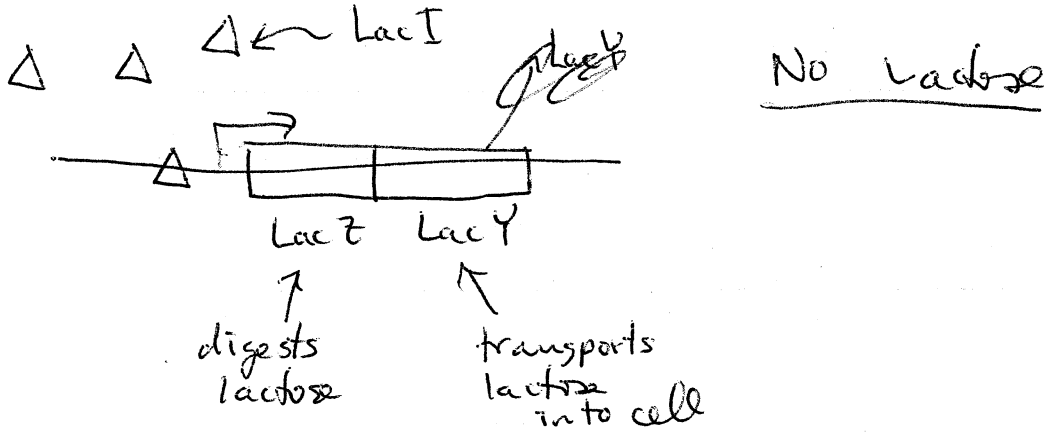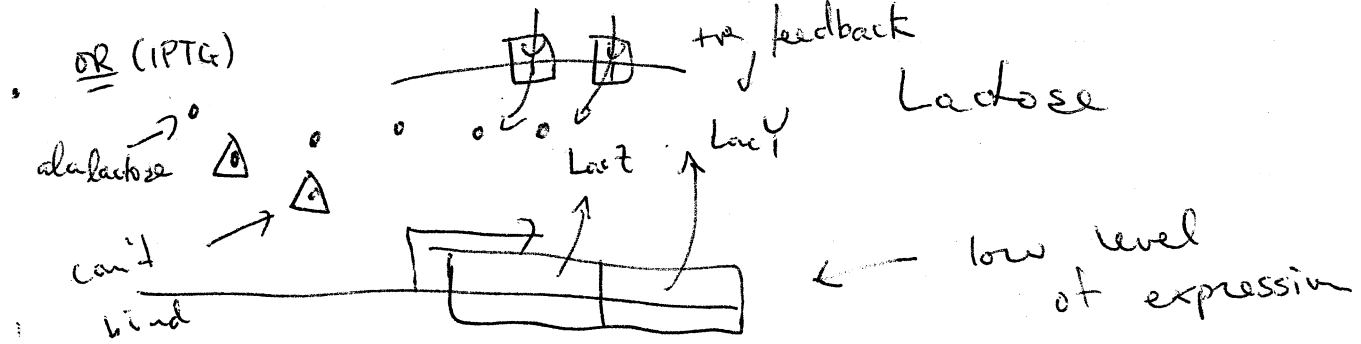# Switches & Cooperativity:

Lac Operon   Monod & Jacob
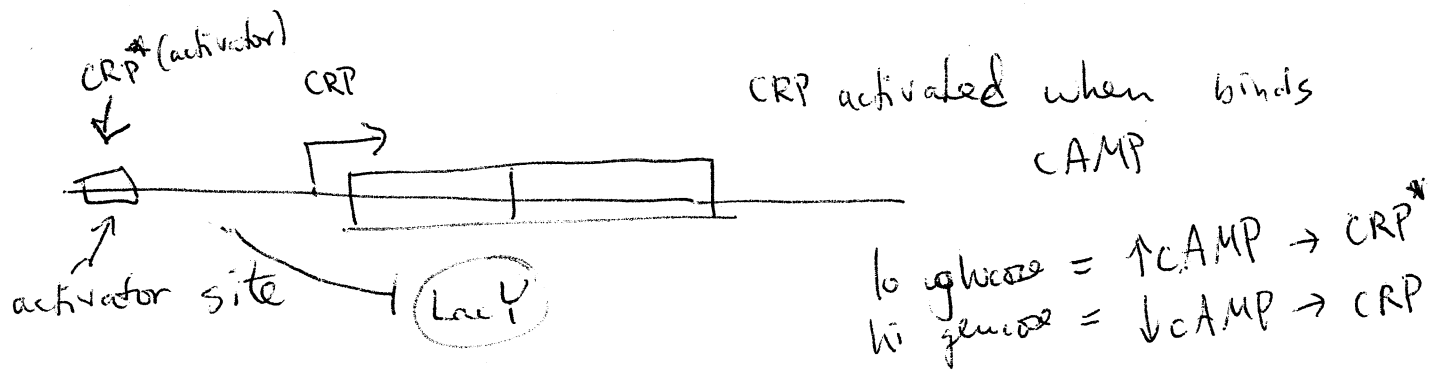
E.Coli: different energy sources. Likes glucose, then lactose. If no lactose then don't make the enzymes that process it

No Lactose

LacI

LacZ   LacY

↑
digests
lactose

↑
transports
lactose
into cell

• LacI - always on - in absence of Lactose binds & represses the lac operon

• OR (IPTG)

+ve feedback

Lactose

alulactose

can't bind

LacZ   LacY

← — low level of expression

Other factor: Low Glucose & Lactose → hi level

CRP* (activator)

CRP

CRP activated when binds cAMP

activator site   LacY

lo glucose = ↑cAMP → CRP*
hi glucose = ↓cAMP → CRP

② Like an ~~AND~~ gate   Lo glucose AND lactose = ON (Hi)
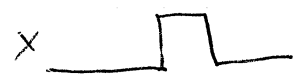
hi glucose AND lactose = ON (Lo)

hi glucose No Lactose = OFF

lo glucose NO Lactose = OFF
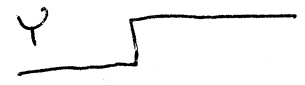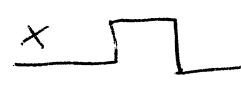
The Switch: there's +ve feedback

No feedback   X → Y   X ⎽⎽⎽�_⎽⎽⎽   Y ⎽⎽⎽�_⎽⎽⎽

+ve feedback   X → Y   X ⎽⎽⎬‾‾‾   Y ⎽⎽⎬‾‾‾‾‾‾

So

stays up in the 'ON' state

→ starve   → Feed

inducer
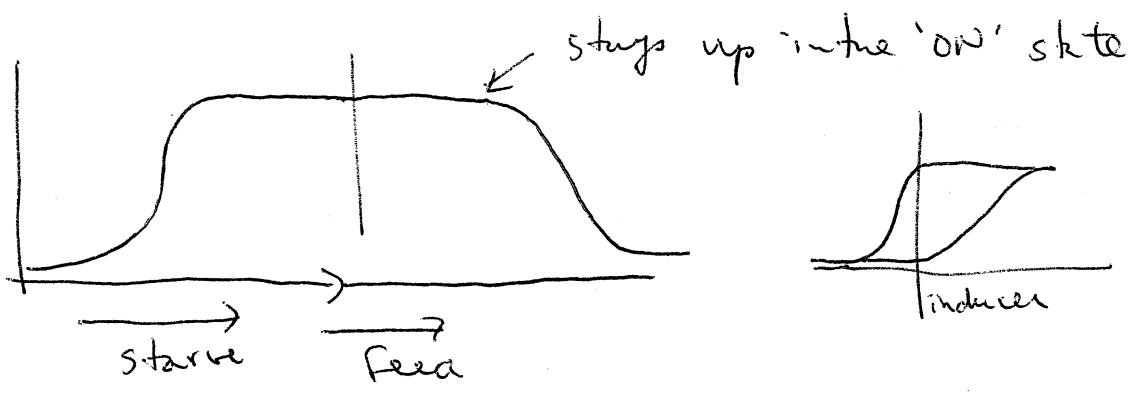
We will develop this on Wed?

DNA

head

tail

chromosome fold (handwritten)

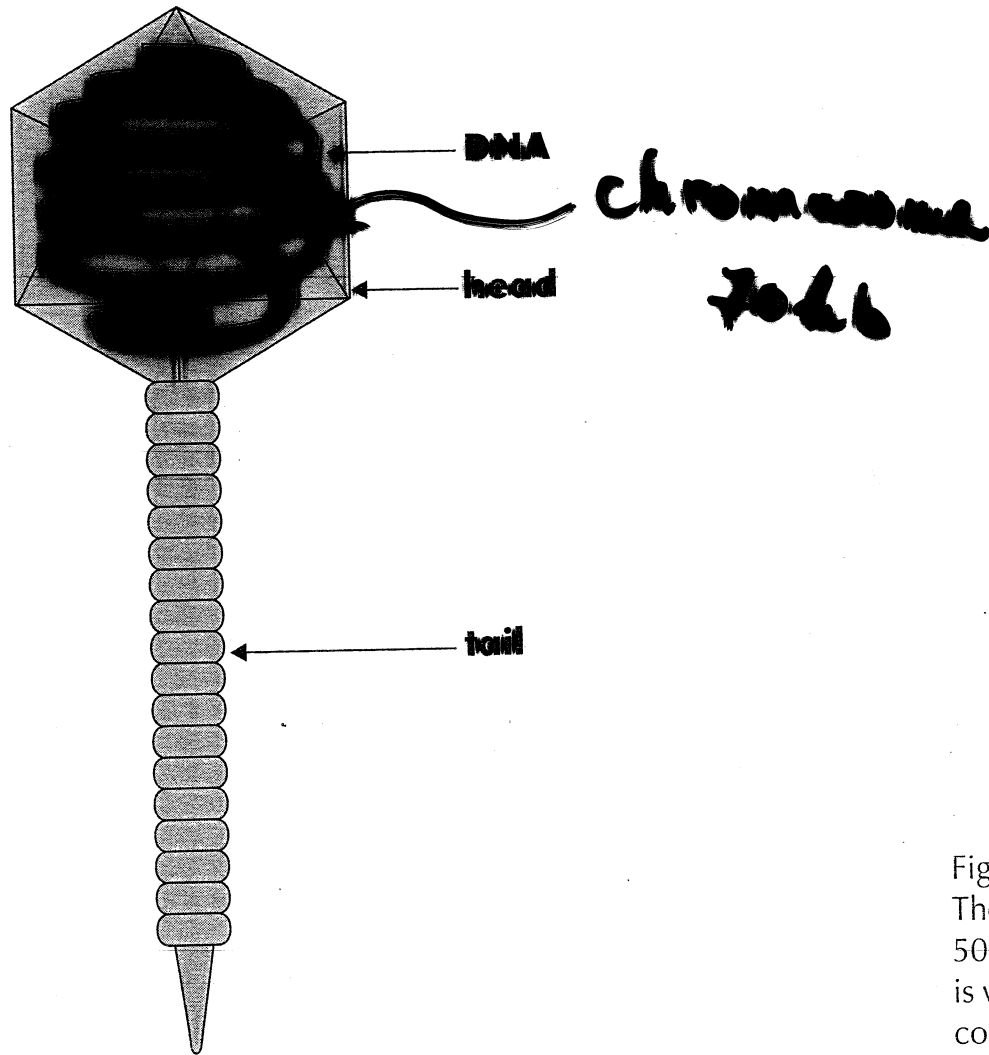Figure 1.1. A λ
The λ chromoso
50,000 base pairs
is wrapped aroun
core in the head.

its own gene. (How the repressor-encoding gene gets turned on in the fir
immediately following infection when there is no repressor present—wi
plained in Chapter 3.)

Although there is only one prophage in a lysogen there are about 1
molecules of repressor, and the excess repressor is free to bind to any ad
chromosome that might be injected into the cell. This has the result illustrated
1.3: λ cannot grow lytically on a λ-lysogen. The lysogen is said to be imn
infection.

Ultraviolet irradiation of lysogens inactivates repressor. As a result a seco
regulatory protein—Cro—is synthesized. Cro, which promotes and is require
growth, also binds DNA—in fact it binds to the same operator sites as does
but with opposite physiological effects. These two regulatory proteins, toge
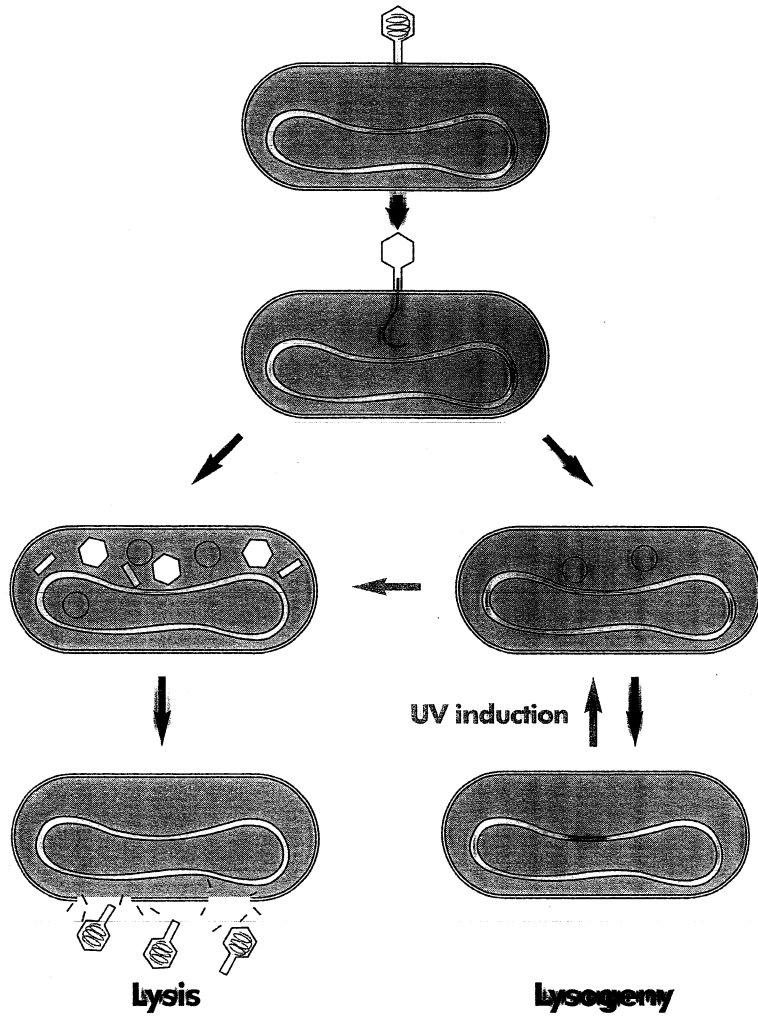RNA polymerase and their promoter and operator sites on DNA, constitute t

Figure 1.2. Growth of phage λ. The injected λ chromosome may either lyse or lysogenize the host. Ultraviolet irradiation of a lysogen induces lytic growth. Induction of lysogens was first demonstrated for a prophage of the bacterium *Bacillus megaterium.*
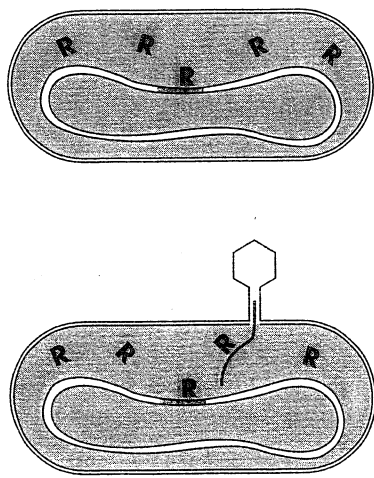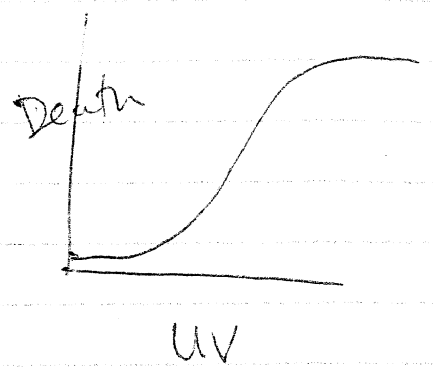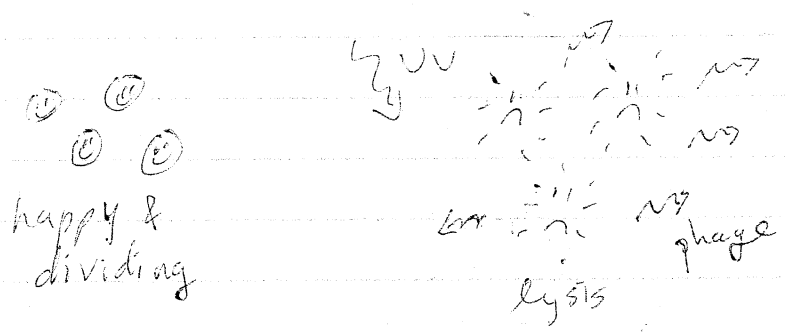
UV induction

Lysis

Lysogeny

partic'
ne–sc
f DNA–
a protein

place–
be ex-

active
ional λ
Figure
ne to λ

phage
or lytic
ressor,
er with
switch.
st (that
and in
r gene
onen'

Figure 1.3. Immunity of a λ-lysogen. Lambda phages inject their chromosomes into a λ-lysogen, but repressor molecules (R) immediately turn off the genes of these "superinfecting" chromosomes, just as they turn off the genes of the prophage. Immunity is thus caused by the same repressor that maintains the prophage in its dormant state.
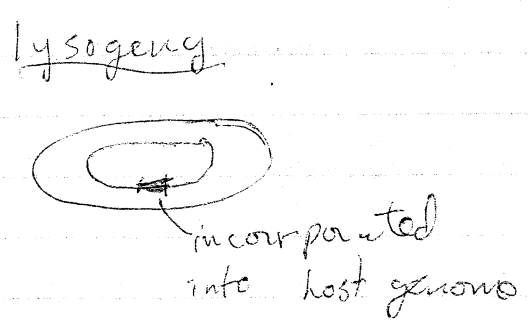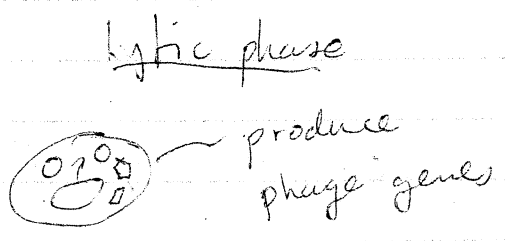
λ-Phage: from M Ptashne, A Genetic Switch
& K. Sheppen

- λ-Phage, a specific virus that affects E.coli

happy &
dividing

error phage
lysis

Depth

UV

- this is a switch in activity

✳ | Fig1.1 | ✳

- show picture of phage - obligate parasite

lytic phase

~ produce
phage genes

lysogeny

incorporated
into host genome

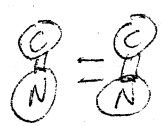- switched on by UV damage - detects ultimate
demise of host ⇒ GET OUT!!

- In lysogen — λ-repressor is only gene
that is produced ⇒ turns off all other
genes
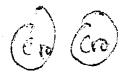
• UV ⊣ λ-rep → turns on Cro   ←cI

λ-DNA
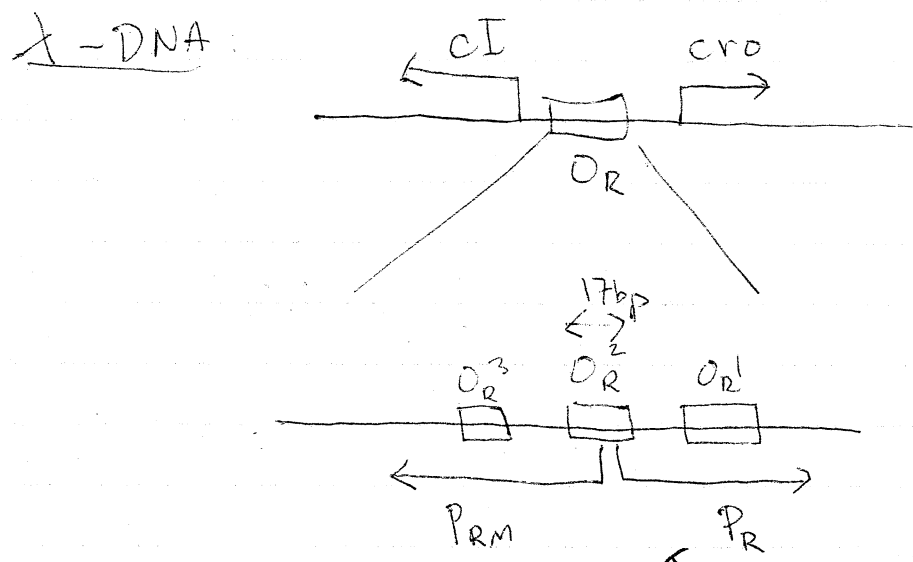
• OR's all bind λ-rep & cro bt @ dif't affinities   ←cI

• P_R doesn't require an activator
• P_{RM} requires an activator

•   ←cI λ-rep forms dimers → cooperativity

        • dimer can bind any of 3 O_R sites

    cro also forms dimers

Switch:
   • if O_R^2 occupied ⊣ P_R & ∴ no cro → lysogen
      → activates P_{RM} → 10 fold ↑ in cI

O_R^1 helps binding
to O_R^2 +ve for
RNAP

Reality: lysogen

$\leftarrow$ PRM | 3 | 2 | 1 | P_R → (with R R labels over boxes)

sometime

R | R | R
3 | 2 | 1

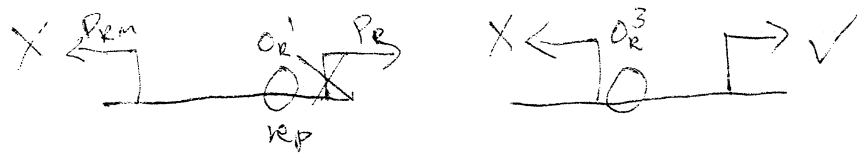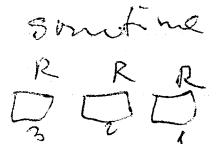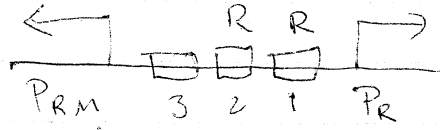OI site affinity: $O_R^1 > O_R^2 > O_R^3$ & cooperativity btwn R's @ O_R^1 & O_R^2 ↑ O_R^2 occupancy

no-coop btwn O_R^2 & O_R^3 since $O_R^2 \to O_R^1$
but if
no O_R^1 then O_R^3 & O_R^2 are cooperative

- Regulation of repressor:
  - O_R^3 acts as negative feedback
  - cI continually being diluted by cell growth & division
  - if cI level is too high then O_R^3 occupied ⊣ cI production

· Induction:
  · UV → DNA damage → turns on RecA ⌐ SOS response ⌐ DNA repair
  · RecA cleaves λ-repressor cI

result: gradual decrease in binding to O_R^1 & O_R^2 by cI → cro gets transcribed!

· cro binds independently & $O_R^3 > O_R^2 > O_R^1$
  → turns on cro & other genes → eventually acts as -ve feedback

Switching & cooperativity
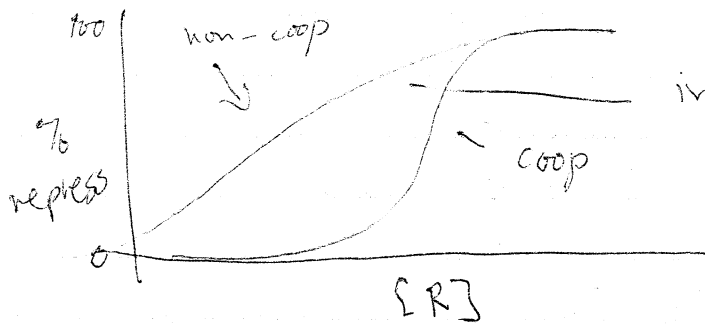
ineffective switch, in fact only single stable state, more on that later

What sets the lysis-lysogeny decision?

40,000 bp
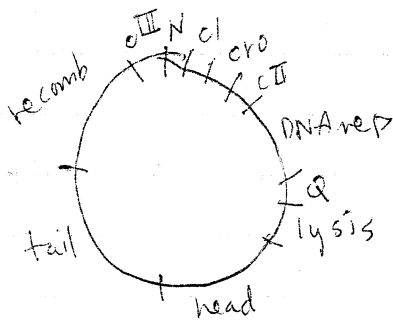
very early: N & cro are on

Early: + recomb & rep genes

N → cII

Late: 1) early genes off & head, tail + lysis → lytic

2) cI & int ≡ recomb → puts in host ≡ lysogen

Lytic: controlled by Q

Lysogenic: cII turns on cI & int, hence in absence of cI, cII will turn on cI which will then stimulate its own synthesis

Decision: affected by cII - activity determined by environment

N
↓
bacterial enzymes/proteases

inactive cII ←
Q ← ↓active
        cI
lysis   lysogeny

— level of proteases depends
  on health of bacteria

↙ weaker suppression of cro 2 orders of mag

3-2-3 mutant ⎱
               ⎰ still switch ?!?
1-2-1 mutant ⎰

|not
a great
switch

↑ shuts itself down sooner
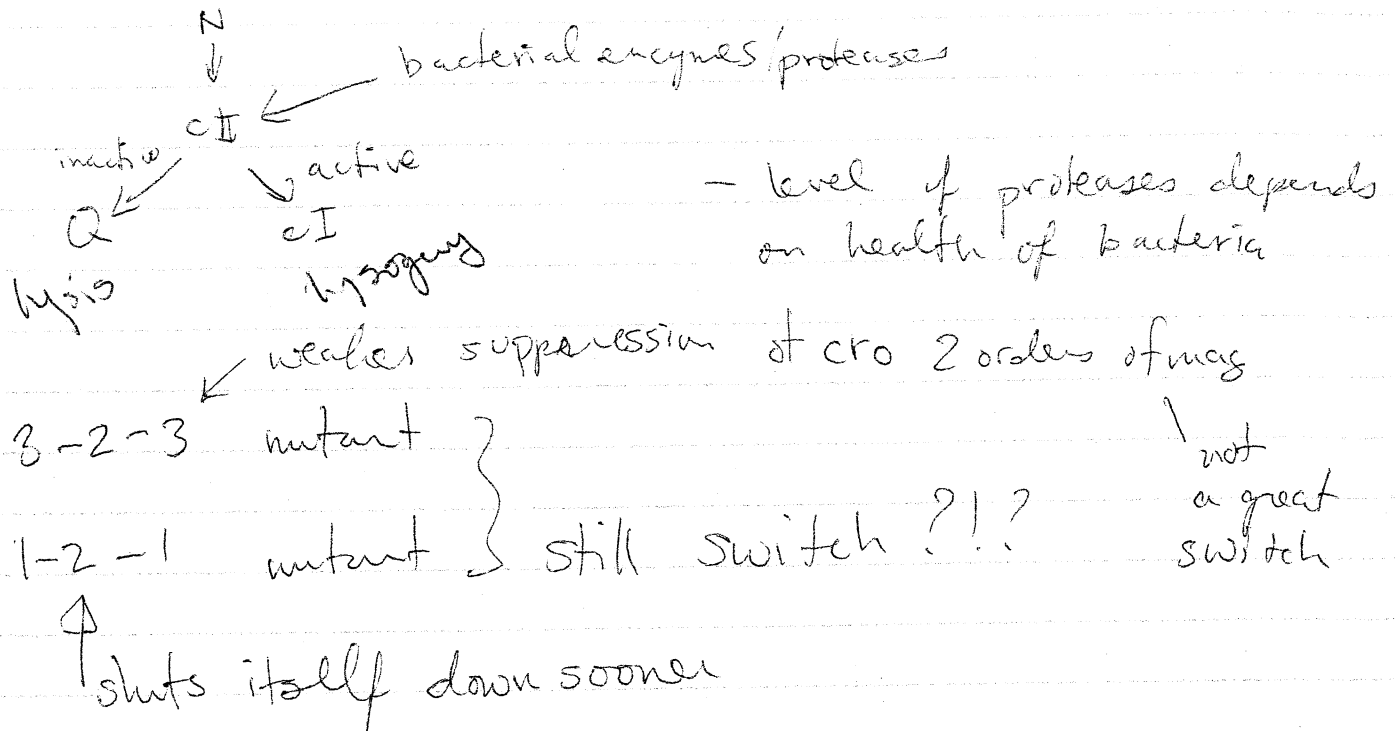
• CII attacked by proteases @ are more active
                            in starving cells

$kT = .6$ kcal/mol

$$P = \frac{1}{1 + n_t/n} = \frac{n}{n + n_t} = \frac{n/n_t}{1 + n/n_t}$$

λ-Phage: (just look @ cI)

$x = [cI]$

Simple:
$$\frac{dx}{dt} = \underbrace{\frac{\overset{f(x)}{\alpha x}}{K+x}}_{} - \underbrace{\gamma x}_{g(x)} \qquad (\text{no coop})$$

unstable   stable → only $\underline{1}$ state
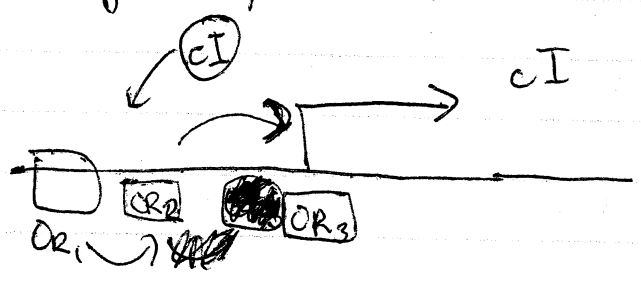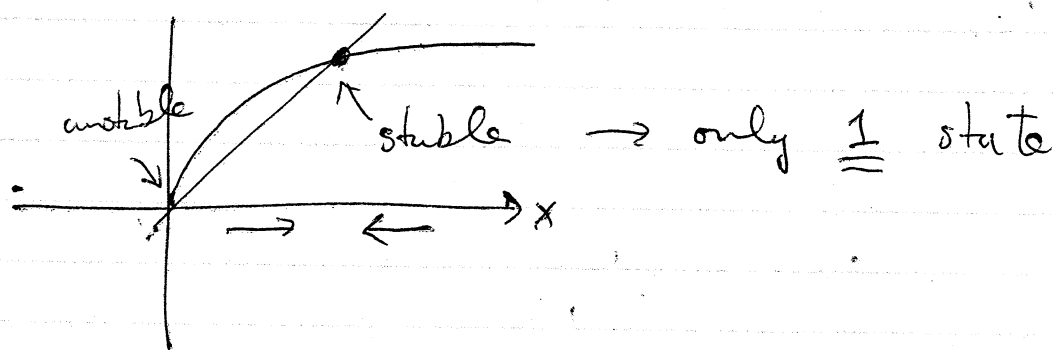
Cooperativity: 2 interacting binding sites

$$\frac{dx}{dt} = \frac{\alpha x^2}{K_D^2 + x^2} - \gamma x$$

Lo   Hi
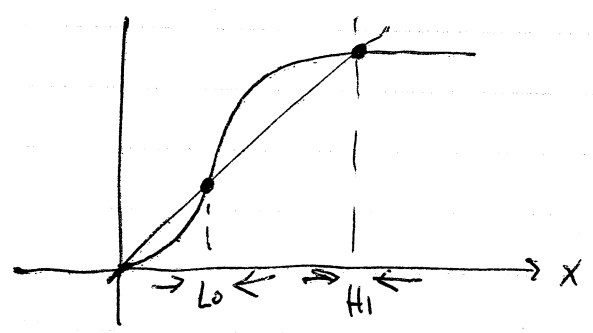
⇒ possibility for 2 stable states!

What about lac operon?   From A. van oudenaarden
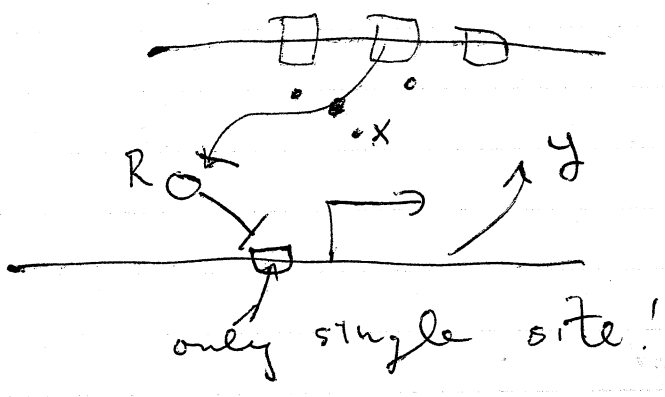
only single site!
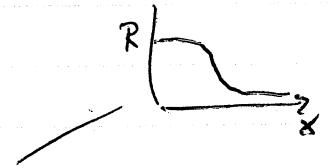
Repressor: $\dfrac{R}{R_T} = \dfrac{1}{K_x^n + x^n}$ ← shutting down of LacI due to binding alc-lactose

$$\frac{dy}{dt} = \frac{\alpha}{K_R + R} - \gamma y \qquad (\text{no -coop})$$

$$\frac{dx}{dt} = \beta y - \mu x \qquad (\text{uptake})$$

• x uptake is fast & @ equilibrium

$$\Rightarrow \quad x = \beta' y$$

so

$$R = \frac{R_T}{K_x^n + (\beta' y)^n}$$

$$\Rightarrow \quad \frac{dy}{dt} = \frac{\alpha'(1 + \beta' y)^n}{\gamma + (\beta' y)^n} - y \; ; \quad n \cong 2$$

∴ switchable

LacY hysteresis

Green fluorescence

initial HIGH state

monostable   bistable   monostable

initial LOW state

Extracellular TMG (µM)

2   4 6 8 10   20   40

100
10
1

25

26

Functions α, β, and ρ are calculated from switching thresholds

$$\frac{R}{R_T} = \frac{1}{1+(x/x_o)^n}$$

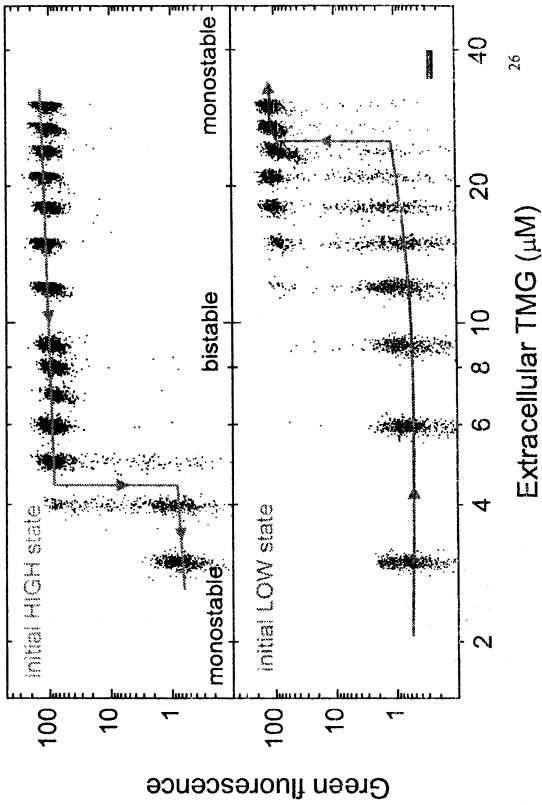$$\tau_y \frac{dy}{dt} = \alpha \frac{1}{1+R/R_o} - y$$

$$\tau_x \frac{dx}{dt} = \beta y - x$$

$$\rho = 1 + R_T/R_o$$

α: maximum lacY synthesis rate obtained if R → 0

α / ρ : minimum lacY synthesis rate obtained if R → R_T

Maximal activity (α)

Repression factor (ρ)

Red fluorescence

28

Mapping the bistable region as a function of TMG and glucose concentration

Extracellular TMG (µM)

monostable (induced)

bistable

monostable (uninduced)

Extracellular glucose (µM)

Green fluorescence

27
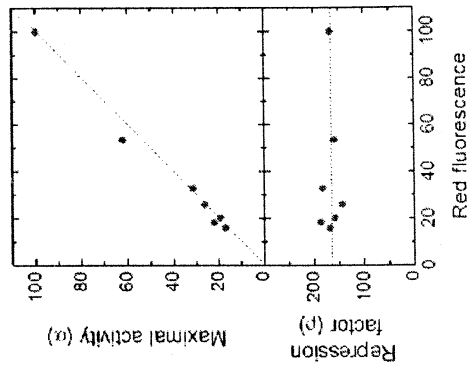
Stability Analysis

S. Strogatz   Nonlinear Dynamics & Chaos

<u>1D</u> $\frac{dy}{dt} = f(y)$ & $f(y^*) = 0$

is y^* <u>stable</u>?

$$y = y^* + \delta y$$

$$\Rightarrow \quad \dot{\delta y} = f'(y^*)\,\delta y \qquad (\text{Taylor})$$

assume $\delta y = A \exp(\lambda t)$
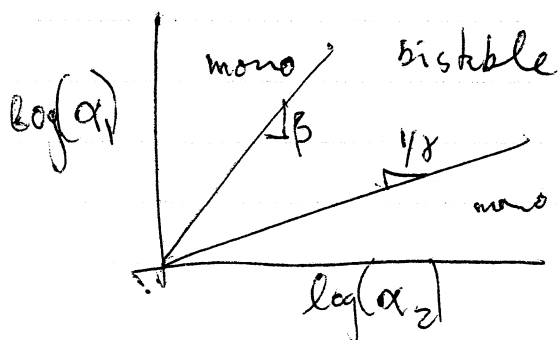
so $\lambda \delta y = f'(y^*)\delta y$

$$(f'(y^*) - \lambda)\, y = 0$$

so $\lambda = f'(y^*)$

if $\lambda < 0$ then decays & is stable
$\lambda > 0$ unstable

<u>2D</u> Want to derive Fig 2cd from Celtius

2D $\quad \dot{x} = f(x, y)$
$\qquad \dot{y} = g(x, y)$

$\underline{\text{nullclines}} \qquad \dot{x} = 0 \quad \& \quad \dot{y} = 0 \quad \to \quad f(x_0, y_0) = 0 \ \& \ g(x_0, y_0) = 0$

$\underline{\text{linearize}}$:

$$\delta \vec{x} = x - x_0 \qquad \& \qquad \delta y = y - y_0$$

$$\dot{x} = \delta \dot{x} = \delta x \left.\frac{\partial f}{\partial x}\right|_{x_0, y_0} + g \left.\frac{\partial f}{\partial y}\right|_{x_0, y_0} = a\,\delta x + b\,\delta y$$

$$\dot{y} = \delta \dot{y} = \delta x \left.\frac{\partial g}{\partial x}\right|_{x_0, y_0} + \delta y \left.\frac{\partial f}{\partial y}\right|_{x_0, y_0} = c\,\delta x + d\,\delta y$$

$\overset{\text{or}}{\quad}$

$$\dot{\delta \vec{x}} = A\,\delta \vec{x} \qquad \text{where} \qquad A = \begin{bmatrix} a & b \\ c & d \end{bmatrix} \equiv \text{Jacobian}$$

$$A_{ij} = \frac{\partial f^i}{\partial x_j} \qquad ; \qquad \begin{aligned} \tau &= \text{Tr}(A) = a + d \\ \Delta &= \det(A) = ad - cb \end{aligned}$$

- Consider ~~$\text{both } \tau = \text{Tr}(A) = a + d \ \& \ \Delta = \det(A)$~~

$$\delta \vec{x}_0 = \vec{a}\,e^{\lambda t} \qquad\qquad \delta \vec{x} = \sum c_i \vec{a}_i\, e^{\lambda_i t}$$

$$\implies \quad A\,\delta \vec{x} = \lambda\,\delta \vec{x}$$

$$\implies \text{eigenvalue problem}$$

$$\det \begin{bmatrix} a-\lambda & b \\ c & d-\lambda \end{bmatrix}$$

$$\Rightarrow \quad \lambda_1 = \frac{\tau + \sqrt{\tau^2 - 4\Delta}}{2} \qquad \Delta = ad - bc$$

$$\tau = a + d$$

$$\lambda_2 = \frac{\tau - \sqrt{\tau^2 - 4\Delta}}{2}$$

<u>diagonal</u>: $\quad \Delta = \lambda_1 \lambda_2 \quad \& \quad \tau = \lambda_1 + \lambda_2$

for stability require λ's < 0

so must have $\boxed{\Delta > 0 \quad \& \quad \tau < 0}$ (for 2D)

<u>Collins</u>:

$$\dot{u} = \frac{\alpha_1}{1 + v^\beta} - u \quad \doteq f(u,v)$$

$$\dot{v} = \frac{\alpha_2}{1 + u^\gamma} - v \quad = g(u,v).$$

<u>fixed points</u> $\quad u = \frac{\alpha_1}{1 + v^\beta} \qquad v = \frac{\alpha_2}{1 + u^\gamma}$
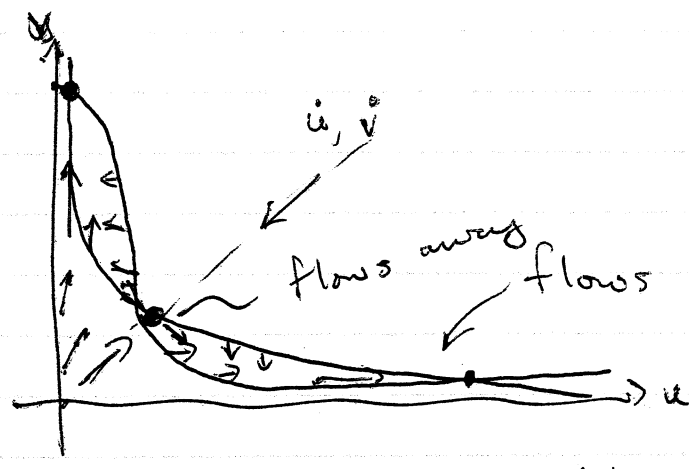
$$A = \begin{bmatrix} -1 & \dfrac{-\alpha_1 \beta v^{\beta-1}}{(1+v^\beta)^2} \\ \dfrac{-\alpha_2 \gamma u^{\gamma-1}}{(1+u^\gamma)^2} & -1 \end{bmatrix}$$

- $\tau < 0$ good!

- $\Delta = 0$ gives boundary

$$\Rightarrow \quad \frac{\alpha_1 \beta v^{\beta-1}}{(1+v^\beta)^2} \frac{\alpha_2 \gamma u^{\gamma-1}}{(1+u^\gamma)^2} = 1$$

use fixed point equs for u & v

$$\Rightarrow \quad \beta \gamma v^{\beta+1} u^{\gamma+1} = \alpha_1 \alpha_2$$

for large α_1 & α_2 & for high state $u \gg v$

so $u \gtrsim \alpha_1$ & $v \approx \alpha_2 / \alpha_1$

so $\beta \gamma \alpha_2^\beta \alpha_1^{-\beta\gamma} = 1$ & symetry $\beta\gamma \alpha_1^\gamma \alpha_2^{-\beta\gamma} = 1$

or $\log(\alpha_1) \approx \frac{1}{\gamma} \log \alpha_2$ (straight line with slope $1/\gamma$)