The role of gene copy number in p53 pathway

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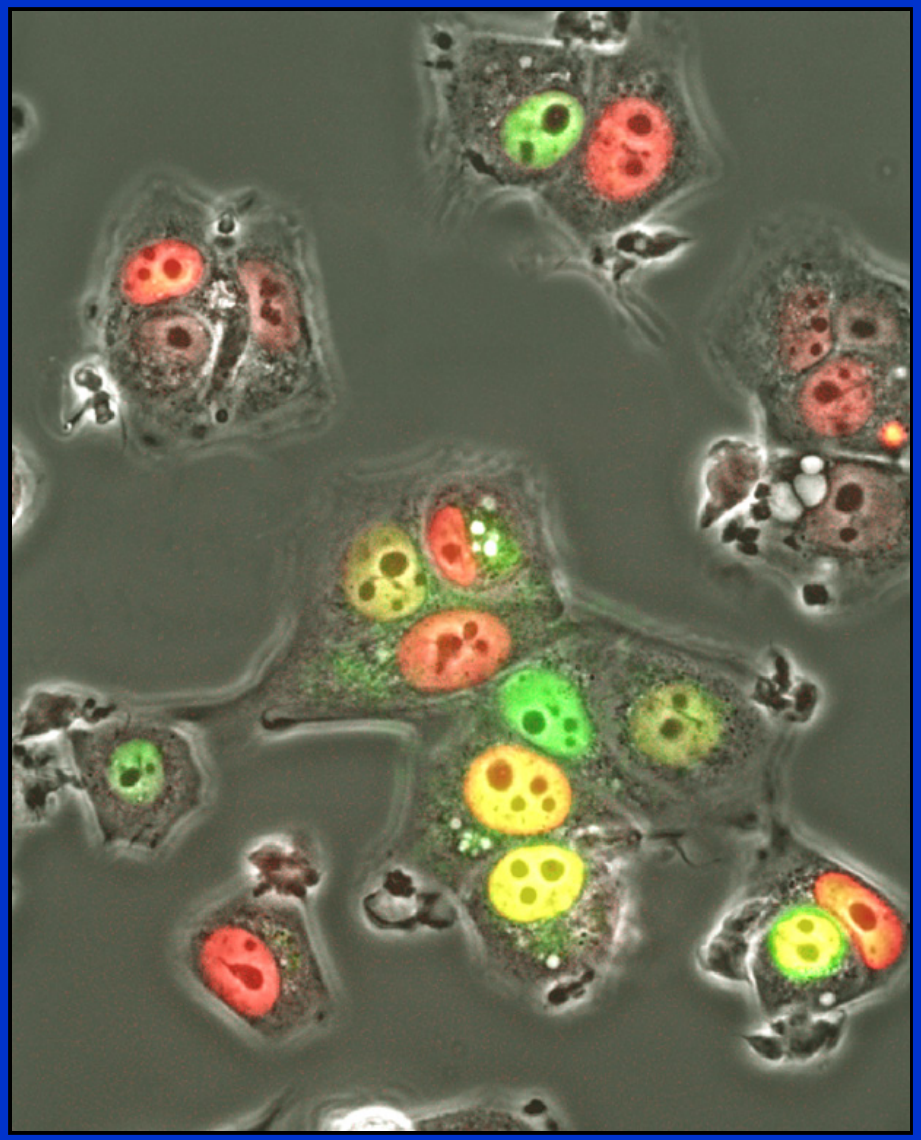
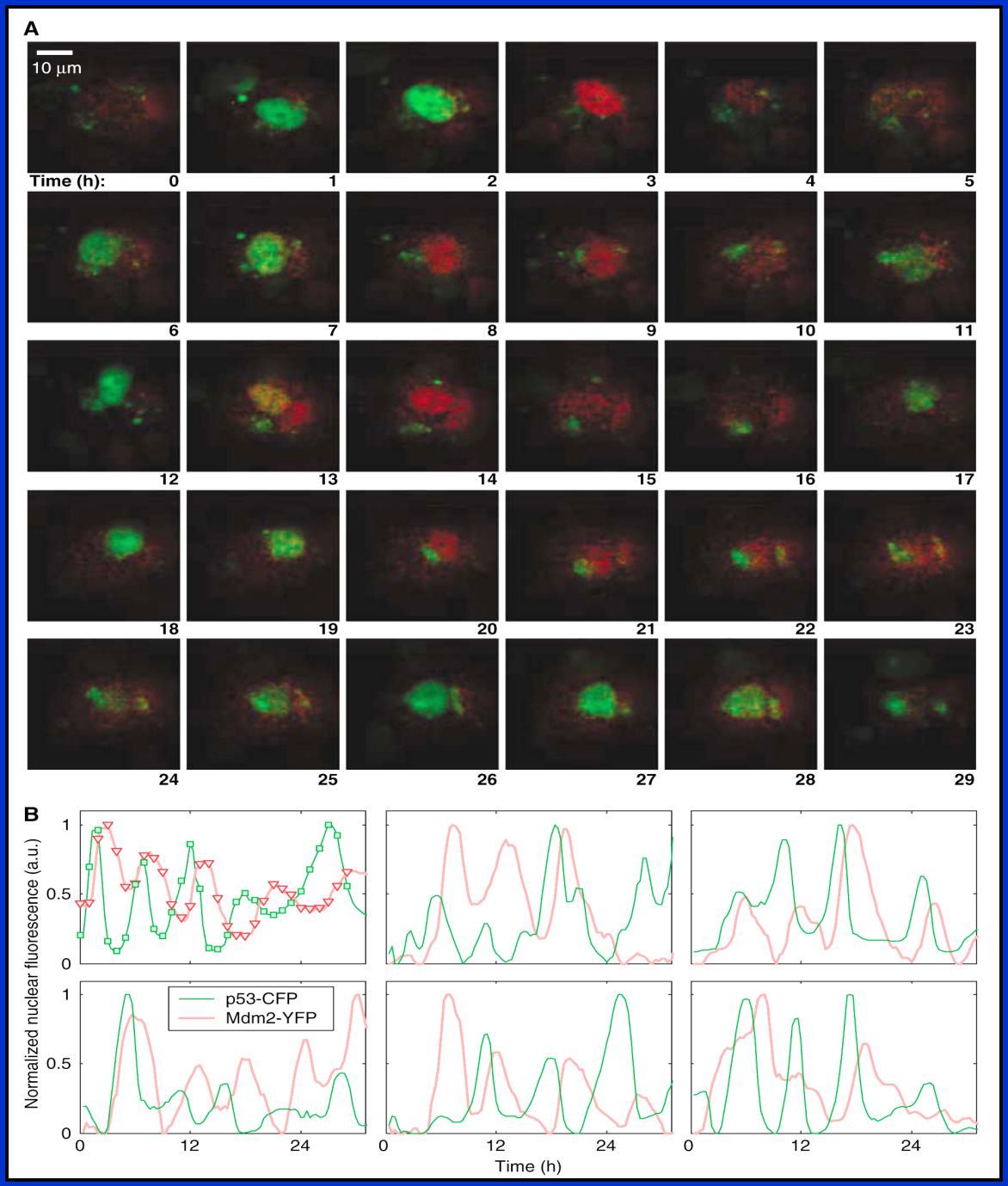
Introduction

- p53 („guardian of the genome”) is a transcription factor controlling genes involved in repair of DNA damage, cell cycle arrest and apoptosis
- in normal cells p53 is usually inactive, kept at low level due to Mdm2 induced degradation
- in response to DNA damage p53 activates, its concentration increases, it triggers synthesis of its inhibitor Mdm2 what leads to prolonged oscillations of p53 and Mdm2 levels
- since these oscillations are not well synhronized across the population the only way to observe them is by means of single cell experiments

Human cells expressing p53-CFP (green) and Mdm2-YFP (red) after DNA damage, Uri Alon group, Harvard Med. School, US





Continuous oscillations are observed over 72 hours in the response to DNA damage by gamma irradiation

Uri Alon experiment

p53-CFP (green)
Mdm2-YFP (red)

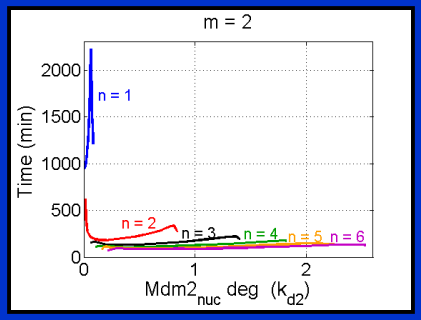
The aim of this work

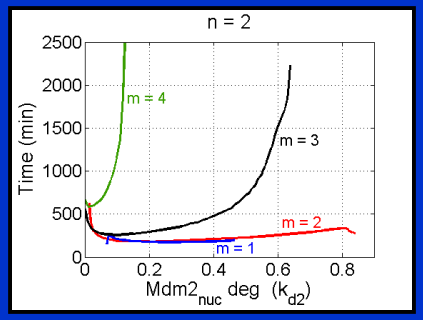
- To analyze how the behavior of cells depends on the number of p53 and Mdm2 gene copies

- How the lack of one p53 allele deregulates the pathway (what may result in tumor development)

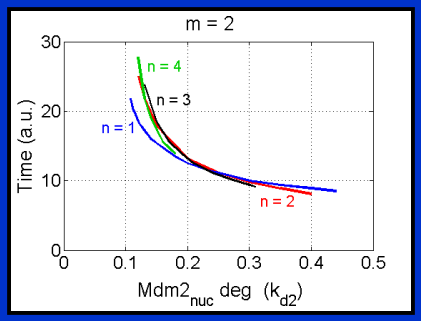
- To deduce the behavior of „normal” cells from experiments performed on the transfected cells

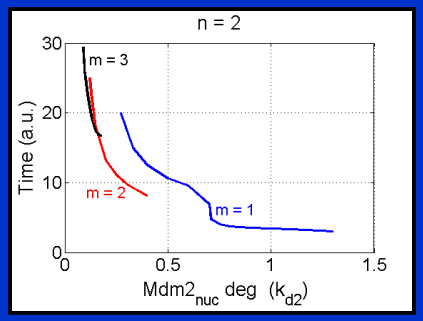
Ranges of Mdm2 deg for which systems have stable limit cycles



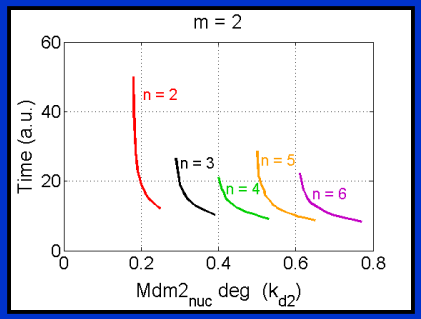


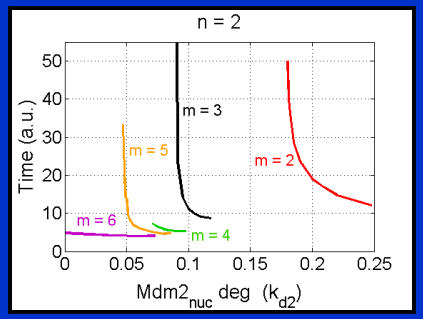
Ciliberto



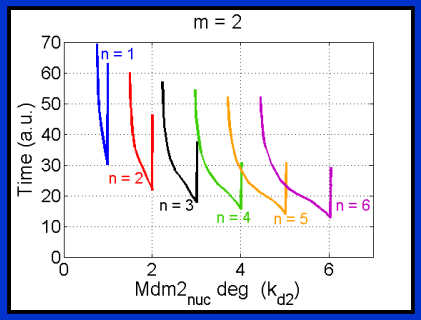


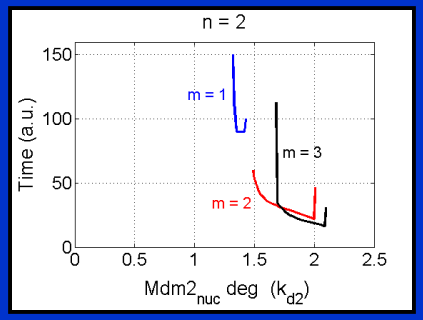
Zhang I





Zhang II





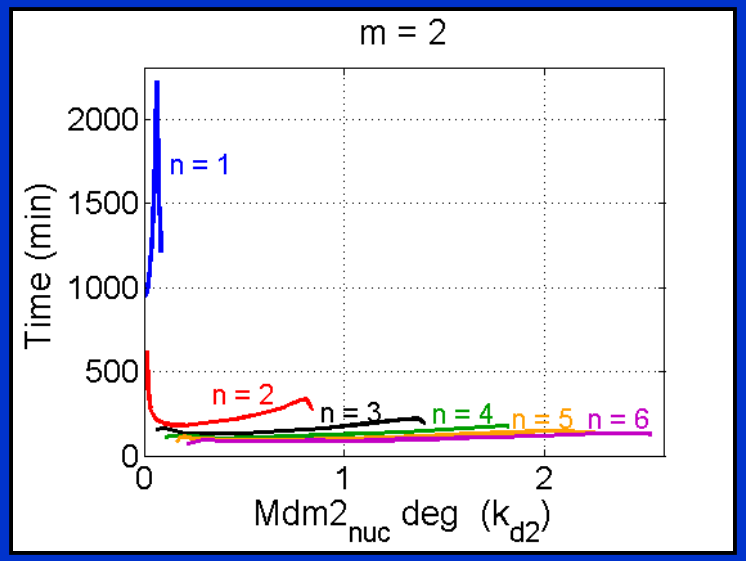
Zhang III

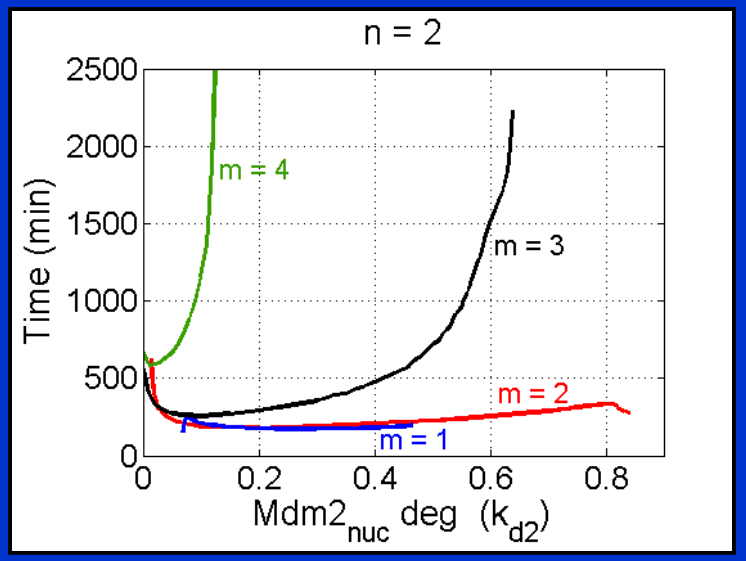
Proper model should satisfy conditions:

- period of oscillations is robust to change in gene copy number
- range of Mdm2 deg for oscillations of p53 and Mdm2 weakly depends on the number of p53 and Mdm2 gene copies

m – number of p53 gene copies
n – number of Mdm2 g. c.

Ciliberto model

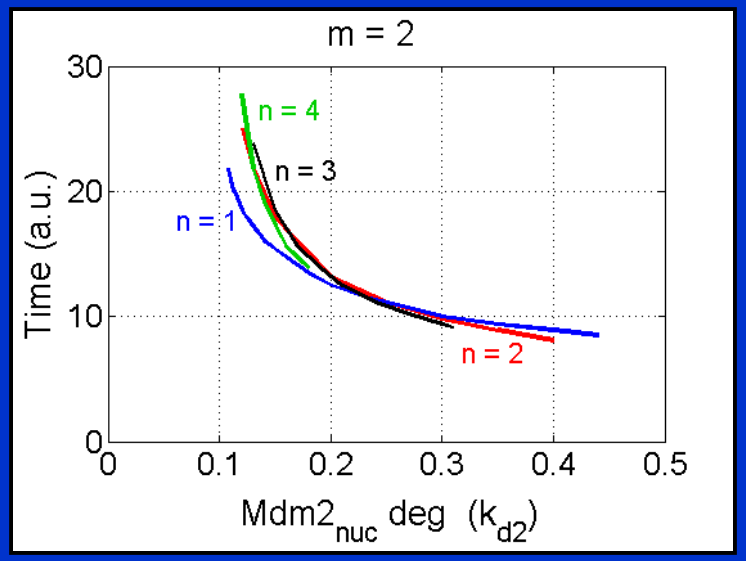


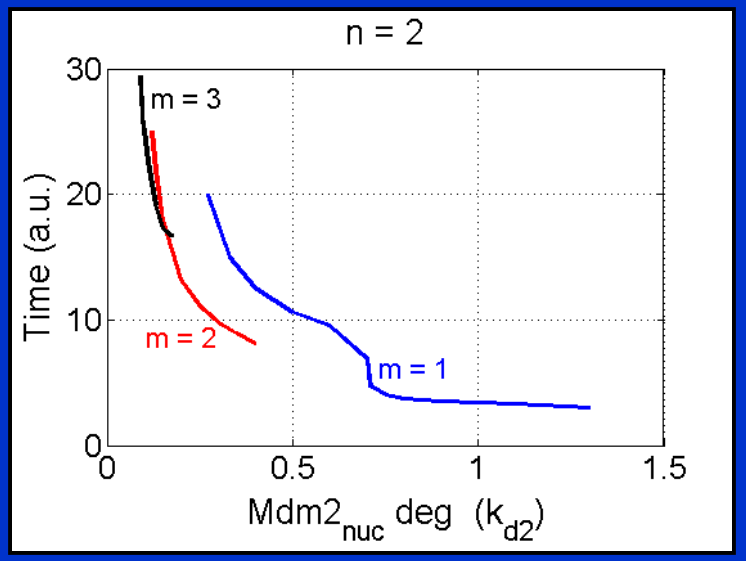


m – number of p53 gene copies

n – number of Mdm2 gene copies

Zhang, model I

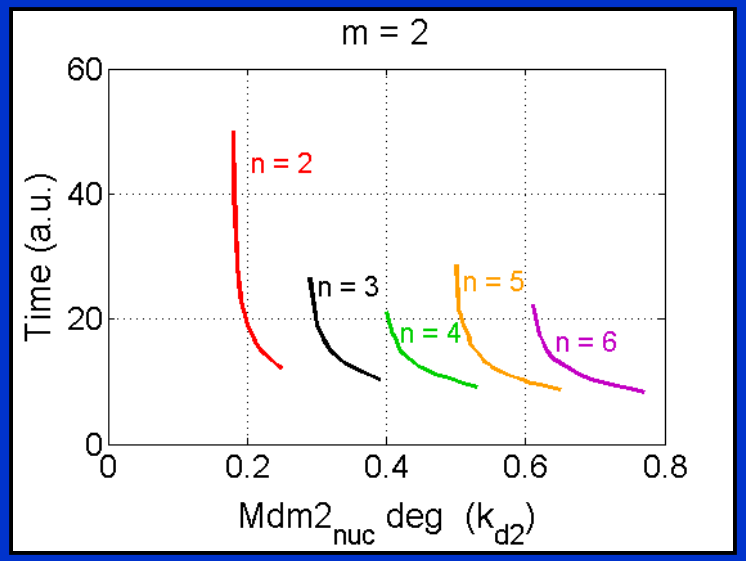


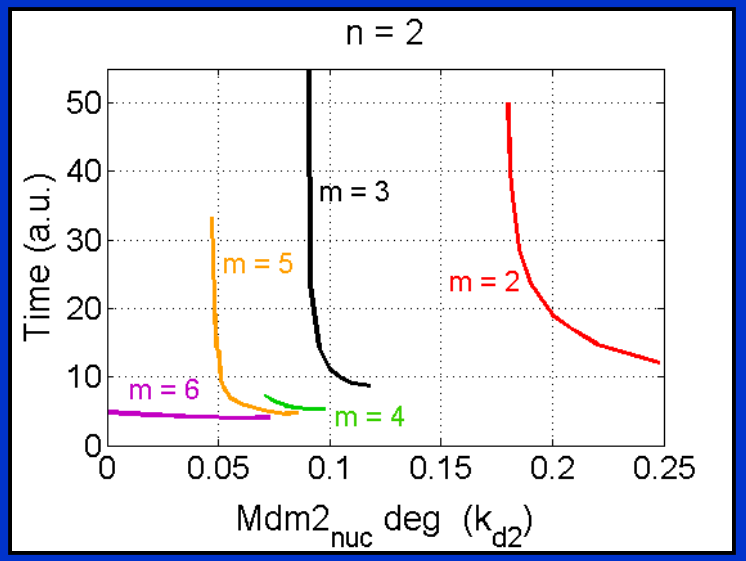


m – number of p53 gene copies

n – number of Mdm2 gene copies

Zhang, model II

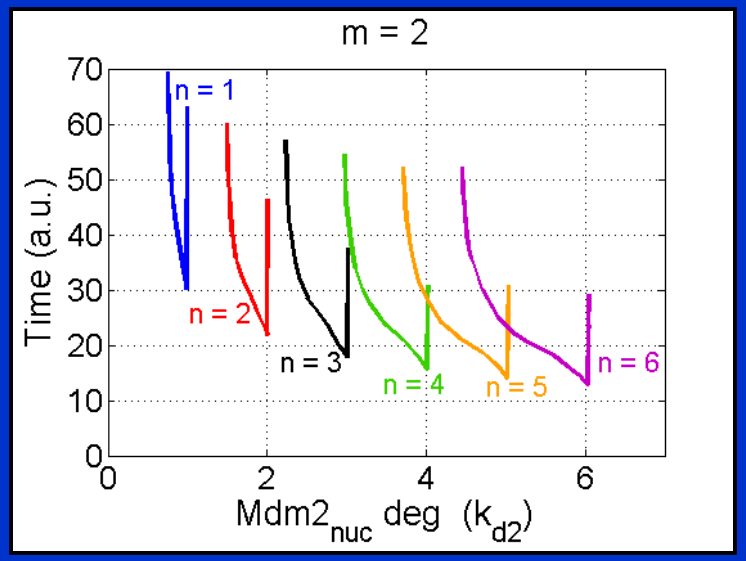


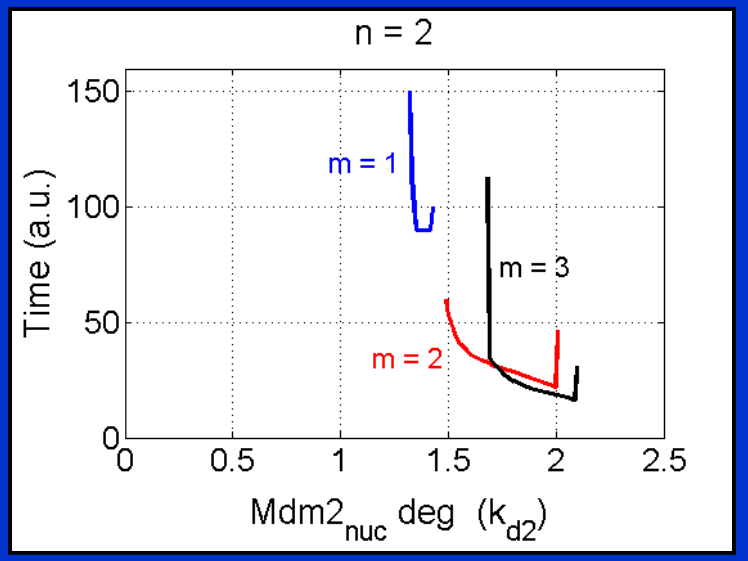


m – number of p53 gene copies

n – number of Mdm2 gene copies

Zhang, model III





m – number of p53 gene copies

n – number of Mdm2 gene copies

Our model of p53/Mdm2 regulatory core, positive and negative feedback

The model consists of three components: total p53, cytoplasmic Mdm2 and nuclear Mdm2

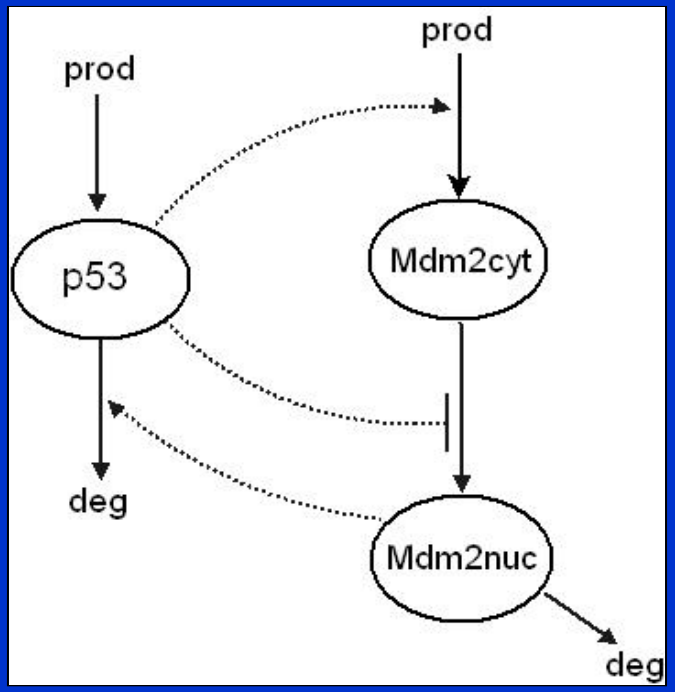


Diagram of p53/Mdm2 regulatory core

The pathway is described by the system of three ordinary differential equations for amounts of total p53, cytoplasmic Mdm2 and nuclear Mdm2

$$\frac{d(p53)}{dt} = m\,s_1 - d_1\,p53\,(Mdm2_{nuc})^2,$$

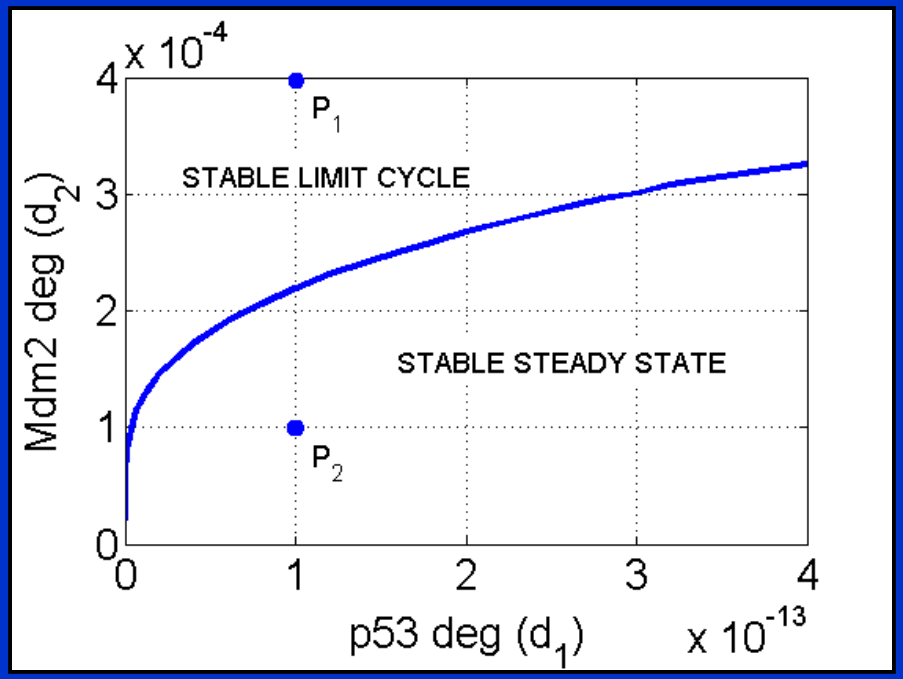
$$\frac{d(Mdm2_{cyt})}{dt} = n\left(s_2 + s_{20}\,\frac{(p53)^3}{(p53)^3 + s_{200}{}^3}\right) - \frac{k_1}{p53 + k_2}\,Mdm2_{cyt},$$

$$\frac{d(Mdm2_{nuc})}{dt} = \frac{k_1}{p53 + k_2}\,Mdm2_{cyt} - d_2\,Mdm2_{nuc},$$

where *m* and *n* are the numbers of p53 and Mdm2 gene copies

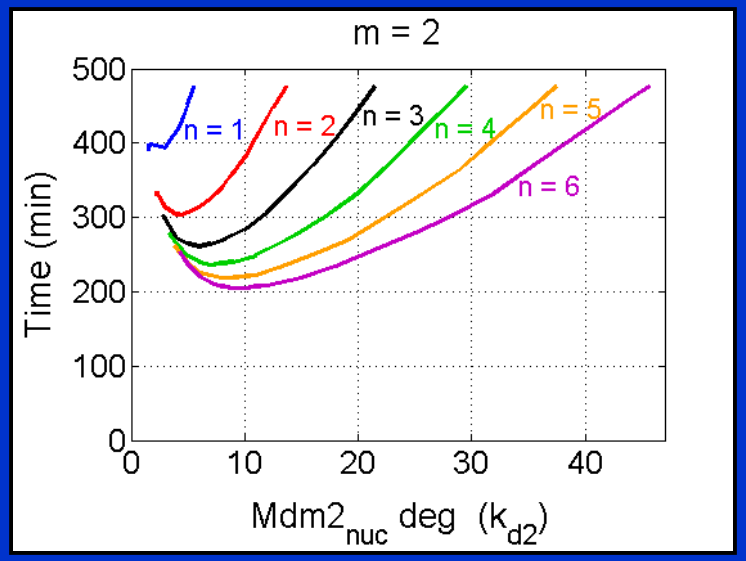
Necessary conditions of the proper model of the p53/Mdm2 regulatory core:

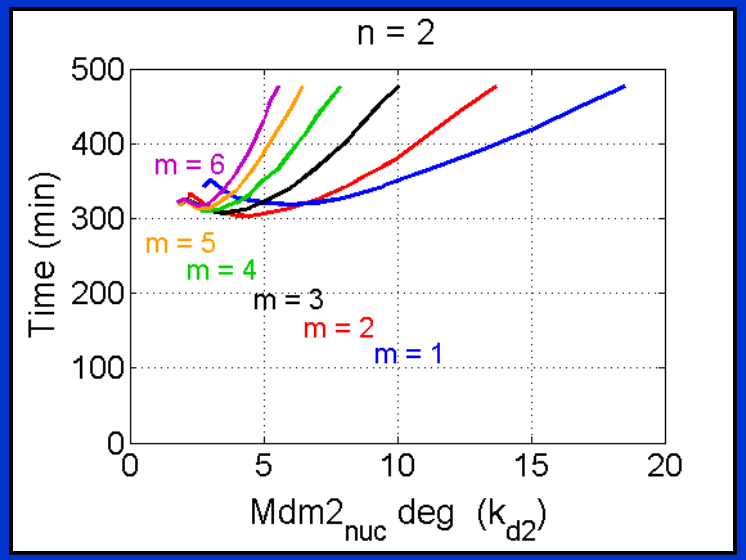
Oscillations result from DNA damage manifested by decreased p53 deg constant and/or elevated Mdm2 deg constant



Regions of stable limit cycles and stable steady states in (d_1 = p53 deg, d_2 = Mdm2 deg)-plane for diploidal cells, $m = n = 2$

Our



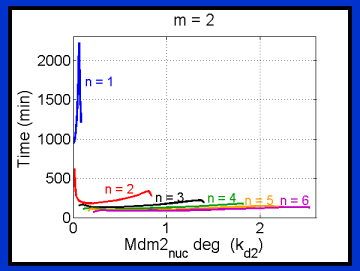


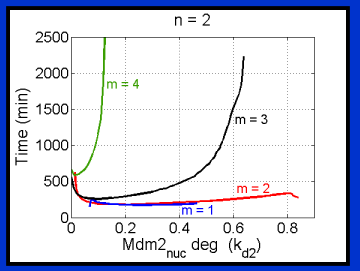
m – number of p53 gene copies

n – number of Mdm2 gene copies

Ranges of Mdm2 deg for which systems have stable limit cycles

Ciliberto





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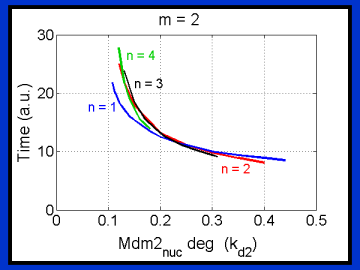

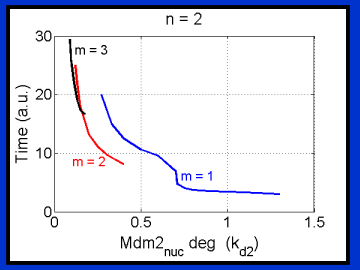

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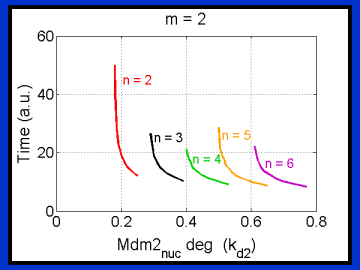
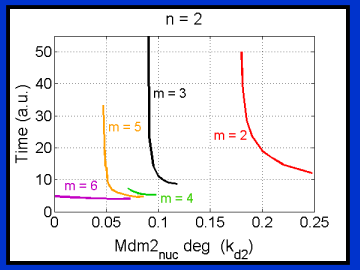
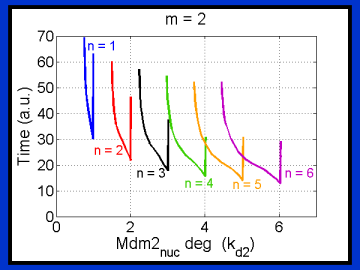
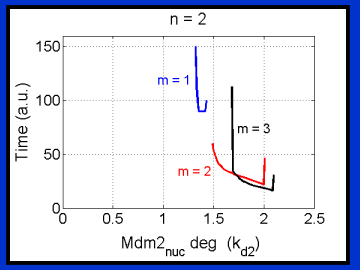




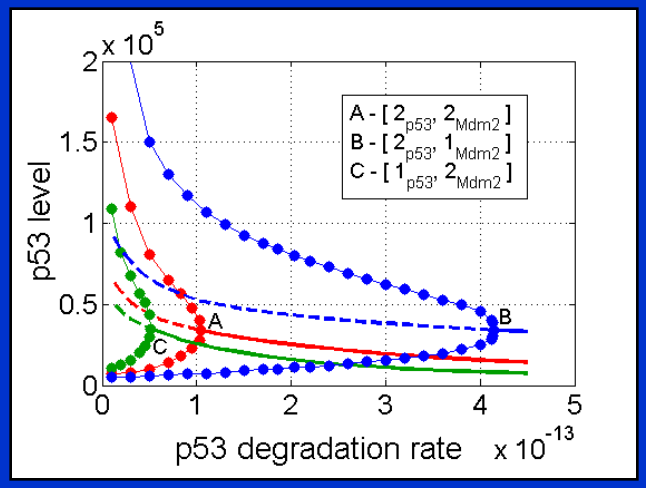
Zhang III

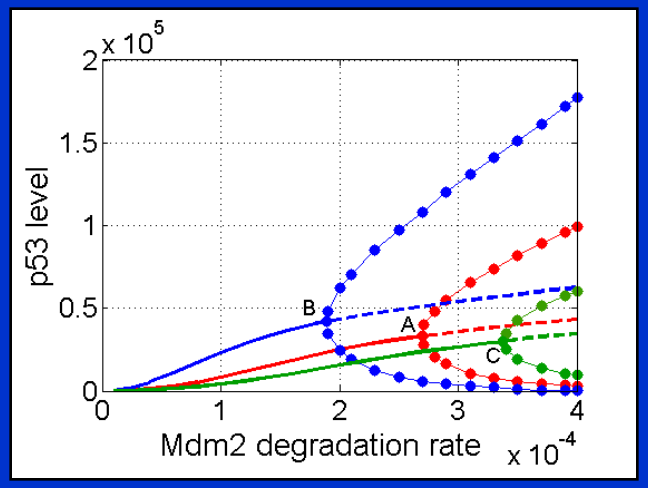




Results

We analyze bifurcation diagrams to investigate how the transition point from stable state to stable limit cycle depends on the number of p53 and Mdm2 gene copies



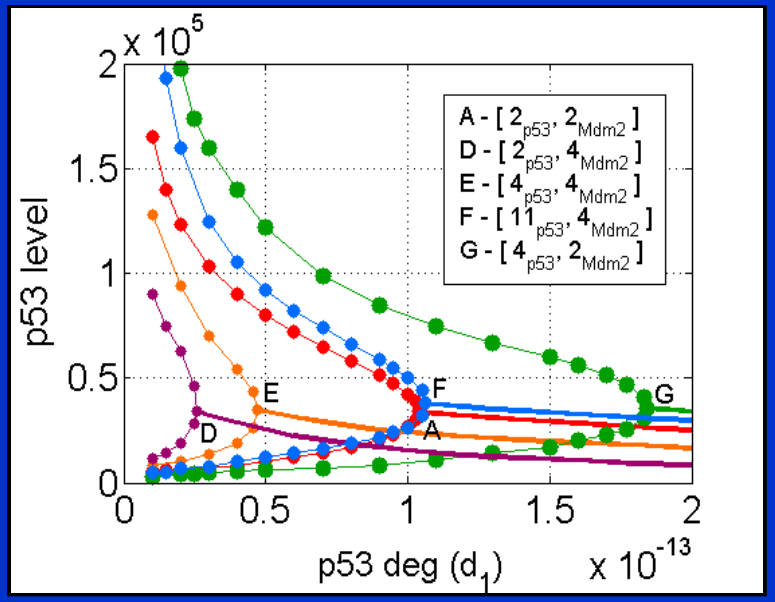


Red: normal cell (2,2)
Green: p53 haploidal (1,2)
Blue: Mdm2 haploidal (2,1)

Bifurcation diagrams for normal (diploidal) and haploidal cells

- loss of p53 gene copy makes the oscillatory region smaller
- loss of Mdm2 gene copy enlarge oscillatory region

To deduce how gene transfection influences cell behavior we investigate systems with elevated gene copy numbers



Red: normal cell (2,2)
Violet: Mdm2 transfection (2,4)
Green: p53 transfection (4,2)
Orange: cotransfection (4,4)
Blue: cotransfection (11,4)

Bifurcation diagram for normal (diploidal) and transfected cells

- p53 transfection forces oscillations
- Mdm2 transfection inhibits oscillations
- cotransfected cells are closer to normal cells than singly transfected cells
- the (11,4) transfection most accurately mimic normal cells (property of this particular model)

Conclusions

Assuming that oscillations observed experimentaly are due to cooperation of positive and negative feedback

- behavior of normal cells is qualitatively different from that of transfected cells

- when one of p53 copies is missing, the system may remain in stable state even when DNA is damaged; this may lead to haploinsufficiency, which can be partialy rescued by loss of Mdm2 allele

- to better resemble normal cells the p53/Mdm2 cotransfection experiments are more reliable and the number of p53 gene copies must be larger then Mdm2 copies

The other possibility is that positive feedback does not play any role in the oscillations, which result from negative feedback and time delay and are thus less sensitive to the number of Mdm2 and p53 gene copies

Robustness

Range of parameters in which the system has a stable limit cycle for $d_2 = 1 \cdot 10^{-4}$ and a stable steady state for $d_2 = 4 \cdot 10^{-4}$

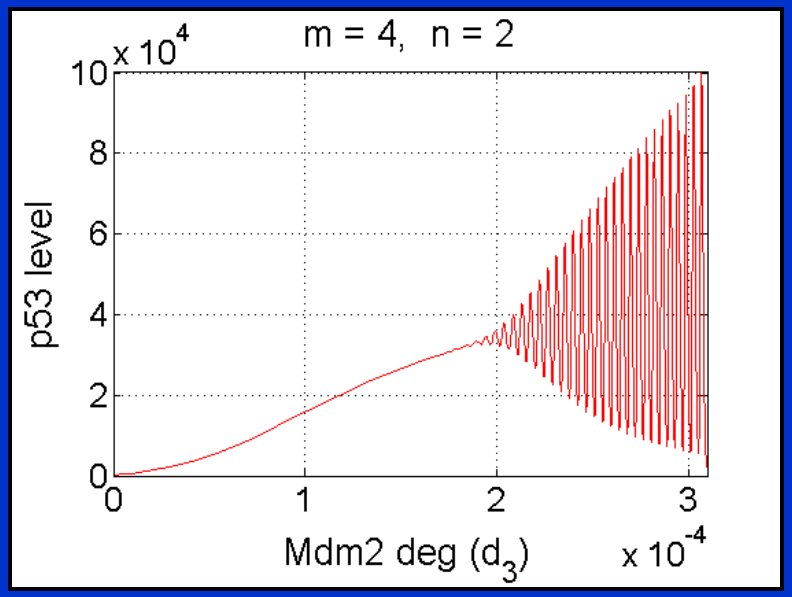
Parameters and definitions	Value	Range
s_1 - p53 production rate	16	(4.1, 90)
s_2 - $Mdm2_{cyt}$ production rate (p53 independent)	8	(0, 22.5)
s_{20} - $Mdm2_{cyt}$ production rate (induced by p53)	80	(20, 250)
s_{200} - saturation term in $Mdm2_{cyt}$ production	100000	$(0.53 \cdot 10^5, 4 \cdot 10^5)$
d_1 - p53 degradation rate (induced by Mdm2)	$1 \cdot 10^{-13}$	$(0.1 \cdot 10^{-13}, 7.3 \cdot 10^{-13})$
k_1 - transition coefficient from $Mdm2_{cyt}$ to $Mdm2_{nuc}$	8	(2.8, 15)
k_2 - inhibition term in quantity of transition from $Mdm2_{cyt}$ to $Mdm2_{nuc}$	2300	(0, 42000)
d_2 - $Mdm2_{nuc}$ degradation rate (P_1 in Fig. 2)	$4 \cdot 10^{-4}$	
d_2 - $Mdm2_{nuc}$ degradation rate (P_2 in Fig. 2)	$1 \cdot 10^{-4}$	
m (n) - number of p53 (Mdm2) gene copies		

$$\frac{d(p53)}{dt} = m\,s_1 - d_1 p53\,(Mdm2_{nuc})^2,$$

$$\frac{d(Mdm2_{cyt})}{dt} = n\left(s_2 + s_{20}\frac{(p53)^3}{(p53)^3 + s_{200}}\right) - \frac{k_1}{p53 + k_2} Mdm2_{cyt},$$

$$\frac{d(Mdm2_{nuc})}{dt} = \frac{k_1}{p53 + k_2} Mdm2_{cyt} - d_2\, AMdm2_{nuc},$$

$$\frac{d(A)}{dt} = 10^{-9}$$



$$\frac{d(p53)}{dt} = m\,s_1 - d_1 p53\,(Mdm2_{nuc})^2,$$

$$\frac{d(Mdm2_{cyt})}{dt} = n\left(s_2 + s_{20}\frac{(p53)^3}{(p53)^3 + s_{200}}\right) - \frac{k_1}{p53 + k_2} Mdm2_{cyt},$$

$$\frac{d(Mdm2_{nuc})}{dt} = \frac{k_1}{p53 + k_2} Mdm2_{cyt} - d_2\, AMdm2_{nuc},$$

$$\frac{d(A)}{dt} = 10^{-12}$$