SI Appendix

Table of Contents

Modeling the integrated cell cycle and DNA damage network

Model construction

The integrated model is comprised of sub-models for the cell cycle and the DNA damage response. The DNA damage signaling model consists of 72 state variables and 135 nonzero parameters, while the cell cycle model and its mechanisms of arrest consist of 31 state variables and 85 nonzero parameters. Both are ordinary differential equation models that describe rates of change of concentrations of proteins and other species resulting from chemical reaction processes. Models are available for download from the PNAS website (SI MATLAB code).

The cell cycle model is based on a recently published model by Tyson and colleagues (1). In the original model, it was assumed that a variety of molecular species react quickly and are at quasi-steady state. Such species are represented by algebraic rather than differential equations. In order to incorporate interactions between these species and the DNA damage model, we expanded the algebraic relations into dynamical equations. In addition, we added protein synthesis and degradation reactions for each species in the model to simulate the decrease in protein synthesis caused by cycloheximide treatment, an experiment that helped define the restriction point in mammalian cells (2). These changes affect the equations governing the dynamics of the model species `Wee1`, `Cdc25C`, `TFE` (E2F1), `TFB` (Mcm), and `APC` (model species are named according to the Tyson model). We ensured that the model's freely cycling dynamics were unchanged by incorporating synthesis and degradation of each cell cycle protein, and by treating variables dynamically rather than at steady state. These changes were essential for

simulating arrest, as this process requires dynamical interaction with cell cycle proteins, and can connect to the cell cycle by reducing protein synthesis rates.

The Tyson model links cell cycle progression to cell size by setting cyclin synthesis rates proportional to an exponentially increasing cell mass (1). While size dependent progression through the cell cycle has been shown in yeast, the existence of such a mechanism in mammalian cells is not well understood (3). In addition, the regulation of cell growth during arrest is poorly understood, and initial simulations of cell cycle arrest without incorporating such regulation resulted in growth to unphysical cell sizes (data not shown). We therefore define a mitogen parameter, M, representing the intracellular signal resulting from extracellular growth factors present in the medium. We model this mitogen parameter's effect as increasing the synthesis rates of `CycD` and `CycE` transcripts, replacing their dependence on cell size. For cell cycle simulations we set M = 1.8 unless otherwise indicated, a value that leads to cyclin synthesis rates comparable to those attained with the original model.

The topology of the DNA damage model was derived from the model of Batchelor *et al*., in which oscillations are driven by a combination of two negative feedback loops: the core p53-Mdm2 loop and a loop in which the upstream checkpoint kinases are inhibited by a p53-inducible gene product, the phosphatase Wip1 [11]. To provide an extensible framework for future modeling of the DNA damage network, we incorporated additional feedback loops involving p53 [14] in our model (Fig. S1A shows a detailed network diagram). With the current parameterization, however, these loops do not significantly affect the network's dynamics.

Modeling cyclin knockouts and the restriction point

To test the model's ability to match known experimental results for mammalian cells, we simulated serum starvation and cycloheximide treatment. To do the former, we modeled an immediate, step-like decrease in *M* from 1.8 to 1 at different times during a normal cell cycle (Fig. S1B,B'). These simulations recapitulate the existence of a restriction point: if *M* is decreased within 3 hours of cell division, cells arrest immediately in G1, while for later serum removal times, a full cell cycle is completed before the cell arrests. Cycloheximide treatment is simulated by a decrease in all protein synthesis rates by 20% at various times, leading to similar results (Fig. S2C,C'). For cyclin D and E knockout simulations, the synthesis rates and initial conditions of these species were set to zero. For $M = 2$, the cyclin $E^{-/-}$ model still cycles, albeit more slowly (Fig. S2D). At this mitogen level, however, the cyclin $D^{-/-}$ model undergoes G1 arrest. The cyclin $D^{-/-}$ model only cycles for values of $M > 10$, consistent with the qualitative observation that cyclin $D^{-/-}$ cells have an increased mitogen requirement for proliferation (Fig. S2E).

Modeling cell cycle arrest

In addition to the cell cycle arrest mechanisms described in detail in the main text, other interactions leading to arrest have been described (for example (4, 5)). To ensure that the interactions we consider are representative of the space of possible G1 and G2 arrest mechanisms, we systematically applied arrest by stoichiometric inhibition, enzymatic inactivation or transcriptional repression to all species of the cell cycle model.

Modeling arrest mechanism biochemistry

We modeled cell cycle arrest using three distinct biochemical interactions: protein inactivation by inhibitor binding, protein inactivation by enzymatic phosphorylation, and a decrease in protein level by repression. In the first of these, species x_i is inhibited by the inhibitor I through reactions modeling inhibitor binding formation of the complex c, as well as the degradation of each constituent species (Equation 1). Any additional species-specific terms governing the dynamics of x_i are kept, and the protein's specific degradation term, denoted by $V_{D,i}$, is assumed to act on both the bound and unbound species. We assume the inhibitor is upregulated in proportion to the level of tetrameric nuclear p53.

$$\begin{aligned} \dot{x}_i &= (\text{other terms}) - k_f x_i I + k_r c - V_{D,i} x_i + k_d I \\ \dot{I} &= k_s p53_{4np}(t) - k_d I + V_{D,i} x_i \\ c &= k_f x_i I - k_r c - V_{D,i} c - k_d I \end{aligned} \tag{1}$$

We model enzymatic phosphorylation by a Michaelis-Menten rate law in which species x_i is modified to an inactive phosphorylated form x_i^P by phospho-Chk2 (Equation 2). The phosphorylated form is assumed to degrade at the same rate as the unmodified form.

$$\begin{aligned} \dot{x}_i &= (\text{other terms}) - k_{cat} Chk2_p(t) \frac{x_i}{K_m + x_i} - V_{D,i} x_i \\ \dot{x}_i^P &= k_{cat} Chk2_p(t) \frac{x_i}{K_m + x_i} - V_{D,i} x_i^P \end{aligned} \tag{2}$$

Finally, protein synthesis inhibition was modeled through a repressive saturation term dependent on tetrameric nuclear p53 (Equation 3). This term modulates the protein's usual production rate, denoted by k_{si}.

$$\dot{x}_i = k_{si} \frac{K_D}{K_D + p53_{4np}(t)} + (\text{other terms}) \tag{3}$$

To vary arrest strength as shown in Figs. 2 and S3, we varied the values of k_s, k_{cat}, and K_D in the equations for the three interactions. The initial value of each was set at the limit required for cell cycle arrest, and varied to two orders of magnitude in the direction of stronger arrest.

Analysis of individual arrest mechanisms

To obtain a comprehensive picture of how cell cycle arrest could be achieved by different biochemical implementations and cell cycle targets, we inhibited each cell cycle protein individually by binding, by phosphorylation and by transcriptional repression. The results of this analysis are shown in Fig. S1F. We find that cyclin profiles can be grouped into different types of arrest: mechanism I-like G1 arrest with high G1 cyclins and low G2 cylins; mechanism II-like G2 arrest with high G2 cyclins and low G1 cyclins; mechanism III-like G2 arrest with low G2 cyclins and high G1 cyclins; M-phase arrest characterized by high cyclin B but low cyclins A and E (6); and finally S-phase arrest characterized by high cyclin A but low cyclins E and B. All of the resulting arrests fall into the three classes of G1 and G2 arrest described in the main text, with the exception of interactions leading to arrest in M- or S-phase, which are less prominent in response to DSBs. Inhibiting some species by any of the three mechanisms did not lead to arrest; these species are not pictured. We find that different choices of biochemical implementation frequently lead to qualitatively similar arrest phenotypes, and that inhibiting proteins at similar points in the cell cycle (e.g. `CycD` and `CycE`; `CycA` and `TFE`; `APC` and `Cdc20`) also leads to similar arrests profiles. Thus, the cell cycle arrest mechanisms discussed in the main text, implemented *via* canonical targets and

biochemical interactions, are representative of larger subsets of targets and interactions that lead to similar arrest states (e.g. (4, 5)).

Model simulation details

Computing cell cycle transitions

For all freely cycling simulations (Fig. 1B and during each optimization run), the G1/S, S/G2, and M/G1 transitions were identified as the first peak times of cyclin E (`CycE`), phosphorylated cyclin B (`pB`) and APC^{Cdc20A} (`Cdc20A`). However, during cell cycle arrest simulations, cyclin trajectories might not peak, or peaks might be significantly lower than during the corresponding free cycling simulation. For arrest simulations, the G1/S transition was defined as the time at which `CycE` reached 90% of its maximal free cycling value. Similarly M/G1 transitions were defined as times at which `Cdc20A` reached 80% of its maximal value. For arrest simulations, we assume that S phase is of fixed duration, equal to the duration in freely cycling cells.

Simulating DNA damage and measuring arrest steady states

We modeled the induction of DNA damage as the presence of an input *S*, representing DSBs as well as accessory proteins such as the MRN complex assembled at the site of damage. This input was modeled as capable of binding inactive ATM_2 dimers and catalyzing their phosphorylation and disassociation. At the time of damage t_D, *S* was set to a value of 1, while at the time of recovery from arrest t_R, *S* was returned to a value of 0. The dynamics of p53, Wip1, and Chk2 cause cyclin levels to oscillate with low amplitude during arrest (Fig. 1C-F). In generating Fig. 2, we computed an approximate steady state by averaging cyclin levels from 180-200 h after constant application of the damage stimulus that causes p53 oscillations. We confirmed that the averages were

unchanged compared to the preceding interval from 160-180 h, indicating that steady state has been reached.

Simulating populations of cells (Figs. 4, S4, and S5)

The distribution of cell ages in a free cycling population is expected to follow the distribution given by Equation 8. Thus, for Figs. 4C,D, 5A, and S8D, 500 cells were simulated with initial ages (and corresponding initial conditions) sampled from this distribution. Damage was induced by applying the damage stimulus to all cells at $t = 0$. To account for damage repair, we implemented a simple model of the stochastic repair of DNA damage (7), parameterized using values found in literature, and measured the distribution of repair times (Fig. S4A). We modeled the initial damage distribution in each cell to be Poisson distributed with a mean of 25 DSBs per Gy (8) and a distribution of 80% easy and 20% difficult breaks. Simple breaks were repaired with a half-life of 15 min, while difficult breaks were repaired with a half-life of 10 h (9, 10). We assumed cell cycle reentry occurs when fewer than three breaks remain (7). Fig. S4B shows the distribution of repair times from 500 simulations of this stochastic process as computed by independent runs of the Gillespie algorithm (11), compared to a Gaussian distribution with mean 35 h and standard deviation 10 h. Despite presumed completion of DNA repair, $p53^{+/+}$ cells remain arrested for the duration of our experiments. We model this process as sustained activation of p53-dependent arrest mechanisms.

Analyzing experimental data for fitting

Quantifying the fold-change in cyclin levels during arrest

Two types of flow cytometry measurements were used to train the model to HCT $p53^{+/+}$ and $p53^{-/-}$ data: (i) the fraction of cells in G1, S, and G2, and (ii) the distribution of cyclin B and E levels during G2 arrest.

To extract typical cyclin E and B levels reached during arrest, we fit log-normal distributions to the data of Fig. 3 at each time point (Fig. S3). A single log-normal distribution was fit to the unimodal G2 cyclin E levels at each time point. G1 cyclin E levels were bimodal, possibly indicating that early-G1 and late-G1 subpopulations vary in cyclin E levels; for these data, a sum of two log-normal distributions was used. The G2 cyclin B1 data was fit by a sum of three log-normal distributions.

We measured the fold change of cyclin E levels after G2 arrest by computing the ratio of the mean of the log-normal cyclin E distribution in arrested G2 cells to the mean of the high cyclin E distribution in freely cycling G1 cells. Similarly, we computed the fold change in cyclin B1 levels from the means of the most populated G2 cyclin B1 peak during arrest to the mean of the highest peak in freely cycling G2 cells. In each case, the mean intensity of the appropriate isotype control was subtracted from each measurement to remove background fluorescence. To capture the levels attained during each cell line's arrest, we performed this analysis at the 48 h time point for HCT $p53^{+/+}$ cells, and the 24 h time point for HCT $p53^{-/-}$ cells (as many $p53^{-/-}$ cells have re-entered the cell cycle at later times).

Quantifying the time spent by HCT cells in G1, S, and G2/M

To measure the amount of time spent by HCT $p53^{+/+}$ and $p53^{-/-}$ cells in G1, S, and G2/M, we assumed that unirradiated cell populations at 0 h are completely asynchronous, and that they maintain a stationary distribution across cell cycle phases. However, because cells undergoing mitosis divide into two daughter cells, the observed distribution does not directly reflect the amount of time spent by an individual cell in each cell cycle phase. Instead, young cells will be over-represented relative to older cells. To properly account for this bias towards observing young cells during proliferation, we derived an analytical distribution of cell age in a stationary population (similar derivations can be found in (12)).

We considered a model in which cells progress through N states before dividing, and all transitions between states occur at the rate a. We represented this process as the linear system of ordinary differential equations (ODEs) shown in Equation 4, where the factor of 2 arises from a parent cell dividing into two daughters.

$$\begin{aligned} \dot{X}_1 &= 2aX_N - aX_1 \\ &\vdots \\ \dot{X}_i &= aX_{i-1} - aX_i \\ &\vdots \\ \dot{X}_N &= aX_{N-1} - aX_N \end{aligned} \qquad \textbf{(4)}$$

While the total number of cells in such a model diverge, the fractions of cells in each phase, computed by normalizing to the total population $X_T = \sum_{i=1}^{N} X_i$, approach a steady state. We denoted these fractions as state variables $x_i = X_i / X_T$. Taking derivatives of x_i terms, we defined a new system of nonlinear ODEs (Equation 5).

$$\begin{aligned}
\dot{x}_1 &= 2ax_N - ax_1 - ax_1x_N \\
&\vdots \\
\dot{x}_i &= ax_{i-1} - ax_i - ax_ix_N \\
&\vdots \\
\dot{x}_N &= ax_{N-1} - ax_N - ax_N^2
\end{aligned} \tag{5}$$

This system was analytically solved at steady state to obtain expressions for each species x_i, and resulted in an exponential distribution (Equation 6).

$$\begin{aligned}
x_i &= x_1 \cdot 2^{-\frac{i-1}{N}} \\
x_1 &= 2\left(1 - 2^{-\frac{1}{N}}\right)
\end{aligned} \tag{6}$$

For the linear transitions in the model of Equation 4, the mean time spent in each state X_i is given by $\tau_i = \frac{1}{a}$, and the total mean cell cycle period is $T = \frac{N}{a}$. From these results we defined the probability distribution of mean cell age $p_N[t]$ in terms of the cell population x_i.

$$\begin{aligned}
p_N\left[t = \frac{i}{a}\right] &= x_i \\
p_N[t] &= p_0 \cdot 2^{\frac{1-at}{N}}
\end{aligned} \tag{7}$$

In the limit of large N, the distributions of ages in each stage become arbitrarily narrow around each state's mean lifetime, representing a deterministic cell spending a fixed amount of time in each stage. In this limit, the distribution of cell ages becomes continuous in time. We non-dimensionalized this distribution by normalizing the age

variable to the total period (i.e. defining $\tau = \frac{a}{N}t$), so that the probability distribution of cell ages in the free cycling population can be written as shown in Equation 8.

$$p_\infty(\tau) = 2\log 2 \cdot 2^{-\tau} \qquad \tau \in [0,1] \tag{8}$$

From this distribution and the relationships of Equation 8, we solved for the time spent in G1, S and G2/M by an individual cell given any observed distribution of cells in these phases.

$$\%\mathrm{G1} = \int_0^{\tau_1} p(\tau)d\tau = 2\left(1 - 2^{-\tau_1}\right)$$
$$\%\mathrm{G2/M} = \int_{1-\tau_2}^{1} p(\tau)d\tau = 2^{\tau_2} - 1 \tag{9}$$

We obtained the fraction of cells in each cell cycle phase from the measured DNA profiles by fitting distributions to a modified Dean-Jett model (13) (see Methods), and computing the number of cells in each fit population. The measured population data, and its conversion to the time spent in each phase, is reproduced in Figure S3C.

Fitting the model to data

Mathematical notation for fitting and sensitivities

Our model takes the form of a system of coupled ODEs that depends on initial conditions $\mathbf{x}_0$, the time *t*, the current value of all state variables $\mathbf{x}$, parameters $\mathbf{p}$, and inputs $\mathbf{u}$.

$$\dot{\mathbf{x}} = \mathbf{f}(t, \mathbf{x}, \mathbf{p}, \mathbf{u})$$
$$\mathbf{x}(0) = \mathbf{x}_0 \tag{10}$$

We define a model output of interest y (*i.e.*, the sum of all cyclin E or B species, active cyclin E or B, or Cdc20A) as some linear combination of state variables, formed by multiplication of the row vector $\mathbf{c}_y^T$ with the state vector $\boldsymbol{x}$. All the outputs we consider in this study are listed in Table S1.

$$y = \mathbf{c}_y^T \mathbf{x} \tag{11}$$

Fitting procedure details

We performed a local optimization procedure on the cell cycle arrest model. The aim of this procedure was to find a set of parameters that would allow our fitted model to simultaneously match: (i) the percentage of time spent in each cell cycle phase by undamaged, freely cycling HCT $p53^{+/+}$ and $p53^{-/-}$ cells, (ii) the ratio of steady state cyclin E and cyclin B1 arrest levels to their maximal freely cycling levels, (iii) the requirement for cyclin $E^{-/-}$ and cyclin $D^{-/-}$ cells to still cycle (14, 15), and (iv) the existence of a restriction point after serum starvation and cycloheximide treatment (2). In our fitting procedure, all parameters in the cell cycle model and arrest mechanisms I-III were allowed to vary.

For fitting, we treated p53 and Chk2 as constant-level inputs to the cell cycle model (see Table S1 for a list of all model inputs and the values taken). $p53^{+/+}$ cells were assumed to arrest with a combination of arrest mechanisms I, II and III, while $p53^{-/-}$ cells were assumed to arrest by mechanism II alone. For the cell cycle model to account for differences in the length of cell cycle phases between cell lines, we assumed that a basal level of p53 activation is responsible for some p21 activation in unirradiated $p53^{+/+}$ cells. This might be due to low levels of damage incurred during the normal cell cycle, or to

p53's involvement in cell cycle checkpoints in undamaged, freely cycling cells. All other parameters were shared by both cell lines. Because the total time scale of all simulations was left unconstrained by data, this degree of freedom in parameter space was removed by assuming a total cell cycle period of 30 h for wild-type ($p53^{+/+}$) cells. This constraint was applied by scaling all parameters that include units of time, and otherwise left all trajectories unchanged.

At each parameterization during fitting, we ran eight independent simulations, shown in Table S2. These simulations allow comparison to restriction point and cyclin knockout results, as well as the free cycling and steady state data collected for HCT $p53^{+/+}$ and $p53^{-/-}$ cells. All simulations were run to a final time $t_F = 200$ h, or until 3 cell cycles were completed. We computed various quantities from these simulations, where superscripts on these quantities indicate the simulations from which they are computed (Table S2). $\tau_{G1}^{(i)}$ and $\tau_{G2}^{(i)}$ represent the fraction of the cell cycle spent in G1 (2*N* DNA content) and G2/M (4*N* DNA content). Because a normal cell cycle is split only between G1, S, and G2/M, the fraction of time spent in S is completely specified by these two times. The $r_y^{(i,j)}$ terms represent the ratios of model outputs during arrest to those attained during normal cell cycling, and is computed as shown in Equation 12.

$$r_y^{(i,j)} = \frac{\mathbf{c}_y^T \cdot \mathbf{x}^{(i)}\left(t_F\right)}{\mathbf{c}_y^T \cdot \mathbf{x}^{(j)}\left(t_y^*\right)} \tag{12}$$

$T^{(i)}$ is the total cell cycle period, and is computed by measuring the time between mitoses. Finally, the degree to which steady state is achieved by the final simulation time is measured by the norm of the time derivative at this time, $\left\|\dot{\mathbf{x}}^{(i)}\left(t_F\right)\right\|^2$.

To measure the deviation of these quantities from their experimental values, we evaluated an objective function, shown in Equation 13. Each line of the objective function represents terms that are computed after the corresponding simulation.

$$
\begin{aligned}
O(\mathbf{p}) = 100 \cdot & \left(\left(\tau_{G1}^{(1)} - \overline{\tau}_{G1}^{(1)} \right)^2 + \left(\tau_{G2}^{(1)} - \overline{\tau}_{G2}^{(1)} \right)^2 \right) \\
& + \left(r_{CycE_T}^{(2,1)} - \overline{r}_{CycE_T}^{(2,1)} \right)^2 + \left(r_{CycB_T}^{(2,1)} - \overline{r}_{CycB_T}^{(2,1)} \right)^2 + 10^3 \left\| \dot{\mathbf{x}}_{ss}^{(2)} \right\|^2 \\
& + 100 \cdot \left(\left(\tau_{G1}^{(3)} - \overline{\tau}_{G1}^{(3)} \right)^2 + \left(\tau_{G2}^{(3)} - \overline{\tau}_{G2}^{(3)} \right)^2 \right) \\
& + \left(r_{CycE_T}^{(4,3)} - \overline{r}_{CycE_T}^{(4,3)} \right)^2 + \left(r_{CycB_T}^{(4,3)} - \overline{r}_{CycB_T}^{(4,3)} \right)^2 + 10^3 \left\| \dot{\mathbf{x}}_{ss}^{(4)} \right\|^2 \\
& + 5 \cdot \theta \left(T^{(5)}, T^{(1)} \right) \\
& + 5 \cdot \theta \left(T^{(6)}, T^{(1)} \right) \\
& + \theta \left(0.5, r_{CycE_T}^{(7,3)} \right) + 10^3 \left\| \dot{\mathbf{x}}_{ss}^{(7)} \right\|^2 \\
& + \theta \left(0.5, r_{CycE_T}^{(8,3)} \right) + \theta \left(r_{CycE}^{(8,3)}, 0.5 \right) + \theta \left(0.9, r_{Cdc20\ A}^{(8,3)} \right) + 10^3 \left\| \dot{\mathbf{x}}_{ss}^{(8)} \right\|^2
\end{aligned}
\tag{13}
$$

This objective takes the form of a weighted sum of squared errors, where individual terms are scaled so that their contributions are of comparable magnitude. Terms containing the function $\theta(a,b)$, defined in Equation 14, only penalize for deviation in one direction.

$$
\theta(a,b) = \begin{cases} 0 & a < b \\ (a-b)^2 & a \geq b \end{cases} \tag{14}
$$

For instance, the term $5 \cdot \theta \left(T^{(5)}, T^{(1)} \right)$ only contributes to the objective function if the period of the cyclin $D^{-/-}$ simulation is longer than that of the normal cell cycle. If computing this objective function was impossible at any parameter set during fitting (for example, if arrested cells failed to arrest at all), the objective function value was set to an arbitrary high number.

After fitting, model parameters had changed from the initial parameterization by no more than roughly an order of magnitude above and below between the initial model parameterization, with no parameters reaching upper or lower bounds (Fig. S5A).

Efficient timing sensitivities for fitting

An adjoint method was used to compute the objective function's parameter sensitivities in a computationally efficient manner (16). For this method to be applied, the partial derivatives $\frac{\partial O}{\partial \mathbf{x}}(t)$ and $\frac{\partial O}{\partial \mathbf{p}}(t)$ must be computed. Because many terms of the objective function involve the times at which the sum of certain species is maximal, we solved for the sensitivities to parameters of these times. We define the times of maxima, t_y^*, as times at which the slope of output *y* is zero, and its second derivative is negative. Mathematically, this is written by the two conditions of Equation 15.

$$\begin{aligned} \mathbf{c}_y^T \cdot \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right) &= 0 \\ \mathbf{c}_y^T \cdot \nabla_{\mathbf{x}} \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right) \cdot \mathbf{c}_y &< 0 \end{aligned} \tag{15}$$

Differentiating Equation 15 and using the chain rule, we derived an expression for the sensitivity of the timing of the i^{th} species' extremum (Equation 16; a similar result is found in Equation 18 of Rand *et al*. (17)).

$$\frac{dt_y^*}{d\mathbf{p}} = -\frac{\mathbf{c}_y^T \cdot \nabla_{\mathbf{x}} \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right)}{\left(\mathbf{c}_y^T \cdot \nabla_{\mathbf{x}} \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right)\right) \cdot \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right)} \frac{\partial \mathbf{x}}{\partial \mathbf{p}} - \frac{\mathbf{c}_y^T \cdot \nabla_{\mathbf{p}} \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right)}{\left(\mathbf{c}_y^T \cdot \nabla_{\mathbf{x}} \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right)\right) \cdot \mathbf{f}\left(t_y^*, \mathbf{x}, \mathbf{p}\right)} \tag{16}$$

Notably, terms in this equation can be grouped into the form of Equation 17. This form permits the application of the adjoint sensitivity method without ever computing $\frac{\partial \mathbf{x}}{\partial \mathbf{p}}$, by providing the partial derivatives $\frac{\partial t_i^*}{\partial \mathbf{x}}$ and $\frac{\partial t_i^*}{\partial \mathbf{p}}$.

$$\frac{dt_y^*}{d\mathbf{p}} = \frac{\partial t_y^*}{\partial \mathbf{x}} \frac{\partial \mathbf{x}}{\partial \mathbf{p}} + \frac{\partial t_y^*}{\partial \mathbf{p}} \tag{17}$$

Fitted model validation

We varied individual parameters in our fitted model to identify parameters that, when varied individually, remained poorly constrained by our data and fitting procedure. We also investigated the fitted model's ability to predict experimental results, including the population-averaged levels of cell cycle species after irradiation and the results of various knockout experiments in freely cycling and arrested scenarios.

Sensitivity analysis of fitted model

To address whether or not the fitting procedure described above is able to constrain the value of individual parameters, we computed the second derivatives of the objective function with respect to each parameter by taking finite differences of the adjoint sensitivity (described above) and used these values to estimate the curvature of the local minimum obtained by fitting. This procedure does not account for any additional parameter identification provided by constraints satisfied at the fit (e.g. the requirement of cyclin knockout cells to cycle). To map these entries to actual changes in parameters, we used the curvature to estimate the change in each parameter required to increase the objective function by a value of 0.1 from the minimum (Equation 18).

$$O(p) \approx O(p^*) + \frac{1}{2}\left.\frac{d^2O}{d(\log p)^2}\right|_{p^*}\left(\log\frac{p}{p^*}\right)^2$$
$$\frac{p}{p^*} = \exp\left(\sqrt{2(0.1)\left(\frac{d^2O}{d(\log p)^2}\right)^{-1}}\right) \quad \textbf{(18)}$$

The results of this analysis are shown in Fig. S5B. We find that many parameters, when varied individually, strongly affect the objective function value. Eleven parameters are sufficiently unconstrained that they have to vary at least twofold to achieve a change in objective function value of 0.1 (Table S3). Many of these parameters are association rates between cyclins and either p21 or p27; binding of these species might be sufficiently tight such that the precise value of this association rate is unconstrained. In addition to the parameters pictured in Fig. S5B, this analysis correctly identified five parameters (kifi_p, kifib_pp, Jafi, kafi, Jifi) that are infinitely unconstrained. These are all parameters affecting TFI, a species that is disconnected from the rest of the cell cycle network in Tyson's mammalian cell parameterization.

Western blot predictions

To further validate the model fitted to the cell cycle timing and steady state arrest data, we simulated populations of $p53^{+/+}$ and $p53^{-/-}$ models initially distributed as a freely cycling population (see previous section) and computationally predicted the levels of different cell cycle proteins at times after the application of damage (Fig. S5C). These results were compared with Western blots of total protein levels from HCT $p53^{+/+}$ and $p53^{-/-}$ cell lines (Fig. S4C). We find similar qualitative trends between prediction and experiment, although some changes in protein levels are predicted to occur earlier than

seen experimentally (e.g. Cyclin A and B dynamics in HCT $p53^{-/-}$ cells; Cdc20 dynamics in HCT $p53^{+/+}$ cells).

Knockout cell line predictions

We also validated the model by predicting the results of experiments in which various cell cycle proteins might be targeted for silencing or knockout. As described earlier, the ability for cyclin D and E knockout cell lines to continue cycling was considered in Tyson's original model and used as part of the fitting process. To test the consequences of other deletion experiments, we computationally eliminated each cell cycle protein individually for low (M = 2) and high (M = 16) mitogen levels, and compared the simulation results to published experimental results obtained in mammalian systems. The results are shown in Table S4. We find that the model accurately reproduces most known phenotypes, with only two notable exceptions. Cyclin $A^{-/-}$ cells are predicted to cycle at both low and high mitogen levels, but experimentally cyclin A is found to be essential. This might be due to unmodeled functions of cyclin A in DNA replication, such as a basic requirement for initiating replication. The model predicts that $Wee1^{-/-}$ cells can still undergo cycling, although Wee1 deletion is embryonic lethal. However, this lethality is due to premature mitosis and subsequent cell death, indicating that cells lacking Wee1 continue cycling, albeit too rapidly. We also tested whether the model predicts normal arrest for knockout cell lines that are still able to cycle. All knockout cell lines are predicted to maintain normal arrest regulation except for the $Cdh1^{-/-}$ model, which arrests transiently by mechanism II but resumes cycling after repair, even in the continued presence of mechanisms I and III. This prediction is consistent with evidence that $Cdh1^{-/-}$

cells are able to cycle in the absence of damage, and are defective in maintaining cell cycle arrest after IR (18).

References

1. Csikasz-Nagy A, Battogtokh D, Chen KC, Novak B, & Tyson JJ (2006) Analysis of a generic model of eukaryotic cell-cycle regulation. *Biophys J* 90(12):4361-4379.
2. Zetterberg A & Larsson O (1985) Kinetic Analysis of Regulatory Events in G1 Leading to Proliferation or Quiescence of Swiss 3T3 Cells. *Proceedings of the National Academy of Sciences* 82(16):5365-5369.
3. Conlon I & Raff M (2003) Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression. *J Biol* 2(1):7.
4. Agami R & Bernards R (2000) Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage. *Cell* 102(1):55-66.
5. Rocha S, Martin AM, Meek DW, & Perkins ND (2003) p53 represses cyclin D1 transcription through down regulation of Bcl-3 and inducing increased association of the p52 NF-kappaB subunit with histone deacetylase 1. *Mol Cell Biol* 23(13):4713-4727.
6. Brito DA & Rieder CL (2006) Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. *Curr Biol* 16(12):1194-1200.
7. Ma L*, et al.* (2005) A plausible model for the digital response of p53 to DNA damage. *Proc Natl Acad Sci U S A* 102(40):14266-14271.
8. Cedervall B*, et al.* (1995) Methods for the quantification of DNA double-strand breaks determined from the distribution of DNA fragment sizes measured by pulsed-field gel electrophoresis. *Radiat Res* 143(1):8-16.
9. Frankenberg-Schwager M (1990) Induction, repair and biological relevance of radiation-induced DNA lesions in eukaryotic cells. *Radiat Environ Biophys* 29(4):273-292.
10. Stewart RD (2001) Two-lesion kinetic model of double-strand break rejoining and cell killing. *Radiat Res* 156(4):365-378.
11. Gillespie D (1977) Exact stochastic simulation of coupled chemical reactions. *J Phys Chem* 81(21):2340-2361.
12. Mitchison JM (1971) *The Biology of Cell Cycle* (University Press, Cambridge [Eng.]) p 313.
13. Dean PN & Jett JH (1974) Mathematical analysis of DNA distributions derived from flow microfluorometry. *J Cell Biol* 60(2):523-527.
14. Kozar K*, et al.* (2004) Mouse development and cell proliferation in the absence of D-cyclins. *Cell* 118(4):477-491.
15. Geng Y*, et al.* (2003) Cyclin E ablation in the mouse. *Cell* 114(4):431-443.

16. Cao Y, Li S, Petzold L, & Serban R (2003) Adjoint sensitivity analysis for differential-algebraic equations: the adjoint DAE system and its numerical solution. *SIAM Journal on Scientific Computing* 24:1076.
17. Rand DA, Shulgin BV, Salazar JD, & Millar AJ (2006) Uncovering the design principles of circadian clocks: mathematical analysis of flexibility and evolutionary goals. *J Theor Biol* 238(3):616-635.
18. Sudo T*, et al.* (2001) Activation of Cdh1-dependent APC is required for G1 cell cycle arrest and DNA damage-induced G2 checkpoint in vertebrate cells. *Embo J* 20(22):6499-6508.
19. Ramalingam S*, et al.* (2007) Quantitative assessment of the p53-Mdm2 feedback loop using protein lysate microarrays. *Cancer Res* 67(13):6247-6252.
20. Murphy M*, et al.* (1997) Delayed early embryonic lethality following disruption of the murine cyclin A2 gene. *Nat Genet* 15(1):83-86.
21. Brandeis M*, et al.* (1998) Cyclin B2-null mice develop normally and are fertile whereas cyclin B1-null mice die in utero. *Proc Natl Acad Sci U S A* 95(8):4344-4349.
22. Nakayama K*, et al.* (1996) Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* 85(5):707-720.
23. Li M, York JP, & Zhang P (2007) Loss of Cdc20 causes a securin-dependent metaphase arrest in two-cell mouse embryos. *Mol Cell Biol* 27(9):3481-3488.
24. Tominaga Y, Li C, Wang RH, & Deng CX (2006) Murine Wee1 plays a critical role in cell cycle regulation and pre-implantation stages of embryonic development. *Int J Biol Sci* 2(4):161-170.
25. Lindqvist A, Kallstrom H, Lundgren A, Barsoum E, & Rosenthal CK (2005) Cdc25B cooperates with Cdc25A to induce mitosis but has a unique role in activating cyclin B1-Cdk1 at the centrosome. *J Cell Biol* 171(1):35-45.
26. Wu L*, et al.* (2001) The E2F1-3 transcription factors are essential for cellular proliferation. *Nature* 414(6862):457-462.
27. Wirth KG*, et al.* (2004) Loss of the anaphase-promoting complex in quiescent cells causes unscheduled hepatocyte proliferation. *Genes Dev* 18(1):88-98.