Modeling of the P53 pathway to Cell Cycle arrest and Apoptosis: Relevance to Cancer

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Abstract:

This project report describes the p53 protein pathways to cell arrest and apoptosis. The essential elements of the cell cycle and the role of various proteins is explained. The p53-Mdm2 feedback loop is modeled using a differential equation model and simulations are conducted using SIMULINK software. A network of interacting proteins is developed and we propose the use of a kinetic logic model to discuss the macroscopic effects due to mutations and parameter variations. The discussion also stresses on the effect and relevance of the p53 pathways to cancer.

1. Introduction:

The fundamental process underlying all biological growth and reproduction is the cell division cycle. It is the sequence of events whereby a living cell duplicates its machinery and distributes it between daughter cells capable of carrying out the whole sequence again. The cell cycle is a very well programmed phenomenon and is grossly divided into 4 phases. These are M-Mitosis phase, G1-growth phase 1, S-synthesis phase, G2- growth phase 2 (Fig1.). DNA is replicated in S phase and the 2 copies are separated in M phase. Resting or senescent cells exit the cell cycle from G1 and enter a quiescent state called G0. In a typical somatic cell cycle, the M phase lasts for 30 min; while S and G2 last for 10 hours and 4.5 hours respectively. The variation in cell-cycle length primarily arises from the length of the G0/G1 phase. **Cyclin/CDK** (Cyclin dependent kinases) complexes (Fig2.) phosphorylate specific substrates at appropriate phases in the cell cycle, driving the cellular events necessary for progress from one phase to the other. In humans, the active cyclins/CDK complexes in the different phases are shown in Fig1. In lower Eukaryotes, there is a smaller number of CDKs but they react with different promoting cyclins to have a similar effect as in the higher eukaryotes. These Cyclin/CDKs are also regulated by phosphorylation and binding of inhibitory molecules. The CAK (CDK activating kinase) positively phosphorylates the Cyclin/CDK at its Thr160 residue whereas, Wee1 and Myt1 kinases negatively phosphorylate the same at the Tyr14 and Thr 15 residues. The inhibitory phosphorylations are removed by Cdc25 phosphatase. The inhibitory molecules belong to the class of INK4 family (p16, p19 etc.) and the Cip1/Kip1 family (p21 etc.). Each **transition** i.e. entry and exit into the respective phases of the cell-cycle, is tightly regulated and there exist surveillance mechanisms to monitor the integrity of DNA and regulate the progression of the cell cycle. These are called **checkpoints**. These are specialized intracellular signaling pathways that are triggered by defects and malfunctions such as DNA damage. The activation of a checkpoint normally leads to one of the following 2 phenomenon. The first is **cell-cycle arrest**, which is accompanied by the activation of the DNA repair machinery (Guardians of

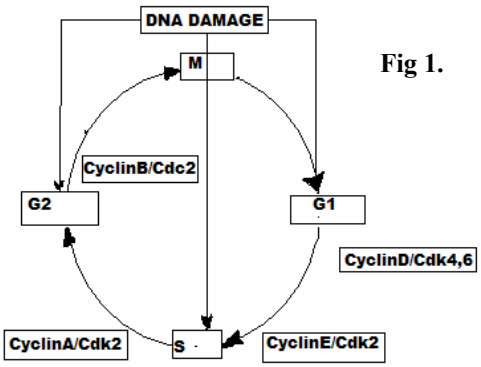


Fig 1.

the Genome). The second outcome is a programmed active cell death, also called **Apoptosis**. Apoptosis is synonymous to intentional cell-suicide; in contrast to **Necrosis**, which a type of cell death that results from a severe injury that affects the physical integrity of the cell. It must be noted that the signaling pathways for arrest and apoptosis, although linked, can function independently as has been proved in knockout studies what have been able to suppress one or the other without affecting the intact phenotype significantly. This is because the cellular

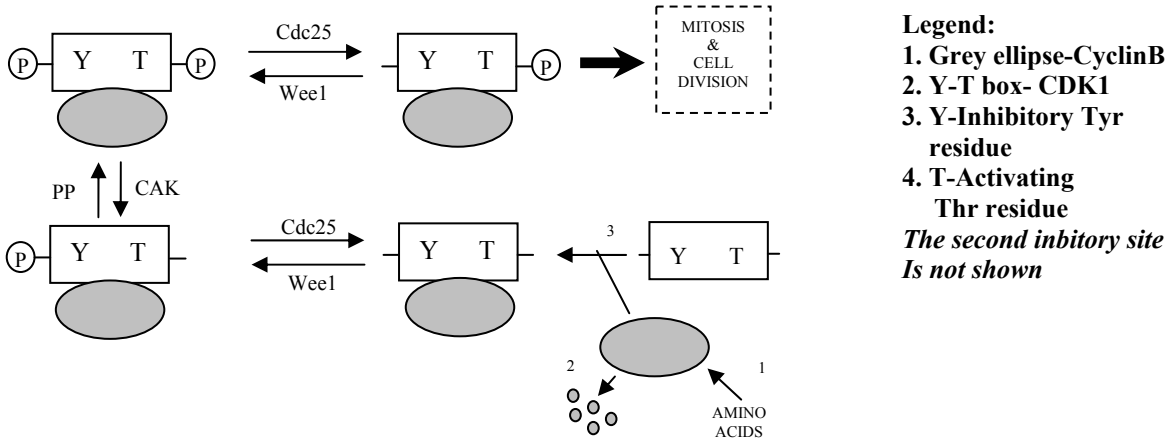


Fig 2: The Phosphorylation States of CyclinB/Cdk1

biomacromolecules involved in each pathway are different. However, the two pathways do have intersections and one such focus is the p53 (an important multifaceted tumor suppressor gene/protein) feedback loop and pathway which is the subject of discussion of this paper. Although, the exact mechanism by which p53 accumulation selects either arrest or apoptosis is not known, some hypotheses are mentioned.

Note: All proteins are named after the corresponding gene except in lower case. e.g. p53 –protein; P53-gene.

2. Checkpoints and Relevance to Cancer:

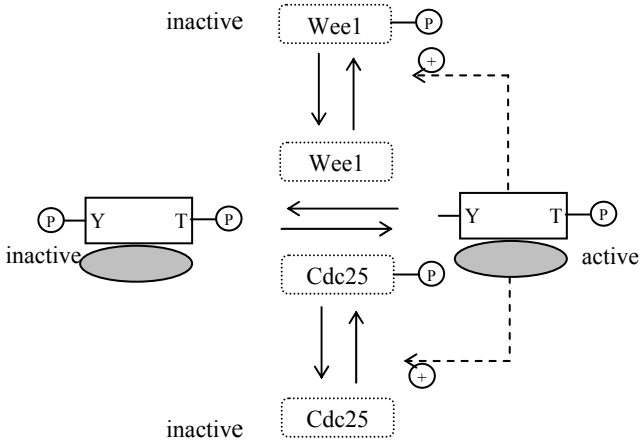
In most Eukaryotic cells, there are 3 checkpoints called the G1 checkpoint, the G2 checkpoint and the M-phase checkpoint, signifying the phase of the cell cycle in which the checkpoint is active. Each checkpoint is explicitly triggered by specific types of abnormalities. The G1 and G2 checkpoints specifically react to DNA damage whereas the M checkpoint, among other things, is found to react to microtubule and kinetochore damage. We will specifically discuss DNA damage checkpoints. The checkpoint pathways and proteins that carry out the responses mainly consist of: **sensor proteins** that detect the abnormality, **signaler proteins** that transduce the signal and **target proteins** that are altered to arrest cell division or cause apoptosis. The direct relevance of checkpoints to cancer is illustrated by the study of two genes, the P53 TS (tumor suppressor) gene and the ATM (Ataxia telangiectasia mutated) gene. Mutant cells fail to arrest in G1 after DNA damage and normally have other checkpoint defects as well. This leads to genomic instability and predisposition to cancer. However, we can also use checkpoint defects to our advantage to trigger apoptosis in cancer cells. This is because checkpoint defects potentially render the cell damage sensitive. However, they do so only in the presence of other defects in the cell's response to DNA damage. E.g. A G1 defect can be compensated by the G2 checkpoint. Nullifying the G2 checkpoint as well, can make the cell more sensitive to cytotoxic and genotoxic stress and hence to apoptosis.

3. Progression through the Cell cycle:

3.1 Entry into G1 and the Restriction point:
Reentry into the cell cycle from the G0 phase is triggered by extracellular growth factors. Continued growth factor stimulation is required for the cell to pass through the **restriction point**, which is defined as the point beyond which the cells are no longer dependent on growth factors and are committed to completing the cell cycle, barring any major disruptions. Many oncogenes (genes that stimulate neoplasia and eventually cancer) regulate the growth factor-mediated signaling that regulates the G0-G1 transition whereas TS genes are found to regulate passage through the restriction point**. Cyclin D/CDK4,6** are activated in the G1 phase. These complexes target the Retinoblastoma (Rb) gene product and phosphrylate (P) it. In the unphosphorylated (UP) state Rb is bound to the **E2F-1 transcription factor** and hence allosterically inhibits it. On (P), Rb dissociates from E2F, thus allowing it to promote transcription of various S phase genes including **Cyclin E and CDK2**. This complex causes complete (P) of the **Rb protein** thus fully activating E2F transcription. This positive feedback leads to passage through the R-point and into the S phase. The G1 Cyclin/CDKs are also regulated by the inhibitory molecules. This is discussed later. A very interesting fact observed in most regulatory proteins in the cell cycle is their dual role. They act as activators and as inhibitors depending on the conditions. A good example in this case is the E2F t.f. Its normal function is to act as a transcriptional activator. However, it also positively regulates the transcription of the **p19ARF/p14ARF** protein which stimulates p53 mediated cell cycle arrest when E2F is produced in excess under abnormal conditions. This kind of duality is found in many elements including many oncogenes which when overexpressed may cause apoptosis in normal cells. This is why cancers are found to have multiple mutations which nullify these proapoptotic agents in synergy with the proliferative effects of the oncogenes.

3.2 Regulation of DNA synthesis and the S phase:
Another CDK complex induced by E2F is the **Cyclin A/CDK2** which peaks in the middle of the S phase. This complex inhibits Cyclin E/CDK2 thereby preventing a direct transition into the M phase and also prevents a rereplication. DNA replication in eukaryotes begins at specific sites called Autonomous replication sequences (ARS). The ARS recognition complex is a six subunit complex that binds to the ARS. It loads CDC6 which is synthesized in the late G1 phase, which in turn loads the **MCM complex** (Minichromosome maintainence). This is the prereplication complex which represents a replication competent state. CDC7/CAK complex which is an S phase CDK complex targets MCM and causes movement of the replication machinery. This displaces MCM from the chromatin and this is believed to be instrumental in preventing a second round of replication in a single cell cycle. Also, towards the end of the G2 phase, the M phase CDK complexes are stimulated. These negatively affect the production of the prereplication complexes which further prevents re-replication.

3.3 Regulation of M phase:
The M phase is mainly induced by and shows active content of the **Cyclin B/CDK1** (CDK1 is also called CDC2 not to be confused with CDK2). In some lower eukaryotes, the cyclin involved is Cyclin A. The **MAPK** (mitogen activating protein kinases) are also an important class of kinases for this phase. All the CDKs discussed so far are activated only on binding to a Cyclin. They are further activated to a 80-300 fold gain by positive phosphorylation by **CAK** which is not particularly regulated through the cell cycle. However, the negatively regulating phosphorylations are intricately regulated by an interplay between the **Wee1/Myt1**, Cdc25 phosphatase and the cyclins. In the M phase, the **CDC25 phosphatase** is activated and removes the inhibitory phosphorylations of the CyclinB/CDK1. The activated complex further phosphorylates Cdc25 to



Cdc25 phosphatase resulting in a positive feedback. It also phosphorylates Wee1 which inactivates Wee1. As is known, in a feeback loop two negative interactions equate to a positive feedback (Fig3). Hence, this further increases the concentration of the activated complex thus ensuring unidirectional progression in the M phase. Exit from the M phase and all other phases are mediated by degradation of the Cyclins specifically as explained below.

Fig 3: Positive Feedback Loops

3.4 Exit out of G1, S and the M phase:

Degradation is the most effective way of ensuring unidirectional and irreversible progression through the cell cycle. The proteins to be degraded by the **26S proteasomes** are targeted by Ubiquitin targeting. **Ubiquitin** is a 76 amino acid protein that targets different kinases at different points in the cell cycle. Ubiquitination is done by two main complexes. The G1,S complex is called the SCF and the M-phase complex is called the APC (Anaphase Promoting complex).

Note: Not to be confused with the APC gene (Adenomatous Polyposis Coli) which is an active gene in Colon Cancer.

The timing of the substrate ubiquitination and hence degradation is determined by the timing of substrate phosphorylation. When these proteins are phosphorylated the F-box protein of the SCF complex identifies the phosphoproteins for ubiquitination. Each F-box protein recognizes its own particular phosphoprotein. The inhibitor proteins and the G1 Cyclins are degraded in this manner. The APC unlike the SCF is not always active. It has a destruction box sequence that needs to be activated by phosphorylation. The APC is directly phosphorylated by CyclinB/CDK1 which is a form of negative feedback since APC degrades this complex. APC also is inhibited by some proteins that need to be degraded before APC can actively ubiquitinate its targets which include non-kinases. The Cdc20 protein also activates APC when complexed with it.

3.5 The Inhibitory Molecules:

The Cyclin/CDK inhibitors play a crucial role in the G1 checkpoint whereas the negative phosphorylations are critical in the G2 checkpoints. The INK4 (Inhibitor of kinases) family specifically act on CDK4,6 complexes while the Cip1/Kip 1 family bind all G1 and S phase CDKs. The main purpose of these inhibitors is cell-cycle arrest and senescence (permanent arrest without death). **p15/INK4B** induced by TGF-beta (transforming growth factor which is an anti-proliferative agent) binds CDK4,6 thus redistributing **p27/Cip1** (also induced by TGF-B) which binds to CDK2. p27 is phosphorylated by active Cyclin E/CDK2 leading to its degradation. Hence, regulation is at the level of translation. **p21/Cip1** is a very important protein involved in cell cycle arrest. DNA damage causes accumulation of p53 which induces p21 transcription. It binds to CDKs and arrests the cell cycle. The **p16/INK4A** is a particularly interesting protein whose gene is found to be mutated in a number of cases. It inhibits CDK4,6 but more importantly it shares its locus with the **p19/p14ARF** gene. p19 stimulates p53 accumulation thereby stimulating p21 which causes cell cycle arrest. A single lesion at this common site could have a 2 fold derogatory effect on cell cycle arrest which is why it is common in cancers.

4. The p53 Protein:

p53 is a 393 amino acid **nuclear phosphoprotein** who gene lies on the short arm of chromosome 12 in humans and contains 11 exons. It can be divided into **3 domains**: an N-terminal acidic trans-activating domain; a central evolutionarily conserved DNA binding domain and a complex C terminal domain that houses, among other things, a homotetramerization domain and a DNA damage recognition site (specifically single stranded DNA). Activation of p53 following DNA damage is associated with accumulation of the normally **short lived protein**. This accumulation is not mediated at the transcriptional level. Rather, it is stabilized after DNA damage as explained later. This accumulation of p53 trans-activates a number of different genes including GADD45, MDM2 and p21. These genes contain the consensus binding site PuPuPuC(A/T)(T/A)GPyPyPy either in the intronic region or the promoter region.

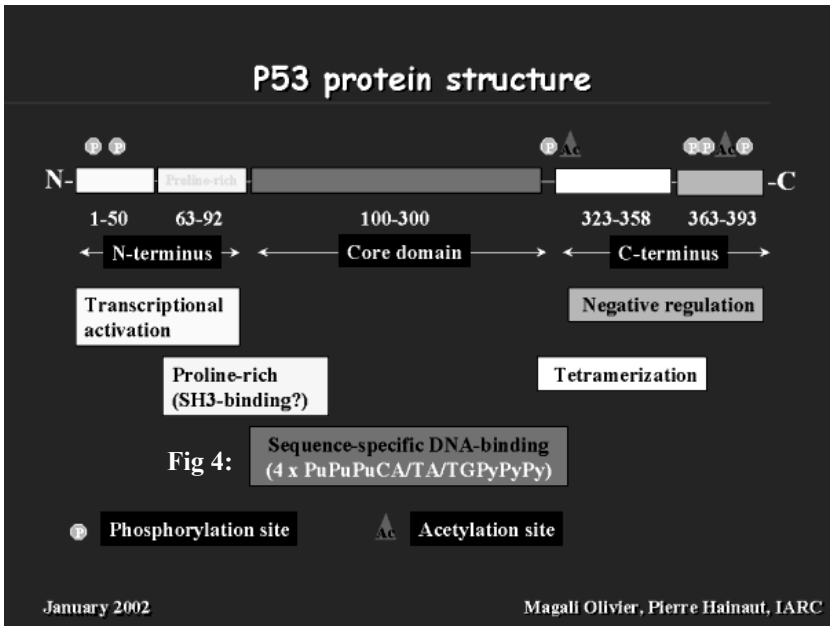


Fig 4:

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5. Signalling the presence of DNA Damage to p53:

It has been shown that DNA strand breaks are sufficient and probably necessary for DNA damaging agents to activate p53. This is probably why base damaging agents induce p53 relatively slowly as compared with gamma radiation. Also, this has been extended to include any **single-stranded DNA** such as linearized plasmid DNA intentionally injected into the cell. However, inevitably most kinds of DNA damage untimately lead to p53 accumulation. The exact mechanism by which p53 is activated by different types of DNA lesions that involve a host of different proteins is not known as yet. Some of the possibilities are mentioned below:

5.1 Relevant Types of DNA damage and repair:

The main types of DNA damage induced by Gamma radiation include, single strand breaks (**SSB**s), double strand breaks(**DSB**s-consisting of two proximal SSBs), base modifications and crosslinks. DSBs mainly lead to chromosomal aberrations and cell death after radiation. Damage induction normally follows Poisson statistics in a cell cluster. Thus, individual cells incur broad ranges of damage.

Repair is found to be of two types namely slow and fast. Cells with fast repair are found to have lower frequency of misrejoining events and higher survival than with slow repair. The rate is also found to depend on the complexity of the lesion.

5.2 p53 Activation by Direct Binding to DNA lesions:

p53 is found to bind to the ends of short single stranded oligonucleotides and to insertion/deletion mismatches through the non-specific binding C domain. This potentiates DNA sequence specific binding by p53.

5.3 p53 Activation by Amplifying Kinases:
Ku proteins have a high affinity for double stranded ends and other abnormal DNA. These recruit the **DNA-PK** (DNA activated protein kinase) subunit to the damage site and activates its kinase activity. DNA-PK is able to phosphorylate p53 in the N-terminal transcriptional activation domain thereby transducing a damage signal to p53.
The **ATM** (Ataxia Telangiectasia) protein is a phosphotidylinositol-3 kinase which is very important in tranducing signals to p53. Cells exposed to ionizing radiation and radiomimetic drugs increase the production of reactive oxygen intermediates (ROIs) such as hydroxyl radicals and hydrogen peroxide leading to oxidative stress. These activate the ATM gene. The ATM protein is found to stabilize p53 by phosphorylation.

5.4 p53 Activation by Interaction with Repair Factors and SS DNA Binding Proteins:
p53 can detect gaps exceeding 30 nucleotides through interaction between its N domain and the **XPB** (Xeroderma Pigmentosum) helicase. XPB is a component of the TFIIH transcription complex and this recruitment mechanism shows a link between transcription, damage within transcribed genes and cell cycle arrest. p53 can also interact with proteins associated with gaps. One such protein is **RPA** (replication protein A) to which p53 binds inhibiting replication.

5.5 Activation by Structural modification due to Redox potentials:
p53 is a zinc containing protein and it is a very flexible molecule whose function may be altered by conformational changes due to redox variations as are common in the case of DNA damage.

6. p53 Activation in the absence of DNA damage:

Cell cycle arrest via p53 can also be induced by Ribonucleotide (rNTP) depletion. Two models have been proposed. The first proposes that normal nucleotide levels restrict p53 to the cytoplasm due to the binding of rRNA (ribosomes/polysomes). rNTP depletion disrupts such interaction due to reduced synthesis of rRNA which allows p53 to enter the nucleus and activate cell cycle inhibitors (CIKs) like p21. Another model is based on the fact that 5.8S rRNA is normally synthesized with p53 and is bound to the C-terminus preventing non-specific binding and tetramerisation to form the active tetramer. rNTP depletion leads to resynthesis of p53 without the the 5.8S rRNA which allows it to transcribe the CIKs and cause cell cycle arrest.

7. p53 Dependent G1 Arrest through p21:

p53 accumulation as indicated leads to transcriptional activation of p21. p21 binds to CyclinE/CDK2 in particular and deactivates it. This CDK complex has a very important role not only in Rb phosphorylation (inactivation) as described in section 3.1 but also in other G1/S promotion pathways. Thus, its inhibition stalls the cell cycle at the G1/S pathway. p21 is also found to inhibit other CDK complexes important for cell cycle progression such as CyclinD1/CDK4, CyclinA/CDK2 and CyclinB/CDK1. There is also an interesting hypothesis that suggests that more than one p21 molecule is needed to inhibit the CDKs completely.
p21 also has the ability to bind to **PCNA** (proliferating cell nuclear antigen). PCNA functions as a processivity factor for DNAP-delta and is required for DNA replication and repair. In vitro experiments show that this binding blocks DNAP-delta dependent DNA replication. It is also found to slow down the rate of the S phase, once again showing its inhibitory effect on cell cycle progression. However, it is not clear as to what extent this interaction is important in the G1

checkpoint. p21 knockout studies have also led to the supposition that it has a role in promoting **nucleotide excision repair** by regulating some specific aspect of PCNA is the repair process.

8. Gadd45 and G1 Arrest:

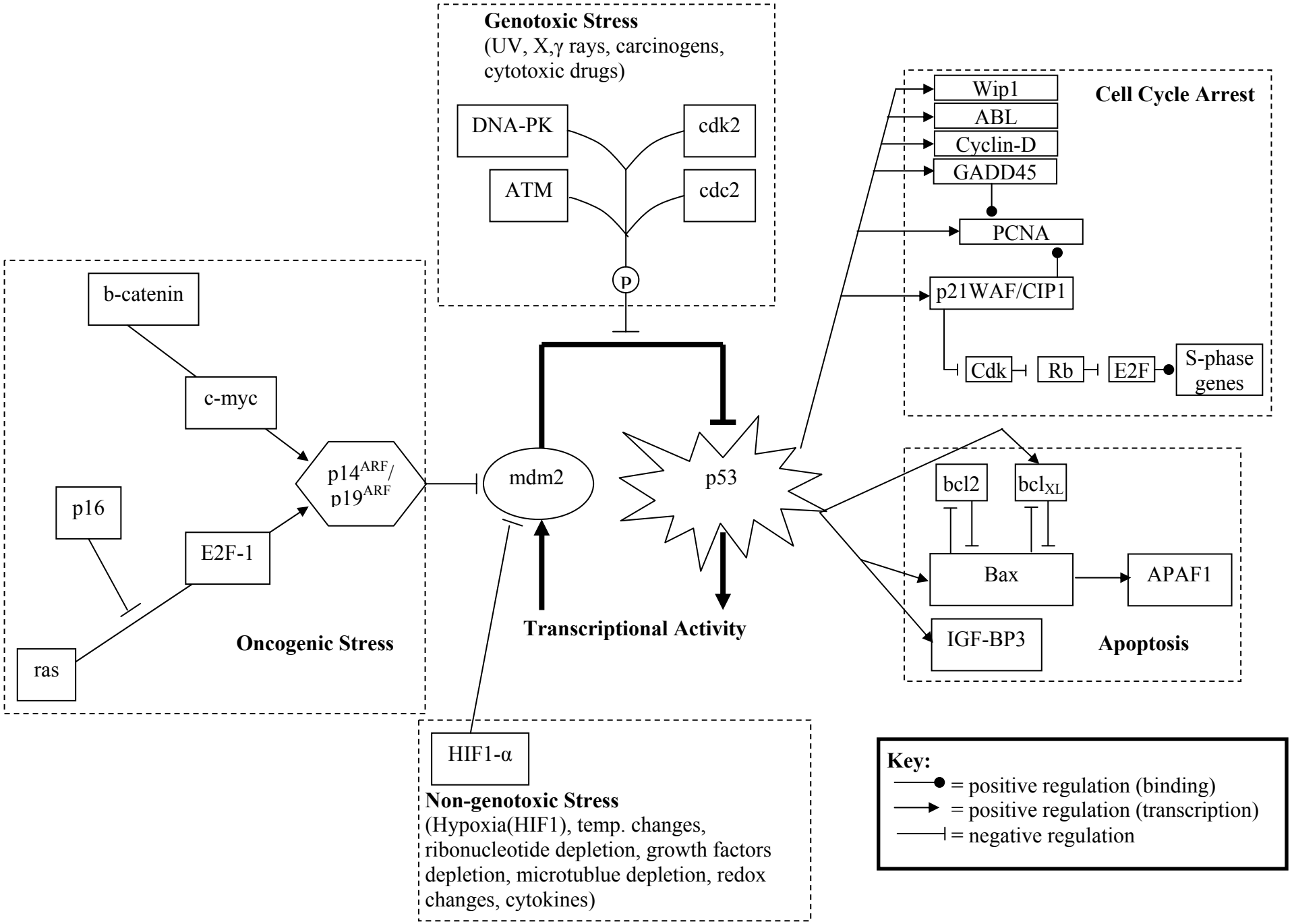
GADD45 is a p53 inducible gene containing a binding site in its third intron. Gadd45 has been found to block entry into S phase and hence may be part of the G1 checkpoint. A potential mechanism by which it accomplishes this is by **binding to PCNA** like p21. Infact, the two compete to bind to PCNA suggesting a common binding site. Gadd45 is also found to stimulate the DNA resynthesis step of the **nucleotide excision repair**.

9. Other Components Contributing to G1 Arrest:

Wip1 is a p53 induced phosphatase which is found to delay cells in G1 phase. It functions independent of the Rb pathway and possibly has a role in dephosphorylating the CDKs at the activating Thr160 site.
CyclinD1 although often associated with growth promotion is found to induce G1 arrest on overexpression once again proving the dual role of the regulatory protein. p53 is found to activate accumulation of CyclinD1. However, this is not by stabilizing it but by directly inducing transcription of CyclinD1 mRNA. There is also an indirect effect by which p53 stimulates CyclinD1 expression. p21 induced by p53, keeps Rb in its active hypophosphorylated state which is found to induce CyclinD1 transcription.
ABL is also implicated in p53 dependent G1 arrest. ABL is able to suppress CDK2. However, to do this it first needs to bind to p53 and also requires ABL kinase activity.

10. Mdm2 and p53:

The most important player in regulation of p53 is the **Mdm2** protein. In the absence of Mdm2, p53 becomes strongly deregulated to the extent that it can lead to apoptosis. In normal cells, p53 levels are kept at a very low value by Mdm2. The half life is around 5 mins. This is because Mdm2 ubiquitinates p53 for degradation. This interaction can be blocked by **phosphorylation** of p53 (e.g. at Ser-15 by the ATM kinase) or by **p19/p14ARF**. Phosphorylation of p53 reduces the affinity of p53 to Mdm2. Other post translational modifications also modify this interaction. p19/p14ARF is induced by E2F-1 which is released from Rb after the latters phosphorylation by G1/S CDKs. Oncoproteins such as Myc and Ras are found to active E2F-1 in access leading to an overexpression of p14/p19 and p53. This normally leads to the apoptosis pathway.
Mdm2 exhibits a dual relation with p53. On one hand, it promotes p53 degradation and also represses its transcriptional function by binding to it. On the other hand, MDM2 gene is transcriptionally activated by p53. This constitutes a **negative feedback loop** which is the major topic of discussion in the remainder of the paper. The levels of the 2 proteins are found to oscillate after a sufficiently strong damage signal. The delay to Mdm2 transcriptional activation by p53 and the period and amplitude of oscillations may play a very important role in the selection of the downstream p53 pathway to temporary cell-cycle arrest, DNA repair and recovery or to senescence or to apoptosis. Mdm2 overexpression is a common observation in tumors which prevent p53 pathways which maintain genomic integrity. It has been observed that a number of pathways leading to cell-cycle arrest and apoptosis converge at this crucial negative feedback loop. Hence, a highly simplified and insolated differential model of this loop is discussed below.


Genotoxic Stress
(UV, X,γ rays, carcinogens, cytotoxic drugs)
DNA-PK
ATM
cdk2
cdc2
P
Wip1
ABL
Cyclin-D
GADD45
Cell Cycle Arrest
PCNA
p21WAF/CIP1
Cdk
Rb
E2F
S-phase genes
b-catenin
c-myc
p14ARF/ p19ARF
p16
E2F-1
ras
Oncogenic Stress
mdm2
p53
Transcriptional Activity
bcl2
bclXL
Bax
APAF1
IGF-BP3
Apoptosis
HIF1-α
Non-genotoxic Stress
(Hypoxia(HIF1), temp. changes, ribonucleotide depletion, growth factors depletion, microtublue depletion, redox changes, cytokines)
Key:
= positive regulation (binding)
= positive regulation (transcription)
= negative regulation

11. The Model:

In the fig above, we see a confluence of important p53 related pathways (Upstream and Downstream). The whole network can be modeled using kinetic logic as explained in section 15. This model can help us observe macroscopic effects of gene mutation and temporal changes. The network encompasses almost everything that has been discussed in this paper.

We now describe the differential model used to characterize the p53-Mdm2 feedback loop. Simulink was used to model the system and the simulink model is shown in the Appendix S.1. and S.2. Each of the equations described below is represented as a separate subsystem. The graphs A.1 – A.4 are the outputs we got from Simulink. The x axes in all graphs represent time in minutes. The y-axes represent the concentrations of substances mostly p53 all scaled with respect to the basal value.

11.1 Model Description:

The core of the model involves the aforementioned central role that mdm2 plays in regulating p53 accumulation. Both the inhibitory influence of mdm2 on p53 transcriptional activity and its promotion of p53 degradation are included in this core. Also included is a hypothetical, but important, intermediary between p53 and mdm2, which models the observed delay in the p53-dependent induction of mdm2, and facilitates the interesting oscillatory behavior observed in p53 and mdm2 levels in response to a stress signal. Secondary, but also important, mechanisms are also included in the model. These include: stress signal resolution by cell repair machinery, effect of stress on transcriptional activity of p53, effect of stress on mdm2-promoted degradation of p53, p53-independent induction of mdm2, degradation of mdm2, and mdm2-independent degradation of p53. Details of the model are presented in the following paragraphs.
The following differential equation models the kinetics of p53 concentration:

$$\frac{dp53}{dt} = source_{p53} - p53(t) \cdot Mdm2(t) \cdot degradation(t) - d_{p53} \cdot p53(t) \qquad [1]$$

Here the coefficient $source_{p53}$ specifies the synthesis rate of the p53 protein. Not included, for the sake of simplicity, is evidence that exposure of cells to p53-activating signals also can lead to increased translation of the p53 mRNA. The second term in Eq. **1** describes Mdm2-dependent degradation of p53, where mass-action binding of Mdm2 to p53 results in p53 ubiquitination and its subsequent proteasomal degradation. The variable *degradation*(*t*) measures the stress signal-dependent rate of degradation (Eq. **6**). The last term in Eq. **1** reflects an Mdm2-independent mechanism for p53 degradation. The kinetics governing the concentration of Mdm2 protein are given by:

$$\frac{dMdm2}{dt} = p1 + p2_{max} \cdot \frac{I(t)^n}{K_m^n + I(t)^n} - d_{Mdm2} \cdot Mdm2(t) \qquad [2]$$

Here the coefficient *p1* denotes the rate of p53-independent *mdm2* transcription and translation, whereas the last term describes Mdm2 degradation. The second term implements p53-dependent transcription and translation of Mdm2 protein. The quantity $I(t)$ measures the strength of an intermediary: a mathematical representation of an unknown mechanism leading to the observed delay in the p53-dependent induction of Mdm2. This intermediary enhances Mdm2 production with step-like kinetics, modeled by a Hill-type function. The value of n that gives correct results is normally quite high signifying a high level of cooperativity and a sharp increase in the promoting action of I(t) once its conc. Exceeds K_m. The kinetics for the intermediary *I* is given by

$$\frac{dI}{dt} = activity \cdot p53(t) - k_{delay} \cdot I(t) \qquad [3]$$

The first term in Eq. **3** reflects a positive effect of active p53 on the Mdm2 intermediary. The coefficient *activity* can include p53's sequence-specific DNA binding activity and the potency of the p53 trans-activation domain, both of which can be augmented by stress signals. Furthermore, Mdm2-p53 binding can inhibit p53's transcriptional activity. Thus, *activity* can be modeled as

$$activity = \frac{c_1 \cdot signal(t)}{1 + c_2 \cdot Mdm2 \cdot p53}. \qquad [4]$$

From Eq. **3** it is seen that the intermediary *I* reaches its steady-state level with a time scale determined by $1/k_{delay}$. Thus, by using a differential equation to determine *I*, we account in a crude fashion for the possible delay between the activation of p53 and the induction of Mdm2. The idea of a ''gearing up'' for Mdm2 protein production relies on evidence according to which, in some situations, *mdm2* transcription is induced later than that of other p53 target genes, and that there may be an even further delay in *mdm2* translation. The equation representing the kinetics of the p53-activating signal is given by

$$\frac{d(signal)}{dt} = -repair \cdot signal(t) \qquad [5]$$

Here we assume an initial pulse of signal that can represent a short exposure of cells to DNA damaging agents, e.g., UV or ionizing radiation (IR). The signal subsequently is resolved by cellular mechanisms of damage repair, with a rate denoted in Eq. **5** by a constant *repair*. Note that, for the sake of simplicity, we do not incorporate in our model: (*i*) specific repair pathways, to reflect the fact that different types of damage are repaired through different pathways and (*ii*) the direct or indirect role that p53 may play in some DNA repair processes. The variable *degradation*(*t*) in Eq. **1** is chosen to be of the form:

$$degradation(t) = degradation_{basal} - [k_{deg} \cdot signal(t) - threshold(t)] \qquad [6]$$

Here $degradation_{basal}$ represents the strength of Mdm2's ability to promote p53 degradation, controlling the basal levels of p53. k_{deg} models the amount of inhibition of degradation caused by damage-derived signals that modify p53 and/or Mdm2. *Threshold*(*t*) relates to a damping effect on this inhibition, owing to an assumed delay between the delivery of the damage signal and the effective establishment of conditions (modifications) that interfere with efficient Mdmd2-mediated p53 degradation. The kinetics of *threshold*(*t*) is given by

$$\frac{d(threshold)}{dt} = -k_{damp} \cdot threshold(t) \cdot signal(t=0); \qquad [7]$$

$$threshold(t=0) = k_{deg} \cdot signal(t=0)$$

Here k_{damp} models the effect of the initial damage signal on the rate of inhibition of mdm2-mediated p53 degradation. Eqs. **6** and **7** reflect the assumption that in the case of a weak damage signal, the activation of damage-induced signaling pathways is likely to be relatively inefficient. For instance, enzymes (protein kinases, phosphatases, and acetyltransferases) that modify p53 and/or Mdm2 may undergo only a limited change in level of activity. Consequently, its is expected that more time will be required to reach a threshold of p53/mdm2 modifications

sufficient for sparing p53 from the destabilizing effects of Mdm2.In our attempt to model p53-Mdm2 interactions, many gross simplifications had to be made, and much biological information was ignored. Notably, the effects of other proteins that interact with Mdm2 and/or p53, such as ARF, are not included. Furthermore, our model does not incorporate the contribution of changes in the sub-cellular localization of p53 and Mdm2, known to be important in controlling the rate of p53 degradation. Moreover, the effect of the cell cycle phase on the prevalence of the p53-Mdm2 interaction is excluded.

11.2 Choice of Parameters:

Current experimental data does not provide for definitive value assignment to most of the model parameters mentioned in the previous section. As such, rough estimations, based on known relationships between mechanisms are used. Where basis for even such estimations are not evident, less tenable though intuitively reasonable values are chosen. A complete list of the parameters and their descriptions can be found in the appendix in Table 1.

As mentioned earlier, under certain conditions, p53 and mdm2 levels undergo damped oscillations *in vivo* in response to a stress signal. Specifically, coordinated oscillation of p53 and mdm2 can be observed in wild-type p53-expressing cells that experience DNA damage, i.e. in mouse NIH/3T3 cells and human breast carcinoma-derived MCF7 cells when exposed to sufficiently high levels of IR. Using the model parameter values listed in the (table1-appendix), the introduction of a stress signal pulse at t = 0 provides model results closely aligned with observed behavior (Fig A.1 appendix).

11.3 Dependence of Oscillations on Model Parameters

A numerical study of the dependence of the amplitude and width of the first wave on the different parameters finds that increasing the values of $p1$ and K_m gives a higher and wider p53 wave and a lower and narrower Mdm2 wave. Increasing $source_{p53}$, d_{Mdm2}, and c_1 results in a lower and narrower p53 wave and a higher and wider Mdm2 wave. Increasing n makes both p53 and Mdm2 waves higher and narrower. Increasing k_{deg} makes both waves higher and wider. Increasing d_{p53} makes the p53 wave lower and narrower, while making the Mdm2 wave lower and wider. Increasing c_2 makes the p53 wave higher and narrower, while making the Mdm2 wave lower and narrower.

11.3.1 Effect of Damage Strength (Signal) on Oscillations:

Within the model, a stress signal below a certain threshold will not generate oscillations, and p53 and Mdm2 will rise to a lower level than in the high damage case. Also, as predicted from Eqs. **6** and **7**, the rise in steady-state p53 levels should be slower in the case of a weak damage signal, because of the longer time required to reach a critical threshold of p53/Mdm2 modifications sufficient for compromising the inherent p53-destabilizing activity of Mdm2. This behavior predicted by the model agrees with experimental data obtained with MCF-7 cells. When these cells were exposed to a low dose of IR, the results showed an extended rise of both p53 and Mdm2, with no observable oscillations within the time frame of the experiment. Further, the time required to reach peak p53 levels was significantly longer than in the case of higher damage. Fig A.2. in the Appendix shows the effect of damage strength. All other parameters are the same as for Fig. A.1. The red graph is for signal(t=0) = 1 whereas the blue one is for signal(t=0) = 0.8. threshold(t=0) needs to be adjusted accordingly since threshold(t=0) = k_{deg} * signal(t=0). As is evident a lower damage signal does not induce oscillations.

11.3.2 Effect of 'Delay' on Oscillations:

The time lag between the maxima of Mdm2 and p53 is controlled by k_{delay} in Eq. **3.** The rationale for this behavior above can be readily seen from Eqs. **1**-**7**. For example, triggering a stress signal decreases the degradation of p53 (Eq. **6**). This makes p53 free to rise above its basal level with a

rate that is (*i*) positively affected by the rate of p53 supply (denoted by $source_{p53}$), and (*ii*) negatively affected by the rate of p53 degradation. The induction of Mdm2 that takes place after a certain time lag enhances the degradation of p53, which then leads to a decrease in p53 protein levels. This, in turn, generates lower production of intermediary I, thus lowering Mdm2 levels. If there is still enough damage to keep p53 degradation weak, a subsequent decrease of Mdm2 after it has reached its first peak leads to a decrease in p53 degradation. Thus p53 levels increase again, as long as there is a time delay in Mdm2 induction. Upon induction of Mdm2, p53 levels subsequently will decrease, causing in turn a decrease in Mdm2. If the conditions that give rise to the second peak still hold, further oscillations will follow.
Importantly, within the model, the **delay** in p53-dependent induction of Mdm2 is essential for an oscillatory behavior (Fig. A.3. Appendix). In addition, for the delay to generate oscillations, the strengths of the p53-Mdm2 interaction mechanisms (and the parameters that govern them, *degradation*, c_1, *delay*, c_2 and $p2_{max}$) have to lie within an intermediate range. A change in one of these parameters that leads to loss of oscillations can sometimes be remedied by an opposite change in an antagonistic parameter. As can be seen from Fig.A.3, only for an intermediate delay (k_{delay} = c1= -4e-3) of 50 min time lag between the p53 and Mdm2 peaks, do we see meaningful oscillations. For the smaller delay of 20 min (k_{delay} = c1= -0.09) the oscillation are too small in amplitude and the time period of p53 peaks is too small to activate downstream pathways. For the large delay case of 5 hours (k_{delay} = c1= -9e-4) we have a very large amplitude of p53 but it dies out after fewer oscillations. All the other parameters are the same as in Fig A.1.

11.3.3 Effect of Repair time on Oscillations:
In addition, the emergence of oscillations requires that the **repair time** (Eq. **5**) be much longer than the period of the oscillations (Fig A.4. Appendix). As shown in Fig A.3 with all parameters the same as in Fig A.1 except for the repair constant, we have ideal oscillations for a repair time of 5 days (red, repair = 1.4e-4) and 2 days (blue, repair = 3.5e-4). For a fast repair time of 12 hours (black, repair = 1.4e-3) we see no oscillations. Alternatively, a similar outcome (oscillations) may be seen also in cases of fast repair, provided that the signal emanating from the damage (e.g., activation of a kinase) persists long enough afterward. This does not necessarily mean that damage repair is slow, but rather that the signal to p53 must persist at a high level.

11.3.4 Effect of 'n' on Oscillations:
It is also noteworthy that, in the model, oscillations depend on a very steep, steplike induction of mdm2 by the intermediate I (modeled by *n* in Eq. **2**). This means that below a certain threshold (measured by K_m) of intermediary amounts, there is no p53-dependent production of Mdm2. Above this threshold, Mdm2 is produced with a saturating value. Such bi-stable behavior might reflect a process of multiple partially rate-determining steps or the effect of a stoichiometric inhibitor. The value of n is found to range between 10 and 50 to obtain experimentally verified results.

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12. p53 And Apoptosis:

Some cell types respond to wild type p53 activation by arresting in the G1 phase. Other cells just undergo apoptosis. The **cell type** has been highlighted as one of the prime selectors of the downstream pathway. Some insight into the bifurcation has been obtained from studies involving overexpression of myc and E2F-1. These transcription factors along with another t.f. called B-myb can drive the cell into the S phase even in the presence of active p53. This bypass has inevitably seen to lead to apoptosis in the S phase leading to the conclusion that activation of S phase genes in the p53 overexpressed state may be a sufficient signal for apoptosis. Loss of the **Rb** product also leads to p53 induced apoptosis.

The **Bcl-2 family** of proteins is very closely linked to apoptosis. Some members of the family, mainly **Bcl-2** and **Bcl-x_L** are anti-apoptotic whereas **Bax, Bcl-x_S** and **Bad** are pro-apoptotic (cytotoxic). The Bax and Bcl-2 play antithetical roles and the mechanism is best described by the **"Rheostat model"** for Bax/Bcl2 in which the propensity to undergo apoptosis depends on the relative ratios of Bax/Bax homodimers, Bcl-2/Bax heterodimers and Bcl-2/Bcl-2 homodimers. An excess of Bax homodimers promotes apoptosis, whereas the other 2 types of dimers favor survival. Bax may induce apoptosis either directly or by inhibiting the downstream action of Bcl-2 by dimerising with it. p53 stimulates Bax expression, but it is **upregulated only in cell lines that are committed to apoptosis** following p53 activation. In this type of cells, p53 is also found to inhibit Bcl-2 expression. However, an important enhancement to this model is the fact that Bcl-x_L is also induced by p53 which also dimerises with Bax. This might be a form of increasing the threshold for Bax induced apoptosis. However, this is not necessarily true since Bcl-2 and Bcl-x_L seem to have different patterns of expression indicating that their physiological roles are distinct even though they affect the same pathway.
Other pathways to apoptosis downstream of p53 include the overexpression of the **TRAIL-DR5** death receptor and the activation of a **CD95** pathway.
There are several ways by which normal and cancerous cells may combat apoptosis.

- P53 overexpression may not be involved with deregulated myc and E2F-1.
- P53 induced apoptosis can be suppressed by growth factors, which lead to a more stable G1 cell-cycle arrest. Growth factors thus tend to favor G1 arrest and survival.
- An alternate mechanism used by many cancer cells to combat apoptosis is over expression of the Bcl-2 protein which is an anti-apoptotic protein and/or the suppression of the Bax induction. Bcl-2 overexpression synergizes very well with myc overexpression in cancer cells, preventing them from undergoing apoptosis.

As mentioned before, the **cellular context** is found to be very important to the outcome of p53 activation (e.g. effect on radiosensitivity). This is particularly important in cancerous cells.
It has been found that cell types that are characterized by apoptosis as the dominant outcome of p53 response (lymphoid cells), require intact wild-type p53 to be sensitized to apoptosis by ionizing radiation and some chemotherapeutic agents. A loss of p53 functionality in such cells leads to decreased sensitivity and such cancer cells are difficult to kill using radiotherapy.
On the other hand, in cells that are inherently not prone to p53-mediated apoptosis (some epithelial cells and fibroblasts), loss of p53 functionality doesn't affect the radiosensitivity but does sensitize the cell to DNA cross linking chemical agents.
Based on the model and the simulations described above, the oscillation characteristics of p53 may also play an important role in deciding the outcome of the pathway. These characteristics depend on the type of stress, level of damage induced and sensitivity. It might be speculated that in cases where the damage should be dealt with successfully without leading to apoptosis, it makes sense for the system to oscillate to achieve a compromise between the state of insufficient p53 to elicit a response of any kind and a state of intolerably high amounts of p53 for a long duration leading to apoptosis. The oscillations may be viewed as an arrangement that allows repetitive repair efforts corresponding to peaks of p53. If the repair is unsuccessful and the damage is severe, the amplitude and duration of the p53 pulse may be high enough to induce the cell to irreversible apoptosis. However, it is not necessary that cell cycle arrest must preclude apoptosis as either of the two pathways can occur independently and non-sequentially.

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13. Temporal Behavior: Damage sensor fails to arrest after G1 restriction point:

The p53 pathway is unable to elicit cell cycle arrest due to DNA damage in the late G1 phase which temporally relates to the restriction point (R-point). Thus, once the cell crosses the R-point is will move into S phase despite DNA damage. This phenomenon may be closely related to the

Rb pathway which activates E2F-1 and the S phase genes. Experiments reveal that p53 accumulation and prevention of Rb phosphorylation must occur before the R-point in the mid G1 phase. This may be explained in several ways. Rb may have additional phosphorylation states which have not been recognized as yet. Alternatively, E2F may be modified in the late G1 phase which may prevent rebinding of Rb regardless of its phosphorylated state. This has been verified to a certain extent by the fact that CyclinA/CDC2 has the ability to phosphorylate E2F leading to its dissociation from Rb even when Rb is in the hypophosphorylated state. This phenomenon may prove Rb to be the most effective and temporally important protein in the arrest pathway This aspect needs to be modeled rather carefully to study the effect of DNA damage on the cell.

14. Conclusion:

The p53 pathway is probably the most important pathway to cell cycle arrest and apoptosis. It is also a major target for mutations in many cancers. Modeling this pathway might shed light on aspects of the pathway not commonly observed in experiments. This may lead to break throughs in Cancer therapy by identifying target molecules and genes that may help manipulate cell cycles and identify and eliminate cancerous cells. Due to the complexity of the pathways and the number of intermediates involved a kinetic logic model of the whole pathway which takes into account time delays may be representative enough to see macroscopic effects of manipulations. However, key regulatory subnetworks such as the p53-Mdm2 feedback loop should be modeled as either differential equation or stochastic models. Based on the outcomes of the kinetic logic models we may be able to manipulate parameters in the differential/stochastic models to obtain meaningful kinetics.

15. Future Directions:

As mentioned in the conclusion, the main aim of this project was to study a simple differential equation model of the p53-Mdm2 feedback loop and understand the reasons behind the formulation of the equations. Section 11 covers this aspect. However, we felt that kinetic logic models for the larger p53 dependent pathways made more sense due to lack of kinetics data and also due to the ability to abstract sections as explained below. This part of the project is as yet unfinished and we intend to work on it over the next few weeks/months. It will be added to this report as an addendum.

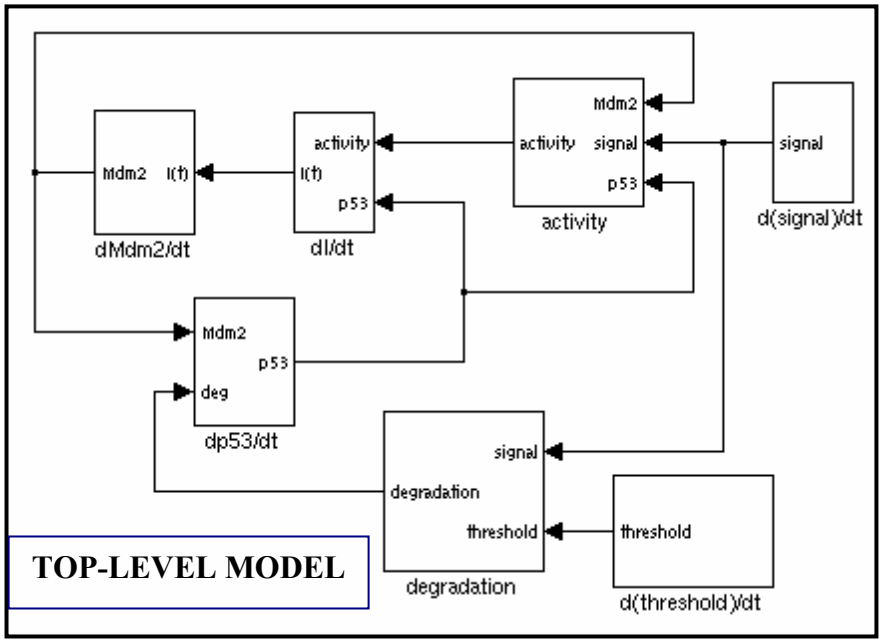
16. References:

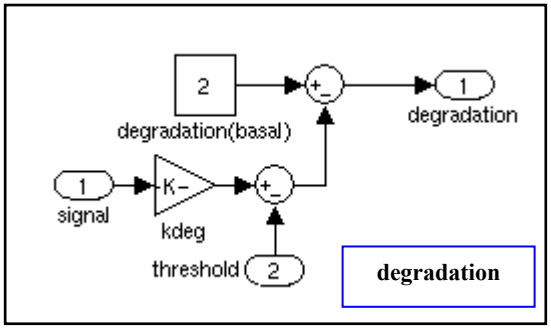
1. Generation of oscillations by the p53-Mdm2 feedback loop: A theoretical and experimental study (Paper) – ***by Ruth Lev Bar et al***.
2. The Molecular Basis of Cancer (Book) – ***by Mendelsohn, Howie, Israel, Liotta***
3. Checkpoint Controls and Cancer: Volume 29 Cancer Surveys (Book) – ***edited by M B Kastan***
4. Checkpoints in the Cell Cycle from a Modeler's perspective (Paper) – ***by John Tyson, Bela Novak et al.***
5. Cell cycle Regulation (URL): http://www.geocities.com/CollegePark/Lab/1580/cycle.html
6. IARC TP53 Mutation Database Resources (URL): http://www.iarc.fr/p53/Resources.html
7. Biological Feedback (Book) – ***by Thomas and D'Ari***
8. Computational Modeling of Genetic and Biochemical Networks (Book): ***by Hamid Bolouri and James Bower***

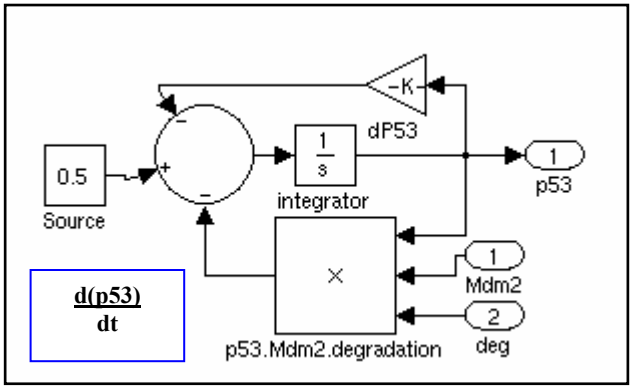
17. Appendix

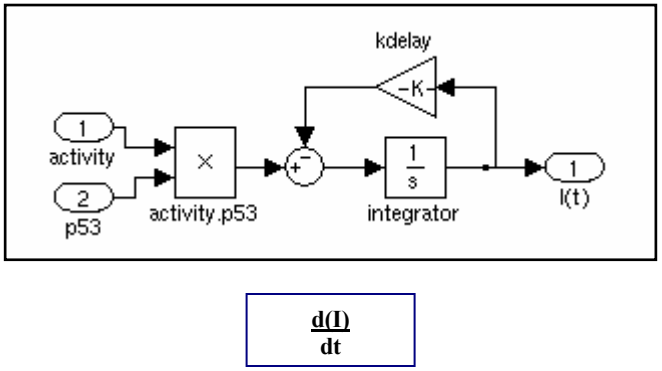
This appendix shows the simulink model we created for the p53-Mdm2 feedback loop based on the differential equation model and the graphical outputs discussed in section 11.

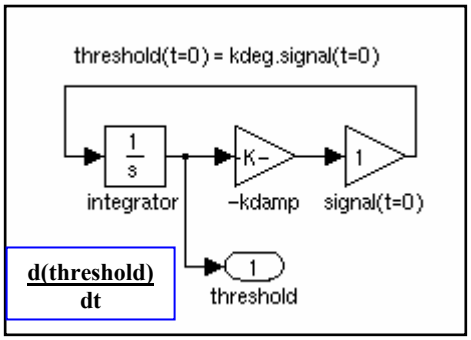
S.1

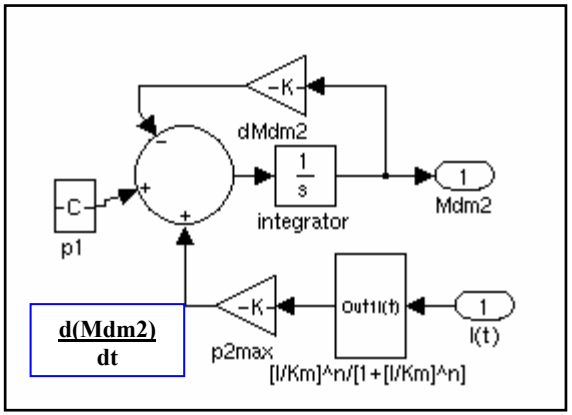








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dMdm2
1
s
integrator
1
Mdm2
-C
p1
d(Mdm2)
dt
-K
p2max
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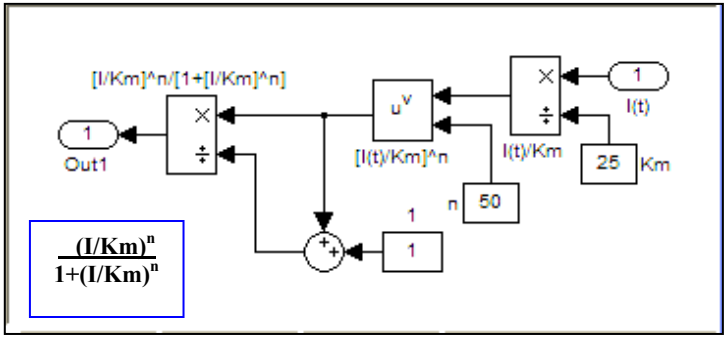
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Out1
u^v
[I(t)/Km]^n
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(I/Km)^n
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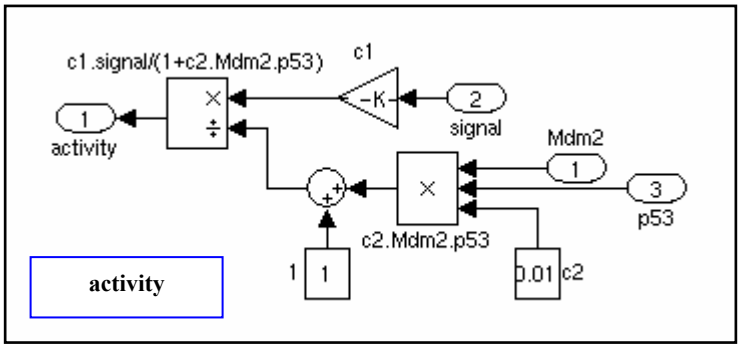
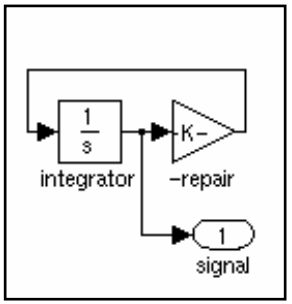

c1.signal/(1+c2.Mdm2.p53)
c1
1
activity
-K
2
signal
Mdm2
1
3
p53
c2.Mdm2.p53
1
1
0.01
c2
activity


1
s
integrator
K-
-repair
1
signal
d(signal)
dt

S.2.

APPENDIX: TABLE 1.

Equation	Explanation
$\frac{dp53}{dt} = source_{p53} - p53(t) \cdot Mdm2(t) \cdot degradation(t) - d_{p53} \cdot p53(t)$	p53 concentration kinetic model accounting for p53 synthesis and both Mdm2-dependent and Mdm2-independent p53 degradation
$\frac{dMdm2}{dt} = p1 + p2_{max} \cdot \frac{I(t)^n}{K_m^n + I(t)^n} - d_{Mdm2} \cdot Mdm2(t)$	Mdm2 concentration kinetic model accounting for both p53-induced and non-p53-induced Mdm2 transcription/translation and degradation
$\frac{dI}{dt} = activity \cdot p53(t) - k_{delay} \cdot I(t)$	Mdm2 intermediary concentration kinetic model accounting for the possible delay between the activation of p53 and the induction of Mdm2
$activity = \frac{c_1 \cdot signal(t)}{1 + c_2 \cdot Mdm2 \cdot p53}$.	Models p53's sequence-specific DNA binding activity and the potency of the p53 transactivation domain, both of which can be augmented by stress signals.
$\frac{d(signal)}{dt} = -repair \cdot signal(t)$	represents the kinetics of the p53-activiating stress signal including the effect of the cellular repair machinery on the stress-induced signal
$degradation(t) = degradation_{basal} - [k_{deg} \cdot signal(t) - threshold(t)]$	models cell stress inhibitions on Mdm2's ability to degrade p53
$\frac{d(threshold)}{dt} = -k_{damp} \cdot threshold(t) \cdot signal(t=0);$ $threshold(t=0) = k_{deg} \cdot signal(t=0)$	models the damping effect of cell stress inhibitions on Mdm2's ability to degrade p53, caused by the assumed delay between damage signal arrival and the effective establishment of conditions that interfere with efficient Mdm2-mediated p53 degradation

Constant	Value	Explanation
$source_{p53}$	0.5	p53 synthesis rate
d_{p53}	$2.5(10^{-4})$	Mdm2-independent p53 degradation rate
p1	$2.35(10^{-3})$	p53-independent Mdm2 transcription and translation rate
$p2_{max}$	0.03	max. p53-dependent Mdm2 transcription and translation rate
d_{Mdm2}	0.05	Mdm2 degradation rate
K_m	25	Hill function constant
n	50	Hill function constant exponent
k_{delay}	$1.52(10^{-2})$	Mdm2 intermediary delay constant
c_1	$1.52(10^{-2})$	effect of stress signals on p53 sequence-specific DNA binding activity and potency of the p53 transactivation domain
c_2	0.01	strength of p53 transcriptional inhibition due to Mdm2-p53 binding
repair	(10^{-4})	rate of stress signal resolution by cellular damage repair mechanisms
$degradation_{basal}$	2	strength of Mdm2's ability to promote p53 degradation
k_{deg}	1.93	amount of degradation inhibition caused by damage-derived signals that modify p53 and/or Mdm2
k_{damp}	0.05	effect of the initial damage signal on the rate of inhibition of mdm2-mediated p53 degradation
p53(t=0)	5.3	p53 initial concentration
Mdm2(t=0)	0.047	Mdm2 initial concentration
signal(t=0)	1	stress signal initial strength

APPENDIX: GRAPHS

Fig A.1

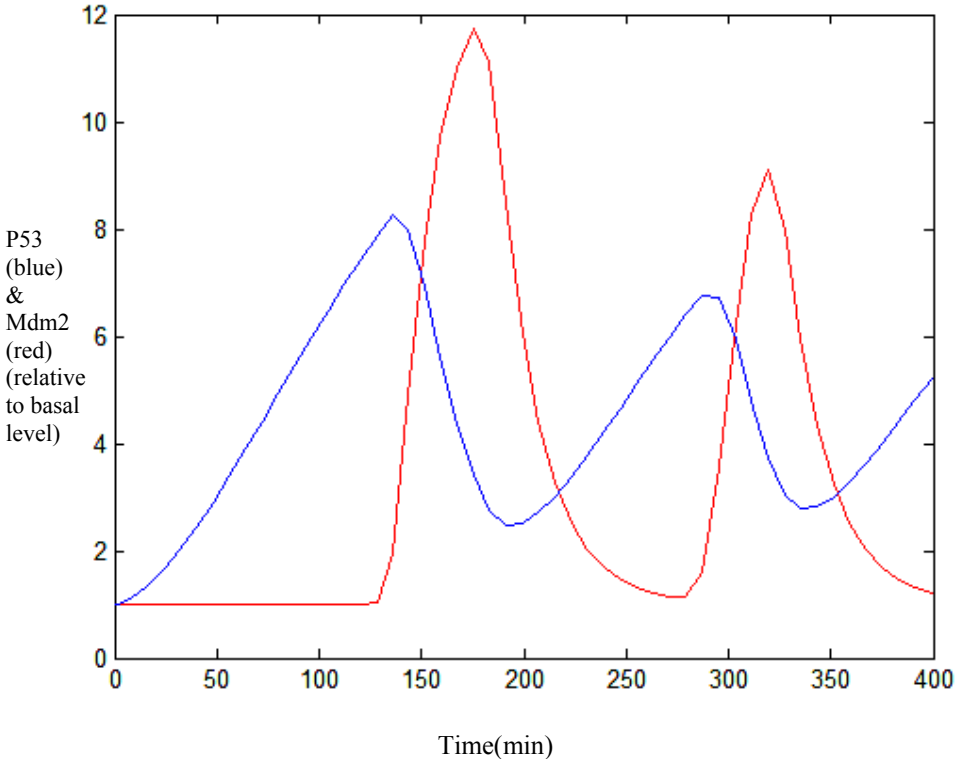


Fig A.1 : P53 and Mdm2 levels (relative to basal amounts) undergo oscillations after an initial pulse of damage signal. Mdm2 (red) peaks with a delay of 1 hour after the p53 peak (blue). The p53 peaks correspond to the Mdm2 minima. Parameters used are mentioned in Table1.

Fig A.2

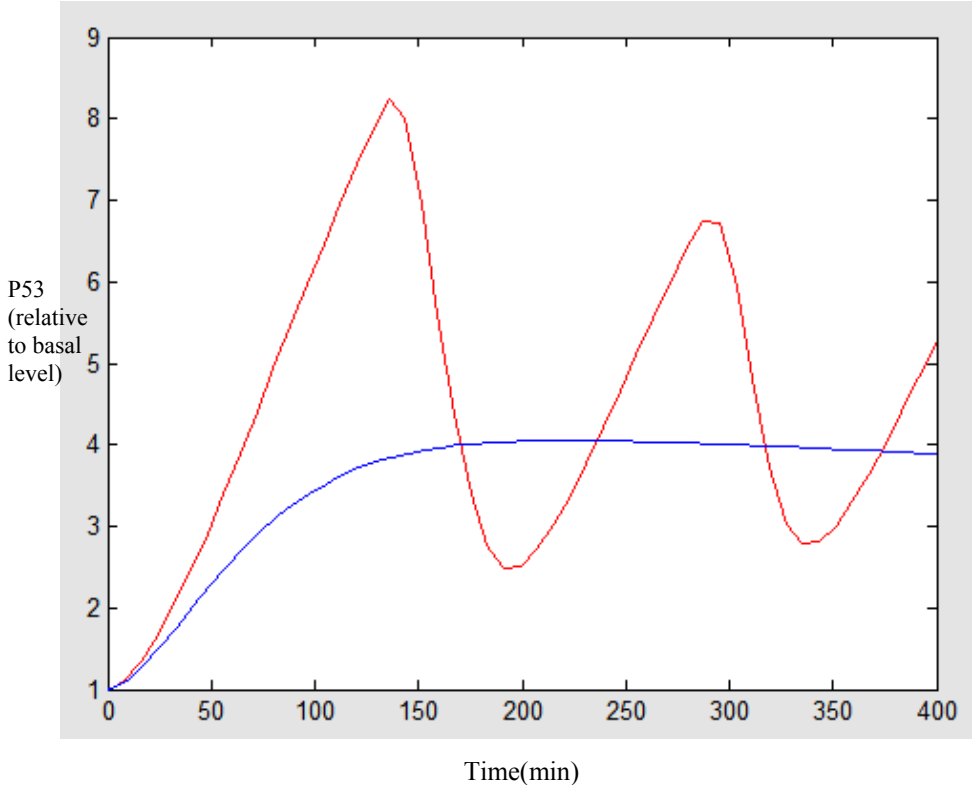


Fig A.2: Effect of low stress signal on p53 levels (relative to basal level). Red curve is same as in FigA.1 with signal(t=0)=1. Blue curve is for low signal signal(t=0)=0.8. All other parameters are the same. Threshold(t=0) must be changed accordingly.

APPENDIX: GRAPHS

Fig A.3

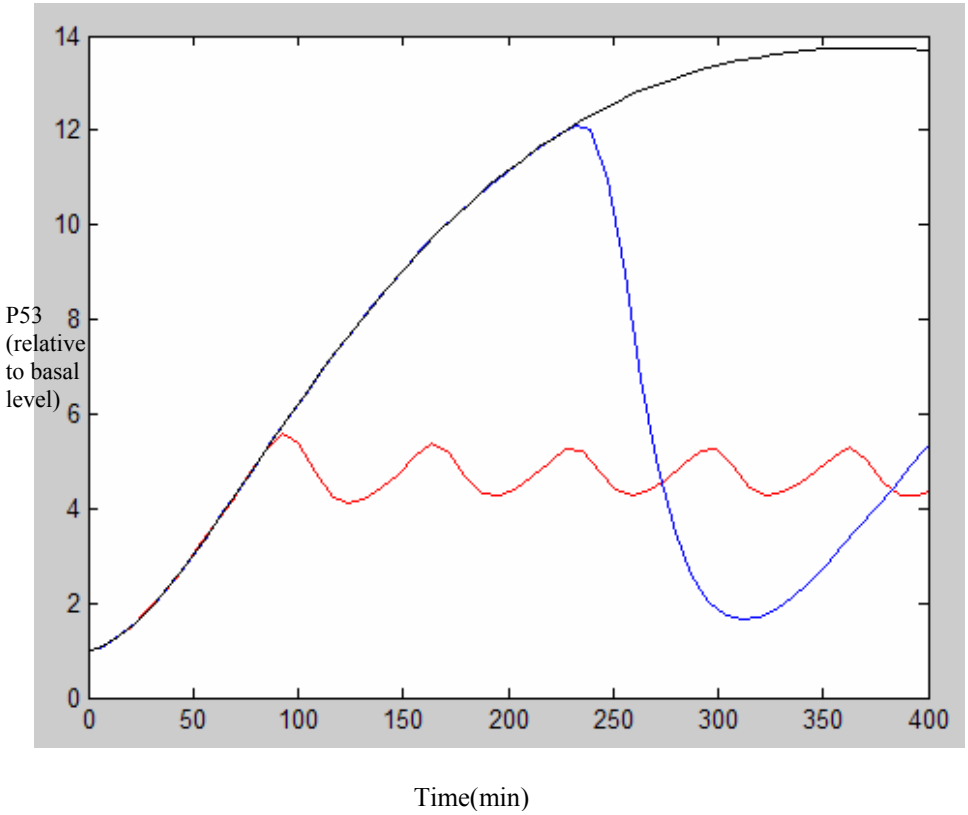


Fig A.3: Effect of delay on p53 levels (relative to basal level) Blue line is for intermediate delay (c1=kdelay=4e-3) i.e. 50 min lag between p53 and Mdm2 peaks. Red line is for small delay (c1=kdelay=0.09) lag is 20 min. Oscillations last for longer than first case with smaller amplitude. Black line if for large delay (c1=kdelay=9e-4) lag is 5 hours. Oscillation has a large initial pulse of p53 but dies out soon.

Fig A.4

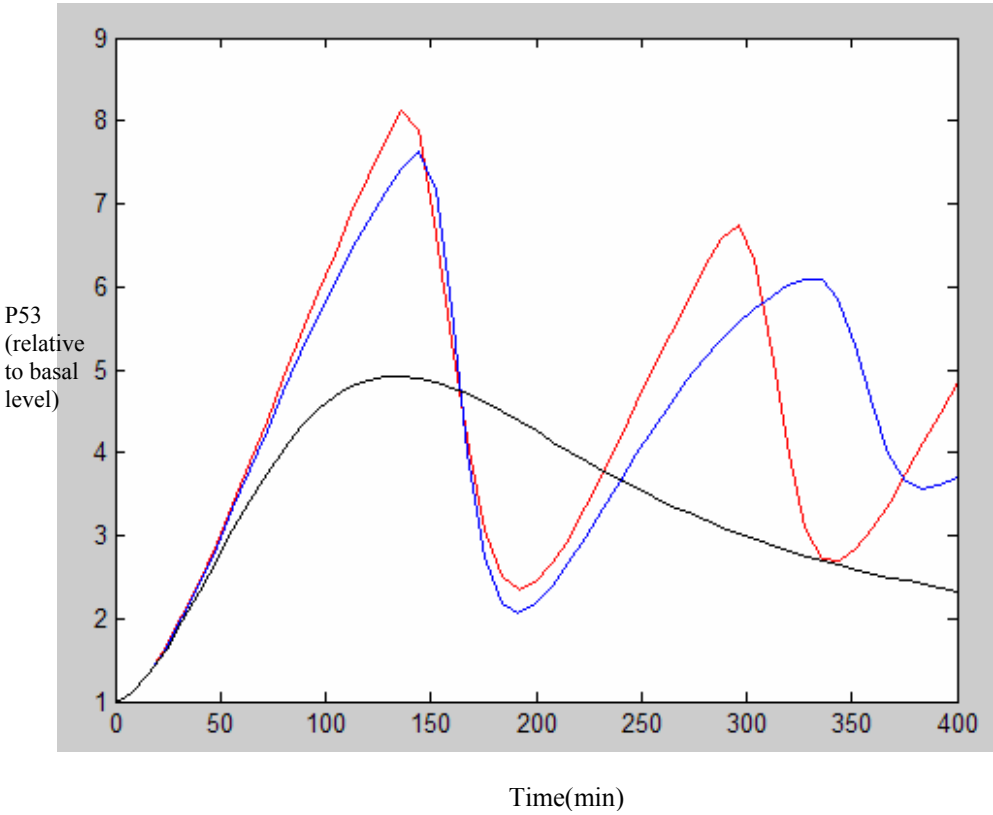


Fig A.4: Dependence of p53 levels (relative to basal amounts) on the damage repair rate (repair). Black line is for repair = -1.4e-3 (12 hours). Blue line is for repair = -3.5e-4 (2days) and red line is for repair = -1.4e-4 (5days). All other parameters same as in Table1.