**Development of a Computational Systems Biology
Approach to Evaluate Hypotheses of Molecular
and Cellular Mechanisms of Adaptive Response to
Low Dose Ionizing Radiation**

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1.0 INTRODUCTION

Ionizing radiation (IR) causes DNA damage that, if not adequately repaired, results in pro-carcinogenic mutations and eventually in cancer (Little, 2003). Low dose (usually refer to less than 0.2Gy) IR exposure is particularly important for human health risk assessment because environmental and occupational exposure to IR lies in this region. To estimate IR risk in low dose region, a linear no threshold (LNT) dose-response model for IR-induced cancer is assumed as a default for low dose IR extrapolation (Upton, 2002). The LNT dose response suggests that the biological effect of IR is proportional to dose even at very low dose. However, research results at low dose IR regions pose challenges to the default linear dose-response assumption. These studies include bystander effects, adaptive responses and low dose hypersensitivity (Bonner, 2004; Mothersill and Seymour, 2004a; Mothersill and Seymour, 2004b). In the adaptive response, for example, the cellular transformation frequency in the low dose region is reduced relative to controls (Azzam et al., 1996; Redpath and Antoniono, 1998; Redpath, Liang et al., 2001). LNT model is used because sound, quantitative, mechanism based computational models are lacking.

Recent reviews emphasize the new opportunities in mechanism based dose response modeling afforded by developments in computational systems biology (Andersen et al., 2005a; Andersen et al., 2005b; Slikker et al., 2005). Systems biology is defined as a comprehensive quantitative analysis of the manner in which all the components of a biology system interact functionally over time (Aderem, 2005). The components can be molecules, signaling networks, organelles, cells, organisms and populations. Putting toxicology in a systems biology context involves the study of perturbations by environmental stressors on the gene and protein expressions and linking these perturbations to toxicological outcome. The incorporation of computational systems biology in dose response modeling requires modular description of the functional interactions of normal biology system and the perturbation of the normal biology by stressors (Andersen et al., 2005b). Incorporation of computational systems biology approach into IR dose response modeling will assist identification and evaluation of the mechanisms for low dose IR specific phenomena, such as adaptive response.

This report describes the development of a computational systems biology approach to evaluate the hypotheses of molecular and cellular mechanisms of adaptive response to low dose ionizing radiation. Our concept is that computational models of signaling pathways can be developed and linked to biologically based dose response models to evaluate the underlying molecular mechanisms which lead to adaptive response. For development of quantitatively accurate, predictive models, it will be necessary to describe tissues consisting of multiple cell types where the different types each contribute in their own way to the overall function of the tissue. Such a model will probably need to incorporate not only cell type-specific data but also spatial information on the architecture of the tissue and on intercellular signaling. The scope of the current model is, however, more limited. Data obtained in a number of different biological systems are synthesized to describe a chimeric, “average” population cell. Biochemical signaling pathways involved in sensing of DNA damage and in the activation of cell cycle checkpoint controls and the apoptotic pathway are described. As with any computational modeling effort, it is necessary to develop such initial descriptions (models) that can be iteratively refined. Our preliminary model thus defines a starting point which, with time, can evolve to a level of refinement where large amounts of detailed biological information are synthesized and a capability for robust predictions of dose- and time-response behaviors is obtained.

For IR-induced DNA damage, checkpoint arrest and apoptosis serve, among other things, as defensive responses. These responses lead ultimately to a binary decision - cell death or cell survival (Fei et al). Both responses contribute to bystander effects (Wang et al, 2004; Azzam et al, 2000) and may also be involved in nonmonotonic dose-response (Conolly and Lutz, 2004). Moreover, checkpoint arrest and apoptosis have significant implications for cancer dose response. Checkpoint-induced delays in the cell cycle increase the cell generation time and thus decrease the cell proliferation rate, which is an essential parameter in clonal growth models of tumor incidence (Moolgavkar and Knudson, 1981). Cell loss due to apoptosis leads to compensatory regenerative proliferation, which is a risk factor for cancer (Tan et al, 2003). The signaling networks that mediate these responses are sufficiently complex that computational models based on systems biology approach are useful (and perhaps even essential) adjuncts to laboratory

studies of pathway structure and dynamic behavior (Bhalla et al, 2002; Zu et al, 2003; Novak et al, 2001). Our modeling work includes the description of signaling pathway for IR induced checkpoint arrest and apoptosis. As checkpoint arrest instead of apoptosis plays a major role in low dose region, our preliminary results focus on the identification of how checkpoint arrest influences the shape of dose response in that region.

The signaling pathways leading from sensing DNA damage to the cell cycle checkpoints and to the apoptotic pathway are redundant (van Vugt et al, 2004; Levrero et al, 2000). Redundancy is achieved by having parallel pathways linked to common downstream endpoints such as checkpoint controls and switching into apoptosis (Tortora et al, 2004; Rosell et al, 2003). Redundancy has significant implications for cancer dose-response, since individuals with non-redundant signaling between DNA damage, cell cycle checkpoints and apoptosis would be susceptible to cancer, while individuals with redundant signaling would be less susceptible. We developed a series of models with increasing degrees of pathway redundancy in accord with our longer-range goal of obtaining insights into the molecular basis of dose-responses for IR-induced cancer.

2.0 METHODOLOGY

In this section, the methodological issues are first addressed regarding how to integrate computational systems biology approach into dose response modeling. The integration of cell cycle checkpoint control into dose response modeling for IR induced transformation frequency is then addressed. Subsequently, the molecular mechanisms of cell cycle regulation and IR-induced checkpoint arrest and apoptosis pathway and the associated mathematical methods are described. Finally, the integration of signaling pathway model into response model – two stage clonal growth model, is introduced.

2.1 Integration of computational systems biology approach in dose response modeling

Traditional dose response modeling development generally implements a linear structure and focuses on toxicology, i.e., the perturbation caused by environmental stressor (Andersen et al., 2005b). Figure 1 shows the schematic of linking physiologically

based pharmacokinetic (PBPK) and physiologically based pharmacodynamic (PD) models together to predict dose response. PBPK models describe exposure to the biologically effective dose and calculate the form of the chemical/metabolite that is causative for the biological effect; mode of action describes early biological response to the active dose; and the PBPD model describes how the toxic dose affects the body to cause altered structure/function and ultimately disease.

Figure 1. Linear structure of linking PBPK and PD models to predict dose response

Compared to the traditional approaches of dose response modeling, computational systems biology approach implements a more complex structure, as shown in Figure 2. Different components in Figure 2 describe different biological processes. Component 1 captures the normal “biology” describing how normal biological functions arise from relevant biological elements that together are called systems inputs; Component 2 captures the “perturbation” and links the perturbation to altered biological targets (e.g. proteins) that have direct influence on the normal biological system; Component 3 focuses on the “response” and disease outcome describing a process linking lower level impaired function to higher level problems and diseases. The “biology” component can be any process of interest which is directly damaged by the mode of action and is deemed to play a significant role on dose response relationship. Therefore, the incorporation of maintenance of normal biological function is the key advantage of integration of computational systems biology approach in dose response modeling. In this manner, this approach enables a direct linkage between normal biology and toxicology. In this report, we take cell cycle checkpoint control as the normal “biology” component.

Figure 2. Schematic of integration of computational systems biology approaches in dose response modeling. Component 1– “biology”; Component 2– “perturbation”; Component 3–“response”. Specific targets in current model are indicated in brackets.

2.2 Inclusion of cell cycle model as the biology model in dose response modeling

Cell cycle control is a basic function of a cell. Study of the dynamics of cell cycle regulation is a subdiscipline of systems biology. Both experimentalists and modelers have made considerable progress in this area (Kohn, 1998; Tyson et al., 2002; Qu et al., 2003; Blais and Dynlacht, 2005). For example, Tyson and his colleagues have established a series of mathematical cell cycle models for budding yeast, fission yeast and frog eggs (Chen et al., 2004; Svecizer et al., 2004; Zwolak et al., 2005).

A normal cell cycle contains G1, S, G2 and M phases. S represents DNA synthesis; M represents mitosis, which includes prophase, metaphase, anaphase and

telophase. S and M phases are separated by two gaps: G1 and G2. Proper progression through the cell cycle is assured by “checkpoints” that guard crucial transitions: G1 to S transition (G1/S checkpoint), G2 to M transition (G2/M checkpoint) and metaphase to anaphase transition (spindle checkpoint). Checkpoint control is a surveillance mechanism in cell cycle regulation that ensures suitable conditions for the transitions to occur. The G1/S checkpoint makes sure that (1) cells are large enough for a new round of DNA synthesis, (2) any damage suffered by the DNA has been repaired, and (3) external conditions are favorable for mitotic cell divisions. The G2/M checkpoint makes sure that (1) DNA is fully replicated, (2) any new damage sustained by the DNA has been repaired, and (3) the cell is large enough to divide. The spindle checkpoint control ensures that chromosomes are properly aligned on the mitotic spindle before the cohesions are degraded (Tyson et al., 2002).

IR, an important environmental stressor, induces DNA damage and disrupts cell cycle through activating several proteins which have direct interactions with cell cycle regulatory genes. These interactions lead to checkpoint arrest and apoptosis. Checkpoint arrest allows time for DNA to repair. The delay at checkpoint lengthens the cell cycle and thus decreases cell proliferation. The cell proliferation rate directly influences the number of mutated neoplastic cells and is usually an essential parameter in computational cancer modeling. Therefore, if checkpoint control is taken as the normal biological function in Figure 2, checkpoint arrest and apoptosis are perturbed functions caused by IR- mediated DNA damage, and DNA repair is an adaptation process. Checkpoint arrest induced decrease in cell proliferation rate will influence organism level response, i.e., cancer incidence.

Hence, it is almost essential to include a cell cycle model as a “biology” component in IR dose response modeling for cancer. In this manner, the influence of checkpoint arrest on cell proliferation rate can be investigated. The inclusion of a cell cycle model as a “biology” component adds more realism to the dose response modeling and helps explore the biological mechanisms of the particular phenomena (e.g. adaptive response) in low dose region, which is difficult with more empirical modeling approaches.

Several mathematical cell cycle models have been developed to study physiology of the normal cell cycle (Novak et al., 1998; Novak et al., 2001; Tyson and Novak, 2001; Qu et al., 2003; Qu et al., 2003). In this study, cell cycle modeling serves as the “biology” component to support dose response modeling. Our implementation is simplified to capture key nodes in the signaling pathway and remain capable of modeling checkpoint control. Furthermore, to support the whole structure of the new approach for dose response modeling (Fig. 2), the systems inputs of checkpoint control must provide a direct linkage to toxicological perturbation. Therefore, appropriate identification of systems inputs in checkpoint control is needed.

The identification of cell cycling inputs was based on the study of the series of cell cycle model developed by Tyson (Tyson and Novak, 2001; Tyson et al., 2002). Molecular antagonism, cell growth and zero-order ultra-sensitivity were considered to be essential for checkpoint control (Tyson and Novak, 2001). With antagonism, a key protein (A) would regulate cell cycle transition, A has a counteractive protein B which represses the activity of A and is also simultaneously inhibited by A. The mutual antagonism between A and B ensures an irreversible transition. Perturbation signals (e.g. proteins activated by IR) that directly interacting with A or B are thus necessary nodes in the “perturbation” component. Progress from one cell phase to next is promoted by cell growth. Based upon existing cell cycle models and associated mathematical analysis, a zero-order ultra-sensitivity switch must exist in the antagonism reaction in order to make the dynamics of the checkpoint regulation occur (Tyson and Novak, 2001; Tyson et al., 2002). In zero-order ultra-sensitivity circuit, which often involves an enzymatic inter-conversion between a substrate and a product, switching occurs when the enzymes are saturated by the substrate and/or product (Goldbeter and Koshland, 1981; Goldbeter and Koshland, 1984). Here the zero-order ultra-sensitivity switch is identified as the third element of systems input essential for checkpoint control.

2.3 Molecular mechanism of cell cycle regulation and IR-induced checkpoint arrest and apoptosis pathway

A modular approach was used to construct the overall computational model linking IR-mediated DNA damage with activation of cell cycle checkpoint controls and

apoptosis. Module 1 describes an empirical linkage of IR with DNA damage; module 2 provides a molecular level description of the signaling pathways linking DNA damage with target proteins that induce checkpoint arrest and apoptosis; and module 3 describes the molecular mechanisms of the G1/S and G2/M cell cycle checkpoint controls. Modules 2 and 3 are the main focus of this report. Three versions of module 2 with increasing levels of biological details corresponding to increasing levels of signaling redundancy were developed (Figs. 3a - 3c). In future work the overall model will be extended by adding additional modules describing bystander effects and tumor incidence.

Module 1

IR causes DNA damage, with double strand breaks (DSBs) being the most significant lesions (Criswell et al, 2003). DSB may arise through direct hits by IR or as secondary damage from reactive oxygen species (ROS) (Feinendegen, 2002; Slupphaug et al, 2003). In Module 1 IR generates ROS and ROS cause DSB (Fig. 3). Direct formation of DSB by IR is not described. Repair of DSB is included in the model but we have not attempted to describe the molecular mechanism of this process.

Fig. 3a

G1/S checkpoint and Chk2-Cdc25A checkpoint arrest pathway

G2/M checkpoint and Chk2-Cdc25C checkpoint arrest pathway

Fig. 3b

Fig. 3c

Figure 3. Signaling Pathway

3a. IR-mediated DNA damage, p53 dependent signaling from ATM, which senses the DNA damage, to the G1/S and G2/M cell cycle checkpoints and to apoptosis. → and —| imply activation and repression, respectively. Note the central role of p53 in the signaling pathways. The left oval indicates proteins and interactions in the G1/S checkpoint and the right oval indicates proteins and interactions in the G2/M checkpoint.

3b. Redundancy in signaling from ATM to the G1/S and G2/M checkpoints is illustrated by the addition of Chk2-dependent signaling in parallel with the p53-dependent signaling shown in Fig. 1a. Chk2 activates the G1/S checkpoint by inhibiting Cdc25A, which is a facilitator of the G1/S transition. Chk2 activates the G2/M checkpoint by inhibiting

Cdc25C, a facilitator of the G2/M transition. The dephosphorylated (dp) and total forms of cyclin E/cdk2 (cycE, cycEdp) and cyclin B/cdk1 (cycB, cycBdp) are shown separately as Cdc25A and Cdc25C dephosphorylate and thereby activate cycE and cycB complexes that respectively promote the G1/S and G2/M transitions. $\longleftrightarrow$ connects phosphorylated and dephosphorylated forms of proteins. $--\rightarrow$ indicates indirect induction to apoptosis through BCL2 and BAX as shown in Figure 1a.

3c. Redundancy of signaling from ATM to the apoptotic pathway is illustrated by addition of the p73-dependent pathway (compare with Fig. 3a). The anti-apoptotic influence of Rb through its inhibitory interactions with E2F and cAbl is also shown.

Module 2

Module 2 is the signal transduction module. The transducer and effector phases of molecular mechanisms that are activated in response to IR are described (Fig. 3). DSB activate ataxia telangiectasia-mutated kinase (ATM) by autophosphorylation (Criswell et al, 2003; Bakkenist and Kastan, 2003; Bartek and Lukas, 2001; Mikkelsen and Wardman, 2003). ATM plays a role in DSB repair by binding to the broken ends of the DNA strands created by DSB. DSB break repair is defective in ATM-deficient cells (Yang et al, 2004). We thus assumed that the rate of DSB repair (Module 1) is proportional to the activity of phosphorylated ATM, and to the amount of DSBs.

Notwithstanding its role in DNA repair, the primary function of ATM is to phosphorylate and activate the transcription factor p53 (Fig. 3). p53 is a critical mediator of cellular responses to DNA damage in IR-exposed mammalian cells (Criswell et al, 2003; Mikkelsen and Wardman, 2003; Pieterpol and Stewart, 2002; Agarwal et al, 1998). p53 signals to the G1/S checkpoint through p21 and to the G2/M checkpoint through 14-3-3 σ and GADD45 (Fei and El-Deiry, 2003; Criswell et al, 2003; Taylor and Stark, 2001).

Cyclin E and its dependent kinase (Cdk2) form a heterodimer (CycE) that stimulates progression of the cell cycle from G1 to S (Fig. 3). p21 inhibits CycE, and p53 mediated transcriptional activation of p21 thereby invokes the G1/S checkpoint arrest

(Taylor and Stark, 2001; Sekiguchi and Huter, 1998). The role of CycE in the G1/S transition is covered in the description of Module 3.

Cyclin B and its dependent kinase (Cdk1) form a heterodimer (CycB) that stimulates progression of the cell from G2 into mitosis (Fig. 3). 14-3-3 σ anchors CycB in the cytoplasm, thereby blocking mitosis (Taylor and Stark, 2001). GADD45 dissociates the CycB heterodimer and therefore also blocks mitosis (Taylor and Stark, 2001). p53 mediated activation of 14-3-3 σ thus invokes the G2/M checkpoint arrest. The role of CycB in the G2/M transition is covered in the description of Module 3.

p53 dependent signaling also mediates entry into the apoptotic pathway through effects on Bax and Bcl-2 (Fig. 3) (Fei and El-Deiry, 2003). Bax is pro-apoptotic and is transcriptionally activated by p53 (Levine, 1997; Chao et al, 1995; Thomas et al, 2000). Bcl-2 is anti-apoptotic and is transcriptionally repressed by p53 (Fussenegger et al, 2000). Both Bax and Bcl-2 signal to caspases, which are proteases at the core of the apoptotic machinery (Fussenegger et al, 2000).

In addition to this simple description of the p53 dependent checkpoint arrest and apoptosis pathway, two more complex versions of modules 2 are described that illustrate how pathway architecture determines the robustness of the system. Here, robustness refers to the ability to maintain a significant level of functionality when pathway components are missing or inoperative due to mutations. Redundancy in signaling pathways can also serve to enhance the degree of biochemical response to an input such as DNA damage. These pathways include Chk2 dependent checkpoint arrest (Fig. 3b) and c-Abl - p73 dependent apoptosis (Fig. 3c).

ATM-mediated activation of Chk2 induces cell cycle arrest at both the G1/S and G2/M checkpoints (Fig. 3b) (McGowan, 2002). These effects of ATM-Chk2 signaling are mediated through Cdc25A and Cdc25C, respectively. The normal function of Cdc25A is to remove inhibitory phosphates from Cdk2 (Iliakis et al, 2003), thereby activating CycE and facilitating the G1/S transition. Similarly, Cdc25C dephosphorylates and activates cyclin B/Cdk1 (CycB) complexes, facilitating the G2/M transition (Iliakis et al, 2003). When mammalian cells are exposed to IR, a rapid decrease, independent of p53 status, is observed in the abundance and activities of Cdc25A and Cdc25C (Iliakis et al,

2003; Sorensen et al, 2003). Activated Chk2 phosphorylates Cdc25A and Cdc25C, leading to their proteolytic degradation (Bartek and Lukas, 2001; Iliakis et al, 2003; Pearce and Humphrey, 2001). ATM-Chk2-mediated loss of Cdc25A and Cdc25C activity thus activates the G1/S and G2/M checkpoint arrest.

c-Abl and p73 form a p53-independent signaling pathway leading from ATM to apoptosis (Fig. 3c). ATM binds to and activates c-Abl, which in turn transcriptionally activates p73. p73 activates initiator caspases and thereby induces apoptosis (Wang and Ki, 2001). Retinoblastoma protein (Rb) is a negative regulator of this pathway. First, transcription of p73 is activated by E2F, which is inhibited by Rb. Second, the c-Abl tyrosine kinase phosphorylates and stabilizes p73 leading to an increase in p73 activity. Rb binds to and inhibits c-Abl tyrosine kinase, leading to down-regulation of p73 (Wang and Ki, 2001; Wang, 2000). Rb thus appears to be an important determinant of the cellular choice between checkpoint arrest and apoptosis (Wang and Ki, 2001).

Module 3

Module 3 describes key aspects of the molecular mechanisms of the G1/S and G2/M cell cycle checkpoints. The term “DNA damage checkpoints” is sometimes used to refer to functional points in the cell cycle where the cell monitors for the presence of DNA damage and, if damage is found, initiates a cascade of events leading to cell cycle arrest until the damage is repaired (Laiho and Latonen, 2003). Both G₁/S and G₂/M checkpoints in the cell cycle are DNA damage checkpoints.

Module 3 was developed to describe the G1/S and G2/M checkpoints. Our approach was based on that used by Tyson and co-workers, where checkpoints are activated by inhibitory phosphorylation of key proteins, and cell growth (i.e., the increase in cell size with time) drives progression around the cycle (Tyson and Novak, 2001). The description of the G1/S checkpoint is a synthesis of information taken from literature review. The description of G2/M checkpoint control is taken from the model developed by Tyson and Novak (Tyson and Novak, 2001).

The key proteins controlling the G1 to S transition in a mammalian cell cycle are the CycEs (Koepp et al, 2001; Moberg et al, 2001), which are thought to initiate DNA

replication (Iliakis et al, 2003). To keep our model as simple as possible, we only considered the heterodimer - cyclin E and cdk2. No description of the interactions between CycE monomers was included. Since cdk is only active when association with its cyclin partner (Koepp et al, 2001), it is acceptable to ignore its monomer form in the model.

E2F, Rb, and p27 all interact with cyclin E (Sherr, 2004; Leng et al, 1997; Russo et al, 1996). E2F is a transcription factor for cyclin E and thus promotes G1/S progression (Bartek and Lukas, 2001; Yamasaki, 2003; Ohtani, 1999). Rb is thought to regulate progression through the G1 phase of the cell cycle (Ohtani, 1999; Gala et al, 2001; Morris and Dyson, 2001) by inhibiting the activity of E2F through formation of Rb-E2F complexes (Morris and Dyson, 2001; Wells et al, 2003; Weintraub et al, 1992; Arroyo and Raychaudhuri et al, 1992).

Formation of the Rb-E2F complex not only determines the activity of cyclin E but also is regulated by CycE. That is, Rb can be phosphorylated by CycE, inhibiting Rb activity and enabling release of active E2F to promote entry into S phase (Leng et al, 1997). CycE can phosphorylate both Rb and Rb-E2F complexes in the model, as experimental observations show (Qin et al, 1992). The net effect of the CycE-Rb-E2F-CycE regulatory loop is to form a molecular switch that synergizes CycE activation when CycE levels are rising (Halaban, 1999). These are the main controlling factors for Rb (Qu et al, 2003a; Degregori, 2004) and the total Rb activity is assumed to be constant and not regulated by other factors in the model.

Activity of CycE is also stimulated by interactions between CycE and p27. CycE is inactive when complexed with p27. Ubiquitylation of p27, however, causes dissociation of the CycE-p27 complex (Akli et al, 2004; Furukawa, 2002; Lane et al, 2001). The trigger for p27 ubiquitylation is phosphorylation of p27 by CycE (Sherr, 2000; Li et al, 2004). As indicated by experimental observations, CycE is assumed in the model to phosphorylate both p27 and p27-cycE complexes (Sherr, 2000; Yang et al, 2004). The result of the CycE-p27-CycE regulatory loop is the same as that of the CycE-Rb-E2F-CycE loop, i.e., to synergize CycE activation as CycE levels are rising. The relative importance of these two loops in increasing CycE levels is not clear, although

most of the literature to date has focused on the loop containing Rb. P27 activity is also assumed to have a zero order synthesis and a first order degradation.

Our depiction of the G2/M checkpoint is based on the model developed by Tyson and Novak (Tyson and Novak, 2001). Entry of the cell into mitosis is promoted by CycB, which is thus the key complex of the G2/M checkpoint (Kaufmann, 1998; Bahassi et al, 2004). Antagonism between CycB and the anaphase promoting complex (APC) is considered to be the basis for the G2/M checkpoint control (Fig. 3a) (Tyson and Novak, 2001). Cdc20 and Cdh1 are two auxiliary proteins of APC. Active (dephosphorylated) APC ubiquitylates and thereby inactivates CycB, while CycB inactivates APC by phosphorylation (Bembenek and Yu, 2001). The cyclin-dependent kinase Cdc20 helps to degrade CycB and is indirectly phosphorylated and activated by cyclin B (Tyson and Novak, 2001). The CycB-Cdc20 regulatory loop independent of CycB-APC therefore, is a down regulator of CycB activity, although its effect is considered to be weak compared to that of APC. While not the focus of the current model, Cdc20 is an effector of the spindle checkpoint, regulating the metaphase to anaphase transition (Reed, 2003).

Therefore, specific targets in the model have been identified for the schematic in Figure 2 and are shown in brackets. ROS is taken as the product of initial biological interaction from IR exposure; Subsequent changes in DSB, activated ATM^p, p53^p, p21, GADD45 and 1433sigma are downstream perturbations to the cell and will finally impair normal biological function – checkpoint control.

2.4 Mathematical methods and software for signaling pathway model

The mathematical model is composed of a set of ordinary differential equations (ODEs). The equations and their derivations are described in the appendix. The dynamical system contains 45 ODEs and 135 parameters. When available, specific parameter values were taken from previously published sources. For example, for the equations taken from Tyson's model of the cell cycle, we used the parameter values as published (Tyson and Novak, 2001). These rate constants were also used in the construction of other equations. Whenever possible, biological mechanisms were considered when assigning values to the kinetic constants. For example, rate constants for transcriptional reactions were assigned relatively small values, as transcription is a

relatively slow process. When such information was not available, parameter values were used that were consistent with the expected qualitative behaviors of the model. This approach to the assignment of parameter values has been used by others (Qu et al, 2003a; Qu et al, 2003b) and is reasonable for the first-generation model we have developed.

The nonlinear ordinary differential equations describe the rates of changes in protein activities and cell mass. The absorbed dose rate of IR, represented by IRR, is the input variable. In the model, the rate of change in cell mass is described as a logistic function,

$$\frac{dm}{dt} = \mu * m * (1 - m / m_{\max}) \quad (1)$$

where,

m = cell mass

μ = growth rate constant

$m_{\max}$ = maximum size to which a cell may grow if it does not divide

To study the predictions of cell cycle behavior from the mathematical model, mathematical methods from nonlinear dynamics were applied. The results are displayed graphically, and illustrated by time course plots, phase plane plots and bifurcation for protein activity.

For a dynamical system with two variables x and y where $\frac{dx}{dt} = f(x, y)$ and

$\frac{dy}{dt} = g(x, y)$, phase plane plots are used to study how x and y change relative to each

other and to determine if the system has one or more steady states, i.e., $f(x, y) = 0$ and $g(x, y) = 0$. In the phase plane plot, a point on the x nullcline is identified by fixing the value of y and running the computational model to identify the corresponding steady state value of x . The entire x nullcline is developed by repeating this steady state analysis over the range of values of y that are of interest. The x nullcline is thus the set defined by $f(x, y) = 0$, or in other words, the set of values of x at which the system is at steady state for a range of values of y . Similarly, the y nullcline denotes steady state values of y for a range of values of x , $g(x, y) = 0$.

Intersections of the x and y nullclines identify steady states of the entire dynamical system. In a system with more than one steady state each steady state is associated with a unique intersection of the nullclines. When the dynamical system has more than two state variables, the variables not represented in the 2-dimensional plot are assumed to be in a “pseudo-steady state”. That is, they change quickly with the variables of interest.

In addition to phase plane plot, bifurcation diagrams can be used to study steady states. Bifurcation theory explains how the behavior of a dynamical system depends upon its parameter values. The basic idea is described as follows: The long-term behavior of a regulatory network is characterized by its attractor(s). The simplest kind of attractor is the fixed-point attractor, which is the system at its steady state (or equilibrium). The basin of attraction of an attractor consists of the sets of initial conditions that allow the system to converge toward the attractor over time. More complicated attractors such as limit cycles that do not correspond to steady states may also exist (Tyson et al., 2001; Tyson et al., 2002).

Dynamical systems may have more than one attractor. For fixed-point attractors, this corresponds to a system with 2 or more stable steady states, such as the MAPK signal transduction system (Bhalla et al., 2002) or the G2 to M steady states of the cell cycle (Tyson and Novak, 2001). When multiple attractors exist we are interested in knowing how transitions between attractors are associated with changes in the values of specific parameters. For example, Tyson’s computational model of the cell cycle (Tyson and Novak, 2001) provides insight into the biological mechanism that control the transition between G2 and M. Bifurcation theory assures us that there are only a limited number of combinations of parameter values that allow such transitions to occur (Tyson and Novak, 2001).

Transitions between attractors can be visualized by choosing a single variable as representative of all the interacting proteins in the network and a single parameter as representative of all the rate constants that are involved in the reactions (Tyson and Novak, 2001). The plot of the proxy variable against the proxy parameter is called a bifurcation diagram (Tyson et al., 2001; Tyson and Novak, 2001; Tyson et al., 2002).

This diagram shows how the parameter of interest influences steady state values of the variable. Transition between attractors appears as sharp inflections in the bifurcation plot.

To draw time course and phase plane plots, we developed MATLAB code in MATLAB 6.5. To draw bifurcation diagrams, we used Matcont (<http://allserv.ugent.be/~ajdhooqe/>), a continuation toolbox for MATLAB ODE which is freely available from Annick Dhooge in Dept. of Applied Mathematics & Computer Science, Ghent University, Belgium.

Data Transformation

The model simulation results can be compared to experimental time course and dose response data in the literature. The experimental data are measured based on specific types of IR and specific cell lines. For purposes of comparing with model predictions, the experimental data are first linearly transformed and scaled. That is, the x and y coordinates of the transformed data are obtained by multiplying a constant to the x and y coordinates of the original data. For example, if the original data are represented by $(X1_o, Y1_o), (X2_o, Y2_o), \dots (Xn_o, Yn_o)$ and the transformed data is represented by $(X1_t, Y1_t), (X2_t, Y2_t), \dots (Xn_t, Yn_t)$, the original data and transformed data have the following

relationship: $\frac{X1_t}{X1_o} = \frac{X2_t}{X2_o} = \dots = \frac{Xn_t}{Xn_o} = a$ and $\frac{Y1_t}{Y1_o} = \frac{Y2_t}{Y2_o} = \dots = \frac{Yn_t}{Yn_o} = b$. Where, a and b

are constants. In this manner, the consistency between simulation and transformed data actually evaluates if the model can qualitatively describe time courses of protein post-IR activities and the shapes of dose response curve.

2.5 Integration of signaling pathway model into response model

As a “response” component in the Schematic in Figure 2, the impaired function simulated by the IR perturbed cell cycle model was linked to a two-stage clonal growth model to investigate the dose response for IR induced cancer. The linkage resides in direct relationship between checkpoint arrest and cell proliferation rate. The two-stage clonal growth model used for this analysis is identical in its biological structure to other two-stage models (Moolgavkar and Knudson, 1981; Cohen and Ellwein, 1990),

describing populations of normal cells and of intermediate cells with one mutation. Mutations occur during the process of cell division, so that the mutation parameter (μ) is a probability of mutation per cell division. A tumor cell arises when an intermediate cell acquires a second mutation. The equations of the two-stage clonal growth model are represented by Equations (2), (3) and (4):

Figure 4. Schematic of two stage clonal growth model

$$\frac{dN}{dt} = N(\alpha_N - \beta_N) \quad (2)$$

$$\frac{dI}{dt} = I(\alpha_I - \beta_I) + N\alpha_N\mu_N \quad (3)$$

Where, $p(tumor) = 1 - e^{-\int I\alpha_I\mu_I}$ (4)

N is the number of normal cells

α_N is the division rate constant for normal cells (h^{-1})

β_N is the death rate constant for normal cells (h^{-1})

μ_N is the probability of mutation per division for normal cells

I is the number of intermediate cells

α_I is the division rate constant for intermediate cells (h^{-1})

β_I is the death rate constant for intermediate cells (h^{-1})

μ_I is the probability of mutation per division for intermediate cells

The product of α_N and μ_N represents mutational fraction per unit time. The cell division rate α_N , inverse of cell cycle time, is directly influenced by checkpoint arrest. In this study, as an initial effort to implement a computational systems biology approach in dose response modeling, we focused on exploration of the relationship between IR dose and the quantity of $\alpha_N\mu_N$.

Neoplastic transformation is an important endpoint associated with IR carcinogenesis (Redpath et al., 2001) and strongly correlated with mutation and cancer (Mills et al., 2003; Ishikawa et al., 2004). Here $\alpha_N\mu_N$ is the surrogate of neoplastic transformation and is compared to experimental data.

Cell proliferation rate α_N , inverse of cell cycle time, can be directly simulated from the IR perturbed cell cycle model. When applying this computational systems biology approach, models that survive initial testing can then be used to make predictions (Kitano, 2002). IR perturbed cell cycle model was tested by comparing simulation results to extensive experimental data. The consistency between the simulation and data provided justification for these subsequent analyses.

The cell cycle time is a function of the lengths of G1/S and G2/M checkpoint arrests, as shown in Equation 5:

$$T = c + A_1 + A_2 \quad (5)$$

Where,

T = the cell cycle time under IR exposure

c = the normal cell cycle length without IR exposure, including G1, S, G2 and M phases, we assume it is 450 time unit in the study

A_1 = the G1/S checkpoint arrest time

A_2 = the G2/M checkpoint arrest time

Two modeling approaches can be used to identify the checkpoint arrest time - time course plots and bifurcation diagrams. In a time course plot, the key proteins regulating checkpoint control have switch-like behaviors during G1 to S and G2 to M transitions (Zhao and Conolly, 2005, in review). For example, when CycE jumps from a lower state to a higher state, the G1 to S transition is activated; when CycB jumps from a lower state to a higher state, the G2 to M transition is activated. The simulated switch-

like behaviors of the proteins in G1/S and G2/M checkpoints are shown in Results. Protein behaviors under normal conditions are defined as basal activity; protein behaviors under a certain IR exposure are defined as induced activity. The switch can be used to identify the G1 to S transition. For example, we can assume that when CycE's activity increased greater than 0.1, G1 to S transition happens. The time lag of the switches between induced activity and basal activity can be used to identify the checkpoint arrest time.

The bifurcations of a dynamical system can be characterized by a one-parameter bifurcation diagram. These diagrams single out one variable (say, x_1) as a representative of all the dynamic variables in the control system and one parameter (say, p_1) as a representative of all the rate-determining factors in the model. The steady state value of x_1^* is then plotted versus p_1 . Bifurcation points are noted by the complex behavior of the function (Tyson et al., 2002).

Taking G2/M checkpoint as an example, we illustrate the bifurcation diagram and its utilization to identify the checkpoint arrest time. In bifurcation diagram of G2/M checkpoint simulated from the signaling pathway model under no IR exposure (Fig. 5), the representative variable is CycB and the representative parameter is cell mass m . When m is small, the corresponding steady state CycB activity is low, indicating that the cell is in G2. When m reaches a critical value (the arrow in Fig. 5), CycB activity jumps to a higher steady state, indicating that the cell transits to M phase. This qualitative change is a "bifurcation". Therefore, the bifurcation point is the cell mass at which the transition happens, referred to as critical mass. The time of the transition, and thus, the checkpoint arrest time can be calculated based on the critical m value using Equation 1.

Because bifurcation diagrams provide a graphical indication of the critical cell mass, as well as its relationship to the checkpoint regulating protein (e.g. CycB), it is a useful tool to investigate the mechanism. Cell mass plays a key role in the model as it connects the mathematical modeling of the system to the biology of cell replication.

Figure 5. Bifurcation diagram of G2/M checkpoint under no IR exposure. The critical mass is indicated by a black solid arrow.

The mutational rate μ_N is assumed to be a linear function of IR. Some investigators have taken μ_N as a linear function of DNA damage, and DNA repair may shape the relationship between μ_N and environmental dose to nonlinear with a threshold or even “J-shaped” (Conolly et al., 2003). The main purpose here is to explore how checkpoint arrest time influences mutational fraction through proliferation rate, so an assumption of a simple linear relationship between μ_N and IR is appropriate. Therefore, μ_N has the following function:

$$\mu_N = \mu_{bas} + k \bullet IRD \quad (6)$$

where,

μ_{bas} = basal probability of mutation per division for normal cells, 0.01 in the study

k = slope constant, three cases are evaluated in the study: 5.27×10^{-5} (Case 1), 3.69×10^{-5} (Case 2), 2.64×10^{-5} (Case 3)

IRD = dose of IR, the product of IRR and exposure time

3.0 RESULTS

In this section, the simulation results from the signaling pathway model are first presented. The integration of the signaling pathway model into two-stage clonal growth model to predict dose response of IR induced tumor incidence is then addressed.

3.1 Simulation results from the signaling pathway model

Model predictions compared with experimental data

1) Qualitative comparisons

The proteins involved in the G1/S and G2/M checkpoints work independently and, therefore, can be studied separately (Fig. 3). Signaling from IR induced DSB through p53 to the G1/S checkpoint is described by Equations A-1 to A-21 (Appendix). Setting $IR = 0$ in the model, a normal cell cycle behavior is obtained with switch-like changes in activities denoting the transition from G1 into S (Fig.6). The model-predicted increase in activity of CycE in the transition is consistent with published descriptions of the role of cyclin E in the mammalian cell cycle (Koepp et al, 2001; Moberg et al, 2001).

Figure 6. Time course of protein activities for the transition from G1 to S. Changes in protein activities are switch-like. The time when the transition occurs can be identified approximately as the point when protein activities switches from one state to another (e.g. when CycE activity reaches 0.1 from below). (Refer to Table A-2 for abbreviations used in the legend.)

The predicted changes in activities of other proteins involved in the G1/S transition are also consistent with their regulating functions in a normal cell cycle. For example, Rb is phosphorylated by CycE to form Rbp. Hence, the activity of Rb decreases while the activity of Rbp increases. Similarly, the complex Rb-E2F is phosphorylated by CycE to form Rbp-E2F, leading to release of free E2F and further enhancement of CycE activity. In response, the activity of Rb-E2F drops and the activities of Rbp-E2F and E2F increase. The activity of p27, an antagonist of CycE, decreases as the transition progresses.

Linkage of p53-mediated ATM signaling to the G2/M checkpoint is described by Equations A-11 to A-15 and A-18 to A-27 (Appendix). The predicted protein activities associated with G2 to M transition (Fig. 7) are consistent with their known functions in the cell cycle (Reed, 2003). Because Tyson's eukaryotic cell cycle model was the basis

for our description of the G2/M checkpoint, these results are similar to those obtained by Tyson and Novak (Tyson and Novak, 2001).

Figure 7. Time course of cell mass and protein activities from G2 to M transition

- (a) Time course of cell mass
- (b) Time course of CycB and Cdh1 activities
- (c) Time course of total Cdc20 (Cdc20T), activated Cdc20 (Cdc20A) and a hypothetical intermediary enzyme (IEP) (Refer to Table A-2 for abbreviations used in the legend.)

2) Semi-quantitative comparisons

The model can be used to simulate post-irradiation effects of IR on protein activities. IRR in the model is the absorbed dose rate. Therefore the total exposure equals $IRR \times \Delta t$. Δt is the exposure time. For a given exposure, the model can simulate the post-irradiation protein activity. Some post-irradiation experimental data for protein time courses are available, such as phosphorylated p53 and p21 in liver and confluent fibroblast cultures (Reynolds et al, 2004; Fournier et al, 2004). Here, the simulated phosphorylated p53 post irradiation activity and p21 post-irradiation activity are compared with transformed experimental data for carbon-ion-irradiated confluent fibroblast cultures in Figs 8 and 9, respectively (Fournier et al, 2004). The modeling behaviors in terms of the trends of the time courses, for both p53 and p21 activities are consistent with experimental data.

Figure 8. Comparison of model simulation and experimental data time course of p53p activity. The simulation results are consistent to the transformed experimental time course data for p53p activity, indicating the model results are able to track the trend of the post IR p53p activity.

Figure 9. Comparison of model simulation and experimental data time course of p21 activity.

The simulation results are consistent to the transformed experimental time course data for p21 activity, indicating the model results are able to track the trend of the post IR p21 activity.

The model can also be used to predict the shape of the dose response curve for protein activity versus IR, DSB, or other upstream protein activity. Fig. 10 compares the simulation of a dose response curve of caspase 3 activity versus IR to transformed experimental data generated for normal cell line by Fernet et al. (Fernet et al, 2003). The model simulations are highly consistent with the transformed data.

The parameter values used for the simulations in Figs 8, 9 and 10 were kept constant while the exposure values were adjusted for the different cases.

Figure 10. Comparison of model simulation and experimental data for dose response curve of Caspase 3 activity versus IR. The simulation results are consistent to the transformed dose response data.

Analysis of checkpoint arrest with phase plane plots

Phase plane plots are utilized to make predictions for checkpoint arrest following exposure to IR.

Figure 11 shows a phase plane comparison of CycB and Cdh1 nullclines in G2/M checkpoints without and with exposure to IR (Figs. 11a and 11b, respectively). As m (cell mass) increases from 0.30 to 0.53 to 0.70, the Cdh1 nullcline moves to the left while CycB nullcline changes only for small values of Cdh1 (Fig. 11a). At $m = 0.30$, the CycB and Cdh1 nullclines intersect 3 times. These intersections define two stable steady states separated by an unstable steady state (the unstable steady state is also called a saddle point). The two stable steady states correspond to G2 and M. G2 is defined by high Cdh1 and low CycB activities, while M is defined by low Cdh1 and high CycB activities.

Figure 11. Phase plane plot of CycB and Cdh1 nullclines in the G2/M checkpoint illustrates how the G2 to M transition is driven by cell growth (increasing values of m). For $IR = 0$, when $m = 0.3$, the cell is stable in G2, as indicated by the intersecting nullclines near the top of Fig. 11a. At $m = 0.53$, the Cdh1 nullcline has moved to the left,

causing the intersection that defines G2 to merge with its adjacent saddle point intersection. At any value of m larger than 0.53 (i.e., $m = 0.7$ in Fig. 11a), the one remaining intersection defines the new steady state at M. Fig. 11b indicate when $IR = 0.1$, the critical $m = 0.82$, indicating a checkpoint arrest when the cell is exposed to IR.

As m increases from 0.3 to 0.53, the intersection of nullclines defining G2 state converges with the saddle point. As m increases to 0.7, the intersection of nullclines that defines G2 disappears and the cell switches into the M steady state. Thus, in the absence of exposure to IR, 0.53 is a critical value of m . The G2/M transition does not occur until the cell grows to this critical size.

The critical cell size associated with the transition from G2 to M increases in the presence of DNA damage subsequent to IR exposure (Fig. 11b). In this case, with $IR = 0.1$, the critical value of m is 0.82. Recall that the critical value for the control case is 0.53. Activation of the G2/M checkpoint is thus evidenced as the extra time spent in G2 as the cell grows to this larger size ($m = 0.82$ vs. $m = 0.53$). This extra time provides time for DNA repair.

Figure 12 shows a bifurcation diagram of the G2/M system for $IR = 0$ and $IR = 0.1$, respectively. The representative variable is CycB and the representative parameter is m . G2 is the steady state corresponding to a lower CycB activity while M corresponds to the higher CycB activity. The cell is in G2 at small values of m . As m reaches the critical value of 0.53 (for $IR = 0$), the cell switches to the M phase. The bifurcation diagram also indicates an increase in the critical m (0.82; $IR = 0.1$) when the cell is exposed to IR.

Figure 12. Bifurcation diagram showing G2/M checkpoint arrest after exposure to IR. The transition from G2 to M occurs when a cell reaches a critical value of m , as indicated by arrows. Checkpoint arrest occurs because the critical cell size increases when the cell is exposed to IR.

Phase plane plots comparing the CycE and Rb nullclines in the G1/S checkpoint also indicate that exposure to IR increases the critical value of m associated with the G1/S transition (Fig. 13).

Figure 13. Phase plane plots of the CycE and Rb nullclines in G1/S checkpoint without (a) and with (b) exposure to IR.

Analysis of the choice of cell survival or death given various doses of IR

IR exposure and its consequent DNA damage lead ultimately to a binary decision - the cell either enters the apoptotic pathway and dies or else it attempts to survive. Here, we examine the biochemical mechanism by which this decision is made at the G1/S checkpoint. As CycE and Rb have switch-like behaviors at the G1 to S transition, a comparison of the IR-induced and basal activities of CycE and Rb was used to identify how the checkpoint is activated (Fig. 14). High caspase activity, which is associated with entry into the apoptotic pathway, was used as a surrogate measure of cell death.

(a)

(b)

(c)

(d)

Figure 14. Analysis of protein activities at G1/S checkpoint for different doses of IR for CycE, Rb, DSB, Caspase 3 (dashed line: exposed to IR; solid line: no IR; Δt : Exposure Time)

- (a) Case1: IR = 0.01, $\Delta t = 10$
- (b) Case 2: IR = 0.3, $\Delta t = 10$
- (c) Case 3: IR = 0.5, $\Delta t = 20$
- (d) Case 4: IR = 1, $\Delta t = 20$

Exposure to a low dose of IR for a short duration (Case 1, IR = 0.01, $\Delta t = 10$) was predicted to cause only small, transient changes in the amount of DSB (Fig. 14a-2), while no conspicuously visible effect was predicted for the activities of CycE and Rb (Fig. 14a-1). These behaviors are consistent with rapid repair of a low level of DSB and with a consequent lack of significant propagation of signal to the cell cycle checkpoint.

Exposure to a higher dose of IR (Case 2, IR = 0.3, $\Delta t = 10$) was predicted to delay the switch-like transitions in the activities of CycE and Rb, indicating arrest at the

G1/S checkpoint (Fig. 14 b-1). In this case the level of DNA damage is sufficient to activate the signal transduction pathway and alter the time-course activities of the key regulatory activities at the checkpoint, providing additional time for DNA repair. This dose of IR was not predicted to increase caspase activity to a level that conspicuously improves the possibility of apoptosis.

Exposure to a still higher dose of IR (Case 3, IR = 0.5, $\Delta t = 20$) was predicted to trigger a correspondingly longer checkpoint arrest (Fig. 14b-1 and 14c-1). The duration of the checkpoint arrest was predicted to be sufficient in this case to allow caspase activity to increase to a level associated with an apparently improved possibility of entry into the apoptotic pathway (Fig. 14c-2). With a sufficiently long checkpoint arrest, it is reasonable to think that some of the cells go into apoptosis during the arrest period; thus this prediction is consistent with data showing that cell survival is reduced in association with p53-dependent G1 arrest (Gupta et al, 1996).

At the highest dose evaluated (Case 4, IR = 1, $\Delta t = 20$), caspase activity was predicted to increase the likelihood for apoptosis dramatically (Fig. 14d). An increased likelihood of apoptosis in the presence of high levels of DNA damage has been seen experimentally (Sun et al, 2005).

Figure 15 shows the time the cells stay in G1/S and caspase 3 activity as a function of IR dose. The G1/S time initially increases with IR and then saturates when IR reaches 0.5; caspase 3 activity increases with IR.

Figure 15. G1/S time and Caspase 3 activity as a function of IR dose.

Robustness of cellular functions in checkpoint arrest pathway

In general, “robustness” means the persistence of a system’s characteristic behavior under perturbations or conditions of uncertainty (Stelling et al, 2004). Robustness of signaling from ATM to the G1/S checkpoint was examined by simulating the time courses of key regulatory proteins associated with the G1/S transition using the versions of module 2 without and with the Chk2-dependent pathway (Figs. 3a and 3b, respectively). The duration of checkpoint arrest was predicted to be much longer when ATM to G1/S signaling was both p53- and Chk2- dependent (Fig. 16) than when only p53-dependent (Fig. 14c). Redundant signaling from ATM to the G1/S checkpoint thus allows more time for DNA repair and would also provide some functionality of the pathway in the presence of mutations to the p53 or Chk2 genes. This result from the computational modeling is consistent with experimental observations showing that Chk2 activation contributes to S phase cell cycle arrest (Bougeret et al, 2001).

Figure 16. Model-predicted protein activities in G1/S checkpoint with incorporation of Chk2-dependent signaling in addition to p53-dependent signaling. Solid lines indicate basal protein activities. Dashed lines represent activities for an IR dose of 0.5 with duration of 20. The duration of checkpoint arrest is much longer when both p53-and Chk2-dependent signaling are included (compare with Fig. 14c, which shows predicted activities when only p53-dependent signaling is considered).

Robustness of cellular functions in apoptosis pathway

The robustness of the apoptosis pathway was evaluated by studying the abilities to induce the level of caspase in response to IR exposure without and with a redundant p73 pathway (Fig. 17). In Case 1, only the p53 dependent apoptosis pathway was considered. In Case 2, the p73-dependent apoptosis pathway was added.

Predicted caspases activity was higher in Case 2 than in Case 1 because p73-dependent signaling is proapoptotic. These predicted behaviors are qualitatively consistent with experimental data regarding p73's function in apoptosis (Welch and Wang, 1993; Welch and Wang, 1995).

Figure 17. Comparison of predicted caspase activities without (Case 1) and with (Case 2) inclusion of redundant pathways. In Case 1, only the p53 dependent apoptosis pathway was considered. In Case 2, the p73-dependent apoptosis pathway was included.

3.2 Integration of the Signaling Pathway Model into Two-stage Clonal Growth

Model

In this section, we first described the simulated results of dose response relationship. Then mathematical methods are implemented to gain deeper biological insights into the mechanisms leading to the specific shapes of the dose response.

Simulation of dose response relationship

For $IRR = 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6$, G1/S and G2/M checkpoint arrest time are identified using time course plot. The cell cycle time are calculated based on Equation 5. The proliferation rate is calculated as inverse of cell cycle time. The IR dose is calculated by IR dose rate multiplying cell cycle time. Table 1 summarizes the estimated G1/S checkpoint arrest time, G2/M checkpoint arrest time, cell cycle time, IR dose and

proliferation rate. Then the mutational fraction per hour, as surrogate for transformation frequency, is calculated by multiplying ∂_N and μ_N for Cases 1, 2 and 3. Figures 18(a), 18(b) and 18(c) show the predicted shapes of dose response for IR induced transformation frequency for three cases, respectively. Figure 18 captured monotonically increasing (Case 1), nonmonotonically increasing or threshold (Case 2) and J-shaped (Case 3) dose response curves.

Table 1. G1/S checkpoint arrest, G2/M checkpoint arrest, cell cycle time and proliferation rate

IRR	0	0.1	0.2	0.3	0.4	0.5	0.6
A1 (min)	0	44	74	84	90	93	93.3
A2 (min)	0	52	75	82	84	89	89
T (min)	450	546	599	616	624	632	632.3
IRD	0	54.6	119.8	184.8	249.6	316	379.4
∂_N (h^{-1})	0.1333	0.1099	0.1002	0.0974	0.0962	0.0949	0.0949

Figure 18 Predicted dose response shapes for IR induced transformation frequency for Cases 1, 2 and 3

Biological mechanisms for specific dose response curves

In order to gain insights with respect to the biological mechanisms, dose response of checkpoint arrest time and proliferation rate are studied. Figure 19 plots the G1/S and G2/M checkpoint arrest time versus IR dose. Figure 20 plots the proliferation rate versus IR dose. The arrest time in both checkpoints tends to saturate when increasing IR to a certain level. The corresponding proliferation rate decreases in a sublinear manner with the increment of IR dose. Therefore, for Case1, when the slope of μ_N function is large, the value of μ_N dominates the result of the product of ∂_N and μ_N in low dose region,

resulting in a monotonically increasing dose response curve. For Case 2, when the slope of μ_N function is moderately small, the increase of μ_N and decrease of ∂_N offset each other in low dose region, leading to a threshold dose response curve. For Case 3, when the slope of μ_N function is small, the value of ∂_N dominates the result of the product of ∂_N and μ_N in low dose region, while μ_N dominates the result in high dose region in which ∂_N tends to saturate, thus resulting in a J-shaped dose response curve. Therefore, the J-shaped dose response is fundamentally caused by the saturation of the checkpoint arrest time when the dose of IR increases.

Figure 20. Dose response curve of IR induced G1/S and G2/M checkpoint arrest time

Figure 21. Dose response curve of proliferation rate vs. IR

The saturation of checkpoint arrest time is also indicated by the degree of the increment of critical cell mass under various doses of IR. Taking G2/M checkpoint control as an example, bifurcation diagram is used to identify the critical cell mass when the rate of IR is equal to 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6, as shown in Figure 22. The critical cell mass is found to increase in a sublinear manner with increasing IR dose.

Figure 22. Bifurcation diagram to identify the critical cell mass in G2/M transition under various doses/dose rates of IR

One advantage of application of computational systems biology approach is that it allows for the use of advanced engineering and mathematical methods to characterize the mechanistic constraints that lead to the “sublinear increment” of the critical cell mass. We can ask “is saturation of the checkpoint arrest time an innate nature determined by the cell cycle regulation system *per se* or by the toxicological perturbation signal?” To answer this question, we simplified the model and used the essential systems inputs and a toxicological perturbation term “R” to represent the model. The inhibition of CycB by R is assumed to be a first order reaction. For example, the G2/M checkpoint and perturbation can be represented by Equations (7) and (8) together with Equation (1):

$$\frac{d[\text{CycB}]}{dt} = k_1 - (k_2 + k_2'[\text{Cdh1}])[\text{CycB}] - R[\text{CycB}] \quad (7)$$

$$\frac{d[\text{Cdh1}]}{dt} = \frac{k_3(1 - [\text{Cdh1}])}{J_3 + 1 - [\text{Cdh1}]} - \frac{k_4 m[\text{CycB}][\text{Cdh1}]}{J_4 + [\text{Cdh1}]} \quad (8)$$

Equations (7) and (8) describes the antagonism between CycB and APC, represented by APC’s auxiliary protein Cdh1, based on Tyson’s work (Tyson and Novak, 2001). Cdh1 is assumed to have the first-order influence on CycB’s degradation. Here the removal of [CycB] is assumed to be dominated by [Cdh1] mediated degradation, that is, $k_2' \gg k_2$ and R. Equation (8) is based on Michaelis-Menton law, describing CycB as an enzyme to phosphorylate Cdh1 (the total Cdh1 activity is scaled to 1 here). The zero-order ultrasensitivity switch is indicated by J_3 and $J_4 \ll 1$ (Tyson and Novak, 2001).

To study steady state, $\frac{d[\text{CycB}]}{dt}$ and $\frac{d[\text{Cdh1}]}{dt}$ are set to zero. Critical m can be calculated by $\frac{d[\text{CycB}^*]}{dm_{\text{critical}}} = 0$, where [CycB*] represents the steady state value of CycB activity. Therefore,

$$m_{\text{critical}} = \frac{k_3 k_2'}{k_1 k_4} \left[1 + 2 \left(\frac{k_2 + R}{k_2'} \right)^{1/2} \right]^{-1} \quad (9)$$

where, m_{critical} is the critical cell mass.

It is easy to prove that the second derivative of m_{critical} with respect to R, $(m_{\text{critical}})_{\text{RR}} < 0$ regardless of the values of the rate constants. That is, critical m increases in a

superlinear manner with respect to **R**. Hence, in order to have a sublinear increment of critical mass when increasing IR dose, there must be a nonlinear relationship (J-shape or threshold relationship) between toxicological perturbation signal **R** and exposure **IR**. The nonlinearity can be obtained by DNA repair (Conolly and Lutz, 2004). Detailed discussion regarding the mechanisms leading to nonlinear relationship between **R** and external exposure is elsewhere (Conolly and Lutz, 2004).

4.0 CONCLUSIONS

This study demonstrates the approach of application of computational systems biology approach in dose response modeling through integration of cell cycle model as the “biology” module. It accounts for molecular mechanisms in dose response model and thus is capable of exploring how transformation frequency is influenced by cellular checkpoint arrest and the associated molecular signaling network. Hence the model includes multiple-level components in biology system. Furthermore, because the direct interaction between toxicological signal and systems biology regulator occurs in the molecular level, the model provides a more detailed description regarding the manner how stressor and genetics interacts directly to impair the function and cause adverse health effects.

The model is developed based upon the complex signaling network in the literature. The newly emerging omics technology dramatically facilitates dissecting and establishing these kinds of network circuit. The application of systems biology approach in dose response modeling is in fact a contemporary approach of the emerging omics technology.

As applying a computational systems biology approach in dose response modeling, mathematical models of signaling pathways of checkpoint arrest and apoptosis induced by IR-mediated DNA damage are first developed. In its current form, module 1 (Fig. 3) links IR exposure to DNA damage but does not contain detailed information about specific kinds of IR, doses and dose-rates, the production of ROS, the kinds of DNA damage produced, and the repair of the damage. While these are all important components of the IR-exposure-health effect continuum, detailed descriptions were beyond the scope of the current investigation. Much of the biochemical detail in the

G2/M checkpoint in module 3 (Fig. 3) is due to Tyson et al. (Tyson and Novak, 2001). We therefore focused our efforts largely on the development of module 2 (Fig. 3) and on the G1/S checkpoint component of module 3 (Fig. 3) in order to obtain a detailed description of the overall pathway that senses DNA damage and transmits signals to cell cycle checkpoints and the apoptotic pathway.

The biochemical detail in these modules, including signaling pathways and regulatory switches at the checkpoints, allows the computational model to recapitulate established qualitative behaviors: DNA damage activates checkpoints; more severe damage leads to extended delays at checkpoints and greater probabilities that cells will apoptose. In addition to these qualitative behaviors, the model successfully reproduces measured time-courses of key proteins (e.g., p53, p21, and caspase 3, Figs. 8, 9 and 10, respectively).

To the extent that the model structure is qualitatively and quantitatively accurate, model-generated predictions of dose- and time-responses should be accurate. Model structure can be no more accurate, however, than the database of mechanistic information used in its development. To assert that the structure of the model is reliable, we assume that the data we have used for model development are accurate and we have extracted a meaningful subset from all of the available relevant information to use for constructing the model. However, as measured values are lacking for many of the parameters of the model (Table A-3), its predictive capabilities are best categorized as qualitative but not quantitative. Development of a quantitatively accurate version of the model will require (1) for each parameter either a data-derived value or a formal sensitivity analysis showing that model behaviors are not sensitive to its value and (2) additional dose-response and time course datasets for the activities of key signaling and regulatory proteins.

The data used to develop the signaling pathway model were obtained from a variety of test systems. Ideally, data would be obtained under uniform experimental conditions using a single biological model. At the level of the biology it describes, the computational model is thus a chimera. Much of the biology in the model is evolutionarily conserved, leading us to think that the model structure is likely to be robust

across cell types and species. The chimerical nature of the model should, however, be viewed as a source of uncertainty in the evaluation of its predictions.

It is interesting to note that Albert and Othmer (Albert and Othmer, 2003) found that the spatial and temporal pattern of expression of the segment polarity genes in *Drosophila melanogaster* is determined by the topology of the gene network and by whether components are absent or present. The behavior of the network is relatively insensitive to the absolute levels of the mRNAs and proteins and the functional details of their interactions. There is thus some hope that insight into key features of the behavior of the signaling pathways and regulatory activities that coordinate DNA repair, cell cycle checkpoints and apoptosis will not require exhaustive enumeration of protein concentrations and reaction rate constants. Additional work will be required to tell whether or not this is the case.

The version of module 2 incorporating parallel signaling pathways (e.g., Figs. 1b and 1c) provided a capability for examination of robustness, an essential feature of complex biological systems (Aderem and Smith, 2004). For example, parallel signaling was predicted to increase the duration of the delay at the G1/S checkpoint for a given exposure to IR (Fig. 11), allowing more time for both DNA repair and for caspases activation leading to apoptosis.

The current signaling pathway model is effectively a model of average behavior of cell population. In subsequent work we plan to extend the model to describe tissue - a population of cells, thereby providing a capability for modeling cells in different phases of the cell cycle as well as modeling intercellular signaling of bystander effects (Matsumoto et al, 2004). While several models for bystander effects have been described (Brenner et al, 2001; Nikjoo and Khvostunov, 2003; Little et al, 2005), none of them incorporate biological detail to the degree it is found in the current model. The tissue-level model is essential for developing reliable predictions of stochastic effects induced in vivo by ionizing radiation. We recognize that a fully mature model would include descriptions of all the quantitatively significant pathways leading from IR exposure to cell death.

Entry into the apoptotic pathway is determined by the activity of biochemical signaling pathways while necrosis is due to relatively large amounts of damage associated with generalized disruption of signaling and of cellular architecture. At its current, early stage of development we are primarily interested in describing intracellular signaling pathways that link detection of DNA damage with activation of cell cycle checkpoint controls and with the apoptotic pathway. We think that these kinds of signaling-mediated events are most likely to be the important determinants of the shape of the dose-response curves at low doses. At somewhat higher doses necrosis will become more important. We will include an option for necrotic cell death in later versions of the model.

While the current model was motivated by an interest in the health effects of IR, its structure is relevant to other environmental stressors that generate DNA DSB, since the signaling pathways from DSB to the cell cycle and to apoptosis are presumably independent of the character of the stressor inducing the DSB. Furthermore, other protein activities and signaling pathways can be incorporated as relevant new data become available or to allow the model to address new questions. For instance, the description of DNA repair in the current version of the model is simple. More detailed descriptions could be developed to describe, for example, the actual biochemical mechanisms of repair of DSB.

One characteristic of computational systems biology approach is that it uses engineering and mathematical simulation method to assist to understand the complex biological system. The current study demonstrates this characteristic –component approaches enables simplification of the complicated system to representative key elements to facilitate investigation; sophisticated mathematical methods allows for more insights into the underlying mechanisms.

A significant contribution of this report in carcinogenic mechanism study is that it captured the nonmonotonic dose response curve for transformation frequency, as identified in the adaptive response experiment. The dominating factor leading to the predicted nonmonotonic dose responses was the increase in cell cycle time associated with activation of checkpoint arrest. The checkpoint arrest time saturates when IR reaches a certain level. This kind of dose response relationship is determined by the

behavior of toxicological signal which directly interacts with the checkpoint control regulating protein. Modeler toxicologists have spent a decade to study the shape of the dose response for low dose region for chemicals and IR, which is particularly important for the risk assessment purpose. An interesting argument emerging is that the hormetic J-shaped dose-response curve depicting low-dose reduction and high-dose enhancement of adverse effects for carcinogenesis, mutagenesis and disease incidence should be taken as the default dose response model in toxicology in particular (Calabrese, 2005). Our study suggested checkpoint arrest can be one of the factors that lead to the nonmonomial shape of these adverse effects. Future study should focus on how apoptosis pathway in the model influences the endpoint of survival fraction as well as transformation frequency through compensatory increase in cell division rate due to cell death. A tissue-level model will finally be developed to incorporate intercellular communication-bystander effect.

A main obstacle of developing systems model is the “parameter identifiability” problem. That is, as the parameter number increase, a situation often arises in which many different sets of model inputs fit the available data equally well. The signaling pathway model in this study has been initially validated by comparing modeling behavior and experimental behavior, focusing on qualitative modeling structure. The more than a hundred parameters in the model were assigned based on the biological mechanism whenever possible. Although the “parameter identifiability” problem more or less exists in the model, the interest of the current study is in the qualitative shape of dose response curve, for which the mechanism (modeling structure) plays a key role and the “parameter identifiability” problem is not a significant factor (Conolly and Lutz, 2004). That different parameter values may shape the dose response curve reflects the influence on dose response relationship by inter-individual variability (Conolly et al., 2005). This problem will exist unless sufficient data are available to identify each parameter value and it should not be the excuse of not developing systems models. In brief, experimental data are an important need in order to use the “systems” model to make quantitative predictions.

In conclusion, we have illustrated with a preliminary example of a computational systems biology approach that can improve our ability to predict dose- and time-response

curves for adverse health effects related to IR exposure. Future research leading to refinements in model structure and to laboratory measurements of key parameter values will lead to mature models and provide an ability to predict health risks on the basis of the relevant biological mechanisms. Replacement of policy- or default-based approaches to risk assessment will help to ensure protection of the public health and alignment of the regulatory standards with more accurate estimates of health risk.

The following paragraphs address limitations of the model based on the recent peer review comments of the associated journal papers of this report. Our development of the G2/M checkpoint module was based on the simple version of the NT model (Tyson and Novak, 2001). The simple version of the NT model was developed for the earliest eukaryotes and has only two checkpoints — Start (G1 to S) and Finish (metaphase to anaphase), and one cyclin — Cyclin B. The Start checkpoint is based on the antagonism between Cyclin B and its cyclin dependent kinase Cdk (referred to in our model as CycB) and APC/Cdh1. We have only recently realized that use of this description from the NT model for our model of the mammalian cell cycle is not fully consistent with the biology of the mammalian cell cycle.

The mammalian cell cycle has three checkpoints: G1/S, G2/M and metaphase to anaphase (Tyson et al. 2002). Since only the mammalian G1/S and G2/M checkpoints are sensitive to DNA damage (Tyson et al. 2002), we omitted the metaphase to anaphase description from our model. Checkpoint regulation in the mammalian cell is complicated, with seven families of cyclins. Among them, Cyclin B and its cyclin dependent kinase Cdk, cyclinB/Cdk, is the regulator and inducer of mitosis (Taylor and Stark 2001), while Cyclin A, Cyclin D and Cyclin E and their dependent kinases are the inducers of S phase (Bartek and Lukas 2001). This is the reason why we took the antagonism between CycB and APC/Cdh1 as the basis for G2/M checkpoint in the current mammalian cell cycle model, rather than for the G1/S checkpoint as described in the NT model of Tyson and Novak (2001).

However, based on experimental biological knowledge, when APC/Cdh1 (CycB's antagonist) activity jumps from a lower stable steady state to a higher stable steady state, it corresponds to G1 to S transition instead of G2 to M transition (Rape and Kirschner 2004). In fact, in the mammalian cell, APC/Cdh1 is inhibited by Cyclin A and its cyclin

dependent kinase in the G1/S phase entry through autonomous regulation and APC/Cdh1 will further influences the activity of CycB in G2/M checkpoint (Rape and Kirschner 2004). The details of the autonomous regulation are described in Rape and Kirschner.

We are currently working on a revised version of this model on G2/M checkpoint. This revised model will include the biology of the autonomous regulation of S-phase entry and the effect on how the autonomous regulation influences G2/M checkpoint. The difference between the existing model and proposed revised model is described in Table 2. However, the modification of the model will not be expected to influence the simulations significantly for the following reasons. First, the revised description of the G2/M checkpoint module is still intended to make the switch of CycB activity as the basis for the G2 to M transition. Second, the IR-induced perturbation pathway model will remain the same as the current modeling effort. The revised version of this model will be a highly mechanistically defensible one.

Table 2. Difference between Protein Activity of Existing Model and Proposed Revised Model

Existing Model		Proposed Revised Model	
G1/S	G2/M	G1/S	G2/M
CycE (low to high) Rb (high to low)	CycB (low to high) APC/Cdh1 (high to low)*	CycE (low to high) Rb (high to low)	CycB (low to high) There is time lag between CycB (low to high) activity and APC/Cdh1 activity (high to low) APC/Cdh1 activity happens (high to low) in G1/S checkpoint, which is promoted by autonomous regulation with CycA (low to high)

* This point causes problem since the switch of APC/Cdh1 activity corresponds to G1/S checkpoint based on biological knowledge (Rape and Kirschner 2004)

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APPENDIX

MATHEMATICAL MODELING

The mathematical model consists of a set of nonlinear ordinary differential equations (ODEs):

$$\frac{dx_i}{dt} = f_i(x_1, \dots, x_n; p_1, \dots, p_m), i = 1, \dots, n$$

(1)

where,

x_i = concentration or activity of the i^{th} protein in the reaction network

f_i = the ODE that describes the i^{th} protein's activity

p_j = value of the j^{th} parameter (e.g. rate constant)

n = number of proteins in the network

Biochemical basis for mathematical equations

As discussed in the Model Development Section, the biochemical reactions in Figs. 1 to 3 include phosphorylation, binding, transcriptional activation, transcriptional repression, induction, repression, and ubiquitylation. The corollaries to phosphorylation and binding are dephosphorylation and dissociation.

The biochemical reactions are mathematically represented by ODEs. Table A-1 summarizes the different reaction types, biochemical reactions and the corresponding ODEs.

Table A-1. Summary of Different Reaction Types, Biochemical Reactions and ODEs

Reaction Types	Biochemical Reactions	Mathematical Equations
Phosphorylation and dephosphorylation	$A + B \xrightleftharpoons[k_b]{k_f} A_B \xrightarrow{k_c} A^p + B$ $A^p + E \xrightleftharpoons[k_b]{k_f} A^p_E \xrightarrow{k_c} A + E$	$\frac{d[A^p]}{dt} = \frac{k_f[A][B]}{[A] + (k_c + k_b)/k_f} - \frac{k_f[A^p][E]}{[A^p] + (k_c + k_b)/k_f}$
Binding and dissociation	$A + B \xrightleftharpoons[k_d]{k_s} AB$	$\frac{d[AB]}{dt} = k_s[A][B] - k_d[AB]$
Transcriptional activation (B transcriptionally activates A)	$\xrightarrow{k_s B} A$	$\frac{d[A]}{dt} = k_s[B]$
Transcriptional repression (B transcriptionally represses A)	$\xrightarrow{k_s(1-B)} A$	$\frac{d[A]}{dt} = k_s(1-[B])$
Induction (B induces A)	$\xrightarrow{k_s B} A$	$\frac{d[A]}{dt} = k_s[B]$
Repression (B represses A)	$\xrightarrow{k_s/B} A$	$\frac{d[A]}{dt} = k_s/[B]$
Degradation/ Ubiquitylation	$A \xrightarrow{k_u}$	$\frac{d[A]}{dt} = -k_u[A]$

Based on Table A-1, the mathematical dynamical system describing the pathways and interactions in Figures 1a to 1c was developed (equations A-1 to A-32). For instance, equation A-26 defines the rate of change of p27 activity. Equation A-7 consists of:

- (1) zero order synthesis of p27 (k_{s7})
- (2) a loss of activity due to binding to CycE ($-k_{s1}[p27][CycE]$)
- (3) an increase of activity due to the dissociation of the CycE-p27 complex ($k_{d1}[p27CycE]$)
- (4) a loss of activity due to phosphorylation by CycE ($-\frac{k_{c5}m[CycE][p27]}{[p27] + \frac{(k_{b5} + k_{c5})}{k_{f5}}}$)
- (5) an increase in activity due to dephosphorylation by a hypothetical enzyme, and it is represented by EN3 ($\frac{k_{c6}[EN3][p27^p]}{[p27^p] + \frac{(k_{b6} + k_{c6})}{k_{f6}}}$)
- (6) a first-order loss of p27 ($-k_{d7}[p27]$).

Another typical example is Equation A-10. In the equation, the ratio of BAX to Bcl-2 is a contributing factor in the rate of change of caspase activity, which is based on experimental results (92).

Following each equation, an explanation of the equation is given. Definition of variables is given in Table A-2. Default parameter values are given in Table A-3. Table A-4 summarizes the equation number corresponding to different modules.

Ionizing radiation (IR)

The rate of change of ROS activity is assumed to be linear with IRR. In the model, IRR is the absorbed dose rate, (e.g. gray/min). Using Δt to represent exposure duration, the total IR exposure is $IRR \times \Delta t$.

Apoptosis

Apoptosis is connected to the model by caspase activity. No description was developed for the current version of the model of the actual numbers of cells entering the apoptotic pathway. We simply assumed that the rate of entry of cells into apoptosis is proportional to caspase activity.

Equations

$$(A-1) \frac{d[ROS]}{dt} = k_1 + k_2[IRR] - k_3[ROS]$$

(The rate of change of ROS activity is equal to the sum of a zero-order production, a first-order production induced by IRR and a first-order degradation)

$$(A-2) \frac{d[DSB]}{dt} = k_4[ROS] - k_5[DSB] - k_{s10}[ATM^p][DSB] + k_{d10}[Drepair]$$

(The rate of change of DSB is equal to the sum of a first-order production, a first-order degradation, a second order decrease due to repair, a first order increase due to degradation of repair)

$$(A-3) \frac{d[Drepair]}{dt} = k_{s10}[ATM^p][DSB] - k_{d10}[Drepair] - k_{d11}[Drepair]$$

(The rate of change of DNA repair is equal to the sum of a second-order production, a first-order induced degradation and a first-order spontaneous degradation)

$$(A-4) \frac{d[ATM^p]}{dt} = k_{c9}[DSB](1 - [ATM^p]) - \frac{k_{c10}[E2][ATM^p]}{[ATM^p] + \frac{(k_{b10} + k_{c10})}{k_{f10}}} + k_{d10}[Drepair]$$

(The rate of change of phosphorylated ATM activity is equal to a second-order production induced by DSB and a dephosphorylation part based on Michaelis-Menten enzymatic reaction; the sum of dephosphorylated ATM and phosphorylated ATM is equal to 1)

$$(A-5) \frac{d[p53^p]}{dt} = \frac{k_{c11}[ATM^p](1 - [p53^p])}{1 - [p53^p] + \frac{(k_{b11} + k_{c11})}{k_{f11}}} - \frac{k_{c12}[E3][p53^p]}{[p53^p] + \frac{(k_{b12} + k_{c12})}{k_{f12}}}$$

(The rate of change of phosphorylated p53 is equal to the sum of a phosphorylation part and a dephosphorylation part; both of them are based on Michaelis-Menten enzymatic reactions)

$$(A-6) \frac{d[p21]}{dt} = k_{s11} + k_{s12}[p53^p] - k_{d12}[p21] - k_{s4}[CycE][p21] + k_{d4}[CycEp21]$$

(The rate of change of p21 is equal to the sum of a zero-order production, a first-order transcriptional activation activated by phosphorylated p53, a first-order degradation, a second-order binding and a second-order dissociation)

$$(A-7) \frac{d[CycEp21]}{dt} = k_{s4}[CycE][p21] - k_{d4}[CycEp21] - k_{de13}[CycEp21]$$

(The rate of change of CycEp21 is equal to the sum of a second-order binding, a first-order dissociation and a first-order degradation)

$$(A-8) \frac{d[BAX]}{dt} = k_{s014} + k_{s14}([p53^p] + [p73^p]) - k_{d14}[BAX]$$

(The rate of change of BAX is equal to the sum of a zero-order production, a first-order transcriptional activation and a first-order degradation)

$$(A-9) \frac{d[Bcl-2]}{dt} = k_{s015} + k_{s15}(1 - [p53^p] - [p73^p]) - k_{d15}[Bcl-2]$$

(The rate of change of Bcl-2 is equal to the sum of a zero-order production, a first-order repression caused by phosphorylated p53 and p73 and a first-order degradation)

$$(A-10) \frac{d[Caspase]}{dt} = k_{s016} + k_{s16} \frac{[BAX]}{[Bcl-2]} - k_{d16}[Caspase]$$

(The rate of change of Caspase is equal to the sum of a zero-order production, a first-order production proportional to the ratio of BAX to Bcl-2 activity and a first-order degradation)

$$(A-11) \frac{d[inhibitor]}{dt} = k_{s017} + k_{s17}[p53^p] - k_{d17}[inhibitor]$$

(The rate of change of inhibitor is equal to the sum of a zero-order production, a first-order transcriptional activation and a first-order degradation)

(A-12)

$$\frac{d[CycEdp]}{dt} = \frac{k_{end1}[Cdc25A]([CycE] - [CycEdp])}{J_{end1} + [CycE] - [CycEdp]} - \frac{k_{end2} \bullet End1[CycEdp]}{J_{end2} + [CycEdp]}$$

(The rate of change of dephosphorylated CycE is equal to the sum of a dephosphorylation part and a phosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-13) \frac{d[Chk^p]}{dt} = \frac{k_{end3}[ATM^p](1 - [Chk^p])}{J_{end3} + 1 - [Chk^p]} - \frac{k_{end4} \bullet E_{end2}[Chk^p]}{J_{end4} + [Chk^p]}$$

(The rate of change of phosphorylated Chk is equal to the sum of a phosphorylation part and a dephosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-14) \frac{d[Cdc25A]}{dt} = k_{end5} - k_{end6}[Chk^p][Cdc25A]$$

(The rate of change of Cdc25A is equal to the sum of a zero-order production and a second-order dissociation)

(A-15)

$$\frac{d[cAbl]}{dt} = -k_{s18}[Rb][cAbl] + k_{d18}[RbcAbl] - \frac{k_{c13}[ATM^p][cAbl]}{[cAbl] + \frac{(k_{b13} + k_{c13})}{k_{f13}}} + \frac{k_{c14}[E19p73][cAbl^p]}{[cAbl^p] + \frac{(k_{b14} + k_{c14})}{k_{f14}}}$$

(The rate of change of cAbl is equal to the sum of a second-order binding, a first-order dissociation, a phosphorylation part and a dephosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-16) \frac{d[cAbl^p]}{dt} = \frac{k_{c13}[ATM^p][cAbl]}{[cAbl] + \frac{(k_{b13} + k_{c13})}{k_{f13}}} - \frac{k_{c14}[E19p73][cAbl^p]}{[cAbl^p] + \frac{(k_{b14} + k_{c14})}{k_{f14}}}$$

(The rate of change of phosphorylated cAbl is equal to the sum of a phosphorylation part and a dephosphorylation part based on Michalis-Menton enzymatic reactions)

(A-17)

$$\frac{d[p73]}{dt} = k_{s019} + k_{s19}[E2F] - k_{d19}[p73] - \frac{k_{c15}[cAbl^p][p73]}{[p73] + \frac{(k_{b15} + k_{c15})}{k_{f15}}} + \frac{k_{c16}[E22p73][p73^p]}{[p73^p] + \frac{(k_{b16} + k_{c16})}{k_{f16}}}$$

(The rate of change of p73 is equal to the sum of a zero-order production, a first-order production, a first-order degradation, a dephosphorylation and a phosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-18) \frac{d[p73^p]}{dt} = \frac{k_{c15}[cAbl^p][p73]}{[p73] + \frac{(k_{b15} + k_{c15})}{k_{f15}}} - \frac{k_{c16}[E22p73][p73^p]}{[p73^p] + \frac{(k_{b16} + k_{c16})}{k_{f16}}}$$

(The rate of change of phosphorylated p73 is equal to the sum of a phosphorylation and a dephosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-19) \frac{d[RbcAbl]}{dt} = k_{s18}[Rb][cAbl] - k_{d18}[RbcAbl]$$

(The rate of change of RbcAbl is equal to the sum of a second-order binding and a first-order dissociation)

(A-20)

$$\begin{aligned} \frac{d[CycE]}{dt} = & -k_{s1}[p27][CycE] + k_{d1}[p27CycE] - k_{s2}[p27^p][CycE] + k_{d2}[p27^pCycE] \\ & + k_{s3}[E2F] - k_{d3}[CycE] - k_{s4}[CycE][p21] + k_{d4}[CycEp21] \end{aligned}$$

(The rate of change of CycE is equal to the sum of a second-order binding, a first-order dissociation, a second-order binding, a first-order dissociation, a first-order degradation, a second-order binding and a first-order dissociation)

(A-21)

$$\frac{d[E2F]}{dt} = -k_{s5}[Rb][E2F] + k_{d5}[RbE2F] - k_{s6}[Rb^p][E2F] + k_{d6}[Rb^pE2F]$$

(The rate of change of E2F is equal to the sum a second-order binding, a first-order dissociation, a second-order binding and a first-order dissociation)

$$(A-22) \frac{d[Rb]}{dt} = -k_{s5}[Rb][E2F] + k_{d5}[RbE2F] - \frac{k_{c1}m[CycE][Rb]}{[Rb] + \frac{(k_{b1} + k_{c1})}{k_{f1}}} + \frac{k_{c2}[EN8][Rb^p]}{[Rb^p] + \frac{(k_{b2} + k_{c2})}{k_{f2}}}$$

(The rate of change of Rb is equal to the sum of a second-order binding, a first-order dissociation, a dephosphorylation and a phosphorylation part based on Michalis-Menton enzymatic reactions)

(A-22')

$$\begin{aligned} \frac{d[Rb]}{dt} = & -k_{s5}[Rb][E2F] + k_{d5}[RbE2F] - \frac{k_{c1}m[CycE][Rb]}{[Rb] + \frac{(k_{b1} + k_{c1})}{k_{f1}}} + \frac{k_{c2}[EN8][Rb^p]}{[Rb^p] + \frac{(k_{b2} + k_{c2})}{k_{f2}}} \\ & - k_{sp73}[Rb][cAbl] + k_{dp73}[RbcAbl] \end{aligned}$$

(The rate of change of Rb is equal to the sum of a second-order binding, a first-order dissociation, a dephosphorylation and a phosphorylation part based on Michalis-Menton enzymatic reactions and a second-order binding and a first-order dissociation)

(A-22'')

$$\begin{aligned} \frac{d[Rb]}{dt} = & -k_{s5}[Rb][E2F] + k_{d5}[RbE2F] - \frac{k_{c1}m[CycEdp][Rb]}{[Rb] + \frac{(k_{b1} + k_{c1})}{k_{f1}}} + \frac{k_{c2}[EN8][Rb^p]}{[Rb^p] + \frac{(k_{b2} + k_{c2})}{k_{f2}}} \\ & - k_{sp73}[Rb][cAbl] + k_{dp73}[RbcAbl] \end{aligned}$$

(The rate of change of Rb is equal to the sum of a second-order binding, a first-order dissociation, a dephosphorylation and a phosphorylation part based on Michalis-Menton enzymatic reactions and a second-order binding and a first-order dissociation)

(A-23)

$$\frac{d[Rb^p]}{dt} = \frac{k_{c1}m[CycE][Rb]}{[Rb] + \frac{k_{b1} + k_{c1}}{k_{f1}}} - \frac{k_{c2}[EN8][Rb^p]}{[Rb^p] + \frac{k_{b2} + k_{c2}}{k_{f2}}} - k_{s6}[Rb^p][E2F] + k_{d6}[Rb^pE2F]$$

(The rate of change of phosphorylated Rb is equal to the sum of a phosphorylation and dephosphorylation part, a second-order binding and a first-order dissociation)

(A-23'')

$$\frac{d[Rb^p]}{dt} = \frac{k_{c1}m[CycEdp][Rb]}{[Rb] + \frac{k_{b1} + k_{c1}}{k_{f1}}} - \frac{k_{c2}[EN8][Rb^p]}{[Rb^p] + \frac{k_{b2} + k_{c2}}{k_{f2}}} - k_{s6}[Rb^p][E2F] + k_{d6}[Rb^pE2F]$$

(The rate of change of phosphorylated Rb is equal to the sum of a phosphorylation and dephosphorylation part based on Michalis-Menton enzymatic reactions, a second-order binding and a first-order dissociation)

$$\begin{aligned}
\frac{d[RbE2F]}{dt} &= k_{s5}[Rb][E2F] - k_{d5}[RbE2F] - \frac{k_{c3}m[CycE][RbE2F]}{[RbE2F] + \frac{(k_{b3} + k_{c3})}{k_{f3}}} \\
&+ \frac{k_{c4}[EN10][Rb^pE2F]}{[Rb^pE2F] + \frac{(k_{b4} + k_{c4})}{k_{f4}}}
\end{aligned}
\tag{A-24}$$

(The rate of change of RbE2F is equal to the sum of a second-order binding, a first-order dissociation, a phosphorylation and dephosphorylation part based on Michalis-Menton enzymatic reactions)

$$\begin{aligned}
\frac{d[RbE2F]}{dt} &= k_{s5}[Rb][E2F] - k_{d5}[RbE2F] - \frac{k_{c3}m[CycEdp][RbE2F]}{[RbE2F] + \frac{(k_{b3} + k_{c3})}{k_{f3}}} \\
&+ \frac{k_{c4}[EN10][Rb^pE2F]}{[Rb^pE2F] + \frac{(k_{b4} + k_{c4})}{k_{f4}}}
\end{aligned}
\tag{A-24''}$$

(The rate of change of RbE2F is equal to the sum of a second-order binding, a first-order dissociation, a phosphorylation and dephosphorylation part based on Michalis-Menton enzymatic reactions)

$$\begin{aligned}
\frac{d[Rb^pE2F]}{dt} &= k_{s6}[Rb^p][E2F] - k_{d6}[Rb^pE2F] + \frac{k_{c3}m[CycE][RbE2F]}{[RbE2F] + \frac{(k_{b3} + k_{c3})}{k_{f3}}} \\
&- \frac{k_{c4}[EN10][Rb^pE2F]}{[Rb^pE2F] + \frac{(k_{b4} + k_{c4})}{k_{f4}}}
\end{aligned}
\tag{A-25}$$

(The rate of change of Rb^pE2F is equal to the sum of a second-order binding, a first-order dissociation, a phosphorylation and dephosphorylation part based on Michalis-Menton enzymatic reactions)

(A-25'')

$$\begin{aligned} \frac{d[Rb^p E2F]}{dt} = & k_{s6}[Rb^p][E2F] - k_{d6}[Rb^p E2F] + \frac{k_{c3}m[CycEdp][RbE2F]}{[RbE2F] + \frac{(k_{b3} + k_{c3})}{k_{f3}}} \\ & - \frac{k_{c4}[EN10][Rb^p E2F]}{[Rb^p E2F] + \frac{(k_{b4} + k_{c4})}{k_{f4}}} \end{aligned}$$

(The rate of change of Rb^pE2F is equal to the sum of a second-order binding, a first-order dissociation, a phosphorylation and dephosphorylation part based on Michalis-Menton enzymatic reactions)

(A-26)

$$\frac{d[p27]}{dt} = k_{s7} - k_{s1}[p27][CycE] + k_{d1}[p27CycE] - \frac{k_{c5}m[CycE][p27]}{[p27] + \frac{(k_{b5} + k_{c5})}{k_{f5}}} + \frac{k_{c6}[EN3][p27^p]}{[p27^p] + \frac{(k_{b6} + k_{c6})}{k_{f6}}} - k_{d7}[p27]$$

(The rate of change of p27 is equal to the sum of a zero-order production, a second-order binding, a first-order dissociation, a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions and a first-order degradation)

(A-26'')

$$\frac{d[p27]}{dt} = k_{s7} - k_{s1}[p27][CycE] + k_{d1}[p27CycE] - \frac{k_{c5}m[CycEdp][p27]}{[p27] + \frac{(k_{b5} + k_{c5})}{k_{f5}}} + \frac{k_{c6}[EN3][p27^p]}{[p27^p] + \frac{(k_{b6} + k_{c6})}{k_{f6}}} - k_{d7}[p27]$$

(The rate of change of p27 is equal to the sum of a zero-order production, a second-order binding, a first-order dissociation, a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions and a first-order degradation)

$$\begin{aligned} \frac{d[p27^p]}{dt} = & \frac{k_{c3}m[CycE][p27]}{[p27] + \frac{(k_{b3} + k_{c3})}{k_{f3}}} - \frac{k_{c6}[EN3][p27^p]}{[p27^p] + \frac{(k_{b6} + k_{c6})}{k_{f6}}} + k_{d2}[p27^p CycE] \\ (A-27) \quad & - k_{s2}[p27^p][CycE] - k_{d9}[p27^p] \end{aligned}$$

(The rate of change of phosphorylated p27 is equal to the sum of a phosphorylation and dephosphorylation part based on Michalis-Menton enzymatic reactions, a first-order dissociation, a second-order binding and a first-order degradation)

$$(A-27'') \quad \frac{d[p27^p]}{dt} = \frac{k_{c3}m[CycEdp][p27]}{[p27] + \frac{(k_{b3} + k_{c3})}{k_{f3}}} - \frac{k_{c6}[EN3][p27^p]}{[p27^p] + \frac{(k_{b6} + k_{c6})}{k_{f6}}} + k_{d2}[p27^p CycE] - k_{s2}[p27^p][CycE] - k_{d9}[p27^p]$$

(The rate of change of phosphorylated p27 is equal to the sum of a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions, a first-order dissociation, a second-order binding and first-order degradation)

$$(A-28) \quad \begin{aligned} \frac{d[p27CycE]}{dt} = & k_{s1}[p27][CycE] - k_{d1}[p27CycE] - \frac{k_{c7}m[CycE][p27CycE]}{[p27CycE] + \frac{(k_{b7} + k_{c7})}{k_{f7}}} \\ & + \frac{k_{c8}[EN4][p27^p CycE]}{[p27^p CycE] + \frac{(k_{b8} + k_{c8})}{k_{f8}}} \end{aligned}$$

(The rate of change of p27CycE is equal to the sum of a second-order binding, a first-order dissociation, a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-28'') \quad \begin{aligned} \frac{d[p27CycE]}{dt} = & k_{s1}[p27][CycE] - k_{d1}[p27CycE] - \frac{k_{c7}m[CycEdp][p27CycE]}{[p27CycE] + \frac{(k_{b7} + k_{c7})}{k_{f7}}} \\ & + \frac{k_{c8}[EN4][p27^p CycE]}{[p27^p CycE] + \frac{(k_{b8} + k_{c8})}{k_{f8}}} \end{aligned}$$

(The rate of change of p27CycE is equal to the sum of a second-order binding, a first-order dissociation, a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions)

(A-29)

$$\frac{d[p27^p \text{CycE}]}{dt} = \frac{k_{c7}m[\text{CycE}][p27\text{CycE}]}{[p27\text{CycE}] + \frac{(k_{b7} + k_{c7})}{k_{f7}}} - \frac{k_{c8}[\text{EN4}][p27^p \text{CycE}]}{[p27^p \text{CycE}] + \frac{(k_{b8} + k_{c8})}{k_{f8}}} + k_{s2}[p27^p][\text{CycE}] - k_{d2}[p27^p \text{CycE}]$$

(The rate of change of p27^pCycE is equal to the sum of a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions, a second-order binding and a first-order dissociation)

$$(A-29'') \quad \frac{d[p27^p \text{CycE}]}{dt} = \frac{k_{c7}m[\text{CycEdp}][p27\text{CycE}]}{[p27\text{CycE}] + \frac{(k_{b7} + k_{c7})}{k_{f7}}} - \frac{k_{c8}[\text{EN4}][p27^p \text{CycE}]}{[p27^p \text{CycE}] + \frac{(k_{b8} + k_{c8})}{k_{f8}}} + k_{s2}[p27^p][\text{CycE}] - k_{d2}[p27^p \text{CycE}]$$

(The rate of change of p27^pCycE is equal to the sum of a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions, a second-order binding and a first-order dissociation)

(A-30)

$$\frac{d[\text{CycB}]}{dt} = kt - (kt_{21} + kt_{22}[\text{Cdh1}] + kt_{23}[\text{Cdc20A}])([\text{CycB}]) - kt_{add}[\text{Inhibitor}][\text{CycB}]$$

(The rate of change of CycB is equal to the sum of a zero-order production, a first-order production, a second-order production, a second-order production and a second-order binding)

$$(A-31) \quad \frac{d[\text{Cdh1}]}{dt} = \frac{(kt_{31} + kt_{32}[\text{Cdc20A}])(1 - [\text{Cdh1}])}{Jt3 + 1 - [\text{Cdh1}]} - \frac{kt_4m[\text{CycB}][\text{Cdh1}]}{Jt4 + [\text{Cdh1}]}$$

(The rate of change of Cdh1 is equal to the sum of a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-31'') \quad \frac{d[\text{Cdh1}]}{dt} = \frac{(kt_{31} + kt_{32}[\text{Cdc20A}])(1 - [\text{Cdh1}])}{Jt3 + 1 - [\text{Cdh1}]} - \frac{kt_4m[\text{CycBdp}][\text{Cdh1}]}{Jt4 + [\text{Cdh1}]}$$

(The rate of change of Cdh1 is equal to the sum of a dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions)

$$(A-32) \frac{d[Cdc20T]}{dt} = kt_{51} + kt_{52} \frac{\left(\frac{m[CycB]}{Jt5}\right)^n}{1 + \left(\frac{m[CycB]}{Jt5}\right)^n} - kt_6[Cdc20T]$$

(The rate of change of Cdc20T is equal to the sum of a zero-order production, a production based on Hill function and a first-order degradation)

$$(A-32'') \frac{d[Cdc20T]}{dt} = kt_{51} + kt_{52} \frac{\left(\frac{m[CycBdp]}{Jt5}\right)^n}{1 + \left(\frac{m[CycBdp]}{Jt5}\right)^n} - kt_6[Cdc20T]$$

(The rate of change of Cdc20T is equal to the sum of a zero-order production, a production based on Hill function and a first-order degradation)

(A-33)

$$\frac{d[Cdc20A]}{dt} = \frac{kt_7[IEP]([Cdc20T] - [Cdc20A])}{Jt7 + [Cdc20T] - [Cdc20A]} - \frac{kt_8[Mad][Cdc20A]}{Jt8 + [Cdc20A]} - kt_6[Cdc20A]$$

(The rate of change of Cdc20A is equal to the sum of dephosphorylation and phosphorylation part based on Michalis-Menton enzymatic reactions and a first-order degradation)

$$(A-34) \frac{d[IEP]}{dt} = kt_9m[CycB](1 - [IEP]) - kt_{10}[IEP]$$

(The rate of change of IEF is equal to the sum of a second-order production and a first-order degradation)

$$(A-35) \frac{dm}{dt} = \mu * m * (1 - m / m_{\max})$$

(Cell mass is assumed to follow a logistic function)

Table A-2. Definition of Variables

Variables	Definitions	Variables	Definitions
CycE	Total Cyclin E/Cdk 2 dimer	CycEp21	P21_Cyclin E_Cdk trimer
CycEdp	Dephosphorylated form of CycE	BAX	---
E2F	E2F transcription factor	BCL-2	---
Rb	Dephosphorylated (p-) Retinoblastoma	Caspase	---
Rb ^p	Phosphorylated (p+) Rb	cAbl	cAbl (p-)
RbE2F	Rb_E2F complex	cAbl ^p	cAbl (p+)
Rb ^p E2F	Rb_E2F complex with Rb (p+)	p73	---
P27	p27 (p-)	p73 ^p	p73 (p+)
P27 ^p	p27 (p+)	RbcAbl	Rb_cAbl complex
P27CycE	P27_Cyclin E_Cdk trimer	m	Cell mass
P27 ^p CycE	P27_Cyclin E_Cdk trimer with p27 (p+)	CycB	Total Cyclin B/Cdk1 dimer
ROS	Reactive Oxygen Species	CycBdp	Dephosphorylated form of CycB
DSB	Double Strand Break	Cdh1	---
Drepair	Repaired DSB	Cdc20T	Total Cdc20
ATM ^p	Ataxia Telangiectasia mutated (p+)	Cdc20A	Activated Cdc20
P53 ^p	Phosphorylated p53	IEP	Hypothetical “Intermediary enzyme”
P21	p21	Inhibitor	The sum of GADD45 and 1433 sigma

Table A-3. Default parameter values

Parameter	Name	Equation No.	Value (min ⁻¹)	Source
k ₁	zero-order rate constant	1	0.01	-
k ₂	first-order rate constant	1	0.1	-
k ₃	first-order rate constant	1	0.04	-
k ₄	first-order rate constant	2	0.04	-
k ₅	first-order rate constant	2	0.04	-
k _{d11}	first-order rate constant	3	0.002	-
k _{c9}	second-order rate constant	4	0.1	-
k _{s10} ¹	second-order rate constant	2, 3, 4	0.8 or 2	-
k _{d10}	first-order rate constant	2, 3, 4	0.02	-
k _{c10}	dissociation rate constant	4	1	-
k _{b10}	backward rate constant	4	1	-
k _{f10}	forward rate constant	4	5	-
k _{c11}	dissociation rate constant	5	1	-
k _{b11}	backward rate constant	5	1	-
k _{f11}	forward rate constant	5	5	-
k _{c12}	dissociation rate constant	5	1	-
k _{b12}	backward rate constant	5	1	-
k _{f12}	forward rate constant	5	5	-
E2	hypothetic enzyme	4	0.1	-
E3	hypothetic enzyme	5	0.1	-
k _{s12}	first-order rate constant	6	0.04	-
k _{d12}	first-order rate constant	6	0.04	-
k _{s11}	zero-order rate constant	6	0	-
k _{de13}	first-order rate constant	7	0.1	-
k _{s014}	zero-order rate constant	8	0	-
k _{s015}	zero-order rate constant	9	0	-
k _{s15}	first-order rate constant	9	0.1	-
k _{s14}	first-order rate constant	8	0.1	-

Table A-3. Continued

Parameter	Name	Equation No.	Value (min ⁻¹)	Source
k _{d14}	first-order rate constant	8	0.001	-
k _{s016}	zero-order rate constant	10	0	-
k _{s16}	first-order rate constant	10	0.001	-
k _{d15}	first-order rate constant	9	0.01	-
k _{d16}	first-order rate constant	10	0.01	-
k _{s017}	zero-order rate constant	11	0	-
k _{s17}	first-order rate constant	11	0.04	-
k _{d17}	first-order rate constant	11	0.04	-
k _{end1}	dissociation rate constant	12	20	-
J _{end1}	Michalis-Menton constant	12	0.04	-
k _{end2}	dissociation rate constant	12	1	-
E _{end1}	hypothetic enzyme	12	0.1	-
J _{end2}	Michalis-Menton constant	12	0.04	-
k _{end3}	dissociation rate constant	13	0.04	-
J _{end3}	Michalis-Menton constant	13	0.04	-
k _{end4}	dissociation rate constant	13	0.001	-
E _{end2}	hypothetic enzyme	13	0.1	-
J _{end4}	Michalis-Menton constant	13	0.04	-
k _{end5}	zero-order rate constant	14	0.001	-
k _{end6}	second-order rate constant	14	0.45	-
k _{s18}	second-order rate constant	15	0.1	-
k _{d18}	first-order rate constant	19	0.01	-
k _{c13}	dissociation rate constant	15, 16	0.2	-
k _{b13}	backward rate constant	15, 16	0.2	-
k _{f13}	forward rate constant	15, 16	10	-
k _{c14}	dissociation rate constant	15, 16	0.02	-
k _{b14}	backward rate constant	15, 16	0.02	-
k _{f14}	forward rate constant	15, 16	10	-
E19p73	hypothetic enzyme	16	0.01	-
E22p73	hypothetic enzyme	17	0.01	-
k _{s019}	zero-order rate constant	17	0.0	-
k _{s19}	first-order rate constant	17	0.005	-
k _{d19}	first-order rate constant	17	0.005	-
k _{c15}	dissociation rate constant	17, 18	0.02	-
k _{b15}	backward rate constant	17, 18	0.02	-
k _{f15}	forward rate constant	17, 18	20	-
k _{c16}	dissociation rate constant	17, 18	0.2	-
k _{b16}	backward rate constant	17, 18	0.2	-
k _{f16}	forward rate constant	17	10	-
k _{s1}	second-order rate constant	20, 26, 26'', 28, 28''	1	-
k _{d1}	first-order rate constant	20, 26, 26'', 28, 28''	1	-
k _{s2}	second-order rate constant	20, 27, 27'', 29, 29''	1	-
k _{d2}	first-order rate constant	20, 27, 27'', 29, 29''	1	-
k _{s3}	first-order rate constant	20	0.35	-
k _{d3}	first-order rate constant	20	0.5	-
k _{s4}	second-order rate constant	20, 6, 7	10	-
k _{d4}	first-order rate constant	20, 6, 7	1	-
k _{s5}	second-order rate constant	21, 22, 22', 22''	1	-
k _{d5}	first-order rate constant	21, 22, 22', 22''	0.01	-
k _{s6}	second-order rate constant	21, 23, 23'', 25, 25''	1	-
k _{d6}	first-order rate constant	21, 23, 23'', 25, 25''	1	-

Table A-3. Continued

Parameter	Name	Equation No.	Value (min ⁻¹)	Source
k_{c1}	Michalis-Menton rate constant (the dissociation rate constant of the substrate and enzyme complex, later on called dissociation rate constant)	22, 22', 22'', 23, 23''	1	-
k_{b1}	Michalis-Menton rate constant (the backward rate constant of the substrate and enzyme binding reaction, later on called backward rate constant)	22, 22', 22'', 23, 23''	1	-
k_{f1}	Michalis-Menton rate constant (the forward rate constant of the substrate and enzyme binding reaction, later on called forward rate constant)	22, 22', 22'', 23, 23''	0.1	-
k_{c2}	dissociation rate constant	22, 22', 22'', 23, 23''	0.1	-
k_{b2}	backward rate constant	22, 22', 22'', 23, 23''	0.1	-
k_{f2}	forward rate constant	22, 22', 22'', 23, 23''	50	-
EN8	hypothetic enzyme	22, 22', 22''	0.1	-
EN10	hypothetic enzyme	24, 24''	0.1	-
k_{sp73}	second-order rate constant	22', 22''	0.1	-
k_{dp73}	first-order rate constant	22', 22''	0.01	-
k_{s7}	zero-order rate constant	26, 26''	0.0004	-
k_{d7}	first-order rate constant	26, 26''	0.0001	-
k_{c3}	dissociation rate constant	24, 24'', 25, 25''	1	-
k_{b3}	backward rate constant	24, 24'', 25, 25''	1	-
k_{f3}	forward rate constant	24, 24'', 25, 25''	10	-
k_{c4}	dissociation rate constant	24, 24'', 25, 25''	1	-
k_{b4}	backward rate constant	24, 24'', 25, 25''	1	-
k_{f4}	forward rate constant	24, 24'', 25, 25''	10	-
k_{c5}	dissociation rate constant	26, 26'', 27, 27''	0.1	-
k_{b5}	backward rate constant	26, 26'', 27, 27''	0.1	-
k_{f5}	forward rate constant	26, 26'', 27, 27''	1	-
k_{c6}	dissociation rate constant	26, 26'', 27, 27''	0.25	-
k_{b6}	backward rate constant	26, 26'', 27, 27''	0.5	-
k_{f6}	forward rate constant	26, 26'', 27, 27''	0.5	-
k_{s8}	second-order rate constant	27, 27''	1	-
k_{d8}	first-order rate constant	27, 27''	2	-
k_{c7}	dissociation rate constant	28, 28'', 29, 29''	0.001	-
k_{b7}	backward rate constant	28, 28'', 29, 29''	0.001	-
k_{f7}	forward rate constant	28, 28'', 29, 29''	1	-
k_{d9}	first-order rate constant	27, 27''	1	-
k_{c8}	dissociation rate constant	28, 28'', 29, 29''	1	-
k_{b8}	backward rate constant	28, 28'', 29, 29''	1	-
k_{f8}	forward rate constant	28, 28'', 29, 29''	5	-
μ^2	mass rate constant	35	0.005 or 0.01	Ref. 48
m_{max}	maximal cell mass	35	10	Ref. 48
kt_{23}	second-order rate constant	30	1	Ref. 48
Kt	zero-order rate constant	30	0.04	Ref. 48
kt_{21}	first-order rate constant	30	0.04	Ref. 48
kt_{22}	second-order rate constant	30	1	Ref. 48

Table A-3. Continued

Parameter	Name	Equation No.	Value (min ⁻¹)	Source
Jt_3	Michalis-Menton constant equal to $(k_b+k_c)/k_f$, later on called Michalis-Menton constant	31, 31''	0.04	Ref. 48
kt_{add}^3	second-order rate constant	30	0.1 or 0.9	Ref. 48
kt_{31}	dissociation rate constant	31, 31''	1	Ref. 48
kt_{32}	dissociation rate constant	31, 31''	10	Ref. 48
Jt_5	Michalis-Menton constant	32, 32''	0.3	Ref. 48
Jt_4	Michalis-Menton constant	31, 31''	0.04	Ref. 48
kt_{51}	zero-order rate constant	32	0.005	Ref. 48
kt_{52}	first-order rate constant	32	0.2	Ref. 48
Jt_7	Michalis-Menton constant	33	0.001	Ref. 48
N	Hill coefficient	32	4	Ref. 48
Kt_6	first-order rate constant	32	0.01	Ref. 48
Kt_7	dissociation rate constant	33	1	Ref. 48
Kt_9	second-order rate constant	34	0.1	Ref. 48
Kt_8	dissociation rate constant	33	0.5	Ref. 48
Jt_8	Michalis-Menton constant	33	0.001	Ref. 48
Mad^*	Hypothetic enzyme	33	1	Ref. 48
kt_{10}	first-order rate constant	34	0.02	Ref. 48

¹ For the simulation of Figure 9, $k_{s10} = 2$; for others, $k_{s10} = 0.8$.

² For the simulation of Figure 8, $\mu = 0.01$; for others, $\mu = 0.005$.

³ For the simulation of Figures 7 and 8, $kt_{add} = 0.9$; for others, $kt_{add} = 0.1$.

Table A-4. Summary of Equation Number for Different Modules

Module	Equation
Module 1	A-1~A-2
Module 2, p53	A-3~A-11
Module 2, Chk2-Cdc25	A-3, A-4, A-12~A-14
Module 2, p73	A-3, A-4, A-15~A-19, A-22'
Module 3, G1/S, p53	A-20~A-29
Module 3, G1/S, Chk2-Cdc25	A-20, A-21, A-22''~A-29''
Module 3, G2/M, p53	A-11, A-30~A-34
Module 3, G2/M, Chk2-Cdc25	A-11, A-30, A-31'', A-32'', A-33, A-34