Exploring a minimal two-component p53 model

This article has been downloaded from IOPscience. Please scroll down to see the full text article.

2010 Phys. Biol. 7 036008

(http://iopscience.iop.org/1478-3975/7/3/036008)

View the table of contents for this issue, or go to the journal homepage for more

Download details:
IP Address: 202.121.180.253
The article was downloaded on 17/09/2013 at 14:58

Please note that terms and conditions apply.
Exploring a minimal two-component p53 model

Tingzhe Sun\(^1\), Ruoshi Yuan\(^2\), Wei Xu\(^3\), Feng Zhu\(^1\) and Pingping Shen\(^{1,4}\)

\(^1\) State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, 210093, People’s Republic of China

\(^2\) Laboratory for Biocomputing and Bioinformatics, Department of Computer Science and Engineering, Shanghai Jiao Tong University, Shanghai, 200240, People’s Republic of China

\(^3\) Shanghai Center for Systems Biomedicine, Key Laboratory of Systems Biomedicine of Ministry of Education, Shanghai Jiao Tong University, Shanghai, 200240, People’s Republic of China

E-mail: ppshen@nju.edu.cn

Received 30 April 2010
Accepted for publication 19 August 2010
Published 10 September 2010
Online at stacks.iop.org/PhysBio/7/036008

Abstract
The tumor suppressor p53 coordinates many attributes of cellular processes via interlocked feedback loops. To understand the biological implications of feedback loops in a p53 system, a two-component model which encompasses essential feedback loops was constructed and further explored. Diverse bifurcation properties, such as bistability and oscillation, emerge by manipulating the feedback strength. The p53-mediated MDM2 induction dictates the bifurcation patterns. We first identified irradiation dichotomy in p53 models and further proposed that bistability and oscillation can behave in a coordinated manner. Further sensitivity analysis revealed that p53 basal production and MDM2-mediated p53 degradation, which are central to cellular control, are most sensitive processes. Also, we identified that the much more significant variations in amplitude of p53 pulses observed in experiments can be derived from overall amplitude parameter sensitivity. The combined approach with bifurcation analysis, stochastic simulation and sampling-based sensitivity analysis not only gives crucial insights into the dynamics of the p53 system, but also creates a fertile ground for understanding the regulatory patterns of other biological networks.

Online supplementary data available from stacks.iop.org/PhysBio/7/036008/mmedia

Introduction
The transcription factor p53 protein is a famous tumor suppressor [1]. Wild-type p53 remains at low levels under the homeostatic condition, and if present at high levels, as occurs in response to DNA damage, oncogene activation, it initiates cell cycle arrest when cells are confronted with mild damage, while induces apoptosis when the damage is irreparable [1–3]. A major function of p53 is to dictate the expression of many target genes. Differential selection of target genes appears to be an essential aspect of the mechanisms that determine which cellular response will finally emerge. One of the well-known targets is MDM2, which controls p53 stabilities [4].

MDM2 is an E3 ubiquitin ligase which targets p53 and itself for degradation [4–6]. The reciprocal regulations ensure a negative feedback loop [7]. Meanwhile, p53 is also under control of multiple positive feedback loops [8–10]. The p53-MDM2 axis integrates the upstream signals to elicit appropriate downstream effects and dictates cell fate. Thus, it is one of the core regulatory circuits, which motivates considerable investigations.

Early dynamical studies found that p53 performs damped oscillation at a cell population level [11] and a simplified mathematical model was constructed to describe these dynamics [11]. Later experiments further revealed a dynamic pattern called ‘digital oscillation’ [12], although more recent experiments raised some questions. However, this finding inspired admirable models [13, 14]. Noticeably, the first model that describes sustained p53 pulses with a time delay...
was constructed by Tiana et al [15]. However, only until recently confidential experiments showed that p53 performs undamped pulses [16], while Batchelor et al later reconfirmed the sustained pulses and emphasized the importance of an upstream negative feedback [17]. Those findings motivated several other theoretical models [18–20]. Meanwhile, Zhang et al from Tyson’s group [21, 22], Zhang et al [23] and we [24] also provide plausible mechanisms to unravel the underlying physiological role of p53 pulses.

Although numerous models focus on p53 oscillations or pulses, several models also evaluated the plausible bistability of the p53 system. Wee et al constructed a hierarchy of qualitative models and demonstrated that p53-Akt network possesses the potential to exhibit bistability [25]. Their later model further refined their previous work to recapitulate that oscillations decrease the threshold at which transition to high p53 levels occurs and the bifurcation diagram fundamentally implies the bistability [26]. Puszyski et al further depicted a bistable scenario albeit an ambiguous positive feedback loop was incorporated in their model [18].

Despite diverse bifurcation patterns in p53 modeling, few models provide a linkage between oscillation and bistability. Abou-Jaoudé et al [27] and Wee et al [26] shed shimmering light on this issue albeit they described seemingly contradictory bifurcation patterns. To explore the dynamics in the p53 system, we developed a minimal two-component model to capture essential feedback loops. Given that the actual regulatory network is not fully characterized, a minimal model allows a systematic analysis without loss of generality. Furthermore, the core regulatory architecture can be incorporated with other regulatory motifs to generate more sophisticated models. In addition, the minimal model can be extrapolated into genetic circuit with similar architectures.

Three essential aspects are investigated in current work. First, we identified the irradiation dichotomy (ultraviolet light and γ-irradiation) in p53 modeling. We further proceed to bridge the gap between oscillation and bistability in the p53 system. Oscillations arise with a strong negative feedback (p53-induced MDM2 expression) while the model generates bistability with a relatively weak negative feedback. Distinct negative feedback strengths give rise to copious bifurcation patterns. Secondly, stochastic simulation qualitatively fitted experimental observations. Notwithstanding the counterintuitive idea on p53 bistability, we proposed that bistability might be plausible based on experimental observations. Finally, we performed parameter sensitivity analysis to systematically demonstrate that the experimentally observed higher variations in amplitude could be derived from overall parameter sensitivity biased toward amplitude regulation.

Materials and methods

Model construction

The minimal model captures essential feedback regulation in p53 networks. The first two terms in equation (1) represent basal production and degradation rate of p53, respectively. The third term in equation (1) donates positive feedback effects in p53 systems. Multiple positive feedback loops are embedded in the p53 regulatory network [8–10]. Positive feedback loops indicate that when the changes in a variable occur, the systematic response tends to change that variable even more at the same direction. For this reason, the positive feedback can be regarded as ‘self-induction’ and we adopted a simplified mathematical description with a Hill function. Incorporation of positive feedback loops makes it more feasible to evaluate the influence of the positive feedback effects in the p53 system. The fourth term in equation (1) represents MDM2-mediated degradation of p53. The first two terms in equation (2) donate basal production and degradation for MDM2. The third term means p53-induced MDM2 expression (negative feedback more exactly), and the fourth one implies the effects of MDM2-catalyzed self-degradation. Many simplifications are made in the two-component model. The minimal model does not explicitly delineate the mRNA species. Transcription and translation are incorporated into a single step of protein synthesis. The diverse post-translational modifications, translocations and interactions with other signaling networks are not considered here for simplicity. Compartmentation and other detailed mechanisms are also not taken into account. P53 and MDM2 donate protein levels in equations, respectively. A schematic illustration of the minimal model is shown in figure 1. For nominal parameter values, please refer to supplementary table S1 (available at stacks.iop.org/PhysBio/7/036008/mmedia):

\[
\begin{align*}
\frac{dp53}{dt} &= r_1 - d_1 \cdot [p53] + V_p \cdot \frac{[p53]^4}{[p53]^4 + k_1^4} - k_{\text{max}} \cdot \frac{[p53] \cdot [\text{MDM2}]}{[p53] + k_p}, \\
\frac{d\text{MDM2}}{dt} &= r_2 - d_2 \cdot [\text{MDM2}] + V_m \cdot \frac{[p53]^4}{[p53]^4 + k_2^4} - m_{\text{deg}} \cdot \frac{[\text{MDM2}]^2}{[\text{MDM2}] + k_m}.
\end{align*}
\]

Figure 1. Schematic representation of the p53-MDM2 feedback loop.

Stochastic modeling and potential construction

We implemented a binomial τ-leap-based stochastic simulation algorithm. For detailed descriptions, please refer to
Relative sensitivity

Sensitivity analysis probes systematic responses to an infinitesimal disturbance in nominal model parameters. A dynamic system is given by $x' = F(x, p)$, where $x$ and $p$ donate the state vector and parameter vector for the system, respectively. Amplitude sensitivity and period sensitivity capture the variations of amplitude and period upon parameter changes. The relative amplitude sensitivity $S_A$ and the period $S_\tau$ sensitivity are defined as

$$S_A = \frac{\partial A / A}{\partial p / p} = \frac{\partial \ln(A)}{\partial \ln(p)}, \quad (4)$$

$$S_\tau = \frac{\partial \tau / \tau}{\partial p / p} = \frac{\partial \ln(\tau)}{\partial \ln(p)}. \quad (5)$$

$A$ and $\tau$ donate (peak) amplitude and period, respectively. Note that these sensitivity measures are normalized and only locally valid with respect to parameter space (i.e. not robust in the presence of stochasticity). Therefore, a global visualization of related sensitivities requires robust methodology (e.g. constructing the potential landscape).

Sensitivity rankings

To explore the robustness properties of dynamic systems, we implemented a rank-based method to assign sensitivity rankings to individual parameters in a bistability scenario. The algorithm embedded in the BioSens Toolkit [33] is employed to compute the sensitivity rankings and explore the systematic dependence of parameters; detailed analyses were performed in the custom code.

Mutual information index (MII)

The mutual information of two variables characterizes their mutual dependence. A general measure of input–output associations can be provided by mutual information regardless of the shape of underlying distributions [34]. Randomized perturbations of input parameters $(X_1, X_2, \ldots, X_n)$ in parameter space within the predefined range create a random output $Y$ (e.g. oscillation periods or amplitude) with a probability density $p(y)$. The entropy associated with $Y$ is defined as

$$H(Y) = -\sum_y p(y) \cdot \log_2 p(y). \quad (6)$$

$H(Y)$ provides a measure for the uncertainty of $Y$ due to randomized inputs.

Conditional entropy can further be defined with one fixed $X_i$

$$H(Y | X_i) = \sum_x p(x) \cdot H(Y | X_i = x). \quad (7)$$

The mutual information is defined as the difference between total entropy $H(Y)$ and one preferred conditional entropy $H(Y | X_i)$ to characterize the impact $X_i$ exerts on $Y$:

$$I(X_i; Y) = H(Y) - H(Y | X_i). \quad (8)$$

Mutual information index (MII) can be defined to be

$$m_i = \frac{I(X_i; Y)}{H(Y)}. \quad (9)$$

MII provides a first-order sensitivity measure for all inputs. MII was applied in an oscillation scenario to give a sensitivity measure for period and amplitude.

Results and discussion

The strength of the p53-induced MDM2 expression determines bifurcation patterns

We first explore the bifurcation patterns with varying strength of the p53-induced MDM2 expression ($V_m$). As MDM2 targets p53 for degradation, $V_m$ may also dictate the negative feedback strength. The parameter $r_1$ (p53 production rate) was selected as the second bifurcation parameter because p53 translation rate is elevated in response to UV or IR irradiation [35, 36], which can be regarded as increased protein production.

In figure 2(a), bistability arises with a fairly weak strength ($V_m = 0.001$). Three steady states coexist, while the middle branch is unstable. Upon increasing $V_m$ to 0.005 (figure 2(b)), unstable oscillations come forth from the upper branch of the steady state at a subcritical Hopf point (sub-H). These limit cycles coalesce with an unstable steady state to generate a homoclinic bifurcation (HC). When $V_m$ reaches 0.008 (figure 2(c)), sub-H has already moved beyond SN2. At the LP-PO, stable and unstable limit cycles coalesce and become semi-stable. As $V_m$ increases to a 0.12 (figure 2(d)), sub-H transits to supercritical Hopf point (sup-H, figure 2(d)). Thus, as $V_m$ increases, SN1 evolves into a sup-H.

We further came to explore how SN2 evolves into a sup-H. SN2 retains its status if $V_m$ stays below the critical value (BT: Bogdanov–Takens, figure S1 (available at stacks.iop.org/PhysBio/7/036008/mmedia)). When $V_m$ grows further, unstable limit cycles emerge from the lower steady state. Increasing $V_m$ to 0.01095 yields a bifurcation diagram shown in figure 3(a). Unstable limit cycles emanating from sub-H coalesce with the unstable steady state of the middle
Figure 2. Bifurcation diagrams of p53. The solid line denotes a stable steady state, while the dashed line denotes an unstable steady state. A stable limit cycle is marked by a solid circle and an unstable limit cycle by an open circle. The parameter values for $V_m$ are (a) 0.001, (b) 0.005, (c) 0.008 and (d) 0.12, respectively. Other parameter values are listed in table S1, available at stacks.iop.org/PhysBio/7/036008/mmedia. SN, saddle-node point; sub-H, subcritical Hopf bifurcation point; sup-H, supercritical Hopf bifurcation; HC, homoclinic point; SNIC, saddle-node invariant circle and LP-PO, limit point on the periodic orbit.

Figure 3. Bifurcation diagrams of p53 versus $r_1$. Line and circle types are described as in figure 2. The parameter values for $V_m$ are (a) 0.01095, (b) 0.0111 and (c) 0.0112, respectively. Right panels indicate the corresponding period. We further investigate bifurcation properties by a two-parameter diagram (figure 4(a)). The region inside the curved-edge triangle denotes bistability, and the area surrounded by a dashed arc indicates the limit cycle. The topology of this two-parameter bifurcation pattern preserves within twofold change of all parameters, while for specific ones even tenfold change is allowed (data not shown). We then inspected the region marked by a solid rectangle which is amplified in figure 4(b). SNIC and SN2 overlap because they occur at identical $r_1$. CUSP and BT, two codimension-2 bifurcation points, emerge (figure 4(b)). At the CUSP bifurcation point, two branches (SN1 and SN2) meet tangentially and

branch generating a HC bifurcation point (figure 3(a)). Also, an SNIC can be detected, upon crossing which the other set of unstable limit cycles arises. Further increasing $V_m$ to 0.0111 (figure 3(b)), the bistable region with three unstable steady states completely resides in the LC region and two sets of unstable limit cycles become continuum compared with figure 3(A). Finally, elevating $V_m$ to 0.0112 yields a bifurcation diagram shown in figure 3(c). We found that bistability vanishes within the oscillation region. Therefore, during these evolutionary stages, SN2 undergoes evolution into a sup-H.
coalesce. For the BT point, a saddle node and a Hopf point coalesce. The rectangle-bounded region in figure 4(b) can be further divided into topologically differentiable regions in the ($r_1, V_m$) plane, and phase portraits are shown in figure S2 (available at stacks.iop.org/PhysBio/7/036008/mmedia). But this contributes little and for details we refer readers to supplementary materials. In summary, the system is relatively robust to parameter variation. Furthermore, it implies that diverse bifurcation properties can be derived by manipulating feedback strength ($V_m$).

Stochastic simulations qualitatively explain experimental data

We further applied stochastic simulation to characterize the stochasticity of biochemical processes.

For low $V_m$ values (0.001), 1000 independent runs were computed and then averaged (figure 5(a), black). Results show that the transition time at which the system flips to the high state differs significantly (sometimes the transition does not occur at least in a limited time scale). Averaging over all runs generates a gradually increasing pattern (red, figure 5(a)). Upon increasing $V_m$ to 0.005, most runs show irregular fluctuations (gray, figure 5(b)), but a detectable fraction does show pulsatile dynamics (black, figure 5(b)). Furthermore, averaging over all runs also indicates approximately a gradual increasing pattern (red). For a larger $V_m$ (0.02, for bifurcation diagram, figure S3 (available at stacks.iop.org/PhysBio/7/036008/mmedia)), significant fractions of simulation runs show sustained pulses (dark gray in figure 5(c)), but random fluctuations can also be detected (black in figure 5(c)).

However, a formal approach in stochastic simulation requires the construction of underlying potential landscape. More importantly, the methodology of constructing potential landscape is robust in the presence of stochasticity [29, 32] and outcompetes traditional stochastic simulation and local
Figure 6. Potential landscape. Deterministic parameter values for each panels are (a) $r_1 = 0.0064$, $V_m = 0.001$; (b) $r_1 = 0.0065$, $V_m = 0.005$; (c) $r_1 = 0.0072$, $V_m = 0.02$, respectively. The colormap denotes potential. The final representation is smoothed by convolution with a Gaussian kernel.

sensitivity analysis methods. This formal approach covers standard nonlinear analyses and may serve as a framework in an analyzing dynamical system [32]. By constructing potential landscape, we can identify systematic properties (e.g. robustness, stability, sensitivity) of the underlying network. Therefore, it helps to unravel the hidden layer of complexity and provides crucial insights into the network regulation [32]. We further explore correspondingly the dynamic evolution of the system with underlying potential landscape. In figure 6(a), we found that there exist two local minima in the potential landscape. The two minima correspond to two states where the system tends to relax under noisy conditions. The visible valley that connects the two minima reveals that there exists a fraction of ‘cells’ that transits from the low to high state. Meanwhile, the swift transitions may contribute to less residence time and relatively higher potential along the valley. The high steady state defines a lower potential minimum compared with the low steady state. Therefore, the system tends to relax to the higher steady states although at different time scales (figures 5(a) and 6(a)). When the negative feedback strength is increased, the potential barrier between these two minima decreases significantly and more frequent transitions may occur (figure 6(b)). Noticeably, fluctuations also induce a visible ‘closed ring’ along the valley that corresponds to a (probable) closed orbit in the phase plane. The closed orbit may denote an oscillatory or pulsatile pattern in time series (e.g. in comparison with figure 5(b), at least in a limited time scale). Furthermore, the basin of attraction that delineates the high steady state in the potential landscape becomes shallower and extended. Therefore, the trajectories along the basin will be more fluctuated and may also exhibit plausibly ‘oscillations’ or ‘pulses’ in a probabilistic manner (figures 5(b) and 6(b)). For larger $V_m = 0.02$, the minimum corresponding to the high state disappears and a visible ‘closed ring’ also emerges which denotes an oscillation as described above (figure 6(c)). Besides, the system may also fluctuate around the potential minimum which might correspond to random fluctuations in figure 5(c). Note that the rates at which the system passes the troughs are much slower (data not shown) and therefore the potential may favor the lower states using our construction method (see Materials and methods). Taken together, the qualitative features of stochastic trajectories can be revealed in light of underlying potential landscape.

Geva-Zatorsky et al [16] showed that on IR irradiation, some cells perform sustained pulses while others show either no response or fluctuation. Lev Bar-Or et al [11] found that high dose IR leads to p53 oscillation (i.e. western blotting, see figure 5 in [11]), while low dose IR will not generate oscillations at cell population levels (see figure 6 in [11]). Note that a low dose 0.3 Gy was used in both experiments from the same group (figure 3 in [16] and figure 6 in [11]). In single cell experiment, some fractions of cells performed sustained oscillations with 0.3 Gy [16], while at cell population levels,
conditions, the effects of p53-induced MDM2 production 

Following these experiments, we assume that under unstressed (i.e. elevated production) in DNA damage response [35, 36]. The mdm2 promoter is characterized as a non-B-DNA supercoiling and prevents promoter activation [38, 39]. Upon exposing to IR, double strand breaks will promote local DNA relaxation and transcription, whereas DNA cross-linking treatment, such as UV, will slow down or inhibit p53-mediated transcription [37, 38]. Meanwhile, numerous experiments also showed that post-translational modifications of p53 will promote the transcriptional capacity by recruiting co-activators [1, 3, 40, 41]. These two effects might counteract each other in regulating transcriptional efficiency under UV. In short, IR promotes the p53-induced MDM2 expression. As described above, p53 promotes mdm2 transcription at low levels of UV, while MDM2 induction is severely attenuated on exposure to high dose UV compared with unstressed condition, and this has been demonstrated by Levine’s lab [41, 42] and other workers [43–46]. More exactly, low dose UV elevates MDM2 induction compared with an unstressed state. As UV dose increases, MDM2 induction is gradually reduced and even drops below the control group [45]. Secondly, however unstable in unstressed cells, p53 is not inert and capable of inducing MDM2 [40, 47, 48]. Finally, it has also been demonstrated that p53 mRNA incurs increased translation (i.e. elevated production) in DNA damage response [35, 36]. According to these experiments, we assume that under unstressed conditions, the effects of p53-induced MDM2 production situate at an intermediate level (e.g. Vm = 0.003 or 0.004), but r1 is small in the unstressed state, thereby p53 remains at low state. Upon exposure to extremely low levels of IR, as post-translation modification and IR-induced DNA relaxation both contribute to transcription promotion, Vm gets slightly larger (e.g. 0.005, as in figure 5(b)) and generates a gradual increasing pattern at cell population levels. Further increasing IR (e.g. figure 5(c)), bifurcation property changes and ensures a damped oscillation at cell population. For relatively low doses UV, as MDM2 induction is relatively efficient (which defines a relatively high Vm), damped oscillation emerges at the cell population levels (e.g. figure 5(c)). Following high UV, however, MDM2 induction is severely attenuated (for extremely low Vm values, e.g. figure 5(a)) and a gradual increasing pattern is generated at cell population. By mining experimental results, however, we did find a p53 response pattern at least in a limited time scale [3, 11, 49–56]. It shows that p53 oscillates in response to relatively high dose IR (figure 5 in [11], figure 3(b) in [49], figure 3 in [50], figure 5 in [56]) or low dose UV (figure 6 in [50], figure 6 in [51], figure 3(c) in [55]), while approximately increase gradually upon exposure to extremely low dose IR (figure 6 in [11]) and relatively high dose UV (figure 3(b) in [49], figure 6 in [51], figure 1(a) in [52], figure 6(b) in [53], figure 3(c) in [54], figure 3(c) in [55], figure 5 in [56]) at the cell population level. Figure 5(c) is referred to as response curves for both low UV and high IR irradiation. We proposed that high dose IR and low dose UV probably correlate to oscillation scenarios, which are integrated into one panel for simplicity. Taken together, we characterize the irradiation dichotomy in p53 models (i.e. UV and IR elicit distinct responses in p53 dynamics). Our stochastic simulations give plausible credence to characterized experimental results.

It seems counterintuitive to discuss bistability in the p53 system. But some reports suggested that the p53 level increases gradually and the stabilization is long-lasting even more than 24 h [49, 55, 56]. Meanwhile, the dynamics of p53 upon IR irradiation was also investigated in identical cellular environment and showed astonishingly distinct patterns compared with UV [55, 56]. To our knowledge, noise-induced transition contributes to the heterogeneous responses. We might recall the stochastic oscillations even with a deterministic bistable scenario (figures 2(b) and 5(b)). In experiments one could not easily distinguish stochastic fluctuations around stable foci from limit cycle oscillations in a limited time scale, because their stochastic trajectories are similar. On the other hand, a negative feedback is prerequisite for oscillation. As suggested (figure 4), sustained oscillation is only allowed under the situation when Vm exceeds some critical value. For the p53-MDM2 oscillator, a positive feedback may function in a way to maintain the undamped oscillation [7], and this mechanism has been employed in many previous models [13, 21, 24, 26]. If negative feedback strength is attenuated as in the case with high dose UV or extremely low IR (i.e. insufficient negative feedback strength), the positive feedback probably overcomes its rivals and confer bistability, although stochastic oscillations preserve (figure 5(b)). Thus, we supposed (by extrapolation) that once cells confront severe UV irradiation, induction of MDM2 by p53 or more exactly the negative feedback strength would be severely attenuated or even compromised and finally the positive feedback plays dominant roles, and therefore bistability might occur. A major concern clarifies that instead of bistability, graded increases in p53 might represent the low probability of p53 activation and lack of oscillation synchronization across the population. To our knowledge, the single cell experiments that present the sustained p53 pulses are all stimulated with IR [16, 17]. We have distinguished IR from UV as described above. As the nature of these two types of irradiation differs, we cannot easily conclude that p53 performs oscillations in a single cell irradiated with UV. Furthermore, Kaeser et al showed that MDM2 induction is strongly attenuated upon UV irradiation [45], and sometimes MDM2 induction is completely impaired [44]. Meanwhile, Batchelor et al showed that Wip1-mediated dephosphorylation of ATM constitutes another negative feedback which is essential for sustained p53 pulses [17] and they also identified that UV does not allow p53 pulses (see Discussion in [17]). As we know that ATM primarily responds to DSB (i.e. IR-induced DNA damage) but not UV-induced DNA lesions [39, 57], the negative feedback strength will be further attenuated upon UV irradiation; therefore, sustained oscillation would be more or less suspicious with high dose UV. Note that even though oscillation might disappear with high dose UV, we still cannot conclude that bistability definitely exists because ensemble of
stochastic runs with sigmoidal curves can also lead to a gradual increasing pattern.

Identifying key control parameters in apoptotic response

To our knowledge, there can also be uncertainty in kinetic parameters [58]. To capture the correlation between the parameter and output uncertainty, we implemented a sampling-based method to evaluate the bistable performance. A Latin hypercube sampling is used to generate 200 parameter sets with 10% perturbation from nominal values. Each set is propagated to the equations to analyze the systematic response. This process was repeated for three times and each run is ensured to reach the steady state. In figure S4 (available at stacks.iop.org/PhysBio/7/036008/mmedia), as parameter \( r_1 \) increases, the fraction of the parameter sets which lead to high p53 levels follows the same tendency and a statistic scheme is also shown (bottom-right panel). In deterministic bifurcation diagrams, SN2 corresponds to high p53 levels, while the other leads to low p53 levels. Because wild-type p53 protein is a potent death inducer [1, 3], we assume that a high p53 level correlates to apoptosis for simplicity. In figure 7, \( r_1 \) is set 0.006, and for other cases which ensure bimodal responses the results are qualitatively comparable. The parameters are ranked from 1 to 12 (see Materials and methods, and table 1 for parameter number), with 12 the most sensitive. To determine whether the variations in sensitivity rankings are statistically significant, \( p \)-values are listed. Most of the parameter rankings are not preserved between apoptosis inducing and survival groups. For both groups, the system is substantially sensitive to the p53 production (\( r_1 \), parameter 1) and catalytic degradation (\( k_{\text{max}} \), parameter 5) parameters, and only rankings of these two parameters are central to cellular control and thus deserve the highest sensitivity [1, 3, 35, 36, 40]. The p53 system is increasingly sensitive to the parameters 3 and 4 in the apoptosis group (these two parameters represent the positive feedback in the p53 system). The increased sensitivity shows that the positive feedback loops will play more important roles once apoptosis is triggered. Sensitivity rankings decrease significantly for the parameters 8 (\( d_2 \), basal MDM2 degradation rate), 11 and 12 (these two parameters denote MDM2-mediated self-degradation) once apoptosis occurs. The decline in sensitivity rankings indicates that regulation of MDM2 degradation becomes less important once apoptosis occurs. From another point of view, we proposed that at a survival state, regulation of MDM2 degradation becomes a fundamental issue and might be a much more critical step in survival maintenance. The parameters, which depict the effects of the p53-induced MDM2 expression, are considerably less sensitive (parameters 9 and 10) in both apoptosis inducing and survival groups.

Impact of parameter uncertainty on oscillation

We then applied the sampling-based method to explore the parametric effects on oscillation. Random parameter sets were generated and analyzed. For first two \( r_1 \) values (0.006, 0.007), although they do not reside in limit cycle regions, significant fractions show LC

![Figure 7. Mean parameter rankings. Parameter sets are divided into two groups: apoptotic group (leading to high p53 levels) and survival group (leading to low p53 levels). Parameters are ranked from 1 to 12, with 12 being the most sensitive. Triangle: apoptosis inducing group; square: survival group.](image-url)
oscillations (for non-oscillations, see figure S5, available at stacks.iop.org/PhysBio/7/036008/mmedia). In figure S6 (available at stacks.iop.org/PhysBio/7/036008/mmedia), as $r_1$ increases, initially the fraction that leads to oscillation increases and reaches the maximum around 0.009 (91.7 ± 1.3%). Then, the fraction of oscillations declines. Note that sustained oscillation preserves even $r_1$ reaches 0.02, which is far away from the limit cycle region (figure S3, available at stacks.iop.org/PhysBio/7/036008/mmedia). Simulations suggest that oscillating fractions have a local maximum as $r_1$ passes through the LC region.

We further performed relative sensitivity analysis (for the parameter number, see table 1). Basically, $r_1$ has a strong positive effect on the amplitude (parameter 1). $d_2$ also positively regulates the amplitude although less effective (parameter 8, figure S7(a) (available at stacks.iop.org/PhysBio/7/036008/mmedia) $V_m = 0.02$, $r_1 = 0.008$). The basal degradation for p53 (parameter 2) and basal production of MDM2 (parameter 7) both negatively regulate the amplitude. Positive feedback loops elicit a positive effect (parameters 3 and 4). Special attention should be paid to the parameter 4 ($k_1$). $k_1$ has negative correlation with positive feedback effects. That means increased $k_1$ decreases the amplitude, but simultaneously with reduced feedback strength, leading to the conclusion that positive feedback loops positively regulate the amplitude. Comparably, the processes of the p53-induced MDM2 expression (parameters 9 and 10) and MDM2-dependent p53 degradation (parameters 5 and 6) both have negative effects. For period sensitivity, p53 production (parameter 1) and MDM2 basal degradation (parameter 8) elicit strong negative effects (figure S7(b) available at stacks.iop.org/PhysBio/7/036008/mmedia). Other parametric effects can be assessed as above. Together, simulations highlight that p53 upregulation and stabilization negatively regulate period and positively regulate the amplitude (parameters 1–6). But functions of MDM2 regulatory processes diverge in amplitude and period regulation, although only basal production and degradation of MDM2 always play essential roles. As local sensitivity only records the slope of global sensitivity curves at origin, we further performed wide range parameter analysis (figure S8, available at stacks.iop.org/PhysBio/7/036008/mmedia). We found that most local effects (figure S7, available at stacks.iop.org/PhysBio/7/036008/mmedia) are qualitatively consistent with the global effect (figure S8, available at stacks.iop.org/PhysBio/7/036008/mmedia). However, the inconsistencies reveal that there exists substantial deficiency in simply calculating relative sensitivity (i.e. the relatively sensitivity measures are not robust under stochastic fluctuations). Therefore, these results again demonstrate that formulating a mathematical framework which is robust in the presence of stochasticity (e.g. the methodology regarding potential construction [29, 32]) is strongly needed.

Finally, a sensitivity measure based on Shannon’s entropy was used. MII is based on random sampling; thus, it will be more informative in characterizing environmental noises. Comparing MIIs in figures 8(a) and (b), we found that the amplitude seems to be more sensitive to parameter variation. Amplitude and period MIIs were then summed. We found that amplitude summation (0.5185) is greater than period summation (0.3749). Results above suggest that there exists much stronger mutual dependence between total parameters and amplitude; in other words, the amplitude is more sensitive to total parameter variations and much more variable than the period. Furthermore, plotting sensitivity measures against each other enables us to evaluate the oscillation properties that are more significantly influenced by parameter perturbation (figure 8(c)). The parameters below the diagonal primarily impact the period. Some parameters reside in the vicinity of the diagonal, while only $r_1$ has a strong period bias. Four parameters predominantly impact the amplitude (figure 8(c)). Results again imply that total parameters are biased toward changing amplitude. Therefore, we proposed that ‘variable-amplitude, concise-period’ might be a design principle in our characterized genetic circuits. Geva-Zatorsky et al [16] found that amplitudes showed more variations, while periods are more concise. Proctor et al [19], Cai et al [59], Jolma et al [60] and Ouattara et al [20] recently identified the high variations...
in amplitude independently. We demonstrated that global parameter uncertainty toward amplitude regulation might be another source.

**Conclusion and outlook**

We developed a minimal model for the p53 system and investigated its bifurcation and parameter property. We first identified irradiation dichotomy in p53 models and further proposed that bistability and oscillation could collaborate well. Furthermore, we found that the period is more concise than the amplitude. The ‘variable-amplitude, concise-period’ oscillator might be inherent properties in systems with similar structure described here. Also, our model and related analyses could be further extrapolated to other signaling networks with similar structures. With experimental data coming in torrents, a detailed dynamic model will finally achieve its goal, in quantitative terms, to describe the dynamic system behavior of the p53 system.

**Acknowledgments**

This work is supported by the National Natural Science Foundation of China (project nos 30870588 and 30821006) and the National Basic Research Program of China (973 program, no 2006CB910103).

**References**

[5] Honda R and Yasuda H 2000 Activity of MDM2, a ubiquitin ligase, toward p53 or itself is dependent on the ring finger domain of the ligase *Oncogene* 19 1473–6


