Stochastic modelling of eukaryotic cell cycle

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STOCHASTIC MODELLING OF EUKARYOTIC CELL CYCLE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Chemical Engineering

in

The Department of Chemical Engineering

by

Murali Krushna Koneru
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DEDICATION

To,

My Parents

For their love, patience, sacrifice
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ABSTRACT

Stochastic models are developed to capture the inherent stochasticity of the biochemical networks associated to many biological processes. The objective of the present thesis is to present a detailed picture of stochastic approach for the mathematical modeling of eukaryotic cell cycle, to demonstrate an important application of such model in chemotherapy and to present a methodology for selecting the model parameters. The stochastic cell cycle model, developed using stochastic chemical kinetics approach, leads to the formation of an infinite dimensional differential equation in probabilities of system being in a specific state. Using Monte Carlo simulations of this model, dynamics of populations of eukaryotic cells such as yeasts or mammalian cells are obtained. Simulations are stochastic in nature and therefore exhibit variability among cells that is similar to the variability observed in natural populations. The model’s capability to predict heterogeneities in cell populations is used as a basis to implement it in a chemotherapeutic modeling framework to demonstrate how the model can be used to assist in the drug development stage by investigating drug administration strategies that can have different killing effect on cancer cells and healthy cells.

Finally, basic cell cycle model is refined in a systematic way to make it more suitable for describing the population characteristics of budding yeast. Selection of model parameters using an evolutionary optimization strategy referred to as insilico evolution is described. The benefits of this approach lie in the fact that it generates an initial guess of reasonable set of parameters which in turn can be used in the least squares fitting of model to the steady state distributions obtained from flow cytometry measurements. The Insilco evolution algorithm serves as a tool for sensitivity analysis of the model parameters and leads to a synergistic approach of model and experiments guiding each other.
To conclude, the stochastic model based on single cell kinetics will be useful for predicting the population distribution on whole organism level. Such models find applications in wide areas of biological and biomedical applications. Evolutionary optimization strategies can be used in parameter estimation methods based on steady state distributions.
CHAPTER 1. INTRODUCTION

1.1 Eukaryotic Cell Cycle - Overview

Cell cycle is an orderly sequence of events by which cells replicate and divide into two daughter cells with identical genetic material. The eukaryotic cell cycle is divided into four different phases: gap phase -G1, synthesis (S) phase, gap phase -G2 and mitosis (M) phase. DNA is replicated once per cell cycle in the S phase, duplicated chromosomes are separated and moved to two opposite poles of mitotic spindle in the M phase. S and M phases are separated in time by the gaps G1, G2. G1 precedes S phase and prepares cell for DNA replication; G2 precedes M phase and prepares cell for division. M phase is separated into five sub phases: prophase - the extended duplicated genome condenses into chromosomes, which are highly ordered structures and metabolic activity is reduced; prometaphase - the nuclear envelope breaks and microtubules elongate from centromeres; metaphase - condensed chromosomes align properly in the metaphase plane; anaphase - the duplicated chromosomes are separated into two identical parts and move towards the opposite poles of the spindle; telophase - the chromosomes decondensate, metabolic activity is restored, and the nuclear envelope is reconstructed. The cell cycle ends with cytokinesis where the cell divides into two each with same copies of genetic material. The duration of the total cell cycle varies for different species and for different tissues in the same species.

Cell cycle has surveillance mechanisms that control the progress of cell through the four phases and ensure the crucial processes of replication and division are performed with high fidelity. These are called checkpoint controls which include both internal and external control mechanisms. The internal mechanisms monitor the timely completion of critical cell cycle events in proper order. External mechanisms control the progress of the cell cycle mainly in response to
three important events – DNA damage, replication blocks and mitotic spindle damage. Main sources of DNA damage are environmental agents - genotoxic chemicals, UV and ionizing radiation; highly reactive free radicals, by products of cellular metabolism; cell physiological conditions that disintegrate the chemical bonds in DNA. These damages occur as single strand breaks (SSBs), double strand breaks (DSBs), point mutations. Replication blocks occur during DNA synthesis due to insufficient nucleotides and proteins or lesions on the DNA templates halting synthesis of DNA strands. Inactivation or under-expression of the proteins involved in the spindle formation causing misalignment of chromosomes resulting in mitotic spindle defects.

Checkpoints not only detect DNA damage and replication blocks, but also form an integral part of multifaceted network of repair pathways that arrest the cell cycle progression to allow sufficient time for repair, initiate the transcription of proteins involved in repair and apoptosis or programmed cell death. Apoptosis occurs in multi-cellular organisms like mammals to prevent the defective cell from harming the whole organism when the damage is too severe to repair. A variety of proteins play crucial roles in DNA damage response pathways at various stages by sensing the defects in cell cycle, transferring these signals to activate or transcribe effector-proteins which carry out the corrective actions. Also, if there are spindle defects, cell is arrested in the metaphase to allow time for related proteins to align the chromosomes properly.

Although there are differences in the number of molecules involved in the cell cycle control system (CCCS) that regulates the eukaryotic cell cycle of different species, it is well-preserved through the evolutionary history and all eukaryotes share similar set of key compounds and interactions. The core of CCCS is a special group of enzymes called cyclin-dependent kinases (Cdks). Cdks are maintained at constant level throughout the cell cycle and are in their active state only when bound to cyclins. A variety of cyclins are synthesized during different
phases of cell cycle progression and their Cdk/cyclin complexes control the transition between the phases. Cyclin levels are maintained by controlled proteolysis and transcription, two regulatory processes that are important for cell cycle progression. If DNA damage is detected, or if the cell growth is not sufficient to have synthesized required amounts of cyclins, the cell cycle will be arrested in G1 phase. Cell enters into M-phase from G2 only if the DNA replication is complete and the duplicated chromosomes are properly attached to opposite poles of mitotic spindle. The transition from metaphase to anaphase which drives the separation of chromosomal spindle and exit of mitosis, occurs when M-phase Cdk/cyclin complexes phosphorylate anaphase promoting complex (APC) that in turn causes the proteolytic degradation of cyclins and of proteins that hold the sister chromatids (duplicated chromosomes) together. Cdns and APC are antagonistic in nature - the activated Cdk/cyclin activates conversion of APC to its inactive form while active APC inactivates the Cdns by promoting the degradation of cyclins and the activation of Cdk-inhibitors (CKIs).

1.2 Significance of Studying the Cell Cycle

Excellent reviews on the cell cycle regulation and its significance are presented in the literature (Nurse, 2000; Tyers, 2004; Morgan, 2006). All the living organisms should maintain a balance between the growth and division of cells for having control over cell size, for surviving under different nutrient conditions and environment, and for performing all other key biological processes. The imbalance between the growth and division is the key feature of tumors. Cell cycles have great influence on neuronal, cardiovascular activities. Thus, many biological functions are cell cycle dependent and understanding and controlling the cell cycle is highly important in disease treatment. Inappropriate activation of apoptosis will lead to the killing of
normal cells causing diseases like Alzheimer’s. Inactivation of genes involved in apoptosis is another cause of growing tumors.

Occurrence of many diseases is related to the defects in the cell cycle regulatory system. Any defects to the genes involved in the cell cycle checkpoint pathways, repair mechanisms result in chromosomal instability, mutations to crucial genes in the cell cycle growth regulation and physiology leading to cancer, immunodeficiency and many disorders (Hanahan and Weinberg, 2000). Cancer is caused by the genetic imbalance between the proliferation and suppression mechanisms of normal cells resulting in their uncontrolled growth. Various types of DNA damage discussed earlier, when unrepaired, will cause mutations to genes involved in the mechanisms that control proliferation and cell death which will be carried to many generations. These permanent mutations cause the activation of oncogenes that stimulate the proliferation or protection against cell death and the inactivation of tumor suppressor genes which would normally inhibit proliferation. This uncontrolled growth of cells leads to tumors in many cancer cells.

Detailed study of cell cycle physiology helps in better understanding the biological processes. Developmental biology is the study of the processes that give rise to tissues, organs and organisms in specific shapes and patterns. These processes are controlled by biochemical reactions which are closely related to the cell cycle. Cell migration which is a phenomenon important in wound healing, tumor growth or apoptosis is influenced by two factors, cell cycle phase and the cell size (Fuss et al., 2005).

1.3 Mathematical Modeling as a Tool for the Study of Cell Cycle

Earlier research in cellular processes involved identifying the important components of the regulatory network and the interactions among each other. These interactions among the cell
cycle components occur at different spatial and temporal scales. With the advances in experimental technology, experimental observations are possible to obtain at these different scales. However, to better understand how these molecular interactions act in a system to regulate the biological functions responsible for disease progression, development and other cellular activities, a systems level analysis is required. Mathematical modeling provides a better analysis tool for gaining system level understanding of the interactions of many components acting in diverse ways.

Mathematical modeling can be used as a valuable tool in hypothesis testing. Hypotheses are made about the mechanisms and functions of the cell cycle control system and a mathematical model formed based on this hypothesis with proper assumptions will generate predictions. The predictions from the model also will be helpful in estimating the key kinetic parameters which are sometime difficult to measure. Based on the agreement of the model predictions with the experimental observations, the hypothesis can be accepted, rejected or modified to form a new hypothesis which gives directions to the design of new experiments. In this way, mathematical modeling in synergism with the experiments will be useful as a tool for identifying more realistic representations of the cell cycle molecular network and to achieve better predictions of the biological phenomena.

Another application of mathematical modeling is to predict the behavior of cells subject to different environmental conditions. The behavior of cells is generally measured in terms of the concentrations of the cell cycle components or the growth of a cell population. One major application is in the cancer chemotherapy, where mathematical model is used to predict the response of cells to chemotherapeutic drugs. The predictive capabilities of mathematical models help in reducing the financial burden of carrying out expensive experimental techniques.
1.4 Mathematical Models – Deterministic and Stochastic Approaches

Mathematical modeling involves converting the interactions among the proteins, enzymes in the biochemical network to mathematical relationships which are mainly rates of synthesis and degradation of proteins, association or dissociation reactions between two proteins. It is important to know the mathematical form of these relationships. Mass action kinetics, Michaelis-Menten kinetics or simple switch-like functions such as Goldbeter-Koshland function are some of the forms used in variety of models. Two main approaches for modeling cellular networks are deterministic and stochastic. The deterministic approach assumes average concentrations of the cell cycle proteins and such approach will always give the same time trajectory of molecular concentrations for specific parameters and initial system state expressed in terms of molecular concentrations. The relationships among the reacting components are converted to differential equations. Once the kinetic rate parameters are assigned and the initial conditions are specified, the differential equations can be solved using numerical integration techniques to obtain the concentration of various proteins with time. There are many models proposed in the literature that follow this deterministic approach which are described in the literature review presented in the next chapter.

The other approach is using stochastic modeling for describing the cellular processes which are inherently stochastic. Molecular components of cellular processes often involve low copy numbers and due to the complex interactions among these components, fluctuations in the number of molecules become very important in deciding the outcome of the associated biological processes. For example, stochastic events such as mutations play an important role in the fate of a cell, which may convert to a cancer cell from a healthy cell. Cellular differentiation is another stochastic phenomenon in which depending on the molecular state of the system, a
particular cell may convert to cell of any of different types of tissues. Other biological phenomena that are stochastic in nature include apoptosis and plaque formation. It is not always possible to identify the complete interactions and external environmental effects on the biochemical system. Stochastic models are developed to capture these stochastic phenomena.

1.5 Objective

Since the stochastic events occurring at the molecular level in a cell cycle network influence the response of whole organism to external stimuli, a stochastic model developed based on a cell cycle network, when accurately represented with the parameters will be helpful in predicting the population responses to the stochastic events occurring at the molecular level. One objective of the present work is to describe the development of a stochastic model of eukaryotic cell cycle and present the scope of its practical application.

Budding yeast *Saccharomyces cerevisiae* is studied extensively for eukaryotic cell cycle research. The yeast genome is well documented, and because yeast cells are unicellular they can be cultured and genetically manipulated much more readily than mammalian cells. Additionally, yeasts are eukaryotic and their gene regulation and biochemical pathways related to cell cycle progression are similar to those found in higher organisms including humans. The model development is based on the cell cycle network of budding yeast.

Stochastic models, even those that are simplified beyond biological reasonableness, contain a large number of model parameters that must be determined for the model to be of value. Stochastic models require data that captures fluctuations in concentrations at single cell level for estimating the parameters. Another approach that can be employed to estimate the parameters is by fitting the population distributions obtained from the model to the measured distributions using least squares techniques. Another objective of the present work is to present a
strategy that can be used to identify the significance of different model parameters in model fitting and to obtain reasonable values of the model parameters using an evolutionary optimization method, which is called in this work as insilico evolution.

1.6. Thesis Organization

This thesis contains six chapters and is organized as described below.

**Chapter 2** – Literature review of deterministic and stochastic cell cycle models is presented

**Chapter 3** – Description of stochastic chemical kinetics followed by the application of the stochastic formulation to develop basic cell cycle model of eukaryotic system is presented. Using Monte Carlo strategy, the cell cycle model is used to obtain the solution to population balance equations of cell growth.

**Chapter 4** – The application of stochastic cell cycle model in predicting the treatment effect of a chemotherapeutic drug on two types of cells, cancer and healthy is demonstrated. Also, a detailed overview of mathematical modeling in assisting the chemotherapy, various approaches for modeling and the associated challenges for developing accurate models are discussed.

**Chapter 5** – Methodology to systematically select model parameters from the steady state distributions of states of the cell cycle reaction network. Refinement of basic cell cycle model presented in chapter 2 to make it more suitable for budding yeast is described and the insilico evolution algorithm is applied to select more reasonable parameter values for the model.

**Chapter 6** – Conclusions and future directions to extend the knowledge gained through this modeling approach to more complex models is outlined.
CHAPTER 2. LITERATURE REVIEW

There have been many studies dedicated to the modeling of eukaryotic cell cycle. These studies started with simple models that do not depend on the molecular description of the cell cycle. Later, when the discoveries are made about the molecular components and their interactions controlling the cell cycle, more detailed studies are made. These mathematical models of cell cycle are made to serve different goals. One of them is to understand the working of the cell cycle and characteristics of different phases and transitions between those phases. For this purpose, attempts are made to describe the cell cycle network in terms of differential equations and using the dynamic systems theory to understand the qualitative features of cell cycle such as limit cycle, hysteresis, bistability, cell size homeostasis, linking of growth to cell cycle progression, creation of phenotypes based on mutations at the crucial transitions which are controlled by checkpoints. A more sought after goal is to build comprehensive models that combine individual studies of sub systems of cell cycle network to predict the behavior of different phenotypes of cells under study. Comprehensive models of cell cycle of eukaryotes exist that combine many features of eukaryotic cell cycles and many species and interactions. Here, some of the mathematical models developed for each of the above goals are presented. Both deterministic and stochastic models are presented in the literature, even though most of the studies followed deterministic approach. First, deterministic models are discussed followed by stochastic models. The general mathematical formulation of deterministic models involves transforming the reaction network into differential equations and solving for average molecular concentrations.

Mathematical modeling of cell cycle has a long history. It started with simple models of cell cycle that does not contain many components in the network. Tyson (Tyson, 1991)
developed a model for fertilized frog eggs cell cycle which is described by an autonomous oscillatory behavior based on the levels of maturation promoting factor, a dimer of cdc2 and cyclin, and it consists of alternation between S and M phases. Later, models were developed by incorporating molecular interactions in the cell cycle. The focus was mainly on specific phases or transitions between these phases. Obeyesekere et al. (Obeyesekere et al., 1995) described control of G1 phase by a model including compounds cyclin E, cdk2 and retinoblastoma (Rb) protein. Kohn (Kohn, 1998) proposed a model for mammalian cell cycle with an emphasis on the transition at G1 – S interphase, studied the dynamics of the regulatory molecules influencing the transition and provided an approach for synthesizing a realistic complex molecular reaction network. They have followed a quasi-evolutionary approach in which functional capabilities of the molecular components are evaluated by adding small subsets of reactions sequentially towards increasing complexity to the reaction network, a method that can assist in the design and interpretation of experiments. Qu et al. (Qu, 2003) followed a similar approach of dividing the complex reaction network of molecules controlling the G1/S transition of a mammalian cell cycle into small modules analyzed the effect of each module on the dynamics at the G1/S interphase in terms of limit cycles, bistability using bifurcation analysis. Similar studies of G1/S transition dynamics using bifurcation theory also exist in literature (Swat et al., 2004). A more comprehensive cell cycle model for the S phase initiation (Barberis et al., 2007) relates the critical cell size requirement to the G1/S transition by using a differential equation model that stresses on the transport of components between nucleus and cytoplasm and the growth of cells in G1 phase under various nutritional conditions.

A quantitative model for mitotic exit by down-regulation of cyclin dependent kinase (cdk) by cdc14 in budding yeast is presented by Queralt et al. (Queralt et al., 2006). Their work
mainly focused on verifying the molecular network controlling the mitotic exit by comparing deterministic model predictions with experiments. Toth et al. (Toth et al., 2007) proposed another cell cycle model of mitosis exit that explains the presence of two bistable switches in the regulatory network controlling M-phase, using phase plane analysis.

Research group by Tyson and Novak have studied extensively eukaryotic cell cycles of budding yeast and fission yeast, mammalian systems. Starting with models focusing on molecular mechanisms for specific portions of the cell cycle, their studies extended to models of more comprehensive nature. A ordinary differential equations (ODE) based model by Novak et al. (Novak et al., 1999) explains the antagonism between cyclin dependent kinases and anaphase promoting complex (APC) based on the mechanism of mitotic exit of budding yeast cell cycle controls.

Tyson et al. (Tyson et al., 1996) developed a cell cycle model based on a network of chemical reactions controlling the activities of M-phase and S-phase promoting factors to understand the phenomena like limit cycle oscillations, stable steady states such as cell cycle arrest. A deterministic model for morphogenesis checkpoint that controls the progression of cell cycle during the bud formation in budding yeast is presented by Ciliberto et al. (Ciliberto et al., 2003). The model is used to predict the behavior of cells in delaying the cell cycle progression when external stimuli prevent the formation of bud. Bifurcation diagrams are used to analyze how the checkpoint governs the progression of cell cycle.

Models are described that explain the dynamic behavior by concentrating on the entire network of the cell cycle. Csikasz-Nagy et al. (Csikasz-Nagy et al., 2006) presented a generic model of the eukaryotic cell cycle to explain the dynamic behavior of cell cycle concentrating on entire network rather than on an individual phase. Their generic model can be used for describing
the cell cycle behavior of various organisms that include budding yeast, fission yeast, frog eggs, and mammalian cells. They have represented the cell cycle regulation in terms of biochemical reactions of the interacting molecules and built detailed ODE models through proper selection of rate parameters representative of organisms for which they are described. The dynamic analysis of the cell cycle regulation is carried out using single parameter bifurcation diagrams to show the linking of cell growth to cell cycle progression, creation of different phenotypes due to mutations and achieving of cell homeostasis by these organisms.

Stelling and Gilles (Stelling and Gilles, 2004) proposed a modular approach for modeling complex cellular regulatory networks discussed with emphasis on model structure selection, evaluating system dynamics and using experimental support to get realistic models. As an example, control of mitosis in budding yeast is described using the modular approach in terms of positive and negative feedback loops in the network. Each module is represented by differential equations and they used bifurcation theory to demonstrate the bistability and limit cycle oscillations of the budding yeast cell cycle model.

A cell cycle model of budding yeast is presented by Lovrics et al. who have used time scale analysis as a tool to test the cell cycle dynamics at the transition between steady states (Lovrics et al., 2006). This is an alternative to using cell mass as a bifurcation parameter in analyzing the dynamic behavior of cell cycle transitions between steady states that does not depend on cell mass.

Logical methods are proposed to study the qualitative behavior of cell cycle models as an alternative to differential equation based models that require molecular interactions in detail and contain large number of parameters which need to be estimated accurately. These logical models use regulatory graphs to represent the cell cycle network. Faure et al. use a Boolean network
model based on network of a differential equation model of a mammalian cell cycle system that is used to analyze cell cycle behaviors such as limit cycles, stable steady states (Faure et al., 2006).

An important objective envisioned for cell cycle models is to use them to predict the behavior of cells to carry out various biological functions, response to various environments. The model should include all the crucial details of the cell cycle network to be realistic. Based on the understanding of the earlier cell cycle models and detailed experimental observations on yeast cell cycle, Tyson and Novak group came up with a most comprehensive model of eukaryotic cell cycle. Their first comprehensive model of the budding yeast cell cycle (Chen et al., 2000), developed by incorporating detailed biochemistry, genetic interactions of cell cycle control, was able to predict the properties of wild type cells and 50 other phenotypes. This model had adequate description of G1 to S transition; however, more details of the mitotic exit are included in a later version of the model (Chen et al., 2004). This model was able to predict the phenotypes of 120 mutant strains and can predict phenotypes of new mutants, helpful for finding out biochemical rate constants of crucial cell cycle interactions which are difficult to measure experimentally. Thornton et al. presented a mathematical model that models strains lacking APC. The model was shown to accurately simulating APC− strains and 27 other phenotypes (Thornton et al., 2004).

Ribeiro and Pinto have developed a model by combining signal pathways of proteins that are crucial for tumor progression such as p53 pathway (important in causing apoptosis when abnormalities like DNA damage and abnormal growth occur in the normal cell cycle progression) and human cell cycle regulation pathway (Ribeiro and Pinto, 2009). In the deterministic approach, differential equations are formulated in terms of average concentrations
of the cell cycle proteins and solving them using analytical or numerical techniques. However, stochastic models differ from deterministic models.

Stochastic models are developed to capture the fluctuations that are common in cellular processes. For gene networks, the concentrations of the reacting molecules will be very low, the fluctuations are comparable to the number of molecules of that species in the system. The chemical population changes as integral number of molecules and the reaction events occur as discreet random events. If the regulatory protein stays in low molecular concentrations, then fluctuations will influence the timing of the regulatory events in different cells leading to different fates and thus leading to heterogeneities in populations. Another example of stochasticity is at the “Checkpoint” pathways. Signal proteins control these regulatory events and the fluctuations in their expression will cause uncertainty in the timing of those events and not in the outcome. Thus, the duration of cell cycle for different cells varies and causes to the heterogeneity in the population doubling times.

A stochastic model based on stochastic Petri net (SPN) approach is developed by Mura and Csikasz-Nagy (Mura and Csikasz-Nagy, 2008) to include fluctuations to the number of protein molecules in the network. The ODEs from a well-defined deterministic model are converted to stochastic Petri net (SPN). The model predicts the behavior of wild type and many mutant budding yeast cells. The stochastic model predicts some characteristics that could not be observed by deterministic model on which it is built. It provides statistics of cell cycle duration and average cell mass. However, one issue with the model is it gives to negative numbers due to added noise to the number of molecules in less abundance. Their model considers does not consider the randomness in the distribution of cell mass at division as it considers binary division.
For biochemical systems involving molecules in low numbers, Langevin-type equations are used which give rise to stochastic differential equations in the model formulation as in another work (Steuer, 2004). It is proposed that the system of differential equation, when introduced noise, is not merely a small deviation from the deterministic behavior rather it will have a different dynamic behavior. One issue with this type of models is that they are applicable only when the molecules exist in large numbers.

Braunewell and Bornholdt investigated the stability of a cell cycle model of budding yeast in the presence of intrinsic noise in the gene regulatory network (Braunewell and Bornholdt, 2007). Their stochastic model uses a generalization of Boolean network dynamics (Thomas, 1973).

Some stochastic models use stochastic formulation of chemical kinetics for the biochemical reactions representing the cell cycle network. Stochastic approach to chemical kinetics is described in the later chapters of this report. The evolution of the reacting system in terms of the discreet reaction events is a discreet markov process (Gillespie, 1992) and is described by the chemical master equation (Gillespie, 1992). The solution to the CME is calculated using SSA presented by Gillespie (Gillespie, 1976). The stochastic chemical kinetics approach is discussed in the next chapter of this report.

Arkin et al. (Arkin et al., 1998) emphasizes stochasticity in gene expression by studying phase Lambda lysis – lysogeny decision circuit. Although, this model is not based on eukaryotic cell cycle, the stochastic formulation of chemical kinetics used here is employed in other stochastic models of eukaryotic cell cycles.

A software program is developed to simulate biochemical systems using stochastic simulation approach which can be used for simulating cell division processes (Kierzek, 2002).
They have tested the computational capabilities of the Gillespie’s algorithm with their model taking the examples of two cellular processes, one with large number of reactions and another with small number of reactions. One limitation is the reaction set in any network, for this program to be implemented, should be either first order or second order.

A stochastic model based on a probabilistic Boolean network on the protein interaction network of the yeast cell cycle is presented in another work (Zhang et al., 2006). Stochasticity is introduced by adding noise in terms of a temperature like parameter to the nodes representing proteins or protein complexes in the Boolean network. They have found out that the biological pathway, which is a cell cycle sequence of protein states, is stable for stochastic fluctuations and for large noise, the network behaves randomly. Thus, they demonstrate that the network of interacting proteins is robust under noise.

Sveiczer et al. present a stochastic model for fission yeast cell cycle based on a deterministic model (Sveiczer et al., 2001). They have introduced stochasticity by incorporating asymmetry of cell sizes at the time of division and assuming unequal volumes for nuclei of newly divided cells. The model is able to predict the population distribution of wild type cells.

In another work, stochasticity is introduced into a deterministic model by adding deviations to the deterministic rate of protein concentrations (Ullah and Wolkenhauer, 2009). Thus, all the biochemical reactions rates and concentrations of proteins are described by stochastic variables and the mathematical model is expressed in terms of stochastic differential equations.

In the present work, a cell cycle model is developed based on the stochastic chemical kinetics approach.
CHAPTER 3. BASIC CELL CYCLE MODEL USING STOCHASTIC APPROACH

3.1. Stochastic Chemical Kinetics

In the stochastic approach, probability theory is applied to describe the kinetics of the stochastic processes associated with the biochemical network. In the stochastic formulation, the reaction propensity or the probability for that reaction to occur depends on the current states of the system described by the number of molecules of each species in the network. The states of the system evolve according to the discrete Markov process governed by a probability density function which follows a chemical master equation (CME) (Gillespie, 1992).

CME is a deterministic differential equation in the probability of the system being in any state of the system. Generally, reactions systems exist in many possible states and this leads to as many differential equations in probability as there are possible states of the system.

The CME is given by

\[
\frac{dP(x, t| x_0, t_0)}{dt} = \sum_{\mu=1}^{M} [a_{\mu}(x - v_{\mu})P(x - v_{\mu}, t| x_0, t_0) - a_{\mu}(x)P(x, t| x_0, t_0)]
\]

\(x = [x_1, ..., x_N]\) = state of the system, in terms of the number of molecules of ‘N’ reacting species

\(P(x, t| x_0, t_0)\) = the probability that the system will be in state ‘x’ at time ‘t’ starting with an initial state x0 at time t0.

\(a_{\mu}dt\) \(\equiv c_{\mu}dt \times h_{\mu}, (\mu = 1, ..., M, number\ of\ reactions\ in\ the\ system)\)

= Probability that a \(\mu^{th}\) reaction will occur in (t, t+dt), given that the system is in the state ‘x’ at time t

\(c_{\mu}\) is the reaction propensity of \(\mu^{th}\) reaction, which is stochastic equivalent of reaction rate constant. \(c_{\mu}dt\) = Probability that a particular combination of reactant molecules of \(\mu^{th}\) reaction will occur in (t, t+dt).
\[ h_\mu = \text{number of distinct molecular combinations of the reactants of the } \mu^{\text{th}} \text{ reaction.} \]

For example, for a first order reaction, \( h_\mu \) simply equals the number of reactant molecules \( N \), for a second order reaction it equals \( \frac{N(N-1)}{2!} \), etc.

Clearly, the probability of reaction occurring depends on the state of the system given by the number of molecules of the reacting species. A detailed derivation of CME is presented in (Gillespie, 1992).

According to the CME, the state of the system at any time is a random vector that follows the cumulative probability distribution evolving from the CME. However, analytical solutions of the CME are possible only for simple cases. McQuarrie (1967) present examples of various simple reacting systems for which analytical solutions are possible (McQuarrie, 1967). The authors review the approach of Master equation for chemical kinetics. In most of the reaction systems, numerical solutions are desired for the CME. One such method to exactly simulate the stochastic behavior of the reaction system according to the CME is presented in the form of exact stochastic simulation algorithm (SSA) by Gillespie (1976) (Gillespie, 1976). Through this method, it is possible to deduce the time trajectories of reacting species resulting from discreet molecular events. The algorithm is based on a joint probability function that determines the reaction that occurs next and the time at which it occurs in a discreet stochastic process.

The joint probability function is given by,

\[ P(\tau, \mu) d\tau \equiv \text{Probability that, given the state } x \text{ at time } t, \text{ the next reaction will occur in the infinitesimal time interval } (t + \tau, t + \tau + d\tau), \text{ and it will be } \mu^{\text{th}} \text{ reaction.} \]

\[ P(\tau, \mu) = a_\mu \exp(-a_0 \tau) \]

Where, \( a_0 \equiv \sum_{\mu=1}^{M} a_\mu \equiv \sum_{\mu=1}^{M} h_\mu c_\mu \)
There are various numerical algorithms available that are exact or approximate to simulate the time course trajectories of the reacting system. Gillespie (Gillespie, 1976, 1977) presents two methods to simulate the SSA which are direct method and next reaction method.

**3.1.1. Direct Method**

In the combined probability density function, the probability of any reaction to occur depends on the reaction propensity and on distinct combinations of the reactant molecules. The higher the reaction propensity is the more likely it is that the reaction will happen next. The algorithm for this method is presented in Table 1. It starts with the initialization of the state of the system, i.e. number of molecules of each species, cell mass etc. Reaction propensities are calculated for each reaction based on the current state of the system. Random numbers are generated using reaction propensities to determine the next occurring reaction and the time of its occurrence. State of the system is updated to reflect the occurrence of a selected reaction event. The time interval between two successive state updates is called the interval of quiescence during which the state of the system is assumed not changing. Since the time steps are relatively small, any changes in the state like cell mass will not affect the propensities much in the interval of quiescence. This procedure of selecting random time and reaction followed by state update continues till the end of the simulation and the time course trajectory of the state of the system is obtained.

**3.1.2 First Reaction Method**

This is exactly same as the direct method, but the implementation to obtain the solution of SSA is different. For each of the reactions, time of occurrence is calculated as if no other occurred first. The difference between the two methods is that more random numbers are used with the first reaction method. The algorithm for the first reaction method is presented in Table 2.
### Table 1: Direct Method - Algorithm

**Step 0:**
- Initialize the state \( x = [x_1, \ldots, x_N] \)
- Input the values of the propensities, \( c_\mu \)
- Initialize the time \( t = t_0 \)

**Step 1:**
- Calculate \( a_\mu = h_\mu c_\mu (\mu = 1, \ldots, M) \)
- Calculate \( a_0 = \sum_{\mu=1}^{M} h_\mu c_\mu \)

**Step 2:** Generate uniformly distributed random numbers in \([0,1]\), \(r_1\) and \(r_2\)
- Select \( \tau = \left( \frac{1}{a_0} \right) \ln \left( \frac{1}{r_1} \right) \)
- Select \( \mu \) as an integer satisfying \( \sum_{l=1}^{\mu-1} a_l < r_2 a_0 \leq \sum_{l=1}^{\mu} a_l \)

**Step 3:**
- Advance \( t = t + \tau \)
- Update the state of the system according to the execution of reaction \( \mu \).
- Repeat step 1 to step 3 until final time.

### Table 2: First Reaction Method - Algorithm

**Step 0:**
- Initialize the state \( x = [x_1, \ldots, x_N] \)
- Input the values of the propensities, \( c_\mu \)
- Initialize the time \( t = t_0 \)

**Step 1:**
- Calculate the reaction propensities, \( a_\mu (\mu = 1, \ldots, M) \)

**Step 2:**
- Select \( M \) random numbers \( r_1, \ldots, r_M \) from the uniform distribution \([0,1]\)
- \( \tau_i = -\frac{\ln(r_i)}{a_\mu}, i = 1, \ldots, M \)
- Select \( \tau \) such that \( \tau = \min(\tau_1, \ldots, \tau_M) \)
- Select \( \mu \) as the index \( i \) of \( \tau_i \) for which \( \tau_i \) is minimum.

**Step 3:**
- Advance \( t = t + \tau \)
- Update the state of the system according to the execution of reaction \( \mu \).
- Repeat step 1 to step 3 until final time.
These exact stochastic simulation algorithms will be efficient when used for reaction systems with small set of reactions and reacting species. The biochemical reaction networks used to represent the intracellular processes contain large number of reactions and components. For such cases, exact simulation algorithms become computationally expensive. There are approximate algorithms available that improve the computational speeds.

Gibson and Bruck (2000) present next reaction method that is developed based on first reaction method to reduce the use of random numbers and to reduce the computational load by introducing the concept of priority index queue (Gibson and Bruck, 2000). Number of calculations of the reaction propensities is reduced drastically with the use of clever data storage structures, by modifying and reusing the values of propensities in the priority queue.

An optimized direct method is used by Cao et al. (2004) (Cao et al., 2004) which is an optimized version of the direct method proposed by Gillespie (1977) (Gillespie, 1977). The search depth for identifying the next reaction that occurs in the algorithm is reduced by arranging the reactions in decreasing order of the number of times each reaction fires in a time span of interest.

Gillespie (Gillespie, 2001) presented an explicit tau-leaping method according to which number of times each of the reaction fires is decided by a poisson random variable, in a leap or subinterval of time that does not result in much changes to the reaction propensities. The method is explicit in the sense that the propensities for the next time step are evaluated based on the current state. This method is advantageous when there are moderately large numbers of reacting species. As each reaction event may result in the change of only one or two molecules, a large number of firings of the reactions will avoid the many steps required by the execution of SSA.
The selection criterion that does not result in much change in the propensities is improved in another approach (Gillespie and Petzold, 2003).

Binomial leap methods for stochastic chemical kinetics use binomial random variable instead of Poisson random variable for determining the number of firings of each reaction in a time leap (Tian and Burrage, 2004). This way, possible reaction firings can be restricted and negative molecular numbers are avoided when large time steps are used.

By characterizing some of the reactions as critical reactions for which the reacting species are in the danger of exhaustion, a modified tau-leap method is used to avoid negative molecular numbers during the simulation (Cao et al., 2005b). It is proposed as an improvement over the binomial leap methods.

To avoid the instability for stiff reaction systems at larger time steps of the explicit tau-leaping method, an implicit tau-leaping method is proposed that has greater numerical stability (Rathinam et al., 2003). In the implicit version, the propensities are evaluated based on the estimated future state of the system that ensures stability. Limitation of these tau-leap methods is that they require the values of propensity functions to be approximately constant. This requirement is realizable only when the species population is large compared to 1.

In the context of the limitations of tau-leap methods in applying to systems with low copy number of molecules, hybrid methods have been proposed. Hybrid methods combine the deterministic regimes expressed by chemical Langevin equation with the discreet stochastic regime expressed by SSA. Hybrid methods account for multiscale nature of reacting systems. Multiscale phenomena occur at time scales and population scale. On time scale, some reactions occur very fast and always will be in equilibrium state while slow reactions dominate the reaction dynamics. On population scale, some of the species exist in small number which is to be
dealt using discreet approach whereas some species exist in large numbers that can be accurately represented by deterministic approach.

A simplification to the stochastic kinetic model is made by applying quasi steady state approximation (QSSA) to the SSA (Rao and Arkin, 2003). QSSA assumes the rates of change of intermediate species to be zero for time scales of interest. Using QSSA, they have reduced the complexity of the model by reducing the number of reactions and molecular species that have fast dynamics and are not of much interest.

Cao et al. (Cao et al., 2005a) describe a partial equilibrium assumption that executes the SSA for slow reactions with the propensity functions calculated based on the partial equilibrium values of the fast species molecular concentrations. This method avoids the expensive simulation of the fast reactions.

All these exact or approximate algorithms greatly help to simulate the stochastic behavior of many biochemical systems of interest.

3.1.3. Monte Carlo Strategy

Using any of the above algorithms, it is possible to traverse along the evolving state of the system by following similar procedure. The procedure simulates a sequence of state dependent random variables which is equivalent to the evolution of the state of the biochemical reaction system of the cell cycle. Starting with an initial state, time for the next reaction and the index of the next reaction is generated in terms of the state of the system and these two random variables determine the next updated state of the system. This procedure is continued till a desired final value of time.

Under identical initial conditions, different independent stochastic simulations will give different realizations of the state. This principle is used in Monte Carlo strategies to obtain the
distributions of states. The strategy is to repeat stochastic simulation up to a final time, large number of times and the desired properties obtained in each trial are summarized to deduce the distribution of properties.

3.2. Stochastic Cell Cycle Model

3.2.1. Solution to Population Balance Models Using Cell Cycle Model

One interest in obtaining the population distributions of cell populations is in the chemotherapeutic drug applications where researchers want to study the response of cell populations to various drug actions. Due to the heterogeneity among the cell population, each cell responds differently to a given action of chemotherapeutic drug and thus a mathematical model to describe the population behavior of cells will be useful to estimate the curing potential of a drug. Population balance models are used to deduce the population distribution of properties of various particulate systems.

Population balance models (PBM) are number balances on population of particles which include biological cells. Population balance models for describing biological cell populations are different from those used for other particulate systems such as crystallizers, bubbles or aerosols. Dependent variable of a population balance is the distribution of states (Eakman, 1966; Fredrickson, 1967; Ramkrishna, 2000; Hjortso, 2006). For a population of cells that follow a cell cycle model of biochemical network of cell cycle proteins, the distribution of states is a probability distribution indicating the frequency of cells with a given cell mass and a given number of molecules of the molecular components of the cell cycle network which may involve hundreds or even thousands of metabolites and similarly large complex biochemical reactions. This leads to a large number of state variables in the population balance equations the solutions of which are difficult to obtain due to the high dimensionality. Solution methods such as moment
methods and methods of weighted residuals will work well only for low dimensional problems. Monte Carlo simulations are suggested as an alternative method to obtain the solution of population balance problems of high dimensionality (Shah et al., 1976; Shah et al., 1977; Ramkrishna, 2000). Previously, Sherpa and Hjortso (unpublished work) described how the distribution of cell states can be obtained from the Monte Carlo simulations of a cell cycle model using stochastic chemical kinetics approach. The implementation and solution of this cell cycle model is presented here.

3.2.2. Description of the Basic Cell Cycle Model

A simple model adapted from the deterministic model of Tyson and Novak (Tyson, 2000) and not specific to any organism is used as the biochemical network representing the interactions among the proteins involved in cell cycle regulatory system.

The cell cycle network is shown in Fig. 1 and it is not specific to any particular organism. Several cyclin-kinase complexes that normally take part in the chosen model are lumped into a pseudo compound, cyclin/Cdk as shown in Fig. 1. Activated cyclin/Cdk activates conversion of APC to its inactive form while active APC activates degradation of cyclin, thereby removing the activated cyclin/Cdk complex. Increased accumulation of cyclin/Cdk initiates production of an activator protein which activates conversion of APC from the inactive form to the active form.

The cell cycle model contains two checkpoints or irreversible transition points. The first checkpoint is called the “start event” and is triggered when the number of molecules of cyclin/Cdk exceeds a specified threshold value. This event occurs when the cell switches to a state with a high amount of cyclin/Cdk and corresponds roughly to the transition from the G1 – phase to the S – phase. The second checkpoint is passed only if the cell has passed the start event and when the number of molecules of cyclin/Cdk drops below a specified value. This point
corresponds roughly to the state after DNA replication has finished and the chromosomes are aligned on the mitotic spindle. In this model it signifies cell division.

Figure 1: Schematic of the eukaryotic cell cycle network. (Sherpa and Hjortsø – unpublished)

Individual reactions and expressions for propensities are shown in Table 3. The binding between cyclin and Cdk is assumed fast such that rate of cyclin/cdk formation is equal to the rate of cyclin synthesis. Degradation of cyclin/Cdk is modeled as two parallel reactions, one which is constitutive another which is activated by active APC. The model does not include reactions for synthesis and turnover of Cdk and APC as both are assumed to be maintained at constant concentrations in the cell through the cell cycle.

3.2.3. Implementation and Solution of the Cell Cycle Model

The model contains 4 discreet variables, number of molecules per volume of cyclin/Cdk (X), of active and inactive APC (Y_a, Y_i) and of activator protein (A) and one deterministic and continuous variable, the cell mass (m). Formation of cyclin/Cdk is assumed to be rapid compared to the other reactions and free cyclin is therefore not considered in the model.
Table 3: The reactions of the eukaryotic cell cycle model

‘m’ is the cell mass, X – number of molecules per volume of cyclin/Cdk; Y_a – of active APC; Y_i – of inactive APC; A – activator protein

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Stoichiometry</th>
<th>Reaction propensity, c_n</th>
<th>Reactant combinations, h_n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>→ cyclin/Cdk</td>
<td>c_1 = k_1 m</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>cyclin/Cdk →</td>
<td>c_2</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>cyclin/Cdk →</td>
<td>c_3 = k_3 Y_a</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>Active APC → inactive APC</td>
<td>c_4 = \frac{k_4 m X}{1 + Y_a}</td>
<td>Y_a</td>
</tr>
<tr>
<td>5</td>
<td>inactive APC → active APC</td>
<td>c_5 = \frac{k_5' A}{1 + Y_i}</td>
<td>Y_i</td>
</tr>
<tr>
<td>6</td>
<td>→ Activator protein</td>
<td>c_6 = k_6' + \frac{k_6''(m X)^n}{1 + (m X)^n}</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Activator protein →</td>
<td>c_7</td>
<td>A</td>
</tr>
</tbody>
</table>

It is assumed that all the cells divide continuously without considering dormant cells, differentiated cells and mature cells. Cell mass is the only continuous state variable and it is assumed to be growing according to first order growth kinetics and it is independent of the values of the discreet state variables. Cell mass increases exponentially with time as

\[ m = m(t_0) \exp(\nu(t - t_0)) \]

Where, \( m(t_0) \) is the initial cell mass at time \( t_0 \); \( \nu \) – specific growth rate.

Due to the stochastic nature of the cell cycle oscillator in this model, there is always a possibility that the trajectory of the system can escape from the oscillatory state. In that case, cell never passes the second checkpoint and therefore never divides. Consequently, cell mass increases without bounds and a cell is assumed dead when its mass exceeds a specified value. In one way, it represents the natural death that occurs in cells due to errors in the regulatory system. Hence, cell deaths due to any drug action on the cell population will be in addition to the natural deaths occurring in the normal cell cycle.
Asymmetric division of mother cells is considered using a unimodal probability distribution function as one source of cell cycle variability. Cell mass is assumed to partition according to the function \( P(m, M) \) at the time of cell division, where \( P(m, M)dm \) is the probability that a dividing cell of mass \( M \) will give rise to a new cell with mass between \( m \) and \( m + dm \).

\[
P(m, M) = \begin{cases} 
0 & , m < M_{min} \\
\frac{30(m - M_{min})^2(m - (M - M_{min}))^2}{(m - 2M_{min})^5} & , M_{min} < m < M - M_{min} \\
0 & , m > M - M_{min}
\end{cases}
\]

The function is a bell shaped curve situated between a minimum possible cell mass \( M_{min} \) and \( (M - M_{min}) \), the maximum possible cell mass that can result from division of a cell with mass \( M \). Perfect binary fission is a special case of this distribution. The concentrations of all other model variables are assumed preserved in a division, i.e. the number of molecules of each of the discrete variables does not change.

3.2.4. Monte Carlo Solution for Population Distributions

The reactions among the four discreet variables are modeled using stochastic chemical kinetics. Gillespie’s algorithm for direct method described in the previous chapter is used to simulate the time course trajectory of state of the system in the cell cycle. Monte Carlo strategy is used to obtain the population distribution. Because of the stochastic nature of the model, different simulations of the cell cycle model with same initial state will have different outcomes and the distribution of the cell states follows the probability distribution given by the CME for the assumed cell cycle biochemical network.

All the parameters and state variables are given arbitrary units as the model does not represent any organism. In the absence of parameters representing any particular organism,
model parameters are systematically tuned to obtain reasonable oscillatory dynamics qualitatively resembling eukaryotic cell cycle.

The stochastic model for simulating the cell cycle progression of a single cell is modified to include multiple cells at the start of the simulations. The total number of molecules of each species involved in the cell cycle is specified for all the cells as random initial states of the cell population. By using these random initial states for different cells, another source of extrinsic heterogeneity of cell population is included in addition to the asymmetric division of cell mass. As time increases, cells continuously undergo division or death thereby changing the size of the population. Thus, these simulations once started can be viewed as cell population that is changing over time. From these simulations, it is possible to obtain the time trajectories of total number of live cells present and the total number of cell deaths occurred in the cell population.
CHAPTER 4. APPLICATION OF STOCHASTIC CELL CYCLE MODEL IN CHEMOTHERAPY

4.1. Introduction

Chemotherapy is the usual treatment strategy in the earlier stages of tumor development or after the surgery. Conventional chemotherapy involves strategic administration of drug that kills tumor with less toxicity to normal cell tissues (Sanga et al., 2006). Chemotherapeutic drugs are mainly classified as cell cycle non-specific, cell cycle specific drugs. Cell cycle specific drugs facilitate the design of treatment strategies that exploit the difference in the cell cycle kinetics of cancer cells and healthy cells to selectively kill cancer cells. Thus, there has been growing interest in the development of more cell cycle specific drugs in treating tumors.

There are many challenges and goals that need to be addressed for making the field of chemotherapy successful. Among these challenges, toxicity to the normal cells receiving the same treatment is a primary concern. Since healthy cells and cancer cells both live in the same microenvironment utilizing the nutrients, application of chemotherapy targeting cancer cells will also affect healthy cells which leads to varieties of toxicities. Thus the primary challenge of chemotherapy is to kill cancer cells with minimal toxic effects to normal tissues. Both biochemical and kinetic resistance to the drugs affect treatment outcome (Norton and Simon, 1986) which should be handled carefully. Biochemical resistance is the resistance developed by the tumor cells to the acting chemotherapeutic drug. Kinetic resistance is observed for phase specific drugs which are only effective in particular phases of the cell cycle and are ineffective in the other phases. Multiple drug resistance is another problem of chemotherapy where a single mutated gene results in resistance to many drugs of different biological composition and configurations and understanding its evolution and control is another important challenge (Swierniak et al., 2009). Delivered drug needs to overcome various resistances and barriers
before it reaches its target sites on tumors and a successful delivery of the drug to its target requires detailed understanding of the effect of these barriers which is separately studied as drug pharmacokinetics. Individuals have different drug pharmacokinetic properties and variations in the immune systems which result in differences in birth and death rates of cells, evolution of drug resistance. Due to this highly heterogeneous nature of the tumors among individuals, same type of drug and its administration strategy may not work well for all the individuals in a similar manner and hence chemotherapeutic strategies should be tailored to each patient. Even for a given patient, due to the inherent heterogeneities among the tumor cells, not all the cells respond uniquely to given drug and thus it is important to identify the best treatment strategy that is characterized by the dose, exposure time, periodic or continuous administration of the drug without inducing more toxicity to the patient. Using multiple drugs is usual practice due to toxicity considerations and due to evolving resistance to a single drug, many novel, minimally cross-resistant drugs have been identified. Identifying the best combination of these drugs and their administration regimens is crucial for achieving best results from the treatment which is difficult to accomplish by experience from clinical practice alone. During the drug development stage, probability of cure of a newly developed drug should be known before deciding on its introduction into the market. Millions of dollars are spent by pharmaceutical companies on costly clinical trials and lab experiments to screen potential drug candidates with high probability to achieve cure. However, the drug development process is considered to be highly inefficient due to the high failure rate (>75%) of most of the drug candidates (Sanga et al., 2006).

Mathematical modeling and bio computation in combination with experiments and clinical trials is considered to be a valuable tool by the cancer research community to address the challenges hampering the success of chemotherapy. Mathematical models are useful for
generating predictions about the optimum treatment strategies to achieve treatment objectives which can be tested through accurately designed experimental and clinical studies (Goldie and Coldman, 1986). Thus, Time, money, energy, animals can be spent on conducting more statistically significant clinical trials and experiments.

In this chapter, application of the stochastic cell cycle model, described in the previous chapter, in evaluating the effect of scheduling and doses of cell cycle phase specific drugs on tumor cells and healthy cells is presented. The stochastic model described in the previous chapter for obtaining the distributions for cell population based on the dynamics at single cell level is used as tumor growth model. The chemotherapeutic model based on the stochastic cell cycle model formulation in its preliminary stage is aimed at a long term goal of addressing the previously mentioned challenges hampering the progress of chemotherapy field.

In the next section, general features of chemotherapeutic models and a review of previous models in literature is presented along with an outline of the present model development. It is followed by a section on the development of chemotherapeutic model based on cell cycle model is described and the simulation methodology for evaluating various chemotherapeutic strategies is delineated. Results from drug testing simulations are presented and discussed followed by conclusions.

4.2. Literature Review on Mathematical Models in Chemotherapy

Mathematical approach for chemotherapy has a long history. Even though theories were formed long ago, due to the lack of sufficient computational power and experimental and clinical data for tumor growth, research in this field gained momentum only since late 90s. With the advent of super computing power and of high end experimental techniques to trace the tumors from biopsies at gene level, extensive research is devoted for developing simulators that can
predict response to multiple drugs, tailor treatments to individuals by integrating details at multiple scales (Gardner and Fernandes, 2003). A pictorial representation of multiple scales at which chemotherapy modules are developed is presented in other studies (Sanga et al., 2006; Ribeiro and Pinto, 2009). Models exist in literature, developed for individual modules and also those developed for integrating different modules. References to different mathematical models involved in the description of tumor growth for cancer chemotherapy are found in (Swan, 1990; Gardner and Fernandes, 2003; Sanga et al., 2006; Venkatasubramanian et al., 2008; Swierniak et al., 2009). These models are classified in many ways – deterministic and stochastic; spatially homogeneous and heterogeneous; cell cycle specific and non-specific. Most of the mathematical models follow deterministic approach where behavior of cancer cells is averaged rather than considering individual variations among the cells which cannot be neglected when describing the cancer phenomena. Stochastic models are developed to address the randomness prevailing in the origin and progression of cancer. Spatially heterogeneous models focus on the detailed vasculature in the tumors and on the distribution of nutrients, drugs in the highly irregular structures inside the tumor. A detailed review of modeling approach for spatial variations is given in (Sanga et al., 2006). Cell cycle specific models are specifically designed to facilitate the testing of cell cycle specific drugs.

Mathematical models in chemotherapy differ in the way they include the details to represent the tumor systems and the effect of chemotherapy viz. growth pattern of tumors, drug pharmacokinetics, drug resistance, toxicity, type of killing effect of the drug on tumor cells, cell cycle specificity, combined action of multiple drugs and also they differ in the goals they are designed to achieve. These categories are combined into two main features of the chemotherapic
models, one is to describe the growth of tumor and the other is to incorporate the effect of drug that kills some or all of these cancer cells.

4.2.1. Describing the Growth of Tumor

Models representing tumor growth can be broadly classified into two categories: homogeneous models that consider tumor as homogeneous population of cells and models that consider spatial heterogeneity of tumors (Venkatasubramanian et al., 2008).

In the literature, many models are found that use homogeneous assumptions. There are simple exponential, Gompertzian and logistic growth models that does not relate to the biological details of the tumor system (Norton and Simon, 1986; Martin et al., 1992; Iliadis and Barbolosi, 2000), (Gardner, 2002). Tumors contain relatively less number of cancer cells immediately after the surgery or in early stages of cancer and the tumor growth is approximately exponential. As the tumor grows larger in size as in late stage of the cancer, transport limitations of blood supply cause the cell population to reach a plateau after certain size which is generally modeled using Gompertz and logistic equations. Multiple compartmental models differentiate various phases of the cell cycle into homogeneous compartments to obtain cell population dynamics. Different phases of the cell cycle viz. G0, G1, S, G2, M and their sub phases and a separately added death phase are denoted by these compartments for cell cycle specific models. Cell cycle non-specific models generally use proliferating and quiescent compartments. The progression of the cell through the compartments is described by parameters such as transition rates between the compartments, cell birth rates and decay rates, cell kill rates due to drugs. Depending on whether these are parameters are fixed or variable, compartments may be deterministic or probabilistic. Some studies use both deterministic and probabilistic compartments in their models (Dibrov et al., 1985). The number of compartments considered depended on types of drugs considered in
the chemotherapy. For example, models that consider only cytotoxic drug propose two compartment models, whereas those including the additional cytostatic drug or recruiting agent propose three compartmental models (Ledzewicz and Schattler, 2002).

Another approach is to use cell population balance models (PBM) which can be structured or unstructured for describing tumor growth. For unstructured population balance models, cells will undergo transitions at different phases or stages at prespecified rates. Unstructured population balance models result in ODEs which may not represent the cell cycle phase delays (Gaffney, 2004). Structured models of cell population dynamics account for the variability among the cells in age, mass, RNA content, volume and other physically measurable properties (Webb, 1990). The transition rates between the phases depend on the structured variable and these models generally result in partial differential equations or integro-partial differential equations in the structured variables. Webb (1990) developed a cell cycle specific model with age and size structured population balance model. Brikci et al. (2008) (Brikci et al., 2008) used age and cyclin structured PBM to simulate the growth of cancer and normal cells in parallel using two compartments – proliferating and quiescent. Some growth models are developed based on theory of branching processes (Goldie and Coldman, 1983; Day, 1986).

Due to the transport limitations of oxygen and nutrients, tumors form a heterogeneous mass that have wide variations in space. Modeling these factors interacting in spatial domain is considered in some studies as a more realistic approach than homogeneous assumption of the tumor mass (Sanga et al., 2006; Swierniak et al., 2009). These models consider transport effects of drugs, nutrients in the micro environment surrounding cells. The microenvironment surrounding these cells comprises of mass of other cancer cells, a network of blood vessels that supplies nutrients, oxygen and drug compounds to the cancer cells which is referred to as
vasculature. Some studies do not incorporate tumor vasculature in their models which are referred to as avascular tumor growth models (Venkatasubramanian et al. 2008 and the references therein) and other studies include vasculature in their models (Sanga et al., 2006). More complex models describe tumor growth by incorporating angiogenesis, process by which new blood vessels are formed from the existing vasculature (Sanga et al. 2006 and the references therein). Generally these tumor models are described mathematically in terms of partial differential, ordinary differential, and algebraic equations and solved using numerical simulations. Stamatakos et al. developed a 3D model for tumor growth based on the 3D structure of the tumor and functional gene data.

Another approach to describe the growth of the cells is using mechanistic models that describe the growth of individual tumors in relation to biochemical network of various proteins and genes that are involved in cell cycle regulatory network errors of which are linked to the origin and progression of cancer (Chappell et al., 2008; Ribeiro and Pinto, 2009).

4.2.2. Describing the Effect of Drug on Cancer Cells

When it comes to the cell kill due to drug, there are many ways it is implemented. Response of cancer cells in monolayer cultures (in vitro) is different to that in vivo. The interaction of drug with blood and bodily environment into which it is injected plays a crucial role in the final response of cancer cells to drug. Effect of micro and macro environment surrounding cells on drug is studied as drug pharmacokinetics and the effect of drug on cancer cells is referred to as drug pharmacodynamics.

Pharmacodynamics (PD)

When PD is modeled, an additional cell kill term is added to the tumor growth model. This cell kill term may follow the traditional log-kill concept which uses first order dynamics, a
Norton-Simon type model, or a bilinear law that multiplies both the drug concentration and cell concentration. According to log-kill concept, a given dose kills a constant proportion of a tumor cell population irrespective of the size of the tumor (Ledzewicz and Schattler, 2002). Norton-Simon’s hypothesis (Norton and Simon, 1986) says that rate of cell kill is also proportional to the growth rate of the unperturbed tumor according to which proportion of tumor cells killed by a given dose of drug decreases with increase in size of the tumor. Cell kill terms of bilinear expression (Ledzewicz and Schattler, 2007; Ribeiro and Pinto, 2009) are considered mainly when drug pharmacokinetics cause changes in drug concentration with time. Effect of cell kill is included in these terms in the form of cell kill rates. The relationship between the cell kill effect and the drug dose are given by dose response curves. The dose is obtained from the “Area under the concentration versus time of exposure of the drug (AUC)” curve to measure the cell kill effect of the drug. AUC gives the cumulative dose of the drug during the total drug exposure time. Other than dose response curves, empirical models are also used to relate the cell kill effect to the drug dose. A simplified relationship considers cell kill effect to be “on- off” type, that results in killing of all the cells during the drug effective period (Webb 1990, Augur 1988). Hill model and Exponential kill models (Goldie and Coldman, 1983; Gardner, 2000) are often used for this purpose. In Ledzewicz & Schattler (2005), PD is given by Emax, Sigmoidal relations. More realistic functions which describe the saturation effects include Michaelis–Menten formula which is more suitable for fast acting drugs that saturate at high concentrations or the sigmoid function that more accurately approximates the effectiveness at both lower and higher concentrations of the drug (Swierniak et al., 2009). According to above relations, response to the chemotherapeutic drug by using the value of dose obtained from AUC will be same for any amount of drug exposure time. However, it is mentioned that factors like cell cycle specificity of the
drug, cell cycle time, duration and concentration of the drug exposure at the site of action, level of drug resistance affect the shape of the dose-response curves (Gardner, 2000). By incorporating such factors, Laveuser et al. (1998) (Levasseur et al., 1998) proposed Modified Hill model and Gardner (2002) (Gardner, 2002) used a modified exponential kill model both resulting in sigmoidal dose response curves. Some researchers speculate that dose of the drug may not be the proper measure to introduce the effect of cell kill and they propose peak DNA bound drug concentration to replace the total dose of the drug (Sanga et al., 2006).

**Pharmacokinetics (PK)**

Intensity of the effect of the drug is influenced by the ease with which it can be delivered to the target. Drug pharmacokinetics plays a crucial role in this. Drug has to overcome many barriers before it is delivered to the target location. The cumulative effect of these barriers is described by compartmental modeling (Godfrey, 1983; Jacquez, 1996) in which different components are interconnected in the overall process of drug delivery to the target from the drug intake into the extra cellular environment. Various components that are generally represented by the compartments in pharmacokinetic models include lesion interstitium, cell membranes, intracellular organelles, blood-brain barrier and other barriers obstructing successful drug delivery to the target. One of these compartments gives the concentration at the tumor site and it appears as the drug concentration in PD equations.

Different multi-compartmental models are proposed that are either linear or non-linear PK models. Simple linear models are proposed in the form of first order linear differential equations. An accurate representation is done by introducing nonlinearities into the models. Simple nonlinearities are introduced in the form of bilinear systems (Ledzewicz and Schattler, 2005, 2007). There are more complicated nonlinear models used in literature.
Venkatasubramanian et al. (2008) use both linear and non-linear pharmacokinetics in a three compartmental model: Central compartment represents blood plasma and two peripheral compartments represent organs and tissues that have poor distribution of drug. Ribeiro (2009) use a three compartment model that represents blood, extracellular environment and tumor cell as the compartments.

**Drug Resistance**

Drug resistance is another phenomenon by which cancer cells evade the action of drug and it is an important challenge to be overcome before making chemotherapy successful. Mutations to crucial genes involved in the cell cycle regulatory pathways such as DNA repair, apoptosis and to those involved in the drug related activities like absorption, distribution, metabolism, excretion are considered to be main reason for the evolution of drug resistance. The drug resistance is described by a single, random irreversible mutation (or point mutation) (Coldman and Murray, 2000) to a crucial gene or by reversible multistep mechanism of gene amplification through which multiple copies of the mutated gene are acquired in leading to different levels of drug resistance. A general approach in point mutation models is to divide the cell population into different clones each having a different type of mutated single gene along with the non-mutated drug sensitive cells. During the cell growth, cells from one clonal category to another transform through single random mutation event characterized by mutational probability rates. Drug resistance models built based on the mutational probability rates obtained from patient biopsies (Gardner, 2002) will help to describe this phenomenon accurately. For describing gene amplification, a similar approach is followed where cells are categorized according to the number of mutated gene copies and reversible transformation of cells from one category to its adjacent category with probabilities of gene amplification and de-amplification.
4.2.3. Previous Models of Chemotherapy

An extensive literature review of studies for chemotherapeutic models is presented in the Appendix E. Some of the studies relevant to the present work are mentioned here. Mathematical models that describe the controlling and regulatory mechanisms at the single cell level will help to explain the patient specific treatment effect of drugs. In response to treatment, cells may undergo apoptosis or develop defense mechanisms such as drug resistance or differentiation. If a mathematical model is developed based on these regulatory processes at the single cell level to obtain the population characteristics, if it is tractable computationally or mathematically, will greatly aid in the patient tailored treatment. Ribeiro & Pinto (2009) has developed a deterministic biochemical network based multi-compartmental model that depends on the molecular mechanisms of cancer development, neglecting drug resistance, cell cycle specificity and toxicity to normal cells. The deterministic approach follows as forming the differential equations for various proteins involved and solving them analytically or numerically assuming average concentrations. They combined signal pathways of proteins that are crucial for tumor progression such as p53 pathway (important in causing apoptosis when abnormalities like DNA damage and abnormal growth occur in the normal cell cycle progression), targeted by drug action, and cell cycle regulation pathway. They have combined this network module with a compartmental PK/PD model following a multi-scale integrative approach. Another network based deterministic model is presented by Chappel et al. (2008) (Chappell et al., 2008) a single drug kinetics is linked directly to the cell cycle reaction network and thus avoiding the necessity for using separate PD model. Without considering drug resistance, toxicity they have combined this complete network dynamics with PK following an integrated approach for evaluating the effect of varying doses on cancer cells.
4.2.4. Present Model

The stochastic cell cycle model is combined with PD and PK laws to form an integrated chemotherapic model. The model is formulated to test the effect of multiple cell cycle phase specific drugs. The model formulation facilitates the evaluation of multiple toxicities in response to treatment along with tumor response to chemotherapic drugs. As more information becomes available about specific genes involved in oncogenesis and drug responses and as methods to measure cell kinetics become feasible to perform in a clinical setting, it appears that a network based model can be used to account for individual patient specific models. In contrast to the network based models mentioned earlier (Chappell et al., 2008; Ribeiro and Pinto, 2009) which are deterministic, a stochastic approach is to account for variable cell cycle kinetics of cancer cells. The intrinsic and extrinsic heterogeneities that are commonly observed in cancer cell population can be handled suitably using stochastic approach. The development of the overall chemotherapic model is described in the next section.

4.3. Using the Stochastic Model for Drug Screening and Efficacy Tests

It was discussed earlier about obtaining the growth of cell population based on Monte Carlo simulation of the stochastic cell cycle model. Here, the same model is used to obtain the growth of tumor cells and healthy cells. Repeating simulations with different initial number of tumor cells and their random initial states, it is possible to simulate the intra and inter heterogeneities in tumor growth among patients. By combining these simulations with cell kill due to drugs and with a proper measure of cure of cancer, the probability of cure obtained will represent results from actual clinical trials. Description of various modules of the chemotherapic model is presented below.
4.3.1. Incorporating the Effect of Drug into the Model (Pharmacodynamics, PD)

Drug dosage is an important parameter in optimizing the drug treatment strategies for any disease. In a conventional sense, drug dosage denotes concentration or amount of the drug that is administered into the body. Generally, cell cycle phase specific chemotherapic drugs act by obstructing the natural progression of cancer cells through their cell cycle. Since the proposed reactions in the model are crucial for multiplication of cells through the processes of replication and division in cell cycle, inhibition of some of the reactions affects the growth of the cell population either by killing the cells or by delaying their progression through cell cycle. Thus, it is proposed here that a chemotherapic drug acts on the cells by inhibiting any of the biochemical reactions that are part of the cell cycle regulatory network. Inhibition of any reaction is carried out by reducing the values of the corresponding rate parameters by multiplying with a factor which named as inhibition factor. Extent of inhibition of targeted reaction varies with the concentration or amount of drug present in the body. Thus, in the present model, effect of a drug is incorporated by the degree of inhibition of the reaction that the drug targets. This is different from the generally followed PD relations to incorporate the effect of drug on the cancer cells, for example dose-response curves that relate dose to the killing effect of drug on the cells. Since, the model is in its primitive stages and there is not enough complexity in the model, any exact correlations between drug dosage and degree of inhibition are not introduced. So, comparison of two types of treatments for different drug dosages is done by comparing different degrees of inhibition of the target reaction. Furthermore, the term ‘dosage’ is used frequently in place of ‘inhibition factor’ in the later discussions.

4.3.2. Types of Drug Administration (Drug Pharmacokinetics, PK)

Continuous and periodic types of drug administration are considered in the present study.
In the continuous administration, the degree of inhibition of a reaction is constant while the drug is being administered and falls to zero when the drug is no longer administered. This would be a simple model of e.g. a drug that is administered by a continuous drip or by a time formulation and removed quickly from the body once administration of the drug ceases. For simulations involving continuous administration, rate constant of the target reactions of the drug is multiplied by corresponding inhibition factors and their values kept constant from the time of drug administration till the end of the simulation.

A more complex scenario involves periodic administration of a drug which is modeled as periodic changes in the degree of inhibition of the target reaction, combined with removal of the drug from the body following administration. After the drug administration, it can be removed from the body by being metabolized or by excretion (or by both). This type of time varying drug concentration is incorporated by different pharmacokinetic models that mainly use single or multiple compartment models as described in earlier sections. Here, a simple model is adopted where the first of the two mechanisms mentioned earlier will be modeled as a first order reaction, the latter as a zero order reaction. Doing so suggests that the degree of inhibition versus time can be modeled as an exponential decay or a linear decay. Hence, three cases of periodic administration are considered, viz. constant degree of inhibition (no decay of the drug during injection period), exponential decay and linear decay of the degree of inhibition of the drug. Fig. 2 shows periodic administration characterized by the starting time of the drug injection, time gap between the two successive drug injections, effective time of the drug during which only drug shows its inhibitory effect and finally the degree of inhibition. During the exponential and linear decay cases, the inhibitory effect of the drug is assumed to fall to 10% of the value that is at the
start of the injection and upon which action of the drug is neglected till the start of the next periodic injection.

Figure 2: Schematic of the periodic drug administration.

4.3.3. Classification into Target Cells and Normal Cells

Cell population is classified into normal cells and target cells by selecting two separate sets of kinetic and growth parameters of the stochastic cell cycle model as shown in Tables 5 & 6. Based on the type of cancer, cancer cells may divide at a faster or slower rate than the normal cells do. In the simulations of the present model, abnormal cells are assumed to be having higher growth rate than the normal cells which can be noticed from the values of the specific growth rate in the Tables 5 & 6. Monte Carlo simulation of the model for each set of parameters set will give the population growth dynamics for both types of cells the results of which are shown in the Fig. 3 and population average of some cell cycle properties for normal cells and target cells are shown in Table 4. Normal cells have almost equal durations of G1 and (S+G2+M) phases whereas the target cells have very small duration of (S+G2+M) phase compared to the G1 phase. The predicted difference in the properties of the two types of cells is due to the assumed difference in the specific growth rate and rate parameters for the mechanisms of cyclin synthesis (reaction 1), APC inactivation (reaction 4) and degradation of activating protein (reaction 7).
Generally, cell cycle phase specific drugs exploit these differences in the cell cycle properties of normal and abnormal cells and a similar strategy is followed in the current model simulations.

In practice, inhibition by any particular drug of the reaction mechanism in the cell cycle network depends on the biochemistry of the drug and the genes involved in the mechanism. In the absence of information about biochemistry of the drug, equal inhibition effect is assumed for both types of cells for a given action of drug. In some compartmental models, researchers provide higher selectivity of the drug towards target cells by giving lesser value of cell kill rate for healthy cells than cancer cells. One such provision can be made in the present model by adjusting the cell death threshold value for the target cells. To selectively have higher action of the drug for target cells, the threshold value is reduced in some of the simulations. These are denoted as the target cells of type 2. There are oscillations that resemble qualitatively the cell cycle. There is antagonism between APC and cyclin levels.

Table 4: Cell cycle properties for normal cells and target cells

<table>
<thead>
<tr>
<th>Type of Cell</th>
<th>Mass at Birth</th>
<th>Mass at Division</th>
<th>Mass after Division</th>
<th>G1 length</th>
<th>(S+G2+M) length</th>
<th>Cycle Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cells</td>
<td>0.163552</td>
<td>0.297337</td>
<td>0.163545</td>
<td>1.473451</td>
<td>1.533916</td>
<td>3.007367</td>
</tr>
<tr>
<td>Target cells</td>
<td>0.698833</td>
<td>1.443708</td>
<td>0.713909</td>
<td>1.558247</td>
<td>0.147951</td>
<td>1.699369</td>
</tr>
<tr>
<td>target cells</td>
<td>0.562545</td>
<td>1.116099</td>
<td>0.586075</td>
<td>1.624644</td>
<td>0.104821</td>
<td>1.725776</td>
</tr>
</tbody>
</table>

Table 5: Parameters and Trigger values for slow growing populations

<table>
<thead>
<tr>
<th>Trigger Values</th>
<th>k_1: 1500</th>
<th>k_2^*: 200</th>
<th>J_5: 4</th>
<th>c_2: 0.02</th>
<th>k_3^*: 200</th>
<th>n: 5</th>
<th>k_3: 2</th>
<th>k_4^*: 1</th>
<th>c_7: 6</th>
<th>k_4: 800</th>
<th>k_5^*: 2000</th>
<th>J_4: 0.04</th>
<th>J_6: 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific growth rate</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cyclin/Cdk molecules needed to trigger start</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cyclin/Cdk molecules needed to trigger division</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell mass at which cell is considered dead</td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6: parameters and trigger values for fast growing populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>800</td>
</tr>
<tr>
<td>$c_2$</td>
<td>0.02</td>
</tr>
<tr>
<td>$k_3$</td>
<td>2</td>
</tr>
<tr>
<td>$k_4$</td>
<td>280</td>
</tr>
<tr>
<td>$k_6$</td>
<td>201</td>
</tr>
<tr>
<td>$k_7$</td>
<td>20000</td>
</tr>
<tr>
<td>$J_4$</td>
<td>0.04</td>
</tr>
<tr>
<td>$J_6$</td>
<td>50</td>
</tr>
<tr>
<td>$J_5$</td>
<td>4</td>
</tr>
<tr>
<td>$n$</td>
<td>5</td>
</tr>
</tbody>
</table>

Trigger Values

- Number of cyclin/Cdk molecules needed to trigger start: 40
- Number of cyclin/Cdk molecules needed to trigger division: 10
- Cell mass at which cell is considered dead: 2.5

Figure 3: Monte Carlo solution of cell cycle model for normal and target cells

4.3.4. Concept of Toxicity

Toxicity of the drug towards normal cells limits the total dosage of the drug that can be administered in a given treatment period (also known as maximum tolerated dosage – MTD) and also limits the dosage of the drug at any instance during the treatment (known as dose limiting toxicity - DLT). Thus, toxicity is incorporated by simulating the effect of drug on healthy cells also. In the simulated results toxicity is measured in terms of surviving healthy cells fraction. If the effect of drug action results in reduction of healthy cell fraction more than the acceptable limits, then it cannot be a suitable drug treatment. This approach is very convenient to handle multiple toxicities. A single drug can lead to multiple toxicities, for example toxicities associated with paclitaxel include peripheral neuropathy, neutropenia, alopecia, mucositis, arthralgias, and
myalgias (Fetterly et al., 2008). If the model parameters are available for different types of cells related to these toxicities, it is easy to simulate the effect of the drug on those normal cells and thus to evaluate multiple toxicities in parallel to the tumor response and hence to control the scheduling and doses of drugs. In the simulation results, toxicity is discussed qualitatively in terms of the fraction of live healthy cells predicted during the treatment.

4.3.5. Assumptions

1. The model is assumed to be homogeneous in that all the cells are in a highly vascularized tissue and all these cells will be exposed to the same drug environment. Hence, during the drug effective period, the reaction parameters affected by the drug are modified with same value of inhibition factor for all the cells.

2. The drug is assumed to be administered through intravenous injection and it is assumed to reach the tumor target site instantaneously.

3. Evolution of drug resistance is not considered at this time.

4. In case of multiple drug treatment, synergism between two drugs acting simultaneously is neglected and the drugs are assumed to have independent effect on cells. For including such interactions among the drugs, detailed biochemistry is needed which is not considered in this model.

4.3.6. Reporting the Results of Drug Action Simulations

Simulation results are reported in two different ways. One is the time course of total number of live cells in the population with or without the influence of drug. The simulations without the action of drug are referred to as WT (stands for Wild Type) case. In some of the results, logarithm of the total number of live cells is used for convenience. Expressing the results in terms of the total number of live cells gives a direct measure of the size of the cell population.
Another convenient way is to express the results in terms of the time course of proportion of live cells compared to the WT type case, expressed as the ratio of the total number of live cells after any time period of a selected drug treatment strategy to the total number of live cells during the same time when there is no drug action (WT case). This ratio will give the survival fraction of the targeted cell population from the treatment if this treatment results in the reduction of the total number of live cells compared to the WT case.

4.4. Results and Discussion

Simulations are designed to demonstrate the usefulness of the model in identifying treatments that can simultaneously satisfy two important goals of chemotherapy viz. effectiveness in reducing the tumor size and keeping the toxicity to the normal cellular tissues in acceptable limits. First, set of reactions in the proposed cell cycle model are identified that can be used as potential drug targets for the selected cancer cell population. Second, for the drugs targeting the identified target reactions, various administration strategies are tested for a single drug by varying drug dosage, time interval between drug injections, and effective period of the drug. Finally, simulation results are presented for identifying combination of multiple drugs that are effective in satisfying both efficacy and toxicity requirements.

Effect of treatment on curing the tumor is evaluated by determining the number of tumor (target) cells and the toxicity is determined by evaluating the number of normal cells after the treatment. The target reactions are identified in such a way that the treatment strategy satisfies both the requirements adequately.

Simulation results of the time course of population growth of normal cells and target cells for continuous inhibition of each of the seven reactions for wide ranging inhibition factors are
obtained to identify good target reactions. The results of the identification of the target reactions are presented in the Appendix B.

Based on the results of the identification of target reactions, reactions 4 and 7 are identified as possible target reactions from the chosen cell cycle reaction network. The drugs that act on cells by targeting these reactions are denoted as drug 4 and drug 7 respectively. From the continuous inhibition simulations, the results for drug 7 show that, it is very effective for continuous inhibition, in selectively killing the target cells leaving the normal cells unaffected. It is effective even at small dosages and it has the tendency to increase the growth of the normal cells by speeding up their progression through cell cycle at low dosages. For Drug 4 to be effective in killing the target cells it should be applied at high dosage which will also severely affect the growth of normal cells.

Prolonged continuous infusion, however, may facilitate the evolution of drug resistance by gradual processes such as gene amplification (Gardner and Fernandes, 2003) and also causes severe toxicity problems and thus chemotherapeutic drugs are administered in periodic cycles. Cyclic treatment gives rest intervals for the normal cells to recover as well as for pulling quiescent cells to proliferating state. The disadvantage is that cancer cells start regrowth and the treatment duration increases. For that matter, the time gaps between the drug injections, time of drug exposure, and dose of the drug are important parameters in the treatment design considerations to achieve greater tumor control while limiting the toxicities. In the next section, the results of various periodic administration strategies involving drug 4 and drug 7 are presented that evaluate tumor control and toxicity to normal cells simultaneously.
4.4.1. Periodic Administration of Drug 4

Same Gap - Dose Variation

For same gap and effective time, dosages (or degrees of inhibition of reaction 4) are varied and the effect on target cells and normal cells of this treatment is shown in Fig. 4.

Figure 4: Periodic administration of drug 4 for various inhibition factors. Numeric notation (e.g. 0.2,10,5,10) as shown in labels represent inhibition factor, time gap, effective time, drug injection time.

The total number of live cells is simulated for both types of cells for inhibition factors 0.16, 0.2, 0.232 and 0.3. Simulations are done for effective time = 5 and time gap = 10 with the treatment started at time = 10. These simulations are similar to the dose escalating experiments generally conducted in the clinical trials for finding out the dose limiting toxicity (DLT).

From the Fig. 4, live cell fractions for the target cells decreases considerably with increasing dosage. The fraction drops to 0.05 after treatment time = 20 for 0.16 dose whereas it is equal to 0.2 for the dose 0.2. Increasing the dose has considerable advantage in killing the target cells as in the case of continuous inhibition. However, it is interesting to see if periodic administration helps normal cells to recover in the drug ineffective periods. From the Fig. 4, normal cell fraction fluctuates from 1 to 0.4 during the treatment time at high dosage of 0.16 and this fraction further reduces away from 1 as there are some cell deaths observed in these simulations. Whereas for lesser dosages, this fraction fluctuates around 1 and the deviations are
small with lesser dosage than the deviations observed for the dosage 0.16. Model predicts no cell
deaths for normal cells for doses 0.2 and higher. The selected gaps at these doses are sufficient
for normal cells to recover and hence their live cell fraction oscillates around 1. Based on the
toxicity tolerance limits, we can either go for high dosage of 0.16 that has severe effect on
normal cells or for a slightly lesser dosage of 0.2 that is not severe on normal cells. Through
these results we demonstrate that the model predictions can be used to find out suitable dosages
for a given periodic drug administration with known pharmacokinetics.

**Same Dose - Gap Variation**

Time interval between the two drug infusions is another parameter that can be varied to
improve the treatment effectiveness. From the earlier discussion, dose 0.16 was effective in
controlling the target cell population, but it has caused large fluctuations in the normal cell
populations at time gap between injections of 10. Simulations are performed by varying the time
interval between the drug infusions for values of 6, 8, 12 and 15 units and the response of the
normal cells and target cells to the treatment is shown in the Figure 7 along with the response for
gap = 10. Drug injection started at time = 10 and the effective time for drug is kept at 5. WT
(wild type) represents the response of the cells when there is no action of drug.

For same dosage, increasing the gap, its effect on the treatment efficacy is examined. At
the selected dosage, all the target cells have died for gaps 6 and 8. Increasing the gap between the
injections allows cancer cells to regrow and this is reflected in the results for higher gaps shown
in Fig. 5. As the gap is increased to values of 10, 12, 15 increase in the target cell population is
evident. However, there will be maximum tolerated toxicity (MTD) for any drug limiting the
amount of drug that can be given in a single day. If such limitations exist on the periodic
administration of the drug, more frequent injection (small gap between the successive injections)
may not be possible. The effect of gap on the normal cell population is also shown in Fig. 5. Simulations predicted no deaths of normal cells for gap of value 6 and some deaths for all the higher gaps. As long as the gap does not result in any cell deaths, normal cells oscillate around the average value of around 0.8. This is better than the case of continuous inhibition at the same dose where live cell fraction for normal cells is maintained closed to 0.6 as in Fig. 5(b) of Appendix B without any cell deaths. As the gap is increased between the injections, some cell deaths have occurred resulting in a constant drifting away of the total live cell fraction from average value. Since periodic injection at dose 0.16 with small gaps is causing target cells to die and normal cells to have fewer oscillations around mean value, more frequent periodic administration of drug 4 is preferred to a less frequent administration with large gaps. Again, if there is a problem with the frequent application because of the portability of the drug injection equipment, these simulations help us to identify periodic administration strategy with the next best suitable gap.

Figure 5: Periodic administration of drug 4 for various time gaps between drug injections
For continuous administration for same dosage, all the target cells are dead as observed in simulations (not shown here), but normal cells have live cell fraction close to 0.6. By employing periodic drug administration, the impact on normal cells is reduced. The above results show that, keeping the frequency of drug administration high will keep the effectiveness of the treatment closer to that of continuous administration.

**Different Doses - Different Gaps**

Sometimes, it is important to decide on how to distribute a total dosage in periodic administration. Whether to apply the drug in more frequent intervals with less dose each time or to apply the drug less frequently with large dose each time. Generally, AUC is used to find out the combinations of dose and frequency each giving rise to same total dosage over a total treatment time. Simulations are performed for various dose – frequency combinations to study their effect on cancer and normal cells. From the Figure 8(a), high dose (0.16) of drug 4 for less frequent injection is effective than small dose (0.232) with more frequent (continuous) injection. From Figure 6(b), more frequent injection at smaller dose (0.2) will be better than high dose (0.16) of drug at large gap between the injections for normal cells. Considering both toxicity and effectiveness of the drug 4, the intermediate dose 0.2 appears to be preferable to the other two doses (0.16 and 0.232).

From Fig. 4, dose of 0.2 is better than the dose of 0.16 from the toxicity point of view. For killing the target cells, 0.16 dose is preferred to 0.2 dose. Considerable improvement for 0.2 dose is obtained by reducing the gap from 10 (Fig. 4) to 7 as in Fig. 6. More frequent injection at dose 0.2 has improved its effectiveness while keeping its toxicity to normal cells acceptable. Through this model, it is possible to find out suitable dose-frequency combinations that can simultaneously satisfy the requirements of reducing the cancer burden and reducing the toxicity.
Figure 6: Dose frequency combinations of administration of drug 4

**Effect of Drug Decay (Half Life)/Drug Pharmacokinetics**

All the previous results are presented for continuous inhibition without considering the pharmacokinetics of the drug in the body. Fig. 7 shows the results for administration of drug 4 in periodic pulses accompanied by exponential and linear drug decay instead of periodic administration with constant inhibition.

Figure 7: Periodic administration of drug 4 with linear and exponential decay

A dose of 0.16, gap of 10, effective time of 5 are considered and drug is administered at time = 10. For the selected effective time which indirectly signifies the half-life of the drug, this treatment has negligible effect on the target cell population for both types of drug decay. To have its effect, the drug should act for longer effective times in the body. In other words, half-life of the drug in the body should be very high. Fig. 8 shows the results for drug 4 with longer effective time of value 20, where there is not much effect on normal cells (Fig. 8(b)). There is not much inhibitory effect on target cells for exponential decay of drug 4, only linear decay case shows...
some improvement with higher effective time (Fig. 8(a)). Thus, drug 4 may not be an effective choice when its pharmacokinetics is such that its action stays for less time in the body. These dependencies on pharmacokinetics suggest better formulations of this drug that will allow drug to stay for longer times in the body.

Figure 8: Periodic administration of drug 4 with linear and exponential decay with increased effective time of drug

In the case of drug 4, the less effectiveness is due to the less retention time of the drug. If there was drug for more time, as in the case of uniform drug infusion, efficacy was more. So, the drug should be designed in such a way that there will be more retention time for the drug.

4.4.2. Periodic Administration of Drug 7

In the case of drug 4, pharmacokinetics played an important role in the effectiveness of the drug in killing the cancer cells. Drug 4 was needed to stay for a long time in the body to show its effect on target cell population. Similar simulations to see the influence of pharmacokinetics on the effectiveness of the drug 7 in killing the target cells are performed at the dosage 0.6. Separate simulations are carried out for exponential, linear and no decay of the drug 7 with time gap = 10, effective time = 5 and injection starts at time = 7. The results of the simulations are shown in Fig. 9. Fraction of the live target cells predicted in the case of no decay of the drug is less compared to the cases when exponential and linear decay are considered. However, for both
the cases of drug decay, unlike for drug 4, there is considerable decrease in the fraction of the target cells in the periodic treatment for drug 7.

![Graph showing the effect of drug decay on target cells](image1.png)

**Figure 9:** Efficacy of drug 7 with exponential and linear decay compared to no decay

The amount of time drug stays in the body was enough to bring in a reduction in the size of the target cell population. In Fig. 10, fraction of cells with the exponential and linear decay for high dose of 0.3 is compared with the no decay case of drug 7 acting at low dose of 0.8. Increasing the dose of the drug has compensated for the lesser retention time due to the drug pharmacokinetic effects and the efficacy is more compared to the no decay case. Normal cells are not affected by this increase in the dose. These results demonstrate the differences in the effectiveness of the two drugs, drug 4 and drug 7 with different pharmacokinetic properties in killing cancer cells.

![Graph showing drug decay with increasing dose](image2.png)

**Figure 10:** drug decay with increasing dose of drug 7
Dose - frequency simulations are performed for drug 7 as it is done for drug 4. For a drug effective time of 5 units, doses of 0.6, 0.75 and 0.8 are applied for 11 gap, 7 gap and continuous injection respectively. These values have been selected arbitrarily and the purpose is to demonstrate the use of the model for the case of drug 7 for various dose-frequency combinations. From the Fig. 11(a), 0.6 with gap 11 has high efficacy compared to the other high dose cases with frequent injection. However, the continuous injection of dose 0.8 is slightly better than the treatment with higher dose 0.75 with gap 7 between the injections. Since, the exact relationships are not defined for dose and the amount of inhibition it is not possible in the present model to choose the gaps and dose in a way to conserve the total amount of dose for a specific length of treatment period. As a consequence of this, the efficacies may vary for a different set of gaps for the chosen doses. For example, in the Fig. 11(b), simulation results for target cells of type 2 are presented for the same doses (0.6, 0.75, 0.8), but, with different set of gaps between the injections. From Fig. 11(b), frequent injection of drug 7 with dose 0.8 gives high efficacy than the other two cases which are more or less equally effective in killing the target cells. Combining such predictions from the model with the practical considerations like portability issues will result in best choice among a variety of dose-frequency combinations.

Figure 11: Dose frequency combinations of drug 7 on target cells (a) and target cells of type 2(b).
Periodic Administration - Drugs Targeting Reactions 5 and 6

From the continuous inhibition simulations, it was seen that drugs targeting reactions 5 and 6 are less effective in killing target cells at low doses but at higher doses those drugs affect normal cells also. Periodic administration strategies may ease the toxicity as is observed in the case of drug 4. Simulation results for the case of periodic administration of the drug 5 and drug 6 are shown in the Fig. 12.

![Figure 12](image)

Figure 12: Effects of periodic administration of (a) drug 5 on target cells (b) drug 5 on normal cells and (c) drug 6 on both target and normal (shown as slow on the legend) cells.

All the simulations are done by starting the drug injection at time = 10 and effective time = 5. At doses 0.7, 0.75, 0.725 the fraction of target cells does not drop much with the periodic treatment, but the efficacy of the drug improves if the dose is increased to 0.6. At the doses 0.725, 0.75, fraction of the normal cells stays close to 0.8 during the drug effective time and the cells recover to the actual population size during the gap between the successive injections. As the drug dose increased to 0.7 and 0.6, normal cells are also killed with the treatment which can
be observed by the gradually decreasing fraction of the normal cells with the treatment time. Similar result is obtained for periodic inhibition of the reaction 6 as shown in Fig. 12(c). At small dosages, there were not many target cells killed (results not shown here) and at the dose of 0.6, fraction of cells for normal cells is very less and the drug acts almost as severely on normal cells as it does on target cells.

These simulations suggest that treatment strategies by compounds that target reactions 5 and 6 cannot be advisable from the considerations of their toxicity to normal cells. Hence, the present modeling approach based on the regulatory mechanisms controlling the division cycle of cell populations will give insights to the drug developers on what pathways to target and what pathways not to target for cell cycle based chemotherapy.

**Combined Action of Two Drugs**

Conventionally, most of the chemotherapeutic drugs are administered in combination with other drugs to increase the effectiveness of the treatment. The present model is easily extended to include the action of multiple drugs. In the absence of detailed biochemistry of the drugs, it will be difficult to include any interactions among the drugs which can affect the overall inhibition effect by the combined action of the drugs. Hence, it is assumed that the inhibitory effect of any drug on a particular reaction is independent of the action of any other drug on the same reaction. Simulations are performed for the case of continuous inhibition, to demonstrate the combined effect of two drugs targeting different reactions. Corresponding rate constants are multiplied by inhibition factors to represent each drug action. Fig. 13 shows the examples of drug combinations for continuous drug administration where both drugs act synergistically in improving the efficacy of the treatment by killing more number of target cells than their individual drug action. Their combined effect results in negligible effect on the normal cell
population. In Fig. 13(b), drugs 4 and 7 are combined with doses 0.3 and 0.85 respectively. In Fig. 13(a), drug 6 and 7 are combined with doses 0.8 and 0.85 respectively. In both the cases, live cell fraction of normal cells stayed close to 1, whereas the combined effect of treatment on target cells is highly significant.

![Drugs combination - Drug 6 & Drug 7](image1)
(a)  
![Drugs combination - Drug 4 & Drug 7](image2)
(b)

Figure 13: (a) Combined effect of drug 6 and drug 7 on target cells and normal cells; (b) Combined effect of drug 4 and drug 7 on target cells and normal cells.

A different scenario where the combined action of drugs does not show significant improvement in the treatment is shown in Fig. 14. In Fig. 14(a), Drug 5 and Drug 4 are combined with doses 0.75 and 0.3 respectively and this combination does not succeed in reducing the target cell population even though the live cell fraction of normal cells stays close to 1. For the combination of drugs and doses shown in Fig. 14(b), (drug 5 and Drug 6 are combined with doses 0.8 and 0.8 respectively), both normal cells and target cells are affected severely and thus this combination cannot be used for a successful treatment.

![Drugs combination - Drug 5 & Drug 4](image3)
(a)  
![Drugs combination - Drug 5 & Drug 6](image4)
(b)

Figure 14: (a) Combined effect of drug 5 and drug 4 on target cells and normal cells; (b) Combined effect of drug 5 and drug 6 on target cells and normal cells.
4.4.3. Summary of the Results

Continuous inhibition of each reaction in the cell cycle network is simulated to identify target reactions. Drug 4, Drug 7 which indirectly contribute for keeping active APC at higher levels by inhibiting reactions 4, 7 respectively in the cell cycle network are identified as suitable for controlling the selected tumor type. Such predictions about target reactions for drugs will be helpful in development of new drugs targeting those pathways. These simulations are not only helpful in identifying target reactions, but also helpful in finding out the limits on the dosage of drugs. These are similar to the dose escalation studies performed in clinical trials. However, continuous inhibition simulations also reveal the effect of cell cycle specific drugs on normal cells which is a measure of toxicity and suggest periodic drug administration. Periodic administration allows cells to recover in the gaps between successive drug injections. Using drug 4 and drug 7, periodic drug administration strategies are tested by varying drug dose, gap between the injections, different dose – gap combinations and by including drug pharmacokinetics for both drugs 4 and drug 7. The results for suitability of other drugs reveal their ineffectiveness even when periodic administration is considered. Same model is used to find the combination of drugs that can work in synergy to cure tumor without affecting normal tissues. Continuous inhibition simulations are performed on both cancer and normal cells for synergistic action of drug combinations.

4.3. Conclusions

A chemotherapeutic model is developed based on stochastic chemical kinetics of cell cycle regulatory network and Monte Carlo simulations are used to compute the time course of the growth of cancerous and healthy cell populations in response to chemotherapeutic treatment. Effect of drug is included in the model by inhibiting one of the reactions that are crucial for regulating
the cell cycle progression. This approach helps in predicting the action of multiple drugs on tumor cell growth. Toxicity is considered by simulating the inhibitory effect of drug on healthy cell population growth. By including the effect of the drug action on the growth of both healthy and cancer cells, the model demonstrates the pathway to determine favorable treatment strategies for varying dosages, drug effective times and time gaps between drug injections. A simple linear or exponential decay of the drug is included in the drug testing simulations.

Model’s predictability is helpful and useful in clinical trials to identify administration strategies that are best suited to each individual. If the parameters are obtained from the patient specific biopsies, tailoring treatments to each individual is possible. If the parameter space is available that represents a population of random patients, this model can predict the result of drug treatment for each such combination of parameters which averaged on the whole population gives probability of cure for a specific drug during the drug development stage. Hence, in silico screening of drugs is possible with the present modeling approach.

Compared to many deterministic models available in the literature, the present stochastic formulation has the benefits of dealing with intra-patient variability. – Tumors are associated with variability among cell population and each of them will respond independently to chemotherapeutic drugs which is an important factor when cell cycle phase specific drugs are used.

Cell cycle specific drug action is easily handled in the current modeling formulation as the model is based on the progression of the cells through cell cycle. However, cell cycle non-specific drug action also can be easily implemented. Cell cycle non-specific drugs target genes (for example p53) that act outside the cell cycle, which are involved in DNA damage, apoptosis pathways. By simply adding another module that includes these reaction networks, it is easily possible to link the cell cycle to the extra-cell cycle regulatory network and checkpoint
mechanisms in the stochastic chemical kinetics formulation. This is similar to the integrated approach described in Ribeiro & Pinto (2009) except that they followed deterministic approach.

The present modeling approach is very useful as it follows an integrated methodology in which multiple modules can be added independently. In the present study, a simple cell cycle model representing a model eukaryotic organism is used. A more detailed and more realistic reaction network can be used in future work. Similarly, it is easy to use different drug pharmacokinetics in place of simple linear and exponential decay. Single and multiple compartmental models are generally used to represent various barriers for representing drug transport from the drug intake till it acts on its gene target on the cellular and subcellular region. Similar provisions are possible for modifying drug pharmacodynamics by incorporating well defined correlations between the dose and degree of inhibition of the target reaction such as Emax, sigmoidal relationships. Another important module in the integrated model is the genetic resistance to the acting chemotherapeutic drugs. It is not considered in the present model in its preliminary stage. It can be incorporated as another module. Gene amplification, random mutations to the genes involved in drug metabolism and alternate metabolic pathways are identified as some of the possible mechanisms for evolution of drug resistance. These genetic interactions and reaction pathways can be combined with the cell cycle network as another module for describing drug resistance. The effect of drug on cells manifests in terms of the reduced inhibition factor of the selected drug on its target reaction.

With the current approach it is possible to evaluate toxicity and tumor control independently which is useful considering the fact that each patient will have different objectives and concerns (i.e., cure or palliation) related to the treatment. Also, there are many types of toxicities viz. neurological, cardiac, gastrointestinal in addition to the toxicity to the blood stem
cells that may affect the treatment (Gardner and Fernandes, 2003). These toxicities can be handled by using parameters specific to the corresponding normal tissues, in the tumor growth model. Using this methodology, we do not have to resort to simultaneous optimization of toxicity and tumor cell kill as described in optimal control models of (Swierniak et al., 2003) where only one type of toxicity is considered.

Through the present model, simultaneous action of multiple drugs can be tested by making changes to inhibition factors of their respective target reactions in the cell cycle reaction network. One way to obtain the exact relationship between drug dose and resulting inhibition factor is by combining drug kinetics with the cell cycle network as is done by Chappel et al. (2008) using a deterministic formulation. Any interactions among the drugs acting simultaneously is not considered at the moment, as it demands more biochemical details and reaction kinetics of drug molecules.
CHAPTER 5. SELECTION OF MODEL PARAMETERS

5.1. Introduction

5.1.1. Background and Motivation

Parameter estimation for stochastic models needs a special consideration. One way of finding the stochastic model parameters is by using the rate parameters obtained from deterministic models. Deterministic models are fit to experimental data by classical least squares optimization tools to obtain the rate parameters and these can be converted to the equivalent rate parameters for stochastic kinetics. However, such conversions are not always possible, especially when the rate expressions are not in the form of simple mass action kinetics. Another issue is that stochastic kinetic models of a given reaction network will have more model parameters than the deterministic model of the same network. All the reaction propensities must be determined to complete the model. One possibility to determine these parameters is by fitting the model to transient distribution of reactant molecules which can be used to estimate the parameters of the stochastic model. Since there will be large number of species involved in most of the biochemical networks, real time measurements of molecular concentrations for large enough number of cells is needed to calculate the required transient distribution. Obtaining such a huge amount of real time data is not always an easy task.

An alternative strategy is to fit the model to steady state distributions of states of cell population that can be easily obtained from flow cytometry measurements. Obtaining the property distributions for cell population from Monte Carlo simulation of the present stochastic cell cycle model is described earlier. The distributions obtained from the model will depend on the values of the reaction propensities and other model parameters. Thus, biologically reasonable values of the model parameters can be obtained by fitting the model to the distribution of states
obtained experimentally using the usual least squares techniques. This is a well-known technique applied for DNA distributions (Dean and Jett, 1974; Johnston D. A., 1978; Bailey, 1981). Fitting the distributions to models with large number of parameters is a challenging problem as it may result in over fitting. Thus, a wise strategy is to seek the solution of least squares problem in terms of only those parameters that have considerable impact on the distributions. In the present work, a method is developed that will be helpful in identifying the importance of different parameters on the observed distributional properties and also helpful in obtaining a good starting guess of the parameters for the least squares problem.

Budding yeast *Saccharomyces Cerevisiae* is considered as model organism for eukaryotic cell cycle studies and the characteristics of its cell population distributions are widely understood. The gene regulation and biochemical pathways related to cell cycle progression of yeasts are similar to those found in higher eukaryotes. Thus, logical step to handle the parameter selection problem for stochastic models is to start with the distributions of budding yeast cell populations and then to extend the understanding to higher organisms.

### 5.1.2. Objective

The main objective of the current chapter is to present a methodology to refine the basic cell cycle model described earlier such that the population characteristics derived from the model resemble the characteristics of budding yeast cell populations and to implement a parameter selection algorithm that selects values of the parameters that optimize a specific characteristic of the population derived from the model. Specifically an evolutionary optimization strategy, termed here as insilico evolution is proposed that selects the stochastic model parameters representative of a cell population with largest growth rate.
The idea of selecting parameters that optimize a particular property of distributions is developed similar to the idea of flux balance analysis (FBA) that has been widely used in metabolic pathway analysis (Schilling et al., 2000; Orth et al., 2010). The objective of FBA is to select the optimal distribution of metabolic fluxes of each reaction in the network to maximize a phenotype or production of a particular metabolite. There might be many combinations of the individual reactions that give optimal solution. The range of values of contribution from a specific reaction gives the sensitivity of the objective function to changes in fluxes from that reaction, which is determined by flux variability analysis. In the present work, insilico evolution is employed as the optimization strategy to select the combination of parameters contributing to a maximize population growth rate. Maximum population growth rate of cells can be easily obtained from the cells growing in exponential phase.

Insilico evolution is initially applied to the basic cell cycle model to gain insights into the salient features of the proposed methodology. The stochastic cell cycle model is then reformulated to include more realistic features of budding yeast cell population. To make the model more realistic, model parameters with arbitrary units are assigned proper units of mass and time based on the comparison of properties of distributions obtained from the model with those collected from the literature data. At this stage, the model with its realistic parameters is in a more refined form than the basic model. The insilico evolution is then applied on the refined model to improve the values of model parameters corresponding to cells with maximum growth rate.

5.2. Parameter Selection by Insilico Evolution

The insilico evolution method which is described in the next section requires initial set of model parameters that give cell cycle oscillations. To obtain the initial set of parameters, a
systematic approach to tune the stochastic cell cycle model parameters to cause oscillations, characteristic of cell cycle dynamics, is discussed by Sherpa and Hjortso (unpublished work) and the parameter set used in the basic cell cycle model is obtained from the same approach which is briefly explained here. First, reactions that are not essential for onset of oscillations are turned off. Even though a rigorous mathematical analysis is possible using center manifold analysis, this process can be carried out by inspection for simple reaction networks. The rate constants for the still active reactions are then determined by turning off all reactions except those determine the dynamics in the early part of the cell cycle. Reaction parameter values are adjusted by hand until one obtains a response that is in qualitative and, to the extent possible, quantitative agreement with experimental observations. Reactions that determine the dynamics of next sequence of events in the cell cycle oscillator are then turned on and their associated parameters adjusted by hand as before. This continues until all reactions are activated at which point the model oscillates and the parameter set is ready to be used in the insilico evolution algorithm.

5.2.1. Insilico Evolution Algorithm

The final parameters obtained from the insilico evolution will result in cell cycle oscillations and represent the cells with maximum population growth rate. The description of the algorithm is as follows.

The basic idea of the insilico evolution is built on the process of natural evolution. Initially, there will be a specified number of cells in the system all having the same set of parameters of the cell cycle model.

The parameters of the model which affect the propensities of various cell cycle pathways are perturbed randomly (characteristic of mutation) at each cell division and each of the newly born cells will get two different sets of perturbed parameters and their growth is described based
on the new set of model parameters. The extent of perturbation (or mutation) of the existing value of any parameter depends on the mutation rate which is assumed to take values between 0 and 2, where a value of 0 corresponds to no mutation and a value of 2 corresponds to maximum range of possible mutated values which is double the magnitude of the parameter value. The perturbation of the parameter is related to the mutation rate by the following expression which is schematically represented in the Fig. 15.

\[ k_{after} = k_{before} + k_{before}(random\ number - 0.5)(mutation\ rate) \]

Where, \(k_{after}\) represents the parameter after division and \(k_{before}\) represents the parameter before division occurred. The ‘random number’ is a real number uniformly distributed in the interval [0,1].

After the population increases to a pre-specified size, some cells will be eliminated from the system to bring the population back to the original size and the process of mutation and elimination of the cells is repeated. After many repetitions of the procedure, cells that have a low population growth rate and maximum likelihood of death will be eliminated and the existing cells in the system will have a high population growth rate. The set of parameters corresponding to the existing cells are selected as the improved parameters compared to the initial parameter set that gave oscillations.

Figure 15: Schematic explanation of “mutation rate” applied to model parameter (“k” as illustration)
5.2.2. Linking Perturbations in Parameters to the Change in Population Growth Rate During Insilico Evolution

Perturbations to the parameters of the cell cycle model influence the cell cycle progression. By knowing the relationship between cell cycle progression and the single cell specific growth rate, it is possible to relate the perturbations in the parameters and the resulting effect on the population growth rate. For zero order single cell growth kinetics, specific growth rate of cell continuously changes during the cell cycle from maximum at the start to minimum at the end. Based on this fact, a population with smallest average cell mass will have highest population growth rate. Hence, by assuming zero order growth kinetics of single cell, parameters corresponding to the lowest possible cell mass in the evolution can be selected as those giving a population of maximum growth rate.

5.2.3 Insilico Evolution Simulations

In the present work, all the simulations of insilico evolution are started with 100 new born cells and 10 cells are selected randomly and eliminated from the system once the total cell population reaches a number of 110 cells. These simulations are performed till 10000 iterations whenever possible to resemble the natural evolution in which new traits occur due to mutations over many generations.

Evaluation of the Algorithm

To evaluate the algorithm, it is tested with an initial condition of an equal number of fast growing and slow growing cells and a zero mutation rate. For this system, the algorithm should select for the faster growing cells and eventually eliminate the slower growing population completely. These cells are classified as slow growing and fast growing cells based on the difference in their doubling times. Different values to one of the cell cycle parameters
(Parameter k4 in this case) are assigned to these cells growing in zero order kinetics. The cell cycle properties of these two types of cells are listed in Table 7. The differences in the values of cell mass after and before cell division highlight that fast growing cells divide at lower cell mass than the slow growing cells which is expected when zero order single cell growth kinetics are used.

Table 7: Fast and slow growing cells

<table>
<thead>
<tr>
<th>Average values</th>
<th>Fast Cells</th>
<th>Slow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>mass at birth</td>
<td>0.403929</td>
<td>0.497314</td>
</tr>
<tr>
<td>mass just before division</td>
<td>0.807758</td>
<td>0.99277</td>
</tr>
<tr>
<td>G1 duration</td>
<td>2.01007</td>
<td>2.462842</td>
</tr>
<tr>
<td>G2 duration</td>
<td>0.009074</td>
<td>0.01444</td>
</tr>
<tr>
<td>Doubling time</td>
<td>2.019144</td>
<td>2.477282</td>
</tr>
<tr>
<td>Reaction parameter, k4</td>
<td>400</td>
<td>85</td>
</tr>
</tbody>
</table>

The results of insilico evolution are shown in Fig. 16 in terms of number of fast cells and slow cells in the system which started with 50 cells of each type. When 10 cells are randomly eliminated from the system, only fast growing cells remain after 146 iterations eliminating all the slow cells. These results give confidence in the effectiveness of the insilico evolution algorithm in selecting cells with maximum growth rate.

Figure 16: Evolution of system into fast growing cells and elimination of slow cells in insilico evolution
5.2.4. Application of the Insilico Evolution for the Basic Cell Cycle Model

Insilico Evolution Results for a Single Mutation Rate

The insilico evolution algorithm is then applied to the basic cell cycle model to get insights into the parameter selection problem which can be used to the refined budding yeast cell cycle model discussed later. Simulations are carried out for different mutation rates. Results of average values of the parameters and cell mass for the 100 cells remaining after each iteration of the insilico evolution for a mutation rate of 0.4 are shown in Fig. 17.

For this mutation rate, convergence in mass was reached rapidly around 2000 iterations. All the rate parameters, after initial transient, will continue fluctuating even after the cell mass has converged. These fluctuations are very high for some parameters and are small or moderate for some parameters and some parameters remain constant with negligible fluctuations. These fluctuations in the parameters can be seen as different combinations that give same optimal solution as is done using flux balance analysis (FBA) of metabolic networks. From the Fig. 16, parameter k1 reaches a very high value compared to the initial value it was started with. Parameters k1 and k4 change up to magnitude of 10 times, whereas parameter k5 changes up to several orders of magnitude. The parameter Nx converges to a constant value of 2 and remains constant. The insilico evolution simulations qualitatively perform sensitivity analysis of the model parameters. The parameters that fluctuate to large extent will have less effect on the population distribution than those parameters that fluctuate by small amount. However, the magnitudes of these fluctuations are very high for the parameters shown in Fig. 17. It is not convincing to think that they are different combinations of parameters giving the same optimal solution. It happens when the rate at which mutations to the parameters are occurring is very high compared to the rate at which the change is occurring in the population in terms of growth.
rate. Simulations results from two different mutation rates are compared to gain some insights into these aspects.

Figure 17: Insilico evolution simulation results for Zero order kinetics, mutation rate 0.4. The starting values for the parameters are, \( k_1 = 800; k_4 = 280, k_5'' = 200 \).

**Influence of Mutation Rate in Insilico Evolution**

Simulations are performed for different mutation rates ranging from very small to high mutation rates. At higher mutation rates, all the cells are died in very small number of iterations.
Results for some mutation rates of 0.4 and 1.2 are presented in Fig. 18. It appears that the convergence has reached at around 2000 iterations for mutation rate of 0.4 and it has not yet reached for the rate of 1.2 even after 5000 iterations. There is still some transient in the average mass curve for mutation rate of 1.2. The value of parameter k1 when the cell mass is close to the convergence value appears to be 10000 for mutation rate of 0.4 and then it has increased to large values.

Figure 18: Effect of mutation rate on convergence to the final solution.

After reaching the convergence for the rate 0.4, further perturbations to the parameter k1 did not cause any improvement in the value of optimum cell mass. Hence, the selection process is not in pace with the mutation process. For the case of high mutation rate of 1.2, even for large fluctuations, there is still some transient in the cell mass. The selection process is still responding to the large changes in the parameter values suggesting that there should be a definite relationship between mutation rate and selection pressure. Selection pressure can be defined
simple terms as the ratio of maximum specific growth of an individual in the population to the average specific growth of all the cells.

The concept of mutation and selection in the present case can be explained as followed. There are two rates of changes in the system, one is the rate of change of parameter values and another is the rate of change in the population composition in terms of specific growth rate of each cell. The rate of change of parameter values can be viewed as characteristic rate of mutation and the rate of change of population composition can be viewed as characteristic rate of selection.

If the characteristic rate of mutation is significantly higher than the characteristic rate of selection, then the selection process cannot keep up and there is chance of best traits being lost in the evolution. This should result in traits that are not the best possible. In the current optimization problem, the best trait we are looking for is the maximum population growth rate which is reflected in minimum average cell mass of the cells in the system, and if we compare the values of the converged mass for low mutation rate and high mutation rate, the above point is reflected. Average cell mass for low mutation rates is less compared to that for high mutation rate and this difference increases with the difference in the mutation rates.

Only if the mutation rate is slow enough does the selection process have a chance to have an effect and only then do we see meaningful results. If the characteristic rate of mutation is too high, there is danger of extinction of the species in the evolution. Similar results are reflected in the present scenario of parameter selection through evolution. For the simulations started with high mutation rates, the initial perturbations in parameters caused lot of cell deaths and the insilico evolution ceased soon. Hence, it is a matter of further investigation to quantify the
mutation rate and selection rate so that mutation rate can be adjusted in the evolution process to match the selection rate.

5.3. Refining the Basic Cell Cycle Model

5.3.1 Model Description

With the insilico evolution algorithm working properly, the next logical step is to start with realistic parameters that lead to population characteristics that are close to the experimentally observed values. A literature survey on yeast models and experiments to identify properties characteristic of the yeast cells is done (Hartwell, 1974; Hjortso and Bailey, 1982; Barberis et al., 2007; Di Talia et al., 2007; Barik et al., 2010; Bryan et al., 2010). Many observations are made in the literature regarding the cell division of budding yeast and some of which are mentioned below. A schematic of the cell division model based on these observations is shown in Fig. 19.

Figure 19: Schematic of the model of cell division in budding yeast
M: cell cycle time of mother cell
D: cell cycle time of daughter
m₀: mass of daughter cell immediately after cell division
m*: critical mass (cell mass required for start event to occur)
mₐ₀: cell mass at the time of division

- Mother cells will divide into a new cell of mass close to a constant value known as critical cell mass and the daughter cell will obtain a mass less than critical cell mass.
- The mother cell cycle time is roughly constant, independent of growth conditions, while the daughter cell cycle time varies strongly with growth rate, being shorter at higher growth rates and longer at lower growth rates.
- The start event which is considered as the checkpoint for starting the DNA synthesis, will not occur until the critical cell mass is reached.

The stochastic model is modified to incorporate these changes. The modifications are as followed.

- The “start event” will not occur until cell grows to the critical mass in addition to the condition of the earlier model in which start event occurs only after cyclin/Cdk molecules reach a threshold value.
- Cell will not divide until it attains a mass that is at least equal to the sum of critical cell mass and minimum required cell mass. Earlier condition is for the cell to attain a mass that is double the minimum mass.
- At the time of division, partition of the total cell mass occurs in such a way that mother cell will attain a cell mass equal to critical cell mass and daughter cell gets the remaining mass.
• Daughter cell will not synthesize any cyclins until it reaches the size of the critical cell mass. This provision is to prevent multiple oscillations during the cell cycle of daughter cell before the threshold value of mass for division is reached as shown in the Fig. 20.

![Figure 20: An erroneous behavior of multiple oscillations of daughter cell in cell cycle](image)

**5.3.2 Evaluation of the Refined Model**

The qualitative behavior one should observe with this kind of model is that the mass distribution obtained for the cell population should give a curve with double peak with the distribution shifting towards lower cell mass at lower growth rates and moving to higher cell masses at high growth rates with almost unimodal distribution. The results depicting the same behavior are as shown in Fig. 21. Here, parameter ‘nu’ is the specific growth rate of single cell that is used in the cell cycle model simulations with first order kinetics.

At very low specific growth rates, a large fraction of the population consists of cells of small cell mass and as the specific growth rate is increased, distribution shifted towards higher cell mass. The second peak in the above distribution occurs at the exact value equal to 0.88, which is the critical cell mass chosen, at all the specific growth rates. In practice, this peak will not occur at a fixed value for all the growth rates, instead it shifts towards right as the growth rate is increased. To include such realistic behavior, value of the mother cell mass is not taken exactly as critical mass; instead it follows a probability distribution centered on critical cell mass.
Figure 21: Mass distribution for various specific growth rates; at the time of division, mother cell attains a mass equal to critical cell mass and the daughter cell gets the remaining mass.

A simple 4\textsuperscript{th} order algebraic equation is used to get the distribution of mother cell mass at the time of division which is given by the following equation.

\[ P(m) = \frac{(m - a)^2(m - b)^2}{A} \]

Where, \( a \) = lower bound for cell mass = \textit{Mother Mass}(1 - \varepsilon)

\( b \) = upper bound for cell mass = \textit{Mother Mass}(1 + \varepsilon)

\( \varepsilon \) = Maximum allowed deviation of new cell mass from the value of critical mass

\[ \frac{(a+b)}{2} = \text{critical cell mass} \]

\( A \) = area of the curve \((m - a)^2(m - b)^2\) in the interval \((a, b)\)

The results for the modified model are presented below in Fig. 22 for various growth rates.
Figure 22: Mass distribution for various specific growth rates, mother cell divides exactly according to a probability distribution centered on critical cell mass.

Apparently, the peak on the right side of the distribution shifts towards higher cell mass rather than staying at the critical cell mass, as the specific growth rate is increased which is reasonable with the realistic budding yeast cell mass distributions.

For higher specific growth rates, it is observed in the simulations that daughter cells will attain larger mass than the mother cell at the time of division which is not reasonable. Thus, the specific growth rate should be limited to some value above which it is not feasible to have a biologically reasonable distribution.

5.3.3. Assigning Units to the Parameters

Another step towards making the model more realistic is to assign reasonable parameters with units assigned to the rate parameters. The approach to adjust the parameters in such a way
that the simulation results match the experimental data is to compare specific properties of the
distribution obtained both from experiments as well as from the model simulations and to use
this comparison as to estimate the values of the model parameters. The two properties of the
distribution that are used for obtaining units to the initially dimensionless parameters are average
cell cycle time of mother cell and the critical cell mass of the cell population. It is proposed and
experimentally verified in an earlier study that cell cycle times of mother cells are almost
constant at the time of division, whereas variability exists among the cycle times of daughter
cells (Hartwell and Unger, 1977).

Value of average cell cycle time of mother cells is calculated for dimensionless
parameters and equated to the experimentally observed values to obtain the conversion factor for
arbitrary time units to minutes. Similarly, the critical cell mass of mother cells is equated to the
average cell mass of the distribution of cell masses to obtain the conversion factor for cell mass.

Values of critical cell mass and the constant mother cell generation time are taken from
the experimental data of (Hartwell and Unger, 1977).

Average volume of the newly born mother cell at the time of division = 79.4±15.2 µm³.
Approximate value taken in the calculations = 80 µm³ = 80×10⁻¹² cc
Density of yeast is taken as 10% more than that of water
Density of yeast cell = 1.1 g/cc
Average mass of a mother cell at the time of division = 80×10⁻¹² cc × 1.1 g/cc = 0.88 pg
(pico gram)
Approximate value for the mother cell generation time = 135 time (Hartwell and Unger, 1977)
Simulations are carried out for the parameters used in the basic cell cycle model. The properties of the distribution for the arbitrary units (AU) using first order kinetics and zero order kinetics are given below.

**First order kinetics**

Average cell mass from the distribution = 0.35 mass AU

Mother cell generation time (or cell cycle time) = 1.65031 time AU

Specific growth rate, $\nu = 0.2 \text{ time}^{-1}$

Conversion factors:

$0.35 \text{ AU} = 0.88 \text{ pg (pico gram)}$

$1.65031 \text{ AU} = 135 \text{ min}$

**Zero order kinetics**

Average cell mass from the distribution = 0.324417 mass AU

Mother cell generation time (or cell cycle time) = 1.62 time AU

Specific growth rate = $0.2 \text{ (mass AU)} (\text{time AU})^{-1}$

Conversion factors:

$0.324417 \text{ AU} = 0.88 \text{ pg (pico gram)}$

$1.62 \text{ AU} = 135 \text{ min}$

Resulting parameters for both the kinetics are shown in Tables 8 & 9.

**Table 8: Parameters and growth rate for zero order kinetics**

<table>
<thead>
<tr>
<th>$k_1$: $3.54 \text{ min}^{-1} \text{ pg}^{-1}$</th>
<th>$k_5^*$: $2.4 \text{ min}^{-1}$</th>
<th>$J_5$: $4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_2$: $0.00024 \text{ min}^{-1}$</td>
<td>$k_5^*$: $2.4 \text{ min}^{-1}$</td>
<td>$n$: $5$</td>
</tr>
<tr>
<td>$k_3$: $0.024 \text{ min}^{-1}$</td>
<td>$k_6^*$: $0.012 \text{ min}^{-1}$</td>
<td>$c_7$: $0.024 \text{ min}^{-1}$</td>
</tr>
<tr>
<td>$k_4$: $1.2387 \text{ min}^{-1} \text{ pg}^{-1}$</td>
<td>$k_7^*$: $24 \text{ min}^{-1}$</td>
<td>$J_4$: $0.04$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$J_6$: $50$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\nu = 0.00651 \text{ pg min}^{-1}$</td>
</tr>
</tbody>
</table>
Table 9: Parameters and specific growth rate for first order kinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>3.889619 min⁻¹ pg⁻¹</td>
</tr>
<tr>
<td>$k_2^5$</td>
<td>2.444903 min⁻¹</td>
</tr>
<tr>
<td>$c_2$</td>
<td>0.000244 min⁻¹</td>
</tr>
<tr>
<td>$k_3$</td>
<td>0.024449 min⁻¹</td>
</tr>
<tr>
<td>$k_4^5$</td>
<td>1.361366 min⁻¹ pg⁻¹</td>
</tr>
<tr>
<td>$k_6^5$</td>
<td>24.44903 min⁻¹</td>
</tr>
<tr>
<td>$c_3$</td>
<td>0.012225 min⁻¹</td>
</tr>
<tr>
<td>$c_7$</td>
<td>0.024449 min⁻¹</td>
</tr>
<tr>
<td>$f_4^5$</td>
<td>0.04</td>
</tr>
<tr>
<td>$f_5$</td>
<td>4</td>
</tr>
<tr>
<td>$f_6^5$</td>
<td>50</td>
</tr>
<tr>
<td>$n$</td>
<td>5</td>
</tr>
<tr>
<td>$\nu$</td>
<td>0.002445 min⁻¹</td>
</tr>
</tbody>
</table>

5.4. Insilico Evolution Simulations for the Modified Model with Units

Insilico evolution algorithm is now applied for the updated model of the basic cell cycle model. All the cell cycle parameters are given units as shown in the conversion calculations earlier. The parameters used in the simulations are as shown in Table 9. Simulations are carried out for different mutation rates varying from low 0.2 to high range 1.0 and the results are presented below and discussed.

5.4.1 Results and Discussion

Average mass of cells in the system stays constant after sufficiently large number of iterations of insilico evolution for different mutation rates. Fig. 23 shows the convergence of mass for two mutation rates, one is a low rate 0.2 and another is a higher rate 0.4. Convergence reached quickly (around 2000 iterations) for low rate compared to the higher rate (around 3000 iterations). The idea behind the insilico evolution is based on the linking of growth rate to the cell mass using zero order growth kinetics. For zero order kinetics, the smaller the mass of the cell, the higher is its specific growth rate. Thus, when the convergence in mass is achieved during the insilico evolution, the cells in the system have reached maximum possible growth rate. In the later discussion, the term “convergence” used frequently to refer to the condition of maximum population growth rate and not to any other parameters. The characteristics of the population in terms of cell cycle time and population growth rate determined from the cycle time are shown in Table 9 for the two mutation rates. These values are obtained from the simulation
of the refined model using the model parameters found out from the insilico evolution for the case of two mutation rates, at the time of convergence. The parameters used are shown in Table 9. The calculated population characteristics are in reasonable agreement with the values observed for the yeast strains used in our research lab.

![Cell mass convergence for mutation rates 0.2 (Left) and 0.4 (Right)](image)

**Figure 23:** convergence of cell mass for mutation rates 0.2 (Left) and 0.4 (Right)

**Table 10:** Parameter values at the time of convergence for mutation rates, 0.4 and 0.2

<table>
<thead>
<tr>
<th>Mutation Rate</th>
<th>0.2</th>
<th>0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convergence considered after Iteration</td>
<td>2000</td>
<td>3000</td>
</tr>
<tr>
<td>k1</td>
<td>8.136948</td>
<td>61.91434</td>
</tr>
<tr>
<td>c2</td>
<td>3.92E-05</td>
<td>8.61E-05</td>
</tr>
<tr>
<td>k3</td>
<td>0.018778</td>
<td>0.250774</td>
</tr>
<tr>
<td>k4</td>
<td>1.305939</td>
<td>3.538061</td>
</tr>
<tr>
<td>J4</td>
<td>0.010195</td>
<td>0.014855</td>
</tr>
<tr>
<td>k5_1</td>
<td>0.366755</td>
<td>0.143234</td>
</tr>
<tr>
<td>k5_11</td>
<td>4.601016</td>
<td>405.8477</td>
</tr>
<tr>
<td>J5</td>
<td>1.511774</td>
<td>0.182309</td>
</tr>
<tr>
<td>k6_1</td>
<td>0.01643</td>
<td>0.000514</td>
</tr>
<tr>
<td>k6_11</td>
<td>23.40614</td>
<td>60.04024</td>
</tr>
<tr>
<td>J6</td>
<td>139.1396</td>
<td>10.92971</td>
</tr>
<tr>
<td>Nx</td>
<td>5.041864</td>
<td>2.016789</td>
</tr>
<tr>
<td>c7</td>
<td>0.143474</td>
<td>0.198089</td>
</tr>
</tbody>
</table>
Table 11: Population characteristics calculated for the optimal parameters from insilico evolution

<table>
<thead>
<tr>
<th>Mutation Rate</th>
<th>Population doubling time, min</th>
<th>Population growth rate, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>98.666</td>
<td>0.007025</td>
</tr>
<tr>
<td>0.2</td>
<td>97.6</td>
<td>0.0071</td>
</tr>
</tbody>
</table>

For both the mutation rates, similar trends are observed for the evolution of parameters of cells in the system. Plots showing the variation of parameters in the insilico evolution for mutation rate 0.2 are shown in Fig. 24, Fig. 25 and those for mutation rate 0.4 are shown in Fig. 26, Fig. 27. One typical behavior observed is that those parameters either increase or decrease towards improved values as the cell mass approaches its convergence and then they start fluctuating, remain constant or follow the same direction of increase or decrease with intermittent fluctuations. Fig. 24 shows the results for parameters – k1, k3, k4, k5”, c7 and k6” for a mutation rate of 0.2. For example, parameter k5” smoothly reaches a value close to 5 at the time of convergence, and then fluctuates rarely falling below this value. It fluctuates by a factor of 7 to reach 35. The range of these fluctuations indicates the sensitiveness of the parameter k5” in the model. Parameter c7 also follows a similar behavior of reaching a value of 0.15 at the time of convergence and then fluctuates around this value with a maximum factor of 2. Fluctuations are observed in parameters k1, k3, k4 and k6”. However, the magnitude of deviations of the values of these parameters from the values observed at convergence varies. It depends on the sensitivity of the model to each parameter.

Fig. 25 shows some parameters which do not show many fluctuations. They either move towards a final value asymptotically or stay constant. Parameter Nx remains constant at the starting value of 5 with minor fluctuations. Parameters c2, k5’ and k6’ approach values close to zero.
Figure 24: Parameters in insilico evolution for mutation rate, 0.2. There are small fluctuations in these parameters till the point of convergence, fluctuations of different magnitude are observed afterwards. Insilico evolution serves as a measure of sensitiveness of the model to these parameters.
Figure 25: Parameters of the insilico evolution for mutation rate, 0.2. Parameter Nx, fluctuates slightly around 5. J5 is increasing with fluctuations. Other parameters show asymptotic behavior.
The insilico evolution results for mutation rate 0.4 are shown in Fig. 26, Fig. 27. Parameter $k_1$ reaches a value close to 50 at the time of convergence, and then fluctuates rarely falling below this value of 50. It changes by a factor of 7 to reach 350. Parameter $k_3$ also follows a similar behavior of reaching a value of 0.1 at the time of convergence and then starts fluctuating up to a factor of 7. Parameter $k_5'''$ also reaches a value at the time of convergence and undergoes fluctuations of several orders of magnitude in line with the fluctuations observed for parameters $k_1$ and $k_3$. Similar trend is observed for parameter $c_7$.

Results for parameters $J_6$, $N_x$, $k_6'''$, $c_2$, $J_5$ are shown in Fig. 27. Parameter $J_6$ reached convergence in the case of high mutation rate of 0.4 whereas it fluctuated for low mutation rate of 0.2. $N_x$ remains constant for the case of high mutation rate also, however, it attains a different constant value of 2 compared to a value of 5 observed for low mutation rate. Similarly, parameter $k_4$ does have fluctuations of small magnitude despite large fluctuations in parameters $k_1$, $k_3$, $c_7$, $k_5'''$. Parameters like $J_6$, $N_x$, $k_4$ whose values stay in a close range in the insilico evolution seem to be important for the parameter estimation problem because their possible values for a given growth rate are in small range. Thus, insilico evolution algorithm gives an idea about the sensitivity of the model to different parameters.

**Effect of mutation rate**

Many nonlinear optimization search algorithms, when they are far away from optimal solution use large magnitudes of perturbations in the optimization variables and the perturbations become small when near the optimum. It is a point of investigation to see how mutation rate which is an indication of perturbation made to the parameters, changes with the distance from the optimal solution. It is mentioned in the earlier discussion about the parameters that show considerable magnitude of fluctuations after reaching convergence in mass (all the cells in the
Figure 26: Parameters in insilico evolution for mutation rate, $0.4$. Parameter $k_5$ fluctuates to several orders of magnitude, while $k_1$, $k_3$, $c_7$ fluctuate to a factor of 7. Model appears sensitive to fluctuations in parameter $k_4$. 
Figure 27: Insilico evolution of parameters for mutation rate, 0.4. Parameters J6, Nx reached convergence. The other parameters stay close to zero due to large fluctuations in parameters k5", k1, k3 at this mutation rate.
system have reached their maximum possible growth rates), will not show much fluctuations before reaching the convergence. They moved in a smooth manner towards the optimal solution where cells grow at a maximum rate, only then started fluctuating to considerable magnitude without causing any cell deaths. At low growth conditions, where the parameters are far away from the optimal solution, the perturbations in the parameters should be very small. Hence, for the present initial parameters, it appears that we should start with small mutation rate. The results of the insilico evolution simulations performed for large mutation rates confirm this point. All the cells have died after a few iterations of the insilico evolution for very high mutation rates.

After reaching the optimal solution, the magnitude of fluctuations is large for higher mutation rates than for small mutation rate. For example, parameter $k_5''$ fluctuates to several orders of magnitude for mutation rate of 0.4 compared to fluctuations in other parameters $k_1$, $k_3$, $c_7$ which change by a factor of 7. This shows that the parameter $k_5''$ may not be that important in fitting the model to steady state distributions using least square technique. However, for the mutation rate of 0.2, the magnitude of fluctuations of $k_5''$ is comparable to the fluctuations in the values of parameters $k_1$, $k_3$, $c_7$. This makes it equally important in the model fitting even though it is shown to be less important from the high mutation rate observation. Thus, using higher mutation rates after reaching the optimal solution helps in identifying sensitivity of parameters.

The time to reach convergence and also execution time of the algorithm may change with mutation rate. The algorithm is executed for mutation rate of 0.6 and the result of convergence of cell mass is shown in Fig. 28. It has reached the lowest value of cell mass, which is the optimum, in less than 2000 iterations, but do not appear to be converged as it continues fluctuating. Also, the average cell mass for the population is slightly higher than that observed for lower mutation rates of 0.4 and 0.2. The results of execution time for each of the mutation rates are shown in
Table 5. It took less time for the execution of algorithm to 10000 iterations for low rate of 0.2. Even though the execution time for the rate 0.6 is less than that for the rate 0.4, the doubling time of cells is high which gives a less population growth. Thus, mutation rate is an important factor to be investigated to accomplish many improvements to the insilico evolution algorithm proposed here, when applied for more complex models.

Figure 28: Convergence of cell mass for mutation rate of 0.6

Table 12: Execution time and average cell mass for different mutation rates

<table>
<thead>
<tr>
<th>Mutation Rate</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Execution Time, min</td>
<td>65</td>
<td>248</td>
<td>208</td>
</tr>
<tr>
<td>Average time for iteration, milli sec</td>
<td>396</td>
<td>1490</td>
<td>1247</td>
</tr>
<tr>
<td>Doubling time, min</td>
<td>83.5</td>
<td>87.13</td>
<td>97.1</td>
</tr>
<tr>
<td>Average mass, pg</td>
<td>0.856</td>
<td>0.9168</td>
<td>1.04</td>
</tr>
</tbody>
</table>

5.4.2 Summary

Using insilico evolution algorithm, optimal parameters are obtained that gave the population doubling time in reasonable agreement with the experimental value. These parameters can be selected as an initial estimate of the model parameters. Cell mass converges towards a minimum value and parameters either tend towards a lower value or higher value at the point of convergence. When iterations are continued even after the convergence of mass, fluctuations of
small to large range in the parameters are observed. The extent of fluctuations gives an idea about the sensitivity of the model to these parameters when the selection rate is keeping up with the mutation rate. If the mutation rate is fast enough that there is no improvement in the population composition, then all the perturbations in the parameters are random variations and we cannot draw many conclusions from that without investigating further on mutation rate. Large fluctuations of the parameters mean that the parameter will have less influence on the dynamics of the population. Mutation rates affect the speed of achieving the solution and these are to be intelligently adjusted to get to the final solution minimizing the fluctuations.

5.5. Conclusions

The basic cell cycle model is modified to include the features observed for experiments of budding yeast. Mother cells are assumed to acquire a constant cell mass known as critical cell mass and daughter cells will have large variations in their mass at the time of birth. Simulations are performed by including the feature of critical cell mass in the cell cycle model and the shape of mass distributions obtained for specific growth rates of large range give reasonable result.

Further direction from the insilico evolution simulations is to use a least square fit of distributions of states of key components of cell cycle oscillator those are obtained from flow cytometry. There are least square techniques available in the literature to fit DNA distributions to the models (Dean and Jett, 1974; Johnston D. A., 1978; Bailey, 1981). However, even a moderately realistic cell cycle model will have many parameters some of which may not be having great impact on the goodness of the fit of the distributions. The parameters of importance for the fitting problem can be identified from the insilico evolution simulations by the range over which they fluctuate. In addition to the insilico evolution simulations which give an idea about the sensitivity of the model to various parameters, sensitivity analysis of the parameter set can be
carried out using other methods (Gunawan et al., 2005) to identify the important model parameters.
CHAPTER 6. CONCLUSIONS AND FUTURE DIRECTIONS

A stochastic model developed based on a cell cycle model that is representative of a simple eukaryotic system is described. The kinetics of biochemical interactions among the species involved in the chosen cell cycle network are described by stochastic formulation and stochastic simulation algorithm is used to simulate the time course trajectories of the state of the system. Using a Monte Carlo strategy, a large number of independent simulations of the system with the same initial state will give different realizations of the state and thus give a distribution of states. All the cells in the same initial state will attain different states in time and thus the model depicts the loss of cell synchrony and the population heterogeneity in terms of their states is predicted from the stochastic model based on single cell kinetics. Thus, the stochastic cell cycle model presents Monte Carlo solution to the population balance equations.

Model’s ability to predict the population growth and to represent the heterogeneity of population is used in developing an integrated chemotherapeutic model that combines the growth of cells described by cell cycle model with simple pharmacokinetics describing the decay of drug in the body and pharmacodynamics describing the killing effect. Killing effect of a cancer drug on healthy cells and cancer cells will be different and selection of a type of drug injection in terms of various dose-frequency combinations to maximize the killing on cancer cells and minimizing the effect on healthy cells are tested using the basic cell cycle model with different parameters for each type of cells. Such a methodology has great scope in predicting the outcome of a drug treatment and drug administration protocol on a heterogeneous patient population and thus to assist in in silico screening of cancer drugs and testing clinical trial protocols.

Budding yeast is a model organism frequently studied for cell cycle research and the basic cell cycle model is refined in a systematic way to make it more realistic representation of
the budding yeast. The mass distributions predicted from the model are in qualitative agreement with the experimental observations. At high growth conditions, the mass distribution contains a double peak and it becomes unimodal at very low growth conditions which is predicted by the model. The qualitative agreement is not sufficient to use it in making predictions about the behavior of organism and thus a systematic methodology is developed in a direction to make the model accurate in quantitative aspects as well. Plausible values of the model parameters with proper units are assigned by making comparisons between the average mass and doubling time of the population of cells with the predictions obtained from model parameters with arbitrary units. Insilico evolution, an optimization strategy, is used to predicting the evolution of model parameters through generations of mutation and selection towards the parameter values that correspond to maximum growth rate of the population. There are many combinations of the model parameters that are selected by the evolution algorithms as to give maximum growth rate. Insilico evolution simulations show fluctuations to several orders of magnitude for some parameters, fluctuations in a close range for some indicating different sensitivities of the model parameters. The exact set of parameters can be obtained by using nonlinear least squares techniques to fit the distribution of important proteins in the biochemical network obtained from the model to the measured distributions. Insilico evolution, however, serves to identify the important parameters in terms of goodness of fit to the distributions, which only can be used for model fitting problem. From the computational aspects, further investigation of effect of mutation rate on improving the speed of selection, optimal solution.

A methodology presented for budding yeast with small set of compounds and reactions can be extended to more complex networks containing large number of reactions. Presently, work is going on to extend the learning gained through the experience of formulating stochastic
model for a simple eukaryotic system of biochemical network to more realistic networks of budding yeast cell cycle containing 10 components and 42 components respectively. The challenges lie ahead in handling more diverse set of reaction kinetics concepts such as quasi steady state assumption, partial equilibrium, and stiffness in the reaction set for which implementation of approximation algorithms is to be investigated. The original exact stochastic simulation algorithm (SSA) of Gillespie (1976, 1992) will perform satisfactorily for small systems and will be computationally demanding when it has to deal with large set of reactions as discussed in a study (Kierzek, 2002). Approximate algorithms discussed in the chapter 2 of this report are developed to speed up the simulations. Combining these approximations with parallel computing power of clusters of computers and multiprocessors should make the computational requirements less demanding and this is another direction for future work. Another task is to improve the features of insilico evolution strategy. At present, a single mutation rate is used to obtain the optimum solution starting from an initial set of parameters. Based on the discussion in the previous chapter, mutation rate might influence the speed of convergence of the optimization problem and also it may lead to different optimal solutions.

Developing stochastic models with more realistic features and combining them with the advanced computing technologies will help us in realizing many applications of those models in the fields of biology and medicine and thus for the better serving of the mankind.
REFERENCES


APPENDIX A. DOCUMENTATION FOR THE INSILICO EVOLUTION PROGRAM IN VB.NET

A.1. Overview

Insilico evolution is a type of evolutionary optimization method that we employed for selecting the best parameter set for the stochastic cell cycle model, that give maximum population growth rate of cells. Like other optimization search algorithms, the execution of algorithm requires an initial guess of the optimization variables. Here, the optimization variables are the parameters of the cell cycle model. The working principle of the algorithm and the cell cycle model on which the insilico evolution program is built is described in chapter 5 of this report. Here, the procedure for the execution of the user interface developed in VB.Net platform is explained for convenience of the users of this program.

Various windows in the user interface are explained below.

A.2. Main Window

As is shown in the window, it asks for the seed to start the random number generator in the program. Entering a negative number, the program will generate the seed according to the timer in the system. User can give any value for the seed. Clicking on the “Exit” tab, terminates the program.
A.3. Model Parameters

Figure A2: Opening window after clicking on “Model Parameters” tab.

Clicking on the “Model Parameters”, the main window will like above. There are five different sub menus appear.

A.4. Model Parameters - Cell Cycle Reactions

Clicking on the “Cell Cycle Reactions” will open a new window as shown below.

Figure A3: Opening window after clicking on “Cell Cycle Reactions” in “Model Parameters” tab of main window.

The window is self-explanatory. These are the initial cell cycle parameters for all the cells existing at the start of the insilico evolution algorithm. It has tabs “Load Parameters” and
“Save Parameters” clicking on which the program asks for the file name to load or save the model parameters respectively.

Clicking on “Done” will close the window.

A.5. Model Parameters – Trigger Values

Clicking on the “Trigger Values” will open a new window as shown below.

![Trigger Values Window](image)

Figure A4: Opening window after clicking on “Trigger Values” in “Model Parameters” tab of main window.

As written on the window, it asks for entering the trigger values for the start, division and cell death events.

Clicking on “Done” will close the window.

A.6. Model Parameters – Division

Clicking on the “Division” will open a new window as shown below.
Minimum Cell Mass is the minimum biologically possible value of cell mass for budding yeast.

ε is the maximum deviation allowed from the value of critical cell mass that is assigned to the mother cell after division.

Constant Mother Mass is the value of the critical cell mass. In the model, start event occurs only when cell mass crosses this threshold.

Clicking on “Done” will close the window.

A.7. Model Parameters – Single Cell Growth

Clicking on the “Single Cell Growth” will open a new window as shown below.

- Clicking on the button “First Order Single Cell Kinetics” will ask for specific growth rate, with units as time$^{-1}$
- Clicking on the button “Zero Order Single Cell Kinetics” will ask for growth rate for zero order kinetics. The units here should be in “mass/time”
Figure A6: Opening window after clicking on “Single Cell Growth” in “Model Parameters” tab of main window.

Clicking on “Done” will close the window.

A.8. Model Parameters – Mutation Rates

Clicking on the “Mutation Rates” will open a new window as shown below.

Figure A7: Opening window after clicking on “Mutation Rates” in “Model Parameters” tab of main window.

- **Mutation Range** is the range by which the model parameter can be perturbed from the existing value. Mutation range should have a mean value equal to one.

Clicking on the “Mutation Rates” will open a new window as shown below.

A.9. Main Window – Enter Seed Value for Random Number Generator

After completing all the inputs in the Model Parameter, click on “Done” in the main window to proceed further.
After entering the “seed” value for the random number generator, click on “Done” and the main window appears as shown below.

A.10. Main Window – Enter Inoculum Size

- User should enter “Inoculum Size” which by default uses a value of 100 in the algorithm.

  This is the number of cells to start with in the insilico evolution algorithm.

  After clicking on “Enter Inoculum Size”, the main window looks like below.
A.11. Main Window – Load Inoculum File

Figure A10: Main Window after clicking on “Enter Inoculum Size”.

After entering the size of the inoculum, user needs to select the file from which the initial cells should be selected. This file is called inoculum file in our model terminology.

- **Inoculum File** – This is the file where states of a large set of new born cells are stored. State of the cell is in terms of the mass, and number of molecules of each of the four components and the status of the cell in terms of start event occurred or not. Since all these cells are newly born, the ‘start event’ is stored as FALSE, a Boolean variable.

A.12. Main Window – Save Parameter Values after Each Iteration

Figure A11: New Window after finishing entering the inoculum file
After supplying the program with the ‘inoculum file’ and clicking OK, a new window opens as shown above. It will ask for the user if the parameters after each iteration of the insilico evolution simulations should be saved.

These parameters are the average of each of the parameters for the 100 cells in the system after eliminating some of the cells randomly.

If clicked ‘yes’, user need to specify the file name in which the parameters will be stored and proceeds to the next step.

If clicked ‘no’, the program proceeds to next step.

### A.13. Main Window – Save Final Distribution

User will be asked then if the final distribution of states should be saved. The window is as shown below.

<table>
<thead>
<tr>
<th>Insilico Evolution Four Component Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exit</td>
</tr>
</tbody>
</table>

Figure A12: New Window opens after clicking on ‘yes’ or ‘no’ to the save parameters window.

- **Final Distribution of States** – This is the distribution of the states of the 100 cells existing in the system after each iteration. By default, program stores the states of five of the 100 cells. It can be changed in the program.
A.14. Main Window – Start

After responding ‘yes’ or ‘no’ for saving the final distribution, the Main Window will look like this.

![Figure A13: Main Window after clicking ‘yes’ or ‘no’ on the save final distribution window.](image)

Clicking on the “Start” button, Main Window appears as shown below.

![Figure A14: Main Window while the program is being executed.](image)

The value in the box for “Iteration No” is the number of iterations of insilico evolution program execution that have been completed.

A.15. Data Files

The results of the insilico evolution simulation are stored in two files. One is the file for storing the average model parameters, cell mass and doubling time of the cells existing after each iteration and execution time of the iteration. It also contains the input values supplied to the program at the beginning of the simulation. The file appears as shown below.
Figure A15: Output file in which parameters at the end of each iteration are shown

Another output file is for saving the final states of a selected number of cells after each iteration. This will be helpful if we need to check how the states of the cells change as the parameters are changed. The output file for the final distribution of states is shown next.
Figure A16: Output file for saving the final distribution of states of cells existing in the system after each iteration.
APPENDIX B. IDENTIFICATION OF TARGET REACTIONS FOR CHEMOTHERAPY MODEL

B.1. Identifying the Target Reactions for Chemotherapeutic Drug

Simulation results of the time course of population growth of normal cells and target cells for continuous inhibition of each of the seven reactions for wide ranging inhibition factors are obtained to identify good target reactions. In Figure B1, natural logarithm of number of live cells (Nc) is plotted with time for the two cyclin degradation reactions (reactions 2, 3). The population of the target cells has increased as a result of inhibition and thus these two reactions are eliminated as the target reactions for drug.

![effect of c2 on population growth of target cells](image1.png)

![effect of k3 on population growth of target cells](image2.png)

Figure B1: inhibitory effect of reaction 2 (a) and reaction 3 (b) on target cell population growth

The corresponding results for the other reactions are shown in Figure B2 expressed in terms of the time course of the fraction of total live cells in normal and target populations at different inhibition levels (denoted by different inhibition factors). An ideal drug target reaction should have the value of the live cell fraction approaching zero for target cells and one for the normal cells after inhibition. However, from Figure B2(a), it appears that both types of cells are equally affected by inhibiting the cyclin synthesis reaction (reaction 1). For low inhibition factors, only a small reduction in the fraction of target cells is obtained and at higher inhibition more number of cells are killed and the fraction moves close to zero. But, the same trend is obtained for normal cells as well. In Figure B2(a), for the same dosage (or inhibition factor), live
cell fraction of healthy cells is less than that of the target cells. For example, at 0.333 dosage (k1 = 500 slow) healthy cells fraction is close to 0.6, whereas at 0.31 dosage, fraction of the target population is close to 0.6 (between doses 0.31 and 0.35). Hence, the inhibition effect is acting almost equally on normal and target cells which reveals that targeting reaction 1 is suitable for reducing tumor burden but not recommended from the toxicity considerations. Periodic injection of the drug that facilitates the normal cells to recover from the slow growth because of the inhibition may help the treatment to be effective. On the basis of continuous inhibition, reaction 1 may not be suitable target for the present target cells.

APC inactivation (Reaction 4) (Figure B2(b)) appears to be a target reaction for a chemotherapeutic drug. For normal population, at 0.2 dosage (k1 = 160), the fraction is close to 0.7 and will increase for higher doses. At nearly the same dosage (0.23), the fraction of target cells is very less.

Figure B2: inhibitory effect of reactions 1,4,5,6,7 (a,b,c,d,e respectively) on population growth of target cells (left side) and normal cells (right side). In the labels in figures, fast denotes target cells and slow denotes normal cells.
For continuous inhibition the inhibition factor (or dosage) can be varied between 0.2 – 0.325 without causing toxicity to the normal cells. For this dosage range, live cell fraction of the normal cells varies from 0.7 to 0.825 and for target cells it is 0.1 to 0.7. These simulation results
are identical to the dose escalation studies conducted in clinical trials to establish safe dose limits for chemotherapeutic drug administration strategies.

APC activation (reaction 5) (Figure B2(c)) is not a target reaction as it appears as equivalently toxic to both target cells and normal cells. At 0.725 dosage, the toxicity of the drug targeting reaction 5 is more dominant than its effectiveness in killing the target cells. A similar observation can be made for reaction 6 (activator protein synthesis) (Figure B2(d)). Hence, both these reactions fail to be good targets when acted alone on body of cells containing both normal and target cells.

Reaction 7 (activator protein degradation) (Figure B2(e)) is a possible target reaction. At the dosage 0.25, the fraction of healthy cells is around 0.8. For higher doses, this fraction is slightly above 1 which is an indication of reduced cell cycle length of normal cells. For target cells, for 0.75 dosage, the target cell fraction is very less and if the dosage is increased up to 0.5, almost all the cells will die. The large negative slope of the time course plot of target cells while the slope for normal cells is close to zero indicates that continuous action of the drug at specific dosages will contribute to the high rate of killing of target cells without affecting normal cells. Since the fraction of healthy cells is close to 0.8 even at 0.25 dosage, the range of drug dosage for continuous inhibition of reaction 7 can be selected as 0.25 – 0.8 (for this dosage, live cell fraction for normal cells is 0.8 - 1.1; for target cells is 0 - 0.2)
APPENDIX C. FORTY THREE COMPONENT MODEL

C.1. Description of the Model and Molecular Mechanism

Stochastic cell cycle model is developed based on the deterministic model presented in (Chen et al., 2004). A brief description of the molecular mechanism proposed in the deterministic version is described here. A single cyclin-dependent kinase (Cdk), cdc28 combining with two families of cyclins, Cln1-3 and Clb1-6 controls the major cell cycle events in the budding yeast. It is approximated that cdc28 is present abundantly and rapidly combines with cyclins as soon as they are synthesized. Cln1-2 play major role in bud formation and their concentration peaks in late G1 phase. Cln3 governs the size of the cell and the initiation of Start event (license to start DNA synthesis). Clb5-6 are responsible for initiating DNA replication whereas Clb3-4 assist in the replication process. At least one of Clb1-Clb2 pair is necessary for completing mitosis. In the model some of these cyclins are lumped together and represented as a single cyclin. Cln2 represents Cln1-2, Clb2 represents Clb1-2, Clb5 represents Clb4-5. Clb3-4 are not included in the model as they are considered to be assisting the activities of other cyclins.

Most of the earlier part of the G1 phase is abundant in proteins (Cdh1, Sic1 and Cdc6) whose role is to keep S/G2/M phase cyclins, Clb5 and Clb2 in low concentrations. Cdh1/APC degrades the cyclins whereas Sic1 and Cdc6 together referred to as cyclin-dependent kinase inhibitors (CKIs), inhibit the activity of cyclins. Cell grows to a critical size in G1 phase to have enough Cln3 and Bck2 that activate the transcription factors SBF and MBF for synthesizing Cln2 and Clb5. Cln2 and Cln3 are not inhibited by Cdh1 and CKI activities. When the abundance of Cln2 reaches sufficient level, it inhibits the activities of Cdh1 and CKIs leading to the synthesis of Clb5. Clb5 and Cln2 together shut off the activities of CKI and Cdh1 and this will allow the synthesis of Clb2 which activates its own transcription factor Mcm1. DNA synthesis is initiated
after Clb5 and Clb2 are reached to sufficient levels. After the cell enters S-phase, synthesis activities of Cln2, Cln3 and Clb5 are turned off and Clb2 drives the cell into mitosis. Clb2 phosphorylates the components of anaphase promoting complex (APC) and stimulates the synthesis of Cdc20 which is crucial for the cell’s exit from mitosis in combination with mitotic exit network (MEN) pathway.

MEN pathway contains proteins Lte1, Bub2, Tem1, Cdc15 and Cdc14 with the ultimate result of releasing Cdc14 in active form from RENT complex. Cdc14 is required for the exit of mitosis. Cdc14 plays role in the activation of CKIs, Cdh1 and inactivation of Cdks. Cdc15 is the end point of the MEN pathway and is responsible for the release of Cdc14. Cdc15 is activated by Tem1. Bub2 is a checkpoint protein which prevents the exit from mitosis if there are defects in the mitotic spindle pole formation. Lte1 is involved in the activation of Tem1.

APC/cdc20 becomes active when Mad2-dependent checkpoint signal is released after the chromosomes are aligned properly and it initiates the exit from mitosis. First, it degrades Pds1 releasing Esp1 prompting the sister chromatid separation. Active Cdc20 promotes degradation of phosphatase PPX that keeps Net1 in its active unphosphorylated form which holds on to Cdc14 forming RENT complex. When PPX is degraded, Net1 gets phosphorylated by Cdc15 releasing Cdc14 which acts against Cdks in the activation of Cdh1 (degrades cyclin Clb1-2) and Swi5 (transcription factor for Cdk-inhibitor Sic1) and returns the cell to G1 phase.

C.2. List of Biochemical Reactions Representing the Cell Cycle Network

The set of biochemical reactions and corresponding reaction propensities and reactant combinations are listed in the Table B2. Different species involved in the model are listed in
Table B3. These are derived from the deterministic model of (Chen et al., 2004). However, modifications are made in the formulation of some of the reactions and reaction propensities.

Production of both Cln3 and Bck2 are taken as continuous variables and proportional to the cell mass in the deterministic model.

\[ [Cln3] = \frac{C_0 D_{n3} m}{J_{n3} + D_{n3} m}; \quad [Bck2] = B_0 m \]

In the stochastic formulation, these expressions are included as reaction propensities for synthesis reactions of Cln3 and Bck2 respectively.

Table B1: some differences in the reaction kinetic expressions between the deterministic and stochastic versions of the budding yeast cell cycle model

<table>
<thead>
<tr>
<th>No.</th>
<th>Reaction</th>
<th>Reaction Propensity</th>
<th>No. of reactant combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Cln3 synthesis</td>
<td>( C_3 = \frac{C_0 D_{n3} m}{J_{n3} + D_{n3} m} )</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( \rightarrow Cln3 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Bck2 synthesis</td>
<td>( C_4 = B_0 m )</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( \rightarrow Bck2 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Cdh1, Net1, Swi5, is synthesized in active form, but degraded in both active and inactive forms.
- Cdc14 is synthesized in active form and degraded in inactive form.
- Cdc20 is synthesized in inactive form.
- The rate expressions for activating [SBF], [MBF],[Mcm1] contain Goldbeter functions that act as switches in the deterministic model. Michaeli’s Menten formulae are used in the stochastic model to make the implementation of switch type activity easier.
### Table B2: List of Reactions used in the stochastic model

<table>
<thead>
<tr>
<th>No.</th>
<th>Reaction</th>
<th>Reaction Propensity, $C_n$</th>
<th>No. of Reactant Combinations, $h_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cln2 degradation</td>
<td>$C_1 = k_{d,n2}$</td>
<td>[Cln2]</td>
</tr>
<tr>
<td>2.</td>
<td>Cln2 synthesis</td>
<td>$C_2 = {k'<em>{s,n2} + k''</em>{s,n2}[SBF]}m$</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Cln3 synthesis</td>
<td>$C_3 = \frac{c_0 D_n m}{n_3 + D_n m}$</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>Bck2 synthesis</td>
<td>$C_4 = B_0 m$</td>
<td>1</td>
</tr>
<tr>
<td>5.</td>
<td>Clb5 synthesis</td>
<td>$C_5 = {k'<em>{s,b5} + k''</em>{s,b5}[MBF]}m$</td>
<td>1</td>
</tr>
<tr>
<td>6.</td>
<td>Clb5 degradation</td>
<td>$C_6 = k'<em>{d,b5} + k''</em>{d,b5}[Cdc20]_a$</td>
<td>[Clb5]</td>
</tr>
<tr>
<td>7.</td>
<td>Inhibition of Clb5 by Sic1</td>
<td>$C_7 = k_{as,b5}$</td>
<td>[Sic1][Clb5]</td>
</tr>
<tr>
<td>8.</td>
<td>Inhibition of Clb5 by Cdc6</td>
<td>$C_8 = k_{as,f5}$</td>
<td>[Cdc6][Clb5]</td>
</tr>
<tr>
<td>9.</td>
<td>Clb2 synthesis</td>
<td>$C_9 = {k'<em>{s,b2} + k'</em>{s,b2}[Mcm1]_a}m$</td>
<td>1</td>
</tr>
<tr>
<td>10.</td>
<td>Clb2 degradation</td>
<td>$C_{10} = k'<em>{d,b2} + k''</em>{d,b2}[Cdh1]<em>a + k''''</em>{d,b2}[Cdc20]_a$</td>
<td>[Clb2]</td>
</tr>
<tr>
<td>11.</td>
<td>Inhibition of Clb2 by Sic1</td>
<td>$C_{11} = k_{as,b2}$</td>
<td>[Clb2][Sic1]</td>
</tr>
<tr>
<td>12.</td>
<td>Inhibition of Clb2 by Cdc6</td>
<td>$C_{12} = k_{as,f2}$</td>
<td>[Clb2][cdc6]</td>
</tr>
<tr>
<td>13.</td>
<td>Activation of promoting factor SBF</td>
<td>$C_{13} = \frac{k_{a,SBF}}{J_{a,SBF} + [SBF]<em>i} {\varepsilon</em>{sbfn2}[Cln2]<em>i + \varepsilon</em>{sbfb5}[Clb5]<em>a + \varepsilon</em>{sbfn3}([Cln3]_i + [Bck2])}$</td>
<td>[SBF]_i</td>
</tr>
<tr>
<td>14.</td>
<td>Inactivation of promoting factor SBF</td>
<td>$C_{14} = \frac{k'<em>{l,SBF} + k''</em>{l,SBF}[Clb2]}{J_{l,SBF} + [SBF]_a}$</td>
<td>[SBF]_a</td>
</tr>
<tr>
<td>15.</td>
<td>Activation of promoting factor Mcm1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
<td>Equation</td>
<td>[Parameter]</td>
<td>(k_{i,j}^{\text{prom}})</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>-------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>(Mcm1)(_i) → (Mcm1)(_a)</td>
<td>(C_{15} = \frac{k_{a,mcm}[Clb2]}{j_{a,mcm} + [Mcm1]_i})</td>
<td>[Mcm1]i</td>
<td></td>
</tr>
<tr>
<td>(Mcm1)(_a) → (Mcm1)(_i)</td>
<td>(C_{16} = \frac{k_{i,mcm}}{j_{i,mcm} + [Mcm1]_a})</td>
<td>[Mcm1]a</td>
<td></td>
</tr>
<tr>
<td>Swi5 synthesis</td>
<td>(C_{17} = k_{s,swi}^{'} + k_{s,swi}^{''}[Mcm1]_a)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Degradation of active Swi5</td>
<td>(C_{18} = k_{d,swi})</td>
<td></td>
<td>[Swi5]a</td>
</tr>
<tr>
<td>Inactivation of CKI promoting factor Swi5</td>
<td>(C_{19} = k_{i,swi}[Clb2])</td>
<td></td>
<td>[Swi5]a</td>
</tr>
<tr>
<td>Degradation of inactive Swi5</td>
<td>(C_{20} = k_{d,swi})</td>
<td></td>
<td>[Swi5]i</td>
</tr>
<tr>
<td>Activation of CKI promoting factor Swi5</td>
<td>(C_{21} = k_{a,swi}[Cdc14]_a)</td>
<td></td>
<td>[Swi5]i</td>
</tr>
<tr>
<td>Sic1 synthesis</td>
<td>(C_{22} = k_{s,c1}^{'} + k_{s,c1}^{''}[Swi5]_a)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cdc6 synthesis</td>
<td>(C_{23} = k_{s,f6}^{'} + k_{s,f6}^{''}[Swi5]_a)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Phosphorylation of Sic1</td>
<td>(C_{24} = k_{d1,c1} + \frac{k_{d2,c1}}{j_{d2,c1} + [Sic1]<em>T}$$\epsilon</em>{c1,n3}[Cln3] + \epsilon_{c1,n2}Cln2 + \epsilon_{c1,b2}Clb2 + \epsilon_{c1,b5}Clnb5 + \epsilon_{c1,k2Bck2})</td>
<td></td>
<td>[Sic1]</td>
</tr>
<tr>
<td>Dephosphorylation of Sic1P</td>
<td>(C_{25} = k_{pp,c1}[Cdc14]_a)</td>
<td></td>
<td>[Sic1P]</td>
</tr>
<tr>
<td>Degradation of Phosphorylated Sic1</td>
<td>(C_{26} = k_{d3,c1})</td>
<td></td>
<td>[Sic1P]</td>
</tr>
<tr>
<td>Dissociation of inactive trimer C2 to Clb2, Sic1</td>
<td>(C_{27} = k_{d1,b2})</td>
<td></td>
<td>[C2]</td>
</tr>
<tr>
<td>Degradation of Clb2 in inactive trimer C2</td>
<td>(C_{28} = C_{10})</td>
<td></td>
<td>[C2]</td>
</tr>
<tr>
<td>Phosphorylation of Sic1 in trimer C2</td>
<td>(C_{29} = C_{24})</td>
<td></td>
<td>[C2]</td>
</tr>
<tr>
<td>Dephosphorylation of Sic1P in trimer C2P</td>
<td>(C_{30} = C_{25})</td>
<td></td>
<td>[C2P]</td>
</tr>
<tr>
<td>Degradation of Sic1P in trimer C2P</td>
<td></td>
<td></td>
<td>126</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>$C2P \rightarrow Clb2$</td>
<td>$C_{31} = C_{26}$</td>
<td>[C2P]</td>
<td></td>
</tr>
<tr>
<td>32. Clb2 degradation in trimer C2P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C2P \rightarrow Sic1P$</td>
<td>$C_{32} = C_{28}$</td>
<td>[C2P]</td>
<td></td>
</tr>
<tr>
<td>33. Dissociation of inactive trimer C5 to Clb5, Sic1</td>
<td>$C_{33} = k_{di,b5}$</td>
<td>[C5]</td>
<td></td>
</tr>
<tr>
<td>$C5 \rightarrow Clb5 + Sic1$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34. Degradation of Clb5 in inactive trimer C5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C5 \rightarrow Sic1$</td>
<td>$C_{34} = C_{6}$</td>
<td>[C5]</td>
<td></td>
</tr>
<tr>
<td>35. Phosphorylation of Sic1 in trimer C5</td>
<td>$C_{35} = C_{24}$</td>
<td>[C5]</td>
<td></td>
</tr>
<tr>
<td>$C5 \rightarrow C5P$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36. Dephosphorylation of Sic1P in trimer C5P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C5P \rightarrow C5$</td>
<td>$C_{36} = C_{25}$</td>
<td>[C5P]</td>
<td></td>
</tr>
<tr>
<td>37. Degradation of Sic1P in trimer C5P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C5P \rightarrow Clb5$</td>
<td>$C_{37} = C_{26}$</td>
<td>[C5P]</td>
<td></td>
</tr>
<tr>
<td>38. Clb5 degradation in trimer C5P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C5P \rightarrow Sic1P$</td>
<td>$C_{38} = C_{6}$</td>
<td>[C5P]</td>
<td></td>
</tr>
<tr>
<td>39. Phosphorylation of Cdc6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Cdc6 \rightarrow Cdc6P$</td>
<td>$C_{39} = k_{d1,f6} + k_{d2,f6} + \frac{v_{f6,n3}[Cln3] + v_{f6,n2Cln2} + v_{f6,b2Clb2} + v_{f6,b5Clb5} + v_{f6,k2Bck2}}{J_{d2,f6} + [Cdc6]_T}$</td>
<td>[Cdc6]</td>
<td></td>
</tr>
<tr>
<td>$[Cdc6]_T = [Cdc6] + [Cdc6P] + [F2] + [F5] + [F2P] + [F5P]$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40. Dephosphorylation of Cdc6P</td>
<td>$C_{40} = k_{pp,f6}[Cdc14]_a$</td>
<td>[Cdc6P]</td>
<td></td>
</tr>
<tr>
<td>41. Degradation of Phosphorylated Cdc6</td>
<td>$C_{41} = k_{d3,f6}$</td>
<td>[Cdc6P]</td>
<td></td>
</tr>
<tr>
<td>$Cdc6P \rightarrow Cdc6$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42. Dissociation of inactive trimer F2 to Clb2, Cdc6</td>
<td>$C_{42} = k_{di,f2}$</td>
<td>[F2]</td>
<td></td>
</tr>
<tr>
<td>$F2 \rightarrow Clb2 + Cdc6$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43. Degradation of Clb2 in inactive trimer F2</td>
<td>$C_{43} = C_{10}$</td>
<td>[F2]</td>
<td></td>
</tr>
<tr>
<td>$F2 \rightarrow Cdc6$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44. Phosphorylation of Cdc6 in trimer F2</td>
<td>$C_{44} = C_{39}$</td>
<td>[F2]</td>
<td></td>
</tr>
<tr>
<td>$F2 \rightarrow F2P$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45. Dephosphorylation of Cdc6P in trimer F2P</td>
<td>$C_{45} = C_{40}$</td>
<td>[F2P]</td>
<td></td>
</tr>
<tr>
<td>$F2P \rightarrow F2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46. Degradation of Cdc6P in trimer F2P</td>
<td>$C_{46} = C_{26}$</td>
<td>[F2P]</td>
<td></td>
</tr>
<tr>
<td>$F2P \rightarrow Clb2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47. Clb2 degradation in trimer F2P</td>
<td>$C_{47} = C_{43}$</td>
<td>[F2P]</td>
<td></td>
</tr>
<tr>
<td>$F2P \rightarrow Cdc6P$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

127
<table>
<thead>
<tr>
<th></th>
<th>Dissociation of inactive trimer F5 to Clb5, Cdc6</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>( F5 \rightarrow Clb5 + Cdc6 ) ( C_{48} = k_{d1, f6} ) [F5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Degradation of Clb5 in inactive trimer F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>( F5 \rightarrow Cdc6 ) ( C_{49} = C_6 ) [F5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Phosphorylation of Cdc6 in trimer F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>( F5 \rightarrow F5P ) ( C_{50} = C_{39} ) [F5]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Dephosphorylation of Cdc6P in trimer F5P</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>( F5P \rightarrow F5 ) ( C_{51} = C_{40} ) [F5P]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Degradation of Cdc6P in trimer F5P</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>( F5P \rightarrow Clb5 ) ( C_{52} = C_{41} ) [F5P]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Clb5 degradation in trimer F5P</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>( F5P \rightarrow Cdc6P ) ( C_{53} = C_6 ) [F5P]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Inactivation of hypothetical protein IE (may be the phosphorylated form of APC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>((IEP)<em>a \rightarrow (IEP)<em>i ) ( C</em>{54} = \frac{k</em>{l, iep}}{f_{l, iep} + [IEP]_a} ) [IEP]_a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Activation of hypothetical protein IE</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>((IEP)<em>i \rightarrow (IEP)<em>a ) ( C</em>{55} = \frac{k</em>{a, iep}[Clb2]}{f_{a, iep} + [IEP]_i} ) [IEP]_i</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Synthesis of Cdc20 (in inactive form)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>((Cdc20)<em>i \rightarrow (Cdc20)<em>i ) ( C</em>{56} = k'</em>{s, 20} + k''_{s, 20}[Mcm1]_a ) 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Degradation of inactive-Cdc20</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>((Cdc20)<em>i \rightarrow ) ( C</em>{57} = k_{d, 20} ) [Cdc20]_i</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Activation of Cdc20</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>((Cdc20)<em>i \rightarrow (Cdc20)<em>a ) ( C</em>{58} = k'</em>{a, 20} + k''_{a, 20}[IEP]_a ) [Cdc20]_i</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Inactivation of Cdc20</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>((Cdc20)_a \rightarrow (Cdc20)<em>i ) ( C</em>{59} = k_m ad2 ) [Cdc20]_a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Degradation of active-Cdc20</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>((Cdc20)<em>a \rightarrow ) ( C</em>{60} = C_{57} ) [Cdc20]_a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Synthesis of Cdh1 (in active form)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>((Cdh1)<em>a \rightarrow ) ( C</em>{61} = k_{s, cdh} ) 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Degradation of active-Cdh1</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>((Cdh1)<em>a \rightarrow ) ( C</em>{62} = k_{d, cdh} ) [Cdh1]_a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Inactivation of Cdh1</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>((Cdh1)<em>a \rightarrow ) ( C</em>{63} = k_{d, cdh} ) [Cdh1]_a</td>
</tr>
<tr>
<td>Reaction</td>
<td>Rate Constant</td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
</tr>
<tr>
<td>(Cdh1)$_a$ → (Cdh1)$_i$</td>
<td>$C_{63} = \frac{1}{\frac{1}{I_{i,cdh} + [Cdh1]<em>a} + k</em>{i,cdh}'} + k_{i,cdh}''[Cln3] + \epsilon_{cdh,n3}[Cln3] + \epsilon_{cdh,n2}[Cln2] + \epsilon_{cdh,b2}[Clb2] + \epsilon_{cdh,b5}[Clb5]}$</td>
</tr>
<tr>
<td>(Cdh1)$_i$ → (Cdh1)$_a$</td>
<td>$C_{64} = \frac{k_{a,cdh}' + \epsilon_{a,cdh}'}{I_{a,cdh} + [Cdh1]_i}$</td>
</tr>
<tr>
<td>(Tem1)$_a$ → (Tem1)$_i$</td>
<td>$C_{66} = \frac{k_{bub2}}{I_{i,tem} + [Tem1]_a}$</td>
</tr>
<tr>
<td>(Tem1)$_i$ → (Tem1)$_a$</td>
<td>$C_{67} = \frac{k_{ite1}}{I_{a,tem} + [Tem1]_i}$</td>
</tr>
<tr>
<td>(Cdc15)$_i$ → (Cdc15)$_a$</td>
<td>$C_{68} = k_{a,15}'[Tem1]<em>i + \epsilon</em>{a,15}'[Tem1]<em>a + \epsilon</em>{a,15}''[Cdc15]_a$</td>
</tr>
<tr>
<td>(Cdc15)$_a$ → (Cdc15)$_i$</td>
<td>$C_{69} = k_{i,15}$</td>
</tr>
<tr>
<td>(Cdc14)$_a$ → (Cdc14)$_i$</td>
<td>$C_{70} = k_{s,14}$</td>
</tr>
<tr>
<td>(Cdc14)$_i$ → RENT</td>
<td>$C_{71} = k_{d,14}$</td>
</tr>
<tr>
<td>(Cdc14)$_a$ + Net1 → RENT</td>
<td>$C_{72} = k_{as,rent}$</td>
</tr>
<tr>
<td>(Net1P)$_a$ + Net1P → RENTP</td>
<td>$C_{73} = k_{as,rentp}$</td>
</tr>
<tr>
<td>Net1 synthesis</td>
<td>$C_{74} = k_{s,net}$</td>
</tr>
<tr>
<td>Net1 degradation</td>
<td>$C_{75} = k_{d,net}$</td>
</tr>
<tr>
<td>Phosphorylation of Net1</td>
<td></td>
</tr>
<tr>
<td>Step</td>
<td>Reaction</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
</tr>
<tr>
<td>77.</td>
<td>Dephosphorylation of Net1P</td>
</tr>
<tr>
<td>78.</td>
<td>Degradation of Net1P</td>
</tr>
<tr>
<td>79.</td>
<td>Degradation of Net1 in RENT complex</td>
</tr>
<tr>
<td>80.</td>
<td>Degradation of Net1P in RENTP complex</td>
</tr>
<tr>
<td>81.</td>
<td>Dissociation of RENT complex</td>
</tr>
<tr>
<td>82.</td>
<td>Dissociation of RENTP complex</td>
</tr>
<tr>
<td>83.</td>
<td>Degradation of Cdc14 in RENT complex</td>
</tr>
<tr>
<td>84.</td>
<td>Degradation of Cdc14 in RENTP complex</td>
</tr>
<tr>
<td>85.</td>
<td>Dephosphorylation of Net1P in RENTP complex</td>
</tr>
<tr>
<td>86.</td>
<td>Phosphorylation of Net1 in RENT complex</td>
</tr>
<tr>
<td>87.</td>
<td>PPX synthesis</td>
</tr>
<tr>
<td>88.</td>
<td>PPX degradation</td>
</tr>
<tr>
<td>89.</td>
<td>Pds1 synthesis</td>
</tr>
<tr>
<td>90.</td>
<td>Association of Pds1 and Esp1 to form</td>
</tr>
</tbody>
</table>
Dissociation of PE to Pds1 and Esp1

\[ Pds1 + Esp1 \rightarrow PE \]

\[ C_{90} = k_{as,esp} \]

[Pds1][Esp1]

Degradation of Pds1 in PE

\[ PE \rightarrow Pds1 + Esp1 \]

\[ C_{91} = k_{dt,esp} \]

[PE]

Pds1 degradation

\[ PE \rightarrow Esp1 \]

\[ C_{92} = k'_{d,pds} + k''_{d,pds}[Cdc20]_a + k'''_{d,pds}[Cdhl]_a \]

[PE]

\[ Pds1 \rightarrow [Esp1]_T = [Esp1] + [PE] \]

\[ C_{93} = C_{92} \]

[Pds1]

Table B3: List of variables in the model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(1)</td>
<td>Cln2</td>
<td>Cyclin involved in budding</td>
</tr>
<tr>
<td>N(2)</td>
<td>Cln3</td>
<td>Cyclin initiating start event</td>
</tr>
<tr>
<td>N(3)</td>
<td>Bck2</td>
<td>Protein initiating start event</td>
</tr>
<tr>
<td>N(4)</td>
<td>Clb5</td>
<td>Cyclin appearing in the late G1, involved in the synthesis of DNA</td>
</tr>
<tr>
<td>N(5)</td>
<td>Sic1</td>
<td>Stoichiometric inhibitor of Clb2, Clb5</td>
</tr>
<tr>
<td>N(6)</td>
<td>C5</td>
<td>Trimer of Clb5/Cdk and Sic1</td>
</tr>
<tr>
<td>N(7)</td>
<td>Cdc6</td>
<td>Stoichiometric inhibitor of Clb2, Clb5</td>
</tr>
<tr>
<td>N(8)</td>
<td>F5</td>
<td>Trimer of Clb5/Cdk and Cdc6</td>
</tr>
<tr>
<td>N(9)</td>
<td>Clb2</td>
<td>Cyclin essential for exit from mitosis, present in S/G2/M phase</td>
</tr>
<tr>
<td>N(10)</td>
<td>C2</td>
<td>Trimer of Clb2/Cdk and Sic1</td>
</tr>
<tr>
<td>N(11)</td>
<td>F2</td>
<td>Trimer of Clb2/Cdk and Cdc6</td>
</tr>
<tr>
<td>N(12)</td>
<td>(SBF)\textsubscript{i}</td>
<td>Transcription factor for Cln2, in inactive form</td>
</tr>
<tr>
<td>N(13)</td>
<td>(SBF)\textsubscript{a}</td>
<td>Transcription factor for Cln2, in active form</td>
</tr>
<tr>
<td>N(14)</td>
<td>(Mcm1)\textsubscript{i}</td>
<td>Transcription factor for Clb2, Cdc20, Swi5 in inactive form</td>
</tr>
<tr>
<td>N(15)</td>
<td>(Mcm1)\textsubscript{a}</td>
<td>Transcription factor for Clb2, Cdc20, Swi5 in active form</td>
</tr>
<tr>
<td>N(16)</td>
<td>(Swi5)\textsubscript{i}</td>
<td>Transcription factor for Sic1 and Cdc6, in inactive form</td>
</tr>
<tr>
<td>N(17)</td>
<td>(Swi5)\textsubscript{a}</td>
<td>Transcription factor for Sic1 and Cdc6, in active form</td>
</tr>
<tr>
<td>N(18)</td>
<td>Sic1P</td>
<td>Phosphorylated form of Sic1</td>
</tr>
<tr>
<td>N(19)</td>
<td>C2P</td>
<td>Trimer of Clb2/Cdk and Sic1P</td>
</tr>
<tr>
<td>N(20)</td>
<td>C5P</td>
<td>Trimer of Clb5/Cdk and Sic1P</td>
</tr>
<tr>
<td>N(21)</td>
<td>Cdc6P</td>
<td>Phosphorylated form of Cdc6</td>
</tr>
<tr>
<td>N(22)</td>
<td>F2P</td>
<td>Trimer of Clb2/Cdk and Cdc6P</td>
</tr>
<tr>
<td>N(23)</td>
<td>F5P</td>
<td>Trimer of Clb5/Cdk and Cdc6P</td>
</tr>
<tr>
<td>N(24)</td>
<td>(IEP)\textsubscript{i}</td>
<td>Intermediary enzyme, a hypothetical protein involved in activating Cdc20, in inactive form</td>
</tr>
<tr>
<td>N(25)</td>
<td>(IEP)\textsubscript{a}</td>
<td>Intermediary enzyme, a hypothetical protein involved in activating Cdc20, in active form</td>
</tr>
<tr>
<td>N(26)</td>
<td>(Cdc20)\textsubscript{a}</td>
<td>Activator of the APC; protein involved in Clb2, Clb5 and Pds1 proteolysis, and required for exit from mitosis, in active form</td>
</tr>
<tr>
<td>N(27)</td>
<td>(Cdc20)\textsubscript{i}</td>
<td>Inactive form of Cdc20</td>
</tr>
<tr>
<td>N(28)</td>
<td>(Cdhl)\textsubscript{a}</td>
<td>Activator of the APC; protein involved in Clb2 and Pds1 proteolysis, in active form</td>
</tr>
</tbody>
</table>
The current model is described by 93 reactions consisting of 121 parameters, 43 state variables including cell mass, as shown in Tables B2 &B3. Cell progresses through various phases of cell cycle as the trigger values are satisfied.

For the cell to progress through the cell cycle, following events should occur in the order. Each event is described with the corresponding trigger variable in the parenthesis.

1. Origin re-licensing (when Clb2+Clb5 drops below threshold) – cell will be ready for next round of replication

2. Origin activation (when Clb2+Clb5 subsequently raises above threshold) – DNA synthesis will start

3. Entry into mitosis when spindle is aligned (when Clb2 rises above threshold)

4. Esp1 activation (when Esp1 rises above threshold, after Pds1 is degraded) – cell is ready for division and waits for the Clb2 level to drop down below a threshold value
5. Nuclear division (when Clb2 drops below a threshold) – cell division occurs

Another condition that is included in the model is the provision for cell death occurrence.

When the cell mass increases above a threshold value, cell is considered dead.

C.3. Modules for the VB code

Figure C1: VB form for supplying the Initial Conditions and Trigger Values
Figure C2: Visual Basic form for Main Window

Figure C3: Visual Basic form for supplying the output parameters
### Reaction Rate Constants

#### CLN2
- $k_{5}^{2}$ / $n^{2} = 0$
- $k_{6}^{2} / n^{2} = 0.15$
- $k_{7}^{2} / n^{2} = 0.12$

#### CLN3 - BCK2
- $k_{10}$ / $B = 0.4$
- $k_{12}$ / $D = 1$
- $k_{13} / B = 0.054$
- $k_{14} / A = 0$

#### CLN5
- $k_{10}^{5} / B = 0.0006$
- $k_{11}^{5} / B = 0.01$
- $k_{12}^{5} / B = 0.16$
- $k_{13}^{5} / C = 0.06$
- $k_{14}^{5} / C = 0.01$
- $k_{15}^{5} / C = 0.01$

#### CDC28
- $k_{6}^{28} / A = 0.006$
- $k_{13}^{28} / B = 0.06$
- $k_{14}^{28} / C = 0.3$
- $k_{15}^{28} / D = 0.05$
- $k_{16}^{28} / E = 0.2$
- $k_{17}^{28} / F = 0.01$

#### SIC1
- $k_{25} / C = 0.012$
- $k_{26} / D = 0.12$
- $k_{27} / E = 0.05$

### CDH1
- $k_{6}^{4} / A = 0.01$
- $k_{10} / A = 0.25$
- $k_{11} / A = 0.01$
- $k_{12} / A = 0.01$
- $k_{13} / A = 0.01$
- $k_{14} / A = 0.01$

### CDC14 - NET1
- $k_{10} / B = 0.2$
- $k_{11} / B = 0.02$
- $k_{12} / B = 0.03$
- $k_{13} / B = 0.05$
- $k_{14} / B = 0.05$
- $k_{15} / B = 0.05$

### Parameters

Single cell growth rate parameter: 0.00762
Figure C4: Visual Basic form for supplying the Parameters for reactions

C.4. Approach for the Selection of Initial Conditions and Trigger Events

For the model to be executed, initial conditions and trigger values should be selected properly along with the parameter values to give oscillations. Ghaemmaghami et al. (Ghaemmaghami et al., 2003) and Cross et al. (Cross et al., 2002) have published the results of their experiments on budding yeast cell cycle network. Their results contain average copies of
different proteins (molecular concentrations) during the cell cycle of a haploid cell growing in asynchronous culture.

Parameters used in the deterministic model of Chen et al. (Chen et al., 2004) do not have units. Matlab codes for the deterministic model are obtained from Prof. Cross’s research group. Using the codes, it is possible to obtain plots for the expression of different proteins during the cell cycle. With slight modifications to the code, average concentrations can be found out in a given cell cycle. Molecular concentrations during different phases of the cell cycle can be estimated by equating the dimensionless average concentrations to the average molecular concentrations. It is proposed that a reasonable estimate of trigger values for the six different transitions in the model can be obtained using this approach.

Assigning units to the parameters is discussed in Chen et al. (Chen et al., 2004) which might be helpful in giving the initial guess of the parameters.

C.5. Trigger events in the VB code

In the deterministic version of the model, three rate constants $k_{bub2}$, $k_{lte1}$, $k_{mad2}$ are designed to have two different values, a higher and a lower value depending on which phase of the cell cycle the cell is in. When there is spindle defect, protein Bub2 will inactivate Tem1 in MEN pathway. Protein Lte1 will try to activate Tem1. Also, when the kinetochores are unattached, Mad2 dependent checkpoint signal will inactivate Cdc20.

- **Replication**

  If Relicence And (N(3) + N(4)) > Replication_Trigger Then
  
  Replication = True
  
  Relicence = False
k117 = kbub2_high
k63 = kmad2_high
End If

When the trigger for replication is satisfied, $k_{bub2}$ and $k_{mad2}$ are kept high to prevent the activation of mitotic exit network.

- **Mitosis**

  If Replication And N(3) > Mitosis_Trigger Then
  Mitosis = True
  Replication = False
  k117 = kbub2_low 'decreased kbub2
  k63 = kmad2_low 'decreased kmad2
  k118 = klte1_high 'increased klte1
  End If

  When the trigger for mitosis is satisfied, $k_{bub2}$ and $k_{mad2}$ are kept low and $k_{lte1}$ is kept high to activate the mitosis exit network.

- **Division**

  If esp1 And N(3) < Division_Triger Then
  Division = True
  esp1 = False
  k118 = klte1_low ' klte1 is decreased
  End If

  After the division occurred, $k_{lte1}$ is again kept low to stop the mitotic exit network activities.
APPENDIX D. TEN COMPONENT MODEL

D.1. Description of the model

Based on the learning from building small model of four cell cycle components, using stochastic formulation, the next step is to extend the understanding to build models that include more details and components of the cell cycle reaction network. In that direction, the stochastic formulation is extended to a cell cycle model containing ten components with many interactions among them. It is developed earlier by Hjortso (unpublished work) based on a deterministic model of Tyson and Novak (Tyson, 2000) and is presented here along with the modifications on the original stochastic model.

A schematic of the cell cycle reaction network which is redrawn based on the model of Tyson and Novak (Tyson, 2000) is shown in Fig. D1.

Various features of the cell cycle model are described as followed. Early in the G1 phase, the S-G2-M cyclin, CycB is low because it is rapidly degraded in the presence of active Cdh1. CycB is required to initiate DNA replication. Cdh1 is abundant in the early G1 phase. Cyclin-dependent kinase inhibitor (CKI), which is also abundant in the early G1 phase keeps small amount of active CycB inactive. As cell mass increases, it results in an increased rate of formation of starter kinase (SK) transcription factor, TF. This in turn results in an increase of starter kinase, SK. The starter kinase causes the CKI to be phosphorylated and degraded driving the equilibrium between CKI, CycB and the Trimer (which is actually a dimer of Cdk/CycB and CKI) towards free CycB. Free CycB phosphorylates CKI and inactivates Cdh1 thus further increasing the CycB concentration and activates degradation of TF causing the concentration of the starter kinase to drop. CycB activates the synthesis of Cdc20 in its inactive form. Cdc20 is a
Figure D1: Reaction network of budding cell cycle model. Active compounds are shown as circles or large pie pieces, inactive compounds as start and degraded compounds as small pieces. Reactions are shown by solid arrows, activation by broken arrows. (Hjortso, unpublished work)

protein that activates degradation reaction of CycB and also activates Cdh1 from its inactive state thus driving the cell towards division. Finally, CycB causes phosphorylation of intermediary enzyme, IE, leading to the formation of IEP (P for phosphorylation) which activates Cdc20. As CycB drops below a threshold value, the cell divides and will return to the initial state with low CycB and high Cdh1, required for starting another cell division cycle.

The model is formulated in the same way as the four component model presented earlier using stochastic kinetics approach. Various variables in the model are listed in the Table 1. The variables are the number of molecules of the different molecular species of the reaction network and the continuous variable, cell mass. The molecular species are all represented by non-negative integer values.
Table D1: List of variables in the model. Intermediary enzyme, IE is not used in the model explicitly

<table>
<thead>
<tr>
<th>Position</th>
<th>Symbol</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SK</td>
<td>Starter kinase. Dimer of Cdk, called Cln2 in budding yeast, Cig2 in fission yeast and Cyclin D in vertebrates.</td>
</tr>
<tr>
<td>2</td>
<td>TF</td>
<td>Transcription factor for SK</td>
</tr>
<tr>
<td>3</td>
<td>CKI</td>
<td>Cyclin-dependent kinase inhibitor. Binds to Cycb to form Trimer. A quasi steady state equilibrium is assumed between this compound, CycB and Trimer.</td>
</tr>
<tr>
<td>4</td>
<td>CycB</td>
<td>S-G2-M phase cyclin. Binds to CKI to form Trimer. A quasi steady state equilibrium is assumed between this compound, CycB and Trimer.</td>
</tr>
<tr>
<td>5</td>
<td>Cdh1</td>
<td>The active or unphosphorylated form of Cdh1</td>
</tr>
<tr>
<td>6</td>
<td>Cdc20I</td>
<td>Inactive form of Cdc20</td>
</tr>
<tr>
<td>7</td>
<td>Cdc20A</td>
<td>Active form of Cdc20</td>
</tr>
<tr>
<td>8</td>
<td>IEP</td>
<td>Active form of intermediary enzyme</td>
</tr>
<tr>
<td>9</td>
<td>Trimer</td>
<td>Compound formed when CKI binds to CycB (which more accurately is a dimer of CyclinB and Cdk. Thus the same trimer). A quasi steady state equilibrium is assumed between CKI, CycB and trimer.</td>
</tr>
<tr>
<td>10</td>
<td>m</td>
<td>Cell mass</td>
</tr>
</tbody>
</table>

The cell cycle network is converted into a set of biochemical reactions associated with propensities which is shown in Table D2.

Table D2: List of reactions in the 10 component model

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Description</th>
<th>Stoichiometry</th>
<th>Propensity, $C_n$</th>
<th>$h_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Constant rate of formation of CycB: $\rightarrow CycB$</td>
<td>$k_1$</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Degradation of CycB. Three first order mechanisms: Unactivated, activated by cdh1 and activated by Cdc20A</td>
<td>$CycB \rightarrow k_2' + k_2''[Cdh1] + k_2''[Cdc20]_A$</td>
<td>$[CycB]$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Formation and activation of Cdh1</td>
<td>$\frac{(k_3' + k_3''[Cdc20]<em>A)[Cdh1]</em>{max} - [Cdh1]}{J_3 + [Cdh1]_{max} - [Cdh1]}$</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Inactivation, i.e. phosphorylation of Cdh1</td>
<td>$Cdh1 \rightarrow \frac{k_4'SK + k_4'[CycB]}{J_4 + [Cdh1]}$</td>
<td>$[Cdh1]$</td>
<td></td>
</tr>
<tr>
<td>Step</td>
<td>Reaction</td>
<td>Reaction Direction</td>
<td>Rate Expression</td>
<td>Concentration</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>-------------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>5</td>
<td>Formation and inactivation of Cdc20</td>
<td>$\rightarrow Cdc20_I$</td>
<td>$k_5' + k_5'' \frac{(m[CycB])^n}{J_5^m + (m[CycB])^n}$</td>
<td>$1$</td>
</tr>
<tr>
<td>6</td>
<td>Degradation of inactivation of Cdc20</td>
<td>$Cdh20I \rightarrow$</td>
<td>$k_6$</td>
<td>$[Cyc20]_I$</td>
</tr>
<tr>
<td>7</td>
<td>Formation of active Cdc20 from inactive Cdc20</td>
<td>$Cdc20_I \rightarrow Cdc20_A$</td>
<td>$\frac{k_7[IEP]}{J_7 + [Cdc20]_I}$</td>
<td>$[Cyc20]_I$</td>
</tr>
<tr>
<td>8</td>
<td>Formation of active Cdc20 from inactive Cdc20 from active Cdc20</td>
<td>$Cdc20_A \rightarrow Cdc20_I$</td>
<td>$\frac{k_8[Mad]}{J_8 + [Cdc20]_A}$</td>
<td>$[Cyc20]_A$</td>
</tr>
<tr>
<td>9</td>
<td>Formation of active IEP</td>
<td>$\rightarrow IEP$</td>
<td>$k_9m[CycB][(IEP)_{max} - [IEP]]$</td>
<td>$1$</td>
</tr>
<tr>
<td>10</td>
<td>Inactivation of IEP</td>
<td>$IEP \rightarrow$</td>
<td>$k_{10}$</td>
<td>$[IEP]$</td>
</tr>
<tr>
<td>11</td>
<td>Formation of CKI</td>
<td>$\rightarrow CKI$</td>
<td>$k_{11}$</td>
<td>$1$</td>
</tr>
<tr>
<td>12</td>
<td>Degradation of CKI through three first order mechanism</td>
<td>$CKI \rightarrow$</td>
<td>$k_{12} + k_{12}''m[CycB]$</td>
<td>$[CKI]$</td>
</tr>
<tr>
<td>13</td>
<td>Formation of SK</td>
<td>$\rightarrow SK$</td>
<td>$k_{13}' + k_{13}''[TF]$</td>
<td>$1$</td>
</tr>
<tr>
<td>14</td>
<td>Degradation of SK</td>
<td>$SK \rightarrow$</td>
<td>$k_{14}$</td>
<td>$[SK]$</td>
</tr>
<tr>
<td>15</td>
<td>Formation of TF</td>
<td>$\rightarrow TF$</td>
<td>$\frac{(k_{15}'m + k_{15}''[SK])\cdot([TF]<em>{max} - [TF])}{J</em>{15} + [TF]_{max} - [TF]}$</td>
<td>$1$</td>
</tr>
<tr>
<td>16</td>
<td>Degradation of TF</td>
<td>$TF \rightarrow$</td>
<td>$\frac{k_{16}' + k_{16}''m[CycB]}{J_{16} + [TF]}$</td>
<td>$[TF]$</td>
</tr>
<tr>
<td>17</td>
<td>Degradation of active Cdc20</td>
<td>$Cdc20_A \rightarrow$</td>
<td>$k_{17}$</td>
<td>$[Cdc20]_A$</td>
</tr>
</tbody>
</table>

CycB, CKI and Trimer are assumed to be in equilibrium and this is incorporated by the quasi-steady state assumption which is handled as follows. Suppose that at $t_0$, a reaction occurs that changes the number of molecules of CKI and CycB. After this reaction has occurred, the state of the system is given by the molecular numbers.
which obviously do not satisfy the equilibrium assumption. The molecular numbers after equilibrium has been attained are determined from the equilibrium equation and two stoichiometric balances

\[
[\text{Trimer}] = K_{eq} [\text{CKI}] [\text{CycB}]
\]

\[
[\text{Trimer}]_0 - [\text{Trimer}] = [\text{CKI}] - [\text{CKI}]_0
\]

\[
[\text{Trimer}]_0 - [\text{Trimer}] = [\text{CycB}] - [\text{CycB}]_0
\]

which have the solution

\[
[\text{CycB}] = \frac{K_{eq}([\text{CKI}]_0 - [\text{CycB}]_0) - 1 + \sqrt{\phi}}{2K_{eq}}
\]

\[
[\text{CKI}] = [\text{CKI}]_0 + [\text{CycB}] - [\text{CycB}]_0
\]

\[
[\text{Trimer}] = [\text{Trimer}]_0 + [\text{CycB}]_0 - [\text{CycB}]
\]

where

\[
\phi = 1 + 2K_{eq} ([\text{CKI}]_0 + [\text{CycB}]_0) + K_{eq}^2 ([\text{CKI}]_0 - [\text{CycB}]_0)^2 + 4K_{eq} [\text{Trimer}]_0
\]

The solution for the number of molecular species obtained from the above relation is not an integer. One approximation included is rounding off the solution to the nearest integer. This is obviously incorrect since the species involved in the quasi-steady state equilibrium must satisfy some probability distribution according to the chemical master equation. As a first step, model is simulated using this approximation.

The parameter values used in the simulation are shown in Table D3. The simulation of the cell cycle model using Gillespie’s algorithm resulted in cell cycle oscillations and one such result showing the time course trajectories of different state variables including the cell mass is shown in Fig. D2.
Table D3: Parameter values and Trigger values used in the simulations

<table>
<thead>
<tr>
<th>Parameter and Trigger Values for the 10 component model</th>
</tr>
</thead>
<tbody>
<tr>
<td>k1</td>
</tr>
<tr>
<td>k2_1</td>
</tr>
<tr>
<td>k2_11</td>
</tr>
<tr>
<td>k2_111</td>
</tr>
<tr>
<td>k3_1</td>
</tr>
<tr>
<td>k3_11</td>
</tr>
<tr>
<td>k4_1</td>
</tr>
<tr>
<td>k4_11</td>
</tr>
<tr>
<td>k5_1</td>
</tr>
<tr>
<td>k5_11</td>
</tr>
<tr>
<td>k6</td>
</tr>
<tr>
<td>k7</td>
</tr>
<tr>
<td>k8</td>
</tr>
<tr>
<td>k9</td>
</tr>
<tr>
<td>k10</td>
</tr>
<tr>
<td>k11</td>
</tr>
<tr>
<td>k12_1</td>
</tr>
<tr>
<td>k12_11</td>
</tr>
<tr>
<td>k12_111</td>
</tr>
<tr>
<td>k13_1</td>
</tr>
<tr>
<td>k13_11</td>
</tr>
<tr>
<td>k14</td>
</tr>
<tr>
<td>k15_1</td>
</tr>
</tbody>
</table>
D.2. Insilico evolution of 10 component model

The insilico evolution simulations are run for two cases for the 10 component model with the quasi-steady state assumption described earlier.

- Binary fission, with mother and daughter getting same mass at the time of division
- A more refined approach where mother attains a mass close to critical cell mass and daughter gets the remaining. Start event will not occur until, the cell mass reaches a value equal to the critical cell mass

Simulation results for the case of unequal division for a mutation rate of 0.2 are presented in Fig. D3.
Figure D3: Insilico evolution simulations for the 10 component model
General observations from the simulations are

- There is no convergence in the cell mass
- The average number of trimer molecules is continuously increasing with each iteration
- Execution times have increased drastically with the number of iterations
- Some of the parameters have approached values close to zero and some have moved towards very high values
- Starter Kinase (SK) molecules kept on increasing as the number of iterations in the insilico evolutions has increased.

The rate parameters corresponding to the formation, degradation of TF for the SK and also, the formation of SK reaction have attained very high values (Reactions 15, 16, 13 respectively). This resulted in very high values for the number of starter kinase (SK) molecules as the iterations are continued longer and the execution time of the algorithm also increased proportionately.
The insilico evolution results did not give any convergence values for cell mass. The only change observed is the increase in the parameters corresponding to the formation and degradation of SK, TF.

The results of the cell cycle model are analyzed and it is observed that the number of trimer molecules in a cell have gradually increased as the number of cell divisions increased for the same set of parameters and also cell mass has increased.

It is suspected that the problem might be in the quasi-steady state assumption.

Hence, the handling of QSSA in the model is reconsidered.

D.3. Original Stochastic Algorithm and Simulation of the 10 Component Model

The equilibrium reaction is re-written as two separate reactions and the resulting reaction set is simulated using stochastic simulation algorithm of Gillespie with high propensity values for the two equilibrium reactions. Hence, the two reactions corresponding to the equilibrium occur very fast and thus have the chance of being fired frequently than the other reactions which are slow reactions. However, the dynamics of the system can be described only if large numbers of slow reactions have occurred.

As expected, the computational time is wasted by simulating the fast reactions most frequently and the simulations took long time to obtain the time course of different cell cycle proteins. The two additional reactions along with their propensities are shown in the Table D4. The resulting cell cycle oscillations are shown in the Fig. D4.
Table D4: Two reactions added to the stochastic chemical kinetic model, by avoiding the quasi-steady state assumption

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Description</th>
<th>Stoichiometry</th>
<th>Propensity, $C_n$</th>
<th>$h_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Formation of Trimer</td>
<td>$\text{CycB} + \text{CKI} \rightarrow \text{Trimer}$</td>
<td>$k_{18}$</td>
<td>$[\text{CKI}][\text{CycB}]$</td>
</tr>
<tr>
<td>19</td>
<td>Dissociation of Trimer</td>
<td>$\text{Trimer} \rightarrow \text{CycB} + \text{CKI}$</td>
<td>$k_{19}$</td>
<td>$[\text{Trimer}]$</td>
</tr>
</tbody>
</table>

Figure D4: the simulation results for the 19 reactions. The execution time is 80832 sec for three completed cell cycles. (10 time units in the model)

D.4. Slow Scale Stochastic Simulation

It is thought that the QSSA assumption needs to be reconsidered. Cao et al. (2005) proposed a slow-scale stochastic simulation approach to handle the reactions involving slow and fast reactions occurring on widely different time scales. The description of the approach and its application to the 10 component model is presented here.

The reactions are classified into slow and fast reactions depending on the reaction propensities. The reactions for which propensities are usually much larger than those the other
reactions are fast reactions and the rest are slow reactions. Reacting species are also classified as slow and fast. Any species whose population gets changed by the occurrence of fast reaction is defined as fast species and the species that does not get changed by the fast reaction is defined as slow species.

The fast and slow reaction propensity functions depend in general on both the fast and slow species which are represented as:

\[ a^f_j(x) = a^f_j(x^f, x^s), j = 1, ..., R_f \]

\[ a^s_j(x) = a^s_j(x^f, x^s), j = 1, ..., R_s \]

\( x \) is the generic representation of the state of the system which contains slow \( (x^s) \) variables and fast variables \( (x^f) \).

\( R_f \) is the number of fast reactions and \( R_s \) is the number of slow reactions.

According to slow-scale SSA, if two conditions are satisfied, the state of the system evolution can be described in terms of the occurrence of slow reactions alone. Those are,

- The time scales of fast reactions are very small compared to the time scales of slow reactions. Then, a large number of fast reactions occur between two successive slow reactions.
- The fast variables reach a steady state between the occurrence of two slow reactions. When a slow reaction occurs, state of the system is updated and this change in the state of the system affects the propensities of fast reactions. As a result, many fast reactions occur and generally, a stable state is reached before another slow reaction occurs.
If the above conditions are satisfied, slow reactions and fast reactions can be considered as two discrete Markov processes occurring on different time scales.

Fast reactions follow a discrete Markov process with slow reactions turned off. The stable state of the system after a series of fast reactions is obtained by solving the steady state CME. Steady state solutions of CME are available for simple reactions as described in McQuarre (McQuarre, 1967).

The slow reactions also follow a discrete Markov process. The propensities required for simulating the slow reactions are calculated based on the weighted average of the propensities of the steady state distribution of states obtained from the solution of CME for fast reactions.

**D.5. Application of the Slow-Scale SSA to the Ten Component Model**

The steps of the algorithm are explained by using the example of the 10 component model.

1. Initialize the state of the system as \((x_0^f, x_0^s)\) in terms of fast and slow variables.

   For the 10 component model,
   \[
   x^f = [CycB, CKI, Trimer] \\
   x^s = [SK, TF, Cdh1, Cdc20l, Cdc20a, IEP]
   \]

2. Calculate the propensity function \(\bar{a}_{ij}^s(x^f, x^s)\) for each slow reaction \((R_j)\). \(\bar{a}_{ij}^s(x^f, x^s)\) is the average of the regular propensity functions of \(R_j\) over the distribution of the fast variables obtained from the steady state CME of the fast reactions.

   \[
   \bar{a}_{ij}^s(x^f, x^s) = \sum_{x'^f} \hat{P}(x'^f, \infty \mid x^f, x^s) \cdot a_{ij}^s(x'^f, x^s)
   \]

   \(\hat{P}\) is the steady state probability distribution of the fast variables, when the system is initially in the state \((x^f, x^s)\). This distribution can be derived for trivial systems.
For the present equilibrium reaction,

\[ \text{CycB} + \text{CKI} \rightleftharpoons \text{Trimer} \]

\[ K = \frac{k_f}{k_r}, \]

is the equilibrium constant of the dimerization reaction. \( k_f, k_r \) are the equivalent forward and reverse rate constants for the dimerization reactions.

The probability distribution is derived by solving the steady state master equation.

\[ P_n = \frac{1}{K^n n! (\gamma + \alpha - n)! (\beta - \alpha + n)!} P_0 \]

\( \alpha, \beta, \gamma \) are the initial state of the fast variables, \( \text{CycB}, \text{CKI}, \text{Trimer} \) respectively, from which final steady state distribution of the state is evaluated from the steady state CME of fast reactions. \( K \) is the equilibrium constant of the dimerization reaction.

\( P_n \) is the probability of finding ‘\( n \)’ number of molecules of CycB when the state of the system is evolving from the initial state \( (\alpha, \beta, \gamma, x^s) \) according to the CME of the equilibrium dimerization reaction.

\[ P_0 = \frac{1}{\sum_0^N \frac{1}{K^n n! (\gamma + \alpha - n)! (\beta - \alpha + n)!}} \]

\( N \) is the maximum number of molecules of CycB that can be observed for the given initial state.

\[ N = \alpha + \gamma \]

The other fast variables are calculated from the stoichiometric relations,

\[ [\text{CKI}] = [\text{CycB}] + \beta - \alpha \]

\[ [\text{Trimer}] = \gamma + \alpha - [\text{CycB}] \]

3. The next slow reaction (\( \mu \)) and the time (\( \tau \)) at which it occurs is calculated from the average propensities of each of the slow reactions calculated in the earlier step.
Calculate, $\overline{a_0^s}(x^f, x^s) = \sum_{j=1}^{\tau_s} a_j^s(x^f, x^s)$

Generate uniformly distributed random numbers in $[0,1]$, r1 and r2

Select $\tau = \left(\frac{1}{\overline{a_0^s}(x^f, x^s)}\right) \ln \left(1/r_1\right)$

Select $\mu$ as an integer satisfying, $\sum_{i=1}^{\mu-1} \overline{a_j^s}(x^f, x^s) < r_2 \overline{a_0^s}(x^f, x^s) \leq \sum_{i=1}^{\mu} \overline{a_j^s}(x^f, x^s)$

4. Update the system according to the occurrence of the slow reaction, $\mu$.

5. The new state after the update, $(x^f, x^s)$ changes the propensities of the fast reactions and the system attains a new set of stationary values of fast variables. The new state is selected randomly following the steady state distribution of fast variables.

   $$x^f_{new} = \text{a sample of } \hat{p}$$

Then, $x = (x^f_{new}, x^s)$

6. The procedure is repeated until the end time.

In the above procedure, selecting the state of the fast variable according to the probability distribution given by $\hat{p}(x^f', \infty| x^f, x^s)$ is a difficult task.

A simplification is suggested by Cao et al. (2005) in their slow-scale SSA work. If the distribution is unimodal, it can be represented by an equivalent normal distribution with the mean and variance calculated from the discrete Markov process representation of the fast reactions.

The mean of the state, $\hat{x}$ is calculated as the root of the equation,

$$a(\hat{x}) = W_+(x - 1) - W_-(x) = 0$$

Where, $W_\pm(x)dt$ is the probability that the state will be $(x \pm 1)$ in the time interval $(t + dt)$.
For the present system,

\[ W_{-}([\text{CycB}]) = k_f [\text{CycB}](\beta - \alpha + [\text{CycB}]) \]

\[ W_{+}([\text{CycB}]) = k_r (\gamma + \alpha - [\text{CycB}]) \]

From the above relationships, the mean value of the final state in fast variables, starting from the initial state \((\alpha, \beta, \gamma)\) is given by,

\[ [\tilde{C}\text{ycB}] = \frac{-(k_r + k_f(\beta - \alpha \sigma))^2 + 4k_fk_r(\gamma + \alpha + 1)}{2k_f} \]

Variance of the equivalent normal distribution is given by,

\[ \sigma^2 = \frac{W_{-}(x)}{-\alpha'(x)} \]

For the present case,

\[ \sigma^2 = \frac{k_f [\text{CycB}](\beta - \alpha + [\text{CycB}])}{2k_f [\text{CycB}] + k_r + k_f(\beta - \alpha)} \]

These expressions of mean and variance provide an approximate way to choose the stable state of fast variables between two successive slow reactions, without using the cumbersome probability distribution mentioned earlier.

**D.5.1. Calculation of the Average Reaction Propensities for the Slow Reactions**

Calculation of the weighted average of the reaction propensity function given by

\[ \bar{a}_j^s(x^f, x^s) = \sum_{x^f} \hat{P}(x^f', \infty | x^f, x^s) a_j^s(x^f', x^s) \]

Simplifications can be made for trivial reactions such as simple unimolecular, bimolecular reactions.

When \(a_j^s(x^f, x^s) = k x^f_l\), then \(\bar{a}_j^s(x^f, x^s) = k <x^f_l>\)
When \( a_j^s(x^f, x^s) = k x^f_i x^f_k \), then \( \bar{a}_j^s(x^f, x^s) = k(x^f_i x^f_k) \)

By knowing the first and second moments of the fast variables above expressions can be evaluated.

The similar adjustments are made to the propensity functions of the ten component model, using the mean value of CycB, \([\hat{\text{CycB}}]\). For example, propensity function for the 9\textsuperscript{th} reaction in the reaction network is calculated as:

\[
a_9 = k_9 m ([IEP]_{max} - [IEP])[\hat{\text{CycB}}]
\]

Whenever, such adjustments are not possible, a deterministic approximation is made. For example, propensity function for 5\textsuperscript{th} reaction is calculated as:

\[
a_5 = k'_5 + k''_5 \left[ \frac{m[\hat{\text{CycB}}]^n}{J_5^n + [m[\hat{\text{CycB}}]^n} \right]
\]

It is not quantified, how much such adjustments can induce errors in the model predictions. It is taken as an initial approximation towards implementing the multi-scale simulation approach for the cell cycle model.

The simulation results of the model are presented in Fig. D5.
Figure D5: Simulation results for the slow scale stochastic simulation approach. The execution time is 214 sec for three completed cell cycles. (10 time units in the model). The accuracy of the model still needs to be evaluated.
There are many models described in the literature and they have different objectives and goals in the context of chemotherapy studies. These models vary from simple tumor growth models that focus on finding out the administration strategies without including details like pharmacokinetics, drug resistance to complex models that follow an integrated approach with aiming for tailoring the treatments to individuals and for in silico screening of potential drugs.

Norton and Simon (1986) developed a mathematical model assuming non-exponential growth for tumors without considering drug resistance. They propose that the rate of regression of tumor is proportional to the growth fraction of an unperturbed tumor of the same size and conclude that the dose of the drug should be intensified as the size of the tumor decreases. This concept of late intensification is well known as the Norton and Simon Hypothesis. Jansson (1975) developed cell cycle phase specific model that includes cell differentiation and heterogeneity with capability to obtain growth dynamics of cancer cells, normal cells in parallel with a separate use of cell cycle kinetic parameters. Through the model, the researcher identifies treatment protocols that show improvement for cancer reduction and toxicity control. Webb (1990) studies the resonance phenomenon discovered by Dibrov (Dibrov et al., 1985) considered to be significant in periodic administration of chemotherapeutic drugs according to which toxicity to normal cells can be minimized by adjusting the treatment period close to the mean cell cycle length of normal cells. An age and size structured population balance model accounting for cell cycle variability is used for cancer cells and normal cells with different parameters. Drug action is instantaneous and drug PD is introduced by including periodic cell loss function that is ‘on-off’ destruction of cells. Augur et al. (1988) (Agur et al., 1988) develop a cell cycle specific model for heterogeneous cell population based on a Malthusian form.
(Sundareshan and Fundakowski, 1984) for intrinsic population growth and dividing cell cycle into drug resistant and sensitive phases. The model based on different parameters for normal and cancer cells evaluates periodic treatment protocols based on elimination times of both types of cells under treatment.

Many models are described by applying optimal control theory to obtain best drug administration profiles during the treatment period. These models focus on different objectives such as minimizing the tumor size, maximizing the bone marrow population under treatment constraints generally using compartmental models. A review of cell cycle non-specific models using optimal control is presented by Swan (1990) and Swierniak et al. (Swierniak et al., 2003) presents cell cycle specific models. Ledzewicz & Schattler (2007) derive optimal drug schedules based on a cell cycle non-specific model to minimize the bone marrow depletion using simple PD and PK. Ledzewicz & Schattler (2002) present a cell cycle specific model for minimizing cancer cells at the end of the treatment. Fister and Panetta (2000) (Fister and Panetta, 2000) apply optimal control theory to maximize both bone marrow and the amount of drug dose to kill cancer cells using cell cycle specific three compartmental model. In the model by Dua et al. (2008) (Dua et al., 2008), growth dynamics of both normal cells and cancer cells are described using different growth equations and optimal control problem is formulated to minimize the total tumor size after some time while restricting the toxicity to the normal cells. Two different models are solved for cell cycle specific and cell cycle non-specific treatments by coupling to PK/PD models. Ledzewicz & Schattler (2005) proposed a cycle non-specific model using a two compartmental model to demonstrate that addition of PK/PD models does not change the optimal solution of drug doses and scheduling. Dibrov (1985) discovered the phenomenon of resonance in chemotherapeutic applications studies the phenomenon using optimal control methods through
their age structured model for heterogeneous cell population used for both normal and cancer cells with different model parameters. They suggest that the drug administration should be periodic rather than continuous and also the period of drug administration should be small and more frequent. They determine the optimum period is close to the value of the cell cycle length when large variations exist between the normal and cancer cells.

Models are developed with the aim to determine the drug schedules to minimize the evolving drug resistance during the treatment. (Goldie and Coldman, 1983) presented their point mutational stochastic model for drug resistance for non cell cycle specific drugs by assuming instantaneous action of drug. Their model considers two non-cross resistant identical drugs which can be extended for multiple drugs. Later, Day (1986) relax the symmetry assumption for drugs and suggest administration strategies based on a multi type branching process model (Day, 1986). These models do not consider cell cycle effects and only study chemotherapy scheduling in the context of drug resistance. Gaffney (2004) combine cell cycle effects with drug resistance using their age structured population balance model and suggest schedules to reduce the probability of development of drug resistance. A branching random walk model is developed by Kimmel and Swiernaik (2006) (Kimmel and Swierniak, 2006) in which they apply optimal control theory for designing chemotherapeutic scheduling by including cell cycle effects and drug resistance through gene amplification mechanism.

More complex models include features like stochasticity, quiescence, drug pharmacokinetics, pharmacodynamics, drug resistance, spatial variations in a detailed formulation, in some cases based on experimentally derived parameters. Sherer et al. (2006) (Sherer et al., 2006) examined the resonance effect in chemotherapy using a multi-staged age structured population balance model for cell cycle specific drugs with variable transition and
death rates. Dividing cells into six different phases, their model is used to study the influence of in vivo effects such as quiescence, drug metabolism, drug properties, and transport considerations on the resonance chemotherapy. Sherer et al. (2007) (Sherer et al., 2007), through an age structured binary cell division population balance model, present probabilistic interpretations for obtaining treatment durations that result in complete cancer cure. The model considers variable response of patients to treatment and presence of dormant population while not considering the cell cycle effects, toxicity to normal cells and drug pharmacokinetics. Another model (Coldman and Murray, 2000) applies optimal control techniques to consider multiple determinant of chemotherapy – toxicity, drug resistance, tumor control, which are separately handled separately by many other optimal control models. A birth and death type of stochastic model is used neglecting PK/PD effects. Model by Panetta et al. (2008) (Panetta et al., 2008) combines PK/PD models with models for cancer growth and neutrophil production in the bone marrow to demonstrate its usefulness in the clinical practice. Based on the experimentally derived parameters, they aim to identify the schedules and exposure times for improved tumor response with minimized toxicities. In another study (Stamatakos et al., 2006), a spatiotemporal Monte Carlo simulation model of tumor response to chemotherapy is developed based on three dimensional structural and functional information from tumors of individual patients. Toxicity and drug resistances are neglected.

An integrative approach that combines different modules at different scales for chemotherapy studies will be useful for predicting invivo tumor growth and response to chemotherapy and thus helpful in achieving the much desired goals of cancer research community – for screening potential drugs in silico and for tailoring treatments to individual patients. Sanga et al. (2006) described the framework for development of such a simulator that
integrates tumor growth in spatial domain, tumor induced angiogenesis, drug resistance, PD and PK that is suitable for application of multiple drugs. Venkatasubramanian (2008) developed an integrated model where cell cycle transitions are described based on energy metabolism and spatial heterogeneity of tumor is considered. Neglecting toxicity, drug resistance their integrated model studies the effect of PK, PD on treatment efficacy. (Kolokotroni, 2008) describe a multi-scale spatiotemporal, patient specific model that integrates PK, PD and cell cycle kinetics by considering different types of cells such as stem cells, cells with limited mitotic capability, differentiated cells, necrotic cells, dead cells. Their model based on the data from individual patients is aimed at translating the integrated model into clinical practice for optimizing cancer treatment in the patient individualized context.

Another model that has integrated many modules of integrated chemotherapy model framework is OncoTCap (Oncology Thinking Cap) developed by Day and colleagues (Day R, 1998; Gardner and Fernandes, 2003). This simulation tool, developed based on the multi-type branching process model by (Day, 1986), is capable of describing phenomena such as heterogeneity in cell kinetics, random mutations, cell death due to chemotherapeutic treatment, drug resistance, quiescence, stem-cell differentiation, metastasis, spatial heterogeneity and gene amplification, however with the high dependence on computational power. The model derives a cumulative probability generating function of different types of cells which can be used to find out the probability of cure of a given treatment, effect of different treatment schedules on the heterogeneous cell population. However, drug action is assumed to be instantaneous and cell cycle specificity, drug pharmacokinetics are neglected. OncoTCap simulation tool was used for educating medical students about clinical trials (Day et al., 2004). In another study, Gardner (2002) develop an integrated model, Kinetically Tailored Treatment (KITT), that includes
exponential and gompertzian growth, drug resistance, multi drug testing capability, PK, PD, intra-tumor heterogeneity, apoptosis, quiescence based on experimentally derived parameters. The same model can be used for tumor cells and for different types of normal cells accounting for toxicities, by using different parameters. The model is demonstrated to tailor the treatments to individuals when combinations of cell cycle specific, non-specific and cytostatic drugs are used.
VITA

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