Lecture 2: Modeling intracellular dynamics (introductory)

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Regulation

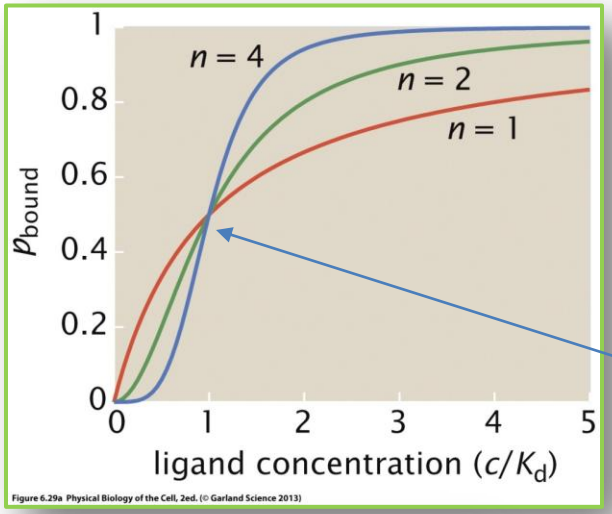
- **Feedback loops**
- **Cooperativity**
- **Alostery**

→ **Relation of output to input**

Input changes with time (e.g. concentration of TF increaes) → **Output changes with time** → **Regulatory dynamics**

Example of an inherently dynamical system: Circadian oscillators

Reminder/excercise first lecture



$$p = \frac{\left([A]/K_d\right)^n}{1+\left([A]/K_d\right)^n}$$

$$\frac{dp}{dA} = \frac{n}{4K_d} \qquad \text{(calculated at } A = K_d\text{)}$$

$$\frac{dp}{dA} = \frac{n}{4} \qquad \text{for } K_d = 1$$

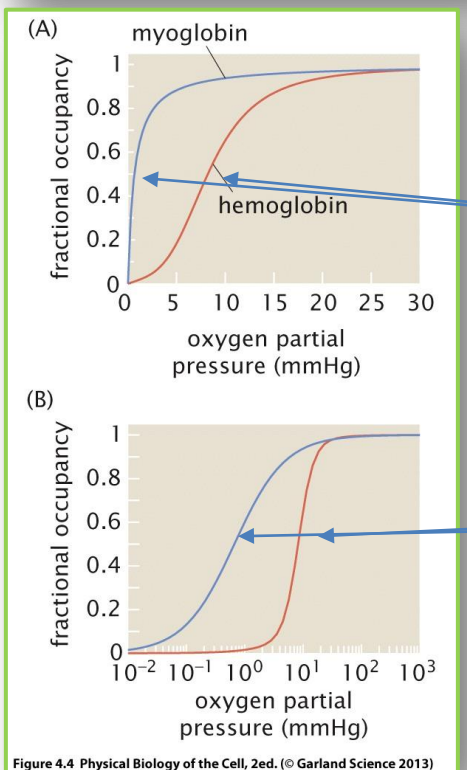


Figure 4.4 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$\frac{dp}{dA} = \frac{n}{4K_d}$$

Note that dissociation constant is much smaller in the case myoglobin compared to hemoglobin

$$\frac{dlog(p)}{dlog(A)} = \frac{n}{2}$$

Plotting things on log scale eliminates K_d and "isolates" the effect of differences in cooperativity (n)

Change of concentration with time

$$\frac{dc_i(t)}{dt} = f\left(c_1, c_2, \ldots, c_n; k_1, k_2, \ldots, k_m\right)$$

$c_1, c_2, \ldots, c_n$ **Relevant concentrations**

$k_1, k_2, \ldots, k_m$ **Rates of chemical reactions**

What is the form of *f* ?

Degradation of molecules

$$\frac{dc(t)}{dt} = -kc(t)$$

Dependence of concentration on time

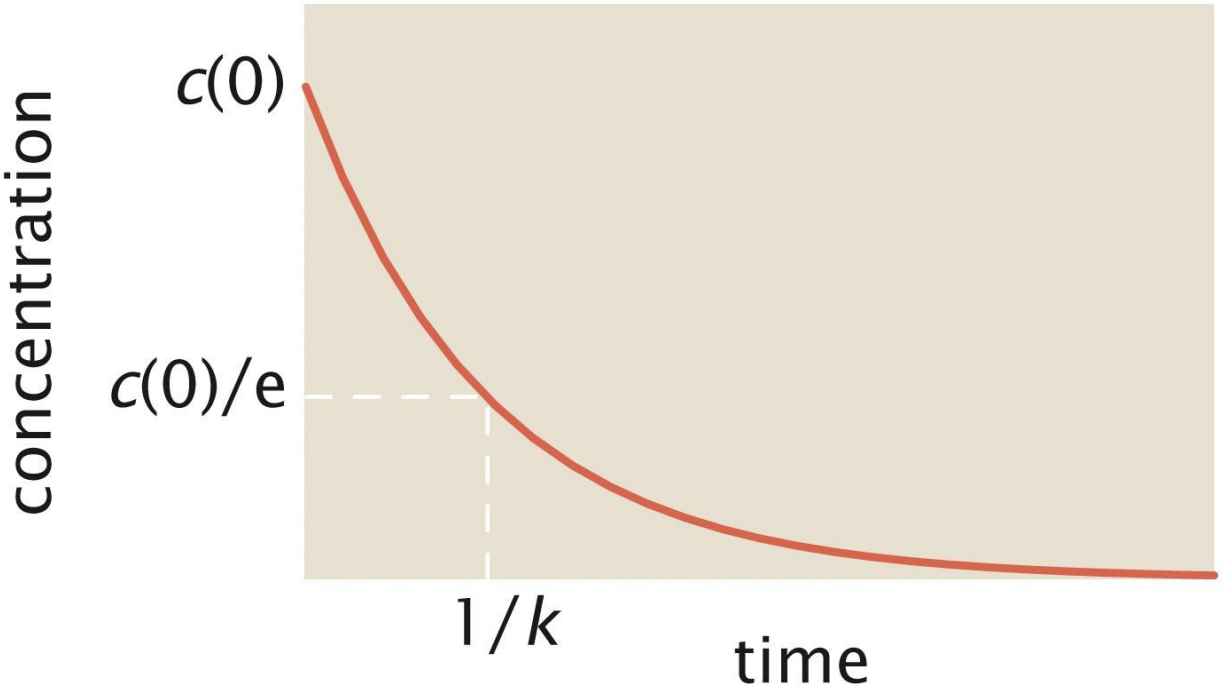


Figure 15.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$c(t) = c_0 \exp(-t/\tau)$$

$$\tau = 1/k$$

Characteristic decay time

Reversible reaction

$$A \Leftrightarrow B$$

$$\frac{dc_A}{dt} = -k_+ c_A + k_- c_B \qquad \frac{dc_A}{dt} = -\frac{dc_B}{dt}$$

Bimolecular reactions

$$A + B \rightarrow AB$$

$$\frac{dc_{AB}(t)}{dt} = k_{AB} c_A c_B$$

From dynamics to equilibrium

$$L + R \Leftrightarrow LR$$

$$\frac{d[LR]}{dt} = -k_{off}[LR] + k_{on}[L][R]$$

In the case of equuilibrium:

$$-k_{off}[LR]_{eq} + k_{on}[L]_{eq}[R]_{eq} = 0$$

$$K_d = \frac{[L]_{eq}[R]_{eq}}{[LR]_{eq}} = \frac{k_{off}}{k_{on}}$$

Raction dissociation constant

Approximation of unequilibrium system by equilibrium

$$A \underset{k_-}{\overset{k_+}{\rightleftarrows}} B \xrightarrow{r} C$$

$$k_+, k_- \gg r$$

Unequilibrium process

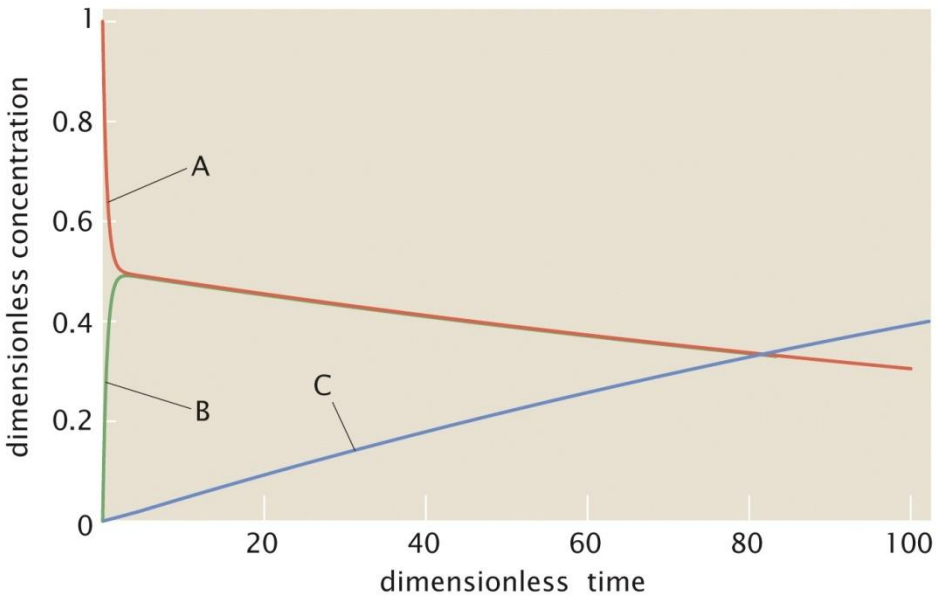
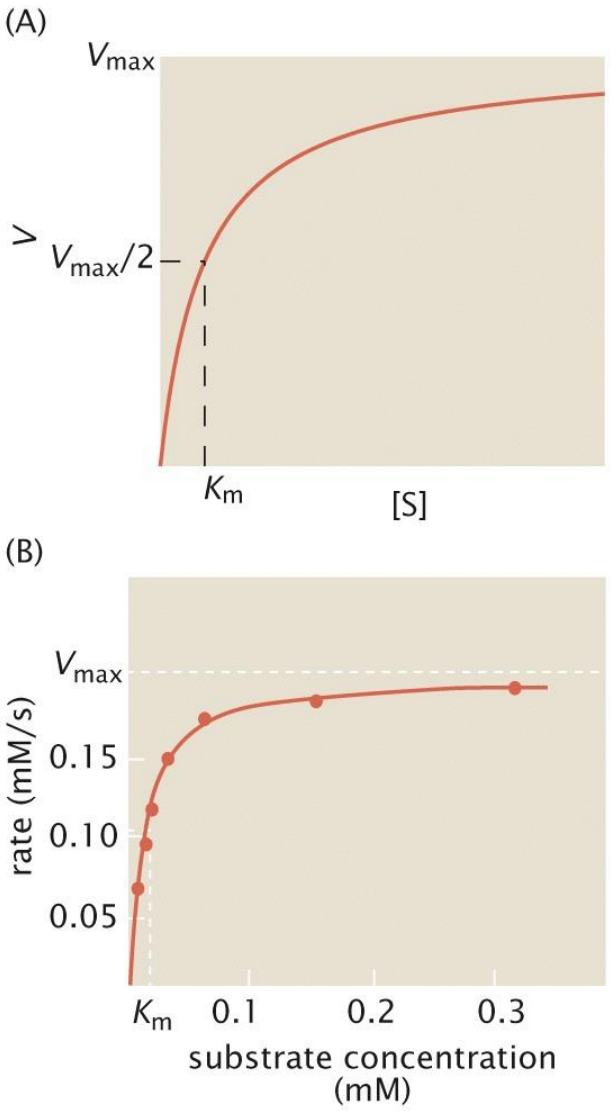


Figure 15.14 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

However, A and B make equilibrium very fast

$$B(t)/A(t) = k_+/k_-$$

Michaelis-Menten kinetics



$$E + S \underset{k_-}{\overset{k_+}{\rightleftarrows}} ES \xrightarrow{r} E + P$$

Assumption: $k_+, k_- \gg r$

$$\frac{d[P]}{dt} = r[ES] = V_{\max}\frac{[S]/K_m}{1+[S]/K_m}$$

Michaelis Menten kinetics

$$[S] \ll K_m \Rightarrow \frac{d[P]}{dt} = V_{\max}[S]/K_m$$

The mass action low

Speed of ATP hydrolisis by myosine

Example 1: CRISPR/Cas – Advanced bacterial immune systems

CRISPR transcript processing

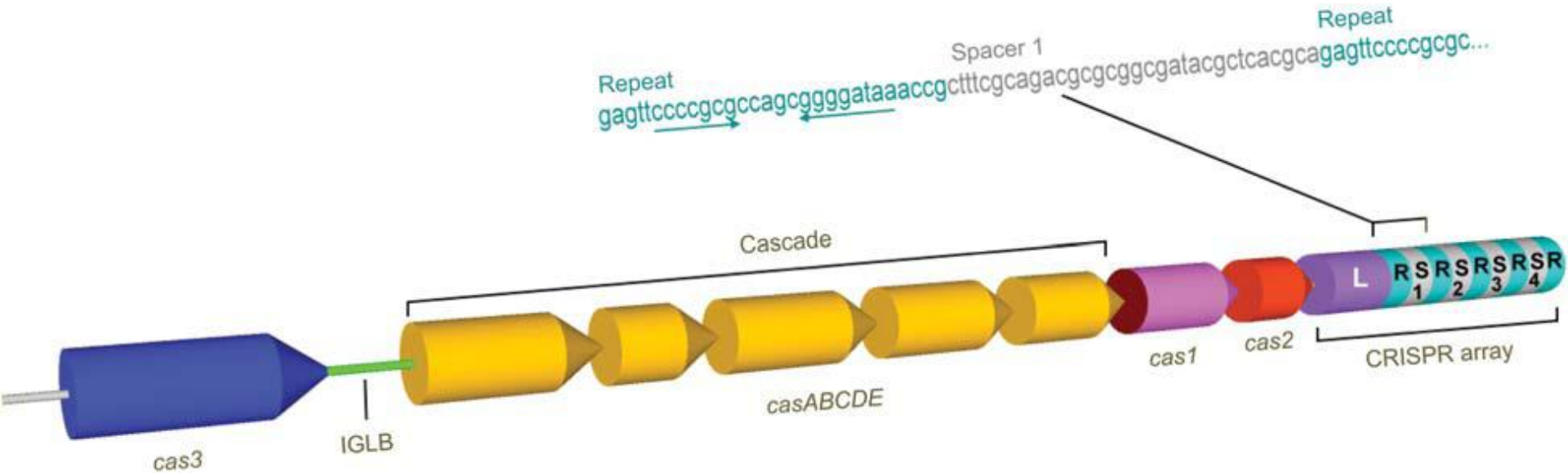
CRISPR/Cas

Advanced bacterial immune system which is based on expression of small RNA molecules

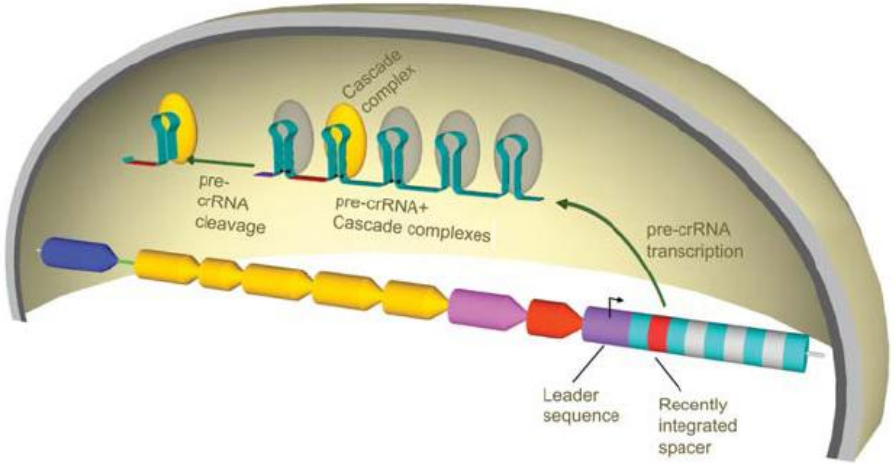
First theoreticaly predicted (bioinformatics + specific model) and subsequently experimentaly confirmed

CRISPR/Cas system

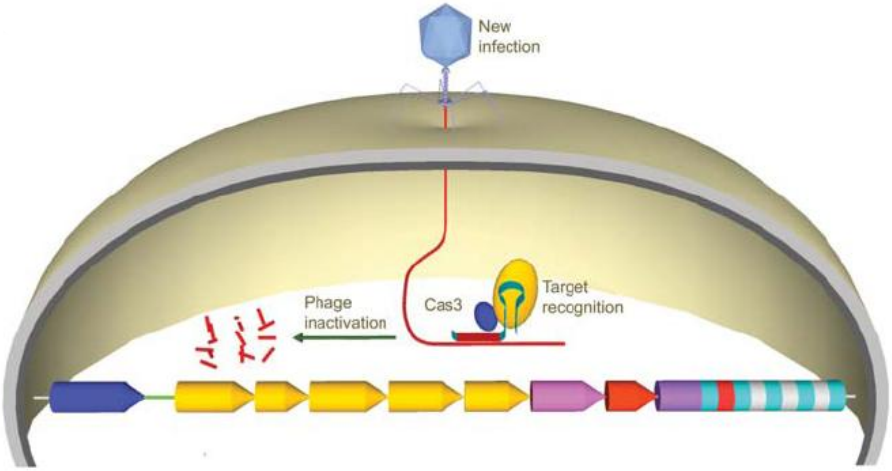
- **CRISPR/Cas = CRISPR + Cas proteins**
- **CRISPR array: sequences which are repeated “R” are seprated by variable sequences “S”**



Mechanism of resistance to infection



CRISPR array is transcribed as a long transcripts (pre-crRNA), which are then processed to small RNA molecules (crRNA) by Cas proteins.

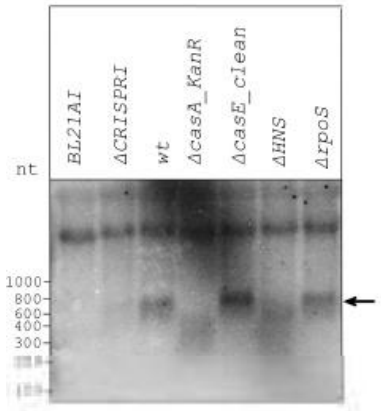


As soon as crRNA recognizes virus sequence, Cas proteins will be recruited to the target, and the virus DNA is destroyed.

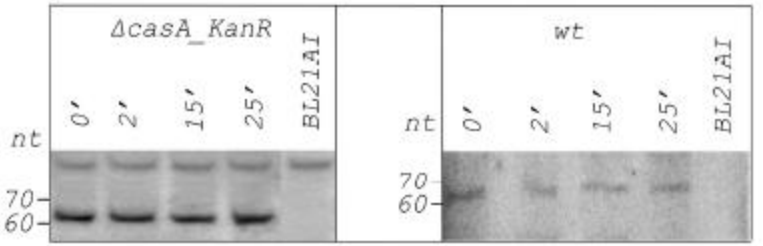
CRISPR transcript processing

Cas gene expression

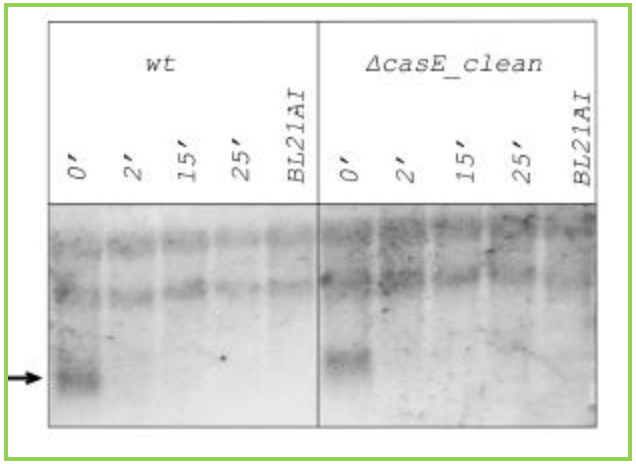
pre-crRNA decreases for factor less than 10



Much larger (more than two orders of magnitude) increase of crRNA.



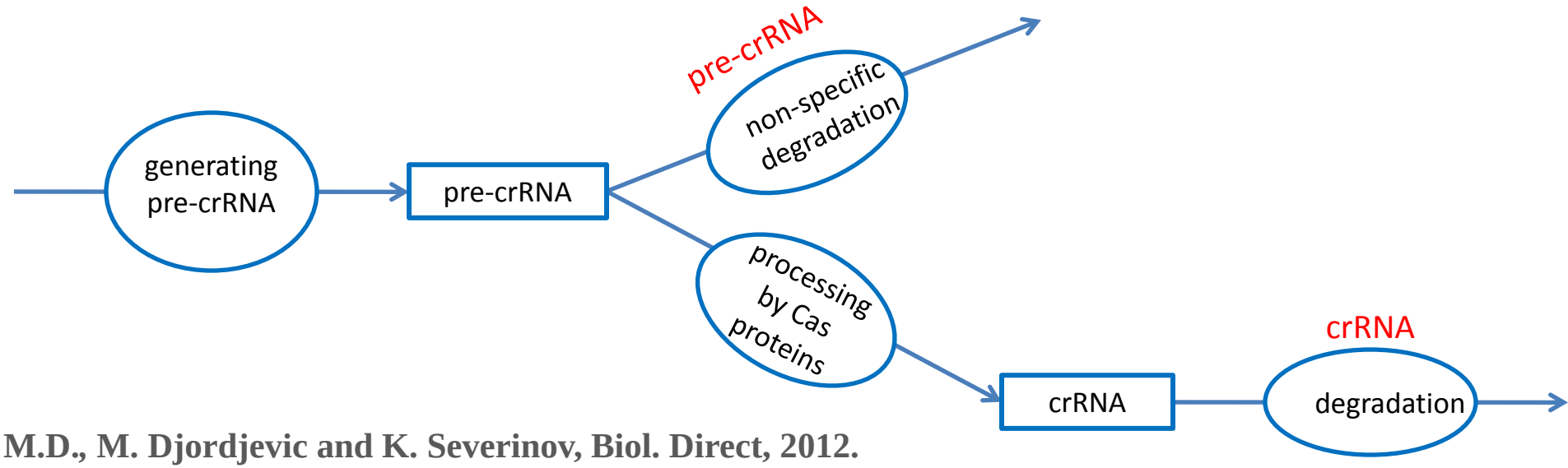
crRNAs are very stable!



pre-crRNAs are very stable, which is a consequence of non-specific degradation.

Pougach et. al., Mol. Microbiol., 2010

Model of CRISPR transcript processing



M.D., M. Djordjevic and K. Severinov, Biol. Direct, 2012.

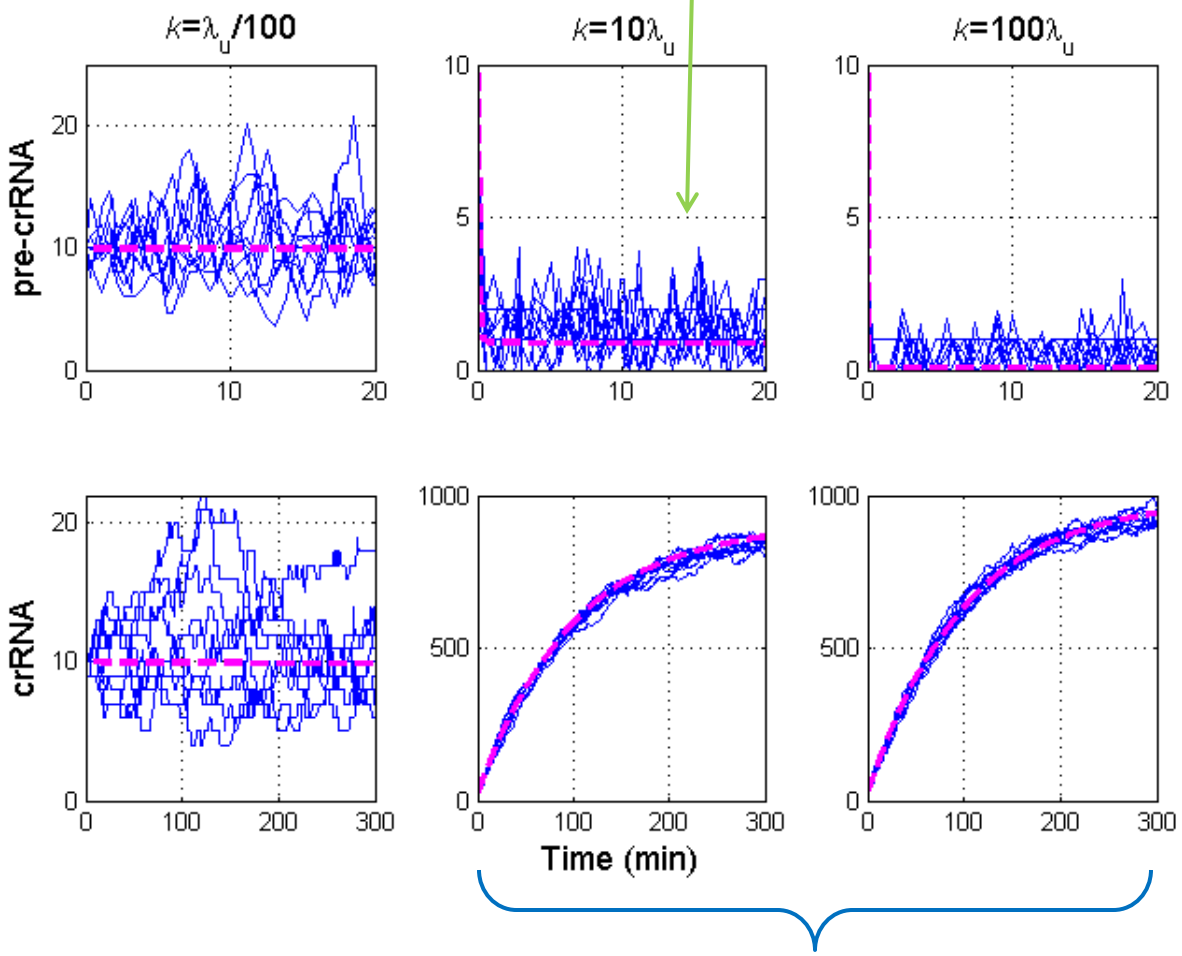
Expression of *cas* genes leads to strong linear amplification of transcripts:

$$\Delta[crRNA] = -\frac{\lambda_{pre-crRNA}}{\lambda_{crRNA}}\Delta[pre-crRNA]$$

Interestingly this strong amplifications crucialy depends on non-specific processing of pre-crRNA transcripts.

Expression of *cas* genes

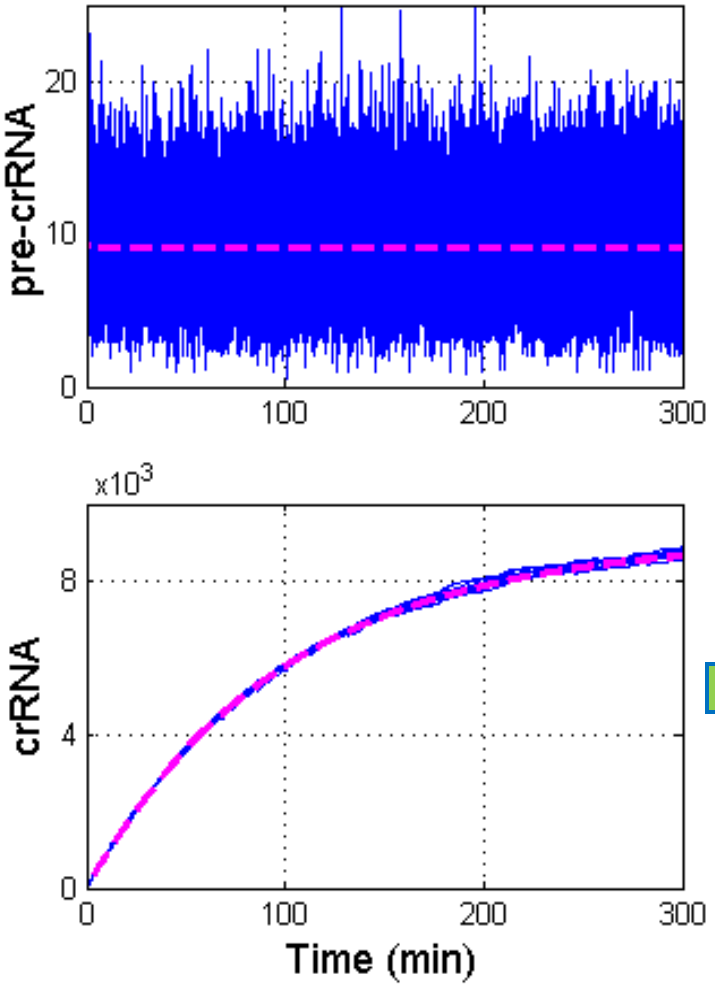
Experimental conditions



saturation of crRNA amount

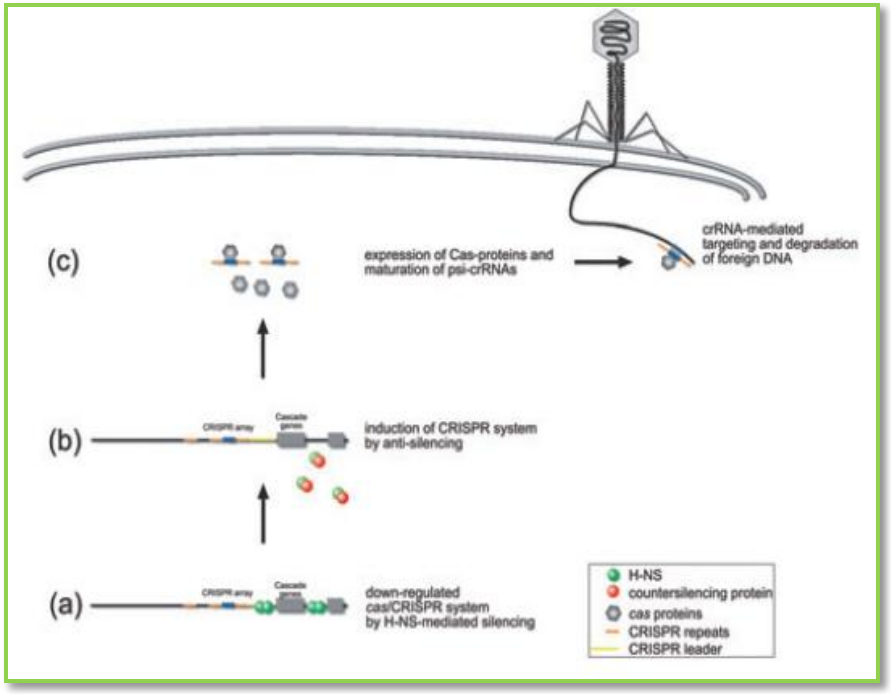
M.D., M. Djordjevic and K. Severinov, Biol. Direct, 2012.

Joint increase of transcription and processing rates



Saturation in crRNA can be reliewed if pre-crRNA production is also increased. This joint increase can lead to very large production of crRNA.

Repression by H-NS



Promoters for CRISPR and *cas* genes are repressed by H-NS-a.

Joint increase of processing and pre-crRNA generation rate is probably directly relevant for functioning of the system in natural conditions.

Conclusion CRISPR/Cas

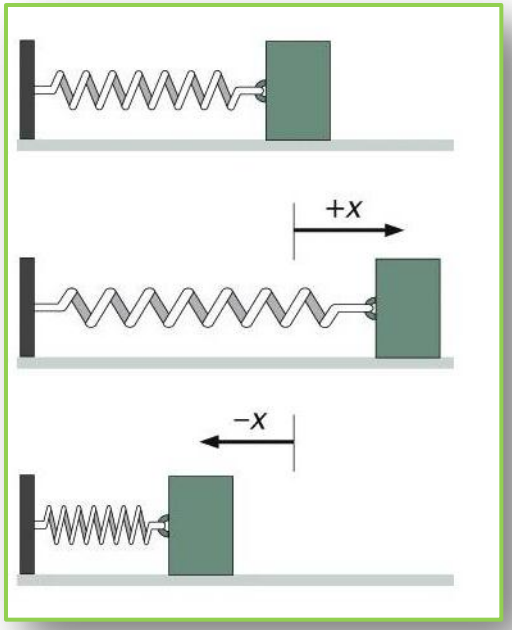
- **System can generate very large quantities of crRNA very fast.**
- **This fast production is based on control of the system at the level of RNA processing, and on tuning the system parameters (e.g. on extreme transcript stability values).**
- **Unindentified nuclease which is responsible for fast pre-crRNA degradation is probably the most important control element of CRISPR/Cas.**

Example 2: Oscillatory systems

Oscillatory systems

- **Many cell processes are repeated in regular manner.**
- **Two important examples are cell cycle and circadian rhythms (track change between day and night).**
- **Biological oscillators are typically complex, but can often be reduced to feedback between activator and receptor.**

Inertia/Oscillations



Mechanical oscillations are based on inertia.

Inside cell (in cytoplasm) inertia plays a minor role, due to a large viscosity.

Oscillations inside cell are based on biochemical reactions and feedback loops

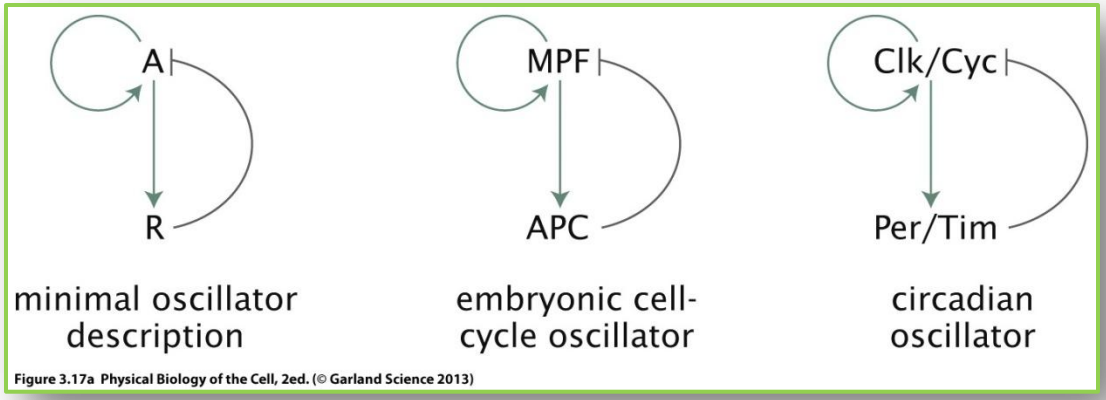


Figure 3.17a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Oscillator in cell cycle

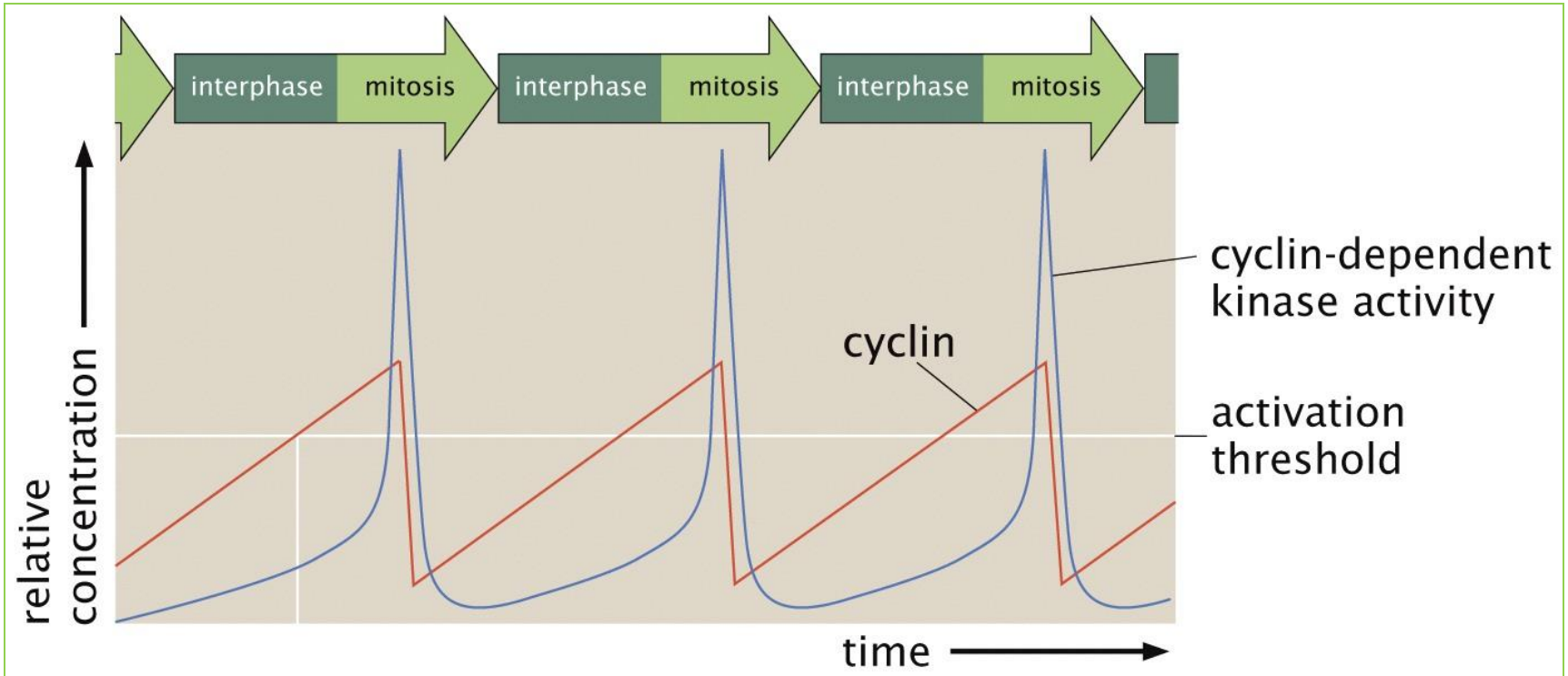


Figure 3.16a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

What is crucial is existance of a positive regulator (cyclin) and negative regulator (cyclin dependent kinase), and feedbacks between them.

Circadian oscillators

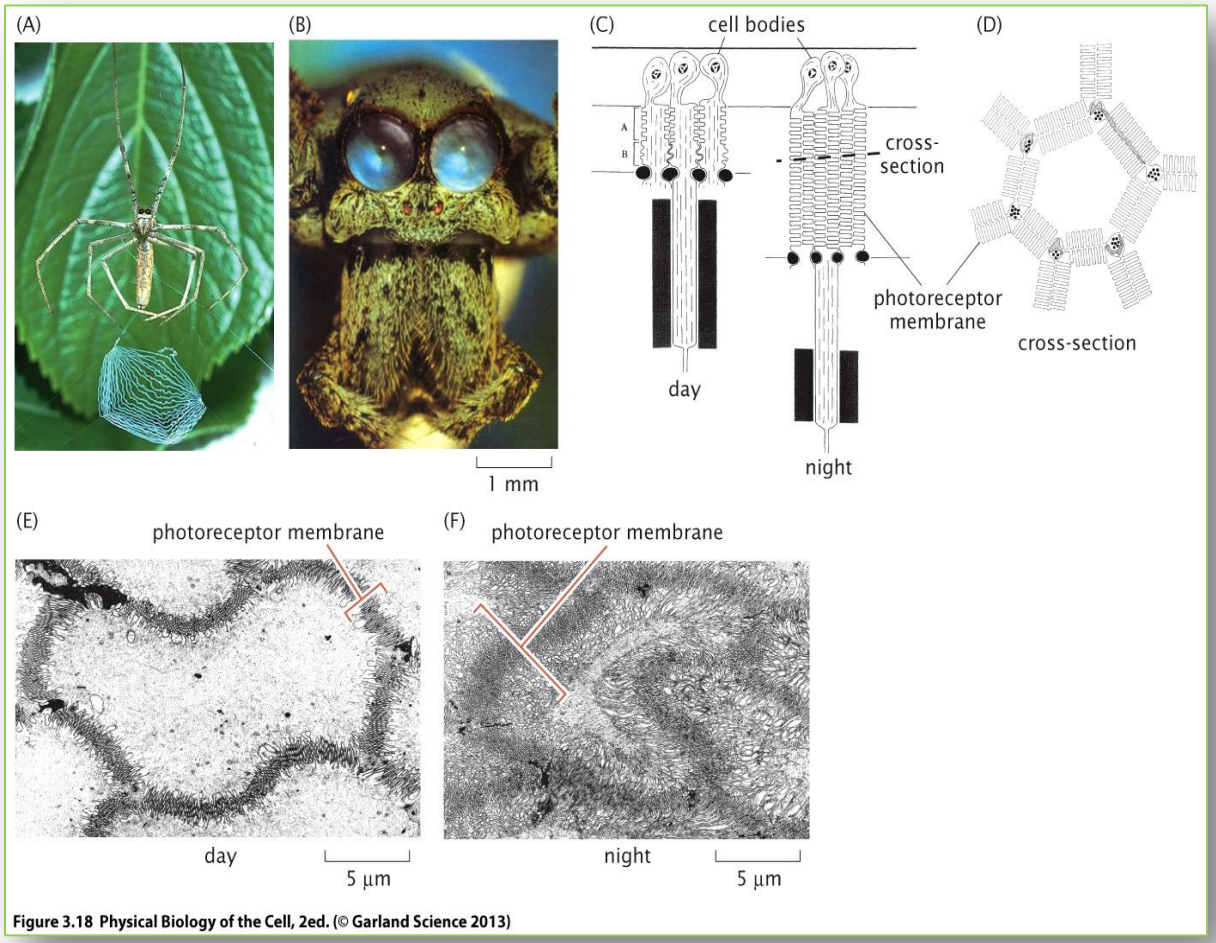


Figure 3.18 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Make track of changes between day and night – an extreame example are photoreceptor cells in this spider

Different biological processes – the same mechanism

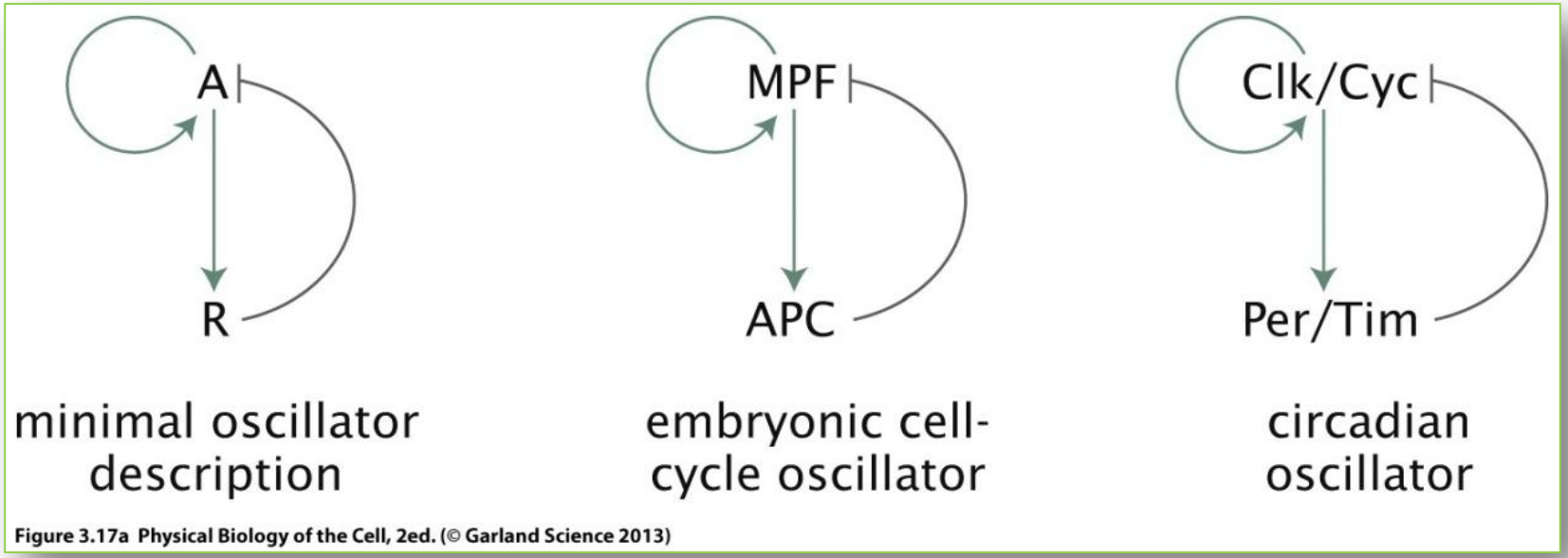


Figure 3.17a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Feedback loop between activator and repressor leads to oscillations.

One of the major goals of systems biology: Understand common design principles behind mechanistically otherwise different biological systems.

"For the greatest benefit to mankind"
Alfred Nobel

The Nobel Assembly at Karolinska Institutet has today decided to award the

2017 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

to:

Illustrations: Niklas Elmehed. Nobel Prize Medal: © Nobel Foundation. Photo: Lovisa Engblom.

Jeffrey C. Hall
Michael Rosbash
Michael W. Young

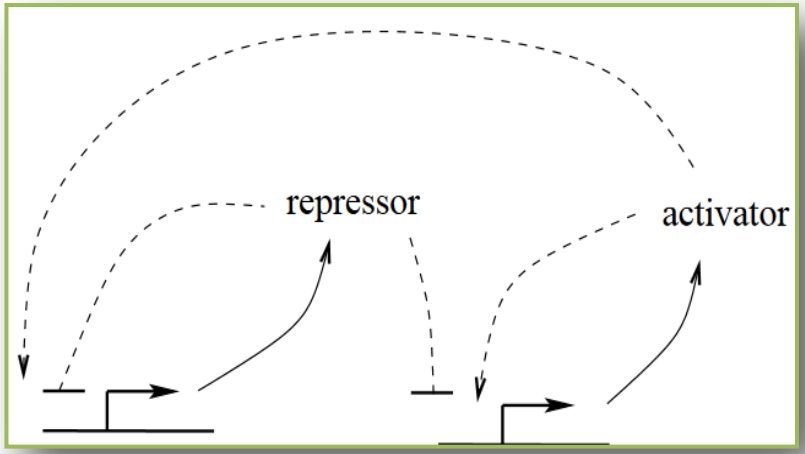
"for their discoveries of molecular mechanisms controlling the circadian rhythm"

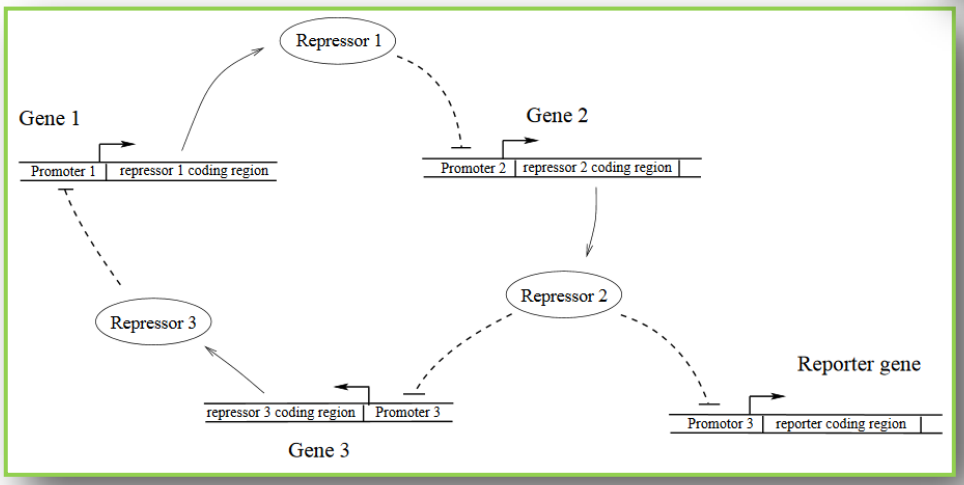
Nobelprize.org

More on oscillators

Working session 2: Biological rhythms (genetic oscillators): delay and relaxation oscillator

Relaxation oscillator – combination of positive and negative feedback, basically the type of oscillator that we talked about today.





Delay oscillator – just negative feedback, but with the delay

Literature:

General: The same as in the first lecture

CRISPR transcript processing:

Djordjevic, M., Djordjevic, M., & Severinov, K. (2012). *Biology direct*, *7*(1), 24.

Djordjevic, M., & Djordjevic, M. (2012). *Physical biology*, *9*(5), 056004.

Biological oscillators (more on this on Working session 3 – Friday):

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.

Bistable switches&bistability&bifurcations (Working session 2-today):

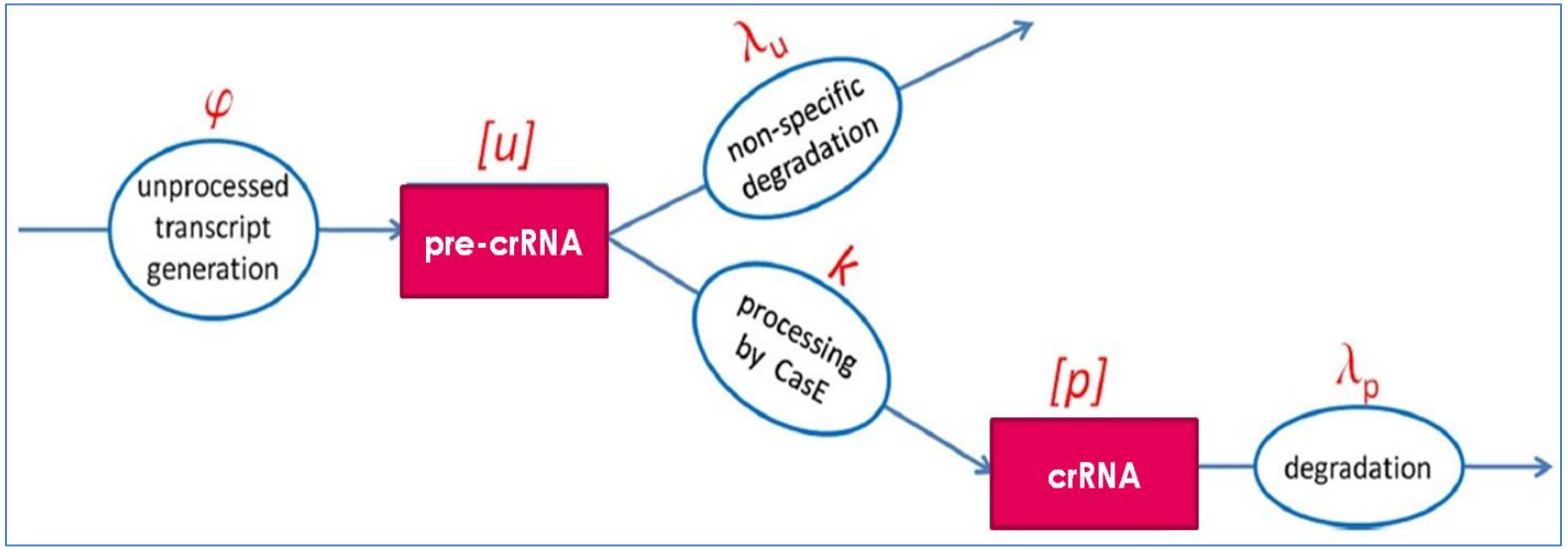
Gardner, T. S., Cantor, C. R., & Collins, J. J. (2000). Construction of a genetic toggle switch in Escherichia coli. Nature, 403(6767), 339-342.

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.

Problem

The following kinematic scheme is given:



a) Write differential equations that determine amounts of pre-crRNA and crRNA transcripts. Assume the following:

$u - pre - crRNA\,amount$

$p - crRNA\,amount$

$\lambda_u - pre - crRNA\,decay\,rate$

$\lambda_p - crRNA\,decay\,rate$

$\varphi - pre - crRNA\,transcription\,rate$

$k - pre - crRNA\,y\,crRNA\,transcription\,rate$

b) Write equations that determine equilibrium pre-crRNA and crRNA amounts.

c) Assume that the system is induced so that Cas proteins are expressed so that the degradation rate of pre-crRNA to crRNA is increased from k to k', while all other parameters remain the same. Show that there is the following relationship between the change of pre-crRNA and crRNA.

$$\Delta[p] = -\frac{\lambda_u}{\lambda_p}\Delta[u]$$

d) Simulate the system dynamics, that is reproduce the crRNA dynamics shown in slide 15. The parametes are:

$\varphi = 10$ nM/min $\quad \lambda_u = 1$ 1/min $\quad \lambda_p = 1/100$ 1/min