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IBM Research

Digital response of the p53 to DNA damage: A tale of limiting resources, negative feedback and time delays

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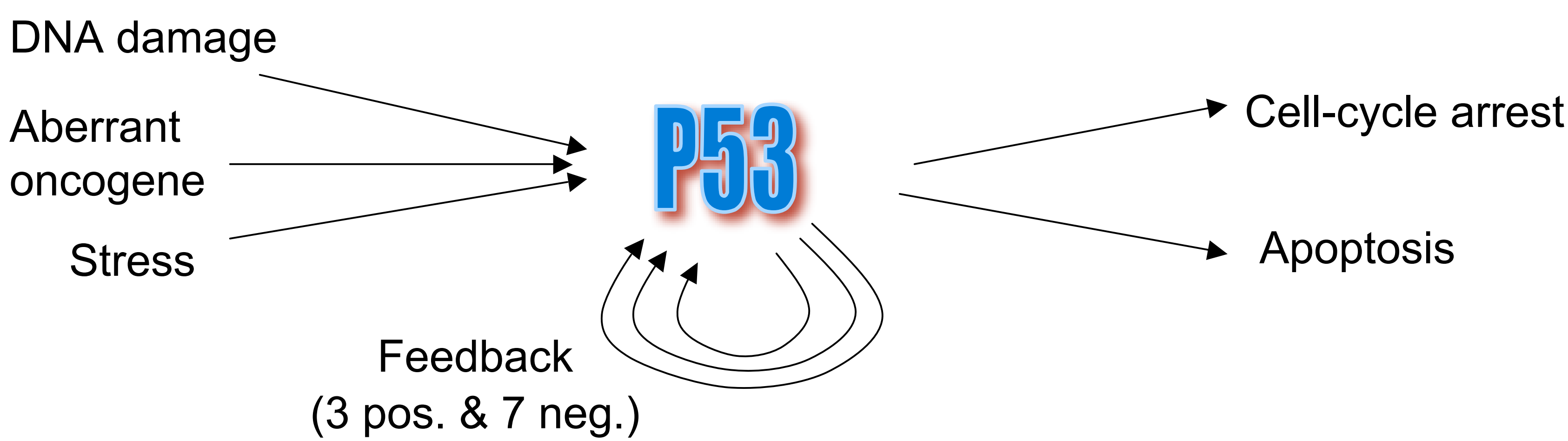
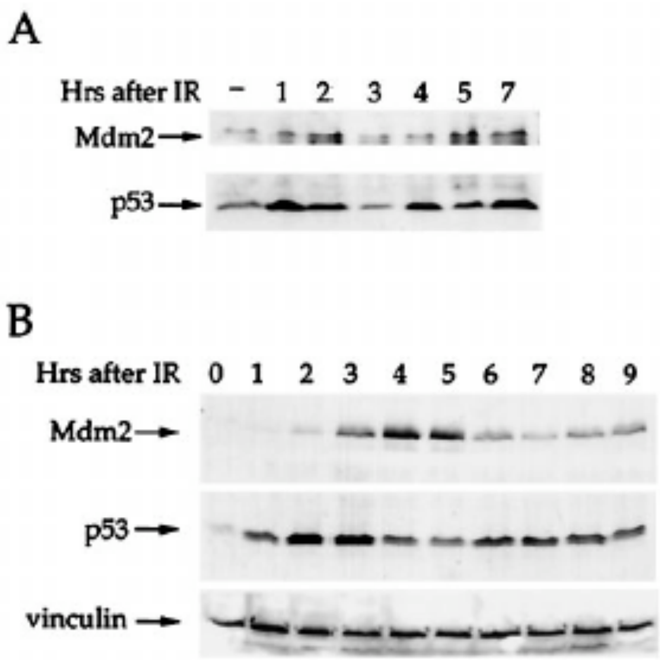
Roles of P53

- Transcription factor
- Central role in defending genomic stability
- Decides on DNA repair and possibly apoptosis
- Implicated in over 50% of cancers
- Highly regulated in positive and negative feedback circuits

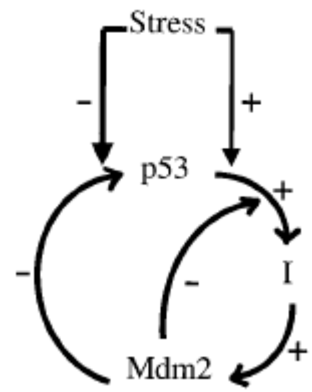


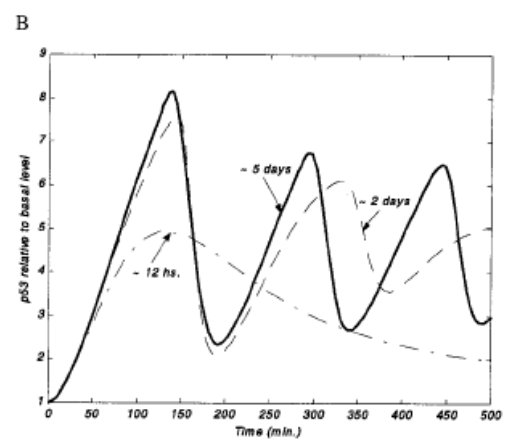
p53 – MDM2 auto-regulation

Protein level after irradiation (IR)



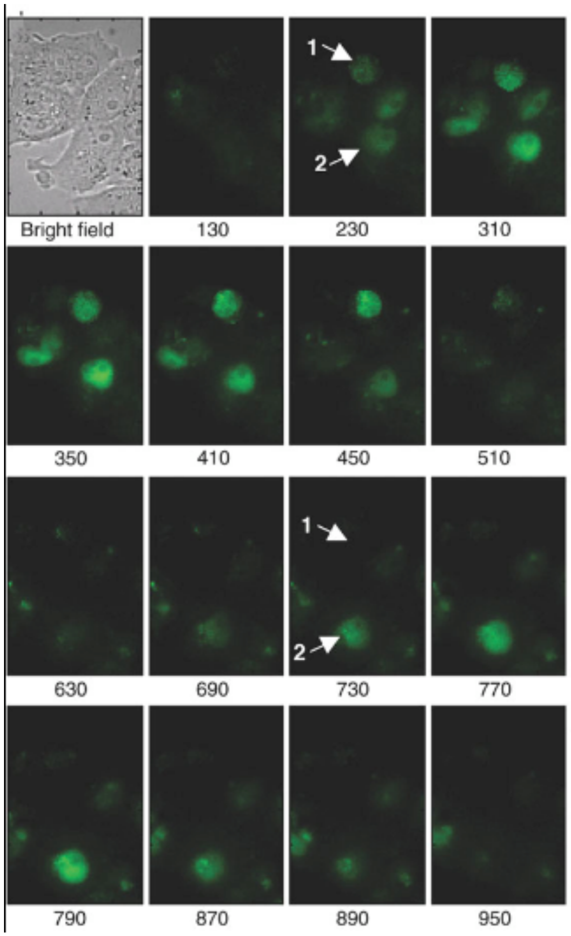
Bar-Or *et al., PNAS,* 2000



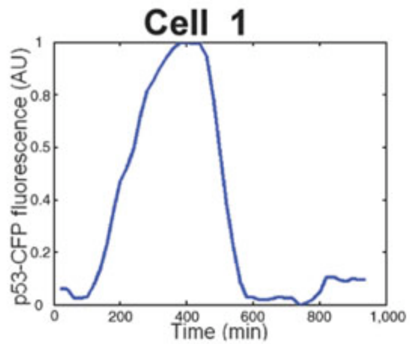


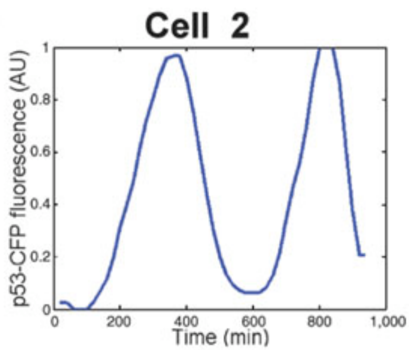
Reflects population but not single cells

Digital Clock: individual cells

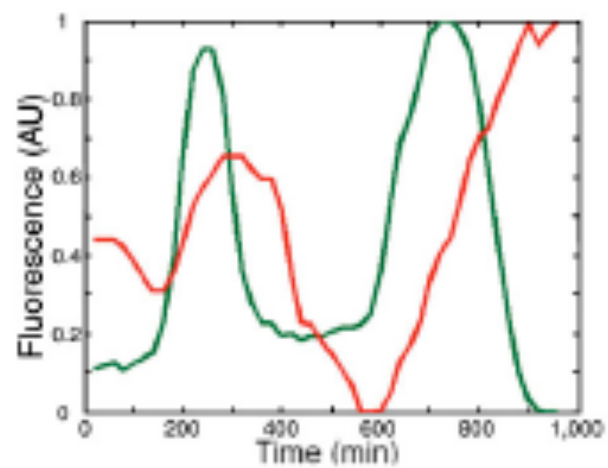


P53 protein





Coordinated oscillations of P53 and MDM2

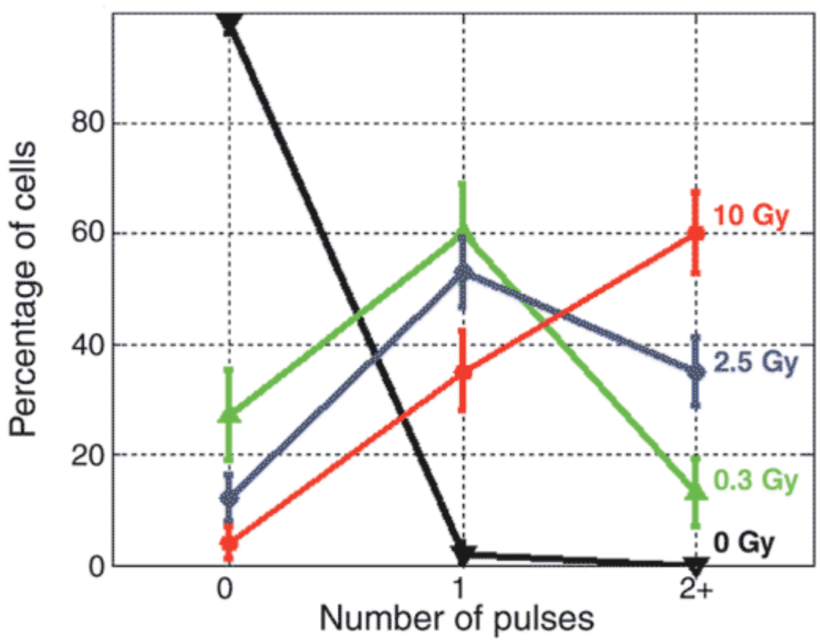


Lahav et al., *Nature Genetics,* 2004

Oscillations are not damped at single cell level

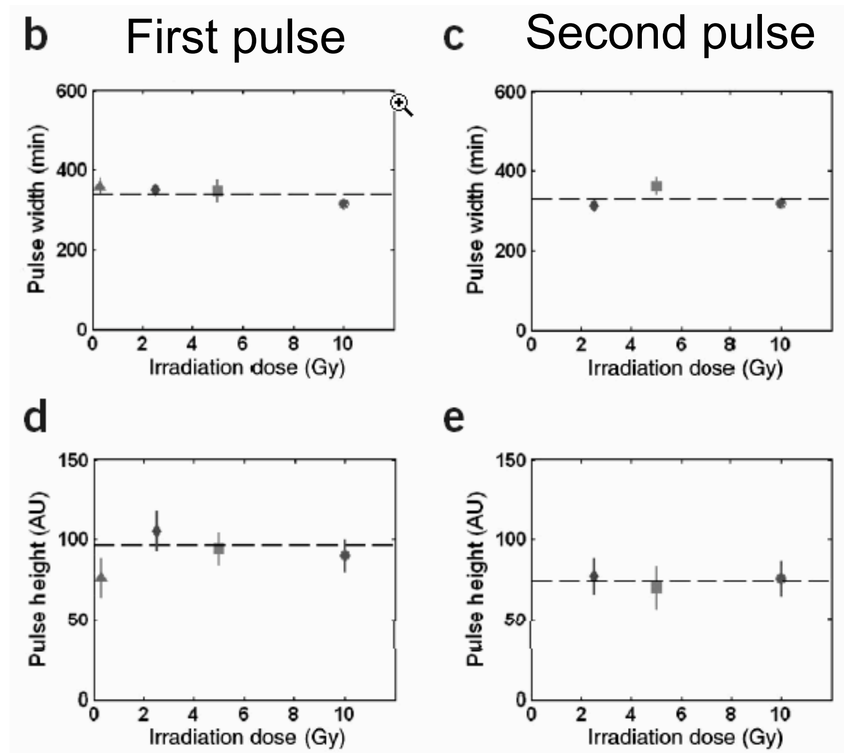
Digital Clock: individual cells

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



Lahav et al., *Nature Genetics*, 2004

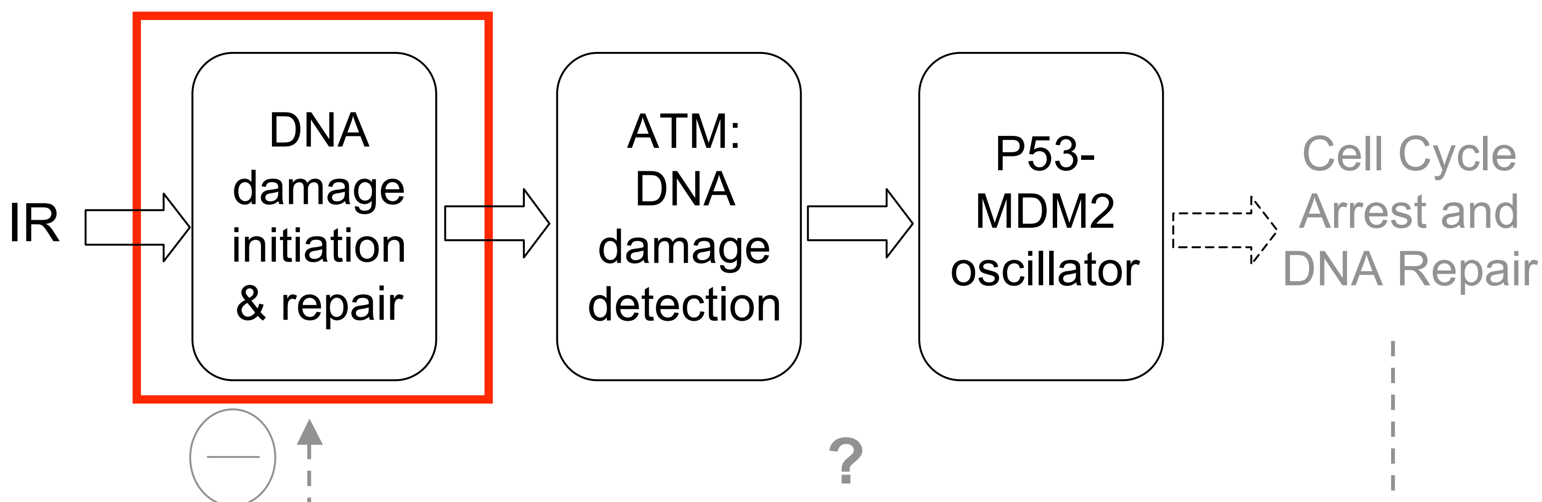
Pulse width and height as a function of γ-IR dose:



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

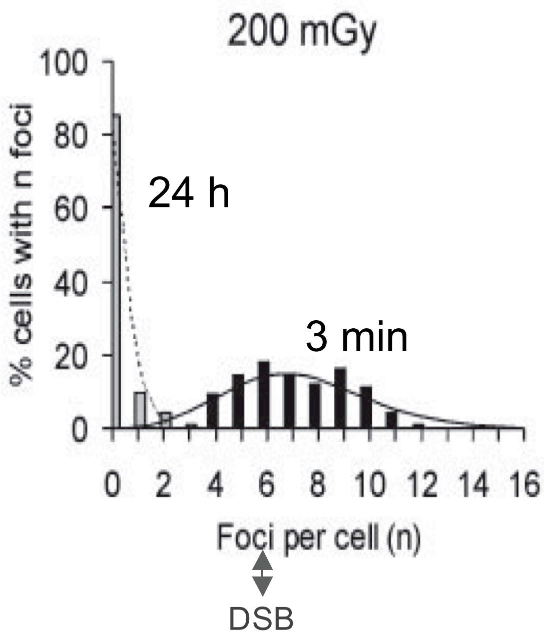
Modeling digital behavior

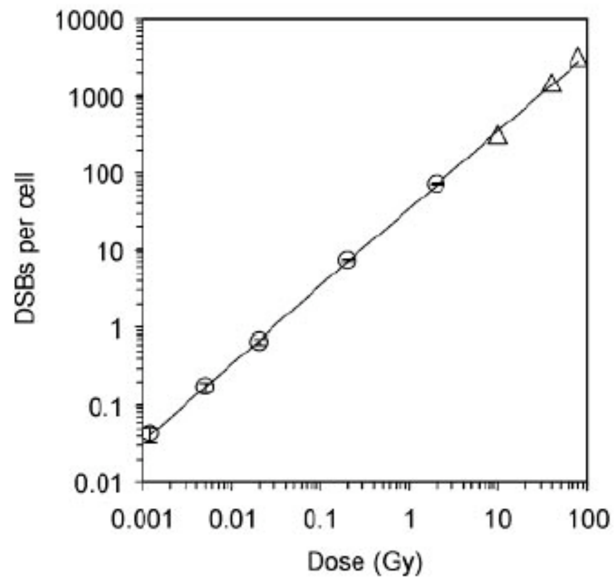
Basic structure of the model



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} $cell^{-1}$)

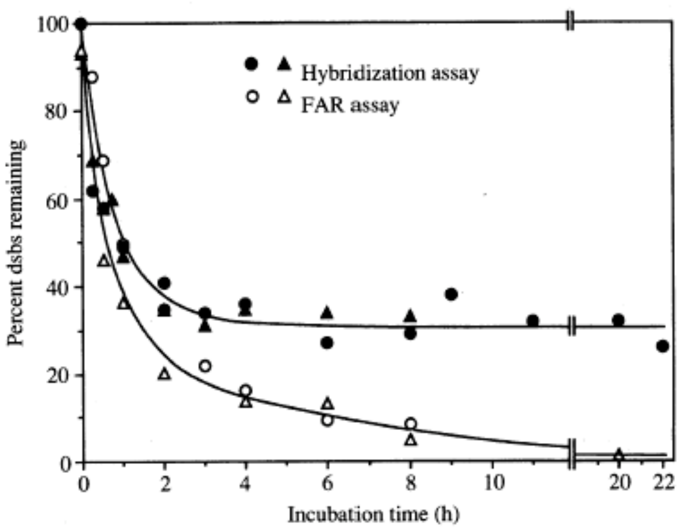




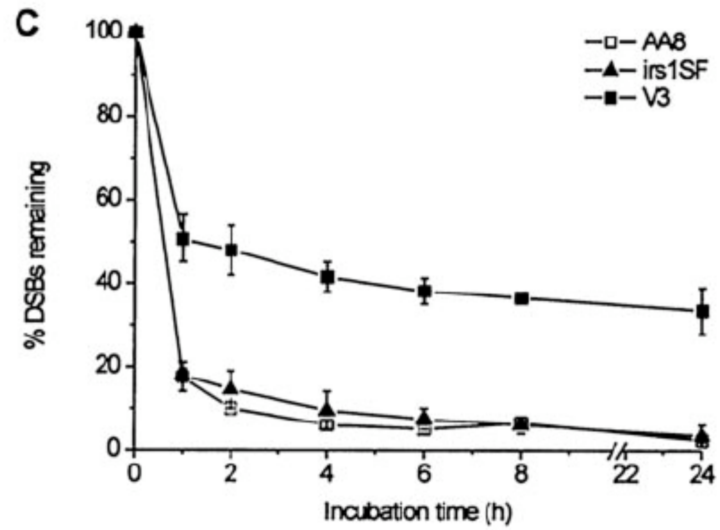
Rothkamm & Löbrich, *PNAS,* 2003

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)



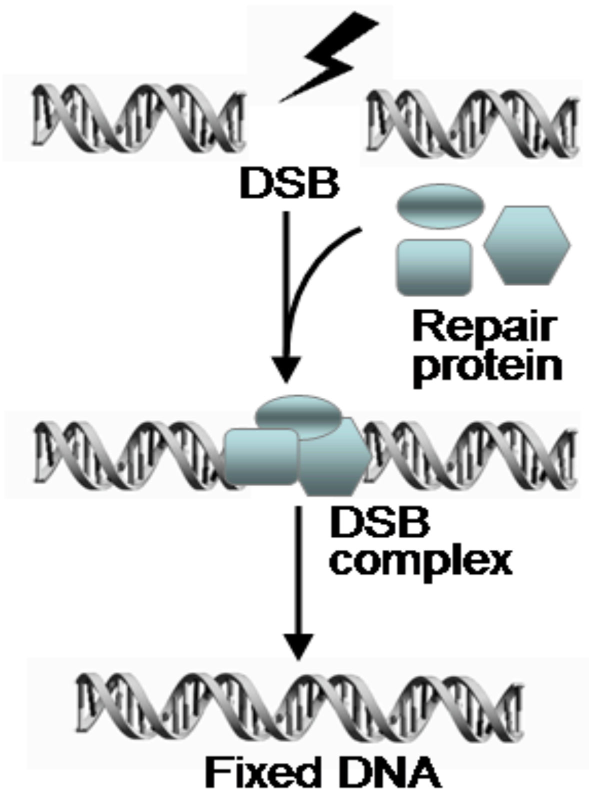
Löbrich *et al.*, *PNAS,* 1995



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: <u>fast</u> DSB lesion repair

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

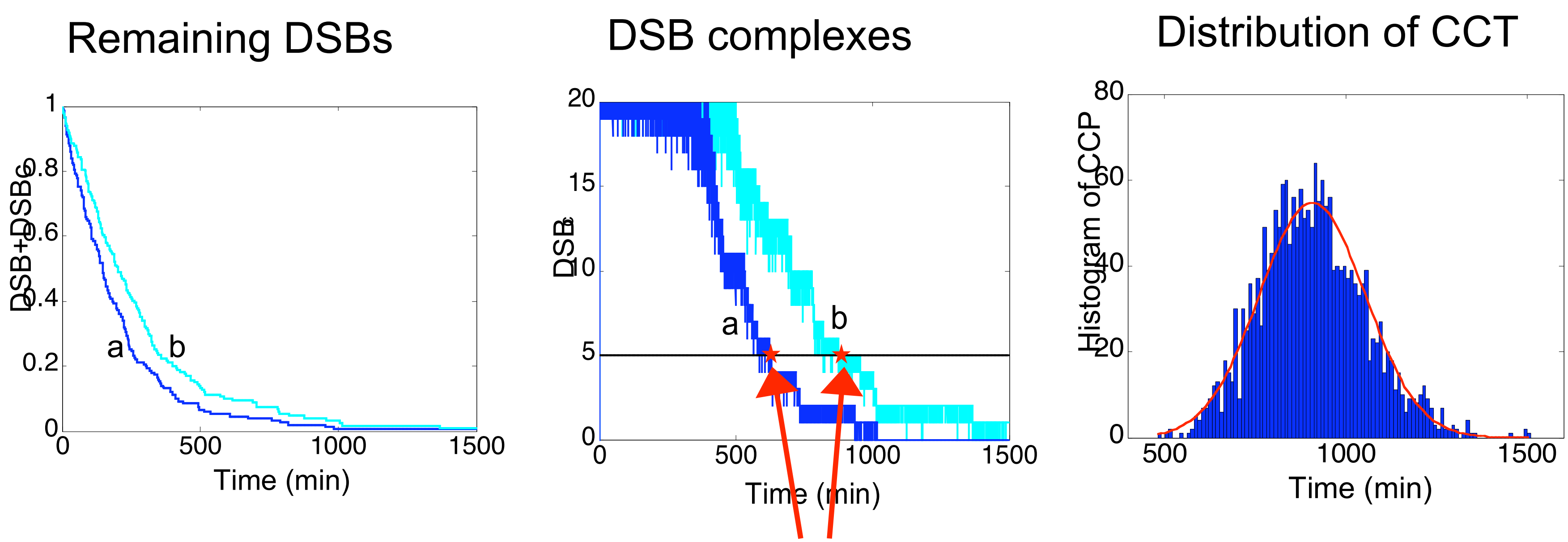
Pathway 2: <u>slow</u> DSB lesion repair:

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

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

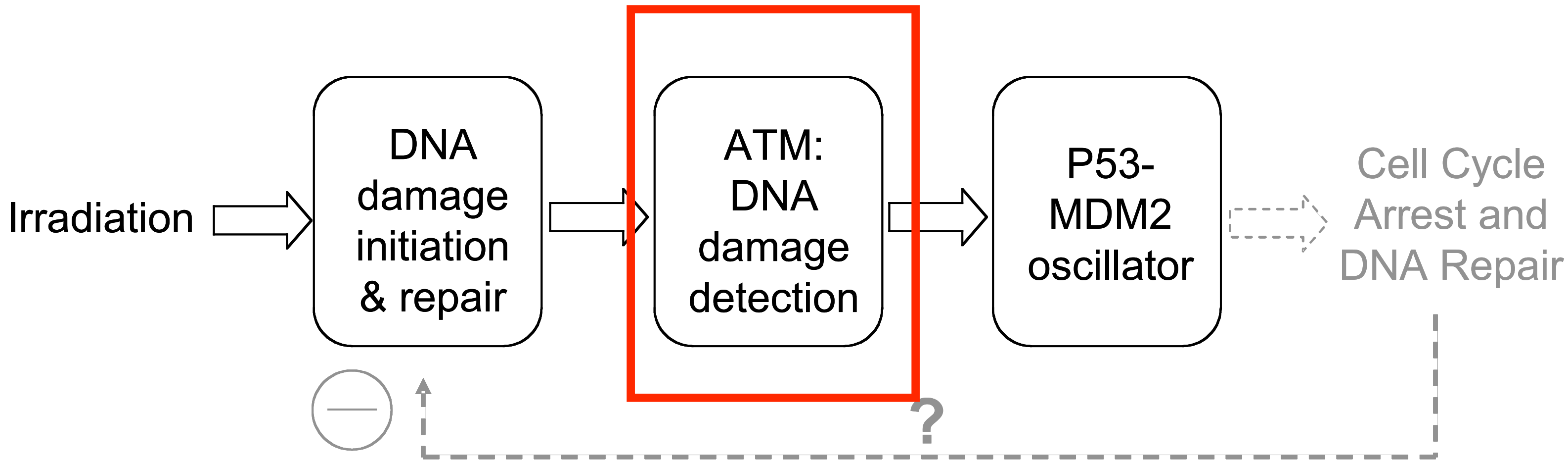
Simulation: DNA repair process

Implemented using Monte-Carlo method:



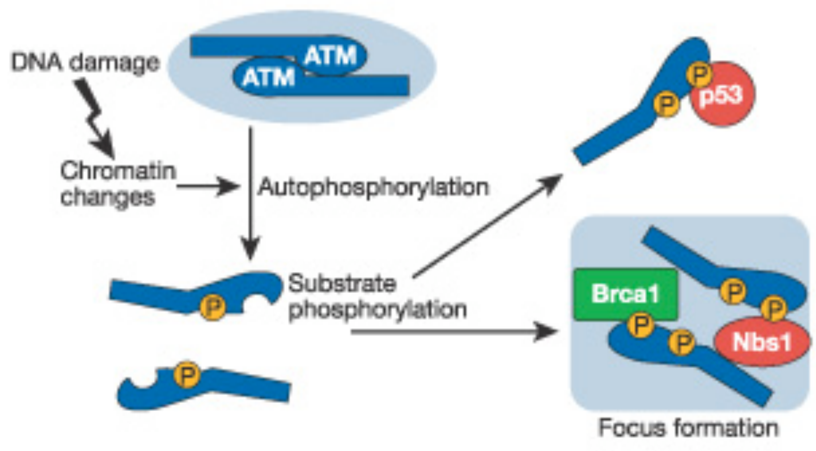
CCT: critical crossing times

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.

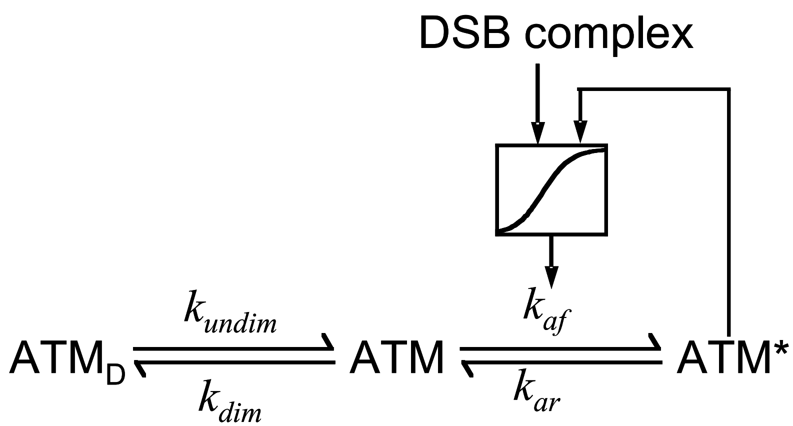
ATM activation



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

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

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

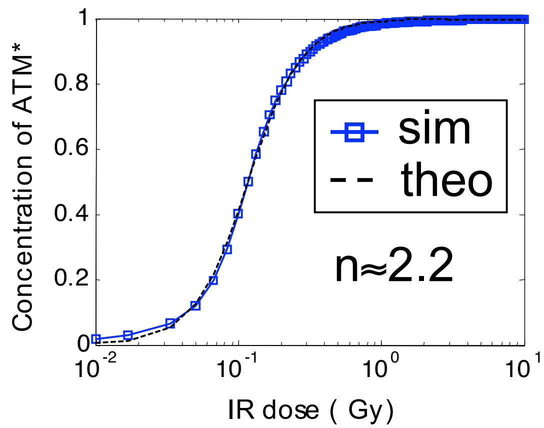
$$\frac{d\mathrm{ATM}}{dt} = 2k_{un\dim} \mathrm{ATM_D} - k_{\dim} \mathrm{ATM}^2 - k_{af} f(\mathrm{C},\ \mathrm{ATM}^*)\mathrm{ATM} + k_{ar} \mathrm{ATM}^*$$

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

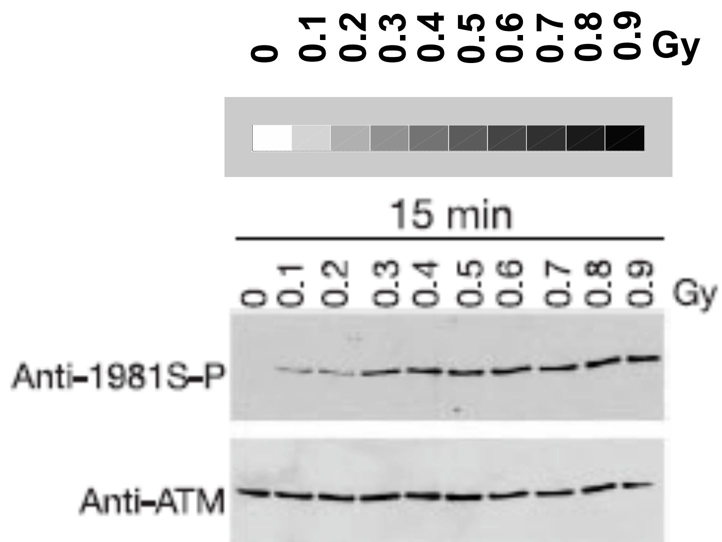
Where $f(\mathrm{C},\ \mathrm{ATM}^*) = (\alpha_1 C + \alpha_2 \mathrm{C} * \mathrm{ATM}^* + \alpha_3 \mathrm{ATM}^*)$ and C is DSB complex

Simulation: Switch like behavior of ATM*

Correlation b/w ATM* & IR

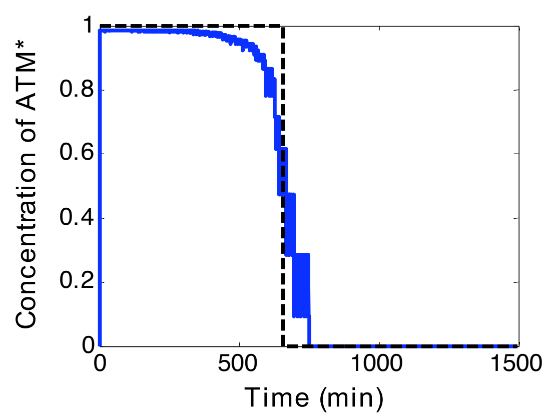


Normalized by ATM^T

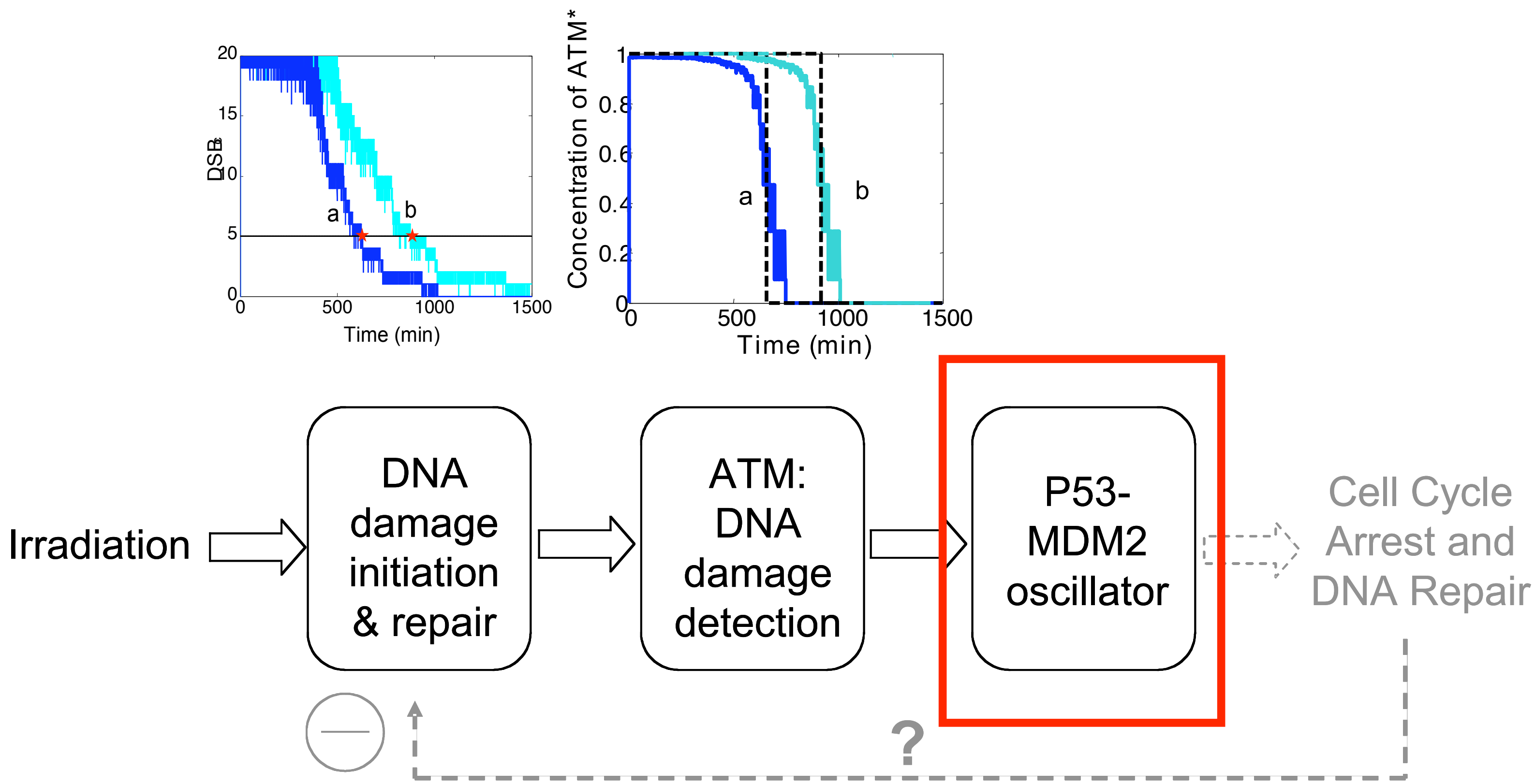


Bakkenist & Kastan, Nature 2003

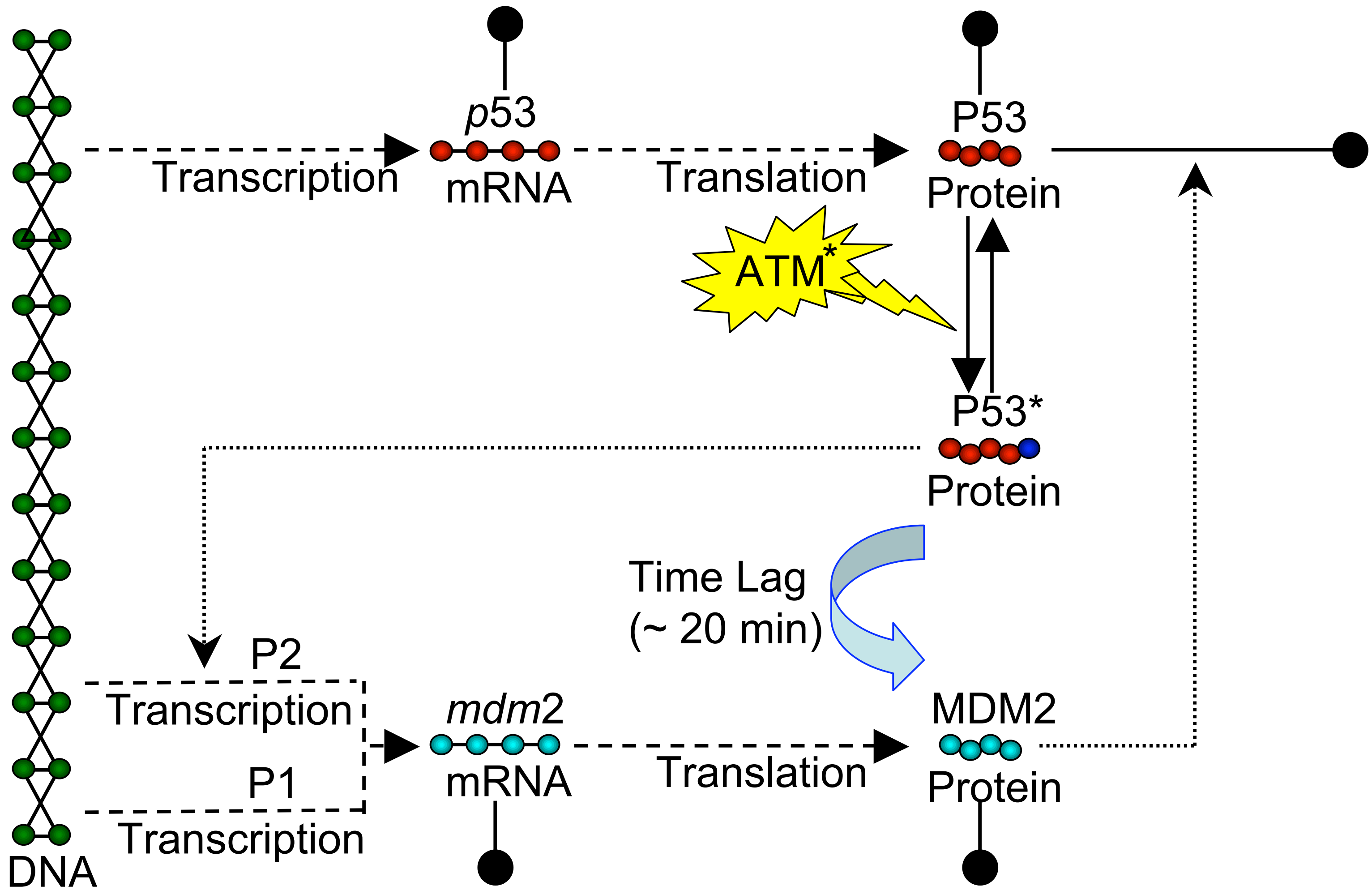
Time response:
ON-to-OFF
signal



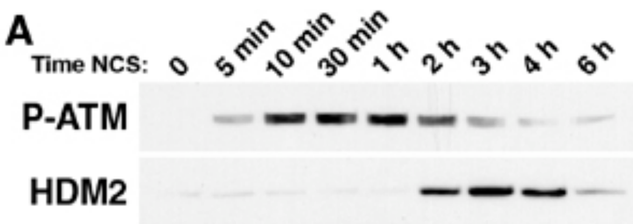
Basic structure of model



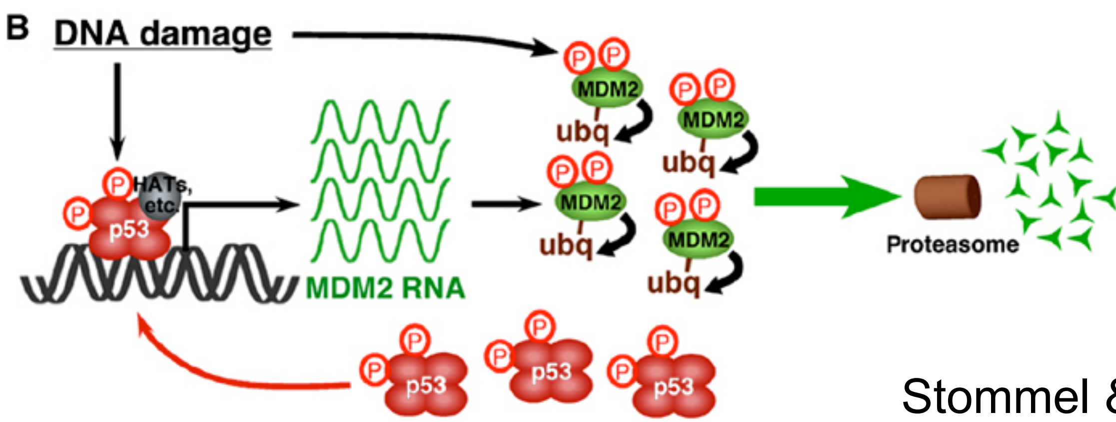
The p53-MDM2 Negative Feedback Loop



Signal transduction to oscillator



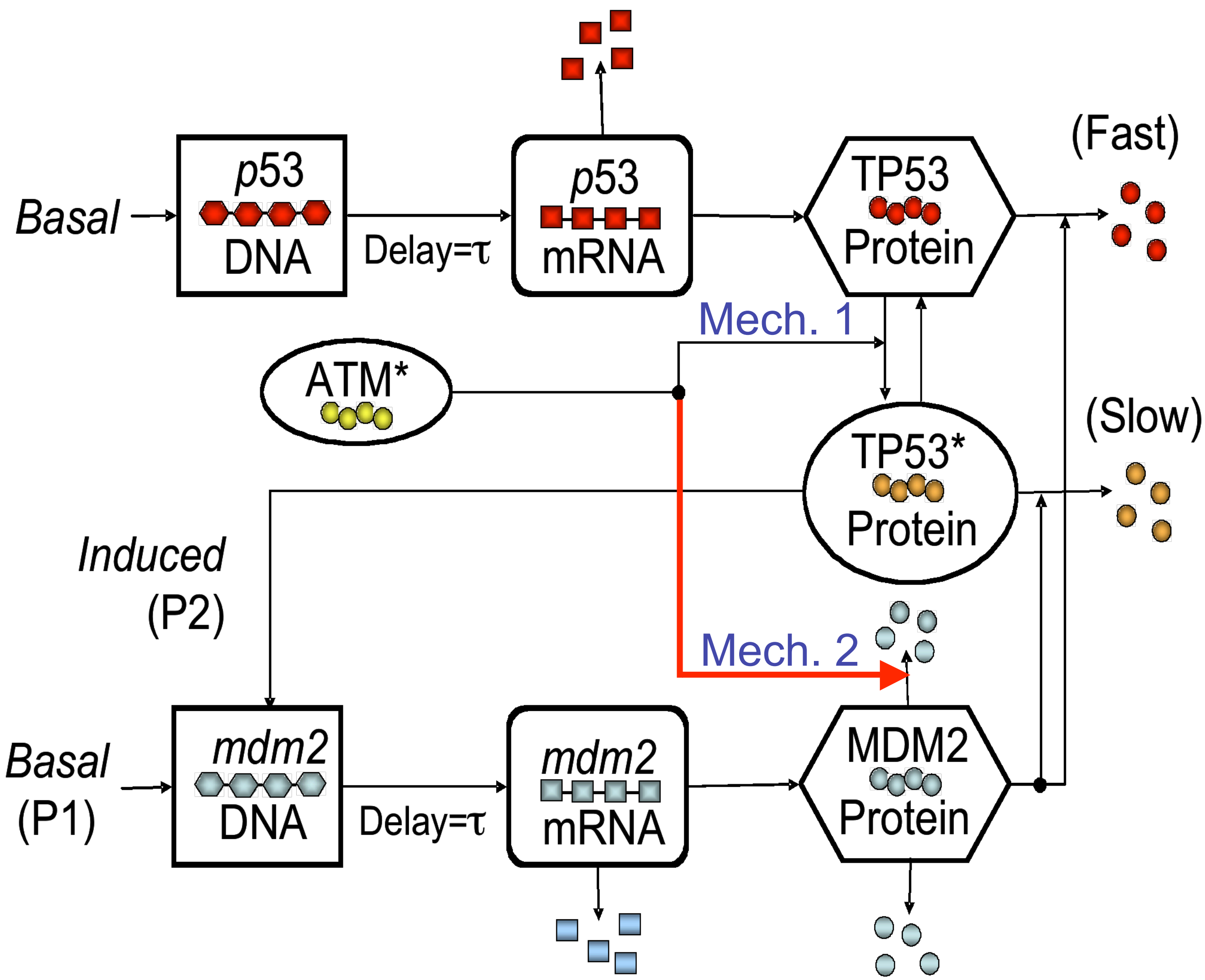
Accelerated autodegradation of MDM2 by DNA damage kinase is necessary for p53 activation



Stommel & Wahl, *EMBO* 2004

ATM* directly activates MDM2 auto-degradation

Modified p53 – Mdm2 oscillator



p53 – Mdm2 oscillator: equations

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

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

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

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

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

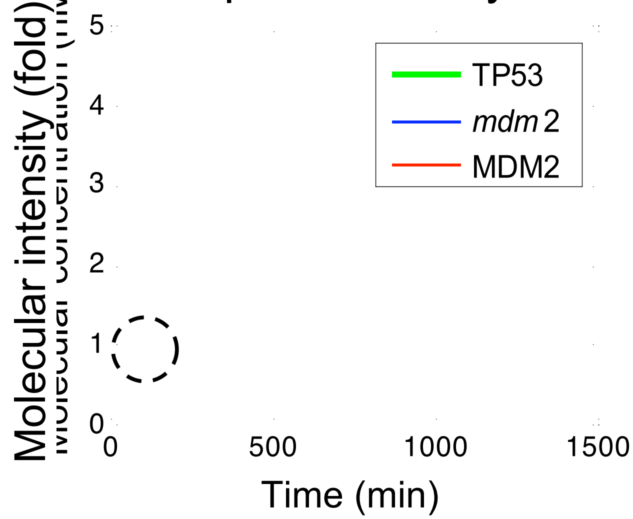
n=4

mRNA: p*53, mdm*2

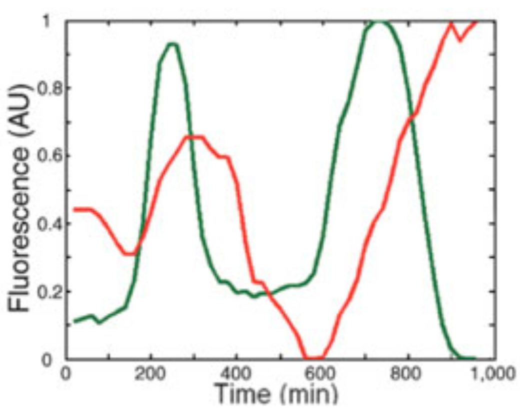
Protein: TP53 (inactive), TP53* (active / phosphorylated), MDM2

Complete Model Results

Response to 5Gy

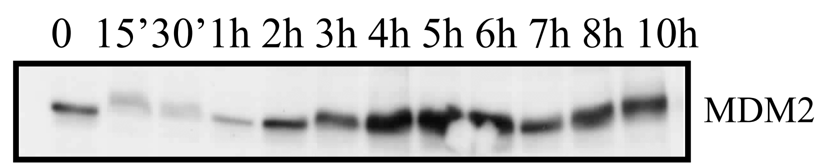


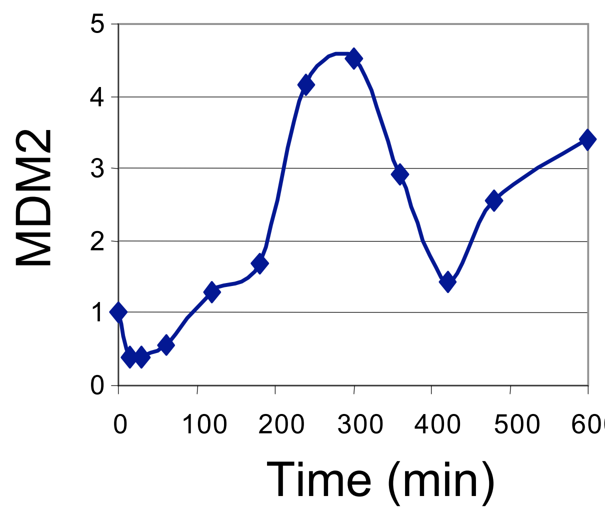
Predict drop of MDM2 at the beginning of time course



Lahav *et al.*, Nature Genetics 2004

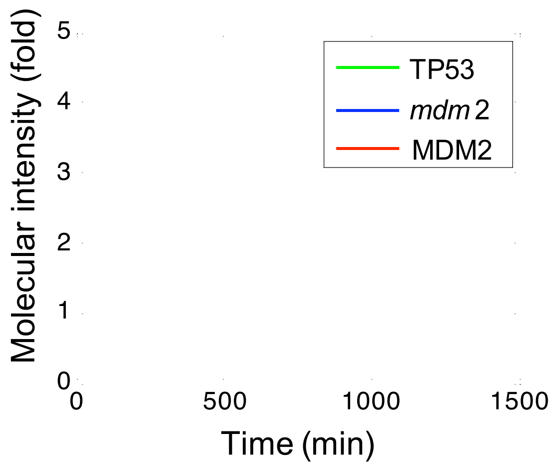
Experiment

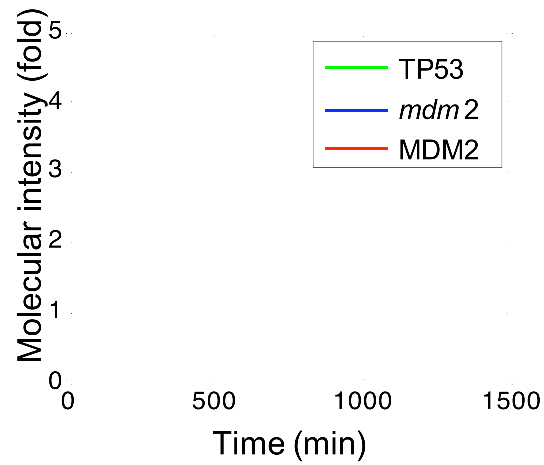


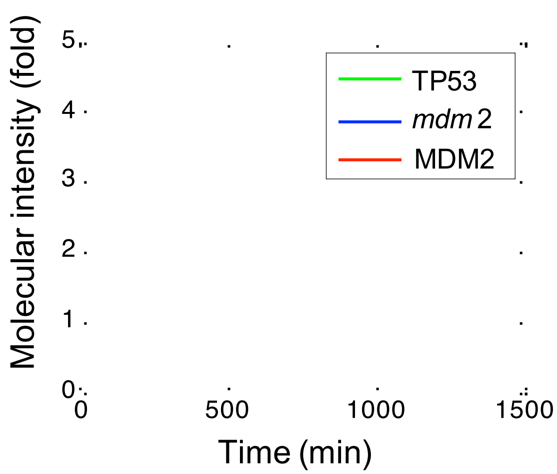


Complete Model Results

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

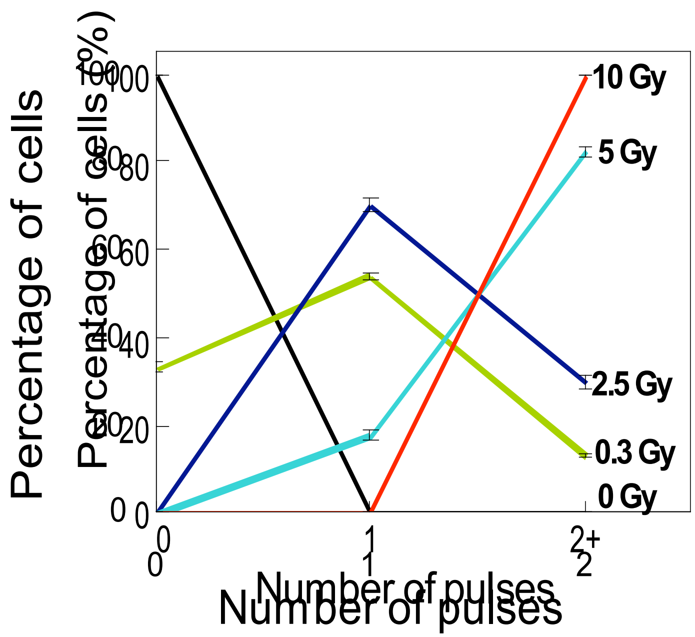


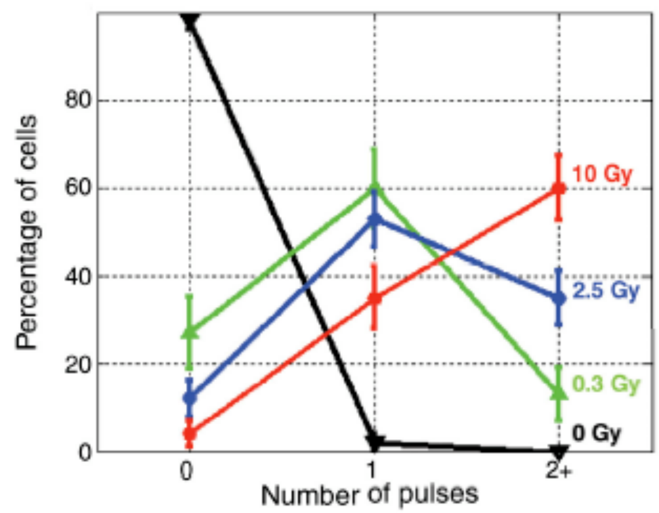




Note: molecular intensity is normalized by respective basal concentration.

Complete Model Results



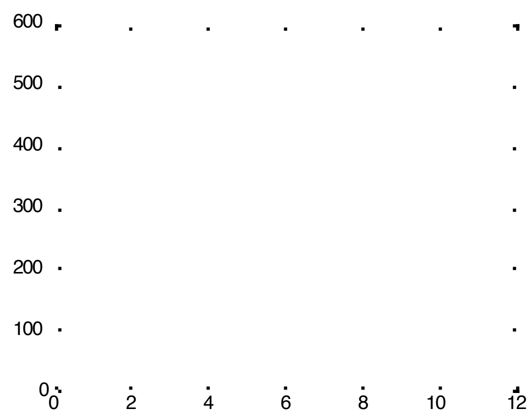


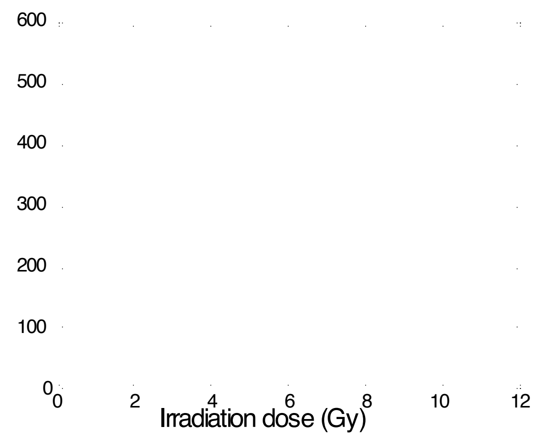
Lahav *et al.*, Nature Genetics 2004

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

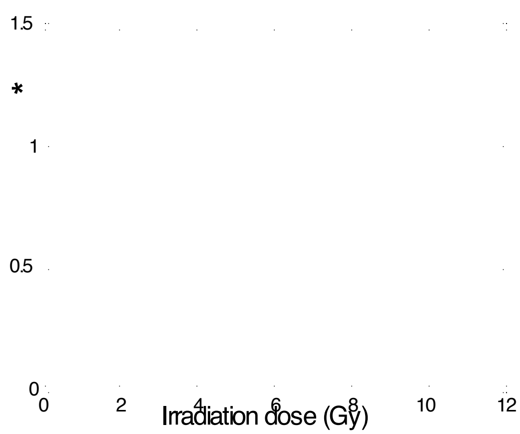
Digital behavior

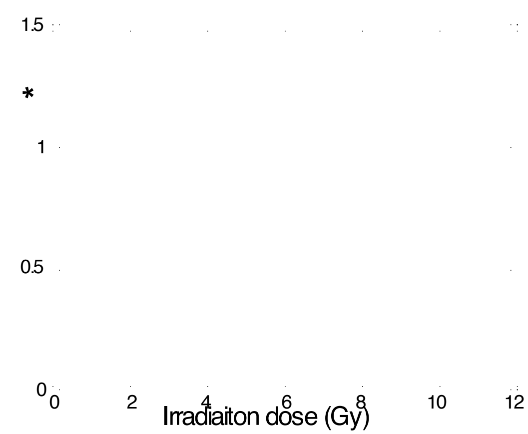
Period as function of IR dose:





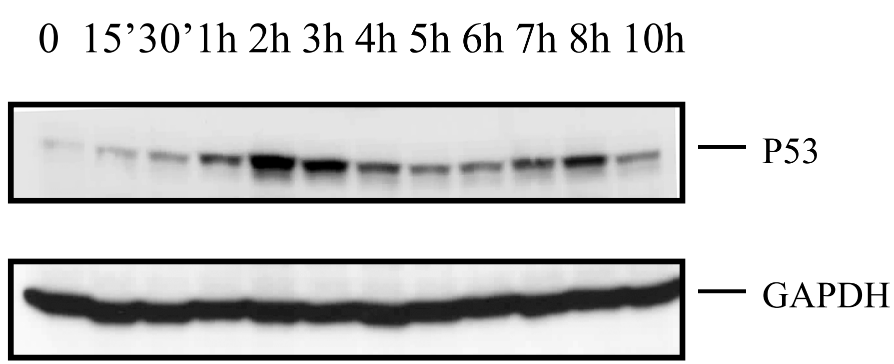
Pulse height as function of IR dose:

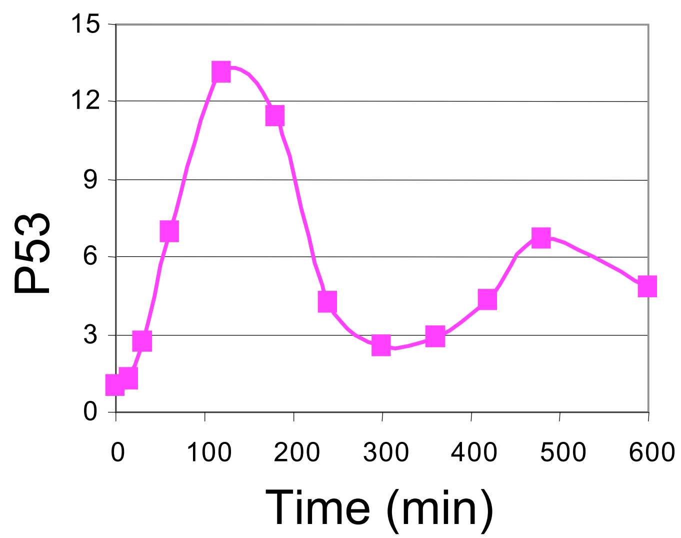




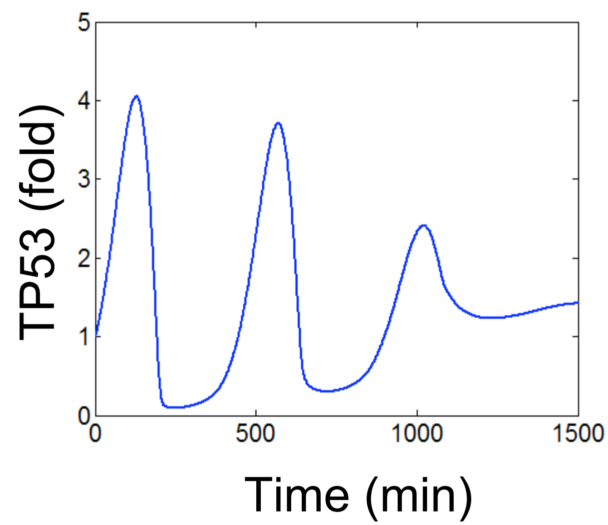
Simulating a cell population

Experiment

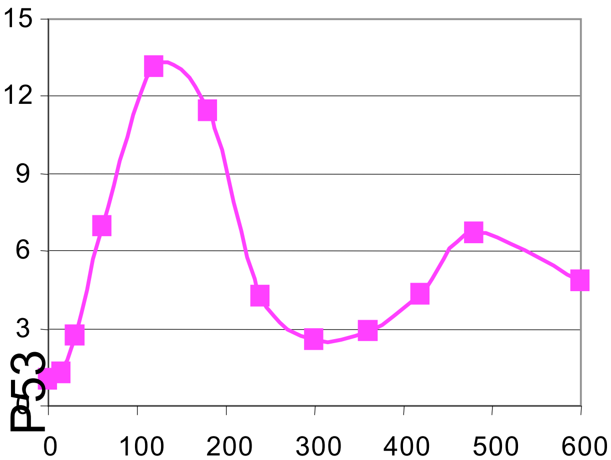


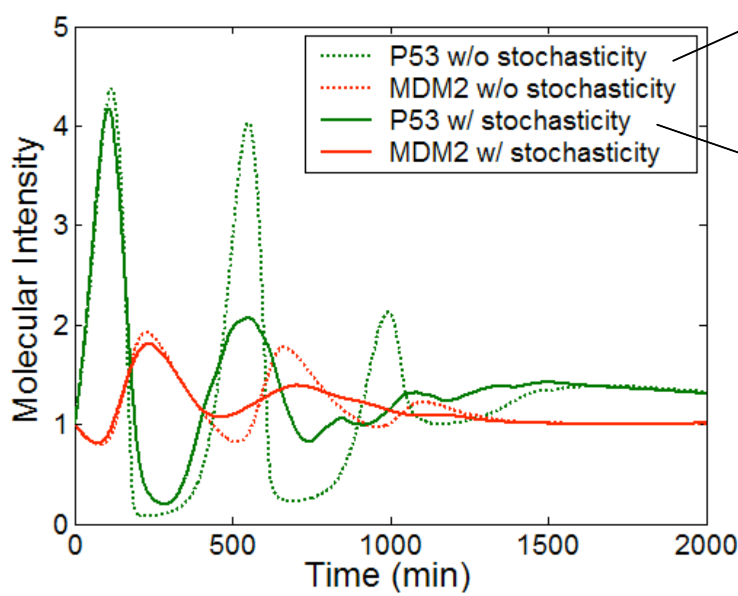


Simulation

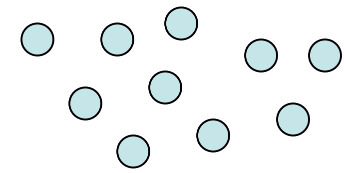


Smarter simulation of cell populations

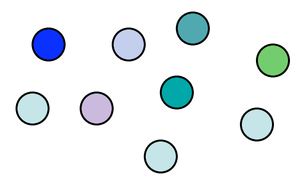




Uniform Population



Stochastically Varied Population

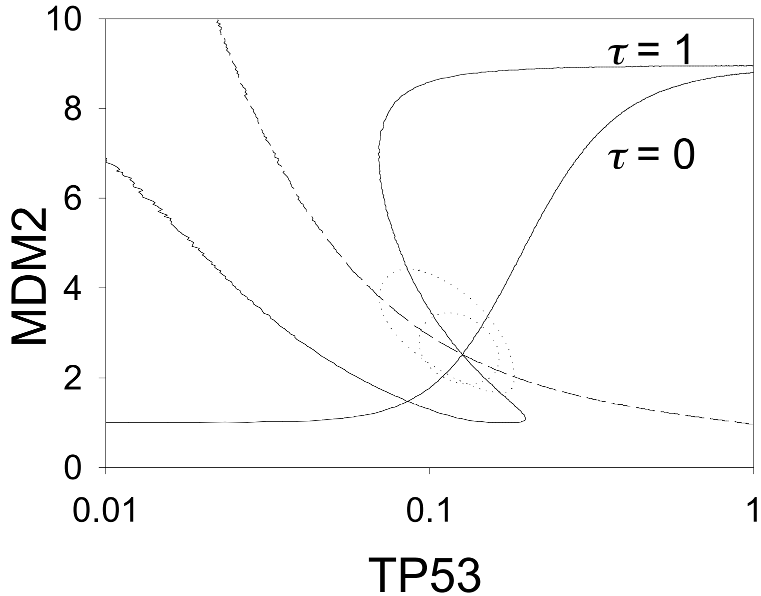


0.5 l and 1.5 l where l is a reference parameter value for time delay, no. of repair proteins and the total amount of ATM

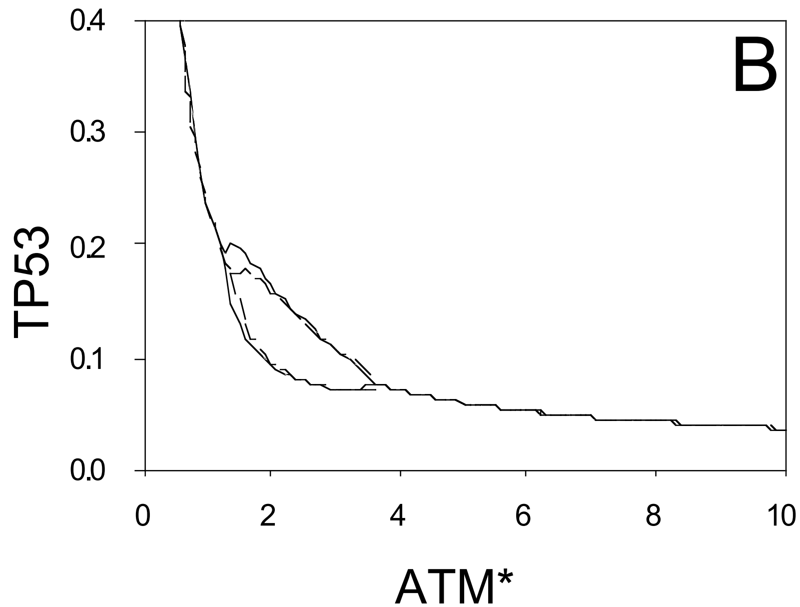
Hopf Bifurcation

Dimensionless oscillator

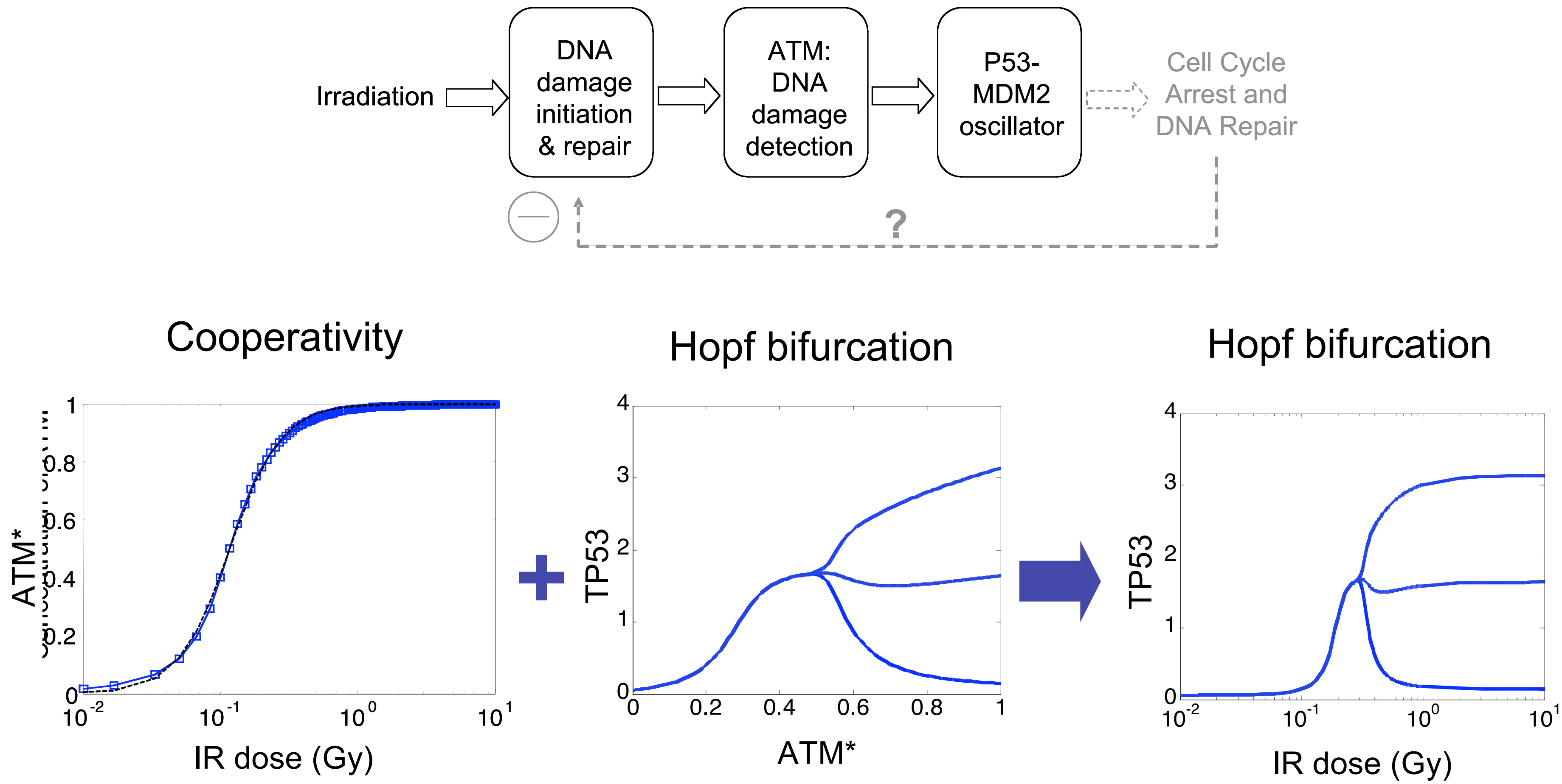
Phase plane: reduced 2D oscillator



Bifurcation diagram w.r.t. ATM*



System as highly sensitive switch



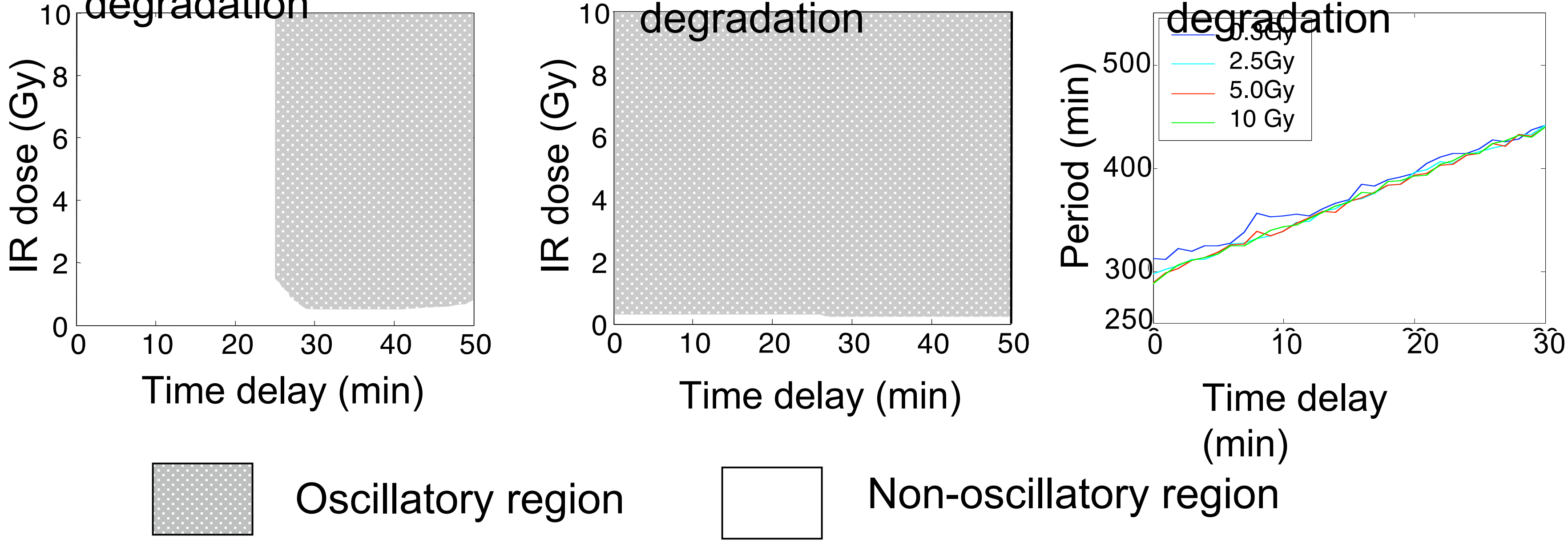
ATM*: normalized to total concentration
TP53: normalized to basal concentration

Stability analysis

w/o ATM* induced MDM2 degradation

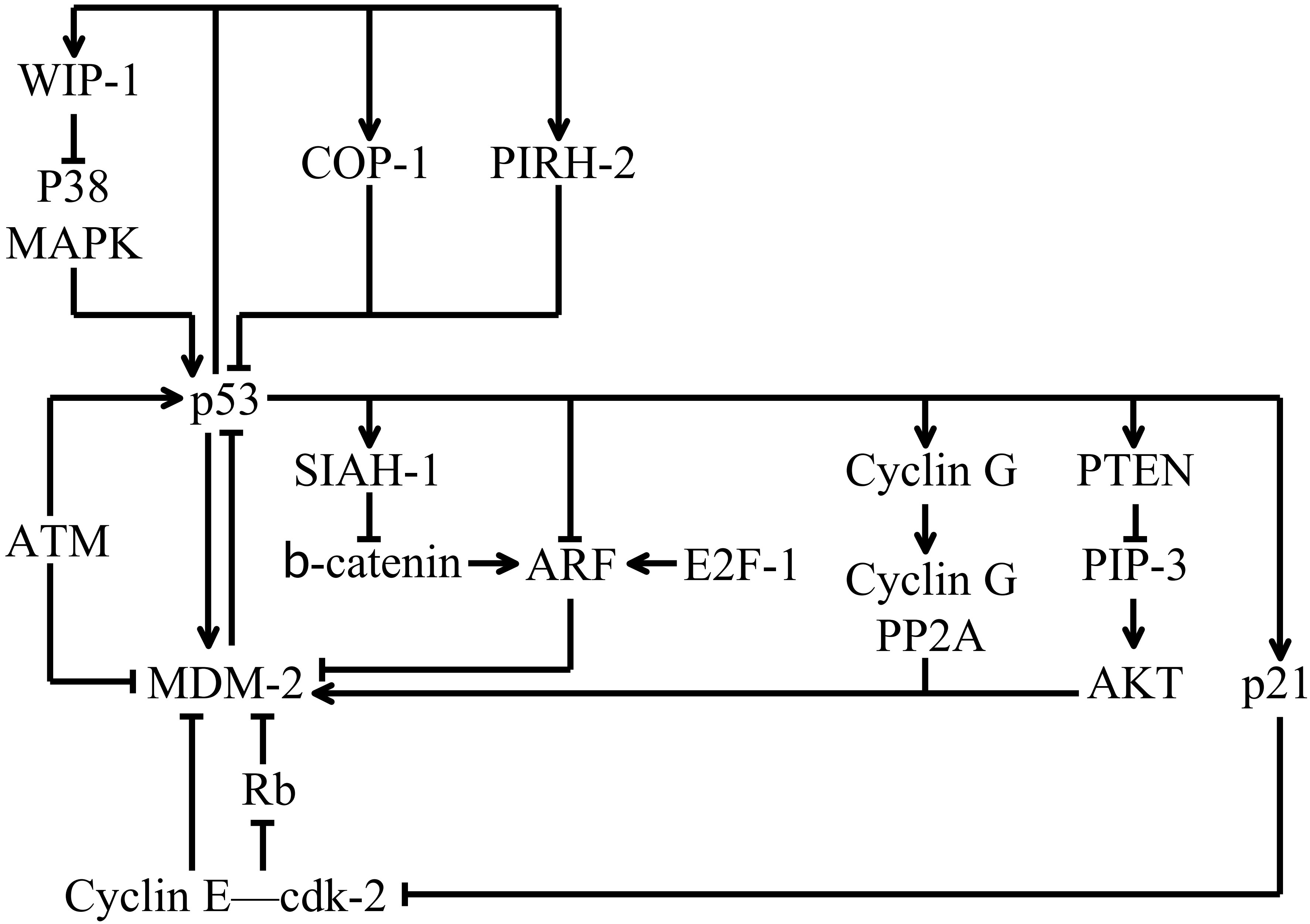
w/ ATM* induced MDM2 degradation

w/ ATM* induced MDM2 degradation



With DNA damage induced degradation of MDM2 by ATM*

- improves the robustness of oscillation
- Time delay not required
- But time delay helps to set period of oscillation


WIP-1
COP-1
PIRH-2
P38
MAPK
p53
SIAH-1
Cyclin G
PTEN
ATM
b-catenin
ARF
E2F-1
Cyclin G
PIP-3
PP2A
MDM-2
AKT
p21
Rb
Cyclin E—cdk-2

Conclusion

- Propose model for digital behavior of p53-mdm2 system to replicate “digital behavior”
- Stable oscillator results from negative feedback loop with time delay
- Initial number and repair process are stochastic processes – sets number of pulses
- ATM is cooperative sensor
- Future work to verify model and extend to apoptosis