Introduction to Systems Biology of Cancer
Lecture 4

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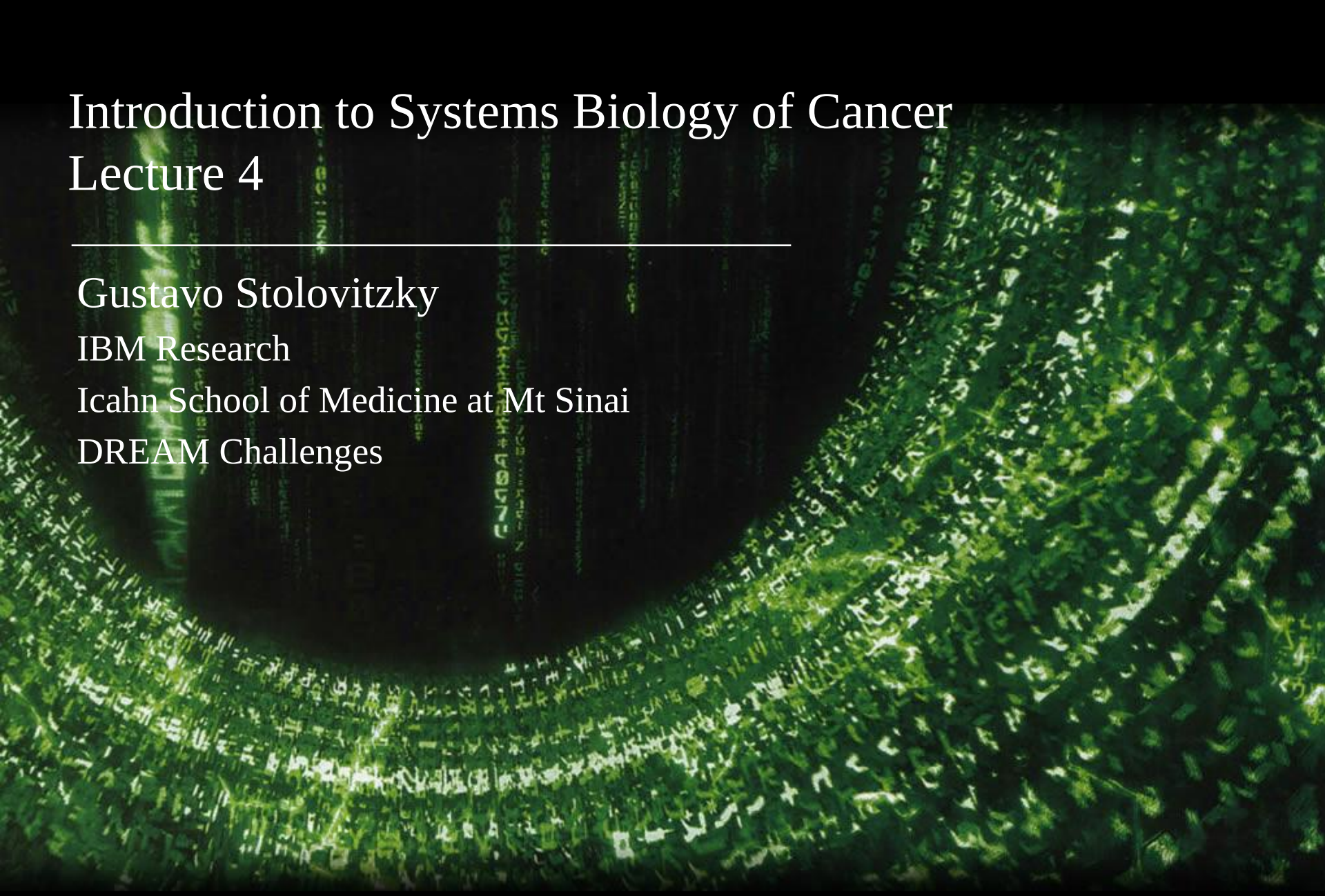
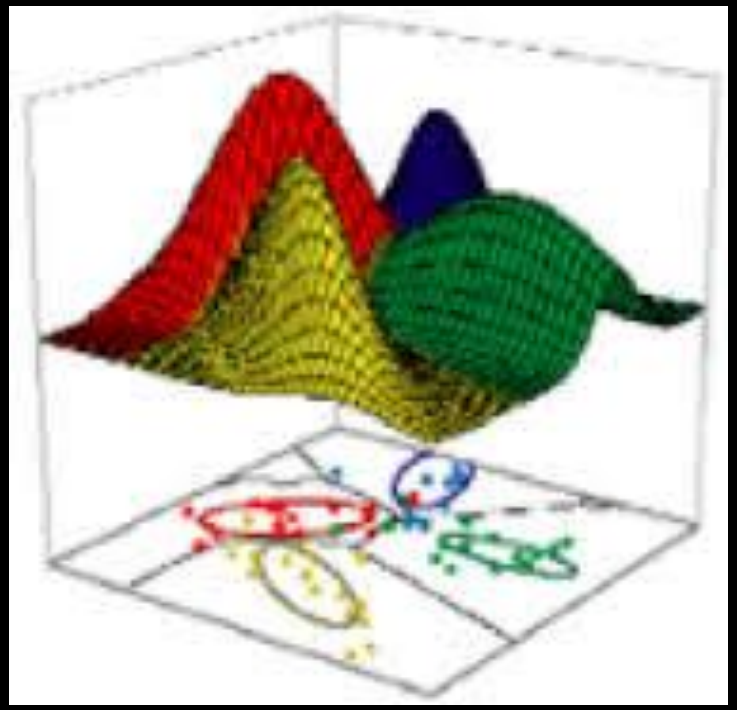
DREAM Challenges





Modeling in biological processes of relevance in cancer

Modeling

"… In that Empire the Art of Cartography was so Perfect that the map of a single province occupied a whole city, and the map of the Empire, a whole Province. With the passing of time, these Huge Maps wouldn't be enough and the Colleges of the Cartographers erected a Map of the Empire that equaled in width the Empire itself…"

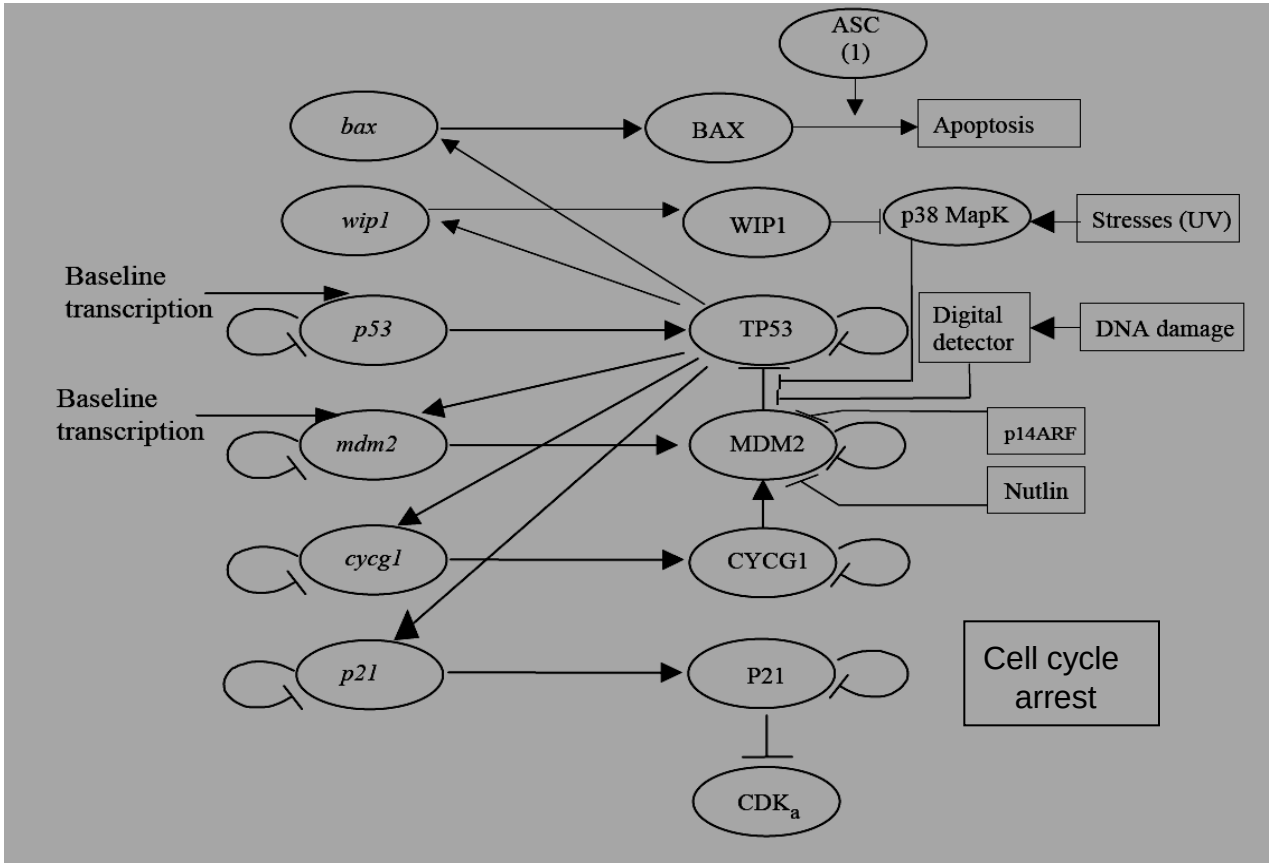
J.L. Borges: El Hacedor, 1960.

All models are wrong … but some are useful.

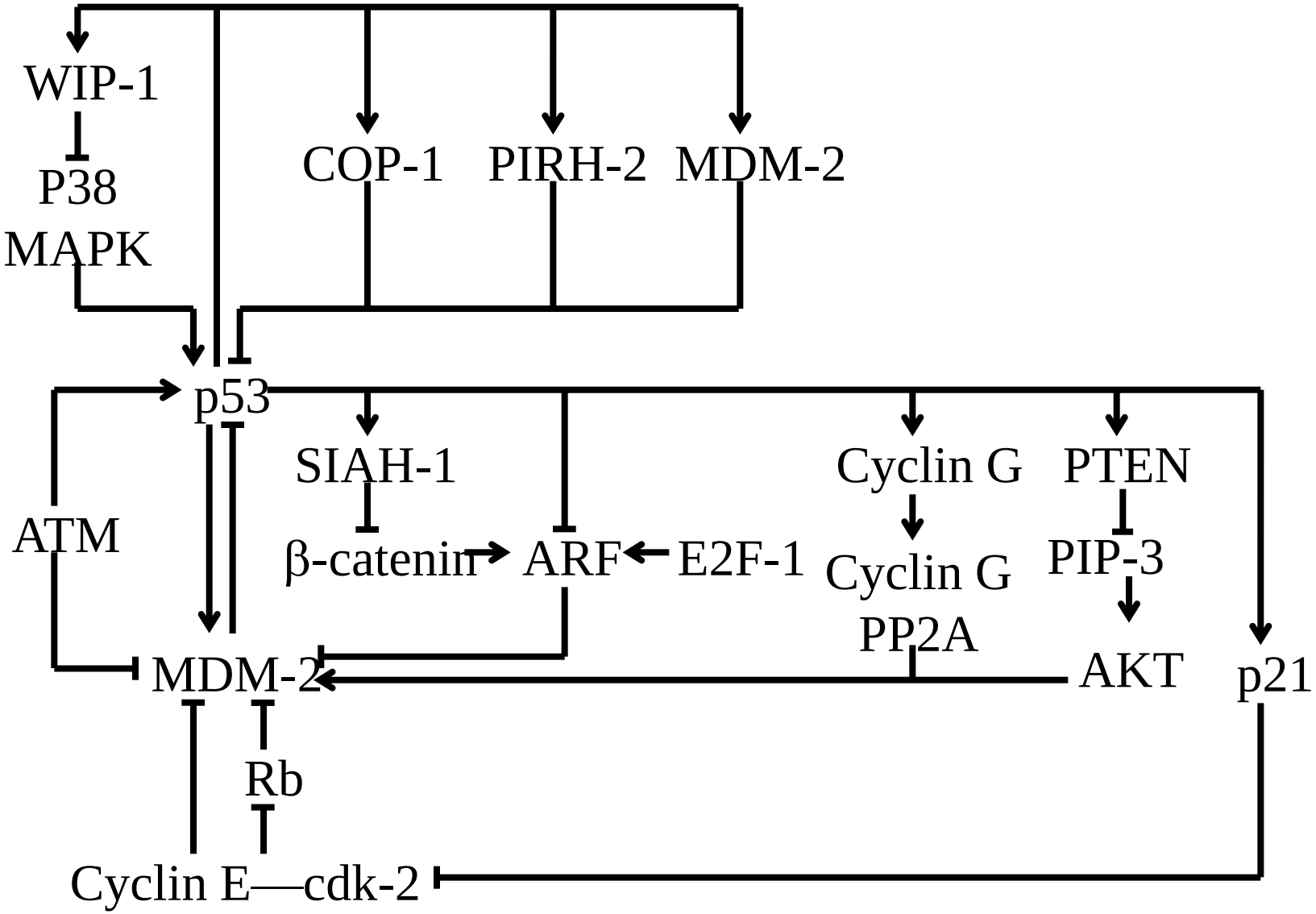
Attributed to George Box

Roles of p53

- Transcription factor
- Central role in defending genomic stability
- Decides on cell cycle arrest and apoptosis
- Implicated in over 50% of cancers

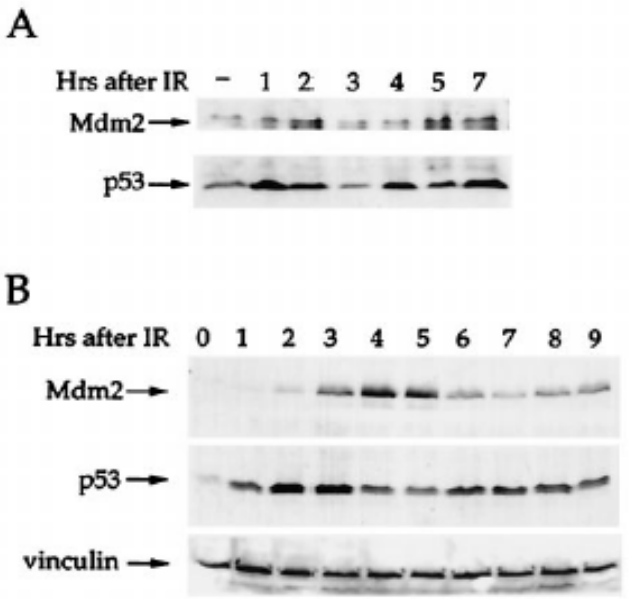


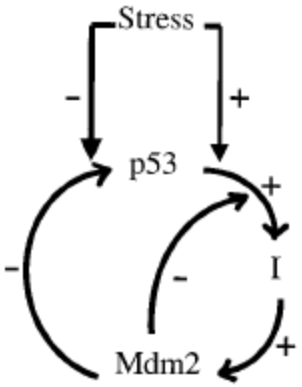
p53 is highly regulated

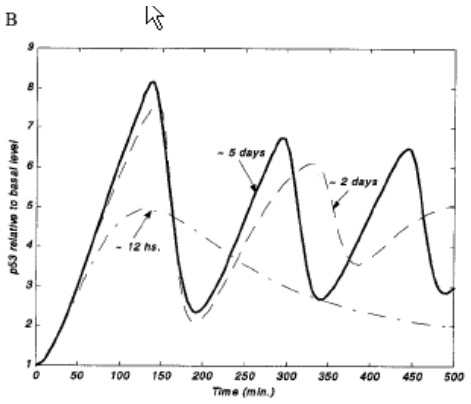


p53 – MDM2 auto-regulation

Protein level after irradiation (IR)



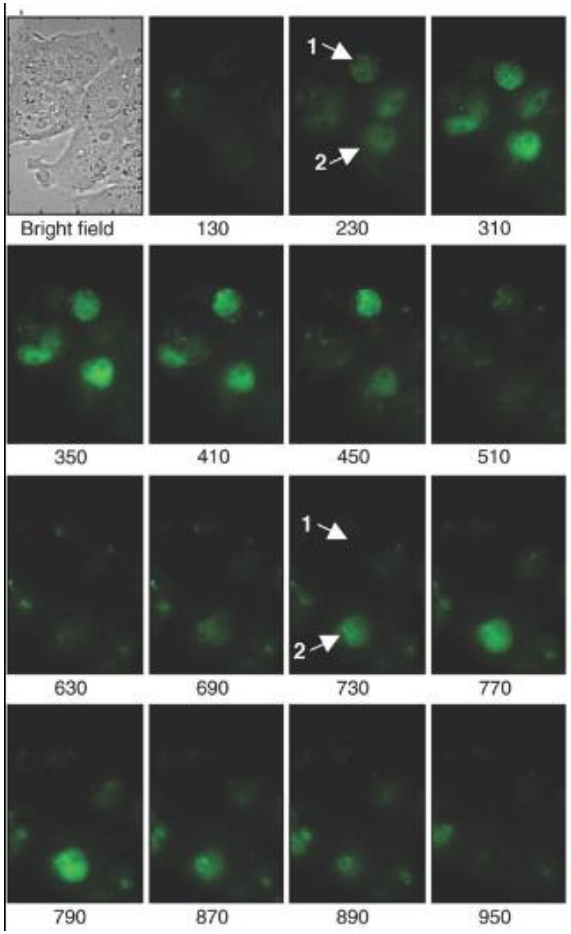




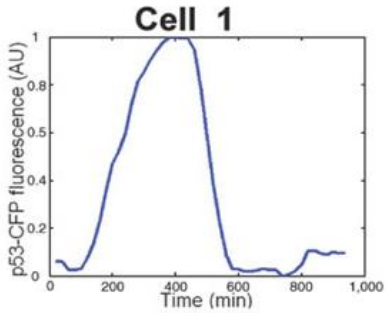
Bar-Or *et al., PNAS,* 2000

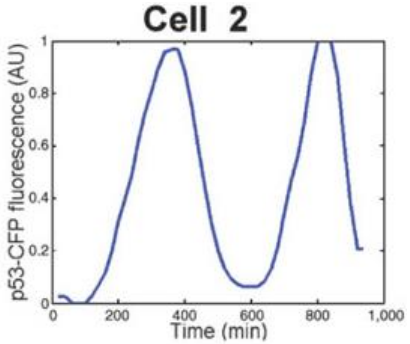
Reflects population but not single cells

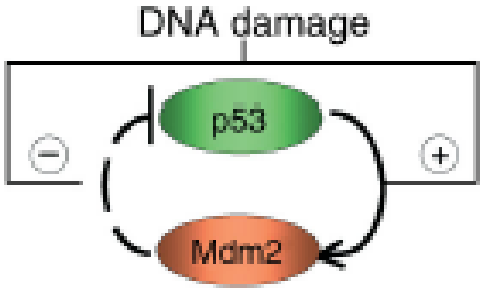
Digital Clock: individual cells



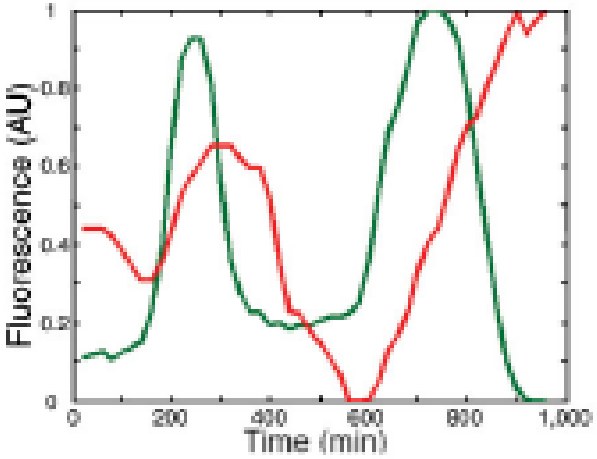
P53 protein







Coordinated oscillations of P53 and MDM2

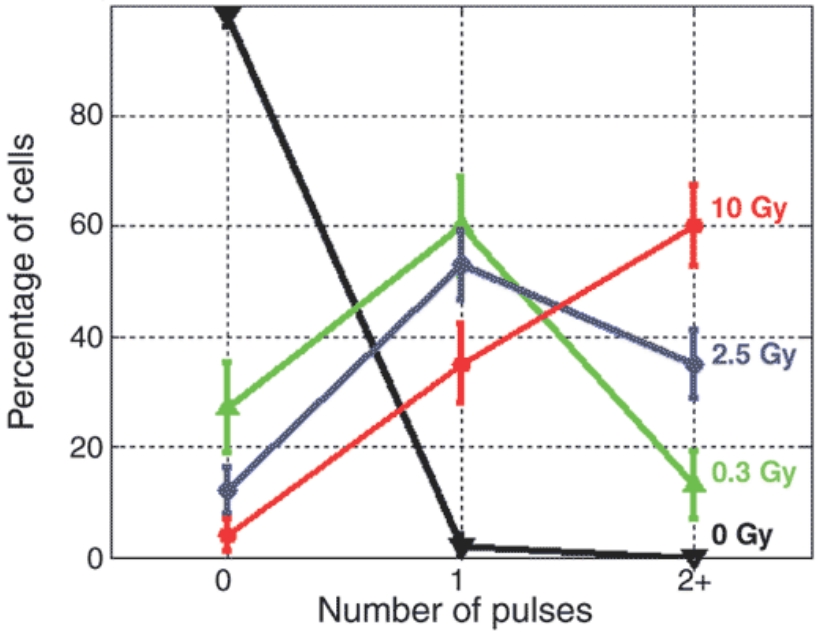


Lahav et al., *Nature Genetics,* 2004

Oscillations are not damped at single cell level

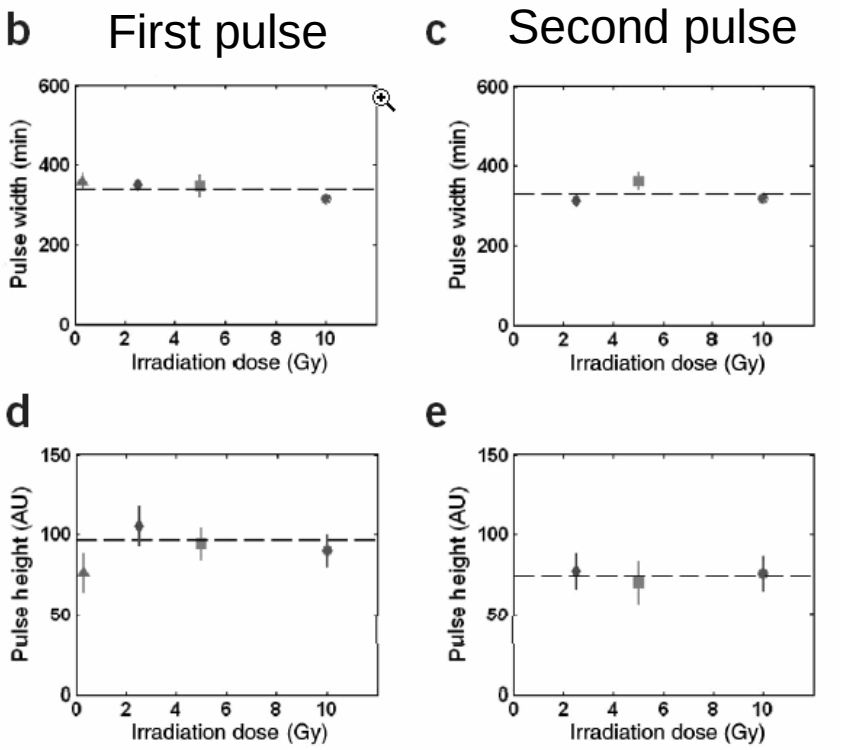
Digital Clock: individual cells

Fraction of cells with zero, one, two or more pulses as a function of γ-IR dose:



Lahav et al., *Nature Genetics,* 2004

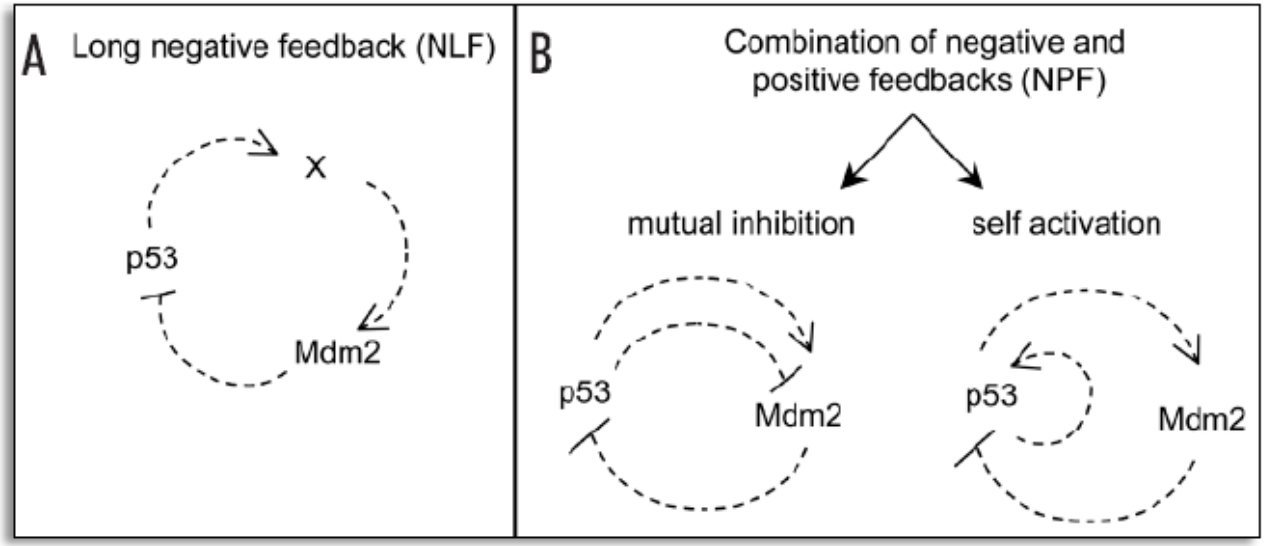
Pulse width and height as a function of γ-IR dose:

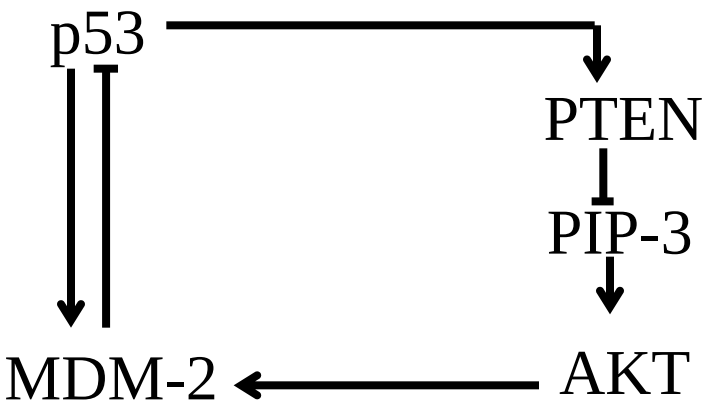


Digital behavior at single cell level: mean number of pulses but not the amplitude or frequency depends on input signal.

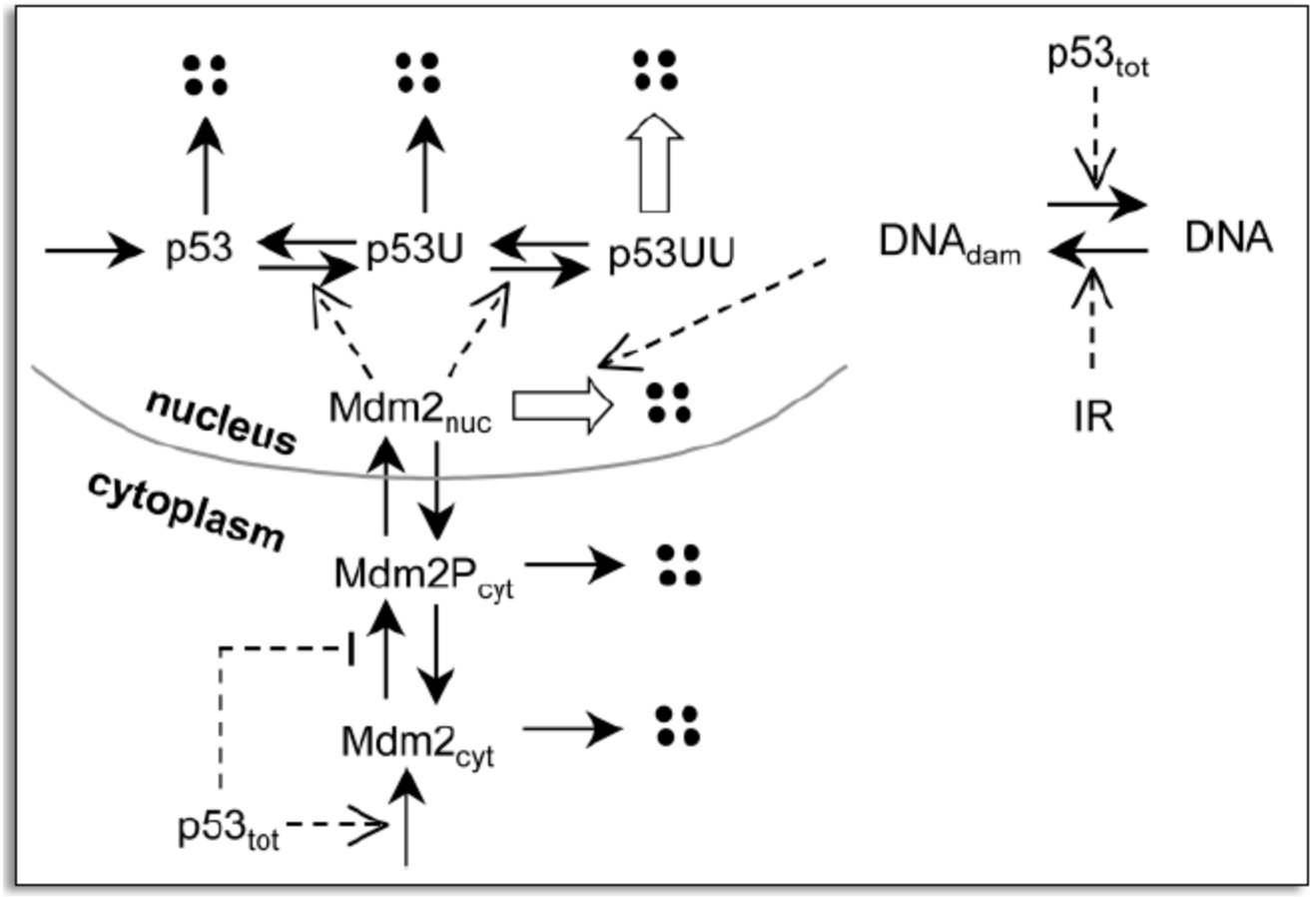
Pathways to oscillations

From Ciliberto, Novak, Tyson model, cell-cycle, 2005



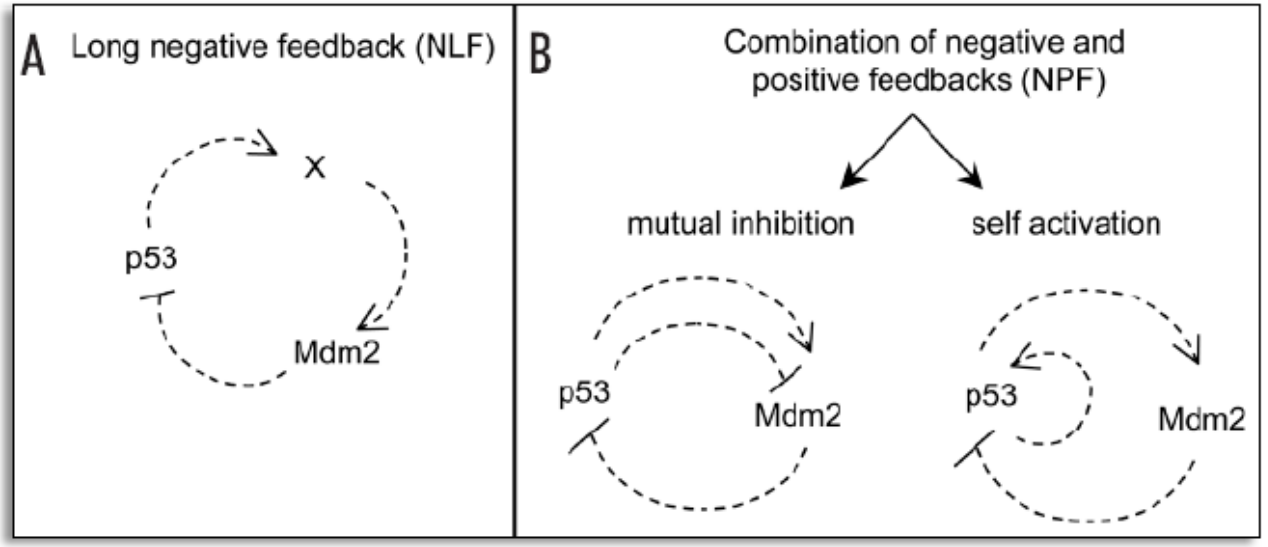


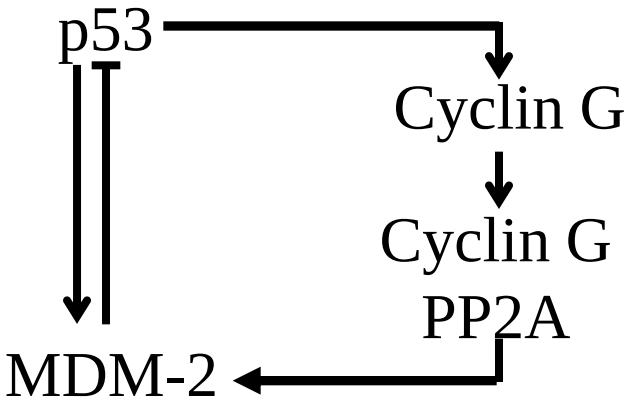
Modeling digital behavior



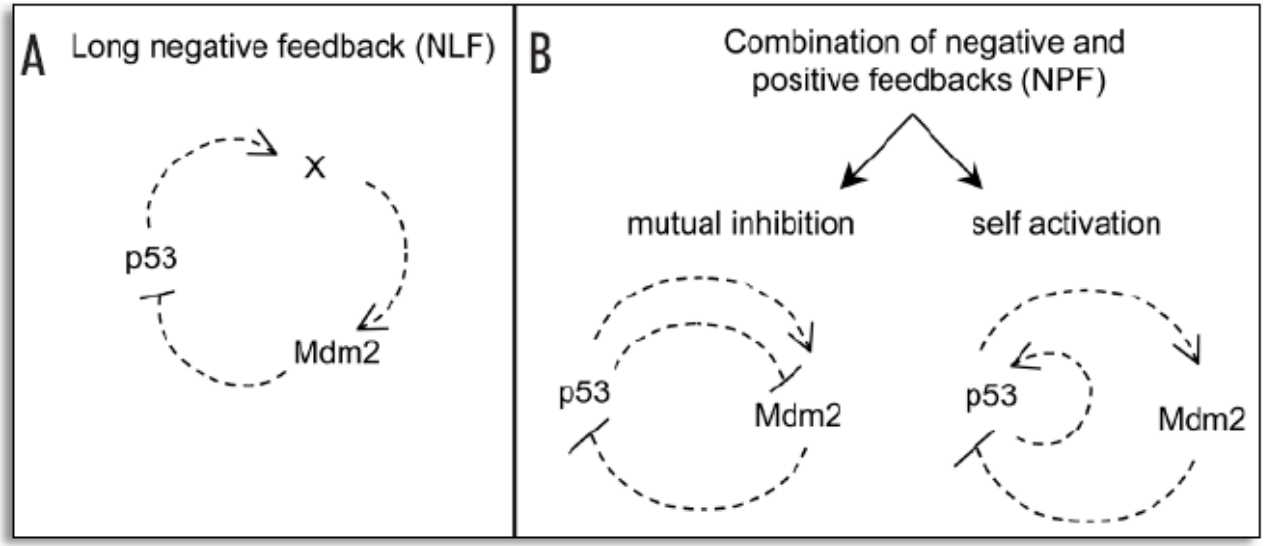
From Ciliberto, Novak, Tyson model, cell-cycle, 2005

Pathways to oscillations





Pathways to oscillations

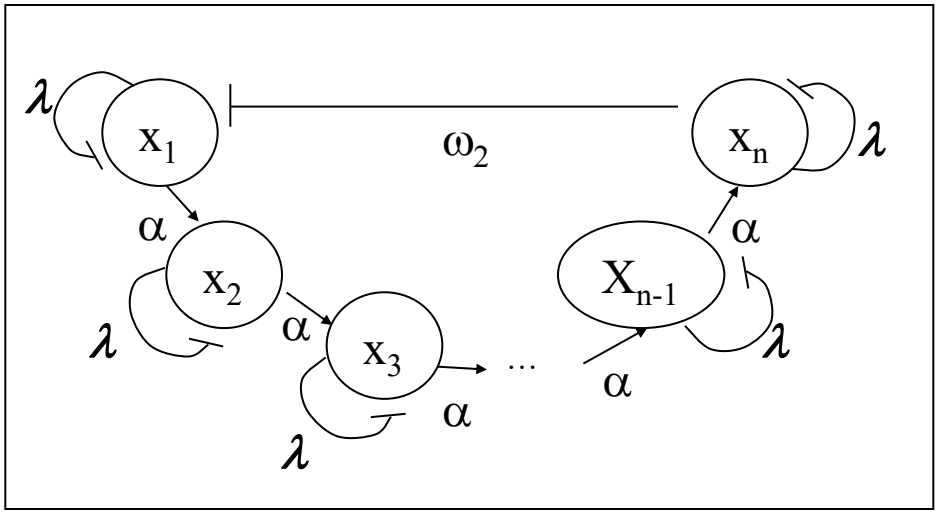


p53

mdm2 rna – transport out of nucleus

MDM2 translation – transport into nucleus

MDM-2



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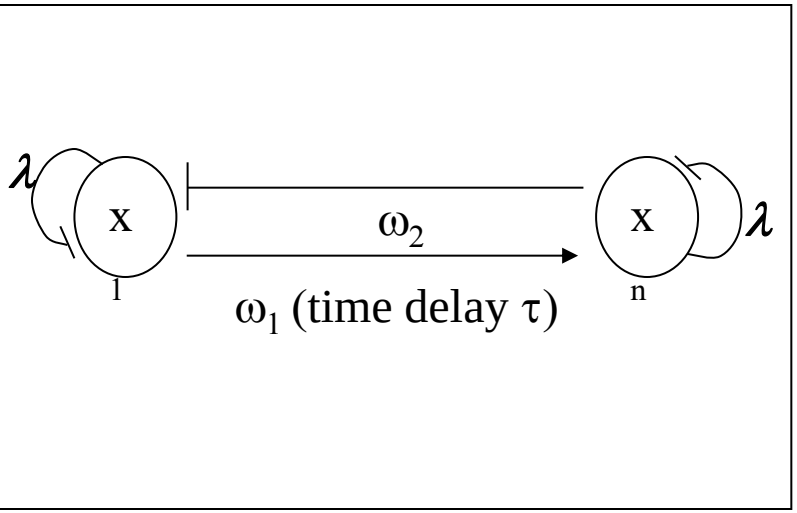
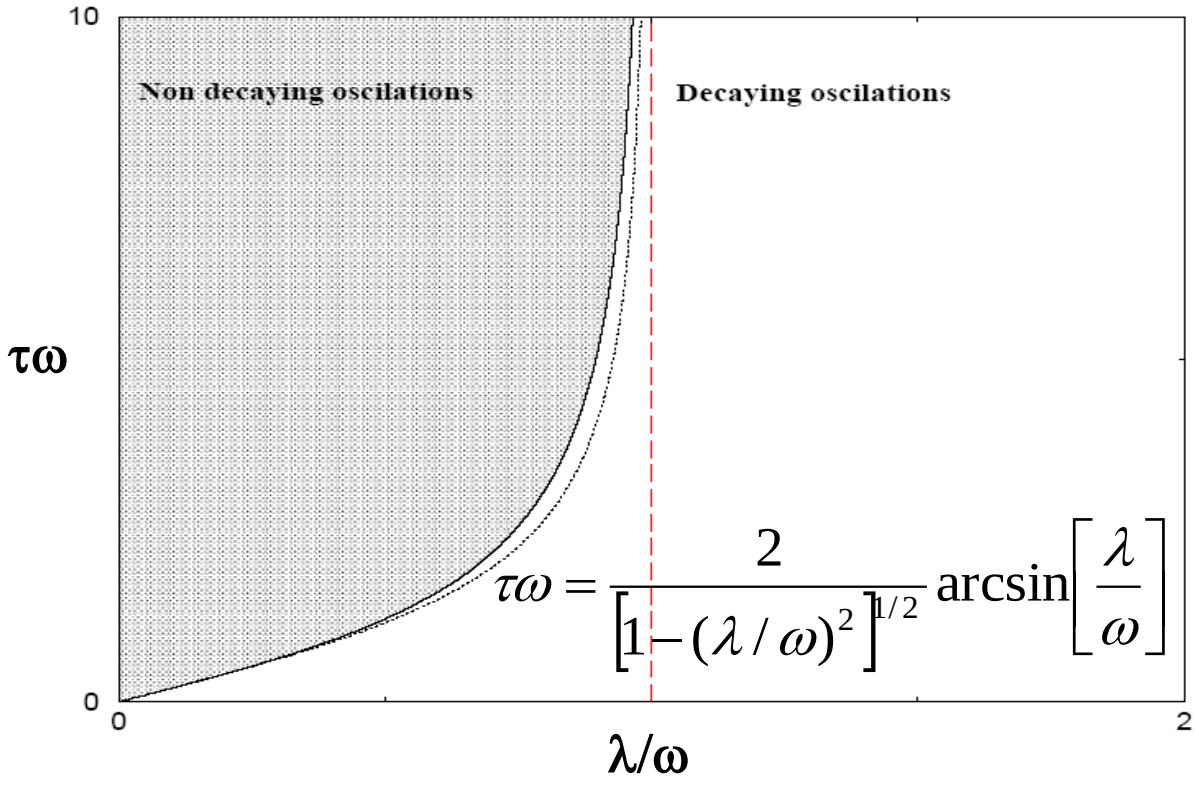


$$\tau = \frac{n-2}{n}\,\frac{\pi}{\omega\sqrt{1-(\lambda/\omega)^2}}$$

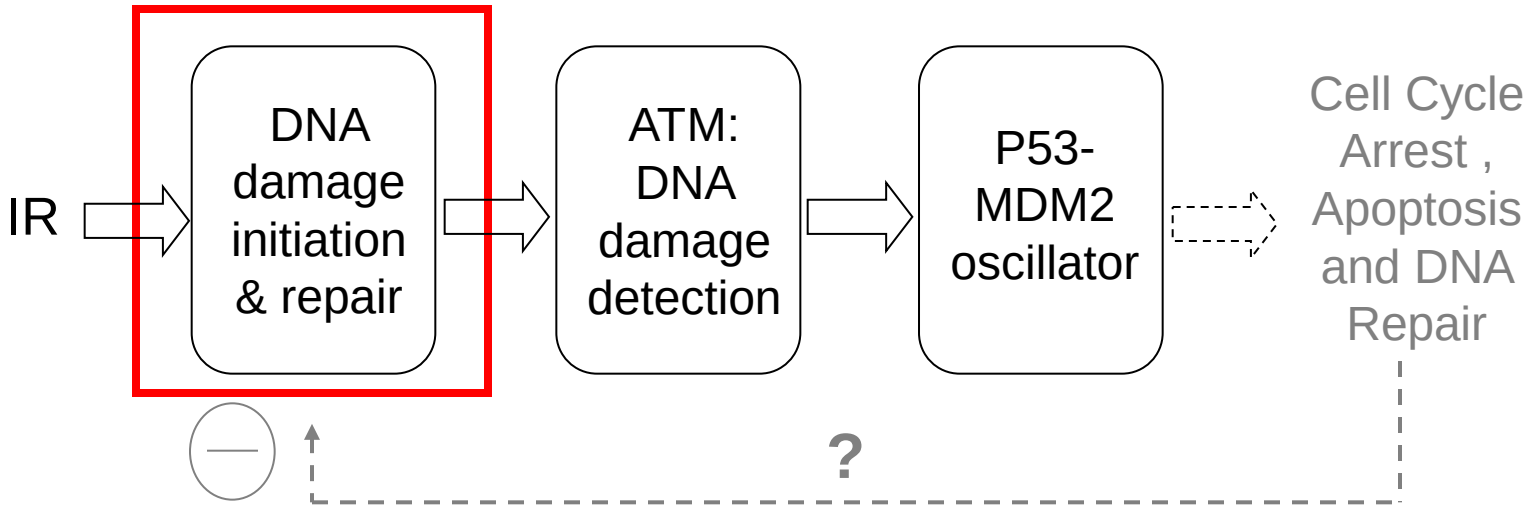
$$\omega = \alpha\left(\frac{\omega_2}{\alpha}\right)^{1/n}$$

$$\omega_1 = \alpha\left(\frac{\alpha}{\omega_2}\right)^{(n-2)/n}$$



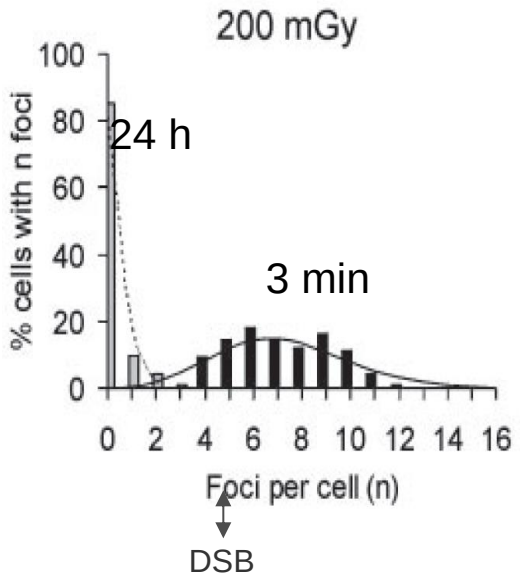
Modeling digital behavior

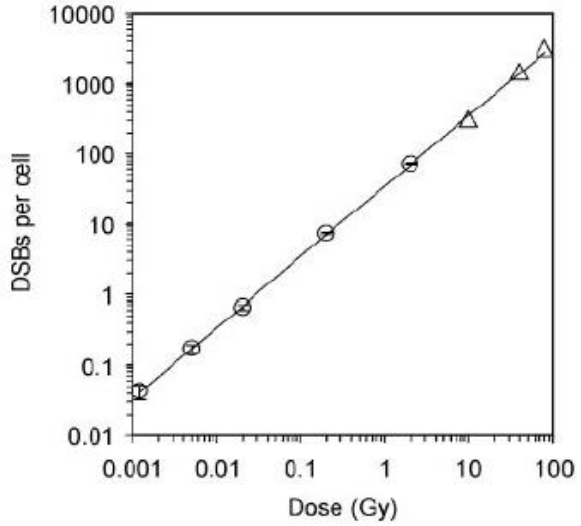
Basic structure of the model



Repair of double strand breaks (DSBs)

- Distribution of initial DSBs ~ Poisson Distribution
- Mean of number of DSBs proportional to IR dose (30-40 Gy^{-1} cell $^{-1}$)



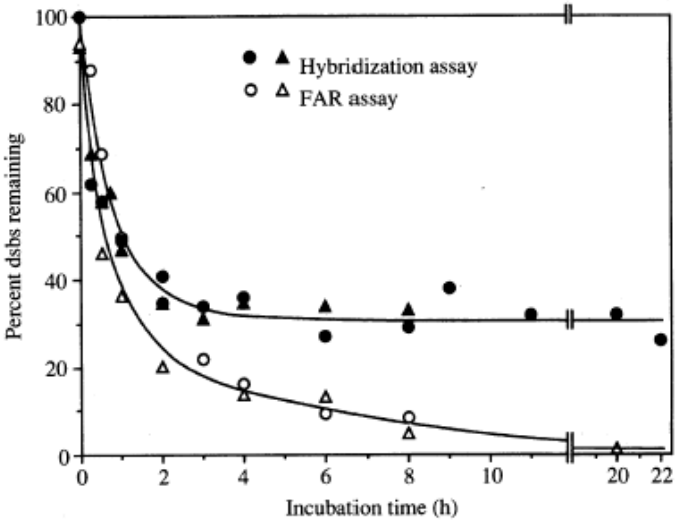


Rothkamm & Löbrich, *PNAS,* 2003

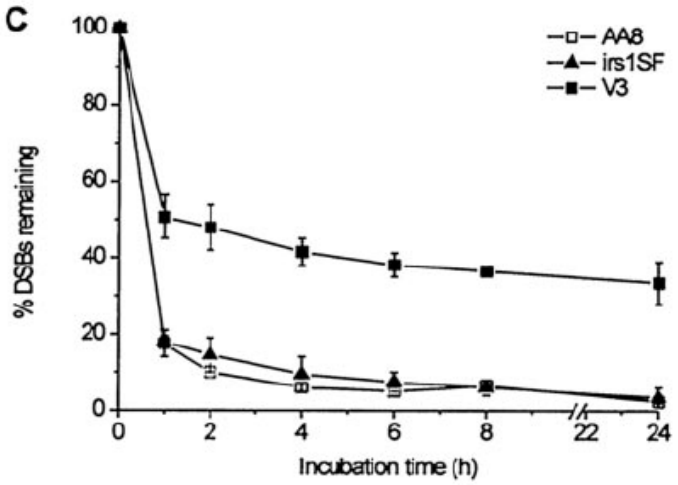
Two-Lesion-Kinetics (TLK)

- Biphasic repair process: rapid repair of simple lesions + slower repair of complex lesions

- Two repair mechanisms: NHEJ (Non-Homologous End-Joining) & HR (Homologous Recombination)



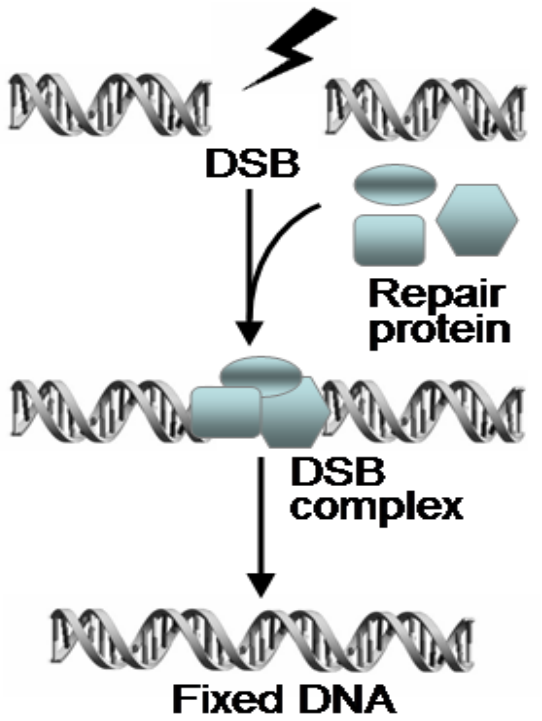
Löbrich *et al.*, *PNAS,* 1995



Rothkamm *et al.*, MCB, 2003

Model: stochastic TLK of DSB repair

- Limiting pool of repair proteins
- DSB-enzyme complexes necessary for DNA damage repair



Pathway 1: fast DSB lesion repair

$$D_1 \underset{k_{rb1}}{\overset{RP*(k_{fb1}+k_{cross}*(D_1+D_2))}{\rightleftarrows}} C_1 \xrightarrow{k_{fix1}} F_1$$

Pathway 2: slow DSB lesion repair:

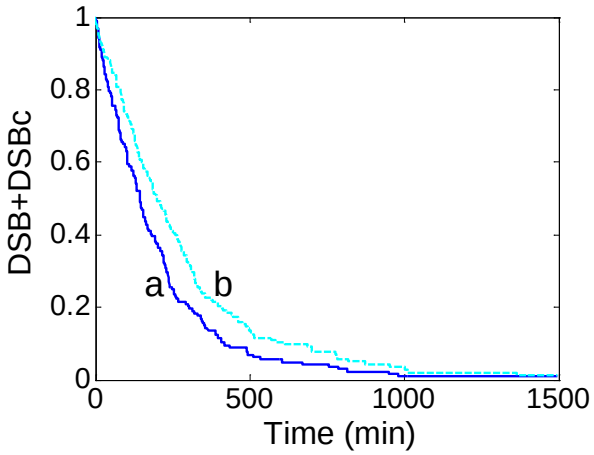
$$D_2 \underset{k_{rb2}}{\overset{RP*(k_{fb2}+k_{cross}*(D_1+D_2))}{\rightleftarrows}} C_2 \xrightarrow{k_{fix2}} F_2$$

RP: repair protein (Mre11/Rad50/Nbs1 cmplx)
D: intact DSB
C: DSB-enzyme complex
F: fixed DSB

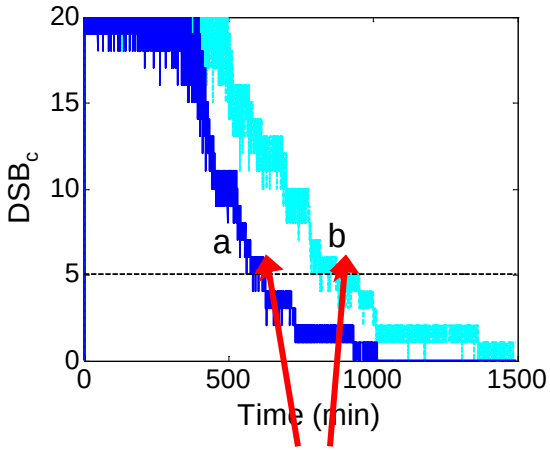
Simulation: DNA repair process

Implemented using Monte-Carlo method:

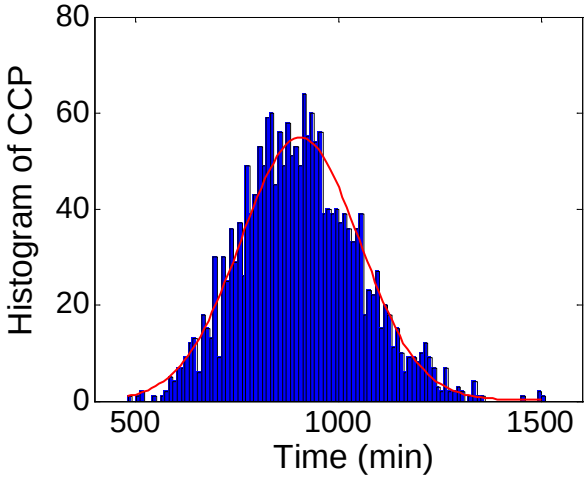
Remaining DSBs



DSB complexes

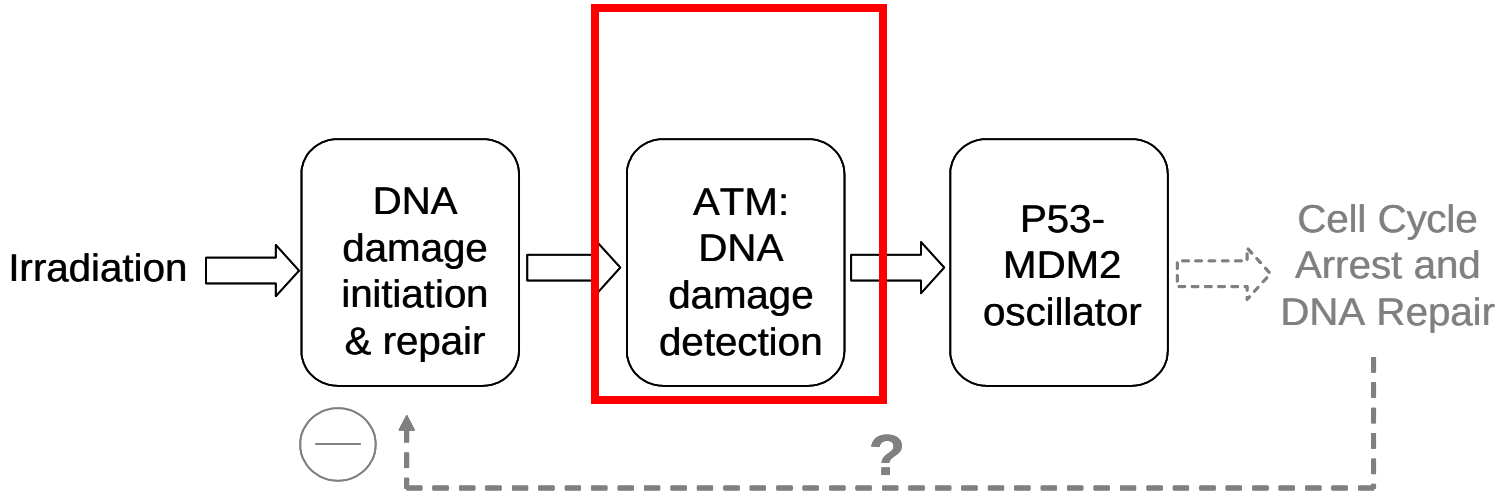


Distribution of CCT



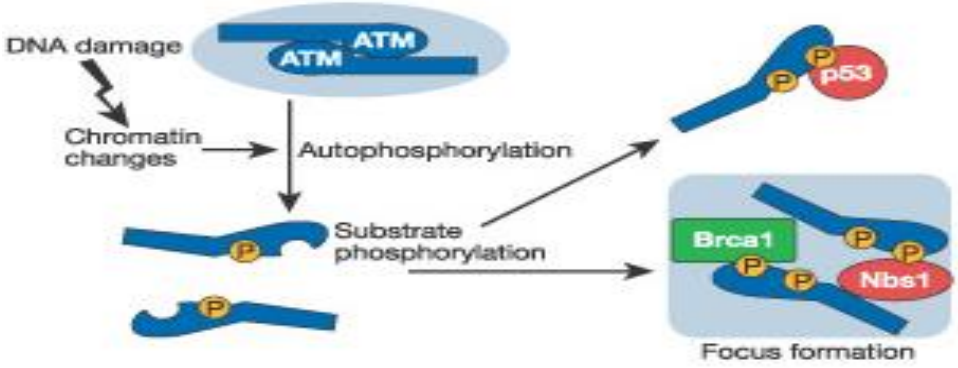
CCT: critical crossing times

Basic structure of model



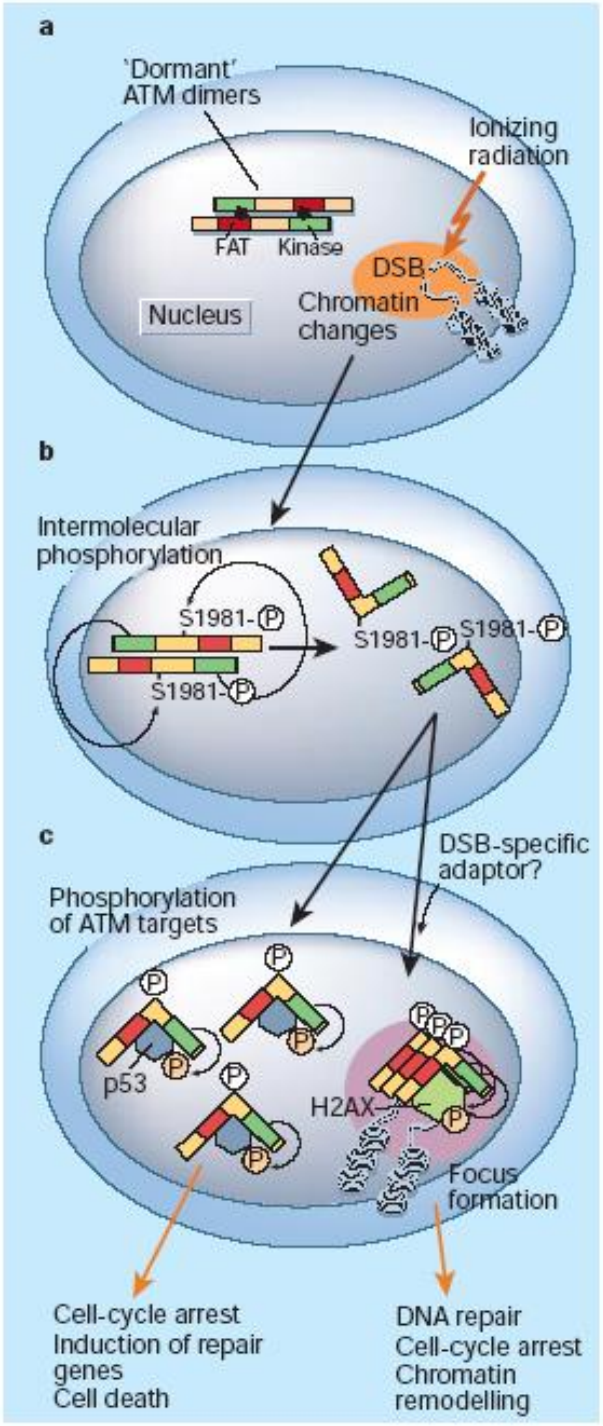
Ataxia telangiectasia mutated (ATM): mutated in disease AT, a human genetic disorder characterized by neural degeneration, immunodeficiency, sterility, cancer predisposition, etc.

ATM activation

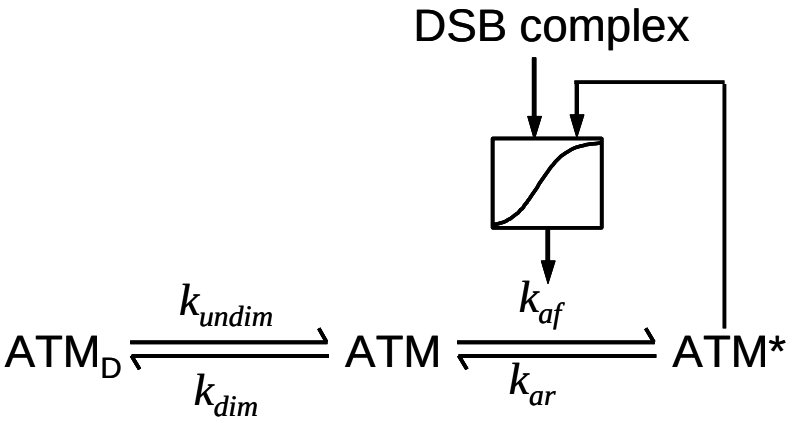


Bakkenist & Kastan, Nature 2003

- Dimer in normal cells
- Intermolecular autophosphorylation
- Direct activation by DSBs
- Nucleation formed by DSB and ATM*



Model: ATM activation



ATM$_D$: ATM dimer

ATM : inactive ATM monomer

ATM* : active ATM monomer

$2ATM_D+ATM+ATM^*=ATM^T$

$$\frac{d\mathrm{ATM_D}}{dt} = \frac{1}{2}k_{\dim}\mathrm{ATM}^2 - k_{un\dim}\mathrm{ATM_D}$$

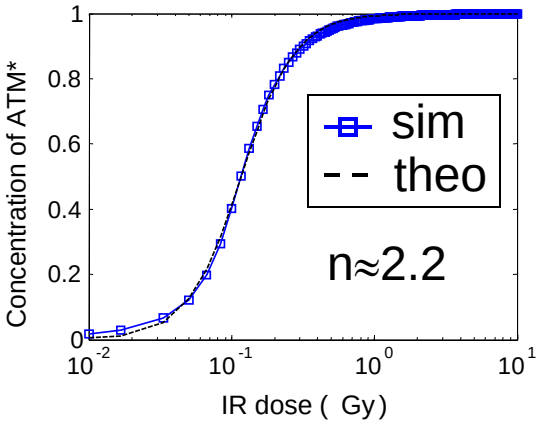
$$\frac{d\mathrm{ATM}}{dt} = 2k_{un\dim}\mathrm{ATM_D} - \frac{1}{2}k_{\dim}\mathrm{ATM}^2 - k_{af}f(\mathrm{C},\ \mathrm{ATM}^*)\mathrm{ATM} + k_{ar}\mathrm{ATM}^*$$

$$\frac{d\mathrm{ATM}^*}{dt} = k_{af}f(\mathrm{C},\ \mathrm{ATM}^*)\mathrm{ATM} - k_{ar}\mathrm{ATM}^*$$

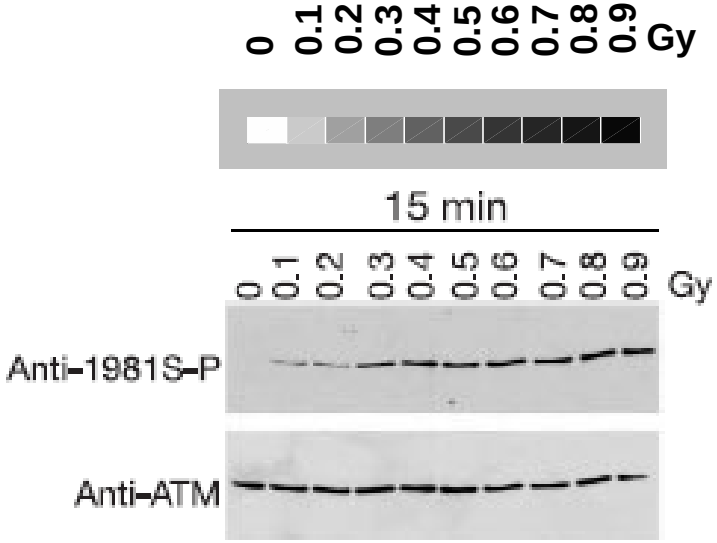
Where $f(\mathrm{C},\ \mathrm{ATM}^*) = (\alpha_1 C + \alpha_2 \mathrm{C} * \mathrm{ATM}^* + \alpha_3 \mathrm{ATM}^*)$ and C is DSB complex

Simulation: Switch like behavior of ATM*

Correlation b/w ATM* & IR

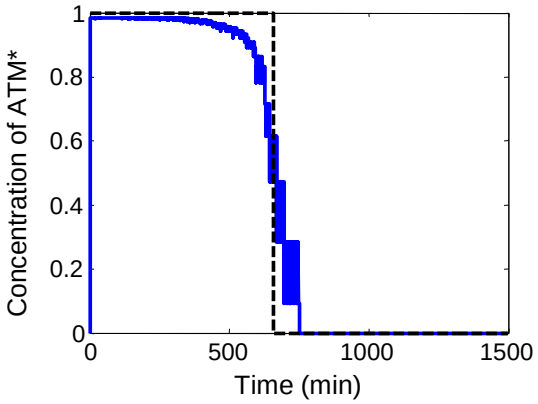


Normalized by ATM^T

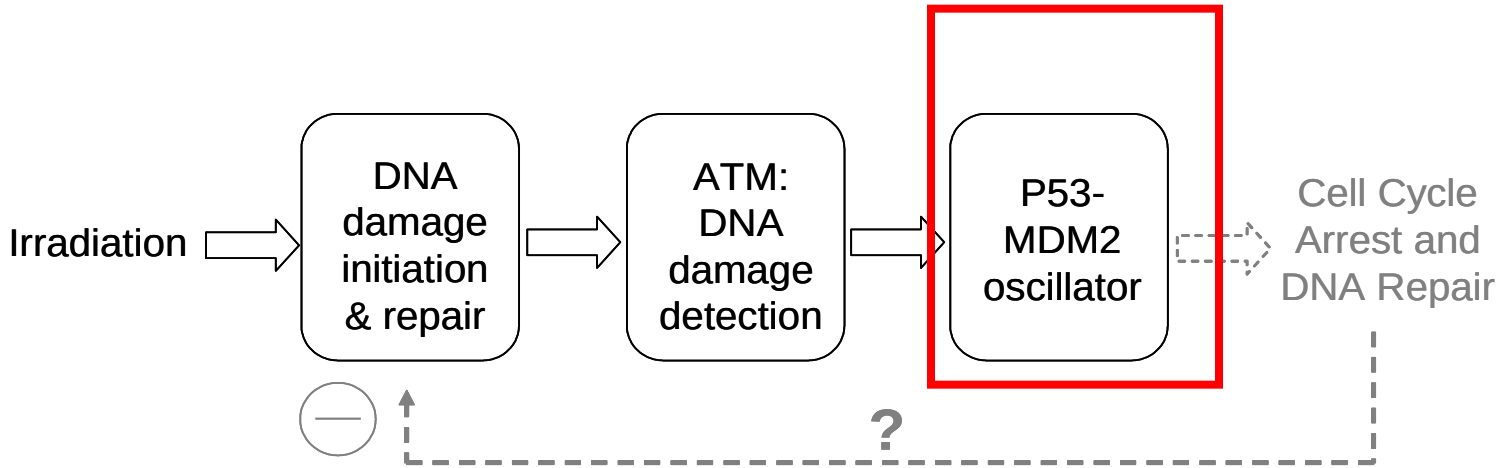


Bakkenist & Kastan, Nature 2003

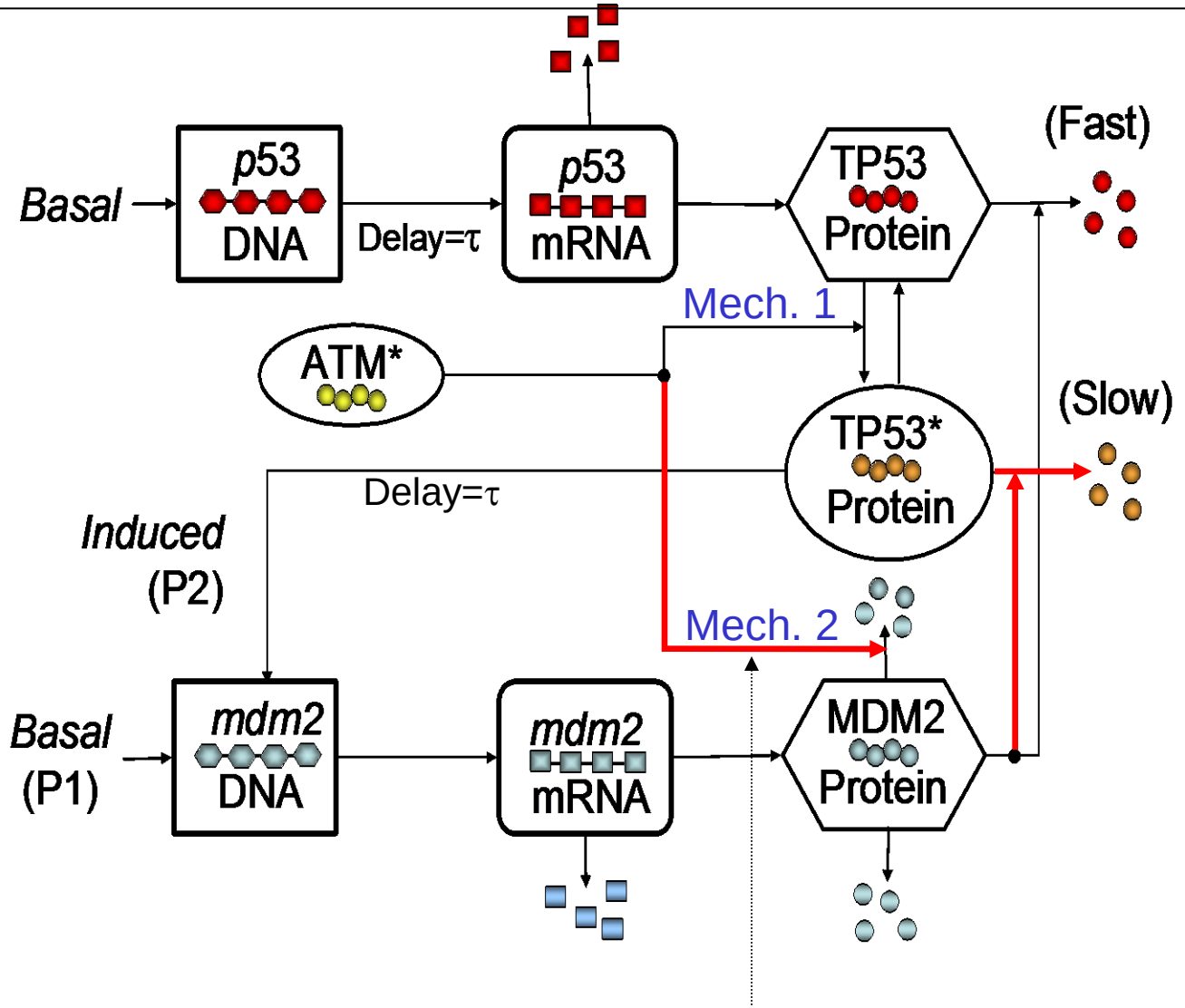
Time response:
ON-to-OFF signal



Basic structure of model



Modified p53 – Mdm2 oscillator



Stommel & Wahl, *EMBO* 2004

p53 – Mdm2 oscillator: equations

$$\frac{dp53}{dt} = s_{p53} - \delta_{p53} p53$$

$$\frac{dmdm2}{dt} = s_{mdm2} + k_{mdm2} \frac{[TP53^{*}(t-\tau)]^{n}}{[TP53^{*}(t-\tau)]^{n} + K^{n}} - \delta_{mdm2} mdm2$$

$$\frac{dTP53}{dt} = r_{TP53} p53 - \mu_{TP53} TP53 - \nu_{TP53} MDM2 \frac{TP53}{TP53 + K_d} + k_{rp} TP53^{*} - k_{fp} ATM^{*} \frac{TP53}{TP53 + K_p}$$

$$\frac{dTP53^{*}}{dt} = k_{fp} ATM^{*} \frac{TP53}{TP53 + K_p} - k_{rp} TP53^{*} - \nu_{TP53^{*}} MDM2 \frac{TP53^{*}}{TP53^{*} + K_d^{*}}$$

$$\frac{dMDM2}{dt} = r_{MDM2} mdm2 - [\mu_{MDM2} + (\nu_{MDM2} - \mu_{MDM2}) \frac{ATM^{*}}{ATM^{*} + K_a}] MDM2$$

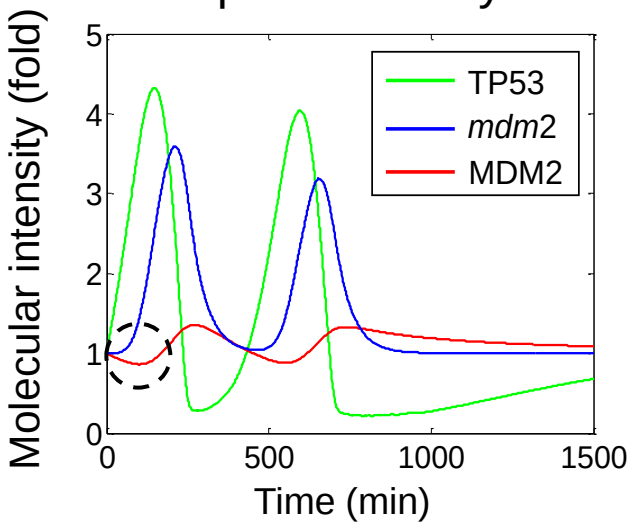
n=4

mRNA: p*53*, *mdm2*

Protein: TP53 (inactive), TP53* (active / phosphorylated), MDM2

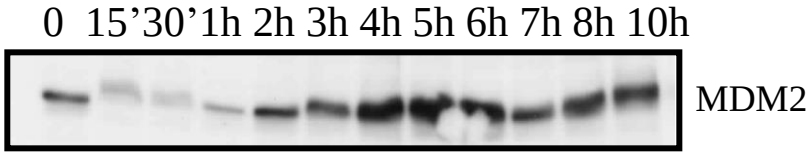
Complete Model Results

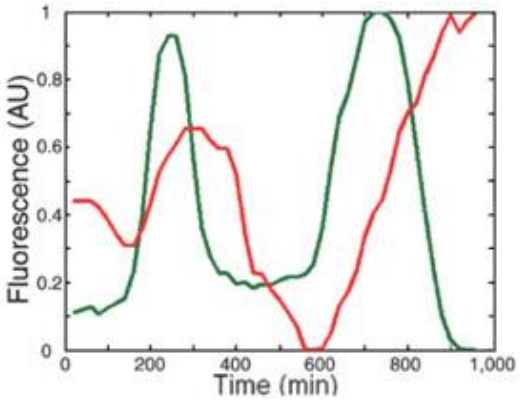
Response to 5Gy



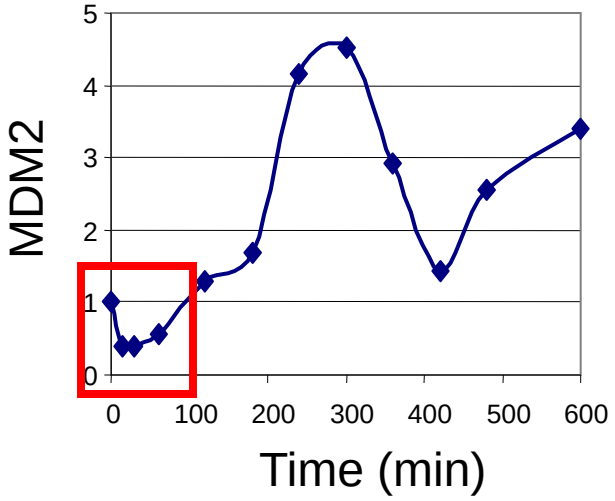
Predict drop of MDM2 at the beginning of time course

Experiment



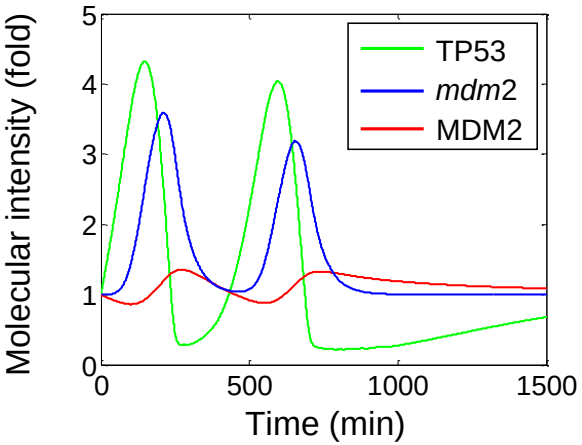


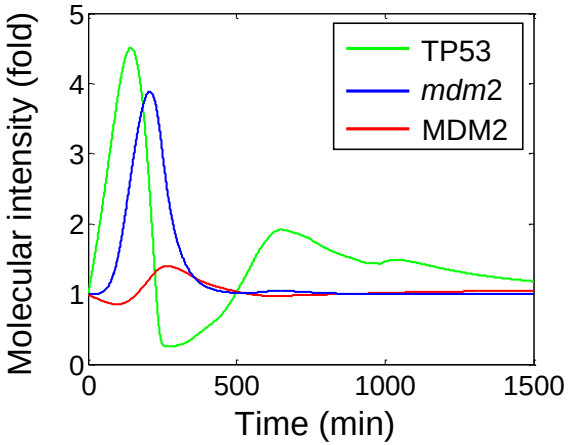
Lahav *et al.*, Nature Genetics 2004

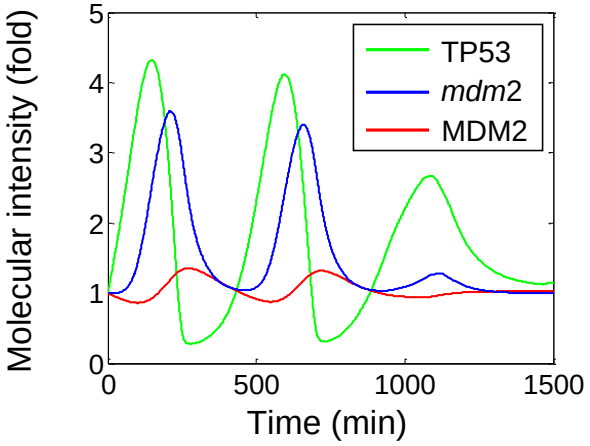


Complete Model Results

Stochasticity in oscillation: IR of 5 Gy induces one, two or three oscillations

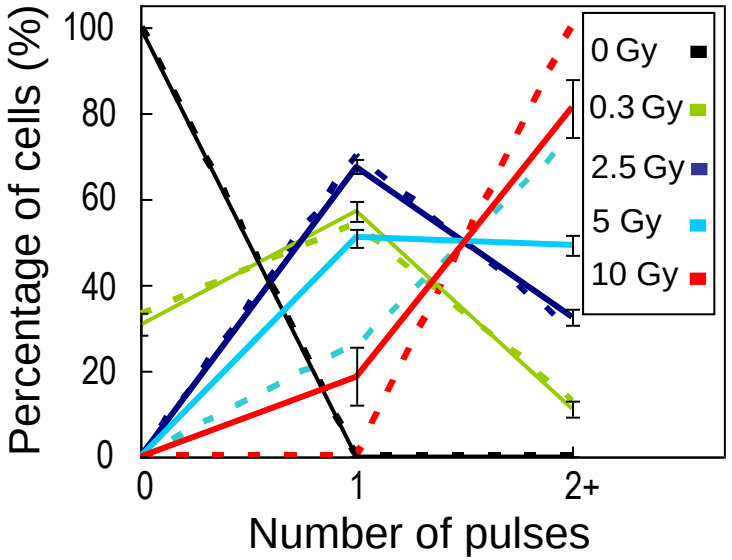


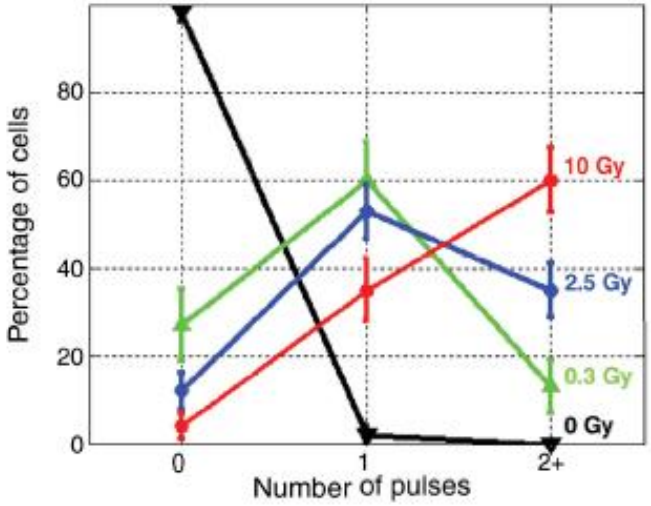




Note: molecular intensity is normalized by respective basal concentration.

Complete Model Results



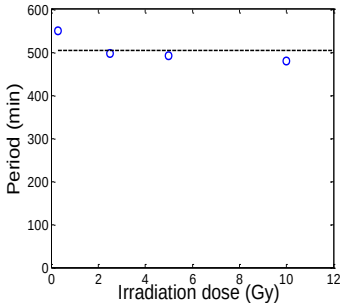


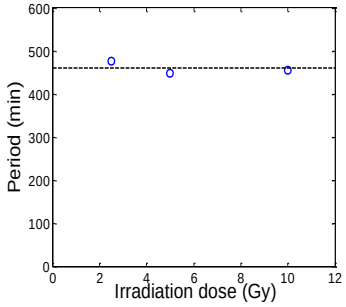
Lahav *et al.,* Nature Genetics 2004

- Number of pulses increases as IR dose increases
- Less stochasticity than experiment

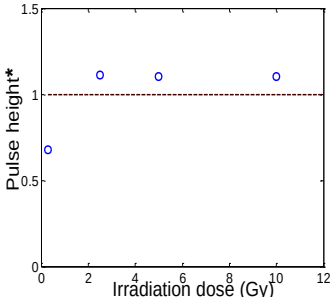
Digital behavior

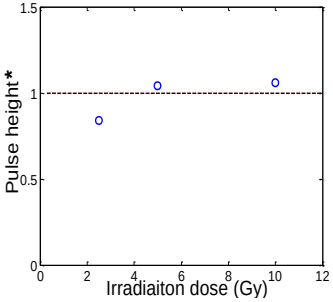
Period as function of IR dose:



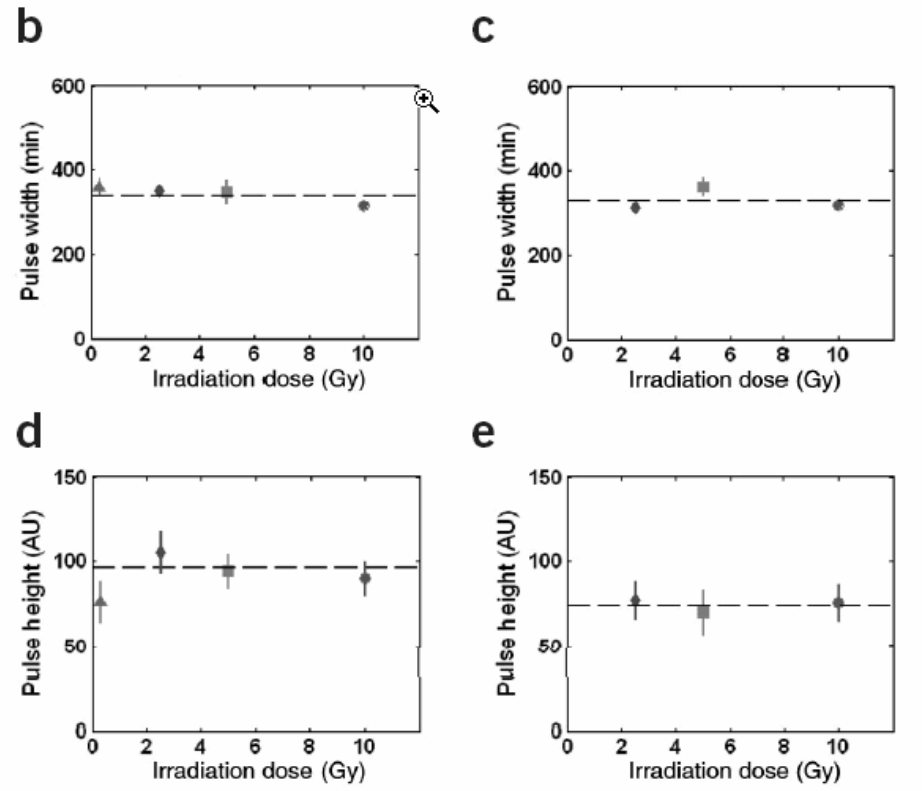


Pulse height as function of IR dose:

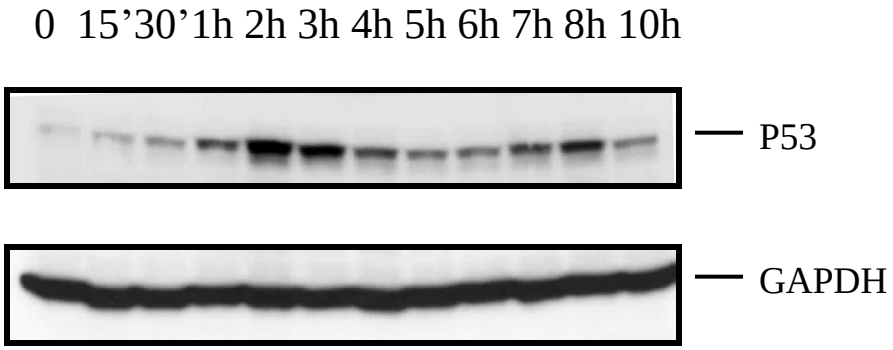


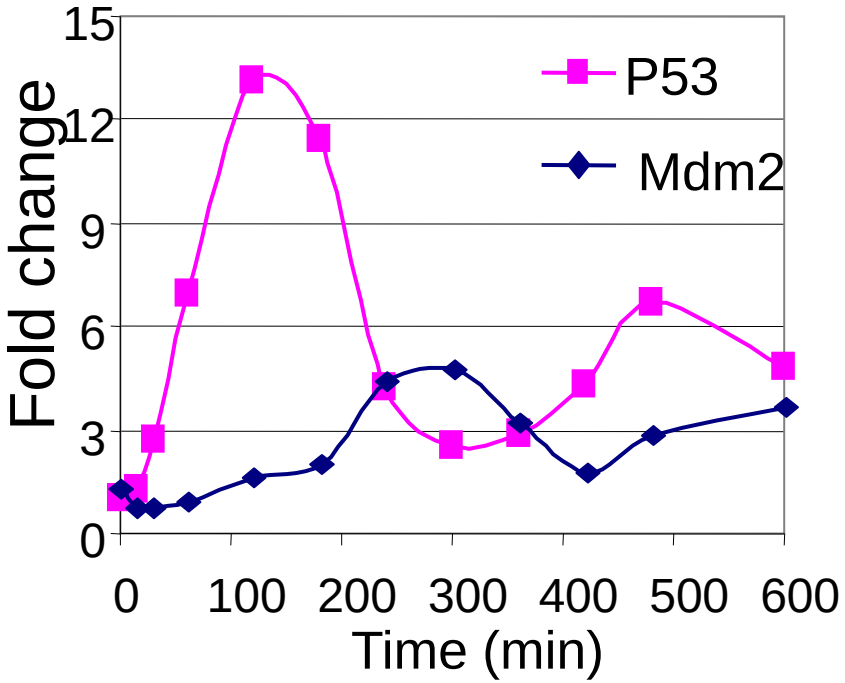


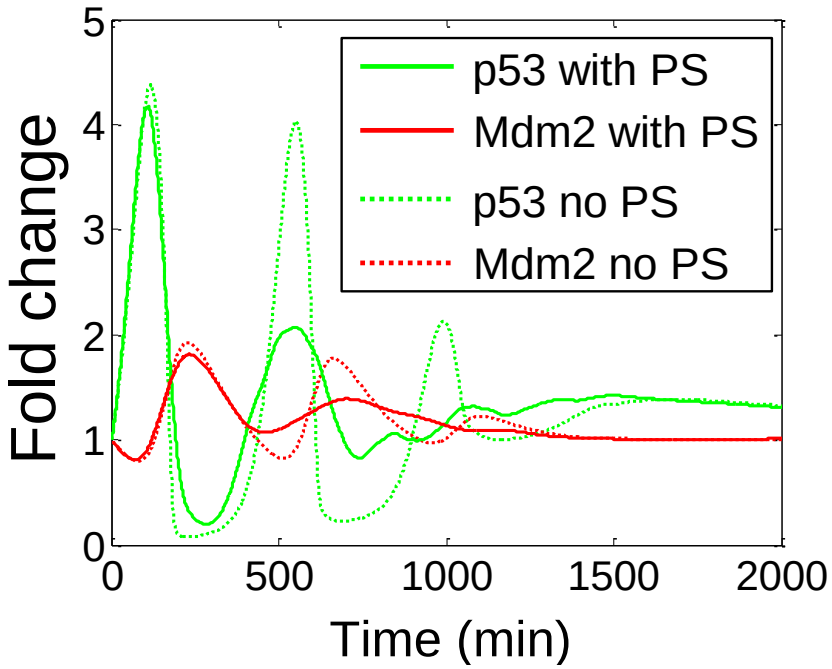
Pulse width and height as a function of γ-IR dose:



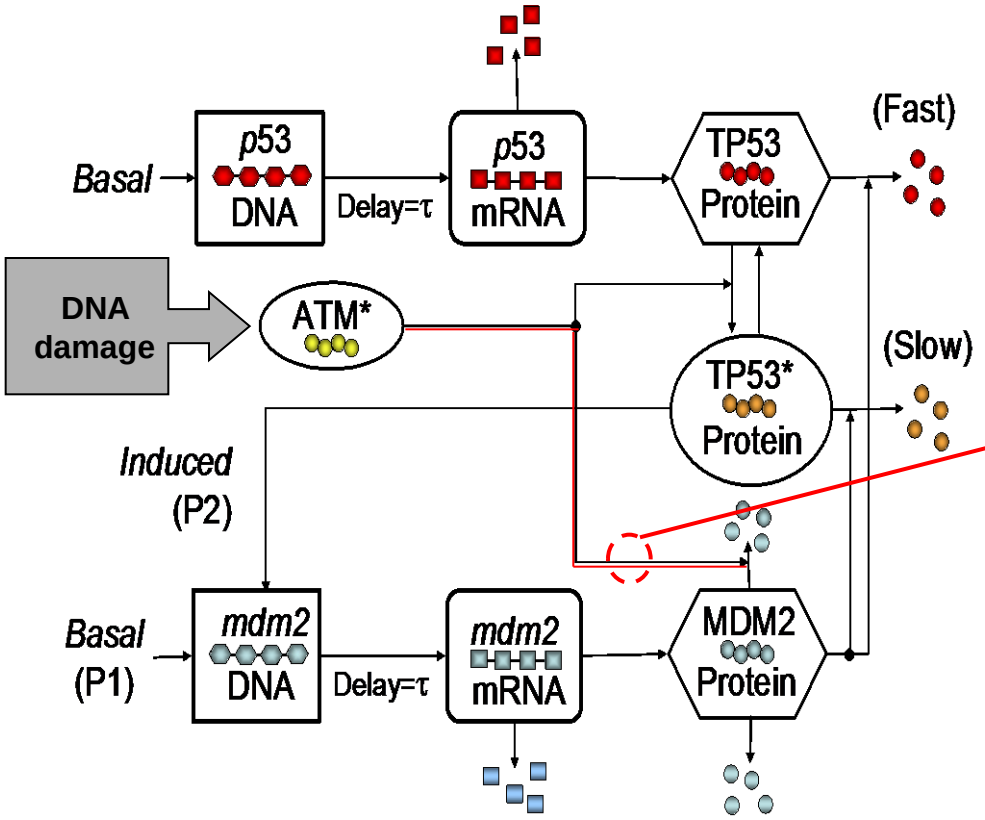
Simulating a cell population

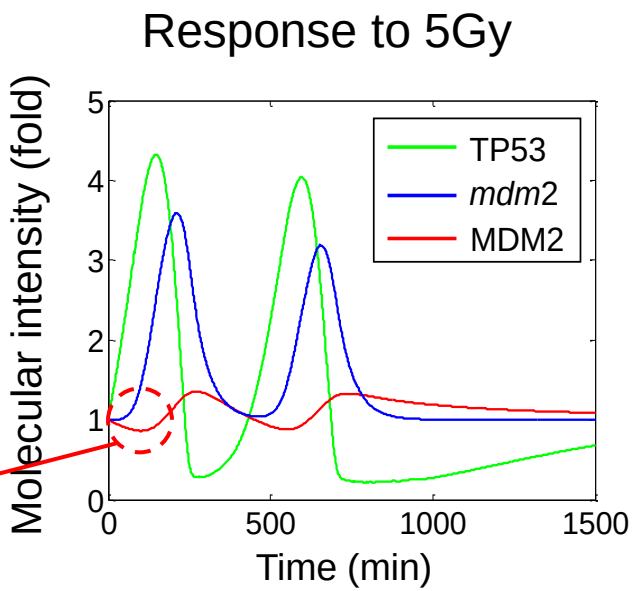


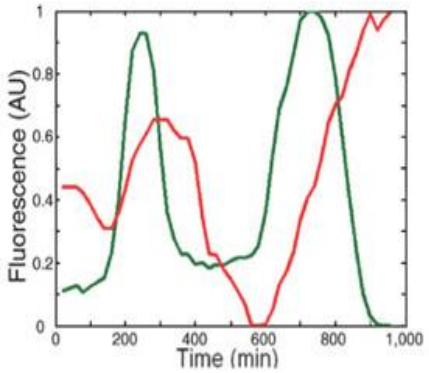




Digital response of tumor suppressor p53 to IR







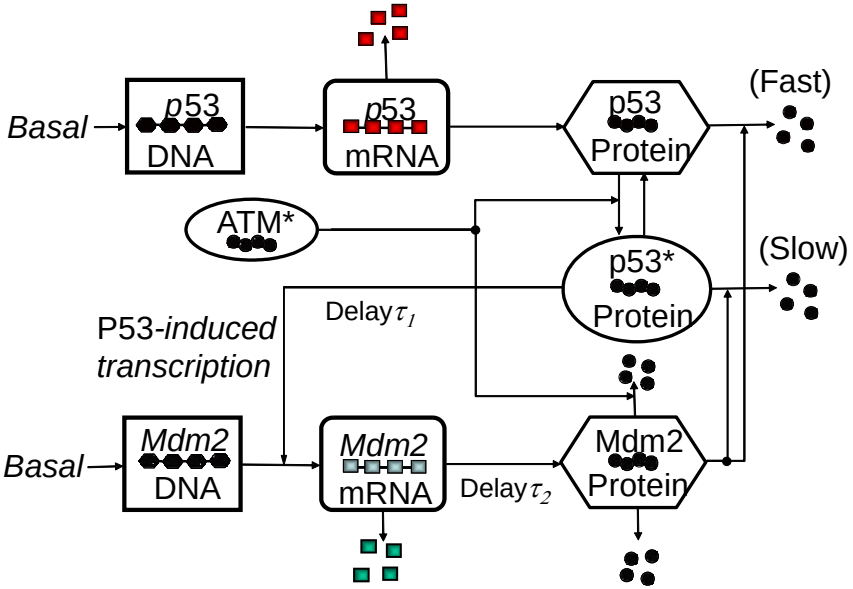
Lahav *et al.*, Nature Genetics 2004

Ma, Wagner, Rice, Hu, Levine and Stolovitzky, A plausible model for the digital response of p53 to DNA damage, Proc. Natl. Acad. Sci. U S A. 102, 14266 (2005).

Wagner; Ma; Rice; Hu; Levine; Stolovitzky, p53-Mdm2 loop controlled by a balance of its feedback strength and effective dampening using ATM and delayed feedback, IEE PROCEEDINGS SYSTEMS BIOLOGY, 152, 3, 109-118 (2005).

Predictions

Figures 4 and 8 from Ma, Wagner *et al*.,



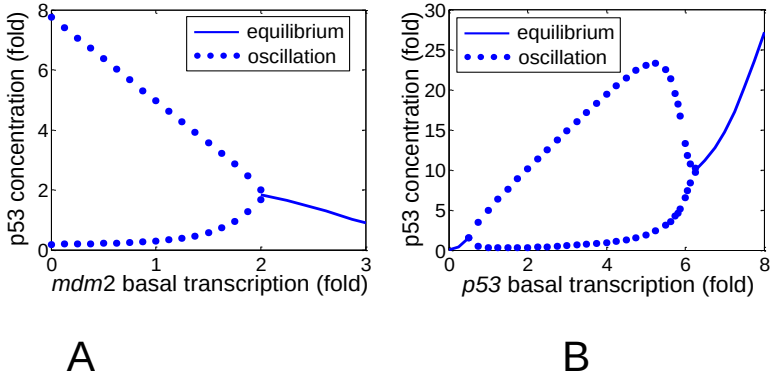
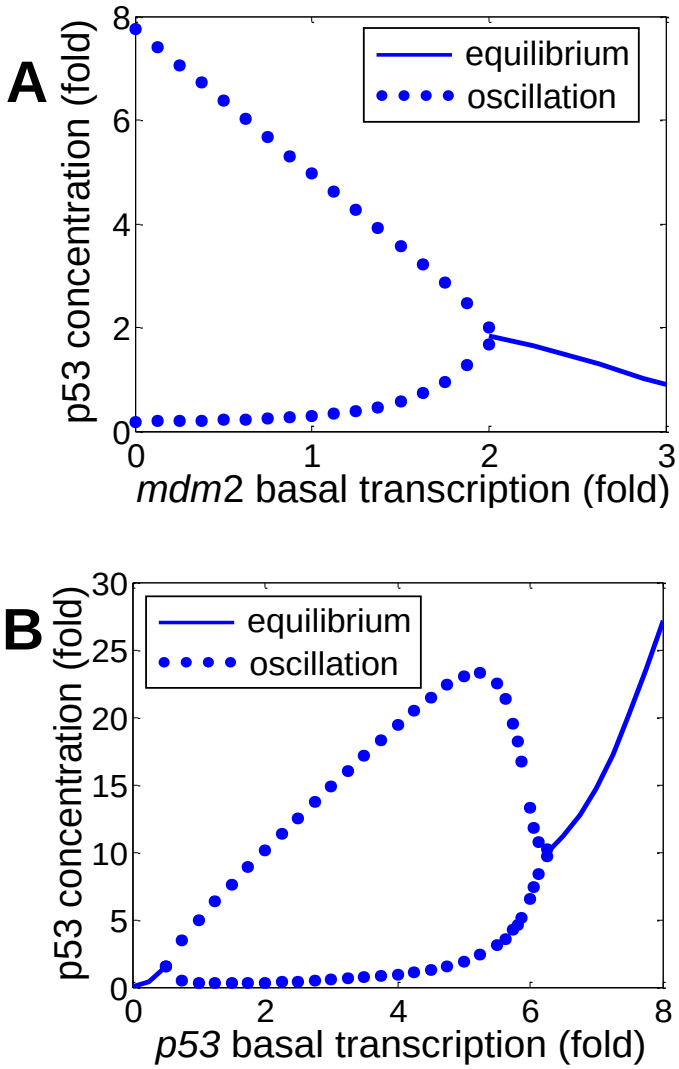


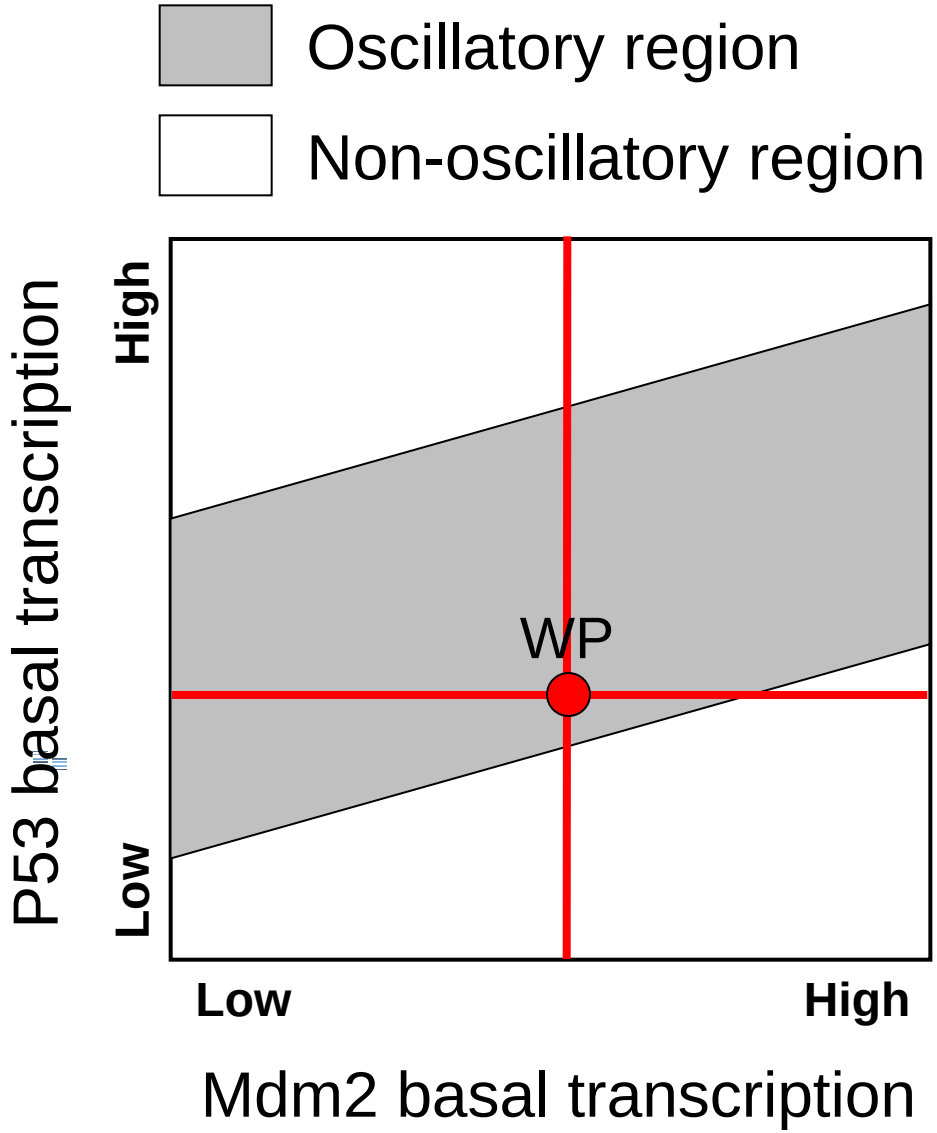
Figure 4 (From Ma, Wagner et.al.) - Diagram of the p53-Mdm2 oscillator. p53 is translated from *p53* mRNA and inactive for induction of its targets. Phosphorylated by ATM*, p53 becomes active (p53*), and able to transcribe (after a time delay) *Mdm2* which also has a basal transcription rate. Mdm2 protein promotes a fast degradation of p53 and a slow degradation of p53*. In addition to a basal self-degradation, Mdm2 is degraded by a mechanism stimulated by ATM*.

Figure 8 (From Ma, Wagner, et. al.) - One-dimensional bifurcation diagrams of steady-state p53 versus single parameter variation of *Mdm2* basal transcription rate (A) or *p53* basal transcription rate (B). The stable equilibrium is represented by solid line. The lower and upper bounds of stable oscillation are represented by paired dotted lines.

Specific Predictions About Bifurcations

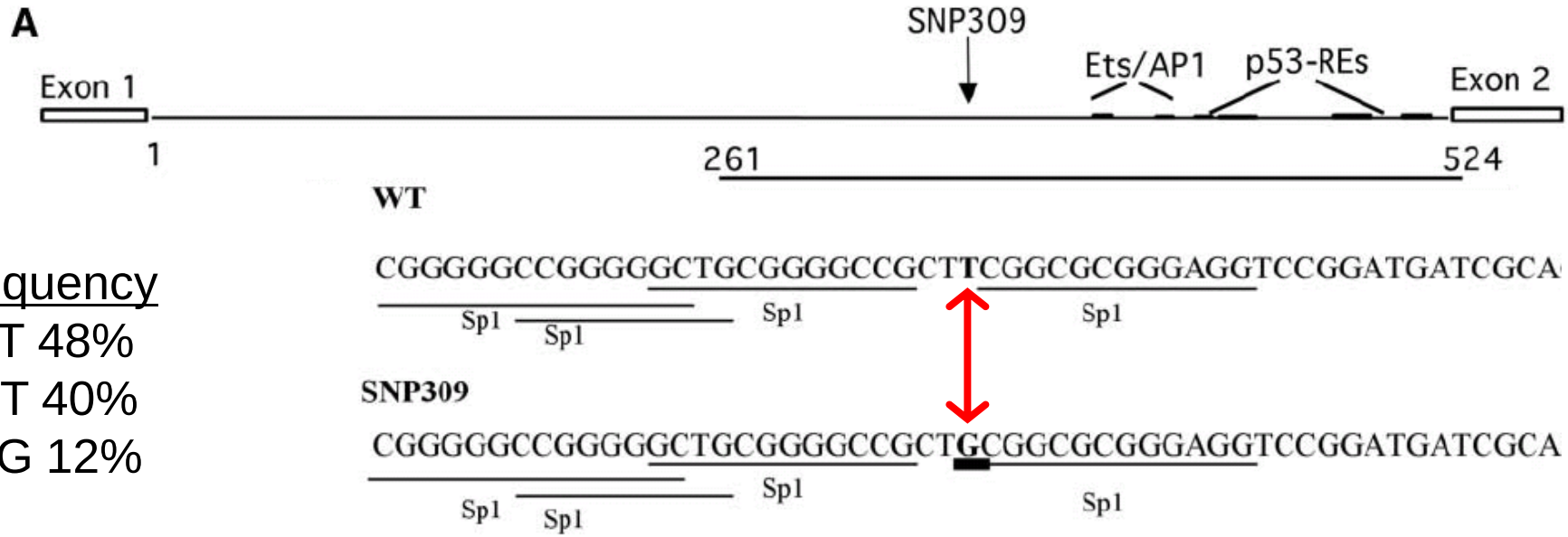
Fig. 8 of Ma et al., *PNAS*, 2005





Mdm2 SNP309: T → G SNP Increases Mdm2

Bond et al, *Cell*, 2004.

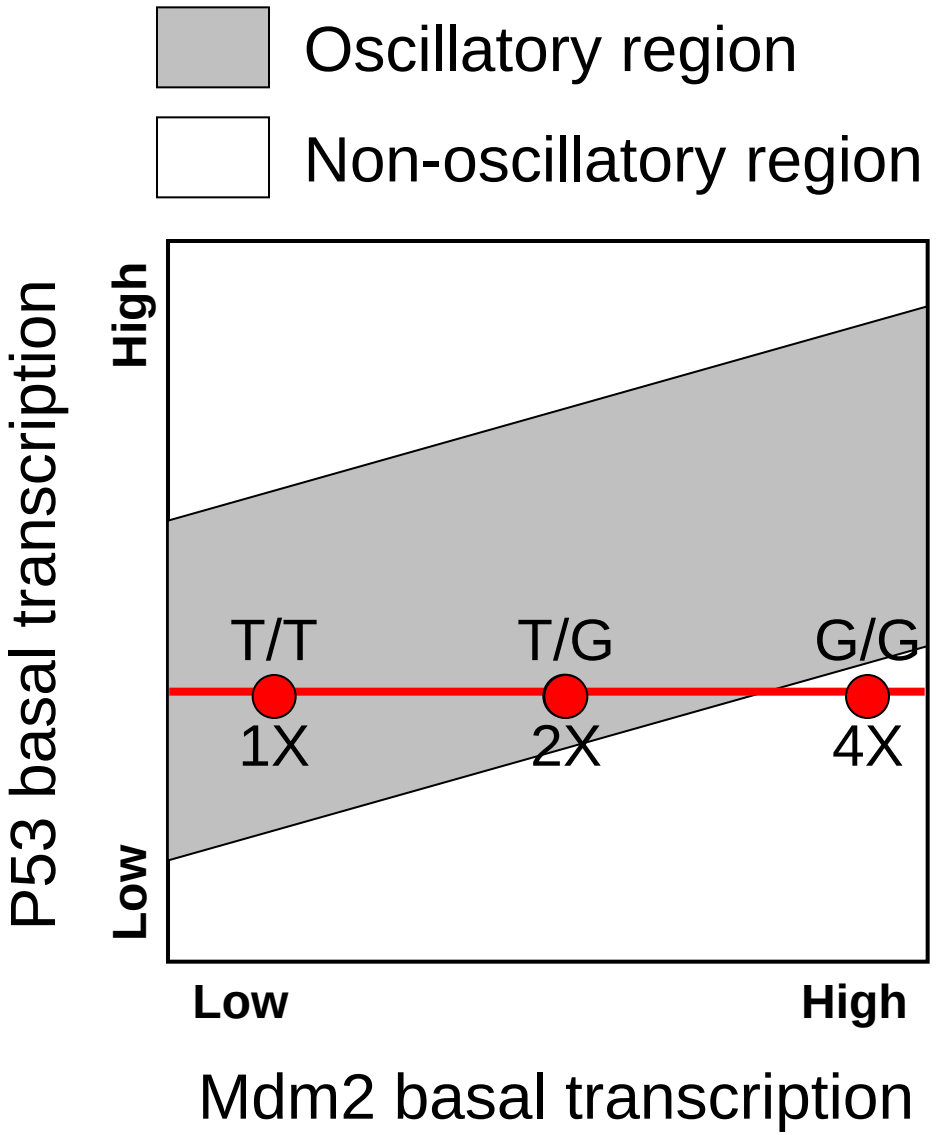


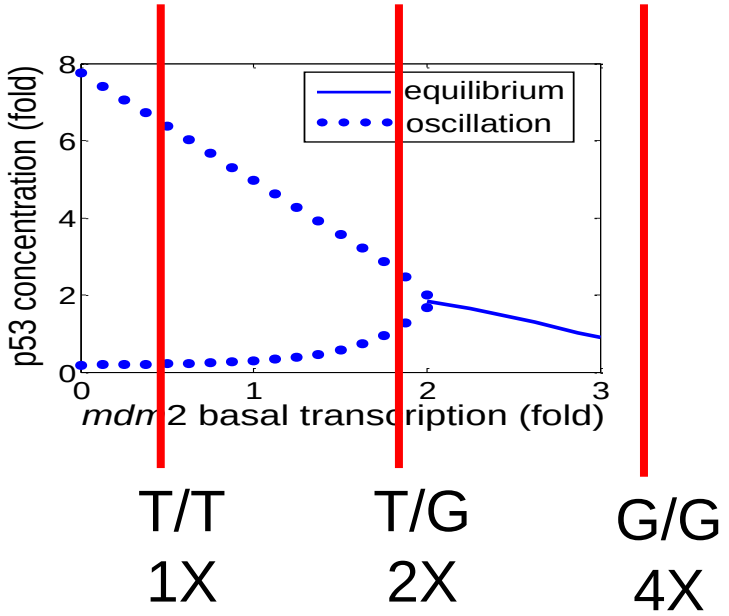
Frequency
T/T 48%
G/T 40%
G/G 12%

	T/T	T/G	G/G
Mdm2 mRNA	1X		8X
Mdm2 Protein	1X	2X	4X

- Quantitative data at cellular level explained by a point mutation.
- T → G increases affinity for Sp1
- MCF-7 cell line is T/G
- SNP status correlates with cancer risk

Predictions T/T & T/G Oscillate, G/G Does Not



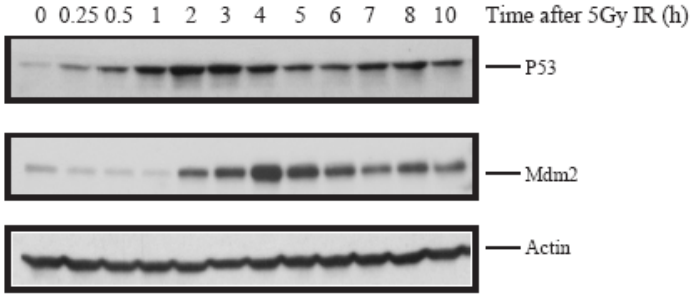


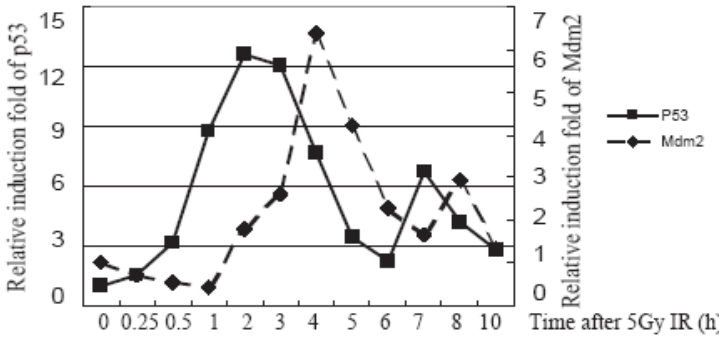
T/T & T/G Cell Lines H460 and MCF-7 Oscillate

Cells wild type (T/T, H460) or heterozygous (T/G, MCF-7) for SNP309 oscillate in response to 5Gy IR.

A) H460

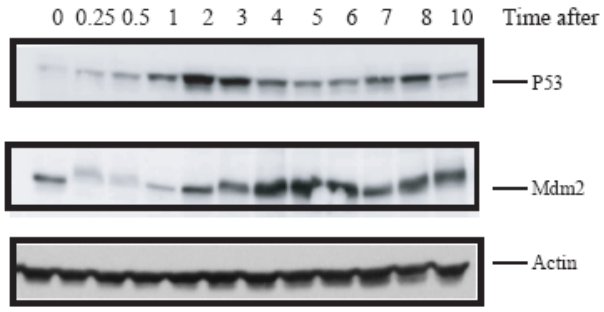
T/T

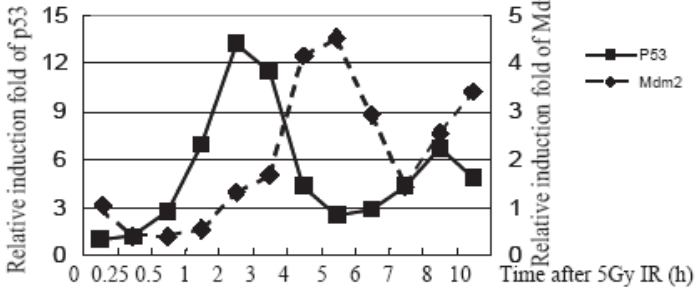




B) MCF-7

T/G

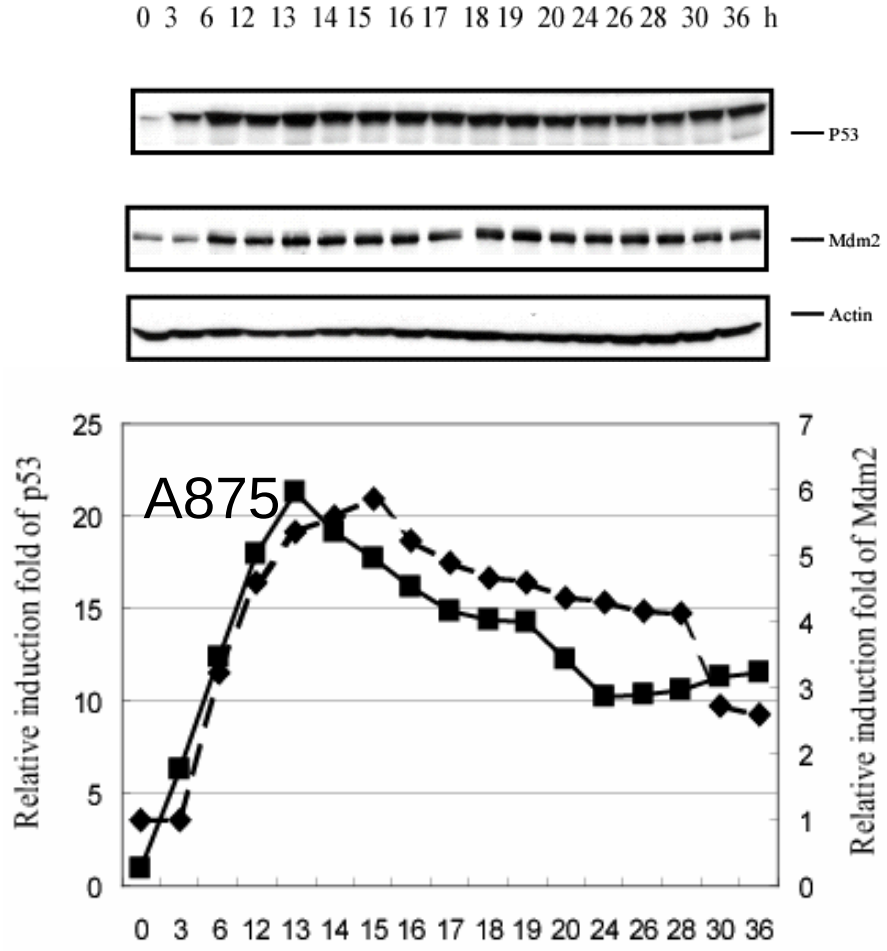


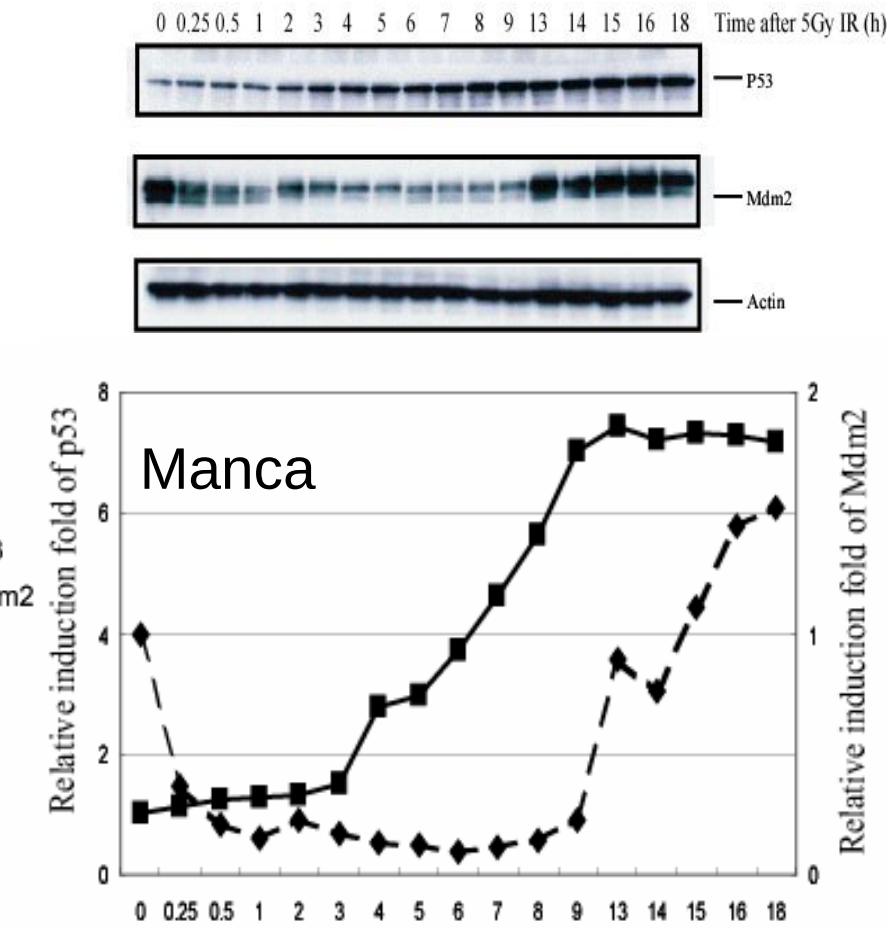


NB: First p53 peak at 2 hours, second peak at 7 to 8 hours.

G/G Cell Lines A875 and Manca Do Not Oscillate

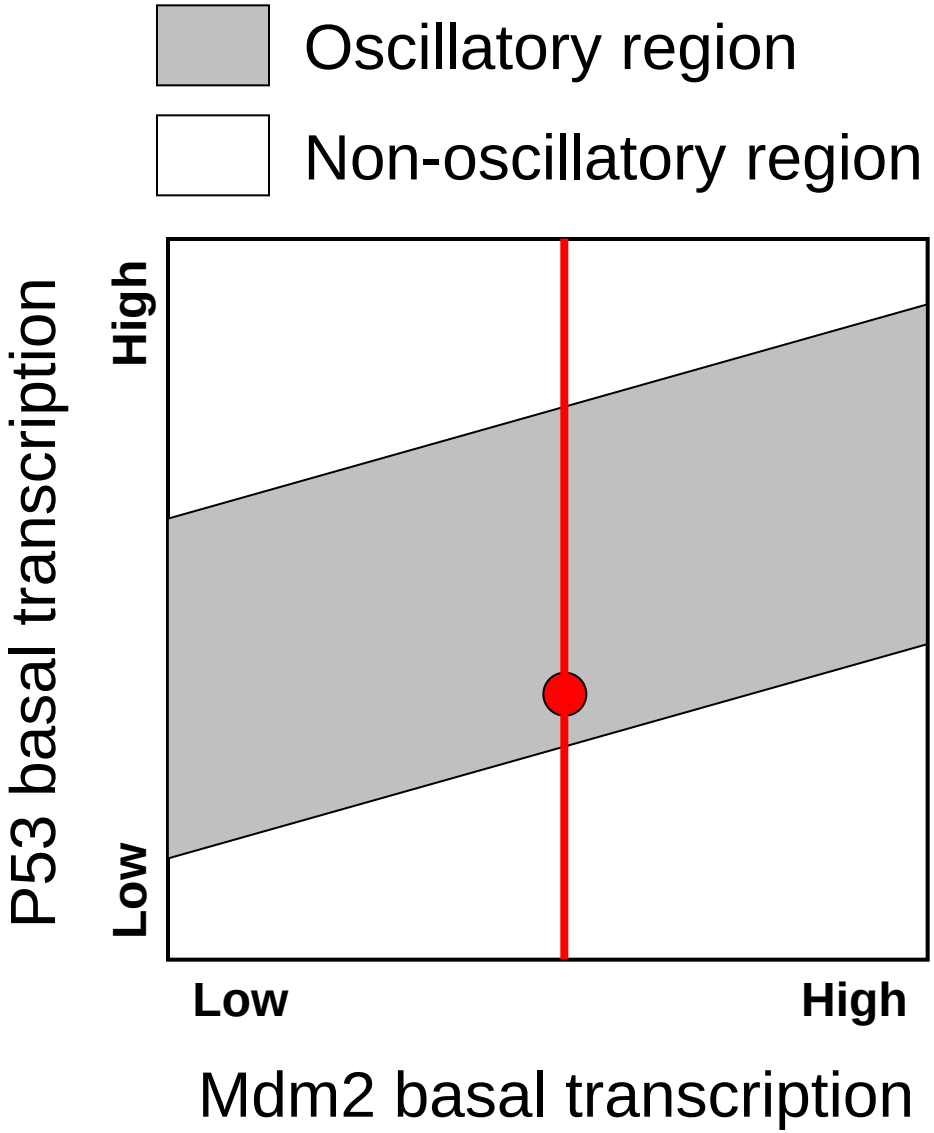
Two cell lines homozygous (G/G) for SNP309 do NOT oscillate in response to 5Gy IR.

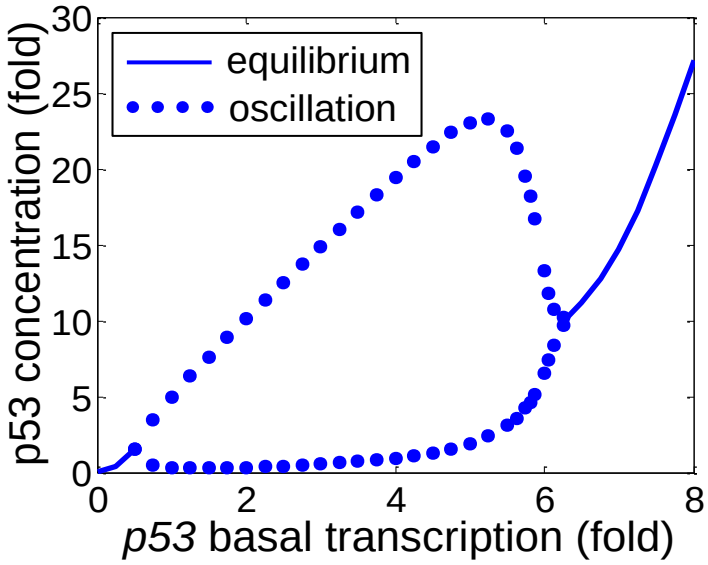




NB: p53 peaks at 10 to 12 hours.

Model Predicts Hopf Bifurcations WRT p53 Production



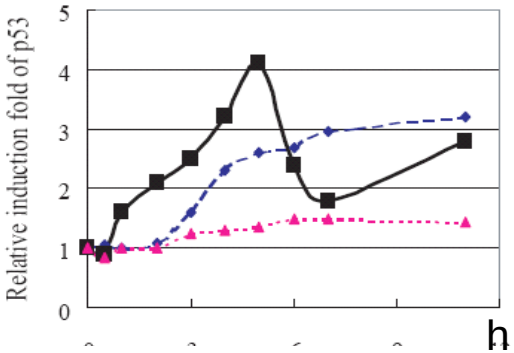


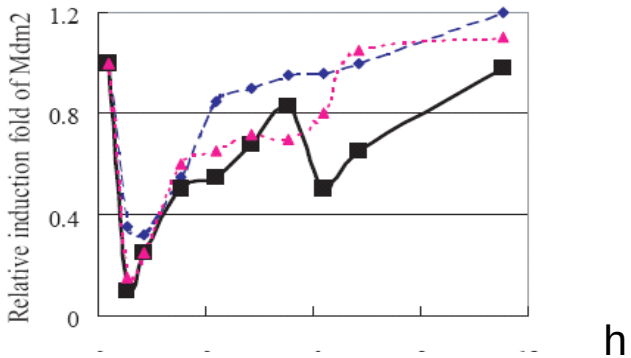
Oscillations Require Intermediate p53 Production Rates

P53-null H1299-SW24 cells expressing p53 under tetracycline-dependent promoter.

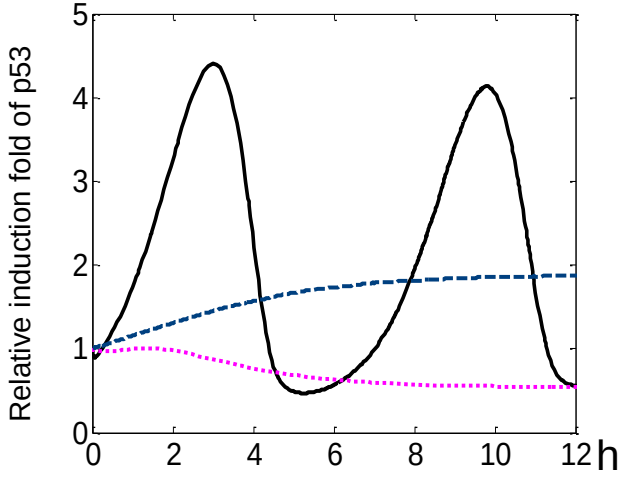
p53 Mdm2

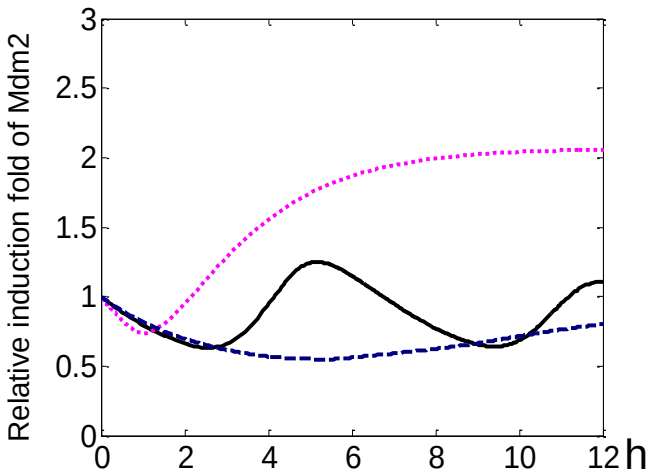
Experiment





Model





Low Tetracycline
High P53

Medium Tetracycline
Medium P53

High Tetracycline
Low P53