Supporting Text

Stochastic Process of Double Strand Break (DSB) Generation and Repair. In eukaryotic cells, DSBs trigger two major repair mechanisms: homologous recombination (HR) and nonhomologous end joining (NHEJ) (1, 2). Although the NHEJ and HR pathways predominate in different stages of the mammalian cell cycle and may use independent repair enzymes (3), it has been reported that the DNA complex formed by DSB and repair proteins Mre11, Rad50, and NBS1 activates ATM upon IR in both the NHEJ and HR repair of DNA DSBs. Our model describing DSB repair is based on the experimental evidence and the assumptions put forward in the two lesion kinetics (TLK) model (4) that radiation creates both rapidly and slowly repaired DSBs. In the TLK model, the fast dynamics refers to repair of simple DSBs, envisioned as a section of the DNA 10 - 20 bp in length with a break in each strand of the DNA. The slower dynamics, on the other hand, refers to the repair of complex DSBs, envisioned as a simple DSB that contains additional elementary damage sites (base damage, strand breaks, base deletion, etc.) within the same section of DNA. Fig. 9 (extracted from Fig. 2) illustrates our implementation of the TLK model, which differs from the original TLK model on two important counts. First, our model explicitly includes the formation of DSB-repair enzyme complexes. Second, there is a finite supply of repair enzymes, and these are treated as dynamic variables. The latter feature of our model is necessary if we consider high radiation doses when the number of unrepaired DSBs is much greater than the number of available repair enzymes. Each of the fast and slow pathways in the model contains a first-order reaction (represented by the rates k_{fb1} and k_{fb2} in Fig. 9) and a second-order repair process (represented by the rate k_{cross}). DSB repair is a first-order process if break ends associated with the same DSB are rejoined and a second-order process if the break ends associated with two different DSBs are involved in the repair event. In our model, we do not need to distinguish between correct repair and misrepair of DSBs (a distinction made in the original TLK model), even though this has profound consequences for the subsequent viability of the cell. Instead, we are only interested in the signaling from the DSB to the downstream p53-Mdm2 system through ATM, and this signal will subside whether the DSB was correctly or incorrectly repaired.

The two pathways envisioned in the TLK model should not be confused with the HR or the NHEJ repair pathways known to be essential for DSB repair in mammalian cells. In our model, HR is primarily responsible for the first-order repair process, whereas NHEJ is at work in the second-order component of our model, in which a DSB can be repaired (or misrepaired) by ligating one end of the break with the end of any other break. In our model, we assume that the DSB sites form complexes C_1 and C_2 with the same repair enzymes, and we have *RP* number of them. This is a simplifying assumption given that the HR and NHEJ pathways are known to use independent repair enzymes. However, it has been reported that the Rad50/NBS1/Mre11 nuclease complex plays an important role in both the NHEJ and HR repair of DNA DSBs (5).

Earlier studies suggest that ≈ 60-80% of DSBs are quickly rejoined, whereas the remaining 20-40% of DSBs rejoin more slowly (4), with the precise relative contributions of slow and fast processes depending on the cell type. In our simulations, it is assumed that 70% of the initial DSB yield is processed by fast repair. Previous fitting of the TLK model with experimental data indicated that the rates in fast kinetics are ≈ 6-40 times those in the slow kinetics (4, 6). For our simulations, the ratio of all the fast rates over their respective slow rates is chosen to be ≈10 ($k_{fb1}/k_{fb2} = k_{rb1}/k_{rb2} = k_{fix1}/k_{fix2} = 10$). We also assume that the binding of repair proteins to DSBs is much faster than the process of lesion repair (e.g., $k_{fb1}/k_{fix1} = k_{fb2}/k_{fix2} = 5\text{-}10$). Once these ratios are chosen, the remaining parameters are adjusted so that the downstream trends evolved from DSBs in our simulations agree with those obtained from the experimental observations in Lahav *et al.*'s (7) work. The parameters used in the simulations reported in the main paper are listed in Table SM1. The simulation time step Δt is set to be 10^{-3} min, 25 times smaller than the fastest time scale in the repair process given by $k_{fb1}RP$ (0.025 min).

Given the small number of DSBs generated for typical radiation doses and the relatively small number of repair proteins, the DNA repair process was simulated stochastically. In the stochastic representation of the DNA repair dynamics, each locus in which a DSB is created can be in one of three states corresponding to intact DSB (state 1), DSB in

complex with repair proteins (state 2), and (correctly or incorrectly) fixed DSB (state 3). At time step k, the number of DSBs in states 1, 2, and 3 are represented by $D(k)$, $C(k)$, and $F(k)$, respectively. By using subscripts '1' and '2' to differentiate simple DSBs (fast kinetics) and complex DSBs (slow kinetics), we have $D(k) = D_1(k) + D_2(k)$, $C(k) = C_1(k) + C_2(k)$ and $F(k) = F_1(k) + F_2(k)$. We will assume that we have a fixed total number RP_T of repair proteins, or more precisely, at most RP_T number of DSB foci that can be repaired simultaneously. At any time, a variable RP out of RP_T number of repair proteins are free to bind to DSBs. The Monte Carlo algorithm for the evolution of DSBs induced by an irradiation dosage of x Gy during time $[0, t_T]$ is as follows:

1. Set the initial conditions. Set $t = 0$. The initial number D_T of total DSBs is generated from a Poisson distribution with mean value $35x$. This assumes that on average 1 Gy produces 35 DSBs per cell. The initial split of DSBs into simple and complex breaks is $D_{T1} = 0.7D_T$, and $D_{T2} = 0.3D_T$. Initially, all the DSBs are in state 1, that is for $t = 0$, we set $D_1(0) = D_{T1}$, $D_2(0) = D_{T2}$ and $C_1(0) = C_2(0) = F_1(0) = F_2(0) = 0$. We choose the number of total repair proteins to be $RP_T = 20$. Given that initially all repair proteins are free, the simulation starts with RP=20.
2. Increment time. Set $t = t + \Delta t$. Let $k = t/\Delta t$.
3. Update the states for each of the D_{T1} damages sites controlled by fast repair. Compute the transition probabilities as follows: from state 1 to state 2, $P_{D1\to C1} = RP\left[k_{\text{fb1}} + k_{\text{cross}}(D_1(k-1) + D_2(k-1))\right]\Delta t$; from state 2 to state 1, $P_{C1\to D1} = k_{\text{rb1}}\Delta t$; and from state 2 to state 3, $P_{C1\to F1} = k_{\text{fix1}}\Delta t$. For each damage locus i, with $1 \le i \le D_{T1}$, draw a value X from a uniform distribution with support between 0 and 1. If the damage at locus i is in state 1, a transition to state 2 occurs if $0 \le X < P_{D1\to C1}$, while it stays in state 1 if $P_{D1\to C1} \le X \le 1$. If the damage is in state 2, a transition to state 1 occurs if $0 \le X < P_{C1\to D1}$, or a transition to state 3 occurs if $P_{C1\to D1} \le X < P_{C1\to D1} + P_{C1\to F1}$, or no transition occurs if $P_{C1\to D1} + P_{C1\to F1} \le X \le 1$. If the damage is in state 3, it stays in state 3 (i.e., state 3 is absorbing). Set $RP = RP - 1$ if transition from state 1 to state 2 occurs; set $RP = RP + 1$ if transition from state 2 to state 1 occurs; otherwise RP remains the same. When the last damage site $i = D_{T1}$ has

been updated, count the number of fast repaired breaks at time t in states 1, 2, and 3 to be $D_1(k)$, $C_1(k)$, and $F_1(k)$, respectively.

4. Update the states for each of the D_{T2} damages sites controlled by slow repair. Compute the transition probabilities as follows: from state 1 to state 2, $P_{D2\to C2} = RP[k_{\text{fb2}} + k_{\text{cross}}(D_1(k-1) + D_2(k-1))]\Delta t$; from state 2 to state 1, $P_{C2\to D2} = k_{\text{rb2}}\Delta t$; and from state 2 to state 3, $P_{C2\to F2} = k_{\text{fix2}}\Delta t$. For each damage locus j, with $1 \le j \le D_{T2,}$ draw a value X from a uniform distribution with support between 0 and 1. If the damage at locus j is in state 1, a transition to state 2 occurs if $0 \le X < P_{D2\to C2}$, while it stays in state 1 if $RP > 0$ or $P_{D2\to C2} \le X \le 1$. If the damage is in state 2, a transition to state 1 occurs if $0 \le X < P_{C2\to D2}$, or a transition to state 3 occurs if $P_{C2\to D2} \le X < P_{C2\to D2} + P_{C2\to F2}$, or no transition occurs if $P_{C2\to D2} + P_{C2\to F2} \le X \le 1$. If the damage is in state 3, it stays in state 3 (i.e., state 3 is absorbing). Set $RP = RP - 1$ if transition from state 1 to state 2 occurs; set $RP = RP + 1$ if transition from state 2 to state 1 occurs; otherwise RP remains the same. When the last damage site $j = D_{T2}$ has been updated, count the number of slowly repaired breaks at time t in states 1, 2, and 3 to be $D_2(k)$, $C_2(k)$ and $F_2(k)$, respectively.
5. Let $D(k) = D_1(k) + D_2(k)$, $C(k) = C_1(k) + C_2(k)$, and $F(k) = F_1(k) + F_2(k)$.
6. Repeat steps 2-5 until $t = t_T$.

Typical results of these stochastic simulations are shown in Fig. 5.

Equations for the ATM Activation Module. The processes of autophosphorylation of ATM and the activation of ATM by signaling of DSBs in complex with repair proteins are schematized in Fig. 10. Its implementation in terms of ODEs is:

$$\begin{aligned}
\frac{d[\text{ATM}_\text{D}]}{dt} &= \frac{1}{2}k_{\text{dim}}[\text{ATM}]^2 - k_{\text{un dim}}[\text{ATM}_\text{D}] \\
\frac{\text{d}[\text{ATM}]}{\text{dt}} &= 2k_{\text{un dim}}[\text{ATM}_\text{D}] - k_{\text{dim}}[\text{ATM}]^2 - k_{\text{af}}\,f(C,[\text{ATM}^*])[\text{ATM}] + k_{\text{ar}}[\text{ATM}^*] \qquad \textbf{[1]} \\
\frac{\text{d}[\text{ATM}^*]}{\text{dt}} &= k_{\text{af}}\,f(C,[\text{ATM}^*])[\text{ATM}] - k_{\text{ar}}[\text{ATM}^*]
\end{aligned}$$

where [ATM], [ATM*], and [ATM$_D$] represent the concentrations of ATM, active ATM, and ATM dimer molecules, respectively; C counts the number of DSBs in complex with repair proteins (obtained from the simulations described above); k_{dim} and k_{undim} are the ATM dimerization and the undimerization rates, respectively; k_{ar} is the ATM inactivation rate; and k_{af} is a parameter representing the strength of ATM activation per unit time. The reactions of dimerization, undimerization, and dephosphorylation follow the principle of mass action. Because the precise mechanism of activation of ATM is not well understood, we model ATM activation with a simple function $f(C, [\mathrm{ATM}^*]) = (\alpha_1 C + \alpha_2 C\ [\mathrm{ATM}^*] + \alpha_3 [\mathrm{ATM}^*])$ that captures some of the observed dependencies. The functional form of f (C, [ATM*]) was chosen because of its parsimonious simplicity (it is a simple bilinear relation) and in an attempt to interpret mathematically previous qualitative experimental results. For example, the term α_1C is conjectured from the results of ref. 8, which suggests that DSBs must somehow activate ATM molecules at a distance, given that the fast activation of many ATM molecules by only a few DSBs seems inconsistent with the possibility that ATM has to bind directly to DSBs to become activated. This mechanism may possibly be initiated by chromatin restructuring (8), produced by phosphorylation of the minor histone H2AX over a region of 2 Mbp proximal to the DSB (9). Furthermore, it has also been reported that the MRN complex, which plays a central role in DSB repair both in HR and NHEJ, stimulates the kinase activity of ATM toward its targets (10) and is required to activate ATM by DNA damage (11). The third term $\alpha_3[\mathrm{ATM}^*]$ expresses the mechanism of autophosphorylation of ATM (8). The cross term $\alpha_2 C[\mathrm{ATM}^*]$ indicates an interaction between the DSB-repair protein complexes and activated ATM, suggested by the appearance of localized foci of ATM* around the repair complexes (8). The observed foci of active ATM are consistent with a diffuse activation of ATM and migration of a fraction of ATM* protein to the sites of DNA strand breaks introduced by IR, presumably to phosphorylate substrates at the breaks, possibly including ATM itself.

The functional dependence between the number of DSB bound complexes and [ATM*] at steady state can be solved analytically. By setting the right-hand side of any two of the three differential equations in Eq. **1** to be zero and using the conservation equation

$2[\mathrm{ATM}_D]+[\mathrm{ATM}]+[\mathrm{ATM}^*]=[\mathrm{ATM}^T]$, where $[\mathrm{ATM}^T]$ is the total concentration of all forms of ATM molecules, we obtain ATM^* as a function of *C*. The result is a lengthy formula, which we omit here.

In ref. 8 it was shown that the activation of ATM saturated about 5-10 min after irradiation. Comparing this time scale to the kinetics of downstream oscillations with a period > 400 min, the reactions of the ATM module are extremely fast. Accordingly, the rate constants for ATM phosphorylation were chosen to be ≈ 50 times the rate of oscillations (Table 1).

Equations for the p53-Mdm2 Oscillator Module. The schematic diagram of the p53-Mdm2 oscillator is illustrated in Fig. 11 (the same as Fig. 4). The p53 protein is translated from *p53* mRNA and is inactive for transactivation of its targets unless phosphorylated by ATM^*. In its active state, $\mathrm{p53}^*$ transcribes *Mdm2* mRNA in a process that involves a time delay. *Mdm2* also has a constant basal transcription rate, accounting for the p53-independent promoter (12). Mdm2 protein promotes a fast degradation of p53 and a slow degradation of $\mathrm{p53}^*$. Mdm2 has a basal degradation rate, but in the presence of active ATM, Mdm2 degradation is further stimulated. The equations used in the paper to model the p53-Mdm2 oscillatory module of Fig. 11 are

$$\frac{d[p53]}{dt}=s_{\mathrm{p53}}-\delta_{\mathrm{p53}}[p53]$$

$$\frac{d[Mdm2]}{dt}=s_{\mathrm{Mdm2}}+\varepsilon_{\mathrm{Mdm2}}\frac{[\mathrm{P53}^*(t-\tau_1)]^n}{[\mathrm{P53}^*(t-\tau_1)]^n+K^n}-\delta_{\mathrm{Mdm2}}[Mdm2]$$

$$\frac{d[\mathrm{P53}]}{dt}=r_{\mathrm{P53}}[p53]-\mu_{\mathrm{P53}}[\mathrm{P53}]-\nu_{\mathrm{P53}}[\mathrm{MDM2}]\frac{[\mathrm{P53}]}{[\mathrm{P53}]+K_{\mathrm{d}}}+k_{\mathrm{rp}}[\mathrm{P53}^*]-k_{\mathrm{fp}}[\mathrm{ATM}^*]\frac{[\mathrm{P53}]}{[\mathrm{P53}]+K_{\mathrm{p}}}$$

$$\frac{d[\mathrm{P53}^*]}{dt}=k_{\mathrm{fp}}[\mathrm{ATM}^*]\frac{[\mathrm{P53}]}{[\mathrm{P53}]+K_{\mathrm{p}}}-k_{\mathrm{rp}}[\mathrm{P53}^*]-\nu_{\mathrm{P53}^*}[\mathrm{MDM2}]\frac{[\mathrm{P53}^*]}{[\mathrm{P53}^*]+K_{\mathrm{d}}^*}$$

$$\frac{d[\mathrm{MDM2}]}{dt}=r_{\mathrm{MDM2}}[Mdm2(t-\tau_2)]-(\mu_{\mathrm{MDM2}}+(\nu_{\mathrm{MDM2}}-\mu_{\mathrm{MDM2}})\frac{[\mathrm{ATM}^*]}{[\mathrm{ATM}^*]+K_{\mathrm{a}}})[\mathrm{MDM2}]$$

In writing these equations, we have modified slightly our notation of the gene products to avoid confusion. In the equations above, [P53] and [MDM2] represent protein concentrations of p53 and of Mdm2, respectively, and [*p53*] and [*Mdm2*] represent their respective mRNA concentrations. We will discuss the meaning and estimates of the parameters below. The actual parameter values used in the simulation can be found in Table 1. An in-depth analysis of this set of equations as a dynamical system can be found in ref. 13. Here we emphasize only a few key aspects of the model:

(A) The transcription rate of *Mdm2* mRNA activated by $p53^*$ depends on the concentration of $p53^*$ at time $t-\tau_1$. The rate of production of nuclear Mdm2 depends on the concentration of *Mdm2* mRNA at time $t-\tau_2$. The delays τ_1 and τ_2 are convenient representations of the time required for some processes that we have omitted in our model. Delay τ_1 corresponds to the transcription and splicing processes of the *Mdm2* gene into mature *Mdm2* mRNA, while τ_2 corresponds to the translocation of *Mdm2* mRNA to the cytosol, the translation of *Mdm2* mRNA into Mdm2 protein, and the transport of Mdm2 back to the nucleus to become nuclear Mdm2 at time *t,* Mdm2(*t*). It is this Mdm2(*t*) that interacts with p53(*t*), tagging it for degradation. But it is the p53 τ_1 units of time earlier, p53($t-\tau_1$), that promoted the transcription of *Mdm2*, and *Mdm2* τ_2 units of time earlier, *Mdm2*($t-\tau_2$), that gets transported out of the nucleus, translated, and translocated back to the nucleus. In other words, the transcriptional and translational/translocation time delays are the price we have to pay in order to reduce the dimensionality of the problem and describe only nuclear concentrations. Note that these time delays are incorporated based on the assumption that there is no degradation during the delayed processes. We estimate the transcriptional time delay τ_1 to be 30 min based on the assumption that elongation rate is 20 nucleotides per second (25 min of elongation) (14, 15) and that splicing rate is 25 seconds per intron (5 min of splicing) (16). The translational/translocation delay τ_2 is assumed to be 10 min assuming a translation rate of four amino acids per second (2 min of translation) (16) and nuclear import and export time of 4 min each (16, 17).

(B) The transcription rate of *Mdm2* mRNA is an *n*th order Hill function of $p53^*(t-\tau_1)$. The Hill coefficient *n* is chosen to be 4 to account for the cooperativity of the tetrameric form of $p53^*$ as a transcription factor (18).

(C) The ratio between the maximal $p53^*$-activated *Mdm2* transcription rate ε_{Mdm2} and the basal *Mdm2* transcription rate s_{Mdm2} is set to be around 10 (12).

(D) The degradation of both p53 and $p53^*$ promoted by Mdm2 are described by Michaelis-Menten kinetics because the catalytic role of Mdm2 as an ubiquitin ligase in this process is similar to the role played by an enzyme in the conversion of a substrate to a product. Previous experiments indicate that ubiquitination of p53 may follow alternative pathways depending on the concentration of Mdm2, with p53 being polyubiquitinated and subsequently degraded in the nucleus for high levels of Mdm2, whereas low levels of Mdm2 activity induce monoubiquitination and nuclear export of p53 (19). After p53 is phosphorylated at serine 15, the interaction between p53* and Mdm2 is reduced drastically (20), and the effective activity of Mdm2 experienced by p53* is lower than that experienced by p53. Therefore, the results on the control of mono- versus polyubiquitination of p53 by Mdm2 (19) suggest that p53* and p53 may follow different degradation pathways, with p53* having a lower degradation rate compared to p53, probably due to a slower polyubiquitination pace in face of inhibited interaction with Mdm2 (19, 21). Therefore, we assume that the binding affinity between Mdm2 and $p53^*$ is 10-fold less than that between Mdm2 and p53 (20, 22) and that the Mdm2-mediated degradation rate of $p53^*$ is 5-fold less than that of p53. Specifically, $\nu_{p53*}/\nu_{p53} = 0.2$ and $K_d^*/K_d = 10$.

(E) The phosphorylation of p53 catalyzed by ATM^* is modeled as a first-order Michaelis-Menten mechanism.

(F) The degradation rate of Mdm2 is composed of a basal self-degradation rate μ_{MDM2} and an ATM*-dependent accelerated rate of maximal value $\nu_{MDM2.}$ The ratio ν_{MDM2}/μ_{MDM2} is assumed to be 5-fold, in the spirit of the experimentally measured reduction of half-life of Mdm2 in DNA damaged cells (23).

(G) With respect to the remaining model parameters, the decay rates of proteins and mRNA are set to be in the range of 0.02-0.008 min^{-1} under basal conditions,

corresponding to ~35- to 87-min half-lives (24, 25). The reactions of p53 phosphorylation and dephosphorylation are assumed to be about 10 times faster than other reactions of transcription, translation and degradation in this module. Notice that the transcription rate of *p53* mRNA is invariant, leading to a constant mRNA steady state as well as to a constant protein synthesis rate. This is consistent with previous modeling and experimental work (25, 26).

Measurement and Calibration of p53 Basal Concentration. Various cell lines growing logarithmically were collected, counted, and lysed in cell extraction buffer (10mM Tris, pH7.4/100 mM NaCl/1 mM EDTA/1 mM EGTA/1 mM NaF/20 mM Na4P2O7/2 mM Na3VO4/1% Triton X-100/10% glycerol/0.1% SDS/0.5% deoxycholate/1 mM PMSF/ protease inhibitor cocktail). The basal concentration of p53 was measured using human p53 Elisa kit (Biosource, Camarillo, CA) according to the manufacturer's instruction. In brief, cell lysis of each cell line was added to a microtiter well precoated with human p53 antibody and incubated at room temperature for 2 hours. The well was then washed thoroughly with wash buffer followed by incubation with rabbit anti-p53 antibody at room temperature for another 1 hour. After washing with wash buffer, anti rabbit-HRP working conjugate was added to each well and incubated at room temperature for 30 minutes. The well was washed with wash buffer, and stabilized chromogen was added to each well for 30 minutes in dark to produce the color; the intensity of this colored product is directly proportional to the concentration of p53 present in the original sample. After adding stop solution to each well, the absorbance at 450 nm was read by Wallac Victor 3 multilabel counter (PerkinElmer), and the concentration of p53 of each cell line was read from a standard curve plotted by p53 standard with known concentration. The p53 concentration of each cell line was measured 3 times. As shown in Fig. 12, the p53 concentrations in various cell lines range from 2.1-19.57 $\times 10^4$ molecules per cell. Specifically, the basal p53 concentration in MCF7 cell is 15.84 $\times 10^4$ molecules per cell.

Assuming that basal p53 molecules are uniformly distributed in a spherical cellular volume of radius 5-10 μm, we estimate the molar concentration of basal p53 in MCF7 cell to be between 0.06 and 0.5 μM.

Cooperativity of the Modeled ATM Module in Comparison with Actual Data. As mentioned in the main text, the mechanisms of phosphorylation of ATM, and in particular its detailed dependence on IR dose, has not been characterized to a high level of quantitative detail, and even less so in single cell experiments. Our present understanding of the speed of ATM activation and its IR dependence comes fundamentally from immunoblotting studies from the work of Bakkenist and Kastan (8). The two key plots in ref. 8 in relation to the present work is reproduced in Fig. 13 here. A rather abrupt onset of activated ATM that starts at 0.1 Gy and saturates at ≈ 0.4 Gy can be easily inferred from Fig. 13. In the same figure, we plot in a similar grayscale format the data from our model, which shows the qualitatively resemblance between the actual data and our model prediction, although the latter seems to be less cooperative than the experimental data. For example, our model has not yet saturated at 0.4 Gy, which may indicate that the Hill coefficient of 2 yielded by our model underestimates the actual cooperativity in the real system.

Characterization of the Pulses Arising from the Model and Comparison with Experiments. As discussed in the main text, the time taken by the DNA repair mechanism to fix enough DSBs and reduce its number to a value smaller than the threshold of ATM activation is a fluctuating quantity that varies from cell to cell. Consequently, the time when p53 and Mdm2 stop oscillating, as well as the number of pulses, fluctuate. Fig. 14 shows three simulation results representing three single cells, for which the same set of parameters were used. The resulting response exhibits either one (Fig. 14*A*), two (Fig. 14*B*), or three (Fig. 14*C*) pulses of p53. Notice that the last pulse in the oscillation series may be cut off and its peak and period become smaller if ATM is turned off before the last cycle is complete. This variability in amplitude and width is within the range of stochasticity observed in single cells (7). Fig. 14*D*, adapted from ref. 7, shows the case of the experimental single cell response at the same IR dose of 5 Gy as in the simulations. A comparison between Fig. 14 *B* and *D* shows a similarity in behavior between the model and the corresponding experimental results. In order to test system response to step input of DNA damage simulating the effect of continual presence of

DNA damage agent such as MMC, a constant input of DSBs causing continual activation of ATM is applied. Not surprisingly, the output is sustained oscillations with constant amplitude and period (Fig. 14).

Besides the number of pulses at a given radiation dose, there are two other features of the pulses that are worth studying: the period and the amplitude. The periods of the first and second pulses are shown in Figs. 15 *A* and *B*, respectively (diamonds). These figures show the same constant trend with respect to changes of IR dose as the equivalent results in the single cell experiments of Lahav *et al.* (7) (squares). Fig. 15 *C* and *D* shows the amplitude of first and second pulse, respectively, as a function of IR dose. The ordinates in the figure represent the percentage of the pulse in reference to the average amplitude of the respective pulse. The solid diamonds are the simulation results, and the squares are the experimental data reproduced from ref. 7. The trends are qualitatively similar.

Role of the ATM-Dependent Autodegradation of Mdm2, and the Transcriptional and Translational/Translocation Time Delays. In separate work (13), we have studied the role of the time delays as a central component of the p53-Mdm2 self-regulatory model in relation to the ability of the system to sustain stable oscillations. The time delays in the p53-Mdm2 module represent the time taken by a number of processes not explicitly considered in the model, such as transcription, splicing, translation, and transport to and from the nucleus. In this section, we present some results on the stability of oscillations in relation to the time delay and the role of the ATM^*-dependent degradation of Mdm2.

Simulations show that if we do not incorporate ATM^*-induced self-degradation of Mdm2 (in other words, if we "cut the wire" connecting ATM^* and Mdm2 in Fig. 11), the system cannot sustain stable oscillations but settles at stable equilibrium in response to IR no matter how big the transcriptional time delay τ_1 and the translational/translocation time delay τ_2 are (data not shown). However, the model proposed in the present paper does include the experimentally observed ATM^*-stimulated Mdm2 degradation. This additional link allows for the existence of sustained oscillations if the total explicit time

delay $\tau = \tau_1 + \tau_2$ is above a threshold of around 16 min (Fig. 16*A*). Note that, mathematically, it is the total amount of delay τ that determines the existence of oscillation; that is, the system dynamics does not differentiate whether τ appears in the transcriptional delay or in the translational/translocation delay (13).

It is therefore clear that time delays play a central role in the maintenance of oscillations. A generic, intuitive reason for the importance of time delays in negative feedback systems is that a time delay can destabilize what would otherwise be a stable fixed point. In a linear system, this creates a divergent behavior. In a nonlinear system, this divergence can be stabilized by nonlinearities, generating, as is the case in our oscillator, a limit cycle. The period of this limit cycle in the p53-Mdm2 oscillator of this paper is shown in Fig. 16*B* as a function of the total time delay τ and for several IR doses. The period of the oscillations follows a linear trend with the total time delay and is relatively insensitive to the irradiation dose. Notice that even though there is a direct relationship between the time delay and the period of oscillations, the time delay is not equal to the period. Rather, the period of oscillations is about one order of magnitude larger that the time delay, reaching for a delay of 40 min the period of around 420 min observed in the actual p53-Mdm2 system.

ATM Cooperativity and Hopf Bifurcation as a Strategy for Threshold Detection. In order to arrest the cell cycle in response to a small number of DSBs, it is desirable for the system to be able to start its response abruptly after a minimum threshold of DSBs is reached. When studying the p53-Mdm2 module as a dynamical system (13), we observed that increasing the ATM^* level produces an abrupt (but continuous) onset of oscillations (Fig. 17*A*). In dynamical systems parlance, the system undergoes a supercritical Hopf bifurcation in which a single stable solution turns unstable (specifically at the solid red circle in Fig. 17*A*), giving rise to a limit cycle (i.e., stable oscillatory behavior). The abrupt onset suggests a possible role of this instability as a threshold sensor.

To investigate the potential role of this threshold detection behavior, we have quantified the global input-to-output response (that is, the IR-p53 response) of the complete model.

The stochastic aspects have been removed by adding a deterministic function for the number of DSBCs per unit of IR. This function could be interpreted as the maximum number of DSBs induced for a given IR level. We then modeled the transfer function that determines the fraction of ATM^* as a function of the number of DSBCs with a Hill function of Hill coefficient n_H. This is necessary because the exact cooperativity of the ATM transduction system has not yet been quantified, and several mechanisms could potentially generate high cooperativity. The ATM^* signal is then used as input to the p53-Mdm2 oscillator, and the output is plotted as a function of IR dose. To measure and compare two qualitatively different dynamics of equilibrium point and limit cycle, we define the output quantity as Δp53, the normalized difference between maximum and minimum p53 levels. The correlation results are illustrated in Fig. 17*B* for n_H at values 1, 2, 4, and 8, showing switching behavior of the output. When low cooperativity is assumed for the ATM activation ($n_H = 1$), the threshold behavior produced by the Hopf bifurcation exhibits an abrupt onset of oscillations at 0.2 Gy. However, the size of the oscillation does not saturate until 10 Gy. Because activation of ATM is assumed to be more cooperative, the transition occurs at slightly higher IR doses but the transition becomes sharper. These results show that a steeply cooperative activation function of ATM can produce a system that transits very abruptly through the Hopf bifurcation. As a result, the whole model behavior of ATM and the p53-Mdm2 oscillator closely resembles an on-off switch with an abrupt onset that quickly reaches the saturation level (for $n_H > 1$). Hence, once the IR dose is sufficiently large to produce oscillations, the amplitude of the oscillations is essentially fixed. This mechanism presents a biologically plausible framework for understanding the basis of the observed digital behavior.

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