Bistability: a building block for cellular decisions

Sandeep Krishna

Simons Centre for the Study of Living Machines, NCBS
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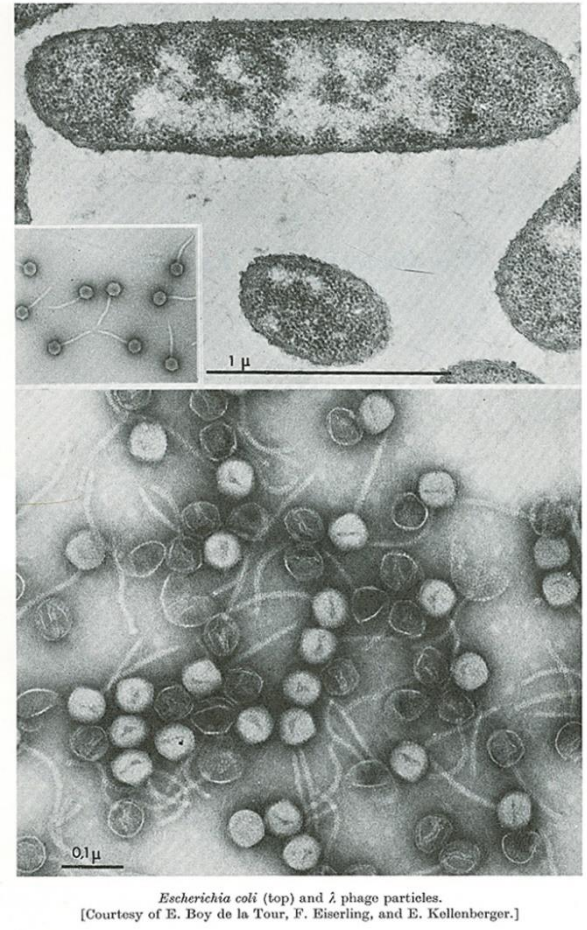
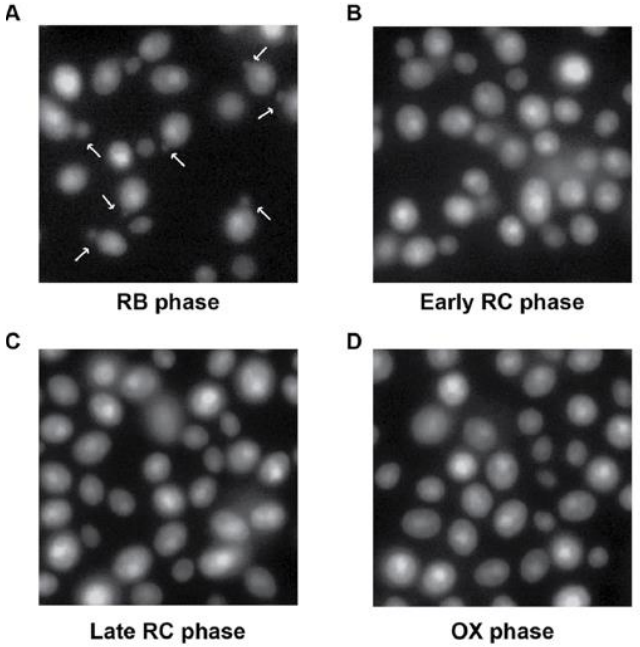


Escherichia coli (top) and λ phage particles.
[Courtesy of E. Boy de la Tour, F. Eiserling, and E. Kellenberger.]

Today: temperate bacteriophage (like phage λ)

Tomorrow(with Sunil): budding yeast



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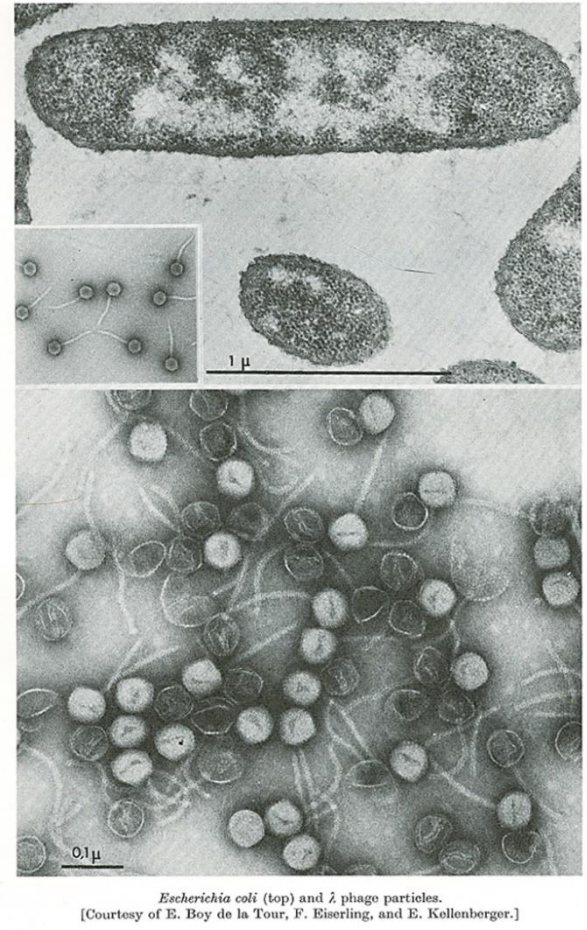

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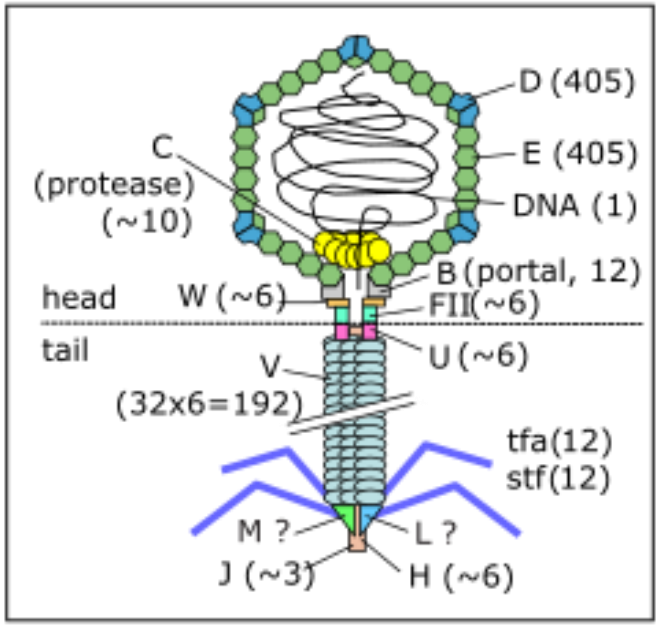
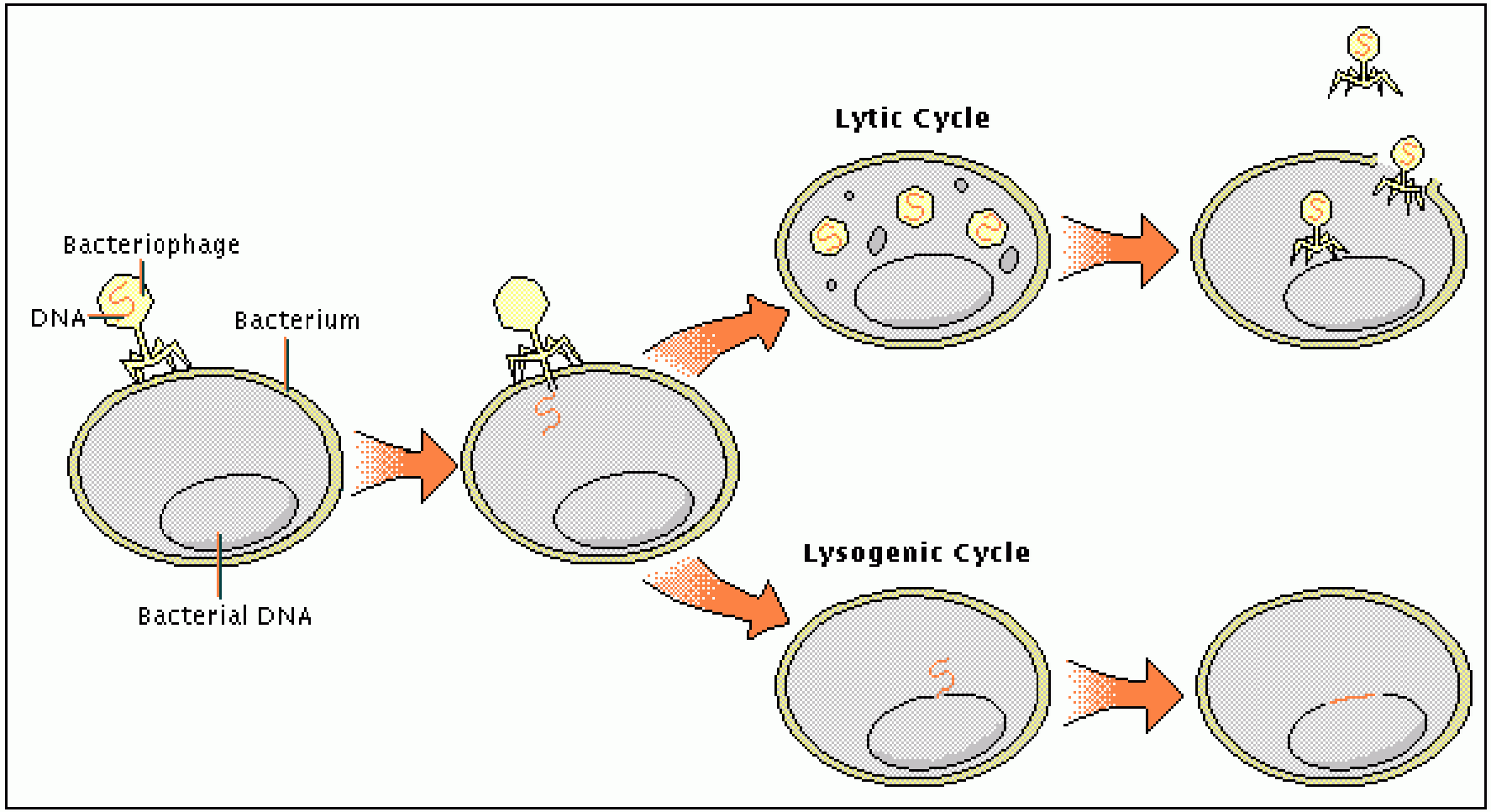


Temperate bacteriophage: Lysis-lysogeny decisions



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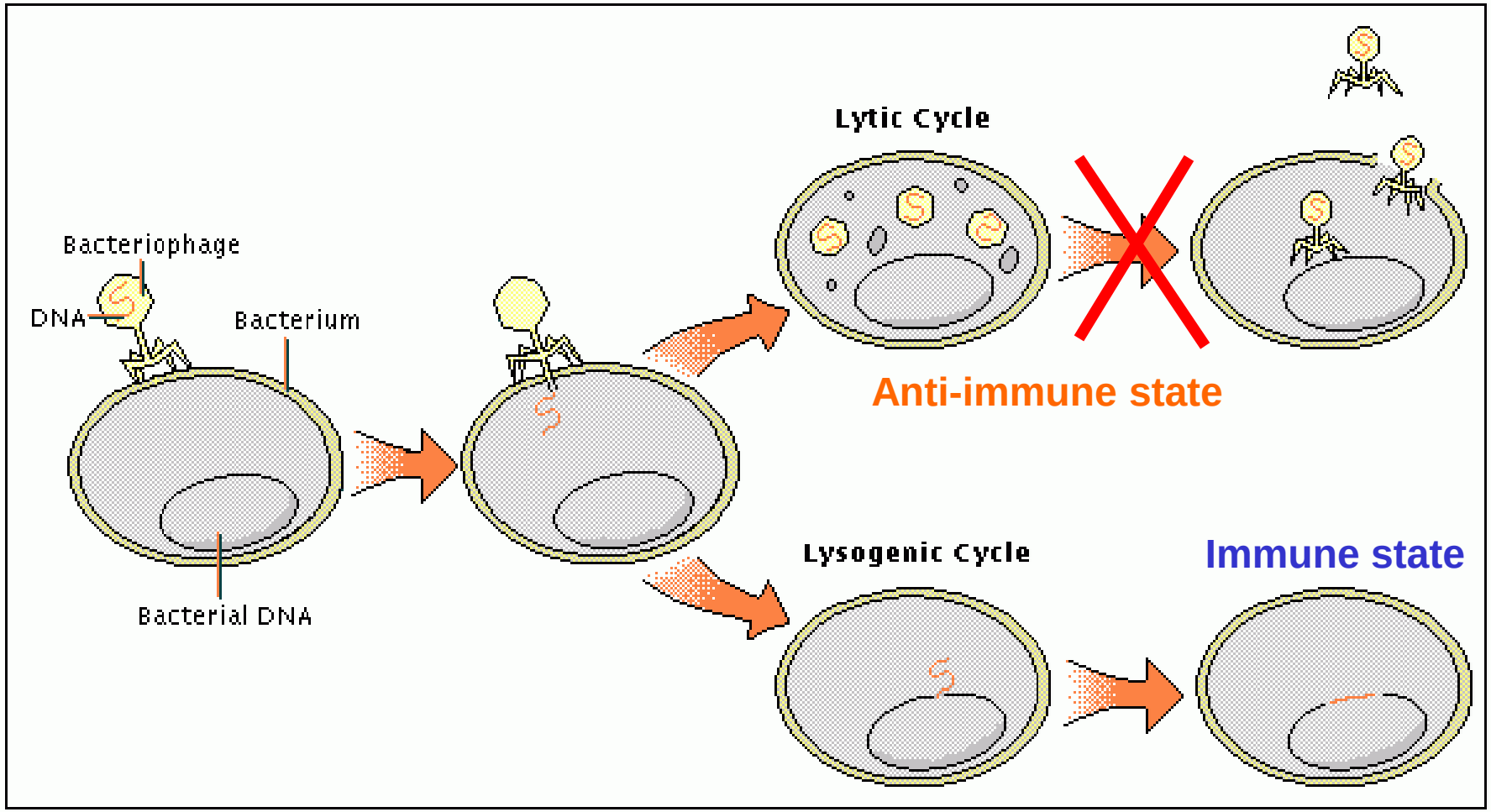


Two very stable states: probability of exiting ~O(10^{-5}-10^{-6}) per cell per generation
Stable even with a single copy of the genome left

Genome of phage λ

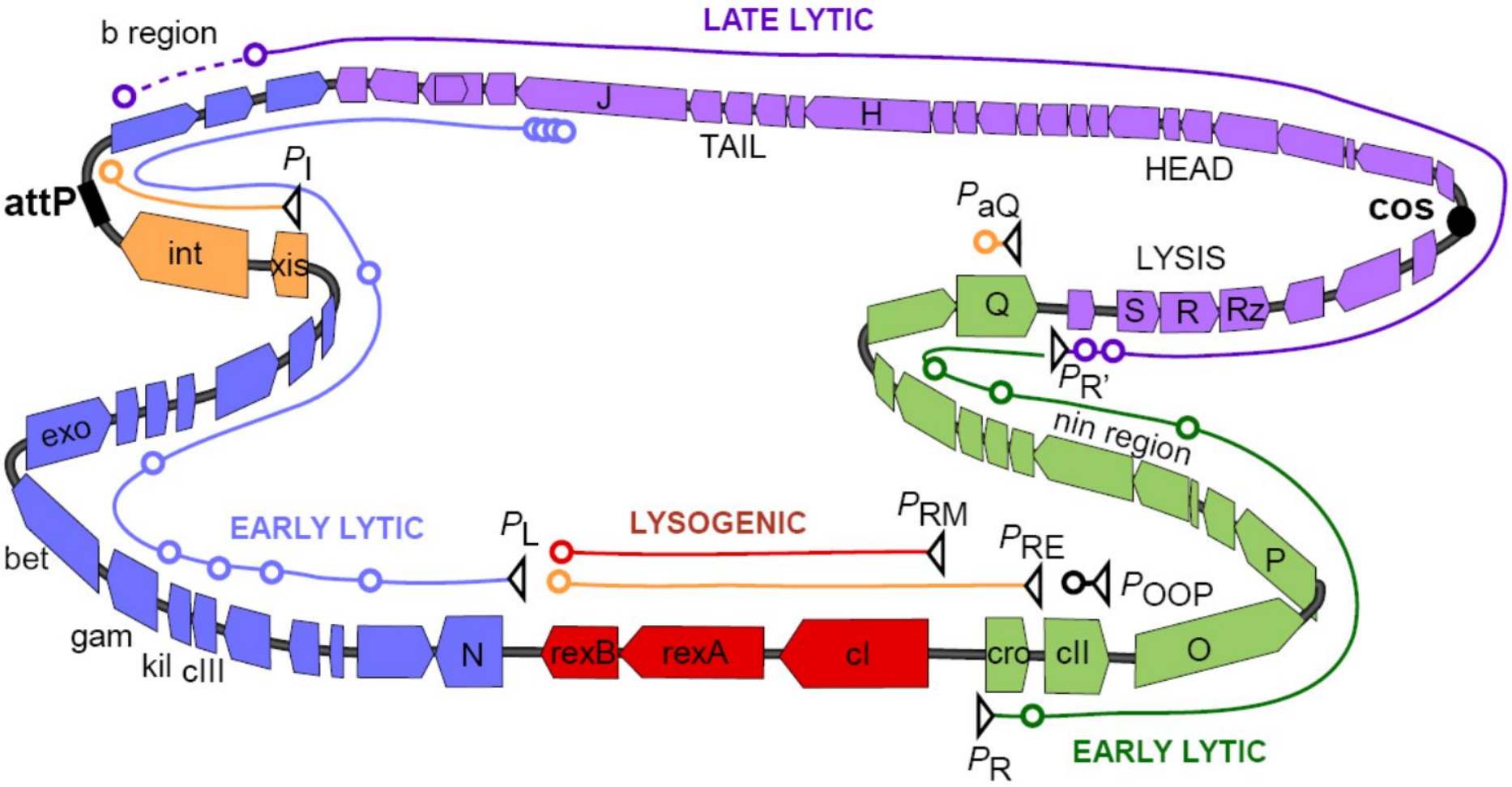
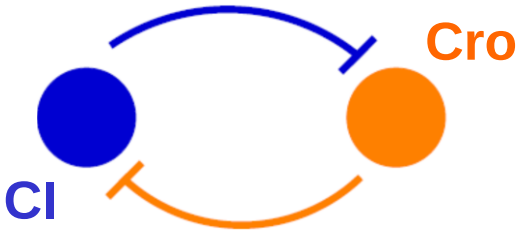


Image courtesy Keith Shearwin, Adelaide Univ.

“Standard model” of λ

Ptashne, A Genetic Switch: Phage Lambda Revisited

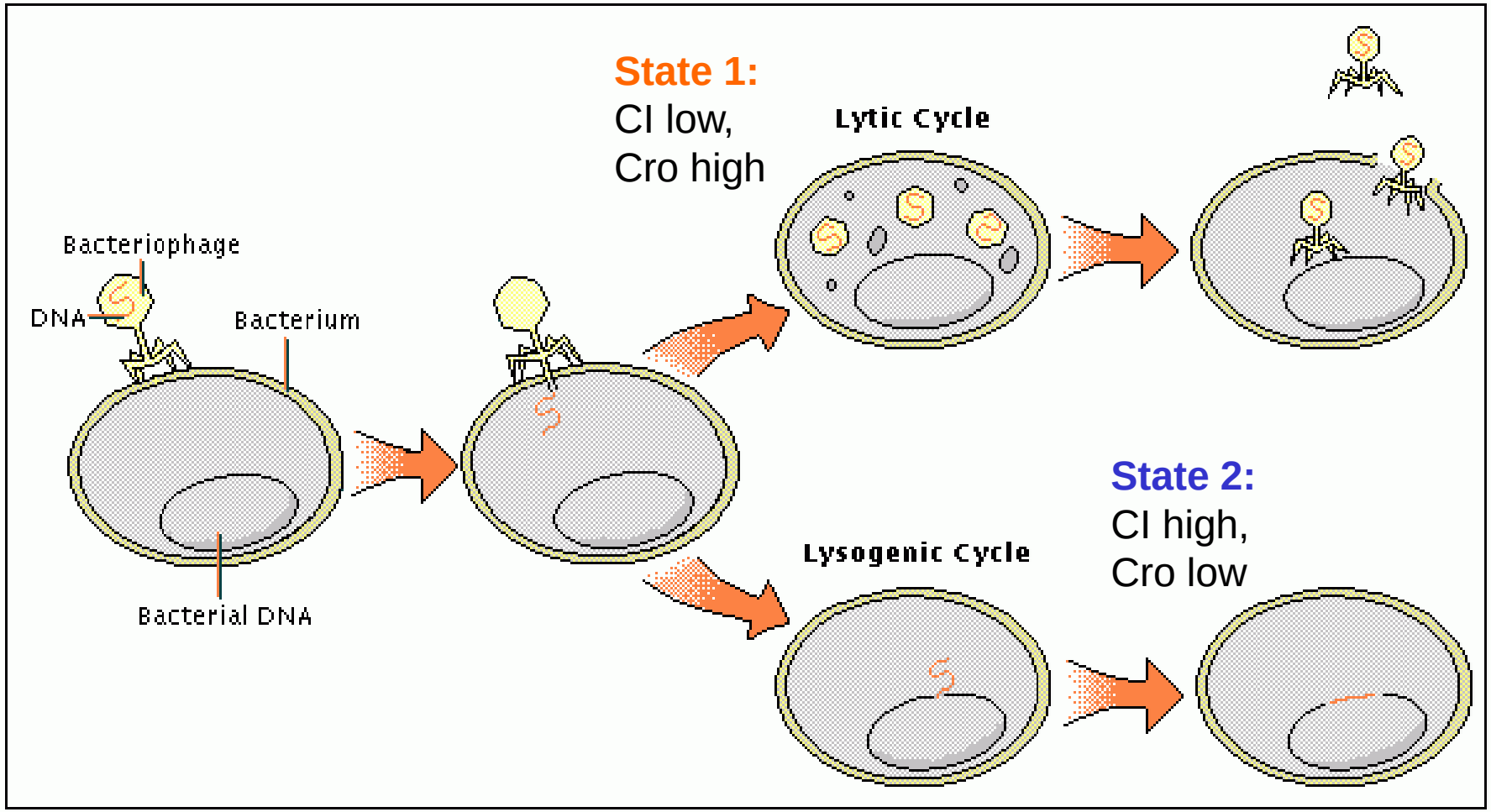
Ptashne & Gann, Genes and Signals

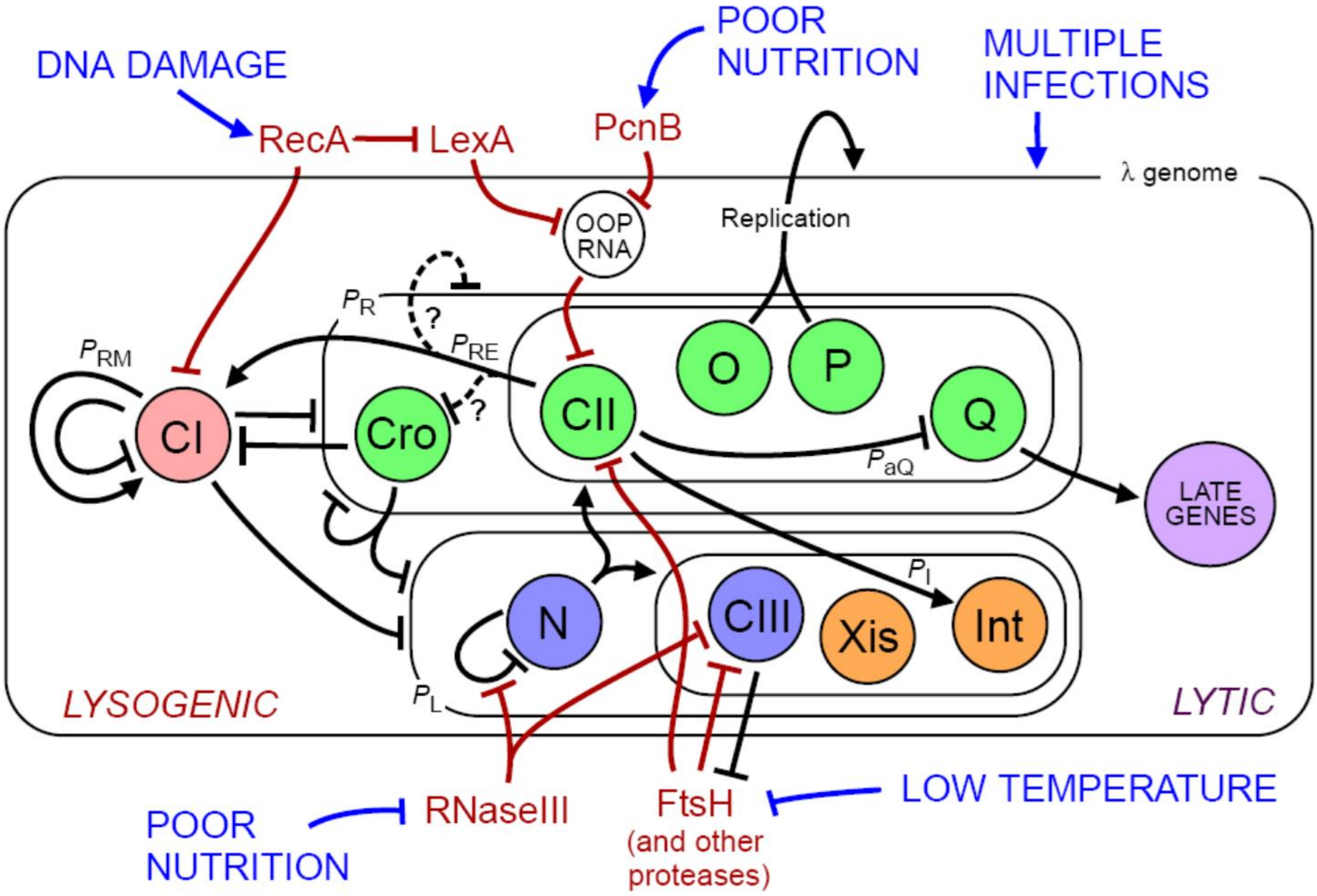


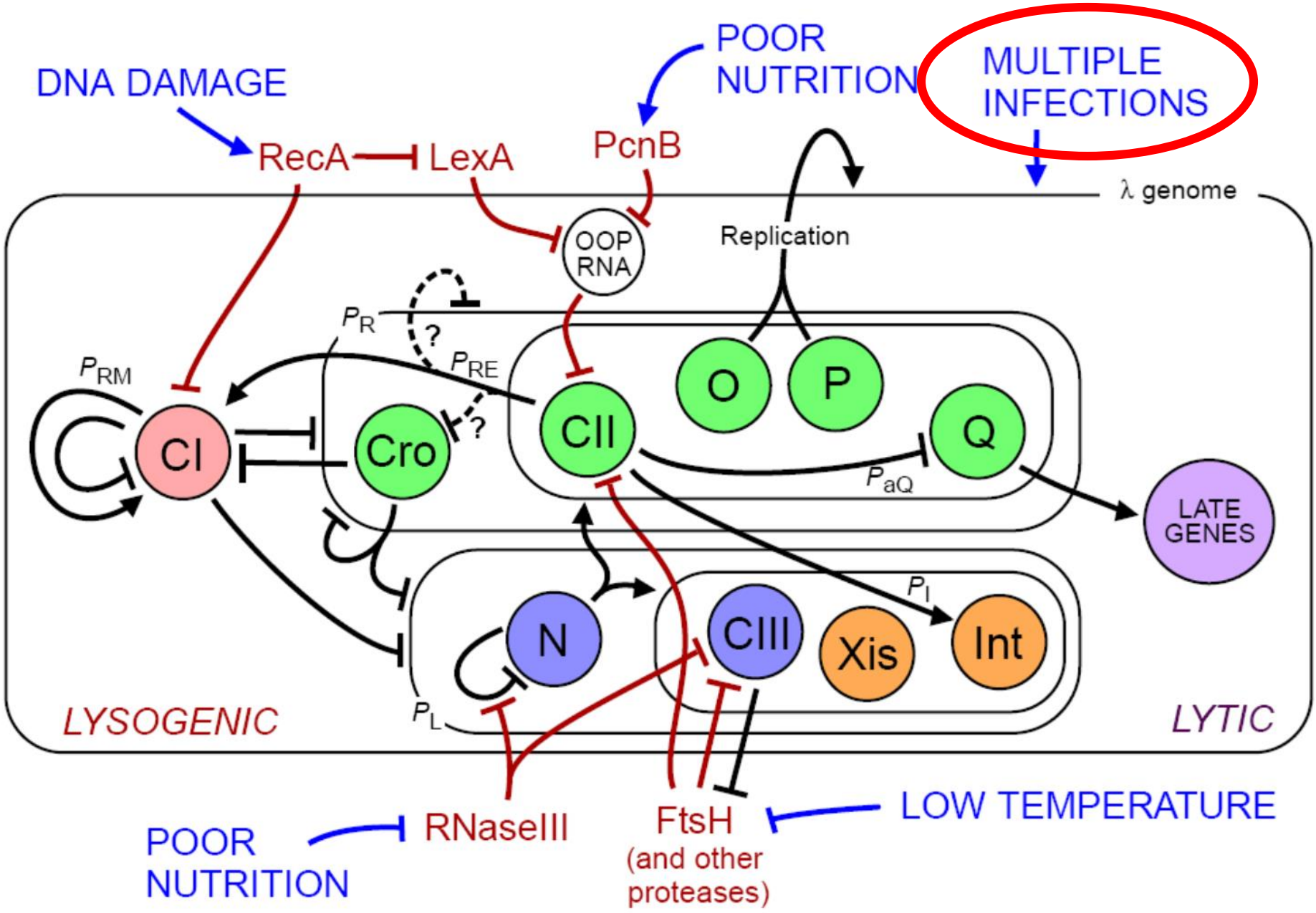
- Simple bistable switch (A represses B; B represses A)
- Two states:

1. Lytic (**CI** low, **Cro** high)
2. Lysogenic (**CI** high, **Cro** low)

Temperate bacteriophage: Lysis-lysogeny decisions




DNA DAMAGE
RecA
LexA
POOR
NUTRITION
PcnB
MULTIPLE
INFECTIONS
λ genome
OOP
RNA
Replication
P_R
P_{RE}
P_{RM}
?
?
CI
Cro
CII
O
P
Q
P_{aQ}
LATE
GENES
N
CIII
Xis
Int
P_I
P_L
LYSOGENIC
LYTIC
POOR
NUTRITION
RNaseIII
FtsH
(and other
proteases)
LOW TEMPERATURE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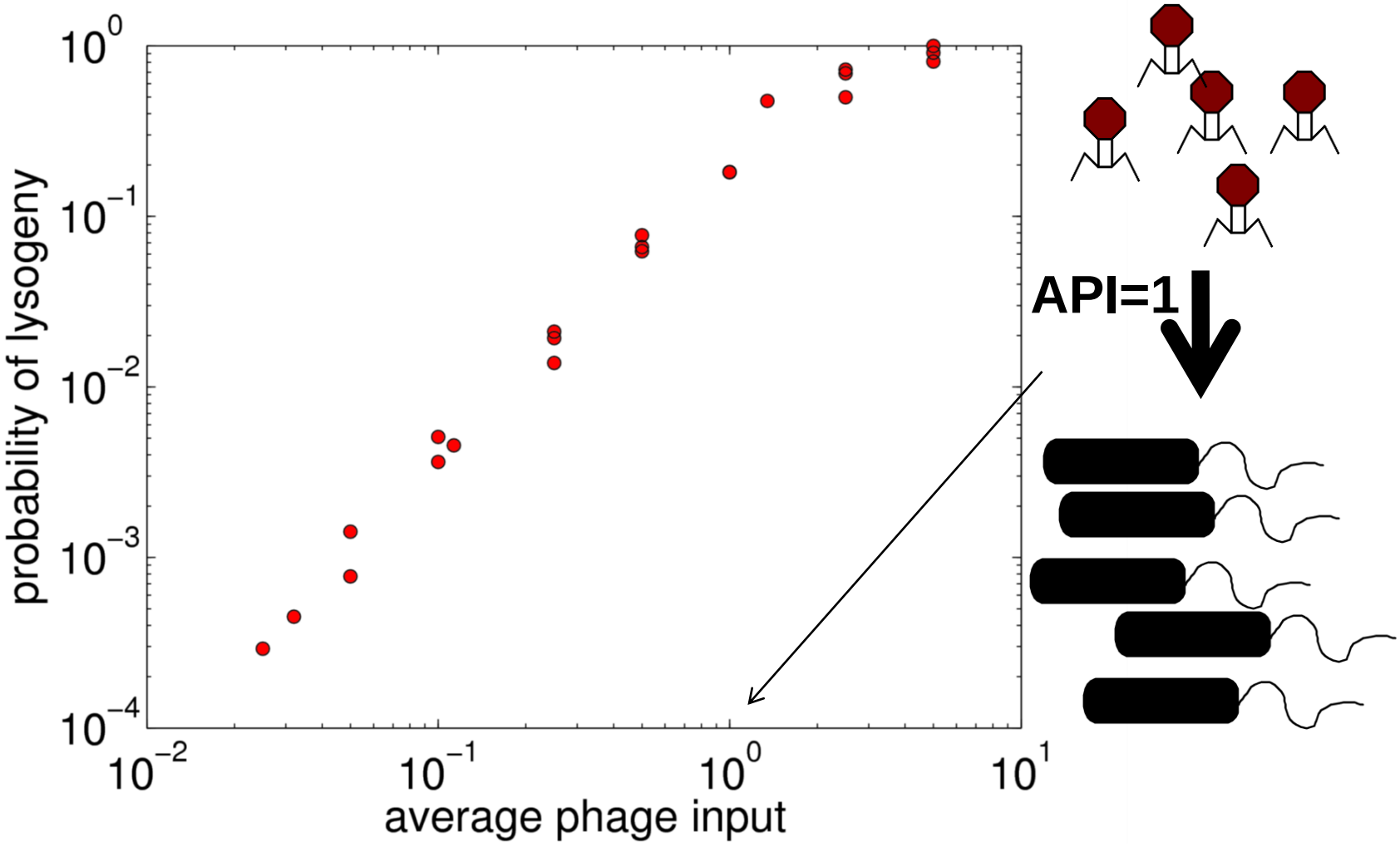
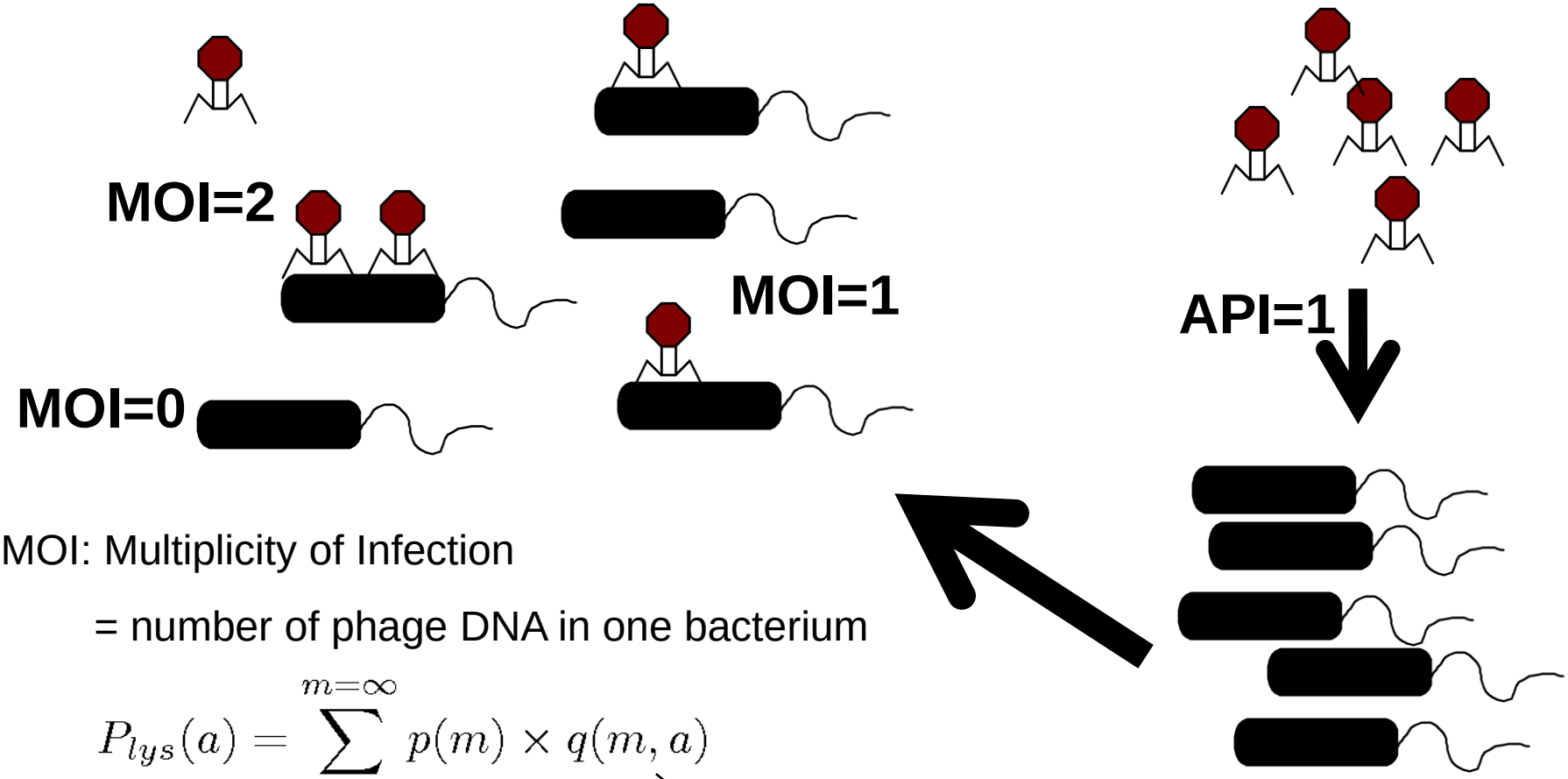
Kourilsky's experiment



(API: ratio of total phage to total bacteria)

P. Kourilsky: Molec. gen, Genet. 122, 183-195 (1973); Biochimie 56, 1517-1523 (1974).

Kourilsky's experiment



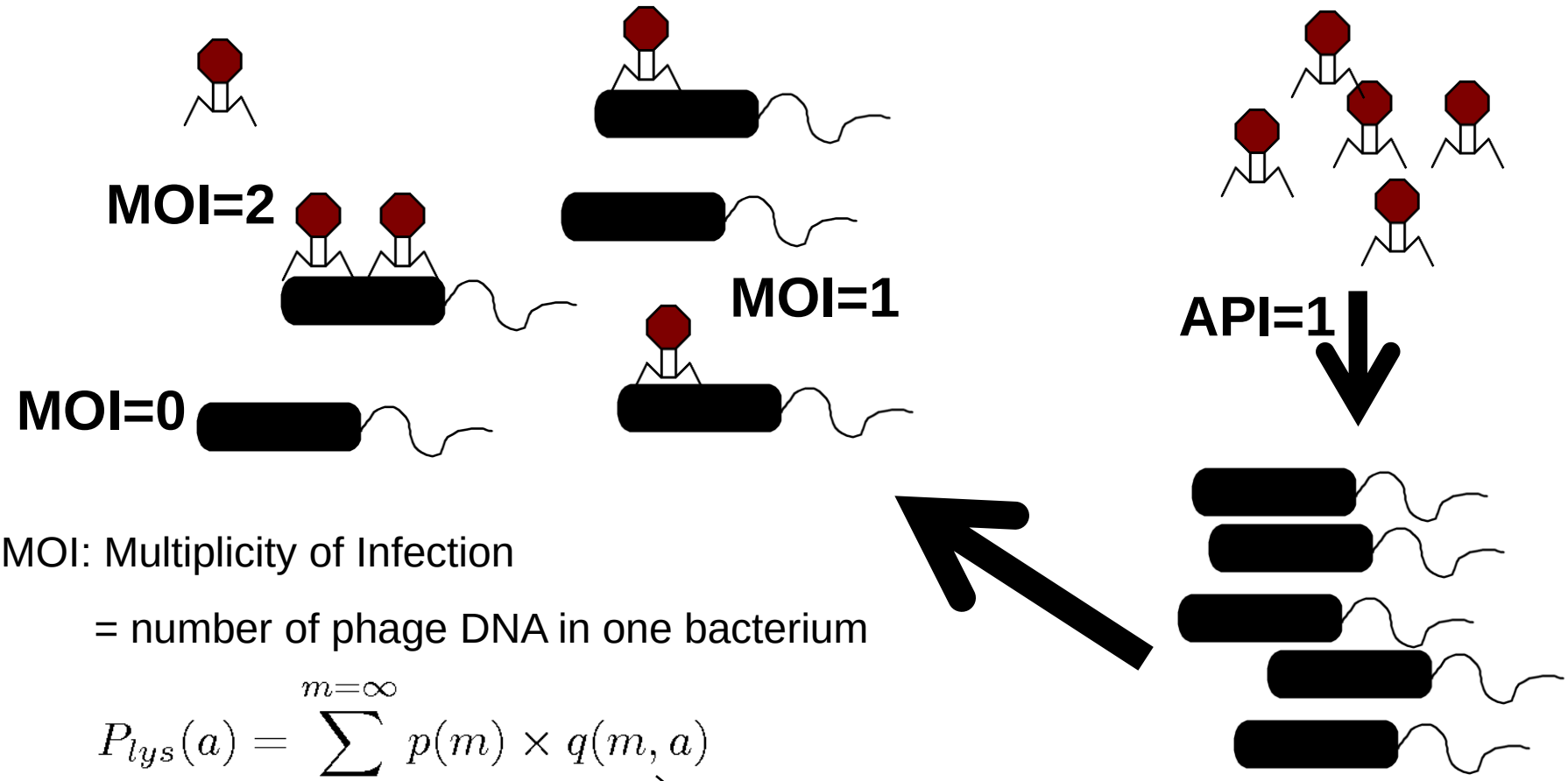
MOI: Multiplicity of Infection

= number of phage DNA in one bacterium

$$P_{lys}(a) = \sum_{m=0}^{m=\infty} p(m) \times q(m,a)$$

Prob. Lysogeny if API is *a* = **Prob. Lysogeny if MOI is *m*** X **Prob. of getting MOI *m* if API is *a***

Kourilsky's experiment



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$$P_{lys}(a) = \sum_{m=0}^{m=\infty} p(m) \times q(m,a)$$

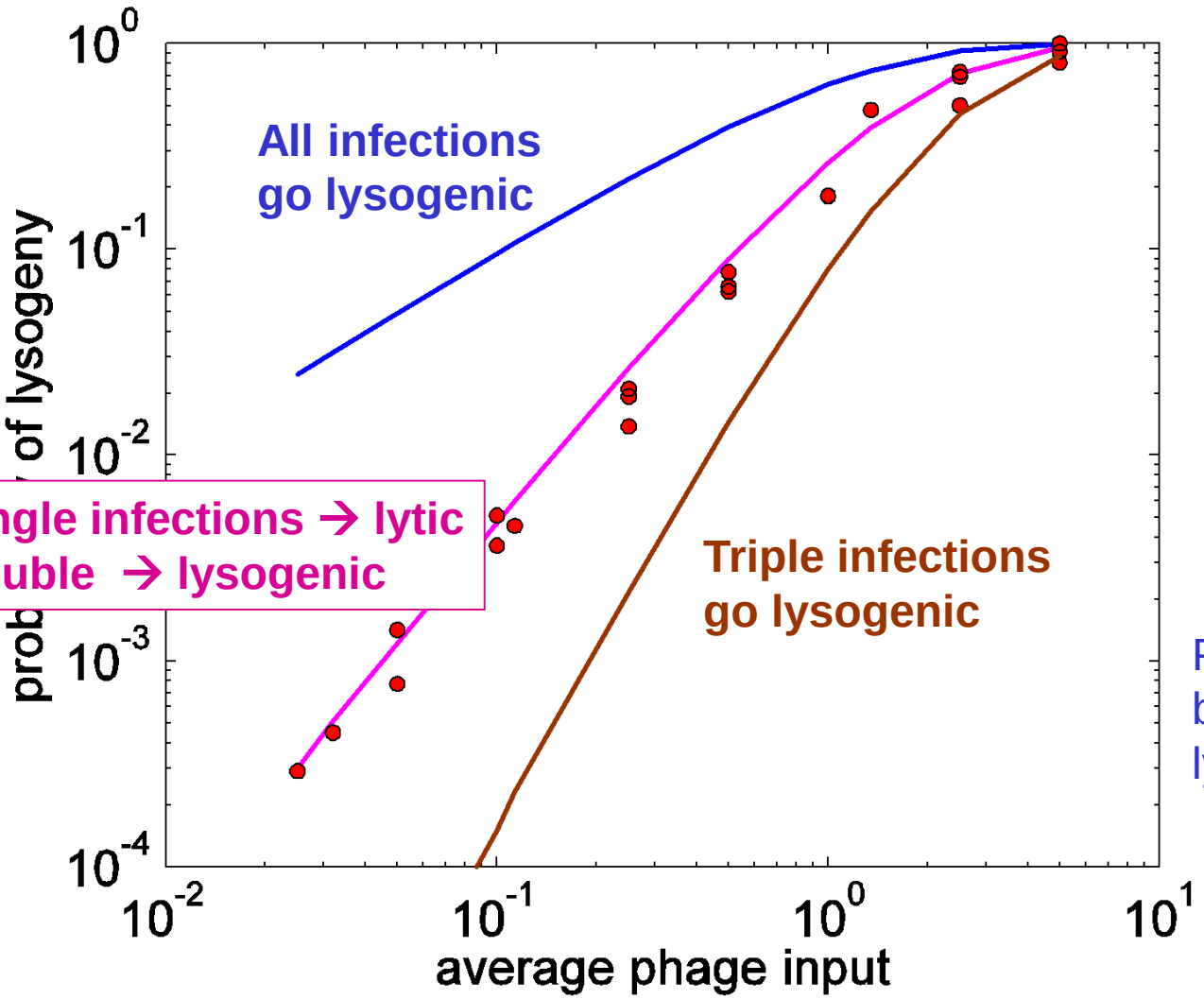
Prob. of getting MOI *m* if API is *a*

$$q(m,a) = \frac{a^m}{m!} e^{-a}$$

Poissonian assumption:

Each phage randomly & independently finds a bacterium to infect

Kourilsky's experiment



Number of phage DNA in one bacterium

m =	1	2	>2	
	1	1	1	
p(m)=	0	1	1	
	0	0	1	

Probability that bacterium will go lysogenic

M. Avlund, I. B. Dodd, S. Semsey, K. Sneppen, S. Krishna
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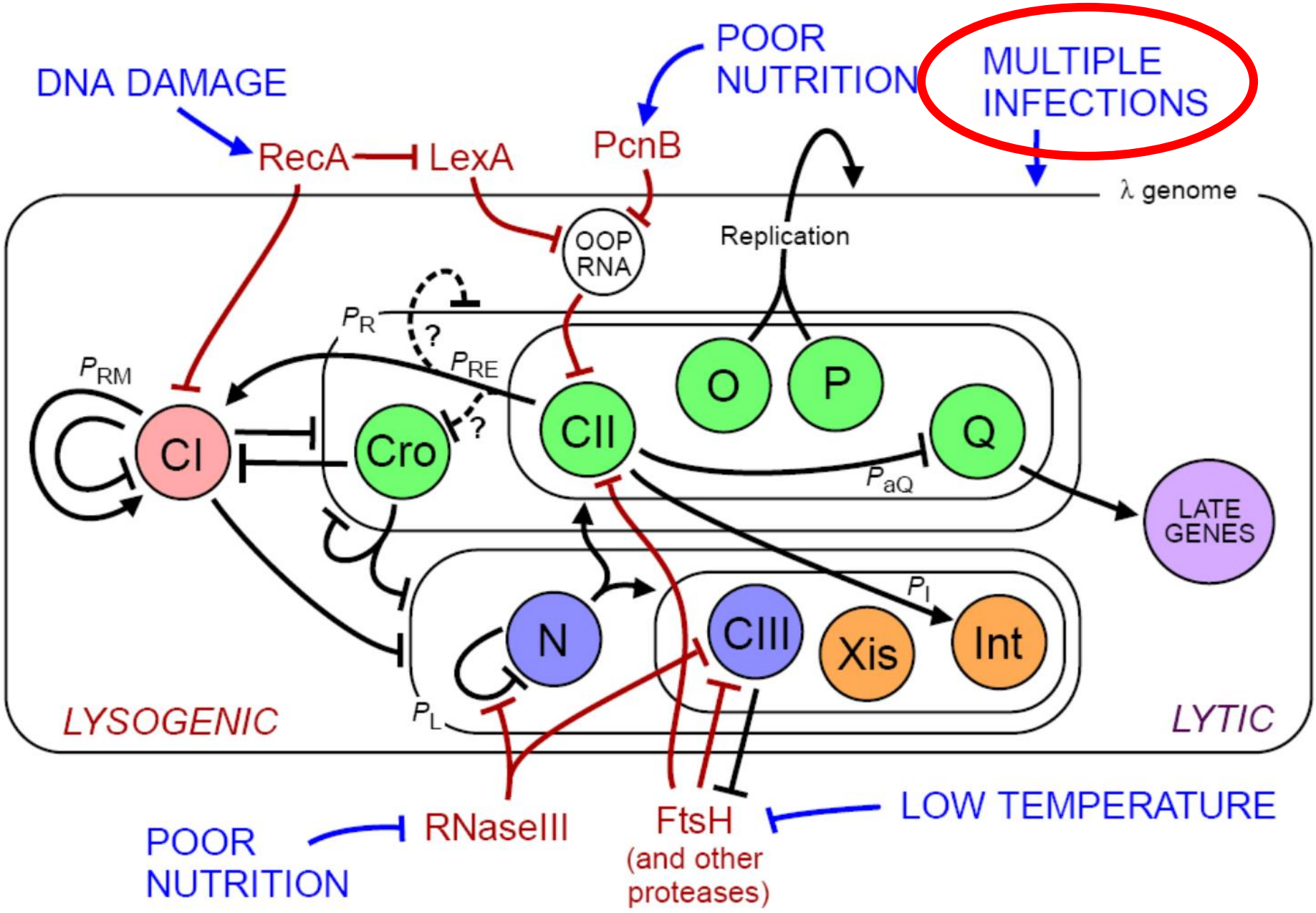
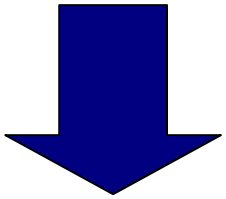


Detailed models of lambda: Deterministic models: McAdams & Shapiro (1995) Science 269, p 650.; **Stochastic models:** Arkin et al. (1998) Genetics 149, p 1633.

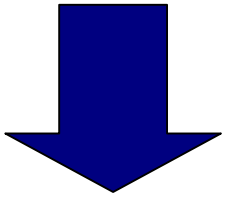
An alternate approach

Choose the building blocks



Build a class of dynamical systems

Subject them to some functional task



What range of behaviour is possible?

How would one construct a given behaviour?

Are there many ways of doing so?

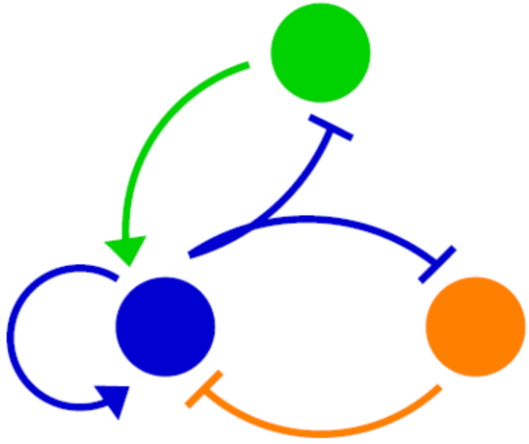
1-protein motifs

e.g. self-activator

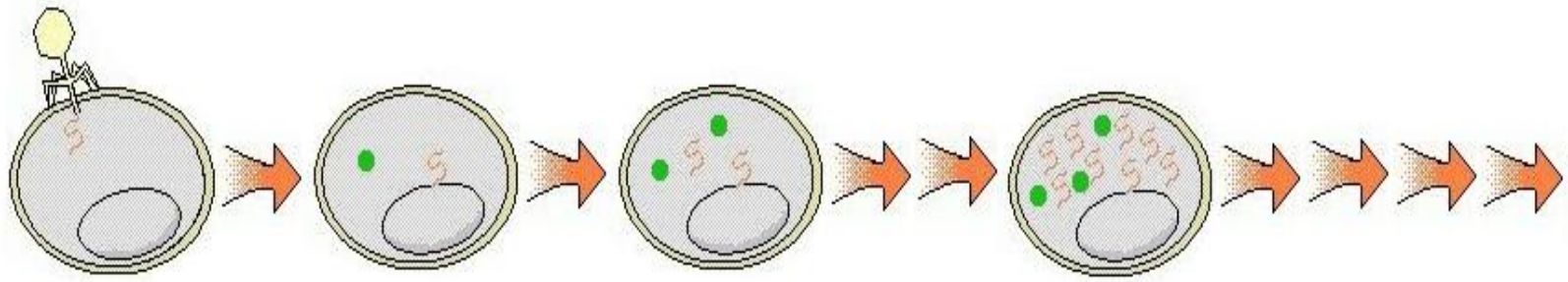
2-protein motifs

e.g. mutual repressors or mutual activators

3-protein motifs

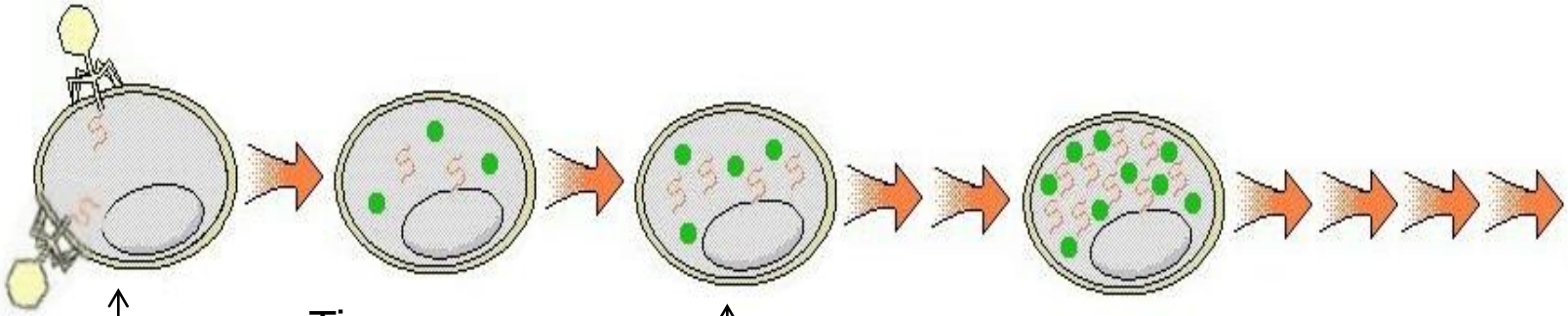


MOI=1



State 1

MOI=2



State 2

Initially all phage proteins are at zero

Time passes: proteins are produced

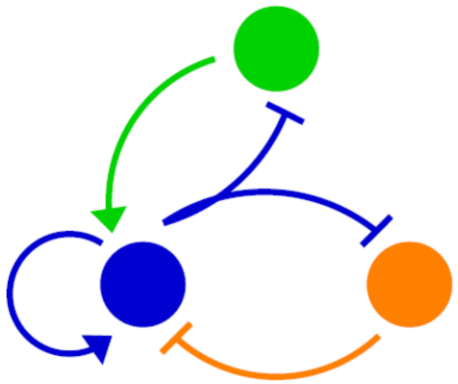
3 min

Phage genomes replicate

Task: find motifs that are bistable and can count genomes.

Network =

Motifs



x

Parameter sets

- Protein stabilities
- Production rates
- Repression, activation strengths and cooperativities

100 million ~ **100** **x** **1 million**

Example motif

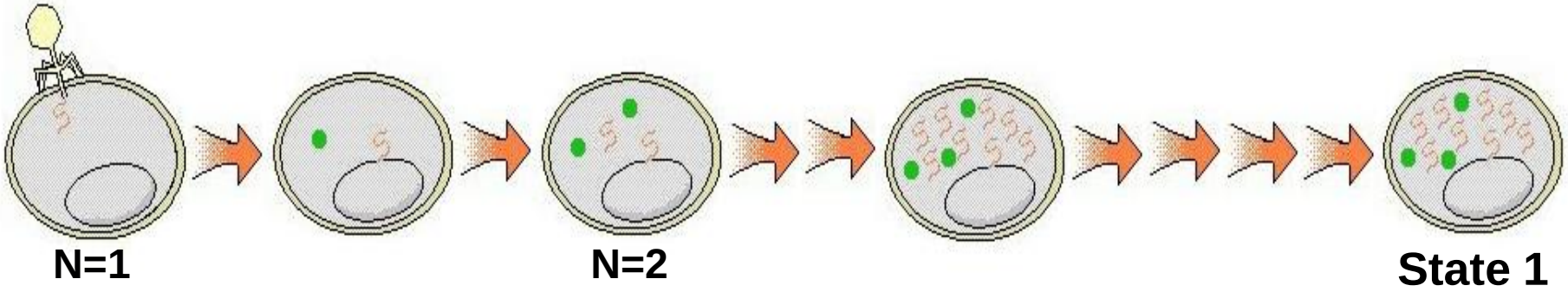
One self-activating protein

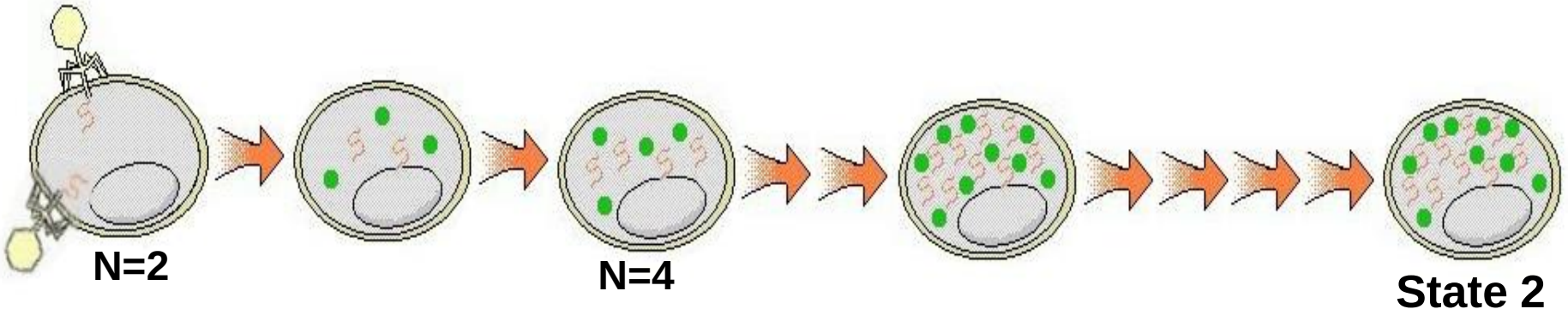
$$\frac{d(CI)}{dt} = N\left[\frac{(CI)^h}{(CI)^h + K^h}\right] - \gamma(CI)$$

Degradation rate of protein

Number of phage genomes

Concentration of protein





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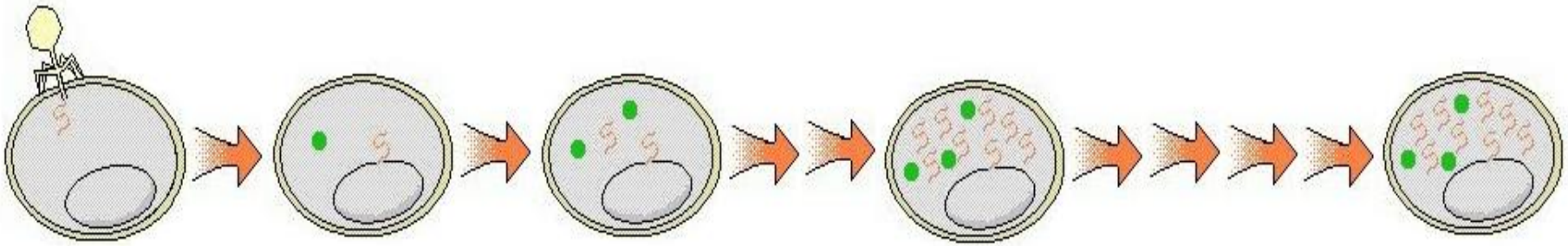
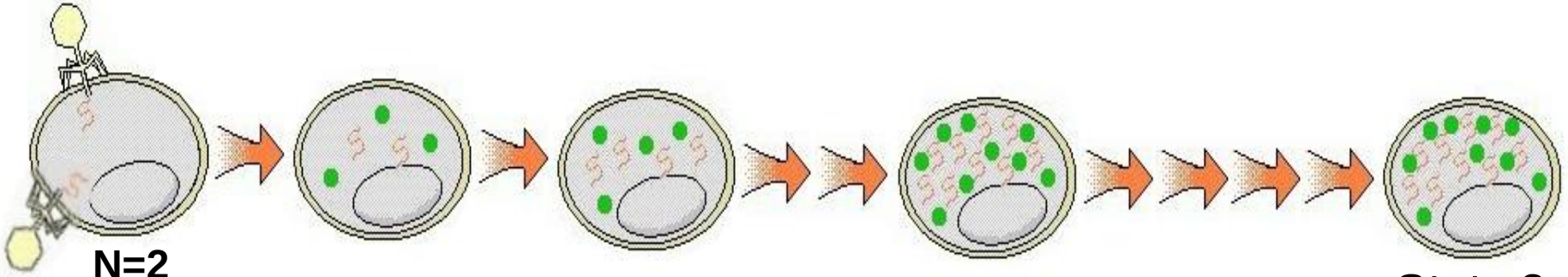


N=1

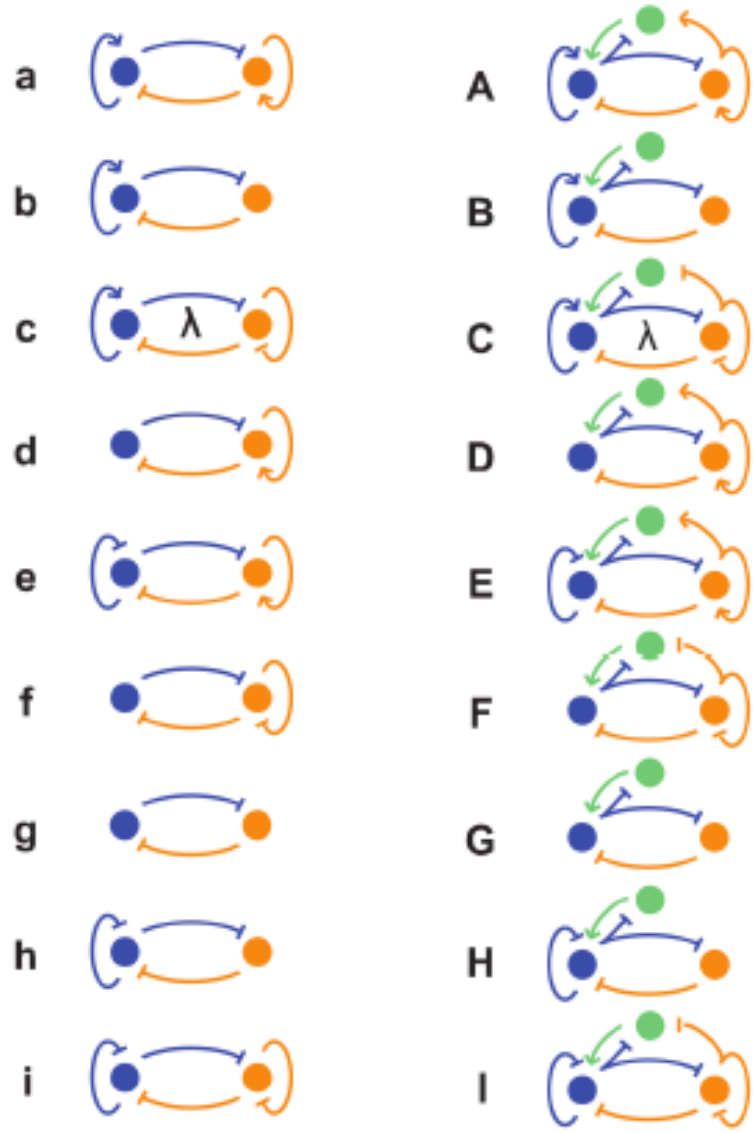
State 1



N=2

State 2

- Is state 1 sufficiently distinct from state 2?
- Are states 1 and 2 stable when N is brought down to 1?



There are many ways to make a bistable circuit that can also count.

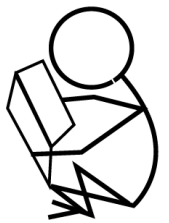
Motifs without positive feedback don't work

1 protein motifs don't work

2 protein mutual activators don't work

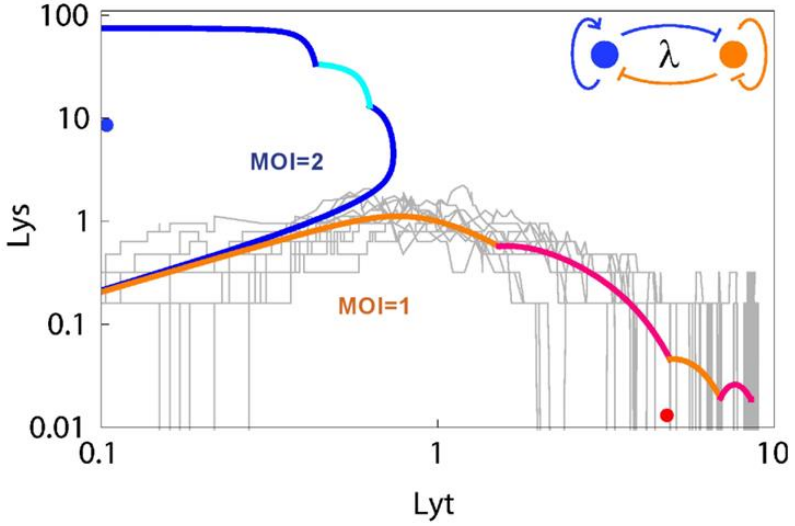
2 protein mutual repressors do work

Avlund, Dodd, Sneppen, Krishna (2009) J. Mol. Biol. 394, 681
Avlund, Krishna, Semsey, Dodd, Sneppen (2010) PLoS ONE 5(12): e15037



	Motif	Determ.	Stochastic
a		1112	0
b		1054	2
c		563	1
d		462	0
e		127	0
f		447	0
g		753	0
h		295	0
i		171	0
Total		5142	3

Two-protein motifs are very sensitive to noise



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g	753	0
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i	171	0
Total	5142	3

Motif	Determ.	Stochastic		
		p.lys.cII	*p.lys/p.cII*	*cII shut-off*
A	273	2	0	6
B	397	37	13	80
C	326	29		
D	78	1		
E	33	1		
F	186	12	3	16
G	267	21	7	36
H	89	8	2	13
I	67	4	3	6
Total	1808	117	39	211

Three-protein motifs are more robust to noise than two-protein networks

Why are three protein motifs more robust than two protein motifs?

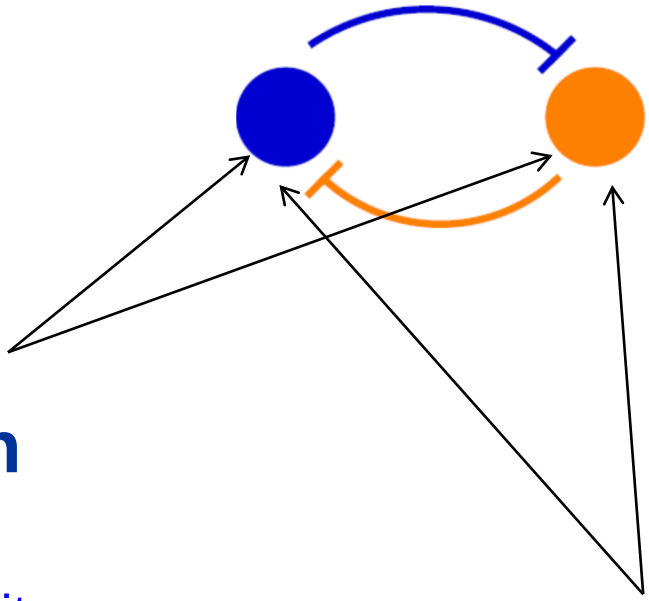
Tasks:

1. Do N=1 and N=2 go to two distinct states?

2. Are the two states stable?

	Short half-lives	Long half-lives
	✓	✗
	✗	✓

Why are three protein motifs more robust than two protein motifs?



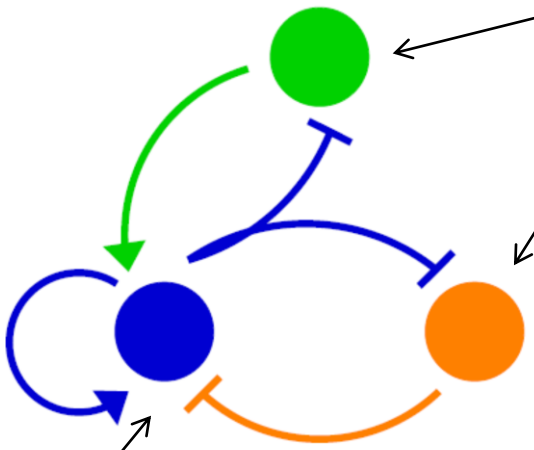
Maintaining the decision

Longer half-lives provide more stability

Making the decision

Short half-lives help

Why are three protein motifs more robust than two protein motifs?



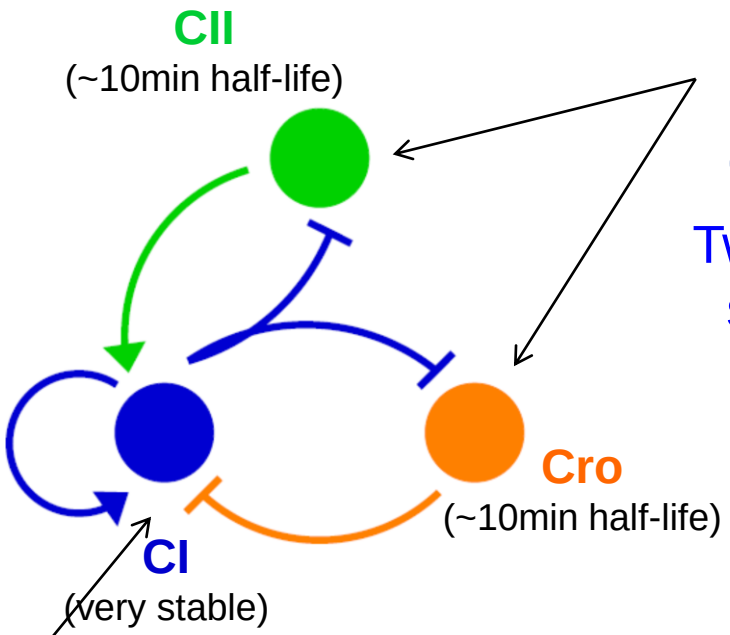
Making the decision

Two proteins with short half-lives

Maintaining the decision

Third protein with a long half-life

Why are three protein motifs more robust than two protein motifs?



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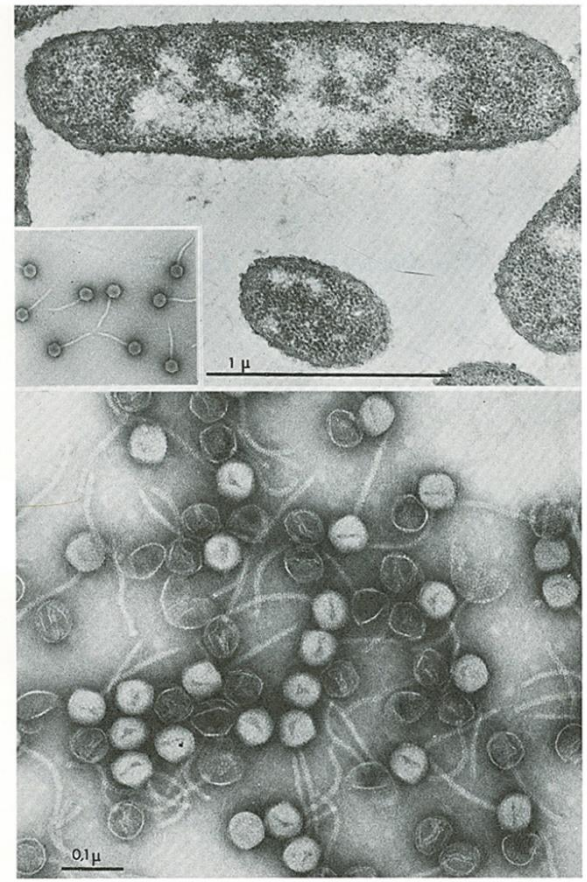

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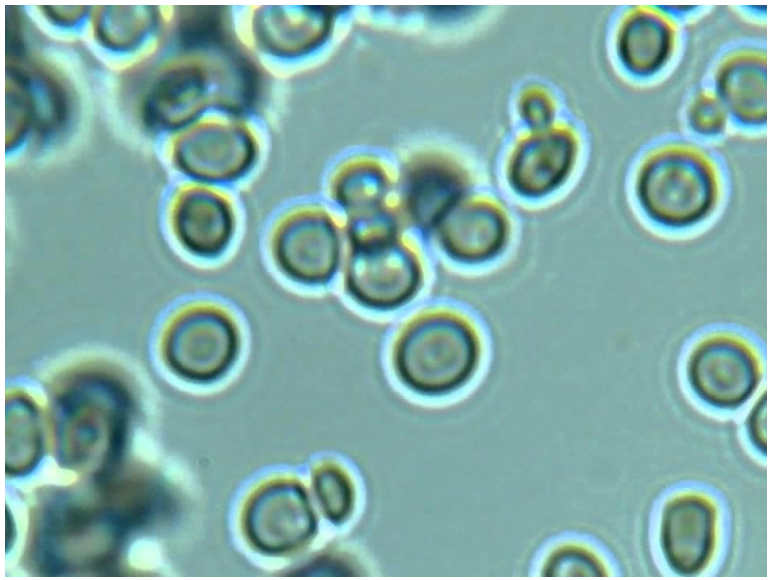


Summary

Data:

- Some idea of the basic gene circuits of phage lambda, 186
- Some data on parameter ranges
- Some data on noise and fluctuations (measurements on phage lambda)
- Kourilsky experiment

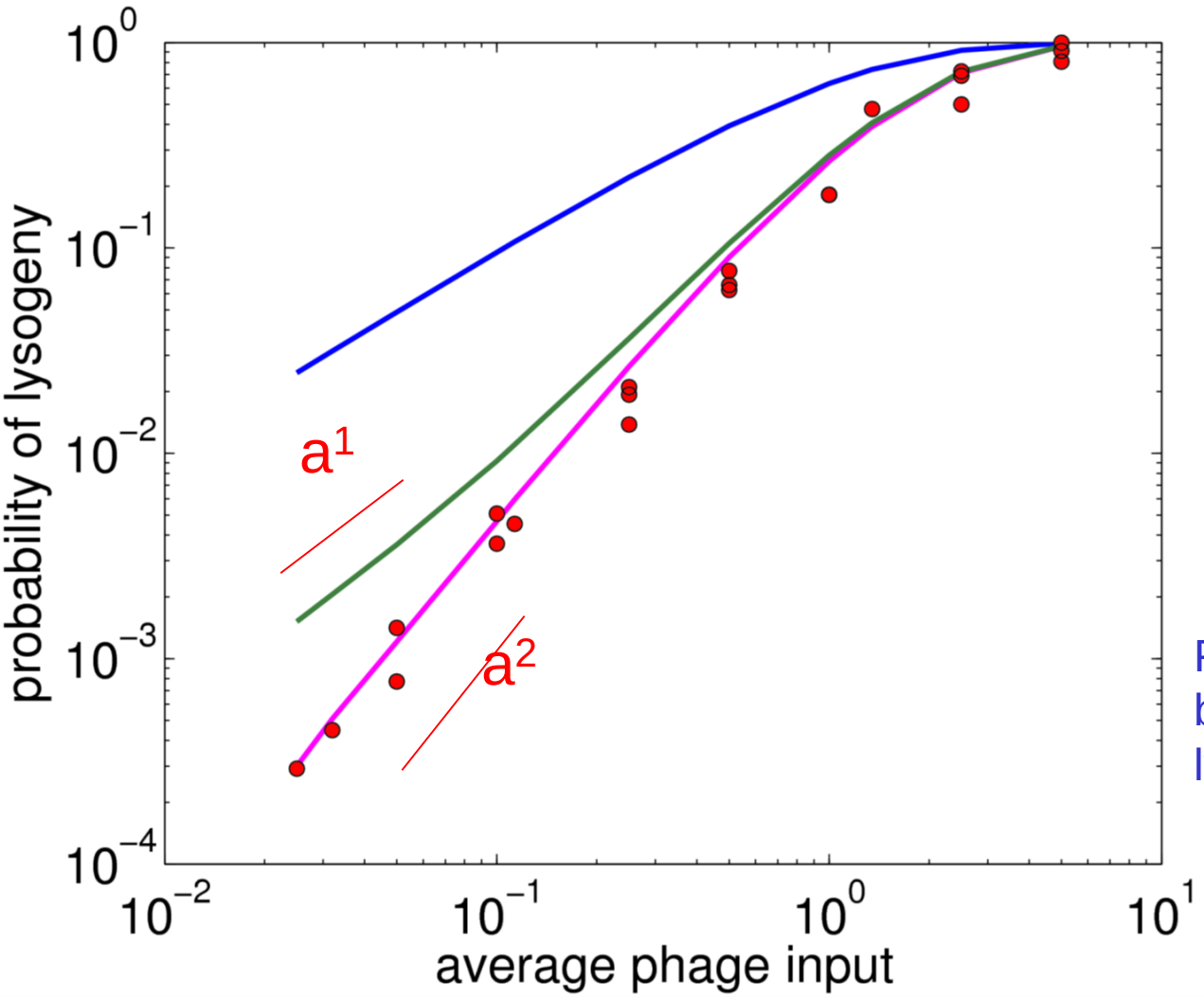
Approach:

- Differential equations modeling the dynamics of small genetic networks
- Exhaustively numerical sweep of possible circuit topologies & parameters

Insights:

- What doesn't work in the deterministic case
- What doesn't work with noise
- The idea that making a decision and maintaining a decision may need to be separated, especially in the presence of noise
- The connection with the half-life of proteins

Kourilsky's experiment



Number of phage DNA in one bacterium

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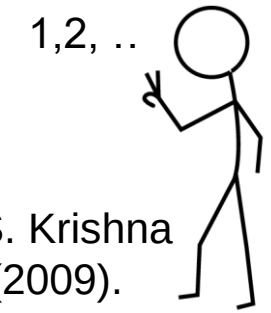
p(m)=	0	1	1
	0.05	1	1

Probability that bacterium will go lysogenic



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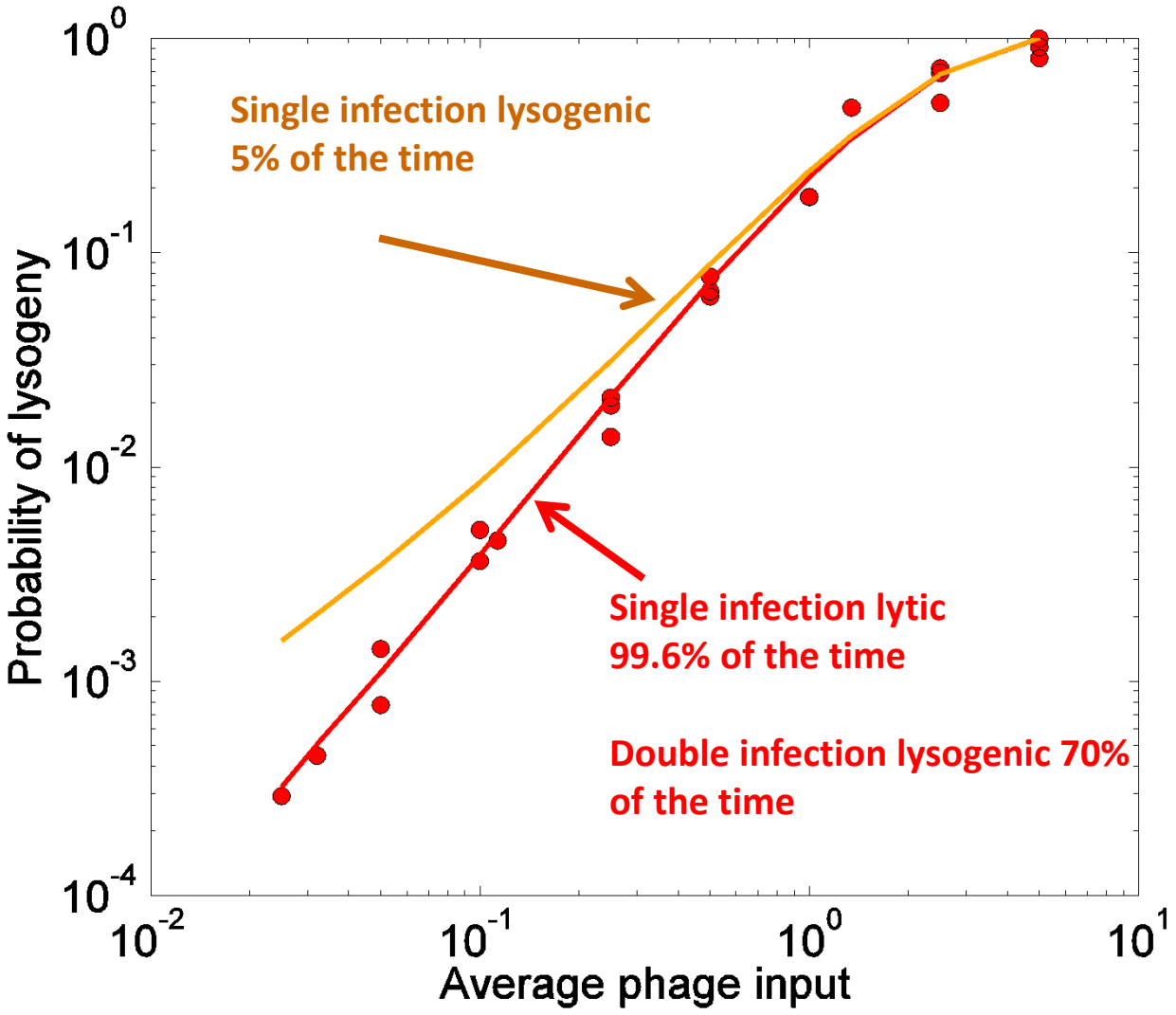
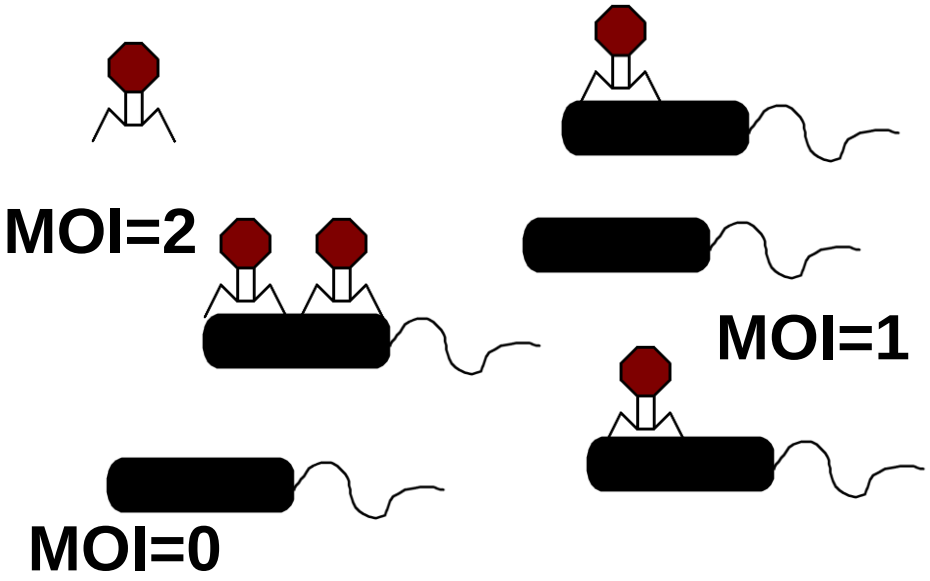
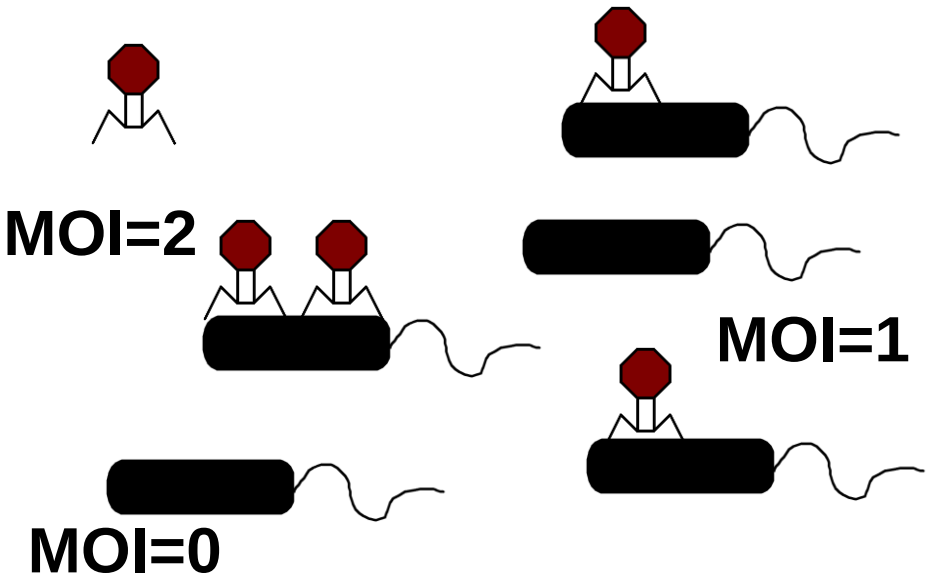


Multiple infections?



What is the most competitive strategy if lysogeny % is allowed to be different for different MOI?

Multiple infections?



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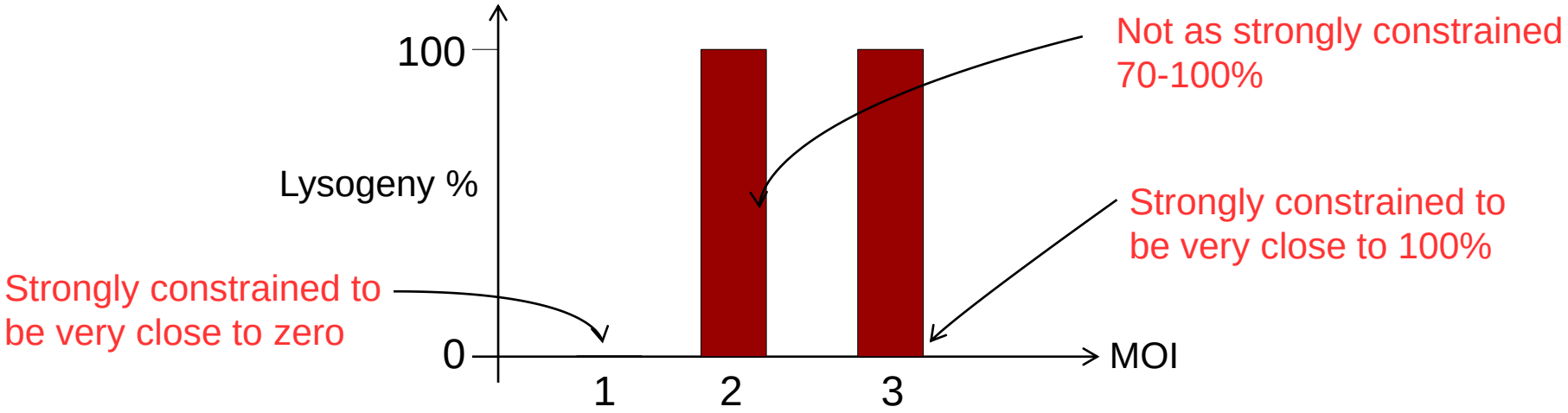

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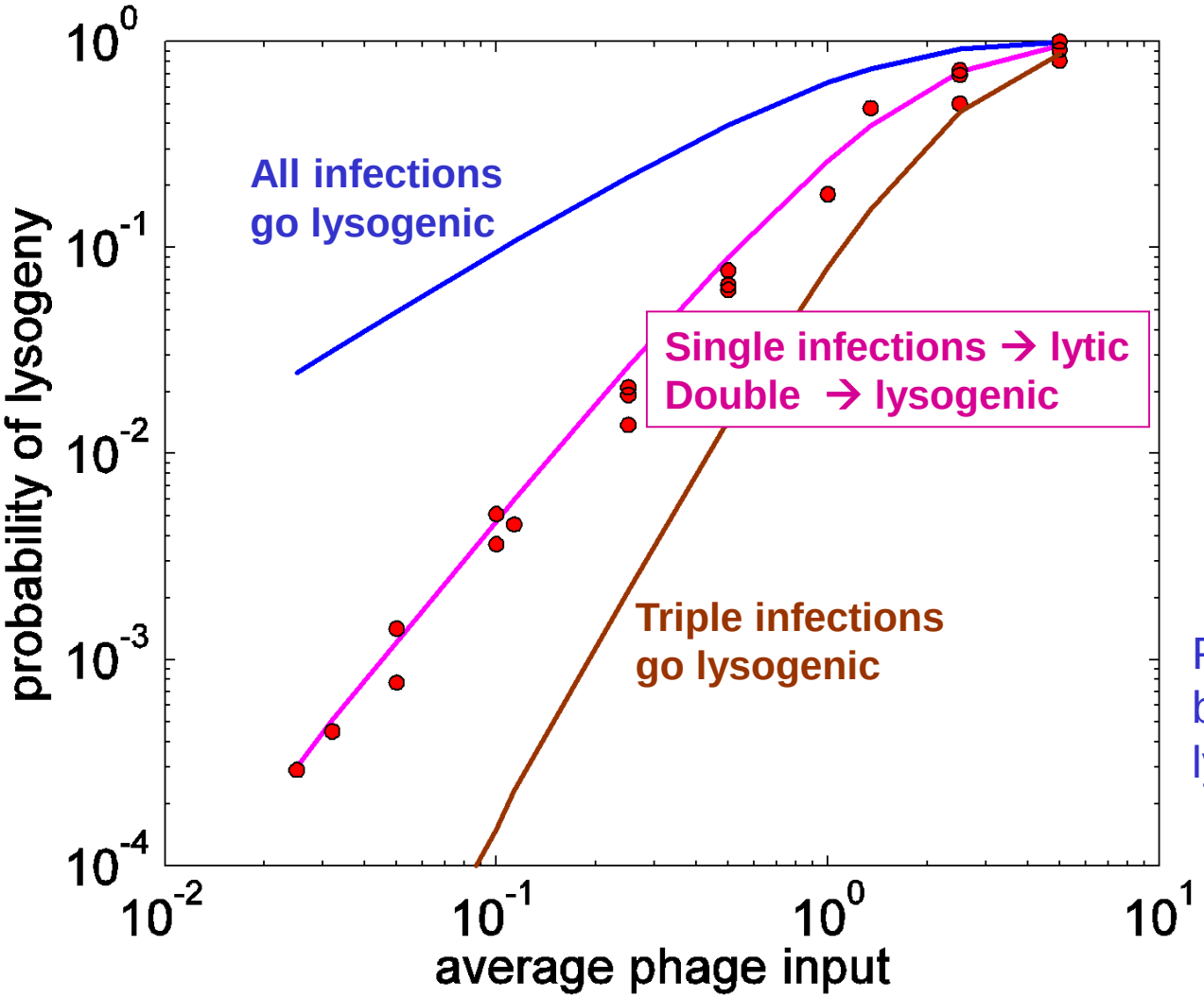

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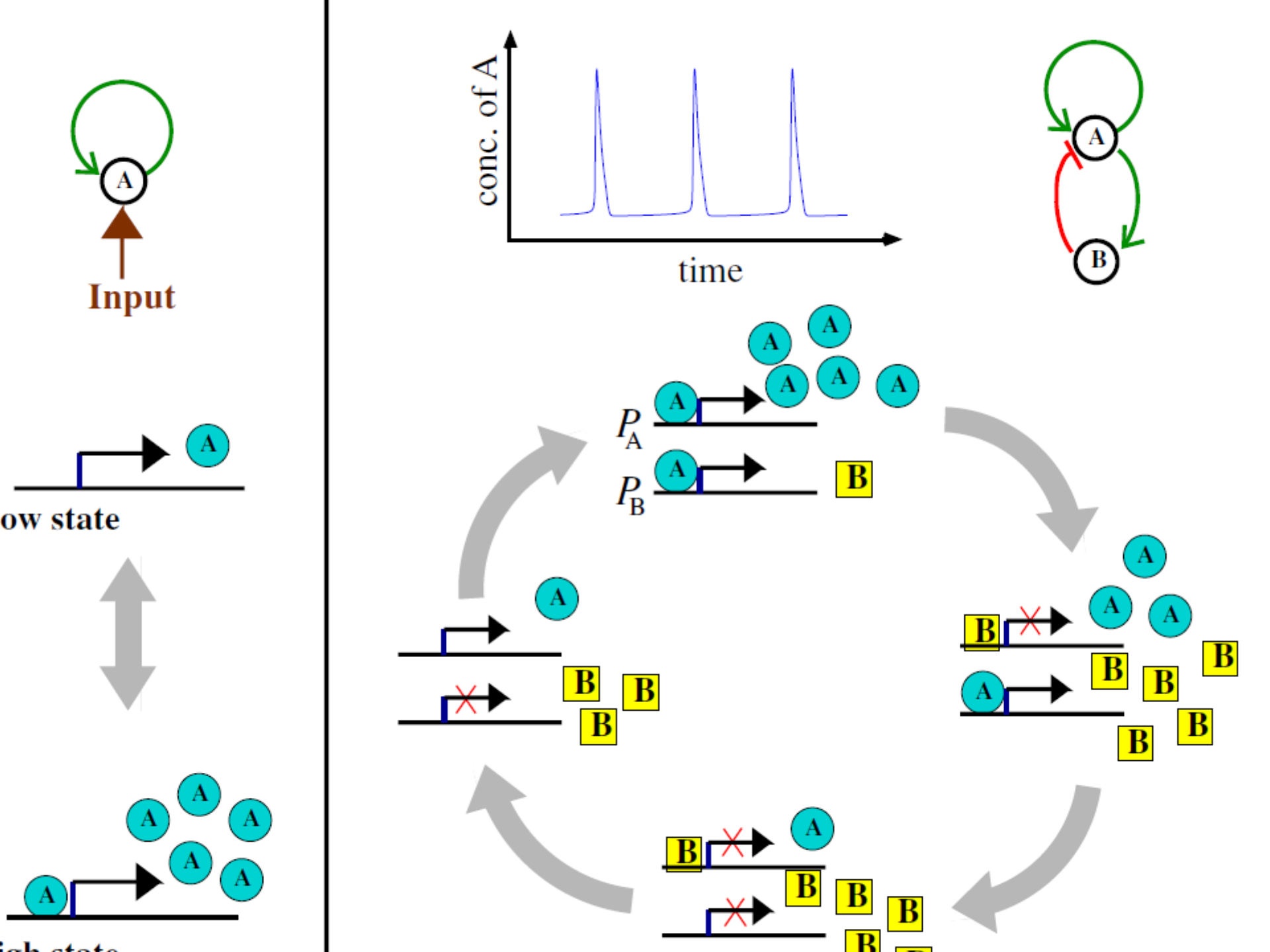
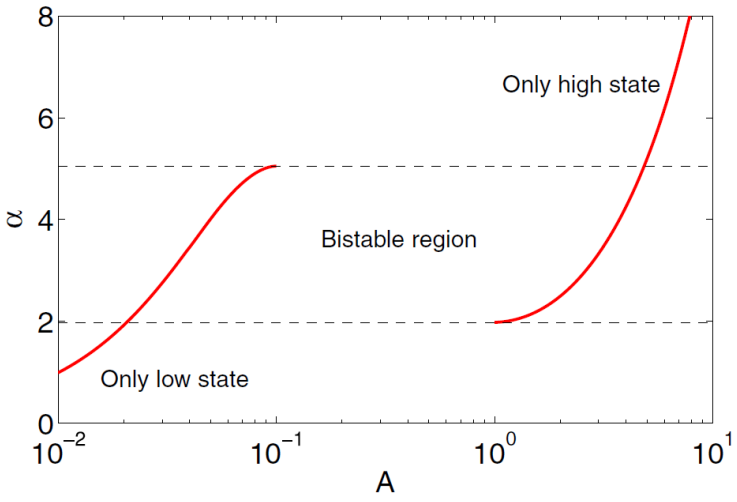
p(m)=	0	1	1
	0	0	1

Probability that bacterium will go lysogenic

M. Avlund, I. B. Dodd, S. Semsey, K. Sneppen, S. Krishna
Why do phage play dice? J. Virology 83, 11416 (2009).

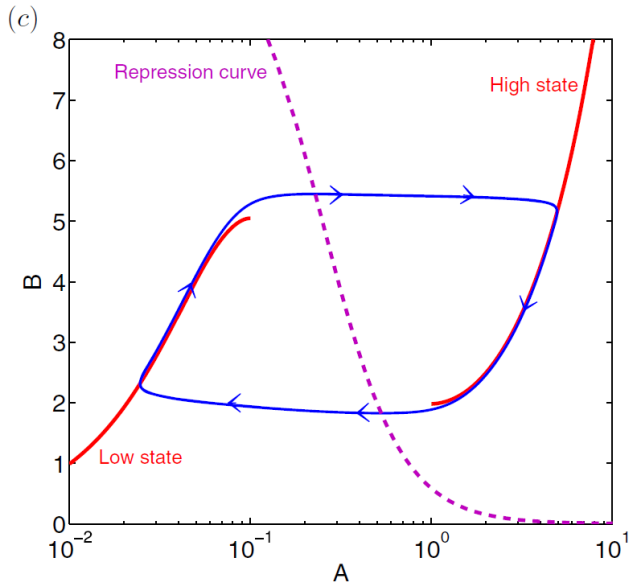

A
Input
ow state
A
A
A
A
A
conc. of A
time
A
B
A
A
A
A
A
P_A
P_B
B
A
B
B
B
A
A
A
B
B
B
B
B
A
B
B
B
B
B

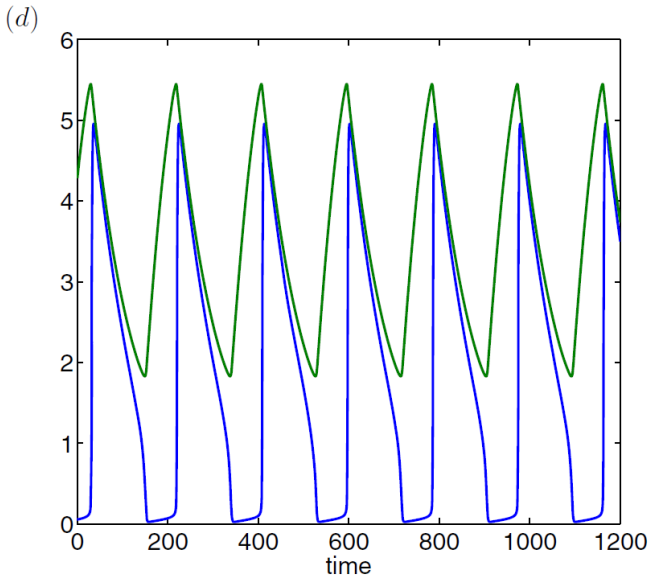
$$\frac{\mathrm{d}A}{\mathrm{d}t} = \alpha \frac{b + A^2}{1 + A^2} - A.$$



$$\frac{\mathrm{d}A}{\mathrm{d}t} = B\left(\frac{b + A^2}{1 + A^2}\right) - A.$$

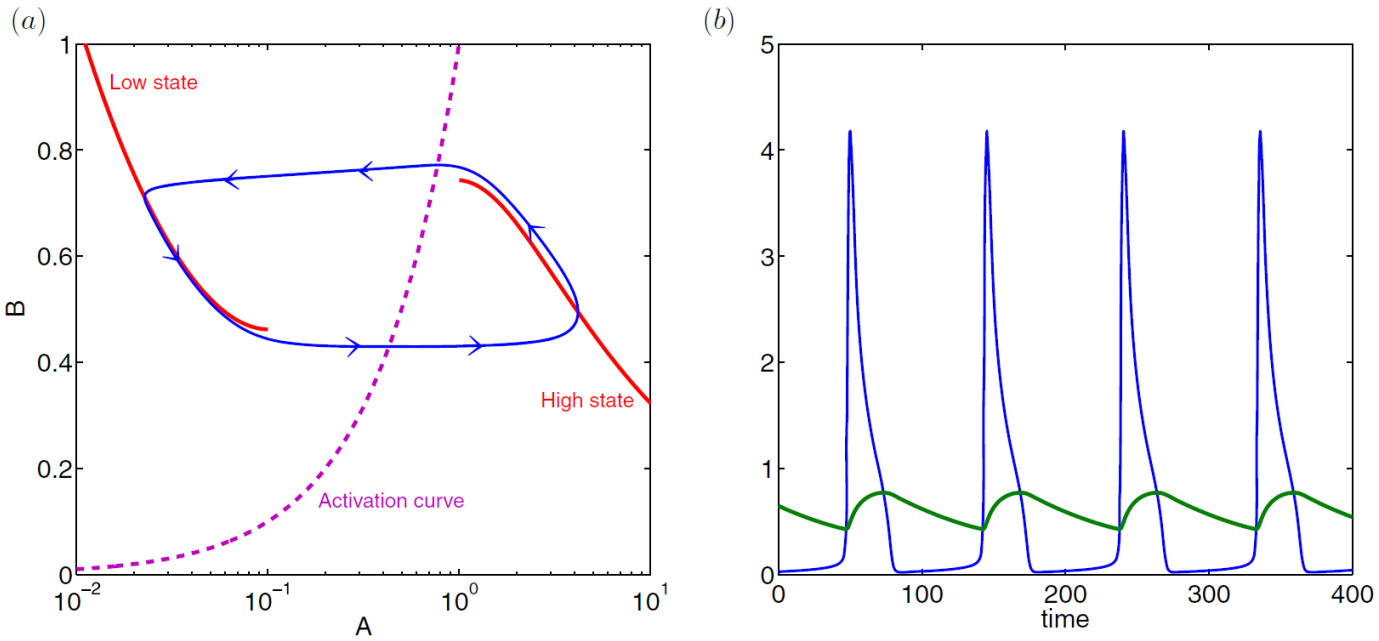
$$\frac{\mathrm{d}B}{\mathrm{d}t} = \frac{\alpha}{1 + (A/K)^2} - \gamma B.$$

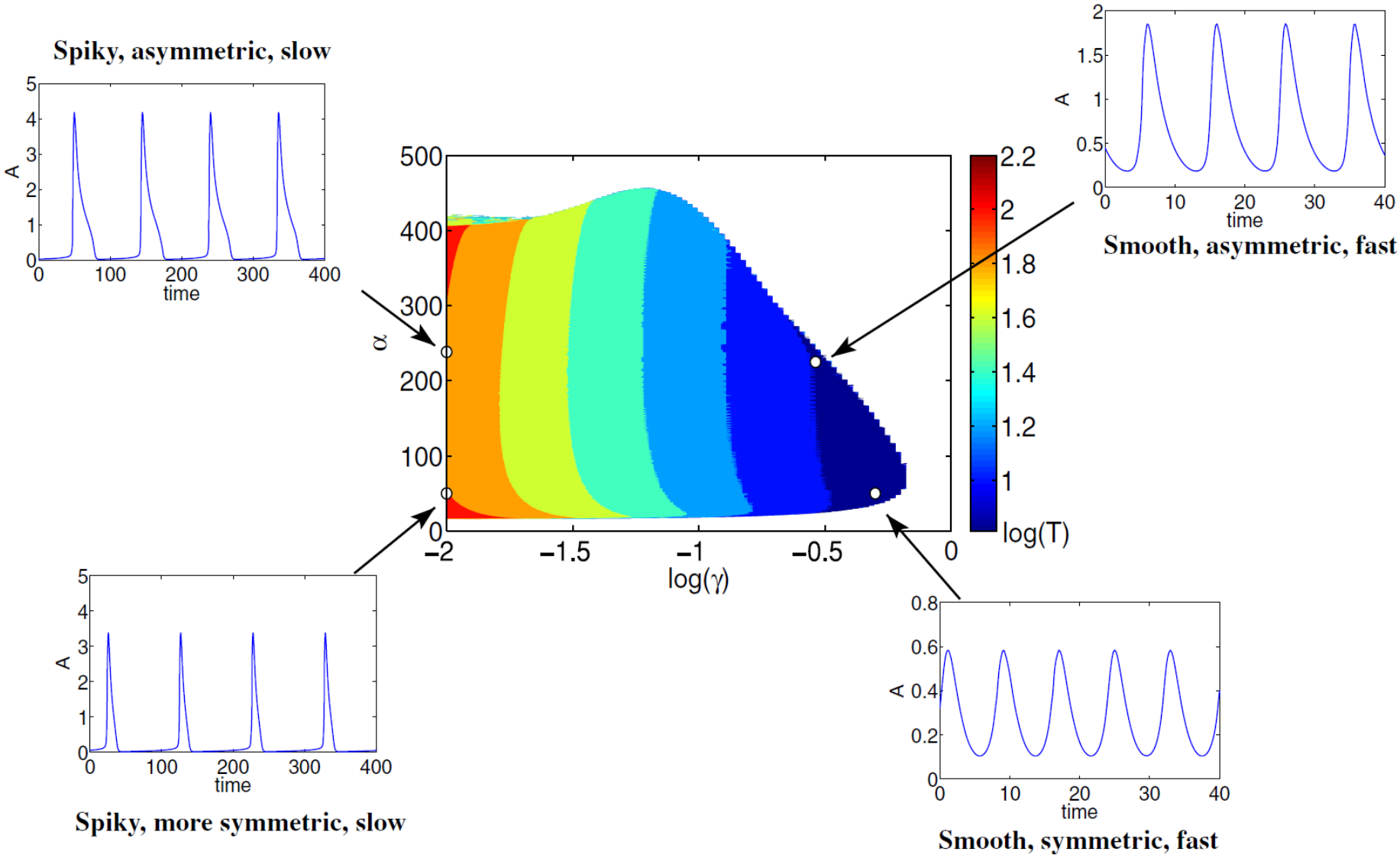




$$\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{\alpha}{1+(B/K)^2} \times \left(\frac{b+A^2}{1+A^2}\right) - A,$$

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \gamma A - \gamma B.$$

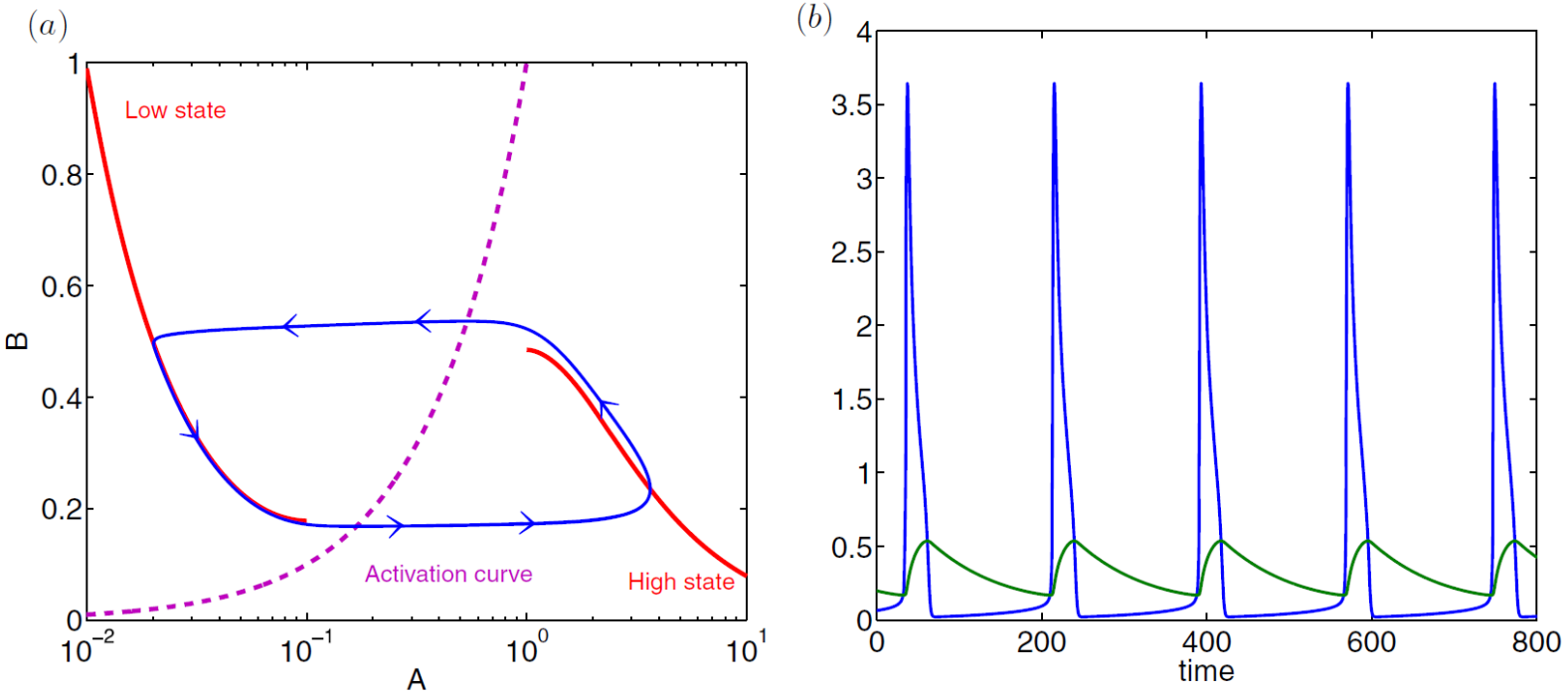



Spiky, asymmetric, slow
A
time
Smooth, asymmetric, fast
A
time
α
log(γ)
log(T)
Spiky, more symmetric, slow
A
time
Smooth, symmetric, fast
A
time

$$\frac{dA}{dt} = \frac{\alpha}{1 + B/K} \times \left(\frac{b + A^2}{1 + A^2} \right) - A.$$

$$\frac{dB}{dt} = \gamma A - \gamma B.$$

Hill coeff = 1



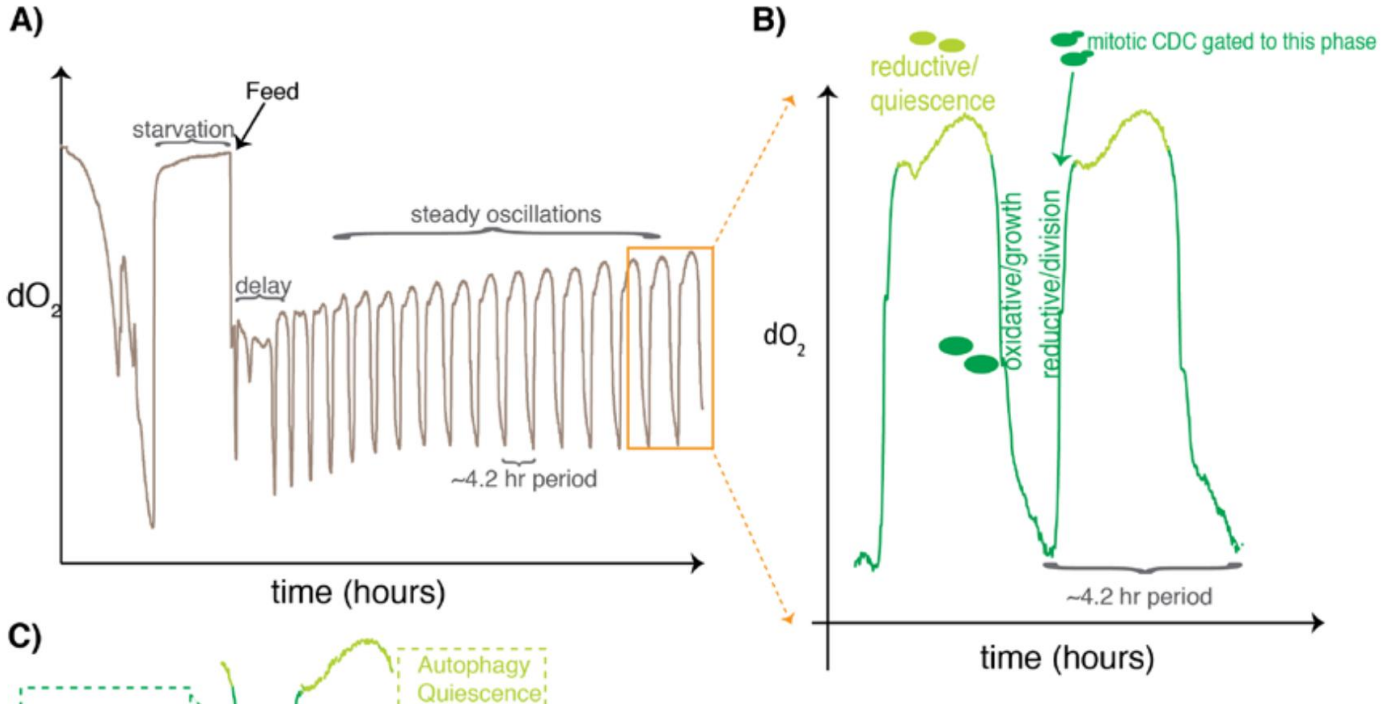
$$\frac{\mathrm{d}A}{\mathrm{d}t} = \alpha A_m - A.$$

$$\frac{\mathrm{d}A_m}{\mathrm{d}t} = \gamma_m \left(\frac{b + A^2}{1 + A^2} \right) - \delta A_m B - \gamma_m A_m.$$

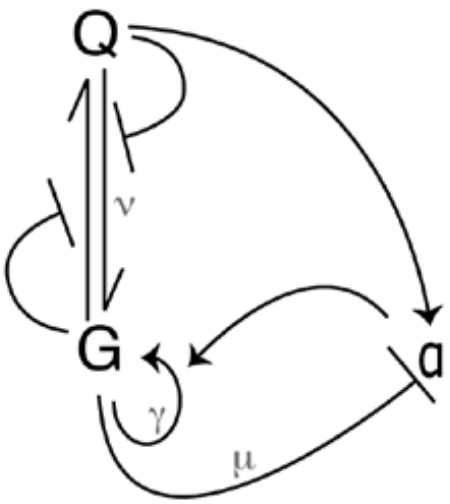
$$\frac{\mathrm{d}B}{\mathrm{d}t} = \gamma A - \gamma B.$$

$$\frac{\mathrm{d}A}{\mathrm{d}t} = \frac{\alpha}{1 + \delta B/\gamma_m} \times \left(\frac{b + A^2}{1 + A^2} \right) - A.$$

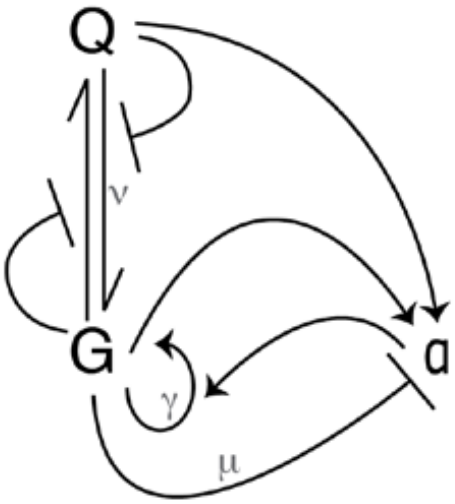
$$\frac{\mathrm{d}B}{\mathrm{d}t} = \gamma A - \gamma B.$$


A)
Feed
starvation
steady oscillations
delay
dO2
~4.2 hr period
time (hours)
B)
reductive/
quiescence
mitotic CDC gated to this phase
oxidative/growth
reductive/division
dO2
~4.2 hr period
time (hours)
C)
Autophagy
Quiescence

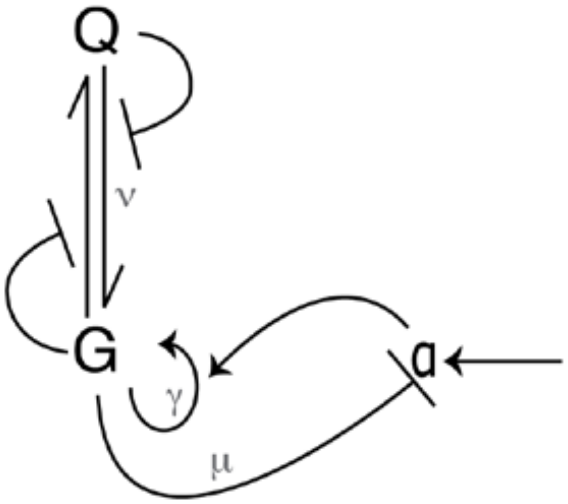
B) scenario 1

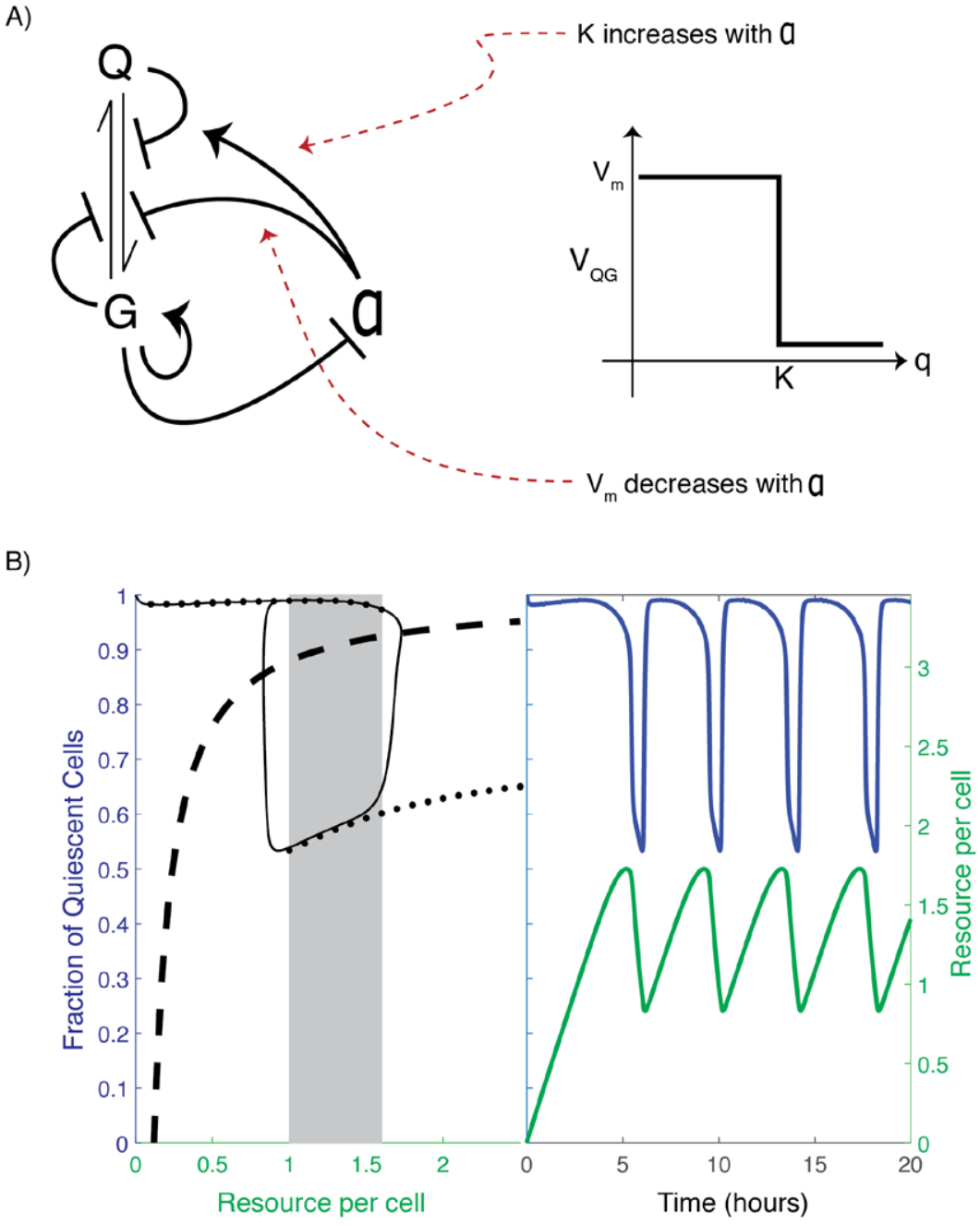


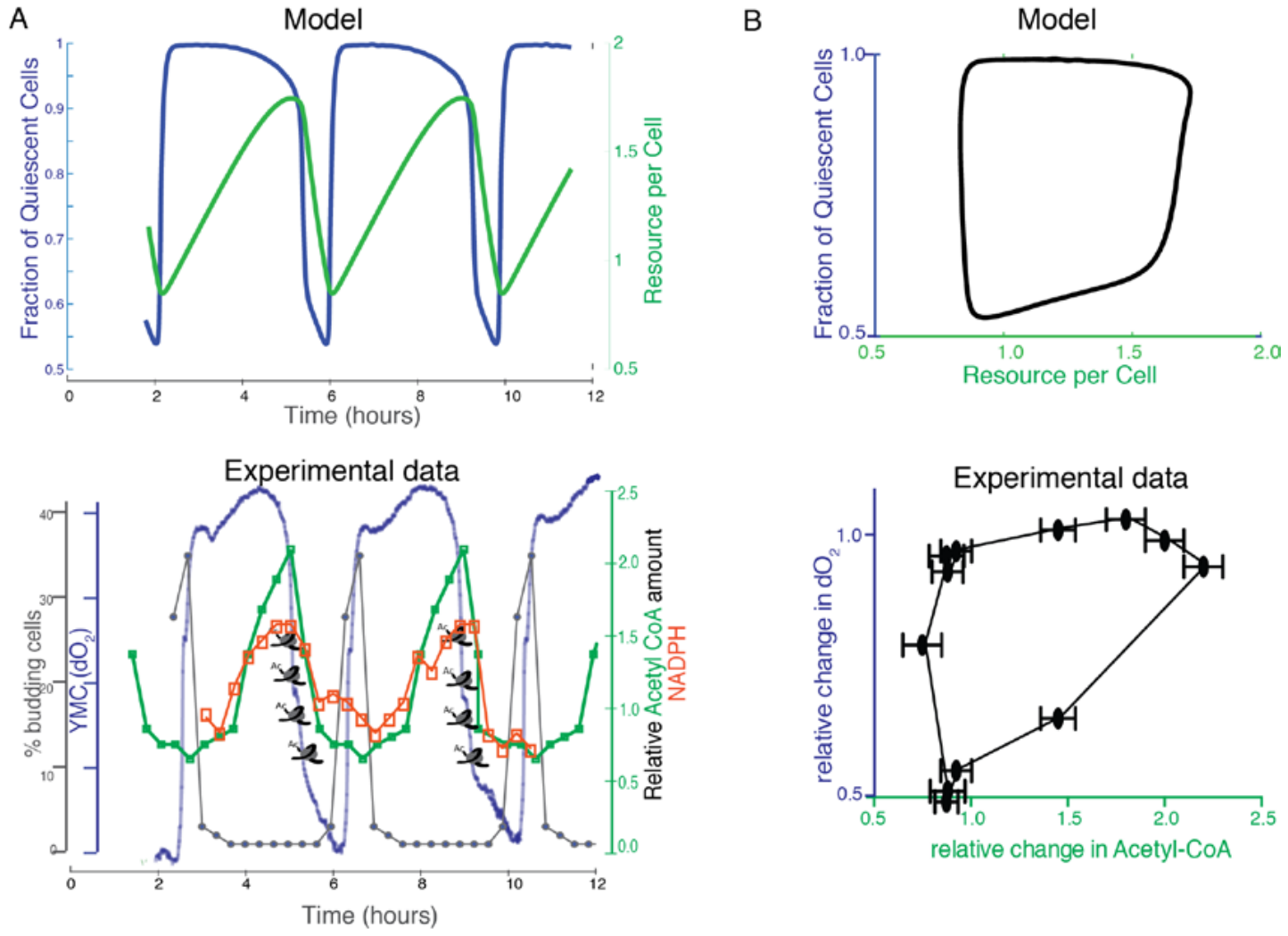
scenario 2



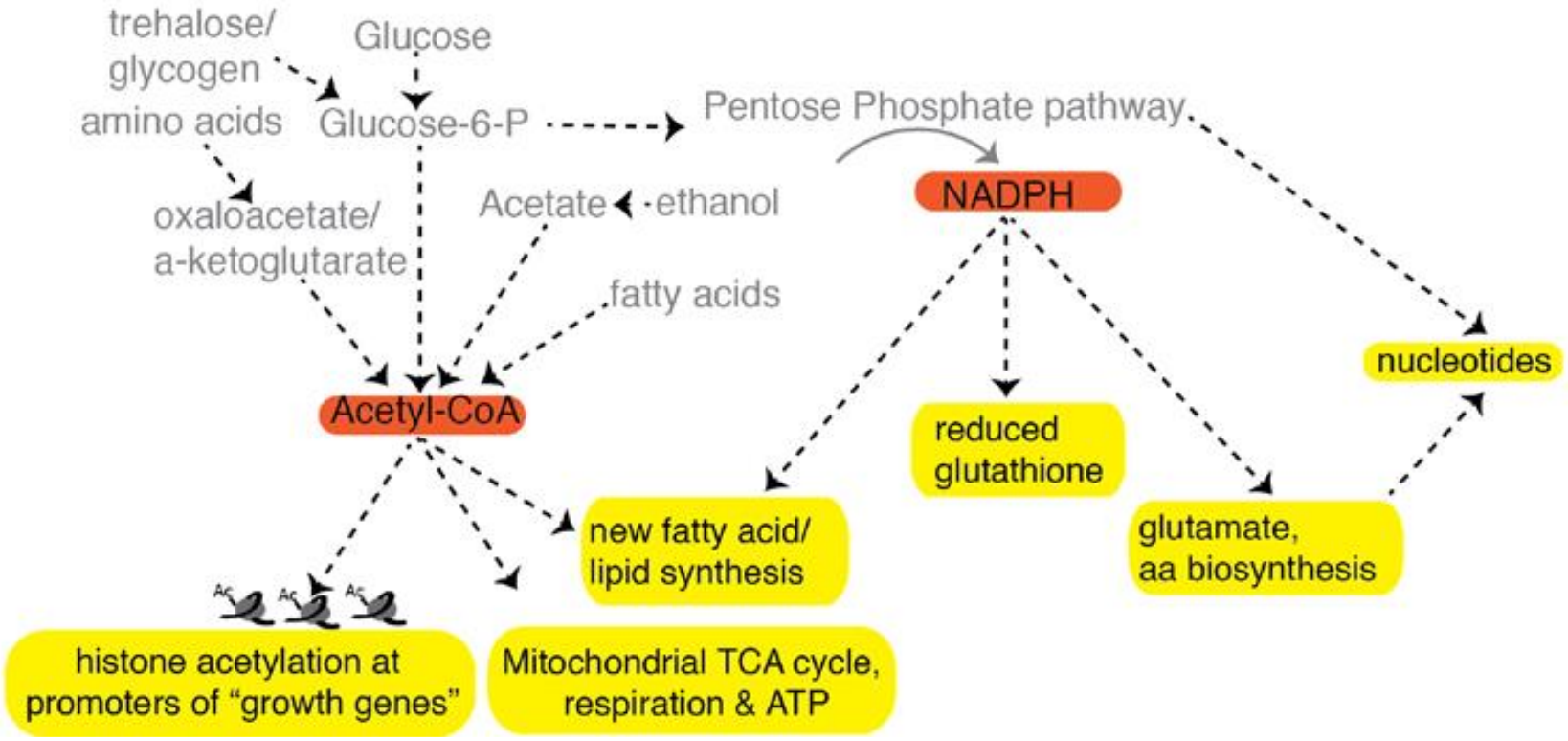
scenario 3

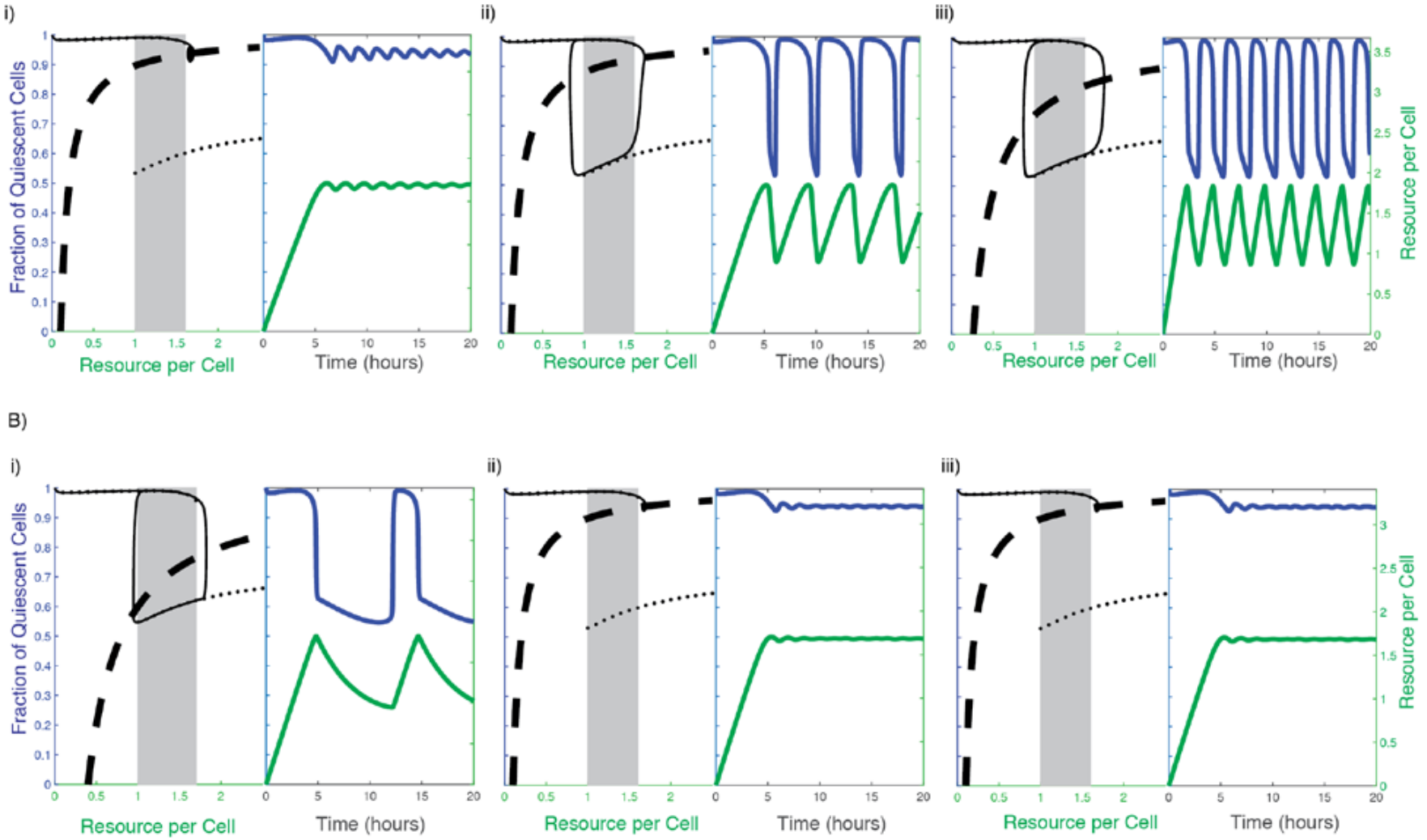


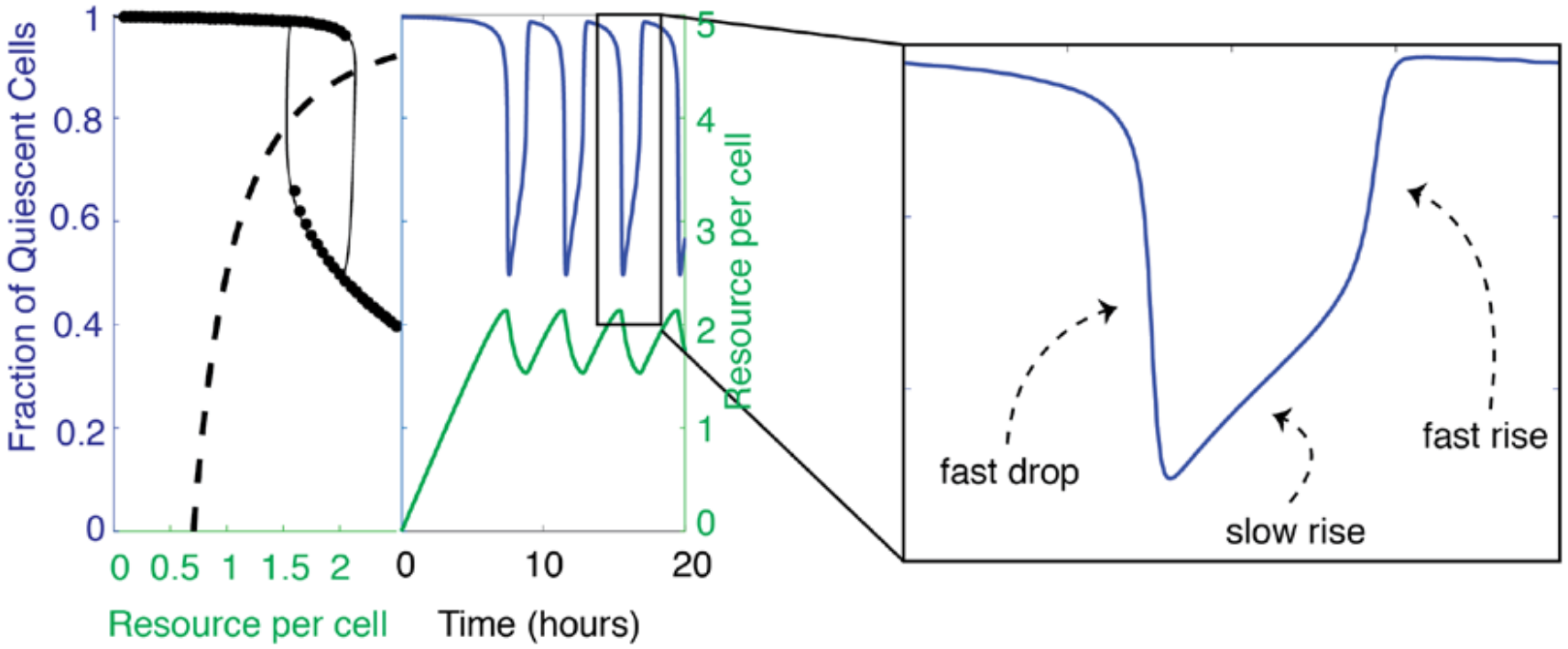

A)
Q
G
q
K increases with q
V_m
V_QG
K
q
V_m decreases with q
B)
Fraction of Quiescent Cells
Resource per cell
Resource per cell
Time (hours)


A
Model
Fraction of Quiescent Cells
Resource per Cell
Time (hours)
Experimental data
% budding cells
YMC1(dO2)
Relative Acetyl CoA amount
NADPH
Time (hours)
B
Model
Fraction of Quiescent Cells
Resource per Cell
Experimental data
relative change in dO2
relative change in Acetyl-CoA

C


trehalose/
glycogen
amino acids
Glucose
Glucose-6-P
Pentose Phosphate pathway
NADPH
oxaloacetate/
a-ketoglutarate
Acetate
ethanol
fatty acids
Acetyl-CoA
nucleotides
reduced
glutathione
new fatty acid/
lipid synthesis
glutamate,
aa biosynthesis
histone acetylation at
promoters of "growth genes"
Mitochondrial TCA cycle,
respiration & ATP


i)
ii)
iii)
Fraction of Quiescent Cells
Resource per Cell
Time (hours)
Resource per Cell
B)
i)
ii)
iii)
Fraction of Quiescent Cells
Resource per Cell
Time (hours)
Resource per Cell


Fraction of Quiescent Cells
1
0.8
0.6
0.4
0.2
0
0 0.5 1 1.5 2
Resource per cell
0
10
20
Time (hours)
Resource per cell
5
4
3
2
1
0
fast drop
slow rise
fast rise

$$\frac{dQ}{dt} = \nu_{GQ}G - \nu_{QG}Q - \phi(t)Q,$$

$$\frac{dG}{dt} = \gamma G - \nu_{GQ}G + \nu_{QG}Q - \phi(t)G,$$

$$\frac{dq}{dt} = \nu_{GQ}(1-q) - \nu_{QG}q - \gamma q(1-q),$$

$$\frac{da}{dt} = \sigma - \mu\gamma(1-q)a - \gamma(1-q)a,$$

To produce Fig 3B, 5A and 5B, we make the following choices (within scenario 3c): $\gamma = 1.665\ hr^{-1}$, $\sigma = 0.3996\ hr^{-1}$, $\mu = 1$, $\nu_{QG} = v[1 - 0.99\times\theta(q-K)]$, $K = a^2/(0.75^2 + a^2)$, $v = (0.165 - 0.125K)\ hr^{-1}$, $\nu_{GQ} = 16.65\ hr^{-1}$, where $\theta(x)$ is the step-function, which is zero for $x < 0$ and unity for $x > 0$ (approximated as above). Figure 4 panels are made using the same equations, but varying the values of σ and γ.

Some thoughts on the role of (this kind of) theory in biology

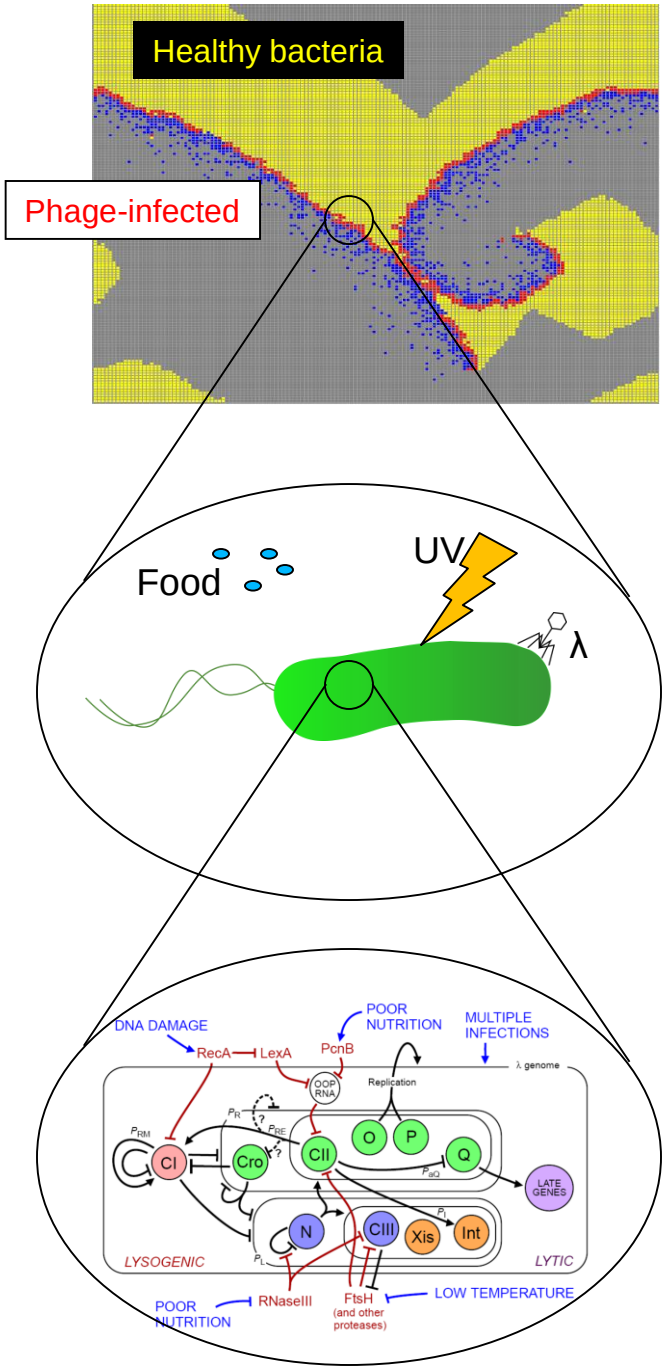
1. Big data vs small data
(models can be useful even when there is little experimental information)

2. The tension between making models specific vs general
(One approach that appeals to me: study a class of dynamical system made from well known building blocks and ask what is the range of behaviour that it is capable of)

3. The point of this kind of modelling is not to be “correct” or “wrong”, but to raise interesting questions

4. Back and forth between experiments and theory

5. Finding equations vs finding solutions to equations – or, what kind of “laws” are we looking for in biology?
(Discussions of “physics” approaches tend to emphasise the discovery of fundamental equations in the history of physics and forget that at least an equally important part of the history of physics has been the exploration of solutions of these equations)



Population/Ecosystem level

What are good lysis-lysogeny strategies for a phage when, say, it is competing with other phage species for a bacterial host?

How are the population (and evolutionary) dynamics of phage-bacteria ecosystems influenced by different bacterial defences against phage?

Cellular level

Why is only a narrow 5-15% lysogeny percentage observed in laboratory phage infections?

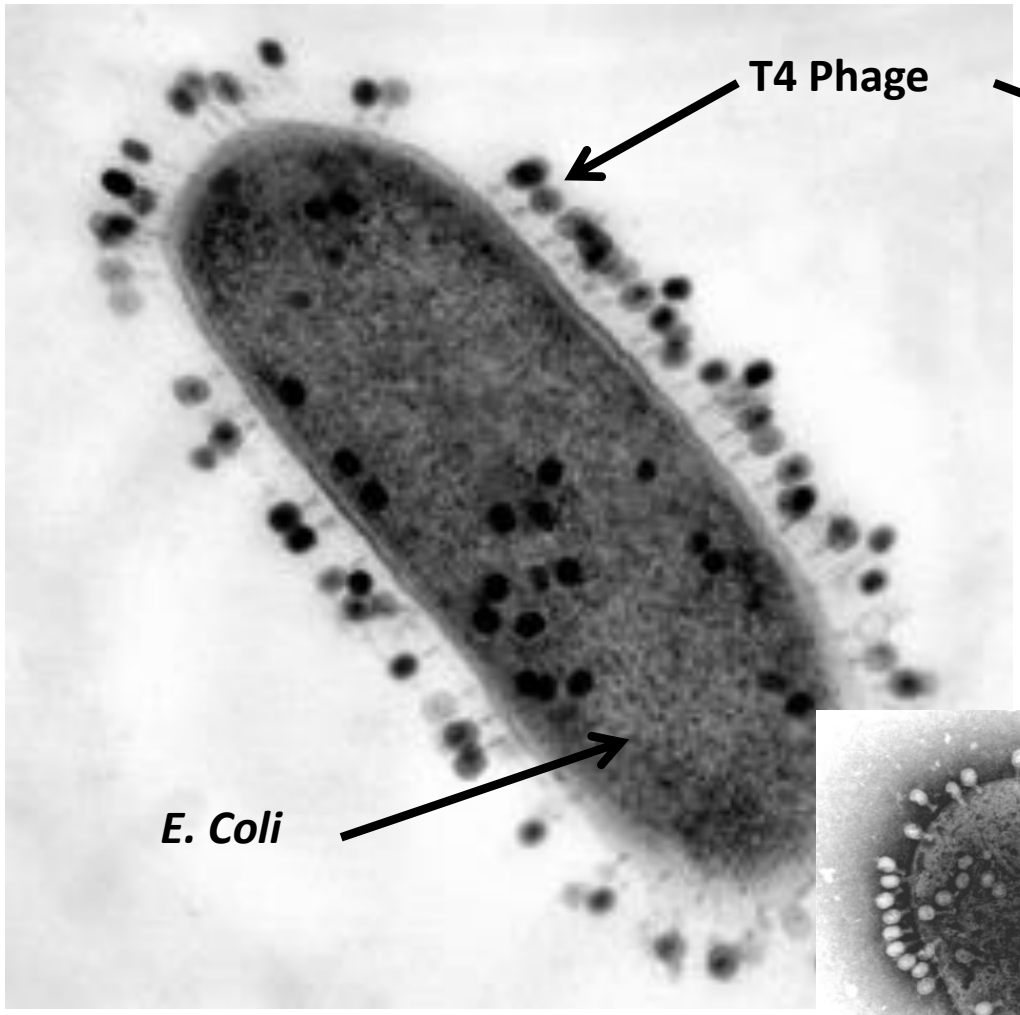
What conditions make a phage-infected bacterium go preferentially lytic, or lysogenic?

What aspects of the bacterial cell state bias the decision?

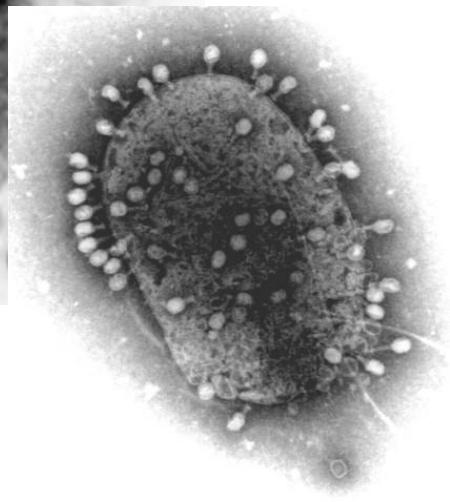
Subcellular level

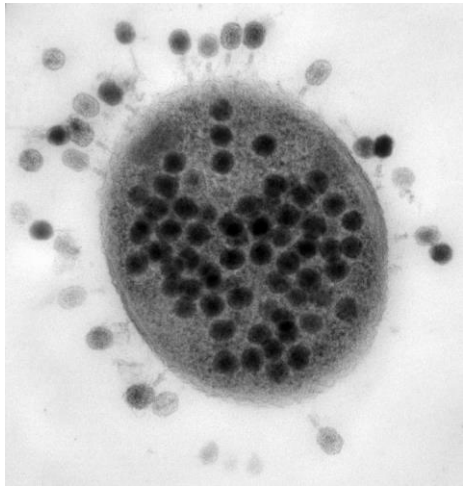
How is the lysis-lysogeny decision regulated?
What produces bistability?
What makes the network robust to noise?

How does the phage network integrate information about the environment (e.g. does it use bacterial quorum sensing)?



Ref: http://www.absoluteastronomy.com
/topics/Bacteriophage





Ref: http://viromag.wordpress.com/2009/03/13/
bacteriophages-viruses-of-bacteria/

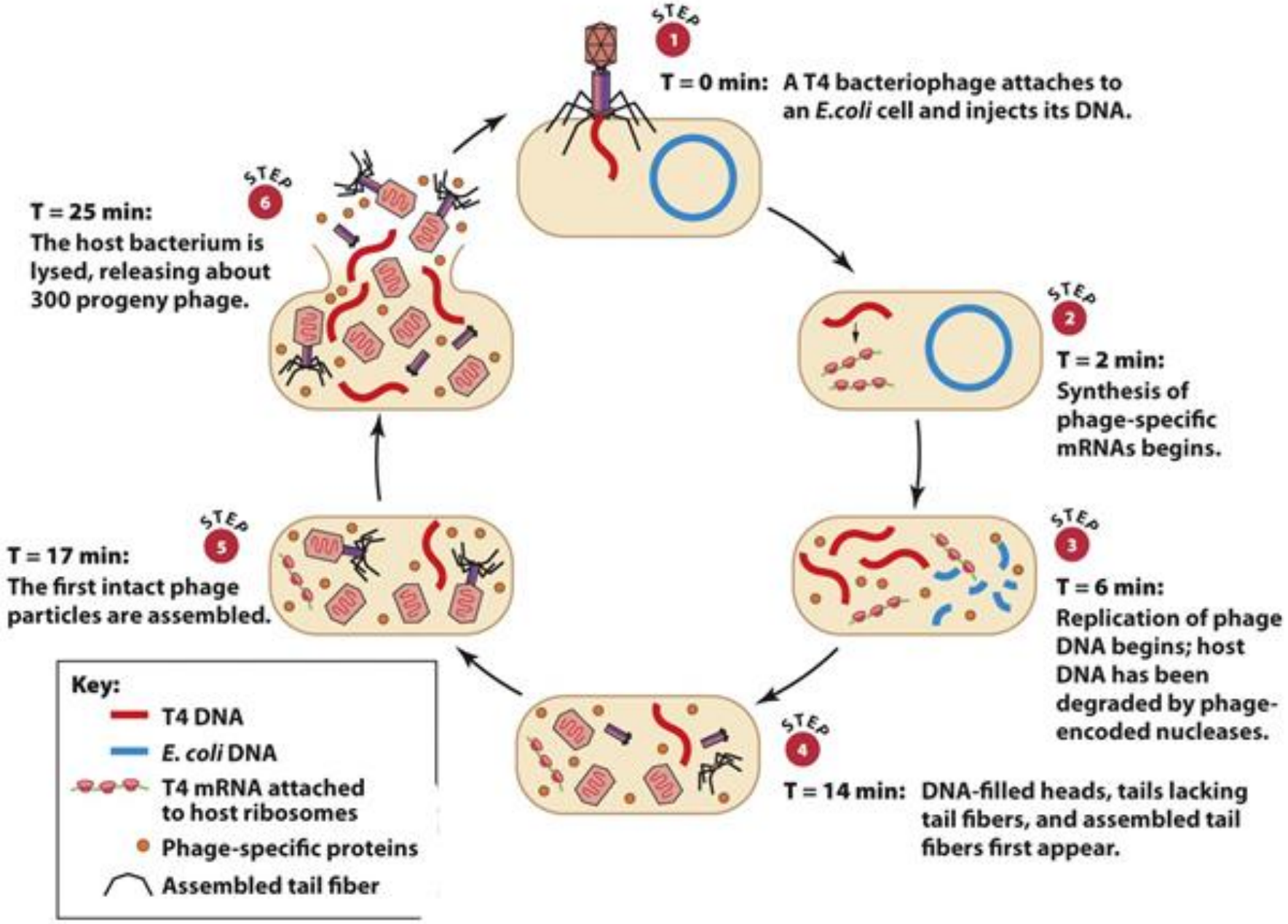


Image courtesy Aileen L. https://classconnection.s3.amazonaws.com/20/flashcards/2047020/png/lytic_phase1365650202676.png

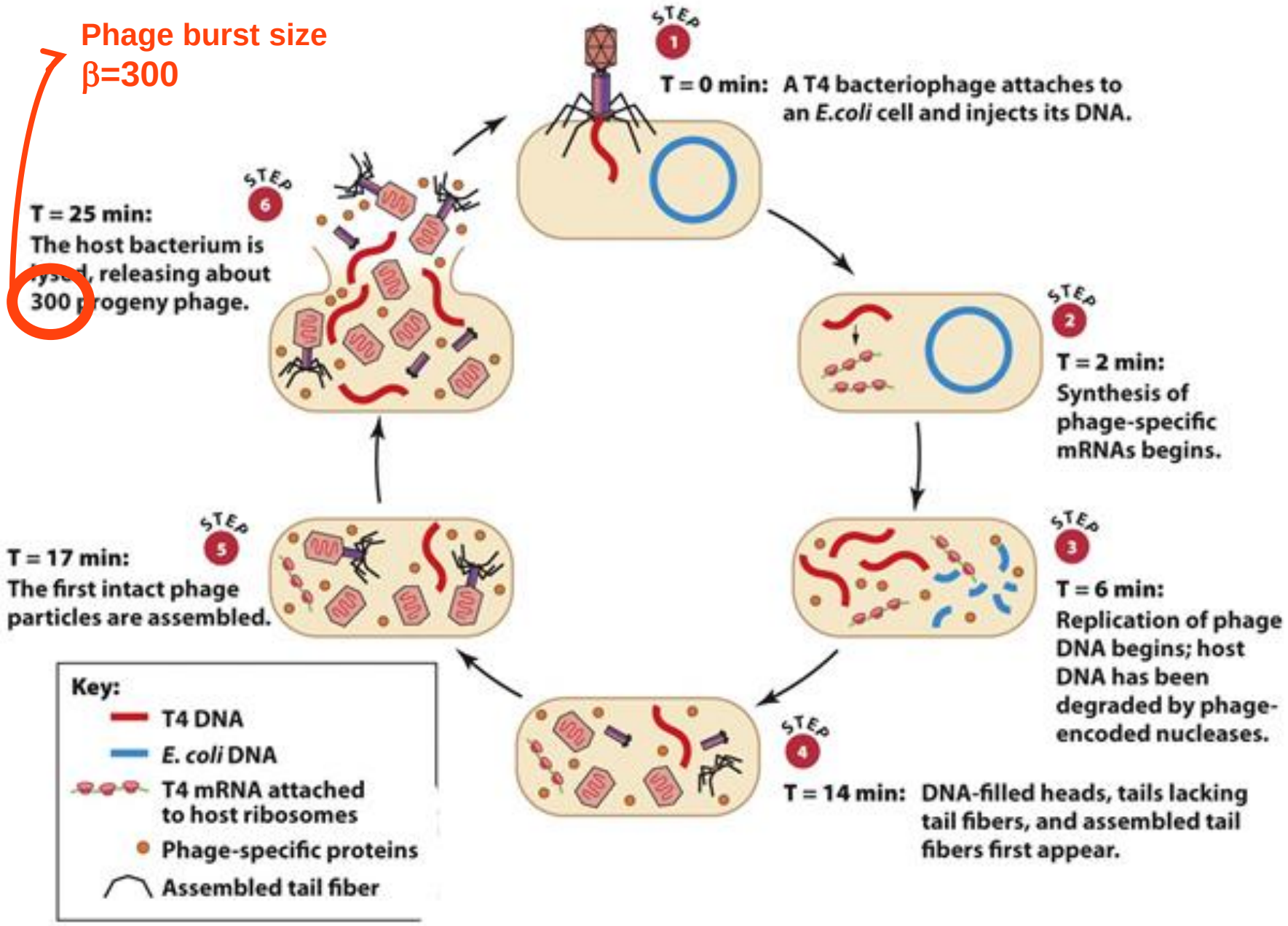
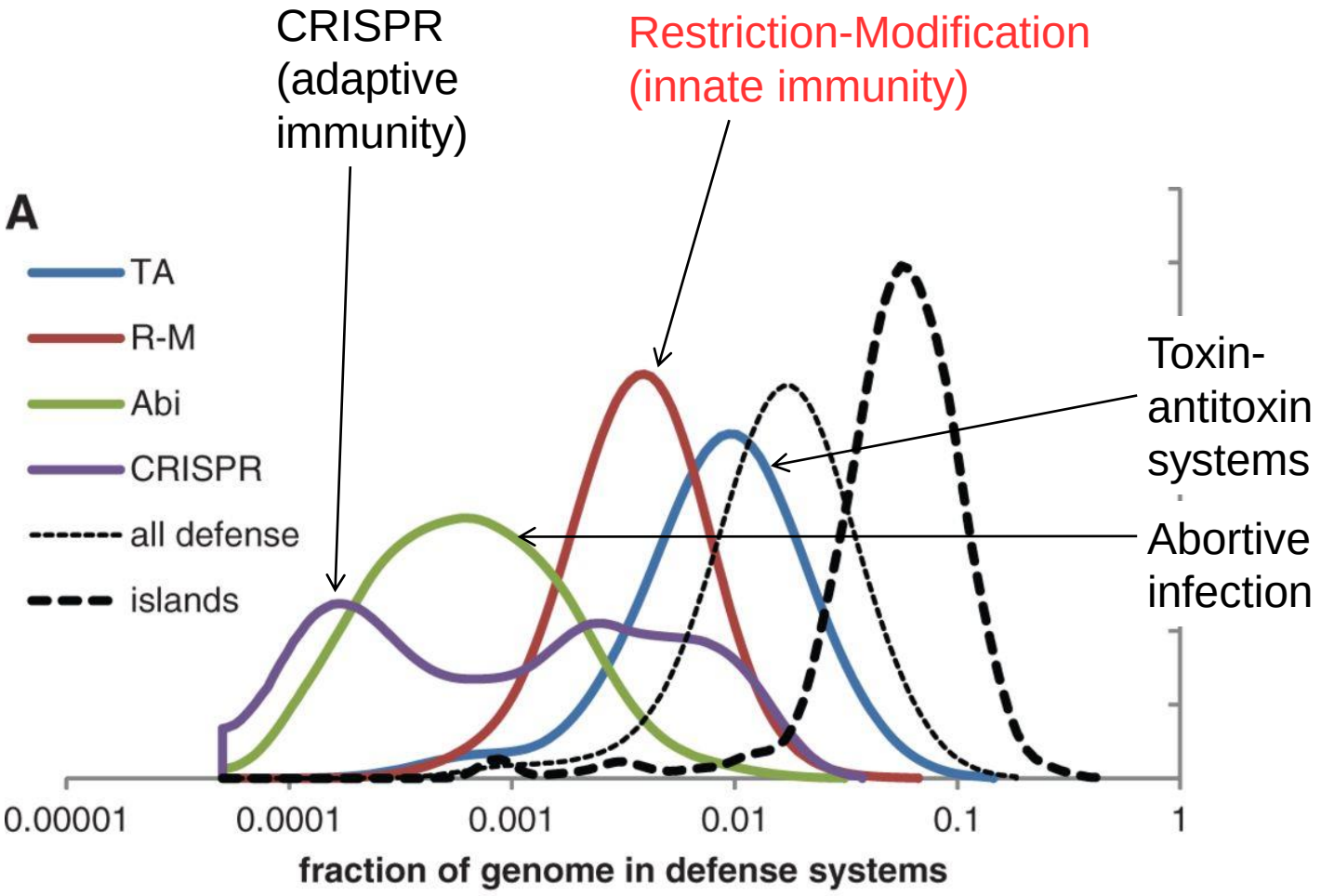


Image courtesy Aileen L. https://classconnection.s3.amazonaws.com/20/flashcards/2047020/png/lytic_phase1365650202676.png

The major types of defense systems in bacterial and archaeal genomes



Makarova K S et al. (2013) Nucl. Acids Res. 41, 4360

Restriction-Modification systems in bacteria

a ubiquitous (but weak) defence against phage

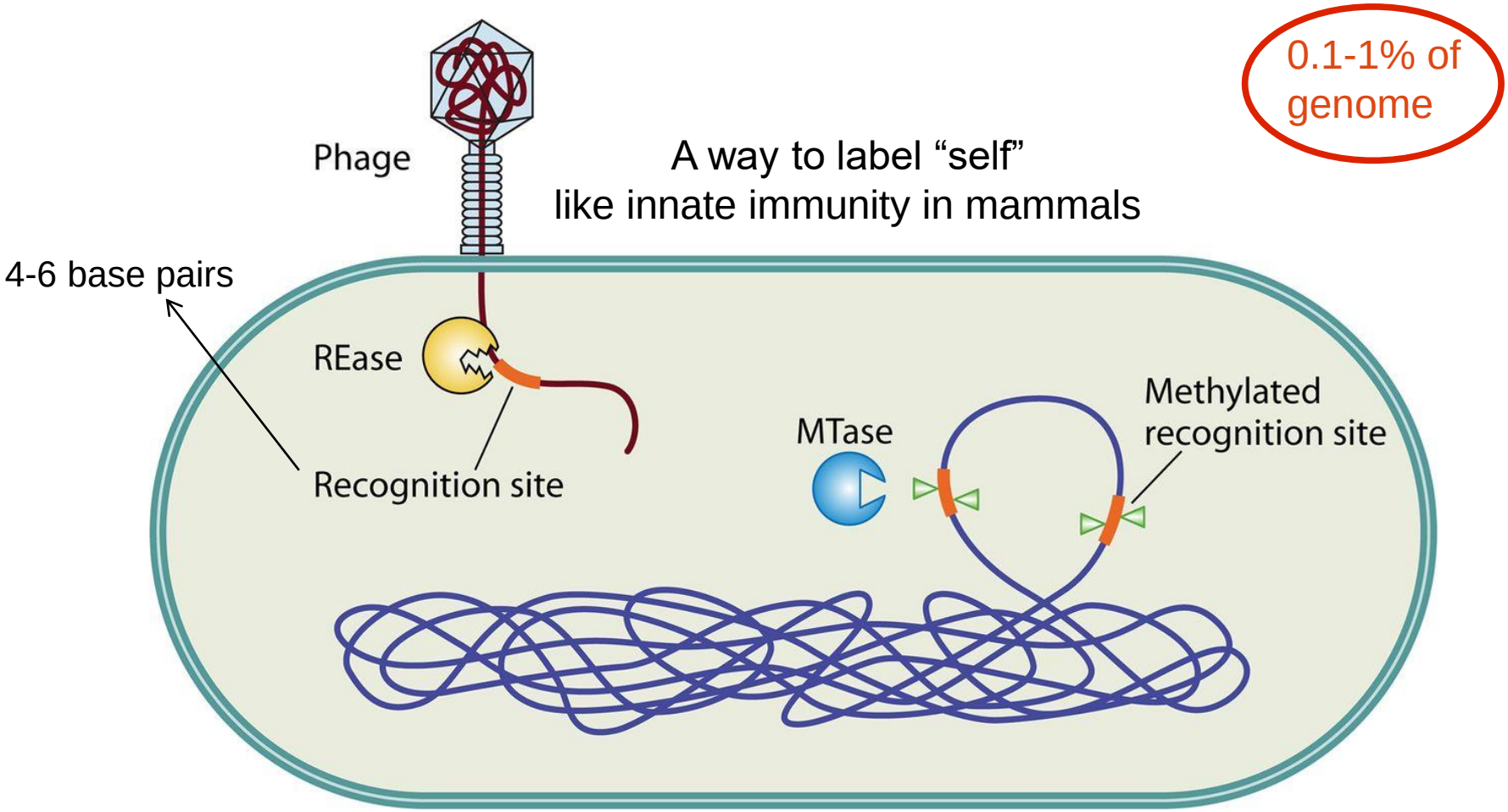
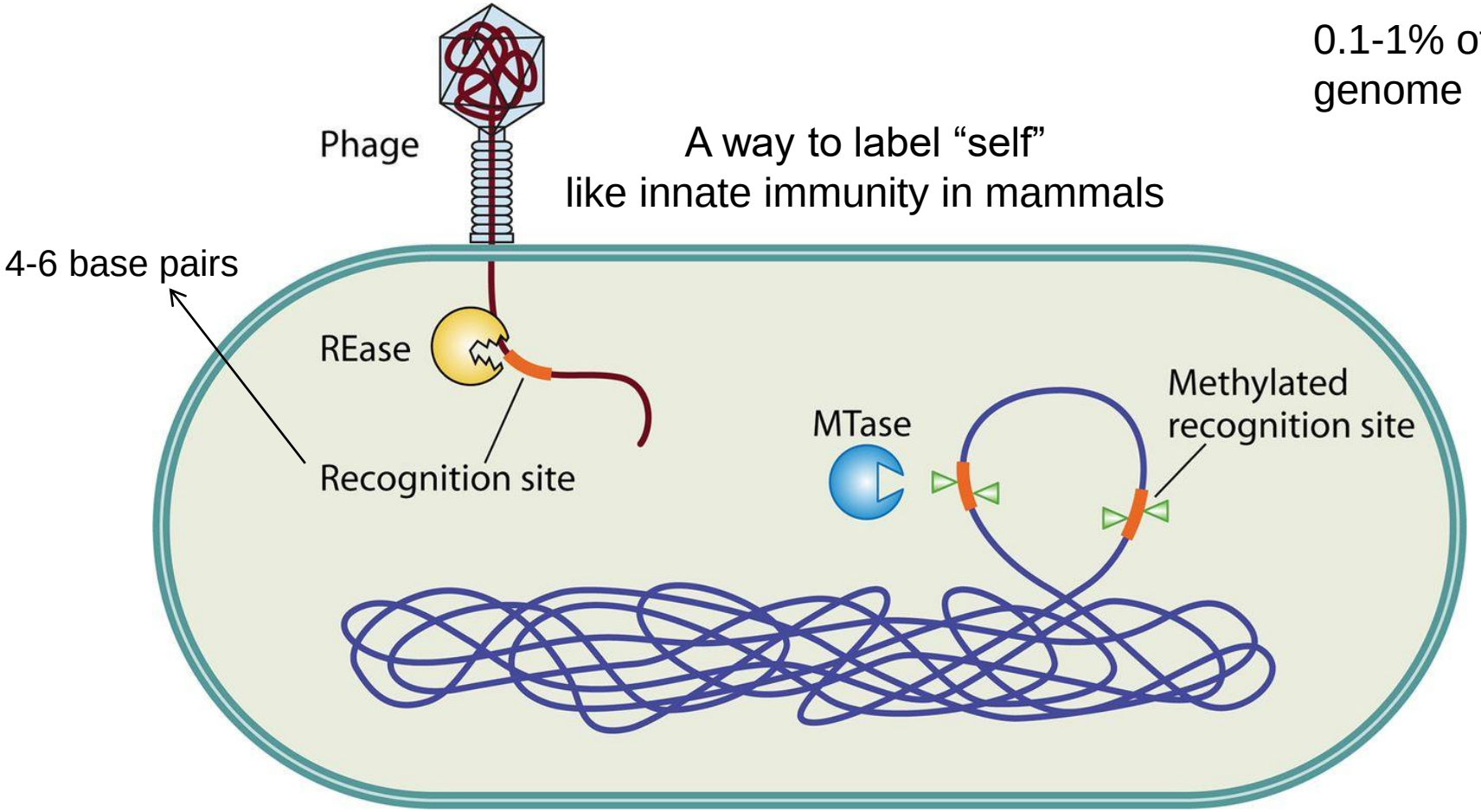


Image from: Vasu & Nagaraja (2013) Microbiol. Mol. Biol. Rev. 77, 53-72

Restriction-Modification systems in bacteria

a ubiquitous (but weak) defence against phage



A weakness: phage DNA may get “mistakenly” labelled before it gets chopped up

Probability of this can be as high as 0.01

Notice: once a phage escapes, it and all its future offspring escape!