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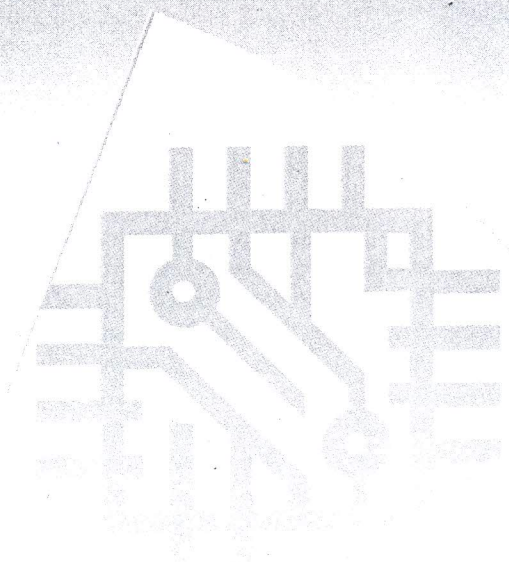

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A Computational Modeling of Apoptosis Signaling using VHDL and MATLAB Simulator

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Abstract: *Cell death is an essential strategy for the control of the dynamic balance in living systems, and two fundamentally different forms of cell death, apoptosis and necrosis, have been defined. In general, necrosis occurs in response to any severe injury, which leads to the biochemical collapse of the cell. Milder forms of the same types of injury cause apoptosis. At the cellular level there are fundamental differences between necrosis and apoptosis. Necrosis results from the additive effect of a number of independent biochemical events that are activated by severe depletion of cell energy stores. By contrast, apoptosis occurs via a coordinated, predictable and pre-determined pathway. These biochemical differences between apoptosis and necrosis have important therapeutic implications. Once a cell has been severely injured, necrosis is difficult to prevent. By contrast, the apoptotic pathway can potentially be modulated to maintain cell viability. The model for cell death has been implemented using Very High Speed Integrated Circuit Hardware Description Language (VHDL) (Xilinx Tool) and MATLAB simulator taking three input signals: Tumor necrosis factor-á (TNF), Epidermal growth factor (EGF) and Insulin. Tumor necrosis factor (TNF) is a cytokine which induces cytotoxicity in some but not all tumor cells. EGF and Insulin is a growth factor that plays an important role in the regulation of cell growth , proliferation and differentiation.*

Index Terms: *Tumor necrosis factor-á (TNF), Epidermal growth factor (EGF), Insulin, Apoptosis, Necrosis*

I. INTRODUCTION

Cell signaling pathways interact with one another to form networks. Such networks are complex in their organization and exhibit emergent properties such as bistability and ultrasensitivity. Analysis of signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of models.

The signaling system underlying apoptosis allows the cell to process input signals capturing information coming from the environment of the cell to lead to one of two possible outputs: cell survival or cell death. This work examines signaling networks that control the death decision treated with combinations of three primary signals [1]; the prodeath cytokine, *tumor necrosis factor-á* (TNF), and the prosurvival growth factors, *epidermal growth factor* (EGF) and *insulin*. The system output is typically a phenotypic readout (death or survival); however, it can also be determined by measuring "early" signals that perfectly correlate with the death/ survival output. Examples of such early signals include phosphatidylserine exposure, membrane permeability, nuclear fragmentation and caspase substrate cleavage. Figure 1 illustrate the system under study linking the three input signaling such as TNF, EGF and Insulin and four output signals phosphatidylserine exposure, membrane permeability, nuclear fragmentation and caspase substrate cleavage leading to cell death/ survival.

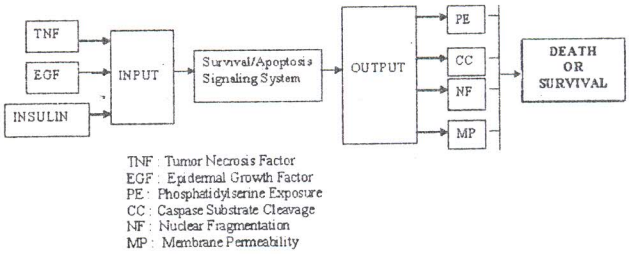

Figure 1: Illustration of the System

Necrosis and apoptosis are best differentiated by the distinct morphologic differences between these two forms of cell death [1]. Cells dying by necrosis become rapidly swollen. They increase in size because of an accumulation of sodium and water within the cytosol. This typical feature of necrosis sometimes referred to as called "oncosis" [2]. Soon thereafter, the mitochondria become progressively enlarged and the normal folding of the mitochondrial cristae is lost. In the final stages of necrosis, the plasma membrane of the cell disintegrates and cytosolic contents leak from the cell. This causes a reactive inflammatory reaction and injury to surrounding cells. For this reason, necrosis of cells is easily identified in histologic sections because of surrounding injury and inflammation [3, 4]. Cells undergoing apoptosis become progressively smaller in distinct contrast to necrotic cells [2, 5, 6] and rapidly lose cell-cell and cell-matrix adhesion. Another distinct feature of apoptosis is that the plasma membrane remains structurally intact. Nuclear chromatin becomes condensed into a small featureless mass DNA. The nuclear membrane disappears and the condensed chromatin fragments into multiple pieces [2, 5, 6]. The

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terminal stages of apoptosis are characterized by the cell disintegrating into "apoptotic bodies", which are comprised of pieces of condensed chromatin and a small amount of cytosol containing normal appearing mitochondria which are surrounded by the relatively intact plasma membrane [2, 3, 4] The nuclear changes associated with apoptosis are due to endonuclease activation and DNA fragmentation [7, 8]. The DNA fragments into nucleosomal-sized fragments that are multiples of the size of a nucleosome Apoptotic bodies are rapidly phagocytosed and degraded by macrophages as well as by surrounding epithelial cells [3, 4]. Phagocytosis of apoptotic cells and apoptotic bodies provides an efficient mechanism for the removal of dead cells without incurring any of the surrounding tissue inflammation and injury that is associated with necrosis [1]. The rapid clearance of apoptotic cells accounts for the lack of any obvious injury in tissue sections. The inconspicuous morphologic nature of apoptosis probably accounts for the fact that the importance of apoptosis in ischemic and toxic injury to organs including the kidney was not recognized until relatively recently [9, 10] Thus, necrosis and apoptosis differ fundamentally from each other, not only morphologically, but biochemically as well [3, 4] Apoptosis has long been recognized as an essential component of embryologic development [4]. More recently it has become recognized that the same biochemical pathway can be induced by a wide variety of different forms of stress and cellular injury. Thus understanding has led to the current interest in the role of apoptosis in injury to many organs including brain, heart, liver and kidney [11]. In general, the mechanism of cell death induced by cytotoxic events is determined by the severity of injury, with extremely severe insults causing metabolic collapse and necrosis, and milder insults of the same sort causing apoptosis [3, 11, 12, 13].

The process of apoptosis can be divided into two clear phases as shown in Figure 2[6, 14, 15] During the first phase, called the "*commitment phase*", an individual cell "decides" whether or not to enter the execution phase and die or whether to remain viable. The ultimate result of exposure to an apoptotic trigger, life or death, is determined by a balance between a numbers of pro- and anti-apoptotic pathways present in every cell. If a cell is destined to die, the second stage of apoptosis, called the "*execution phase*" is activated. The execution of apoptosis entails the controlled activation of a number specific effector mechanisms that lead to the classic morphologic features of apoptosis described above [5, 14].

II. THE EXECUTION PHASE OF THE APOPTOTIC PATHWAY

Role of caspases : Caspases, the family of proteases largely responsible for the execution phase of apoptosis. The caspases are present in cells in an inactive form (pro-caspases) and form a tightly regulated, sequential, and self-amplifying cascade. Caspases are responible for almost all the biochemical and morphologic features of apoptosis and act by the proteolytic cleavage of a host of cellular proteins[4, 9, 10]. All caspases share a number of structural and functional features, two of which are reflected in the name caspase itself. The "c" refers to the fact that caspases are cysteine proteases, with the catalytic site cysteine contained

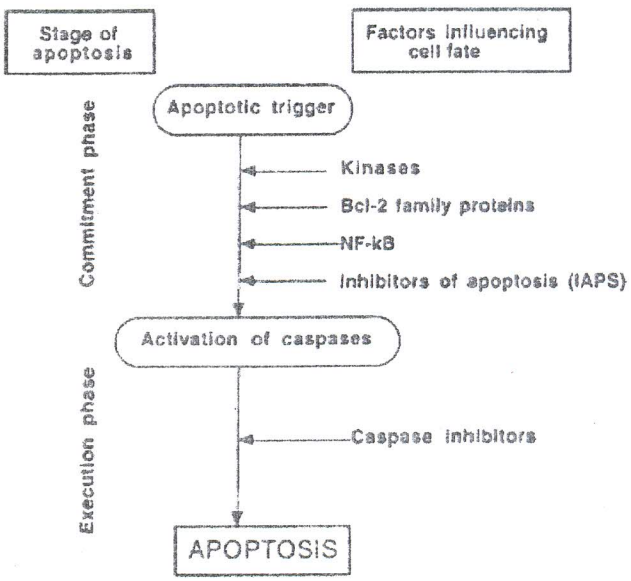

Figure 2: The Apoptotic Pathway: The Apoptotic Pathway Consists of a Commitment Phase, during which the Fate of the Cell is Decided, and an Execution Phase During which Caspases are Activated and Mediate Cell Death. Many Factors Determine Influence the Cell Survives or Dies. All these Factors Represent Potential Targets of Therapeutic Intervention that can be used to Promote or Prevent Cell Death

within a conserved QACXG motif (single letter amino acid code), whereas the "aspase" refers to the unique and absolute predilection of all caspases for cleaving proteins after aspartic acid residues [4, 9, 10, 16]. While is not yet possible to provide a complete flow diagram of the precise cascade of reactions by which caspases mediate the apoptotic pathway, caspases can be divided into two main functional classes; "*initiator*" caspases and "*effector*" caspases [4, 17]. Initiator caspaces are activated at the beginning of the execution phase and activate the effector caspases which target cell proteins and are responsible for the morphological changes. While many of the cytoplasmic and nuclear targets cleaved by capsases during the execution phase of apoptosis have been identified, the majority probably remains to be discovered [4, 9, 10, 16]. Caspase-mediated celavage of apoptosis-specific endonuclease CAD (caspase-activated DNase) is responsible for the "ladder" pattern of DNA fragmentation typical of apoptosis [18, 19]. Caspases also are responsible for proteolysis of the nuclear lamins therby facilitating nuclear condensation. Caspases that target cytoskeletal proteins such as α-fodrin, β-actin, and keratins mediate dissasembly of the cell cytoskeleton [20]. Other

classes of caspase substrates cleaved during apoptosis include DNA repair enzymes, signal transduction molecules, as well as transcriptional and cell cycle regulators.17

Role of mitochondria: The mitochondria play a major role in the execution phase of apoptosis [21, 22] Apoptosis is an active, energy-requiring process [2, 3, 15]. For this reason, as mentioned above, a key factor in determining whether a cell dies by necrosis or by apoptosis is whether the mitochondria can produce enough ATP to allow the apoptotic pathway to occur [13]. Mitochondria also play an essential role in the execution phase of apoptosis by releasing cytochrome c into the cytosol [23, 24, 25]. Cytochrome c is normally part of the mitochondrial electron transport chain. However, once it gains access to the cytoplasm, cytochrome c acts as an essential co-factor for activation of one of the initiator capsases, caspase 9. Cytochrome c binds to a protein known as apoptosis protease-activating factor 1 (Apaf-1), an event that requires deoxyadenosine 5*0*-triphosphate (dATP) [23, 24, 25]. The interaction of cytochrome c and Apaf-1 allows Apaf-1 to bind to and activate procaspase-9 [23, 24, 25]. The complex of cytochrome c, Apaf-1, and procaspase-9 is referred to as the "apoptosome". Active caspase 9 activates procaspase 3, an important effector caspase [23, 24, 25]. In addition to cytochrome c, mitochondria contain a caspase-like protein called apoptosis-inducing factor (AIF) which when released into the cytosol also activates procaspase-3 [26]. In addition, the mitochondria of certain cell types contain procaspase-3 that can be released during apoptosis [27]. It is important to emphasize that, despite the essential role of mitochondria in apoptosis, the mitochondria of cells undergoing apoptosis appear morphologically normal in contrast to the mitochondria of necrotic cells that are usually morphologically normal. While the role of the mitochondrion as an arbiter of cell fate is well supported, the precise mechanisms by which the mitochondrion senses damage and integrates the disparate signals reaching it from throughout the cell are much less clear [21, 22]. As discussed above, mitochondrial events are among the earliest in the execution of apoptosis, and events such as release of cytochrome c into the cytosol may in fact represent a "point of no return" in the cell's decision to undergo apoptosis [21, 22]. Moreover, mitochondrial events, such as loss of mitochondrial transmembrane potential, seem to trigger events within other cell compartments, so that interventions which prevent loss of mitochondrial transmembrane potential can also prevent apoptotic nuclear and cell membrane changes [21, 22].

III. THE COMMITMENT PHASE OF APOPTOPSIS

After exposure to an apoptotic trigger, a cell enters phase of variable duration during which the fate of the cell, apoptosis or survival, is decided. This is called the commitment phase of apoptosis. The outcome of the commitment phase depends upon the balance of a number of different factors, some of which are anti-apoptotic and others that promote precipitation of the execution phase of apoptosis.

Bcl-2 family of proteins: Bcl-2 and related proteins can be divided on a functional basis into two distinct groups, those with anti-apoptotic activity and those that promote apoptosis [28, 29]. Pro-survival members of this family can inhibit apoptosis induced by an extremely wide range of triggers, including survival factor deprivation [30] Fas and TNF-R1 receptor activation, cytotoxic stimuli such as hydrogen peroxide or oxidative free radicals, [31] and DNA damage-inducing agents such as chemotherapeutic drugs or γ-irradiation [32]. Bcl-2 family members play an important role in influencing cell fate during the commitment phase of apoptosis. Bcl-2 family members bind to one another to form heterodimers [28, 29]. Dimerization of Bcl-2 members with opposite effects on apoptosis results in a titration of the effects of each of the interacting proteins [28, 29]. In addition to direct interaction, Bcl-2 proteins may influence the effects of other members by competing for common downstream targets [28, 29]. Thus, the outcome of the commitment phase appears to depend, at least in part, on the relative concentrations of pro-survival and pro-apoptotic Bcl-2 family members [28, 29]. Bcl-2 proteins appear to regulate apoptosis in two major ways. The first involves the direct interaction of Bcl-2 family members with procaspase activating complexes. As discussed above, activation of procaspase-9 occurs within a so-called "apoptosome", in which cytoplasmic Apaf-1 binds via separate domains to procaspase- 9 and cytochrome c [25, 26] Pro-survival Bcl-2 family members, such as Bcl-xL, by binding to Apaf-1, can inhibit the cytochrome c-induced change in Apaf-1 that leads to the recruitment and activation of procaspase-9 [33, 34]. Thus, pro-survival Bcl-2 family members may block initiation of the execution phase of apoptosis, even after mitochondrial permeability transition and release of cytochrome c has occurred [35]. Pro-apoptotic Bcl-2 family members may counteract the protective effects of Bcl-xL by binding to and sequestering Bcl-xL [33, 34]. The second mechanism of action of Bcl-2 family members involves alterations in the permeability of mitochondrial membranes. In response to several triggers of apoptosis, pro-survival proteins Bcl-2 or Bcl-xL stabilize the mitochondrial membrane thereby inhibiting mitochondrial permeability transition and the release of apoptosispromoting substances such as cytochrome c and AIF [36]. It is still uncertain how Bcl-2 and Bcl-xL stabilize the mitochondrial membrane. In any case, these proteins protect cells by acting at two discrete but sequential steps in the apoptotic pathway; by inhibiting the release of mitochondrial cytochrome c, and, once cytochrome c is released, by interfering with cytochrome c-mediated activation of procaspase 9. In contrast to the stabilizing effects of pro-survival Bcl-2 on the mitochondrial membrane, some pro-apoptotic proteins such as Bax, and Bid directly induce mitochondrial permeability transition and

the release of cytochrome c [37, 38]. These proteins appear to act by inserting into the mitochondrial membrane and forming pores and ionconducting channels [39].

The Bcl-2 proteins have a role both in the commitment phase and the execution phase of apoptosis. During the commitment phase, Bcl-2 proteins are regulated primarily via phosphorylation events. Phosphorylation events can increase or decrease the activity of pro-survival proteins such as Bcl-2 and Bcl-xL [28, 29]. On the other hand, phosphorylation of the pro-apoptotic member BAD by PKB/ Akt leads to the sequestration of BAD within the cytosol, thereby preventing access of BAD to mitochondria where it can heterodimerize with and inactivate prosurvival Bcl-2 family members [40]. During the execution phase, the activity of these proteins can be altered by caspase-mediated cleavage. In some cases, caspase-mediated cleavage can convert proteins from pro-survival to pro-apoptotic activity. Thus, cleavage of Bcl-2 abrogates its pro-survival activity and converts the protein into a Bax-like pro-apoptotic factor [41]. Also, caspase-8 activates the pro-apoptotic protein Bid, and converts it from a latent cytoplasmic form to an active moiety which moves to mitochondria and promotes the release of cytochrome c [37]. These examples highlight the complexity of regulation not only among Bcl-2 family members but also between Bcl-2 family members and other components of the apoptotic machinery.

NF-κB: A powerful anti-apoptotic transcription factor: The term NF-κB refers to a family of nuclear transcription factors that regulate the transcription of genes involved in the immune response and apoptosis [42, 43]. It has become evident that activation of NF-κB plays an important role in opposing apoptosis and therefore in determining cell fate in response to a number of apoptotic triggers [44]. When inactive, NF-κB is present within the cytoplasm complexed to one of several inhibitory proteins known collectively as IκB, which prevent NF-κB from entering the nucleus. An extremely wide range of stimuli, including some apoptotic triggers, activates NF-κB-Inducing Kinase (NIK) which phosphorylates IκB thereby leading to its rapid proteasomal degradation. This allows the active NF-κB to translocate to the nucleus where it binds to specific motifs in the promoter regions of its multiple target genes [42, 43]. The anti-apoptotic effect of NF-κB was first clearly demonstrated for TNF-α [44]. TNF-α *binds* with high affinity to two distinct receptors, TNF-R1 and TNF-R2, with opposing effects on cell fate [45]. The death receptor TNFR1 induces apoptosis through recruitment and activation of the death domain-containing proteins TRADD and FADD, ultimately leading to the activation of the apoptotic initiator pro caspase 8 [45]. By contrast, signaling through TNF-R2, which lacks a death domain and so does not activate TRADD and FADD, generally promotes survival and proliferation. However, the division in signaling pathways induced by TNF-R1 versus TNFR2 is not absolute. NF-κB is activated via engagement of both receptors [43, 46]. The strong induction of NF-κB by both TNF-R1 and TNF-R2 accounts for the fact that induction of apoptosis by TNF-α generally requires the concomitant addition of a protein synthesis inhibitor such as cycloheximide [45]. NF-κB protects against apoptosis in a number of ways. These include the transcriptional induction of various members of the IAP family of apoptosis inhibitors [47] as well as of the anti-apoptotic Bcl-2 family member Bfl- A1; the induction of a novel inhibitor of apoptosis, named IEX-1L; the inhibition of p53 activity through competition for a limiting shared co-factor; and an increase in the expression of TNF receptor-associated proteins such as TRAF2 [47]. It is likely that the contribution and magnitude of these pro-survival effects of NF-κB will depend on the cell type and inducing stimulus. It is important to note that the pro-survival effect of NF-κB is unlikely to be restricted to TNF-induced apoptosis since other stimuli, such as growth factors and oxidant stress are known to activate NF-κB. Thus NF-κB may turn out be potent anti-apoptotic response to a ubiquitous array of apoptotic triggers.

IV. RESULTS AND DISCUSSIONS

On the basis of block diagram we have made truth tables of every possible path for cell survival based on individual inputs i.e. TNF, EGF and Insulin. Than we realize the truth tables by Karnaugh Map (K-Map) and get the expression for each input and its individual possible paths. With the help of VHDL tool and MATLAB simulator we simulate the results of each path and then combine all the results and get final result of TNF, EGF and Insulin for its cell death(as shown in Figure 3 and Figure 4).

V. CONCLUSIONS

We had successfully made computational model for cell death using three inputs such as TNF, EGF and insulin With that model we had made truth table, Boolean expression and logical circuit for each possible pathway. We than simulate the results of each path and then combine all the results and get final result of TNF, EGF and Insulin for its death. Later we proceed for cell survival.

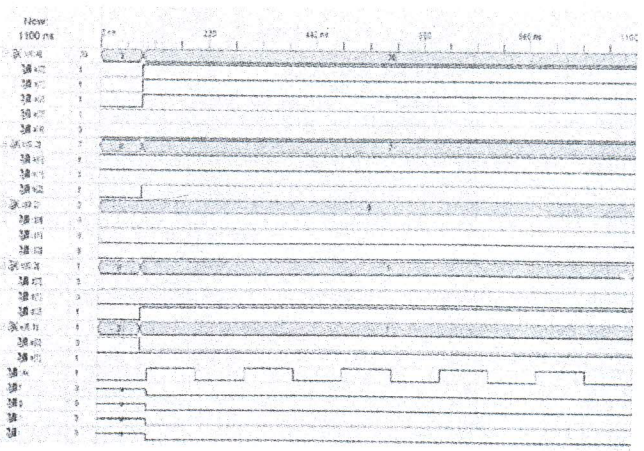

Figure 3: Final Output of TNF, EGF and Insulin using VHDL

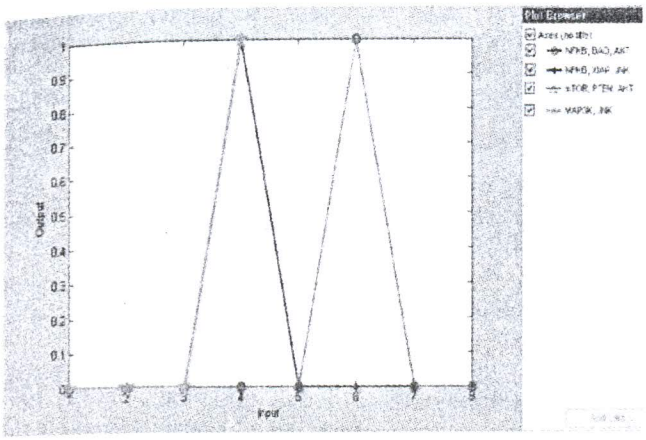

Figure 4: Two Dimensional MATLAB Output for Four Possible Combinations of TNF, EGF and Insulin

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