# Dynamics of the "life or death" decision circuitry: the p53 DNA-damage responses and intrinsic apoptotic pathway


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# Outline

- Biological background
- P53 response to DNA damage (mechanism driven approach)
- Mechanism of p53 ubiquitination (data driven approach)
- Dynamics of the intrinsic apoptotic pathway

# Basics about the p53 protein

- The p53 protein is a **transcription factor** controlling expressions of many genes involved in: cell cycle regulation, repair of DNA damage, and programmed cell death
- p53 lies at the **hub of** a complex **network** of signaling pathways that integrate a variety of intracellular and extracellular inputs
- The **mutations** of the p53 gene are found in more than 50% of human cancers
- The gene coding for the p53 protein is **constitutively expressed** in normal cells
- Activity of the p53 protein is controlled by **post-transcriptional modifications** and interactions with other proteins
- Response of p53 to genomic stress demonstrates **nontrivial dynamics**
- Understanding how p53 accomplishes its functions **requires quantitative analysis**

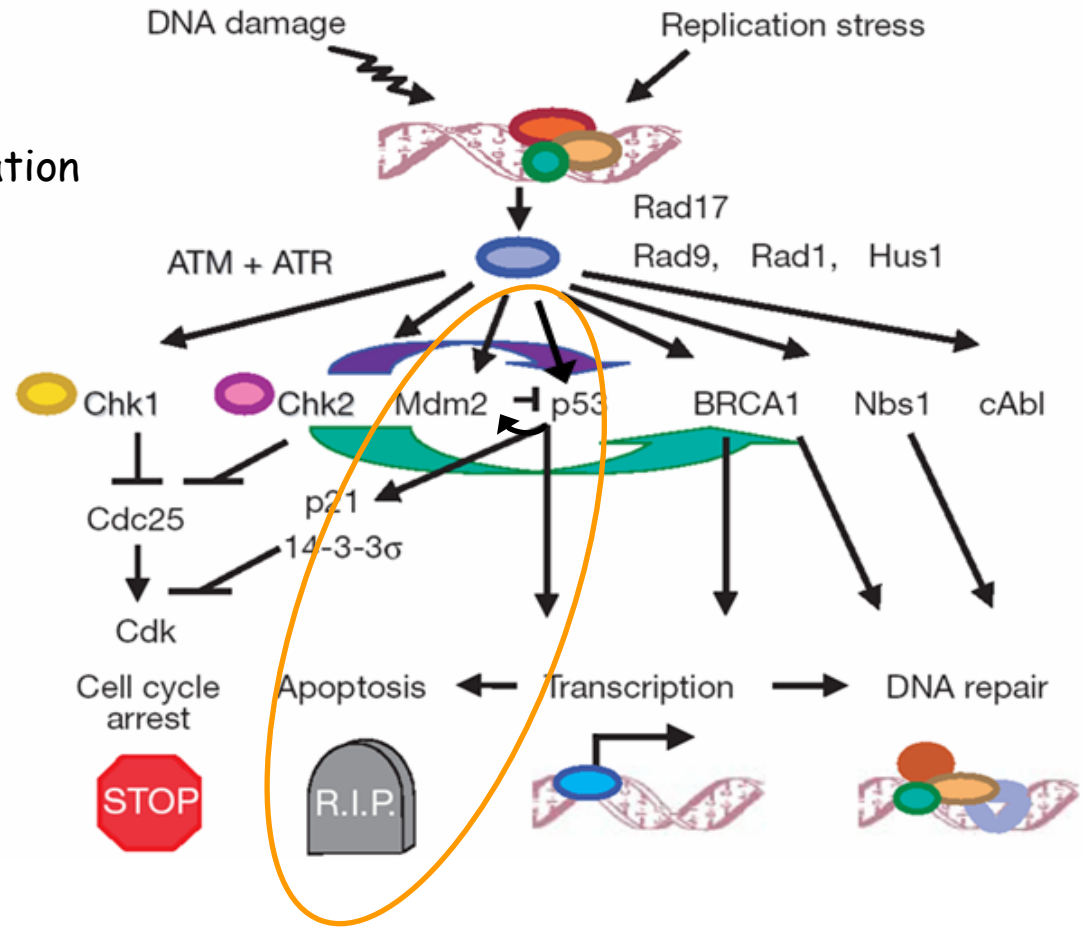
# p53 roles in determining cell fate

- In undamaged cells,
  the p53 level is kept low by Mdm2,
  a protein that promotes p53 degradation
- In response to a DNA damage,
  the ATM kinase is activated
  what leads to elevation of p53
- High p53 induces genes:

  (1) to arrest cell division cycle
  and block DNA synthesis,

  (2) to repair the damage, or

  (3) to commit the cell to apoptosis




*Adapted from: Zhou & Elledge, Nature 2000*

# Big question being asked

How p53 regulated multiple events:

(1) cell cycle arrest

(2) DNA repair

(3) apoptosis ?

IMPORTANCE: Disruption of p53 regulation leads to cancer.

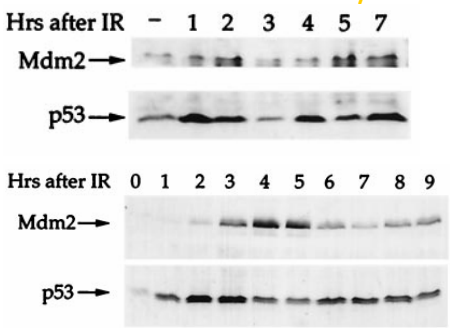
# Experimental observations of p53 variations

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

  Cell populations

  γ-irradiation

Western blot analysis




Mouse fibroblast NIH 3T3 cells

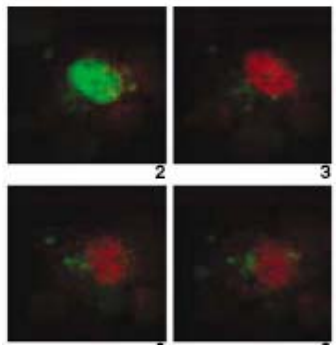
Human breast cancer epithelial MCF-7 cells

- **Lahav** et al, 2004, Nature Genetics
  **Geva-Zatorsky** et al, 2006 MSB

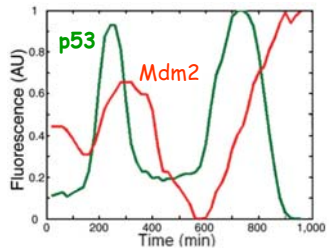
  Individual living cells

  γ-irradiation, MCF-7 cells

Fluorescence microscopy



MCF-7 cells

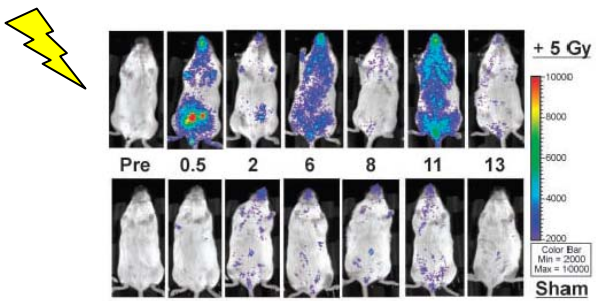


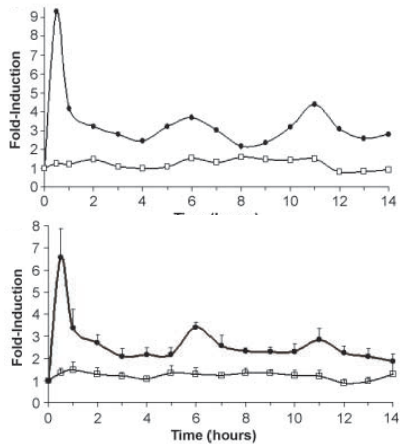

- **Hamstra** et al,
  Cancer Research 2006

  Whole body live animals

  γ-irradiation

Mdm2 P2 promoter-dependent
Luciferase bioluminescence activity



# Two questions

- What mechanism produces observed variations of p53 in response to the DNA damage?

- How might apoptosis be triggered by repeated pulses of p53?

# P53 interaction network

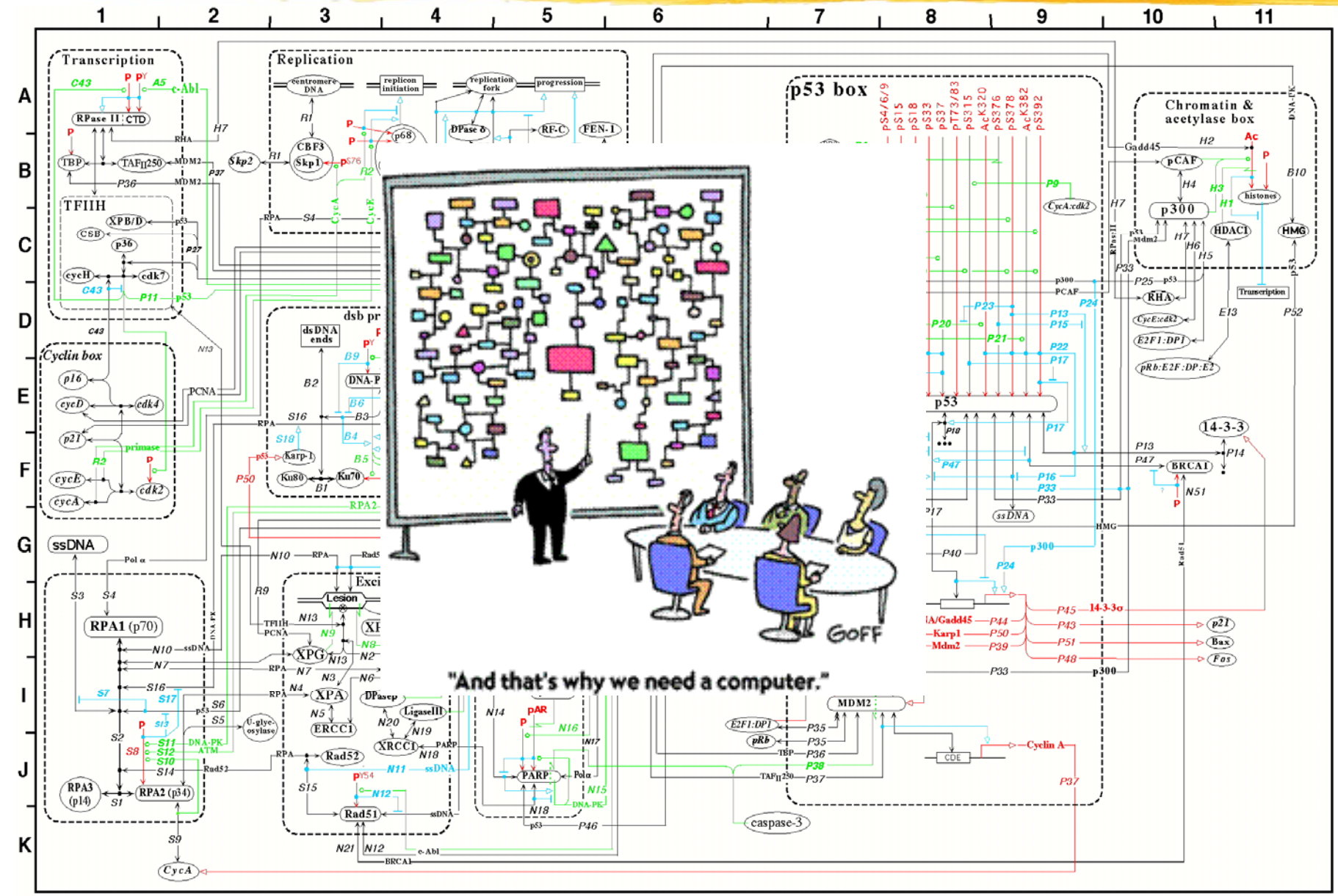


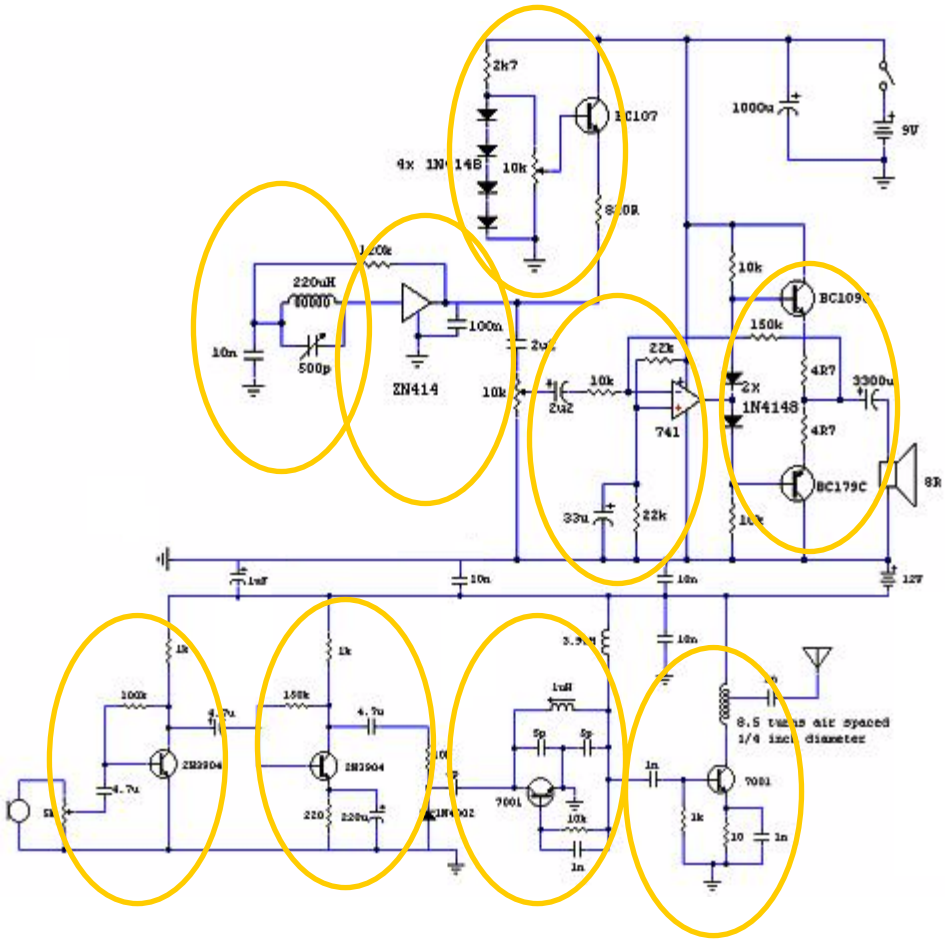

Figure 6B: The p53-Mdm2 and DNA repair regulatory network (version 2p - May 19, 1999)

Kohn, Mol. Biol. Cell 1999

# Why brute-force computation is insufficient…

- 500 ODEs would carry with them 2000 kinetic parameters. How are we to assign numerical values to these parameters?
- Even if we knew the right parameter values, the output from such a large system of equations would be just as mysterious as the behavior of the intact cell !
- We need a theoretical approach that reveals the logic of molecular regulatory systems.

# Molecular networks look like electronic circuits




Complex molecular networks, like electrical circuits, are constructed from simpler modules: sets of interacting genes and proteins that carry out specific task and can be hooked together.

# P53 interaction network

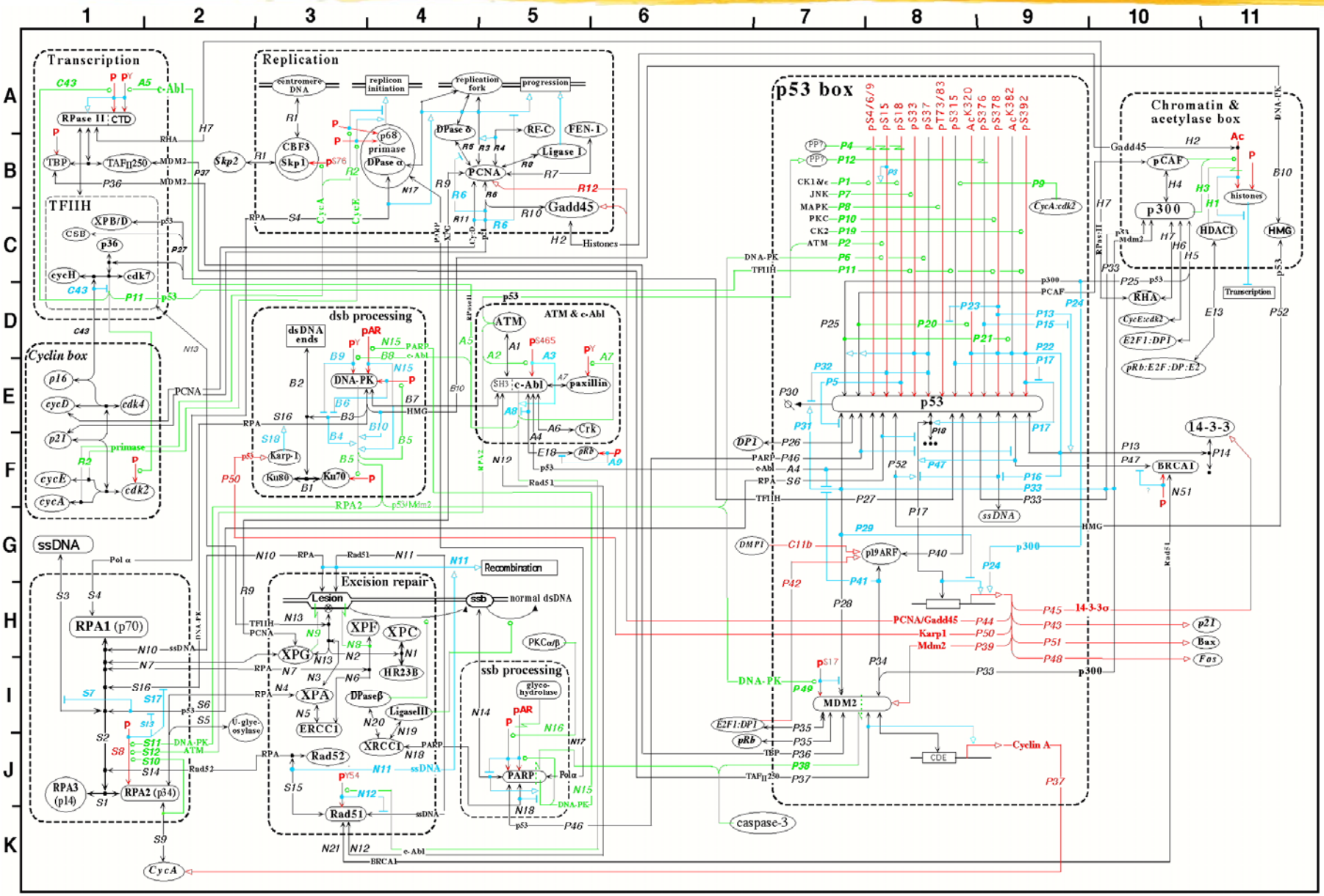
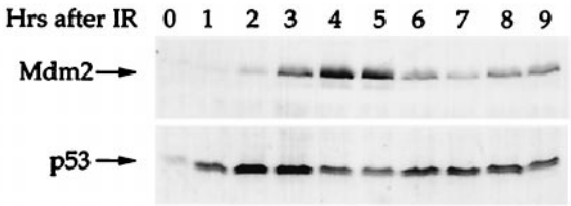



Figure 6B: The p53-Mdm2 and DNA repair regulatory network (version 2p - May 19, 1999)

Kohn, Mol. Biol. Cell 1999

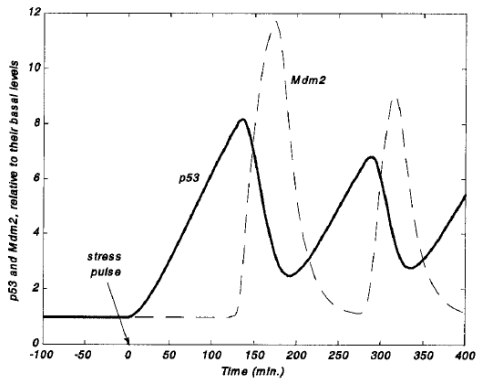
# Negative feedback model

## Western blot




**Lev Bar-Or** et al, PNAS 2000
population of cells
γ-irradiation

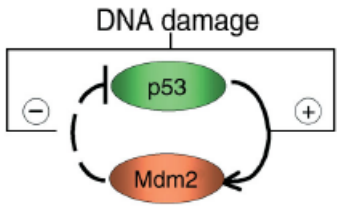
## Numerical solution

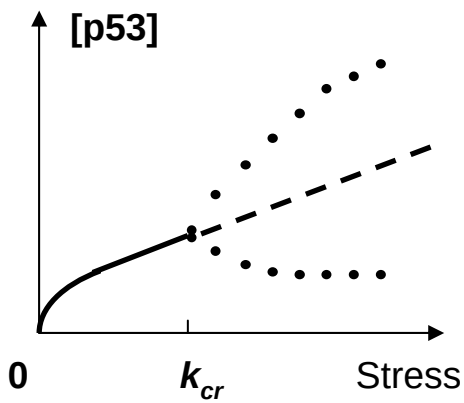



## Shortcomings

- Biology is not captured quite right
- Parameter values do not correspond measurable; some are not realistic, e.g. Hill coefficient >10.
- No feedback from the DNA state to signal
- Did not study bifurcation

## Negative feedback



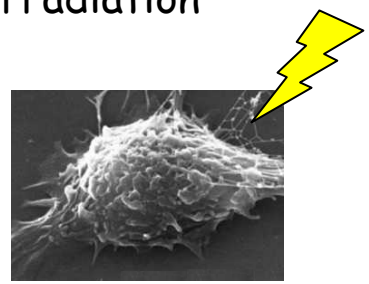

Supercritical Hopf bifurcation

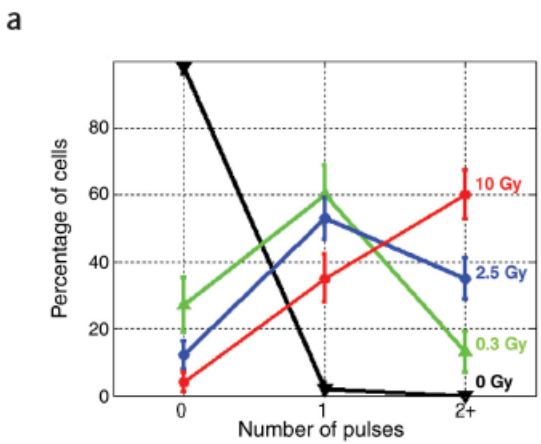
# Experimentally observed pulses of p53

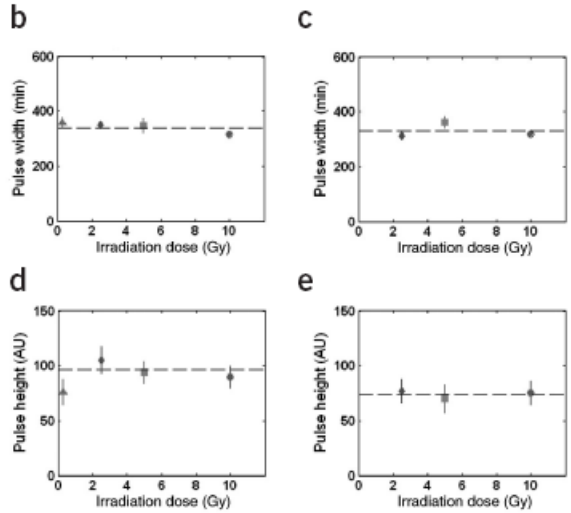
**Lahav** et al, 2004, Nature Genetics

Individual living cells

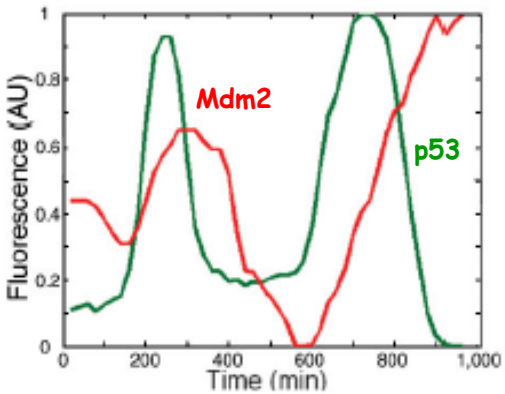
γ-irradiation





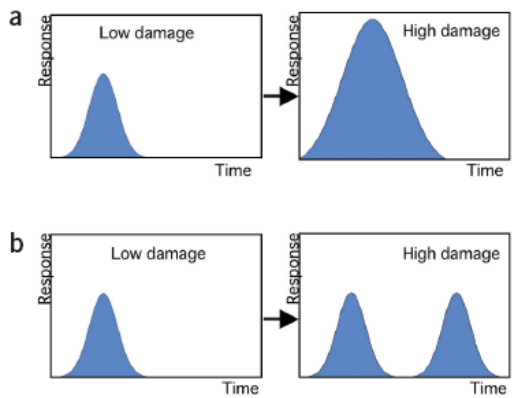

Levels in the nucleus of the cell



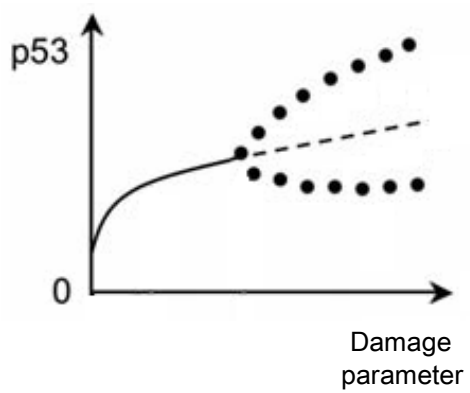

"The mean height and duration of each pulse were fixed and did not depend on the amount of DNA damage. The mean number of pulses, however, increased with DNA damage."

Response:

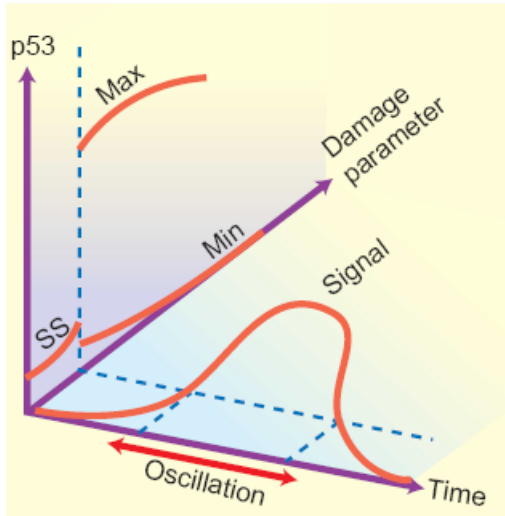
- Not switch-like
- Not graded
- Digital?



# Change of the paradigm




Supercritical Hopf bifurcation




From NFL to NPL

# Negative and positive feedbacks

## Theoretical possibilities

Mutual inhibition


Mutual activation


Self-activation



## Biological evidences

- Through PTEN, PIP3 and Akt pathway, p53 inhibits indirectly the Mdm2 phosphorylation and consequently its nucleus entry, reducing therefore the Mdm2 effect on p53 degradation:

  P53 --> PTEN --| PIP3 --> Akt --> Mdm2nuc --| p53

- The p53 mRNA translation is enhanced by the cytoplasmic Mdm2. Combined with the p53-induced Mdm2 transcription, this results in a positive feedback loop, through which Mdm2 can enhance its own synthesis.

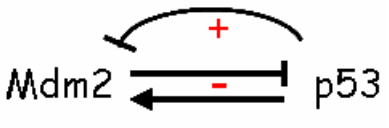
- Phosphorylation of p53 can enhance its specific DNA binding and increase this way its transcriptional activity. The signal transduction protein c-Ha-Ras can enhance p53 phosphorylation, through JNK, MAPK and PKC, and c-Ha-Ras gene expression is itself positively regulated by p53. Thus, p53 can induce its own activation via the above positive feedback.

- Besides by p53, Mdm2 transcription can also be induced by the p53 homologue p63. It turns out that Mdm2 can in turn increase the transcriptional activity and the protein level of p63. These create the possibility for Mdm2 to activate itself independently of p53.

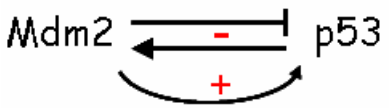
# Negative and positive feedbacks

## Theoretical possibilities

Mutual inhibition



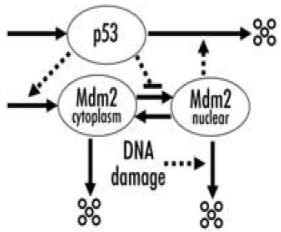

Mutual activation

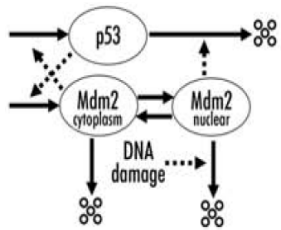


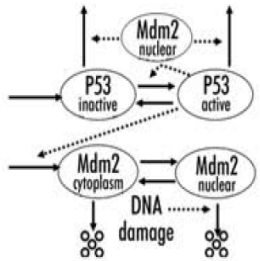

Self-activation

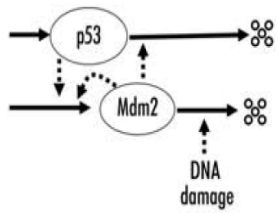


## Wiring diagrams



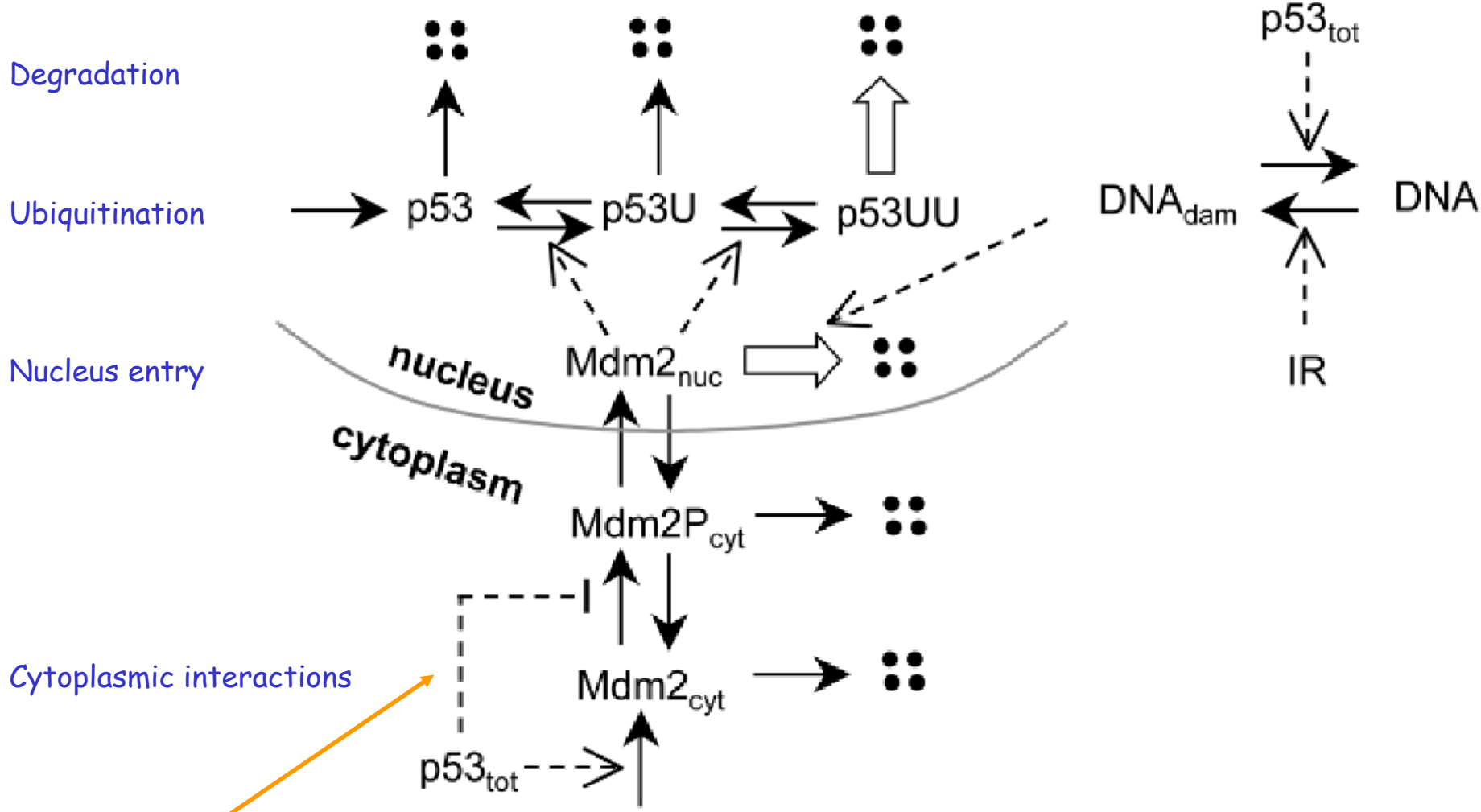




### Modeling assumptions

- Models are constructed at the proteins level.
- Transcriptional regulation is replaced by regulation of corresponding proteins
- Mathematical framework: rate equations; Hill functions in rhs, where appropriate.
- Details of the Mdm2-mediated p53 degradation are not being modeled
- DNA damage increases Mdm2 degradation in the nucleus
- DNA is being repaired with a constant rate (no feedback) except for the model (1)

# Wiring Diagram of the Mutual-Inhibition Model




P53 --> PTEN --| PIP3 --> Akt --> Mdm2nuc --| p53

# Equations of the Model

$$\frac{d[p53_{tot}]}{dt} = k_{s53} - k_{d53}'[p53_{tot}] - k_{d53}\cdot[p53UU]$$

$$\frac{d[p53U]}{dt} = k_f\,[Mdm2_{nuc}]\,[p53] + k_r\,[p53UU] - [p53U]\,(k_r + k_f\,[Mdm2_{nuc}]) - k_{d53}'[p53U]$$

$$\frac{d[p53UU]}{dt} = k_f\,[Mdm2_{nuc}][p53U] - [p53UU]\,k_r - [p53UU]\,(k_{d53}'+k_{d53})$$

$$\frac{d[Mdm2_{nuc}]}{dt} = V_{ratio}(k_i\cdot[Mdm2P_{cyt}] - k_o\cdot[Mdm2_{nuc}]) - k_{d2}\cdot[Mdm2_{nuc}]$$

$$\frac{d[Mdm2_{cyt}]}{dt} = k_{s2}' + \frac{k_{s2}\,[p53_{tot}]^m}{Js^m + [p53_{tot}]^m} - k_{d2}'\,[Mdm2_{cyt}] + k_{deph}\,[Mdm2P_{cyt}] - \frac{k_{ph}}{J_{ph} + [p53_{tot}]}[Mdm2_{cyt}]$$

$$\frac{d[Mdm2P_{cyt}]}{dt} = \frac{k_{ph}}{J_{ph} + [p53_{tot}]}[Mdm2_{cyt}] - k_{deph}\,[Mdm2P_{cyt}] - k_i\,[Mdm2P_{cyt}] + k_o\,[Mdm2_{nuc}] - k_{d2}'\,[MdmP_{cyt}]$$

$$\frac{d[DNA_{dam}]}{dt} = k_{DNA}[IR] - k_{dDNA}\,[p53_{tot}]\,\frac{[DNA_{dam}]}{J_{dna} + [DNA_{dam}]}$$

$$k_{d2} = k'_{d2} + \frac{[DNA_{dam}]}{J_{dam} + [DNA_{dam}]}\,k''_{d2}$$

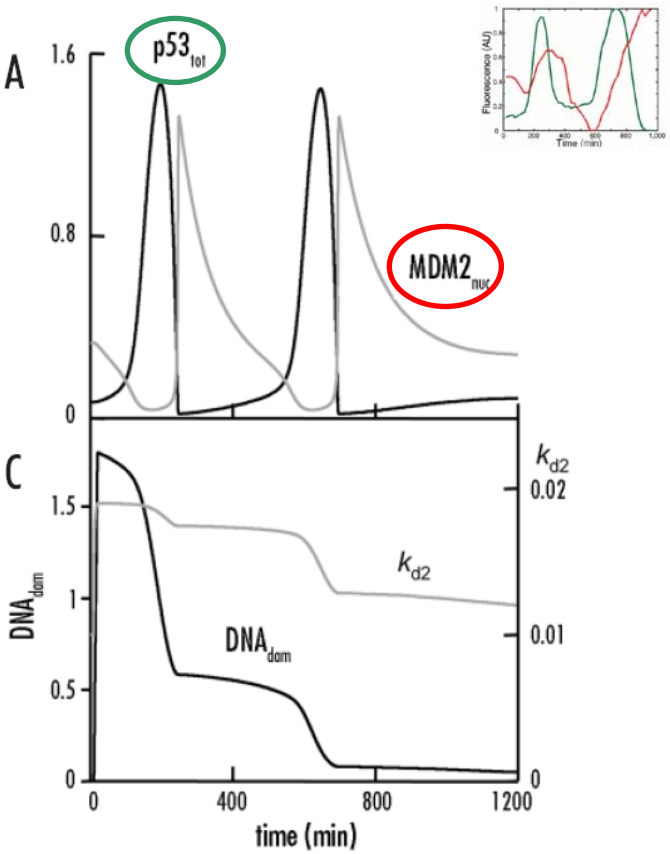
$$[p53] = [p53_{tot}] - ([p53U] + [p53UU])$$

$$[Mdm2_{tot}] = [Mdm2_{cyt}] + \frac{1}{V_{ratio}}[Mdm2_{nuc}] + [Mdm2P_{cyt}]$$

$$IR = ampl\cdot heav(10 < t < 20)$$

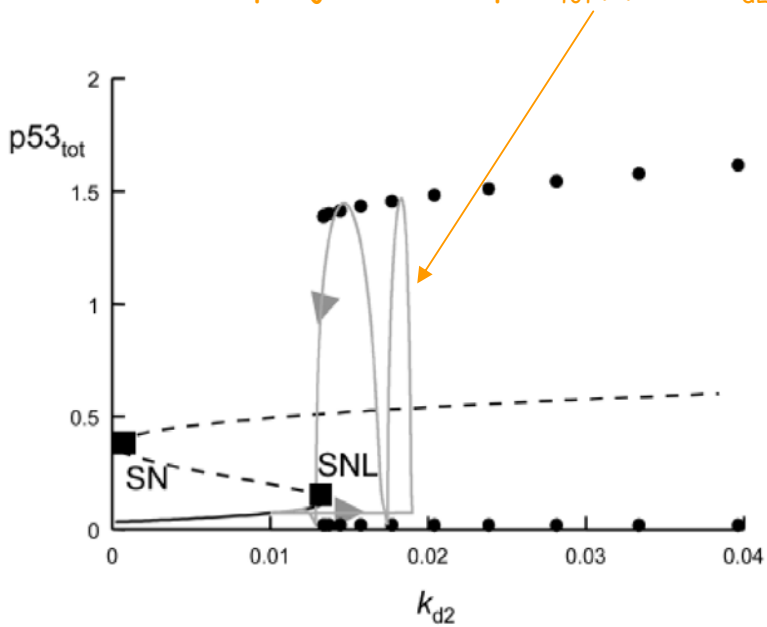
# Responses generated by the model

P53 and Mdm2 levels after DNA damage




Dynamics of the DNA repair

"Orbit" = projection of $p53_{tot}(t)$ and $k_{d2}(t)$




Bifurcation diagram

p53 pulses have large and almost constant amplitude

Every oscillation brings the system closer to the original resting state, as p53 induces Repair

# Equations of the models

DNA damage and degradation rates:

(1) $\frac{dDNAdamage}{dt} = -k_{repair} \cdot H(DNAdamage)$

(2) $k_{d2} = k'_{d2} \cdot (1 + DNAdamage)$

(3) $k_{d53} = k'_{d53} + k''_{d53} \cdot G([\text{Mdm2*}], \theta, J_1/[\text{p53*}], J_2/[\text{p53*}])$

Goldbeter-Koshland Function (Goldbeter and Koshland, 1981):

$$G(u,v,q,r) = \frac{2 \cdot u \cdot r}{(v-u+v \cdot q+u \cdot r+\sqrt{(v-u+v \cdot q+u \cdot r)^2 - 4 \cdot u \cdot r \cdot (v-u)}}$$

Heaviside Function:

$$H(x) = \begin{cases} 1 & \text{if } x > 0 \\ 0 & \text{if } x \le 0 \end{cases}$$

## Model One

Equations 1, 2, 3; [Mdm2*] = [$\text{Mdm2}_{nuc}$], [p53*] = [p53]

(4) $\frac{d[\text{p53}]}{dt} = k'_{s53} + k''_{s53} \cdot \frac{[\text{Mdm2}_{cyt}]^4}{J_{s53}^4 + [\text{Mdm2}_{cyt}]^4} - k_{d53} \cdot [\text{p53}]$

(5) $\frac{d[\text{Mdm2}_{cyt}]}{dt} = k'_{s2} + k''_{s2} \cdot \frac{[\text{p53}]^4}{J_{s2}^4 + [\text{p53}]^4} - k_i \cdot [\text{Mdm2}_{cyt}] + k_o \cdot [\text{Mdm2}_{nuc}] - k''_{d2} \cdot [\text{Mdm2}_{cyt}]$

(6) $\frac{d[\text{Mdm2}_{nuc}]}{dt} = k_i \cdot [\text{Mdm2}_{cyt}] - k_o \cdot [\text{Mdm2}_{nuc}] - k_{d2} \cdot [\text{Mdm2}_{nuc}]$

## Model Two

Equations 1, 2, 3; [Mdm2*] = [$\text{Mdm2}_{nuc}$], [p53*] = [$\text{p53}_{total}$]

(7) $[\text{p53}_{total}] = [\text{p53}_{active}] + [\text{p53}_{inactive}]$

(8) $\frac{d[\text{p53}_{active}]}{dt} = k_{activation} \cdot [\text{p53}_{inactive}] - k_{inactivation} \cdot [\text{p53}_{active}] - k_{d53} \cdot [\text{p53}_{active}]$

(9) $\frac{d[\text{p53}_{inactive}]}{dt} = k_{s53} - k_{activation} \cdot [\text{p53}_{inactive}] + k_{inactivation} \cdot [\text{p53}_{active}] - k_{d53} \cdot [\text{p53}_{inactive}]$

(10) $k_{activation} = k'_{activation} + k''_{activation} \cdot \frac{[\text{p53}_{active}]^3}{J_{activation}^3 + [\text{p53}_{active}]^3}$

(11) $\frac{d[\text{Mdm2}_{cyt}]}{dt} = k'_{s2} + k''_{s2} \cdot \frac{[\text{p53}_{active}]^3}{J_{s2}^3 + [\text{p53}_{active}]^3} - k_i \cdot [\text{Mdm2}_{cyt}] + k_o \cdot [\text{Mdm2}_{nuc}] - k''_{d2} \cdot [\text{Mdm2}_{cyt}]$

(12) $\frac{d[\text{Mdm2}_{nuc}]}{dt} = k_i \cdot [\text{Mdm2}_{cyt}] - k_o \cdot [\text{Mdm2}_{nuc}] - k_{d2} \cdot [\text{Mdm2}_{nuc}]$

## Model Three

Equations 1, 2, 3; [Mdm2*] = [Mdm2], [p53*] = [p53]

(13) $\frac{d[\text{p53}]}{dt} = k_{s53} - k_{d53} \cdot [\text{p53}]$

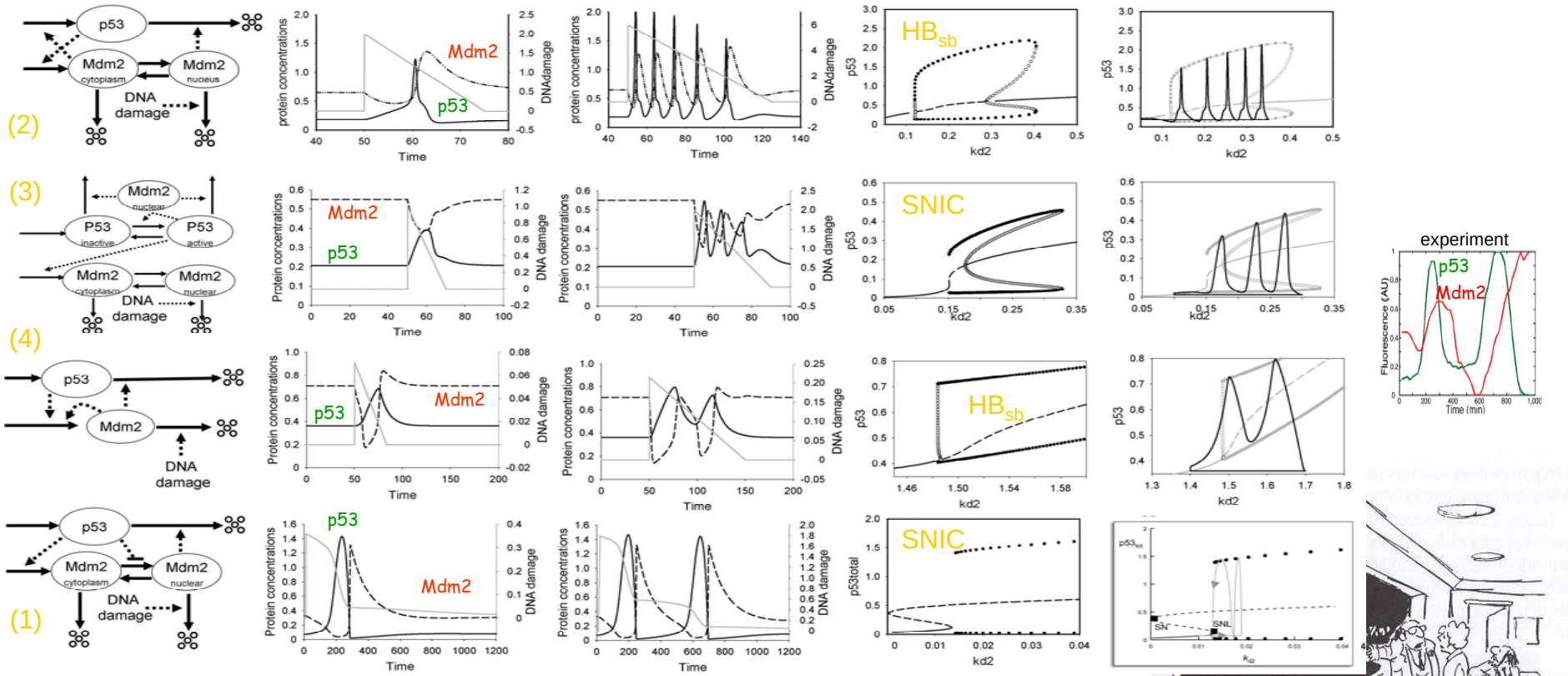
(14) $\frac{d[\text{Mdm2}]}{dt} = k'_{s2} + k''_{s2} \cdot [\text{p53}] + k'''_{s2} \cdot \frac{[\text{Mdm2}]^4}{J_{s2}^4 + [\text{Mdm2}]^4} - k_{d2} \cdot [\text{Mdm2}]$

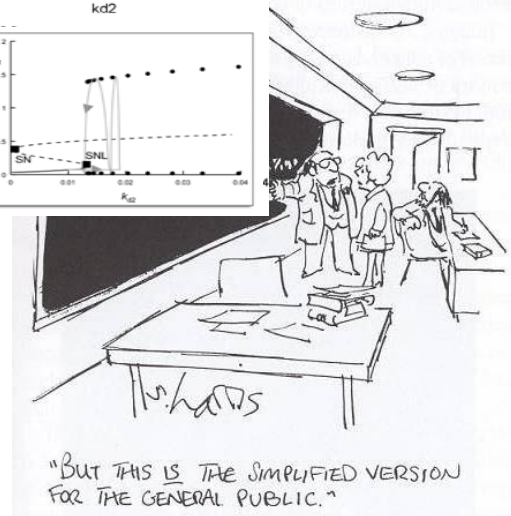
## Model Four

See Ciliberto et al. Cell Cycle, 2005

# Generation of the pulsatile response

Wiring diagrams | Time series | Bifurcation diagrams | Time series plotted on the bifurcation diagrams

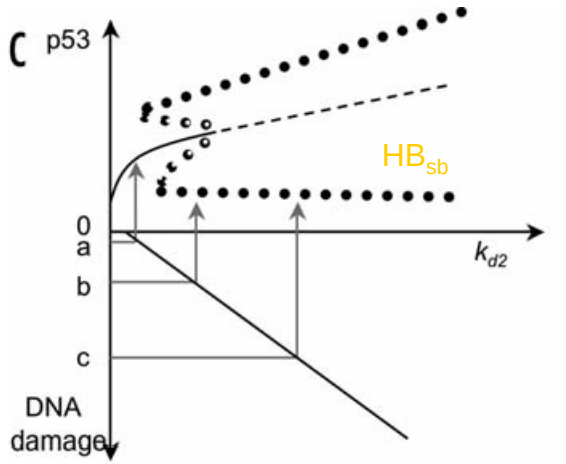


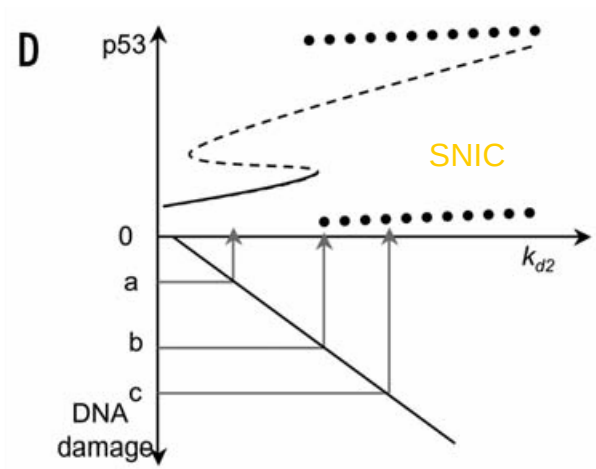




- In normal cells, when DNA damage is absent, kd2 (the Mdm2 degradation rate) is small, and the system remains at a homeostatic stable steady state with low level of p53
- DNA damage increases kd2. The stable steady state is lost due to a large amplitude bifurcation. As a result, the control system is pushed into a stable limit cycle regime
- The system stays in this oscillatory region and generates pulses until the DNA damage is repaired and kd2 is decreased well below the bifurcation point. Presumably, larger DNA damage keeps the system in the oscillatory region for a longer time allowing it to generate a larger number of p53 pulses

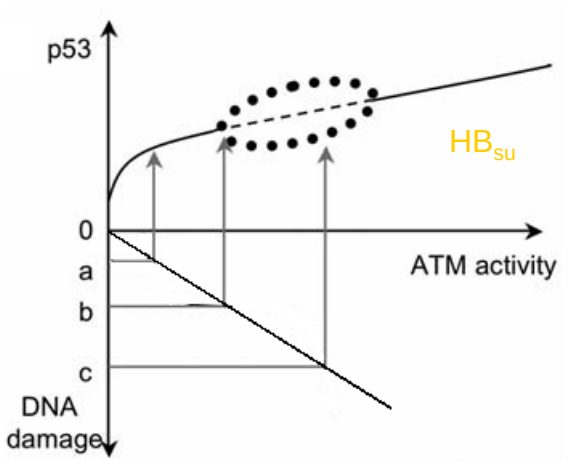
# Schematic comparison of published models

Ciliberto, Zhang,Tyson, 2004,2005,2007

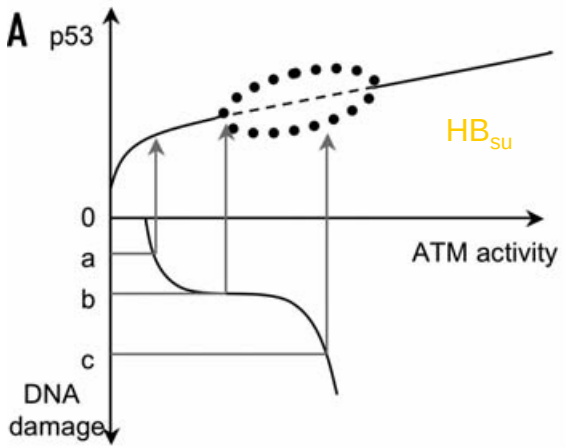


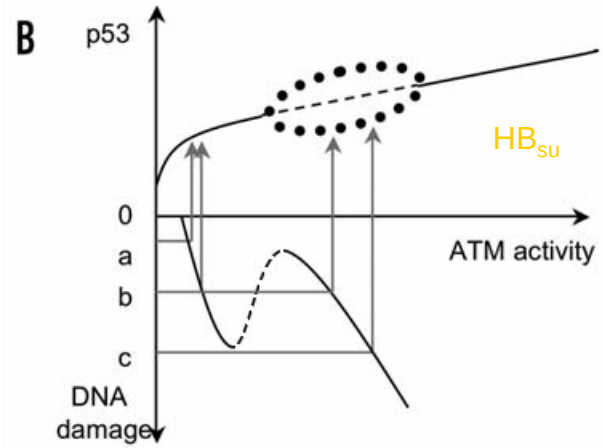

Lev Bar-Or et al, 2000. NFL




Ma et al, 2005. NFL




Chickarmane et al, 2005. NFL




What experiments can help to distinguish oscillations arising from NFL or NPF?

# Conclusions

- Proposed the mechanism for the p53 pulse generation
- Identified biological evidences for the mechanism
- Constructed corresponding quantitative models
- Showed that there can be 2 different bifurcations
- Proposed how to distinguish the mechanisms in experiments

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## Collaborators

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- Tongli Zhang, Virginia Tech
- Kurt W. Kohn, NCI, NIH

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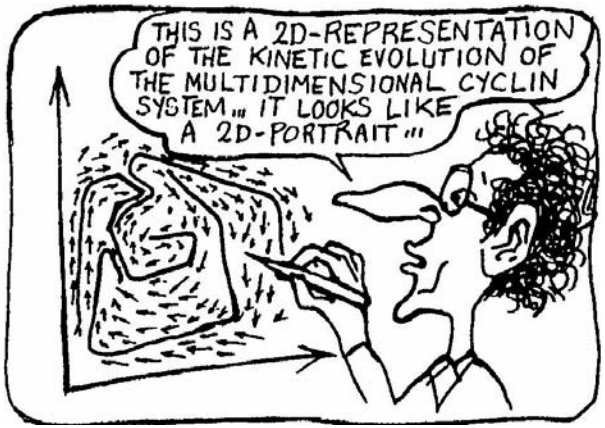
- National Institute of Health
- Commonwealth of Virginia

# The End

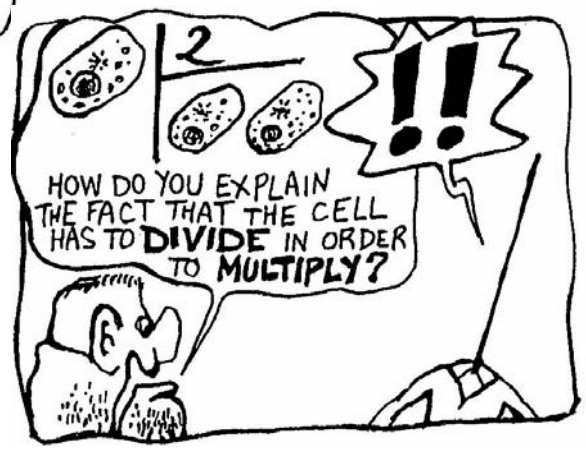
There is never enough time …..

….. Thank you for yours

# Questions


THIS IS A 2D-REPRESENTATION OF THE KINETIC EVOLUTION OF THE MULTIDIMENSIONAL CYCLIN SYSTEM... IT LOOKS LIKE A 2D-PORTRAIT!!!


ARE THERE QUESTIONS ON MATHEMATICAL ASPECTS?
YES!


2
!!
HOW DO YOU EXPLAIN THE FACT THAT THE CELL HAS TO DIVIDE IN ORDER TO MULTIPLY?