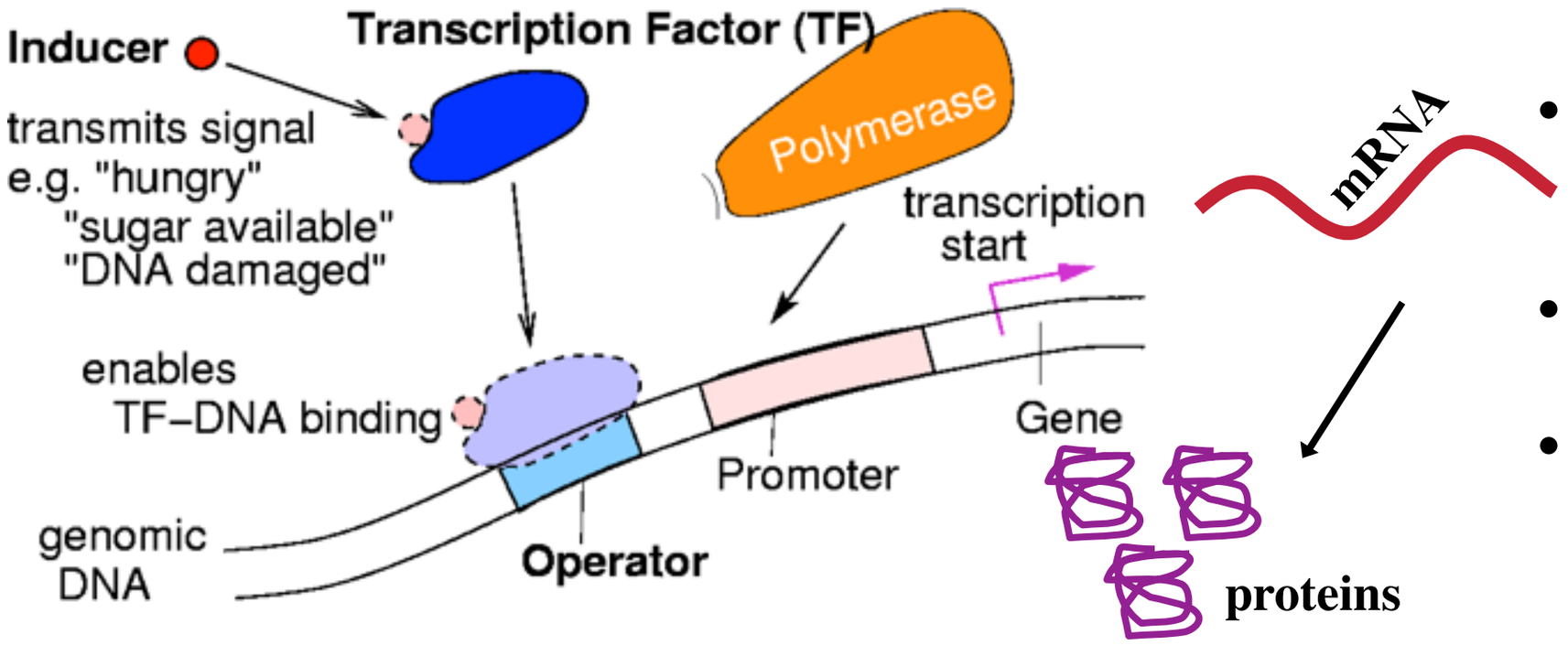
Regulation of Transcription

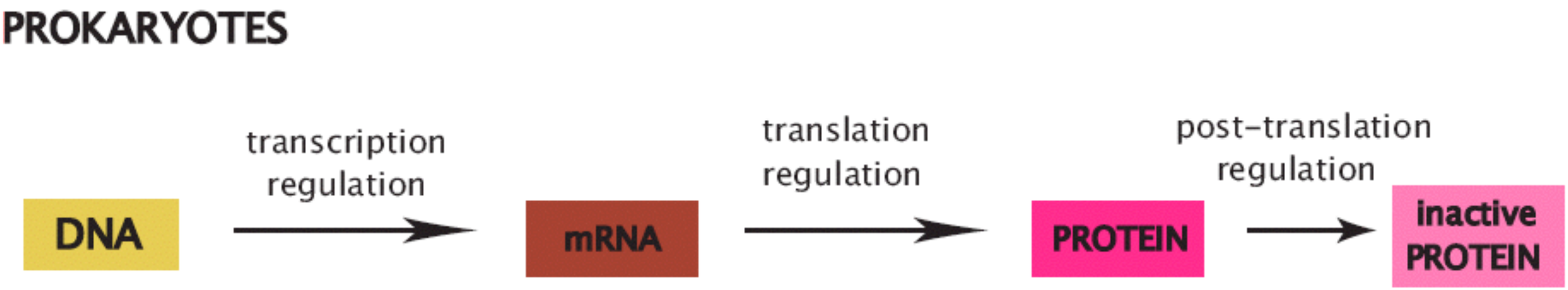


- activation of transcription factors (TF) - repressors and/ or activators
- binding of TF(s) to DNA targets (~10 bp)
- enhance transcription by recruiting RNA polymerase (RNAP) to its target ("promoter")

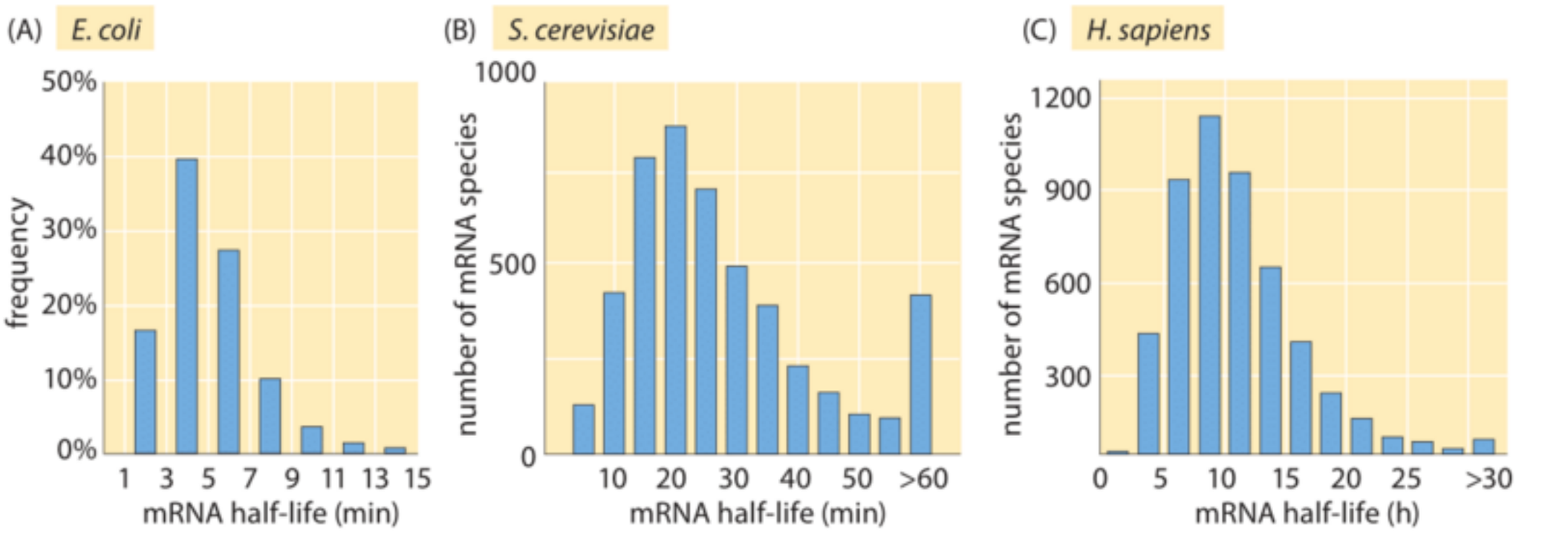
➔ one TF can regulate (activate or repress) multiple promoters

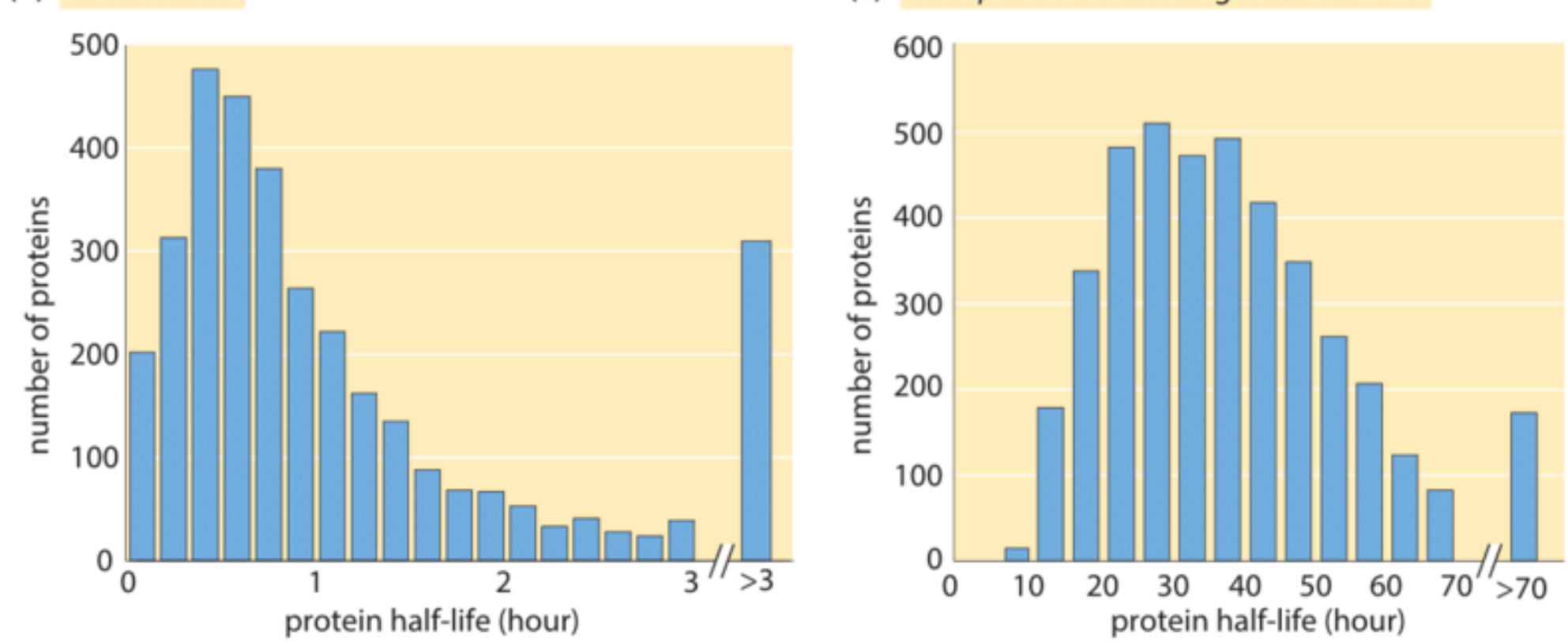
➔ some promoters regulated by multiple TFs

➔ regulated gene may encode another regulator

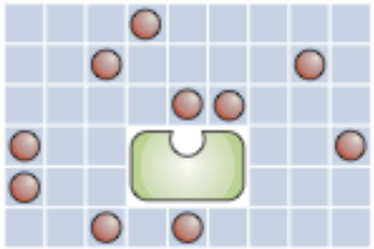
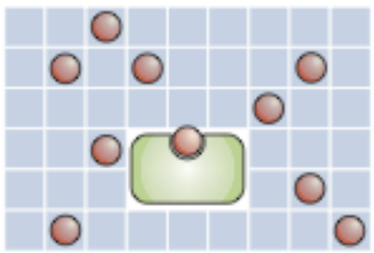


A, adapted from J. A. Bernstein et al., Proc. Natl Acad. Sci. USA 99:9697, 2002; B, adapted from Y. Wang et al., Proc. Natl Acad. Sci. USA 99:5860, 2002; C. adapted from B. Schwanhausser, Nature, 473:337, 2013



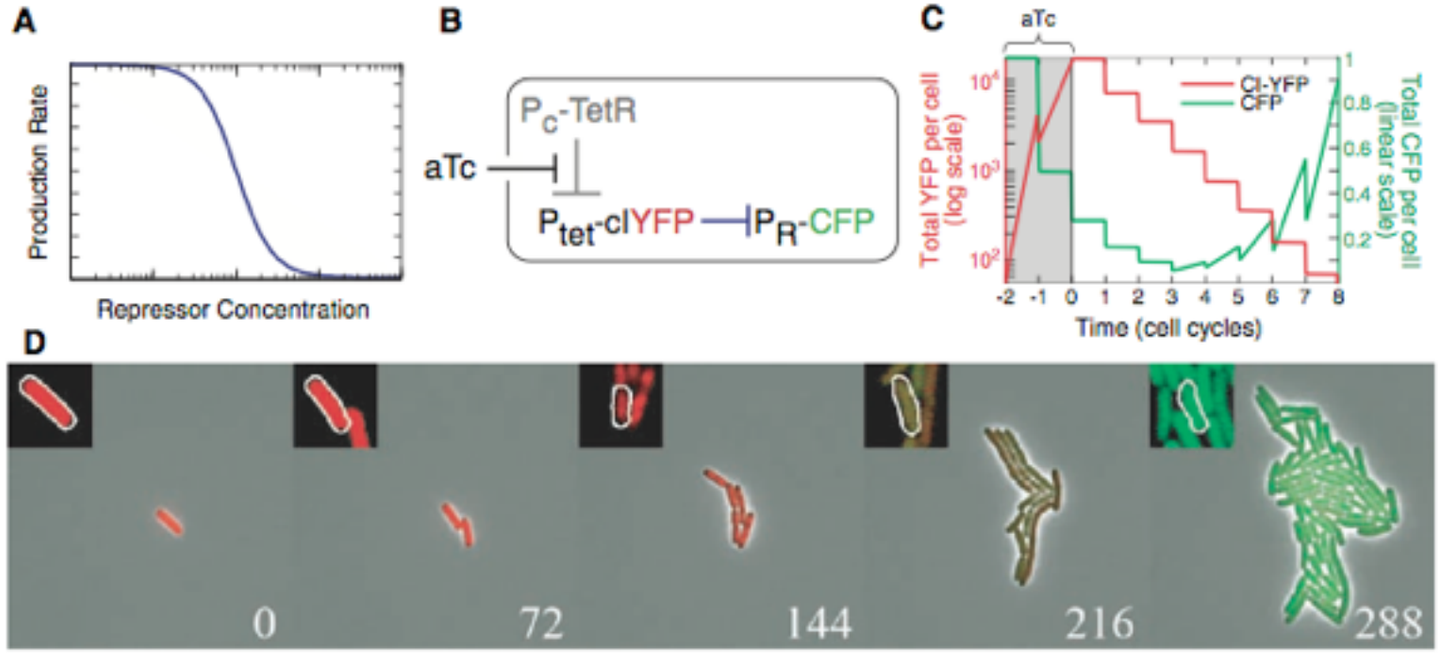


A, adapted from A. Belle et al., Proc. Natl Acad. Sci. USA 103:13004, 2006; B, adapted from S. Cambridge et al, J. Proteome Res. 10:5275, 2011.

STATE	ENERGY	MULTIPLICITY	WEIGHT
(A) 	$L\varepsilon_{sol}$	$\frac{\Omega!}{L!(\Omega-L)!} \approx \frac{\Omega^L}{L!}$	$\frac{\Omega^L}{L!}\, e^{-\beta L\varepsilon_{sol}}$
(B) 	$(L-1)\varepsilon_{sol} + \varepsilon_b$	$\frac{\Omega!}{(L-1)!(\Omega-L+1)!} \approx \frac{\Omega^{L-1}}{(L-1)!}$	$\frac{\Omega^{L-1}}{(L-1)!}\, e^{-\beta[(L-1)\varepsilon_{sol} + \varepsilon_b]}$

Measuring regulatory functions quantitatively

the setup:



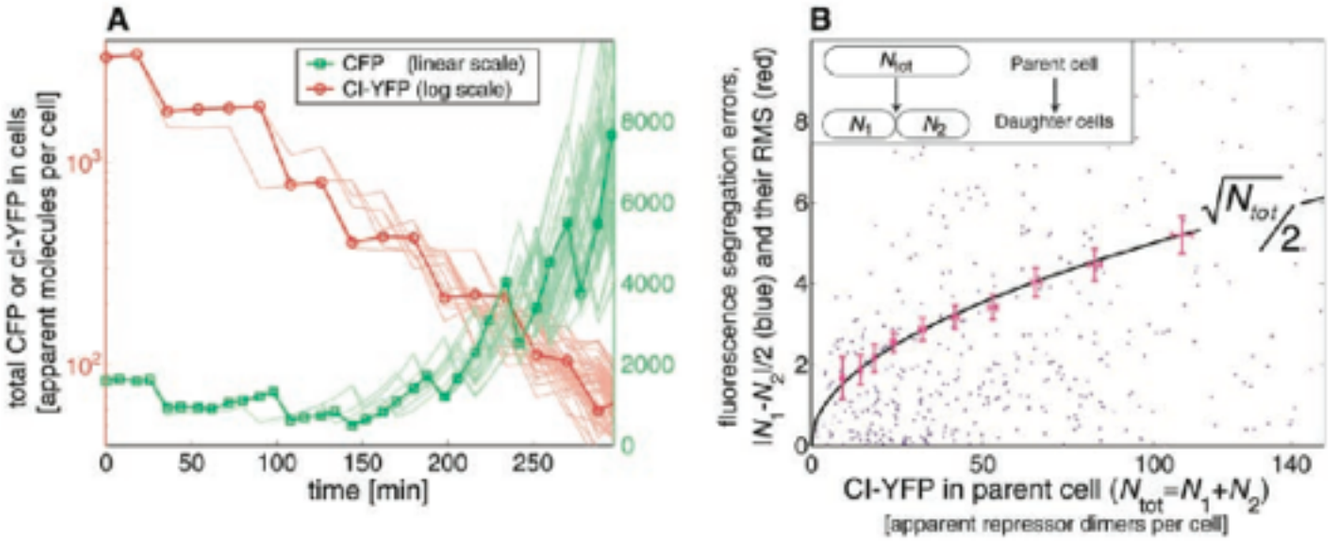
assumptions:

- fluorescence ~ concentration
- well isolated system

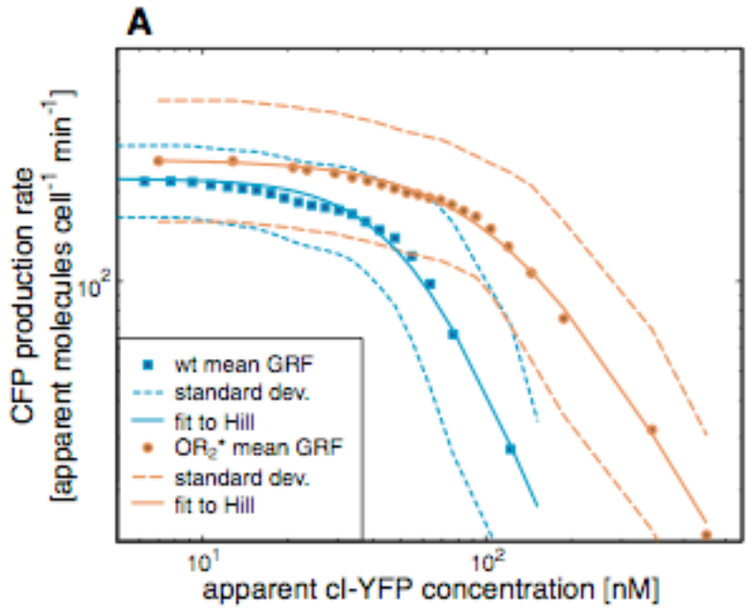
need to:

- calibrate expression
- quantify parameters
- make sure degradation is negligible
- measure cI and gene expression simultaneously

checks:

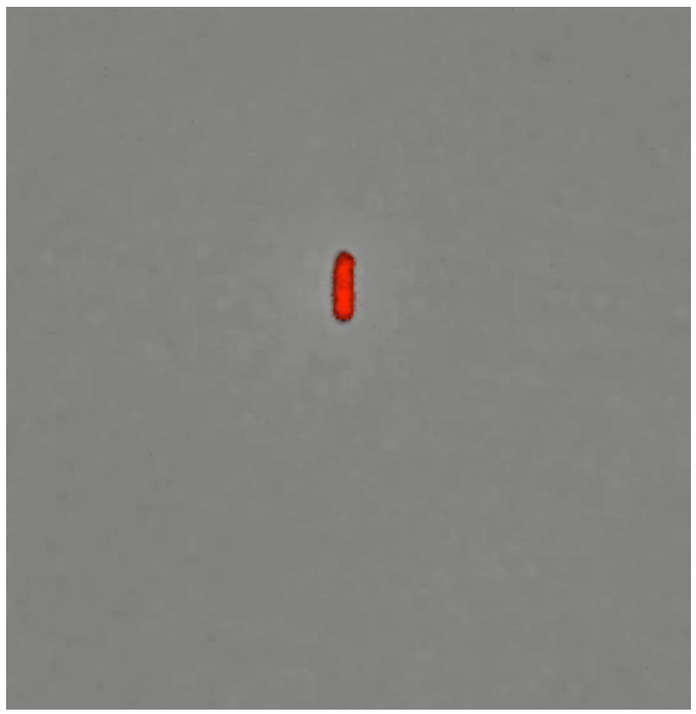


results:



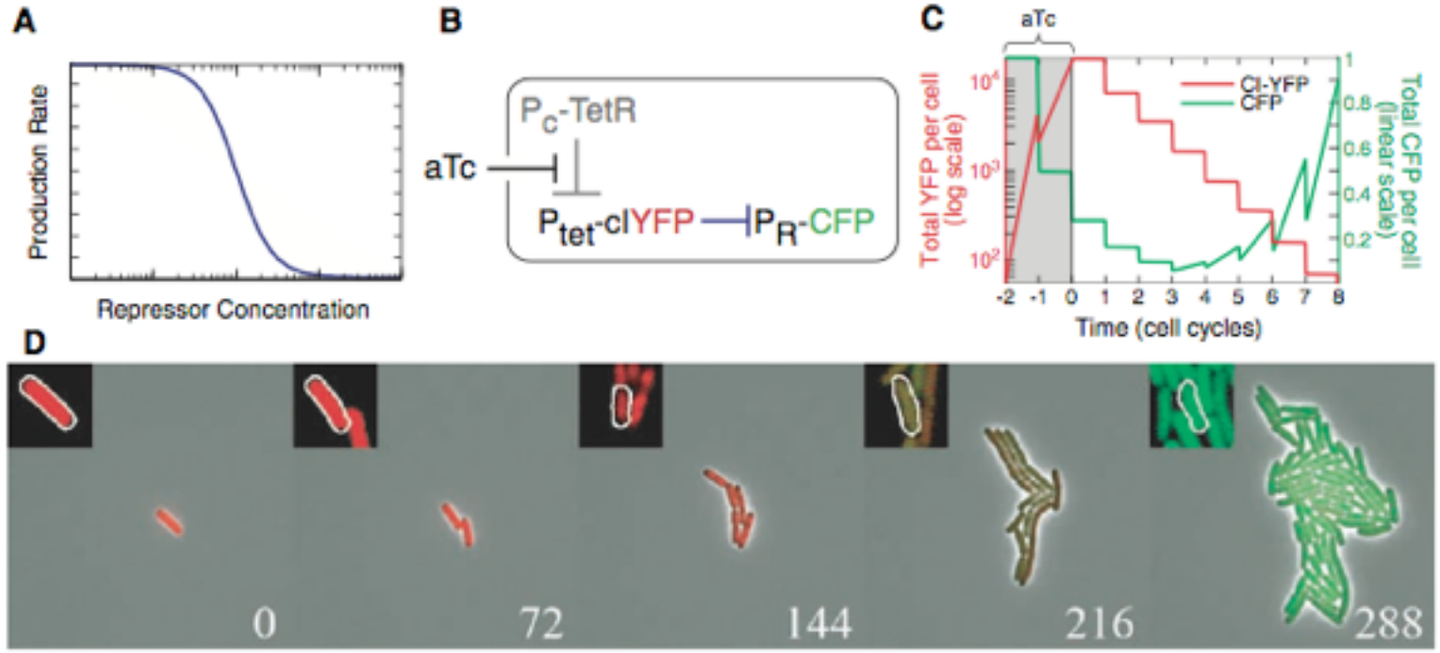
N Rosenfeld, JW Young, U Alon,PS Swain,MB Elowitz 25 MARCH 2005 VOL 307 SCIENCE

Measuring regulatory functions quantitatively



Measuring regulatory functions quantitatively

the setup:



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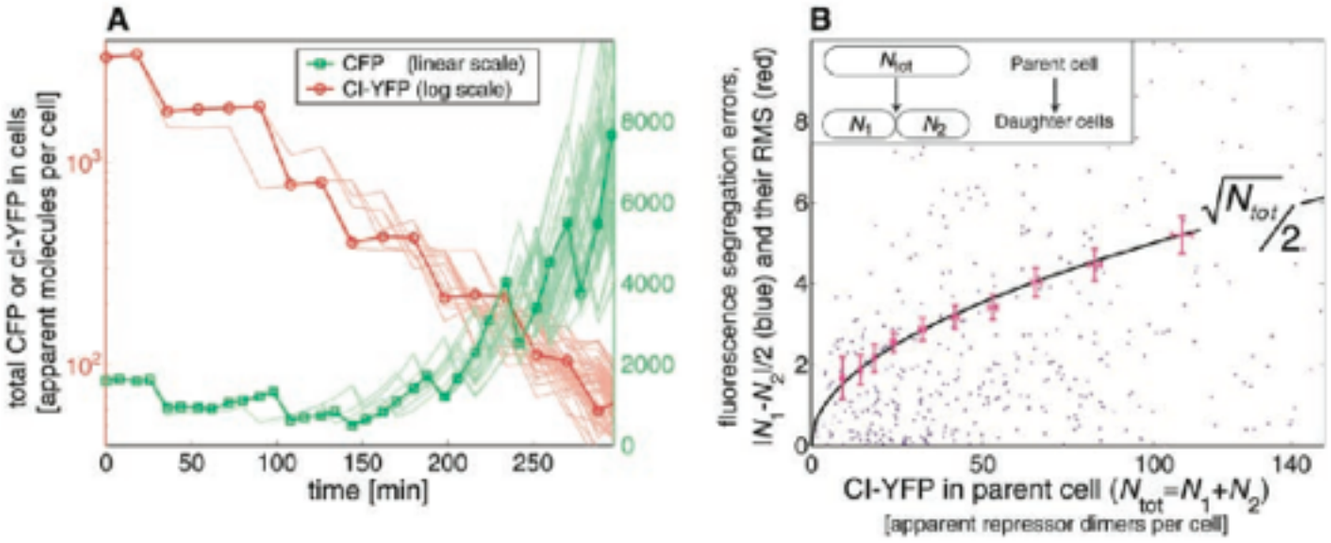
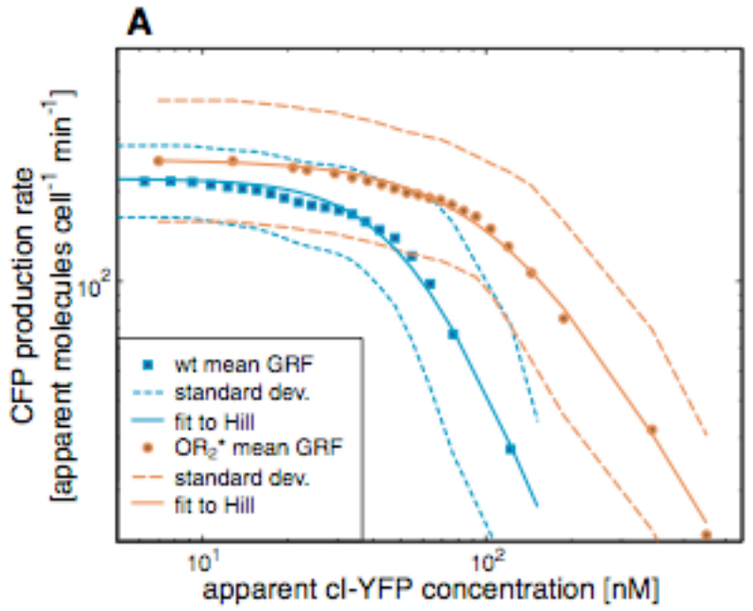


results:



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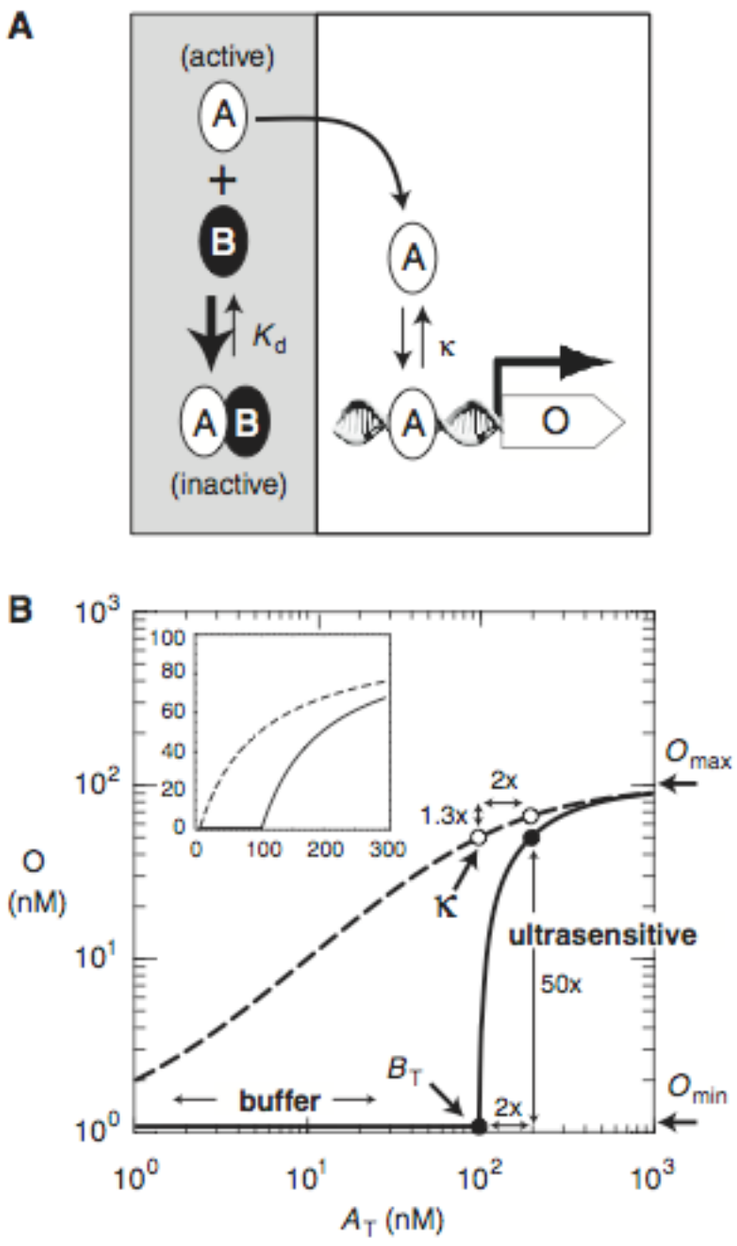
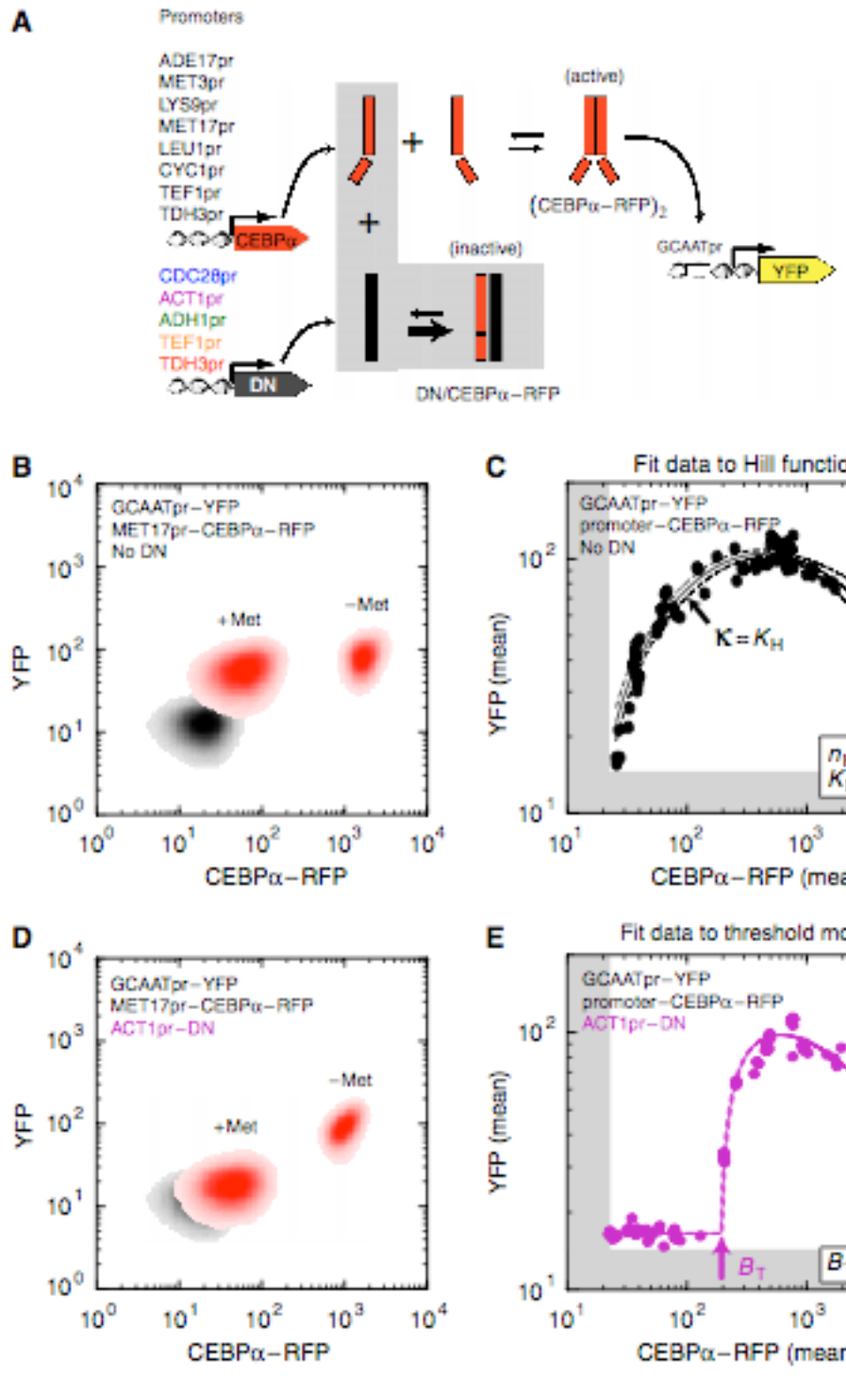
Sequestering of TFs in complexes

- yeast
- synthetic circuit - but common motif proteins
- 1 binding site (no cooperativity)
- measure active TF and gene expresssion (YFP)
- also achieved by small RNAs, enzymes, ...



ultrasensitivity

NE Buchler and FR Cross Molecular Systems Biology 5:272 (2009)



no sequesteration

with sequesteration

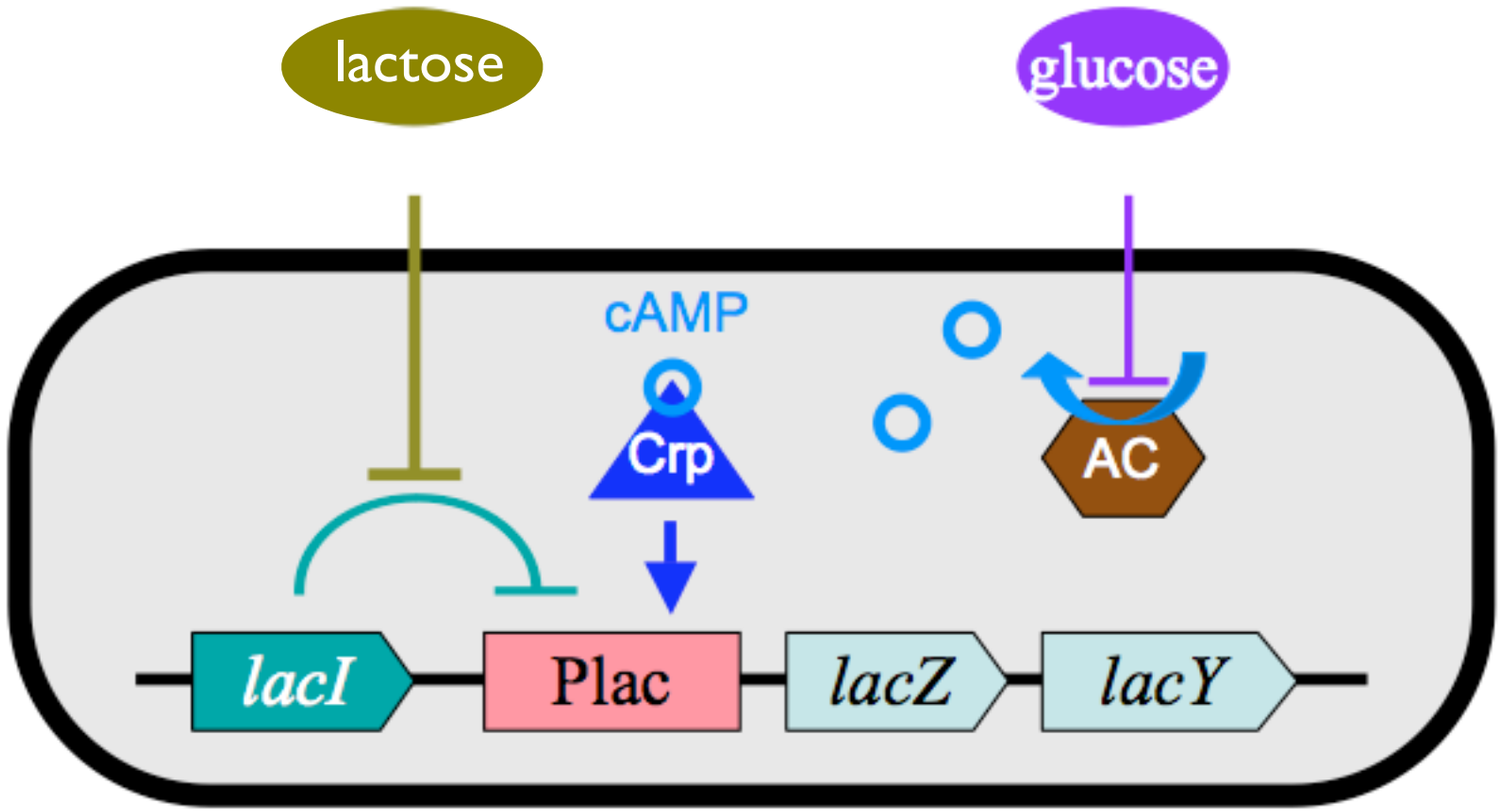
The lac operon

Function: break up **lactose**

Jacob, Monod, Lwoff and collaborators (1950s)

....

presentation taken from Terry Hwa



lacZ - encodes intracellular enzyme that cleaves lactose into glucose and galactose

lacY - encodes a membrane-bound transport protein that pumps lactose into the cell

lacA- encodes another enzyme

Expressed only in the **presence** of **lactose** and **absence** of **glucose**

- repressed by the Lac Repressor (encoded by lacI)
- repression alleviated by allo-lactose (by-product of lactose metabolism)
- synthetically allo-lactose replaced by IPTG
- activated by the global regulator Crp; requires the inducer cAMP
- cAMP synthesized endogenously by Adenylate Cyclase (encoded by cyaA)
- activity of AC repressed by glucose uptake

IPTG	glucose	expression
low	high	OFF
low	low	OFF
high	high	OFF
high	low	ON

1960's

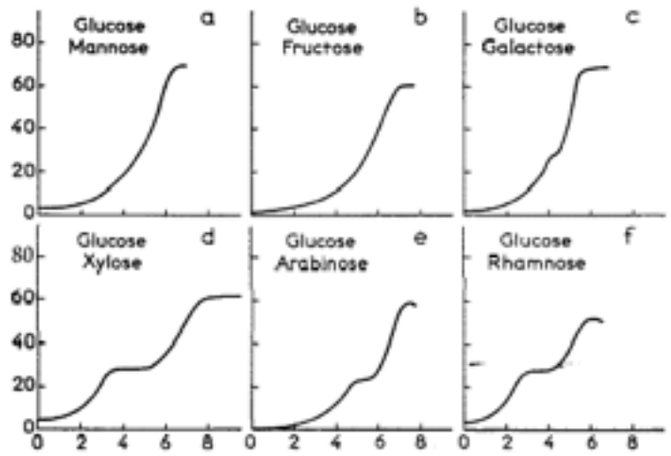
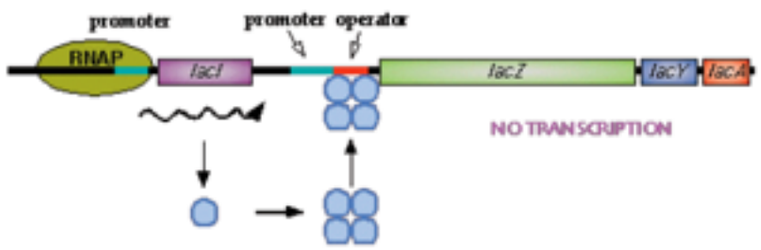
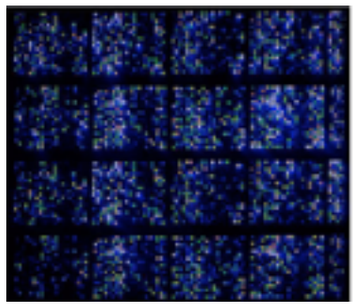


Fig.1. Growth of *Escherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[8].

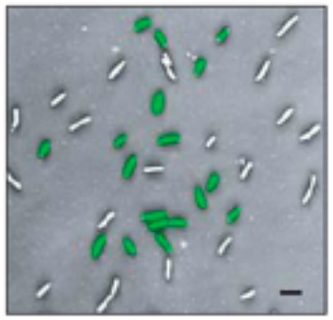


[Jacob,Monod]

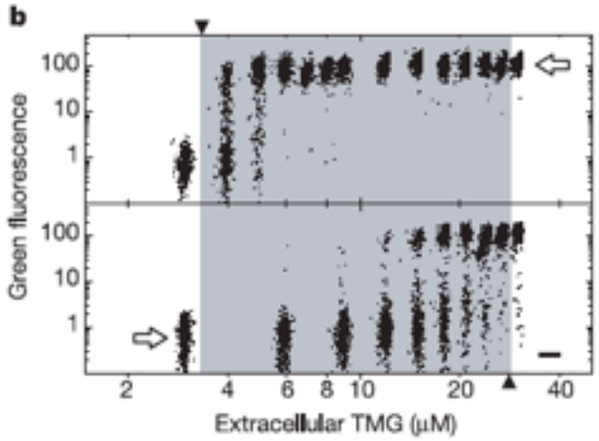
2000's



microarrays



fluorescent proteins

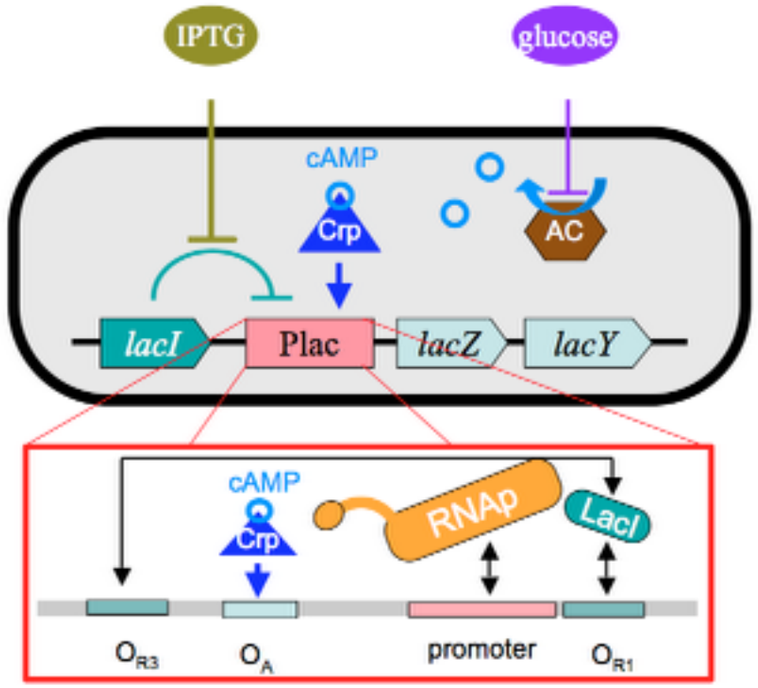


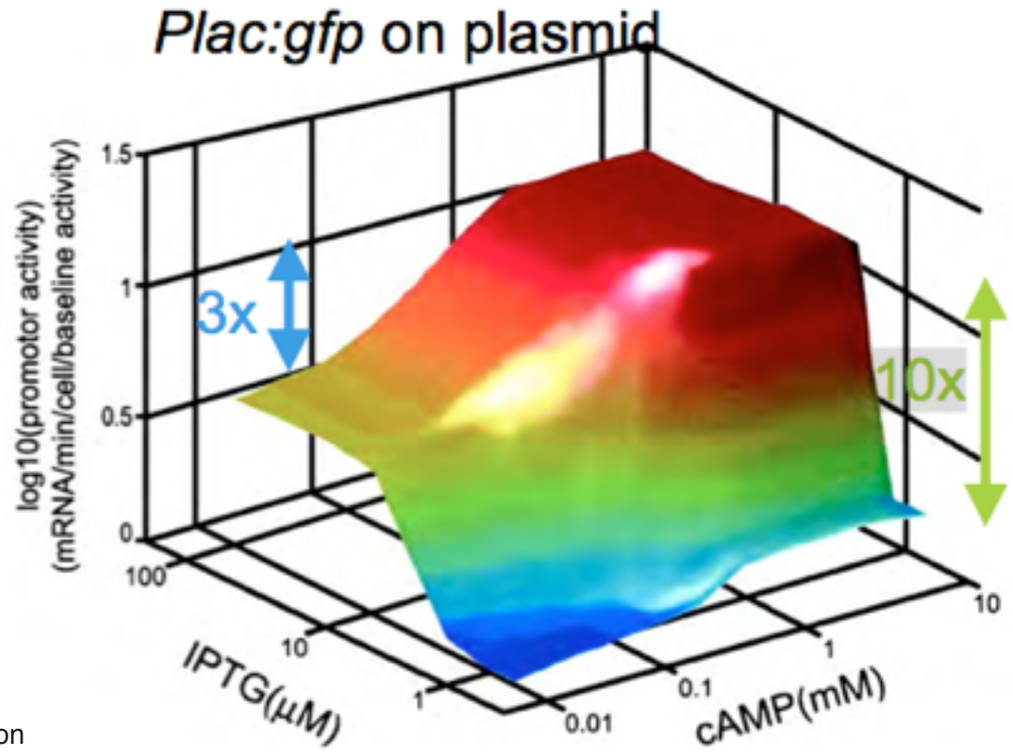
[Ozbudak et al]

The lac operon

molecular complexity:

- protein-protein interactions
- specific protein-DNA binding
- looping





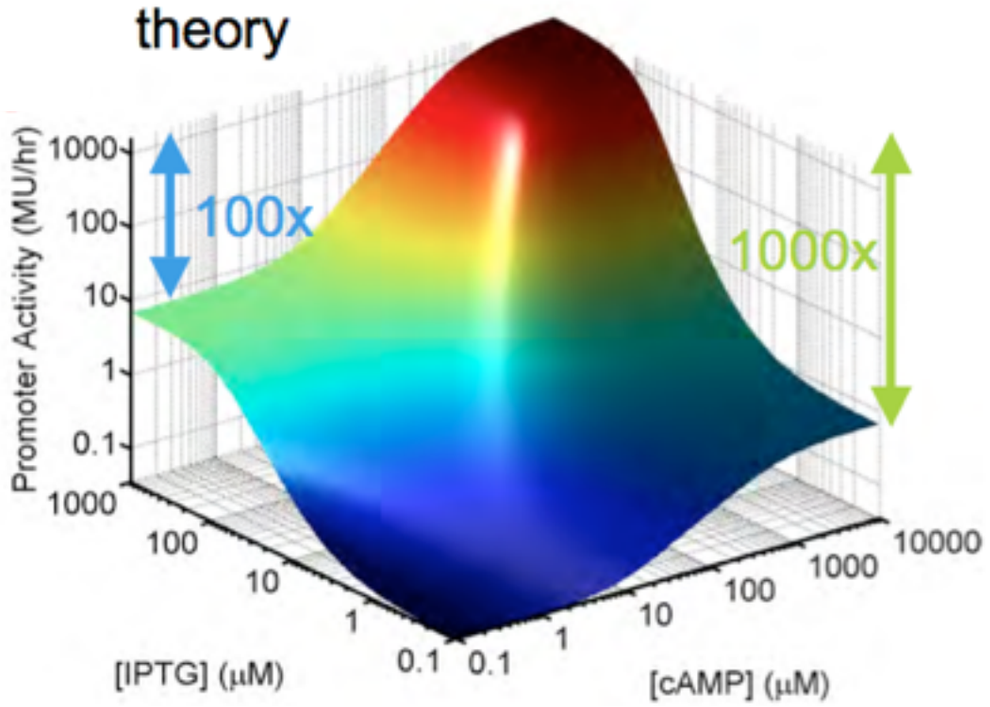
Y. Setty, A.E. Mayo, M.G. Surette, U. Alon
Detailed map of a cis-regulatory input function
PNAS June 24, 2003 vol. 100 no. 13 7702

Initial experiments:

- grow cells in medium with **cAMP** and **IPTG**
- use **glucose** to suppress intracellular **cAMP** synthesis
- control **cAMP**-level extracellularly

Problem:

- inconsistency with theory of mutants

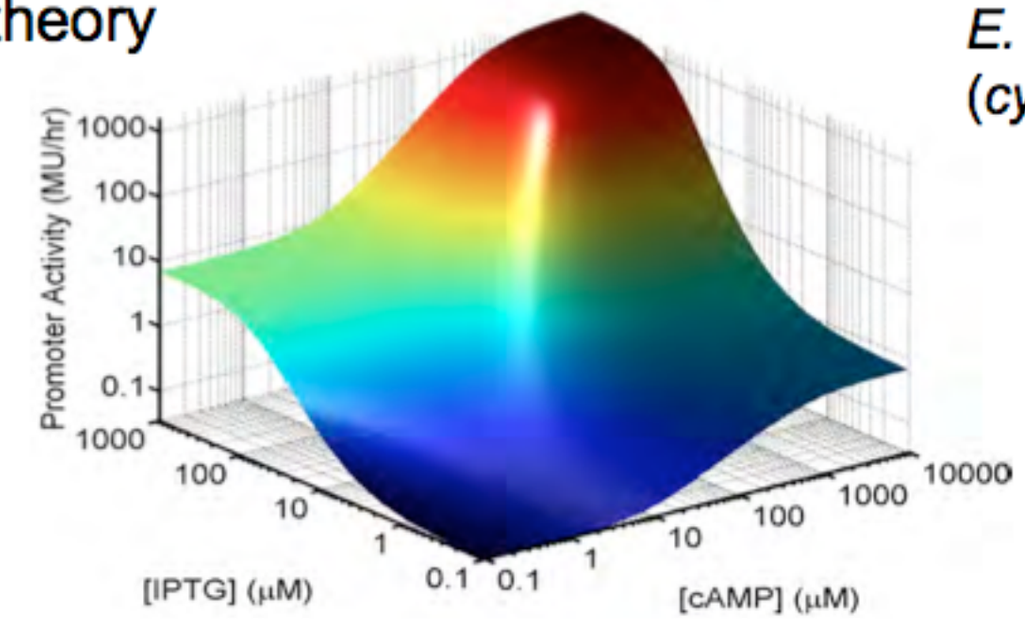


Possible reason:

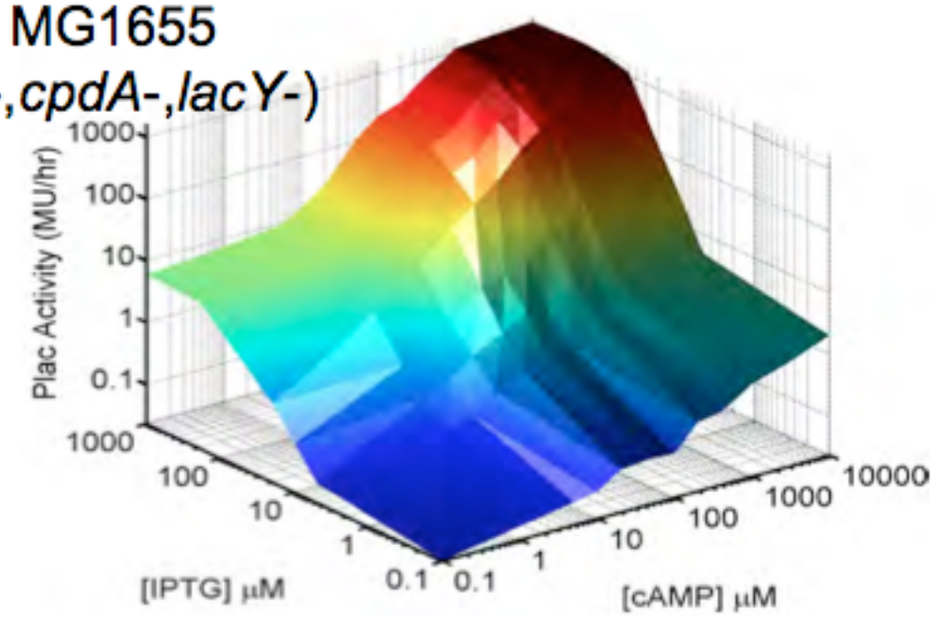
- extracellular **cAMP** and **IPTG** concentrations (the ones that are controlled) not equal to intracellular ones (the ones that matter)

The lac operon

theory



E. coli MG1655
(*cyaA*-,*cpdA*-,*lacY*-)

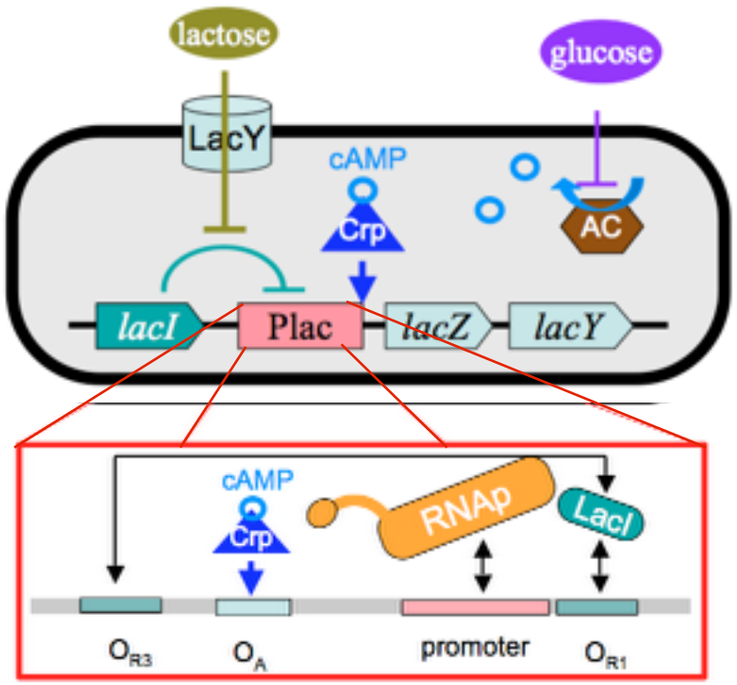


Solutions of problem:

- the sensitivity of the inducer response results from LacI-mediated DNA looping
- DNA looping is significantly enhanced by **Crp**.
- **IPTG** intake increased by LacY
- difference between **IPTG** and **lactose** uptake mechanisms

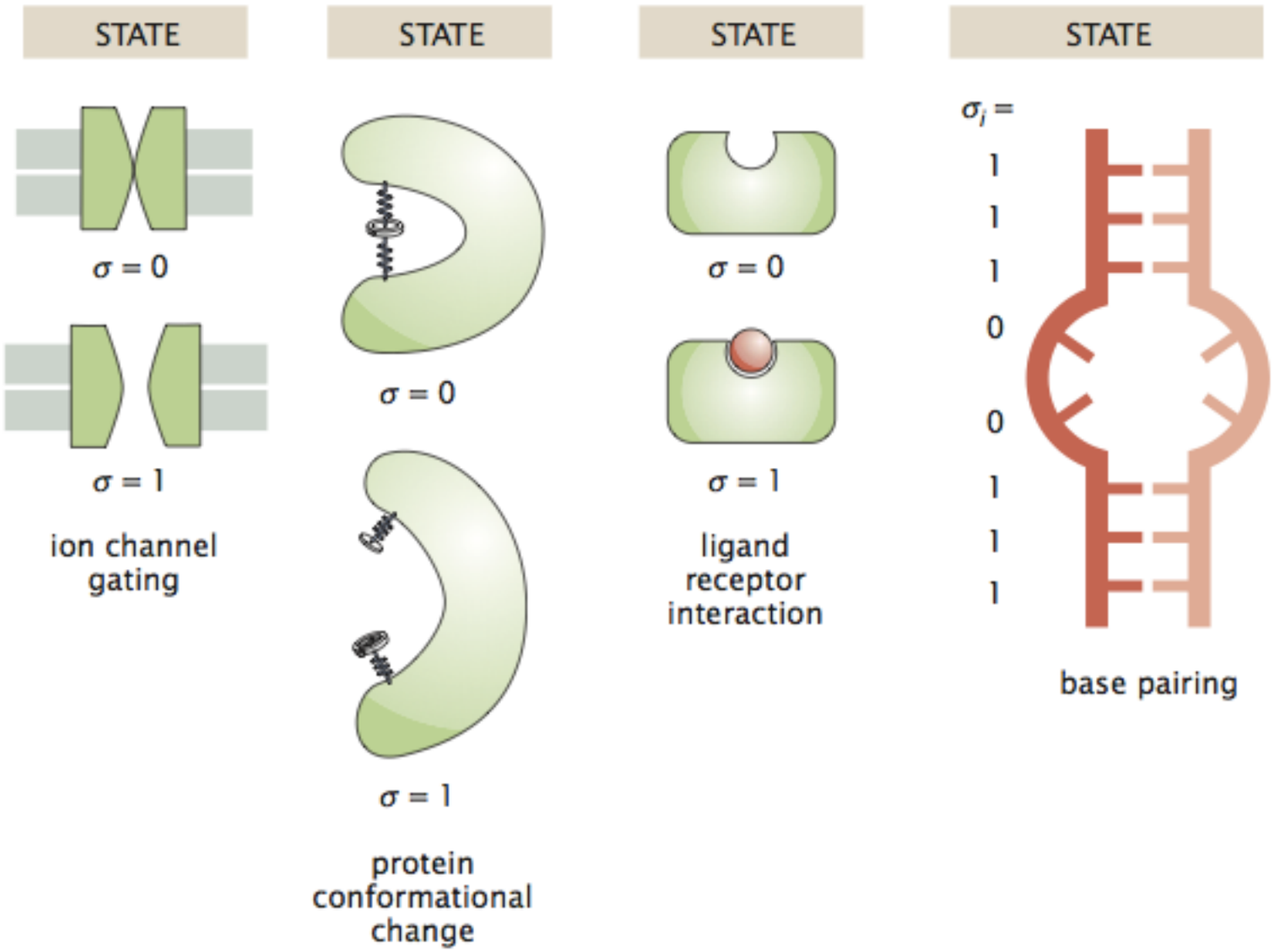
Lesson:

- quantitative study leads to correct mechanistic understanding



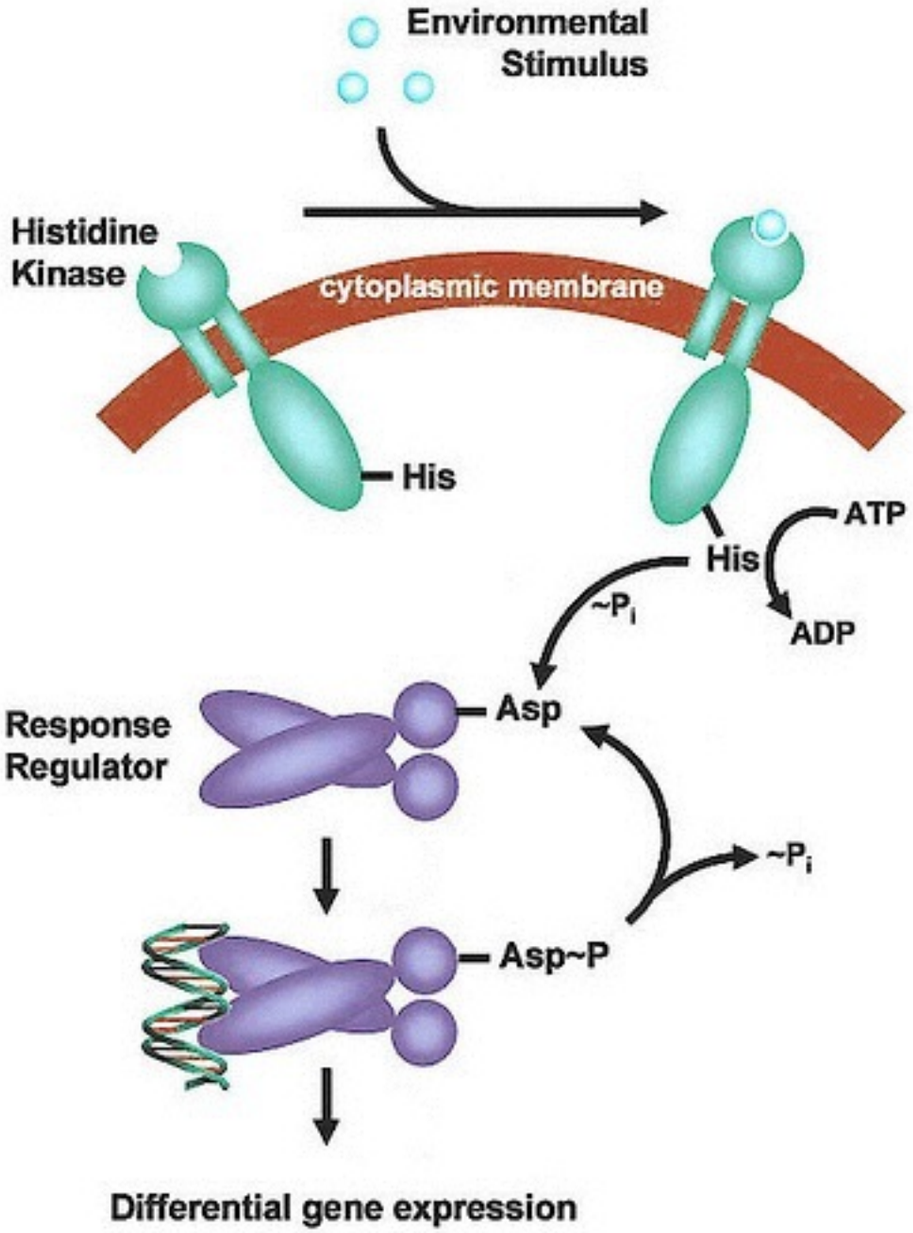
Thomas Kuhlman, Zhongge Zhang, Milton H. Saier, Jr., and Terence Hwa
Combinatorial transcriptional control of the lactose operon of Escherichia coli
PNAS April 3, 2007 vol. 104:6043

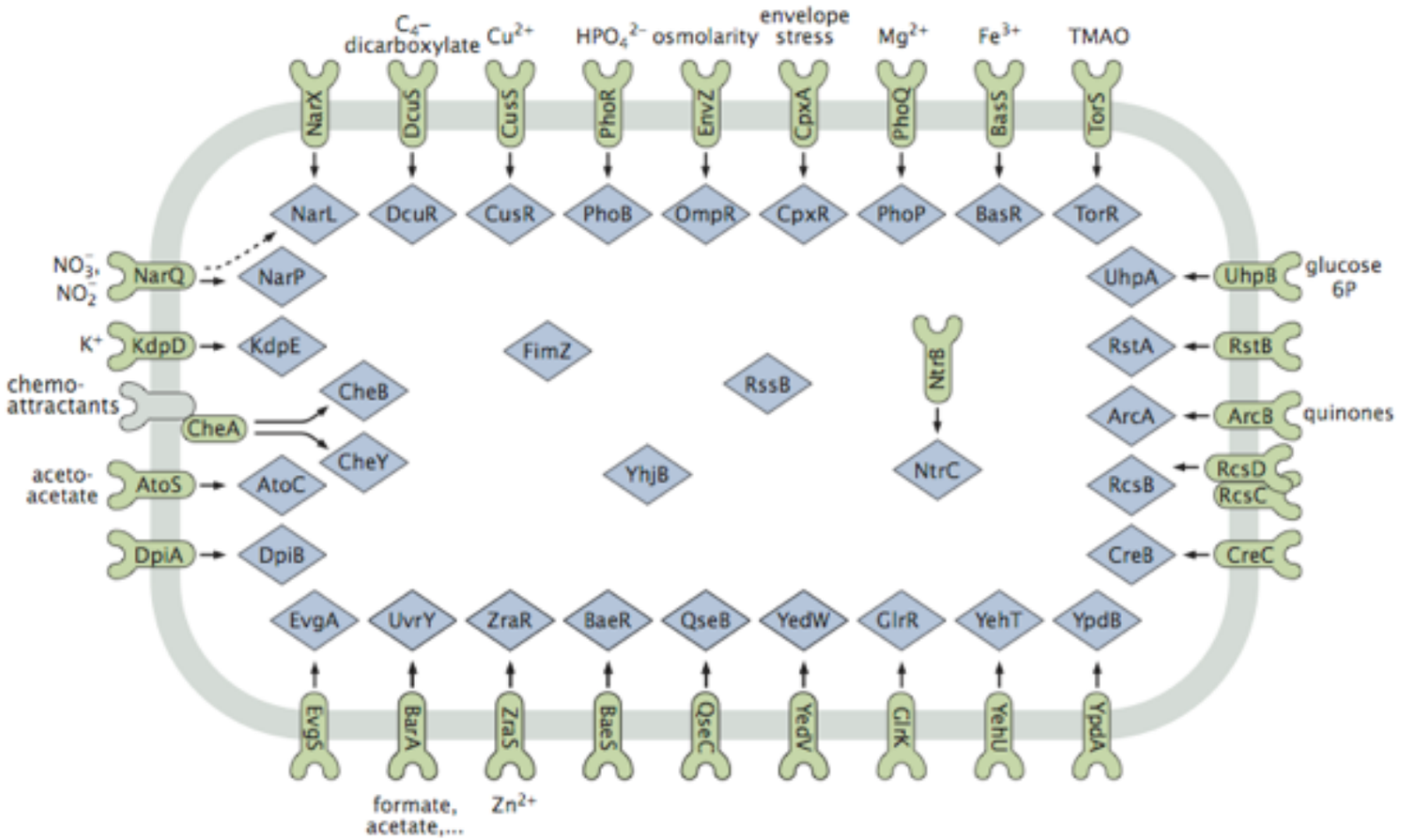
Cooperativity

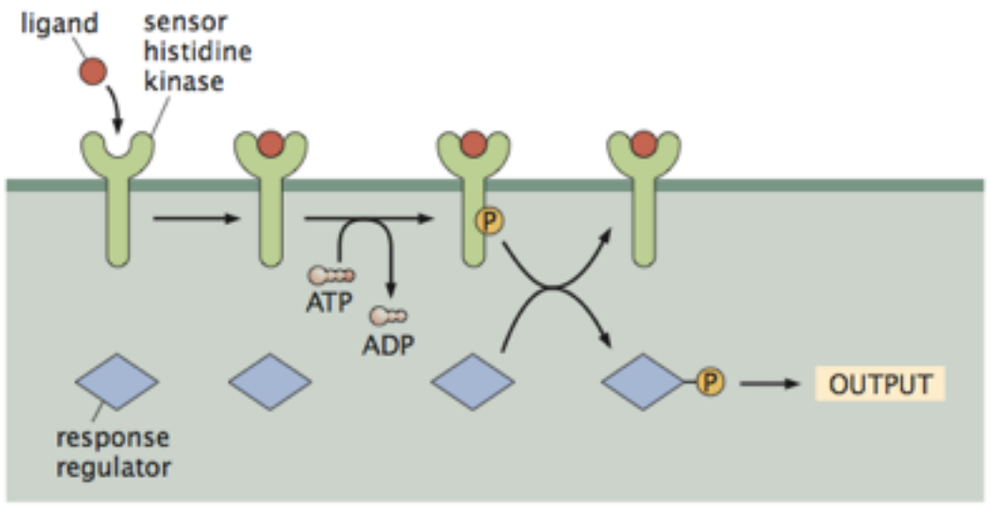


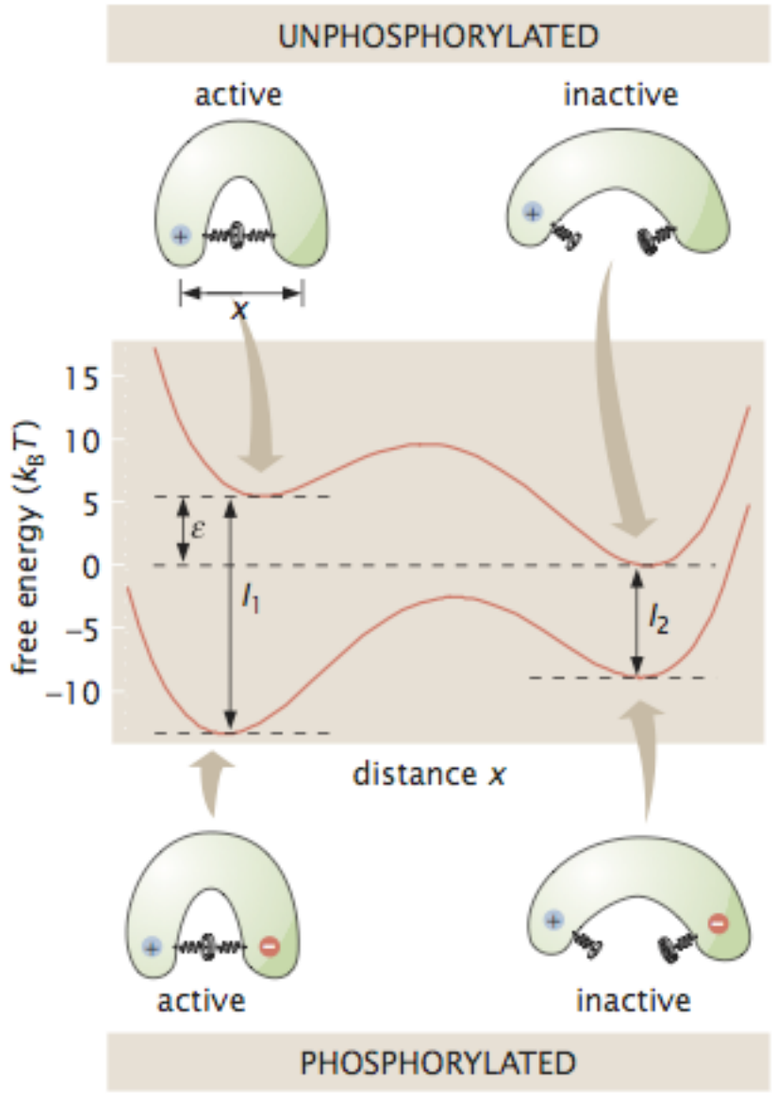
Monod-Wyman-Changeux (MWC) model

Post-translational regulation

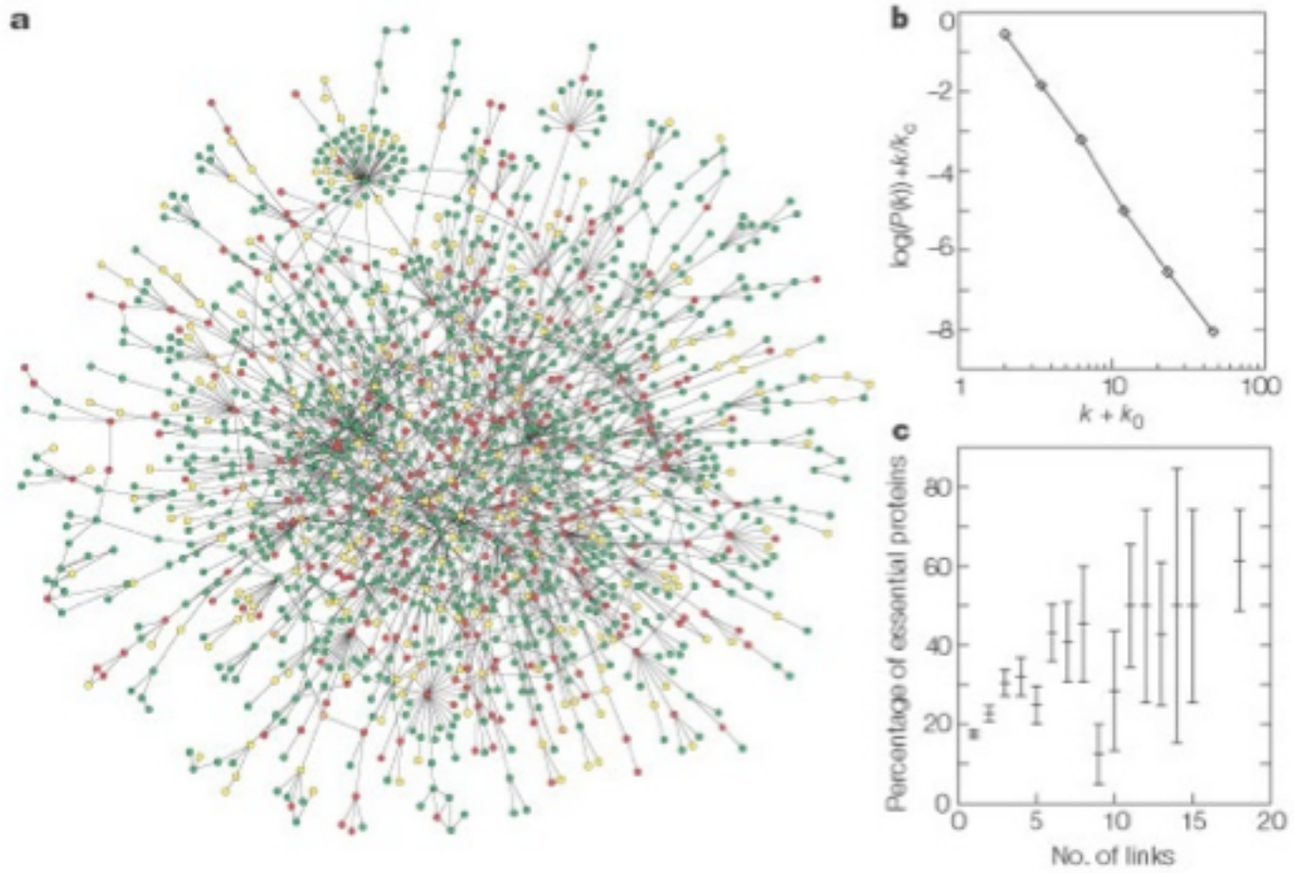







UNPHOSPHORYLATED
active
inactive
x
15
10
5
0
−5
−10
free energy (k_BT)
ε
l_1
l_2
distance x
active
inactive
PHOSPHORYLATED

Yeast protein-protein interaction network



red - lethal

green - non-lethal

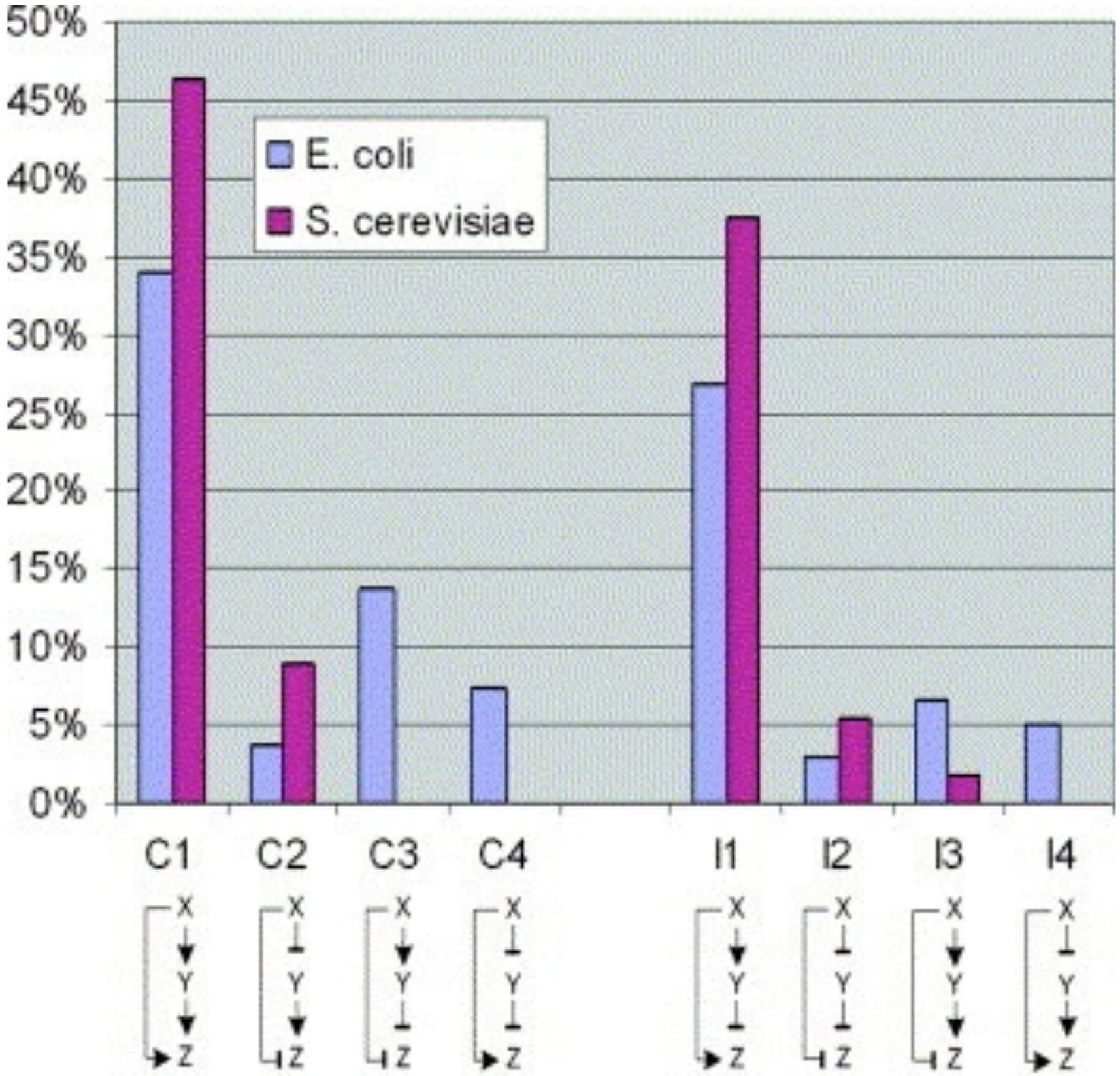
orange - slow growth

yellow - not determined

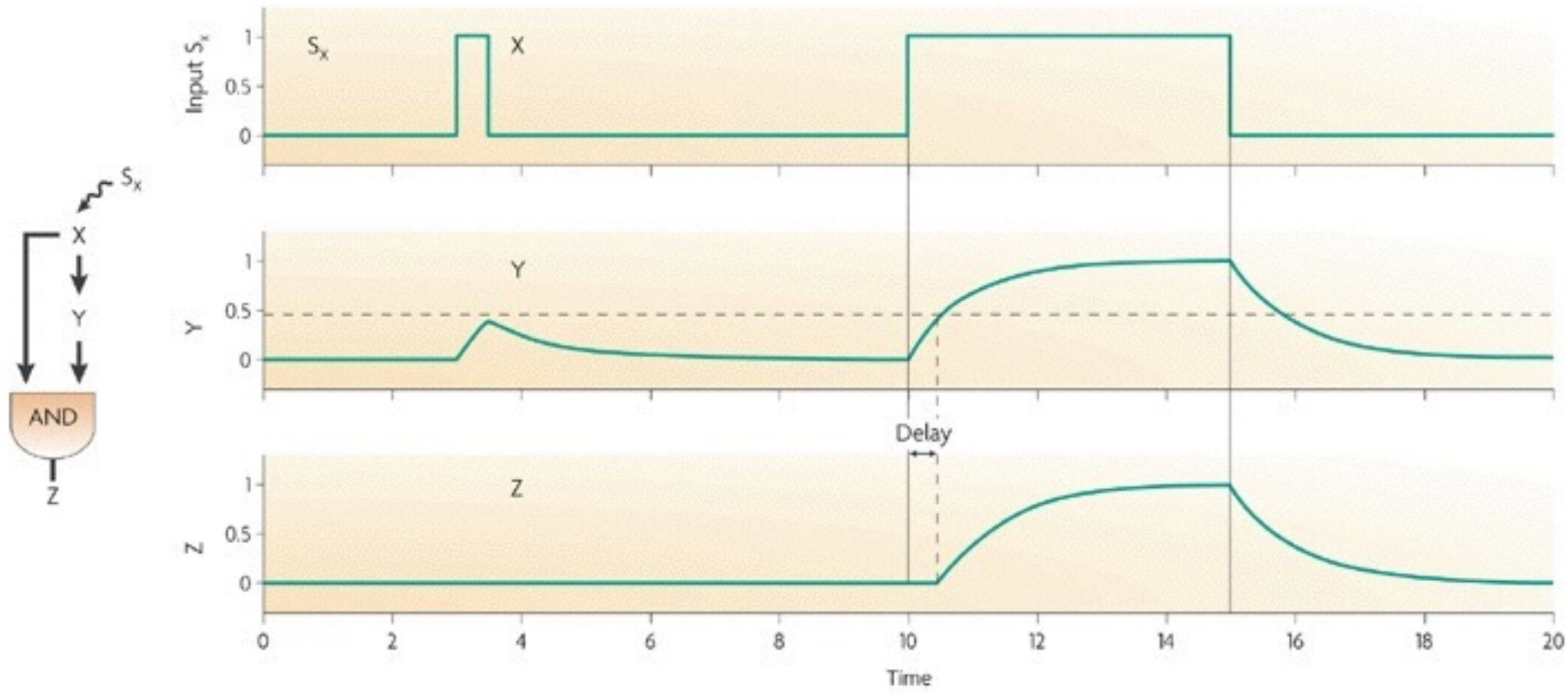
Yeast protein interaction network: 1870 proteins, 2240 interactions

H. Jeong et al, Nature 411,41 (2001)

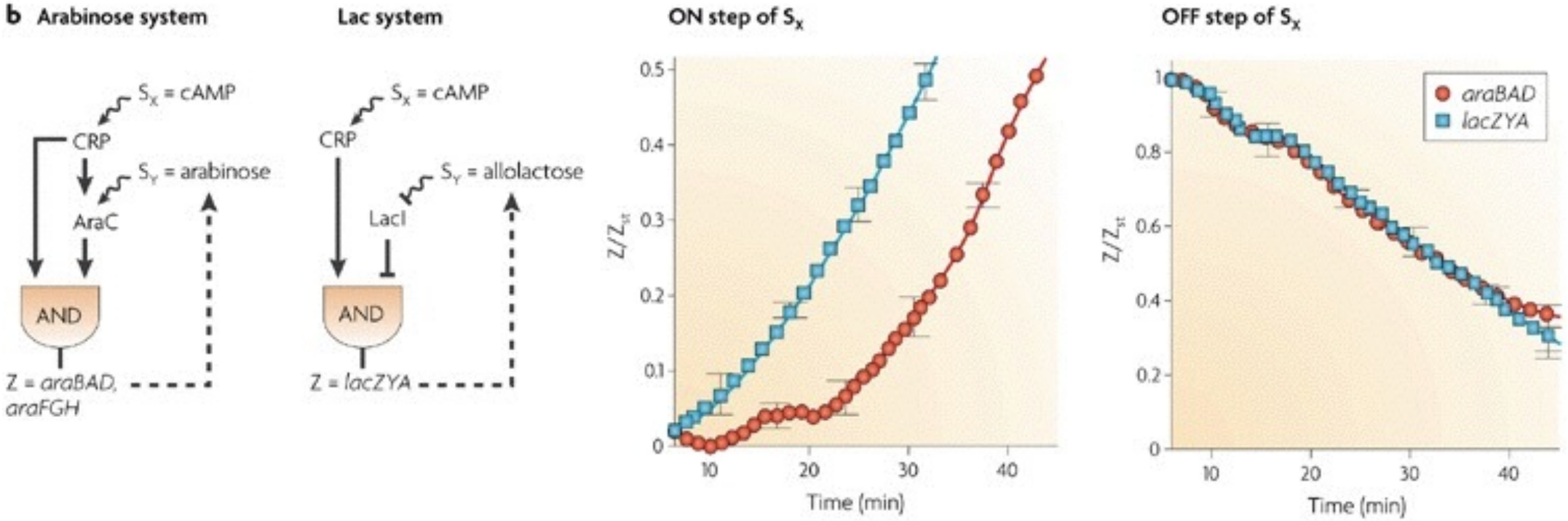
Relative proportion of different FFL, Mangan et al J. Mol. Biol (2006)



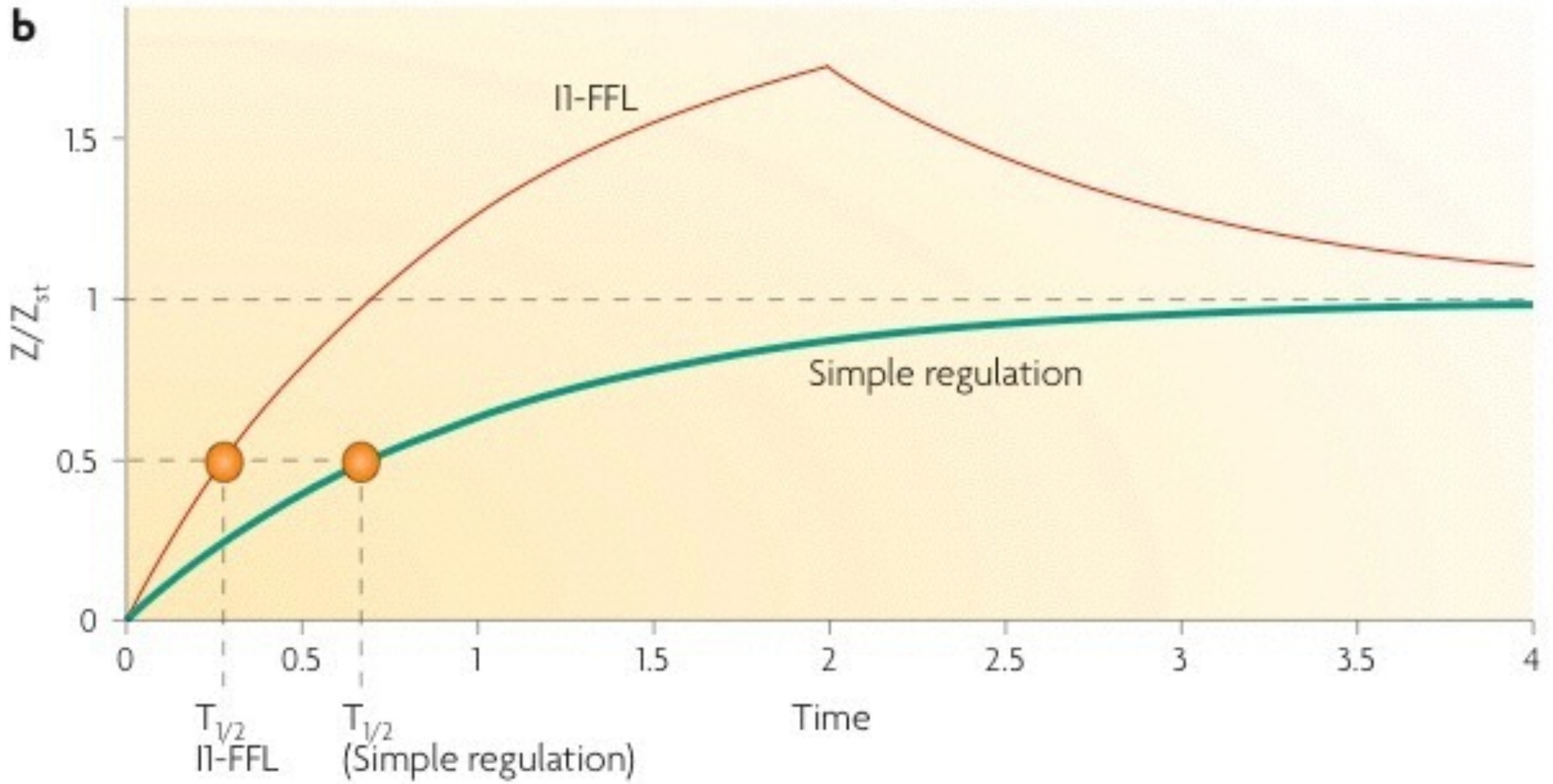
Coherent feed-forward loop



Alon, Nat Rev Gen (2007)

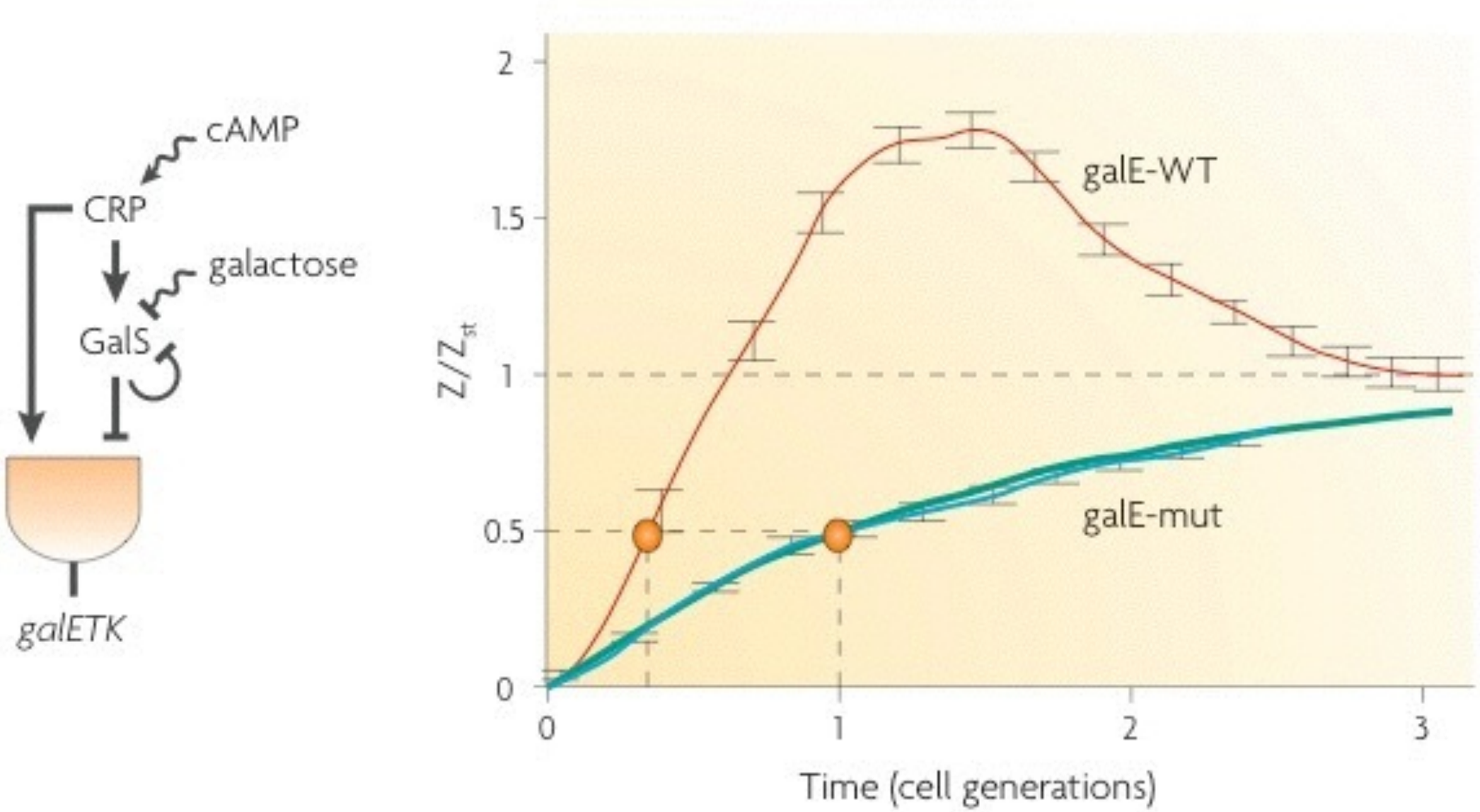


Alon, Nat Rev Gen (2007)



Alon, Nat Rev Gen (2007)

Incoherent feed-forward loop or feed-forward inhibition

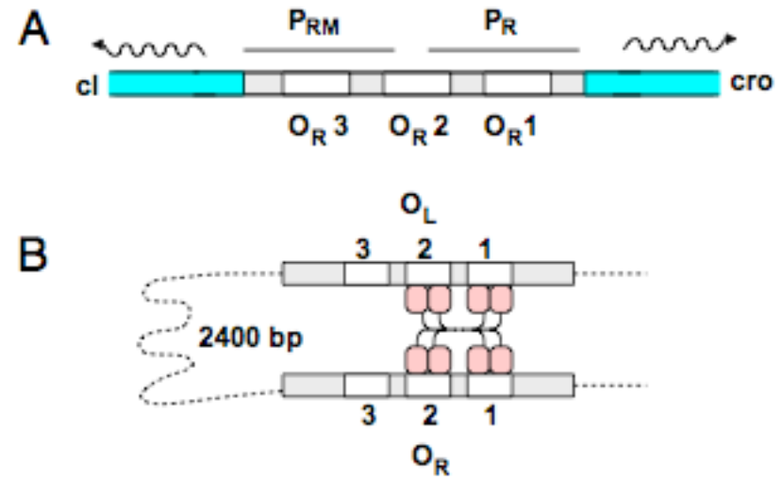


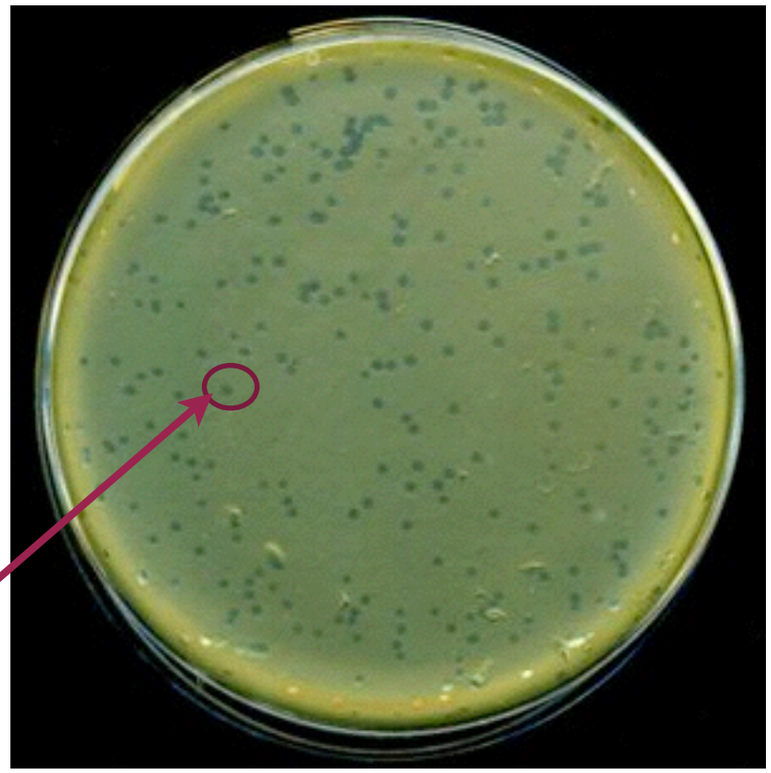
Alon, Nat Rev Gen (2007)

The λ phage

XOR promoter!

looping
dimer binding



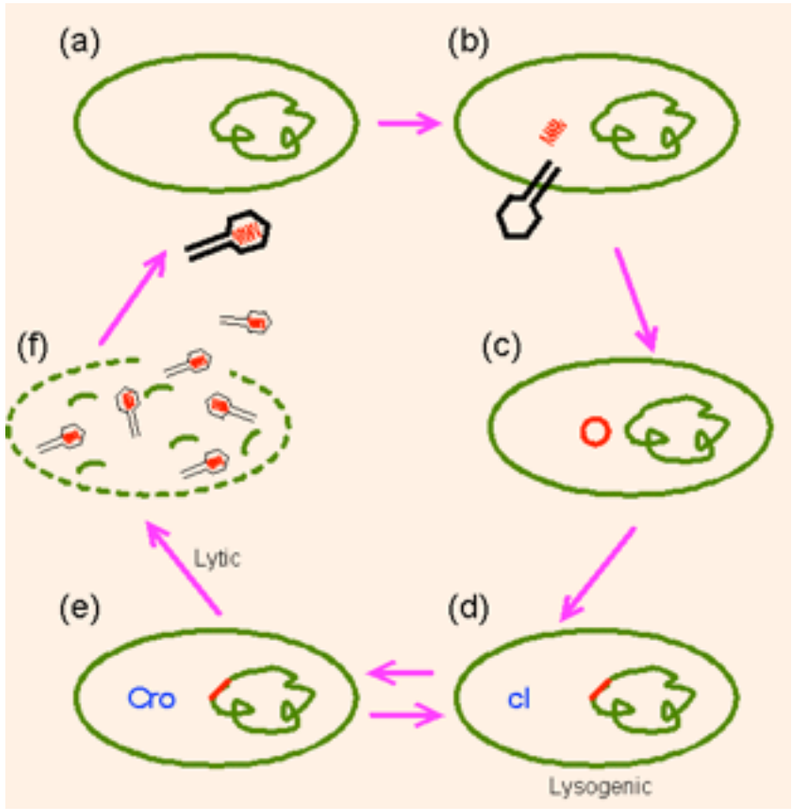


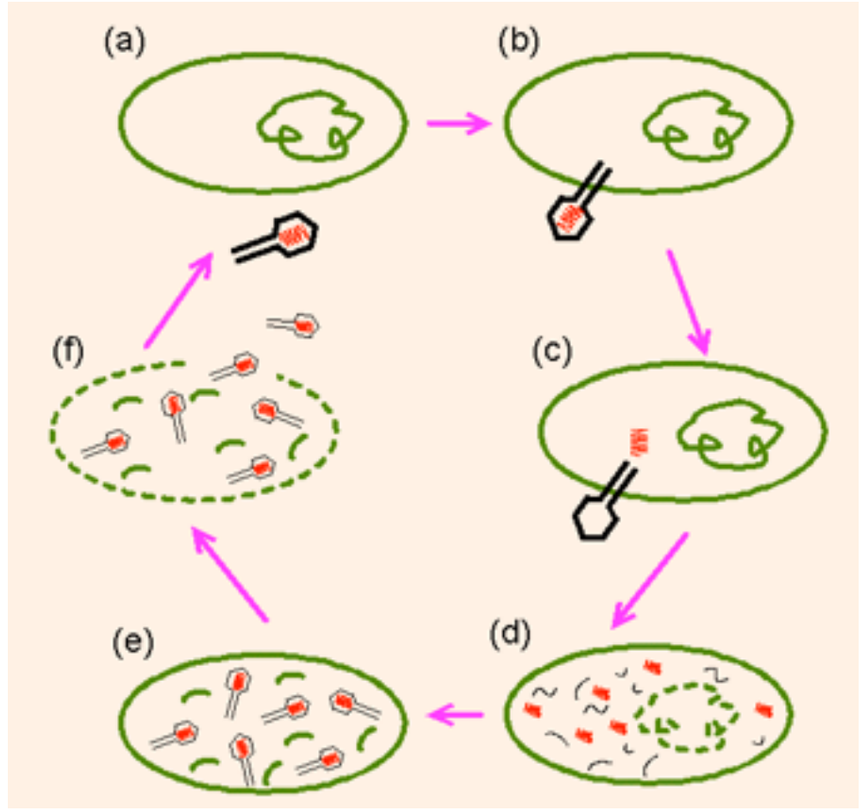
Lysogeny

cro - low
cI - high

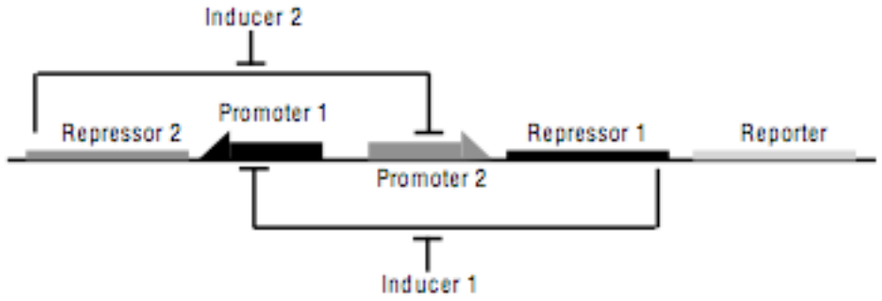
cro - high
cI - low

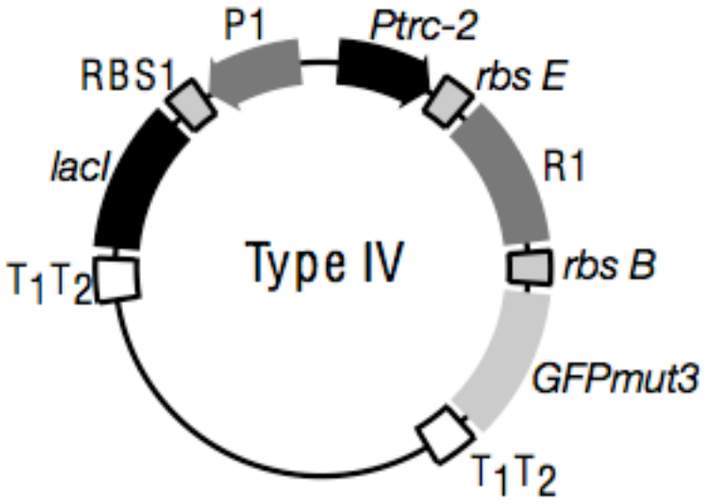
Lysis

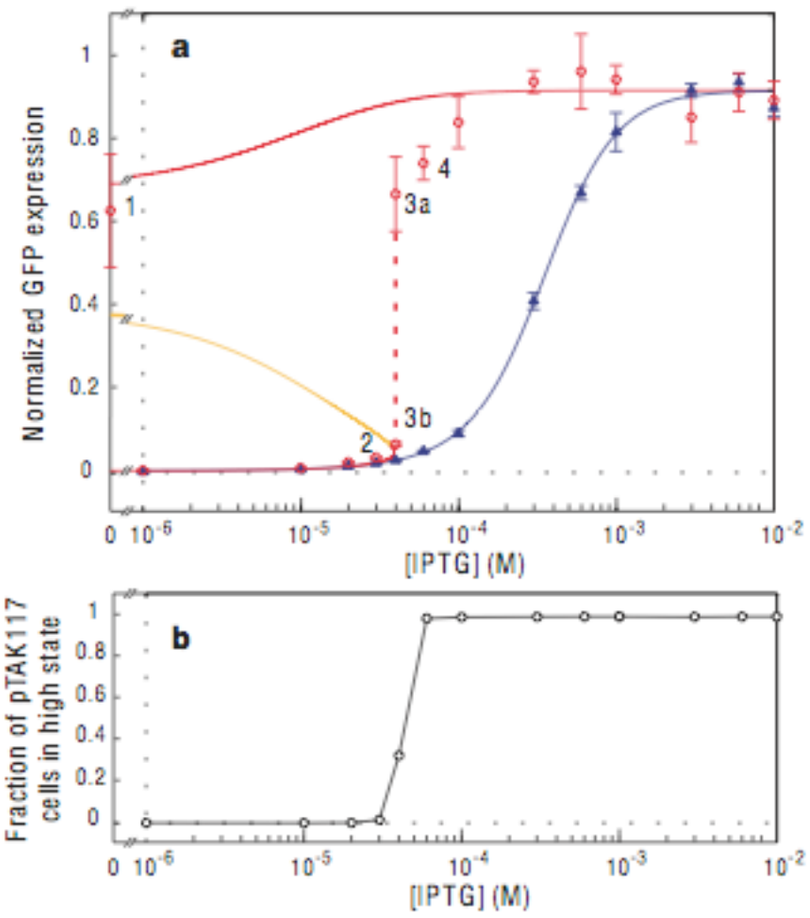


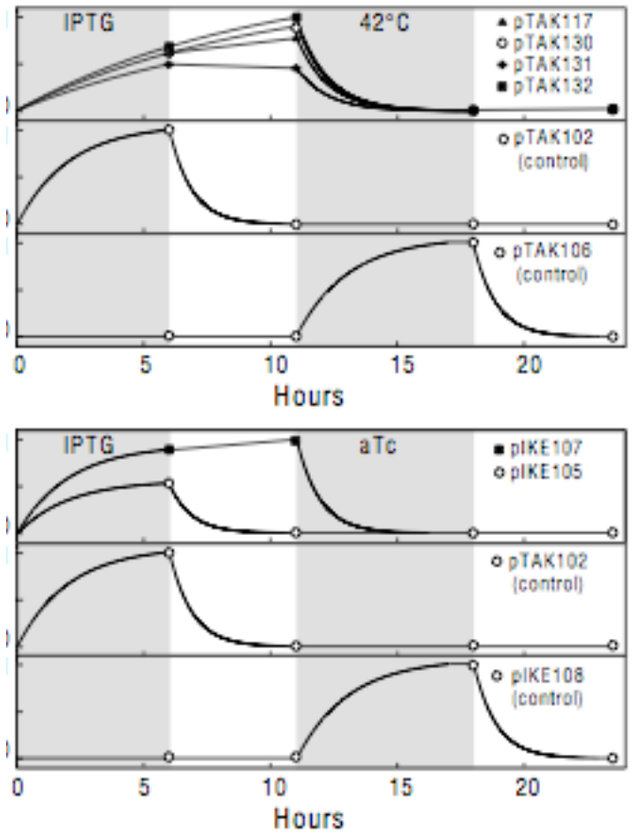


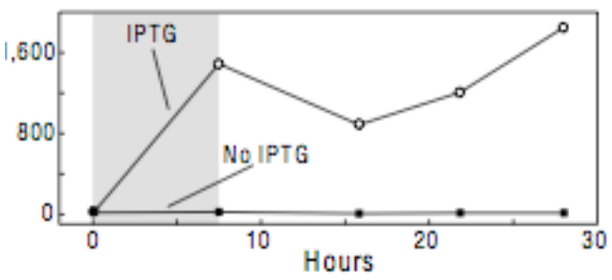
The synthetic toggle switch

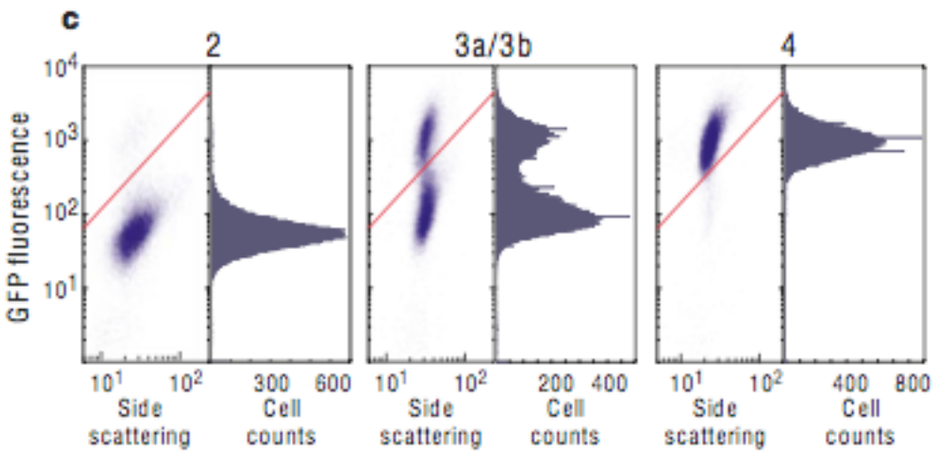












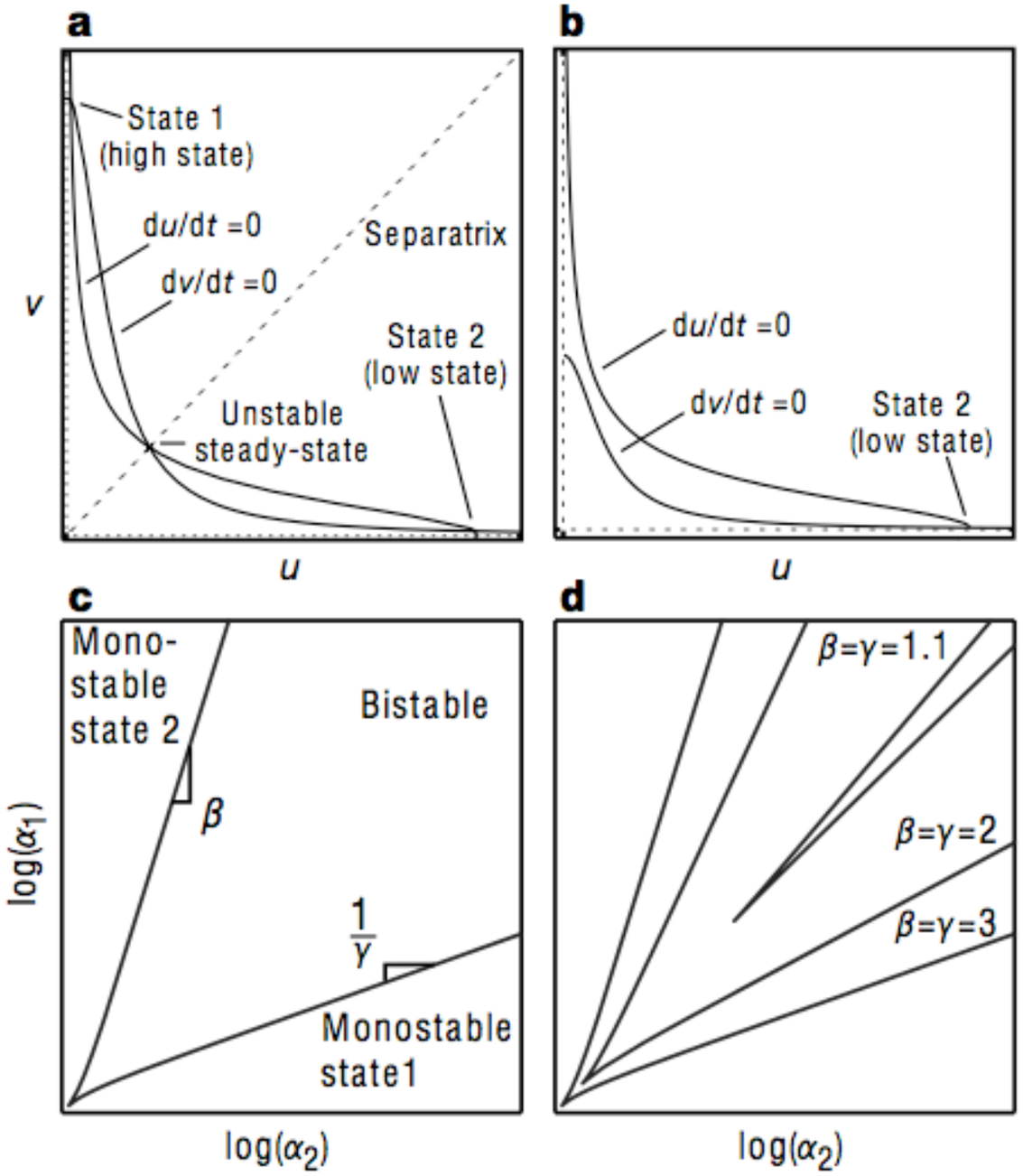
Timothy S. Gardner, Charles R. Cantor & James J. Collins

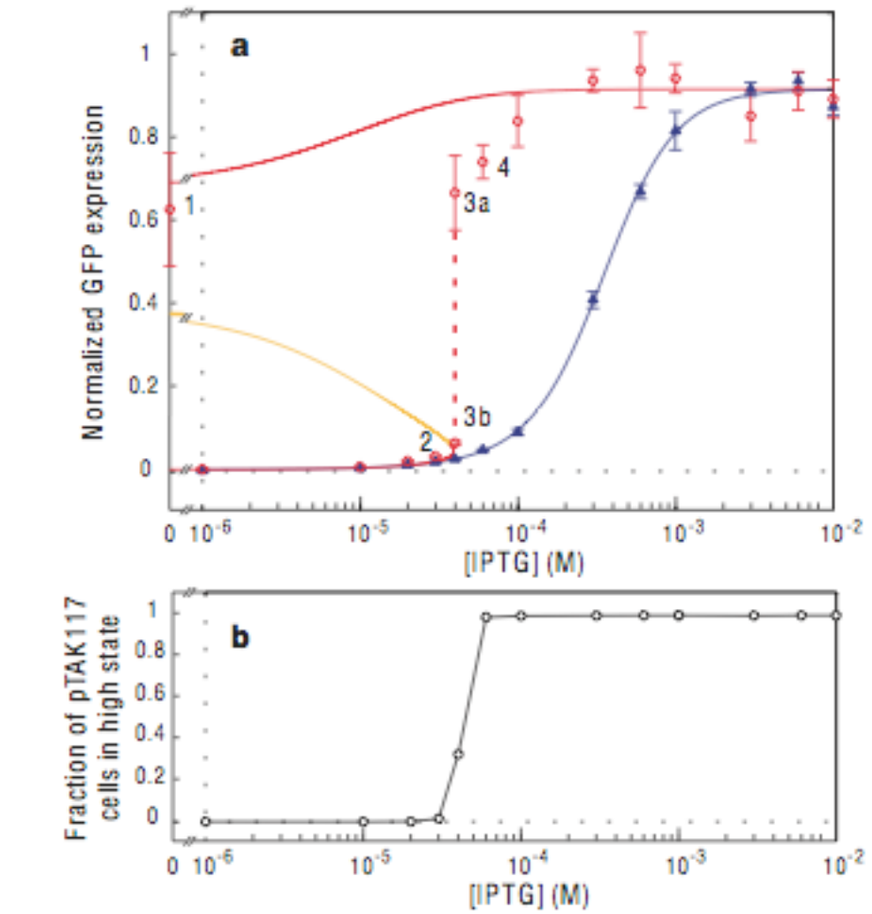
Construction of a genetic toggle switch in Escherichia coli

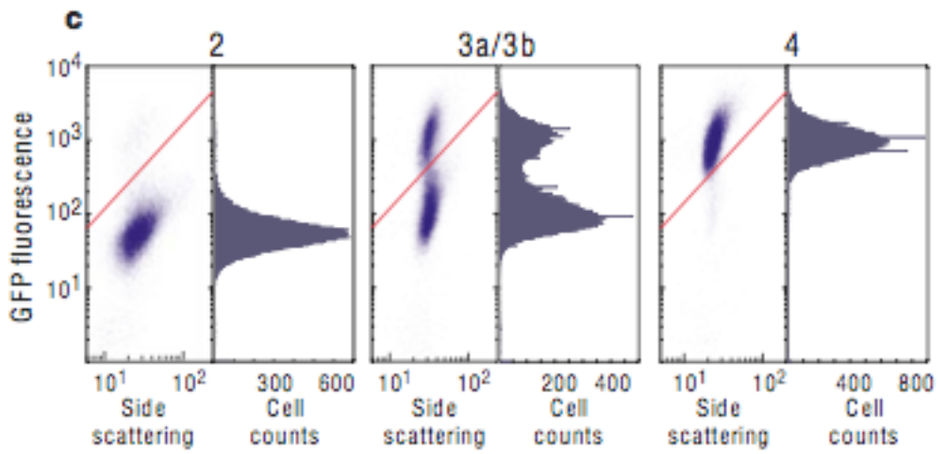
NATURE | VOL 403 | 20 JANUARY 2000

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v$$





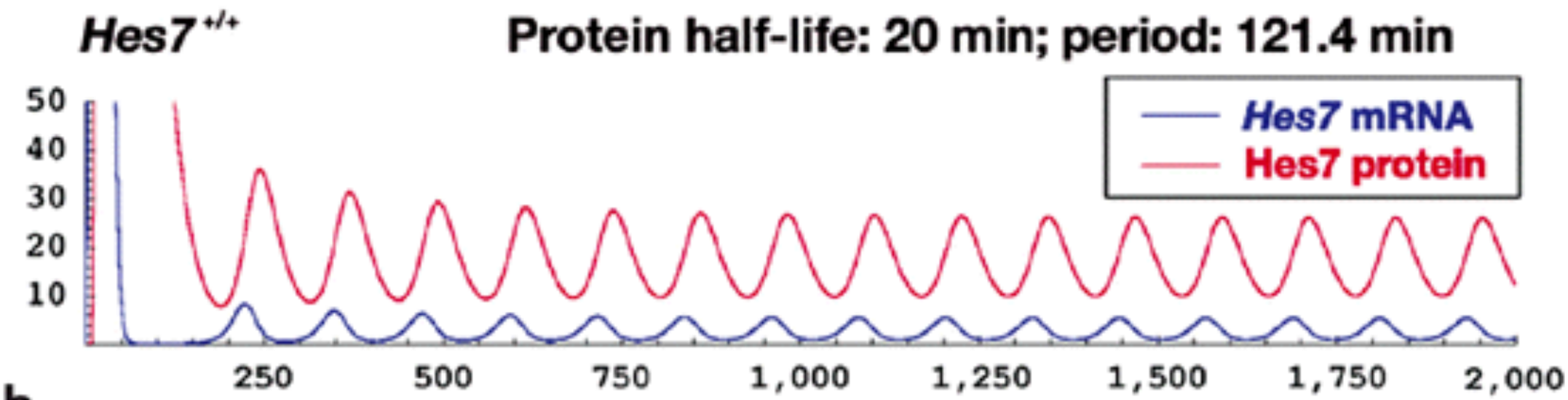


Timothy S. Gardner, Charles R. Cantor & James J. Collins

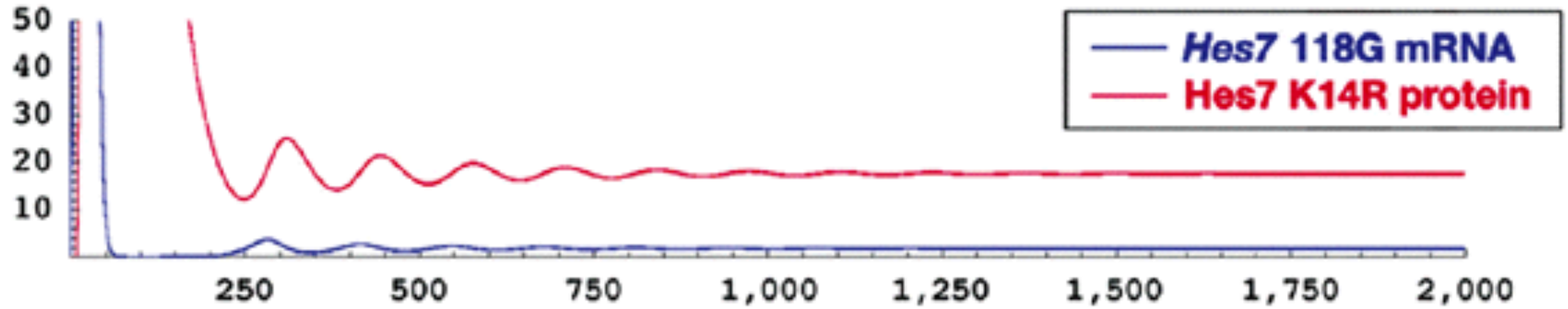
Construction of a genetic toggle switch in Escherichia coli

NATURE I VOL 403 I 20 JANUARY 2000

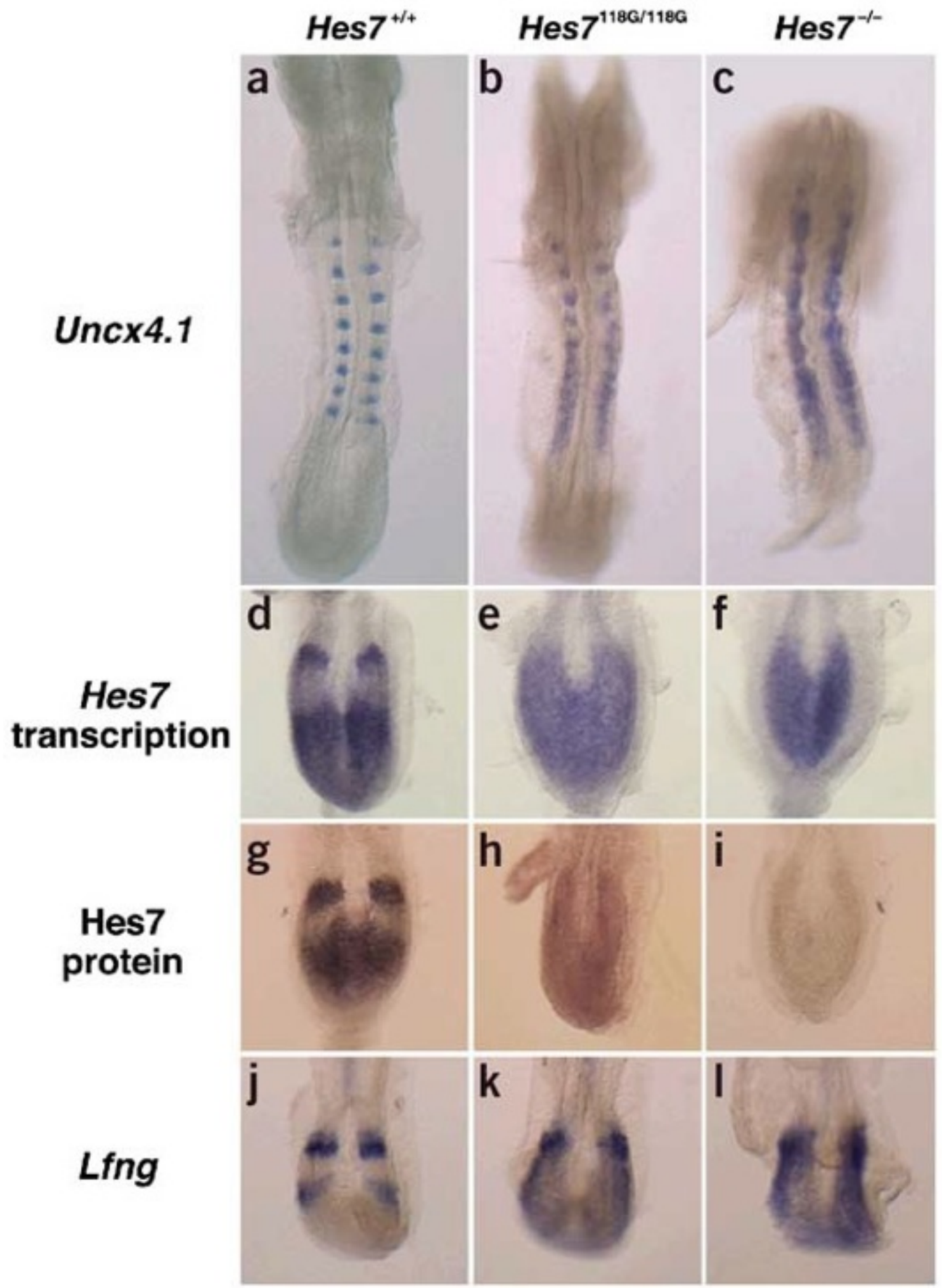
a



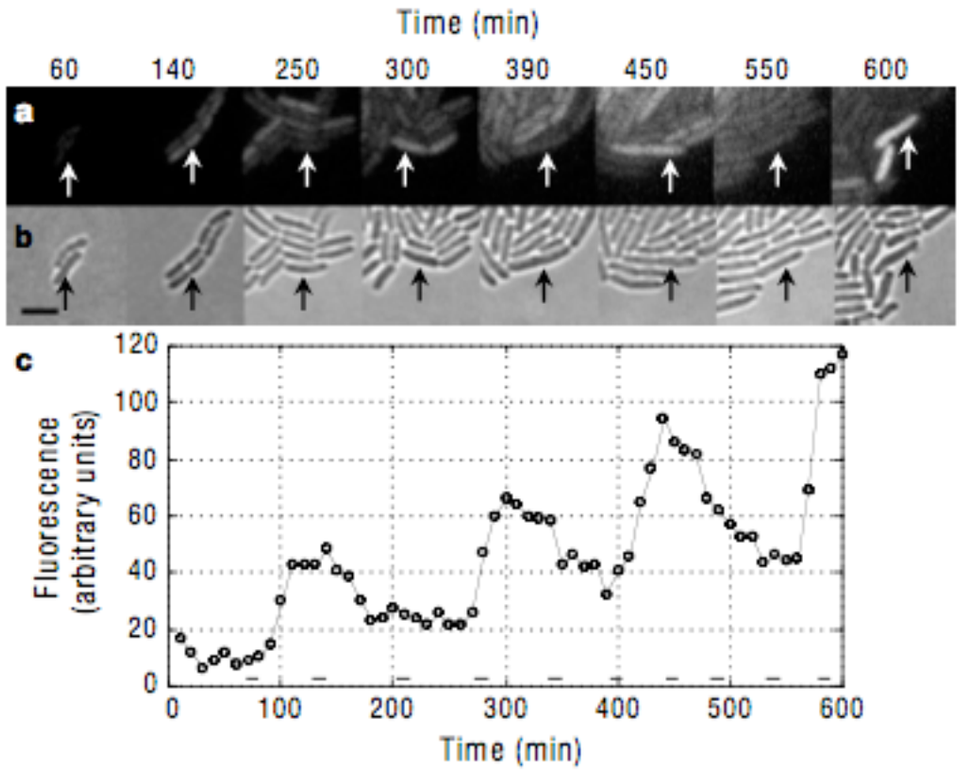
b



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

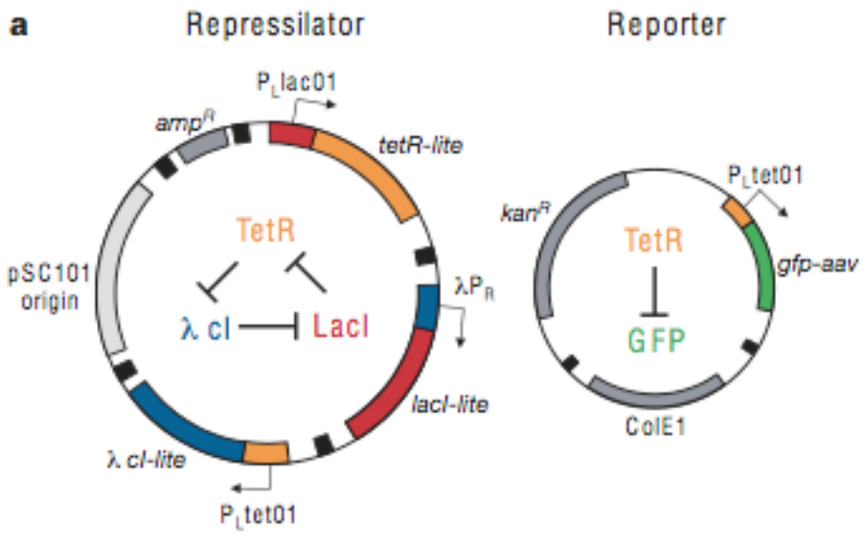

Hes7+/+
Hes7118G/118G
Hes7−/−
a
b
c
Uncx4.1
d
e
f
Hes7
transcription
g
h
i
Hes7
protein
j
k
l
Lfng

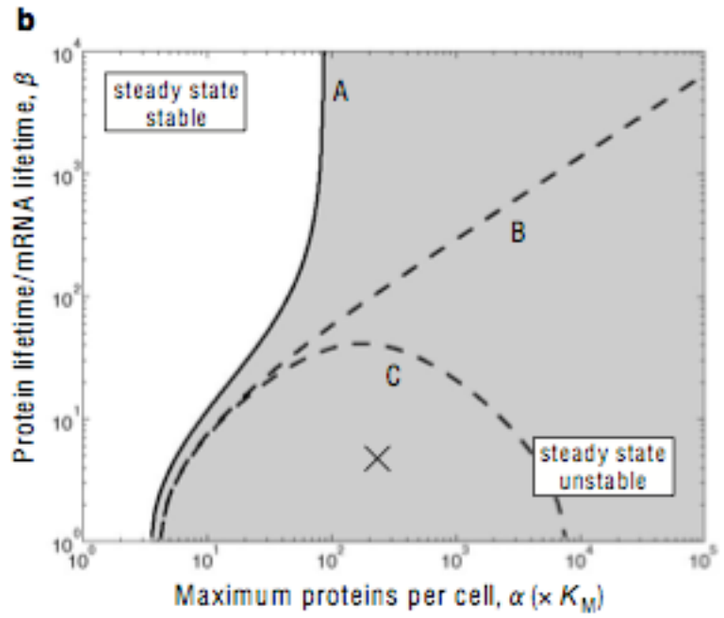
The repressilator

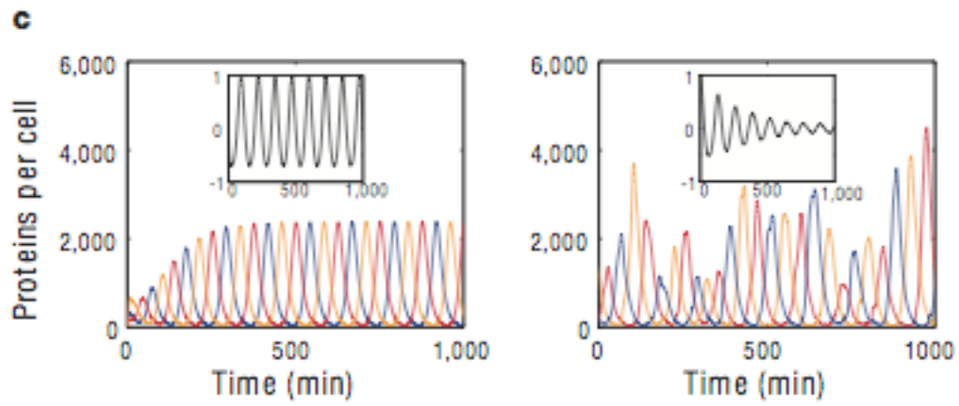


$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_j^n)} + \alpha_0 \qquad \begin{pmatrix} i = lacI, tetR, cI \\ j = cI, lacI, tetR \end{pmatrix}$$

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$





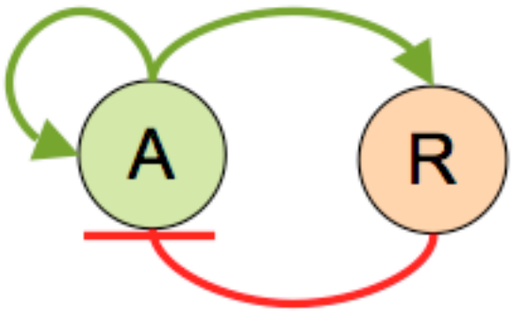


Michael B. Elowitz & Stanislas Leibler

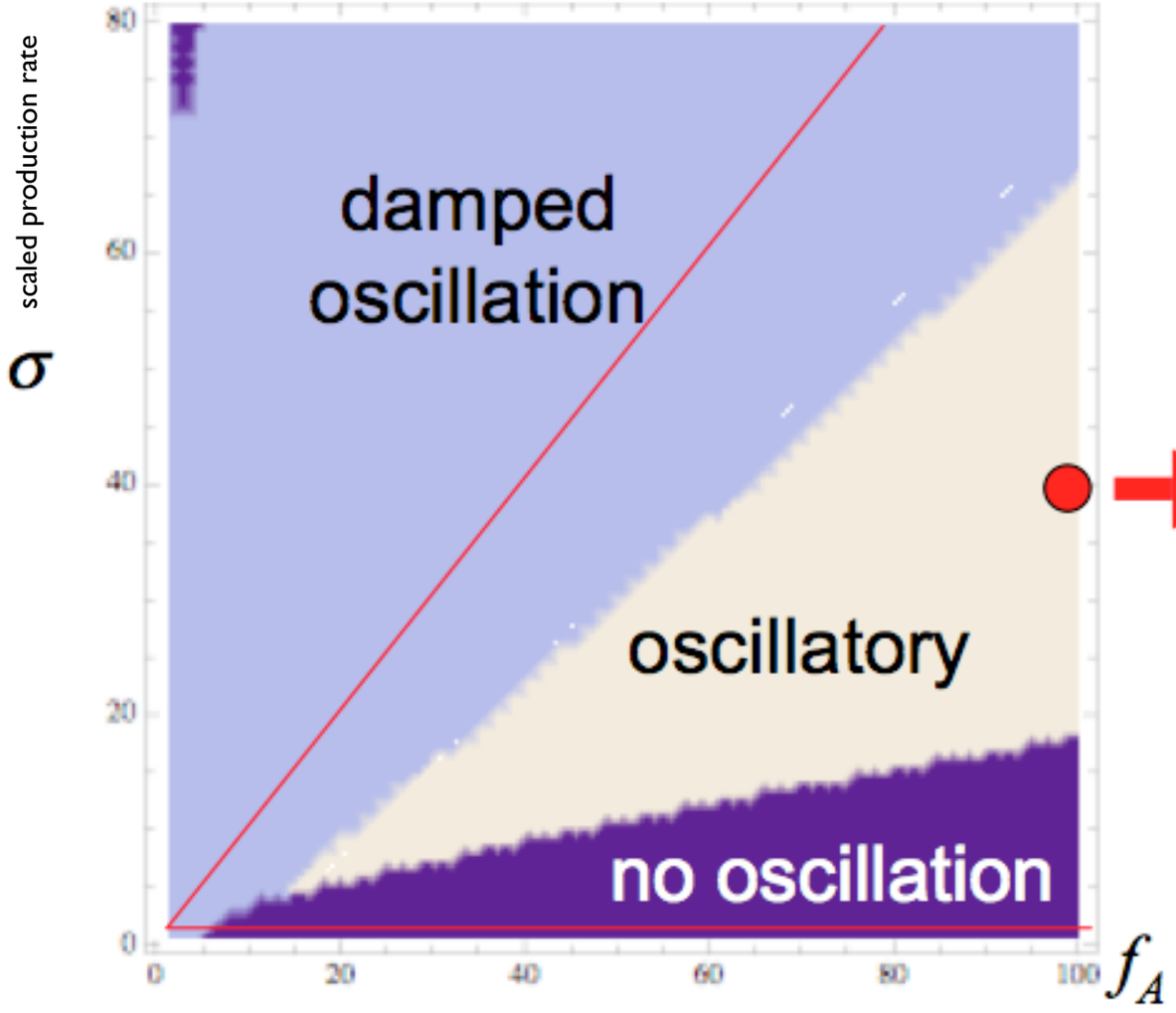
A synthetic oscillatory network of transcriptional regulators

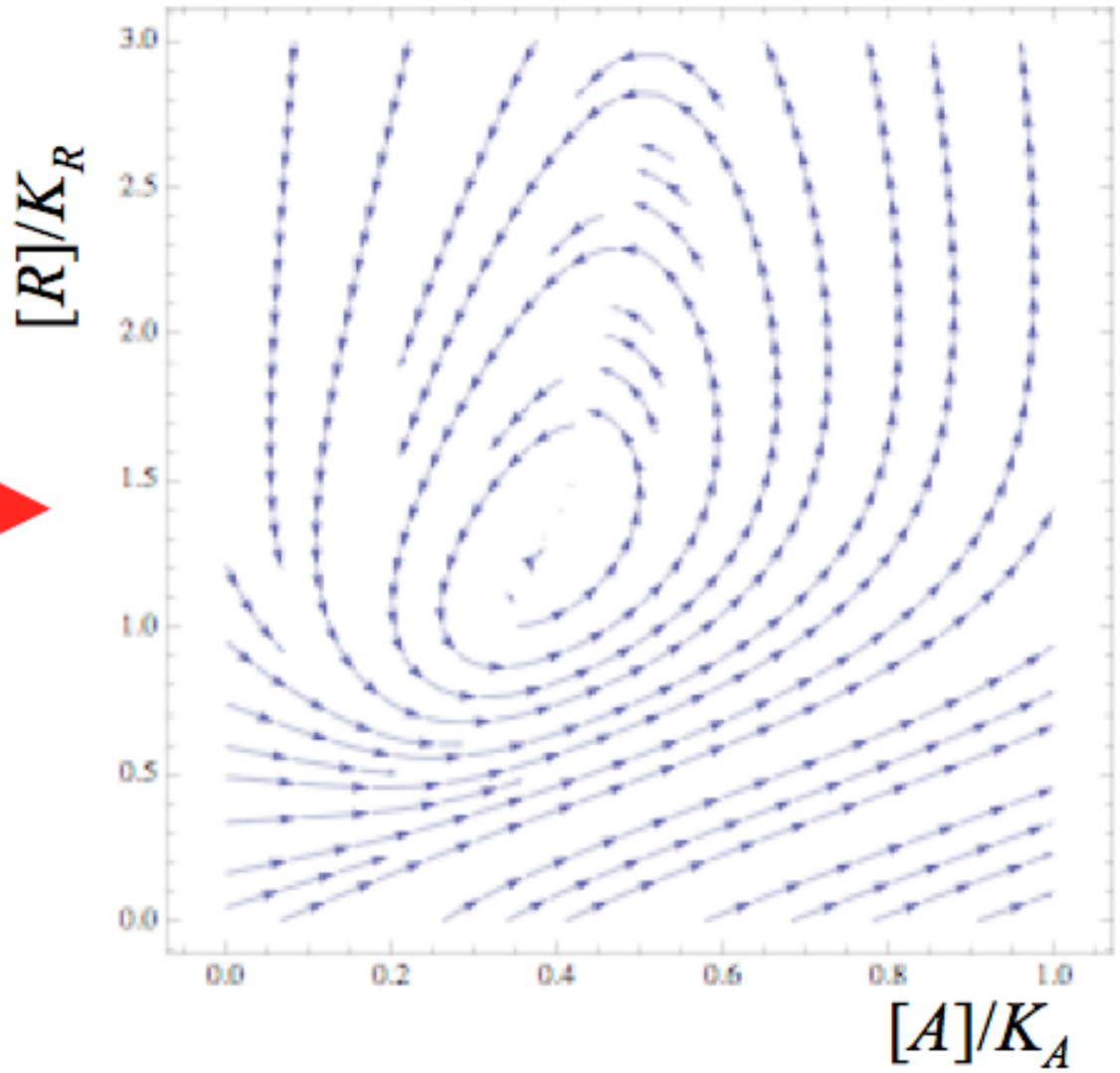
NATURE | VOL 403 | 20 JANUARY 2000

Predator-prey oscillations

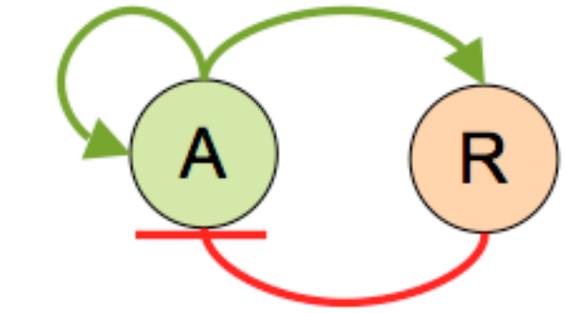


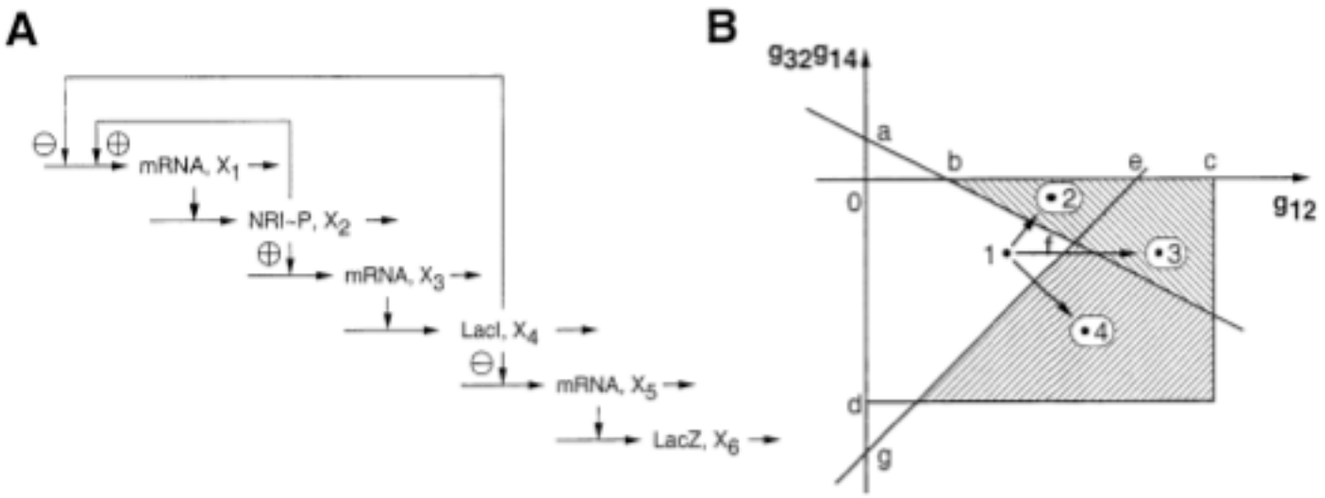
$$\begin{cases} \dfrac{d[A]}{dt} = \alpha \cdot \mathcal{G}_A\left([A]\right) \cdot \mathcal{G}_R\left([R]\right) - \beta \cdot [A] \\ \dfrac{d[R]}{dt} = \alpha \cdot \mathcal{G}_A\left([A]\right) - \beta \cdot [R] \end{cases}$$

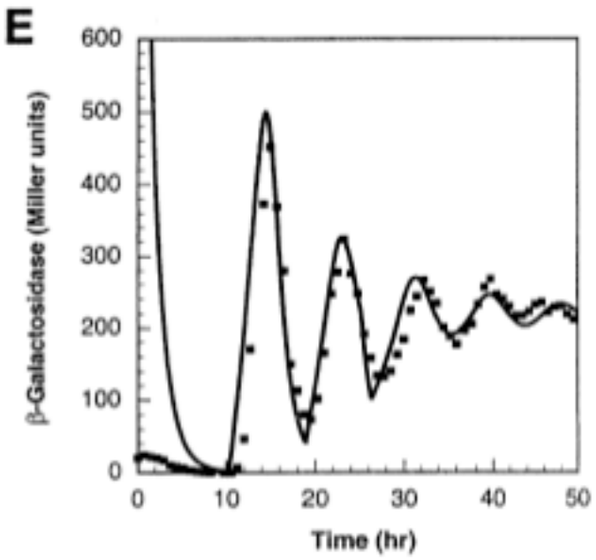


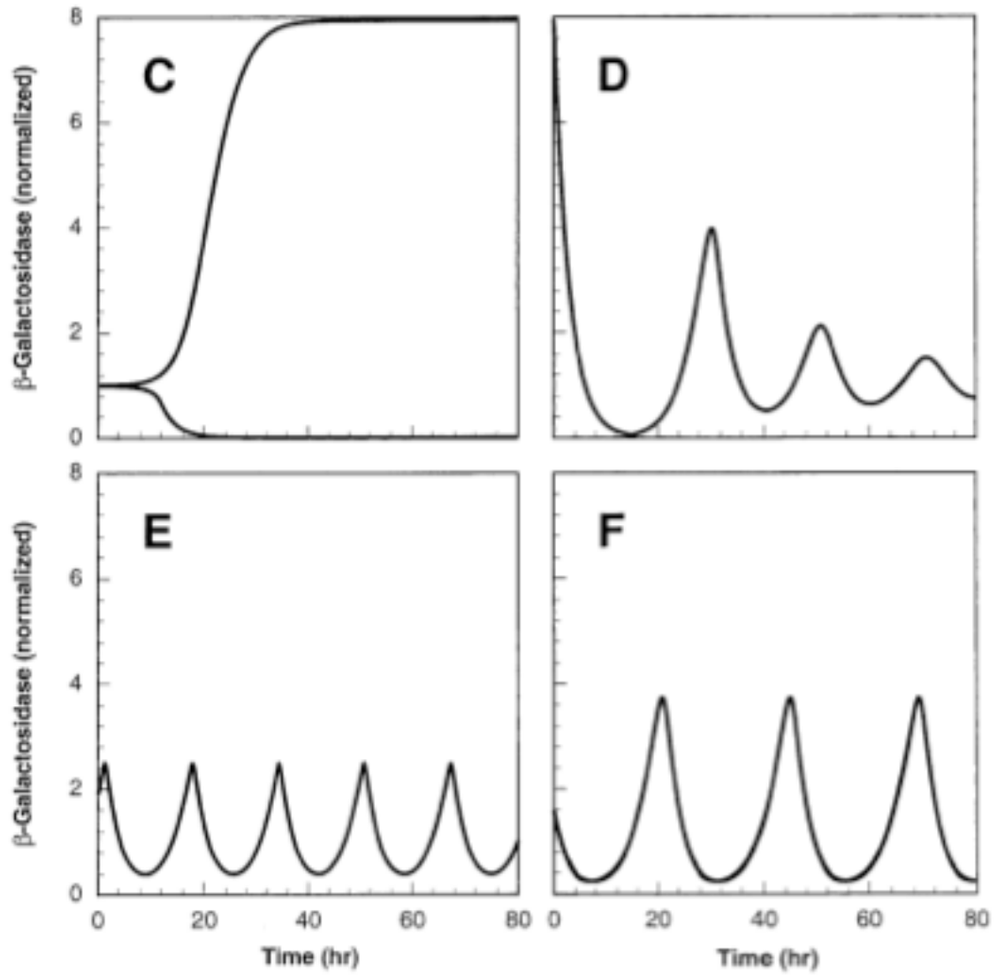


Predator-prey oscillations







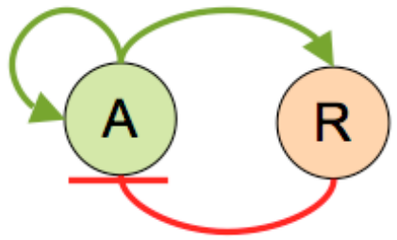


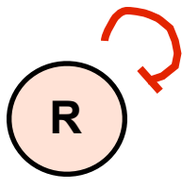
Mariette R. Atkinson, Michael A. Savageau, Jesse T. Myers, and Alexander J. Ninfa

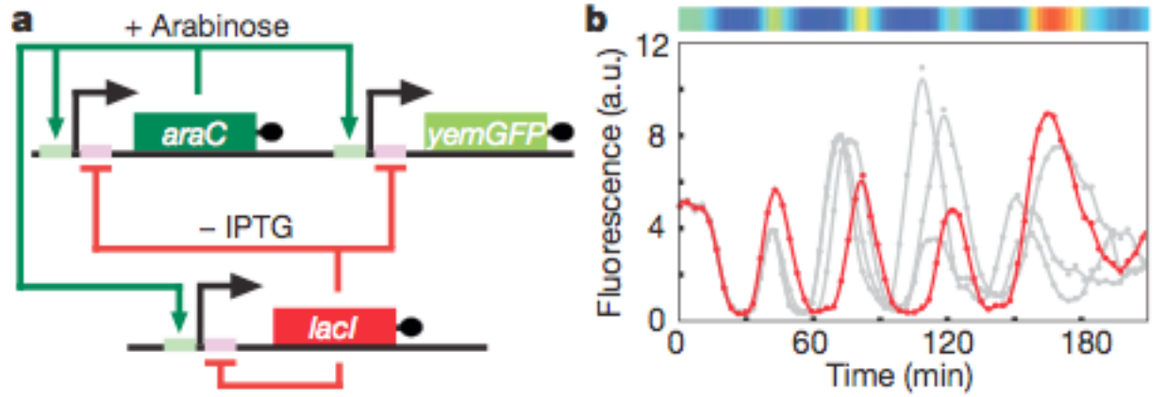
Development of Genetic Circuitry Exhibiting Toggle Switch or Oscillatory Behavior in Escherichia coli

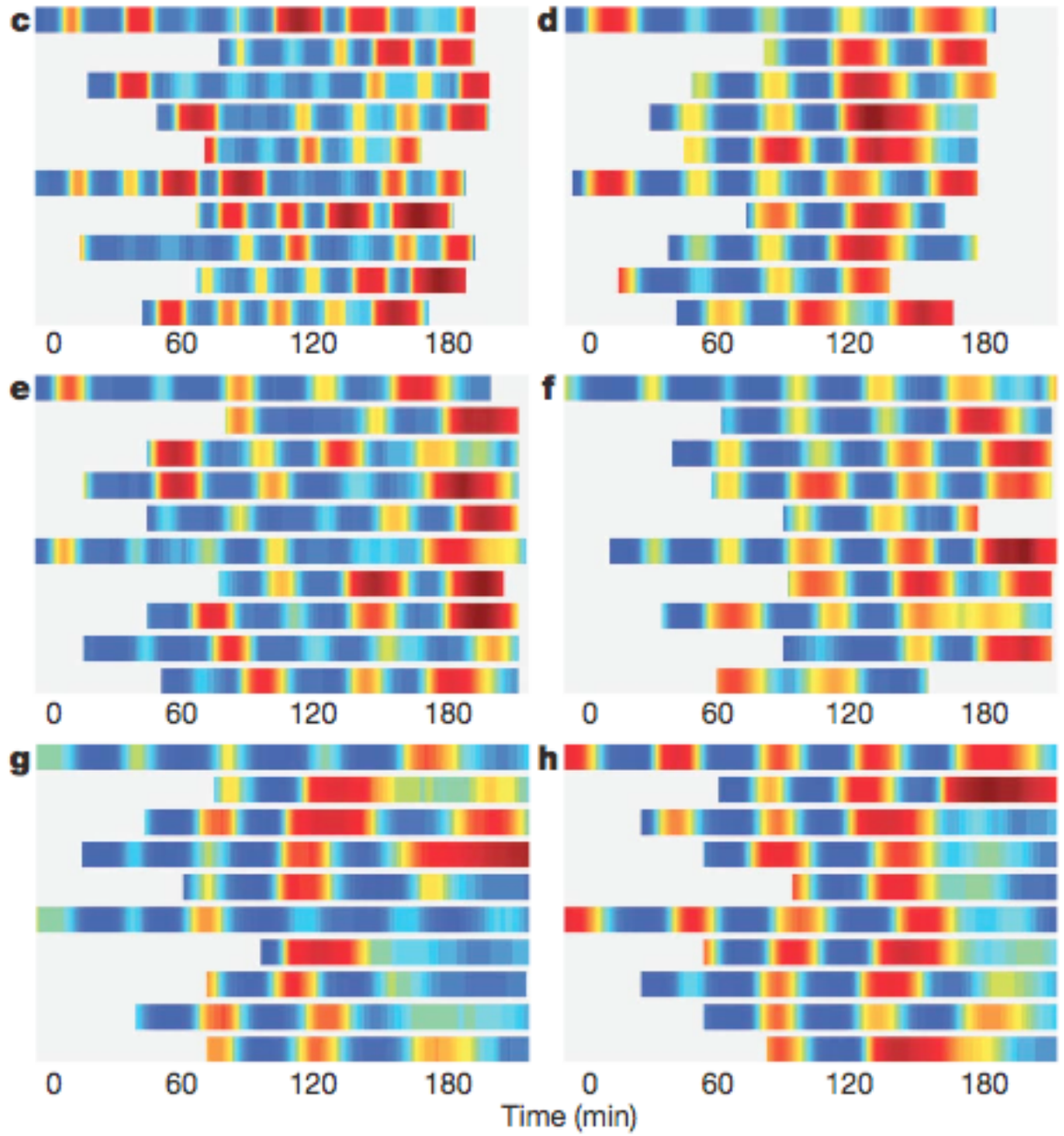
Cell, Vol. 113, 597–607, May 30, 2003

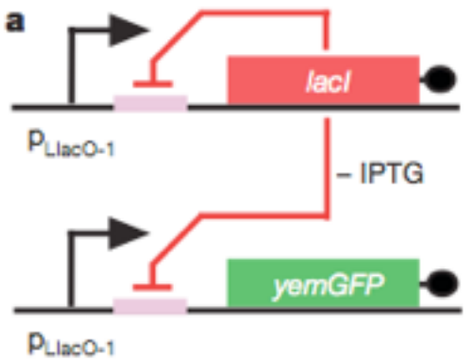
Predator-prey oscillations

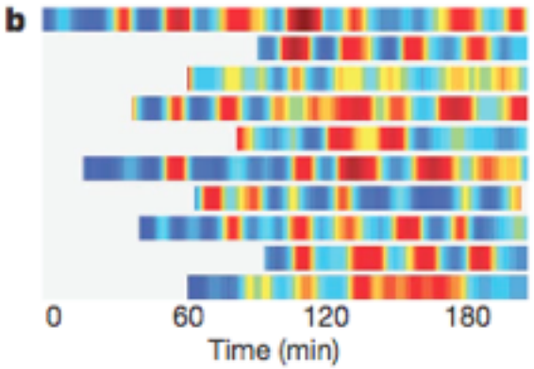












Jesse Stricker, Scott Cookson, Matthew R. Bennett, William H. Mather, Lev S. Tsimring & Jeff Hasty

A fast, robust and tunable synthetic gene oscillator

NATURE Vol 456|27 November 2008

$$
\begin{aligned}
\frac{d\left[\mathrm{Cdc13_T}\right]}{dt} &= k_1\,M - \left(k_2' + k_2''\left[\mathrm{Ste9}\right] + k_2'''\left[\mathrm{Slp1}\right]\right)\left[\mathrm{Cdc13_T}\right], \\
\frac{d\left[\mathrm{preMPF}\right]}{dt} &= k_{\mathrm{wee}}\left(\left[\mathrm{Cdc13_T}\right] - \left[\mathrm{preMPF}\right]\right) - k_{25}\left[\mathrm{preMPF}\right] \\
&\quad - \left(k_2' + k_2''\left[\mathrm{Ste9}\right] + k_2'''\left[\mathrm{Slp1}\right]\right)\left[\mathrm{preMPF}\right], \\
\frac{d\left[\mathrm{Ste9}\right]}{dt} &= \left(k_3' + k_3''\left[\mathrm{Slp1}\right]\right)\frac{1-\left[\mathrm{Ste9}\right]}{J_3 + 1 - \left[\mathrm{Ste9}\right]} \\
&\quad - \left(k_4'\left[\mathrm{SK}\right] + k_4\left[\mathrm{MPF}\right]\right)\frac{\left[\mathrm{Ste9}\right]}{J_4 + \left[\mathrm{Ste9}\right]}, \\
\frac{d\left[\mathrm{Slp1}\right]}{dt} &= k_5' + k_5''\frac{\left[\mathrm{MPF}\right]^4}{J_5^4 + \left[\mathrm{MPF}\right]^4} - k_6\left[\mathrm{Slp1_T}\right], \\
\frac{d\left[\mathrm{Slp1^*}\right]}{dt} &= k_7\left[\mathrm{IEP}\right]\frac{\left[\mathrm{Slp1_T}\right] - \left[\mathrm{Slp1}\right]}{J_7 + \left[\mathrm{Slp1_T}\right] - \left[\mathrm{Slp1}\right]} - k_8\frac{\left[\mathrm{Slp1}\right]}{J_8 + \left[\mathrm{Slp1}\right]} \\
&\quad - k_6\left[\mathrm{Slp1}\right], \\
\frac{d\left[\mathrm{IEP}\right]}{dt} &= k_9\left[\mathrm{MPF}\right]\frac{1-\left[\mathrm{IEP}\right]}{J_9 + 1 - \left[\mathrm{IEP}\right]} - k_{10}\frac{\left[\mathrm{IEP}\right]}{J_{10} + \left[\mathrm{IEP}\right]},
\end{aligned}
$$

$$
\begin{aligned}
\frac{d\left[\mathrm{Rum1_T}\right]}{dt} &= k_{11} - \left(k_{12} + k_{12}'\left[\mathrm{SK}\right] + k_{12}''\left[\mathrm{MPF}\right]\right)\left[\mathrm{Rum1_T}\right], \\
\frac{d\left[\mathrm{SK}\right]}{dt} &= k_{13}\left[\mathrm{TF}\right] - k_{14}\left[\mathrm{SK}\right], \\
\frac{d\left[\mathrm{M}\right]}{dt} &= \mu\,M, \\
\left[\mathrm{Trimer}\right] &= \frac{2\left[\mathrm{Cdc13_T}\right]\left[\mathrm{Rum1_T}\right]}{\Sigma + \sqrt{\Sigma^2 - 4\left[\mathrm{Cdc13_T}\right]\left[\mathrm{Rum1_T}\right]}}, \\
\left[\mathrm{MPF}\right] &= \frac{\left(\left[\mathrm{Cdc13_T} - \left[\mathrm{preMPF}\right]\right]\right)\left(\left[\mathrm{Cdc13_T}\right] - \left[\mathrm{Trimer}\right]\right)}{\left[\mathrm{Cdc13_T}\right]}, \\
\left[\mathrm{TF}\right] &= G\left(k_{15}M, k_{16}' + k_{16}''\left[\mathrm{MPF}\right], J_{15}, J_{16}\right)
\end{aligned}
$$

- 9 ODEs
- 3 algebraic equations
- ~30 parameters

$$
\begin{aligned}
k_{\mathrm{wee}} &= k_{\mathrm{wee}}' + \left(k_{\mathrm{wee}}'' - k_{\mathrm{wee}}'\right)G\left(V_{\mathrm{awee}}, V_{\mathrm{iwee}}\left[\mathrm{MPF}\right], J_{\mathrm{awee}}, J_{\mathrm{iwee}}\right), \\
k_{25} &= k_{25}' + \left(k_{25}'' - k_{25}'\right)G\left(V_{\mathrm{a25}}\left[\mathrm{MPF}\right], V_{\mathrm{i25}}, J_{\mathrm{a25}}, J_{\mathrm{i25}}\right), \\
\Sigma &= \left[\mathrm{Cdc13_T}\right] + \left[\mathrm{Rum1_T}\right] + K_{\mathrm{diss}}.
\end{aligned}
$$

$$
G(a,b,c,d) = \frac{2ad}{b - a + bc + ad + \sqrt{(b - a + bc + ad)^2 - 4ad(b-a)}}
$$

Novak et al, Chaos 11(1):277–286, 2001

mammalian cell cycle oscillations