Lecture 3: Modeling gene expression regulation

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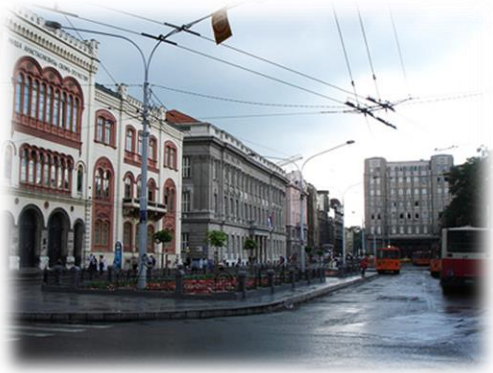


Example of gene expression regulation

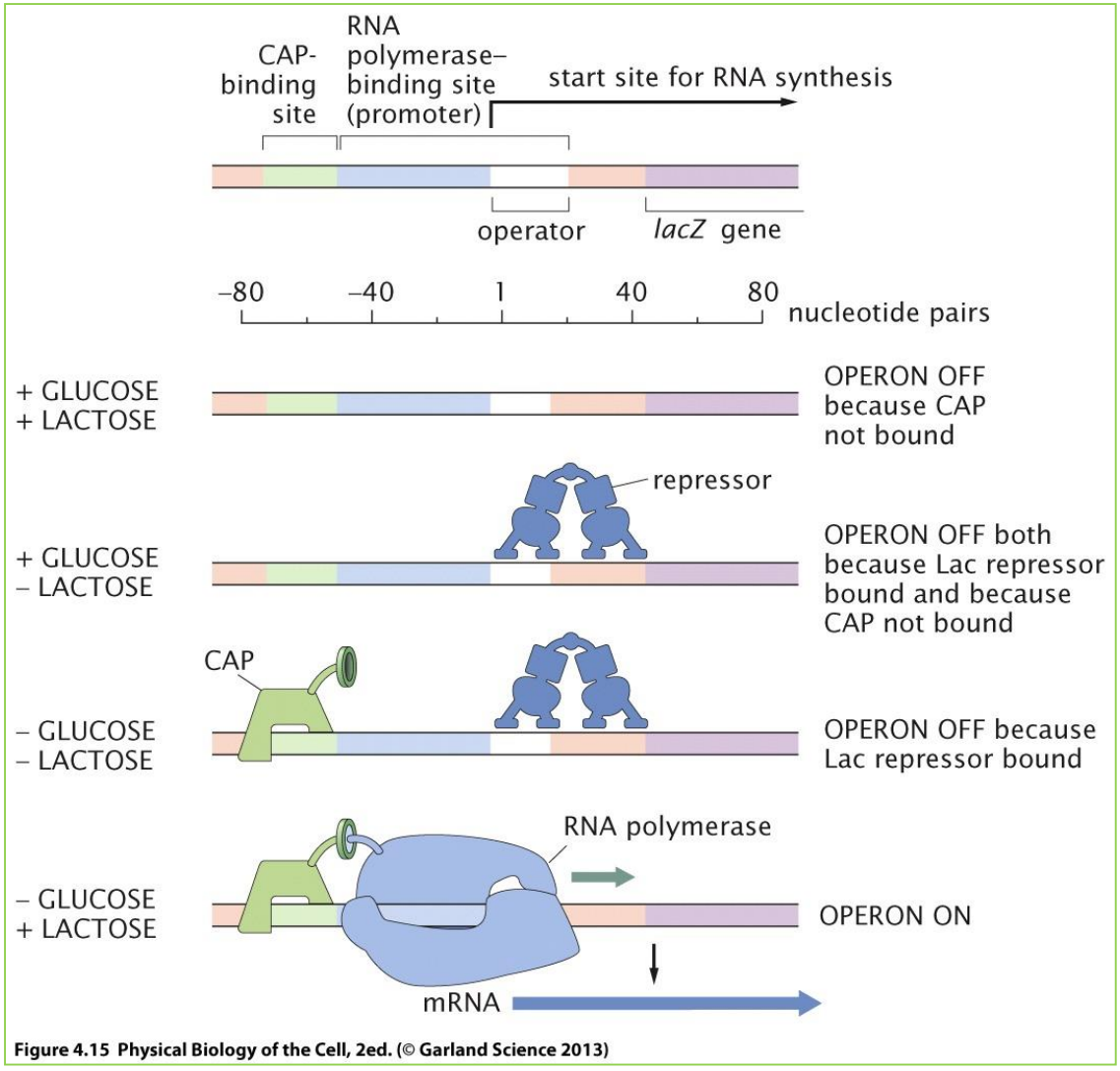


Figure 4.15 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Lac operon:

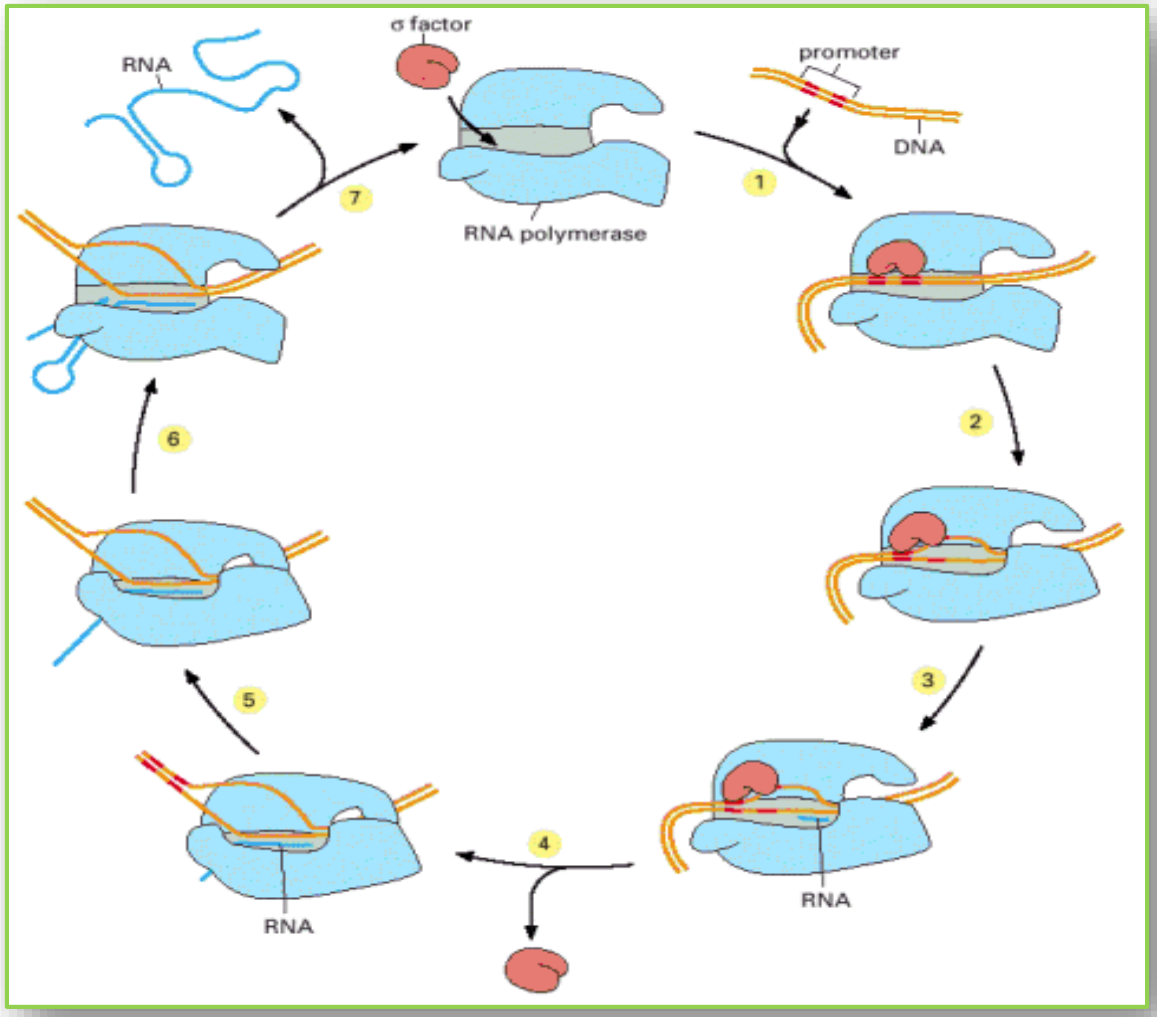
Significant expression only when lactose is present and glucose is absent.

Regulation is exhibited through interaction of proteins (transcription factors) with DNA.

Dependence of transcription activity (output) from transcription factor concentration (input)

- **You sketch all possible configurations of proteins (regulators) and RNA polymerase (RNAP) on DNA.**
- **For every configuration you write the statistical weight (see the examples).**
- **Transcriptional activity is proportional to *equilibrium* binding probability of RNAP to promoter.**
- **That is, to sum of statistical weights which correspond to activation configurations, divided by the sum of all statistical weights.**

Why equilibrium?



Gene activation

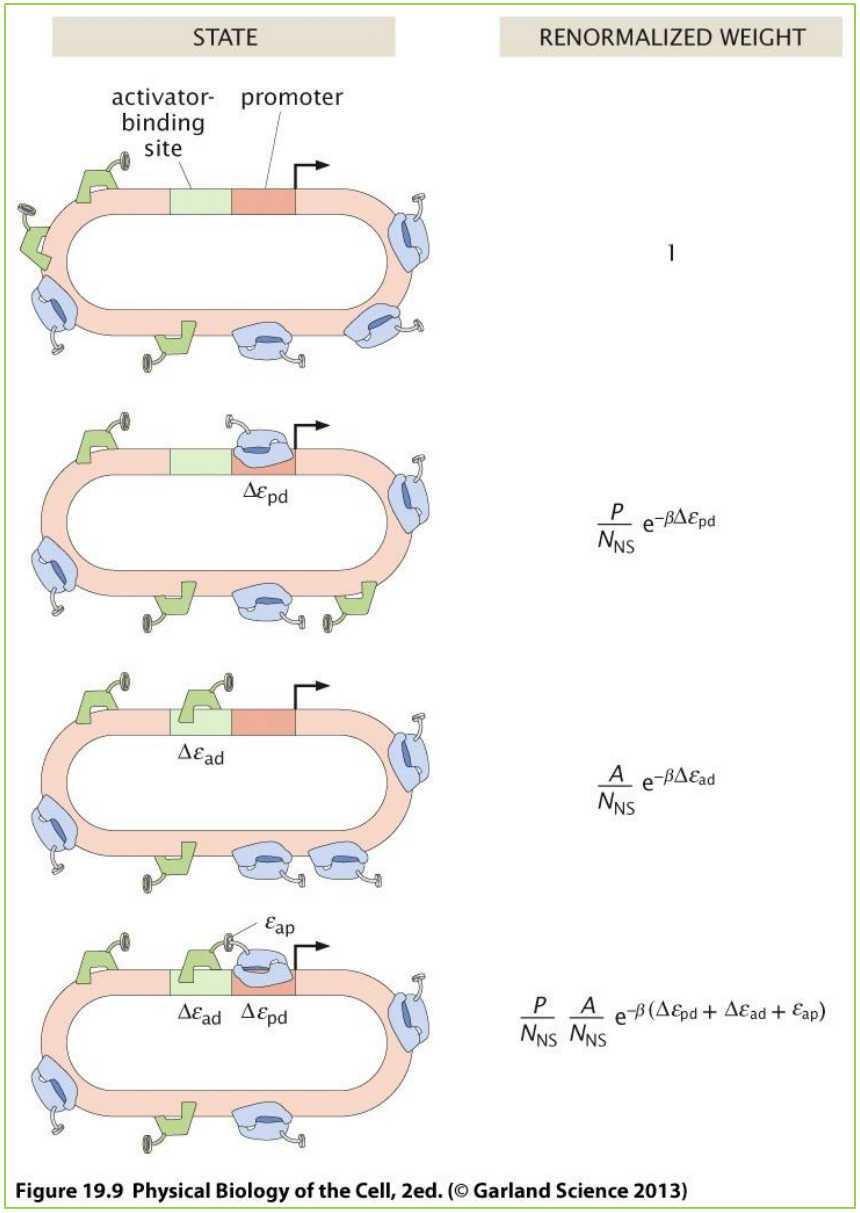


Figure 19.9 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$\varphi = \varphi_0 \frac{P/N_{NS}\exp\left(-\beta\Delta\varepsilon_{pd}\right) + (P/N_{NS})(A/N_{NS})\exp\left(-\beta\left(\Delta\varepsilon_{pd} + \Delta\varepsilon_{ad} + \Delta\varepsilon_{ap}\right)\right)}{1 + P/N_{NS}\exp\left(-\beta\Delta\varepsilon_{pd}\right) + R/N_{NS}\exp\left(-\beta\Delta\varepsilon_{rd}\right) + (P/N_{NS})(A/N_{NS})\exp\left(-\beta\left(\Delta\varepsilon_{pd} + \Delta\varepsilon_{ad} + \Delta\varepsilon_{ap}\right)\right)}$$

Gene repression

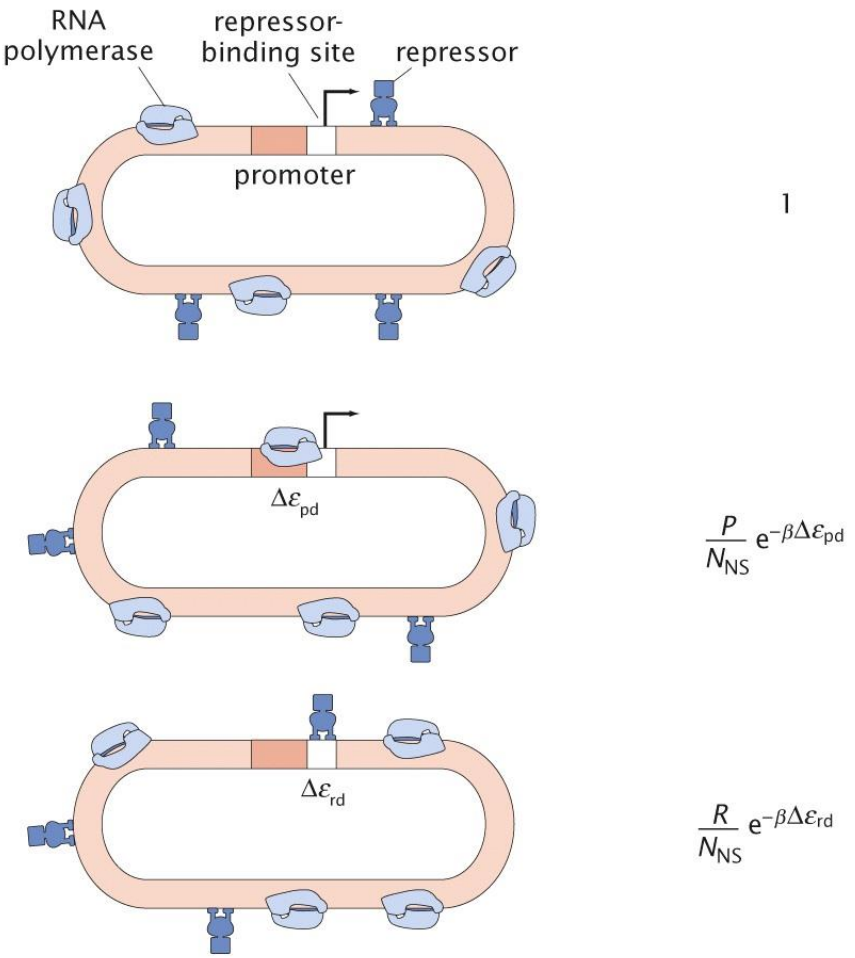


Figure 19.14 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Transcription activity

(according to Shea-Ackers model proportional to occupancy of promoter by RNA polymerase)

$$\varphi = \varphi_0 \frac{P/N_{NS}\exp\left(-\beta\Delta\varepsilon_{pd}\right)}{1+P/N_{NS}\exp\left(-\beta\Delta\varepsilon_{pd}\right)+R/N_{NS}\exp\left(-\beta\Delta\varepsilon_{rd}\right)}$$

Lac operon

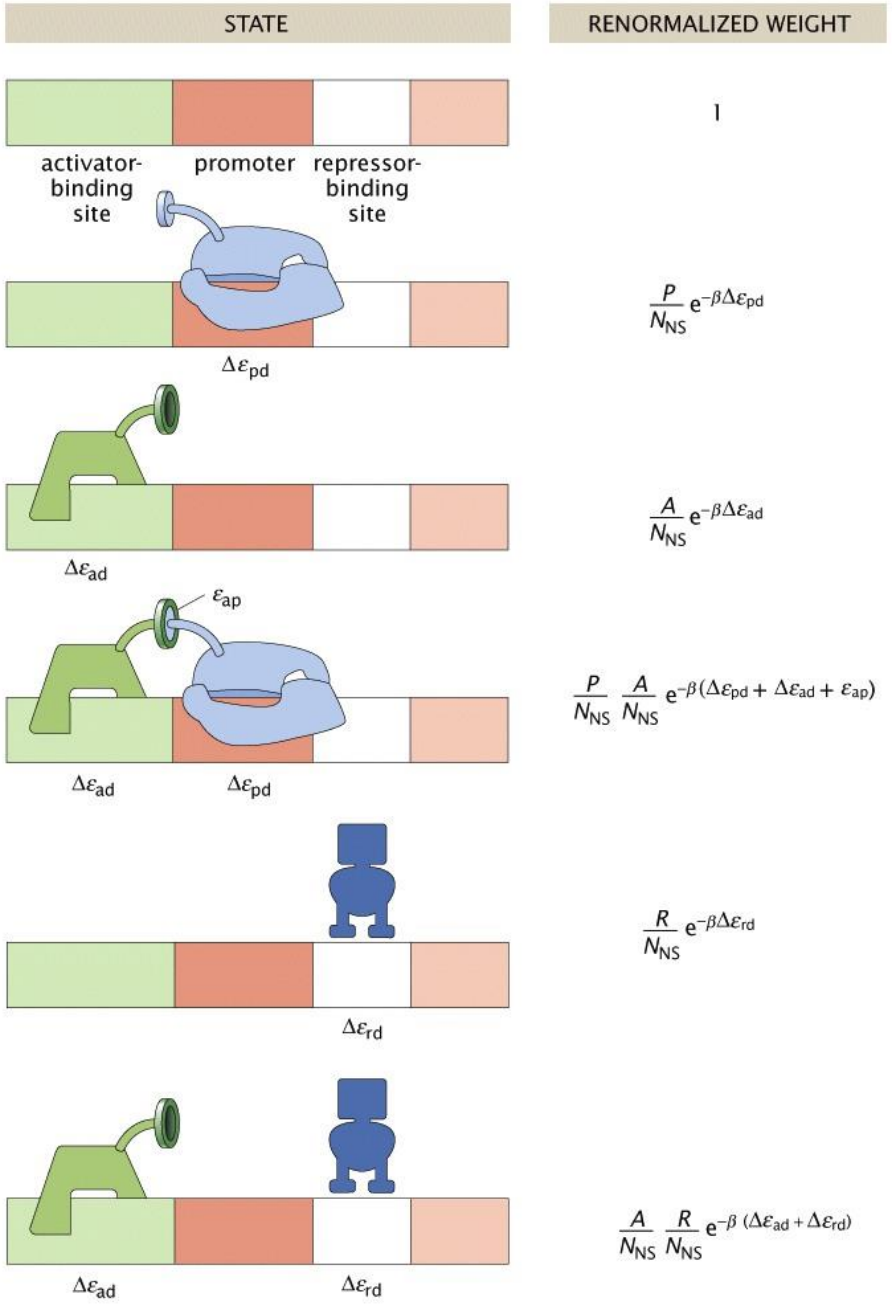


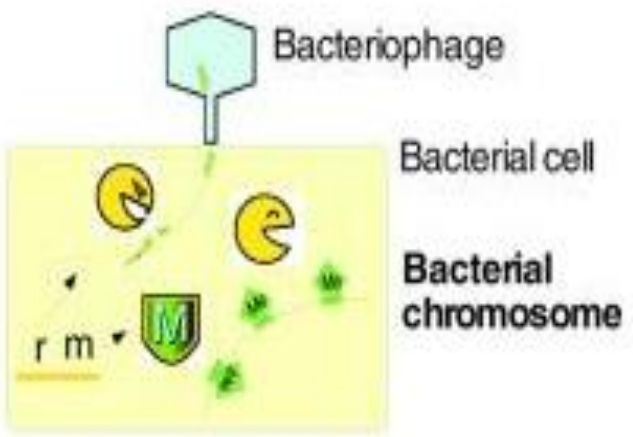
Figure 19.18 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

For excercise find expression for transcription activity!

Example 1: Modeling rudimental immune system

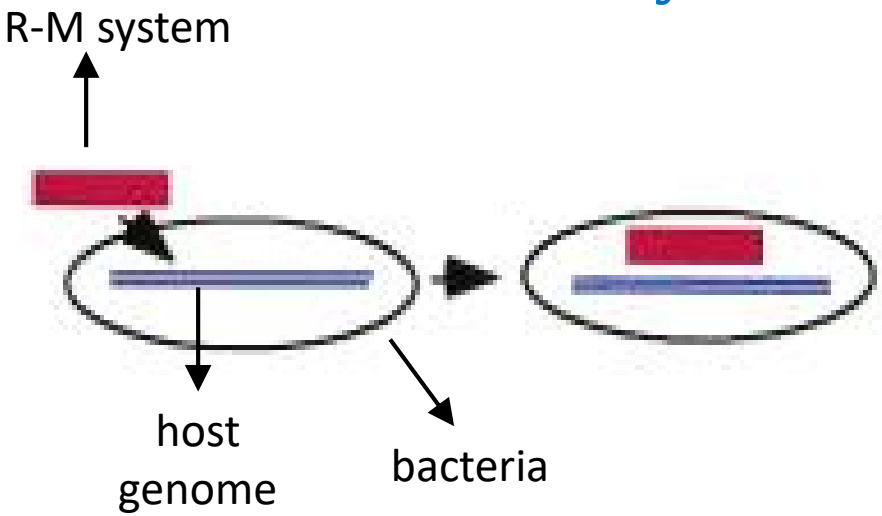
Restriction-modification system in bacteria

Restriction-modification is a rudimental immune system



DNA of the host cell is methylated and will not be cut by restriction enzyme, but foreign DNA (e.g. virus that attacks bacteria) is not methylatted, and becomes destroyed.

R-M systems are often mobile

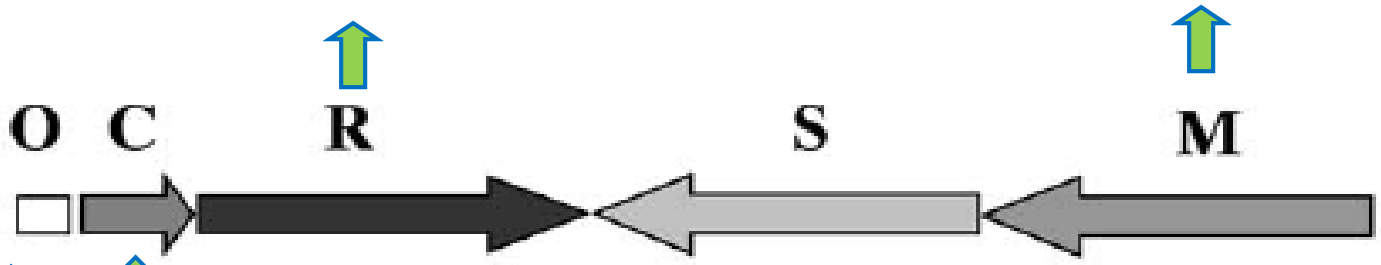


R-M genes can go from one host cell to another, and in this way spread through bacterial population.

Bacterial restriction-modification (R-M) system of type II

Endonulease (R) recognizes and cuts DNA sequences

Methylase (M) methylates the same DNA sequences that are cut by the endonuclease, and consequently protects them from cleaving.



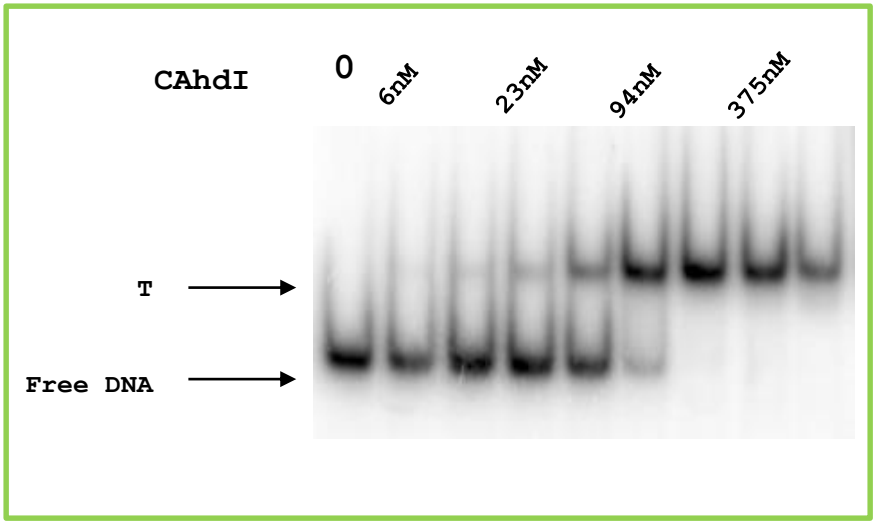
Operator sequence

Control protein (C) coordinates expression of R and M genes so that it is bound upstream of CR gene and regulate their expression.

Regulation by the control protein

The control protein exhibits very large cooperativity:

- **Only dimmer can bind to DNA**
- **Only tetramer is bound to DNA in the absence of RNA polymerase**

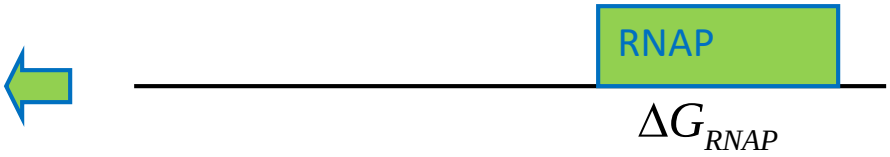


Over the entire range of control protein concentrations two dimmers are simultaniously bound

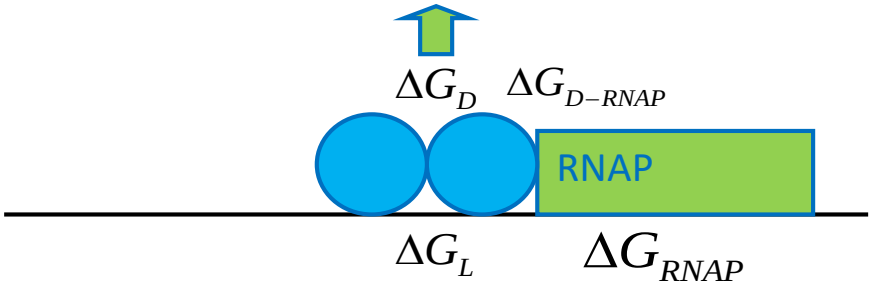
Large cooperativity in binding

Quantitative model

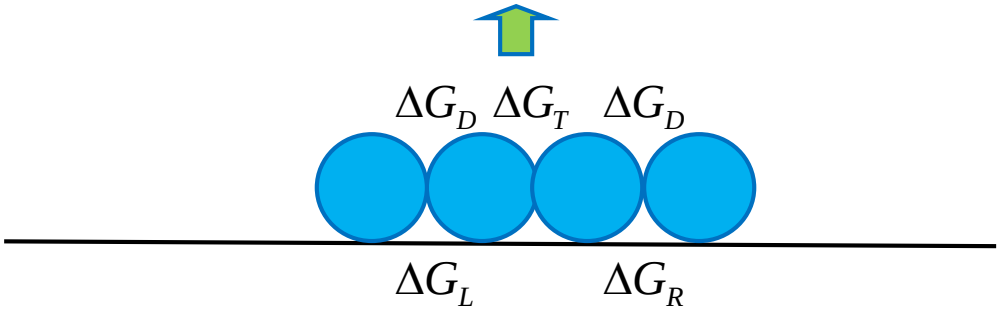
$Z_{RNAP} = ??$



$Z_{D-RNAP} = ??$



$Z_T = ??$

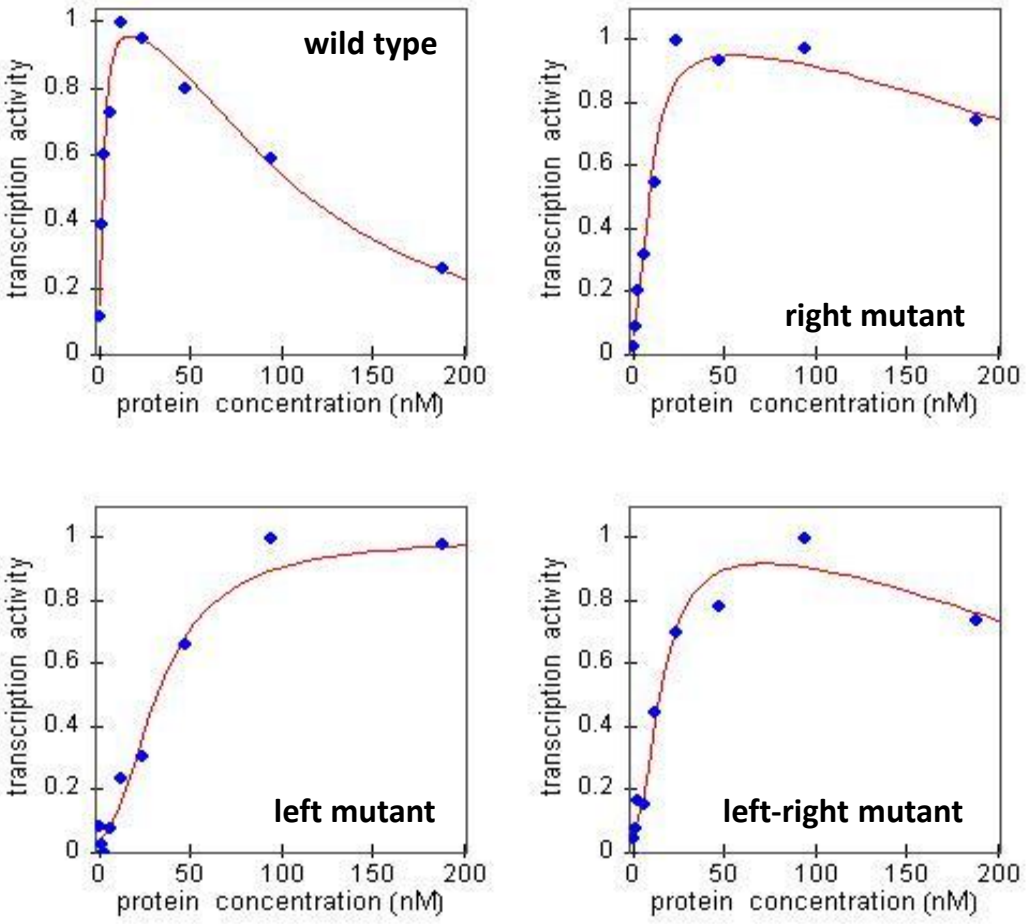


$\varphi = ??$ ⇒ **transcription activity**

a, b and c are directly related with the biophysical properties of the switch.

Comparison with experiment

E. Bogdanova, M.D. *et al.*, NAR 36,1429 (2008)



Model agrees well with measured transcription activity, in the case of "wild-type" and „mutant" sequences.

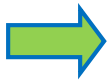
Modeling the system dynamics

$$\frac{dc}{dt} = \gamma\left(C_C\right) - \lambda c$$

generating RC transcript

Transcription activity versus C protein concentration

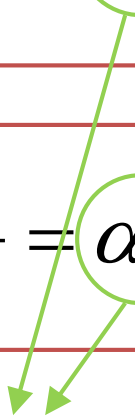
$$\frac{dC_C}{dt} = \alpha_C c - \beta C_C$$



synthesis of C protein

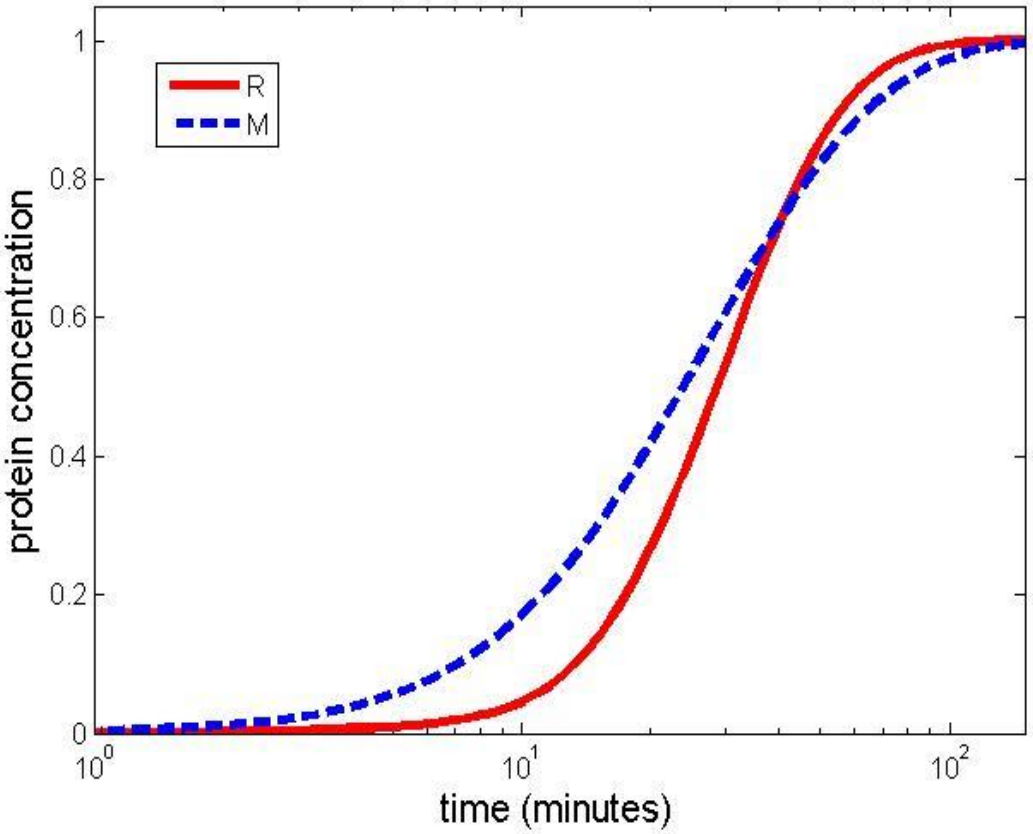
$$\frac{dC_R}{dt} = \alpha_R c - \beta C_R$$

synthesis of R protein



C transcripts are translated less efficiently than R transcripts.

Dynamics of establishing equilibrium



E. Bogdanova, M.D. *et al.*, NAR 36,1429 (2008)

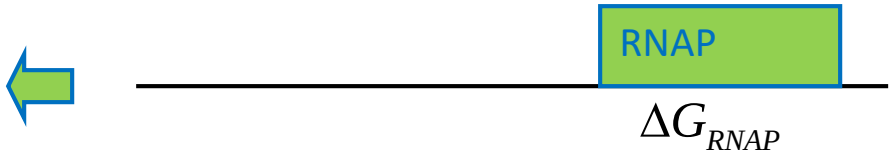
Endonuclease is synthesized with , и у уском временском интервалу.

Conclusion R-M system

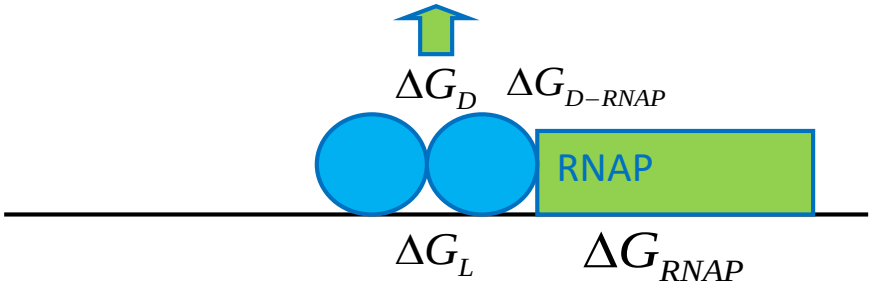
- **Toxic molecule is expressed in a narrow time interval, and with delay with respect to antidote.**
- **This is exhibitied through large cooperativity in binding and modulation of translation efficiency.**

Problem

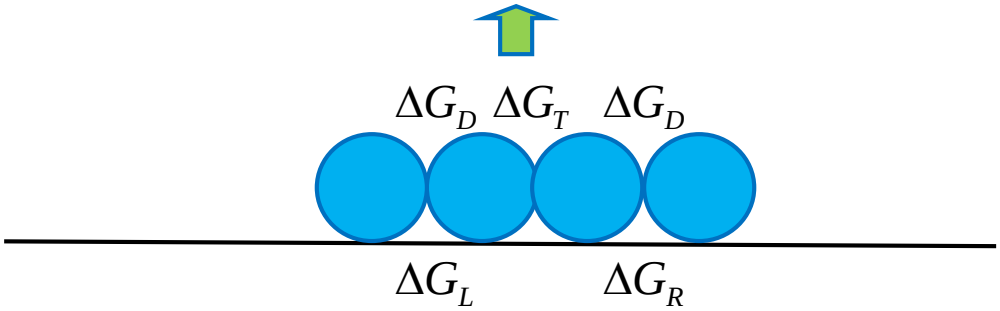
$Z_1 = ??$



$Z_2 = ??$



$Z_1 = ??$



$\varphi = ??$ ⇒ **transcription activity**

On the figure is provided the scheme of the promoter with restriction -modification system.

a) **Find statistical weights** Z_1, Z_2, Z_3 **as a function of transcription factor concentration, RNA polymerase and interaction energies shown in the figure.**
b) **By using Shea-Ackers model express транскрипциону активност of promoter in terms of** Z_1, Z_2, Z_3
c) **Write transcription activity in terms of C protein concentration and parameters suitable for the fit.**
d) **Graphycally sketch transcription activity in terms of concentration of C protein**

e) Write differential equations that determine concentration of transcript and protein for gene which is transcribed from this promoter. Assume the following parameters:

p – *концентрација протеина*

m – *концентрација транскрипта*

λ_p – *константа распада протеина*

λ_m – *константа распада транскрипта*

k – *стопа транслације*

$\varphi(C)$ – *транскрипциона активност промотера*

f) Under assumption that the gene which is transcribed from the promoter is itself a transcription factor (i.e. C protein regulates its own transcription) find equation which determines equilibrium concentration of C protein.

g) Graphically sketch solution of this equation – use answer d)

Example 2: Modeling biological oscillators

Represilator, relaxation oscillator

Goodwin oscillator (1965, Brian Goodwin)

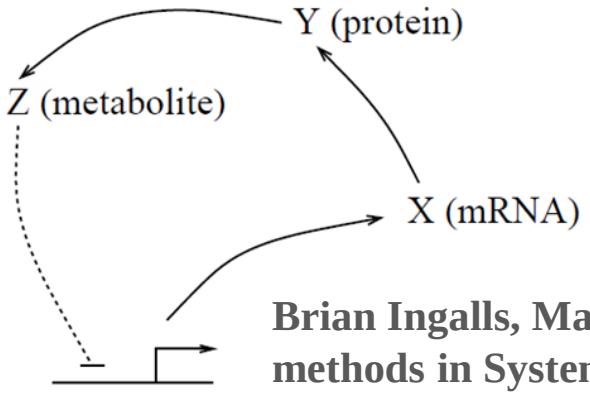


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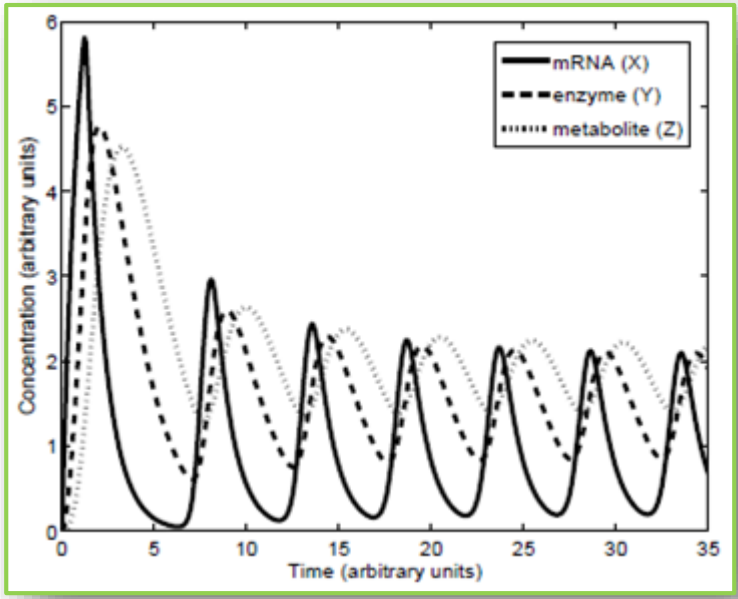
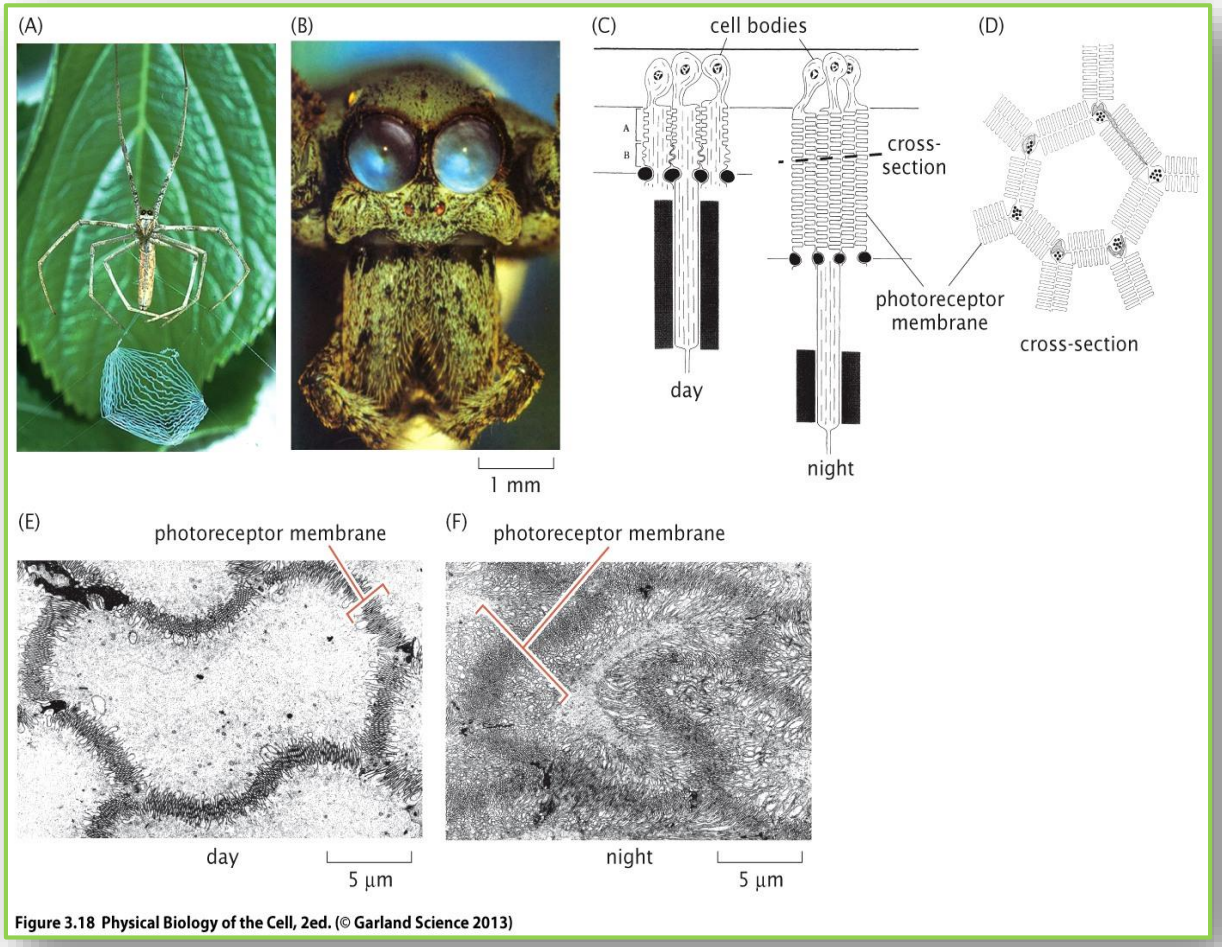
Figure 7.16: The Goodwin oscillator. (The dashed blunted arrow indicates repression.) The mRNA (X) is translated into an enzyme (Y), which catalyses production of a metabolite (Z), which (indirectly) represses gene expression. This negative feedback, coupled with the delay inherent in the three-step loop, can result in oscillatory behavior.

Large cooperativity is necessary,
n=8 so that there are oscillations

$$\frac{d}{dt}x(t) = \frac{a}{k^n + (z(t))^n} - bx(t)$$
$$\frac{d}{dt}y(t) = \alpha x(t) - \beta y(t)$$
$$\frac{d}{dt}z(t) = \gamma y(t) - \delta z(t).$$



Circadian oscillators



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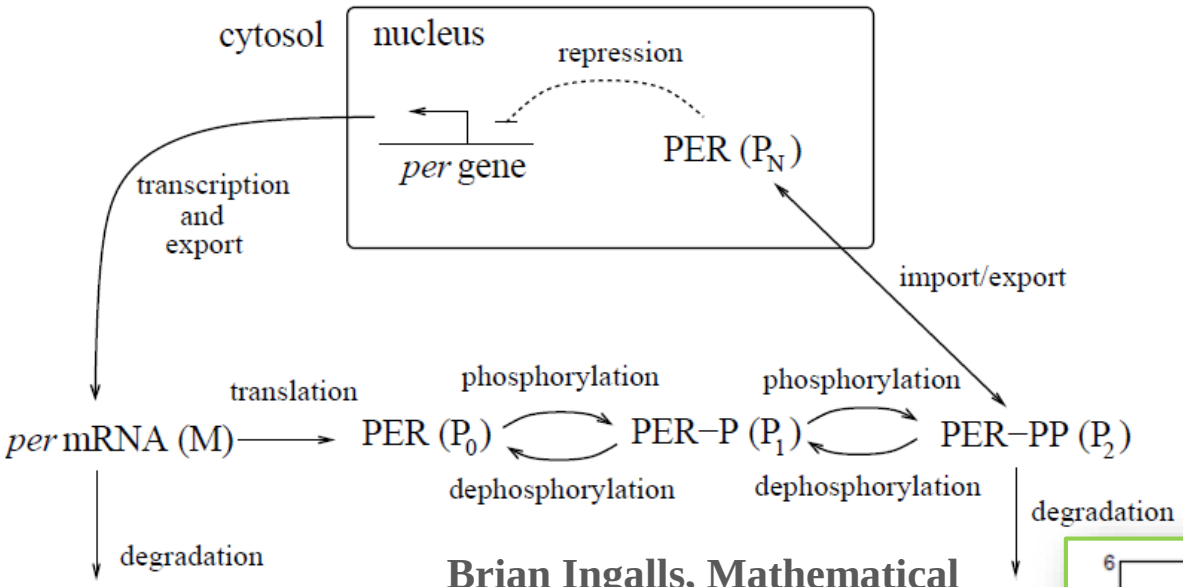
Take note about change of day and night – an extreme example are photoreceptor cells in spider.

Goldblater model of circadian oscillator



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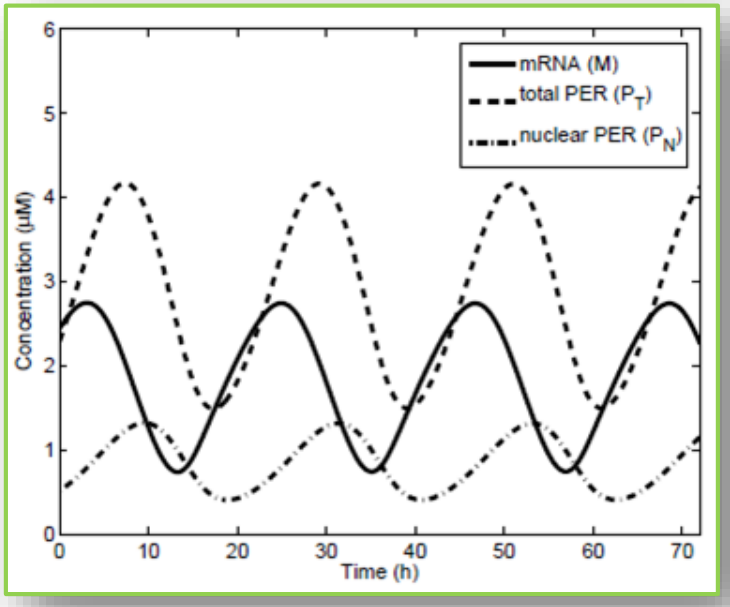
Again large cooperativity (non-linarity), n=4

$$\frac{d}{dt}m(t) = \frac{v_s}{1+(p_N(t)/K_I)^n} - \frac{v_m m(t)}{K_{m1}+m(t)}$$



Synthetic oscillators

... Two synthetic gene circuits that show oscillatory behaviour:

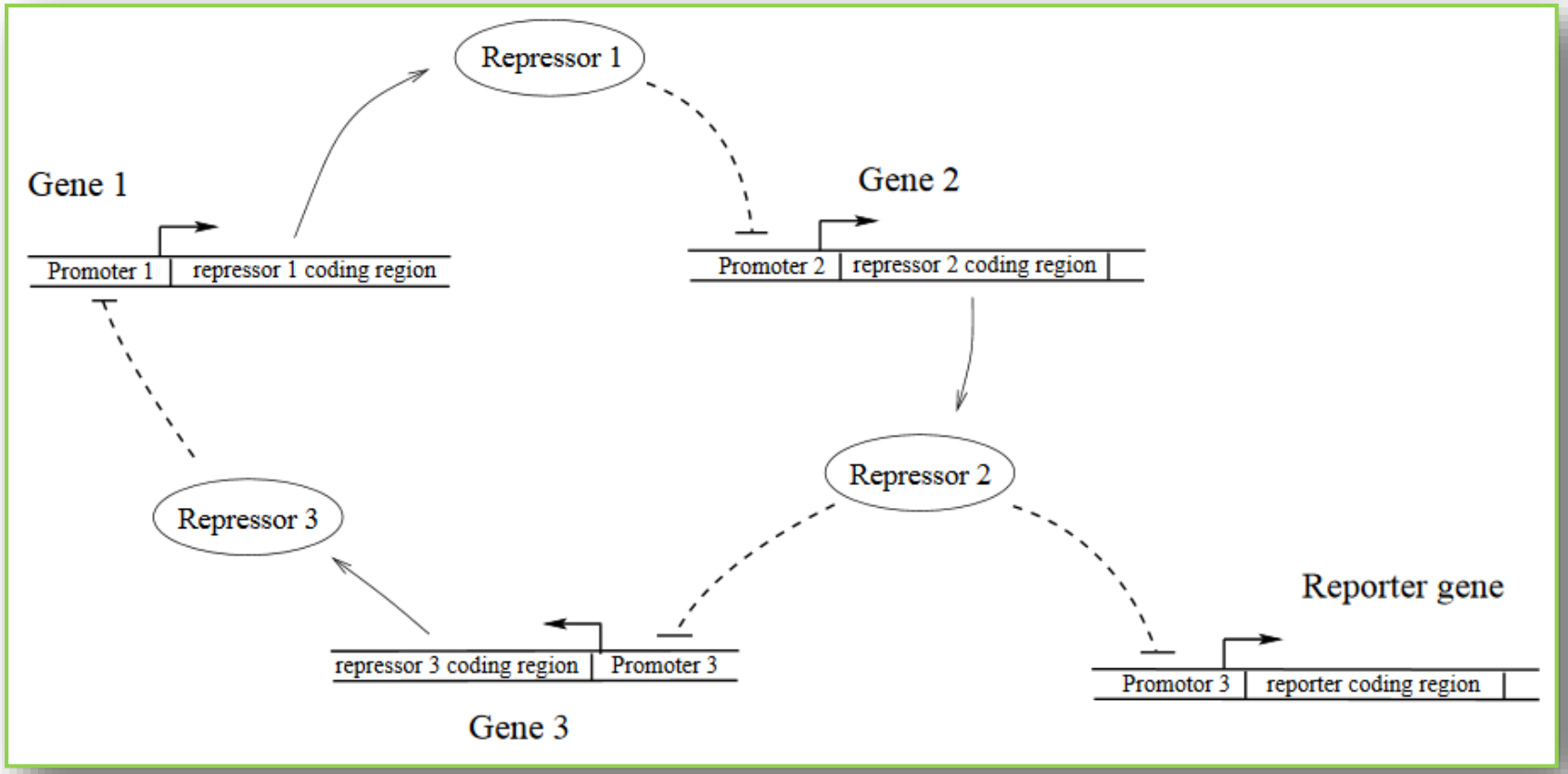
i. **Repressilator – an example of oscillatory circle with delay**

ii. **Oscillator with relaxation oscillations**

Repressilator

- **Elowitz and Leibler, 2000.**
- **3 pairs of promoters and genes that code repressors**
- **system can lose its stable state, and exhibit oscillatory behaviour**
- **delay oscillator since every protein in a loop inhibits its own expression (with a delay) in 3 steps**

Схема кола



Model: **describes time dynamics of changing the concentration of proteins in a loop;**

Assumption: **all 3 genes have identical characteristic**

p_3 *represses expression of* m_1

$$\frac{d}{dt}m_1(t) = \alpha_0 + \frac{\alpha}{1+[p_3(t)]^n} - m_1(t)$$

$$\frac{d}{dt}m_2(t) = \alpha_0 + \frac{\alpha}{1+[p_1(t)]^n} - m_2(t)$$

$$\frac{d}{dt}m_3(t) = \alpha_0 + \frac{\alpha}{1+[p_2(t)]^n} - m_3(t)$$

Dynamics of chage [transcripts]

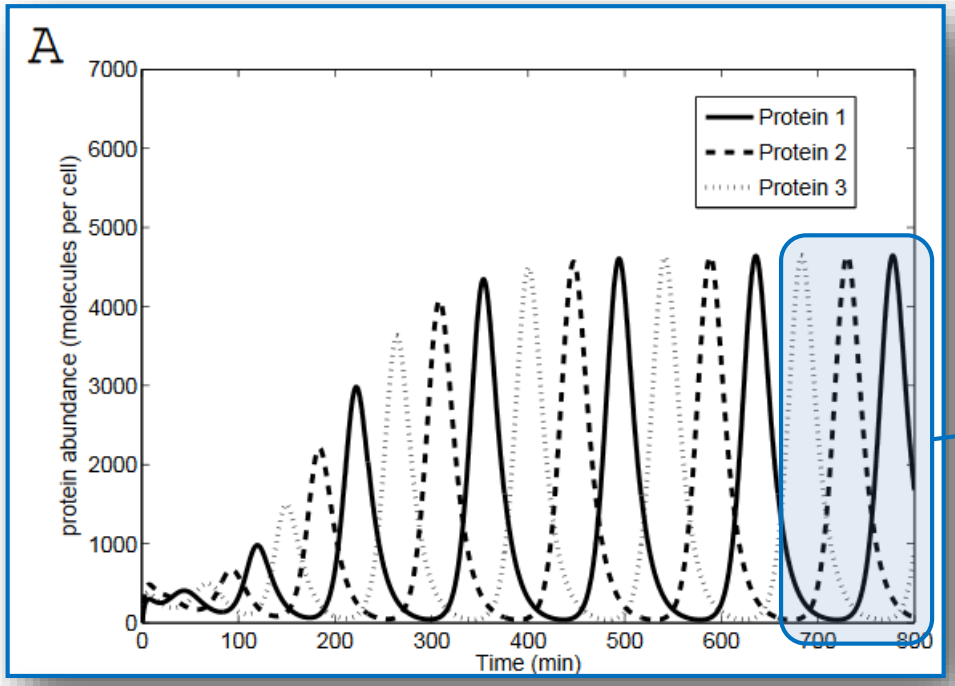
$$\frac{d}{dt}p_1(t) = \beta m_1(t) - \beta p_1(t)$$

$$\frac{d}{dt}p_2(t) = \beta m_2(t) - \beta p_2(t)$$

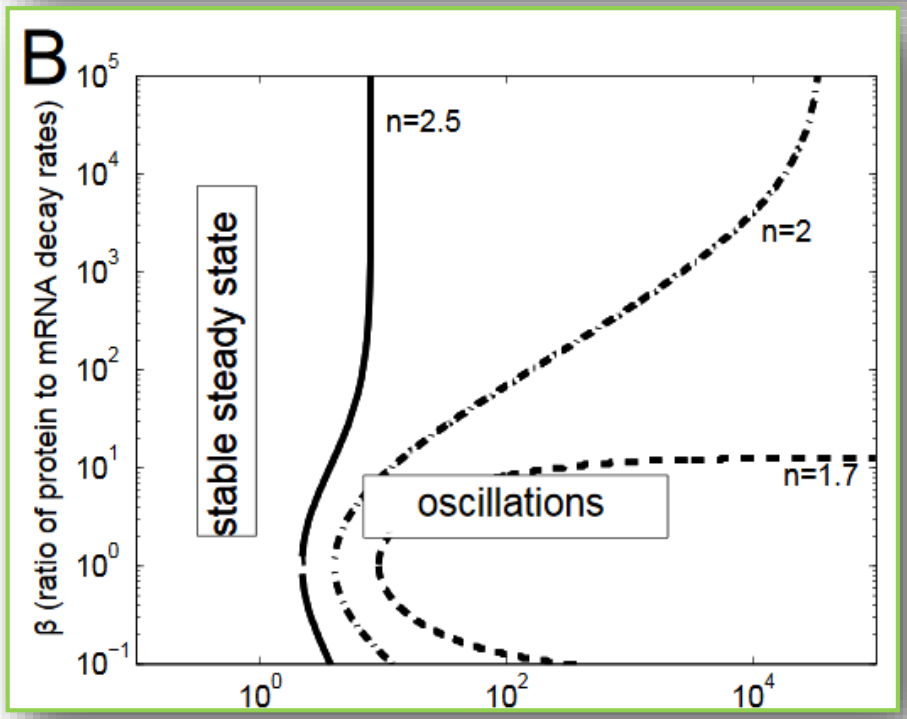
$$\frac{d}{dt}p_3(t) = \beta m_3(t) - \beta p_3(t).$$

Dynamics of change [proteins]

Симулација модела



Notice stable oscillations that appear after some transition period (when the system makes a transition to stable limit cycle)



Bifurcation analysis:

- Oscillations are favoured by high level of cooperativity, high level of expression, approximately the same degradation rate of transcripts and proteins

Changing the circuit component!
(LacI, TetR – E. coli; cI – λ phage)

Oscillator in cell cycle

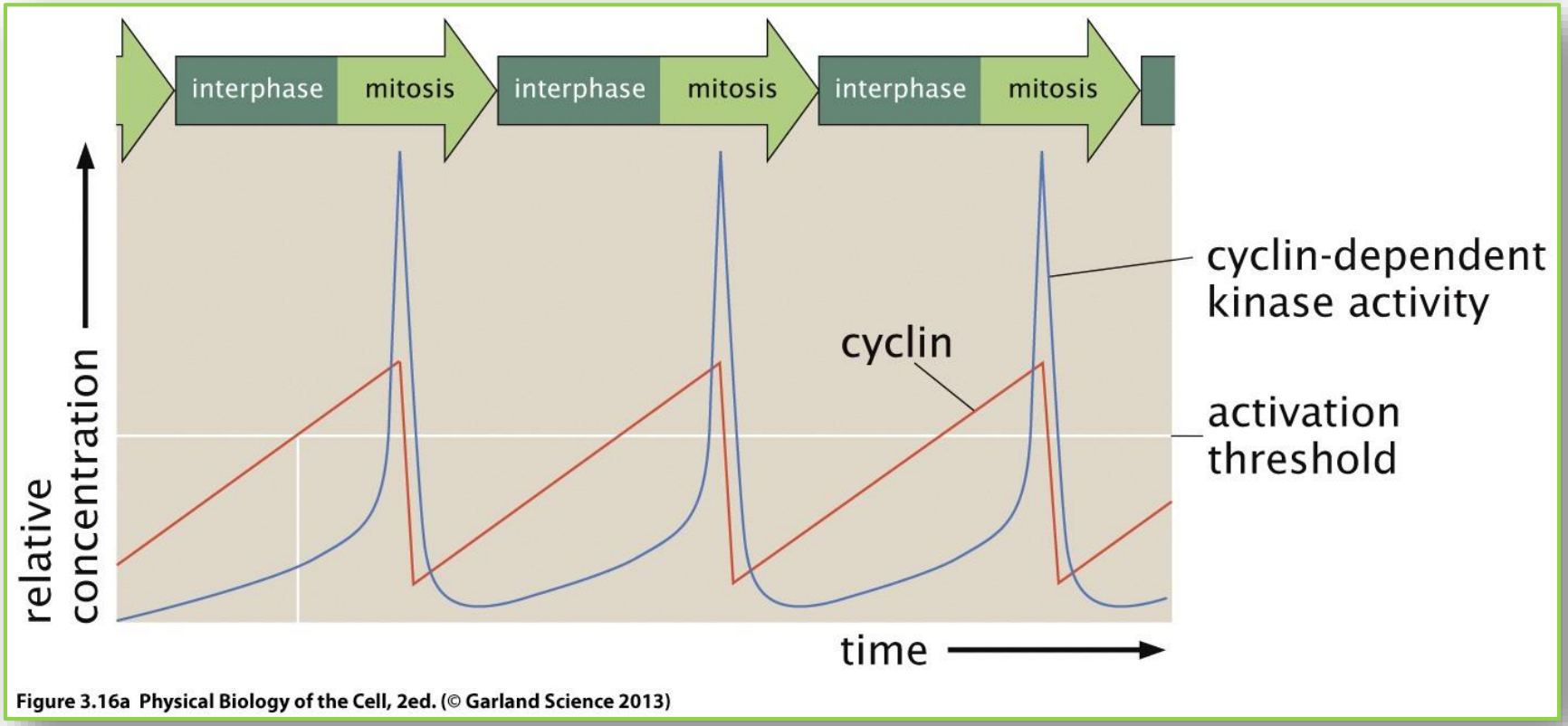


Figure 3.16a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Existance of positive regulator (cyclin) and negative regulator (cyclin dependent kinase), and feedback loop between them.

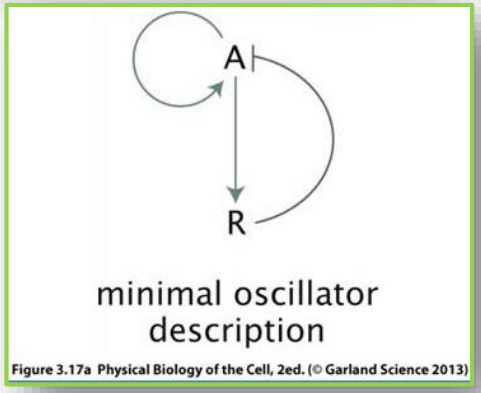
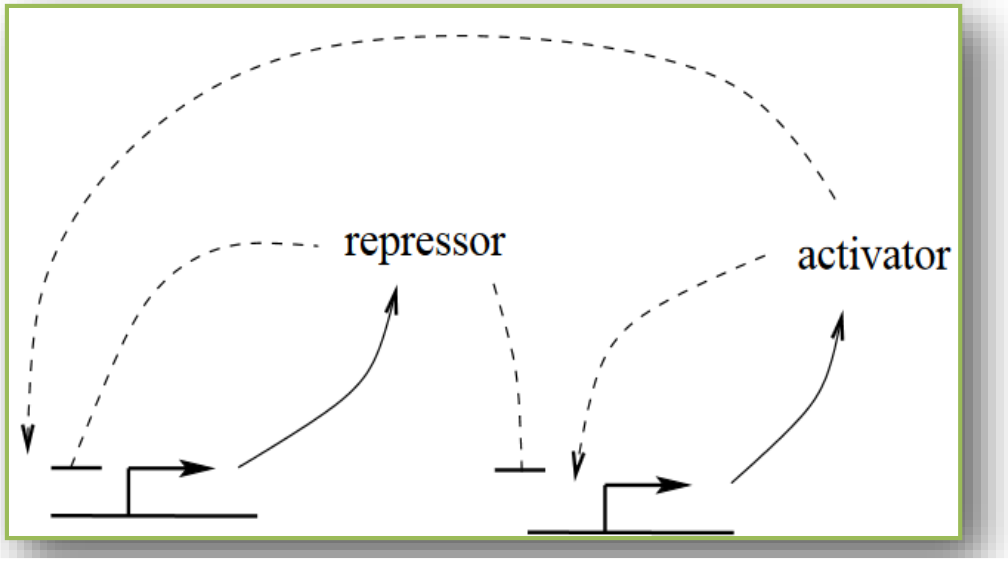


Figure 3.17a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Synthetic circle with relaxaton oscillations – larger robustness of the system with respect to delay oscilators



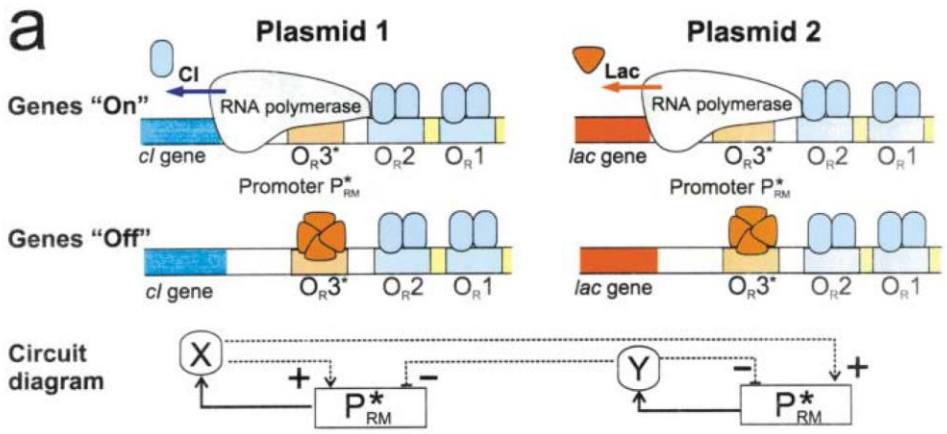


FIG. 1 (color). (a) Schematic for the synthetic gene oscillator. The P^*_{RM} promoter is a mutant of the P_{RM} promoter that naturally exists in the virus λ phage [16]. In its natural state, the state of the virus is regulated by CI dimers which bind to the three operator sites OR1, OR2, and OR3; in our design, the OR3 operator is replaced with an operator region OR3* which has an affinity only for Lac tetramers. The depicted position of the Lac operator site is for illustrative purposes only, since the ideal placement

Model:

$$\frac{d}{dt}x(t) = \frac{1 + x(t)^2 + \alpha\sigma x(t)^4}{(1 + x(t)^2 + \sigma x(t)^4)(1 + y(t)^4)} - \gamma_x x(t)$$

$$\frac{d}{dt}y(t) = a_y \frac{1 + x(t)^2 + \alpha\sigma x(t)^4}{(1 + x(t)^2 + \sigma x(t)^4)(1 + y(t)^4)} - \gamma_y y(t)$$

X – activator

y - repressor

$$O + 4Y \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} OY_4 \quad \Rightarrow \quad [OY_4] = [O][Y]^4(k_1/k_{-1}).$$

SS

$$O + 2X \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} OX_2 \qquad OX_2 + 2X \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} OX_4$$

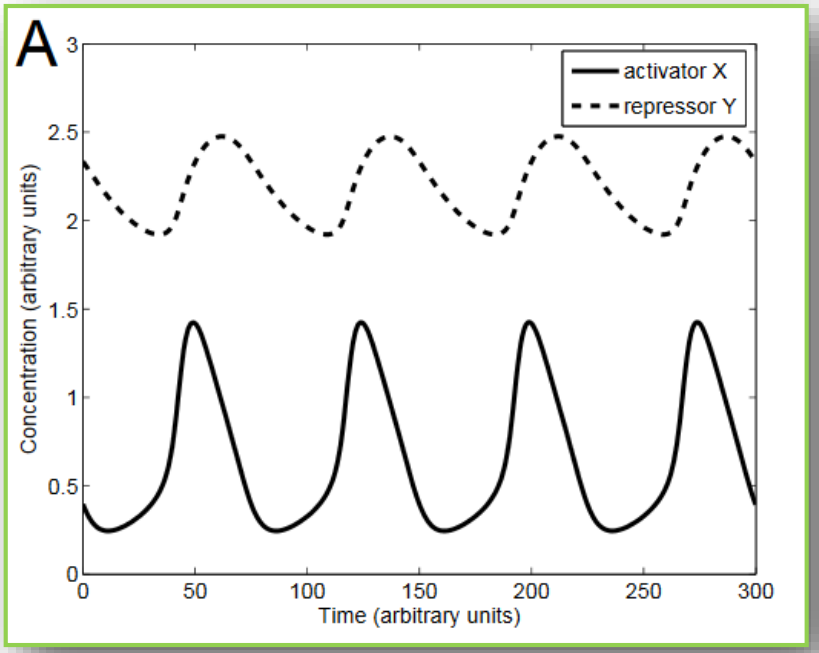
$$OY_4 + 2X \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} OY_4X_2 \qquad OY_4X_2 + 2X \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} OY_4X_4$$

$$\begin{aligned}
[OX_2] &= [O][X]^2(k_2/k_{-2}) \\
[OY_4X_2] &= [OY_4][X]^2(k_2/k_{-2}) = [O][Y^4][X]^2(k_2/k_{-2}) \\
[OX_4] &= [OX_2][X]^2(k_3/k_{-3}) = [O][X]^4(k_2/k_{-2})(k_3/k_{-3}) \\
[OY_4X_4] &= [OY_4X_2][X]^2(k_3/k_{-3}) = [O][Y]^4[X]^4(k_2/k_{-2})(k_3/k_{-3})
\end{aligned}$$

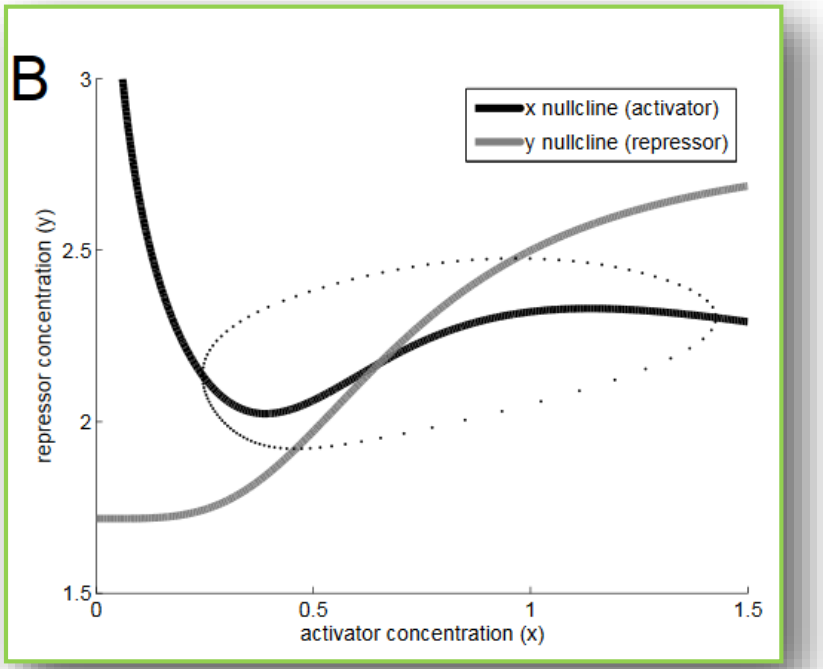
SS

σ

Model simulation



Persistent oscillations of activator and repressor



Limit cycle

Literature:

General:

Ingalls, B. P. (2013). *Mathematical modeling in systems biology: an introduction*. MIT press.

Strogatz, S. (2001). Nonlinear dynamics and chaos: with applications to physics, biology, chemistry, and engineering.

Restriction modification regulation:

Bogdanova, E., Djordjevic, M., Papapanagiotou, I., Heyduk, T., Kneale, G., & Severinov, K. (2008). *Nucleic acids research*, 36(5), 1429-1442.

Morozova, Natalia, et al. *Nucleic acids research* 44.2 (2016): 790-800.

Klimuk, E., Bogdanova, E., Nagornykh, M., Rodic, A., Djordjevic, M., Medvedeva, S., ... & Severinov, K. (2018). *Nucleic acids research*, 46(20), 10810-10826.

Oscilators: Elowitz, M. B., & Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. Nature, 403(6767), 335-338.

Hasty, J., Dolnik, M., Rottschäfer, V., & Collins, J. J. (2002). Synthetic gene network for entraining and amplifying cellular oscillations. Physical Review Letters, 88(14), 148101.

Stricker, J., Cookson, S., Bennett, M. R., Mather, W. H., Tsimring, L. S., & Hasty, J. (2008). *Nature*, *456*(7221), 516-519.