

## Report

# Steady States and Oscillations in the p53/Mdm2 Network

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## KEY WORDS

network dynamics, signal transduction, feedback control, tumor suppressors, p53

## ABBREVIATIONS

SNL Saddle-Node-Loop bifurcation  
NPF negative and positive feedback loop  
NFL negative feedback loop

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## ABSTRACT

p53 is activated in response to events compromising the genetic integrity of a cell. Recent data show that p53 activity does not increase steadily with genetic damage but rather fluctuates in an oscillatory fashion.<sup>7</sup> Theoretical studies suggest that oscillations can arise from a combination of positive and negative feedbacks or from a long negative feedback loop alone. Both negative and positive feedbacks are present in the p53/Mdm2 network, but it is not known what roles they play in the oscillatory response to DNA damage. We developed a mathematical model of p53 oscillations based on positive and negative feedbacks in the p53/Mdm2 network. According to the model, the system reacts to DNA damage by moving from a stable steady state into a region of stable limit cycles. Oscillations in the model are born with large amplitude, which guarantees an all-or-none response to damage. As p53 oscillates, damage is repaired and the system moves back to a stable steady state with low p53 activity. The model reproduces experimental data in quantitative detail. We suggest new experiments for dissecting the contributions of negative and positive feedbacks to the generation of oscillations.

## INTRODUCTION

p53 is a transcriptional activator that plays an important role in preserving genomic integrity in mammalian cells.<sup>1</sup> When active and present in high concentration, it induces the transcription of genes involved in cell cycle arrest and apoptosis. Not surprisingly, p53 activity is restrained in normal cells. By stimulating p53 degradation, the E3 ubiquitin ligase Mdm2 plays a key role in maintaining p53 levels low in normal cells.<sup>2</sup> Interestingly, Mdm2 transcription is induced by p53 itself.<sup>3</sup> The resulting negative feedback loop (p53 → Mdm2 —| p53) guarantees that p53 is kept to a low, stable steady-state concentration in undamaged cells.<sup>4-6</sup>

Although deleterious in normal cells, p53 activity plays a crucial role when a cell's genomic integrity is in danger. In response to environmental stresses (e.g., DNA damage or oncogene activation), the negative feedback loop with Mdm2 is weakened, and p53, no longer under the strict control of Mdm2, accumulates in the nucleus, starting a transcriptional program that leads to arrest of cell cycle progression, repair of DNA damage, and, if repair is impossible, apoptosis (programmed cell death). The negative feedback is fully restored only if the damage is fully repaired. Considering its central role as a guardian of genomic integrity, it is not surprising that p53 is mutated in more than 50% of human cancers.<sup>1</sup>

A recent study from Alon and collaborators sheds new light on the dynamics of stress-induced activation of p53.<sup>7</sup> They observed that Mdm2 and p53 concentrations in the nuclei of gamma irradiated cells undergo one or more oscillations (depending on the extent of damage) with constant amplitude and interpulse interval. The fact that the p53/Mdm2 network reacts to an increase in stress intensity with an increasing number of discrete signals (oscillations) led Alon and collaborators to suggest that the p53/Mdm2 network behaves like a digital control system.<sup>7</sup>

The generation of oscillations in the p53/Mdm2 network poses a challenge to modelers. Oscillatory behaviors are widespread in molecular systems,<sup>8</sup> and modelers have long been aware that negative feedback is necessary for oscillations, yet negative feedback is not sufficient.<sup>9</sup> For example, a negative feedback composed of only two elements, such as p53 → Mdm2 —| p53, cannot oscillate. Lev Bar-Or et al.<sup>10</sup> considered the possibility of a negative feedback loop composed of three components (Mdm2, p53 and a putative

intermediate), which can oscillate. Here, we explore the possibility that oscillatory behavior in the p53/Mdm2 network emerges from a combination of negative and positive feedbacks. Our model, despite its simplicity, suggests new experiments that can help to understand the molecular mechanisms underlying oscillations in the p53/Mdm2 network.

## RESULTS

**Experimental basis of the model.** First, we summarize the biological data upon which the model is built.

- When cells are treated with proteasome inhibitors, Mdm2 and p53 accumulate predominantly in the nucleus.<sup>11,12</sup> From these observations, Stommel and Wahl suggested that Mdm2 and p53 are degraded mainly in the nucleus,<sup>12</sup> and we adopt this suggestion.
- p53 is degraded in a ubiquitin-dependent manner in a reaction catalyzed by Mdm2.<sup>2</sup> Although Mdm2 is clearly responsible for attaching the first ubiquitin, other enzymes (e.g., p300) can be more efficient in catalyzing subsequent ubiquitinations.<sup>11,14</sup> However, Mdm2 is able to polyubiquitinate p53 *in vivo*<sup>11</sup> and is required for the reaction catalyzed by p300.<sup>14</sup> For these reasons, in the model we assume that Mdm2 catalyzes all the ubiquitination steps.
- p53 is recognized and degraded most efficiently by the proteasome when it has at least five ubiquitin moieties attached. In the model, we assume for the sake of simplicity that the nuclear form of Mdm2 (Mdm2<sub>nuc</sub>) attaches only two ubiquitins to p53, first converting p53 into a “monoubiquitinated” form (p53U), and then converting p53U into “polyubiquitinated” p53 (p53UU). p53UU is degraded faster than p53 and p53U, which are both subject only to a slow background degradation.
- Mdm2 transcription is induced by p53.<sup>3</sup> We assume that all three forms of p53 (p53<sub>tot</sub> = p53 + p53U + p53UU) induce transcription of Mdm2 with the same efficiency. Mdm2 is produced in a nonphosphorylated, cytoplasmic form (Mdm2<sub>cyt</sub>). It is known that p53 transcriptional activity is greatly enhanced when four molecules of p53 form a tetramer.<sup>16,17</sup> Hence, we assume that p53<sub>tot</sub> induces Mdm2 transcription following a Hill function, with exponent 3.
- To be translocated into the nucleus, Mdm2<sub>cyt</sub> must first be phosphorylated by the Akt protein kinase.<sup>18,19</sup> In the model, this reaction converts Mdm2<sub>cyt</sub> into Mdm2P<sub>cyt</sub>.
- The phosphorylated cytoplasmic form, Mdm2P<sub>cyt</sub>, shuttles freely into and out of the nucleus. Because the nuclear volume is between 1/10 and 1/20 of the cytoplasmic volume, the nuclear concentration of Mdm2 changes 10–20 times faster than the cytoplasmic concentration. In the model, we set the volume ratio = 15.

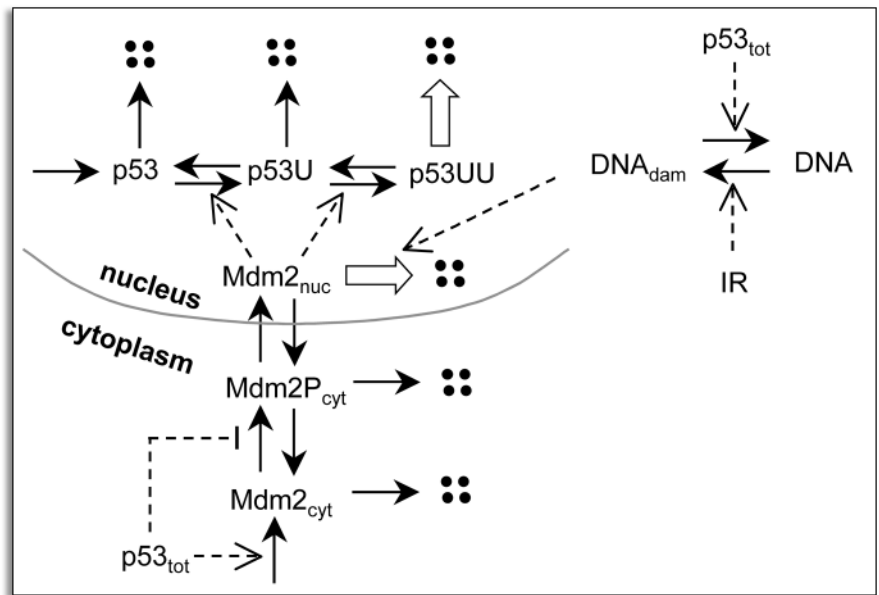


Figure 1. Wiring diagram for the p53/Mdm2 network. p53, p53U and p53UU represent the forms of p53 with 0, 1 or 2 ubiquitin moieties attached, respectively. The sum of the three forms is called p53<sub>tot</sub>. We assume that the “polyubiquitinated” form, p53UU, is degraded faster than the other forms. Ubiquitination reactions are carried out by the nuclear form of Mdm2, Mdm2<sub>nuc</sub>. Two cytoplasmic forms of Mdm2 are also considered, a phosphorylated one, Mdm2P<sub>cyt</sub>, which is able to enter the nucleus, and an unphosphorylated form, Mdm2<sub>cyt</sub>, whose synthesis is induced by p53. The phosphorylation step is inhibited by p53<sub>tot</sub>. Damaged DNA, DNA<sub>dam</sub>, increases Mdm2<sub>nuc</sub> degradation rate. Its production is induced by ionizing radiations (IR), and reverted by p53<sub>tot</sub>. See text for details.

Table 1 **Differential equations for the p53/Mdm2 network model**

$$\begin{aligned} \frac{d[p53_{tot}]}{dt} &= k_{s53} - k_{d53} [p53_{tot}] - k_{d53} [p53UU] \\ \frac{d[p53U]}{dt} &= k_{\tau} [Mdm2_{nuc}] [p53] + k_{\tau} [p53UU] - [p53U] (k_{\tau} + k_{\tau} [Mdm2_{nuc}]) - k_{d53} [p53U] \\ \frac{d[p53UU]}{dt} &= k_{\tau} [Mdm2_{nuc}] [p53U] - [p53UU] k_{\tau} - [p53UU] (k_{d53} + k_{d53}) \\ \frac{d[Mdm2_{nuc}]}{dt} &= V_{ratio} (k_i [Mdm2P_{cyt}] - k_o [Mdm2_{nuc}]) - k_{d2} [Mdm2_{nuc}] \\ \frac{d[Mdm2_{cyt}]}{dt} &= k_{s2} + \frac{k_{s2} [p53_{tot}]^m}{J_{s2} + [p53_{tot}]^m} - k_{d2} [Mdm2_{cyt}] + k_{deph} [Mdm2P_{cyt}] - \frac{k_{ph}}{J_{ph} + [p53_{tot}]} [Mdm2_{cyt}] \\ \frac{d[Mdm2P_{cyt}]}{dt} &= \frac{k_{ph}}{J_{ph} + [p53_{tot}]} [Mdm2_{cyt}] - k_{deph} [Mdm2P_{cyt}] - k_i [Mdm2P_{cyt}] + k_o [Mdm2_{nuc}] - k_{d2} [Mdm2P_{cyt}] \\ \frac{d[DNA_{dam}]}{dt} &= k_{DNA} [IR] - k_{dDNA} [p53_{tot}] \frac{[DNA_{dam}]}{J_{dna} + [DNA_{dam}]} \\ k_{d2} &= k'_{d2} + \frac{[DNA_{dam}]}{J_{dam} + [DNA_{dam}]} k''_{d2} \\ [p53] &= [p53_{tot}] - ([p53U] + [p53UU]) \\ [Mdm2_{tot}] &= [Mdm2_{cyt}] + \frac{1}{V_{ratio}} [Mdm2_{nuc}] + [Mdm2P_{cyt}] \\ IR &= ampl \cdot heav(10 < t < 20) \end{aligned}$$

Table 2 Parameters for the p53/Mdm2 network model

Rate constants (min <sup>-1</sup> )			
$k_{s2}^1 = 0.0015$	$k_{s2} = 0.006$	$k'_{d2} = 0.01$	$k''_{d2} = 0.01$
$k_{ph} = 0.05$	$k_{deph} = 6$	$k_i = 14$	$k_o = 0.5$
$k_{s53} = 0.055$	$k_{d53} = 8$	$k_{d53}^1 = 0.0055$	$k_f = 8.8$
$k_r = 2.5$	$k_{DNA} = 0.18$	$k_{dDNA} = 0.017$	
Other constants (dimensionless)			
$m = 3$	$J_s = 1.2$	$J_{dam} = 0.2$	$V_{ratio} = 15$
$J = .01$	$J_{dna} = 1$	$ampl = 1$	

• Stommel and Wahl recently reported that DNA damage shortens the half-life of Mdm2.<sup>12</sup> We implement this piece of evidence in the model, although many other hypotheses have been proposed to explain how p53 escapes the control of Mdm2.<sup>5,6,23,24</sup> We introduce a generic variable DNA<sub>dam</sub> (damaged DNA) whose synthesis depends upon IR (ionizing radiation), a step-function which is 1 during stress induction and 0 otherwise. In the simulation, damage is exerted for 10 minutes, between time 10 and 20. DNA<sub>dam</sub> increases the degradation rate of Mdm2<sub>nuc</sub>.

• Finally, we do not aim to explain in molecular terms the relationship between p53 expression and damage repair, we simply assume that DNA<sub>dam</sub> decreases proportionally to p53<sub>tot</sub> activity, following Michaelis-Menten kinetics.

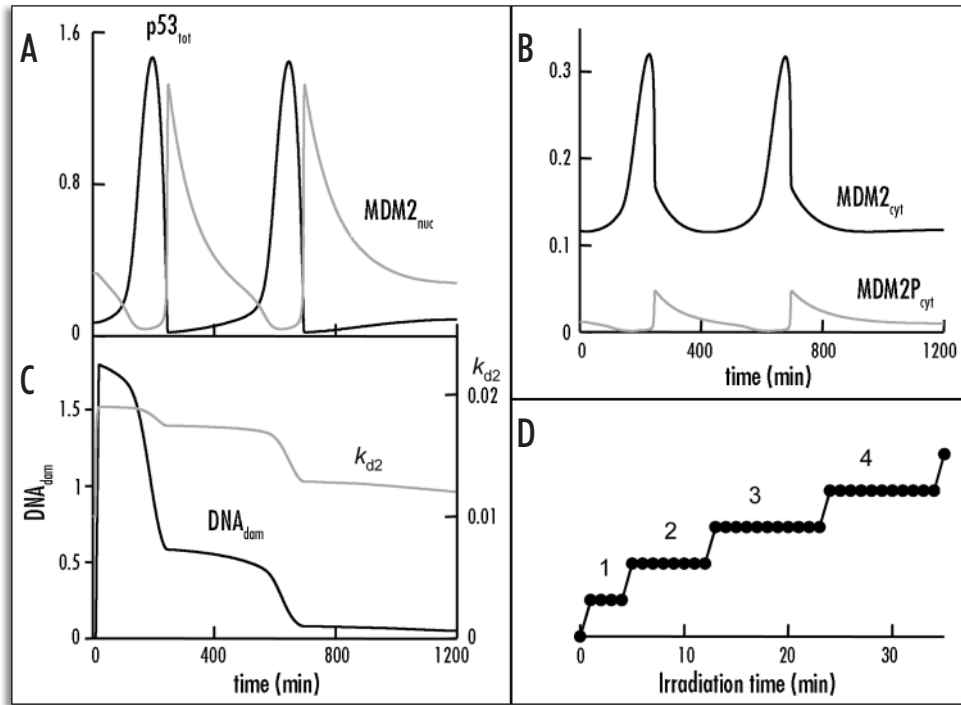


Figure 2. Simulation of gamma-irradiation experiment. At the beginning of the simulation, the system is at steady state. (A) Between time 10 and 20, the control system is exposed to a transient damaging agent, which induces two large amplitude oscillations in p53<sub>tot</sub> and Mdm2<sub>nuc</sub>. (B) The oscillations of the two cytoplasmic forms of Mdm2 have a smaller amplitude compared to Mdm2<sub>nuc</sub> concentration in panel (A). (C) The oscillations are initiated as a consequence of  $k_{d2}$  increase, which is induced by IR. As the damage is repaired,  $k_{d2}$  decreases back to its basal value. (D) The number of oscillations increases with the amount of damage. In the simulation, we count the number of oscillations as a function of the irradiation time. In (A–C) irradiation time = 10.

• Mdm2 nuclear entry is opposed indirectly by p53, via a long pathway involving PTEN, PIP3 and Akt.<sup>18,20</sup> Akt is activated by PIP3 (Phosphatidylinositol-tris-phosphate) but not by PIP2. PIP3 is produced by PI3 kinase, and hydrolyzed to PIP2 by the protein phosphatase PTEN. Therefore, PTEN indirectly inhibits Akt, thereby reducing Mdm2 entry into the nucleus and hence stabilizing p53. Recently, PTEN has been shown to inhibit Mdm2 in other ways, e.g., through transcriptional control.<sup>21</sup> Interestingly, PTEN transcription is induced by p53.<sup>15</sup> As noticed by Mayo,<sup>22</sup> these interactions introduce a positive feedback loop in the system ( $p53 \rightarrow PTEN \rightarrow PIP3 \rightarrow Akt \rightarrow Mdm2_{nuc} \rightarrow p53$ ). We simplify this loop by assuming that phosphorylation of Mdm2<sub>cyt</sub> is inhibited by p53<sub>tot</sub>.

steady state ( $0 < t < 10$ ) and introduce a dose of ionizing radiation ( $IR = 1$  unit for  $10 < t < 20$ ). In response (Fig. 2C), DNA<sub>dam</sub> increases abruptly as does  $k_{d2}$ , the rate constant for degradation of Mdm2<sub>nuc</sub>. As a result, the steady state loses stability and oscillations in p53 and Mdm2 commence. As Mdm2<sub>nuc</sub> decreases, p53<sub>tot</sub> increases (Fig. 2A), which causes an initial drop in Mdm2P<sub>cyt</sub> and a steady increase in Mdm2<sub>cyt</sub> (Fig. 2B). When a sufficient amount of Mdm2 accumulates in the cytoplasm, it initiates a change of regime: phosphorylated Mdm2 enters the nucleus, causing increased degradation of p53, which relieves the inhibition of Mdm2 phosphorylation in the cytoplasm, allowing more Mdm2 to enter the nucleus. The positive feedback loop causes the abrupt drop in p53<sub>tot</sub> and rise in Mdm2<sub>nuc</sub>. The drop in p53<sub>tot</sub> cuts off the synthesis of Mdm2<sub>cyt</sub>, and consequently Mdm2<sub>cyt</sub> and Mdm2<sub>nuc</sub> drop. The system is back to the original state, p53<sub>tot</sub> starts to accumulate again due to the low level of Mdm2<sub>nuc</sub> and a new oscillation starts.

Every time p53<sub>tot</sub> increases, DNA<sub>dam</sub> decreases, hence  $k_{d2}$  decreases (Fig. 2C); eventually it goes back to its original value, and the system is

**Model.** Based on these assumptions, we propose a mechanism for p53/Mdm2 oscillations in Figure 1. The diagram is translated into differential equations (Table 1) using standard principles of biochemical kinetics. Parameter values (Table 2) have been adjusted to fit the experimental data presented in Figure 2E in ref. 7.

In the model, p53<sub>tot</sub> and Mdm2<sub>nuc</sub> are involved in both a positive and a negative feedback loop. The positive feedback originates from two opposing negative effects: nuclear Mdm2 induces p53 degradation, while p53 inhibits nuclear entry of Mdm2, by inhibiting phosphorylation of Mdm2 in the cytoplasm. The negative feedback loop is the well-known fact that p53 induces the synthesis of Mdm2 ( $p53_{tot} \rightarrow Mdm2_{cyt} \rightarrow Mdm2_{nuc} \rightarrow p53_{tot}$ ). We study the combined effects of these loops by performing numerical simulations of the full model and by applying bifurcation theory to the nonlinear ordinary differential equations.

**Simulations.** In the absence of DNA damage ( $IR = 0$ ,  $DNA_{dam} = 0$ ), the system of differential equations in Table 1 has a unique stable steady-state solution given by

$$[p53_{tot}] = 0.07, [p53U] = 0.02, [p53UU] = 0.01,$$

$$[Mdm2_{nuc}] = 0.33, [Mdm2_{cyt}] = 0.12, [Mdm2P_{cyt}] = 0.01$$

for the parameter values given in Table 2.

In Figure 2, we start the system at this steady state ( $0 < t < 10$ ) and introduce a dose of ionizing radiation ( $IR = 1$  unit for  $10 < t < 20$ ). In response (Fig. 2C), DNA<sub>dam</sub> increases abruptly as does  $k_{d2}$ , the rate constant for degradation of Mdm2<sub>nuc</sub>. As a result, the steady state loses stability and oscillations in p53 and Mdm2 commence. As Mdm2<sub>nuc</sub> decreases, p53<sub>tot</sub> increases (Fig. 2A), which causes an initial drop in Mdm2P<sub>cyt</sub> and a steady increase in Mdm2<sub>cyt</sub> (Fig. 2B). When a sufficient amount of Mdm2 accumulates in the cytoplasm, it initiates a change of regime: phosphorylated Mdm2 enters the nucleus, causing increased degradation of p53, which relieves the inhibition of Mdm2 phosphorylation in the cytoplasm, allowing more Mdm2 to enter the nucleus. The positive feedback loop causes the abrupt drop in p53<sub>tot</sub> and rise in Mdm2<sub>nuc</sub>. The drop in p53<sub>tot</sub> cuts off the synthesis of Mdm2<sub>cyt</sub>, and consequently Mdm2<sub>cyt</sub> and Mdm2<sub>nuc</sub> drop. The system is back to the original state, p53<sub>tot</sub> starts to accumulate again due to the low level of Mdm2<sub>nuc</sub> and a new oscillation starts.

again at steady state. For the parameter values we have chosen, two oscillations are sufficient to bring the system back to its original state. Were the damage heavier, a larger number of oscillations would be produced (Fig. 2D), as experimentally observed by Lahav et al. (Fig. 3A).<sup>7</sup>

**Bifurcation diagram.** A more complete picture of the dynamical system is provided by its bifurcation diagram. In Figure 3 we plot the system's recurrent solutions—steady states (when all variables are unchanging in time) or periodic solutions (when all variables repeat themselves periodically in time)—as functions of  $k_{d2}$ , the rate of degradation of nuclear Mdm2. In the resting condition ( $k_{d2} = 0.01$ ), the system has only one stable steady state, with low  $p53_{tot}$ . When  $k_{d2}$  increases beyond  $k_{SNL}$  (when  $k_{d2} = k_{SNL}$  the qualitative behavior of the system changes: this is called a Saddle-Node-Loop (SNL) bifurcation point), the steady state becomes unstable and is surrounded by a stable limit cycle. Every oscillation brings the system closer to the original resting state, as p53 induces repair.

It seems reasonable that cells would react to damage either fully activating the transcriptional program orchestrated by p53 or remaining silent, since an intermediate response would only give rise to a series of aborted starts of the transcriptional program. If translated in mathematical terms, this argument implies that oscillations should be born with large amplitude.<sup>25</sup> For example, in our model a sufficiently strong signal (i.e.,  $k_{d2} > k_{SNL}$ ) will fully activate the transcriptional program, otherwise the system will stay in its original steady state (i.e.,  $k_{d2} < k_{SNL}$ ); no intermediate outcomes are possible. Other bifurcations, which share this property with the SNL, are possible alternatives to (Fig. 3), for example a subcritical Hopf bifurcation (Fig. 4A). The case of supercritical Hopf bifurcations (Fig. 4B) is quite different: the amplitude of p53 oscillations increases from zero as  $k_{d2}$  increases beyond the bifurcation point. In response to high damage levels, the amplitude of p53 oscillations will decrease dramatically as the damage is repaired, in contradiction to the observations of Lahav et al.<sup>7</sup> In response to low damage levels, small pulses of p53 are unlikely to trigger effective repair. Hence, we consider supercritical Hopf bifurcations unlikely to be used by the p53/Mdm2 network.

**Comparison to experimental data.** The model (Fig. 2A) reproduces in quantitative detail the results published by Alon and colleagues (Fig. 2E in ref. 7). In both model and experiments, as a consequence of gamma-irradiation, the nuclear form of Mdm2 initially drops while nuclear p53 increases. Mdm2 and p53 continue to oscillate out of phase, with a period of approximately 400 minutes. In the data published by Lahav et al.,<sup>7</sup> It is clear that most of the signal is localized in the nucleus. In our model, although Mdm2 is present also in two cytoplasmic forms (Mdm2<sub>cyt</sub> and Mdm2P<sub>cyt</sub>), the signal coming from the nucleus (i.e., Mdm2<sub>nuc</sub>) exceeds considerably the cytoplasmic one, (Figs. 2A and B). The main discrepancy between model and experiment is that in the model Mdm2<sub>nuc</sub> is kept very low when p53<sub>tot</sub> is high while experimental results show that Mdm2<sub>nuc</sub> is already high when p53 reaches its maximum. Apparently, the antagonism exerted by p53 on Mdm2 is stronger in the model than in real cells. It is possible that at least a fraction of cytoplasmic Mdm2 is measured during the experiment, either Mdm2<sub>cyt</sub> or Mdm2P<sub>cyt</sub> or both, which would make the simulations more similar to the experimental data (not shown).

## DISCUSSION

Recent observations have shown that the p53/Mdm2 network responds to environmental stresses, such as gamma irradiation, by generating pulses of p53.<sup>7</sup> As the extent of the stress increases, the p53/Mdm2 network produces an increasing number of oscillations with constant amplitude and interpulse interval. We present a mathematical model of the p53/Mdm2 network that produces similar oscillations. The model consists of (1) the well-known negative

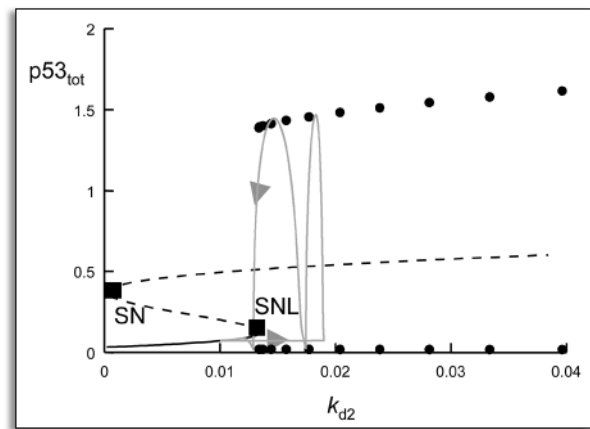


Figure 3. Bifurcation diagram. Recurrent states (steady states and limit cycles) for  $p53_{tot}$  are plotted as functions of  $k_{d2}$ , the degradation rate of Mdm2<sub>nuc</sub>. The solid line represents stable steady states, the dotted line unstable steady states. Black dots are the maxima and minima of the stable limit cycles. The grey solid line represents  $p53_{tot}$  as a function of  $k_{d2}$  from the simulation shown in Figure 2. Notice that in Figure 2,  $k_{d2}$  is a variable, see equations in Table 1, while here it is a parameter (all other equations and parameter values as in Tables 1 and 2). When the qualitative behavior of the system changes, it is said to undergo a bifurcation. In the p53/Mdm2 model there is a Saddle-Node (SN) bifurcation at  $k_{d2} = 0.0018$  and a Saddle-Node-Loop bifurcation at  $k_{d2} = 0.0135$ . Before the SNL bifurcation there is only one stable steady state, with low p53 ("p53 OFF"); after the SNL the steady state becomes unstable, surrounded by a stable limit cycle. The family of stable limit cycles disappears at a Hopf bifurcation at  $k_{d2} = 0.8532$  (not shown on the diagram).

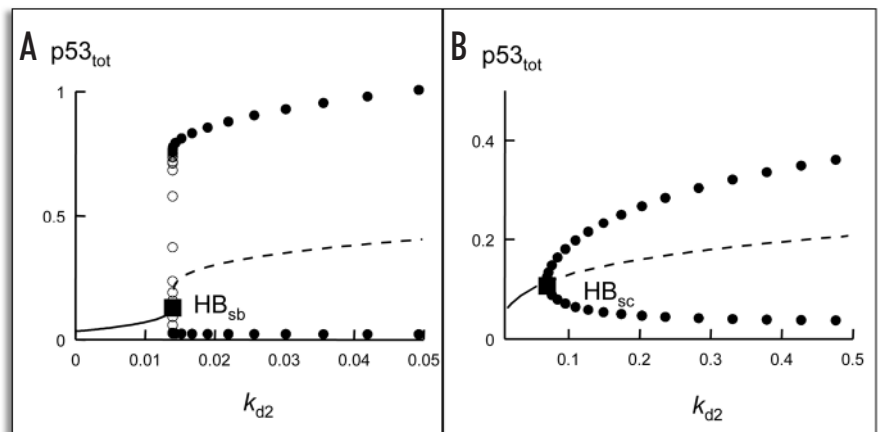


Figure 4. Supercritical and subcritical Hopf bifurcations. It is possible to distinguish between two classes of Hopf bifurcations according to the amplitude of the stable oscillations in the vicinity of the bifurcation point. (A) Oscillations are born with large amplitude at a subcritical Hopf bifurcation (HB<sub>sb</sub>). Equations from Table 1 and parameters from Table 2 except for  $k_{s2} = 0.0155$ . (B) At a supercritical Hopf bifurcation (HB<sub>sc</sub>), oscillations are born with small amplitude. Equations from Table 1 and parameters from Table 2 except for  $k_{s2} = 1$ . The solid line represents stable steady states, the dotted line unstable steady states. Black squares mark the Hopf bifurcations. Black dots are the maxima and minima of the stable limit cycles, while open circles represent the maxima and minima of unstable limit cycles.

feedback between Mdm2 and p53, whereby p53 induces Mdm2 transcription and Mdm2 induces p53 degradation, and (2) a positive feedback loop that originates from a negative effect on nuclear localization of Mdm2 exerted by p53 via PTEN and Atk kinase.

In the model, p53 level is kept low by degradation induced by Mdm2. Guided by experimental evidence, we simulate DNA damage by increasing Mdm2 degradation in the nucleus. When this

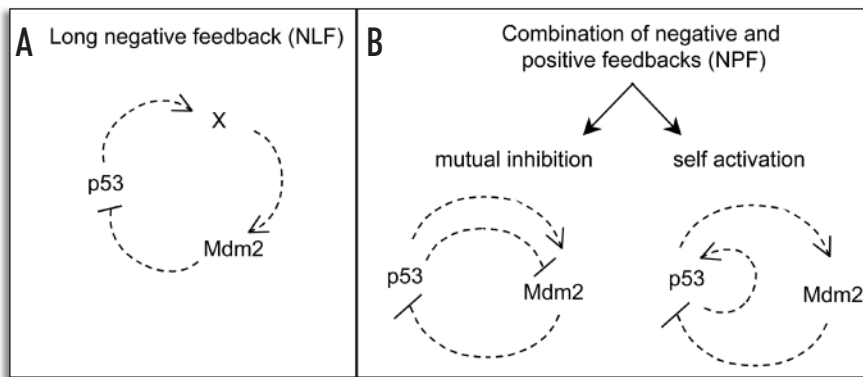


Figure 5. Pathways to oscillations. Oscillations can be found in two different types of circuits; (A) negative feedback loops with three or more components (NFL), and (B) combinations of negative and positive feedbacks (NPF). NPF comes in two different classes, according to the nature of the positive feedback: either mutual inhibition or self-activation.

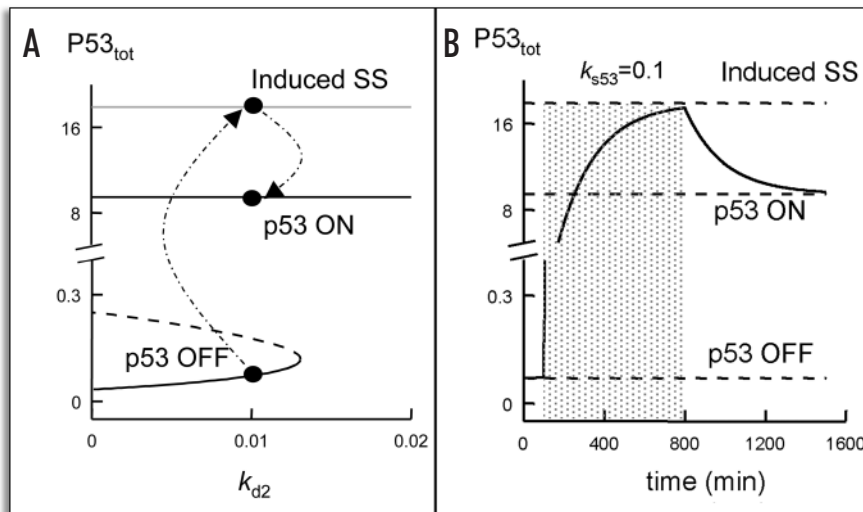


Figure 6. Transient increase of p53 transcription rate in a model without negative feedback. The p53/Mdm2 model with antagonism but without negative feedback relaxes to a high p53 level after a transient increase in p53 expression. (A) Bifurcation diagram. In a model where the negative feedback has been removed (equations and parameters in Table 1, except for  $k_{s2} = 0$ ), there are two stable steady states (p53 ON and p53 OFF), separated by unstable steady states (solid and dotted black lines, respectively). When p53 is overexpressed, the system has only one stable steady state, solid grey line. Dashed-dotted line represent the transitions among the different attractors during a transient overexpression of p53. (B) Time course of the full model without negative feedback. In the shaded area, p53 is overexpressed ( $k_{s53} = 0.1$ ). Once the inducer is removed, the system settles down on a high p53 state.

happens, the “p53 OFF” steady state loses its stability, and the system enters a region characterized by stable limit cycles (Fig. 3). Every oscillation of p53 level decreases the extent of the damage, pushing the system back towards the original steady state, where it comes to rest once Mdm2 degradation rate is back to normal.

Although our model is consistent with many qualitative and quantitative features of the experiments of Lahav et al.<sup>7</sup> it is admittedly primitive and could be expanded and improved in many obvious ways. For example, p19<sup>Arf</sup>, p21, Rb, E2F are involved in an additional negative feedback loop with p53.<sup>24</sup> At this stage, more important than specific details of the model is the general principle we propose, that the “digital output” of the p53-Mdm2 network<sup>7</sup> is a consequence of both positive and negative feedback in the net-

work, which create a global bifurcation to large amplitude oscillations in response to DNA damage. Moreover, the model can be used to formulate two experiments that might discriminate whether oscillations are based on a negative feedback loop alone<sup>10</sup> or on a combination of positive and negative feedback loops (as we propose).

**Pathways to oscillations.** Oscillations impose quite strict constraints on the topology and kinetics of molecular networks (Fig. 5). One way to get oscillations, which is explored in this paper, requires the simultaneous presence of negative and positive feedbacks (NPF) (Fig. 5B). In the NPF case, the smallest possible network is composed of two elements, and we find a dichotomy concerning the nature of the positive feedback. It can be produced either via mutual inhibition, as in our model, or through autocatalysis (e.g., it has been reported that in some cells p53 induces its own transcription<sup>26</sup>). Whatever the origin of the positive feedback, the general mechanism which initiates oscillations is similar to the one we address in this work: the positive feedback gives rise to a bistable system where p53 can be either high (“ON”) or low (“OFF”), while the negative feedback makes the steady states unstable and generates oscillations between them.

It is well known that oscillations can also arise from negative feedback alone (NFL), if it is composed of at least three elements (Fig. 5A). A model of this sort (composed of p53, Mdm2 and a putative intermediary) has been presented by Oren and collaborators<sup>10</sup> to explain damped p53 oscillations in a population of cells. Without going into further details, we point out that in this case oscillations do not require a potential bistability. The NFL has only one unstable steady state, surrounded by a stable oscillatory solution. If the negative feedback is weakened, oscillations disappear, and the steady state becomes stable.

**Predictions.** Can we conceive an experiment to distinguish oscillations arising from NFL or NPF? One obvious experiment would be to eliminate the positive feedback (in our model the loop  $p53 \rightarrow PTEN \rightarrow Akt \rightarrow Mdm2 \rightarrow p53$ ). For example, this loop would be absent from an Mdm2 mutant which does not require phosphorylation to enter the nucleus. If p53/Mdm2 oscillations were not observed in such a mutant, we could conclude that the positive feedback is required for oscillation, and the network belongs to the NPF class. If oscillations persisted in such a mutant, we could not rule out the presence of other sources of positive feedback (e.g., autocatalytic production of p53).

A more definitive experiment consists in removing the negative feedback loop, for example creating a mutant where Mdm2 is expressed at a basal rate in a p53-independent manner (in the model,  $k_{s2} = 0$ ). Although this modification will prevent oscillations in both an NFL and an NPF, the two systems will respond very differently to a transient increase in p53 expression. Let's assume that p53 is

overexpressed for a short time, using an inducible promoter (in the model, a twofold increase of  $k_{53}$ ). In an NFL without negative feedback, p53 level will increase in response to p53 overexpression and then relax to the original p53 OFF state after the inducer is removed. If the negative feedback is removed from the NPF, the positive feedback is still operational, and it gives rise to a bistable system, (Fig. 6A). During the transient increase of p53, the p53 OFF state is lost and the cell is forced to move to the only possible, temporary steady state, with high p53. After removal of the inducer, the system is bistable again, but it does not return to the original p53 OFF state because the p53 ON state is closer to the temporary steady state. Summarizing, the model predicts that a pure NFL without negative feedback will relax to the original p53 OFF state after inducer removal, while an NPF without negative feedback will relax to a p53 level higher than the original, (Fig. 6B). This difference can be exploited to determine which mechanism (NFL or NPF, in Fig. 5A and B, respectively) is responsible for generating oscillations in the p53/Mdm2 network.

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