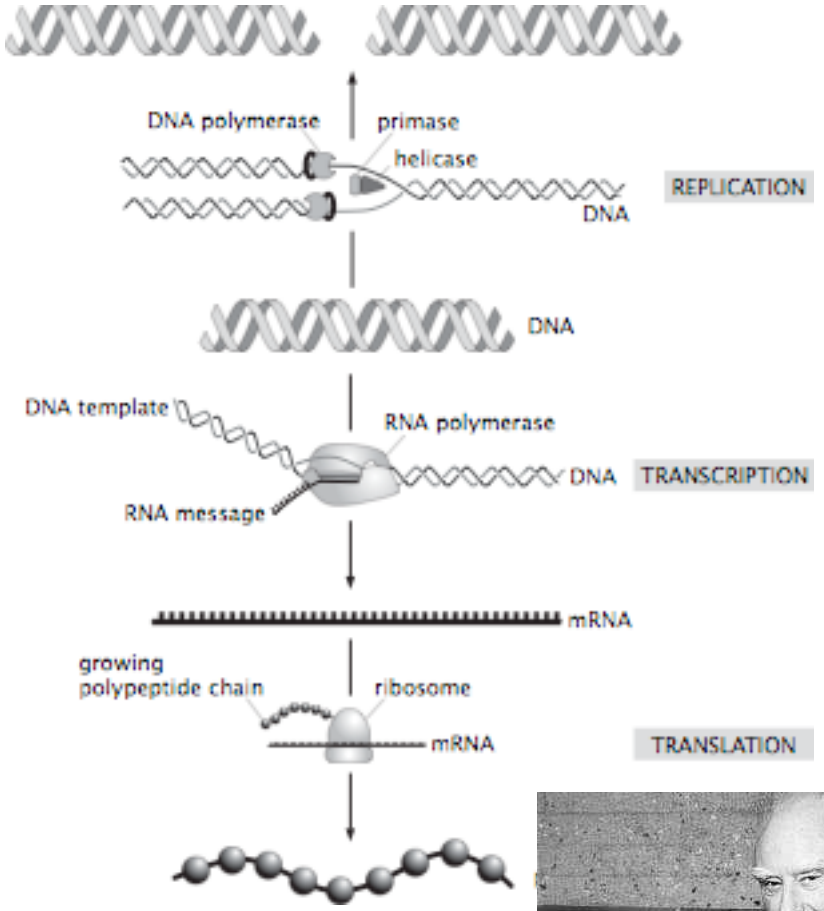
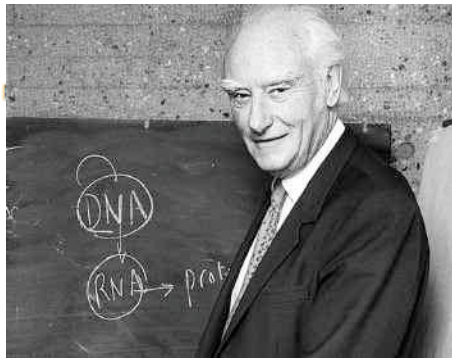
The Central Dogma of Molecular Biology: How Genes Lead to Proteins

- *Crick and others mused over the ``two great polymer languages".*
- *Central dogma explains the chain of events relating them.*
- *The ribosome is the universal translating machine that speaks both languages.*
- *We have seen what genes are and how they serve as the informational memory of organisms. But we have NOT said how they are controlled.*

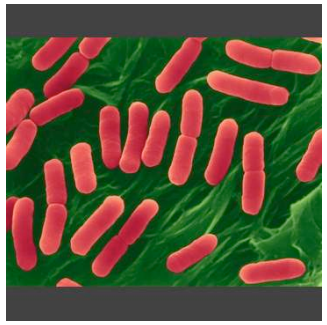




Now we have the background to tackle the question we started with: how do cells make decisions?

Not All DNA Codes for Proteins

- *The E. coli genome is a circle with roughly 4.7 million base pairs.*
- *How many genes? An estimate.*
- *The genes related to sugar usage have been one of the most important stories in the history of modern biology and biochemistry (and take us right back to the great debate on vitalism played out with Pasteur in the 1800s).*
- *"Promoter" region on DNA is subject to intervention by various molecular bouncers that govern the gene.*

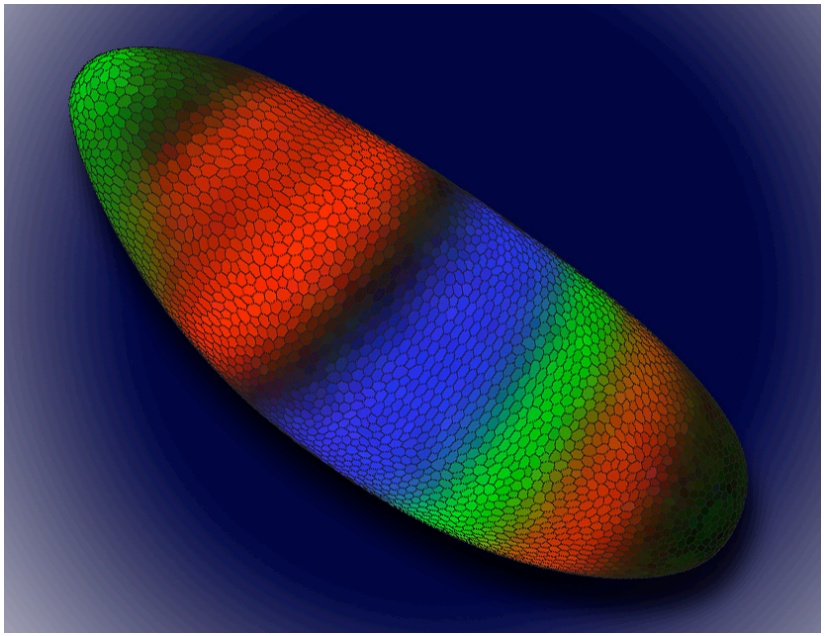


The regulatory landscape

Ways to Measure Gene Expression

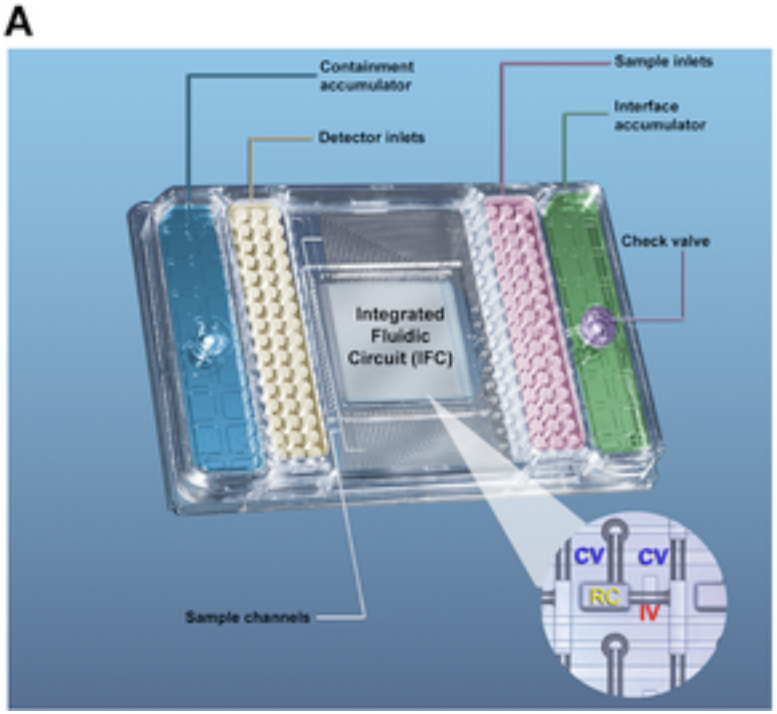
- ***Basic point: looking for "reporters" of the level of expression of gene of interest.***
- ***Can ask the system to report on the level of gene expression at various steps in the processes linking DNA to active protein.***
- ***Promoter occupancy, level of mRNA, level of active protein.***

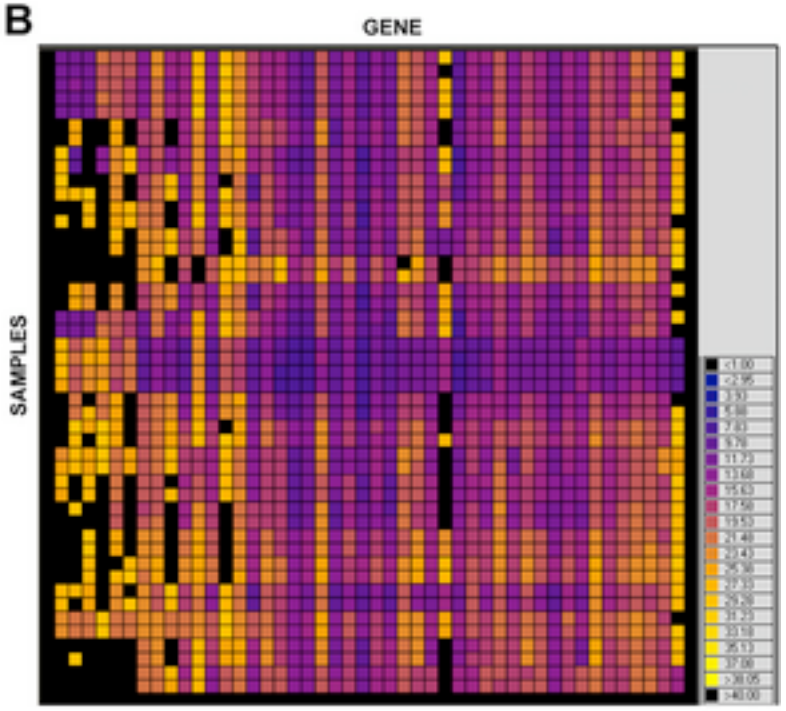
http://www.lbl.gov/Science-Articles/Archive/sabl/2008/Feb/genome-mystery.html



This image shows a Drosophila embryo colored to show the expression patterns of early gene regulators. Each color represents the level of expression of one of three gene regulators, Knirps (green), Kruppel (blue), and Giant (red). Color intensity reflects a higher level of expression. The darker areas of the embryo are cells where none of these gene regulators are expressed, and the yellowish areas indicate that both Knirps and Giant are being expressed.

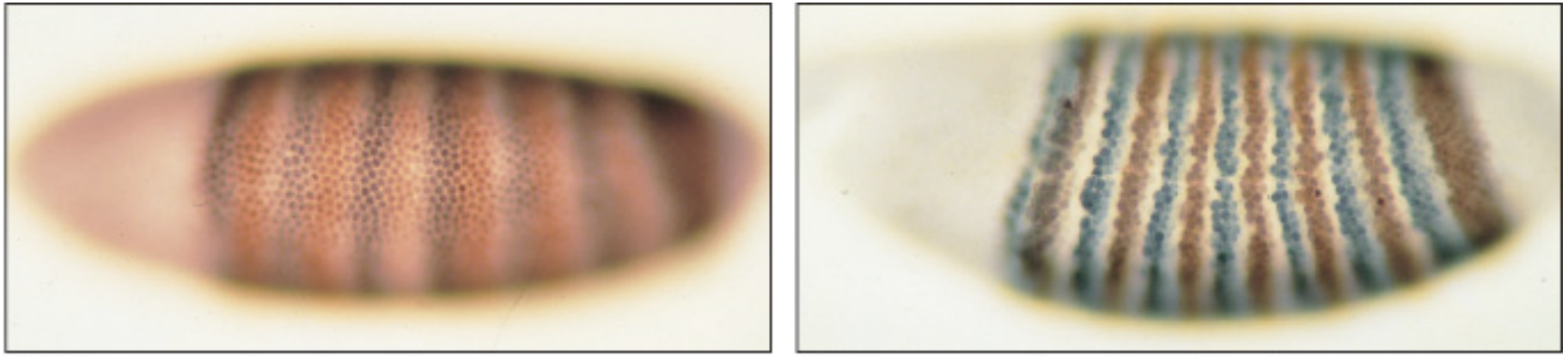
Count the Messenger RNA Molecules





Enzymatic Assay or In-Situ Hybridization

- ***Enzymatic assays – promoter leads to the production of a protein that then does some enzymatic action on the substrate which yields a product that can be visualized.***
- ***In-situ hybridization – described the other day – probe is complementary to the RNA of interest and is labelled for detection.***



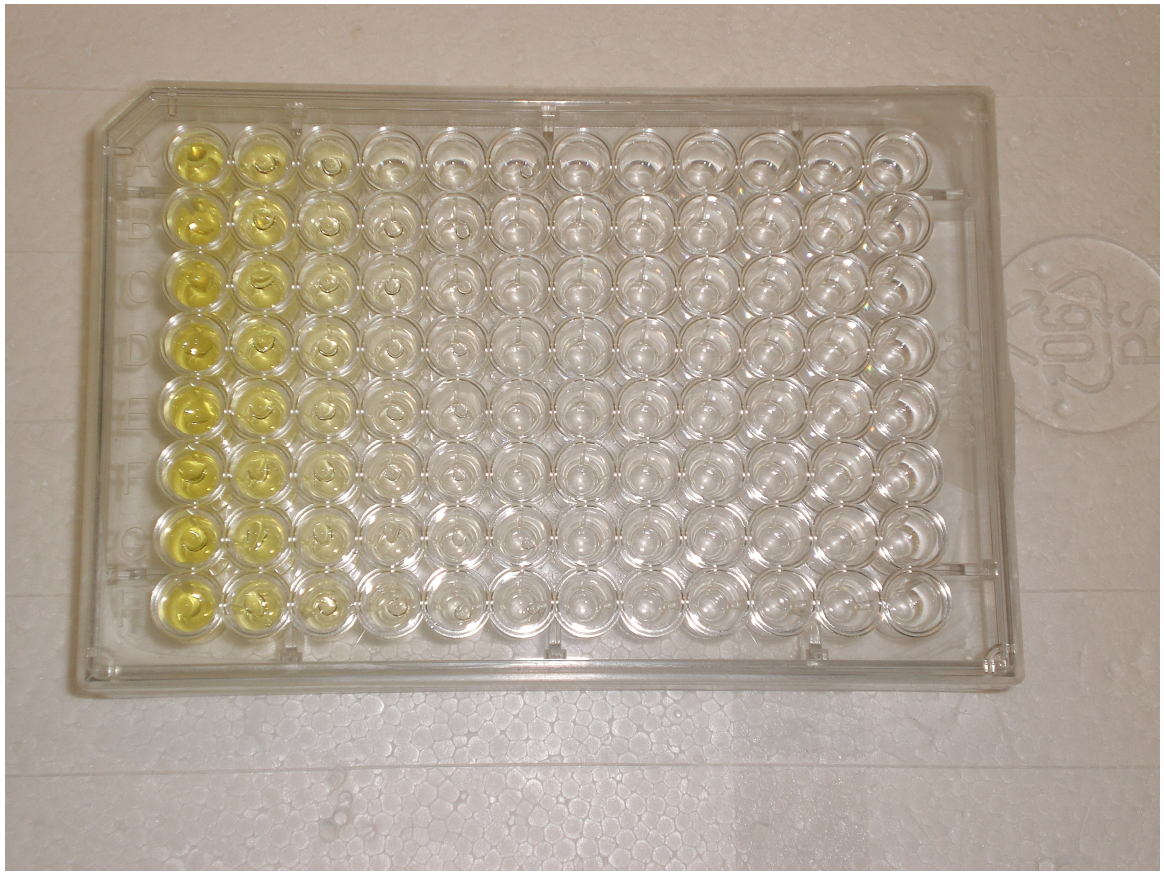
2.7 hours after fertilization

3.5 hours after fertilization

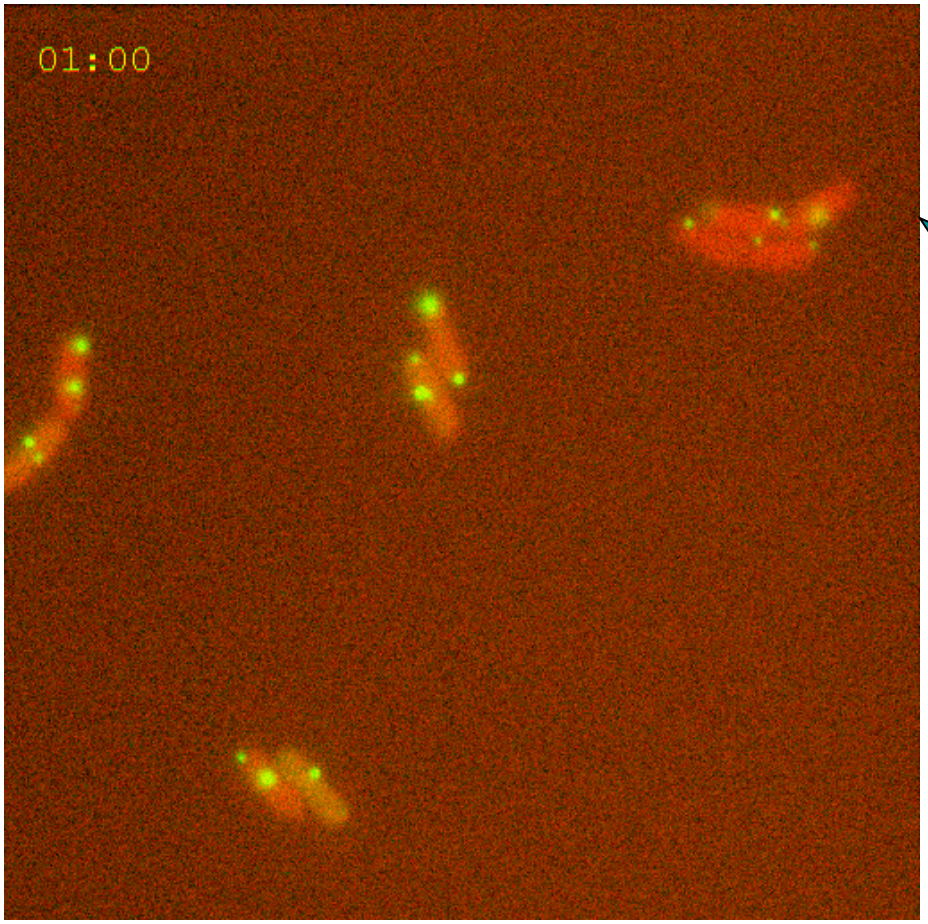
Figure 21–39. Molecular Biology of the Cell, 4th Edition.

Enzymatic Assay or In-Situ Hybridization

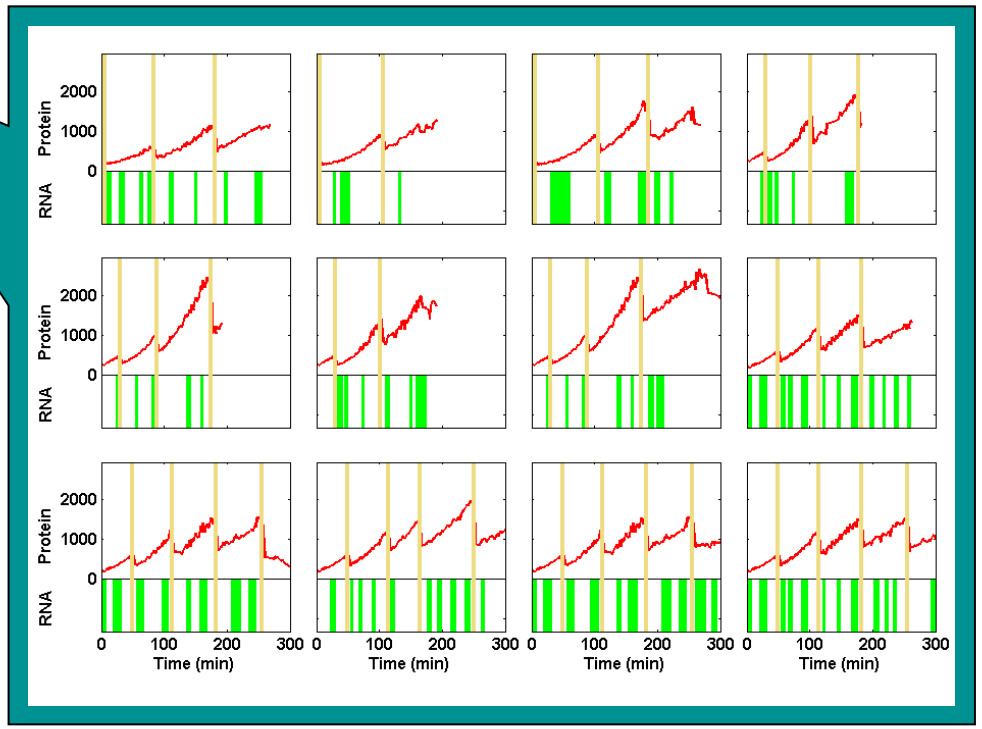
- ***Enzymatic assays – promoter leads to the production of a protein that then does some enzymatic action on the substrate which yields a product that can be visualized.***
- ***In-situ hybridization -***



Information Processing in Living Cells: Beyond First Approximations



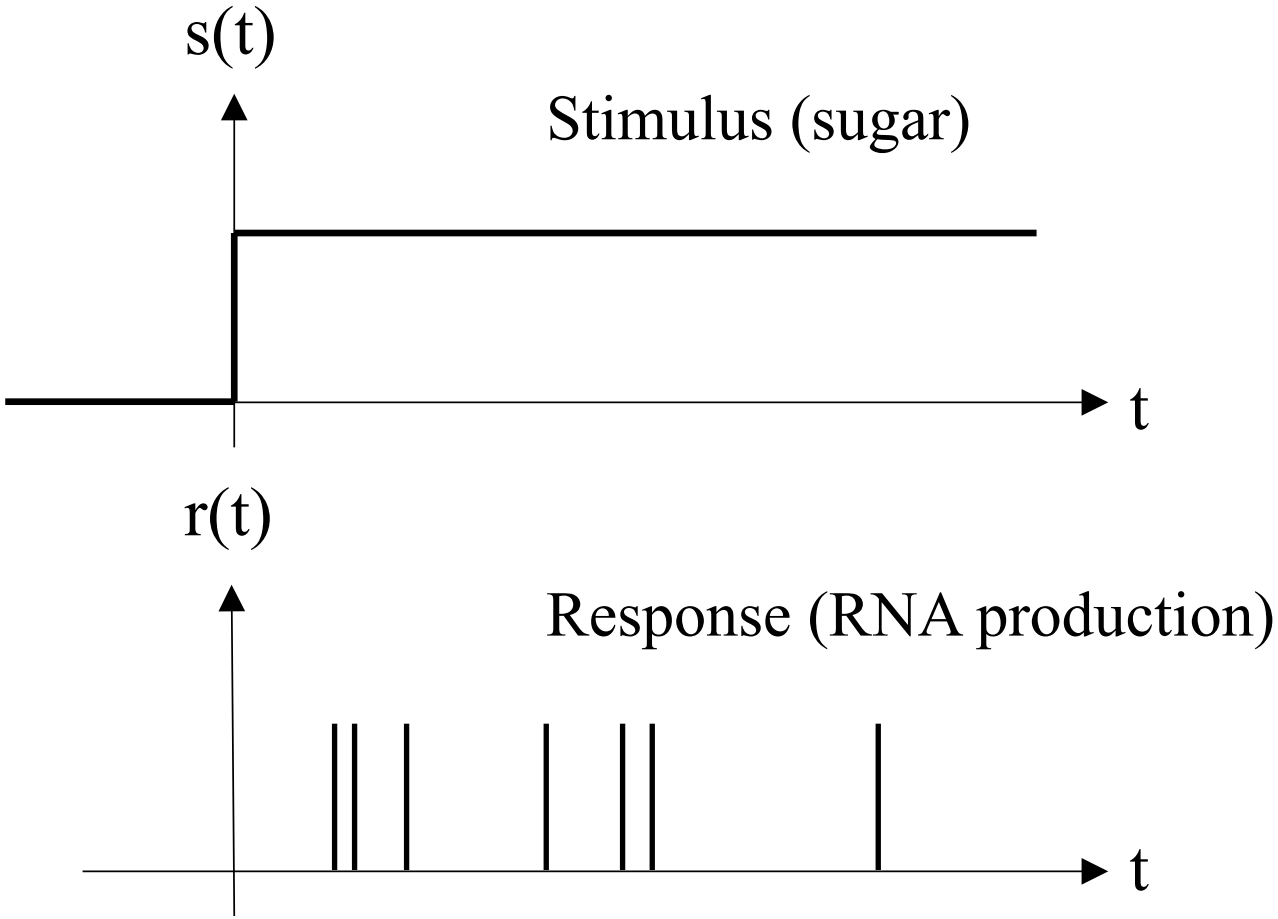
Ido Golding



Department of Physics
ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

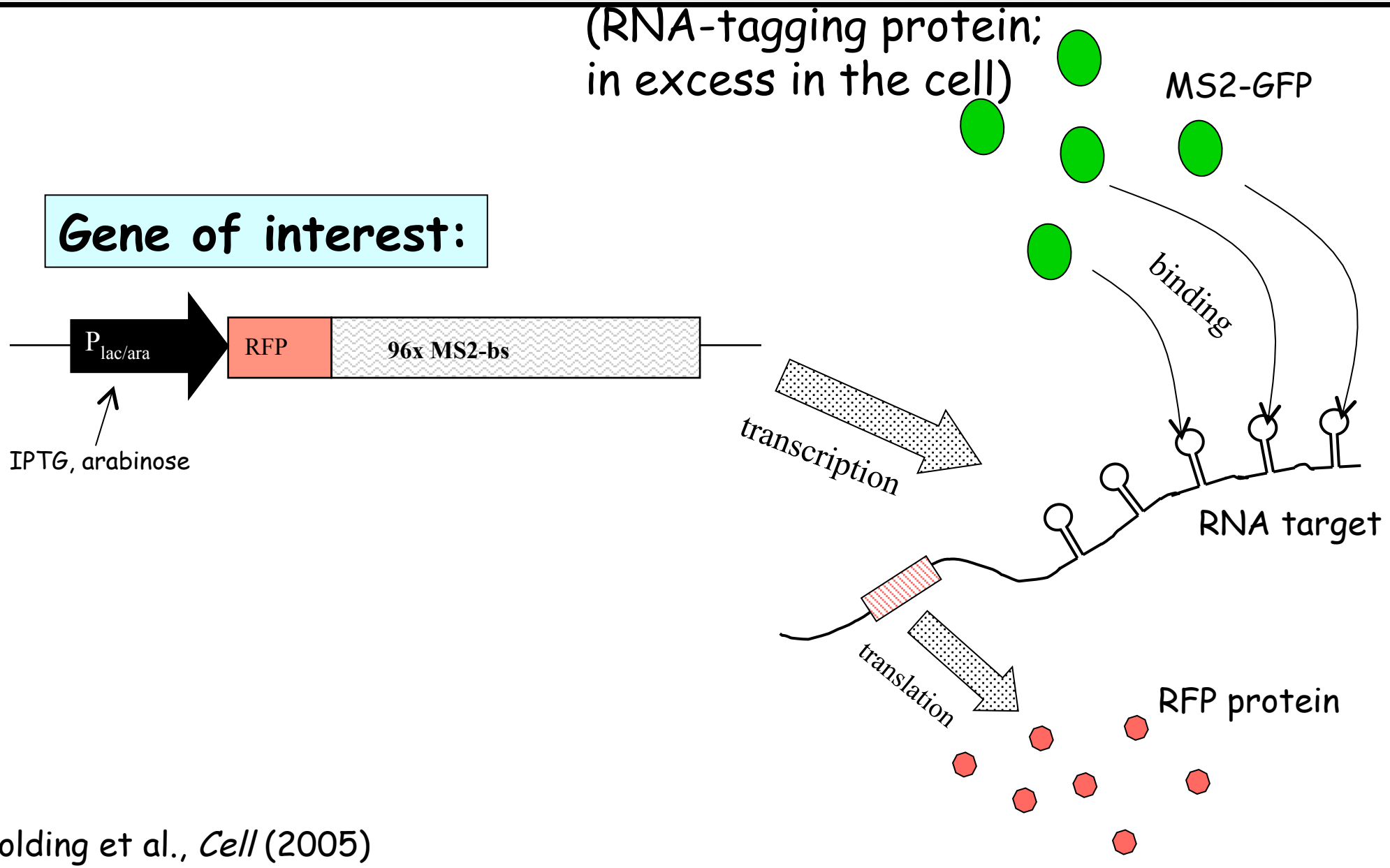
Center for the Physics of Living Cells

Simple Case of Turning a Gene "On"



Approximations used to describe the process…

Engineering bacteria to report on gene activity



Golding et al., *Cell* (2005)

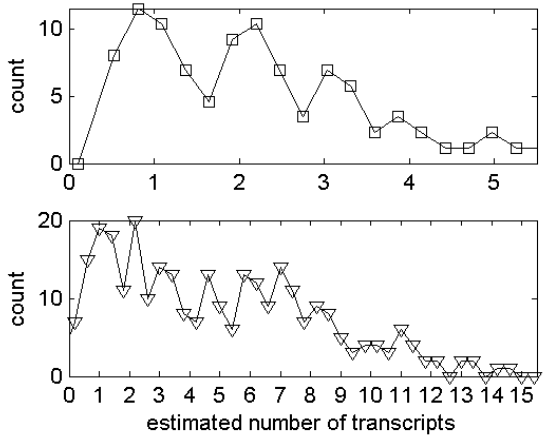
Measuring mRNA & protein numbers

mRNA ∝ number of bound MS2-GFPs
∝ photon flux from localized green fluorescence

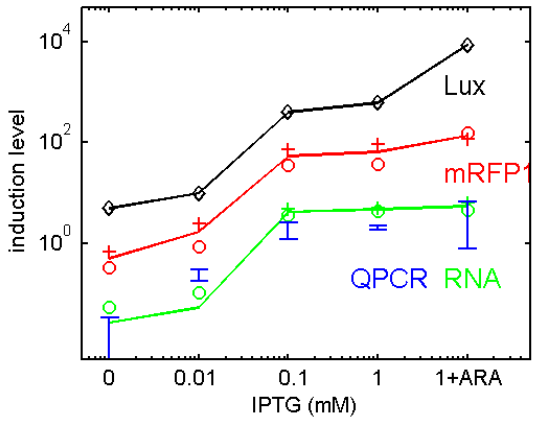
Protein ∝ number of RFPs
∝ photon flux from whole-cell red fluorescence

Histogram of
RNA copy number:

1st peak =
inter-peak interval ≈
50-100 X GFP =
1 transcript

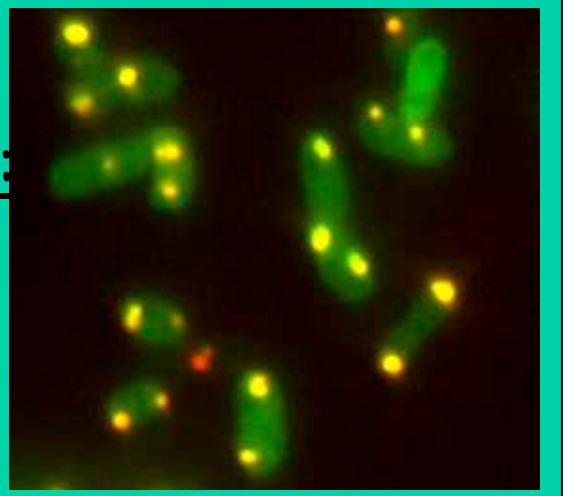


Controls:
QPCR
Protein levels



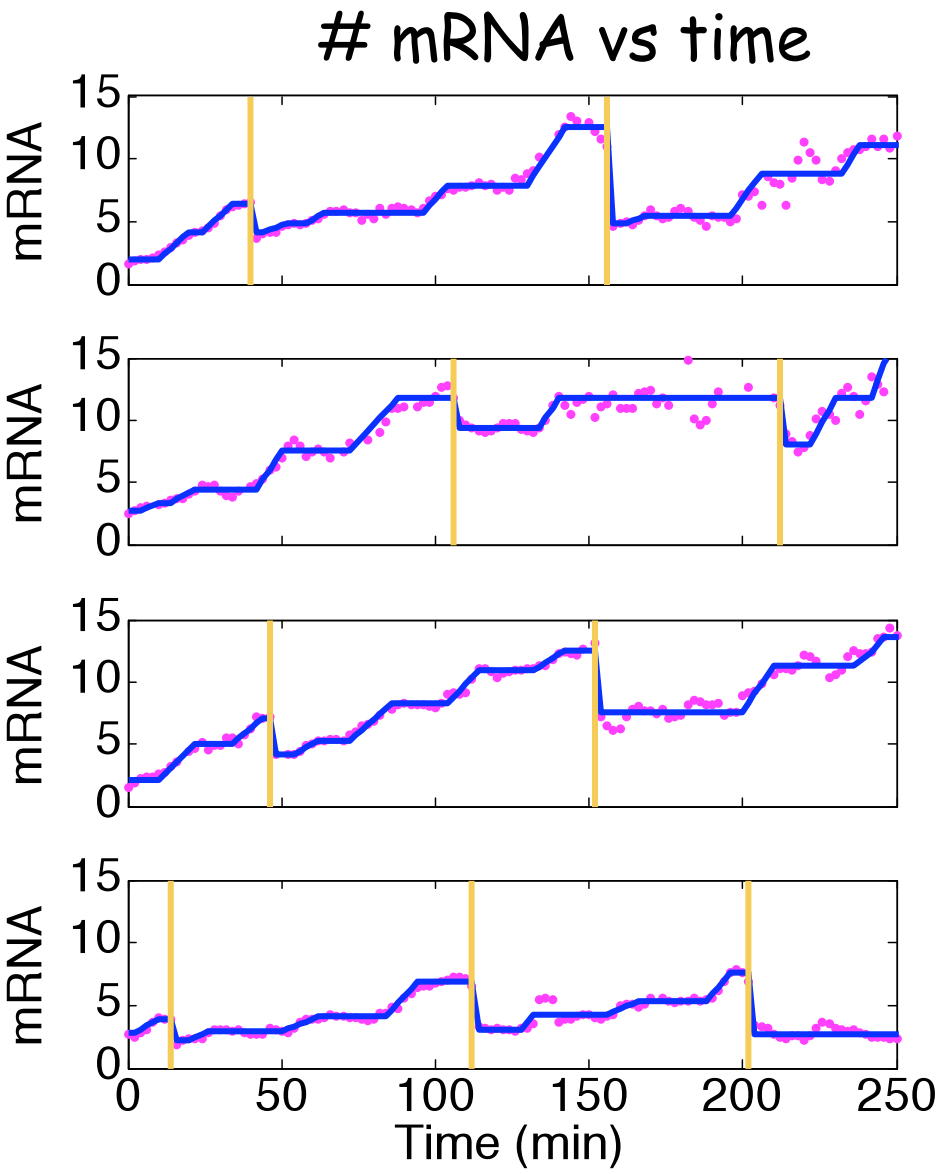
Lux: Lutz & Bujard 1997

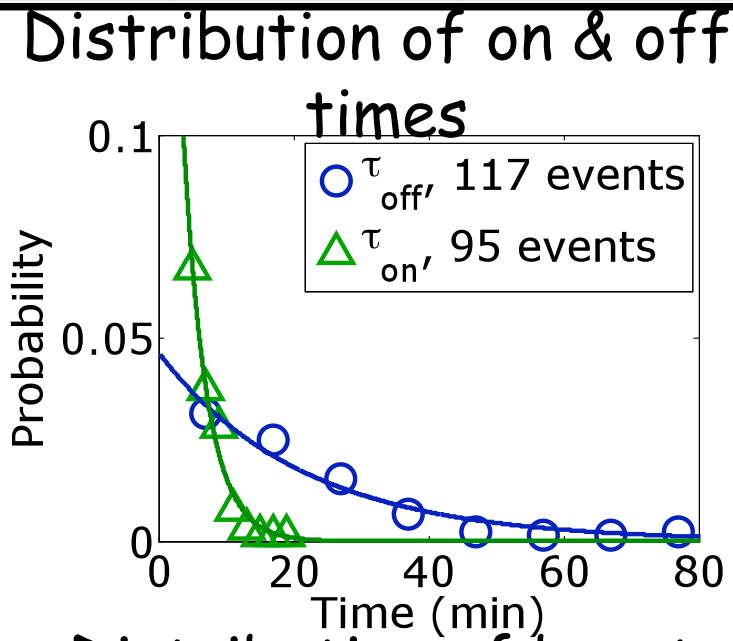
Controls:
FISH

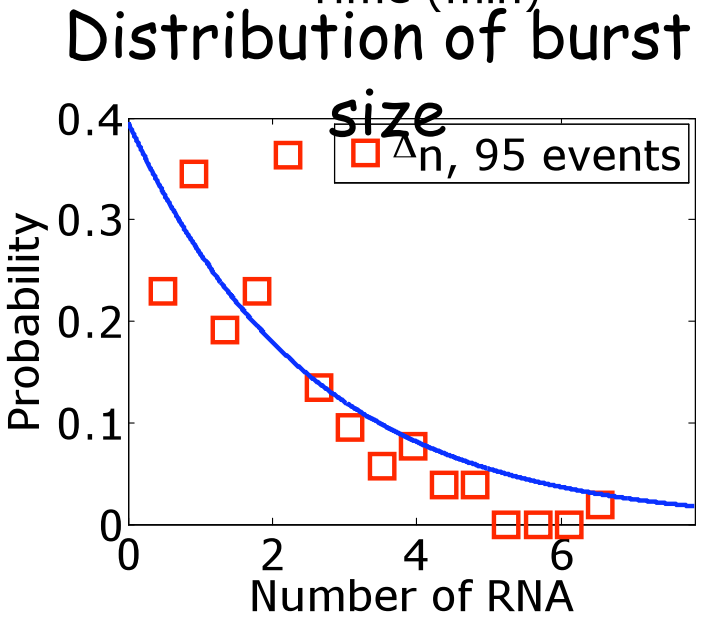


(Thanks to: A. Raj, A. van Oudenaarden)

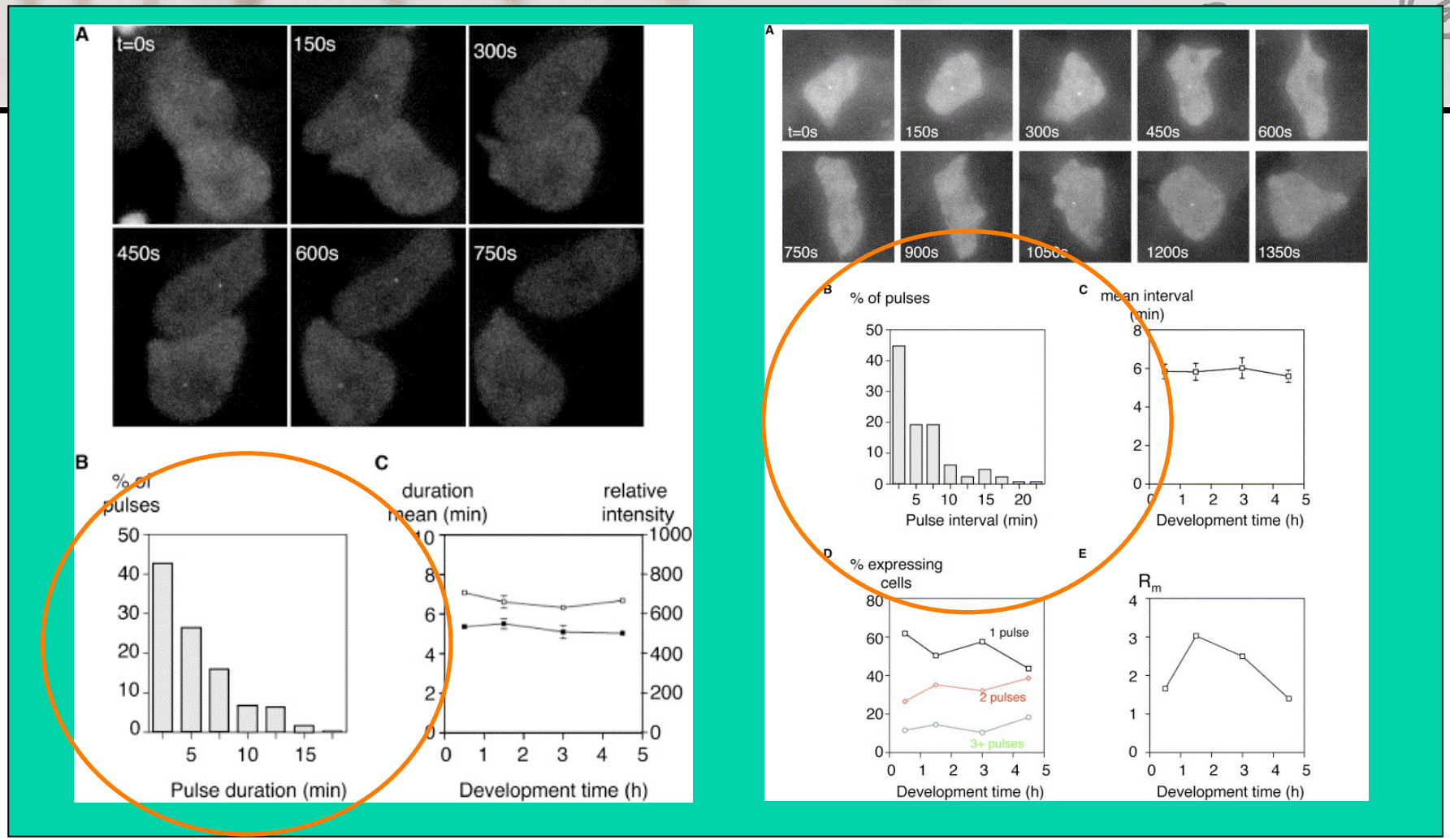
RNA kinetics in individual cells







Transcriptional bursting in eukaryotes



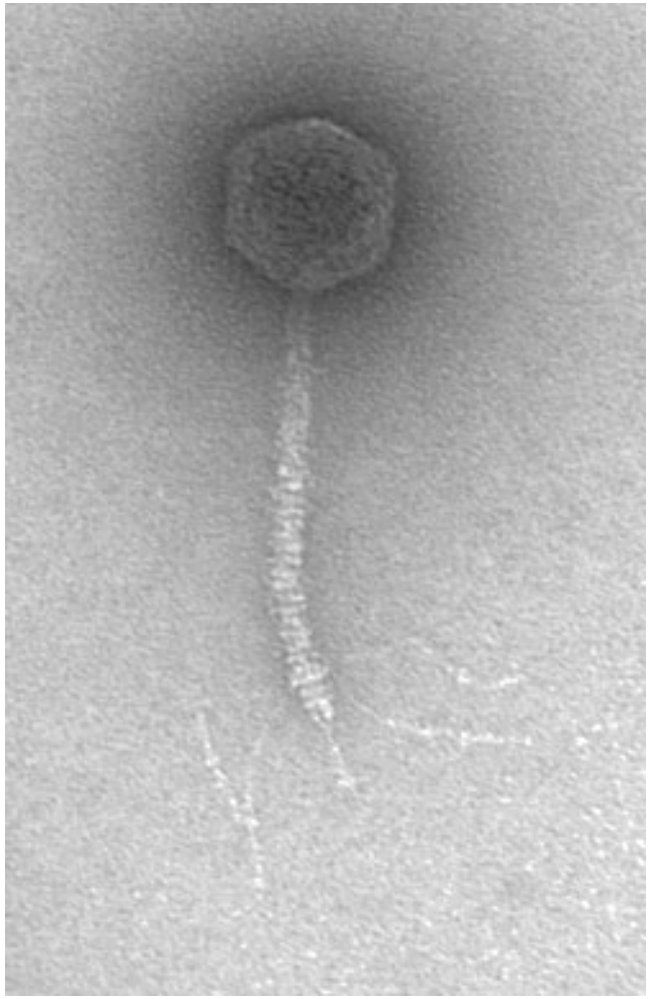
Chubb JR, Trcek T, Shenoy SM, Singer RH. *Curr. Biol.* (2006)

See also: Golding & Cox, *Curr. Biol.* (2006)

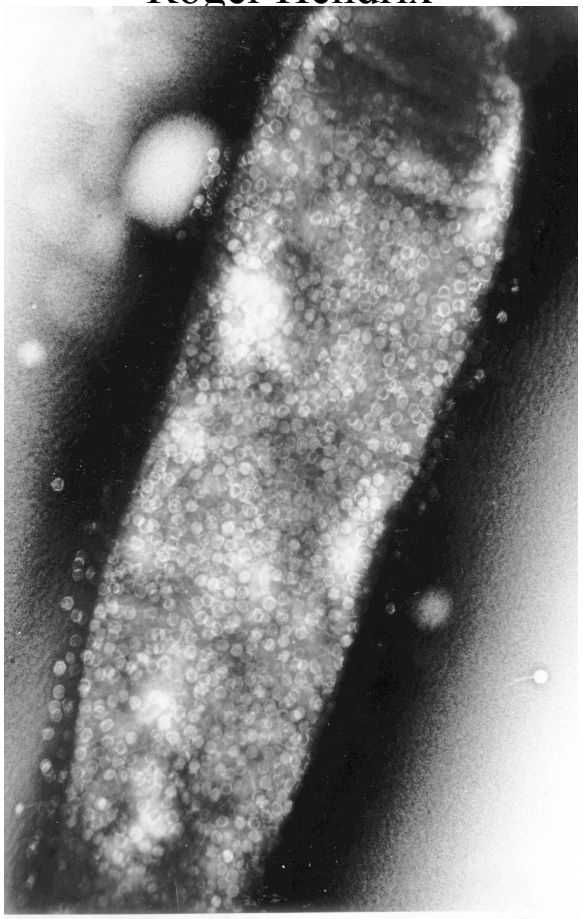
Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S, *PLoS Biol.* (2006)

"Stochastic mRNA Synthesis in Mammalian Cells".

The Lambda Switch: The Other Hydrogen Atom of Gene Regulation

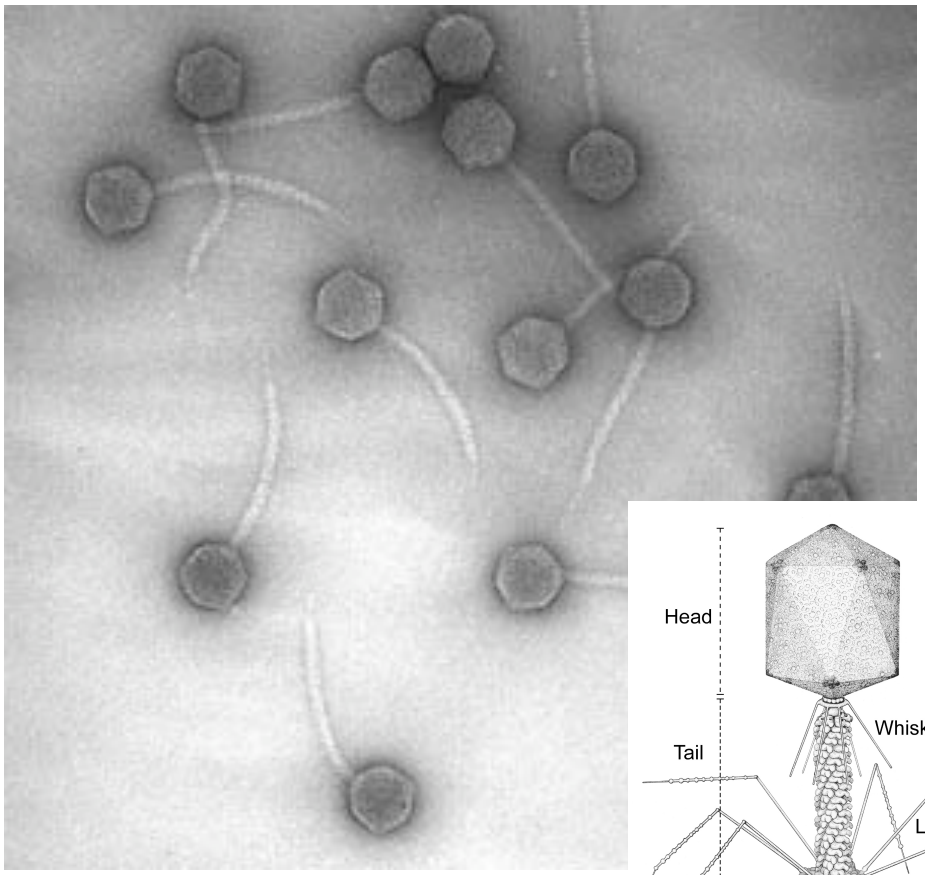


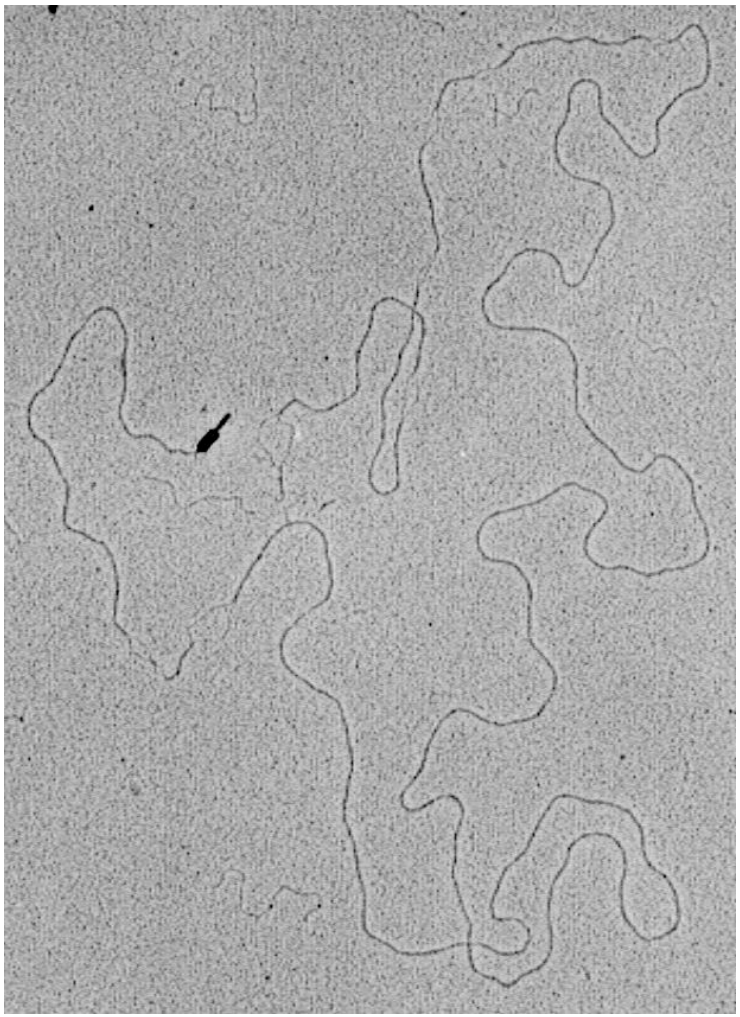
Roger Hendrix



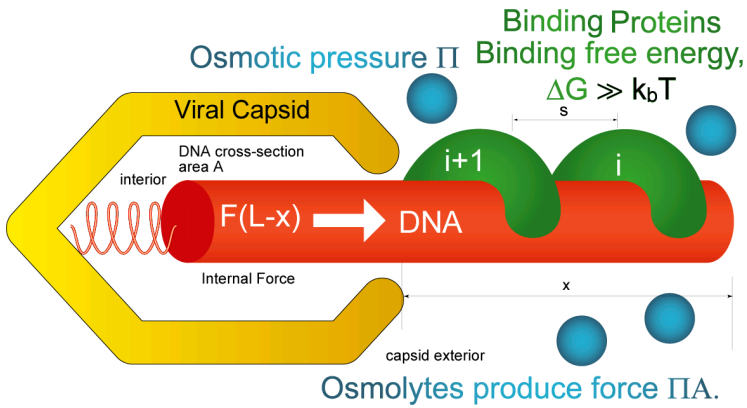
Bacteriophage and Their Genomes

http://www.biochem.wisc.edu/inman/empics/0020b.jpg



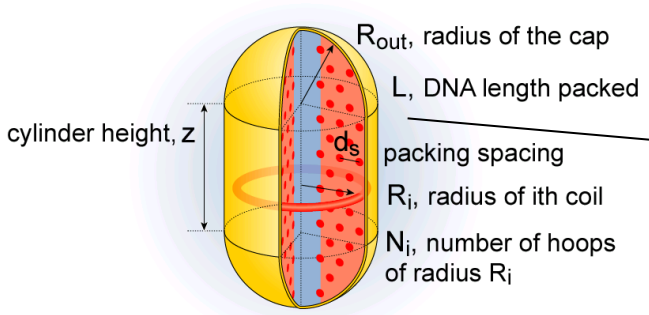


Physical Consequences of the Tight Squeeze in the Life Cycle of a Bacteriophage

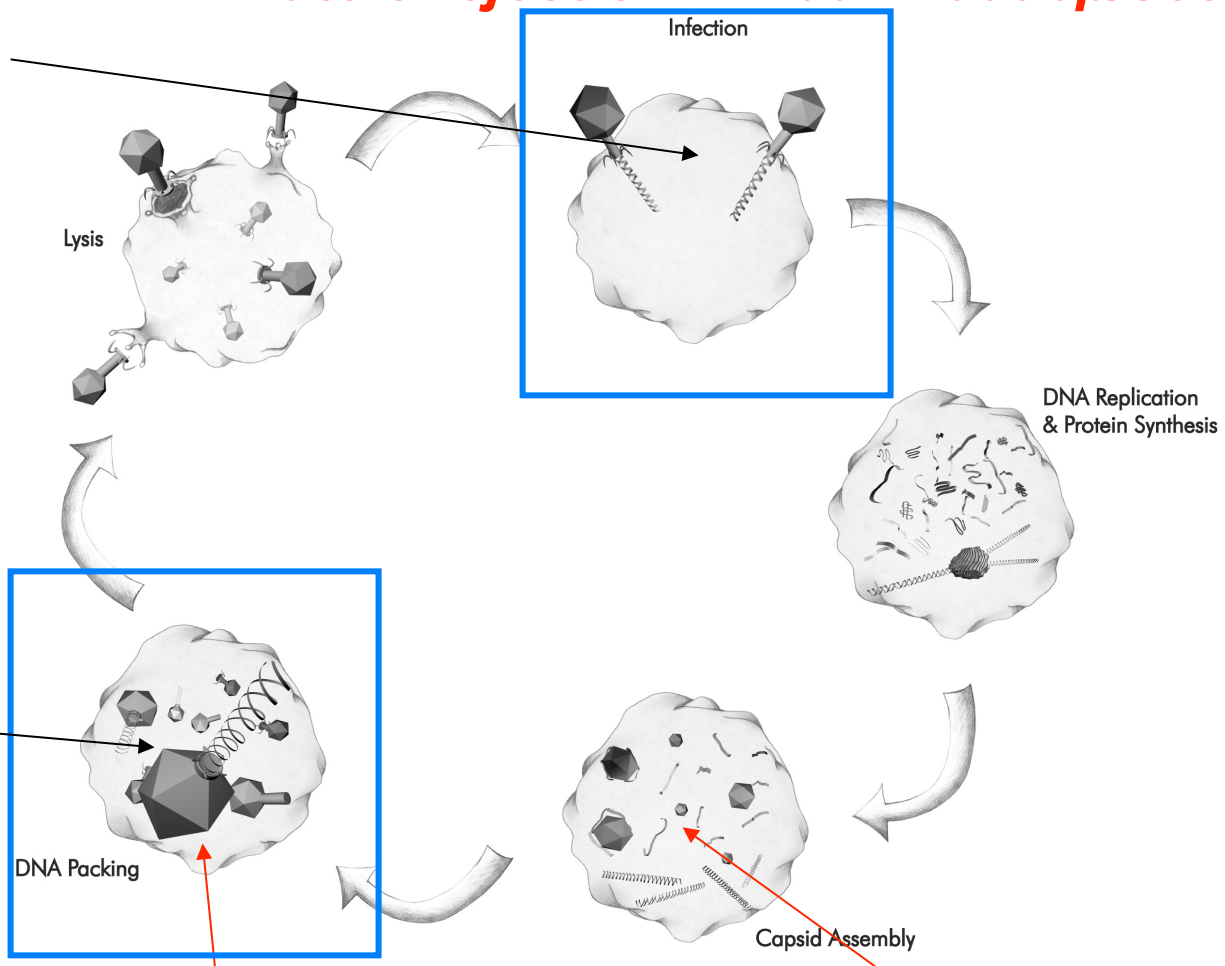


Forceful ejection

Rate of ejection: ≈ 100 - 1000bp/sec



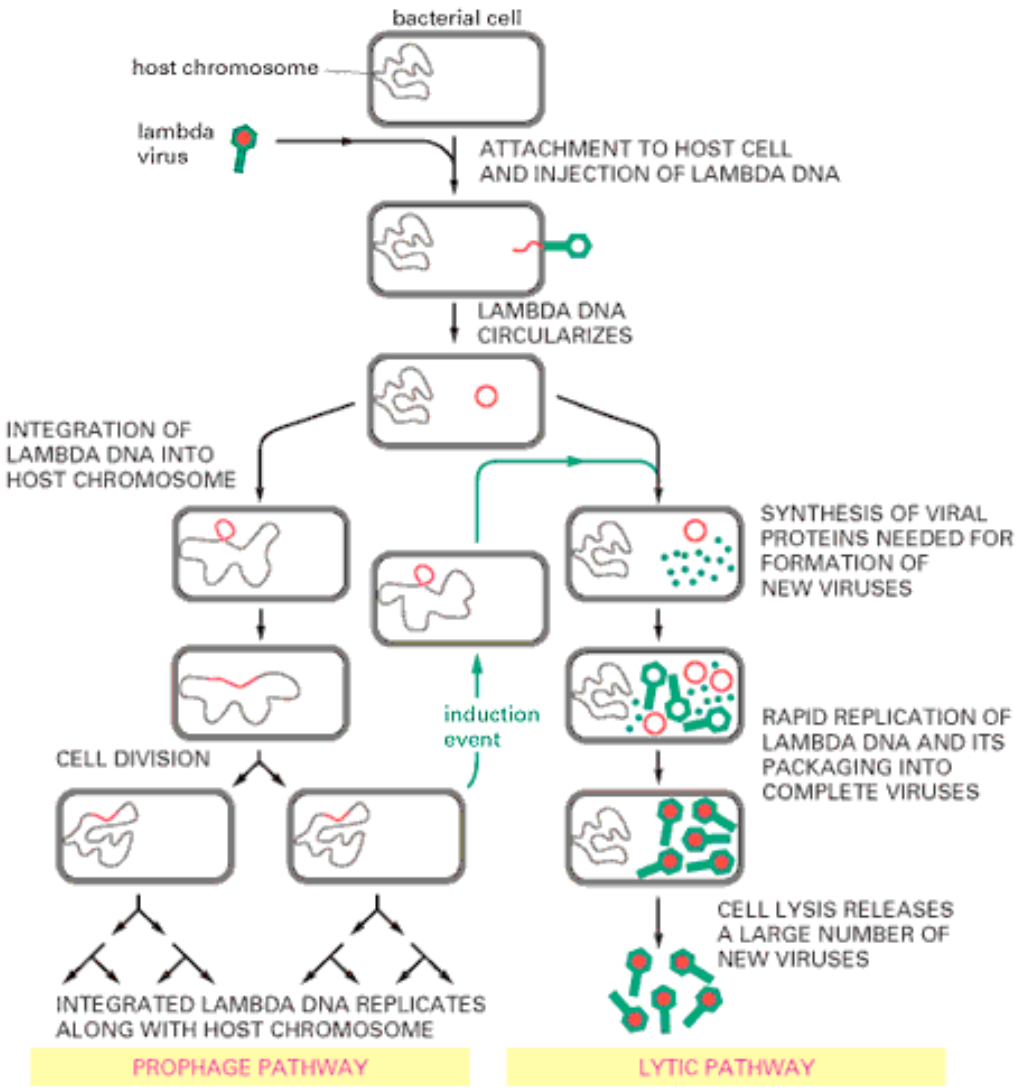
Construct a physical model of these processes.



Rate of packing: 100bp/sec
"Some assembly required"

Self-assembly

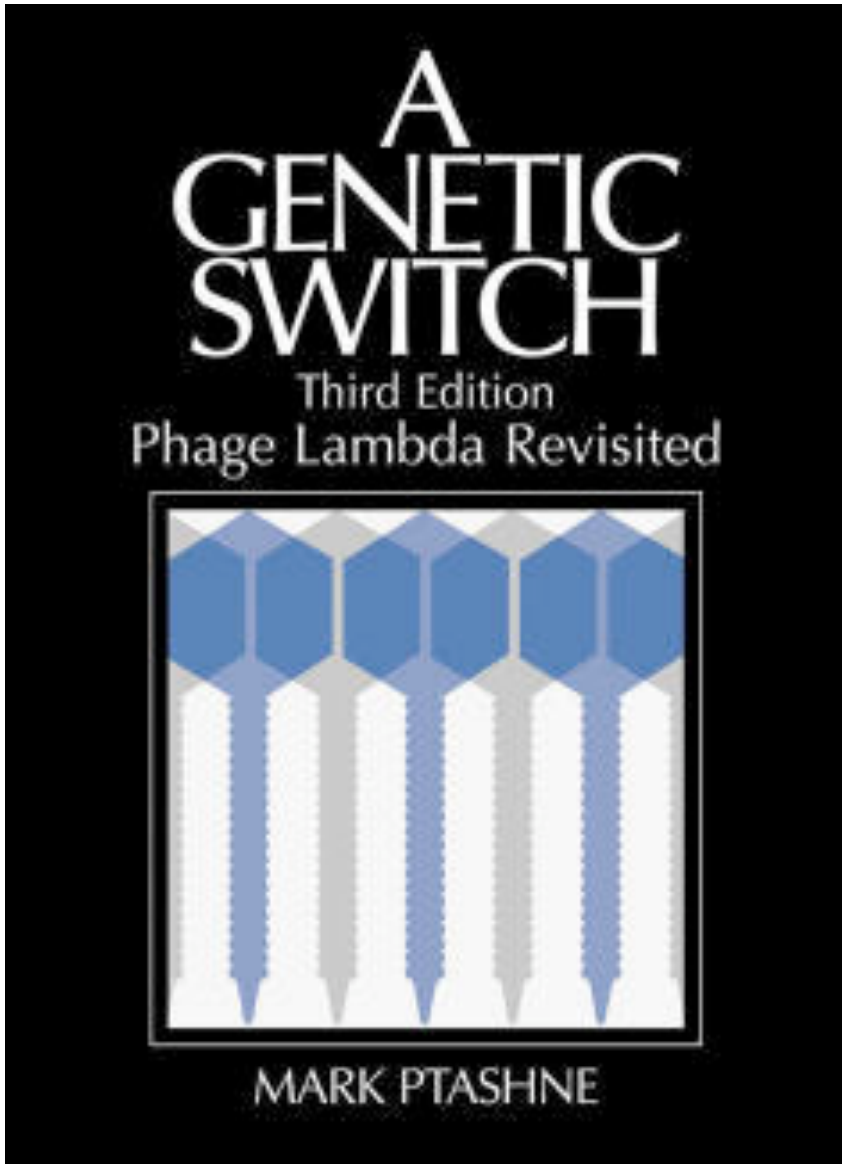
The Life Cycle of Bacteriophage Lambda

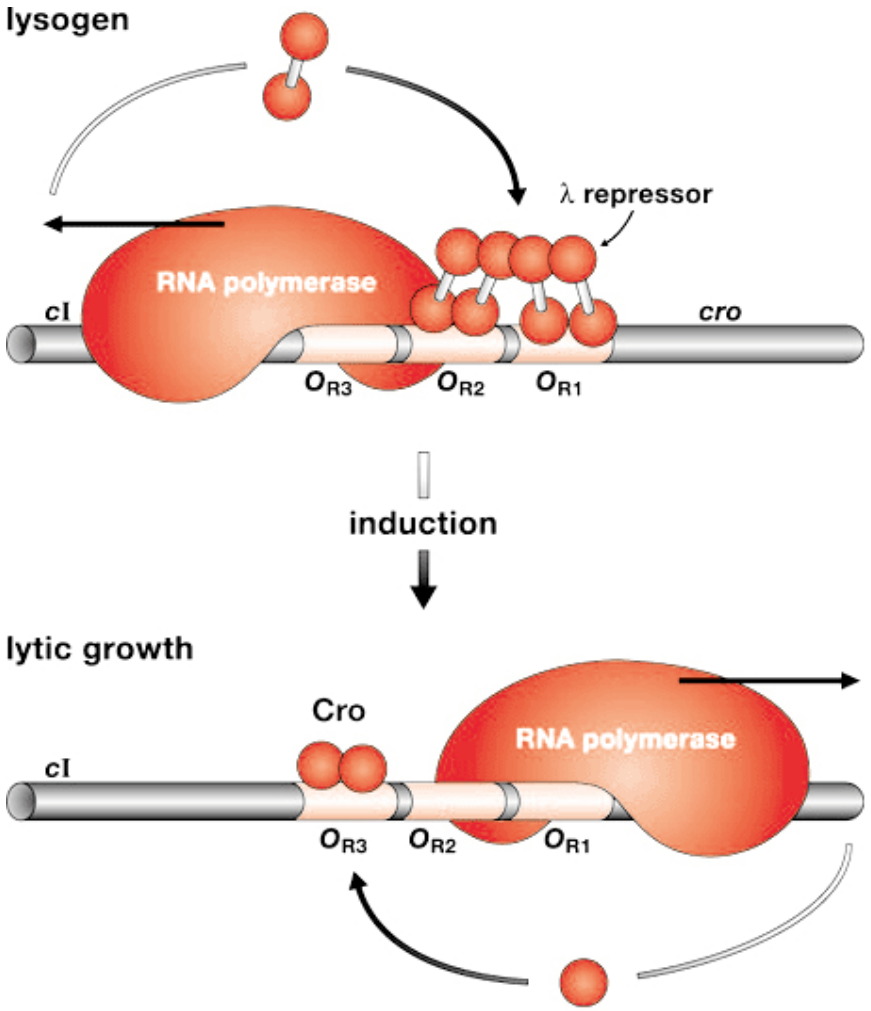


$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$

A Genetic Switch

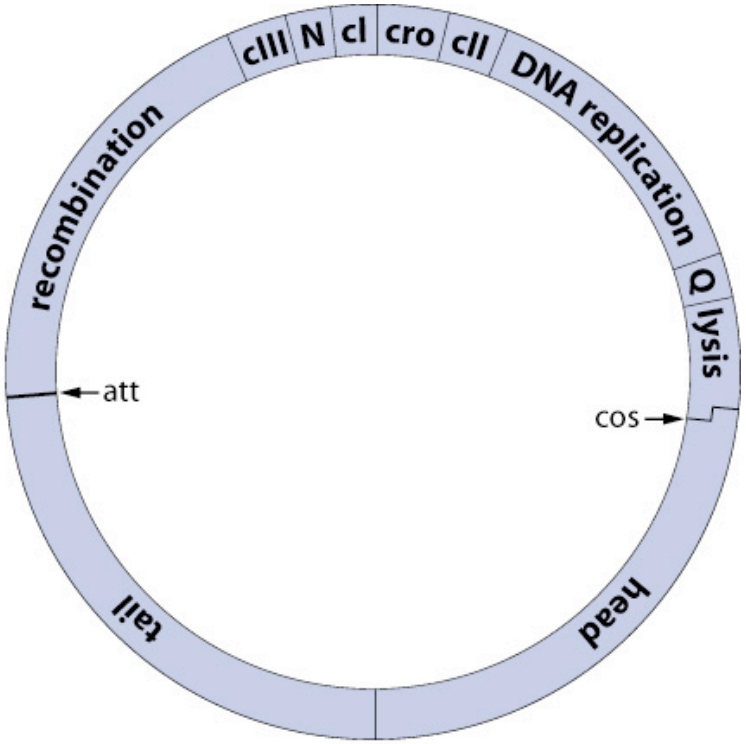
$E_{bend} = \frac{\pi \xi_p k_B T}{R}$





Genes and Signals, © 2002 by Cold Spring Harbor Laboratory Press, Chapter 1, Figure 13.
(Modified, with permission, from Blackwell Science http://www.blacksci.co.uk/.)

The Lambda Genome



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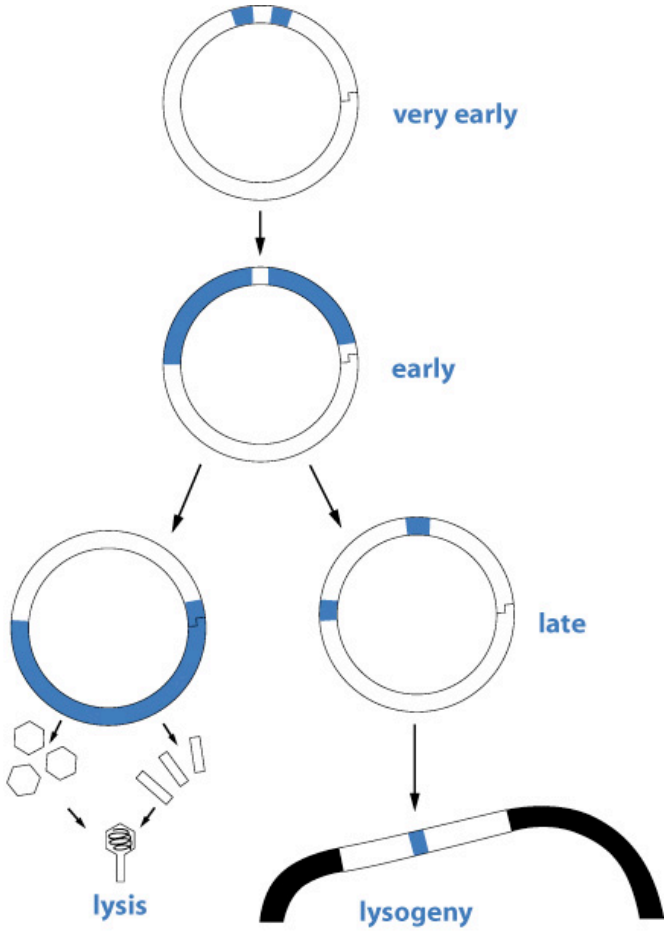
Chapter 3, Figure 1



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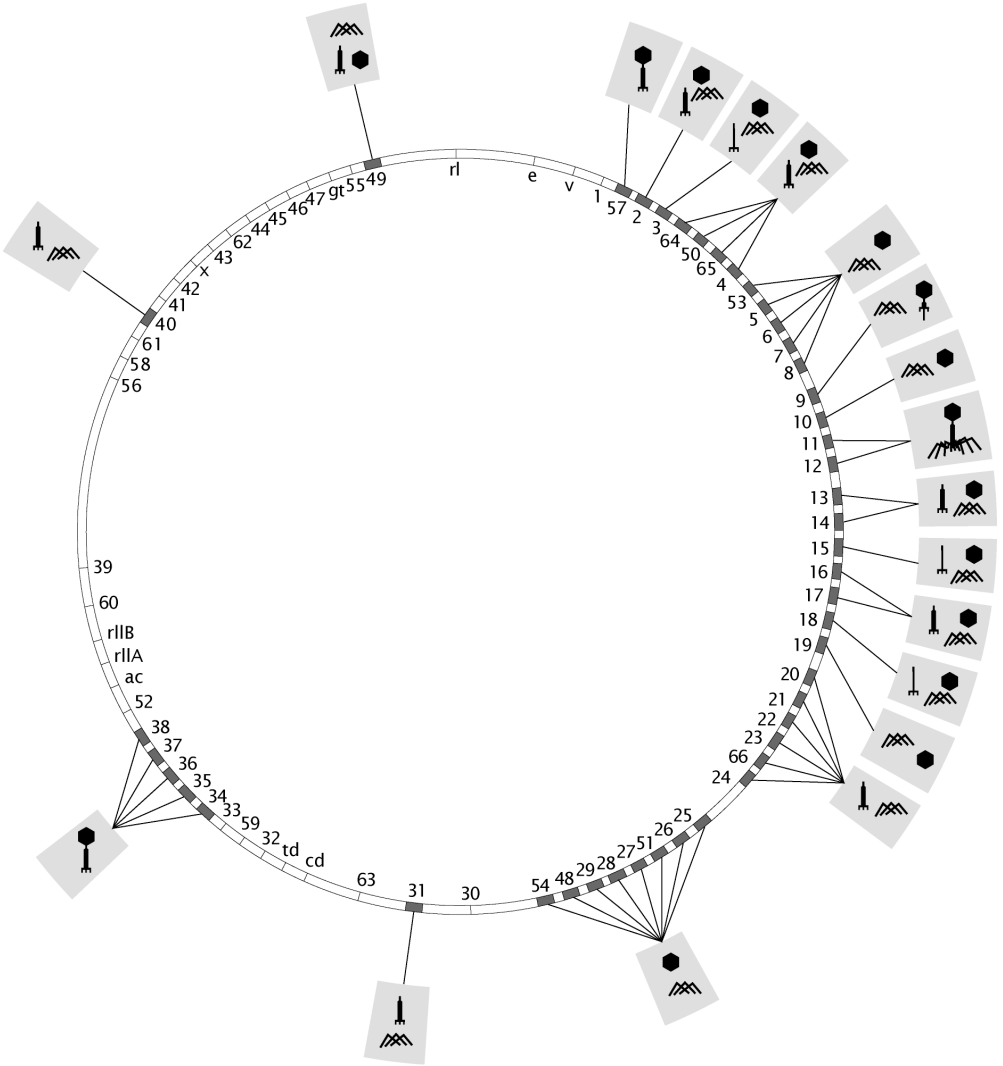
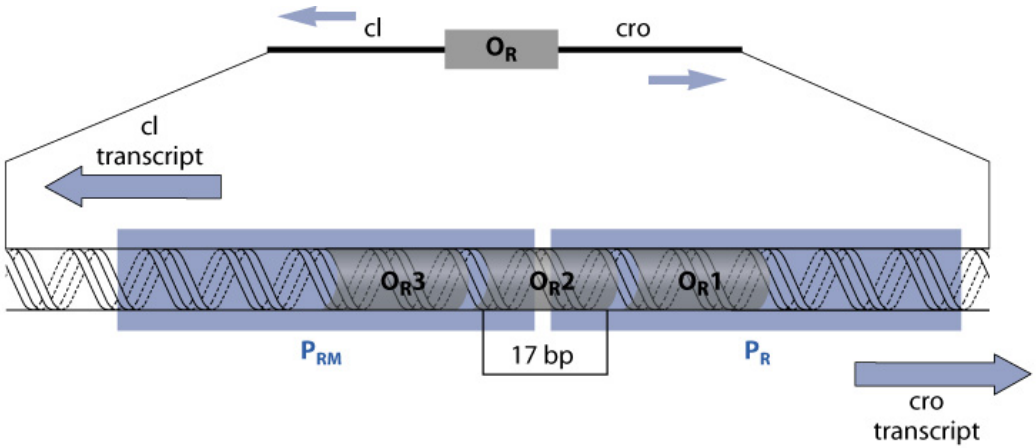
Chapter 3, Figure 3

A More Detailed Example of the Parrts List



The Lambda Switch: Lysogenic State



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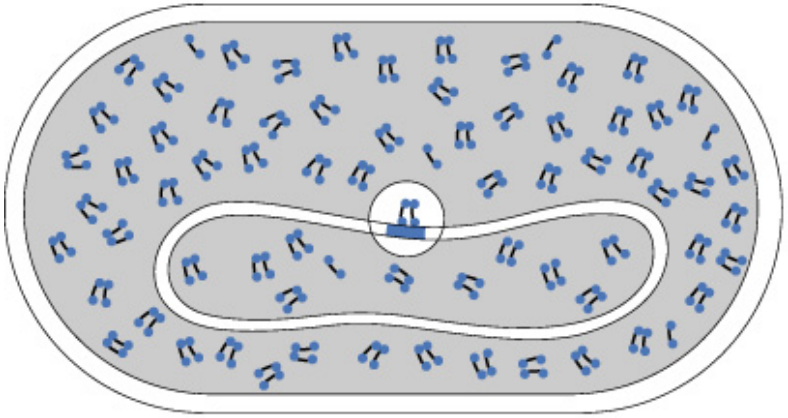
Chapter 1, Figure 4



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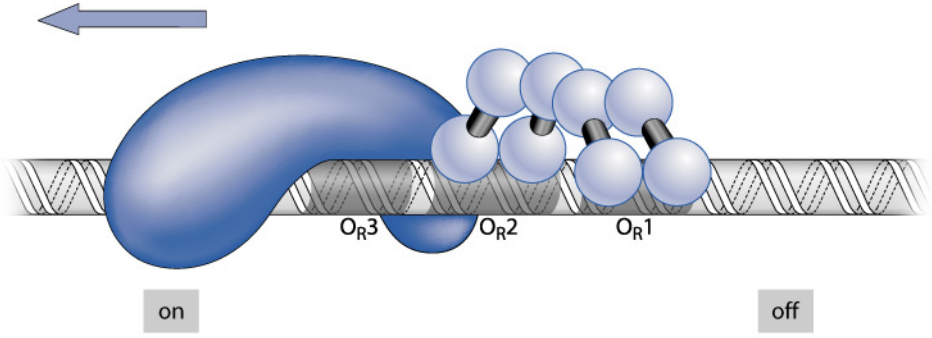
Chapter 1, Figure 8



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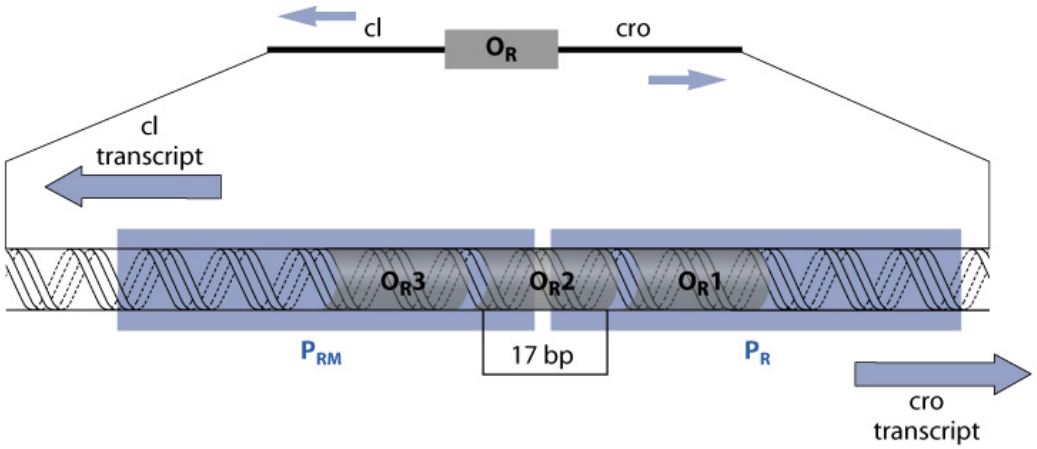
Chapter 1, Figure 19

The Lambda Switch: Lytic State



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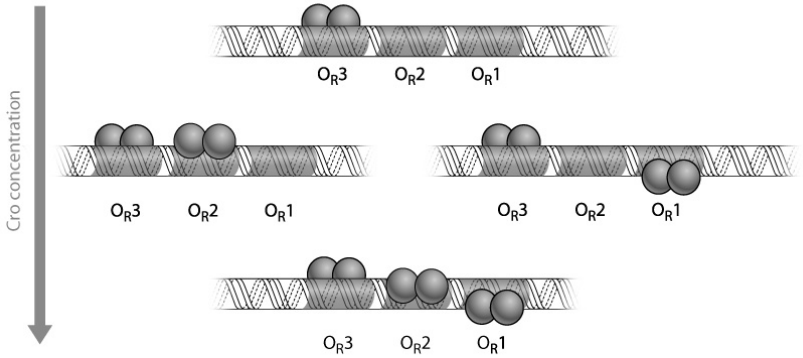
Chapter 1, Figure 4



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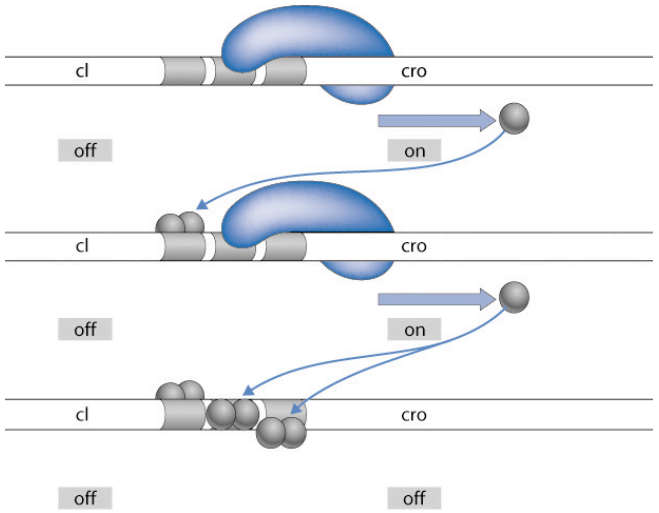
Chapter 1, Figure 23



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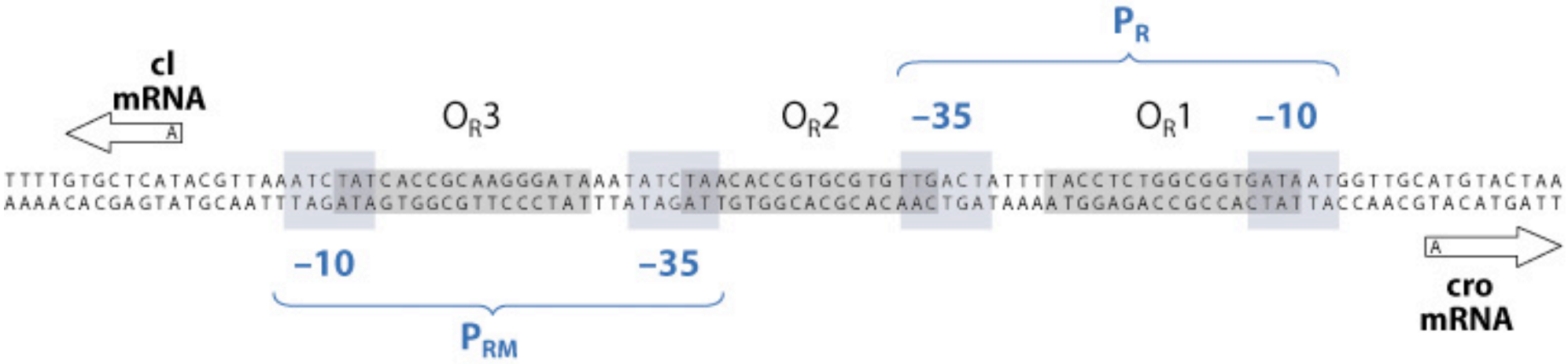
Chapter 1, Figure 24

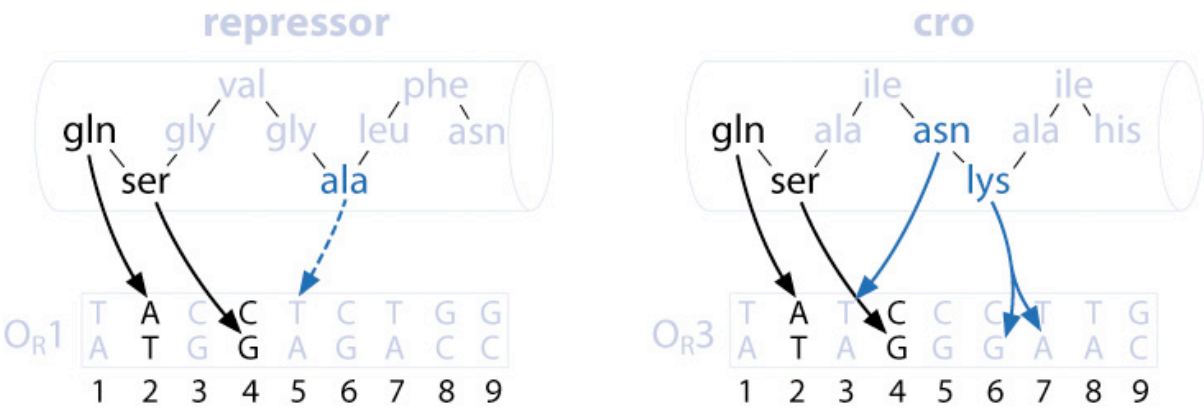
DNA Geography of the Switch



A Genetic Switch, 3rd edition, 2004

Chapter 2, Figure 16

Binding of Transcription Factors



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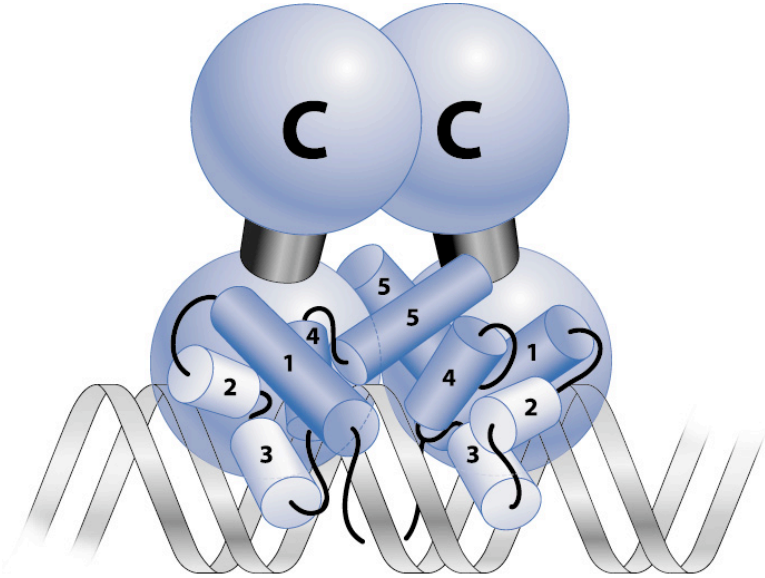
Chapter 2, Figure 11a



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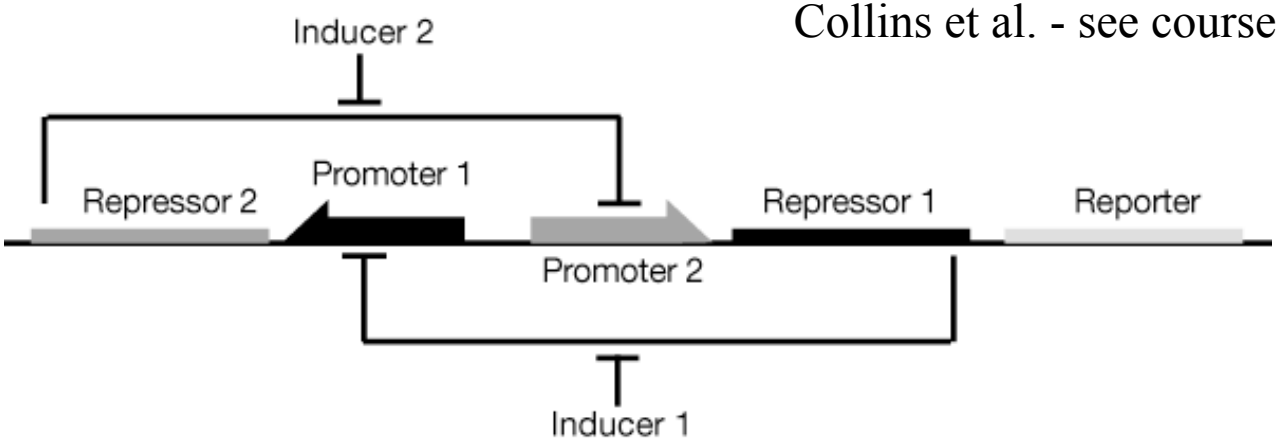
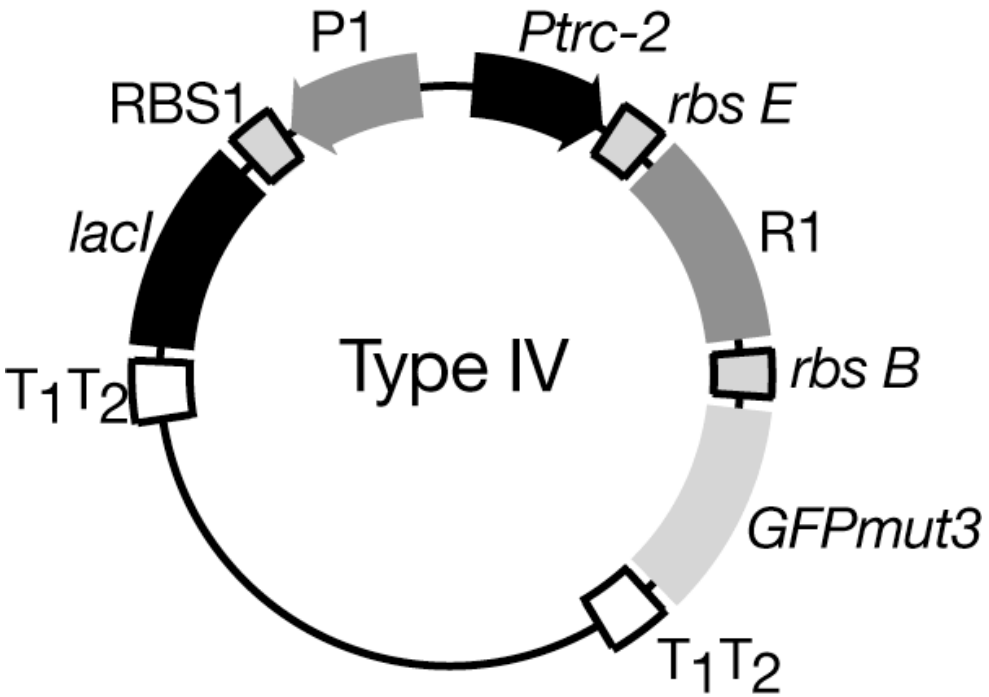
Chapter 2, Figure 6

Synthetic Genetic Switch

Collins et al. - see course website



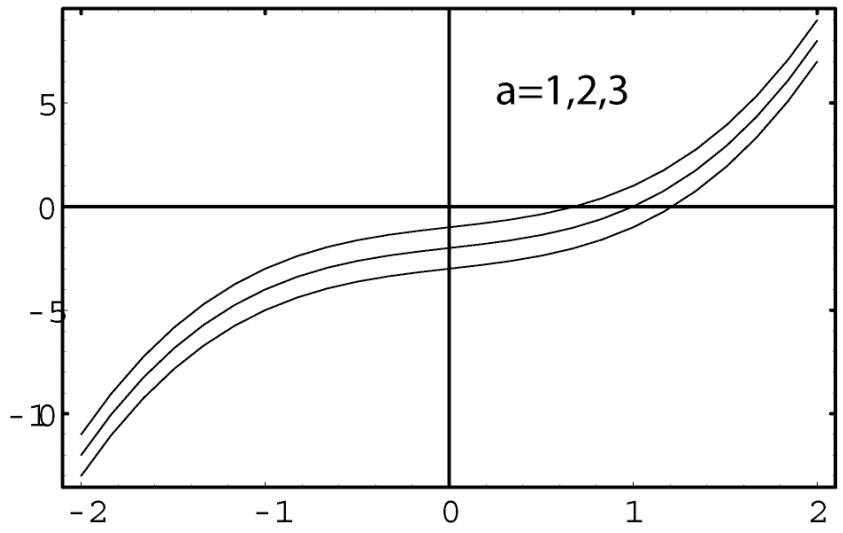


$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$

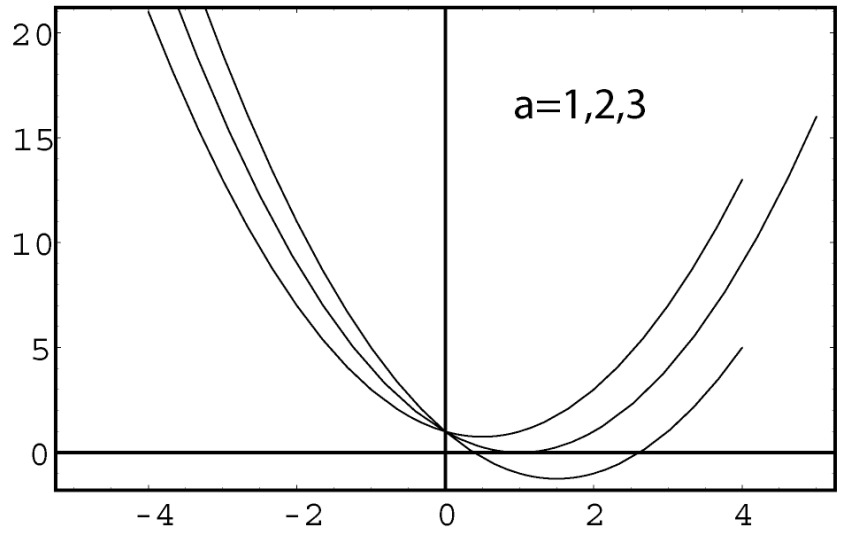
Stable Solutions

$E_{bend} = \frac{\pi \xi_p k_B T}{R}$

a)

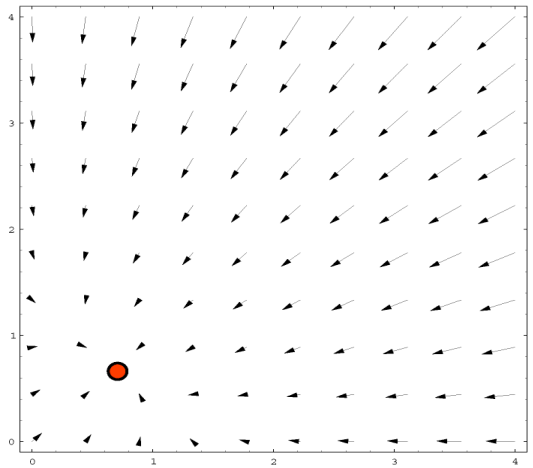


b)



Phase Portrait for the Switch

a)



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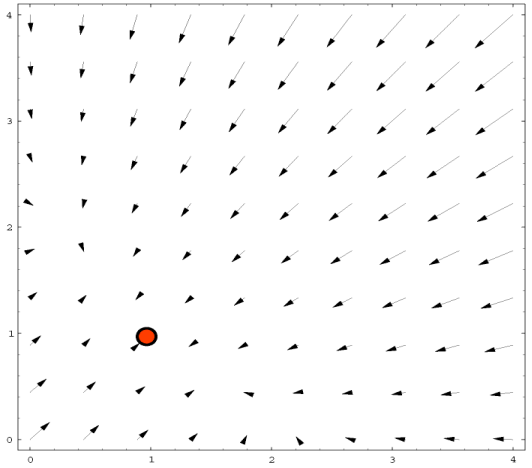

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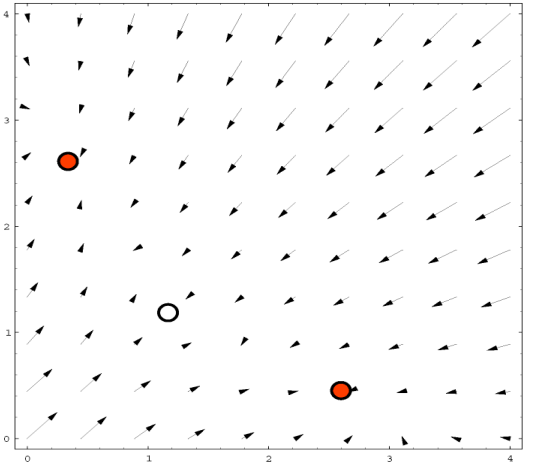
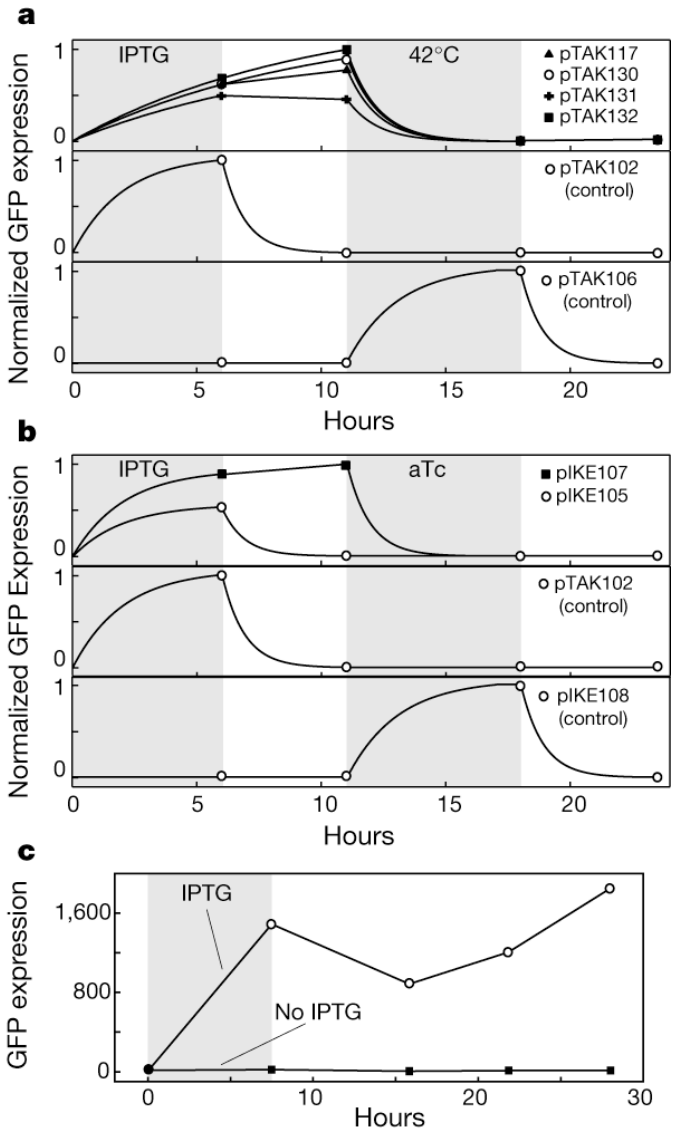
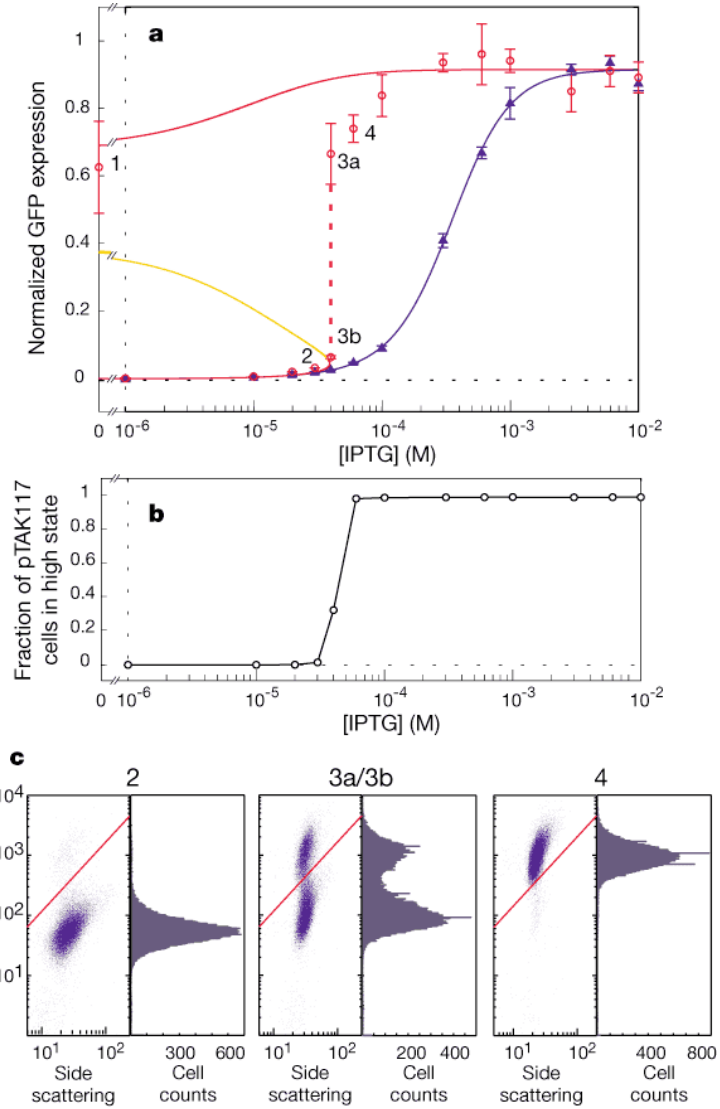


Synthetic Genetic Switch

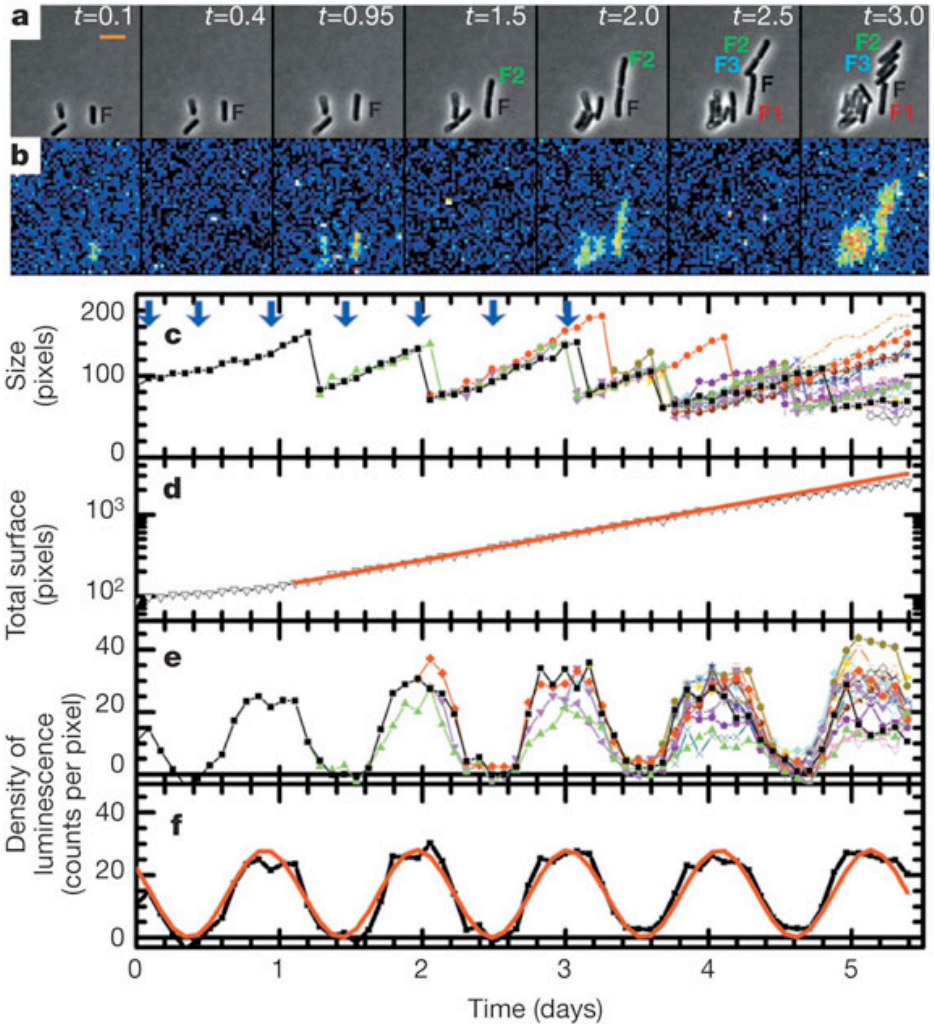
Collins et al. - see course website





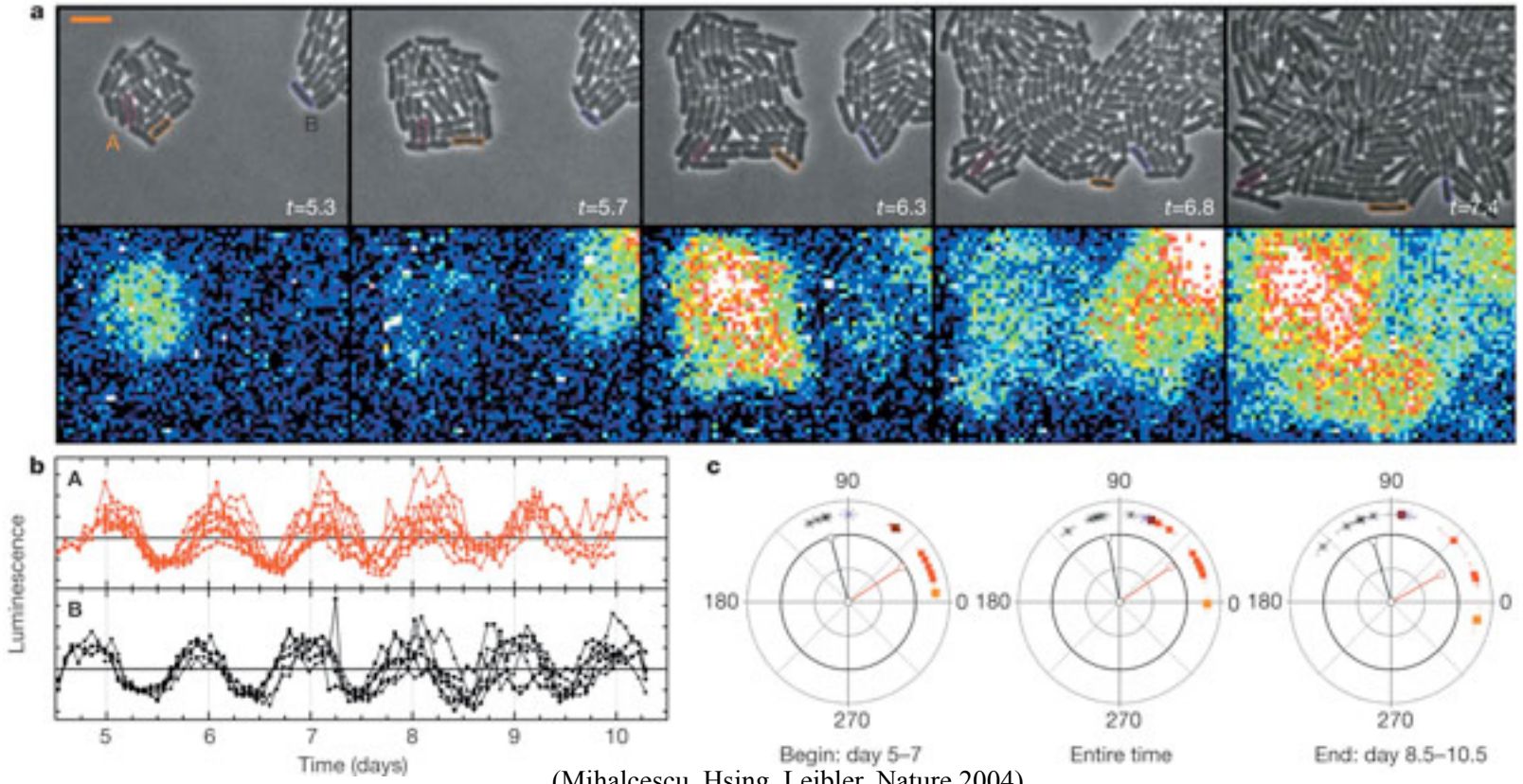
Gene Expression in Cyanobacteria

(Mihalcescu, Hsing, Leibler, Nature 2004)



a, Snapshots of phase-contrast image showing cell F and its progeny and b, related bioluminescence image at different times t (given in days, a 24 h period of time) from the beginning of the measurement. Pixels in the bioluminescence images were binned 3 times 3 (pseudo-colour, where red is high signal intensity and blue is low signal intensity). Scale bar, 5 microm. c, The size of the cell F and all its progeny as a function of time measured from the phase-contrast images (non-binned pixels). The arrows point to the time where the snapshots in (a) and (b) were taken. d, The total number of pixels occupied by F and its all progeny versus time (black line) plotted in a logarithmic scale. The red line is the corresponding exponential growth fit: total size (t) = initial size times 2t/tau with tau = 23.04 plusminus 0.17 h. e, Density of bioluminescence for the same cell and all its progeny versus time. f, The average density of bioluminescence versus time (black line) and its fit (red line) with: left fenced(t)right fence = B + A cos(2pit/T0 + phi0). The resulting period is T0 = 25.4 plusminus 0.12 h, the initial phase phi0 = 52 plusminus 2.8°, the amplitude A = 12.9 plusminus 0.3 counts per pixel and the offset B = 14.8 plusminus 0.3 counts per pixel.

Gene Expression in Cyanobacteria

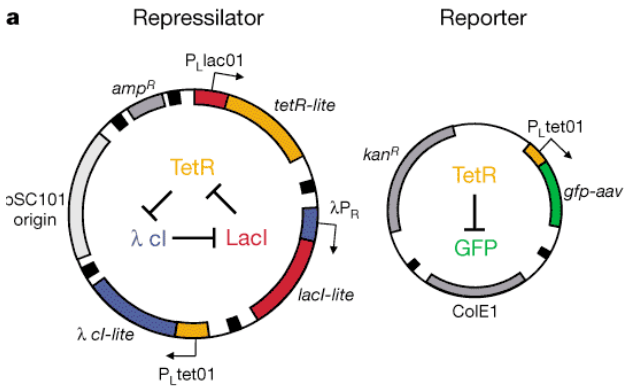


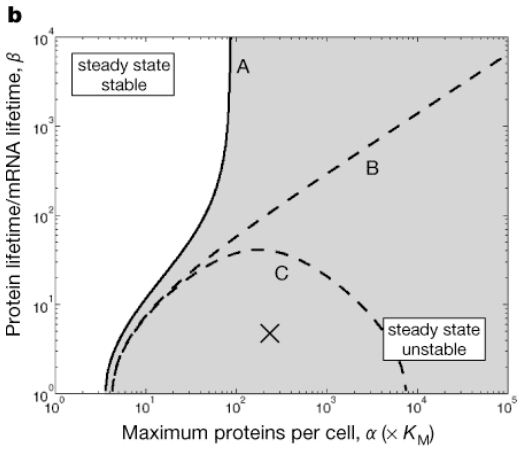
(Mihalcescu, Hsing, Leibler, Nature 2004)

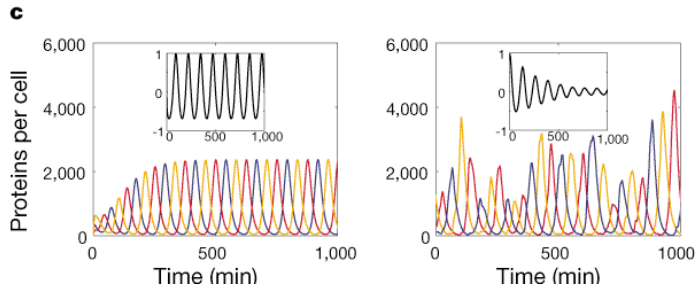
a, Upper part shows the phase-contrast snapshots of colonies A and B; lower part shows the related bioluminescence images. Scale bar, 5 microm. b, Normalized density of bioluminescence of individual cyanobacterial cells. Each colour corresponds to the progeny from one of the initial cells: red line, colony A; black line, colony B. c, Phase of individual oscillators as a function of their original colony and their evolution in time: red square, colony A; asterisk, colony B. An example of the exact location for three of the cells tracked and their phase evolution is shown, marked by the corresponding coloured lines: magenta, orange and purple. The change of the phase in time was quantified by a fit over a different period of time: the first 2 days (days 5–7), the entire time (days 5–10.5) and the last 2 days of the measurement (days 8.5–10.5). The fit function is left fenced(t)right fence = B + A cos(2pit/T0 + phi), with T0 = 24.78 h. The line segments in each graph, with corresponding colours, represent the resulting vector Pres = sumPi, where Pi is the unit vector whose orientation is the measured angle of the same colony cell i.

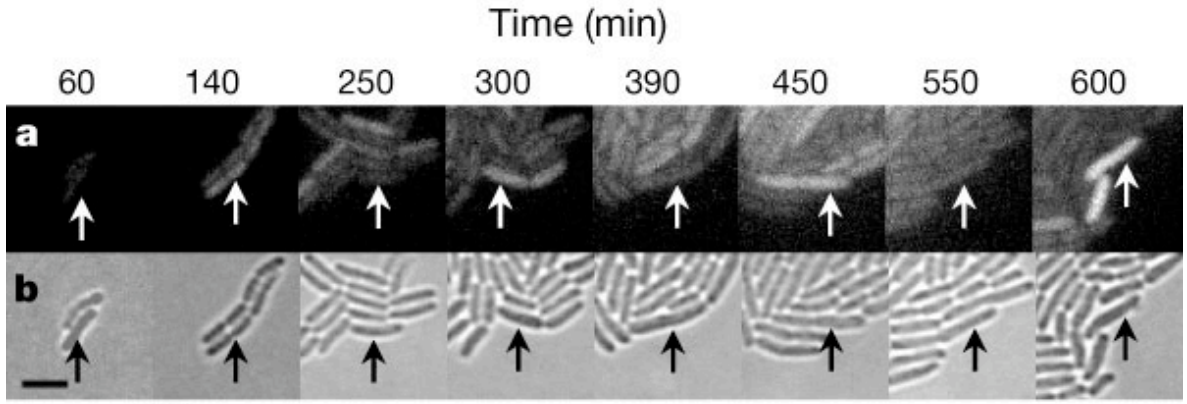
Synthetic Transcriptional Oscillator

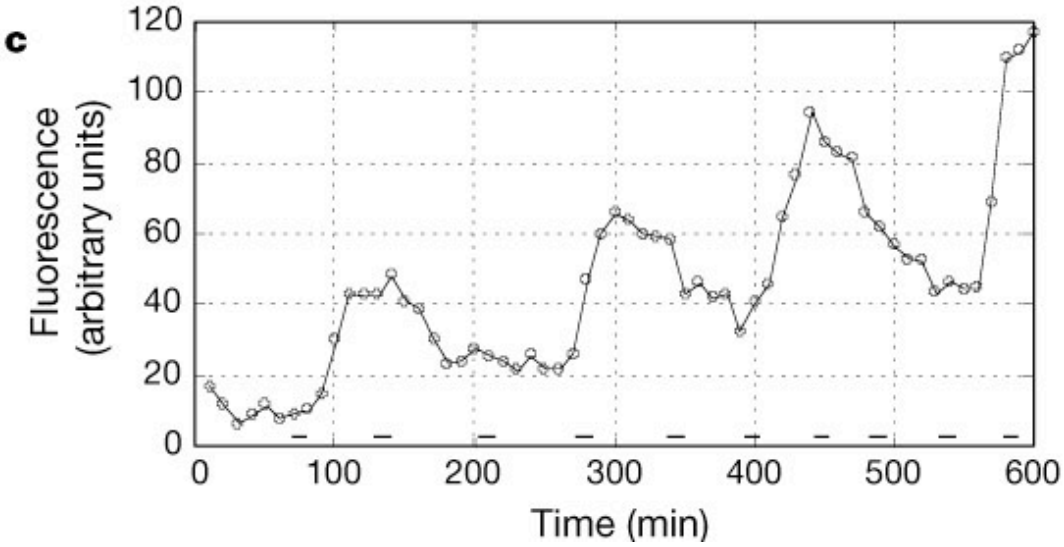
(Elowitz, Leibler, Nature 2002)











$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$

Coupling of Genes in Networks

$F_{bend} = \frac{\pi \xi_p k_B T}{R}$