

Physics of multicellular systems 2023

14h-15h30

Lecture

15h30-17h

TD/practical session (Nicolas Ecker)

Please be on time!

Lecture I-III, V (23/01,30/01, 06/02, 20/02) :

gene networks, pattern formation,...(Vincent Hakim)

Lecture IV, VI-IX (13/02, 06/03, 13/03, 20/03, 27/03).:

active mechanics, morphogenesis,..

The last three sessions will be devoted to work on a paper
in small (2-3 persons) groups

Exam (03/04) : presentation of the paper and work done.

Physics of multicellular systems

**Lecture I : Introduction, modeling and dynamics of
genetic networks**

A quantitative cell-centered approach to biology

-Intra-cellular dynamics :

how a cell state should be characterized?

how does it evolve in time, respond to external signals,...

-Inter-cellular dynamics :

how do cell cooperate to create functioning organs, organisms

-Dynamical processes can be hard to understand:

-see the details, perturb

- Why physics/math ?

qualitative reasoning is difficult, mathematical/physical analysis help

Statistical physics/nonlinear dynamics ideas/techniques useful

assess the robustness of the process, regulation and control, optimal solution, information processing, physical limits...

-> new physics (out-of equilibrium structures, active matter),

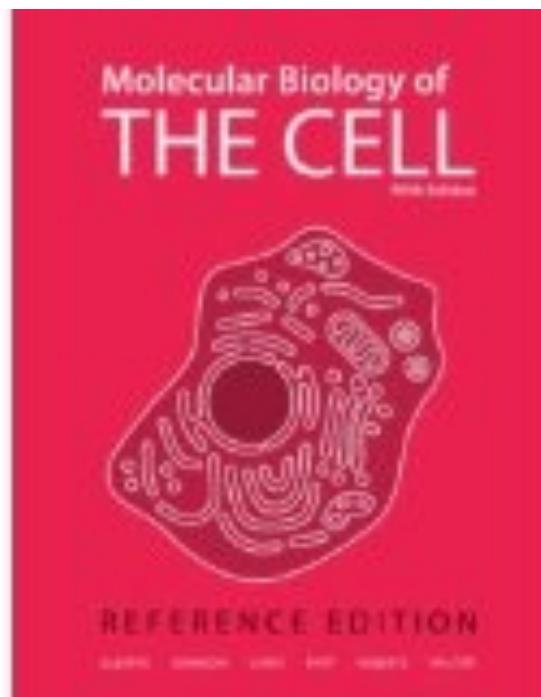
new questions (not asked for physical systems)

-> A very active area of research now and certainly for many years to come

(plenty of open questions).

Today:

A few fundamental notions of molecular biology + basic modeling of gene regulation
+ some dynamical phenomena



Genomes

Species	Genome size	Protein coding genes	Chromosomes
Yeast	13.5 Mb	5800	16
Drosophila	165 Mb	14000	X/Y, 2, 3, 4 (very small)
C elegans	100 Mb	20000	5 autosomes + X
Chicken	1Gb	15000	10+28 (small) + Z/W
Mouse	2.7 Gb	22000	19 autosomes + X/Y
Human	3.2 Gb	21000	22 autosomes + X/Y

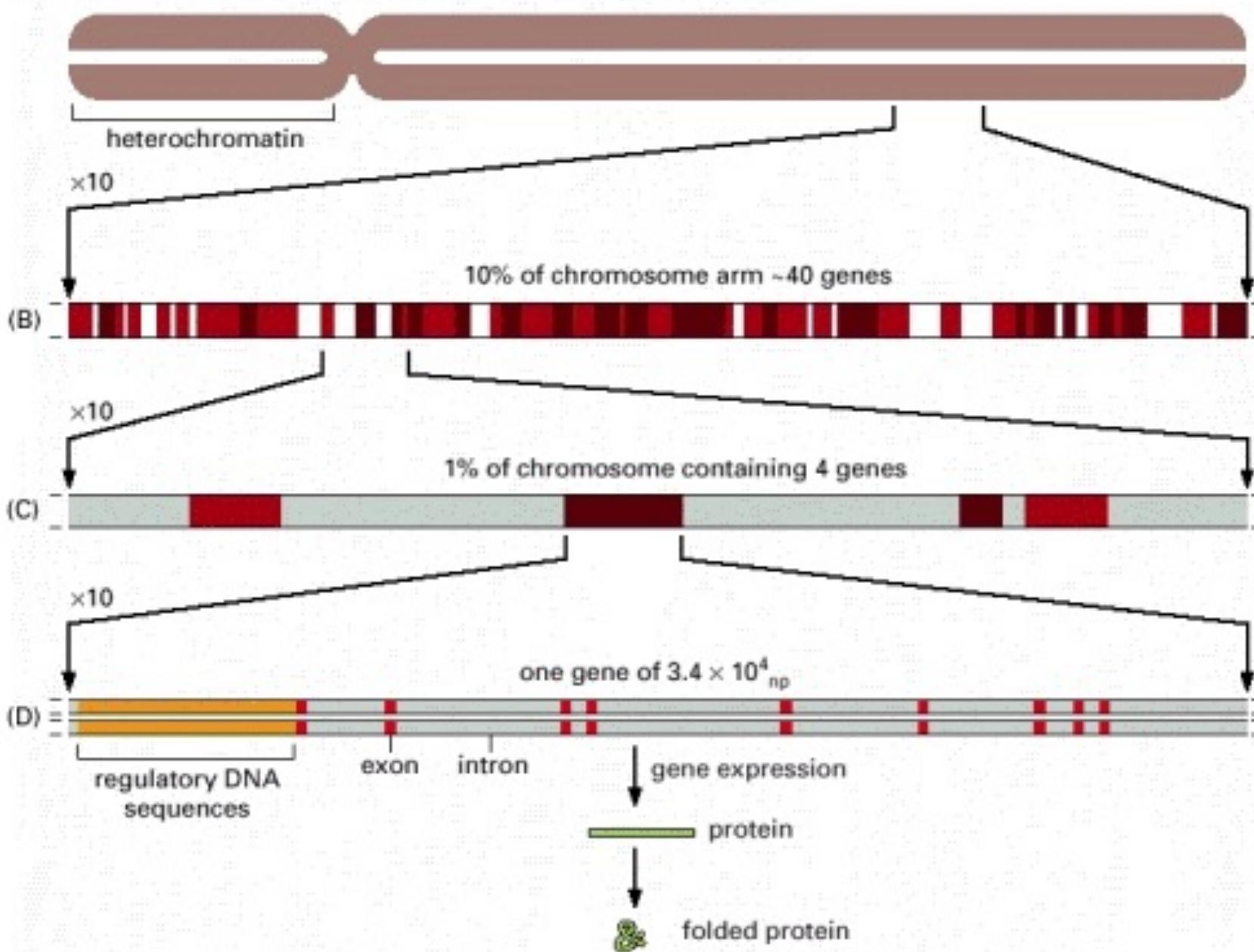
Human :

Mean gene size: 27 Kb

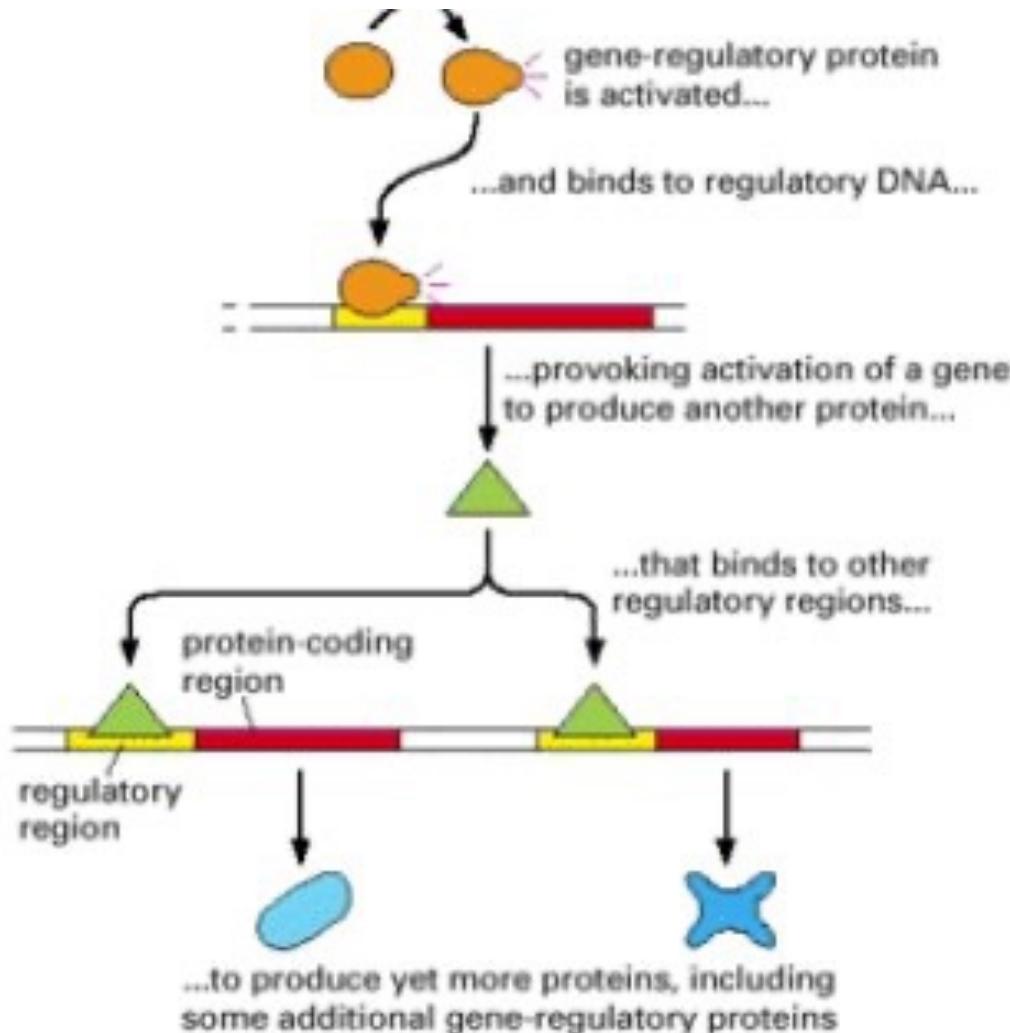
Mean number of exons: 10.4

Mean exon size: 145 nt (largest 17Kb)

(A) human chromosome 22— 48×10^6 nucleotide pairs of DNA.

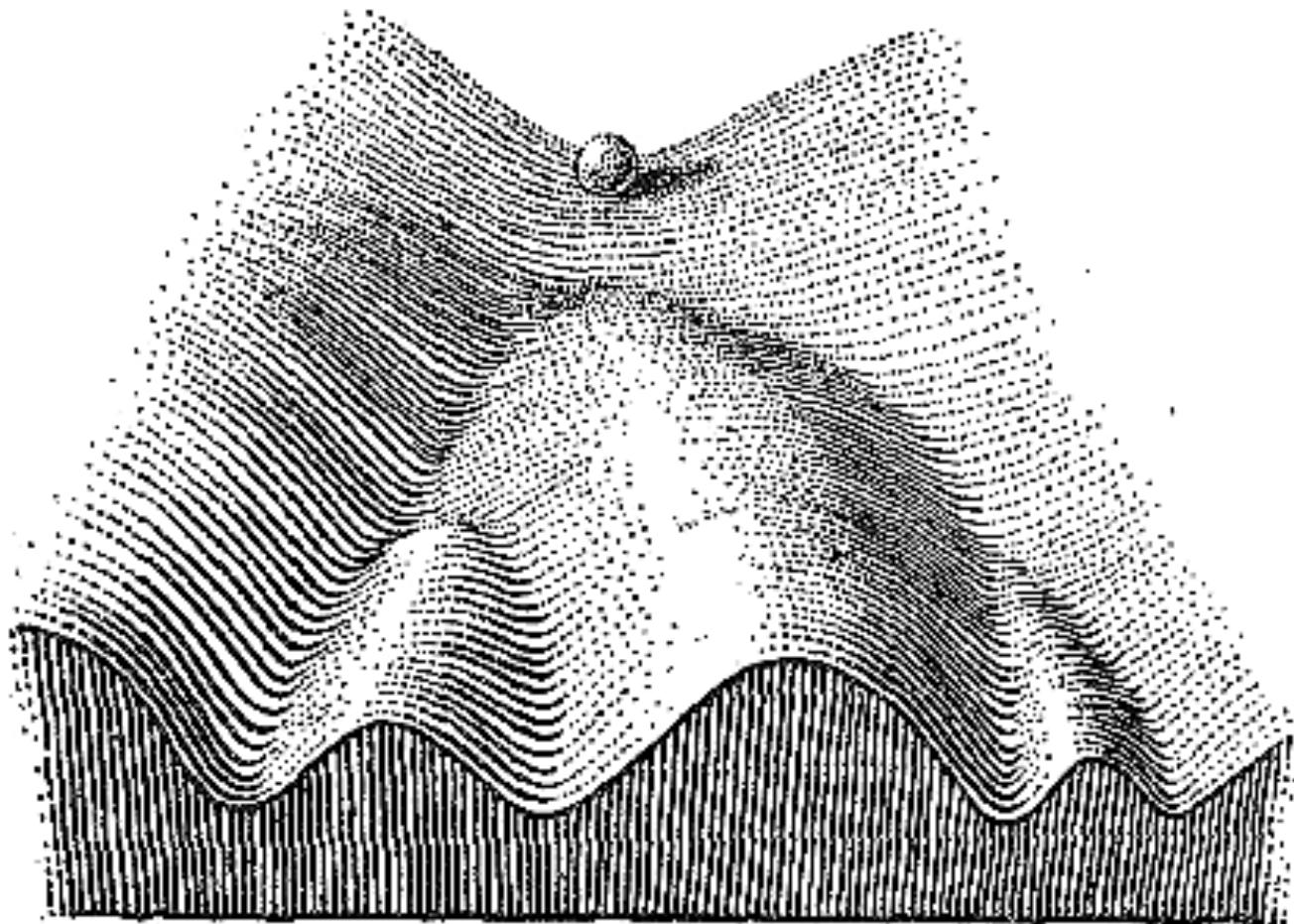


Gene-protein interaction networks :



Very simplified
Wikipedia view
(but useful)

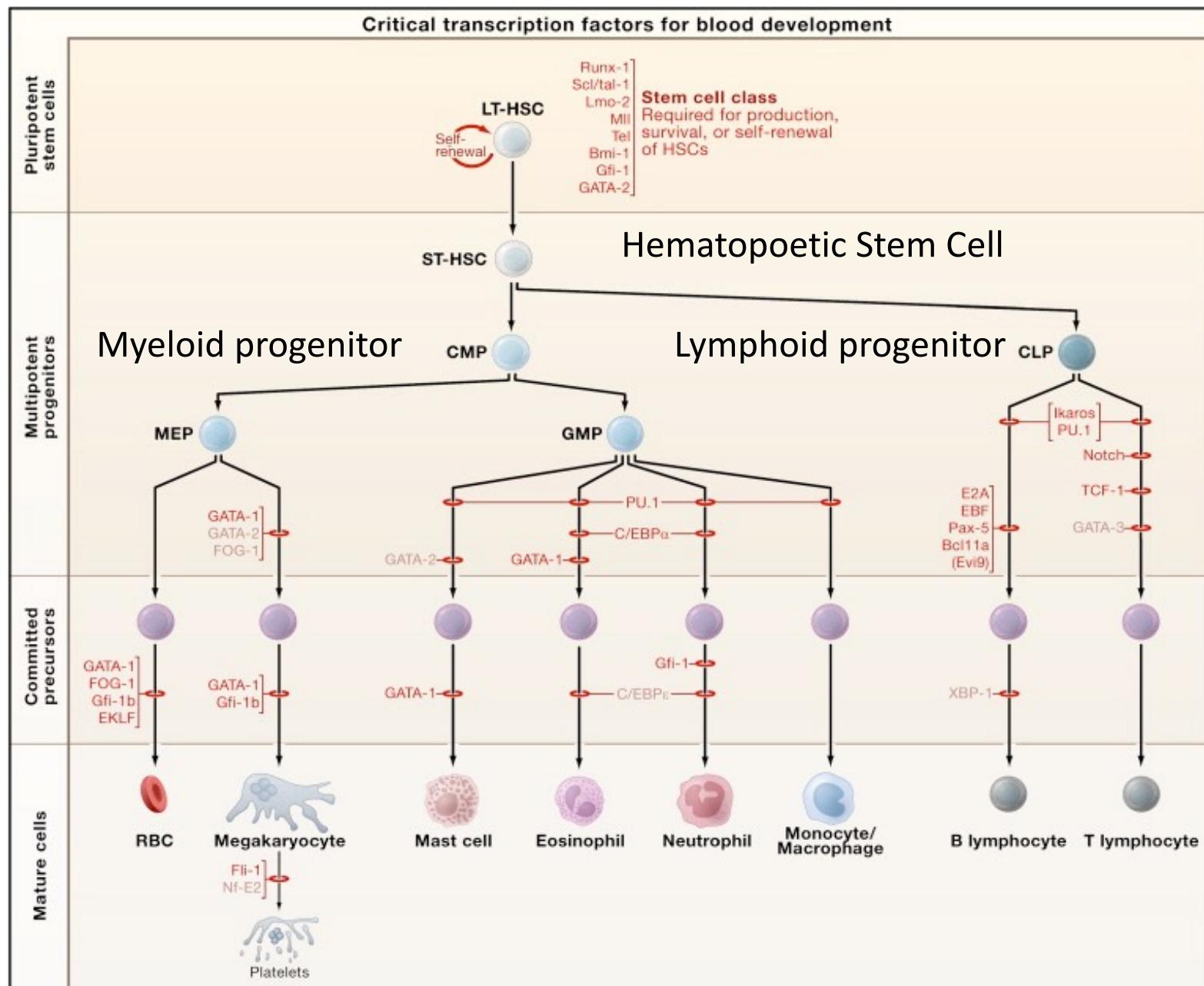
Cells are different



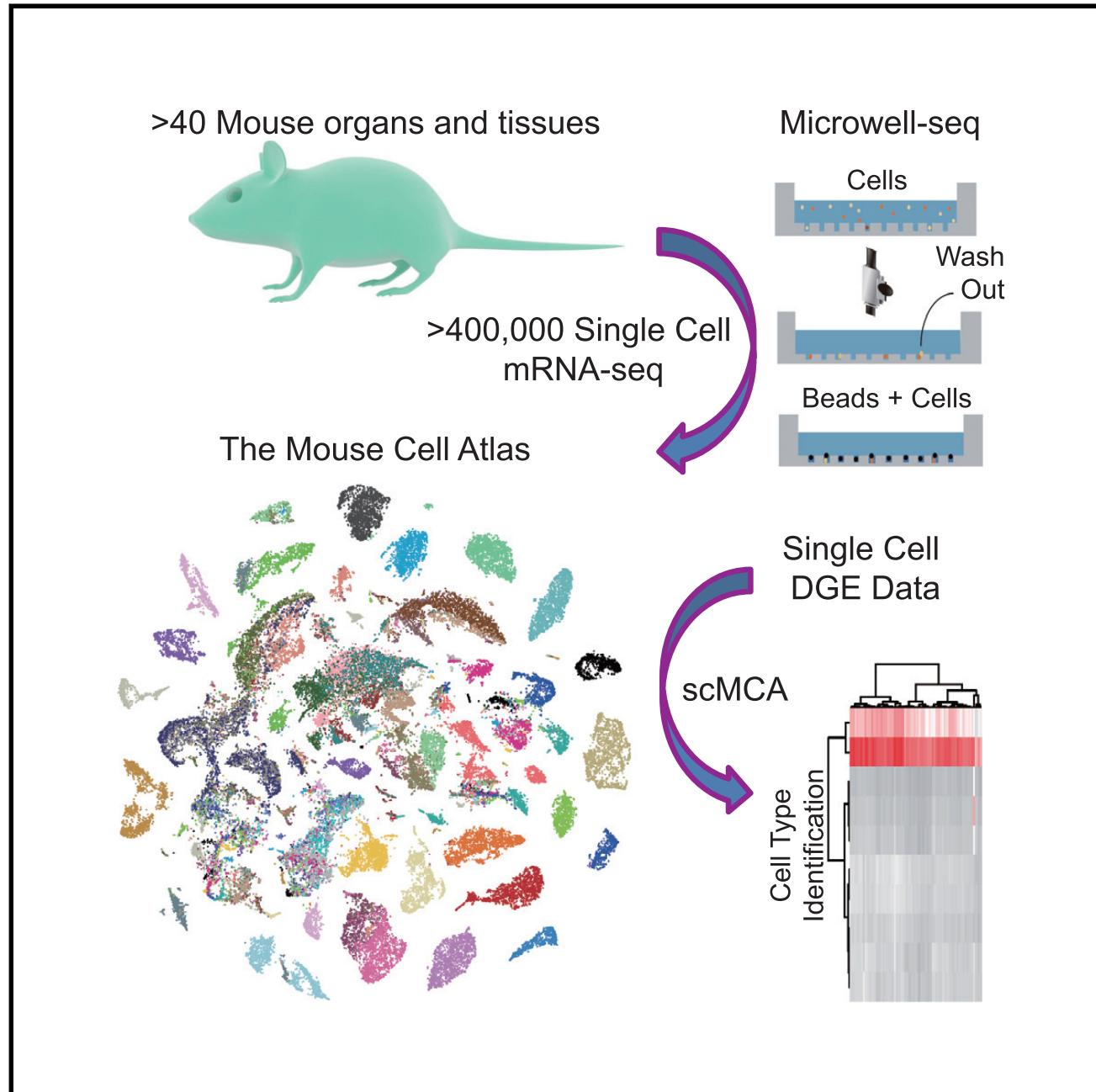
Waddington's picture (1957) of cell differentiation : series of **bifurcations**

- How do these bifurcations arise?
- How are cells pushed one way or another?
- How is this organized in space?

Cells are different



Cells are different: single cell RNA sequencing

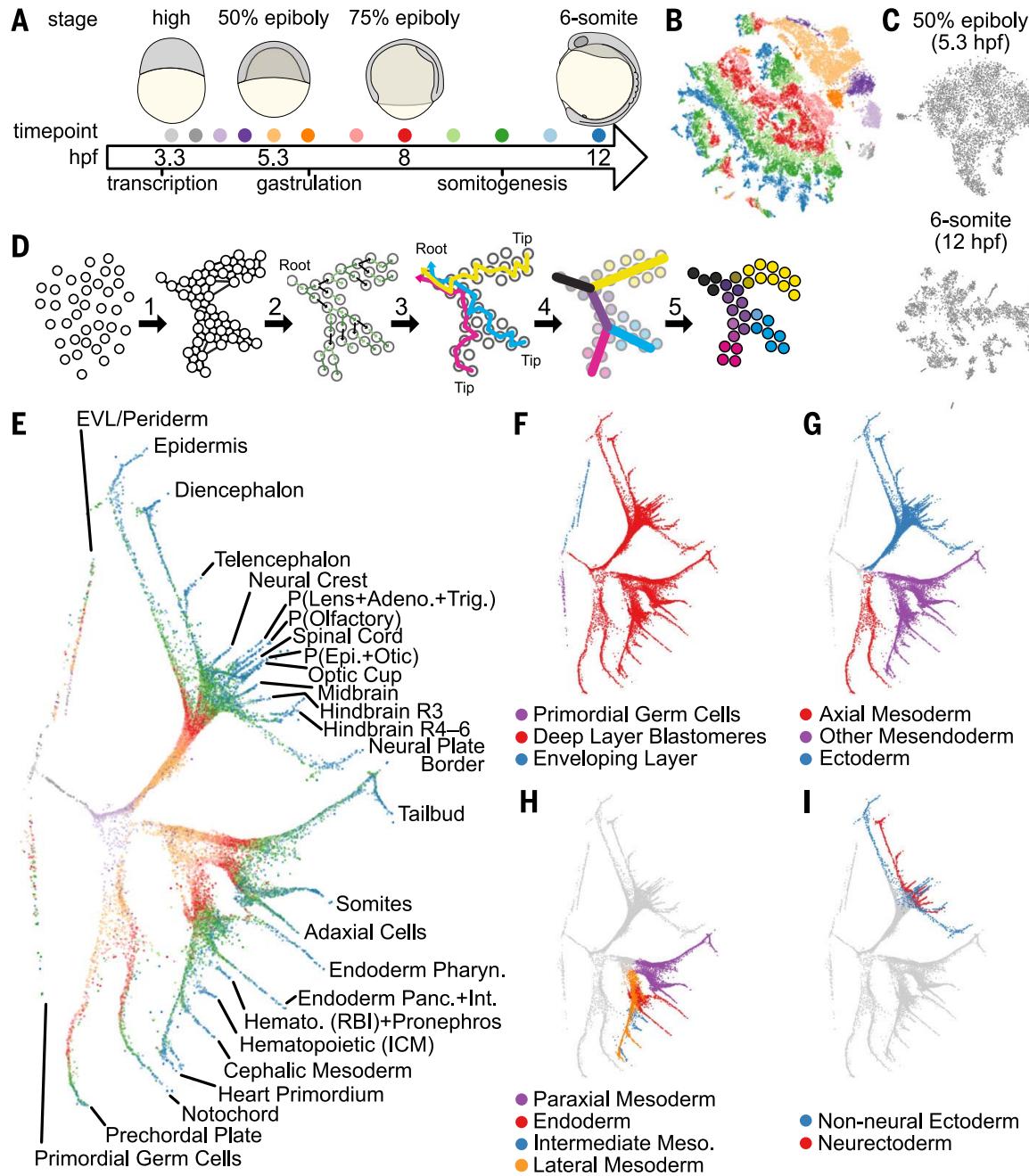


Han et al, Cell (2018)

400.000 cells from
>50 tissues

98 major clusters
>800 cell types

Single cell RNAseq and developmental paths



Farrell et al, Science (2018)

Zebrafish development

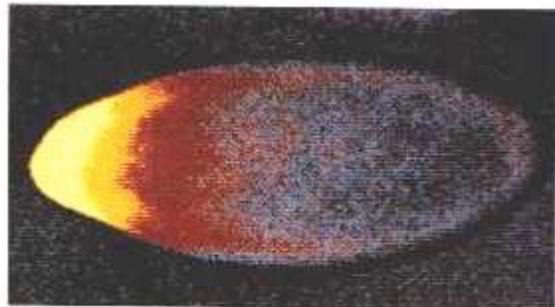
-12 developmental stages
(3.3hr pf ->12hr pf)

~39000 cells from ~700 embryos

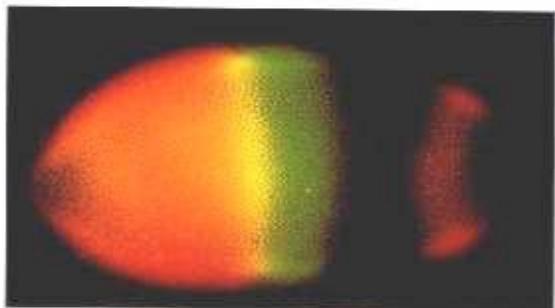
Mouse see
Cao,..., Shendure, Science (2019)

Spatial patterns

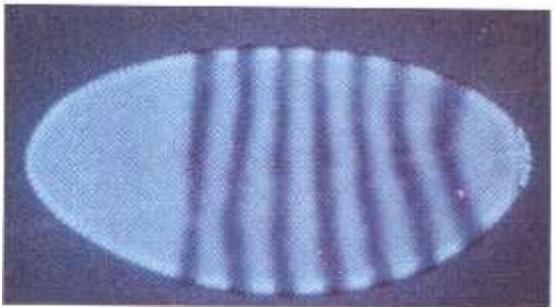
Drosophila early embryogenesis



Gradient(s)



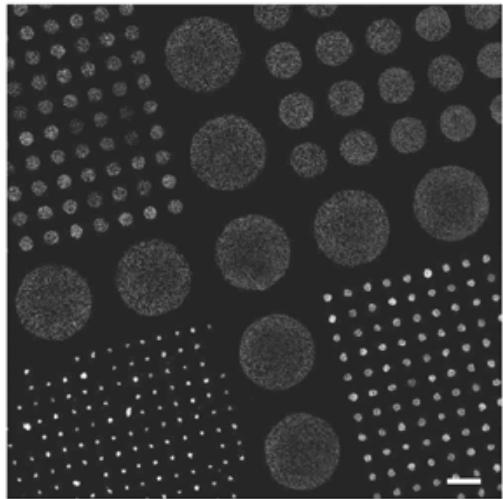
Localized gene expression



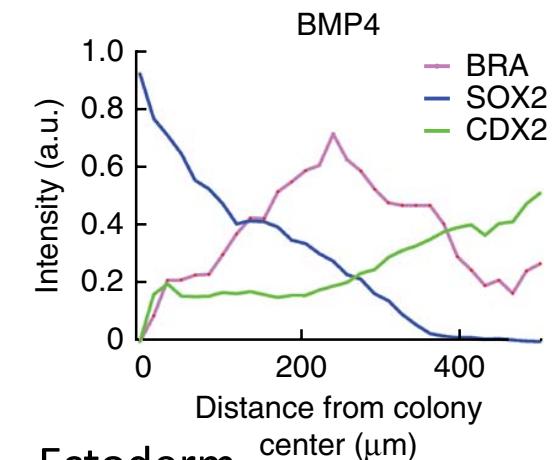
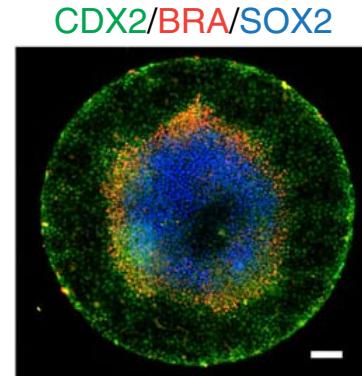
Repeated bands

Spatial patterns

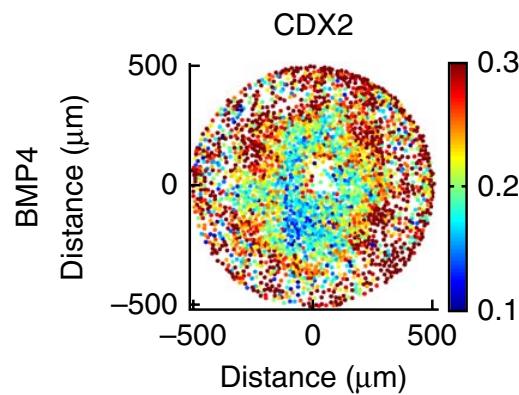
Human embryonic stem cells in vitro



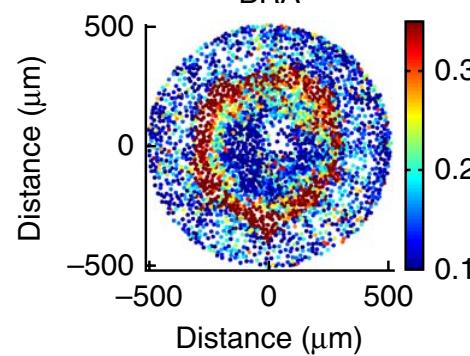
Micropatterned culture



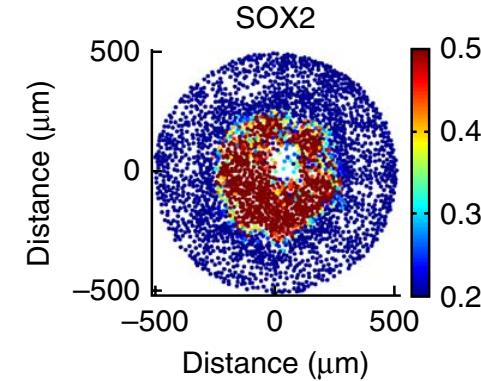
Extra-embryonic



Mesendoderm



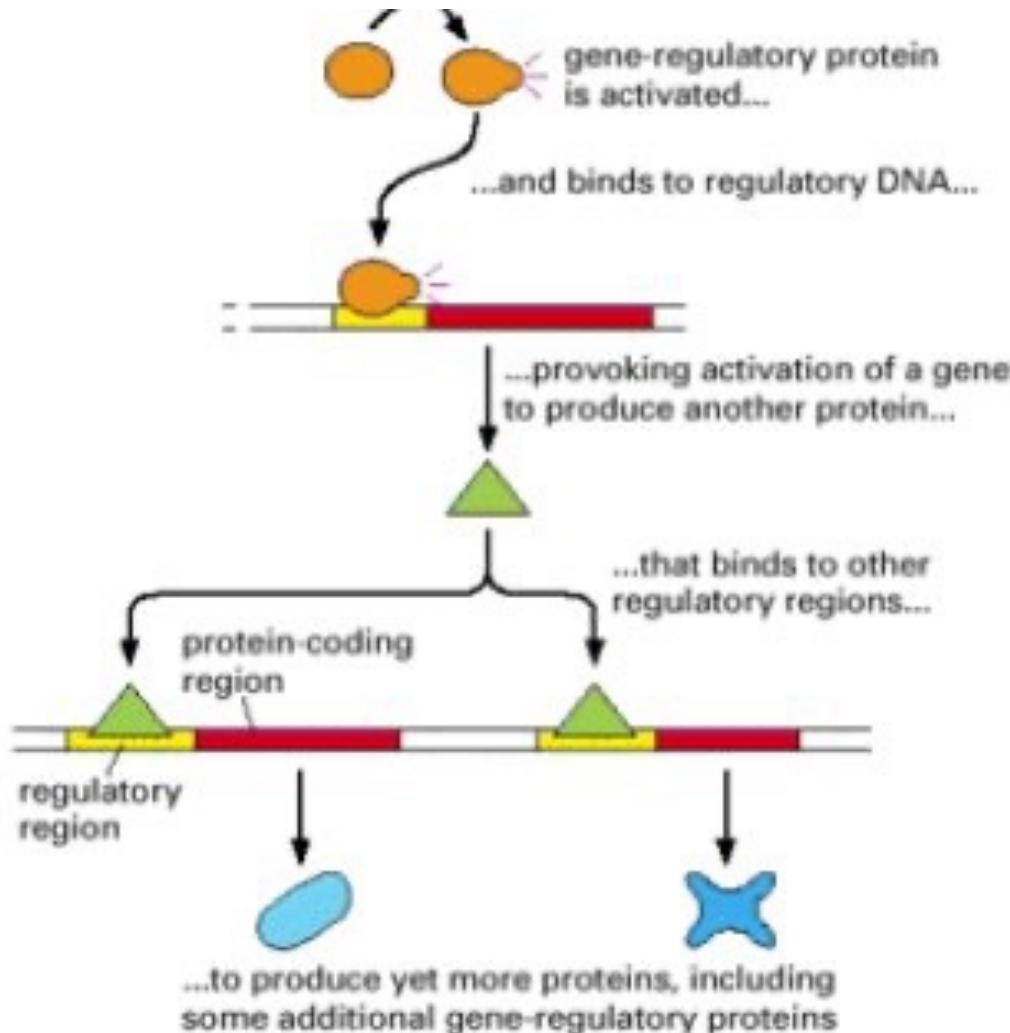
Ectoderm



Warmflash, Sorre, Etoc, Siggia, Brivanlou, Nature Methods (2014)

Modeling the dynamics of genes and proteins

Gene-protein interaction networks :



Very simplified
Wikipedia view
(but useful)

The simplest description of mRNA and protein dynamics

I. mRNA dynamics

$$\frac{dm}{dt} = \rho_m - \delta_m m$$

If $m = 0$ at $t = 0$ then,

$$m(t) = \frac{\rho_m}{\delta_m} [1 - \exp(-\delta_m t)], \quad t > 0$$

Half-life $t_{m,1/2} = \frac{\ln(2)}{\delta_m} \simeq \frac{0.7}{\delta_m}$

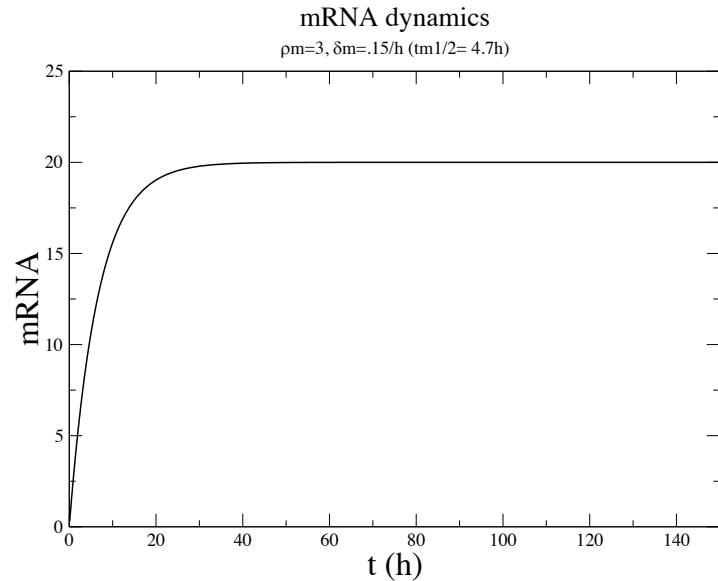
Extremely simplified :

- details of transcription activation, Pol II, splicing,...are completely absent
- nonetheless useful !

m =mRNA concentration

ρ_m =production rate

δ_m =degradation rate



Reaction time controlled by $1/\delta_m$, to react fast degradation rate should be large, costly.

The simplest description of mRNA and protein dynamics

I. Protein dynamics

$$\frac{dP}{dt} = \rho_P - \delta_P P$$

P = protein concentration

ρ_P = production rate

δ_P = degradation rate

Translation : the protein production rate is proportional to the mRNA concentration.

$$\rho_P(t) = \beta m(t)$$

If $\delta_P \ll \delta_m$, mRNA concentration reacts faster than protein concentration.

To describe $P(t)$, m can be supposed to have reached its steady state

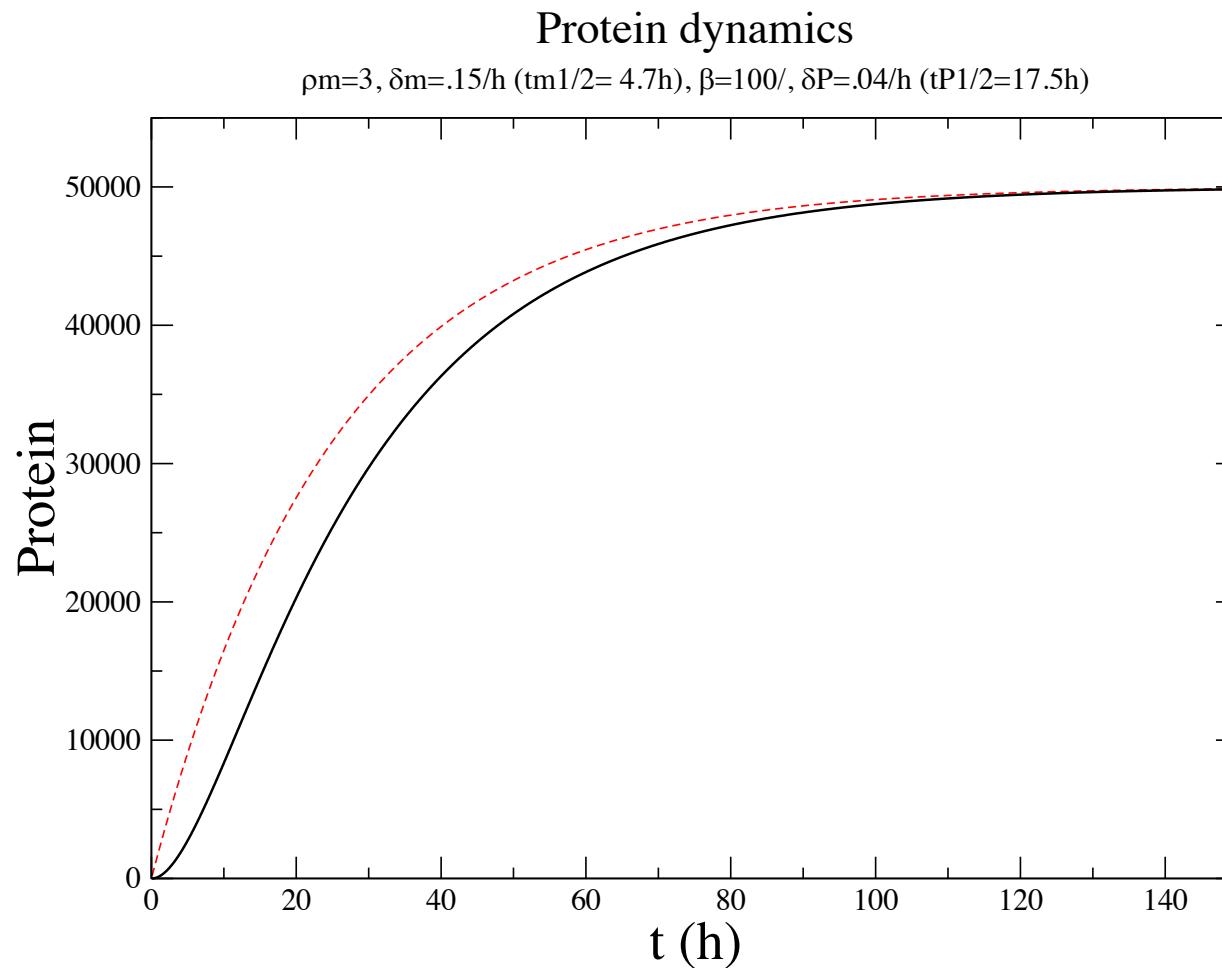
$$\rho_P = \beta \rho_m / \delta_m. \quad \text{« adiabatic approximation »}$$

If $P = m = 0$ at $t = 0$ then,

$$P(t) = \frac{\beta \rho_m}{\delta_m \delta_P} \left\{ 1 - \exp(-\delta_P t) - \frac{\delta_P}{\delta_m - \delta_P} [\exp(-\delta_P t) - \exp(-\delta_m t)] \right\} \quad (\text{exact integration})$$

Adiabatic approximation : simple exponential relaxation

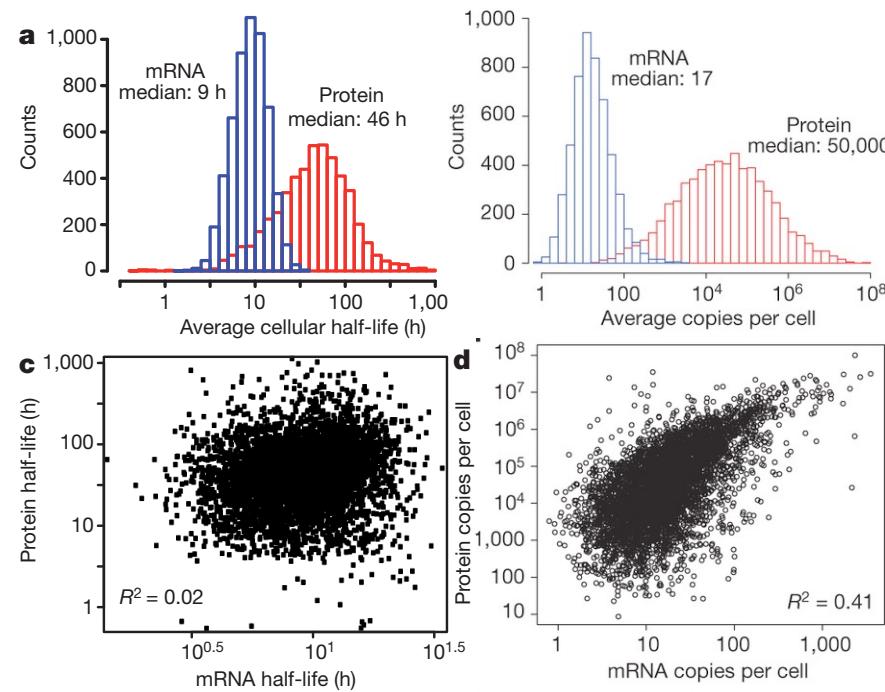
Protein relaxation to steady state



$P(t) \sim t^2$ instead of $P(t) \sim t$ at short times (boundary layer!)

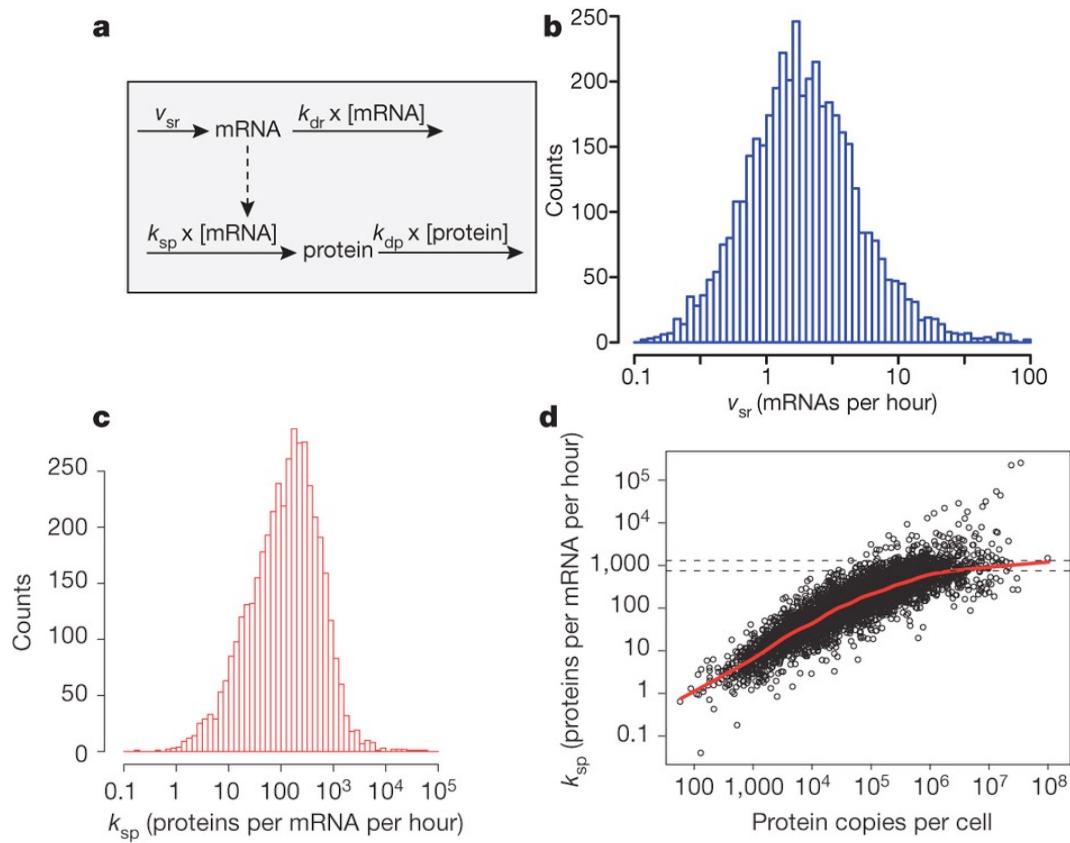
A feeling for the number of mRNA and proteins

B Schwandhäusser et al, Nature 473, 337 (2011); corrected 495, 126-127 (2013)



Mouse fibroblasts (NIH3T3)

Quantitative model of gene expression in growing cells.



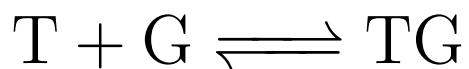
B Schwanhäusser *et al.* *Nature* **473**, 337-342 (2011) + correction (2013)

nature

The simplest description of mRNA and protein dynamics

III. Regulation of transcription

(extremely simplified : add other features if needed)



$$\rho([T]) = \rho_0[G] + \rho_1[TG]$$

$$\frac{[T][G]}{[TG]} = K_d \quad \text{K}_d \text{ concentration nM, few tens of TF/cell}$$

$$[G] + [TG] = 1 \quad (\text{one gene considered})$$

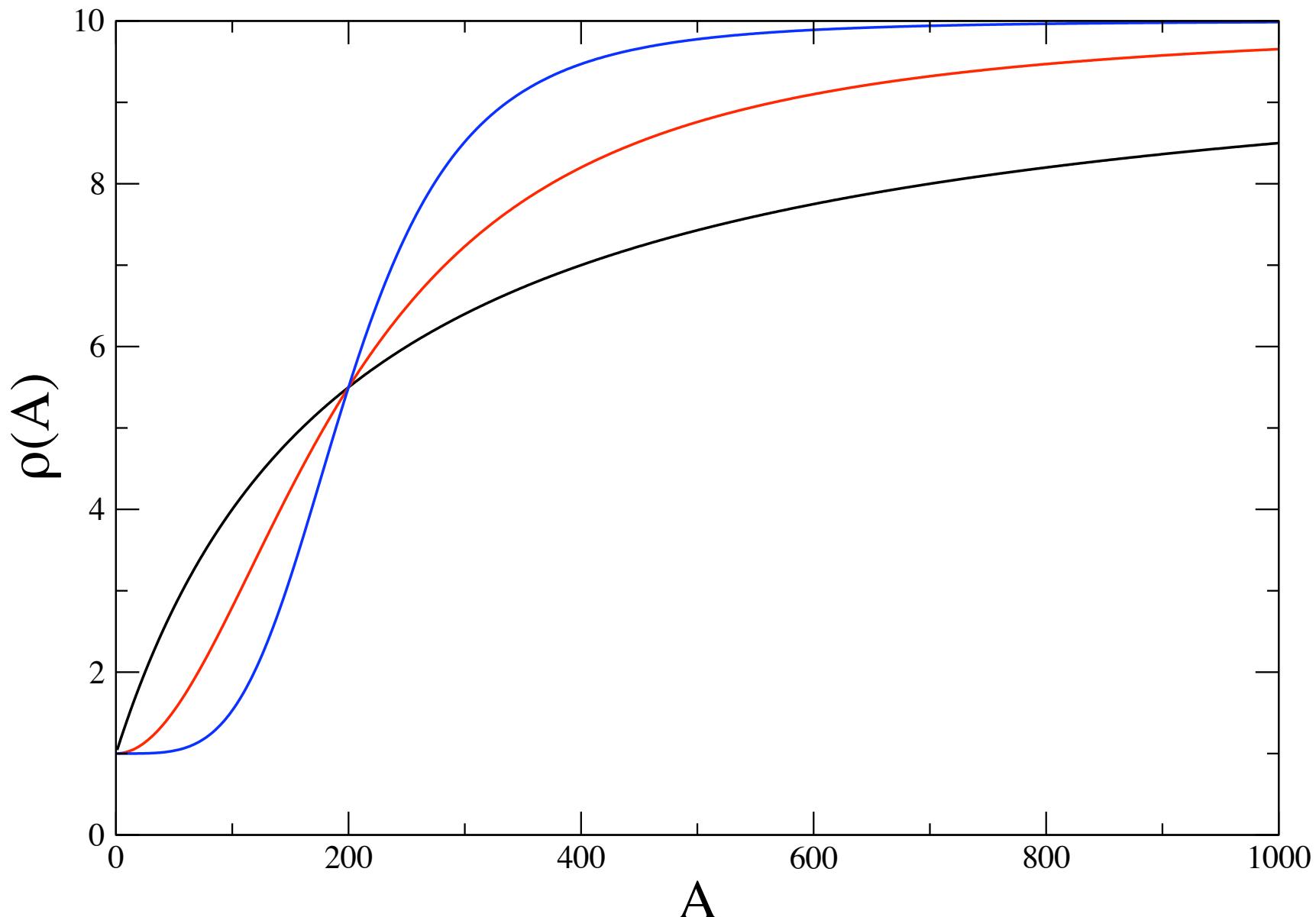
$$K_d \frac{[TG]}{T} + [TG] = 1 \quad \text{i.e.} \quad [TG] = \frac{[T]}{K_d + [T]}$$

Finally : $\rho([T]) = \rho_0 \frac{K_d}{K_d + [T]} + \rho_1 \frac{G}{K_d + [T]} = \frac{\rho_0 + \rho_1 [T]/K_d}{1 + [T]/K_d}$

More general : $\rho_m = \frac{\rho_0 + \rho_T ([T]/K_d)^h}{1 + ([T]/K_d)^h}$ h : Hill coefficient

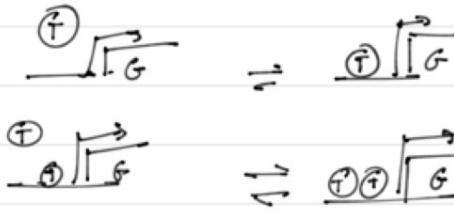
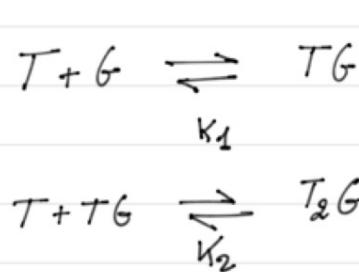
Hill activation

(black, h=1; red, h=2; blue h=4)



Where does the Hill exponent come from ?

- General idea : cooperativity between transcription factors
- One example : **sequential attachment**



$$\frac{[T][G]}{[TG]} = K_1 \quad \frac{[T][TG]}{[T_2G]} = K_2$$

$$[T] + [TG] + [T_2G] = 1$$

$$[TG] = [T_2G] \cdot \frac{K_2}{[T]} \quad , \quad [G] = [TG] \cdot \frac{K_1}{[T]} = [T_2G] \cdot \frac{K_1 K_2}{[T]^2}$$

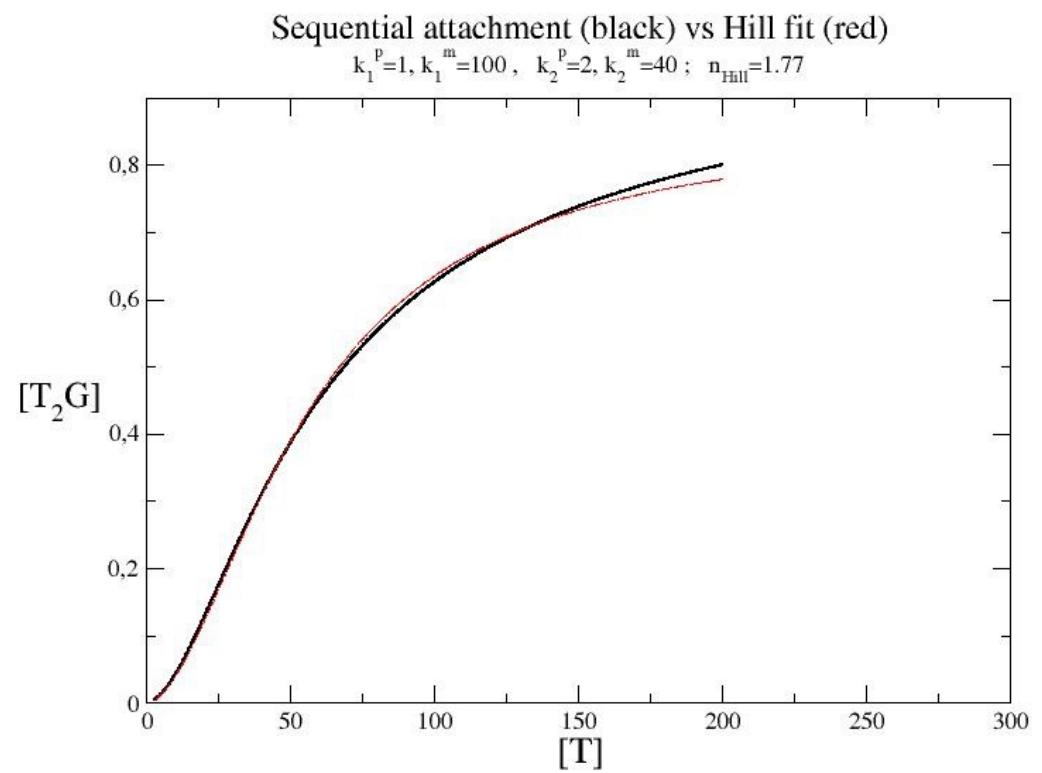
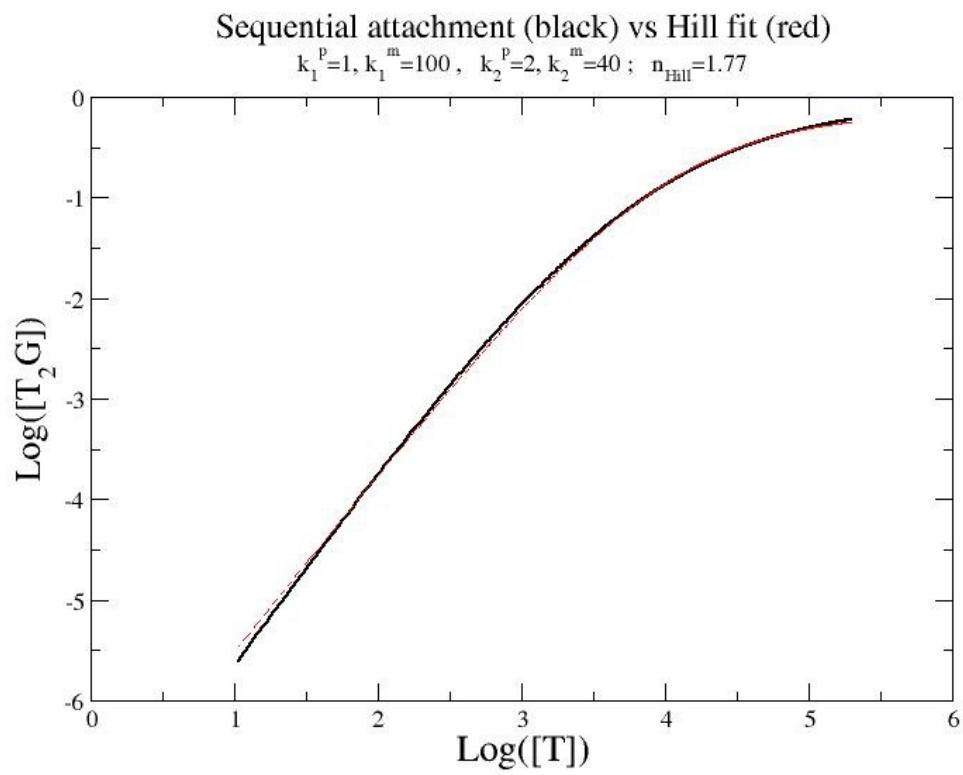
$$[T_2G] = \frac{[T]^2 / (K_1 K_2)}{1 + [T]/K_1 + [T]^2 / (K_1 K_2)}$$

Hill if for $T^* = \sqrt{K_1 K_2}$, $T^*/K_1 \ll 1$

i.e. $K_2 \ll K_1$

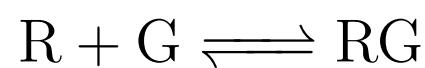
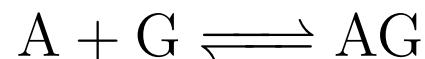
When the concentration $[T]$ is sufficient for the first factor to bind, it is well the concentration necessary for the second T to bind => **cooperativity of binding**

Hill formula is an **effective description** of more complex mechanisms



More complex functions with multiple transcription factors

Ex. : Competitive antagonistic binding of an activator and a repressor



$$\frac{[A][G]}{[AG]} = K_A, \quad \frac{[R][G]}{[RG]} = K_R$$

$$[G] + [AG] + [RG] = 1$$

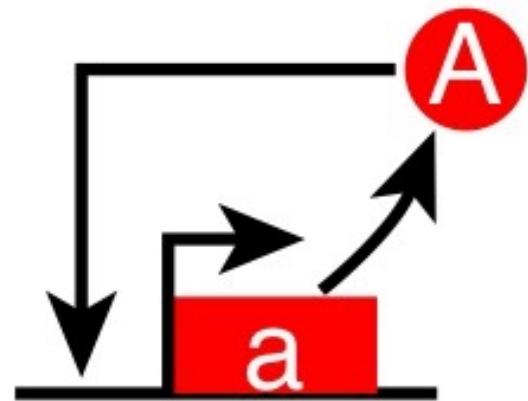
$$[G] = \frac{1}{1 + [A]/K_A + [R]/K_R}, \quad [AG] = \frac{[A]/K_A}{1 + [A]/K_A + [R]/K_R}, \quad [RG] = \frac{[R]/K_R}{1 + [A]/K_A + [R]/K_R}.$$

$$\rho([A], [R]) = \frac{\rho_0 + \rho_A[A]/K_A + \rho_R[R]/K_R}{1 + [A]/K_A + [R]/K_R}$$

With $\rho_0 = \rho_R = 0$, transcription function (A AND NOT R)

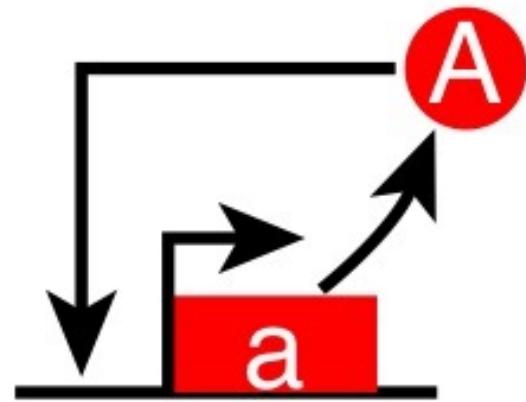
Bistability

The simplest network



Can this network
be bistable?

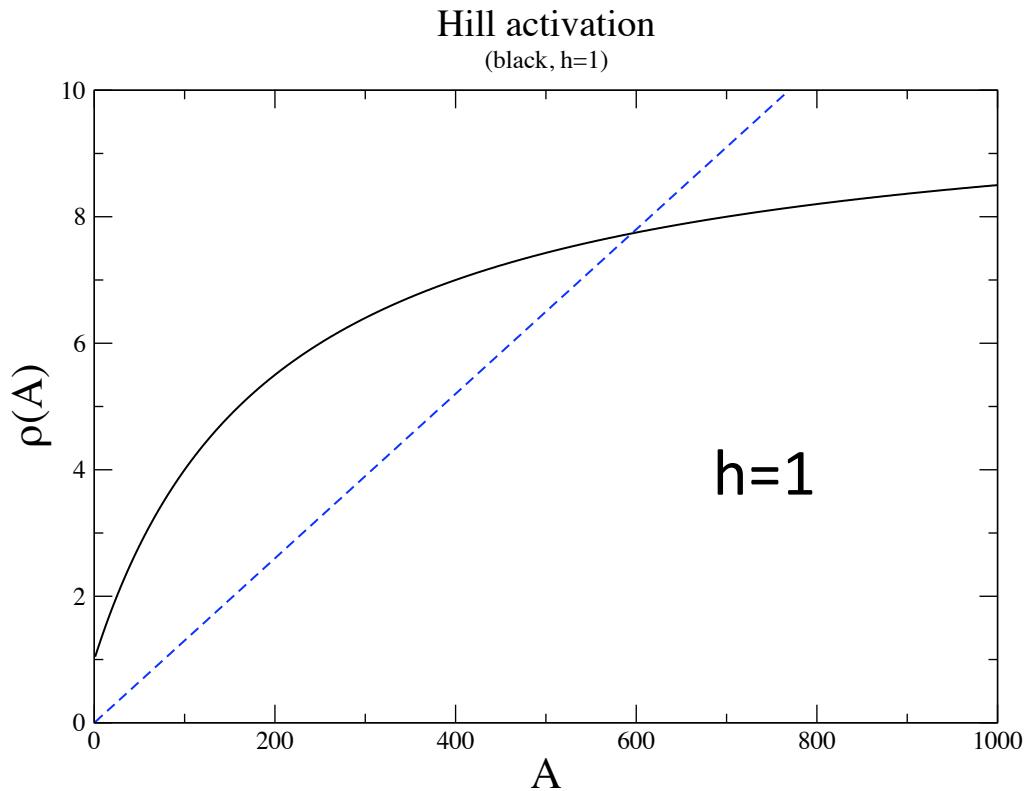
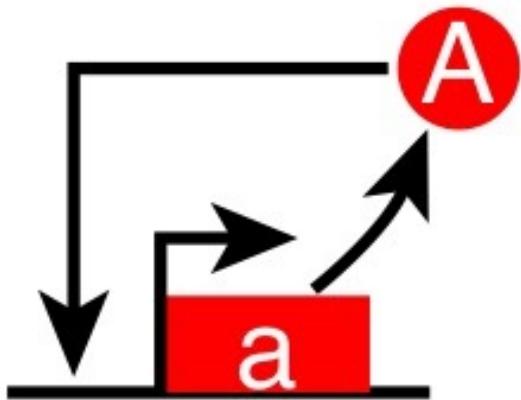
The simplest network



Can this network
be bistable?

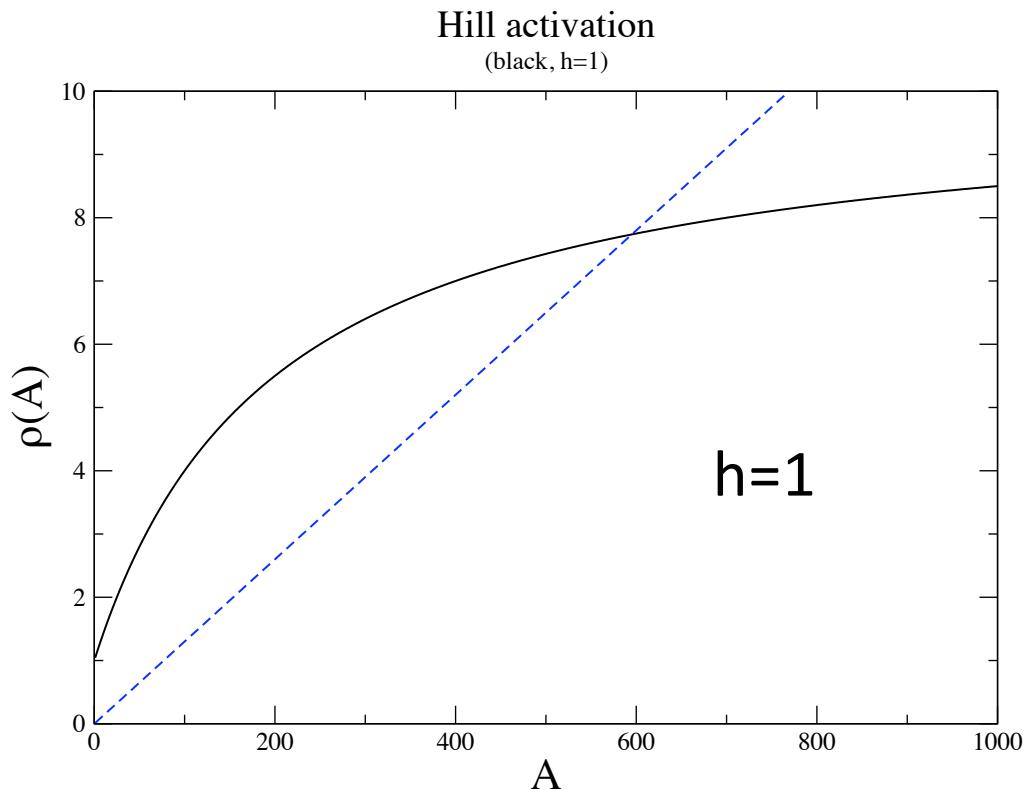
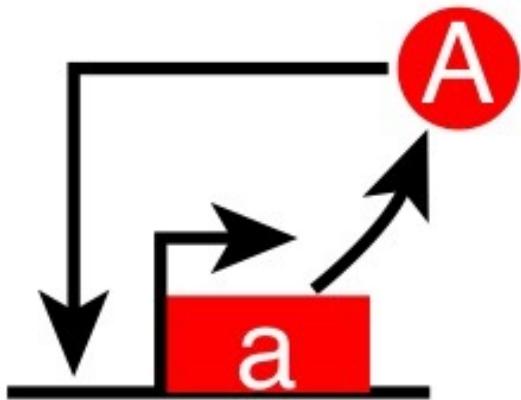
$$\frac{dA}{dt} = \frac{\rho_0 + \rho_1 (A/A_0)^h}{1 + (A/A_0)^h} - \delta_A A$$

The simplest network



$$0 = \frac{\rho_0 + \rho_1 (A/A_0)}{1 + (A/A_0)^h} - \delta_A A$$

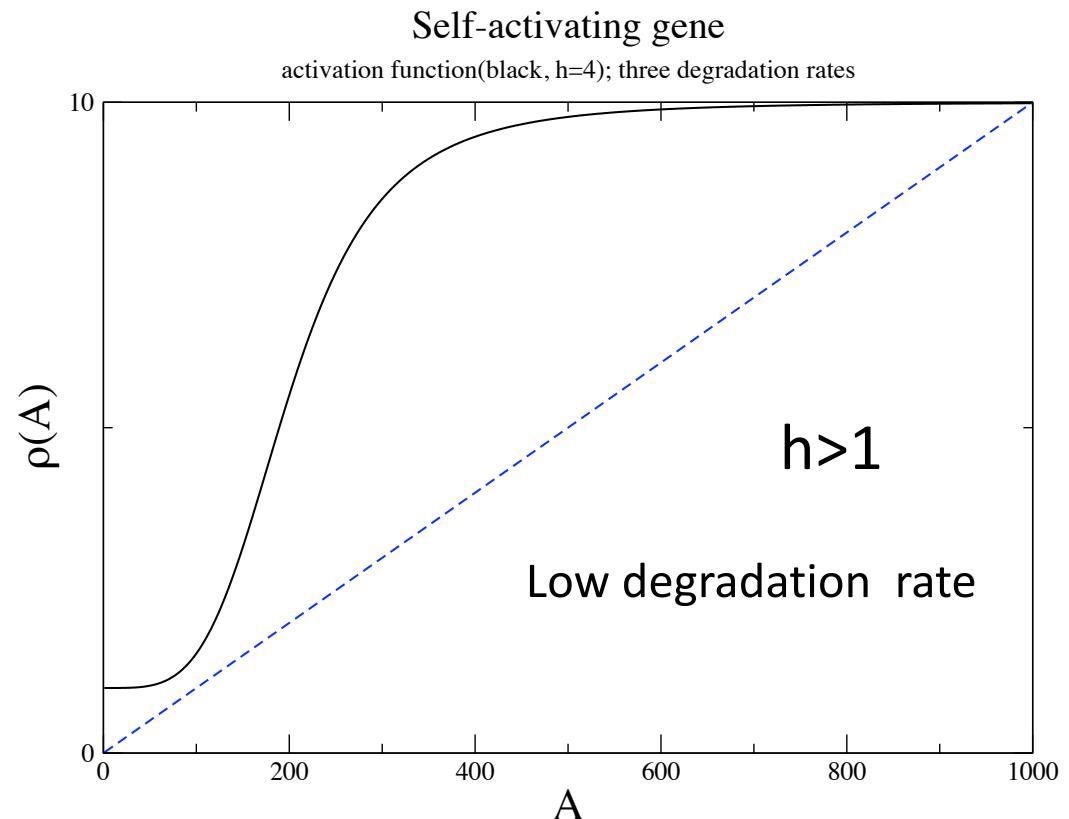
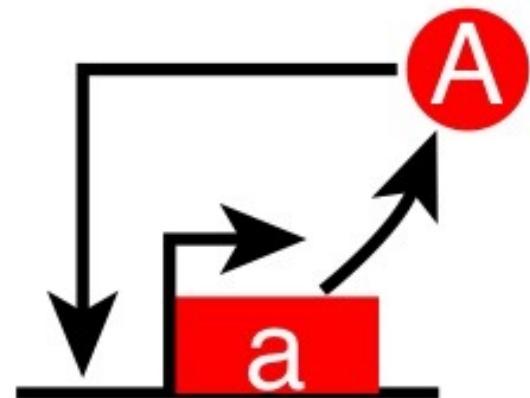
The simplest network



$$0 = \frac{\rho_0 + \rho_1 (A/A_0)}{1 + (A/A_0)^h} - \delta_A A$$

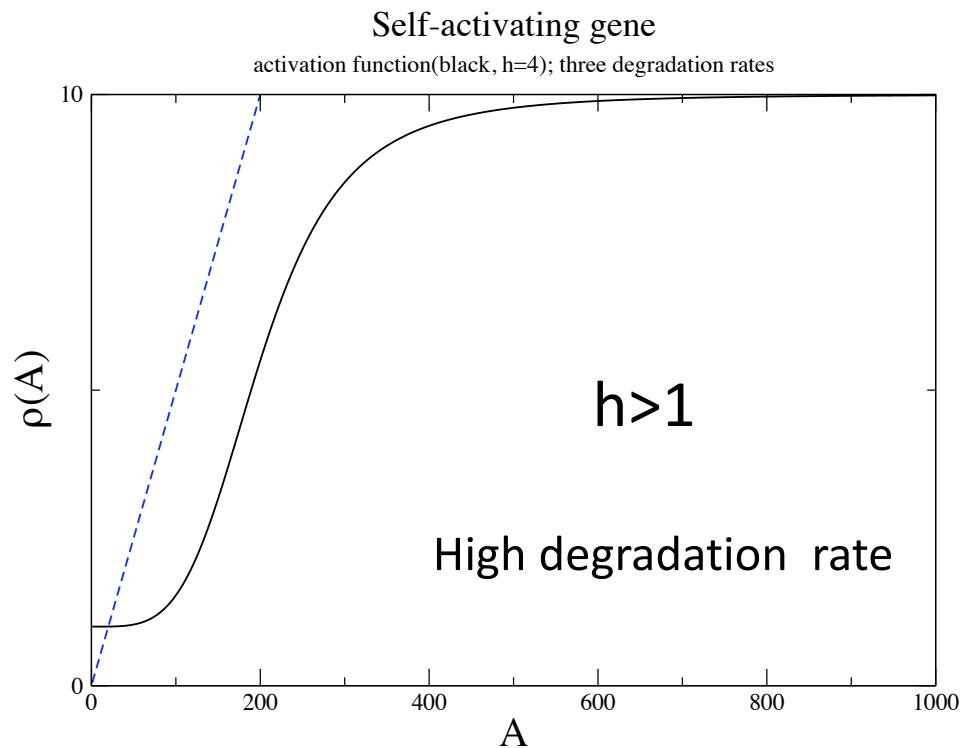
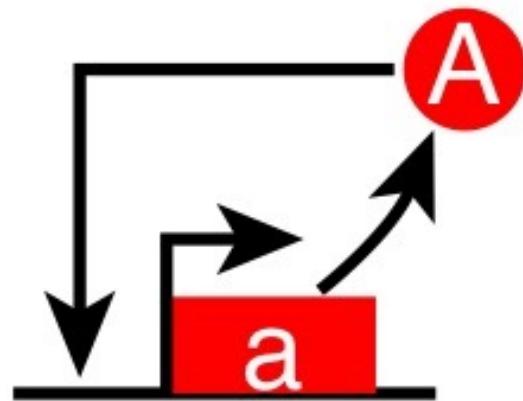
$h=1 \Rightarrow$ a single stationary state, no bistability.

The simplest network



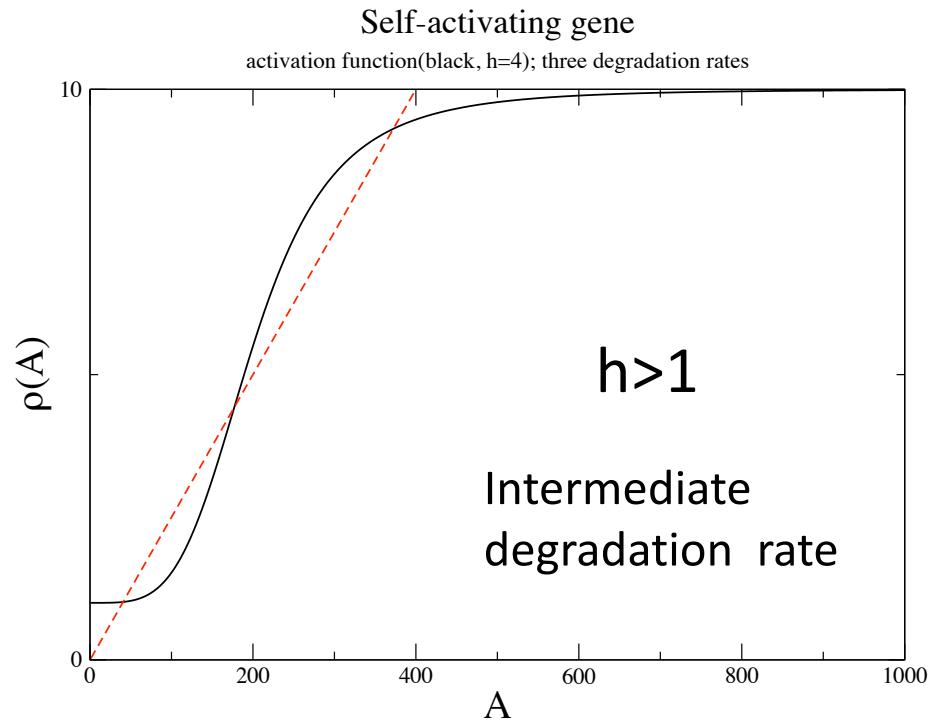
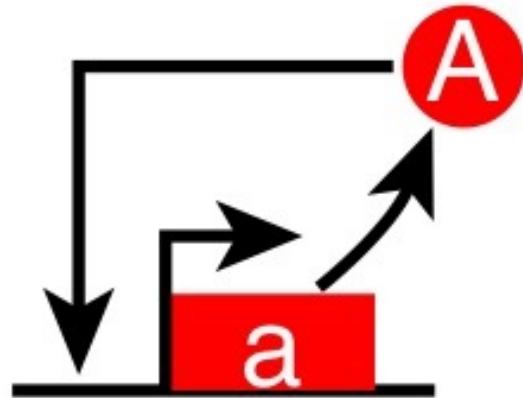
$$0 = \frac{\rho_0 + \rho_1 (A/A_0)^h}{1 + (A/A_0)^h} - \delta_A A$$

The simplest network



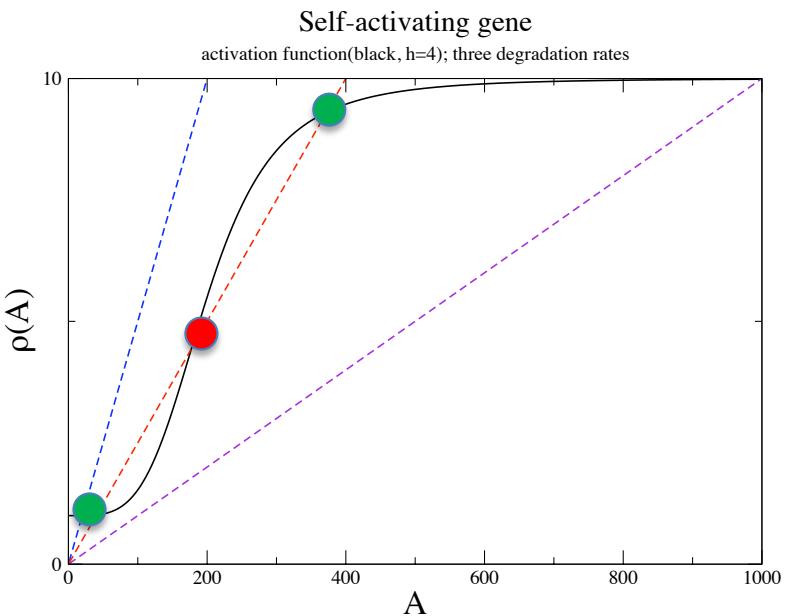
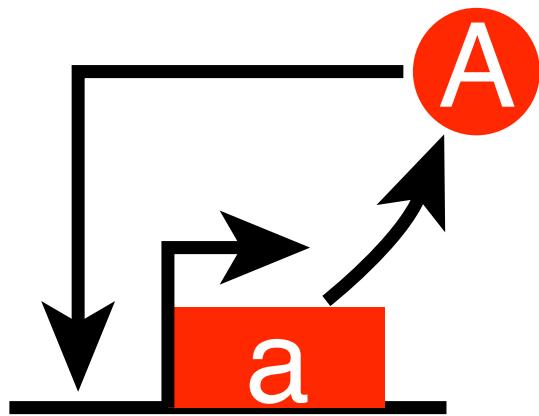
$$0 = \frac{\rho_0 + \rho_1 (A/A_0)^h}{1 + (A/A_0)^h} - \delta_A A$$

The simplest network



$$0 = \frac{\rho_0 + \rho_1 (A/A_0)^h}{1 + (A/A_0)^h} - \delta_A A$$

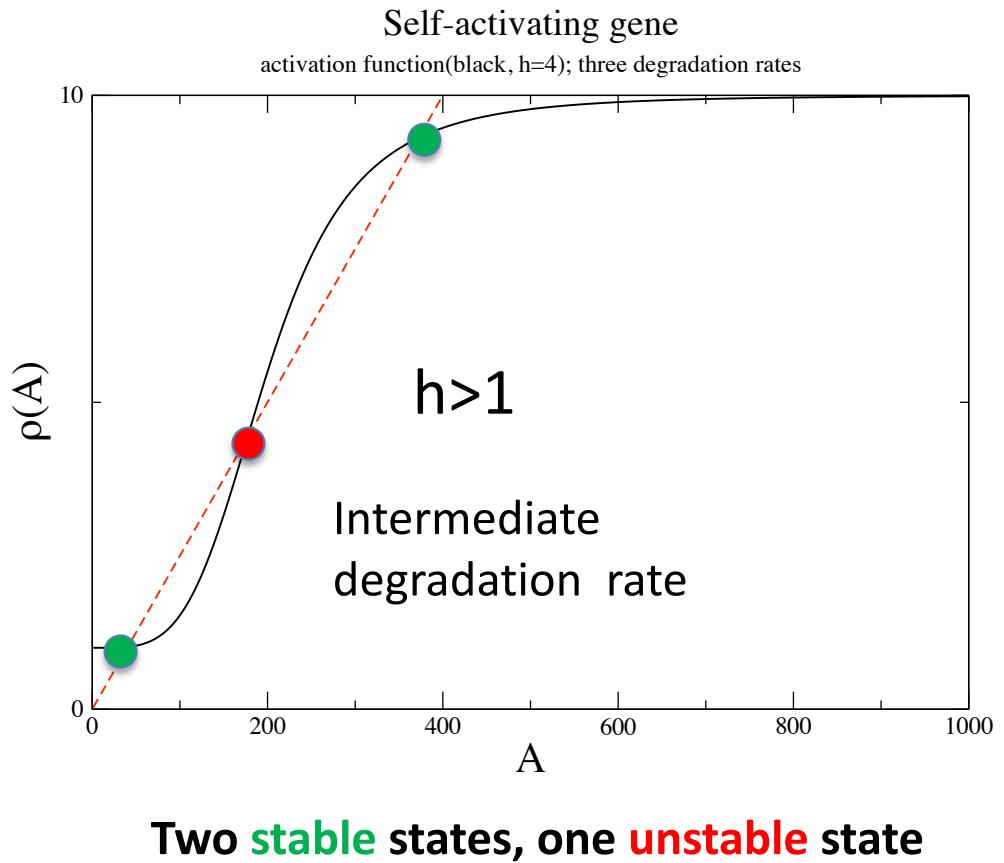
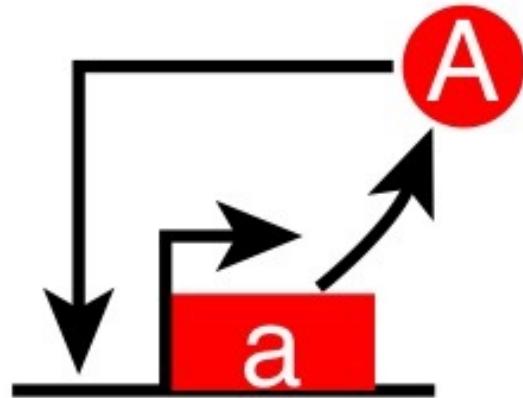
The simplest bistable network



Two stable states, one unstable state

Hill >1 required;
two stable fixed points (+ one unstable)
for intermediate degradation rate (red dashed-line)

The simplest network

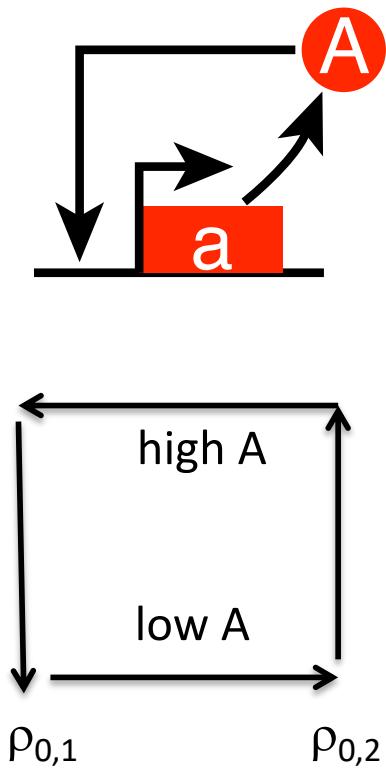


Bistability requires Hill coefficient $h > 1$

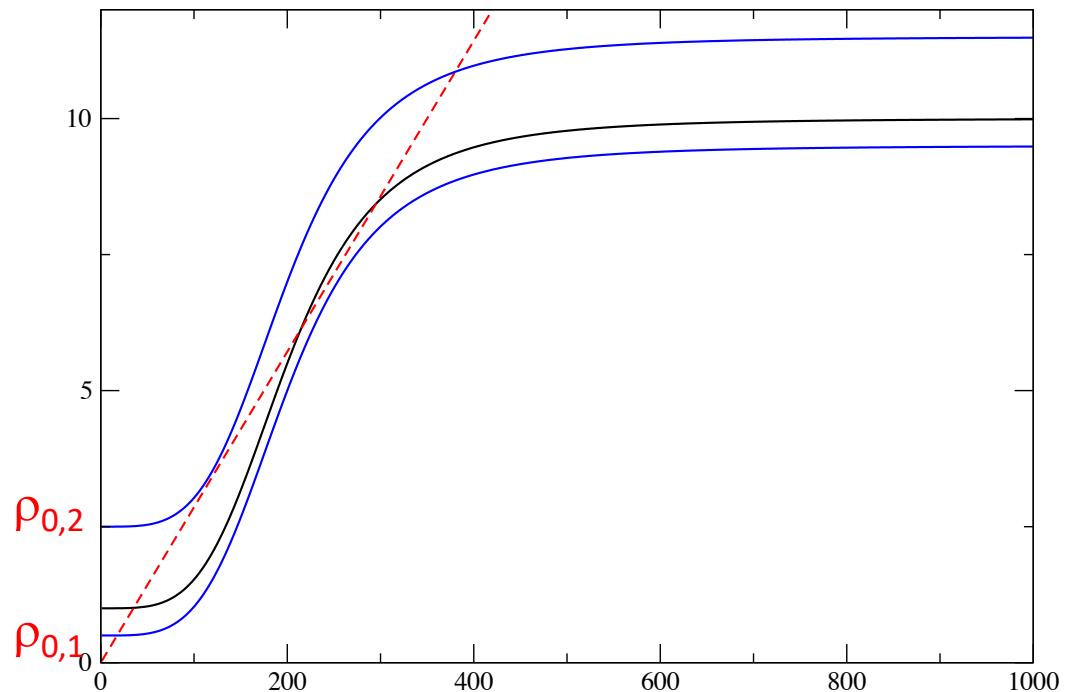
It is interesting to quantitatively measure gene regulation functions

$$\frac{\rho_0 + \rho_1 (A/A_0)^h}{1 + (A/A_0)^h}$$

Bistability and hysteresis



Bistability: jump between the two states by changing ρ_0



In the bistable region, when changing ρ_0 :

- jump at $\rho_{0,1}$ from high concentration to low and
- jump at $\rho_{0,2}$ from low to high concentration

with

$$\rho_{0,1} < \rho_{0,2}$$

« hysteresis »

« Saddle-node »
bifurcation

Cross-repression between two genes and bistability



$$a = A/A_0$$

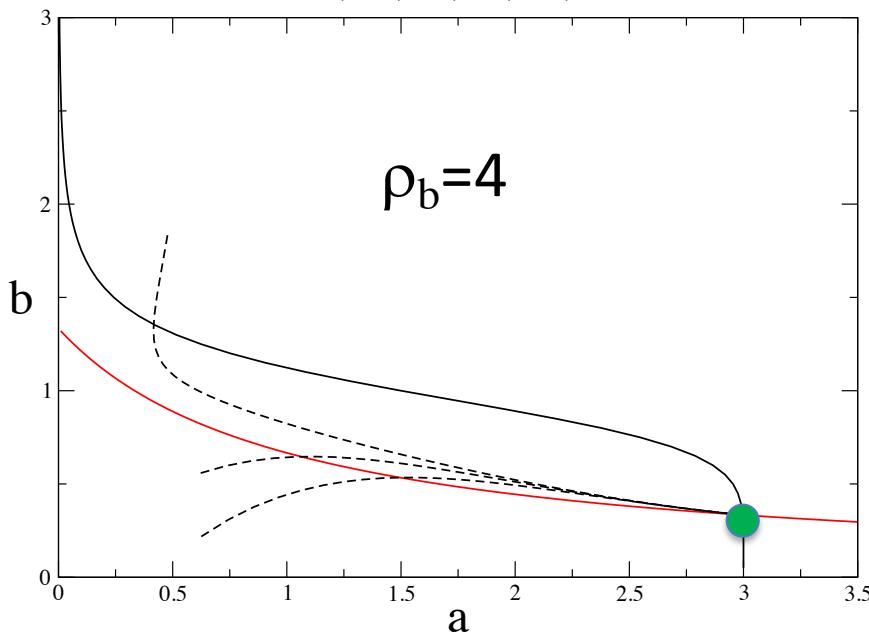
$$b = B/B_0$$

$$\frac{da}{dt} = \frac{\rho_a}{1 + b^{h_a}} - \delta_a a$$

$$\frac{db}{dt} = \frac{\rho_b}{1 + a^{h_b}} - \delta_b b$$

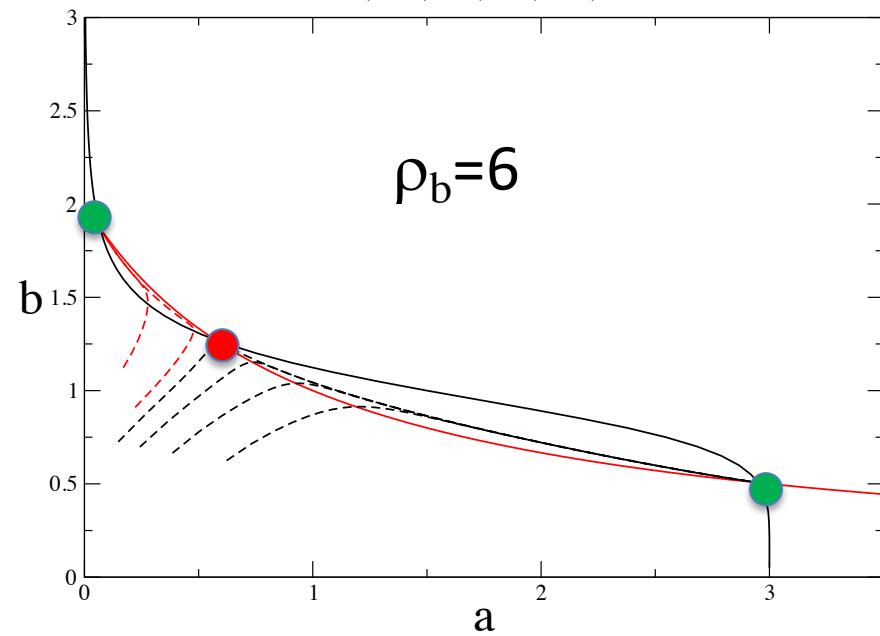
Two-gene network with cross inhibition

$ra=3, da=1, ha=6; rb=4, db=3, hb=1$



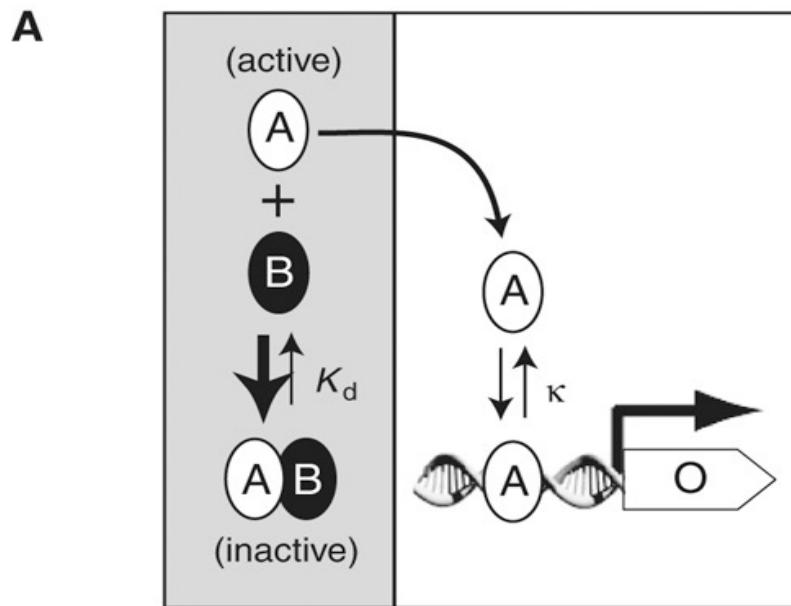
Two gene network with cross inhibition

$ra=3, da=1, ha=6; rb=6, db=3, hb=1$

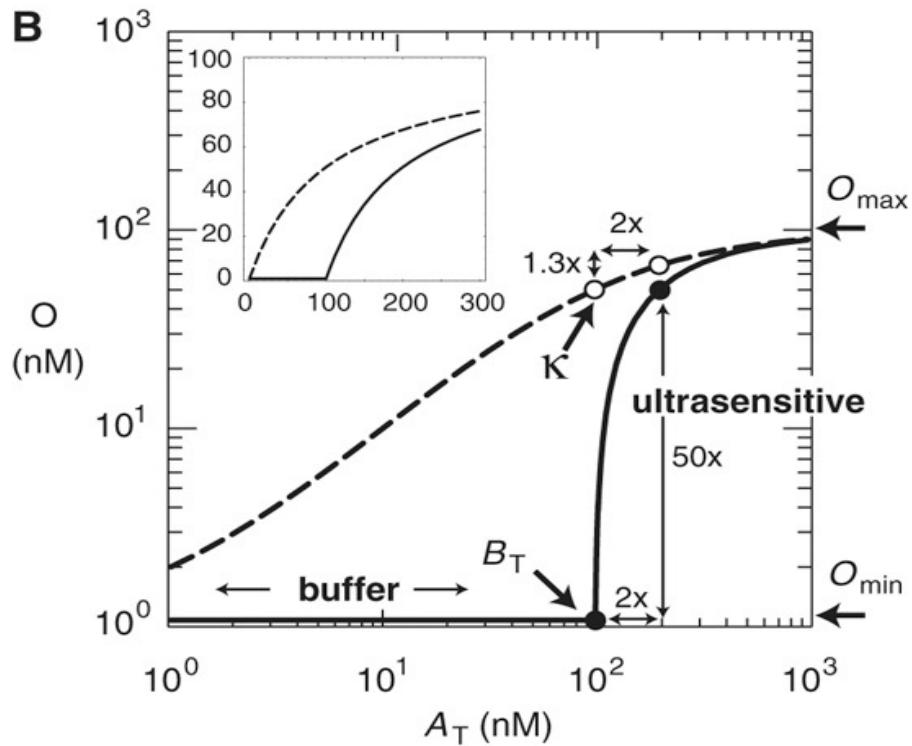


Imperfect « pitchfork » bifurcation

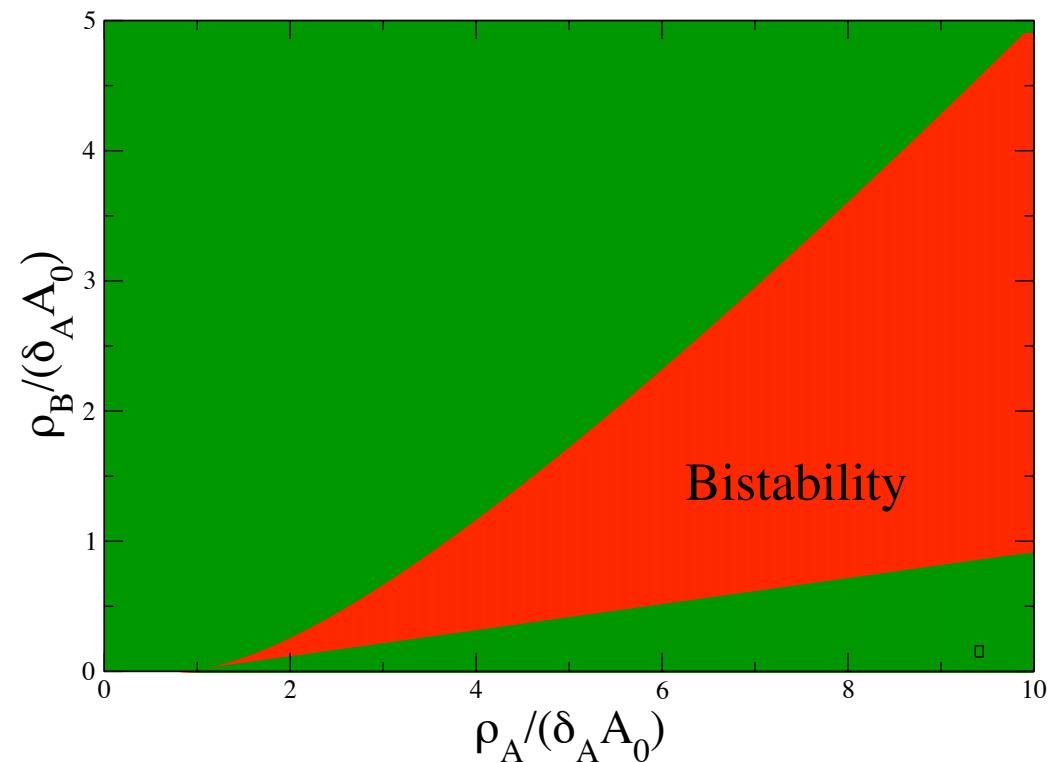
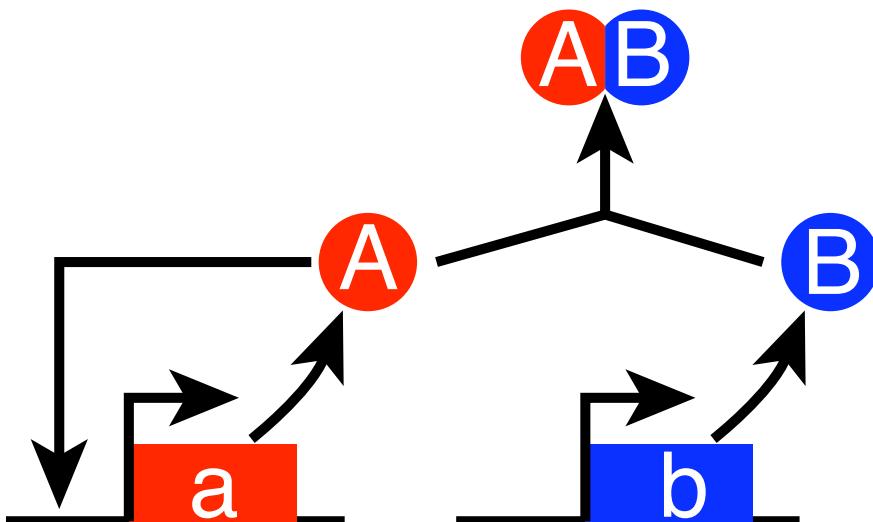
Effective high Hill coefficient from protein complexation



N. E. Buchler and F Cross
MSB 5:272 (2009)



A switch based on auto-activation and complexation



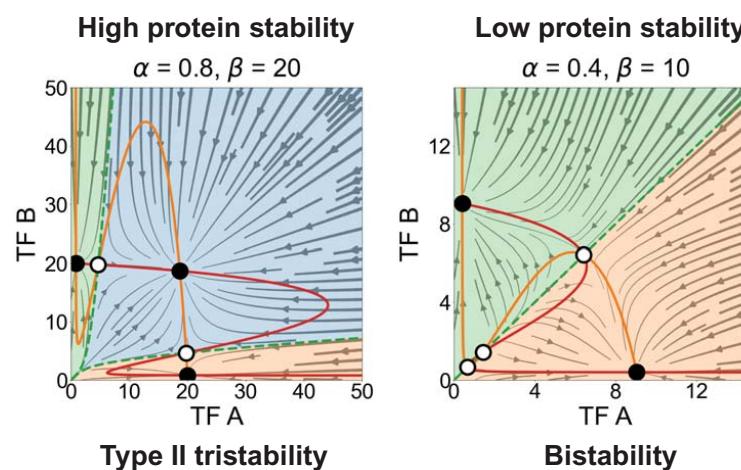
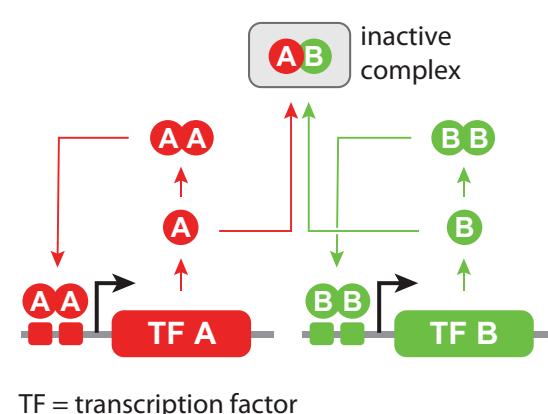
Works with Hill=1

Synthetic multistability in mammalian cells

Ronghui Zhu¹, Jesus M. del Rio-Salgado¹, Jordi Garcia-Ojalvo², Michael B. Elowitz^{1,3*}

Science 375 (2022)

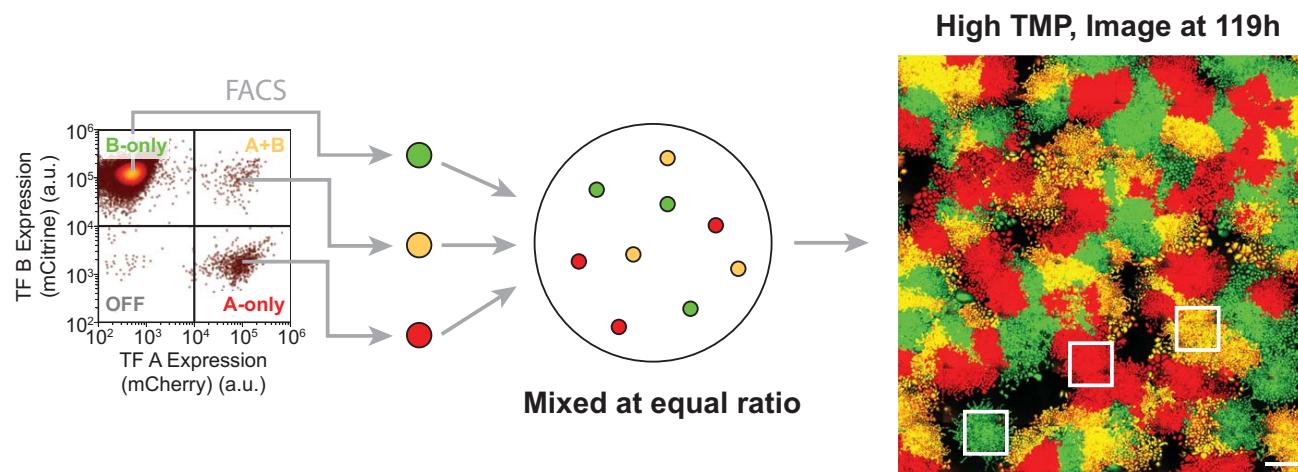
C MultiFate-2 circuit



Non-dimensionalized parameters
 α = basal protein production rate
 β = maximal activated protein production rate

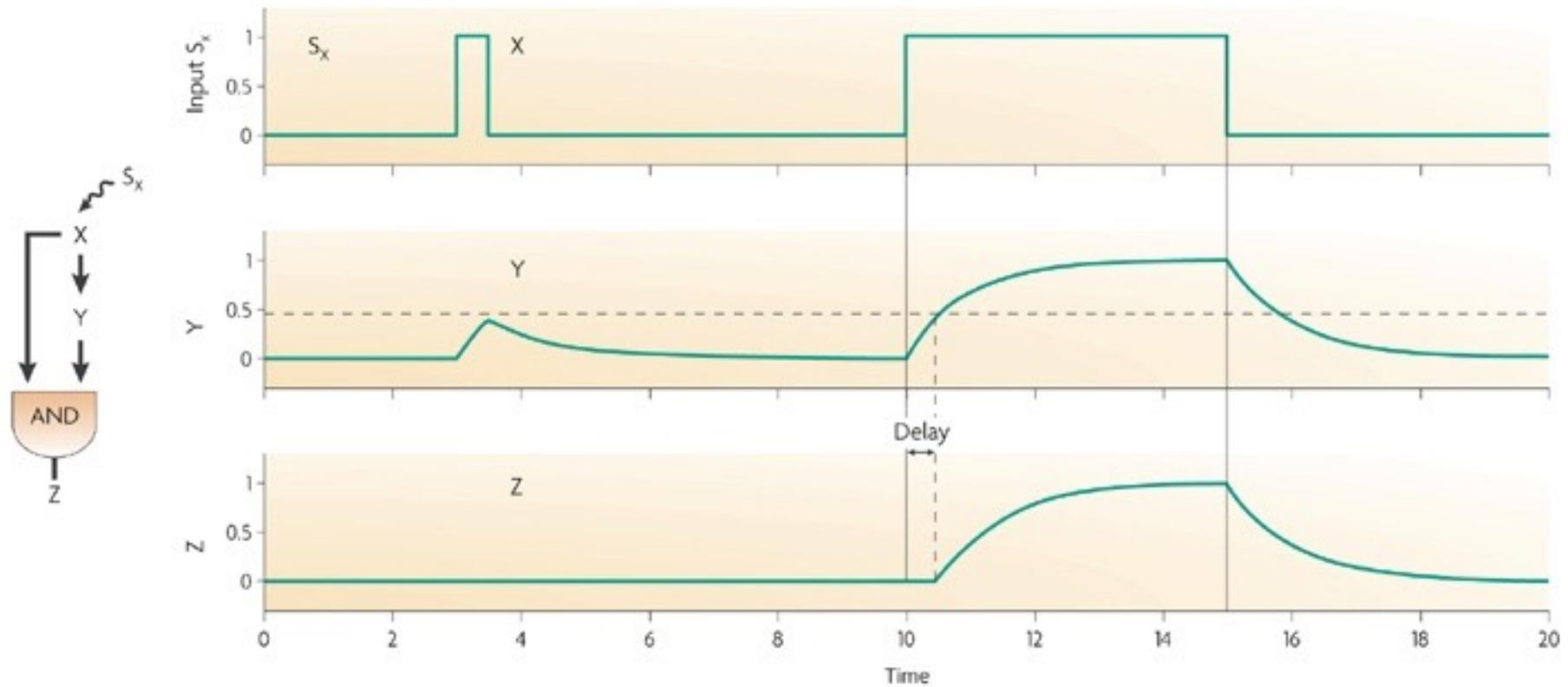
Phase portrait legends
— Nullclines
● Stable fixed point
○ Unstable fixed point
— Separatrix
■ Attractor basins

D Time-lapse imaging reveals MultiFate-2.3 tristability



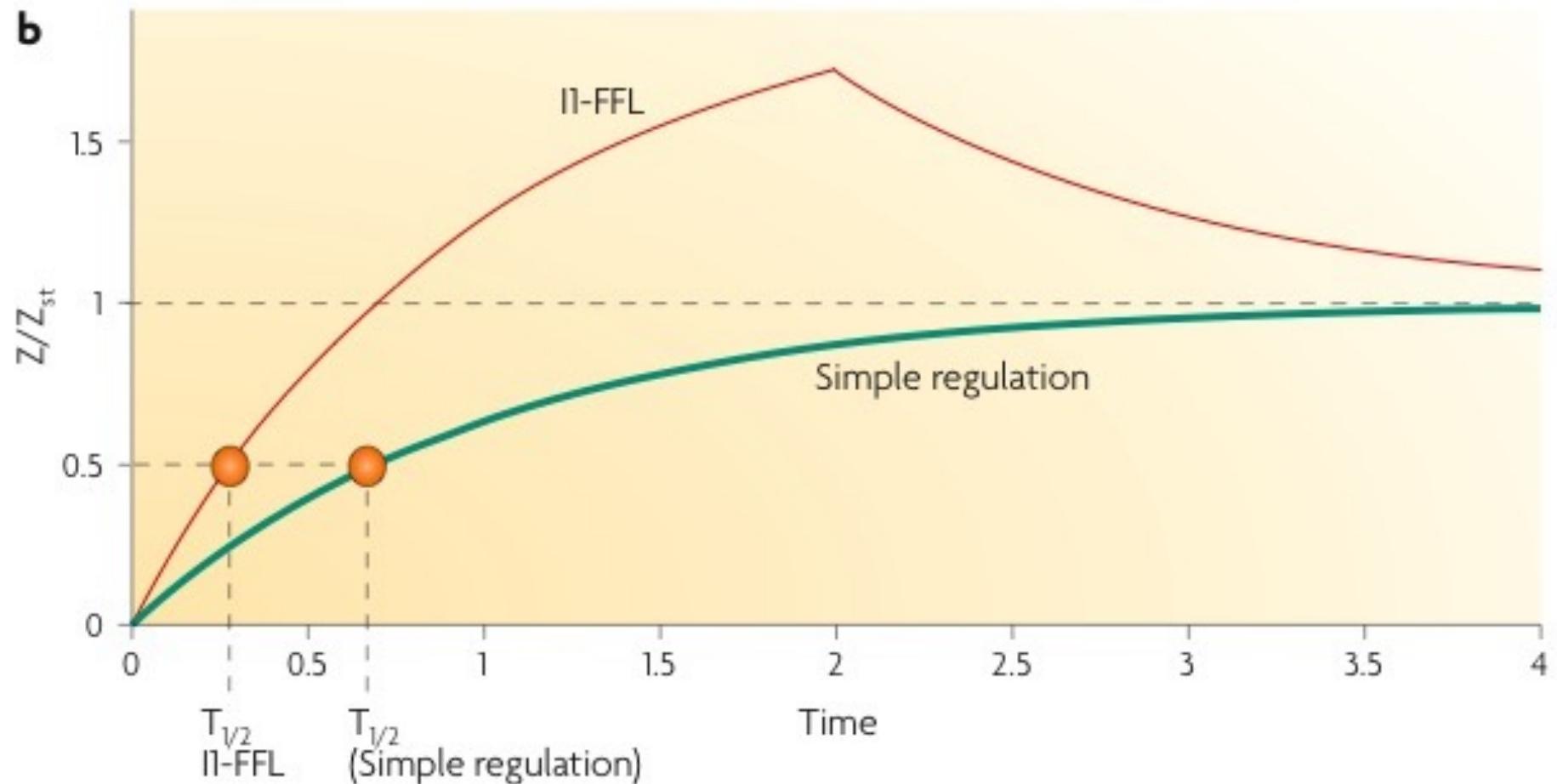
Two other simple circuits

Coherent feed-forward loop



Alon, Nat Rev Gen (2007)

Incoherent feed-forward loop or feed-forward inhibition

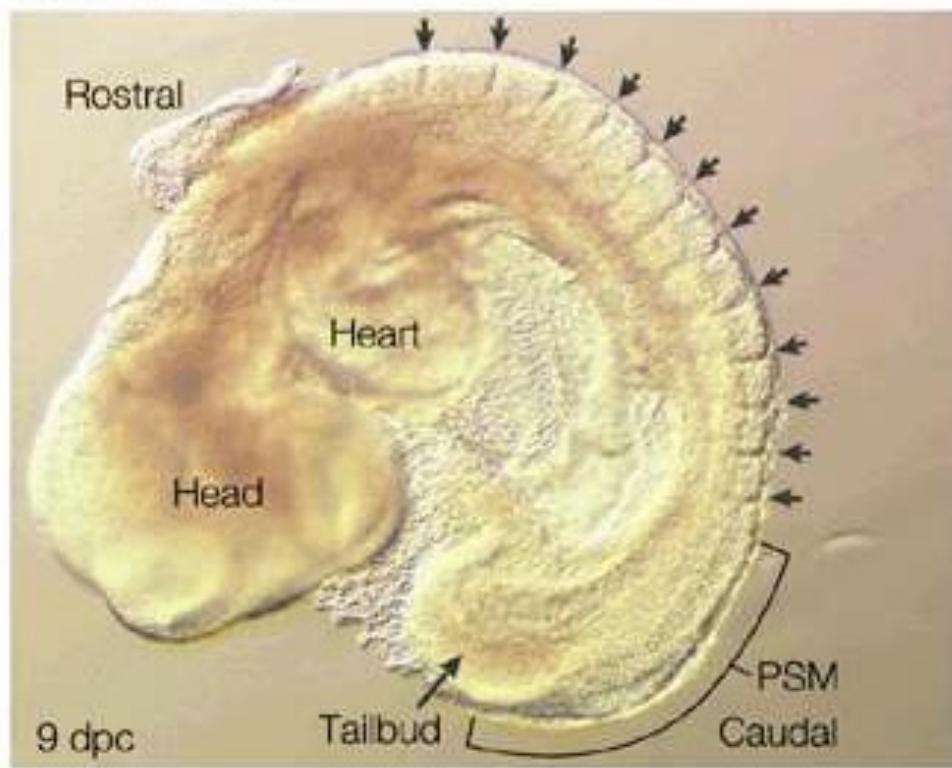


Alon, Nat Rev Gen (2007)

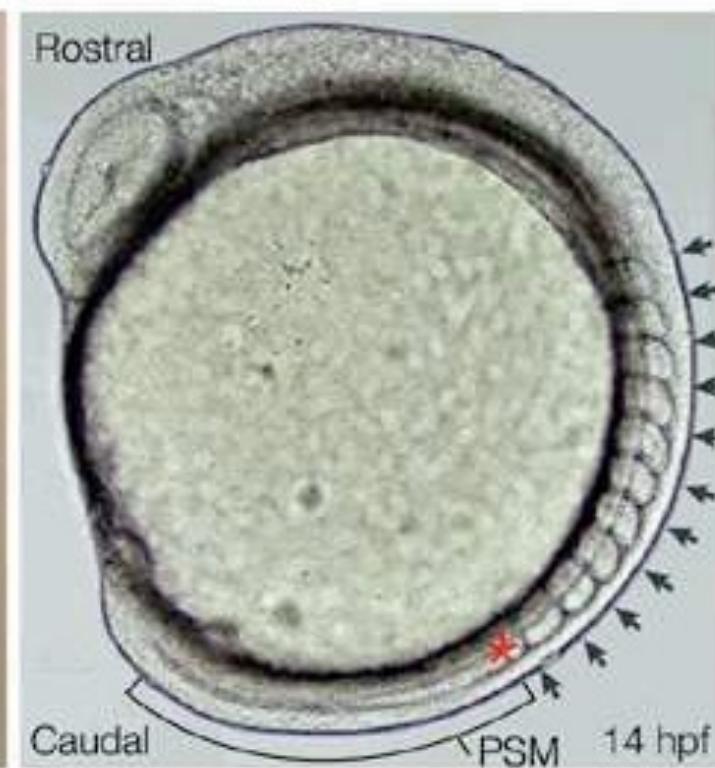
Oscillations

Oscillations and segmentation : somite formation in vertebrates

Mouse embryo

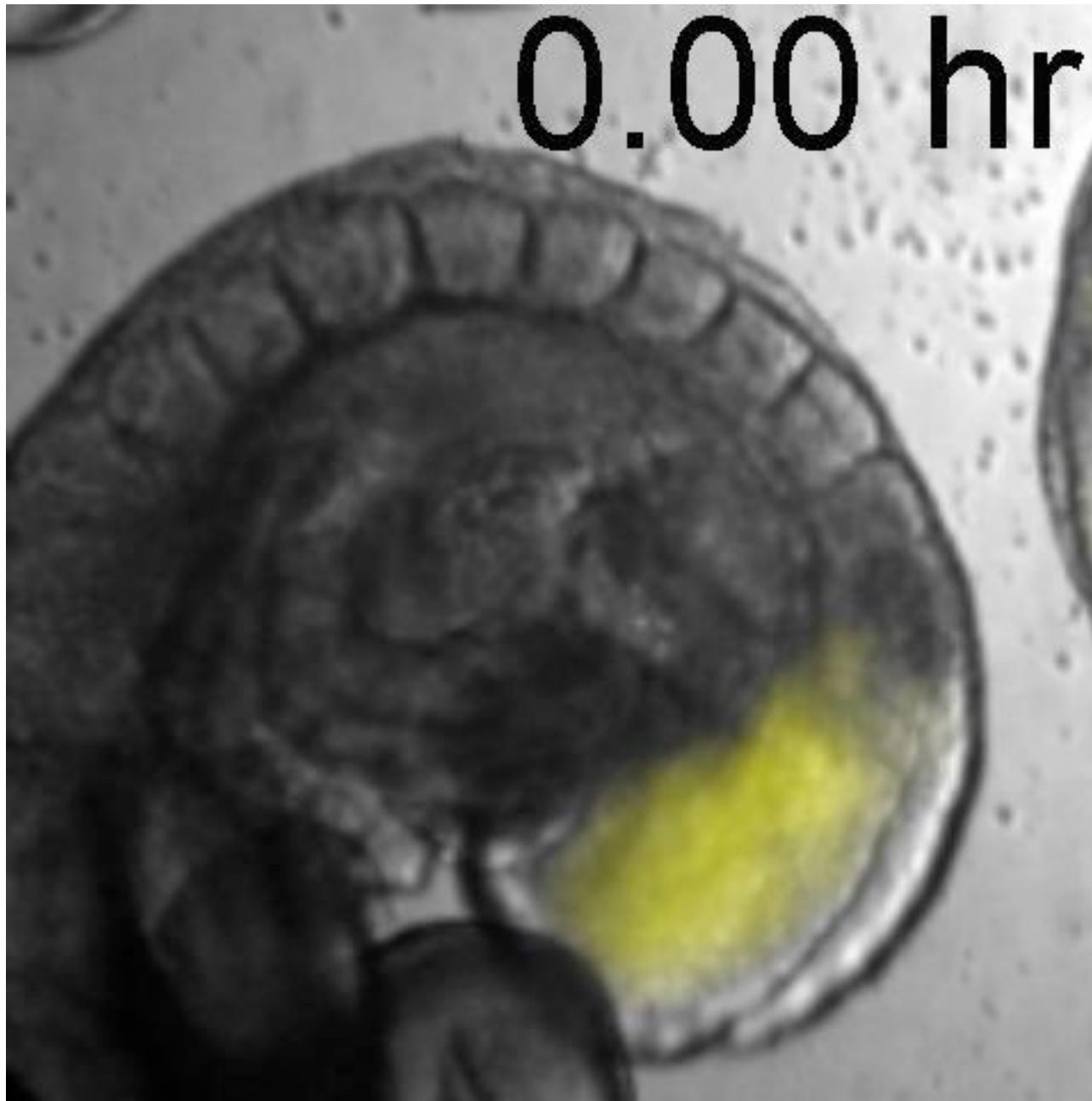


Zebrafish embryo



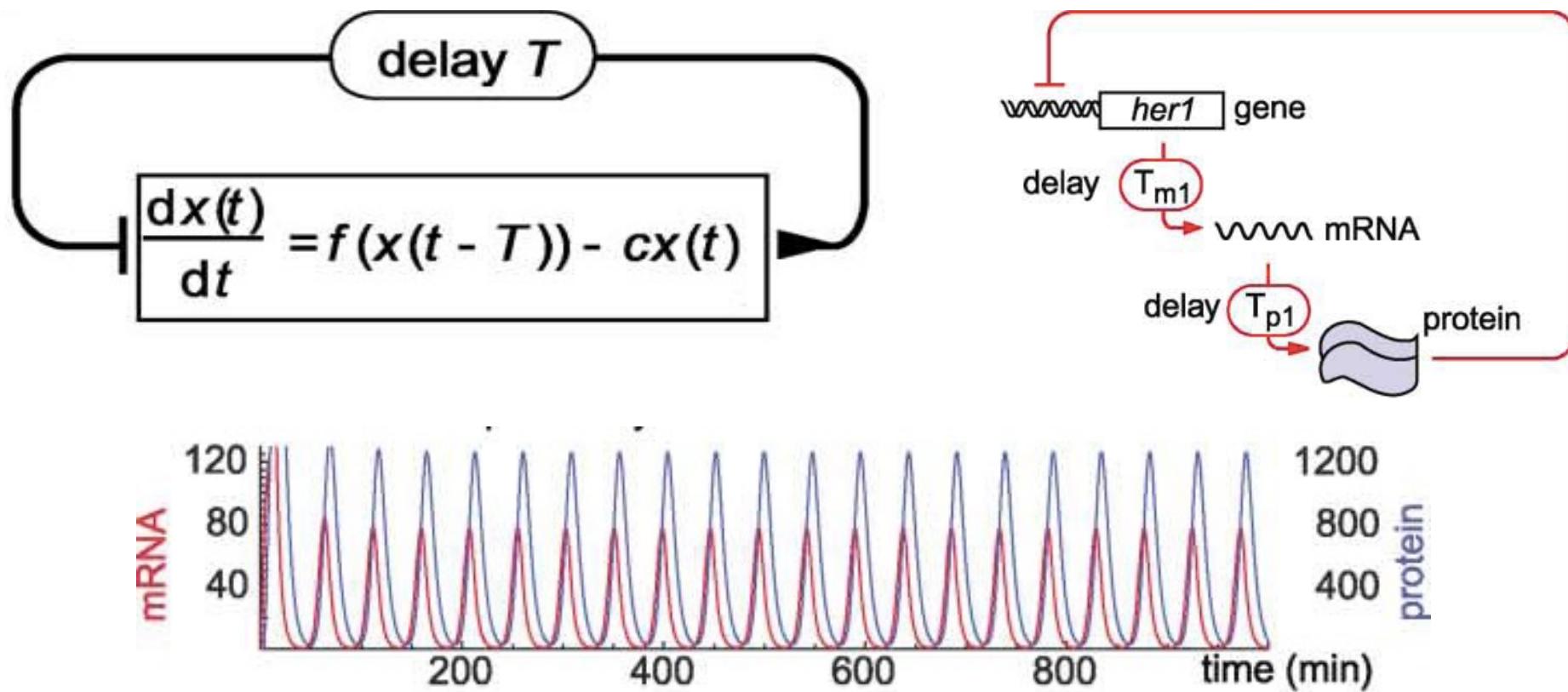
Saga, Nat Rev Gen (2001)

0.00 hr



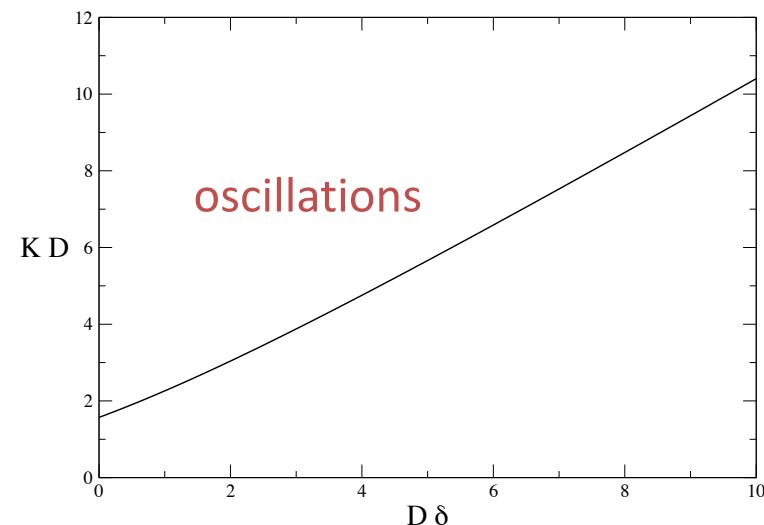
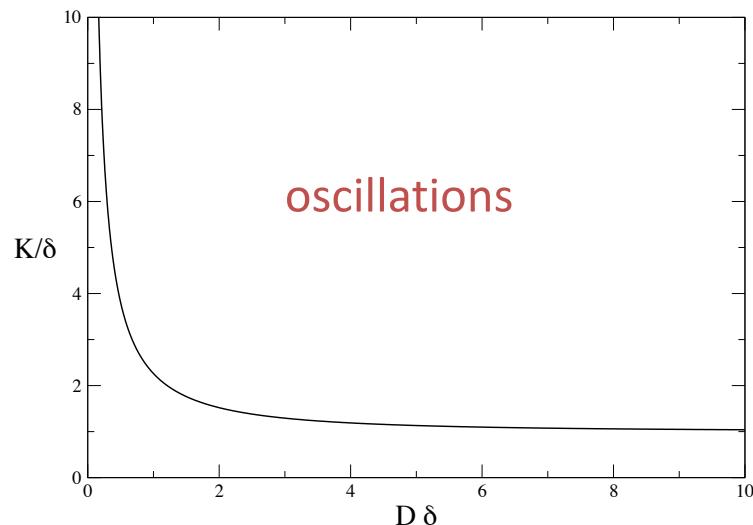
Auto-inhibition with delay

..., Mackey, ..., Goldbeter, ..., Monk, ..., Sneppen & Jensen, ...

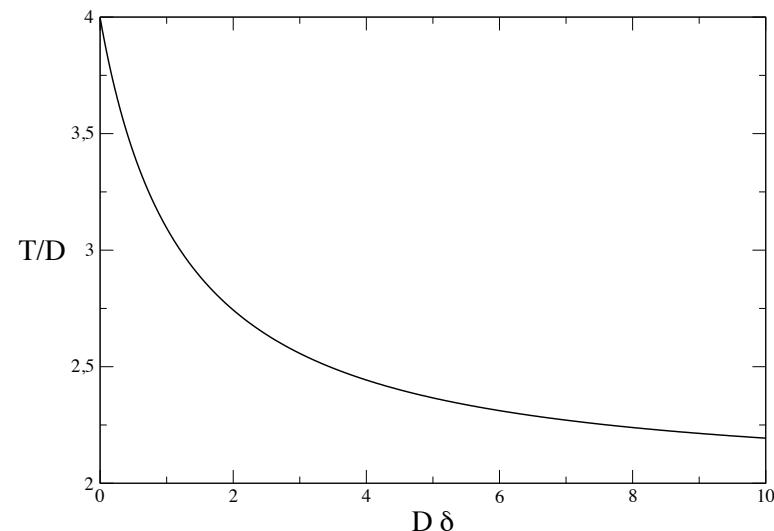
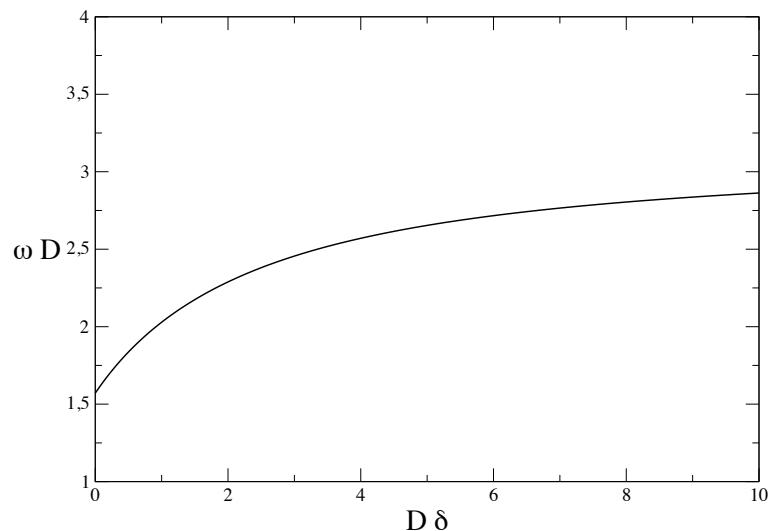


J Lewis, Current Biology (2003)

The self-repressing gene oscillatory regime.



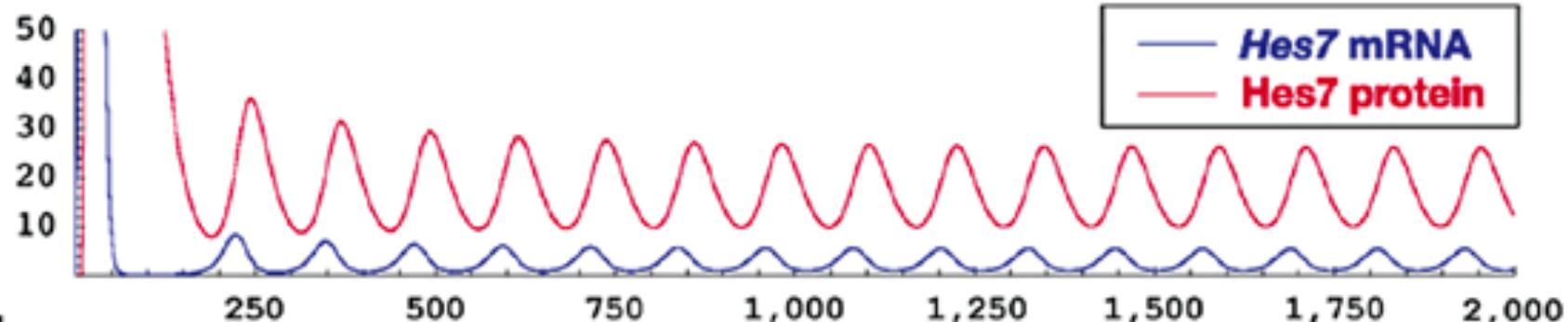
« (Poincaré-Andronov-)Hopf » bifurcation



a

Hes7^{+/+}

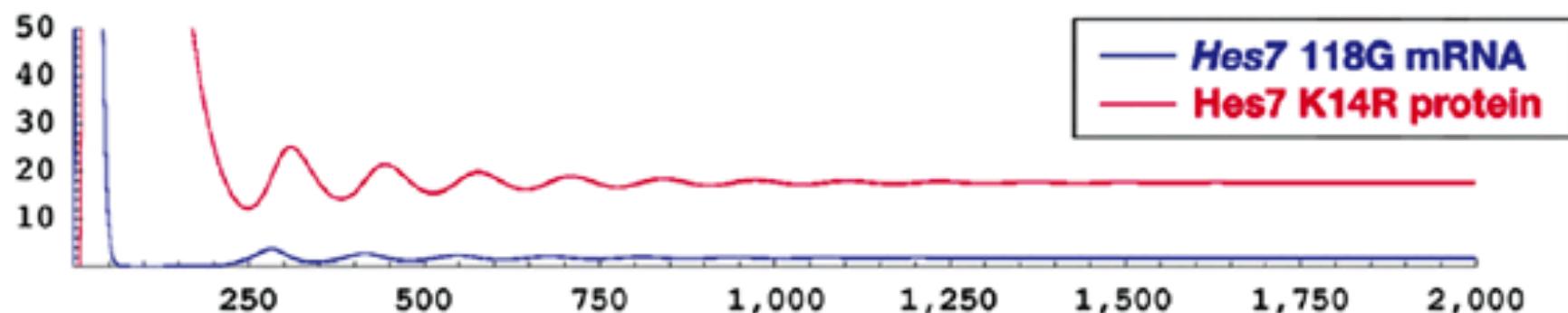
Protein half-life: 20 min; period: 121.4 min



b

Hes7^{118G/118G}

Protein half-life: 30 min; period: 131.6 min

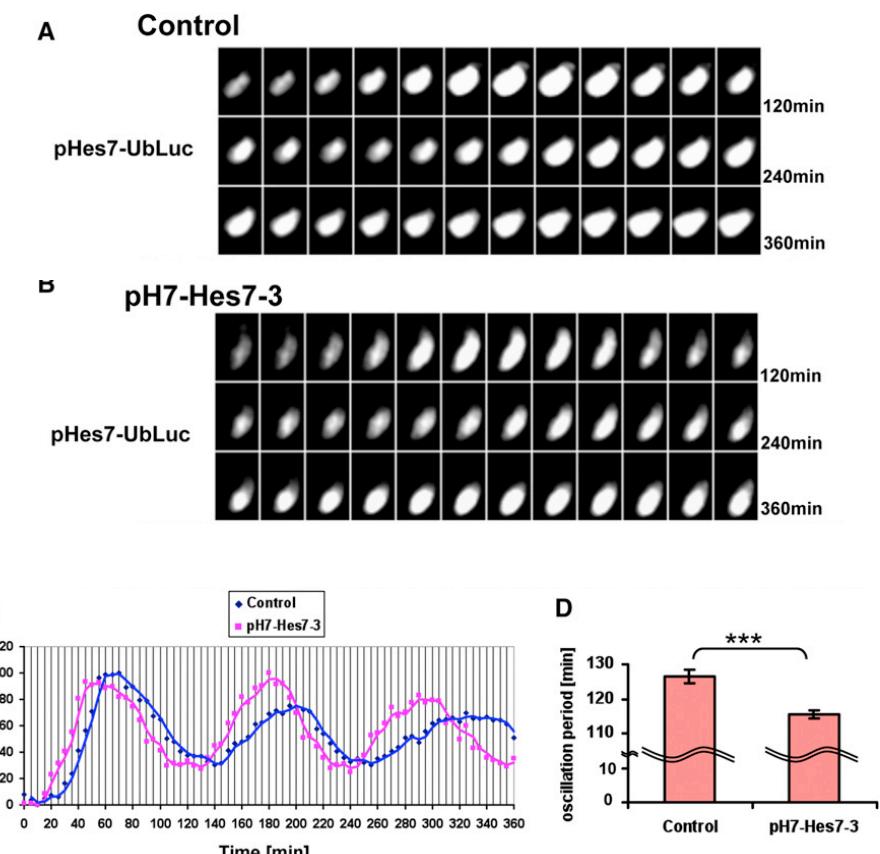
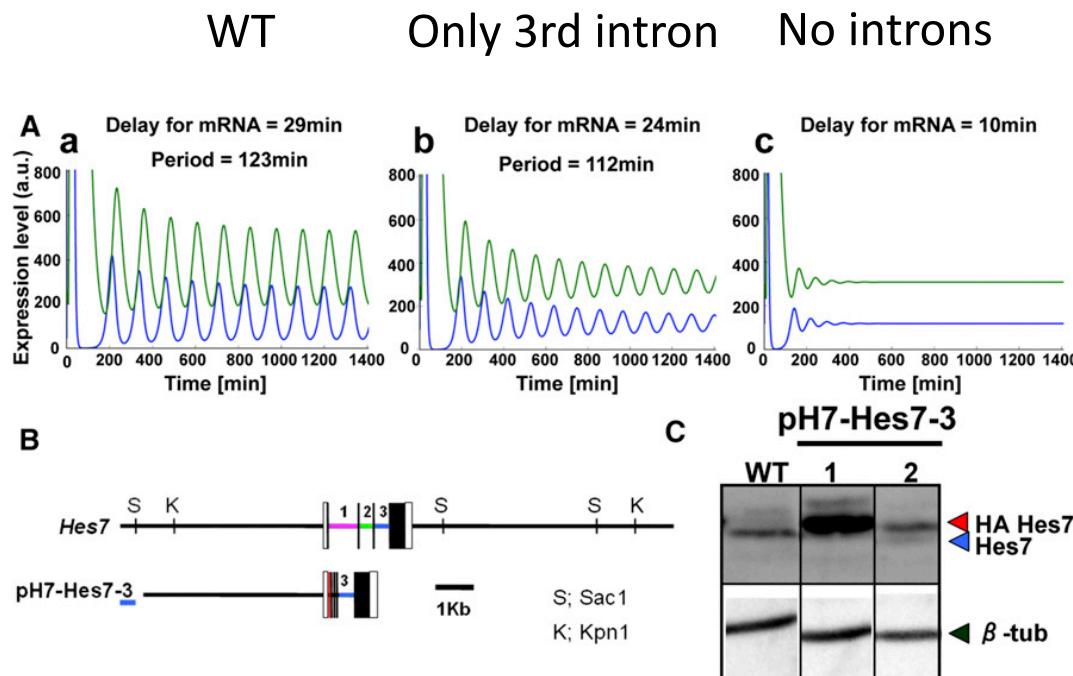


Hirata et al, Nat. Gen. 36, 750 (2004)

Accelerating the Tempo of the Segmentation Clock by Reducing the Number of Introns in the *Hes7* Gene

Yukiko Harima,^{1,3} Yoshiki Takashima,^{1,5} Yuriko Ueda,^{1,2} Toshiyuki Ohtsuka,^{1,4} and Ryoichiro Kageyama^{1,4,*}

Cell Reports 3, 1–7, January 31, 2013



The End

(for this afternoon...)

Thank you!