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P53 dynamics: single-cell imaging data analysis and modeling

Mengyao Li  
*Hong Kong Baptist University*

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P53 Dynamics: Single-cell Imaging Data Analysis and Modeling

LI Mengyao

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Philosophy

Principal Supervisor: Prof. TANG Lei Han

Hong Kong Baptist University

September 2014
DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of MPhil at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature: ______________________

Date: September 2014
ABSTRACT

The p53 protein plays a central role in controlling the fate of cancer cells. At moderate levels of DNA damage, the concentration of the phosphorylated form of p53 undergoes temporal oscillation with a period of a few hours. In Dr. Shi’s lab, single-cell measurements were carried out using the p53-YFP fusion proteins and time-lapse fluorescence microscopy. We report here a detailed study of the image data. From the time series of the p53 concentration in individual cells, we deduce the amplitude and period of the oscillation. The pulse-to-pulse and cell-to-cell variability of the oscillation is characterized. We then carry out a computational study of a mathematical model that involves a negative feedback loop between p53 and Mdm2 proteins. We have determined the phase diagram of the model, and studied the sensitivity of the properties of the oscillating state against the model parameters. Although only p53 concentration is measured in the experiment, we show that careful analysis of the pulse shape can nevertheless yield valuable information on the underlying molecular processes, and shed light on the possible origin of the observed cell-to-cell variations.
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# TABLE OF CONTENTS

Declaration ........................................... i

Abstract ............................................ ii

Acknowledgements ................................... iii

Table of contents ................................... vi

List of figures ....................................... ix

List of tables ....................................... x

1 Introduction ...................................... 1
   1.1 The Biology of p53 ................................. 1
       1.1.1 The discovery of p53 and its role in tumorigenesis . . . . . . 2
       1.1.2 The core activation network of p53 .................. 2
       1.1.3 The p53 dynamics and cell fate ................. 3
       1.1.4 Single-cell measurements .................... 7
   1.2 Scope of This Work ................................. 8
   1.3 Organization of the Thesis ..................... 9

2 Oscillatory Solution of Coupled Nonlinear ODEs 10
   2.1 Dynamical System, Phase Space Trajectory, Fixed Points and
       Limit Cycles ....................................... 10
   2.2 The Hopf Bifurcation .............................. 13
   2.3 Numerical Integration ............................. 16
3 Single-cell Imaging of p53 Intensity 18
   3.1 The Experimental Setup ........................................ 19
   3.2 Method to Extract p53 Fluorescence Intensity of Single Cells .. 20
   3.3 Time Course of Nuclear p53 Concentration ........................ 23

4 Model Construction and Analysis 28
   4.1 Model Construction .............................................. 29
   4.1.1 A two-component model ....................................... 29
   4.1.2 A 4-component model ......................................... 34
   4.2 Main Results ................................................... 36
   4.2.1 The bifurcation diagram .................................... 36
   4.2.2 Analysis of one oscillation cycle ............................ 40
   4.2.3 Sensitivity analysis against model parameters ............. 44

5 Comparison of Model and Experiment 46
   5.1 Damage Dependent p53 Dynamics ............................... 46
   5.2 Variations in the Period of Single Cell p53 Oscillation ...... 48
   5.2.1 Period variation at different damage levels ............... 48
   5.2.2 Period variation at the same damage level ................ 51
   5.3 Variation in the Peak Value of Single Cell p53 Oscillation .. 53
   5.3.1 Peak value variation at different damage levels .......... 53
   5.3.2 Peak value variation at the same damage level .......... 55
   5.3.3 Correlation between p53 rising speed and peak value ..... 57
   5.4 Summary and Discussion ....................................... 60

6 Conclusions 62

Appendices 64

A Model parameter table 64

B Flow chart of the image program 66

Bibliography 71
# LIST OF FIGURES

1.1 Two typical p53 network models ........................................... 4  
1.2 A complex p53 network model ............................................. 6  

2.1 An example of trajectory in phase space ................................. 11  
2.2 Phase space diagram ....................................................... 12  
2.3 The examples of Hopf bifurcation ........................................ 15  
2.4 An example of supercritical Hopf bifurcation .......................... 16  

3.1 Typical microscope images from the experiment ....................... 19  
3.2 Time course of nuclear p53 concentration ............................... 20  
3.3 The distribution of pixel values of each image at five time points. 22  
3.4 Identification of cell nucleus at different background cutoff ....... 23  
3.5 Time courses of nuclear p53 concentration at 1 µM etoposide ..... 24  
3.6 Time course of nuclear p53 concentration at 5 µM etoposide .... 24  
3.7 Time courses of nuclear p53 concentration at 5 µM etoposide with the big initial pulse ................................. 25  
3.8 Time course of nuclear p53 concentration at 50 µM etoposide .... 25  
3.9 Period statistics of p53 oscillation under different etoposide conditions ....................................................... 26  
3.10 Peak value (amplitude) statistics of p53 oscillation under different etoposide conditions .............................................. 27  
3.11 Slope of monotonic increase of p53 concentration for 50 µM etoposide condition ....................................................... 27  

4.1 A simple 2-component p53-Mdm2 network .............................. 30  

vii
4.2 Two component model with changing Mdm2-dependent p53 degrada-
dation $\alpha_p$. ................................................. 34
4.3 The DNA damage dependent p53 dynamics network .......... 35
4.4 Bifurcation diagrams against the damage level. ............... 37
4.5 Hopf bifurcation characteristics around the transition points. .. 38
4.6 Phase space trajectories of the Batchelor et al. model near the
first bifurcation at various initial conditions. ....................... 39
4.7 Phase space trajectories of the 4-component model near the first
bifurcation at various initial conditions. ........................ 40
4.8 The concentration of the four component proteins in one oscil-
lation cycle. .................................................. 41
4.9 The limit cycle in the Mdm2 and total p53 plane. ............... 41
4.10 The effect of the delay time $\tau_m$ on p53 oscillation. .......... 43
4.11 Dependence of p53 peak value on each parameter. ............ 44
4.12 Dependence of p53 oscillation period on each parameter. ...... 45

5.1 Model predictions for different damage levels. ................. 47
5.2 Time courses of nuclear p53 concentration at different etoposide
levels. .................................................................. 48
5.3 The average oscillation periods at different etoposide concentra-
tions. .................................................................. 49
5.4 Duration of p53 quiescent phase in the data ...................... 50
5.5 Duration of p53 quiescent phase at different damage levels in
the model .......................................................... 50
5.6 Oscillation period statistics of the experimental data at 1$\mu M$
etoposide. ....................................................... 51
5.7 Oscillation period statistics of the experimental data at 5$\mu M$
etoposide. ....................................................... 52
5.8 Duration of $T_1$ and $T_p$ at different drug dosages .............. 53
5.9 The average peak values at different etoposide concentrations. .. 54
5.10 The relationship between p53 peak value and damage level. ... 55
5.11 Peak value statistics of experimental data at 1µM etoposide. . . 56
5.12 Peak value statistics of experimental data at 5 µM etoposide. . 56
5.13 Relationship between peak values of the oscillation and $\beta_p$ or $\alpha_{ampa}$. . . . . . . . . . . . . . . . . . . . . . . . . . . . 57
5.14 Statistics of the rising time distribution in the experimental data. 58
5.15 Scatter plot of peak value against rising slope. . . . . . . . . . 59
5.16 p53 pulse shapes at 1 µM etoposide. . . . . . . . . . . . . . . . 59
5.17 p53 pulse shapes at 5 µM etoposide. . . . . . . . . . . . . . . . 59
B.1 Flow chart . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 66
LIST OF TABLES

A.1 Parameters used in simulation except otherwise specified. . . . . 64
A.2 An alternative parameter set . . . . . . . . . . . . . . . . . . . . . . . . 65
Chapter 1

Introduction

1.1 The Biology of p53

The mammalian protein p53 has received much attention in the last four decades due to its important role in tumorigenesis. However, the complexity of its interactions with other cellular components has prevented full understanding of its biological functions even today [1]. Upon its initial discovery, p53 was recognized as middle T-antigen of the simian virus SV40. Afterwards, p53 was found to function independently of SV40 and thus it was supposed to be an oncoprotein. This was in line with the prevailing paradigms dominating molecular biology at the time: tumor is mainly caused by oncoproteins. As more and more in-vitro experiments and studies of human tumor cells focused on the role of p53, the understanding shifted from being a simple oncoprotein\(^1\) to a tumor suppressor protein\(^2\) [2]. Nowadays, it is generally accepted that p53 is an important tumor suppressor protein, which integrates various stress signals to determine cell fate, e.g. cell cycle arrest\(^3\) or apoptosis\(^4\).

\(^{1}\)The oncoprotein has the potential to cause cancer.
\(^{2}\)The activity of tumor suppressor protein stops the formation of tumor.
\(^{3}\)Cell cycle arrest is a stopping point in the cell cycle, where processes of duplication and division come to a halt.
\(^{4}\)Apoptosis is the process of programmed cell death.
1.1.1 The discovery of p53 and its role in tumorigenesis

The first glimpse of p53 came along during the investigation of virus SV40 [3], [4] and it was discovered as a co-precipitation product by using the antisera to immunoprecipitation\(^5\) of simian virus SV40. Besides, researchers believed that SV40 virus was similar to a virus named Polyoma Virus and naturally thought that it constitutes of three T antigens: large T, middle T, small T [2]. At the same time, the molecular mass of middle T was almost the same as p53, about 53-55 kDa. Therefore, p53 was recognized as middle T-antigen of the simian virus SV40 at that time.

The breakthrough happened in 1979: p53 was expressed not only in simian virus SV40 transformed cells but also in SV40 uninfected tumors [5–9]. Further experiments also confirmed that p53 was an independent protein leading to the tumorigenesis [10], [11]. Therefore, p53 was thought as an oncoprotein at that time [12].

However, during subsequent experiments to further confirm p53 as an oncoprotein, a puzzling observation was made in 1989: the new p53 clone was unable to reproduce the same transforming effects as the previous clone. DNA sequence analysis suggested that no two p53 clones had the same sequences, specifically, single-nucleotide polymorphism (SNP) occurred in every part of the TP53 gene [13]. Besides, many researchers found that tumor cells frequently lost the p arm of the chromosome where the TP53 gene is located. Thus, cells with mutated or lost TP53 gene are more likely to develop cancer. These findings cast doubt on the role of p53 as an oncogene. Consequently, prospects for p53 as a tumor suppressor were explored.

To further confirm the tumour suppressing character of p53, the Li-Fraumeni syndrome, an inheritable cancer disease, was used [14]. Humans with Li-Fraumeni syndrome have mutated TP53 genes, which induces tumor growth, and mutated genes are passed to their offsprings. Finally, several groups found that, when the TP53 gene of mice is knocked out, cancer develops [15]. All

---

5\(^{\text{Immunoprecipitation is a chemical technique using specific antibodies to extract protein antigens out of a solution.}}\)
these experiments suggested p53 as a tumor suppressor protein.

By now, it is generally accepted that p53 functions to maintain the structural integrity of cell’s DNA, and hence acquired the nickname “guardian of the genome”. Even though the root cause of cancer varies, about 50 percent of cancer cells have mutations in the p53 protein and almost in all cancer cells, the p53 regulatory network is not activated [16]. Under normal conditions, cellular concentration of the p53 protein is low. However, when the cell suffers from DNA damage (e.g. induced by UV [17]/IR radiation, chemical agents [18]) or oxidative stress [19], p53 is activated and its concentration rises. In some cases, downstream proteins such as p21 (cyclin-dependent kinase inhibitor 1) and XPC (Xeroderma pigmentosum, complementation group C) are then activated to bring growth arrest and to support DNA repair. In other cases, p53 protein stimulates apoptosis by interacting with other pro-apoptotic proteins like BAX (Bcl-2-associated X protein) and APAF1 (Apoptotic protease activating factor 1), thereby preventing cancer [20]. The details of how cells choose to follow one path versus the other, under different types of cellular and environmental conditions, are still under active investigation.

1.1.2 The core activation network of p53

The cellular concentration and activity of p53 is controlled by a molecular network whose components and interactions have been studied extensively in the past two decades. At the core of the network is a negative feedback loop between the transcription factor p53 protein and the E3 ubiquitin ligase Mdm2. p53 promotes the transcription of Mdm2 while Mdm2 targets both itself and p53 for degradation. Expression of Mdm2 promoted by p53 is a relatively slow process (on the timescale of hours) while the degradation of p53 with the help of Mdm2 only takes a few minutes [21].

Under normal conditions, the basal p53 expression is low due to the rapid ubiquitination process and downstream proteasome activity [22], [23]. Several stress-response proteins can trigger elevation of the p53 protein concentration. One such protein is the kinase ATM (Ataxia Telangiectasia Mutated). ATM
normally exists as a dimer with constrained activity. When DSBs (Double Strand Breaks) occurs, the dimeric ATM molecules are converted to monomers through a complex process. These ATM monomers acquire kinase activity to phosphorylate p53 proteins which in turn significantly slow down p53 ubiquitination. As a result, the concentration of p53 rises rapidly. In brief, ATM is activated by DSBs (Double Strand Breaks) and then transmits the damage signal to p53 [24].

Figure 1.1A depicts the key players of p53 activation in response to DSBs caused by e.g. γ-irradiation or chemical agents. This simplified model was proposed by Batchelor et al. in 2008. [25] In addition to Mdm2 and ATM introduced above, there is a third protein Wip1 phosphatase. Its expression is induced by p53 but once activated, it dephosphorylates p53 which in turn restores the ubiquitination process. In addition, Wip1 dephosphorylates the upstream ATM to lower its activity. Simulation studies of the model showed persistent oscillations of p53 when the damage level is sufficiently high, as shown in Fig. 1.1C. [25]

Figure 1.1: Figure (A) The p53 molecular mechanism in response to DSBs. (B) The p53 molecular mechanism in response to irradiation with UV light. (C) DSB simulation results; p53 level response to a single pulse (left) and multiple pulses (right). (D) UV condition simulation results for different levels of UV light. (Figures taken from [26])

DNA under UV light develops singe-strand breaks (SSBs). In this case,
the kinase ATM is replaced by its relative ATR (ataxia telangiectasia and Rad3-related protein) which is activated by DNA SSBs. Figure 1.1B shows the molecular network of p53 in this case. The only difference to Figure 1.1A is the absence of direct inhibition of ATR by Wip1.

Simulations of the two models carried out by Batchelor et al. [25, 26] gave different dynamical patterns of p53, in agreement with their experiments. For the network depicted in Figure 1.1A, persistent oscillations of p53 are reproduced. In comparison, the network in Figure 1.1B yielded a single graded pulse. Mathematical analysis carried out by the group also showed that dephosphorylation of ATM by Wip1 is required for repeated pulses.

1.1.3 The p53 dynamics and cell fate

It has been generally established that elevated levels of p53, in response to various types of cellular stresses, trigger cell cycle arrest, senescence, apoptosis, and other downstream pathways. Some of these downstream activities are mutually exclusive and are the outcome of different cell fate decisions. There is still considerable debate on how such decisions are connected to the temporal behavior of p53 protein concentration, e.g., whether it stays at a high level or undergoes large amplitude oscillations. There is also the possibility that the downstream pathways are affected directly by the stress signals.

Ref. [27] summarizes the experimental knowledge on the interactions between p53 and the downstream pathways that lead to either cell cycle arrest or apoptosis. As shown in Fig 1.2, the model consists of three modules: one for the activation of p53 and two for the downstream pathways leading to either cell cycle arrest or apoptosis. Based on the network topology and other experimental observations, the authors proposed a mathematical model for the cell fate decisions. In their model, the threshold time for the cell to undergo programmed cell death is inversely proportional to the damage level. For a low damage level, the time to arrive the threshold is too large and thus the cell keeps dividing. For an intermediate damage level, the cell repairs the DNA damage before the threshold time is reached. For a high damage level however,
the threshold time is reached quickly and the cell initiates apoptosis.

Figure 1.2: A complex p53 molecular network in response to DNA damage. (From reference [27])

A related but different proposal for the cell fate decision was put forward in Ref. [20]. There, it is suggested that different cell fates are directly related to the presence or absence of p53 oscillation. As shown in Fig 1.1, γ and ultraviolet-irradiation induce different dynamic patterns of p53: γ-irradiation induces a succession of pulses with fixed amplitude and period. Experimentally, it was found that an increase of γ-irradiation leads to an increase in the number of pulses. UV radiation on the other hand, results in a single pulse with amplitude and duration depending on the strength of the radiation used. If the damage caused by the radiation is severe enough, these dynamics finally lead to cell cycle arrest in the case of γ-radiation, and apoptosis in the case of UV radiation. Their group followed up on these observations by interrupting the p53 oscillations and instead inducing a steady level of p53. This was done by using Nutlin-3, one protein which represses the Mdm2 dependent degrada-
tion of p53. It was found that the protein levels of key genes related to cell cycle arrest, apoptosis, and senescence significantly differ depending on p53 signalling. The artificially sustained expression of p53 led to a significantly larger number of cells going to senescence rather than cell cycle arrest.

Xi Chen et al. [28] reported that different levels of DNA damage caused by etoposide leads to different temporal patterns of p53 protein concentration in mammalian cells. Under low damage conditions, time series of p53 concentration shows oscillation, causing the cell to go into cell cycle arrest. While at high levels of DNA damage, p53 concentration increases monotonically leading to apoptosis of the cells. They found that the dose-dependent bimodal switching was caused by discriminating Mdm2 concentration.

In addition, M Kracikova et al. [29] proposed a threshold mechanism which regulates the cell fate decision. They found that in order to induce apoptosis, the cell needs to overcome an apoptotic threshold. Specifically, below this threshold, cell usually undergoes cell-cycle arrest while above this threshold, apoptosis is induced. This threshold is mainly controlled by expression levels of p53 and its integral over time.

1.1.4 Single-cell measurements

Single-cell measurements have played a pivotal role in providing the actual temporal patterns of p53 protein concentration in a given cell and how they are related to cell fate. In early studies using western blot, Bar-Or et al. [30] found that after suffering DNA damage induced by γ-irradiation, the expression of p53 first increased and then decreased. At intermediate levels of damage, damped oscillation of p53 was observed. Since this technique averages over the cell population, people began to cast doubt on the result. With the development of new experimental techniques, Lahav’s group quantitatively studied the p53 dynamics in individual cells [31] and produced much more informative results as shown in Fig 1.1.

The asynchronous nature of p53 oscillations in a population was discussed by Lan [21] and Ciliberto [32]. However, since the number of molecules during
the reactions are small, it is important to consider the stochasticity of gene expression. Geva-Zatorsky et al. quantitatively measured the p53 dynamics for single cells under $\gamma$-irradiation. They found that the oscillation amplitude varies in a large regime while the oscillation period is relatively stable. In further studies about p53 dynamics, researchers also found excitability and bistable properties of the p53 dynamics at different levels of $\gamma$-irradiation [33].

The single-cell measurements were made possible by the development of time-lapse microscopy technology. Here, functional p53-YFP fusion proteins is used. Special care has to be taken in order to avoid any influence of the protein fusion on the dynamics of p53. Using this technique, concentration of the fluorescently labelled protein in individual cells can be followed quantitatively during its functional process.

1.2 Scope of This Work

The single-cell measurements, particularly those in Shi’s lab, generated a wealth of data which has the potential to allow more quantitative characterization of how different cells, or even a single cell in the course of its lifetime, respond differently to the same environmental stress. We will attempt to answer this question by concentrating on the well-established interplay between the p53 and its suppressor Mdm2.

On the theoretical side, we will re-examine some of the mathematical models in the literature, and investigate how various features of the p53 dynamics are controlled by the assumed interactions among the main components of the molecular network. We will then quantify the time-lapse microscope images collected in Dr. Shi’s lab for cells at different DNA DSB levels. The time course of nuclear p53 concentration from individual cells will be extracted. Statistics on the time course data will be performed to yield information on how individual cells respond to the same drug treatment. Detailed comparisons will then be carried out between the theoretical models and the experimental findings to gain a better understanding of the origin of cell-to-cell variability.
1.3 Organization of the Thesis

In this Chapter, we have provided a brief survey of the experimental studies on the tumor suppressor p53 protein. Chapter 2 presents a summary of key concepts and tools for the analysis of coupled ordinary differential equations which are used in our modelling work. In Chapter 3, the experimental setup in Dr. Shi’s lab and a method to quantify the single-cell microscope image data are described. In Chapter 4, a 4-component model for the p53 dynamics is introduced and its properties analyzed. In Chapter 5, a comparison of the results from theoretical modelling and experimental data analysis is presented to suggest possible origins of the cell-to-cell variability. Finally, we will summarize our findings and give a short outlook on possible future work.
Chapter 2

Oscillatory Solution of Coupled Nonlinear ODEs

The diffusion of molecules inside a cell or a particular compartment of it is usually fast compared to the chemical transformations or binding/unbinding events that modify the chemical state of these molecules. Therefore the description of biochemical reactions can often be approximated by a set of ordinary differential equations (ODEs) governing only the total number or the concentration of the molecules involved, without keeping track of their spatial position (apart from, in some cases, the compartment they are located in). This is the approach taken in later chapters of this thesis. For completeness, we present a brief review of the basic mathematical concepts and numerical techniques to obtain the solution of the ODEs, from the dynamical systems point of view.

2.1 Dynamical System, Phase Space Trajectory, Fixed Points and Limit Cycles

Let us consider a system characterized by \( n \) independent variables. Its state at any time \( t \) is given by value of all these \( n \) variables, i.e.

\[
s(t) = (x_1(t), x_2(t), ..., x_n(t)).
\]

When these variables form a complete description of the system, the change
of these variables is governed by a set of first order ODEs of the form,

\[
\begin{align*}
\frac{dx_1}{dt} &= f_1(x_1, \ldots, x_n) \\
\vdots \\
\frac{dx_n}{dt} &= f_n(x_1, \ldots, x_n)
\end{align*}
\]  

These equations, with specific choice of the functions \( f_1(x_1, \ldots, x_n) \) etc., define an \( n \)-dimensional dynamical system.

Solutions to the Eqs. (2.1) can be represented geometrically as trajectories in the space spanned by the variables \( x_1, \ldots, x_n \), known as the phase space. Figure 2.1 shows such a trajectory for a 2-dimensional system. In classical mechanical system, phase space is constructed using the positions and momenta of the particles. Given a point i.e. position and momentum at one time, the trajectory describes the time evolution of the system from the initial state.

![Figure 2.1: An example of trajectory in phase space. (From reference [34])](image)

The phase space description is an important and effective way to gain an overall understanding of the solutions to a given set of ODEs, even when the solutions themselves cannot be obtained analytically. The basic mathematical reason behind it has to do with continuity, i.e., for sufficiently well-behaved functions \( f_1(x_1, \cdots, x_n) \) etc., two nearby initial states will yield trajectories
that are nearby at least after a short time. In addition, for deterministic dynamical systems, the trajectories never cross each other except after infinite time, as otherwise the evolution of the crossing point would be non-unique. However, different trajectories may converge onto a lower dimensional object such as a fixed point or a limit cycle. These are illustrated below.

Consider first a one-dimensional dynamical system defined by the equation:

\[
\frac{dx}{dt} = \sin x
\] (2.2)

The analytic solution of the above equation is

\[
t = \ln \left| \csc x_0 + \cot x_0 \csc x + \cot x \right|
\]

with \( x_0 \) being the initial value of \( x \) at \( t = 0 \). However, plotting it will not give much insight into the inherent dynamical properties of the system. Instead we can use a phase plot to visualize the dynamics in a more intuitive way, as shown in Figure 2.2.

Figure 2.2: Phase space plot. (From reference [34])

With the aid of the above figure, the analysis of the problem became easier. The sinusoidal curve shows \( \dot{x} \) as a function of \( x \) according to Eq. (2.2). In regions of positive \( \dot{x} \), the particle moves to the right on the real axis. In contrast, the particle moves to left when the velocity is negative. Furthermore, when \( \dot{x} = 0 \), there is no flow along the axis, and these points are called fixed
points. Near the solid black dots, the flow is towards them and these dots are called stable fixed points. Near the open circles, the flow is away from them and these points are call unstable fixed points.

In two and higher dimensions, trajectories may converge onto (or diverge from) not only fixed points, but also closed contours known as limit cycles. Generally speaking, a limit cycle corresponds to a periodic solution of the PDEs. If it is a stable limit cycle, all the neighboring trajectories tend towards the limit cycle. However, if neighboring trajectories tend towards the limit cycle as time goes to negative infinity, it is called an unstable limit cycle.

### 2.2 The Hopf Bifurcation

The Hopf bifurcation describes a local change of the phase space flow around a previously stable fixed point. Continuous change in the form of the functions on the right-hand-side of Eq. (2.1) may turn a stable fixed point into an unstable one and, at the same time, an encircling limit cycle emerges in its neighborhood. This phenomenon is also called Poincaré-Andronov-Hopf bifurcation.

The stability of a fixed point can be determined via a linear analysis. The basic procedure is: Firstly, find the fixed point by using the ODEs of the system. Secondly, compute the corresponding Jacobian at the fixed point. Thirdly, calculate the eigenvalues of the Jacobian matrix. If all the eigenvalues have negative real parts, then the fixed point is stable. Otherwise the fixed point is unstable.

Consider for example Eqs 2.1 at \( n = 2 \) with a fixed point at \((x_1^*, x_2^*)\). Linearizing the equations near the fixed point yields,

\[
\frac{d(x_1 - x_1^*)}{dt} = \frac{\partial f_1(x_1^*, x_2^*)}{\partial x_1}(x_1 - x_1^*) + \frac{\partial f_1(x_1^*, x_2^*)}{\partial x_2}(x_2 - x_2^*) \\
\frac{d(x_2 - x_2^*)}{dt} = \frac{\partial f_2(x_1^*, x_2^*)}{\partial x_1}(x_1 - x_1^*) + \frac{\partial f_2(x_1^*, x_2^*)}{\partial x_2}(x_2 - x_2^*)
\]
The Jacobian has the form:

\[
A = \begin{pmatrix}
\frac{\partial f_1(x^*_1, x^*_2)}{\partial x_1} & \frac{\partial f_1(x^*_1, x^*_2)}{\partial x_2} \\
\frac{\partial f_2(x^*_1, x^*_2)}{\partial x_1} & \frac{\partial f_2(x^*_1, x^*_2)}{\partial x_2}
\end{pmatrix}.
\]

Since the matrix is real, its eigenvalues \(\lambda_1\) and \(\lambda_2\) are complex conjugates of each other. If the real parts of both \(\lambda_1\) and \(\lambda_2\) are negative, then \((x^*_1, x^*_2)\) is a stable fixed point. On the other hand, if the real part of \(\lambda_1\) and \(\lambda_2\) is positive, the fixed point is unstable.

The imaginary part of the eigenvalues yields the oscillation frequency of the trajectory around the fixed point. In the case of a Hopf Bifurcation, the two eigenvalues, which are symmetrically located about the real axis on the complex plane, cross the imaginary axis as certain parameter of the equations is varied. As the real part of the eigenvalues changes sign, the fixed point changes from being stable to being unstable [35].

The Hopf bifurcation is accompanied by the appearance of a limit cycle in the neighborhood either before or after the fixed point loses its stability. In the former case, the bifurcation is called subcritical (Fig. 2.3a); otherwise it is called supercritical (Fig. 2.3b). The size of the limit cycle vanishes at the critical point of stability.
The generic mathematical representation is a dynamical equation on the complex plane:

\[
\frac{dz}{dt} = z\left(\lambda + i\right) + b|z|^2
\]

Here \( \lambda \) is the controlling parameter, and both \( z \) and \( b = \alpha + i\beta \) are complex. The equation has a fixed point at \( z = 0 \) and a limit cycle solution \( z(t) = re^{i\omega t} \) with \( r = \sqrt{-\lambda/\alpha} \) and \( \omega = 1 + \beta r^2 \). The fixed point is stable for \( \lambda < \lambda_c = 0 \) and unstable for \( \lambda > \lambda_c \). On the other hand, the limit cycle solution exists only when \( \lambda \) and \( \alpha \) have opposite sign, i.e., for a given \( \alpha \), either on the subcritical or the supercritical side, but not both. A negative \( \alpha \) yields a supercritical Hopf bifurcation, and the system goes into a stable limit cycle when \( \lambda \) exceeds the critical value \( \lambda_c = 0 \) (Fig. 2.3b). On the other hand, a positive \( \alpha \) yields a subcritical Hopf bifurcation, and the system has an unstable limit cycle when \( \lambda \) is smaller than \( \lambda_c \) (Fig. 2.3a).

Figure 2.3: The examples of subcritical and supercritical Hopf bifurcation (From reference [34]).
Figure 2.4 illustrates the behavior of a component variable when the system undergoes supercritical Hopf bifurcation as a system parameter $\mu$ increases beyond its threshold value $\mu_c$. On the subcritical side (i.e., $\mu < \mu_c$), the variable exhibits damped oscillation. It changes to periodic oscillation when $\mu > \mu_c$, with an amplitude that increases as the square root of the distance to the threshold.

![Figure 2.4: An example of a supercritical Hopf bifurcation. (a) When the system parameter $\mu$ is smaller than $\mu_c$, the system goes through damped oscillation and approaches a fixed value. (b) When the parameter $\mu$ is larger than $\mu_c$, the system exhibits stable oscillations.](image)

2.3 Numerical Integration

The ODEs defined by Eqs (2.1) can be solved numerically using various methods such as the Euler method, Runge-Kutta methods etc. During numerical integration, the value of the variable at the next time step is determined by the value at the present time step. Time is discretised as $t_1, t_2, ..., t_n$, where $t_i = t_i + h$ for step size $h$. In the modelling work described in later chapters, we also consider delay differential equations (DDEs). In those cases, the value at the next time step is determined by the values at multiple earlier time points.

The program dde23 in Matlab is used in the numerical integration of our model. The algorithm is an extension of the one used in ode23 to solve the ODEs [36]. ode23 is implemented by using the Bogacki-Shampine method which is a third order Runge-Kutta method with an adaptive step size. Three function evaluations are performed in each time step.
Take for example the ordinary differential equation $y'(t) = f(t, y(t))$. Let $y_n$ be the value of $y(t)$ at time $t_n$ and $h_n$ is the step size between two adjacent times $h_n = t_{n+1} - t_n$. In one step, the Bogacki-Shampine goes as:

$$
k_1 = f(t_n, y_n)
$$

$$
k_2 = f(t_n + \frac{1}{2} h_n, y_n + \frac{1}{2} h_n k_1)
$$

$$
k_3 = f(t_n + \frac{3}{4} h_n, y_n + \frac{3}{4} h_n k_2)
$$

$$
y_{n+1} = y_n + \frac{2}{9} h_n k_1 + \frac{1}{3} h_n k_2 + \frac{4}{9} h_n k_3
$$

$$
k_4 = f(t_n + h_n, y_{n+1})
$$

$$
z_{n+1} = y_n + \frac{7}{24} h_n k_1 + \frac{1}{4} h_n k_2 + \frac{1}{3} h_n k_3 + \frac{1}{8} h_n k_4
$$

The expression for $y_{n+1}$ is based on a third order approximation while the one for $z_{n+1}$ is from a second-order approximation. The difference of $y_{n+1}$ and $z_{n+1}$ is used to obtain an appropriate step size $h_{n+1}$ in the next time step.

As mentioned above, dde23 \cite{37} is an extension of ode23. Because of the time delay, the time stepping is more involved and also requires interpolation due to the discreteness of the time points used in the integration.
Chapter 3

Single-cell Imaging of p53 Intensity

In this Chapter, firstly, the experimental procedures to obtain the time courses of p53 protein fluorescence intensity are described. All the experiments were carried out by the students and postdocs in Dr. Jade Shi’s lab. The obtained experimental results are series of snapshots of groups of cancer cells. Secondly, based on the snapshots, the computational procedures to extract the amount of p53 protein in the nuclei of a single cell are presented. Cell tracking and nuclei identification algorithms are discussed in details. Thirdly, time series of p53 fluorescence intensity are analysed for each cancer cell or compared between different cancer cells. In the analysis, we focused on the variations in oscillation period and amplitude for each cancer cell or between different cells. It is observed that the variation of oscillation amplitude is generally higher than that of oscillation period. Furthermore, we showed that the variation of oscillation amplitude correlates very well with the rising speed of p53 in the oscillation. These observations serve as the basis for the discussions on the comparison between experiments and model studies in Chapter 5.
3.1 The Experimental Setup

In this section, the experimental protocols in Dr. Shi’s lab to obtain the nuclear p53 fluorescence intensity is briefly described. Experiments were done using the A549 cell line from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured at 37°C in McCoy’s 5A or F-12K medium, supplemented with 10% fetal calf serum (FCS), 100U/ml penicillin and 100 ug/ml streptomycin. The partial pressure of CO$_2$ is kept at 5%. Besides, in order to obtain the nuclear p53 concentration, Yellow Fluorescence Protein is knocked into part of the p53 gene in the genome. Thus, the intensity of the fluorescence can reflect the concentration of p53 in the cell nucleus.

An anticancer chemical agent etoposide is applied to the cell culture to cause DNA double strand breaks. With the constructed p53-YFP complex, the concentration of p53 is tracked by YFP fluorescence intensity, as shown in Fig 3.1(b). Note that p53 exists both in nucleus and in cytoplasm. However, upon application of the drug treatment, change of the fluorescent signal from the nuclei is the most prominent. On the other hand, the luminance cannot tell whether the p53 is in its phosphorylated form or not.

![Labeled protein in cytoplasm.](image1)

![Nuclear p53 intensity.](image2)

Figure 3.1: Typical microscope images from the experiment. (a) The Red Fluorescent image of a cytoplasmic protein in single cells which can be used to mark the nuclei position. (b) The Yellow Fluorescent image of the p53 proteins taken at the same time, showing elevated concentration inside nuclei. Each view frame typically contains between 20-30 cells.

Each experiment typically lasts about 72 hours. Two sets of images at the
same location on the culture plate were obtained every 10 minutes. As shown in Figure 3.1 (a), the red fluorescence intensity reflects the concentration of a cytoplasmic protein. This set of images can help us to identify the nucleus of the cell. Figure 3.1 (b) shows the yellow fluorescence intensity in the cell, which reflects the concentration of p53 proteins.

In order to extract the time dependent p53 concentration when the cell suffers from DNA damage, we use the captured images to track each cell and document the yellow fluorescence intensity of each cell nuclei at each time point. Figure 3.2 gives one example of the raw image data used in the analysis.

![Figure 3.2: An example of the time course of nuclear p53 fluorescence intensity from a single cell under etoposide treatment starting at time 0 hr.](image)

### 3.2 Method to Extract p53 Fluorescence Intensity of Single Cells

The original experimental data were obtained as image files. Each image frame consists of a two-dimensional array of pixels, which are the smallest single component of a digital image. The whole image is defined by a matrix of pixel intensities.

To construct the time course of nuclear p53 fluorescence intensity from these images, we first track individual nuclei by identifying the region on the image belonging to a given nucleus on each frame. Secondly, a weighted average of the intensities of the pixels within the identified region (which we call mask) is assigned to the nucleus as its average p53 concentration. By following this procedure, the time course of the nuclear p53 concentration for selected cells are obtained.

The general procedure to build a sequence of masks for a given nucleus is as follows. First, we convert each original image file into a new image file by
resetting the pixel values based on a chosen threshold. For each pixel, if the original value is larger than the threshold, it is set to 1 (occupied), otherwise it is set to 0 (empty). Pixels with value 1 form clusters in the transformed image frame. The threshold is chosen such that the size (i.e., pixel number) of the largest cluster in the transformed image frame is smaller than a certain value, reflecting the fact that each cluster is supposed to approximate an illuminated nucleus. To automate the procedure, we use the Hoshen-Kopelman algorithm to identify clusters in the transformed image frame. The desired cluster sizes can be reached by gradually ramping up the threshold in an iterative fashion. Finally, after the clusters are identified for each frame, we compare successive frames to track individual nuclei. If two clusters in two consecutive frames overlap with each other by more than, say 90%, we assign them to the same nucleus.

Figure 3.3 shows the distribution of the pixel intensity for five selected image frames during the course of an experiment. Due to a somewhat variable background fluorescent intensity, cell migration, and cell division events etc., the success rate of the above procedure for cell tracking is limited to less than 50%. This can be improved by keeping a window around the chosen cell to reduce the field of view. Manual intervention is sometimes required to complete the tracking process for selected cells.
Figure 3.3: The distribution of pixel values of each image at five time points. The horizontal axis represents the pixel values. The vertical axis corresponds to the frequency on logarithmic scale.

For cells tracked successfully, the next step is to identify the fluorescence intensity in the cell nucleus. As shown in Fig 3.4, intensity variation in the region identified is significant. One of the reasons is that during cell tracking, a relatively low threshold is chosen to cover those nuclei with low fluorescence intensity. Therefore, for the nucleus of interest, the identified region may extend to part of the cytoplasm where the p53 concentration is lower. In order to obtain the average nuclear p53 fluorescence intensity, we consider subsets of pixels whose intensity lies in top 25%, top 50%, or top 75% of all pixels in the identified region, respectively. As shown in Fig 3.4, their average values represent much better estimates of the average fluorescent intensity inside the nucleus. From our experience, average over the top 50% pixel values is a good approximation to the nuclear p53 concentration.
3.3 Time Course of Nuclear p53 Concentration

In this section, the time courses of the average nuclear p53 fluorescence intensity under different experimental conditions are shown, and some basic statistical analysis about those time courses are carried out.

The data are collected from the experiment in Dr. Shi’s lab where cells of the cell line A549 were challenged with etoposide at concentrations 1 µM, 5 µM, and 50 µM, respectively. The basic feature is as follows: at low concentrations of etoposide, most cells exhibit an oscillating p53; with increasing level of etoposide, more cells show monotonic induction of p53 instead of oscillation. This has been reported in [28].

Typical time courses of p53 from individual cells are shown in Fig 3.5-3.8. In Fig 3.5a (1 µM etoposide) and 3.6a (5 µM etoposide), p53 concentration
shows regular oscillations from very early on but with significant variations in oscillation amplitude. On the other hand, in Figure 3.5b and 3.6b, the behavior in the first 30 hours or so is quite irregular, but the cell switches to strong and regular oscillations afterwards. These features happen at both 1\(\mu\)M and 5\(\mu\)M etoposide conditions, although the oscillation amplitude of p53 is about a factor of 2 greater in the 5\(\mu\)M case. Figure 3.7 shows that some cells at 5\(\mu\)M etoposide exhibits a big pulse in the beginning and then a series of small irregular pulses later. Such behavior are not observed under 1\(\mu\)M etoposide condition. At 50\(\mu\)M etoposide concentration, most cells show monotonic p53 induction as shown in Figure 3.8.

Figure 3.5: Time courses of nuclear p53 concentration at 1\(\mu\)M etoposide. (a) cells show regular oscillations with increased amplitude after about 30 hours. (b) cells that switch from irregular to regular oscillation.

Figure 3.6: Time course of nuclear p53 concentration at 5\(\mu\)M etoposide. (a) cells show regular oscillations with increased amplitude after about 30 hours. (b) cells that switch from irregular to regular oscillation.
Figure 3.7: Time courses of nuclear p53 concentration at 5 µM etoposide. p53 concentration in these cells shows a big pulse in the beginning, followed by successive small pulses. Duration of the first pulse is about 6 times that of the successive pulse.

Figure 3.8: At 50 µM etoposide, the majority of cells show monotonic induction of nuclear p53 before bursting after about 36 hours.

We now look at the statistical features of variations in the p53 oscillation period and amplitude. For each cell with p53 oscillation, we extracted the period and peak value for each oscillation. Deducting the average background value from the peak of each pulse is the definition of peak value here. In Figure
3.9 and 3.10, we show the average period and peak value of each cell during the course of the experiment. The error bars indicate the standard deviation in each case. The pink bar gives the average value over all the cells shown. From the figures, variations for a single cell during the course of the experiment is comparable to variations across different cells, both for the amplitude and the period of oscillation. Obviously, variation in the period is much less than that of the amplitude. Compare the results at 1 µM and 5 µM, we see that the average amplitude increases significantly. On the other hand, the period of oscillation, changing from slightly below to slightly above 6 hours on average, is remarkably stable.

For the p53 time courses at 50 µM etoposide, we have computed the slope of each curve shown in Fig 3.8. The slope of each line is obtained by fitting the data with a linear relation. Figure 3.11 shows the result. More discussions will be carried out in Chapter 5.

Figure 3.9: (Unit: Hours) Each blue column on the hist displays the average period of single cell p53 oscillation. The error bar on each column represents the standard deviation of period for single cell p53 oscillation. Under 1 µM etoposide condition, the average period of the all the single cells is 5.9, the standard variation is 0.77 by calculating single cell deviation for the whole average (represented by the pink column and its error bar on the left hand side figure). Under 5 µM etoposide condition, the average period of the all the single cells is 6.27, the standard variation is 0.7 by calculating single cell with the whole average (represented by the pink column and its error bar on the right hand side figure).
Figure 3.10: Each blue column on the histogram displays the average peak value of single cell p53 oscillation. The error bars on each column represent the standard deviation of peak value for single cell p53 oscillation. Under 1 $\mu$M etoposide condition, the average peak value of the all the single cells is 17, the standard deviation is 6.8 by calculating single cell with the whole average (represented by the pink column and its error bar on the left hand side figure). Under 5 $\mu$M etoposide condition, the average peak value of the all the single cells is 22.6, the standard variation is 8.7 by calculating single cell with the whole average (represented by the pink column and its error bar on the right hand side figure).

Figure 3.11: Slope of monotonic increase of p53 concentration for 50 $\mu$M etoposide condition.
Chapter 4

Model Construction and Analysis

Batchelor et al. [25, 26] developed an ODE model for the p53 dynamics with the key components and interactions summarised in Fig 1.1. In their model, p53 can exist in either inactive or active form. Activation of p53 requires either ATM or ATR. The degradation of p53 by Mdm2 (through the process of ubiquitination) is taken to be about 3.5 times slower when it is in the active form. In addition, a time delay (0.7 hours) is introduced in the p53 promoted expression of Mdm2 proteins.

A central component in the Batchelor et al. model is the phosphatase Wip1. In their model, dephosphorylation of ATM by Wip1 is required to generate sustained oscillations. Although their model predictions fit quantitatively with single-cell experiments carried out in their lab under both γ and UV irradiation, it cannot be directly applied to the cell line (A549) studied in Dr. Shi’s lab, where the protein Wip1 is essentially dysfunctional. In this Chapter, we introduce a modified interaction between p53 and Mdm2 and show that, even in the absence of Wip1, oscillatory state can still appear at intermediate levels of damage, in agreement with the experimental data presented in Chapter 3.

To find a suitable mathematical representation for the ubiquitination of p53 by Mdm2, we shall first investigate a 2-component model involving only p53 and Mdm2. We show that a Michaelis-Menten type kinetics is important for achieving sustained oscillations. Biologically, Mdm2 can be seen as the enzyme that facilitate p53 degradation. Hence it is meaningful to introduce this form
into the ODE model instead of the mass action form used by Batchelor et al. Other models that show sustained oscillations also employed this form such as [27].

In the real system, p53 degradation is affected not only by Mdm2 concentration, but also by its phosphorylation state which itself is the result of the upstream signalling process involving ATM and others kinase proteins. To provide a more complete description of the experiments, we return to the 4-component model by Batchelor et al., but replace the ubiquitination of p53 by Mdm2 by the Michaelis-Menten form. The effect of etoposide is introduced through ATM concentration, which sets the rate of conversion from inactive to active p53. At intermediate levels of damage, the enhanced conversion rate effectively decreases the ubiquitination rate of p53, which in turn activates its oscillation. On the other hand, high damage level triggers ATM-dependent Mdm2 degradation, leading to monotonic induction of p53. Therefore, the signalling kinase ATM is able to drive two bifurcations: one is from low level state to oscillation state and another one from the oscillation state into high level state.

4.1 Model Construction

4.1.1 A two-component model

The basic dynamical properties of the negative feedback module p53-Mdm2 can be studied in a 2-component model illustrated by Figure 4.1. Here, Mdm2 production is enhanced by p53 with a time delay $\tau$, associated with the time from p53 binding to the promoter site to the completion of Mdm2 synthesis in the cytosol. Meanwhile, Mdm2 acts as a E3 ubiquitin to help the degradation of p53. In the following, we consider two mathematical presentations for the latter process, one in the mass action form and the other in the Michaelis-Menten form.
Mdm2 feedback on p53 in a mass action form cannot generate sustained oscillation

We let $[p]$ represents the p53 concentration and $[m]$ represents the Mdm2 concentration. First, for the Mdm2 dependent p53 degradation in the mass action form, the rate equations take the form:

$$\frac{d[p(t)]}{dt} = \beta_p - \alpha_p[p(t)][m(t)] \quad (4.1)$$
$$\frac{d[m(t)]}{dt} = \beta_m[p(t - \tau)] - \alpha_m[m(t)] \quad (4.2)$$

Here the $\beta$’s are production rates and the $\alpha$’s are degradation rates.

The physiological fixed point of the above equation is:

$$p^* = \sqrt{\frac{\beta_p \alpha_m}{\beta_m \alpha_p}} \quad (4.3)$$
$$m^* = \frac{\beta_m p^*}{\alpha_m} = \sqrt{\frac{\beta_p \beta_m}{\alpha_p \alpha_m}} \quad (4.4)$$

To investigate the stability of the fixed point, one can linearize Eqs. (4.1)
and (4.2) to obtain,

\[
\frac{d}{dt} \begin{bmatrix} \hat{p}(t) \\ \hat{m}(t) \end{bmatrix} = \begin{bmatrix} -\alpha_p m^* & -\alpha_p p^* \\ 0 & -\alpha_m \end{bmatrix} \begin{bmatrix} \hat{p}(t) \\ \hat{m}(t) \end{bmatrix} + \begin{bmatrix} 0 & 0 \\ \beta_m & 0 \end{bmatrix} \begin{bmatrix} \hat{p}(t - \tau) \\ \hat{m}(t - \tau) \end{bmatrix} \tag{4.5}
\]

where \( \hat{p}(t) = p(t) - p^* \) and \( \hat{m}(t) = m(t) - m^* \).

Let \( \hat{p}(t) = p_0 e^{\lambda t}, \hat{m}(t) = m_0 e^{\lambda t} \), the above equation gives:

\[
0 = \begin{bmatrix} -\alpha_p m^* - \lambda & -\alpha_p p^* \\ \beta_m e^{-\lambda \tau} & -\alpha_m - \lambda \end{bmatrix} \begin{bmatrix} p_0 \\ m_0 \end{bmatrix}
\]

Set the determinant of the matrix to zero, we obtain the following equation for \( \lambda \),

\[
\lambda^2 + (\alpha_m + \alpha_p m^*)\lambda + \alpha_m \alpha_p m^* + \beta_m \alpha_p p^* e^{-\lambda \tau} = 0 \tag{4.6}
\]

It is clearly to see that when \( \tau = 0 \), \( \lambda \) must be a real negative number, which means that the fixed point is stable. In addition, since all the coefficients in Eq (4.6) are positive, there is no real positive solution for \( \lambda \) when all the rate constants are nonzero. To determine whether the equation has complex solutions with a positive real part, one strategy is to consider the evolution of the solutions as \( \tau \) increases from 0. At the onset of such instability, \( \lambda \) must be purely imaginary. Writing \( \lambda = i\alpha \), Eq. (4.6) becomes:

\[
-\alpha^2 + b + c \cos(\alpha \tau) = 0 \\
\alpha a - c \sin(\alpha \tau) = 0 \tag{4.7}
\]

where \( a, b \) and \( c \) are given by:

\[
a = \alpha_m + \alpha_p m^*, \quad b = \alpha_m \alpha_p m^*, \quad c = \beta_m \alpha_p p^* = b.
\]

Eliminating \( \tau \), we obtain:

\[
\alpha^4 + (a^2 - 2b)\alpha^2 = 0 \tag{4.8}
\]
Since \( a^2 - 2b = \alpha_m^2 + (\alpha_p m^*)^2 > 0 \), there is no real solution except the trivial case \( \alpha = 0 \). Thus, for this particular model, it is not possible to have unstable fixed points (sustained oscillation).

**Mdm2 feedback on p53 in a Michaelis-Menten form can generate sustained oscillation**

For the Mdm2 dependent p53 degradation in the Michaelis-Menten form, the equations are written as follows:

\[
\frac{dp(t)}{dt} = \beta_p - \alpha_p [m(t)] \frac{[p(t)]}{[p(t)] + K_m} \tag{4.9}
\]
\[
\frac{dm}{dt} = \beta_m [p(t - \tau)] - \alpha_m [m(t)] \tag{4.10}
\]

Here \( K_m \) is known as the Michaelis constant. The main difference of Eq (4.9) to (4.1) is that the degradation rate of p53 levels off when its concentration exceeds \( K_m \).

The fixed point of the above equations are easily obtained:

\[
p^* = \frac{\beta_p \alpha_m + \sqrt{\beta_p^2 \alpha_m^2 + 4 \beta_p \beta_m \alpha_p \alpha_m K_m}}{2 \beta_m \alpha_p} \tag{4.11}
\]
\[
m^* = \frac{\beta_m p^*}{\alpha_m} \tag{4.12}
\]

The linear stability analysis can be carried out in the same way as above. The equation satisfied by \( \lambda \) now reads,

\[
\lambda^2 + \left( \alpha_m + \alpha_p \frac{m^* K_m}{(p^* + K_m)^2} \right) \lambda + \frac{\beta_m \alpha_p p^* K_m}{(p^* + K_m)^2} + \frac{\beta_m \alpha_p p^*}{p^* + K_m} e^{-\lambda \tau} = 0 \tag{4.13}
\]

Again, there is no real positive solution for \( \lambda \) at any \( \tau \). To look for the onset of complex solution with a positive real part, we again consider the case \( \lambda = i\alpha \). Equation (4.13) then yields Equation (4.7) with \( a, b \) and \( c \) now given by:

\[
a = \alpha_m + \alpha_p \frac{m^* K_m}{(p^* + K_m)^2}, \quad b = \frac{\beta_m \alpha_p p^* K_m}{(p^* + K_m)^2}, \quad c = \frac{\beta_m \alpha_p p^*}{p^* + K_m}.
\]
Eliminating $\tau$ from Eqs (4.7) yields:

$$\alpha^4 + (a^2 - 2b)\alpha^2 - \left[1 - \left(\frac{K_m}{p^* + K_m}\right)^2\right]c^2 = 0$$ \hspace{1cm} (4.14)

Here again $a^2 > 2b$ but due to the negative constant term, real solutions for $\alpha$ exist. The onset value for $\tau$ can then be determined from Equation (4.7) accordingly. Thus in this case, it is possible to have unstable fixed points (sustained oscillation).

In the experiments, the external control parameter is the concentration of the etoposide, which can change the DNA damage level of the cells. It is observed that when the etoposide concentration is high enough, there will be sustained oscillations of p53 fluorescence intensity. Mechanistically, it is believed that at high DNA damage level, significant amount of p53 should be phosphorylated by ATM and hence be less sensitive to Mdm2 dependent ubiquitination and degradation. Consequently, sustained oscillations can be maintained. Here in the 2-component model, for a fixed time delay $\tau$ and other parameters, we simulate the p53 dynamics when the Mdm2 dependent p53 degradation rate ($\alpha_p$) is changed. The model with higher $\alpha_p$ corresponds to the dynamics of non-phosphorylated p53 and vice versa. As shown in Figure 4.2(b), for the Michaelis-Menten type 2-component model, when $\alpha_p$ decreases, there exists a transition of p53 dynamics from a non-oscillatory state to an oscillatory one. In the 4-component model studied in the following, ATM is responsible to boost the production of p53 to initiate the oscillation.
Figure 4.2: (a) For the 2-component model with the mass action form of Mdm2-dependent p53 degradation, when the parameter $\alpha_p$ (Mdm2-dependent degradation of p53) decreases, p53 always arrives to a stable state with a higher value. (b) For the 2-component with the Michaelis-Menten form of Mdm2-dependent p53 degradation, when $\alpha_p$ decreases, p53 can go from a stable state into a sustained oscillatory state.

4.1.2 A 4-component model

With the above preparation, we now introduce a modified version of the 4-component model of Batchelor et al. [25], with the Michaelis-Menten form for the p53 degradation. There are altogether four types of molecules, ATM, Mdm2, and the inactive (unphosphorylated) and active (phosphorylated) forms of p53. Figure 4.3 illustrates the interaction between them, with the corresponding rate constants indicated.

In terms of the protein concentrations within a cell, the rate equations take
Figure 4.3: The DNA damage dependent p53 dynamics network.

The model equations are:

\[
\frac{dp53_{inactive}}{dt} = \beta_p - \alpha_{mpi}[Mdm2] \frac{[p53_{inactive}]}{[p53_{inactive}] + T_{in}} - \beta_{sp}[p53_{inactive}] \frac{[ATM]^{n_s}}{[ATM]^{n_s} + T_{ns}} - \alpha_{mpi}[p53_{inactive}] \tag{4.15}
\]

\[
\frac{dp53_{active}}{dt} = \beta_{sp}[p53_{inactive}] \frac{[ATM]^{n_s}}{[ATM]^{n_s} + T_{ns}} - \alpha_{mpa}[Mdm2] \frac{[p53_{active}]}{[p53_{active}] + T_{ac}} \tag{4.16}
\]

\[
\frac{d[Mdm2]}{dt} = \beta_{mi} + \beta_{m}[p53_{active}(t - \tau_m)] - \alpha_{sm}[ATM][Mdm2] - \alpha_{m}[Mdm2] \tag{4.17}
\]

\[
\frac{d[ATM]}{dt} = \beta_s D - \alpha_s[ATM] \tag{4.18}
\]

Here, both Mdm2 and the inactive p53 have a basal expression rate \(\beta_{mi}\) and \(\beta_p\), respectively. The DNA damage signal \(D\) increases the phosphorylation level of ATM, and all the active p53 comes from inactive p53 catalysed by phosphorylated ATM. This interaction is important to elevate the overall p53 concentration when the damage level increases. The term \(\beta_m[p53_{active}(t - \tau_m)]\) in (4.17) describes enhanced expression of Mdm2 activated by active p53.
The delay time $\tau_m$ accounts for the relatively slow process of mRNA nuclear transport and cytosolic protein synthesis compared to the protein-protein interaction. The Michaelis-Menten type interaction in Eqs. (4.15) and (4.16) is used for Mdm2-mediated degradation of inactive p53 and active p53. The Michaelis-Menten constants are chosen according to Ref [21]. Besides, all the proteins have a basal degradation rate except for active p53. There is another important interaction of ATM on Mdm2. Phosphorylated ATM promotes the degradation of Mdm2 at a rate $\alpha_{sm}$. This interaction is needed to eliminate the oscillation at high damage level. Default values of the parameter set used in the simulation are given in Table A.1. Most parameter values are similar to those used in [25].

4.2 Main Results

In this section, the main numerical results on the 4-component model introduced above are presented. Unless stated, otherwise, the parameters in the model are assigned values listed in A.1. In subsection 4.2.1, we consider the effect of damage level D on the p53 dynamics. As D increases, the system goes from non-oscillatory steady state at low p53 level into the oscillatory states and back to non-oscillatory states at high p53 level. To identify the key parameters that control the oscillation period and amplitude we examine in subsection 4.2.2 more closely the 3 phases in an oscillation cycle defined by the low and high values of p53 and Mdm2. We derive approximate formulas for the duration of each phase and estimate the oscillation amplitude. Finally, a systematic parameter sensitivity analysis is carried out in subsection 4.2.3. Each parameter is perturbed by 10 fold around its original value to evaluate its effects on both oscillation period and amplitude.

4.2.1 The bifurcation diagram

We have carried out extensive simulations of the four-component model at different levels of DNA damage. The blue dots in Figure 4.4 shows the total
p53 concentration as a function of the damage level $D$ after the system has reached the steady or the periodic oscillating state. At small values of $D$, p53 stays at a low and constant level. As the damage level increases, oscillations start to occur. In this case, we plot the high and low values of p53 in each oscillating period. At even higher levels of damage, oscillation disappears, with the steady-state p53 concentration at a much higher value. Thus, the model indeed has two transitions.

![Figure 4.4: Bifurcation diagrams obtained from numerical solutions of the ODEs. Blue dots are for the 4-component model while the red dots are for the original model proposed by Batchelor et al. [25] At low DNA damage levels, p53 reaches a low steady-state value. When the damage level increases, p53 shows oscillations. At still higher damage levels, the oscillation disappears and the system reaches a steady-state at a high p53 concentration. Transitions into and out of the oscillating state are all of the Hopf bifurcation type.](image)

To gain a better understanding of the bifurcation process, we examine more closely the oscillation amplitude (the difference between the high and low values) of p53 near the onset and termination of the oscillation state (i.e., the beginning and the end of the bubble in Fig 4.4). Figure 4.5(a) shows the square of the oscillation amplitude versus damage level near the onset. The data follows a straight line. This linear relationship between the two suggests that the transition is a Hopf bifurcation. A least-square fit to a linear functions yields a threshold value $D_{c1} \simeq 0.5434$. 

37
For the second transition, Fig 4.5(b) shows that the square of the oscillation amplitude against the damage level can also be well-fitted by a straight line. This confirms that the transition is again a Hopf bifurcation at $D_{c2} \simeq 3.7911$.

Figure 4.5: The square of oscillation amplitude plotted against the damage level around the two transition points. In both cases, data lie on a straight line, in agreement with the Hopf bifurcation. Linear fit of the data yields the transition points: the low threshold is 0.5434 and the high threshold is 3.7911.

We have also compared the bifurcation diagram of the current model and that of the original model by Batchelor et al. [25]. The original model is defined by the following ODEs:

$$
\frac{dp_{53_{\text{inactive}}}}{dt} = \beta_p - \alpha_{mpl}[Mdm2][p53_{\text{inactive}}] - \alpha_{pl}[p53_{\text{inactive}}]
- \beta_{sp}p_{53_{\text{inactive}}} \frac{[ATM]^{n_s}}{[ATM]^{n_s} + T_s^{n_s}}
$$  
(4.19)

$$
\frac{dp_{53_{\text{active}}}}{dt} = \beta_{sp}[p53_{\text{inactive}}] \frac{[ATM]^{n_s}}{[ATM]^{n_s} + T_s^{n_s}}
- \alpha_{mfa}[Mdm2][p53_{\text{active}}]
$$  
(4.20)

$$
\frac{d[Mdm2]}{dt} = \beta_{mi} + \beta_m[p53_{\text{active}}(t - \tau_m)] - \alpha_{sm}[ATM][Mdm2]
- \alpha_m[Mdm2]
$$  
(4.21)

$$
\frac{d[Wip1]}{dt} = \beta_w[p53_{\text{active}}(t - \tau_w)] - \alpha_w[ATM]
$$  
(4.22)

$$
\frac{d[ATM]}{dt} = \beta_s D - \alpha_{ws}[ATM] \frac{[Wip1]^{n_w}}{[Wip1]^{n_w} + T_w^{n_w}} - \alpha_s[ATM]
$$  
(4.23)
Values for the model parameters used in their study are given in Table A.1 in Appendix A except $\tau_m = 0.7 h$ and $\alpha_{mpi} = 5 h^{-1} C s^{-1}$, $\alpha_{mpa} = 1.4 h^{-1} C s^{-1}$.

In addition, the kinetic parameters involving Wip1 are: $\beta_w = 0.25 h^{-1}$, $\alpha_w = 0.7 h^{-1}$, $\tau_w = 1.25 h$, $\alpha_{ws} = 50 h^{-1}$, $T_w = 0.2 C s$, and $n_w = 4$.

The red dots in Figure 4.4 give the steady-state or the peak and valley values of the p53 concentration from numerical solutions of the Batchelor et al. model. Here, the oscillation bubble is significantly smaller in size as compared to the 4-component model, with smaller oscillation amplitudes and an elevated minimum value of p53 concentration. Nevertheless, as in the 4-component model, the oscillating state exists only at intermediate levels of the DSB.

Figures 4.6 and 4.7 give the time trajectories of the two models in the reduced phase space spanned by the total p53 concentrations and the Mdm2 concentration, before and after the first bifurcation point on the bifurcation diagram Figure 4.4. As can be seen from the plots, transition to the oscillating state exhibits the typical characteristics of a supercritical Hopf bifurcation as shown in Figure 2.3b.

![Phase space trajectories](image)

(a) Phase space trajectories of the Batchelor et al. model just before the first bifurcation.

(b) Phase space trajectories of the Batchelor et al. model just after the first bifurcation.

Figure 4.6: Phase space trajectories of the Batchelor et al. model near the first bifurcation. Different colours are used for different initial conditions. (a) When the damage level is under the threshold, all the trajectories converge to the fixed point at long times. (b) When the damage level is over the threshold, all the trajectories converge to the limit cycle.
(a) Phase space trajectories of the 4-component model just before the first bifurcation.

(b) Phase space trajectories of the 4-component model just after the first bifurcation.

Figure 4.7: Phase space trajectories of the 4-component model near the first bifurcation. Different colours are used for different initial conditions. (a) When the damage level is under the threshold, all the trajectories converge to the fixed point at long times. (b) When the damage level is over the threshold, all the trajectories converge to the limit cycle.

4.2.2 Analysis of one oscillation cycle

We now take a closer look of the solution in the middle of the oscillation bubble in Fig 4.4. Figure 4.8 shows the time dependence of the four variables as well as the total p53 concentration at $D = 1.2$, well after the system has relaxed from its initial state. Figure 4.9 shows the corresponding limit cycle in the total p53-Mdm2 plane. Note that, at a constant damage level, ATM reaches its steady state value $\beta_s D/\alpha_s$ in a time $\alpha_s^{-1}$. 
Figure 4.8: The concentration of the four component proteins in one oscillation cycle. Here $D = 1.2$. ATM stays at a constant value, while the inactive p53 concentration remains low in the whole period. The parameters used are shown in Table A.1.

Figure 4.9: The limit cycle in the Mdm2 and total p53 plane. Different colors are used for the three time intervals in one cycle as defined in Fig 4.8. The parameters used are shown in Table A.1.

With the help of Fig 4.8 and 4.9, we may decompose each cycle into approximately three phases with their main characteristics described below.

- Phase 1: Mdm2 decay.
Phase 1 starts at the maximum of Mdm2 and ends at a point where p53 begins its linear rise. From Eq (4.17), we see that this phase is entered due to the continuing fall of p53 at a time interval $\tau$ earlier, as the other terms in the equation do not change appreciably near the maximum of Mdm2. At small values of $\beta_{mi}$ and small values of $\tau$, the parabolic-and-then-exponential fall lasts over a time of the order

$$T_1 \simeq \frac{1}{\alpha_m + \alpha_{sm}[ATM]} \quad (4.24)$$

However, the decay process may be pre-empted by the rise of p53 which signals the beginning of Phase 2.

- Phase 2: Linear rise of p53.

Phase 2 begins when p53 start to rise linearly in time and ends when it reaches its peak value. From Fig 4.8, the inactive p53 does not change appreciably in this time interval. According to Eq (4.16), the rate of production of the active form of p53 is nearly a constant, while the rate of degradation is low due to the low value of Mdm2. This yields a linear rise of the active form of p53. The rise terminates when Mdm2 comes back to a sufficient level. To estimate the time for this process to complete, let us assume that all inactive p53 is converted to active p53. Since $T_{ac} \ll [p53_{active}]$ in this phase, and taking the delay $\tau$ to be small, we may approximate Eq (4.16) and Eq (4.17) by,

$$\frac{d[p53_{active}]}{dt} = \beta_p - \alpha_{mpa}[Mdm2] \quad (4.25)$$
$$\frac{d[Mdm2]}{dt} = \beta_m[p53_{active}] \quad (4.26)$$

With the “initial condition” $[p53_{active}](0) = [Mdm2](0) = 0$, we obtain,

$$[p53_{active}] = (\beta_p/\omega_2) \sin(\omega_2t) \quad (4.27)$$
$$[Mdm2] = (\beta_p/\alpha_{mpa})[1 - \cos(\omega_2t)] \quad (4.28)$$
Here $\omega_2 = \sqrt{\alpha_{mpa}\beta_m}$. Thus the length of this phase is approximately given by,

$$T_2 \simeq \frac{\pi}{2\omega_2} = \frac{\pi}{2\sqrt{\alpha_{mpa}\beta_m}} \quad (4.29)$$

Note that $\beta_p$, which approximates the phosphorylation flux of p53, sets the amplitude of active p53 and Mdm2, but does not affect $T_2$. This is an important result and will be used in the interpretation of the experimental data.

The above analysis assumed that $\tau_m$ is much shorter than the value of $T_2$ predicted by Eq (4.29). We have investigated numerically what happens when $\tau_m$ is comparable or bigger than this value. Figure 4.10a shows our result of $T_2$ against $\tau_m$. The data falls on a straight line with a slope one, i.e., $\tau_m$ simply delays the rise of Mdm2. As Fig 4.10b shows, the oscillation amplitude also increases with the time delay.

Figure 4.10: (a) The period of oscillation against the delay time. The data falls on a straight line with a slope close to one. (b) p53 temporal profile for delay times ranging from 2hr to 8 hr. Note that, for this set of model parameters, longer delay prolongs the rising time of p53 but not the slope. Consequently, the peak value also increases linearly with the delay time.

- **Phase 3: Fall of p53.**

Phase 3 begins at the maximum active p53 and ends at the maximum of Mdm2. Examining the Eqs (4.16) and (4.17), we see that the assumptions that lead to Eqs (4.25) and (4.26) are still approximately valid when $\tau_m$ is small. Also note that concentration of the inactive form of
p53 does not change dramatically in this time interval. Therefore the phosphorylation flux to produce active p53 does not change by a big amount. The form of the solution shown in Fig 4.8 also support a solution of the form (4.27) and (4.28), starting from the peak of active p53. Based on these observations, we may estimate the length of this phase to be approximately $T_2$ when $\tau_m \ll T_2$.

4.2.3 Sensitivity analysis against model parameters

To further validate the above approximate analysis, we have performed sensitivity tests on the peak value of p53 and the period of oscillation against parameters of the model. Each time, the value of a chosen parameter is changed from its default value by a certain factor while other parameters are kept fixed. The numerically determined peak value and period are plotted against the specific parameter. The slope of these curves yield the sensitivity of the quantity in question against these parameters.

![Figure 4.11: Each parameter is changed to different folds of its default value while other parameters are fixed. Then the peak value of the oscillations is then plotted against the specific parameter. Both the values on horizontal and vertical axes represent the natural logarithm of the fold change. All these relations are obtained by using the parameters in Table A.1.](image-url)
Figure 4.12: Each parameter is changed to different folds of its default value while other parameters are fixed. Then the oscillation period is then plotted against the specific parameter. Both the values on horizontal and vertical axes represent the natural logarithm of the fold change. All these relations are obtained by using the parameters in Table A.1.

In the Figure 4.11, the basal inactive p53 generation rate $\beta_p$ and time delay $\tau$ are two very important parameters for the oscillation peak value. The results are in good agreement with our approximate analysis of Phase 2 where p53 rises to attain its peak value. In particularly, Eq (4.27) predicts a linear growth of p53 amplitude with $\beta_p$, in very good agreement with the data (blue crosses). On the other hand, as seen in Fig 4.12, the period is controlled by the time delay $\tau$ as well as $\alpha_{m_p}$ and $\beta_m$ in the way predicted by Eq (4.29).
Chapter 5

Comparison of Model and Experiment

In this Chapter, we re-examine various aspects of the experimentally measured time courses of p53 concentration in light of the theoretical studies in Chapter 4. Although only p53 concentration is measured in the experiment, we show that careful comparison of the pulse shape in the two cases can nevertheless yield valuable information on the underlying molecular processes. The study allows us to make suggestions on the possible origin of the observed cell-to-cell variations, as well as variations within a single cell at different times.

5.1 Damage Dependent p53 Dynamics

In both the experiment and the model, the oscillating phase exists at intermediate levels of the DNA DSBs. From the shape of the bubble in Fig 4.4 we see that, as the damage level passes the onset threshold, the pulse peak initially increases sharply and then turns into a much slower climb before ceding eventually. On the other hand, the floor level of p53 stays at a nearly constant low value except at the very beginning and towards the end. Assuming the model provides a reasonable description of the real cells, the observations made in Sec 3.3 put the damage levels at 1 µM etoposide and 5 µM etoposide to be within the bubble region not too close to the two transitions, while the
higher damage level at 50 μM etoposide is above the termination point of the oscillating phase.

We now compare the two systems not only in the long-time regime, but also their initial response upon administration of the drug agent. Figure 5.1 shows numerical solutions of the four-component model at different damage levels. The initial condition corresponds to low values of all four components. All curves show similar initial rise of p53. As we mentioned earlier, at moderate levels of damage, ATM reaches a level high enough to boost the production of active p53, but not enough to suppress Mdm2 activity. As the damage level increases, the latter effect picks up, eventually eliminating the oscillation generated by the negative feedback loop of Mdm2 and p53. This scenario is in general agreement with the experimental data and other studies in the literature.

Figure 5.1: (a) The model predicts that when damage level is moderate, time courses of p53 concentration show sustained oscillations. When damage level is high, time courses of p53 concentration show monotonic induction. (b) From the same initial condition, the low damage level yields a limit cycle (oscillation feature) while high damage level yields a high stable state. The relations are obtained by using the parameters in Table A.1.

Figure 5.2 shows examples of time courses of p53 from experiments in Shi’s lab. While the general trend of the data is in agreement with the model behavior, there are also noticeable differences particularly in the first 30 hours when the etoposide is applied.
Figure 5.2: The experimental data show two different p53 dynamical features. At 1 $\mu$M etoposide or 5 $\mu$M etoposide, nuclear p53 concentration undergoes oscillations. At 50 $\mu$M etoposide, nuclear p53 concentration undergoes monotonic induction.

5.2 Variations in the Period of Single Cell p53 Oscillation

5.2.1 Period variation at different damage levels

Figure 5.3 shows the mean oscillation period at 1 $\mu$M etoposide and 5 $\mu$M etoposide, respectively. The error bar in each case indicates the standard deviation across different pulses and over different cells. The original data have been presented in Chapter 3. Although the average value changed from about 5.84 hours to about 6.3 hours, the standard deviation is too large to be sure that the oscillation period depends significantly on the damage level.
According to the model analysis in Chapter 4, the damage level can affect the duration of phase 1 ($T_1$, p53 quiescent phase) because $T_1 \approx \frac{1}{\alpha_m + \alpha_{sm}[ATM]}$.

To see if this prediction is borne out by the experiment, we define the quiescent phase in each cycle to be the valley between two dashed vertical lines shown in Fig 5.4b (a). Each dashed line marks the point where p53 level is at 15% of the interval defined by the floor and peak values on a rising or declining curve. As one can see in Fig 5.4b (b), the average of the time $T_1$ where p53 stays near its low value indeed decreases a bit, though the pulse-to-pulse variation is still large.

We have also computed the duration $T_1$ at different damage levels in the four-component model, with the result given in Fig 5.5. With the increase of damage level, a moderate decrease of $T_1$ is seen for the parameters used in the calculation.

For the parameters used to generate Figure 5.5, most of the parameters are from Table A.1 except for $\alpha_{mpa}$, $\alpha_{mpi}$, $\tau$ and $\beta_p$.

In order to match the p53 degradation rate at the same Mdm2 concentration between our model and Batchelor et al, $\alpha_{mpa}$ and $\alpha_{mpi}$ are adjusted according to the average p53 concentration (around 0.5 Cs) in their model.
The time delay is tuned to 0.9 hours from 3 hours in Table A.1 to match the oscillation period in the experiments. At last, sufficiently high $\beta_p$ is needed to generate sustained oscillations for the new parameter set. In Table A.2, it is 3 fold of the value in Table A.1.

The theoretical values for $T_1$ are somewhat below the averages obtained from the experiments. One plausible explanation is that, due to ambient fluorescence, the actual floor value in the experiments is slightly lower than what is extracted using our procedure. This has the effect of increasing the apparent $T_1$. On the other hand, there may also be uncertainties in the parameters assigned by Batchelor et al. Table A.2 gives a slightly modified set of parameter values which yield better agreement between the model and experiment in terms of the time intervals.

Figure 5.4: (a) The example image to show pulse interval and quiescent interval. (b) Blue bars show the average duration of p53 quiescent phase ($T_1$) for cells under 1 $\mu$M or 5 $\mu$M etoposide treatment. Green error bars show the standard deviation of $T_1$.

Figure 5.5: Duration of the p53 quiescent phase ($T_1$) against damage level from the 4-component model. The parameters used are given in Table A.2.
5.2.2 Period variation at the same damage level

The experimental time courses also show significant variations in the cycle length under the same experimental condition, from pulse-to-pulse and from cell-to-cell, as mentioned in Chapter 3. Here we present further details. Figure 5.6(a) gives the length of individual cycles from eight selected cells labeled from 1 to 8 during the course of the experiment at 1µM etoposide. The corresponding histogram is shown in Fig 5.6(b). Cycle lengths from 12 cells at 5µM etoposide are collected in Fig 5.7(a) and the histogram in Fig 5.7(b).

![Figure 5.6](image1.png)

(a) The periods of each cell at 1 µM etoposide.

![Figure 5.7](image2.png)

(b) Distribution of the period of p53 pulses at 1 µM etoposide.

Figure 5.6: The periods of each cell at 1 µM etoposide condition are shown here. (a) The data for eight cells studied are presented. The average period of the pulses for each cell is denoted by red cross and the average period of the oscillations for all cells is denoted by red line (5.84 hours). (b) Histogram is used to illustrate the period distribution of all p53 pulses at 1 µM etoposide (68 pulses). Majority of the periods of the cycles are between 4 to 9 hours.
Figure 5.7: (a) The periods of each cell for 5 \( \mu M \) etoposide condition are shown here. Twelve labeled cells are chosen. The average period of the pulses for each cell is denoted by red cross and the average period of the overall pulses is denoted by red line (6.30 hours). (b) Histogram is used to illustrate the period distribution of all p53 pulses at 5 \( \mu M \) etoposide (91 pulses). Majority of the periods of the pulses are between 4 to 9.

The model study of Chapter 4 shows that the pulse duration is mainly affected by three parameters: the delay time \( \tau_m \), the rate of Mdm2 transcription \( \beta_m \) induced by p53, and the rate of p53 ubiquitination \( \alpha_{mpa} \) by Mdm2. In addition, the length of Phase 1, \( T_1 \), is set by the degradation rates \( \alpha_m \) and \( \alpha_{sm} \) of Mdm2. In Figure 5.8 we plot separately \( T_1 \) and the duration of the pulse \( T_p \), defined by the length of the peak region delineated by the vertical dashed lines in Fig 5.4a. It is found that most of the variation in the period comes from \( T_1 \). It can be seen that the relative standard deviation of the \( T_1 \) is higher than that of \( T_p \). This result may imply that the parameters \( \alpha_m \) and \( \alpha_{sm} \) of Mdm2 can fluctuate more, whereas \( \tau_m \), \( \beta_m \) and \( \alpha_{mpa} \) assume similar values in the same cell or across different cells.
5.3 Variation in the Peak Value of Single Cell p53 Oscillation

In this section, we focus on variations in the oscillation amplitude. As briefly shown in Figure 3.10, there are differences in oscillation amplitude under different experimental conditions and also amplitude variations for a single cell or among different cells under the same experimental condition. Since the valley of the oscillation is close to the image background in the experiments and its value is close to 0 most of the time in the model, we simply use peak value instead of amplitude in the analysis. The change in peak value under 1µM and 5µM etoposide concentration is examined. We also characterize in detail variations of peak value for a single cell or different cells under the same condition and discuss their possible origin.

5.3.1 Peak value variation at different damage levels

Figure 5.9 shows the average and standard deviation of the peak value at 1 µM etoposide and 5 µM etoposide conditions, respectively. Although the peak
value has a broad distribution in each case, the average peak value increases significantly as the etoposide concentration increased.

Figure 5.9: The average peak values at different etoposide concentrations. The average peak value at 1 µM etoposide condition is 16.36 with the standard variation 10.69. The average peak value at 5 µM etoposide condition is 23.87 with the standard variation 12.13.

The dependence of the peak value on the damage level in the model is shown in Figure 5.10. When the oscillation occurs, because the production of active p53 is promoted by ATM, the peak value first increases as the damage level increases. However, as the damage level increases further, the fast degradation of Mdm2 by ATM dominates, which stabilises Mdm2 concentration by preventing it from over excited by p53. The system eventually exits from the oscillatory state with weaken feedback and a lowered peak value.
Figure 5.10: Steady or peak value of p53 at different damage levels predicted by the model. Parameters are taken from Table A.1.

5.3.2 Peak value variation at the same damage level

In this section, we analyse variations in the peak value of single cell p53 oscillation at a given experimental condition. Figure 5.11a and 5.12a give the peak values of each cell investigated at 1µM or 5µM etoposide, respectively. Figure 5.11b and 5.12b consider all the oscillation peaks of cells at 1µM or 5µM etoposide, respectively. It can be seen from the figures that the variation is quite significant.
(a) The peak values of each cell at 1 µM etoposide. (b) The peak value distribution of p53 pulses at 1 µM etoposide.

Figure 5.11: (a) The peak values of each cell at 1 µM etoposide are shown here. Eight cells are investigated. The average peak value of the pulses of each cell is denoted by red cross and the average of the overall pulses is denoted by red line (16.3605). (b) Histogram is used to illustrate the peak value distribution of all p53 pulses at 1 µM etoposide (68 pulses).

(a) The peak values of each cell at 5 µM etoposide. (b) The peak value distribution of p53 pulses at 5 µM etoposide.

Figure 5.12: (a) The peak values of each cell at 5 µM etoposide are shown here. Twelve cells are investigated. The average of the pulses of each cell is denoted by red crosses and the average of the overall pulses is denoted by red line (23.8701). (b) Histogram is used to illustrate the peak value distribution of all p53 pulses at 5 µM etoposide condition (91 pulses).

From the model calculation, as we can see from Figure 4.11 and 5.13a, the basal generation rate of p53 ($\beta_p$) is the most important parameter for the peak value. At the same time, $\beta_p$ almost does not affect the period as shown in Figure 4.12. On the other hand, the time delay $\tau$ also significantly influences the peak value, and it also changes the period significantly at the same time as shown in Figure 4.11 and 4.12. Furthermore, higher degradation rate of active
p53 $\alpha_{m_{pa}}$ reduces the peak value as shown in Figure 5.13b.

![Graph a](image1.png) ![Graph b](image2.png)

(a) Relationship between peak values of the oscillation and basal p53 generation rate $\beta_{p}$.

(b) Relationship between peak values of the oscillation and degradation rate of active p53 $\alpha_{m_{pa}}$.

Figure 5.13: Each parameter is varied to generate corresponding oscillation peak value. The figures only show the peak values when the system is in the oscillating state. For very small $\beta_{p}$ and $\alpha_{m_{pa}}$, the model does not have an oscillatory phase. Besides, both the values on horizontal and vertical axes represent the natural logarithm of the fold change. The other parameters used in the above two figures are shown in Table A.1.

### 5.3.3 Correlation between p53 rising speed and peak value

In the discussions above, it has been shown that variations in amplitude is quite a bit more significant than the period. We now examine the shape of individual pulses to gain a better idea about the pulse-to-pulse and cell-to-cell variations.

#### Rising time statistics

The rising time here means the duration between the 15% of the amplitude (peak minus valley) from the valley of p53 pulse and its peak of the same pulse. Experimentally, we discovered that the average rising time increases from 1.94 to 2.23 hours with the elevation of etoposide concentration from 1$\mu$M to 5$\mu$M. The relative standard deviation (RSTD) for each condition is 22% or 16%, respectively. Compared with the RSTD of the peak value (65% or 51%), the variation is less significant. Therefore, we suppose the rising time is more or less the same for each oscillation.
Figure 5.14: These two figures demonstrate the distribution of the rising time under two different conditions. The average rising time at 1 µM etoposide is 1.94 hours and its standard deviation is 0.42 (left side). The average rising time at 5 µM etoposide is 2.23 hours and its standard deviation is 0.37 (right side).

The relationship between rising slope and peak value

Since the p53 rising time is similar for all the oscillations, it should be expected to observe that there is a linear relationship between the rising slope of p53 and its peak value. Here, in the data analysis, the rising slope is defined as the maximum positive difference in the p53 time courses.

From the data analysis, as we can see from Figure 5.15a and Figure 5.15a, there exists a positive correlation between the rising slope of p53 and its peak value. Moreover, in order to give a more intuitive picture, we normalised each pulse by its peak value (Figure 5.16a and Figure 5.17a) and align them according to their peak positions. The largest value of p53 concentration corresponds to 1 and the time point of the largest p53 concentration equals to 0. If there is a perfect linear relationship between the rising slope and the peak value, the curves are expected to collapse into one on this plot as shown in Figure 5.16b and 5.17b. For the real data, due to variations in rising time, it can be seen that the curves do not collapse perfectly onto each other but are still close to each other as shown in Figure 5.16a and 5.17a.
Figure 5.15: Scatter plot of peak value against rising slope at (a) 1 \( \mu M \) etoposide; (b) 5 \( \mu M \) etoposide.

\[
y = 1.9589x + 13.702
\]

\[
y = 1.7303x + 20.533
\]

Figure 5.16: (a) Normalised p53 pulses at 1 \( \mu M \) etoposide. Each pulse is normalised by its peak value. Different colours are used for different cells. (b) Normalised p53 pulse shape from the model when damage level equals to 0.6.

Figure 5.17: (a) Normalised p53 pulses at 5 \( \mu M \) etoposide. Each pulse is normalised by its peak value. Different colours are used for different cells. (b) Model prediction of the pulse shape when damage level equals to 0.7.
5.4 Summary and Discussion

In this Chapter, the experimental data and the model results are compared in some detail. In the experiments, two types of p53 behaviour were observed: oscillation and monotonic induction under relatively low or high etoposide treatment, respectively. The model can reproduce the basic features of experimental data by changing the parameter $D$, which stands for the DNA damage level.

Each p53 oscillation cycle can be decomposed into a quiescent period of length $T_1$ at low p53 concentration, and a pulsing period of length $T_p$. The pulsing period is characterized in addition by the peak value of p53. We have analyzed the statistics of all three quantities from the data gathered at two different etoposide concentrations. It is observed that the mean values of the two time intervals $T_1$ and $T_p$ do not depend significantly on the etoposide dosage, while the mean peak value shows a close to 50% increase. This behavior is qualitatively consistent with the model prediction.

At a given concentration of etoposide, all three quantities introduced above show significant pulse-to-pulse and cell-to-cell variations. However, variations in the peak value are more pronounced than the length of each of the two time intervals, in agreement with previous studies [38]. Interestingly, when we compare the shape of these pulses normalized by their peak value, a reasonable data collapse was seen in each of the two etoposide concentrations, with the one at higher damage arguably better. The normalized shapes are quite similar to what we obtain from the 4-component model, although in the latter case none of its features vary from pulse to pulse when the system has relaxed from its initial state.

The origin of the observed variation in pulse peak value is still puzzling. One plausible explanation is that the actual DNA damage level within the cell nucleus varies with time even under a given experimental condition. Therefore the parameter $D$ in the model is not a constant, but changes with time. At this point we do not have information on the typical amplitude of such variations.
Nevertheless, from Fig 5.10 we see that, depending on where we are on the curve, a 20% variation in the damage level can certainly produce the 50-70% variation in peak value as observed in the experiments. Also note that, as seen in Figs 3.5 and 3.6, there are significant positive correlations between the peak value of successive pulses. This may be taken as evidence for slow time scales associated with the change in DNA damage rate or the rate of repair processes.

Noise within the p53-Mdm2 circuit itself, e.g., noise in the p53 transcription rate $\beta_p$, may also give rise to the observed variations, as suggested in the literature [38]. However, we are not aware of direct experimental evidence to support such a scenario, particularly given the slow yet large fluctuations of the peak value.
Chapter 6

Conclusions

p53 is one of the most important proteins in cancer research. It responds to various intracellular stress signals by dramatically extending its turnover time. The elevated p53 level in the cell nucleus switches on a large number of genes, including its antagonist Mdm2. At moderate levels of double-strand DNA damage, the p53-Mdm2 negative feedback loop yields an oscillatory state where the nuclear p53 concentration varies nearly periodically with time. This pulsation phenomenon is well-known and quantitative models have been proposed to describe the dynamics of the underlying molecular circuit. In particular, the model proposed by Bachelor et al. was shown to agree well with a set of single-cell measurements carried out in Lahav’s group at Harvard.

In this thesis, we have presented a detailed comparison between a modified version of Batchelor et al. model and the single-cell fluorescent p53 imaging data collected recently in Shi’s lab at HKBU. The modified model was necessary in order to silence the ineffective Wip1 protein in the particular cell line used in the experiment. As we have shown, the model correctly reproduces the oscillatory and monotonic induction of p53 levels at moderate and high levels of the DNA damage, respectively. Furthermore, detailed features of the oscillation cycle, including the length of the quiescent period and the shape of the p53 pulse, can be accounted for by the model when the parameters are chosen appropriately.

The main contribution of this work, however, is in the analysis and in-
interpretation of the pulse-to-pulse and cell-to-cell variations of the oscillation cycle. The experimental data show that, after an initial period of about 30 hours, the time interval between successive pulses is fairly regular and, on average, depends only weakly on damage level. On the other hand, the peak height varies significantly from pulse to pulse even for the same cell under a constant experimental condition. Therefore the p53 concentration time course is better described as a sequence of pulses of different strengths at regular time intervals. In some cases, successive pulses have similar strength, giving the appearance of regular oscillation.

Theoretical analysis of the 4-component model also shows weak dependence of the oscillation period on damage level. On the other hand, the peak height varies significantly with the damage level, particularly near the onset and termination of the oscillatory phase. Our approximate analysis shows that the peak value is mainly determined by the p53 flux into the nucleus, which itself is controlled by the rate of p53 phosphorylation in the cytosol upon its translation. Therefore the p53 peak value directly reflects the level of cellular stress via the concentration change of the relevant kinase proteins. Comparison between the model behavior and the experimental data led us to the hypothesis that large variations in the peak value of pulses from the same cell at different times, or from different cells under the same experimental condition, are due to temporal variations in the intracellular stress level. The latter may take place on a time scale of one or more pulses in the experiment. Note also that, according to our model, the peak value reflects accumulated stress over the rising time of the p53 concentration within one cycle, i.e., it is doing integration of damage events during such a time interval.

In future work, it would be desirable to confirm details of the model assumptions through direct measurement of other components of the molecular circuit, in particular the time course of Mdm2 concentration. This will give us more confidence in the model and allow us to introduce amendments if necessary. Together, it will give us a deeper understanding of the p53 as a stress hub and a controller of cell fate.
## Appendix A

### Model parameter table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_p$</td>
<td>Basal production rate of inactive p53</td>
<td>$0.9Cs * h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_{mpi}$</td>
<td>Mdm2-dependent degradation rate of inactive p53</td>
<td>$5h^{-1}$</td>
</tr>
<tr>
<td>$\beta_{sp}$</td>
<td>Saturating production rate of active p53</td>
<td>$10h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_{pi}$</td>
<td>Basal degradation rate of inactive p53</td>
<td>$2h^{-1}$</td>
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<tr>
<td>$\alpha_{mpa}$</td>
<td>Mdm2-dependent degradation rate of active p53</td>
<td>$1.4h^{-1}$</td>
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<tr>
<td>$\beta_{mi}$</td>
<td>Basal production rate of Mdm2</td>
<td>$0.2Cs * h^{-1}$</td>
</tr>
<tr>
<td>$\beta_m$</td>
<td>P53-dependent Mdm2 production rate</td>
<td>$0.9h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_{sm}$</td>
<td>ATM-dependent degradation rate of Mdm2</td>
<td>$0.5Cs^{-1} * h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_{m}$</td>
<td>Basal degradation rate of Mdm2</td>
<td>$1h^{-1}$</td>
</tr>
<tr>
<td>$\beta_s$</td>
<td>Damage dependent of ATM production rate</td>
<td>$10Cs * h^{-1}$</td>
</tr>
<tr>
<td>$\alpha_s$</td>
<td>Basal degradation rate of ATM</td>
<td>$7.5h^{-1}$</td>
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<tr>
<td>$T_s$</td>
<td>ATM concentration for half-maximal active p53 production</td>
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</tr>
<tr>
<td>$n_s$</td>
<td>Hill coefficient of active p53 production by ATM</td>
<td>4</td>
</tr>
<tr>
<td>$T_{ac}$</td>
<td>Active p53 concentration when Mdm2-mediated degradation rate is half-maximal</td>
<td>$0.1Cs$</td>
</tr>
<tr>
<td>$T_{in}$</td>
<td>Inactive p53 concentration when Mdm2-mediated degradation rate is half-maximal</td>
<td>$0.1Cs$</td>
</tr>
<tr>
<td>$p53_{inactive0}$</td>
<td>Initial inactive p53 concentration</td>
<td>$0.1Cs$</td>
</tr>
<tr>
<td>$p53_{active0}$</td>
<td>Initial active p53 concentration</td>
<td>$0Cs$</td>
</tr>
<tr>
<td>$Mdm2_{0}$</td>
<td>Initial Mdm2 concentration</td>
<td>$0.8Cs$</td>
</tr>
<tr>
<td>$ATM_{0}$</td>
<td>Initial ATM concentration</td>
<td>$0Cs$</td>
</tr>
<tr>
<td>$\tau_m$</td>
<td>Time delay in Mdm2 production</td>
<td>$3h$</td>
</tr>
</tbody>
</table>

Table A.1: Parameters used in simulation except otherwise specified.
Note: Cs = simulated concentration units. All kinetic rates are the same as Ref. [25] except $\alpha_{mpi}$ and $\alpha_{mpa}$, due to introduction of the Michaelis-Menten form for Mdm2-mediated p53 degradation.

<table>
<thead>
<tr>
<th>$\beta_p$</th>
<th>$\alpha_{mpi}$</th>
<th>$\beta_{sp}$</th>
<th>$\alpha_{pi}$</th>
<th>$\alpha_{mpa}$</th>
<th>$\beta_{mi}$</th>
<th>$\alpha_{sm}$</th>
<th>$\alpha_m$</th>
<th>$\beta_{s}$</th>
<th>$\alpha_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.5</td>
<td>10</td>
<td>2</td>
<td>0.7</td>
<td>0.2</td>
<td>0.5</td>
<td>1</td>
<td>10</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Table A.2: An alternative parameter set
Note: The numbers are chosen to match the time intervals $T_1$ and $T_p$ obtained in the experiment. Units are the same as Table A.1.
Appendix B

Flow chart of the image program

Figure B.1: Flow chart
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Alex Sigal, Erez Dekel, Talia Yarnitzky, Yuvalal Liron, Paz Polak, Galit
Curriculum Vitae

Academic qualifications of the thesis author, Ms. LI Mengyao:

• Received the degree of Bachelor of Science from Beijing Normal University, June 2012.

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