Real Time Modelling of CEACAM1 Related Gene Regulatory Network

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Submitted By

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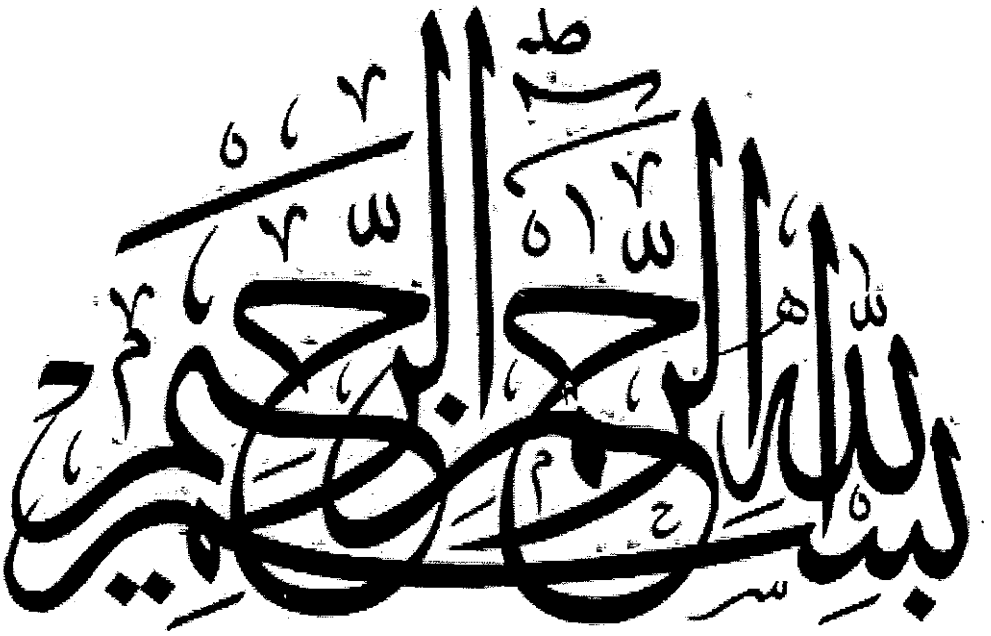

In the name of Allah Most Gracious and Most Beneficial

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Dated: 07-2-2012

FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. Saba Munawar and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Bioinformatics

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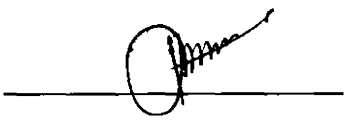

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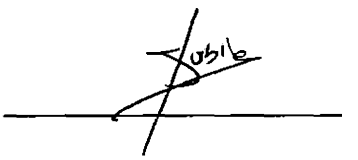

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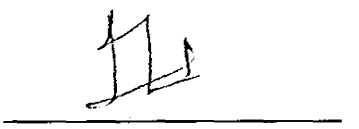

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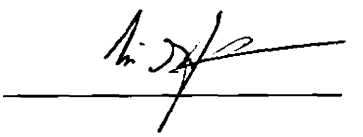

A thesis submitted to Department of Environmental Sciences, International Islamic University, Islamabad as a partial fulfillment of requirement for the award of the degree of MS Bioinformatics.

Dedicated to my loving Father
Munawar Hussain Tahir and my
mother Shehzadi Gulnaz Tabassum
Their support and love have
always been a source of strength
for me.

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

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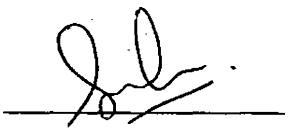

Saba Munawar

Table of Contents

Acknowledgment

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Saba Munawar

List of Abbreviations

T1DM	Type 1 diabetes mellitus
IDDM	insulin-dependent diabetes mellitus
T2DM	Type 2 diabetes mellitus
rER	rough endoplasmic reticulum
IR	Insulin Receptor
IRS	Insulin receptor substrate proteins
SH2	src homology 2
CEACAM1	Carcino Embryonic Antigen-related Cell Adhesion Molecule 1
BRNs	Biological Regulatory Networks
PADEs	Piecewise-Affine Differential Equations
LHA	Liner Hybrid Atomata
INS	Insulin

List of Figures

List of Tables

Abstract

Insulin resistance is the main cause of Diabetes Miletus type 2. Major sites of insulin clearance are liver and kidney, while 80% of portal insulin clearance occurs in liver. CEACAM1 is immunoglobulin related membrane glycoprotein found in human liver is a substrate of insulin receptor tyrosine kinase (IR) that regulates hepatic insulin internalization. Knockout of CEACAM1 resulted in blockage of IR uptake, leading to insulin resistance. In this study, discrete model of hepatic insulin clearance was designed using kinetic logic of René Thomas to investigate the insulin turn over. The molecular connection between CEACAM1 phosphorylation, IR endocytosis and insulin degradation was modelled. Present study proposed biological regulatory network of insulin clearance from Hepatocytes and also focused on elucidate role of CEACAM1 models which are based on the discrete approach of René Thomas. This study also automatised and refined the use of delays in activation and inhibition of proteins associated with the model in order to specify which protein quickly affected by change of its regulators. A linear hybrid automaton is designed for the model by introduction of such delays and it allows the model for such refinements. Oscillatory behaviour of the BRN showed the regulation of insulin facilitated by CEACAM1 which exhibited normal behaviour while some states lead towards the dead lock state (stable steady state) that represents the diseased behaviour in which CEACAM1 does not respond to the active IR. This qualitative modelling would help to generate all possible set of states showing behavior of the system, Modeling satisfies qualitative properties arisen from the biological network structure and delays associated with the dynamics of genes or gene products. Hybrid modeling

technique was also used on biological models to obtain the qualitative and partial temporal data. Hytech was used to find automatically all paths from a specified initial state to another one and to synthesize constraints on the delay parameters in order to follow any specific path that may lead to stable steady state or dead lock state. This model was allowed to find the therapeutic targets for the insulin resistance and CEACAM1 abnormal behavior. With the help of invariance kernel analysis of the cycle the conditions to remain the system in the state of homeostasis is also obtained.

In Conclusion the modeling of insulin clearance from Hepatocytes is a modest contribution to the theory of Biological Regulatory Networks in this study computer-aided method of qualitative mathematical modeling, models that match the mixed continuous and discrete character of real biology more realistically produced. By introduction of time in modeling such networks, allowing the modeler to verify temporal properties,the predictions obtained from the model are highly significant and delay constraints helps to find therapeutic targets.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Major intimidation to human health in 21st century is due to obesity, unhealthy diet, mantel stress, and inactive everyday life. World health organization declared that 177 million people worldwide were diabetic in 2000 and by 2025 this number is predictable to increase up to 300 million (King *et al.*, 1998; Zimmet *et al.*, 2001). In Pakistan almost 9 million people are diabetic and International Diabetes Federation guesstimate that by 2025 this number will grow to 11.5 million unless preventive measures are taken to control the disease.

1.1 Biological Problem

Diabetes has been classified into two types: type 1 and type 2. Type 1 diabetes mellitus (T1DM) found in 5 to 10 % of all cases and is known as childhood-onset diabetes and also referred as insulin-dependent diabetes mellitus (IDDM), it is caused due to distraction of pancreatic β cells by autoimmune action and patient have to depend on exogenous insulin for survival. It normally occurs in childhood or in young age but can develop at any age. Obesity and family history or genetic linkage not found in T1DM. T1DM results from defective pancreatic insulin secretion (Salsali and Nathan 2006).

Type 2 diabetes mellitus (T2DM) found in 90% of patients of diabetes and is also known as adult-onset diabetes or noninsulin dependent diabetes mellitus (NIDDM). In this case patients may have high plasma level of insulin but have very low response to insulin simulation because insulin hormone loss its sensitivity. In last stage of disease hypoinsulinemia occurs due to the β-Cell failure and patient depends on insulin treatment, like patients having T1DM. Insulin resistance is the major cause of T2DM and its main factors are genetic and acquired that decrease the sensitivity of insulin at

different levels. Insulin resistance is caused due to impaired signaling of receptor and post receptor signaling molecule and genetic abnormality can also be a major factor. (Kahn *et al.*, 2000; Nandi *et al.*, 2004).

1.1.1 Insulin (INS)

Diabetes research and treatment starts with the discovery of insulin in 1921 by Banting and Macleod in 1923. The size of insulin is 5.8 kDa and it is globular protein containing 2 chains α and β these are linked by disulfide bonds. In the Islets of Langerhans β-cells of pancreas synthesized and secrete this hormone. In rough endoplasmic reticulum (rER) the preproinsulin is synthesized and in lumen of rER it rapidly cleaved into proinsulin. After post translational modification the mature insulin molecules are packed into vesicles, and when the glucose level raise in the circulation insulin secrete in response to it. When insulin attached or binds to the surface receptor of target tissue like liver, muscle and adipose on their membrane, Insulin mediate actions like uptake of nutrients, storage of energy and also increases the growth of somatic cells (White and Kahn, 1994; White, 1997).

Cellular response are regulated by insulin clearance it keep control tightly over circulating insulin level, it also specify insulin signaling and mediate its compartmentalization (Duckworth *et al.*, 1997; Valera Mora *et al.*, 2003).

All sensitive tissues have feature of uptake and degradation of insulin hormone (Castillo *et al.*, 1994; Canas *et al.*, 1995; Duckworth *et al.*, 1998); but liver and kidney are the major sites for insulin clearance they are also known as primary sites of insulin clearance. 50% of the portal insulin clears in circulation during first pass transit id done by liver, and kidney remove 50% of systemic circulation of peripheral insulin approximately.

1.1.2 The Insulin Receptor (IR)

Insulin Receptor (IR) regulates a number of cellular functions like glucose transport, protein synthesis, mitogenesis, glycogen synthesis, and fatty acid synthesis. IR is the most extensively studied protein due to its significance in regulation of cellular functions. IR is encoded by the IR gene that is located on human chromosome 19p13.2. The gene consists of 22 exons, from which 11 exons encode α-subunit and the remaining 11 exons encode the β subunit (Seino *et al.,* 1989). IR is most highly expressed in insulin target tissues such as the liver, adipose tissue and skeletal muscle; however it is translated in all body tissues. Insulin mediates its action when insulin binds to the α-subunit of the IR (Rosen, 1987). The action of insulin start when the insulin binds to the IR with its α-subunit (Rosen, 1987) when transmembrane of insulin receptor bind with insulin the action of insulin starts and in response the enzyme tyrosin kinase activate in receptor β subunit which cause the conformational changes in the receptor (Kasuga *et al.*, 1982). Insulin receptor substrate proteins (IRS) serve as the main ligands for the IR and are phosphorylated in response to insulin action (Myers *et al.*, 1994). There are four known IRS proteins, IRS-1, IRS-2, IRS-3, and IRS-4 which serve to amplify and transmit the insulin signal to downstream pathways through the recruitment and phosphorylation of src homology 2 (SH2) domain containing proteins.

1.1.3 CEACAM1

Carcino Embryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM1) is a 120kDa plasma membrane glycoprotein (Rees-Jones and Taylor, 1985). The gene that encodes the CEACAM 1 is located on human chromosome 19q13.2, and is composed of

approximately 15kb of DNA, containing 9 exons. CEAMCAM1 was first identified as an endogenous substrate of the IR tyrosine kinase in a cell free system consisting of partially purified IR preparations from rat hepatocytes (Rees-Jones and Taylor, 1985).

Follow up studies found that this association was specific to the liver and was not seen in muscle or fat tissues (Accili *et al.*, 1986). The promoter region lacks a functional TATA box but contains multiple Sp1 binding sites and transcription initiation sites at nucleotide positions -101, -71, -41, and -27 (Najjar *et al.*, 1993; Najjar *et al.*, 1996). CEACAM1 found in many tissues. It is most highly expressed in the liver but is also found in the pancreas, kidney, endometrium, epithelial cells of the intestine, and hematopoietic cells (Thompson, 1995; Hammarstrom, 1999) CEACAM1 has been found to be a substrate of both the IR (Rees-Jones and Taylor, 1985) and EGFR (Rjaily *et al.*, 2004).

In liver insulin clearance is carried out by receptor-mediated insulin endocytosis which leads to its degradation. Experimentally the role of CEACAM1-4L has been studied in the cultured Hepatocytes to check insulin clearance and in knockout and transgenic mouse models (Formisano *et al.*, 1995; Poy *et al.*, 2002). It has been proved that receptor mediated insulin endocytosis increased in rat hepatoma cells due to the over expression of CEACAM1-4L (Formisano *et al.*, 1995). Impaired insulin clearance developed with the over expression of a liver specific dominant negative Ser503 to Ala mutant of CEACAM1-4L, to an L-SACC1 mouse (Poy *et al.*, 2002a). Impaired insulin clearance cause hyperinsulinemia and insulin Resistance.

In hepatocytes, insulin uptake and removal is aided by phosphorylation of insulin receptor of (CEACAM1), by forming an endocytosis complex with the insulin-receptor (Najjar *et al.*, 1998, Poy *et al.*, 2002) for the activation of CEACAM1 the activation of

serine phosphorylation is required at the site of CEACAM1 in response to insulin. (Najjar, 1998). For the phosphorylation of CEACAM1 on Tyr488 a serine phosphatase is required and Insulin receptor require Tyr513 for the activation of serine phosphatase that later phosphorylate the CEACAM1 on Tyr488 (Najjar *et al.*, 1998).

Phosphorylation of interacellular domain residues is the reason of CEACAM1 activation. CEACAM1 found in hepatics membrane play its essential role in the internalization and degradation of receptor-mediated insulin (Najjar *et al.*, 1997, Najjar *et al.*, 1998).

Another study demonstrated the ability of CEACAM1 to acutely bind to and inhibit fatty acid synthetase (FAS) in the liver (Najjar *et al.*, 2005). These assumptions have been investigated by performing experiment on transgenic mice with deficient CEACAM1 and to check the response of insulin internalization and its expression in the dominant negative CEACAM1. Transgenic mice developed the metabolic syndrome and hyperinsulimea because the phosphorylation site mutated (Poy *et al.*, 2002).

1.1.4 Insulin Clearance and Degradation

Hepatocytes are involved in the uptake and degradation of insulin hormone. A major risk factor of type2 diabetes is reduced hepatic insulin uptake and degradation (Duckworth *et al.*, 2011). Clearance of insulin is a critical process of insulin regulation. The response of peripheral insulin is seen impaired insulin clearance (Duckworth *et al.*, 1998; Rabin *et al.*, 1984).

CEACAM1 play an important role in the mediating of insulin clearance in the liver and that disruption in CEACAM1 function can have serious metabolic consequences. The mechanism by which CEACAM1 mediates insulin endocytosis begins when insulin bind to its receptor on the cell surface and the phosphorylation of subsequent residues of

tyrosine begins on the β-subunit. Following this event, Tyr1316 located on the C-terminal end of the IR phosphorylates CEACAM1 on Tyr488 and enables it to bind to the SH2 domain of SHC, which serves to link CEACAM1 to Tyr960 of the IR. Ceacam1 required Tyr960 phosphorylation to its site for the insulin endocytosis effect on insulin endocytosis (Najjar, Choice *et al.*, 1998).

To investigate the extended role of CEACAM1, A (L-SACC1) CEACAM1 S503A phosphorylation-defective transgenic mice with over expression of phosphorylation defected were generated subsequent phenotypic characterization revealed these mice developed altered fat metabolism, insulin resistance, impaired insulin clearance, visceral adiposity and hyperinsulinemia (Poy *et al.*, 2002).

1.1.5 Insulin Resistance

Insulin cannot properly metabolize in the condition of insulin resistance. For the mediation of insulin action it is necessary to bind the insulin with its receptor on the cell surface (Accili *et al.*, 2001). A group of risk factor simultaneously associated with the insulin resistance condition and it is referred as metabolic syndrome, for eg. Increasing the risk of coronary artery disease, stroke and type2 diabetes. Insulin resistance is the cause of impaired insulin clearance and poor signaling. Insulin resistance is caused due to the abnormal expression of insulin genetically or can be due to the signaling of receptor and post receptor signaling molecule. Primary response of insulin resistance is generally considered as hyperglycaemia and it also trigger a down regulation secondary response to regulation to insulin signaling pathway (Kahn *et al.*, 2000; Nandi *et al.*, 2004).

1.1.6 Biological Regulatory Network of Insulin Clearances from Hepatocytes

BRN of insulin clearance in hepatocytes is constructed as follows 1.Through mediation of IR uptake of insulin in hepatocytes starts. 2. IR is activated in response to ligand binding. 3. IR substrates regulate cellular signaling process such as activation of Phosphatidylinositol-3(PI-3) kinase (White, 2003). The IR phosphorylates a single tyrosine residue (Tyr-488) in the cytoplasmic domain of CEACAM1. Serine phosphorylation (ser- 503) in CEACAM1 is required for the phosphorylation by IR. So, the presence of CEACAM1 has a major role in the uptake of insulin. CEACAM1 is involved in the internalization of IR, down regulation of CAECAM1 expression in hepatocytes decrease internalization of insulin via the IR and degrades the insulin hormone (Formisano *et al.*, 1995). The cartoon diagram of the whole process depicting the clear picture is shown in figure (1.1).

The objective of the study

1. Identification of regulatory networks of insulin associated with CEACAM1 pathway.
2. Discrete modeling/ Hybrid modeling formalism will then be applied on identified network, by using Model Checker tools for the Qualitative analysis of the discrete/ Hybrid Models developed in study.
3. Based on the qualitative characterization of the biological regulatory pathways Qualitative Models will build. Development of the Hybrid model of the system using regulation Delays (Production/Degradation delays).

4. Analysis of the system behaviour for both normal pathways i.e. Oscillation and abnormal (diseased) condition i.e. dead lock state.
5. By performing in silico experiments and adjusting similar biological observed characters, parameters will be identified.
6. Introducing delays constraints and clocks the real time modeling has been done by using Hytech software.

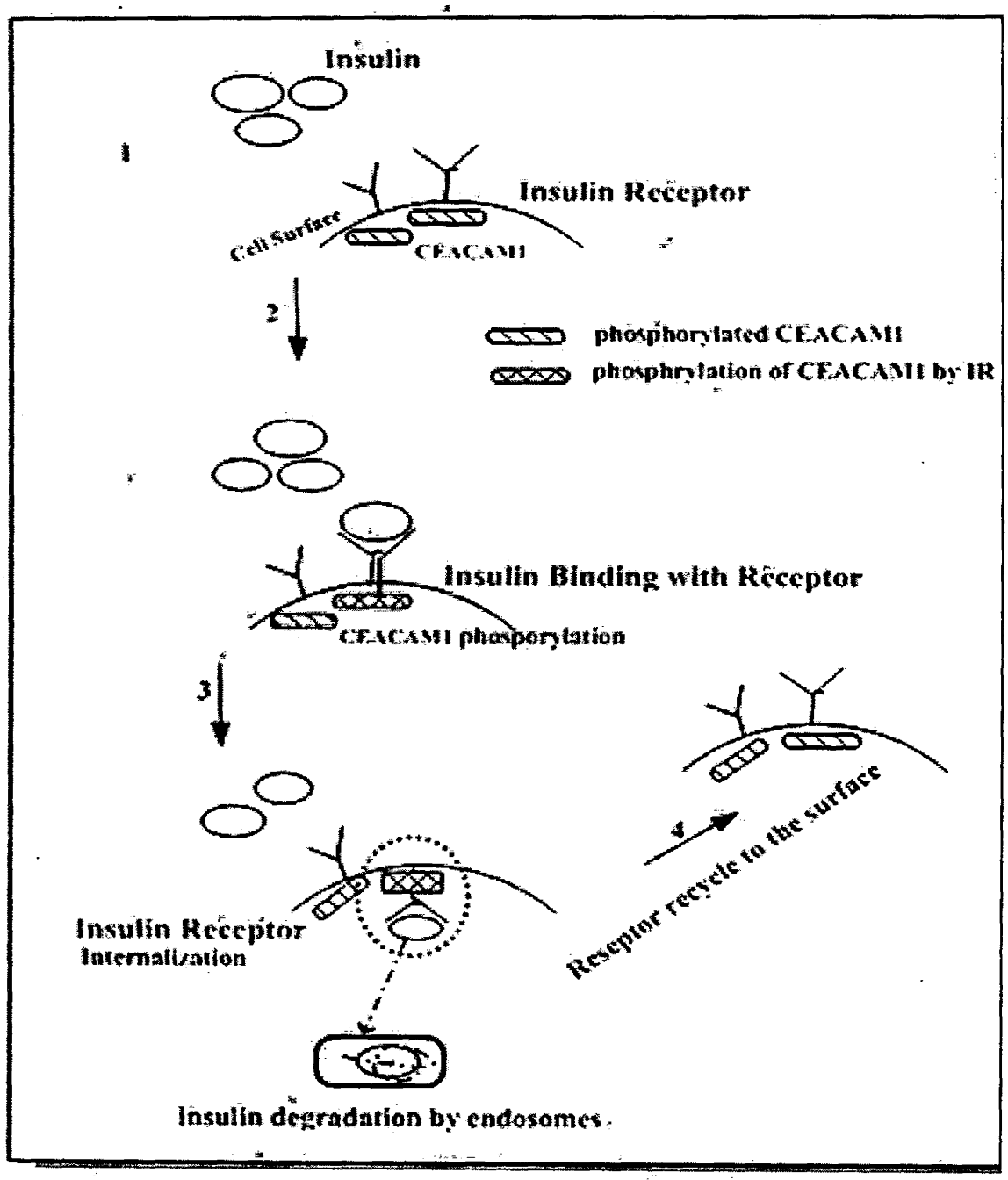


Figure 1.1: Cartoon diagram of insulin clearance from Hepatocytes

1.2 Qualitative Modeling

Biological processes are very hard to comprehend. Computer modeling give an aid to elucidate biological complexity to make novel and abstract concepts(Huang, 2001). Understanding the behavior of biological systems through modeling is a painless, economical and efficient process.

Biological Regulatory Networks (BRNs) show the interaction between the elements of the biological process (genes, proteins, etc) (De Jong, 2002). For simplification and understanding biologists present their knowledge in terms of graph. A BRN presented in the form of directed graph, the nodes refer to biological entities for example gene, protein, and mRNA etc and edges represent the interactions between them. These interactions show activation or inhibition of the biological elements and characterized by a '+' or '—' sign respectively (Ahmed *et al.*, 2006). Each node assigns a numeric value which shows its concentration of element (Bernot *et al.*, 2004). Biologists use regulatory graph for generating the dynamical model which can be continuous or discrete (Ahmed *et al.*, 2006).

BRN can be model by using different approaches the most classic approach is differential equation which is a non-linear approach but it requires heaps of parameters and their initial values are unknown. Discrete modelling approach is a well known formalism of René Thomas (Thomas and D'Ari, 1990) next approach is piecewise linear differential equation by Glass and Kaufman (Glass and Kaufman, 1973). The advantage of using discrete (qualitative) modelling is that we can set threshold to discretize the concentration

and can obtain dynamics parameters on discrete values. Qualitative models predict the response to knock-out or over-expression of mechanism (NCBI web 2010).

To model insulin clearance in hepatocytes, we use approach of René Thomas and will use the semantics of Bernot et al 2003 and drive the qualitative model (Thomas, 1991). BRN shows cyclic or non-cyclic behaviour, cycle predicts oscillatory behaviour of the system and it shows homeostasis which require negative feedback loop and positive feedback loop shows multistationarity.

Due to the Presence of unknown biological parameters abundantly it is very difficult to model the behavior of gene regulatory network (GRN) i.e. Dynamic features of numerical values for the biochemical reactions. Several approaches have been designed to overcome the lack of parameters values by proposing dedicated qualitative modeling approaches. In all these methods the gene interaction was considered as the cornerstone of a biological behavior. From a computational perspective, these modeling approaches employ the structure of the network (e.g. interlocked feedback loops) rather than relying on the numerical values of biological compound concentrations during chemical interactions.

When the qualitative modeling techniques are applied on concrete biological systems, approaches that are based on Piecewise-Affine Differential Equations (PADEs) (de Jong et al., 2004) or the René Thomas's formalism (Thomas and Thieffry, 1995) gave astonishing results. These techniques can be used for a class of hybrid systems (Ghosh *et al.*, 2004) and these powerful techniques can help us in verification and control of these hybrid systems. Particularly, they facilitate us to automatically investigate the qualitative properties of the genetic regulatory networks (Batt *et al.*, 2005).

New experimental techniques like micro-arrays (Bennett *et al.*, 2009) that examine the gene expressions over time. It drags our attention towards the temporal aspect of a biological phenomenon that takes place in all biological scales. Thus all biological systems modeling must take into account this new parameter that is called 'the time delay'. This parameter was often neglected before, although variations of specific products over time were documented.

The existing qualitative models can be refined by the representation qualitative properties that verify experimental temporal constraints. In this way the time delay represents a unique opportunity to refine existing qualitative models. So we can say that for modeling it satisfies both qualitative properties, arisen from the biological network structure, and delays associated with the dynamics of genes or gene products. For this purpose, a new hybrid modeling technique is developed that allows the biological society to directly use the qualitative and partial temporal experimental data. It abstracts the structure of the biological network by positive and negative feedback loops in order to focus on the variation of signs of the gene products as a result of qualitative behaviour. In this qualitative abstraction, some constraints on delays are added for the purpose of natural refinement of the qualitative behavior (Fromentin *et al.*, 2010).

In modern genomic techniques the simultaneous measurement of the expression levels of all genes in an organism has taken a qualitative leap to study the gene regulatory networks (Panday and Mann 2000, Lockhart and Winzeler, 2000). Likewise the formal methods for the modeling and simulation of gene regulation processes will be essential.

In most networks many genes are connected through interlocking positive and negative feedback loops, their dynamics is difficult to obtain and may lead to invalid conclusions. Formal modeling and simulation methods supported by computer tools allow us to study the behavior of large and complex networks to be predicted in a systematic way (Mcadams and Arkin 1998, Endy and Brent, 2001).

To study the qualitative behavior of dynamical systems a phase space representation is suitable introduced by Poincaré (Strogatz *et al.*, 1994). A phase space represents all possible states (Gagneur and Casari *et al.*, 2005).

1.3 Hybrid systems

In hybrid system modeling discrete event are combined with the continuous differential equation to obtain the varying real life behavior like observed inside the cell. Hybrid modeling is used for the verification of embedded and real systems. The natural and simpler formalism for hybrid modeling is time automata formalism (Ahmed *et al.*, 2007) in this formalism the state of the system are describe as discrete locations and the continuous values of variables evolve with time synchronously. These variables are known as clock. These clocks are used to test for the verification of constraints and reset when pass from one discrete location to another. The analysis of the hybrid refinement of the BRN is performed by using a linear hybrid model checker HyTech (Henzinger *et al.*, 1997).

1.3.1 Clock

The natural and simpler formalism for hybrid modeling is time automata formalism (Ahmed *et al.*, 2007) The state of the system are described as discrete locations, and the

continuous values of variables evolve with time synchronously. These variables are known as clock. Clocks are used to test for the verification of constraints and reset when pass from one discrete location to another.1, 0,-1 are the possibilities of having derivative of graphs (these values can be negative), subclass of LHA (Ahmed *et al.*, 2007).

Given a set of variable V let C (V) be a set of simple constraints i.e. of the form

$$a - b \# c \text{ or } a \# c \text{ with } c \in Q,\ a,\ b \in V \text{ and } \# \in \{=, <, \leq, >, \geq\}.$$

Clock is defined as the vector of continuous variables. Any gene *as* is associated with a clock and denoted as h_a where x shows the evolution rate of protein concentration and *ha* is the evolution rate of the clock *ha* is associated with variable *a.* Clock intervals represent the continuous system.

1.3.2 Delays:

The phenomenon of activation/inhibition occurs during a biological process and biological machinery response to this process by increasing/decreasing the concentration of corresponding proteins in order to accomplish this task it requires time. The time that elapses between each interval of biological transition is termed as time delay. Delays are defined as perameters with unknown values.

We use two types of time delays

i) Positive delays d+$v(x)$

ii) ii) Negative delays d-$v(x+1)$,

In order to represent the change of gene/protein expression level from x to x+1 and from x to x-1 respectively as depicted in figure (1.2) and (1.3) (Ahmad *et al.*, 2008). We associate a continuous clock *ha* with each variable a, having slop at state μ is wv (μ). At a given state μ, in case $Ha\ (\mu) = +1$ (resp. $Ha\ (\mu) = -1$), then, when *ha* reaches *da* (μ (*a*))

(resp. $d- a(\mu(a))$), the level of a becomes $\mu(a) + 1$ (resp. $\mu(a) - 1$) and the clock ha is reset i.e set to 0. The guard $ha == dav$, where $\alpha \in (+, -)$ is a condition which means that the delay has accomplished (Ahmed *et al.*, 2008).

1.3.3 Bioliner Hybrid Automata

(Liner Hybrid Automata) LHA are finite state automata based on real values and their values evolve continuously in a discrete state. Discrete transition in discrete state can affect the continuous values of the variables. LHA depicts that the solutions to the differential equations are lines. LHA can be subject to accessibility analysis to verify that a given set of parameters is true. However, commonly, the accessibility problem for linear hybrid automata is undecidable (Thomas *et al.*, 1998).

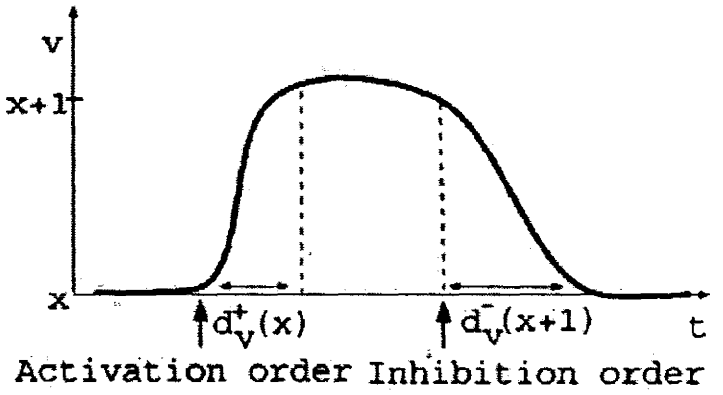


Figure 1.2: The actual evolution of a gene expression (Ahmad *et al.*, 2008)

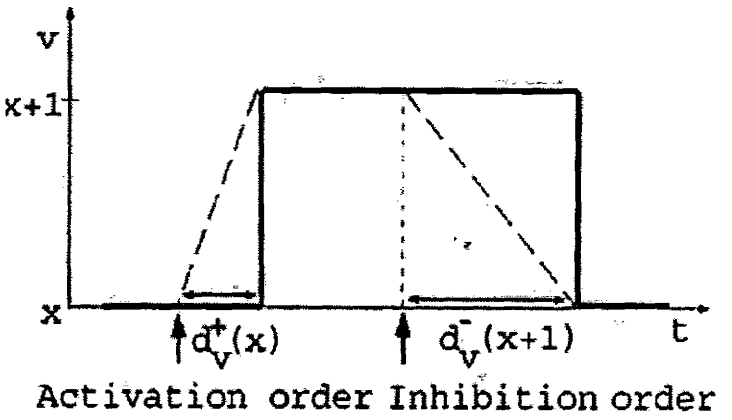


Figure 1.3: The discrete Model (Ahmad *et al.*, 2008)

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

To study dynamical behavior of regulatory networks models are built. Modeling predicts how their components are controlled, and these prediction are a set of not observable conclusions that can be later addressed experimentally (Cára *et al* ., 2007). To understand the phenomenon insulin clearance model by using kinetic logic of Rene Thomas are build so the behaviour of the regulatory pathway can comprehend. The model building steps are:

2.1 Construction of Biological Regulatory Network (BRN) from literature Survey

At the start of the study extensive literature survey about the problem searched and find out all the interaction of the proteins involve in the system and make its BRN. For example A is the protein when it achieves its certain threshold level it activates B. and when concentration of protein B goes to a certain threshold level it inhibits A.B activates itself upon a certain threshold level.

Based upon the concentration levels the activation or inhibition of protein depends on proteins concentration as shown in figure (2.1)

2.1.1 Steps of BRN Construction by Genotech:

BRN of insulin clearance in hepatocytes was constructed as follows. Through mediation of IR uptake of insulin in hepatocytes starts and IR is activated in response to ligand binding. IR substrates regulate cellular signalling process such as activation of Phosphatidylinositol-3(PI-3) kinase (White, 2003). CEACAM1 is phosphorylated by the active IR (Najjar *et al.*, 1995). The IR phosphorylates a single tyrosine residue (Tyr-488)

in the cytoplasmic domain of CEACAM1. Serine phosphorylation (ser- 503) in CEACAM1 is required for the phosphorylation by IR. So, the presence of CEACAM1 has a major role in the uptake of insulin. CEACAM1is involved in the internalization of IR, down regulation of CAECAM1 expression in hepatocytes decrease internalization of insulin via the IR and degrades the insulin hormone (Formisano *et al.*, 1995). For BRN construction the following formalism apply in our study to construct the model of insulin clearance from Hepatocytes using software Genotech.

2.1.2 Formalism

To construct the model of BRN we use discrete modelling approach of René Thomas in 1991(Thomas *et al.*, 1991). This formalism gives us the qualitative states of our BRN (Ahmed *et al.*, 2006). Some definitions related to discrete modelling formalism are given below. A directed graph G= (N, I), we note $G^-(a)$ and $G^+(a)$ the set of predecessors and successors of a node a $\in$ N respectively.BRN is constructed by using software Genotech (Ahmed *et al* ., 2009)

Definition 1: **(Biological Regulatory Network)**

A Biological regulatory Network is a graph G= (N, I) where N is representing the set of nodes which are biological entities and I is the set of edges which shows the interaction between them. Each edge a $\rightarrow$ b is labelled by a pair (t_{ab}, U_{ab}), where t_{ab} is a positive integer and U_{ab} (+,-) is the sign of interaction + for the activation and for the inhibition. Every node has a limit which is equal to its out-degree.

Transition among the states tells us about the behaviour of BRN so first we need to find the states of our BRN.

Definition 2: **(States of BRN)**

States of BRN is a tuple m where $m=(m_1,m_2,...,m_x)$, where x is the number of vertices and n_i is the abstract expression sign of interaction and threshold value tell about the current state of target node. Influence of regulator on target can be known by the current state with sign of interaction and threshold value.

Threshold T_{xy} for interaction $(x \rightarrow y)$ and a for states. For positive interaction we see if x stimulates y. if x has level below T_{xy} then it is not able to activate y and same is the case in negative interaction.

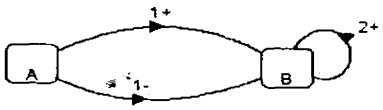


Figure 2.1: Toy example of Biological Regulatory Network

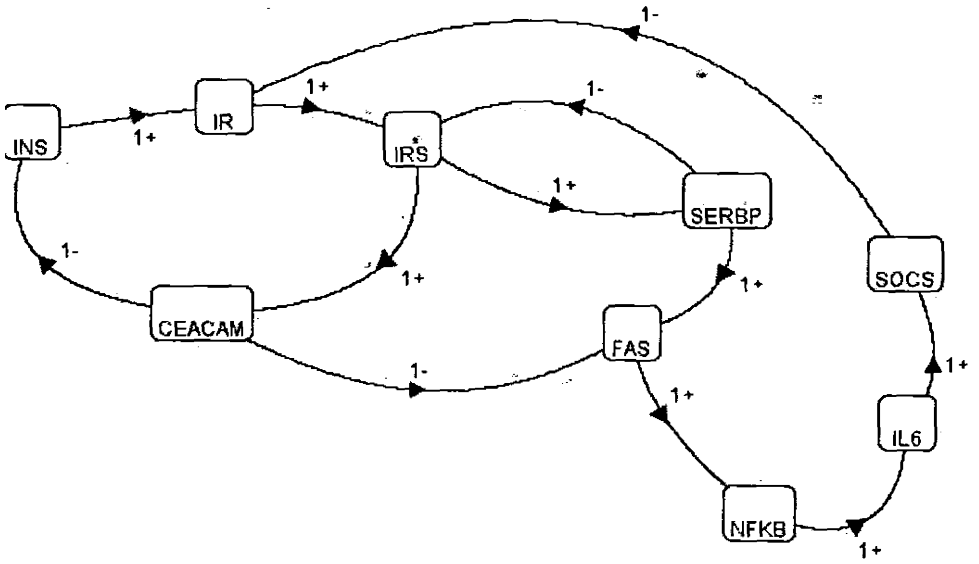


Figure 2.2: BRN drawn from survey by Genotech

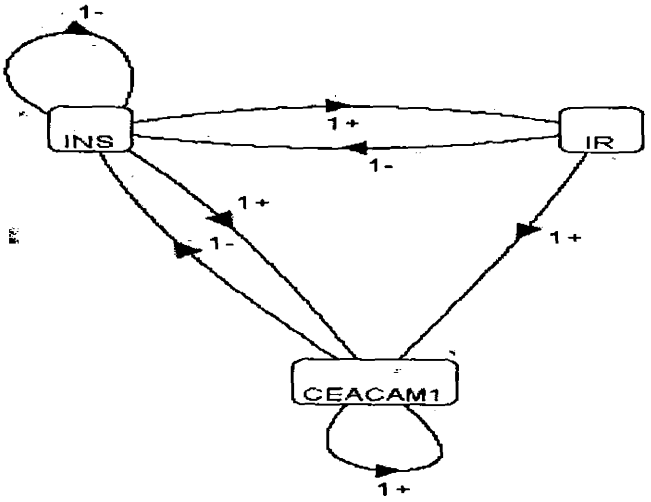


Figure 2.3: Abstracted BRN of insulin clearance model of Boolean logic

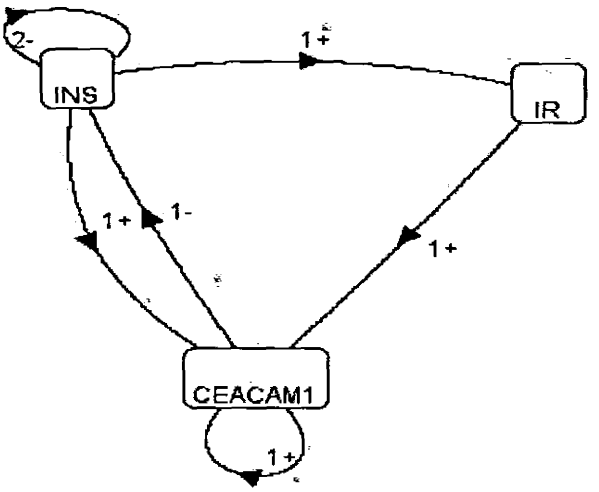


Figure 2.4: Abstracted BRN of insulin clearance model with high level of insulin

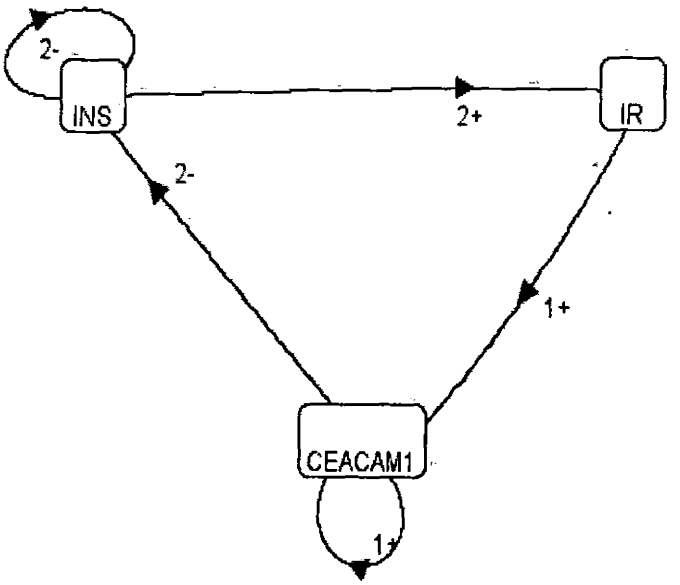


Figure 2.5: BRN of insulin clearance model with multivalued

To describe the system dynamics target will attract to certain level for this identification of each state m is required which regulator are effective for the target x or that need to find the resources of m in state m.

2.2 Abstraction of BRN

If A activates B and B activates C then it can be assumed that A activates C and the entity of B removed if its concentration is not affect any other proteins activation or inhibition initial BRN drawn from digging the literature. Analyzing the figure (2.2) it is seen that IR is activating the CEACAM1 via IRS so a direct activation is drawn. Likewise unnecessary entities removed and Figure (2.3) shown the abstracted BRN of the system.

2.3 Resource Function Assignment

Definition 3: **(Resource Function of BRN)**

Let $G(N, I)$ be a BRN. The set of resources R_{xa} of a variable $a \in N$ at level x is define as:

$R_{xa} = \{b \in G\text{-}(a) \mid (x_b \geq t_{ab} \text{ and } U_{ab} = +) \text{ or } (x_b < t_{ab} \text{ and } U_{ab} = -)\}$

According to this definition the absence of an inhibitor treated as activator. Logical parameters of BRN also called target. Set of logical parameters describe as

$K(G) = \{K_a, Rxa \in \{0, ma\} \mid x_a \in C_a \forall a \in N\}$.

At a level x of a, K_a, R_{xa} gives the level towards which the variable a tends to evolve. We consider three cases, (a) if $x_a < K_a, R_{xa}$ then x_a can increase by one unit,(b) if $x_a > K_a, R_{xa}$ then x_a can decrease by one unit and (c) if $x_a = K_a, R_{xa}$ then x_v cannot evolve. Transition that a BRN follow represents by a state graph.

Asynchronous state graph of BRN, Let x and $k \in Z \geq 0$, then

$$x \uparrow k = \begin{cases} x + 1 \text{ if } x < k \\ x - 1 \text{ if } x > k \\ x \text{ if } x = k \end{cases}$$

The parameter assignment of the variables of the model has been shown in table 2.1.

Table 2.1: Boolean Variable Assignment of the Variables.

Protein	**Activator**	**Inhibitor**	**Parameter**
INSULIN (INS)	NILL	CEACAM1 ,IR, INS	K (INS , { }) = 0 K (INS,{INS }) = 1 K (INS ,{CEACAM1}) = 1 K (INS , { IR}) = 1 K (INS , { INS , CEACAM1 }) = 1 K (INS , { IR , CEACAM1 }) = 1 K (INS,{INS,IR})=1 K(INS,{ INS , IR , CEACAM1 }) = 1
INSULIN RECEPTOR (IR)	INSULIN (INS)	NILL	K (IR , { }) = 0 K (IR , { INS }) = 1
CEACAM1	INS, CEACAM1, IR	INS	K (CEACAM1,{}) = 0 K (CEACAM1 , { CEACAM1 }) = 0 K (CEACAM1 , { IR }) = 0 K (CEACAM1 , { INS }) = 0 K (CEACAM1 , { INS , IR }) = 0 K (CEACAM1 , { IR,CEACAM1})=0 K(CEACAM1,{ INS,IR,CEACAM1 })=1

Table 2.2: Values Exhibiting High Level of Insulin Resources.

Protein	Activator	Inhibitor	Parameter
INSULIN (INS)	NIIL	CEACAM1 , INS	K (INS , { }) = 0 K (INS,{INS }) = 2 K (INS ,{CEACAM1}) = 2 K (INS , { IR}) = 2 K (INS , { INS , CEACAM1 }) = 2 K (INS , { IR , CEACAM1 }) = 2 K (INS,{INS,IR})=2 K(INS,{ INS , IR , CEACAM1 }) = 2
INSULIN RECEPTOR (IR)	INSULIN (INS)	NILL	K (IR , { }) = 0 K (IR , { INS }) = 1
CEACAM1	NILL	INS, CEACAM1,IR	K (CEACAM1,{}) = 0 K (CEACAM1 , { CEACAM1 }) = 0 K (CEACAM1 , { IR }) = 0 K (CEACAM1 , { INS }) = 0 K (CEACAM1 , { INS , IR }) = 0 K(CEACAM1,{IR,CEACAM1})=0 K(CEACAM1,{ INS,IR,CEACAM1 })=1

Table 2.3: Values of Multilevel Resources.

Protein	**Activator**	**Inhibitor**	**Parameter**
INSULIN (INS)	NILL	CEACAM1 ,IR, INS	K (INS , { }) = 0 K (INS,{INS }) = 2 K (INS ,{CEACAM1}) = 2 K (INS , { INS , CEACAM1 }) = 2
INSULIN RECEPTOR (IR)	INSULIN (INS)	NILL	K (IR , { }) = 0 K (IR , { INS }) = 1
CEACAM1	INS, CEACAM1, IR	INS	K (CEACAM1,{}) = 0 K (CEACAM1 , { CEACAM1 }) = 0 K (CEACAM1 , { IR }) = 0 K (CEACAM1 , { IR,CEACAM1})=2

2.4 Analysis of state Graph

Definition 4: **(State graph of BRN)**

Let $G = (N, I)$ be a BRN and s_a represent the level of a gene a in a state $s \in S$. The state graph of a BRN is a directed graph $G = (S, T)$ with a transition relation $T \in S \times S$ such that $s \rightarrow s' \in T$ iff: There exists a unique $a \in N$ such that $s_a \neq s_a'$ and $s_a' = s_a \uparrow K_a, R_{xa}$ and $s_b' = s_b \; \forall b \in V \mid \{a\}$.

Only one component differs from a successor in state graph .from state s there will be n successor from n components in the state graph.

2.4.1 Cycles

Definition 5: (Trajectories and cycles).

A trajectory is a sequence of states related by discrete and continuous transitions. A cycle is a trajectory that starts in a given location and returns to this same location further on. In the hybrid model of a GRN, we respectively denote $\square(t)$ for $t \in R \geq 0$ and S the sequence of points of a trajectory and the set of all points in its state space

2.4.2 Dead lock State

Definition 6: **(Dead lock state of BRN)**

A dead lock state is a state where system gets stuck and no outer transition is possible from this stage.

2.5 Graphical view of Model by using Graphiz:

Graphiz (software) package contains graph visualization software. Graph visualization is a way of representing structural information as diagram of abstract network and graph

graphviz-2.26.3 is used for the graph generation and analysis and from these graph cycle and deadlock state is easily analyzed.

2.7 Real time Modeling/ Hybrid Modeling

2.7.1 Hybrid Modeling of BRN

Discrete and continuous features are mixed in hybrid modeling to obtain distinct biological properties. Hybrid modeling focuses to find the time delay pass from a gene expression level to the other. By introducing time delays a subgraph which is showing characterized state of model can be convert into a hybrid model.

Definition of Linear Hybrid Automata (LHA)

A Linear Hybrid Automaton is a 6-tuple (Q, q_0, F, A, Inv, Dif) where

- Q is a finite set of locations.
- q_0 is the initial location.
- F is a finite set of real-valued variables
- $A \subset Q \times C(F) \times 2^F \times Q$ is a finite set of edges. If $a = (q, \alpha, R, q') \in A$, a is the Edges between the locations q and q', with the guard and the set of variables to be reset R.
- $Inv \in (C(F))^Q$ maps an invariant to each location .
- $Dif \in (Z^F)^Q$ maps an evolution rate to each (continuous) variable in each location, dX/dt being the set of derivatives of the variables wrt. time.

$dX/dt = (Dif(q,f))_{f \in F}$. For short, given a location q, a continuous variable x and $n \in Z$, we will denote

$Dif(q,f) = n$ by $dx/dt = n$ when the location considered is not ambiguous.

Note that in our BRN models, *Dif (q,f)* will always be 0, 1 or –1

2.7.2 HyTech:

HyTech is the model-checker chosen in this study (Henzinger et al., s1997) for real time modeling it has the ability to manage parameters through synthesizing constraints relative to these parameters, thus satisfying necessary conditions for the existence of the behaviors analyzed. Hytech file includes the clocks and delays clocks measure the time of delays. Hytech file is generated from Genotech software and after amending delay constraints the output file gives behavior with delays..

2.7.3 Invariance Kernel Analysis

Automatic symbolic analyses such as detection of cyclic behaviours or identification of the so-called *invariance kernel* can be performed using a symbolic model-checker.

Definition 4.3 *(Invariance kernel)*

A trajectory □(t) is viable in A if □(t) ∈ A for all t ≥ 0. S subset *K* of A is said to be invariant if for any point $p \in K$, a trajectory starting in p is viable in *K*. An invariance kernel *K* is the largest invariant subset of A.

CHAPTER 3

RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSIONS

Half life of insulin in circulation is about five minutes. Insulin binds to its receptor and internalize and it is degraded in endosome, formed in the process of endocytosis. About 80% of insulin degrades in liver and kidney. The action of insulin is initiated by binding with cell surface receptor. This number of receptor varies in different type of cells e.g. only 40 in erythrocytes and 300,000 per cell on hepatocytes and adipose tissues. Insulin resistance occurs in liver and adipose tissues, whilst not properly responding to insulin, glucose and fats released into circulation. Sustained elevation of circulatory glucose and triglyceride causes hyperinsulimia, a condition in which there are excess levels of circulating insulin in the blood, cause pre-diabetes, insulin resistance, cardiovascular diseases and obesity. Insulin resistance is the leading cause of many metabolic syndromes. Resistance of insulin is a major cause of Diabetes. Diabetes is a chronic metabolic disease wherein the human body does not produce or properly use insulin. Each year, approximately 4 million deaths are attributed to diabetes. 20 million people in Pakistan are Diabetic patients. Type 2 diabetes accounts for approximately 90-95% of all diabetes. Globally, there are approximately 257 million people with type 2 diabetes.

For the study dynamical behavior of regulatory networks we built model, modeling predicts how their components are controlled, these predict a set of not observable conclusions that can be later addressed experimentally (Cara *et al.*, 2007). Insulin clearance is critical insulin regulatory action. Impaired insulin clearance is seen in response to peripheral insulin clearance is seen in response to peripheral insulin resistances (William *et al.*, 1998: Rabkin *et al.*, 1984). Insulin clearance in the liver is mediated by receptor-mediated insulin edocytosis followed by degradation .Upon its phosphorylation by the

insulin receptor the Carcino Embryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM1) take part of the insulin-receptor endocytosis complex to promote insulin uptake and removal in the Hepatocytes (Najjar *et al.*, 1995: Poy *et al.*, 2002).

To construct the model of BRN discrete modeling approach of René Thomas was used in 1991 (R. Thomas, 1991). This formalism gives us the qualitative states of the BRN (Ahmed *et al.*, 2006). According to René Thomas logic, cycle predicts oscillatory behavior of the system and it shows homeostasis which require negative feedback loop and for multistationary, it requires positive feedback loop. The model of hepatic insulin clearance contains three variables INSULIN, INSULIN RECEPTOR, and CEACAM1.

In current study 3 models have been constructed. Model 1 is the original abstracted model from the extensive literature survey and use Boolean values only 0,1 as shown in parameter Table (2.1). Second model was build in which only the concentration of insulin set at high level and finally to conform the results as table (2.2) depicts another model was the maltivalued approach in which the level inceresed up to 2 shown in table (2.3). The activation levels of the variables in model was set and described here graphically.

3.1 Activation Levels

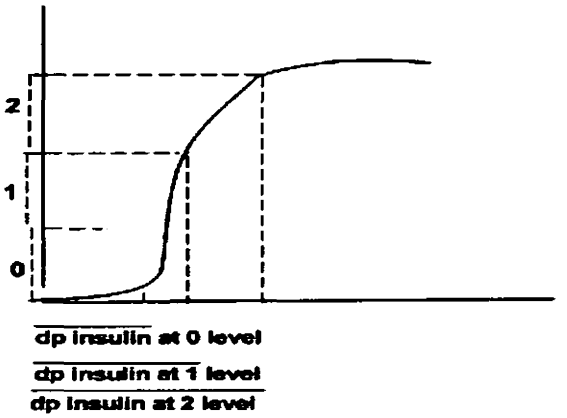
3.1.1 Insulin Activation Level

The figure (3.1) depicting the levels of insulin use to set the perametric values of insulin which assign to the model and these values will help to understand the Graph obtain from the model which will show the behavior of the model either it will remain in cycle or go to the steady stable state. When the level of insulin becomes 0 it means that insulin not present or it get digested mean 0 level shows the absence of insulin at level 1 it produce

and found in normal concentration and level 2 showed that high level of insulin that means the level of insulin was more than the normal level.

3.1.2 Insulin Receptor (IR) Activation Level

Activation of Receptor can either be ON (1) or OFF (0) it means that when insulin bind with the receptor it will be activate and when it will free it doesn't respond to insulin due to some abnormality the threshold levels are depicted in figure (3.2)

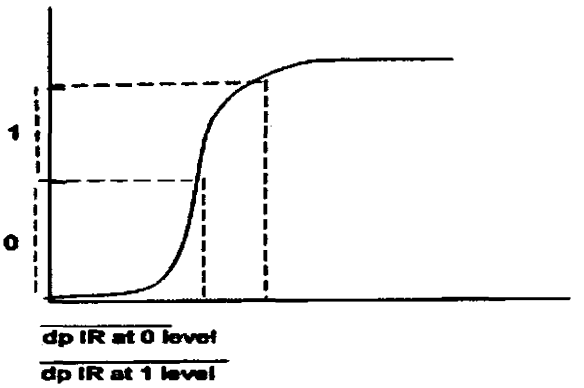


0 insulin is either abnormal or absent

1 normal level of insulin

2 high level of insulin

Figure 3.1 Insulin Activation Level.



0 IR is not responding to the insulin

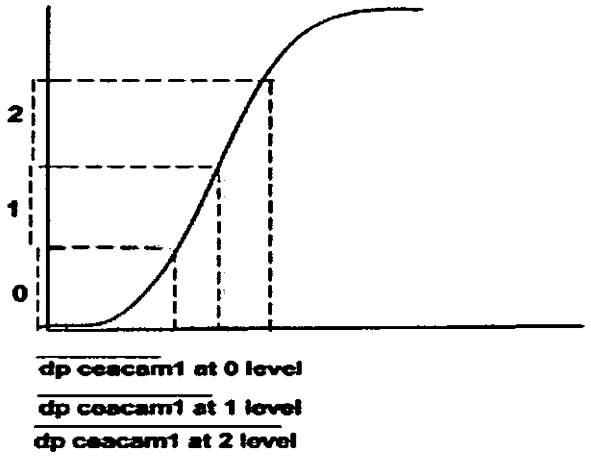
1 IR is normaly binding with the IR

Figure 3.2 Insulin Receptor (IR) activation level

3.1.3 CEACAM1 Levels of Activation

The figure (3.3) shows the activation levels of CEACAM1 and shows that at 0 level it behaves as abnormal or absent. It is not activating by activation signals from insulin receptor due to any mutation or inhibition from any inhibitor. The level 1 shows its pre phosphorylation on (Tyr-488) in the cytoplasmic domain of CEACAM1 and level 2 depicted Serine phosphorylation (ser- 503) in CEACAM1 required for the phosphorylation by IR and at this stage the CEACAM1 will be fully functional to internalize the ligand binded IR. These are the threshold level that was decided for the concentration level of variables.

For the normal process of insulin clearance insulin should properly oscillate. Its oscillation is due to the response of the receptor and activation of CEACAM1. This activation leads to complex internalization. This phenomenon is necessary for the proper regulation and if CEACAM1 does not activated in response to IR, phosphorylation the internalization process will not possible. Insulin cannot be properly degraded which lead to the condition of hyperinsulemia and the study obtain the same results that depicts where CEACAM1 get off means absent, mutated or do not respond to the IR it leads the system to the disease condition of the body or in terms of system biology to the dead lock state.



0 CEACAM1 is either abnormal or absent

1 CEACAM1 is partialy activated

2 CEACAM1 is phosphorylated by active IR

Figure 3.3 Activation levels of CEACAM1

3.2 DYNAMICAL MODELS

3.2.1 Boolean Model of Insulin Clearance

The order of BRN: INS, IR, and CEACAM1

The BRN in chapter 2 figure 2.2 was constructed by assuming the following Boolean logical expressions from extensive literature study,

1. $INS=\overline{INS}+\overline{CEACAM1}+\overline{IR}$
2. IR=INS
3. CEACAM1=INS.CEACAM1.IR

In these expressions '+' sign represents logical OR operation while '.' represents logical AND operation. The logical constraints used in the BRN are listed in the parameter table no (2.1). The optimisation of these parameters done for obtaining the required results

$INS=\overline{INS}+\overline{CEACAM1}+\overline{IR}$ shows that the insulin will be produced and reached to its threshold when previous insulin get utilized or cleared. When insulin is not available in blood and CEACAM1 is also inactive it means, it does not internalizing the ligand bind IR because no insulin binds with IR and same in the case with IR that IR if not in its activated form means no insulin attached with it. A logical OR relation between that it means any of the condition if true the expression will work truly for it.

IR=INS means that the IR will only work in the presence of insulin means it will activate when insulin will present. The equality relations show that condition must be valid when true.

CEACAM1=INS.CEACAM1.IR depicts the proper working of CEACAM1 the presence of insulin and insulin receptor and properly activated CEACAM1 all are required and truly a summarize expression for whole study that when these three element will present and make complex the CEACAM1 facilitate to internalize ligand bond IR and after internalization degradation process of insulin and recycling of IR start but complex formation is the main key point of internalization. A logical and is between all the variables of the system which means that all the variable should must be present for the proper response of CEACAM1.

The parameter assigned to the model and after optimization these are the best fitted values for the variable to show the highly significant results.

The state graph obtained on the basis of these values shown in figure (3.4) which shows the complete picture of whole model graphically. This state graph clearly showing the transitions of the sates it also shows the cycle and deadlock state in the table (3.1) to see these transitions clearly obtained this model graph from Graphivz as depicted in figure (3.5).

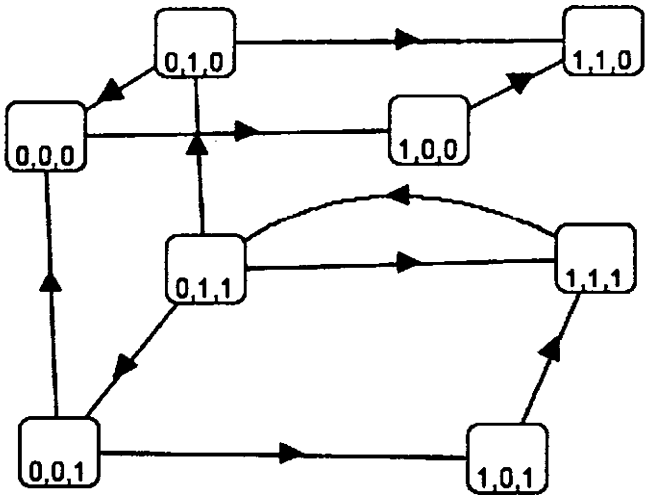


Figure 3.4: Boolean model of insulin clearance

Table 3.1: Sate Table Showing Cycle and Deadlock State

INS,IR,CEACAM1	wINS,wIR,wCEACAM1	kINS,kIR,kCEACAM1	Transition State
000	{INS,IR, CEACAM1},{}, {}	1 0 0	[1, 0, 0]
001	{INS,IR},{}, {CEACAM1}	1 0 0	[0, 0, 0][1, 0, 1]
010	{INS, CEACAM1} {} {IR}	1 0 0	[0, 0, 0][1, 1, 0]
011	{INS},{},{IR, CEACAM1}	1 0 0	[0, 1, 0][0, 0, 1] [1, 1, 1]
1 0 0	{IR, CEACAM1} {INS} {INS}	1 1 0	[1, 1, 0]
1 0 1	{IR} {INS} {INS, CEACAM1}	1 1 1	[1, 1, 1]
1 1 0	{CEACAM1} {INS} {INS, IR}	1 1 0	[]
1 1 1	{},{INS},{INS,IR, CEACAM1}	0 1 1	[1, 1, 0]

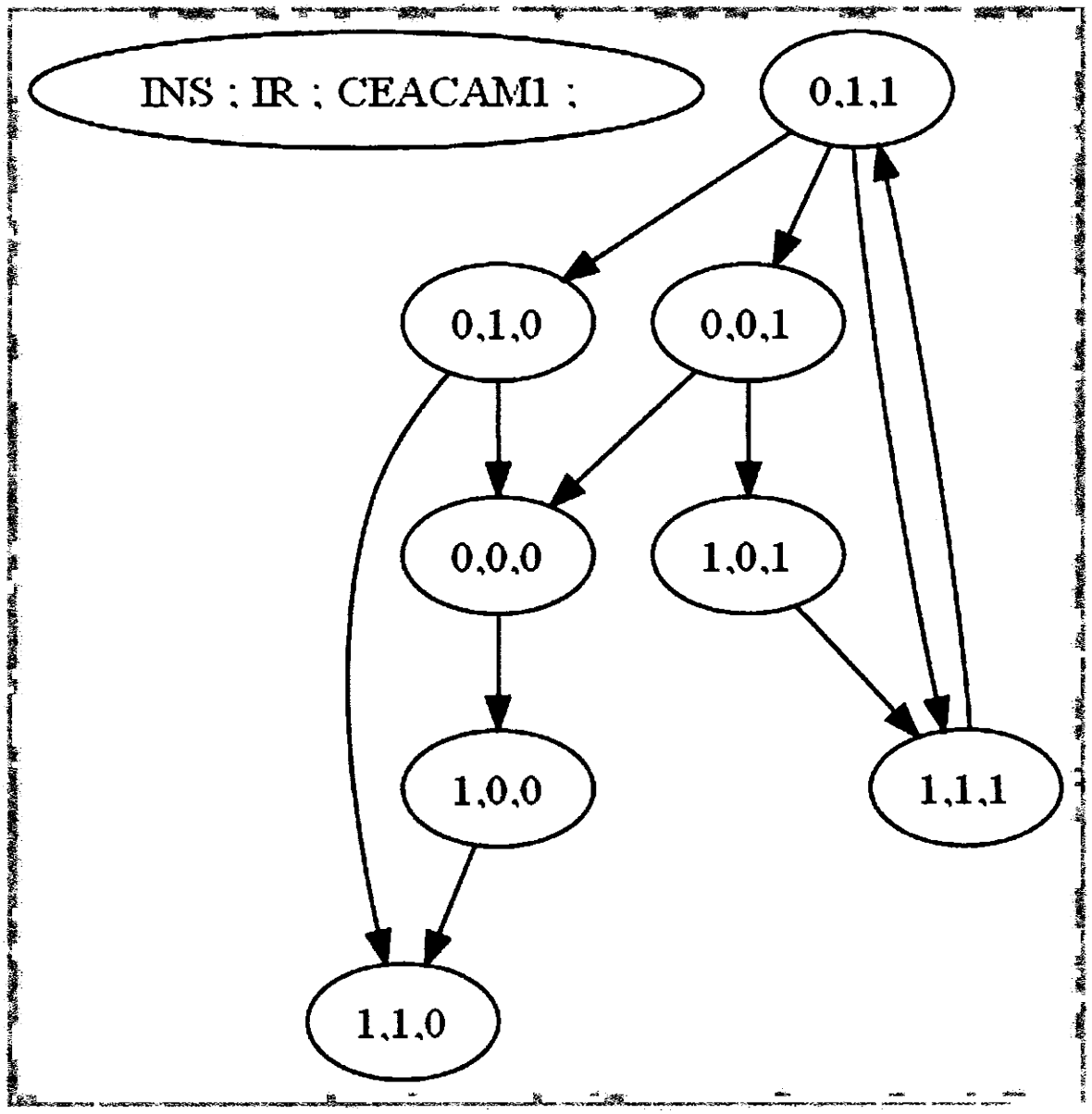


Figure 3.5: Directed graph of the Boolen model drawn by graphviz

Graph of the insulin clearance in hepatocytes shows the complete picture of model by presenting normal as well as disease behaviour of the system. Cyclic behaviour shows the proper oscillation of the entry of insulin and its degradation while the steady state show the condition where system get stuck and system shows abnormal (disease) behaviour.

In homeostasis insulin attaches to the insulin receptor, then receptor activates the CEACAM1 which makes complex with INS and IR and allow the complex to internalize, this leads to the degradation of the hormone. This process is showing in cycle n°1obtain from the model from Genotech (Ahmed J ., 2009) in cycle figure (3.6) from state [0,1,1] to [1,1,1] in these states insulin come and make complex and get degrade and system go back to 0,1,1 its insulin receiving stage or initial stage then a new insulin hormone come and cycle goes on as shown in figure (3.6).

In state [0,1,1], Insulin receptor and cecam1 are partially activated. In state [1,1,1], insulin hormone came and bound with IR. Insulin, IR and CEACAM1 undergo internalization process. In state [0,1,1], CEACAM1 degraded the hormone. This cycle clearly explain the regulation of insulin, insulin hormone came attached to the surface receptor and degraded by CEACAM1. Second cycle generated from the model, In [0, 0, 1], INS and IR are inactive while CEACAM1 partially activated which is required for its activation by IR. In [1,0,1] INS hormone is released in circulation and come to the receptor surface. State [1,1,1] shows INS bind to its receptor and activate the receptor which phosporylate CEACAM1. CEACAM1 make a complex with INS and IR and involve in their internalization. All these players are activated in this phase. [0,1,1] insulin hormone degrades under receptor mediated endocytosis with the help of

CEACAM1. At state [0,0,1] receptor get deactivated and a new insulin will switch on next insulin signal and the cycle reach its initial stage and the cycle continues.

Qualitative model generates one steady state. State [1, 1, 0] shows system goes to disease state when the CEACAM1 get mutated or knockout mean it doesn't properly responding to IR. It happens when system follow the states from [0,1,1] to [0,1,0], system moves towards the disease state but if it moves from [0,1,1] to [0,0,1] it can head for cycle. If CEACAM1 impassive the system will go in [0,0,0] state now the whole system is in unresponsive state. Insulin came in next state i.e. [1,0,0] and activate its receptor but due to CEACAM1being mutated or knocked out the complex is inefficient to be formed and internalized as a result the system will stuck here it's the dead lock state of the system. Indicating no internalization and regulation is possible now. This state predicts that insulin and IR both are active but CEACAM1 is off. So the insulin and its receptor are incompetent to internalize without CEACAM1. As a result insulin hormone unable to enter the cell and cannot undergo degradation cyclic and abnormal state shows in figure (3.8).The steady state or deadlock state causes insulin resistance, here the problem arise when CEACAM1 does not respond to the receptor which is tested in wet lab (Formisano *et al.*, 1995) and CEACAM1 model offers the true representation of the basic problem by using the kinetic logic. Constructed model predicts accurate results and response to the abnormal function of CEACAM1.

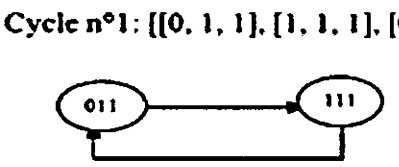


Figure 3.6: Insulin Degradation Cycle.

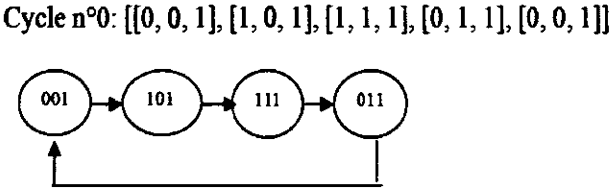


Figure 3.7: Insulin Internalization and Degeradation Cycle.

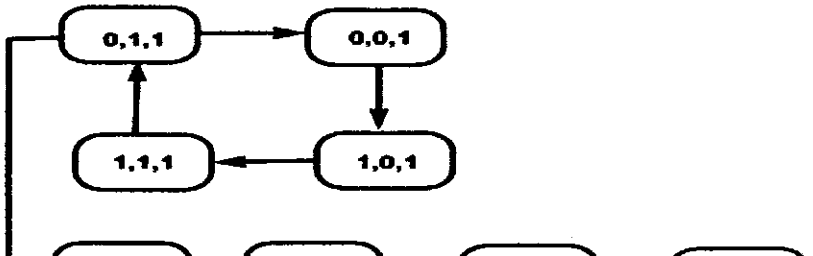


Figure 3.8: Cyclic states and abnormal states deviation.

Table 3.2: States leads to dead lock states in Boolean model.

Index	Pathway to Stable Steady State
1	011,001,000,100,110
2	011,010,000,100,110
3	011,010,110

3.2.2 Insulin Clearance Model with High Level of Insulin

The BRN with high level of insulin is based on the three variables like the Boolean model but the concentration level of insulin in this case is optimised at high level. Insulin level raise up to 2, rest of the BRN was same as Boolean but in this case insulin was inhibiting its self and also by CEACAM1 in a sense of uptake and degradation further it actives IR by its binding and IR in turn activates CEACAM1. CEACAM1 partially activated by its pre phosphorlation so regulation of positive1 shows to it INS also regulated CEACAM1 as found in literature survey. BRN showed in figure (2.3).

In this study optimized perameters are adjusted like in Boolean model but insulin level set at threshold level 2 so it becomes multivalued model. Model obtained using these parameter is shown in figure (3.9).

The model generated by Genotech in figure 3.9 is clearly shown the raise of insulin level upto 2 and stable steady state visibly shown in red {2, 1, 0} which means that when insulin level in blood becomes high and reach to maximum level (level 2 is maximum as adjusted in parameters) receptor also responding but if CEACAM1 does not respond to this legand bound IR the disease condition will arise and system will go to dead lock state and body will face the insulin resistance the pre-diabetic condition and gradually goes towards diabetes. The directed graph showing theses conditions obtain by using Graphiz which also shows the homeostatic as well as the disease condition of the system in figure (3.11).

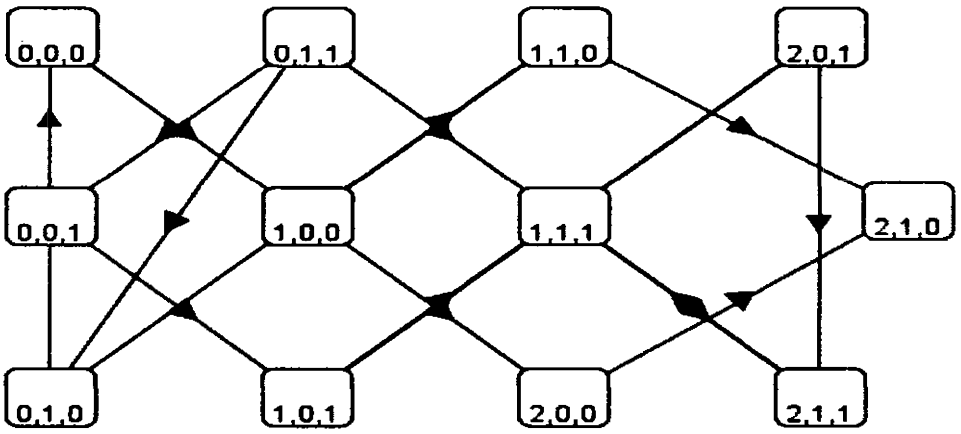


Figure 3.9: Insulin clearence model with high level of insulin.

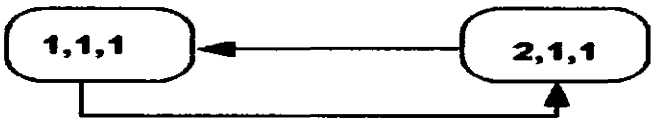


Figure 3.10: Homeostatic enternalization cycle

Table 3.3: State table showing transition states and dead lock state.

INS,IR,CEACAM1	wINS,wIR,wCEACAM1	kINS,kIR,kCEACAM1	Transition State
0 0 0	{INS, CEACAM1} {} {}	2 0 0	[1, 0, 0]
0 0 1	{INS} {} {CEACAM1}	2 0 0	[1, 0, 1], [0, 0, 0]
0 1 0	{INS, CEACAM1} {} {IR}	2 0 0	[1, 1, 0], [0, 0, 0]
0 1 1	{INS} {} {IR, CEACAM1}	2 0 0	[1, 1, 1], [0, 0, 1] [0, 1, 0]
1 0 0	{INS, CEACAM1} {INS} {INS}	2 1 0	[2, 0, 0], [1, 1, 0]
1 0 1	{INS} {INS} {INS, CEACAM1}	2 1 0	[2, 0, 1], [1, 1, 1]
1 1 0	{INS, CEACAM1} {INS} {INS, IR}	2 1 0	[2, 1, 0]
1 1 1	{INS} {INS} {INS, IR, CEACAM1}	2 1 1	[2, 1, 1]
2 0 0	{CEACAM1} {INS} {INS}	2 1 0	[2, 1, 0]
2 0 1	{} {INS} {INS, CEACAM1}	0 1 0	[1, 0, 1], [2, 1, 1]
2 1 0	{CEACAM1} {INS} {IR}	2 1 0	[]
2 1 1	{} {INS} {INS, IR, CEACAM1}	0 1 1	[1, 1, 1]

The graph drawn by Graphviz in figure (3.11) clearly shows that 2 cycles are found in the transition state, the large one started from 1,1,1 to 2,1,1 and back to 1,1,1 as in figure (3.10) and second one is 1,0,1 to 2,0,1 and back to 1,0,1.

This cycle depicts in figure (3.10) shows the homeostatic or cyclic behavior of insulin clearance that was initially on normal level of 1 but as its level rise to 2, the internalization performs and it returns again to its normal level. The cycle showed the normal homeostatic behavior of the degradation process. These results are another conformation of the model resemblance to the natural system of body.

Dead lock state of the model is {2, 1, 0} where CEACAM1 does not respond to the active or legand bond IR and system moves toward stable state it is obviously confirmed from these models that where ever CEACAM1 level goes to 0 mean mutated or abnormal system will lead to the dead lock state from that point states moves from cyclic path and lead towards a stable state. The transition states and dead lock states shown in table (3.3) and the table (3.4) shows all the states that lead to the stable state.

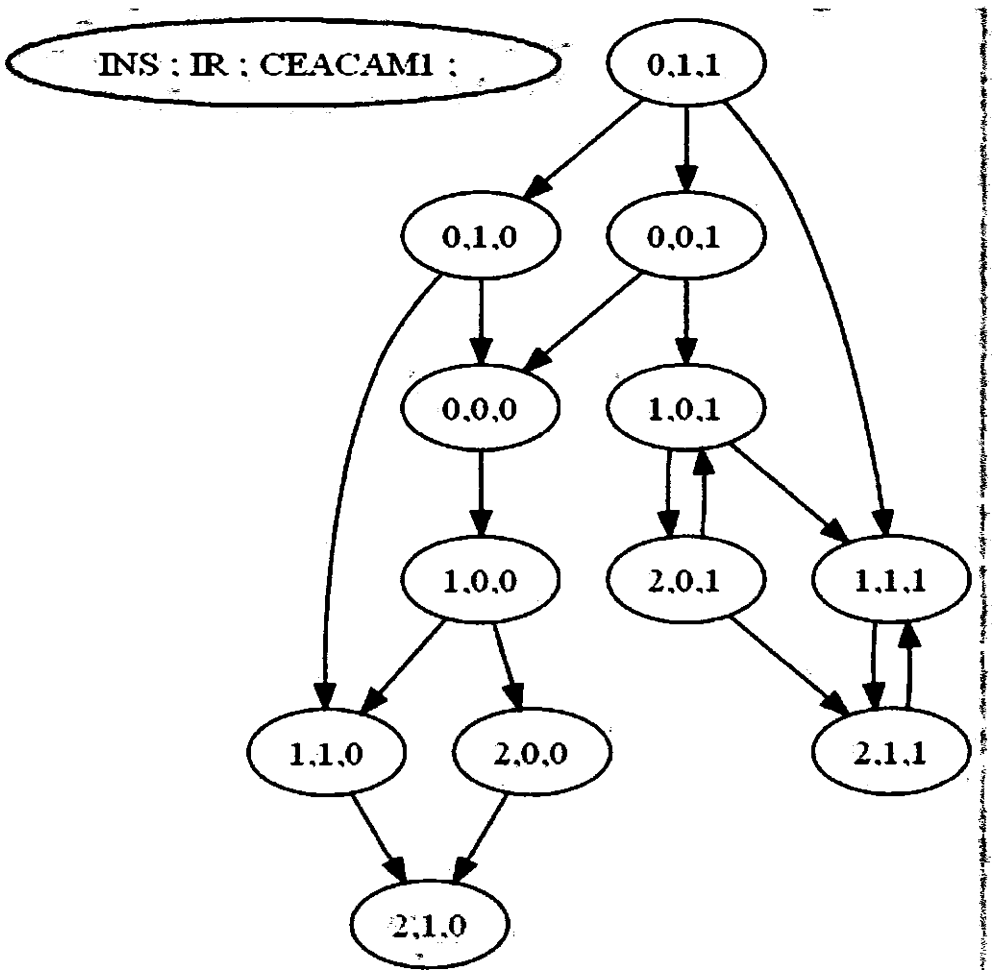


Figure 3.11: Directed graph of the model with high level of insulin

Table 3.4: High level insulin model states leads to dead lock states.

Index	Pathway to Stable Steady State
1	011,010,000,110,210
2	011,001,000,100,110,210
3	011,001,000,100,200.210

3.2.3 Completely Multivalued Model

The BRN depicted in the figure (2.4) was constructed according to the laws of multivalued in which the threshold level increase upto the maximum possible outgoings from a variable. The model in which the threshold level of the insulin has been set upto level 2, it means that the insulin level is higher than normal level whereas level 1 is considered as the normal level of insulin. CEACAM1 is also optimized up to the three levels 0, 1 and 2. These level shows the activation levels as described in figure (3.3) and shows that at level 0, 0 is the abnormal or inactive and can be mutated form of CEACAM1, level 1 depicts the pre-phosphorylated form of CEACAM1 is mandatory required for the phosphorylation by active (ligand bound IR) IR and level 2 is supposed to ideal threshold level for CEACAM1 when it is ready for the internalization of insulin and IR. At the level 2 IR the insulin already attached to IR and is capable to phosphorylate CEACAM1. This process allows the complex to internalize inside the cell so it's the fully functional state of CEACAM1 whole process in shown in cartoon diagram shown in figure (1.1).

The BRN in the figure (2.4) showed the interactions of insulin binding with IR and then the activation of the pre-phosphorylated CEACAM1. The internalization of the complex and degradation of the insulin, concentration levels of the insulin and CEACAM1 thresh hold levels and its effect at the fluctuation of the molecules engage with the system. The behavior of the network is closely resembled with the Biological observation that observed and used in the study for model building that has been used in following studies. In hepatocytes, insulin uptake and removal is aided by phosphorylation of insulin receptor of (CEACAM1), by forming an endocytosis complex with the insulin-receptor

(Najjar *et al.*, 1998, Poy *et al.*, 2002) Intact phosphorylation at this site is required for CEACAM1 to activate a serine phosphatase in response to insulin (Najjar, 1998). Moreover, CEACAM1 phosphorylation on Tyr488 by the insulin receptor activated a serine phosphatase that requires Tyr513 (Najjar *et al.*, 1998).

The hepatic membrane protein CEACAM1 has been shown to participate in the process of receptor-mediated insulin internalization and degradation. CEACAM1 activation depends on the phosphorylation of residues in its intracellular domain (Najjar *et al.*, 1997, Najjar *et al.*, 1998).

Exactly the same behavior is exhibited by the model results in state graph in figure (3.12) shows the steady state of the system or disease state. The state confirmed that the insulin concentration at high level and receptor also activated but CEACAM1 does not respond either due to mutation or some abnormality. So the complex is not able to internalize and the degradation process of the insulin will stop here. Insulin level reach to its maximum but the proper metabolism of insulin will not take place due to the abnormal response of the CEACAM1. The disease state obtained from any model either Boolean model or model in which only increased value of insulin is observed or completely multivalued is same that means that results are highly significant and is not changed by changing the threshold or concentration level of the system.

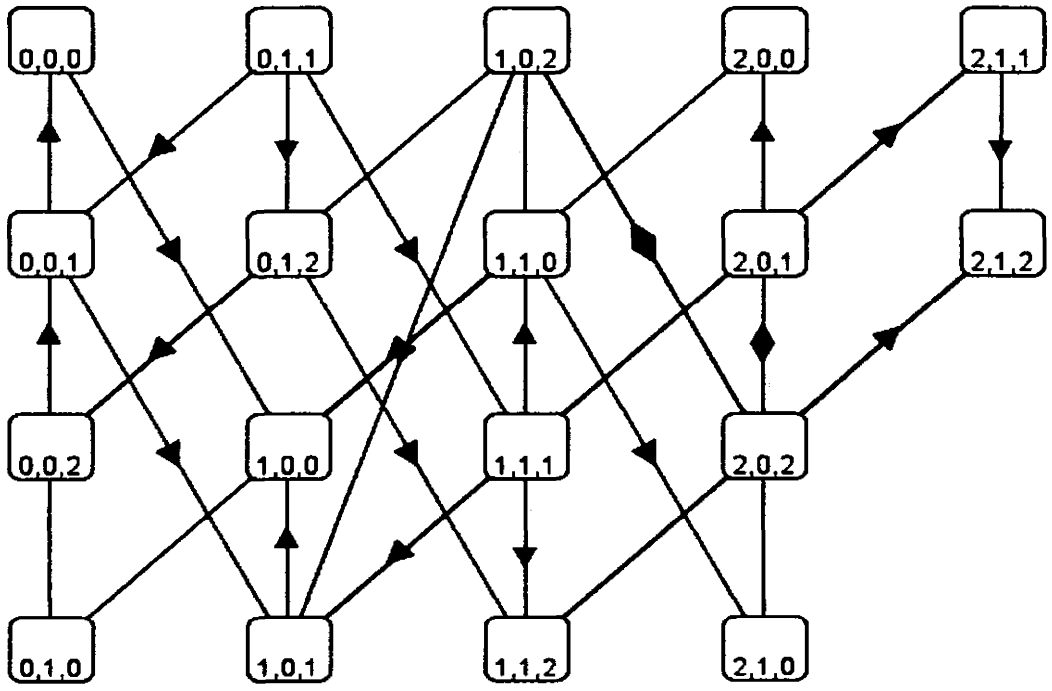


Figure 3.12: Multivated Insulin Clearence Model of Insulin Clearence.

Table 3.5: Multivalued state table transition states and dead lock state

INS,IR,CEACAM1	wINS,wIR,wCEACAM1	kINS,kIR,kCEACAM1	Transition State
0 0 0	{INS, CEACAM1} {} {}	2 0 0	[1, 0, 0]
0 0 1	{INS ,CEACAM1} {} {CEACAM1}	2 0 0	[1, 0, 1], [0, 0, 0]
0 0 2	{INS}{}{CEACAM1}	2 0 0	[1, 0, 2], [0, 0, 1]
0 1 0	{INS, CEACAM1} {} {IR}	2 0 0	[1, 1, 0], [0, 0, 0]
0 1 1	{INS, CEACAM1} {} {IR, CEACAM1}	2 0 2	[1, 1, 1], [0, 0, 1], [0, 1, 2]
0 1 2	{INS} {} {IR, CEACAM1}	2 0 2	[1, 1, 2], [0, 0, 2]
1 0 0	{INS, CEACAM1} {} {}	2 0 0	[2, 0, 0]
1 0 1	{INS, CEACAM1} {} {CEACAM1}	2 0 0	[2, 0, 1], [1, 0, 0]
1 0 2	{INS} {} {CEACAM1}	2 0 0	[2, 0, 2], [1, 0, 1]
1 1 0	{INS, CEACAM1} {} {IR}	2 0 0	[2, 1, 0], [1, 0, 0]
1 1 1	{INS, CEACAM1} {} {IR, CEACAM1}	2 0 2	[2, 1, 1], [1, 0, 1], [1, 1, 2]
1 1 2	{INS} {} {IR, CEACAM1}	2 0 2	[2, 1, 2], [1, 0, 2]
2 0 0	{CEACAM1} {INS}	2 1 0	[2, 1, 0]

			{}				
2	0	1	{CEACAM1} {CEACAM1}	{INS}	2	1	0	[2, 1, 1], [2, 0, 0]
2	0	2	{} {CEACAM1}	{INS}	0	1	0	[1, 0, 2], [2, 1, 2], [2, 0, 1]
2	1	0	{CEACAM1} {IR}	{INS}	2	1	0	[]
2	1	1	{CEACAM1} {IR, CEACAM1}	{INS}	2	1	2	[2, 1, 2]
2	1	2	{} CEACAM1}	{INS}	{IR,	0	1	2	[1, 1, 2]

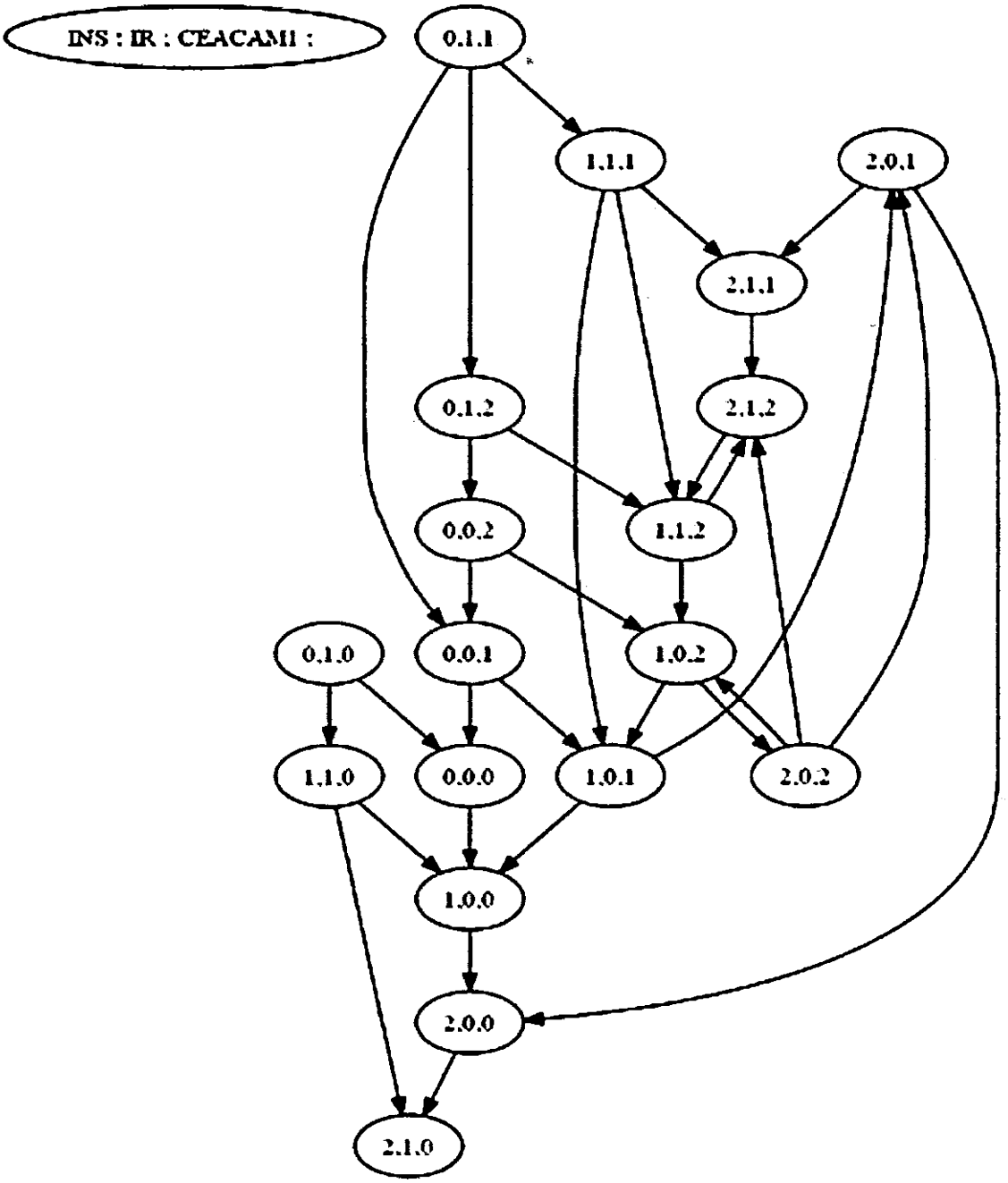


Figure 3.13: Directed graph of the multivalued insulin clearance model.

The comprehensible picture of all states can be viewed by Graph drawn from Graphiz figure (3.13). Cycles are also obtained from the model which shows the homeostasis of the system. Homeostatic or cyclic behavior depicts the proper metabolism from model 4 cycles are obtained and the biggest cycle of insulin metabolism model is

Cycle n°0 : [[1, 0, 1], [2, 0, 1], [2, 1, 1], [2, 1, 2], [1, 1, 2], [1, 0, 2], [1, 0, 1]]

In this cycle, figure (3.14) shows that initial state 1,0,1 in this state the insulin is present and CEACAM1 is pre activated. At state 2,0,1 the concentration of insulin get high and at state 2,1,1 IR is activated in response to insulin at next state 2,1,2 the active IR activate the CEACAM1 and its threshold reach to the level 2 as it is now completely ready for the internalization of complex and at next state complex will internalize and insulin will be degraded and its level becomes low and back from 2 to 1 the phenomenon is clear from the high insulin model and after the degradation of insulin the IR becomes free and show as inactive or off at level 0. After the internalization process CEACAM1 is also gone to its normal phosphorlated level.

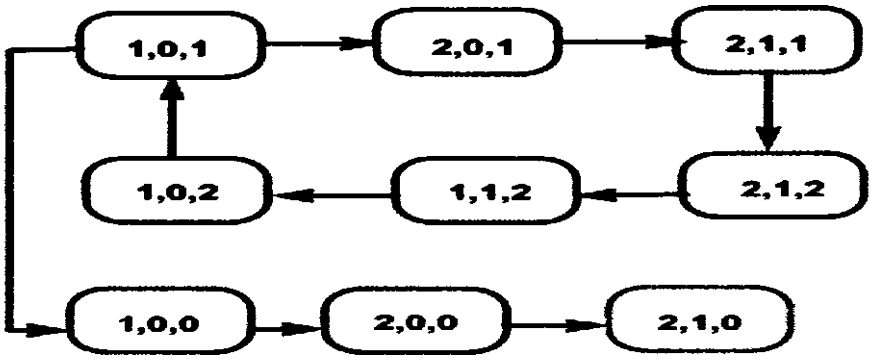


Figure 3.14: Cyclic states and abnormal states deviation to deadlock in mutivalued.

Table 3.6: States leads to dead lock states in generalized model

Index	Pathway to Stable Steady State
1	011,111,211,212,112,102,101,100,200,210
2	011,111,112, 102,101,100,200,210
3	011,111, 101,100,200,210
4	011,012,002,001,000,100,200,210
5	011, 001,000,100,200,210

3.3 Real Time modeling

After modeling from Genotech the time delays introduce to the states obtain from the model to find the time delay of a system to remain in the cyclic state or leading towards the dead lock state. Its hybrid modeling and by using Hytech software Real time model can be obtained for the system.

The introduction of time delays for both Boolean as shown in table (3.8) and multivalued model as shown in table (3.7) depicted the time elapse between transition from one state to another and cover the whole path that lead to stable state. These tables cover all the path and their delays of each model that lead to dead state. Automatic symbolic analyses such as detection of cyclic behaviors or identification of the *invariance kernel* can be achieved using a symbolic model-checker. *HyTech* is the model-checker chosen in current study (Henzinger et al., 1997): it has the ability to manage parameters through synthesizing constraints relative to these parameters Hytech file is generated from genotech, adding the required conditions, the delay constraints obtained that shows thel necessary conditions for the existence of the model to remain in cycle and move towards the dead lock state these behaviors of model analyzed.

Table 3.7: Pathway with delay leads to Stable Steady State of multivalued model.

Index	Pathway with delay leads to Stable Steady State
1	$011 \xrightarrow{\text{dpINS101} <= dpIR101} 111, \xrightarrow{\text{2dpINS111} <= dpIR101 + \lvert dnIR111 \rvert} 211,$ $\xrightarrow{\text{dpCEACAM1111} <= dnCEACAM1102 + dpINS112} 212, \xrightarrow{\text{dpCEACAM1111} + \text{dnINS211} <= 2dpINS111} 112,$ $\xrightarrow{\text{dnIR112} <= dnIR111 \ \& \ dnCEACAM1112} 102 \xrightarrow{\text{dnCEACAM1112} <= dpIR101 + dnIR111 + dpCEACAM1111},$ $101, \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} 100 \xrightarrow{\text{dpINS100} <= dpIR200} 200, \xrightarrow{\text{dpIR100} <= dpINS} 210$
2	$011 \xrightarrow{\text{dpINS101} <= dpIR101} 111, \xrightarrow{\text{dpCEACAM1111} <= dnCEACAM1102 + dpINS112} 112,$ $\xrightarrow{\text{dnIR112} <= dnIR111 \ \& \ dnCEACAM1112} 102, \xrightarrow{\text{dnCEACAM1112} <= dpIR101}, 101$ $\xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} 100, \xrightarrow{\text{dpINS100} <= dpIR200} 200, \xrightarrow{\text{dpIR100} <= dpINS} 210$
3	$011 \xrightarrow{\text{dpINS101} <= dpIR101} 111, \xrightarrow{\text{dnIR112} <= dnIR111} 101, \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} 100,$ $\xrightarrow{\text{dpINS100} <= dpIR200} 200, \xrightarrow{\text{dpIR100} <= dpINS} 210$
4	$011, \xrightarrow{\text{dpCEACAM1111} <= dnCEACAM1102 + dpINS111} 012, \xrightarrow{\text{dnIR110} <= dpIR100 + \lvert dpINS110 \rvert} 002,$ $\xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} 001, \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002}$ $000, \xrightarrow{\text{dpINS001} <= \lvert dnCEACAM1101 \rvert} 100, \xrightarrow{\text{dpINS100} <= dpIR200} 200, \xrightarrow{\text{dpIR100} <= dpINS} 210$
5	$011, \xrightarrow{\text{dnIR110} <= dpIR100 + \lvert dpINS110 \rvert} 001, , \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002}$ $000, \xrightarrow{\text{dpINS001} <= \lvert dnCEACAM1101 \rvert} 100, \xrightarrow{\text{dpINS100} <= dpIR200} 200, \xrightarrow{\text{dpIR100} <= dpINS} 210$

Table 3.8: Pathway with delay leads to Stable Steady State in Boolean model

Index	Pathway with delay leads to Stable Steady State
1	011, , $\xrightarrow{\text{dnIR110 <= } dpIR100 \text{ +| } dpINS110|}$ 001 $\xrightarrow{\text{dnCEACAM1001 <= } dnCEACAM1002}$,000, , $\xrightarrow{\text{dpINS001 <= | } dnCEACAM1101|}$ 100, , $\xrightarrow{\text{dpIR100 <= } dpINS}$ 110
2	011, $\xrightarrow{\text{dnCEACAM1001 <= } dnCEACAM1002}$ 010, $\xrightarrow{\text{dnIR110 <= } dpIR100 \text{ +| } dpINS110|}$ 000, $\xrightarrow{\text{dpINS100 <= } dpIR200}$ 100, $\xrightarrow{\text{dpIR100 <= } dpINS}$ 110
3	011, $\xrightarrow{\text{dnCEACAM1001 <= } dnCEACAM1002}$ 010, $\xrightarrow{\text{dpINS100 <= } dpIR200}$ 110

Table 3.9: Pathway with delay leads to Stable Steady State with high insulin concentration.

Index	Pathway with delay leads to Stable Steady State
1	$011, \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} 010, \xrightarrow{\text{dnIR110} <= dpIR100 + \mid dpINS110\mid} 000, \xrightarrow{\text{dpINS100} <= dpIR200} 110, \xrightarrow{\text{dpINS001} <= \mid dnCEACAM1101\mid} ,210$
2	$011 \xrightarrow{\text{dnIR110} <= dpIR100 + \mid dpINS110 \mid} 001 \quad , \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} ,000, \xrightarrow{\text{dpINS001} <= \mid dnCEACAM1101 \mid} 100, \xrightarrow{\text{dpIR100} <= dpINS} 110 \xrightarrow{\text{dpINS001} <= \mid dnCEACAM1101\mid} ,210$
3	$011 \xrightarrow{\text{dnIR110} <= dpIR100 + \mid dpINS110 \mid} 001 \quad , \xrightarrow{\text{dnCEACAM1001} <= dnCEACAM1002} ,000, , \xrightarrow{\text{dpINS001} <= \mid dnCEACAM1101\mid} 100, \xrightarrow{\text{dpINS001} <= \mid dnCEACAM1101\mid} 200, \xrightarrow{\text{dpIR100} <= dpINS} 210$

3.4 Invariance kernel Analysis

Constraints obtained from the Hytech file is given below, 2 cycles are obtained from the Genotech results both cycles overlapped. The presence of equality and inequality determines the instability or stability of the cycle respectively. Invariance kernel of the cycle shows the condition in which cycle will exist and condition to remain in cycle and also the stability of cycle.

After obtaining all constraints the refined is selected, which explain the required result.

$$dpIR101=dpINS001+|dnINS111|$$

This constraint is the invariance kernel of the cycle shown in figure (3.14) and explains that the positive delay of IR is equal to the positive and negative delay of insulin which means that the activation time of IR is equivalent to the attachment time of Insulin and its degradation time. These results are same as the natural behavior, the regulation of IR that attach which Insulin and after internalization release the insulin to the endosomes and recycle to the surface. This process takes equivalent time of insulin attachment and degradation time. The constraints are highly significant and results are depicting the natural phenomenon as observed receptor take time in attachment with insulin its the receptor activation time and then it internalize insulin start degrading by lysosomes and receptor get recycle. The phenomenon is described graphically in figure (3.15).

This constraint shows the equality which means that cycle is unstable, means their condition can not disturb with even minor change. Reason of unstability can be

- Boolean level abstraction.
- Sensitive Cycle

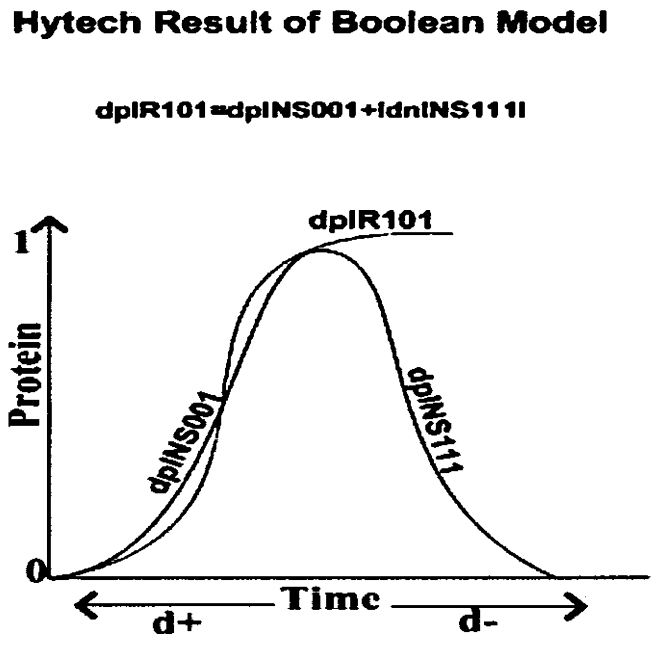


Figure 3.15: Graphical representation of delay constraints of Boolean model.

3.5 Multivalued Hytech file

To verify the model increases the concentration of the entities and make maltivalued model the Hytech results are shown below dp shows the positive delay while dn shows the negative delay.

In these results damping behavior was observed, damping means that the cycle has nested loops in it and the behavior oscillates as shown in figure (3.16).

The figure (3.17) explains the damping also called expanding cyclic behaviors the constant select from this behavior is

$$dpINS112+IdnINS212I<=|dnIR111|$$

these constraint shows that the insulin attachment and degradation process is less then equal to the negative delay of IR which means the recycle period of the IR and it conforms the significance of the results. Graph is shown in figure (3.18) which depicts the protein concentration relatation with time and max threshold goes from 0 to 1.

These results shown the highly significant Insilco work that confirms the normal as well as disease behavior of the system and these results are ready to test on living model and clinical trials.

The present study also observed the knockout behavior of CEACAM1 which confirms that disease condition will arise if at any point CEACAM will not respond properly and insulin knockout behavior will also conform that if the production of insulin does not meet the required concentration level it will go towards the disease behavior.

Insulin increases the synthesis and storage of carbohydrates and lipids in liver and blocks their break down and release in blood circulation (Saltiel and Kahn ., 2001). Blockage of

FAS activity by CEACAM1 provides the understanding of insulin resistance which is studied in number of obesity and diabetes model (Shimomura *et al* ,. 2000). Modeling of insulin clearance in hepatocytes by CEACAM1 also shows its role in fat synthesis and their adverse function. It will also investigate the inhibition of over expression of FAS activity. Behaviors associated with FAS activity will be modeled in the extended version of the model. Normal and abnormal behaviors in the case of obesity and heart disease will also be studied with the help of enhanced version of model. Making this model a useful therapeutic tool is a land mark to be achieved.

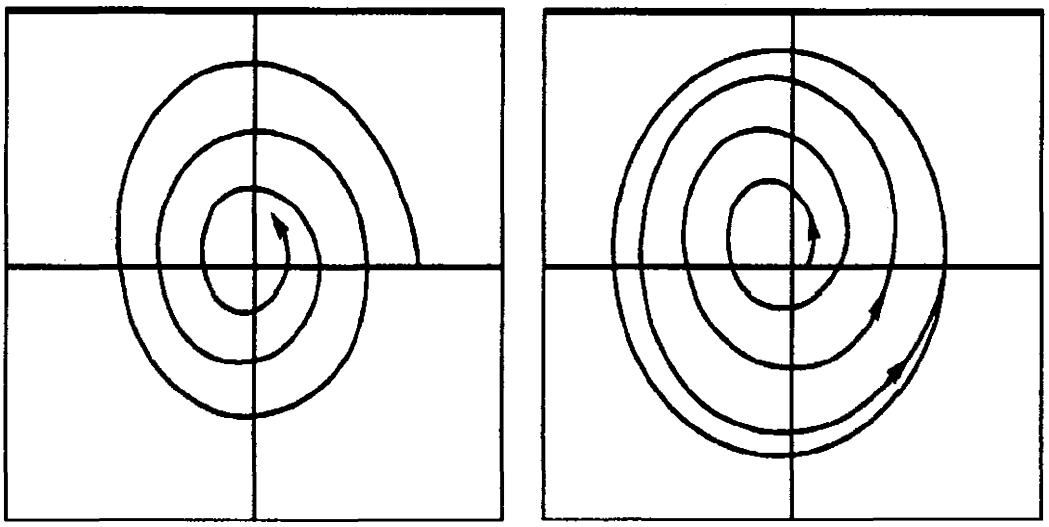


Figure 3.16: Focusing cyclic behavior (left) and expanding cyclic behavior (right)

(Ahmed *et al.*, 2009)

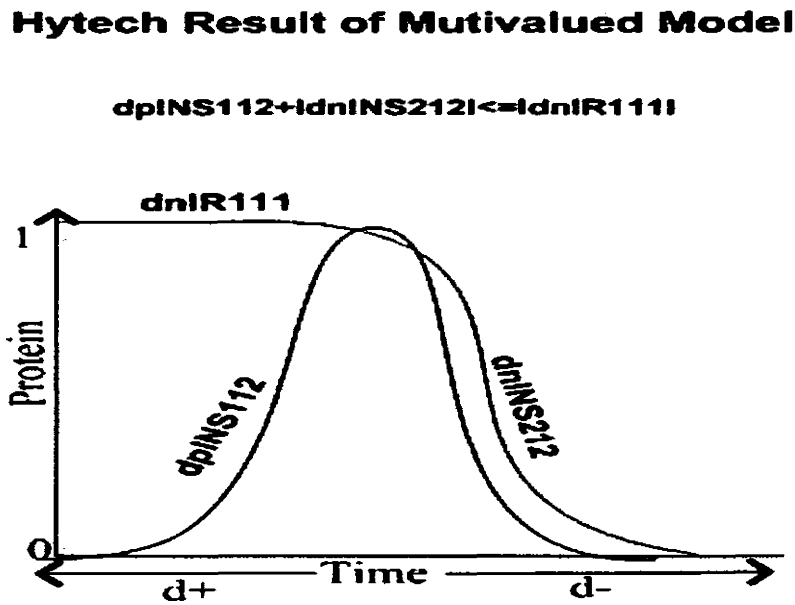


Figure 3.17: Graphical representation of delay constraints of Boolean model

Conclusion

The Results obtained from Genotech model either Boolean or multivalued clearly shows that whatever the reason either silencing, mutation, knockout if CEACAM1 does not respond to the lignad bound IR it will lead to the hyperinsulimea condition or disease condition of the body. Present results depicts that where ever ceacam1 trun off or 0 means either absent or mutated the system will lead towards deadlock state which perfectly matched to the experimental study performed by Deangelis (2008) on mouse that was based on the effect of CEACAM1 on insulin clearance which depends on the cytoplasmic domain of glycoprotein and on the integrity of Tyr-488 and Ser-503. Tissue-specific expression of a non-phosphorylatable mutant of CEACAM1 in the mouse liver impairs insulin develop secondary insulin resistance and show visceral adiposity.

In present study the cycles shows the homeostasis exactly like the normal process follow both the cycle obtain form Boolean and multivalued model. BRN drawn by GenoTech best explains the natural phenomenon of the internalization and degradation of the hormone, insulin properly regulated and the fluctuation of IR explaining its behavior naturally on the uptake or recycle after the degradation of CEACAM1 in both models. CEACAM1 is exhibiting its normal behavior, up take the insulin with receptor and internalization and then degradation of the hormone by endocytosis also conforming that it will show the same results even on generalized levels. Results support the study and prove that the accuracy of the model and significant finding make it can also helpful for the further diagnostics.

Likewise hybrid modeling results also showed very significant results even on real time the delay constraints conforms the relation of protein concentration and activation level

Boolean and multivalued both produce the value able result and these results are ready to test in living model and for clinical trials.

Future Prospect

Modelling of hepatic insulin clearance would be helpful to find the concentration and delay constraints and to achieve the therapeutic target for this life threatening disease. The model will extend this model and will add more related variables and their normal and abnormal behavior which will help model to explain the whole system of insulin clearance in hepatocytes. Models of the regulatory pathways of insulin will lead to the identification of drug targets. Benefits of study can be classified into three broad areas:

1. Therapeutic tools development (Based on regulatory networks)
2. Advances in therapy of Diabetes.
3. Real-world practical applications such as predictive and preventive medicine.

CHAPTER 4

REFERENCES

REFERENCES

1. Abou-Rjaily G. A., Lee S. J., May D., Al-Share Q. Y., Deangelis A. M., Ruch R .J., Neumaier M., Kalthoff H., Lin S. H., and Najjar S. M., (2004). CEACAM1 modulates epidermal growth factor receptor--mediated cell proliferation, *J Clin Invest*, 114, p.944-52.
2. Accili D., Kido Y., Nakae J., Lauro D., and Park B. C., (2001). Genetics of type 2 diabetes: insights from targeted mouse mutants, *Curr. Mol. Med*, 1, p. 9–23.
3. Accili D., Perrotti N., Rees J. R., and Taylor S. I., (1986). Tissue distribution and subcellular localization of an endogenous substrate (pp120) for the insulin receptor-associated tyrosine kinase, *Endocrinol*, 119, p.1274-1280
4. Ahmad J., (2009). Hybrid Modeling and Dynamical Analysis of Biological Regulatory Networks with Delays, *Ecole Centrale de Nantes*, France.
5. Ahmad J., Bernot G., Comet J. P., Lime D., and Roux O., (2007). Hybrid Modelling and Dynamical Analysis of Gene Regulatory Networks with Delays, *ComPlexUs*, 3, p.231–251.
6. Ahmad J., Bourdon J., Eveillard D., Fromentin J., Roux O., and Sinoquet C., (2009). Temporal constraints of a gene regulatory network: Refining a qualitative simulation, *BioSystems*, 98, p. 149–159.
7. Ahmad J., Roux O., Bernot G., CometJ. P., and Richard A., (2008). Analysing formal models of genetic regulatory networks with delays, *Int. J. Bioinformatics Research and Application*, 4, p.240–262.
8. Ahmad J.,Richard A., and Bernot G., (2006). Delays in Biological Regulatory Networks (BRN), *Computational Science Lecture Notes in Computer Science*, 3992, p. 887-894.

9. Batt G., Ropers D., de Jong H., Geiselmann J., Mateescu R., Page M., and Schneider D., (2005). Validation of qualitative models of genetic regulatory networks by model checking: analysis of the nutritional stress response in Escherichia coli, *Bioinformatics*, 21, p.19-28.

10. Bennett M., and Hasty J., (2009). Microfluidic devices for measuring gene network dynamics in single cells, *Nature reviews Genetics*, 10, p.628-38.

11. Bernot G., Comet J. P., Richard A., and Guespin J., (2004). Application of formal methods to biological regulatory networks: extending Thomas' asynchronous logical approach with temporal logic, *Journal of Theoretical Biology*, 229, p.339–347.

12. Canas X., Fernandez-Lopez J. A., Ardevol A., Adan C., Esteve M., Rafecas I., Remesar X., And Alemany M., (1995). Rat insulin turnover in vivo, *Endocrinology*, 136, p.3871-3876.

13. Cara Di A., Garg A., Micheli De G., and Mendoza L., (2007). Dynamic simulation of regulatory networks using SQUAD, *BMC Bioinformatics*, 8, p. 462.

14. Castillo M. J., Scheen A. J., Letiexhe M. R., and Lefebvre P. J., (1994). How to measure insulin clearance, *Diabetes Metab Rev*, 10, p.119-15.

15. Choice C. V., Howard M. J., Poy M. N., Hankin M. H., and Najjar S. M., (1998). Insulin stimulates pp120 endocytosis in cells co-expressing insulin receptors, *J. Biol. Chem*, 273, p. 22194-22200.

16. De Jong H., (2002). Modeling and simulation of genetic regulatory systems: a literature review, *J. Comput. Biol*, 9, p. 67–103.

17. De Jong H., Gouzé J. L., Hernandez C., Page M., Sari T., and Geiselmann J., (2004). Qualitative simulation of genetic regulatory networks using piecewise-linear models, *Bull Math Biol*, 66 p.301-40.

18. DeAngelis A. M., Heinrich G., Dai T., Bowman T. A., Patel P. R., Lee S. J., Hong E. G., Jung D. Y., Assmann A., Kulkarni R. N., Kim J. K., and Najjar S. M., (2008). Carcinoembryonic antigen-related cell adhesion molecule 1: a link between insulin and lipid metabolism, *Diabetes*, 57, p.2296-303.

19. Duckworth W. C., Bennett R. G., and Hamel F. G., (1998). Insulin Degradation: Progress and Potential, *Endocrine Reviews*, 19, p. 608-624.

20. Duckworth W. C., Abraira C., Moritz T. E., Davis S. N., Emanuele N., Goldman S., Hayward R., Huang G. D., Marks J. B., Reaven P. D., Reda D. J., Warren S. R., and Zieve F. J., (2011). The duration of diabetes affects the response to intensive glucose control in type 2 subjects: the VA Diabetes Trial, J Diabetes Complications, [Epub ahead of print].

21. Endy D., and Brent R., (2001). Modeling cellular behavior, *Nature*, 409, 391-395.

22. Formisano P., Najjar S. M., Gross C. N., Philippe N., Oriente F., Kern B. C. l., Accili D., and Gorden P., (1995). Receptor-mediated internalization of insulin. Potential role of pp120/HA4, a substrate of the insulin receptor kinase, *J. Biol. Chem*, 270, p.24073-24077.

23. Fromentin J., Eveillard D., and Roux O., (2010). Hybrid modeling of biological networks: mixing temporal and qualitative biological properties, *BMC Syst Biol*, 4, p.79.

24. Ghosh R., and Tomlin C., (2004). Symbolic reachable set computation of piecewise affine hybrid automata and its application to biological modelling: Delta-Notch protein signaling, *Syst Biol (Stevenage)*, 1, p.170-183.

25. Glass L., and Kauffman S.A., (1973). The logical analysis of continuous non linear biochemical control networks, *J. Theor. Biol*, 1 (39), p.103–129.

26. Hammarstrom S., (1999). The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues, *Semin Cancer Biol*, 9, p. 67-81.

27. Henzinger T. A., Ho P. H., and Wong-Toi H., (1997). HyTech:a model checker for hybrid systems, *Int J Soft ware Tools Technol Transfer*, 1, p.110–122.

28. Huang S., (2001). Genomicscomplexity and drug discovery: insights from boolean network models of cellular regulation, *Pharmacogenomics*, 2, p.203–222.

29. Kahn C. R., Bruning J. C., Michael M. D., and Kulkarni R. N., (2000). Knockout mice challenge our concepts of glucose homeostasis and the pathogenesis of diabetes mellitus, J. Pediatr. *Endocrinol. Metab*, 13(S6), p.1377-1384.

30. Kasuga M., Karlsson F. A., and Kahn C. R., (1982). Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor, *Science*, 215, p.185-187.

31. King H., Aubert, R., and Herman W., (1998). Global burden of diabetes, 1995-2025. Prevalence, numerical estimates and projections. *Diabetes Care*, 21, p. 1414-1431.

32. Lockhart D. J., and Winzeler E.A., (2000). Genomics, gene expression and DNA arrays, *Nature*, 405, p.827-836.

33. Mcadams H. H., and Arkin A., (1998). Simiulation of prokaryotic genetic circuits, *Annual Rewiew of biophysics and biomolecular structure*, 27, p.199-224.

34. Myers M. G., Sun X. J., and White M. F., (1994). The IRS-1 signaling system, *Trends Biochem Sci*, p. 289-93.

35. Najjar S. M., Accili D., Philippe N., Jernberg J., Margolis R., and Taylor S. I., (1993). pp120/ecto-ATPase, an endogenous substrate of the insulin receptor tyrosine kinase, is expressed as two variably spliced isoforms, *J Biol Chem*, 268, p.1201-6.

36. Najjar S. M., Boisclair Y. R., Nabih Z. T., Philippe N., Imai Y., Suzuki Y., Suh D. S., and Ooi G. T., (1996). Cloning and characterization of a functional promoter of the rat pp120 gene, encoding a substrate of the insulin receptor tyrosine kinase, *J Biol Chem*, 271, p.8809-17.

37. Najjar S. M., (1998). pp120, a substrate of the insulin receptor tyrosine kinase, is associated with phosphatase activity, *Biochem Biophys Res Commun*, 247, p.457-61.

38. Najjar S. M., Blakesley V. A., Li Calzi S., Kato H., LeRoith D., and Choice C. V., (1997). Differential phosphorylation of pp120 by insulin and insulin-like growth factor-1 receptors: role for the C-terminal domain of the beta-subunit, *Biochemistry*, 36, p.6827-34.

39. Najjar S. M., Choice C. V., Soni P., Whitman C. M., and Poy M. N., (1998). Effect of pp120 on receptor-mediated insulin endocytosis is regulated by the juxtamembrane domain of the insulin receptor, *J Biol Chem*, 273, p.12923-8.

40. Nandi A., Kitamura Y., Kahn C. R., and Accili D., (2004). Mouse models of insulin resistance, *Physiol. Rev*, 8, p.623-47.

41. Panday A., and Mann M., (2000). Proteomics to study genes and genomics, *Nature*, 405, p.837-846.

42. Poy M. N., Yang Y., Rezaei K., Fernström M. A., Lee A. D., Kido Y., Erickson S. K., and Najjar S. M., (2002). CEACAM1 regulates insulin clearance in liver, *Nat Genet*, p.270-6.

43. Rabin D., Bloomgarden Z. T., Feman S. S., and Davis T. Q., (1984). Development of diabetic complications despite the absence of growth hormone in a patient with post-pancreatectomy diabetes, *N Engl J Med*, 310, p.837-9.

44. Rees-Jones R. W., and Taylor S. I., (1985). An endogenous substrate for the insulin receptor-associated tyrosine kinase, *J Biol Chem*, 260, p.4461-7.

45. Rosen O. M., (1987). After insulin binds, Science, 237, p. 1452-8.

46. Salsali A., and Nathan M., (2006). A review of types 1 and 2 diabetes mellitus and their treatment with insulin, *Am J Ther*, 13(4), p.349-61.

47. Saltiel A. R., and Kahn C. R, (2001). Insulin signalling and the regulation of glucose and lipid metabolism, *Nature*, 414, p.799–806.

48. Seino S., Seino M Nishi S., and Bell G. I., (1989). Structure of the human insulin receptor gene and characterization of its promoter, *Proc Natl Acad Sci*, 86, p.114–118.

49. Shimomura M. M. I., Hammer R. E., Bashmakov Y., Brown M. S., and Goldstein J. S., (2000) Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice, *Mol Cell*, 6, p.77-86.

50. Strogatz S. H., (1994). Nonlinear Dynamics and Chaos, Addison-Wesley Co., Reading.

51. Thomas A. P., Henzinger A., Kopke P. W., and Varaiya P., (1998). What's decidable about Hybrid Automata, EECS Department, University of California, Berkeley Online available.

52. Thomas R., (1991). Regulatory networks seen as asynchronous automata: A logical description, *J. Theor . Biol,* 153, p.1–23.

53. Thomas R., and d'Ari R., (1990). Biological Feedback. Boca Raton, Florida, U.S.A

54. Thomas R., Thieffry D., and Kaufman M., (1995). Dynamical behaviour of biological regulatory networks, Biological role of feedback loops and practical use of the concept of the loop-characteristic state, *Bull Math Biol*, 57, p.247-76.

55. Thompson J. A., (1995). Molecular cloning and expression of carcinoembryonic antigen gene family members, *Tumour Biol*, 16, p.10-6.

56. Valera Mora M. E., Scarfone A., Calvani M., Greco A. V., and Mingrone G., (2003). Insulin clearance in obesity, *J Am Coll Nutr*, 6, p.487-93.

57. White M. F., (1997). The insulin signalling system and the IRS proteins, *Diabetologia*, 40, p.2–1.

58. White M. F., (2003). Insulin signalling in health and disease, *Science*, 302, p.1710-1711.

59. White M. F., and Kahn C. R., (1994). The insulin signaling system, *J Biol Chem*, 269(1), p.1-4.

60. Zimmet P., Alberti K. G., and Shaw J., (2001). Global and societal implications of thediabetes epidemic, *Nature*, 414, p.782-787.

Annexure

Hytech File results of Boolean Model

The constraints obtain are given below for Boolean model cycle

Cycle = 001→101→111→011→001

```
==========================================================

Constrained region of the Invariance Kernel in the zone:

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Delay constraintes:

Location: loc_011

     dpIR101 + dnINS111 = dpINS001   & dnIR011 = 0   & dpIR011 >= 0

& dnIR111 <= 0   & dpINS001 <= dpIR001   & dpINS011 >= 0   &

dnINS111 <= 0   & dnINS101 <= dnINS111   & dpINS001 >= 0

==========================================================

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Max memory used =       4772 pages = 19546112 bytes =  18.64 MB

Time spent      =       0.26u +      2.04s =       2.30 sec total
```

Hytech File Results of Multivalued Model

Location: loc_201

dnIR112 <= dnIR111 & dpIR102 >= 0 & dnINS212 <= dnIR111 + dpINS112 & dnIR212 <= dnIR111 + dpINS112 & dpINS112 >= 0 & dnCEACAM1112 <= 0 & dnIR111 + dnCEACAM1201 <= dpCEACAM1111 + dnINS212 & dnIR111 + dnINS211 <= dnINS212 & dnIR111 + dnCEACAM1211 <= dpCEACAM1111 +

dnINS212 & dnIR111 <= 0 & dnCEACAM1102 <= 0 & dpINS102 >= 0 & dnINS212 <= dpINS102 + dnIR111 & dnCEACAM1102 + dnINS212 <= dnIR111 & dnINS212 <= 0 & dnCEACAM1211 <= dpCEACAM1111 & dnINS211 <= 0 & dnCEACAM1201 <= dpCEACAM1111 & dnCEACAM1112 + dnINS212 <= dnIR111 & dnIR212 <= dpINS102 + dnIR111 & dnCEACAM1102 + dnIR212 <= dnIR111 & dnIR212 <= 0 & dnCEACAM1211 + dnIR212 <= dpCEACAM1111 + dnINS212 & dnINS211 + dnIR212 <= dnINS212 & dnCEACAM1201 + dnIR212 <= dpCEACAM1111 + dnINS212 & dnCEACAM1112 + dnIR212 <= dnIR111 & dpIR201 >= 0 & dpCEACAM1111 + dnINS212 <= dnIR111 + dpINS112 & dpCEACAM1111 + dnINS212 <= dpINS102 + dnIR111 & dnCEACAM1102 + dpCEACAM1111 + dnINS212 <= dnIR111 & dpCEACAM1111 + dnINS212 <= 0 & dnCEACAM1211 <= 0 & dpCEACAM1111 + dnINS211 <= 0 & dnCEACAM1201 <= 0 & dpCEACAM1111 + dnCEACAM1112 + dnINS212 <= dnIR111.....