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Mathematical modelling of core regulatory mechanism in p53 protein that activates apoptotic switch

Ket Hing Chong^a, Sandhya Samarasinghe^b, Don Kulasiri^{c,*}, Jie Zheng^d

^a*Biomedical Informatics Lab, School of Computer Science and Engineering, Nanyang Technological University, 639798, Singapore*

^b*Complex Systems, Big Data and Informatics Initiative (CSBII) and Centre for Advanced Computational Solutions (C-fACS), Lincoln University, Christchurch, New Zealand*

^c*Department of Molecular Biosciences, and Centre for Advanced Computational Solutions (C-fACS), Lincoln University, Christchurch, New Zealand*

^d*School of Information Science and Technology, ShanghaiTech University, Pudong District, Shanghai 201210, China*

Abstract

The p53 protein, a tumour suppressor, is a key player in the DNA damage response. The activation of apoptosis by p53 involves the intrinsic apoptotic pathway to eliminate stressed cells that contain DNA lesions. Recent experiments have found that apoptosis happen in an all-or-none switch like manner (Albeck et al., 2008; Rehm et al., 2002). We focus on modelling the mechanism of p53 activation of apoptosis in response to sustained high DNA double-strand breaks. The aim of the research is to investigate the design principles behind the regulation of p53 activation of apoptotic switch. Building on previous models (Chong *et al.*, 2015; Zhang *et al.*, 2009), we developed a mathematical model that incorporated the molecular interactions in the core regulation of p53 and the apoptosis initiation module involving Puma, Bcl2 and Bax. Activation of Bax was assumed to be an indicator of apoptosis initiation. Chen et al. (2013) suggested that one of the components in the p53 pathway may control a threshold activation of apoptosis. We hypothesized that ATM auto-activation is the component that controls p53 threshold activation of apoptosis with ATM's multi-site autophosphorylation depending on damage intensity. The constructed

*Corresponding author

Email address: don.kulasiri@lincoln.ac.nz (Don Kulasiri)

model demonstrated how molecular interactions and stress signalling molecule ATM's auto-activation of the p53 network dictate cell fate decisions. Our simulation results are qualitatively consistent with the experimental findings of all-or-none activation of apoptosis and predicted overexpression of Bcl2 as a factor in causing malfunction of the apoptotic switch. We present a simplified yet plausible model of molecular mechanism that controls p53 activation of apoptotic switch.

Keywords: p53; apoptosis; mathematical modelling; DNA damage response; apoptotic switch

1. Introduction

P53 is a major tumour suppressor that regulates the balance of cell growth and death, and prevents the growth of tumours (Vousden and Lu, 2002). The well-known key functions of p53 activation include DNA damage repair, cell cycle arrest, senescence (permanent cell cycle arrest) and apoptosis (programmed cell death) (Vousden and Lane, 2007). One question that remains open is: How does p53 activate apoptosis to eliminate stressed cells? The answer to this question is crucial for understanding the mechanism underlying the p53 activation of apoptosis, and investigating possible strategies for p53-based cancer therapy, e.g. activating the p53 cell death induction to kill cancerous cells.

There are two major apoptotic pathways: The first one is the intrinsic pathway where DNA damage or stress signal invokes the mitochondrial outer membrane permeabilization (MOMP), which releases apoptogenic proteins that cause a cascade of caspase activation and destruction of cell; the second one is the extrinsic or death receptor pathway that requires cell surface receptor activation, such as tumour necrosis factor receptor (TNFR), TNF-related apoptosis inducing ligand receptor (TRAILR) and Fas-receptor (FasR), etc. to trigger apoptosis (Dewson and Kluck, 2009). It is known that either pathway alone can activate cell death (Rehm et al., 2002). P53 is a transcription factor that plays important roles in the nucleus of cell in the activation of apoptotic

21 genes for the intrinsic apoptotic pathway (Vousden and Lu, 2002).

22 Notably, malfunction of apoptosis causes cancer and hampers cancer ther-
23 apy (Cory and Adams, 2002) because the control system has been destroyed
24 or deactivated in some way. A major reason for the failure of apoptotic switch
25 in cancer cells is the inactivation of p53 due to p53 mutations in the majori-
26 ty of human tumours which prevents p53 from activating the transcription of
27 target genes (Vogelstein et al., 2000). p53 mutations happen in about 50% of
28 all cancers and the rest involve malfunction of p53 signaling pathway such as
29 over-expression of Mdm2 or inactivation of positive feedback through PTEN (O-
30 livier et al., 2010; Muller and Vousden, 2013; Kozłowska and Puszynski, 2016).
31 Conceptually p53 mutation is analogous to a bus’s faulty mechanical door that
32 cannot be closed even though the bus driver has repeatedly pushed the button
33 for door closure many times; for example, the signal is successfully transmitted,
34 but the mechanism that executes door closure fails to operate properly due to
35 some defect. Malfunction of the p53 pathway is similar to the case where the
36 door works properly but the appropriate signal is not delivered to it. An or-
37 ganism’s survival depends on a highly reliable apoptosis function to monitor its
38 cellular activities and eliminate unwanted or potentially threatening cells.

39 p53 is capable of regulating apoptosis induction by a transcription-dependent
40 pathway, activating target genes such as *Puma* and *Noxa* that can induce apop-
41 tosis (Vousden and Lu, 2002). Here, we propose a mathematical model of p53
42 activation of apoptosis through the intrinsic (or mitochondrial) pathway caused
43 by transactivation of pro-apoptotic genes, particularly *Puma*, a potent activa-
44 tor of apoptosis (Yu and Zhang, 2008). *Puma* is a Bcl-2 homology domain 3
45 only (BH3-only) protein that was found to act as a direct activator of apop-
46 tosis, activating Bax (a multidomain pro-apoptotic protein) directly (Gallenne
47 et al., 2009; Letai, 2009). The activation of Bax leads to MOMP, which allows
48 the apoptogenic proteins such as cytochrome c (Goldstein et al., 2000; Marti-
49 nou et al., 2000), SMAC/DIABLO and AIF to be released from mitochondria,
50 thereby ensuring an all-or-none and irreversible process of apoptosis induction.
51 The meaning of all-or-none apoptosis induction is that when a cell decides to die

52 it is a road with no return; once determined to die it will commit to apoptosis.
 53 Single-cell experiments have shown that the activation of MOMP is rapid and
 54 varies in activation time even in a population of cells that was exposed to the
 55 same death stimulus (Albeck et al., 2008; Rehm et al., 2002).

56 The study of the p53 activation of apoptotic switch has been the focus of
 57 molecular biologists because of its importance to understanding the cell biology
 58 of the disease of cancer, and its potential for leading us to a novel strategy for
 59 better cancer treatment (Yu and Zhang, 2003). In the thirty years of research
 60 on p53, there have been great advancements in our knowledge of p53 regulation
 61 based on its structure and function (Lane et al., 2010), but how p53 and Bcl-
 62 2 proteins induce MOMP-based apoptosis still remains controversial (Chipuk
 63 and Green, 2008; Jiang et al., 2010; Oren, 2003). Furthermore, through a re-
 64 cent experiment using a drug called etoposide as DNA damage stimulus, Chen
 65 *et al.* (2013) discovered different dynamics of p53 that is correlated with cell
 66 fate decisions (Chen et al., 2013). At a high dose of drug, p53 increases to
 67 a high concentration with one and a half p53 pulses and subsequently leads
 68 to apoptosis in 14-39 hours (Chen et al., 2013). Chen *et al.* pointed to the
 69 fact that there must be a controlling factor for the apoptotic switch. The un-
 70 known controlling factor of apoptosis induction motivated us to construct a
 71 mathematical model to investigate this problem. We hypothesised that AT-
 72 M auto-phosphorylation (Bakkenist and Kastan, 2003) or ATM auto-activation
 73 k_{auto} as the controlling factor in the apoptotic switch. Thus, we explore the
 74 dynamics of p53 activation of apoptosis under high DNA damage.

75 1.1. Mathematical modelling of p53 regulation of apoptosis induction

76 p53 regulation of apoptosis is critical for the development and homeostasis
 77 of a multicellular organism. Although molecular cell biologists have gathered
 78 enormous amounts of data about p53 regulation of apoptosis, and much has
 79 been uncovered at the molecular level, the complexity of the regulation involv-
 80 ing feedback loops make it difficult to gain insights by analyzing these biological
 81 networks and diagrams alone. Thus, quantitative dynamical models play an im-

portant role in exploring the mechanism theoretically and generating hypotheses that contribute to new experimental testing and deeper understanding (Ideker et al., 2001). To understand the roles played by p53 in activating apoptosis and cell fate decisions, a few mathematical models have been constructed (Pu et al., 2010; Puszyński et al., 2008; Sun et al., 2009; Tian et al., 2012; Zhang et al., 2007, 2009a,b, 2010). For example, Zhang et al. (2009a) modelled the intrinsic pathway of apoptosis which depends on a threshold stress signal so that when stress signal exceeds a specific threshold, cells commit suicide.

A recent hybrid model combining stochastic and deterministic methods also investigated ATM, p53, Wip1 and NF- κ B function in determining cell fate (Jonak et al., 2016). In their model Wip1 negatively controls ATM and they have defined two thresholds for cell cycle arrest and apoptosis but how they specifically relate to ATM activation is not clear. Their model suggests that Wip1 plays the gatekeeper role in controlling apoptosis. Meanwhile, Hat et al. proposed a three module-model that contains the core module, cell cycle arrest module and apoptotic module in the p53 system to study cell fate decisions (Hat et al., 2016). Their bifurcation analysis suggests the existence of a Neimark-Sacker bifurcation revealing the possibility of a transition from oscillations to higher steady state levels of p53 due to a positive feedback loop through PTEN. Batchelor et al. has proposed a delay differential equations model to study the UV dependent stimulus in activating ATR role in p53 response (Batchelor et al., 2011). Their single cell experiments revealed that UV radiation activates through ATR a dose dependent p53 single pulse with larger dosage of UV resulting in larger amplitude and duration of p53 pulse.

In this paper, a model of p53-induced apoptosis is proposed based on the p53 transcription-dependent pathway as an extension of our previous mathematical model (Chong et al., 2015). Our previous p53 model system contains ATM, p53, Mdm2, MdmX, Wip1 and p21. The p53 core model with p53 auto-regulation has captured the p53 threshold excitable dynamics. Bifurcation analysis reveals a saddle-node combined with Hopf bifurcation diagram suggesting how p53 protein dynamics control cell fate decisions in activating DNA damage repair,

113 cell cycle arrest and apoptosis. Here, we include the *Puma* gene activated by
 114 p53, Bax (apoptosis induction protein) and the inhibition of cell death by the
 115 Bcl2 protein. The model constructed in this paper incorporates the apoptosis
 116 initiation module (i.e., Bax activation by BH3-only protein) proposed by Zhang
 117 *et al.* (2009) with modified parameter values for realistic model simulation in-
 118 stead of dimensionless concentrations as used by Zhang *et al.* (2009a). The
 119 model is constructed to explore the molecular control of apoptosis mediated by
 120 p53 and it must satisfy some of the key features of apoptosis: 1) all-or-none,
 121 switch-like manner; 2) irreversible commitment to cell death; 3) very rapid; and
 122 4) variable timing to MOMP. Chen *et al.* (2013) experimental results suggest
 123 that one component of p53 pathway may control p53 threshold activation of
 124 apoptosis (Kracikova *et al.*, 2013). We hypothesized that ATM auto-activation
 125 or ATM multi-site autophosphorylation may be the component that induce p53
 126 activation of apoptosis in a switch-like manner.

127 p53 pathway has been identified as a target for cancer therapy. For example,
 128 experimental and theoretical studies have shown the ability of small interfer-
 129 ing RNA (siRNA) to reduce Mdm2 protein level (Davis, 2009; Kozłowska and
 130 Puszynski, 2016) or small molecule such as Nutlin binding to Mdm2 to induce
 131 p53 activation (Vassilev *et al.*, 2004; Puszynski *et al.*, 2014). Puszynski *et al.*
 132 (2014) predicted that it is more effective to administer a few large dosages of
 133 Nutlin than low dose-splitting and they attributed this to nonlinear behaviour
 134 of the regulatory system where a p53 threshold needs to be exceeded to in-
 135 duce apoptosis (Puszynski *et al.*, 2014). The understanding of p53 regulation of
 136 apoptosis is crucial for designing new therapies, particularly *p53* (Lane *et al.*,
 137 2010) or *Puma* (Yu *et al.*, 2006) gene therapies that may be more effective in
 138 treating certain cancer patients than current methods.

139 2. Materials and Methods

140 2.1. Model description for the extended model of apoptosis initiation

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

158 A schematic diagram of the model of p53 activation of apoptotic switch is il-
159 lustrated in Fig. 1. In our previous model we constructed a model for explaining
160 the p53 pulsatile dynamics (or oscillations) and excitability property observed
161 in single cell experimental studies (Loewer et al., 2010) and the results suggest
162 how p53 dynamics control cell fate decision (Chong et al., 2015). We did not in-
163 clude the detail mechanism of Puma and Bcl2 regulation of apoptosis. Building
164 on the model proposed in our previous work (Chong et al., 2015), of which the
165 model equations and parameters are summarised in Appendix, the model of p53
166 activation of apoptosis is extended here to incorporate the apoptosis initiation
167 network from Zhang *et al.* (Zhang et al., 2009a) (see the bottom part of Fig. 1).
168 The key differences in our model are that the upstream stress signal is represent-

169 ed by ATM auto-activation (whereas in Zhang *et al.* (Zhang et al., 2009a) model
 170 stress is represented by a constant between 0 and 1) and the down stream stress
 171 response is represented by Puma mRNA. The downstream caspases-based acti-
 172 vation of apoptosis is not modelled as we assume that the commitment to cell
 173 death follows Bax activation through Puma (Dewson and Kluck, 2009). Simpli-
 174 fications were made as Zhang *et al.* (Zhang et al., 2009a): Puma represents the
 175 BH3-only proteins; Bcl2 represents the pro-survival proteins; and Bax represents
 176 the Bax/Bak pro-apoptotic proteins. It is assumed that Puma (as a BH3-only
 177 protein) serves as a direct activator of Bax and inhibitor of pro-survival Bcl2
 178 (refer to lower part of Fig. 1), which is in line with a direct activator model
 179 proposed by Chipuk and Green (Chipuk and Green, 2008). Puma, Bax and Bcl2
 180 are assumed to reside in cytoplasm. Baxm represents the active form of Bax ac-
 181 cumulated at the mitochondrion, which is assumed to be able to initiate MOMP
 182 and apoptosis. Bcl2 that located in the outer mitochondrial membrane (OMM)
 183 binds to Baxm to form complexes Baxm:Bcl2, and thereby Baxm is inactivated
 184 by the Bcl2 binding in non-stressed situations so that apoptosis is restrained.
 185 Bcl2 in the cytoplasm also binds to Puma to form complexes Puma:Bcl2 and
 186 keeps Puma in the non-active form.

187 The cellular localizations of Puma, Bax, Bcl2, Puma:Bcl2, Baxm:Bcl2 and
 188 Baxm are illustrated in Fig. 2. Bax is predominantly cytoplasmic and Baxm
 189 represents the active form of Bax accumulated at the mitochondrion that initi-
 190 ate MOMP and cause cell death. The activation of Bax in the form of Baxm
 191 is assumed to be an indicator of the initiation of apoptosis. Puma and Bcl2
 192 are assumed to be in the cytoplasm. Baxm:Bcl2 and Puma:Bcl2 are the pro-
 193 tein complexes inactivated by the pro-survival protein Bcl2 under non-stressed
 194 conditions. These need to be activated in apoptosis. Based on these assump-
 195 tions, the equations below describe the total concentrations of mitochondrion
 196 Bax (Baxm), Bax, Bcl2 and Puma, respectively (T stands for total).

$$BaxmT = [Baxm] + [Baxm : Bcl2] \quad (1)$$

Figure 1: A schematic diagram of p53 activation of apoptotic switch (apoptosis initiation module in the lower part). For illustration, few model interactions of complex association and dissociation are shown in Fig. 3.

Figure 2: A diagram illustrating the cellular localization of the modelled proteins in the cell.

$$BaxT = [Bax] + [Baxm] + [Baxm : Bcl2] \quad (2)$$

$$Bcl2T = [Bcl2] + [Puma : Bcl2] + [Baxm : Bcl2] \quad (3)$$

$$PumaT = [Puma] + [Puma : Bcl2] \quad (4)$$

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

2.2. Model equations

The model equations are a combination of those from Appendix (19 delay differential equations (DDEs)) and model equations for p53 activation of apoptotic switch (5 DDEs) listed below. We used Hill function with Hill coefficient equals 4 as it is a modelling assumption used in the literature (e.g (Sun et al., 2011) and Chong et al. (2015)). The coefficient of 4 is for p53 activation of a gene as a tetramer.

Equations for the mRNA:

puma: The first term in Equation (5) describes the basal synthesis, the second

term describes gene transcription from P53pp with a time delay $\tau_{11} = 70$ minutes (as a representation of E2F1 activation of ASPP, and subsequent ASPP promotion of the p53 activation of apoptotic genes such as *Puma* (Sullivan and Lu, 2007)) and the third term describes degradation of Puma mRNA.

$$\frac{d[puma]}{dt} = s_{puma} + \frac{e_6[P53pp(t - \tau_{11})]^4}{K_{puma}^4 + [P53pp(t - \tau_{11})]^4} - \delta[puma] \quad (5)$$

Equations for Proteins:

Puma: The first term in Equation (6) describes the basal synthesis of Puma not dependent on p53 as reported in (Wang et al., 2009), the second term describes translation of p53 dependent Puma mRNA and the third term describes basal degradation of PumaT.

$$\frac{d[PumaT]}{dt} = k_{s3} + r_{puma}[puma(t - \tau_{12})] - k_{dPumaT}[PumaT] \quad (6)$$

Puma-Bcl2 complexes: The first term on the right-hand side of Equation (7) describes the association of Puma-Bcl2 complexes, the second term describes the dissociation of Puma-Bcl2 complexes, and the third term describes the basal degradation of Puma-Bcl2.

$$\begin{aligned} \frac{d[Puma : Bcl2]}{dt} &= k_{as3}[Puma][Bcl2] - k_{ds3}[Puma : Bcl2] \\ &\quad - k_{d3}[Puma : Bcl2] \end{aligned} \quad (7)$$

Baxm-Bcl2 complexes: The first term on the right-hand side of Equation (8) describes the association of Baxm-Bcl2 complexes, the second term describes the dissociation of Baxm-Bcl2 complexes, and the third term describes the basal degradation of Baxm:Bcl2.

$$\begin{aligned} \frac{d[Baxm : Bcl2]}{dt} &= k_{asx}[Baxm][Bcl2] - k_{dsx}[Baxm : Bcl2] \\ &\quad - k_{bx}[Baxm : Bcl2] \end{aligned} \quad (8)$$

BaxmT: The first term in Equation (9) describes the auto-activation of Baxm, the second term describes the Puma-induced activation of Baxm, and the third

term describes the basal degradation of BaxmT.

$$\frac{d[BaxmT]}{dt} = k_{fx}[Bax] + k_{fx'}[Bax][Puma] - k_{dbx}[BaxmT] \quad (9)$$

From Equations (1)-(4), we derived the following formulae (use in Equations 7-9):

$$[Bcl2] = Bcl2T - [Puma : Bcl2] - [Baxm : Bcl2] \quad (10)$$

$$[Puma] = [PumaT] - [Puma : Bcl2] \quad (11)$$

$$[Bax] = BaxT - [BaxmT] \quad (12)$$

$$[Baxm] = [BaxmT] - [Baxm : Bcl2] \quad (13)$$

It is assumed that Bcl2T and BaxT are constants with the values: Bcl2T = 0.6 μ M and BaxT = 0.5 μ M.

2.3. Model parameters and initial conditions

The parameters used are those under stressed conditions in the model from Chong et al. (2015) (see Appendix). Two important parameters are the number of DSBs (M) set to 300, which represents sustained high damage, and the ATM auto-activation rate k_{auto} set to 0.4 indicating a high level of ATM signalling. The model parameters for the apoptosis initiation module (from Section 2.2) are estimated using trial-and-error (same approach as Zhang et al. (2009a)) to generate simulations that are consistent with experimental findings of all-or-none activation of apoptosis initiated by Baxm activation (Rehm et al., 2002; Martinou et al., 2000; Dewson and Kluck, 2009). These model parameters and initial conditions are listed in Table 1 and Table 2, respectively.

There are two sets of model parameters. The first set (as in Table 3 and Table 4) is based on the model published in Chong et al. (2015). The model

262 in Chong et al. (2015) is an extension of the model proposed by Sun et al.
 263 (2011) and the parameter values in the former are mostly from Sun et al. The
 264 second set of model parameters is for the apoptotic module. This set (as in Ta-
 265 ble 1 and Table 2) is estimated to capture the all-or-none switch like activation
 266 of Baxm with biologically observed time for apoptosis to happen after DNA
 267 damage is induced. Local parameter sensitivity analyses for all the 98 param-
 268 eters (combined total of two sets of parameters) were performed by increasing
 269 (or decreasing) by 20% the actual parameter value one at a time while holding
 270 the other parameter values at the nominal value. It shows that the parameter
 271 values still produce model simulations where p53 activation leads to triggering
 272 Baxm switch-like activation of apoptosis (see Supplementary Figures for Local
 273 Parameter Sensitivity Analysis and Supplementary Table 1). The local param-
 274 eter sensitivity analyses show that this robust behaviour could be due to the
 275 model structure together with the model parameter values that reveal the p53
 276 all-or-none activation of apoptosis.

277 *2.4. Software used*

278 The mathematical model equations were implemented and analysed using the
 279 software listed below:

- 280 1. XPPAUT software for dynamical systems analysis (from
 281 <http://www.math.pitt.edu/~bard/xpp/xpp.html>)
- 282 2. MATLAB R2012b
- 283 3. MATLAB software package DDE-BIFTOOL for bifurcation analysis
 284 (Engelborghs et al., 2002) (from
 285 <http://twr.cs.kuleuven.be/research/software/delay/ddebiftool.shtml>)

286

Figure 3: A diagram illustrating the association and dissociation of protein complexes.

3. Results and Discussion

3.1. Cell simulation: p53 and Baxm dynamics in the presence of high DNA damage

First, we carried out the simulation of a cell's activation of apoptotic switch under stressed conditions with high DNA damage where the number of double strand breaks M is set to 300. Fig. 4 shows the network dynamics under stressed conditions. After DNA damage, the total p53 pulses and then reaches a high concentration at the end of 25 hours. In the process, p53 induces the expression of Puma which activates Bax as follows: Part of the Puma protein produced binds to Bcl2 forming Puma:Bcl2 inactive complexes. Consequently, Bcl2 (pro-survival) protein expression level decreases sharply and approaches 0 after 3 hours. This low level of Bcl2 (see Fig. 4 olive colour) allows Puma to activate Bax directly and cause Bax accumulation at the mitochondrion (Baxm). Thus, Baxm concentration increases rapidly in a switch-like, all-or-none manner and results in MOMP within 6 hours (Fig. 4 Baxm red curve sharply increases). The characteristic of all-or-none switch-like execution of apoptosis was measured in experiments by cytochrome c releases after MOMP (Goldstein et al., 2000; Martinou et al., 2000) and caspase-3 activation (Rehm et al., 2002; Tyas et al., 2000). In our model, we focus on the all-or-none Baxm activation and initiation of cell death. The death signal from MOMP is executed in an irreversible way in

the downstream events after MOMP, which include cytochrome c release from mitochondrial outer membrane channels, and subsequently causing caspase-3 activation that dismantles the cell.

This simulation result (as shown in Fig. 4) of Puma rapidly inducing apoptosis is consistent with the experimental finding that morphological signs of apoptosis take place within 6-9 hours after Puma expression (Nakano and Vossen, 2001; Yu et al., 2001). In addition, our model correctly recapitulates an experimental observation that the Puma activation of apoptosis is dependent on Bax (Yu and Zhang, 2003).

In the model of Zhang et al. (2009a), the stress signal is represented by a value between 0 and 1 which is held constant for the simulation of different levels of stress. According to their model, there is a saddle-node bifurcation with a threshold value of stress 0.5401; when the stress signal is greater than this threshold value, their model simulated that cell activates the bistable apoptotic switch in an all-or-none manner that triggers irreversible cell death. Instead, we assumed that the upstream stress signal is represented by ATM auto-activation and the downstream stress response can be represented by the abundance of Puma mRNA. This is a more realistic assumption for the DNA damage response inside a cell as Puma gets synthesised after p53 triggers apoptosis induction. With this assumption in our model, can we see a threshold activation of bistable apoptotic switch activated by p53? This is the question we will explore next.

3.2. Bifurcation analysis of p53 and Baxm dynamics in the presence of extrinsic stress

ATM plays critical roles in p53 modulation of the DNA damage response. For instance it detects DNA damage inside a cell and invokes downstream activities of DNA damage response including the phosphorylation of p53 and Mdm2 (Cheng and Chen, 2010). In the case of DNA damage, DNA double-strand breaks in particular are detected by ATM. ATM is normally kept inactive as a dimer when there is no DNA double-strand breaks (Bakkenist and Kastan, 2003). However, when there is DNA double-strand breaks present

Figure 4: p53 dynamics (blue) induces Puma (black dashed line) and leads to the activation of Bax in a switch-like manner (red). Also shown is, how Puma engages Bcl2 (olive) and initiates the activation of Bax. In this simulation, ATM auto-activation rate is set to $k_{auto} = 0.4$. P53T and PumaT represent total p53 and total Puma protein levels, respectively. Baxm represents Bax protein accumulated at the mitochondrion and constant high level of Baxm is an output indicator for cell death.

337 ATM become phosphorylated and the stress signal is amplified by ATM auto-
 338 phosphorylation (Bakkenist and Kastan, 2003). In order to investigate how
 339 stress signal and the corresponding p53 dynamics leads to Bax activation of
 340 MOMP, a bifurcation analysis was done with respect to the parameter of ATM
 341 auto-activation rate, k_{auto} . Fig. 5 shows a bifurcation diagram with a saddle-
 342 node bifurcation that illustrates a threshold value at $k_{auto} = 0.04$. This saddle-
 343 node bifurcation characterizes a bistable apoptotic switch controlled by p53.
 344 That is, for $k_{auto} \leq 0.04$, Baxm stays at the basal low level or “off” state, but
 345 once k_{auto} exceeds this threshold value of 0.04, there is an abrupt increase in
 346 Baxm which causes cell death as indicated in Fig. 5. When the apoptotic switch
 347 is turned “on” in this way, it is assumed to transmit the death signal by causing
 348 MOMP which releases cytochrome c, and subsequently triggers a cascade of
 349 caspases activation.

350 Fig. 5 also shows a Hopf bifurcation, i.e., Baxm oscillates with small fluctu-
 351 ations. Meanwhile Baxm protein is maintained at a high level for k_{auto} greater
 352 than 0.04 (e.g., Baxm is more than $0.1 \mu\text{M}$) and it is assumed to activate apop-
 353 tosis. This activation of apoptosis is indicated by the time-course simulation for
 354 Baxm as shown in Fig. 6. For example, $k_{auto} = 0.1$ (black thin line), $k_{auto} =$
 355 0.2 (blue line) and $k_{auto} = 0.4$ (green line without oscillations) indicate apop-
 356 tosis. With these three k_{auto} scenarios, bifurcation analysis shows that Baxm
 357 turns on when k_{auto} is above the threshold value. This activation of apoptosis
 358 is consistent with one of the key characteristics of apoptosis where a threshold
 359 value is required such that cell is protected from premature activation of apop-
 360 tosis (Zhang et al., 2009a). Experimental evidence suggests that p53 activation
 361 of apoptosis is regulated by a threshold value of p53 protein level (Kracikova
 362 et al., 2013). Our model assumes that the threshold value corresponds to the
 363 upstream stress signal from ATM autophosphorylation (or ATM auto-activation
 364 k_{auto}).

Figure 5: Bifurcation diagram illustrating the bistable switch activated by p53. Solid dots represent stable steady states, the dashed line represents unstable steady states and open circles represent an unstable limit cycle.

Figure 6: Time-course simulations of Baxm level for three values of k_{auto} at 0.1, 0.2 and 0.4. For these values of k_{auto} , Baxm is maintained at a concentration higher than $0.1 \mu\text{M}$ indicating apoptosis induction.

365 3.3. Bax activation switch depends on the ATM auto-activation rate k_{auto}

366 Next we investigate how k_{auto} controls the timing of Bax activation of apop-
 367 totic switch. To demonstrate how stress signal controls Bax activation we simply
 368 changed k_{auto} in the range of 0.041 to 0.4 and did simulation one at a time to
 369 generate Baxm time-course data. The time for Bax activation to “switch on”
 370 apoptosis was recorded. We found that the timing of the switch-like activation
 371 of Baxm depends on the value of k_{auto} : The larger the k_{auto} value, the shorter
 372 time it takes to turn “on” Baxm. For example, Fig. 7 shows that for k_{auto}
 373 = 0.045 (red), the time for Baxm activation (which indicates MOMP) is 17
 374 hours and for $k_{auto} = 0.4$ (green) the time for Baxm activation is just 7 hours.
 375 Based on these data gathered, it can be said that the time to MOMP through
 376 Baxm activation of apoptosis depends on the ATM auto-activation rate, k_{auto} .
 377 There is a possibility of ATM multi-stage activation. Specifically, that larger
 378 k_{auto} in terms of multi-stage ATM activation could be explained by multi-site
 379 autophosphorylation in ATM activation: ATM auto-phosphorylation at Serine
 380 1981 (Bakkenist and Kastan, 2003), Serine 367 and Serine 1893 (Kozlov et al.,
 381 2006). In our model, larger k_{auto} means larger ATM auto-activation or ATM
 382 autophosphorylation. We assume that high stresses cause increased ATM auto-
 383 activation or multi-site autophosphorylation. Future models and experiments
 384 can explore if multi-stage activation supports this kind of instant increase.

385 These simulation results demonstrate that when cells are treated with the
 386 same death stimulus (or agent), the cell death signal in individual cells may
 387 vary as represented by different values of k_{auto} , thus causing variability in the
 388 length of time to MOMP. This feature of variability in apoptosis induction was
 389 experimentally observed in single-cell studies (Rehm et al., 2002). For example,
 390 in Rehm et al. (2002), it was shown that even though the same death stimulus
 391 was given in the form of 10 μ M etoposide, a DNA-damaging agent, some cells
 392 died earlier than others in a time frame ranging from 12 to 15 hours. However,
 393 we can not rule out other possibilities for the observed variability in apoptosis
 394 induction for the same stimuli such as stochastic gene switching (Puszynski
 395 et al., 2014) or noise in protein production rates (Geva-Zatorsky et al., 2006).

Figure 7: Simulations of Bax activation for different values of k_{auto} .

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

To further explore variability in time to apoptosis, a range of simulated time to Baxm activation for k_{auto} values between 0.041 and 0.4 were collected, and these data are plotted in Fig. 8. It shows that for a small value of k_{auto} the time to MOMP (or Baxm activation) is larger, and the larger the value k_{auto} , the shorter the time is to turn on Baxm. This curve may explain the heterogenous responses in a population of cells that activate apoptosis at different rates or time points due to distinct states of individual cells.

After p53 is expressed in a population of cells, there is heterogeneity in the times to apoptosis which was observed in experiments (i.e., once death stimulus is given, some cells died earlier than others). One possible reason for the different times to apoptosis observed in individual cells is the existence of a wide range

Figure 8: Time to Baxm activation of MOMP in p53-induced apoptosis is dependent on the parameter of ATM auto-activation rate, k_{auto} . Simulation results show that the larger the value k_{auto} , the shorter the time is to turn on Baxm.

of stress signals in the cell population corresponding to their states of the cell cycle (Feng et al., 2007). To account for this stress signal variation, we assume that k_{auto} is normally distributed with mean 0.15 and standard deviation of 0.1. A population of 2000 cells is drawn from this distribution and is illustrated by the histogram in Fig. 9. Estimates of times to MOMP for cells based on the values of k_{auto} from Fig. 9 are given in Fig. 10. It also shows that the interpolated time estimates for the 2000 cells fit well to our original simulation (Fig. 8). We can use this information to find the percentage of apoptotic cells in the population at any given time as shown in Fig. 11. It shows that after 5 hours some cells start to commit suicide and the percentage increases very quickly until it reaches 90% after 50 hours. Fig. 11 has captured similar results as the experimental observations that, when p53 was expressed, approximately 90% of cells die through apoptosis after 48 hours (Yu et al., 2001). This result indicates that apoptosis is a fast acting process where 80% dies off rapidly as in experimental observations (Tyas et al., 2000) and also suggests that ATM auto-activation k_{auto} is the determining factor in the timing of the decision to

Figure 9: Histogram of the k_{auto} distribution drawn from a population of 2000 cells (y-axis represents the number of cells).

429 commit to cell death. The role of intrinsic noise as in our model simulation of
 430 variability of ATM auto-activation rate may be important for cell fate decisions;
 431 e.g., Puszynski et al. (2016) has studied the role of gene switching in cell-
 432 response-curves.

433 3.4. P53 protein dynamics when a cell dies

434 The next question we asked was: What is p53 protein dynamics when a cell
 435 dies after induced by a DNA damage agent? The answer to this question can
 436 be found in our computer simulation whereby we observe simulated p53 protein
 437 levels until the time to MOMP. For example, Fig. 12 shows the simulation of
 438 three cells, each corresponding to one k_{auto} value (0.06, 0.043 and 0.041). The
 439 simulation results indicate that apoptosis can occur one and a half pulses after
 440 p53 activation. Recently, there was one study that used U-2 OS and A549 cell
 441 lines in single-cell analysis and measured p53 protein dynamics in individual
 442 cells until apoptosis was observed (Chen et al., 2013). Their data show that the
 443 p53 protein increases monotonically until apoptosis happens and one cell shows
 444 one and a half pulses of p53. The times to apoptosis for the three cells from

Figure 10: The estimation of the times to MOMP (black dots) for the k_{auto} values in Fig. 9. Blue circles are the time to Baxm activation of MOMP as in Fig. 8.

Figure 11: The percentage of apoptotic cells in a population of 2000 cells based on estimation of times to MOMP for varying k_{auto} values as given in Fig. 10.

Figure 12: Simulation of p53 protein levels in three individual cells until the occurrence of MOMP for three different k_{auto} values ($k_{auto} = 0.06, 0.043$ and 0.041).

445 their data are 15, 19 and 39 hours (in Chen et al. (2013)); they compare well
 446 with our simulation results with the times to MOMP of 12, 22 and 38 hours
 447 (Fig. 12). Thus, our simulation has captured some of the qualitative behaviour
 448 in the recent experimental findings of Chen et al. (2013).

449 3.5. Cancer cells with Bcl2 overexpression - Simulated p53 and Baxm dynamics 450 in the presence of severe stress

451 The overexpression of Bcl2 (a pro-survival protein) in cancer, impairing
 452 apoptosis, is a key factor in resistance to chemotherapy (Adams and Cory,
 453 2007). To investigate the 50-70% of solid organ malignancies with Bcl2 overex-
 454 pression (Fahy et al., 2005), we simulate this condition by setting a higher level
 455 of total Bcl2 with $Bcl2T = 1 \mu M$ (66.7% increase from $0.6 \mu M$). Fig. 13 shows
 456 the simulation results for Bcl2 overexpression. The difference in cancer cells
 457 compared to normal cells is that the Bcl2 level does not approach 0 and this
 458 result shows that Puma (BH3-only protein) can not neutralise Bcl2 completely.
 459 As a result, our simulation demonstrates that the Bax activation switch fails
 460 to be activated in cancer cells as indicated by the basal level of Baxm (red)

Figure 13: Overexpression of Bcl-2 protein prevents Bax activation as shown by the low level of Baxm (red line) throughout the simulation. In this simulation, ATM auto-activation rate is set to $k_{auto} = 0.4$.

461 throughout the simulation period of 25 hours shown in Fig. 13. This simulation
 462 shows that the p53-induced apoptosis can fail even when it can still activate
 463 Puma.

464 The breast cancer MCF7 cell line used in the study of single-cell dynam-
 465 ics of p53 was shown to have a high level of Bcl2 (Fahy et al., 2005) and this
 466 overexpression of Bcl2 could explain why some cells experienced repeated pulses
 467 of p53 for days after DNA damage by gamma irradiation, even for high gam-
 468 ma irradiation of 10 Gy (Geva-Zatorsky et al., 2006). Over 50% of colorectal,
 469 prostate, pancreas, breast, and lung cancers that account for major incidences
 470 of death by cancer are associated with Bcl2 overexpression (Fahy et al., 2005)
 471 and thus inactivating p53 function. Our model simulations are consistent with
 472 these observations which suggest that cancer cells employ some mechanisms to
 473 overexpress Bcl2 and thereby evade apoptosis (Adams and Cory, 2007).

Figure 14: Overexpression of Bcl2 protein in cancer cells preventing apoptosis can be avoided by increasing p53-dependent Puma expression that reactivates Bax (apoptotic switch). In this simulation, ATM auto-activation rate is set to $k_{auto} = 0.4$.

3.6. Prediction of pharmacological intervention for Puma gene therapy in cancer cells overexpressing Bcl2

In order to find pharmacological means to sensitise cell death in cancer cells that overexpress Bcl2 protein, we set p53-dependent *Puma* transcription rate, e_6 , to a higher value of 0.036 (100% increase from 0.0179) thereby mimicking the interventions such as *Puma* gene therapy that increases Puma expression, to see if this strategy can reactivate the apoptotic switch. The simulation results are shown in Fig. 14. With the *Puma* transcription rate doubled, the Puma protein level increases to a higher value ($1.2 \mu\text{M}$) and Baxm is activated. This result is in line with the pharmacological intervention of *Puma* gene therapy in activating apoptosis in cancer cells and other BH3 mimetic drugs such as ABT-737 to perform the same function as Puma (BH3-only protein), which has shown very promising pharmacological effects (Wang et al., 2012; Adams and Cory, 2007).

488 4. Conclusions

489 The study by Chen et al. (2013) reported novel observations on p53 activa-
490 tion of apoptosis under high DNA damage where p53 increases to a high level
491 and induced apoptosis. They suggested that one of the components in the p53
492 pathway may control a threshold activation of apoptosis which requires theo-
493 retical investigation and analysis. In this paper, we proposed a mathematical
494 model of the core regulatory feedback mechanism that regulates p53 activation
495 of apoptosis. Using this model we investigated the design principles behind the
496 regulation of p53-activation of apoptotic switch. To this end, we incorporat-
497 ed the molecular interactions in the core regulation of p53 and the apoptosis
498 initiation module involving Puma, Bcl2 and Bax. The activation of Bax (in
499 the form of Baxm) is assumed to be an indicator of the initiation of apopto-
500 sis. The constructed model demonstrated how molecular interactions and the
501 auto-activation of the stress signalling molecule ATM in the p53 network dictate
502 cell fate decisions. The model proposed provides a quantitative explanation of
503 all-or-none apoptosis activation and suggests ATM auto-activation as the com-
504 ponent that controls the threshold of p53 activation of apoptosis. Our model
505 analysis shows that there is a signal threshold for the activation of apoptotic
506 switch controlled by ATM signal transduction. This apoptotic signal activa-
507 tion controls p53 protein level and induces cell death once the stress signal
508 (ATM auto-phosphorylation) has exceeded a critical value. The k_{auto} controls
509 the switch through the activation of p53 that regulates *Puma* that activates
510 the switch. Most importantly, our simulation results are qualitatively consis-
511 tent with the experimental findings of an all-or-none activation of apoptosis
512 and predicted the overexpression of Bcl2 as a factor in causing malfunction of
513 the apoptotic switch. Our model simulation also predicted that increasing the
514 Puma transcription rate can induce cell death in cancer cells in the case of Bcl2
515 overexpression.

516 In our study we focus on the scenario of high DNA damage and we assume
517 that for high DNA damage, there is no DNA repair and the system directly

invokes apoptosis. Recently, (Kozłowska and Puszynski, 2016) found a positive feedback in the p53-Mdm2 module (through PTEN, PIP2 and Akt) that makes apoptosis irreversible even in the case of attempted DNA repair - which also points to the fact that high damage essentially invokes apoptosis regardless of repair attempts. Therefore, our use of constant stress signal is justified as a simplification. The above authors have also found that without PTEN feedback system in the model, if the stress signal is made to go down as in repair, then cells do not commit apoptosis. Therefore, if DNA repair is actually attempted by a cell with highly damaged DNA as reported by these authors, our constant stress signal can be assumed to cover for the effect of attempted DNA repair and the PTEN positive feedback system. Future studies can explore DNA repair and PTEN feedback system together with potential ATM auto-activation to assess how the system activates p53 responses and what changes would ensue. In conclusion, we present a simplified yet plausible model of molecular mechanisms that control p53 activation of apoptotic switch. The model gives insight into the mechanism underlying p53 regulation of apoptosis and prediction of pharmacological interventions for p53-based therapy.

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Appendix. Model equations from (Chong et al., 2015)

We assume that the kinetics of gene expression up-regulated by p53 is represented by a Hill function with Hill coefficient 4. For example, the mRNA synthesis rate $f(x)$ based on p53 protein level (x) is given by:

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

540

541 where K denotes the Michaelis constant of p53-dependent mRNA transcription
 542 and e_i represents p53-dependent transcription rate. Most of the ODEs formu-
 543 lated in the following describe the rates of reactions for production, degradation,
 544 association, dissociation, activation and inhibition based on mass action kinetics.

545

The equations of the model in our previous work (Chong et al., 2015) are given below:

Equations for mRNAs:

$$\begin{aligned} \frac{d[p53]}{dt} = & s_{p53} + e_5 \frac{[P53p(t - \tau_5) + P53pp(t - \tau_5)]^4}{K_{p53}^4 + [P53p(t - \tau_5) + P53pp(t - \tau_5)]^4} \\ & - \delta_{p53}[p53] \end{aligned} \quad (A.1)$$

$$\frac{d[p21]}{dt} = e_4 \frac{[P53a(t - \tau_4)]^4}{K_{p21}^4 + [P53a(t - \tau_4)]^4} - \delta_{p21}[p21] \quad (A.2)$$

$$\begin{aligned} \frac{d[mdm2]}{dt} = & s_{mdm2} + e_1 \frac{[P53p(t - \tau_1) + P53pp(t - \tau_1)]^4}{K_m^4 + [P53p(t - \tau_1) + P53pp(t - \tau_1)]^4} \\ & - \delta_{mdm2}[mdm2] \end{aligned} \quad (A.3)$$

$$\begin{aligned} \frac{d[mdmx]}{dt} = & s_{mdmx} + e_3 \frac{[P53p(t - \tau_3) + P53pp(t - \tau_3)]^4}{K_x^4 + [P53p(t - \tau_3) + P53pp(t - \tau_3)]^4} \\ & - \delta_{mdmx}[mdmx] \end{aligned} \quad (A.4)$$

$$\frac{d[wip1]}{dt} = s_{wip1} + e_2 \frac{[P53p(t - \tau_2) + P53pp(t - \tau_2)]^4}{K_w^4 + [P53p(t - \tau_2) + P53pp(t - \tau_2)]^4}$$

$$-\delta_{wip1}[wip1] \quad (A.5)$$

Equations for Proteins:

$$\begin{aligned} \frac{d[ATMp]}{dt} = & k_{DSB} \frac{M}{M + K_{DSB}} [ATM] + k_{auto}[ATMp][ATM] \\ & - k_{basal}[ATMp] - k_{wip4}[Wip1][ATMp] \end{aligned} \quad (A.6)$$

$$\begin{aligned} \frac{d[P53]}{dt} = & r_{p53}[p53(t - \tau_6)] - \mu_{p53}[P53] - k_1[C1] - k_2[P53][C2] \\ & - k_{atm1}[ATMp][P53] + k_{wip1}[P53p][Wip1] \\ & - k_{f1}[Mdm2][P53] + k_{b1}[C1] - k_{f3}[Mdmx][P53] \\ & + k_{b3}[C3] \end{aligned} \quad (A.7)$$

$$\begin{aligned} \frac{d[P53p]}{dt} = & k_{atm1}[ATMp][P53] - k_{wip1}[P53p][Wip1] - k_{phos}[P53p] \\ & + k_{dephos}[P53pp] - \mu_{p53p}[P53p] \end{aligned} \quad (A.8)$$

$$\frac{d[P53pp]}{dt} = k_{phos}[P53p] - k_{dephos}[P53pp] - \mu_{p53pp}[P53pp] \quad (A.9)$$

$$\begin{aligned} \frac{d[P53a]}{dt} = & V_{max1} \frac{[P53]}{[P53] + K_{m1} + K_{m1} \frac{[Mdm2]}{K_{i1}}} \\ & + V_{max2} \frac{[P53]}{[P53] + K_{m2} + K_{m2} \frac{[MdmX]}{K_{i2}}} - \mu_{p53a}[P53a] \end{aligned} \quad (A.10)$$

$$\frac{d[P21]}{dt} = r_{p21}[p21(t - \tau_7)] - \mu_{p21}[P21] - k_{d21}[C2][P21] \quad (A.11)$$

$$\begin{aligned} \frac{d[Mdm2]}{dt} = & r_{mdm2}[mdm2(t - \tau_8)] - \mu_{mdm2}[Mdm2] \\ & - k_{atm2}[ATMp][Mdm2] - k_{f1}[Mdm2][P53] + k_{b1}[C1] \\ & - k_{f2}[Mdm2][MdmX] + k_{b2}[C2] + k_{wip2}[Mdm2p][Wip1] \\ & - k_{d22} \frac{M}{J + M}[Mdm2] - k_4[Mdm2p][Mdm2] \end{aligned} \quad (A.12)$$

$$\begin{aligned} \frac{d[Mdm2p]}{dt} = & k_{atm2}[ATMp][Mdm2] - k_{wip2}[Mdm2p][Wip1] \\ & - \mu_{mdm2p}[Mdm2p] \end{aligned} \quad (A.13)$$

$$\begin{aligned} \frac{d[MdmX]}{dt} = & r_{mdmx}[mdmx(t - \tau_9)] - \mu_{mdmx}[MdmX] \\ & - k_{atm3}[ATMp][MdmX] + k_{wip3}[MdmXp][Wip1] \\ & - k_{f2}[Mdm2][MdmX] + k_{b2}[C2] - k_{f3}[MdmX][P53] \\ & + k_{b3}[C3] \end{aligned} \quad (A.14)$$

$$\begin{aligned} \frac{d[MdmXp]}{dt} = & k_{atm3}[ATMp][MdmX] - k_{wip3}[MdmXp][Wip1] \\ & - k_3[MdmXp][Mdm2p] - \mu_{mdmXp}[MdmXp] \end{aligned} \quad (A.15)$$

$$\frac{d[Win1]}{dt} = r_{win1}[win1(t - \tau_{10})] - \mu_{win1}[Win1] \quad (A.16)$$

$$\frac{d[C1]}{dt} = k_{f1}[Mdm2][P53] - k_{b1}[C1] \quad (A.17)$$

$$\frac{d[C2]}{dt} = k_{f2}[Mdm2][MdmX] - k_{b2}[C2] \quad (A.18)$$

$$\frac{d[C3]}{dt} = k_{f3}[MdmX][P53] - k_{b3}[C3] \quad (A.19)$$

The ATM level is assumed constant at 1 μ M . Thus, the concentration for ATM is given by:

$$[ATM] = 1 - [ATMp]$$

546 Note: Additional model assumptions as in Chong et al. (2015) are listed
547 below

- 548 1. Mdm2 protein (associates with p53 and forms complex C1) and het-
549 erodimer C2 promotes P53 protein degradation.
- 550 2. P21 protein degradation is mediated by heterodimer C2.
- 551 3. DSBs directly induce Mdm2 protein degradation.
- 552 4. Mdm2p promotes auto-ubiquitination and degradation of Mdm2.

553 *Table 3 and Table 4 are model parameters as in (Chong et al., 2015)*

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Table 1: Model parameters

Parameter	Meaning	Value	Unit
k_{as3}	Puma-Bcl2 complex association rate	33	$\mu M^{-1} min^{-1}$
k_{ds3}	Puma-Bcl2 complex dissociation rate	0.0264	min^{-1}
k_{d3}	Basal degradation rate of Puma-Bcl2	0.005	min^{-1}
k_{asx}	Baxm-Bcl2 complex association rate	297	$\mu M^{-1} min^{-1}$
k_{dsx}	Baxm-Bcl2 complex dissociation rate	0.07	min^{-1}
k_{bx}	Basal degradation rate of Baxm-Bcl2	0.2	min^{-1}
k_{fx}	Bax auto-activation rate	1	min^{-1}
$k_{fx'}$	Puma-dependent activation of Bax	3	$\mu M^{-1} min^{-1}$
BaxT	Total Bax	0.5	μM
Bcl2T	Total Bcl2	0.6	μM
e_6	P53-dependent Puma transcription rate	0.0179	$\mu M min^{-1}$
K_{puma}	Michaelis constant of p53-dependent Puma transcription	0.0132	μM
δ_{puma}	Degradation rate of Puma mRNA	0.0235	min^{-1}
r_{puma}	Translation rate of Puma	0.0245	min^{-1}
k_{dbx}	Degradation rate of Baxm	0.7081	min^{-1}
K_{dPumaT}	Degradation rate of PumaT	0.0331	min^{-1}
s_{puma}	Basal production rate of Puma mRNA	0.002	$\mu M min^{-1}$
k_{s3}	Basal translation rate of Puma	0.0001	$\mu M min^{-1}$
τ_{11}	Puma transcription delay	70	min
τ_{12}	Puma translation delay	10	min

Table 2: Initial conditions used in the model

Molecular species	Meaning	Value
puma	Puma mRNA	0
Puma:Bcl2	Puma:Bcl2 complexes	0.005
Baxm:Bcl2	Baxm:Bcl2 complexes	0.02
PumaT	Total Puma protein	0.006
BaxmT	Total Bax protein accumulated at the mitochondrial	0.033

Table 3: Initial conditions used in (Chong et al., 2015)

Molecular species	Meaning	Value
p53	P53 mRNA	0.05
p21	P21 mRNA	0.05
mdm2	Mdm2 mRNA	0.05
mdmx	MdmX mRNA	0.01
wip1	Wip1 mRNA	0.04
ATMp	Phosphorylated ATM	0
P53	P53 protein	0.0258
P53p	Phosphorylated P53 protein	0
P53pp	Multiple Phosphorylated P53 protein	0
P53a	Acetylated P53 protein	0.01
P21	P21 protein	0.01
Mdm2	Mdm2 protein	0.15
Mdm2p	Phosphorylated Mdm2 protein	0.0178
MdmX	MdmX protein	0.08
MdmXp	Phosphorylated MdmX protein	0.01
Wip1	Wip1 protein	0
C1	P53-Mdm2 protein complex	0.06
C2	Mdm2-MdmX protein complex	0.05
C3	P53-MdmX protein complex	0.05

Table 4: Model parameters used in (Chong et al., 2015)

Parameter	Meaning	Value	Unit
τ_1	mdm2 transcription delay	30	min
τ_2	wip1 transcription delay	30	min
τ_3	mdmx transcription delay	30	min
τ_4	p21 transcription delay	30	min
τ_5	p53 transcription delay	30	min
τ_6	P53 translation delay	10	min
τ_7	P21 translation delay	10	min
τ_8	Mdm2 translation delay	10	min
τ_9	MdmX translation delay	10	min
τ_{10}	Wip1 translation delay	10	min

Table 4: Model parameters used in (Chong et al., 2015) (continue)

Parameter	Meaning	Value	Unit
s_{p53}	Basal production rate of P53 mRNA	0.0005	$\mu M \min^{-1}$
s_{mdm2}	Basal production rate of Mdm2 mRNA	0.002	$\mu M \min^{-1}$
s_{mdmx}	Basal production rate of MdmX mRNA	0.001	$\mu M \min^{-1}$
s_{wip1}	Basal production rate of Wip1 mRNA	0.002	$\mu M \min^{-1}$
e_1	P53-dependent mdm2 transcription rate	0.02	$\mu M \min^{-1}$
e_2	P53-dependent Wip1 transcription rate	0.014	$\mu M \min^{-1}$
e_3	P53-dependent mdmx transcription rate	0.005	$\mu M \min^{-1}$
e_4	P53-dependent p21 transcription rate	0.015	$\mu M \min^{-1}$
e_5	P53-dependent p53 transcription rate	0.02	$\mu M \min^{-1}$
K_{p53}	Michaelis constant of p53-dependent p53 transcription	0.017	μM
K_{p21}	Michaelis constant of p53-dependent p21 transcription	0.017	μM
K_m	Michaelis constant of p53-dependent mdm2 transcription	0.16	μM
K_x	Michaelis constant of p53-dependent mdmx transcription	1.5	μM
K_w	Michaelis constant of p53-dependent Wip1 transcription	0.2	μM
δ_{p53}	Degradation rate of P53 mRNA	0.03	$\min^{-1}$
δ_{p21}	Degradation rate of P21 mRNA	0.04	$\min^{-1}$
δ_{mdm2}	Degradation rate of Mdm2 mRNA	0.05	$\min^{-1}$
δ_{mdmx}	Degradation rate of MdmX mRNA	0.03	$\min^{-1}$
δ_{wip1}	Degradation rate of Wip1 mRNA	0.05	$\min^{-1}$
r_{p53}	Translation rate of P53	0.01	$\min^{-1}$
r_{p21}	Translation rate of P21	0.02	$\min^{-1}$
r_{mdm2}	Translation rate of Mdm2	0.04	$\min^{-1}$
r_{mdmx}	Translation rate of MdmX	0.01	$\min^{-1}$
r_{wip1}	Translation rate of Wip1	0.02	$\min^{-1}$
μ_{p53}	Basal degradation rate of P53	0.03	$\min^{-1}$
μ_{p53p}	Basal degradation rate of P53p	0.01	$\min^{-1}$
μ_{p53pp}	Basal degradation rate of P53pp	0.004	$\min^{-1}$
μ_{p53a}	Basal degradation rate of P53a	0.001	$\min^{-1}$
μ_{p21}	Basal degradation rate of P21	0.03	$\min^{-1}$
μ_{mdm2}	Basal degradation rate of Mdm2	0.033	$\min^{-1}$
μ_{mdmx}	Basal degradation rate of MdmX	0.03	$\min^{-1}$

Table 4: Model parameters used in (Chong et al., 2015) (continue)

Parameter	Meaning	Value	Unit
μ_{wip1}	Basal degradation rate of Wip1	0.035	min^{-1}
μ_{mdm2p}	Basal degradation rate of Mdm2p	0.1	min^{-1}
μ_{mdmxp}	Basal degradation rate of MdmXp	0.2	min^{-1}
k_1	Mdm2-dependent P53 degradation through C1	0.2	min^{-1}
k_2	C2 (Mdm2-MdmX)-dependent P53 degradation	0.01	$\mu M^{-1} min^{-1}$
k_3	Mdm2-dependent MdmX degradation	1.5	$\mu M^{-1} min^{-1}$
k_4	Mdm2-dependent Mdm2 degradation (auto-ubiquitination)	0.1	$\mu M^{-1} min^{-1}$
k_{atm1}	ATM induced P53 phosphorylation	0.8	$\mu M^{-1} min^{-1}$
k_{atm2}	ATM induced Mdm2 phosphorylation	0.02	$\mu M^{-1} min^{-1}$
k_{atm3}	ATM induced MdmX phosphorylation	0.02	$\mu M^{-1} min^{-1}$
k_{wip1}	Wip1 induced P53p dephosphorylation	1.3	$\mu M^{-1} min^{-1}$
k_{wip2}	Wip1 induced Mdm2p dephosphorylation	0.5	$\mu M^{-1} min^{-1}$
k_{wip3}	Wip1 induced MdmXp dephosphorylation	0.2	$\mu M^{-1} min^{-1}$
k_{wip4}	Wip1 induced ATMp dephosphorylation	1.5	$\mu M^{-1} min^{-1}$
k_{DSB}	DSB induced ATM activation rate	0.0005	min^{-1}
M	The number of DSBs (300 approximately 10 Gy γ -irradiation)	300	Unit of 1
K_{DSB}	Activation scaling parameter	200	Unit of 1
k_{auto}	ATM auto-activation rate	0.07	$\mu M^{-1} min^{-1}$
k_{basal}	ATMp basal inactivation rate	0.02	min^{-1}
k_{f1}	P53-Mdm2 complex association rate	552	$\mu M^{-1} min^{-1}$
k_{f2}	Mdm2-MdmX complex association rate	600	$\mu M^{-1} min^{-1}$
k_{f3}	P53-MdmX complex association rate	552	$\mu M^{-1} min^{-1}$
k_{b1}	P53-Mdm2 complex dissociation rate	123.6	min^{-1}
k_{b2}	Mdm2-MdmX complex dissociation rate	18	min^{-1}
k_{b3}	P53-MdmX complex dissociation rate	123.6	min^{-1}
k_{phos}	P53 further phosphorylation by other enzymes (e.g. Chk2)	0.3	min^{-1}
k_{dephos}	P53 further dephosphorylation by other enzymes (e.g. PP2A)	0.05	min^{-1}
V_{max1}	The maximal rate	0.0001	$\mu M min^{-1}$
V_{max2}	The maximal rate	0.001	$\mu M min^{-1}$
K_{m1}	The half-saturation constant	0.000025	μM
K_{m2}	The half-saturation constant	0.5	μM

Table 4: Model parameters used in (Chong et al., 2015) (continue)

Parameter	Meaning	Value	Unit
K_{i1}	The dissociation constant (e.g. p300-Mdm2)	0.00001	μM
K_{i2}	The dissociation constant (e.g. p300-MdmX)	0.0001	μM
k_{d21}	C2 induced P21 degradation	0.5	$\mu M^{-1} min^{-1}$
J	Degradation scaling parameter	0.2	Unit of 1
k_{d22}	DSB induced Mdm2 degradation	0.01	min^{-1}