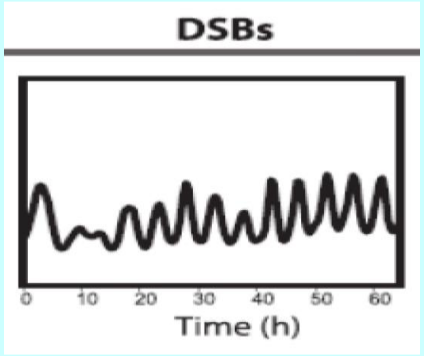
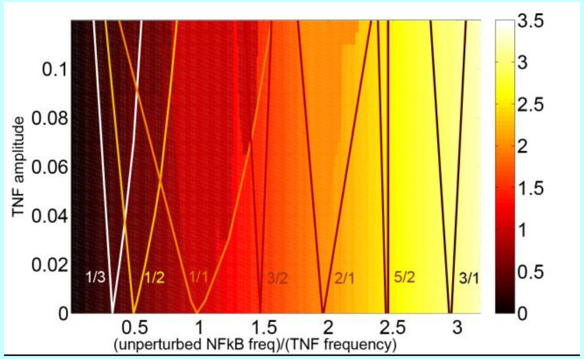
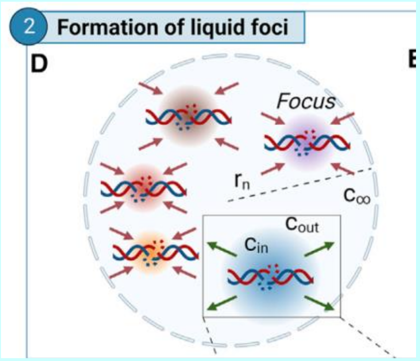
Modelling and Data Analysis for Biological Oscillators

KSMB-SMB Satellite Workshop, 27 June 2024

Mogens H. Jensen, Niels Bohr Institute







1. **When healthy and sick cells are damaged or inflamed:**

   Protein densities oscillate over days:

   p53 (cancer), NF-kB (diabetes)

   → The cell is repaired on foci or dies

2. **Oscillations of p53 and NF-κB:**

   regulated by negative feed-back loops

3. **Add external protein oscillation coupled to internal oscillation:**
   Oscillations synchronize →
   Arnold tongues! Chaos! → Gene control
   (collaboration with Harvard Medical)

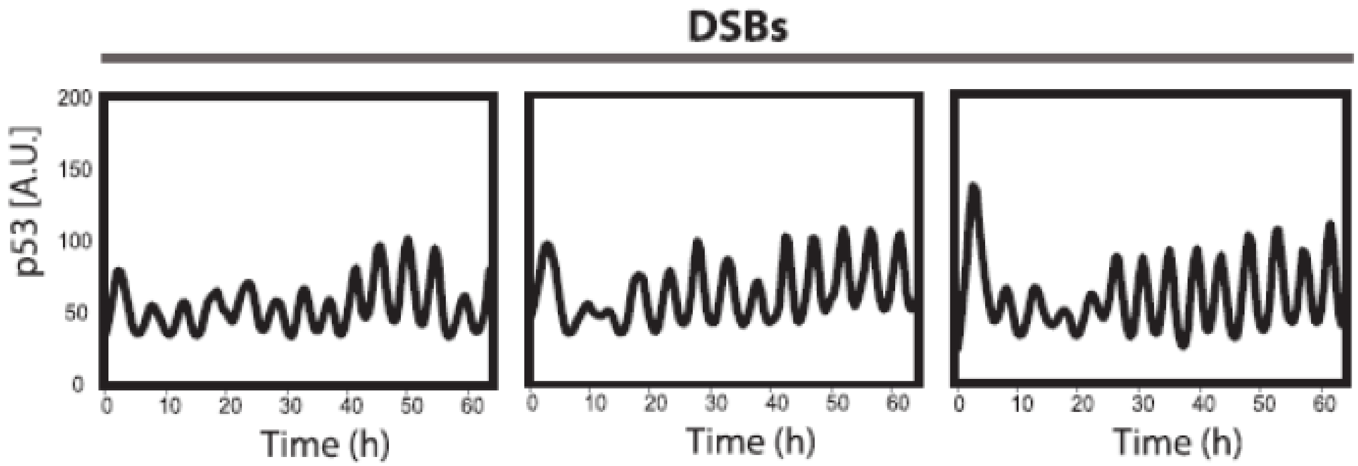
4. **Oscillations important for DNA-repair:**
   Through foci/droplet formation (collaboration with Taipei)

5. **Genetic resonance: Excite cell dynamics with maximal amplitude response**
   (collaboration with Harvard Medical)

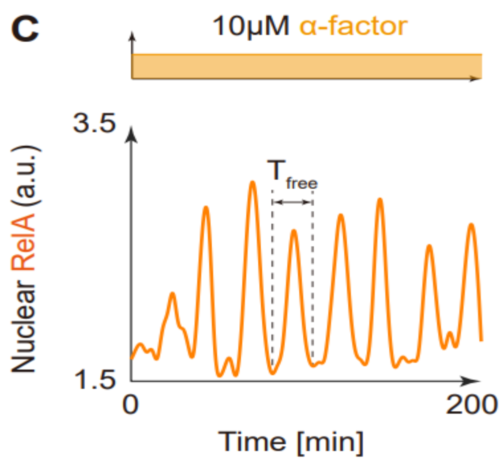
6. **Two external oscillator to internal oscillator: Chronobiology!**
   Enhance control and tunability
   (collaboration with Peking/Shenzhen University)

7. **Is deterministic chaos relevant for gene productions ? Yes!**

Gamma radiation on human (MCF7) cancer cells: p53 oscillate (Lahav, Harvard)



Alpha-factor on yeast cells: NF-kB oscillate (Beijing/Shenzhen, Ping Wei)

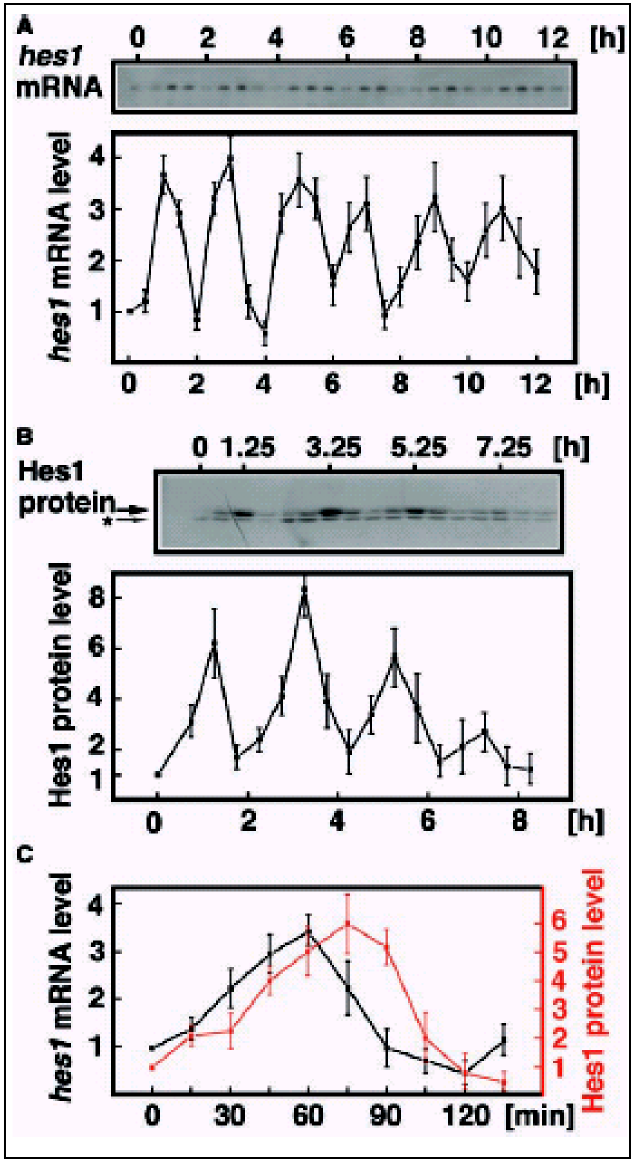


Our research:

Can we control and understand it?

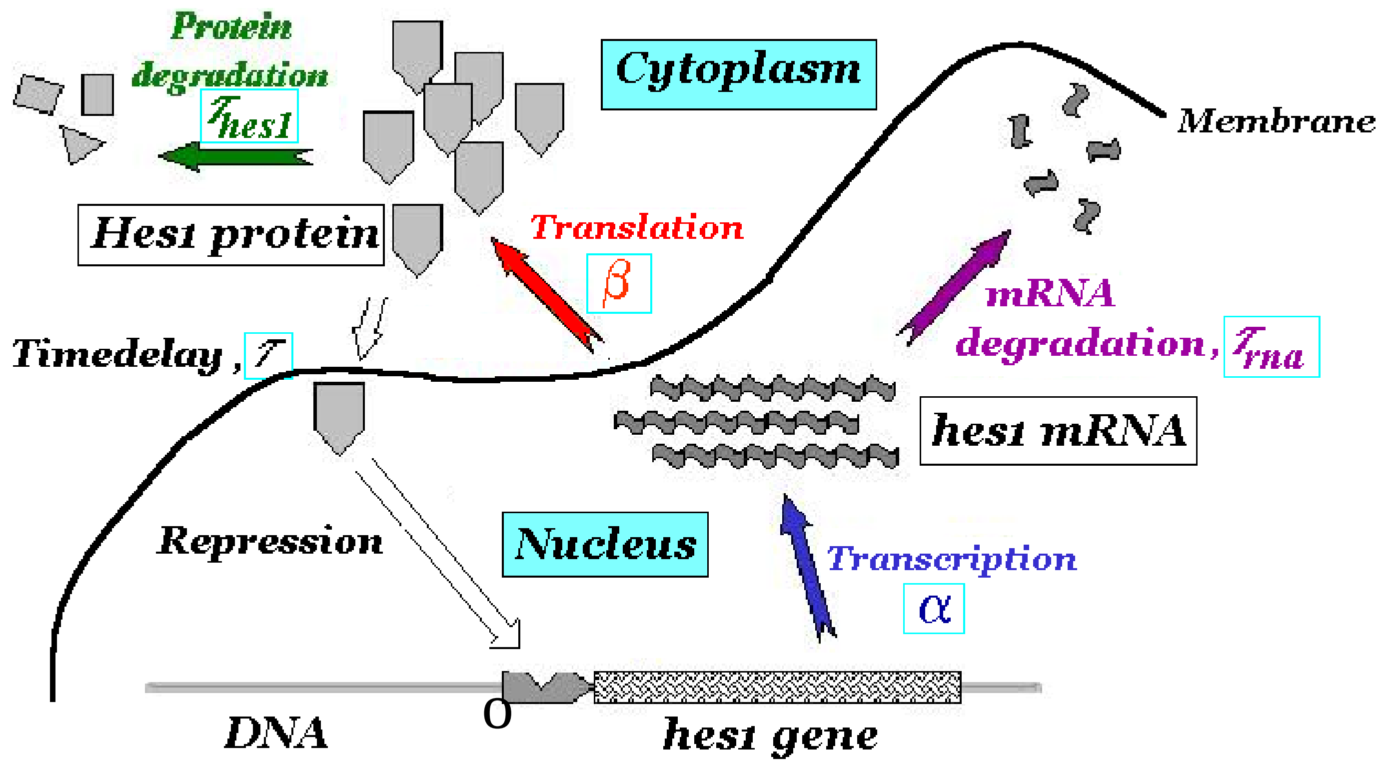
Can it be used for gene regulation and control and DNA repair?

‘Typical’ Oscillating data: Hes1 - segmentation



(Hirata et al, 2002)

Simplest negative feed-back loop: Hes1



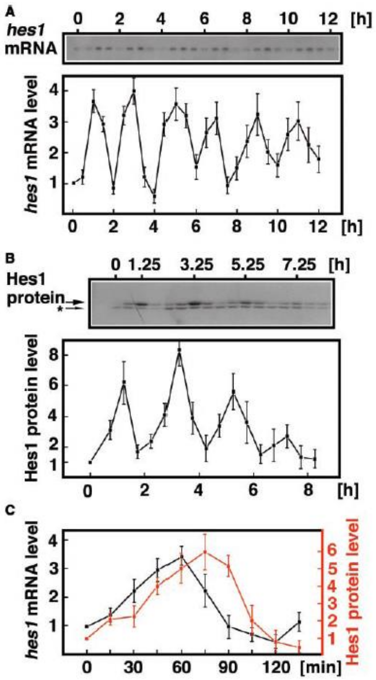
$$\frac{d[mRNA]}{dt} = \alpha \cdot [o_{free}] - \frac{[mRNA(t)]}{\tau_{rna}}$$

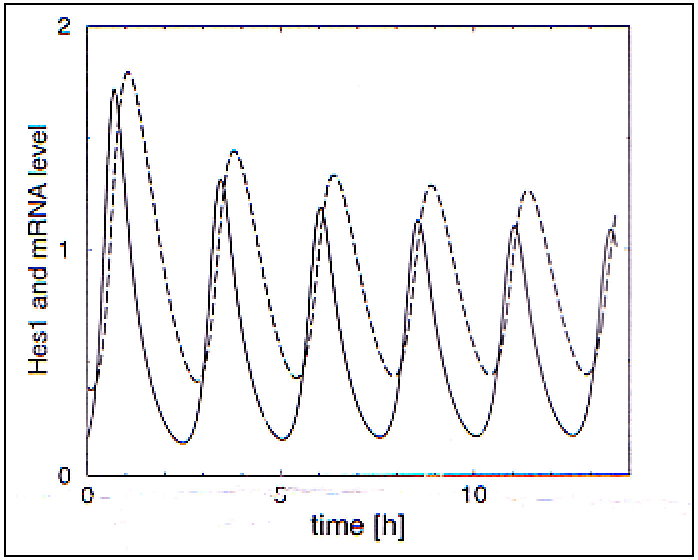
$$\frac{d[Hes1]}{dt} = \beta \cdot [mRNA(t)] - \frac{[Hes1(t)]}{\tau_{hes1}}$$

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

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

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

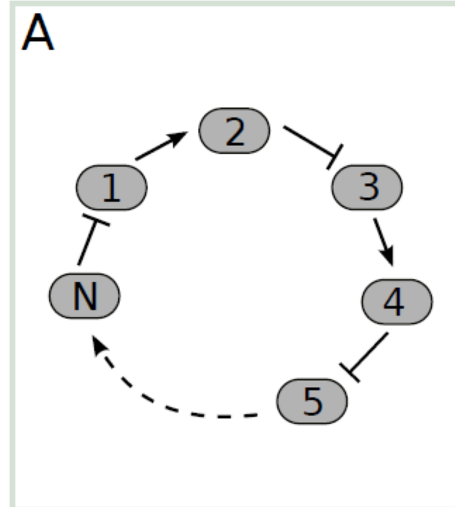


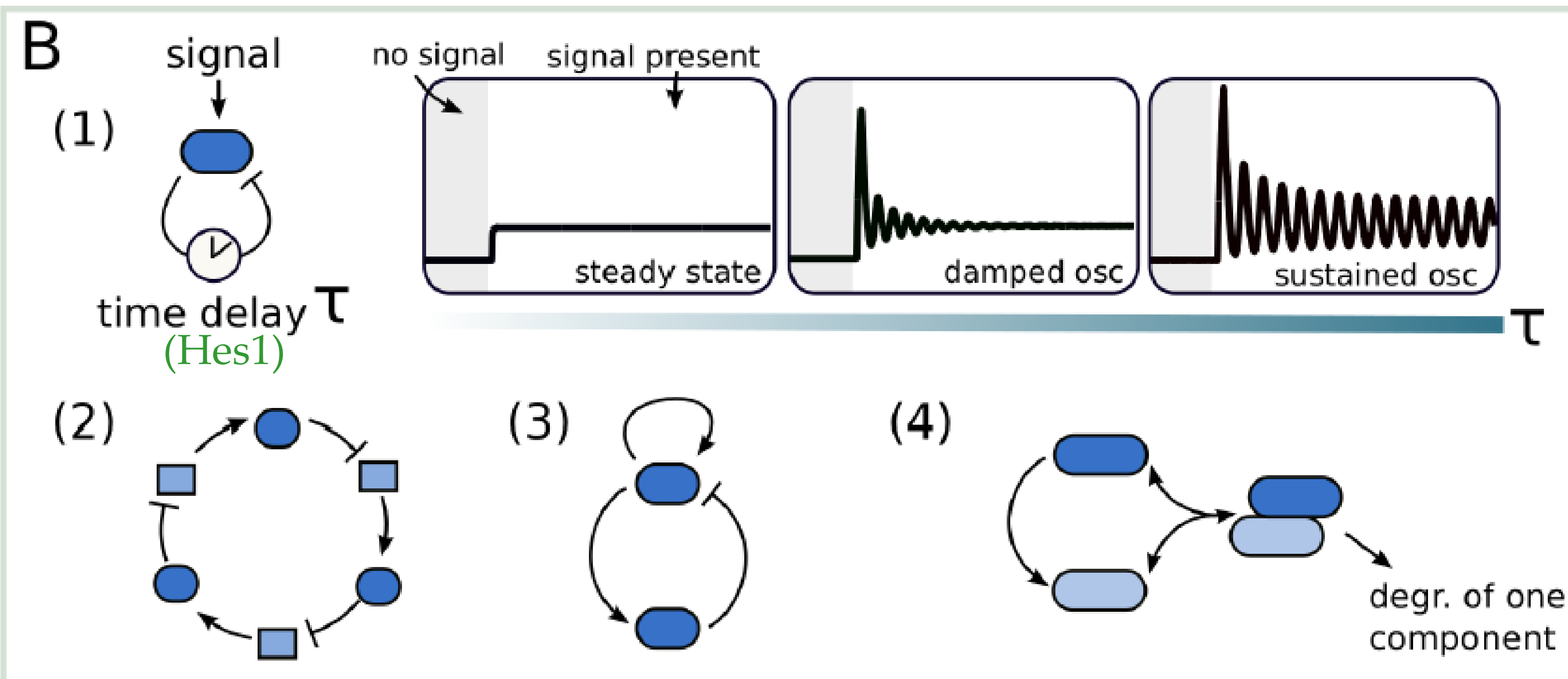


- τ_{rna} = 24.1 min
- τ_{hes1} = 22.3 min
- τ = 24 min
- $\alpha = 20\ [R]_0\ \text{min}^{-1}$
- $\beta = 1/20\ \text{min}^{-1}$
- $K_M = (0.1[R]_0)^n$
- n = 4

Tiana, Sneppen , Jensen

Basic oscillator: Negative Feed-Back loops:





(Hes1)

(Repressilator)

(NF-kB, p53)

Two very fundamental transcription factors oscillates:

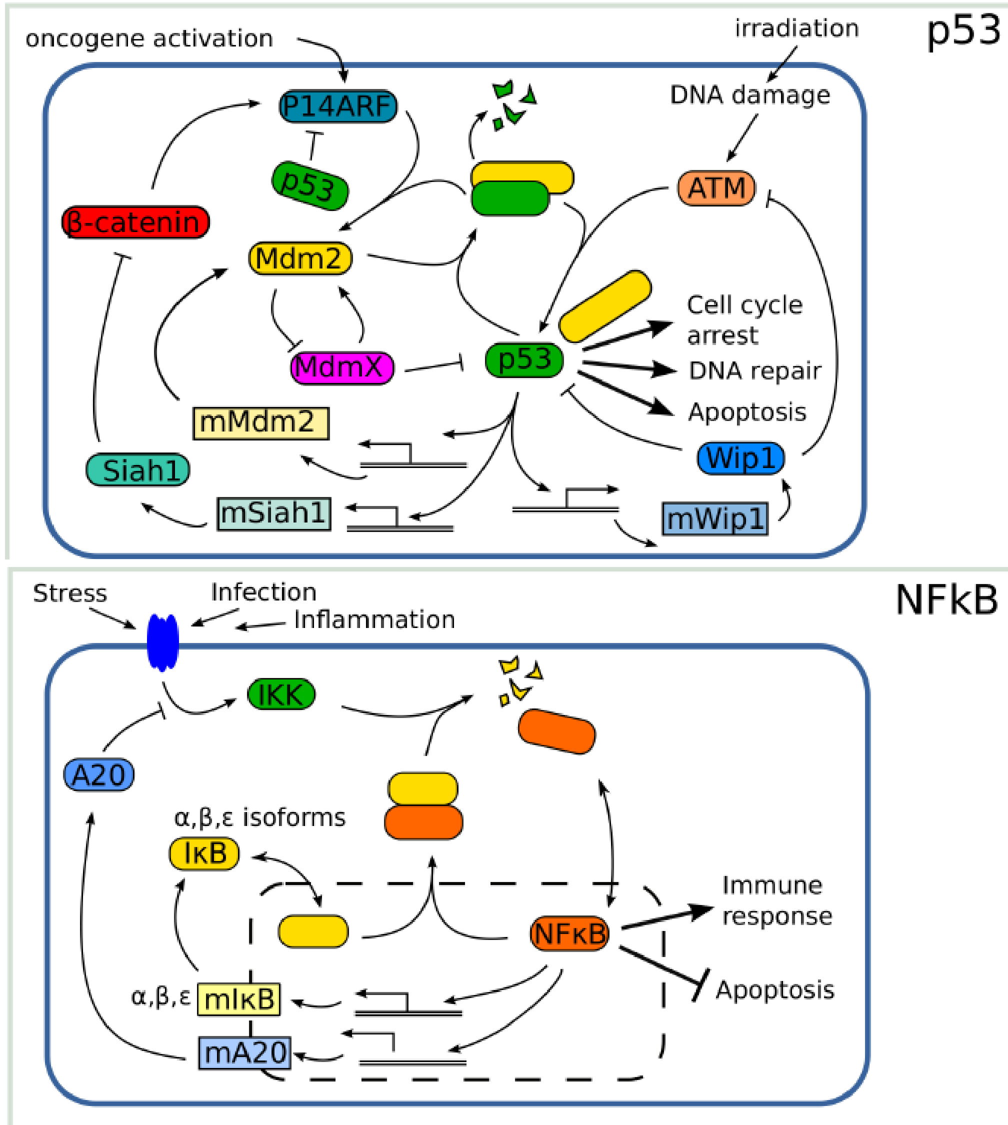
p53:

- cancer gene
- Mutated in nearly all cancers
- High value of p53 prevents cancer
- Control over 3500 genes!

NF-kB:

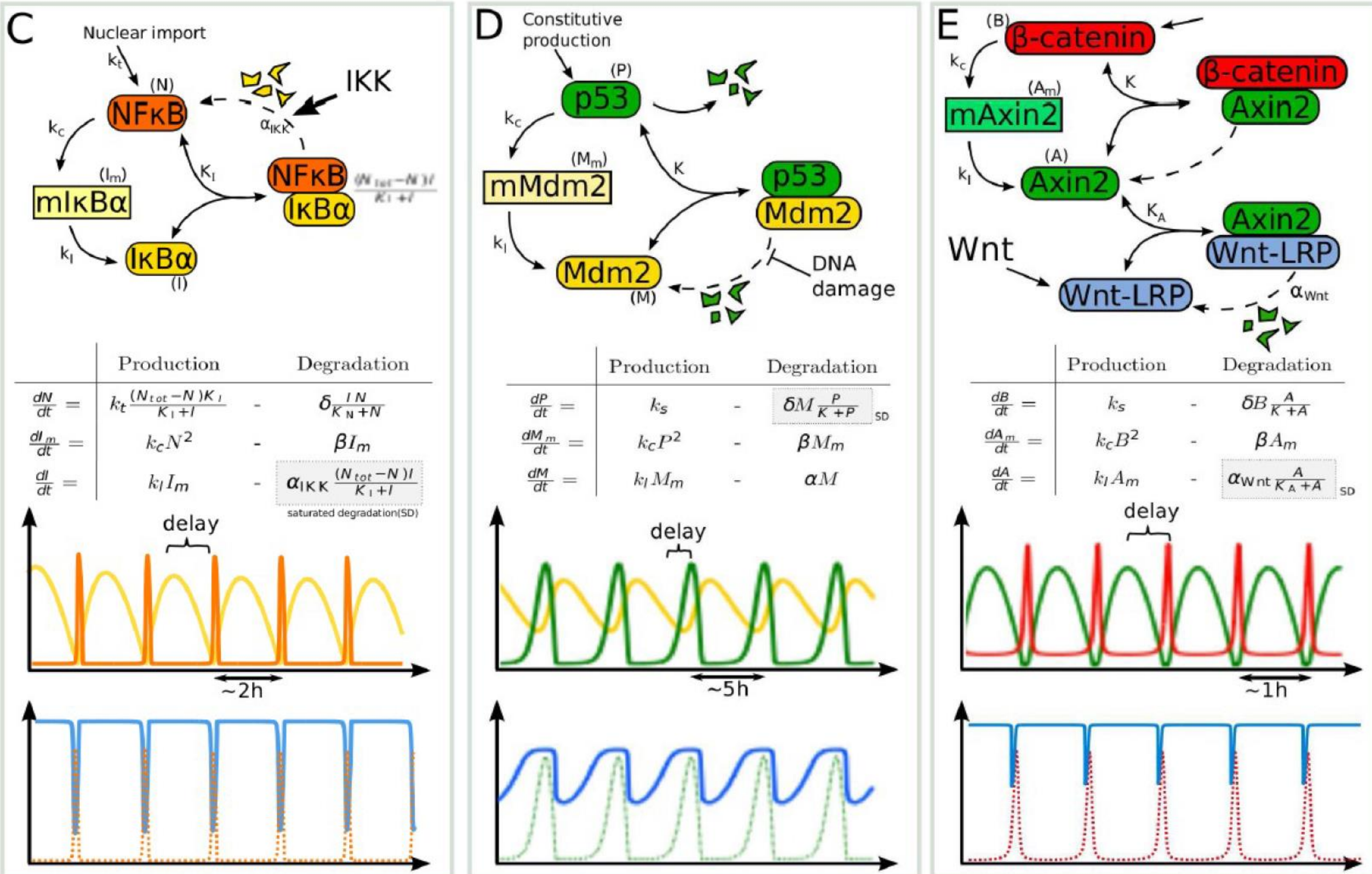
- Control over >500 genes
- Upregulated by inflammation
- Very important in diabetes

Genetic networks for p53 and NF-kB!



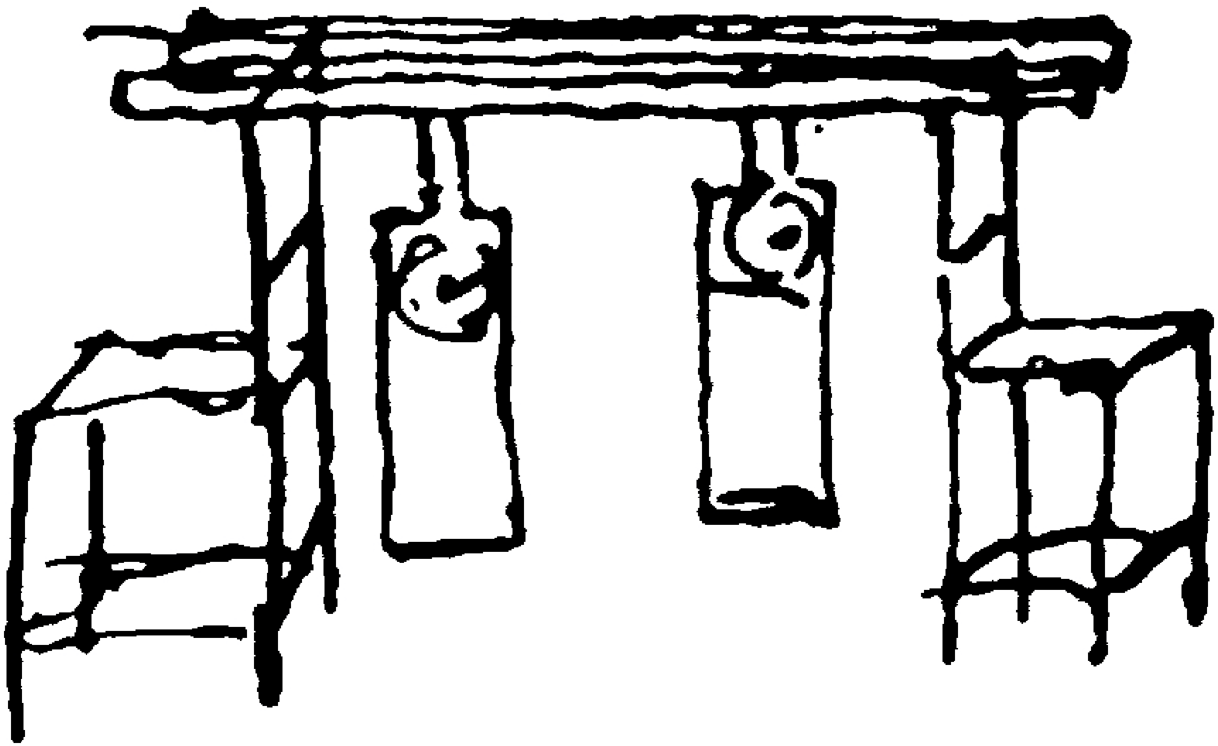
We simplify them (according to Niels Bohr!)

NF-kB p53 Wnt



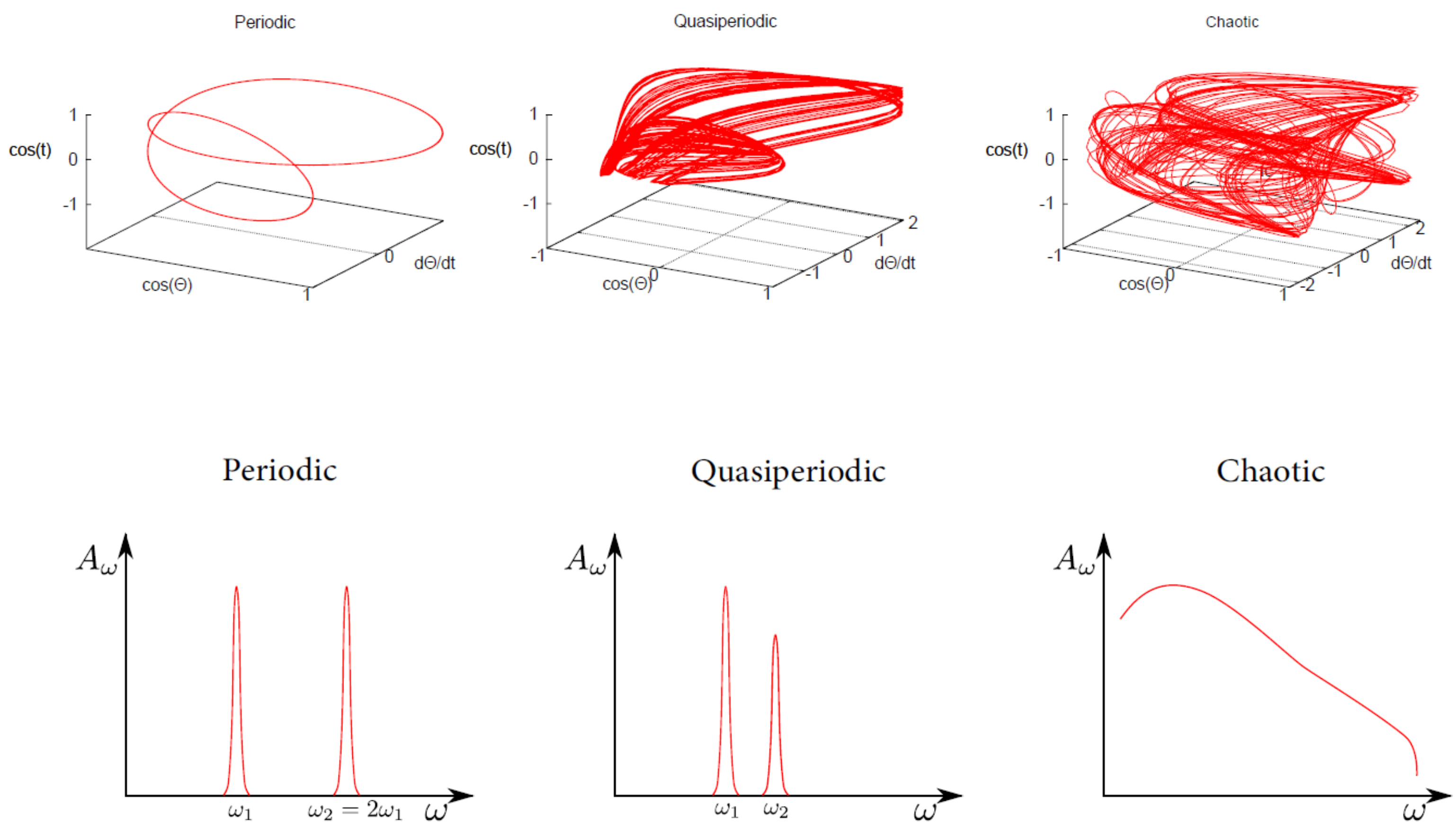
Krishna, Jensen, et al

A short physics tour:
Synchronization of two oscillators



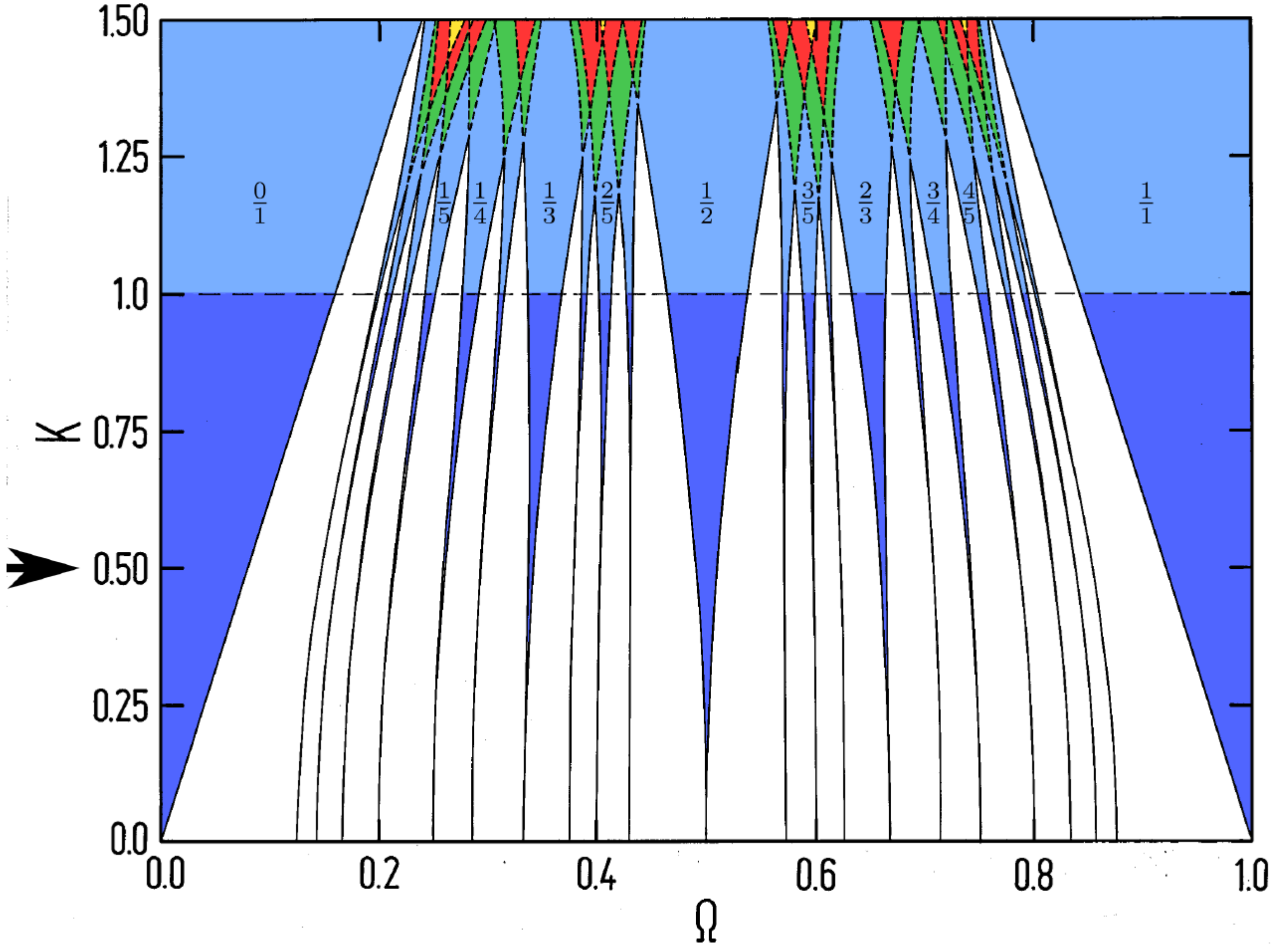
Huygens' clocks 1665

Two independent oscillators:
Three different non-linear dynamics



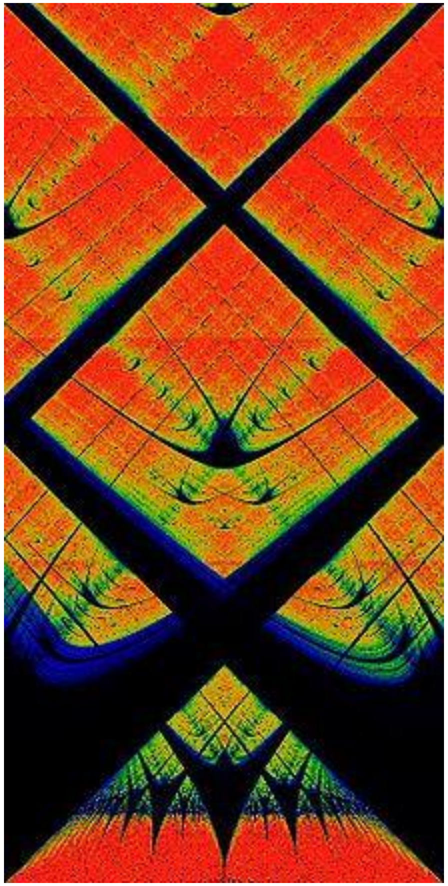
Two coupled oscillators: Arnold tongues

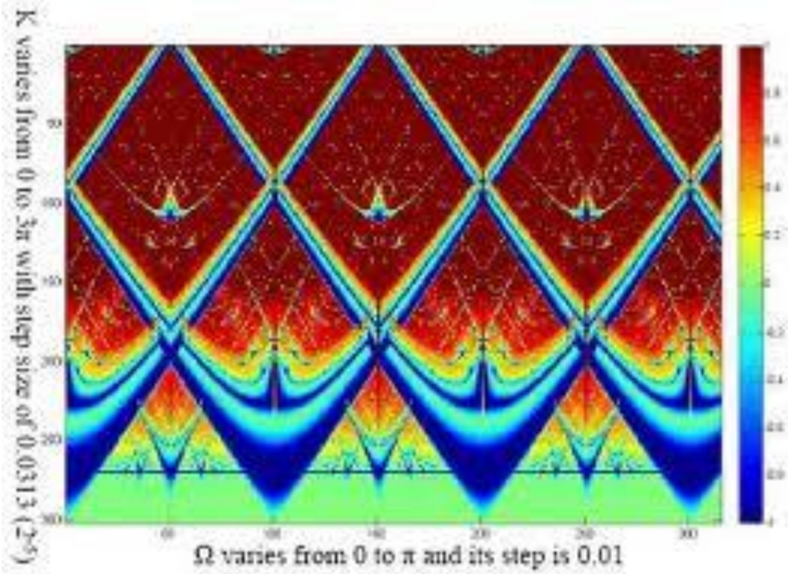
$$\omega/\Omega = P/Q$$

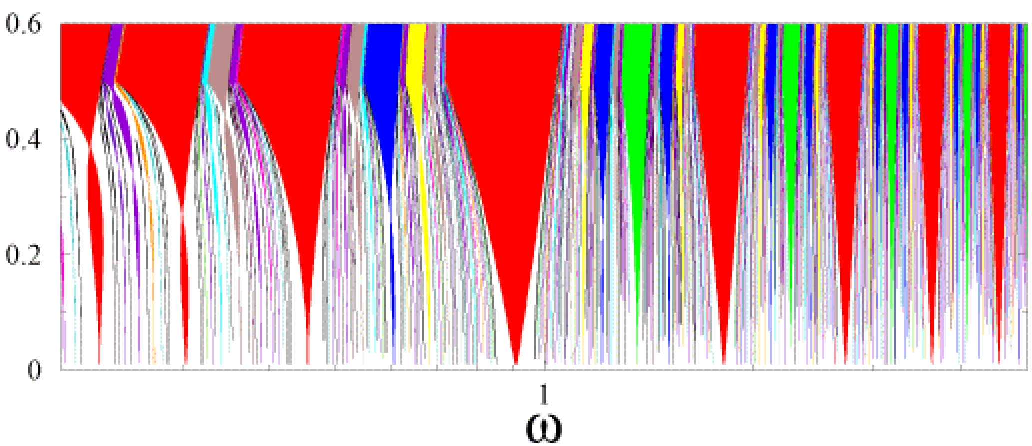


Per Bak, Tomas Bohr, MHJ (1983, 1984)

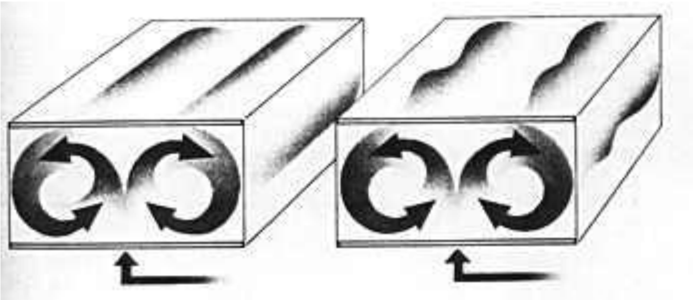
Examples of Arnold tongues !







Chicago basement convection (1984)!



Libchaber, Stavans, Glazier:
External oscillating current !

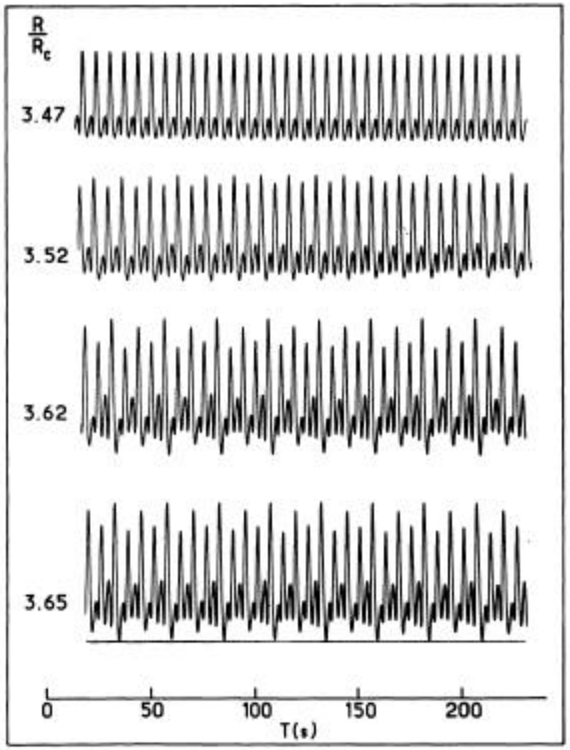


Fig. 2. — Direct time recordings of temperature for various stages of the period doubling cascade showing the onset of $f/4$ ($R/R_c = 3.52$), $f/8$ ($R/R_c = 3.62$), $f/16$ ($R/R_c = 3.65$).

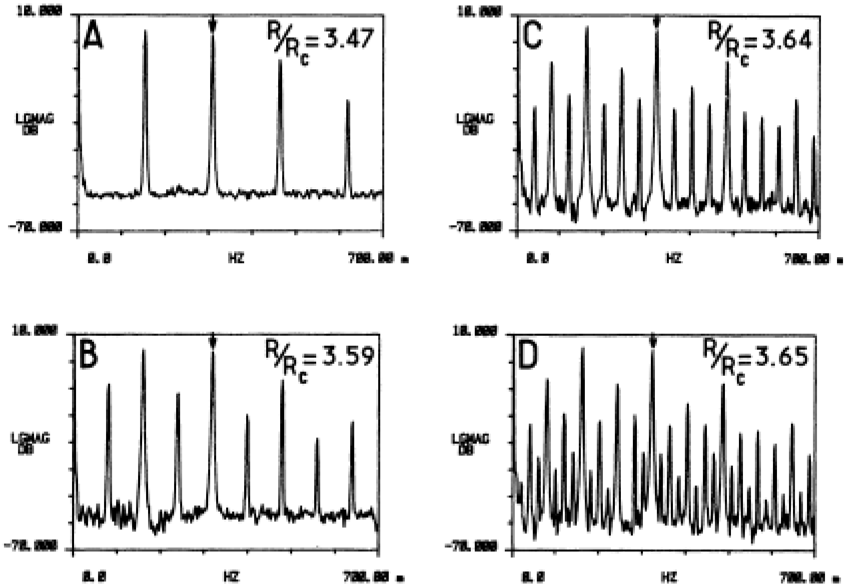
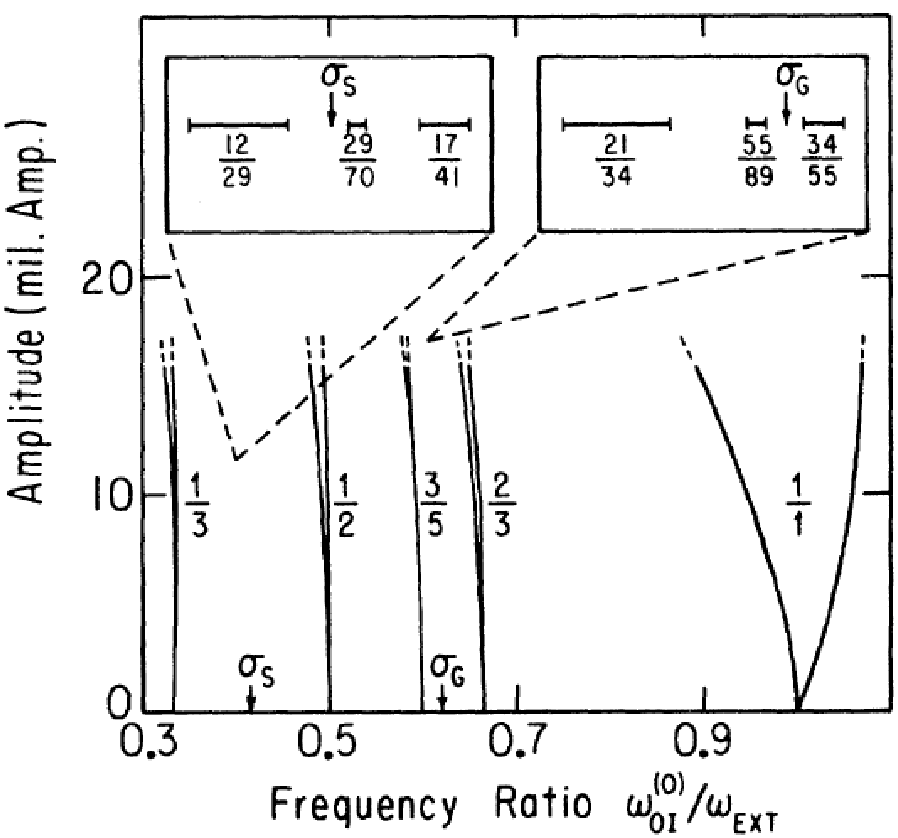
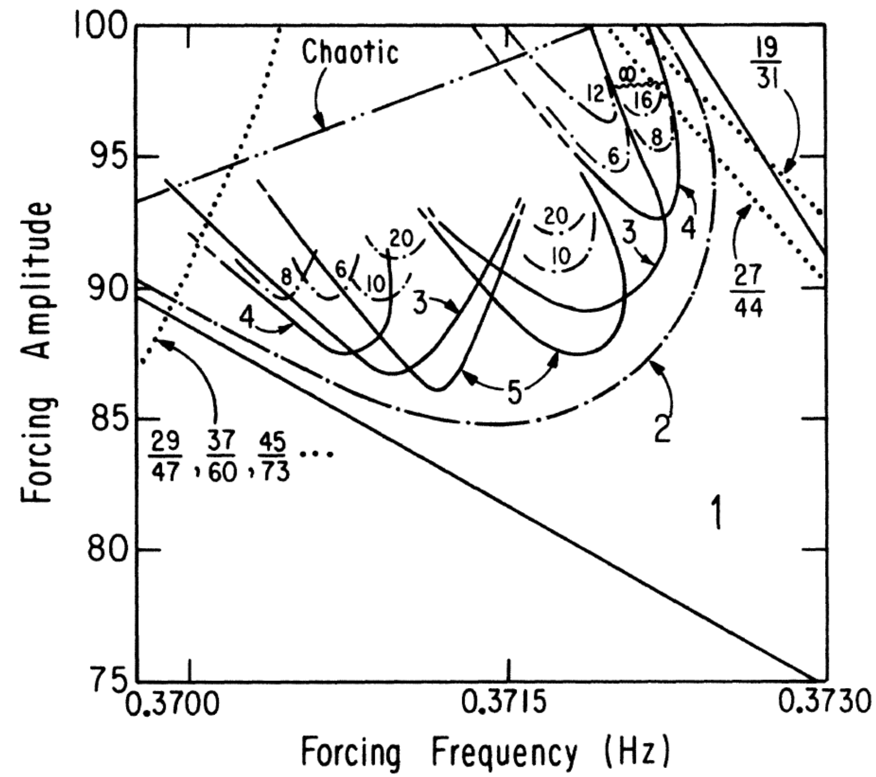


Fig. 3. — The Fourier spectrum. Arrows indicate the peak at the frequency f_1.

Chicago basement convection !

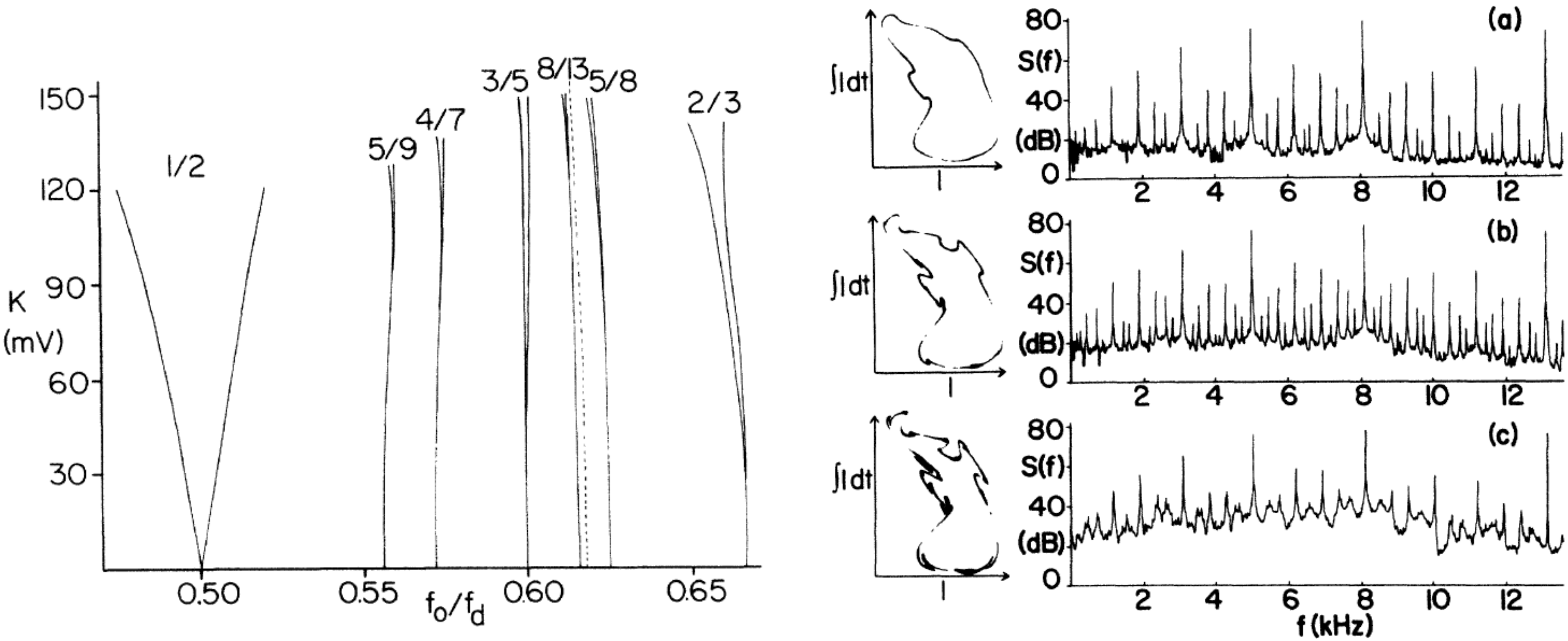


Stavans, Heslot, Libchaber



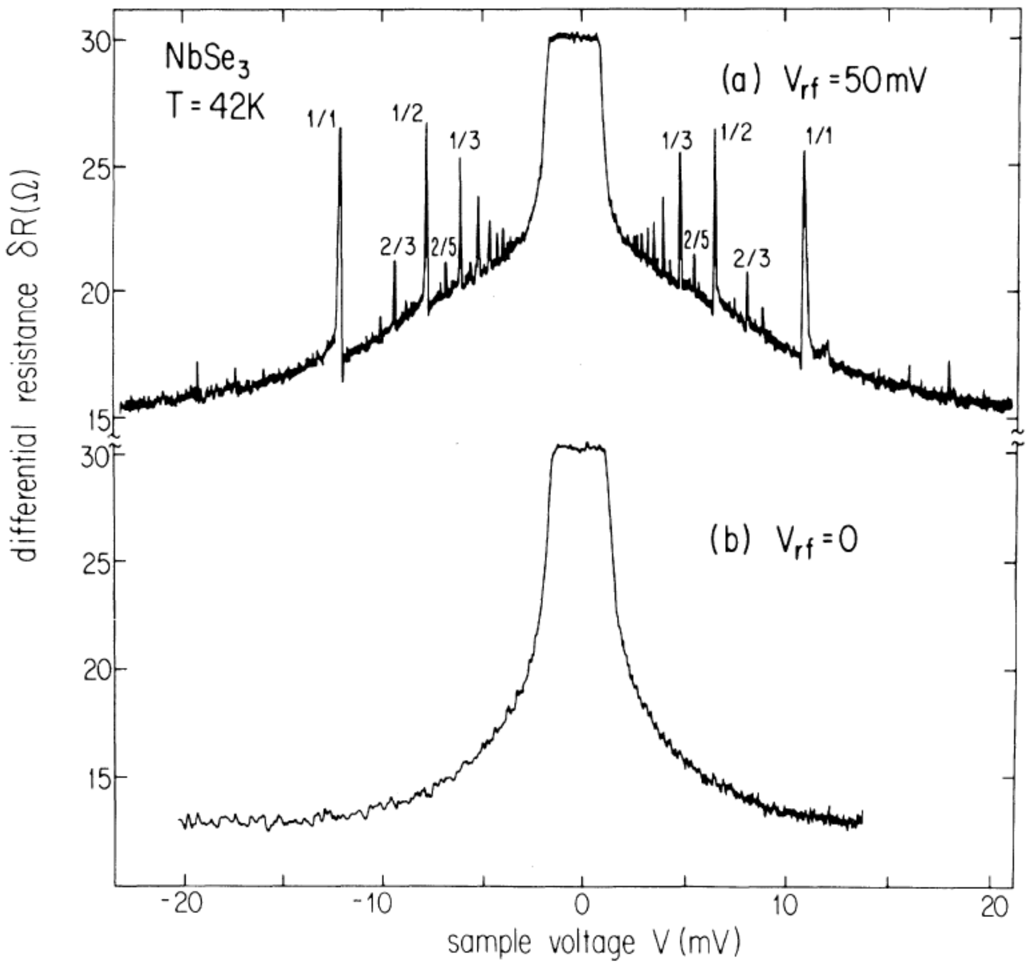
Glazier, Jensen, Libchaber, Stavans

Semiconductors at Harvard : Gwinn, Westervelt



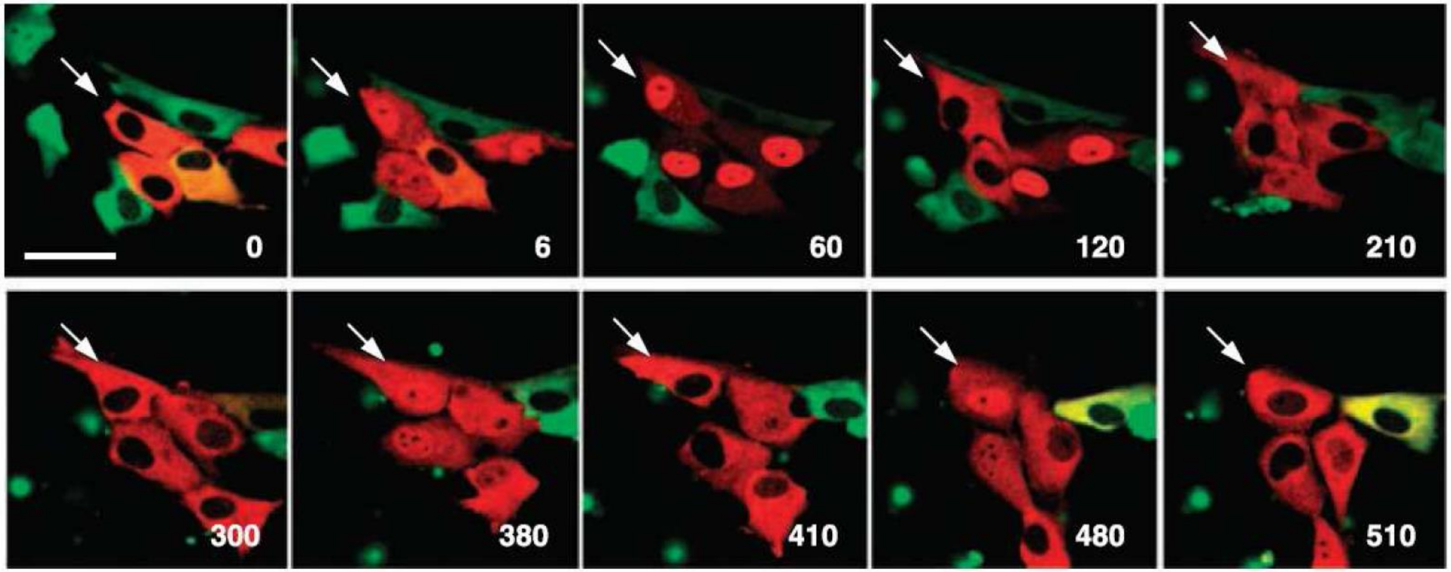
(p-type Ge: Phys Rev Lett. 57)

Sliding CDW's at UCLA:
Brown, Mozurkewich, Gruner



(Phys Rev Lett. 52)

'Direct' observations of oscillations in nucleus



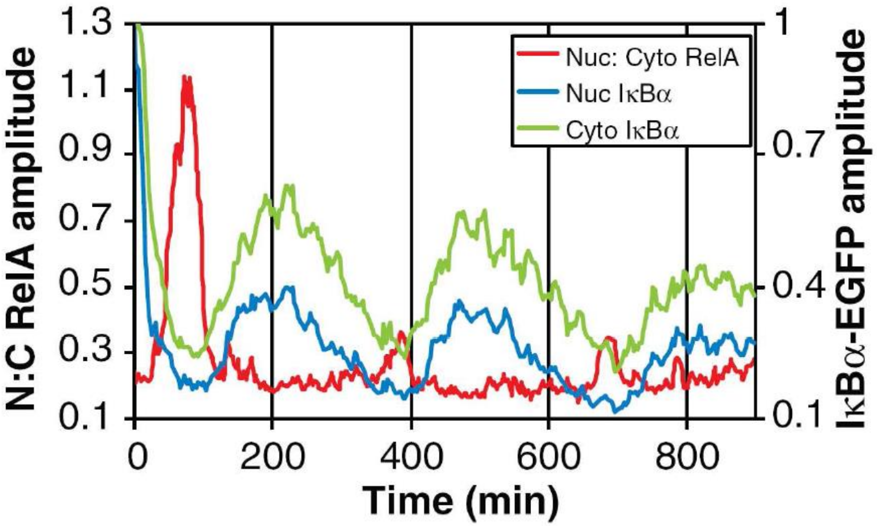
Oscillations in the nuclear localization of an NF-κB transcription factor in human cells

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

The NF-κB 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.

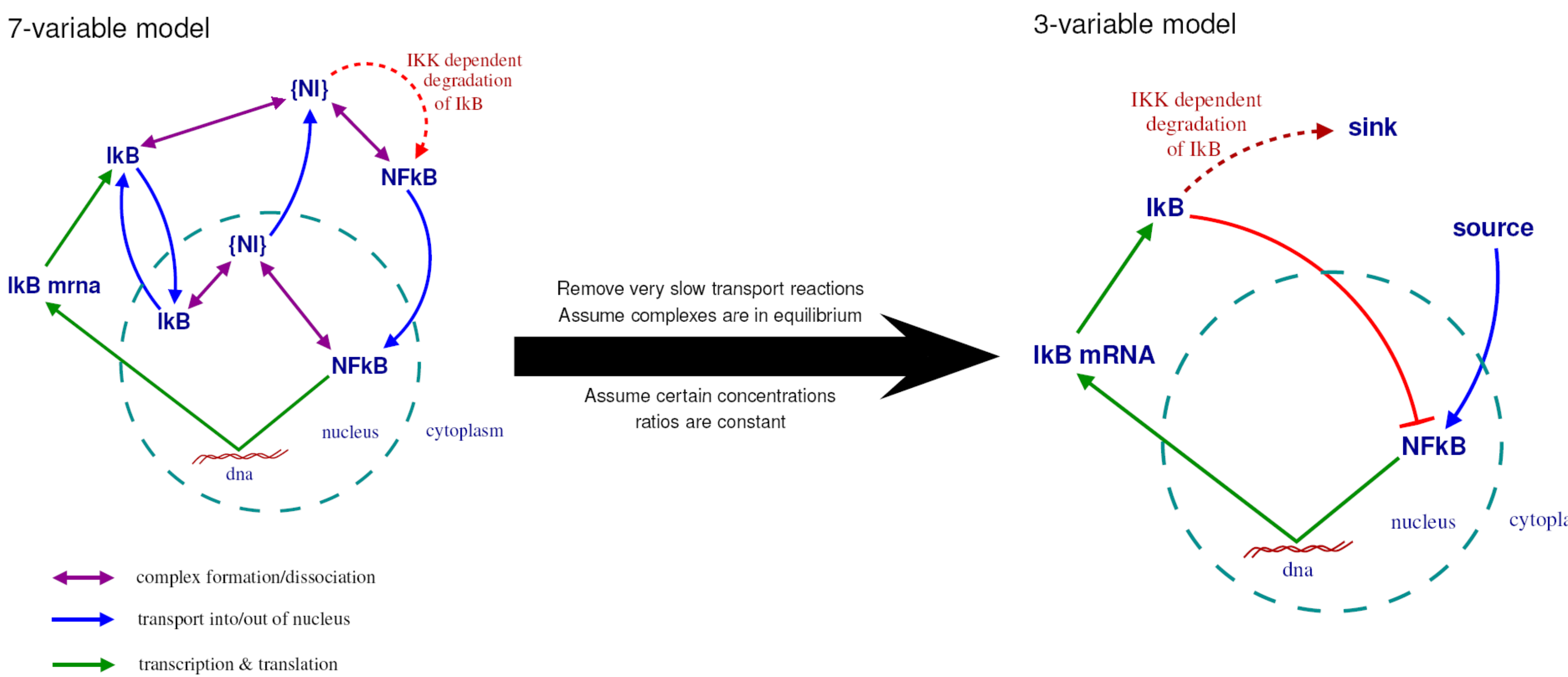
Nelson et al. (2004) Science 306, 704.



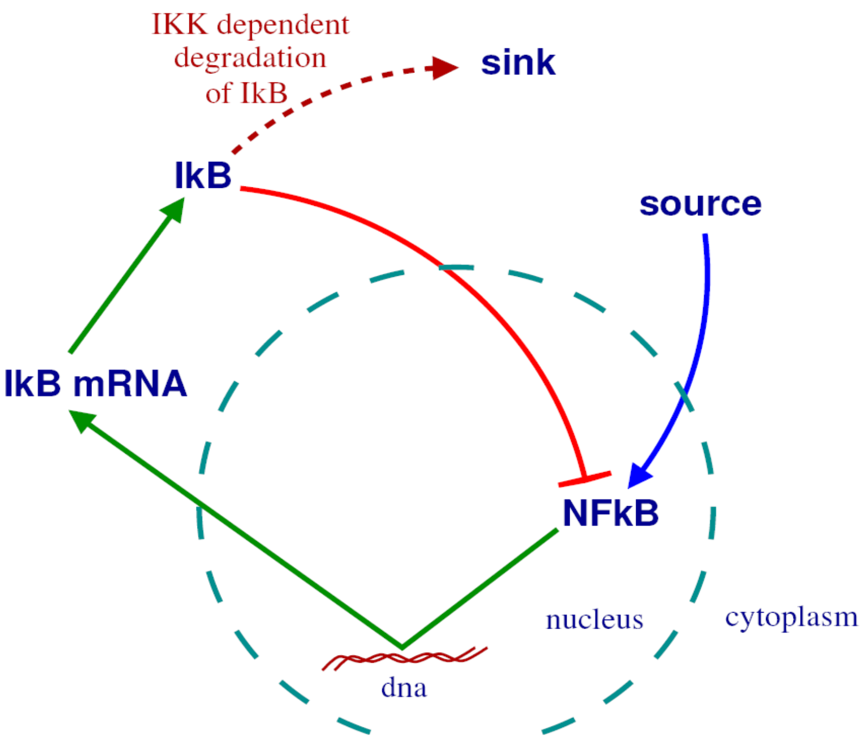
How does the network produce oscillations?

Why does the cell need the oscillations?

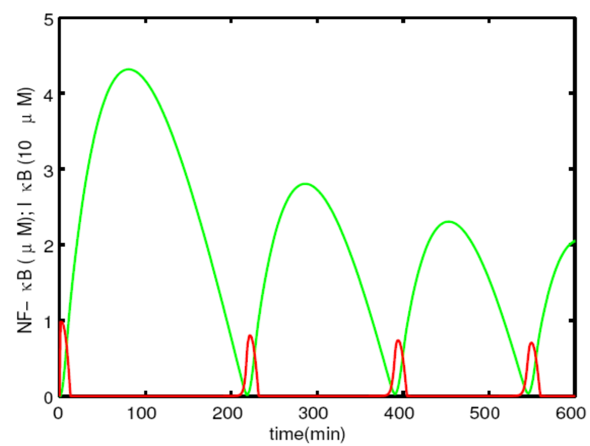
Reduction of the NF-κB system



Simple Model for Protein Oscillations



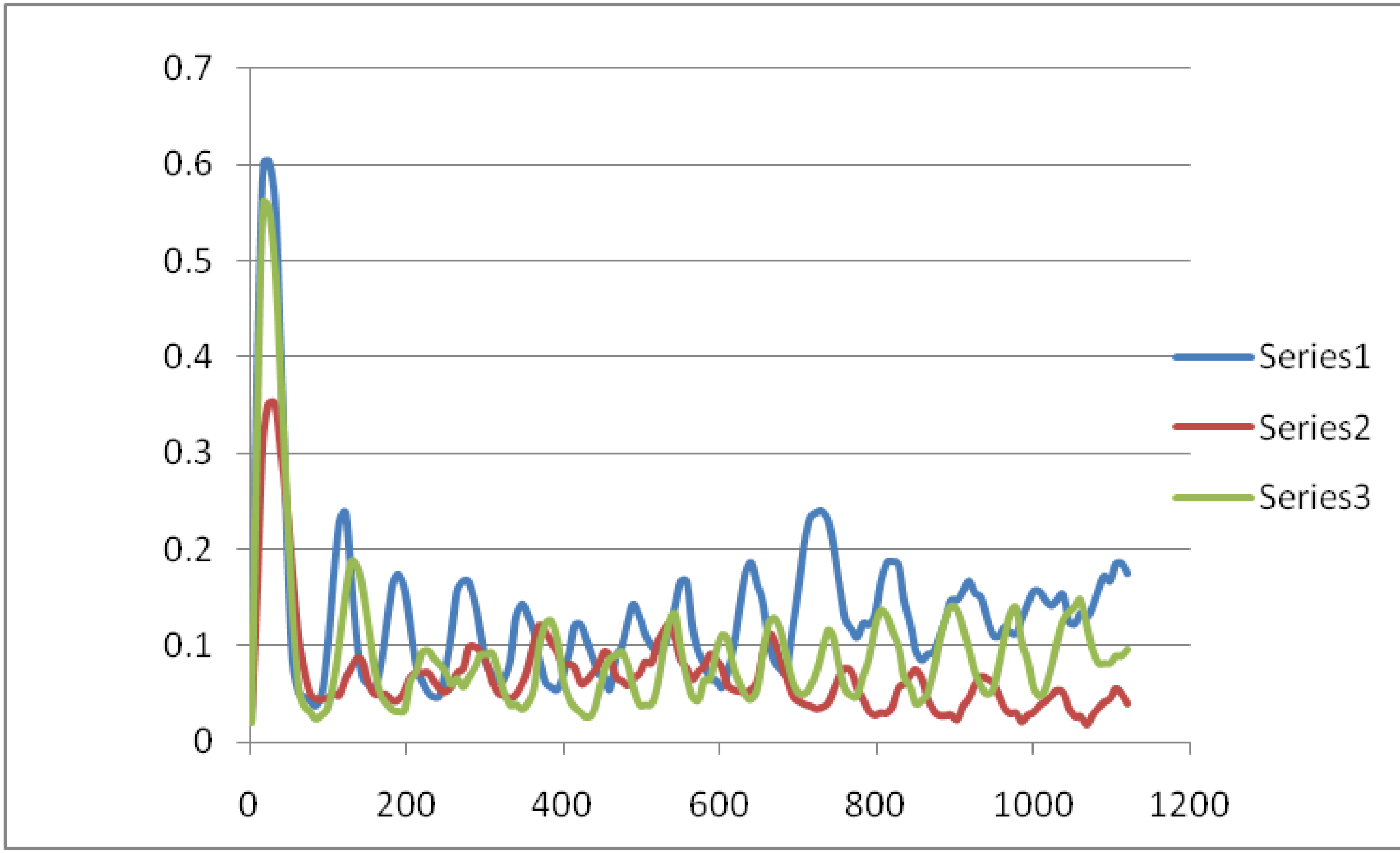
$$
\begin{aligned}
\frac{dN_n}{dt} &= A\frac{(1-N_n)}{\epsilon+I} - B\frac{IN_n}{\delta+N_n}, \\
\frac{dI_m}{dt} &= N_n^2 - I_m, \\
\frac{dI}{dt} &= I_m - C\frac{(1-N_n)I}{\epsilon+I}.
\end{aligned}
$$



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

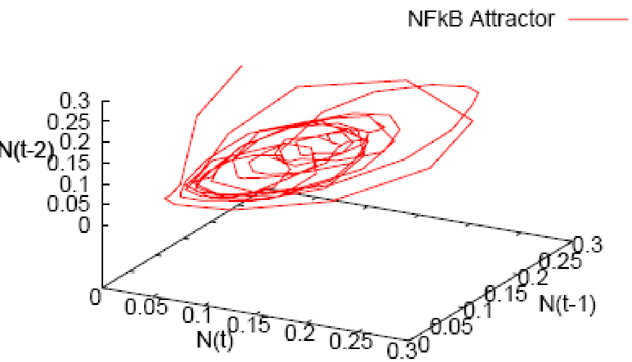
$$\delta = 0.029,\ \epsilon = 2\times10^{-5}$$

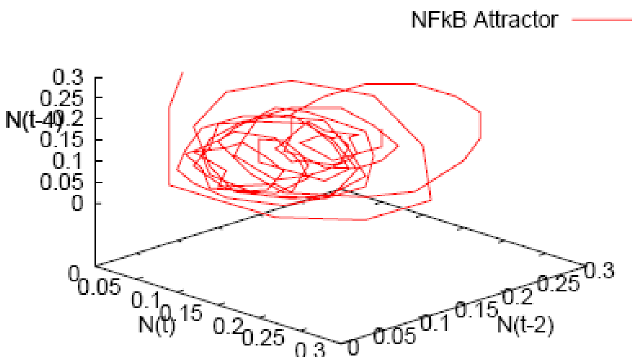
Oscillations of protein densities in a single cell

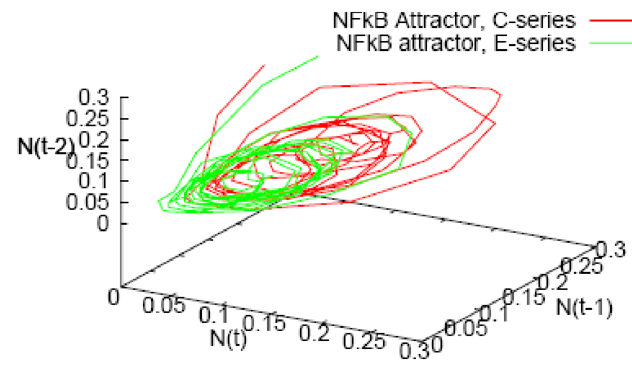


(M. Covert, Stanford, unpublished)
(Savas Tay, Zurich)

Embedded attractors:
Chaos ??







Externally ‘forced’ NF-κB system

External modulation of TNF cytokine signal

$\rightarrow$ Transformed into IKK signal (C)

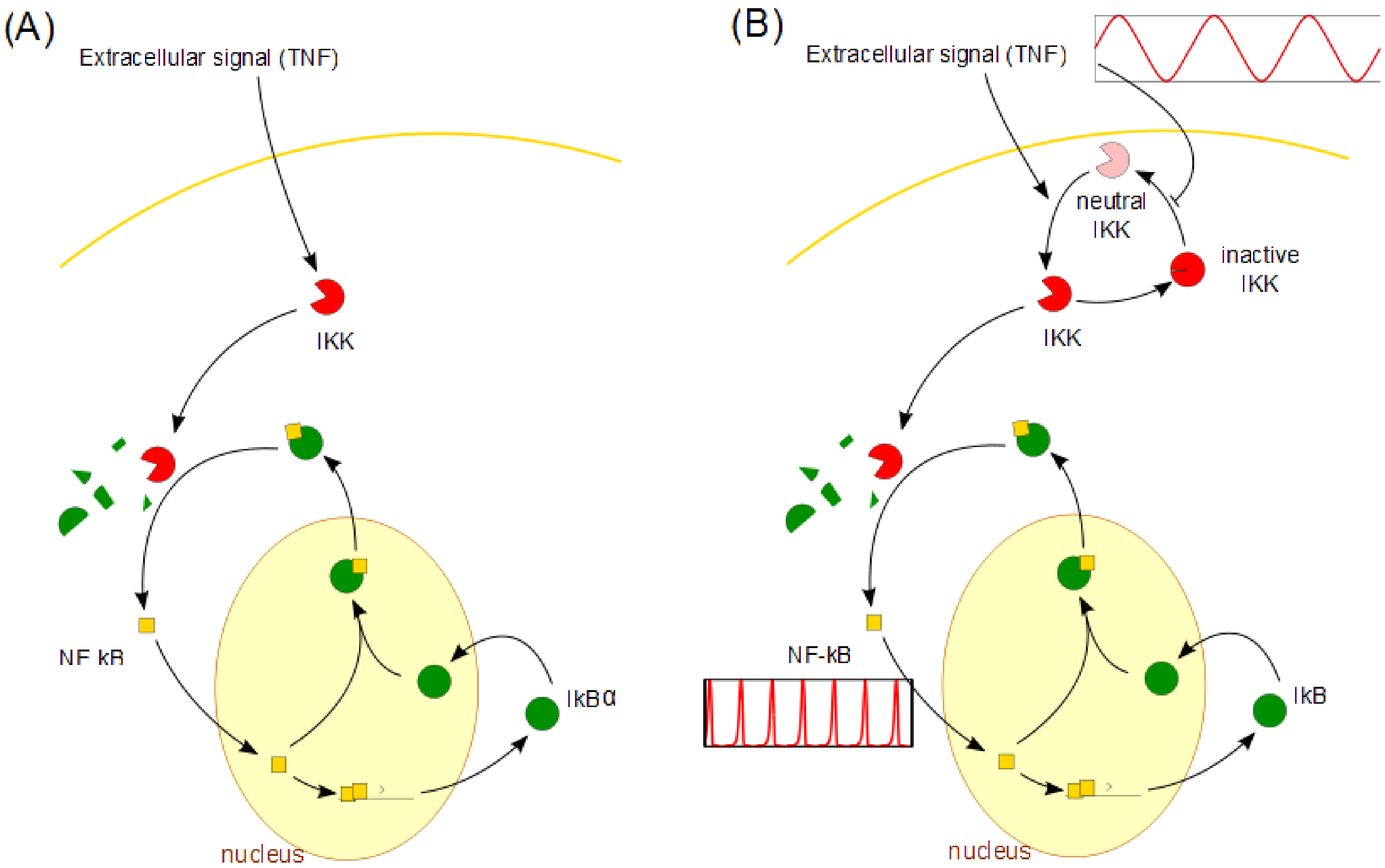
Arnold tongues:

Can synchronize the dynamics of a single cell:

Maybe a way to control DNA damage/DNA repair

(M. Heltberg, S. Krishna, MHJ)

Externally ‘forced’ NF-κB system



(S. Krishna, MHJ)

NFκB model, driven by TNF:

NFκB

$$\frac{dN_n}{dt} = k_{Nin}(N_{tot} - N_n)\frac{K_I}{K_I + I} - k_{Iin}I\frac{N_n}{K_N + N_n}$$

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

IκBα

$$\frac{dI}{dt} = k_{tl}I_m - \alpha[IKK]_a(N_{tot} - N_n)\frac{I}{K_I + I}$$

IKK

$$\frac{d[IKK]_a}{dt} = k_a[TNF]([IKK]_{tot} - [IKK]_a - [IKK]_i) - k_i[IKK]_a$$

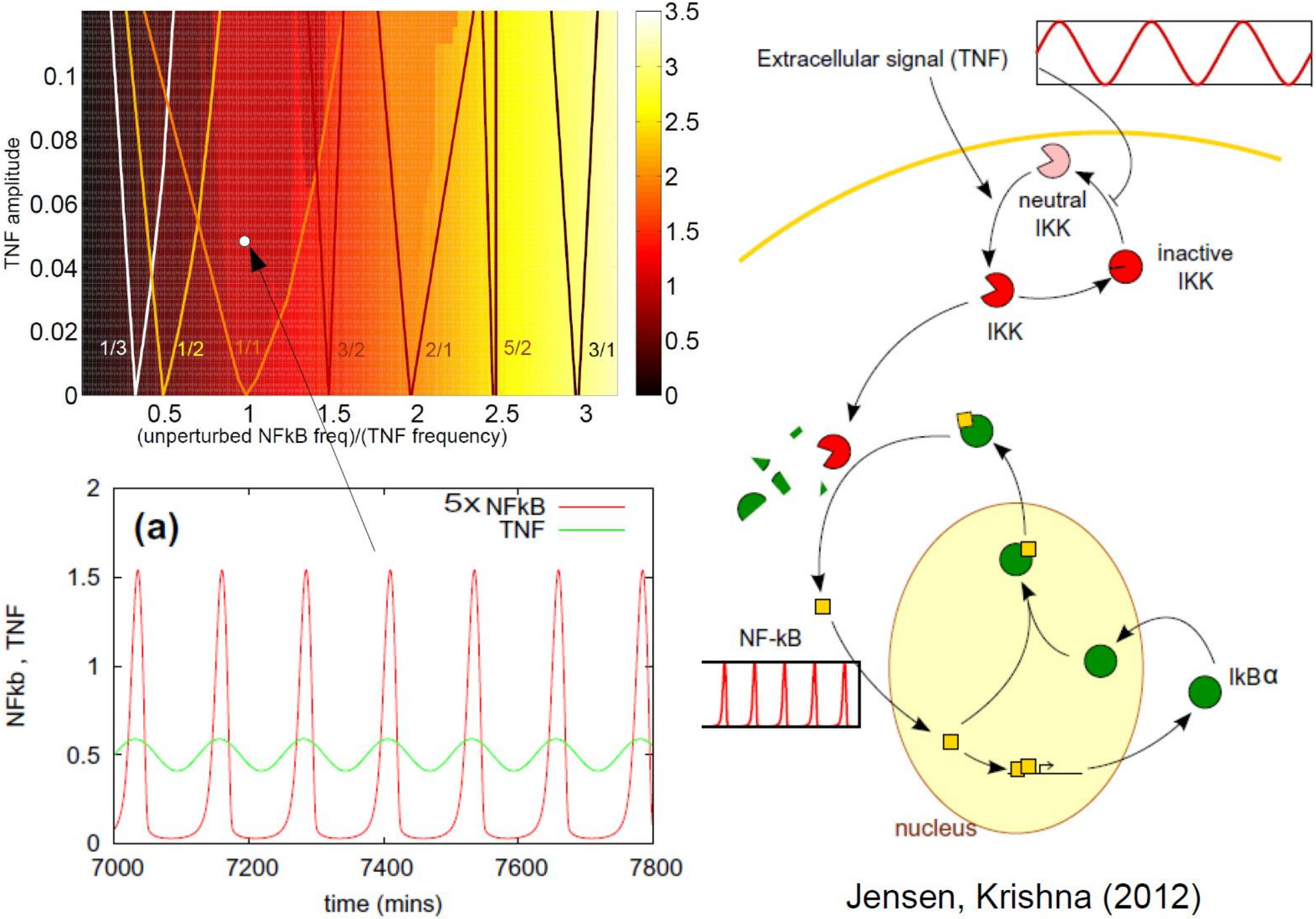
TNF

$$\frac{d[IKK]_i}{dt} = k_i[IKK]_a - k_p[IKK]_i\frac{k_{A20}}{k_{A20} + [A20][TNF]}$$

A20

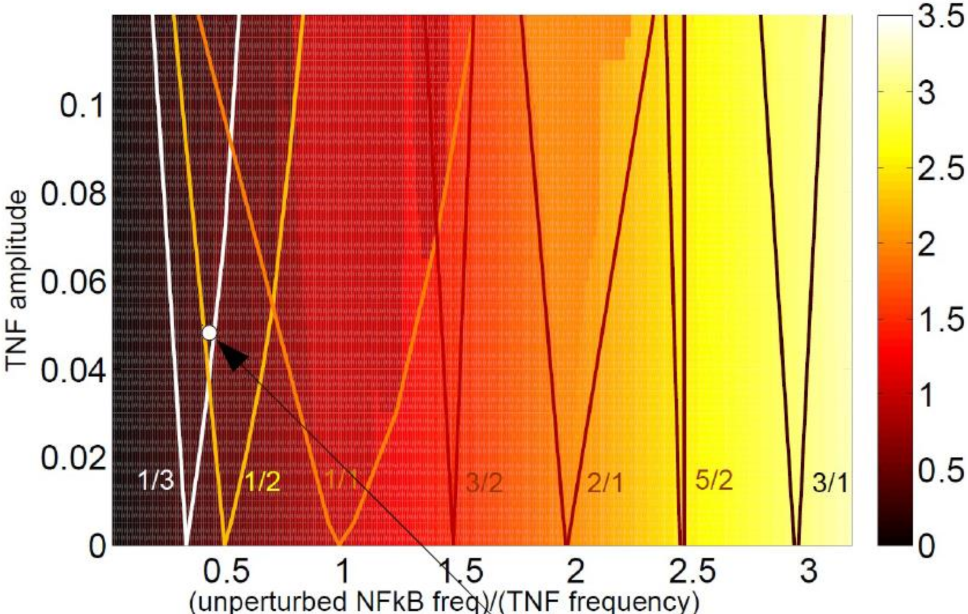
IKK, TNF, A20: Ashall, Rand, White, et al…. Science (2009)

Sinusoidally driven NF-kB oscillations



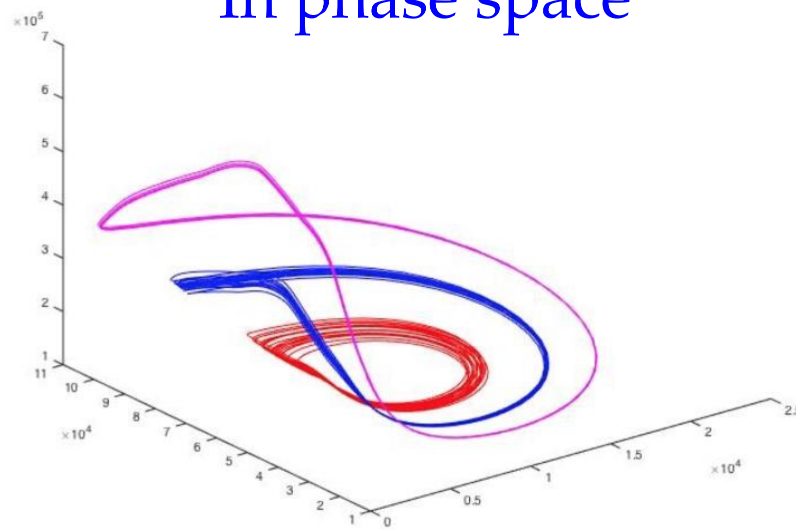
Jensen, Krishna (2012)

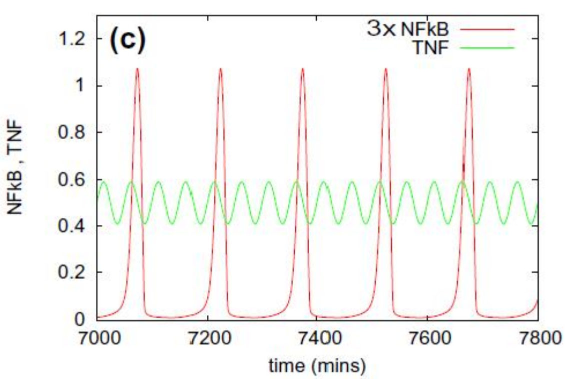
Sinusoidally driven NF-kB oscillations

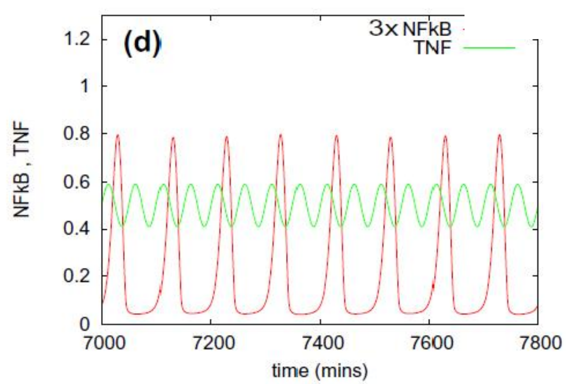


Tongues overlap !

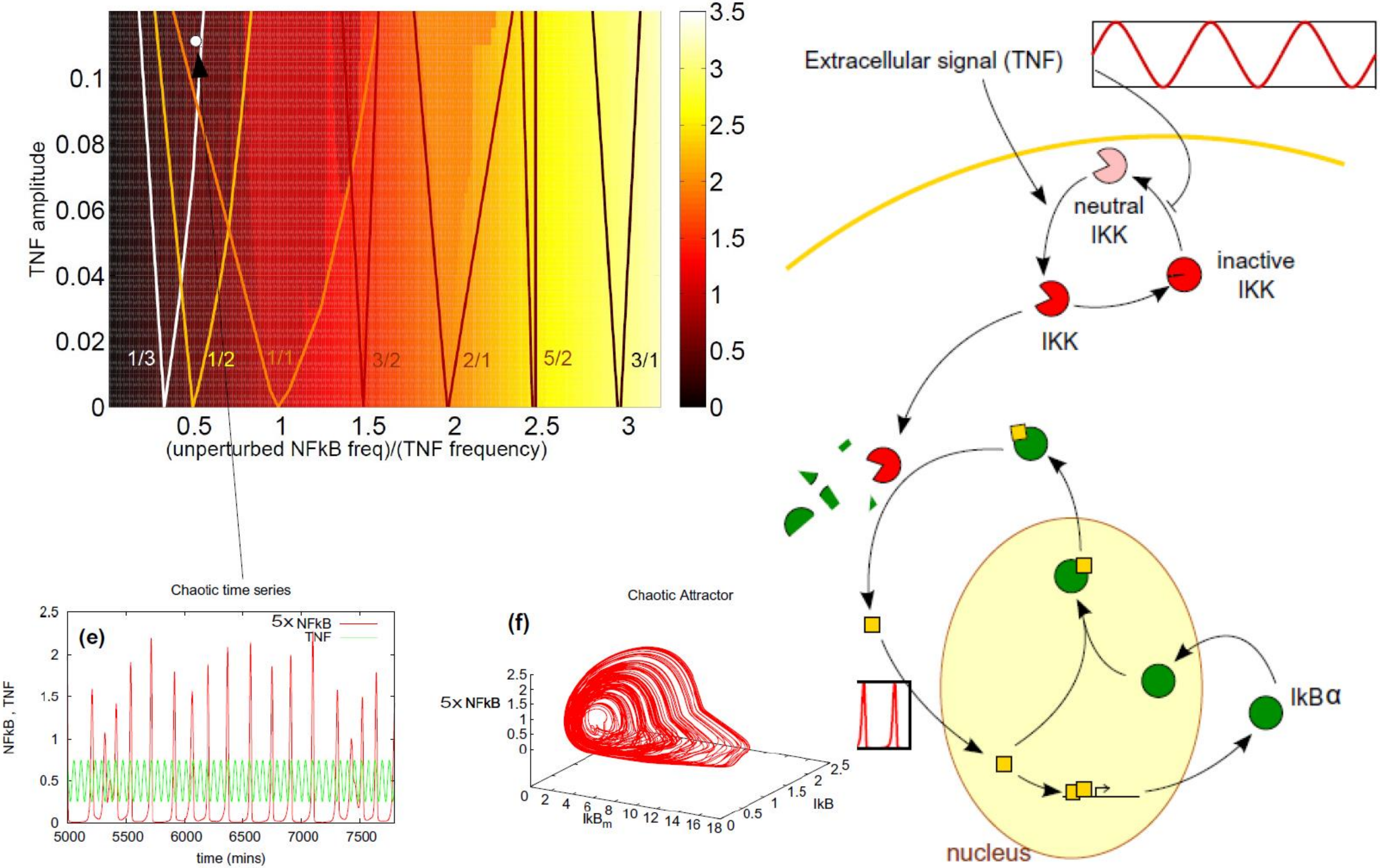
In phase space





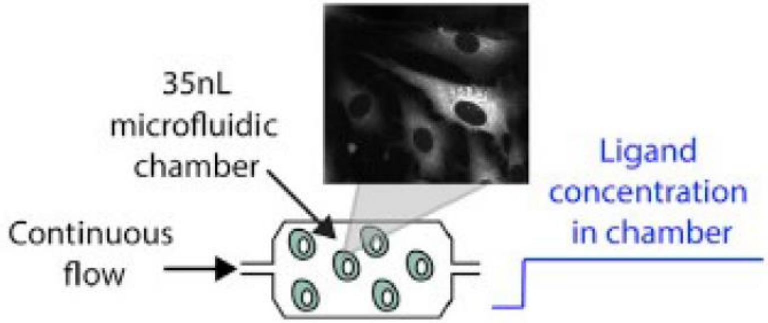


Sinusoidally driven NF-kB oscillations



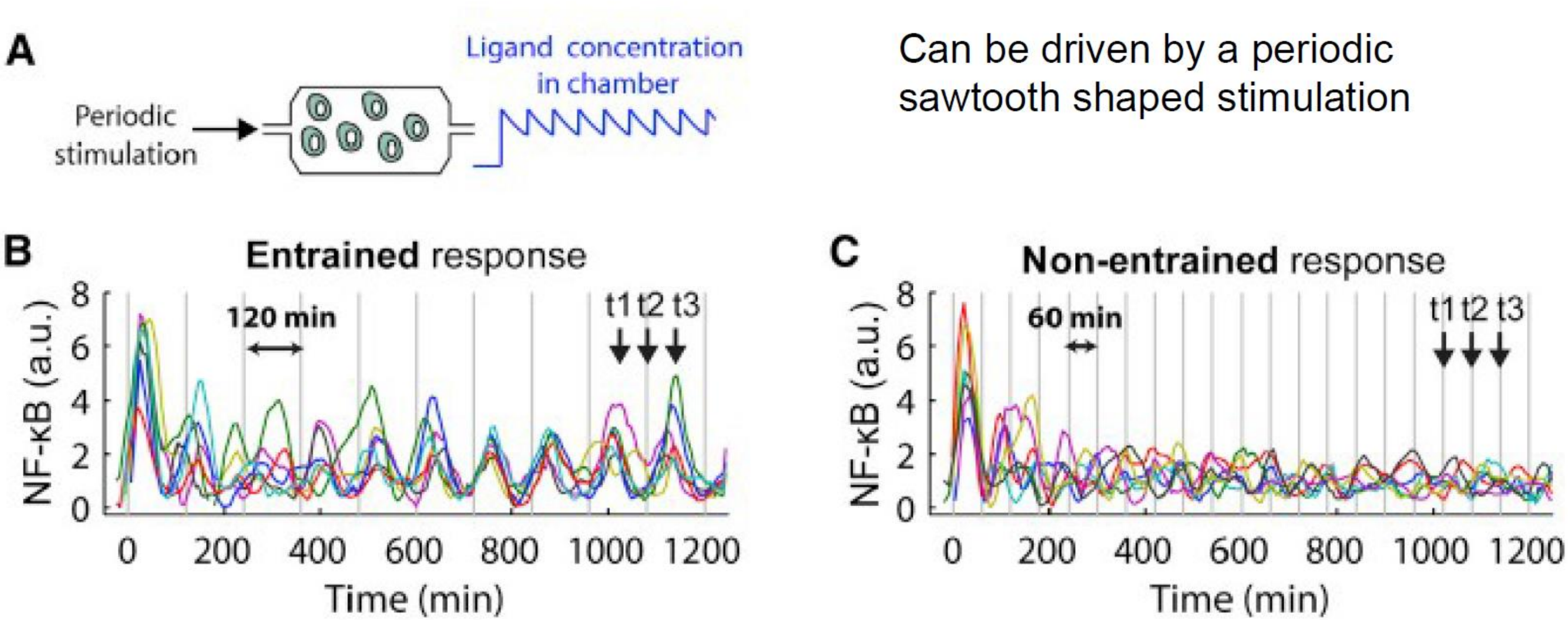
Jensen, Krishna (2012)

Sinusoidally driven NF-kB oscillations



Ryan Kellog, Savas Tay (2015)

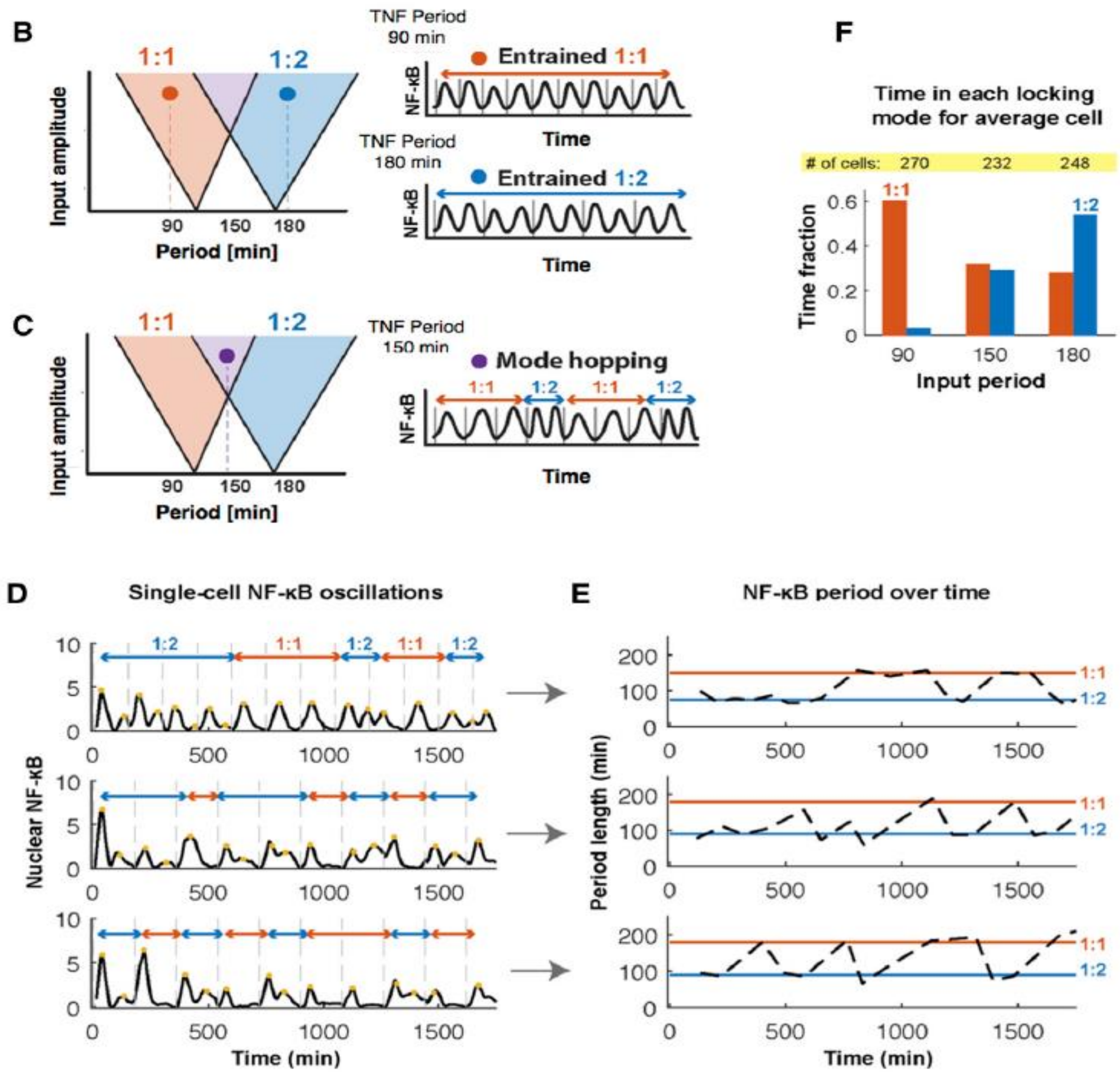
Microfluidic chamber with mouse fibroblast cells



Can be driven by a periodic sawtooth shaped stimulation

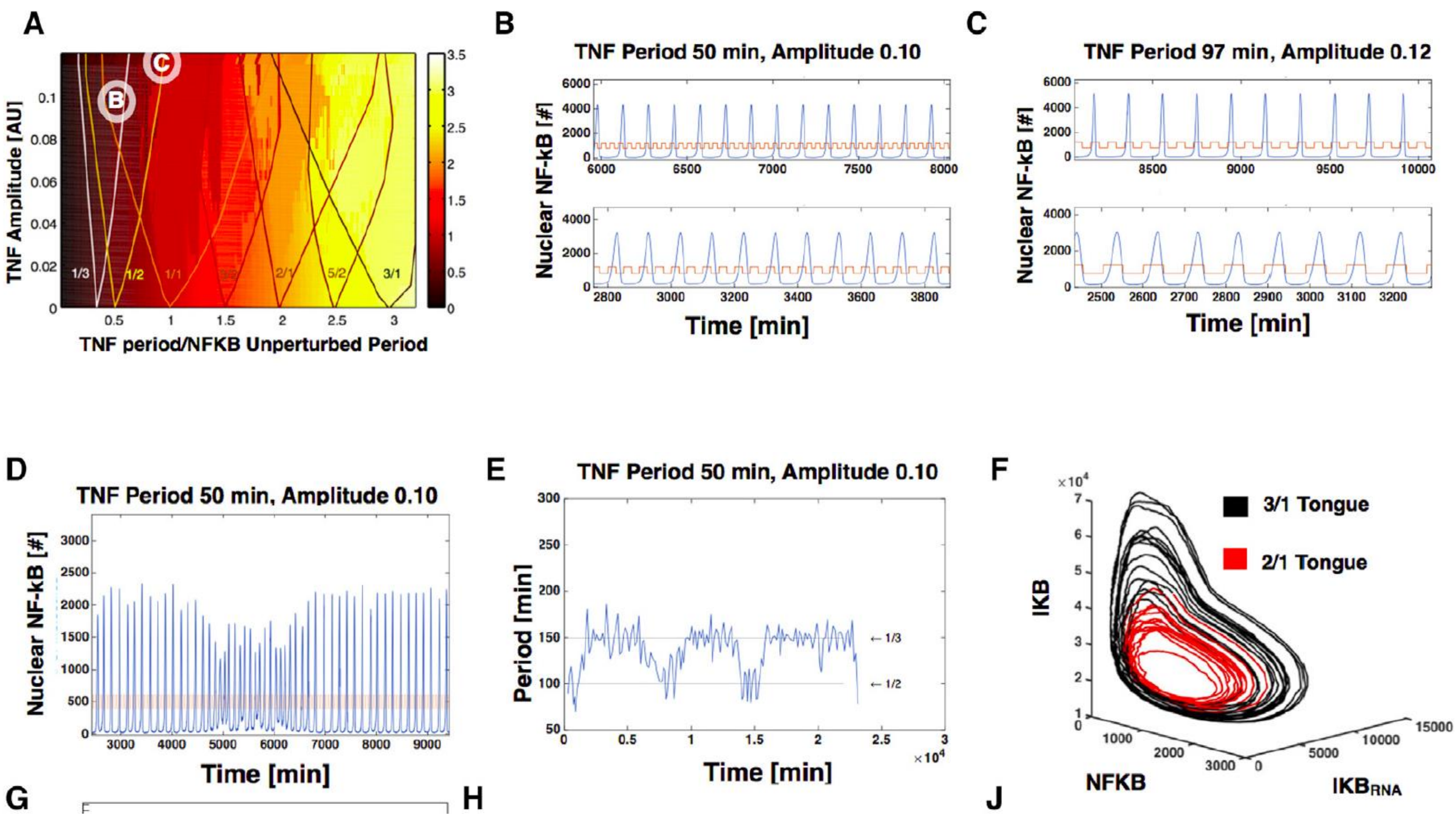
(Zurich/Chicago data: Savas Tay, Ryan Kellog)

When tongues overlap: Experimentally observed mode hopping between entrained states



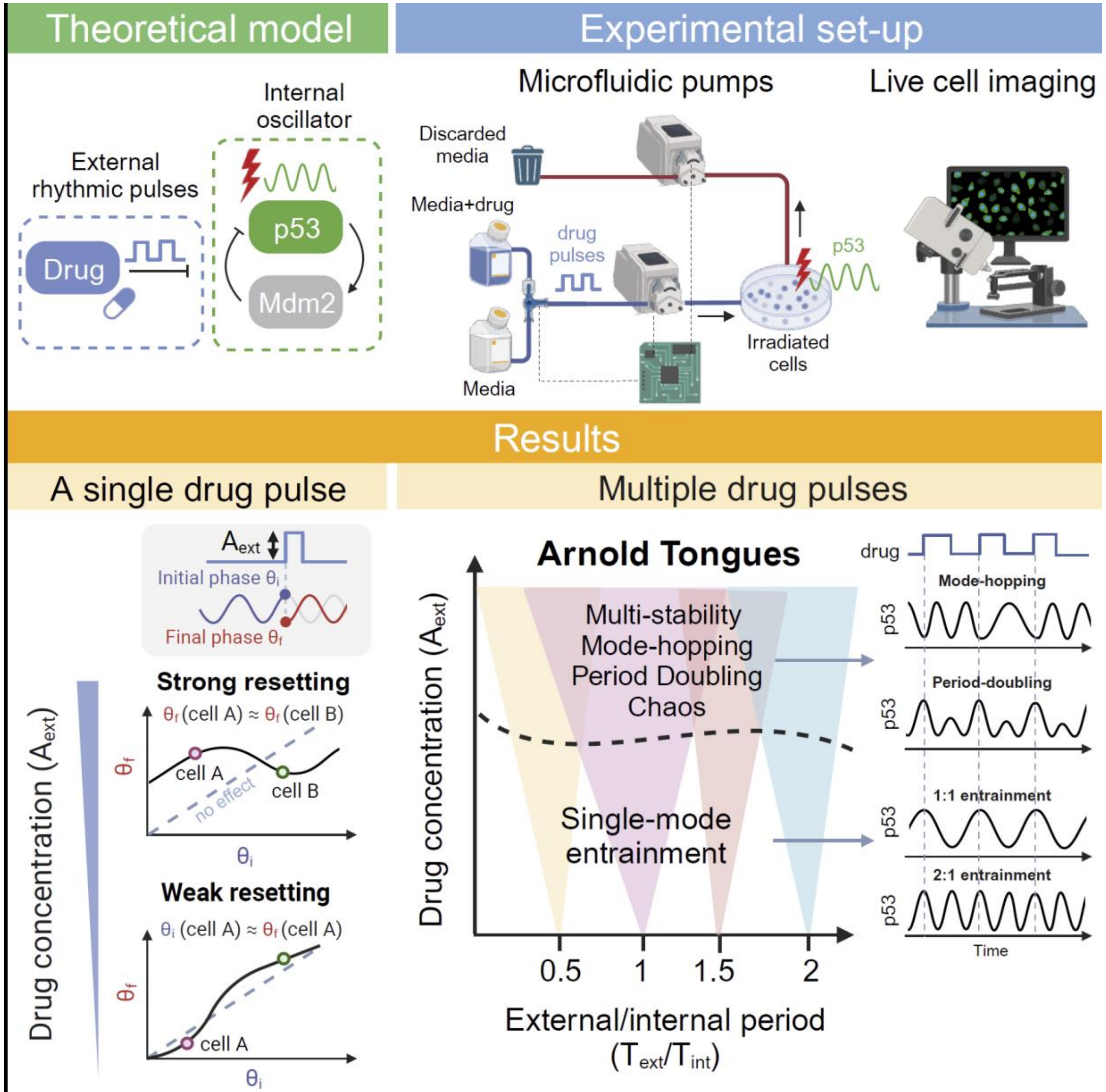
(Heltberg, Kellogg, Tay, Jensen, Cell Systems (2016))

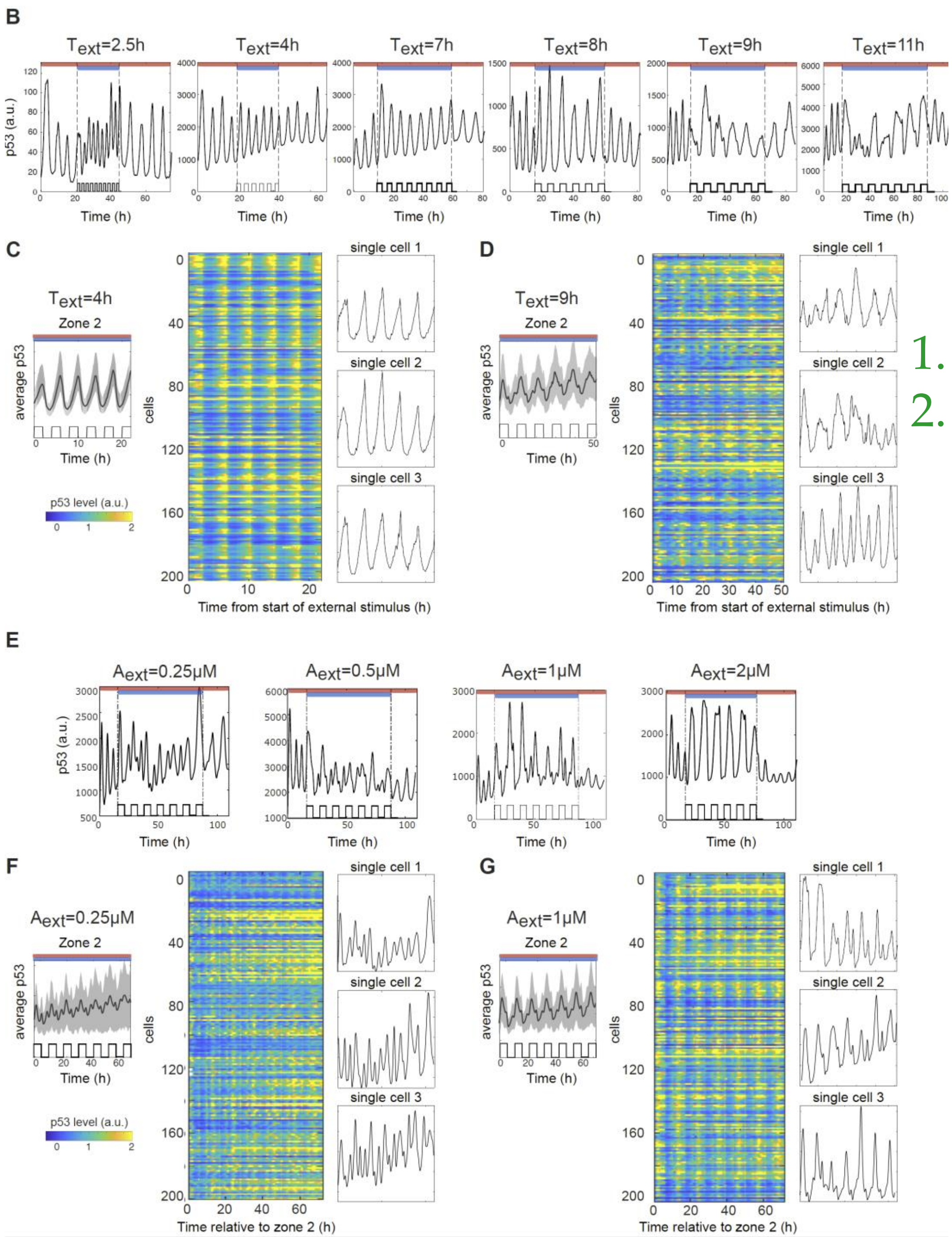
Stochastic Gillespie simulations: manifest as modehopping between entrained states



With Harvard Medical School (Lahav lab):

1. DNA damage (X-ray), sustained p53 oscillations for a week
2. Adding external oscillator (nutlin)



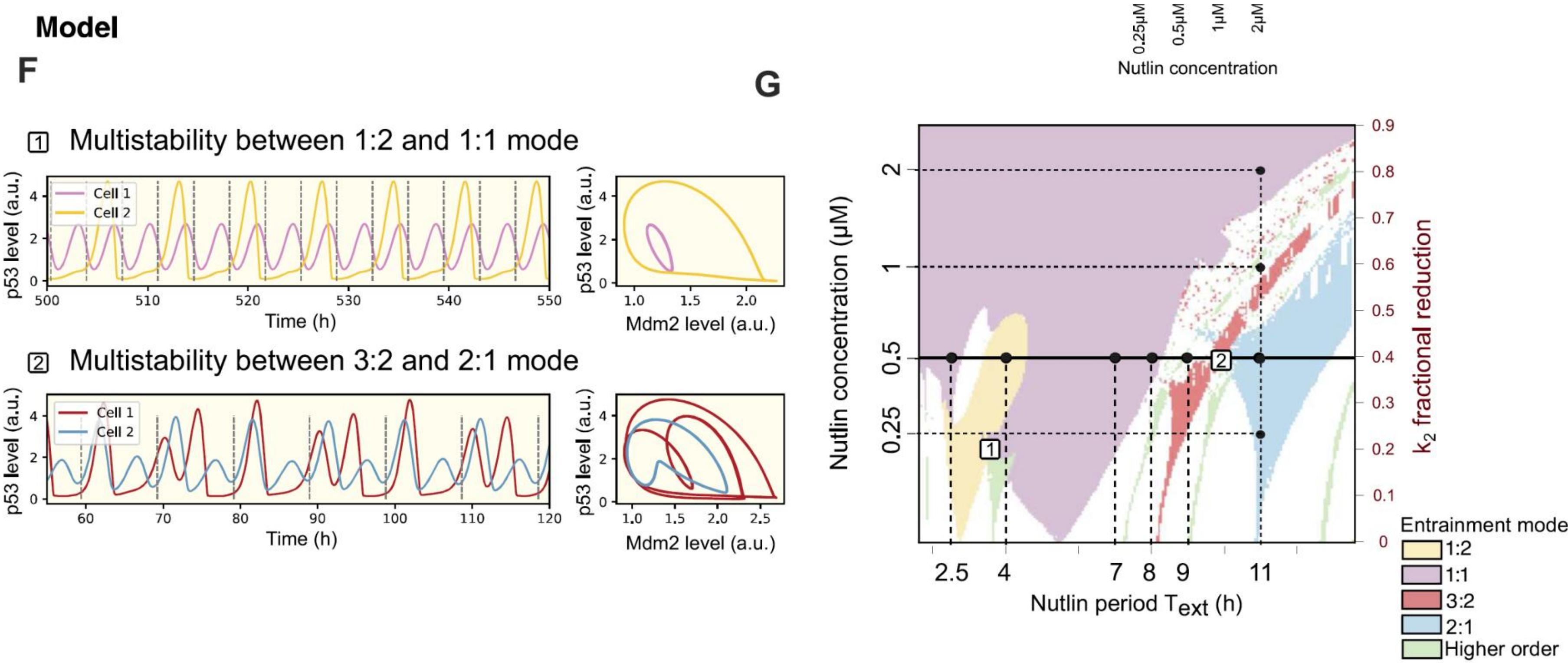


Harvard experiments:

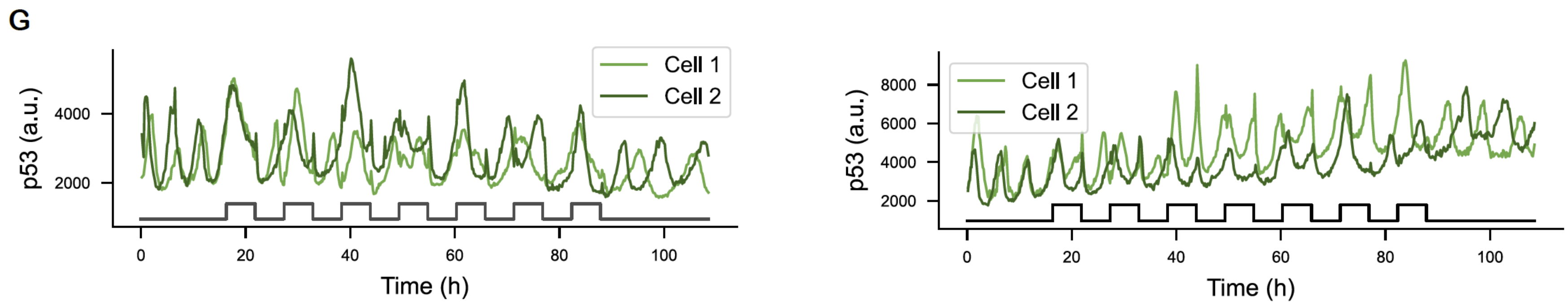
1. Nutlin period: 2.5h -> 11h
2. Nutlin amptude: 0.25μM -> 2 μM

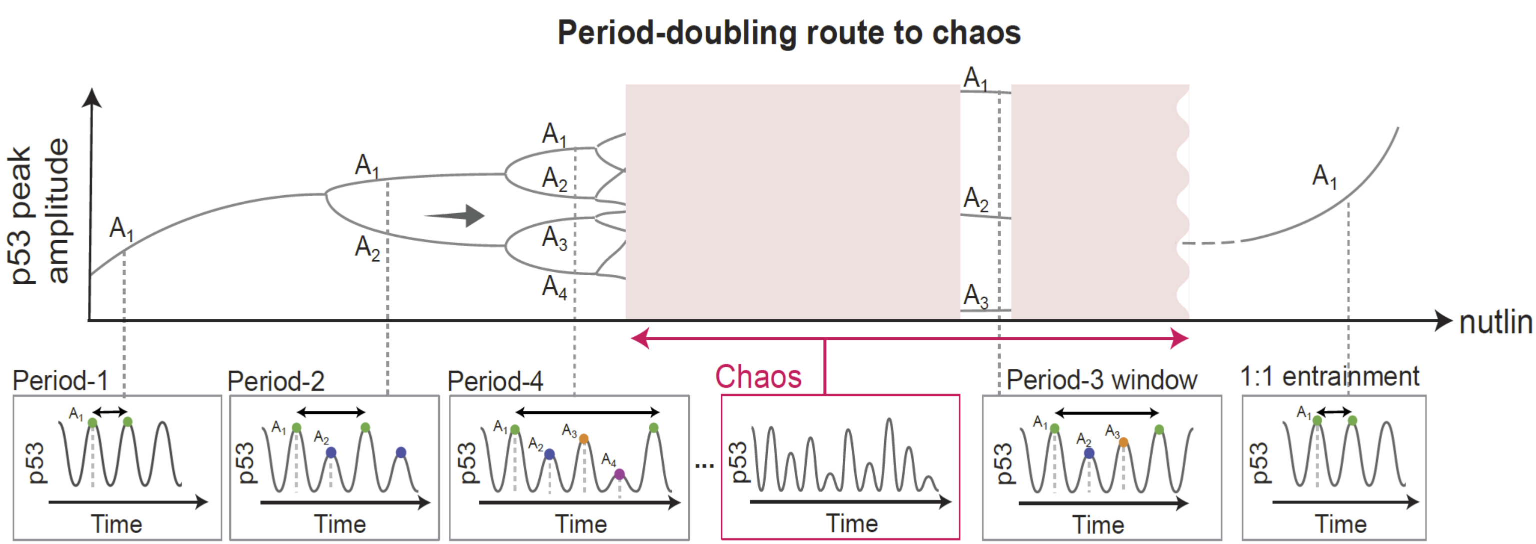
(Galit Lahav, Alba Jimenez)

Multistability and Arnold Tongues: Model

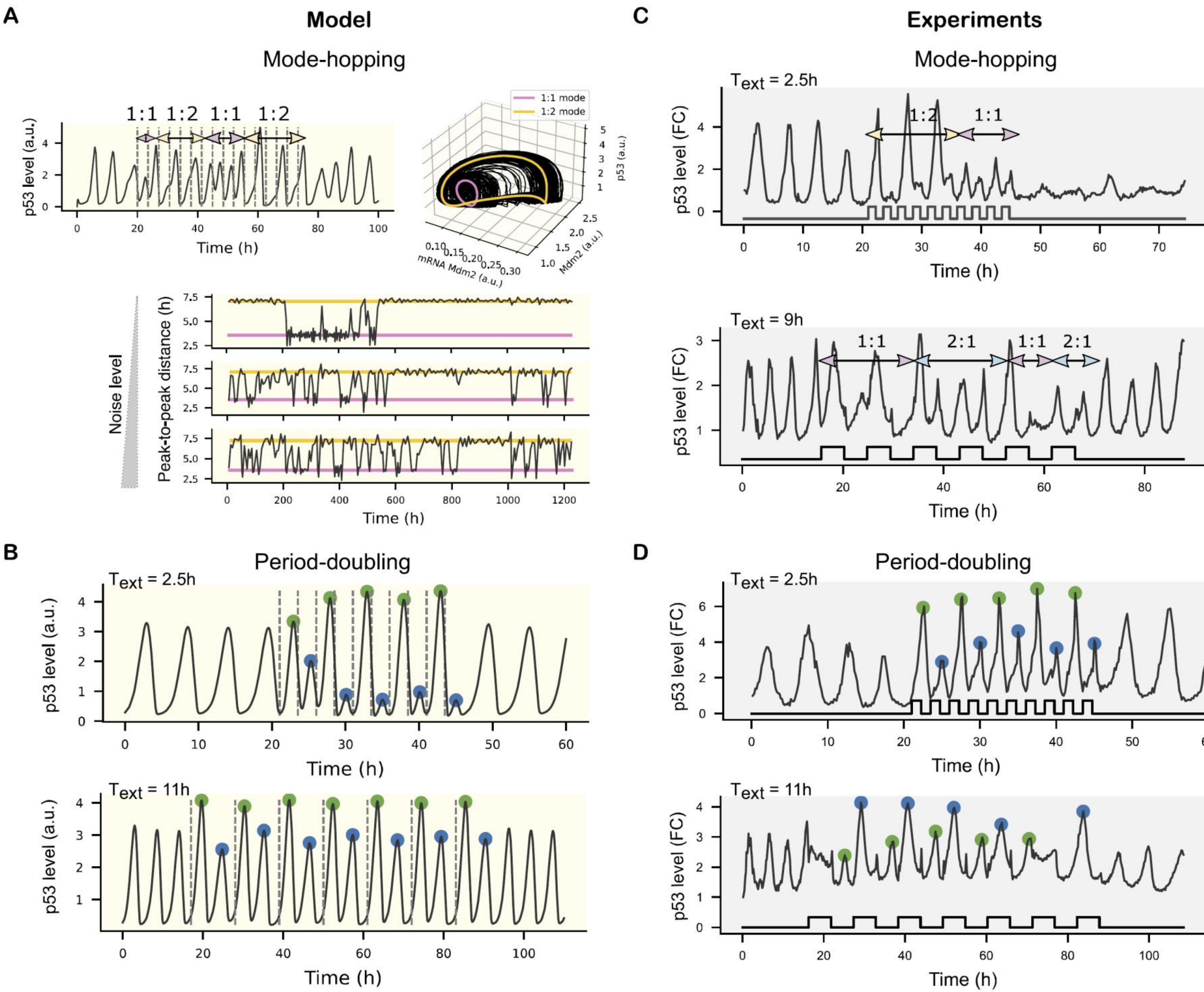


Indications of chaos:
Sensitive dependence on initial conditions

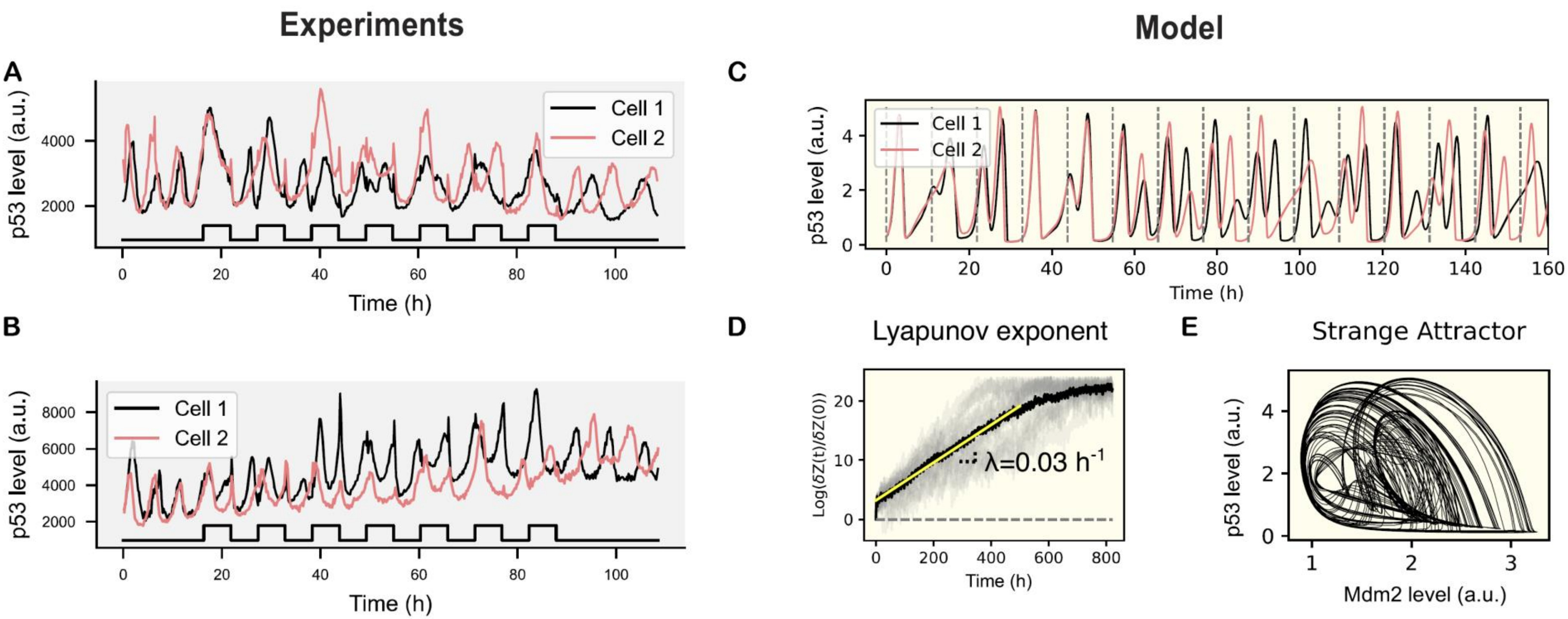




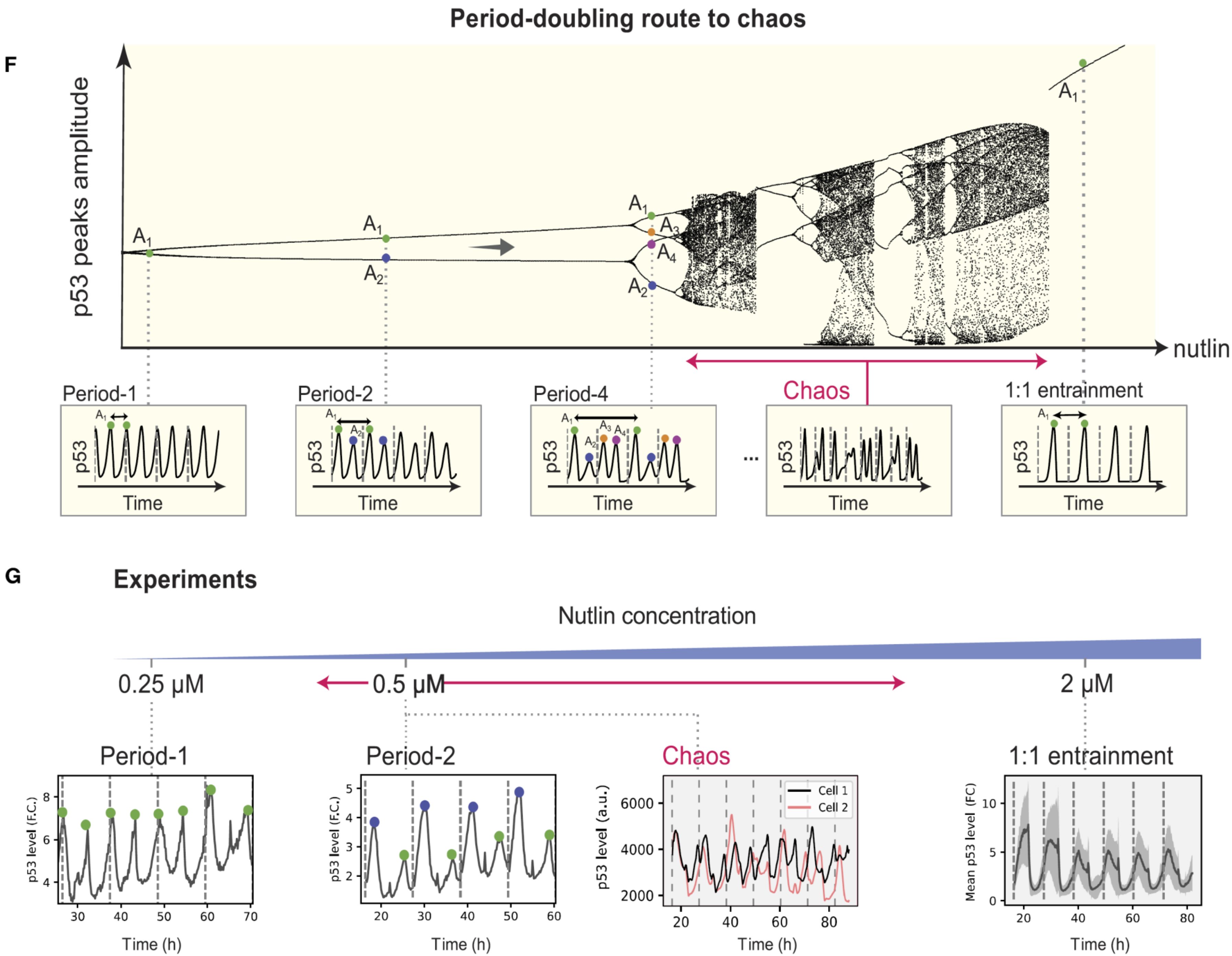
(Heltberg, Jimenez, Lahav, Jensen, Cell Systems, in print)


A
Model
Mode-hopping
1:1 1:2 1:1 1:2
p53 level (a.u.)
Time (h)
1:1 mode
1:2 mode
p53 (a.u.)
mRNA Mdm2 (a.u.)
Mdm2 (a.u.)
Noise level
Peak-to-peak distance (h)
Time (h)
B
Period-doubling
T_{ext} = 2.5h
p53 level (a.u.)
Time (h)
T_{ext} = 11h
p53 level (a.u.)
Time (h)
C
Experiments
Mode-hopping
T_{ext} = 2.5h
1:2 1:1
p53 level (FC)
Time (h)
T_{ext} = 9h
1:1 2:1 1:1 2:1
p53 level (FC)
Time (h)
D
Period-doubling
T_{ext} = 2.5h
p53 level (FC)
Time (h)
T_{ext} = 11h
p53 level (FC)
Time (h)

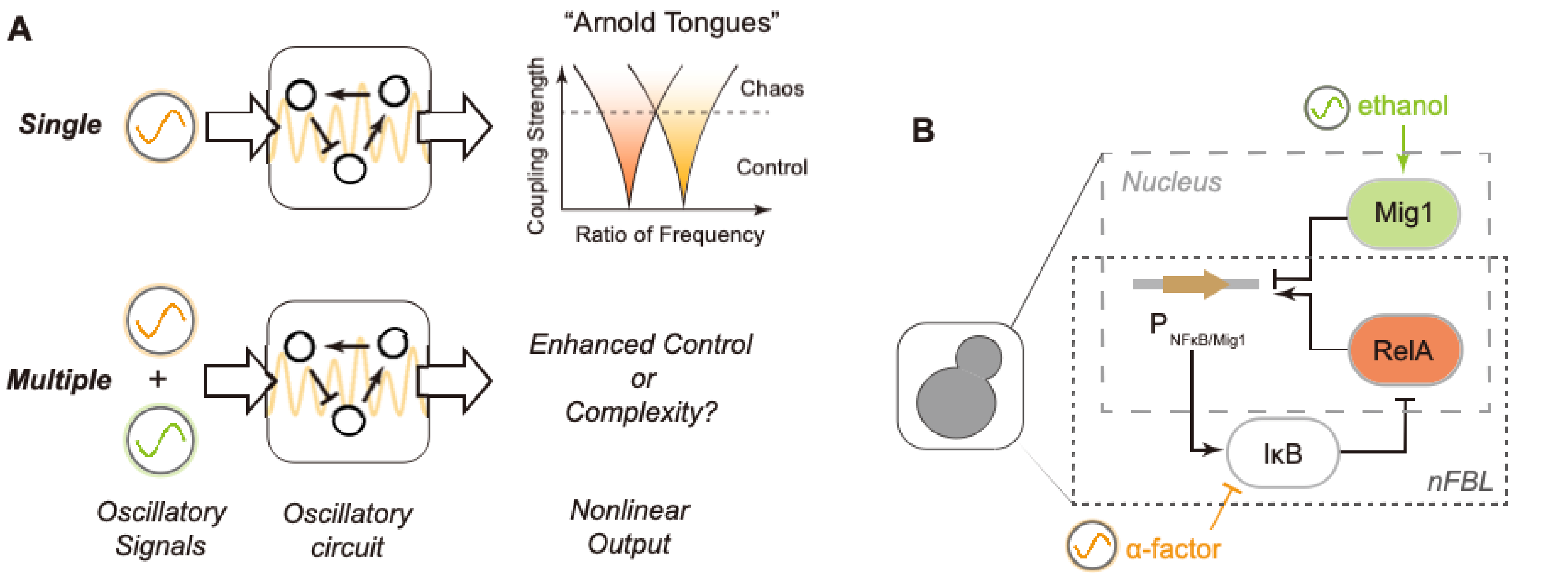
Indications of chaos:
Sensitive dependence on initial conditions



(Heltberg, Jimenez, Lahav, Jensen, Cell Systems, in print)

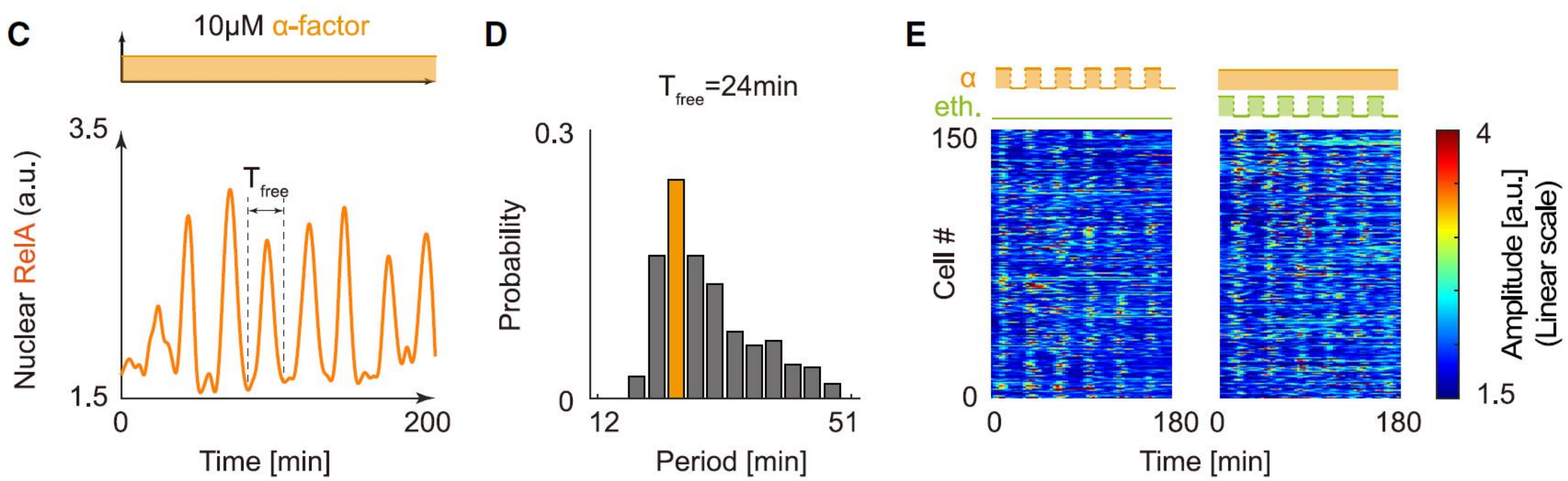

Period-doubling route to chaos
F
p53 peaks amplitude
nutlin
A1
A2
A3
A4
Period-1
Period-2
Period-4
Chaos
1:1 entrainment
p53
Time
...
G
Experiments
Nutlin concentration
0.25 μM
0.5 μM
2 μM
Period-1
Period-2
Chaos
1:1 entrainment
p53 level (F.C.)
p53 level (a.u.)
Mean p53 level (FC)
Time (h)
Cell 1
Cell 2

With two oscillatory inputs: TNF and ethanol in NF-kB: Elegant way to study **chronobiology**



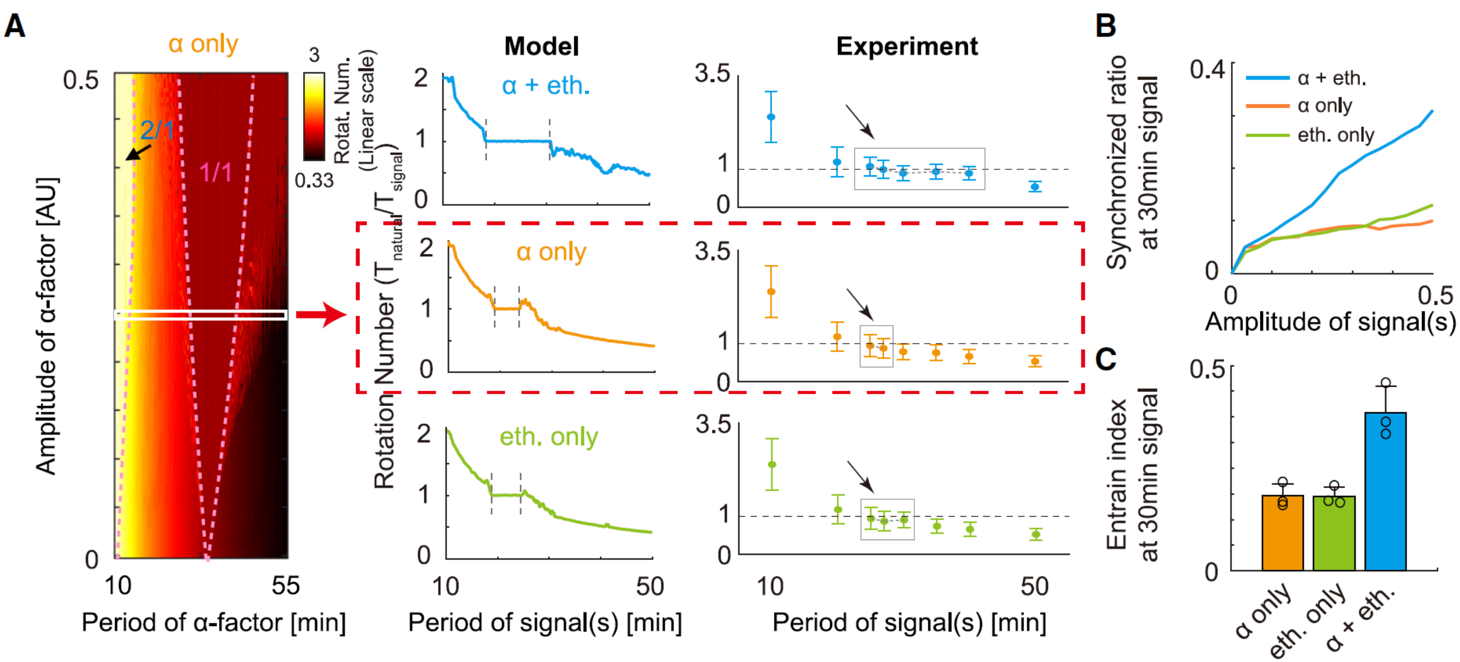
Ping Wei (Beijing/Shenzhen), Heltberg, Jensen etc (Cell Systems 14, 382-391 (2023))

With two oscillatory inputs: TNF and ethanol in NF-kB

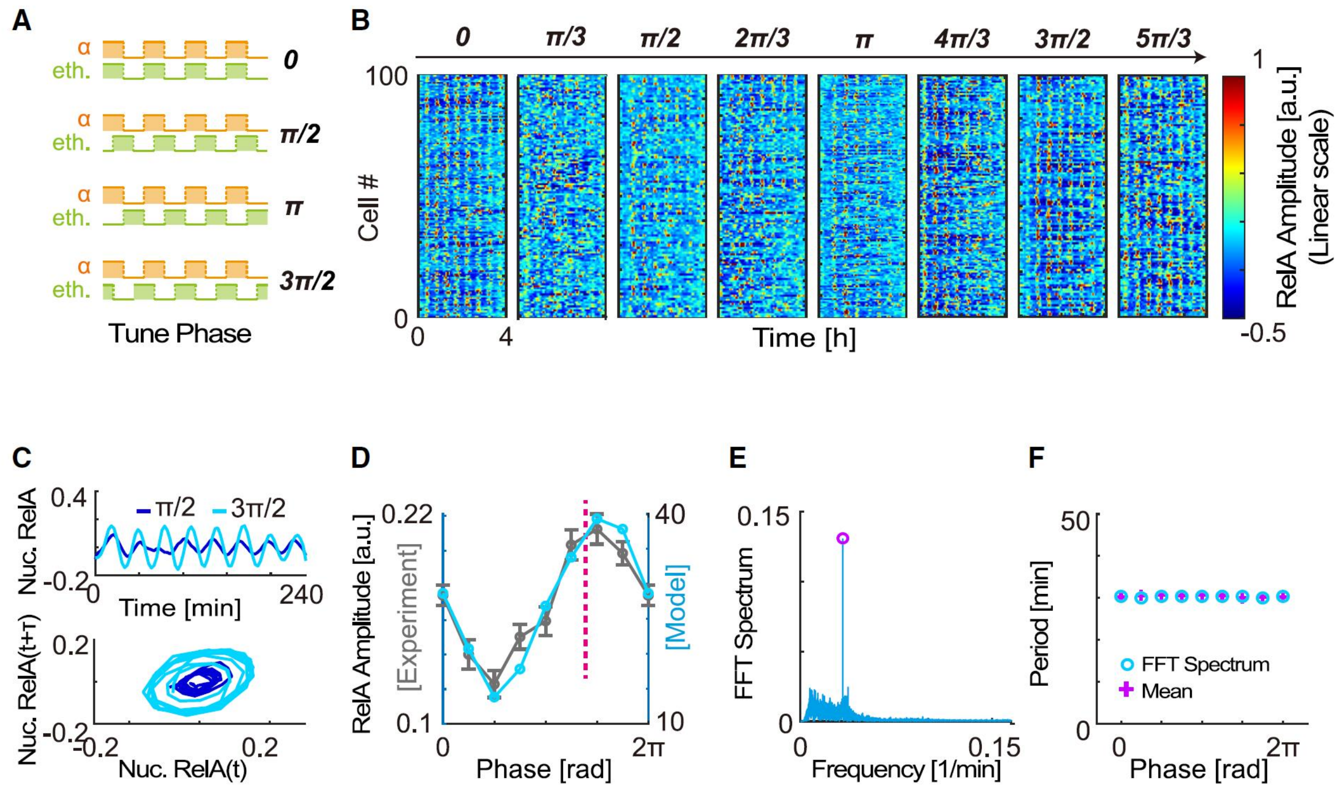


Ping Wei (Beijing/Shenzhen), Heltberg, Jensen etc (Cell Systems 14, 382-391 (2023))

Oscillatory robustness with double stimuli

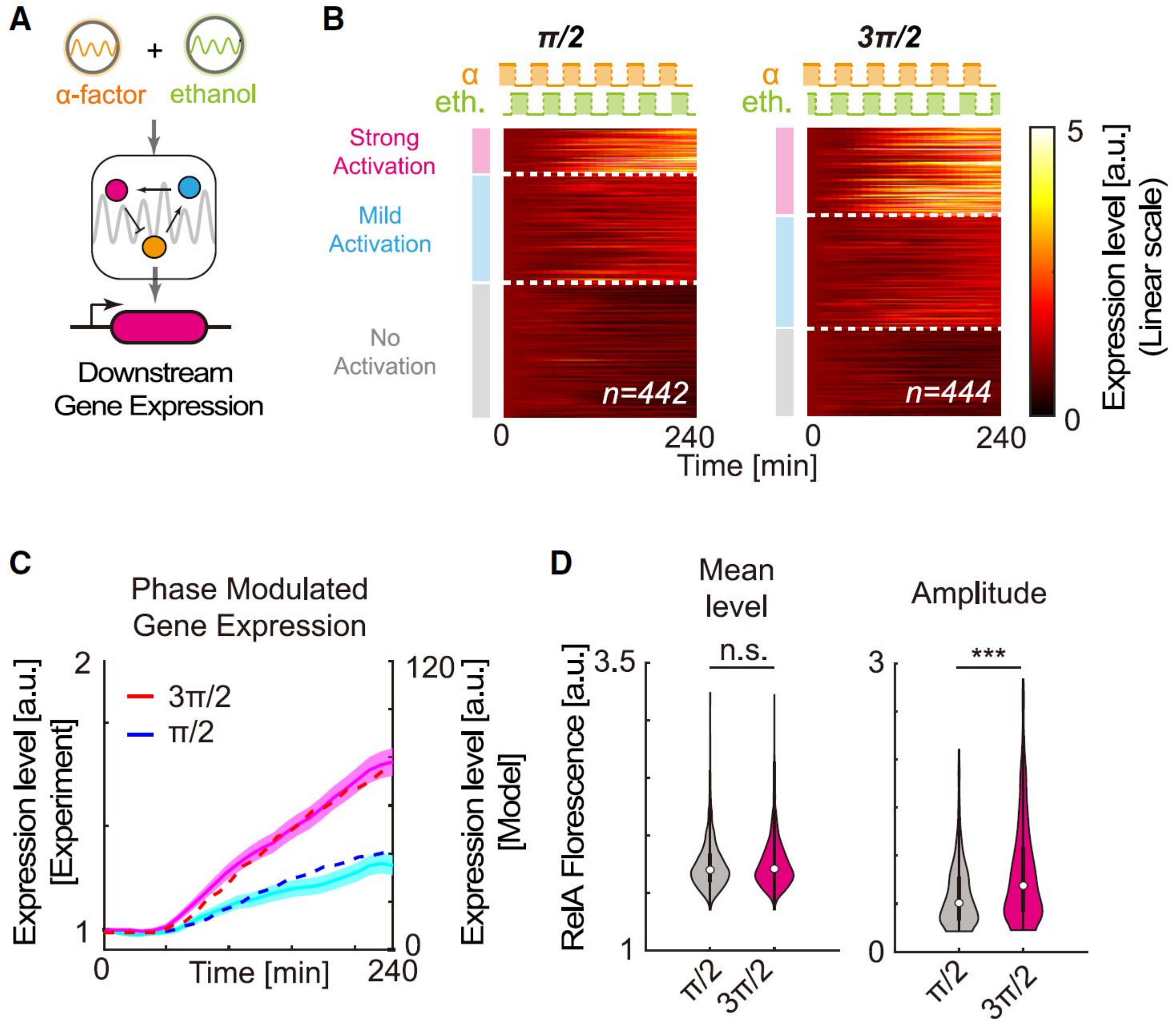


Tunability of RelA dynamics through phase variation



Highest RelA amplitude for phase 3π/2 !

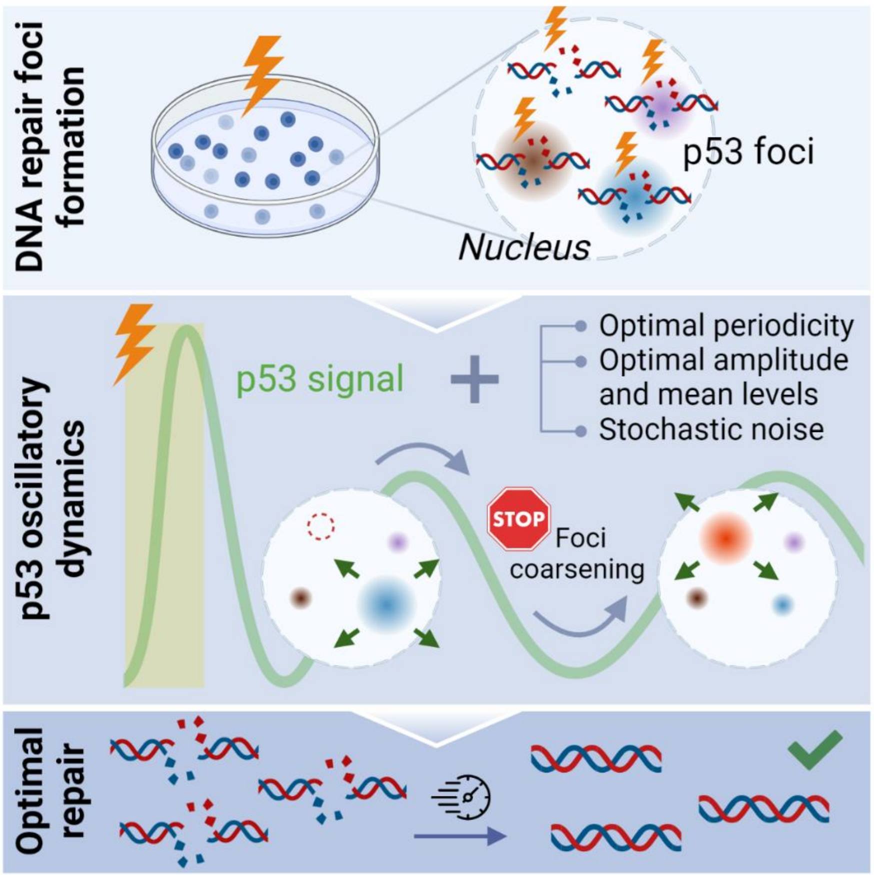
Downstream stimulation



Downstream production depends on phase difference!

Highest for phase $3\pi/2$!

DNA-damage repair through foci and p53 oscillations



In brief:

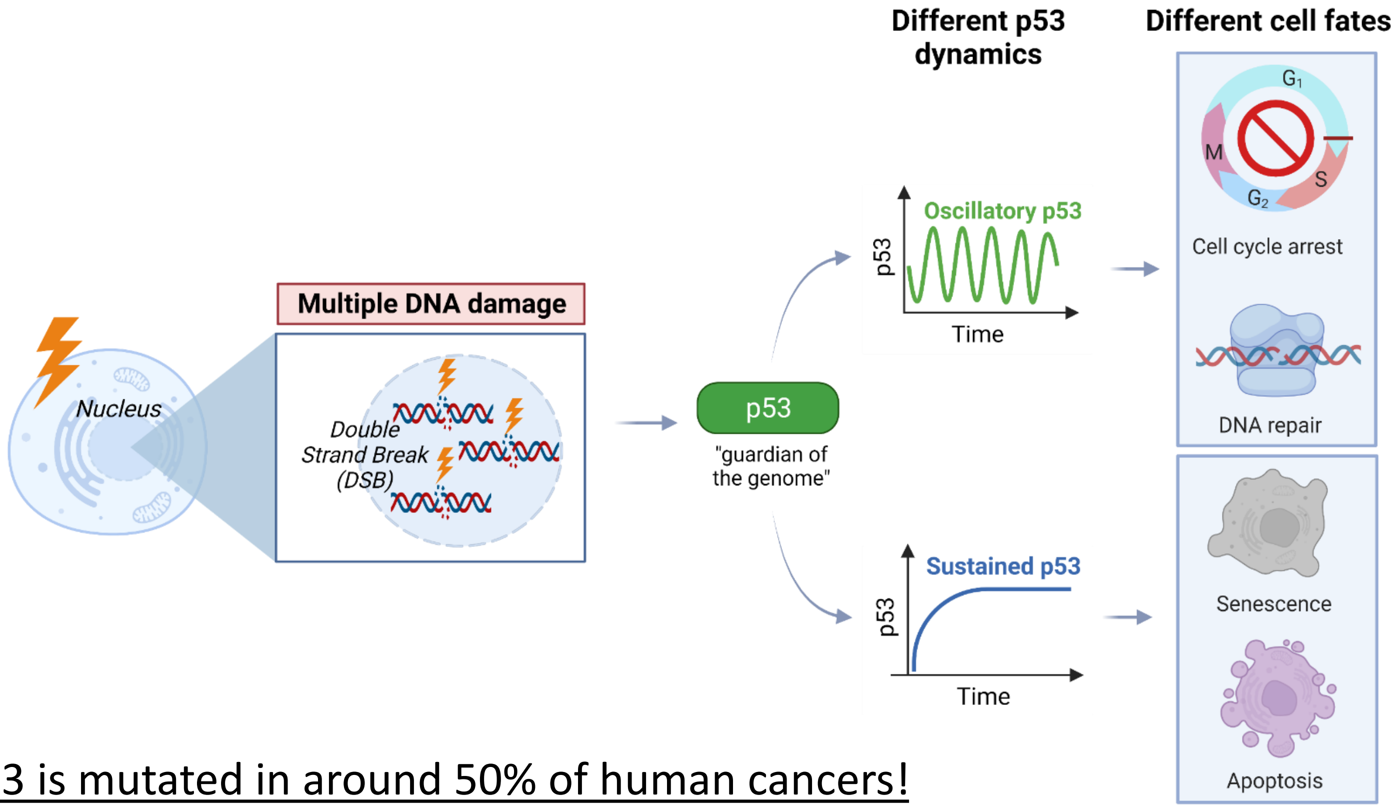
- Our theory explains how oscillations in p53 nuclear abundance optimize DNA damage repair.

Main points

- **DNA repair foci** form around the sites of DNA damage creating an inhomogeneous landscape
- **p53 regulates the formation and dissolution** of these foci
- p53 oscillations guarantee an optimal **spatio-temporal distribution of repair material**, leading to optimal DNA repair

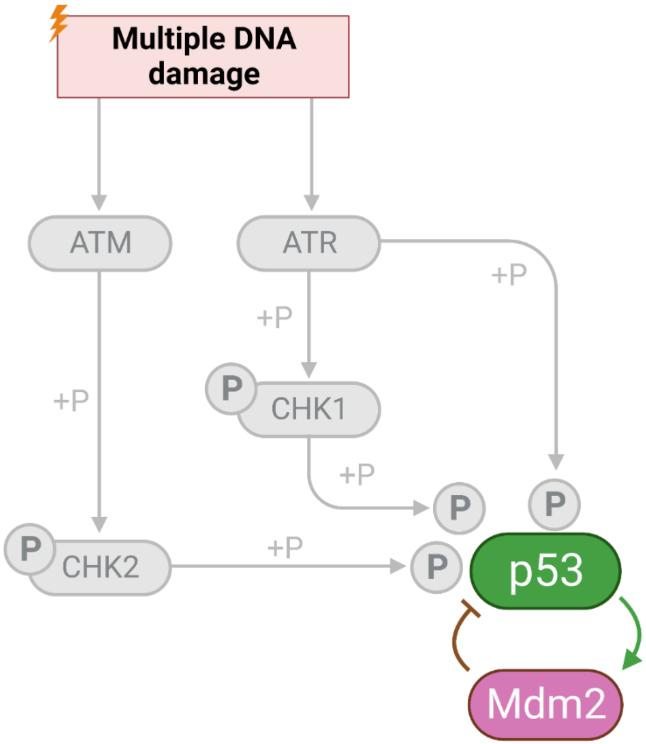
(Heltberg, Lucchetti, Sheng (Taiwan), Jensen, Cell 'Theory'185, 4394-4408, 2022)

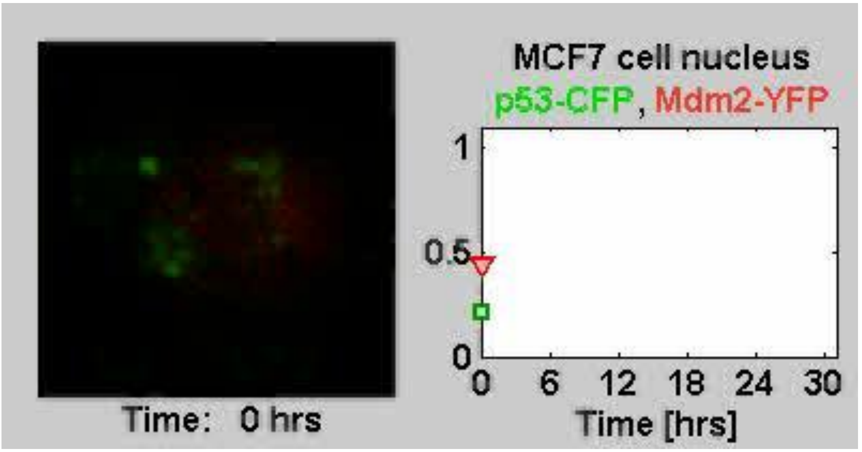
p53 regulates ~200 genes for DNA repair



<u>p53 is mutated in around 50% of human cancers!</u>

p53 nuclear oscillates after DNA damage





Geva-Zatorsky N, *et al.*, (2006), Mol Syst Biol.

- The oscillations arise as a result of the negative feedback loop with p53 inhibitor, Mdm2
- The **role** of p53 specific **frequency** (5.5 hours) is still largely **unknown**!

Model of the p53 network

$$\frac{dp}{dt} = k_1 - k_2 M \frac{p}{k_3+p}$$

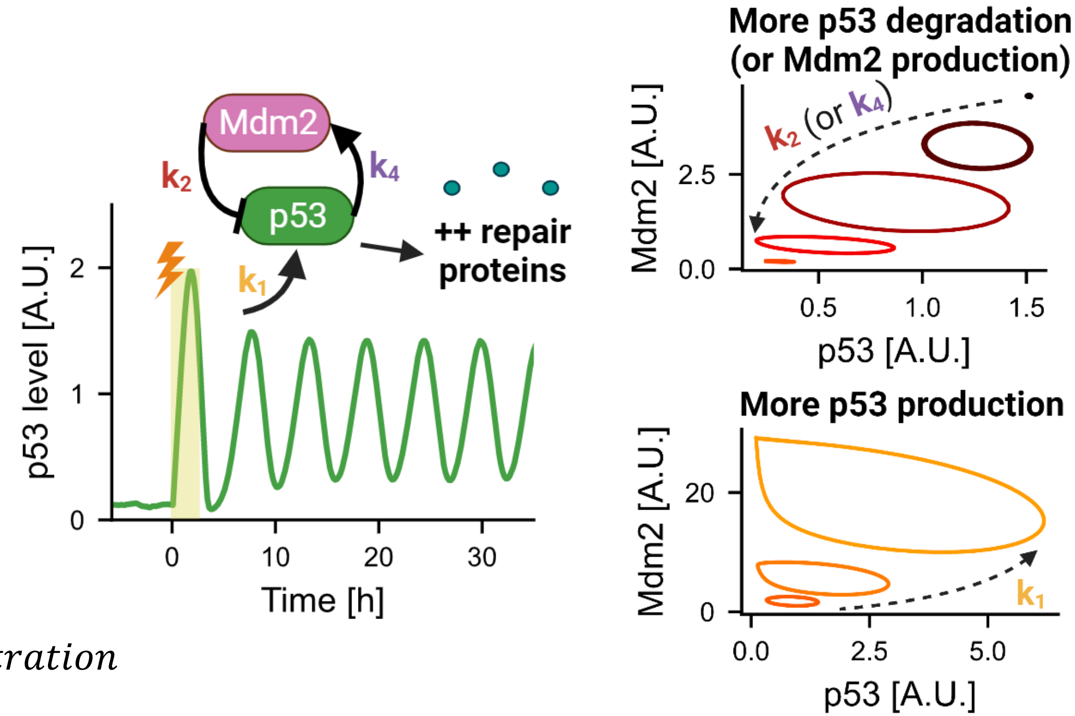
$$\frac{dm}{dt} = k_4 p^2 - k_5 m$$

$$\frac{dM}{dt} = k_6 m - k_7 M$$

$p = p53\ concentration$

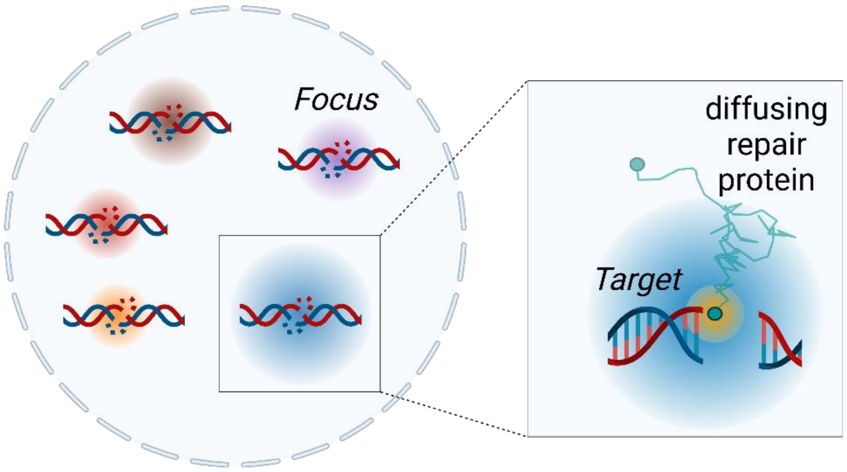
$m = \ Mdm2 - mRNA\ concentration$

$M \ = \ Mdm2\ concentration$



Formation of liquid foci

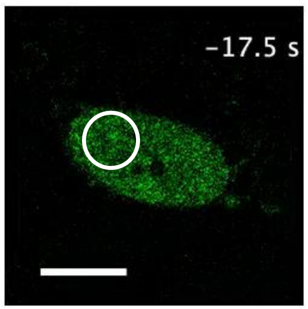
In mixtures of two or more components (e.g., proteins and water molecules), it may be **energetically favorable** for the components to separate into two liquid phases of **different relative concentrations**.



- Following multiple DSBs, **microenvironments rich of repair proteins** organize around the sites of damage
- These biomolecular condensates are believed to be **liquid foci**
- A molecule will need to **navigate this inhomogeneous environment** to find its target to repair.

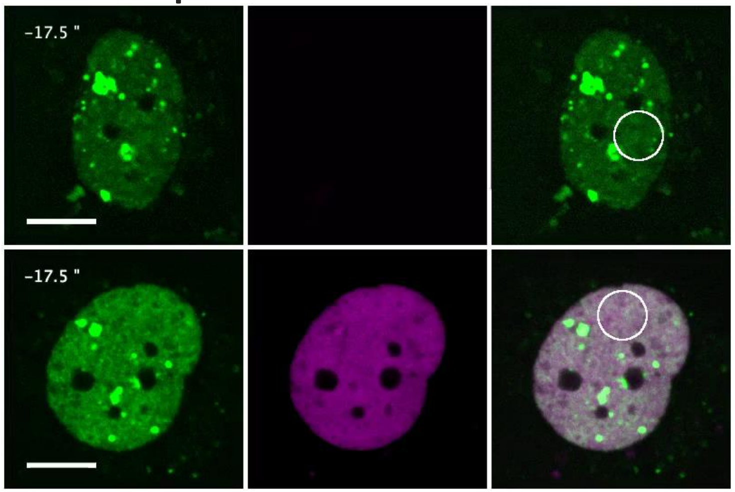
Supporting evidence

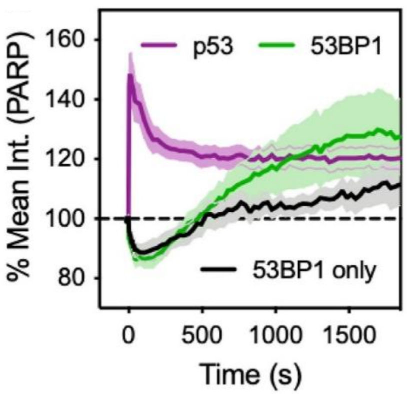
Rapid accumulation of p53 in RPE1 cells after localized DNA damage.



Wang *et al*, (2022). PNAS

P53-dependent recruitment of 53BP1



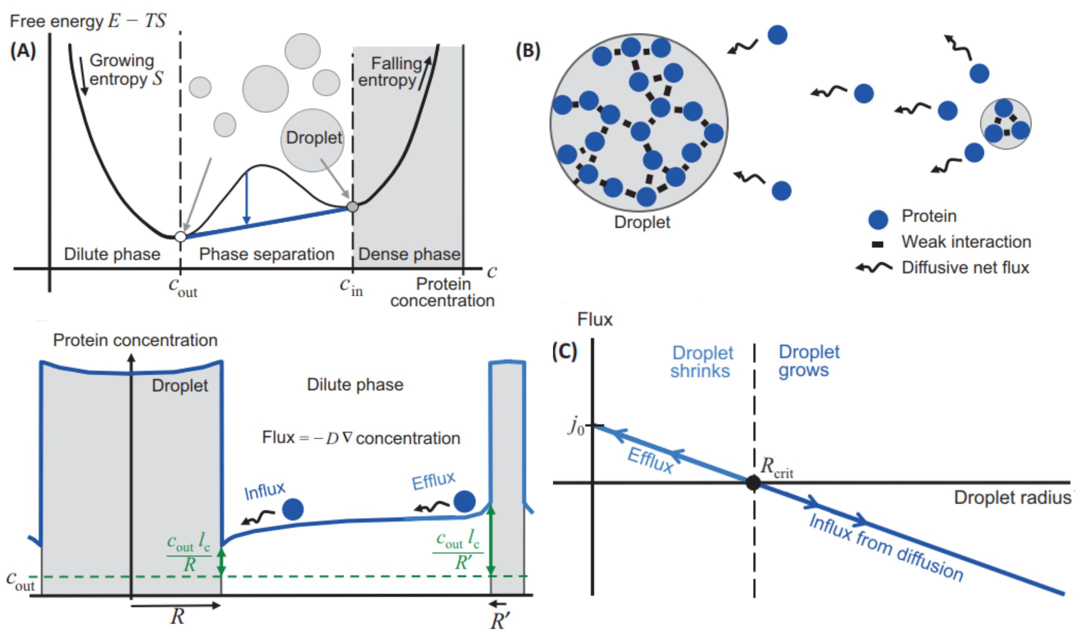


Wang *et al*, (2022). PNAS

+ p53 could form a liquid droplet state itself.

Kamagata et al, (2020); Petronilho et al., (2021)

Space is important: Formation of droplets



Soding *et al*., (2020). Trends in Cell Biology

A. When protein–protein and solvent–solvent interactions are more favorable than protein–solvent interactions, **demixing into two phases can occur**: a dilute phase with **low protein concentration c_{out}** and a **dense phase with high concentration c_{in}**.

B. c_{out} is the limiting concentration for infinite condensate droplet radius. The concentration just outside a condensate increases with decreasing droplet radius, as **small droplets cannot hold on to their proteins as well as large ones**. This creates a concentration gradient, which fuels a diffusive flux from small to large droplets.

C. As a result, **condensates below a radius R_{crit} will shrink and larger ones will grow**.

Ostwald ripening!!

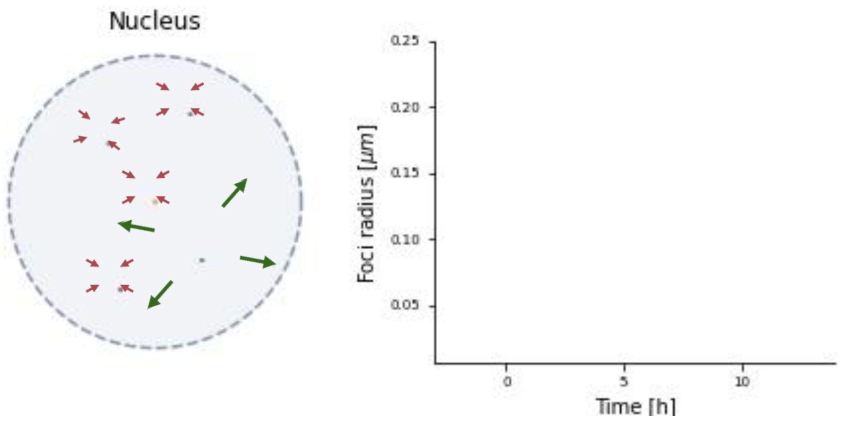
(Lifshitz, Slyozov, 1961)

Ostwald Ripening

$$\frac{dR_i}{dt} = \frac{Dc_{out}}{c_{in}}\frac{1}{R_i}\left(\frac{c_\infty}{c_{out}} - 1 - \frac{l_\gamma}{R_i}\right)$$

$$\bar{c}V_{tot} = c_{in}\sum_{i=1}^{N} V_i + c_\infty(V_{tot} - \sum_{i=1}^{N} V_i)$$

(mass conservation)



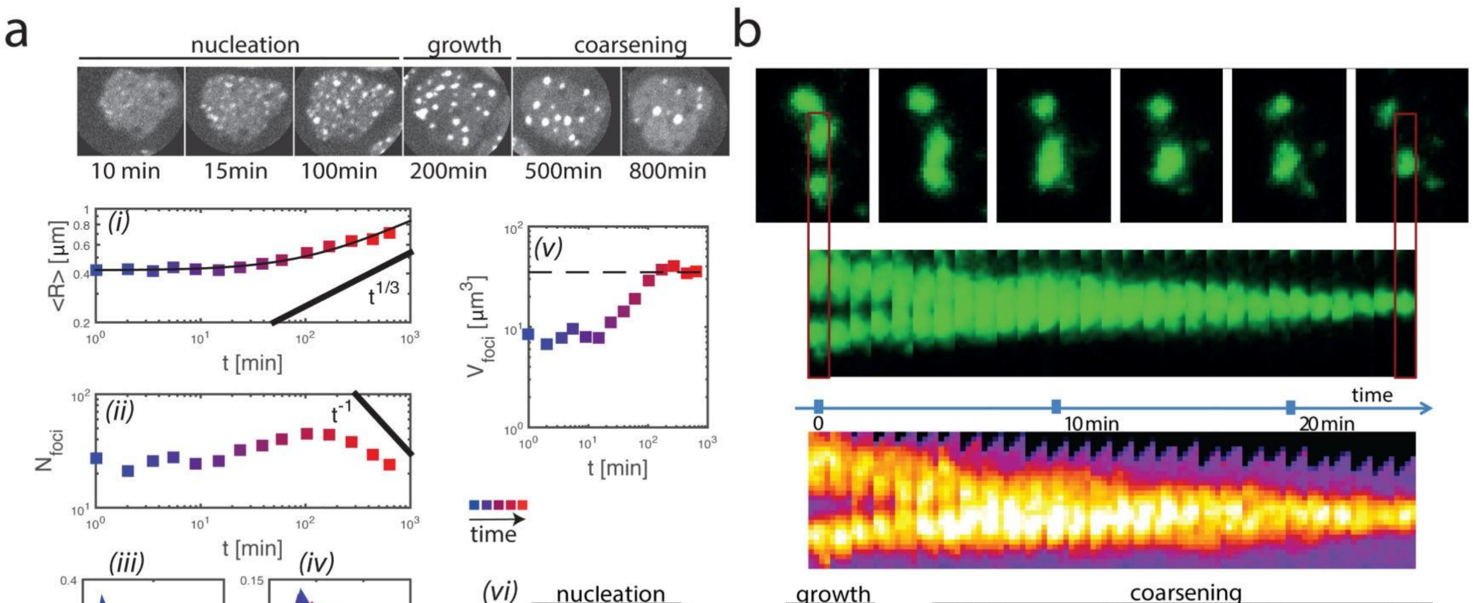
Lifshitz I. M., & Slyozov V. V.. (1961). J. Phys. Chem. Solids
Wagner, C. (1961) . *Zeitschrift für Elektrochemie, Berichte der Bunsengesellschaft für physikalische Chemie*

Pessina et al Nat. Cell. Bio (2019): Irradiation of cancer cells:

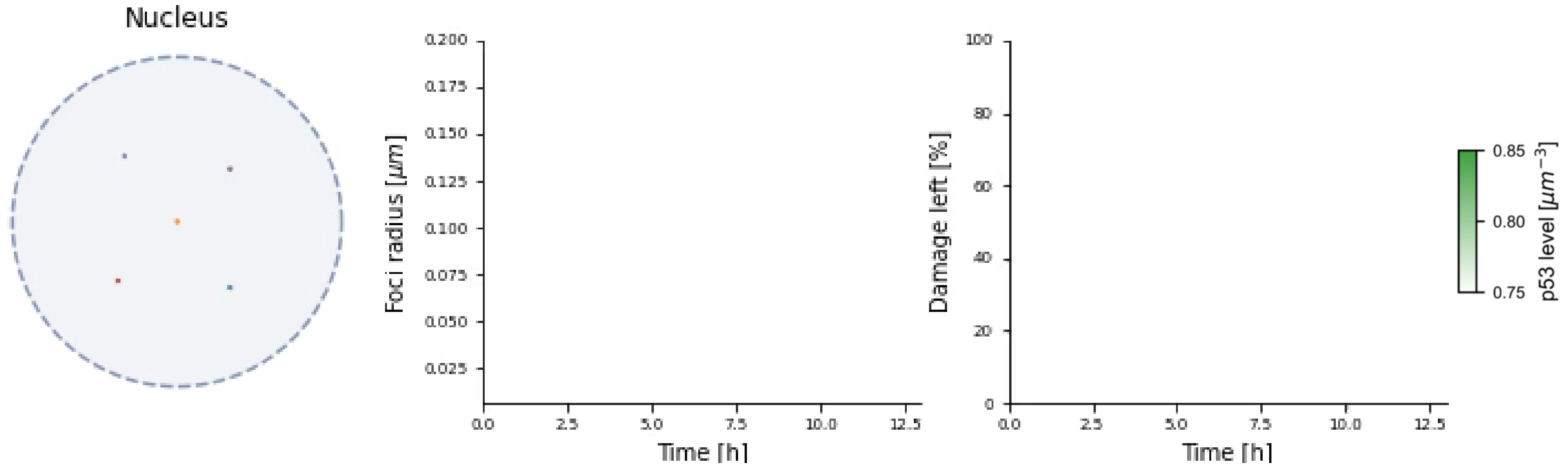
Liquid Droplets forming!

One droplet might dominate after some time: Ostwald ripening?

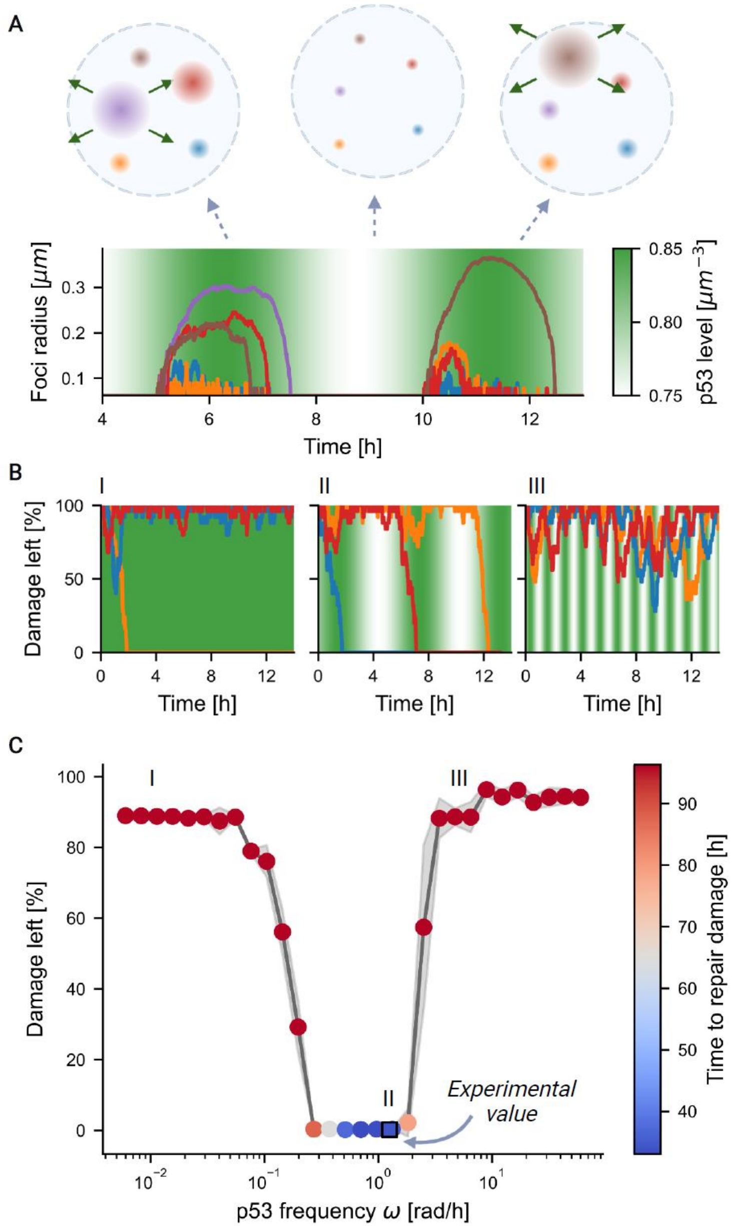
(we don't know about p53!)



With p53 oscillations: suppression of Ostwald Ripening



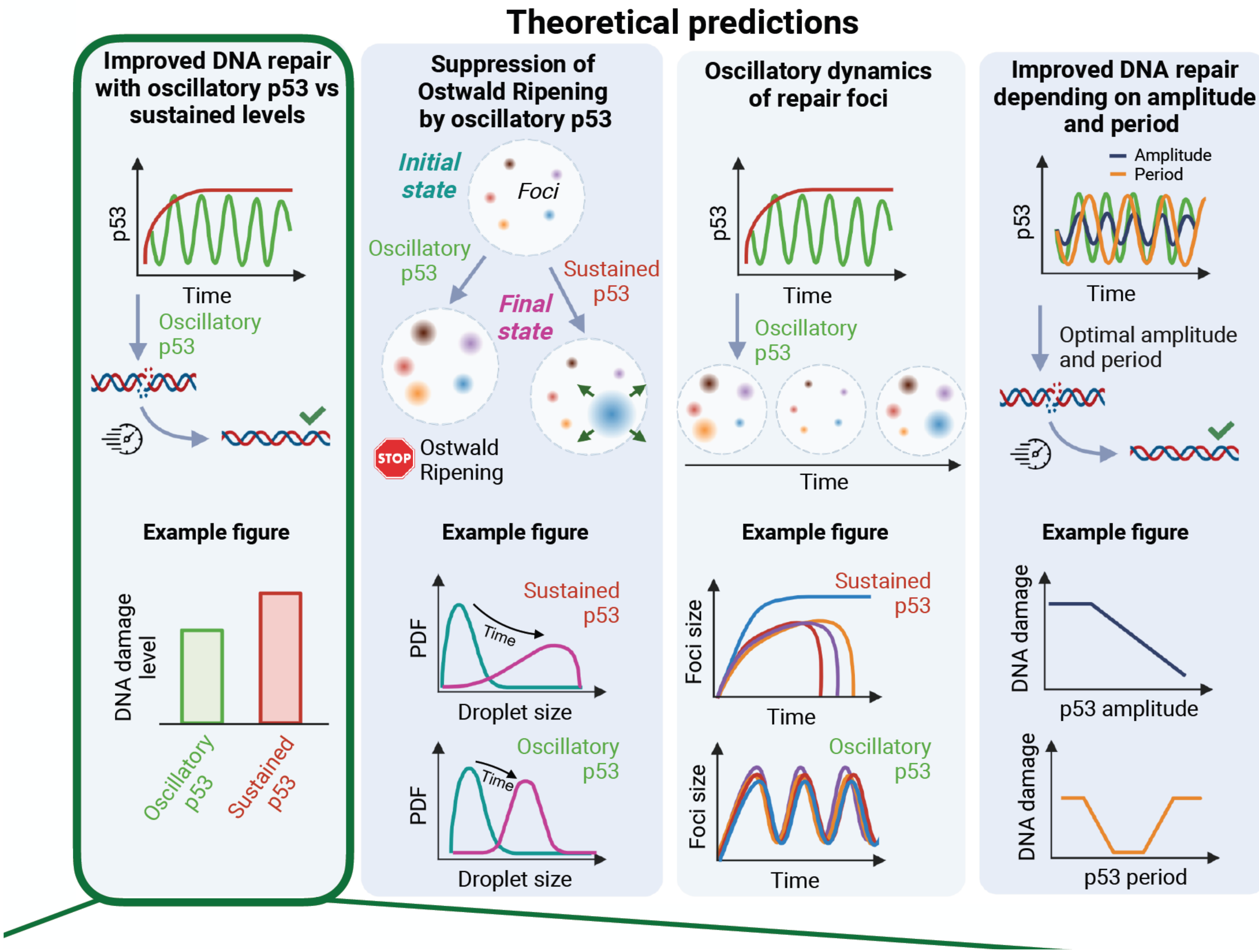
An optimal frequency of p53 oscillations for fast repair



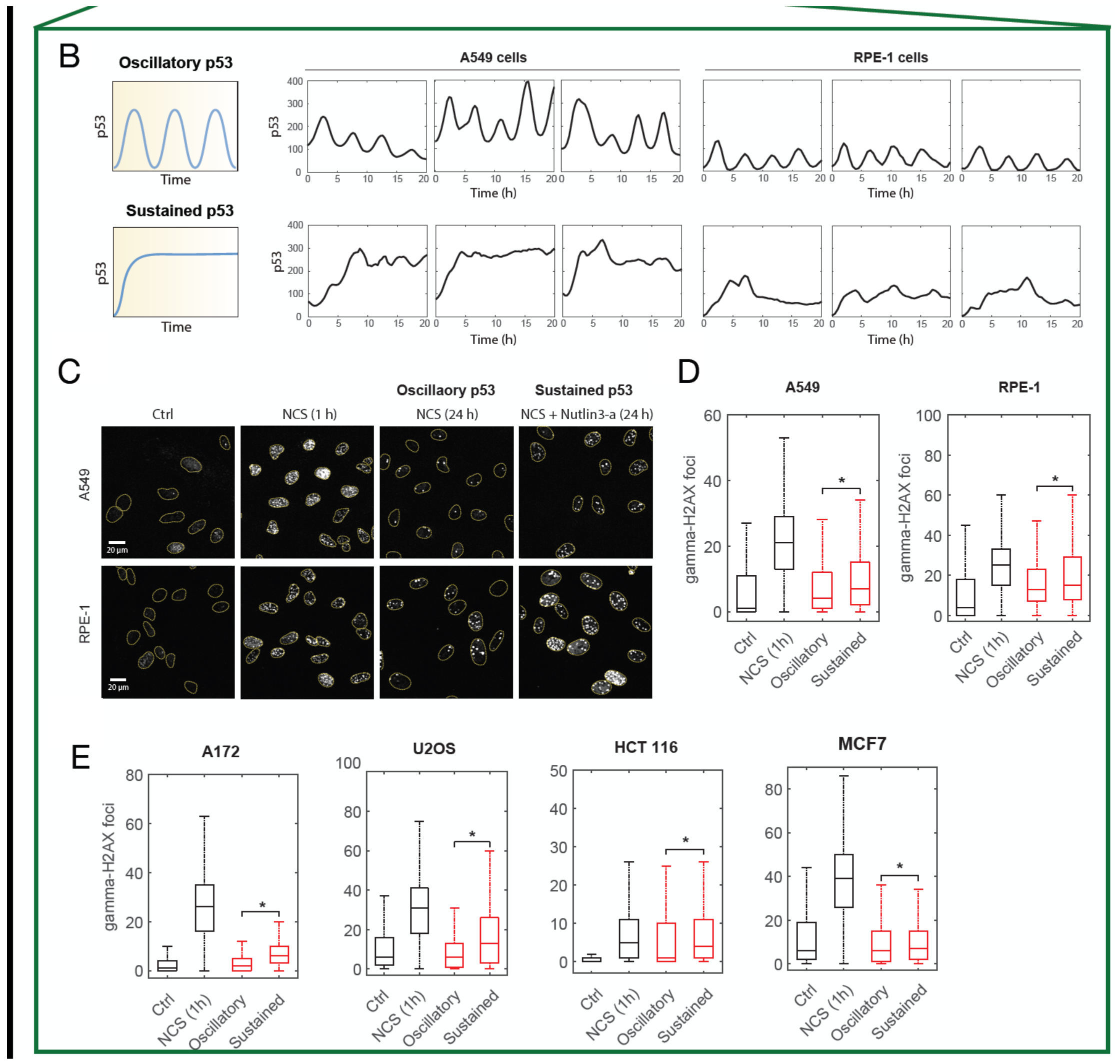
Oscillations helps for efficient DNA-repair!
Suppress Ostwald ripening

Optimal time scale is the experimental values ~5.5 hours!

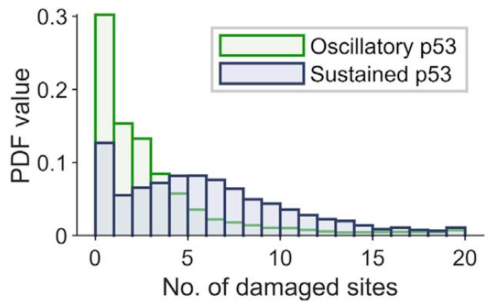
Theoretical hypotheses



New experiments in Taiwan: oscillatory p53 less DNA damage than sustained!



(γ-H2AX level)

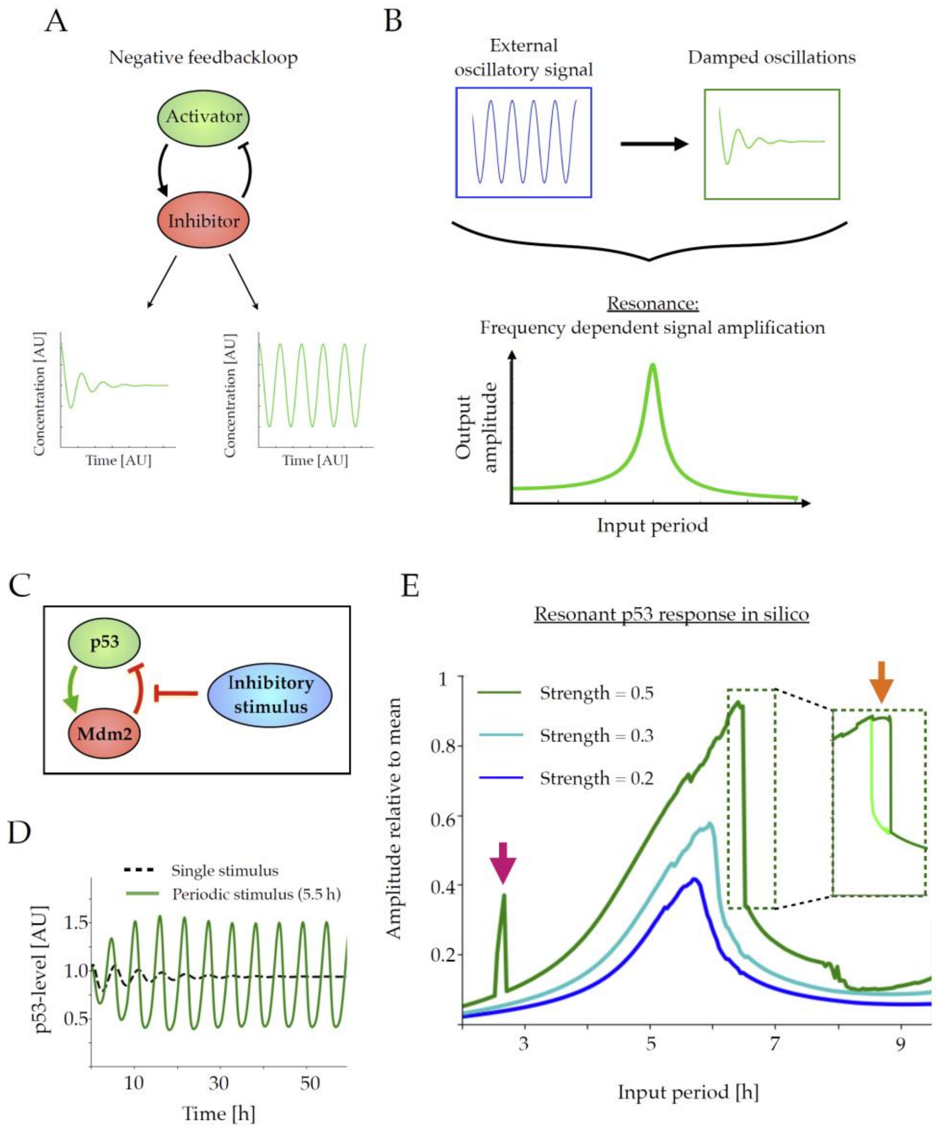


(Heltberg, Lucchetti, Sheng (Taiwan), Jensen, Cell, 185, 4394-4408, 2022)

Genetic resonance

Above Hopf bifurcation: Arnold tongues

Below Hopf bifurcation: Resonance (internal frequency)



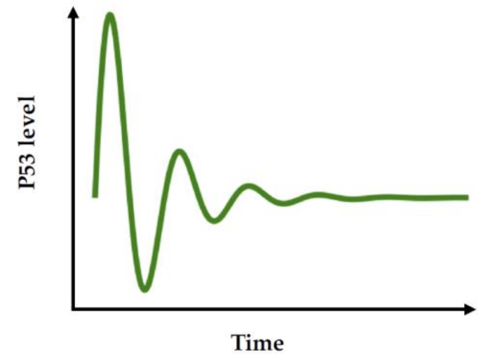
Non-linear resonance curve:

Bi-stability and possibly chaos

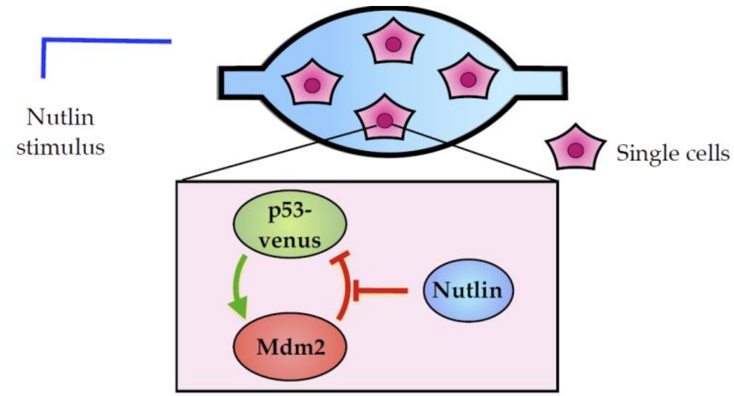
Experiments on p53 dynamics, Harvard Medical School

Alba Jimenez, Galit Lahav

A

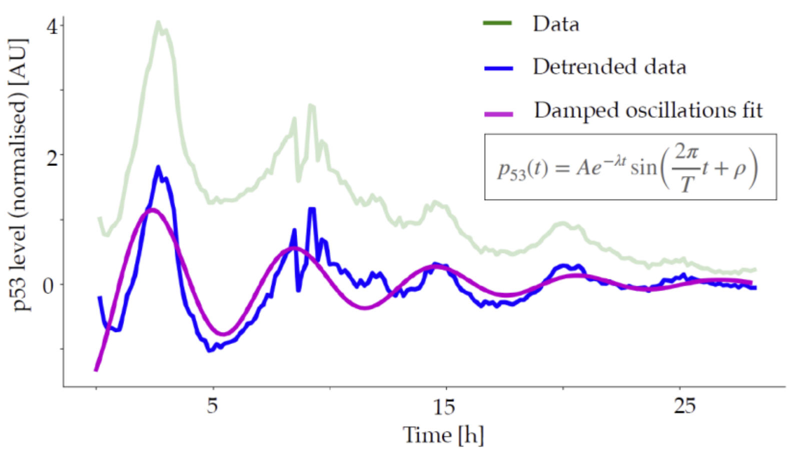


B



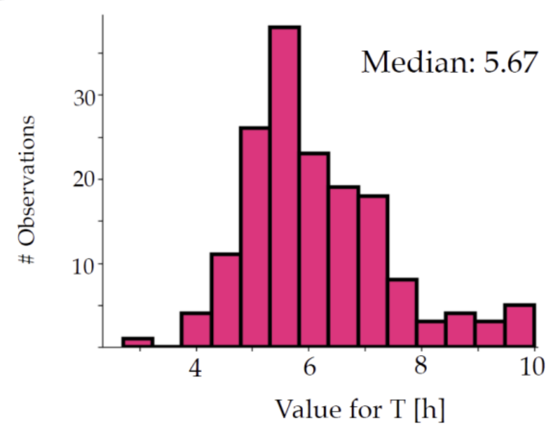
E

Fit to damped oscillator

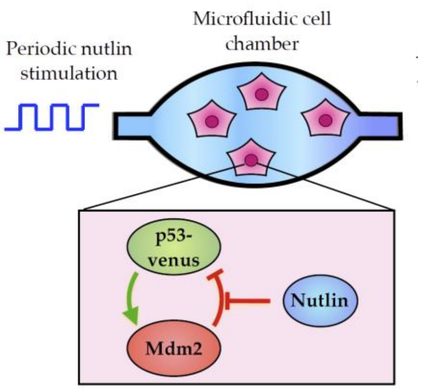


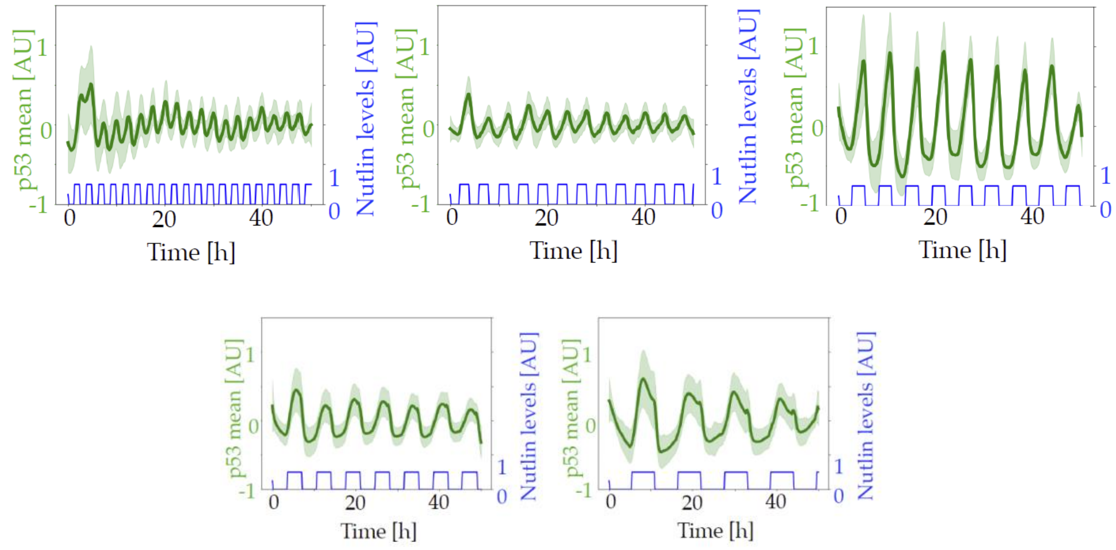
G

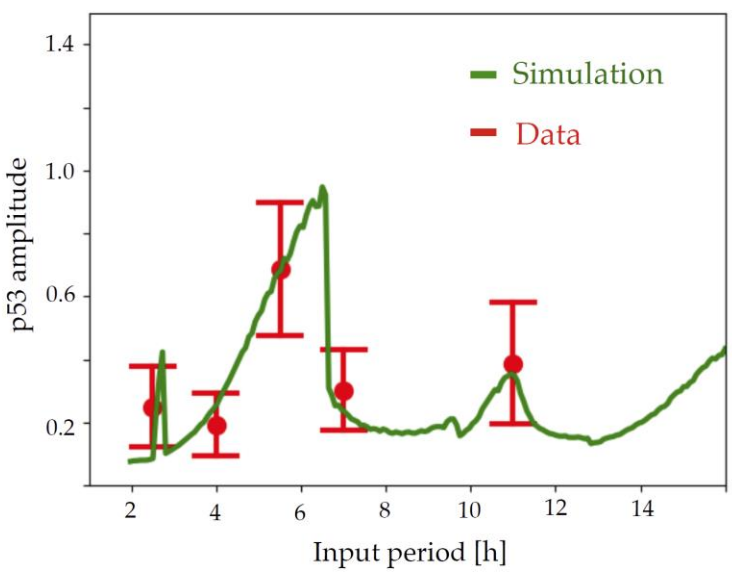
Period of oscillation



Experiments and Theory compared

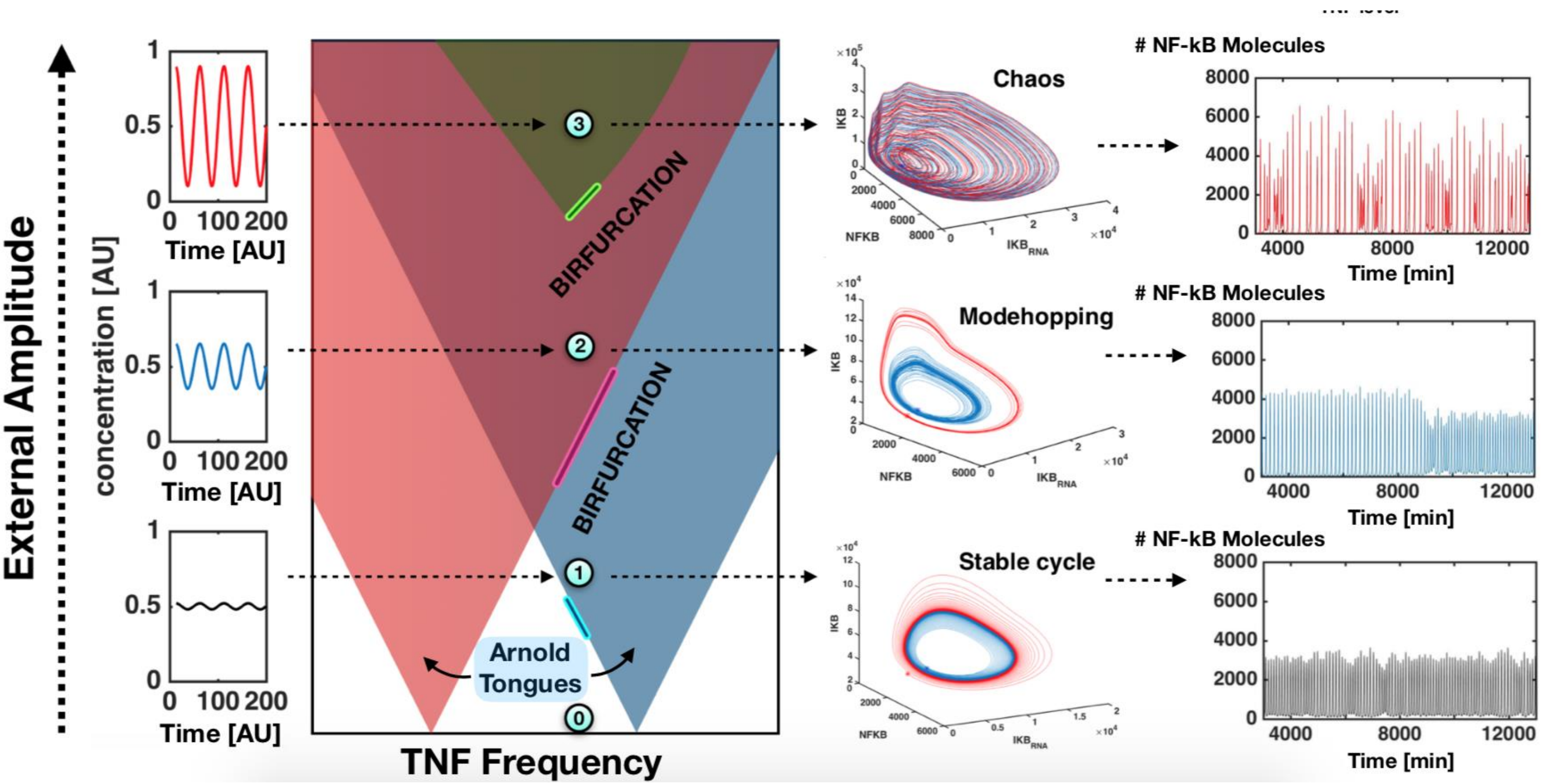




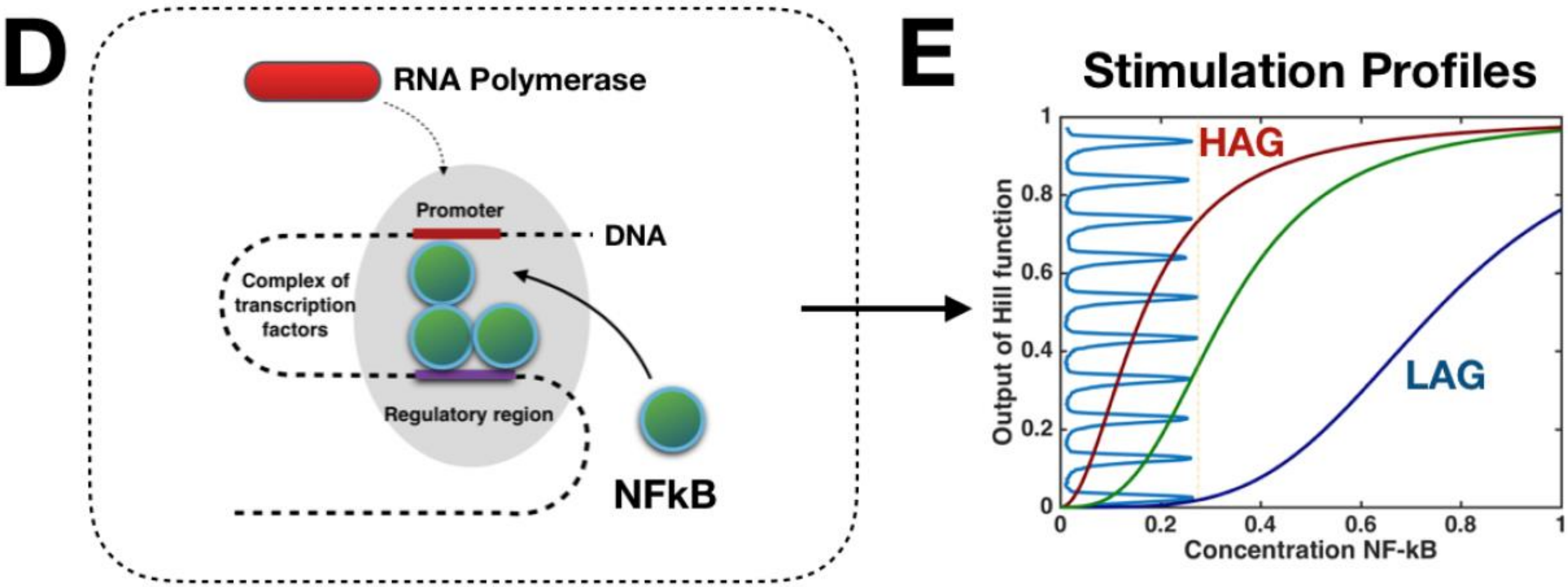


(Nature, Submitted, Mathias Heltberg, Alba Jimenez, Galit Lahav, MHJ)

Strongly coupled oscillations: Overlap of tongues ! Transitions to chaos !



Is chaos relevant for gene production ?

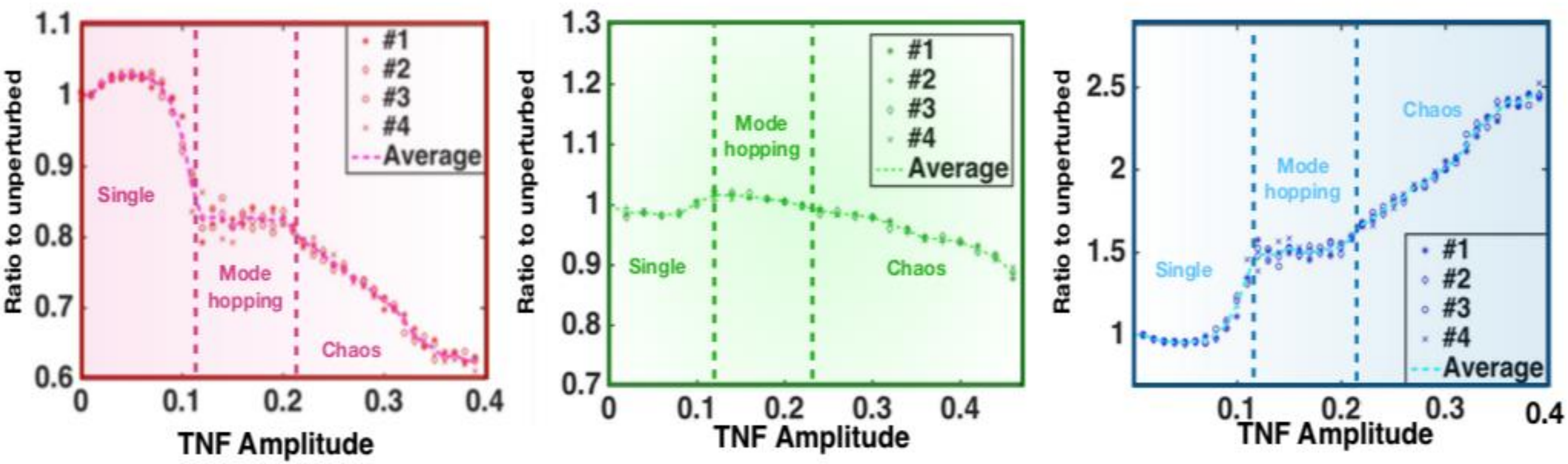


Hill function –
h cooperativity,
K affinity:

$$\dot{m}_i = \gamma_i \frac{N^{h_i}}{N^{h_i} + K_i^{h_i}} - \delta_i m_i,$$

$$\dot{P}_i = \Gamma_i m_i - \Delta_i P_i.$$

For low affinity: High gene production in chaos!



Medium cooperativity
High affinity
(h=2, K=1)

High cooperativity
Low affinity
(h=4, K=4.5)

(M. Heltberg, S. Krishna and M.H. Jensen, Nature Comm. (2019)) .

Fractals, Arnold tongues, Chaos

Collaborators:

Leo Kadanoff (Chicago), Galit Lahav (Harvard), Sheng Chen (Taiwan), Ping Wei (Beijing/Shenzhen), Sandeep Krishna (Bangalore), Savas Tay (Chicago), Ryan Kellogg (Stanford), Uri Alon (Weizmann), Simone Pigolotti (Okinawa)

NBI: Mathias Heltberg, Alessandra Lucchetti, N. Mitarai, L. Oddershede, K. Sneppen, Guido Tiana, many students and post docs

Weekly data from Beijing/Shenzhen, Harvard and Taiwan!

M.S. Heltberg, A. Lucchetti, F.-S. Hsieh, D.P.M. Nguyen, S.-h.Chen and M.H. Jensen, "Enhanced DNA repair through droplet formation and p53 oscillations", Cell (Theory) 185, 4394-4408 (2022).

M.S. Heltberg, Y. Jiang, Y. Fan, Z. Zhang, M.S. Nordentoft, W. Lin, L. Qian, Q. Ouyang, M.H. Jensen and P. Wei "Coupled oscillator cooperativity as a control mechanism in chronobiology", Cell Systems 14, 382-391 (2023).

M.L. Heltberg, S. Krishna, L.P. Kadanoff and M.H. Jensen, "A tale of two rhythms: Locked clocks and chaos in biology (Review)", Cell Systems 12, 291-303 (2021).

M. Heltberg, S.-h. Chen, A. Jiménez, A. Jambhekar, M.H.Jensen and G. Lahav, "Inferring Leading Interactions in the p53/Mdm2/Mdmx Circuit through Live-Cell Imaging and Modeling", Cell Systems 9, 548-558 (2020).

M. Heltberg and M.H. Jensen, "Locked Body Clocks", Nature Physics 15, 989-990 (2019).

M. Heltberg, S. Krishna and M.H. Jensen, "On chaotic dynamics in transcription factors and the associated effects in differential gene regulation", Nature Communication 10, 71 (2019) .

M. Heltberg, R. Kellogg, S. Krishna, S. Tay and M.H. Jensen, "Noise-induced NF-kB Mode Hopping Enables Temporal Gene Multiplexing", Cell Systems 3, 532-539 (2016).

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