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Computational Modeling of Cell Survival Using VHDL

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ABSTRACT
The model for cell survival has been implemented using Very High Speed Integrated Circuit Hardware Description Language (VHDL) (Xilinx Tool) taking three input signals: Tumor necrosis factor-α (TNF), Epidermal growth factor (EGF) and Insulin. Cell survival has been regulated by the interaction of five proteins viz P13K, TNFR1, EGFR, IRS and IKK in a network. In the absence of any one, in protein network leads to cell death. For the EGF input signal the proteins like MEK, ERK, AkT, Rac & JNK have been important for regulation of cell survival. Similarly for TNF and Insulin input signal proteins like NFκB, AkT, XIAP, JNK, MAP3K & MK2 and MEK, ERK, AkT, Rac, mTOR & JNK respectively have been important for regulation of cell survival.

KEYWORDS
Tumor necrosis factor-α, Epidermal growth factor, Insulin, AkT (PKB).

ABBREVIATIONS
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; 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 NH2 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; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; PKB, Protein Kinase B; p38, P38 mitogen-activated protein kinases; ptEGFR, phospho-to-total EGFR; ptAkT, phospho-to-total AkT; Rac, Ras-related C3 botulinum toxin substrate; SOS, Son of Sevenless; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TRADD, Tumor necrosis factor receptor associated via death domain; TRAF2, TNF receptor associated factor 2, VHDL, Very High Speed Integrated Circuit Hardware Description Language; XIAP, X-linked Inhibitor of Apoptosis Protein;.

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 ultra sensitivity [1]. Analysis of signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of models. This work examines signaling networks that control the survival decision treated with combinations of three primary signals [2, 3]; the prodeath cytokine, tumor necrosis factor-α (TNF), and the prosurvival growth factors, epidermal growth factor (EGF) and insulin. TNF induce apoptosis and survival [1, 2, 4], 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 [5, 6] results in receptor trimerisation and clustering of intracellular death domains. This allows binding of an intracellular adapter molecule called TNFR-associated death domain (TRADD) 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 [6, 7]. EGF is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation. It also increases cancer risk. EGF acts by binding with high affinity to epidermal growth factor receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor [8, 9]. Activation of the EGF receptor tyrosine kinase (EGFR) [9, 10, 11] occurs through receptor dimerization, conformational change, and autophosphorylation. Phosphorylated receptors recruit adaptor proteins, and these then activate multiple signaling proteins including extracellular-regulated kinase (ERK) via Ras [12] and the Akt [13] kinase via phosphatidylinositol 3- kinase (PI3K). In general insulin being a peptide hormone plays important role in both metabolic and mitogenic (growth promoting) pathways [14, 15]. Signaling through the insulin pathway is critical for the regulation of intracellular and blood glucose levels and the avoidance of diabetes. Insulin binds to its receptor leading to the auto phosphorylation of the ß-subunits and the tyrosine phosphorylation of insulin receptor substrates (IRS). The role of insulin in cell survival has been proved by Insulin/Glucose Phospho-Antibody Array. It induces cell survival by interacting with Insulin receptors or the cell membrane through several cell signaling pathways such as PI3K/ AkT and MAP kinase [16, 17]. The binding of insulin [18, 19] to the insulin receptor also activates ERK and Akt, but in contrast to EGFR, the insulin receptor is constitutively dimerized, and most insulin

induced signaling involves modification of insulin receptor substrate 1 (IRS1) [19, 20], a multidomain adaptor protein.
Regulation of cell survival and cell death are very complicated physiological processes involving a large number of proteins which interact in protein networks. The induction of specific network is executed by input signals like EGF, Insulin and TNF. Therefore, it is very difficult to define and measure the protein- protein interactions in a network leading to cell survival and cell death experimentally. However, the computational model is useful as a means to assemble and test what we know about proteins networks regulating a physiological process. In this study we have implemented the system model of cell survival considering three input signals (TNF, EGF and Insulin) and 16 proteins using VHDL simulation.

2. MATERIALS AND METHODS

Experimental

The experimental observation of cell survival from cells treated with ten cytokine combinations of tumor necrosis factor-α (TNF), a pro apoptotic cytokine, in combination with epidermal growth factor (EGF) or insulin, two pro survival growth factors has been worked out by Gaudet et al [3]. They have predicted with the response of cell survival as well as cell death with 94 % accuracy by including eleven different proteins such as MK2, JNK, FKHR, MEK, ERK, IRS, AkT, IKK, pAkT, ptAkT and ptEGFR. All the eleven proteins forms signaling network as represented in Figure1 leads to cell survival. The response of signaling network is regulated by the concentration of cytokines like TNF, EGF and Insulin. Therefore, it is possible to built self consistent compendia cell signaling data based on the above eleven proteins that can be simulated computationally to yield important insites into the control of cell survival.

Computational Model

The prediction model for cell survival has been implemented using VHDL programming language. We have implemented the signaling system heading by three input signals such as TNF, EGF and Insulin (inputs are same as that of experimental). The block diagram of the signaling system that was modeled is shown in Figure 1.
TRADD recruits TRAF2 and RIP. TRAF2 in turn recruits the multicomponent protein kinase IKK, enabling the serine-threonine kinase RIP to activate it. An inhibitory protein, IκBα, that normally binds to NF-κB and inhibits its translocation, is phosphorylated by IKK and subsequently degraded, releasing NF-κB. NF-κB is a heterodimeric transcription factor that translocates to the nucleus and mediates the transcription of a vast array of proteins involved in cell survival and proliferation, inflammatory response, and anti-apoptotic factors. NF-κB induces the caspase inhibitors IAP1 and IAP2 and pro-survival Bcl-2 family members. Of the major MAPK cascades, TNF induces a strong activation of the stress-related JNK group, evokes moderate response of the p38-MAPK, and minimal activation of the classical ERKs. A general activation scheme involves the activation of receptor tyrosine kinases by growth factors, such as EGF [2, 3], which provides the binding site of the adapter protein Grb2 [19] that in turn localizes Sos to the plasma membrane. Sos activates Ras by exchange of GTP for GDP. The Ras-GTP binds directly to a serine-threonine kinase Raf [17, 18], forming a transient membrane-anchoring signal. Active Raf kinase phosphorylates a dual specificity kinase, MEK, [15, 16] and activates it. MEK can also be phosphorylated by Mos, a protein kinase expressed during meiotic maturation of oocytes and by MEKK1. The mono phosphorylated ERK then rebinds to an active MEK1 for dual phosphorylation and complete activation. The activated MEK phosphorylates ERK1/ERK2. Within the cell, at any time, one may find three low active forms of ERKs: one unphosphorylated enzyme, and two singly phosphorylated forms that contain phosphate either at the tyrosine or threonine residue. Activation of Akt involves growth factor binding to a receptor tyrosine kinase and activation of PI 3-K, which phosphorylates membrane bound PIP2 to generate PIP3. The binding of PIP3 to the PH domain anchors Akt to the plasma membrane and allows its phosphorylation and activation by PDK1. Akt is fully activated following its phosphorylation at two regulatory residues, a threonine residue on the kinase domain and a serine residue on the hydrophobic motif, which are structurally and functionally conserved within the AGC kinase family. Phosphorylation of a threonine residue on the kinase domain, catalyzed by PDK1, is essential for Akt activation. It causes a charge-induced conformational change, allowing substrate binding and increased rate of catalysis. Akt activity is augmented about 10-fold by phosphorylation at the serine residue by PDK2. The activity of Akt is negatively regulated by PTEN and SHIP. The activation of these MAP kinases is mediated by Rac and cdc42, two small G-proteins. The activated cdc42 binds to PAK65 protein kinase and activates it. The activated PAK65 can activate MEKK, which in turn phosphorylates SEK/JNKK and activates it. The active SEK/JNKK phosphorylates JNK/SAPK (at the TPY motif) that in turn binds to the N-terminal region of c-Jun and phosphorylates it. JNKK, an activator of JNK/SAPK, is reported to activate p38, whereas MKK3 activates only p38 and not JNK/SAPK. MEKK1 that stimulates SEK/JNKK1 in the JNK/SAPK cascade has only a trivial effect on p38 activation. In the upstream signaling, Sos stimulates only the ERK pathways without affecting either JNK or p38 cascade. 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. 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. Cell cycle arrest by the mammalian Target of Rapamycin (m TOR) complex requires the presence of the intact kinase domain of mTOR and, in particular, a conserved serine within this domain, which has been identified as an Akt/PKB-mediated phosphorylation site. Biomarkers indicate that the mTOR pathway is hyperactive in certain types of cancers, suggesting that mTOR could be an attractive target for cancer therapy. Activated mTOR may provide tumor cells with a growth advantage by promoting protein synthesis, which is the best-described physiological function of mTOR signaling. mTOR regulates Akt activity, a crucial downstream effector in the PI-3K–PTEN pathway, which controls cell proliferation and survival.

3. RESULTS AND DISCUSSIONS

On the basis of block diagram (Figure 1) we have made truth tables of every possible path for cell survival based on individual inputs i.e. TNF, EGF and Insulin. Than we realized the truth tables by Karnaugh Map (K-Map) and got the expression for each input and its individual possible paths. With the help of VHDL tool, we simulated the results of each path and then all the results were combined and got final result of TNF, EGF and Insulin for its cell survival (as shown in Figure 2, Figure 3 and Figure 4). For cell survival the five different proteins i.e. P13K, TNFR1, EGFR, IRS and IKK should present. If any one of them is absent, than cell will die. Figure 2 g, h, i, j and k shows the output signal considering b, c, d, e and f as possible paths taken TNF as input. Path b shows that it consists of three proteins, and for cell survival combination is 101. 101 means first and third protein is present and second protein is absent. Similarly, for path c, d, e and f combinations are 011, 11, 01 and 0 respectively. If any of the path is present than it will lead to cell survival. 'a' shows the five proteins which are compulsory proteins i.e. P13K, TNFR1, EGFR, IRS and IKK. Similarly for Figure 3 f, g, and h are output signals for b, c and d paths. For path b, c and d possible combinations are 01, 11 and 11, respectively. Similarly for Figure 4 f, g, h and i are output signals for b, c, d and e paths. For path b, c, d and e possible combinations are 101, 111, 11 and 101 respectively.

4. CONCLUSION

We have demonstrated that the VHDL programming language can be applied to predict the cell survival with a high level of accuracy using three inputs such as TNF, EGF and Insulin. The computational model has reproduced experimental data with fairly accurate. Understanding the nature of signaling networks that control the cell survival is very significant and theoretical calculations, in particular the simulation process developed using VHDL, seen to be a proper tool for gaining such understanding. The results obtain will give information on how the input signals inducing cell survival should be modulated to achieve desire outputs and thus helps the experimentalists to design proposals regarding possible improvements to cell survival/ cell death.

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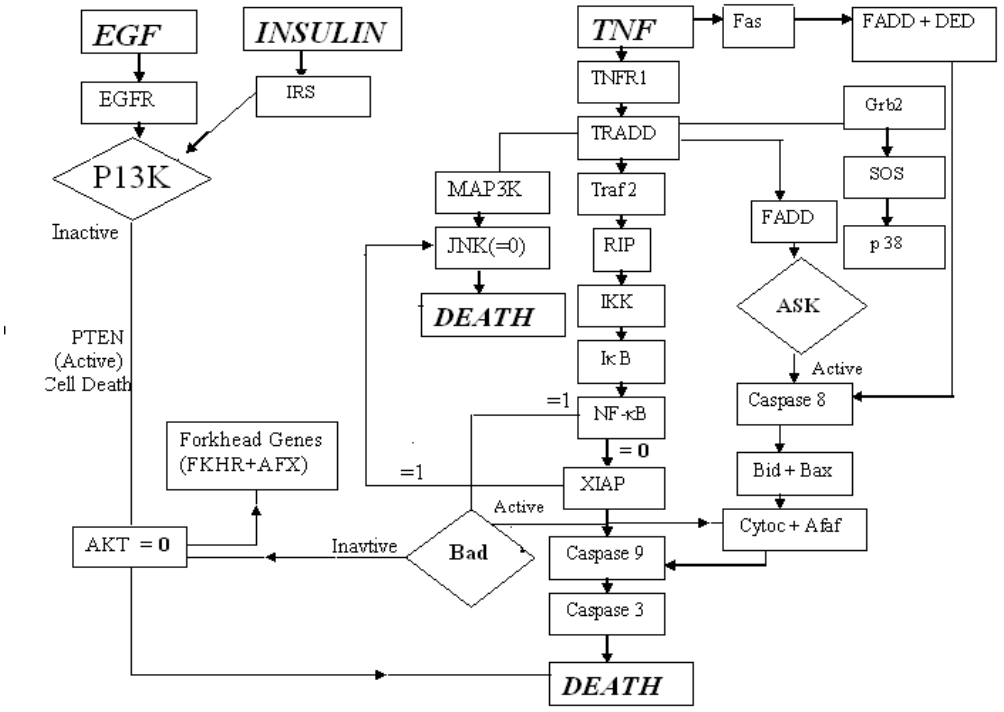

Figure 1: Model for Cell Survival

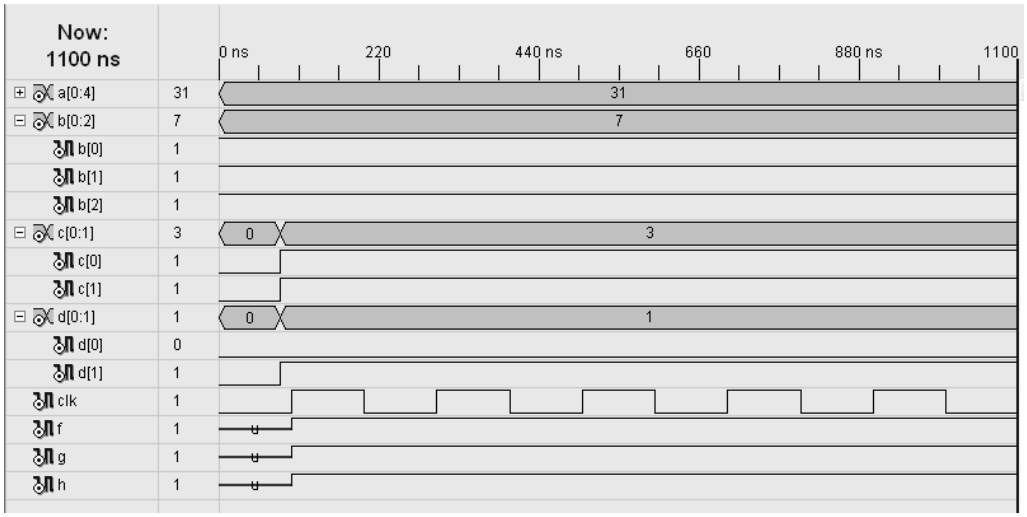

Figure 3: Output signal of cell survival from VHDL simulation considering EGF as input

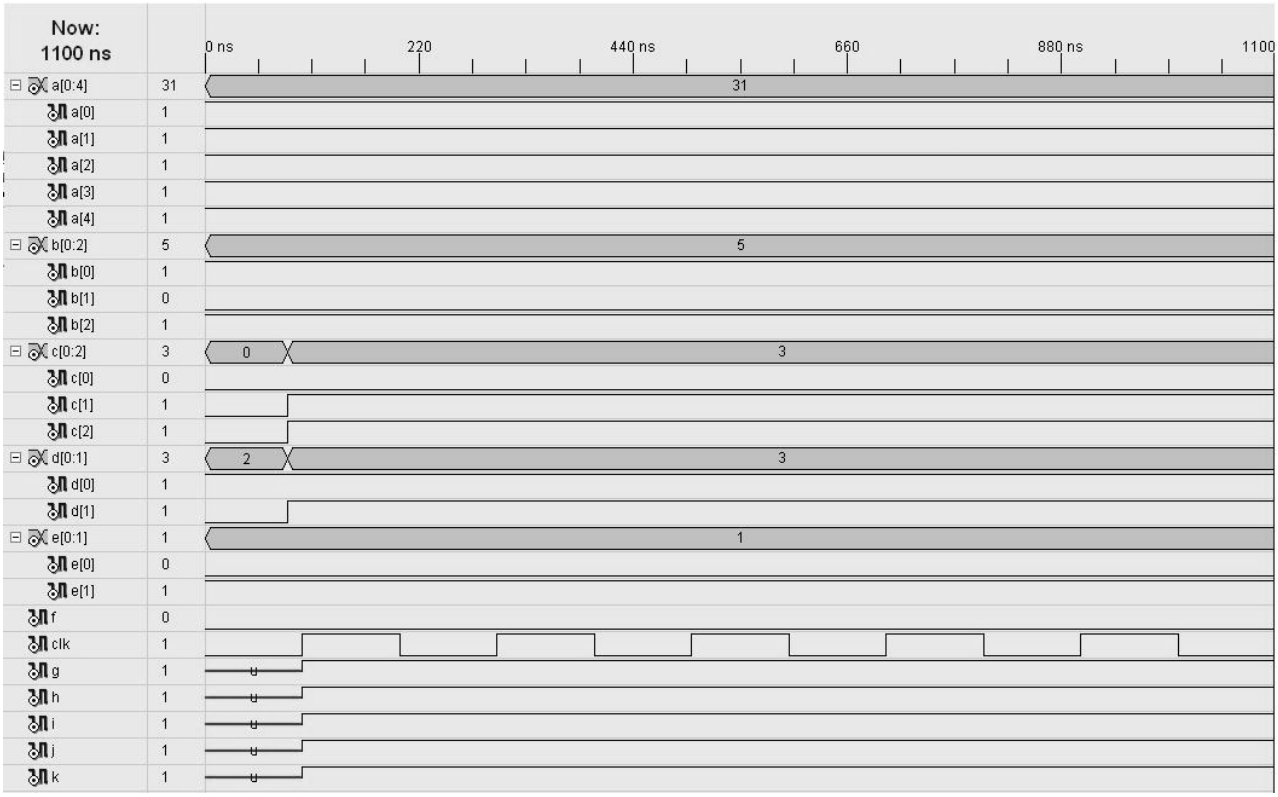

Figure 2: Output signal of cell survival from VHDL simulation considering TNF as input

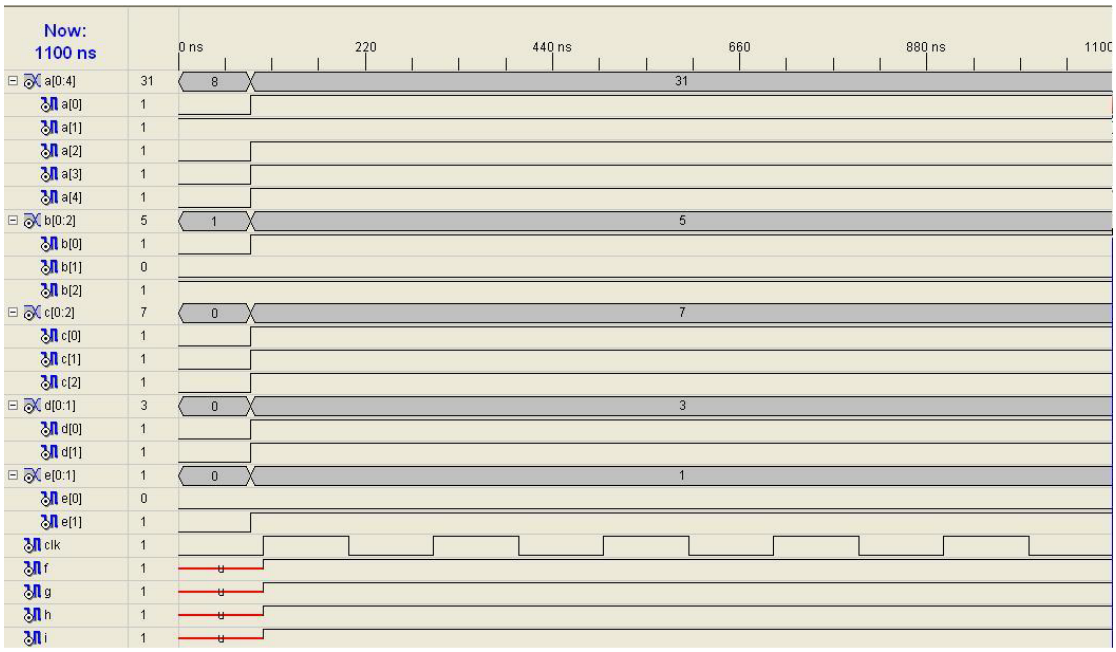

Figure 4: Output signal of cell survival from VHDL simulation considering Insulin as input