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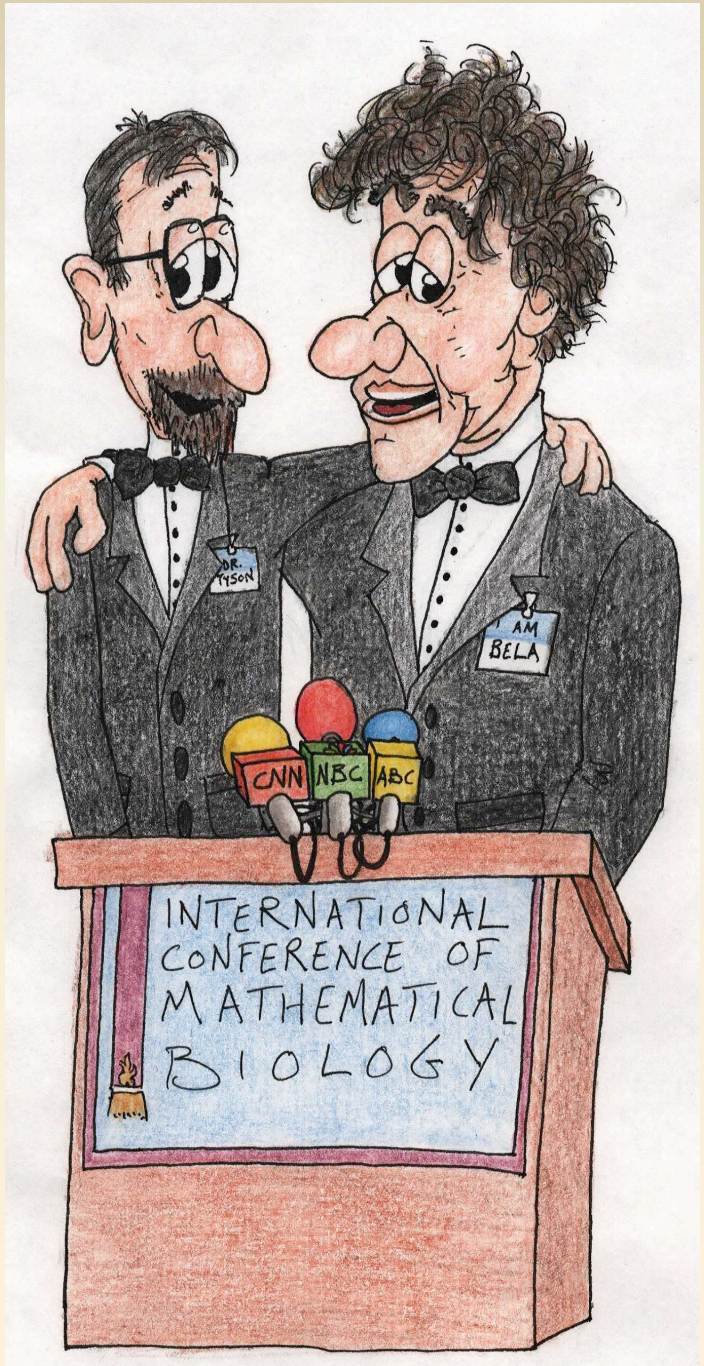
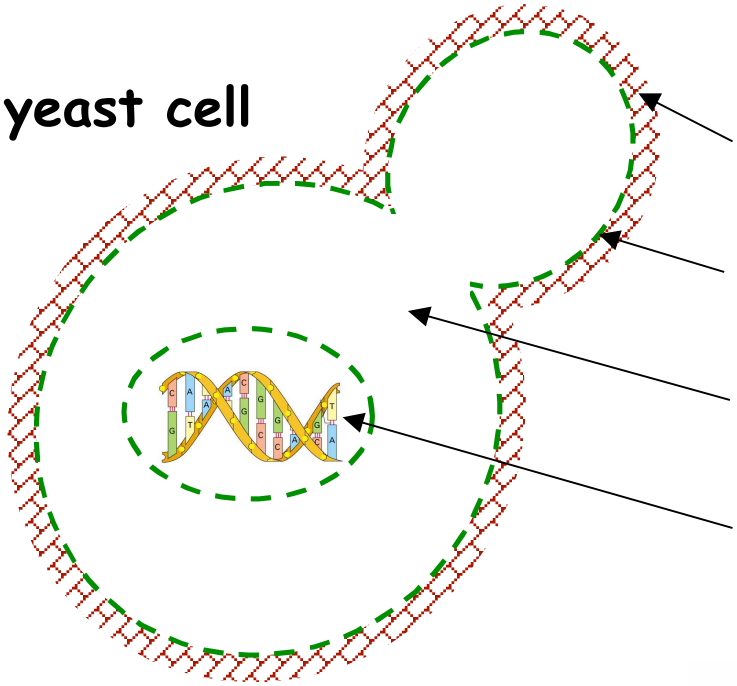


Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell

John J Tyson[*†], Katherine C Chen[*‡] and Bela Novak[§]

Current Opinion in Cell Biology 2003, **15**:221–231

What is a cell?

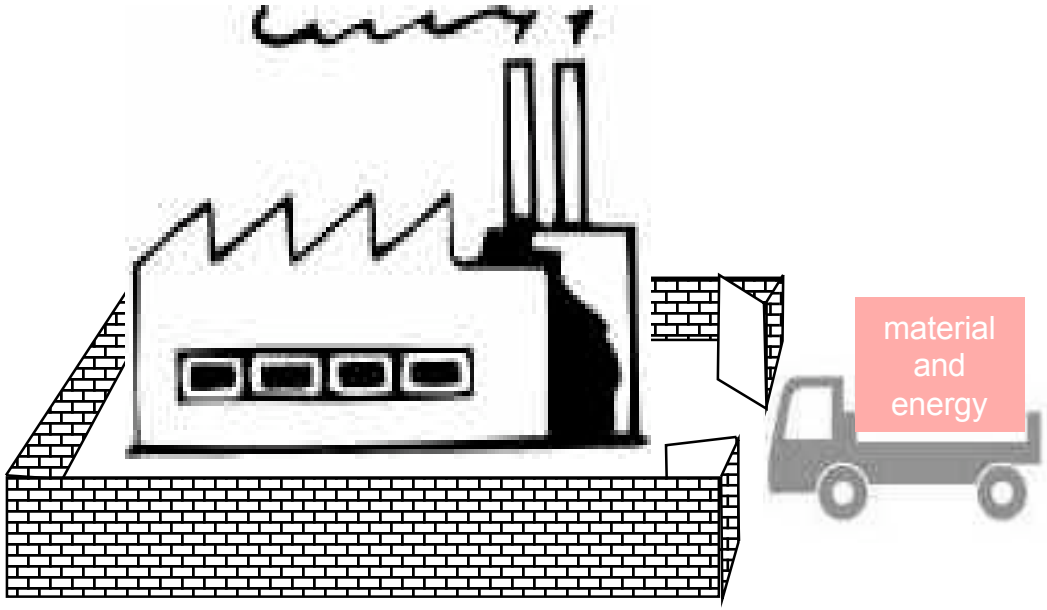


cell wall (protection)

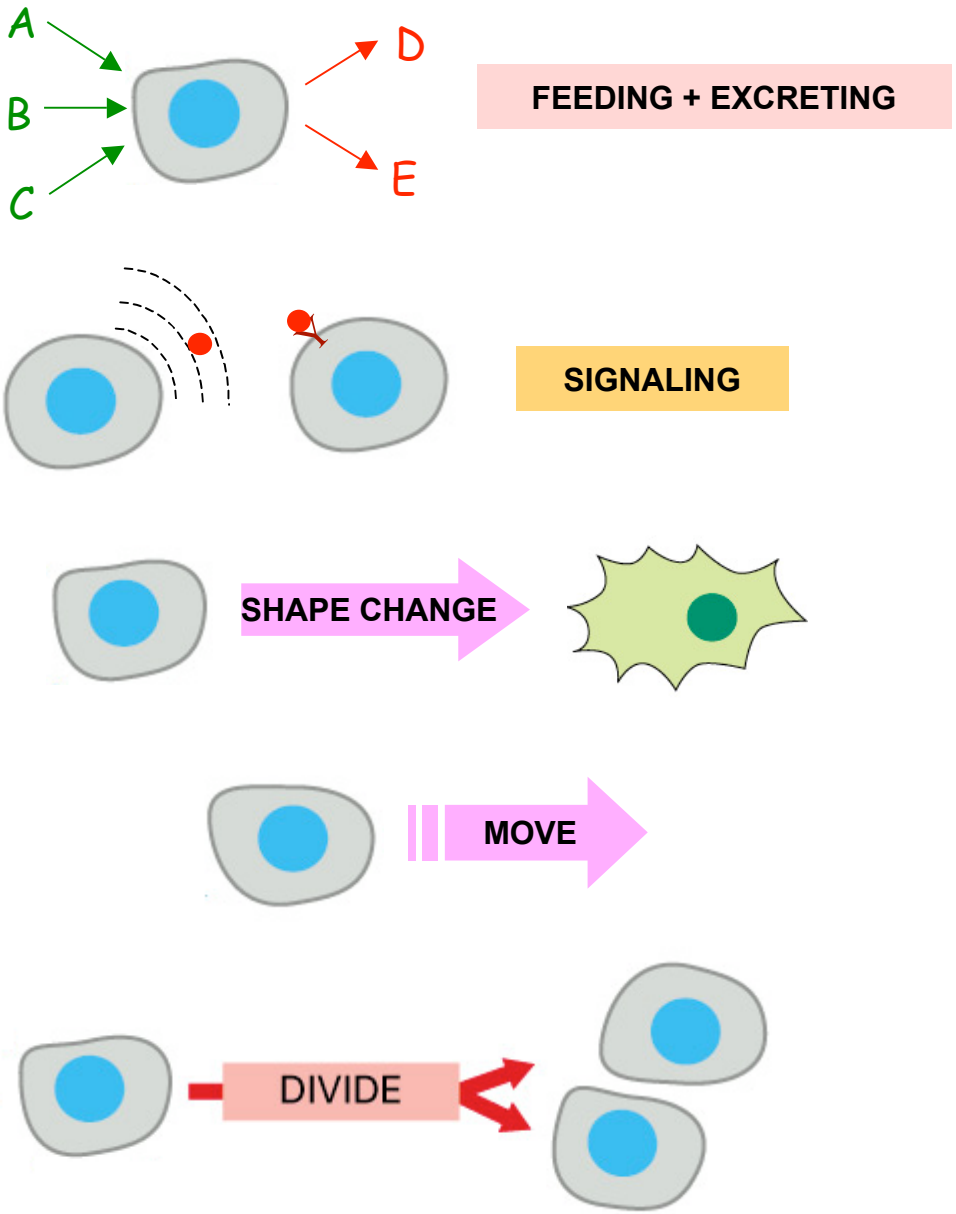
cell membrane (import + export, signaling)

cytoplasm (energy production, growth)

nucleus (heredity)

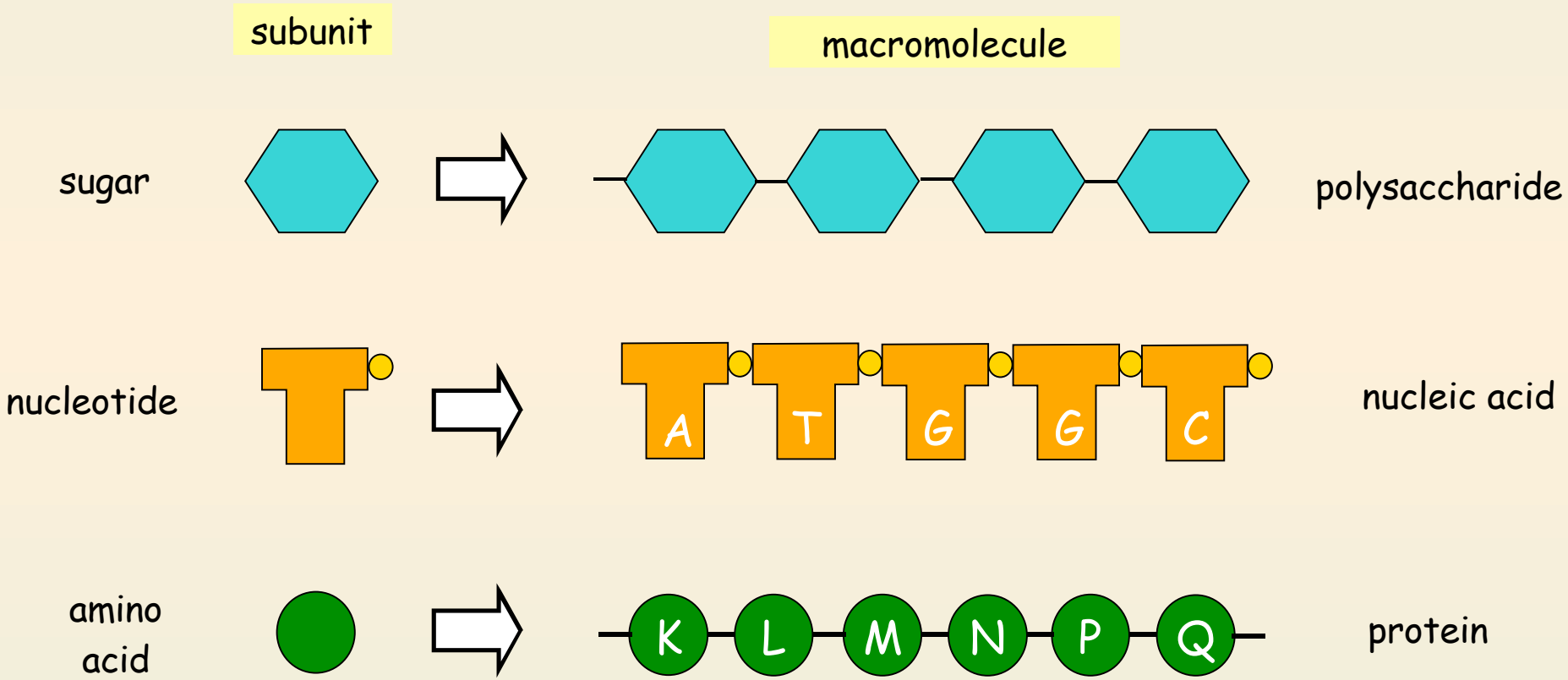


What can cells do?

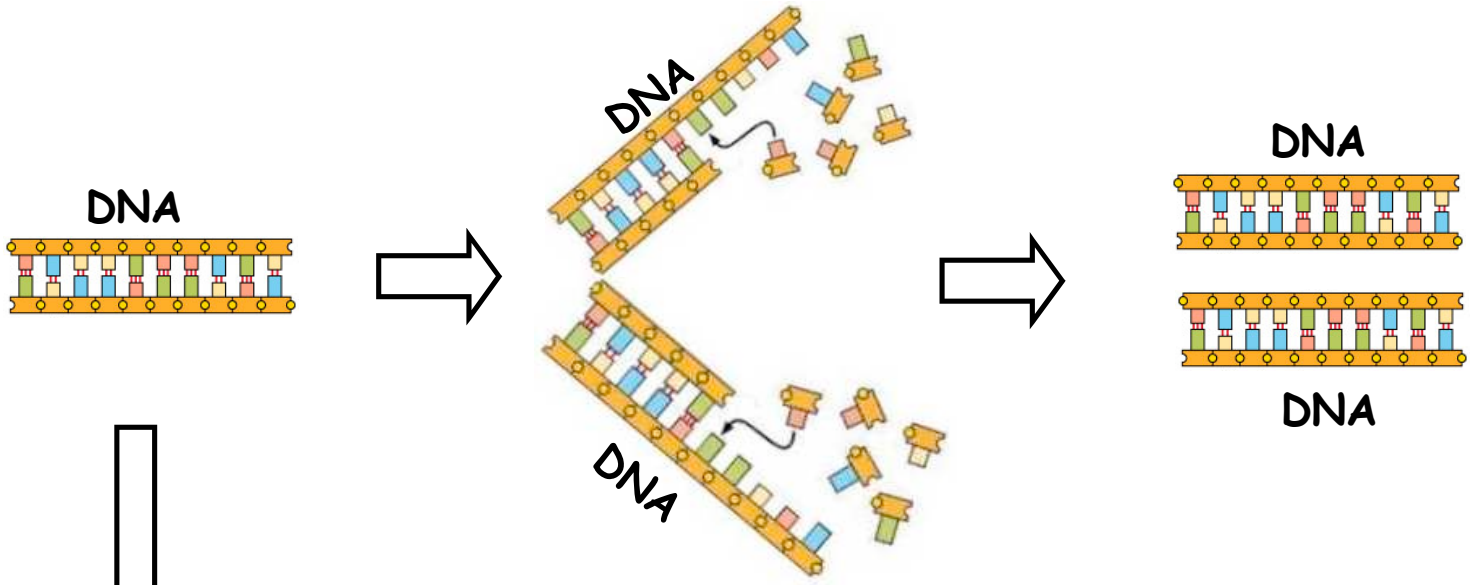


The molecular basis of life

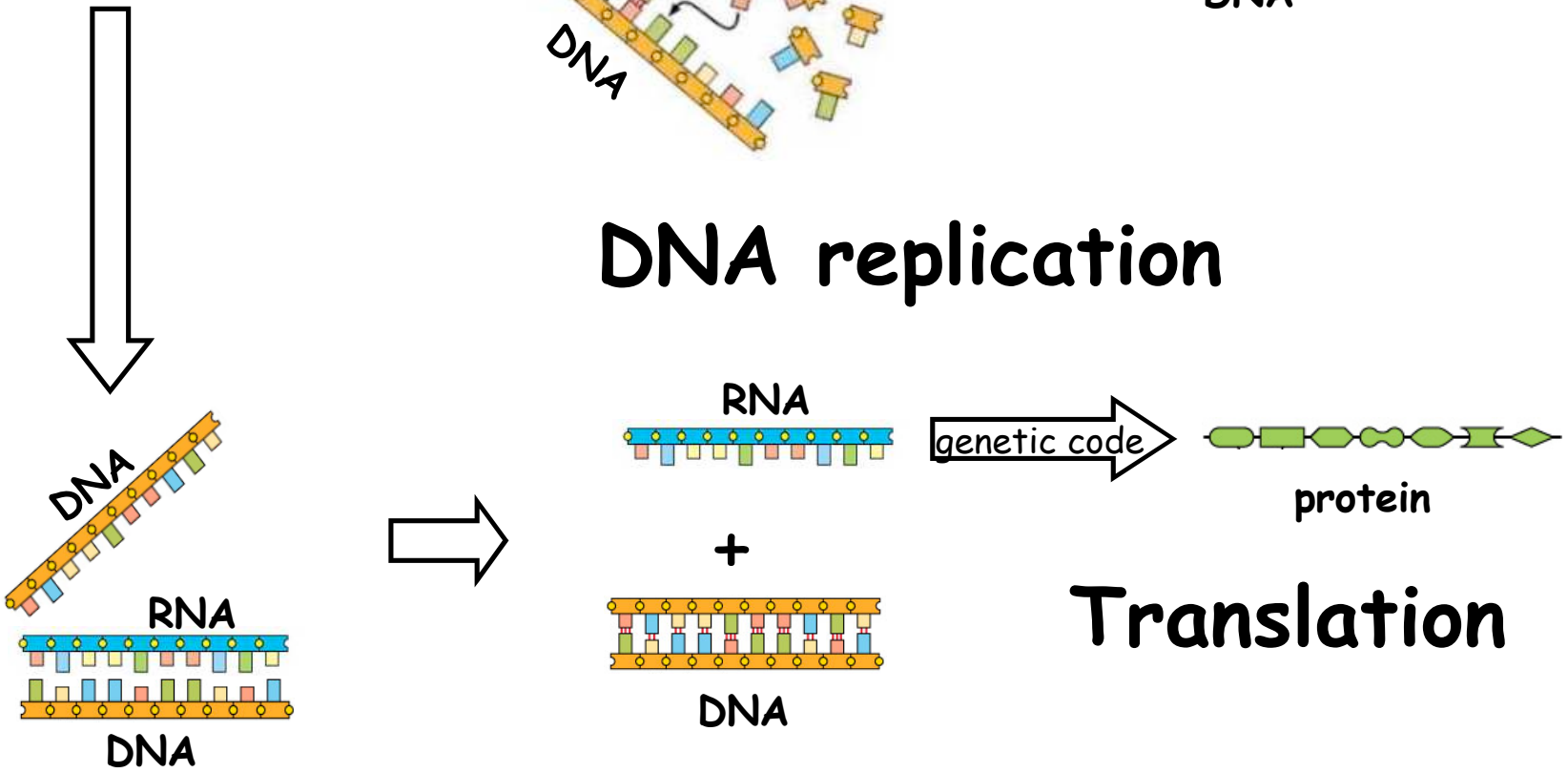
Cells are made of macromolecules



The information flow in cells



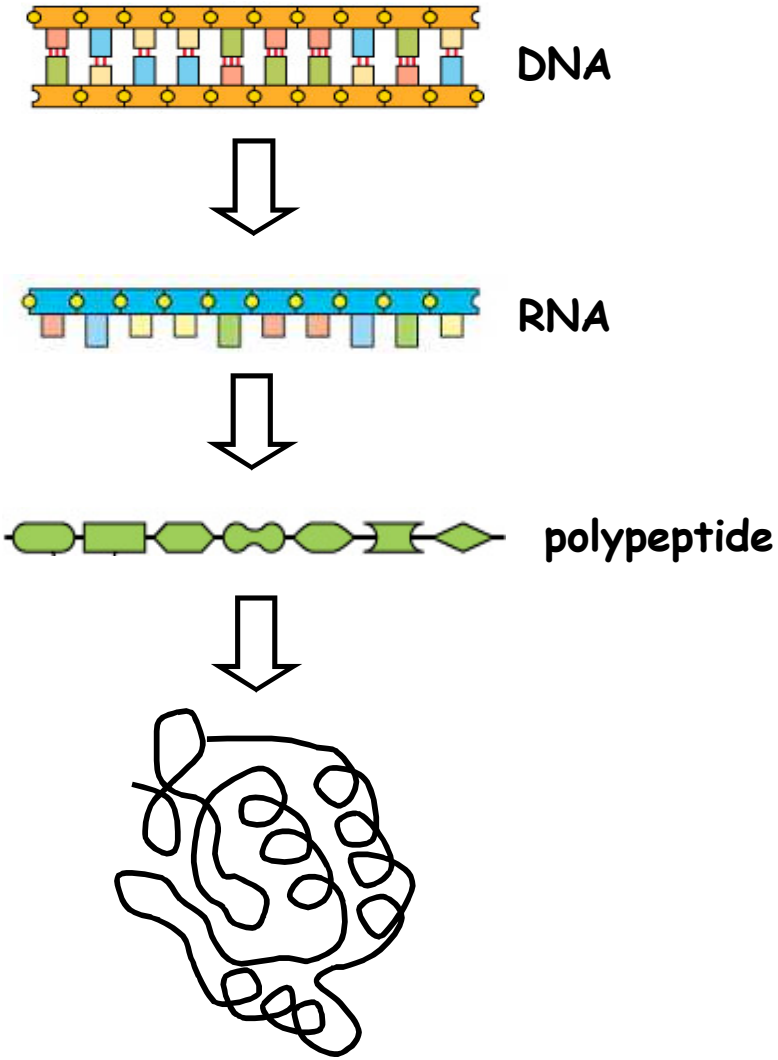
DNA replication



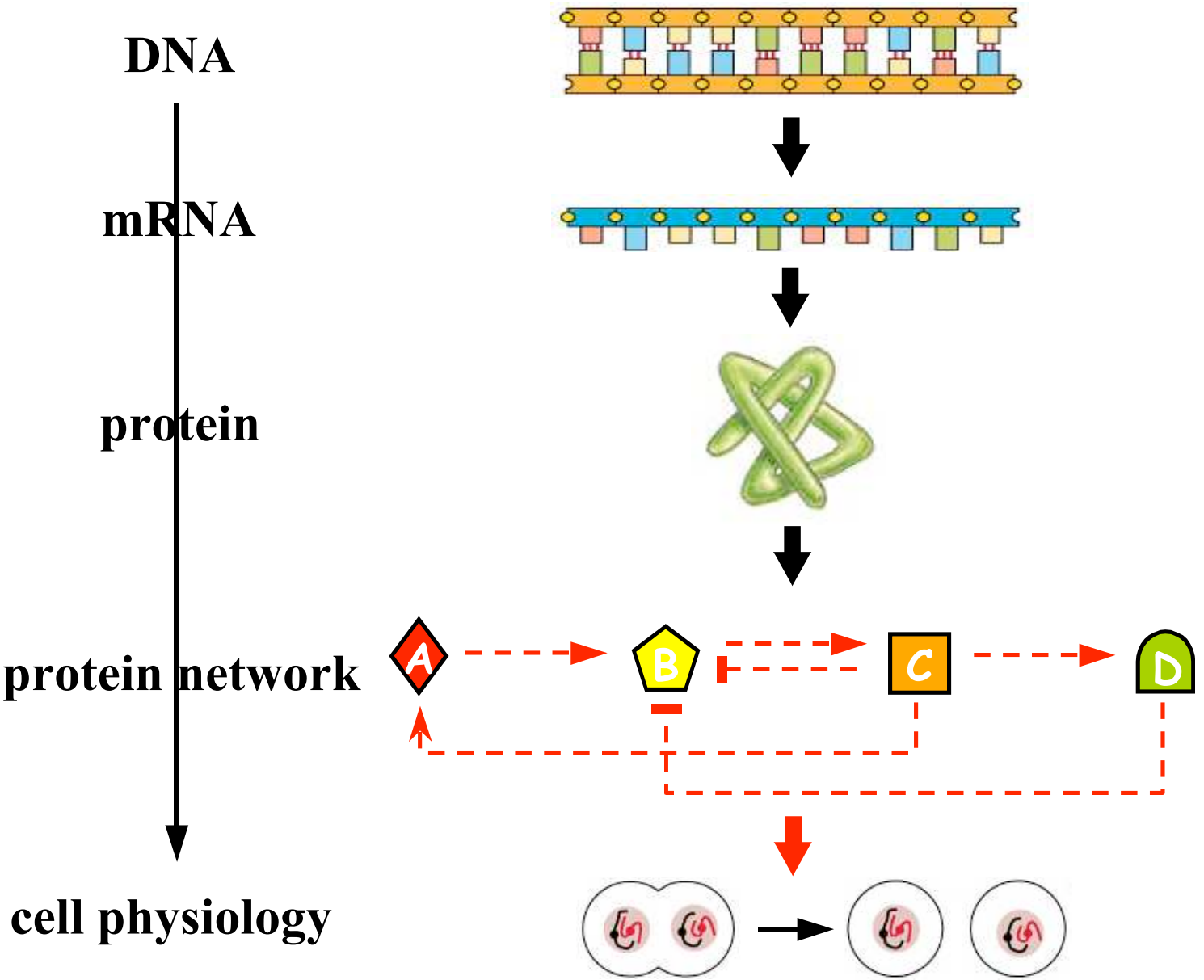
Translation

Transcription

The central dogma

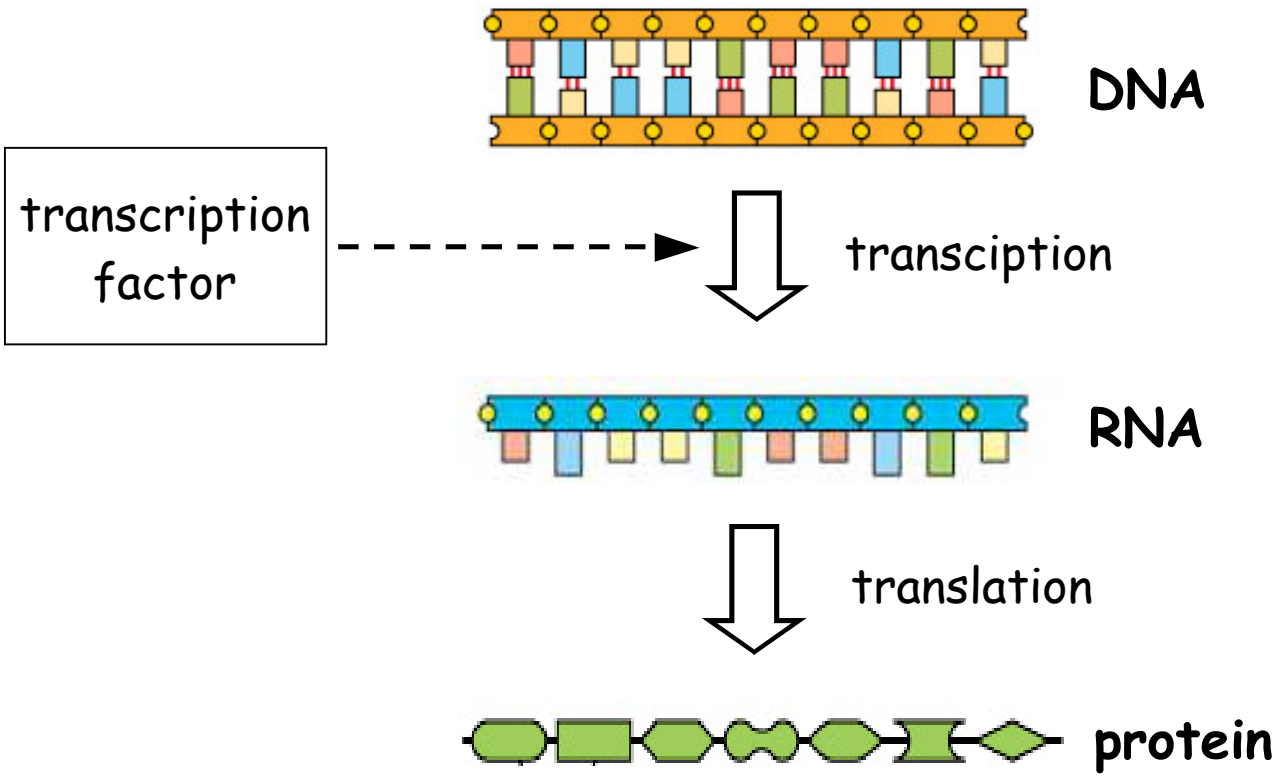


Beyond the central dogma

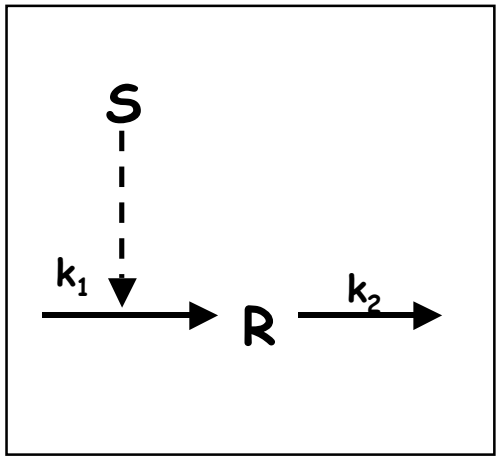


'Birth control' for proteins

$$\frac{d\,[\text{protein}]}{dt} = \text{synthesis} - \text{degradation}$$



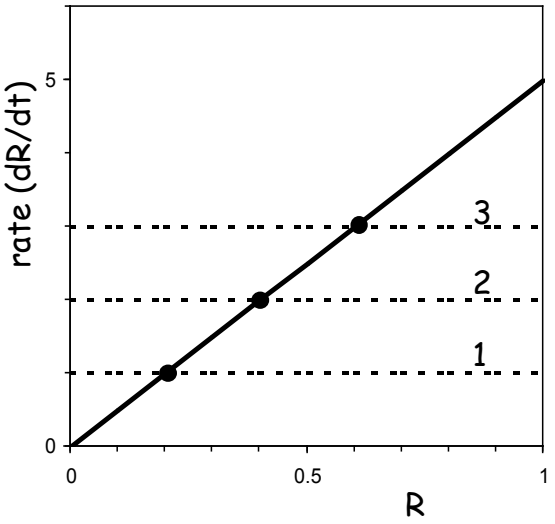
Synthesis and degradation of a protein



$$\frac{dR}{dt} = \underbrace{k_1 \cdot S}_{\text{synthesis}} - \underbrace{k_2 \cdot R}_{\text{degradation}}$$

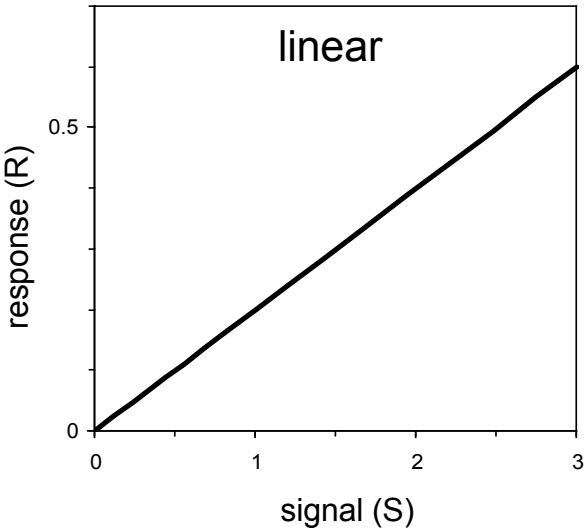
S = mRNA
R = protein concentration

$k_1 = 1$
$k_2 = 5$

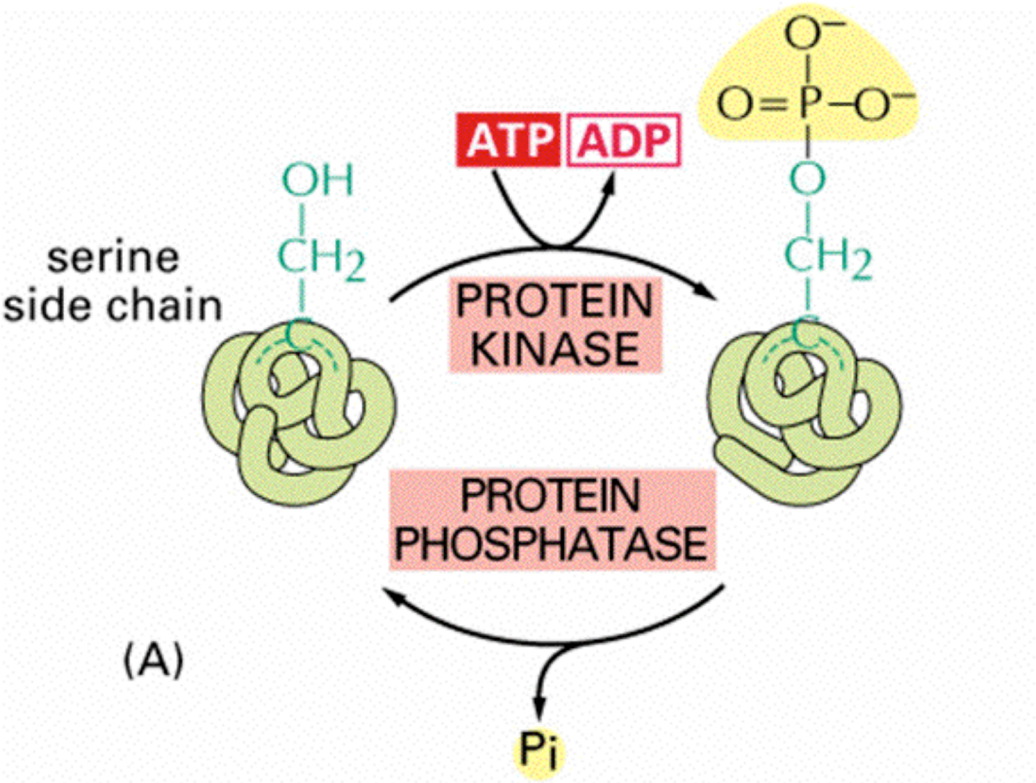


production
removal

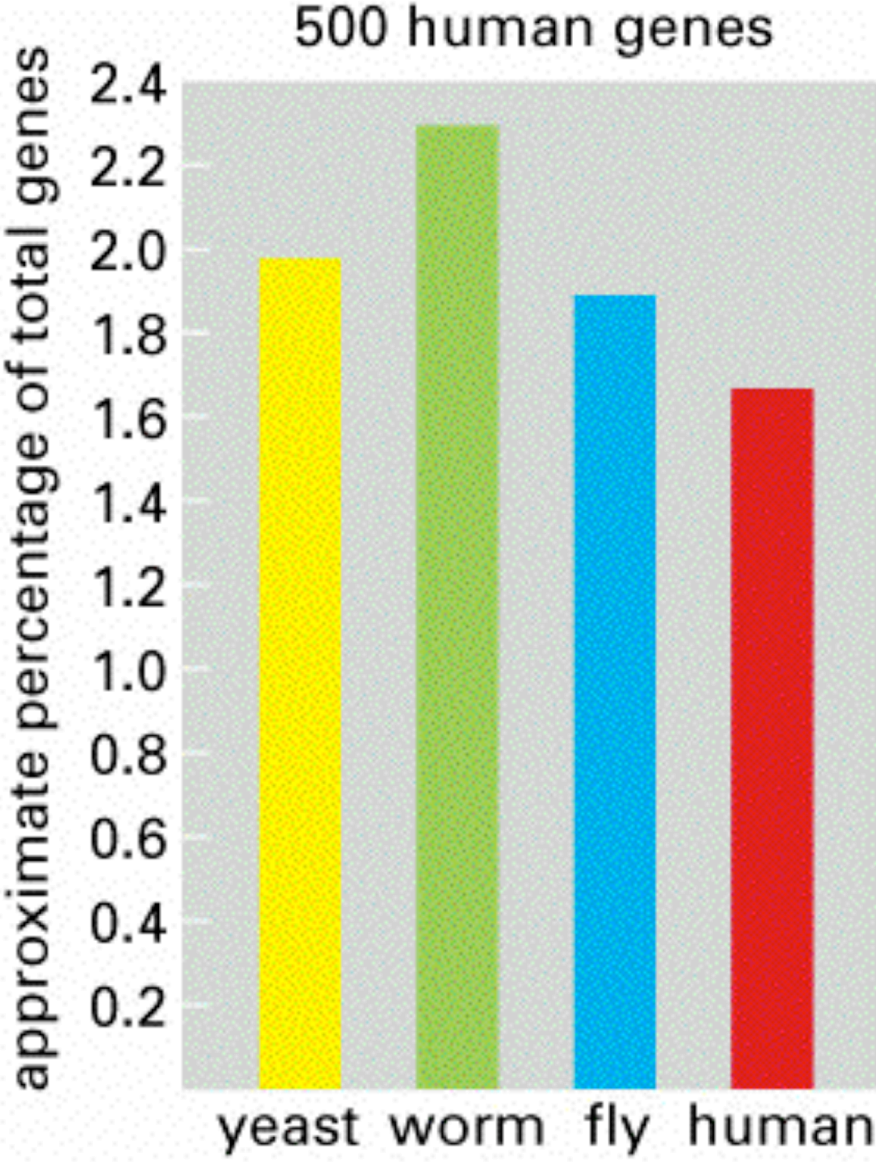
$$R_{ss} = \frac{k_1 \cdot S}{k_2}$$



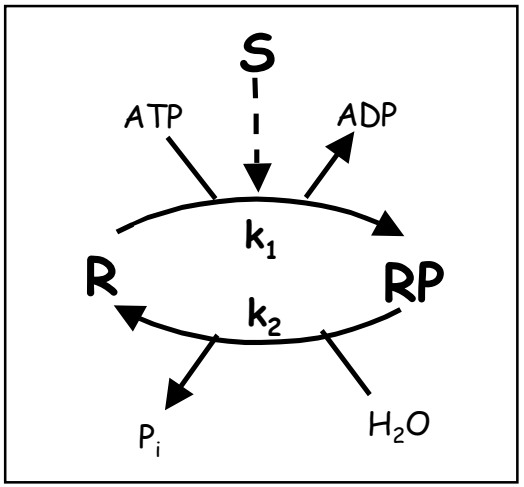
Regulation of activity



The abundance of protein-kinases in eukaryotes



Protein phosphorylation-dephosporylation

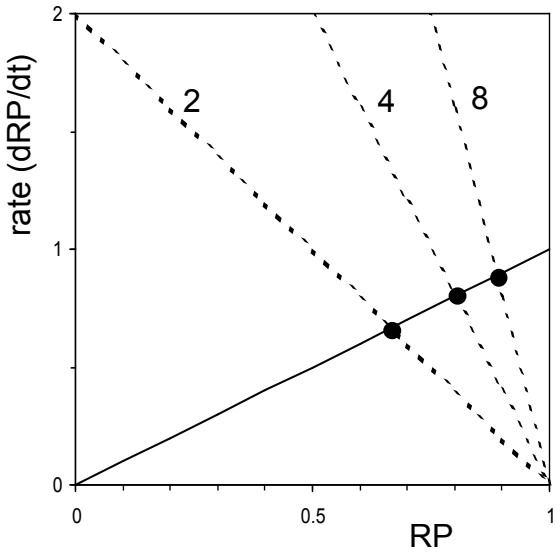


$$\frac{dR^P}{dt} = k^1 \cdot S \cdot (R^T - R^P) - k^2 \cdot R^P$$

phosphorylation dephosphorylation

$k_1 = k_2 = 1$

$R_T = 1$

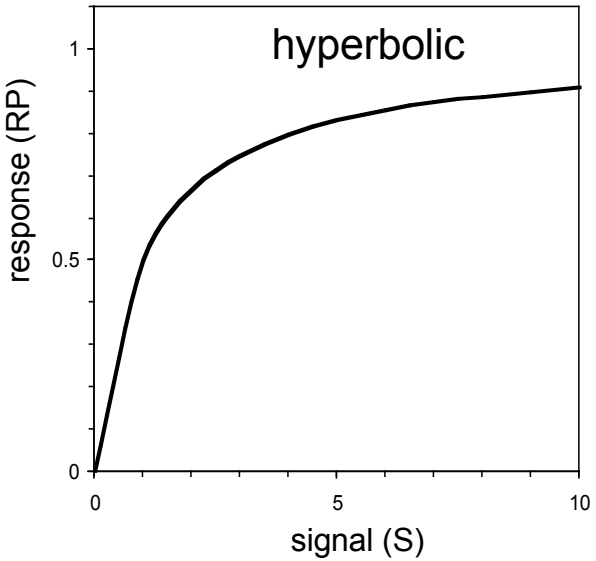


production

removal

$$R^{ss} = \frac{R^T \cdot S(k^2/k^1}{}$$

like Michaelis-Menten !!!



STEADY STATE ENZYME KINETICS

Many enzymes have only one substrate, which they bind and then process to produce products according to the scheme outlined in Figure 3–50A. In this case, the reaction is written as

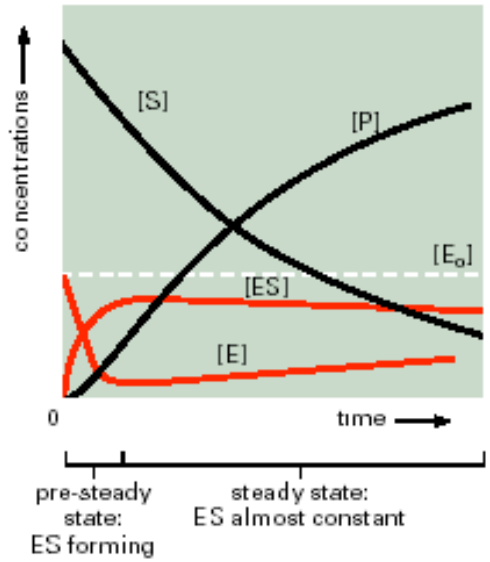
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

Here we have assumed that the reverse reaction, in which E + P recombine to form EP and then ES, occurs so rarely that we can ignore it. In this case, we can express the rate of the reaction – known as its velocity, V, as

$$V = k_{cat}[ES]$$

where [ES] is the concentration of the enzyme substrate complex, and k_{cat} is the turnover number: a rate constant that is equal to the number of substrate molecules processed per enzyme molecule each second.

But how does the value of [ES] relate to the concentrations that we know directly, which are the total concentration of the enzyme, $[E_o]$, and the concentration of the substrate, [S]? When enzyme and substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so-called steady state level, as illustrated below



At this steady state, [ES] is nearly constant, so that

rate of ES breakdown $k_{-1}[ES] + k_{cat}[ES]$ = rate of ES formation $k_1[E][S]$

or, since the concentration of the free enzyme, [E], is equal to $[E_o] - [ES]$

$$[ES] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)[E][S] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)\left([E_o] - [ES]\right)[S]$$

Rearranging, and defining the constant K_m as

$$\frac{k_{-1} + k_{cat}}{k_1}$$

we get

$$[ES] = \frac{[E_o][S]}{K_m + [S]}$$

or, remembering that $V = k_{cat}[ES]$, we obtain the famous Michaelis-Menten equation

$$V = \frac{k_{cat}[E_o][S]}{K_m + [S]}$$

As [S] is increased to higher and higher levels, essentially all of the enzyme will be bound to substrate at steady state; at this point, a maximum rate of reaction, V_{max}, will be reached where $V = V_{max} = k_{cat}[E_o]$. Thus, it is convenient to rewrite the Michaelis-Menten equation as

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

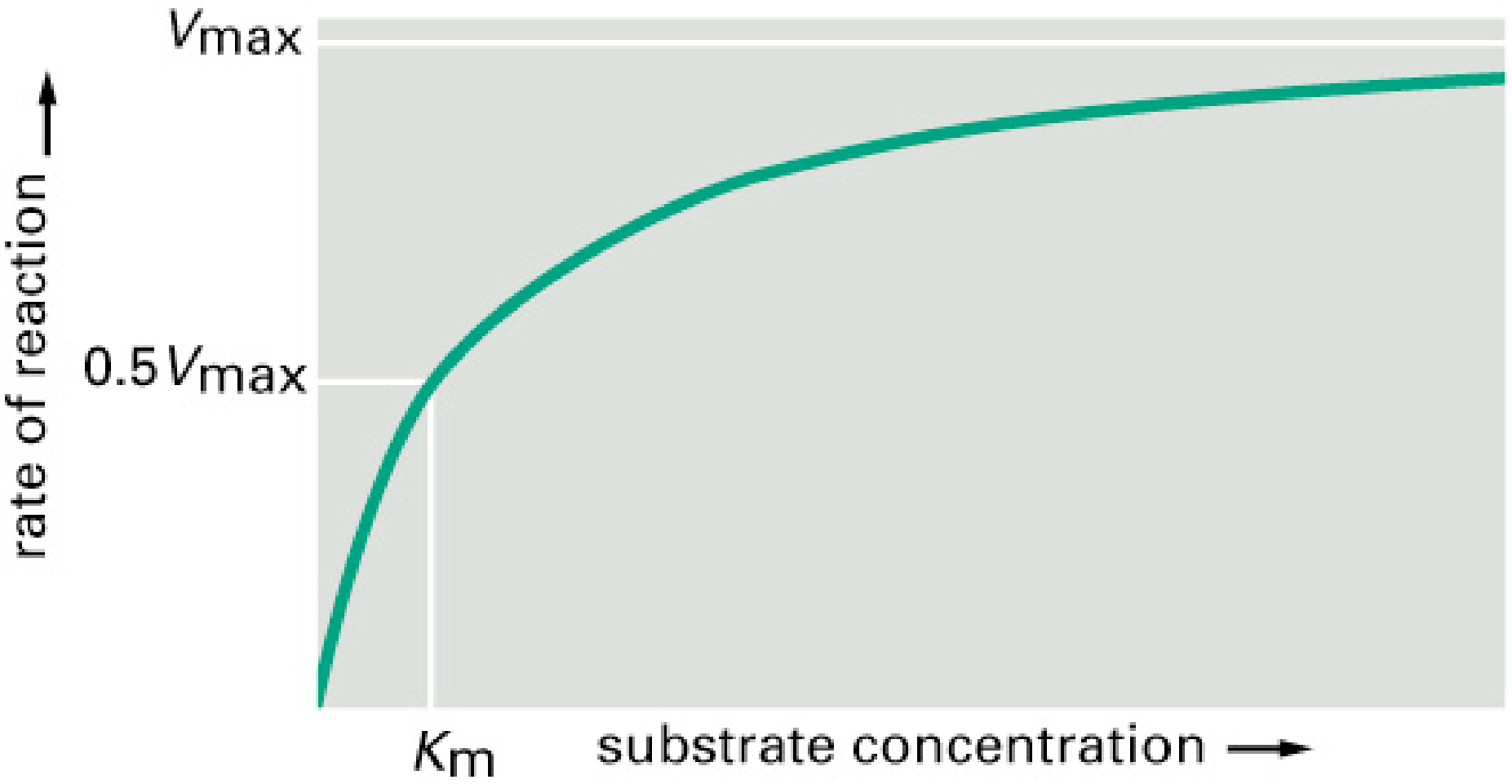
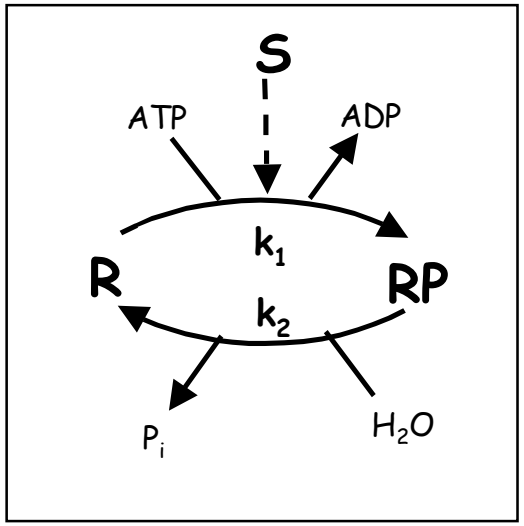


Figure 3–45. Molecular Biology of the Cell, 4th Edition.

Sigmoid signal-response curve (zero order ultrasensitivity)

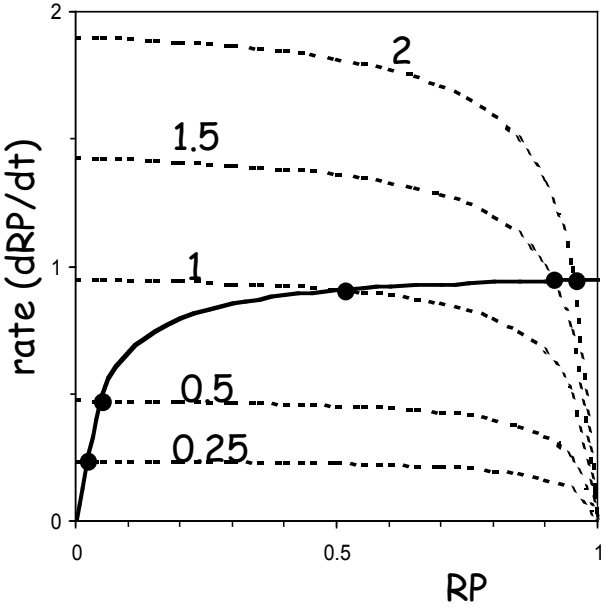


$$\frac{dR_P}{dt} = \underbrace{\frac{k_1 S(R_T - R_P)}{K_{m1} + R_T - R_P}}_{\text{phosphorylation}} - \underbrace{\frac{k_2 R_P}{K_{m2} + R_P}}_{\text{dephosphorylation}}$$

$k_1 = k_2 = 1$

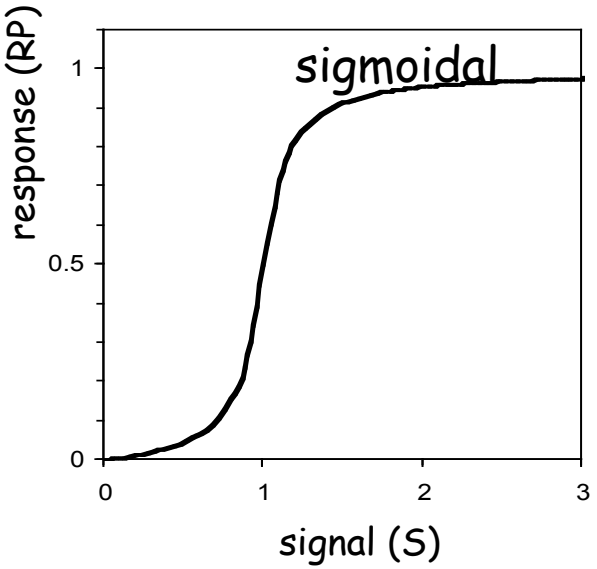
$R_T = 1$

$K_{m1} = K_{m2} = 0.05$



production

removal

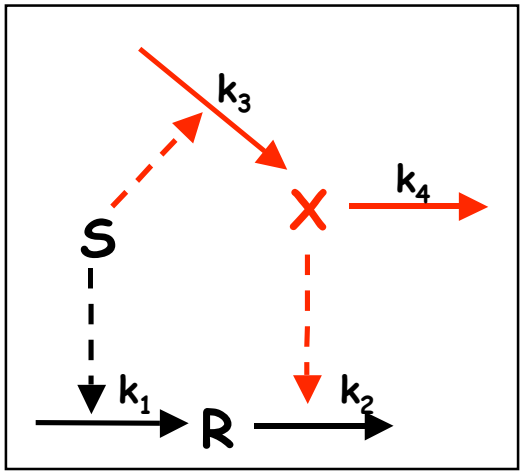


$$\frac{R_{P,ss}}{R_T} = G\left(k_1 S, k_2, \frac{K_{m1}}{R_T}, \frac{K_{m2}}{R_T}\right)$$

$$G(u,v,J,K) = \frac{2uK}{v-u+vJ+uK+\sqrt{(v-u+vJ+uK)^2 - 4(v-u)uK}}$$

Graded and reversible

Perfect adaptation

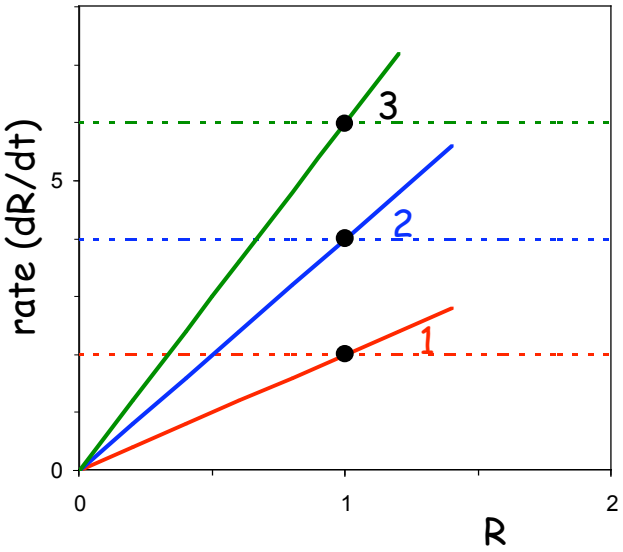


$$\frac{dR}{dt} = k_1 S - k_2 X R$$

$$\frac{dX}{dt} = k_3 S - k_4 X$$

$k_1 = k_2 = 2$

$k_3 = k_4 = 1$

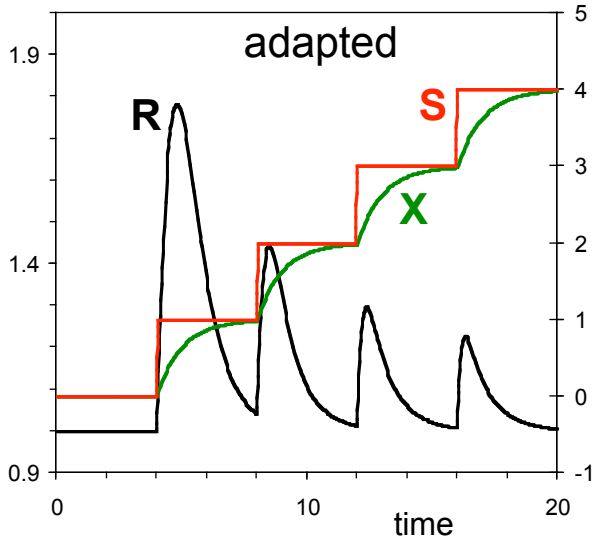


production

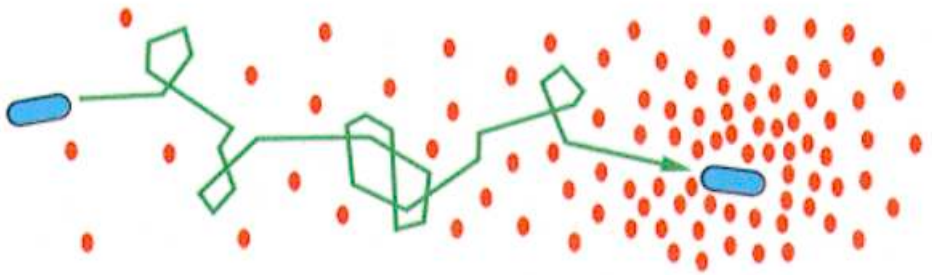
removal

$$R_{ss} = \frac{k_1 k_4}{k_2 k_3} \qquad X_{ss} = \frac{k_3 S}{k_4}$$

value of R is independent of S

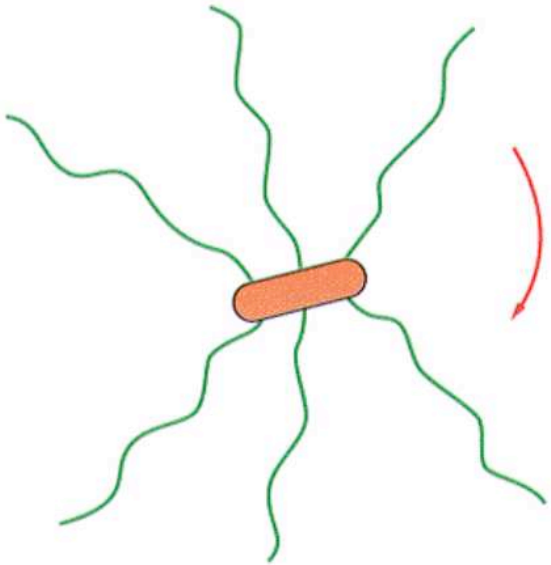


Bacterial chemotaxis



counterclockwise

swimming

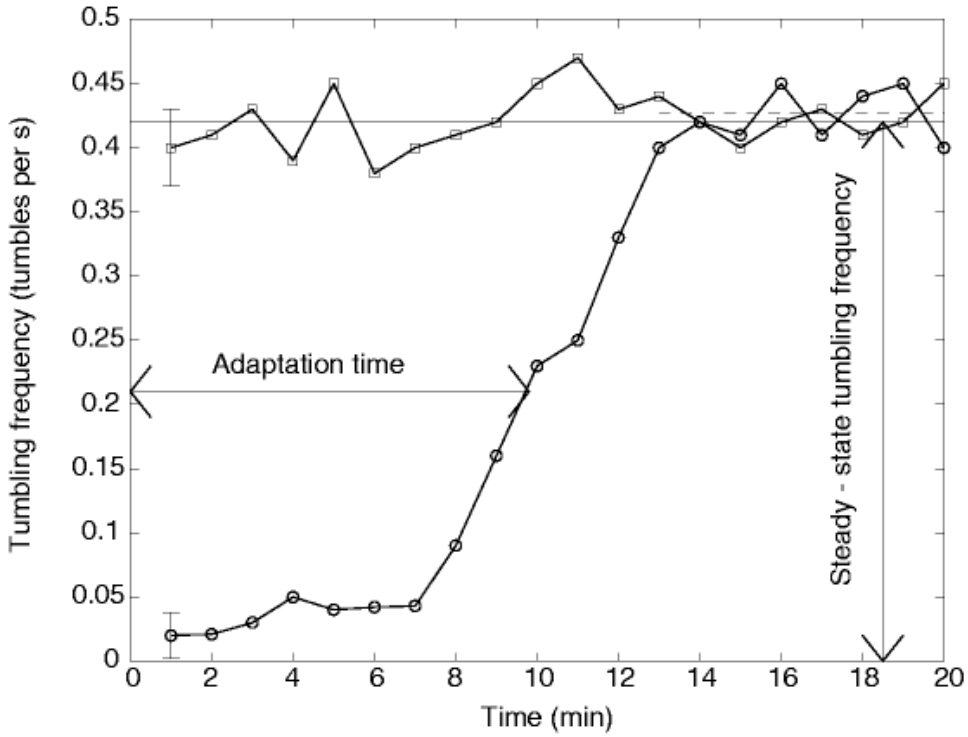


clockwise

tumbling

Robustness in bacterial chemotaxis

U. Alon*†, M. G. Surette‡, N. Barkai† & S. Leibler*†



cells stimulated with attractor (○)
control cells (□)

Biophysical Journal Volume 82 January 2002 50–63

Models of Eukaryotic Gradient Sensing: Application to Chemotaxis of Amoebae and Neutrophils

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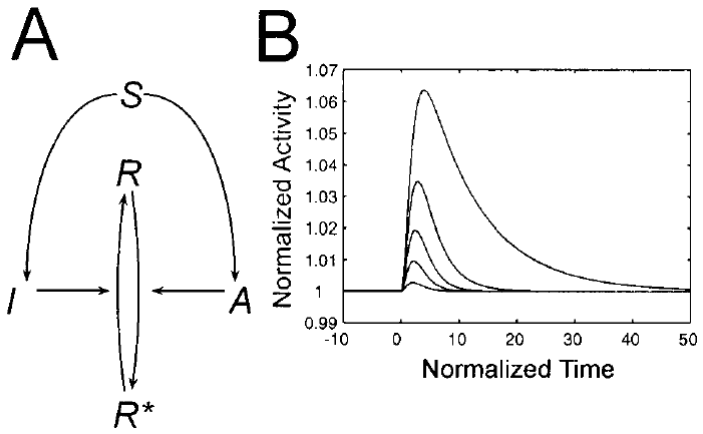
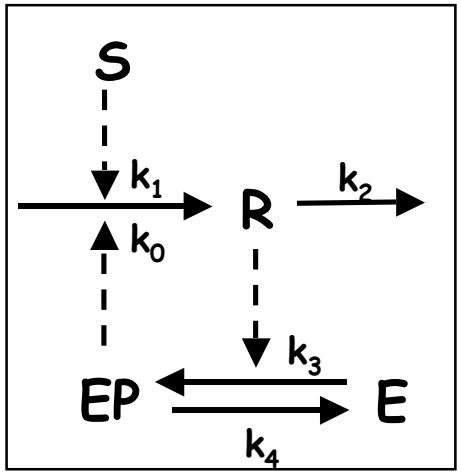
*Divisions of Biology, California Institute of Technology, Pasadena, California 91125 and †Department of Electrical and Computer Engineering, Johns Hopkins University, 105 Barton Hall, Baltimore, Maryland 21218 USA

ABSTRACT Eukaryotic cells can detect shallow gradients of chemoattractants with exquisite precision and respond quickly to changes in the gradient steepness and direction. Here, we describe a set of models explaining both adaptation to uniform increases in chemoattractant and persistent signaling in response to gradients. We demonstrate that one of these models can be mapped directly onto the biochemical signal-transduction pathways underlying gradient sensing in amoebae and neutrophils. According to this scheme, a locally acting activator (PI3-kinase) and a globally acting inactivator (PTEN or a similar phosphatase) are coordinately controlled by the G-protein activation. This signaling system adapts perfectly to spatially homogeneous changes in the chemoattractant. In chemoattractant gradients, an imbalance between the action of the activator and the inactivator results in a spatially oriented persistent signaling, amplified by a substrate supply-based positive feedback acting through small G-proteins. The amplification is activated only in a continuous presence of the external signal gradient, thus providing the mechanism for sensitivity to gradient alterations. Finally, based on this mapping, we make predictions concerning the dynamics of signaling. We propose that the underlying principles of perfect adaptation and substrate supply-based positive feedback will be found in the sensory systems of other chemotactic cell types.

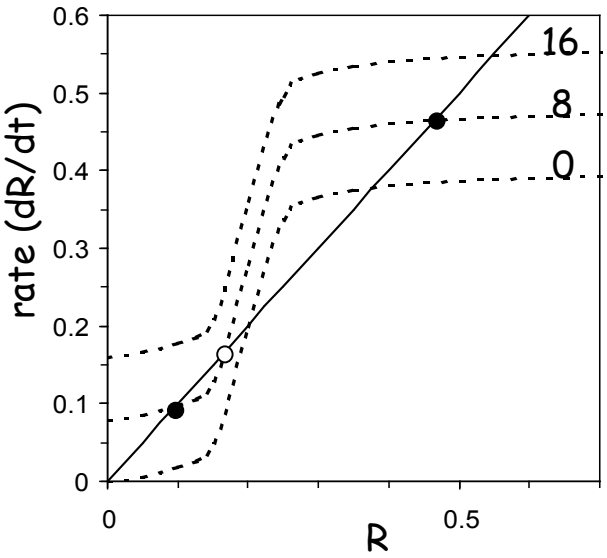


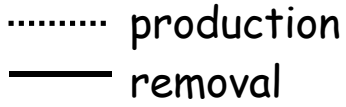
Feedback controls

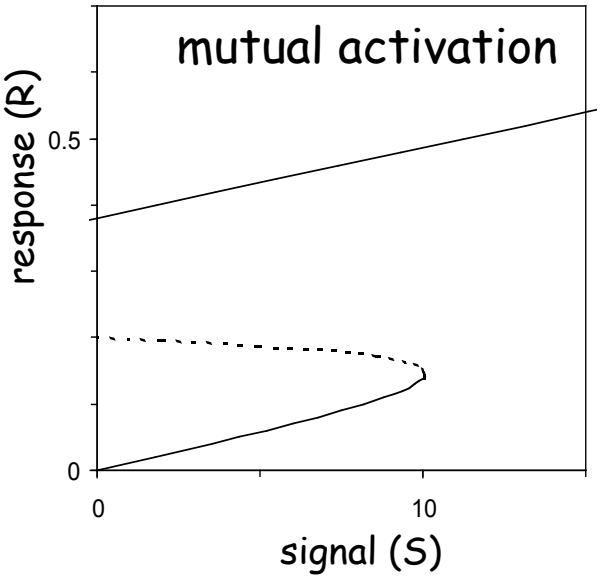
Positive feedback (mutual activation)



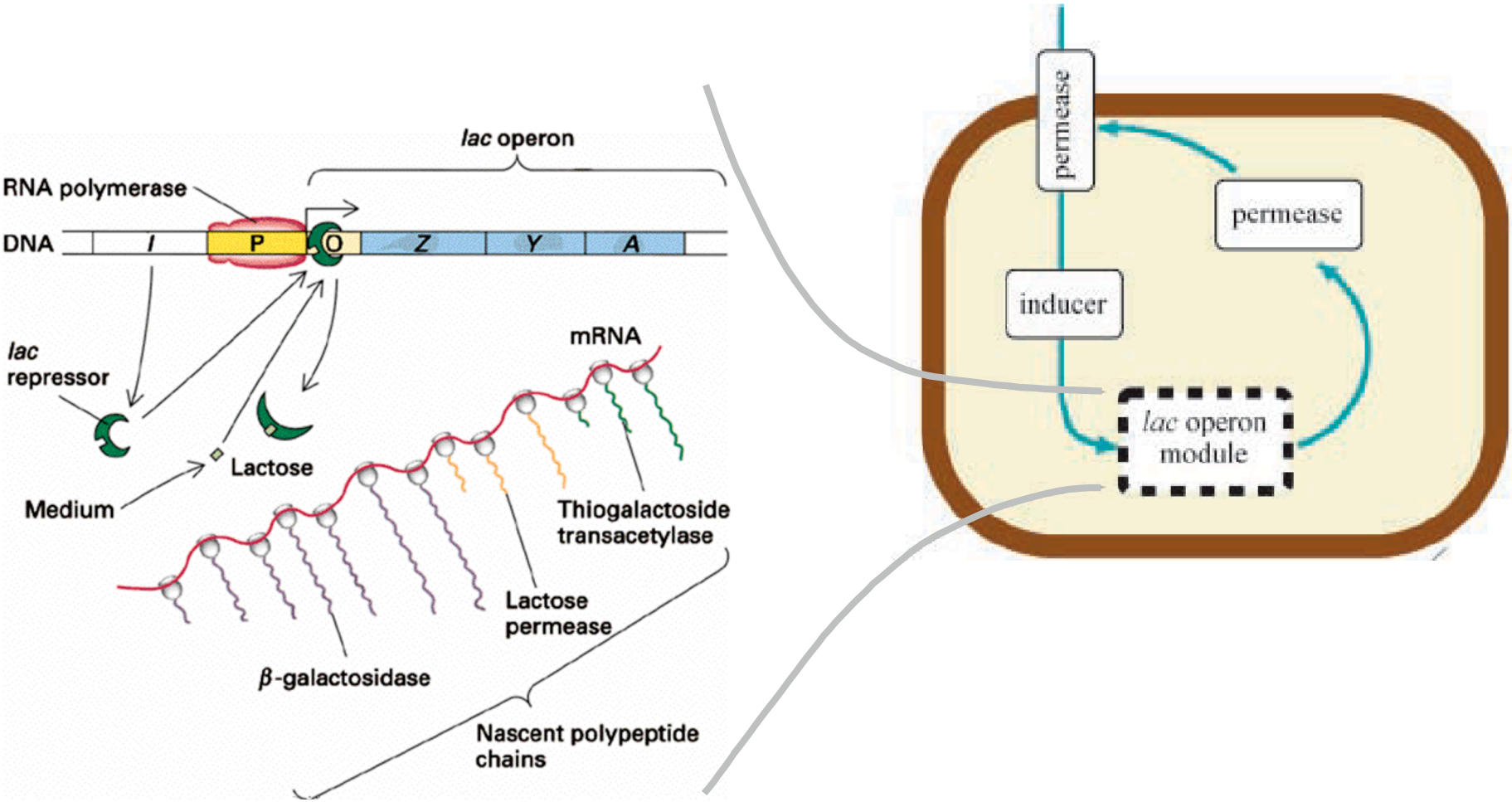
$k_0 = 0.4$
$k_1 = 0.01$
$k_2 = 1$
$k_3 = 1$
$k_4 = 0.2$
$J_3 = J_4 = 0.05$



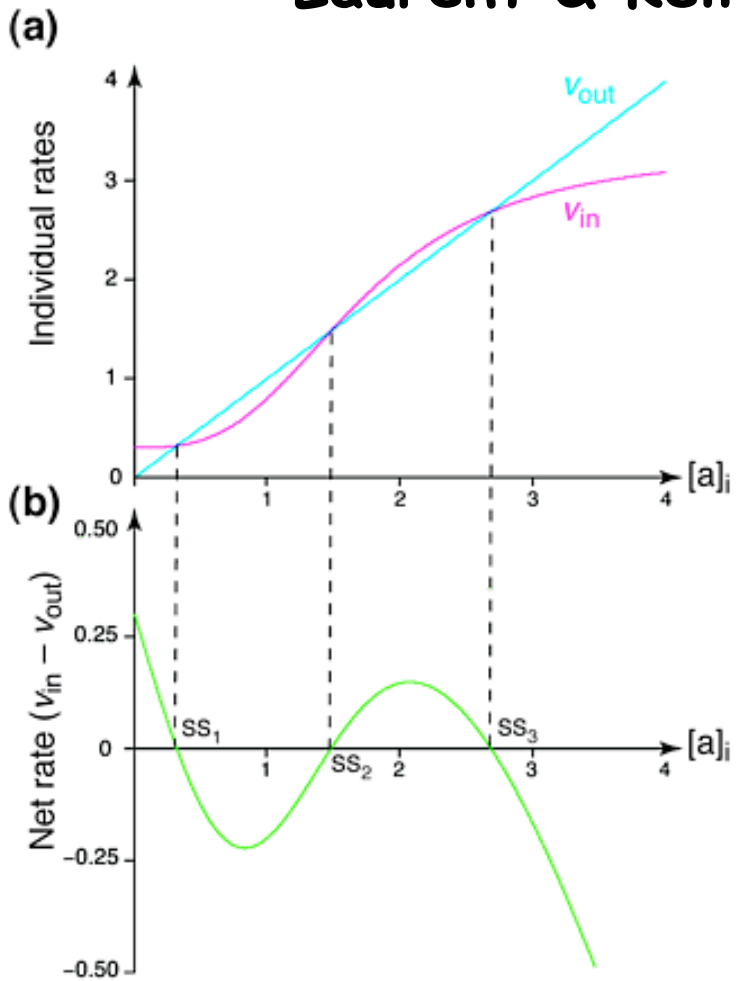


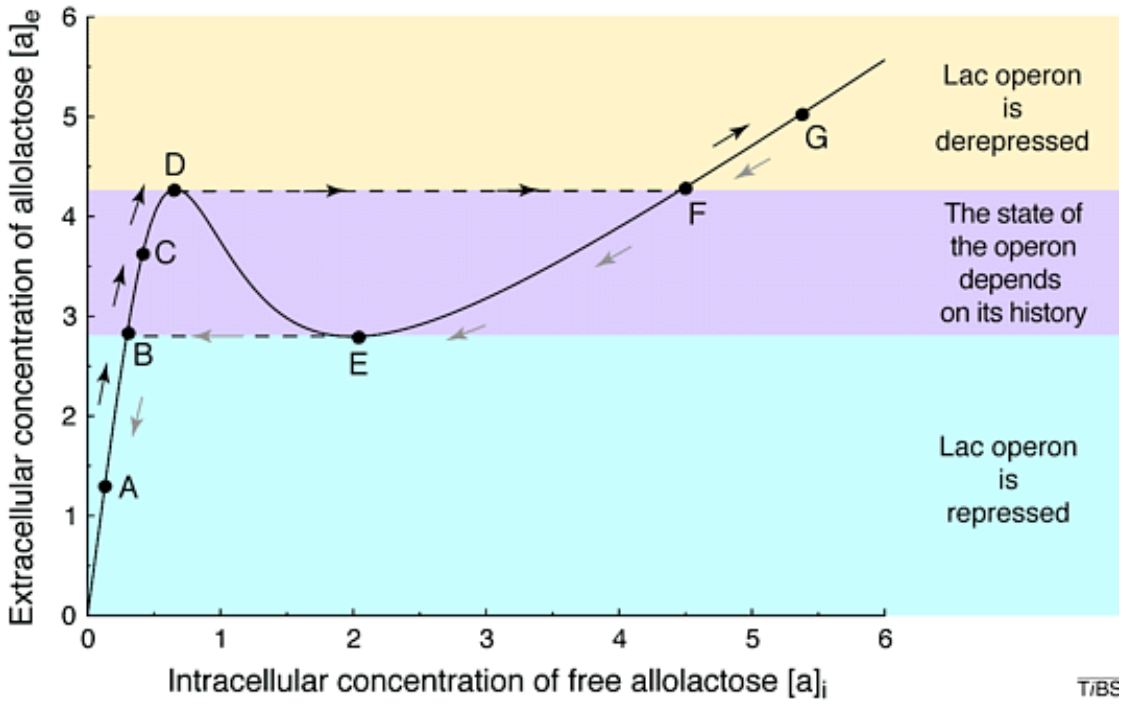


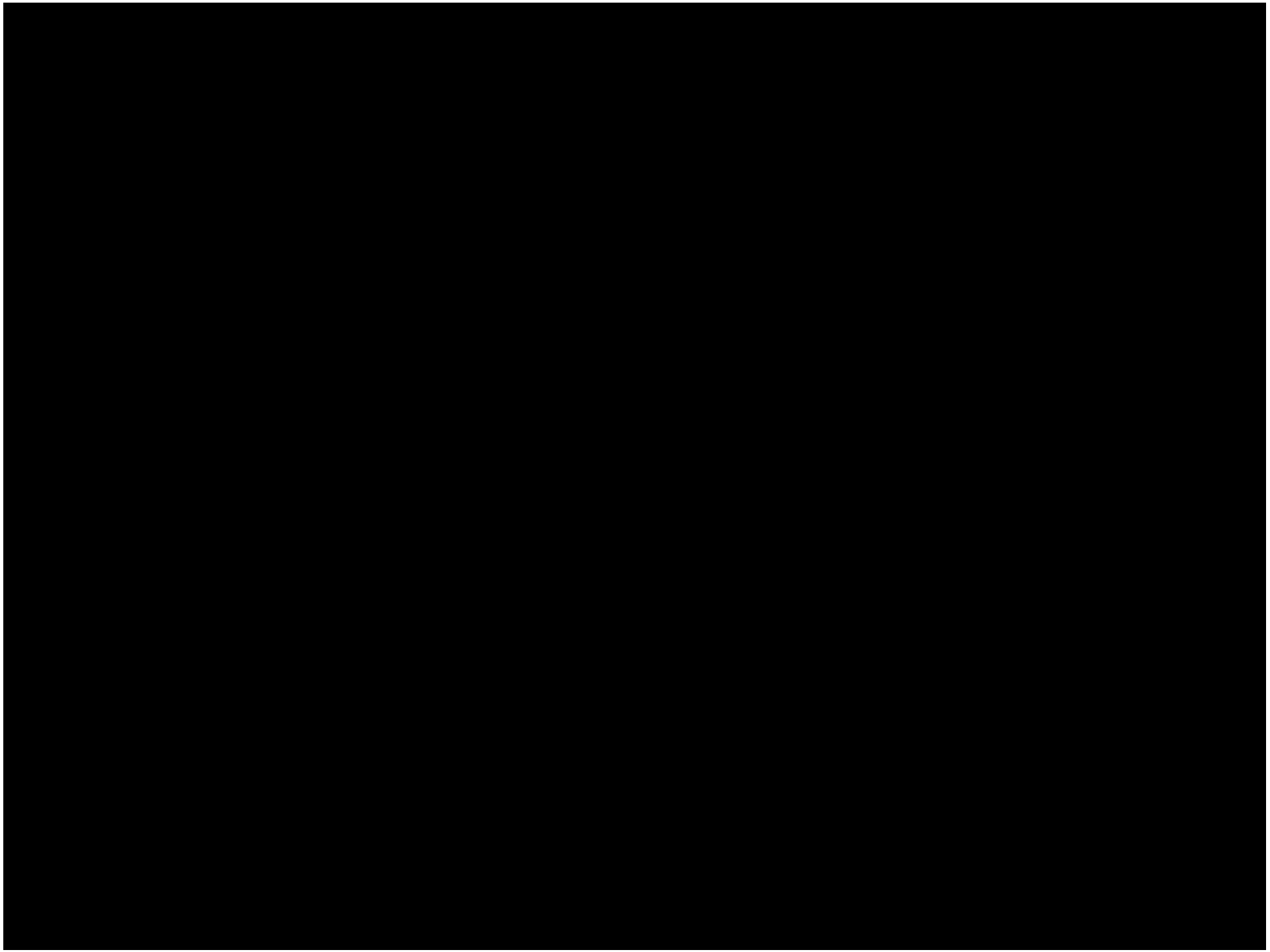
The *lac* operon



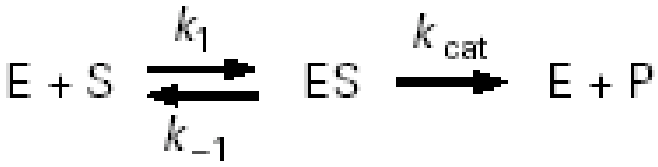
Laurent & Kellershohn (1999): TiBS







Michaelis-Menten enzyme kinetics



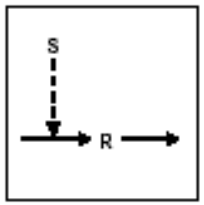
$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_{cat}[ES]$$

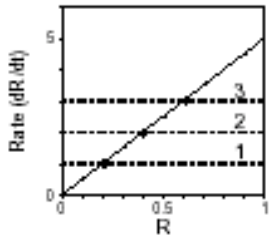
since $[E_o] = [E] + [ES]$

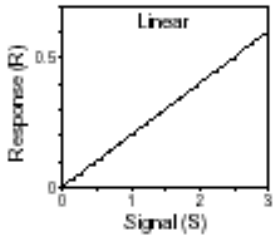
$$\frac{d[ES]}{dt} = k_1([E_o] - [ES])[S] - k_{-1}[ES] - k_{cat}[ES] = 0$$

$$[ES] = \frac{[E_o][S]}{\frac{k_{-1} + k_{cat}}{k_1} + [S]}$$

$$V = \frac{d[P]}{dt} = k_2[ES] = \frac{k_{cat}[E_o][S]}{\frac{k_{-1} + k_{cat}}{k_1} + [S]} = \frac{V_{\max}[S]}{K_M + [S]}$$

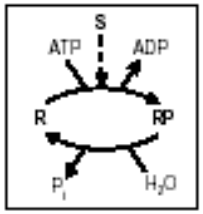


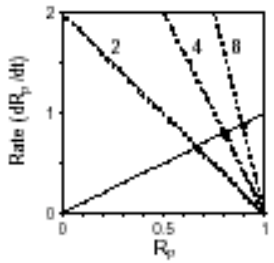


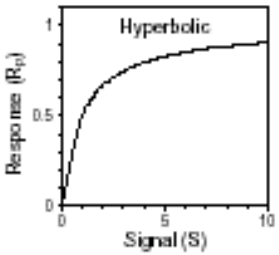


S = mRNA
R = protein

Phsophorylation / dephosporylation

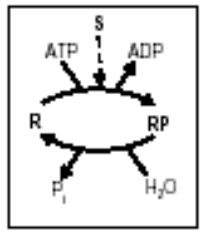


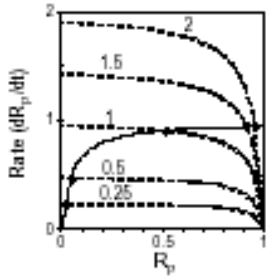


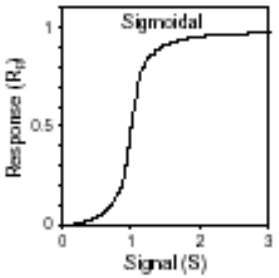


$$\frac{dR_P}{dt} = k_1 S(R_T - R_P) - k_2 R_P$$

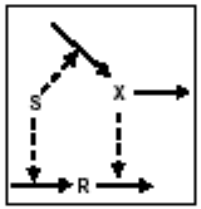
Buzzer:
Reversible switch

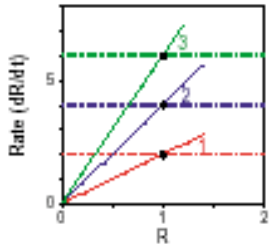


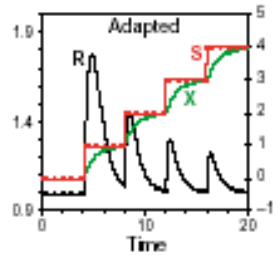




$$\frac{dR_P}{dt} = \frac{k_1 S(R_T - R_P)}{K_{m1} + R_T - R_P} - \frac{k_2 R_P}{k_{m2} + R_P}$$

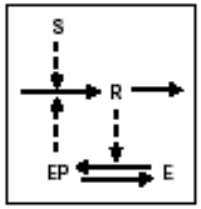


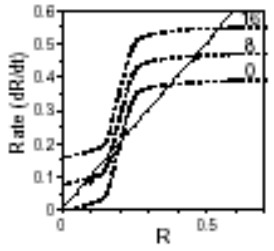


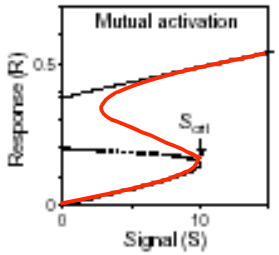


„sniffer":
bacterial
chemotaxis

Irreversible switch:
Toggle switch





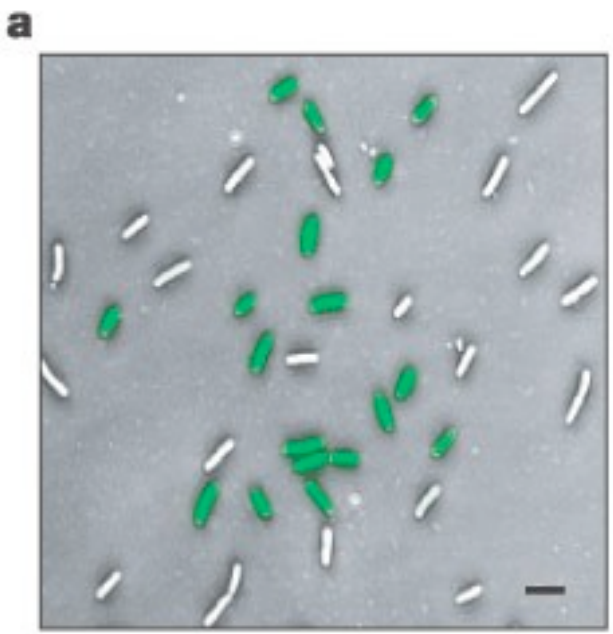


e.g. lac operon

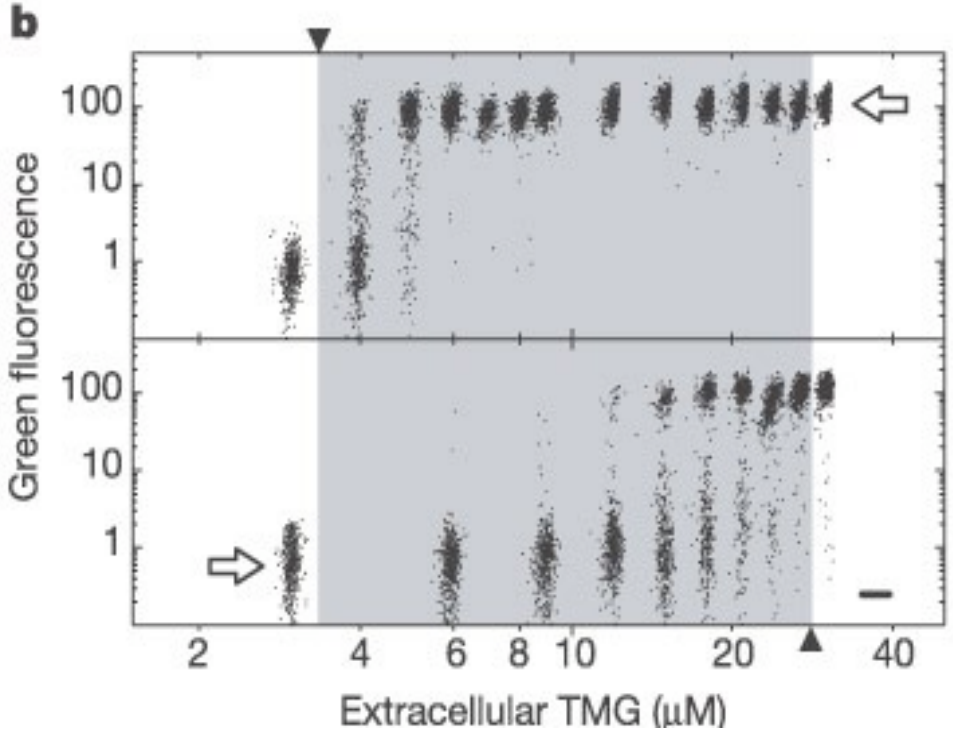
Multistability in the lactose utilization network of *Escherichia coli*

ERTUGRUL M. OZBUDAK[1,*], MUKUND THATTAI[1,*], HAN N. LIM[1], BORIS I. SHRAIMAN[2] & ALEXANDER VAN OUDENAARDEN[1]

Nature **427**, 737 - 740 (19 February 2004)



Initially uninduced cells grown for 20 hrs in 18 μM TMG

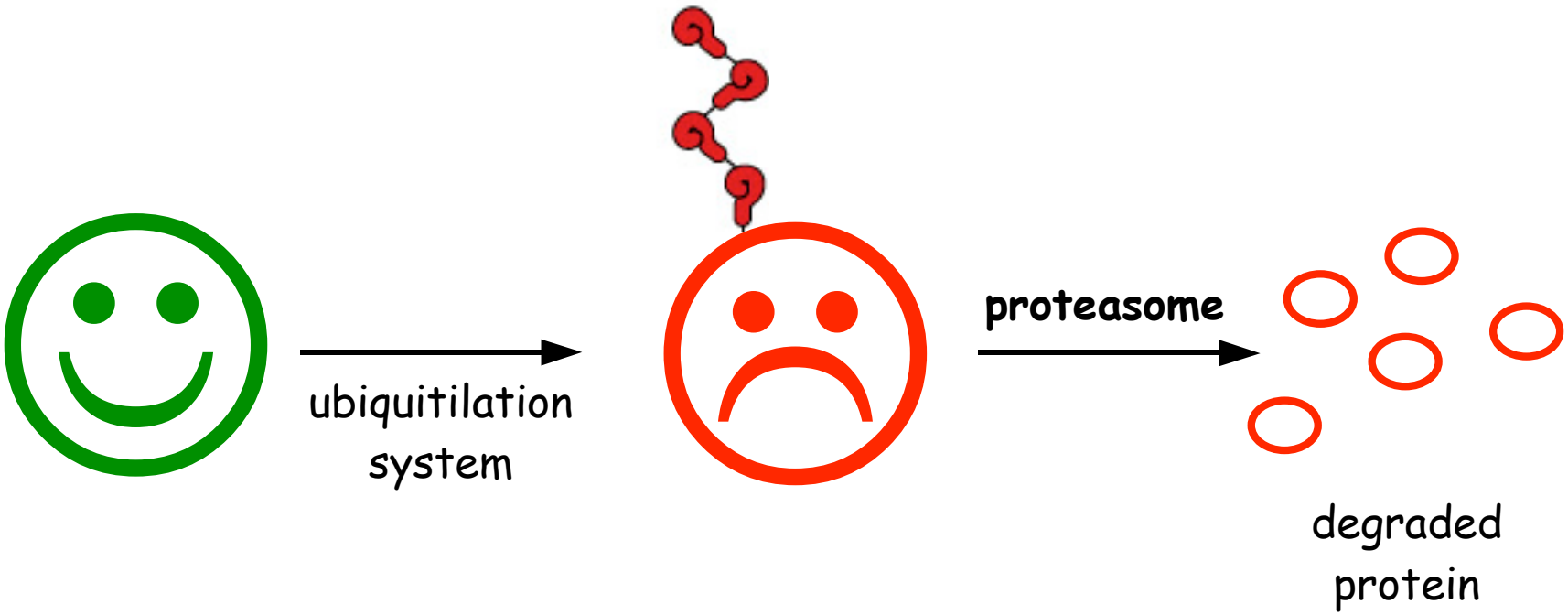


Initially uninduced cells (lower panel) and induced cells (upper panel) grown in media containing different concentration of TMG

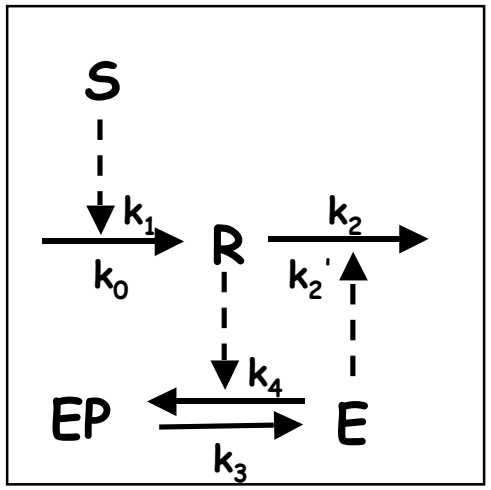
TMG = thio-methylgalactoside

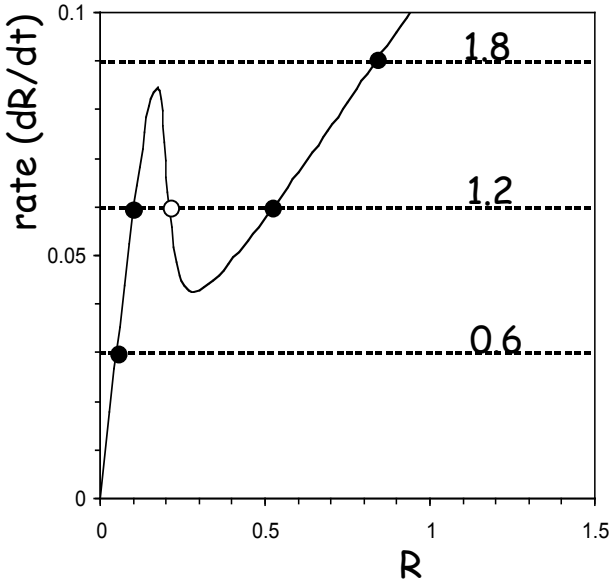
'Death control' for proteins

$$\frac{d\,[\text{protein}]}{dt} = \text{synthesis} - \text{degradation}$$



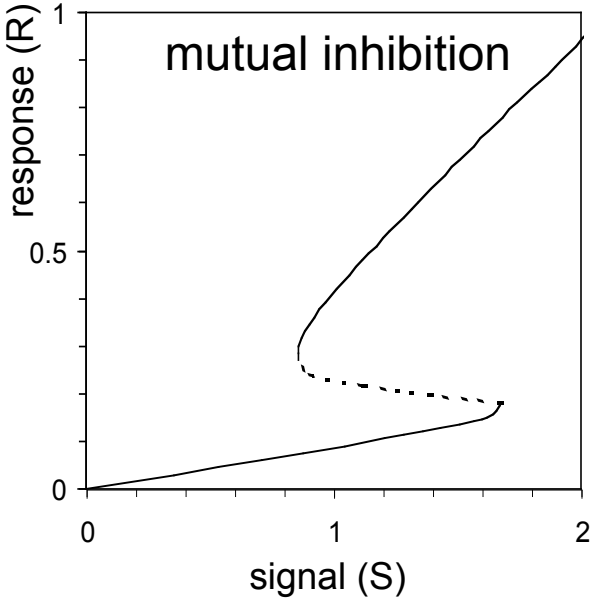
Positive feedback (antagonism)



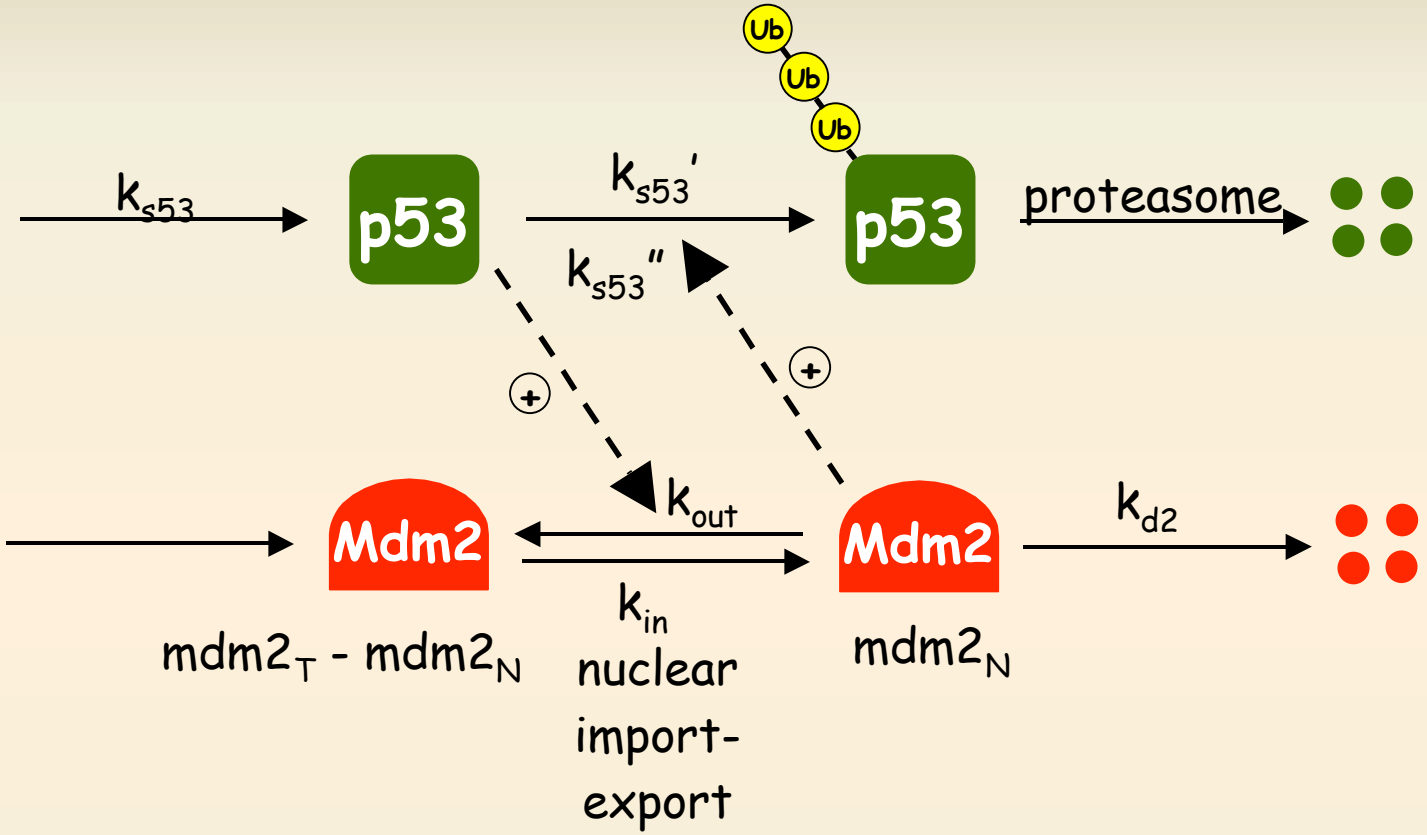


production
removal

$k_0 = 0$
$k_1 = 0.05$
$k_2 = 0.1$
$k_2' = 0.5$
$k_3 = 1$
$k_4 = 0.2$
$J_3 = J_4 = 0.05$



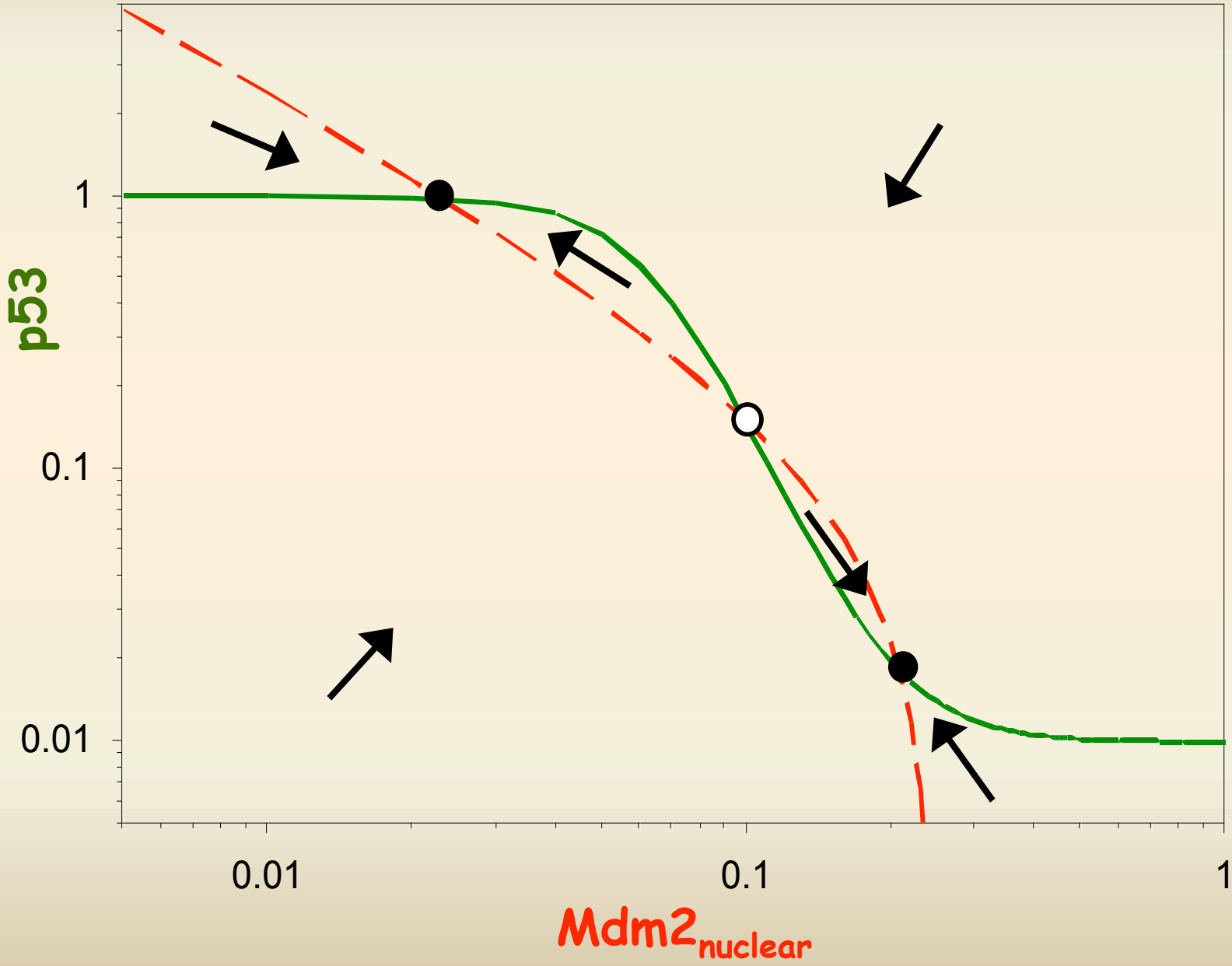
Antagonism (positive feedback) between p53 and Mdm2



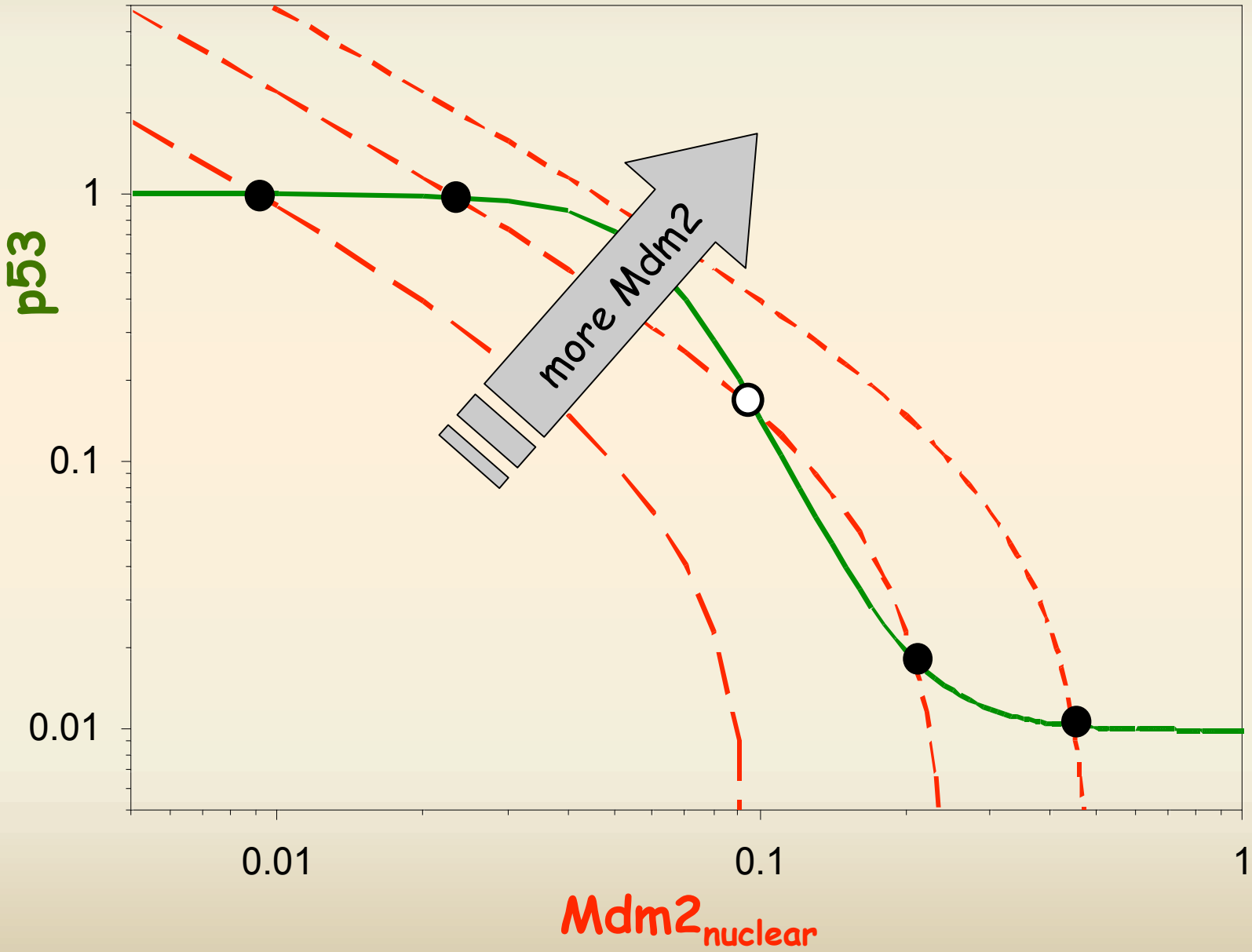
$$\frac{dp53}{dt} = k_{s53} - (k_{d53}' + k_{d53}'' \frac{mdm2_N{}^Z}{J^Z + mdm2_N{}^Z}) p53$$

$$\frac{dmdm2_N}{dt} = k_{in}(mdm2_T - mdm2_N) - k_{out} \cdot p53 \cdot mdm2_N - k_{d2} \cdot mdm2_N$$

The antagonism on the PhasePlane



The number of steady states depends on total Mdm2 level



Another view of antagonism (bifurcation diagam)

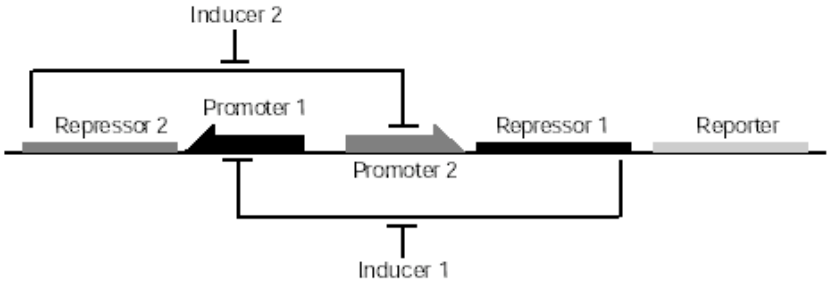
Another example for antagonism:

'Artificial' genetic networks

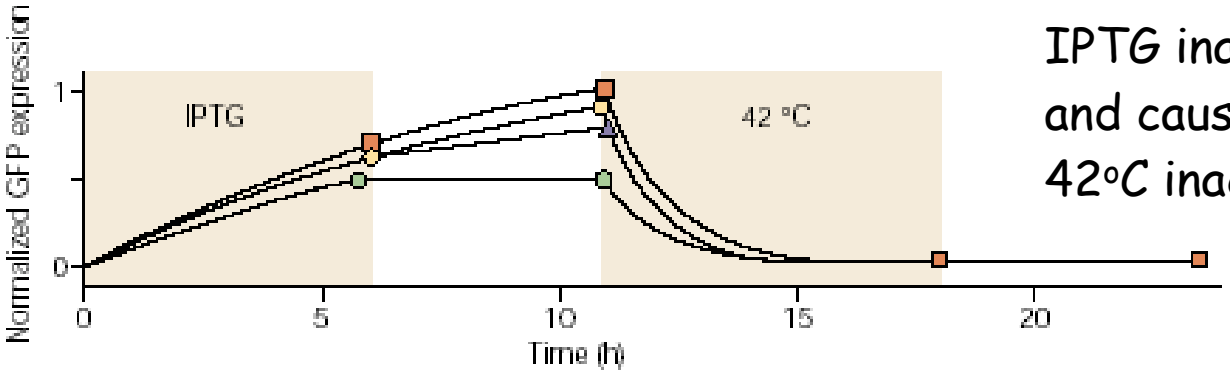
Construction of a genetic toggle switch in *Escherichia coli*

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

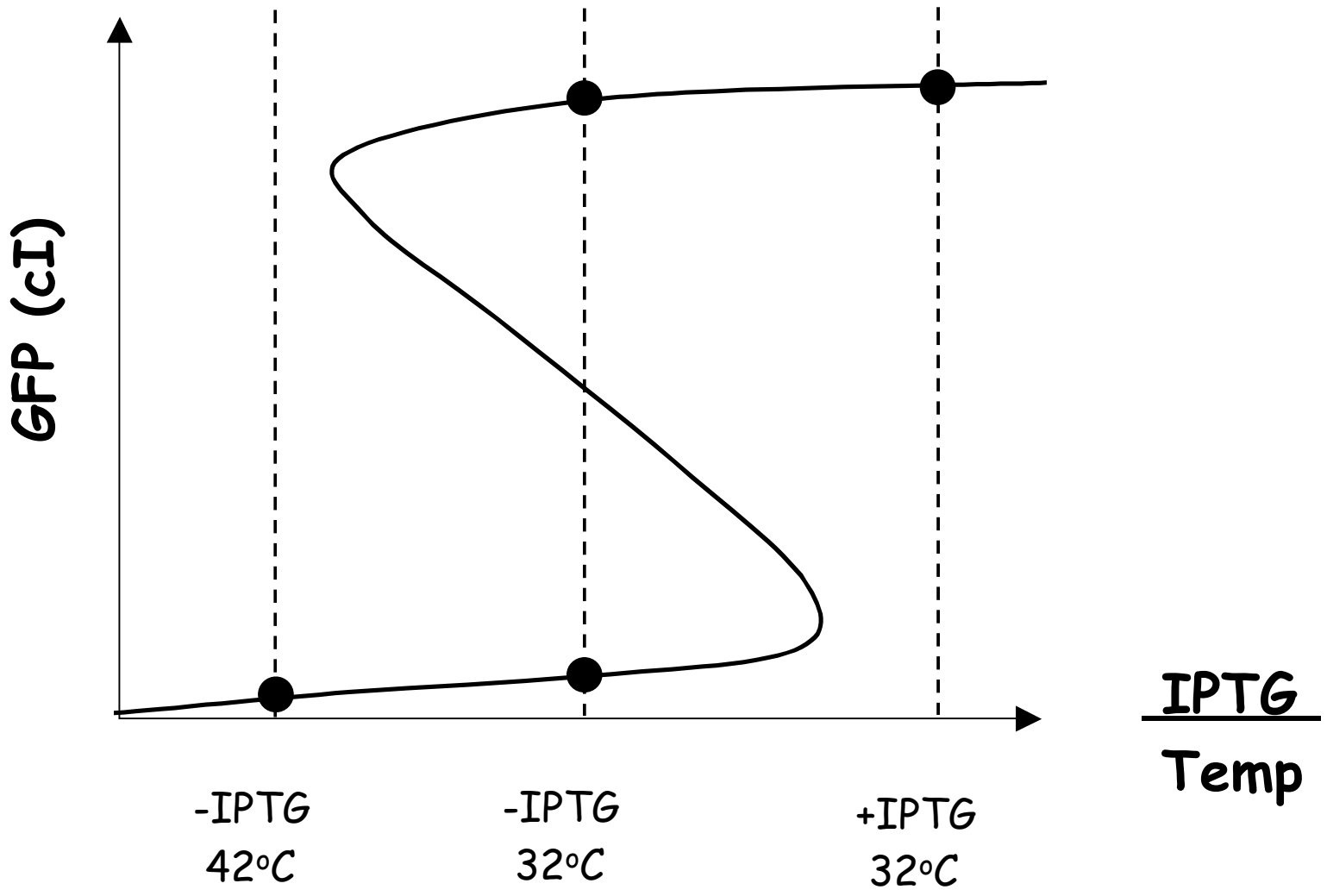
** Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*



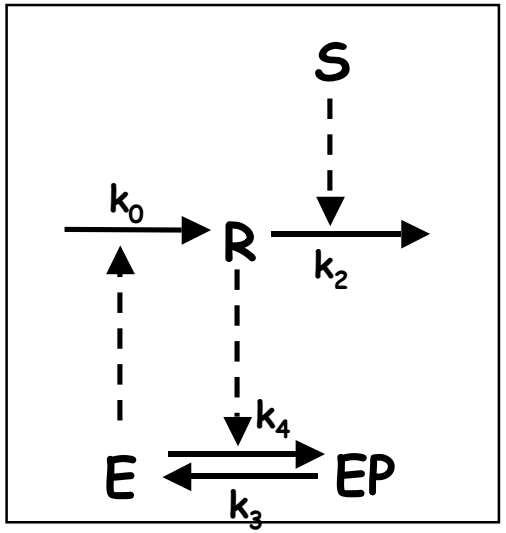
lacI & cI repressors
GFP behind the cI gene
(GFP is proportional to cI expression)



IPTG inactivates lacI
and causes cI expression
42°C inactivates the cI repressor

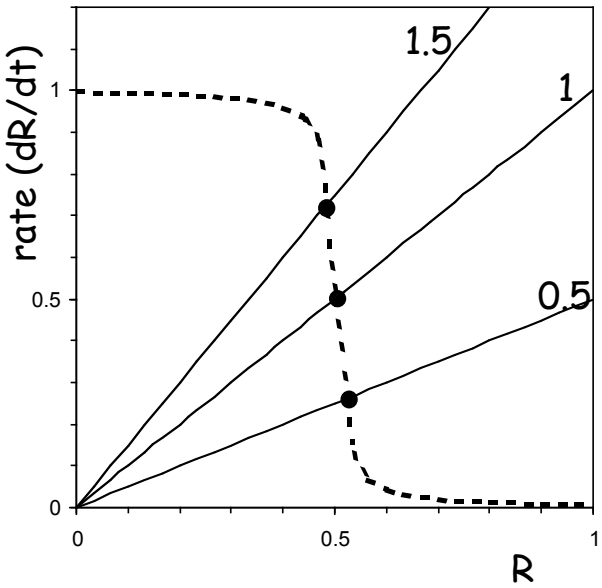

GFP (cI)
IPTG
Temp
-IPTG
42°C
-IPTG
32°C
+IPTG
32°C

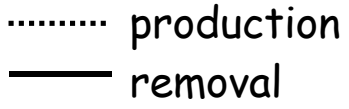
Negative feedback and homeostasis

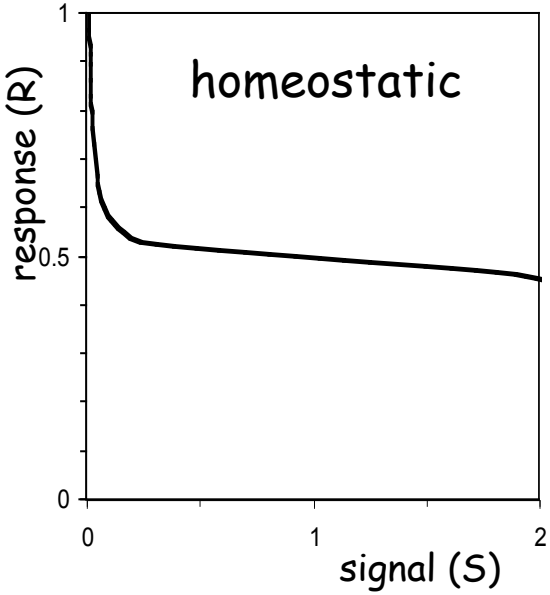


$$E(R) = G(k_3, k_4 R, J_3, J_4)$$

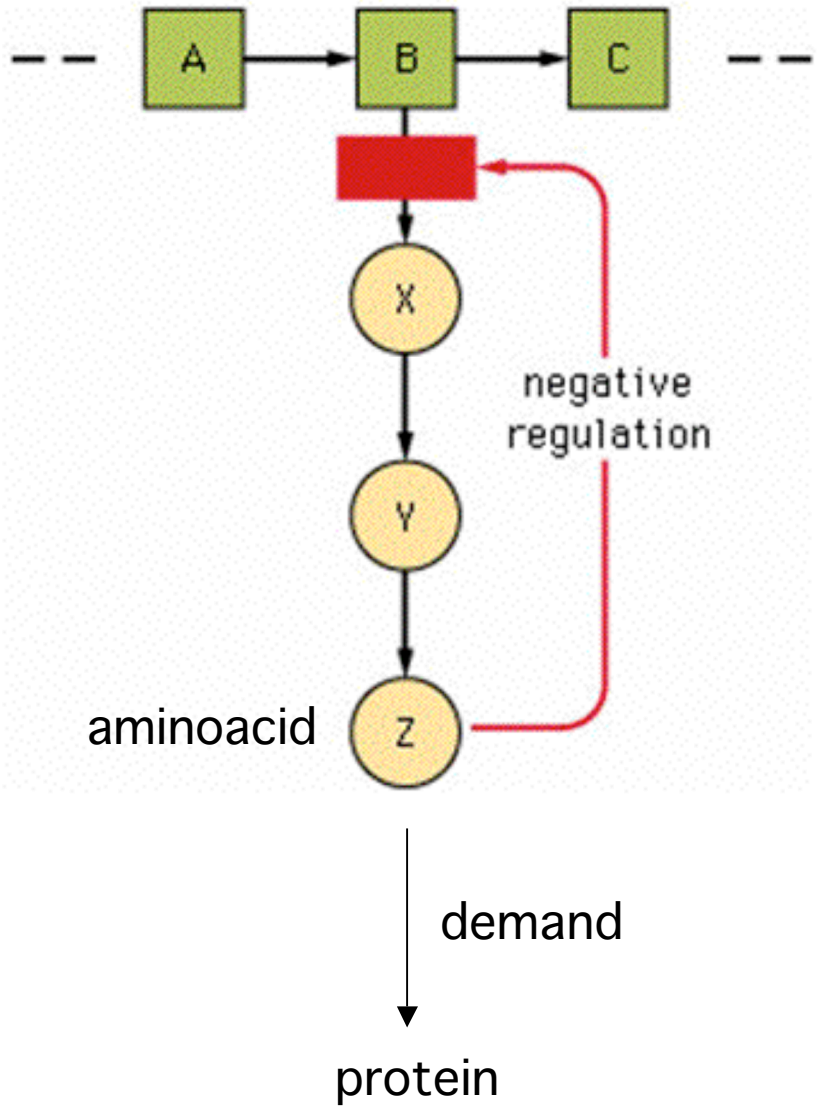
k_0=1
k_2=1
k_3=0.5
k_4=1
J_3=J_4=0.01







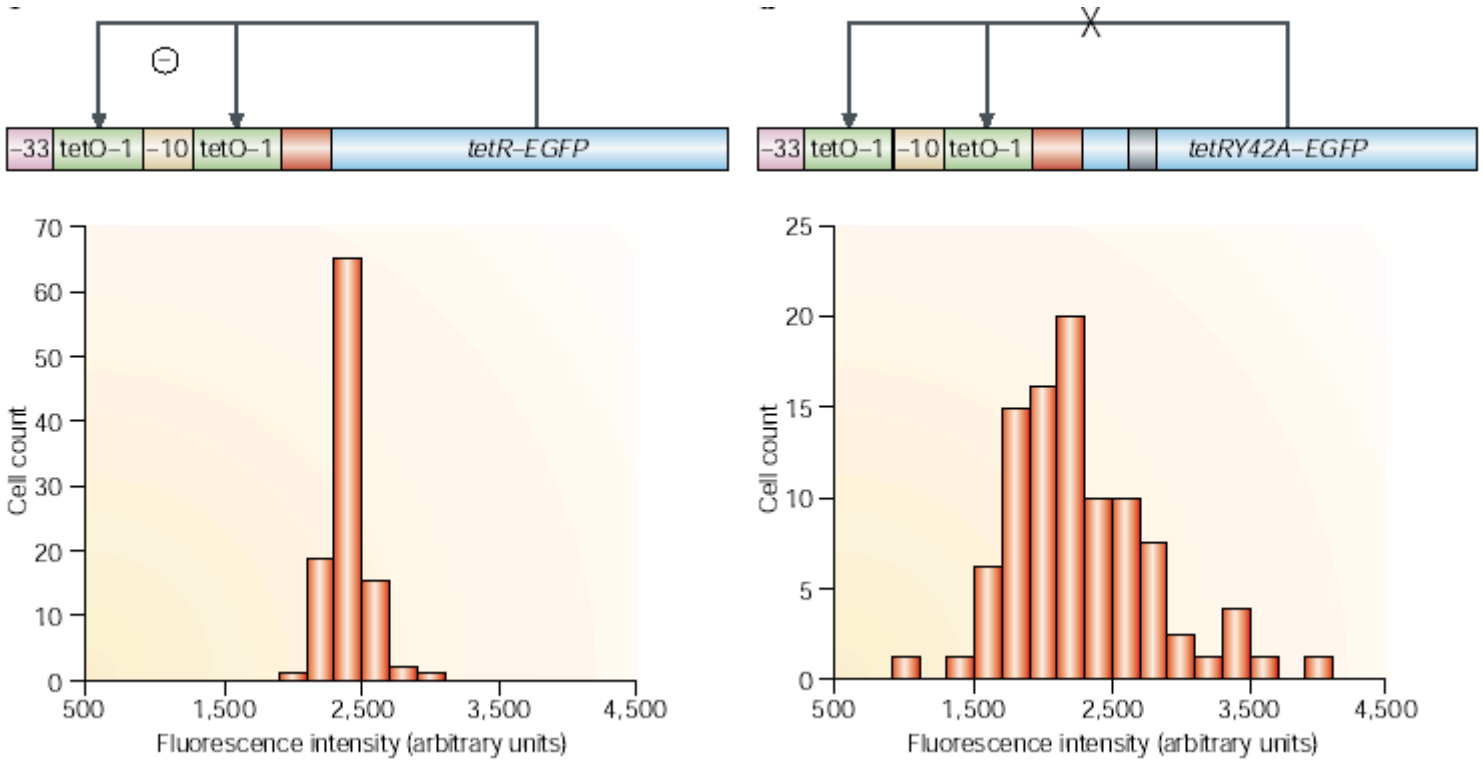
Typical biosynthetic pathway



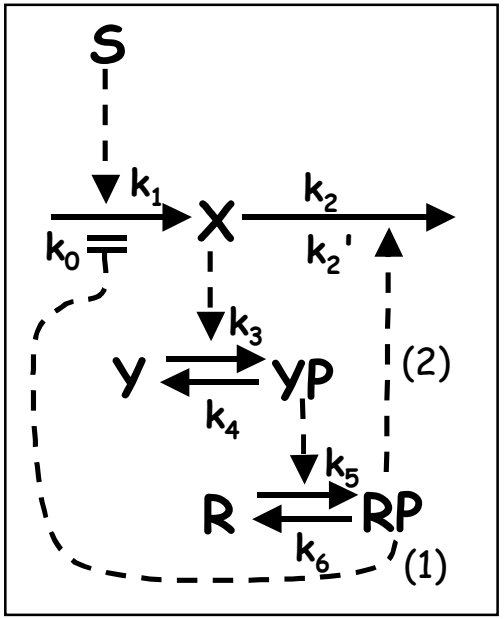
Engineering stability in gene networks by autoregulation

Attila Becskei & Luis Serrano

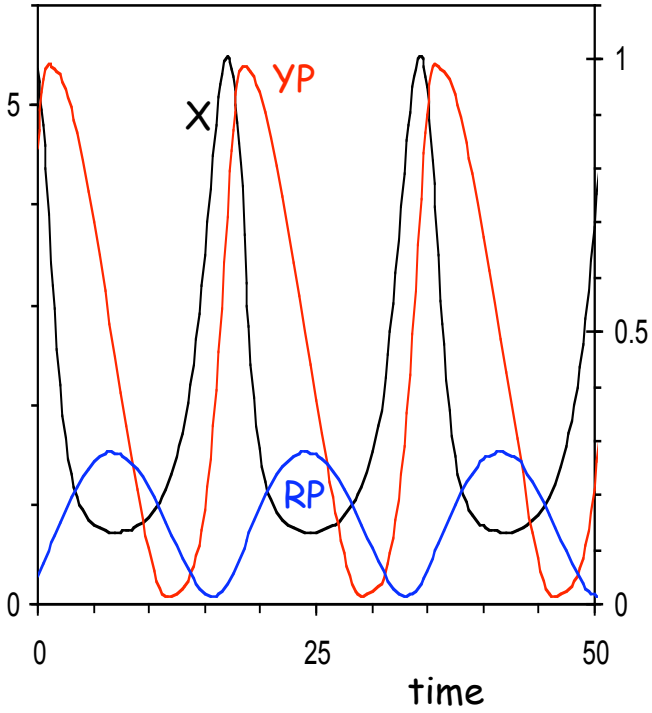
EMBL, Structures & Biocomputing, Meyerhofstrasse 1, Heidelberg D-69012, Germany

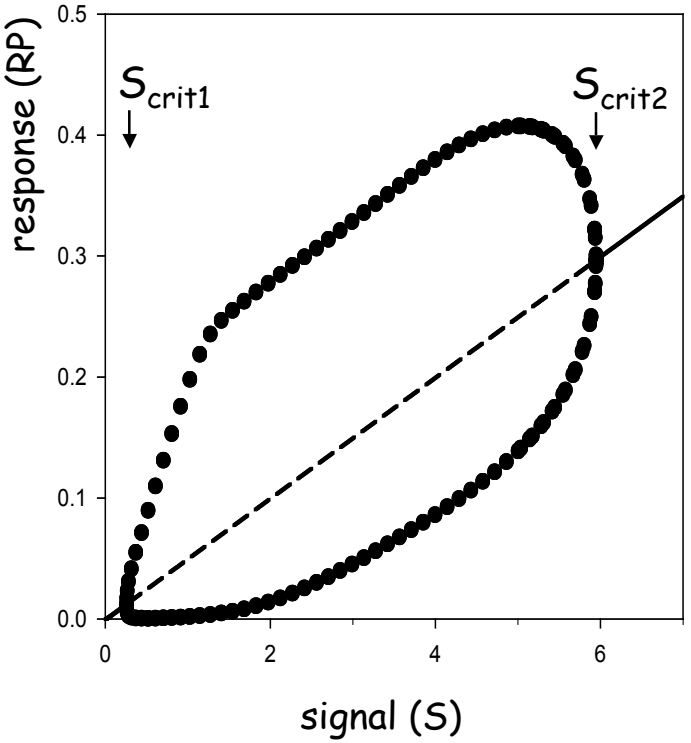


Negative feedback and oscillation



$k_0 = 0$
$k_1 = 1$
$k_2' = 10$
$k_2 = 0.01$
$k_3 = 0.1$
$k_4 = 0.2$
$k_5 = 0.1$
$k_6 = 0.05$
$Y_T = 1$
$R_T = 1$
$K_{m3} = K_{m4} = K_{m5} = K_{m6} = 0.01$

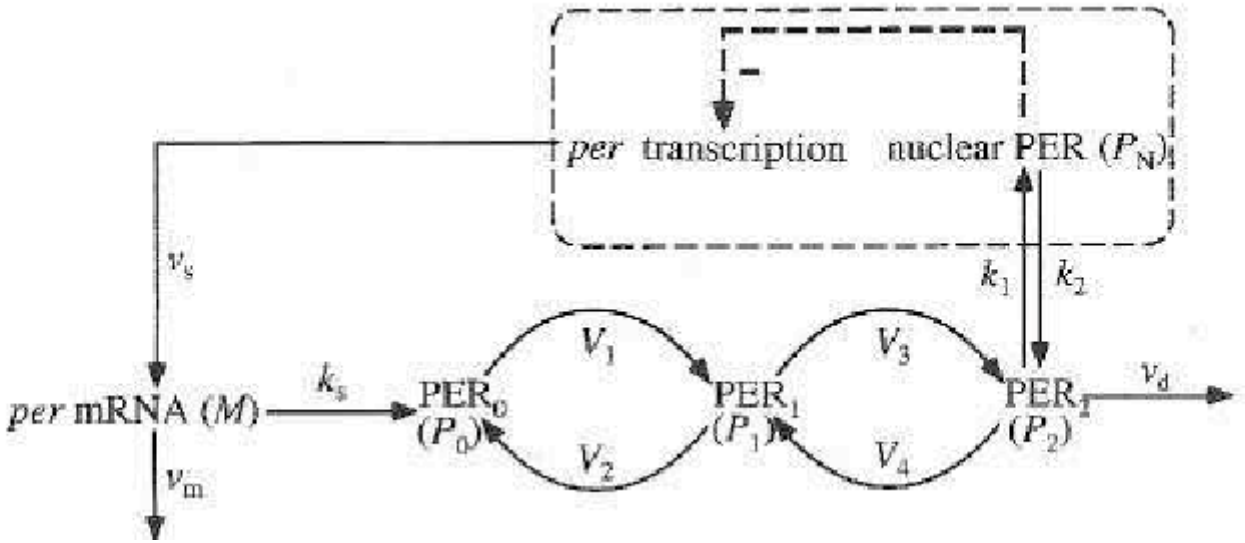




A model for circadian oscillations in the *Drosophila* period protein (PER)

ALBERT GOLDBETER

Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C. P. 231, B-1050 Brussels, Belgium



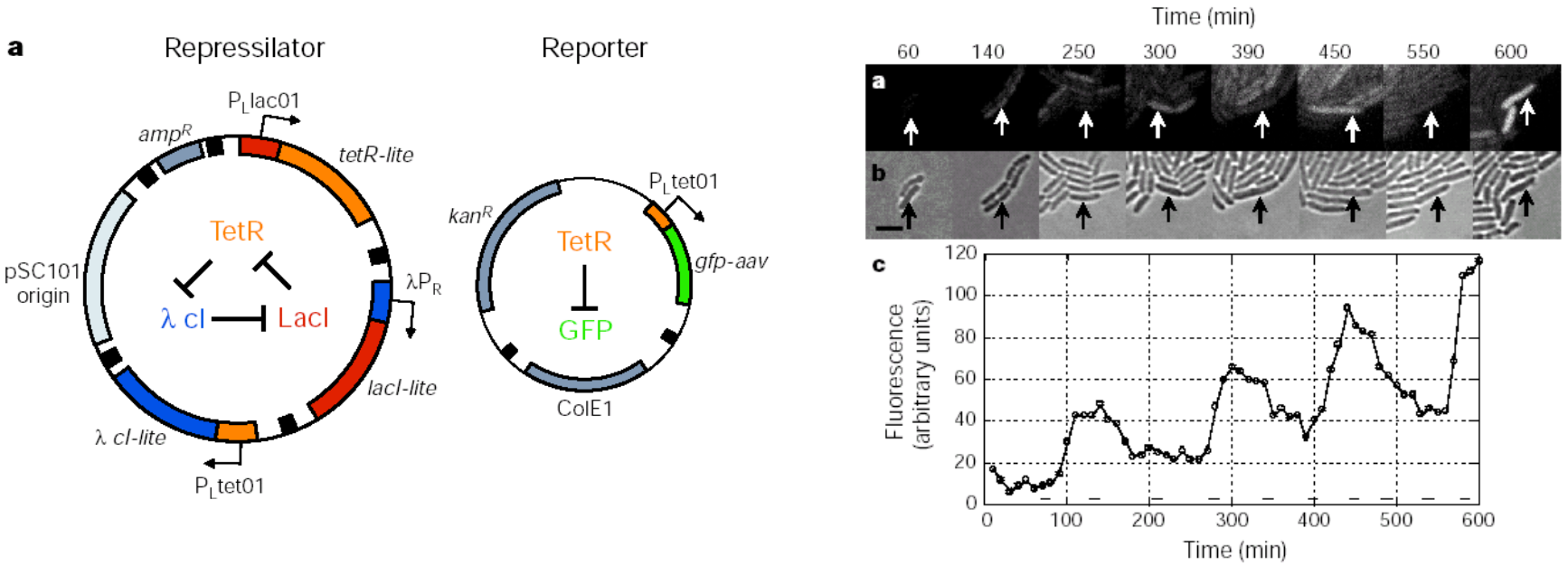
Proc. R. Soc. Lond. B (1995) 261, 319–324
Printed in Great Britain

The repressilator

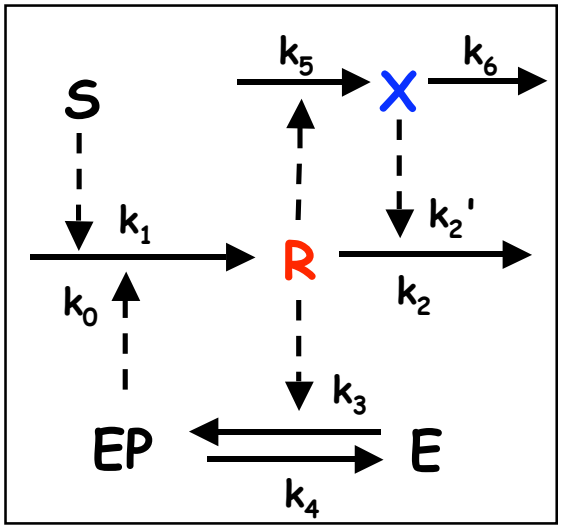
A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler

Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA

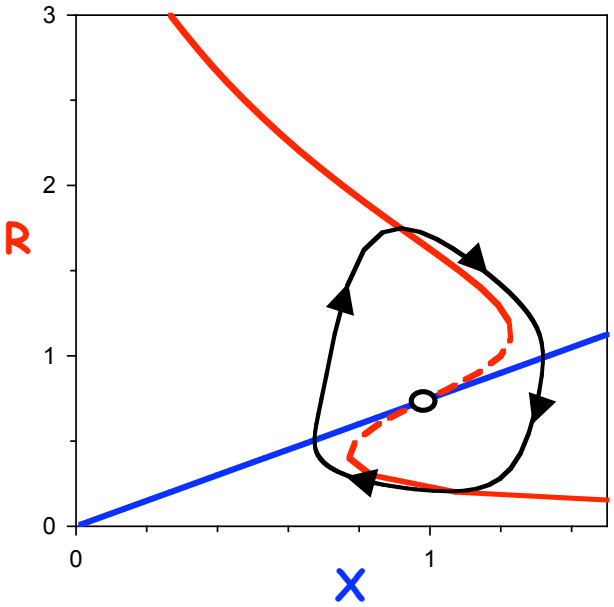


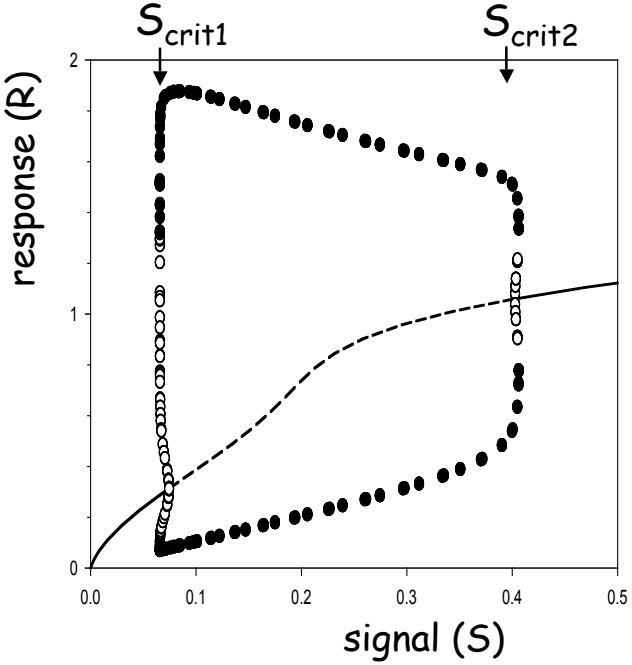
Positive and negative feedback oscillations (activator-inhibitor)



$k_0 = 4$
$k_1 = 0.2$
$k_2 = 1$
$k_2' = 1$
$k_3 = 1$
$k_4 = 1$
$k_5 = 0.1$
$k_6 = 0.075$
$J_3 = J_4 = 0.3$

PhasePlane





LETTERS

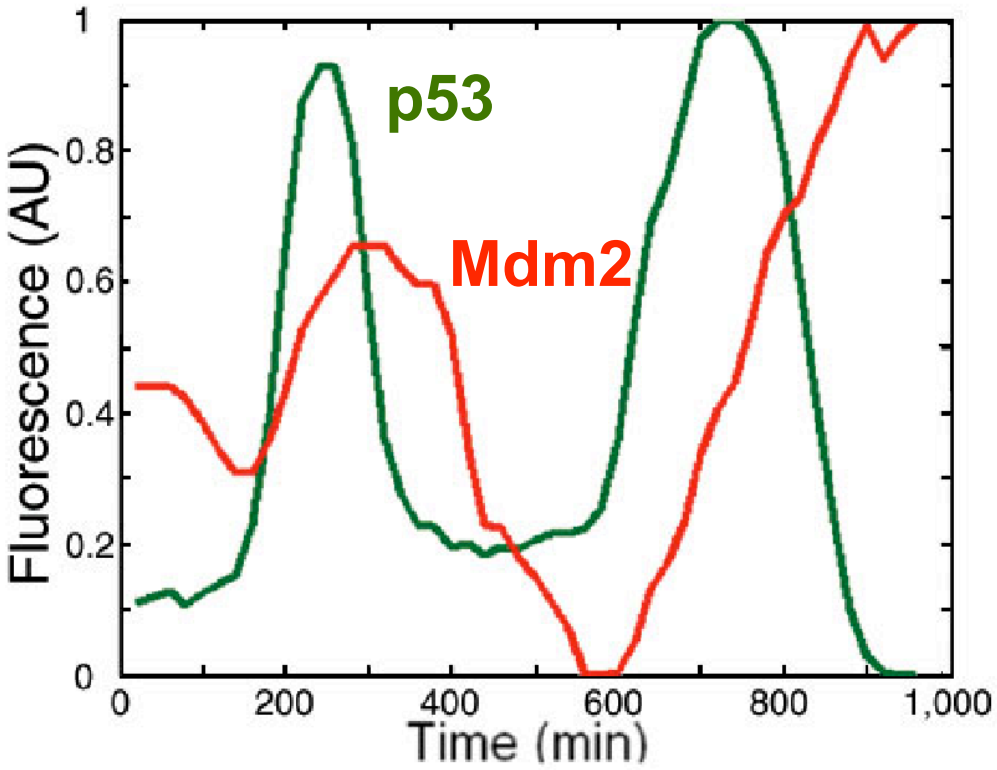
nature genetics

Dynamics of the p53-Mdm2 feedback loop in individual cells

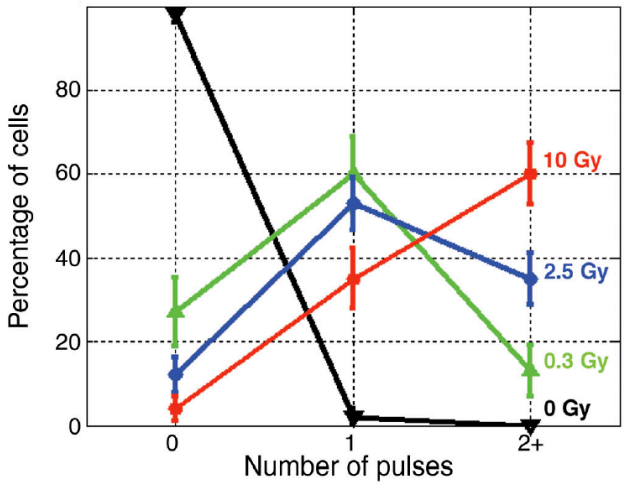
Galit Lahav[1], Nitzan Rosenfeld[1], Alex Sigal[1], Naama Geva-Zatorsky[1], Arnold J Levine[2], Michael B Elowitz[3] & Uri Alon[1]

NATURE GENETICS VOLUME 36 | NUMBER 2 | FEBRUARY 2004 147

p53-CFP and Mdm2-YFP levels in the nucleus after γ-irradiation



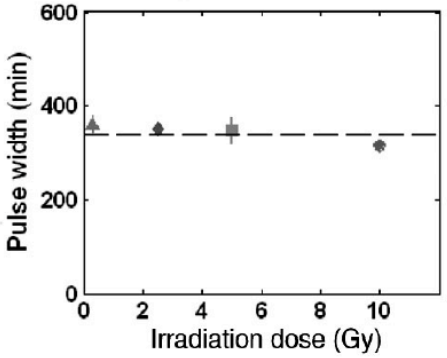
Period of oscillation: 440 ± 100 min

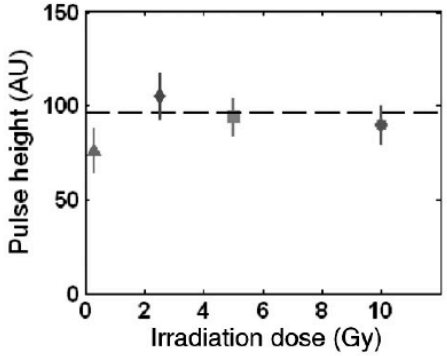


The fraction of cells with more pulses increased with irradiation dose

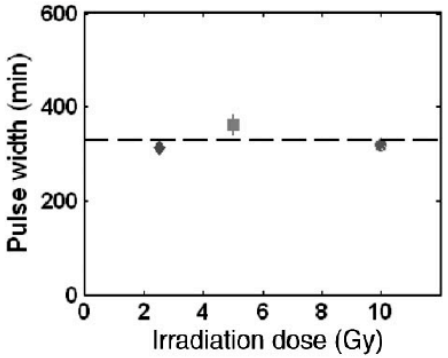
the mean height and width of each pulse did not depend on irradiation dose

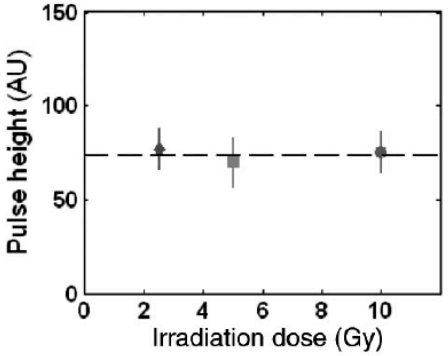
First pulse



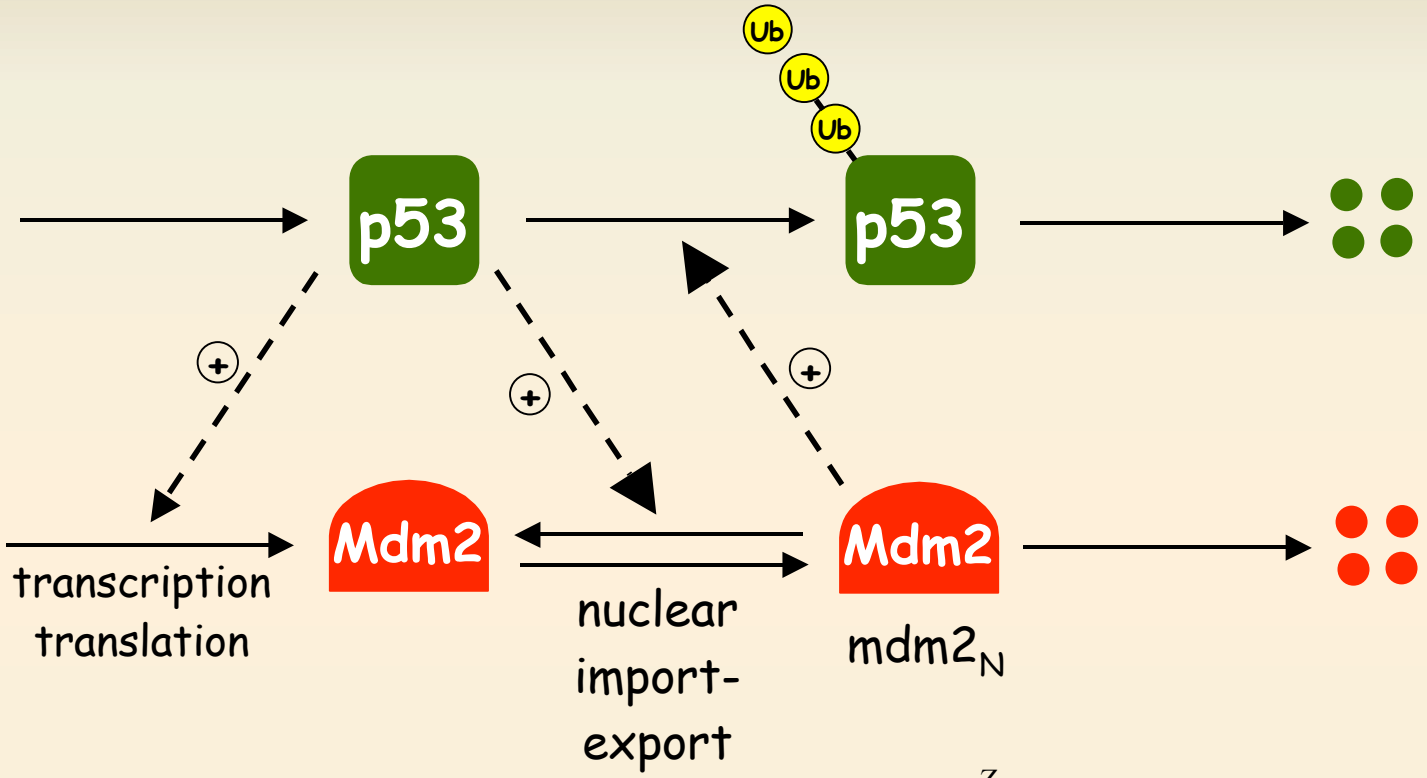


Second pulse





Negative feedback between p53 and Mdm2

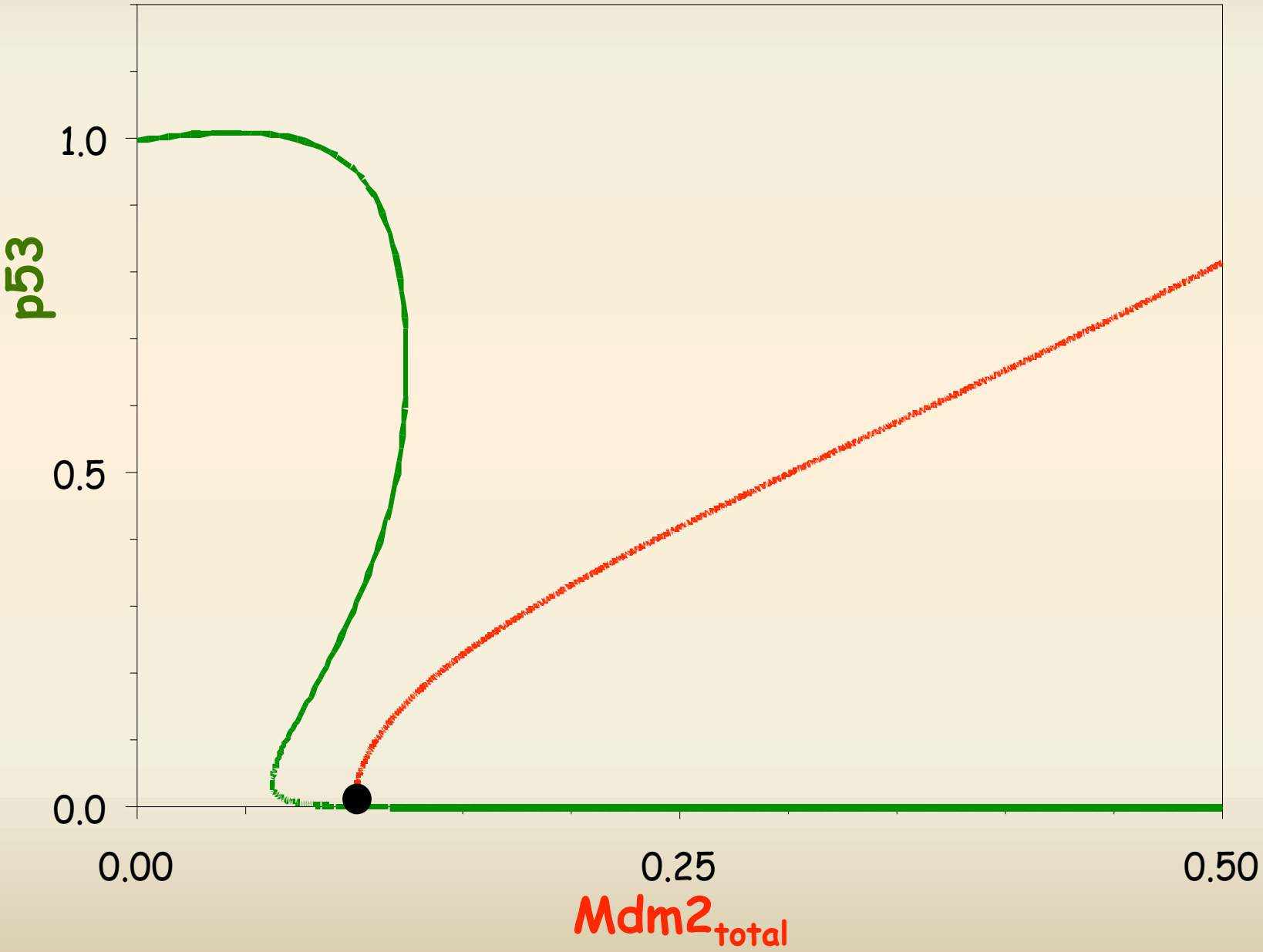


$$\frac{dp53}{dt} = k_{s53} - (k_{d53}' + k_{d53}'' \frac{mdm2_N{}^Z}{J^Z + mdm2_N{}^Z}) p53$$

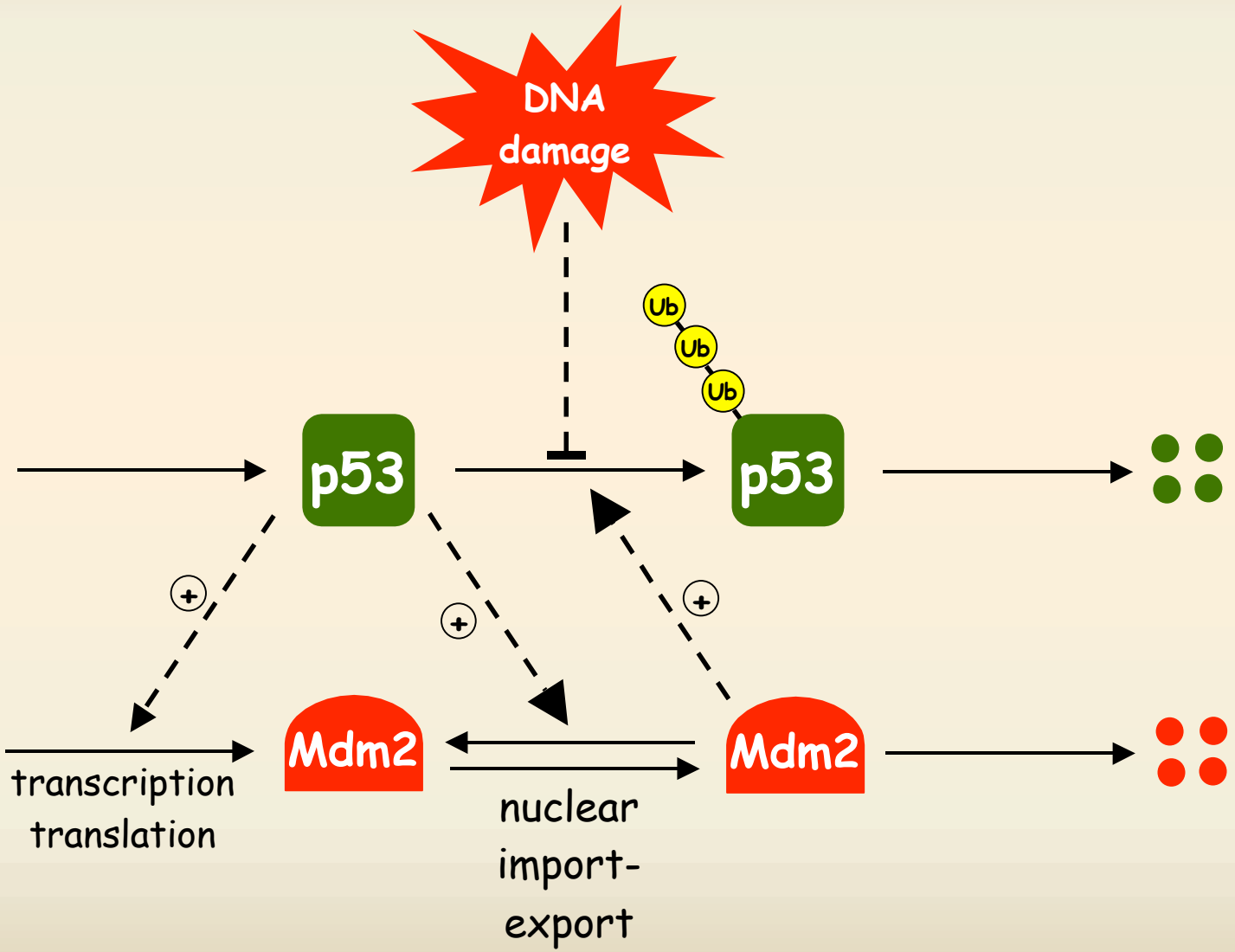
$$\frac{dmdm2_T}{dt} = k_{s2}' + k_{s2}'' \frac{p53^m}{J_S{}^m + p53^m} - k_{d2} \cdot mdm2_T$$

$$mdm2_N = \frac{k_{in} \cdot mdm2_T}{k_{in} + k_{out} \cdot p53 + k_{d2}}$$

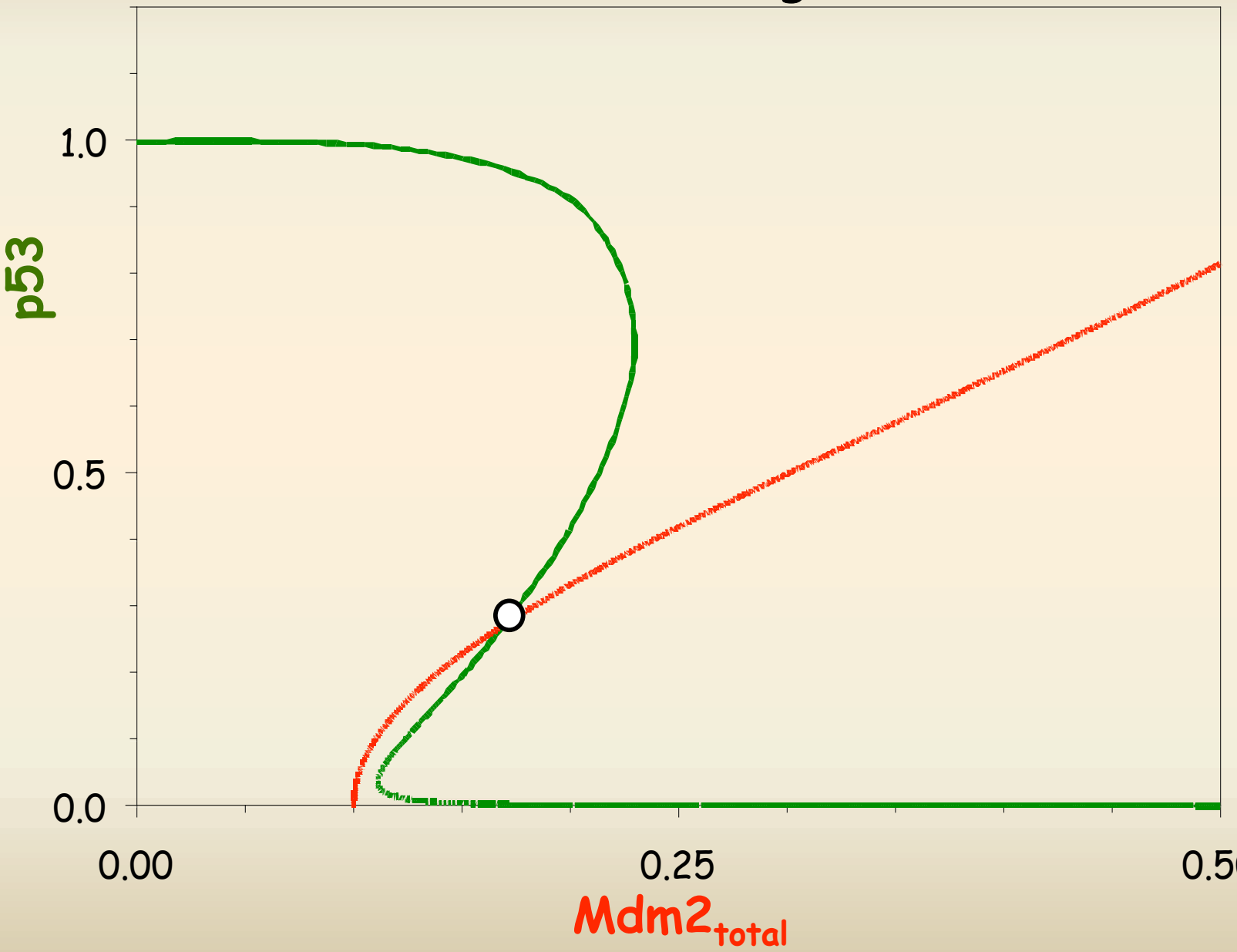
p53 - $Mdm2_T$ PhasePlane



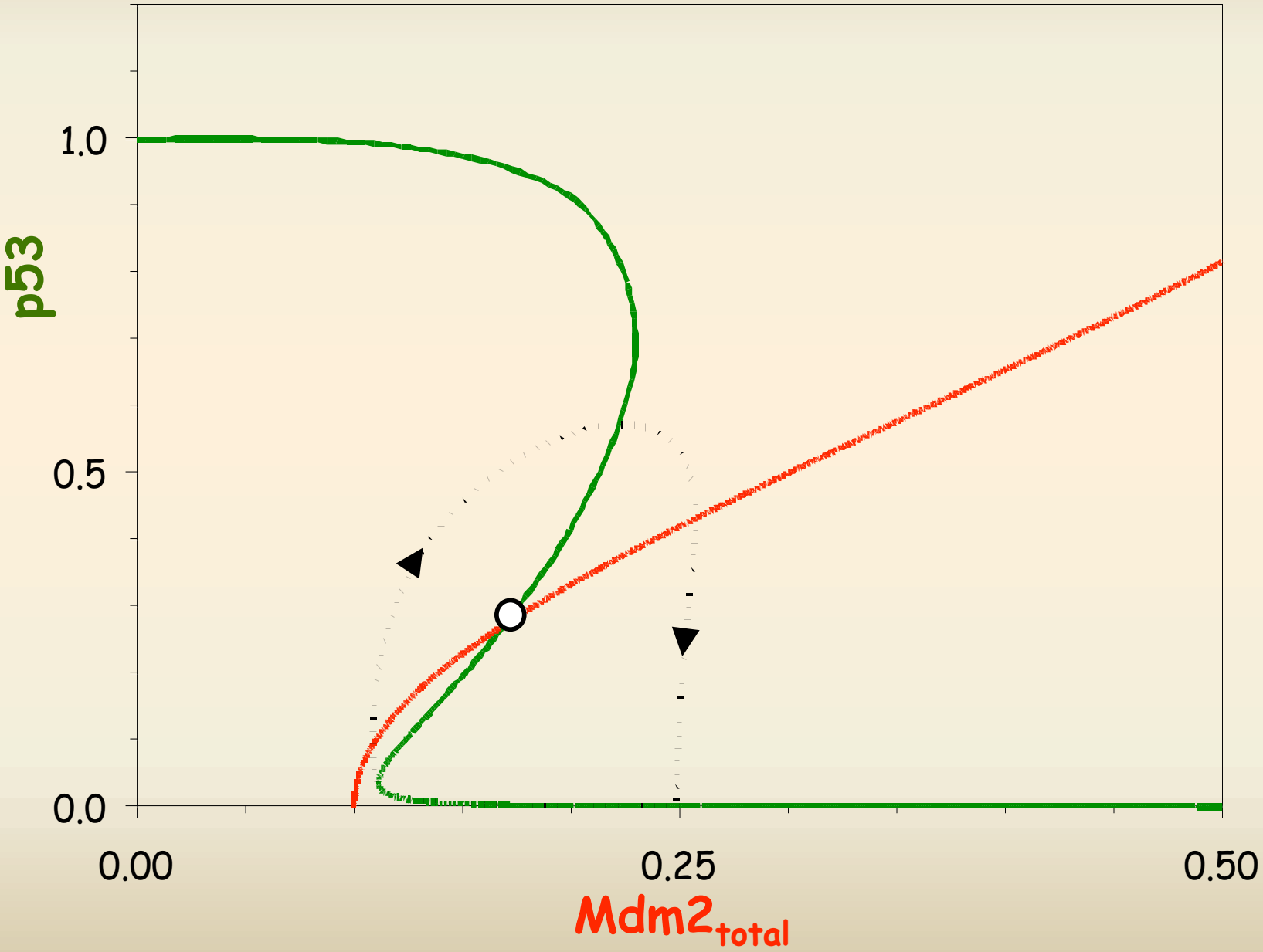
DNA damage makes p53 less accessible for Mdm2 dependent degradation

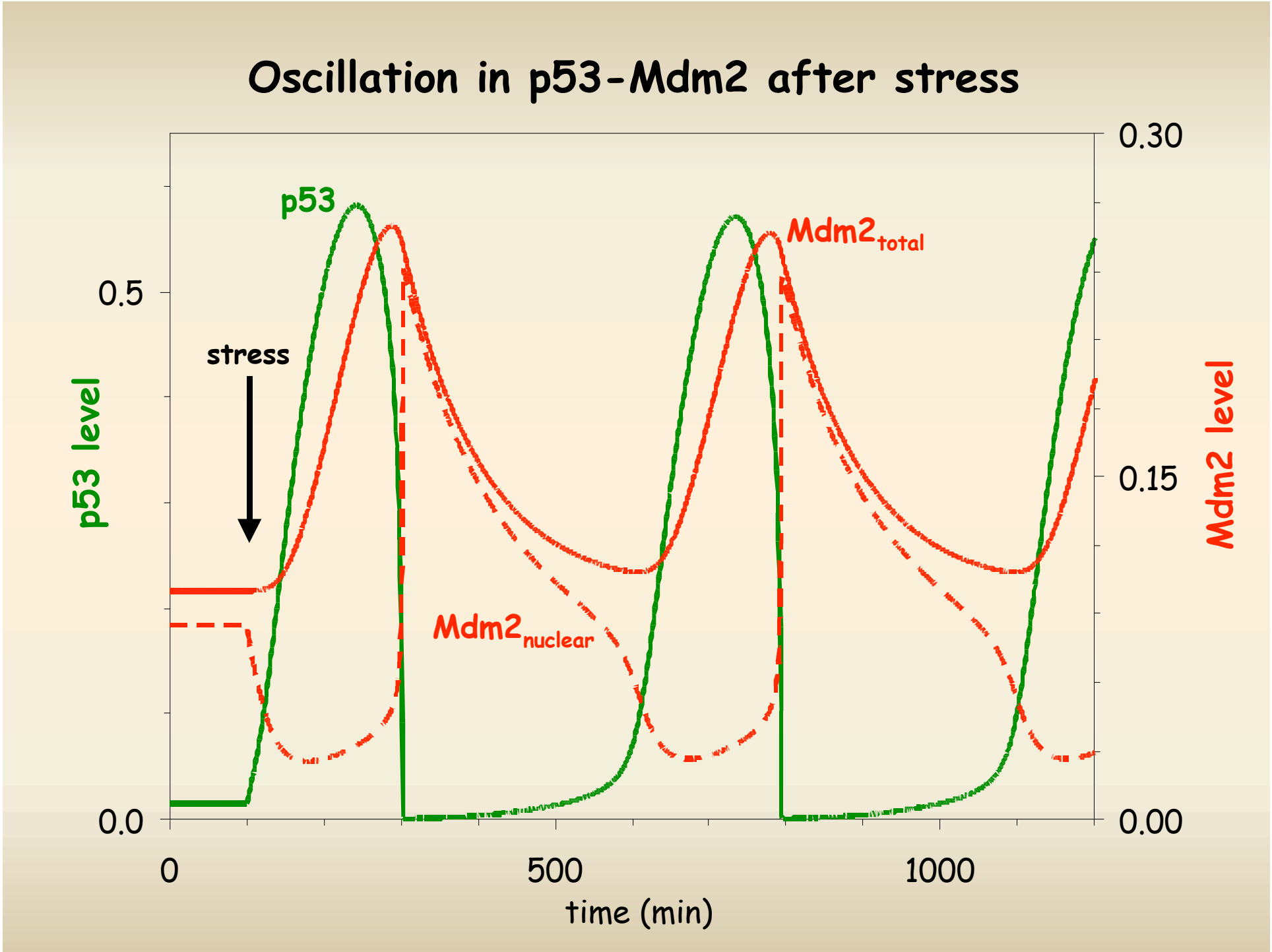


p53 degradation rate constant is decreased after DNA damage

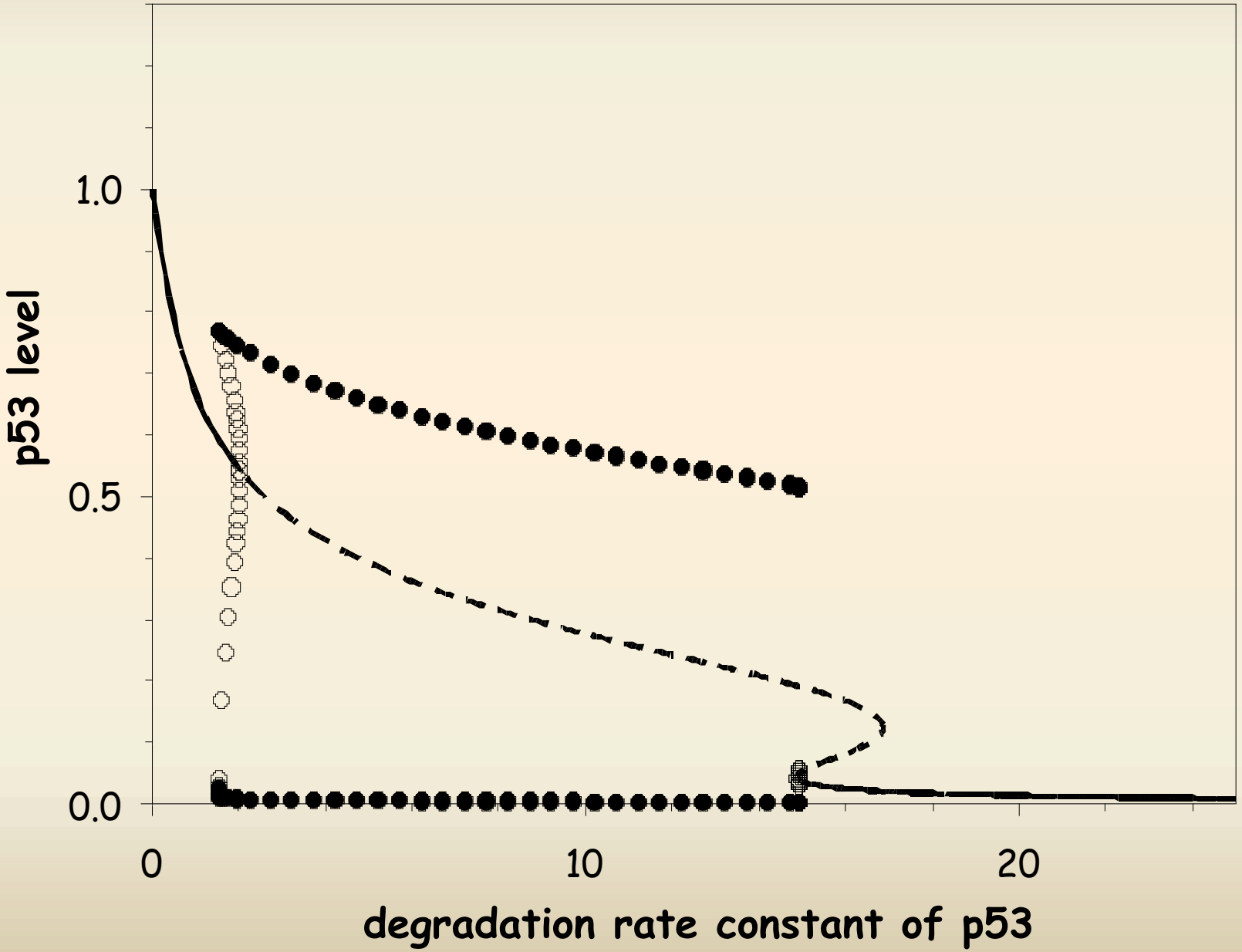


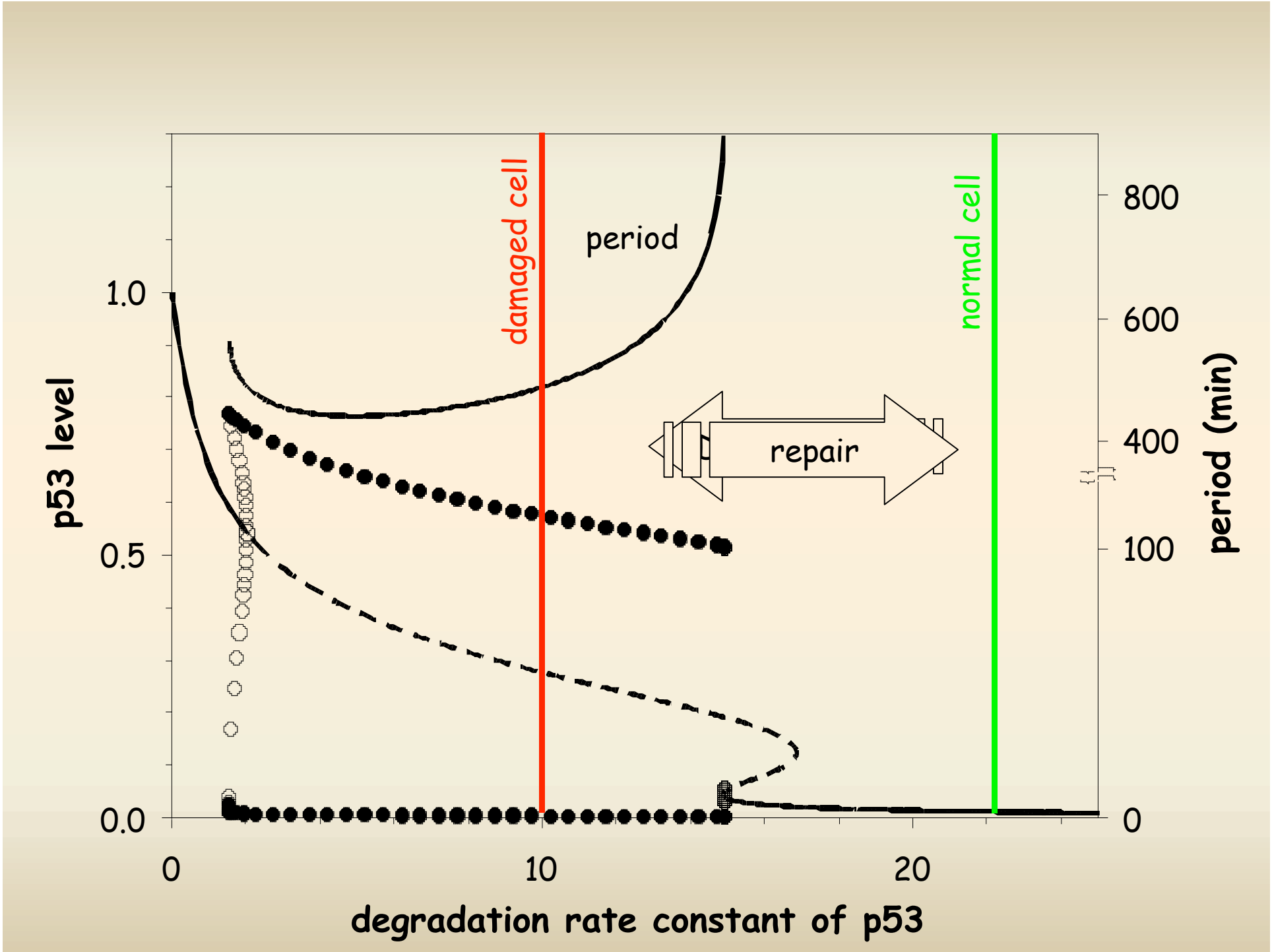
Limit cycle oscillation on the PhasePlane



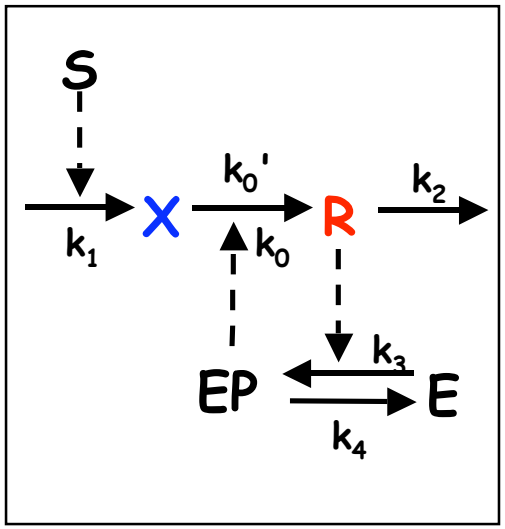

Oscillation in p53-Mdm2 after stress
p53
Mdm2total
Mdm2nuclear
stress
p53 level
Mdm2 level
0.0
0.5
0.00
0.15
0.30
0
500
1000
time (min)

A bifurcation diagram

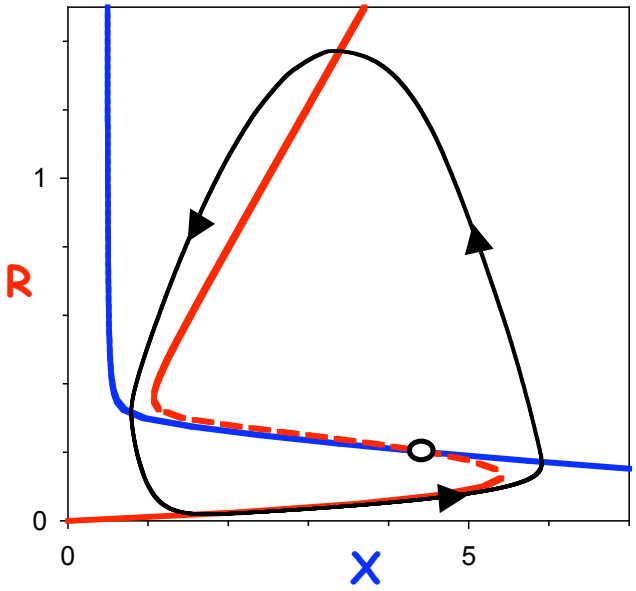


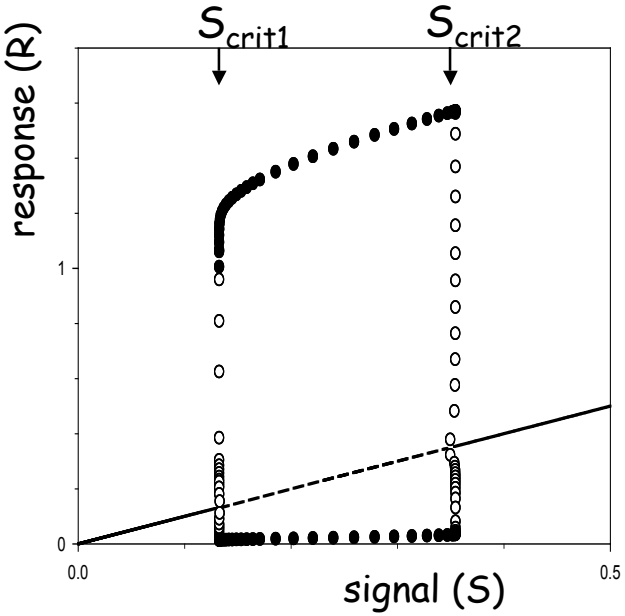

damaged cell
period
normal cell
repair
p53 level
period (min)
1.0
0.5
0.0
800
600
400
100
0
0
10
20
degradation rate constant of p53

Positive and negative feedback oscillations (substrate depletion)

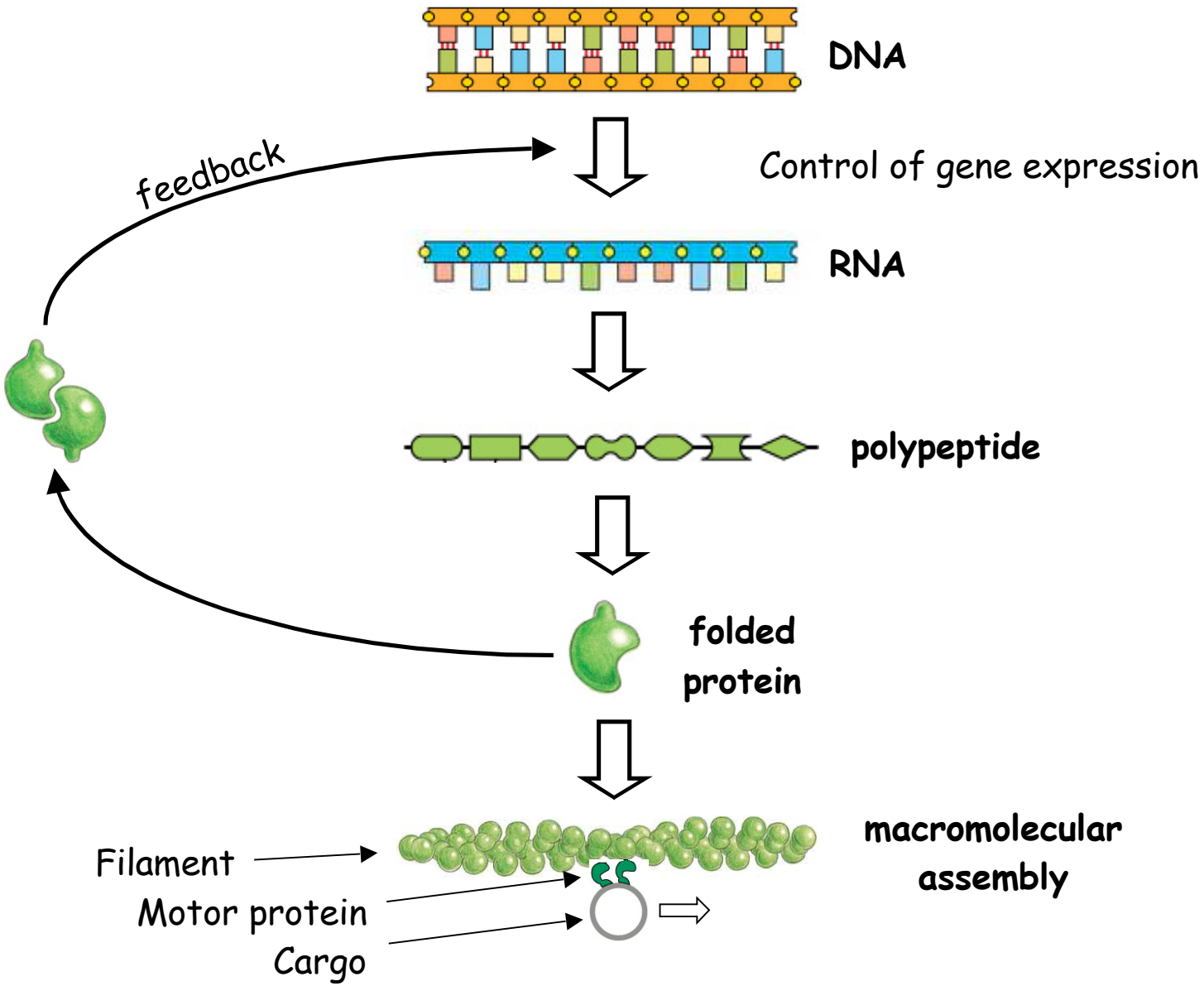


$k_0' = 0.01$
$k_0 = 0.4$
$k_1 = 0.2$
$k_2 = 1$
$k_3 = 1$
$k_4 = 0.3$
$J_3 = J_4 = 0.05$

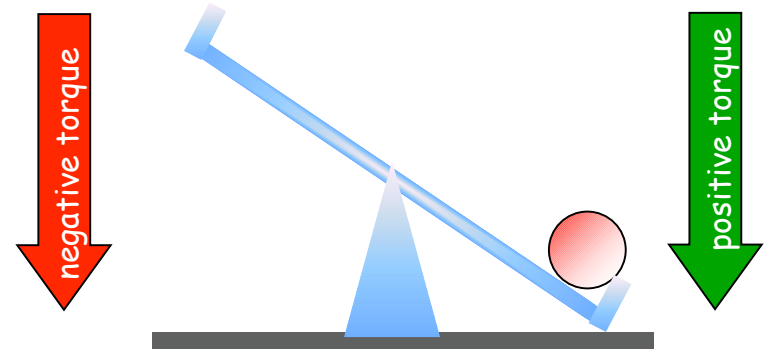


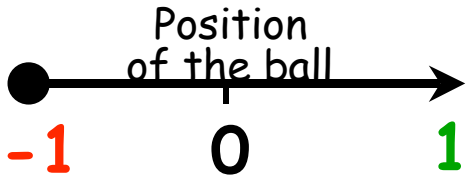


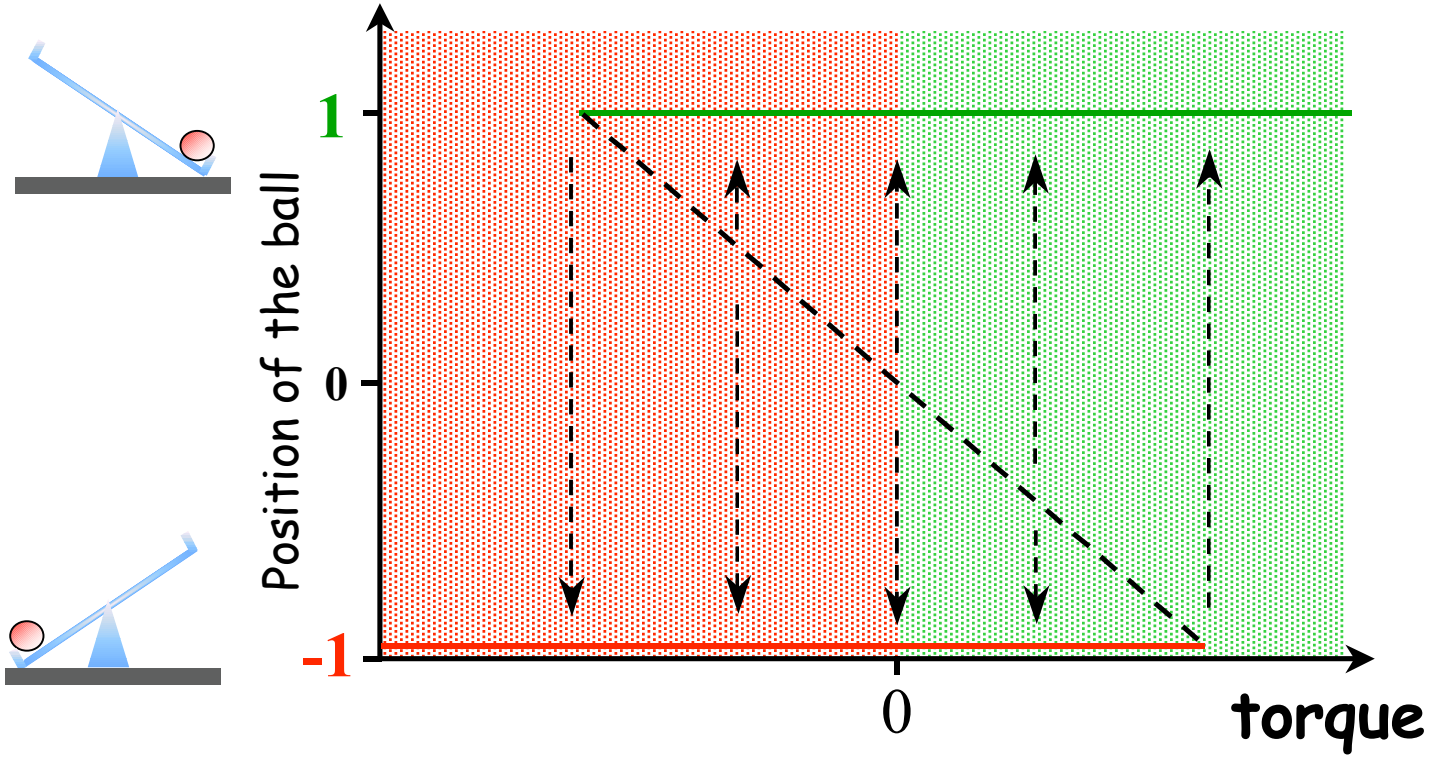
Beyond the central dogma



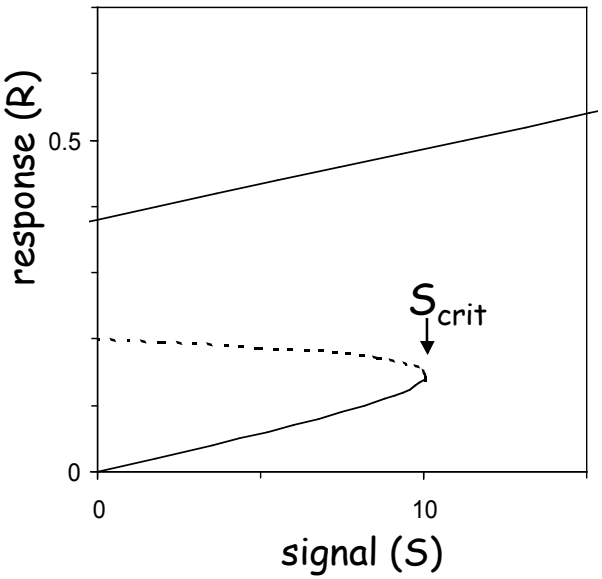
Antagonistic relationship creates two self-maintaining steady states



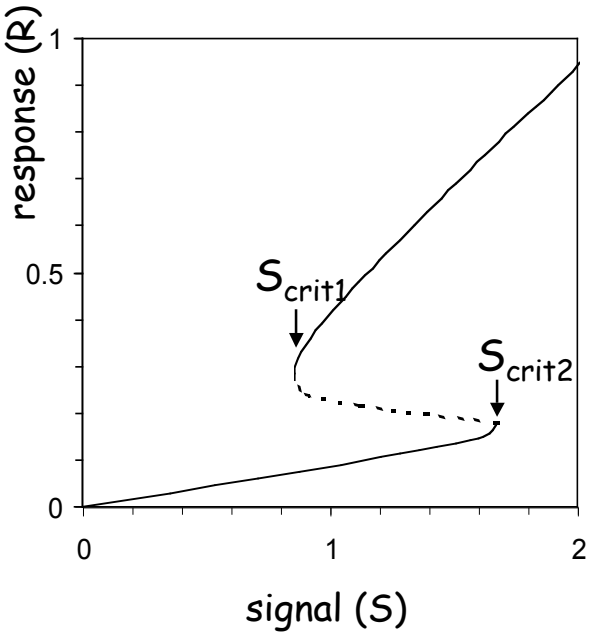




Bifurcation diagram



One-way switch



Toggle switch

Fejl_dési folyamatok: point-of-no-return
pl. béka petesejt érés

Apoptosis: programozott sejthalál

Lac operon:
lactose --| lacI --| lac-permease → lactose

Sejtciklus:
MPF → Cdc25 → MPF és
MPF --|| Wee1 --|| MPF