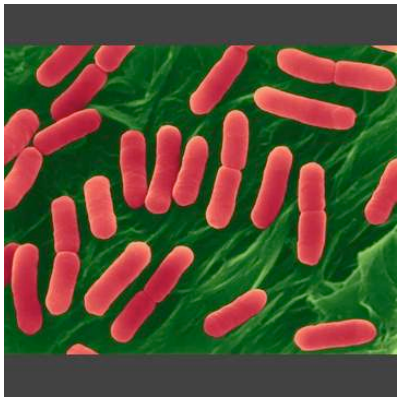
BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

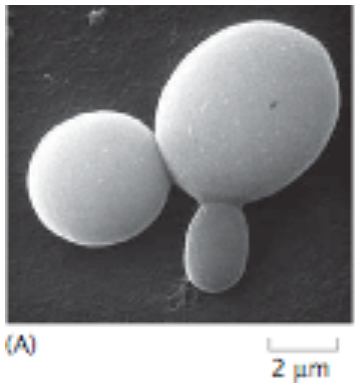
Rob Phillips
Applied Physics and Bioengineering
California Institute of Technology

How cells decide as seen through three hall of fame ``model'' organisms

E. coli



yeast

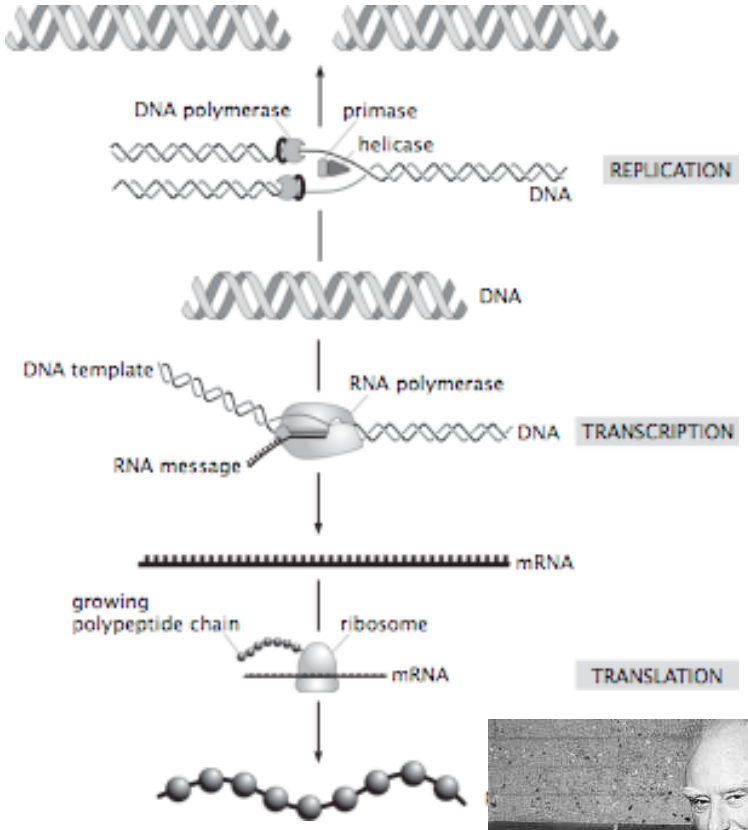


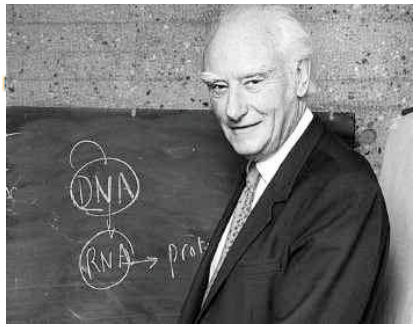
Fruit fly

- *Various organisms are accorded hall of fame status as ``model'' organisms either because they are specialists at some particular process of interest or they are experimentally convenient (grow fast, easily accessible).*
- *Each of these organisms offers something extremely important on the question of how cells decide.*

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.***

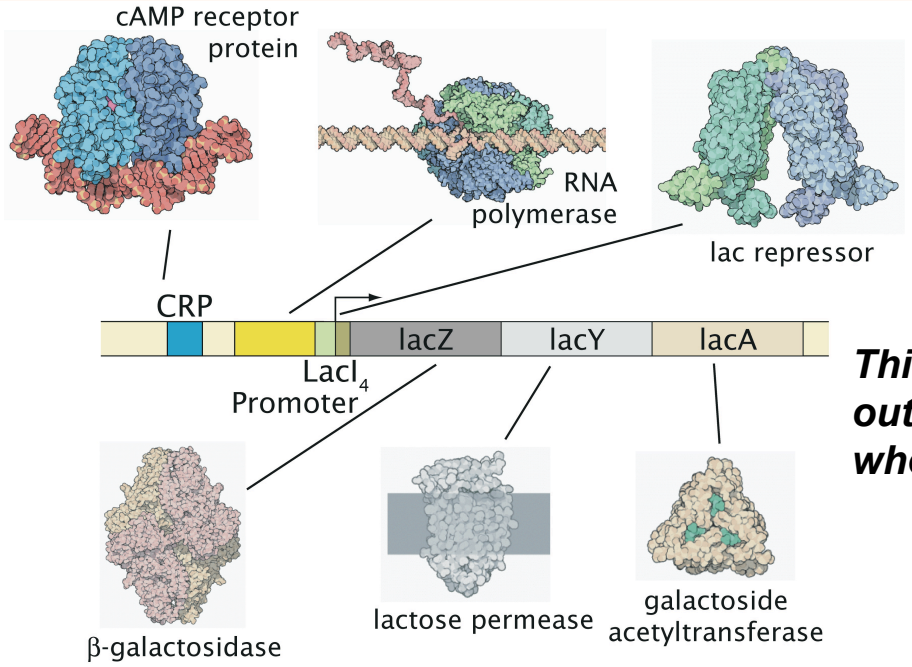




Now we have the background to tackle the question we started with: how do cells make decisions?

THE BIG MESSAGE

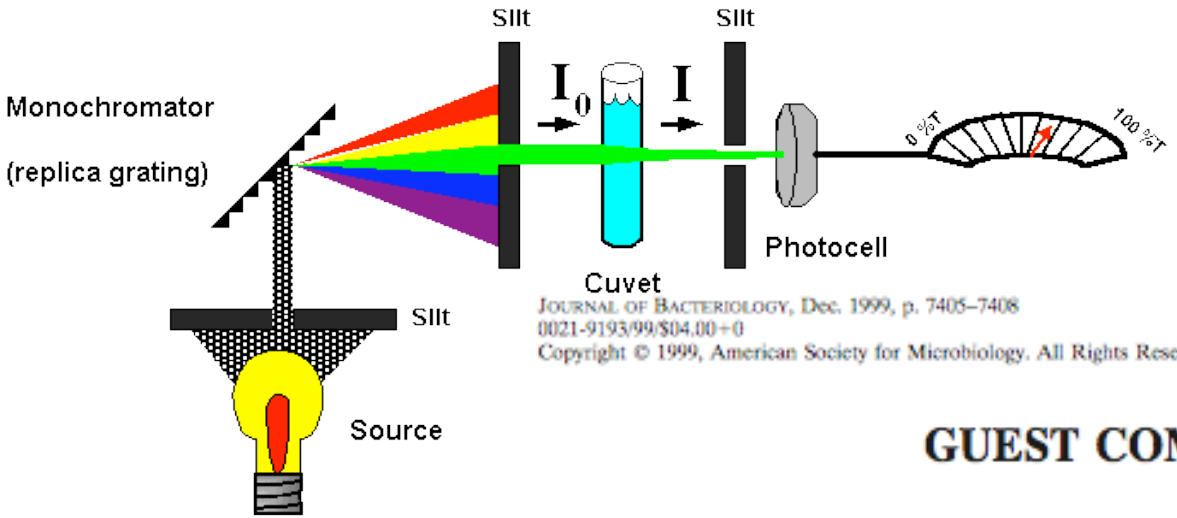
The Puzzle: All the cells in a given organism (almost) carry the same genetic information. And yet, depending upon where they are within the organism, they turn out quite differently.



This lecture: how we found out, some beautiful examples, where we stand now.

The Insight: The genome (i.e. genetic material) is under exquisite control. Genes are turned on and off in response to environmental cues.

Measuring the Diet of a Bacterium



JOURNAL OF BACTERIOLOGY, Dec. 1999, p. 7405–7408
0021-9193/99/$04.00+0

Vol. 181, No. 24

GUEST COMMENTARY

Bacterial Growth: Constant Obsession with *dN/dt*

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One of life's inevitable disappointments—one felt often by scientists and artists, but not only by them—comes from expecting others to share the particularities of one's own sense of awe and wonder. This truth came home to me recently when I picked up Michael Guillen's fine book *Five Equations That Changed the World* (4) and discovered that my equation—the one that shaped my scientific career—was not considered one of the five.

tantly, its invitation to explore—affected me profoundly. The first-order rate constant k in the growth equation seemed to me the ideal tool by which to assess the state of a culture of cells, i.e., the rate at which they were performing life, as it were. I elected to pursue my Ph.D. studies with Boris Magasanik, studying the molecular basis of diauxic growth. Over the ensuing half-century, close analysis of growth curves was to be a central feature of my work, as I followed my intense

- ***Growth curves have served a central role in dissecting the physiology of cells of all types.***
- ***In particular, we know much about how cells decide based upon watching them grow and seeing what they like to eat.***

DECIDING WHAT TO EAT: GIANT DISCOVERIES OFTEN ARISE FROM SEEMINGLY ARCANE TOPICS

- ***Fascinating twist of history of science: human curiosity leads to investigation of seemingly arcane topics (spectral lines of atoms, specific heats of solids, peculiarities in the orbits of Uranus or Mercury, etc.) from which emerge hugely important insights.***
- ***An example: nutrition of single cells like yeast and bacteria.***
- ***Yeast cells express preferences about which sugar to use.***
- ***Interestingly, the proteins used to digest the less preferable sugars are only synthesized when those sugars are present and the more preferable sugars are absent.***

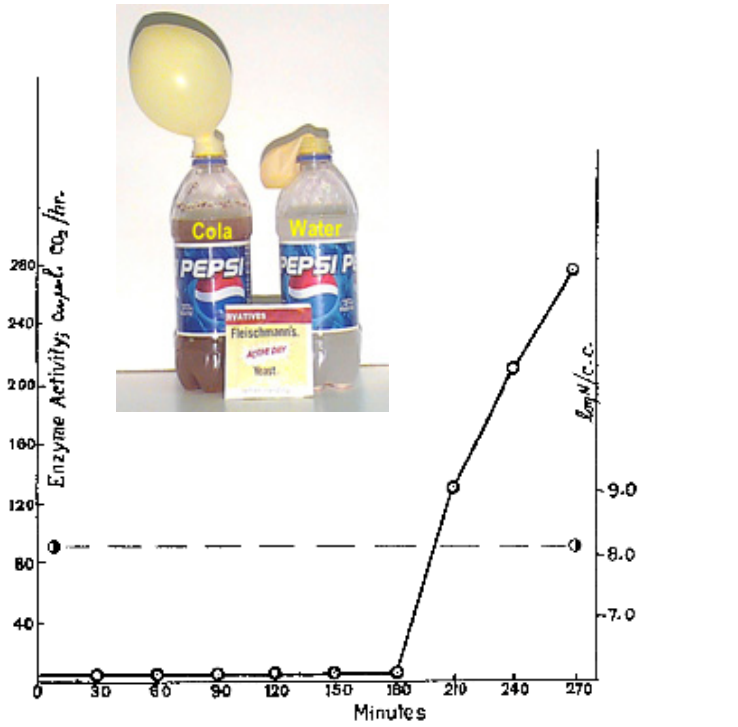
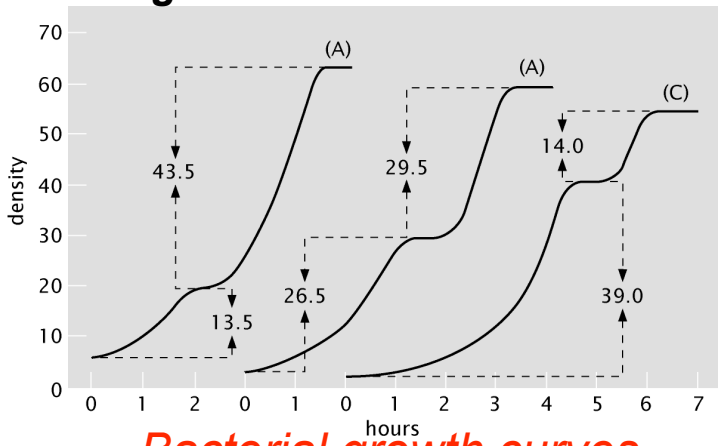


Figure 6. Adaptation to D-galactose without cell multiplication by a strain of *S. cerevisiae*: an experiment of Spiegelman and his colleagues carried out in 1943. Cells grown on D-glucose were washed in 67 mM KH_2PO_4, then resuspended in phosphate under nitrogen and D-galactose added. Carbon dioxide produced anaerobically was measured manometrically for 5 h. ○, CO_2 production ($\mu l^3/h$); ◑, log of number of cells/cm^3 ([254] Figure 2). Reproduced by permission

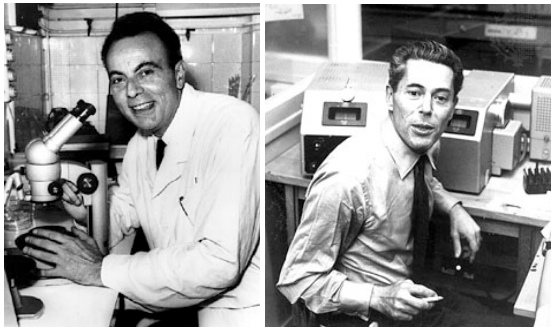
(Spiegelman et al., PNAS, 1944)

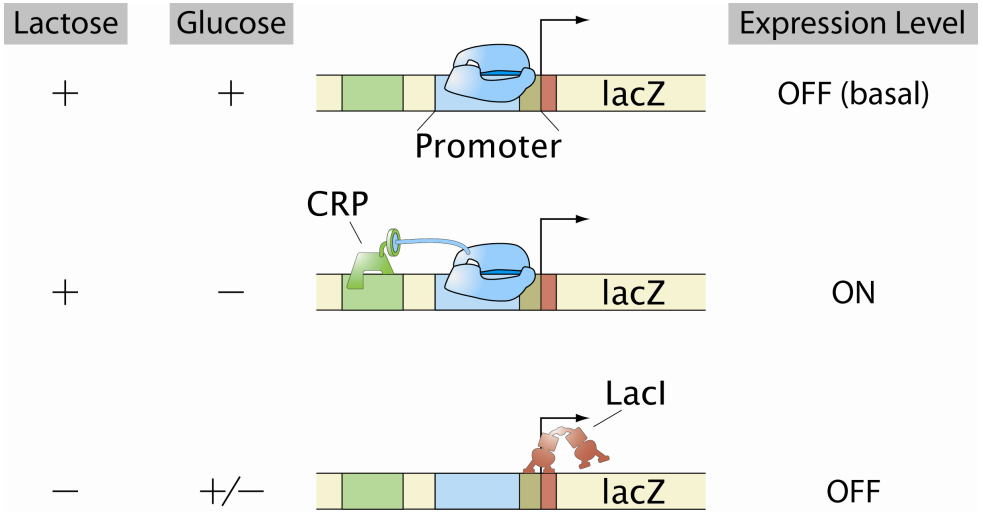
A MODEL SYSTEM FOR MATHEMATICALLY DIALING IN TRANSCRIPTION

- ***The way all of this works was first figured out in the context of a very specific question in bacteria. How do cells implement the decision that they prefer some sugar sources (i.e. glucose) over others (i.e. lactose)?***
- ***What emerged was a picture in which genomic DNA is controlled by an army of molecular bouncers (transcription factors) that activate or repress expression of their genes of interest.***



Bacterial growth curves





REPRESSORS: THE CARTOON

- ***Repressor molecules inhibit action of RNA polymerase.***
- ***Repressors can be under the control of other molecules (i.e. inducers) that dictate when repressor is bound and not.***

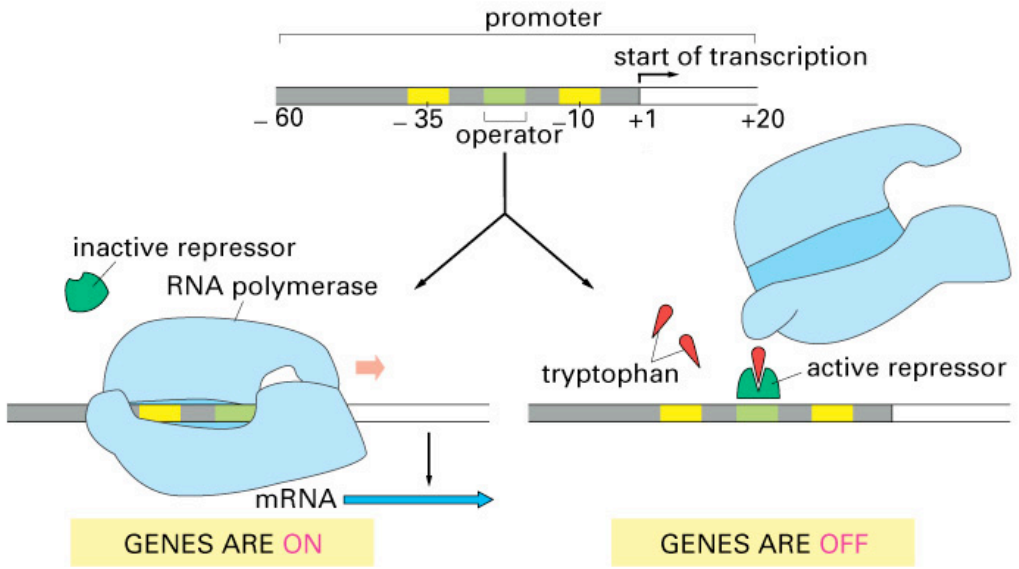
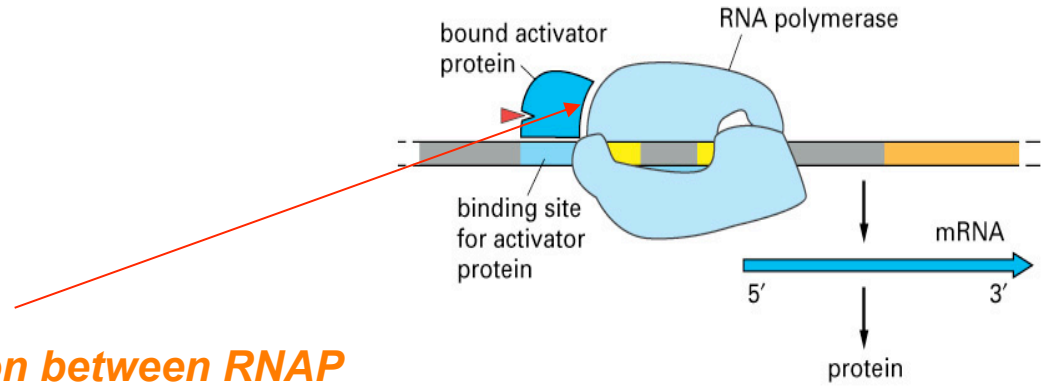


Figure 8-7 Essential Cell Biology, 2/e. (© 2004 Garland Science)

ACTIVATORS: THE CARTOON

- ***Activator molecules enhance the action of RNA polymerase.***
- ***Activators can be under the control of other molecules (i.e. inducers) that dictate when activator is bound and not.***
- ***Activators "RECRUIT" the polymerase.***

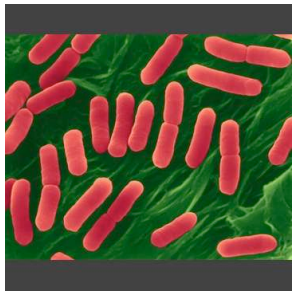


Adhesive interaction between RNAP and activator

Figure 8-8 Essential Cell Biology, 2/e. (© 2004 Garland Science)

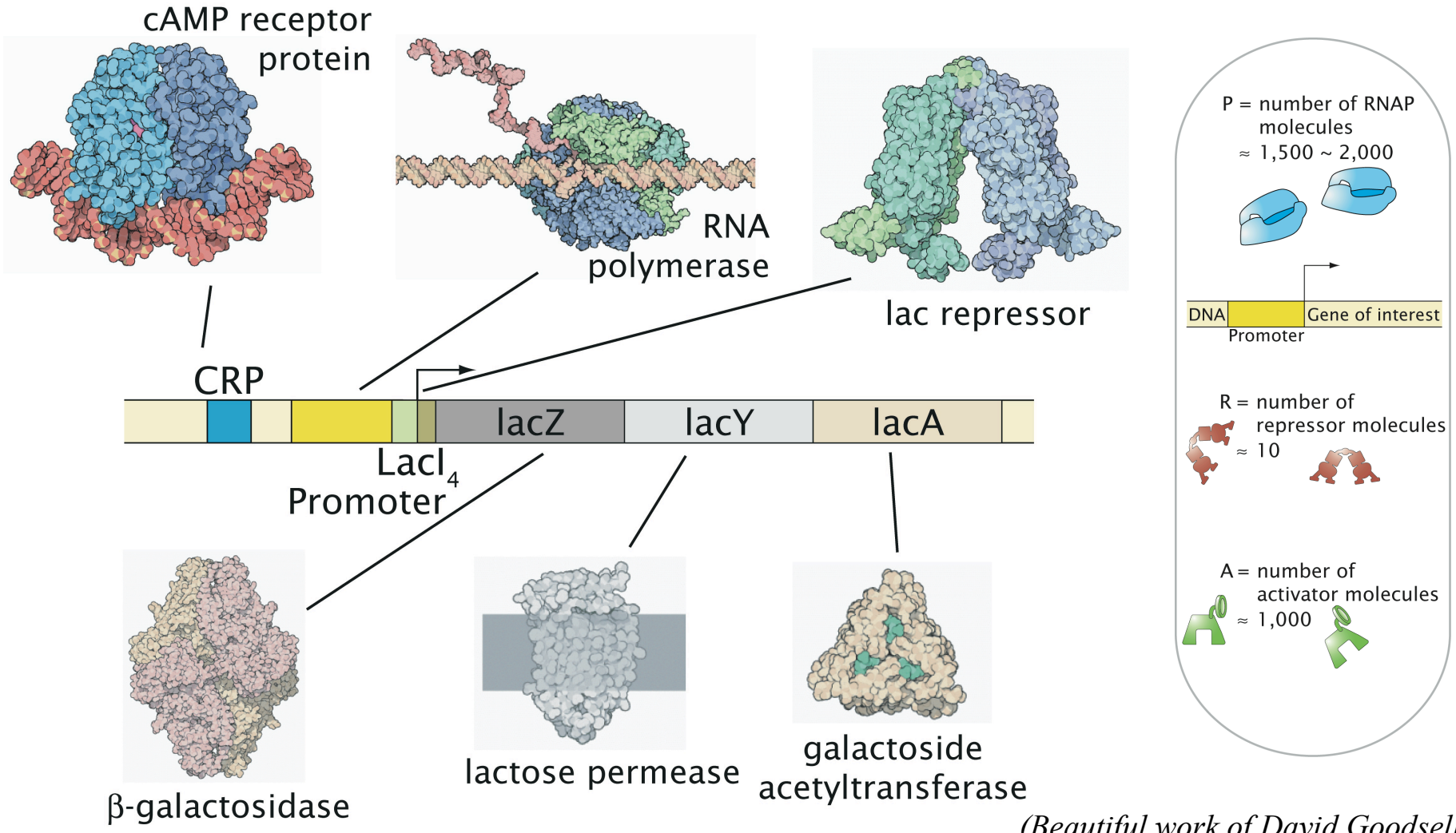
NOT ALL DNA CODES FOR PROTEINS

- ***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.***



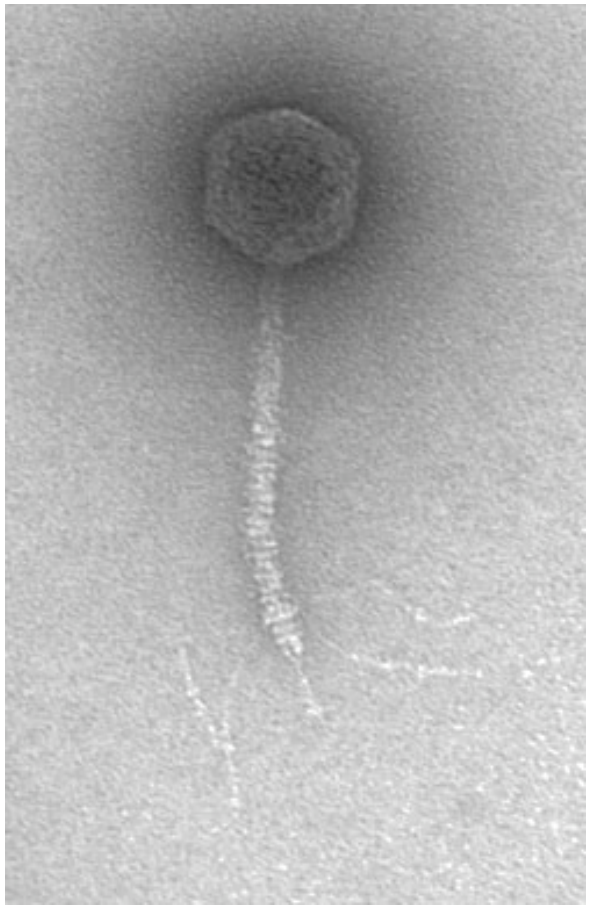
The regulatory landscape

LAC OPERON: THE SINGLE MOLECULE CENSUS

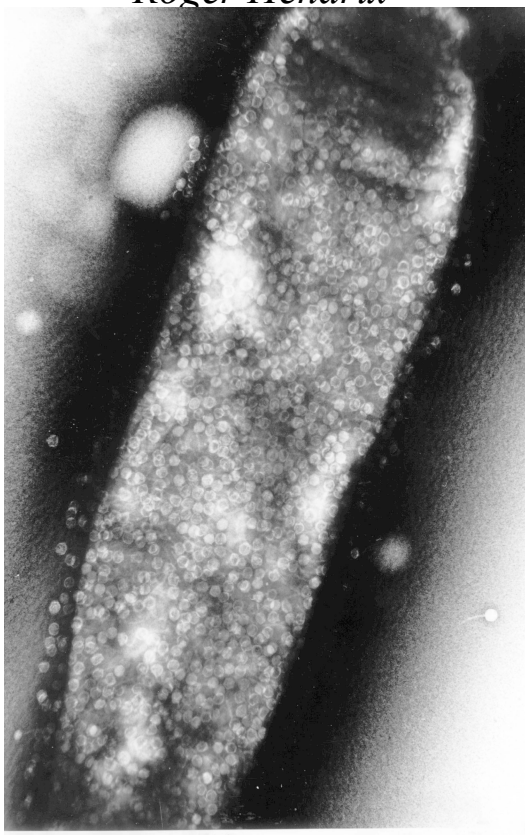


(Beautiful work of David Goodsell)

THE LAMBDA SWITCH: THE OTHER HYDROGEN ATOM OF GENE REGULATION

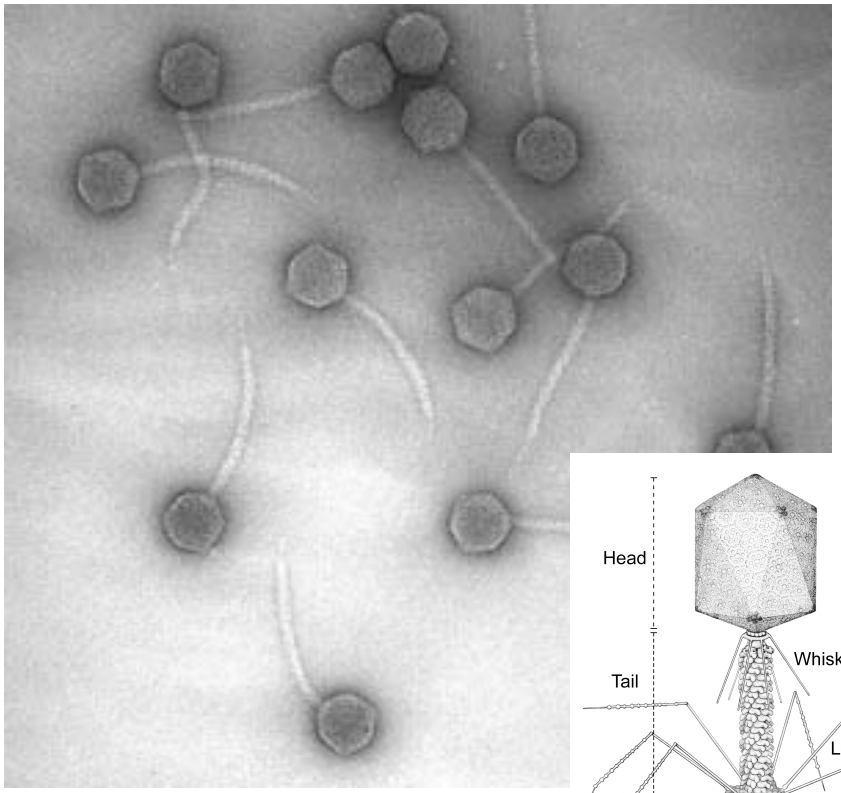


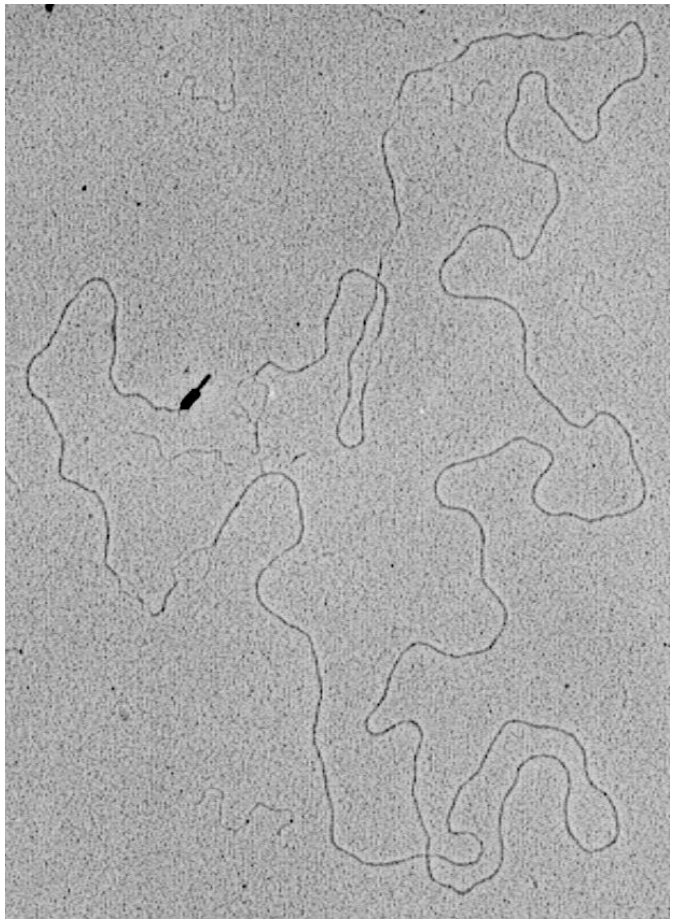
Roger Hendrix



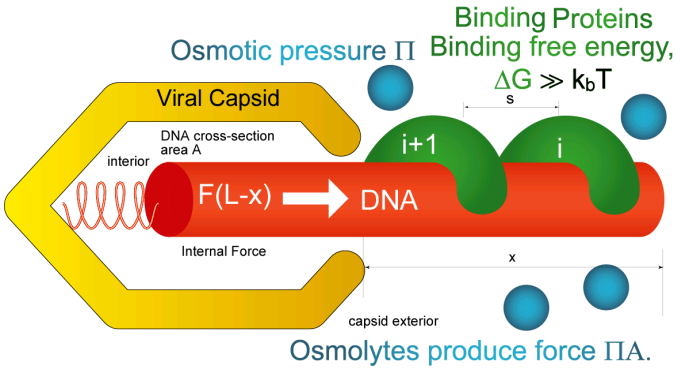
BACTERIOPHAGE AND THEIR GENOMES

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



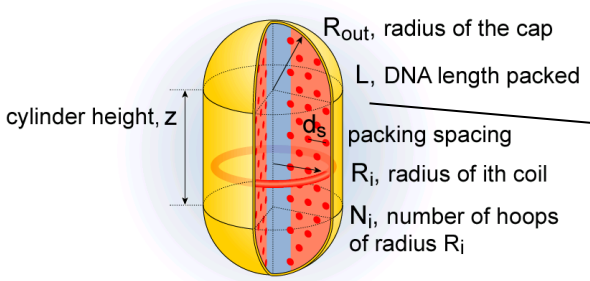


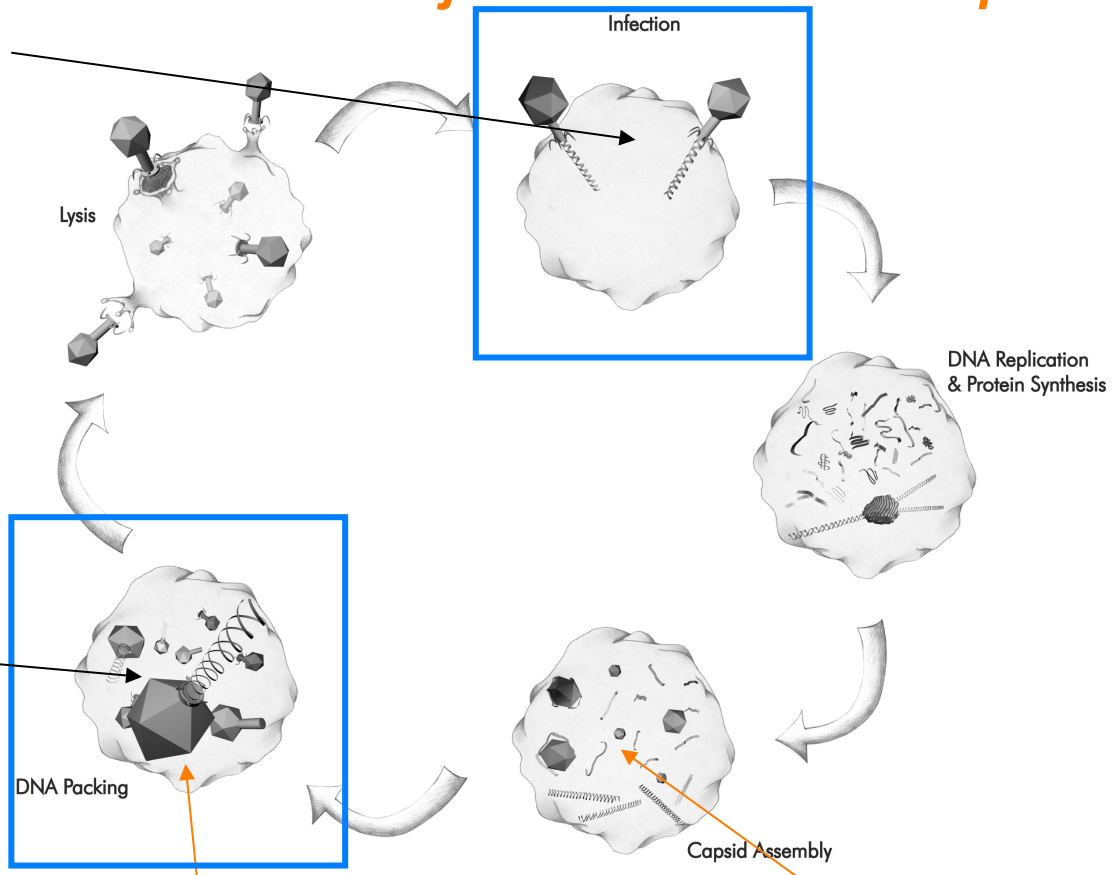
BACTERIOPHAGE LIFE CYCLE 1



Forceful ejection

Rate of ejection: ≈ 100 - 1000bp/sec



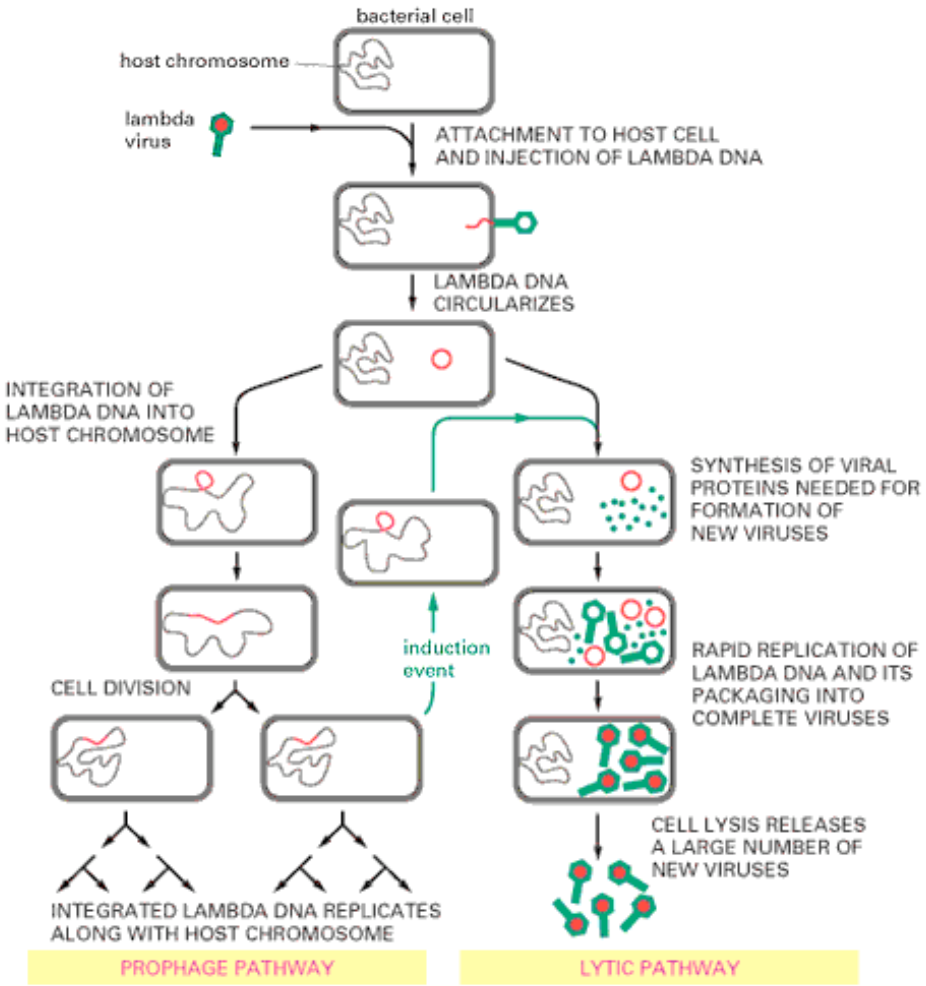


Rate of packing: 100bp/sec

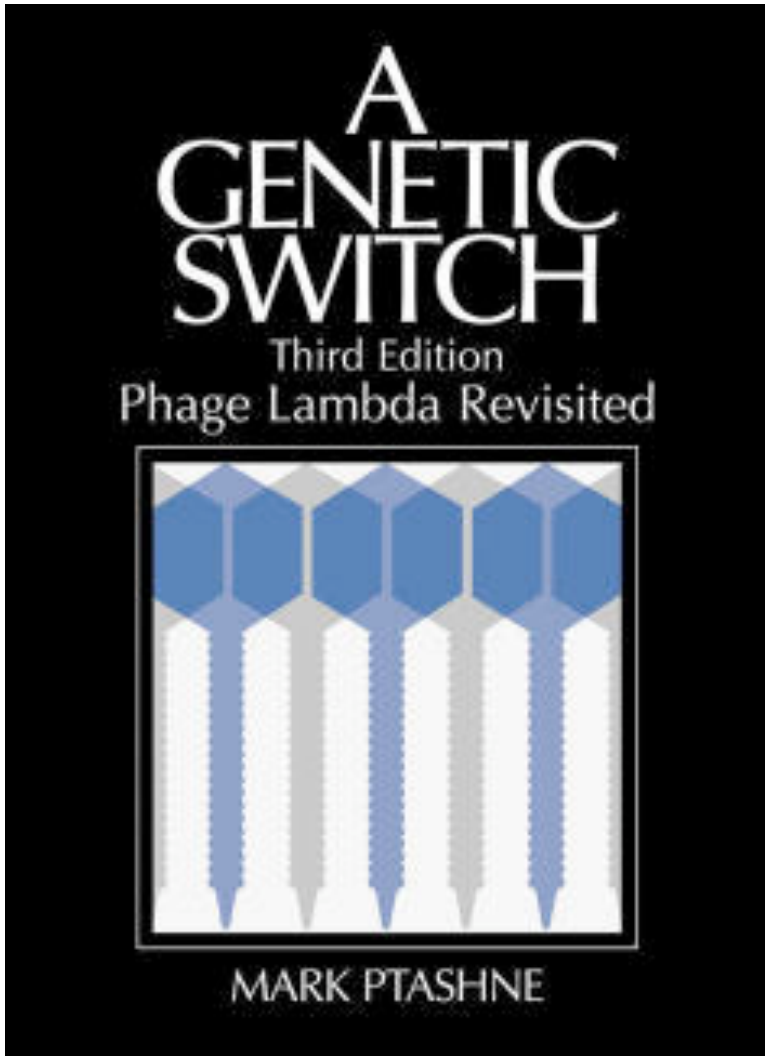
"Some assembly required"

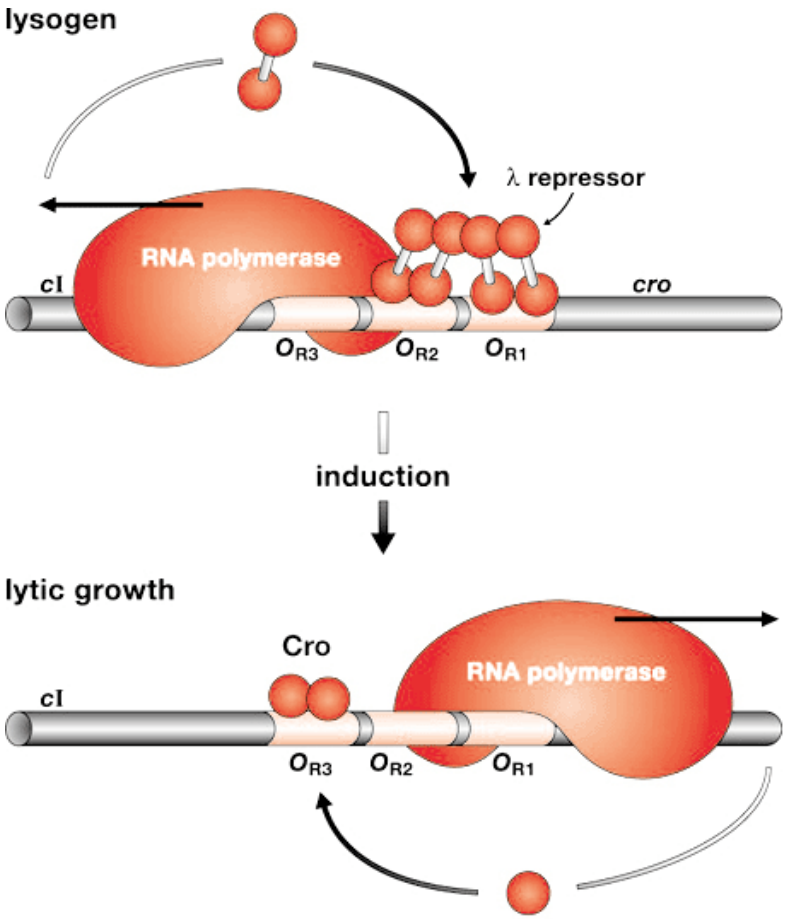
Self-assembly

THE LIFE CYCLE OF BACTERIOPHAGE LAMBDA



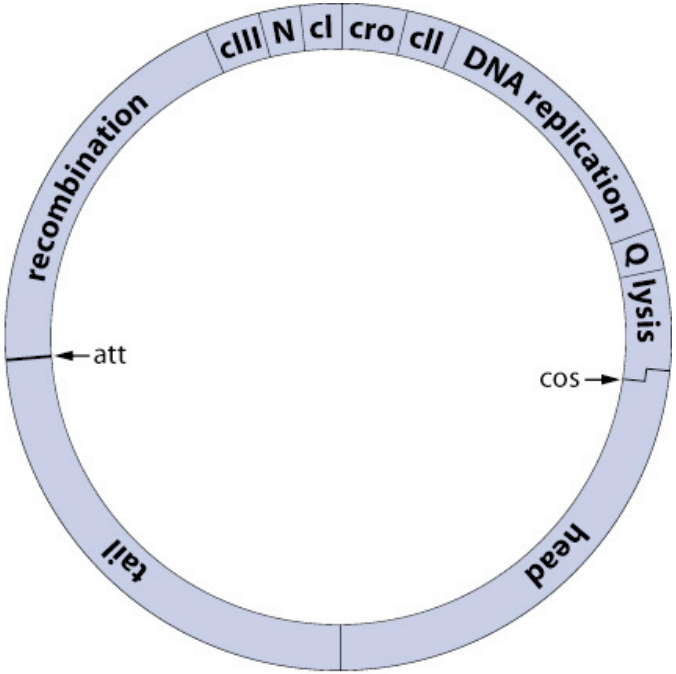
A GENETIC SWITCH





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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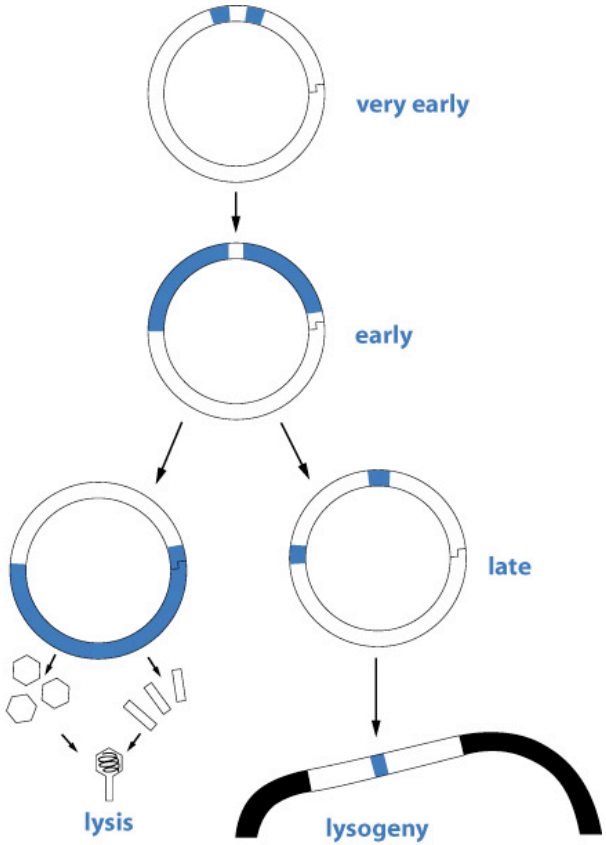
Chapter 3, Figure 1



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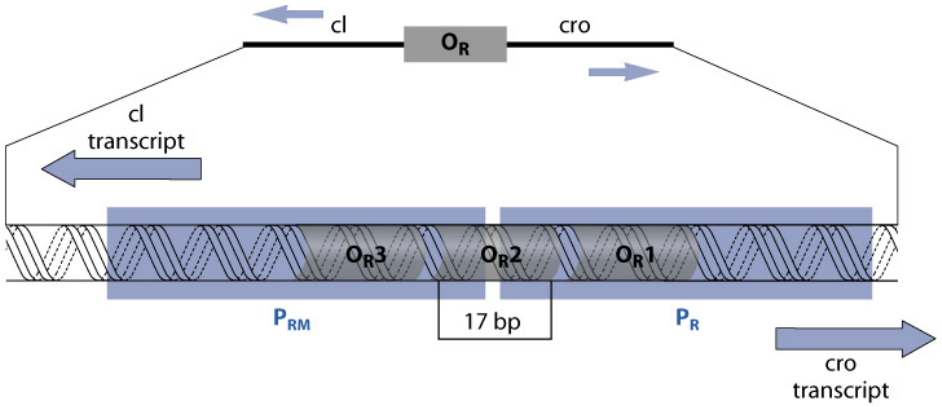
Chapter 3, Figure 3

THE LAMBDA SWITCH: LYSOGENIC STATE



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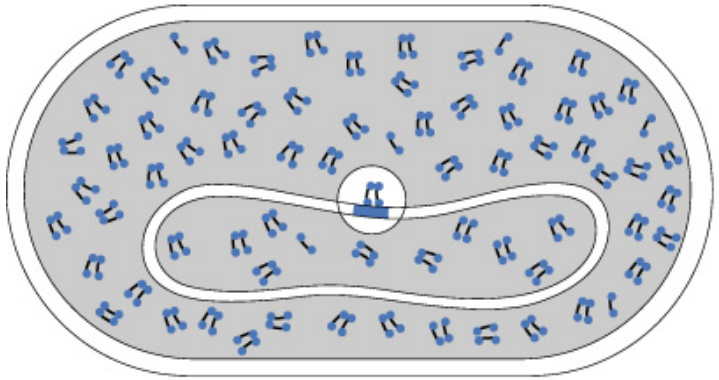
Chapter 1, Figure 4



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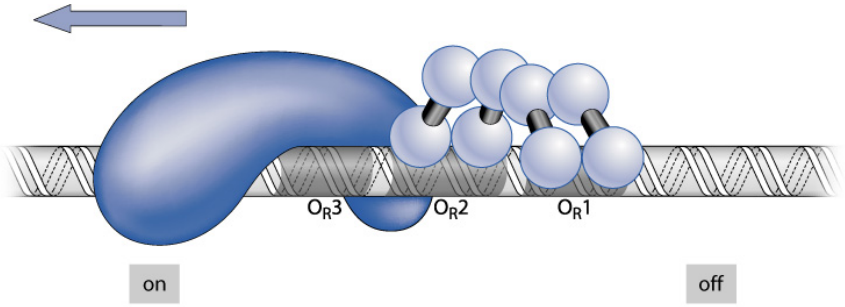

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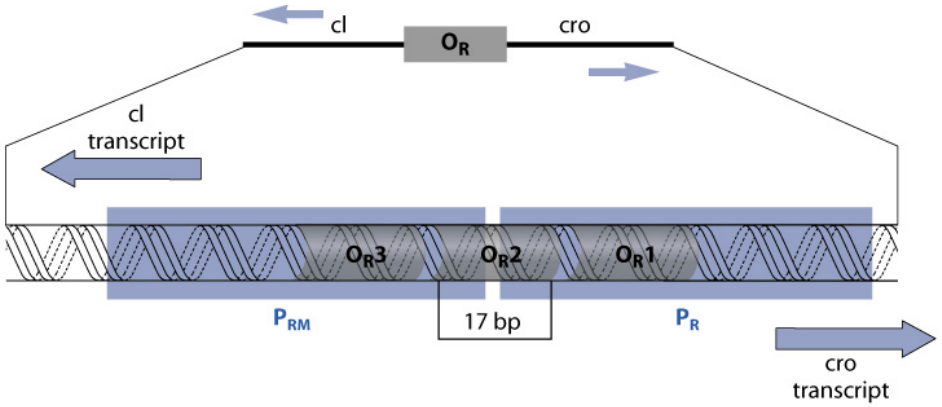
Chapter 1, Figure 19

THE LAMBDA SWITCH: LYTIC STATE



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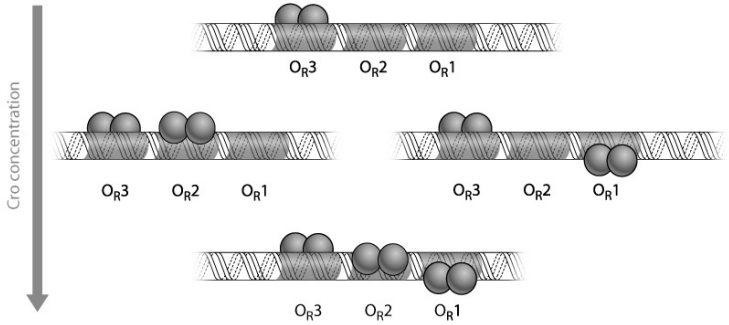
Chapter 1, Figure 4



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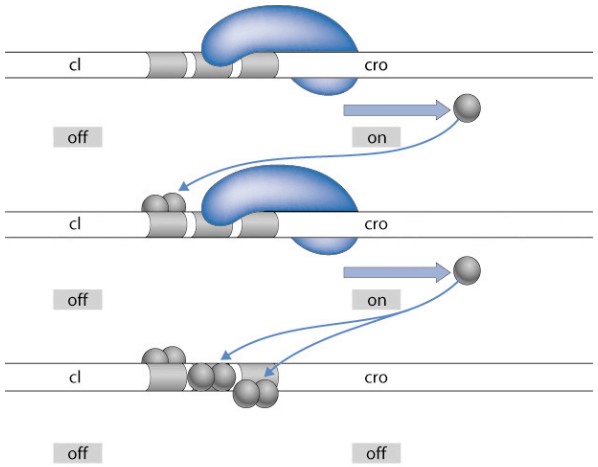
Chapter 1, Figure 23



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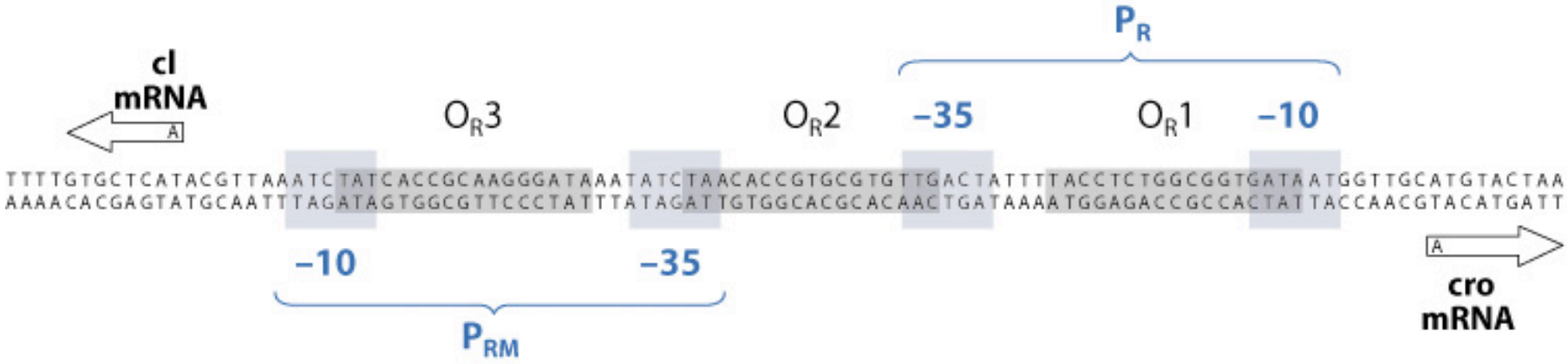
Chapter 1, Figure 24

DNA GEOGRAPHY OF THE SWITCH



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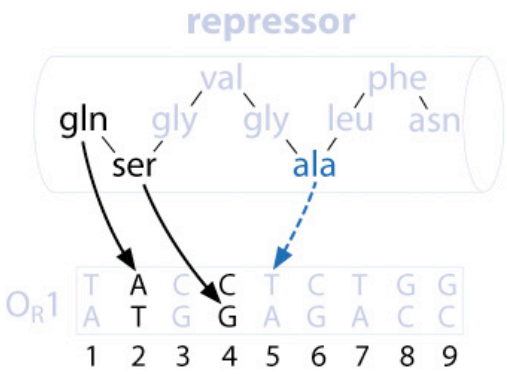
Chapter 2, Figure 16

BINDING OF TRANSCRIPTION FACTORS



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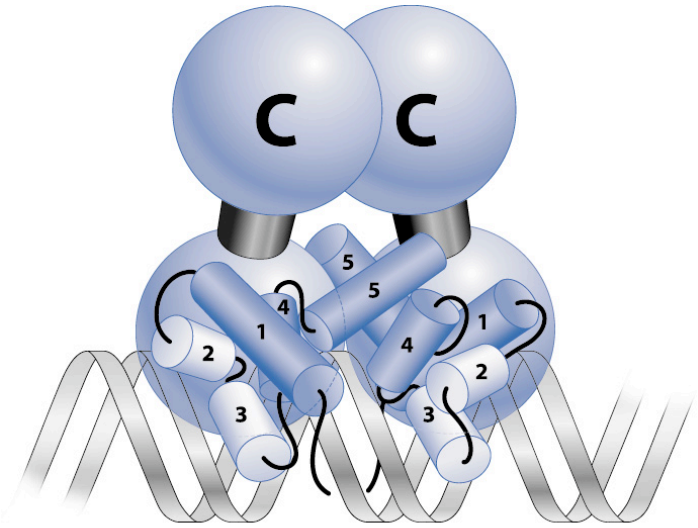
Chapter 2, Figure 11a



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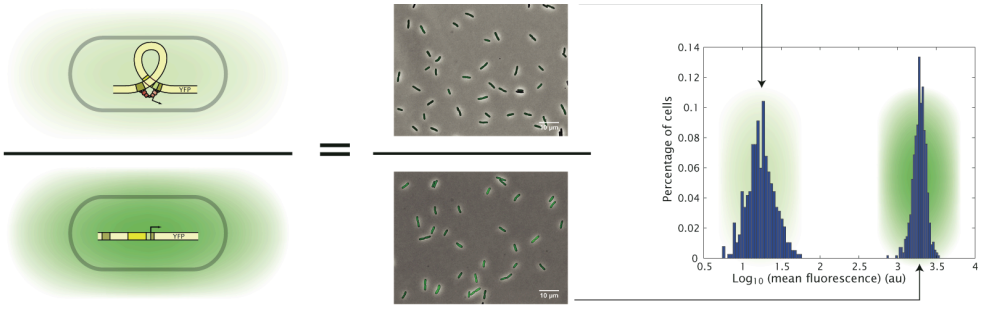
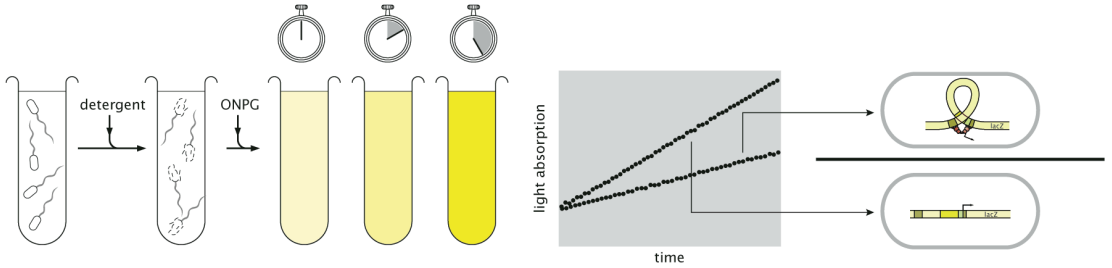
Chapter 2, Figure 6

Measuring Fold Change: The Cell as a Test Tube

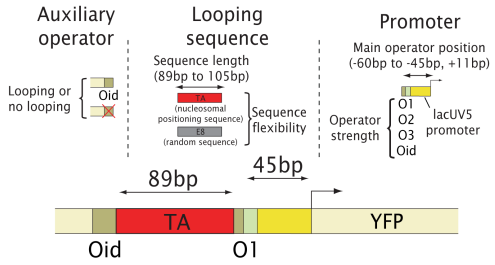
Fold-change(YFP) =



Fold-change(lacZ) =

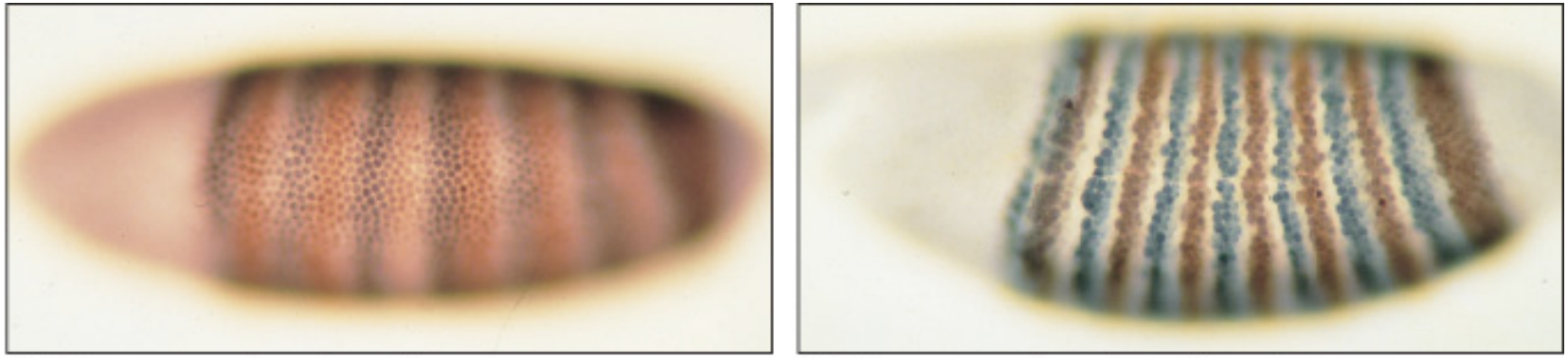


- ***Install the architecture of interest in the cell and then "read out" the state of the DNA and its battery of attendant proteins using gene expression.***



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.***



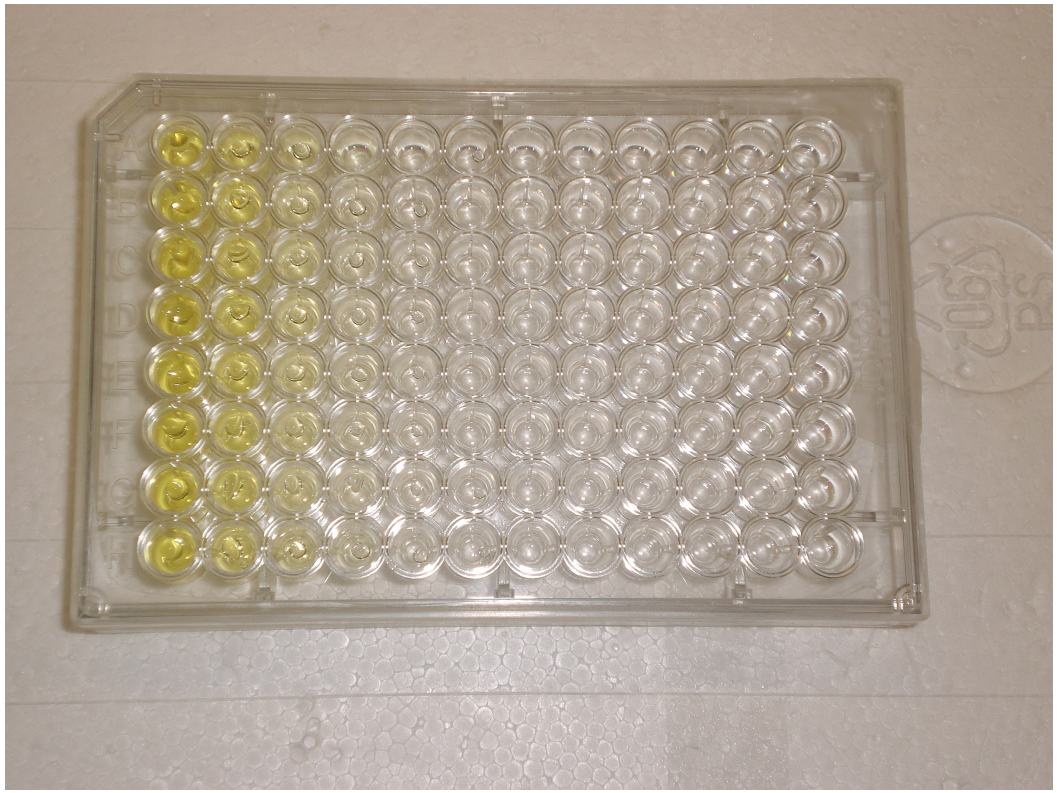
2.7 hours after fertilization

3.5 hours after fertilization

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

ENZYMATIC ASSAY OR IN-SITU HYBRIDIZATION

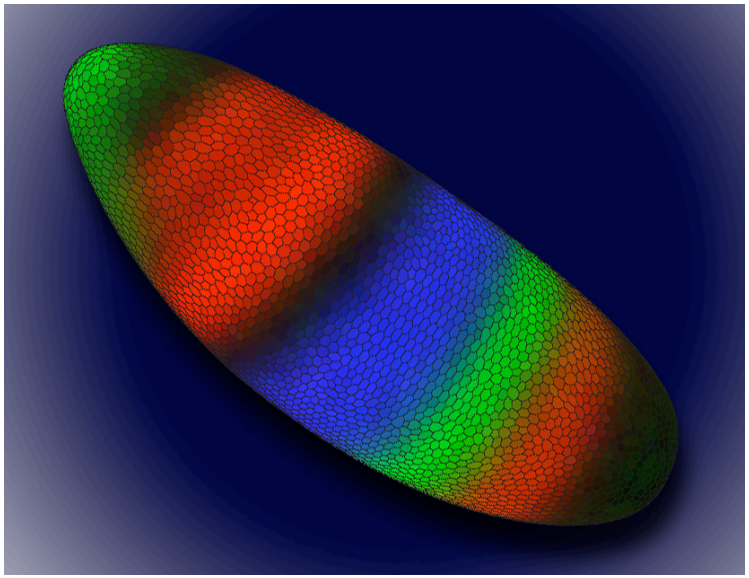
- ***Enzymatic assays – promoter leads to the production of a protein that then does some enzymatic action on the substrate which yields a product that can be visualized.***
- ***In-situ hybridization -***



Ways to Measure Gene Expression

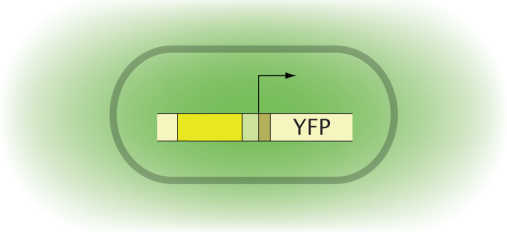
- ***Basic point: looking for "reporters" of the level of expression of gene of interest.***
- ***Can ask the system to report on the level of gene expression at various steps in the processes linking DNA to active protein.***
- ***Promoter occupancy, level of mRNA, level of active protein.***

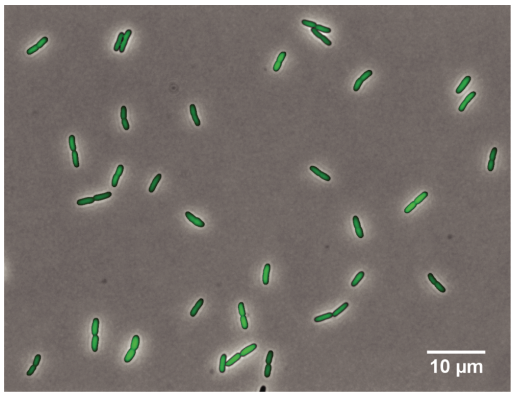
http://www.lbl.gov/Science-Articles/Archive/sabl/2008/Feb/genome-mystery.html



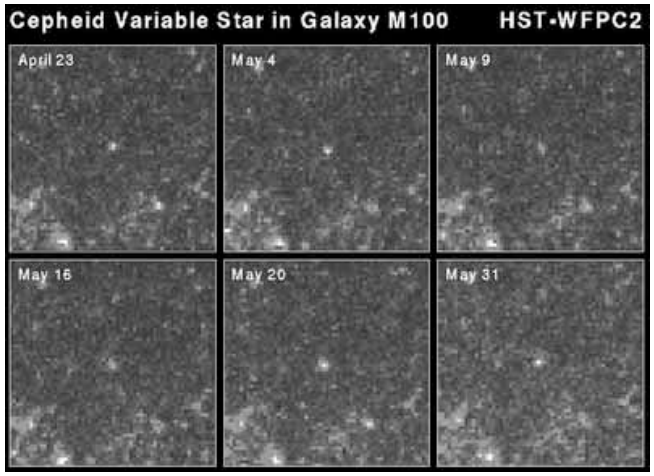
This image shows a Drosophila embryo colored to show the expression patterns of early gene regulators. Each color represents the level of expression of one of three gene regulators, Knirps (green), Kruppel (blue), and Giant (red). Color intensity reflects a higher level of expression. The darker areas 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.

A STANDARD CANDLE FOR GENE EXPRESSION





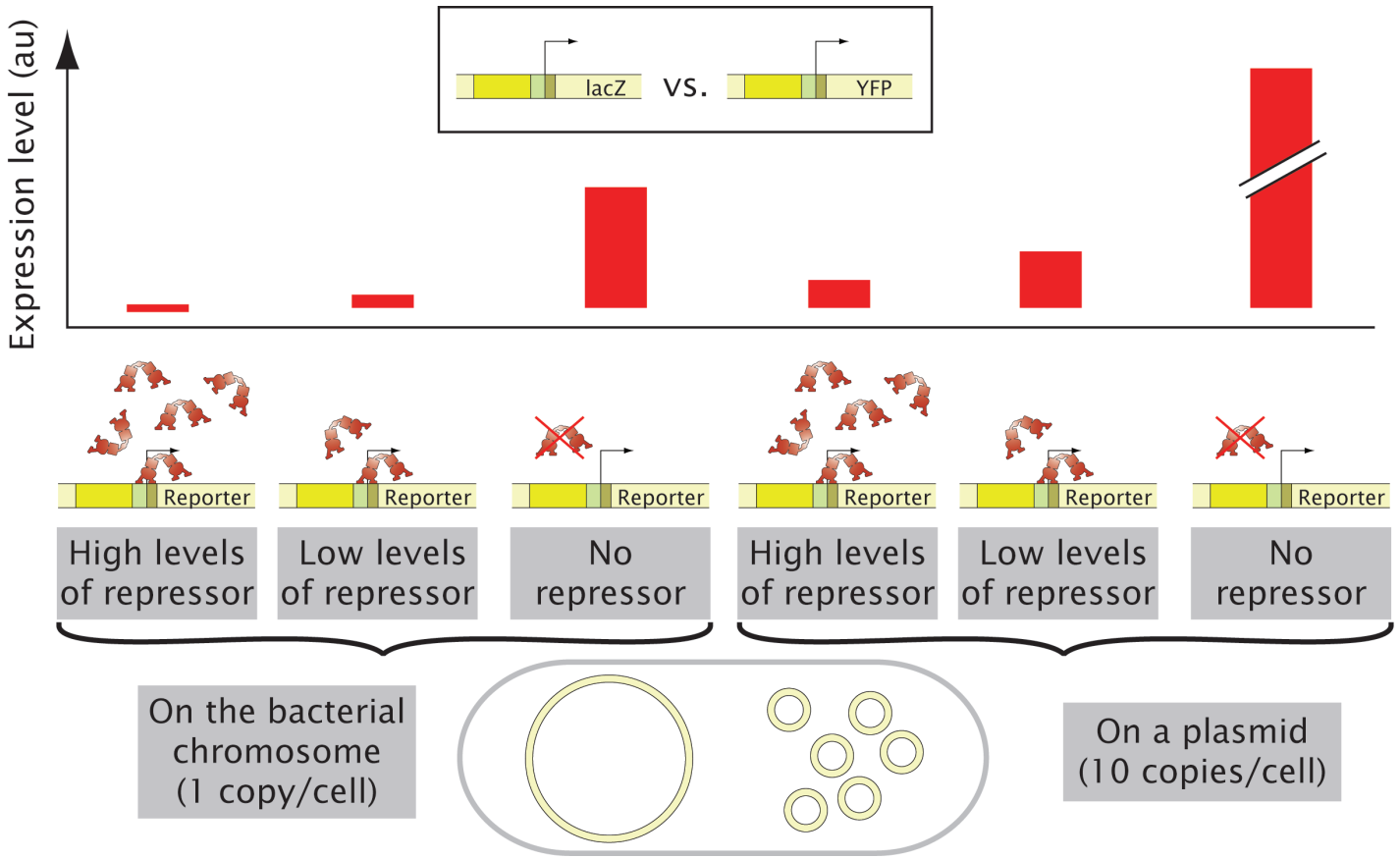
Cepheids variables



Hubble Space Telescope

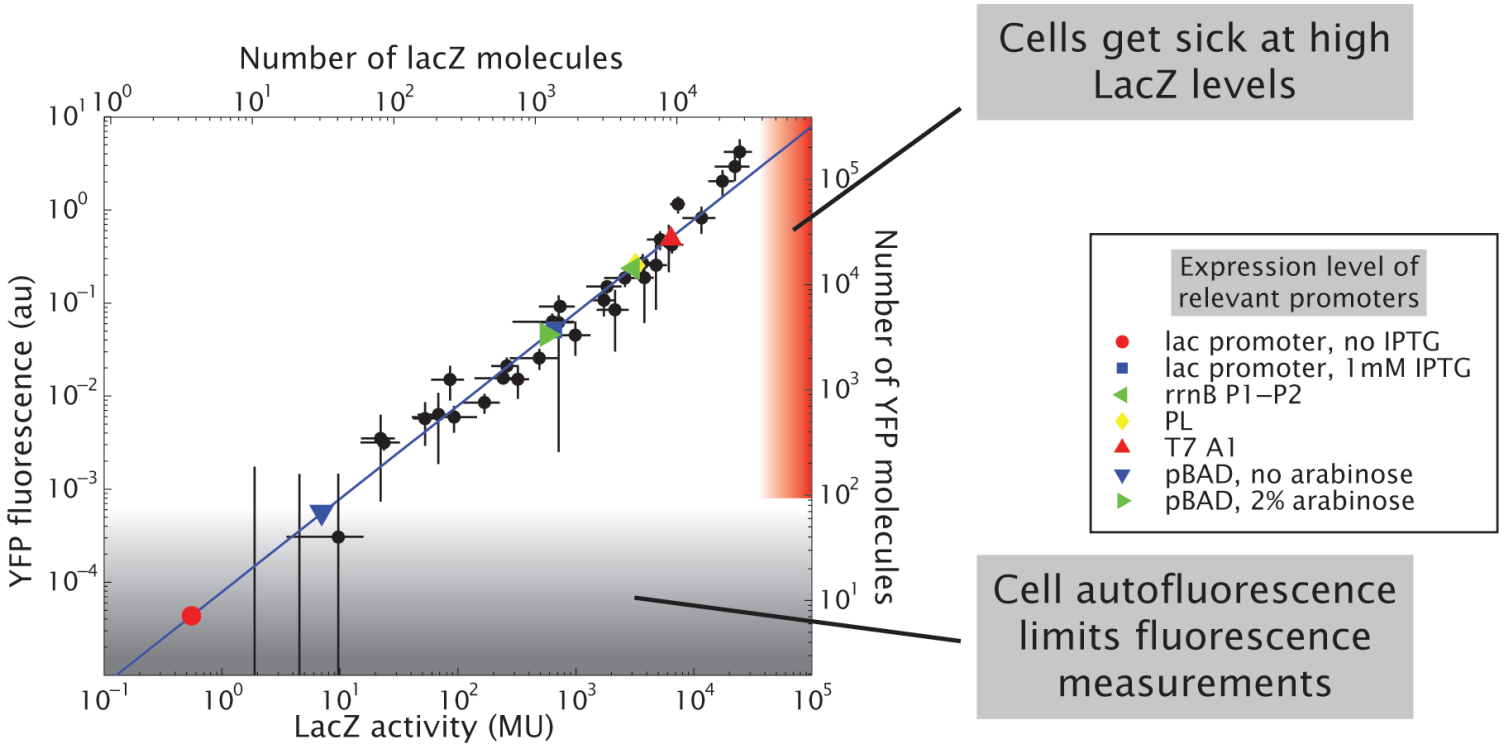
- ***A prerequisite for doing the theory-experiment comparison in the way advocated here is that one has to really know the meaning of the readout of the expression level.***
- ***In particular, is the response linear and do different measurement techniques tell the same story?***

TUNING THE REGULATORY APPARATUS AND COMPARING THE REPORTERS



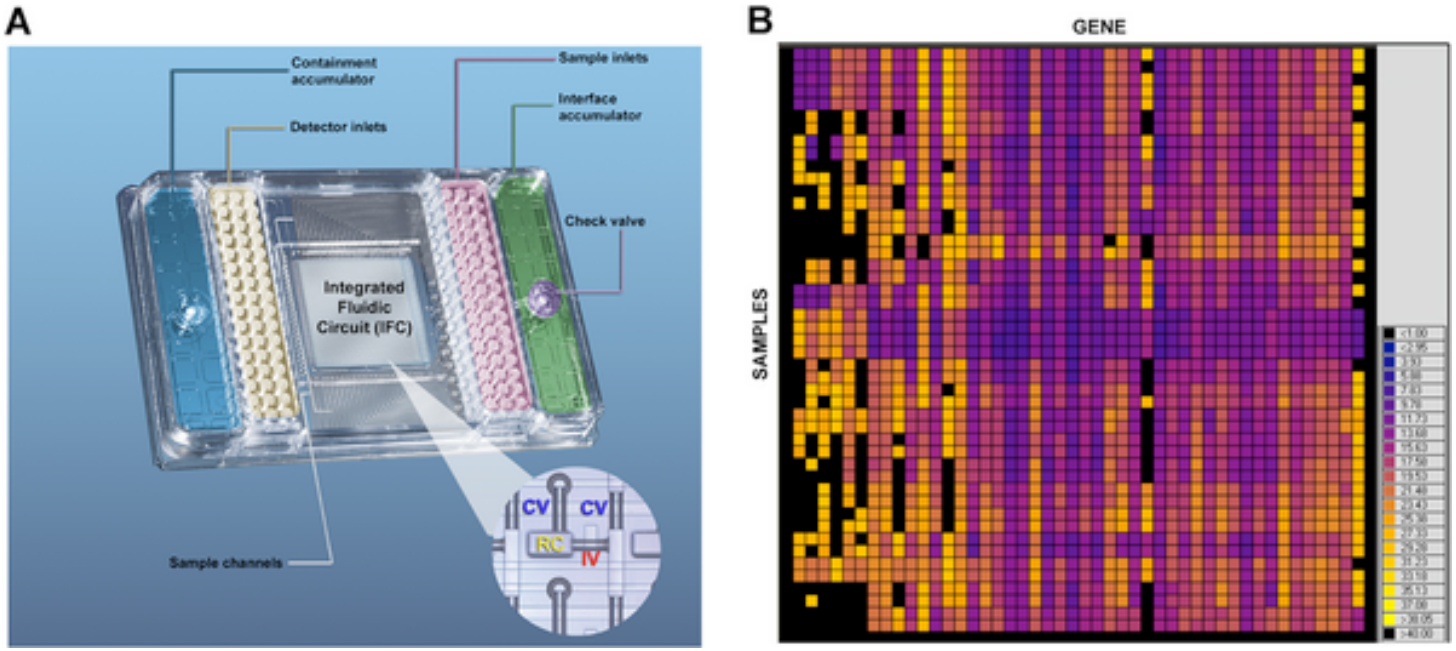
- ***Explore wide range of different regulatory responses (i.e. expression levels) by controlling parameters such as the gene dosage and the number of repressors.***
- ***Note: The census is not so easy.***

Does the measurement depend upon the technique used?



- ***One of our concerns in adopting the quantitative mindset was to see to what extent all measurements on these systems are in agreement.***
- ***But, there are many different readouts of gene expression.***
- ***With this result in hand, we turn to exploring how the expression changes in a case where we tune various parameters for the single-site repression architecture.***
- ***Useful to attempt to make an absolute count of molecules.***

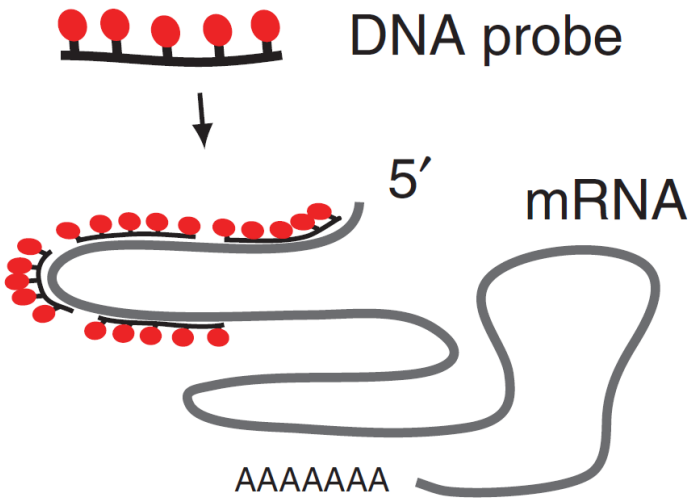
COUNT THE MESSENGER RNA MOLECULES

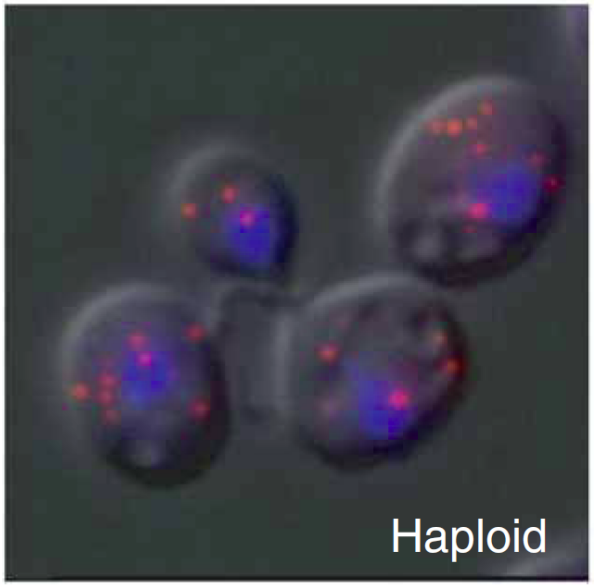


COUNTING MESSENGER RNAS IN CELLS

- *Fixed cells*

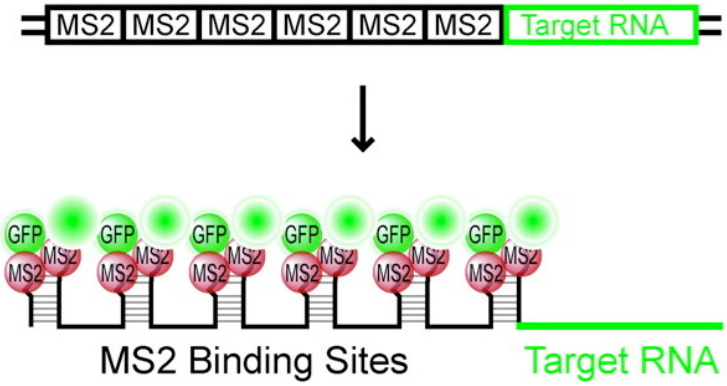
  Zenklusen et al. '08

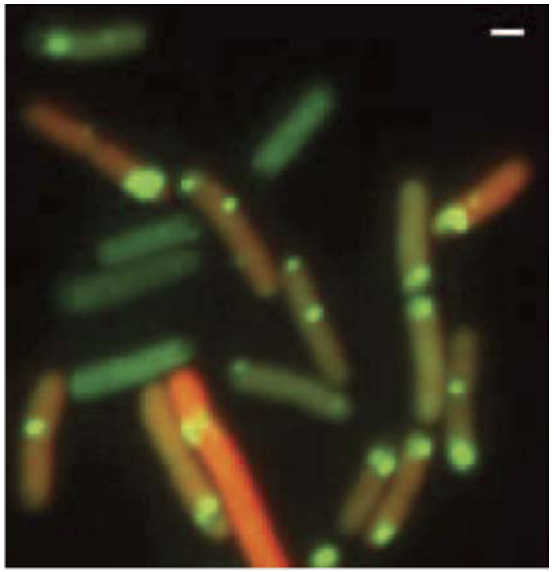




- *Live cells*

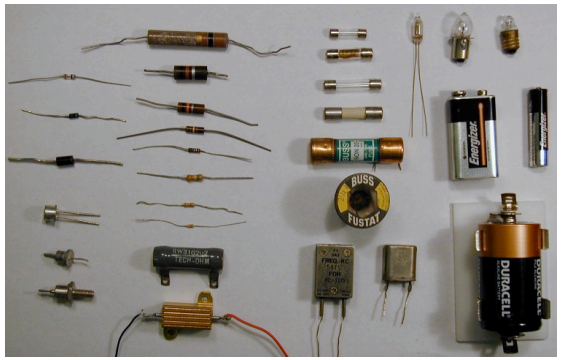
  Golding et al. '05



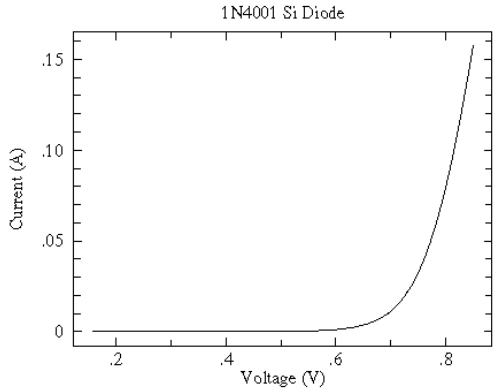


INPUT-OUTPUT CURVES: AN ANALOGY

Electronic Devices



http://www.physics.csbsju.edu/trace/CC.html



Regulatory Devices

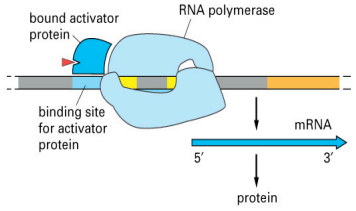
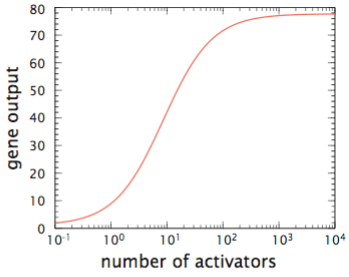
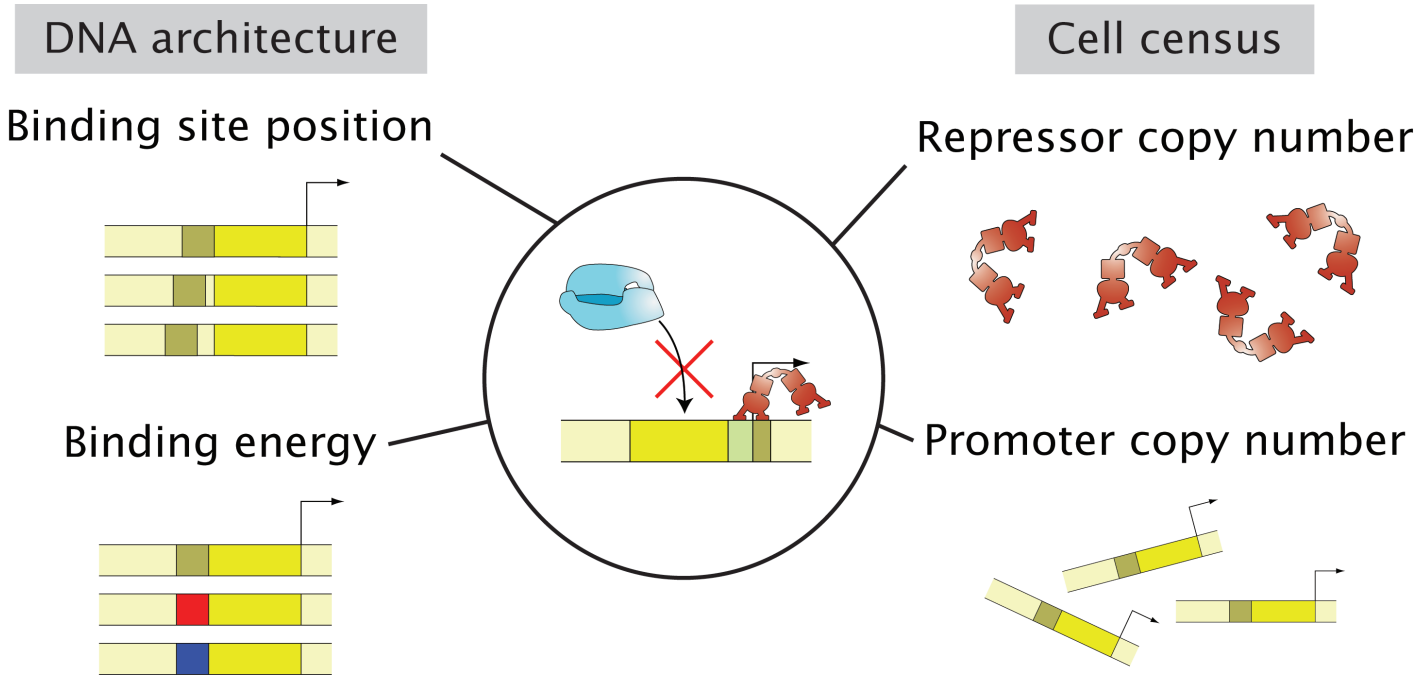


Figure 8-8 Essential Cell Biology, 2/e. (© 2004 Garland Science)



How do we know the number of molecules controlling the decision?

DIALING IN DIFFERENT RESPONSES IN SIMPLE REPRESSION



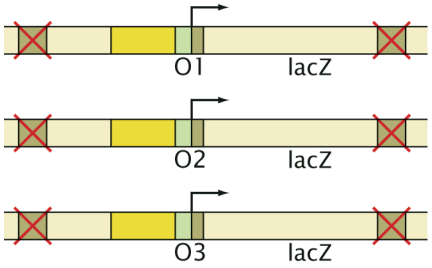
- ***As a starting point for our in vivo story, we begin with the "simpler" case of simple repression.***
- ***The idea is to tune the same parameters that we can control in the theoretical analysis in our experiments.***

CONFRONTING THEORY AND EXPERIMENT: A SIMPLE CASE

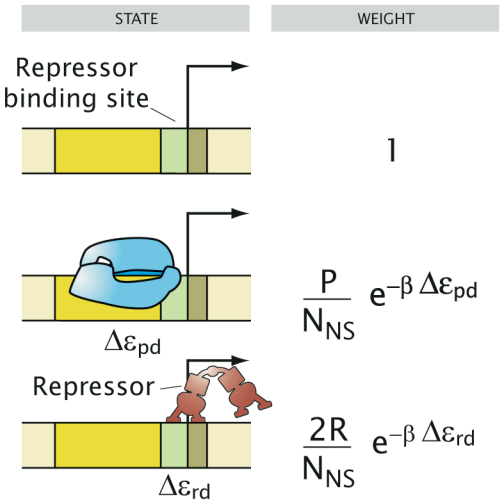
$$fold\ change = \left(1 + \frac{R}{N} e^{-\beta \Delta\varepsilon}\right)^{-1}$$

Fold-change

50 Number of repressors 900 $\Delta\varepsilon_{rd}$ (k_BT)



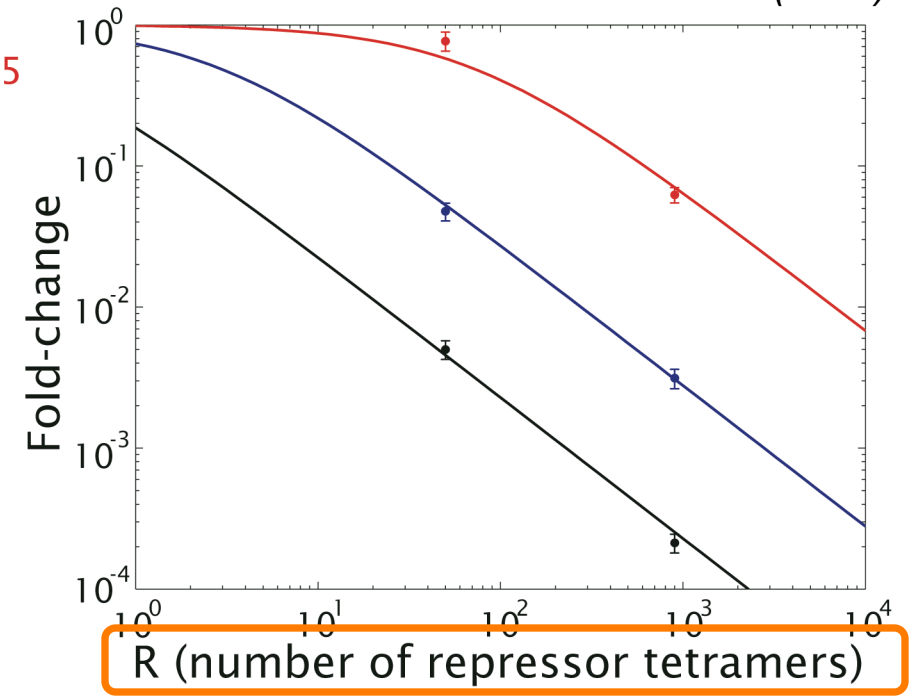
O1	5×10^{-3}	2.1×10^{-4}	-16.2
O2	0.048	3.1×10^{-3}	-13.7
O3	0.77	0.063	-10.5



Oehler et al. (1994)

Becker et al. (2005)
Vilar and Leibler (2003)

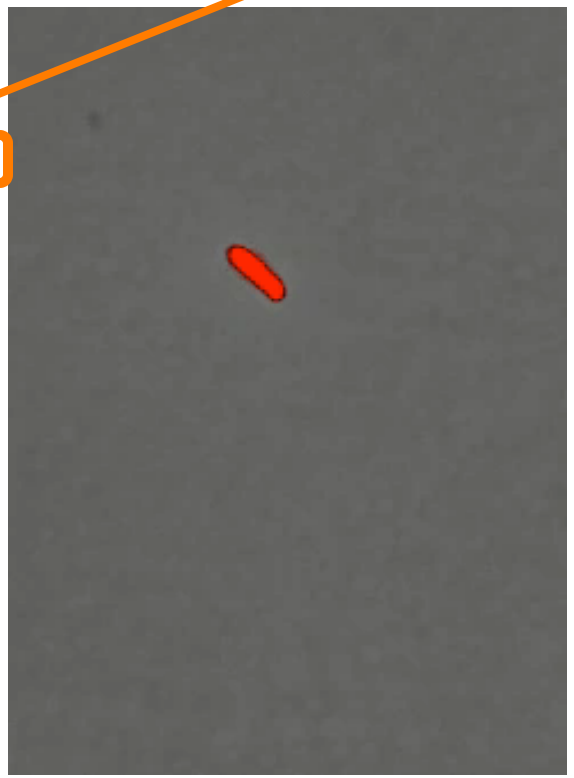
Bintu et al. (2005)

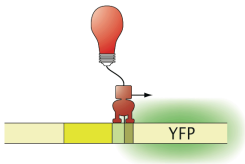


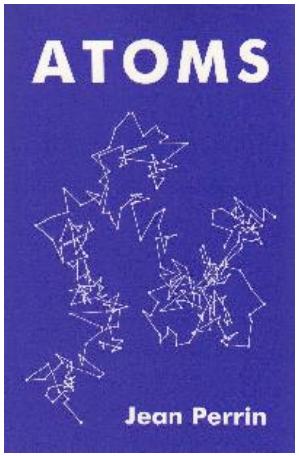
Using drunks to count proteins and measure expression

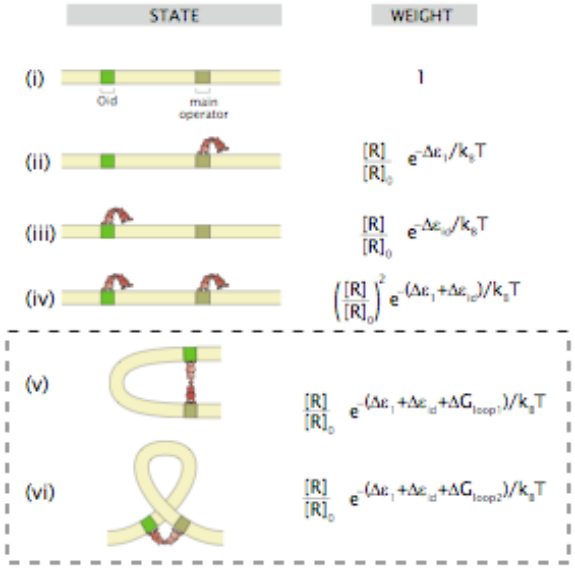
- ***Find new ways to count, new watches, new rulers. Perrin gave us more than 10 ways to measure Avogadro's number, many involve fluctuations.***
- ***Key point: in order to use the statistical mechanical theory, we must know the number of transcription factors. This suggested a cool new way to count and to measure the whole fold-change function.***

$$fold\ change = \left(1 + \frac{R}{N} e^{-\beta \Delta\varepsilon}\right)^{-1}$$









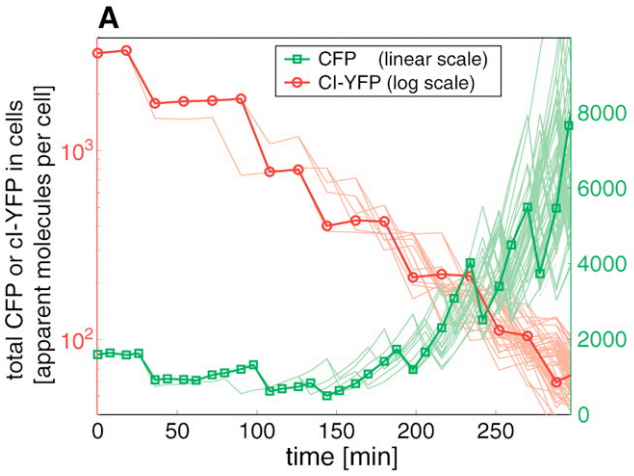
(Rosenfeld, Young, Alon, Swain, Elowitz, Science, 2005)

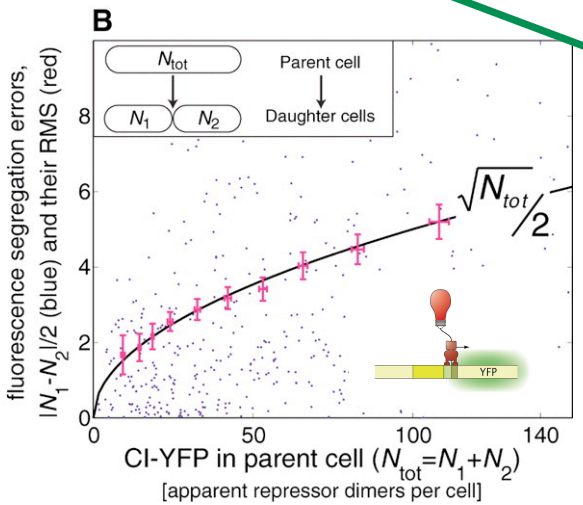
A MATHEMATICAL DESCRIPTION: THE STATISTICS OF DILUTION

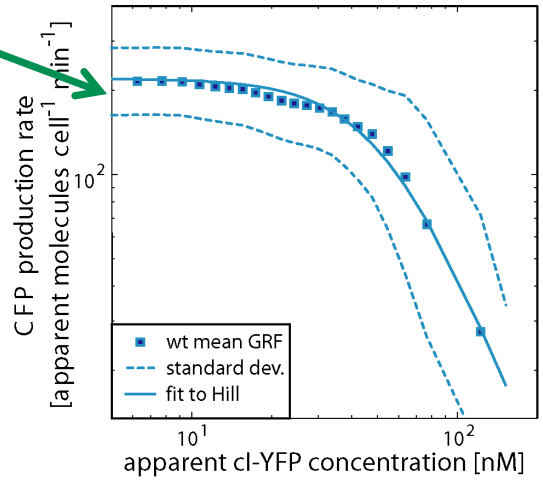
- ***One video of dividing cells can be used to perform multiple measurements on the gene regulation function.***

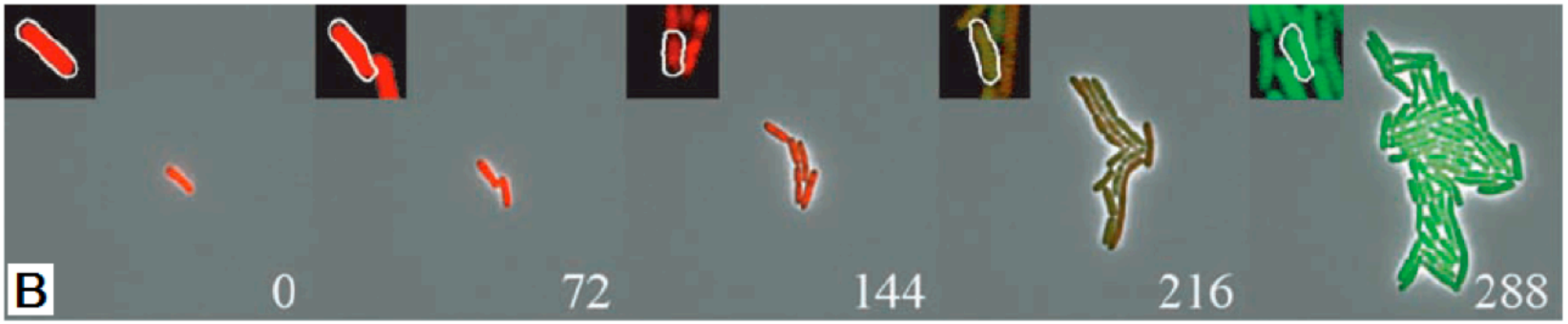
$$fold\ change = (1 + \frac{R}{N} e^{-\beta \Delta\varepsilon})^{-1}$$

(Rosenfeld, Young, Alon, Swain, Elowitz, Science, 2005)









THERMODYNAMIC MODELS OF GENE REGULATION

- ***This table shows the outcome of performing exactly the same kind of calculations we have just done for simple repression, but for other classes of regulatory architecture.***

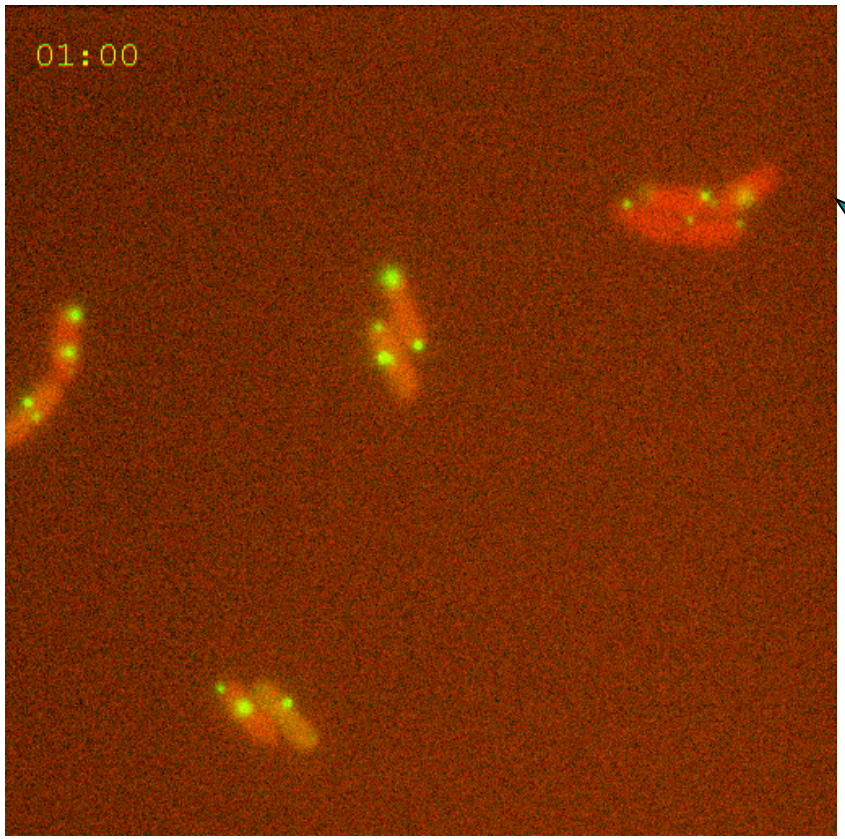
Table 1

Regulation factors for several different regulatory motifs.

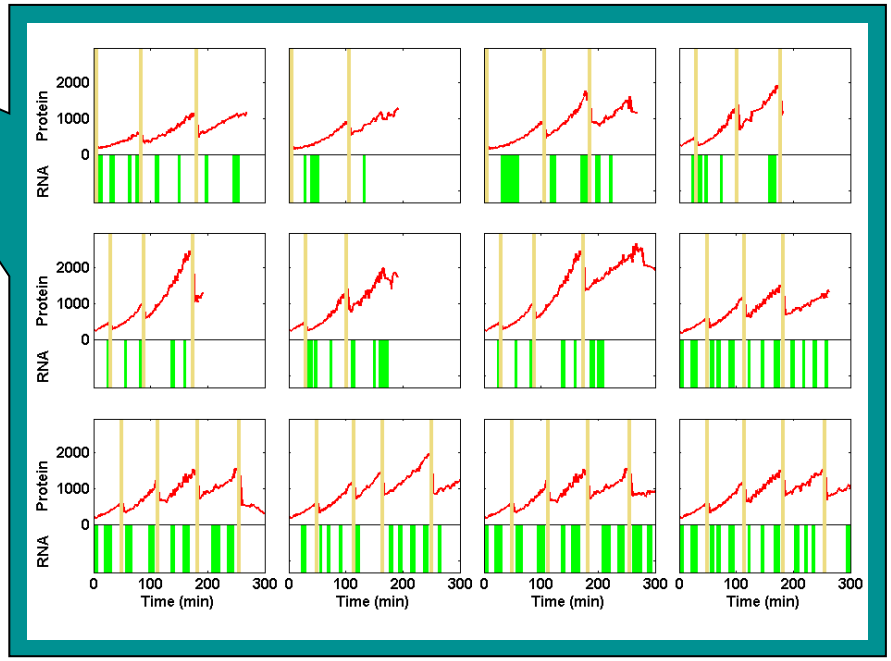
Case	Regulation factor (F_{reg})	
1. Simple repressor	$(1+r)^{-1}$	$\left(1+\frac{[R]}{K_R}\right)^{-1}$
2. Simple activator	$\frac{1+ae^{\frac{\varepsilon_{ap}}{k_BT}}}{1+a}$	$\frac{1+\frac{[A]}{K_A}f}{1+\frac{[A]}{K_A}}$
3. Activator recruited by a helper (*H*)	$\frac{1+a\frac{1+he^{\frac{\varepsilon_{ha}}{k_BT}}}{1+h}e^{\frac{\varepsilon_{ap}}{k_BT}}}{1+a\frac{1+he^{\frac{\varepsilon_{ha}}{k_BT}}}{1+h}}$	$\frac{1+\frac{[H]}{K_H}+\frac{[A]}{K_A}f+\frac{[A]}{K_A}\frac{[H]}{K_H}f\omega}{1+\frac{[H]}{K_H}+\frac{[A]}{K_A}+\frac{[A]}{K_A}\frac{[H]}{K_H}\omega}$
4. Repressor recruited by a helper (*H*)	$\left(1+\frac{1+he^{\frac{\varepsilon_{hr}}{k_BT}}}{1+h}r\right)^{-1}$	$\frac{1+\frac{[H]}{K_H}}{1+\frac{[H]}{K_H}+\frac{[R]}{K_R}+\frac{[R]}{K_R}\frac{[H]}{K_H}\omega}$
5. Dual repressors	$(1+r_1)^{-1}(1+r_2)^{-1}$	$\left(1+\frac{[R_1]}{K_{R_1}}\right)^{-1}\left(1+\frac{[R_2]}{K_{R_2}}\right)^{-1}$
6. Dual repressors interacting	$\left(1+r_1+r_2+r_1r_2e^{\frac{\varepsilon_{r_1r_2}}{k_BT}}\right)^{-1}$	$\left(1+\frac{[R_1]}{K_{R_1}}+\frac{[R_2]}{K_{R_2}}+\frac{[R_1]}{K_{R_1}}\frac{[R_2]}{K_{R_2}}\omega\right)^{-1}$
7. Dual activators interacting	$\frac{1+a_1e^{\frac{\varepsilon_{a_1}p}{k_BT}}+a_2e^{\frac{\varepsilon_{a_2}p}{k_BT}}+a_1a_2e^{\frac{\varepsilon_{a_1}p+\varepsilon_{a_2}p+\varepsilon_{a_1a_2}}{k_BT}}}{1+a_1+a_2+a_1a_2e^{\frac{\varepsilon_{a_1}p+\varepsilon_{a_2}p}{k_BT}}}$	$\frac{1+\frac{[A_1]}{K_{A_1}}f_1+\frac{[A_2]}{K_{A_2}}f_2+\frac{[A_1]}{K_{A_1}}\frac{[A_2]}{K_{A_2}}f_1f_2\omega}{1+\frac{[A_1]}{K_{A_1}}+\frac{[A_2]}{K_{A_2}}+\frac{[A_1]}{K_{A_1}}\frac{[A_2]}{K_{A_2}}\omega}$
8. Dual activators cooperating via looping	$\frac{1+a_1e^{\frac{\varepsilon_{a_1}p}{k_BT}}+a_2e^{\frac{\varepsilon_{a_2}p}{k_BT}}+a_1a_2e^{\frac{\varepsilon_{a_1}p+\varepsilon_{a_2}p+F_{loop}}{k_BT}}}{(1+a_1)(1+a_2)}$	$\frac{1+\frac{[A_1]}{K_{A_1}}f_1+\frac{[A_2]}{K_{A_2}}f_2+\frac{[A_1]}{K_{A_1}}\frac{[A_2]}{K_{A_2}}f_1f_2\omega}{\left(1+\frac{[A_2]}{K_{A_2}}\right)\left(1+\frac{[A_1]}{K_{A_1}}\right)}$
9. Repressor with two DNA binding units and DNA looping	$\left(1+r_m+\frac{r_m}{1+r_a}e^{\frac{\Delta\varepsilon_{ra}d+F_{loop}}{k_BT}}\right)^{-1}$	$\frac{1+\frac{[R]}{K_a}}{\left(1+\frac{[R]}{K_m}\right)\left(1+\frac{[R]}{K_a}\right)+\frac{[R]}{K_m}\frac{[L]}{K_a}}$
10. *N* non-overlapping activators and/or repressors acting independently on RNAP	$F_{reg1}\cdot F_{reg2}\cdot\bullet\bullet\bullet\cdot F_{regN}$	$F_{reg1}\cdot F_{reg2}\cdot\bullet\bullet\bullet\cdot F_{regN}$

Regulation factors for several different regulatory motifs. In the schematics of the motifs appearing in the first column, the inverted 'T' symbol indicates repression, arrows represent activation, and a dashed line is for DNA looping. The second column gives the regulation factor in terms of the number of transcription factors (TFs) in the cell and their binding energies, and the third column provides a translation of the regulation factor into the language of concentrations and equilibrium dissociation constants (used in the following paper [1**]). For an arbitrary TF we introduce the following notation: in the second column, x is the combination $\frac{X}{N_{NS}}e^{\Delta\varepsilon_{xd}/k_BT}$, and $[X]$ in the third column denotes the concentration of transcription factor X. $K_X=[X]/x$ is the effective equilibrium dissociation constant of the TF and its operator sequence on the DNA. Furthermore, in the third column we introduce $f=e^{\varepsilon_{xp}/k_BT}$ for the 'glue-like' interaction of a TF and RNAP, and $\omega=e^{\varepsilon_{x_1x_2}/k_BT}$ for the interaction between two TFs. In cases 8 and 9, F_{loop} is the free energy of DNA looping, ω in case 8 is defined as e^{F_{loop}/k_BT}, while $[L]$ in case 9 is the combination $\frac{N_{NS}}{V_{cell}}e^{F_{loop}/k_BT}$, V_{cell} being the volume of the cell.

INFORMATION PROCESSING IN LIVING CELLS: BEYOND FIRST APPROXIMATIONS



Ido Golding



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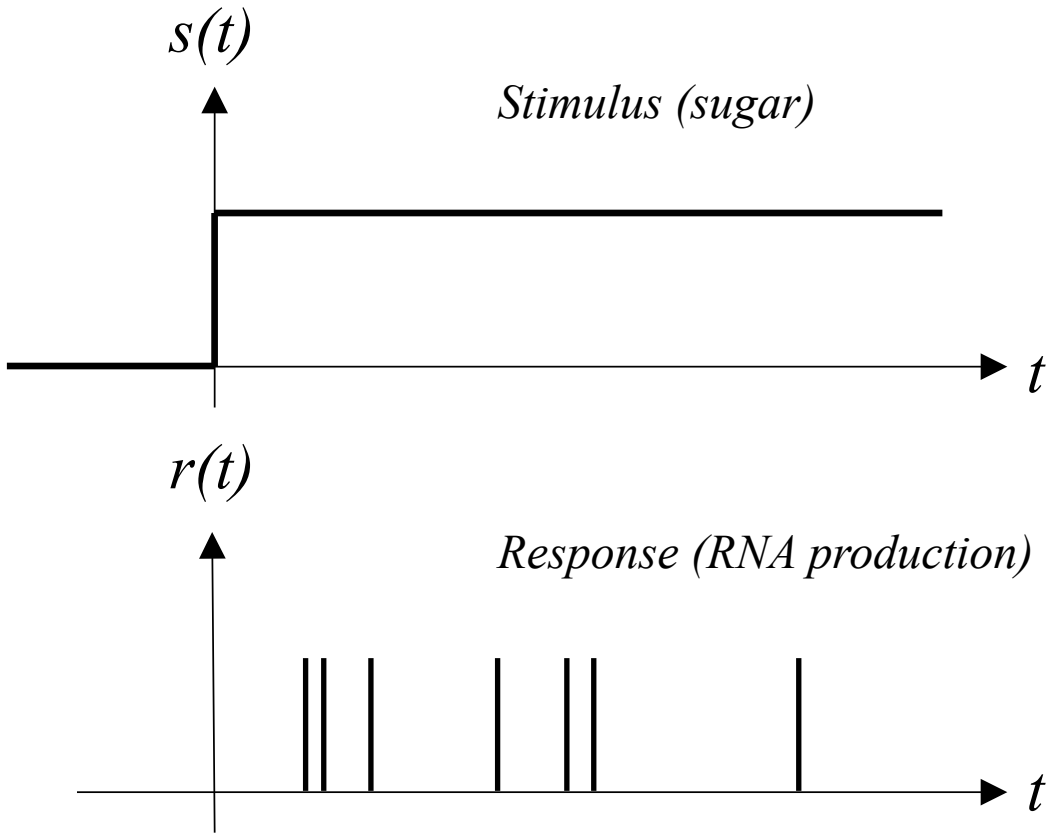
ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

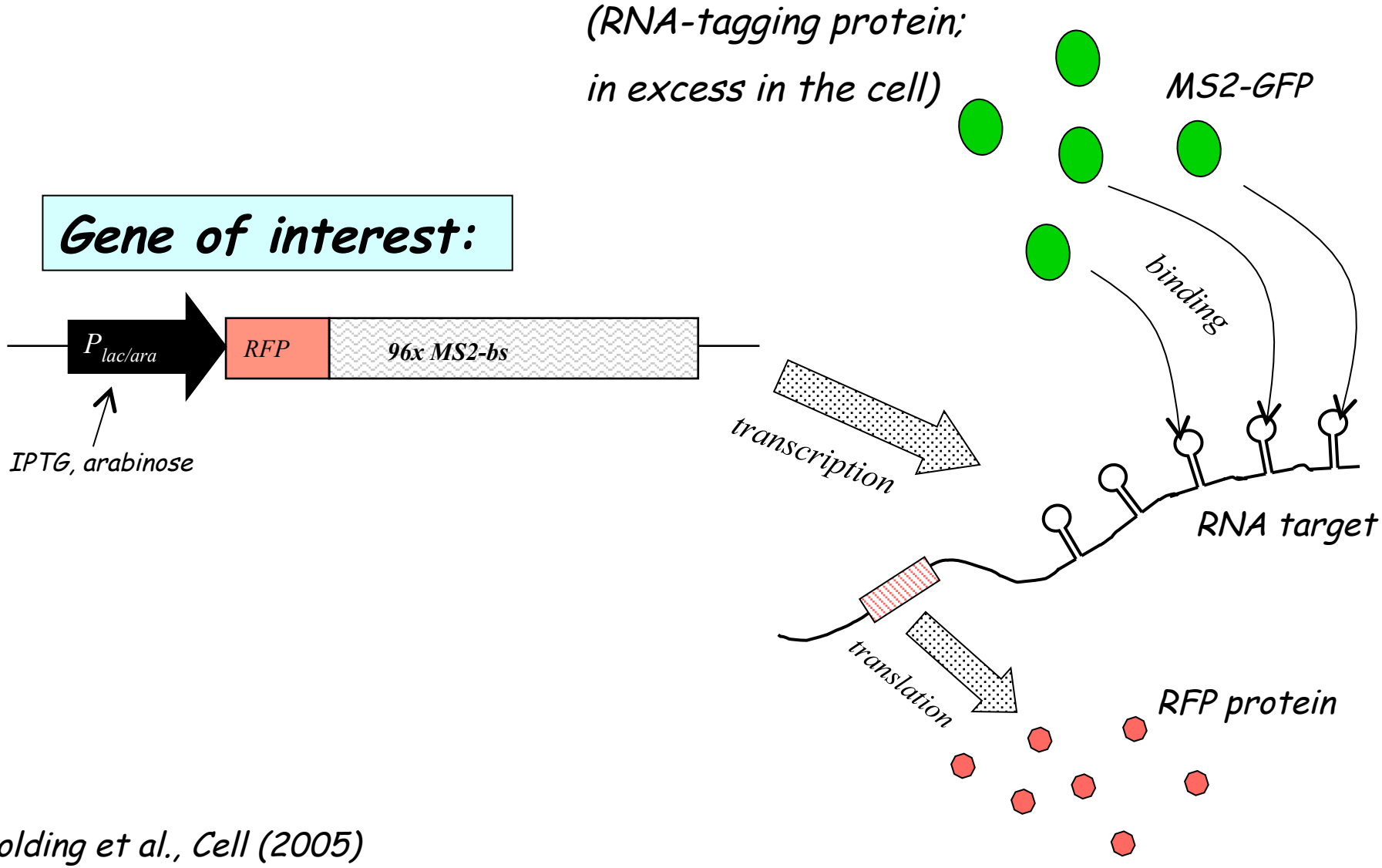
Center for the Physics of Living Cells

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)

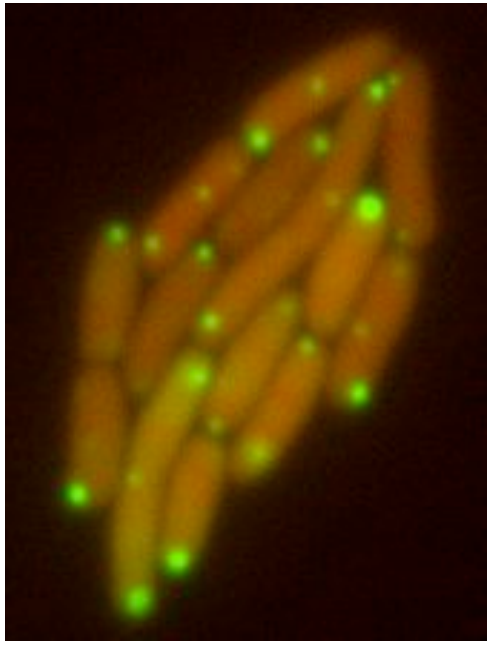
MEASURING MRNA & PROTEIN NUMBERS

mRNA ∝ *number of bound MS2-GFPs*

∝ *photon flux from localized green fluorescence*

Protein ∝ *number of RFPs*

∝ *photon flux from whole-cell red fluorescence*



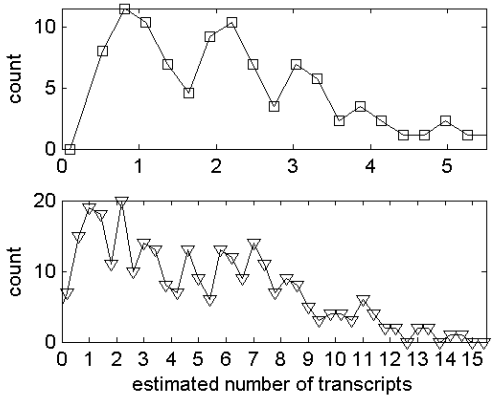
Histogram of RNA copy number:

1st peak =

inter-peak interval ≈

50-100 X GFP =

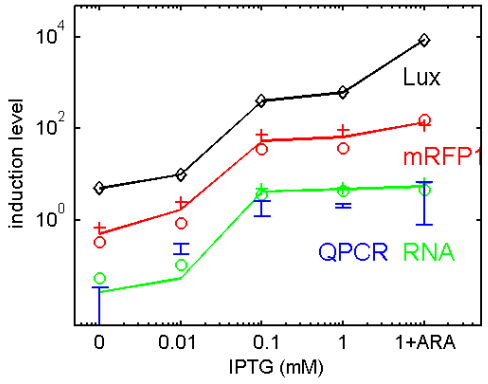
1 transcript



Controls:

QPCR

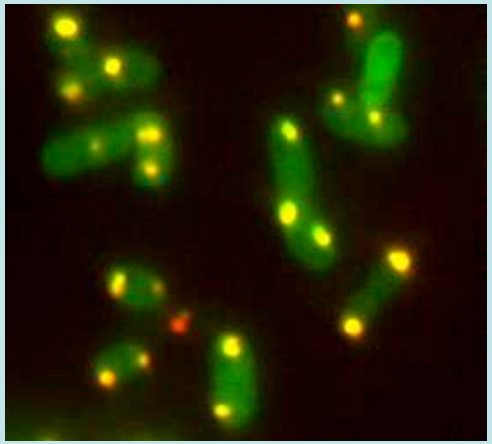
Protein levels



Lux: Lutz & Bujard 1997

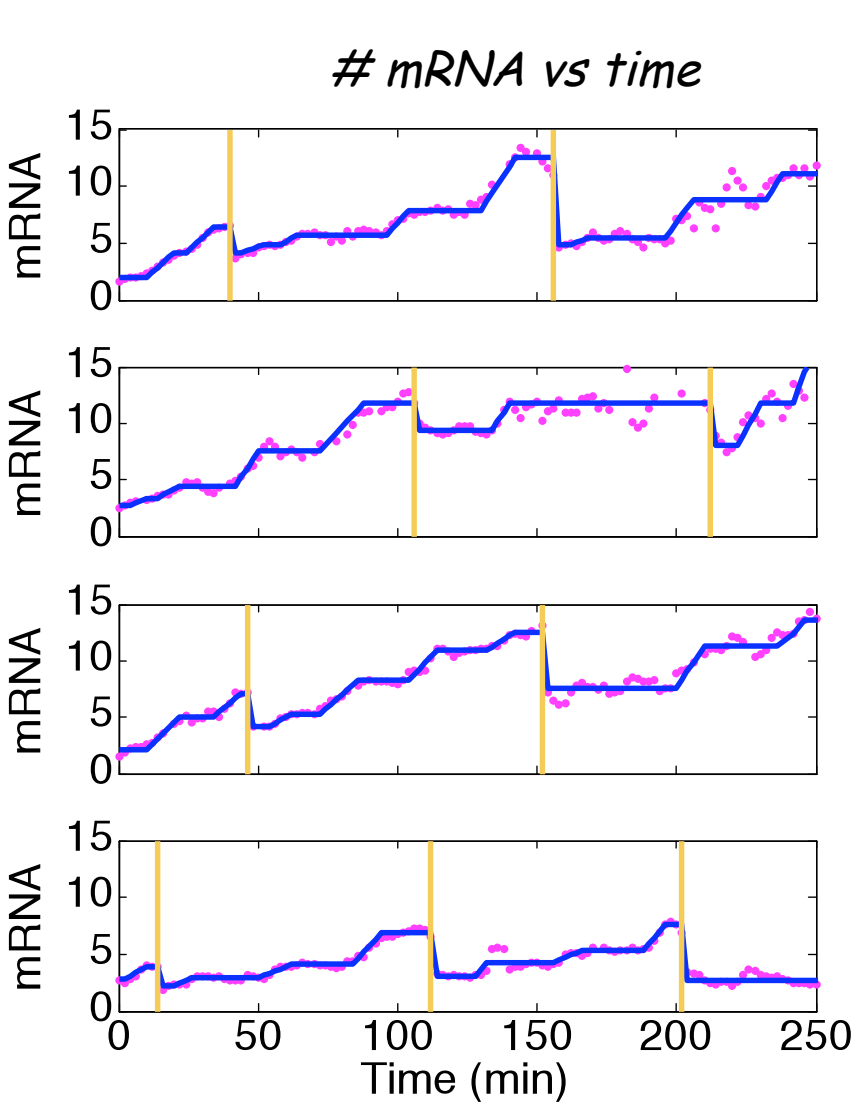
Controls:

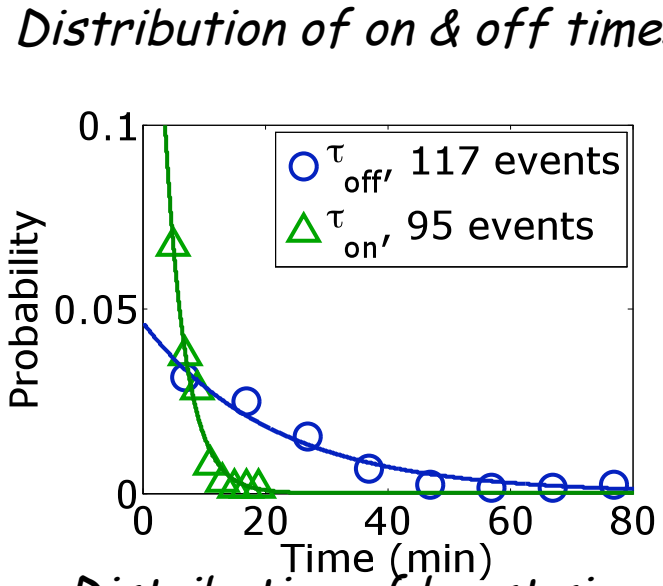
FISH

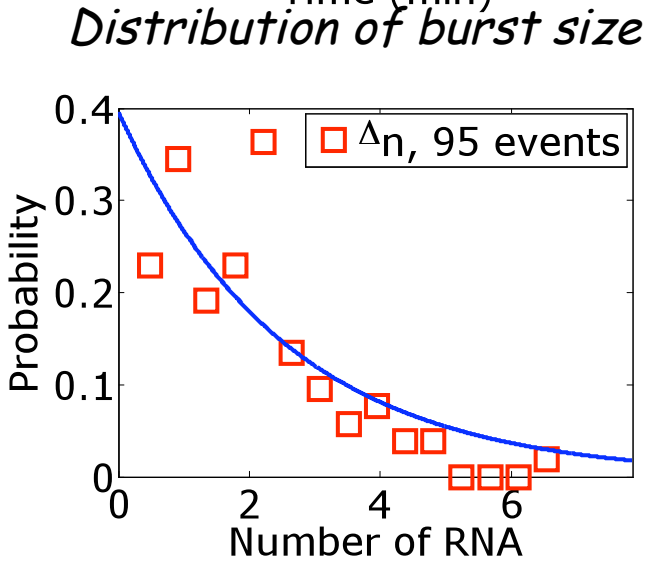


(Thanks to: A. Raj, A. van Oudenaarden)

RNA KINETICS IN INDIVIDUAL CELLS







MRNA PRODUCTION IN *E. COLI*

Golding et al. '05