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Mathematical modelling of the core regulatory feedback mechanisms of p53 protein that decide cell fate

A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Computational Systems Biology at Lincoln University by Ket Hing Chong

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by

Ket Hing Chong

Cells defence against stresses that can cause DNA damage (single-strand breaks, double-strand breaks) is crucial in safeguarding the integrity of the genome and the survival of the organism as a whole. One of the genes that plays a pivotal role in maintaining the stability of the genome in humans is p53, which encodes its product p53 protein. The regulation of p53 activation is extremely complex, and molecular cell biology has gathered parts and pieces of the whole pathway. Mental intuition of this complex regulation is challenging; therefore, it requires a different method to quantitatively model and analyse to enhance the current understanding. This thesis has attempted to create two quantitative models of the mechanisms that regulate p53 basal levels and its appropriate activation as a stress response in deciding cell fate by either cell cycle arrest (to stop proliferation of DNA-damaged cells) or apoptosis (programmed cell death) to eliminate damaged cells.

In the first part of the research, a modified and improved model from Sun et al. (2011) deterministic model is proposed to explain the p53 basal dynamics and its response to stress due to DNA double-strand breaks. This model in the form of delay differential equations incorporates the most recently found molecular interactions and hypothesis: the core regulators consist of ATM, Mdm2, MdmX, Wip1 and p53. ATM as a stress transducer, amplifies the stress signal and activates p53 and inhibits its regulators Mdm2 and MdmX. The network structure consists of two positive feedback loops (p53 auto-regulation and ATM auto-activation), three negative feedback loops (Mdm2, MdmX and Wip1) and the interplay of p53, Mdm2 and MdmX that have successfully captured the basal dynamics (spontaneous pulses under non-stressed conditions) and stress response (repeated pulses or oscillations under stressed conditions). The model simulation results show that p53 spontaneous pulses are due to intrinsic DNA damage involving low number of DNA double-strand breaks; and p53 auto-regulation is an important positive feedback contributing to a threshold activation of p53 in generating pulses whether spontaneous or repeated. It also shows that p53 dynamics are excitable,
in that once initiated, it completes the pulse even if stress signal is inhibited. Bifurcation analysis revealed a spectrum of p53 behaviour under stressed and non-stressed conditions and characterised p53 dynamics as Type II excitability (oscillations arises from non-zero frequency). Most importantly, we reveal some novel findings on the mechanism of threshold activation of p53 pulsatile and oscillatory dynamics that are crucial for its physiological function as a transcription factor and guardian of the genome.

The second model is an extension of the first model by incorporating the apoptosis initiation module structure from Zhang et al. (2009a) with modified parameter values for modelling the core regulatory mechanism of p53 protein that activates apoptotic switch in response to high DNA double-strand breaks. The apoptosis initiation module includes Puma, Bcl2 and Bax. p53 activates the transcription of Puma (BH3-only protein that is pro-apoptotic) as a trigger of apoptosis that inhibits Bcl2 protein (pro-survival) and directly activates Bax. Activation of Bax was assumed to be an indicator of apoptosis initiation. The constructed model demonstrated how molecular interactions and stress signal amplification from ATM auto-activation in the p53 network control cell life and death decisions. Particularly, the model simulation results are qualitatively consistent with the experimental findings of an all-or-none activation of apoptosis and predicted overexpression of Bcl2 as a factor in causing the malfunction of the apoptotic switch. This model presents a simplified yet plausible model for molecular mechanism that regulates p53 activation of the apoptotic switch. The model gives insight into the design principles underlying p53 regulation of apoptosis.

In summary, the two models presented in this thesis have proposed plausible design principles of p53 basal dynamics and DNA damage response, and activation of apoptotic switch. These models provide novel theoretical insights into p53 regulation.

**Keywords:** Mathematical modelling, p53, Mdm2, MdmX, Wip1, p53 basal dynamics, Excitable system, Type II excitability, p53 oscillations, p53 pulses, DNA damage response, Cell fate decisions, Cell cycle arrest, Apoptosis, Biological switch, Systems biology, Design principles, Delay differential equations, Bifurcation analysis
Publications and presentations

Publications


Presentations

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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>anaphase-promoting complex (APC)</td>
<td>Mitosis (M)</td>
</tr>
<tr>
<td>ataxia telangiectasia mutated (ATM)</td>
<td>Murine double minute clone 2 (Mdm2)</td>
</tr>
<tr>
<td>B-cell lymphoma 2 (Bcl2)</td>
<td>neocarzinostatin (NCS)</td>
</tr>
<tr>
<td>Bcl2 homology (BH)</td>
<td>nuclear factor KB (NF-KB)</td>
</tr>
<tr>
<td>Bcl-2 homology domain 3 only (BH3-only)</td>
<td>Ordinary Differential Equations (ODEs)</td>
</tr>
<tr>
<td>check point kinase 2 (Chk2)</td>
<td>p53 fused to cyan fluorescent protein (p53-CFP)</td>
</tr>
<tr>
<td>cyclin-dependent kinase 1 (CDK1)</td>
<td>p53 upregulated modulator of apoptosis (Pum)</td>
</tr>
<tr>
<td>Delay differential equations (DDEs)</td>
<td>Partial Differential Equations (PDEs)</td>
</tr>
<tr>
<td>Deoxyribonucleic acid (DNA)</td>
<td>phosphorylation of histone H2AX (γH2AX)</td>
</tr>
<tr>
<td>DNA double-strand breaks (DSBs)</td>
<td>Quantitative and Systems Pharmacology (QSP)</td>
</tr>
<tr>
<td>Fas-receptor (FasR)</td>
<td>Quantitative reverse transcription-PCR (RT-PCR)</td>
</tr>
<tr>
<td>Green Fluorescent Protein (GFP)</td>
<td>saddle-node (SN)</td>
</tr>
<tr>
<td>Hopf bifurcation (HB)</td>
<td>saddle-node on an invariant circle (SNIC)</td>
</tr>
<tr>
<td>insulin-like growth factor (IGF-1)</td>
<td>TNF-related apoptosis inducing ligand receptor (TRAILR)</td>
</tr>
<tr>
<td>Interphase (I)</td>
<td>tumour necrosis factor receptor (TNFR)</td>
</tr>
<tr>
<td>Massachusetts Institute of Technology (MIT)</td>
<td>ultra violet (UV)</td>
</tr>
<tr>
<td>Mdm2 fused to yellow fluorescent protein (Mdm2-YFP)</td>
<td>wild-type p53-induced phosphatase 1 (Wip1 or PPM1D)</td>
</tr>
<tr>
<td>mitochondrial outer membrane permeabilisation (MOMP)</td>
<td></td>
</tr>
</tbody>
</table>
Cancer has been known as a genetic disease for decades (Hanahan & Weinberg, 2000; Hanahan & Weinberg, 2011; Vogelstein & Kinzler, 2004) and cancer biology has advanced our understanding about the causes of cancer and improving cancer treatments, for example, by targeted therapies (Petrelli et al., 2009). Better understanding also provides a great deal of potential strategies to fight against this disease. One of the most crucial genes in protecting the integrity of our genome is p53. p53, known as “the guardian of the genome” (Lane, 1992), prevents us from getting cancer. p53 was found mutated in over half of all cancers and has since been a target of cancer research (Vogelstein et al., 2000). This thesis focuses on mathematical modelling of p53 system and investigates the regulation of p53 from a theoretical perspective. In this chapter, the study of the p53 network from the perspective of systems biology using mathematical modelling is explained in detail and the motivation of the research is presented. Following that the objectives of this research are given and finally an overview of the chapters of the thesis is given.

1.1 Modelling Biological Systems with a Systems Biology Approach

Living systems such as cells can be considered as a network of molecular components consisting of Deoxyribonucleic acid (DNA) molecules, genes, proteins, metabolites, and a wide range of other molecules (Bray, 2003; Ideker et al., 2001). The advances in molecular biology and biochemistry enable us to view all these components inside cells as molecular machines (Kitano, 2001). These molecular machines can be represented abstractly as a dynamical system (Tyson et al., 2001). A system in general can be represented by a few key interacting components or proteins that control the physiological function of the system such as immune response (Hood & Perlmutter, 2004). The advancement of knowledge and technology, particularly after the completion of the human genome project (Collins et al., 2003; Collins et al., 2004; Venter et al., 2001), rapidly gave rise to a new field of biology called systems biology (Ideker et al., 2001) that “aims at system level understanding of biological systems” (Kitano, 2001). According to Hiroaki Kitano the system-level understanding of a biological system can be defined by four main properties: system structures; systems dynamics; the control method; and the design method. These biological systems properties are closely link to engineering systems in terms of their function and performance, and system properties such as robustness, modularity and use of recurring circuit elements (Alon, 2003).

One of the most challenging aspects of systems biology approach is that it is an interdisciplinary field merging areas “from mathematics to molecular biology” (Hood & Perlmutter, 2004). Most biological
systems are extremely complex and cannot be understood with intuition alone (Sobie et al., 2011). Thus, systems biology approach, through the construction of mathematical models, is considered to play an important role in understanding the emergent behaviour in the form of cell fate decisions and the physiology of the biological system as a whole, and how the system functions as a result of the molecular interactions of the components in the system (Ideker et al., 2001; Sobie et al., 2011).

Cellular oscillatory behaviours like circadian rhythm, oscillations in certain mRNAs and proteins with a period of 24 h, have shown us that living things on this planet including humans live in a consistent rhythm that is aligned with the rhythms of nature or environment with alternate day and night changes (Goldbeter, 2002). Generally, it is known as “cellular rhythms” and these rhythms in an organism are controlled by a connected circuit of gene regulatory networks (Goldbeter, 2002). A cell contains an “integrated circuit” (Hanahan & Weinberg, 2000); if one of the major components is out of tune, it could cause catastrophe or abnormal growth like cancer. The studies of cancer research from a systems biology perspective focus on the details of the design principles using quantitative models. This methodology was proposed by biologists, for example, Douglas Hanahan and Robert A. Weinberg from Massachusetts Institute of Technology (MIT) who “foresee cancer research developing into a logical science, where the complexities of the disease, described in the laboratory and clinic, will become understandable in terms of a small number of underlying principles.” (Hanahan & Weinberg, 2000).

One natural question that arises is: why do we need to do mathematical models of biological systems? There are many answers to this question: first and foremost is the answer given by Albert Goldbeter, who has given a very clear reason, that is, considering the number of components involved in biological systems and the complexity of the feedback regulations, “mathematical models and numerical simulations are needed to fully grasp the molecular mechanisms and functions of biological rhythms.” (Goldbeter, 2002). Another answer is “most biological functions arise from interactions among many components” and thus it is important to integrate experimental study with the construction of mathematical models that can make testable predictions and give insights into the molecular mechanisms or general design principles (Hartwell et al., 1999). In other words, the value of a model, whether simple or complex, is judged not by the number of equations, but by what can be learned from the model (Mogilner et al., 2012), or by what the model teaches us that we do not know now (Tyson et al., 2002). From the literature, we observe that the theoretical approach has produced many successful examples: the seminal work of Hodgkin and Huxley provided the basis for understanding the action potential in the nerve system (Hartwell et al., 1999; Hodgkin & Huxley, 1952); the prominent work of Alan Turing on a conceptual model of pattern formation (Mogilner et al., 2012; Turing, 1952); Novak and Tyson (1993) model on Xenopus cell cycle made accurate
predictions of the mechanisms underlying cell cycle division and estimated some parameter values before these biological data were available (Novak & Tyson, 1993; Tyson et al., 2002).

The attention to the new field of study in systems biology is evident when some renowned experimental based journals, such as Cell and Cancer Research, started to publish a theoretical section on modelling cancer biology (Cobb, 2007). The systems biology approach is predicted to contribute to systems medicine in the near future as anticipated by Leroy Hood and Roger Perlmutter, the pioneers of systems biology:

“In our view, systems biology will inevitably change the rules that govern the selection and development of new therapeutics and will catalyze the development of personalized, predictive and preventive medicine in the next decade (Hood & Perlmutter, 2004).”

This prediction is very likely to happen. The changes are clearly seen in increased funding allocated to systems biology research and the establishment of systems biology departments in universities around the world; with different names such as Integrative Systems Biology at the University of Oxford, Department of Chemical and Systems Biology at Stanford University School of Medicine, Department of Pharmacology and Systems Therapeutics and the Systems Biology Center New York at Mount Sinai School of Medicine, etc. Even first-year graduate students at Mount Sinai School of Medicine attend systems biology courses on Biomedical Modelling (Sobie et al., 2011).

Recently, the systems biology approach to the study of cancer biology has attracted much attention in the scientific community (Abbod et al., 2009; Blair et al., 2012; Kreeger & Lauffenburger, 2010; Materi & Wishart, 2007). In 2008, scientists from Europe and United States gathered and discussed the role played by systems biology in the future of cancer research (Aebersold et al., 2009). One of the issues discussed was on the “systems biology analysis of the cellular pathways in cancer” and emphasised the value of mathematical methods for supporting experimental design by generating alternative hypotheses of network structures based on experimental data (Aebersold et al., 2009). These perspectives have motivated the theoretical approach of this thesis to construct mathematical models of the p53 system in response to DNA damage, which plays a pivotal role in safeguarding us from cancer.

1.2 Mathematical Modelling of p53 Regulation

Modelling biological networks is a vital research area for understanding the signalling pathways in living cells. Cells constantly sense extrinsic (from external environment) and intrinsic (within cellular environment) signals, and activate appropriate responses. Genes stored in DNA are transcribed into mRNA and then translated to make necessary proteins. Many genes and proteins have been identified that work together to keep cell division and growth under control. Uncontrolled cell
growth leads to malignant tumours or cancer. The tumour suppressor protein, p53, is regarded as “the guardian of the genome” (Lane, 1992) because p53 is the first point of contact for DNA damage response. In its damage response, p53 acts as a transcription factor that regulates the transcription of over hundreds genes in maintaining the integrity of human cells (Riley et al., 2008). Modelling the p53 system using mathematical models could enhance the current understanding of p53 regulation and unveil a potential avenue for p53-based cancer therapy.

The p53 gene, first discovered in 1979 (Lane & Crawford, 1979; Linzer & Levine, 1979), was originally believed to be an oncogene that stimulates cell growth. But ten years later, it was found that p53 actually is a tumour suppressor gene (Kruse & Gu, 2009). The p53 gene was found to be mutated in about half of human cancers (Vogelstein et al., 2000) and thus has attracted a great deal of attention from scientists around the world to study the p53 network. The mechanism and functional role of p53 activation after DNA damage is still an active area of research.

One of the important research studies is that published by Lev Bar-Or et al. (2000) through cultured cells studies. After induction of DNA damage by gamma irradiation, they observed an oscillatory behaviour of the expression of p53 and Mdm2 (protein that keeps p53 in check) and suggested a mathematical model of the p53-Mdm2 feedback loop for generating the oscillations in the protein levels of p53 and Mdm2. Their model has captured the essential oscillations of the experimental results. Protein p53 is kept low in normal cells because some of the cellular effects activated by p53, such as apoptosis, which is irreversible. When cells are under stress due to DNA damage, p53 is activated triggering p53-mediated signalling pathways for cell cycle arrest, repair of damaged DNA, or, in the case of serious damage or irreparable damage, apoptosis for committing programmed cell death (Vogelstein et al., 2000). Failure of p53 function leads to proliferation of cancerous cells.

After thirty years of p53 research, we still do not fully understand the functional role of these oscillations in human cells (Lane & Levine, 2010). Two of p53 core negative regulators Mdm2 and MdmX are considered to play an important role in keeping p53 in low levels during cell homeostasis (Wang et al., 2009). However, p53 levels were recently found to display one or few pulses even in non-stressed cell growth that resemble the pulses under radiomimetic drug neocarzinostatin (NCS) induced DNA damage (Loewer et al., 2010). Loewer et al.’s experimental findings throw a new question as to why and how p53 gets activated this way under no DNA damage stress (normal growth conditions). Another open question is the molecular mechanism controlled by p53 in inducing apoptosis through activation of a biochemical bistable switch (Tyson et al., 2011).

After reviewing the relevant literature, there are two current issues that require further investigation: (i) modelling the basal dynamics of p53 under normal growth conditions and how p53 responds to DNA damage in activating cell cycle arrest and (ii) modelling the apoptosis
(programmed cell death) induction by p53 through a bistable apoptotic switch. There are two computational modelling approaches in systems biology based on the availability of data sets: 1) top-down modelling is used for large-scale Omics data sets with statistical models (also known as knowledge discovery or data-mining (Kitano, 2002b)) and 2) bottom-up modelling is for high-quality data with smaller-scale systems described by a dynamical system (Sobie et al., 2011), also known as simulation-based analysis (Kitano, 2002b). In this thesis, the bottom-up modelling approaches is used to explore p53 regulation based on the available data gathered from experiments; one reason why bottom-up modelling is chosen is because of the availability of high-quality data in the form of time course evolution of protein levels in the literature. In this research, we proposed to model p53 system from the core molecular interactions in the network by using non-linear ordinary differential equations that incorporated time delays for transcription (producing mRNA from gene) and translation (producing protein from mRNA) processes.

1.3 Motivation for the Study in the Thesis

The complexity of biological networks has prompted the need for a different (Quantitative) approach as proposed by many renowned scientists such as Robert A. Weinberg, Leroy Hood and Douglas A. Lauffenburger. In particular, an NIH white paper, produced from the workshop on “Quantitative and Systems Pharmacology (QSP) in the Post-genomic Era: New Approaches to Discovering Drugs and Understanding Therapeutic Mechanisms”, suggested the need to train more trainees and PhD graduates equipped with systems pharmacology knowledge and quantitative approaches to drug discovery and development (Sorger et al., 2011). Therefore, this PhD research study is motivated by this report and aims to meet the need for appropriately trained scientists in this field. For this reason, mathematical and computational modelling of the p53 network in cancer research was chosen as the topic of research.

The main motivation of the study is to investigate the signalling pathway in the p53 network that regulates cell responses to stress signals from DNA damage, particularly, DNA double-strand breaks. DNA can be damaged by extrinsic stress such as ultra violet (UV) radiation from the sun and intrinsic stress from reactive oxygen species as a by product from normal cellular metabolism (De Bont & van Larebeke, 2004). Intrinsic stresses cause intrinsic DNA damage that can activate the DNA damage response (Woodbine et al., 2011). The p53 pathway is inactivated in cancer, and the p53-based cancer therapy is to reactivate the p53 function in inducing apoptosis. Therefore, conceptual and theoretical research in this thesis is crucial in understanding the p53 network and function.
1.3.1 Research Questions:

After a thorough literature review of the current understanding of the p53 network (A literature review will be discussed in Chapter 2: Background and Literature Review), we identified nine research questions for constructing two mathematical models. In the first model proposed in Chapter 4, we explored and attempted to answer the following questions:

1. What are the core regulators that control p53 DNA damage response?
2. What are the design principles behind the delicate activation of p53 that results in a series of pulses under stressed conditions and spontaneous pulses (one or two) under non-stressed conditions (basal dynamics)?
3. What is the significance of the p53 spontaneous pulses?
4. How does p53 achieve precise activation of p21 induction in arresting cell cycle under stressed conditions?
5. What is the model structure in the p53 system that contributes to p53 excitability in response to DNA double-strand breaks?
6. How do p53 dynamics decide cell life (proliferation) and death?
7. Does the p53 system work as an oscillator or a pulse generator?

In the second model proposed in Chapter 5, we attempted to answer the following questions:

8. What are the core regulatory mechanisms of p53 protein that control p53 apoptotic switch?
9. Under high DNA double-strand breaks, how does p53 turn on the bistable apoptotic switch to eliminate damaged cells?

1.4 Objectives

To answer all the nine research questions stated above, we plan to construct two mathematical models. The main objective is to achieve two conceptual models that capture the core feedback mechanisms of p53 protein that control cell fate. This study aims to form and implement mathematical models of p53 basal dynamics and DNA damage response based on non-linear ordinary differential equations with time delays. To display a unique response in non-stressed conditions and to achieve a more complex activation in response to DNA damage, p53 requires exquisite mechanisms of signal transduction and protein-protein interactions. Therefore, this study has the following objectives:
1. To identify the core regulators of p53 DNA damage response and signal transduction involving protein-protein interactions

2. To use schematic diagrams of the reaction networks in p53 and formulate a set of non-linear ordinary differential equations

3. To develop two mathematical models, one for p53 basal dynamics and p53 activation of cell cycle arrest and the other for p53 bistable apoptotic switch, which entails the following:
   a) Define appropriate hypotheses for the proposed mathematical models
   b) Find the parameter values and initial conditions for the models

4. To implement the models (on MATLAB and XPPAUT (dynamical system software)) and to analyse the models as a dynamical system. In addition, bifurcation theory is used to investigate the dynamics of p53 responses, and local parameter sensitivity analysis is performed to identify the core parameters in the system.

5. To perform in silico perturbation and generate testable predictions.

The objectives have been achieved with the constructed models presented in Chapter 4 and Chapter 5, respectively. The results and discussions will be covered in the respective chapters. Overall, these two models have successfully reproduced simulation results consistent with experimental findings and the analyses performed have provided novel theoretical insights into the mechanisms underlying p53 regulation of basal dynamics and DNA damage response, and apoptosis.

1.5 Overview of the Chapters

This thesis consists of six chapters. The first chapter gives an introduction of the thesis. The second chapter presents the background knowledge and a detailed literature review of p53 pathway and previous mathematical models and identifies and elaborates the two issues investigated in this thesis. The third chapter presents the methods used in this research in formulating and analysing the constructed models. The fourth chapter presents and discusses the results of the proposed model for p53 basal dynamics and DNA damage response. The fifth chapter presents the second proposed model on p53 activation of apoptosis. Finally, the last chapter gives a summary of the research, conclusions, contributions and future research directions.
Chapter 2

Background and Literature Review

This research involves the construction of mathematical models of p53 regulation from the known molecular interactions and incorporation of the latest experimental findings. The model hypotheses are based on molecular interactions gathered by molecular biologists on p53 regulation in the literature. Thus, before we could build any computer model we need to understand the current state of understanding in the field of p53 biology and modelling. This chapter provides a review of the background information about p53 regulation and a review on previous mathematical models. The review is followed by a discussion on the two current issues addressed in this thesis.

2.1 Overview

p53 is one of the most studied proteins because p53 plays a major role in modulating a wide range of cellular responses to stress signals present in cells, and these include DNA damage repair, cell cycle arrest, senescence (permanent cell cycle arrest) and apoptosis (programmed cell death). There have been a considerable number of experimental studies that concentrate on p53 activation under stressed conditions in cultured cells and mouse models. In general, a conventional model of p53 activation involves three steps – p53 stabilization, DNA binding, and transcriptional activation (Kruse & Gu, 2009).

However, recent studies have suggested that p53 activation is more complex than this classical model; the activation of p53 also relies on its main negative regulators Mdm2 and MdmX (Kruse & Gu, 2009; Toledo & Wahl, 2006; Wade et al., 2010). Mary Perry, a scientist from National Institutes of Health, USA, emphasised that “Exquisite control of the activity of p53 is necessary for mammalian survival” (Perry, 2010). Therefore, it is indispensable to have a flawless control of p53 function in protecting cells from developing cancer and p53 activation needs a precise mechanism that responds rapidly in accordance with the stress signals in maintaining genome fidelity and at the same time avoiding wrong activation that induces programmed cell death.

The p53 pathway involves many proteins interacting with p53—positively or negatively regulating p53, and directly and indirectly controlling p53 transcriptional activation (Harris & Levine, 2005; Lu, 2010). In addition, modes of p53 regulation are also governed by different post-translational modifications, for example, phosphorylation, ubiquitination, methylation and acetylation (Kruse & Gu, 2009).
In explaining the growing complexity of molecular interactions and effects on p53 regulation, an increasing number of mathematical models use ordinary differential equations to formulate hypotheses and quantitative analyses of the models for explaining p53 oscillations after induction of DNA damage. There is only one model that models the p53 basal dynamics (i.e. under normal growth or non-stressed conditions) in individual cells (Sun et al., 2011). It was discovered in experiments that individual cells show one or few spontaneous pulses in non-stressed conditions (Loewer et al., 2010). It is totally different to what was known about p53 basal dynamics until then, where p53-Mdm2 negative feedback loop controls p53 concentration level to keep it at a low steady state in homeostasis or under non-stressed conditions. As a result, it is necessary to understand p53 regulation both in non-stressed and stressed conditions in order to make predictions and to gain insights into the excitable (solitary pulse) mechanism of p53 basal dynamics as well as the molecular mechanism involved in controlling cell cycle arrest (that produces repeated p53 pulses or oscillations) and apoptosis.

2.2 The p53 Pathway

The p53 pathway is essential for tumour suppression function, and modulation of the p53 response to various stress signals is crucial for maintaining cell genomic integrity. Before we can model the p53 system, we need to understand the functioning of the p53 pathway. There are a few review articles (such as Harris and Levine (2005) and Levine et al. (2006)) that provide comprehensive information about the p53 pathway. The regulation of the p53 pathway is still an active area of research and more detailed information about the p53 pathway will be discovered in the coming years with greater “layers of complexity” (Braithwaite et al., 2005). The p53 pathway consists of five parts (Levine et al., 2006) as shown in Figure 2-1:

1. The input stress signals
2. The mediators or modifying enzymes (signal transducers)
3. The p53 core regulators (feedback regulators)
4. The p53 transcriptional activation of target genes (output regulators)
5. The cellular output of p53 activation
2.3 Molecular Biology Based Experimental Measurements of Gene Expression of p53 and Its Transcriptional Activities

Here a brief review of the molecular cell biology based experimental studies on the biology of p53 is presented.

The advent of DNA sequencing technology and the completion of the human genome project marked a new era of advancement in genetics, genomics, high-throughput biochemistry and bioinformatics, and provided biologists with valuable research tools to understand the molecular machineries of how life works on earth (Collins et al., 2003). However, the discovery of p53 was back in 1970s, before DNA sequencing technology was available. With the available technology like cloning and the pull down (or immunoprecipitation) techniques, p53 was discovered in 1979 by David Lane and his co-workers while investigating substances that bind to T antigen as described by David Lane in an
interview (Ng, 2013). Since the discovery of p53 gene, and after thirty years of research on p53, nearly 50,000 research articles have been published on p53 (Lane & Levine, 2010). Thus, it is impossible to list all these articles here, but to provide a relevant review of the literature on p53.

The discovery of antibody helped identify p53 protein in the nucleus (Dippold et al., 1981). For example, the DO-1 monoclonal antibody (Santa Cruz Biotechnology) was used to quantify the presence of p53 protein (Batchelor et al., 2008; Wang & El-Deiry, 2006). Western blot uses p53 monoclonal antibody to quantify p53 response after gamma radiation induced DNA damage. By applying Western blot analysis, it was shown that p53 protein levels in a population of cells followed damped oscillations (Lev Bar-Or et al., 2000) as illustrated in Figure 2-2 Left panel (Batchelor et al., 2009).

A transcription factor is a special protein that can transactivate gene expression of other genes under its control by binding to the response element (also known as promoter) of its target gene and activates gene expression. p53 is the most well studied transcription factor that regulates over hundreds of genes (Beckerman & Prives, 2010) and a comprehensive list of genes (129 genes) activated by human p53 was published based on the genes that satisfied three out of four specific criteria: 1) the presence of a p53 response element; 2) the evidence of the target gene expression at the mRNA and protein level; 3) to be able to clone the p53 response element from that gene and upon insertion into luciferase gene, evidence of luciferase gene expression; 4) the positive response to chromatin immunoprecipitation with a p53-specific antibody to confirm p53 binding to the response element in the DNA (Riley et al., 2008). These criteria are based on causal relationship of the presence of p53 binding to the response element and evidence of the target gene expression.

To provide an example of how experiments have confirmed p53 activates MdmX, we refer to Philips et al. (2010) work. For criteria 1, bioinformatics analysis was conducted and found the presence of a p53 response element for MdmX gene. Quantitative reverse transcription-PCR (RT-PCR) and Western blot (also called Immunoblotting) are common techniques used to quantify mRNA and protein levels, respectively. For criteria 2, after induction of DNA damage, they showed that in the presence of p53, there is an increase in mRNA and protein levels of MdmX, and it suggests that p53 activates MdmX. For criteria 3, the activation of p53 in transactivating MdmX gene is confirmed by the genetic technique that cloned p53 response element from MdmX gene into a luciferase reporter vector. The demonstration of a functional gene expression of luciferase suggests MdmX as a p53-inducible gene (Phillips et al., 2010). For criteria 4, chromatin immunoprecipitation analysis was conducted and confirmed the presence of p53 protein at the response element. All these four criteria were used by Philips et al. (2010) to confirm p53 activation of MdmX.
Some techniques such as immunoprecipitation and yeast two-hybrid are used to investigate protein-protein interactions. Immunoprecipitation was used to discover Murine double minute clone 2 (Mdm2) that bound to p53 and inhibits p53 transactivation activity (Momand et al., 1992). Later, an Mdm2-related protein, based on the structural similarity, was found and named MdmX; co-immunoprecipitation has shown that MdmX also binds to p53 (Shvarts et al., 1996). A comprehensive list of proteins that interact with p53 has been compiled and published in a review paper (Toledo & Wahl, 2006) and it reveals why p53 regulation is such a complicated network.

The discovery of the Green Fluorescent Protein (GFP) from jellyfish has been deployed to quantify the gene expression of specific genes of interest at single cell level because the colour of this protein enable us to see its presence in living organisms (Chalfie et al., 1994). The gene that encodes GFP can be attached to the promoter of the gene of interest and GFP protein synthesis represents a readout of gene activation. The GFP can be captured by a time lapse microscopy movie as a powerful visualisation tool that provides a detailed characterisation of the activity in a single cell and thus allows us to collect high-quality time series data (Purvis & Lahav, 2013). The use of GFP has been well established and it has been widely used by biologists to investigate certain gene and protein activity of interest and to understand the dynamics of the events of interest without disturbing its natural activities (Tsien, 1998).

Recently, GFP fusion protein has been used in studying the dynamics of p53 and Mdm2 in individual MCF7 cells (Breast cancer cell line) (Geva-Zatorsky et al., 2006; Lahav et al., 2004), and the characterisation of p53 responses as discrete pulses as illustrated in Figure 2-2 Right panel (reproduction of Figure 3b and 3c from Batchelor et al. (2009) with permission from the publisher).
Figure 2-2 The response of p53 to DNA double-strand breaks (DSBs). Left panel shows measurements averaged over population of cells showing damped oscillations of p53. Right panel illustrates single cell measurements showing a series of undamped pulses with different cells showing different number of pulses. Reproduced with permission from Nature Reviews Cancer.

A detailed analysis from individual cell measurements for different levels of DNA damage by gamma irradiation that causes DNA double-strand breaks concluded that p53 responses as a series of discrete pulses (Lahav et al., 2004) as illustrated in Figure 2-3 (Left graph). It shows that, the larger the doses of gamma radiation, the higher the number of pulses. However, when UV radiations were used as an agent that causes single-strand DNA breaks, it displays a longer single pulse depending on the magnitude of the radiation (Batchelor et al., 2011). Batchelor et al. (2011) concluded that larger UV radiation causes a higher and longer single pulse as shown in Figure 2-3 Right panel.
Current understanding indicates that p53 levels and dynamics are closely linked to the cellular responses as shown in Figure 2-4; p53 repeated pulses or oscillations result in cell cycle arrest and sustained levels of p53 causes apoptosis (Purvis & Lahav, 2013). More about p53 dynamics will be discussed in the following sections of this chapter.

2.4 Mathematical Models of p53 Regulation

Recently, p53 system modelling has become a dynamic research area. A wide range of mathematical models has been published in the literature (Table 2-1), and this section briefly reviews some of the interesting models. The studies of the p53 system from these mathematical models are consistent with the main task of systems biology, applied specifically to p53 system, as put forth by the authors in a review article (Ideker et al., 2001); that is, to gather information on the p53 system and to integrate the available data and generate predictive mathematical models of the p53 system.
As discussed earlier, p53 functions as a sequence specific DNA binding transcription factor to activate expression of genes that have protective effects (Vousden & Lu, 2002). p53 is activated in response to stress signals, and mediates the gene induction that could result in DNA damage repair, cell cycle arrest, senescence (permanent cell cycle arrest) or apoptosis (programmed cell death). Thus, p53 function is like a “guardian of the genome” (Lane, 1992) or “cellular gatekeeper for growth” (Levine, 1997). Mathematical models and the time course data from experiments not only enable us to describe the levels of p53 protein and its activity, but also make inference on how p53 decides cell fate in response to various stress signals.

The first mathematical model constructed by Mihalas et al. (2000), with a simple model of four equations that described the p53 and Mdm2 interactions with negative feedback regulation, managed to produce computer simulations of damped and sustained oscillations (Mihalas et al., 2000). The Mihalas et al. (2000) model explored the importance of the p53-Mdm2 negative feedback interactions by using: i) p53 dimer or p53 tetramer in activating Mdm2; ii) with or without time delay; iii) different rate of Mdm2 mediated p53 degradation. This simple model provided the foundation for later models on modelling p53 and Mdm2 oscillatory dynamics.

Table 2-1 Some Mathematical models of the p53 system

<table>
<thead>
<tr>
<th>Year</th>
<th>Type of model</th>
<th>p53 dynamics</th>
<th>with delay</th>
<th>References</th>
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<tr>
<td>2000</td>
<td>ODE</td>
<td>Oscillations</td>
<td>Yes &amp; No</td>
<td>(Mihalas et al., 2000)</td>
</tr>
<tr>
<td>2000</td>
<td>ODE</td>
<td>Damped Oscillations</td>
<td>No</td>
<td>(Lev Bar-Or et al., 2000)</td>
</tr>
<tr>
<td>2002</td>
<td>ODE</td>
<td>Oscillations</td>
<td>Yes</td>
<td>(Tiana et al., 2002)</td>
</tr>
<tr>
<td>2003</td>
<td>ODE</td>
<td>Oscillations</td>
<td>Yes</td>
<td>(Monk, 2003)</td>
</tr>
<tr>
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<td>ODE</td>
<td>Oscillations</td>
<td>No</td>
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<td>2005</td>
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<td>Oscillations</td>
<td>Yes</td>
<td>(Wagner et al., 2005)</td>
</tr>
<tr>
<td>2005</td>
<td>ODE/Monte Carlo</td>
<td>Oscillations</td>
<td>Yes</td>
<td>(Ma et al., 2005)</td>
</tr>
<tr>
<td>2006</td>
<td>ODE</td>
<td>Oscillations</td>
<td>Yes</td>
<td>(Mihalas et al., 2006)</td>
</tr>
<tr>
<td>2006</td>
<td>ODE</td>
<td>Oscillations</td>
<td>Yes &amp; No</td>
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<tr>
<td>2007</td>
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<td>Oscillations</td>
<td>No</td>
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</tr>
<tr>
<td>2007</td>
<td>ODE</td>
<td>Oscillations</td>
<td>No</td>
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<td>Oscillations</td>
<td>Yes</td>
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<td>2008</td>
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<td>Oscillations</td>
<td>No</td>
<td>(Proctor &amp; Gray, 2008)</td>
</tr>
<tr>
<td>2008</td>
<td>Stochastic</td>
<td>Oscillations/Switch-like</td>
<td>No</td>
<td>(Puszynski et al., 2008)</td>
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<td>2008</td>
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<td>No</td>
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</tr>
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<td>Oscillations</td>
<td>Yes</td>
<td>(Cai &amp; Yuan, 2009)</td>
</tr>
<tr>
<td>2009</td>
<td>ODE</td>
<td>Oscillations</td>
<td>Yes</td>
<td>(Kim et al., 2009)</td>
</tr>
<tr>
<td>2010</td>
<td>ODE</td>
<td>Oscillations</td>
<td>No</td>
<td>(Hunziker et al., 2010)</td>
</tr>
</tbody>
</table>
2010 ODE     Switch-like     No     (Pu et al., 2010)
2010 ODE/Monte Carlo     Oscillations     No     (Zhang et al., 2010)
2011 Stochastic     Oscillations     Yes     (Liu et al., 2011)
2011 ODE     Oscillations     No     (Li et al., 2011)
2011 ODE     Long single pulse     Yes     (Batchelor et al., 2011)
2011 ODE/Stochastic     Basal dynamics     Yes     (Sun et al., 2011)
2012 ODE     Sustained p53     Yes     (Purvis et al., 2012)
2012 ODE/Monte Carlo     Switch-like     No     (Tian et al., 2012)
2013 ODE/Stochastic     Oscillations     No     (Kim & Jackson, 2013)
2013 ODE/PDE     Oscillations     No     (Dimitrio et al., 2013)

Note: ODE-Ordinary Differential Equations; Monte Carlo-Monte Carlo simulation of DNA damage repair mechanism; Stochastic-Stochastic simulation; PDE-Partial Differential Equations.

2.4.1 P53-Mdm2 Negative Feedback Loop

A network motif is a biological network that is found in organisms more often than random networks (Alon, 2007). The p53-Mdm2 negative feedback loop is a network motif that consists of a slow transcription arm and a faster protein-interaction arm (Lahav et al., 2004). This network entails two time scales: the slow transcription arm with p53 binding to Mdm2 promoter and activating Mdm2 protein transcription occurring in the time scale of hours, and the fast protein-interaction of Mdm2 binding to p53 and promoting p53 degradation happening in the time scale of seconds to minutes. Mdm2 negatively regulates p53 with its E3 ligase function to degrade p53 and inhibit it by forming a negative regulatory feedback loop shown in Figure 2-5.

![p53-Mdm2 Negative feedback loop](image)

Figure 2-5 p53-Mdm2 Negative feedback loop. Reproduced with permission from Nature Reviews Genetics.

This p53-Mdm2 negative feedback loop is part of the core regulatory circuit of p53, and it is one of the biological systems where oscillatory behaviour has been observed in experiments of mammalian cultured cells after DNA damage induced by ionizing radiation (Lev Bar-Or et al., 2000). The oscillations of p53 and Mdm2 has been seen in the experimental studies using population of cultured cells with Western blot analysis (Lev Bar-Or et al., 2000). Later, a more advanced method was used to measure the p53 and Mdm2 levels using MCF7 breast cancer cell line, where living individual cells
with p53 fused to cyan fluorescent protein (p53-CFP), and Mdm2 fused to yellow fluorescent protein (Mdm2-YFP) were studied (Lahav et al., 2004). Using time-lapse fluorescence microscopy movies to measure p53-CFP and Mdm2-YFP after gamma-irradiation, the data collected in intervals of 20 minutes for a period of 16 hours have shown that p53 oscillates with fixed average amplitude and duration. An example of the measurements of the p53-CFP and Mdm2-YFP in an individual cell is shown in Figure 2-6. The findings that the mean number of pulses directly corresponds to the level of DNA damage led to the conclusion that the p53-Mdm2 feedback loop works behind this control system (Lahav et al., 2004). These discoveries marked the beginning of the quest for understanding the mechanism that gives rise to the oscillations of p53 and Mdm2.

Figure 2-6 p53-CFP (green) and Mdm2-YFP (red) levels in a cell show two pulses. AU, arbitrary units. Reproduced with permission from Nature Genetics.

One of the quantitative methods used to study this oscillatory behaviour is mathematical modelling based on differential equations. The second model proposed to explain the population of cells with damped oscillatory behaviour required a transcriptional time delay of p53-dependent induction of the expression of Mdm2 through an intermediary I (Lev Bar-Or et al., 2000). A later model used positive and negative feedbacks in the p53/Mdm2 network, the positive feedback is based on the PTEN, a target of p53 that inhibits Mdm2-dependent degradation of p53 and hence stabilizes p53 (Ciliberto et al., 2005). The model proposed by Ciliberto et al. (2005) was able to reproduce the oscillations of p53 and Mdm2 as the experimental results from Lahav et al. (2004), and demonstrated that the number of pulses depends on the amount of DNA damage.

Another study applied a family of mathematical models to the p53 system: three delay oscillators, two relaxation oscillators and one novel checkpoint mechanism (Model VI in Geva-Zatorsky et al. (2006)) which includes another longer negative feedback loop from one of the targets of p53 to upstream regulator of p53 (Geva-Zatorsky et al., 2006). According to Geva-Zatorsky et al. (2006),
there is no explicit explanation of the checkpoint mechanism given and it is not referring to the cell cycle checkpoint mechanism; however, it is referring to the signalling downstream (gene activated by p53, here assumed to be having similar dynamics as Mdm2) of p53 that feedback to inhibit the signal upstream of p53 (e.g. the phosphorylated ATM—see Figure 2-1). Most of the models can generate oscillations of p53 and Mdm2. They have demonstrated that the added low-frequency noise in the protein production rates was the factor that attributed to the variability in the amplitude of the observed oscillations in individual cells.

A recent model has extended the same (Model VI in Geva-Zatorsky et al. (2006)) novel checkpoint mechanism, and included an inhibitor of the upstream stress signal transducer ATM to p53 (Figure 2-1). This inhibitor was shown to be Wip1, a target gene of p53 (Batchelor et al., 2008). This model has reproduced the results from their experiments, showing that the pulses were in specific sequence: signal pulses after DNA damage, to p53 pulses followed by Mdm2 pulses, and finally the inhibitor (Wip1) pulses (Figure 2-7). The authors of the same group have suggested that the ATM pulses were involved in the DNA damaged repair, and is reactivated by persistent DNA damage, and proposed that the p53 dynamics work as a pulse generator triggered by an excitable signal from DNA damage, and they favoured the behaviour of the p53 system as a pulse generator rather than a continuous oscillator (Batchelor et al., 2009).

![Simulated protein dynamic response (single cell)](image)

Figure 2-7 Numerical simulations of the extended checkpoint model in response to DNA damage (Batchelor et al., 2008). Reproduced with permission from Molecular Cell.

2.4.2 The Advancement of the Understanding of p53 Regulation

The p53 single-cell DNA damage response is oscillatory (Geva-Zatorsky et al., 2006; Lahav et al., 2004) and has been modelled by many, such as Batchelor et al. (2008), Ciliberto et al. (2005), Geva-
Zatorsky et al. (2006) and Ma et al. (2005), and these models have enhanced our understanding of p53 dynamics and provided useful theoretical insights into p53 DNA damage response. The systems biology approach combining the knowledge from experimental findings and theoretical quantitative models are powerful tools to capture the essential features of biological systems with abstract representation of interactions of the components in the system (Alves & Sorribas, 2011).

Mathematical models contribute to gaining a deeper understanding of complex biological systems, particularly in the p53 system. Few examples are the elucidation of feedback loops capturing the stimulus-dependent dynamics of a long single pulse of p53 in response to DNA damage (stress) caused by UV radiation (Batchelor et al., 2011) and the use of mathematical models in predicting the timing of the doses of Nutlin-3 drug treatment after gamma-radiation to achieve a sustained p53 response to activate senescence pathway inducing permanent cell cycle arrest that may have potential benefits for cancer treatment (Purvis et al., 2012). However, mathematical modelling of p53 network will still be a popular research field in years to come into the future because much remains to be done to understand the full spectrum of p53 behaviour and function in its varied forms in cell cycle arrest, senescence and apoptosis. In particular, how p53 decides cell fate navigating the complexity of the feedback regulations remains an attractive avenue that may lead to development of new therapies for cancer.

Three common models of p53 dynamics that decides cell fate have been proposed in literature. The first model is according to cell population studies. Most molecular biologists assume that the level of p53 decides the state of the cell: low level for homeostasis; moderate level for cell cycle arrest; and high level for apoptosis (Lai et al., 2007; Moll & Petrenko, 2003; Vousden & Lane, 2007) as shown in Figure 2-8. The second model is according to some recent individual cell studies (Loewer et al., 2010; Purvis, 2012; Purvis & Lahav, 2013; Purvis et al., 2012) suggesting that: in homeostasis (non-stressed conditions) p53 exhibits one or two spontaneous pulses; a series of repeated pulses in cell cycle arrest; and an increased p53 level in apoptosis (Figure 2-9). The third model, as shown in Figure 2-10, is a theoretical prediction from computational biologists who predict that the number of p53 pulses are the key determinant for cell cycle arrest or apoptosis; the number of pulses greater than a threshold number, for example 3, may mean that cell decides to die (Sun et al., 2009; Tyson, 2006; Zhang et al., 2009b).

p53 system is a “protein circuit” (Geva-Zatorsky et al., 2006). However, Batchelor et al. (2008, 2009) emphasised that the p53 network can function as an “oscillator” or “pulse generator” (few pulses). More needs to be done to explore this behaviour. Therefore, a current issue remaining in modelling the p53 system is to investigate whether p53 functions as an oscillator or a pulse generator and how p53 decides cell fate in regulating diverse target gene activations.
Figure 2-8 Model of p53 behaviour from Population studies.

Figure 2-9 Model of p53 behaviour from Individual cell studies.
A general conclusion from these model studies is that the level of p53 protein or the number of p53 pulses decides p53 DNA damage response. These are the possible explanations so far for how p53 as the guardian of the genome decides cell fate. However, there remains a need for further clarification. Therefore, this thesis proposes further investigation on how p53 makes life or death decisions. The above models will be the basis for the model development and analysis that this thesis aims to achieve in Chapters 4 and 5.

2.5 Two Current Issues Addressed in this Thesis

There are two main issues: 1) p53 basal dynamics (spontaneous pulses under non-stressed conditions) and DNA damage response; and 2) p53 all-or-none activation of bistable apoptotic switch.

2.5.1 p53 Basal Dynamics and Response to DNA Damage

For the first main issue, there is a recent experimental finding from Loewer et al. (2010) that requires a mathematical model to investigate the mechanism behind these observations. These observations are:

1) Experimental observation of p53 spontaneous pulses (one or two pulses) under non-stressed conditions and a series of repeated pulses under stressed conditions

In the literature on p53, it is known that in normal unstressed cells the cellular p53 protein is kept at low levels mainly by Mdm2-mediated degradation; thus p53 protein is unstable with a short half-life of less than 30 minutes (Moll & Petrenko, 2003). However, recent experiments using fluorescently tagged p53 in individual cells revealed that most cells showed more than one pulse in a day under non-stressed conditions and suggested that the average p53 levels over a population of cells has...
masked this basal dynamics (Loewer et al., 2010). Surprisingly, the p53 spontaneous pulses in non-stressed conditions were similar to those after DNA damage, with approximately the same amplitude and duration, but with one obvious difference in that the number of spontaneous pulses was less frequent and asynchronous as shown in Figure 2-11. These spontaneous pulses were shown causally related to cell-cycle progression with most cells demonstrating the first pulse in G1 phase (G1 is the growth phase in cell cycle) (Loewer et al., 2010).

Figure 2-11 p53 level in individual cells measured as the average fluorescence. Normalized trajectories of p53-Venus (fluorescently tagged p53) levels are shown. The vertical dashed line indicates the time of cell division. Left two graphs show that DNA damage induced a series of persistent and relatively uniform p53 pulses after treatment with neocarzinostatin (NCS) and right two graphs illustrate that the individual cells with no extrinsic damage showed spontaneous pulses (Loewer et al., 2010). Reproduced with permission from Cell.

One explanation given by the researchers from Harvard Medical School (Loewer et al., 2010) was that in non-stressed conditions, normal cells experience intrinsic DNA double-strand breaks (caused by internal conditions of the cell) and activate the signalling sensors such as ATM or DNA-PK (see Figure 2-1).

2) Experimental observation of p53 excitable pulses

Loewer et al. (2010) results have indicated the p53 pulses were activated by ATM or DNA-PK kinases in an excitable mechanism, where once initiated by low level transient damage (short duration intrinsic stress; see Figure 2-12 Right panel) or high level transient extrinsic damage (see Figure 2-12 Left panel) it could trigger a full p53 pulse even if the stress signal was inhibited by Wortmannin (wm). These p53 excitable pulses were proposed to be similar to the excitable mechanism of action potential in neurons (Loewer et al., 2010).
3) Experimental observation of p53 precisely inducing p21 under stressed conditions

This excitable mechanism was proposed to be highly sensitive to stress signals and at the same time it could tolerate intrinsic DNA damage (not activate cell cycle arrest genes such as p21 or apoptotic genes) that does not require the response of p53 activation such as repeated oscillations. Loewer et al. (2010) findings have clearly shown that spontaneous p53 pulses did not activate the apoptotic genes or cell-cycle arrest genes such as p21 as shown in Figure 2-13. Here, stressed conditions activate p21 and non-stressed conditions do not. The activation of p21, a p53 target gene that could arrest cell-cycle (see figure 2-1), is mediated by a network of post-translational modifications. For example, under stressed conditions, the activation of p21 requires p53 acetylation.

These results raised a few interesting questions: What is the purpose of these excitable pulses? What is the mechanism behind these p53 excitable pulses? To answer these two questions, next a brief review of the definition of excitable systems and action potential excitability in neurons as an example of an excitable system is discussed.
Figure 2-13 Single-cell trajectories from two cells showing p53 dynamics (solid line) and p21 reporter (dashed line). Left graph shows that after DNA damage, the p21 reporter was induced by p53 activation. Right graph shows that p53 pulses in non-stressed conditions do not activate p21 expression (Loewer et al., 2010). Reproduced with permission from Cell.

A Brief Review of Excitable Systems

One of the famous excitable systems in literature is the Hodgkin-Huxley model for the propagation of nerve signal called “action potential” in the axon of a giant squid (Edelstein-Keshet, 1988). This model contains four differential equations that describe four variables \( V, n, m, h \) in the electrochemical mechanism of the excitable system; where \( V \) is the membrane potential, \( m \) the Na\(^+\) activation, \( h \) the Na\(^+\) inactivation, and \( n \) the K\(^+\) activation (Na\(^+\) and K\(^+\) are sodium and potassium ions respectively). This work has demonstrated the importance of modelling as it can give insight into the mechanism of membrane potential. In particular, Edelstein-Keshet (1988) commented “after numerous trial-and-error models”; “the equations were chosen to fit the data, not from a more fundamental knowledge of molecular mechanisms” but however, the model has been able to produce valuable insights. A simplified Hodgkin-Huxley model, with two variables \( V, m \), was introduced later and known as FitzHugh model (Fitzhugh, 1960, 1961). The FitzHugh model presents a tractable model, especially, FitzHugh application of the phase plane analysis of the two variables (FitzHugh, 1961) “played an important role in leading to an understanding of the nature of excitable systems” (Edelstein-Keshet, 1988). In the following, we define the meaning of excitable systems based on excitability and then look at FitzHugh model as an example to illustrate the excitable behaviour.

A widely known definition of an excitable system is given by Arthur T. Winfree (Winfree, 1987):

“A reaction is excitable if it has a unique steady state that the system will approach from all initial conditions, but there exists a locus of initial conditions near which either of two quite different paths may be taken toward the unique steady state. If one of these paths is a lot longer than the other the system is excitable. But no one has yet offered an exact definition: only examples.”
The meaning of an excitable system from a dynamical system perspective is often linked to threshold excitability (McCormick et al., 1991). To understand the meaning of an excitable system, let us look at the example proposed by Richard FitzHugh (FitzHugh, 1961).

The simplified model presented by FitzHugh is given below:

\[
\begin{align*}
\frac{dx}{dt} &= c(y + x - \frac{x^3}{3} + z) \\
\frac{dy}{dt} &= -\frac{(x-a+by)}{c}
\end{align*}
\]

(2.1) (2.2)

where \(x\) represents the voltage of the action potential and \(y\) is a recovery variable; \(a=0.7\), \(b=0.8\) and \(c=3\). Meanwhile \(z\) is the applied stimulus.

For the case of \(z=0\), the phase plane analysis with the nullclines for \(x\) and \(y\) (for \(\frac{dx}{dt} = 0\) and \(\frac{dy}{dt} = 0\)) are shown in Figure 2-14. One specific feature of the model: the \(x\) nullcline is cubic or “N-shaped” and \(y\) nullcline intersect \(x\) nullcline at only one point (Figure 2-14 black dot). This intersection point ensures the system to have a unique steady state, which is a stable. When we refer to the definition given by Winfree (1987), there exists two different initial conditions for \(x\) (\(x=0.6\) and \(x=0.65\)) producing two different trajectories (or paths) that converged to a unique steady state and one of the trajectories (for \(x=0.6\)) is a lot longer than the other. Thus, we say that this system is excitable. Meanwhile when we refer to the simpler definition of the existence of a threshold for excitability, the threshold for this system is represented by a vertical dotted line (purple), which indicates that the activation threshold is between \(x=0.6\) and \(x=0.65\). Although this model is a simple theoretical model, it contributed to the future development of later models of neurons, particularly the Morris-Lecar model which incorporated biologically related parameters (Morris & Lecar, 1981) as will be discussed next for describing some important features of an excitable system.
Figure 2-14 Phase plane analysis demonstrates the excitability of the action potential. The red colour N-shaped curve represents the nullcline for x (when dx/dt=0) and the green line is for y nullcline (when dy/dt=0). The intersection point (labelled as black dot) represents the only stable steady state at (1.199, -0.624). There are four trajectories (black lines) shown in the graph above for four different initial conditions: for y=-0.624, and the values for x are 0.5, 0.6, 0.65 and 0.7 respectively. All four trajectories converge to the only steady state of the system. Diagram adapted from FitzHugh (1961).

The Morris-Lecar model was proposed based on the same concept as FitzHugh model for constructing a simple model of two variables (Morris & Lecar, 1981). This model describes the experimental results for the barnacle muscle fibre and has become a basic model for studying excitable systems (Lecar, 2007). Subsequently, John Rinzel and Bard Ermentrout (1998) illustrated an analysis of the Morris-Lecar model from a dynamical system perspective, using time course simulations, phase plane analyses and bifurcation diagrams, to characterise some of the important features of an excitable system from a biophysically relevant parameters to understand the excitable and oscillatory behaviours of the dynamics of the system (Rinzel & Ermentrout, 1998). In particular, the usage of phase plane and bifurcation analyses has demonstrated a wide range of dynamics (to list a few): excitability with specific threshold value; bistability (one stable steady state and a stable limit cycle oscillation); oscillations emerging from zero frequency (Type I excitability) and oscillations emerging from non-zero frequency (Type II excitability) (Rinzel & Ermentrout, 1998). Rinzel and Ermentrout (1998) showed that by varying a free parameter I_{app} (the applied current) for one set of
parameter values the system exhibits Type I excitability and for another set of parameter values it is Type II excitability.

Next, in summary form, we illustrate threshold excitability, generation of a single pulse or action potential and repeated action potentials (or repeated pulses) using the Morris-Lecar system as an example and introduce the definition of classification of Type I and Type II excitabilities. Interested readers are referred to a neuroscience introductory book by Ermentrout and Terman (2010) for the model equations and parameters, and the details of the analyses. The two model variables are V and W, where V represents membrane potential and W is the fraction of K⁺ channels open. The importance of this model is that it helps us to predict that for certain parameter values the model neuron can generate an action potential or pulse (Ermentrout & Terman, 2010).

In phase plane analysis, the Morris-Lecar model also exhibits excitability with a threshold (Figure 2-15); notice that in a similar system setting as FitzHugh model discussed earlier, the V nullcline is “N-shaped” (red curve in Figure 2-15) and there is one unique stable steady state (one black dot in Figure 2-15). A simpler definition of an excitable system, by referring to this action potential of neurons, is given as follows: an excitable system exhibits excitability, which means “above-threshold initial voltage leads to a rapid response with large changes in the state of the system” (Edelstein-Keshet, 1988). This model demonstrates this property clearly when we look at the phase plane (Figure 2-15) and the time course simulations (Figure 2-16); the voltage V=-20, which is above the threshold of excitability, generates an action potential (the path 1 which is a lot longer than the others and when we view it in time course simulation corresponding to time course 1, it displays an action potential or a pulse in Figure 2-16). Path 2 and 3 are those subthreshold responses that quickly return to the rest state of the model neuron (stable steady state).
Figure 2-15 Phase plane analysis illustrates the threshold excitability (when $W=0.070$, $I_{app}=60$, and for three initial conditions $V$ -22, -20.1 and -20). The threshold is around -20 mV (Ermentrout & Terman, 2010). The red colour N-shaped curve represents the nullcline for $V$ and the green curve is for $W$ nullcline. The intersection point (labelled as black dot) represents the only stable steady state at (-36.755, 0.070198). Path 1 is a lot longer than the others, thus satisfy the definition for excitable system. Diagram adapted from Ermentrout and Terman (2010).
Figure 2-16 The time course of the three trajectories shown in Figure 2-15 (for three initial conditions \( V = -22, -20.1 \) and \(-20\); when \( W = 0.070198 \) and \( I_{\text{app}} = 60 \)). It indicates that the activation threshold is between \( V = -20.1 \) and \( V = -20 \). When \( V = -20 \) which is above the threshold, an action potential is fired. Diagram adapted from Ermentrout and Terman (2010).

In this same example, Ermentrout and Terman (2010) demonstrate the dynamics of single action potential (Figure 2-17) and repeated pulses of action potentials (Figure 2-18) by changing the applied current, \( I_{\text{app}} \) from \( I_{\text{app}} = 60 \) to \( I_{\text{app}} = 100 \). Note that here what we called repeated pulses were also known as sustained oscillations (Ermentrout & Terman, 2010). These simulations also show that the steady state for \( V \) loses stability at higher current and becomes unstable, thus leading to periodic solutions or limit cycle oscillations. In other words, there is a bifurcation from steady state to periodic solutions. These stable steady state and oscillatory dynamics are depicted with a bifurcation diagram when one of the parameters of interest is varied. (Bifurcation analysis will be explained in Chapter 3: Methods). Based on different sets of parameters that produce distinct types of bifurcation and the frequency of the oscillations that arises from the bifurcation, two types of excitability have been proposed (Ermentrout & Terman, 2010; Rinzel & Ermentrout, 1998): Type I excitability (frequency of the oscillations start from 0) and Type II excitability (frequency of the oscillations start from non-zero). This classification based on the frequency when bifurcation arises was compiled and discussed in Rue and Garcia-Ojalvo (2011) (see Figure 2-19). The Type I excitability is based on the existence of a saddle-node on an invariant circle (SNIC) bifurcation where oscillations arise with infinite period.
and thus zero frequency, and the Type II excitability is based on the existence of Hopf bifurcations (Ermentrout & Terman, 2010; Rinzel & Ermentrout, 1998).

Figure 2-17 Time course simulation for $V$ (for initial conditions $V:-20$; when $W=0.070$ and $I_{app}=60$). When $V=-20$ which is above the threshold, an action potential is fired and then stayed at the stable steady state of -36.755. Diagram adapted from Ermentrout and Terman (2010).
Figure 2-18 Time course simulation for V at higher applied current $I_{app}=100$ (same initial conditions: $W=0.070$ and $V=-20$) showing repeated pulses of action potential. These repeated pulses were known as sustained oscillations (Ermentrout & Terman, 2010). Diagram adapted from Ermentrout and Terman (2010).
The discussion above highlights one important point: the information processing in neurons through electrochemical signal propagation in action potentials and cell biochemical reactions in p53 DNA damage response display similar dynamical system behaviours, that is, p53 also shows spontaneous pulses and repeated pulses that may be explained using non-linear dynamical systems theory. But, can we describe p53 excitable dynamics as a threshold excitable system? The excitability of the p53 system is explored in Chapter 4.

### 2.5.2 p53 All-or-none Activation of Bistable Apoptotic Switch

The all-or-none biological switch is important for a living system to make decisions that ensure its survival in the face of varied dynamic stimuli or signals present. For instance, the all-or-none synthesis of an enzyme (β-galactosidase) required for utilising lactose in bacteria, when lactose is the only source of nutrient present, is controlled by a genetic switch now known as “lac operon” (Novick & Weiner, 1957). Since Jacob and Monod well established study on the biochemical switch of lactose operon in *Escherichia coli* (*Jacob & Monod, 1961*), they provided an early example of the importance of theoretical and experimental studies in elucidating a true bistable all-or-none biological switch. This biological switch forms the basis for studying biochemical switches in other living systems. There is evidence from experiments to suggest that apoptosis occurs in an “all or nothing” manner (Goldstein et al., 2000b; Martinou et al., 2000; Rehm et al., 2002), and cells are believed to be...
controlled by an all-or-none apoptotic switch in deciding its life and death fate (Adams & Cory, 2007). An example of a synthetic genetic switch is explained in Chapter 3 (Section 3.6.2) and a discussion of the bistability characteristics conforming to saddle-node bifurcation is given.

One of the important functions of p53 tumour suppression is activation of apoptosis. Molecular biology studies, by using the causal relationship strategy, have established that p53 can activate apoptosis through its transcriptional up-regulation of genes. There are two apoptotic pathways: intrinsic and extrinsic; both of which show switch-like behaviour in apoptosis induction (Albeck et al., 2008; Rehm et al., 2002). Although there have been many models published on the molecular mechanism that regulates the p53 role in activating the intrinsic apoptotic pathway, which is activated at the mitochondria level (Li et al., 2011; Pu et al., 2010; Sun et al., 2009; Tian et al., 2012; Zhang et al., 2007, 2009a; Zhang et al., 2009b), the biochemical control of p53 activation of bistable apoptotic switch is still an open question (Tyson et al., 2011). Thus, in Chapter 5 we propose a model to investigate the activation of p53 apoptotic switch under high DNA damage.

A recent experimental findings by Chen et al. (2013) has discovered novel p53 dynamics correlated to cell fate that exhibit “bimodal switch”: 1) at low dose of drug, p53 level pulses resulting in cell cycle arrest; 2) at high dose of drug, p53 level increases to a high level of 3-8 fold compared to low dose of drug and subsequently leads to apoptosis in 14-39 hours. Therefore, we extended our model constructed in Chapter 4 to explore the second issue of p53 activation of apoptosis under high DNA damage only.

2.6 Summary

In this chapter we review the background knowledge on the p53 pathway. The p53 pathway plays an important role in DNA damage response to stress and is still an active research topic. Molecular cell biology studies have uncovered p53 as a transcription factor that can activate gene expression and the control mechanisms at molecular level, which involved many positive and negative feedback loops. Particularly, the p53-Mdm2 negative feedback loop. Recently, the complexity of the network has been investigated using population cell studies and individual cell studies to capture the p53 protein levels in the form of oscillations (or pulses) after induction of DNA damage by an agent such as gamma radiation or radiometitic drug. These quantitative measurements of p53 dynamics have led to the need to construct mathematical models to simulate and replicate and explain the experimental findings. After reviewing the mathematical models of p53 regulation, we highlighted the advancement of the current understanding of p53 regulation. Then, two current issues were discussed: 1) p53 basal dynamics and DNA damage response in the form of p53 excitable pulses (a brief review of excitable system is presented) and 2) p53 all-or-none activation of apoptosis. These two issues are the problems investigated in this thesis. Before looking at the proposed models, next
chapter presents the methods use in model construction and analysis that can give theoretical insights into p53 regulation.
Chapter 3
Methods

3.1 Overview

This chapter discusses some of the basic concepts in deterministic modelling of biological systems—from the chemical reactions to the formulation of mathematical model equations, model calibration, and model simulation using software such as XPPAUT and MATLAB. Subsequently, model analysis using bifurcation diagrams in the form of saddle-node and Hopf bifurcations is discussed and its interpretation based on the signal-response curve is presented. The corresponding signal in the form of a parameter change and response in the form of an observed variable in terms of a specific protein concentration can give biological insights into certain control parameters affecting gene expression and cell physiology. This method of connecting network dynamics to cell physiology has been proposed and applied successfully in studying cell cycle regulations (Tyson et al., 2001; Tyson et al., 2002) and p53 modelling as discussed in Chapter 2.

3.2 Basic Concepts in Modelling Biological Systems

To start with, we look at conceptual representation of biological systems in schematic diagrams and then review some of the basic chemical reaction laws and formulation for: Mass Action kinetics, Michaelis-Menten kinetics, Michaelis-Menten competitive inhibition and Hill function.

3.2.1 Schematic Diagram of a Biological System

A schematic diagram can be used to describe the molecular interactions in a biological system based on the known experimental findings and is “an important first step” in representing and understanding a system’s structure and dynamics (Kitano, 2002a). It captures the essential components that play an important role in the biological system. It is typically used in this field to illustrate a complex model in a simple way because a diagram can help reader understand the concept or interactions much more easily and quickly. A schematic diagram or wiring diagram is also used to describe the model hypothesis that can then transform into mathematical model equations.

Schematic diagrams have been widely used to present model hypotheses that lead to the construction of kinetic model equations. For this reason, it is very crucial to draw a clear schematic diagram for our biological system of interest and present a clear picture of the problem and model hypothesis for the molecular mechanism that we are investigating. In the next section (Section 3.3), we illustrate how a schematic diagram is used to represent the molecular mechanism that leads to
the construction of model equations using the p53 model from Geva-Zatorsky et al. (2006) as an example. Before that let us look at the basic kinetic laws in modelling chemical reactions.

### 3.2.2 Kinetic Modelling of Chemical Reactions

In this section, a review is given on some of the essential laws for kinetic modelling of chemical reactions that will be used in formulating ordinary differential equations. There are four kinetics laws: 1) Law of Mass Action, 2) Michaelis-Menten, 3) Michaelis-Menten competitive inhibition and 4) Hill function. Cells form complex networks of interacting macromolecules such as DNAs, mRNAs and proteins. These networks can be modelled as a set of chemical reactions that involve substrates (S) being converted to products (P) by enzymes (or proteins). An enzyme (E) acts as a catalyst that accelerates the rate of a reaction.

![Chemical reaction diagram](image)

**1. Law of Mass Action**

Let us consider a reversible reaction below:

\[
\begin{align*}
A + B & \quad \text{\(\rightarrow\)} \quad C \\
& \quad \text{\(\Leftarrow\)} \quad \text{\(\rightarrow\)} \\
& \quad \text{\(\text{\(k_1\)}\)} \quad \text{\(\text{\(k_{-1}\)}\)}
\end{align*}
\]

The mass action kinetics states that the rates of reaction are proportional to the concentrations of the reactants (Aldridge et al., 2006). Therefore, we assume the rate of forward reaction is linearly proportional to the concentrations of A and B, and the backward reaction is linearly proportional to the concentration of C. Thus, the rate equations are:

\[
\begin{align*}
\frac{d[A]}{dt} &= k_{-1} [C] - k_1 [A][B] \\
\frac{d[B]}{dt} &= k_{-1} [C] - k_1 [A][B] \\
\frac{d[C]}{dt} &= k_1 [A][B] - k_{-1} [C]
\end{align*}
\]

where [A], [B] and [C] represent the concentration for molecular species of A, B and C, respectively.
2. Michaelis-Menten Kinetics

For enzyme-catalysed reaction, we consider a reaction given below:

\[
\text{S} + \text{E} \xrightleftharpoons[k_{-1}]{k_1} \text{ES} \xrightarrow{k_2} \text{P} + \text{E}
\]

The Michaelis-Menten mechanism for an enzyme-catalysed reaction: E binds to the substrate S to form an enzyme-substrate complex ES; in the complex, E converts S to P; once the conversion is done, E dissociates from P and is free to bind another molecule of substrate (Conrad & Tyson, 2006). Assuming that the total enzyme concentration \( [E] \) is much less than the initial substrate concentration \( [S]_0 \), the rate of the enzyme-catalysed reaction is given by:

\[
\frac{dP}{dt} = k_2 \frac{[E][S]}{K_m + [S]} \quad (3.4)
\]

where \( K_m = \frac{k_{-1} + k_2}{k_1} \) is called the Michaelis constant and \( k_2 \) is a rate constant.

The Michaelis-Menten formula is also commonly expressed as:

\[
\frac{dP}{dt} = V_{max} \frac{[S]}{K_m + [S]} \quad (3.5)
\]

where \( V_{max} = k_2 [E] \).

3. The Michaelis-Menten Competitive Inhibition Kinetics

For enzyme-catalysed reaction (with competitive inhibition), we consider a reaction given below:

\[
\text{E} + \text{S} \xrightleftharpoons[k_{-1}]{k_1} \text{ES} \xrightarrow{k_2} \text{P} + \text{E}
\]

\[
\text{E} + \text{I} \xrightleftharpoons[K_i]{K_{i}} \text{EI}
\]

The process is almost the same as the above Michaelis-Menten mechanism for an enzyme-catalysed reaction: E binds to the substrate S to form an enzyme-substrate complex ES; in the complex, E converts S to P; once the conversion is done, E dissociates from P and is free to bind another molecule of substrate. However, in addition, there is an inhibitor I binding to E to form EI in a reversible reaction.
The Michaelis-Menten equation for competitive inhibition (Klipp et al., 2008) is expressed as:

$$\frac{dP}{dt} = \frac{V_{max}[S]}{K_m(1+[I]/K_i)+[S]}$$

(3.6)

where [I] is the concentration of inhibitor.

4. Hill Function

A reaction can bind more than one molecule from a given substrate. Usually, binding of the first substrate molecule changes the rate at which the second substrate molecule binds. If the binding rate of the second substrate molecule is increased, it is called positive cooperativity. This property of positive cooperativity is approximated by a Hill function (Klipp et al., 2008) given below:

$$f(x) = \frac{x^n}{K^n + x^n}$$

(3.7)

where $n$ is defined as Hill coefficient and $n$ more than one indicates cooperative binding. Usually, $n$ is assumed to be a positive integer such as 1, 2, 3 or 4. (Note: when $n=1$, it gives the Michaelis-Menten formula)

3.3 Mathematical Modelling of p53 Systems

The p53 pathway involves gene transcriptional networks, protein-protein interactions and signal transduction as previously discussed in Chapter 2. In this context, the p53 network is very complex because the molecular interactions are dynamic and constantly changing according to the cellular processes that respond to stress signals present. Therefore, it is convenient to study the dynamics of the tumour suppressor protein p53 using mathematical models that could represent the molecular interactions or biochemical reactions with a quantitative model. Mathematical models are useful in exploring mechanisms that are not well established where our knowledge is incomplete (Lahav, 2008), and generating new and useful hypotheses (Aldridge et al., 2006).

3.3.1 Steps in Modelling p53 System

1. Model Design

The objective of the model and the modelling method provide a direction for model design. One of the modelling methods is deterministic modelling based on ordinary differential equations. The p53 network can be represented by a schematic diagram that consists of a set of relationships between the molecular species either activated or inhibited which can be described by a set of ordinary differential equations. In general, we assume that there are $n$ species $X_1, X_2, X_3, \ldots, X_n$. The set of ordinary differential equations that describe their fluctuation of concentrations over time due to their interactions with the other species (Conrad & Tyson, 2006) can be written as:
\[ \frac{dx_i}{dt} = \text{synthesis} - \text{degradation} \]  

The rate of each reaction is represented by a rate constant, which is known as a parameter.

For example, Geva-Zatorsky et al. (2006) observed p53 and Mdm2 oscillations in experiments that measured individual cell responses after DNA damage (see Figure 3-1).

Figure 3-1 p53 and Mdm2 oscillations in six individual cells. Reproduced with permission from Molecular Systems Biology.

Here, the objective is to design theoretical model(s) that can generate these oscillations. We look at a model from Geva-Zatorsky et al. (2006), a relaxation oscillator as shown in Figure 3-2 for p53-Mdm2 interaction under damaged conditions:

Figure 3-2 A schematic diagram of the model V from Geva-Zatorsky et al. (2006). Reproduced with permission from Molecular Systems Biology.

Based on this schematic diagram (Figure 3-2), p53 which is represented by x, positively activates its own production with linear feedback (solid arrow from x to x). p53 activates the production or transcription of Mdm2 mRNA y0. Mdm2 mRNA is translated into Mdm2 protein y. On the other hand Mdm2, y, inhibits p53 by promoting p53 degradation (Figure 3-2 dotted arrow with blunt head). The differential equations for this model are:
\[
\frac{dx}{dt} = \beta x - \alpha_{xy} y x
\]  
(3.9)

\[
\frac{dy_0}{dt} = \beta_y x - \alpha_0 y_0
\]  
(3.10)

\[
\frac{dy}{dt} = \alpha_0 y_0 - \alpha_y y
\]  
(3.11)

where the variables represent the concentration of:

- \(x\) = nuclear p53
- \(y_0\) = Mdm2 precursor, representing Mdm2 mRNA
- \(y\) = nuclear Mdm2

The parameters involved are:

- \(\beta\) = linear p53 production rate
- \(\beta_y\) = p53-dependent Mdm2 precursor production rate
- \(\alpha_0\) = Mdm2 maturation rate
- \(\alpha_y\) = Mdm2 degradation rate
- \(\alpha_{xy}\) = Mdm2-dependent p53 degradation rate

The design of the model depends on the objective of the model and the level of detail. The objective of the model is to capture the dynamics of the network in mathematical form with as few essential species and parameters as possible (Aldridge et al., 2006). For the example shown above, the objective is to find a model that could reproduce the undamped oscillations found in experiments (Figure 3-1) (Geva-Zatorsky et al., 2006). The level of detail determines the complexity of the model and the number of species in the model. For instance, the fact that a specific protein like p53 could undergo a number of different post-translational modifications, such as ubiquitination at eleven different residues (Kruse & Gu, 2009), means that each ubiquitination could affect p53 function; but in a simplified model it could be assumed to have three species- p53, p53U and p53UU - representing the forms of p53 with 0, 1 or 2 ubiquitins attached, respectively (Ciliberto et al., 2005). In general, there is no fixed rule and the level of detail is determined by the modeller’s choice and objective.

2. Model Verification, Calibration and Validation

The model constructed needs to be verified with the current understanding of the mechanisms involved in the network and ensure that the equations have incorporated the experimental findings properly (Aldridge et al., 2006). The next step is the mathematical integration of these differential
equations where the model is supplied with a set of initial conditions and a set of parameters that fit the observed experimental data.

The process of finding the right set of parameters is called model calibration and the mathematical integration is usually done with numerical simulation. Some of the parameters can be found from others works in literature, and those that are not available because the rates constant remain unmeasured, have to be estimated using software like Jacobian or Jsim (Aldridge et al., 2006) or COPASI. The parameter estimation is a challenging task and a method that uses a dynamic recursive estimator, called extended Kalman filter, could be applied in estimating the parameters (Lillacci & Khammash, 2010). Tyson Group, proposed the use of trial and error approach to obtain time course simulations and bifurcation diagrams that are consistent with experimental observations (Zhang et al., 2007, 2009a).

For the example discussed above, the set of calibrated parameters and initial conditions are listed in Table 3-1, and the simulated results are shown in Figure 3-3, which shows oscillations of p53 and Mdm2 protein levels (Geva-Zatorsky et al., 2006). For getting results similar to experimental findings in Figure 3-1, Geva-Zatorsky et al. (2006) added low-frequency noise in the protein production rates and exhibited a similar variability in the oscillations (data not shown).

Table 3-1 Parameters and initial conditions for Model V of Geva-Zatorsky et al. (2006).

<table>
<thead>
<tr>
<th>parameters</th>
<th>Estimated best-fit model parameters</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>2.0</td>
<td>$h^{-1}$</td>
</tr>
<tr>
<td>$\beta_y$</td>
<td>1.5</td>
<td>$M_{max}$ $h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_0$</td>
<td>1.1</td>
<td>$h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_y$</td>
<td>0.9</td>
<td>$h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_{xy}$</td>
<td>3.7</td>
<td>$M_{max}^{-1} h^{-1}$</td>
</tr>
</tbody>
</table>

Initial conditions

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Normalised concentration (model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x$</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>$y_0$</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>$y$</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>
In general, there are two modelling approaches for biological systems (Brazhnik & Kohn, 2007). These two approaches are: 1) to simulate and replicate and explain currently available experimental findings; 2) to simulate according to theoretical model assumptions for generating hypothesis or results that may be unknown (or not yet captured in experiments). The example from model V (Geva-Zatorsky et al., 2006) is using the first approach. The objective of the second approach is to find the mechanisms that control some processes “without paying much attention to observational detail” (Brazhnik & Kohn, 2007). According to Brazhnik and Kohn (2007) both approaches are needed for obtaining theoretical understanding of the biological system under study.

The model validation is the process of evaluating the performance of the calibrated model by comparing with experimental data. From the calibrated model, predictions can be simulated and the results can be compared with data from new experiments (Aldridge et al., 2006). In some cases, specifically with the availability of time-lapse microscopy to quantify protein dynamics in individual cells, a comparison of the pattern of the dynamics in terms of period and amplitude can be made. This is the case in p53 dynamics from individual cell studies.

However, some models are simply theoretical simulations that need not be compared to experimental data at all because they are purely theoretical predictions which may not yet have been captured in experiments. However, they may be validated or revisited years or decades later when experiments or technology allows such measurements. If biologists conduct experiments accordingly...
and the numerical simulations and predictions match experimental findings, the model would gain much recognition for predictions and explanations it offered even in the absence of experimental evidence.

3. Model Analysis

Once the calibrated model has been validated, it can be used to analyse parameter sensitivity for assessing the robustness of the model to noise, and dynamical system analysis such as bifurcation. Bifurcation theory enables us to draw one-parameter bifurcation diagram that captures qualitative changes of the steady state(s) of a variable with respect to changes of a parameter of interest. The application of bifurcation diagram to analyse cell physiology is based on the correlation of the qualitative changes in the attractors and repellers of a vector field and the qualitative changes in the state of cell physiology (Tyson, 2011). Attractors are stable steady states and repellers are unstable steady states. For example, a saddle-node bifurcation diagram is used to describe a bistable system.

An example of saddle-node bifurcation diagram is shown in Figure 3-4. It shows the signal-response curve: the changes in the signal (represented by the changes in a parameter, p) with the corresponding response in a gene expression (represented by a variable, u).

![Figure 3-4 A saddle-node bifurcation diagram. For \( p_{\text{inact}} < p < p_{\text{act}} \), there are two stable steady states (nodes represented by two black dots or solid line) and one unstable steady state (also called saddle point represented by an open circle or dotted line). For \( p < p_{\text{inact}} \) the system displays one stable steady state of u small and for \( p > p_{\text{act}} \) the system displays one stable steady state of u large.](image-url)
The saddle-node (SN) bifurcation illustrates qualitative changes from one stable steady state to the behaviour of two stable steady states or bistability. For the parameter values between the two thresholds, $p_{\text{inact}} < p < p_{\text{act}}$, there exist two stable steady states and one unstable steady state. The bistability of a system can be visualised in a two-variable phase plane analysis (Figure 3-5 (b)). For $p_{\text{inact}} < p < p_{\text{act}}$, there are two stable steady states (nodes represented by two black dots in Figure 3-5 (b)), which are attractors and the indication of the existence of bistability. The system can be attracted to either one of the stable steady states, which depends on the state of the system or initial conditions. The unstable steady state (also called saddle point represented by an open circle in Figure 3-5 (b)) is a repeller. At the thresholds, $p = p_{\text{inact}}$ and $p = p_{\text{act}}$, one of the nodes and the saddle point coalesce and disappear (Tyson, 2011). This is the reason why it is called saddle-node bifurcation. For $p < p_{\text{inact}}$ the system displays one stable steady state of $u$ small (Figure 3-5 (a)) and for $p > p_{\text{act}}$ the system displays one stable steady state of $u$ large (Figure 3-5 (c)), which corresponds to the cell physiology of a gene $u$ getting turned off or turned on. Another feature of saddle-node bifurcation is the hysteresis behaviour: the signal required to turn on the gene is $p_{\text{act}}$ which is different than the signal to turn off the gene at $p_{\text{inact}}$ and $p_{\text{inact}}$ is much smaller than $p_{\text{act}}$.

Figure 3-5 Phase planes showing the nullclines and steady state(s). (nullcline is a curve drawn in the phase plane when one of the variables in the system of differential equations is set to zero or does not change in time)

Another important bifurcation diagram is Hopf bifurcation (HB), which is used for characterising oscillatory behaviour when a signal gets large and exceeds a bifurcation point (Tyson, 2011). More about bifurcation analysis is discussed in Section 3.6 on Bifurcation Analysis. In general, model analysis gives insights into concepts or predictions that either have not been or cannot be achieved by experiments and thus adds value to the constructed model because it provides novel insights.
3.4 Delay Differential Equations (DDEs)

Delay differential equations (DDEs) are set of differential equations which include state variables of a previous time. Delay differential equations have been widely used to model various problems in engineering and biology. In particular, the nature of the problems in certain processes or control systems involves time and time lag variables as delayed feedback to the control system. Among the applications of delay differential equations in biological problems include population growth, predator-prey, epidemiology and periodic dynamic diseases (Kuang, 1993; Murray, 2002). For modelling the p53 system, transcription and translation are biochemical processes that require time and thus delay differential equations are needed. The models constructed in this thesis are in the form of DDEs. Therefore, a general definition of DDEs is given next.

The general form of delay differential equations with \( n \) state variables and \( m \) time delays is defined (Engelborghs et al., 2002) as below:

\[
\frac{d}{dt} x(t) = f(x(t), x(t-\tau_1), ..., x(t-\tau_m), p)
\]  
(3.12)

where \( x(t) \in \mathbb{R}^n, f: \mathbb{R}^{n(m+1)} \times \mathbb{R}^q \rightarrow \mathbb{R}^n \) is a non-linear differentiable function depending on a number of parameters \( p \in \mathbb{R}^q \), and the delays \( \tau_i > 0, \ i = 1, ..., m \). In these definitions, \( \mathbb{R} \) is a real line and \( \mathbb{R}^q \) represents the q dimensional vector space. The maximal delay is \( \tau = \max_{i=1, ..., m} \tau_i \).

Once we formulate our model equations, they can be solved numerically using a numerical solver or computer software. Next we look at some of the software for numerical simulation and analysis.

3.5 Software for Simulating and Analysing Mathematical Models

There is a wide range of software for numerical simulation of mathematical model equations such as COPASI (Hoops et al., 2006), CellDesigner (Funahashi et al., 2003), MATLAB solvers (Shampine & Reichelt, 1997), and XPPAUT (Ermentrout, 2002). The common software (or solver) for numerical integration of delay differential equations are dde23 (a solver from MATLAB) and XPPAUT. In this thesis the software XPPAUT was chosen for simulation of time course results because of its graphical user interface features and it is a free software that is widely used in dynamical systems studies in the scientific community (Ermentrout, 2002). Also, XPPAUT is chosen for time integration simulation because it is comparatively more efficient than dde23 and takes less time to complete the simulation tasks required in this thesis. However, XPPAUT simulation can be carried out in MATLAB by using a XPP-MATLAB interface package written by Rob Clewley, which is freely downloadable from http://www2.gsu.edu/~matrhc/XPP-Matlab.html. XPP-MATLAB enables the running of XPPAUT in MATLAB environment or using MATLAB commands. This package provides MATLAB’s flexibility of changing parameter values, for example, when performing sensitivity analysis, and the speed of...
numerical simulation of XPPAUT. (An example of how to use the XPP-MATLAB package in performing local parameter sensitivity analysis is given Appendix B.2). This combination of powers is an attractive solution for sensitivity analysis that requires many time course calculations, and it is particularly useful for global sensitivity analysis when computational cost is a major concern.

Bifurcation analysis of ordinary differential equations without time delay can be performed using AUTO that is included in XPPAUT (Ermentrout, 2001), Oscill8 (Conrad, 2006) and MATCONT (Dhooge et al., 2003). These software with a graphical interface are user friendly and use the numerical continuation method. Among these software, Oscill8 is the easiest to use, and thus is highly recommended for those who are new in drawing bifurcation diagrams. The Oscill8 software is written by Emery Conrad and is freely downloadable from http://oscill8.sourceforge.net/. The bifurcation analysis of the model proposed in this thesis involves time delay and was done with MATLAB codes that use DDE-BIFTOOL (Engelborghs et al., 2002), a package for bifurcation analysis of delay differential equations. The bifurcation analysis of differential equations with time-delays is more complex as mentioned in Waldherr et al. (2010). There is no graphical user interface software for bifurcation analysis of delay differential equations and it requires a good knowledge of delay differential equations, which will be explained in the following section.

### 3.6 Bifurcation Analysis

A biological system represented by a mathematical model of non-linear ordinary differential equations is a dynamical system that can be analysed with bifurcation theory. For detailed theory on bifurcation, readers are referred to a textbook on non-linear dynamical systems (Strogatz, 1994) and an introductory course lectures from John J. Tyson titled “A Primer in Bifurcation Theory for Computational Cell Biologists” (Tyson, 2010). Basically, a bifurcation diagram is a diagram showing the value of steady states and the type of steady states whether stable or unstable (y-axis) with respect to the change of a parameter of interest (x-axis). Steady state is also known as fixed point, which is defined by the solution(s) of the differential equations set to zero(s). When the stability of a steady state changes or steady state is created or destroyed, there is a qualitative change in the dynamics and thus it is called bifurcation (Strogatz, 1994). Bifurcation diagram is a powerful tool for visualising the vector field (of variables) of a dynamical system with higher dimensionality than two-variable model equations (Tyson et al., 2001). It expands the conceptual phase plane analysis as discussed in FitzHugh and Morris-Lecar models on neuron excitability (Section 2.5.1) and enables us to see the steady states and vector field of more than two dimensions and qualitative changes in the system dynamics or bifurcation.

Stable steady states are attractors, whereas unstable steady states are repellers. Some attractors can be in the form of closed orbits or limit cycles, which can be visualised in time course simulations.
as oscillations. The identified attractors and oscillators are compared with the observations of corresponding states of cell physiology (Tyson et al., 2001). Therefore, bifurcation diagram is a useful tool in quantifying the qualitative behaviour change of some variable of interest with respect to certain changes in one specific parameter. As mentioned earlier, a bifurcation diagram illustrates stable and unstable steady states of a variable, and another important information it provides is the minimum and maximum values of an oscillation of the value of the variable when one of the parameter is changed (Goldbeter, 2002).

Two types of bifurcation that are commonly used in analysing biochemical networks are saddle-node and Hopf bifurcation. The saddle-node is used to describe biochemical switches and the Hopf bifurcation is applied to characterise biochemical oscillators (Tyson et al., 2003; Waldherr et al., 2010). In literature, an analogy has been given to describe switches and oscillators (also known as clocks), that is, switches explain multiple steady states and clocks describe periodic behaviours (Tyson et al., 2008). The historical development of biochemical models studying switches and clocks are given in Tyson et al. (2008) and it also highlights the significant contributions of theoretical models and recognition of the need for such models in future advancements. The importance of bifurcation analysis was demonstrated by a prominent work on analysing cell cycle regulation in Xenopus (Novak & Tyson, 1993; Tyson et al., 2002). Particularly, the saddle-node bifurcation was used for the prediction of bistable switch in the cell cycle events from the Interphase (I) to Mitosis (M) phase (Tyson et al., 2002). This theoretical model of cell cycle has successfully predicted the hysteretic behaviour in Xenopus egg extracts (Novak & Tyson, 1993; Tyson et al., 2002). Notable is the prediction of hysteretic (or toggle switch-like) behaviour (two distinct thresholds of cyclin protein concentration: higher one to turn on mitosis with high MPF; and a lower one to turn off and return to interphase with low MPF) in the control of cell division using bifurcation theory, which was verified by experiments from two different laboratories (Pomerening et al., 2003; Sha et al., 2003). On the other hand, the characterisation of Hopf bifurcation was used effectively in Morris-Lecar model analysis of neural oscillations as discussed in Chapter 2 (Rinzel & Ermentrout, 1998).

3.6.1 Bifurcation Analysis of DDEs

In this section, the theory of saddle-node and Hopf bifurcation diagram generation using DDE-BIFTOOL (Engelborghs et al., 2002) for model equations involving time delay is described and one example of each will be discussed in the following sections.

The theoretical and mathematical background of bifurcation in DDEs is given in this section and for convenience the mathematical notation is retained as in Engelborghs et al. (2000a) and Engelborghs et al. (2002). To find the steady state solutions, one needs to do linearization of the non-linear system. The linearisation of delay differential equations is slightly different to that for general ODEs.
in that it does not produce a jacobian matrix; however, the linearization of the general form of delay differential equations listed in (3.12) around a solution \( x^* (t) \) will lead to the variational equations (Engelborghs et al., 2002) defined as follows:

\[
\frac{d}{dt} y(t) = A_0(t) y(t) + \sum_{i=1}^{m} A_i(t) y(t - \tau_i)
\]

(3.13)

Using a notation for \( f(x(t), x(t - \tau_1), \ldots, x(t - \tau_m), p) = f(x^0, x^1, \ldots, x^m, p) \) and

\[
A_i(t) = \frac{\partial f}{\partial x_i} (x^*(t), x^*(t - \tau_1), \ldots, x^*(t - \tau_m), p), i = 0, 1, \ldots, m
\]

(3.14)

and assuming \( x^* (t) \) represents a steady state solution, where \( x^*(t) \equiv x^* \in \mathbb{R}^n \), this steady state solution means \( f(x^*, x^*, \ldots, x^*, p) = 0 \), since \( x^* \) is a steady state point. Then, matrices \( A_i(t) \equiv A_i \) are matrices with some real values and when substituted into the variational equation (3.13) leads to a characteristic function. Then, we define a squared \( s \times s \) matrix \( \Delta \) as

\[
\Delta(x^*, p, \lambda) = \lambda I - A_0 - \sum_{i=1}^{m} A_i e^{-\lambda \tau_i}
\]

(3.15)

Then, the characteristic equation is given by

\[
\det(\Delta(x^*, p, \lambda)) = 0
\]

(3.16)

The characteristic equation gives an infinite number of complex eigenvalues \( \lambda \in \mathbb{C} \) and these eigenvalues are called characteristic roots. The eigenvalues are used to classify the steady state solutions; stable steady state solution is found when all characteristic roots are with negative real parts. However, for unstable steady state one of the characteristic roots has positive real part.

The method of numerical continuation is used to obtain the bifurcation diagram and the details of the theory are given in the paper by Engelborghs et al. (2002). It is of great interest to know how to locate the bifurcation points and classify the type of bifurcation; in this case either saddle-node or Hopf bifurcation. A bifurcation occurs when the characteristic root crosses the imaginary axis as the parameter of interest is varied. In other words, **saddle-node bifurcation (also called fold bifurcation) occurs when an eigenvalue is zero** \( (\lambda = 0) \) and Hopf bifurcation occurs when a pair of eigenvalues is purely imaginary \( (\lambda = \pm i \omega) \) where the real part of the eigenvalues are zero (Engelborghs et al., 2002).

The implementation of the DDE-BIFTOOL is given in a report with an example (Engelborghs et al., 2000b), which is of great help to those who want to obtain a bifurcation diagram for their model. However, the readers are assumed to have some basic knowledge of bifurcation theory and often for those without a strong understanding, it is hard to grasp the steps of the implementation. For instance, Krauskopf has commented that the process of obtaining a bifurcation diagram for DDEs as
“no pain no gain” and “some pain a lot to gain” (Krauskopf, 2005). Hence, for the benefit of understanding the procedures of generating bifurcation diagram of DDEs, the implementation to obtain saddle-node bifurcation and Hopf bifurcation is illustrated using two examples from biology.

### 3.6.2 Saddle-Node Bifurcation

The first example is from a famous synthetic genetic switch (Gardner et al., 2000), which was constructed from two mutually inhibiting genes. In other words, one of the genes U encodes protein u that inhibits the transcription of another gene V, which encodes protein v that inhibits the transcription of gene U. This genetic circuit is shown in Figure 3-6.

![Gene Circuit](image)


The understanding of this synthetic biochemical switch may have biomedical implications in that it can be applied in gene therapy in future as reported in Gardner et al. (2000) study. This is one of the reasons why this model is chosen here. Gardner et al. (2000) proposed a mathematical model for the genetic switch to gain a deeper understanding through predictions from the model that guided their experiments, and it is given by two differential equations below:

\[
\frac{du}{dt} = \frac{a_1}{1+u^n} - u \\
\frac{dv}{dt} = \frac{a_2}{1+u^m} - v
\]

(3.17)  
(3.18)

The u and v are the levels of u and v proteins and a set of model parameters was chosen from Edelstein-Keshet (2012) as an example for bifurcation analysis and is given below:
The differential equations above with the chosen parameter values can produce saddle-node bifurcation because there exist two stable steady states and one unstable steady state, which is a typical requirement for bistability of biological switches. A phase plane with the $u$ nullcline and $v$ nullcline is shown in Figure 3-7; the intersection points from these nullclines are the steady state points of the system. From the vector field or flow of the trajectories, we can see two stable steady states as attractors and one unstable steady state as repeller.

Figure 3-7 A phase plane of the $u$-$v$ axes. The $u$ nullcline defined by $du/dt=0$ (red) intersects with the $v$ nullcline defined by $dv/dt=0$ (green) at three points and these points are called steady states (or fixed points). Two of the steady states are stable steady states represented by black dots ($0.107, 2.99$) and ($2.99, 0.107$), these two points are basin of attractors; however, one unstable steady state is denoted by an empty dot ($1.164, 1.164$) in the middle, this point is a repeller. Diagram adapted from Edelstein-Keshet (2012). (This diagram was later published in a book: “Segel, L.A., & Edelstein-Keshet, L. (2013). A Primer on Mathematical Models in Biology: SIAM” Reproduced with permission from SIAM)
There is a separatrix (cyan line) in Figure 3-7 that separates the two basin of attractors. The initial conditions decide which basin of attractor a trajectory will be moving towards and thus which protein is turned on (with high protein levels). To illustrate an instance of the bistability: for one set of initial conditions $u_1=0.84$ and $v_1=0.87$ (Figure 3-8), the system is attracted to the top left basin of attractor with $u$ low (0.107) and $v$ high (2.99); and for another set of initial conditions $u_2=0.87$ and $v_2=0.84$ (Figure 3-9), the system is attracted to the bottom right basin of attractor with $u$ high (2.99) and $v$ low (0.107). The qualitative analysis of a biochemical switch generally involves drawing the nullclines of a two variables system in the phase plane and to identify the steady state solutions as in Figure 3-7.

![Graph](image)

Figure 3-8 Time course simulation shows the system is attracted to the top left basin of attraction with $u$ low (0.107) and $v$ high (2.99).
Figure 3-9 Time course simulation shows the system is attracted to the bottom right basin of attraction with \( u \) high (2.99) and \( v \) low (0.107).

The model equations for this synthetic biochemical switch are very similar to a mathematical model (Equation 13 in Thornley (1972) paper) for studying a biochemical switch for flower initiation. The two equations proposed by Thornley (1972) in investigating the vegetative and flowering control system in plants are given below:

\[
\frac{de_v}{dt} = \frac{1}{1+(e_f/e)^2} - e_v \quad (3.19)
\]

\[
\frac{de_f}{dt} = \frac{1}{1+(e_v/e)^2} - e_f \quad (3.20)
\]

where \( e_v \) and \( e_f \) are the amounts of vegetative and flowering enzymes, respectively. Here, there is only one parameter given by the constant \( e \) and for obtaining a bistable switch \( e < 0.5 \).

Returning back to the synthetic genetic switch to further analyse bifurcation points associated with steady states in Figure 3-7, let us consider that the parameter of interest for bifurcation analysis is \( \alpha_1 \). Since there is no time delays in the model equations, bifurcation diagram can be generated from Oscill8. The Oscill8 codes are given in Appendix A.1 and the bifurcation diagrams are given in Figure 3-10 and Figure 3-11. The saddle-node bifurcation points are \( \alpha_1 = 1.91 \) at SN7 and \( \alpha_1 = 9.98 \) at SN3.
Figure 3-10 A bifurcation diagram for the variable $u$ (y-axis) against the bifurcation parameter $\alpha_1$ (x-axis) without time delay. SN3 and SN7 are two saddle-node (SN) bifurcation points. Solid line represents stable steady states and dotted line denotes unstable steady states.
The bifurcation diagrams for $u$ and $v$ indicate that for low value of $\alpha_1$ below the saddle-node SN3 $v$ is turned on and $u$ is turned off because $v$ represses $u$. In contrast, for $\alpha_1$ above the saddle-node SN3 $u$ is turned on and $v$ is turned off because $u$ represses $v$. Thus, the bifurcation analysis shows that there is bistability in this system.

To illustrate the saddle-node bifurcation for DDEs, a time delay $\tau=0.01$ for $v$ is introduced in the equation (3.17); however, the equation (3.18) is remained the same and the model equations are modified as below:

$$\frac{du}{dt} = \frac{a_1}{1+v(t-\tau)^h} - u$$  \hspace{1cm} (3.21)$$

$$\frac{dv}{dt} = \frac{a_2}{1+u^m} - v$$  \hspace{1cm} (3.22)$$

With the application of DDE-BIFTOOL and the MATLAB scripts listed in Appendix A.2 (step-by-step instructions and comments are provided), one can obtain bifurcation diagram for the corresponding DDEs of the system. The bifurcation diagrams are given in Figure 3-12 and Figure 3-13 with the
confirmed identification of two saddle-node bifurcation points (zero eigenvalue) at \( \alpha_1 = 1.918 \) and \( \alpha_1 = 9.986 \).

Figure 3-12 A bifurcation diagram for the variable \( u \) (y-axis) against the bifurcation parameter \( \alpha_1 \) (x-axis) with time delay. Thick line represents stable steady states and dotted line denotes unstable steady states.

DDE-BIFTOOL generation of bifurcation diagram is not fully automated, and thus each step given in the example from Engelborghs et al. (2000b) should be followed carefully. The steps for saddle-node bifurcation identification include finding one zero eigenvalue for the characteristic equation (3.16) (or eigenvalue crosses the imaginary axis) as mentioned previously. Analysis was conducted on the point number 312 and 560 (Figure 3-14) for which the real part of \( \lambda \) is zero using two of the functions in DDE-BIFTOOL to locate the saddle-node (or fold) bifurcation point: \texttt{p_tofold()} and \texttt{p_correc()}. Figure 3-15 explicitly indicates (red asterisk) the first saddle-node bifurcation, for \( \alpha_1 = 1.918 \), where one of the eigenvalues is zero (\( \lambda = 0 \)).
Figure 3-13 A bifurcation diagram for the variable $v$ (y-axis) against the bifurcation parameter $\alpha_1$ (x-axis) with time delay. Thick line represents stable steady states and dotted line denotes unstable steady states.
Figure 3-14 Plot of the real eigenvalue against point number. Saddle-node bifurcation occurs when real eigenvalue is zero at point 312 and 560 (indicated by the vertical red dotted line). In this plot, it shows saddle-node bifurcation occurs when real eigenvalue is zero at point number 312 and point number 560.

Figure 3-15 A plot of the eigenvalues showing the Imaginary (y-axis) versus Real (x-axis) for the first saddle-node bifurcation point for $\alpha_1=1.918$. The red asterisk denotes a zero eigenvalue ($\lambda=0$); both complex and real parts are zero.

Similarly, at the second saddle-node bifurcation, $\alpha_1 = 9.986$, and one of the eigenvalues is zero ($\lambda = 0$) (or eigenvalue crosses the imaginary axis) (right asterisk) as confirmed in Figure 3-16.
From this first example, bifurcation analysis provides a theoretical exploration of a simple mathematical model constructed with double-negative feedback loops to create a bistable switch. In fact, double negative feedback loop is a motif that displays bistability. When one of the parameters of interest is varied, either one of the variables is on at any single time. In this case, the variable v is turned on for small value of $\alpha_1$. However, when $\alpha_1$ increases over a critical value of the bifurcation parameter $\alpha_1 = 9.986$, v is suddenly turned off, and u gets turned on simultaneously because the bistability no longer exist and u gets attracted to the only stable steady state with high value (See Figure 3-17). (In order to obtain these time course simulation from XPPAUT, one needs to start from a specified initial conditions and integrate the system of equations. Then change the parameter value and follow by integrating the system of equations using from last or XPPAUT command: InitialConds-Integrate-Last, which uses the end point of the previous integration as the initial point of the current integration; in this way the memory or state of system is retained). Thus, it creates a bistable switch.
Figure 3-17 Time course simulation shows (for $\alpha_1 = 10$; larger than the threshold value 9.986) $v$ is suddenly turned off, and $u$ gets turned on simultaneously.

This bistable switch demonstrates hysteresis property, another important feature of a bistable switch, that is, it has memory and tends to stay in this state, whereby to turn off $u$ it requires $\alpha_1$ value lower than 1.918 (see Figure 3-18). This simple (two variables) example demonstrates that DDE-BIFTOOL enables bifurcation analysis of DDEs; specifically, the biochemical network that is bistable as captured by the existence of saddle-node bifurcation.
Figure 3-18 Time course simulation shows (for $\alpha_1=1.8$; lower than the threshold value 1.918) u gets turned off and v gets turned on simultaneously.

3.6.3 Hopf Bifurcation

For the second example, a simple model for *Xenopus* embryonic cell cycle oscillator with time delay from Ferrell et al. (2011) is chosen to illustrate Hopf bifurcation analysis. The motivation for modelling cell cycle oscillator is based on experimental findings that demonstrated periodic oscillations of cyclin, which is correlated with cell cycle phases (Murray & Kirschner, 1989; Murray et al., 1989). Two of the essential proteins that control cell cycle are the cyclin-dependent kinase 1 (CDK1) and the anaphase-promoting complex (APC); CDK1 activation starts mitosis and APC activation ends mitosis (Ferrell et al., 2011). A two component model of ODEs, CDK1 activates APC and APC inhibits CDK1, is insufficient to produce sustained oscillations as proposed by Ferrell et al. (2011). The network structure is in the form of negative feedback loop as shown in Figure 3-19.
The equations for the two-ODE model proposed by Ferrell et al. (2011) are as below:

\[
\frac{dCDK1a}{dt} = \alpha_1 - \beta_1 CDK1a \frac{APCa^{n1}}{K_1^{n1} + APCa^{n1}} \tag{3.23}
\]

\[
\frac{dAPCa}{dt} = \alpha_2 (1 - APCa) \frac{CDK1a^{n2}}{K_2^{n2} + CDK1a^{n2}} - \beta_2 APCa \tag{3.24}
\]

CDK1a and APCa are the active CDK1 and the active APC, respectively. The above two equations are formulated based on the rate of activation minus the rate of inactivation. Ferrell et al. (2011) assumed Hill functions for the APC inhibition of CDK1 and CDK1 activation of APC in the formulation of these model equations (The detailed formulation of these two equations are explained in Ferrell et al. (2011)). The model parameters chosen are listed below:

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Description</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>( \alpha_1 )</td>
<td>Activation rate of CDK1</td>
<td>0.1</td>
</tr>
<tr>
<td>2.</td>
<td>( \alpha_2 )</td>
<td>Activation rate of APC</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>( \beta_1 )</td>
<td>Inactivation rate of CDK1</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>( \beta_2 )</td>
<td>Inactivation rate of APC</td>
<td>1</td>
</tr>
<tr>
<td>5.</td>
<td>( K_1 )</td>
<td>Michaelis constant for inactivation of CDK1</td>
<td>0.5</td>
</tr>
<tr>
<td>6.</td>
<td>( K_2 )</td>
<td>Michaelis constant for activation of APC</td>
<td>0.5</td>
</tr>
<tr>
<td>7.</td>
<td>( n_1 )</td>
<td>Hill coefficient</td>
<td>8</td>
</tr>
<tr>
<td>8.</td>
<td>( n_2 )</td>
<td>Hill coefficient</td>
<td>8</td>
</tr>
</tbody>
</table>

Based on this set of model parameters, the time course simulations are shown in Figure 3-20 and it is not possible to generate oscillations.
Figure 3-20 Time course simulation from the two-ODE model system (Ferrell et al., 2011) shows damped oscillations based on the parameter values given. Reproduced with permission from Cell.

To illustrate that this two-ODE model cannot produce oscillations, a bifurcation diagram can be plotted for a selected parameter of interest; let us assume the parameter of interest is $\beta_2$. A bifurcation diagram is shown in Figure 3-21 for the variable of interest CKD1a. The bifurcation diagram shows that no matter what values are used for $\beta_2$, CDK1a reached a stable steady state similar to the time course simulation in Figure 3-20. So, what kind of strategy can be used to make this two-ODE model to oscillate will be discussed next. One of the ways is to introduce time delays because “the negative feedback signal must be sufficiently delayed in time so that the chemical reactions do not settle on a stable steady state” (Novák & Tyson, 2008); time delays is one of the design principles for generating oscillations in biochemical reactions (Novák & Tyson, 2008).
Figure 3-21 Bifurcation diagram for CDK1a (CDK1a is the activated form of CDK1) against $\beta_2$ which shows there is no Hopf bifurcation and only stable steady states, thus confirming that oscillations are not possible.

As this minimal model of two-ODE is not able produce sustained oscillations, Ferrell et al. (2011) proposed a modified model equation system with two time delays $\tau_1$ and $\tau_2$ to replace or represent some intermediate processes (presumably unknown multistep mechanism) so that we can obtain robust limit cycle oscillations as shown in Figure 3-22. The model equations are given below:

\[
\frac{dCDK1a[t]}{dt} = \alpha_1 - \beta_1 CDK1a[t] \frac{APCa[t-\tau_1]^{n_1}}{K_1^{n_1} + APCa[t-\tau_1]^{n_1}} \tag{3.25}
\]

\[
\frac{dAPCa[t]}{dt} = \alpha_2 (1 - APCa[t]) \frac{CDK1a[t-\tau_2]^{n_2}}{K_2^{n_2} + CDK1a[t-\tau_2]^{n_2}} - \beta_2 APCa[t] \tag{3.26}
\]

The model parameters chosen (Ferrell et al., 2011) are listed below (all biological parameters are the same as for the model without time delays):

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$\alpha_1$</td>
<td>Activation rate of CDK1</td>
<td>0.1</td>
</tr>
<tr>
<td>2.</td>
<td>$\alpha_2$</td>
<td>Activation rate of APC</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>$\beta_1$</td>
<td>Inactivation rate of CDK1</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>$\beta_2$</td>
<td>Inactivation rate of APC</td>
<td>1</td>
</tr>
</tbody>
</table>
Thus, this example illustrated that time delays are an important strategy to achieve limit cycle oscillations.

The modified model equations become DDEs that successfully produce robust limit cycle oscillations because it changes the dynamical system so that bifurcation happens. The MATLAB scripts that utilised DDE-BIFTOOL to generate Hopf bifurcation diagram are given in Appendix A.3. The chosen bifurcation parameter is $\beta_2$ that is varied while monitoring the stability of the system. The existence
of Hopf bifurcation corresponds to limit cycle oscillations for the variables of the system. The Hopf bifurcation diagram is shown in Figure 3-23.

![Bifurcation diagram for the cell cycle oscillator two-ODE model with two time delays](image)

Figure 3-23 Bifurcation diagram for the cell cycle oscillator two-ODE model with two time delays (Ferrell et al., 2011). The purple graph represents the minimum and maximum of the periodic solutions for CDK1a in the region of limit cycle oscillations.

Next, we demonstrate how to locate Hopf bifurcation points. The Hopf bifurcation occurs when a pair of complex conjugates of characteristic roots of the characteristic equation (3.16) crosses the imaginary axis or when the real part of the eigenvalue is zero or the eigenvalue is purely imaginary ($\lambda = \pm \omega$). This bifurcation can be examined from Figure 3-24.
Figure 3-24 Plot of the real eigenvalue against point number. Hopf bifurcation occurs when real eigenvalue is zero at point 312 and 525 (indicated by the vertical red dotted line). In this plot, it shows Hopf bifurcation occurs when real eigenvalue is zero at point number 312 and point number 525.

Plot of the real eigenvalue against point number guides the identification of Hopf bifurcation (Figure 3-24). In this figure, point 312 and 525 indicates where Hopf bifurcation occurs. Further analysis was done on the point number 312 and 525 using two of the functions in DDE-BIFTOOL to locate the Hopf bifurcation point: p_tohopf() and p_correc(). The parameter value of the Hopf bifurcation are found and confirmed at these two points: first Hopf bifurcation, $\beta_2 = 4.271$ (Figure 3-25); and the second Hopf bifurcation is at $\beta_2 = 0.021$ (Figure 3-26). For both values of $\beta_2$, the eigenvalues are purely imaginary ($\lambda = \pm i \omega$) where the real parts are zeros as shown in Figure 3-25 and Figure 3-26.
Figure 3-25 At the first Hopf bifurcation at $\beta_2 = 4.271$, real parts are zeros or eigenvalues are purely imaginary ($\lambda = \pm i\omega$) as indicated by the pair of characteristic roots on the right.

Figure 3-26 At the second Hopf bifurcation at $\beta_2 = 0.021$, real eigenvalue are zeros or eigenvalues are purely imaginary ($\lambda = \pm i\omega$) as indicated by the pair of characteristic roots on the left.
According to Strogatz (1994), the definition of a supercritical Hopf bifurcation is given as “a supercritical Hopf bifurcation occurs when a stable spiral changes into an unstable spiral surrounded by a small, nearly elliptical limit cycle.” Let us examine the bifurcation diagram obtained in Figure 3-23. Values of parameter $\beta_2$ greater than 4.271, for example $\beta_2 = 4.5$, result in a stable spiral as shown in Figure 3-27 (a). However, when the parameter $\beta_2$ is decreased from $\beta_2 = 4.271$, for example $\beta_2 = 4.2$, a stable limit cycle is born as shown in Figure 3-27 (b) with unstable spiral (unstable spiral is within the limit cycle and is not shown for visual clarity). This characteristic of a stable spiral changing to an unstable spiral with stable limit cycle when a parameter value is varied (shown as flows in phase space in Figure 3-27) indicates the bifurcation is a supercritical Hopf bifurcation.

The qualitative changes for supercritical Hopf bifurcation in terms of the time course simulations can be seen in Figure 3-28.
Figure 3-28 Time course simulations illustrating the qualitative changes about the bifurcation point.

The numerical computation for finding the period of the oscillations can be found in the papers (Engelborghs et al., 2000a; Engelborghs et al., 2002) and manual (Engelborghs et al., 2000b) that basically uses orthogonal collocation. DDE-BIFTOOL provides useful functions that one can easily use to get the characteristics of limit cycle oscillations. For limit cycle oscillations (Figure 3-27 (b)), these characteristics are period, maximum and minimum as shown in the time course oscillations in Figure 3-28 (b). To get these values, DDE-BIFTOOL require as input one of the Hopf bifurcation points and it starts numerical continuation along a branch of the periodic limit cycle oscillations which can then be used to plot the maximum and minimum of the bifurcation diagram (Figure 3-23), and to obtain the period of the oscillations.

Through these two simple examples, it is clear now how we can use DDE-BIFTOOL to locate and find saddle-node and Hopf bifurcations for DDEs. These two types of bifurcation, saddle-node and Hopf bifurcations, are important events to investigate biochemical switches and periodic oscillations, respectively. Generally, drawing of a bifurcation diagram requires numerical computations, and the steps involved in generating a bifurcation diagram are complex and incomprehensible to beginners. This is compounded by the fact that some software (e.g., AUTO or Oscill8) provide automated steps to generate bifurcation diagrams and these steps are hidden behind the scene for convenience of the users. It is hoped that these two examples illustrate the concept and the implementation of the numerical bifurcation theory specifically for biological systems that include time delays. In general, the incorporation of time delays provides a way to generate robust oscillations.
3.7 Summary

In this chapter, we review the basic concepts and steps in formulating a mathematical model equations using as an example p53 relaxation model proposed by Geva-Zatorsky et al. (2006). We highlight the importance of bifurcation analysis as a powerful tool for analysing a biological system as a dynamical system that links to the state of cell physiology. Following that a brief discussion was given on the methods of bifurcation analysis by analysing few other simple biological models constructed in the form DDEs: a synthetic bistable genetic switch modified with time delay from Gardner et al. (2000) and cell cycle oscillator from Ferrell et al. (2011). Particularly, the key steps involved in drawing saddle-node and Hopf bifurcation diagrams using the package DDE-BIFTOOL were elucidated. In the next two chapters, this thesis proposed two models of p53 system to explain the basal dynamics and DNA damage response, and the apoptotic switch activated by p53 in response to sustained high DNA double-strand breaks.
Chapter 4
A Mathematical Model of the Core Regulatory Feedback
Mechanism of p53 Protein that Controls Basal Dynamics and DNA Damage Response Involving Cell Cycle Arrest

4.1 Overview
In this chapter a mathematical model is proposed to address the first issue: p53 basal dynamics and DNA damage response to DNA double-strand breaks (DSBs). Specifically, how p53 responses with a series of repeated pulses with a period of 4-7 hours to externally-induced stress, and with few spontaneous pulses (basal dynamics) under normal growth (Loewer et al., 2010) is the focus of this chapter. In the literature on p53, it is known that p53 is kept at low levels by Mdm2-mediated degradation under non-stressed conditions (Moll & Petrenko, 2003); however, Loewer et al. (2010) demonstrated that some of the individual cells exhibit high p53 levels similar to those in cells with damage induced by radiomimetic drug neocarzinostatin (NCS) (Loewer et al., 2010). Further investigations by this team of researchers have shown that, most of these individual cells display more than one spontaneous pulse in a day.

It is still unclear what mechanism can generate excitability in p53 pulses in response to DNA double-strand breaks (Batchelor et al., 2011). Current understanding from Batchelor et al. (2009) suggests that p53 system is excitable and requires a positive feedback loop (Batchelor et al., 2009) (i.e., signal amplification). Specifically, their hypothesis on p53 network functioning as a pulse generator suggested that “When a stimulus such as DNA damage is present, p53 shows a pulse only if the stimulus is large enough to push p53 over an activation threshold” (Batchelor et al., 2009). This opinion suggests that mathematical models for explaining p53 dynamics as pulses with an activation threshold is lacking. Thus, the proposed model explores p53 excitable dynamics and DNA damage response in terms of p53 dynamics and its function as a transcription factor that activates a number of downstream genes in deciding cell fate.

Sun et al. (2011) modelled the p53 system with a deterministic model as an initial attempt, but it could not explain the spontaneous pulses of p53 basal dynamics; therefore, they converted the deterministic model structure to a delay stochastic model based on binomial tau-leap method and two-lesion-kinetics of DNA damage repair to explain the p53 basal dynamics in great detail. This stochastic model produced the expected behaviour well; however, the model is complex and is based on the assumption that stochasticity in DSBs generation and repair is behind the p53 spontaneous
pulses. Our study attempts to find out if a simpler deterministic model incorporating the most recently found, and hypothesised, molecular interactions can produce the same basal dynamics. To our knowledge there has not yet been a deterministic model to explain them. For this purpose, in this chapter, we aim to modify and extend the Sun et al. (2011) deterministic model to incorporate more accurate design principles of the p53 molecular system. The design principles of the model should generate appropriate defence responses to both stressed and non-stressed conditions including cell cycle arrest induced by p21 activation which is the p53 triggered response to initiate repair of DNA damage. We explore the following questions:

1) What are the core regulators that control p53 DNA damage response?

2) What is the mechanism that regulates p53 activation of cell cycle arrest in stressed conditions?

3) What is the model structure that gives rise to spontaneous p53 pulses in non-stressed conditions?

Are p53 pulses excitable and if so what gives rise to it?

4) How p53 dynamics accurately decide cell fate within a spectrum of possible decisions?

### 4.2 Introduction to the Core Regulators that Control p53 DNA Damage Response

The p53 gene encodes a tumour suppressor protein p53 that plays a critical role in maintaining the stability of the genome in humans (Vogelstein et al., 2000; Vousden & Lane, 2007). In response to various stresses, such as DNA damage and oncogene activation, p53 acts as a transcription factor to activate its target genes (Riley et al., 2008). The activation of p53 protects cells from genotoxic stresses that could lead to tumourigenesis through cell cycle arrest, DNA repair, senescence (permanent cell cycle arrest), and apoptosis (programmed cell death) (Toledo & Wahl, 2006). Since p53 activation as a transcription factor could result in killing cells, p53 activities need to be regulated appropriately to avoid errant activation (Wahl, 2006). One of the effective ways to regulate p53 is: p53 activates some genes such as Mdm2 to regulate its own activity.

Mdm2 is the master feedback regulator that controls p53 stability and activity (Momand et al., 2000). Mdm2 is an E3 ubiquitin ligase that binds to p53, and facilitates the degradation of p53. In addition, Mdm2 binds to p53’s N-terminal transactivation domain and inhibits p53 interaction with co-activators and as a result represses p53 transcriptional function (Toledo & Wahl, 2006). The transcription of mdm2 is up-regulated by p53, thus forming an auto-regulatory feedback loop (Wu et al., 1993). The p53-Mdm2 negative feedback loop is one of the well-known biological networks that shows oscillatory behaviour in individual cells after gamma-irradiation induced stress (Geva-Zatorsky et al., 2006; Lahav et al., 2004).
The activation of p53 is also regulated by another essential regulator, MdmX, an Mdm2-related protein that binds to p53 and inhibits p53 transcription activation function (Marine & Jochemsen, 2004; Shvarts et al., 1996). Genetic studies show that Mdm2 and MdmX are non-redundant regulators that inhibit p53 function during embryonic development, working alone and together in a synergetic manner. Both are required for proper embryonic development (Marine et al., 2006).

Recently, p53 has been shown to activate mdmx transcription, MdmX forms the second p53-MdmX negative feedback loop (Li et al., 2010; Phillips et al., 2010); however, little attention has been paid to incorporating MdmX activation by p53 into a mathematical model.

Furthermore, p53 activates another target gene referred to as wild-type p53-induced phosphatase 1, Wip1 (or PPM1D) (Fiscella et al., 1997). Its product Wip1, protein phosphatase, dephosphorylates (inhibits) p53 (Lu et al., 2005), ataxia telangiectasia mutated (ATM) protein kinase signalling molecule (see Figure 2-1) (Batchelor et al., 2008), Mdm2 (Lu et al., 2007) and MdmX (Zhang et al., 2009c). Thus, Wip1 forms the third p53-Wip1 negative feedback loop that modulates the level of p53 activation.

ATM plays a crucial role in p53 DNA damage response (see Figure 2-1); it detects DNA damage and invokes downstream activities of DNA damage response including phosphorylation of p53, Mdm2, and MdmX (Cheng & Chen, 2010). In the case of DNA double-strand breaks, DSBs are detected by ATM and this stress signal is amplified by ATM auto-phosphorylation (Bakkenist & Kastan, 2003). Hence, ATM activation is essential in stress signal detection for p53 DNA damage response.

4.3 Mathematical Model

We propose a deterministic model for the core regulatory feedback mechanism of p53 protein, consisting of the core regulators ATM, Mdm2, MdmX, Wip1 and p53, and show that it can monitor p53 levels and function appropriately in response to stress with a series of repeated pulses, as well as spontaneous pulses under normal growth with intrinsic DNA damage. Intrinsic DNA damage is small internally induced damage, for example, caused by the reactive oxygen species from metabolic and endogenous processes (De Bont & van Larebeke, 2004).

Quantitative models that can reproduce experimental observations are an important tool for understanding the dynamics of molecular interactions, and offer an explanation to the observed experimental behaviour (Mogilner et al., 2012). We use a mathematical model of delay differential equations to investigate the molecular interactions in the core regulation of p53 in normal proliferating cells and cells under DNA damage stress. This is a deterministic and mechanistic modelling study to explain the design principles responsible for the precise regulation of p53 in normal cell growth and its activation in inducing p21 for arresting cell cycle in the case of DNA damage.
damage. Subsequently, we simulated the dynamics of p53, Mdm2, MdmX, ATMp and Wip1 in response to stress and our results show that this molecular mechanism regulating p53 levels appropriately decide cell fate. Our model simulation results were consistent with the experimental findings from Loewer et al. (2010) in that p53 can generate spontaneous pulses for intrinsic DNA damage and repeated pulses in the presence of DSB requiring cell cycle arrest for initiating damage repair. The details of the methodology, results and interpretations are presented in the rest of the chapter.

4.4 Methods

4.4.1 Model Construction for the p53 Core Regulatory Network

The model is constructed based on the core regulatory network of p53 and is an improved model from the one (deterministic model) proposed by Sun et al. (2011). We aim to develop a model that is consistent with the limited experimental data, and can be used to make further predictions or gain insights into p53 regulation in light of new experimental findings. P53 regulation involves many post-translational modifications such as phosphorylation, ubiquitination, acetylation, methylation and sumoylation (Bode & Dong, 2004). Our model is a simplified representation that includes phosphorylation, acetylation and degradation mediated by Mdm2. The main differences in our model compared to Sun et al. (2011) model are that in our model: 1) p53 auto regulation (positive feedback loop) is included; 2) MdmX is included; 3) Mdm2 and MdmX inhibit p53 acetylation; 4) p53-Mdm2, Mdm2-MdmX and p53-MdmX complexes are represented explicitly as variables. The model (equations) was integrated with XPPAUT, a software program freely downloadable from www.math.pitt.edu/~bard/xpp/xpp.html.

Figure 4.1 shows a schematic diagram of the model. When cells are exposed to stress, for example gamma irradiation, it causes DNA double-strand breaks (DSBs). The DSB is the input into the model. (For simplicity, the number of DSBs is represented by DSB, a model parameter which could be plural or singular). The DSB activate the protein kinase, ataxia telangiectasia mutated (ATM) stress signalling molecule and these stress signals are further amplified by ATM intermolecular auto-phosphorylation at Serine 1981 (Bakkenist & Kastan, 2003). The DSB caused ATM phosphorylation results in a cascade of phosphorylation activities that activates p53 (Figure 4-1 green arrows, turn on p53) (Cheng & Chen, 2010). Firstly, ATM phosphorylation of Mdm2 prevents the ability of Mdm2 binding to p53 and degradation of p53 (Maya et al., 2001). At the same time, Mdm2 switches the target of ubiquitination from p53 to itself and MdmX, and thus facilitates p53 activation (Wade et al., 2010). Secondly, ATM phosphorylation of p53 on the N terminus Serine 15 further disrupts Mdm2 binding and stabilizes p53. Note that ATM also activates check point kinase 2 (Chk2), which then phosphorylates p53 on Serine 20 (Cheng & Chen, 2010). However, Chk2 is not included in our model.
for simplicity since Chk2 concentration is relatively constant (Kastan & Bartek, 2004). Finally, ATM also phosphorylates MdmX. Phosphorylation of MdmX enhances binding, ubiquitination and degradation by Mdm2 (Cheng & Chen, 2010).

Figure 4-1 Schematic representation of the model. A schematic diagram incorporates the molecular interactions of the p53 core regulatory network. For clarity, a few model interactions are not shown.

Phosphorylated p53 (P53p) can be further phosphorylated at different sites, represented by P53pp. It is assumed that both P53p and P53pp activate the transcription of p53 itself (Deffie et al., 1993; Wang & El-Deiry, 2006). P53p and P53pp also activate the transcription of mdm2, mdxm and Wip1. We adopted the transcriptional time delay of 30 min and translation/translocation delay of 10 min proposed by Ma et al. (2005). The up-regulation of Wip1 plays a role in modulating ATM-dependent signalling pathway, and attenuating the p53 response. Wip1 function as a phosphatase that dephosphorylates ATM, p53, Mdm2, and MdmX (Figure 4-1 red arrows, turn off p53) (Wade et al., 2010). Wip1 reverses the stress signal protein ATMp and p53p to un-phosphorylated form, resetting ATM and p53 to non-active state (Lu et al., 2005; Shreeram et al., 2006). Thus, Wip1 creates a p53
negative feedback mechanism that attenuates the stress signal and p53 activation. Moreover, Wip1 dephosphorylates Mdm2 and MdmX; these dephosphorylations stabilize Mdm2 and MdmX and then lead to the inhibition of p53 activities (Lu et al., 2007; Zhang et al., 2009c). Therefore, p53 activation by ATM is rapid because ATM is sensitive to stress signal (Bakkenist & Kastan, 2003), and feedback from Wip1 ensures that p53 activation in general is not in sustained active state that promotes cell cycle arrest and apoptosis, which can have strong anti-growth effect (Vousden & Lane, 2007).

MdmX inhibits p53 mainly by forming a p53-MdmX complex (Cheng & Chen, 2010), and this is represented by a reversible reaction of p53-MdmX complex (C3) formation and dissociation (Figure 4-2). MdmX also regulates p53 levels by modulating Mdm2 levels and E3 ligase activity towards p53 ubiquitination and degradation through the heterodimers Mdm2-MdmX (C2) (Linke et al., 2008), and this reversible reaction is represented by the reaction of Mdm2-MdmX complex (C2) formation and dissociation. The p53-Mdm2 complex (C1) formation and dissociation is also included in this model to represent the binding and unbinding between Mdm2 and p53 protein molecules (Schon et al., 2002).

Moreover, Mdm2 is assumed to inhibit p53 activity by repressing p53 acetylation. This assumption is based on the experimental results that demonstrated that Mdm2 suppresses p300/CBP acetylation of p53, where p300 and CBP are acetyltransferases that function as co-activators to promote p53 acetylation (Ito et al., 2001). Similarly, MdmX was also reported to suppress p300/CBP acetylation of p53 (Sabbatini & McCormick, 2002) and both Mdm2 and MdmX inhibition of p53 acetylation are represented by a barred arrow in Figure 4-1. These inhibitions by Mdm2 and MdmX of p53 acetylation were modelled as competitive inhibition reactions (See Eqn. 4.10). Acetylated p53 (P53a) is assumed to activate p21, a gene that encodes protein P21, which acts as a cyclin-dependent kinase (Cyclin E/cdk2) inhibitor to arrest cell cycle, and causes G1 arrest (Kastan & Bartek, 2004). For clarity, not all model interactions are shown in Figure 4-1 and Figure 4-2. These interactions are listed below:

1. Mdm2 protein and heterodimer C2 promote P53 protein degradation (see Eqn. 4.7)
2. P21 protein degradation is mediated by heterodimer C2 (Jin et al., 2008) (see Eqn. 4.11)

3. DSB induces Mdm2 protein degradation (Ciliberto et al., 2005) (see Eqn. 4.12)

4. Mdm2p promotes auto-ubiquitination and degradation of Mdm2 (see Eqn. 4.13)

### 4.4.2 Model Equations

Model assumption for the gene expression up-regulated by p53 is represented by a Hill function with Hill coefficient 4 as discussed in Section 3.2.2. For example, the mRNA synthesis rate $f(x)$ based on p53 protein level ($x$) is given by:

$$ f(x) = e_i \frac{x^4}{K^4 + x^4} $$

where $K$ denotes Michaelis constant of p53-dependent mRNA transcription and $e_i$ represents p53-dependent transcription rate.

Most of the ODEs formulated describe rate of reactions for production, degradation, association, dissociation, activation and inhibition are based on mass action kinetics as discussed in Section 3.2.2.

The equations of the model are given below:

#### Equations for mRNAs:

**P53 mRNA**: The first term describes the synthesis, the second term describes gene transcription from P53p and P53pp, and the third term describes degradation of p53 mRNA.

$$ \frac{dp53}{dt} = sp53 + \frac{[p53p(t-\tau_3) + p53pp(t-\tau_3)]^4}{K_{p53}^4 + [p53p(t-\tau_3) + p53pp(t-\tau_3)]^4} - \delta_{p53}[p53] \tag{4.1} $$

**P21 mRNA**: The first term describes gene transcription from P53a and the second term describes degradation of p21 mRNA.

$$ \frac{dp21}{dt} = e_4 \frac{[p53a(t-\tau_4)]^4}{K_{p21}^4 + [p53a(t-\tau_4)]^4} - \delta_{p21}[p21] \tag{4.2} $$

**Mdm2 mRNA**: The first term describes the synthesis, the second term describes gene transcription from P53p and P53pp, and the third term describes degradation of Mdm2 mRNA.

$$ \frac{dm2}{dt} = sm2 + \frac{[p53p(t-\tau_1) + p53pp(t-\tau_1)]^4}{K_{m2}^4 + [p53p(t-\tau_1) + p53pp(t-\tau_1)]^4} - \delta_{m2m}[m2] \tag{4.3} $$

**MdmX mRNA**: The first term describes the synthesis, the second term describes gene transcription from P53p and P53pp, and the third term describes degradation of MdmX mRNA.
\[
\frac{d[mdmx]}{dt} = s_{mdmx} + e_3 \left( \frac{[P53p(t-\tau_3)+P53pp(t-\tau_3)]^4}{K_m^3 + [P53p(t-\tau_3)+P53pp(t-\tau_3)]^4} \right) - \delta_{mdmx}[mdmx]
\] (4.4)

**Wip1 mRNA:** The first term describes the synthesis, the second term describes gene transcription from P53p and P53pp, and the third term describes degradation of Wip1 mRNA.

\[
\frac{d[\text{wip1}]}{dt} = s_{\text{wip1}} + e_2 \left( \frac{[P53p(t-\tau_2)+P53pp(t-\tau_2)]^4}{K_m^3 + [P53p(t-\tau_2)+P53pp(t-\tau_2)]^4} \right) - \delta_{\text{wip1}}[\text{wip1}]
\] (4.5)

**Equations for Proteins:**

**ATM\text{p}:** The first term describes the DSB activation of ATM, the second term describes auto activation of ATM, the third term describes basal degradation of ATM\text{p} and the last term describes Wip1 dephosphorylation of ATM\text{p}.

\[
\frac{d[ATM\text{p}]}{dt} = k_{DSB} \frac{DSB}{DSB + K_{DSB}}[ATM] + k_{auto}[ATM][ATMp] - k_{basal}[ATMp] - k_{wip4}[Wip1][ATMp]
\] (4.6)

**P53:** The first term describes the translation of p53 mRNA, the second term describes the degradation of P53, the third term describes the Mdm2-dependent P53 degradation, the fourth term describes the degradation of P53 by C2, the fifth term describes ATM\text{p} phosphorylation of P53, the sixth term describes Wip1 dephosphorylation of P53\text{p}, the seventh term describes the association of P53-Mdm2 complexes, the eighth term describes the dissociation of P53-Mdm2 complexes, the ninth term describes the association of P53-MdmX complexes, and the last term describes the dissociation of P53-MdmX complexes.

\[
\frac{d[P53]}{dt} = r_{p53}[p53(t - \tau_0)] - \mu_{p53}[P53] - k_1[C1] - k_2[P53][C2] - k_{atm1}[ATM][P53] + k_{wip1}[P53p][Wip1] - k_{f1}[MdM2][P53] + k_{b1}[C1] - k_{f3}[mdmx][P53] + k_{b3}[C3]
\] (4.7)

**P53\text{p}:** The first term describes the ATM\text{p} phosphorylation of P53, the second term describes the Wip1 dephosphorylation of P53\text{p}, the third term describes the phosphorylation of P53\text{p}, the fourth term describes the dephosphorylation of P53pp, and the last term describes the P53p degradation.

\[
\frac{d[P53\text{p}]}{dt} = k_{atm1}[ATM\text{p}][P53] - k_{wip1}[P53p][Wip1] - k_{phos}[P53p] + k_{dephos}[P53pp] - \mu_{p53p}[P53p]
\] (4.8)

**P53pp:** The first term describes the phosphorylation of P53p, the second term describes the dephosphorylation of P53pp, and the last term describes the P53pp degradation.
\[
\frac{d[P53pp]}{dt} = k_{phos}[P53p] - k_{depkos}[P53pp] - \mu_{P53pp}[P53pp] \quad (4.9)
\]

**P53a:** The first term describes the acetylation of P53 inhibited by Mdm2, the second term describes the acetylation of P53 inhibited by MdmX and the last term describes the degradation of P53a.

\[
\frac{d[P53a]}{dt} = V_{max1}\frac{[P53]}{[P53]+K_{m1}+K_{m1}} + V_{max2}\frac{[P53]}{[P53]+K_{m2}+K_{m2}} + k_{p53a}[P53a] \quad (4.10)
\]

**P21:** The first term describes the translation of p21 mRNA, the second term describes the degradation of P21 and the last term describes the degradation of P21 by Mdm2-MdmX complexes.

\[
\frac{d[p21]}{dt} = r_{p21}[p21(t - \tau_7)] - \mu_{p21}[P21] - k_{d21}[C2][P21] \quad (4.11)
\]

**Mdm2:** The first term describes the translation of Mdm2 mRNA, the second term describes the degradation of Mdm2, the third term describes the ATMP phosphorylation of Mdm2, the fourth term describes the association of P53-Mdm2 complexes, the fifth term describes the dissociation of P53-Mdm2 complexes, the sixth term describes the association of Mdm2-MdmX complexes, the seventh term describes the dissociation of Mdm2-MdmX complexes, the eighth term describes the Wip1 dephosphorylation of Mdm2p and the last term describes the DSB-dependent degradation of Mdm2.

\[
\frac{d[Mdm2]}{dt} = r_{mdm2}[mdm2(t - \tau_8)] - \mu_{mdm2}[Mdm2] - k_{atm2}[ATMP][Mdm2] - k_{f1}[Mdm2][P53] + k_{b1}[C1] - k_{f2}[Mdm2][MdmX] + k_{b2}[C2] + k_{wip2}[Mdm2p][Wip1] - k_{d22}\frac{DSB}{f+DSB}[Mdm2] \quad (4.12)
\]

**Mdm2p:** The first term describes the ATMP phosphorylation of Mdm2, the second term describes the Wip1 dephosphorylation of Mdm2p, the third term describes the Mdm2-dependent degradation of Mdm2 (auto-ubiquitination) and the last term describes the degradation of Mdm2p.

\[
\frac{d[Mdm2p]}{dt} = k_{atm2}[ATMP][Mdm2] - k_{wip2}[Mdm2p][Wip1] - k_{f4}[Mdm2][Mdm2] - \mu_{mdm2p}[Mdm2p] \quad (4.13)
\]

**MdmX:** The first term describes the translation of MdmX mRNA, the second term describes the degradation of MdmX, the third term describes the ATMP phosphorylation of MdmX, the fourth term describes the Wip1 dephosphorylation of MdmXp, the fifth term describes the association of Mdm2-MdmX complexes, the sixth term describes the dissociation of Mdm2-MdmX complexes, the seventh term describes the association of P53-MdmX complexes and the last term describes the dissociation of P53-MdmX complexes.
\[
\frac{d[MdmX]}{dt} = r_{mdmx}[mdmx(t - \tau_2)] - \mu_{mdmx}[MdmX] - k_{atm3}[ATM_p][MdmX] \\
+k_{wip3}[MdmXp][Wip1] - k_{f2}[Mdm2][MdmX] + k_{b2}[C2] \\
-k_{f3}[MdmX][P53] + k_{b3}[C3]
\] (4.14)

**MdmXp:** The first term describes the ATMp phosphorylation of MdmX, the second term describes the Wip1 dephosphorylation of MdmXp, the third term describes the Mdm2-dependent MdmX degradation (Mdm2 ubiquitination of MdmX) and the last term describes the degradation of MdmXp.

\[
\frac{d[MdmXp]}{dt} = k_{atm3}[ATM_p][MdmX] - k_{wip3}[MdmXp][Wip1] \\
-k_3[MdmXp][Mdm2p] - \mu_{mdmx}[MdmXp]
\] (4.15)

**Wip1:** The first term describes the translation of Wip1 mRNA and the second term describes degradation of Wip1.

\[
\frac{d[Wip1]}{dt} = r_{wip1}[wip1(t - \tau_1)] - \mu_{wip1}[Wip1]
\] (4.16)

**C1 (P53-Mdm2 complexes):** The first term describes the association of P53-Mdm2 complexes and the second term describes the dissociation of P53-Mdm2 complexes.

\[
\frac{d[C1]}{dt} = k_{f1}[Mdm2][P53] - k_{b1}[C1]
\] (4.17)

**C2 (Mdm2-MdmX complexes):** The first term describes the association of Mdm2-MdmX complexes and the second term describes the dissociation of Mdm2-MdmX complexes.

\[
\frac{d[C2]}{dt} = k_{f2}[Mdm2][MdmX] - k_{b2}[C2]
\] (4.18)

**C3 (P53-MdmX complexes):** The first term describes the association of P53-MdmX complexes and the second term describes the dissociation of P53-MdmX complexes.

\[
\frac{d[C3]}{dt} = k_{f3}[MdmX][P53] - k_{b3}[C3]
\] (4.19)

We assumed that the ATM level is constant at 1 \(\mu M\) (Sun et al., 2011) as the expression of ATM is relatively constant (Kastan & Bartek, 2004). Thus, the concentration for ATM is given by:

\[
[ATM] = 1 - [ATM_p]
\] (4.20)
## 4.5 Model Parameters and Initial Conditions

Table 4-1 Parameters and their values used in the model. Same parameter values were used for stressed and non-stressed conditions. For stressed conditions DSB is set to 300 and for non-stressed conditions DSB is changed to one or three.

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$s_{p53}$</td>
<td>Basal production rate of P53 mRNA</td>
<td>0.0005</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>$s_{mdm2}$</td>
<td>Basal production rate of Mdm2 mRNA</td>
<td>0.002</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>$s_{mdmx}$</td>
<td>Basal production rate of MdmX mRNA</td>
<td>0.001</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>$s_{wip1}$</td>
<td>Basal production rate of Wip1 mRNA</td>
<td>0.002</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>5</td>
<td>$e_1$</td>
<td>P53-dependent $mdm2$ transcription rate</td>
<td>0.02</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>6</td>
<td>$e_2$</td>
<td>P53-dependent $Wip1$ transcription rate</td>
<td>0.014</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>7</td>
<td>$e_3$</td>
<td>P53-dependent $mdmx$ transcription rate</td>
<td>0.005</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>8</td>
<td>$e_4$</td>
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<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>9</td>
<td>$e_5$</td>
<td>P53-dependent $p53$ transcription rate</td>
<td>0.02</td>
<td>$\mu M\ min^{-1}$</td>
</tr>
<tr>
<td>10</td>
<td>$K_{p53}$</td>
<td>Michaelis constant of p53-dependent p53 transcription</td>
<td>0.017</td>
<td>$\mu M$</td>
</tr>
<tr>
<td>11</td>
<td>$K_{p21}$</td>
<td>Michaelis constant of p53-dependent p21 transcription</td>
<td>0.017</td>
<td>$\mu M$</td>
</tr>
<tr>
<td>12</td>
<td>$K_m$</td>
<td>Michaelis constant of p53-dependent $mdm2$ transcription</td>
<td>0.16</td>
<td>$\mu M$</td>
</tr>
<tr>
<td>13</td>
<td>$K_x$</td>
<td>Michaelis constant of p53-dependent $mdmx$ transcription</td>
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<td>$\mu M$</td>
</tr>
<tr>
<td>14</td>
<td>$K_w$</td>
<td>Michaelis constant of p53-dependent $Wip1$ transcription</td>
<td>0.2</td>
<td>$\mu M$</td>
</tr>
<tr>
<td>15</td>
<td>$\delta_{p53}$</td>
<td>Degradation rate of P53 mRNA</td>
<td>0.03</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>16</td>
<td>$\delta_{p21}$</td>
<td>Degradation rate of P21 mRNA</td>
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<td>$min^{-1}$</td>
</tr>
<tr>
<td>17</td>
<td>$\delta_{mdm2}$</td>
<td>Degradation rate of Mdm2 mRNA</td>
<td>0.05</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>18</td>
<td>$\delta_{mdmx}$</td>
<td>Degradation rate of MdmX mRNA</td>
<td>0.03</td>
<td>$min^{-1}$</td>
</tr>
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<td>19</td>
<td>$\delta_{wip1}$</td>
<td>Degradation rate of Wip1 mRNA</td>
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<td>$min^{-1}$</td>
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<td>$min^{-1}$</td>
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<td>$min^{-1}$</td>
</tr>
<tr>
<td>22</td>
<td>$r_{mdm2}$</td>
<td>Translation rate of Mdm2</td>
<td>0.04</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>23</td>
<td>$r_{mdmx}$</td>
<td>Translation rate of MdmX</td>
<td>0.01</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>24</td>
<td>$r_{wip1}$</td>
<td>Translation rate of Wip1</td>
<td>0.02</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>25</td>
<td>$\mu_{p53}$</td>
<td>Basal degradation rate of P53</td>
<td>0.03</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>26</td>
<td>$\mu_{p53p}$</td>
<td>Basal degradation rate of P53p</td>
<td>0.01</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>27</td>
<td>$\mu_{p53pp}$</td>
<td>Basal degradation rate of P53pp</td>
<td>0.004</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>28</td>
<td>$\mu_{p53a}$</td>
<td>Basal degradation rate of P53a</td>
<td>0.001</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>29</td>
<td>$\mu_{p21}$</td>
<td>Basal degradation rate of P21</td>
<td>0.03</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>30</td>
<td>$\mu_{mdm2}$</td>
<td>Basal degradation rate of Mdm2</td>
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<td>$min^{-1}$</td>
</tr>
<tr>
<td>31</td>
<td>$\mu_{mdmx}$</td>
<td>Basal degradation rate of MdmX</td>
<td>0.03</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>32</td>
<td>$\mu_{wip1}$</td>
<td>Basal degradation rate of Wip1</td>
<td>0.035</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>33</td>
<td>$\mu_{mdm2p}$</td>
<td>Degradation rate of Mdm2p</td>
<td>0.1</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>34</td>
<td>$\mu_{mdmxp}$</td>
<td>Degradation rate of Mdmxp</td>
<td>0.2</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>35</td>
<td>$k_1$</td>
<td>Mdm2-dependent P53 degradation</td>
<td>0.2</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>36</td>
<td>$k_2$</td>
<td>C1(Mdm2-MdmX)-dependent P53 degradation</td>
<td>0.01</td>
<td>$\mu M^{-1} min^{-1}$</td>
</tr>
<tr>
<td>37</td>
<td>$k_3$</td>
<td>Mdm2-dependent Mdmx degradation (Mdm2 ubiquitination of MdmX)</td>
<td>1.5</td>
<td>$\mu M^{-1} min^{-1}$</td>
</tr>
<tr>
<td>38</td>
<td>( k_4 )</td>
<td>Mdm2-dependent Mdm2 degradation (auto-ubiquitination)</td>
<td>0.1</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>39</td>
<td>( k_{atm1} )</td>
<td>ATM induced P53 phosphorylation</td>
<td>0.8</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
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<td>( k_{atm2} )</td>
<td>ATM induced Mdm2 phosphorylation</td>
<td>0.02</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>41</td>
<td>( k_{atm3} )</td>
<td>ATM induced MdmX phosphorylation</td>
<td>0.02</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
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<td>42</td>
<td>( k_{wip1} )</td>
<td>Wip1 induced P53p dephosphorylation</td>
<td>1.3</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>43</td>
<td>( k_{wip2} )</td>
<td>Wip1 induced Mdm2p dephosphorylation</td>
<td>0.5</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>44</td>
<td>( k_{wip3} )</td>
<td>Wip1 induced MdmXp dephosphorylation</td>
<td>0.2</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>45</td>
<td>( k_{wip4} )</td>
<td>Wip1 induced ATMp dephosphorylation</td>
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<td>( \mu M^{-1} \ min^{-1} )</td>
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<tr>
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<td>( k_{DSB} )</td>
<td>DSB induced ATM activation rate</td>
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<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>47</td>
<td>( DSB )</td>
<td>Double-strand Breaks (300 approximately 10 Gy ( \gamma )-irradiation)</td>
<td>300</td>
<td>Unit of 1</td>
</tr>
<tr>
<td>48</td>
<td>( K_{DSB} )</td>
<td>Activation scaling parameter</td>
<td>200</td>
<td>Unit of 1</td>
</tr>
<tr>
<td>49</td>
<td>( k_{auto} )</td>
<td>ATM auto-activation</td>
<td>0.07</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>50</td>
<td>( k_{basal} )</td>
<td>ATMp basal inactivation rate</td>
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<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>51</td>
<td>( k_{f1} )</td>
<td>P53-Mdm2 complex association rate</td>
<td>552</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
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<td>( k_{f2} )</td>
<td>Mdm2-MdmX complex association rate</td>
<td>600</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
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<tr>
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<td>P53-MdmX complex association rate</td>
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<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
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<td>( k_{p1} )</td>
<td>P53-Mdm2 complex dissociation rate</td>
<td>123.6</td>
<td>( min^{-1} )</td>
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<td>( k_{p2} )</td>
<td>Mdm2-MdmX complex dissociation rate</td>
<td>18</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>56</td>
<td>( k_{p3} )</td>
<td>P53-MdmX complex dissociation rate</td>
<td>123.6</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>57</td>
<td>( k_{phospho} )</td>
<td>P53 further phosphorylation by other enzymes (e.g. Chk2)</td>
<td>0.3</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>58</td>
<td>( k_{dephos} )</td>
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<td>( min^{-1} )</td>
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<td>59</td>
<td>( V_{max1} )</td>
<td>The maximal rate of p53 acetylation (Mdm2)</td>
<td>0.0001</td>
<td>( \mu M \ min^{-1} )</td>
</tr>
<tr>
<td>60</td>
<td>( V_{max2} )</td>
<td>The maximal rate of p53 acetylation (MdmX)</td>
<td>0.001</td>
<td>( \mu M \ min^{-1} )</td>
</tr>
<tr>
<td>61</td>
<td>( K_{m1} )</td>
<td>The half-saturation constant (Mdm2)</td>
<td>0.000025</td>
<td>( \mu M )</td>
</tr>
<tr>
<td>62</td>
<td>( K_{m2} )</td>
<td>The half-saturation constant (MdmX)</td>
<td>0.5</td>
<td>( \mu M )</td>
</tr>
<tr>
<td>63</td>
<td>( K_{i1} )</td>
<td>The dissociation constant for the enzyme-inhibitor interaction (e.g. p300-Mdm2)</td>
<td>0.00001</td>
<td>( \mu M )</td>
</tr>
<tr>
<td>64</td>
<td>( K_{i2} )</td>
<td>The dissociation constant for the enzyme-inhibitor interaction (e.g. p300-MdmX)</td>
<td>0.0001</td>
<td>( \mu M )</td>
</tr>
<tr>
<td>65</td>
<td>( k_{d21} )</td>
<td>C2 induced P21 degradation</td>
<td>0.5</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>66</td>
<td>( J )</td>
<td>Degradation scaling parameter</td>
<td>0.2</td>
<td>Unit of 1</td>
</tr>
<tr>
<td>67</td>
<td>( k_{d22} )</td>
<td>DSB induced Mdm2 degradation</td>
<td>0.01</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>68</td>
<td>( \tau_1 )</td>
<td>mdm2 transcription delay</td>
<td>30</td>
<td>( \mu M^{-1} \ min^{-1} )</td>
</tr>
<tr>
<td>69</td>
<td>( \tau_2 )</td>
<td>wip1 transcription delay</td>
<td>30</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>70</td>
<td>( \tau_3 )</td>
<td>mdmx transcription delay</td>
<td>30</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>71</td>
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<td>30</td>
<td>( min^{-1} )</td>
</tr>
<tr>
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<td>( \tau_5 )</td>
<td>p53 transcription delay</td>
<td>30</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>73</td>
<td>( \tau_6 )</td>
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<td>10</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>74</td>
<td>( \tau_7 )</td>
<td>P21 translational delay</td>
<td>10</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>75</td>
<td>( \tau_8 )</td>
<td>Mdm2 translational delay</td>
<td>10</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>76</td>
<td>( \tau_9 )</td>
<td>MdmX translational delay</td>
<td>10</td>
<td>( min^{-1} )</td>
</tr>
<tr>
<td>77</td>
<td>( \tau_{10} )</td>
<td>Wip1 translational delay</td>
<td>10</td>
<td>( min^{-1} )</td>
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</table>
| 78 | \( n \) | Hill coefficient | 4 | }
Table 4-2 The initial conditions used in the model for stressed and non-stressed conditions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Molecular species</th>
<th>Meaning</th>
<th>Value (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p53</td>
<td>P53 mRNA</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>p21</td>
<td>P21 mRNA</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>mdm2</td>
<td>Mdm2 mRNA</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>mdmx</td>
<td>MdmX mRNA</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>wip1</td>
<td>Wip1 mRNA</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>ATMp</td>
<td>Phosphorylated ATM</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>P53</td>
<td>P53 protein</td>
<td>0.0258</td>
</tr>
<tr>
<td>8</td>
<td>P53p</td>
<td>Phosphorylated P53 protein</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>P53pp</td>
<td>Multiple Phosphorylated P53 protein</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>P53a</td>
<td>Acetylated P53 protein</td>
<td>0.01</td>
</tr>
<tr>
<td>11</td>
<td>P21</td>
<td>P21 protein</td>
<td>0.01</td>
</tr>
<tr>
<td>12</td>
<td>Mdm2</td>
<td>Mdm2 protein</td>
<td>0.15</td>
</tr>
<tr>
<td>13</td>
<td>Mdm2p</td>
<td>Phosphorylated Mdm2 protein</td>
<td>0.0178</td>
</tr>
<tr>
<td>14</td>
<td>MdmX</td>
<td>MdmX protein</td>
<td>0.08</td>
</tr>
<tr>
<td>15</td>
<td>MdmXp</td>
<td>Phosphorylated MdmX protein</td>
<td>0.01</td>
</tr>
<tr>
<td>16</td>
<td>Wip1</td>
<td>Wip1 protein</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>C1</td>
<td>P53-Mdm2 complex</td>
<td>0.06</td>
</tr>
<tr>
<td>18</td>
<td>C2</td>
<td>Mdm2-MdmX complex</td>
<td>0.05</td>
</tr>
<tr>
<td>19</td>
<td>C3</td>
<td>P53-MdmX complex</td>
<td>0.05</td>
</tr>
</tbody>
</table>

4.6 Results

4.6.1 p53 Basal Dynamics under Normal Cell Proliferation (Non-stressed Conditions)

Under non-stressed conditions, p53 levels are known to be maintained at low level during normal cell proliferation based on most previous studies where measurements of p53 protein levels were from population of cells (Berger, 2010). By contrast, Loewer et al.’s (2010) single-cell microscopy approach has revealed that p53 levels are not always at a low basal level, but the basal dynamics of p53 show one or two spontaneous pulses (in 25 hour observations) of similar shape to those under stressed conditions.

First, we ask whether our model can be used to explain these spontaneous pulses under normal cell proliferation. In the normal cellular processes, cells are subjected to intrinsic DNA damage with a small number of DSB recorded by the presence of the phosphorylation of histone H2AX (biomarker for detection of DSB) at Serine 139 (called γH2AX) (Yu et al., 2006b) that still activates the signalling proteins such as ATM kinase (van Gent et al., 2001). To mimic this condition we set the DSB parameter to a small number, for example three, to represent the intrinsic DNA damage. Lahav et al. (2004) and Loewer et al. (2010) experimental data for p53 protein were measured by fluorescent reporter normalised to arbitrary units. Therefore, our parameters were calibrated, using trial-and-
error method, to fit the p53 protein concentration in the range of 0.06 to 0.5 µM, which is the range of p53 protein measurements made by Ma et al. (2005). Table 4-1 and Table 4-2 list the model parameters and initial conditions, respectively. Figure 4-3 shows the simulation results—two spontaneous pulses in p53 levels and this time course simulation is consistent with the results obtained by Loewer et al. (2010).

![Figure 4-3 P53 dynamics under non-stressed conditions. Model simulation for the total p53 levels (concentration in µM) under non-stressed conditions when DSB is set to 3.](image)

4.6.2 p53 Dynamics in the Presence of Extrinsic Stress

Most research studies apply the strategy of inducing DNA damage by gamma irradiation or a radiomimetic agent such as neocarzinostatin (NCS) and measuring the consequent p53 response in the form of p53 protein levels. The regulation of p53 protein dynamics after DNA damage is an important event and can be seen as one of the significant factors that decide cell fate (Purvis et al., 2012).

To model how DSB activate p53 response, we assume DSB as a parameter that represents the extrinsic stress induced on cells. For example, we assume that the number of DSB is 300, which is the
assumed number of DSB that occurs when cells are exposed to 10 Grays of gamma irradiation (Rothkamm & Lobrich, 2003; Sun et al., 2011). Figure 4-4 shows our model simulation results: in the presence of extrinsic stress, p53 levels show a series of pulses (oscillations) of approximately fixed amplitude and duration, which is in good agreement with the experimental findings of Figure 2C in Loewer et al. (2010) (refer to Figure 2-11 (a) in chapter 2). Together with the results shown previously, our model has achieved the goal of elucidating the mechanism that controls p53 basal dynamics with few spontaneous pulses under non-stressed conditions and damage response with a series of pulses under stressed conditions.

Figure 4-4 P53 dynamics under stressed conditions. Model simulation for the total p53 protein levels (concentration in µM) under stressed conditions when the DSB is set to 300.

4.6.3 p53 Activation of Cell Cycle Arrest: Correct Regulation of p21 in Response to Extrinsic and Intrinsic DNA Damage

Common sense tell us that a problem should be taken care of when it is small and not wait until it is too late to deal with – when the problem is unsolvable or has reached a state that is hard to solve. A small crack is easy to repair, for example in an airplane, but when it is left unnoticed, before long it might cause a catastrophic disaster. Cells might apply the same principle in protecting its genome.
p53 as the guardian of the genome protects cells from mutations and cancer due to its function to stop cell cycle progression in the presence of conditions that can cause genetic changes or mutations (Lane, 1992; Wahl et al., 2005). The p53 tumour suppressor protein is the key factor in regulating cell cycle arrest at the cell cycle checkpoints where the presence of any DNA damage is checked (el-Deiry et al., 1993; Kuerbitz et al., 1992). Normal cellular processes in cells can generate intrinsic DNA damage with a smaller number of DSB (van Gent et al., 2001); however, this intrinsic DNA damage does not require p53 activation of cell cycle arrest and apoptosis (Loewer et al., 2010).

We next ask how p53 executes an accurate cell cycle arrest that is required to avoid propagation of damaged DNA templates during DNA replication. Cell cycle arrest happens at the G1/S checkpoint before the onset of replication. The transcriptional activity of p53 is assumed to be controlled by Mdm2 and MdmX inhibition of p53 acetylation. Here, we introduce p53 activation of p21 to the model; P53a activates p21 induction. We assumed competitive inhibition enzyme kinetics (Klipp et al., 2008) of p53 acetylation by p300/CBP, in which p300/CBP are enzymes and Mdm2 and MdmX are two separate inhibitors. We emphasised p21 mRNA as the output as modelled by Sun et al. (2011) and the results from the model are demonstrated in Figure 4-5: under non-stressed conditions where DSB is set to one, p21 was not induced although there is one p53 spontaneous pulse. In contrast, under stressed conditions, p21 induction was activated by p53 as shown in Figure 4-6. These simulation results match the experimental findings from Loewer et al. (2010) for individual cell measurements of p53 and p21 induction in Figures 5E and 5D of Loewer et al. (2010) that reveal that p21 is only activated under stressed conditions. These results indicate that the competitive inhibition of p53 acetylation by Mdm2 and MdmX is the mechanism that regulates p53 activation of cell cycle arrest in stressed conditions.
Figure 4-5 P53 dynamics and p21 induction under non-stressed conditions. The total p53 protein levels (blue) and p21 mRNAs (green) under non-stressed conditions when DSB is set to 1. Simulated protein concentrations are in µM.
4.6.4 Further Validation of the Model

To illustrate how the proposed network structure in this chapter has successfully achieved a model for p53 basal dynamics and DNA damage response, we test with another scheme of DSB from a recent study on dynamics of DNA damage and repair kinetics (Neumaier et al., 2012). Instead of a fixed number of DSB, we represent the DSB kinetics with a mathematical model of DSB where radiation-induced DSB reach a maximum number in 15-30 min and are resolved after repair, as proposed by Neumaier et al. (2012) and approximated as below:

\[
\frac{dDSB}{dt} = a(D)(k_1)e^{-k_1t} - k_2(\text{DSB})
\]  

(4.21)

where \(k_1\) is rate of DSB generation (detected by radiation induced foci) and \(k_2\) is the rate of repair, \(a=35\) (a constant) and \(k_1=0.13863\). D represents the dose of radiation delivered to the cell. We assumed \(k_2=0.0042\) and \(D=0.1\) for non-stressed conditions with a maximum DSB of about three as in Figure 4-7 (a); whereas, \(k_2=0.0026\) and \(D=10\) for stressed conditions with a maximum of roughly 320 DSB (Figure 4-7(b)). The simulation results for p53 and p21 are shown in Figure 4-7 (c) and (d); the
results are qualitatively in good agreement with the experimental findings (similar to Figure 4-5 and Figure 4-6). For non-stressed conditions, we also set D=0.001 and obtained qualitatively similar results (data not shown). Therefore, the network structure has achieved a consistent model for the p53 basal dynamics and DNA damage response. Taken together, our model hypothesis has captured the essential features of the design principles behind the precise activation of p53 as in the experimental findings by Loewer et al. (2010).

4.6.5 p53 Pulses Are Excitable

Experiments have shown that p53 pulses (whether spontaneous pulses or oscillations) are excitable in that once initiated they complete the pulse even if stress signal is inhibited during pulsing (i.e. for transient or short duration damage signal) (Loewer et al., 2010). Specifically, Loewer et al. (2010)
experiments show that p53 completes a pulse even after ATM kinase is inhibited by addition of Wortmannin. We explore the excitable dynamics of p53 pulses theoretically and investigate the causes of these excitable pulses in response to stress signals caused by transient severe damage as well as low damage in normal physiological stress during cell cycle progression. We simulated each of these situations: for the transient low damage we set the DSB induced ATM activation rate $k_{DSB}=0$ at $t=8$ hr (2 hours before the maximum amplitude of spontaneous pulses at which point p53 level has reached roughly 20% of maximum in experiments (Loewer et al., 2010)); and for transient severe damage, the extrinsic stress signals were inhibited by setting $k_{DSB}=0$ at $t=0.5$ hr (30 min after stress at which point p53 level has reached roughly 20% of maximum value as in experiments (Loewer et al., 2010)). The simulation results are shown in Figure 4-8 (a) and (b), respectively (black vertical line indicates the time of termination of stress signal). It shows that both low and high damages complete a full pulse. Also, the transient low damage caused full pulse has the same amplitude and width as the pulse under transient severe damage. Thus, our results show that p53 pulses are excitable both in transient severe stress and low damage signals that are consistent with experimental results. Our model structure has captured these excitable dynamics of p53. The excitable pulses of p53 satisfy the need of the cell to protect against any damage either low level transient damage or severe damage that require further action of DNA repair. Thus, our new model confirms that DNA damage response to a stress signal invoke excitability providing a cell with a mechanism to achieve a quick response.

We are interested in further investigating the possible causes of the excitable pulses of p53; we hypothesized that these excitable dynamics are caused by stress signal amplification. To explore these excitable dynamics, we set both $k_{DSB}=0$ and $k_{auto}=0$ ($k_{auto}$ is the ATM auto-activation rate) at $t=8$ hr, and at $t=0.5$ hr for non-stressed and stressed conditions, respectively. The results are in Figure 4-8 (c) and (d). For non-stressed conditions (Figure 4-8 (c)), it shows excitability but with significantly lower amplitude compared to the situation with default ATM auto-activation rate. For stressed conditions as in Figure 4-8 (d), it shows no excitability. Therefore, stress signal amplification has a decisive effect on p53 levels – it is the cause of excitable pulses. The effect is significant for high damage that appears to require higher level of magnification to produce a pulse.
4.6.6 Bifurcation Analysis on p53 Dynamics and Its Physiological Functions

Cell physiological behaviour controlled by p53 can be analysed using bifurcation theory that views cell behaviour as a dynamical system (Tyson et al., 2001) as discussed in Chapter 3. The bifurcation analysis was performed using a MATLAB package called DDE-BIFTOOL (Engelborghs et al., 2002), a tool for bifurcation analysis of steady state solutions and periodic solutions of delay differential equations with constant delays. A saddle-node bifurcation and a supercritical Hopf bifurcation were obtained from the MATLAB scripts that use DDE-BIFTOOL and are shown in Figure 4-9. As mentioned previously (in Chapter 3), a general description of supercritical Hopf bifurcation from Strogatz’s textbook on nonlinear dynamics (Strogatz, 1994) is that “a supercritical Hopf bifurcation occurs when a stable spiral changes into an unstable spiral surrounded by a small, nearly elliptical [stable] limit cycle.” The meaning of a limit cycle is defined as “any simple oriented closed curve trajectory that does not contain singular points” (singular points are steady states at which the phase flow is
stagnant) (Edelstein-Keshet, 1988). Figure 4-9 shows three distinct responses of p53 depending on the bifurcation parameter ATM auto-activation $k_{\text{auto}}$ (damage signal amplification rate) (we used the same bifurcation parameter as Sun et al. (2011)). These are saddle-node bifurcations SN1 and SN2 and supercritical Hopf bifurcations HB1 and HB2. Time course simulations for p53 in the regions of SN1, HB1 and HB2 are presented in Figure 4-10. Figure 4-10 (a) shows that for low $k_{\text{auto}}$ values ($k_{\text{auto}} \leq 0.04$), the steady state value of the total p53 levels are very low, and starts to pulse (with only few pulses) after the saddle-node SN1 at the threshold value $k_{\text{auto}}=0.04$ with characteristics of damped oscillations that reach a higher steady state value at $k_{\text{auto}}=0.041$. Figure 4-10 (b) shows that p53 pulses are excitable once it crosses the activation threshold. After supercritical Hopf bifurcation HB1 at $k_{\text{auto}}=0.0492$, p53 starts to oscillate (repeated pulses) with a growing stable limit cycle with a maximum and minimum amplitude shown in blue colour in Figure 4-9 and Figure 4-10 (c). It shows for the default model parameter value $k_{\text{auto}}=0.07$ it generates oscillatory behaviour. After a second Hopf bifurcation HB2 point at $k_{\text{auto}}=0.3434$ (Figure 4-9), a stable steady state of the total p53 levels at a much higher level is attained (Figure 4-10 (d)). These results lead us to conclude that p53 dynamics are both pulsatile (few pulses) and oscillatory depending on the strength of the stress signal. (Although a series of repeated pulses with fixed amplitude and duration is the same as limit cycle oscillations, in literature, p53 dynamics with repeated pulses are known interchangeably as pulses (Batchelor et al., 2008; Lahav et al., 2004) and oscillations (Geva-Zatorsky et al., 2006). But, theoretical and computational biologists refer to it as sustained oscillations (Goldbeter, 2002)).
Figure 4-9 Bifurcation diagram of the system for total p53 (in µM) against the parameter $k_{\text{auto}}$, the ATM auto-activation rate. The dotted and dashed lines represent the stable and unstable steady states, respectively. The saddle-node bifurcation occurs at the $k_{\text{auto}}=0.04$ and $k_{\text{auto}}=-0.036$. The supercritical Hopf bifurcation occurs at the $k_{\text{auto}}=0.0492$ and $k_{\text{auto}}=0.3434$. Saddle-node bifurcation was not found in Sun et al. (2011) model.

We hypothesise that the above bifurcation analysis gives insight into four modes of p53 behaviour that link to the ways p53 decides cell physiology. Figure 4-10 (a)-(d) show the behaviour of total p53 protein concentration (in µM) in response to four different values of the parameter $k_{\text{auto}}$. First, for low value of $k_{\text{auto}} = 0.04$, p53 shows a low steady state value of 0.0116 µM that corresponds to homeostasis. Second, for $k_{\text{auto}} = 0.041$, p53 levels pulse (few pulses with damped oscillations) that activates DNA damage repair genes. Third, for $k_{\text{auto}} = 0.07$, p53 levels oscillate with a stable limit cycle that activates cell cycle arrest and DNA damage repair genes. Finally, for high value of $k_{\text{auto}} = 0.4$, p53 levels show a much higher steady state value of 0.259 µM that leads to apoptosis. As proposed by Tyson (2006), exposure of cells to radiation induced DNA damage can first cause ATM auto-activation $k_{\text{auto}}$ to increase and then decrease, as damage is repaired. Theoretically, this increase of parameter value in $k_{\text{auto}}$ crosses the first saddle-node bifurcation point SN1 giving rise to a small number of p53 pulses (with damped oscillations). The existence of saddle-node bifurcation was a novel result that was not found in Sun et al. (2011) model and could be due to the positive feedback loop of p53 auto-regulation introduced in our model (positive feedback loop in general contributes to saddle-node bifurcation or bistability). Then as explained by Tyson (2004, 2006), its increase crosses the first Hopf
bifurcation point HB1 giving rise to robust limit cycle oscillations. We assumed that $k_{auto}$ above the second bifurcation point HB2 leads to a very high steady state of p53 protein levels corresponding to p53 activation of apoptosis in response to severe damage. This analysis is supported by experimental results (Lai et al., 2007) that suggest that high p53 levels induce apoptosis.

Figure 4-10 The time course simulations for: (a) $k_{auto} = 0.04$; (b) $k_{auto} = 0.041$; (c) $k_{auto} = 0.07$; and (d) $k_{auto} = 0.4$.

The illustration of the growing stable limit cycle after the first Hopf bifurcation point at $k_{auto} = 0.0492$ is shown in Figure 4-11. It is a typical behaviour observed in dynamical systems where a stable steady state is lost after the supercritical Hopf bifurcation point and a stable limit cycle arises with growing amplitude (Goldbeter, 2002) for increasing $k_{auto}$ value as shown in Figure 4-11 (a) and (b) where $k_{auto}$ value is 0.05 and 0.06, respectively.
Figure 4-11 Phase plane diagrams showing the growing stable limit cycle after the first Hopf bifurcation point at $k_{auto}=0.0492$. These diagrams data are generated from XPP and redrawn using MATLAB.

The oscillations emanating from the supercritical Hopf bifurcation of stable limit cycle with growing amplitude generated from DDE-BIFTOOL had period ranging from 4 to 6 hours as shown in Figure 4-12 (a), which is consistent with the experimental findings (Geva-Zatorsky et al., 2006; Loewer et al., 2010). Further analysis of the frequency of the oscillations in Figure 4-12 (b) shows that the limit cycle is born at the bifurcation with a non-zero frequency and this feature classifies p53 excitable dynamics as **Type II excitability** (Rue & Garcia-Ojalvo, 2011). Type I excitability typically happens when the limit cycle is born at the bifurcation with zero frequency (Rue & Garcia-Ojalvo, 2011). The classification of Type I and Type II excitability has been discussed in Chapter 2 (please refer to Section 2.5.1 and Figure 2-19). These classifications are based on the study of neuronal excitability. Our results suggest that the information processing in the p53 network's DNA damage response conforms to Type II excitability.
4.6.7 p53, Mdm2 and MdmX Dynamics in the Presence of Extrinsic Stress

Next we explore the p53 response after DNA damage and the effects on the essential regulators Mdm2 and MdmX. It is known that p53 and Mdm2 protein levels oscillate out of phase after treatment with gamma-irradiation; Mdm2 oscillations lag behind p53 oscillations by about 100 min (Lahav et al., 2004). However, there is increasing evidence that MdmX also plays a crucial role in regulating p53 activity (Marine et al., 2007; Marine & Jochemsen, 2005). Therefore, it is of great interest to examine the interplay of p53, Mdm2 and MdmX.
In normal cells, MdmX protein is about ten to twenty percent of Mdm2 level (Wang et al., 2009), and the degradation of Mdm2 (Stommel & Wahl, 2004) and MdmX (Wang et al., 2007) are key steps for p53 activation in response to stress. Our model can be used to examine the protein levels of p53, Mdm2 and MdmX under stressed conditions. Figure 4-13 shows that in response to stress, Mdm2 and MdmX levels decrease, whereas p53 levels increase as p53 stabilizes, and Mdm2 pulses with a delayed peak of around 100 min compared to p53. These results are consistent with experimental results of Lahav et al. (2004). Figure 4-13 also shows that MdmX levels decrease at the beginning, allowing p53 activation. This result is qualitatively consistent with experimental results of Wang et al. (2007) that degradation of MdmX is an important step for p53 activation.

Figure 4-13 Simulation results obtained for P53, Mdm2 and MdmX dynamics under stressed conditions. The p53 (blue), Mdm2 (red) and MdmX (black) protein levels under stressed conditions when DSB is set to 300. The simulated protein concentration results are in µM.

The role of MdmX in the threshold activation, p53 spontaneous pulses, and the p53 and Mdm2 dynamics were investigated in silico by deletion of MdmX from the model. It was found that MdmX
deletion has little impact on the threshold activation, p53 spontaneous pulses, and p53 and Mdm2 dynamics (data not shown). Thus, it confirms that p53 auto-regulation plays a critical role in our model in threshold activation and p53 dynamics. But, experiments have shown that MdmX does play an important role in p53 regulation, especially in p53 cell cycle arrest (Barboza et al., 2008). According to our model assumptions, it seems that normal MdmX expression is low and plays a secondary facilitating role in p53 activation; however, overexpression of MdmX may have significant effects on p53 regulation.

4.6.8 Local Parameter Sensitivity Analysis

In the modelling process, parameter values were estimated and this invariably results in uncertainty in these estimated parameter values. This uncertainty is unavoidable since most of the parameter values are unavailable or not measured in experiments. Parameter sensitivity analysis is required to investigate which parameters are the most important in affecting p53 behaviours. In the literature, period and amplitude of p53 oscillations are the measurements made from the model outputs for parameter sensitivity analysis. Local parameter sensitivity analysis was performed with one of the parameters being increased (or decreased) by 20% from the standard parameter values as in Table 4-1 while holding the other parameter values fixed. The estimation of the period and amplitude of total p53 levels were obtained from spectrum resampling technique (Costa et al., 2011). This technique applies bootstrapping of spectral estimates and gives good estimation of the period of time course data. The results of the local parameter sensitivity analyses are shown in Figure 4-14 and Figure 4-15.

![Image]

**Figure 4-14** Local parameter sensitivity analyses on the period of p53 levels. The results showed that p53 oscillation period is robust to variation of parameters as the periods remain in the range of ±0.2 hours (±3% change) from the default parameter set that has period of 5.8 hours. The most important factor is the parameter index 32 (indicated by the arrows above), which represents the Wip1 protein degradation rate.
Based on the 20% local parameter sensitivity analyses, some of the key parameters that have a major influence on the p53 periods were identified. These parameters are listed in Table 4-3.

Table 4-3 Four of the most important parameters in controlling p53 oscillations.

<table>
<thead>
<tr>
<th>Parameter index</th>
<th>Parameter name</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>$\mu_{\text{wip1}}$ Wip1 protein degradation rate</td>
</tr>
<tr>
<td>69</td>
<td>$\tau_{\text{wip1 transcrip}}$</td>
</tr>
<tr>
<td>19</td>
<td>$\delta_{\text{wip1 mRNA degradation rate}}$</td>
</tr>
<tr>
<td>49</td>
<td>$k_{\text{auto}}$ ATM auto-activation rate</td>
</tr>
</tbody>
</table>

Local sensitivity analysis of parameters on amplitude (Figure 4-15) shows that parameter 49 ($k_{\text{auto}}$ ATM auto-activation rate) has the most effect. Results from both analyses thus indicate the importance of $k_{\text{auto}}$ which can affect strongly both period and amplitude. The 20% local parameter sensitivity analysis shows the period and amplitude of p53 oscillations are robust to perturbations of most parameters and prompted to investigate larger percentage of perturbations. When 50% local parameter sensitivity analyses were performed, oscillations were still observed for all the parameters. The time course simulations for −50% local parameter sensitivity analyses are shown in Figures 4-16 to 4-19. In general, the amplitude of the oscillations was subjected to more variation than period, which is consistent with the behaviour observed in experimental results from Geva-Zatorsky et al. (2006). Only for three parameters p53 lost its oscillations, these parameters are: wip1 mRNA degradation rate (19), Wip1 protein degradation rate (32) and ATM auto-activation rate (49). The loss of p53 oscillations means p53 is inactivated. These analyses suggest these three parameters as possible target(s) for re-activating p53.
Figure 4-15 Local parameter sensitivity analyses on amplitude of p53 levels. The most important parameter is index 49, which represents the ATM auto-activation (indicated by arrows above).

Figure 4-16 Time course simulations (parameters 1-25) for the ~50% local parameter sensitivity analyses. (parameter index 19 is wip1 mRNA degradation rate)
Figure 4-17 Time course simulations (parameters 26-50) for the ~50% local parameter sensitivity analyses. (parameter index 32 is Wip1 protein degradation rate and 49 is ATM auto-activation rate)

Figure 4-18 Time course simulations (parameters 51-75) for the ~50% local parameter sensitivity analyses.
4.6.9 ATMp, Wip1 and p53 Dynamics in the Presence of Extrinsic Stress

Finally, having established the importance of $k_{\text{auto}}$ (ATM auto-activation rate) and parameters related to production and degradation of Wip1, we ask whether the repeated p53 pulses after DNA damage is due to the pulses in the ATM kinase and investigate the function of the feedback regulator Wip1 in attenuating p53 response. DNA damage signalling protein kinase ATM detects the stress signal and passes it to p53. Recent experimental results have shown that ATMp (ATM phosphorylated at Serine 1981) pulses preceding p53 pulses (Batchelor et al., 2008). Meanwhile Wip1, as a phosphatase, dephosphorylates ATM at Serine 1981 resetting ATM to non-active mode (Shreeram et al., 2006).

Figure 4-20 shows the model simulations for the levels of ATMp, p53 and Wip1 under stressed conditions. ATMp pulses first, followed by p53, and finally Wip1, which are qualitatively similar to Batchelor et al. (2008) results. These results show that ATM and Wip1 are crucial proteins in the p53 signalling pathway. Hence, the observed p53 pulses are caused by the upstream pulses of ATMp, and the attenuation of the p53 stress response is modulated by Wip1. In addition, p53 oscillations are lost and stayed at low basal levels (Figure 4-16 and Figure 4-17) when $\mu_{\text{wip1}}$ (Wip1 protein degradation rate - index 32) or $\delta_{\text{wip1}}$ (Wip1 mRNA degradation rate - index 19) is reduced by 50% from its standard value while holding other parameter values fixed at the nominal values as in Table 4-1. These results with low p53 levels mean that perturbation to Wip1, for example stabilising Wip1 or overexpressing Wip1, can inactivate p53.
4.6.10 Simulation of Pharmacological Interventions to Reactivate p53

Mdm2 and MdmX binding to p53 restrain p53 transcriptional activity and keep p53 in the non-active un-acetylated form. P53 unbinding from Mdm2 and MdmX is required to release p53 from Mdm2 and MdmX suppression and these molecular interactions have become one of the targeted strategies for therapy to reactivate p53 in tumours with intact wild-type p53 that overexpressed Mdm2 or MdmX (Toledo & Wahl, 2006, 2007). Recent experiments using Mdm2 and MdmX inhibitors have demonstrated very promising results by targeting Mdm2 and MdmX, either using Mdm2 inhibitor such as Nutlin alone (Vassilev et al., 2004) or targeting both Mdm2 and MdmX which may be more effective when both Mdm2 and MdmX are overexpressed (Bernal et al., 2010).

In order to predict the effect of an Mdm2 inhibitor that blocks the interaction between p53 and Mdm2, for example Nutlin, the binding rate of Mdm2 and p53, $k_{i1}$, was reduced from 552 µM$^{-1}$ min$^{-1}$ to 5.52 µM$^{-1}$ min$^{-1}$ and the simulation results are shown in Figure 4-21. The prediction shows that p53 levels pulse at a higher level than the p53 levels in Figure 4-6 for default $k_{i1}$, and as a result p53...
induces a higher level of p21 mRNA. This *in silico* prediction is consistent with the *in vivo* intervention of Nutlin in reactivating p53 causing cell cycle arrest (Vassilev et al., 2004).

Figure 4-21 p53 and p21 mRNA dynamics under stressed conditions after treatment with Mdm2 inhibitor. The p53 protein levels (blue) and p21 mRNA (green) after treatment with Mdm2 inhibitor (the binding rate k_{11} was reduced from 552 μM⁻¹ min⁻¹ to 5.52 μM⁻¹ min⁻¹) under stressed conditions when DSB is set to 300.

4.7 Discussion

4.7.1 Mechanism of p53 Activation in Stressed and Non-stressed Conditions

Cells have been evolved with the capability to act promptly in response to induced DNA damage and to tolerate intrinsic DNA damage. Typical intrinsic DNA damage is that caused by free oxygen radicals from normal cellular metabolism (Kastan & Bartek, 2004). This property of survival of individual cells was revealed in Loewer et al. (2010) experiments. Here, we proposed a mathematical model of the mechanisms that control p53 activation and suggest that the core regulatory feedback regulators Mdm2, MdmX and Wip1, together with ATM kinase, play a critical role in controlling p53 activation in stressed conditions that require cell cycle arrest as well as in unstressed conditions. Our mathematical model shows the precise p53 activation and induction of *p21* for arresting cell cycle
and stimulation of DNA damage repair, which underscores one of the key roles in p53-mediated tumour suppression (Efeyan et al., 2007).

Our model, which incorporates the most recently found molecular interactions and genes regulated by p53, offers a mathematical hypothesis to explain quantitatively the experimental findings of Loewer et al. (2010). Lahav and co-workers (Loewer et al., 2010) have proposed that p53 signalling followed a coherent feed-forward network motif where p53 protein is excitable by ATM kinase and persistent ATM signalling is required to convert p53 into active forms (Loewer et al., 2010). For example, active ATM phosphorylates and therefore stabilises p53, one arm of the feed-forward connection. Active ATM also phosphorylates MdmX, enables its ubiquitination by Mdm2 and eventual degradation. Since MdmX is an inhibitor of p53 acetylation, this ATM phosphorylation of MdmX constitutes inhibition of a p53 inhibitor, forming the second arm of a coherent feed-forward connection. Here, in addition to the coherent feed-forward network motif, we suggest that p53 induction of $p21$ is controlled by Mdm2 and MdmX, in such an arrangement that p53 acetylation by p300/CBP follows competitive inhibition enzyme kinetics. P53 induction of $p21$ requires p300/CBP to mediate p53 acetylation. This assumption is supported by quantitative ChIP assays that show that p53 is present at the $p21$ promoter in unstressed cells yet inactive, and is proposed to be suppressed by Mdm2 and MdmX (Kruse & Gu, 2009).

### 4.7.2 p53 Excitable Dynamics

Our model structure has demonstrated the excitable dynamics of p53 both under stressed and non-stressed conditions. Our mathematical model of the p53 network with two positive feedback loops (p53 auto-regulation and ATM auto-activation) and three negative feedback loops (Mdm2, MdmX and Wip1) together with the incorporation of the interplay between p53, Mdm2 and MdmX has successfully captured the essential features of the network structure and the design principles behind p53 basal dynamics and DNA damage response, particularly the excitable p53 pulses. Regarding the excitable dynamics of p53 pulses, Loewer et al. (2010) compared it with other biological excitable observations of action potential; the excitable dynamics of electrical pulses of neurons have long been studied by Hodgkin (1948). Hodgkin has characterised excitable dynamics of neuron into two types of excitability: Type I excitability which happens when the limit cycle is born at the bifurcation with zero frequency; Type II excitability which is born at the bifurcation with non-zero frequency (Rue & Garcia-Ojalvo, 2011). Based on the frequency of the limit cycle arising from the bifurcation with non-zero frequency (Figure 4-12 (b)), we characterised the excitable dynamics of p53 as type II excitability. Interestingly, a recent model from Kim and Jackson (2013) has found Type II excitability for p53 pulses, which is similar to Type II neurons. Our results show p53 is excitable both in transient severe stress and low damage signals. In particular, our model shows the p53
spontaneous pulses are excitable due to intrinsic DNA damage that activates the ATM kinase and the stress signal is further amplified to induce a full pulse of p53 with the backing of p53 auto-regulation. Thus, our model simulation results lead us to conclude that the spontaneous pulses of p53 is a sensitive response of the cell to repair the intrinsic DNA damage that happens during normal cell proliferation. This result is in line with the experimental findings of Loewer et al. (2010) that show that p53 spontaneous pulses are closely connected to cell cycle progression.

4.7.3 Biological Conjectures for the Basal Dynamics of p53

The remarkable experimental findings on p53 basal dynamics with one or two spontaneous pulses were reported in 2010 (Loewer et al., 2010). Sun et al. (2011) constructed a purely stochastic model for p53 basal dynamics incorporating stochasticities in DNA damage and repair processes, and stochastic delay simulation of the reactions involved. In contrast to the stochastic model proposed by Sun et al. (2011), which has suggested a possible explanation for the basal dynamics, we used a purely deterministic model that captures basal dynamics. In comparison to Sun et al. (2011) model that assumes the intrinsic DSB are 50 per cell per cell cycle based on estimation from Vilenchik and Knudson (2003), the assumption made in our model for the intrinsic DNA damage and the number of DSB in non-stressed conditions is low, in the range of one to three DSB. This assumption is based on some of the measurements of the average number of DSB under non-stressed conditions (the average numbers of γH2AX from 1.9 to 3.7 for the four lowest cell lines; for the MCF7 cell line the average numbers of γH2AX is 2.1) (Yu et al., 2006b). Vogelstein et al. (2000) suggested that one DSB may be enough “to trigger a rise in p53 protein levels”, which is in line with Huang et al. (1996) results that suggested a single DSB is sufficient to activate p53. Here, our theoretical model simulation shows p53 protein increase in Figure 4-3 where a small number of DSB results in a spontaneous p53 pulse. This simulation result shows that our deterministic model produces realistic p53 basal dynamics.

One interesting question about the p53 spontaneous pulses in non-stressed conditions that does not induce p21 in arresting cell cycle is: what is the implication of intrinsic DNA damage to mutagenesis? The first conjecture is that the purpose of these spontaneous pulses of p53 is to induce DNA damage repair to fix the DSB – p53 is capable of promoting transcription independent role in DSB repair (Gatz & Wiesmüller, 2006; Sengupta & Harris, 2005) – in order to preserve the genomic integrity. Another conjecture is that the intrinsic DSB are allowed by p53 for somatic cells to evolve or generate genetic changes or mutations (Friedberg et al., 2004). In fact, Collaro and Serrano (2010) state that there can be millions of mutations at a given time, and repairing all of them can be exhausting for cells; therefore, the mechanism employed by cells is to allow a large number of mutated cells to pass cell cycle checkpoints. Once the accumulated DNA damage or mutations reach a threshold, it may lead to
the initiation of senescence (permanent cell cycle arrest) or apoptosis (programmed cell death) (Kuilman et al., 2010); if the senescence or apoptosis mechanisms fail, it may predispose the cells to the onset of neoplasms or tumours.

4.7.4 Novel Findings on a Spectrum of p53 Pulsatile Dynamics

A novel aspect of this research is bifurcation analysis on the deterministic model that reveals a spectrum of p53 dynamics: p53 dynamics is pulsatile with a number of modes of behaviour. Whether it is with a limited number of (spontaneous) pulses or repeated pulses (oscillations) depend on the damage signal. Whether p53 network functions as an oscillator or a pulse generator were discussed in Batchelor et al. (2009) and one interesting question about the possibility of p53 system switching between these two types of dynamical behaviour were raised (Batchelor et al., 2009). Our model analysis shows that p53 can change from pulsatile to oscillatory, and vice versa. Saddle-node bifurcation is commonly used to analyse bistable biological switches, but we found that when $k_{auto}$ is less than or equal to 0.04, p53 levels stayed at a steady basal low level (or off state) and gets turned on with a pulse to a higher steady state level when $k_{auto}$ increases over the activation threshold 0.04. As $k_{auto}$ increases, p53 dynamics move towards oscillations with fixed amplitude. This unexpected result from the bifurcation analysis leads us to provide a quantitative model of the p53 system that can function as a (limited) pulse generator or a limit cycle oscillator. The saddle-node bifurcation and pulsatile dynamics were not present in Sun et al. (2011) deterministic model. Our study found that p53 auto-regulation (positive feedback) is required for achieving a model of p53 that displays this spectrum of pulsatile and oscillatory behaviours.

Our model has proposed four modes of p53 dynamical behaviour that closely explain how p53 regulates its gene activation that decides cell fate. In this way, our model shows that the p53 dynamics as a whole is pulsatile favouring the hypothesis given by Batchelor et al. (2009) that p53 network is a pulse generator in response to DSB. They suggest that pulsatile dynamics enable p53 to act as a transcription factor to activate different target genes that can lead to different cellular responses such as cell cycle arrest and apoptosis. These bifurcation analysis findings on the dynamics of p53 that are associated with cellular responses are consistent with a latest view on some transcription factor signalling dynamics that communicate cellular information, particularly that signalling dynamics are closely linked to certain cellular responses (Purvis & Lahav, 2013). The advantages of our model are that it uncovers novel insights into the pulsatile behaviours of p53 compared to previous models.

One curious question is about the source of variability of $k_{auto}$, the parameter for ATM auto-activation that is so crucial in our bifurcation analysis to reveal a spectrum of p53 dynamics and its physiological functions. We assumed that ATM auto-activation value refers to the ATM auto-phosphorylation at
Serine 1981 (Bakkenist & Kastan, 2003) that can vary when cell is induced by stress such as radiation. There are two reasons why we think this assumption is possible. Firstly, one study on ATM auto-phosphorylation kinetics has observed that ATM auto-phosphorylation peaks in 30 minutes after DNA damage induced by ionizing radiation and then decreases over time (Lavaf et al., 2009). After ionizing radiation, the ATM auto-phosphorylation kinetics is in a similar pattern to the number of DSB in Neumaier et al. (2012); for example, it is as in our simulation in Figure 4-7 (b). Secondly, another study has found two novel sites (Serine 367 and Serine 1893) of ATM auto-phosphorylation (Kozlov et al., 2006). Thus, ATMp may represent multiple site auto-phosphorylated forms, and these ATM auto-phosphorylation sites may produce different degrees of ATM auto-activation corresponding to the varying $k_{auto}$ values used in our model. Thus, this model assumption is reasonable and our model analysis supports the pulse generator hypothesis proposed by Batchelor et al. (2009).

### 4.8 Summary

In this chapter, we propose an extension of Sun et al. (2011) deterministic model for capturing the p53 basal dynamics and DNA damage response and our simulation results closely agree with the latest experimental findings. We present an expanded conceptual model of the p53 core regulatory network incorporating realistic assumptions to explain the observed experimental findings – notably, the basal dynamics of p53 involving a limited number of spontaneous pulses, and DNA damage response involving oscillations after stress. We demonstrate the significance of the p53 spontaneous pulses and classify p53 excitable dynamics as type II excitability; our theoretical analysis has shown that the p53 spontaneous pulses are due to the intrinsic double-strand breaks due to normal cellular processes in proliferating cells.

The bifurcation analysis uncovers novel findings on the versatility of p53 dynamics in response to stress that can be either pulsatile or oscillatory. Specifically it suggests a possibility to characterise the whole spectrum of p53 stress response as just p53 pulsatile dynamics (spontaneous as well as repeated pulses) that enable p53 to decide cell fate. The model advances our understanding of the mechanisms underlying p53 regulation and this theoretical model can be used for the prediction of p53-based therapy. We suggest that more research should focus on understanding basal dynamics and p53 activities in individual cells or how p53 functions in normal cells where its protective function is intact; it may reveal a strategy to rectify p53 dynamics in cancerous cells. Three of the feedback regulators Mdm2 (Toledo & Wahl, 2006), MdmX (Danovi et al., 2004; Riemenschneider et al., 1999) and Wip1 (Castellino et al., 2008) have been found over-expressed in cancer that inactivates p53 and can be targets for p53-based therapy; this strategy has been shown by a recent study that reactivated p53 with one small molecule called RITA that inhibits Mdm2, MdmX and Wip1,
leading to killing of cancerous cells (Spinnler et al., 2011). Our model shows that by suppressing overexpressed Mdm2 and Wip1, p53 can be reactivated to its default oscillatory behaviour in stressed conditions. We suggest that the core regulatory model presented here may be extended to a more detailed model of p53 regulation.
Chapter 5  
A Mathematical Model of the Core Regulatory Network of p53  
Protein that Activates Apoptosis by a Bistable Switch in the Intrinsic Apoptotic Pathway

5.1 Overview

In the previous chapter an investigation of the mechanism that regulates p53 function in arresting cell cycle under stressed conditions involving p53 excitable dynamics and DNA damage response was proposed. In this chapter, the focus is on addressing the second issue, another crucial function of p53: how p53 activates apoptosis (programmed cell death) through the transcription of apoptotic target genes such as p53 upregulated modulator of apoptosis (Puma) and initiate a bistable apoptotic switch in response to sustained high DNA double-strand breaks. There are two major apoptotic pathways: the first one is the intrinsic pathway where DNA damage or stress signal invoke the mitochondrial outer membrane permeabilisation (MOMP), which releases apoptogenic proteins that cause a cascade of caspase activation and destruction of cell; the second one is the extrinsic or death receptor pathway that requires cell surface receptor activation, such as tumour necrosis factor receptor (TNFR), TNF-related apoptosis inducing ligand receptor (TRAILR) and Fas-receptor (FasR), to trigger apoptosis (Dewson & Kluck, 2009). It is known that either pathway alone can activate cell death (Rehm et al., 2002). In this chapter, a model of the intrinsic apoptotic pathway controlled by p53 transcriptional activation of apoptosis is proposed.

Notably, malfunction of apoptosis causes cancer and hampers cancer therapy (Cory & Adams, 2002) because the control system has been destroyed or deactivated in some way. One clear reason for failure of the apoptotic switch in cancer cells is the inactivation of p53 due to p53 mutations in majority of human tumours that results in p53 no longer being able to activate transcription of target genes (Vogelstein et al., 2000). In this chapter, a mathematical model of apoptosis induction by p53 is proposed and conceptually it is analogous to a bus’s faulty mechanical door that cannot be closed even though the bus driver has repeatedly pushed the close button many times; for example, the signal was successfully transmitted, but the mechanism that executed door closure failed to operate properly due to some defects. An organism’s survival depends on a highly reliable apoptosis function to monitor its cellular activity and eliminate unwanted or potentially threatening cells.
5.2 Introduction to the Regulation of p53 Activation and Apoptosis Induction

p53 is capable of regulating apoptosis induction by a transcription-dependent pathway, activating target genes such as Puma and Noxa that can induce apoptosis (Vousden & Lu, 2002). Here, we propose a mathematical model of p53 activation of apoptosis through the intrinsic (or mitochondrial) pathway caused by transactivation of pro-apoptotic genes, particularly Puma, a potent activator of apoptosis (Yu & Zhang, 2008). Puma is a Bcl-2 homology domain 3 only (BH3-only) protein that was found to act as a direct activator of apoptosis, activating Bax (a multidomain pro-apoptotic protein) directly (Gallenne et al., 2009; Letai, 2009). The activation of Bax leads to the mitochondrial outer membrane permeabilisation (MOMP), which allows the apoptogenic proteins such as cytochrome c (Goldstein et al., 2000b; Martinou et al., 2000), SMAC/DIABLO and AIF to be released from mitochondria thus ensuring an all-or-none and irreversible apoptosis induction. Individual cell studies have shown that time to MOMP is rapid and varies even in a population of cells with the same stimulus (Albeck et al., 2008; Rehm et al., 2002).

The study of the p53 activation of apoptotic switch has been the focus of molecular biologists because of its importance to understanding cell biology and the disease of cancer, and its potential for leading us to a novel strategy for better cancer treatment (Yu & Zhang, 2003). In the thirty years of research on p53, there has been great advancements in our knowledge of p53 regulation based on its structure and function (Lane et al., 2010), but how p53 and Bcl-2 proteins induce MOMP and apoptosis still remains controversial (Chipuk & Green, 2008; Jiang et al., 2010; Oren, 2003). Furthermore, a recent experimental findings by Chen et al. (2013) using a drug called etoposide as DNA damage stimulus has discovered different dynamics of p53 that are correlated to cell fate decisions. At high dose of drug, p53 increases to a high concentration with one and a half p53 pulses and subsequently leads to apoptosis in 14-39 hours (Chen et al., 2013). Thus, we explore the second issue of p53 activation of apoptosis under high DNA damage.

5.3 Mathematical Modelling of p53 Regulation of Apoptosis Induction

p53 regulation of apoptosis is critical for the development and homeostasis of a multicellular organism. Although molecular cell biologists have gathered enormous amount of data about p53 regulation of apoptosis, and much have been uncovered at the molecular level, it is clear that the complexity of the regulation involving feedback loops make it incomprehensible to gain insights from biological networks and diagrams alone. Thus, quantitative mathematical models play an important role in exploring the mechanism theoretically and generating hypotheses that contribute to new experimental testing and deeper understanding.
To understand the role played by p53 in activating apoptosis and cell fate decisions, a few mathematical models have been constructed to investigate theoretically the mechanism controlling apoptosis (Pu et al., 2010; Puszynski et al., 2008; Sun et al., 2009; Tian et al., 2012; Tiana et al., 2002; Zhang et al., 2007, 2009a; Zhang et al., 2010; Zhang et al., 2009b). For example, Zhang et al. (2009a) has successfully modelled the intrinsic pathway of apoptosis that depends on a threshold stress signal and when stress signal exceeds the specific threshold, cells commit to cell death. In this thesis, a simplified model of p53 apoptosis induction is proposed based on the p53 transcription-dependent pathway as an extension of the mathematical model presented in Chapter 4. The model incorporates the apoptosis initiation module (Bax activation by BH3-only protein) proposed by Zhang et al. (2009a) with modified parameter values for realistic model simulation instead of dimensionless concentrations as used by the said authors. The model is constructed to explore the molecular control of apoptosis mediated by p53 and it must satisfy some of the key features of apoptosis: 1) all-or-none, switch-like manner; 2) irreversible commitment to cell death; 3) very rapid; and 4) variable time to MOMP. The understanding of p53 regulation of apoptosis is crucial for designing new therapy, particularly p53 (Lane et al., 2010) or Puma (Yu et al., 2006a) gene therapy that may be more effective in treating certain cancer patients than current methods.

5.4 Methods

A model of delay differential equations is constructed based on the hypotheses and assumptions made, which is described in this section. The apoptotic switch is controlled by B-cell lymphoma 2 (Bcl2) family of proteins (Adams & Cory, 2007) and p53 activation of apoptotic genes (Vousden & Lu, 2002). The Bcl2 family of proteins are categorised into three groups based on its function, and they share some common amino acid sequence homology from Bcl2 homology (BH) domains one to four (BH1-BH4) (Cory & Adams, 2002; Dewson & Kluck, 2009). The first group is the pro-survival proteins (also known as the Bcl2-like proteins) that contain BH1-BH4; these include Bcl-2, Bcl-xL and Bcl-w (Dewson & Kluck, 2009). The second group is the pro-apoptotic proteins that contain BH1-BH3, for example, Bax and Bak. The activation of Bax (or Bak), also known as Bax-like protein, causes MOMP and the destruction of a cell (Dewson & Kluck, 2009). The third group is the BH3-only proteins, such as Bim, Puma and Noxa that can initiate the intrinsic pathway by antagonising the first group that is pro-survival proteins (Dewson & Kluck, 2009; Willis & Adams, 2005). Under non-stressed conditions, cells are protected by Bcl2-like proteins that are pro-survival proteins.

Building on the model proposed in Chapter 4 and incorporating the apoptosis initiator module network structure from Zhang et al. (2009a), a model of p53 activation of apoptosis is proposed. The key differences in our model are that the stress signal is represented by Puma mRNA (Zhang et al. (2009a) represented stress by a constant between 0 and 1) and the downstream caspases activation
of apoptosis is not modelled as we assumed the commitment to cell death follows from bax activation (Dewson & Kluck, 2009). Simplifications were made as Zhang et al. (2009a) as follows: Puma represents the BH3-only proteins; Bcl2 represents the pro-survival proteins; and Bax represents the Bax/Bak pro-apoptotic proteins. It is assumed that Puma (as a BH3-only protein) serves as a direct activator of Bax and inhibitor of pro-survival Bcl2 (please refer to lower part of Figure 5-2), which is in line with a direct activator model proposed by Chipuk and Green (2008). Puma, Bax and Bcl2 are assumed to reside in cytoplasm. Bcl2 binds to Baxm and form complexes Baxm:Bcl2, and thus Baxm is inactivated by Bcl2 binding in non-stressed situations so that apoptosis is restrained.

Figure 5-1 A diagram illustrating the distribution of the proteins in the model.

The localisation of Puma, Bax, Bcl2, Puma:Bcl2, Baxm:Bcl2 and Baxm are illustrated in Figure 5-1. Bax is predominantly cytoplasmic and Baxm represents the active form of Bax accumulated at the mitochondrion that is assumed to be able to initiate MOMP and apoptosis. Puma and Bcl2 are assumed to be in the cytoplasm. Baxm:Bcl2 and Puma:Bcl2 are the protein complexes inactivated by the pro-survival protein Bcl2 under non-stressed conditions. These need to be activated in apoptosis. Based on these assumptions, the equations below describe the total concentration of mitochondrion Bax (Baxm), Bax, Bcl2 and Puma, respectively (T stands for total).

\begin{align}
BaxmT &= Baxm + Baxm:Bcl2 \\
BaxT &= Bax + Baxm + Baxm:Bcl2
\end{align}

(5.1) (5.2)
Bcl2T = Bcl2 + Puma:Bcl2 + Baxm:Bcl2  \hspace{1cm} (5.3)

PumaT = Puma + Puma:Bcl2  \hspace{1cm} (5.4)

where BaxT and Bcl2T are assumed to be constants; similar to assumptions made by Zhang et al. (2009a).

A schematic diagram of the model of p53 activation of apoptotic switch is illustrated in Figure 5-2.

![Figure 5-2](image)

Figure 5-2 A schematic diagram of p53 activation of apoptotic switch (apoptosis initiation module in the lower part).

### 5.4.1 Model Equations

The model equations are a combination of those from Chapter 4 Section 4.4.2 (19 DDEs) and model equations for p53 activation of apoptotic switch (5 DDEs) listed below:
Equations for mRNAs:

**puma**: The first term in Eqn (5.5) describes the basal synthesis, the second term describes gene transcription from P53pp with a time delay $\tau_{11} = 70$ minutes (as a representation of E2F1 activation of ASPP, and subsequent ASPP promotion of p53 activation of apoptotic gene (Sullivan & Lu, 2007)) and the third term describes degradation of Puma mRNA.

$$\frac{d[puma]}{dt} = s_{puma} + e_6 \frac{[P53pp(t-\tau_{11})]^4}{K_{puma} + [P53pp(t-\tau_{11})]^4} - \delta_{puma} [puma]$$ (5.5)

Equations for Proteins:

**Puma**: The first term in Eqn (5.6) describes the basal synthesis of Puma, the second term describes translation of Puma mRNA and the third term describes basal degradation of PumaT.

$$\frac{d[PumaT]}{dt} = k_{s3} + \tau_{puma}[puma(t-\tau_{12})] - k_{dPumaT}[PumaT]$$ (5.6)

**Puma-Bcl2 complexes**: The first term in Eqn (5.7) describes the association of Puma-Bcl2 complexes, the second term describes the dissociation of Puma-Bcl2 complexes, and the third term describes basal degradation of Puma-Bcl2.

$$\frac{d[Puma:Bcl2]}{dt} = k_{as3}[Puma][Bcl2] - k_{ds3}[Puma:Bcl2] - k_{d3}[Puma:Bcl2]$$ (5.7)

**Baxm-Bcl2 complexes**: The first term in Eqn (5.8) describes the association of Baxm-Bcl2 complexes, the second term describes the dissociation of Baxm-Bcl2 complexes, and the third term describes basal degradation of Baxm-Bcl2.

$$\frac{dBaxm:Bcl2}{dt} = k_{asx}[Baxm][Bcl2] - k_{dsx}[Baxm:Bcl2] - k_{bx}[Baxm:Bcl2]$$ (5.8)

**BaxmT**: The first term in Eqn (5.9) describes the auto-activation of Baxm, the second term describes the Puma induced activation of Baxm, and the third term describes basal degradation of BaxmT.

$$\frac{dBaxmT}{dt} = k_{fx1}[Bax] - k_{fx}[Bax][Puma] - k_{dbx}[BaxmT]$$ (5.9)

From Eqn (5.1)-(5.4), we derived the following formula:

$$Bcl2 = Bcl2T - Puma:Bcl2 - Baxm:Bcl2$$ (5.10)

$$Puma = PumaT - Puma:Bcl2$$ (5.11)

$$Bax = BaxT - BaxmT$$ (5.12)

$$Baxm = BaxmT - Baxm:Bcl2$$ (5.13)
It is assumed that the Bcl2T and BaxT are constants with values: Bcl2T=0.6 μM and BaxT=0.5 μM.

### 5.4.2 Model Parameters and Initial Conditions

The parameters used in the model are those under stressed conditions where DSB is set to 300, which represents sustained high damage. The ATM auto-activation rate is set at $k_{auto}=0.4$ indicating a high level of ATM signalling. The other model parameters are the same as in Table 4-1 and Table 4-2.

The model parameters for the apoptosis initiation module are estimated using trial-and-error to generate simulations that are consistent with experimental findings of all-or-none activation of apoptosis initiated by Baxm activation. These model parameters and initial conditions are listed in Table 5-1 and Table 5-2.

#### Table 5-1 Model parameters

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$k_{ds}$</td>
<td>Puma-Bcl2 complex association rate</td>
<td>33</td>
<td>$μM^{-1} min^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>$k_{ds}^{-1}$</td>
<td>Puma-Bcl2 complex dissociation rate</td>
<td>0.0264</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>$k_{id}$</td>
<td>Basal degradation rate of Puma-Bcl2</td>
<td>0.005</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>$k_{ax}$</td>
<td>Baxm-Bcl2 complex association rate</td>
<td>297</td>
<td>$μM^{-1} min^{-1}$</td>
</tr>
<tr>
<td>5</td>
<td>$k_{ax}^{-1}$</td>
<td>Baxm-Bcl2 complex dissociation rate</td>
<td>0.07</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>6</td>
<td>$k_{bx}$</td>
<td>Basal degradation rate of Baxm-Bcl2</td>
<td>0.2</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>7</td>
<td>$k_{fx}$</td>
<td>Bax auto-activation rate</td>
<td>1</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>8</td>
<td>$k_{fx}^{-1}$</td>
<td>Puma-dependent activation of Bax</td>
<td>3</td>
<td>$μM^{-1} min^{-1}$</td>
</tr>
<tr>
<td>9</td>
<td>BaxT</td>
<td>Total Bax</td>
<td>0.5</td>
<td>μM</td>
</tr>
<tr>
<td>10</td>
<td>Bcl2T</td>
<td>Total Bcl2</td>
<td>0.6</td>
<td>μM</td>
</tr>
<tr>
<td>11</td>
<td>$e_6$</td>
<td>P53-dependent Puma transcription rate</td>
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<td>$μM min^{-1}$</td>
</tr>
<tr>
<td>12</td>
<td>$K_puma$</td>
<td>Michaelis constant of p53-dependent Puma transcription</td>
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<td>μM</td>
</tr>
<tr>
<td>13</td>
<td>$\delta_puma$</td>
<td>Degradation rate of Puma mRNA</td>
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<td>$min^{-1}$</td>
</tr>
<tr>
<td>14</td>
<td>$r_puma$</td>
<td>Translation rate of Puma</td>
<td>0.0245</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>15</td>
<td>$k_ax$</td>
<td>Degradation rate of Baxm</td>
<td>0.7081</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>16</td>
<td>$K_dPumaT^{-1}$</td>
<td>Degradation rate of PumaT</td>
<td>0.0331</td>
<td>$min^{-1}$</td>
</tr>
<tr>
<td>17</td>
<td>$s_puma$</td>
<td>Basal production rate of Puma mRNA</td>
<td>0.002</td>
<td>$μM min^{-1}$</td>
</tr>
<tr>
<td>18</td>
<td>$k_{s3}$</td>
<td>Basal Translation rate of Puma</td>
<td>0.0001</td>
<td>$μM min^{-1}$</td>
</tr>
<tr>
<td>19</td>
<td>$\tau_{i1}$</td>
<td>Puma transcription delay</td>
<td>70</td>
<td>min</td>
</tr>
<tr>
<td>20</td>
<td>$\tau_{i2}$</td>
<td>Puma translational delay</td>
<td>10</td>
<td>min</td>
</tr>
</tbody>
</table>

#### Table 5-2 Initial conditions used in the model

<table>
<thead>
<tr>
<th>No.</th>
<th>Molecular species</th>
<th>Meaning</th>
<th>Value ($μM$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>puma</td>
<td>Puma mRNA</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Puma:Bcl2</td>
<td>Puma:Bcl2 complexes</td>
<td>0.005</td>
</tr>
<tr>
<td>3</td>
<td>Baxm:Bcl2</td>
<td>Baxm:Bcl2 complexes</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>PumaT</td>
<td>Total Puma protein</td>
<td>0.006</td>
</tr>
<tr>
<td>5</td>
<td>BaxmT</td>
<td>Total Bax protein accumulated at the mitochondrial</td>
<td>0.033</td>
</tr>
</tbody>
</table>
5.5 Results and Discussion

5.5.1 Cell Simulation: p53 and Baxm Dynamics in the Presence of Severe Stress

Firstly, we explored the simulation of cell’s activation of apoptotic switch under stressed conditions. Figure 5-3 shows the network dynamics under stressed conditions. After DNA damage, total p53 pulses and then reaches a high concentration at the end of 25 hours. In the process, p53 induces the expression of Puma which activates Bax as follows: Part of Puma binds to Bcl2 forming Puma:Bcl2 inactive complexes. Consequently, Bcl2 (pro-survival) protein level decreases sharply and approaches 0 after 3 hours. This low Bcl2 allows Puma, as a direct activator of Bax, to activate Bax directly and cause Bax accumulation at the mitochondrion. Thus, Baxm concentration increases rapidly in a switch-like, all-or-none manner and results in MOMP within 6 hours (Figure 5-3 Baxm red curve sharply increases). The characteristic of all-or-none switch-like execution of apoptosis was measured by cytochrome c releases after MOMP (Goldstein et al., 2000b; Martinou et al., 2000) and caspase-3 activation (Rehm et al., 2002; Tyas et al., 2000). In our model, we focus on the all-or-nothing Baxm activation and initiation of cell death. The death signal from MOMP is executed in an irreversible way in the downstream events after MOMP, which includes cytochrome c release from mitochondrial outer membrane channels, and subsequently causing caspase-3 activation that dismantles the cell.

This simulation result (as shown in Figure 5-3) of Puma rapidly inducing apoptosis is consistent with experimental findings of morphological signs of apoptosis taking place within 6-9 hours after Puma expression (Nakano & Vousden, 2001; Yu et al., 2001). In addition, our model correctly describes an experimental fact that Puma activation of apoptosis is dependent on Bax (Yu et al., 2003).

In Zhang et al. (2009a) model, the stress signal is represented by a value from 0 to 1 and held constant for the simulation of different levels of DNA damage. According to their model, there is a saddle-node bifurcation with a threshold value of stress=0.5401; when the stress signal is greater than this threshold value, it activates the bistable apoptotic switch in an all-or-none manner that commits irreversible cell death. The DNA damage signal may not be realistic to be fixed to a constant stress value for a long time as in their simulation. Instead, we represented the stress signal as Puma mRNA. This is a more realistic assumption of the DNA damage response inside cell as Puma gets synthesised after p53 triggers apoptosis induction. With this assumption in our model, can we show a threshold activation of bistable apoptotic switch activated by p53? This is the question we will explore next.
5.5.2 Bifurcation Analysis of p53 and Baxm Dynamics in the Presence of Extrinsic Stress

ATM plays a pivotal role in p53 DNA damage response; it detects DNA damage inside cell and invokes downstream activities of DNA damage response including phosphorylation of p53 and Mdm2 (Cheng & Chen, 2010). In the case of DNA damage, DNA double-strand breaks in particular are detected by ATM, and this stress signal is amplified by ATM auto-phosphorylation (Bakkenist & Kastan, 2003). In order to investigate p53 dynamics in deciding Bax activation of MOMP, a bifurcation analysis was done with respect to the parameter ATM auto-activation rate, $k_{\text{auto}}$, which represents ATM auto-phosphorylation. Figure 5-4 shows the bifurcation diagram with a saddle-node bifurcation that illustrates a threshold value at $k_{\text{auto}}=0.04$. This saddle-node bifurcation characterises a bistable apoptotic switch controlled by p53: for $k_{\text{auto}} \leq 0.04$, Baxm stays at basal low level or “off” state. Once $k_{\text{auto}}$ exceeds this threshold value of 0.04, it leads to an abrupt increase in Baxm that causes cell death as indicated in Figure 5-4. When the apoptotic switch gets turned “on” this way, it is assumed to transmit the death signal by causing MOMP that releases cytochrome c, which subsequently causes a cascade of caspases activation.

Figure 5-3 p53 dynamics (blue) induces Puma (black dashed line) and leads to the activation of Bax in a switch-like manner (red). Also shown is, how Puma engages Bcl2 (green) and initiates the activation of Bax. In this simulation, ATM auto-activation rate is set to $k_{\text{auto}}=0.4$. 

![Figure 5-3](image-url)
Figure 5-4 also shows Hopf bifurcation, i.e., Baxm oscillates with small fluctuations. But Baxm protein is maintained at a high level (e.g., Baxm=0.1 $\mu$M) and it is assumed to activate apoptosis. This activation of apoptosis is indicated by the time course simulation for Baxm as shown in Figure 5-5; for example, $k_{\text{auto}}=0.1$ (black thin line) and $k_{\text{auto}}=0.2$ (blue line) and $k_{\text{auto}}=0.4$ (green line) without oscillations indicate apoptosis. With these three $k_{\text{auto}}$ scenarios, bifurcation analysis shows that Baxm gets turned on when $k_{\text{auto}}$ is over the threshold value. This activation of apoptosis is consistent with one of the key characteristics of apoptosis where a threshold value is required such that cell is protected from premature activation of apoptosis (Zhang et al., 2009a).

Figure 5-4 Bifurcation diagram illustrates the bistable switch activated by p53.
5.5.3 Bax Activation Switch Depends on the ATM Auto-activation $k_{auto}$

Next we investigate how $k_{auto}$ controls the timing of Bax activation of apoptotic switch. To demonstrate how stress signal control Bax activation we simply change $k_{auto}$ in the range of 0.041 to 0.4 and do simulation one at a time and observe Baxm time course data. The time of Bax activation to “switch on” apoptosis was recorded. We find that the timing of the switch-like activation of Baxm depends on the value of $k_{auto}$; the larger the $k_{auto}$ value, the shorter the time it takes to turn “On” Baxm. For example, Figure 5-6 shows that for $k_{auto}=0.045$ (red), time for Baxm activation (which indicates mitochondrial outer membrane permeabilisation (MOMP)) is 17 hours and for $k_{auto}=0.4$ (green) time for Baxm activation is 7 hours. Based on these data gathered, it can say that the time to MOMP through Baxm activation of apoptosis depends on ATM auto-activation rate, $k_{auto}$.

These simulation results demonstrate that when cells are treated with the same death stimulus (or agent), the cell death signal in individual cells may vary as represented by different values of $k_{auto}$, thus causing variability in time to MOMP. This feature of variability in apoptosis induction was experimentally observed in individual cell studies (Rehm et al., 2002). For example, in Rehm et al. (2002) [Figure 2 (bottom panel)], even though the same death stimulus was given in the form of 10...
μM etoposide, a DNA-damaging agent, some cells died earlier than others in a time frame ranging from 12-15 hours.

Moreover, these results are also comparable to the variable time delay (or time to MOMP) characteristic of apoptosis obtained from experiments using different doses of death ligand TRAIL in inducing cell death through the death receptor pathway (the second apoptotic pathway). Here, different strengths of the stress leads to a time graded execution of cell death where higher doses of TRAIL lead to quicker MOMP (Albeck et al., 2008).

Figure 5-6 Simulation of Bax activation for different values of \( k_{\text{auto}} \).

To further explore variability in time to apoptosis, a range of simulation results for the time to Baxm activation for \( k_{\text{auto}} \) values between 0.041 and 0.4 were collected, and these data are plotted in Figure 5-7. It shows that for small value of \( k_{\text{auto}} \) the time to MOMP (or Baxm activation) is larger, and the larger the value \( k_{\text{auto}} \) the shorter the time to turn “On” Baxm. This characteristic may explain the heterogeneity response in a population of cells that activate apoptosis at different rates or time points due to variability and distinct state of individual cells.
Figure 5-7 Time to Baxm activation of MOMP in p53 activated apoptosis is dependent on the parameter ATM auto-activation rate, \( k_{\text{auto}} \). Simulation results show that the larger the value \( k_{\text{auto}} \), the shorter the time to turn “On” Baxm.

After p53 expression in a population of cells, there is heterogeneity in the time to apoptosis that was observed in experiments. One possible reason for the different times to apoptosis in individual cells in a population is the existence of a wide range of stress signals in the cell population corresponding to their states of the cell cycle. To account for this stress signal variation, we assume that \( k_{\text{auto}} \) is normally distributed with mean 0.15 and standard deviation of 0.1. A population of cells consisting of 2000 cells is drawn from this distribution and is illustrated by the histogram in Figure 5-8. The negative values for \( k_{\text{auto}} \) in some cells may indicate that ATM is kept as inactive dimers. Estimates of time to MOMP for each cell based on the value of \( k_{\text{auto}} \) from Figure 5-8 are given in Figure 5-9. It also shows time estimates from our original simulation (Figure 5-7) which seems to follow the population trend. We can use this information to find the percentage of apoptotic cells in the population at any given time as shown in Figure 5-10. It shows that after 5 hours some cells start to commit to cell death and the percentage increases very quickly until it reaches 90% after 50 hours. This figure has captured similar results as the experimental observations that when p53 was expressed approximately 90% of cells committed to apoptosis after 48 hours (Yu et al., 2001). This result demonstrates that ATM auto-activation \( k_{\text{auto}} \) is the determining factor in the timing of the decision to commit to cell death.
Figure 5-8 Histogram of the $k_{auto}$ distribution drawn from a population of 2000 cells.
Figure 5-9 The estimation of the time to MOMP (black asterisk) for the $k_{auto}$ values in Figure 5-8. Blue circles are the same as in Figure 5-7.
5.5.4 P53 Protein Dynamics up to Cell Death

The next question we asked was: What is p53 protein dynamics up to the occurrence of apoptosis when cells are induced by a DNA damage agent? The answer to this question can be found in our model computer simulation where we observe simulated p53 protein levels until the time to MOMP. For example, Figure 5-11 shows the simulation of three cells, each corresponding to one $k_{auto}$ value (0.06, 0.043 and 0.041) as in the previous simulation in Figure 5-7. The simulation results indicate apoptosis can occur after one and a half pulses of p53 activation. Recently, there was one study that used U-2 OS and A549 cell lines in single cell analysis and measured p53 protein dynamics in individual cells until apoptosis was observed (Chen et al., 2013). Their data show that p53 protein level increases monotonically until apoptosis happens and one cell show one and a half pulses of p53. The times to apoptosis for the three cells from these data are 15, 19 and 39 hours (Figure 1b in Chen et al. (2013)); they compare well with our simulation results with the time to MOMP of 12, 22 and 38 hours (Figure 5-11). Thus, our simulation has captured some of the qualitative behaviour in Chen et al. (2013) recent experimental findings.
5.5.5 Cancer Cells with Bcl2 Overexpression Simulation: p53 and Baxm Dynamics in the Presence of Severe Stress

Bcl2 (pro-survival proteins) overexpression in cancer, impairing apoptosis, is a key factor in resistance to chemotherapy (Adams & Cory, 2007). To investigate the 50-70% of solid organ malignancies with Bcl2 overexpression (Fahy et al., 2005), we simulate this condition by setting a higher level of total Bcl2 with \( Bcl2T = 1.06 \) (66.7% increase). Figure 5-12 shows the simulation results for Bcl2 overexpression. The differences compared to normal cells are that Bcl2 levels do not approach 0 and this result shows that Puma (BH3-only protein) could not neutralise Bcl2 completely. As a result, our model simulation demonstrates that Bax activation switch fails to be activated in cancer cells as indicated by the basal level of Baxm (red) throughout the simulation period of 25 hours shown in Figure 5-12. This simulation shows that p53 initiated apoptosis can fail even when it can still induce Pumagene expression.

The breast cancer MCF7 cell line used in the study of individual cell dynamics of p53 was shown to have high level of Bcl2 protein (Fahy et al., 2005) and this overexpression of Bcl2 could be one reason why some cells experienced repeated pulses of p53 for days after DNA damage by gamma irradiation, even for high gamma irradiation of 10 Gy (Geva-Zatorsky et al., 2006), because the
apoptotic switch is dysfunctional in these cells. Over 50% of colorectal, prostate, pancreas, breast, and lung cancers that account for major incidences of death by cancer are associated with Bcl2 overexpression (Fahy et al., 2005) and inactivated p53 function. Our model simulations are consistent with this fact that cancers employ some mechanism to overexpress Bcl2 and thus evade apoptosis (Adams & Cory, 2007).

Figure 5-12 Overexpression of Bcl-2 proteins prevents Bax activation as shown by low level of Baxm (red line) throughout the simulation.

5.5.6 Prediction of Pharmacological Intervention to Reactivate p53 in Cancer Cells Overexpressing Bcl2

In order to investigate pharmacological perturbation to reactivate p53 in cancer that overexpressed Bcl2 protein, we chose to set p53-dependent Puma transcription rate, e6, to a higher value of 0.036 (100% increase) for mimicking the interventions such as Puma gene therapy that increases Puma expression, and see if this strategy can reactivate the apoptotic switch. The simulation results are shown in Figure 5-13. With doubling Puma transcription rate, Puma protein increases to a higher level and it shows that Baxm was activated by this change. This result is in line with the pharmacological intervention of BH3 mimetic drug such as ABT-737 to perform the same function as
Puma (BH3-only protein) in activating the p53 apoptotic switch, which has shown very promising results (Adams & Cory, 2007).

![Graph showing protein levels over time](image)

**Figure 5-13** Overexpression of Bcl2 proteins in cancer preventing apoptosis can be restored by increasing p53-dependent Puma expression that reactivates Bax (apoptotic switch).

### 5.6 Summary

In this chapter, we propose a mathematical model of the core regulatory feedback mechanism that regulates p53 activation of apoptosis. This model investigated the design principles behind the regulation of p53 activation of apoptotic switch. Notably, we incorporated the molecular interactions in the core regulation of p53 and the apoptosis initiation module involving Puma, Bcl2 and Bax. Activation of Bax is assumed to be an indicator of apoptosis initiation. The constructed model demonstrated how molecular interactions and stress signalling molecule ATM auto-activation in the p53 network dictate cell fate decisions. Our model hypothesis shows that there is a signal threshold for activation of apoptotic switch controlled by ATM signal transduction. This apoptotic signal threshold activation controls p53 protein level and induces cell death once the stress signal (ATM auto-phosphorylation) has exceeded a critical value. Most importantly, our model simulation results are qualitatively consistent with the experimental findings of an all-or-none activation of apoptosis and predicted overexpression of Bcl2 as a factor in causing malfunction of the apoptotic switch. Our
model simulation also predicted that increased Puma transcription rate can re-activate p53 apoptosis induction in cases of Bcl2 overexpression. In conclusion, we present a simplified yet plausible model of molecular mechanism that controls p53 activation of apoptotic switch. The model gives insight into the mechanism underlying p53 regulation of apoptosis.
Chapter 6
Summary, Conclusions, Contributions and Future Directions

6.1 Overview

p53 is a key node in the p53 network that integrates various stress signals and decides cell life and death. Recent developments in the understanding of p53 network interactions gathered by molecular biologists using causal relationship reasoning may not be able to view the whole picture of the complex network dynamics. Thus, a systems biology approach is used in exploring the design principles of p53 regulation. In this thesis, two mathematical models have been developed to explore the design principles of the p53 DNA damage response: 1) p53 basal dynamics and DNA damage response as an excitable system; 2) p53 regulation of activation of apoptosis. These two mathematical models have provided novel theoretical insights into p53 regulation in response to DNA double-strand breaks. In this last chapter, a general summary is given along with conclusions from this thesis, key contributions are highlighted and future directions are discussed in terms of the p53 network.

6.2 General Summary

The first focus of the work was to review current knowledge of p53 pathway and experimental results gathered in the literature (Chapter 2). Then we explored how well previous mathematical models have been proposed to explain and give insights into p53 regulation. After a thorough review of the literature and background knowledge, we identified two issues remaining to be investigated: 1) p53 basal dynamics and DNA damage response; 2) p53 all-or-none activation of apoptotic switch.

The second focus was on the methods (Chapter 3) used in modelling and analysing biological systems. First, we looked at the steps in modelling p53 system and how biochemical reactions can be written as mathematical model equations. The next step was on how the mathematical equations can be converted into a computer model that can be simulated numerically. The model simulation includes calibration of model parameters. When model simulation results are reasonably consistent with the experimental results, then model analysis such as bifurcation analysis and local parameter sensitivity analysis can be performed to gain insights from this theoretical model. One important knowledge requirement for modeller is to analyse the built model with bifurcation theory and to be able to draw bifurcation diagram and interpret the signal-response curve of the model, particularly in drawing saddle-node and Hopf bifurcations. The application of the package DDE-BIFTOOL for model equations involving time delays is shown in a step by step explanation and the corresponding MATLAB scripts are given in Appendix A.
The third focus was on building a mathematical model to address the first issue: p53 basal dynamics and DNA damage response (Chapter 4). Based on Sun et al. (2011) deterministic model, we modified and improved the model by adding p53 auto-regulation and MdmX, as two new components, into the network structure. Based on the model hypothesis, a model of 19 DDEs with 78 parameters were formulated. The important results are summarised as: 1) The model parameters were calibrated to reproduce the spontaneous pulses under non-stressed conditions and repeated pulses under stressed conditions; 2) The constructed model was then subjected to bifurcation analysis, that provided valuable insights into p53 excitable dynamics and ATM auto-activation controls threshold activation. Based on the bifurcation analysis we obtained a bifurcation diagram with a saddle-node and a Hopf bifurcation. This novel saddle-node characterised the excitability as a bistable switch from homeostasis to pulsatile dynamics. The model highlighted the crucial role of a positive feedback loop (p53 auto-regulation) in controlling p53 excitable dynamics. For higher values of ATM auto-activation, the pulsatile dynamics become stable limit cycle oscillations arising from Hopf bifurcation. The frequency analysis from these oscillations classified p53 excitability as Type II excitability (arising from non-zero frequency). 3) The model also suggested that p53 excitability leads to p53 network functioning as a pulse generator in regulating cell fate decisions. 4) In addition, local parameter sensitivity analysis has identified Wip1 mRNA and protein degradation rate, and ATM auto-activation rate as crucial parameters in controlling p53 oscillations and activation. This analysis makes testable predictions on activating p53 by increasing Wip1 mRNA or protein degradation so as to reduce Wip1 protein levels.

The final focus was on constructing a mathematical model to address the second issue: p53 all-or-none activation of apoptotic switch (Chapter 5). Building on the previous model in Chapter 4, we incorporated the apoptosis initiation module from Zhang et al. (2009a) where we represented the stress signal by Puma mRNA and with different parameter values to explore the p53 activation of a bistable apoptotic switch. There are 24 DDEs formulated from this model, which include 19 DDEs from previous model (Chapter 4). A summary of the important results are: 1) The calibrated model was able to achieve simulation results that are qualitatively consistent with the characteristics of apoptosis: all-or-none and irreversible commitment to cell death; a bistable switch in activation of apoptosis; the timing of the apoptosis initiation. 2) This model proposed a simple design principle in the core regulation and apoptosis initiation by Bax activation at the mitochondrion that leads to the all-or-none commitment to cell death decision. The model demonstrated how interactions from the core regulators and apoptosis initiation module control the emergent behaviour that form cell death decisions. 3) The model suggested that ATM auto-activation is a key factor in controlling the timing of apoptotic activation. 4) The model confirmed that overexpression of Bcl2 is a factor in the
malfuunction of the apoptotic switch and the model prediction suggested that increasing Puma expression can re-activate p53 activation of apoptosis.

6.3 Conclusions

p53 is a transcription factor that plays an important role in DNA damage response in deciding cell life and death. However, recent experimental findings from Loewer et al. (2010) discovered p53 protein dynamics as excitable pulses both under non-stressed and stressed conditions. The mechanism underlying these excitable pulses is not clear. In addition, another study from Chen et al. (2013) uncovered novel observation on p53 activation of apoptosis under high DNA damage. Chen et al. (2013) suggested one of the components in the p53 pathway may control a threshold activation of apoptosis that requires theoretical investigation and analysis. In this thesis, two models of p53 regulation was proposed as plausible models of p53 activation in regulating cell cycle arrest, basal dynamics and apoptosis. The first model proposed in Chapter 4 has captured the important finding of the behaviour of p53 as excitable pulses with a threshold activation and classification of p53 oscillations as Type II excitability. The second model proposed in Chapter 5 provided quantitative explanation of all-or-none apoptosis activation and suggested ATM auto-activation as the component that controls the threshold of p53 activation of apoptosis. These models are considered successful from a theoretical perspective because it has achieved the goals of the research. In particular, it satisfied one of the main purpose of mathematical models, that is, “to provide a unified conceptual framework to account for experimental observations and to generate testable predictions.” (Goldbeter, 2002)

Our understanding of p53 regulation requires on-going studies, both in theoretical models and experimental results, so that knowledge is accumulated over time. We hope that the models proposed in this study will add value to the field in understanding p53 dynamics and function. Iterative modelling of p53 network helps improve mathematical models of p53 regulation and it is hoped that these improved theoretical models can provide useful predictions for various conditions that cannot be explored experimentally. Model simulations and predictions serve as a basis for generation of hypothesis and contribute to a deeper understanding of p53 regulation that will lead to better treatment of cancer in future.

6.4 Contributions

The contributions of this thesis are the exploration of two theoretical models (1) p53 regulation in non-stressed and stressed conditions, and its DNA damage response, and (2) p53 activation of apoptotic switch in making cell life and death decisions.
Due to the complexity of p53 regulation, it is hard to understand the system behaviour intuitively; therefore, contributions of the developed advanced models incorporating the latest known molecular interactions are highlighted below:

(1) p53 regulation in non-stressed and stressed conditions, and DNA damage response

- Constructed a deterministic conceptual model of p53 core regulatory network model that explains the experimental findings on spontaneous pulses under non-stressed conditions and a series of repeated pulses under stressed conditions; i.e., basal dynamics and DNA damage response after stress.
- Presented a bifurcation analysis showing that p53 dynamics are both pulsatile and oscillatory
- Suggested that the pulse generation mechanism in p53 network controls cell fate decisions

(2) p53 activation of apoptotic switch in making cell life and death decision

- A theoretical model that incorporated the known molecular interactions and captured the switch-like behaviour of p53 activation of apoptosis through activation of Puma.
- One of our model hypotheses that was supported by the model is that there is a threshold activation of apoptotic switch controlled by ATM signal amplification.
- Generated computer simulations are comparable to experimental observations and provided some predictions on pharmacological intervention to reactivate p53 in cancer, for example, suppressing Bcl-2 overexpression found in many cancers.

6.5 Future Directions

This work has investigated the p53 network from a theoretical perspective based on the available molecular interactions, chosen model hypothesis and model assumptions. It is an attempt to formulate conceptual models to capture the experimental findings and regulation of p53. Thus, there are many aspects that are unexplored and may require more work to reconcile the theoretical and experimental studies of p53 regulation and cancer. Here, we suggest some directions for future research.

- One of the future directions is modelling p53 activation in kinetic models incorporating actual pharmacological interventions that can give practically realistic predictions of the p53 responses, which is crucial in improving anti-cancer treatment. The model presented in this thesis should be extended as it may be useful in predicting response to drug intervention. For
example, integrated mass action modelling with particle swarm optimisation approach has been used successfully in insulin-like growth factor (IGF-1) signalling network for identifying optimal drug combinations in inhibiting signalling network and reducing cell proliferation (Iadevaia et al., 2010).

• In our model simulation, the number of DNA double-strand breaks is represented by a fixed value of 1 or 3 for non-stressed conditions and 300 for stressed conditions. An important question for future study is to determine if the number of DNA double-strand breaks for stressed conditions as represented by stochastic generation of DNA double-strand breaks and repair with Monte Carlo simulation (Ma et al., 2005) can reproduce experimental findings of a series of repeated pulses under stressed conditions. For non-stressed conditions, the case will be to determine the number of DNA double-strand breaks as represented by a simplified form of DNA double-strand breaks repair as in (Sun et al., 2011) integrated into the model proposed in Chapter 4 can reproduce experimental findings of spontaneous pulses under non-stressed conditions.

• Stochastic modelling approach was not investigated in this thesis and it is worth exploring it in the model in Chapter 4 as noise plays an important role in gene regulation (McAdams & Arkin, 1999; Ribeiro, 2010). What is more, Geva-Zatorsky et al. (2006) individual cell studies also reveal that p53 dynamics are rather noisy, so the stochastic modelling approach is definitely another direction for future investigation.

• The model structure proposed in Chapter 4, especially the existence of a positive feedback loop (p53 auto-regulation) that offers an explanation for p53 pulses may be useful for describing the activity of other transcription factors, for example, the nuclear factor κB (NF-κB). Experimental observations of NF-κB expression also exhibit oscillatory or pulsatile behaviour (Hoffmann et al., 2002; Nelson et al., 2004a; Nelson et al., 2004b).

• One intriguing question has been asked about mental stress or psychological stress that may have an impact on p53 activation (Levine et al., 2006). The diagnosis report given by a doctor to cancer patients is like a death sentence that causes “extreme stress” and trauma to cancer patients. This extreme stress is because of the current perception on cancer is “no cure” and gives “no hope” that forms a mental block to overcome this disease. The fear of death by cancer is the most difficult thing to handle for any person whether a lay person or a doctor who has cancer. A recent study from mice shows that chronic stress can reduce p53 function and promote tumour growth (Feng et al., 2012). Thus, the linkage of psychological stress to p53 function requires further investigation.
Another interesting direction is the effects of food and exercise on p53 activation, for example, green and black tea that have been proposed related to enhancing p53 activation (Bode & Dong, 2004). In addition, there is a study showing that low fat, high fiber diet and daily exercise greatly increases p53 protein and enhances apoptosis (Soliman et al., 2011). The factors of food and exercise in affecting p53 function could be explored in future studies.

In future, scientists will investigate heat shock, another stress signal, that activates p53 DNA damage response. It has been shown that using heat as a stimulus to elevate to a slightly higher (than normal) body temperature of 43°C can induce p53 phosphorylation at Serine 15 and decrease p53 ubiquitination resulting in p53 accumulation (Wang & Chen, 2003). Since heat can be used as a stimulus that activates p53 and causes no toxicity or side effects, it has potential for use as a cancer treatment strategy.
References


Appendix A

Programming Codes for Chapter 3

The Oscill8 model code is listed in Appendix A1 and A.3. Meanwhile, the step-by-step implementation of DDE-BIFTOOL for generating saddle-node and Hopf bifurcation examples in chapter 3 is listed in Appendix A.2 and A.4.

A.1 The Toggle Switch Model Equation for Oscill8

\[
\begin{align*}
    u' &= \frac{\alpha_1}{1+v^n}-u \\
    v' &= \frac{\alpha_2}{1+u^m}-v
\end{align*}
\]

\[\text{param } \alpha_1=3, \alpha_2=3, n=3, m=3\]

Note: The code for Oscill8 is similar to XPPAUT file format.

A.2 DDE-BIFTOOL MATLAB Scripts for Saddle-Node Bifurcation

In order to run DDE-BIFTOOL, one needs to download the package from:


The latest updates and information from the package can be accessed from:


Unzip the package and save it, for example, in the folder D:\ddebiftool. Then create a folder D:\My_ddebiftool and save all your model files (such as files 1-5 below) in this folder. Set the current folder as D:\My_ddebiftool and to create a bifurcation diagram one needs to run file number 5 below at the MATLAB command prompt.

First, definition of the model system files are given below:

1. sys_init.m for initialising the dimension of the model equations.

   \[
   \begin{align*}
   \text{function } [\text{name}, \text{dim}]=\text{sys_init()} \\
   \text{name}='\text{AToggleSwitch}'; \\
   \text{dim}=2; \\
   \text{path(path,'D:\ddebiftool')}; \\
   \text{return;}
   \end{align*}
   \]
2. sys_rhs.m for defining the DDEs.

```matlab
function f=sys_rhs(xx,par)
% par=[alpha1 alpha2 m n tau]
%      par(1) par(2) par(3) par(4) par(5)
% xx=[u u(t-tau)
%     v v(t-tau)];

f(1,1)=par(1)/(1+xx(2,2)^par(4))-xx(1,1);   % u' = alpha1/(1+v^n) - u
f(2,1)=par(2)/(1+xx(1,1)^par(3))-xx(2,1);  % v' = alpha2/(1+u^m) - v

return;
```

3. sys_deri.m (instead of providing your system derivative you can use the DDE-BIFTOOL sys_deri.m)

The sys_deri.m was written by Engelborghs et al. (2000b).

```matlab
function J=sys_deri(xx,par,nx,np,v)
% function J=sys_deri(xx,par,nx,np,v)
% INPUT:
%   xx state variable and delayed state variables columnwise
%   par list of parameter values
%   nx empty or list of requested state-derivatives (numbers of delay or zero)
%   np empty or list of requested parameter-derivatives
%   v matrix to multiply result with
% OUTPUT:
%   J result of derivatives on righthandside multiplied with v
% COMMENT:
%   the numerical derivatives are evaluated using forward differences
%   (c) DDE-BIFTOOL v. 1.00, 11/03/2000
% first order derivative discretisation parameters:
abs_eps_x1=1e-6;
asb_eps_x2=1e-6;
asb_eps_p1=1e-6;
asb_eps_p2=1e-6;
rel_eps_x1=1e-6;
rel_eps_x2=1e-6;
rel_eps_p1=1e-6;
rel_eps_p2=1e-6;
	n=size(xx,1);

J=[];
% first order derivatives of the state:
if length(nx)==1 & length(np)==0 & isempty(v),
  f=sys_rhs(xx,par);
  for j=1:n
    xx_eps=xx;
    eps=abs_eps_x1+rel_eps_x1*abs(xx(j,nx+1));
    xx_eps(j,nx+1)=xx(j,nx+1)+eps;
    J(:,j)=(sys_rhs(xx_eps,par)-f)/eps;
  end;
% first order parameter derivatives:
elseif length(nx)==0 & length(np)==1 & isempty(v),
  f=sys_rhs(xx,par);
  par_eps=par;
  eps=abs_eps_pl+rel_eps_pl*abs(par(np));
  par_eps(np)=par(np)+eps;
  J=sys_rhs(xx,par_eps)-f)/eps;
% second order state derivatives:
elself length(nx)==2 & length(np)==0 & ~isempty(v),
for j=1:n
    J(:,j)=sys_deri(xx,par,nx(1),[],[])'*v;
    xx_eps=xx;
    eps=abs_eps_x2+rel_eps_x2*abs(xx(j,nx(2)+1));
    xx_eps(j,nx(2)+1)=xx_eps(j,nx(2)+1)+eps;
    J(:,j)=(sys_deri(xx_eps,par,nx(1),[],[])'*v-J(:,j))/eps;
end;
% mixed state parameter derivatives:
elseif length(nx)==1 & length(np)==1 & isempty(v),
    J=sys_deri(xx,par,nx(1),[],[]);
    par_eps=par;
    eps=abs_eps_p2+rel_eps_p2*abs(par(np));
    par_eps(np)=par(np)+eps;
    J=(sys_deri(xx,par_eps,nx(1),[],[])'-J)/eps;
end;
if isempty(J)
    [nx np size(v)]
    error('SYS_DERI: requested derivative does not exist!');
end;
return;

4. sys_tau.m for defining the delay terms indexes.

function tau=sys_tau()

    tau=[5]; % There is only one time delay located at par(5)

    return;

Then, the scripts that run the DDE-BIFTOOL and generate the saddle-node bifurcation diagram are shown below.

5. AToggleSwitch_fold_bifurcation.m

%% This example is taken from Keshet
%% www.math.ubc.ca/~keshet/MCB2012/NotesLEK/MCBNotes.pdf
%% A simple example based on the Bistable genetic equations:
%% u'=alpha1/(1+v^n)-u
%% v'=alpha2/(1+u^m)-v
%-------------------
%% modified to delay differential equations as below:
%%
%% u'=alpha1/(1+v(t-tau)^n)-u
%% v'=alpha2/(1+u^m)-v
%% par=[alpha1 alpha2 m n tau]=[3 3 3 3 0.01]
%% xx=[u u(t-tau)
%%     v v(t-tau)];

function tau=sys_tau()

    tau=[5]; % There is only one time delay located at par(5)

    return;

Then, the scripts that run the DDE-BIFTOOL and generate the saddle-node bifurcation diagram are shown below.

5. AToggleSwitch_fold_bifurcation.m

%% This example is taken from Keshet
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%% A simple example based on the Bistable genetic equations:
%% u'=alpha1/(1+v^n)-u
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%-------------------
%% modified to delay differential equations as below:
%%
%% u'=alpha1/(1+v(t-tau)^n)-u
%% v'=alpha2/(1+u^m)-v
%% par=[alpha1 alpha2 m n tau]=[3 3 3 3 0.01]
%% xx=[u u(t-tau)
%%     v v(t-tau)];

function tau=sys_tau()

    tau=[5]; % There is only one time delay located at par(5)

    return;

Then, the scripts that run the DDE-BIFTOOL and generate the saddle-node bifurcation diagram are shown below.

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%% A simple example based on the Bistable genetic equations:
%% u'=alpha1/(1+v^n)-u
%% v'=alpha2/(1+u^m)-v
%-------------------
%% modified to delay differential equations as below:
%%
%% u'=alpha1/(1+v(t-tau)^n)-u
%% v'=alpha2/(1+u^m)-v
%% par=[alpha1 alpha2 m n tau]=[3 3 3 3 0.01]
%% xx=[u u(t-tau)
%%     v v(t-tau)];

function tau=sys_tau()

    tau=[5]; % There is only one time delay located at par(5)

    return;
```matlab
% name =
% AToggleSwitch
% n =
% 2

% define a variable stst with object kind
stst.kind='stst'; % stst is similar to struct in c and has a field called kind
par=[3 3 3 3 0.01]; % par=[1 2 3 4 tau]=[alpha1 alpha2 m n tau]

% setting the parameters
stst.parameter=par;

% define a first steady state
stst.x=[1.16; 1.16 ];
stst.stability=[]; % define a field stability to stst

% define the method
method=df_mthod('stst');
% sets the correction method to look for an equilibrium point
[stst,success]=p_correc(stst,[],[],method.point)

% calculate the stability of the equilibrium point stst and set stability
% as a new field of stst to the calculation output
stst.stability=p_stabil(stst,method.stability);

% plots a locus of roots for the equilibrium point stst
figure(1); clf;
p_splot(stst);
method.stability.minimal_real_part=-20;
stst.stability=p_stabil(stst,method.stability);
figure(2); clf;
p_splot(stst);

disp('Computing branch1, the branch of steady state solutions.')

% a branch of object is created and named branch1
% note that the first entry in the argument is the number for parameter of
% interest in the par (parameter position in par 1 is the par(1)=alpha1)
branch1=df_brnch(1,'stst');

% set the min and max bound of the bifurcation parameter alpha1
branch1.parameter.min_bound(2,:)=[1 0]; % 1 is the parameter position; row 2
because only tau1 one time delay
branch1.parameter.max_bound(1,:)=[1 20];
branch1.parameter.max_step(1,:)=[1 0.05];
% start with the steady state point determined earlier
branch1.point=stst;

disp('To obtain a second starting point we change parameter value g slightly and
correct again.')
stst.parameter(1)=stst.parameter(1)+0.001;
[stst,success]=p_correc(stst,[],[],method.point);
branch1.point(2)=stst; % use as a second branch point,next branch point is as calculated

% runs the continuation routine on the branch, for as many as 2000
% iterations or until the maximum parameter bound is reached.
[branch1,s,f,r]=br_contn(branch1,2000)
branch1=br_rvers(branch1); % turn the branch around
[branch1,s,f,r]=br_contn(branch1,2000) % continue in the other direction

disp('Computing stability of the steady state solutions.')

% determine stability of each point on the branch
branch1=br_stabl(branch1,0,1)

% obtain suitable scalar measures to plot stability along branch
```
% get a measure of the eigenvalues
[ym,=}df_measr(1,branch1);

k = 1;
while (real(branch1.point(k).stability.l0(1)) < 0) && k < length(branch1.point)
    eigenvalue=real(branch1.point(k).stability.l0(1));
    k = k + 1;
end

if (k == length(branch1.point))
    disp('Failed to find the fold bifurcation point in branch1.')
    disp(k)
    return
end

eigenvalue=real(branch1.point(k).stability.l0(1))
str = sprintf('Found the fold bifurcation near point %d in branch1.',k);
disp(str)
figure(3); clf;
br_plot(branch1,xm,ym,'k.);
xlabel('parameter alpha1');ylabel('Re(\lambda)');
% br_plot function will plot the graph for Re(lambda) versus the parameter 
% g
figure(4); clf;
ym.subfield='l0'; % l0= l zero is the approximations 
br_plot(branch1,xm,ym,'c.-');
xlabel('parameter alpha1');ylabel('Re(\lambda)');
% Again approximations and corrections are nearly indistinguishable. 
% The lines where it crosses the zero line, bifurcation occur. 
% In this case bifurcation occurs when alpha1=1.9188,9.9864 (see below)
figure(5); clf;
br_plot(branch1,[],ym,'b');
br_plot(branch1,[],ym,'b.');
xlabel('Point number','fontsize',30);ylabel('Re(\lambda)','fontsize',30);
plot([0 1000], [0 0], '-.','linewidth',2); % this is where the x-axis lies
plot([k k], [-1 1], '-.','linewidth',2); % Found the fold bifurcation near point k in
branch1.
text(k+3, -0.8,'k1=','fontsize',15);
 templ= ['num2str(k)'];
tt1=eval(templ);
text(k+40, -0.8, tt1,'fontsize',15);
k2 = k+1;
while (real(branch1.point(k2).stability.l0(1)) > 0) && k2 < length(branch1.point)
    eigenvalue=real(branch1.point(k2).stability.l0(1));
    k2 = k2 + 1;
end

if (k2 == length(branch1.point))
    disp('Failed to find the Hopf bifurcation point in branch1.')
    return
end

eigenvalue=real(branch1.point(k2).stability.l0(1))
str = sprintf('Found the Hopf bifurcation near point %d in branch1.',k2);
disp(str)
% Found the Hopf bifurcation near point k2 in branch1.
plot([k2 k2],[-1 1], 'r--','linewidth',2); % Found the fold bifurcation near point k2 in branch1.
% observed that point k2 Re(lambda) is zero 
% tell us that we have found fold bifurcation near point k2
text(k2+3, -0.1,'k2=','fontsize',15) % approximate point number is k2=401
 temp2= ['num2str(k2)'];
tt2=eval(temp2);
Computing the first fold bifurcation point:

The function to locate fold points is p_tofold;
% it takes an initial guess of an equilibrium point as input, and return
% output of a machine approximation of the location of fold point
fold = p_tofold(branch1.point(k));
method = df_method('fold');
[fold, success] = p_correc(fold, 1, [], method.point); % p_correc(fold, parameter(alpha1
is 1) number in par, [], method.point);
disp(fold);

% We copy the corrected point to keep it for later use.
first_fold_point = fold;

% Computing and plotting stability of the fold point clearly reveals the
% pair of pure imaginary eigenvalues, see figure(6)
fold.stability = p_stabil(fold, method.stability); % compute stability of hopf point
figure(6); clf;
p_splot(fold); % plot stability of fold
% One of the eigen value is zero at the bifurcation point, so lambda=0
% indicated we have located a saddle-node bifurcation
bifurcation_g1 = fold.parameter(1)

% In order to follow a branch of fold bifurcations in the two parameter
% space (alpha1, par(1)) we again need two starting points.
branch2 = df_branch([1 2], 'fold'); % par(1) = alpha1; par(2) = alpha2
branch2.parameter.min_bound(2, :) = [1 0];
branch2.parameter.max_bound(1:2, :) = {[1 1] [2 1]'};
branch2.parameter.max_step(1:2, :) = {[1 0.05] [2 0.002]'};
branch2.point = fold;
fold.parameter(2) = fold.parameter(2) + 0.001;
[fold, success] = p_correc(fold, 1, [], method.point); % p_correc(fold, parameter(alpha1
is 1) number in par, [], method.point);
disp('Computing the second fold bifurcation parameter:')

% The function to locate Hopf points is p_tohopf;
% it takes an initial guess of an equilibrium point as input, and return
% output of a machine approximation of the location of Hopf point
fold = p_tofold(branch1.point(k2));
method = df_method('fold');
[fold, success] = p_correc(fold, 1, [], method.point);
disp(fold);

second_fold_point = fold;
fold.stability = p_stabil(fold, method.stability); % compute stability of hopf point
figure(7); clf;
p_splot(fold); % plot stability of fold
% One of the eigen value is zero at the bifurcation point, so lambda=0
% indicated we have located a saddle-node bifurcation (the second one)
bifurcation_g2 = fold.parameter(1)

% Drawing the fold bifurcation diagram
disp('Drawing the fold bifurcation diagram for u:')
LL = length(branch1.point);
par_g = zeros(1, LL);
x = zeros(1, LL);

% preparing data for plotting bifurcation diagram
for i = 1:LL
    par_g(i, i) = branch1.point(1, i).parameter(1);
    x(i, i) = branch1.point(1, i).x(1);
end
par_stable = [];

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var_stable=[];
par_unstable=[];
var_unstable=[];

% Group the stable and unstable steady states
for i=1:LL
    if branch1.point(1,i).stability.10 < 0
        par_stable=[par_stable par_g(1,i)];
        var_stable=[var_stable x(1,i)];
    else
        par_unstable=[par_unstable par_g(1,i)];
        var_unstable=[var_unstable x(1,i)];
    end
end

figure(8); clf;
set(gca,'color','w');
set(gca,'linewidth',1.5,'xtick',[0 5 10 15 20],'ytick',[0 5 10 15 20],'fontsize',20,'fontweight','bold');
hold on;
plot(par_stable,var_stable,'b.');
plot(par_unstable,var_unstable,'k--');
axis([0 20 0 20])
xlabel('parameter alpha1','fontsize',30);
ylabel('steady state value for u','fontsize',30);

%% Drawing the fold bifurcation diagram for v
disp('Drawing the fold bifurcation diagram for v:')
LL=length(branch1.point);
par_v=zeros(1,LL);
v=zeros(1,LL);
% preparing data for plotting bifurcation diagram
for i=1:LL
    par_v(1,i)=branch1.point(1,i).parameter(1);
    v(1,i)=branch1.point(1,i).x(2);
end
par_stablev=[];
var_stablev=[];
par_unstablev=[];
var_unstablev=[];
% Group the stable and unstable steady states
for i=1:LL
    if branch1.point(1,i).stability.10 < 0
        par_stablev=[par_stablev par_v(1,i)];
        var_stablev=[var_stablev v(1,i)];
    else
        par_unstablev=[par_unstablev par_v(1,i)];
        var_unstablev=[var_unstablev v(1,i)];
    end
end
figure(9); clf;
set(gca,'color','w');
set(gca,'linewidth',1.5,'xtick',[0 5 10 15 20 25],'ytick',[0 1 2 3 4],'fontsize',20,'fontweight','bold');
hold on;
plot(par_stablev,var_stablev,'b.');
plot(par_unstablev,var_unstablev,'k--');
axis([0 25 0 4])
xlabel('parameter alpha1','fontsize',30);
ylabel('steady state value for v','fontsize',30);
A.3  The Cell Cycle Oscillator Model Equation for Oscill8

\[
\begin{align*}
\text{CDK1a}' &= \alpha_1 \cdot \beta_1 \cdot \text{CDK1a} \cdot \text{APCa}^{n_1} / (K_1^{n_1} + \text{APCa}^{n_1}) \\
\text{APCa}' &= \alpha_2 \cdot (1 - \text{APCa}) \cdot \text{CDK1a}^{n_2} / (K_2^{n_2} + \text{CDK1a}^{n_2}) - \beta_2 \cdot \text{APCa}
\end{align*}
\]

param \( \alpha_1 = 0.1, \alpha_2 = 3, \beta_1 = 3, \beta_2 = 1, K_1 = 0.5, K_2 = 0.5, n_1 = 8, n_2 = 8 \)

A.4  DDE-BIFTOOL MATLAB Scripts for Hopf Bifurcation

First, definition of the model system files are given below:

1. **sys_init.m**

```matlab
function [name,dim]=sys_init()
name='Ferrell_TwoODE_with_Delay_Model';
dim=2;
path(path,'D:\ddebiftool');
return;
```

2. **sys_rhs.m**

```matlab
function f=sys_rhs(xx,par)
% par=[\alpha_1 \alpha_2 \beta_1 \beta_2 K_1 K_2 n_1 n_2 tau1 tau2]=[0.1 3 3 1 0.5 0.5 8 8 0.5 0.5]
% xx=[\text{CDK1a} \text{CDK1a}(t-tau1) \text{CDK1a}(t-tau2) \text{APCa} \text{APCa}(t-tau1) \text{APCa}(t-tau2)];
% f(1,1)=par(1)-par(3)*xx(1,1)*xx(2,2)^par(7)/(par(5)^par(7)+xx(2,2)^par(7)); % CDK1a
f(2,1)=par(2)*(1-xx(2,1))*xx(1,3)^par(8)/(par(6)^par(8)+xx(1,3)^par(8))-par(4)*xx(2,1); % APCa
return;
```

3. **sys_deri.m** (instead of providing your system derivative you can use the DDE-BIFTOOL sys_deri.m)

Same as in Appendix A.1

4. **sys_tau.m**

```matlab
function tau=sys_tau()
tau=[9 10]; % Two time delays located at par(9) and par(10)
return;
```
Then, the scripts that run the DDE-BIFTOOL and generate the Hopf bifurcation diagram are given below.

5. Ferrell_TwoODE_with_Delay_hopf_beta2.m

```matlab
%% This example is taken from
% Ferrell, J. E., Tsai, T. Y.-C., & Yang, Q. (2011).
% Modeling the cell cycle: why do certain circuits oscillate? Cell, 144(6), 874-885.
%-------------------
% modified to delay differential equations as below:
% CDK1a' = alpha1-beta1*CDK1a*APCa(t-tau1)^n1/(K1^n1+APCa(t-tau1)^n1)
% APCa' = alpha2*(1-APCa)*CDK1a(t-tau2)^n2/(K2^n2+CDK1a(t-tau2)^n2)-beta2*APCa
% % par=[alpha1 alpha2 beta1 beta2 K1 K2 n1 n2 tau1 tau2]=[0.1 3 3 1 0.5 0.5 8 8 0.5 0.5]
% % xx=[CDK1a CDK1a(t-tau1) CDK1a(t-tau2)
% %     APCa APCa(t-tau1) APCa(t-tau2)];
% clear all;
close all;
clc;
disp('Setting up starting points')
[name,n]=sys_init
% define a variable stst with object kind
stst.kind='stst';
par=[0.1 3 3 1 0.5 0.5 8 8 0.5 0.5];
% setting the parameters
stst.parameter=par;
% define a first steady state
stst.x=[0.922; 0.331];
stst.stability=[];
% define the method
method=df_method('stst');
% sets the correction method to look for an equilibrium point
% [stst,success]=p_correc(stst,[],[],method.point)
% calculate the stability of the equilibrium point stst and set stability
% as a new field of stst to the calculation output
stst.stability=p_stabil(stst,method.stability);
% plots a locus of roots for the equilibrium point stst
figure(1); clf;
p_splot(stst);
method.stability.minimal_real_part=-20;
stst.stability=p_stabil(stst,method.stability);
figure(2); clf;
p_splot(stst);
disp('Computing branch1, the branch of steady state solutions.')
% a branch of object is created and named branch1
% note that the first entry in the argument is the number for parameter of
% interest in the par (parameter position in par 4 is the beta2)
branch1=df_brnch(4,'stst'); % we obtain an empty branch with free parameter beta2
% set the min and max bound of the bifurcation parameter beta2=par(4)
branch1.parameter.min_bound(2,:)=[4 0]; % 4 is the parameter position; just need to change the right assignment par number
```

155
branch1.parameter.max_bound(1,:)=[4 10]; % just need to change the right assignment
branch1.parameter.max_step(1,:)=[4 0.2]; % just need to change the right assignment

% start with the steady state point determined earlier
branch1.point=stst;

% To obtain a second starting point we change parameter value beta2
% slightly and correct again.
stst.parameter(4)=stst.parameter(4)+0.001;
[stst,success]=p_correc(stst,[],[],method.point)

% branch1.method.continuation.plot=0;

branch1.point(2)=stst; % use as a second branch point, next branch point is as calculated

% runs the continuation routine on the branch, for as many as 800
% iterations or until the maximum parameter bound is reached.
[branch1,s,f,r]=br_contn(branch1,800) % continue in one direction

branch1=br_rvers(branch1); % turn the branch around

[branch1,s,f,r]=br_contn(branch1,750) % continue in the other direction

disp('Computing stability of the steady state solutions.')</n
% determine stability of each point on the branch
branch1=br_stabl(branch1,0,1);

% obtain suitable scalar measures to plot stability along branch
% get a measure of the eigenvalues
[xm,ym]=df_measr(1,branch1);

k = 1;
while (real(branch1.point(k).stability.l0(1)) < 0) & k < length(branch1.point)
eigenvalue=real(branch1.point(k).stability.l0(1));
k = k + 1;
end

if (k == length(branch1.point))
disp('Failed to find the Hopf bifurcation point in branch1.')
return
end

eigenvalue=real(branch1.point(k).stability.l0(1))

str = sprintf('Found the Hopf bifurcation near point %d in branch1.',k);
disp(str)

figure(3); clf;
br_plot(branch1,xm,ym,'k.');

ym.subfield='l0'; % l0 = l zero is the approximations
br_plot(branch1,xm,ym,'c.');
xlabel('parameter beta2');ylabel('Re(\lambda)');

figure(4); clf;
br_plot(branch1,[],ym,'b');
br_plot(branch1,[],ym,'b.');
xlabel('Point number');ylabel('Re(\lambda)');

plot([0 size(branch1.point,2)],[0 0],'-.'); % this is where the axis lies
% observed that point k Re(lambda) is zero
% tell us that we have found hopf bifurcation near point k
plot([k k],[-1.2 0.8],'.-'); % this is a vertical line

k2 = k+1;
while (real(branch1.point(k2).stability.l0(1)) > 0) & k2 < length(branch1.point)
eigenvalue=real(branch1.point(k2).stability.l0(1));
k2 = k2 + 1;
end
if (k2 == length(branch1.point))
    disp('Failed to find the Hopf bifurcation point in branch1.'
return
end

eigenvalue=real(branch1.point(k2).stability.l0(1))
str = sprintf('Found the Hopf bifurcation near point %d in branch1.',k2);
disp(str)
figure(4);
plot([k2 k2],[-1.2 0.8],'r-.'); % this is a vertical line

% The function to locate Hopf points is p_tohopf;
% it takes an initial guess of an equilirium point as input, and return
% output of a machine approximation of the location of Hopf point

hopf=p_tohopf(branch1.point(k))
method=df_method('hopf');
[hopf,success]=p_correc(hopf,4,[],method.point) % p_correc(hopf,parameter number in par, [], method.point);

bifurcation1_beta2=hopf.parameter(4)

first_point=hopf;

hopf.stability=p_stab(hopf,method.stability); % compute stability of hopf point
figure(6); clf;
p_splot(hopf); % plot stability of hopf
% a pair of purely imaginary eigenvalues or Re(lambda)=0 confirm Hopf
% bifurcation occur.

branch2=df_brnch([4 5],'hopf');
branch2.parameter.min_bound(4,:)=4 0]; % bifurcation parameter of interest
beta2=par(4)
branch2.parameter.max_bound(1:2,:)=4 10]; [5 10]'; % par(4) max=10, par(5)
max=10
branch2.parameter.max_step(1:2,:)=4 0.2]; [4 0.2]'; % par(4) max_step=0.2,
par(5) max_step=0.2
branch2.point=hopf;

hopf.parameter(5)=hopf.parameter(5)+0.001; % perturbed in par(5)=K1

[hopf,success]=p_correc(hopf,4,[],method.point) % p_correc(hopf,parameter of
interest is par(4),[],method.point)

% periodic solutions
intervals=15;
degree=2;
[psol,stepcond]=p_topol(first_point,1e-2,degree,intervals);

method=df_method('psol');
[psol,success]=p_correc(psol,4,stepcond,method.point) % p_correc(psol,parameter of
interest is par(4),stepcond,method.point)

branch4=df_brnch(4,'psol'); % df_brnch(parameter of interest is par(4),'psol')
branch4.parameter.min_bound(3,:)=4 0]; % the left hand side row number is equal to
% the no. of delays + 1, here it is 2 delays (tau1 and tau2) defined in sys_tau
% right hand side is for the parameter of interest par(4)
branch4.parameter.max_bound(1,:)=4 11]; % parameter of interest is par(4)
branch4.parameter.max_step(1,:)=4 .1]; % parameter of interest is par(4)

deg_psol=p_topol(first_point,0,degree,intervals);

deg_psol.mesh=[];
branch4.point=deg_psol;
psol.mesh=[];
branch4.point(2)=psol;
figure(7); clf;
[branch4,s,f,r]=br_contn(branch4,121)
xlabel('parameter b');ylabel('amplitude');

branch4=br_stabl(branch4,0,1)

figure(9); clf;
l_b1=length(branch1.point)
for i=1:l_b1
i
par_beta2(1,i)=branch1.point(1,i).parameter(4)
x_cdkla(1,i)=branch1.point(1,i).x(1);
end

hold on;
for i=1:l_b1
if branch1.point(1,i).stability.l0 < 0
plot(par_beta2(1,i),x_cdkla(1,i),'b*');
else
plot(par_beta2(1,i),x_cdkla(1,i),'k--');
end
end

xlabel('parameter beta2','fontsize',20)
ylabel('steady state value for CDK1a','fontsize',20)

hopf=p_tohopf(branch1.point(k2-1))
method=df_mthod('hopf');
[hopf,success]=p_correc(hopf,4,[],method.point) % p_correc(hopf,parameter number in par, [], method.point);
bifurcation2_beta2=hopf.parameter(4)

second_point=hopf;
hopf.stability=p_stabil(hopf,method.stability); % compute stability of hopf point
figure(8); clf;
p_splot(hopf); % plot stability of hopf

LL=length(branch4.point);
Per=zeros(1,LL);
Perpara=zeros(2,LL);
for i=1:LL
Per(1,i)=branch4.point(1,i).parameter(4); % parameter(4)=beta2
Perpara(1,i)=min(branch4.point(1,i).profile(1,:)); % min value
Perpara(2,i)=max(branch4.point(1,i).profile(1,:)); % max value
end

figure(13); clf;
l_b1=length(branch1.point)
for i=1:l_b1
par_beta2(1,i)=branch1.point(1,i).parameter(4);
x_cdkla(1,i)=branch1.point(1,i).x(1);
end

% Plotting the stable and unstable steady states
hold on;
for i=1:l_b1
if branch1.point(1,i).stability.l0 < 0
plot(par_beta2(1,i),x_cdkla(1,i),'b*');
else
plot(par_beta2(1,i),x_cdkla(1,i),'k.','linewidth',3);
end
end

% Plotting the minimum and maximum
plot(Per,Perpara,'linewidth',5,'color',[0.5 0 1]);
set(gcf,'color','w');
set(gca,'linewidth',4,'xtick',[0 1 2 3 4 5 6],'ytick',[0 0.2 0.4 0.6 0.8 1],'fontsize',15,'fontweight','bold');
xlabel('parameter beta2','fontsize',20)
ylabel('steady state value for CDK1*','fontsize',20)
axis([0 6 0 1])

% print legend('stable','unstable')
text(5,0.95,'* Stable','fontsize',15,'color',[0 0 1])
text(5,0.9,'. ','fontsize',25),,'color',[1 1 1])
text(5,0.89,'   unstable ','fontsize',15)

% save Ferrell_TwoODE_with_Delay_hopf_beta2.mat
Appendix B
XPPAUT files for Chapter 4

The XPP codes are given in this Appendix.

B.1 XPPAUT File for Chapter 4 under Stressed Conditions

p53_Mdm2_MdmX_Wip1.ode

```
#1 p53

dp53m/dt=sp53+e5*(delay(P53p,tau1)+delay(P53pp,tau1))^n/((delay(P53p,tau1)+delay(P53pp,tau1))^n+Kp53^n)-del tap53*p53m

#2 mdm2

dmdm2m/dt=smdm2-kd2*Mdm2-katm2*Mdm2*ATMp+kwip2*Mdm2p*Wip1-kf2*Mdmx*Mdm2+kb1*C1-kf3*Mdmx*Mdm2+kb3*C3-k2*PS3+C2-k1*C1

#3 P53

dP53/dt=rp53*delay(p53m,tau2)-uP53*P53-katm1*ATMp*PS3+kwip1*PS3p*Wip1-kf1*Mdm2*PS3+kb1*C1-kf3*Mdmx*PS3+kb3*C3-k2*PS3+C2-k1*C1

#4 P53p


#5 Mdm2


#6 C1

dC1/dt=kf1*PS3*Mdm2-kb1*C1

#7 wip1

dwip1m/dt=swip1-del twip1*wip1m-e2*(delay(P53p,tau1)+delay(P53pp,tau1))^n/((delay(P53p,tau1)+delay(P53pp,tau1))^n+Kw^n)

#8 Wip1

dWip1/dt=rwip1*delay(wip1m,tau2)-uWip1*Wip1

#9 ATM*+kauto*ATMp*(1-ATMp)
```
\[
\text{dATMp}/\text{dt} = k_{DSB} \cdot (\text{DSB} + k_{DSB}) \cdot (1 - \text{ATMp}) + k_{auto} \cdot (1 - \text{ATMp}) \cdot \text{ATMp}
\]

#10. \text{mdmx}

\[
\text{dmdxm}/\text{dt} = s_{mdmx} - \text{delta} \cdot \text{mdxm} + e^3 \cdot \text{delay(P53p,tau1)} \cdot \text{delay(P53pp,tau1)} / (Kx^n + \text{delay(P53p,tau1)} + \text{delay(P53pp,tau1)})^n
\]

#11. \text{Mdmx}

\[
\text{dMdmx}/\text{dt} = r_{mdmx} \cdot \text{delay(mdmxm,tau2)} - u_{Mdmx} \cdot \text{Mdmx} + k_{atm3} \cdot \text{Mdmx} \cdot \text{ATMp} - k_{wip3} \cdot \text{Mdmxp} \cdot \text{Wip1} - k_{f2} \cdot \text{Mdm2} + k_{b2} \cdot C2 - k_{f3} \cdot P53 \cdot \text{Mdmx} + k_{b3} \cdot C3
\]

#12. \text{Mdmxp}

\[
\text{dMdmxp}/\text{dt} = k_{atm3} \cdot \text{Mdmx} \cdot \text{ATMp} - k_{wip3} \cdot \text{Mdmxp} \cdot \text{Wip1} - k_{f3} \cdot \text{Mdmxp} \cdot \text{Mdm2} - u_{Mdmxp} \cdot \text{Mdmxp}
\]

#13. \text{Mdm2p}

\[
\text{dMdm2p}/\text{dt} = k_{atm2} \cdot \text{Mdm2} \cdot \text{ATMp} - k_{wip2} \cdot \text{Mdm2p} - u_{Mdm2p} \cdot \text{Mdm2p}
\]

#14. \text{C2}

\[
\text{dC2}/\text{dt} = k_{f2} \cdot \text{Mdm2} \cdot \text{kb2} \cdot \text{C2}
\]

#15. \text{C3}

\[
\text{dC3}/\text{dt} = k_{f3} \cdot P53 \cdot \text{Mdmx} \cdot \text{kb3} \cdot \text{C3}
\]

#16. \text{P53pp}

\[
\text{dP53pp}/\text{dt} = k_{phos} \cdot P53 \cdot \text{kdephos} \cdot P53pp - u_{P53pp} \cdot \text{P53pp}
\]

#17. \text{p21}

\[
\text{dp21m}/\text{dt} = e^4 \cdot \text{delay(P53a,tau1)} / (\text{delay(P53a,tau1)} + Kp21^n - \text{deltap21} \cdot p21m)
\]

#18. \text{P21}

\[
\text{dp21}/\text{dt} = r_{p21} \cdot \text{delay(p21m,tau2)} - u_{P21} \cdot P21 - k_{d21} \cdot C2 \cdot P21
\]

#19. \text{dP53a}

\[
\text{dP53a}/\text{dt} = V_{max1} \cdot P53 / (P53 + K_{m1} + K_{m1} \cdot \text{Mdm2}/K_{i1}) + V_{max2} \cdot P53 / (P53 + K_{m2} + K_{m2} \cdot \text{Mdmx}/K_{i2}) - u_{P53a} \cdot P53a
\]
\[kd2 = \frac{uMdm2 + DSB}{(J + DSB)^2}\]

\[par kd2 = 0.01, J = 0.2\]

# parameter values

\[par sp53 = 5e-4, smdm2 = 0.002, swip1 = 0.002, smdmx = 0.001\]

\[par deltap53 = 0.03, deltamd2m = 0.05, deltawip1 = 0.05, deltamd2mx = 0.03, deltap21 = 0.04\]

\[par rp53 = 0.01, rmdm2 = 0.04, rwip1 = 0.02, rmdmx = 0.01, rp21 = 0.02\]

\[par uP53 = 0.03, uMdm2 = 0.033, uWip1 = 0.035, uMdmx = 0.03, uP21 = 0.03, uMdm2p = 0.1, uMdmxp = 0.2, uP53p = 0.01, uP53pp = 0.004, uP53a = 0.001\]

\[par e1 = 0.02, e2 = 0.014, e3 = 0.005, e4 = 0.015, e5 = 0.02\]

\[par k1 = 0.2, k2 = 0.01, k3 = 1.5, k4 = 0.1, katm1 = 0.8, katm2 = 0.02, katm3 = 0.02, kwip1 = 1.3, kwip2 = 0.5, kwip3 = 0.2, kwip4 = 1.5\]

\[par kDSBB = 0.0005, kbasal = 0.02, Km = 0.16, Kw = 0.2, KDSB = 200, kauto = 0.07\]

\[par DSB = 300\]

\[par kf1 = 552, kf2 = 600, kf3 = 552, kb1 = 123.6, kb2 = 18, kb3 = 123.6\]

\[par Kx = 1.5\]

\[par kphos = 0.3, kdephos = 0.05\]

\[par Kp53 = 0.017, Kp21 = 0.017\]

\[par n = 4, tau1 = 30, tau2 = 10, kd21 = 0.5\]

\[par Vmax1 = 0.0001, Km1 = 0.000025, K1 = 0.00001, Vmax2 = 0.001, Km2 = 0.5, K2 = 0.0001\]

# initial conditions

\[init p53m = 0.05, mdm2m = 0.05, P53 = 0.0258, P53p = 0, P53pp = 0, Md2m = 0.15, C1 = 0.06, wip1m = 0.04, Wip1 = 0, ATMp = 0, mdmxm = 0.01, Md2mx = 0.08, Md2mp = 0.01, Md2m2p = 0.0178, C2 = 0.05, C3 = 0.05, P53a = 0.01, p21m = 0.05, P21 = 0.01\]

# total protein

\[aux P53t = P53 + P53p + P53pp + P53a\]
aux Mdm2t=Mdm2+Mdm2p
aux Mdmxt=Mdmx+Mdmxp

# To plot four state variables in one graph

 //@ NPLOT=4,XP=t, YP=P53t, YP2=Mdm2t, YP3=Mdmxt, YP4=p21m, TOTAL=1500, METH=sti, XLO=0, XHI=1500, YLO=0, YHI=0.4, delay=3060, maxstor=500000, bound=10000

@ NPLOT=1,XP=t, YP=P53t, TOTAL=1500, METH=sti, XLO=0, XHI=1500, YLO=0, YHI=0.4, delay=3060, maxstor=500000, bound=10000

done

B.2 MATLAB File for Running XPPAUT (Local Parameter Sensitivity Analysis in Chapter 4)

XPPAUT simulation can be carried out in MATLAB as well with a XPP-MATLAB interface package written by Rob Clewley freely downloadable from http://www2.gsu.edu/~matrhc/XPP-Matlab.html. This package can take advantage of the flexibility of changing parameter values, for example, when performing sensitivity analysis, and the speed of numerical integration of XPPAUT. Unzip the package to C:\xppall folder, and save all files below under this folder.

The MATLAB files for running XPPAUT are given below:

1. p53n_xpp.ode

Note that the content of this ode file is same as in Appendix B.1; it is used with different name as a safety measure for sensitivity analysis that changes the parameter values when MATLAB run the XPP ode file.

#1.p53

dp53m/dt=sp53+e5*(delay(P53p,tau5)+delay(P53pp,tau5))^n/((delay(P53p,tau5)+delay(P53pp,tau5))^n+Kp53^n)-deltap53*p53m

#2.mdm2

dmdm2m/dt=smdm2-
deltamdm2*mdm2m+e1*(delay(P53p,tau1)+delay(P53pp,tau1))^n/((delay(P53p,tau1)+delay(P53pp,tau1))^n+Km^n)

#3.P53

dP53/dt=rp53*delay(p53m,tau6)-uP53*P53-katm1*ATMp*P53+kwip1*P53p*Wip1-kf1*Mdm2*P53+kb1*C1-kf3*Mdx*P53+kb3*C3-k2*P53*C2-k1*C1
dP53p/dt = k_{atm1} \cdot A_T M_p \cdot P53p - k_{wi1} \cdot P53p \cdot W1p1 - k_{phos} \cdot P53p + k_{dephos} \cdot P53pp - u_{P53p} \cdot P53p

dMdm2/dt = r_{mdm2} \cdot delay(mdm2, tau8) - k_{d2} \cdot Mdm2 - k_{atm2} \cdot A_T M_p \cdot Mdm2 + k_{wi2} \cdot Mdm2p \cdot W1p1 - k_{f1} \cdot Mdm2 \cdot P53 + k_{b1} \cdot C1

dC1/dt = k_{f1} \cdot P53 \cdot Mdm2 - k_{b1} \cdot C1

dwip1/dt = swip1 - delay(wip1, tau) + e2 \cdot (delay(P53p, tau2) + delay(P53pp, tau2))^n / ((delay(P53p, tau2) + delay(P53pp, tau2))^n + K_{w1}^n)

dW1p1/dt = r_{wip1} \cdot delay(wip1, tau10) - u_{W1p1} \cdot W1p1

\frac{d}{dt} (A_T M_p + k_{auto} \cdot A_T M_p \cdot (1 - A_T M_p))

d{ATMp}/dt = k_{DSB} \cdot DSB/(DSB + K_{DSB}) \cdot (1 - A_T M_p) - k_{wip4} \cdot W1p1 \cdot A_T M_p - k_{basal} \cdot A_T M_p + k_{auto} \cdot (1 - A_T M_p) \cdot A_T M_p

dmdmxm/dt = s_{mdm} - delay(mdmx, tau) + e3 \cdot (delay(P53p, tau3) + delay(P53pp, tau3))^n / ((Kx^n + delay(P53p, tau3) + delay(P53pp, tau3))^n)

dMdmx/dt = r_{mdmx} \cdot delay(mdmxm, tau9) - u_{Mdmx} \cdot Mdmx - k_{atm3} \cdot A_T M_p \cdot Mdmx - k_{wi3} \cdot Mdmxp \cdot W1p1 - k_{f2} \cdot Mdm2 \cdot Mdmx + k_{b2} \cdot C2 - k_{f3} \cdot P53 \cdot Mdmx + k_{b3} \cdot C3

dMdmxp/dt = k_{atm3} \cdot A_T M_p \cdot Mdmx - k_{wi3} \cdot Mdmxp \cdot W1p1 - k_{3} \cdot Mdmxp + Mdm2p - u_{Mdmxp} \cdot Mdmxp

dMdm2p/dt = k_{atm2} \cdot A_T M_p \cdot Mdm2p - k_{wi2} \cdot Mdm2p \cdot W1p1 - u_{Mdm2p} \cdot Mdm2p

dC2/dt = k_{f2} \cdot Mdm2 \cdot Mdmx - k_{b2} \cdot C2

dC3/dt = k_{f3} \cdot P53 \cdot Mdmx

\[
\frac{dC3}{dt} = kf3 \cdot P53 \cdot Mdx \cdot kb3 \cdot C3
\]

#16. P53pp

\[
\frac{dP53pp}{dt} = kphos \cdot P53p - kdphos \cdot P53pp - uP53pp \cdot P53pp
\]

#17. p21

\[
\frac{dp21m}{dt} = e4 \cdot \text{delay}(P53a, \tau4)^n / (\text{delay}(P53a, \tau4)^n + Kp21^n) \cdot \text{deltap21} \cdot p21m
\]

#18. P21

\[
\frac{dP21}{dt} = rp21 \cdot \text{delay}(p21m, \tau7) - uP21 \cdot P21 - kd21 \cdot C2 \cdot P21
\]

#19. dP53a

\[
\frac{dP53a}{dt} = Vmax1 \cdot P53 / (P53 + Km1 + Km1 \cdot Mdm2 / Ki1) + Vmax2 \cdot P53 / (P53 + Km2 + Km2 \cdot Mdx / Ki2) - uP53a \cdot P53a
\]

# kd2

\[
k2 = uMdm2 + DSB / (J + DSB) \cdot kd22
\]

par \ kd22=0.01, J=0.2

# parameters value

par sp53=0.0005, s mdm2=0.002, s wip1=0.002, s mdmx=0.001

par deltap53=0.03, deltamd2=0.05, deltawip1=0.05, deltamd2mx=0.03, deltap21=0.04

par rp53=0.01, rmdm2=0.04, rwip1=0.02, rmdm2mx=0.01, rp21=0.02

par uP53=0.03, uMdm2=0.033, uWip1=0.035, uMdm2=0.03, uP21=0.03, uMdm2p=0.1, uMdm2p=0.1, uP53p=0.01, uP53pp=0.004, uP53a=0.001

par e1=0.02, e2=0.014, e3=0.005, e4=0.015, e5=0.02

par k1=0.2, k2=0.01, k3=1.5, k4=0.1, katm1=0.8, katm2=0.02, katm3=0.02, kwip1=1.3, kwip2=0.5, kwip3=0.2, kwip4=1.5

par DSB=0.0005, kbasa=0.02, km=0.16, kw=0.2, KDSB=200, kauto=0.06

par DSB=300

par kf1=552, kf2=600, kf3=552, kb1=123.6, kb2=18, kb3=123.6
par Kx=1.5
par kphos=0.3, kdephos=0.05
par Kp53=0.017, Kp21=0.017
par n=4, tau1=30, tau2=30, tau3=30, tau4=30, tau5=30, tau6=10, tau7=10, tau8=10, tau9=10, tau10=10, kd21=0.5
par Vmax1=0.0001, Km1=2.5e-05, Ki1=1e-05, Vmax2=0.001, Km2=0.5, Ki2=0.0001

# initial conditions
init p53m=0.05, mdm2m=0.05, PS3=0.0258, PS3p=0, PS3pp=0, Mdm2=0.15, C1=0.06, wip1m=0.04, Wip1=0, ATMp=0, mdmxm=0.01, Mdmx=0.08, Mdmxp=0.01, Mdm2p=0.0178, C2=0.05, C3=0.05, PS3a=0.01, p21m=0.05, P21=0.01

# total protein
aux P53t=P53+P53p+P53pp+P53a
aux Mdm2t=Mdm2+Mdm2p
aux Mdmxt=Mdmx+Mdmxp

@ dt=0.1, NPLOT=1, XP=t, YP=P53t, TOTAL=1500, METH=stiff, XLO=0, XHI=1500, YLO=0, YHI=0.4,
delay=3060, maxstor=500000, bound=10000

done

2. p53_default_parameters.m

newpars=[];
% index=1:1:78; % for 78 parameters
for i=1:78
    newpars(i).type='PAR';
end
% Note: The EndNote software has a problem in using opening and closing % curly bracket.
% So, two pictures representing the curly brackets are used below.
par_label= {'sp53','smdm2','smdmx','swip1','e1','e2','e3','e4','e5','Kp53',...
    'Kp21','Km','Kx','Kw','deltap53','deltap21','deltamdm2','deltamdmx','deltawipl','rp53',...
    'rp21','rmdm2','rmdmx','rwip1','uP53','uP53p','uP53pp','uP53a','uP21','uMdm2',...
    'uMdmx','uWip1','uMdm2p','uMdmxp','k1','k2','k3','k4','katm1','katm2',...
    'katm3','kwip1','kwip2','kwip3','kwip4','kDSBB','DSB','KDSB','kauto','kbasal',...
    'kfl','kf2','kf3','kb1','kb2','kb3','kphos','kdephos','Vmax1','Vmax2',...
for ind=1:78
    newpars(ind).name=par_label{ind};
end

par_val=[0.0005, 0.002, 0.001, 0.002, 0.014, 0.005, 0.015, 0.02, 0.017,...
        0.017, 0.16, 1.5, 0.2, 0.03, 0.04, 0.05, 0.03, 0.05, 0.01,...
        0.02, 0.04, 0.01, 0.02, 0.03, 0.01, 0.004, 0.001, 0.03, 0.033,...
        0.03, 0.035, 0.1, 0.2, 0.2, 0.01, 1.5, 0.1, 0.8, 0.02, ...
        0.02, 1.3, 0.5, 0.2, 1.5, 0.0005, 300, 200, 0.07, 0.02,...
        552, 600, 552, 123.6, 18, 123.6, 0.3, 0.05, 0.0001, 0.001,...
        2.5e-5, 0.5, 0.00001, 0.0001, 0.5, 0.2, 0.01, 30, 30, 30, ...
        30, 30, 10, 10, 10, 10, 10, 10, 4];
for ind=1:78
    newpars(ind).val=par_val(ind);
end

3. Model_p53_Sen20p.m

% Parameter Baseline values
p53_default_parameters;
success2=ChangeXPPodeFile('p53n_xpp.ode',newpars);

newpars(k).val % to print the new parameter value
% change the local parameter value
newpars(k).val=1.2*newpars(k).val; % for +20% to parameter k
% for -20% to parameter k
% change to newpars(k).val=0.8*newpars(k).val;
newpars(k).name % to print the new parameter name
newpars(k).val % to print the new parameter value
success3=ChangeXPPodeFile('p53n_xpp.ode',newpars);

success4=RunXPP('p53n_xpp.ode','','','');
data=load('output.dat');
% save time course simulation data for total p53 in column 21
svl=['save p',num2str(k),'.dat data -ascii'];
eval(svl)
A=[A, data(:,21)];
end

% save time course simulation data with column 1 for time (in min)
B=[data(:,1), A];
save Model_p53_Sen20p.mat

t1=B(1:100:end,1); % t(0)=0; t(1)=10 min; data point for each 10 min interval until
1500 min
y1=B(1:100:end,:); % dt=0.1, so the t(101)=10 min and total 151 row, 1 column for
y1 (151 x 1)
BB=[y1];
4. Once data were collected for total p53 levels as in matrix BB above, it can then be stored in Microsoft Excel file for estimating the period of p53 oscillations using Spectrum Resampling technique from Costa et al. (2013).