BME 5742 Biosystems Modeling and Control

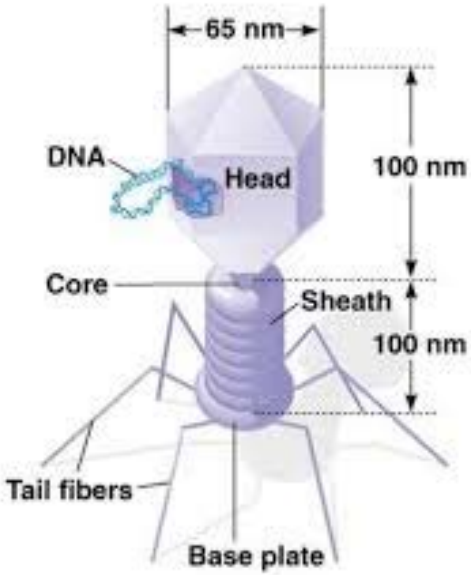
Genetic Switches

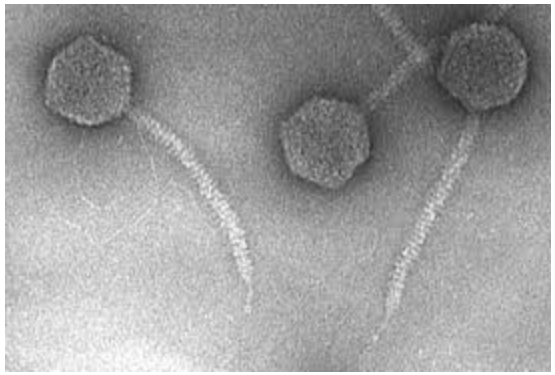
Part 2

Phage Lambda Decision Switch

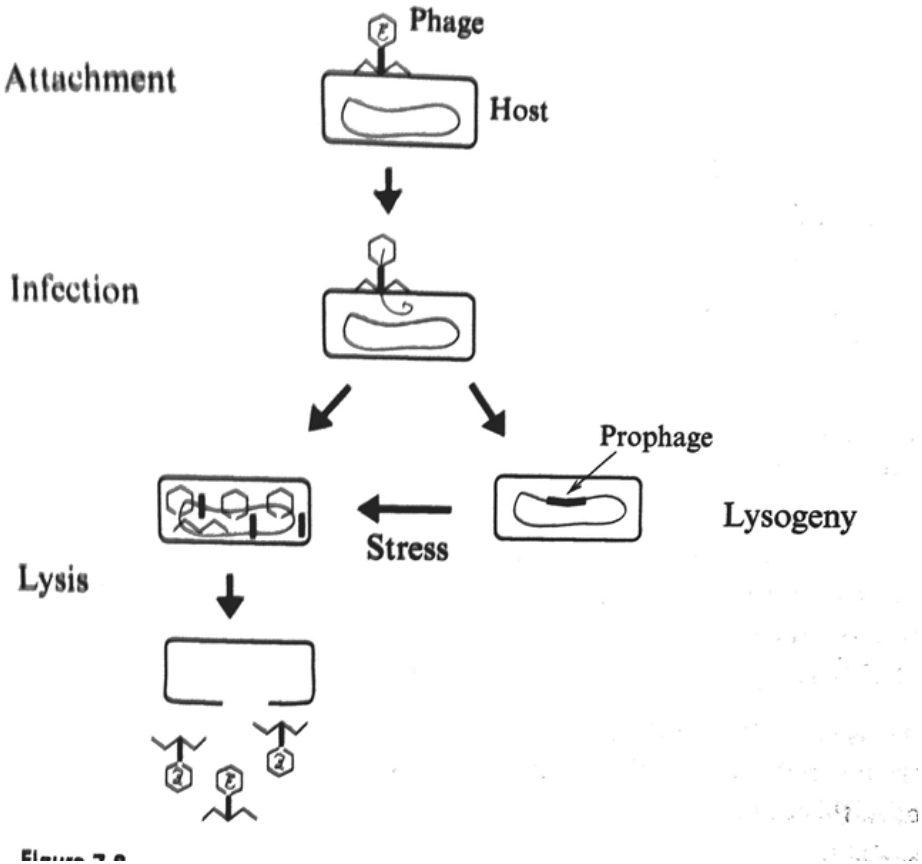
Phage Lambda

- Phage Lambda is a virus that affects bacterial cells.
- Phage particles consist of small genome encased in a protein shell.



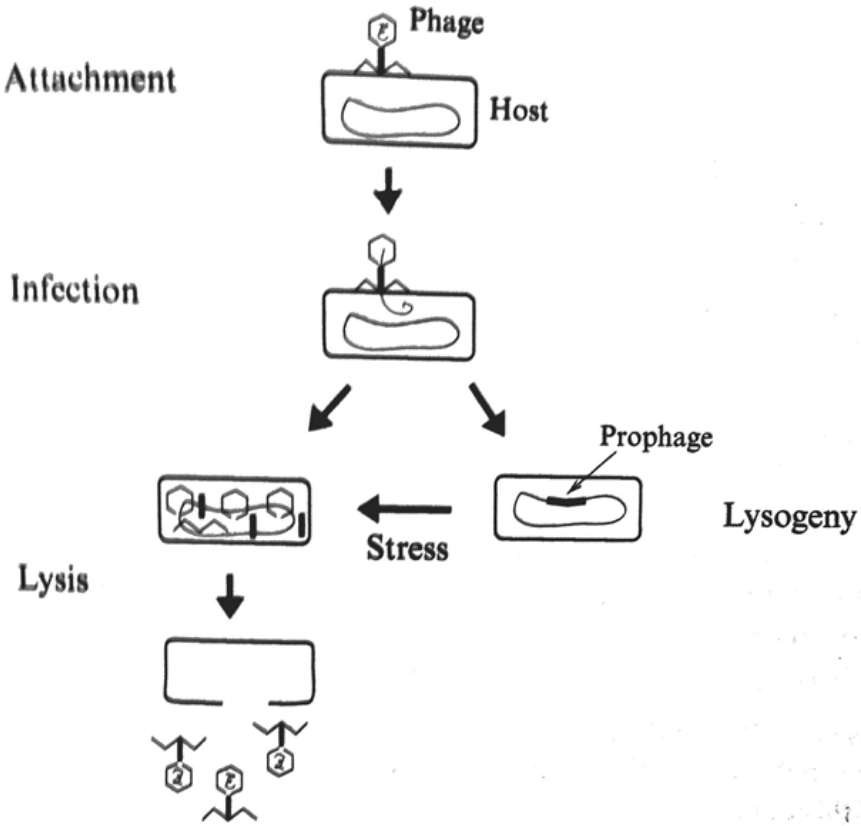


Lysis-Lysogeny Decision-Switch in Phage Lambda: Choice 1 - Lysis



- Lytic Growth: The bacteria host genetic machinery is used to create ~100 phage replicates.
- These new phages then lyse (burst) the host cell.
- Done if host cell is damaged or starving

Lysis-Lysogeny Decision-Switch in Phage Lambda: Choice 2 - Lysogeny

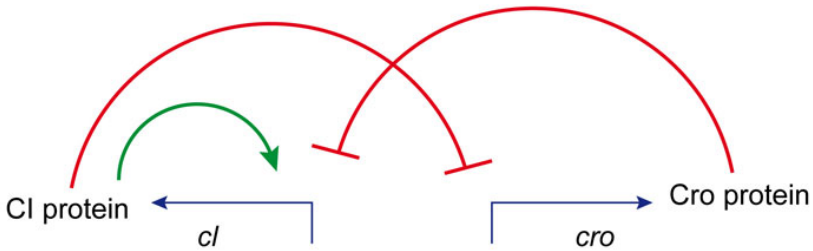


- Lysogenic Growth: Phage's genetic material is integrated into the host cell's genome (becoming a prophage).
- Host cell is allowed to grow normally and differentiate, making copy of the prophage along with own DNA.
- Phage is dormant but exists in all cell's descendants.
- Done if host cell is healthy and in good shape.

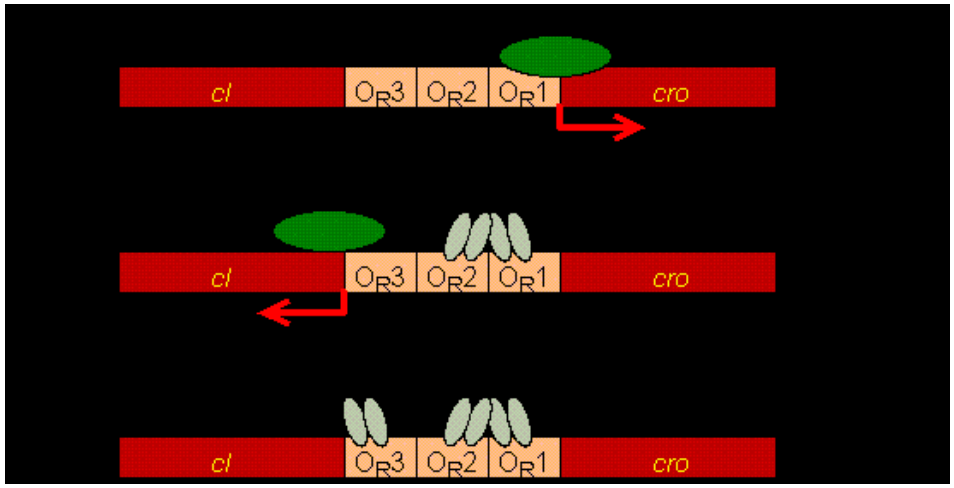
Questions related to the phage decision mechanism

- The phage decides which option to take based on sensing the condition of the host cell. How does it do such sensing?
- Can the environmental conditions that trigger a decision be temporary or should such conditions be persistent?
- Is phage response persistent even if the triggering is short term?

The *cI* and *cro* genes and their cI and cro protein products

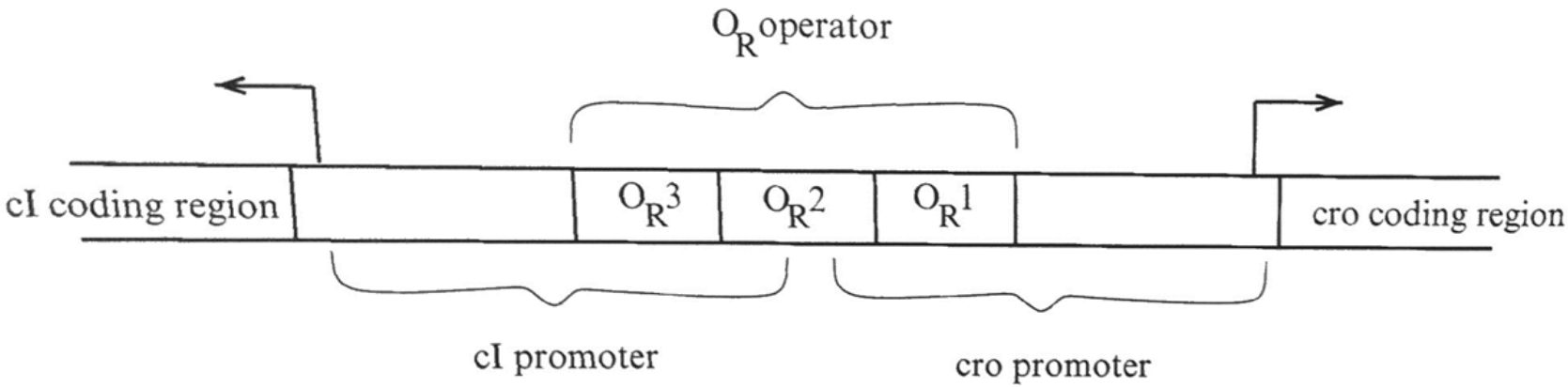


Two antagonist genes: each represses the other



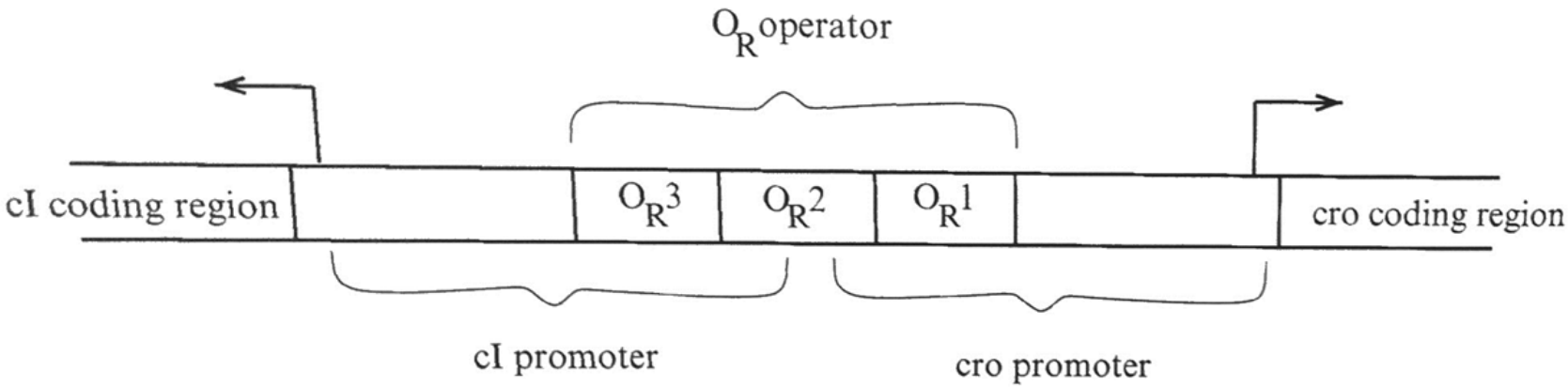
"cro" stands for "control of repressor", referring to "cI" as a repressor

Information about the *cI* and *cro* genes



- Two genes are adjacent on the phage DNA.
- They lie on opposite strands of the double helix → transcribed in opposite directions.
- Their promoter regions lie back-to-back.
- The shared operator overlaps both promoters.

Information about the *cI* and *cro* genes



- The operator region O_R contains 3 binding sites: O_R1, O_R2 and O_R3.
- Each protein cI or cro can bind to one or more of the operator's binding sites.

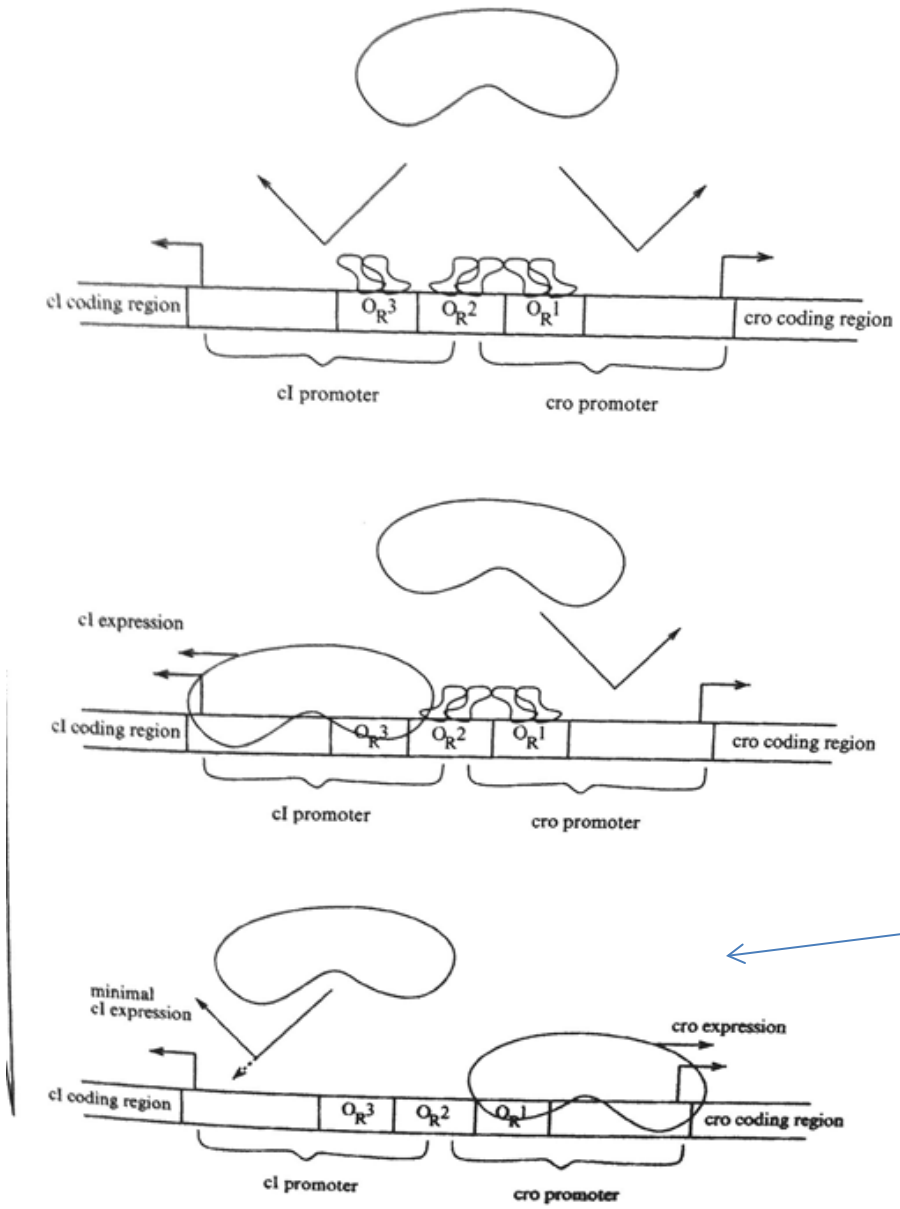
Protein Dimers

- Dimer: macromolecular complex formed by two (typically non-covalently bonded) macromolecules.
- Homodimer: Dimer formed by two identical macromolecules
- Heterodimer: Dimer formed by two different macromolecules.

How does cI binds to the operator sites

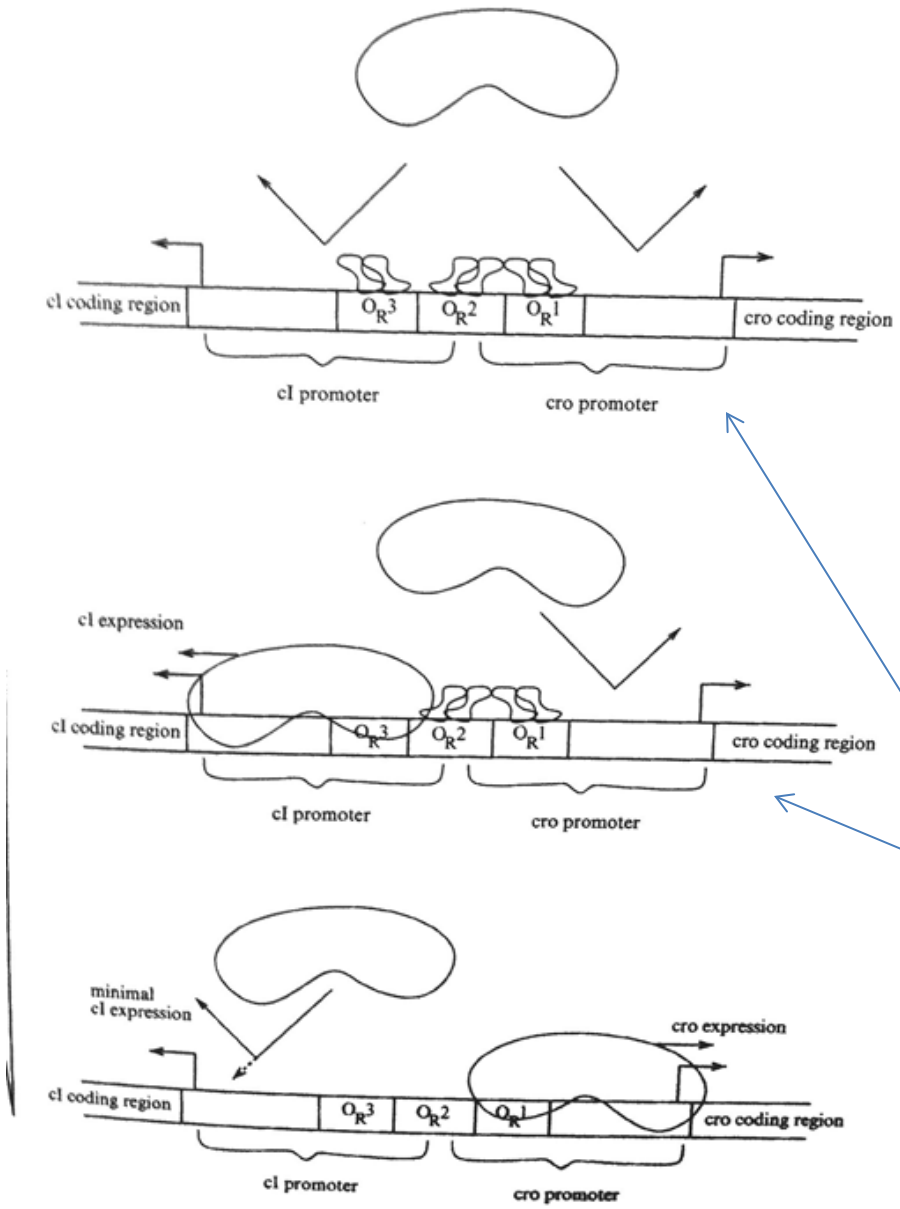
- cI is a homodimer.
- cI binds strongly to O_R1 and weakly (~10 times less affinity) to O_R2 and O_R3.
- A cI dimer bound to O_R1 interacts (with positive cooperativity) with another cI dimer bound to O_R2.
- → at low concentrations cI dimers are found bound to O_R1 and O_R2.
- → at high concentrations cI dimers are bound to all three operator sites.
- What are the effects of such cI binding scenarios?

Regulation by cI



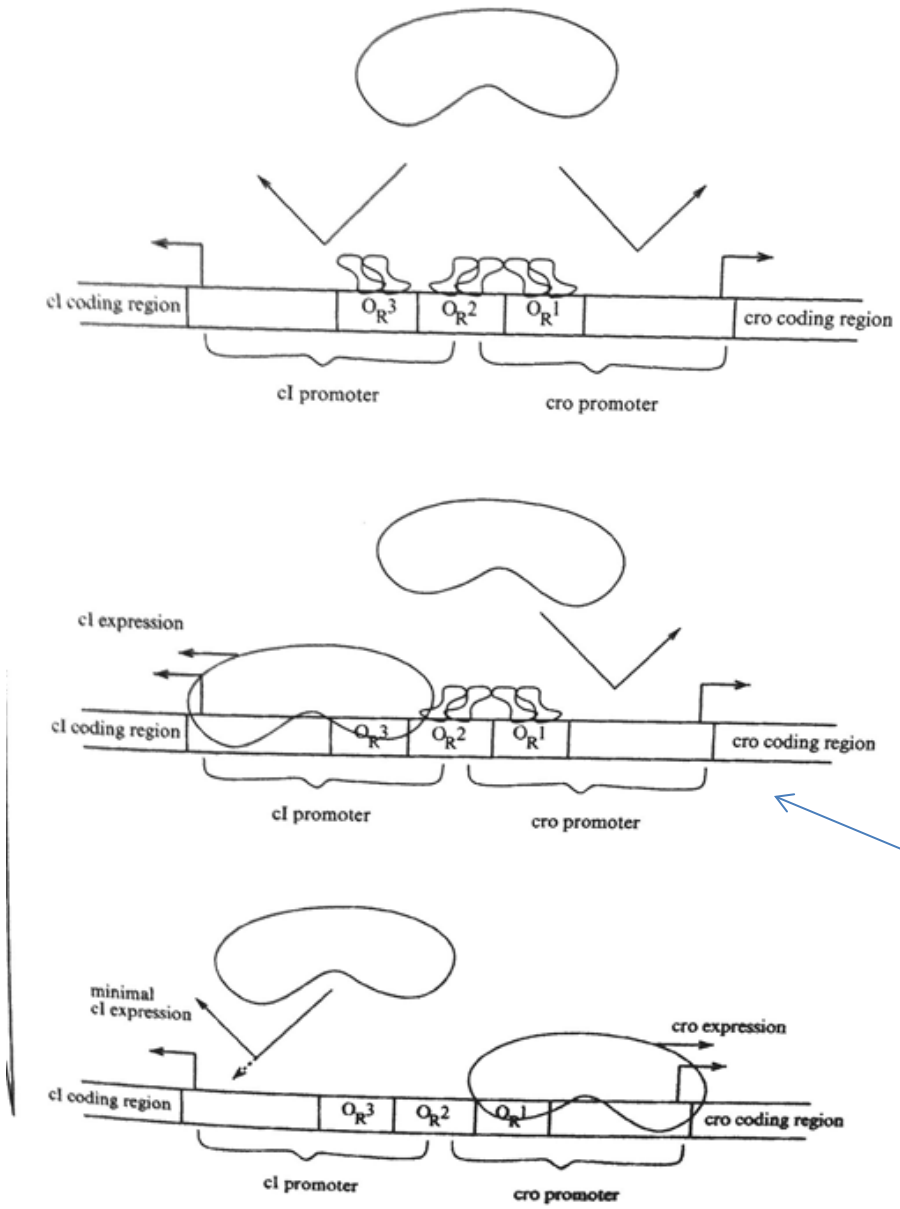
- → at low concentrations cI dimers are found bound to O_R1 and O_R2.
- When no protein is bound to the operator there is strong expression of cro and very little expression of cI.

Regulation by cI



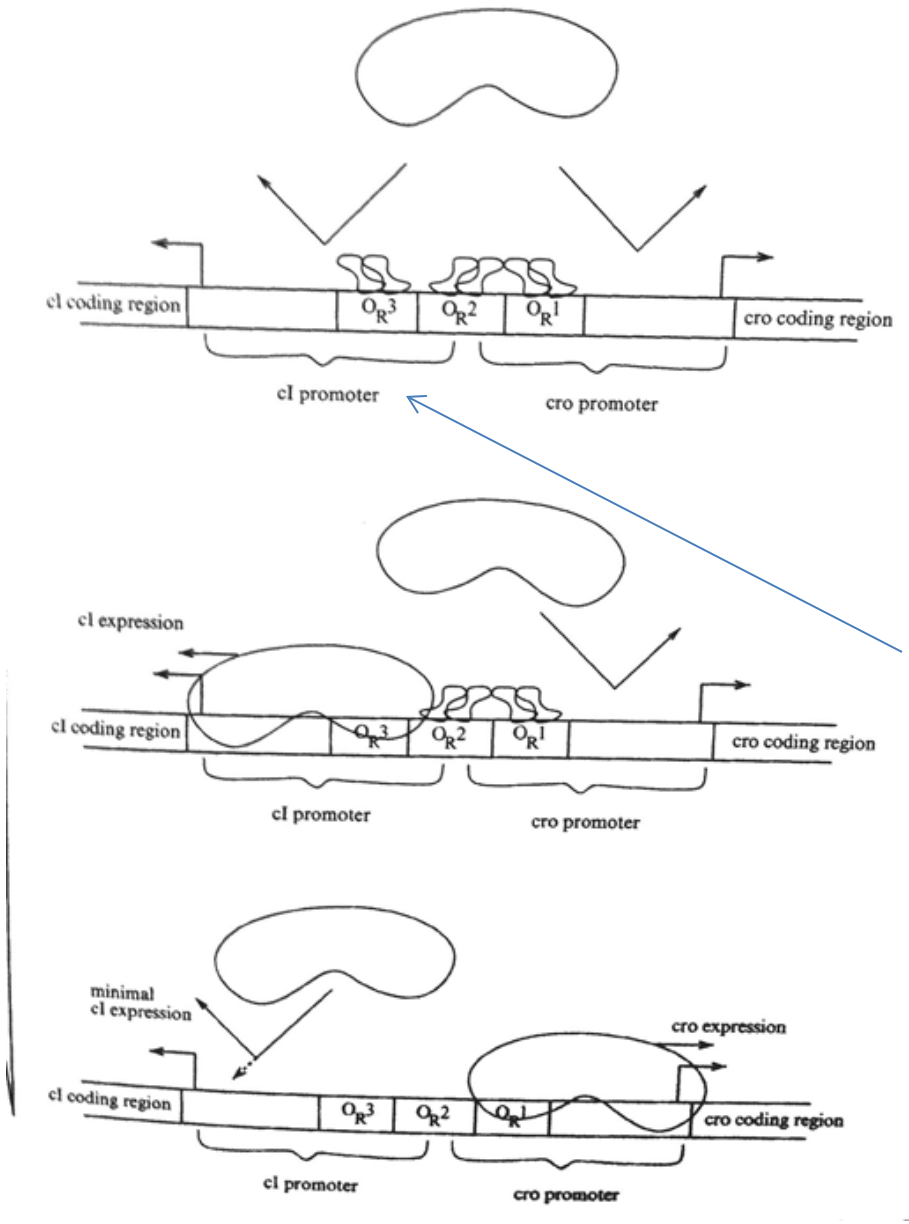
- → at low concentrations cI dimers are found bound to O_R1 and O_R2.
- When cI dimer is bound to O_R1, it inhibits cro expression by blocking polymerase access to the cro promoter.

Regulation by cI



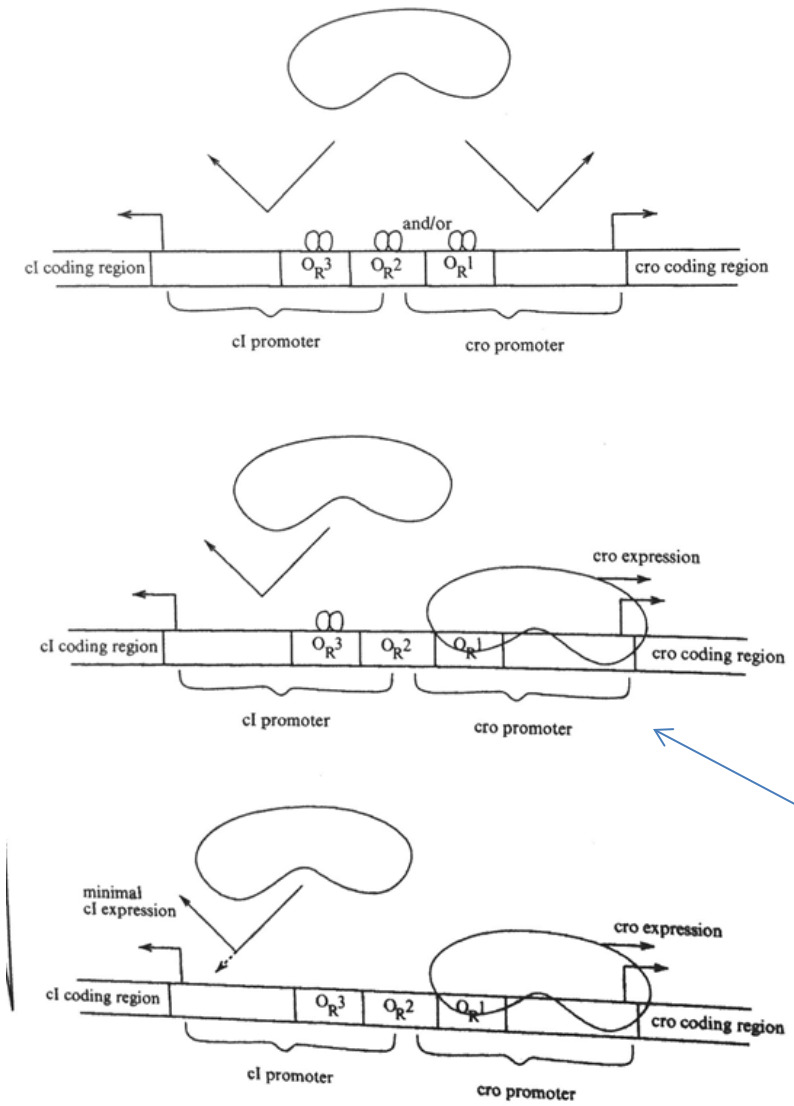
- → at low concentrations cI dimers are found bound to O_R1 and O_R2.
- When cI dimer is bound to O_R2, it up-regulates (~10 fold) expression of cI by increasing polymerase affinity to the promoter site.
- At low concentrations cI becomes an autoactivator.

Regulation by cI



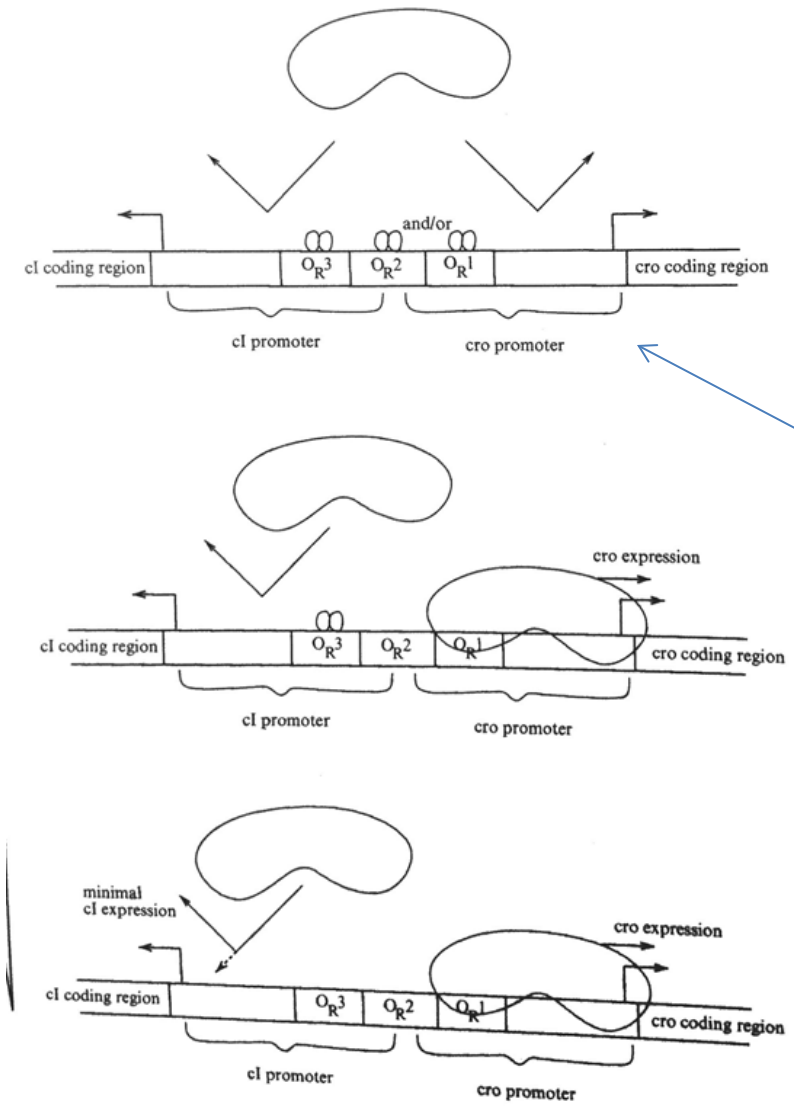
- → at high concentrations cI dimers are bound to all three operator sites.
- When cI dimer is bound to O_R3 it inhibits its own expression by blocking polymerase access to the promoter.
- At high concentrations cI becomes an autoinhibitor.

Regulation by cro



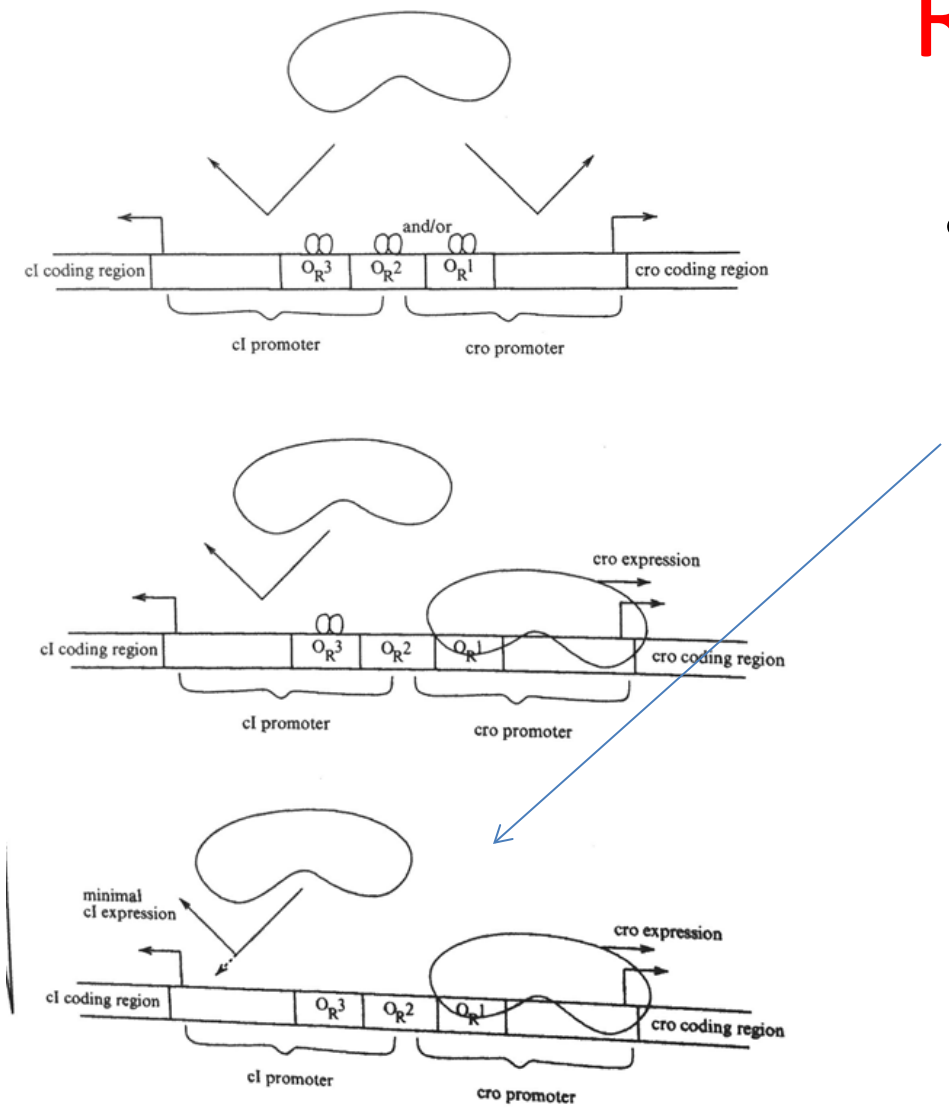
- Also a homodimer.
- Dimers can bind to all three operator sites, with affinity opposite to that of cI, and with no cooperative effects.
- The cro dimer has high affinity for O_R3, and it binds there at low concentrations → inhibits cI.

Regulation by cro



- The cro dimer has lower affinity for O_R2 and O_R1 (about equal) → it is found at these sites only at higher concentrations of cro.
- At high concentrations cro is an autoinhibitor and it also inhibits cI

Regulation by cro

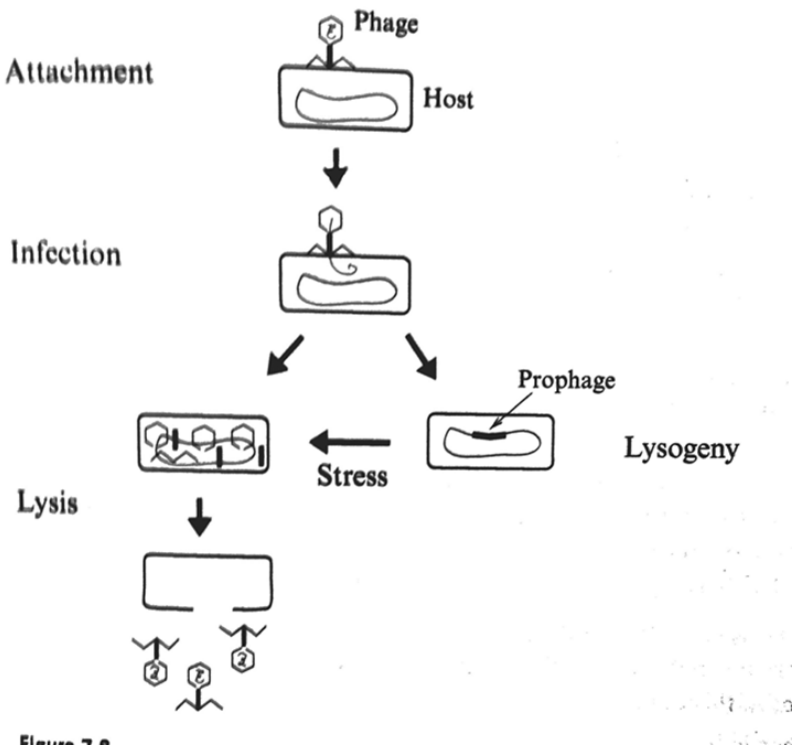


- When no proteins are bound at the operator site, cro is expressed strongly whereas cI is expressed very weakly.

Bistability

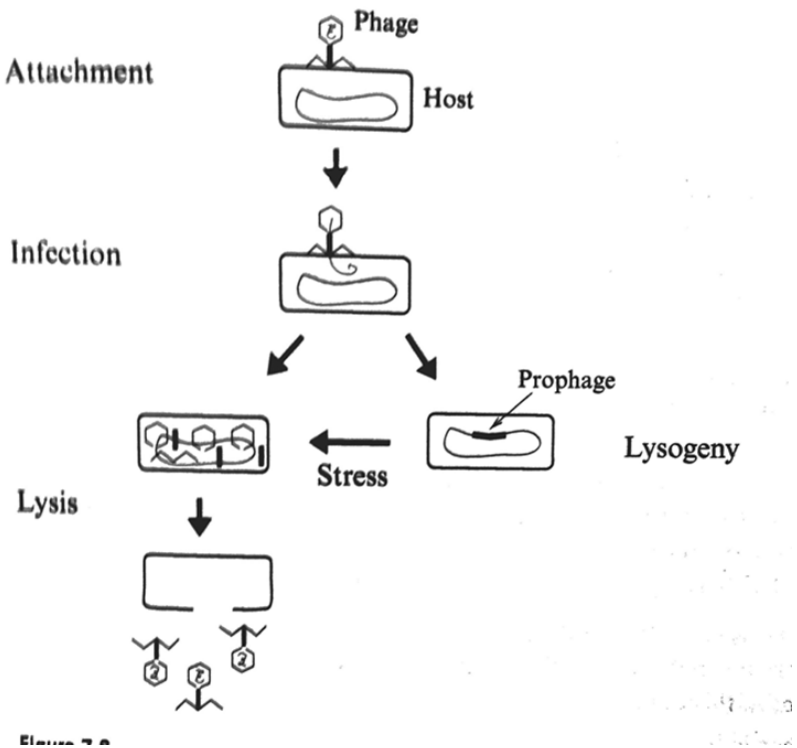
- The *cI* and *cro* genes are antagonists – each represses the other.
- → System exhibits two steady states:
- Either cI will be abundant, repressing cro, or cro will be abundant, repressing cI.
- These two states characterize the two pathways of infection.

The two pathways of infection



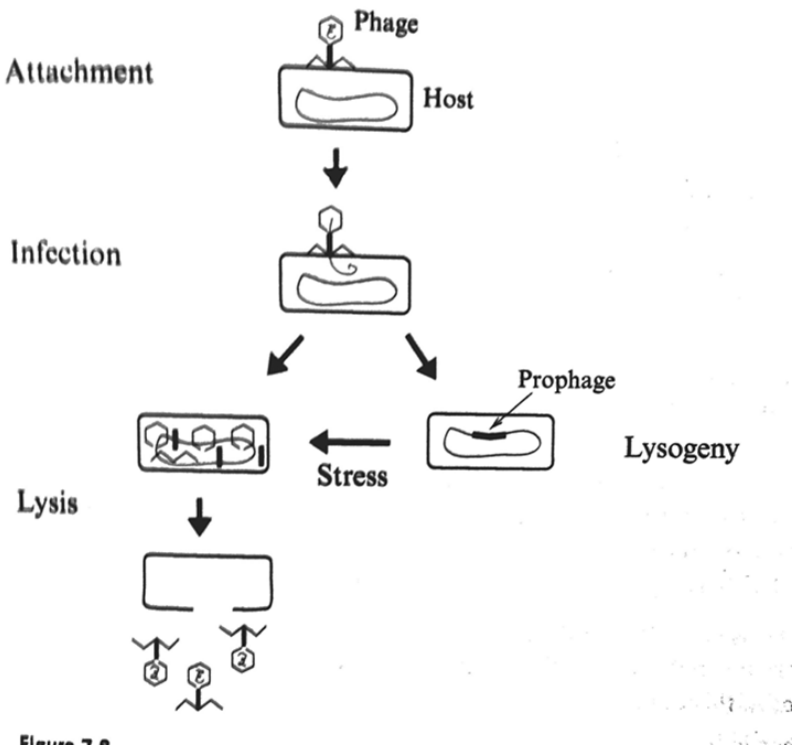
- In the lysogenic state the cI concentration is high and the cro concentration is low.
- Lysis begins when the cI concentration is low and cro concentration is high.

Two time scales



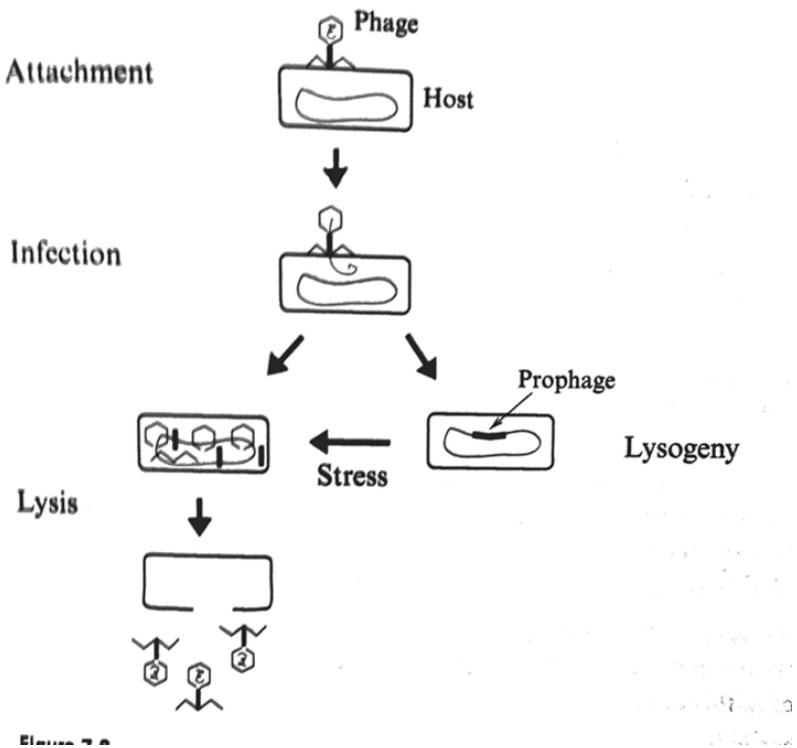
- The lysogenic state is stable on a long timescale – it can be maintained for generations of the host.
- The lytic state is transient – it leads to the host cell's death.
- The decision switch has relatively short timescale.

Flipping the switch



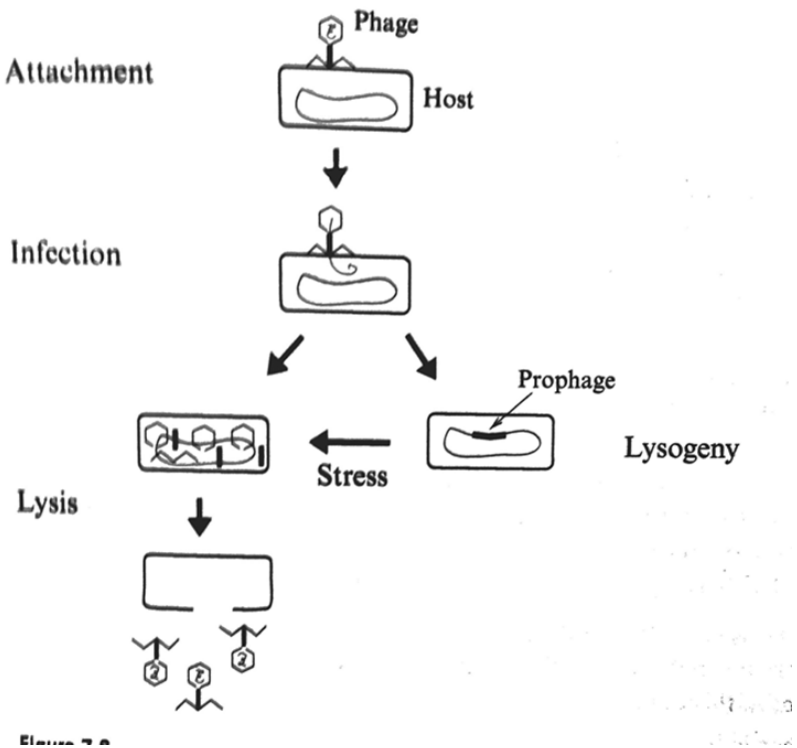
- Once it has integrated into the host's genome, the prophage continuously monitors the state of the cell.
- When it senses that the cell is in bad shape the switch is flipped to begin the lytic growth.
- It is irreversible.

How is cell stress sensed?



- Whenever cell DNA becomes damaged it invokes the expression of number of repair proteins.
- The bacterial repair protein is RecA, which triggers the expression of DNA repair genes.

What does RecA do?



- Once activated, RecA cleaves cI, rendering it unable to bind the O_R sites → This frees *cro* from any repression, leading to lytic phage growth.

Mathematical model of the Phage Lambda decision switch – general structure

$$\frac{dr(t)}{dt} = f_r(r(t), c(t)) - \delta_r r(t)$$

$$\frac{dc(t)}{dt} = f_c(r(t), c(t)) - \delta_c c(t)$$

- We denote [cI]=r and [cro]=c.
- The dynamics of mRNA production is neglected.
- The f() functions describe the rates of expression from the cI and cro promoters.
- The δ coefficients account for dilution and degradation.

Further Simplifying Assumptions -1

State	Notation	Rate of cI Expression	Rate of cro Expression
Unbound operator	O	a	b
cI at O_R1 and O_R2	$O(cI_2)_2$	$10a$	0
cI at O_R1, O_R2, and O_R3	$O(cI_2)_3$	0	0
cro at O_R3	$O(cro_2)$	0	b
cro at O_R3 and O_R1 and/or O_R2	$O(cro_2)_{2+}$	0	0

- Proteins cI and cro never bind the operator simultaneously.
- Strong cooperativity causes the binding of cI at O_R2 to happen concurrently with cI binding to O_R1.
- States in which cro is bound to O_R1 or O_R2 can be lumped together.

Further Simplifying Assumptions -2

State	Notation	Rate of cI Expression	Rate of cro Expression
Unbound operator	O	a	b
cI at O_R1 and O_R2	$O(cI_2)_2$	$10a$	0
cI at O_R1, O_R2, and O_R3	$O(cI_2)_3$	0	0
cro at O_R3	$O(cro_2)$	0	b
cro at O_R3 and O_R1 and/or O_R2	$O(cro_2)_{2+}$	0	0

- Therefore there are five distinct DNA-binding states.
- Table shows the rates of expression of cI and cro from each state.
- We denote by a and b the expression rates from unregulated genes.
- No expression from repressed states (i.e. leakage is negligible).

Table Explained

State	Notation	Rate of cI Expression	Rate of cro Expression
Unbound operator	O	*a*	*b*
cI at O_R1 and O_R2	$O(cI_2)_2$	10*a*	0
cI at O_R1, O_R2, and O_R3	$O(cI_2)_3$	0	0
cro at O_R3	$O(cro_2)$	0	*b*
cro at O_R3 and O_R1 and/or O_R2	$O(cro_2)_{2+}$	0	0

- O (unbound operator): weak expression of *cI* (a is small), and strong expression of *cro* (b is large).
- $O(cI_2)_2$ (cI at O_R1 and O_R2): Strong expression of *cI* (10a), and no expression of *cro*.
- $O(cI_2)_3$ (cI at all sites): Both *cI* and *cro* are repressed.
- $O(cro_2)$ (cro at O_R3): Strong expression of *cro* whereas *cI* is repressed.
- $O(cro_2)_{2+}$ (cro in at least two sites): Both repressed.

Proteins in Dimer Form

$$[cI_2] = \frac{r}{2}$$

$$[cro_2] = \frac{c}{2}$$

- We denote [cI]=r and [cro]=c.
- It is assumed that both proteins are in their dimer form →
- → Half concentration is needed to occupy the operator sites.

DNA-Binding Events and Association Constants

$$O + cI_2 + cI_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O(cI_2)_2$$

$$O(cI_2)_2 + cI_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} O(cI_2)_3$$

$$O + cro_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} O(cro_2)$$

$$O(cro_2) + cro_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} O(cro_2)_{2+}$$

$$K_i = \frac{k_i}{k_{-i}} \qquad i = 1,2,3,4$$

It is assumed that in each such event an equilibrium is reached fast. For instance, $k_1[O][cI_2]^2 = k_{-1}[O(cI_2)_2]$

Rate of Expression from the *cI* Promoter

$$O + cI_2 + cI_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O(cI_2)_2$$

$$O(cI_2)_2 + cI_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} O(cI_2)_3$$

$$O + cro_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} O(cro_2)$$

$$O(cro_2) + cro_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} O(cro_2)_{2+}$$

$$K_i = \frac{k_i}{k_{-i}} \qquad i = 1,2,3,4$$

It is assumed that in each such event an equilibrium is reached fast. For instance, $k_1[O][cI_2]^2 = k_{-1}[O(cI_2)_2]$

How to find the rates of expression for cI and cro – part 1

- There are 5 DNA-binding states.
- Each state has a certain probability of occupancy.
- Example: the probability that at equilibrium the operator remains unbound (Event O) is:

$$\Pr\{O\} = \frac{[O]}{[O]+[O(cI_2)_2]+[O(cI_2)_3]+[O(cro_2)]+[O(cro_2)_{2+}]} =$$

$$= \frac{[O]}{[O]+K_1[O][cI_2]^2+K_2[O(cI_2)_2][cI_2]+K_3[O][cro_2]+K_4[O(cro_2)][cro_2]}$$

How to find the rates of expression for cI and cro – part 2

$$\Pr\{O\} = \frac{[O]}{[O]+[O(cI_2)_2]+[O(cI_2)_3]+[O(cro_2)]+[O(cro_2)_{2+}]} =$$

$$= \frac{[O]}{[O]+K_1[O][cI_2]^2+K_2[O(cI_2)_2][cI_2]+K_3[O][cro_2]+K_4[O(cro_2)][cro_2]}$$

- cI is expressed in O (at a rate a) and at $O(cI_2)_2$ (at a rate 10a).
- The total rate is the sum of products:
- $\Pr\{O\}\cdot a+\Pr\{O(cI_2)_2\}\cdot 10a$
- Likewise the two events for which cro is expressed.

Rates of Expression from *cI* and *cro* Promoters

$$f_r(r,c) = \frac{a + 10aK_1(r/2)^2}{1 + K_1(r/2)^2 + K_2K_1(r/2)^3 + K_3(c/2) + K_4K_3(c/2)^2}$$

$$f_c(r,c) = \frac{b + bK_3(c/2)}{1 + K_1(r/2)^2 + K_2K_1(r/2)^3 + K_3(c/2) + K_4K_3(c/2)^2}$$

Recall:
It is a second order system

$$\frac{dr(t)}{dt} = f_r(r(t), c(t)) - \delta_r r(t)$$

$$\frac{dc(t)}{dt} = f_c(r(t), c(t)) - \delta_c c(t)$$

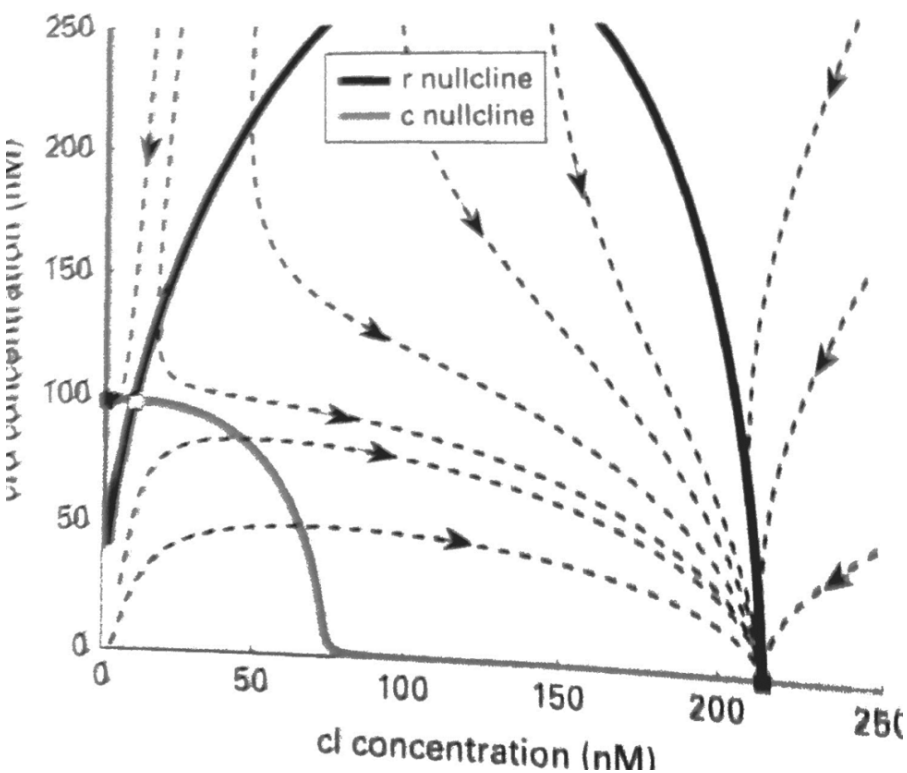
A word about nullclines of second order systems - 1

- Say that we have a second order system:
- Rate of change of x: $dx/dt=f(x,y)$
- Rate of change of y: $dy/dt=g(x,y)$
- We find equilibrium point by making both rates of change zero and solving $f(x,y)=0$ and $g(x,y)=0$.
- If we only look at $f(x,y)=0$, we can plot y against x. The graph is called the x-nullcline.

A word about nullclines of second order systems - 2

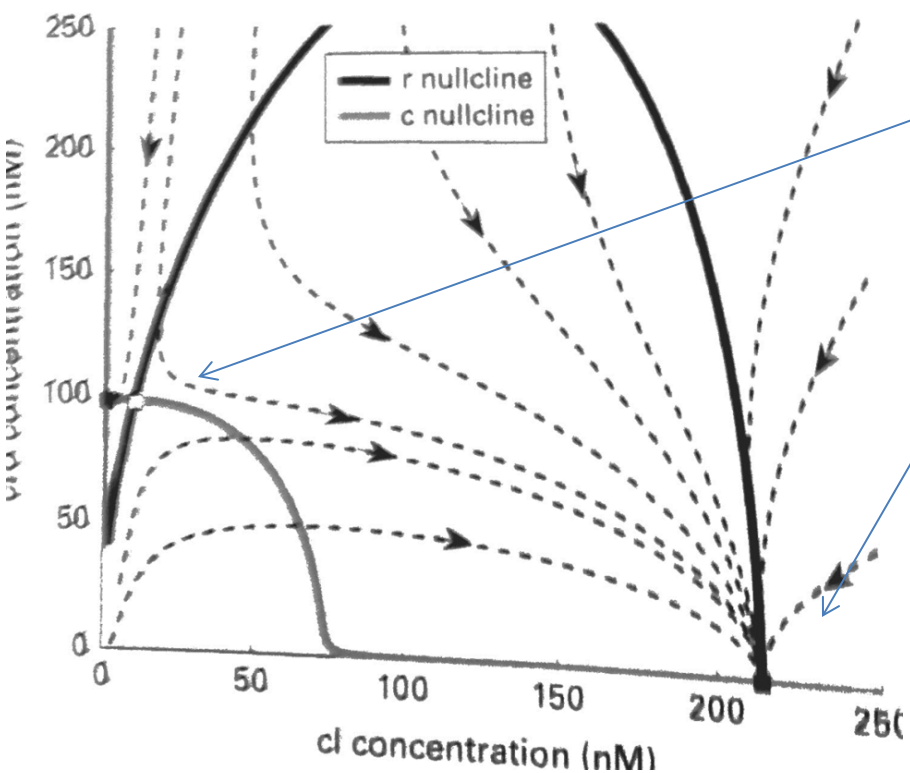
- Rate of change of x: $dx/dt=f(x,y)$
- Rate of change of y: $dy/dt=g(x,y)$
- If we only look at $f(x,y)=0$, we can plot y against x. The graph is called the x-nullcline.
- Likewise, the plot of $g(x,y)=0$ is called the y-nullcline.
- The intersections of the nullclines provides the equilibrium points of the system.

Phase Plane Portrait of the Phage Lambda Model



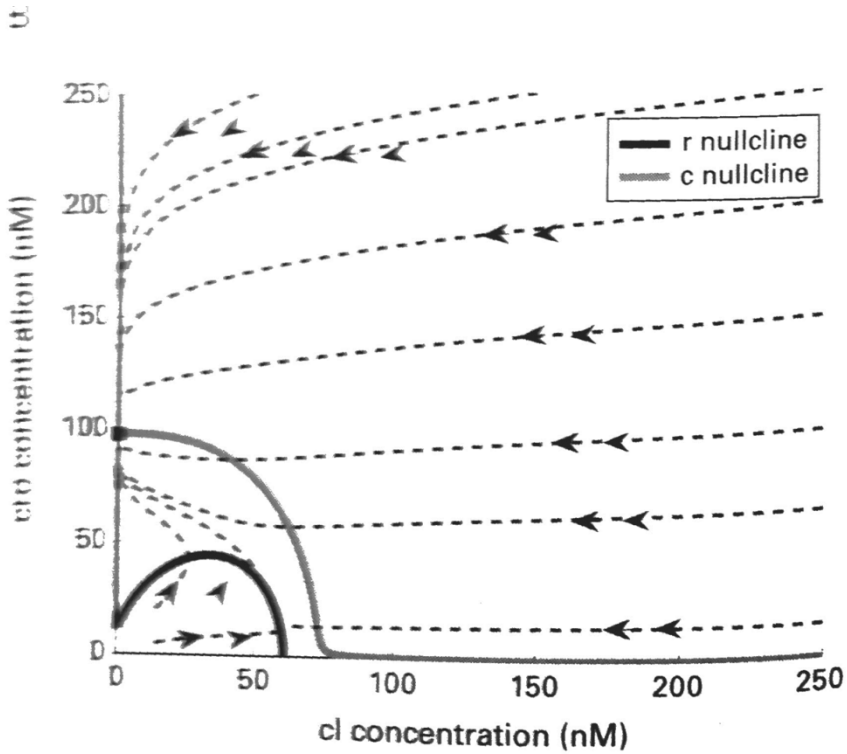
- Simulation parameters: $a=5\ min^{-1}$, $b=50\ min^{-1}$, $K_1 = 1\ nM^{-2}$, $K_2 = 0.1\ nM^{-1}$, $K_3 = 5\ nM^{-1}$, $K_4=0.5\ nM^{-1}$, $\delta_r = 0.02\ min^{-1}$, $\delta_c = 0.02\ min^{-1}$

Phase Plane Portrait of the Phage Lambda Model



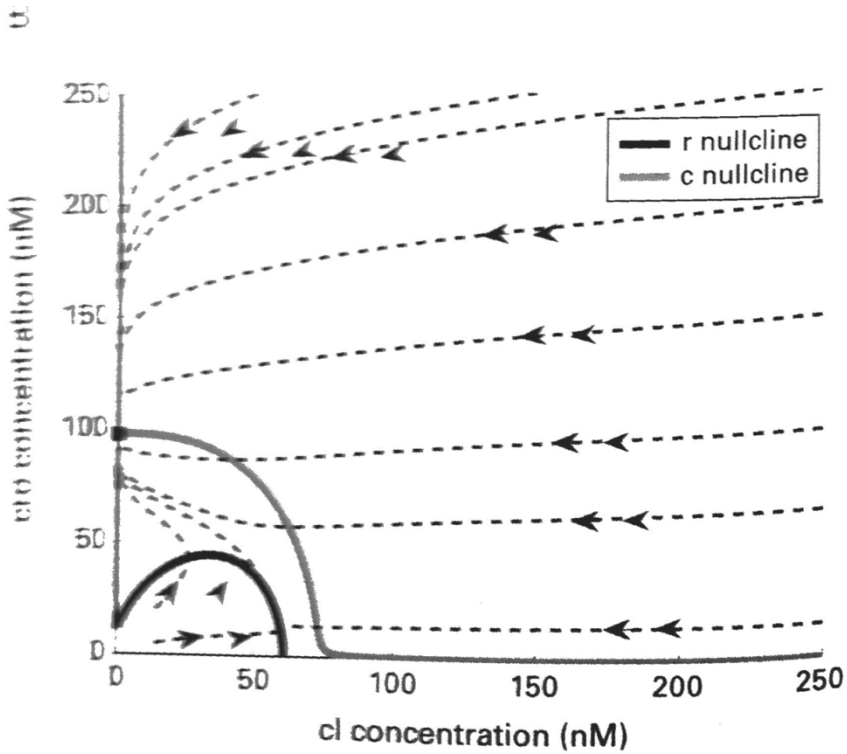
- There are 2 equilibrium points: In one [cI] is low and [cro] is high, in the other [cI] is high and [cro] is close to zero.
- It is a bi-stable system. Depending on the initial concentrations the solution goes to either one of the equilibria.

Transition from Lysogenic to Lytic State



- Recall the bacterial repair protein RecA, that can cleave cI and by that remove the repression for cro expression.
- In this model, RecA action is modelled by simply increasing δ_r 10 fold.

Transition from Lysogenic to Lytic State



- This increase of the dilution/degradation coefficient of cI causes the model to become monostable.
- All solutions (for any initial conditions) converge to an equilibrium in which [cro] is large and [cI] is small.