Journal of Theoretical Biology 254 (2008) 452–465

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Oscillations and bistability in the stochastic model of p53 regulation

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ARTICLE INFO

Article history:
Received 7 March 2008
Received in revised form
27 May 2008
Accepted 27 May 2008
Available online 4 June 2008

Keywords:
Signaling pathways
Positive and negative feedbacks
Mathematical modeling
Stochastic simulations
Apoptosis
p53
Mdm2
PTEN

ABSTRACT

The p53 regulatory pathway controls cell responses, which include cell cycle arrest, DNA repair, apoptosis and cellular senescence. We propose a stochastic model of p53 regulation, which is based on two feedback loops: the negative, coupling p53 with its immediate downregulator Mdm2, and the positive, which involves PTEN, PIP3 and Akt. Existence of the negative feedback assures homeostasis of healthy cells and oscillatory responses of DNA-damaged cells, which are persistent when DNA repair is inefficient and the positive feedback loop is broken. The positive feedback destroys the negative coupling between Mdm2 and p53 by sequestering most of Mdm2 in cytoplasm, so it may no longer prime the nuclear p53 for degradation. It works as a clock, giving the cell some time for DNA repair. However, when DNA repair is inefficient, the active p53 rises to a high level and triggers transcription of proapoptotic genes. As a result, small DNA damage may be repaired and the cell may return to its initial "healthy" state, while the extended damage results in apoptosis. The stochasticity of p53 regulation, introduced at the levels of gene expression, DNA damage and repair, leads to high heterogeneity of cell responses and causes cell population split after irradiation into subpopulations of apoptotic and surviving cells, with fraction of apoptotic cells growing with the irradiation dose.

1. Introduction

1.1. Biological background

The p53 regulatory pathway is composed of hundreds of genes and their products, that respond to a wide variety of stress signals (see Levine et al., 2006; Kohn and Pommier, 2005 for recent reviews). The input signals, that include DNA damage, oncogene activation, heat and cold shock, and others, are transmitted by the upstream mediators and influence p53 level and its transcriptional activity by several posttranslational modifications. The action of p53/Mdm2 core is controlled by a number of positive and negative feedbacks (Harris and Levine, 2005). The downstream events, which are mediated by groups of genes and their products, are regulated by the p53 protein, most commonly by transcriptional activation but in some cases by protein–protein interactions. The cellular outputs of these downstream events include cell cycle arrest, DNA repair, apoptosis or cellular senescence and often result in extensive communication with other transduction pathways, reviewed by Vousden and Lane (2007).

Relevant to the proposed model is the p53 activation in the response to DNA damage. In healthy cells the level of p53 remains typically low under the control of Mdm2, which is responsible for p53 ubiquitination leading to its rapid degradation (Haupt et al., 1997; Kubbutat et al., 1997). In turn, synthesis of Mdm2 transcript is controlled by p53 (Barak et al., 1993), which defines the negative feedback. DNA damage activates the so-called checkpoint proteins, which destabilize Mdm2 and trigger p53 phosphorylation elevating its stability and transcriptional activity (reviewed in Volgenstein et al., 2000). This disturbs homeostatic balance between Mdm2 and p53 leading to oscillations and/or rise of the p53 level. Activated p53, triggers transcription of groups of genes, whose products are responsible for cell cycle arrest and DNA repair and, if the last fails or takes too long, for initiation of apoptosis. Disruption of any of these processes can allow mutations to pass from one cell generation to the next, which is permissive for the development of cancer (Mayo and Donner, 2002). It can be asserted that essentially every solid cancer lacks a normal p53 response; in approximately half of the cancers this is due to alterations in p53 itself, whereas in the other half there are alterations in p53 regulators. One of the key p53 regulators is the p53-responsive phosphatase PTEN, that mediates, via phosphatidylinositol 3-phosphate (PIP3) and Akt/PBK kinase, the positive feedback loop allowing p53 to rise to a high level and initiate apoptosis (Cantley and Neel, 1999). This places PTEN among the most commonly lost tumor suppressors in human cancer.

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doi:10.1016/j.jtbi.2008.05.039

Mutations (or deletions) of PTEN which occur during tumor development block its enzymatic activity leading to increased cell proliferation and reduced cell death (reviewed in Simpson and Parsons, 2001; Li and Ross, 2007).

1.2. Positive and negative feedbacks

The p53-responsive genes produce proteins that interact with a large number of other signal transduction pathways and, as discussed by Harris and Levine (2005), there are at least seven negative and three positive feedback loops regulating p53 level and its transcriptional activity. This complexity poses a difficulty for modelers and makes the p53 regulation far from being resolved. The other difficulty is due to the fact that most of the experimental data available is on cancer cells, which exhibit various malfunctions of the p53 network. In addition, potentially informative experiments on single cells (Lahav et al., 2004; Geva-Zatorsky et al., 2006) are performed on transfected cells with elevated number of p53 or Mdm2 gene copies which, due to high nonlinearity in regulation, may exhibit a different behavior than that of the original cells.

Since Bar-Or et al. (2000) and then (in single cells) Lahav et al. (2004) and Geva-Zatorsky et al. (2006) demonstrated existence of irregular, but persistent oscillations of Mdm2 and p53 nuclear levels in response to DNA damage caused by Γ irradiation, several models have been proposed in order to explain the observed oscillatory behavior. Undamped oscillations are the manifestation of autonomous limit cycle, a recurrent solution of constant amplitude and period. Such solution may appear when negative feedback is combined with time delay, and/or concurrent positive feedback loop (Tyson, 2006; Rateitschak and Wolkenhauer, 2007).

The first case, in which negative feedback coupling p53 and Mdm2 is combined with time delay, has been studied by several authors including Bar-Or et al. (2000), who assumed that p53 induces Mdm2 via an intermediary, then by Ma et al. (2005) and Wagner et al. (2005) who explicitly introduced two time delays associated with transcription and translation of Mdm2.

The group of Tyson explored the second possibility, analyzing four different positive feedback loops which, in cooperation with the negative feedback involving p53 and Mdm2, assure existence of stable oscillations in response to DNA damage. Specifically, Ciliberto et al. (2005) considered the positive feedback loop which blocks Mdm2 nuclear entry, rescuing p53 from Mdm2 mediated degradation. Despite this feedback loop is mediated by PTEN, PIP3 and Akt, the authors neither considered these intermediaries explicitly, nor introduced any time delay to the regulation. This simplification, however, substantially changes the dynamics: since p53 blocks nuclear entry of Mdm2 without any time delay, Mdm2 first accumulates in cytoplasm, then enters the nucleus, which is not observed experimentally. The same two feedbacks have been considered in the work of Wee and Aguda (2006); the scope of their study was to demonstrate existence and robustness of bistability in p53-Akt regulation. Three other positive feedbacks are considered in a recent study by Zhang et al. (2007). The main problem in explaining oscillations by presence of a positive feedback is that these oscillations appear in relatively narrow gap of parameters. Zhang et al. (2007) demonstrated that system passes from the stable point to the limit cycle as Mdm2 degradation coefficient changes, which can be associated with DNA damage. However, these oscillations result also from a relatively modest (in Ciliberto et al., 2005 model a 10% change) change of p53 synthesis rate, which can be due to p53 transfection. In other words, assuming that these models properly describe transfected cells studied by Geva-Zatorsky et al. (2006), one must conclude that the behavior of non-transfected cells is different.

The single cell stochastic model, we introduce in this study, is also based on the negative feedback involving p53 and Mdm2 and on the positive one mediated by PTEN, PIP3 and Akt. However, in contrast to the models considered by Zhang et al. (2007), in our model the oscillations are due solely to the negative feedback and time delay (introduced by explicit presence of intermediaries: Mdm2 transcript, cytoplasmic Mdm2 and cytoplasmic phosphorylated Mdm2). The role of positive feedback is to terminate oscillations, by sequestering most of Mdm2 in cytoplasm, which enables p53 to rise to a high level, such that it may activate proapoptotic genes. The positive feedback acts on a longer time scale than the negative one, and thus becomes important only when DNA repair is inefficient.

1.3. Stochastic effects

Experiment by Geva-Zatorsky et al. (2006) demonstrated oscillations of p53 and Mdm2 nuclear levels continuing over 72 h after Γ irradiation. These oscillations have relatively stable period of about 4–7 h, but highly variable amplitude. In addition, resting cells (and a sizable fraction of the irradiated cells) exhibit irregular bursts or fluctuations. Such behavior suggests existence of some stochastic fluctuations, which cause that the single cell trajectories depart from the stable limit cycle or the stable steady state.

It is natural to expect that reactions involving small number of molecules, but associated with some amplification pathways, are the main source of the overall stochasticity (Lipniacki et al., 2007). The first candidate is the expression of p53-responsive gene. There is a growing experimental evidence that mRNA transcription is quantal, that implies noisy fluctuations of mRNA and protein levels (e.g. Walters et al., 1995; Femino et al., 1998; Rai et al., 2006). Following Ma et al. (2005) we expect also that stochastic DNA damage and repair significantly contribute to the overall stochasticity of the process.

As demonstrated recently by Joo et al. (2007) in NF-κB system, stochasticity strengthen oscillations, i.e. when the stochastic effects are taken into considerations, the range of parameters for which the regulatory system exhibits oscillations is broader. One may thus expect that observed oscillations are, in part, due to the stochastic effects.

2. Model formulation

2.1. Basic assumptions

The model involves two-compartment kinetics of p53, its primary inhibitor Mdm2, phosphatase PTEN, phosphatidylinositol 3-phosphate (PIP3) and Akt kinase. The level and the transcriptional activity of p53 is regulated by two feedbacks, the negative one involving Mdm2 and Akt, and the positive one that involves PTEN, PIP3 and Akt (Fig. 1).

The negative feedback arises since phosphorylated p53 triggers Mdm2 transcription. In turn synthesized Mdm2 is activated in cytoplasm by Akt, enters cell nucleus and ubiquitinates p53, which results in its rapid degradation. The second feedback is positive in that sense that it blocks the negative feedback loop. In short, p53 induces transcription of PTEN, then PTEN deactivates PIP3 hydrolyzing it to PIP2, which may not activate Akt. As a result, p53 inhibits its inhibitor Mdm2, which may not translocate to the nucleus and prime p53 for degradation.

Below we summarize the main biological findings and assumptions on which the model is established.

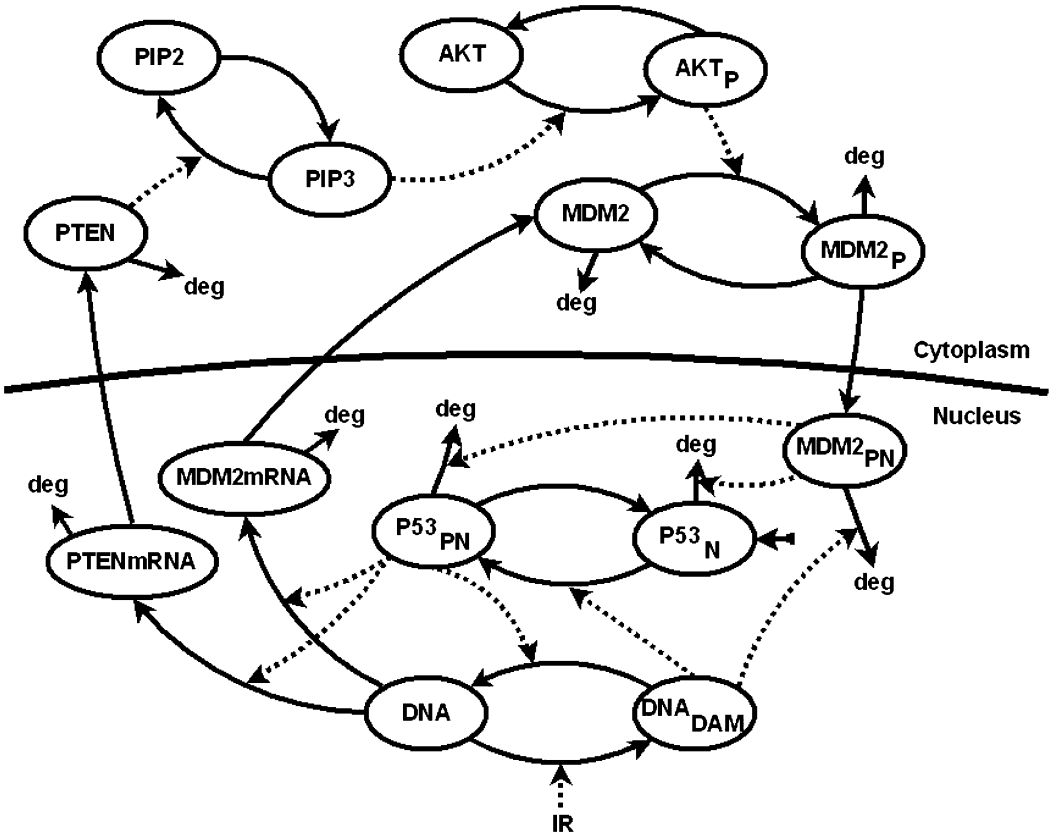

Fig. 1. Schematic of the model.

2.1.1. Negative feedback

The amount of p53 protein in cells is determined mainly by the rate at which it is degraded rather than the rate at which it is made (Michael and Oren, 2003). Biogenesis of p53 involves cotranslational dimerization of monomers and posttranslational dimerization of dimers (Nicholls et al., 2002). The subsequent dimerization of dimers is rather inefficient in the solution with dissociation constant of $\sim 3\,\mu M$ for dimer–tetramer equilibrium; however, p53 dimers exhibit high cooperativity in DNA binding (Weinberg et al., 2004) with a Hill coefficient of 1.8 (dimer:DNA). This finding, together with the observation that mutated p53 with impaired tetramerization binds DNA with six-fold less affinity than wild-type protein, suggests that the fundamental active unit of p53 is the tetramer (Weinberg et al., 2004). In addition, Meek (1998), Meek and Milne (2000) and Brooks and Gu (2003) showed that p53 phosphorylation enhances its activity as a transcription factor.

According to the above we make the simplifying assumption that p53 is produced (with some constant rate) as a dimer, immediately translocates to the nucleus, where it may attain its transcriptional activity due to phosphorylation. We assume also that activation rates of both Mdm2 and PTEN genes are proportional to the level of phosphorylated p53 in power 2.

In order to enter the nucleus Mdm2 must be phosphorylated by Akt, which physically associates with Mdm2 and phosphorylates it at Ser166 and Ser186 (Mayo and Donner, 2001; Zhou et al., 2001). Then the phosphorylated Mdm2 may shuttle between cytoplasm and nucleus. Thus, in the model we assume that in the cytoplasm we have both the phosphorylated and the unphosphorylated Mdm2, while in the nucleus we have only the phosphorylated form. Nuclear Mdm2 is responsible for attaching the first ubiquitin moiety to p53 initializing p53 polyubiquitination (Haupt et al., 1997). Attaching the subsequent ubiquitins to p53 is catalyzed by Mdm2 and other enzymes like p300. Polyubiquitinated p53 translocates to the cytoplasm to be degraded by the 26S proteasome or it is degraded in the nucleus (Stommel and Wahl, 2004). p53 phosphorylation leads to its acetylation (not explicitly included in the model), which protects p53 from Mdm2-dependent ubiquitination and degradation (reviewed in Bode and Dong, 2004).

We assume that the p53 degradation rate is a sum of a constant term and an Mdm2-dependent term following Hill function with Hill coefficient equal to 2, and that phosphorylated p53 has 10-fold lower Mdm2-dependent degradation coefficient than the unphosphorylated p53.

2.1.2. Positive feedback

First, PTEN transcription is regulated by p53, and level of PTEN transcript grows about 3–4-fold over 16 h after 10 Gy irradiation (Stambolic et al., 2001). Next, phosphatase PTEN hydrolyzes PIP3, produced by the activated phosphatidylinositol 3-kinase (PI3K), to PIP2 (reviewed in Cully et al., 2006). Since Akt is activated by PIP3, not by PIP2, PTEN acts as Akt inhibitor. As already said, active Akt is needed to phosphorylate Mdm2, which enables its nuclear import. This accomplishes the positive feedback loop, in which p53 via PTEN, PIP3 and Akt inhibits its own inhibitor Mdm2. We assume that the total amounts of PIP (PIP2+PIP3) and of the Akt kinase remain constant, i.e. that there is a balance between their synthesis and degradation.

Let us note that PTEN regulates p53 protein levels also through Akt-independent mechanisms; it may directly interact with p53 enhancing its stability (Freeman et al., 2003) or downregulates Mdm2 transcription by negatively regulating its P1 promoter (Chang et al., 2004). These interactions, which are not included in the model also provide positive feedback in p53 regulation.

2.1.3. System activation

The p53 system may respond to various stress signals and its activation is mediated by numerous kinases. DNA damage, ultraviolet light or oncogene stress lead to p53 modifications (serine and theorine phosphorylation, acetylation and others) resulting mainly in its enhanced stability and transcriptional activity (reviewed in Bode and Dong, 2004). The other way of p53 activation is mediated by inhibition or degradation of Mdm2. This can be due to the expression of oncogenes (Volgenstein et al., 2000), which may inhibit Mdm2 via activation of $p14^{ARF}$, or DNA damage, which also accelerates Mdm2 auto-degradation.

The most intensively investigated pathway of p53 activation is the one initiated by DNA damage. The irradiation dose of 1 Gy leads to about 35 double strand breaks (DSBs) (Lobrich et al., 1995; Rothkamm and Lobrich, 2003). The damage is sensed by the so-called checkpoint proteins or damage-dependent kinases: ATM, ATR, DNAPK, Chk1, Chk2, JNK and P38 kinase, which sense and signal DNA damage to p53 and Mdm2. Particularly, ATM is a very sensitive and rapid detector of DNA damage. Bakkenist and Kastan (2003) showed that ATM is phosphorylated at Ser 1981 within 5 min after irradiation and that ATM activation starts at irradiation dose of 0.1–0.2 Gy and saturates at 0.4 Gy. As a result, p53 is phosphorylated at several serine residues including Ser 15, 20, 33, 37, 315, 392 (with Ser 15 possibly being the most important), which leads to its stabilization and enhances its transcriptional activity (Appella and Anderson, 2001; Bode and Dong, 2004). Simultaneously in DNA-damaged cells, damage-dependent kinases (particularly ATM) phosphorylate Mdm2 resulting in its accelerated auto-ubiquitination and proteasome-mediated degradation (Stommel and Wahl, 2004, 2005; Meulmeester et al., 2005). As found by Stommel and Wahl (2004) the Mdm2 half-life decreased about seven-fold in the response to DNA damage and may be as low as 5 min.

In the model, these experimental finding are substantially simplified; we assume that p53 may exist only in two forms: active and inactive. Only the active form has a transcriptional activity and simultaneously lower (Mdm2 dependent) degradation rate. We assume that irradiation leads to DNA damage in a Poisson process (see below). Next, we assume that DNA damage influences p53 and Mdm2 (implicitly via ATM or other damage-dependent kinases) and that the strength of this influence follows a Hill function of number of DSB, N (with Hill coefficient equal to 2 and a half-saturation threshold of seven DSB corresponding to $\sim$0.2 Gy). Namely, we assume that transformation rate from inactive to active p53 is

$$\left(a_0 + a_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right) \tag{1}$$

and that Mdm2 degradation rate is

$$\left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right). \tag{2}$$

2.1.4. Transcription and translation

There is a growing experimental evidence that mRNA transcription is quantal, and that transcription factors regulate probability that the given gene is *on* or *off* rather than the transcription rate (e.g. Walters et al., 1995; Femino et al., 1998; Rai et al., 2006). The *on* and *off* gene switching introduces high level of stochasticity to eukaryotic gene expression and cell regulation (e.g. Ko, 1991; Kepler and Elston, 2001; Lipniacki et al., 2006; Paszek, 2007; reviewed in Kærn et al., 2005). A single gene activation event results (if the activation period is sufficiently long) in a sizable burst of mRNA molecules, which is then translated into an even larger burst of proteins. Here, we adopt this approach and assume that the state of each of two p53-dependent genes is a sum of states of its copies: $G_M = G_{M1} + G_{M2}$, $G_M \in \{0,1,2\}$ for Mdm2 gene, and $G_P = G_{P1} + G_{P2}$, $G_P \in \{0,1,2\}$ for PTEN. The activation rate of each gene copy depends on the nuclear level of phosphorylated p53 dimers in a power of 2 (due to the cooperativity in binding). Thus, in an infinitesimal time interval Δt the probability $P^b(t,\Delta t)$ of gene copy activation is

$$P^b(t,\Delta t) = \Delta t \times (q_0 + q_1 \times P53_{np}^2(t)), \tag{3}$$

where $P53_{np}$ is the nuclear amount of phosphorylated p53 dimers, where q_0 represents some basal gene activation rate independent to p53. We assume that inactivation probability is constant, i.e.

$$P^d(t,\Delta t) = \Delta t \times q_2. \tag{4}$$

Let us note that when $P53_{np}(t)$ varies slowly, the probability that the gene copy is active is approximately P_A (it is precisely P_A if $P53_{np}(t) = const$)

$$P_A(t) = \frac{q_0 + q_1 \times P53_{np}^2(t)}{q_2 + q_0 + q_1 \times P53_{np}^2(t)}. \tag{5}$$

Probability $P_A(t)$ may be interpreted as a "transcriptional efficiency of p53"; when there is no active p53, $P_A(t) = q_0/(q_2 + q_0)$, and p53-dependent genes are inactive for most of the time. When the level of active p53 is very high, $P_A(t) \simeq 1$ and p53-dependent genes are transcriptionally active for most of the time. There is no reason to expect that the coefficients q_0, q_1 and q_2 are the same for all p53-dependent genes, nevertheless we assume that they are equal for Mdm2 and PTEN genes in order to reduce the number of parameters.

We will assume that when allele is active then the transcription proceeds with some rate s_0 for Mdm2 gene and rate s_1 for PTEN gene. The speed of mRNA polymerase $\sim$40 nt/s and the characteristic minimum spacing between the neighboring polymerases 250 nt gives the upper limit for transcriptional efficiency from single allele $s_{max} = 40/250 = 0.16$ (mRNA/s). Similarly, we may obtain the upper limit for translation efficiency $t_{max} = 0.5$ (protein/mRNA/s) (see Lipniacki et al., 2007 and references therein).

2.1.5. DNA damage and repair

We assume that during irradiation phase DNA damage arises, and that in an infinitesimal time interval Δt the probability $P^{DAM}(t,\Delta t)$ that new DSB appears is

$$P^{DAM}(t,\Delta t) = \Delta t \times d_{DAM} \times R, \tag{6}$$

where R is the irradiation intensity and $d_{DAM} = 35/\text{Gy}$ is the DNA damage coefficient. As a result of this process (neglecting DNA repair during irradiation phase) the number of DSB follows Poisson distribution with the average equal to $35x$, where x is irradiation dose in Gy.

We do not include any mechanism of DNA repair explicitly in our model. Instead, since p53 regulates transcription of numerous genes involved in DNA repair, we assume that the rate of DSB healing is governed by the p53 transcriptional activity $P_A(t)$, given in Eq. (5).

In an infinitesimal time interval Δt the probability $P^{REP}(t,\Delta t)$ that number of DSBs $N(t)$ decreases by one is

$$P^{REP}(t,\Delta t) = N(t) \frac{\Delta t \times d_{REP} \times P_A(t)}{N(t) + N_{SAT} \times P_A(t)}. \tag{7}$$

This form of P^{REP} reflects the assumption that when the number of DSBs is large, with respect to the amount repair enzymes, they must compete for them.

2.2. Model equations

In this section we give the equations of model following the assumed kinetics. In the computations, amounts of all substrates are expressed in the numbers of molecules. Since we use the ODEs to describe most of the model kinetics, amounts of molecules are not integer numbers, but since these numbers are in most cases much greater than 1, such description is reasonable. To translate concentrations of the substrates to the numbers of molecules, we assume that the cell volume is $2000\,\mu m^3$ and that the ratio of the cytoplasm to nucleus is 5. In such a cell, cytoplasmic concentration of 1 nM corresponds to 1000 molecules, while the same nuclear concentration corresponds to 200 molecules.

Upper-case letters denote levels of mRNAs and proteins. Nuclear levels are denoted by subscript n, while the subscript denoting level of substrate in the cytoplasm is omitted to simplify the notation. Active (phosphorylated) forms are denoted by subscript p, levels of Mdm2 and PTEN transcripts are denoted by subscript t.

Variables:

AKT_p	active form of Akt
MDM_t	Mdm2 transcript
MDM	cytoplasmic Mdm2
MDM_p	phosphorylated cytoplasmic Mdm2
MDM_{pn}	phosphorylated nuclear Mdm2
$P53_n$	inactive form of nuclear p53 dimers
$P53_{pn}$	active form of nuclear p53 dimers
$PTEN_t$	PTEN transcript
$PTEN$	(cytoplasmic) PTEN
PIP_p	active form of PIP (PIP3)
N	number of DSBs
R	irradiation intensity

$G_M = G_{M1} + G_{M2}$ state of Mdm2 gene, $G_M \in \{0,1,2\}$
$G_P = G_{P1} + G_{P2}$ state of PTEN gene, $G_P \in \{0,1,2\}$

Parameters: We estimated the range in which each parameter may be changed (separately), without causing any qualitative difference in the pathway regulation as discussed in the next section, Table 1. Since most of the parameters can be changed in a relatively wide range, the properties of the model are rather due to its structure than a precise tuning of the model parameters.

Equations: (Cytoplasmic) PTEN, *PTEN*: The first term describes *PTEN* synthesis and the second one describes *PTEN* degradation

$$\frac{d}{dt}PTEN(t) = t_1 PTEN_t(t) - d_2 PTEN(t). \tag{8}$$

Active form of PIP, PIP_p: The first term describes *PIP* activation, the second one describes its dephosphorylation by *PTEN*

$$\frac{d}{dt}PIP_p(t) = a_2(PIP_{tot} - PIP_p(t)) - c_0 PTEN(t) PIP_p(t). \tag{9}$$

Active Akt, AKT_p: The first term describes *AKT* activation catalyzed by PIP_p and the second one describes AKT_p inactivation

$$\frac{d}{dt}AKT_p(t) = a_3(AKT_{tot} - AKT_p(t)) PIP_p(t) - c_1 AKT_p(t). \tag{10}$$

Cytoplasmic Mdm2, *MDM*: First term describes *MDM* synthesis, the second term describes MDM_p dephosphorylation, the third term describes *MDM* phosphorylation catalyzed by AKT_p, while last one stands for *MDM* degradation

$$\begin{aligned}\frac{d}{dt}MDM(t) = {} & t_0 MDM_t(t) + c_2 MDM_p(t) \\ & - a_4 MDM(t) AKT_p(t) \\ & - \left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right) MDM(t).\end{aligned} \tag{11}$$

Cytoplasmic phosphorylated Mdm2, MDM_p: The first term represents *MDM* phosphorylation catalyzed by AKT_p, the second term describes MDM_p dephosphorylation, the third and the fourth term represent nuclear import and export, and the last one stands for MDM_p degradation

$$\begin{aligned}\frac{d}{dt}MDM_p(t) = {} & a_4 MDM(t) AKT_p(t) - c_2 MDM_p(t) \\ & - i_0 MDM_p(t) + e_0 MDM_{pn}(t) \\ & - \left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right) MDM_p(t).\end{aligned} \tag{12}$$

Nuclear phosphorylated Mdm2, MDM_{pn}: The first and the second term represent nuclear import and export, while the last term describes spontaneous and DNA damage driven MDM_{pn} degradation

$$\begin{aligned}\frac{d}{dt}MDM_{pn}(t) = {} & i_0 MDM_p(t) - e_0 MDM_{pn}(t) \\ & - \left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right) MDM_{pn}(t).\end{aligned} \tag{13}$$

Inactive (nuclear) p53, $P53_n$: The first term describes $P53_n$ synthesis, the second one its spontaneous and DNA damage driven activation, the third one inactivation of $P53_{pn}$, and last one spontaneous and MDM_{pn}-induced degradation

$$\begin{aligned}\frac{d}{dt}P53_n(t) = {} & p_0 - \left(a_0 + a_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right) P53_n(t) + c_3 P53_{pn}(t) \\ & - (d_3 + d_4 MDM_{pn}^2(t)) P53_n(t).\end{aligned} \tag{14}$$

Active (nuclear) p53, $P53_{pn}$: The first term represents spontaneous and DNA damage driven $P53_n$ activation, the second term represents $P53_{pn}$ inactivation, and last one spontaneous and MDM_{pn} driven degradation

$$\begin{aligned}\frac{d}{dt}P53_{pn}(t) = {} & \left(a_0 + a_1 \frac{N^2(t)}{h_0^2 + N^2(t)}\right) P53_n(t) - c_3 P53_{pn}(t) \\ & - (d_5 + d_6 MDM_{pn}^2(t)) P53_{pn}(t).\end{aligned} \tag{15}$$

Mdm2 transcript, MDM_t: The first term stands for MDM_t transcription, while the second one describes its degradation

$$\frac{d}{dt}MDM_t(t) = s_0(G_{M1} + G_{M2}) - d_7 MDM_t(t). \tag{16}$$

PTEN transcript, $PTEN_t$: The first term stands for $PTEN_t$ transcription, while the second one describes its degradation

$$\frac{d}{dt}PTEN_t(t) = s_1(G_{P1} + G_{P2}) - d_8 PTEN_t(t). \tag{17}$$

Eqs. (8)–(17), in which the discrete variables N, G_{M1}, G_{M2}, G_{P1} and G_{P2} follow the stochastic process defined by transition probabilities given in Eqs. (3), (4), (6) and (7) describe the stochastic kinetic of the model. The numerical implementation

Table 1

Parameter	Description	Value	Range
a_0	Spontaneous $P53_n$ phosphorylation rate	1×10^{-4}/s	$(0.2-5)\times10^{-4}$
a_1	DSB-induced $P53_n$ phosphorylation rate	1×10^{-3}/s	$(0.3-3)\times10^{-3}$
a_2	*PIP* activation rate	5×10^{-5}/s	$(1-10)\times10^{-5}$/s
a_3	*AKT* activation rate	2×10^{-9}/s	$(0.4-4)\times10^{-9}$/s
a_4	*MDM* phosphorylation rate	1×10^{-8}/s	$(0.2-2)\times10^{-8}$/s
c_0	PIP_p dephosphorylation rate (by *PTEN*)	2.5×10^{-9}/s	$(1.25-12.5)\times10^{-9}$/s
c_1	AKT_p inactivation rate	2×10^{-4}/s	$(1.3-10)\times10^{-4}$/s
c_2	MDM_p dephosphorylation rate	1×10^{-4}/s	$(0.2-5)\times10^{-4}$/s
c_3	Spontaneous $P53_{pn}$ de-phosphorylation rate	0	$(0-2)\times10^{-4}$/s
d_0	Mdm2 spontaneous deg. rate (all Mdm2 forms)	3×10^{-5}/s	$(0.6-6)\times10^{-5}$/s
d_1	DSB-induced Mdm2 deg. rate (all Mdm2 forms)	1.5×10^{-4}/s	$(0.75-2.25)\times10^{-4}$/s
d_2	*PTEN* degradation rate	5×10^{-5}/s	$(1-10)\times10^{-5}$/s
d_3	Spontaneous $P53_n$ degradation rate	1×10^{-4}/s	$(0.2-5)\times10^{-4}$/s
d_4	MDM_{pn}-induced $P53_n$ degradation rate	1×10^{-13}/s	$(0.5-4)\times10^{-13}$/s
d_5	Spontaneous $P53_{pn}$ degradation rate	1×10^{-4}/s	$(0.2-3)\times10^{-4}$/s
d_6	MDM_{pn}-induced $P53_{pn}$ degradation rate	1×10^{-14}/s	$(0.2-5)\times10^{-14}$/s
d_7	MDM_t degradation rate	3×10^{-4}/s	$(2-4.5)\times10^{-4}$/s
d_8	$PTEN_t$ degradation rate	3×10^{-4}/s	$(0.6-6)\times10^{-4}$/s
e_0	MDM_{pn} nuclear export	0	$(0-2)\times10^{-4}$/s
i_0	MDM_p nuclear import	5×10^{-4}/s	$(1.6-25)\times10^{-4}$/s
p_0	$P53_n$ production rate	2×10^{2}/s	$(0.4-6)\times10^{2}$/s
s_0	MDM_t transcription rate	6×10^{-2}/s	$(4-9)\times10^{-2}$/s
s_1	$PTEN_t$ transcription rate	6×10^{-2}/s	$(3-30)\times10^{-2}$/s
t_0	*MDM* translation rate	5×10^{-1}/s	$(3-7.5)\times10^{-1}$/s
t_1	*PTEN* translation rate	1×10^{-1}/s	$(0.5-5)\times10^{-1}$/s
h_0	Michaelis const. for DSB-induced $P53_n$ activation and for DSB-induced Mdm2 deg. (all Mdm2 forms)	7	(1.4–35)
q_0	Spontaneous activation of Mdm2 and PTEN genes	1×10^{-4}/s	$(0.2-2.2)\times10^{-4}$/s
q_1	$P53_{pn}$-dependedactivation of Mdm2 and PTEN genes	5×10^{-13}/s	$(1-26)\times10^{-13}$/s
q_2	Mdm2 and PTEN genes inactivation rate	3×10^{-3}/s	$(1.7-16)\times10^{-3}$/s
N_{SAT}	Saturation coefficient in DNA repair	50	(10–250)
d_{DAM}	DNA damage rate	35/Gy	
d_{REP}	DNA repair rate	3×10^{-3}/s	$(0.6-15)\times10^{-3}$/s
AKT_{tot}	Total number of Akt molecules ($AKT+AKT_p$)	2×10^{5}	$(0.4-10)\times10^{5}$/s
PIP_{tot}	Total number of PIP molecules ($PIP+PIP_p$)	1×10^{5}	$(0.2-5)\times10^{5}$/s
a_6	Max DNA damage rate (induced by the apoptotic factor)	1×10^{-1}/s	–
d_9	Apoptotic factors degradation rate	1×10^{-4}/s	–
p_1	Max synthesis rate of apoptotic factor	1×10^{2}/s	–
q_3	Coefficient governing apoptotic factor synthesis	8×10^{-14}/s	–
q_4	Michaelis const. for apoptotic factor synthesis	3×10^{-3}/s	–

The last five parameters are for the extended model only.

follows that of our recent study (Lipniacki et al., 2007) of stochastic dynamics in NF-κB system.

2.3. Dynamics in deterministic limit

As we will demonstrate in next section, stochastic effects of gene expression and DNA damage and repair substantially influence cell kinetics. Nevertheless, it is worth to analyze simultaneously the kinetics in the deterministic limit in which we may use such concepts as steady state or limit cycle. The deterministic limit of the model may be obtained after assuming that

(1) Gene activation and inactivation rates q_0, q_1 and q_2 tend to infinity with q_0/q_2 and q_1/q_2 remaining constant. In such a limit the mRNA transcription rate from single gene copy is $P_A(t)$ (as defined in Eq. (5)) and Eqs. (16) and (17) may be replaced by

$$\frac{\mathrm{d}}{\mathrm{d}t}MDM_t(t)=2s_0P_A(t)-d_7MDM_t(t), \tag{18}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}PTEN_t(t)=2s_1P_A(t)-d_8PTEN_t(t). \tag{19}$$

(2) Number of DSBs $N(t)$ is a continuous variable governed by the following equation:

$$\frac{\mathrm{d}}{\mathrm{d}t}N(t)=d_{DAM}\times R-\frac{N(t)\times d_{REP}\times P_A(t)}{N(t)+N_{SAT}\times P_A(t)}. \tag{20}$$

As a result the system dynamics in the deterministic limit is described by Eqs. (8)–(15) and (18)–(20).

3. Results

3.1. PTEN off, DNA repair off: persistent oscillations

In this section we discuss the case of PTEN knockouted cells in which (in addition) DNA repair is suppressed. PTEN is not induced by p53 in breast cancer MCF-7 cells (Wagner et al., 2005) due to its promoter methylation (García et al., 2004; Krawczyk et al.,

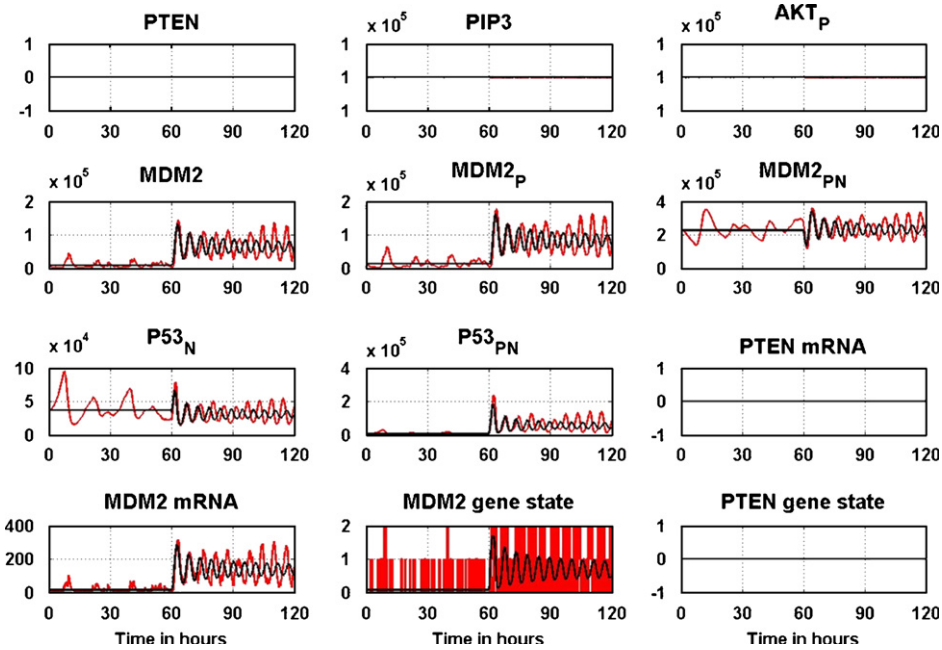

Fig. 2. Deterministic (black) and stochastic simulation (red) of the system with knockouted PTEN and no DNA repair. The deterministic and stochastic simulation started at $t = -60$ h from the fix point of the system in the deterministic limit. At $t = 0$ h the irradiation phase started and lasted 1 h; the total dose is 5 Gy. After irradiation simulation lasted until $t = 60$ h.

2007). These cells have been used in Bar-Or et al. (2000) experiments and then in single cell experiments by Lahav et al. (2004) and Geva-Zatorsky et al. (2006) that demonstrated oscillations in Mdm2 and p53 nuclear levels. In Geva-Zatorsky et al. (2006) experiment irradiated cells exhibited sustained oscillations for at least 72 h and even proliferated. This suggests that in these cells DNA was persistently damaged, but apoptotic and cell cycle arrest mechanisms were inefficient. In part, the resistance to apoptosis of irradiated MCF-7 cells may be caused by lack of caspase-3 (an important effector protein of apoptosis); as demonstrated by Essmann et al. (2004) reexpression of caspase-3 in these cells sensitized them to irradiation-induced apoptosis.

Mdm2 and p53 dynamics observed by Geva-Zatorsky et al. (2006) can be reproduced by our model after assuming that cells do not express PTEN (or that PTEN expression is independent to p53; data not shown) and that DNA repair is suppressed (or inefficient, so that at least about 10 DSBs remain unsealed—data not shown).

In Fig. 2, we show together results from the deterministic (black line) and the stochastic (red line) simulations. Let us first focus on the deterministic approximation; prior to DNA damage the system remains in a steady state, characterized by a low level of active p53. DNA damage enhances transition rate from inactive to active p53, and triggers Mdm2 degradation. Since active p53 is much more stable than inactive, it accumulates and activates Mdm2 transcription. In turn, synthesized and phosphorylated Mdm2 enters the nucleus and leads p53 to degradation. As a result, system tends to the limit cycle of period of about 6 h.

The stochastic dynamic of the system is substantially different. Prior to irradiation, p53 and Mdm2 levels depart from the fix point of the deterministic limit and exhibit highly irregular fluctuations, resembling trajectories of unstimulated and non-oscillatory cells observed in experiment (Geva-Zatorsky et al., 2006, Fig. 2). These irregular fluctuations are due to the stochastic gene activation, but interestingly, the characteristic time scale of these fluctuations is of order of about 15 h, much longer than the expected time of gene activity (~5 min) or inactivity (~150 min). The DNA damage triggers oscillations with well defined period, but varied amplitude, as demonstrated by Geva-Zatorsky et al. (2006, Fig. 2). Both, prior and after DNA damage, the source of stochasticity is the same: Mdm2 gene regulation. However, as showed by our simulations, the limit cycle appears to be a stronger attractor than the fix point.

The difference between the purely stochastic fluctuations prior to DNA damage and semiperiodic oscillations in DNA damaged cells is visualized in Fig. 3A. The averaged over 100 stochastic trajectories (green) exhibits almost no fluctuations prior to DNA damage (despite large fluctuations exhibited by individual cells—red and pink) and quite regular oscillations after the damage. The amplitude of these oscillations slowly decreases to zero (in contrast to the deterministic trajectory, which tends to the limit cycle) due to the progressing desynchronization of cells. The above analysis demonstrates that the deterministic approximation may neither properly reproduce single cell trajectories nor the population average.

As already said, DNA damage stimulates the system in two ways: (1) it destabilizes Mdm2 and (2) activates p53. It is interesting to analyze how cells respond to DNA damage if one of these two activation ways is blocked. We demonstrate that when DNA damage causes solely p53 activation (Fig. 3B) without any Mdm2 destabilization ($d_1 = 0$), the p53/Mdm2 oscillations are not induced, and the level of p53 remains low. Such behavior is in the agreement with the experimental data by Stommel and Wahl (2004, 2005), who found that blocking Mdm2 destabilization in DNA-damaged cells, prevented p53 targeted genes activation. In contrast, the DNA damage-induced p53 activation is not needed for oscillations (Fig. 3C).

3.2. PTEN on, DNA repair off: apoptosis

In this section we analyze the case in which PTEN mediated feedback loop is intact, but DNA repair is suppressed. As showed in Fig. 4, DNA damage results in activation of p53, which triggers PTEN transcription. Phosphatase PTEN accumulates in cytoplasm and dephosphorylates active PIP3 to PIP2, an inactive form, which

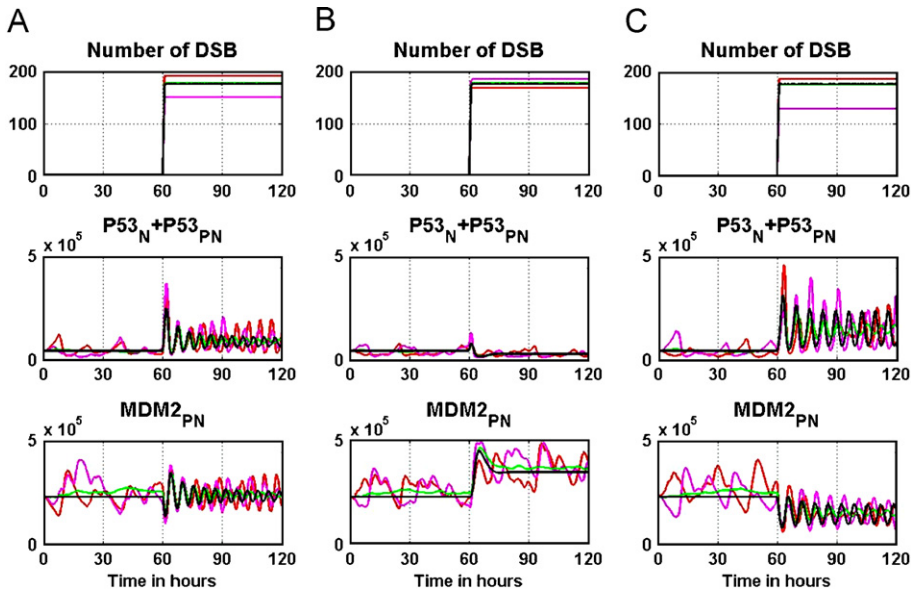

Fig. 3. Deterministic (black), stochastic (red and pink) and the average over 100 stochastic trajectories (green) in the case with PTEN knockout and no DNA repair; the total dose is 5 Gy. Panel A. The case in which DNA damage both augments p53 phosphorylation and destabilizes Mdm2 ($a_1 \neq 0$, $d_1 \neq 0$). Panel B. The case in which DNA damage solely augments p53 phosphorylation ($a_1 \neq 0$, $d_1 = 0$). Panel C. The case in which DNA damage solely destabilizes Mdm2 ($a_1 = 0$, $d_1 \neq 0$). The simulation protocol as in Fig. 2.

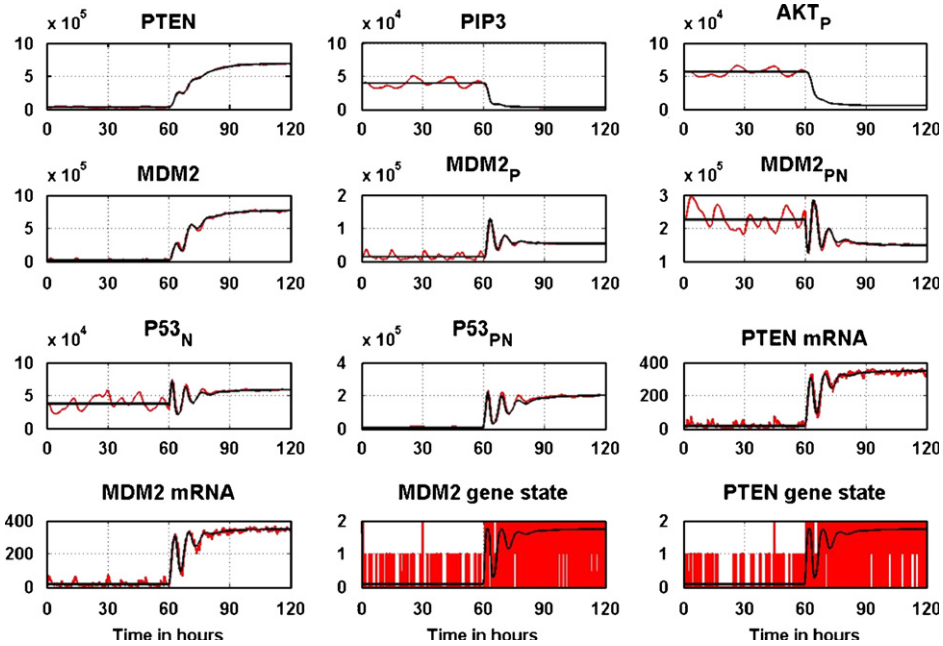

Fig. 4. Deterministic (black) and stochastic simulation (red) of the system with PTEN active and no DNA repair. The deterministic and stochastic simulation started at $t = -60$ h from the fix point of the system in the deterministic limit. At $t = 0$ h the irradiation phase started and lasted 1 h; the total dose is 5 Gy. After irradiation the simulation lasted until $t = 60$ h.

may not phosphorylate Akt. As a result, the level of active Akt decreases and bulk of Mdm2 remains unphosphorylated, and may not enter the nucleus. This way the negative feedback loop (responsible for oscillations) is blocked and p53 escapes out of the control of Mdm2, which accumulates mostly in cytoplasm and thus may not prime p53 for degradation. Finally, the system tends to a steady state, characterized by the high level of active p53 and the relatively low level of the nuclear Mdm2.

These findings are in the agreement with the study by Mayo et al. (2002), who demonstrated that overexpression of PTEN restricts Mdm2 to cytoplasm; Mdm2 was predominantly in the nucleus in U87MG cells and in the cytoplasm in U87MG(PTEN$^{-/-}$) cells. They showed also that PTEN augments the expression of p53 target genes and that the Mdm2 protein level was higher in U87MG(PTEN$^{-/-}$) than U87MG cells. Oppositely, expression of the constitutively active Akt promotes the nuclear entry of Mdm2, diminishes cellular levels of p53, and decreases p53 transcriptional activity (Mayo and Donner, 2001). The steady state, characterized by the high level of p53, may be interpreted as a state in which the apoptotic program is initiated. Such interpretation is justified by the finding that in apoptosis-prone cells, p53-dependent signaling enables Akt downregulation, resulting in elevated level of p53 (e.g. Gottlieb et al., 2002; Vousden and Lane, 2007).

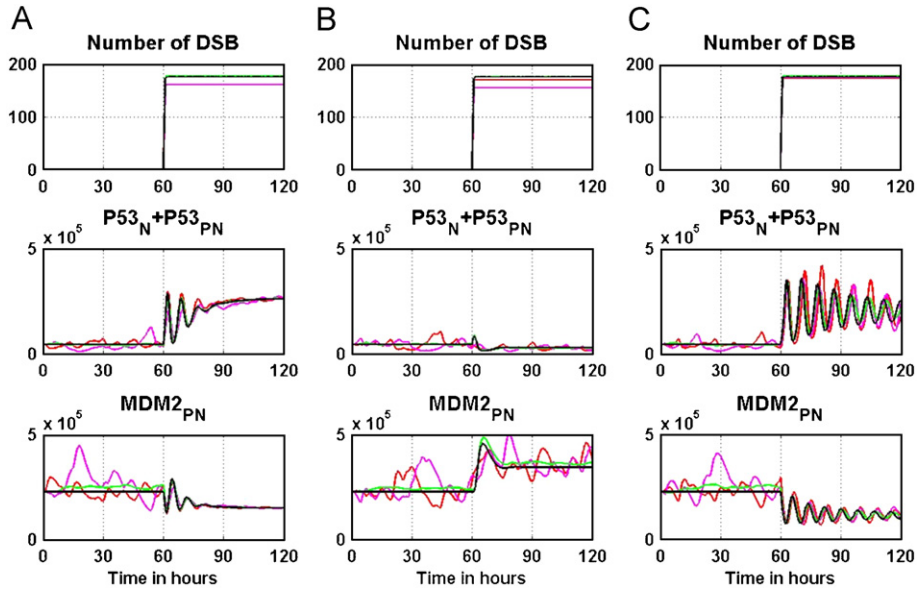

Fig. 5. Deterministic (black), stochastic (red and pink) and the average over 100 stochastic trajectories (green) in the case with PTEN active and no DNA repair; the total dose is 5 Gy. Panel A. The case in which DNA damage augments p53 phosphorylation and destabilizes Mdm2 ($a_1 \neq 0$, $d_1 \neq 0$). Panel B. The case in which DNA damage solely augments p53 phosphorylation ($a_1 \neq 0$, $d_1 = 0$). Panel C. The case in which DNA damage solely destabilizes Mdm2 ($a_1 = 0$, $d_1 \neq 0$). The simulation protocol as in Fig. 4.

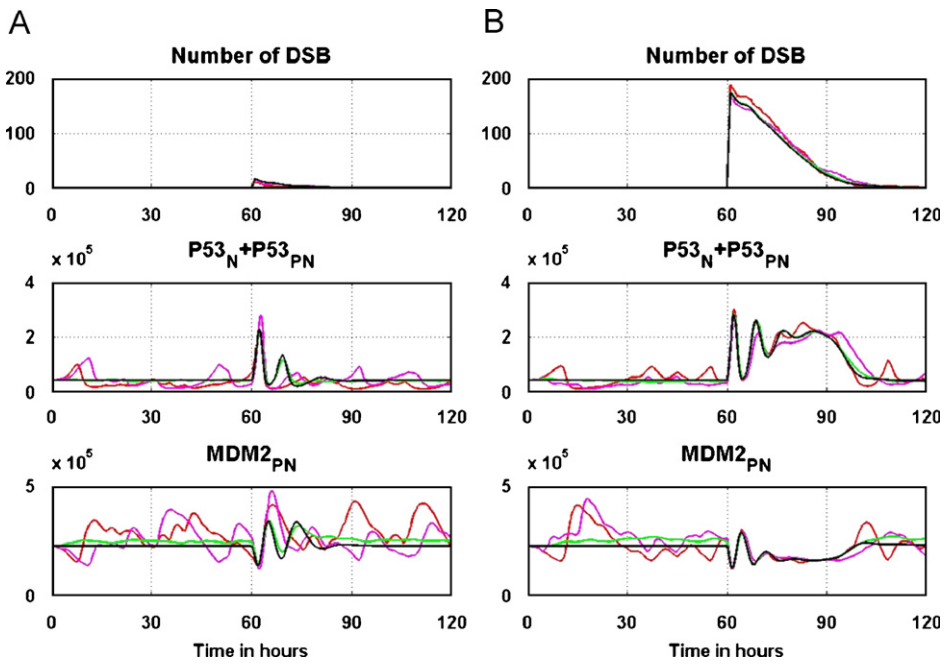

Fig. 6. Deterministic (black), stochastic (red and pink) and the average over 100 stochastic trajectories (green) in the case with PTEN and DNA repair active. At $t = 0$ h the irradiation phase started and lasted 1 h; after irradiation simulation lasted until $t = 60$ h. Panels A and B are for 0.5 Gy and 5 Gy, respectively.

Due to time delay in positive feedback loop (caused mainly by the slowly progressing accumulation of PTEN) the system before reaching the "apoptotic steady state" exhibits two well-pronounced oscillations (Figs. 4 and 5A). In EB1 colon cancer cells the highest, four-fold induction in PTEN expression was observed 20–24 h post-p53 induction (Singh et al., 2002), while in immortalized mouse embryonic fibroblasts PTEN transcript grows about 3–4-fold over 16 h after 10 Gy irradiation (Stambolic et al., 2001). The time delay in positive feedback loop gives chance for DNA repair before the apoptotic decision is taken.

In Figs. 5B and C, we analyze the same two cases as in Figs. 3B and C (where in addition PTEN feedback loop was inactive). In the case, in which DNA damage causes only p53 activation (Fig. 5B), without any Mdm2 destabilization ($d_1 = 0$), no oscillations are induced, and the level of p53 remains low. In the second case, in which DNA damage do not lead to the augmented p53 activation ($a_1 = 0$) the system exhibits persistent periodic oscillations, without reaching the state of high p53 level (Fig. 5C). This analysis demonstrates that for proper apoptotic responses the positive feedback loop (mediated by PTEN) must be active and the DNA damage signaling must assure activation of p53 and destabilization of Mdm2.

3.3. *PTEN on, DNA repair on: cell fate decision*

In this section we consider the most general case, in which DNA repair competes with the positive feedback. At low

irradiation dose of 0.5 Gy (Fig. 6A) system exhibits two oscillations during which DNA is repaired, and then the system (in deterministic approximation) returns to the initial state. For high irradiation dose of 5 Gy, system first reaches the apoptotic state (Fig. 6B—see the plateau in p53 profile), and only after that it goes back to the initial state. Obviously, the commitment to apoptosis is irreversible, and the fact that in the model a cell may return from apoptotic state is solely due to the fact that we have not (yet) included any mechanism of apoptosis.

3.3.1. Extended model

Now, we append our p53 model by a simple, heuristic model of apoptosis. Namely, we assume that p53 regulates synthesis of some apoptotic factor. Since the apoptotic pathway are redundant and there are three groups of proapoptotic genes regulated by p53 that accumulate, respectively, in cell membrane, cytoplasm and mitochondria (reviewed by Fei and El-Deiry, 2003; Vousden and Lane, 2007), there are many candidates for such factor, but we will not attempt to identify here any specific protein. We assume that the level of apoptotic factor, $A(t)$, is given by

$$\frac{\mathrm{d}}{\mathrm{d}t}A(t) = p_1 \frac{q_3 P53_{np}^2(t)}{q_4 + q_3 P53_{np}^2(t)} - d_9 A(t), \tag{21}$$

i.e. for simplicity, we skip its mRNA transcription, and assume that it is produced in a deterministic manner being regulated by active p53. Next, we assume that the apoptotic factor may destroy the integrity of DNA, introducing additional DSBs. That is, in an infinitesimal time interval Δt the probability $P^{DAM}(t, \Delta t)$ that new DSB appears is

$$P^{DAM}(t, \Delta t) = \Delta t \times d_{DAM} \times R + \Delta t \times a_6 \left(\frac{A(t)}{A_{max}}\right)^4, \tag{22}$$

where $A_{max} = p_1/d_9$ is the maximum number of apoptotic factor molecules following from Eq. (21), and a_6 is a measure of their DNA destroying efficiency. Since in any individual cell apoptosis is *Yes* or *NO* event we assume the fourth order interaction, which assures that when the level of apoptotic factor is low it has a negligible effect on DNA integrity. In the deterministic approximation the number of DSBs is given by

$$\frac{\mathrm{d}}{\mathrm{d}t}N(t) = d_{DAM} \times R - \frac{N(t) \times d_{REP} \times P_A(t)}{N(t) + N_{SAT} \times P_A(t)} + a_6 \left(\frac{A(t)}{A_{max}}\right)^4. \tag{23}$$

Finally, the stochastic dynamics of extended model is given by Eqs. (8)–(17) and (21), in which the discrete variables N, G_{M1}, G_{M2}, G_{P1} and G_{P2} follow the stochastic process defined by transition probabilities given in Eqs. (3), (4), (7), (22), while the deterministic limit of the extended system is given by Eqs. (8)–(15), (18), (19), (21), (23). For $a_6 = 0$ dynamics of the extended model is the same as of the initial model; i.e. although the apoptotic factor is produced it does not influence the rest of the system kinetics.

Existence of the apoptotic factor makes the apoptotic decision irreversible: once the level of active p53 reaches the high plateau, the level of apoptotic factor rises and DNA damage takes over DNA repair. As a result (in the model), number of DNA breaks tends to infinity, and the levels of p53, and other variables stabilize. Such an idealized model, has its limitation. Extended DNA damage stops any mRNA synthesis, and activation of "executioner" caspases creates an expanding cascade of proteolytic activity causing protein levels quickly fall down.

Low dose irradiation introduces a small number of DSBs, and the DNA damage may be quickly repaired. High irradiation dose causes that the number of DSBs is higher, and their repair takes more time. As a result, the slow positive feedback loop causes that p53 rises to the high level triggering synthesis of the apoptotic factor, which leads to the further DNA damage. In the deterministic approximation the critical irradiation dose, above which cell tends to apoptosis is approximately 1.88 Gy, below this dose DNA is repaired and the cell returns to its initial state. In the "real" stochastic evolution irradiation dose determines only the probability of apoptosis, which is below 0.1 for dose smaller than 1 Gy and above 0.9 for dose higher than 3 Gy. In Fig. 7, we present both deterministic and stochastic evolution for three irradiation doses 1, 2 and 3 Gy. For 2 Gy, which is just above the critical dose in the deterministic limit, in two, out of three, simulated cells the number of DSBs diverges to infinity (apoptosis), while the third one survives having its DNA repaired. Since, as already said, at latter stages of apoptosis the protein levels fall down, the

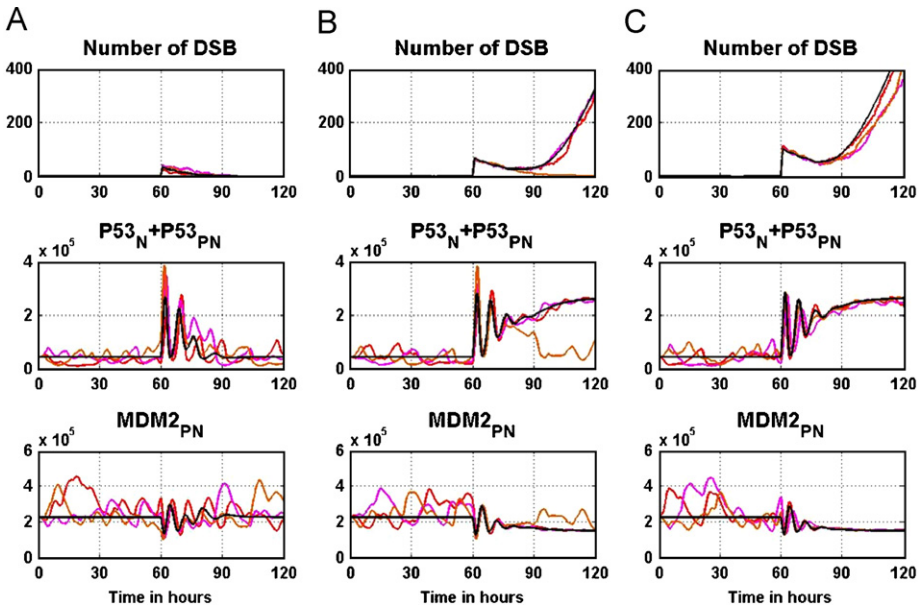

Fig. 7. Deterministic (black) and stochastic (red, pink and brown) trajectories of the extended model: At $t = 0$ h the irradiation phase started and lasted 1 h; after irradiation simulation lasted until $t = 60$ h. Panels A, B and C are for doses 1 Gy, 2 Gy and 3 Gy, respectively.

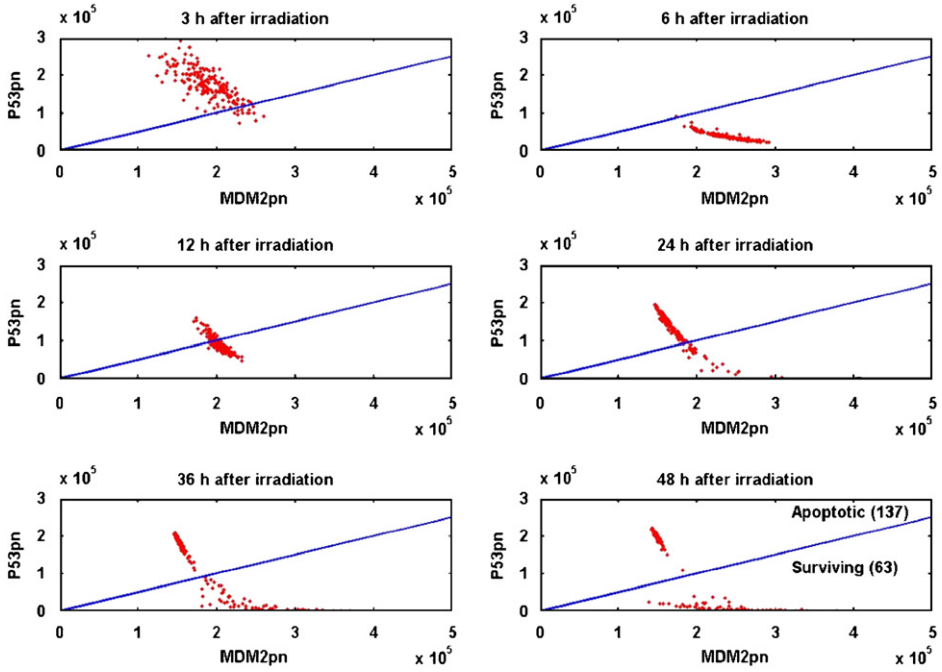

Fig. 8. Analysis of the extended model: distribution of active p53 and nuclear phosphorylated Mdm2 levels in population of 200 cells following 2 Gy irradiation in subsequent time points. After 36 h from irradiation cells can be almost unambiguously separated into apoptotic (with a high level of p53) and surviving (with a low level of p53).

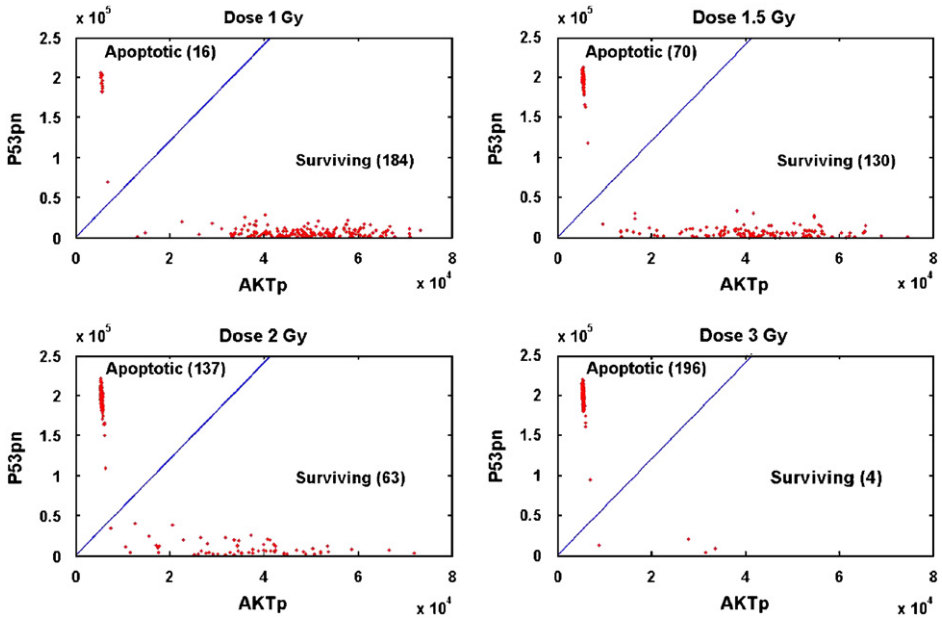

Fig. 9. Analysis of the extended model: distribution of active p53 and active Akt levels in population of 200 cells after 48 h, with respect to the irradiation dose.

discrimination for apoptotic and surviving cells with respect to p53 level makes sense only at first stages of apoptosis.

In Fig. 8, we simulate population of 200 cells in order to visualize how the apoptotic decision is reached. In first three time points (3, 6, 12 h) we may observe oscillatory behavior, but then at 36 h population splits into surviving cells with low level of active p53 and larger fraction apoptotic cells, which are marked by the high level of active p53 and somehow lower level of its inhibitor Mdm2. Fraction of apoptotic cells at 48 h after irradiation, grows rapidly with the irradiation dose, reaching about 30% for 1.5 Gy and 70% for 2 Gy. In Fig. 9, apoptotic cells are marked by high level (proapoptotic) active p53 and low level of (antiapoptotic) active Akt, while the surviving cell have low level of active p53 and high level of active Akt. For 0.5 Gy all of 200 analyzed cells survived and for 4 Gy all of 200 cells died. In contrast, all PTEN knockouted cells irradiated with 2 Gy dose settled below the apoptotic threshold at 48 h (Fig. 10). Even if irradiated with 10 Gy dose PTEN knockouted cells maintain oscillations for 60 h—data not shown. This may explain why MCF-7 cells, which are deficient in PTEN signaling, exhibit prolonged oscillations without commitment to apoptosis as demonstrated by Geva-Zatorsky et al. (2006).

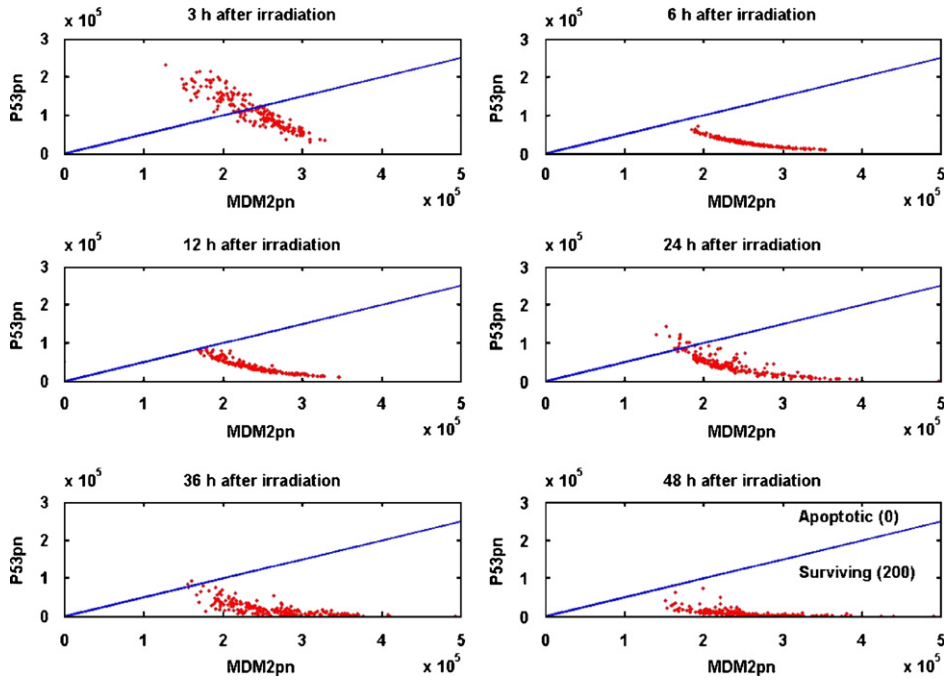

Fig. 10. Analysis of the extended model as in Fig. 8 but for the case with PTEN knockout. Cells are irradiated with 2 Gy dose that causes oscillations in p53 and Mdm2 levels, but in contrast to cells with p53 responsive PTEN (shown in Fig. 8) in these cells the apoptotic state is not reached.

We should note that proapoptotic action of PTEN is not restricted to downregulation of Akt (reviewed by Blanco-Aparicio et al., 2007). Similarly, the antiapoptotic action of Akt is not restricted to downregulation of p53 level via Mdm2 phosphorylation, which enables its nuclear entry; Akt also promotes cell survival by inhibiting proapoptotic proteins (reviewed by Datta et al., 1999; Franke et al., 2003). As a result suppression of apoptosis by PTEN knockout is not restricted to downregulation of p53.

4. Conclusions

In the proposed model, p53 regulation is based on two feedbacks: the negative, which couples p53 with its downregulator Mdm2 and the positive one, involving PTEN, PIP3 and Akt, which may break the negative feedback loop. The negative feedback, together with time delay, resulting from the presence of intermediaries: Mdm2 transcript, cytoplasmic Mdm2 and cytoplasmic phosphorylated Mdm2, assures homeostasis of healthy cells and oscillatory responses to DNA damage.

The main finding of the model is that in untransformed cells (with PTEN mediated positive feedback loop intact), oscillatory responses to DNA damage are terminated by apoptosis preceded by stabilization of p53 on a high level, or by return to homeostasis, characterized by low p53 level. The specific predictions of the model may be summarized as follows:

1. If DNA repair is inefficient, and the positive feedback loop is broken (e.g. PTEN is not expressed, or it is not p53 responsive) the p53 oscillations are persistent as observed by Geva-Zatorsky et al. (2006) in MCF-7 cells, Fig. 2.
2. In the case, in which DNA repair is suppressed, but positive feedback loop is intact, the system, after two oscillations tends (in the deterministic limit) to the second stable state. This state is characterized by the high level of PTEN, the low level of active Akt, Mdm2 mostly trapped in the cytoplasm, that allows for accumulation of active p53 in the nucleus, and upregulation of proapoptotic proteins, Fig. 4.
3. In cells, in which DNA repair and the positive feedback loop mediated by PTEN function properly, there is a competition between DNA repair and apoptosis. The positive feedback acts as a clock allowing about 15 h for DNA repair. If DNA damage is small, and may be quickly repaired, the cell after one or two oscillations returns to its initial state. However, if DNA damage is large, and its repair needs more time, the positive feedback makes that after two oscillations, the system reaches the state of high p53 level and low level of active Akt, in which apoptosis may be initiated, Figs. 6–9.
4. The p53 mediated apoptosis may be induced only when PTEN loop is active and signals from DNA damage simultaneously destabilize Mdm2 and stabilize p53 by phosphorylation, Fig. 5.

Building the model, we assumed that the overall stochasticity of p53 regulation origins from the processes involving the smallest number of molecules, i.e. gene regulation, DNA damage and repair. Performing stochastic simulations, we demonstrated that the "real" stochastic trajectories substantially differ from trajectories obtained in the deterministic limit. Surprisingly, this difference is more significant for resting cells, which exhibit highly irregular fluctuations, similar to those observed by Geva-Zatorsky et al. (2006). In DNA damaged cells, the stochastic oscillations are also irregular regarding amplitude, but with well conserved period, of about 6 h. Interestingly, their amplitude is noticeably larger than amplitude of oscillations in the deterministic limit, confirming the hypothesis that stochasticity is at least in part responsible for oscillations. Finally, considering the extended model, we found that due to stochasticity and bistability, cell population separates distinctively into surviving and apoptotic cells, with fraction of the apoptotic cells growing with the irradiation dose, and reaching about 70% for 2 Gy.

The key idea of the proposed model is based on the conjecture that the role of the negative feedback coupling p53 and Mdm2 is to assure homeostasis of healthy cells and oscillations of p53 level in DNA damage cells (needed for expression of protein involved in DNA repair), while the role of the positive feedback (involving PTEN, PIP3 and Akt) is to terminate these oscillations and provide

bistability, which make apoptotic decision unambiguous. The role of the positive feedback differs our model from models of Ciliberto et al. (2005) and Zhang et al. (2007), in which the positive feedback assures only robustness of oscillations.

In recent years oscillatory responses in p53 system drawn much attention of modelers (e.g. Ma et al., 2005; Ciliberto et al., 2005; Zhang et al., 2007; Tyson, 2006; Batchelor et al., 2008). However, we should remember that all experiments demonstrating persistent oscillations in p53 and Mdm2 levels were performed on transformed cancer cells. Such cells, may have p53 and apoptotic signaling much different from that of normal cells. In particular, MCF-7 breast cancer line, analyzed by Geva-Zatorsky et al. (2006) and Batchelor et al. (2008), expresses PTEN only at a low basal level due its promoter methylation (García et al., 2004; Krawczyk et al., 2007). There is, however, bulk of evidence that PTEN signaling is key for the induction of the apoptotic responses; particularly, in MCF-7 cells, Weng et al. (1999) demonstrated that wild-type PTEN (but not phosphatase dead PTEN mutant) transfection leads to growth suppression (due to G1 cell cycle arrest) and cell death via apoptosis. Accordingly, in PTEN overexpressed cells a significant decrease in phosphorylated Akt was observed at 24 h of PTEN induction. Studying other cell line, immortalized mouse embryonic fibroblast, Stambolic et al. (2001) demonstrated that PTEN null cells are almost completely resistant to apoptosis. These cells, when transfected with PTEN controlled by the wild type, p53-responsive promoter, became sensitized to p53-mediated apoptosis, whereas cells transfected with PTEN under mutated promoter remained resistant to apoptosis. In accordance with this study, Gottschalk et al. (2005) demonstrated that inhibition of PIP3-kinase causes increased sensitivity to irradiation of prostate cancer cell line LNCaP.

The proposed model, together with experimental studies by Weng et al. (1999) and Stambolic et al. (2001), suggests that persistent oscillatory responses, observed by Geva-Zatorsky et al. (2006), and Batchelor et al. (2008) in gamma irradiated MCF-7 cells, can be due to their deficiency in PTEN regulation. It would be thus interesting to repeat these experiments, after transfecting MCF-7 cells with wild-type PTEN controlled by p53-responsive promoter. Based on our model, we would expect that PTEN transfected cells will not exhibit sustained oscillations, but in the response to high dose of gamma irradiation, p53 level, after one or two peaks, will stabilize on a high level inducing apoptosis (cf. Figs. 8 and 10). Effect of this kind has been observed *in vivo* using bioluminescent imaging. Pretreatment of mice with irradiation sensitizer, 5-fluorouracil, 1 h before irradiation causes that after first peak, p53 stabilizes on a high level without exhibiting further oscillations (Hamstra et al., 2006).

Since the proposed model is based on two, out of 10 or more, regulatory feedback loops present in the p53 regulation, we are far from capturing the whole p53 network dynamics. It is, however, tempting to speculate that the negative feedbacks play their role in attenuation of p53 activity during DNA repair phase, while the role of the positive feedbacks is to induce apoptosis if DNA damage is irreparable or its repair proceeds too slow.

Acknowledgments

The MATLAB programs written to perform the simulations are available at our website: http://www.ippt.gov.pl/~tlipnia/. This work was supported by Polish Committee for Scientific Research Grants nos. 4 T07A 001 30, 3 T11A 019 29 and PBZ-MNiI-2/1/2005.

References

Appella, E., Anderson, C.W., 2001. Post-translational modifications and activation of p53 by genotoxic stresses. Eur. J. Biochem. 268, 2764–2772.
Bakkenist, C.J., Kastan, M.B., 2003. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 421, 499–506.
Bar-Or, R., Maya, R., Segel, L.A., Alon, U., Levine, A.J., Oren, M., 2000. Generation of oscillations by the p53–Mdm2 feedback loop: a theoretical and experimental study. Proc. Natl. Acad. Sci. USA 97, 11250–11255.
Barak, Y., Juven, T., Haffner, R., Oren, M., 1993. Mdm2 expression is induced by wild type p53 activity. EMBO J. 12, 461–468.
Batchelor, E., Mock, C.S., Bhan, I., Loewer, A., Lahav, G., 2008. Recurrent initiation: a mechanism for triggering p53 pulses in response to DNA damage, Mol. Cell 30, 277–289.
Blanco-Aparicio, C., Renner, O., Leal, J.F.M., Carnero, A., 2007. PTEN, more than the AKT pathway. Carcinogenesis 28, 1379–1386.
Bode, A.M., Dong, Z., 2004. Post-translational modification of p53 in tumorigenesis. Nat. Rev. Cancer 4, 793–805.
Brooks, C.L., Gu, W., 2003. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. Curr. Opinion Cell Biol. 15, 164–171.
Cantley, L.C., Neel, B.G., 1999. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc. Natl. Acad. Sci. USA 96, 4240–4245.
Chang, C.-J., Freeman, D.J., Wu, H., 2004. PTEN regulates Mdm2 expression though the P1 promoter. J. Biol. Chem. 279, 29841–29848.
Ciliberto, A., Novak, B., Tyson, J.J., 2005. Steady states and oscillations in the p53–Mdm2 network. Cell Cycle 4, 488–493.
Cully, M., You, H., Levine, A.J., Mak, T.W., 2006. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nature 6, 184–192.
Datta, S.R., Anne Brunet, A., Greenberg, M.E., 1999. Cellular survival: a play in three Akts. Genes Dev. 13, 2905–2927.
Essmann, F., Engels, I.H., Totzke, G., Schulze-Osthoff, K., Janicke, R.U., 2004. Apoptosis resistance of MCF-7 breast carcinoma cells to ionizing radiation is independent of p53 and cell cycle control but caused by the lack of caspase-3 and a caffeine-inhibitable event. Cancer Res. 64, 7065–7072.
Fei, P., El-Deiry, W.S., 2003. P53 and radiation responses. Oncogene 22, 5774–5783.
Femino, A.M., Fay, F.S., Fogary, K., Singer, R.H., 1998. Visualization of single RNA transcripts in situ. Science 280, 585–590.
Franke, T.F., Hornik, C.P., Segev, L., Shostak, G.A., Sugimoto, C., 2003. PI3K/Akt and apoptosis: size matters. Oncogene 22, 8983–8998.
Freeman, D.J., Li, A.G., Wei, G., Li, H.-H., Kertesz, N., Lesche, R., Whale, A.D., Martinez-Diaz, H., Rozengurt, N., Cardiff, R.D., Liu, X., Wu, H., 2003. PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. Cancer Cell 3, 117–130.
García, J.M., Silva, J., Peña, C., Garcia, V., Rodriguez, R., Cruz, M.A., Cantos, B., Provencio, M., España, P., Bonilla, F., 2004. Promoter methylation of the PTEN gene is a common molecular change in breast cancer. Genes Chromosomes Cancer 41, 117–124.
Geva-Zatorsky, N., Rosenfeld, N., Itzkovitz, S. Milo, R., Sigal, A., Dekel, E., Yarnitzky, T., Liron, Y., Polak, P., Lahav, G., Alon, U., 2006. Oscillations and variability in the p53 system. Mol. Sys. Biol. 2 (2006), 0033.
Gottlieb, M.T., Leal, J.F.M., Seger, R., Taya, Y., Oren, M., 2002. Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis. Oncogene 21, 1299–1303.
Gottschalk, A.R., Doan, A., Nakamura, J.L., Stokoe, D., Haas-Kogan, D.A., 2005. Inhibition of phosphatidylinositol-3-kinase causes increased sensitivity to radiation through a PKB-dependent mechanism. Int. J. Radiat. Oncol. Biol. Phys. 63, 1221–1227.
Hamstra, D.A., Bhojani, M.S., Griffin, L.B., Laxman, B., Ross, B.D., Rehemtulla, A., 2006. Real-time evaluation of p53 oscillatory behavior in vivo using bioluminescent imaging. Cancer Res. 66, 7482–7489.
Harris, S.L., Levine, A.J., 2005. The p53 pathway: positive and negative feedback loops. Oncogene 24, 2899–2908.
Haupt, Y., Maya, R., Kazaz, A., Oren, M., 1997. Mdm2 promotes the rapid degradation of p53. Nature 387, 296–299.
Joo, J., Plimpton, S., Martin, S., Swiler, L., Faulon, J.L., 2007. Sensitivity analysis of a computational model of the IKK-NF-κB- IκB-A20 signal transduction network. Ann. NY Acad. Sci. 1115, 221–239.
Kærn, M., Elston, T.C., Blake, W.J., Collins, J.J., 2005. Stochasticity in gene expression from theories to phenotypes. Nat. Rev. Genet. 6, 451–464.
Kepler, T.B., Elston, T.C., 2001. Stochasticity in transcriptional regulation: origins, consequences, and mathematical representations. Biophys. J. 81, 3116–3136.
Ko, M.S.H., 1991. Stochastic model for gene induction. J. Theor. Biol. 53, 181–194.
Kohn, K.W., Pommier, Y., 2005. Molecular interaction map of the p53 and Mdm2 logic elements, which control the Off–On switch of p53 in response to DNA damage. Biochem. Biophys. Res. Commun. 331, 816–827.
Krawczyk, B., Rudnicka, K., Fabianowska-Majewska, K., 2007. The effects of nucleoside analogues on promoter methylation of selected tumor suppressor genes in MCF-7 and MDA-MB-231 breast cancer cell lines. Nucleosides Nucleotides Nucleic Acids 26, 1043–1046.
Kubbutat, M.H.G., Jones, S.N., Vousden, K.H., 1997. Regulation of p53 stability by Mdm2. Nature 387, 299–303.

Lahav, G., Rosenfeld, N., Sigal, A., Geva-Zatorsky, N., Levine, A.J., Elowitz, M.B., Alon, U., 2004. Dynamics of the p53|Mdm2 feedback loop in individual cells. Nat. Genet. 36, 147–150.
Levine, A.J., Hu, W., Feng, Z., 2006. The P53 pathway: What questions remain to be explored? Cell Death Differ. 13, 1027–1036.
Li, L., Ross, A.H., 2007. Why is PTEN an important tumor suppressor? J. Cell. Biochem. 102, 1368–1374.
Lipniacki, T., Paszek, P., Marciniak-Czochra, A., Brasier, A.R., Kimmel, M., 2006. Transcriptional stochasticity in gene expression. J. Theor. Biol. 238, 348–367.
Lipniacki, T., Puszynski, K., Paszek, P., Brasier, A.R., Kimmel, M., 2007. Single TNFα trimers mediating NF-κB activation: stochastic robustness of NF-κB signaling. BMC Bioinform. 8, 376.
Lobrich, M., Rydberg, B., Cooper, P.K., 1995. Repair of x-ray-induced DNA double-strand breaks in specific Not I restriction fragments in human fibroblasts: joining of correct and incorrect ends. Proc. Natl. Acad. Sci. USA 92, 12050–12054.
Ma, L., Wagner, J., Rice, J.J., Hu, W., Levine, A.J., Stolovitzky, G.A., 2005. A plausible model for the digital response of p53 to DNA damage. Proc. Natl. Acad. Sci. USA 102, 14266–14271.
Mayo, L.D., Donner, D.B., 2001. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc. Natl. Acad. Sci. USA 98, 11598–11603.
Mayo, L.D., Donner, D.B., 2002. The PTEN, Mdm2, p53 tumor suppressor—oncoprotein network. Trends Biochem. Sci. 27, 462–467.
Mayo, L.D., Dixon, J.E., Durden, D.L., Tonks, N.K., Donner, D.B., 2002. PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. J. Biol. Chem. 277, 5484–5489.
Meek, D.W., 1998. Multisite phosphorylation and the integration of stress signals at p53. Cell. Signal. 10, 159–166.
Meek, D.W., Milne, D.M., 2000. Analysis of multisite phosphorylation of the p53 tumor-suppressor protein by tryptic phosphopeptide mapping. Methods Mol. Biol. 99, 447–463.
Meulmeester, E., Pereg, Y., Shiloh, Y., Jochemsen, A.G., 2005. ATM-mediated phosphorylations inhibit Mdmx/Mdm2 stabilization by HAUSP in favor of p53 activation. Cell Cycle 4, 1166–1170.
Michael, D., Oren, M., 2003. The p53–Mdm2 module and the ubiquitin system. Semin. Cancer Biol. 13, 49–58.
Nicholls, C.D., McLure, K.G., Shields, M.A., Lee, P.W.K., 2002. Biogenesis of p53 involves cotranslational dimerization of monomers and posttranslational dimerization of dimers. J. Biol. Chem. 277, 12937–12945.
Paszek, P., 2007. Modeling stochasticity in gene regulation: characterization in the terms of the underlying distribution function. Bull. Math. Biol. 69, 1567–1601.
Rai, A., Peskin, C.S., Tranchina, D., Vargas, D.Y., Tyagi, S., 2006. Stochastic mRNA synthesis in mammalian cells. PloS Biol. 4, 309.
Rateitschak, K., Wolkenhauer, O., 2007. Intracellular delay limits cyclic changes in gene expression. Math. Biosci. 205, 163–179.
Rothkamm, K., Lobrich, M., 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc. Natl. Acad. Sci. USA 100, 5057–5062.
Simpson, L., Parsons, R., 2001. PTEN: life as a tumor suppressor. Exp. Cell Res. 264, 29–41.
Singh, B., Reddy, P.G., Goberdhan, A., Walsh, C., Dao, S., Ngai, I., Chou, T.C., O-charoenrat, P., Levine, A.J., Rao, P.H., Stoffel, A., 2002. p53 regulates cell survival by inhibiting PIK3CA in squamous cell carcinomas. Genes Dev. 16, 984–993.
Stambolic, V., MacPherson, D., Sas, D., Lin, Y., Snow, B., Jang, Y., Benchimol, S., Mak, T.W., 2001. Regulation of PTEN transcription by p53. Mol. Cell 8, 317–325.
Stommel, J.M., Wahl, G.M., 2004. Accelerated MDM2 auto-degradation induced by DNA-damage kinases is required for p53 activation. EMBO J. 23, 1547–1556.
Stommel, J.M., Wahl, G.M., 2005. A new twist in the feedback loop: stress-activated MDM2 destabilization is required for p53 activation. Cell Cycle 4, 411–417.
Tyson, J.J., 2006. Another turn for p53. Mol. Sys. Biol. 2 (2006), 0032.
Volgenstein, B., Lane, D., Levine, A.J., 2000. Surfing the p53 network. Nature 408, 307–310.
Vousden, K.H., Lane, D.P., 2007. p53 in health and disease. Nat. Rev. Mol. Cell. Biol. 8, 275–283.
Wagner, J., Ma, L., Rice, J.J., Hu, W., Levine, A.J., Stolovitzky, G.A., 2005. p53–Mdm2 loop controlled by a balance of its feedback strength and effective dampening using ATM and delayed feedback. IEE Proc.—Syst. Biol. 152, 109–118.
Walters, M.C., Fiering, S., Eidemiller, J., Magis, W., Groudine, M., Martin, D.I.K., 1995. Enhancers increase the probability but not the level of gene expression. Proc. Natl. Acad. Sci. USA 92, 7125–7129.
Wee, K.B., Aguda, B.A., 2006. Akt versus p53 in a network of oncogenes and tumor suppressor genes regulating cell survival and death. Biophys. J. 91, 857–865.
Weinberg, R.L., Veprintsev, D.B., Fersht, A.R., 2004. Cooperative binding of tetrameric p53 to DNA. J. Mol. Biol. 341, 1145–1159.
Weng, L.-P., Smith, W.M., Dahia, P.L.M., Ziebold, U., Gil, E., Lees, J.A., Eng, C., 1999. PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. Cancer Res. 59, 5808–5814.
Zhang, T., Brazhnik, P., Tyson, J.J., 2007. Exploring mechanisms of the DNA-damage response p53 pulses and their possible relevance to apoptosis. Cell Cycle 6, 85–94.
Zhou, B.P., Liao, Y., Xia, W., Zou, Y., SSpohn, B., Hung, M.C., 2001. HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. Nat. Cell. Biol. 3, 973–982.