (11): 719-727, 2005
25. Steller H., Mechanisms and genes of cellular suicide. Science; 267: 1445–1449, 1995.
26. Ashkenazi A, Dixit V M, Death receptors: Signaling and modulation. Science, 281: 1305–1308, 1998.

ABBREVIATIONS

AP-1, Activation Protein 1; ASK1, Apoptosis signal-regulating kinase 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; FADD, Fas-Associated protein with Death Domain; FKHR, Forkhead transcription factor; Grb2, growth factor receptor-bound 2; GSK 3, Glycogen synthase kinase 3; IGF, insulin-like growth factor; IκB, I Kappa B (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor); IKK, IκB kinase; IR, insulin receptor; IRS1, insulin receptor substrate 1; JNK1, c-jun NH_2 terminal kinase 1; MAP kinases, mitogen-activated protein kinases; MEK, mitogen-activated protein kinase and extracellular-regulated kinase kinase; MK2, mitogen-activated protein kinase-activated protein kinase 2; NF-κB, nuclear factor-κB; PDK, Phi Delta Kappa; PI3K, phosphatidylinositol 3-kinase; p38, P38 mitogen-activated protein kinases**;** Rac, Ras-related C3 botulinum toxin substrate; SAPK/JNK , Stress-activated protein kinase/Jun-amino-terminal kinase; SH2, Src homolgy 2; SODD, Silencer of death domains; SOS, Son of Sevenless; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TRADD, TNFR associated via death domain; TRAF2, TNF receptor associated factor 2, XIAP, X-linked Inhibitor of Apoptosis Protein.