Genetic Oscillations and Feed-Backs in NF-κB, p53 and Wnt Systems

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1. **Four eukaryotic systems with oscillatory gene expressions:**

- ***Hes*1-*m*RNA protein network (Hirata et al (2002))**
- **P53-*mdm*2 network (Oren et al (2000), Lahav et al (2006))**
- **NF-κB transcription factor (Nelson, White et al)**
- **Wnt-Notch segmentation network (Goldbeter, Pourquie)**

→ **Identify the 'simplest' negative feed-back loop.**

→ **Oscillating regimes: Ultradian time period (2-3 hours)**

2. NF-κB, p53, Wnt systems: Regulated by negative feed-back loops: inflammation , apoptosis, segmentation.

3. NF-κB - IκB feed-back loop:
Reduce 26-dimensional dynamics to three/nine variables
Spiky oscillations:
→ Saturated degradation.
→ A20 change period. Chaotic response

4. External stresses and responses in p53-Mdm2:
DNA damage, hypoxia, nutlin, etc

5. Somite segmentation in embryos (space):
Oscillating proteins: Wnt signaling feed-back loop

6. Coupled feed-back loops in space:
Cell-to-cell communications.

Collaborators:

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- Kim Sneppen
- Peter B. Jensen, Lykke Pedersen, Alex Hunziker, Benedicte Mengel,

S. Krishna, M.H. Jensen and K. Sneppen, "Spiky oscillations in NF-κB signalling", Proc.Nat.Acad.Sci. 103, 10840-10845 (2006).

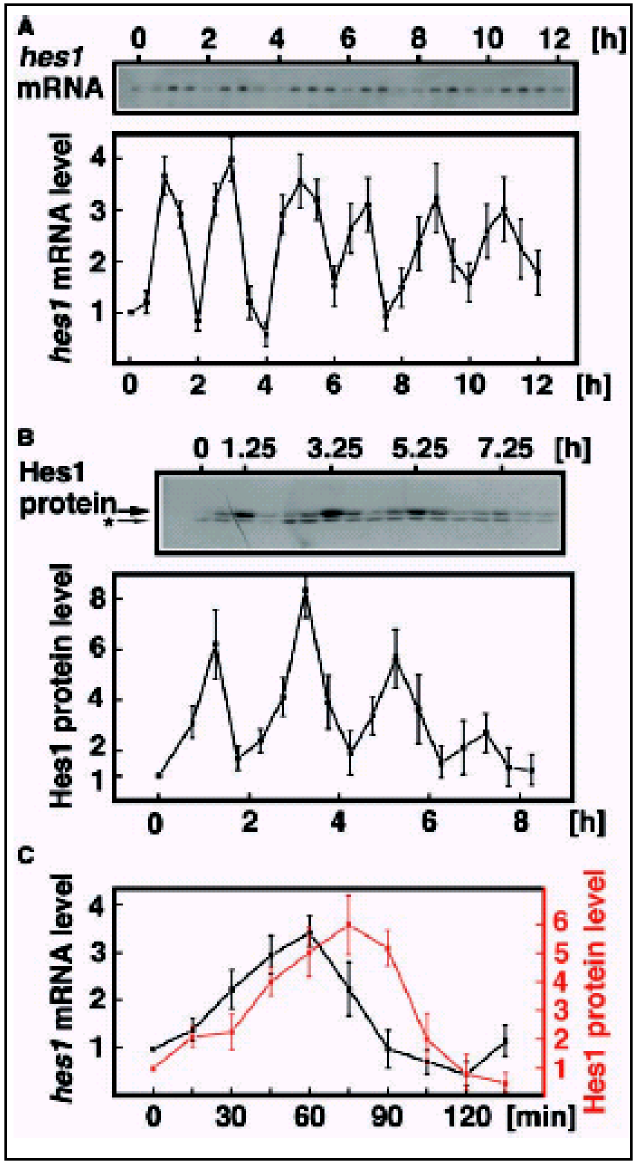
S. Pigolotti, S. Krishna, M.H. Jensen, "Oscillation patterns in negative feedback loops", Proc.Nat.Acad.Sci., 104, 6533-6537 (2007).

G. Tiana, S. Krishna, S. Pigolotti, M.H. Jensen and K. Sneppen, "Oscillations and temporal signalling in cells", Physical Biology 4, R1-R17 (2007).

S. Pigolotti, S. Krishna, M.H. Jensen "Symbolic Dynamics of Biological Feedback Networks", Phys. Rev. Lett. 102, 088701 (2009); 103, 118101 (2009).

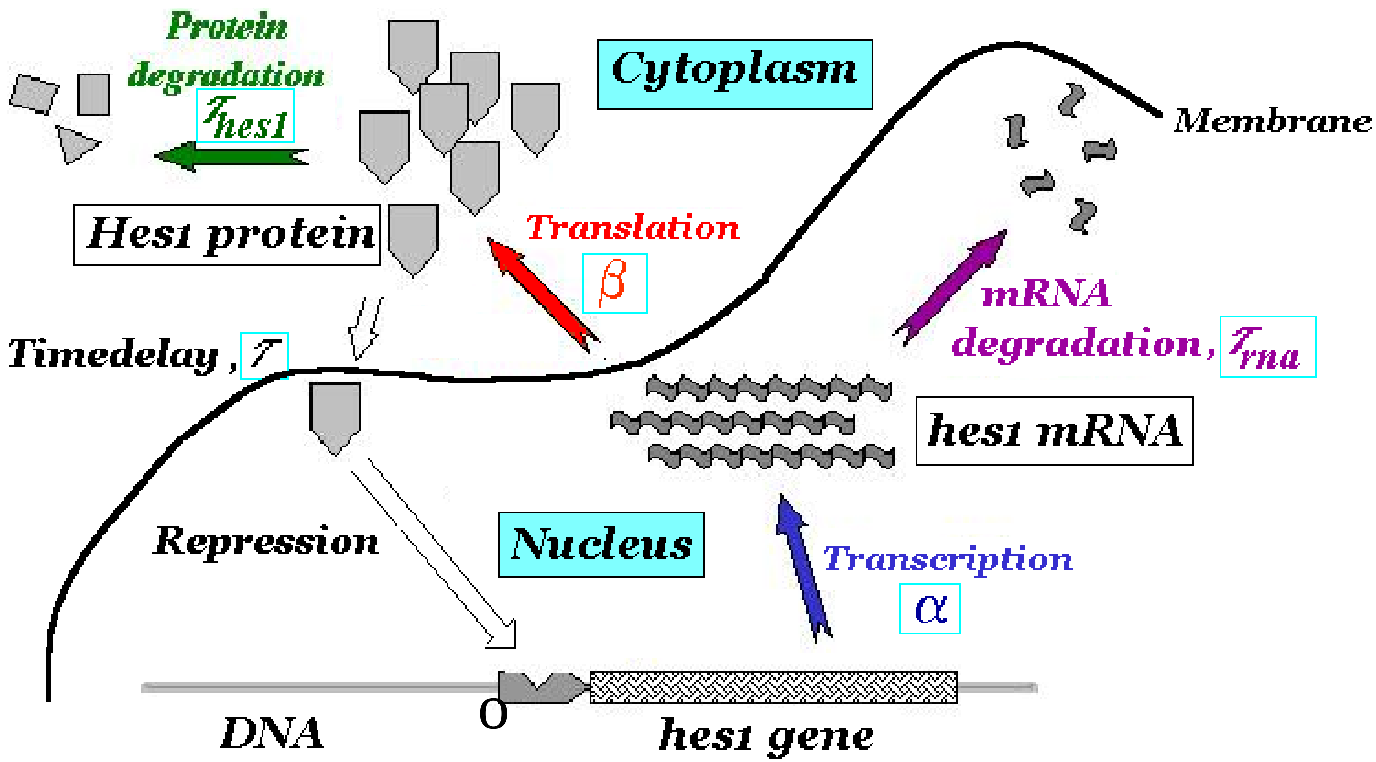
P.B. Jensen, L. Pedersen, S. Krishna, M.H. Jensen "A Wnt Oscillator Model for Somitogenesis" , Biophys. Journ. 98, 943-50 (2010). Several new preprints

‘Typical’ Oscillating data: Hes1 - segmentation



(Hirata et al, 2002)

Simplest negative feed-back loop: Hes1



$$\frac{d[mRNA]}{dt} = \alpha \cdot [o_{free}] - \frac{[mRNA(t)]}{\tau_{rna}}$$

$$\frac{d[Hes1]}{dt} = \beta \cdot [mRNA(t)] - \frac{[Hes1(t)]}{\tau_{hes1}}$$

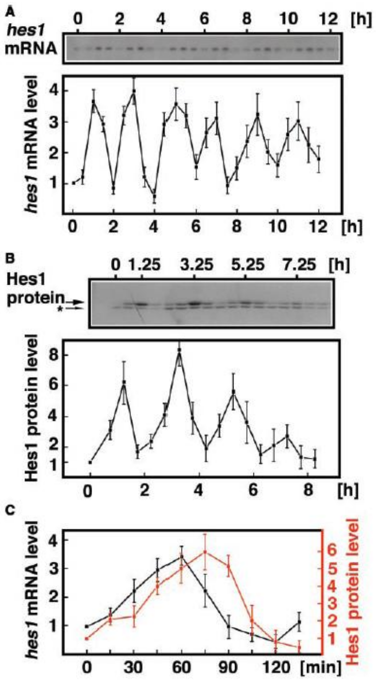
Jensen et al 2003

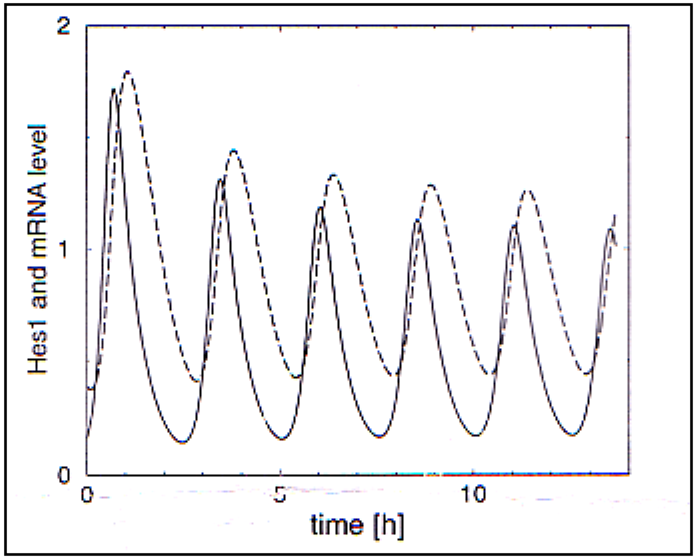
$$\frac{d[mRNA]}{dt} = \alpha \cdot \frac{K_M}{K_M + [Hes1(t-\tau)]^n} - \frac{[mRNA(t)]}{\tau_{rna}}$$

$$\frac{d[Hes1]}{dt} = \beta \cdot [mRNA(t)] - \frac{[Hes1(t)]}{\tau_{hes1}}$$

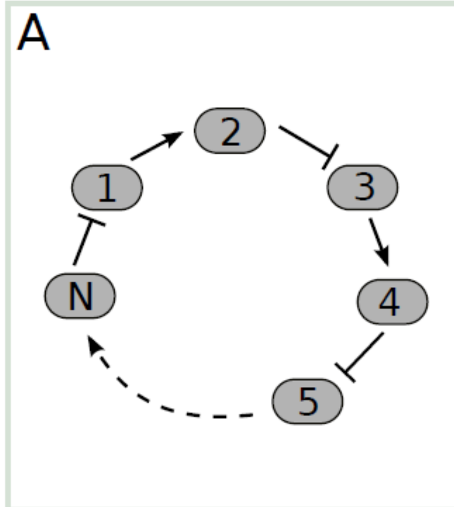
- Dashed curve [Hes1]
- Solid curve [mRNA]

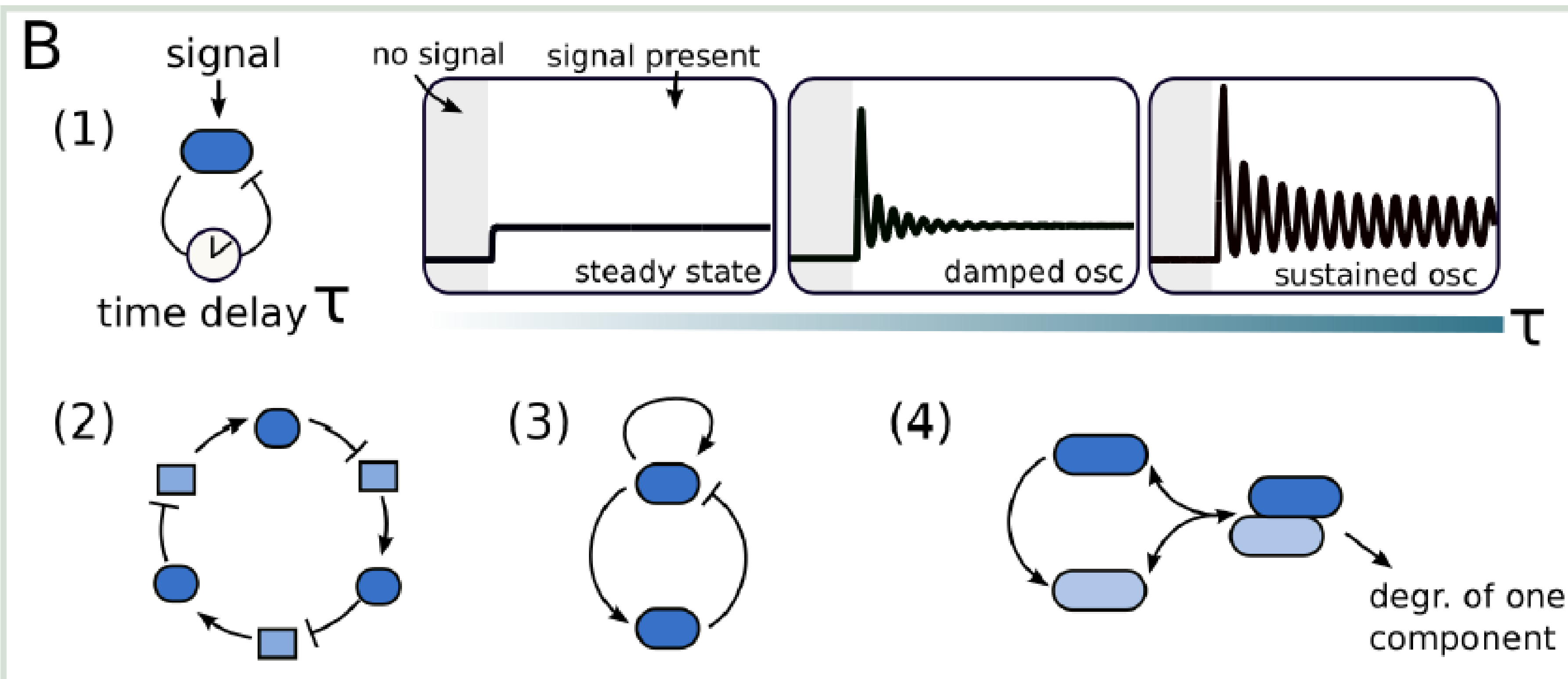
- τ_{rna} = 24.1 min
- τ_{hes1} = 22.3 min
- τ = 24 min
- $\alpha = 20\ [R]_0\ min^{-1}$
- $\beta = 1/20\ min^{-1}$
- $K_M = (0.1[R]_0)^n$
- n = 4

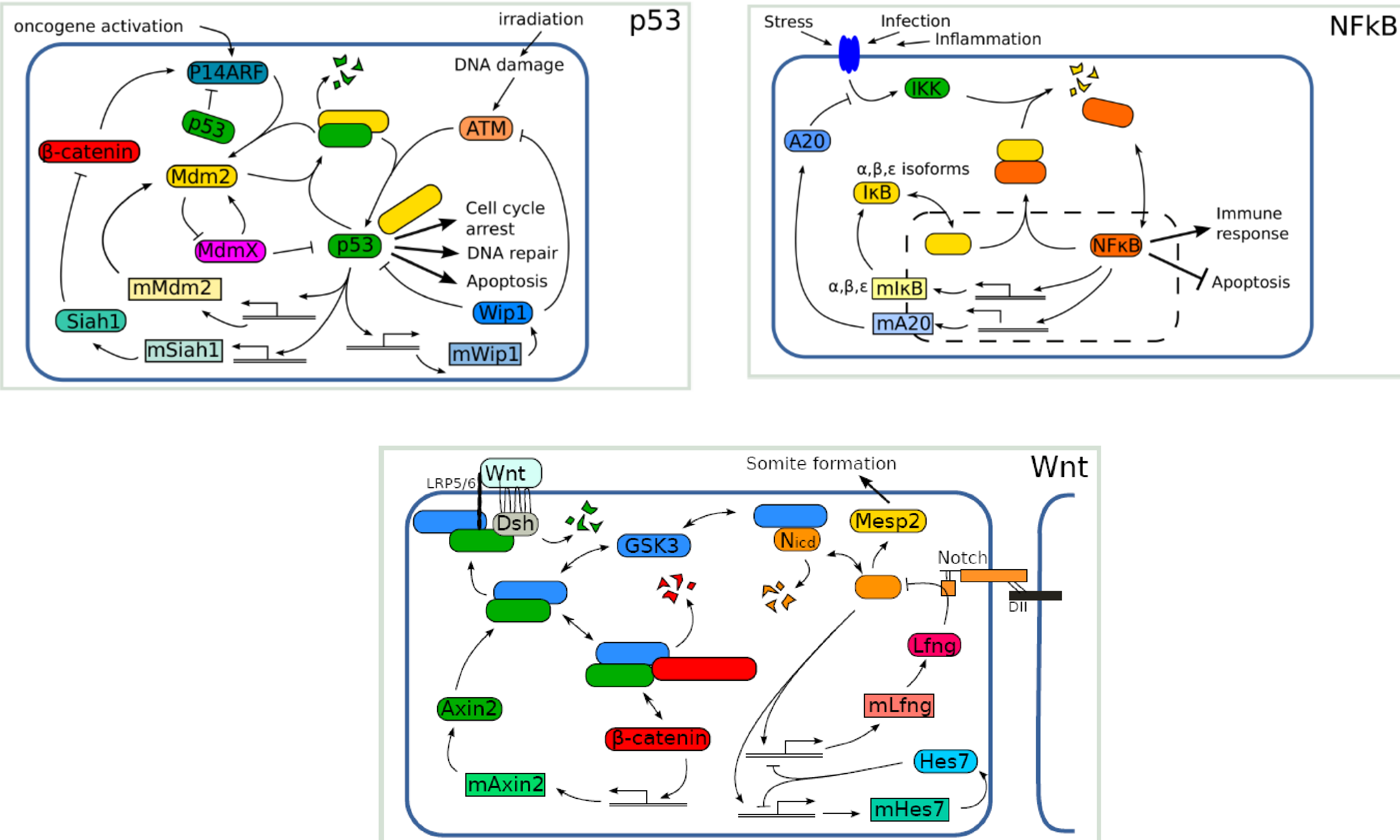


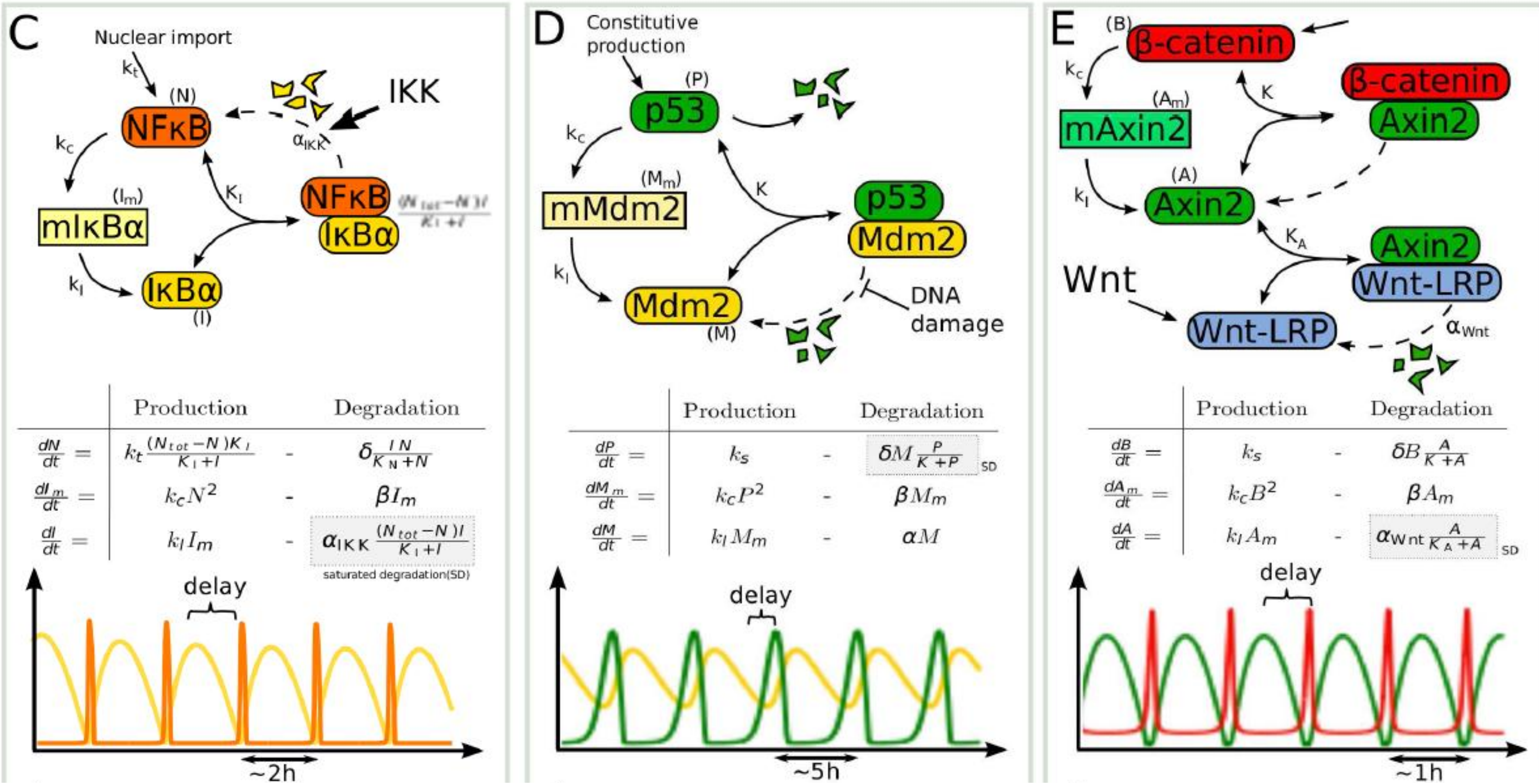


Negative Feed-Back loops:






p53
oncogene activation
irradiation
DNA damage
P14ARF
p53
ATM
β-catenin
Mdm2
MdmX
p53
Cell cycle arrest
DNA repair
Apoptosis
mMdm2
Siah1
mSiah1
Wip1
mWip1
NFkB
Stress
Infection
Inflammation
IKK
A20
α,β,ε isoforms
IκB
NFκB
Immune response
Apoptosis
α,β,ε mIκB
mA20
Wnt
Somite formation
LRP5/6
Wnt
Dsh
GSK3
Nicd
Mesp2
Notch
Dll
Lfng
mLfng
Axin2
β-catenin
Hes7
mAxin2
mHes7



C
Nuclear import
k_t
(N)
NFκB
IKK
α_{IKK}
k_c
K_I
NFκB
IκBα
$\frac{(N_{tot}-N)I}{K_I+I}$
(I_m)
mIκBα
k_I
IκBα
(I)
Production
Degradation
$\frac{dN}{dt} = k_t \frac{(N_{tot}-N)K_I}{K_I+I} - \delta \frac{IN}{K_N+N}$
$\frac{dI_m}{dt} = k_c N^2 - \beta I_m$
$\frac{dI}{dt} = k_I I_m - \alpha_{IKK} \frac{(N_{tot}-N)I}{K_I+I}$
saturated degradation(SD)
delay
~2h
D
Constitutive production
(P)
p53
k_c
K
(M_m)
mMdm2
p53
Mdm2
k_I
Mdm2
(M)
DNA damage
Production
Degradation
$\frac{dP}{dt} = k_s - \delta M \frac{P}{K+P}$ SD
$\frac{dM_m}{dt} = k_c P^2 - \beta M_m$
$\frac{dM}{dt} = k_I M_m - \alpha M$
delay
~5h
E
(B)
β-catenin
k_c
K
β-catenin
Axin2
(A_m)
mAxin2
(A)
k_I
Axin2
K_A
Axin2
Wnt-LRP
Wnt
Wnt-LRP
α_{Wnt}
Production
Degradation
$\frac{dB}{dt} = k_s - \delta B \frac{A}{K+A}$
$\frac{dA_m}{dt} = k_c B^2 - \beta A_m$
$\frac{dA}{dt} = k_I A_m - \alpha_{Wnt} \frac{A}{K_A+A}$ SD
delay
~1h

Why oscillations ?

- Importance for DNA-repair and apoptosis
- Essential in segmentation
- Spiky oscillations →

important for sharp responses,

fast regulations,

high Hill coefficients

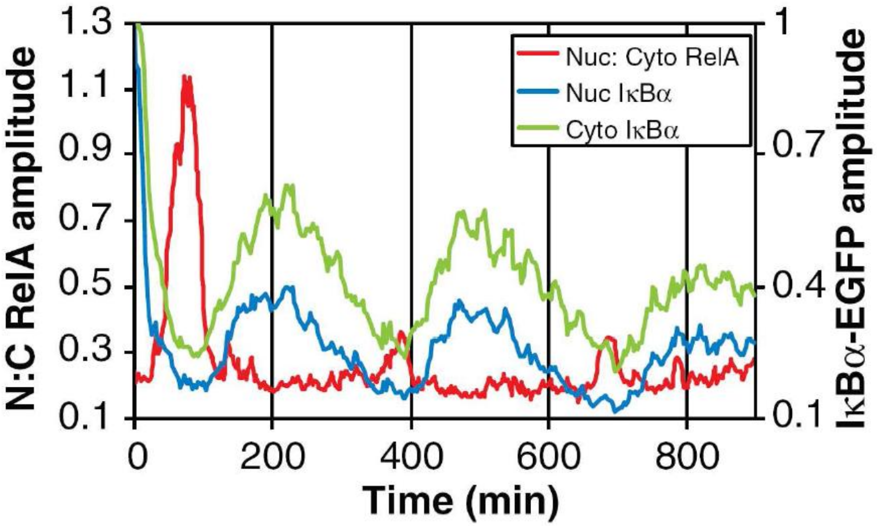
→ hormones also come in spikes

Mathematically: The most `simple' dynamics !

The NF-κB System in Mammalian Cells

- NF-κB family: dimeric transcription factors
- Regulates immune response, inflammation, apoptosis
- Over 150 triggering signals, over 150 targets
- Each NF-κB has a partner inhibitor IκB
- Flourescence imaging of NF-κB and IκB in human S-type neuroblastoma cells.

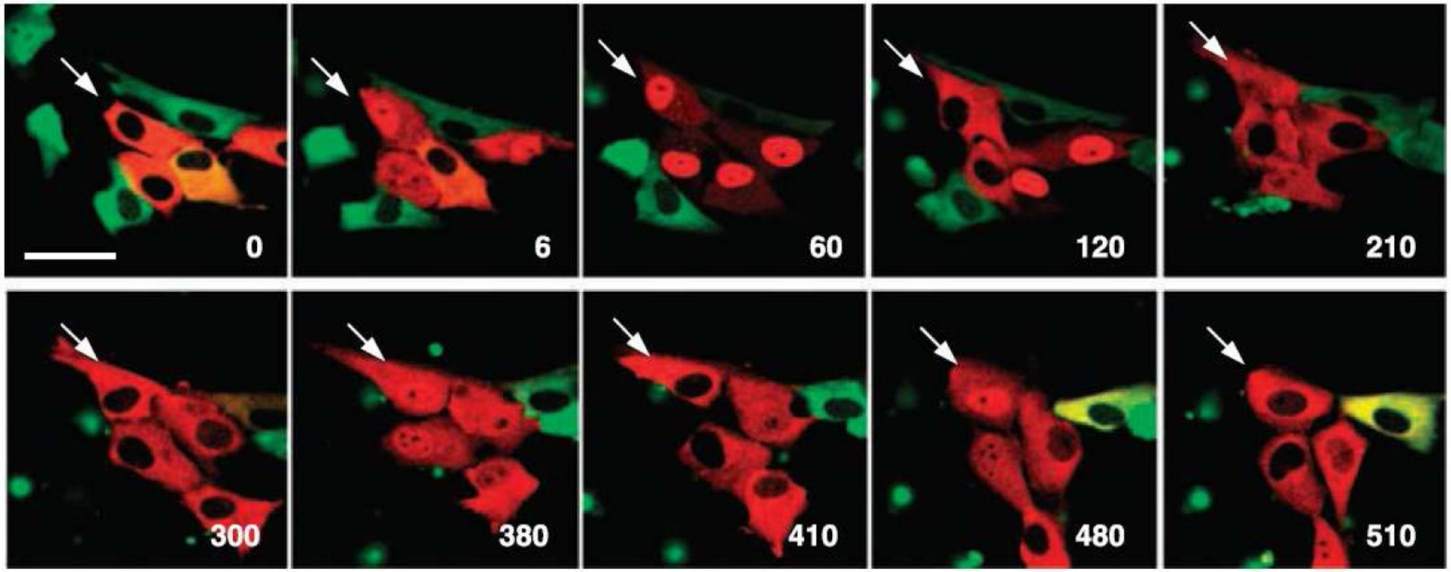
Nelson et al. (2004) Science 306, 704.



How does the network produce oscillations?

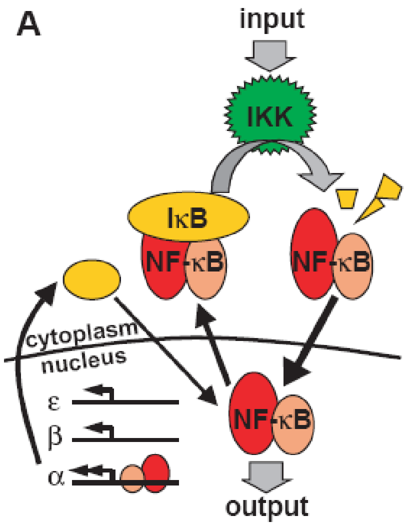
Why does the cell need the oscillations?

‘Direct’ observations of oscillations in nucleus

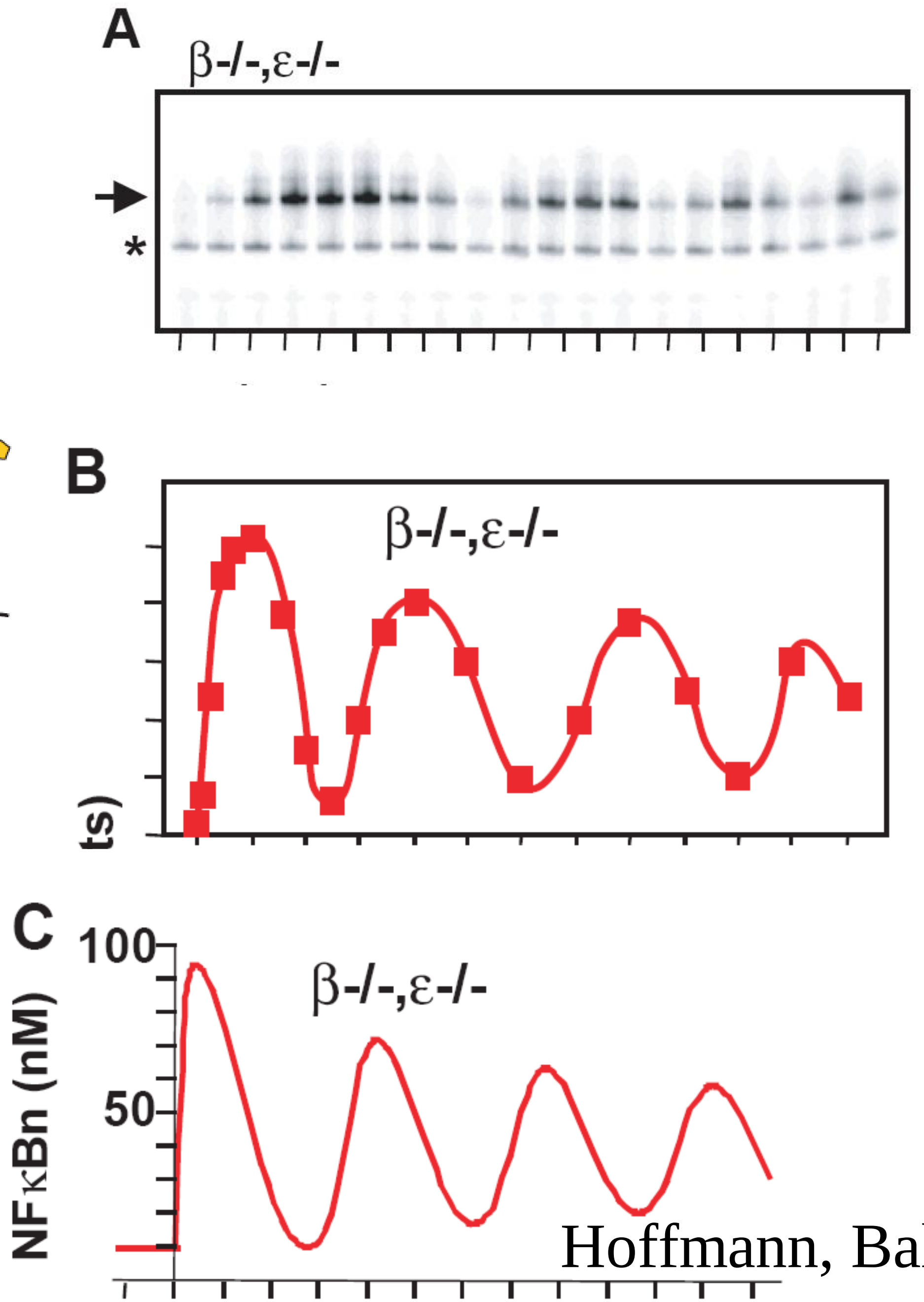


Oscillations in the nuclear localization of an NF-κB transcription factor in human cells

Nelson et al. (2004) Science 306, 704.



The NF-κB feed-back network

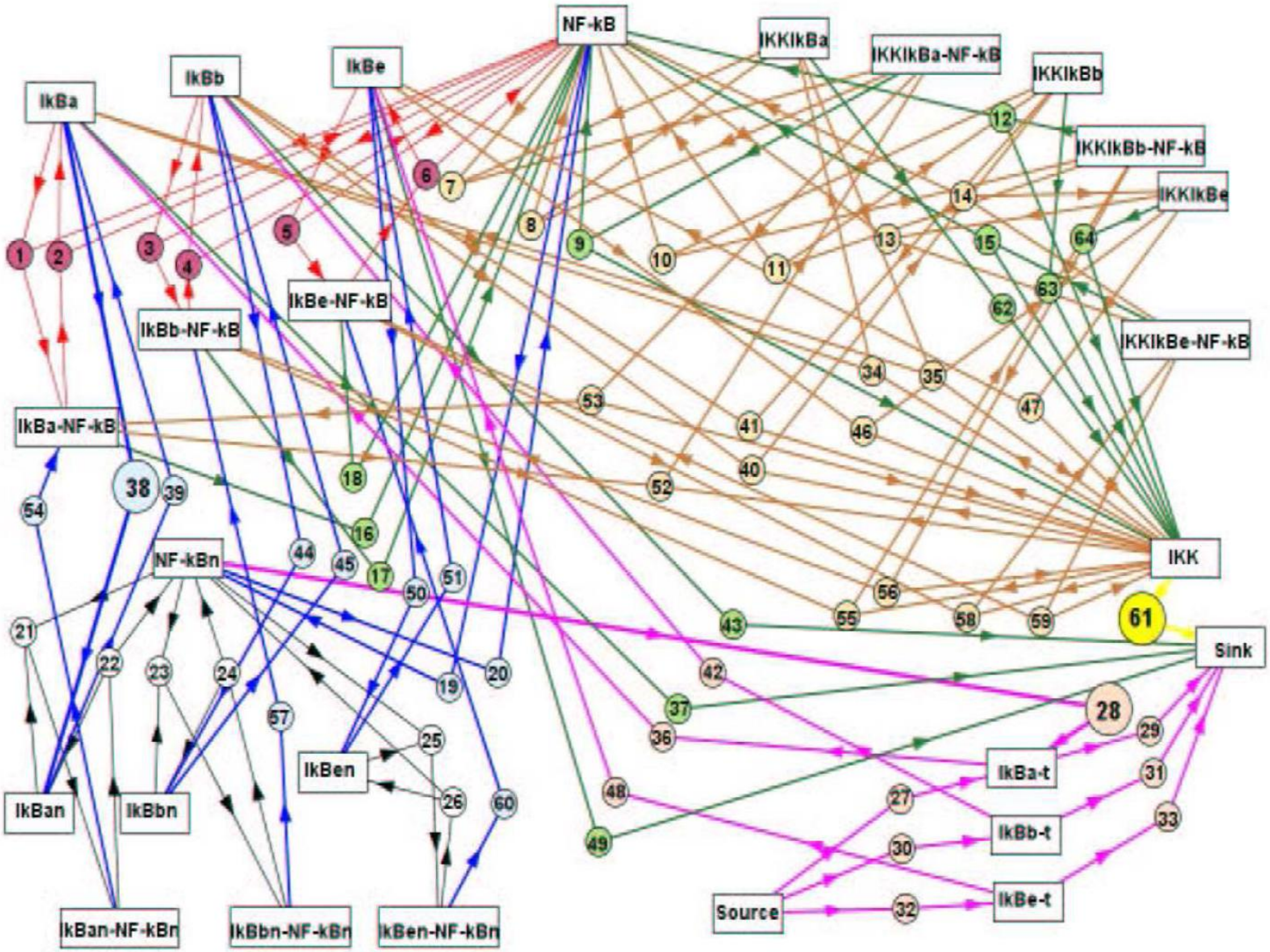


NF-κB oscillations

IκBβ/ε knocked out

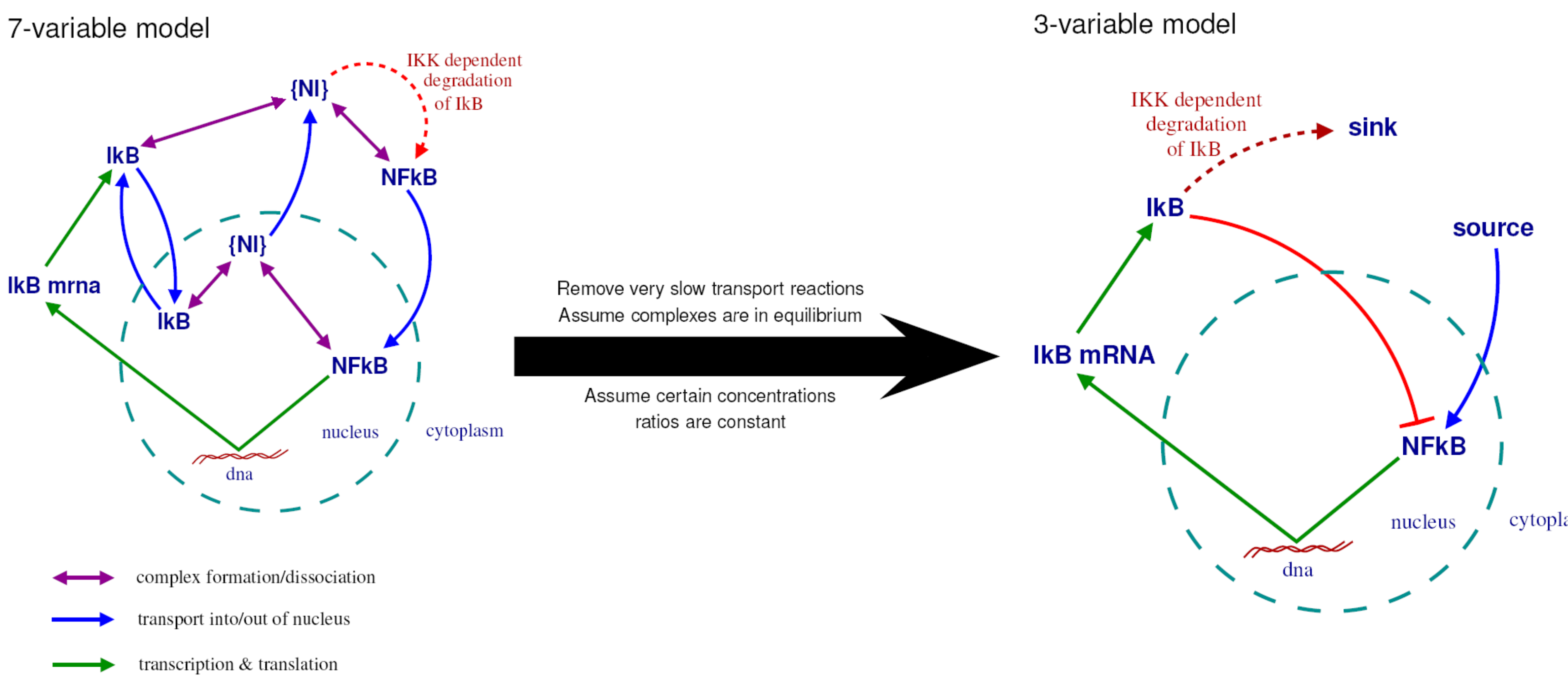
Hoffmann, Baltimore et al (2002)

The NF-κB System in Mammalian Cells



Nelson et al. (2004) Science 306, 704.

Reduction of the NF-κB system

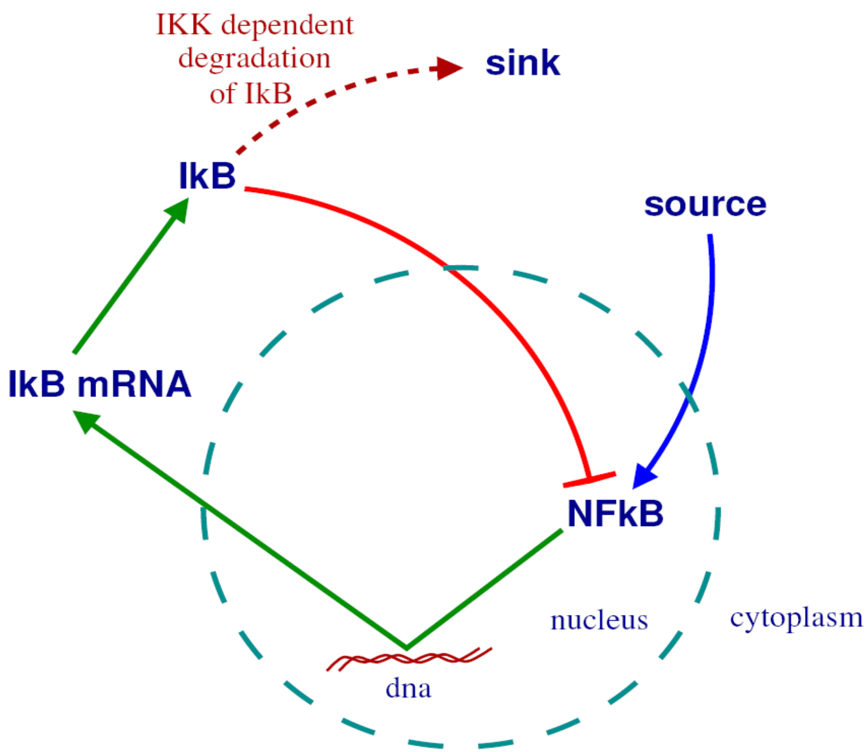


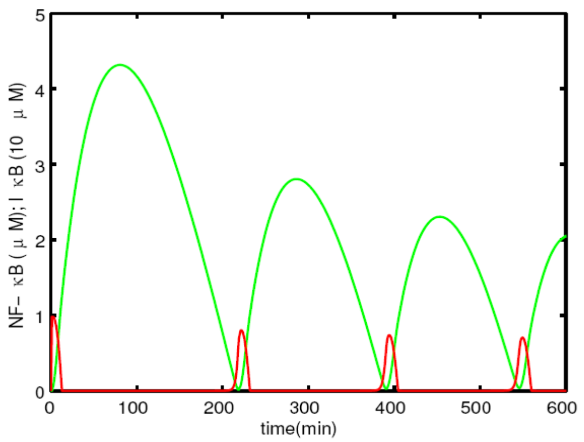
Simple Model for Protein Oscillations

$$\frac{dN_n}{dt} = A\frac{(1-N_n)}{\epsilon + I} - B\frac{I N_n}{\delta + N_n},$$

$$\frac{dI_m}{dt} = N_n^2 - I_m,$$

$$\frac{dI}{dt} = I_m - C\frac{(1-N_n)I}{\epsilon + I}.$$

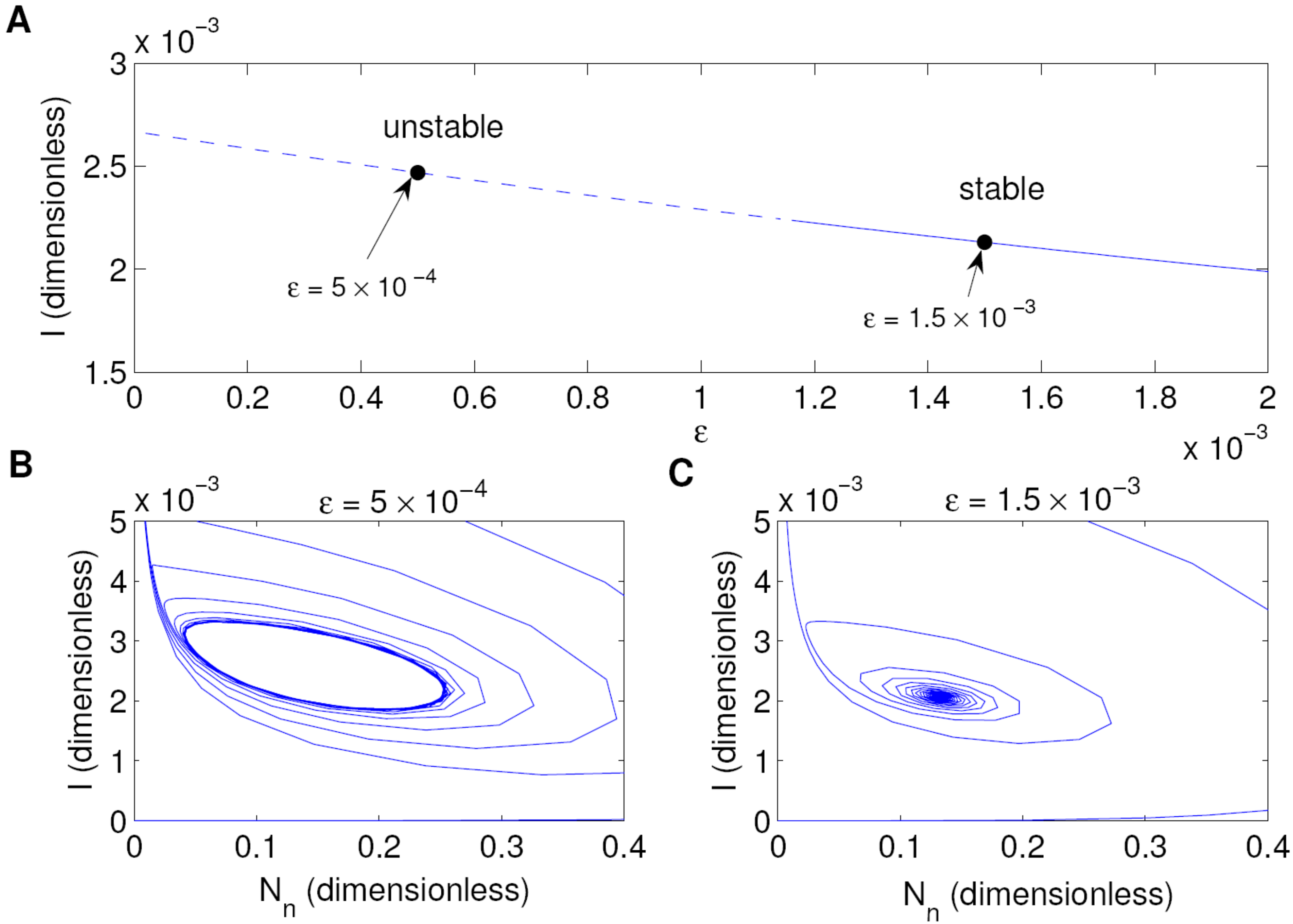




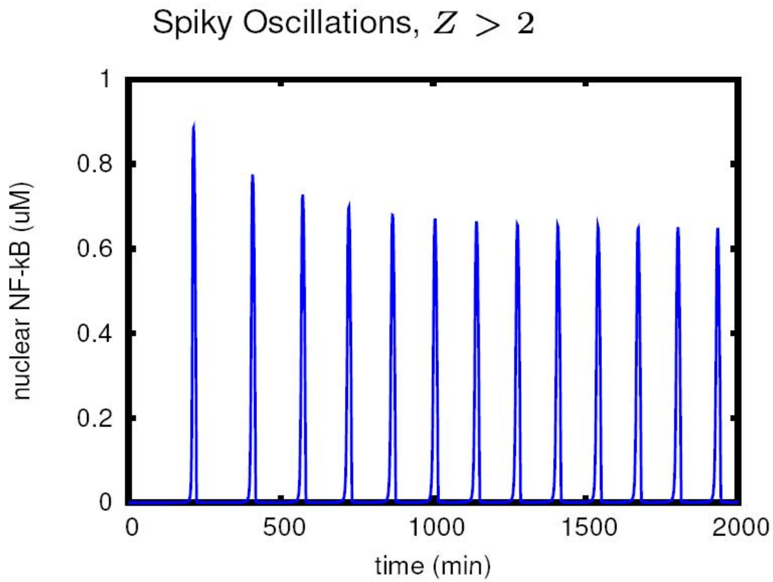
$$A = 0.007,\ B = 954.5,\ C = 0.035,$$

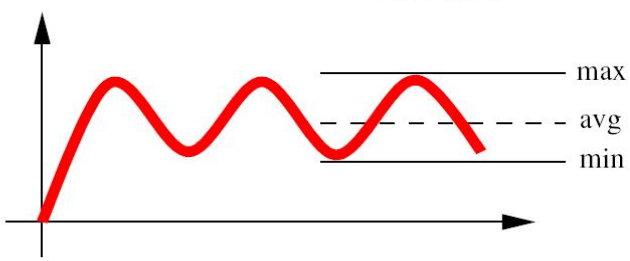
$$\delta = 0.029,\ \epsilon = 2 \times 10^{-5}$$

Hopf bifurcation

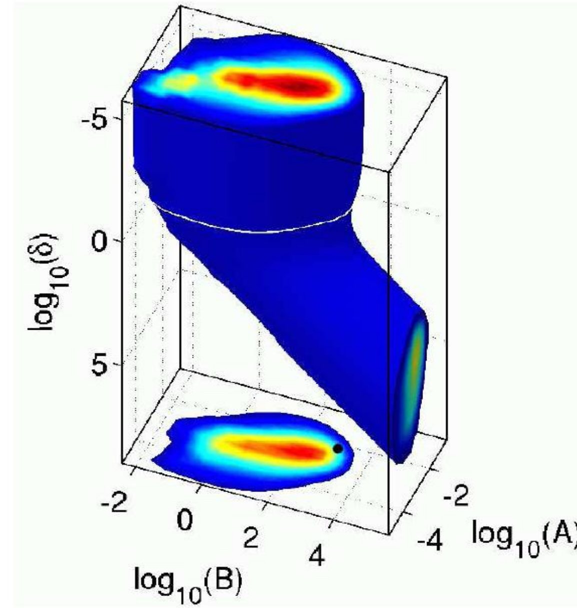


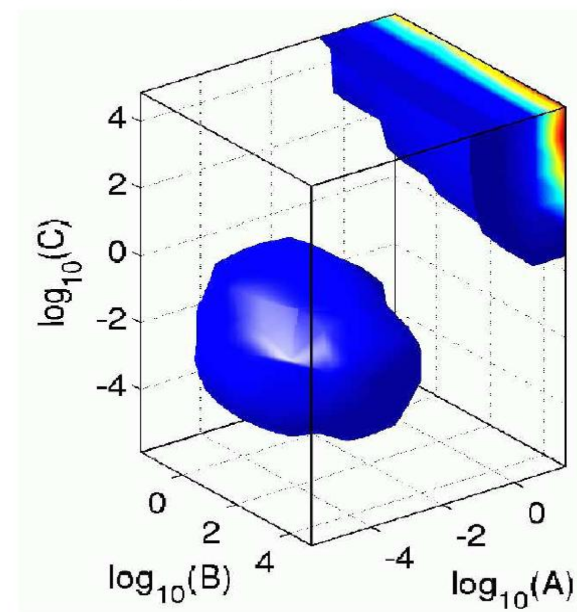
Robust Spiky Oscillations



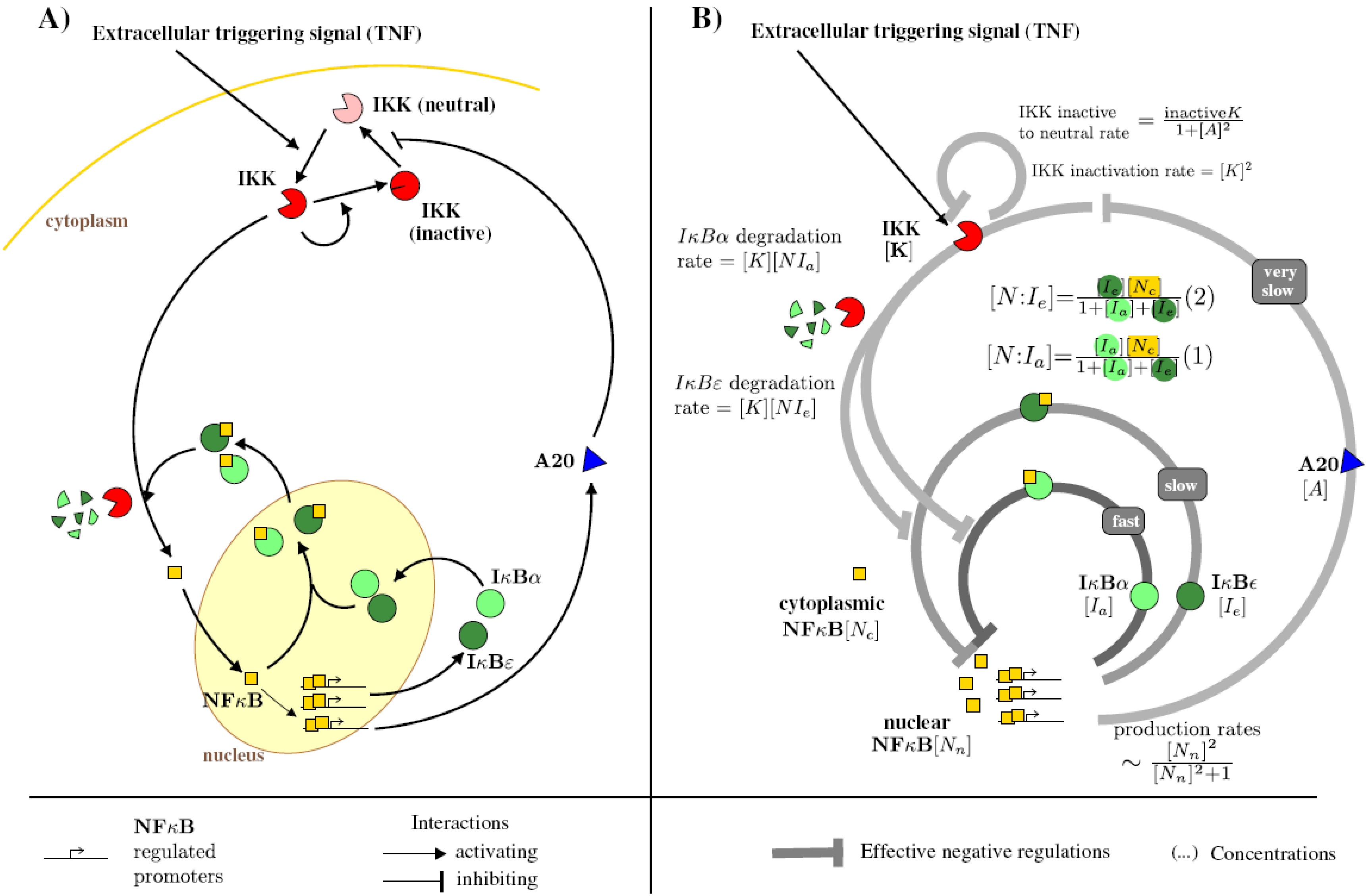


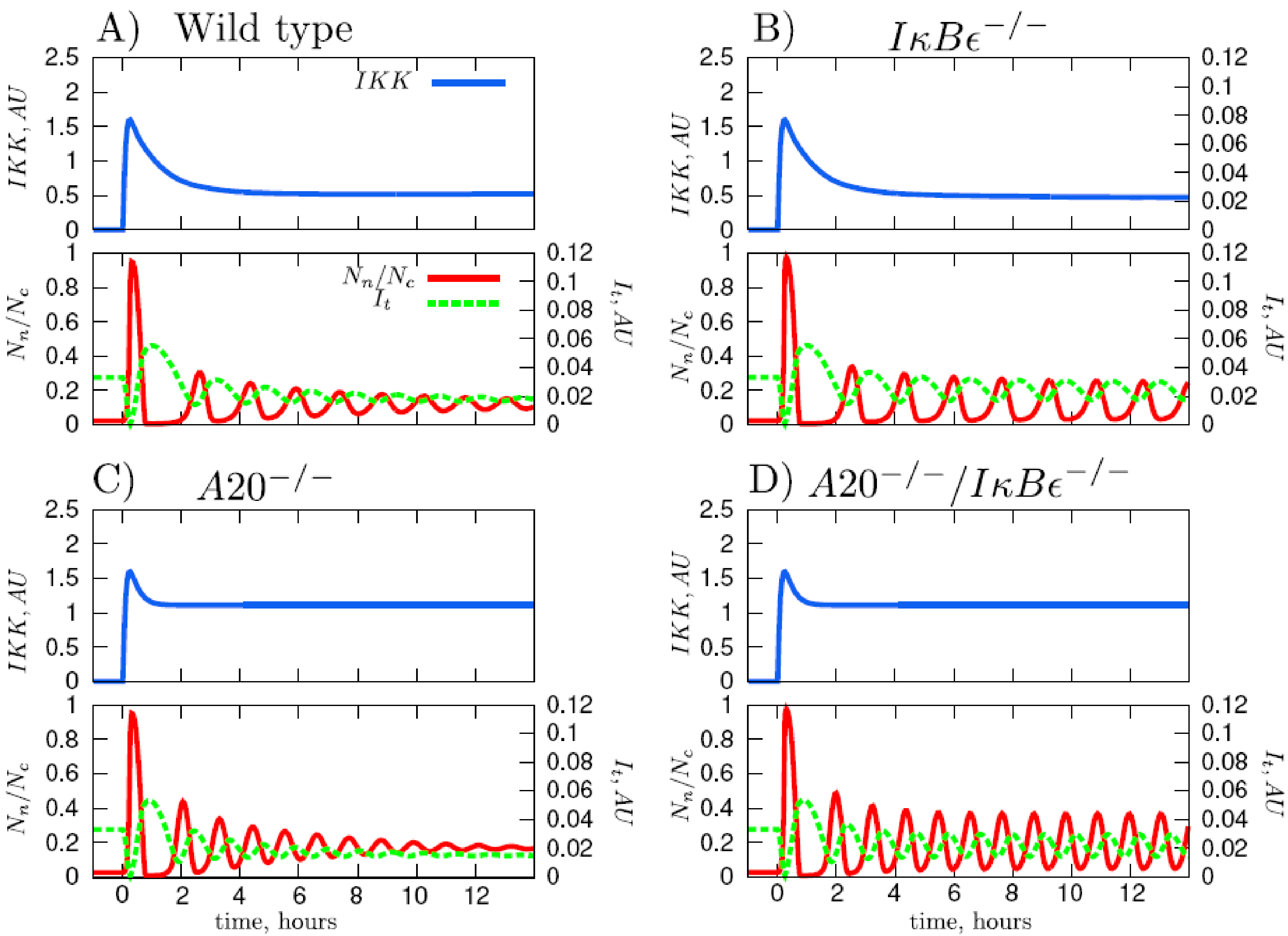
$$Z = \frac{\max - \min}{\text{avg}}$$





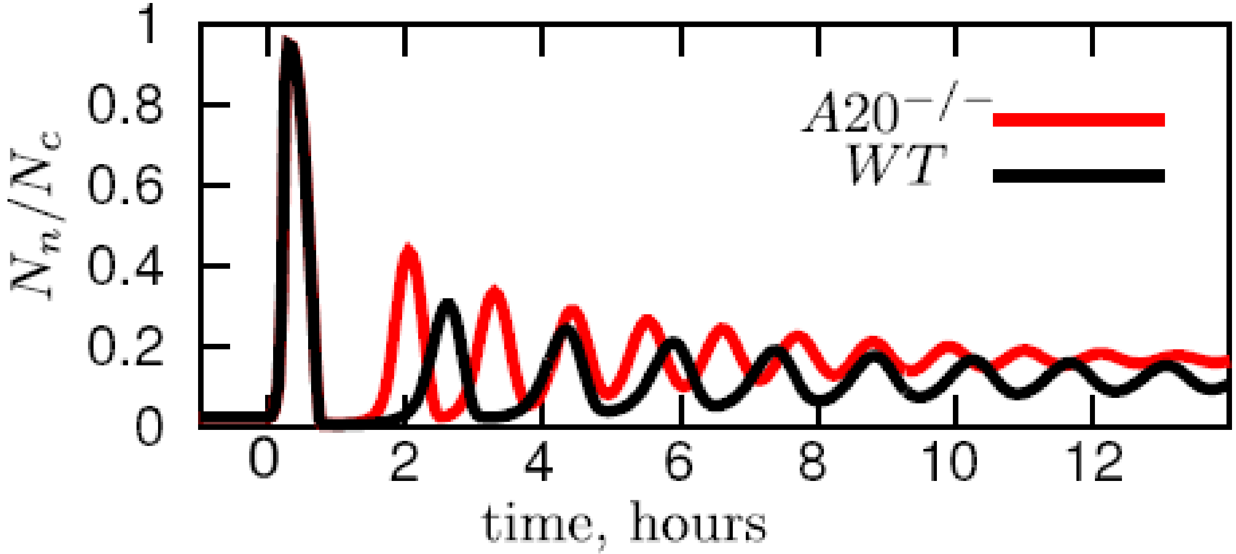
Nested feed-back loops





A) Wild type
B) $I\kappa B\epsilon^{-/-}$
C) $A20^{-/-}$
D) $A20^{-/-}/I\kappa B\epsilon^{-/-}$
IKK
IKK, AU
N_n/N_c
I_t
I_t, AU
time, hours

A20 regulates period of NF-κB oscillations



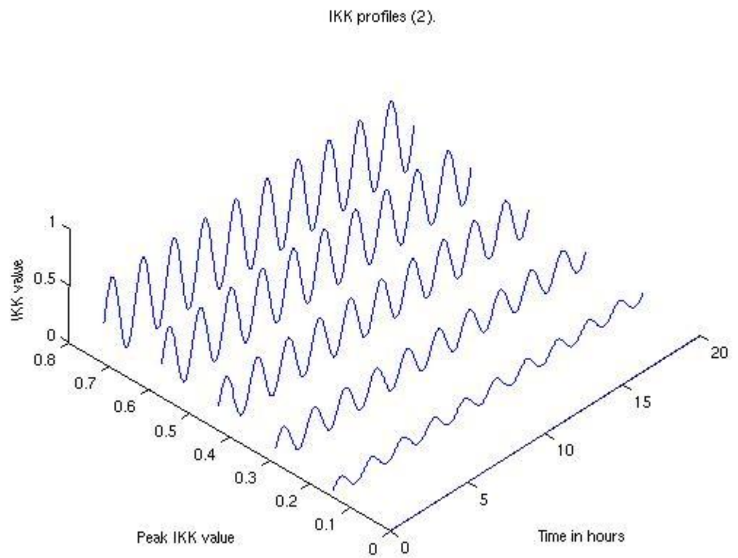
Single cells !

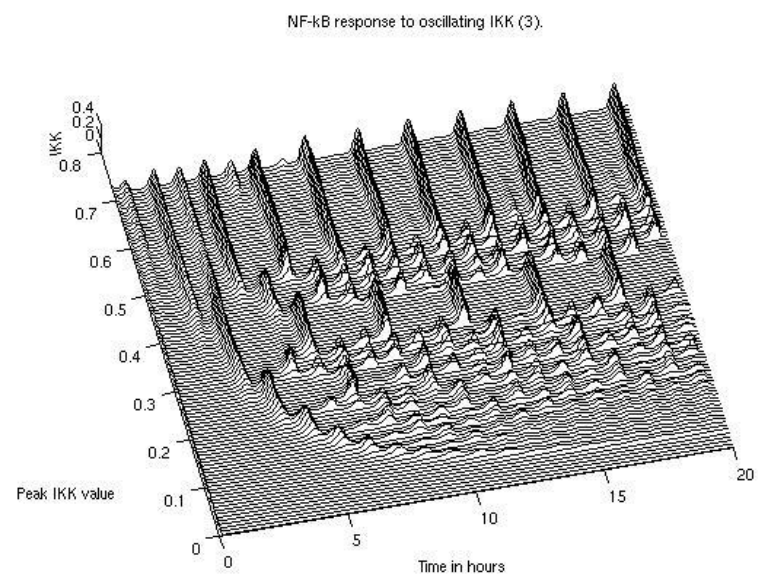
IKK has been observed to oscillate itself

$$\frac{dN_n}{dt} = A\frac{(1-N_n)}{\epsilon+I} - B\frac{IN_n}{\delta+N_n},$$
$$\frac{dI_m}{dt} = N_n^2 - I_m,$$
$$\frac{dI}{dt} = I_m - C\frac{(1-N_n)I}{\epsilon+I}.$$

$$C \to C(1+sin2\pi\omega t)$$

(Kristian Rud, Jesper Fonslet)

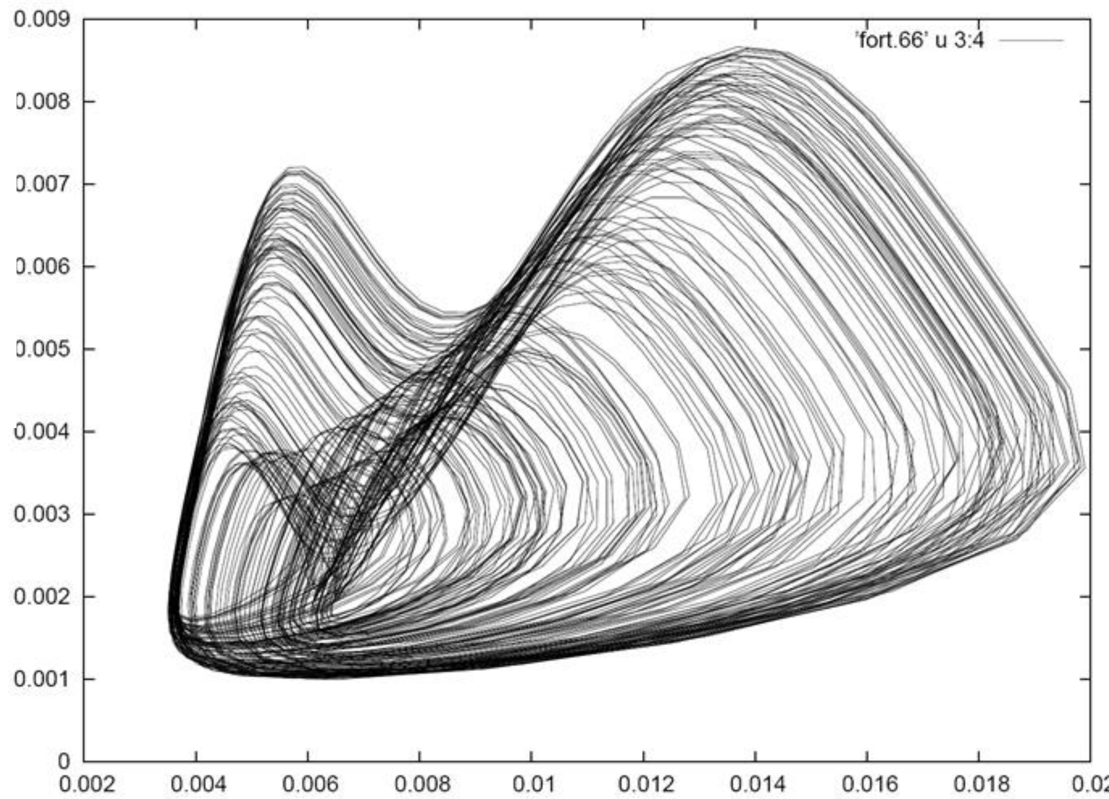




Strange attractor of periodically 'forced' NF-κB system

$$\frac{dN_n}{dt} = A\frac{(1-N_n)}{\epsilon+I} - B\frac{IN_n}{\delta+N_n},$$
$$\frac{dI_m}{dt} = N_n^2 - I_m,$$
$$\frac{dI}{dt} = I_m - C\frac{(1-N_n)I}{\epsilon+I}.$$

$$C \rightarrow C(1+sin2\pi\omega t)$$



ω=1

$A = 0.007$, $B = 954.5$, $C = 0.035$, $\delta = 0.029$ and $\epsilon = 2 \times 10^{-5}$

(2-3 hour period)

Response to irradiation in single cells

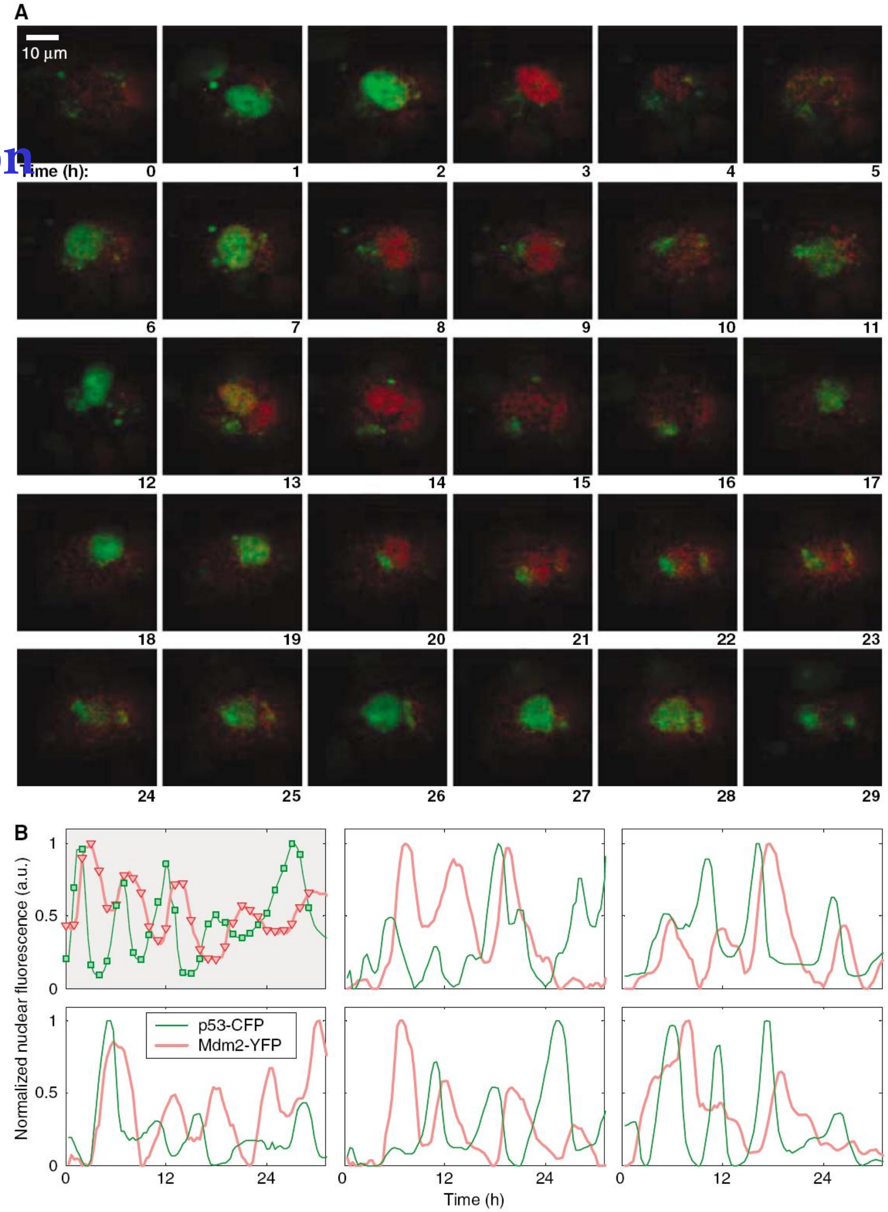


Figure 1 Prolonged oscillations in the nuclear levels of fluorescently tagged p53 and Mdm2 in individual MCF7, U280, cells following gamma irradiation. (**A**) Time-lapse fluorescence images of one cell over 29 h after 5 Gy of gamma irradiation. Nuclear p53-CFP and Mdm2-YFP are imaged in green and red, respectively. Time is indicated in hours. (**B**) Normalized nuclear fluorescence levels of p53-CFP (green) and Mdm2-YFP (red) following gamma irradiation. Top left: the cell shown in panel A. Other panels: five cells from one field of view, after exposure to 2.5 Gy gamma irradiation.

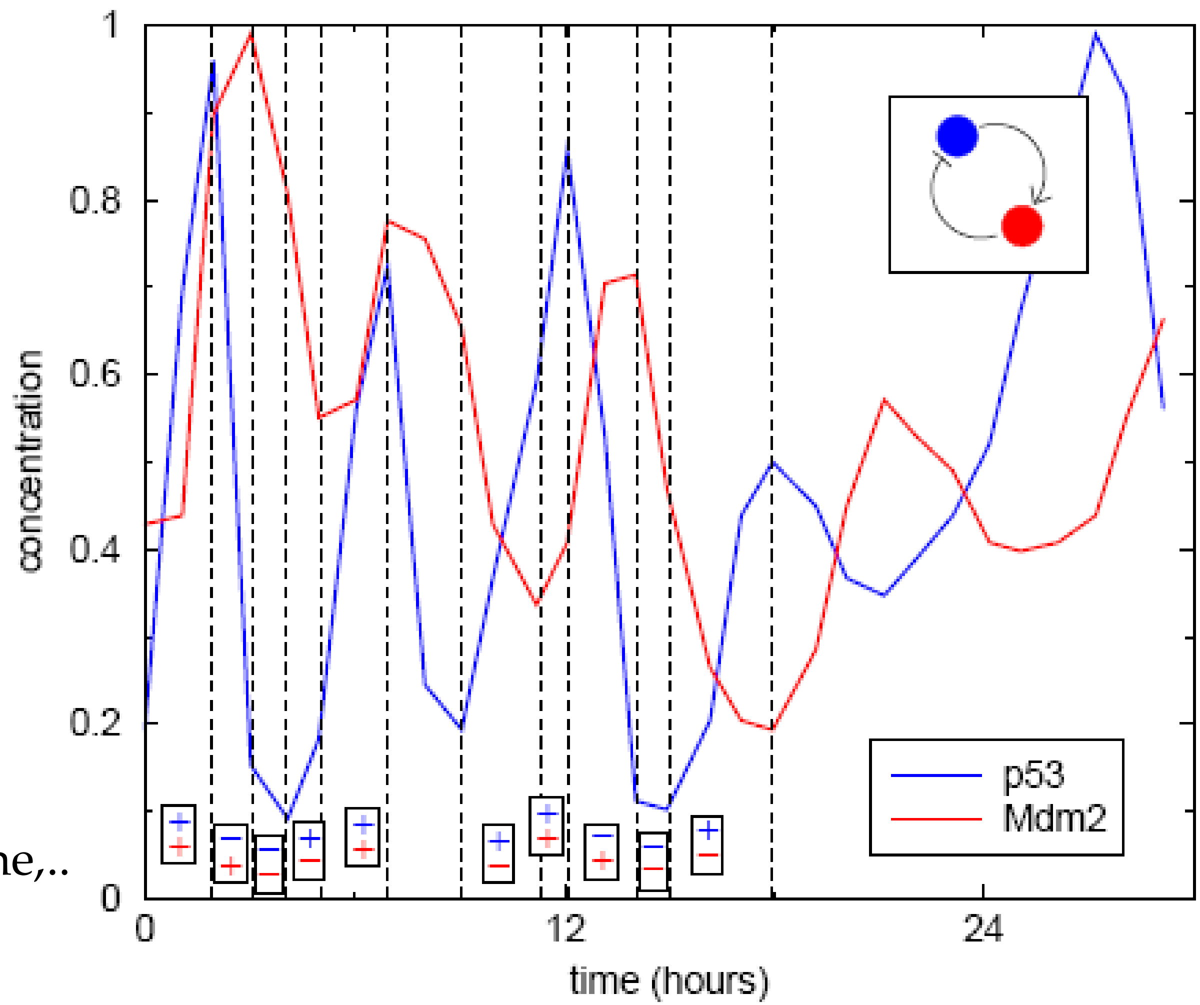
Often time series are very noisy !

→ Then what ?

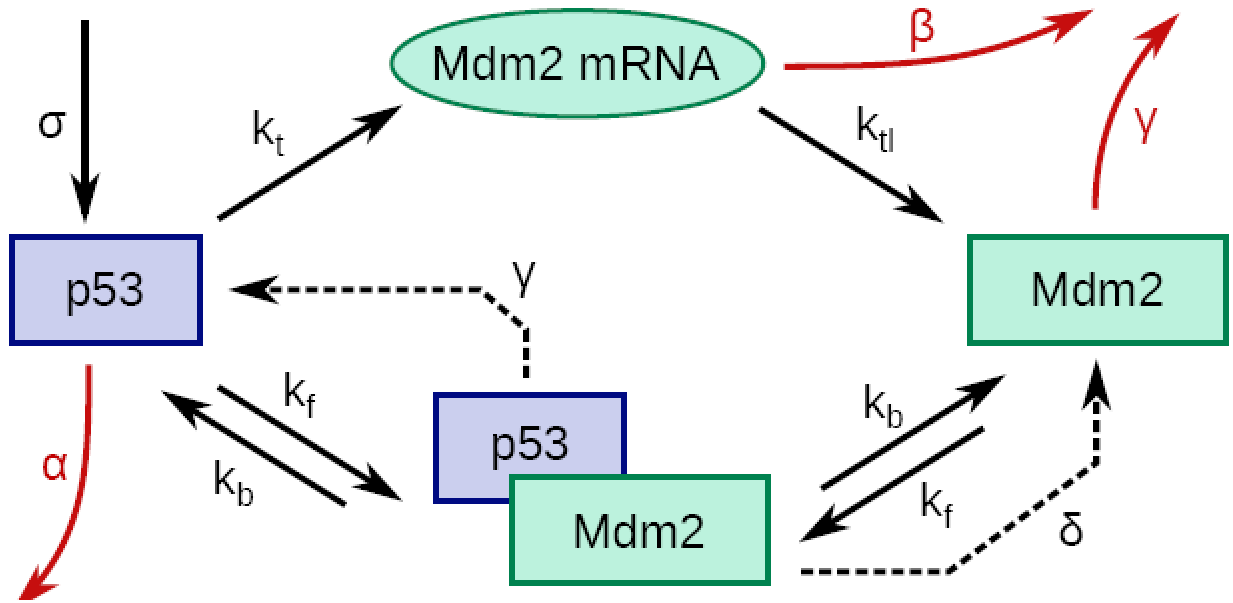
N. Geva-Zatorsky, *Mol. Sys. Biol.* 2:2006.0033 (2006)

Apoptosis

(a) N. Geva-Zatorsky, *Mol. Sys. Biol.* 2:2006.0033 (2006)



Lahav, Alon, Levine,..



$$\frac{dp}{dt} = \sigma - \alpha p - k_f pm + k_b c + \gamma c$$

$$\frac{dm_m}{dt} = k_t p^2 - \beta m_m$$

$$\frac{dm}{dt} = k_{tl} m_m - k_f pm + k_b c + \delta c - \gamma m$$

$$\frac{dc}{dt} = k_f pm - k_b c - \delta c - \gamma c$$

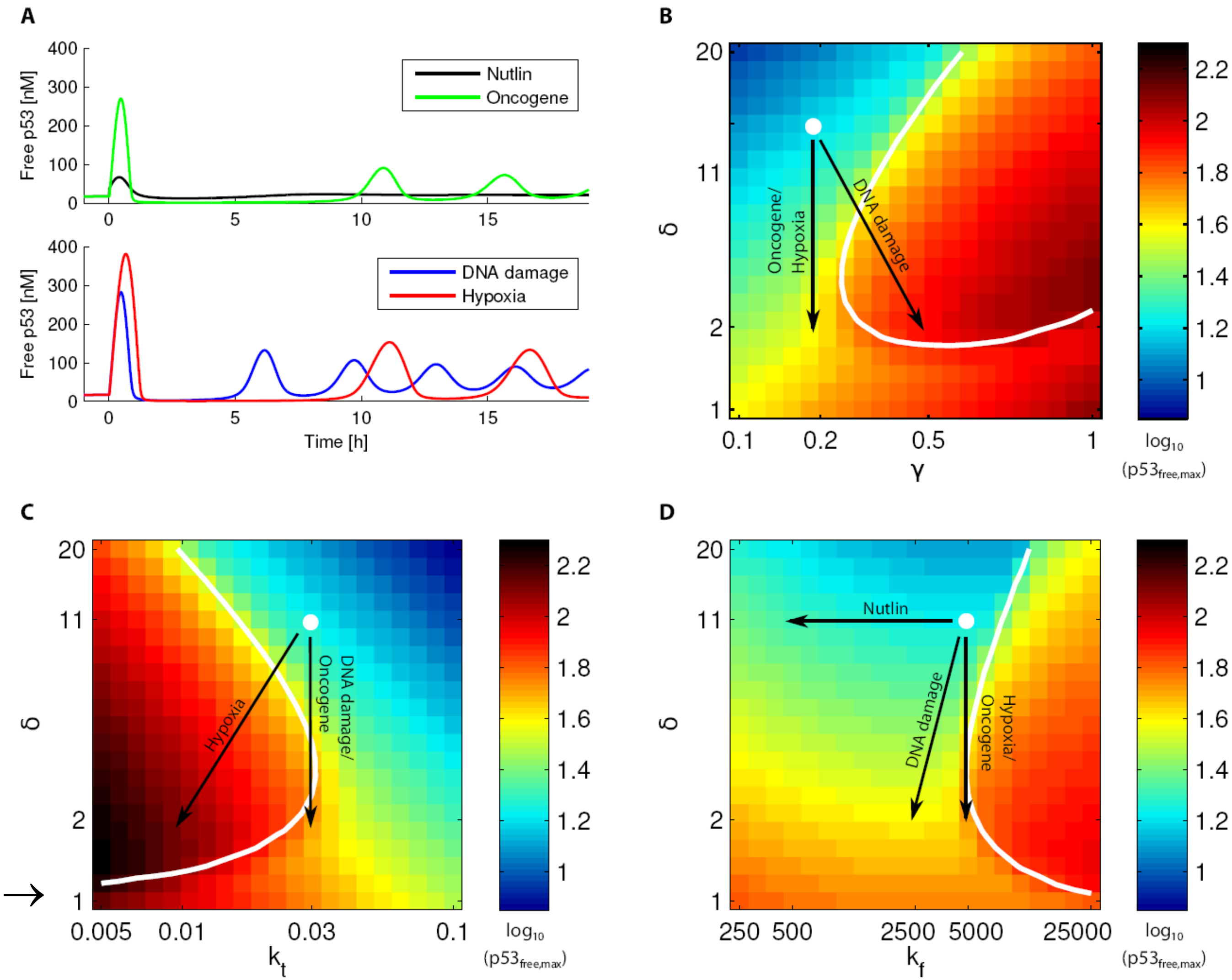
Mdm2 regulates both activity and stability

tions: nuclear-p53, p; Mdm2, m; Mdm2 mRNA, m_m; and the p53-Mdm2 complex, c. The tempo-

Four different stresses

- DNA damage: Irradiation triggers oscillations: increase auto-ubiq. of Mdm2, decrease ubiq. of p53 by Mdm2, weak p53-Mdm2 binding
- Hypoxia: Deprive oxygen, apoptosis: decrease transactivation, prevents degrad. of p53
- Oncogenes: trigger 53 pathway: decrease Mdm2-dependent degrad.
- Nutlin (chemical), cell-cycle arrest (not apoptosis): reduces p53-Mdm2 binding

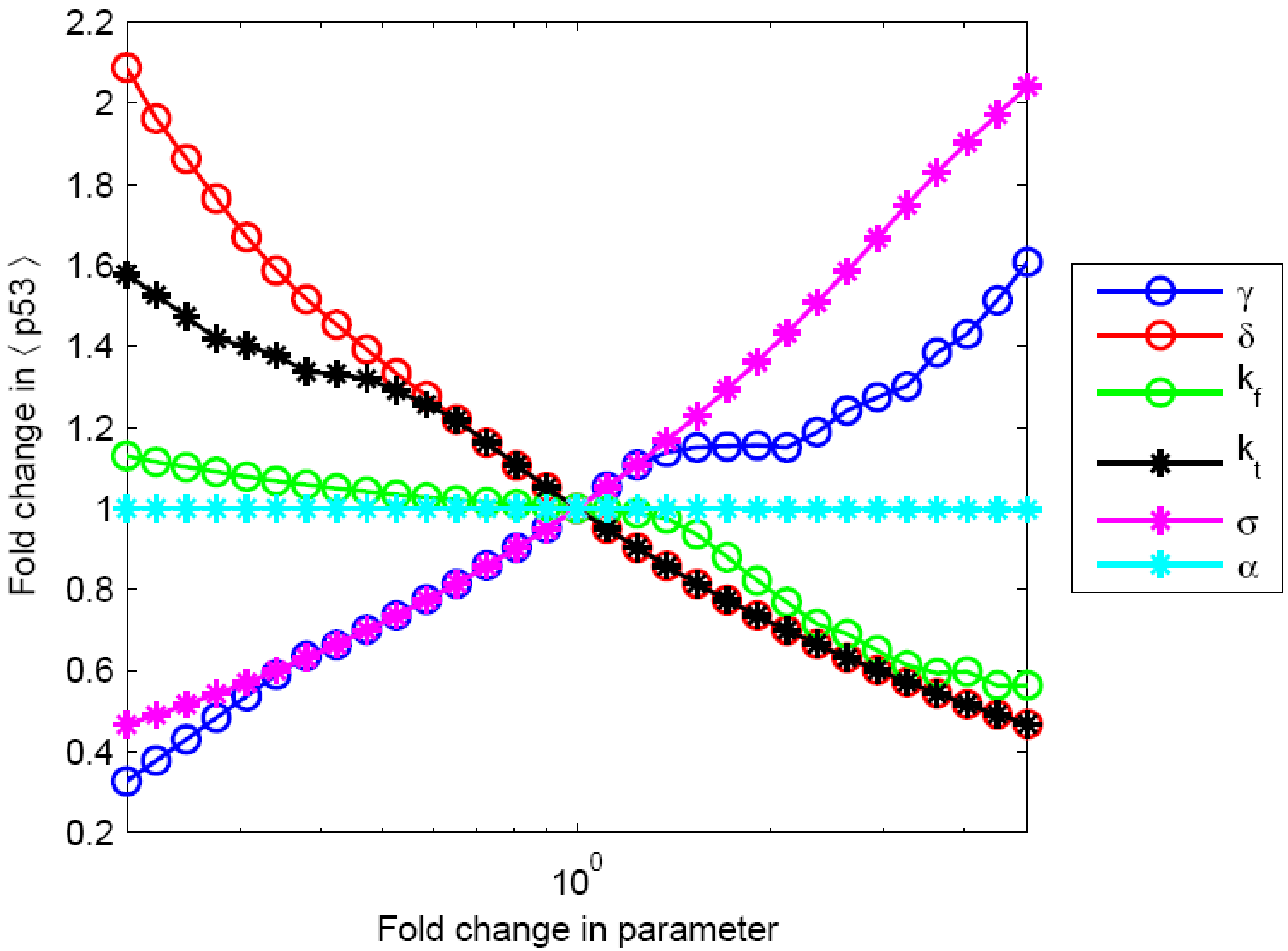
Stress variations in parameters



Spikyness →

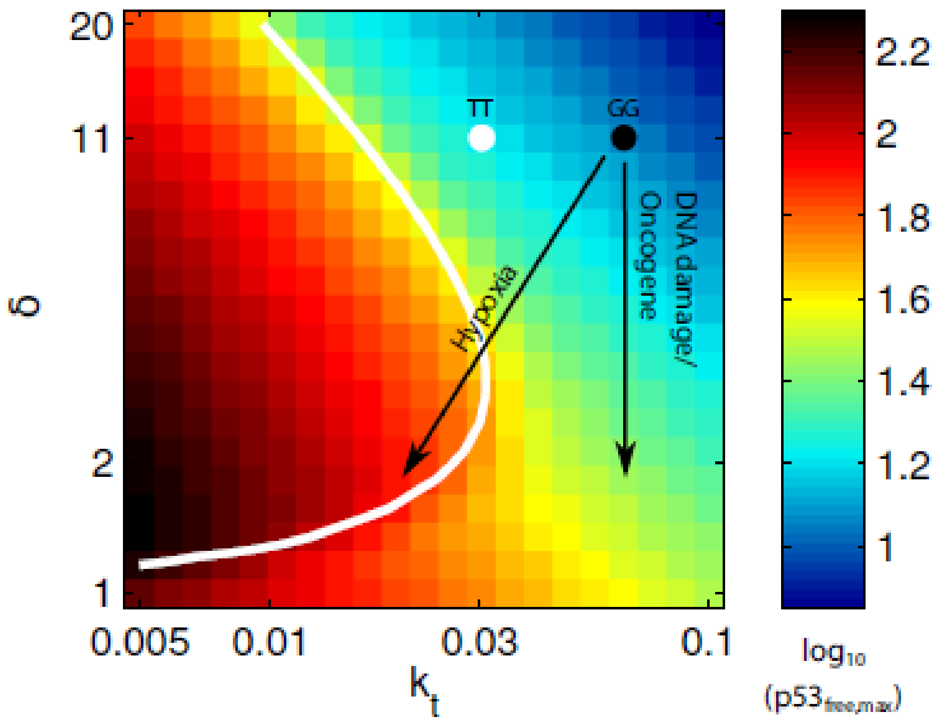
p53 concentrations (peak)

Responses:



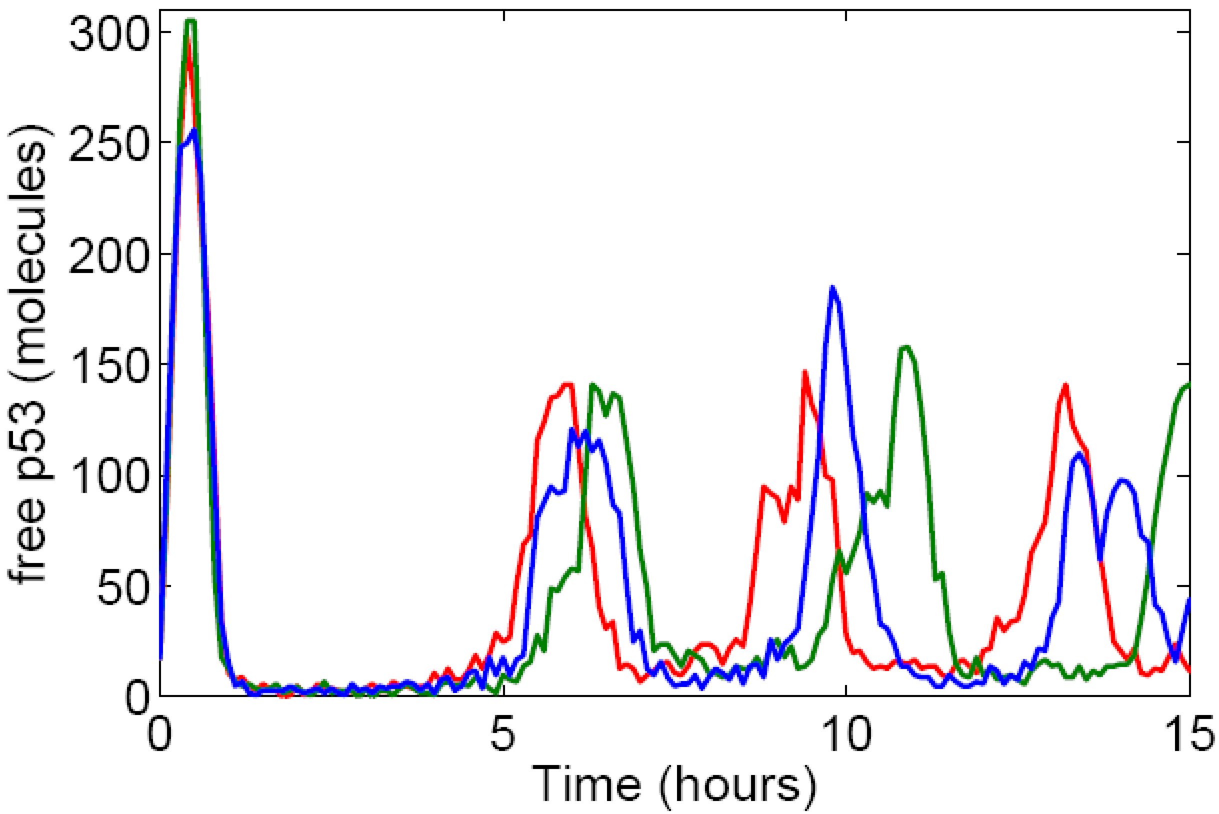
δ: DNA damage, hypoxia, oncogenes

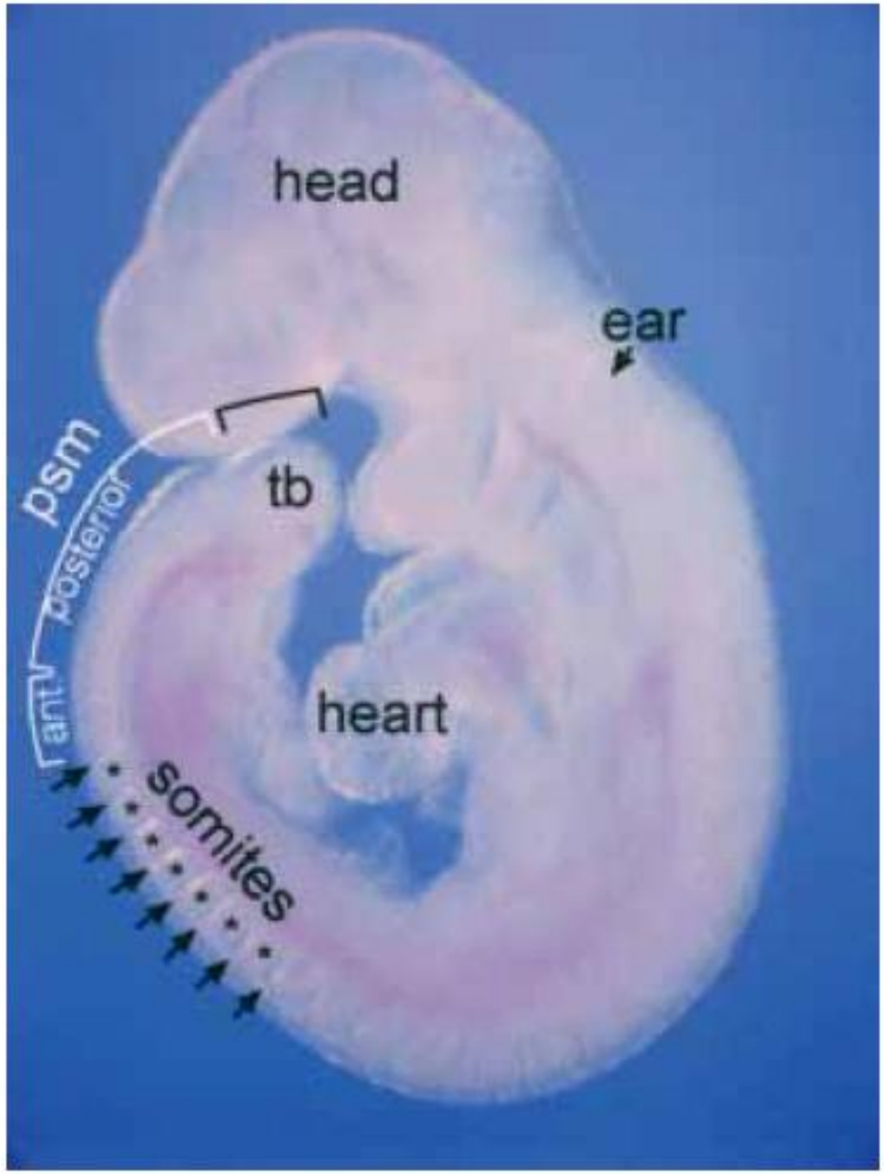
Mdm2 SNP309 allele

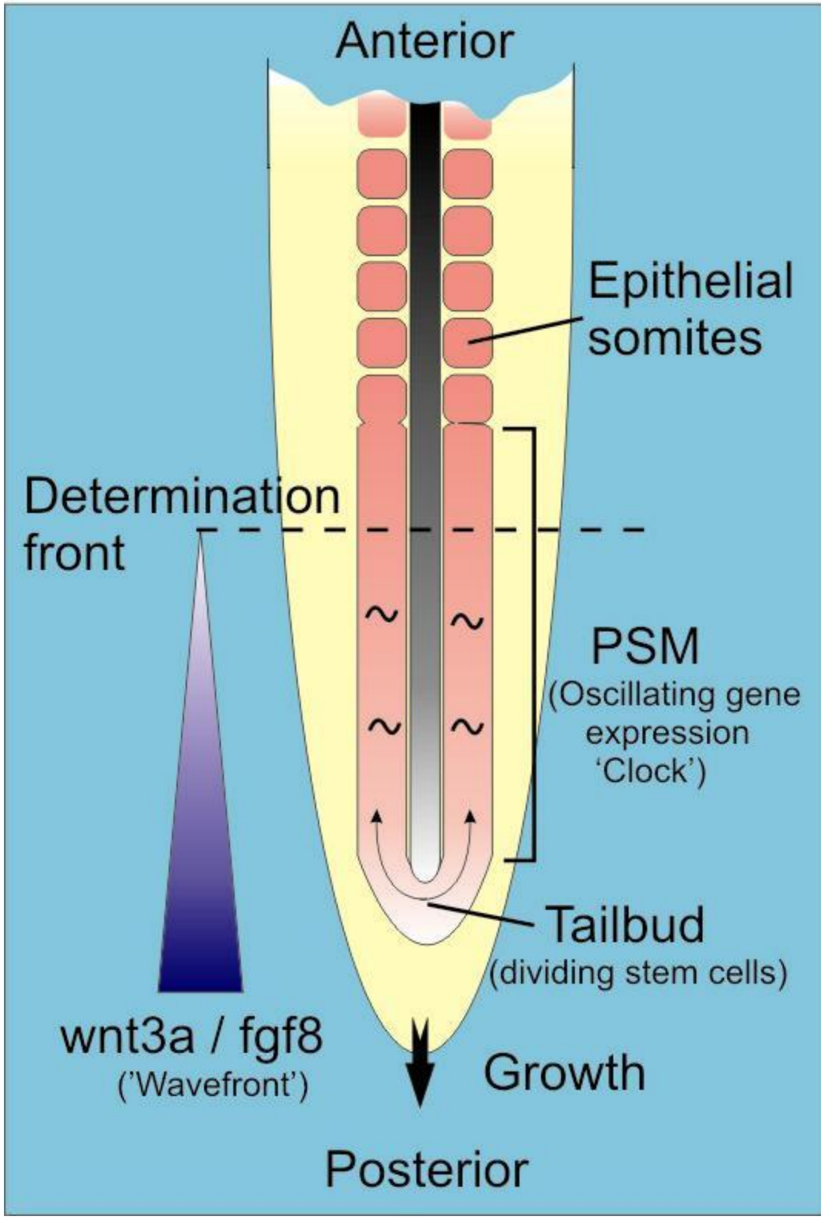


Weaker response for GG than for TT

Stochastic simulations (for p53!!)




head
ear
psm
posterior
ant
tb
heart
somites



A clock and wavefront

(Cooke and Zeeman 1976)

The presomitic mesoderm (PSM) segments anterior-posterior as somites bud off from the anterior end

Dividing stem cells in the tailbud supply cells to posterior PSM and elongates the embryo

PSM cells have locally syncronized oscillating expression patterns with periods matching somite formation (90 min in chick) – **Clock**

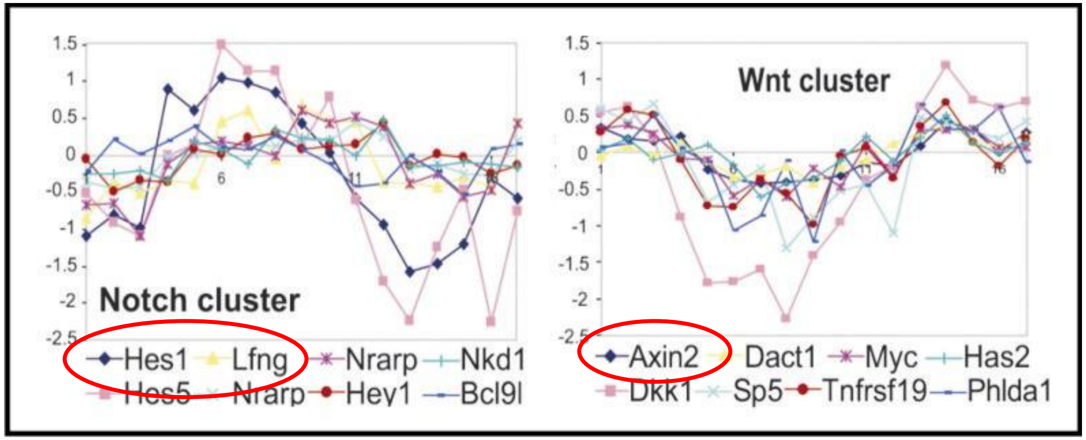
A morphogen gradient (**Wavefront**) determines onset of segmentation program

Clock determines susceptibility to **wavefront**, which ensures groupwise

incorporation into somites

Several signaling pathways are involved

Oscillating transcripts are mainly targets of Notch and Wnt pathways



Dequèant et al. 2006

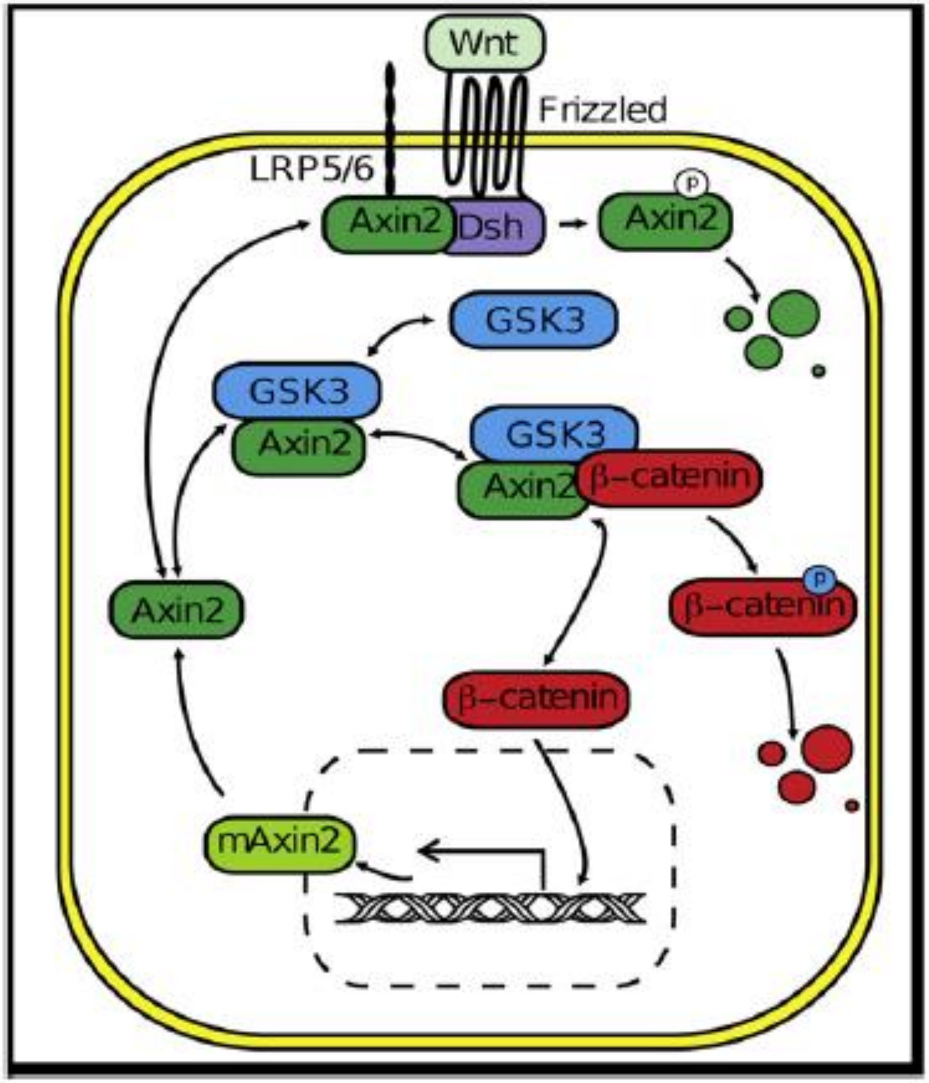
Notch and Wnt targets have same period but are 180 out of phase

➡ Crosstalk & possible hierarchical relationship

Focus is on *Hes1/7, Lfng* and *Axin2* that have all been associated with feedback loops

One crosstalk candidate (out of many possible) is GSK3β, which can bind and phosphorylate both β-catenin and $Notch_{ICD}$

The Wnt systems



Goldbeter, Pourquie

P. B. Jensen, L. Pedersen, S. Krishna, MHJ, Biophys. Journ (2010)

Equations for the Wnt system

Destruction complex

$$\frac{dC}{dt} = c_{\mathrm{fC}} B[\mathrm{GA}] - c_{\mathrm{bC}} C - \alpha C, \tag{1}$$

GA-complex

$$\frac{d[\mathrm{GA}]}{dt} = c_{\mathrm{f[GA]}} GA - c_{\mathrm{b[GA]}}[\mathrm{GA}] - c_{\mathrm{fC}} B[\mathrm{GA}] + c_{\mathrm{bC}} C + \alpha C, \tag{2}$$

β-catenin

$$\frac{dB}{dt} = S - c_{\mathrm{fC}} B[\mathrm{GA}] + c_{\mathrm{bC}} C, \tag{3}$$

GSK

$$\frac{dG}{dt} = -c_{\mathrm{f[GA]}} GA + c_{\mathrm{b[GA]}}[\mathrm{GA}], \tag{4}$$

Axin2

$$\frac{dA}{dt} = -c_{\mathrm{f[GA]}} GA + c_{\mathrm{b[GA]}}[\mathrm{GA}] + c_{\mathrm{tlA}} A_{\mathrm{m}} - c_{\mathrm{f[AL]}} AL + c_{\mathrm{b[AL]}}[\mathrm{AL}], \tag{5}$$

$$\frac{dA_{\mathrm{m}}}{dt} = c_{\mathrm{tsA}} B^2 - \frac{A_{\mathrm{m}}}{\tau_{\mathrm{Am}}}, \tag{6}$$

Axin2-Wnt

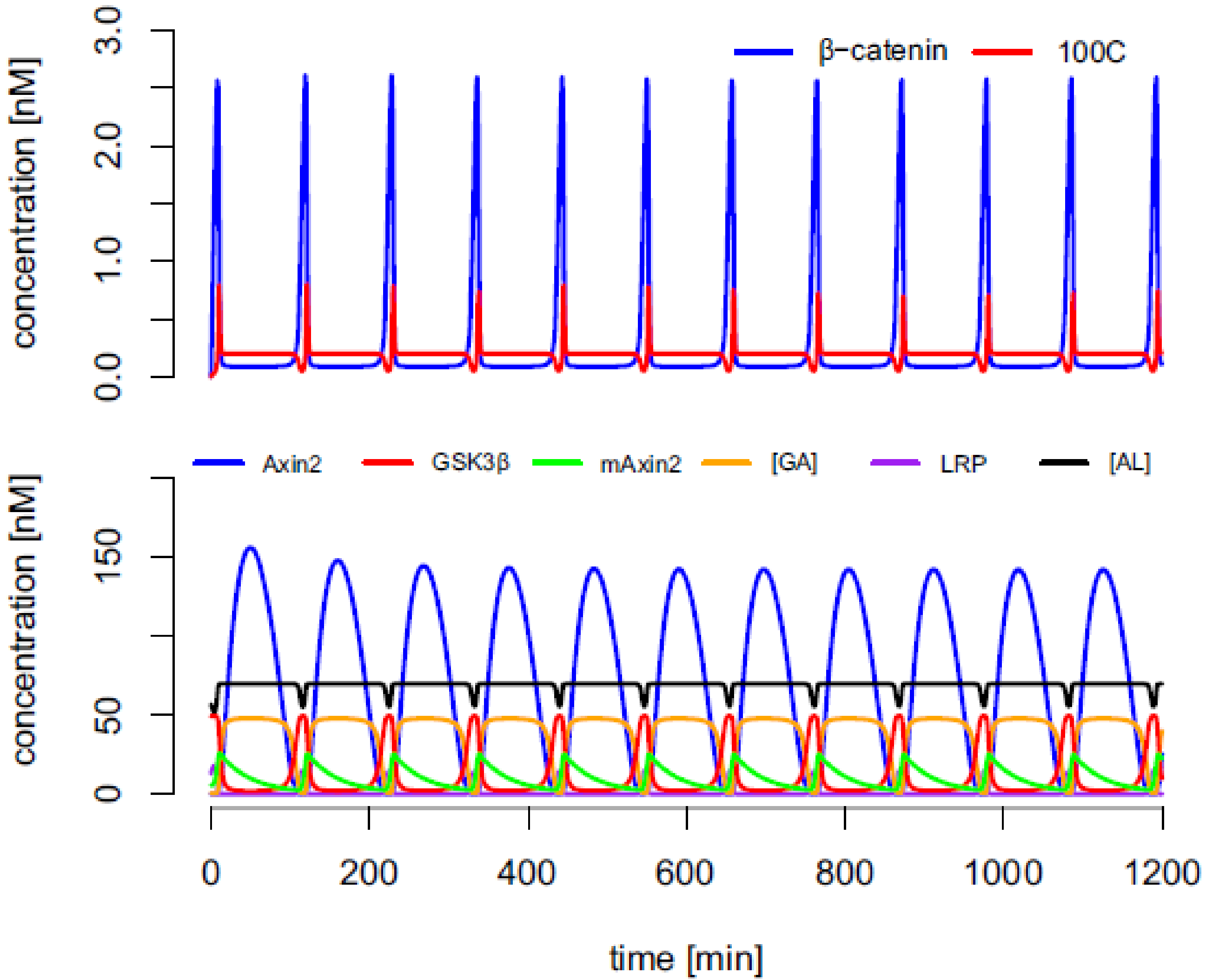
$$\frac{d[\mathrm{AL}]}{dt} = c_{\mathrm{f[AL]}} AL - c_{\mathrm{b[AL]}}[\mathrm{AL}] - \nu[\mathrm{AL}], \tag{7}$$

$$\frac{dL}{dt} = -c_{\mathrm{f[AL]}} AL + c_{\mathrm{b[AL]}}[\mathrm{AL}] + \nu[\mathrm{AL}], \tag{8}$$

TABLE 1 Parameters in the eight-variable model of the Wnt system and their default values

Parameter	Process	Default value
c_{fC}	Binding of B to [GA] to form destruction complex C	$0.1\ nM^{-1}\ min^{-1}$
c_{bC}	Dissociation of C into B and [GA]	$7\ min^{-1}$
α	Dissociation of C due to destruction of β-catenin	$200\ min^{-1}$
$c_{f[GA]}$	Binding of G to A to form [GA]	$0.2\ nM^{-1}\ min^{-1}$
$c_{b[GA]}$	Dissociation of [GA] into G and A	$1.2\ min^{-1}$
S	Constant source of β-catenin	$0.4\ nM\ min^{-1}$
$c_{f[AL]}$	Binding of A to L	$10\ nM^{-1}\ min^{-1}$
$c_{b[AL]}$	Dissociation of [AL] into A and L	$0.08\ min^{-1}$
c_{tsA}	Transcription of *axin2* gene	$0.7\ nM^{-1}\ min^{-1}$
c_{tlA}	Translation of Axin2 mRNA	$0.7\ min^{-1}$
τ_{Am}	Average lifetime of Axin2 mRNA	40 min
ν	Degradation of Axin2 in [AL] complex	$0.1\ min^{-1}$
$GSK3\beta_{tot}$	Total G level	50 nM
L_{tot}	Total L level	70 nM

The Wnt reference state



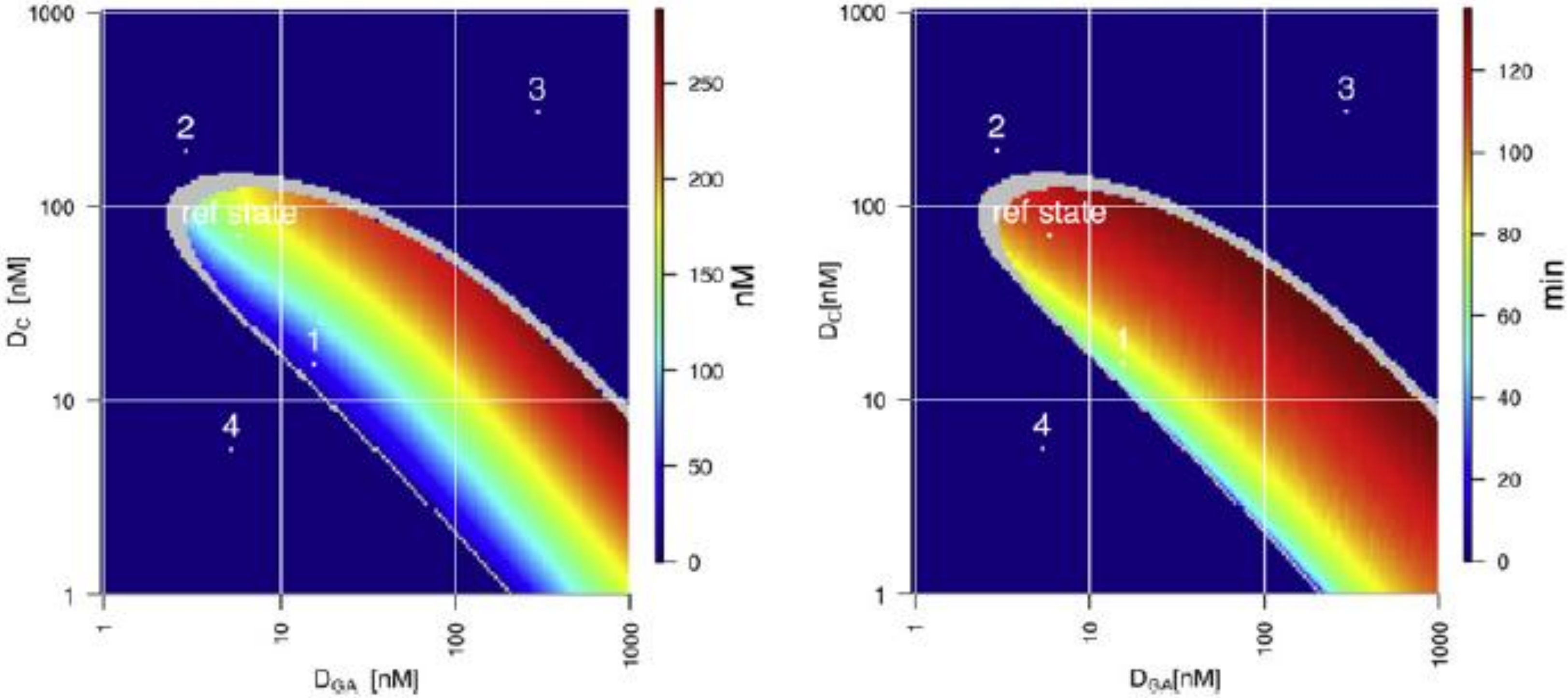
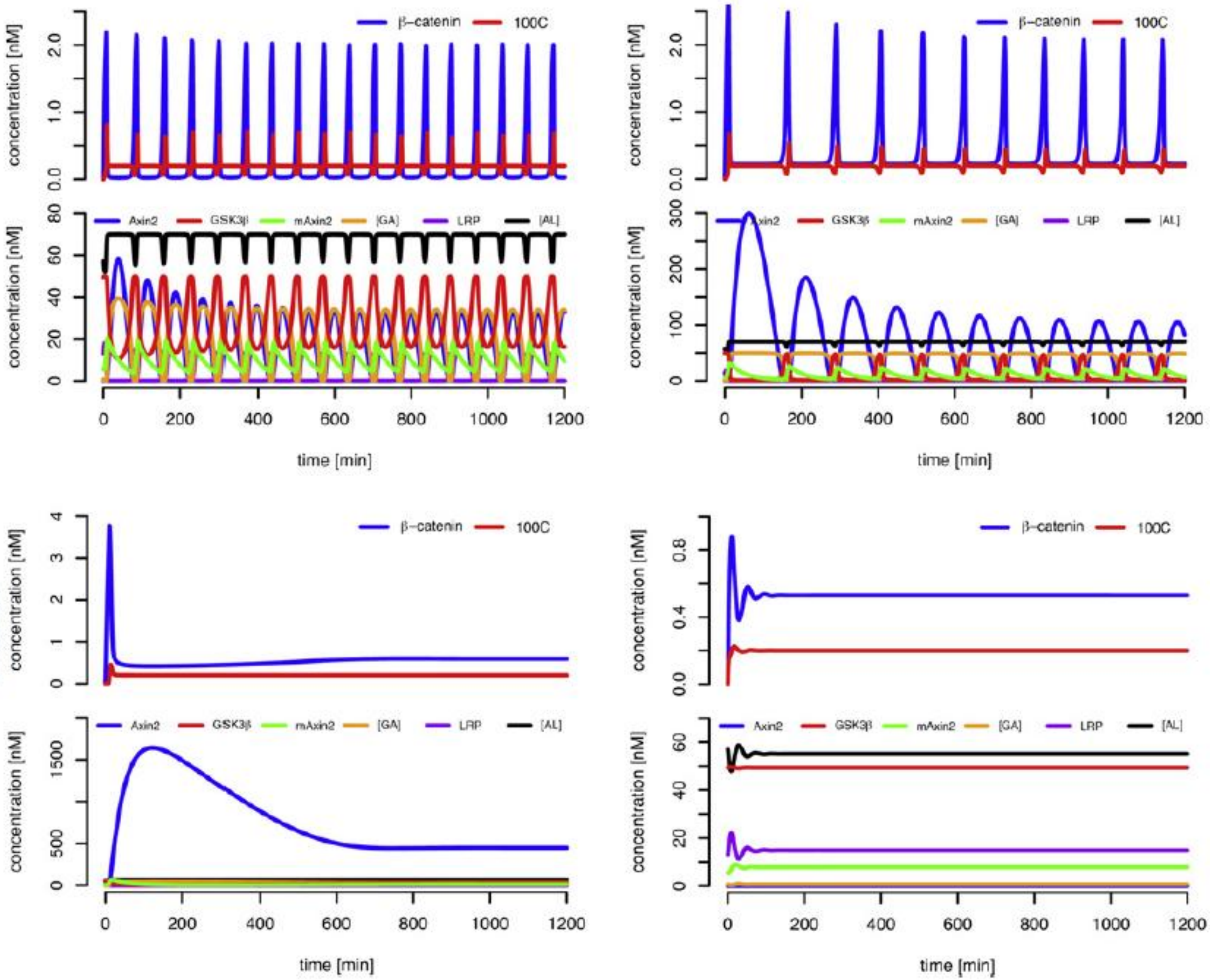


FIGURE 3 The D_C and $D_{[GA]}$ parameter plane. (*Left panel*) The amplitude of the Axin2 oscillations. (*Right panel*) Oscillation period of Axin2. The shaded borderline indicates the boundary of sustained oscillations, derived from a stability analysis of the system. The reference state (see Fig. 2) is indicated by the dot labeled "ref state". Timeseries corresponding to the four numbered dots are shown in Fig. 4.

$$D_C = \frac{c_{bC}}{c_{fC}}$$

$$D_{[GA]} = \frac{c_{b[GA]}}{c_{f[GA]}}$$


β–catenin
100C
Axin2
GSK3β
mAxin2
[GA]
LRP
[AL]
concentration [nM]
time [min]
0
200
400
600
800
1000
1200

Spatial gradient of Wnt:

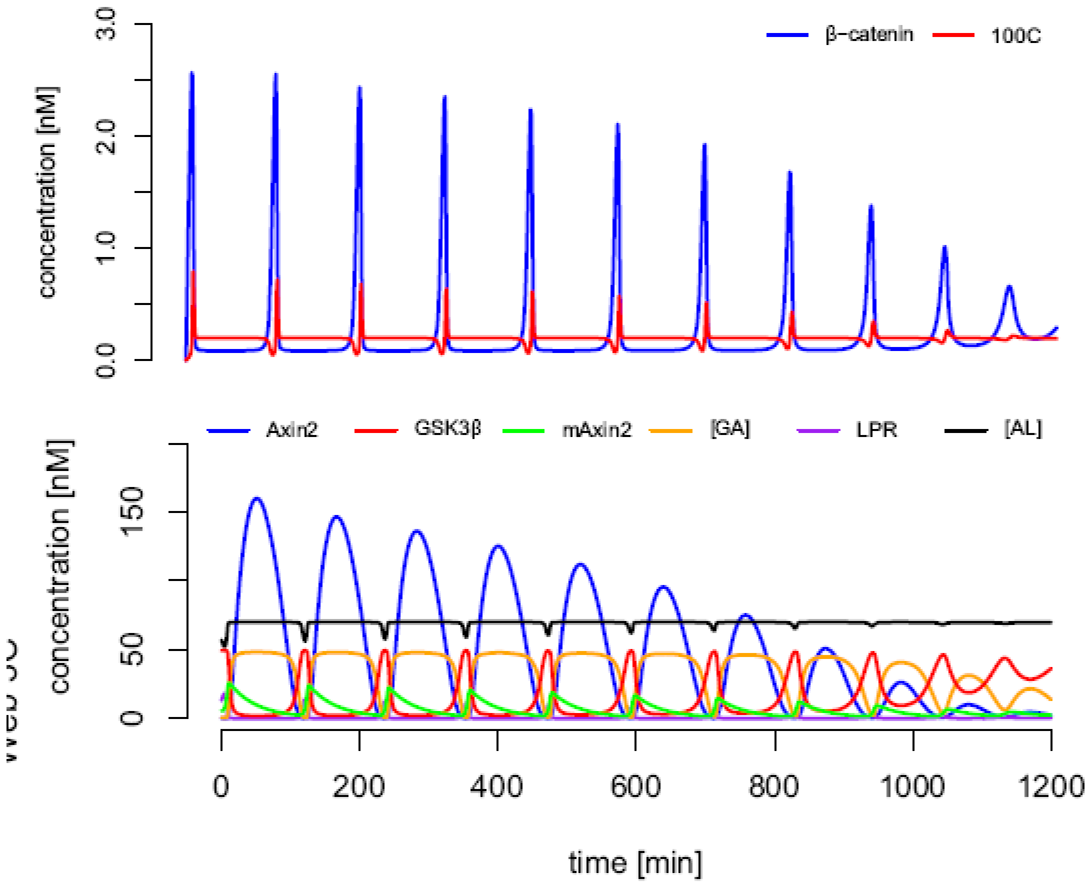
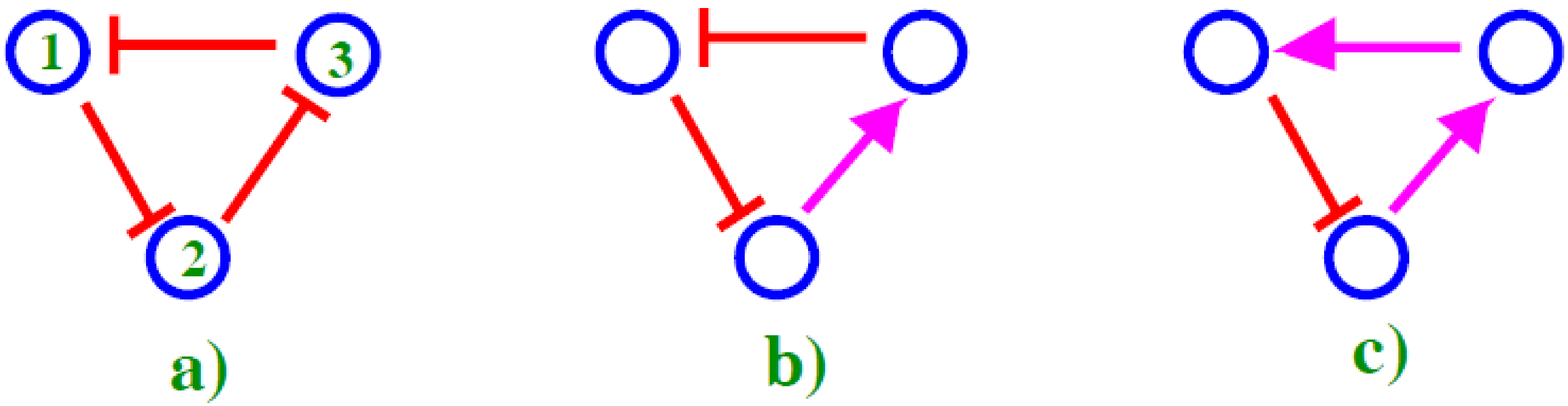


FIGURE 6 Oscillations of the Wnt variables as ν is linearly decreased from 0.1 to 0.03 min^{-1} in 1200 min. Note that the oscillations cease when the Wnt signal falls below a certain threshold level.

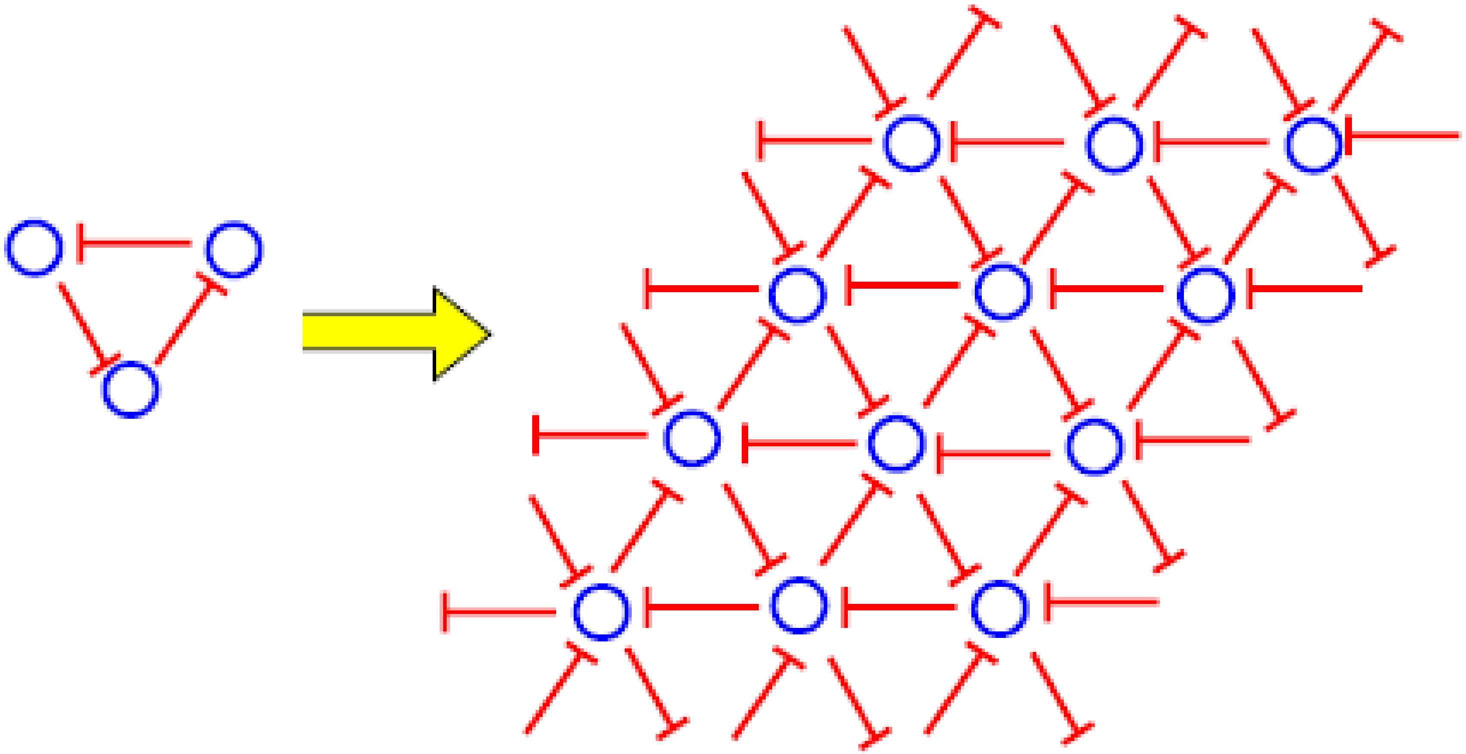
Three node motifs: Oscillations and Switches



Repressilator:
oscillations

$$\frac{dx}{dt} = c - \gamma\, x + \alpha \frac{1}{1 + (\frac{\tilde{x}}{K})^h}$$

A model for cell-to-cell communications:



$$\frac{dx_{m,n}}{dt} = c - \gamma x_{m,n} + \alpha F_{\text{int}}$$

Dynamical equation for site (m,n):

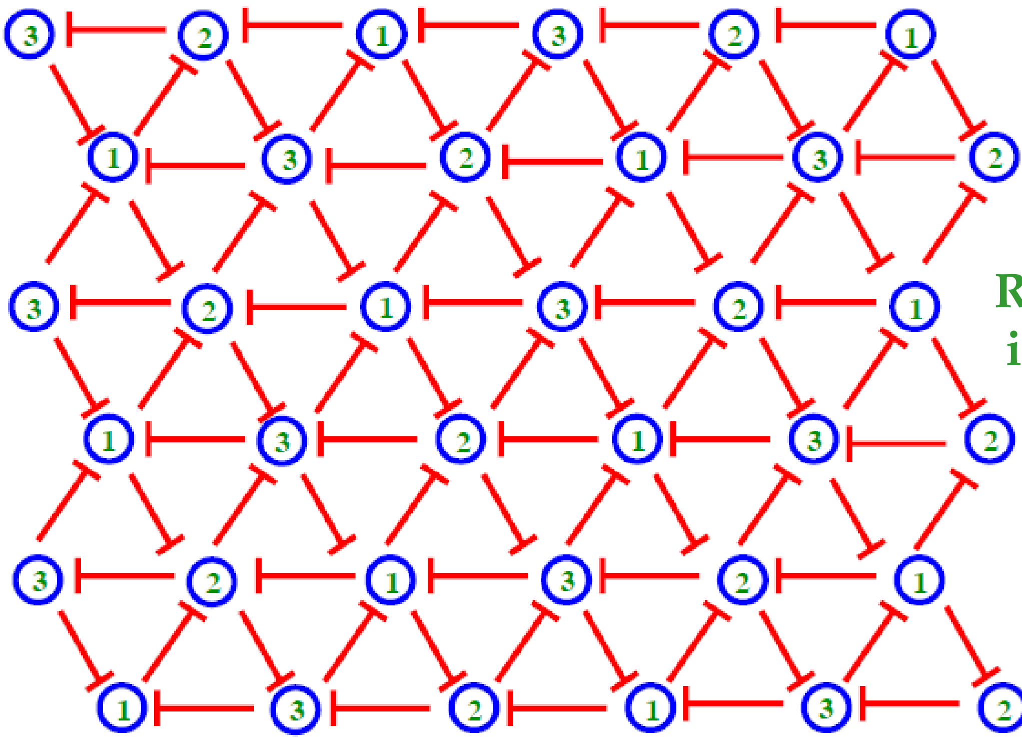
We consider two types of interaction terms—either an additive repression (an "OR gate"),

$$F_{\text{int}} = \frac{1}{1 + (\frac{x_{m+1,n}}{K})^h} + \frac{1}{1 + (\frac{x_{m,n-1}}{K})^h} + \frac{1}{1 + (\frac{x_{m-1,n+1}}{K})^h}, \quad (2)$$

or a multiplicative repression (an "AND gate"),

$$F_{\text{int}} = \frac{1}{1 + (\frac{x_{m+1,y}}{K})^h} \frac{1}{1 + (\frac{x_{m,n-1}}{K})^h} \frac{1}{1 + (\frac{x_{m-1,n+1}}{K})^h}. \quad (3)$$

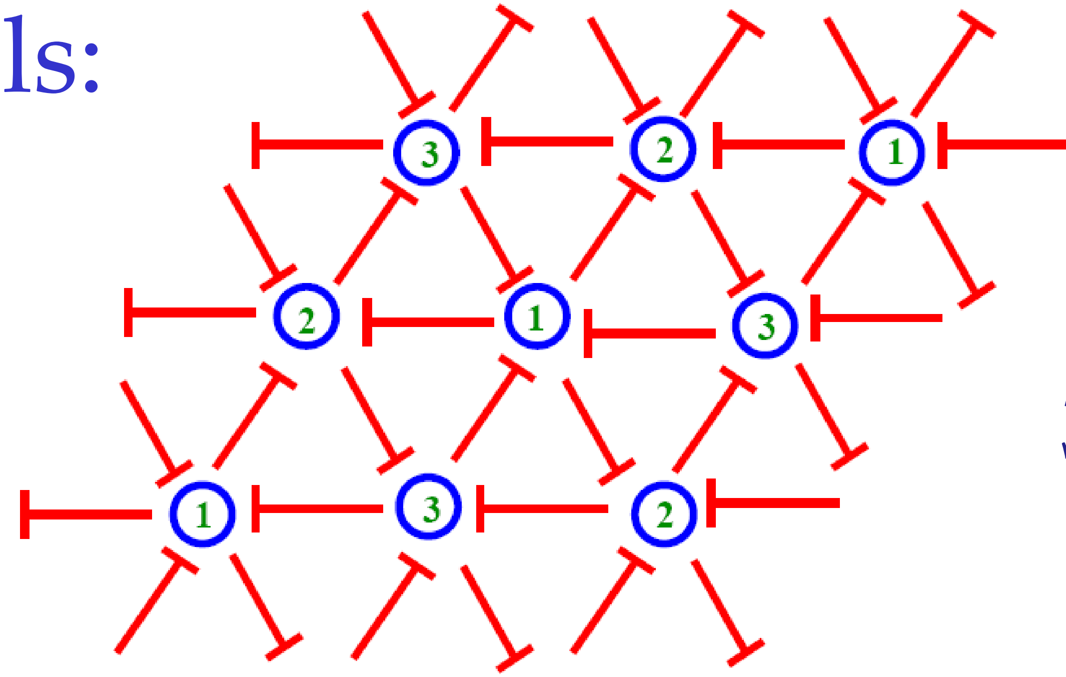
Coupled Repressilators: A Repressor-Lattice



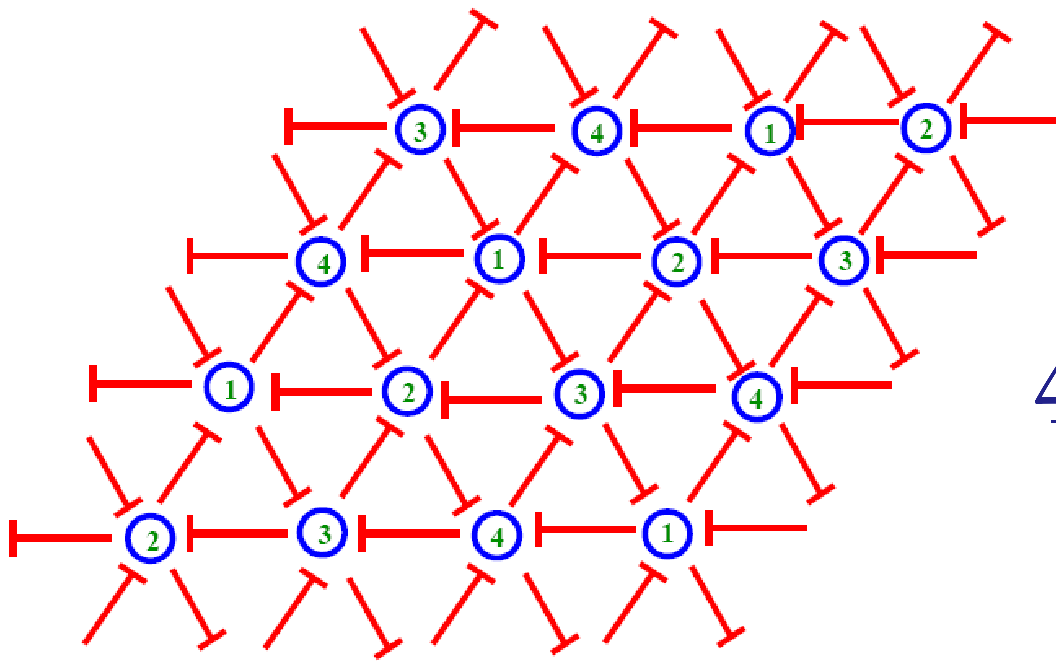
Repressing genes/proteins: in a cell or between cells !

'Natural' phases: No frustration !

Unit cells:

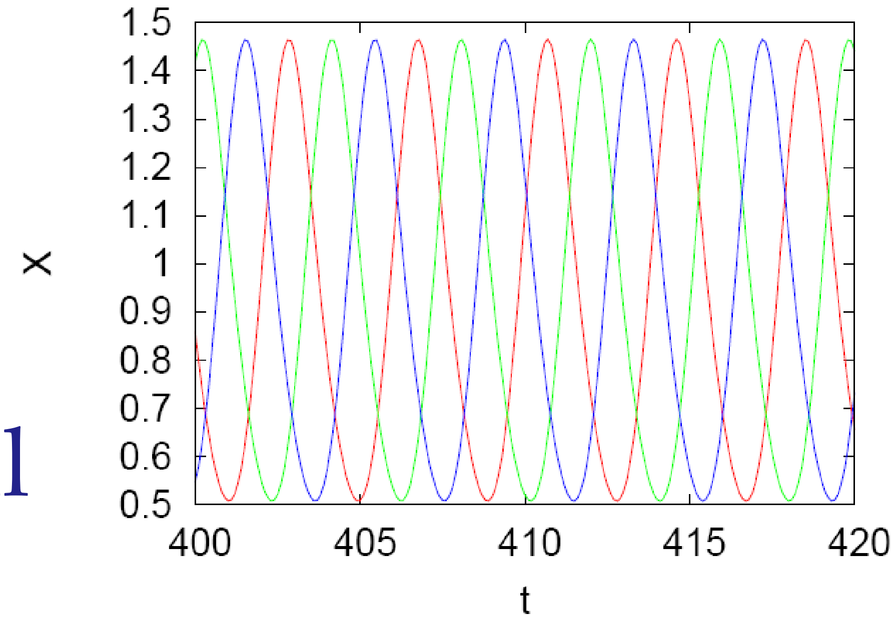


3x3

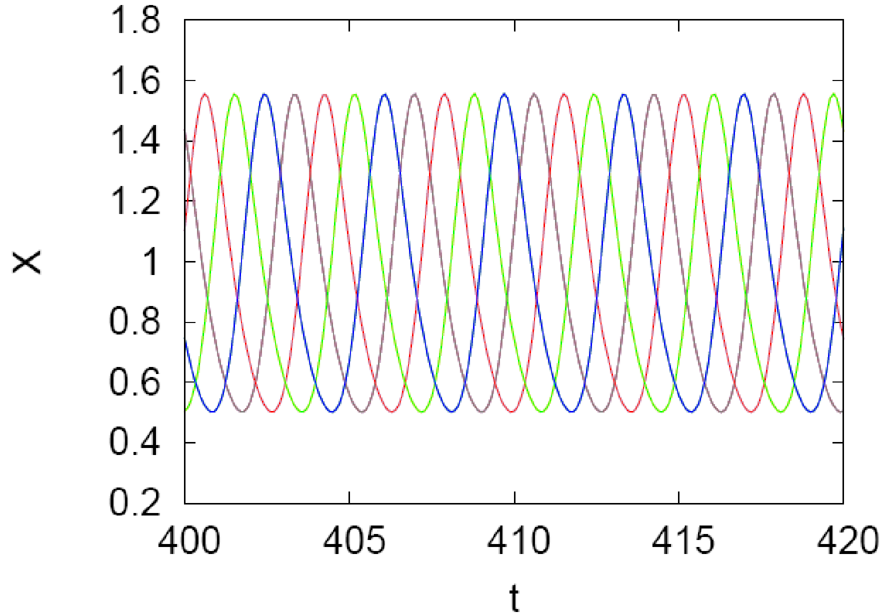


4x4

Dynamical solutions



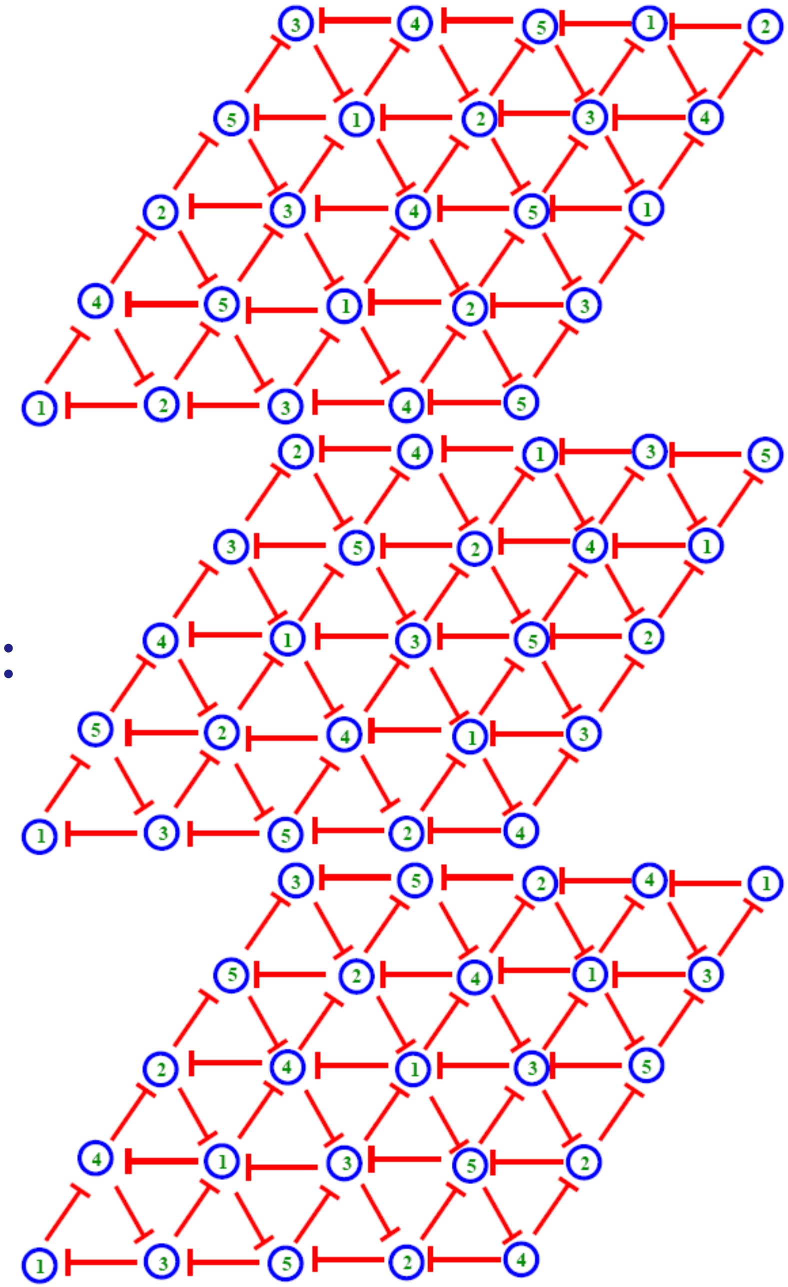
3x3



4x4

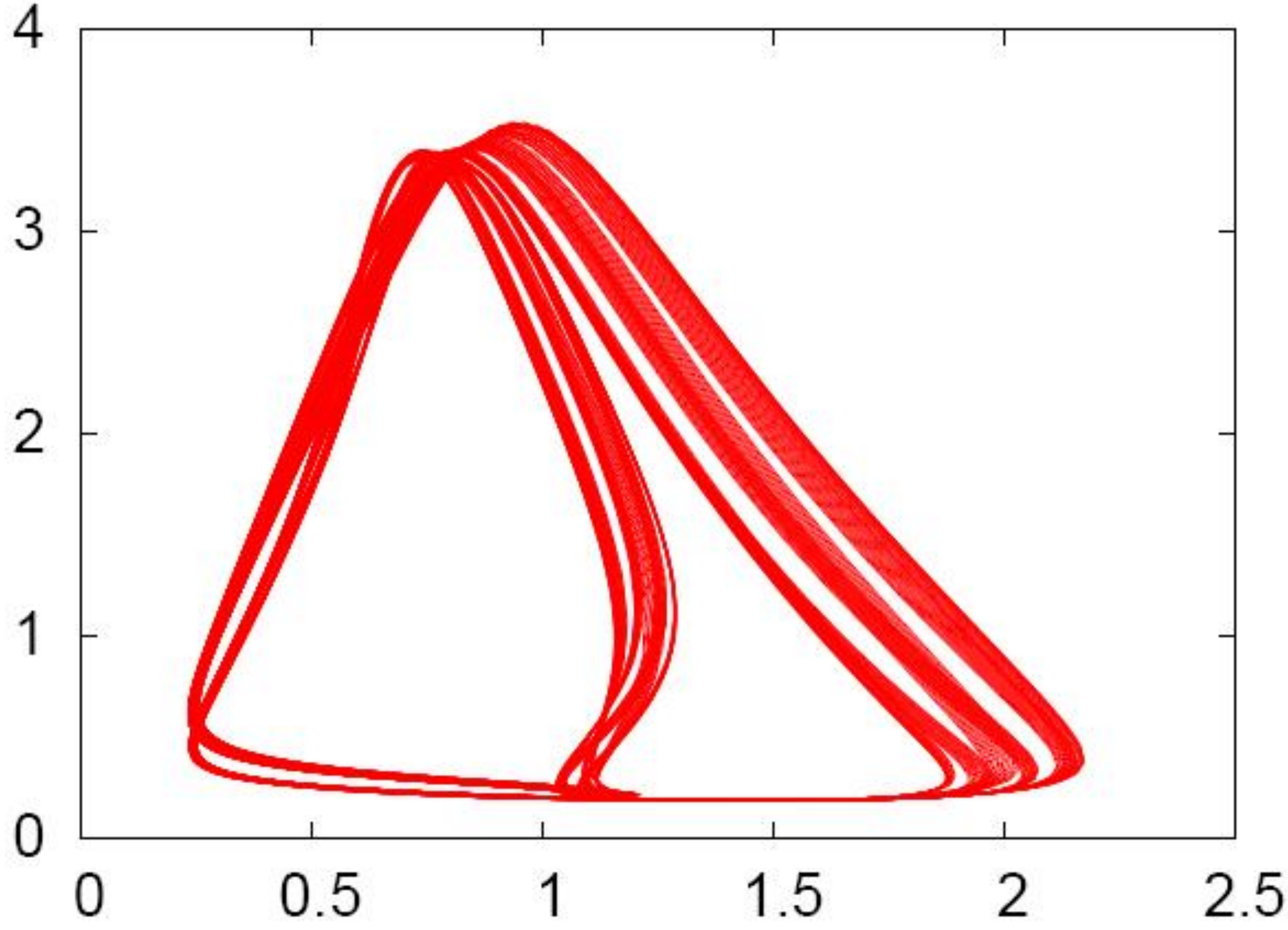
5x5

Solutions from rotational symmetry: Group theory !

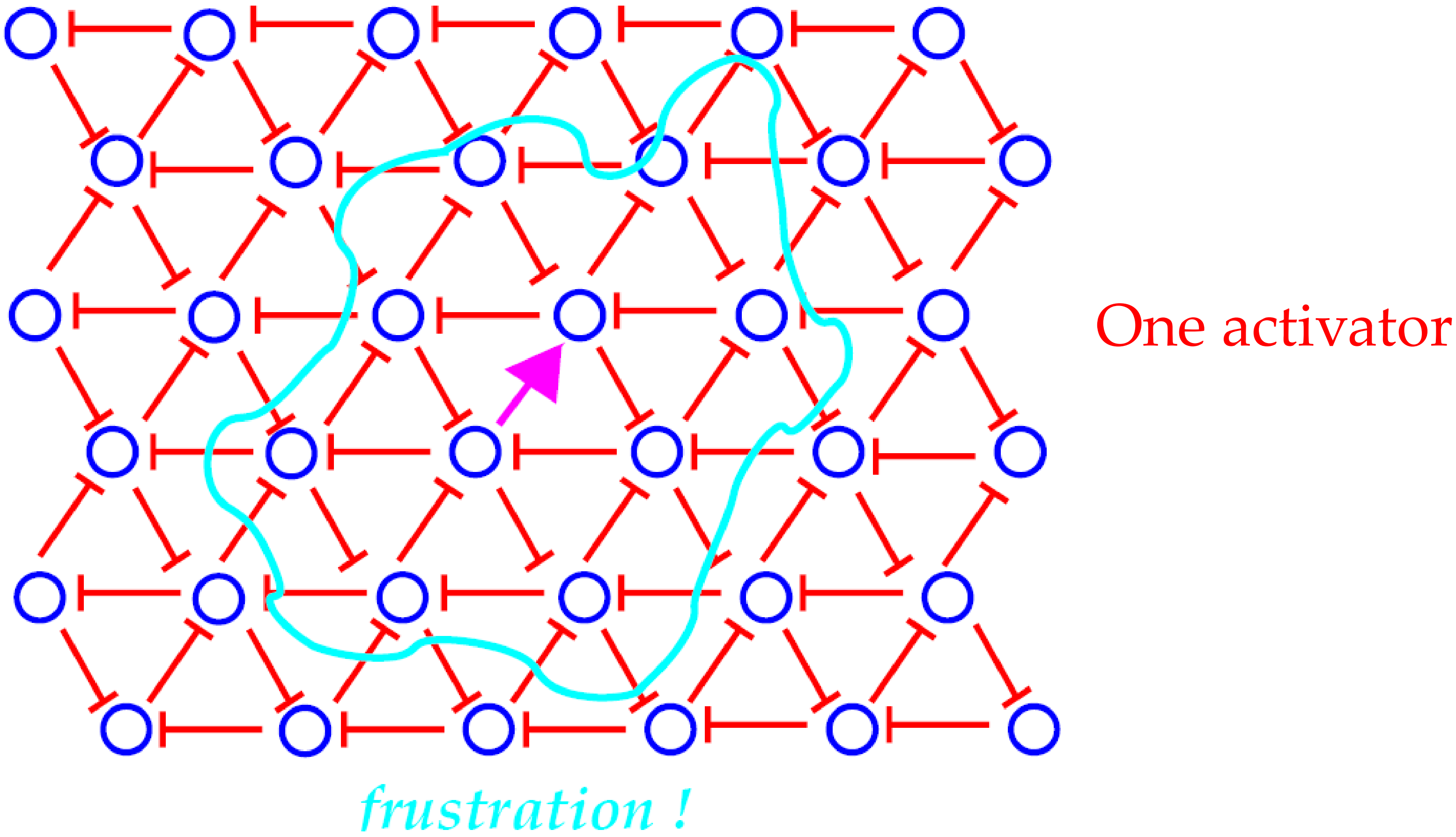


Dynamical frustrations: Chaotic solutions

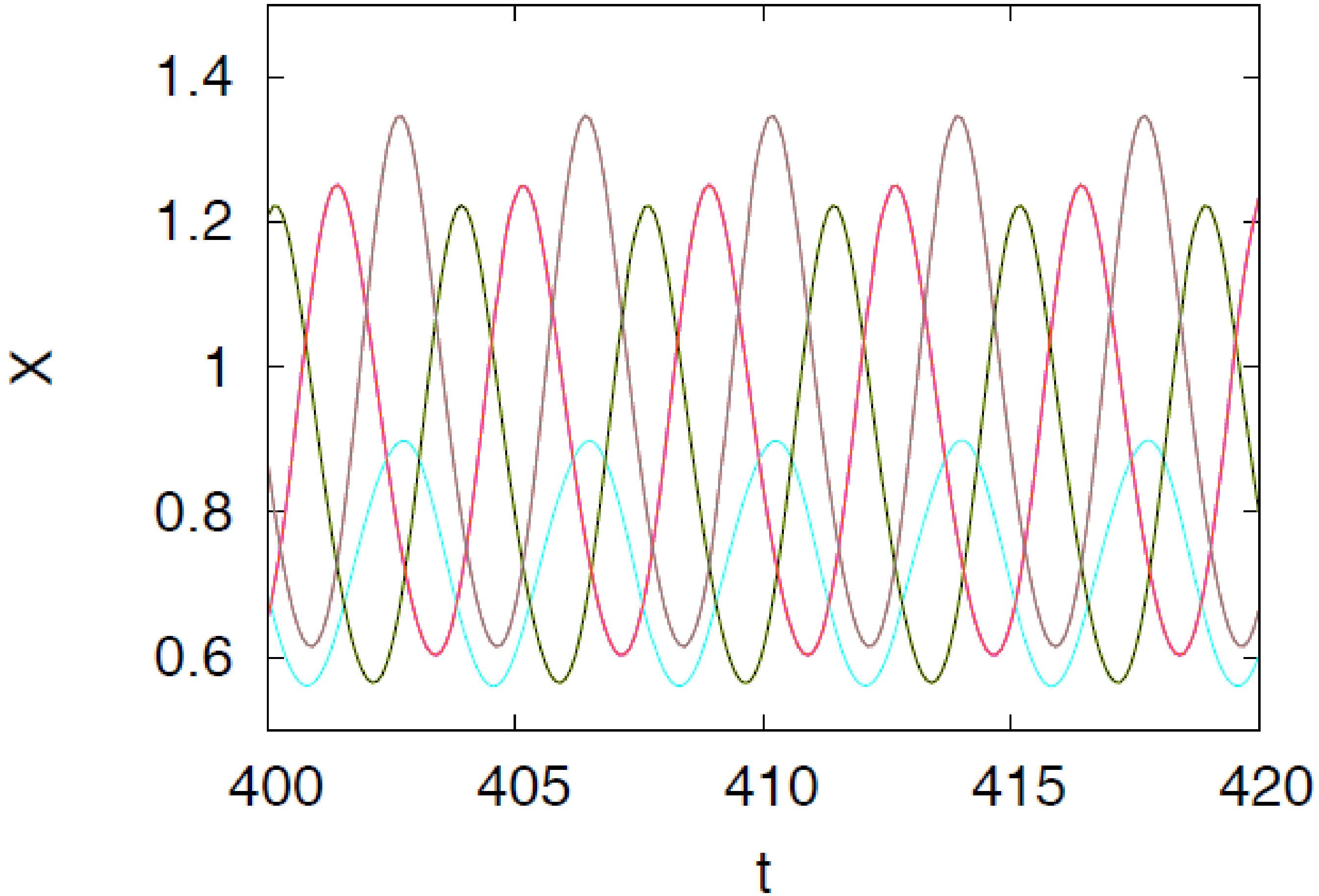
5x5



Non-balanced repressor-lattice



Frustrated solutions:



3 x 3 lattice

Phases are fixed !

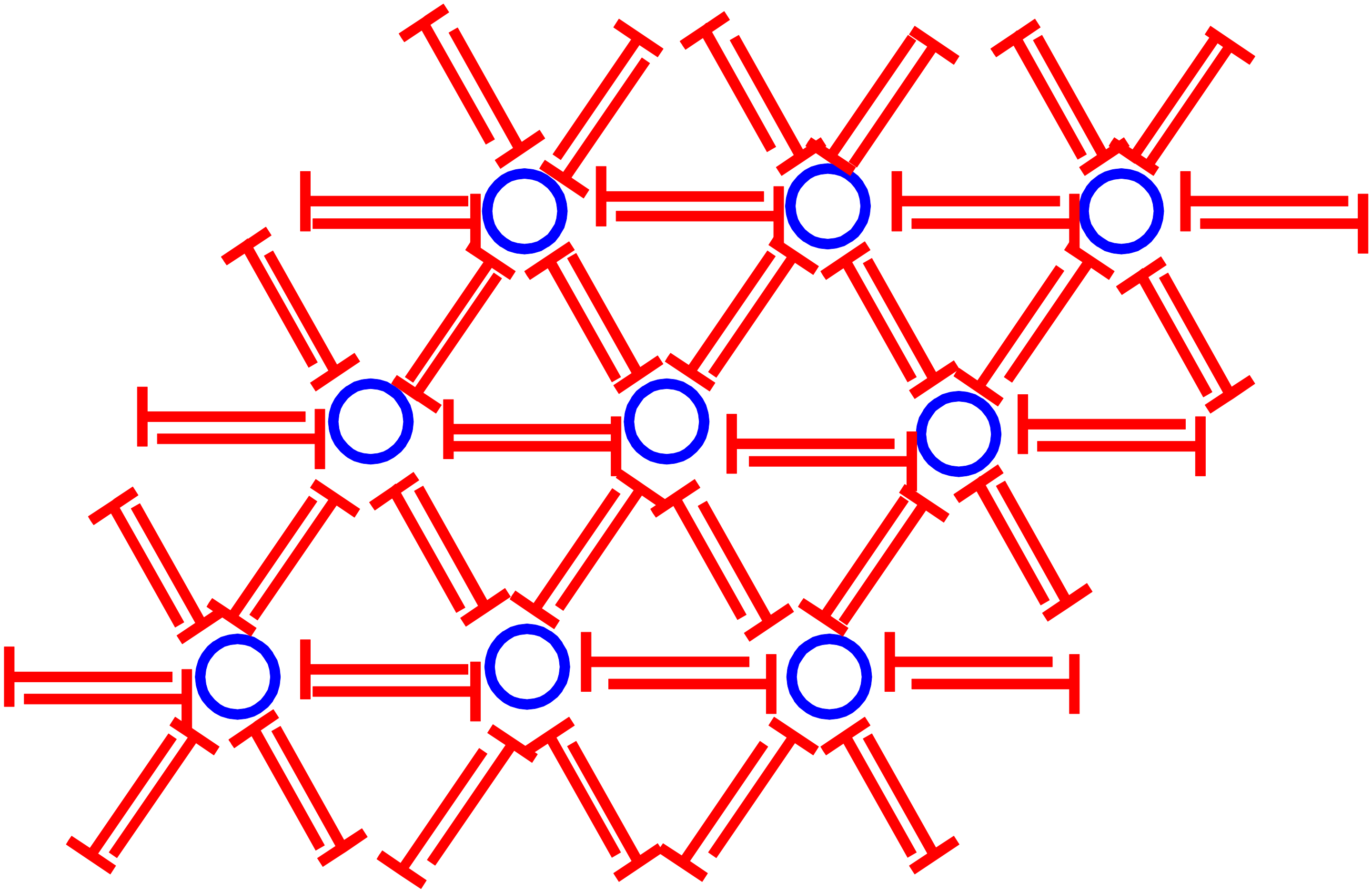
Cell-to-cell communication:

- Cell touches: gap junctions→Directed (or bidirected?) interactions ! Oscillations !
- Cells do not touch: Send out signalling molecules→bi-directed interactions:

  'Local' switches → High or low state !

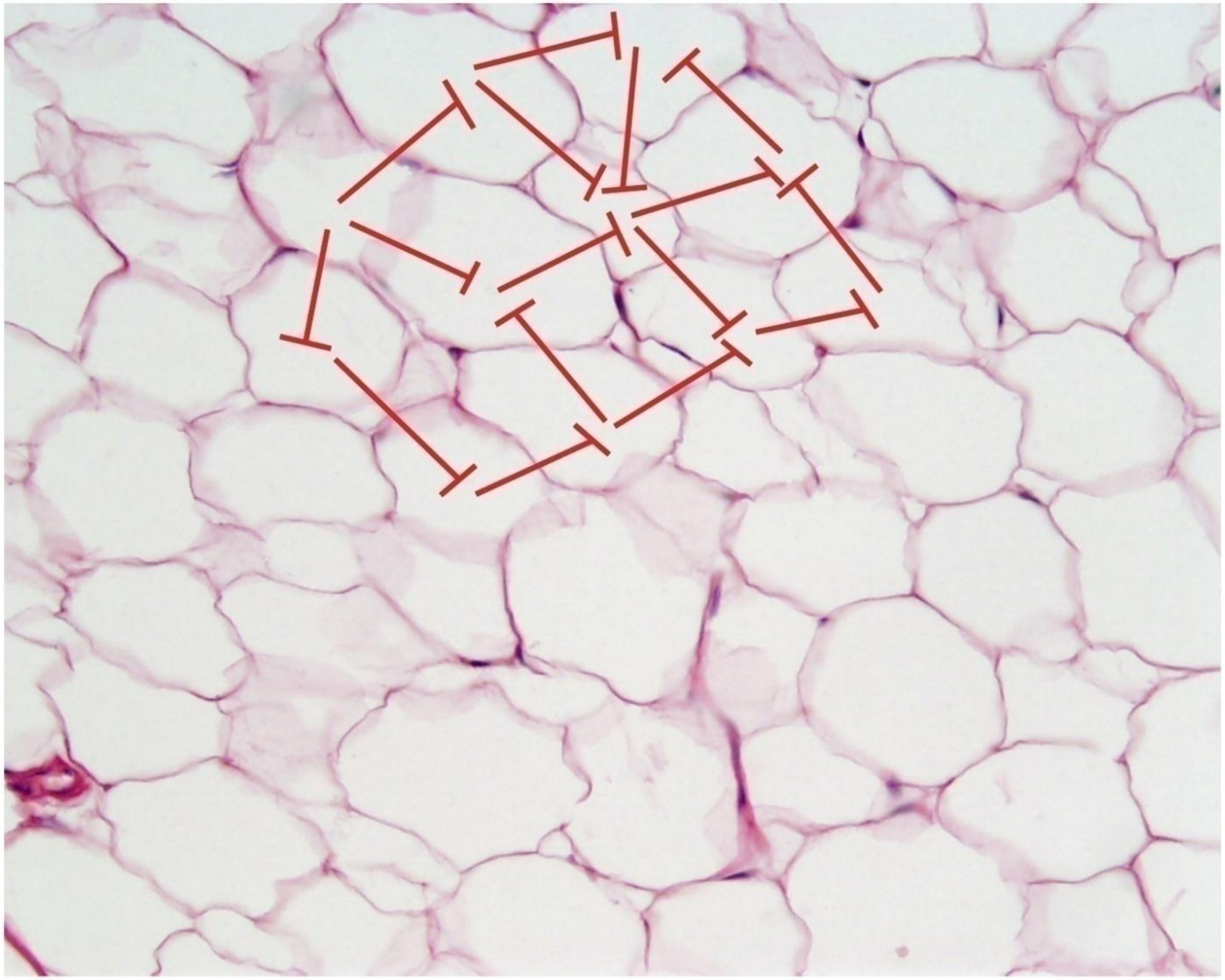
Relevant for ordered tissues: fat tissue, onion skin, human skin, etc.

Bi-directional interactions



No oscillations: hi or low states. Mutations !

Cell-to-cell communicatios in space:
Fatty tissues: organized on a “lattice”



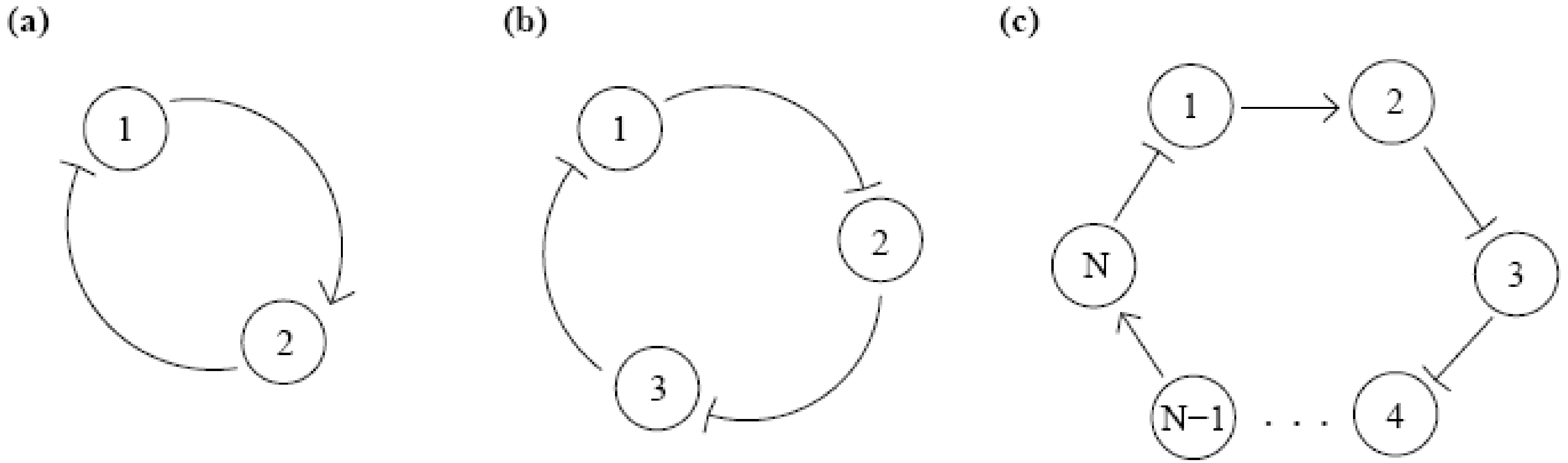
Negative feed-back loops

- Guess the feed-back loop from an experimental time series: Algorithm
- Derive symbolic dynamics
- From fixed points to Hopf bifurcations

with Simone Pigolotti and Sandeep Krishna

Proc. Nat. Acad. Sci. (2007)

Negative Feed-Back Loops:



- **Always odd number of repressor links**
- **A ‘closed’ loop: No cross-links**
- **Node: concentration, expression level, etc**

Determined by the following equation:

$$\frac{dx_i}{dt} = g_i^{(A,R)}(x_i, x_{i-1})$$

Assume $g_i^{(A;R)}$ **are monotonic**

Example:

$$\frac{dx_i}{dt} = c - \gamma x_i + \alpha \frac{1}{1 + (x_{i-1}/K)^h}$$

- c: basal production
- γ: degradation → linear
- α: production rate: activation↔repression

Often time series are very noisy !
$\rightarrow$ Then what ?

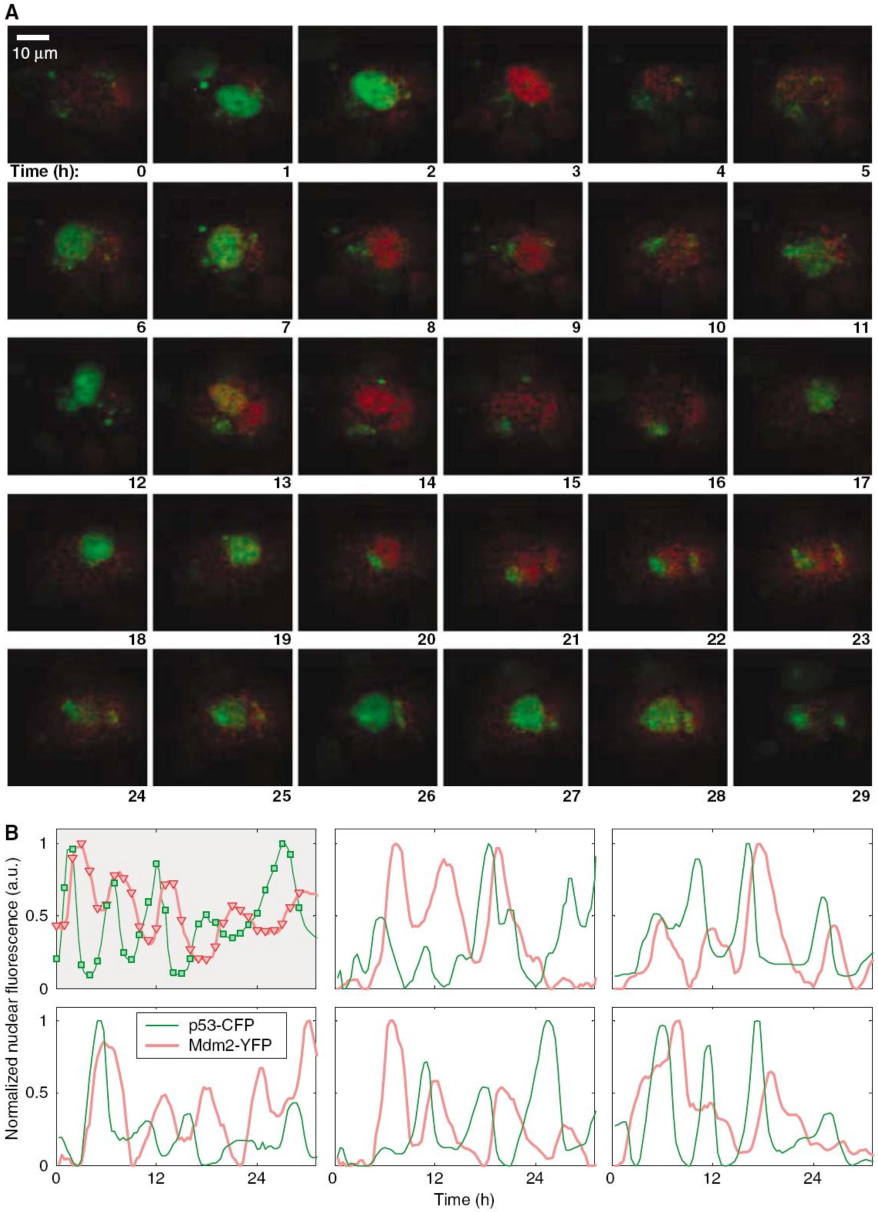
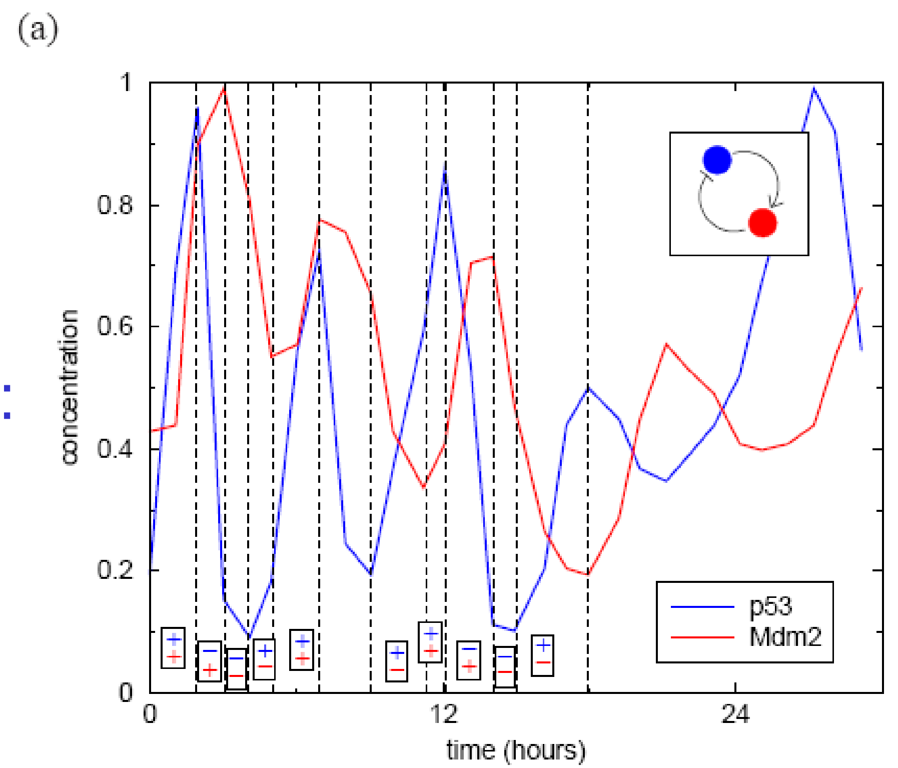


Figure 1 Prolonged oscillations in the nuclear levels of fluorescently tagged p53 and Mdm2 in individual MCF7, U280, cells following gamma irradiation. (**A**) Time-lapse fluorescence images of one cell over 29 h after 5 Gy of gamma irradiation. Nuclear p53-CFP and Mdm2-YFP are imaged in green and red, respectively. Time is indicated in hours. (**B**) Normalized nuclear fluorescence levels of p53-CFP (green) and Mdm2-YFP (red) following gamma irradiation. Top left: the cell shown in panel A. Other panels: five cells from one field of view, after exposure to 2.5 Gy gamma irradiation.

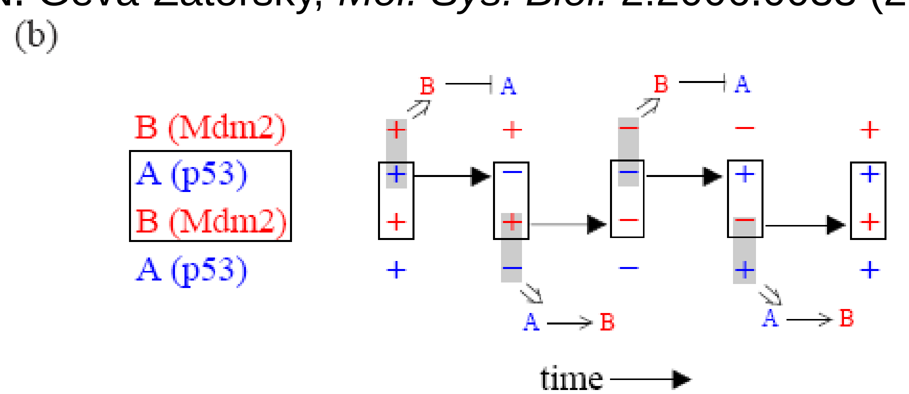
N. Geva-Zatorsky, *Mol. Sys. Biol.*2:2006.0033 (2006)

Experimental time series:
(transient is OK !)



N. Geva-Zatorsky, *Mol. Sys. Biol.* 2:2006.0033 (2006)

Symbolic Dynamics:



Stationary point:

$$g_i^{(A,R)}(x_i^*, x_{i-1}^*) = 0 \quad \Rightarrow \quad x_i^* = f_i^{(A,R)}(x_{i-1}^*)$$

Notice:

$$f_i^{(A,R)} \rightarrow \text{ same monotonocity as } g_i^{(A,R)}$$

$$(\text{when } g_i^{(A,R)} \text{ is decreasing in } x_i)$$

Fixed point equation:

$$x_i^* = f_i(x_{i-1}^*) = f_i(f_{i-1}(x_{i-2}^*)) = \ldots =$$
$$= f_i \circ f_{i-1} \circ f_{i-2} \circ \ldots \circ f_{i+1}(x_i^*) \equiv F_i(x_i^*)$$

→ I.e. measures how species 'i' interacts with itself through the loop

Using chain rule: $F_i'(x) = \prod_j f_j'(x_j)|_{x_i=x}$

Therefore: If even number of repressors $\rightarrow$
positive $\rightarrow$ multiple fixed points (bistability)

If odd number of repressors $\rightarrow$
negative $\rightarrow$ only one fixed point !

Eigenvalue equation:

$$\prod_{i=1}^{N}\left(\frac{\lambda}{h_i}+1\right) = F'(x^*)$$

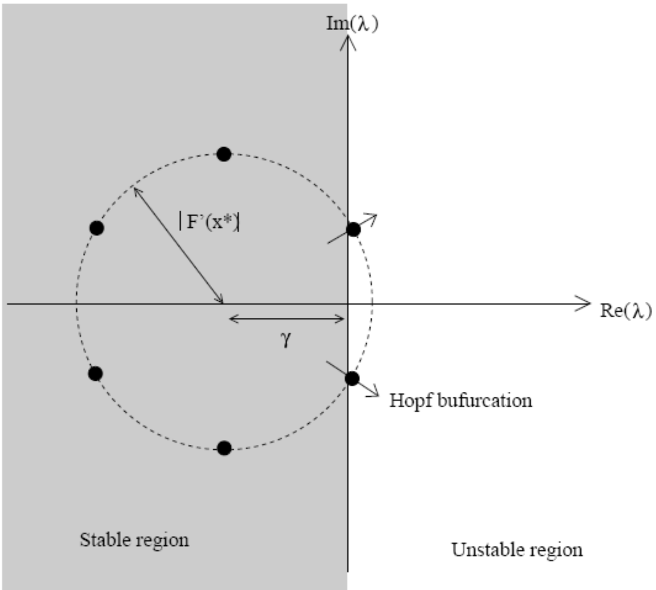
where $h_i = -\partial_x g_i(x_i, x_{i=1})|_{x^*}$ (degradation rates at fixed point)

$$\prod_{i=1}^{N}\left(\frac{\lambda}{h_i}+1\right)=F'(x^*)$$

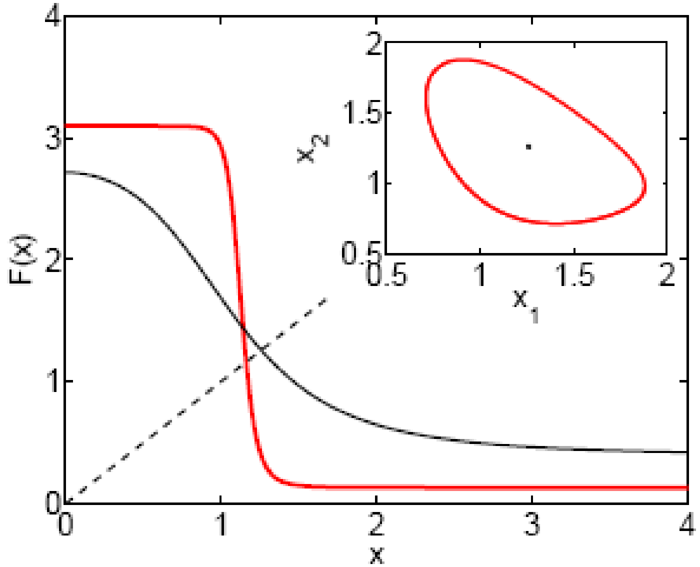
Right hand side: Negative

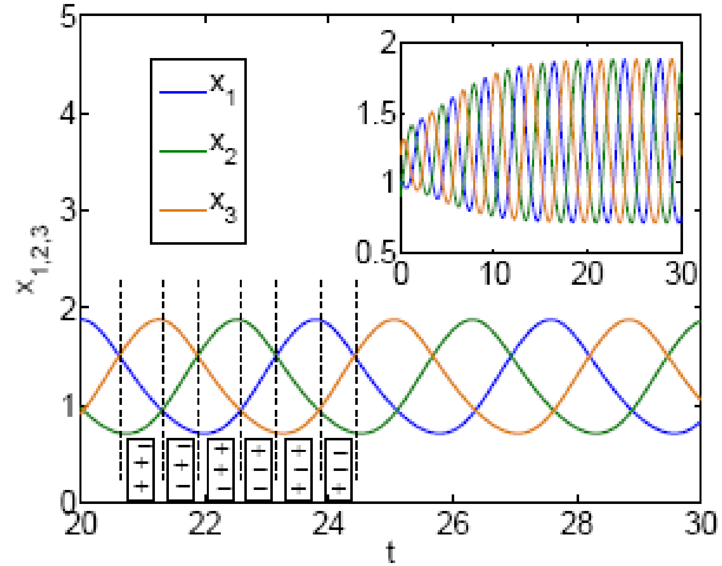
Left hand side: Positive coefficients

: **Hopf bifurcation**



Example:





α=3.0, c=0.1,
K=1, γ=1, h=2
h=4

Three repressors:

$$\frac{dx_i}{dt} = c - \gamma x_i + \alpha \frac{1}{1 + (x_{i-1}/K_i)^h} \qquad i = 1 \ldots 3.$$

We denote by x^* the solution to the equation $\gamma x = c + \alpha/(1 + (x/K)^h)$. Then the characteristic polynomial is simply:

$$(\lambda + \gamma)^3 = -\left(\frac{\alpha}{1 + (x^*/K_i)^h}\right)^3$$

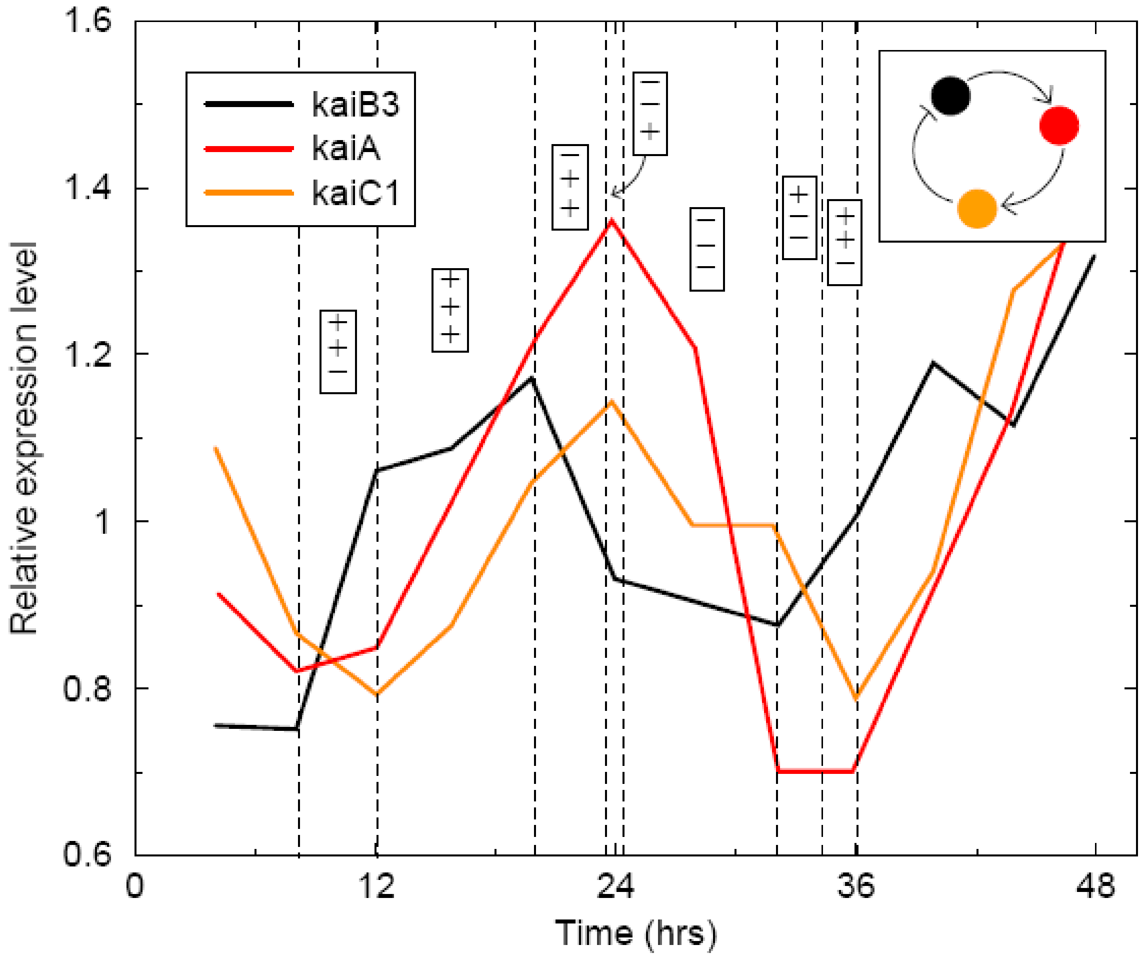
$$\Rightarrow \left(\frac{\lambda}{\gamma} + 1\right)^3 = F'(x^*) \quad \Rightarrow \quad |F'(x^*)| \cos(\pi/3) < \gamma$$

Stability condition

- OK: h=2
- Not OK: h=4

Circadian rhythms of kai genes in cyanobacteria

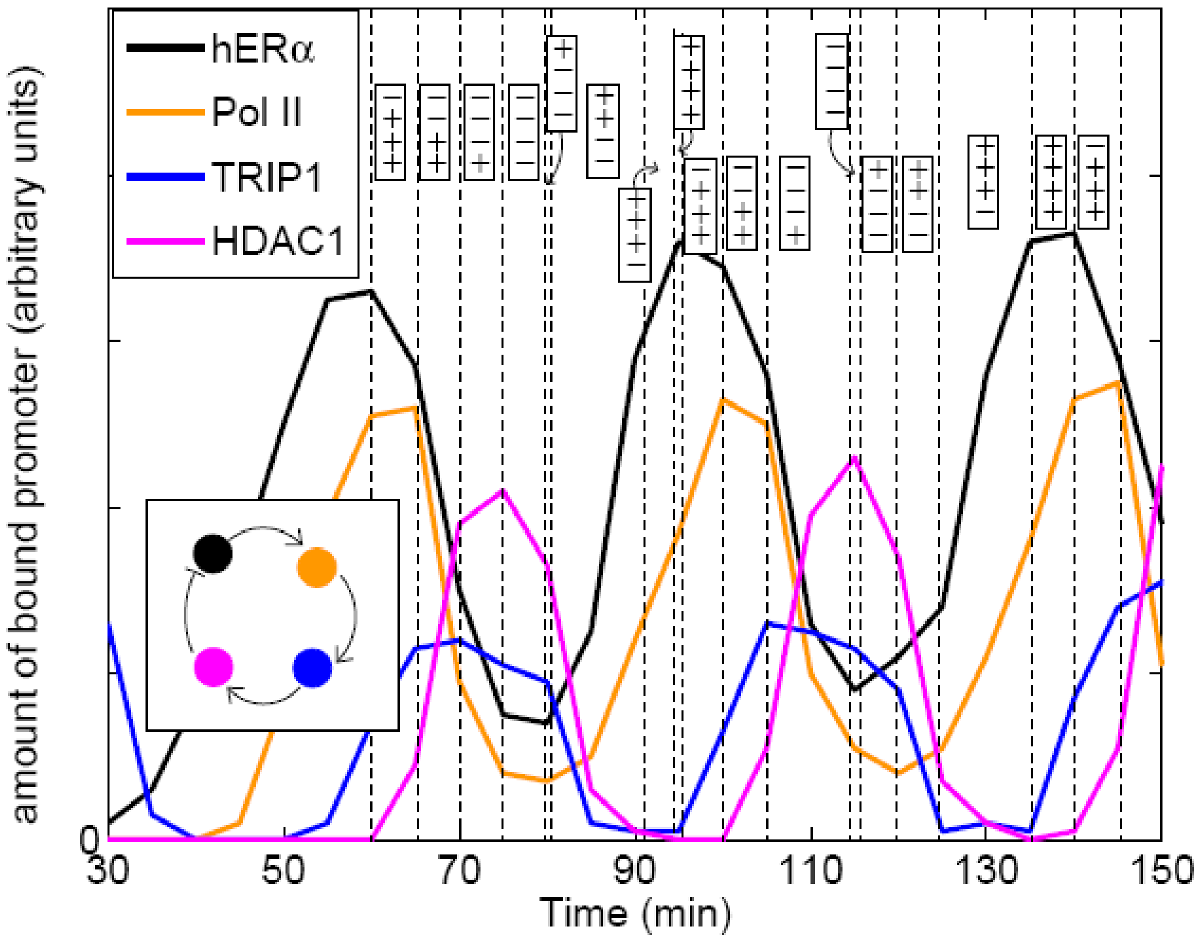
(a)



Kucho et al, *Journ. Bacteriol*. 187, 2190-2199 (2005)

Binding of four proteins to pS2 promoter

(b)



Adding estradiol:

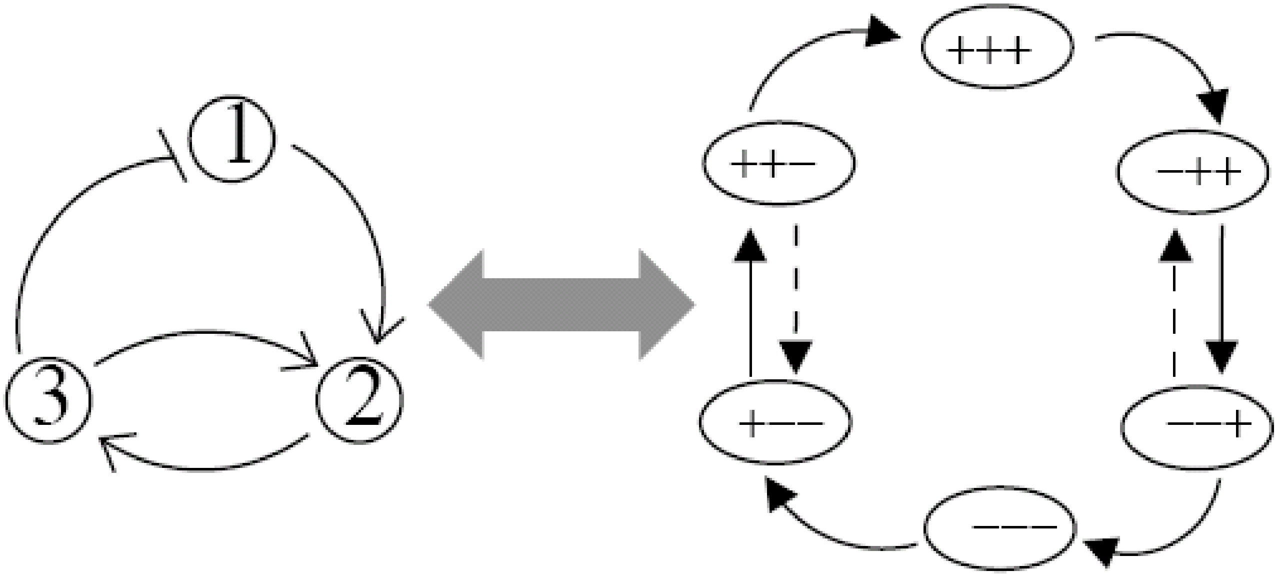
ER: estradiol receptor, initiates transcription

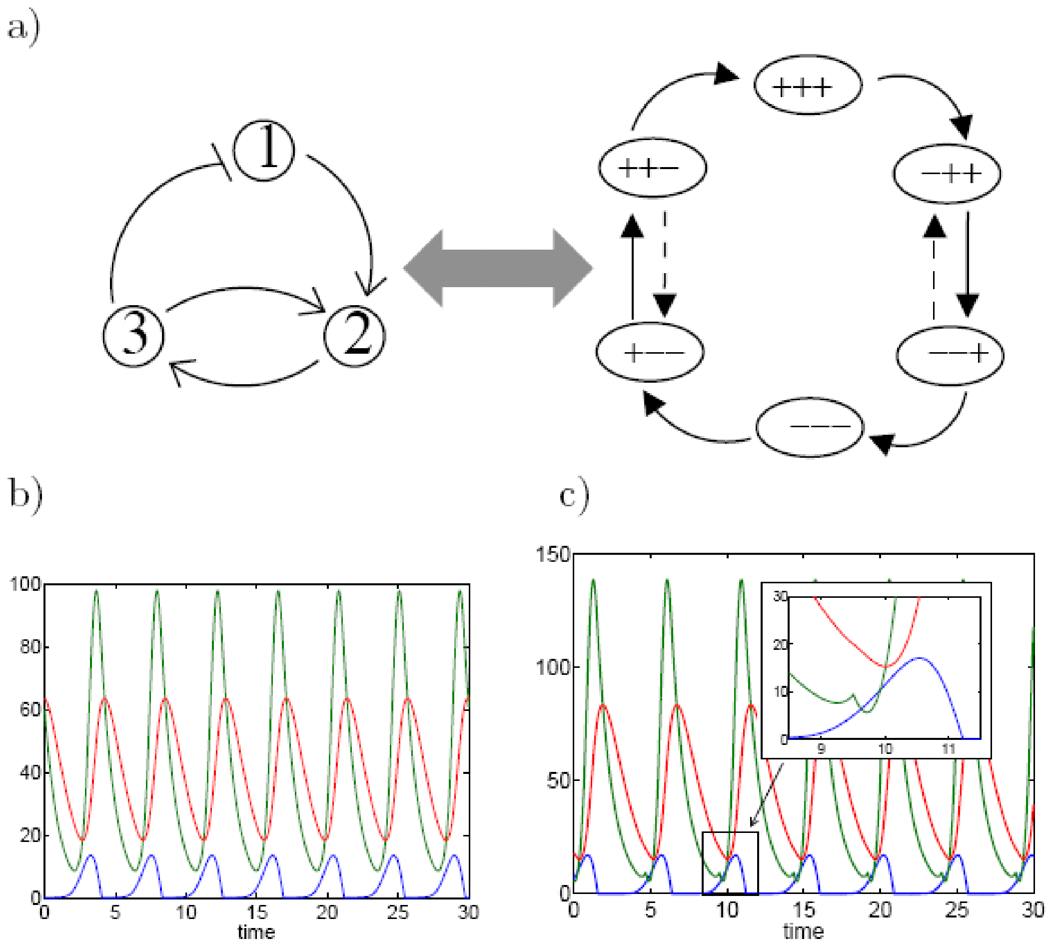
Pol: RNA polemerase

R. Metivier et al, *Cell* 115, 751-763 (2003)

Now with possible cross-links !

a)





‘Normal’ symbolic dynamics

Kick !

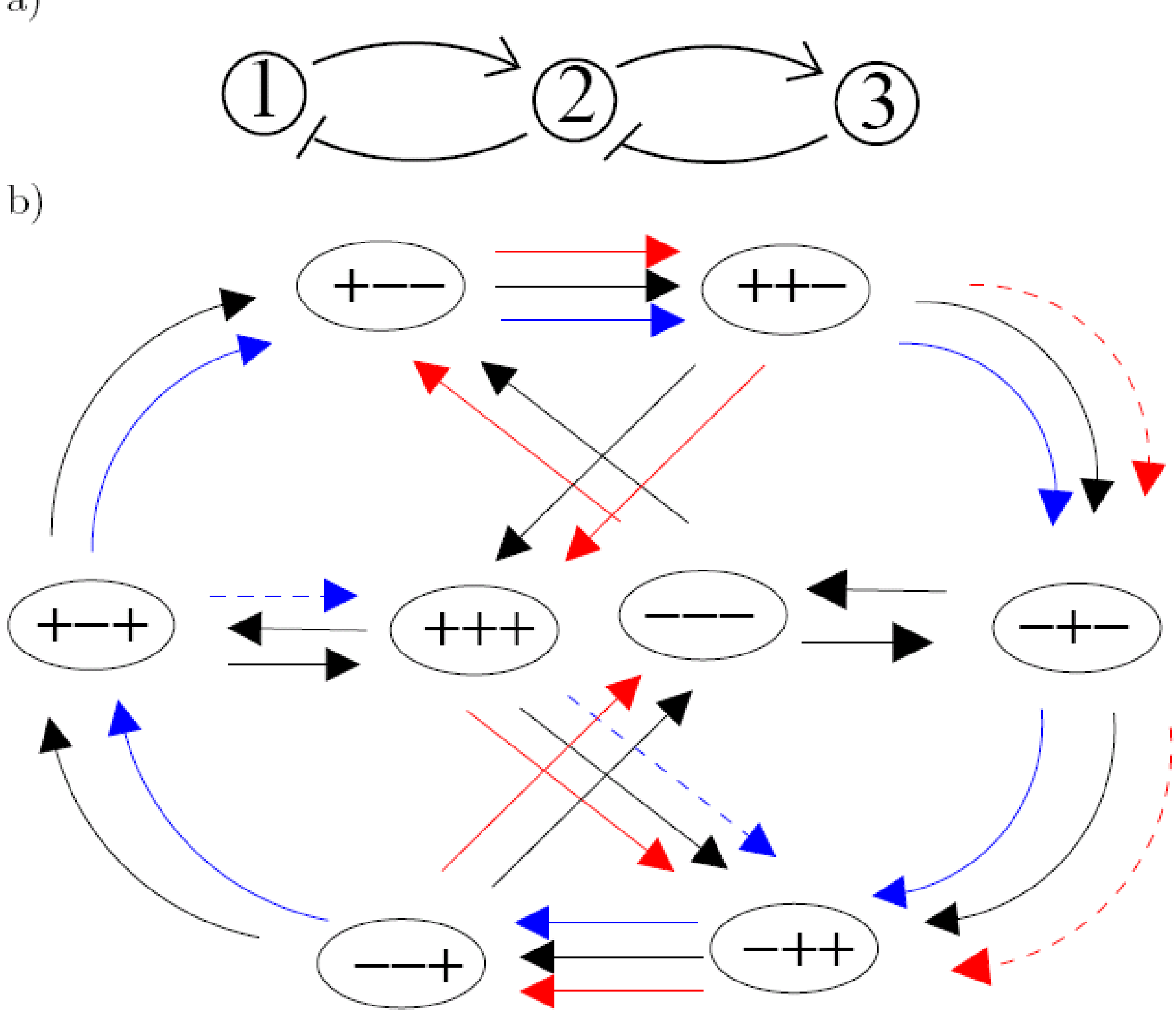


FIG. 2: Network of two coupled two-species oscillators, or a three trophic level ecosystem. (a) Structure of the network. (b) The transition network for this 3-node system. Black arrows indicate all the allowed transitions. Blue arrows are the transitions actually observed in the HP system and red arrows are the transitions observed in the BHS model (see text). In both cases, dashed arrows indicate "kicks", i.e., transitions

Two possible dynamical systems for system

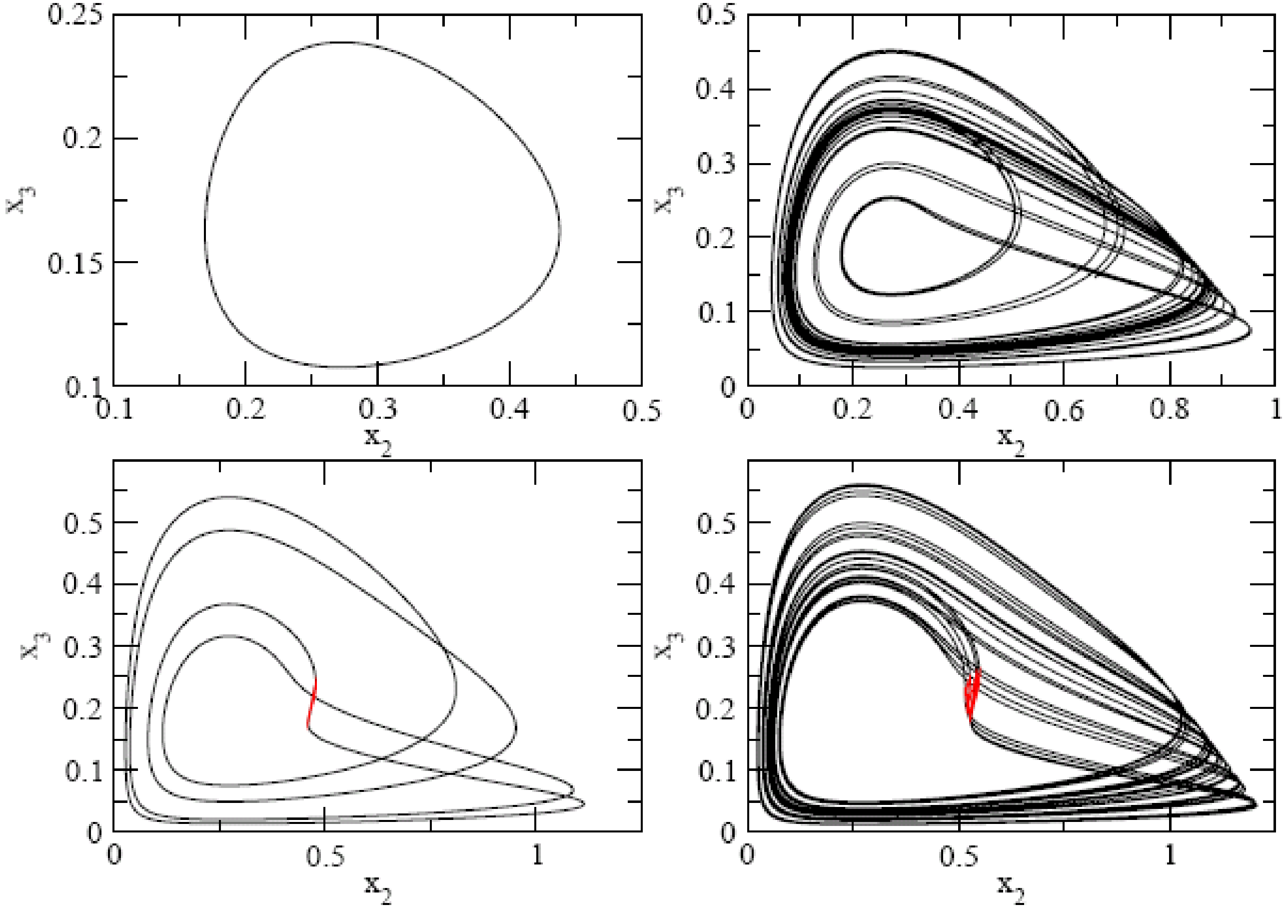
Hastings-Powell

$$
\begin{aligned}
\dot{x}_1 &= r x_1 (1 - k x_1) - \alpha_1 \frac{x_1 x_2}{1 + b_1 x_1} \\
\dot{x}_2 &= -d_1 x_2 + \alpha_1 \frac{x_1 x_2}{1 + b_1 x_1} - \alpha_2 \frac{x_2 x_3}{1 + b_2 x_2} \\
\dot{x}_3 &= -d_2 x_3 + \alpha_2 \frac{x_2 x_3}{1 + b_2 x_2}
\end{aligned}
$$

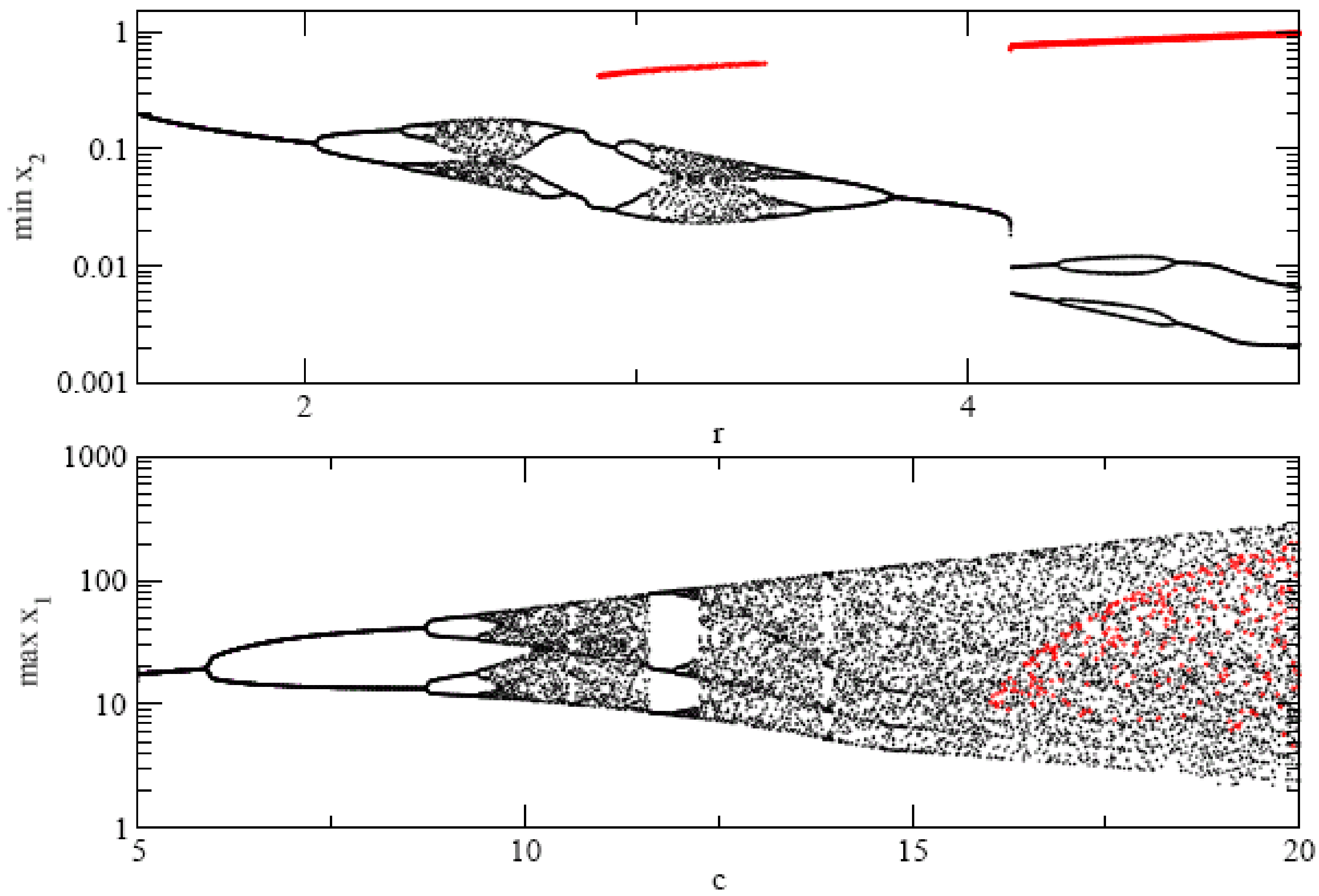
Blasius et al

$$
\begin{aligned}
\dot{x}_1 &= x_1 - \alpha_1 \frac{x_1 x_2}{1 + k x_1} \\
\dot{x}_2 &= -d x_2 + \alpha_1 \frac{x_1 x_2}{1 + k x_1} - \alpha_2 x_2 x_3 \\
\dot{x}_3 &= c(x_3^* - x_3) + \alpha_2 x_2 x_3
\end{aligned}
$$

Attractors of the dynamics of HP model



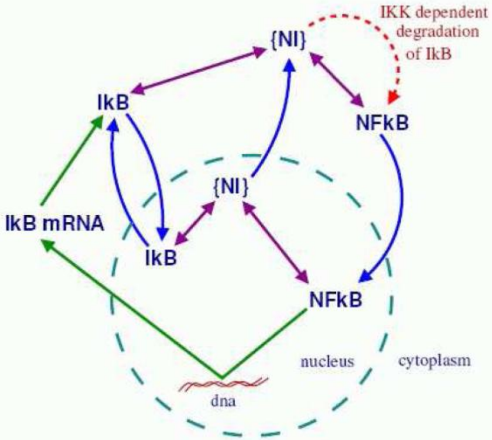
Bifurcation diagrams for HP and Blasius

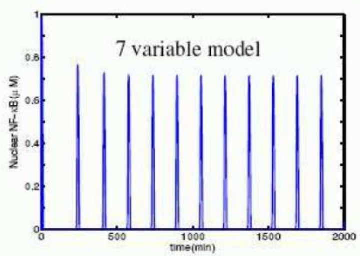


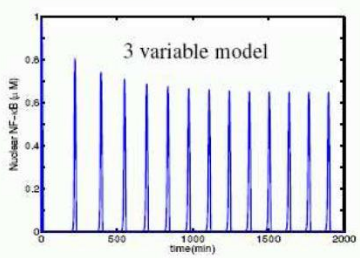
Reduction of the NF-κB System

Sandeep Krishna

7 variable model







$A = 0.007$
$B = 954.5$
$C = 0.035$
$\epsilon = 2 \times 10^{-5}$
$\delta = 0.029$

4 variable model

Equilibrium of complexes:

$$(NI) = (N_{tot} - N_n)\frac{I}{K_I + I}$$
$$N = (N_{tot} - N_n)\frac{K_I}{K_I + I}$$
$$(NI)_n = I_n^{tot}\frac{N_n}{K_N + N_n}$$
$$I_n = I_n^{tot}\frac{K_N}{K_N + N_n}$$

$$\frac{dN_n}{dt} = k_{Nin} K_I \frac{(N_{tot} - N_n)}{K_I + I} - k_{NIout}\frac{I_n^{tot} N_n}{K_N + N_n}$$
$$\frac{dI_m}{dt} = k_t N_n^2 - \gamma_m I_m$$
$$\frac{dI}{dt} = k_{tl} I_m - \alpha \frac{(N_{tot} - N_n) I}{K_I + I} - k_{Iin} I + k_{Iout} K_N \frac{I_n^{tot}}{K_N + N_n}$$

Small terms deleted: $- k_{Iin} I + k_{Iout} K_N \frac{I_n^{tot}}{K_N + N_n}$

$$\frac{dI_n^{tot}}{dt} = k_{Iin} I - k_{Iout} K_N \frac{I_n^{tot}}{K_N + N_n} - k_{NIout}\frac{I_n^{tot} N_n}{K_N + N_n}$$

$I_n^{tot} N_n / (K_N + N_n)$ is significant only when $N_n >> K_n$. At this point I_n^{tot} reaches its minimum
$\Rightarrow I_n^{tot} \approx \frac{k_{Iin}}{k_{NIout}} I$
($dI_n^{tot}/dt = 0$ and $N_n >> K_N$)

$t \to (1/\gamma_m) t$
$N_n \to N_{tot} N_n$
$I_m \to (k_t N_{tot}^2 / \gamma_m) I_m$
$I \to (k_t k_{tl} N_{tot}^2 / \gamma_m^2) I$

3 variable model

$$\frac{dN_n}{dt} = A\frac{(1 - N_n)}{\epsilon + I} - B\frac{I N_n}{\delta + N_n}$$
$$\frac{dI_m}{dt} = N_n^2 - I_m$$
$$\frac{dI}{dt} = I_m - C\frac{(1 - N_n) I}{\epsilon + I}$$

where $A = \frac{k_{Nin} K_I \gamma_m}{k_t k_{tl} N_{tot}^2}$; $B = \frac{k_{Iin} k_t k_{tl} N_{tot}}{\gamma_m^3}$; $C = \frac{\alpha \gamma_m}{k_t k_{tl} N_{tot}}$

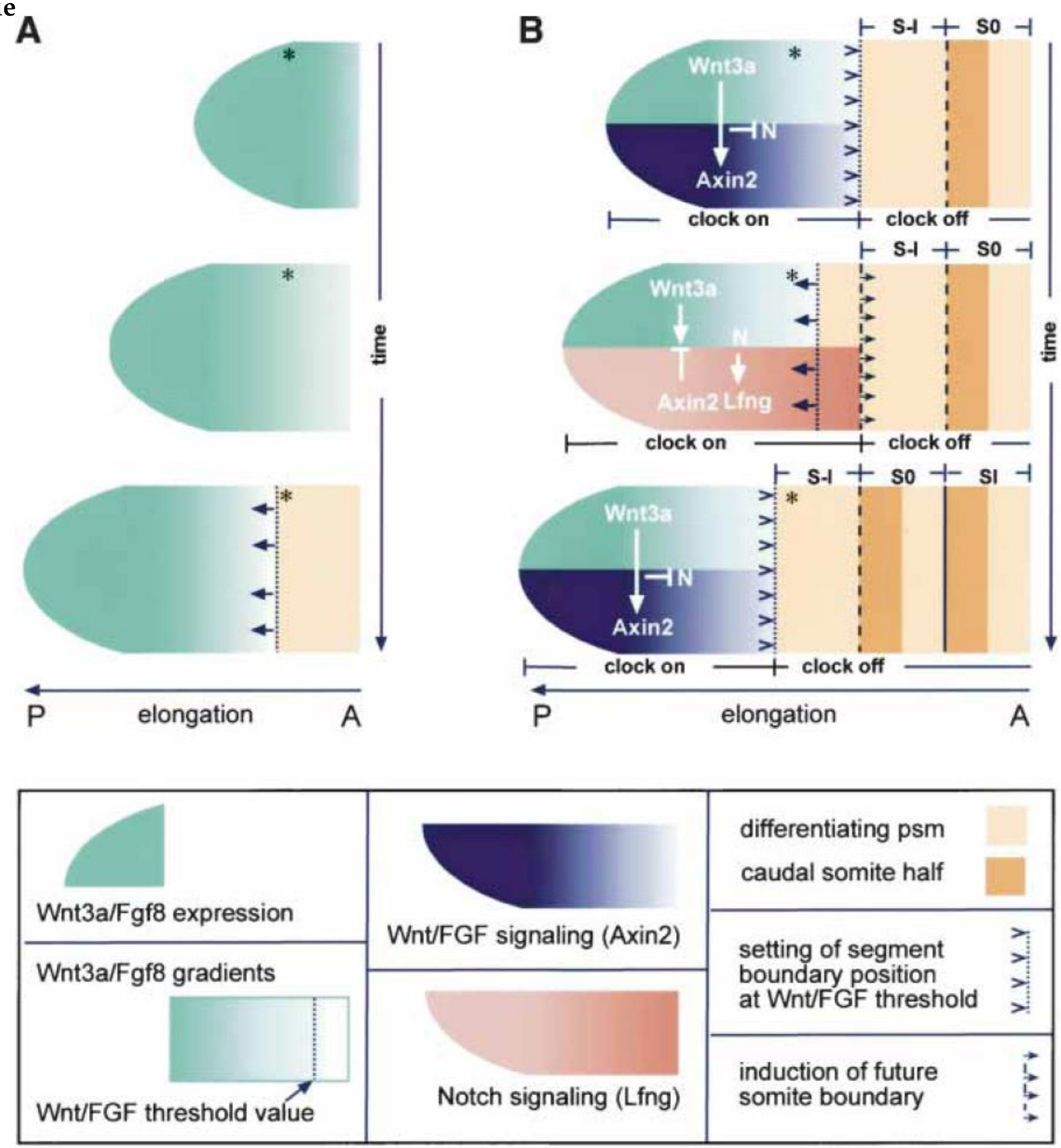
$\epsilon = \frac{K_I \gamma_m^2}{k_t k_{tl} N_{tot}^2}$; $\delta = K_N / N_{tot}$

Two oscillators: Wnt and Notch: out of phase

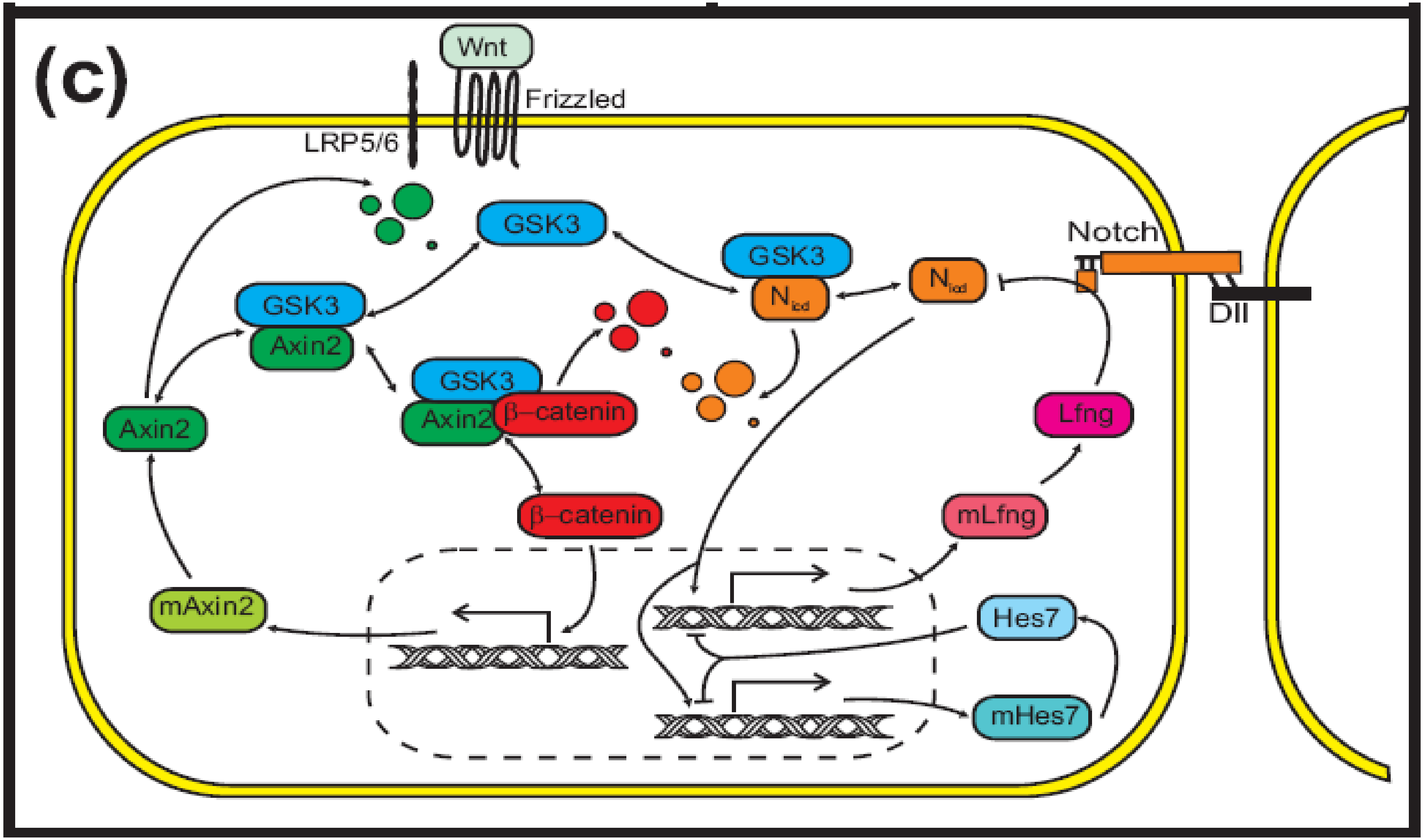
Aulehla and Herrmann

Goldbeter, Pourquie

Wnt gradient and
Clock are coupled



The full systems with Wnt and Noctch and cross-talk



Equations of the Wnt-Notch network

Wnt

$$\frac{dC}{dt} = c_{fC}B[GA] - c_{bC}C - \alpha_1 C \quad (1)$$

$$\frac{dG}{dt} = -c_{f[GA]}AG + c_{b[GA]}[GA] - c_{f[GN]}GN + c_{b[GN]}[GN] + \alpha_2[GN] \quad (2)$$

$$\frac{dB}{dt} = S - c_{fC}B[GA] + c_{bC}C - \frac{B}{\tau_B} \quad (3)$$

$$\frac{dA}{dt} = -c_{f[GA)}AG + c_{b[GA]}[GA] + c_{tlA}A_m - c_A\frac{A}{k_A + A} \quad (4)$$

$$\frac{dA_m}{dt} = c_{tsA}B^h - \frac{A_m}{\tau_{Am}} \quad (5)$$

$$\frac{d[GA]}{dt} = c_{f[GA]}AG - c_{b[GA]}[GA] - c_{fC}B[GA] + c_{bC}C + \alpha_1 C \quad (6)$$

Notch

$$\frac{dN}{dt} = c_s\frac{k_s}{k_s + L} - \frac{N}{\tau_N} - c_{f[GN]}GN + c_{b[GN]}[GN] \quad (7)$$

$$\frac{dHm}{dt} = c_{tsH}\left(\frac{N^{h_{NH}}}{k_{NH} + N^{h_{NH}}} \cdot \frac{k_{HH}}{k_{HH} + H^{h_{HH}}}\right) - \frac{H}{\tau_{Hm}} \quad (8)$$

$$\frac{dH}{dt} = c_{tlH}H_m - \frac{H}{\tau_H} \quad (9)$$

$$\frac{dLm}{dt} = c_{tsL}\left(\frac{N^{h_{NL}}}{k_{NL} + N^{h_{NL}}} \cdot \frac{k_{HL}}{k_{HL} + H^{h_{HL}}}\right) - \frac{L_m}{\tau_{Lm}} \quad (10)$$

$$\frac{dL}{dt} = c_{tlL}L_m - \frac{L}{\tau_L} \quad (11)$$

(Cross talk)

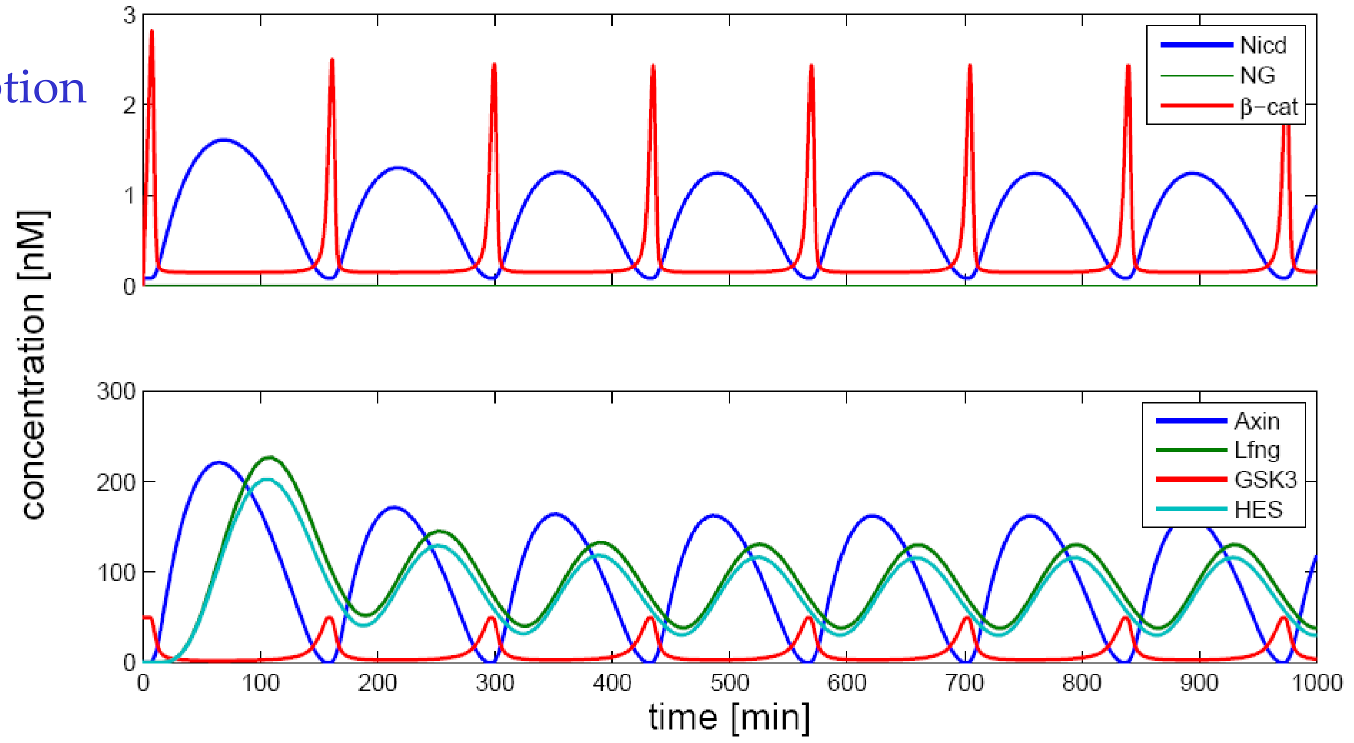
$$\frac{d[NG]}{dt} = c_{f[NG]}NG - c_{b[NG]}[NG] - \alpha_2[NG] \quad (12)$$

A total of approx. 27 constants – Some are poorly defined

Master thesis work by Peter B. Jensen

The full Wnt-Notch system

Transcription factors



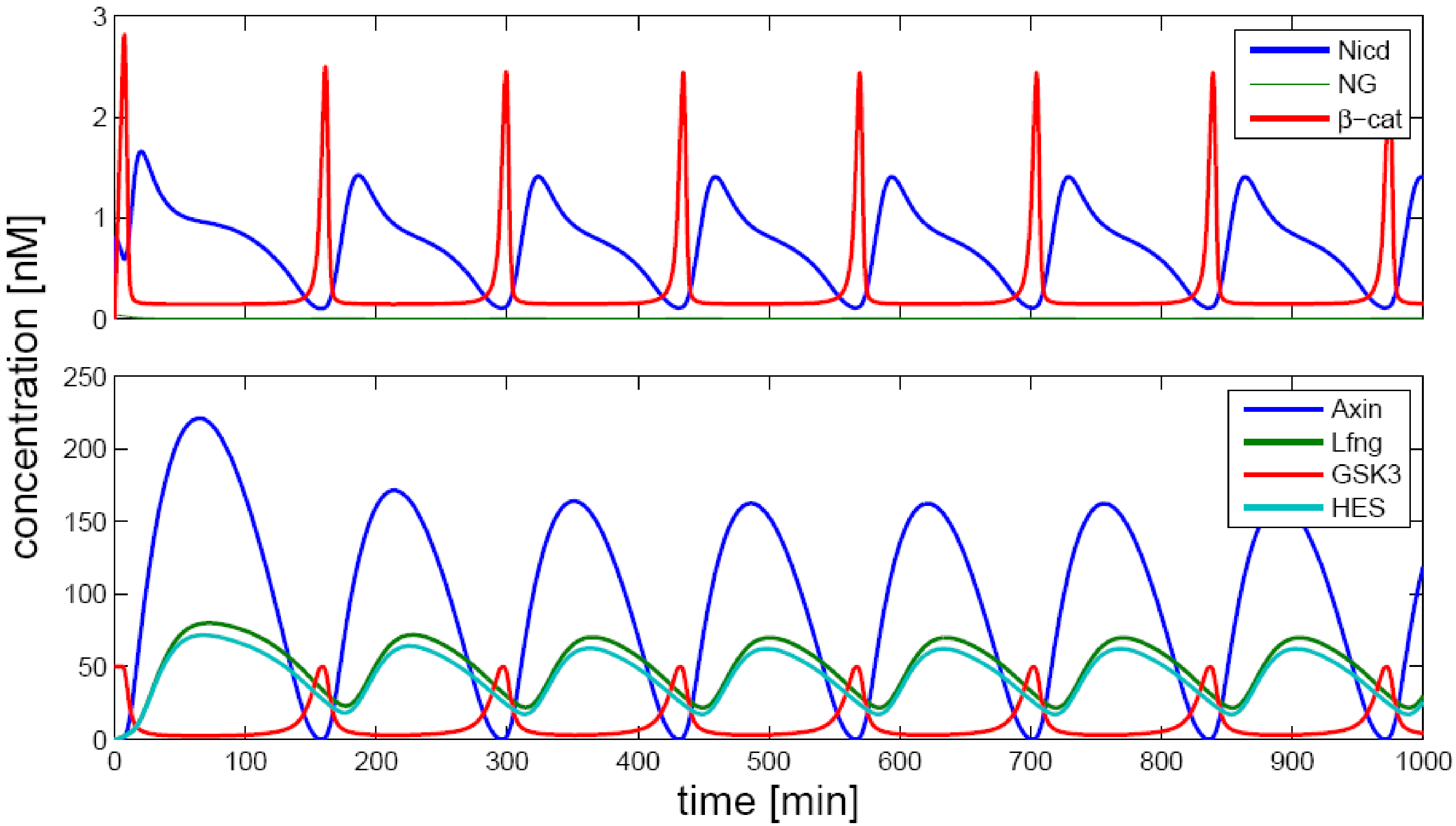
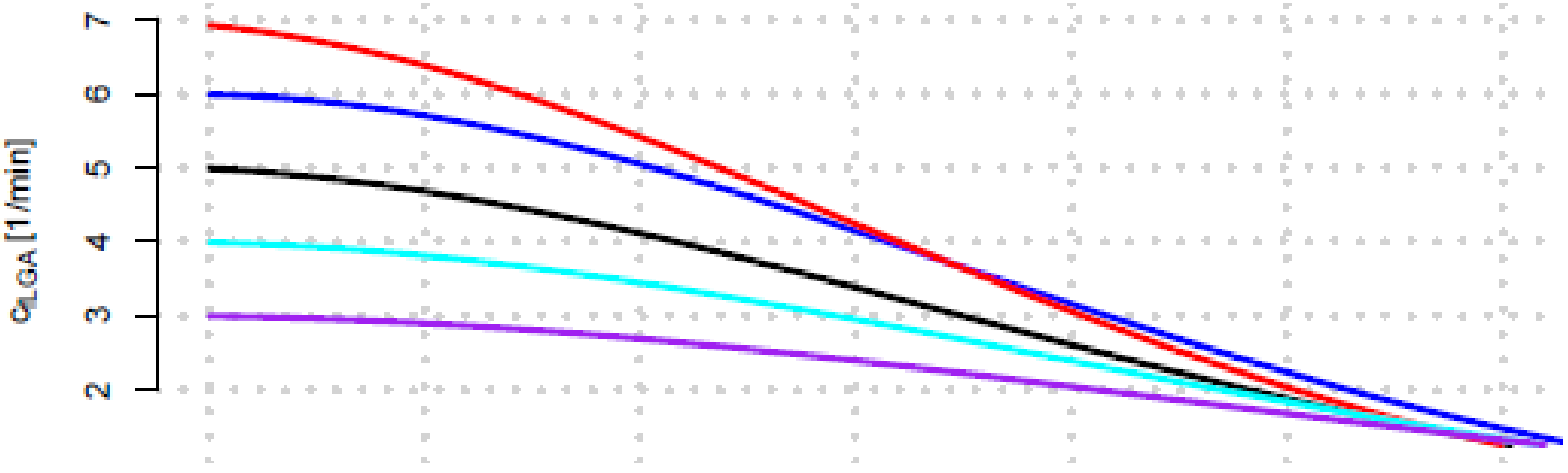


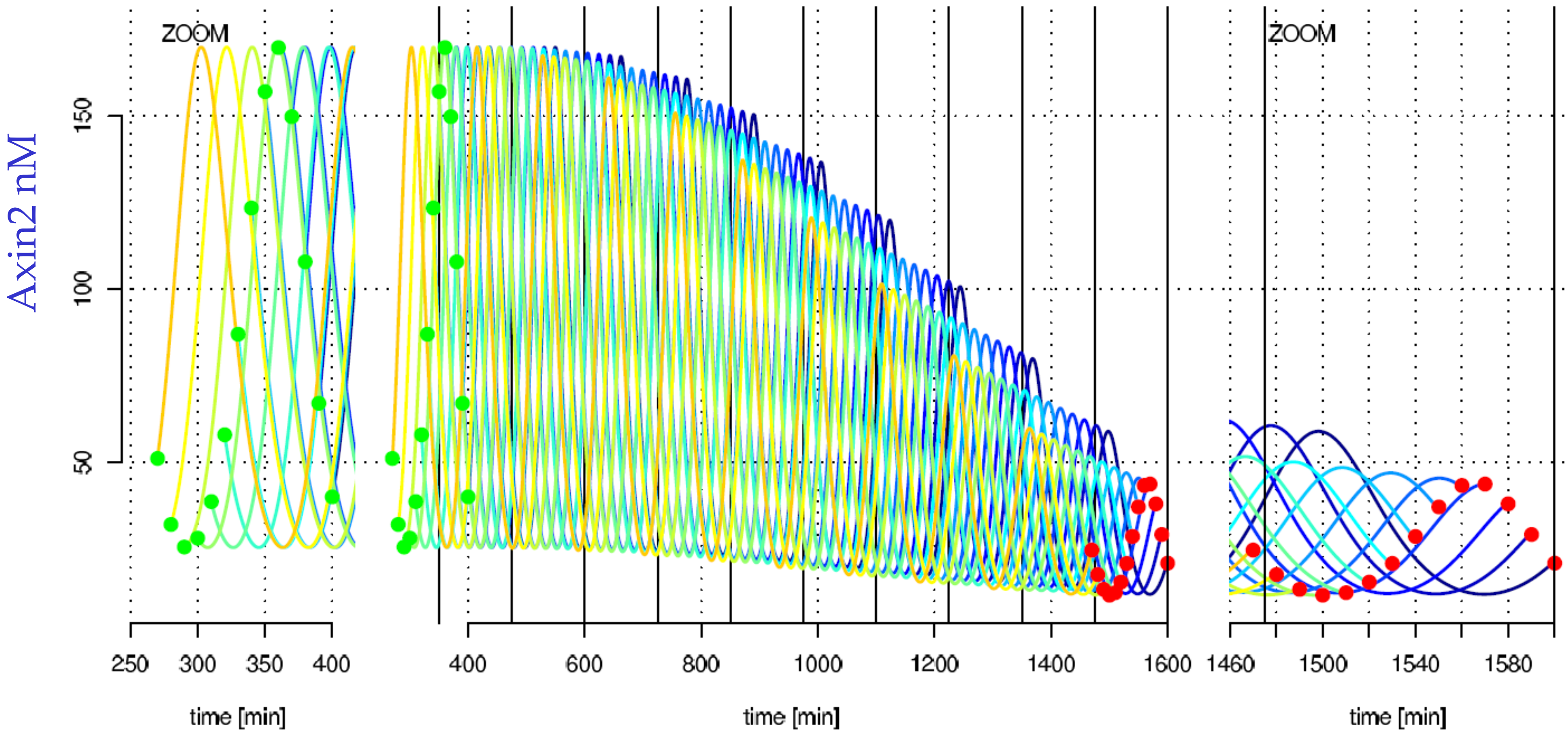
Figure 7.2: Timeseries of the 12 equation system with Lfng negative feedback

Spatial gradient of Wnt:

Gaussian profiles

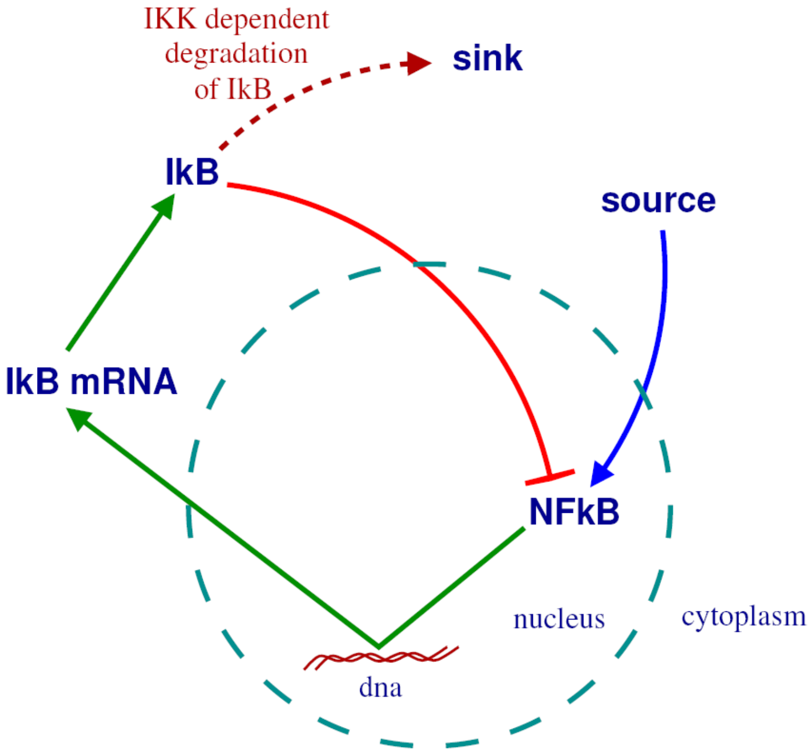


Oscillations stop when Wnt under a threshold



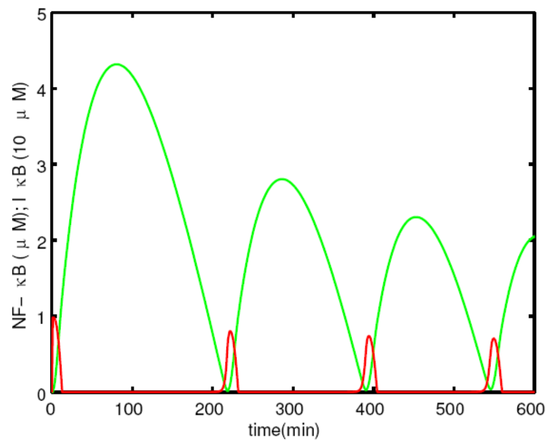
Axin2 nM
ZOOM
ZOOM
50
100
150
250
300
350
400
400
600
800
1000
1200
1400
1600
1460
1500
1540
1580
time [min]
time [min]
time [min]

Simple Model for Protein Oscillations

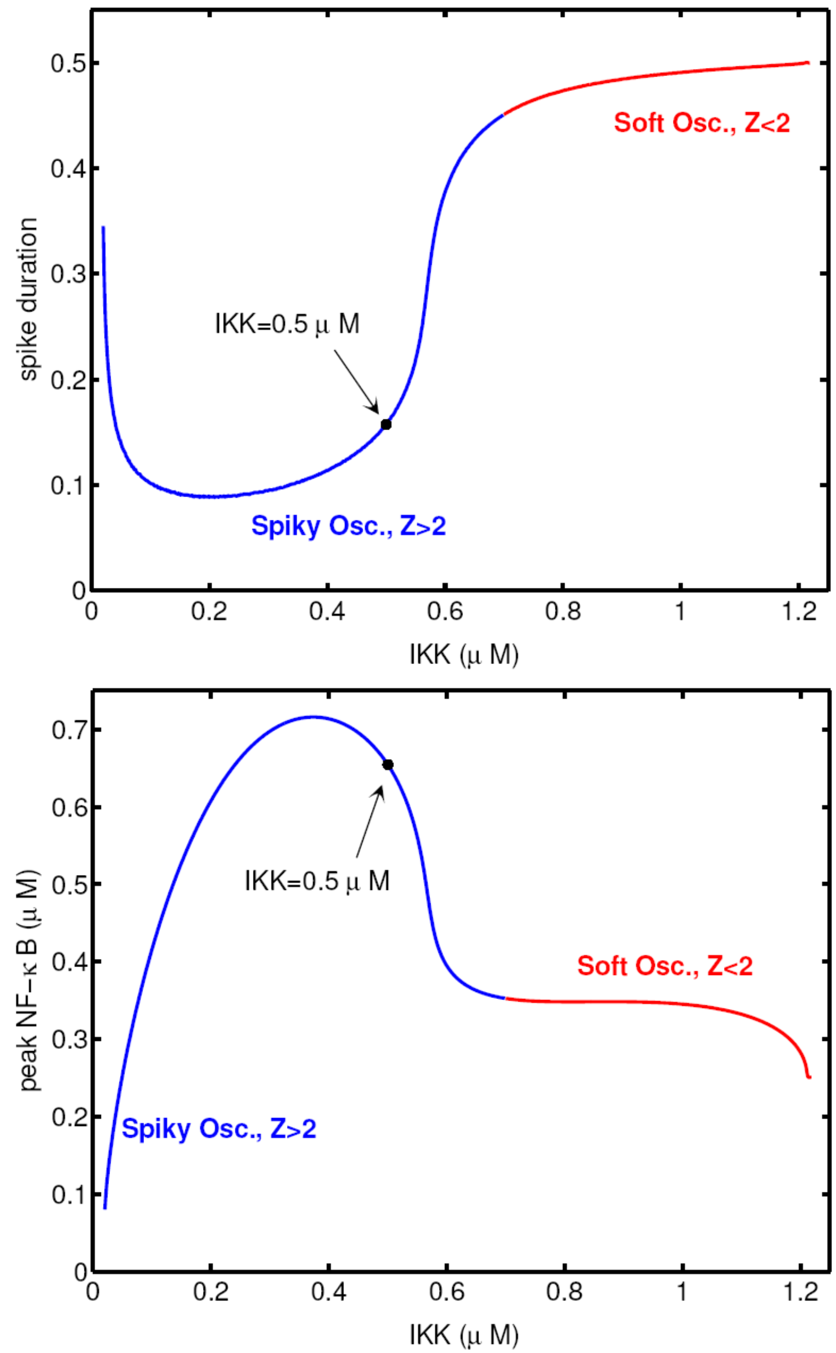


Simple model qualitatively reproduces several features of NF-κB oscillations:

- fact of oscillations
- time period
- shape of oscillations
- phase relationships
- no osc. in the absence of feedback
- increased transcription $\longrightarrow$ lower freq.

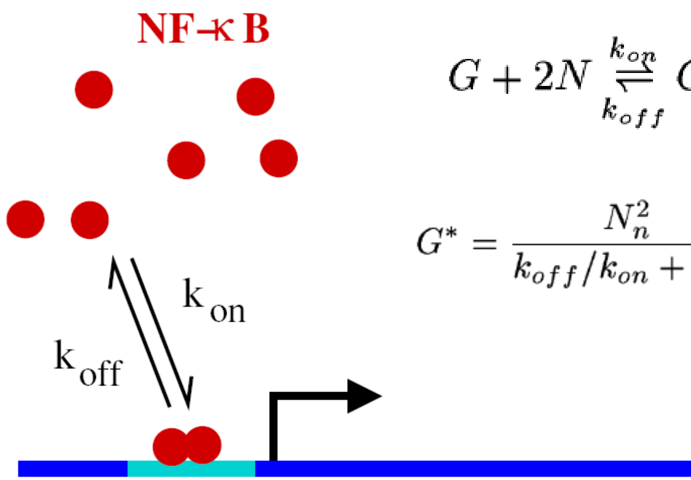


$$A = 0.007,\ B = 954.5,\ C = 0.035,$$
$$\delta = 0.029,\ \epsilon = 2 \times 10^{-5}$$



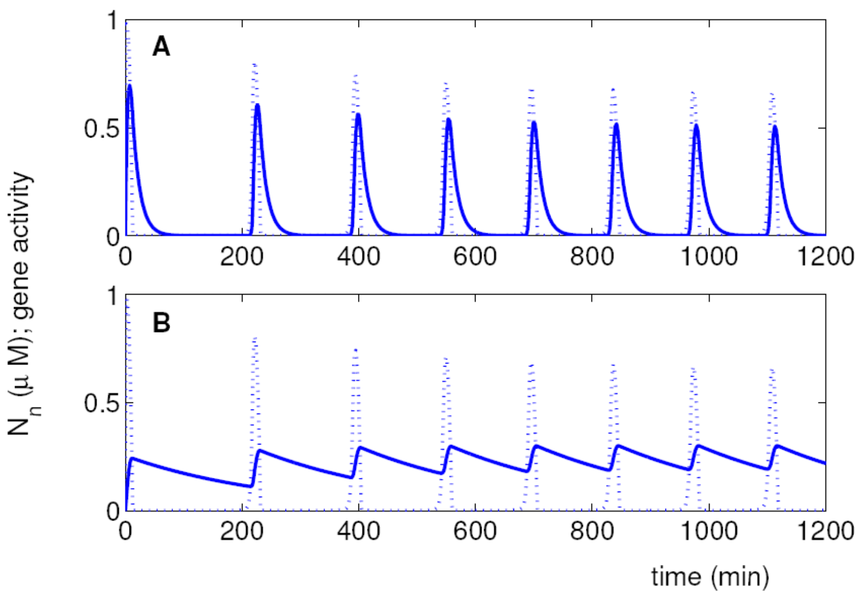
spike duration
0.5
0.4
0.3
0.2
0.1
0
Soft Osc., Z<2
IKK=0.5 μ M
Spiky Osc., Z>2
0
0.2
0.4
0.6
0.8
1
1.2
IKK (μ M)
peak NF−κ B (μ M)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IKK=0.5 μ M
Soft Osc., Z<2
Spiky Osc., Z>2
0
0.2
0.4
0.6
0.8
1
1.2
IKK (μ M)

Coupling NF-κB to a Downstream Gene

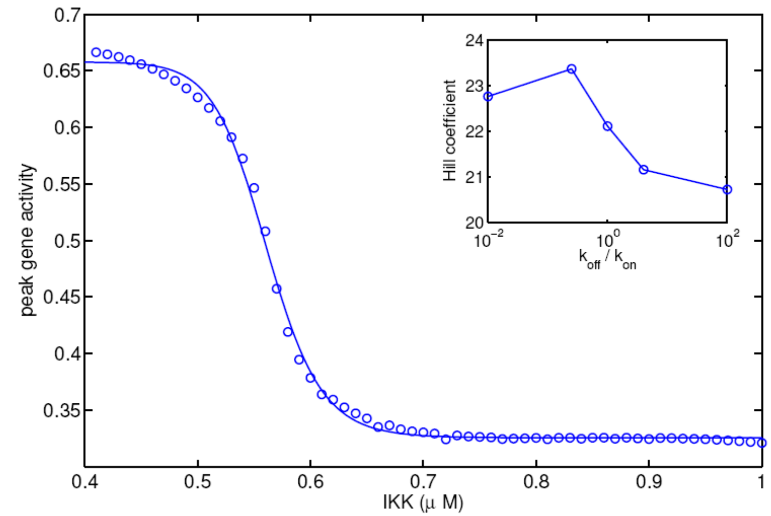


$$G + 2N \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} G^*.$$

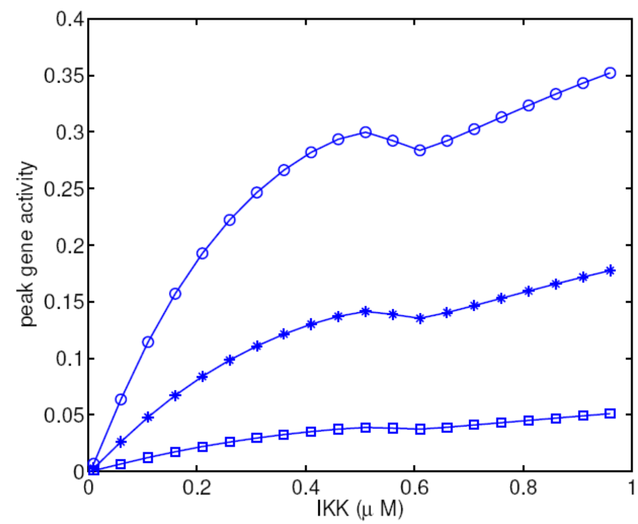
$$G^* = \frac{N_n^2}{k_{off}/k_{on} + N_n^2}.$$



Equilibrium binding, $1/k_{off} \ll$ time period



Non-equilibrium binding, $1/k_{off} >$ time period



Variable	Description	
N_n	nuclear NF-κB	
$I_{\alpha/\varepsilon}$	free IκB	
$I_{m\alpha/\varepsilon}$	IκB mRNA	
A_m	A20 mRNA	
K	active IKK	
K_i	inactive IKK	
Parameter	**Description**	**Value**
T	TNF stimulation	1
B	proportionality factor of the export of nuclear NF-κB	102.6
A	proportionality factor of the import of NF-κB	0.004
η		0.092
K_I	IκB - NF-κB complex dissociation factor	$1.26{\cdot}10^{-5}$ μM
δ	concentration at which half of the IκBα/ε is bound in complex with NF-κB	0.0414 μM
p	NF-κB in-dependent transcription rate of IκBα mRNA	$3.36{\cdot}10^{-5}$ min^{-1}
t_a	NF-κB dependent transcription rate of IκBα mRNA	0.0042 μM min^{-1}
t_e	NF-κB dependent transcription rate of IκBε mRNA	0.084 μM min^{-1}
t_A	NF-κB dependent A20 transciption rate	0.0168 $\mu M^{-1} min^{-1}$
tl_a	translation rate of IκBα	0.0672 min^{-1}
tl_e	translation rate of IκBε	$1.2{\cdot}10^{-5}$ min^{-1}
tl_A	translation rate of A20	0.3024 min^{-1}
$\gamma_{Im\alpha}$	half-life of IκBα mRNA	0.0168 min^{-1}
$\gamma_{Im\varepsilon}$	half-life of IκBε mRNA	0.00168 min^{-1}
$\gamma_{I\alpha/\varepsilon}$	half-life of the IκB's	0.005 min^{-1}
γ_{A20m}	half-life of the A20 mRNA	0.0168 min^{-1}
γ_{A20}	half-life of the A20	0.001 min^{-1}
α_α	IKK dependent degradation of IκBα	0.00025 min^{-1}
α_ε	IKK dependent degradation of IκBε	$7.6{\cdot}10^{-6}$ min^{-1}
μ	rate of IKK self-inactivation	0.063 min^{-1}
σ	strength of A20 negative feedback	0.25
β	proportionality factor of A20 on IKK	1.25

When oscillations in feed-back loops?

- Process that takes a finite (minimum) time

  evt. time delay τ: $dx/dt \sim P(t-\tau)$

- Many intermediate steps

  binding, complex, steps on DNA, etc

  several components: repressilator

- Sharp response: high Hill coefficient

  $$dm/dt \sim p^n/(k^n + p^n)$$

- Saturated degradation: depends on level

  $$dI/dt \sim I_m - cI/(\varepsilon+I)$$

- Autocatalysis: $dx/dt \sim x^n/(k^n + x^n)$

k_g : dissociation constant between p53 and DNA (O-operator site)

$$[p] = [p_f]+[pm] \quad [O] = [O_f]+[pO] \quad ([O] = 1)$$

$$k_g = \frac{[p_f][O_f]}{[pO]} \Rightarrow [pO] = \frac{[p_f][O]}{k_g+[p_f]} = \frac{[p]-[pm]}{k_g+[p]-[pm]}$$

(can add a Hill coefficient)

$[pO]$: "Equilibrium" probability that p53 is bound to DNA

k : dissociation constant between p53 and mdm2

$$k = \frac{[p_f][m_f]}{[pm]} = \frac{([p]-[pm])([m]-[pm])}{[pm]} \Rightarrow$$

$$[pm]^2 - ([p] + [m] + k)[pm] + [p][m] = 0 \Rightarrow$$

$$[pm] = \frac{1}{2}([p]+[m]+k)-\sqrt{([p] + [m] + k) - 4[p][m]}$$

Why oscillations ?

- Importance for apoptosis
- Essential in segmentation
- Spiky oscillations →

important for sharp responses,

fast regulations,

high Hill coefficients

→ hormones also come in spikes

Mathematically: The most `simple' dynamics !