Ultradian Oscillations and Segmentation : The NF-κB and Wnt-Notch Systems

Mogens H. Jensen, Niels Bohr Institute, Copenhagen

KITP, Santa Barbara 3 -7 March 2008

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 (Hoffmann et al (2002))**
- **Wnt-Notch segmentation network (Aulehla, Pourquie)**

→ Identify the ‘simplest’ negative feed-back loop.

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

2. Why oscillations ? When oscillations ?

3. NF-κB: Transcription factor important for regulation of inflammation and apoptosis

4. NF-κB - IκB feed-back loop:
   Reduce 26-dimensional dynamics to three variables
   Spiky oscillations:
   → Saturated degradation.
   → Oscillating external stimuli: chaotic response

5. Oscillations essential for somite segmentation:
   Wnt and Notch signaling loops: out of phase.

6. Coupled model (in progress): 12-dim:
   Wnt drives the Notch: Oscillations

7. From ‘noisy’ biological data:
→ Guess negative feed-back loop from symbolic dynamics !

Collaborators:

- Sandeep Krishna, post doc
- Guido Tiana, visitor (Milan)
- Kim Sneppen
- Simone Pigolotti, post doc
- Peter B. Jensen, Alex Hunziker, B. Mengel M. Avlund,

→ **Bio. Colleagues: A. Levine, A. Hoffmann, G. Lahav**

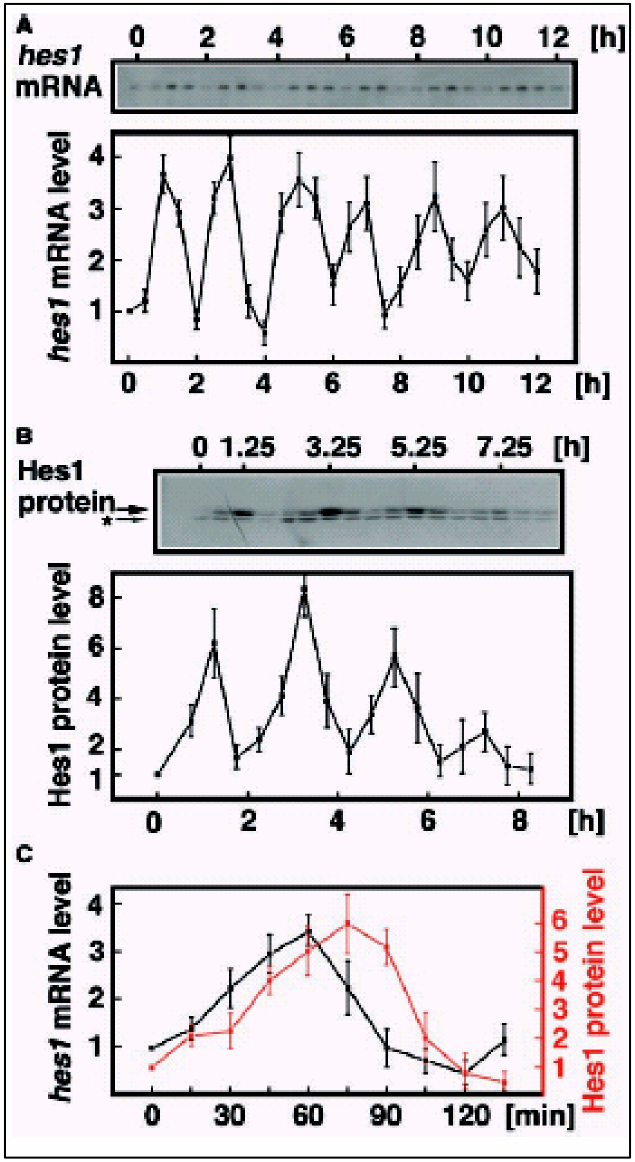
S. Krishna, M.H. Jensen and K. Sneppen, "Spiky oscillations in NF-κB signalling", Proc.Nat.Acad.Sci. 103, 10840-10845 (2006).

S. Pigolotti, S. Krishna and 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).

Peter B. Jensen “Modelling oscillatory gene expressions in the Wnt-Notch system”

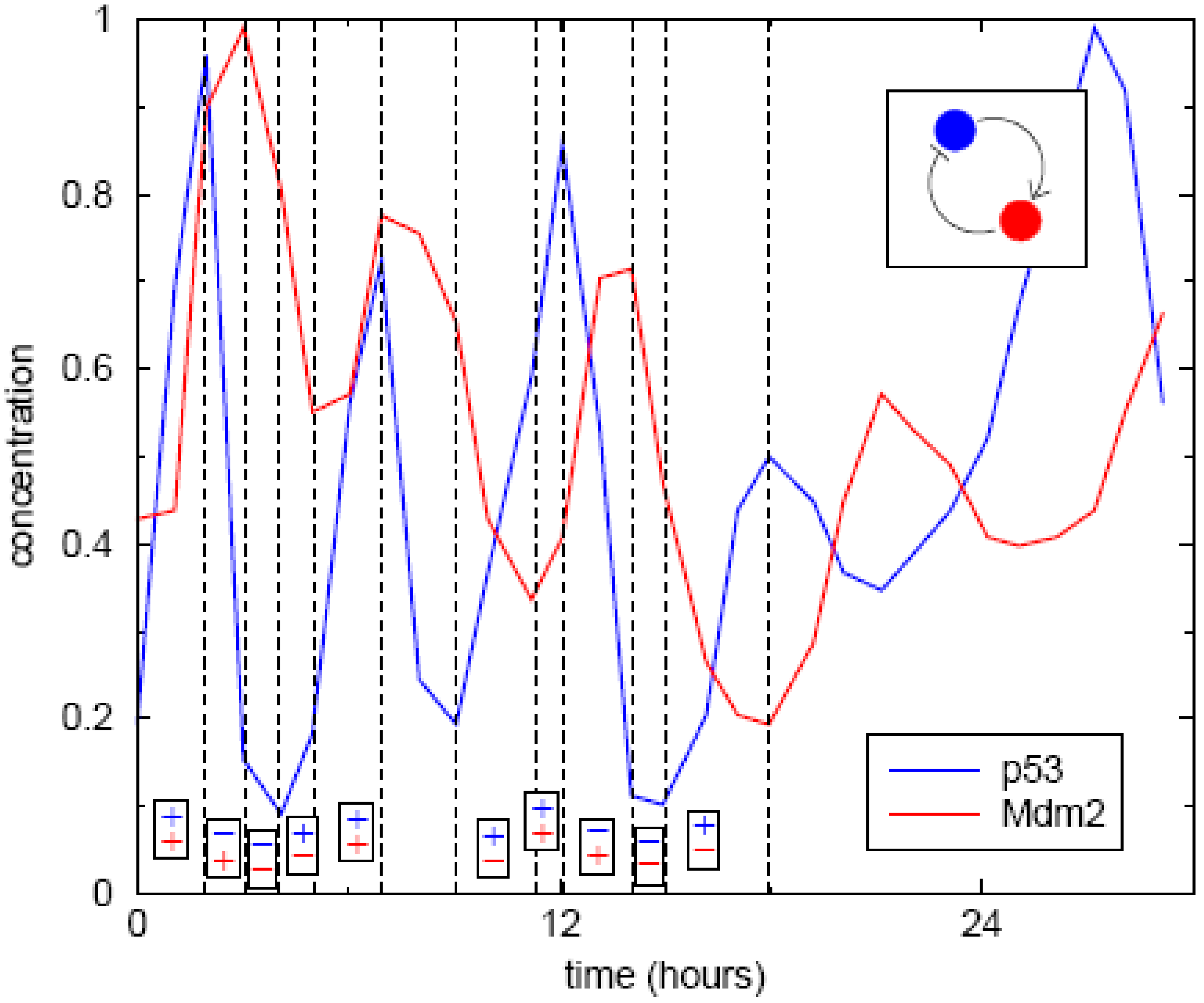
‘Typical’ Oscillating data: Hes1 - segmentation



(Hirata et al, 2002)

Apoptosis

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



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 !

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

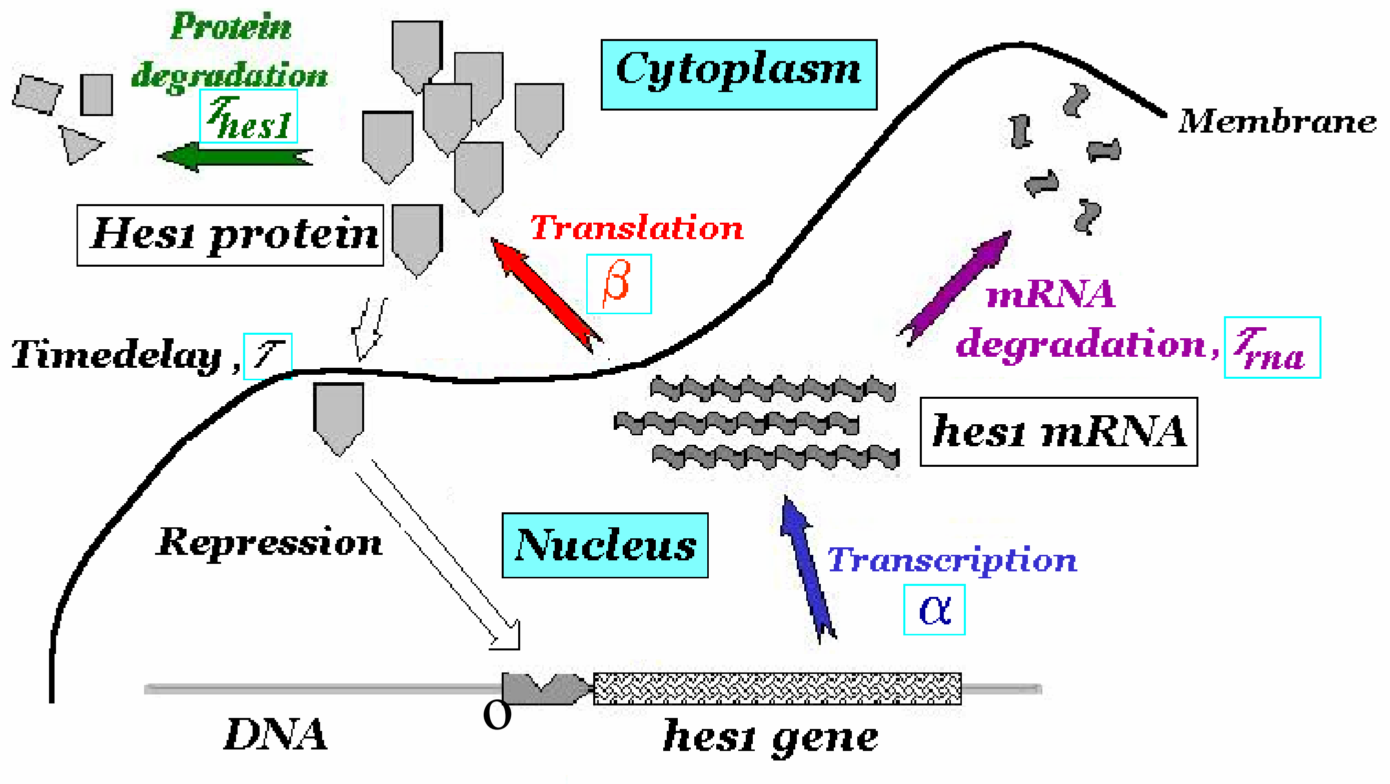
  $dm/dt \sim p^n/(k^n + p^n)$

- Saturated degradation: depends on level

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

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

Model of negative feed-back loop for Hes1



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

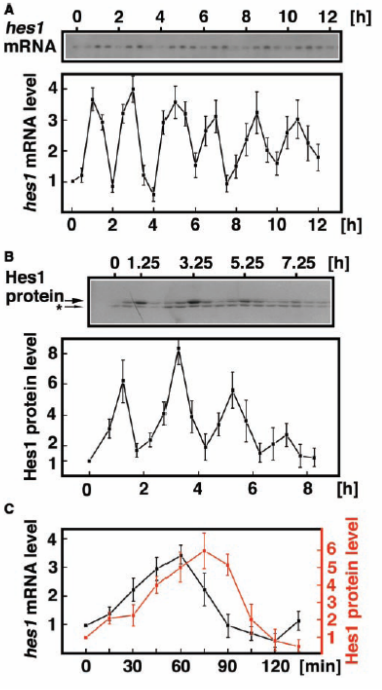
$$\frac{d[Hes1]}{dt} = \beta \cdot [mRNA(t)] - \frac{[Hes1(t)]}{\tau_{hes1}}$$

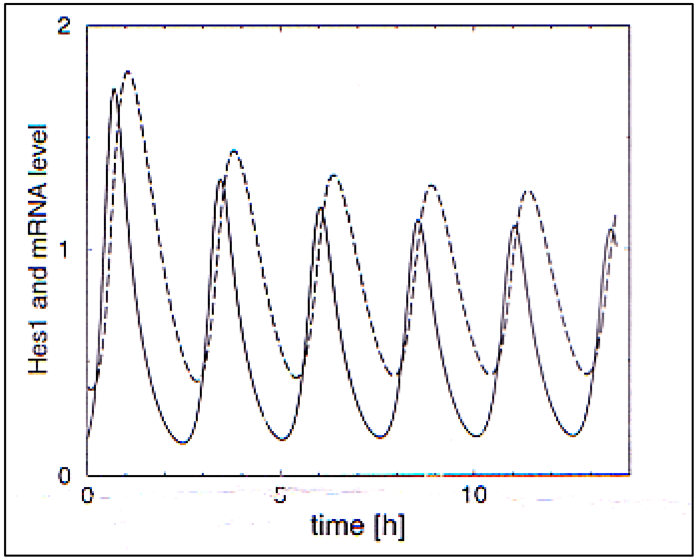
$$\frac{d[mRNA]}{dt} = \alpha \cdot \frac{K_M}{K_M + [Hes1(t-\tau)]^n} - \frac{[mRNA(t)]}{\tau_{rna}}$$

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

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

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

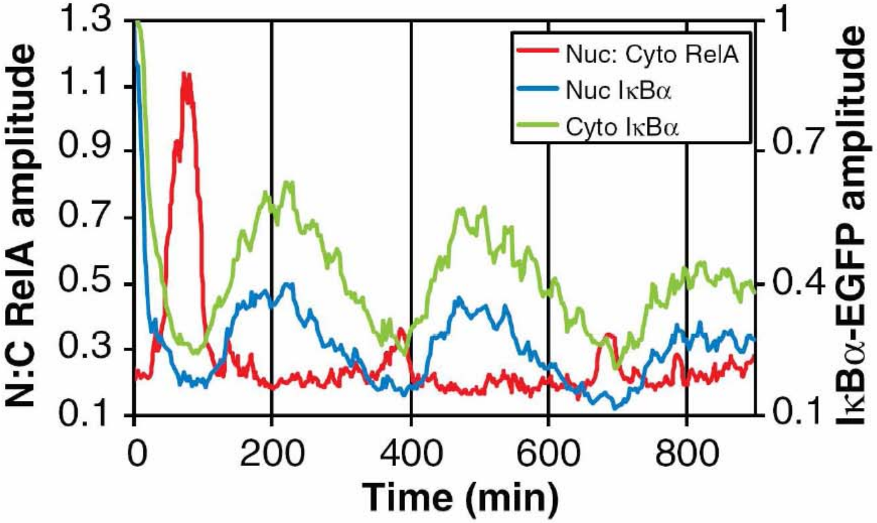




The NF-κB System in Mammalian Cells

- 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.

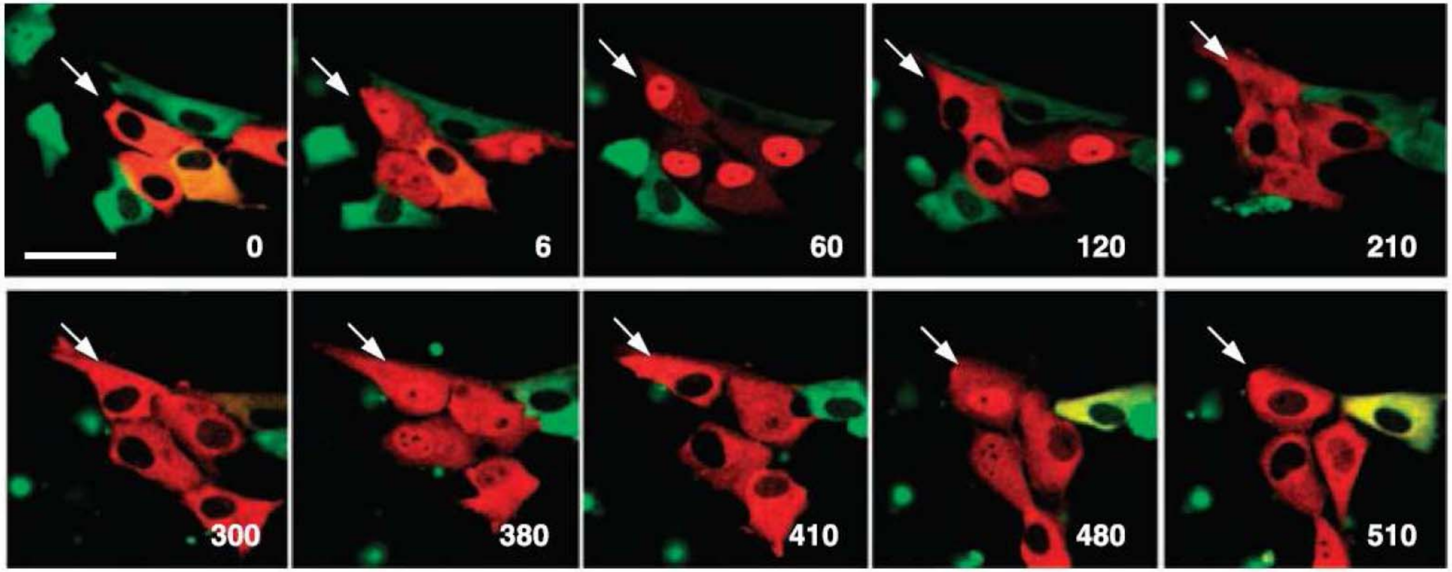
Nelson et al. (2004) Science 306, 704.



How does the network produce oscillations?

Why does the cell need the oscillations?

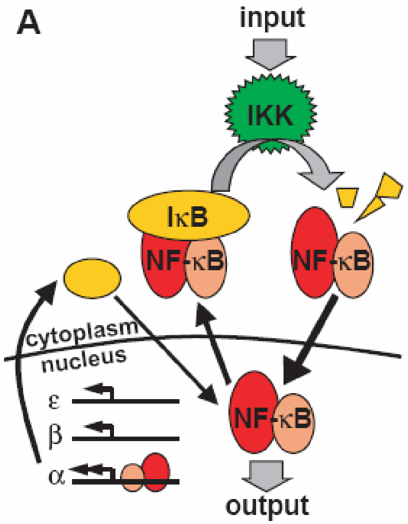
‘Direct’ observations of oscillations in nucleus



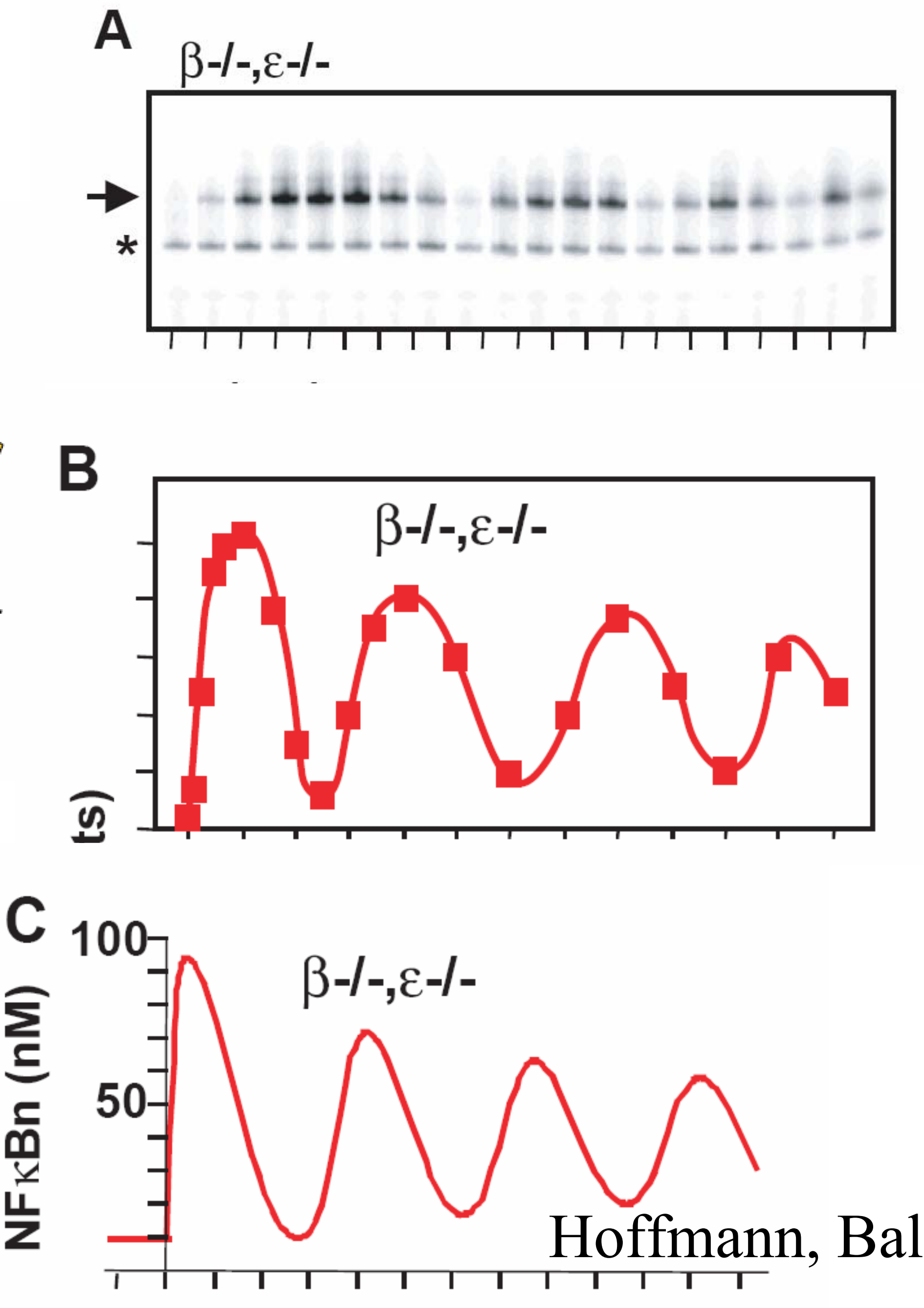
Oscillations in the nuclear localization of an NF-κB transcription factor in human cells

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

NF-κB oscillations



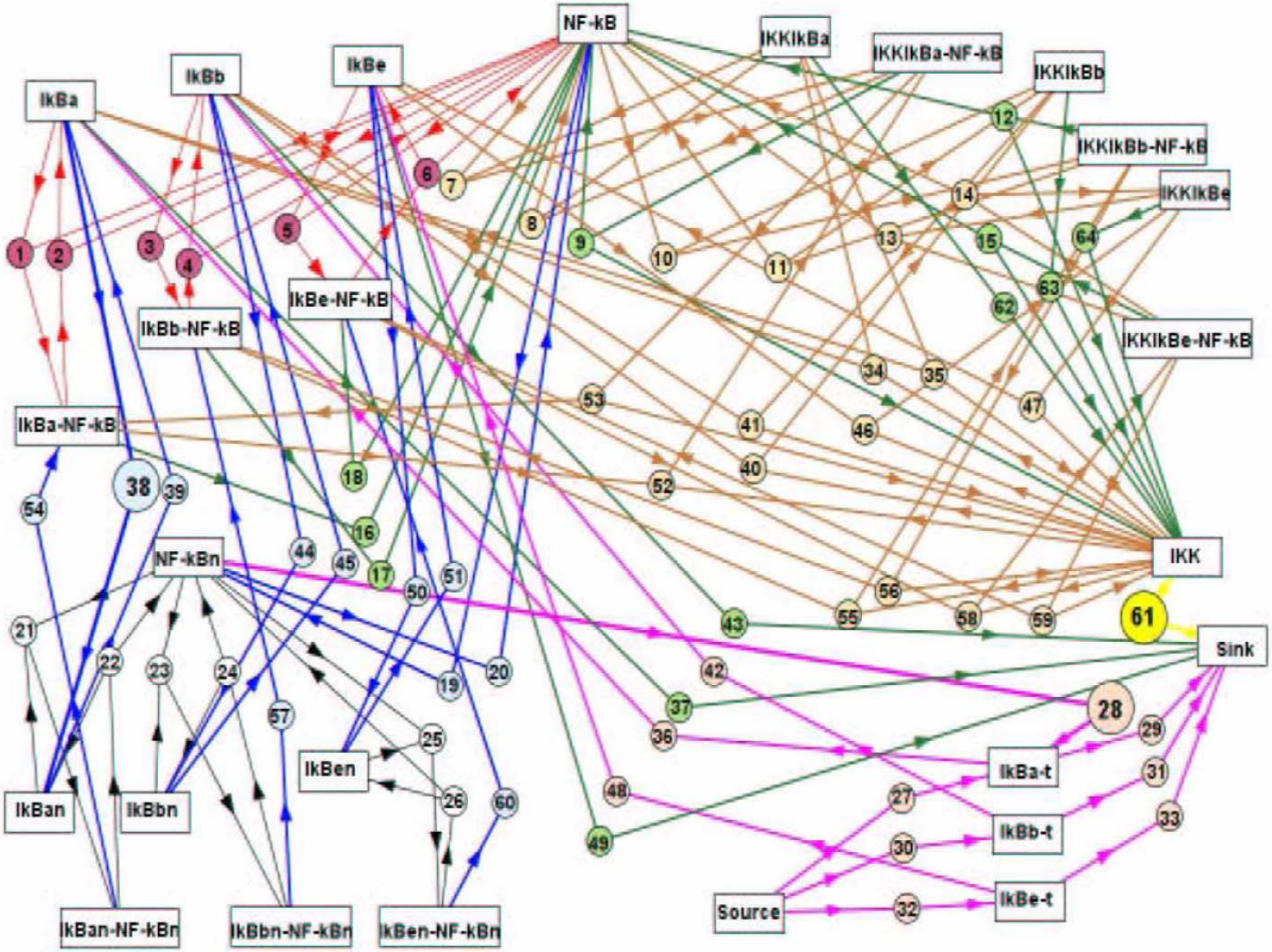
The NF-κB feed-back network



IκBβ/ε knocked out

Hoffmann, Baltimore et al (2002)

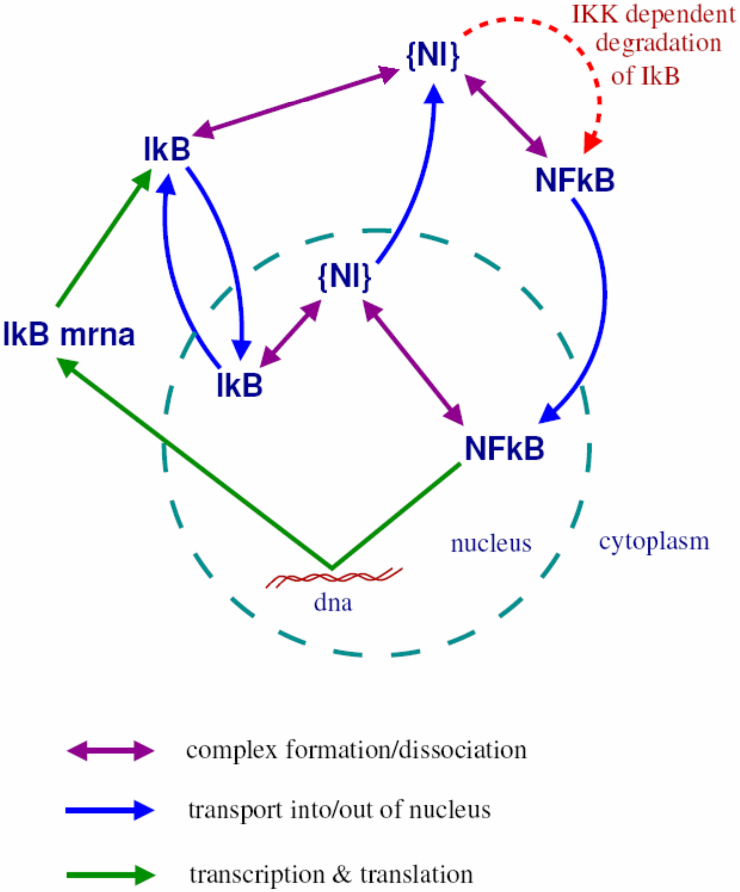
The NF-κB System in Mammalian Cells



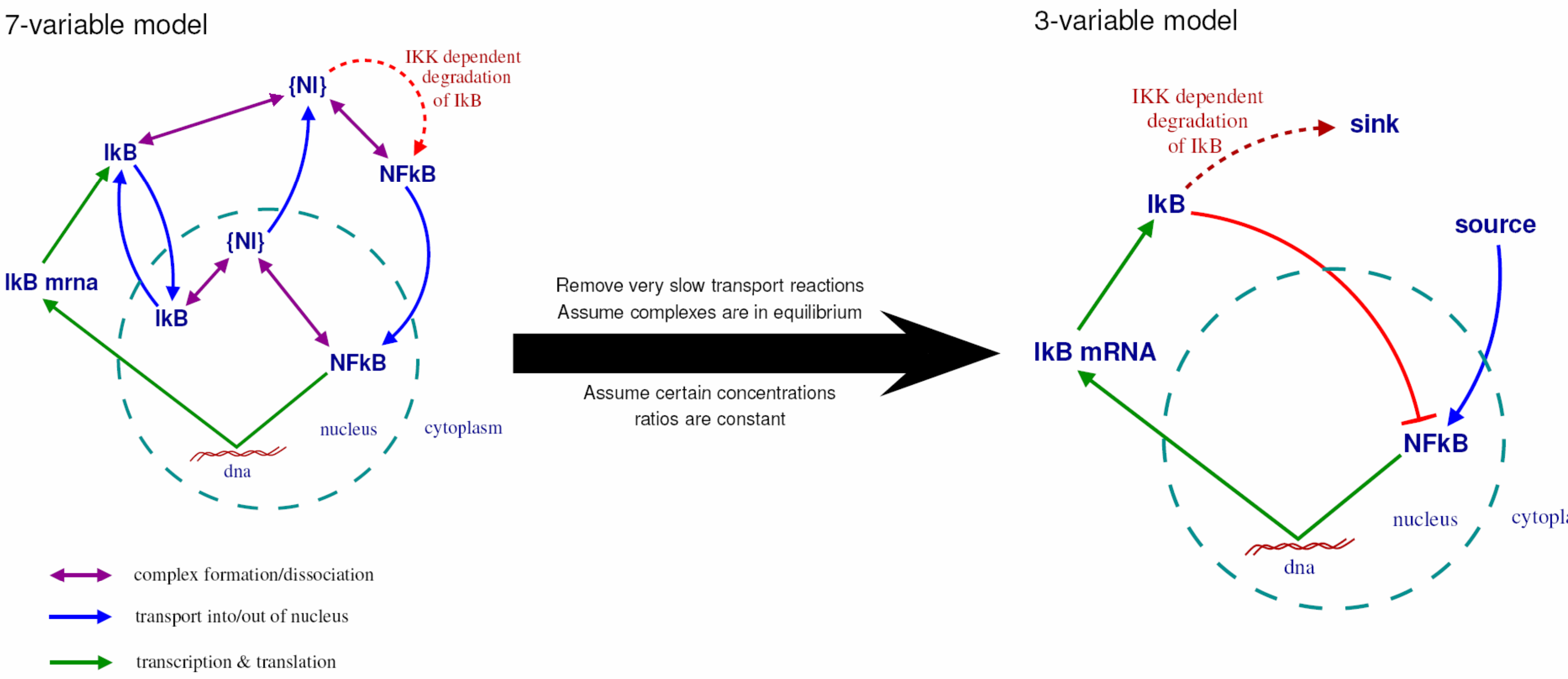
Nelson et al. (2004) Science 306, 704.

Reduction of the NF-κB system

7-variable model



Reduction of the NF-κB system



7-variable model

- complex formation/dissociation
- transport into/out of nucleus
- transcription & translation

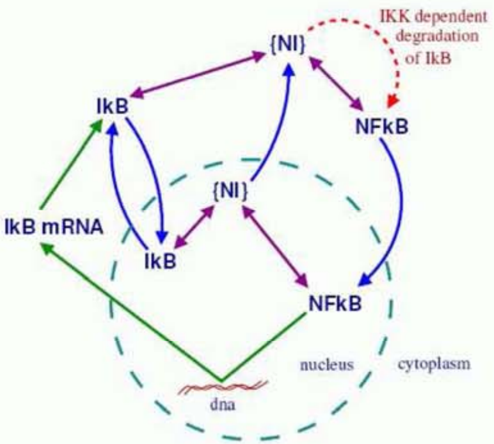
Remove very slow transport reactions
Assume complexes are in equilibrium

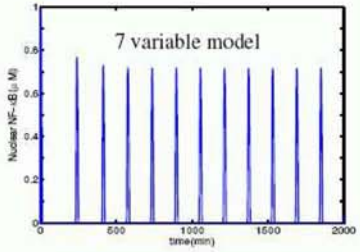
Assume certain concentrations
ratios are constant

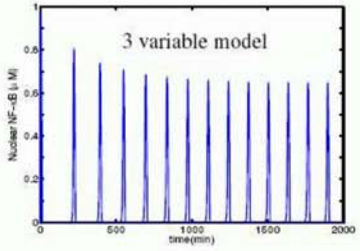
3-variable model

Reduction of the NF-κB System

7 variable model







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

4 variable model

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

Equilibrium of complexes

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

Small terms deleted

$I_n^{tot}N_n/(K_N + N_n)$ 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 $N_n >> K_N$)

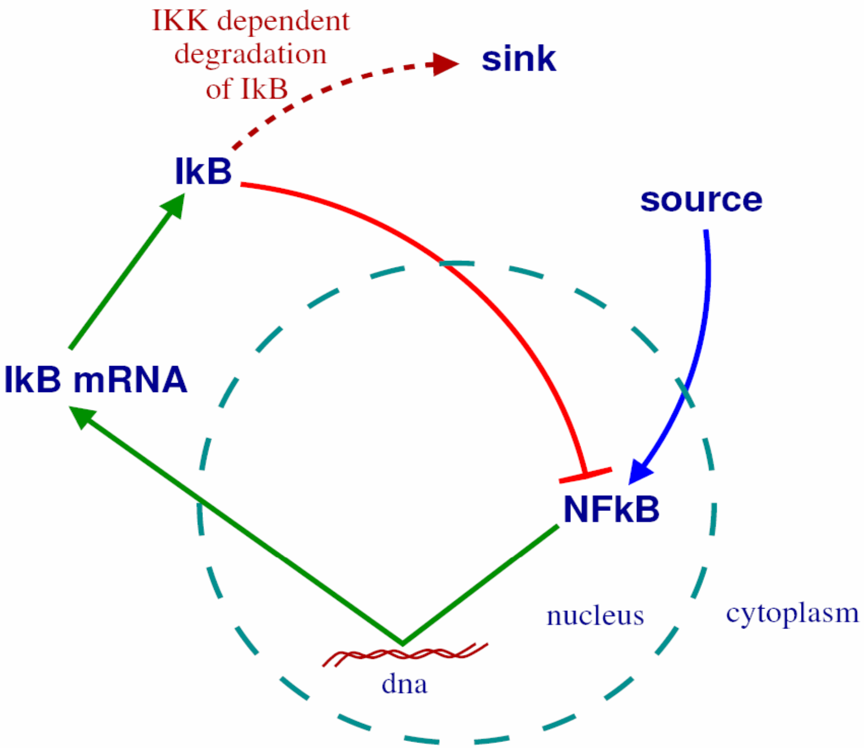
$t \rightarrow (1/\gamma_m)t$
$N_n \rightarrow N_{tot}N_n$
$I_m \rightarrow (k_t N_{tot}^2/\gamma_m)I_m$
$I \rightarrow (k_t k_{tl} N_{tot}^2/\gamma_m^2)I$

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

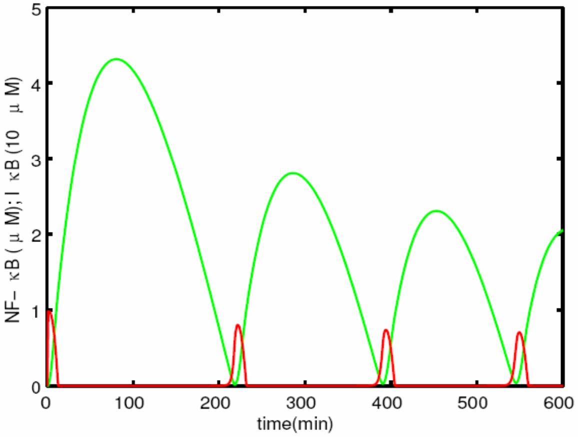
3 variable model

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

Simple Model for Protein Oscillations



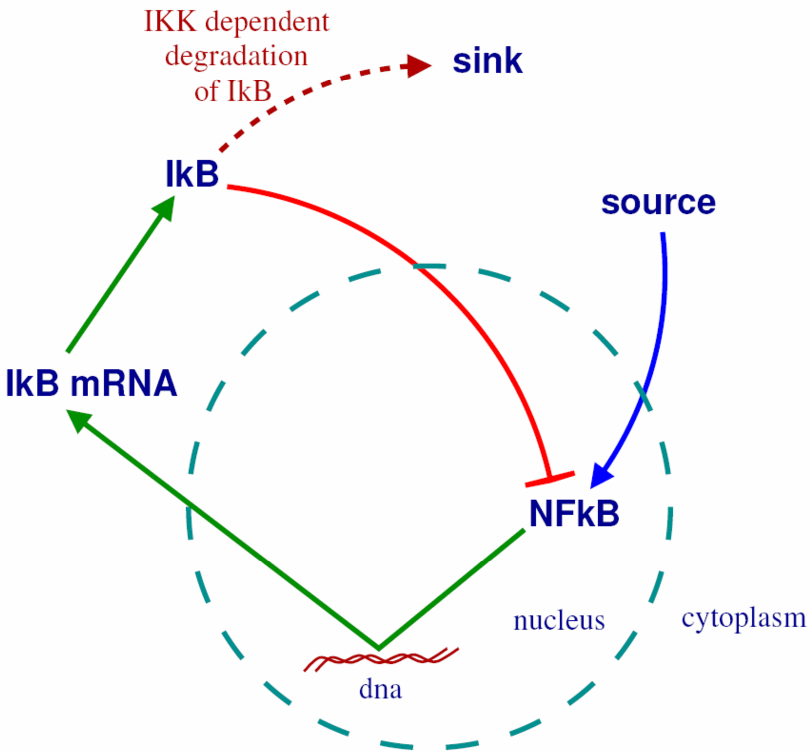
$$
\begin{aligned}
\frac{dN_n}{dt} &= A\frac{(1-N_n)}{\epsilon+I} - B\frac{IN_n}{\delta+N_n},\\
\frac{dI_m}{dt} &= N_n^2 - I_m,\\
\frac{dI}{dt} &= I_m - C\frac{(1-N_n)I}{\epsilon+I}.
\end{aligned}
$$



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

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

Simple Model for Protein Oscillations



IKK dependent degradation of IkB

sink

IkB

source

IkB mRNA

NFkB

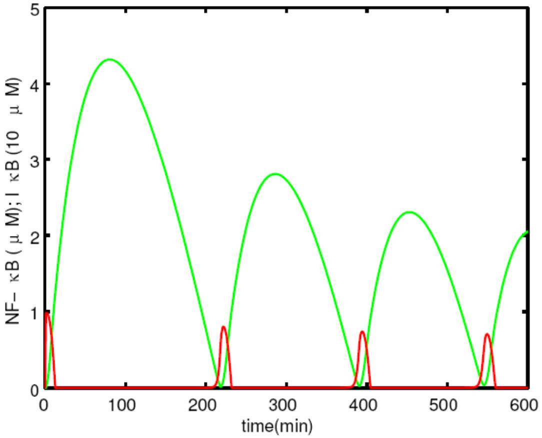
nucleus

cytoplasm

dna

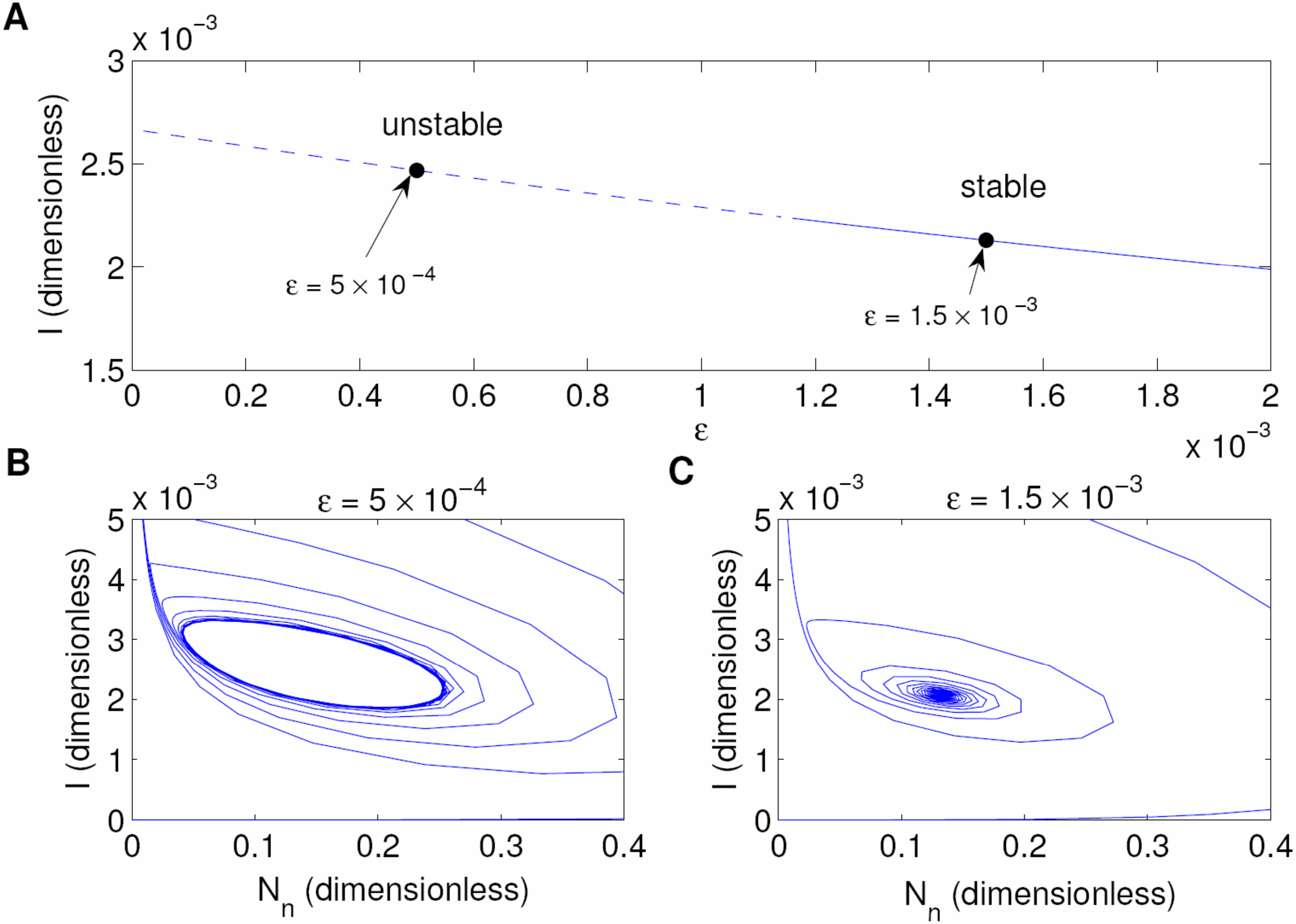
Simple model qualitatively reproduces several features of NF-κB oscillations:

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

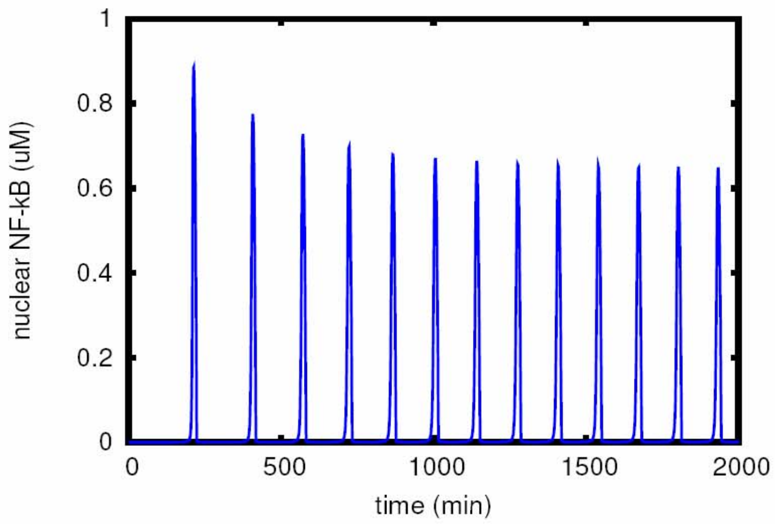


$$A = 0.007,\ B = 954.5,\ C = 0.035,$$
$$\delta = 0.029,\ \epsilon = 2 \times 10^{-5}$$

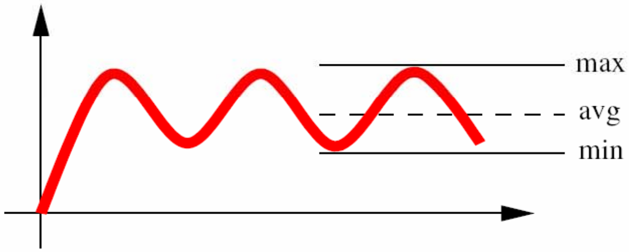
Hopf bifurcation



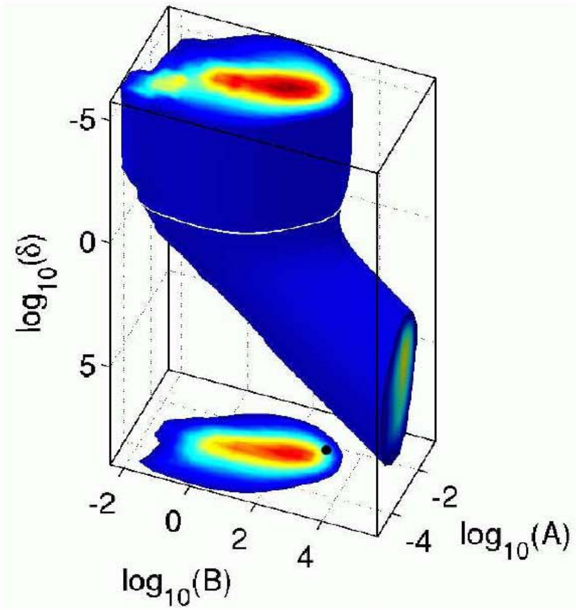
Robust Spiky Oscillations

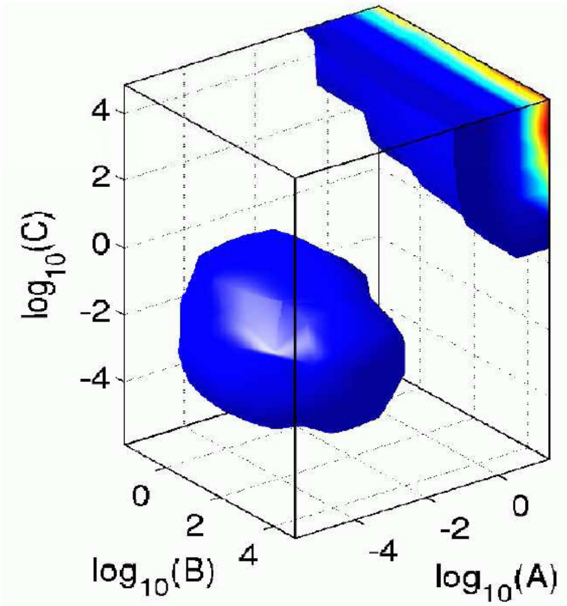


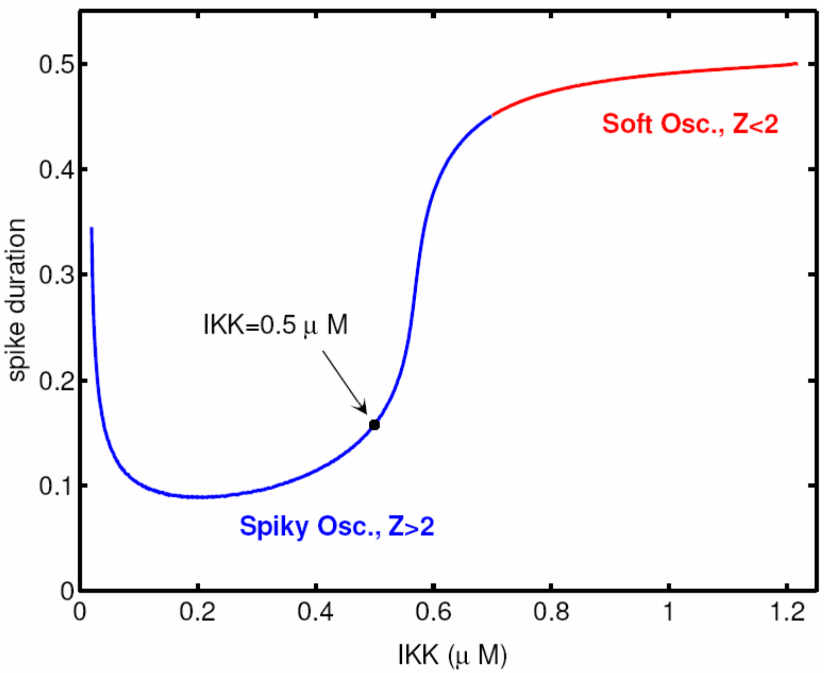
Spiky Oscillations, $Z > 2$

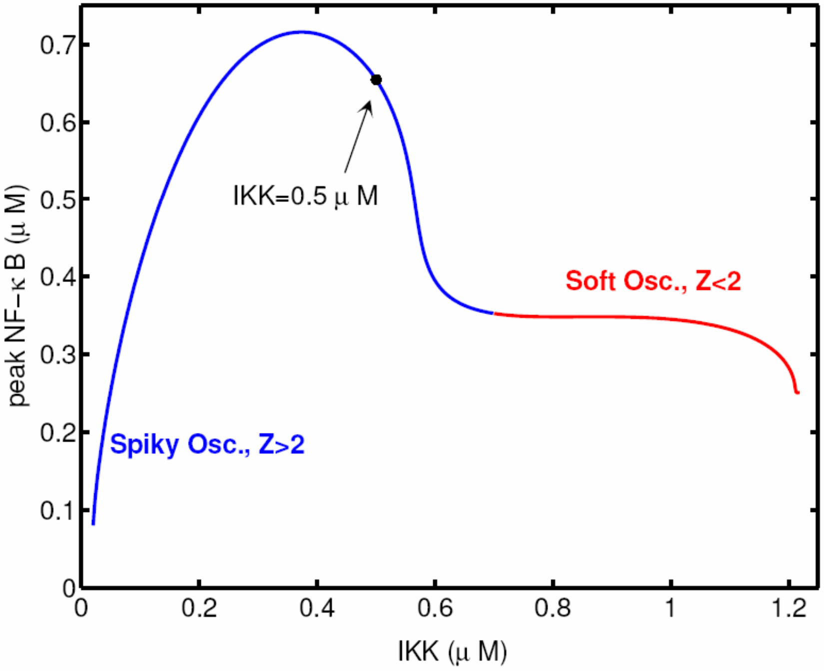


$$Z = \frac{\max - \min}{\text{avg}}$$

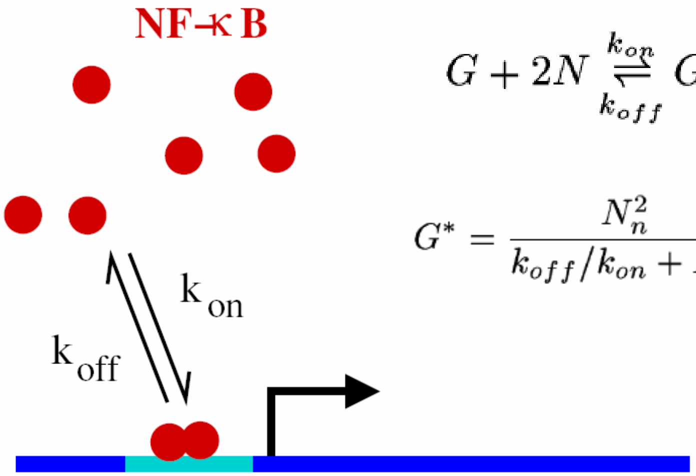





spike duration
IKK (μ M)
0
0.2
0.4
0.6
0.8
1
1.2
0.1
0.2
0.3
0.4
0.5
IKK=0.5 μ M
Soft Osc., Z<2
Spiky Osc., Z>2

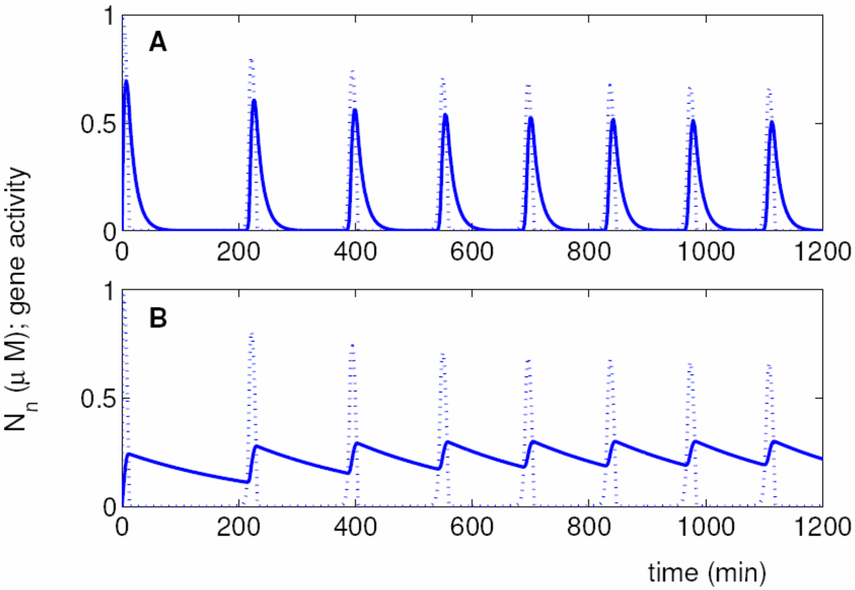

peak NF−κ B (μ M)
IKK (μ M)
0
0.2
0.4
0.6
0.8
1
1.2
0.1
0.2
0.3
0.4
0.5
0.6
0.7
IKK=0.5 μ M
Soft Osc., Z<2
Spiky Osc., Z>2

Coupling NF-κB to a Downstream Gene

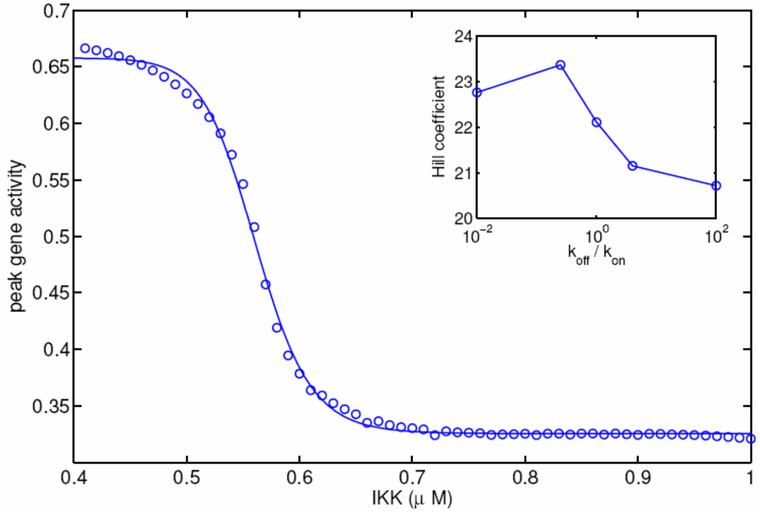


$$G + 2N \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} G^*.$$

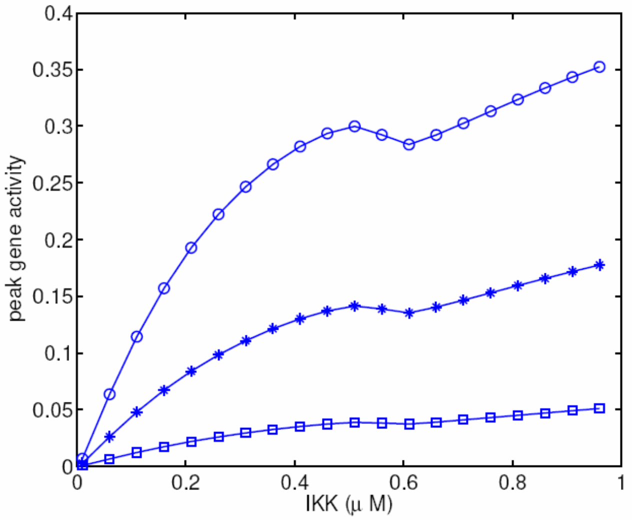
$$G^* = \frac{N_n^2}{k_{off}/k_{on} + N_n^2}.$$



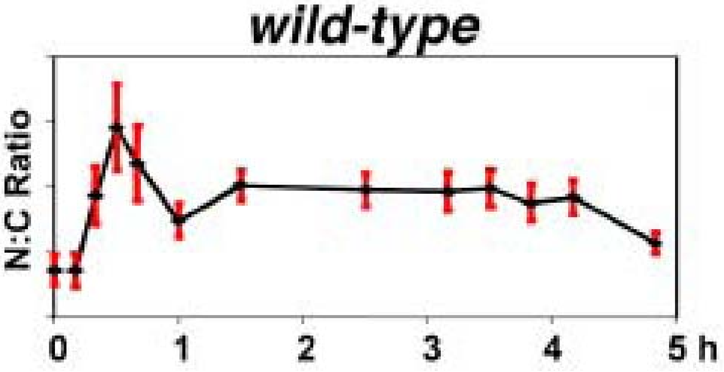
Equilibrium binding, $1/k_{off} \ll$ time period

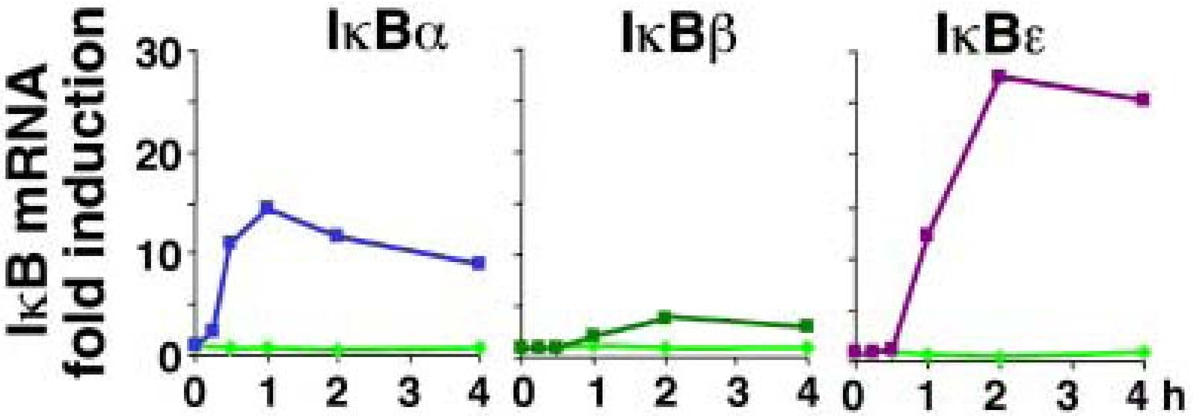


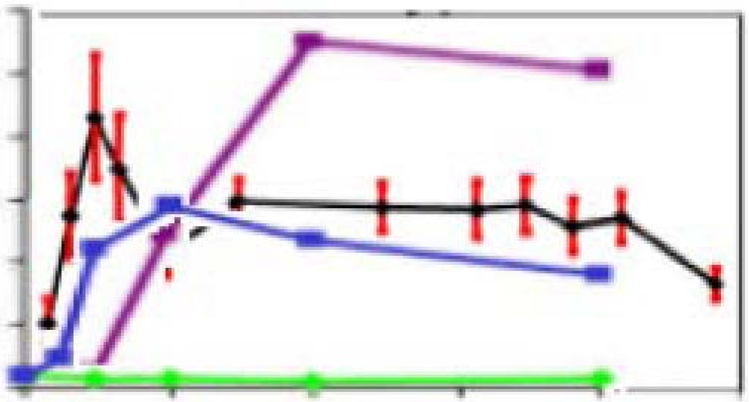
Non-equilibrium binding, $1/k_{off} >$ time period



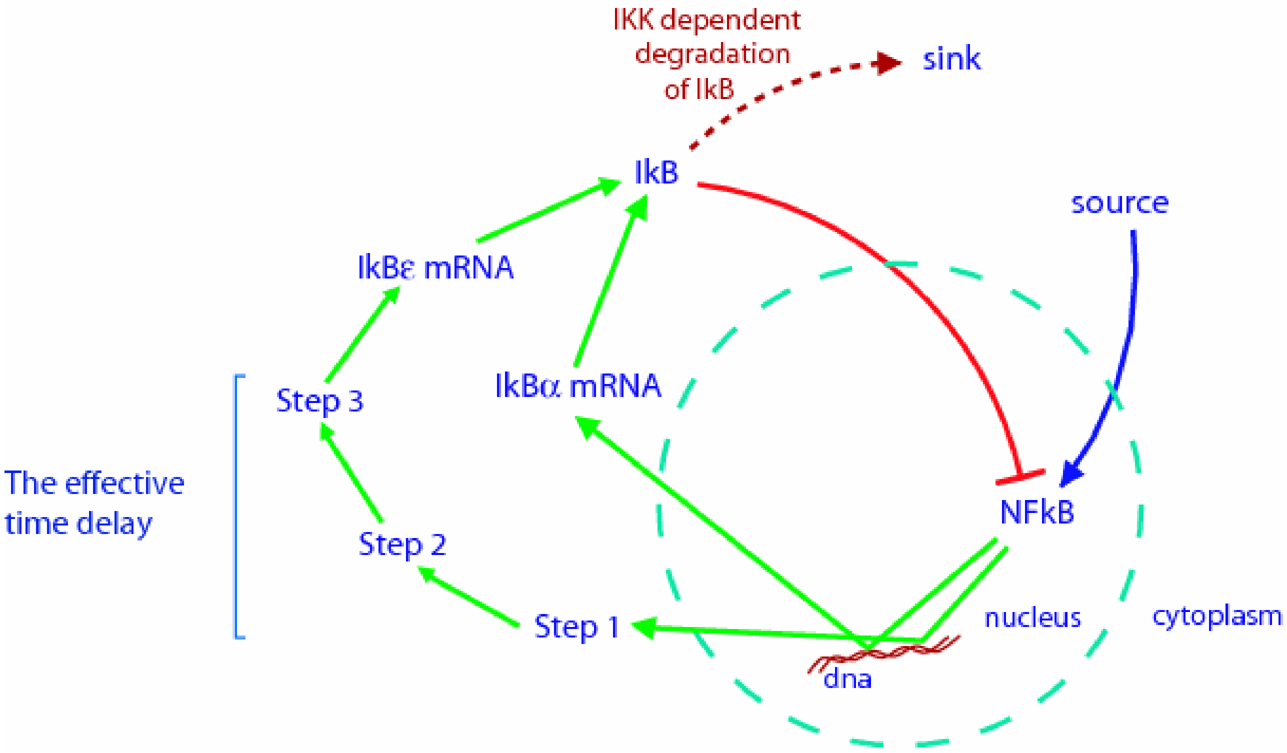
Hoffmann et al
Kearns et al







Improved NF-kB model



Benedicte Mengel, Sandeep Krishna, MHJ

Equations for extended model

$$\frac{dN_n}{dt} = k_{Nin} K_I \frac{(N_{tot} - N_n)}{K_I + I_\alpha + I_\varepsilon} - 0.5 * k_{Iin} \frac{I_\alpha N_n}{K_N + N_n} - k_{Iin} \frac{I_\varepsilon N_n}{K_{N\varepsilon} + N_n}$$

$$\frac{I_{m\alpha}}{dt} = k_t N_n^2 - \gamma_m \cdot I_{m\alpha}$$

$$\frac{dx1}{dt} = k_{te} \cdot N^2 - \gamma_m \cdot x1$$

$$\frac{dx2}{dt} = \gamma_m \cdot x1 - \gamma_m \cdot x2$$

$$\frac{dx3}{dt} = \gamma_m \cdot x2 - \gamma_m \cdot x3$$

$$\frac{I_{m\varepsilon}}{dt} = 10\ x3 - I_{m\varepsilon}$$

$$\frac{I_\alpha}{dt} = ktl\alpha \cdot I_{m_\alpha} - \alpha_\alpha \frac{(N_{tot} - N_n) I_\alpha}{K_I + I_{\alpha + I_\varepsilon}} - 0.005\ I_\alpha$$

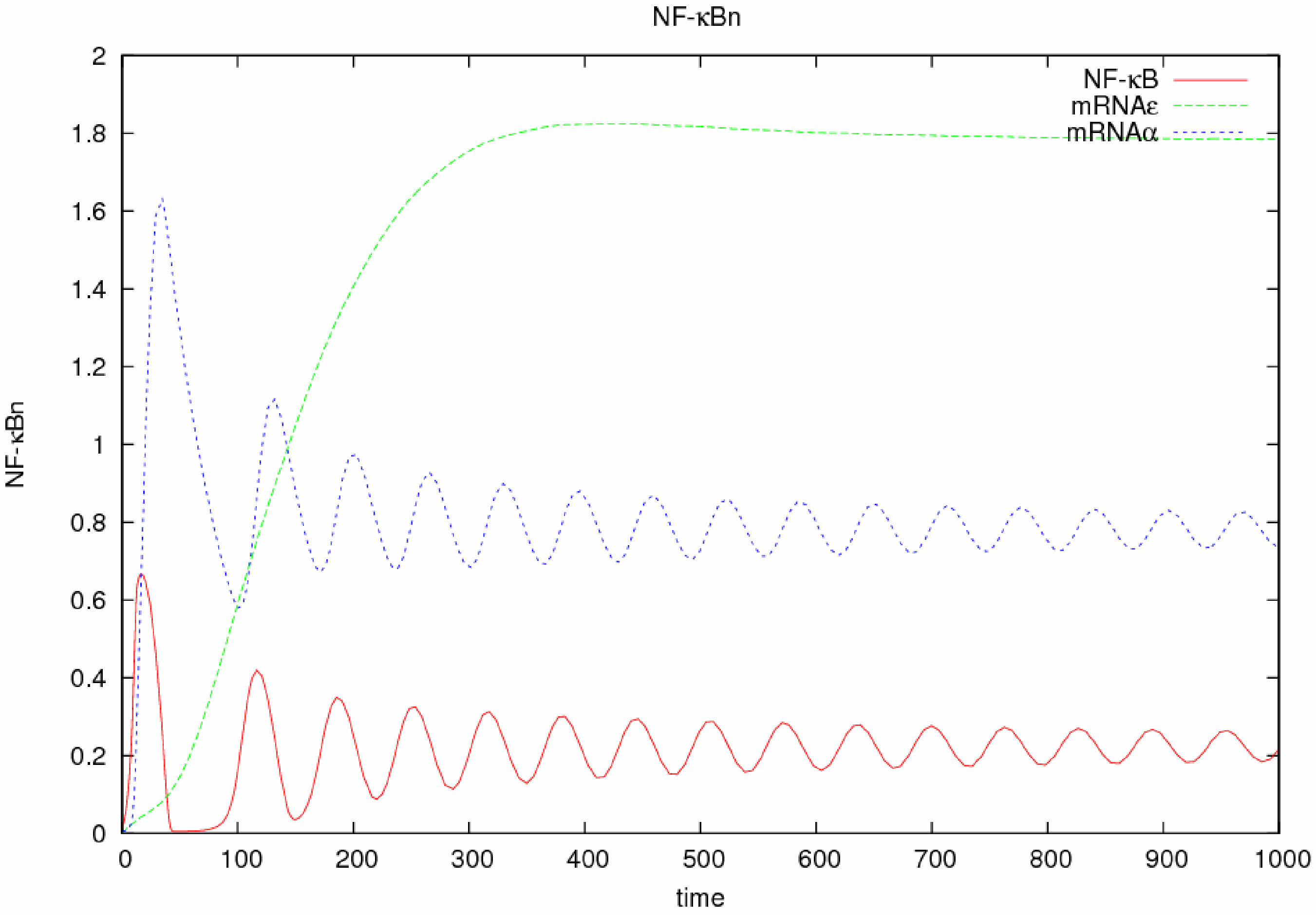
$$\frac{I_\varepsilon}{dt} = 0.05 + ktl\varepsilon \cdot I_{m_\varepsilon} - \alpha_\varepsilon \frac{(N_{tot} - N_n) I_\varepsilon}{K_I + I_{\alpha + I_\varepsilon}} - 0.005\ I_\varepsilon$$

$$N_{tot} = N_c + (NI_\alpha) + (NI_\beta) + (NI_\epsilon)$$

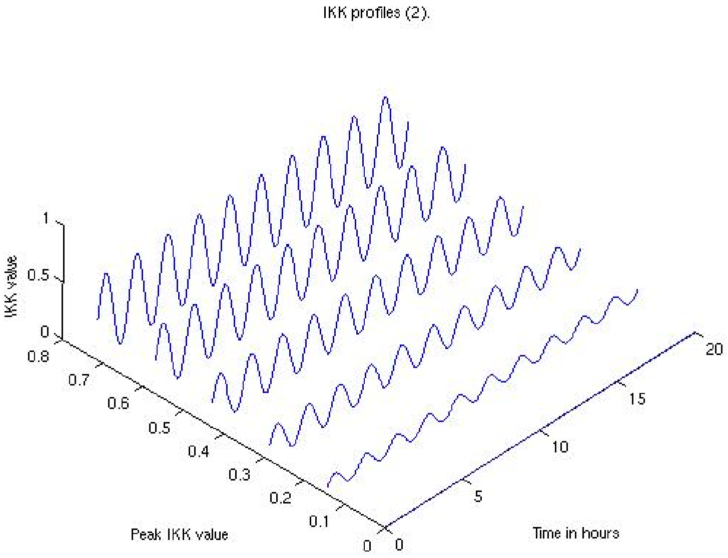
$$K_I = \frac{(k_b + \alpha)}{kf}$$

$$K_N = \frac{(k_{bn} + k_{NIout})}{kfn}$$

WT: delay in Iε

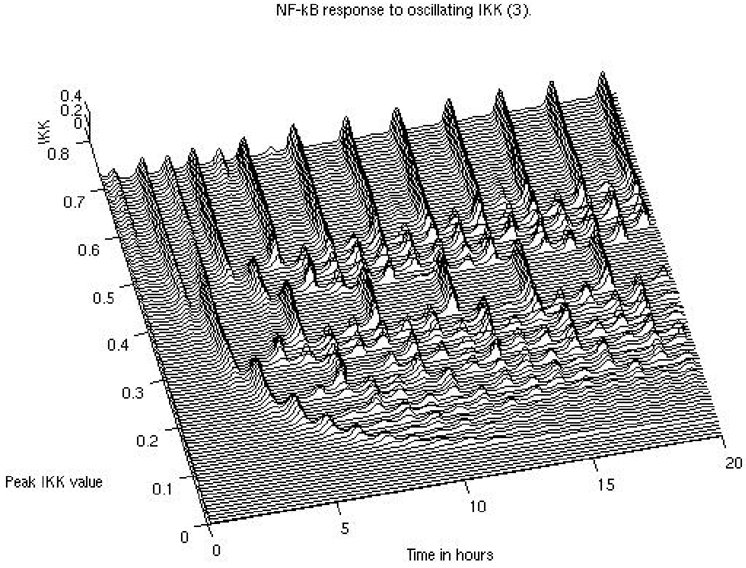


IKK has been observed to oscillate itself

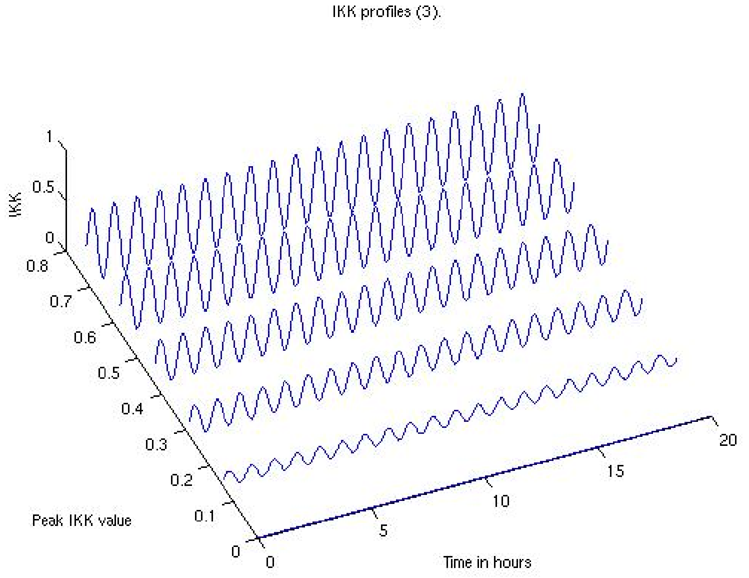


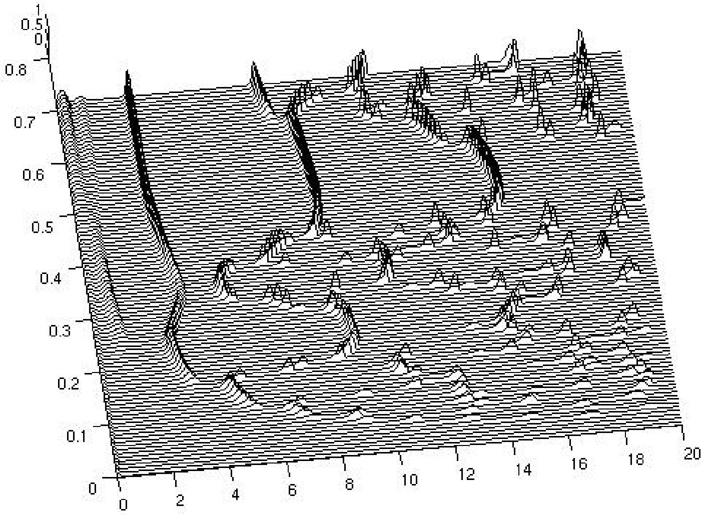
$$
\begin{aligned}
\frac{dN_n}{dt} &= A\frac{(1-N_n)}{\epsilon+I} - B\frac{IN_n}{\delta+N_n}, \\
\frac{dI_m}{dt} &= N_n^2 - I_m, \\
\frac{dI}{dt} &= I_m - C\frac{(1-N_n)I}{\epsilon+I}.
\end{aligned}
$$

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



(Kristian Rud, Jesper Fonslet)



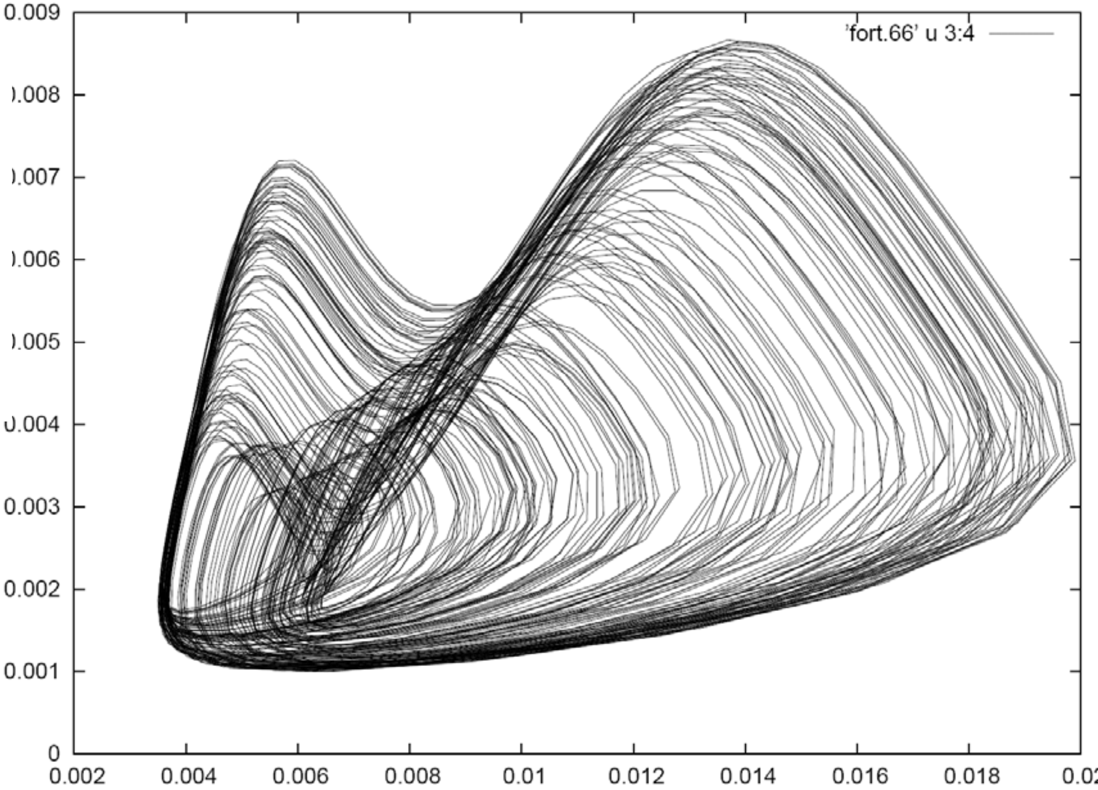


Maybe chaotic dynamics ?

Strange attractor of periodically ‘forced’ NF-κB system

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

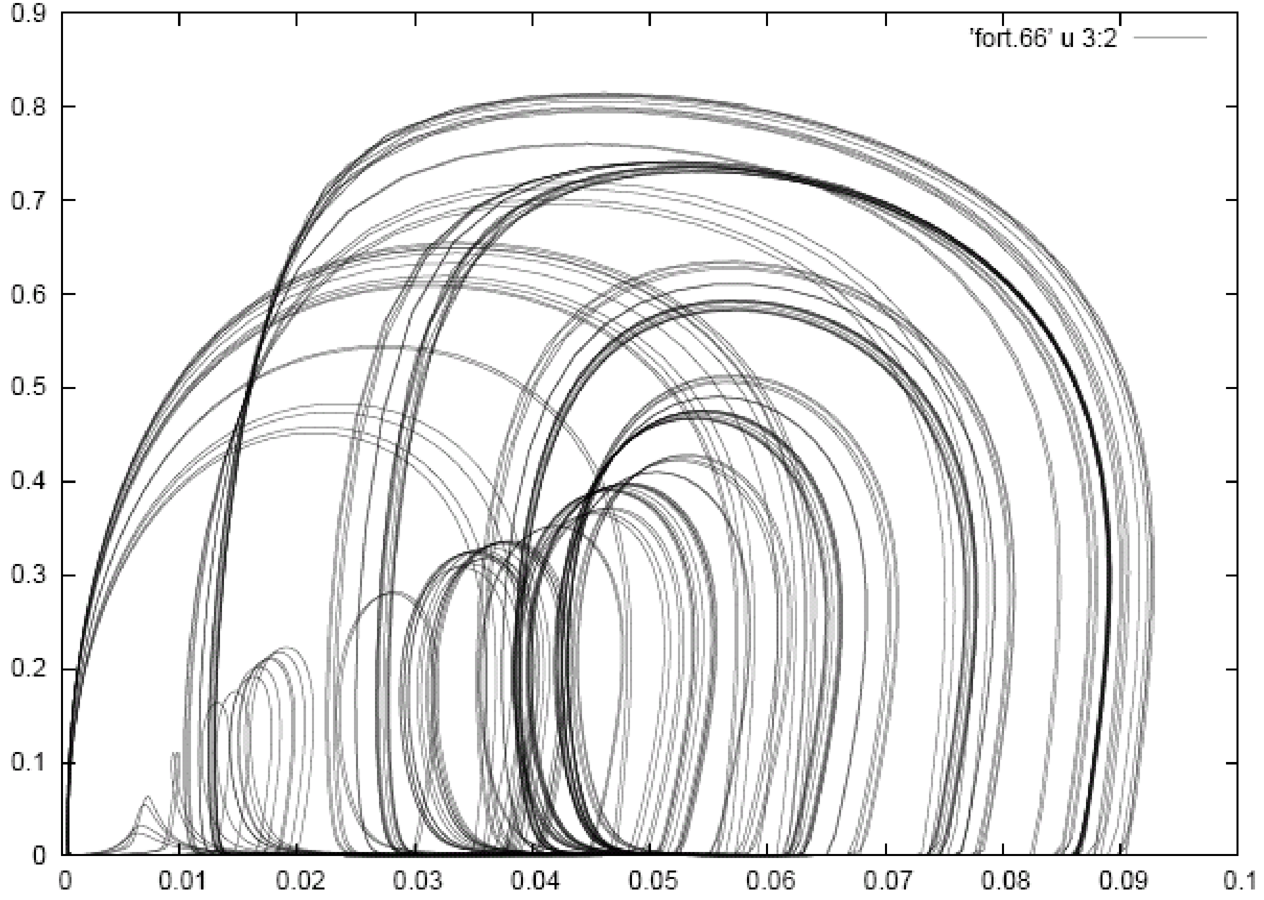
$$C \rightarrow C(1 + sin2\pi\omega t)$$



ω=1

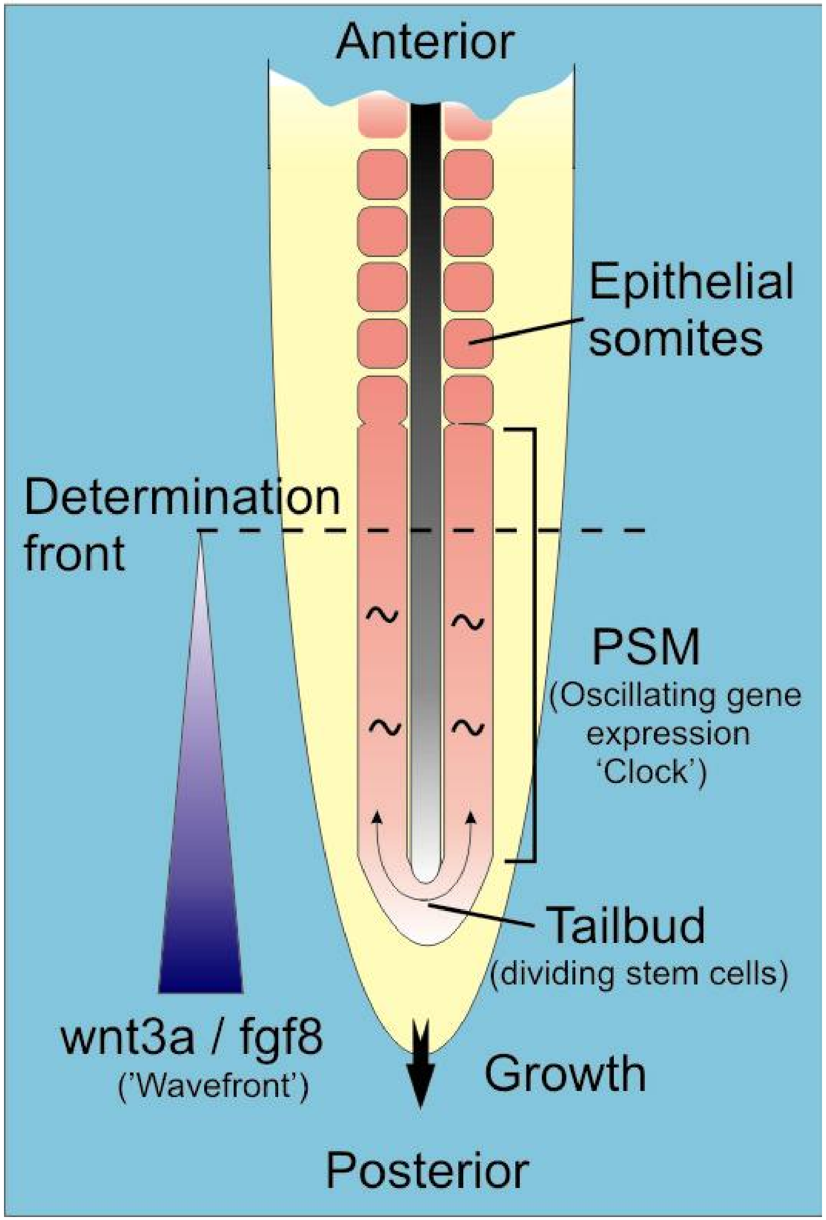
$A = 0.007$, $B = 954.5$, $C = 0.035$, $\delta = 0.029$ and $\epsilon = 2 \times 10^{-5}$

(2-3 hour period)



ω=0.08

(circadian period)



A clock and wavefront

(Cooke and Zeeman 1976)

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

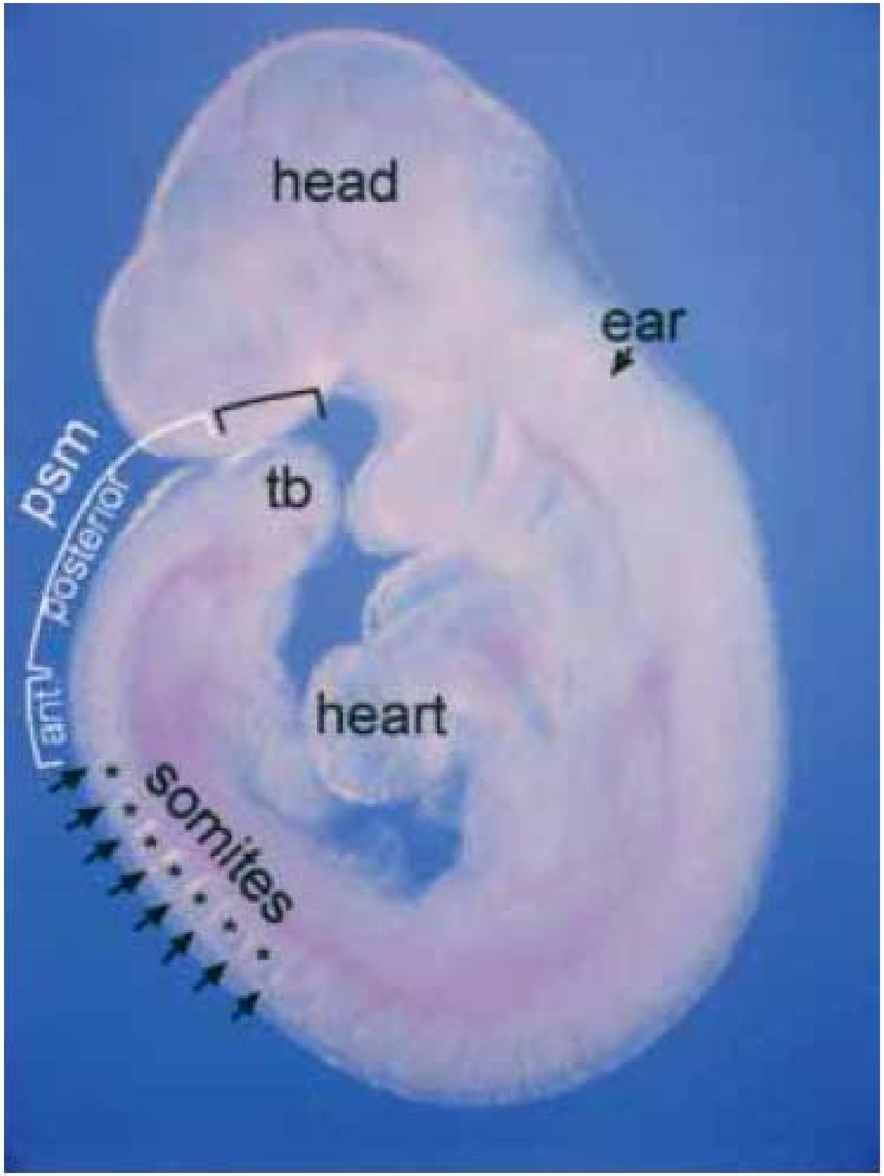
Dividing stem cells in the tailbud supply cells to posterior PSM and elongates the embryo

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

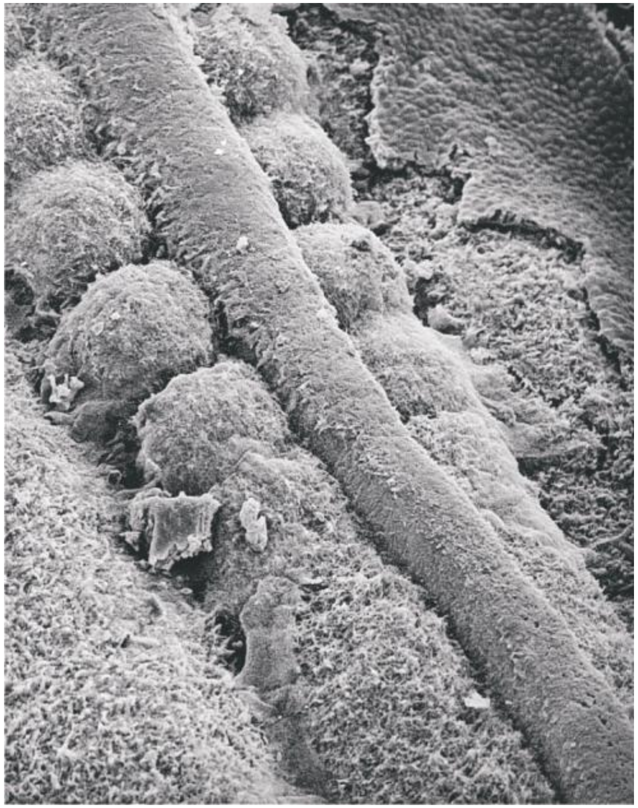
A morphogen gradient (**Wavefront**) determines onset of segmentation program

Clock determines susceptibility to **wavefront**, which ensures groupwise incorporation into somites

Master thesis work by Peter B.Jensen


head
ear
tb
psm
posterior
ant
heart
somites

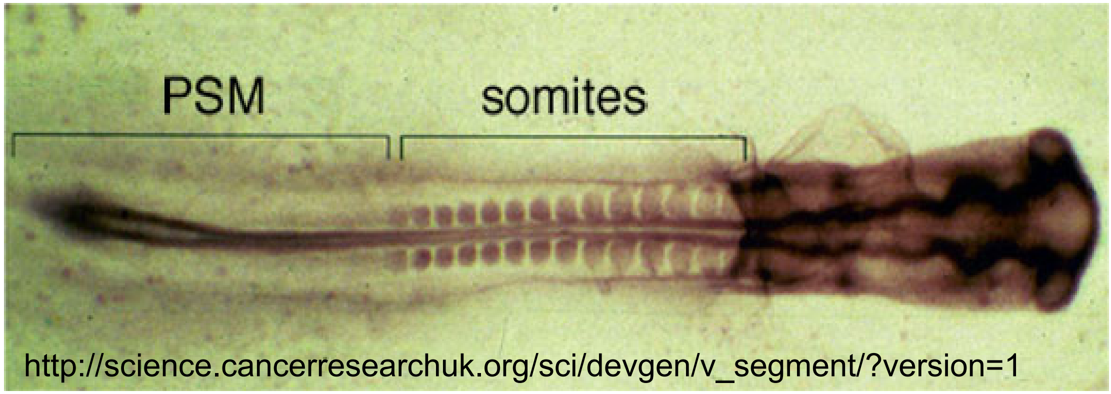
Vertebrate segmentation



The early embryo is segmented into blocks of cells called somites

Somitogenesis is pivotal for the layout of the segmented body plan

The vertebrae of the axial skeleton and associated muscles are derived from the somites



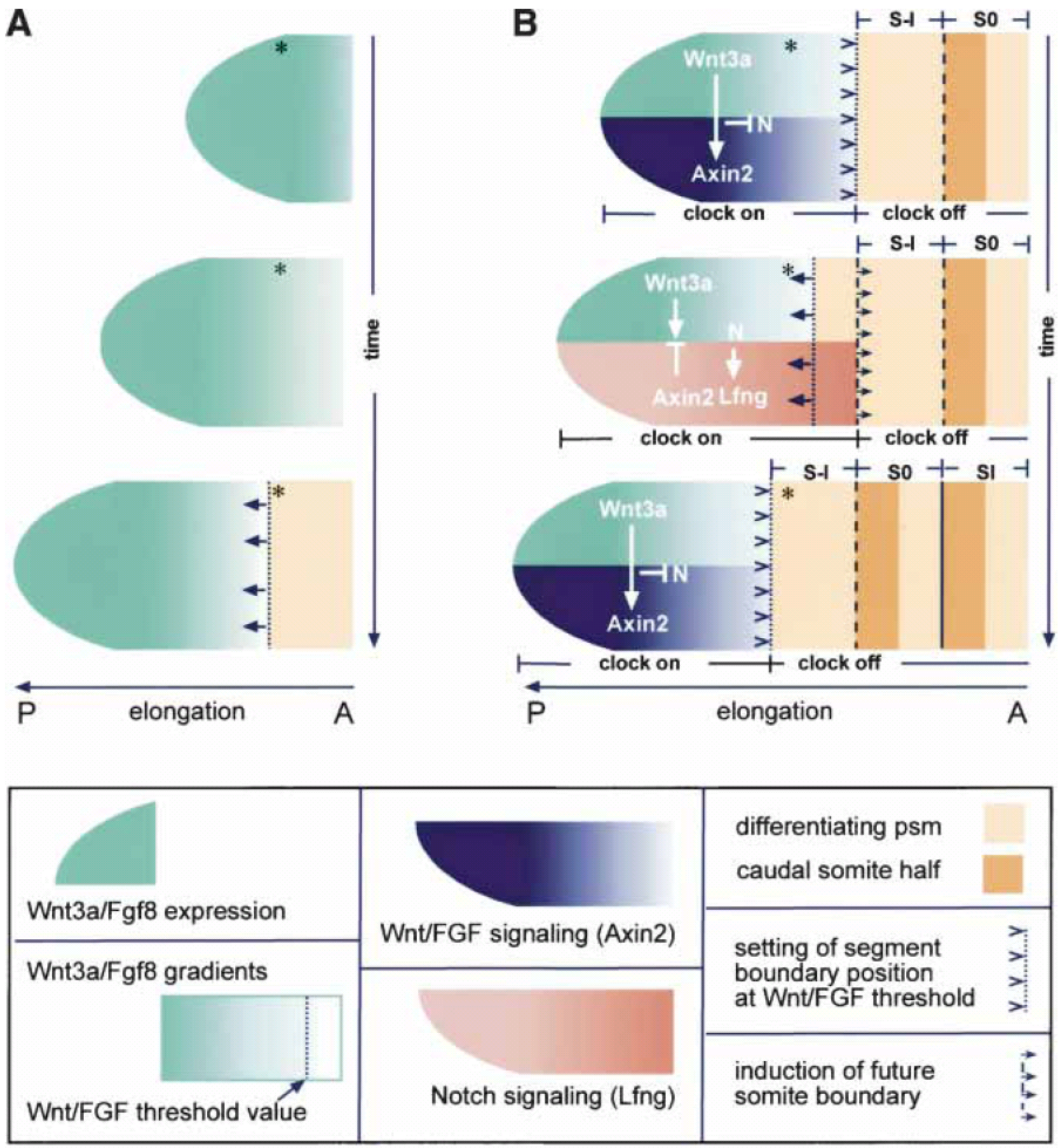
http://science.cancerresearchuk.org/sci/devgen/v_segment/?version=1

The proces relies heavily on the dynamics of molecular oscillators !!!

Two oscillators: Wnt and Notch: out of phase

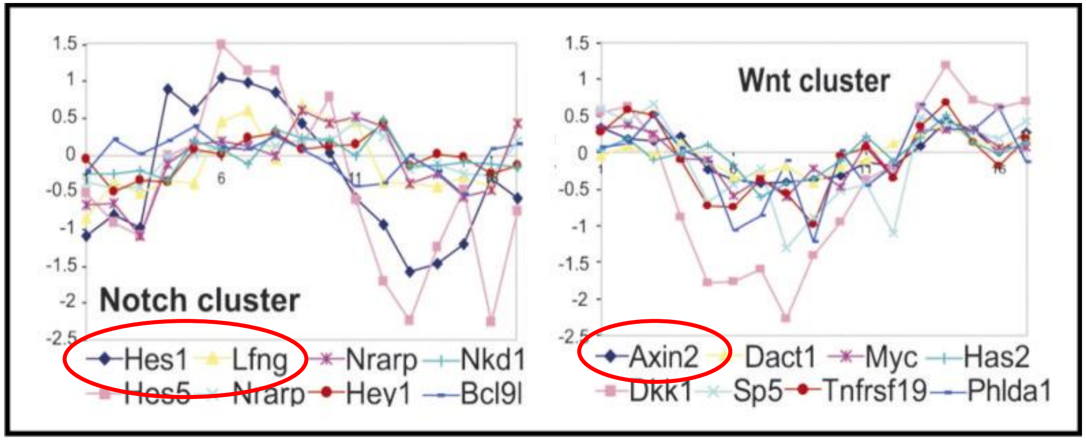
Aulehla and Herrmann

Wnt gradient and
Clock are coupled



Several signaling pathways are involved

Oscillating transcripts are mainly targets of Notch and Wnt pathways



Dequèant et al. 2006

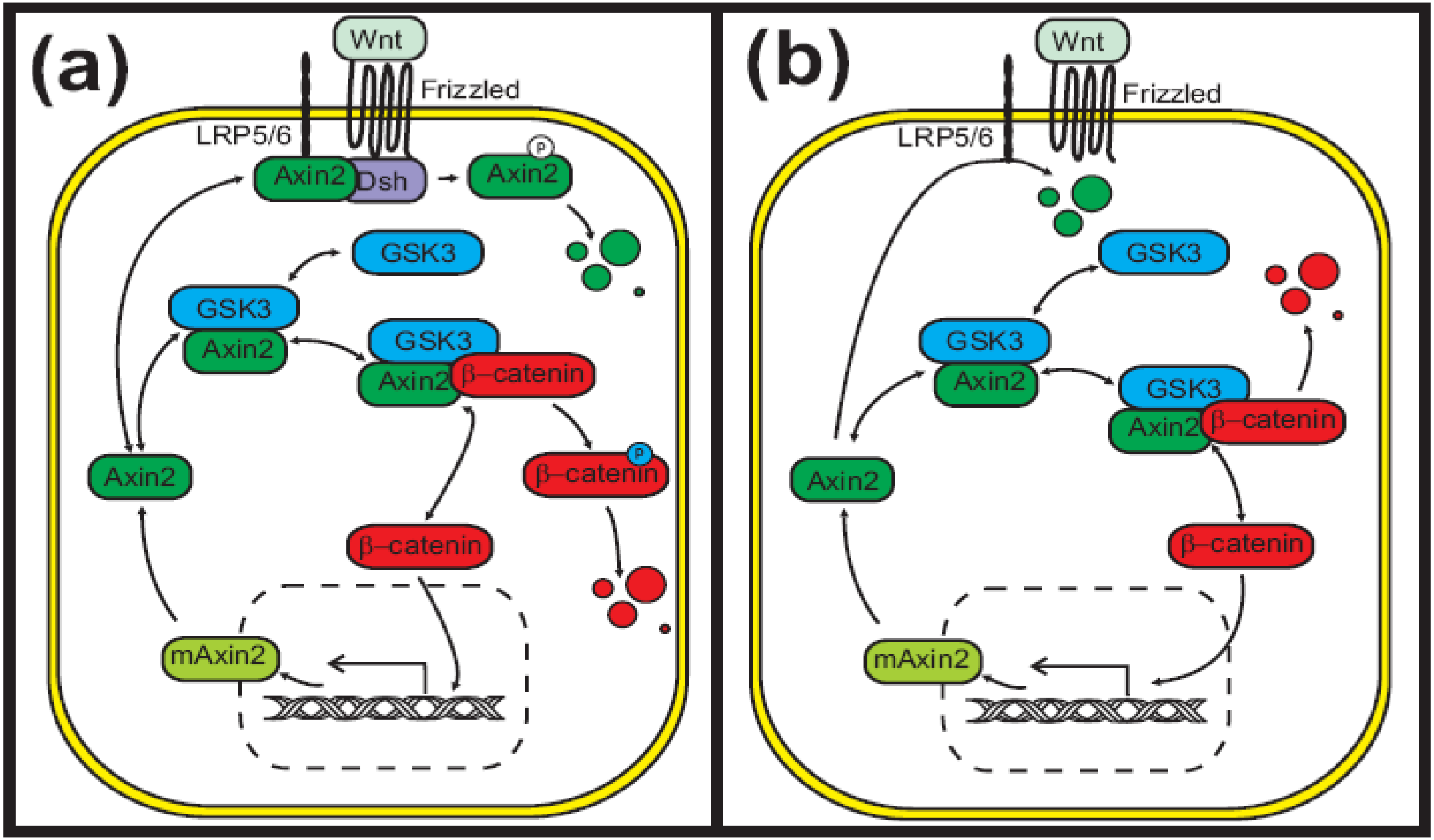
Notch and Wnt targets have same period but are 180 out of phase

→ 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



Equations for the Wnt system

$$\frac{dC}{dt} = c_{fC}B[GA] - c_{bC}C - \alpha C \tag{5.1}$$

$$\frac{d[GA]}{dt} = c_{f[GA]}GA - c_{b[GA]}[GA] - c_{fC}B[GA] + c_{bC}C + \alpha C \tag{5.2}$$

$$\frac{dB}{dt} = S - c_{fC}B[GA] + c_{bC}C \tag{5.3}$$

Unidirectional

$$\frac{dB_p}{dt} = \alpha C - \frac{B_p}{\tau_{B_p}} \tag{5.4}$$

$$\frac{dG}{dt} = -c_{f[GA]}GA + c_{[GA]}[GA] \tag{5.5}$$

$$\frac{dA}{dt} = -c_{f[GA)}GA + c_{b[GA]}[GA] + c_{tlA}A_m - c_{f[AL]}AL + c_{b[AL]}[AL] \tag{5.6}$$

$$\frac{dA_m}{dt} = c_{tsA}B^h - \frac{A_m}{\tau_{Am}} \tag{5.7}$$

Complex equilibrium

$$\frac{d[AL]}{dt} = c_{f[AL]}AL - c_{b[AL]}[AL] - \theta[AL] \tag{5.8}$$

L constant

$$\frac{dL}{dt} = -c_{f[AL]}AL + c_{b[AL]}[AL] + \theta[AL] \tag{5.9}$$

Unidirectional

$$\frac{A_p}{dt} = \theta[AL] - \frac{A_p}{\tau_{A_p}} \tag{5.10}$$

Reduction: only feed-back and equilibrium

$$
\begin{aligned}
\frac{dC}{dt} &= c_{fC}B[GA] - c_{bC}C - \alpha C && (5.15)\\
\frac{d[GA]}{dt} &= c_{f[GA]}GA - c_{b[GA]}[GA] - c_{fC}B[GA] + c_{bC}C + \alpha C && (5.16)\\
\frac{dB}{dt} &= S - c_{fC}B[GA] + c_{bC}C && (5.17)\\
\frac{dG}{dt} &= -c_{f[GA]}GA + c_{b[GA]}[GA] && (5.18)\\
\frac{dA}{dt} &= -c_{f[GA)}GA + c_{b[GA]}[GA] + c_{tlA}A_m - c_A\frac{A}{k_A + A} && (5.19)\\
\frac{dA_m}{dt} &= c_{tsA}B^h - \frac{A_m}{\tau_{Am}} && (5.20)
\end{aligned}
$$

Table 5.1: Rate terms in the 6 eqn. Wnt system

Rate term	Process
$c_{fc}B[GA]$	Binding of B to [GA] to form destruction complex C
$c_{bC}C$	Breaking of C into B and [GA]
αC	Breaking of C due to destruction of β-catenin
$c_{f[GA]}GA$	Binding of G to A to form the subcomplex [GA]
$c_{b[GA]}[GA]$	Breaking of [GA] into G and A
S	Constant source of β-catenin
$c_{tlA}A_m$	Translation of Axin2 protein
$c_A\frac{A}{k_A+A}$	Saturated degradation of Axin2 protein
$c_{tsA}B^h$	Transcription of Axin2 mRNA
$\frac{A_m}{\tau_{Am}}$	Standard degradation of Axin2 mRNA

Values of parameters: the Wnt reference state

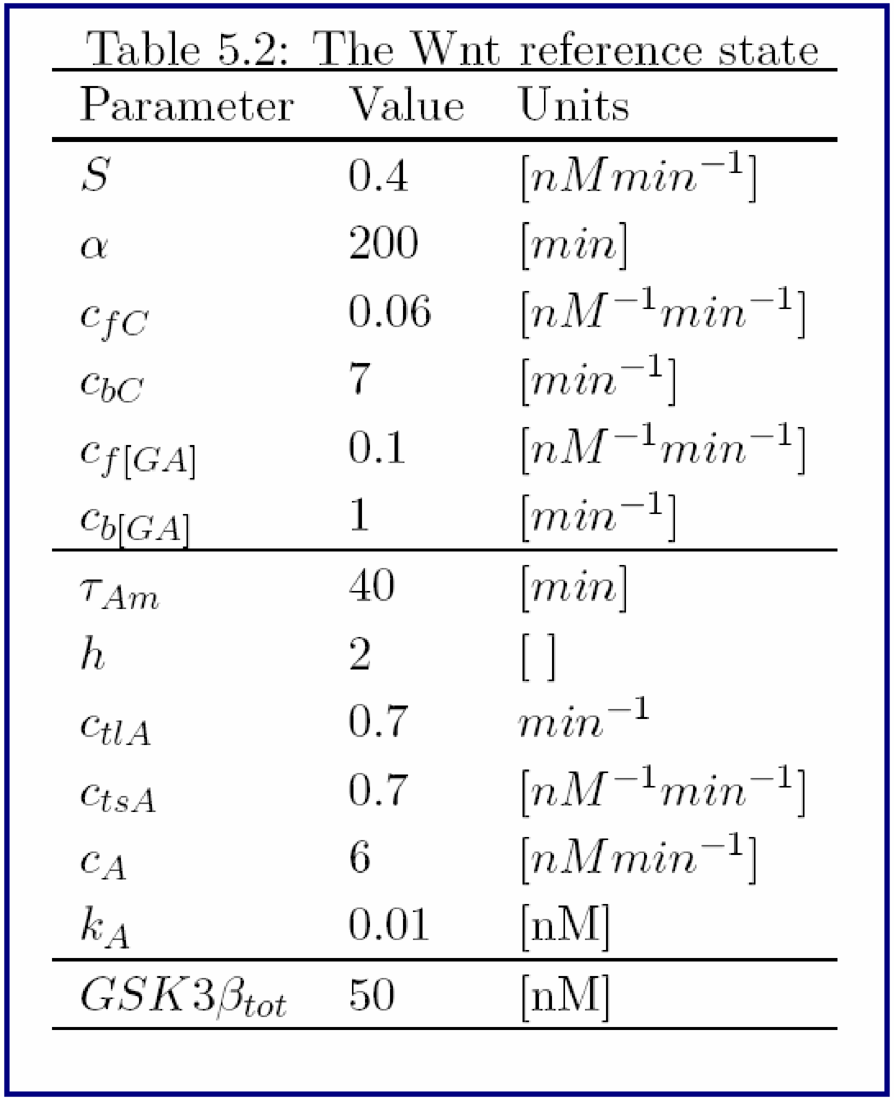


Table 5.2: The Wnt reference state

Parameter	Value	Units
S	0.4	$[nM min^{-1}]$
α	200	$[min]$
c_{fC}	0.06	$[nM^{-1} min^{-1}]$
c_{bC}	7	$[min^{-1}]$
$c_{f[GA]}$	0.1	$[nM^{-1} min^{-1}]$
$c_{b[GA]}$	1	$[min^{-1}]$
τ_{Am}	40	$[min]$
h	2	[ ]
c_{tlA}	0.7	min^{-1}
c_{tsA}	0.7	$[nM^{-1} min^{-1}]$
c_A	6	$[nM min^{-1}]$
k_A	0.01	[nM]
$GSK3\beta_{tot}$	50	[nM]

The six first parameter values are from Lee[44]. From the values of $D_{[GA]}$ and D_C given in [44] the values of c_{fC},c_{bC},$c_{f[GA]}$ and $c_{b[GA]}$ were estimated.

The Wnt reference state

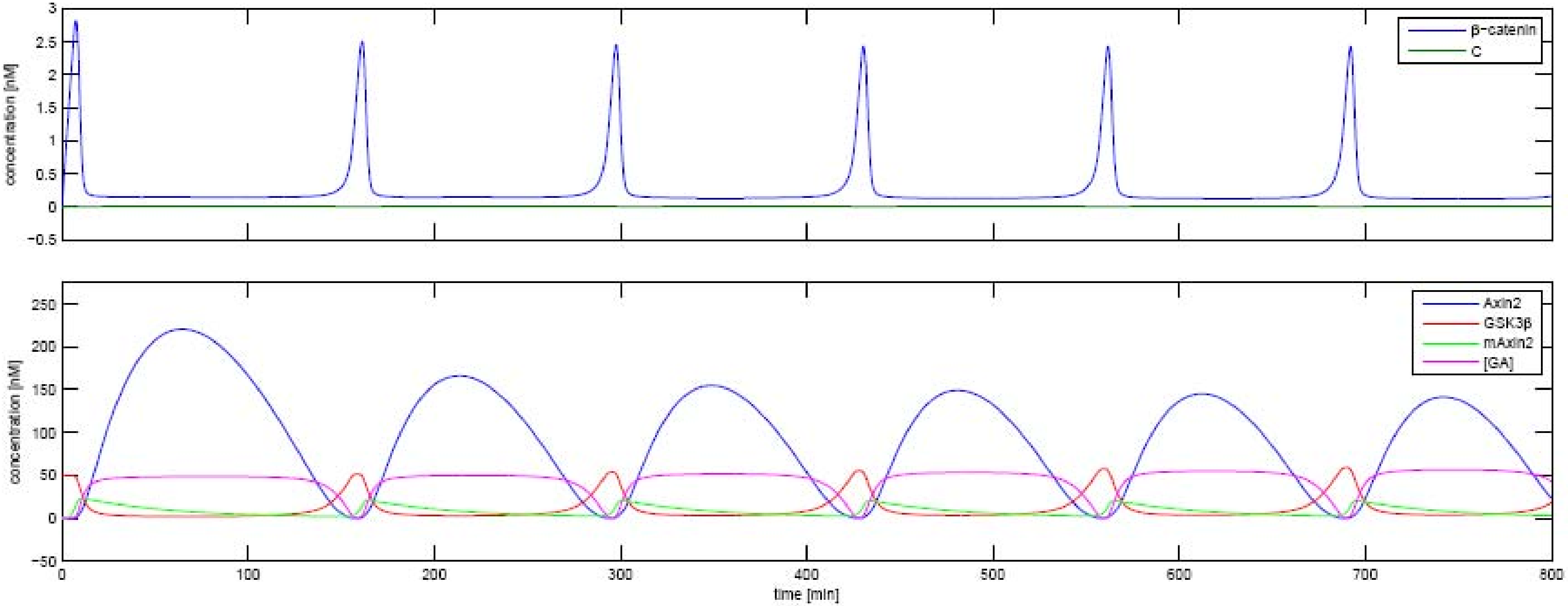
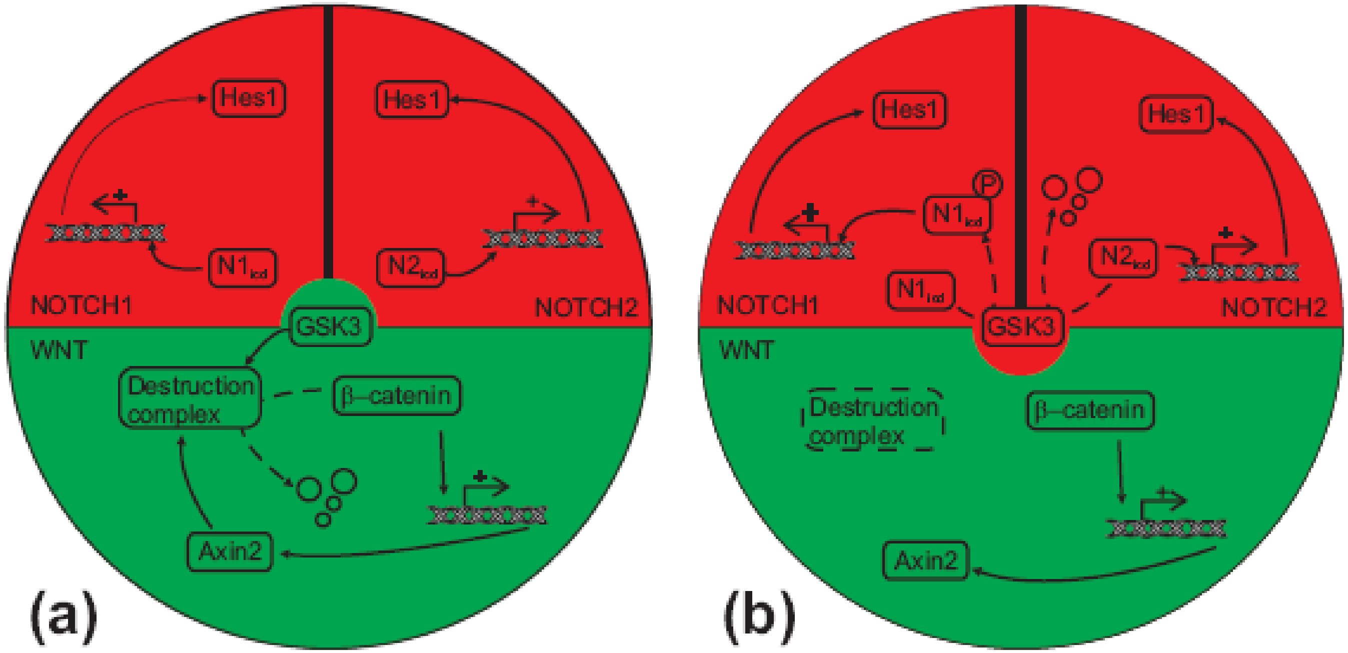


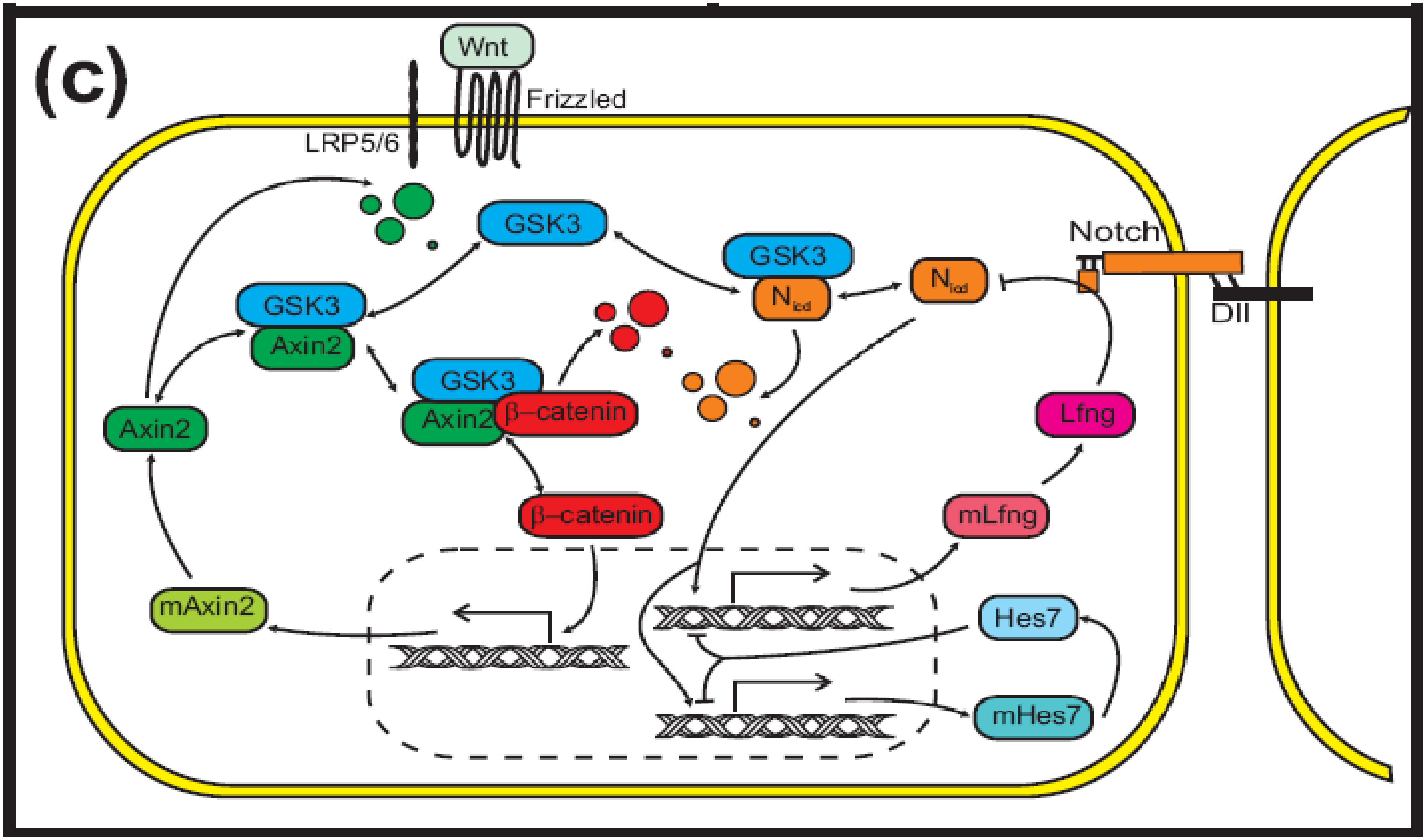
Figure 6.1: Oscillations in the Wnt reference state

The cross-talk between Wnt and Notch:

GSK3 is the mediator



The full systems with Wnt and Noctch and cross-talk



The Notch equations

$$
\begin{aligned}
\frac{dN}{dt} &= S_N \frac{k_s}{k_s + L} - \frac{N}{\tau_N} \\
\frac{dHm}{dt} &= c_{tsH} \left(\frac{N^{h_N}}{k_N + N^{h_N}} \cdot \frac{k_H}{k_H + H^{h_H}} \right) - \frac{H}{\tau_{Hm}} \\
\frac{dH}{dt} &= c_{tlH} H_m - \frac{H}{\tau_H} \\
\frac{dLm}{dt} &= c_{tsL} \left(\frac{N^{h_N}}{k_N + N^{h_N}} \cdot \frac{k_H}{k_H + H^{h_H}} \right) - \frac{L_m}{\tau_{Lm}} \\
\frac{dL}{dt} &= c_{tlL} L_m - \frac{L}{\tau_L}
\end{aligned}
$$

Equations of the Wnt-Notch network

Wnt

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

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

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

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

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

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

Notch

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

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

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

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

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

(Cross talk)

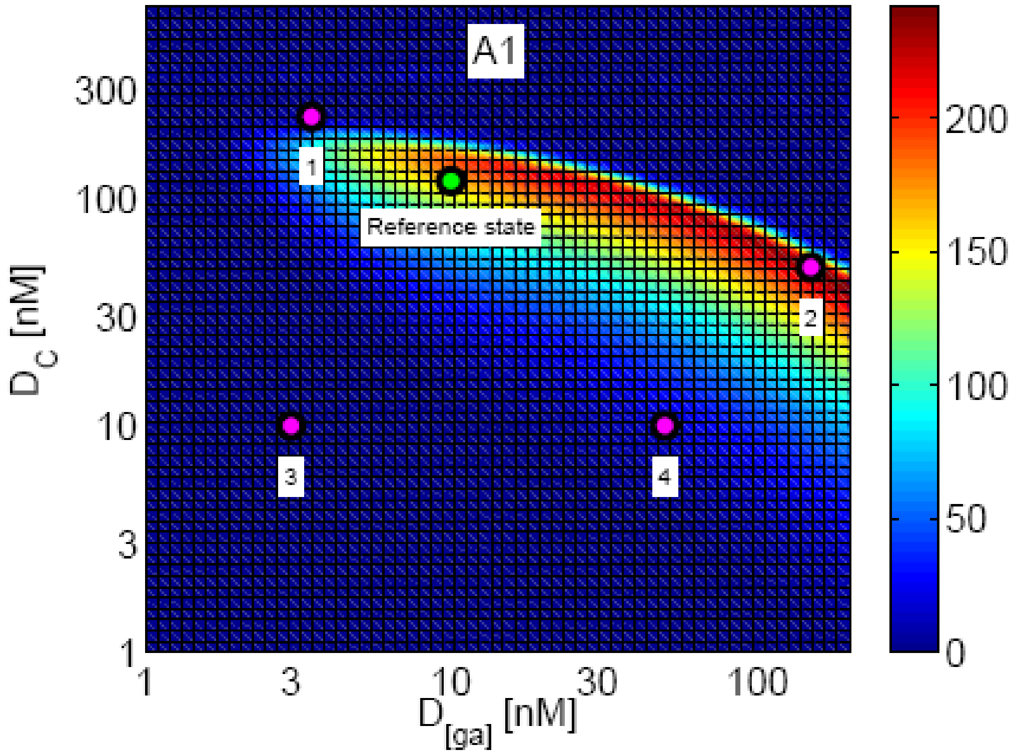
$$\frac{d[NG]}{dt} = c_{f[NG]}NG - c_{b[NG]}[NG] - \alpha_2[NG] \qquad (12)$$

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

Master thesis work by Peter B. Jensen

Exploring parameter space (only Wnt)

Oscillations amplitude
In Axin2



Dissociation constants: $D_C = \frac{c_{bC}}{c_{fC}}$ and $D_{[GA]} = \frac{c_{b[GA]}}{c_{f[GA]}}$

Four different parameter sets:

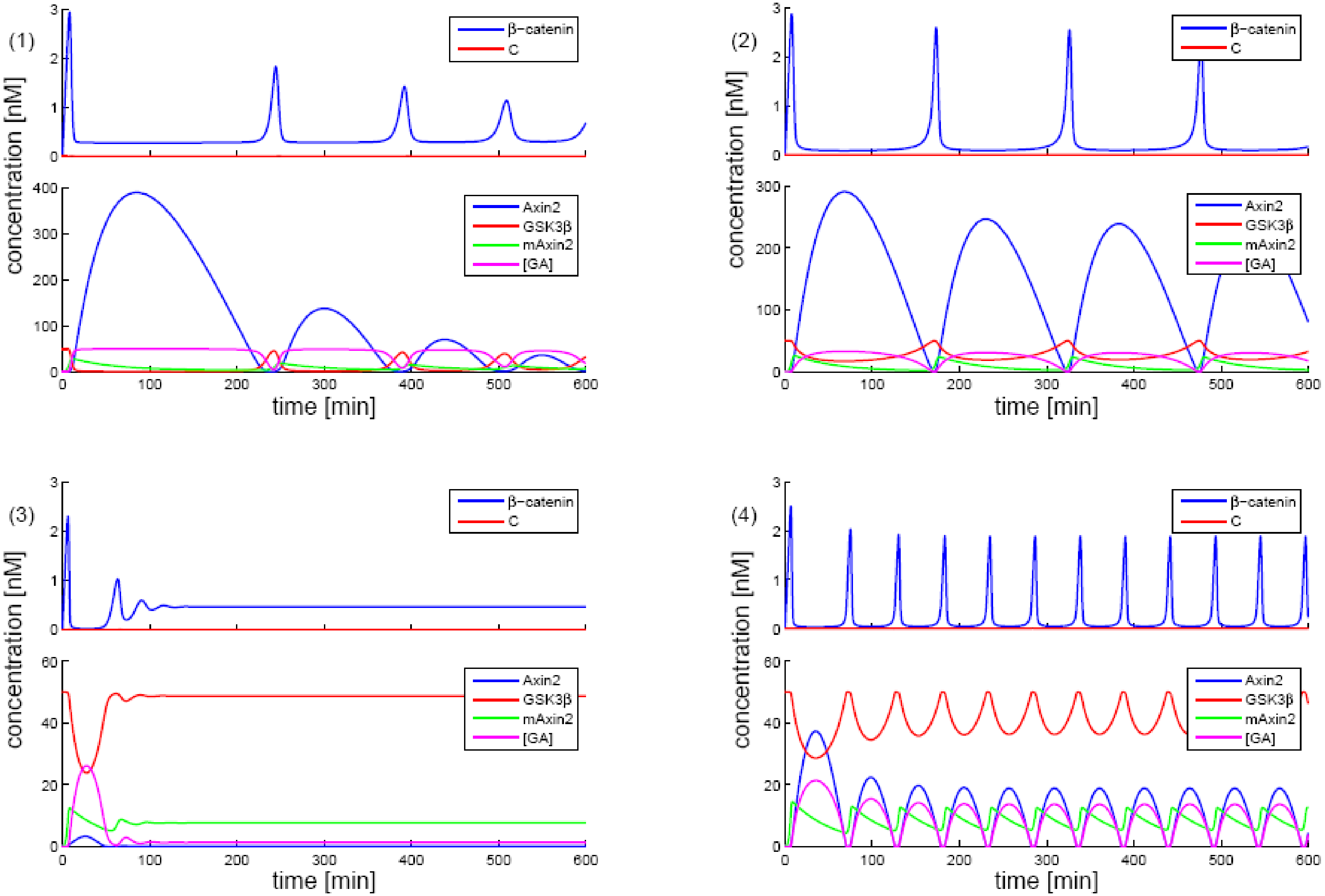
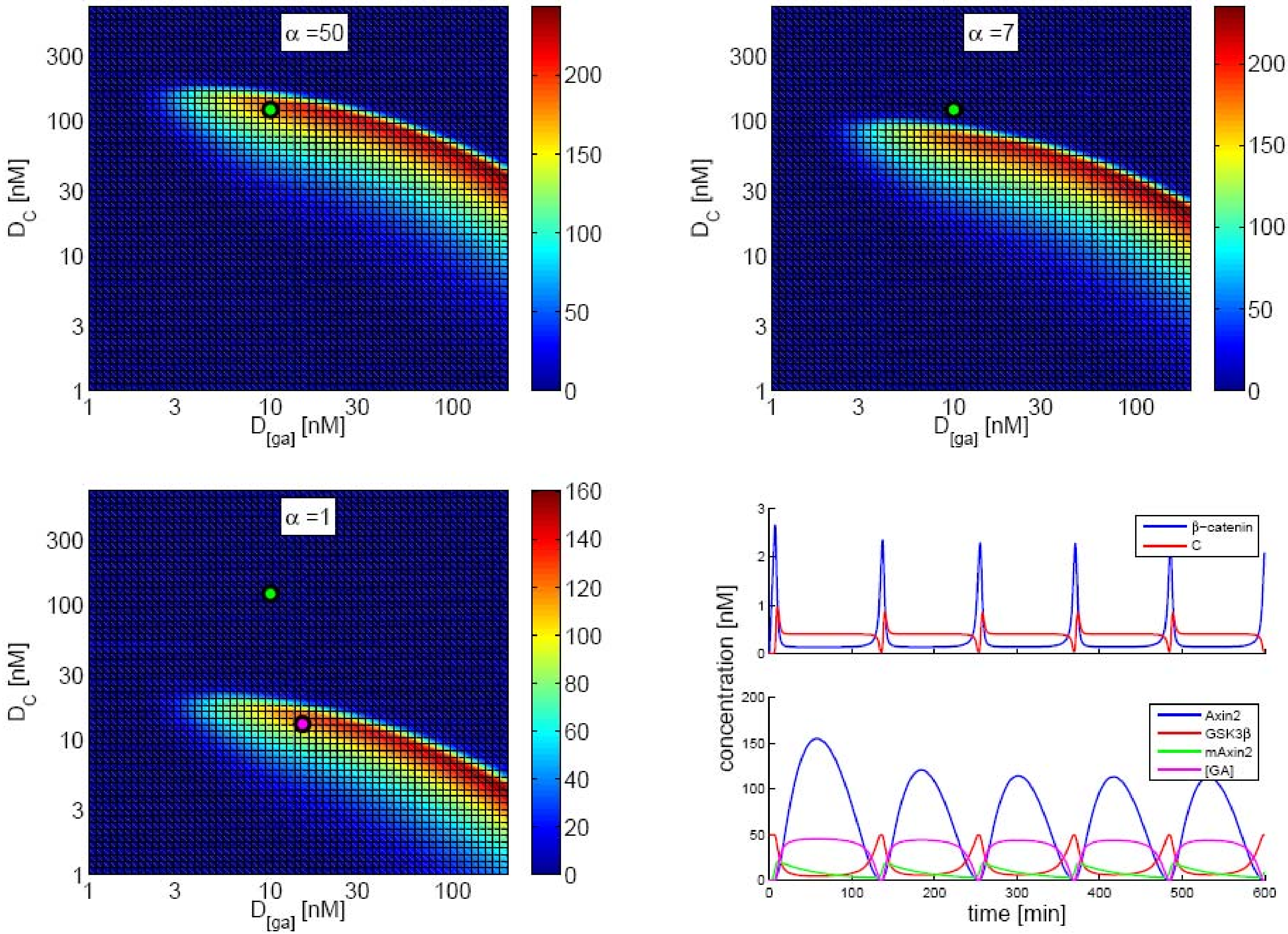
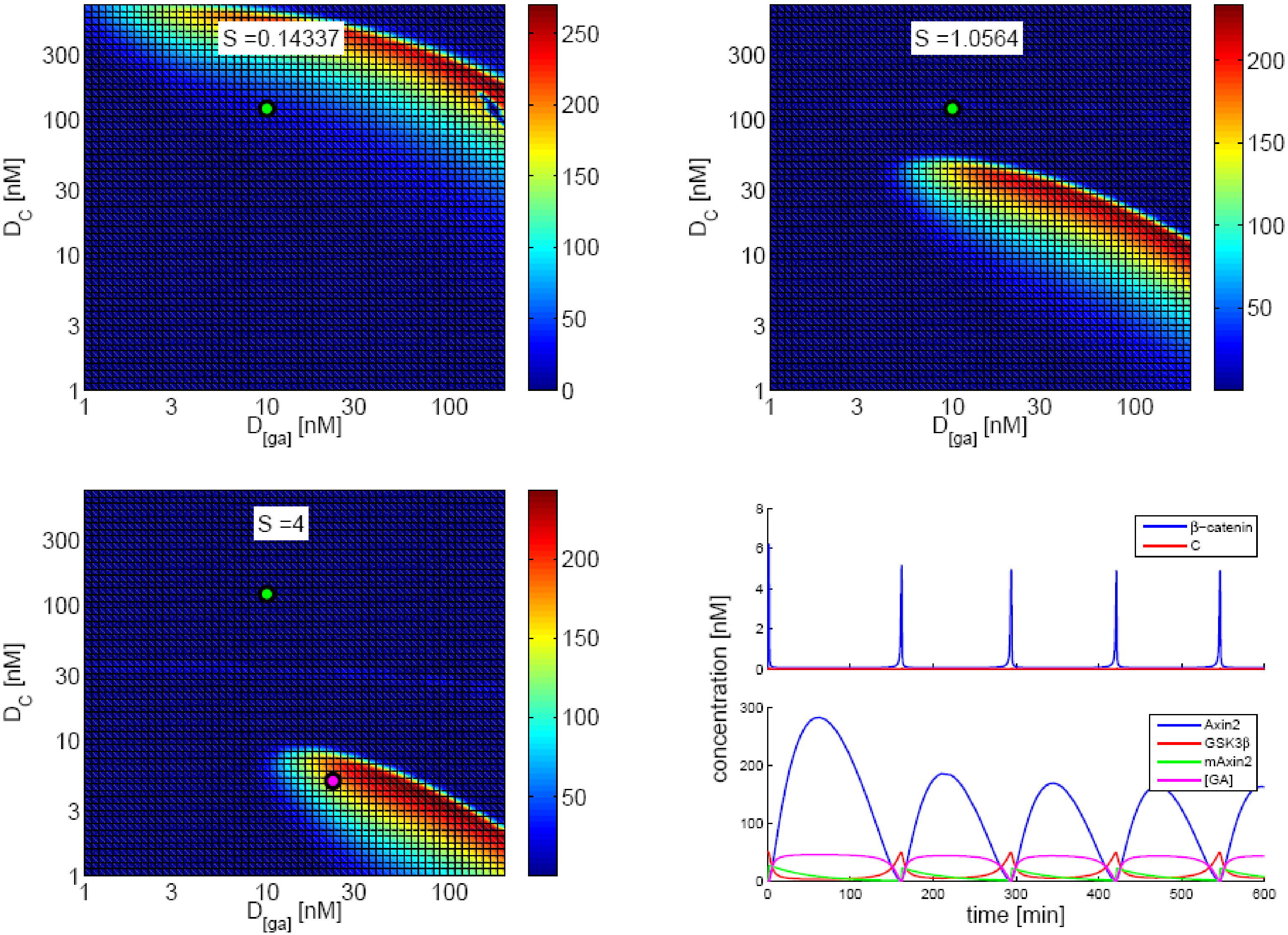


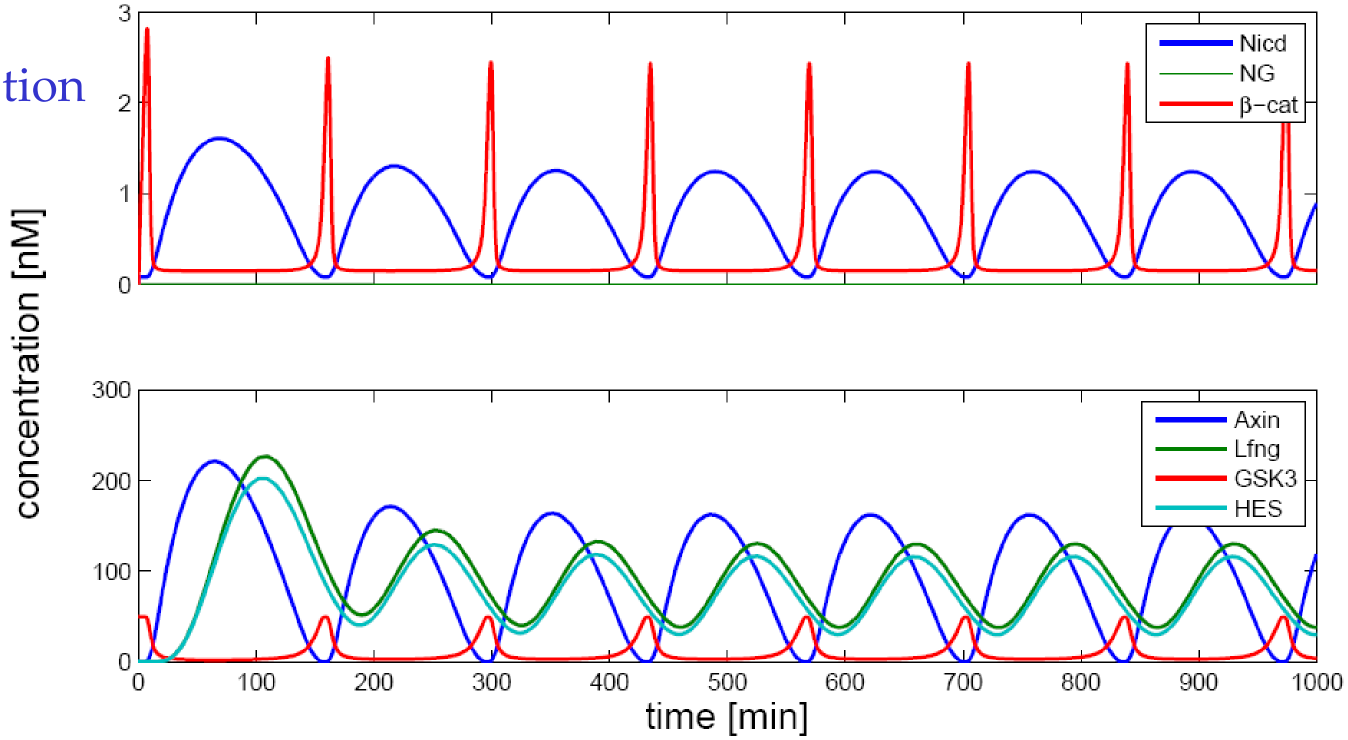
Figure 6.4: 4 selected timeseries matching the four dots in fig.6.3


α =50
α =7
α =1
D_C [nM]
$D_{[ga]}$ [nM]
1
3
10
30
100
300
0
50
100
150
200
20
40
60
80
120
140
160
β−catenin
C
Axin2
GSK3β
mAxin2
[GA]
concentration [nM]
time [min]
0
100
200
300
400
500
600
2
3


S =0.14337
S =1.0564
S =4
D_C [nM]
D_[ga] [nM]
1
3
10
30
100
300
0
50
100
150
200
250
concentration [nM]
time [min]
β−catenin
C
Axin2
GSK3β
mAxin2
[GA]
0
2
4
6
8
0
100
200
300
0
100
200
300
400
500
600

The full Wnt-Notch system

Transcription factors



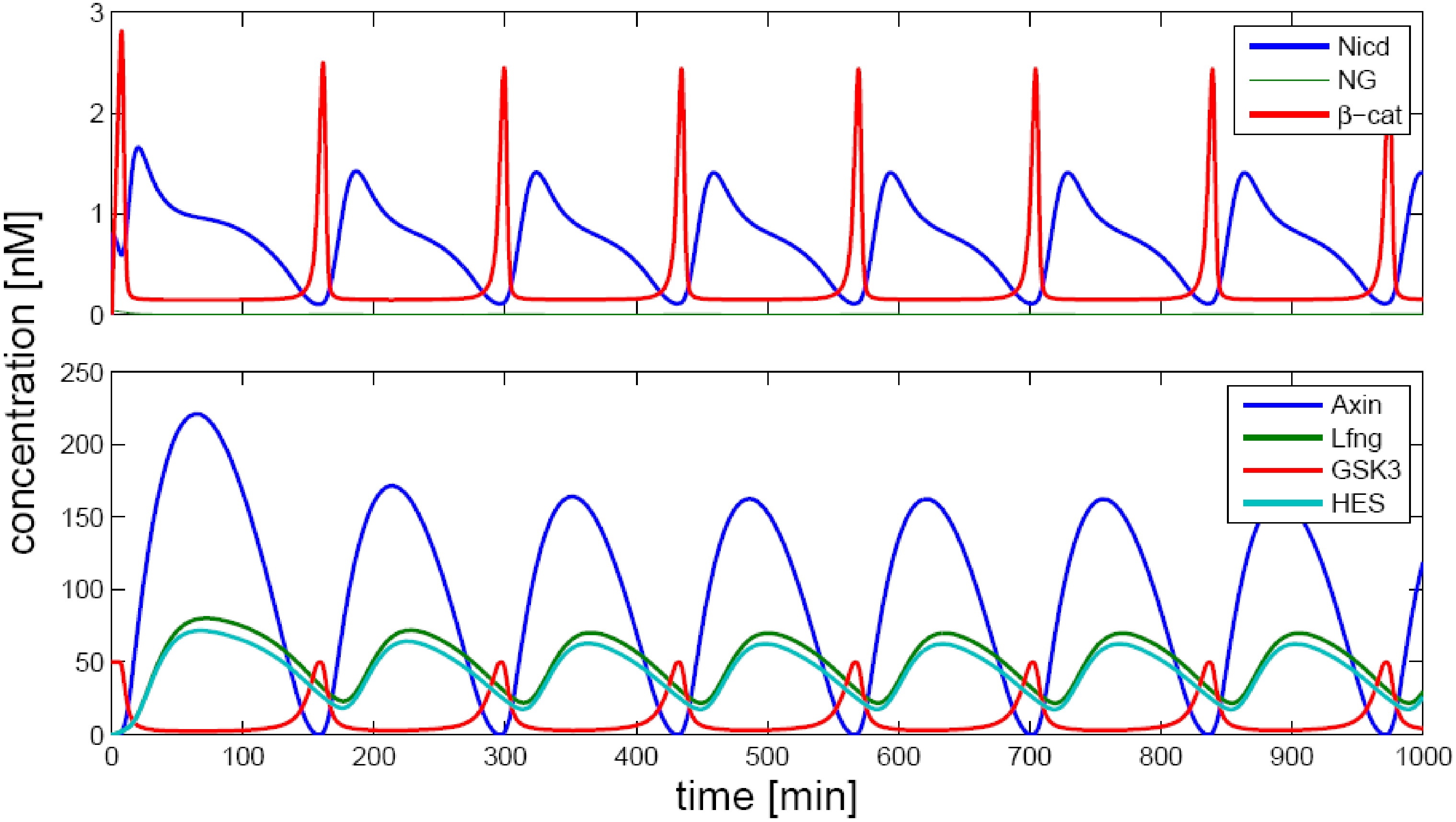


Figure 7.2: Timeseries of the 12 equation system with Lfng negative feedback

Can we put in "spatial" dependence ?
Effect of the Wnt gradient through PSM

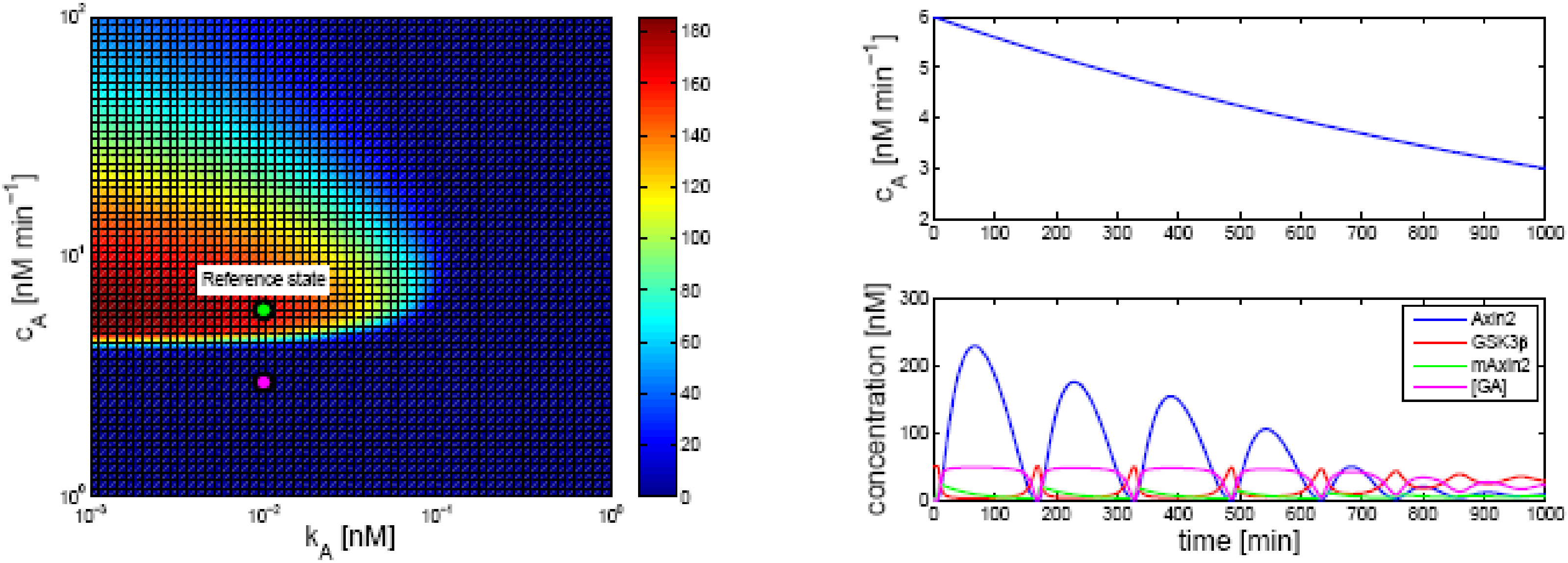


Figure 6.9: left: The $k_A - c_A$ parameterplane of A1. Green dot marks the reference state and the red dot marks the end state. Right: Timeseries as the value of c_A is lowered exponentially to 3 over 1000 min.

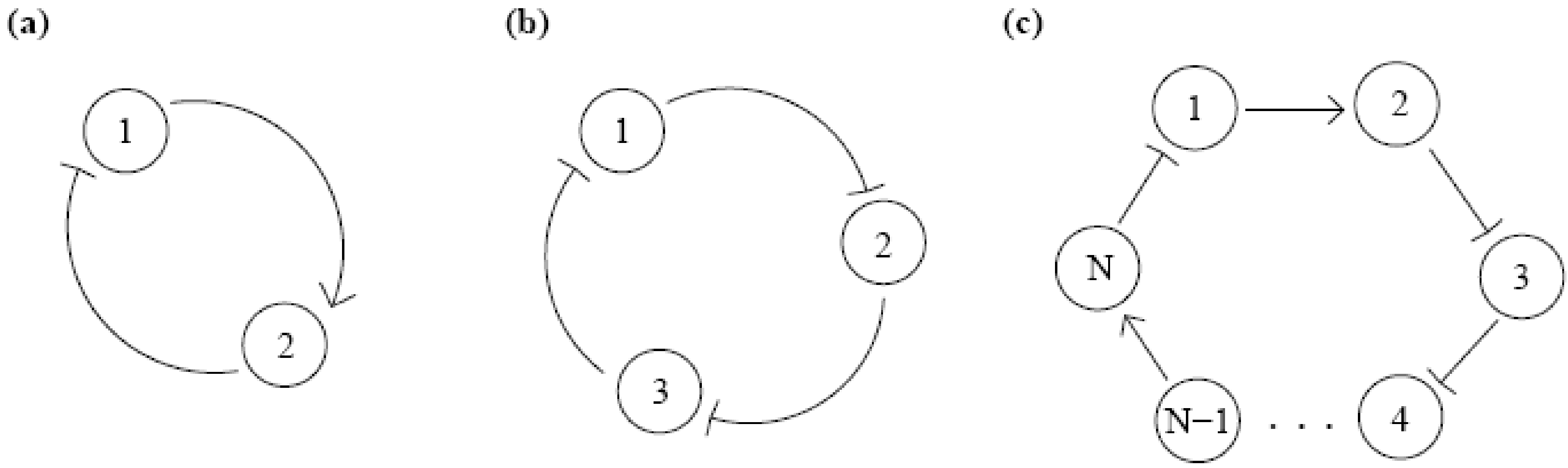
Negative feed-back loops

- 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 !
→ Then what ?

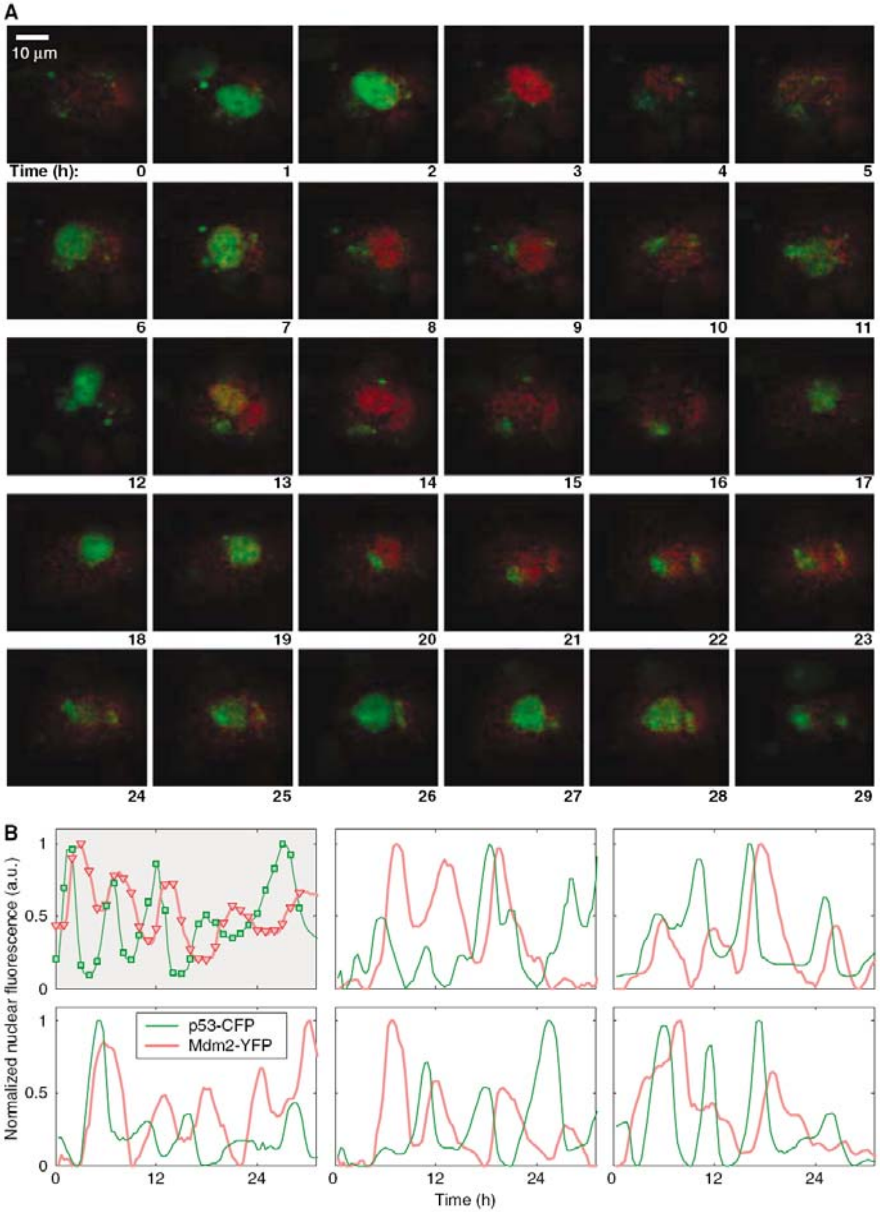
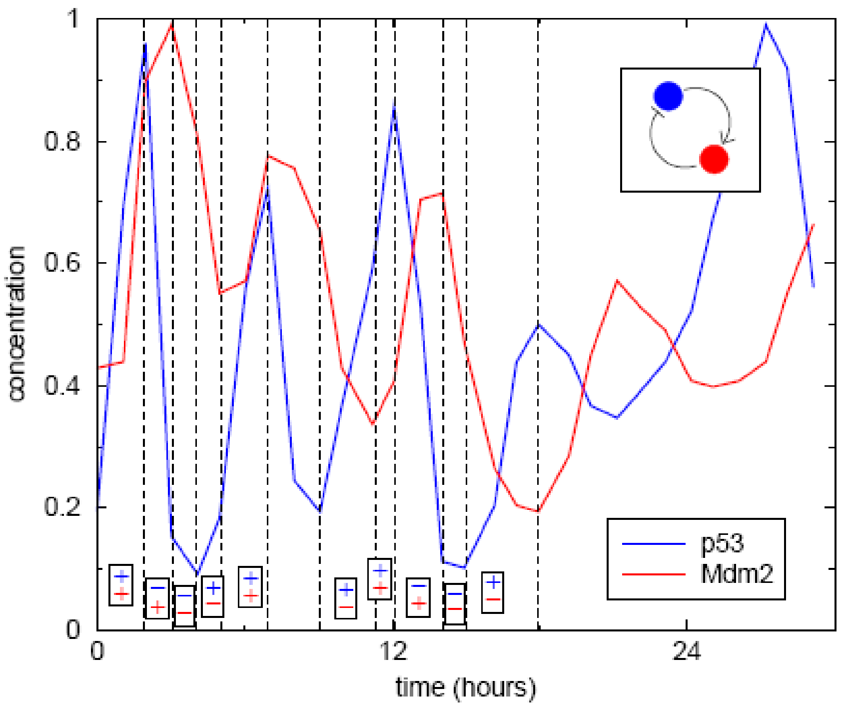


Figure 1 Prolonged oscillations in the nuclear levels of fluorescently tagged p53 and Mdm2 in individual MCF7, U280, cells following gamma irradiation. (**A**) Time-lapse fluorescence images of one cell over 29 h after 5 Gy of gamma irradiation. Nuclear p53-CFP and Mdm2-YFP are imaged in green and red, 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 !)

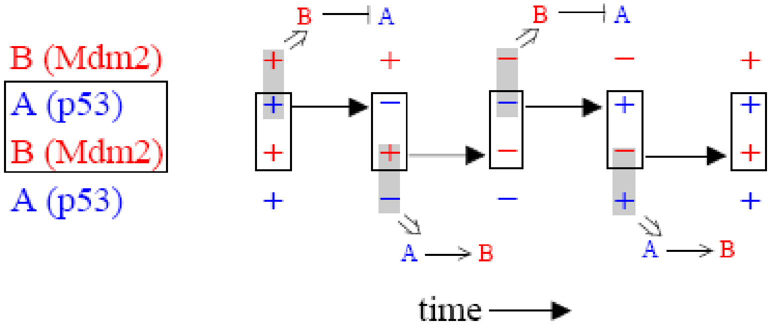
(a)



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

(b)

Symbolic Dynamics:



The algorithm

- List order of maxima and minima of genes/proteins
- Check if cyclic order is unchanging
- Construct symbolic dynamics:+ up/- down
- Symbolic order should be periodic:

  Then: ‘i-1’ activates ‘i’

  Or: ‘i-1’ represses ‘i’
- Procedure repeated for each variable in the loop
- Negative feed back loop: Only if number of repressors is odd

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 →
positive → multiple fixed points (bistability)

If odd number of repressors →
negative → only one fixed point !

Eigenvalue equation:

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

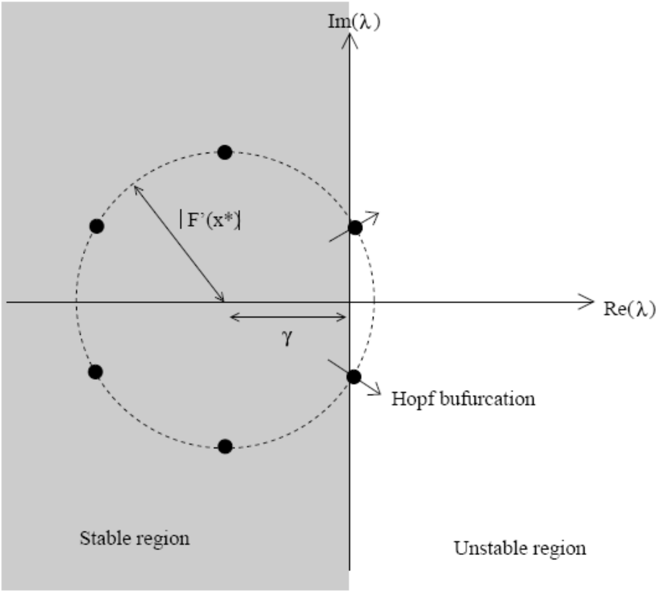
where $h_i = -\partial_x g_i(x_i, x_{i=1})|_{x^*}$ (degradation rates at fixed point)

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

Right hand side: Negative

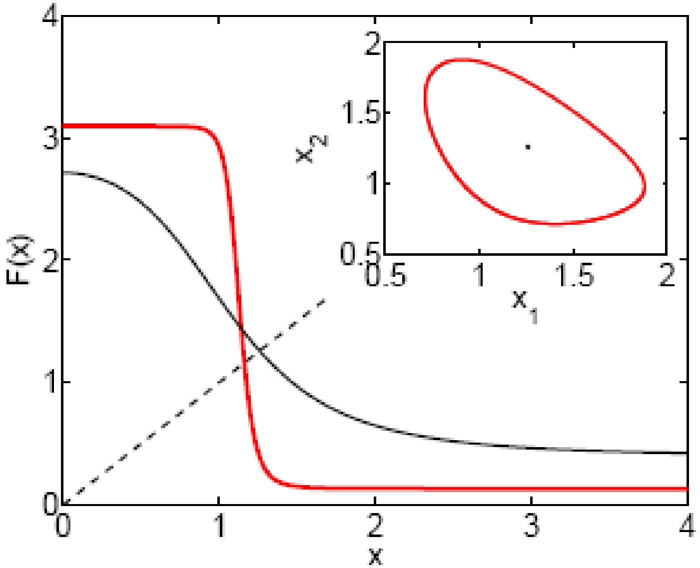
Left hand side: Positive coefficients

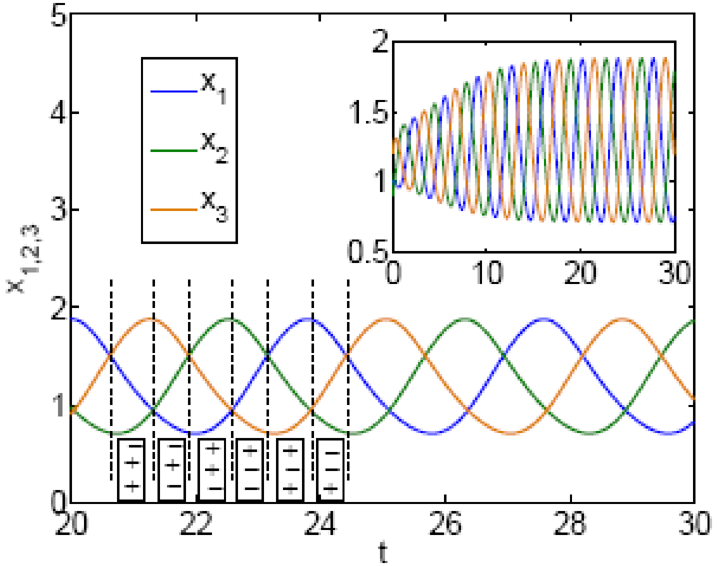
: Hopf bifurcation



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:

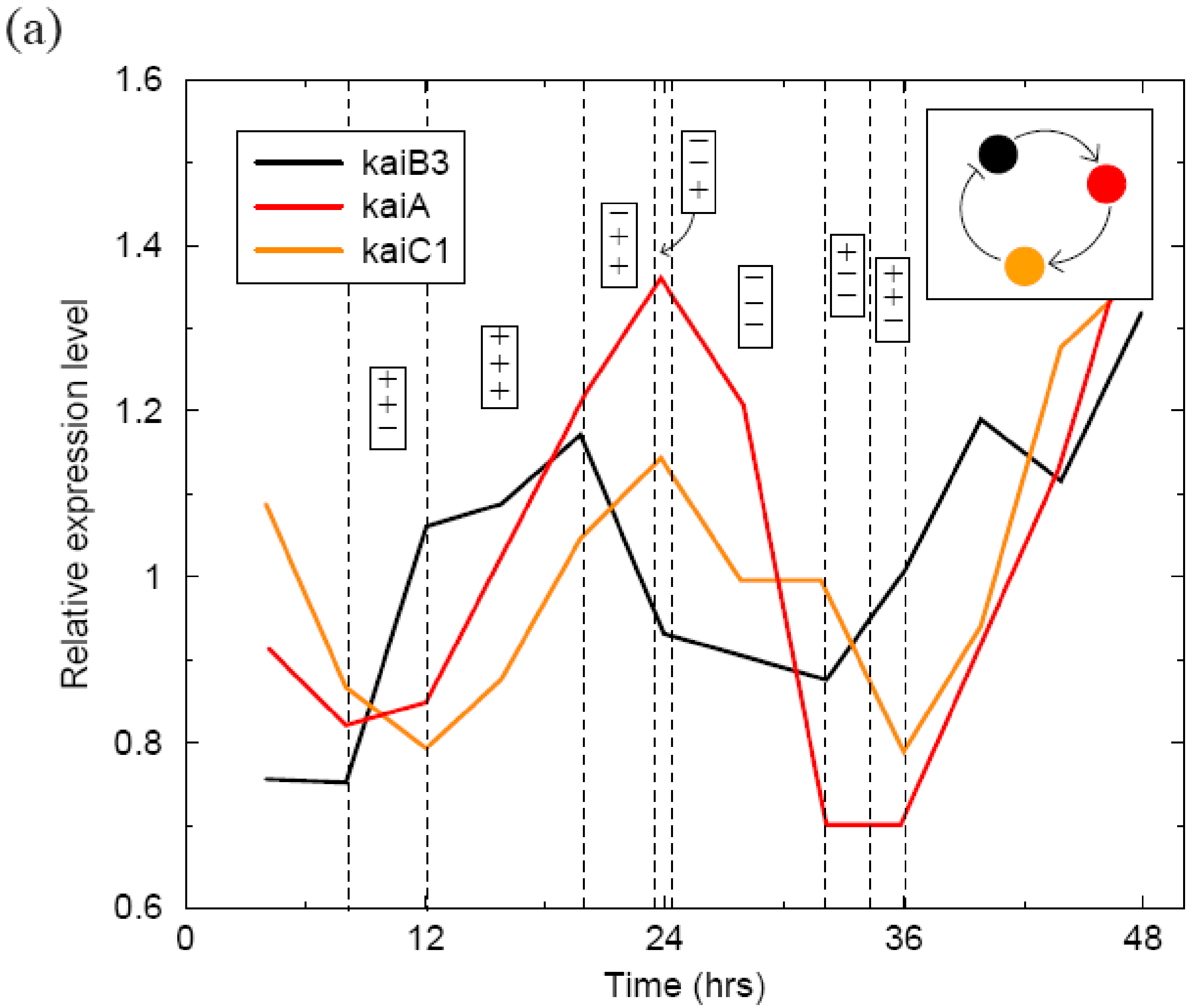
$$(\lambda + \gamma)^3 = -\left(\frac{\alpha}{1 + (x^*/K_i)^h}\right)^3$$

Stability condition

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

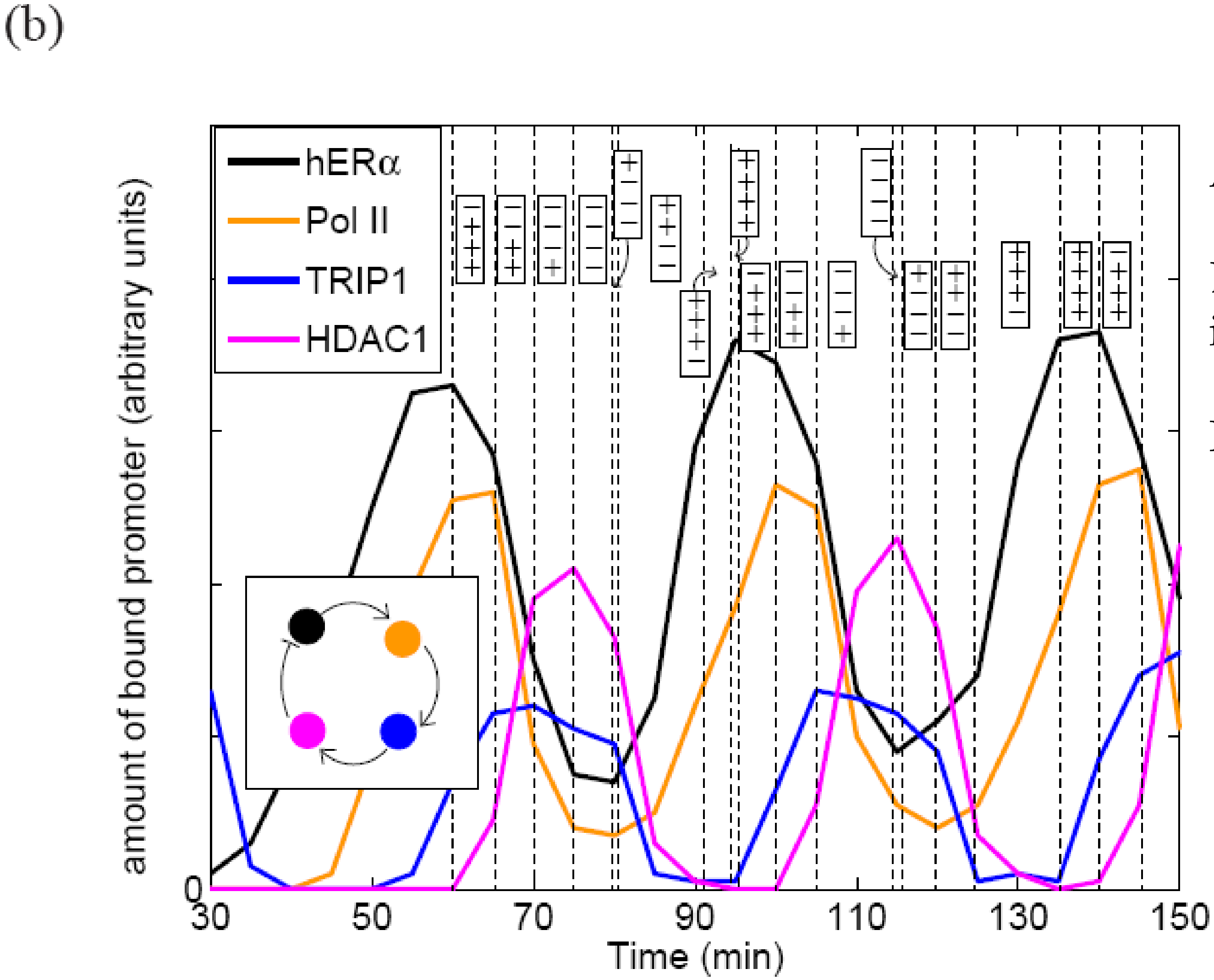
$\Big\{$ OK: h=2
Not OK: h=4

Circadian rhythms of kai genes in cyanobacteria



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

Binding of four proteins to pS2 promoter



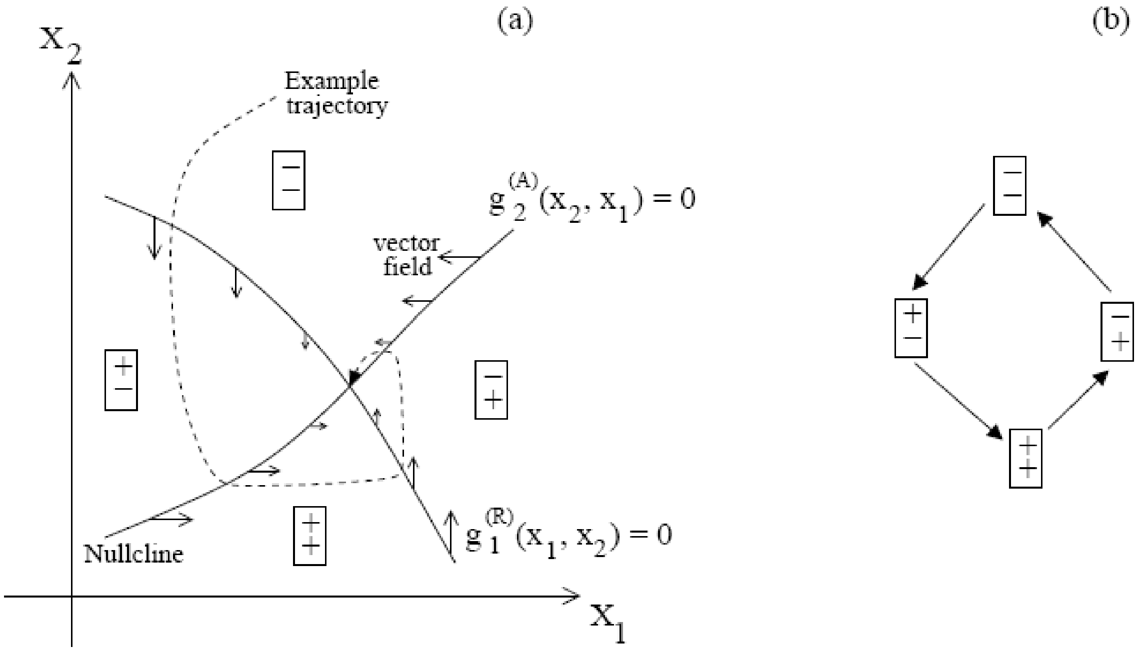
Adding estradiol:

ER: estradiol receptor, initiates transcription

Pol: RNA polemerase

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

Symbolic Dynamics:



Dynamical Rules:

- If the variable $(i-1)$ represses i, the nullcline i can be crossed if g_i and g_{i-1} have the same sign.
- If the variable $(i-1)$ activates i, the nullcline i can be crossed if g_i and g_{i-1} have opposite signs.

Unobserved variables:

Lets take the repressilator:

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

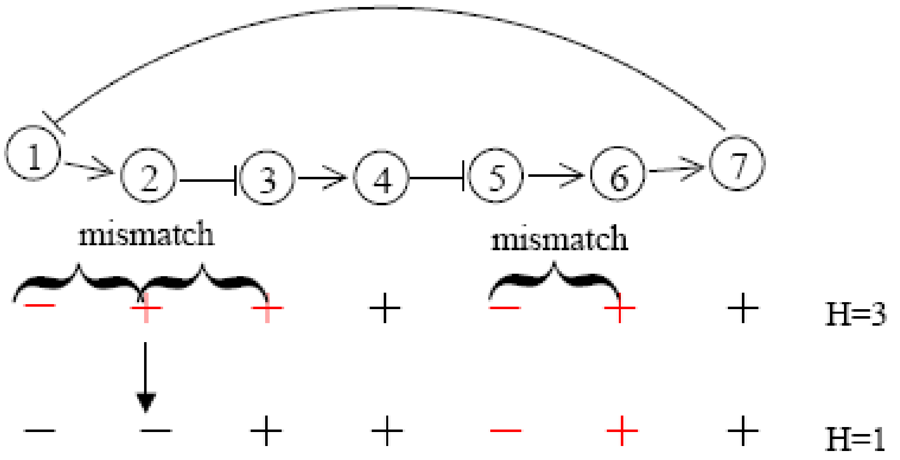
Symbolic dynamics: $\begin{array}{c} x_1 \\ x_2 \\ x_3 \end{array} \begin{pmatrix} - \\ + \\ - \end{pmatrix} \begin{pmatrix} + \\ + \\ - \end{pmatrix} \begin{pmatrix} + \\ - \\ - \end{pmatrix} \begin{pmatrix} + \\ - \\ + \end{pmatrix} \begin{pmatrix} - \\ - \\ + \end{pmatrix} \begin{pmatrix} - \\ + \\ + \end{pmatrix}$

Variable 2 unobserved $\longrightarrow \begin{pmatrix} - \\ - \end{pmatrix} \begin{pmatrix} + \\ - \end{pmatrix} \begin{pmatrix} + \\ + \end{pmatrix} \begin{pmatrix} - \\ + \end{pmatrix} \begin{array}{c} x_1 \\ x_3 \end{array}$

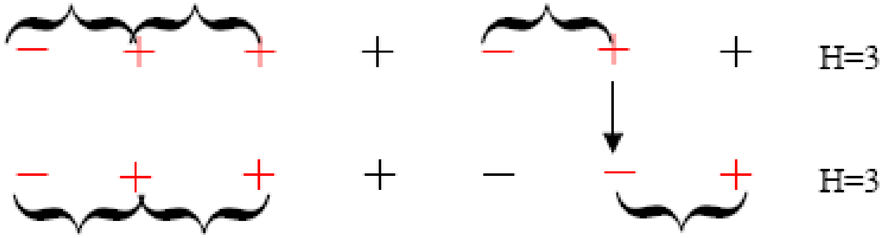
Two repressors become one activator

Resulting symbolic dynamics: two species loop

→ **Still gives correct answer**



Reduction of H
by removal of
two mismatches



No reduction of H
Movement of one
mismatch