Part II

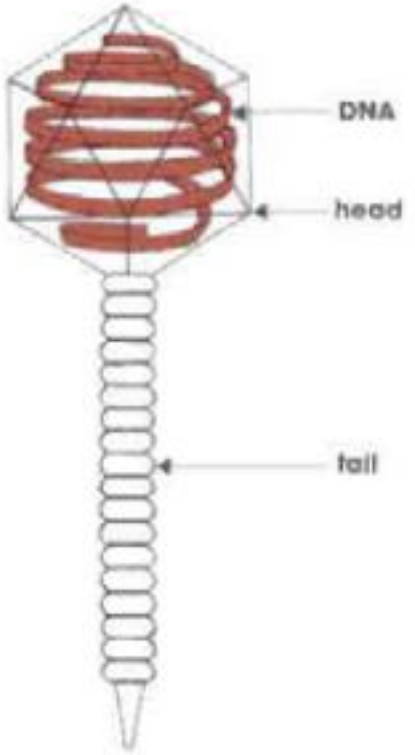
Simple network, complex function

Positive feedback and multistability

weeks 2-3,2019

The lysis-lysogeny switch in λ phage

The goal of this section is to apply our knowledge of reaction kinetics and equilibrium binding to a real biological problem: the lysis-lysogeny decision of the bacteria λ phage.

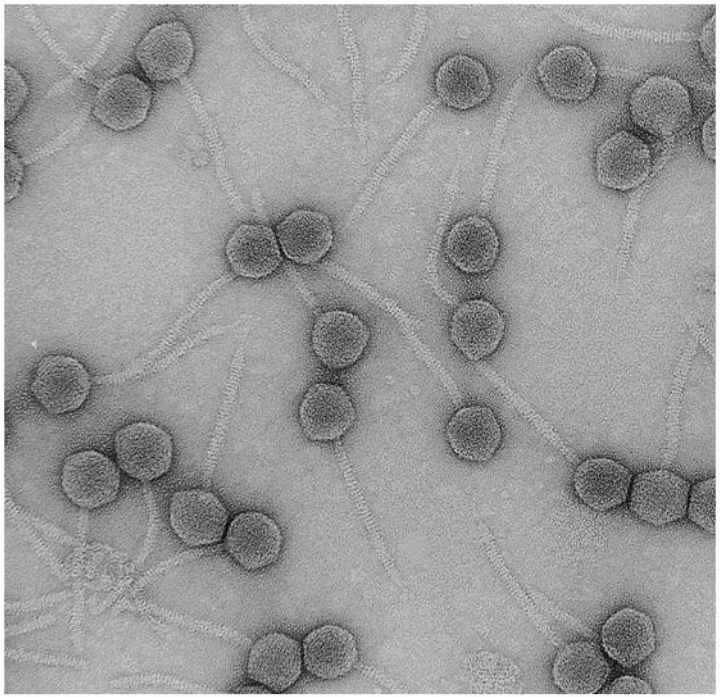


Excellent book:

A genetic switch by Mark Ptashne

λ phage

λ phage is a bacteriophage -- a virus which infects *E. coli*.



A Genetic Switch, 3rd edition, 2004
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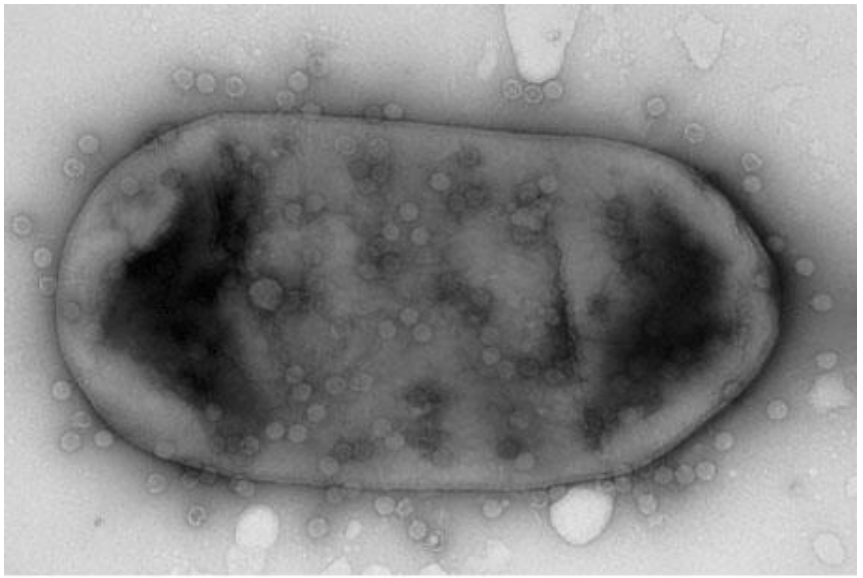
Introduction, Figure 1a



A Genetic Switch, 3rd edition, 2004
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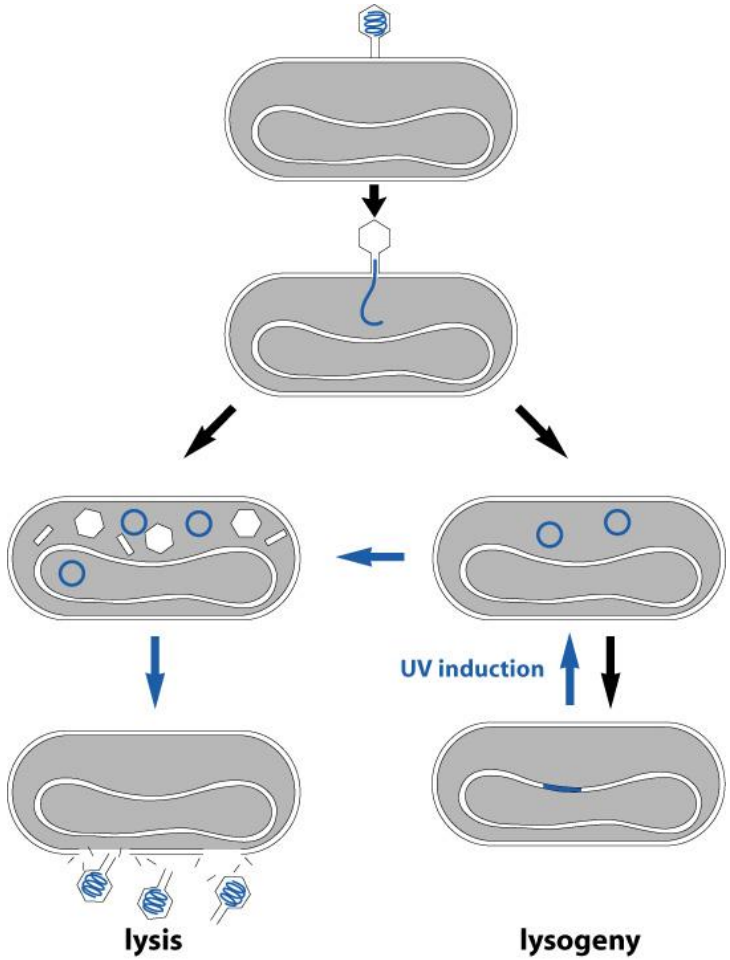
Introduction, Figure 1b

Upon infection, the phage has two mechanisms of action:

Lytic Growth, in which the host's genetic machinery is used to produce ~100 new phages, and then the host cell is lysed (broken).



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Chapter 1, Figure 2

Lysogeny, in which the phage chromosome is integrated into the host's genome. The phage then dormantly infects all progeny, as its genome (called the **prophage**) is replicated when the host divides.

The phage “chooses” between these two mechanisms based on a “reading” of the host’s behavior.

If the host is growing well the phage lysogenizes the host and subsequently infects all of its progeny.

If the host is not growing well (e.g. starving), the phage grows lytically - an 'abandon ship' response.

This “decision” is based on a genetic switch

The switch is composed of two genes and their products:

Gene *cI* which codes for **repressor**;

high *cI* lead to lysogeny

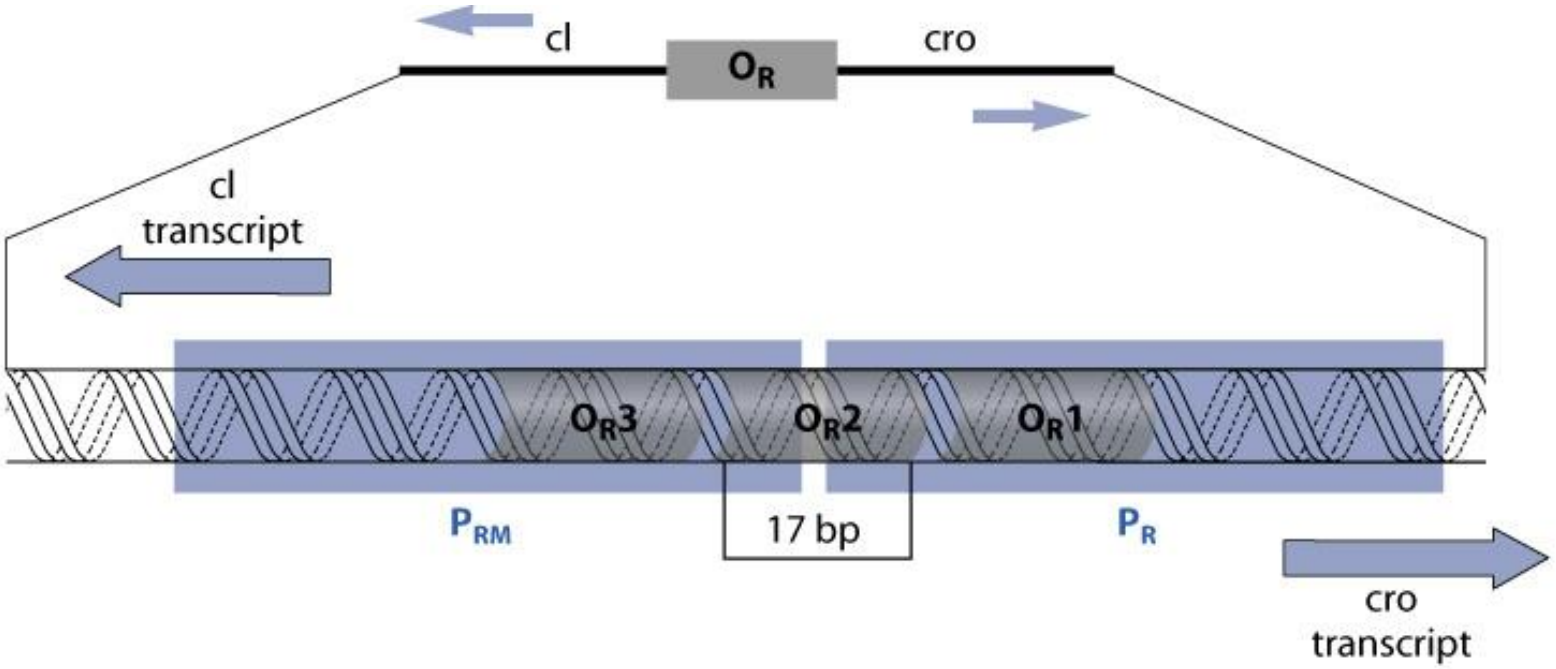
Gene *cro* which codes for **Cro** (**c**ontrol of **r**epressor and **o**thers)

high *cro* leads to lysis

These genes are adjacent to one another on the phage genome:

P_R (the right promoter) is the promoter for *cro*

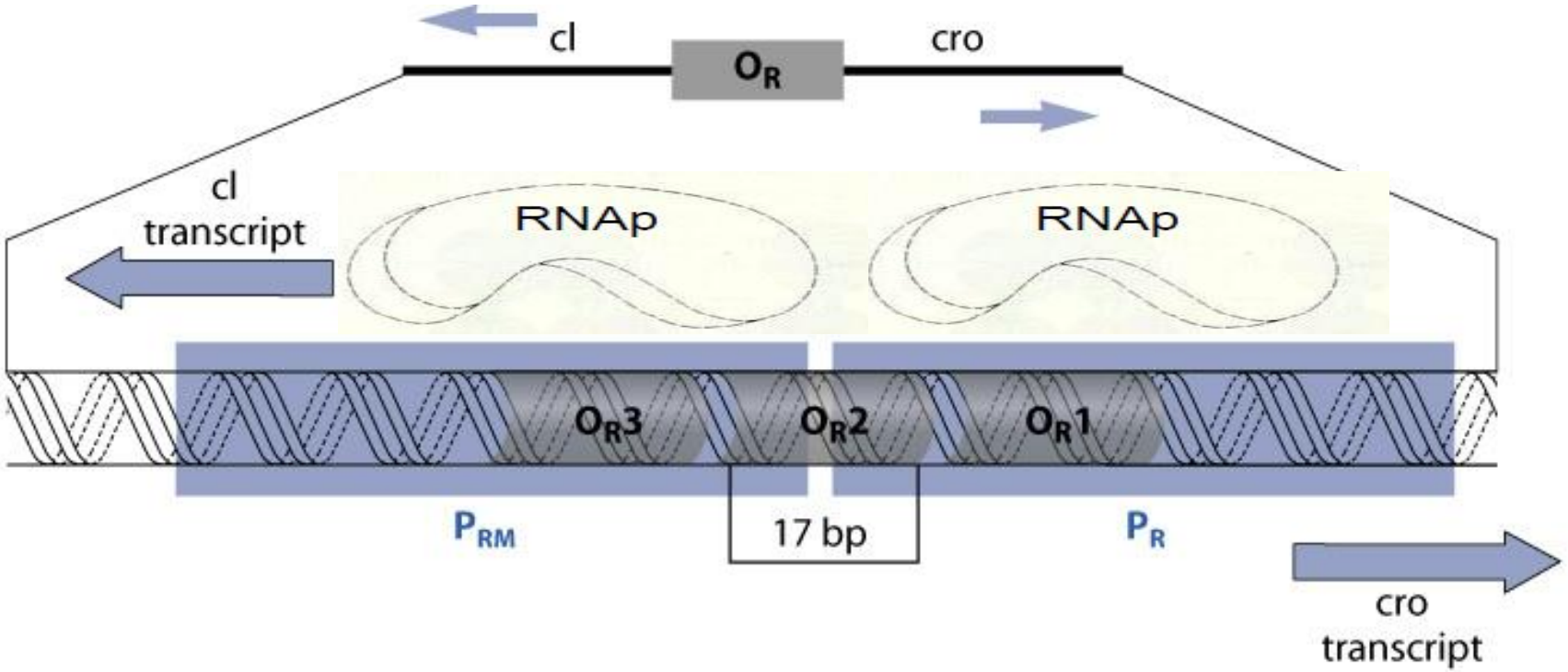
P_{RM} (repressor maintenance) is the promotor for *cI*



A Genetic Switch, 3rd edition, 2004

Chapter 1, Figure 4

Both *cI* and *cro* regulate gene expression by binding to the **right operator** O_R, which is divided into three operator regions: O_R1, O_R2 and O_R3



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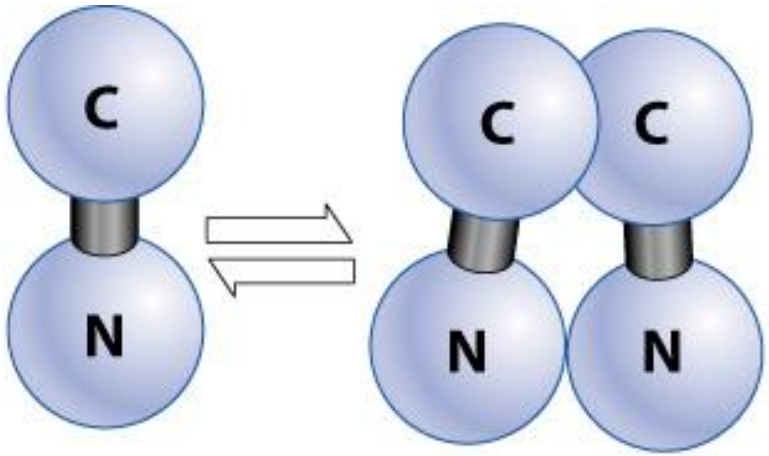
Chapter 1, Figure 4

Only space for one RNA polymerase in each blue area (mutual exclusion)

Repressor protein c1 is predominantly present as a dimer.



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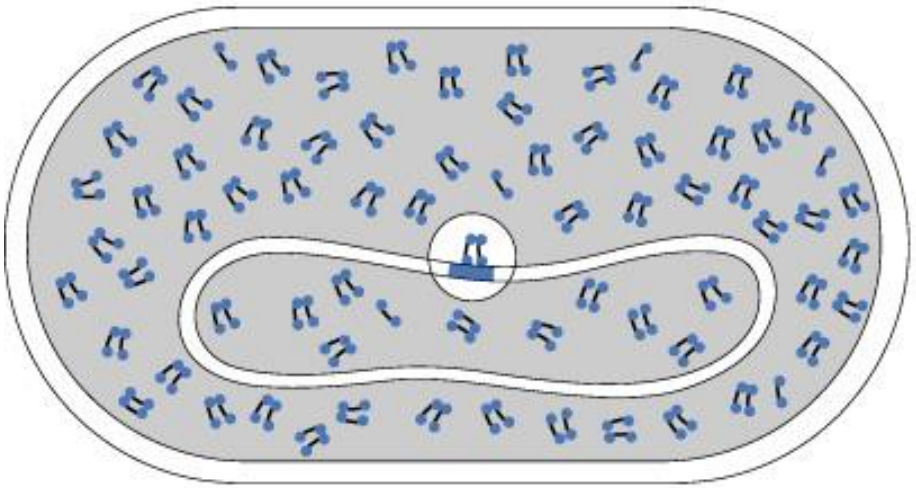
Chapter 1, Figure 7



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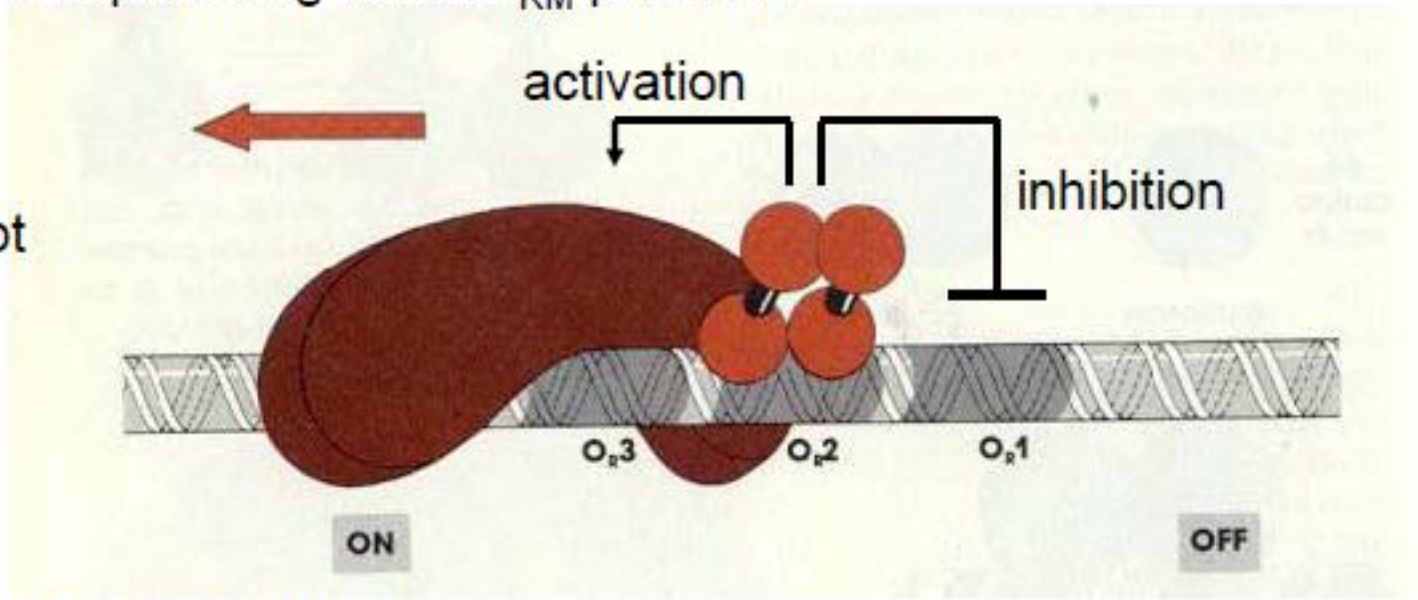
Chapter 1, Figure 8

Single repressor dimer bound - three cases:

I Negative control, dimer binding to OR2 inhibits RNAp binding to right P_R promoter.

Positive control, dimer binding to OR2 enhances RNAp binding to left P_{RM} promoter.



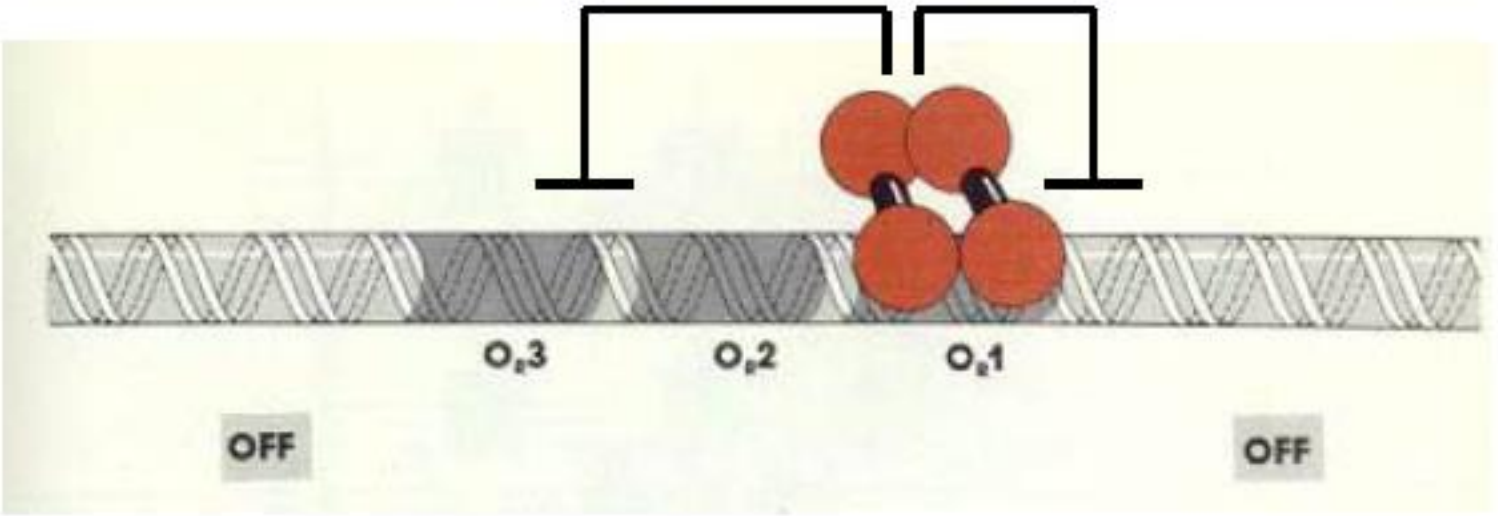
cI transcript

more repressor monomers

more repressor dimers

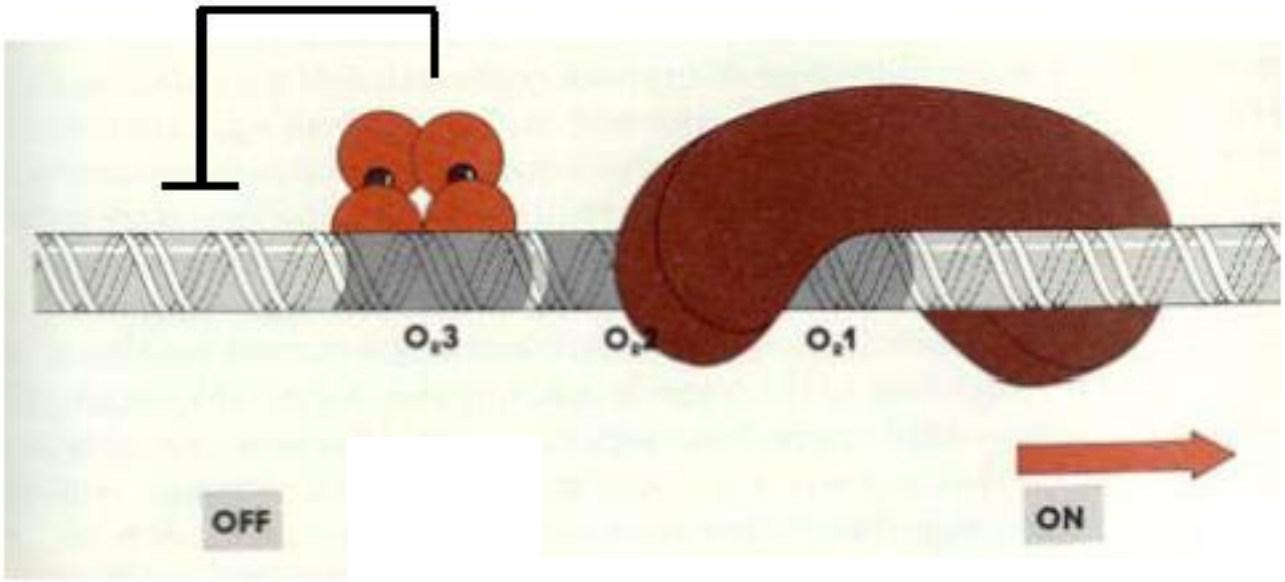
II Negative control, dimer binding to OR1 inhibits RNAp binding to right P_R promoter.

Negative control, dimer binding to OR1 inhibits RNAp binding to left P_{RM} promoter (too distant).



III Negative control, dimer binding to OR3 inhibits RNAp binding to left P_{RM} promoter.

Positive control, dimer binding to OR3 allows RNAp binding to right P_R promoter.

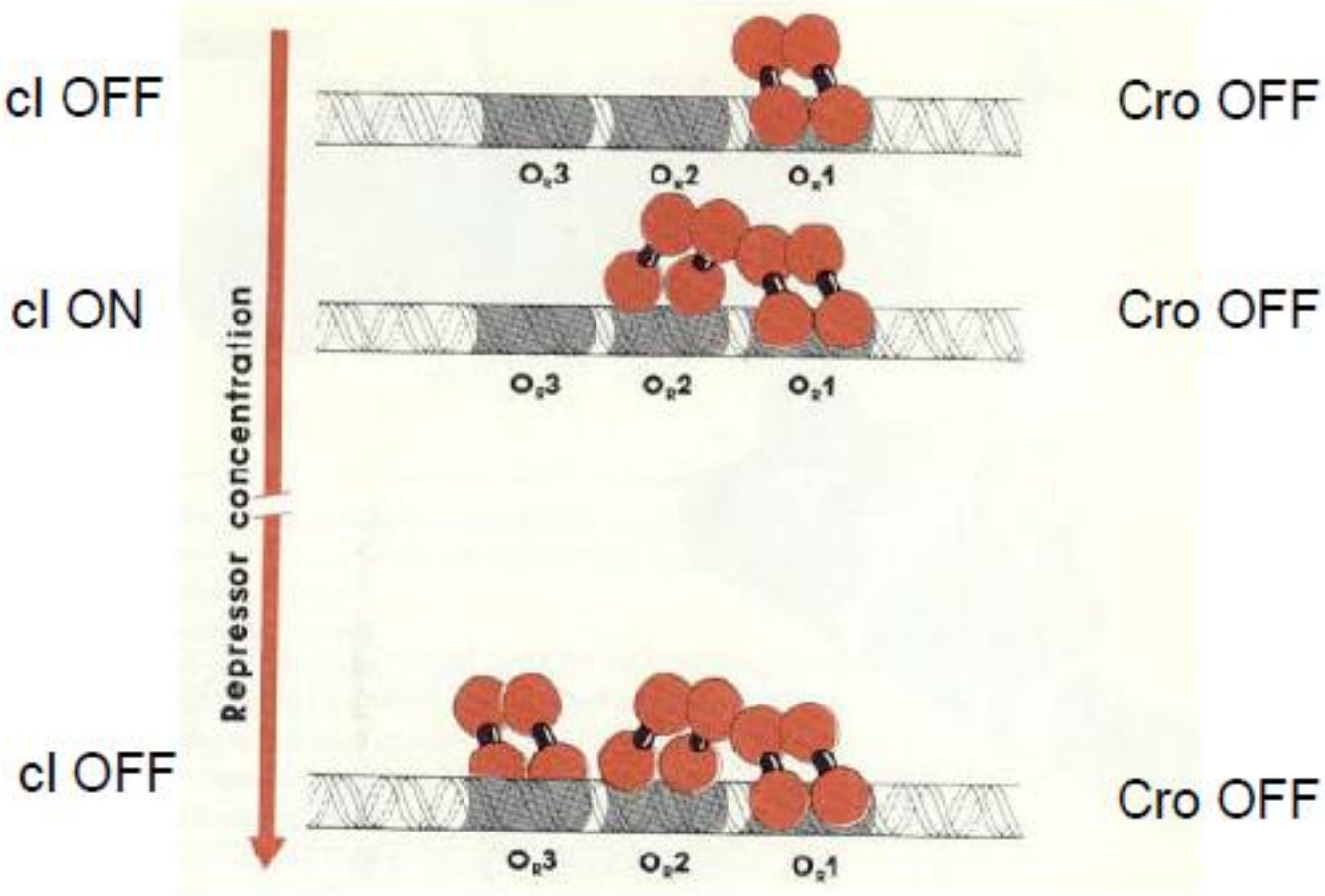


Repressor-DNA binding is highly cooperative

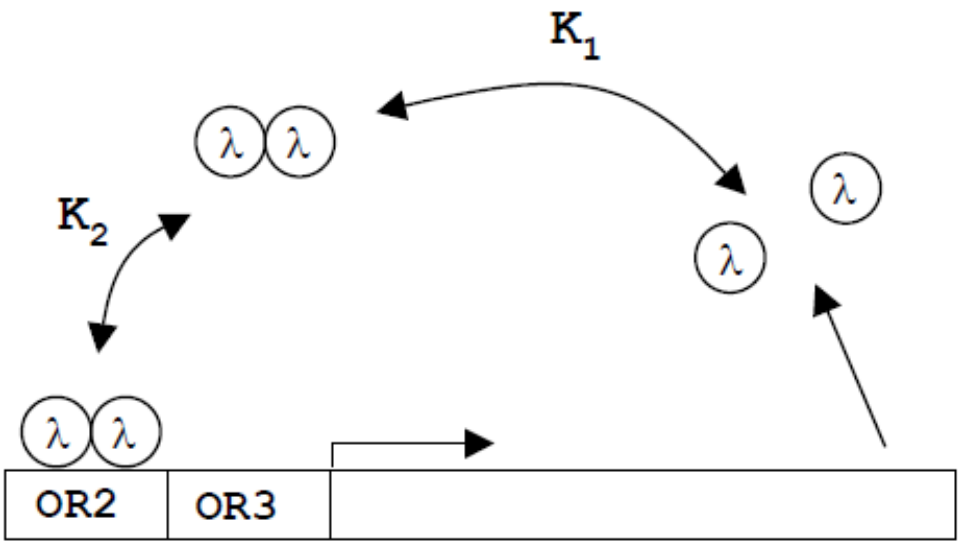
intrinsic association constants:

$K_{OR1} \sim 10\ K_{OR2} \sim 10\ K_{OR3}$

However $K_{OR2}^{*} >> K_{OR2}$ (positive cooperativity)

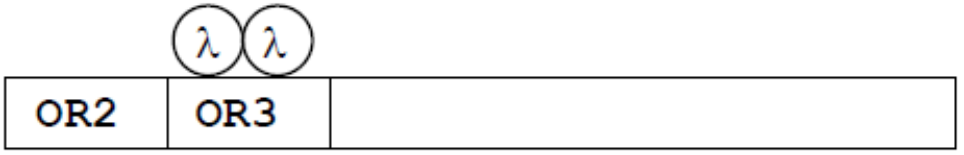


Jeff Hasty (2000, PNAS) proposed a simplified version

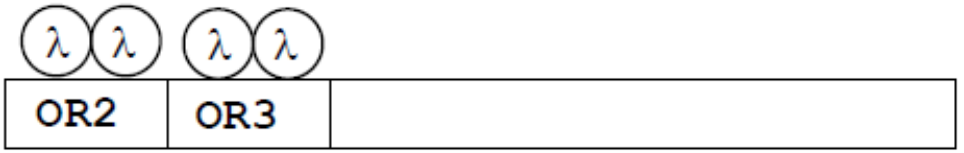


Possible binding states for the λ promoter considered by Hasty. In the unbound state the gene is switched off.

ON

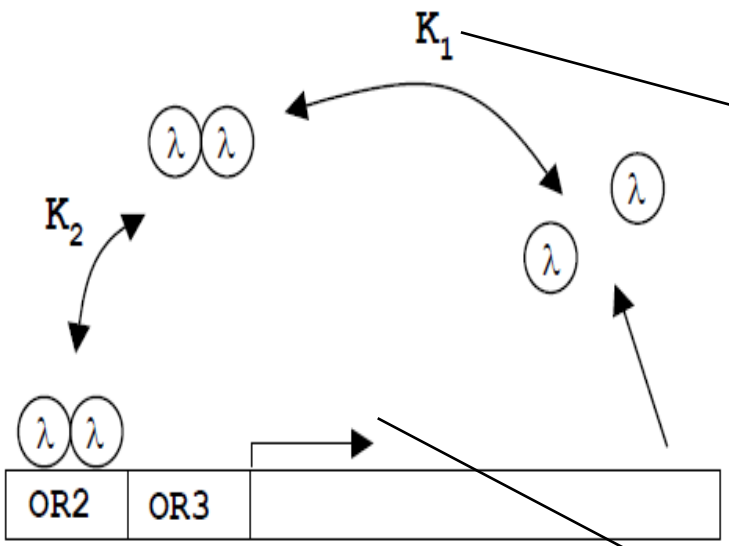


OFF



OFF

Derive the reactions from gene regulation model



λ: X

λλ: X_2

D: promoter

DX_2

DX_2*

DX_2X_2*

$$2X \xleftrightarrow{K_1} X_2$$

$$D + X_2 \xleftrightarrow{K_2} DX_2$$

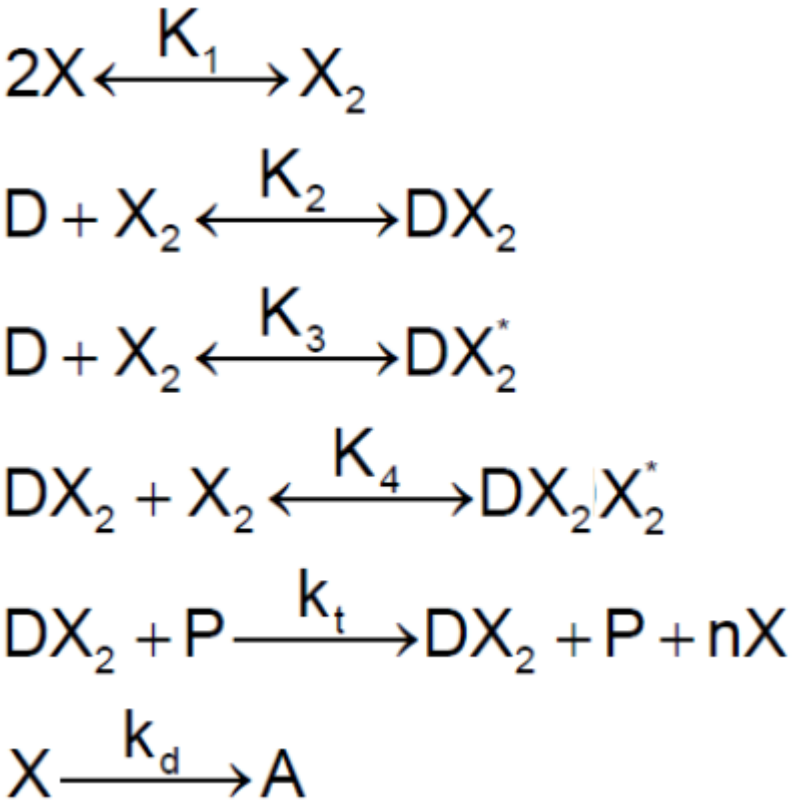
$$D + X_2 \xleftrightarrow{K_3} DX_2^*$$

$$DX_2 + X_2 \xleftrightarrow{K_4} DX_2X_2^*$$

$$DX_2 + P \xrightarrow{k_t} DX_2 + P + nX$$

$$X \xrightarrow{k_d} A$$ *degradation*

Simplify the mathematic model



Protein-protein and protein-DNA binding are fast: ~seconds

Protein synthesis and degradation is slow: ~min-hour

Reaction 1-4 can be simplified as steady-state. Only ODE is for X.

Simplify the mathematic model

X=[X];y=[X_2]; d=[D]; u=[DX_2];v=[DX_2*];z=[DX_2X_2*]; p_0=[P]
$\sigma_1=K_3/K_2;\sigma_2=K_4/K_2$

$$2X \xleftrightarrow{K_1} X_2 \qquad y=K_1x^2$$

$$D + X_2 \xleftrightarrow{K_2} DX_2 \qquad u=K_2dy=K_1K_2dx^2$$

$$D + X_2 \xleftrightarrow{K_3} DX_2^* \qquad v=K_3dy=\sigma_1K_1K_2dx^2$$

$$DX_2 + X_2 \xleftrightarrow{K_4} DX_2X_2^* \qquad z=K_4uy=\sigma_2(K_1K_2)^2dx^4$$

$$DX_2 + P \xrightarrow{k_t} DX_2 + P + nX$$

$$X \xrightarrow{k_d} A$$

$$\frac{dx}{dt} = nk_t p_o u - k_d x + r$$

Simplify the mathematic model

$$d_T = d + u + v + z = d[1 + (1 + \sigma_1)K_1K_2x^2 + \sigma_2K_1^2K_2^2x^4]$$

$$\frac{dx}{dt} = \frac{nk_T p_0 d_T K_1K_2x^2}{1 + (1 + \sigma_1)K_1K_2x^2 + \sigma_2K_1^2K_2^2x^4} - k_d x + r$$

Clean up the equation by setting $\bar{x} = x\sqrt{K_1K_2}, \quad \bar{t} = t(r\sqrt{K_1K_2})$

$$\frac{dx}{dt} = \frac{\alpha x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} - \gamma x + 1$$

$$\alpha = \frac{nk_t p_0 d_T}{r}, \qquad \gamma = \frac{k_d}{r\sqrt{K_1K_2}}$$

Ratio of synthesis rate relative to the basal expression

Ratio of degradation rate relative to the basal expression

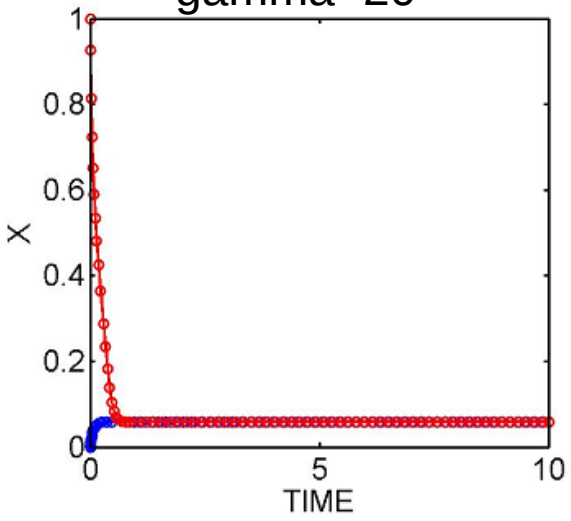
Matlab code for solving the equation

```
% filename: hastyfunc.m

function dydt = f(t,y,flag,alpha,gamma,sigma1,sigma2)
% [x] = y(1)

dydt = [alpha*y(1)^2/(1+(1+sigma1)*y(1)^2+sigma2*y(1)^4)-gamma*y(1)+1];
```

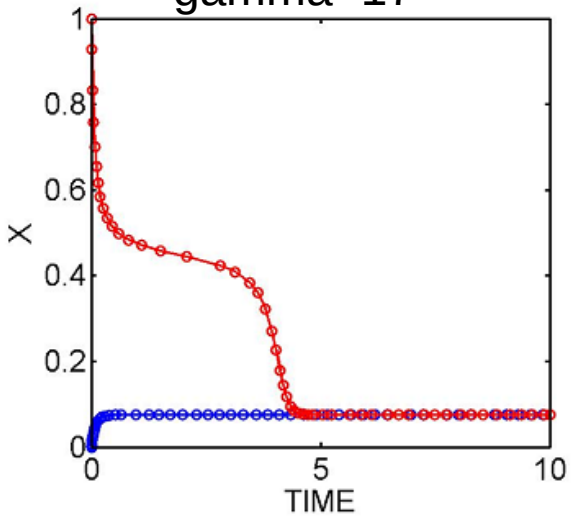
```
% filename hasty.m  solving ODE with two initial c1 values
alpha=50;
gamma=13.8;
%gamma=13.828; %gamma=16.96;
sigma1=1; % both OR2 and OR2 has same affinity
sigma2=3; % binding to OR2 increase binding affinity to OR3: coopertivity
options=[];

[t1 y1]=ode23('hastyfunc',[0 10],[0],options,alpha,gamma,sigma1,sigma2);
[t2 y2]=ode23('hastyfunc',[0 10],[1],options,alpha,gamma,sigma1,sigma2);
plot(t1,y1(:,1),'o-b',t2,y2(:,1),'o-r');
ylabel('X');
xlabel('TIME');
```

Lysis or lysogeny?
depends on degradation and initial c1 value

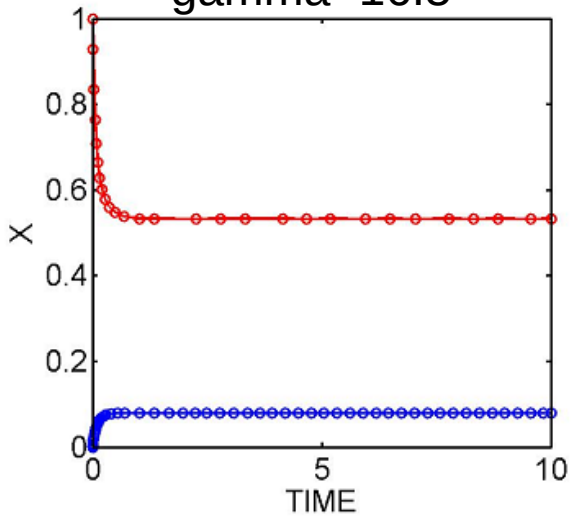
gamma=20



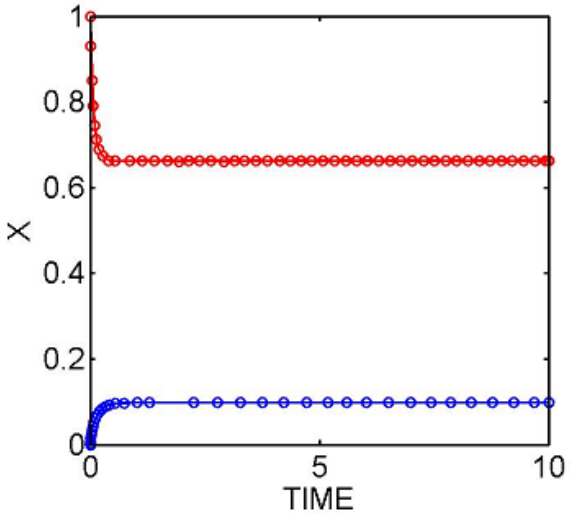
gamma=17



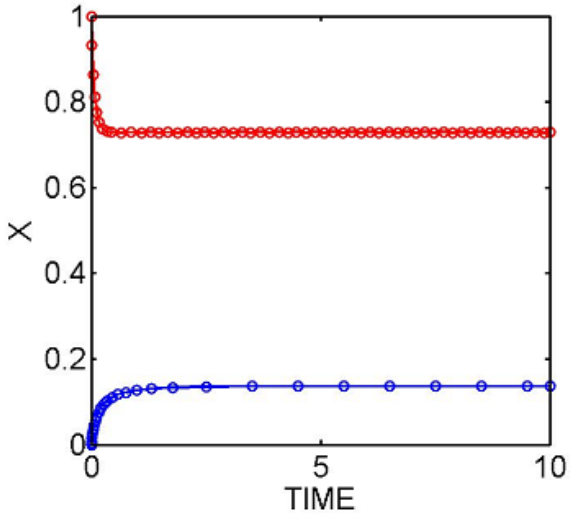
gamma=16.5



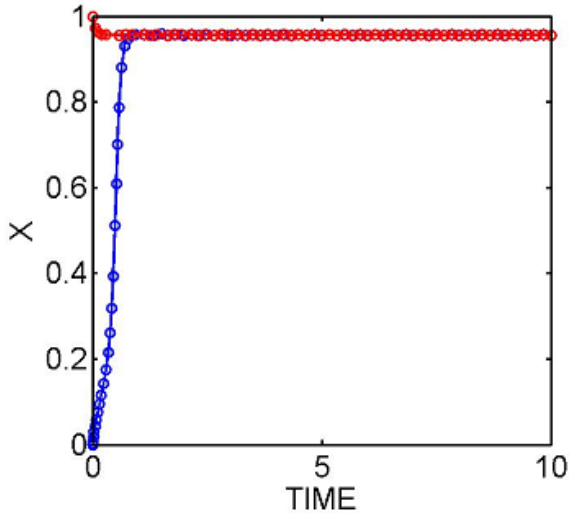
gamma=15



gamma=13.9



gamma=10



Stability analysis

Rewrite the equation as:

$$\frac{dx}{dt} = f(x) - g(x)$$

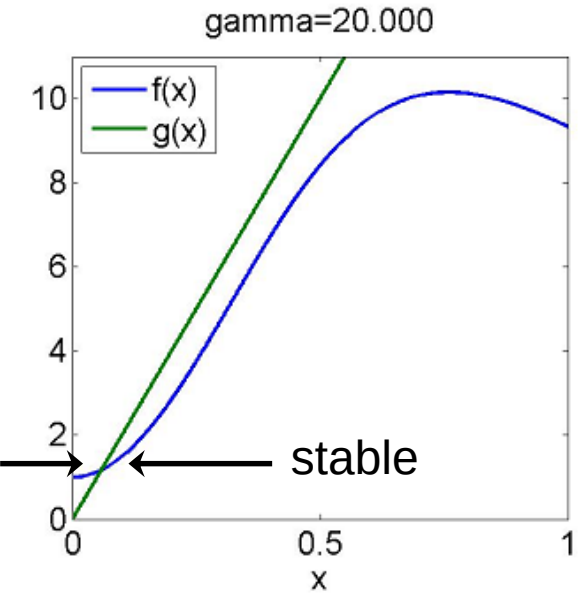
$$f(x) = \frac{\alpha x^2}{1 + (1 + \sigma_1)x^2 + \sigma_2 x^4} + 1$$

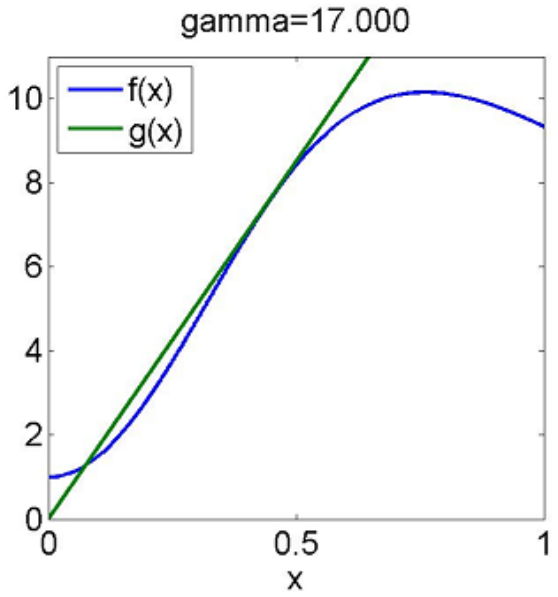
$$g(x) = \gamma x$$

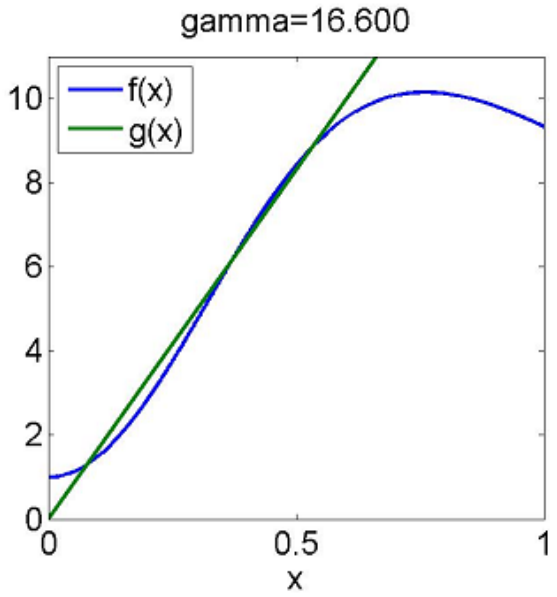
Matlab code for stability analysis

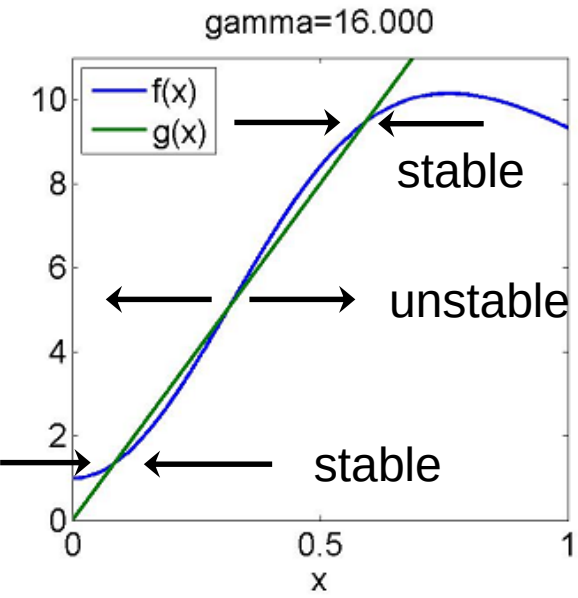
```
%filename hastybs.m plot bistability

function x=hastybs(gamma)
alpha=50;
%gamma=13.828; %gamma=16.96;
sigma1=1; % both OR2 and OR3 has same affinity
sigma2=3; % binding to OR2 increase binding affinity to OR3: coopertivity
    % f=alpha*y(1)^2/(1+(1+sigma1)*y(1)^2+sigma2*y(1)^4)+1;
    % g=gama*y(1)
x=0:1e-4:1;
fx=fofx(x,alpha,sigma1,sigma2);
gx=gofx(x,gamma);
plot(x,[fx;gx],'LineWidth',2)
axis([0 1 0 11]);
xlabel('x');legend('f(x)','g(x)',2);

function [fx]=fofx(x,a,s1,s2)
fx=(a*x.^2)./(1+(1+s1)*x.^2+s2*x.^4)+1;

function [gx]=gofx(x,gamma)
gx=gamma*x;
```

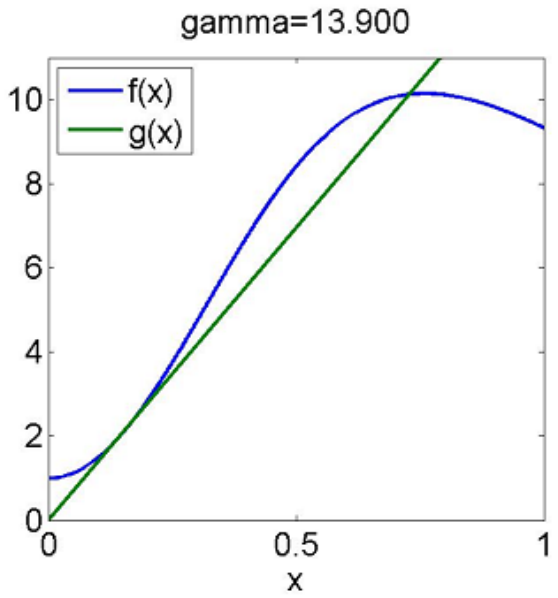
Stability analysis

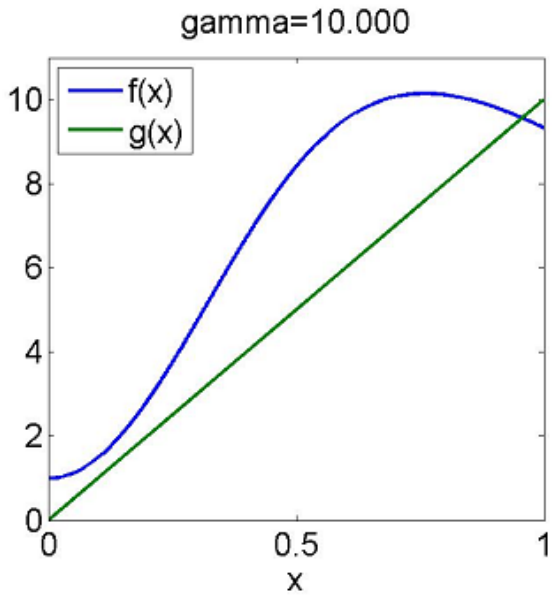




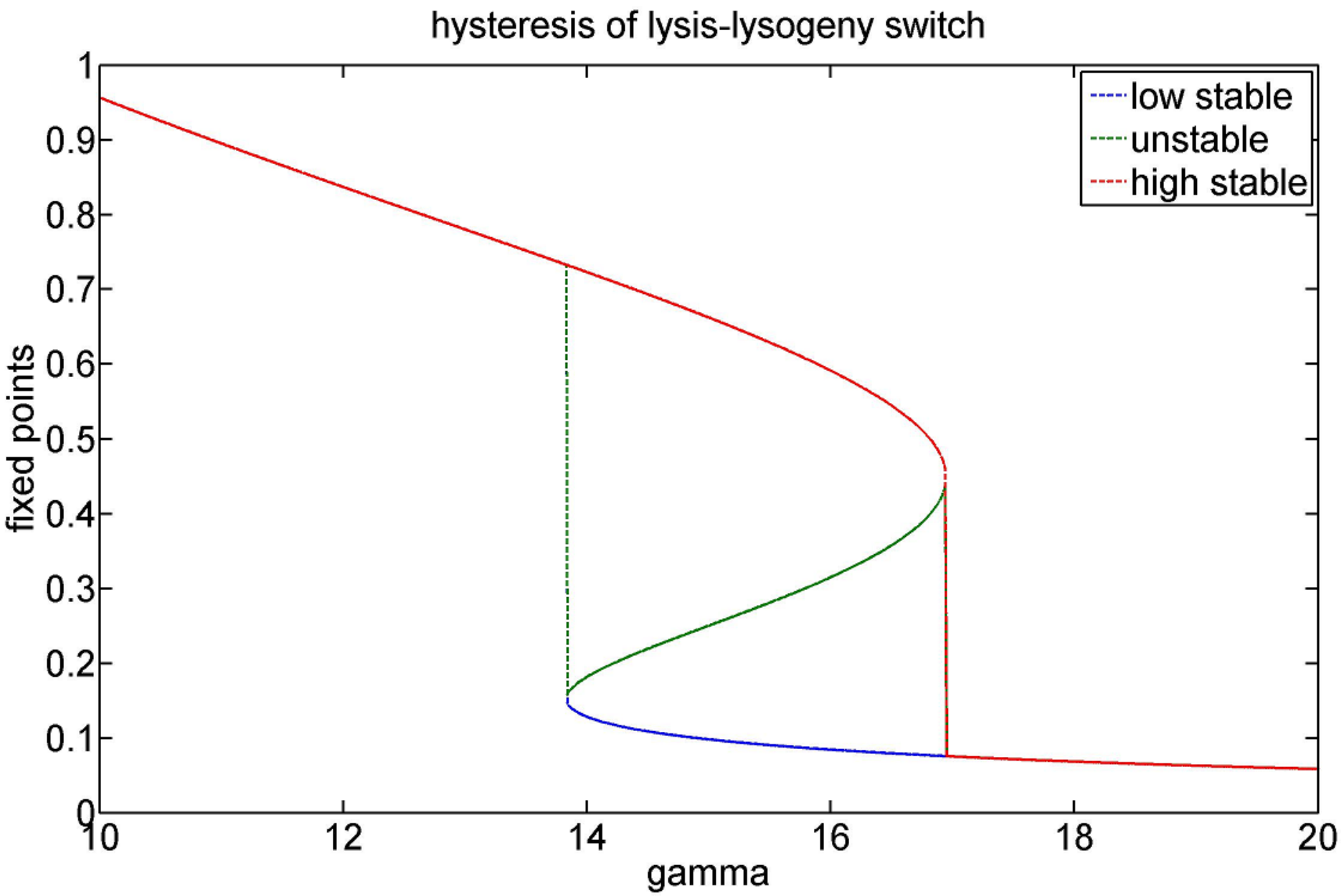








```
% filename bistabilityplot.m     plot bifurcation
function bistabilityplot(gamma1,gamma2)
gamma=gamma1:0.01:gamma2; n=length(gamma);xs=zeros(n,3);
for i1=1:n
    xs(i1,:)=hastyfnzero(gamma(i1));
end
plot(gamma,xs,'.--');
xlabel('gamma');ylabel('fixed points');
legend('low stable','unstable','high stable');
title('hysteresis of lysis-lysogeny switch')

function x0=hastyfnzero(gamma)
alpha=50;sigma1=1; sigma2=3;
x=0:1e-4:1; y=fmg(x,alpha,sigma1,sigma2,gamma);
z=y(1:end-1).*y(2:end); j1=find(z<0);z0=x(j1)+5e-5;
if length(z0)==3
    x0=z0;
elseif length(z0)==2
    x0=[z0 z0(end)];
else
    x0=[z0 z0 z0];
end

% f(x)-g(x)
function [y]=fmg(x,a,s1,s2,g)
y=(a*x.^2)./(1+(1+s1)*x.^2+s2*x.^4)+1-g*x;
```

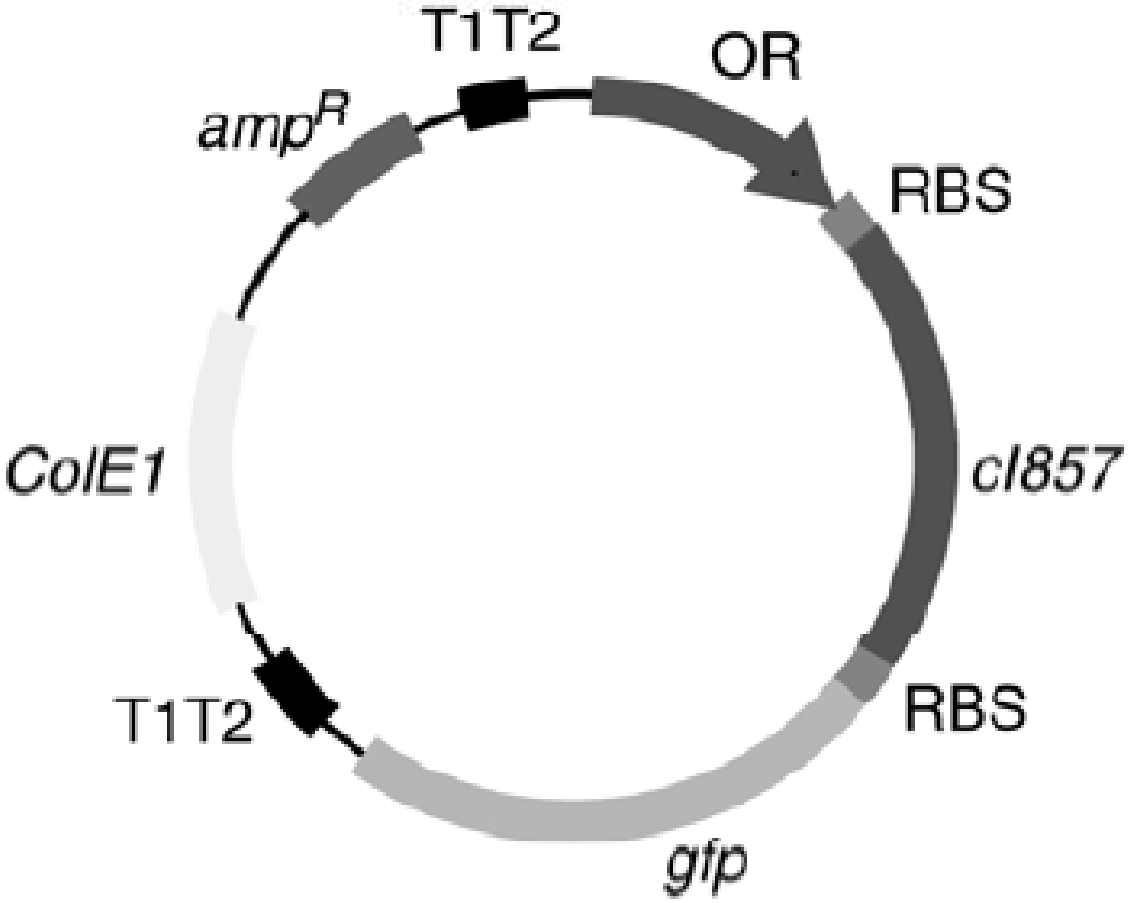

hysteresis of lysis-lysogeny switch
low stable
unstable
high stable
fixed points
gamma
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
12
14
16
18
20

How to experimentally verify these ideas ?

Synthetic Biology

Build your own designed network 'from scratch'
and test your model

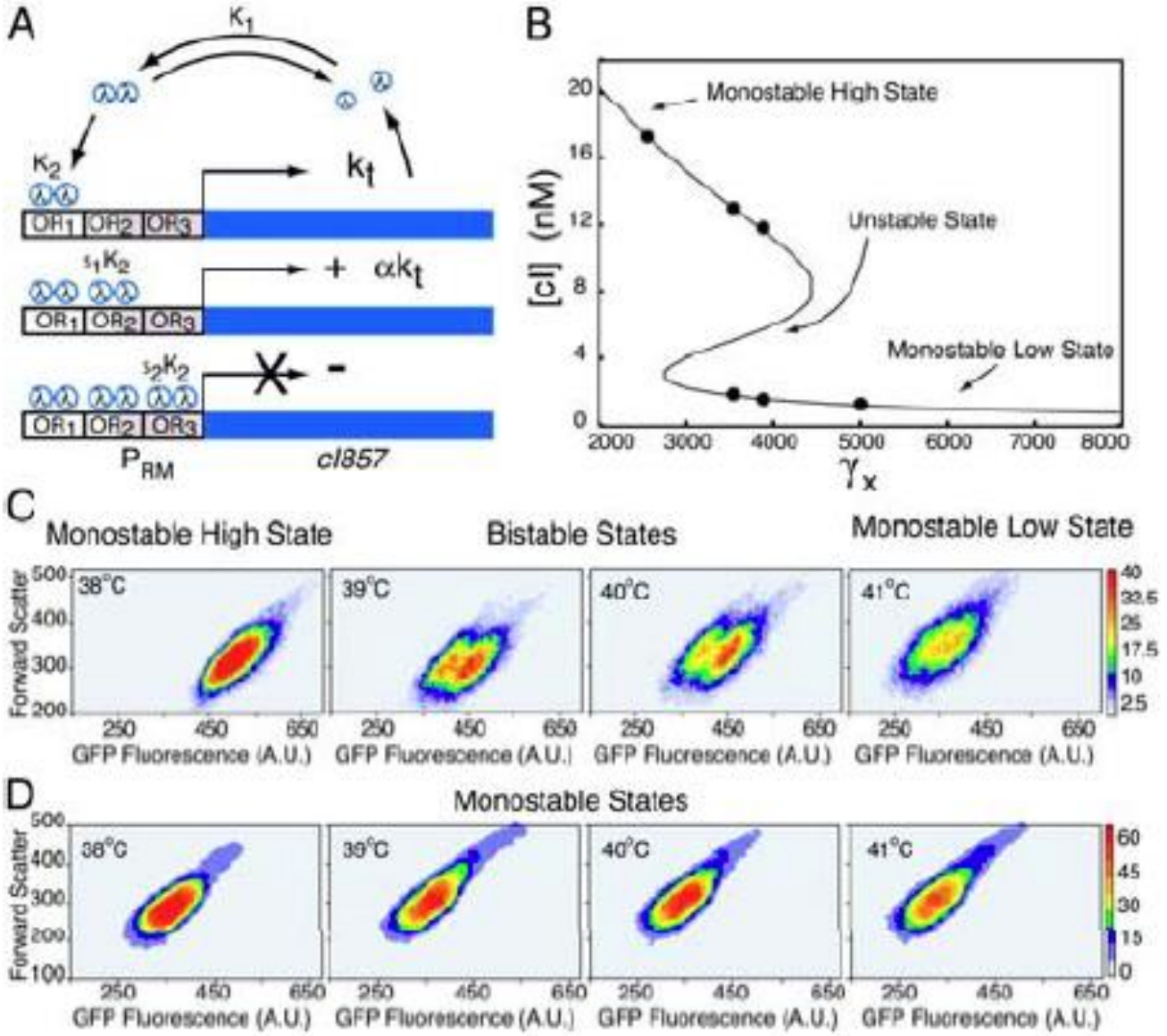
Isaacs *et al.* Prediction and measurement of an autoregulatory genetic module. PNAS **100**, 7714 (2003)



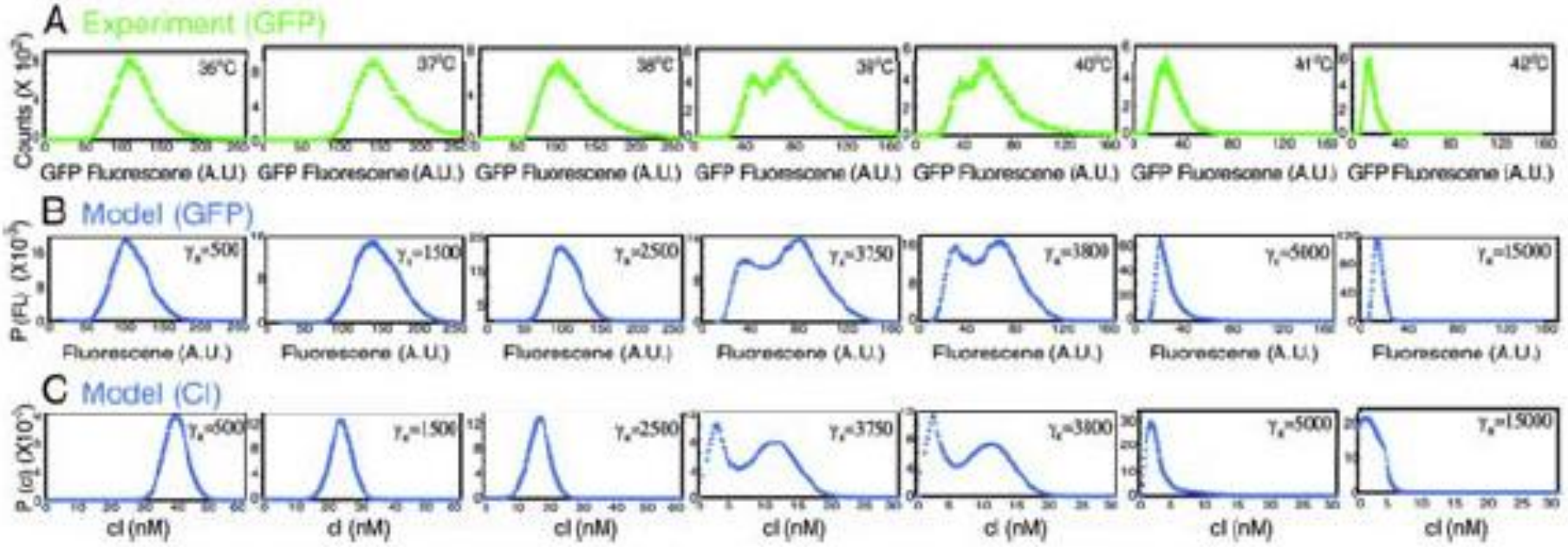
Isaacs *et al.* Prediction and measurement of an autoregulatory genetic module. PNAS **100**, 7714 (2003)

Experimental observation of bistability

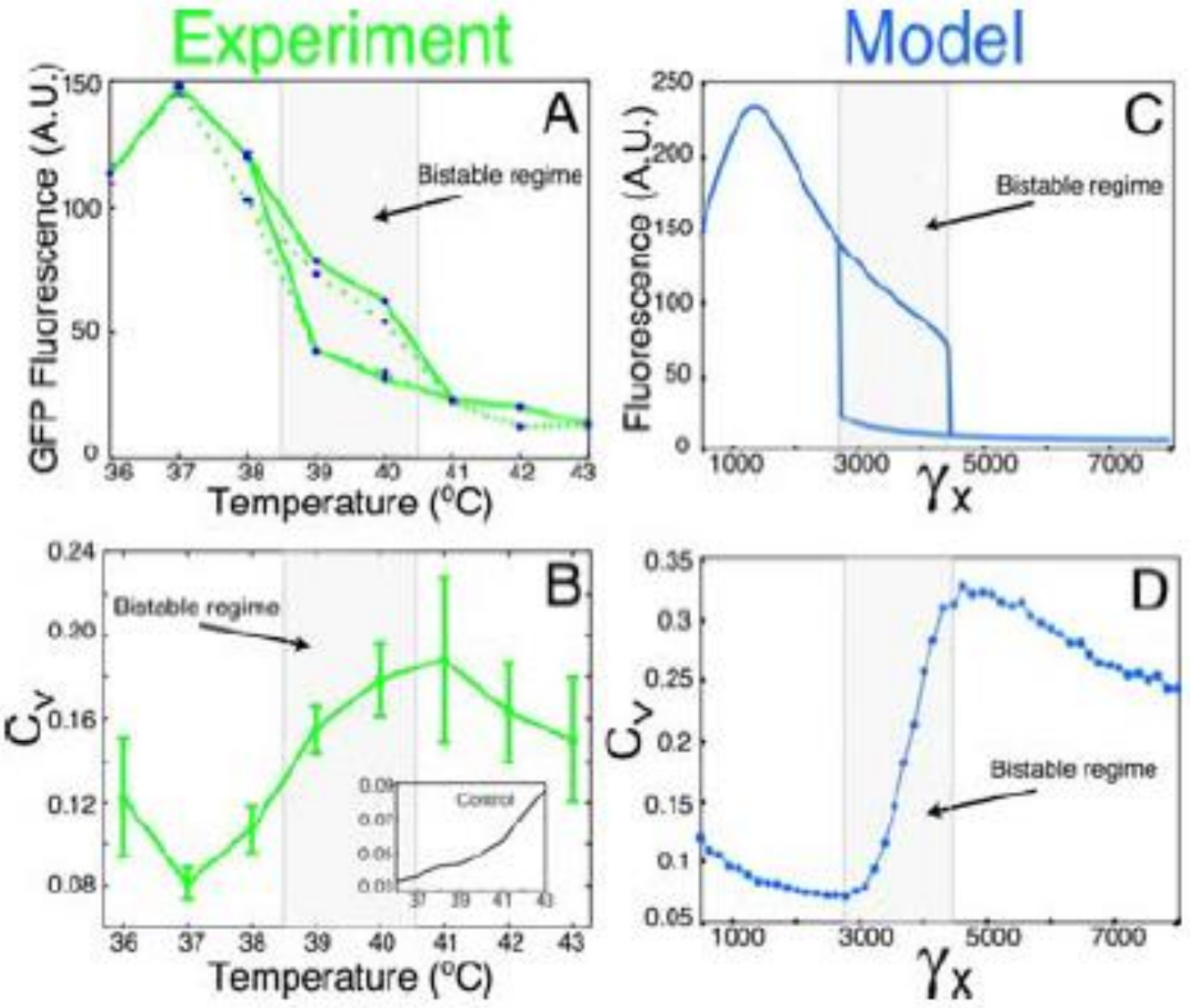
tuning temperature to tune degradation (gamma)



Comparing experiment with model

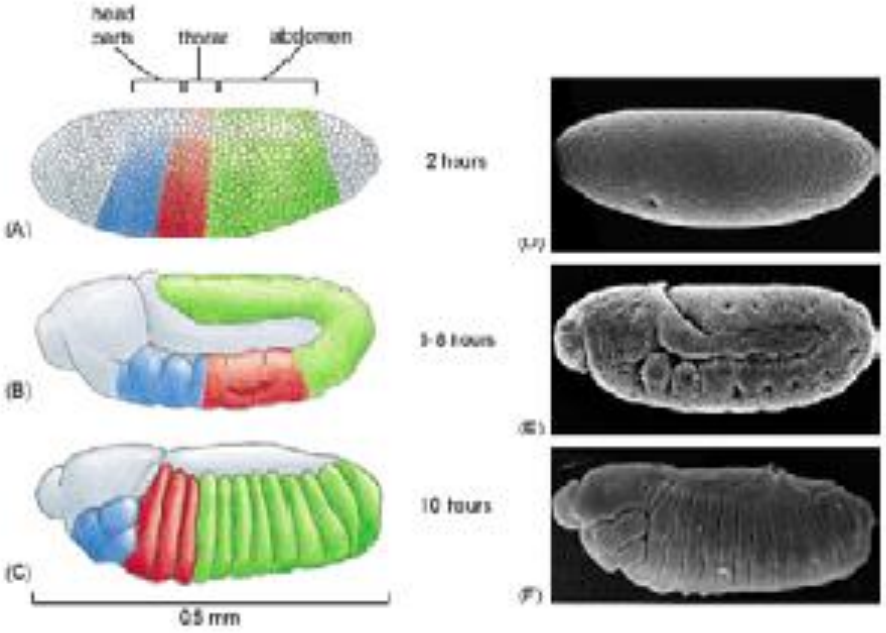


Comparing experiment with model



Biological relevant questions

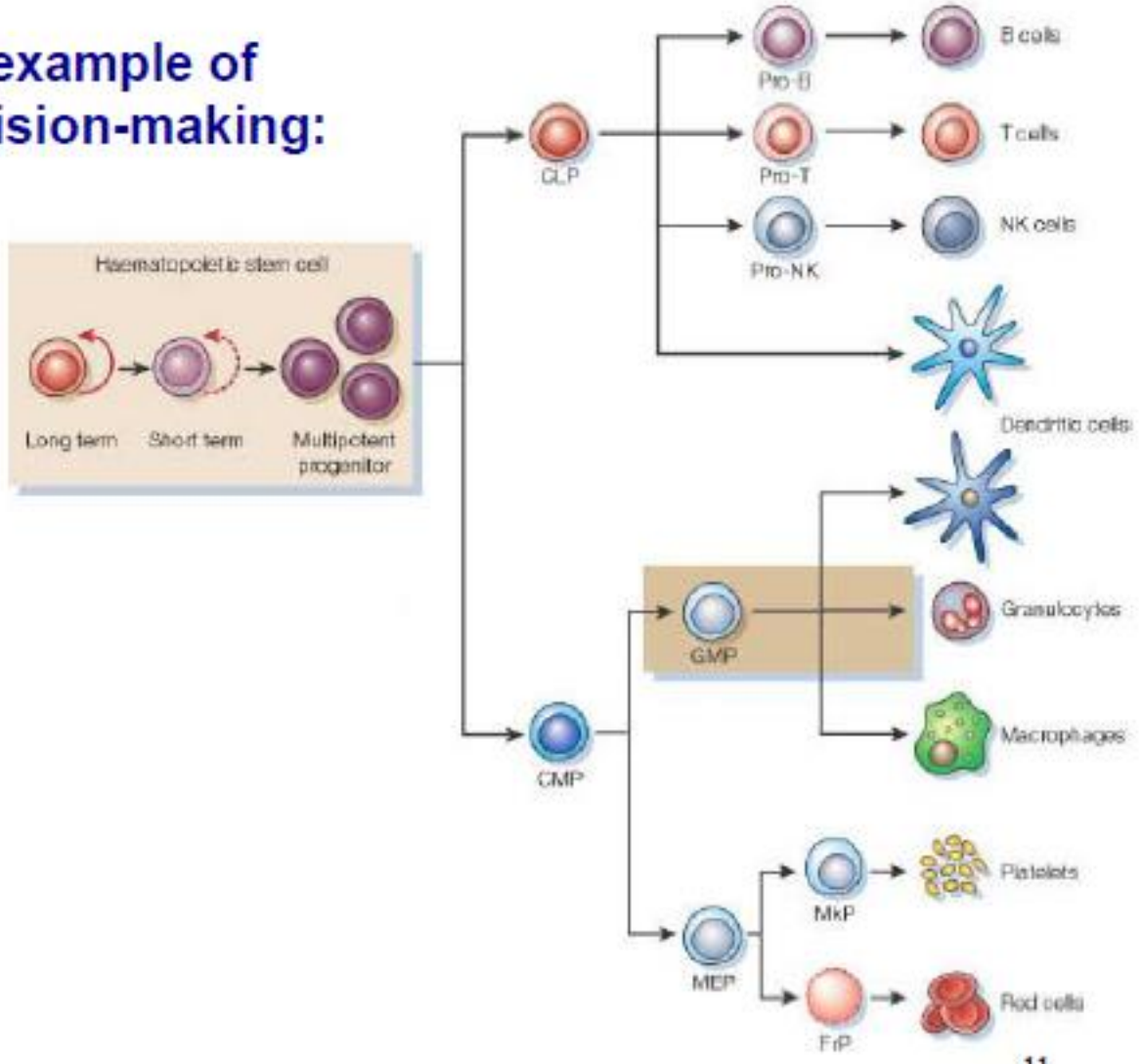
Example of cellular memory: embryonic development



Transient stimuli produce persistent responses
How is this memory stored?

Biological relevant questions

A dramatic example of cellular decision-making: stem cells



Reya *et al. Nature* **414**, 105 (2001)

Origin of positive feedback- alternatives

REPORTS

Positive Feedback Between PU.1 and the Cell Cycle Controls Myeloid Differentiation

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Regulatory gene circuits with positive-feedback loops control stem cell differentiation, but several mechanisms can contribute to positive feedback. Here, we dissect feedback mechanisms through which the transcription factor PU.1 controls lymphoid and myeloid differentiation. Quantitative live-cell imaging revealed that developing B cells decrease PU.1 levels by reducing PU.1 transcription, whereas developing macrophages increase PU.1 levels by lengthening their cell cycles, which causes stable PU.1 accumulation. Exogenous PU.1 expression in progenitors increases endogenous PU.1 levels by inducing cell cycle lengthening, implying positive feedback between a regulatory factor and the cell cycle. Mathematical modeling showed that this cell cycle–coupled feedback architecture effectively stabilizes a slow-dividing differentiated state. These results show that cell cycle duration functions as an integral part of a positive autoregulatory circuit to control cell fate.

Kueh et al Science 2013

Origin of positive feedback: through cell cycle regulations