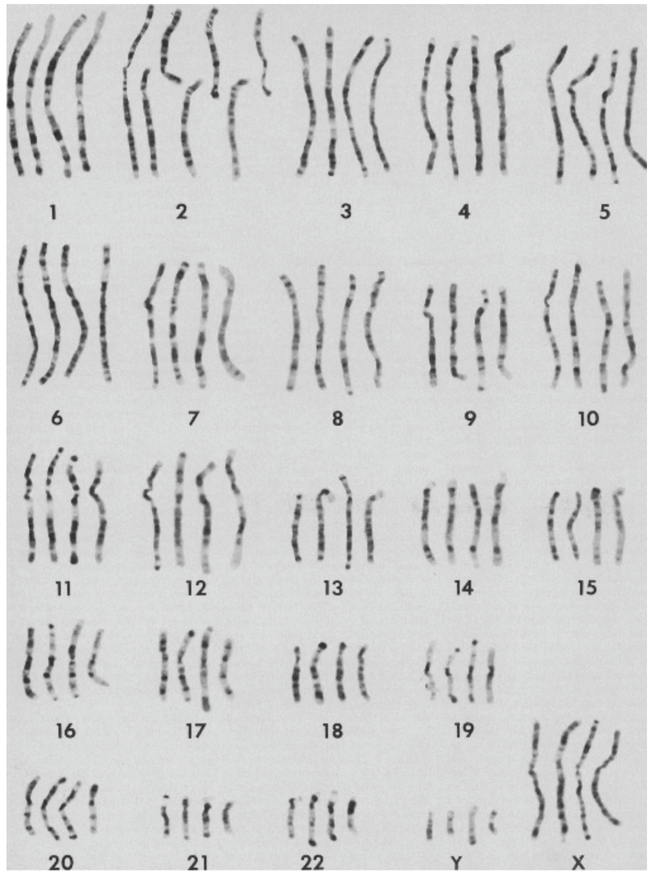
Genes, Chromosomes and Genomes



APh/BE161: Physical Biology of the Cell

Winter 2009

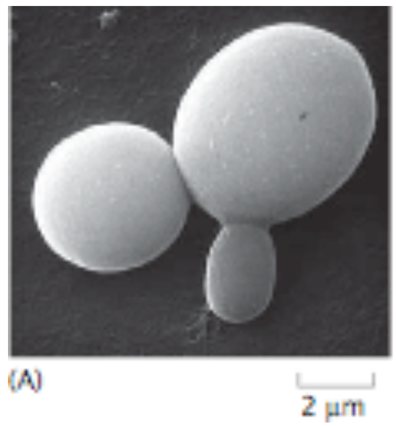
“Lecture 6”

Rob Phillips

How Cells Decide as Seen Through Three Hall of Fame ``Model'' Organisms

E. coli

yeast

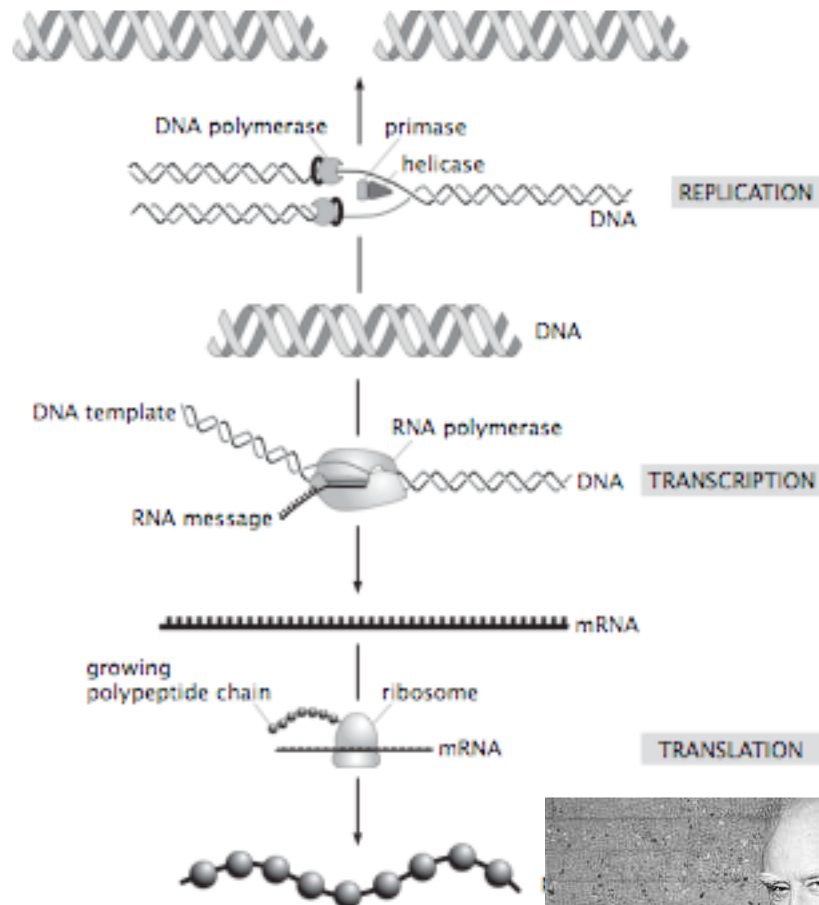


Fruit fly

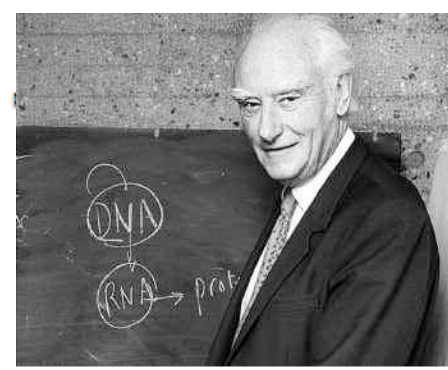
- *Various organisms are accorded hall of fame status as ``model'' organisms either because they are specialists at some particular process of interest or they are experimentally convenient (grow fast, easily accessible).*
- *Each of these organisms offers something extremely important on the question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



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

How Are Genes Related to What an Organism Is Like?

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

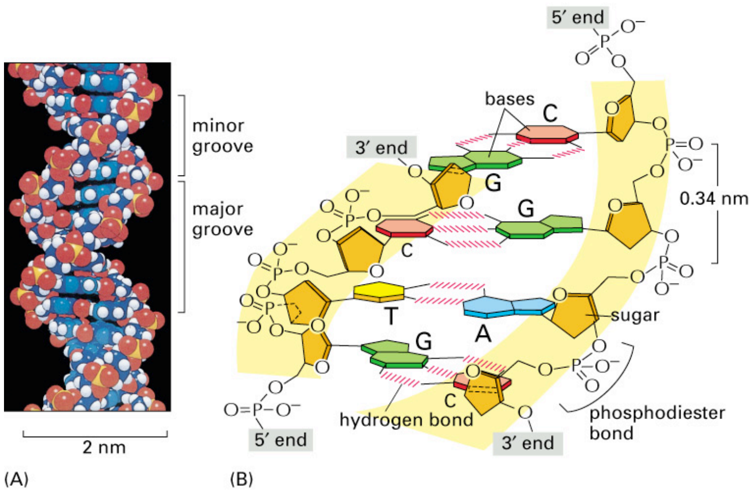
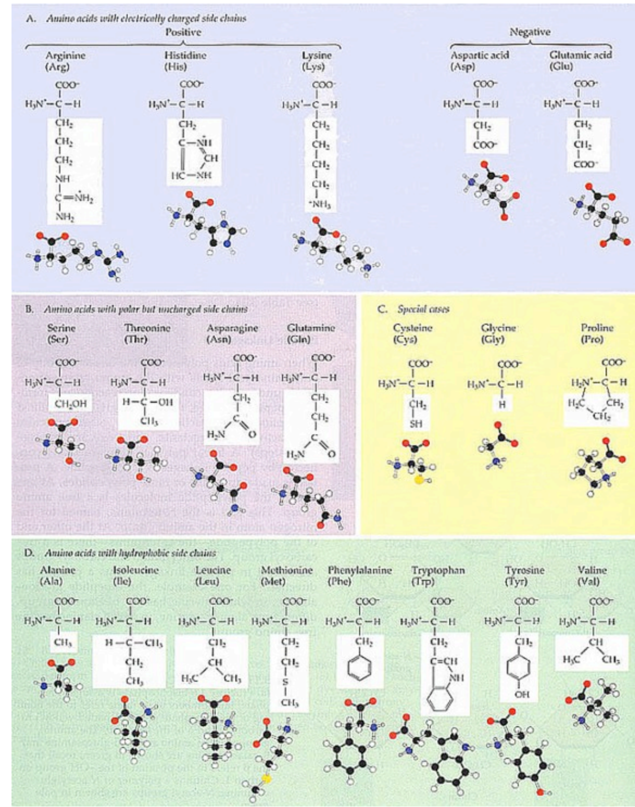


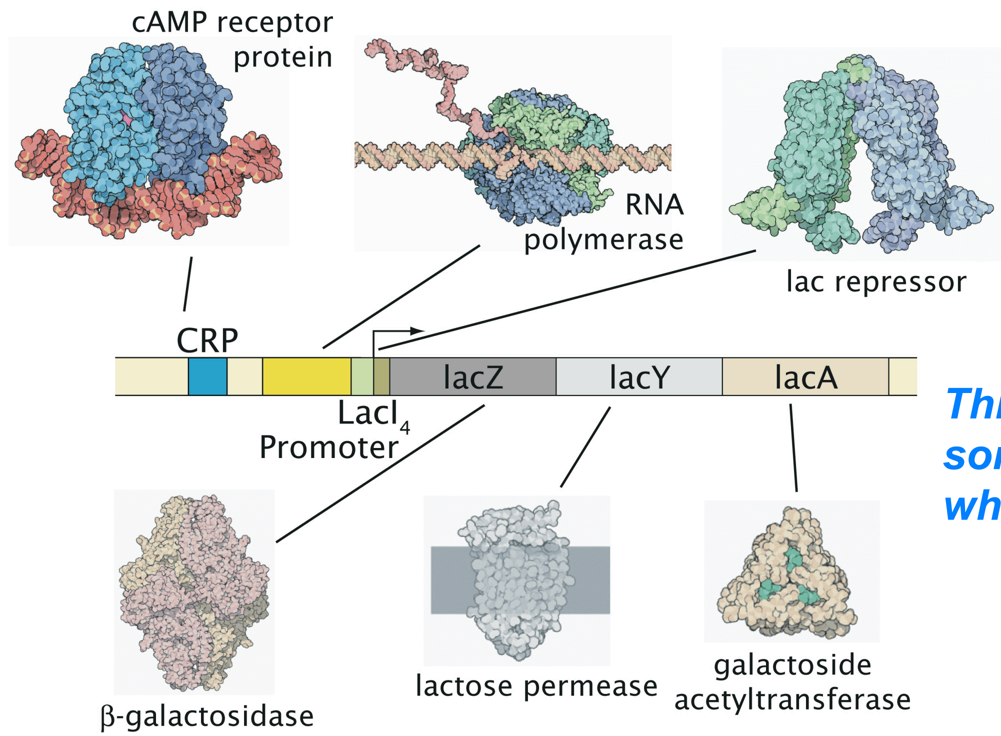
Figure 4–5. Molecular Biology of the Cell, 4th Edition.



- *How are the DNA and protein alphabets related?*
- *The sequence of A, T, G, C in the DNA is turned into a sequence of 20 amino acids strung together to make a protein.*

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.



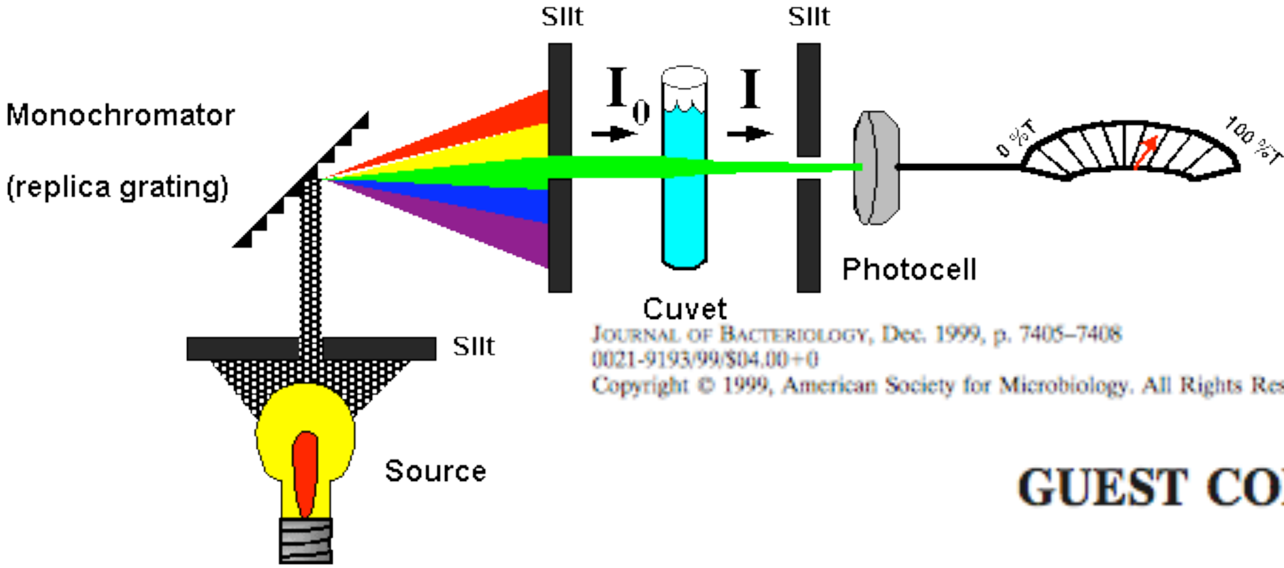
This lecture: how we found out, some beautiful examples, where we stand now.

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

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

Measuring the Diet of a Bacterium

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



JOURNAL OF BACTERIOLOGY, Dec. 1999, p. 7405–7408 Vol. 181, No. 24
0021-9193/99/$04.00+0
Copyright © 1999, American Society for Microbiology. All Rights Reserved.

GUEST COMMENTARY

Bacterial Growth: Constant Obsession with *dN*/*dt*

FREDERICK C. NEIDHARDT*

Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan 48109-0620.

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

$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$ $E_{bend} = \frac{\pi \xi_p k_B T}{R}$

- *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.*

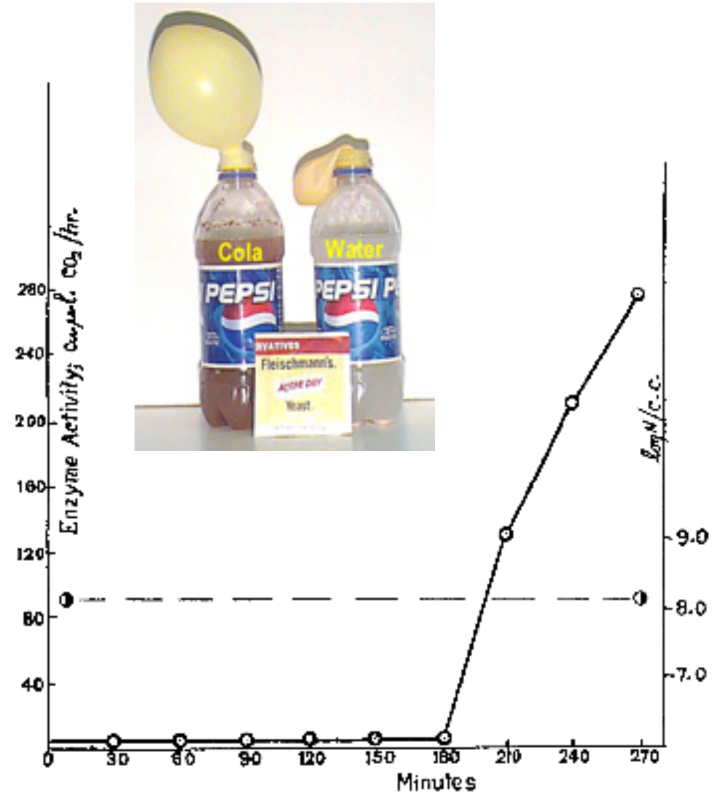


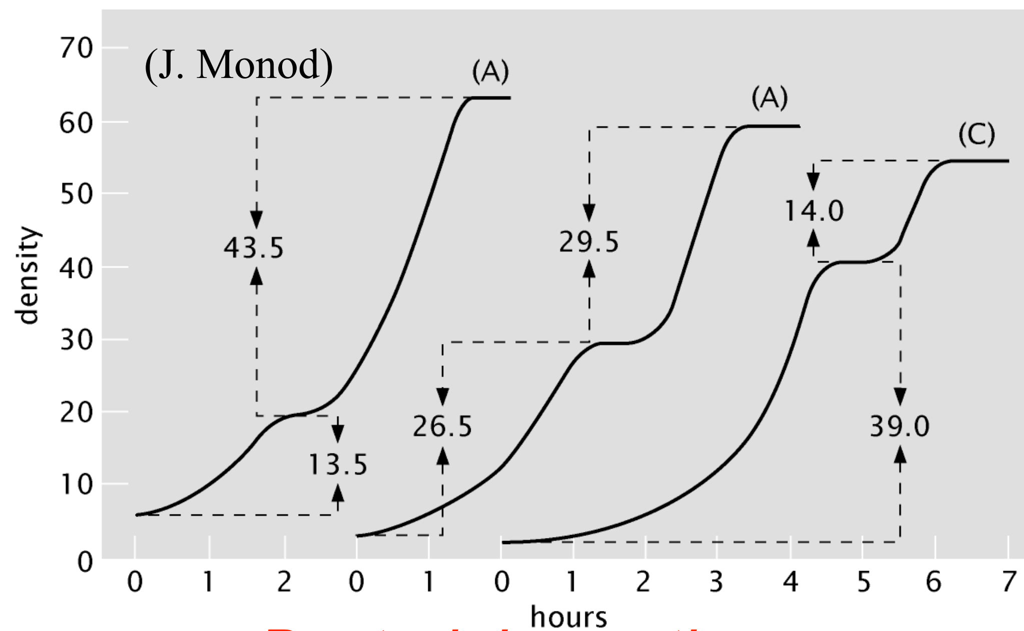
Figure 6. Adaptation to D-galactose without cell multiplication by a strain of *S. cerevisiae*: an experiment of Spiegelman and his colleagues carried out in 1943. Cells grown on D-glucose were washed in 67 mM KH_2PO_4, then resuspended in phosphate under nitrogen and D-galactose added. Carbon dioxide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

(Spiegelman et al., PNAS, 1944)

$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$ $E_{bend} = \frac{\pi \xi_p k_B T}{R}$

The Development of the Operon Concept: What Cells Eat and When They Die

- Studies of the cultures of bacterial cells sufficed to lead Jacob and Monod to formulate the operon concept - the revolutionary view that there are certain genes whose mission is to control other genes.
- This superseded the dictum of Beadle and Tatum which was ``one gene, one enzyme".
- The Mendelian revolution taken to the next stage.



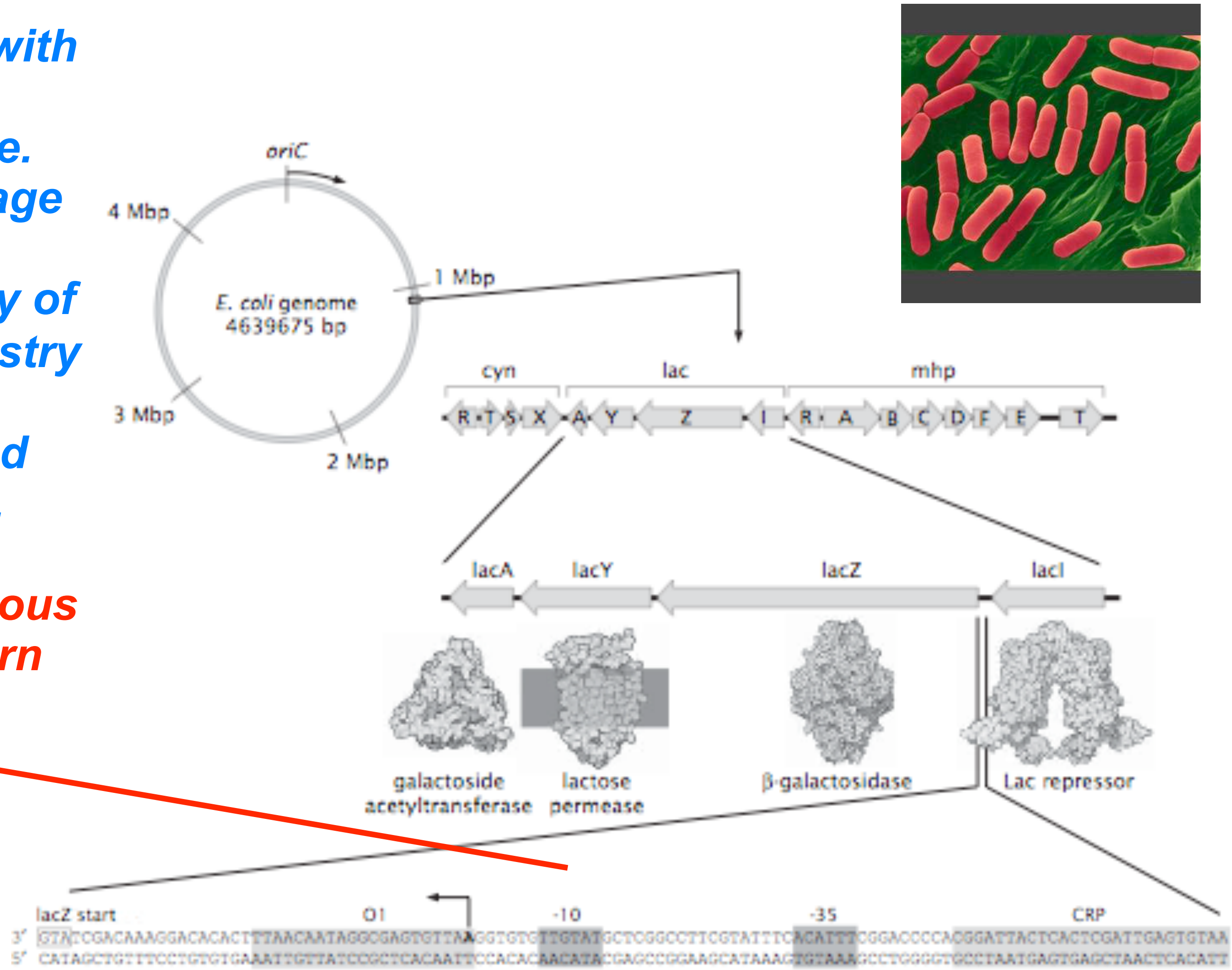
Bacterial growth curves

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

Not All DNA Codes for Proteins

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

- *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

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

Repressors: The Cartoon

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

- ***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.***

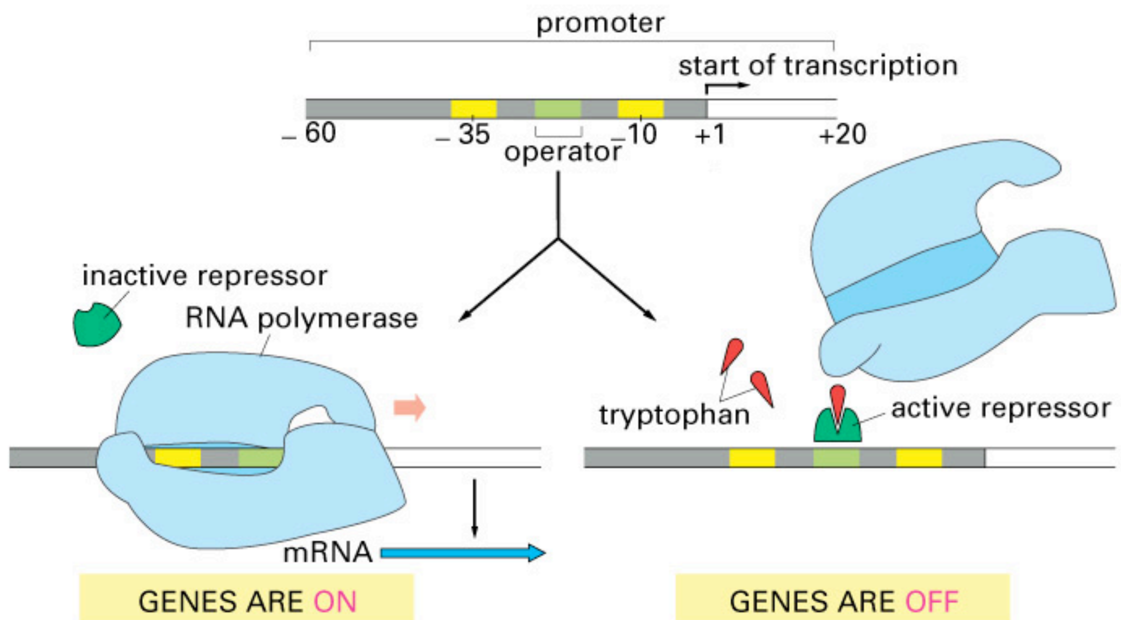


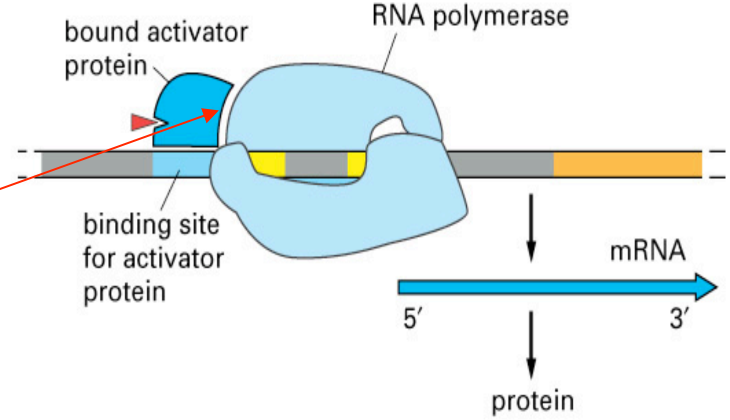
Figure 8-7 Essential Cell Biology, 2/e. (© 2004 Garland Science)

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

Activators: The Cartoon

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

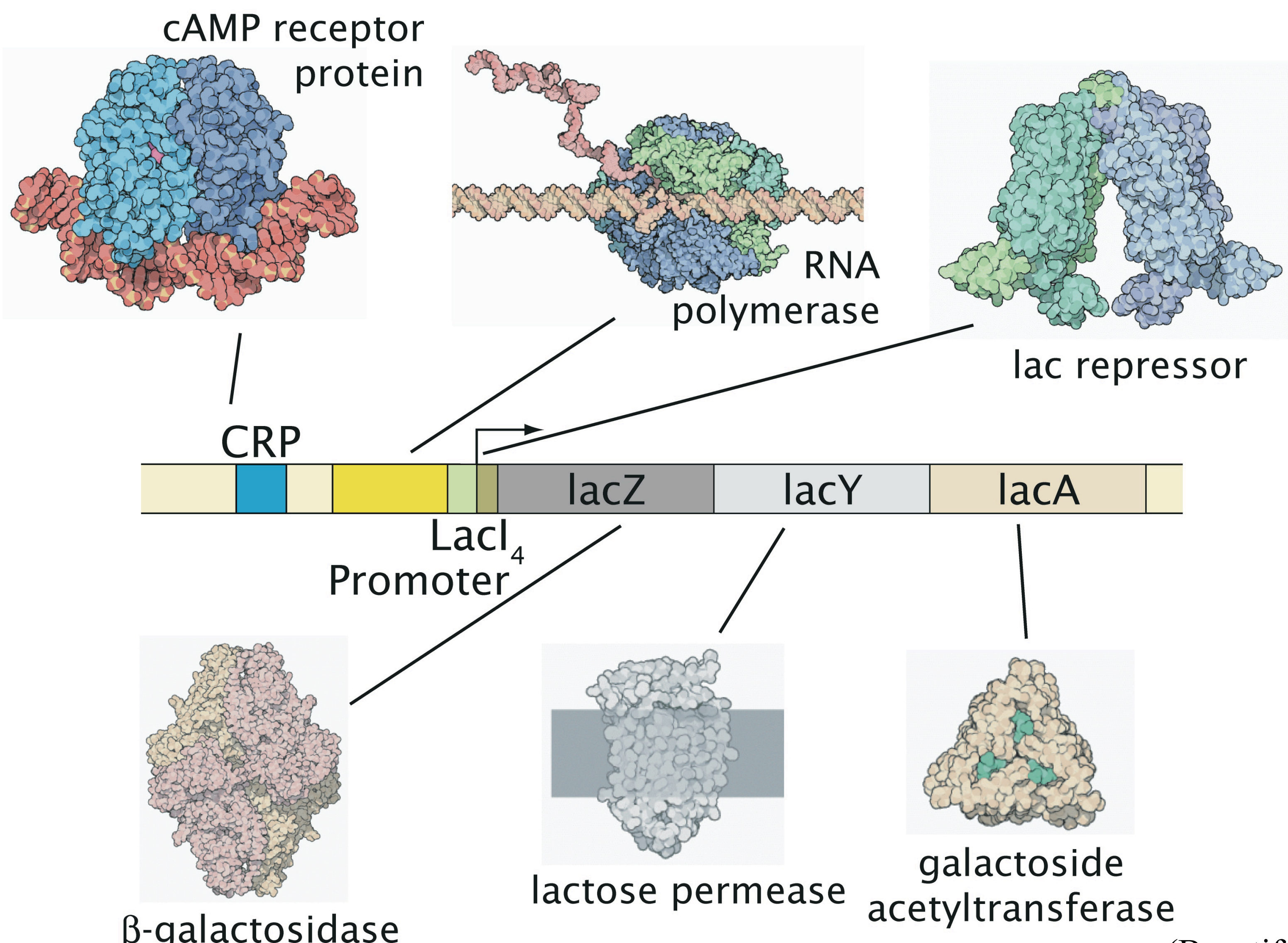
- *Activator molecules enhance the action of RNA polymerase.*
- *Activators can be under the control of other molecules (i.e. inducers) that dictate when activator is bound and not.*
- *Activators “RECRUIT” the polymerase.*

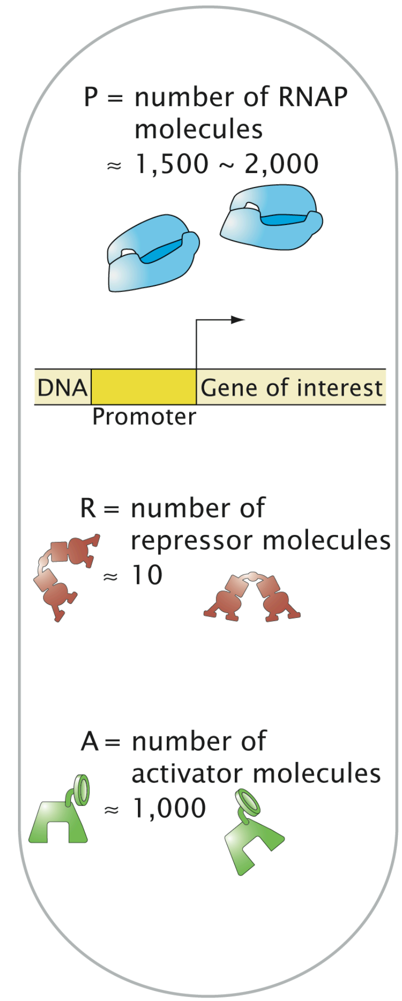


Adhesive interaction between RNAP and activator

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

The Single Molecule Census



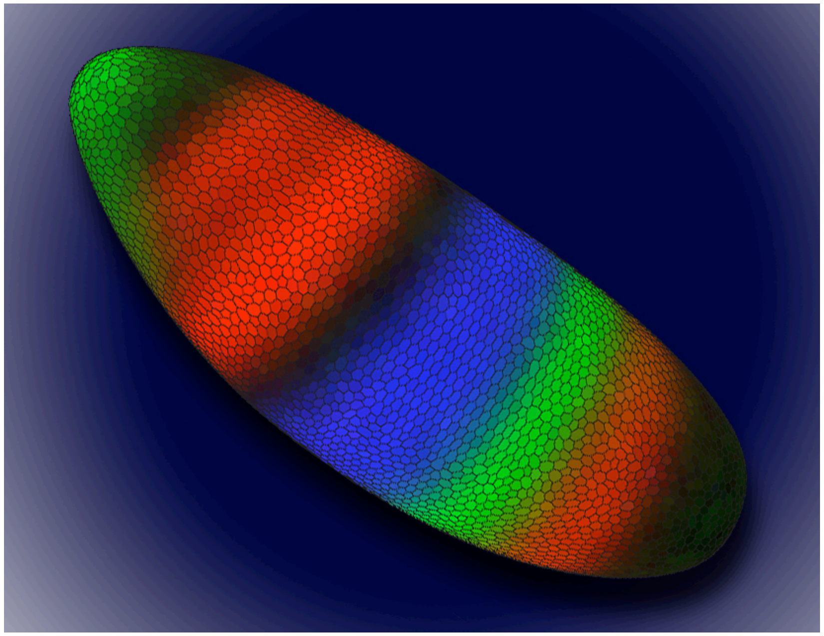


(Beautiful work of David Goodsell)

Ways to Measure Gene Expression

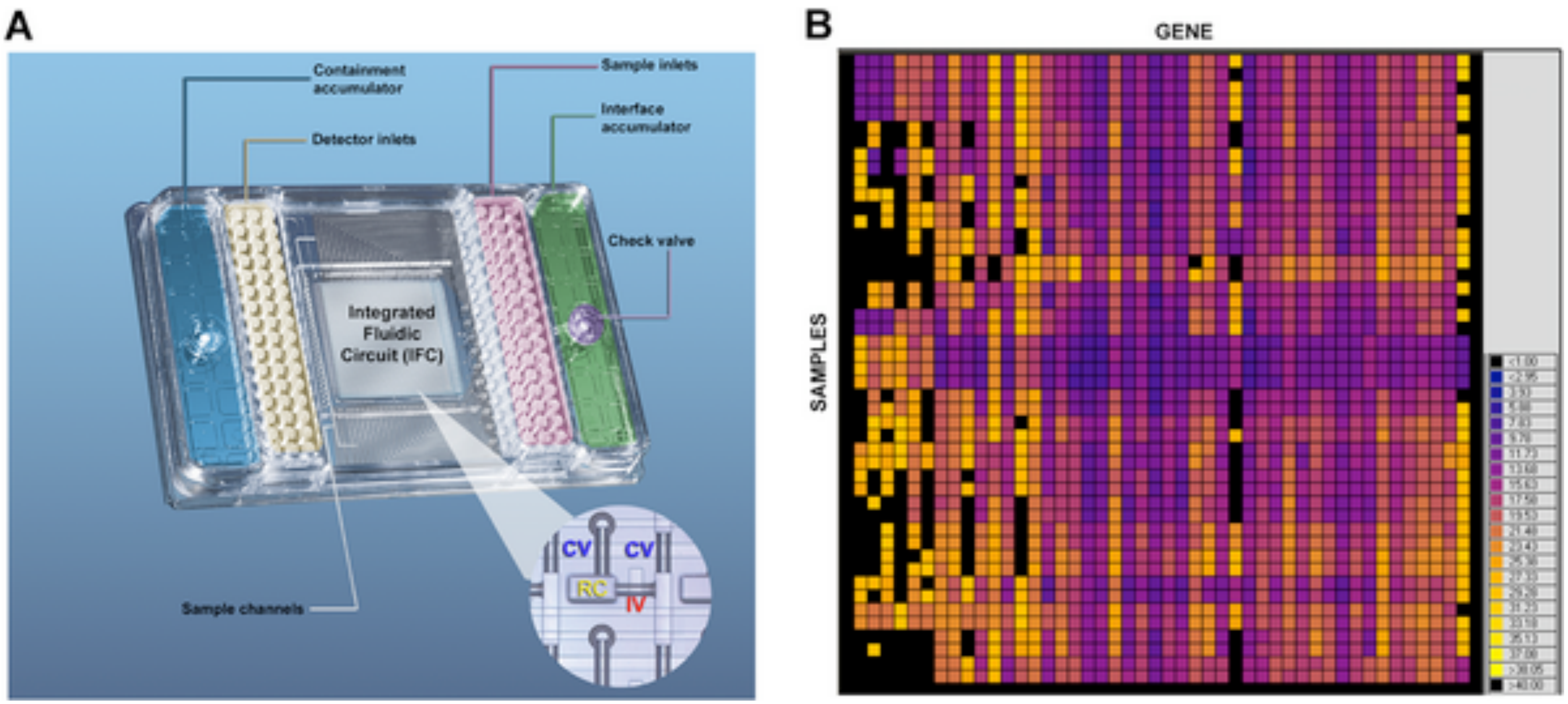
- ***Basic point: looking for "reporters" of the level of expression of gene of interest.***
- ***Can ask the system to report on the level of gene expression at various steps in the processes linking DNA to active protein.***
- ***Promoter occupancy, level of mRNA, level of active protein.***

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

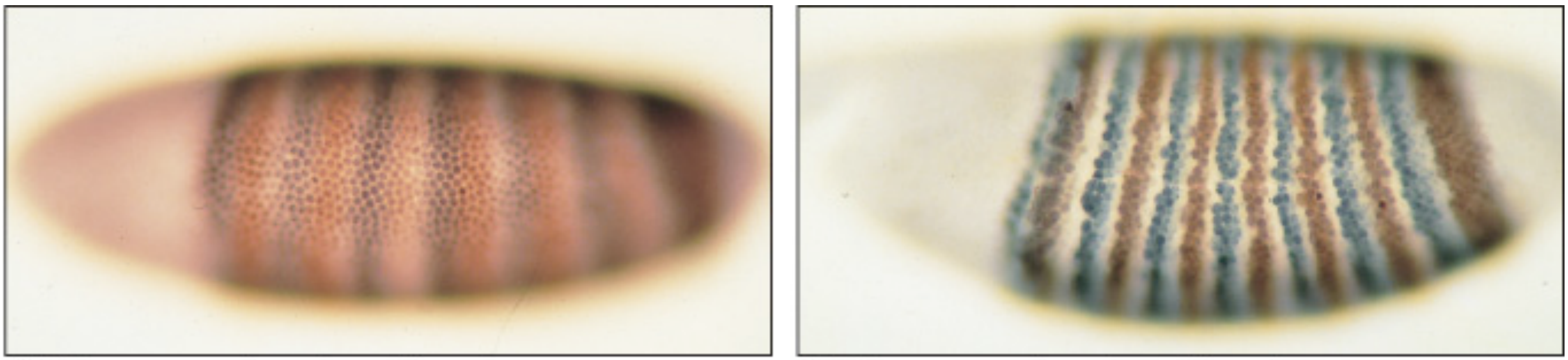


$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$

Enzymatic Assay or In-Situ Hybridization

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

- ***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.***

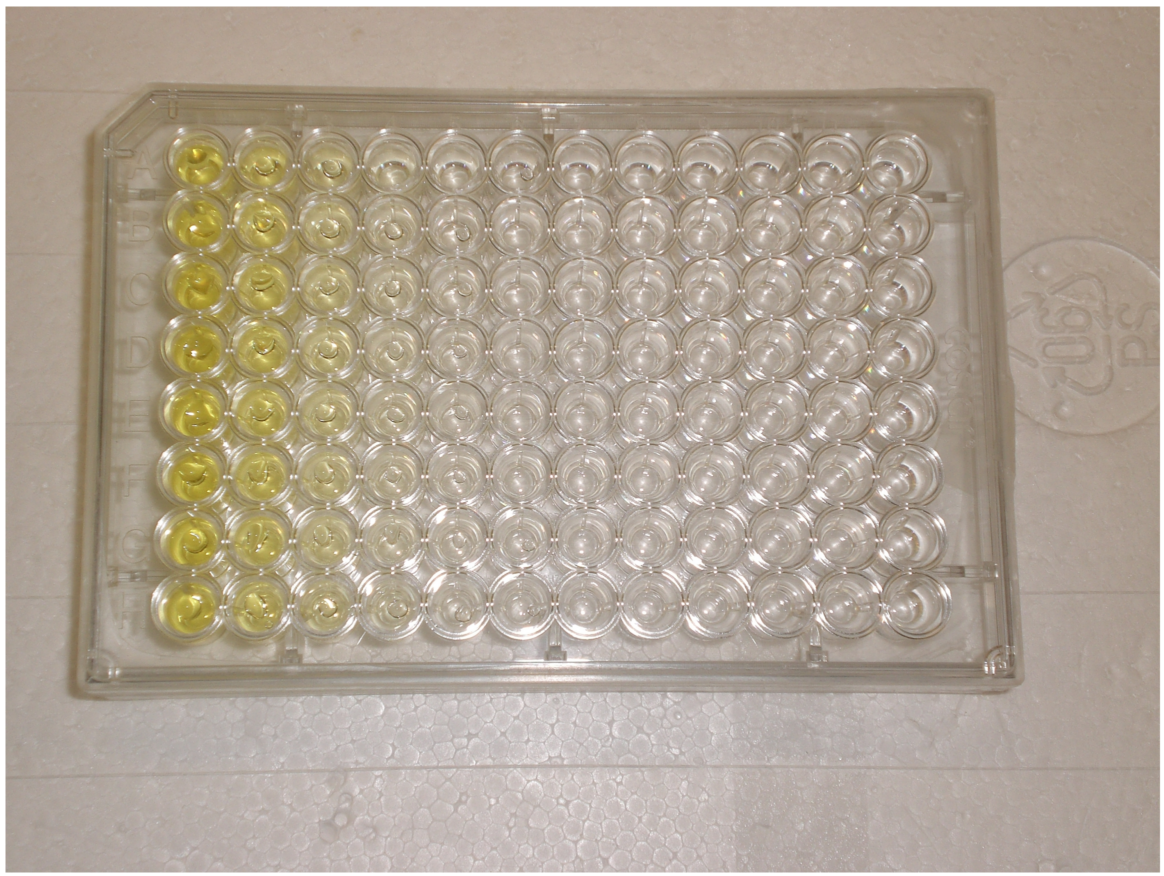


2.7 hours after fertilization   3.5 hours after fertilization

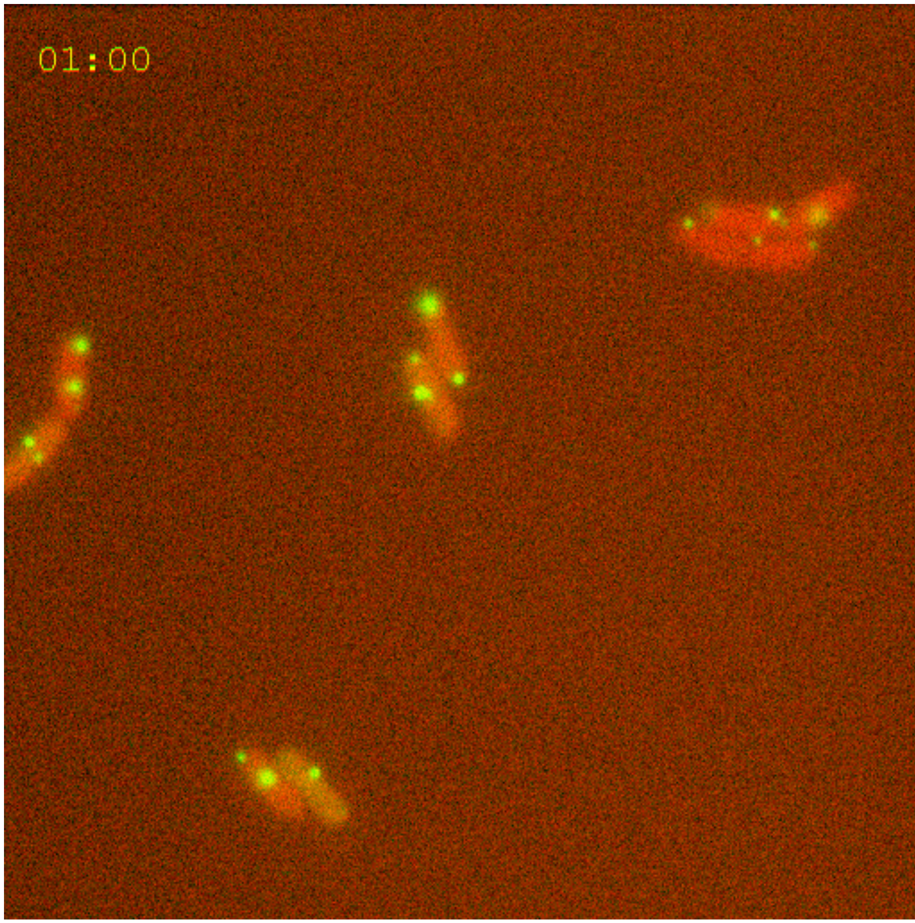
Figure 21–39. Molecular Biology of the Cell, 4th Edition.

Enzymatic Assay or In-Situ Hybridization

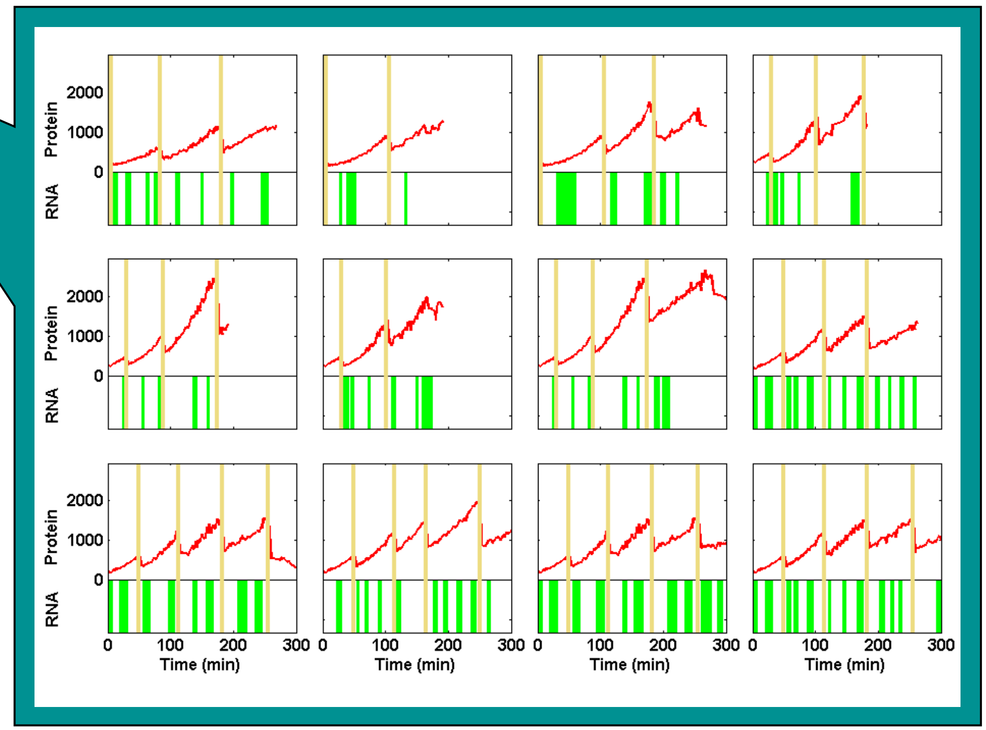
- ***Enzymatic assays – promoter leads to the production of a protein that then does some enzymatic action on the substrate which yields a product that can be visualized.***
- ***In-situ hybridization -***



Information Processing in Living Cells: Beyond First Approximations



Ido Golding



Department of Physics
ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

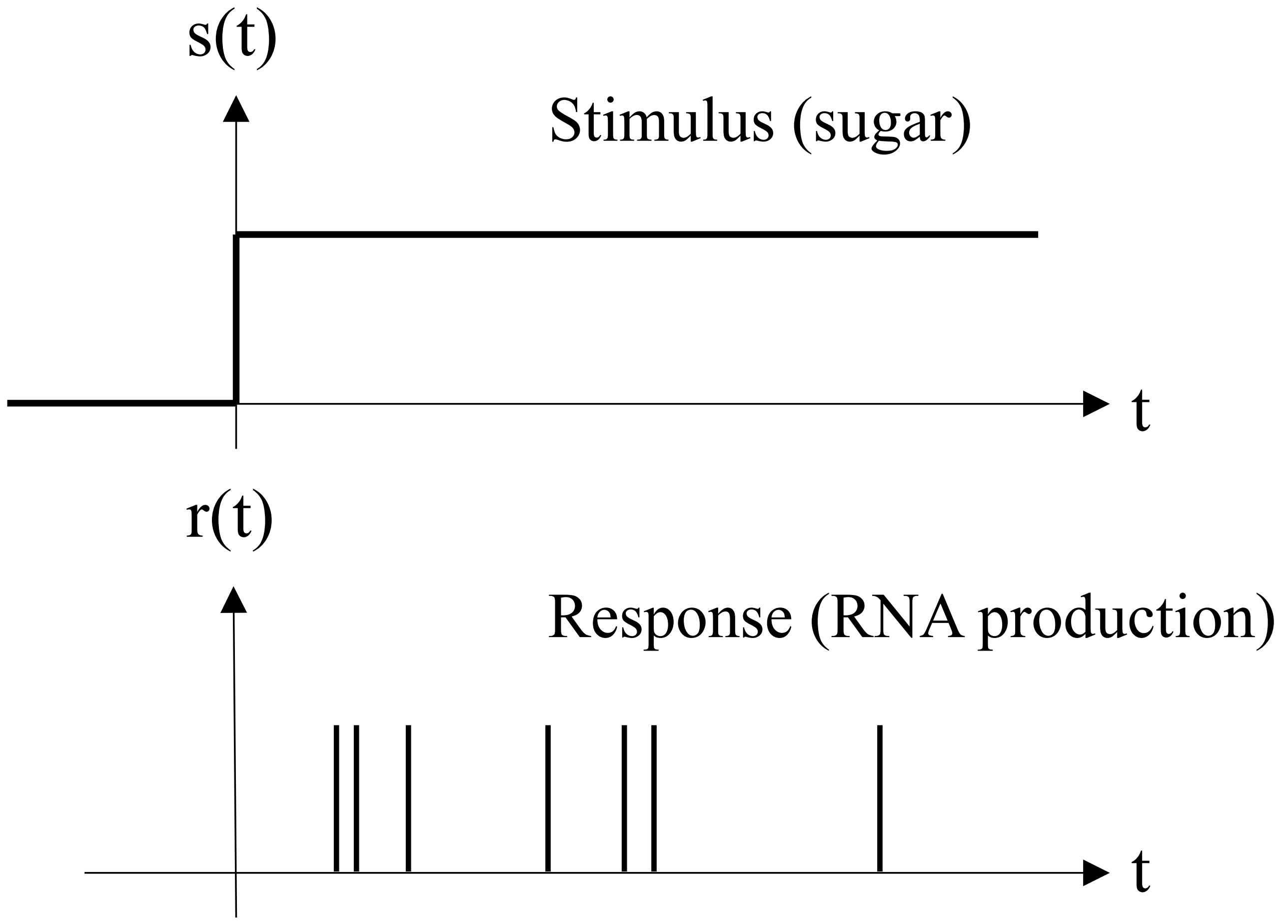
Center for the Physics of Living Cells

Caltech 11/2008

Simple Case of Turning a Gene "On"

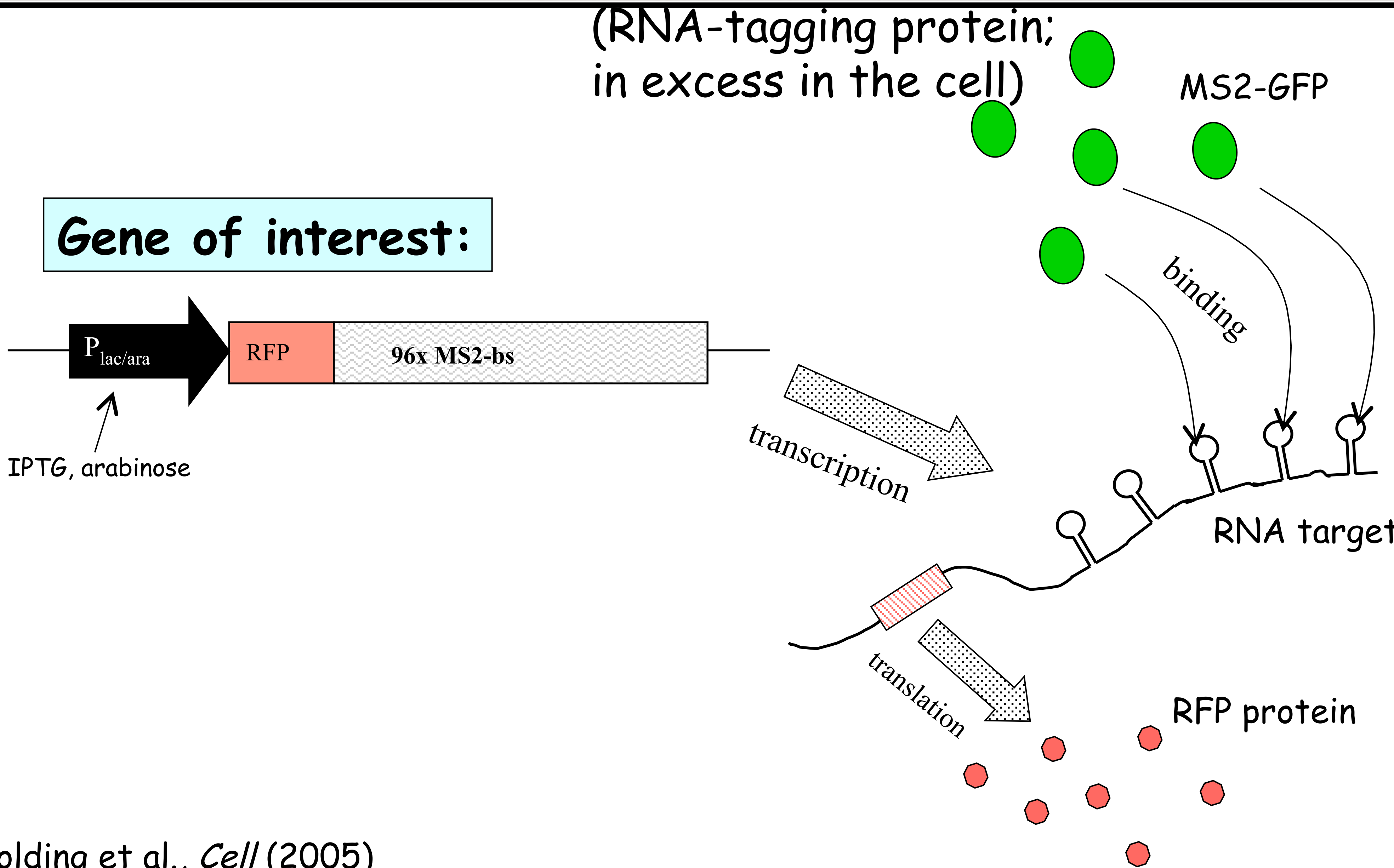
$F(n_{bp}) = \frac{\alpha}{n_{bp}} + \gamma \ln N_{bp}$

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



Approximations used to describe the process…

Engineering bacteria to report on gene activity

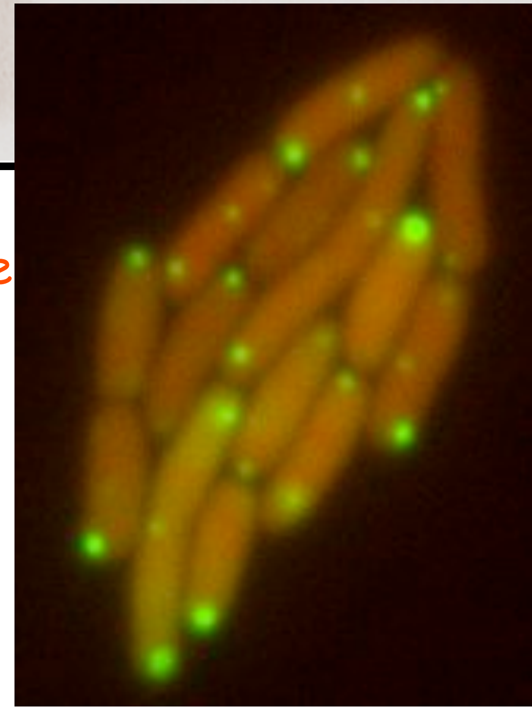


Golding et al., *Cell* (2005)

Measuring mRNA & protein numbers

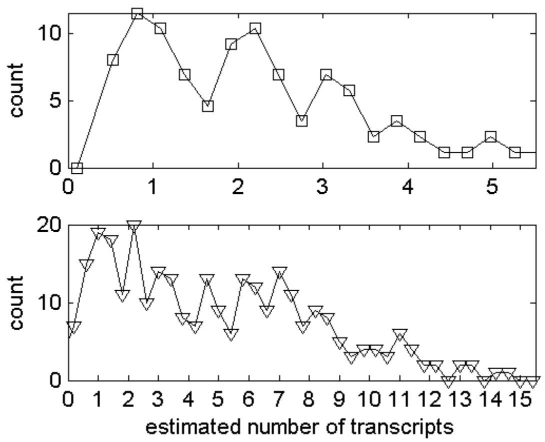
mRNA ∝ number of bound MS2-GFPs
∝ photon flux from localized green fluorescence

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

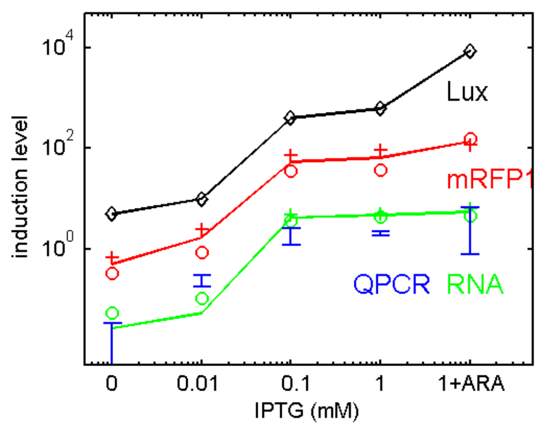


Histogram of
RNA copy number:

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

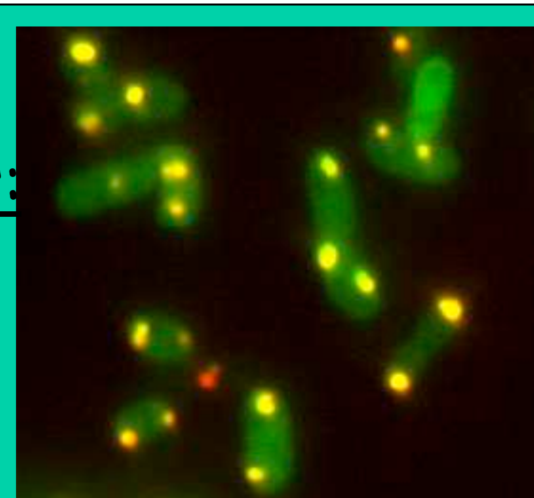


Controls:
QPCR
Protein levels



Lux: Lutz & Bujard 1997

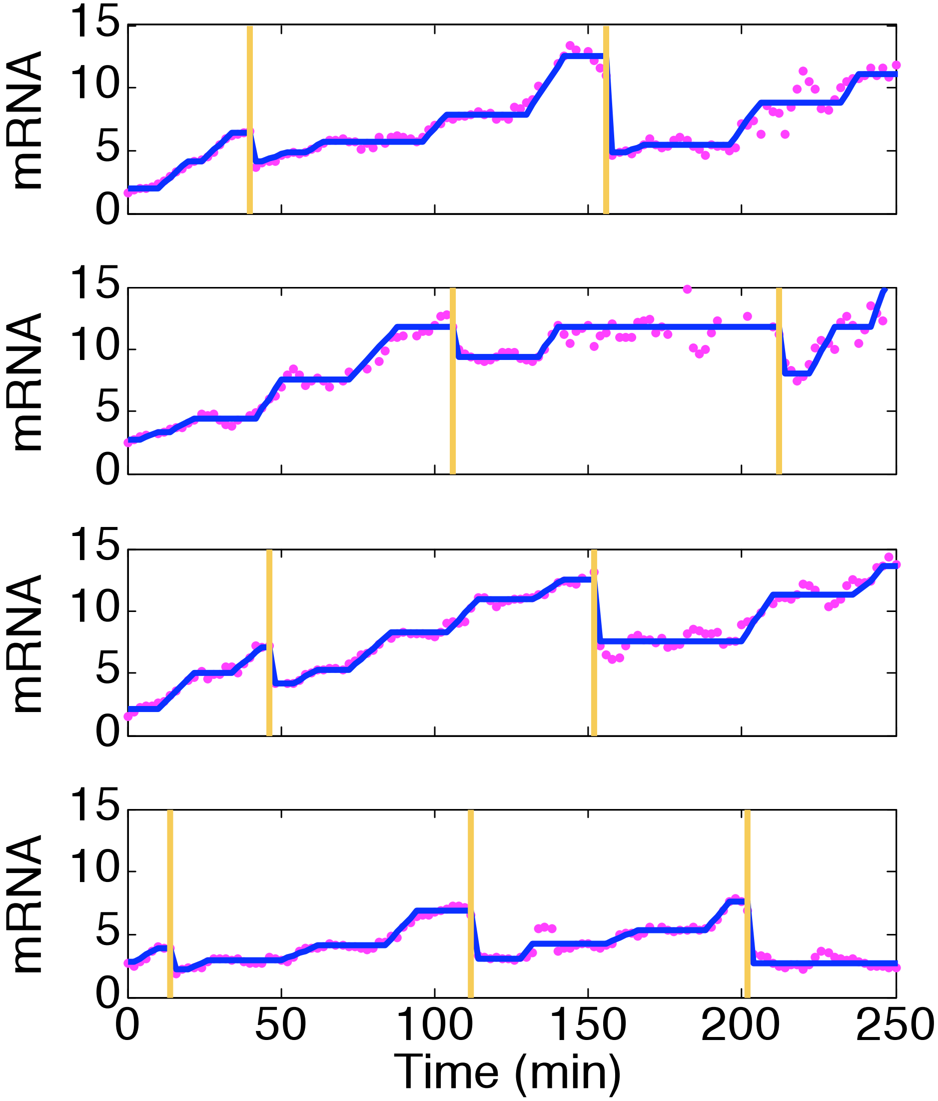
Controls:
FISH



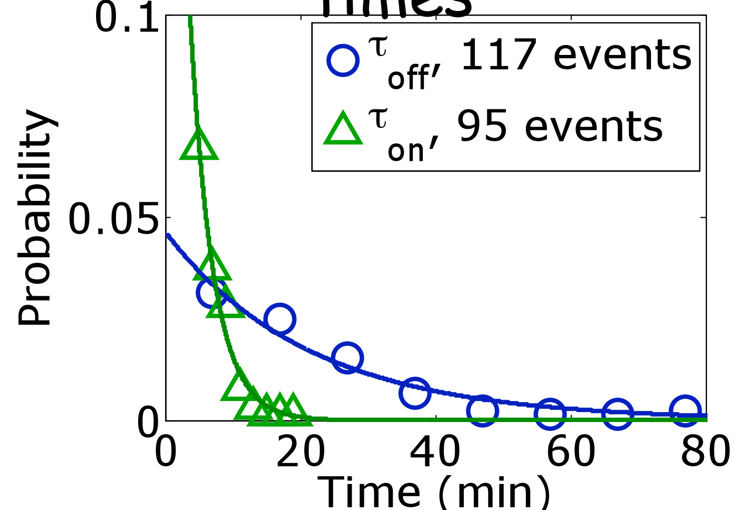
(Thanks to: A. Raj, A. van Oudenaarden)

RNA kinetics in individual cells

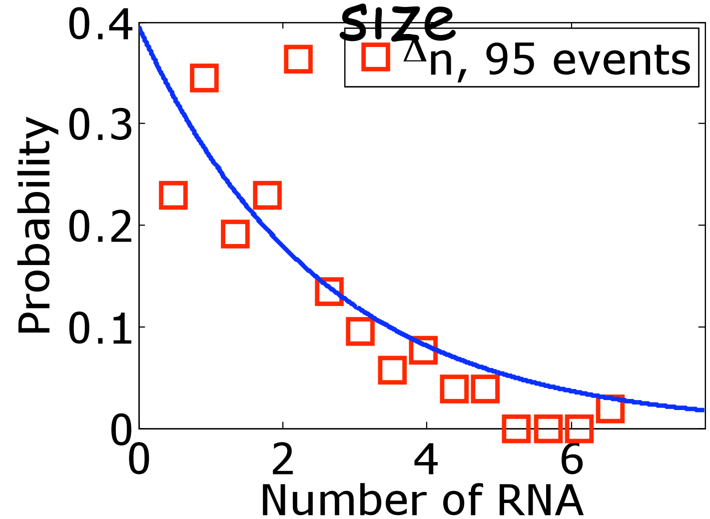
mRNA vs time



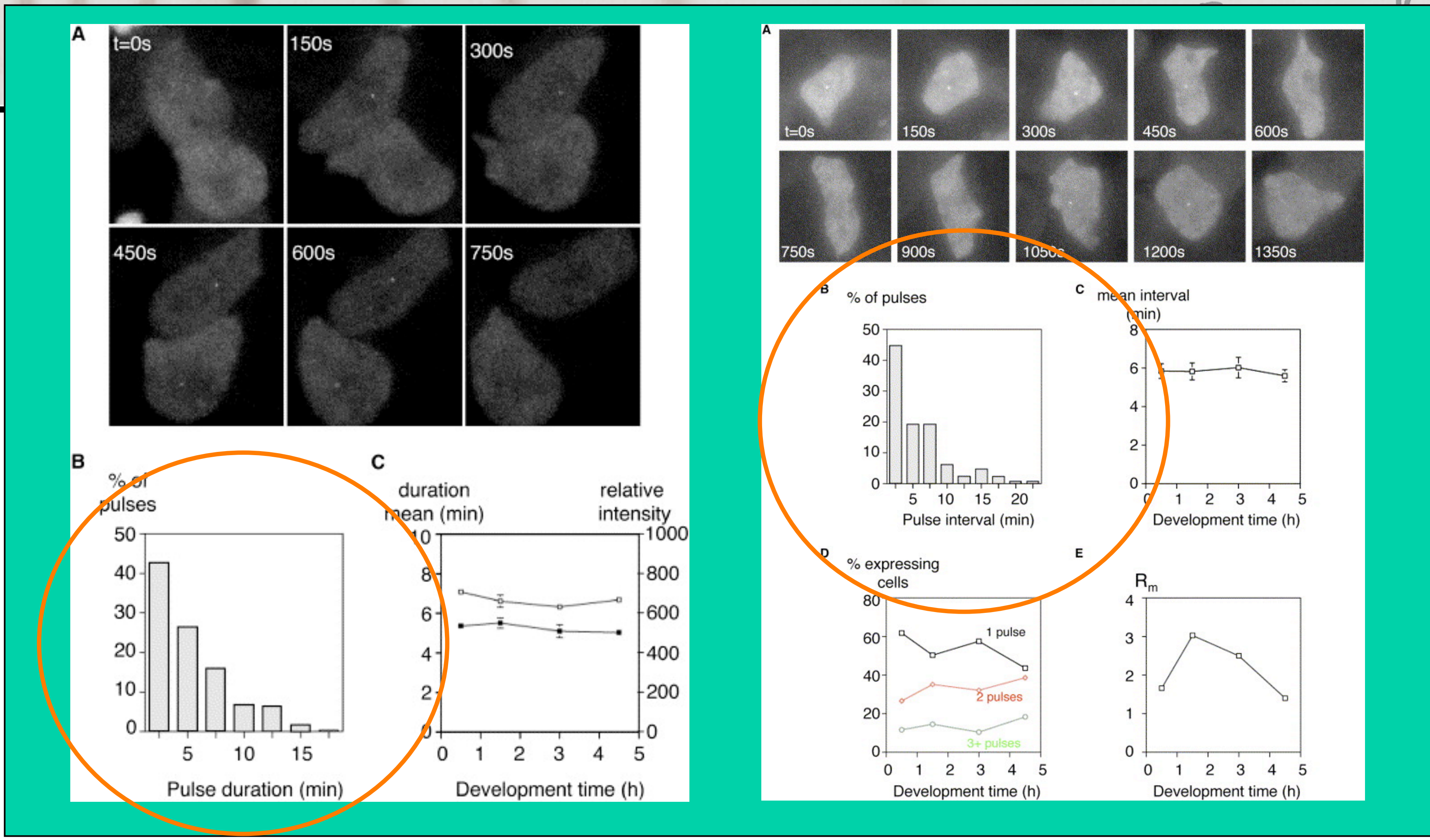
Distribution of on & off times



Distribution of burst size



Transcriptional bursting in eukaryotes



Chubb JR, Trcek T, Shenoy SM, Singer RH. *Curr. Biol.* (2006)
See also: Golding & Cox, *Curr. Biol.* (2006)

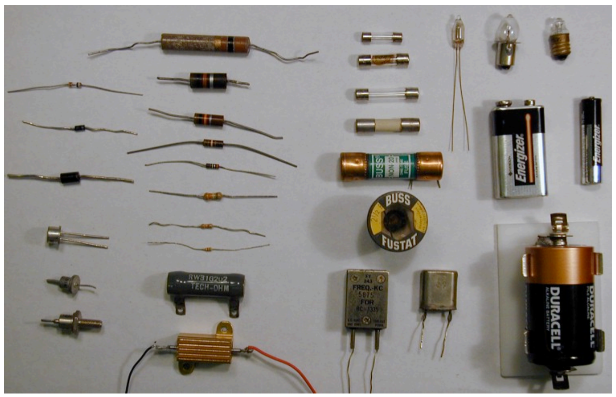
Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S, *PLoS Biol.* (2006)
"Stochastic mRNA Synthesis in Mammalian Cells".

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

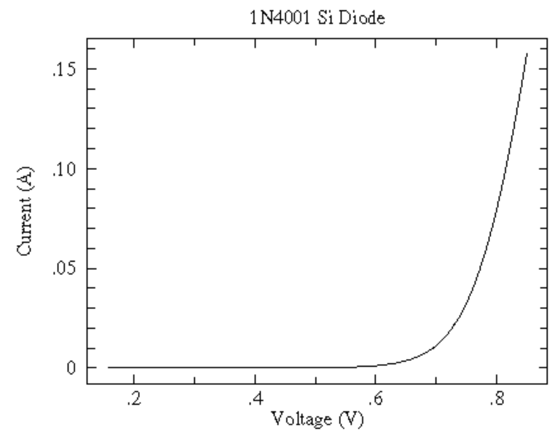
Input-Output Curves: An Analogy

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

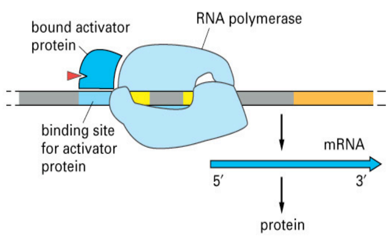
Electronic Devices

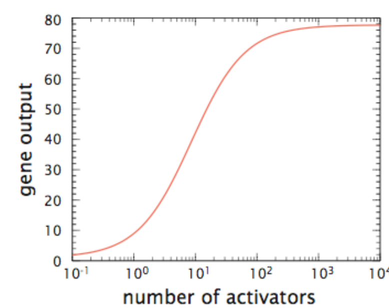


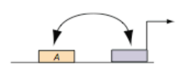
http://www.physics.csbsju.edu/trace/CC.html



Regulatory Devices







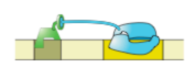


Figure 8-8 Essential Cell Biology, 2/e. (© 2004 Garland Science)

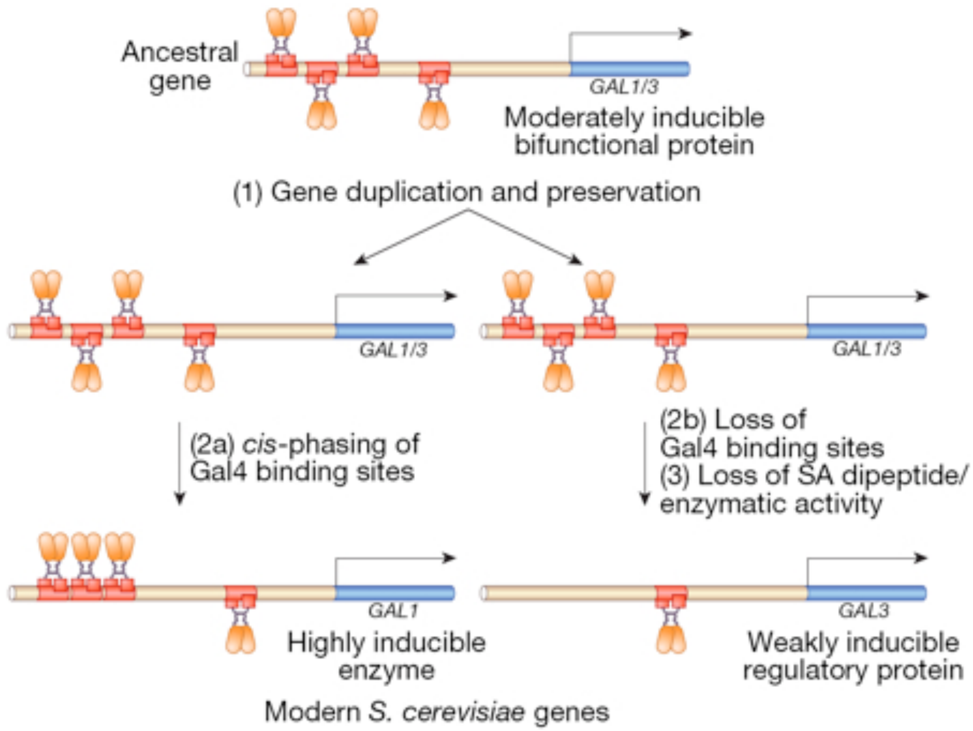
How do we know the number of molecules controlling the decision?

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

Yeast Care What They Eat Too - Galactose vs Glucose

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

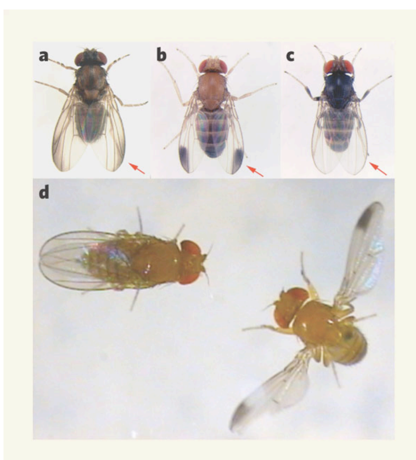
- ***Studies of the cultures of bacterial cells sufficed to lead Jacob and Monod to formulate the operon concept - the revolutionary view that there are certain genes whose mission is to control othe genes.***
- ***Amusing aside: both of the examples fr Jacob and Monod involve DNA looping form of biochemistry on a leash.***



Chance Caught on a Wing: Decision Making and Evolution

- ***Concept: study of wing patterns of gene expression a model system for probing how genes are deployed in space and time and, more importantly, a particular evolutionary mechanism.***
- ***The concept: compare different species of fly that have different patterns on their wings.***
- ***The conclusion: Not changes in the proteins themselves, just changes in the decision about which genes are turned on or off.***

(Prud'homme, Gompel and Carroll, PNAS, 2007)

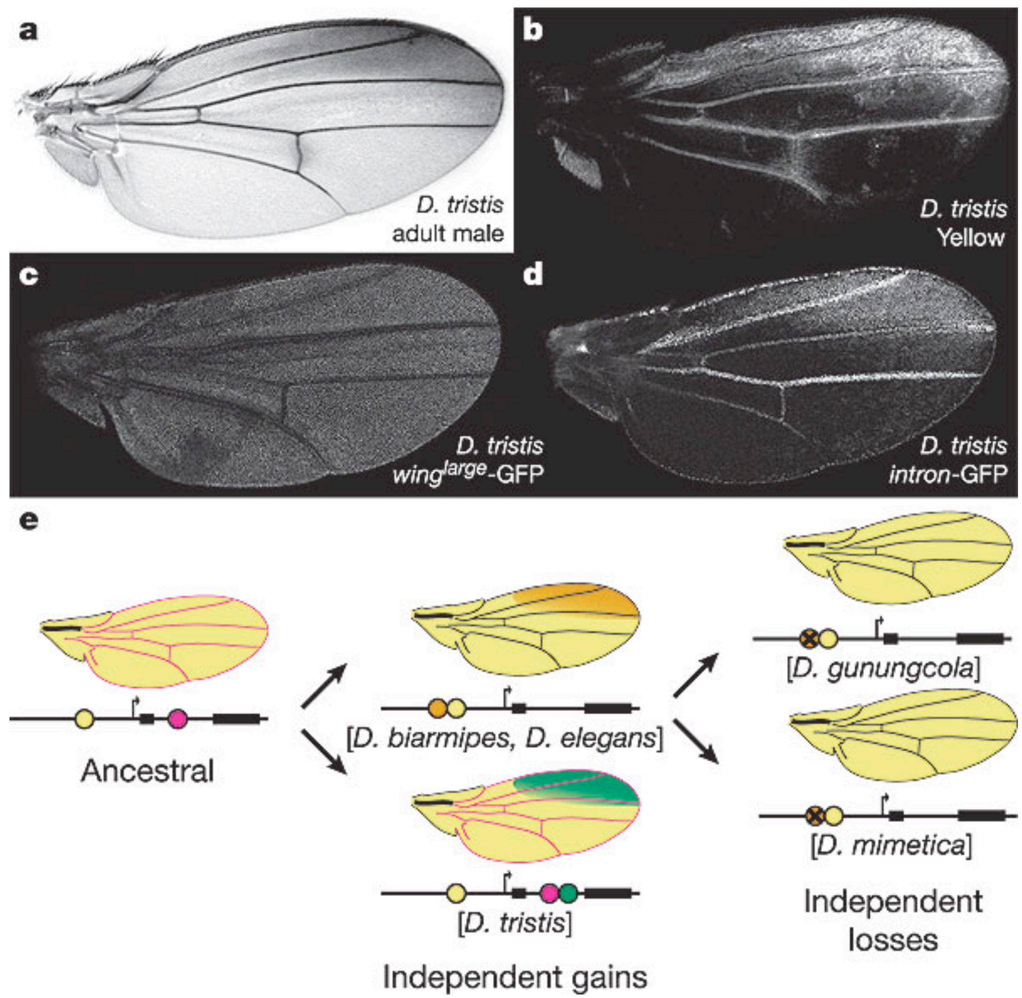


(Wray, Nature, 2006)

Chance Caught on a Wing: Decision Making and Evolution

- *This figure illustrates how the regulatory genome changes over evolutionary time.*
- *Physical question that remains as fun challenge: what is the mechanism of action at a distance?*

(Prud'homme, Gompel and Carroll, et al., Nature, 2006)



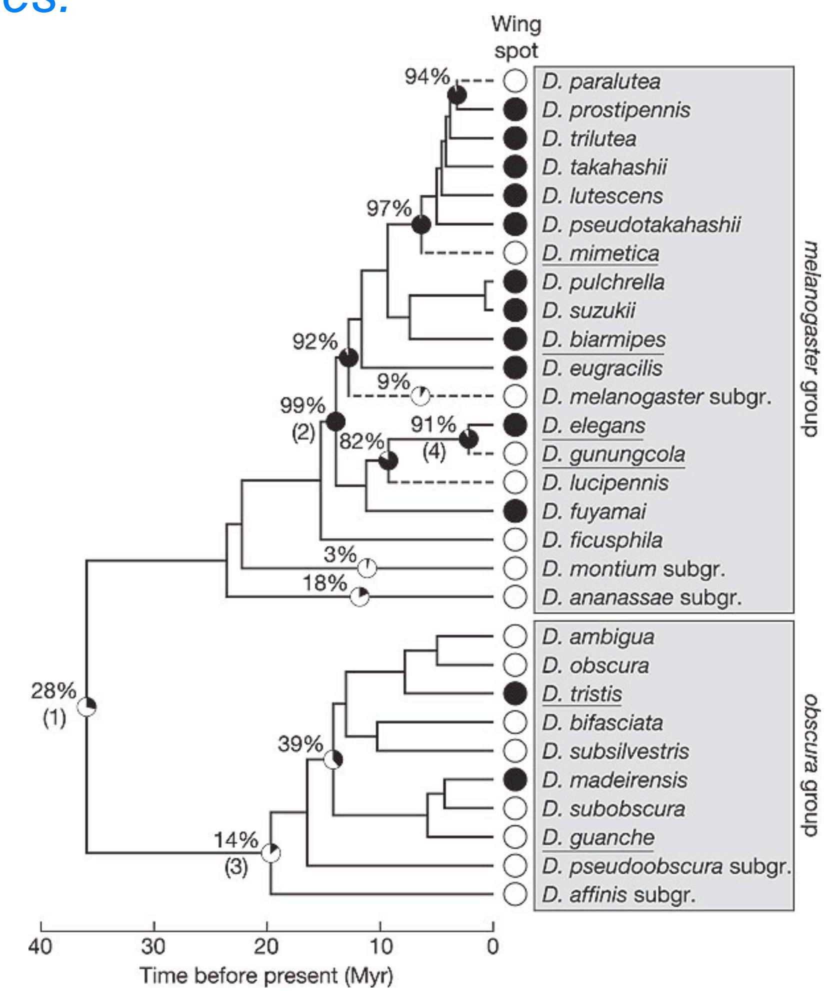
DNA molecules as "documents of evolutionary history" - these sequences are a stopwatch!

Chance Caught on a Wing: Decision Making and Evolution

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

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

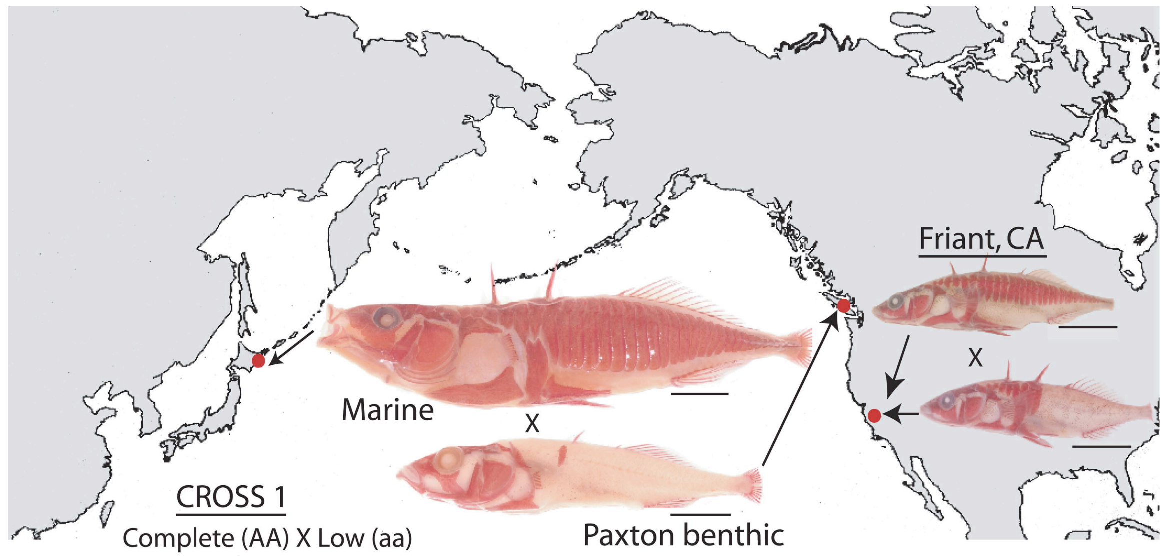
- *Molecules as "documents of evolutionary history" can be used to examine relatedness of flies.*



(Prud'homme, Gompel and Carroll, et al., Nature, 2006)

Fun Case Studies: Changes in Body Plan in Fish

- *Stickleback fish separated after last ice age more than 10,000 years ago.*
- *Morphologically, fresh water and salt water versions all differ. Some of the interesting features include pelvic reduction and the nature of their armor.*
- *Fantastic conclusion - changes not necessarily induced by changes in protein coding regions.*
- *These studies merge genetics with molecular analysis and evolutionary biology. Stunning window onto how novelty is generated in body plans.*



CROSS 1

Complete (AA) X Low (aa)

↓

F1 (Aa) X F1 (Aa)

↓

360 F2 progeny

Genotype @ Gac4174	Plate Morph: Low	Partial	Complete
AA	0	3	79
Aa	6	66	104
aa	90	2	0

CROSS 2

Complete (Aa) X Low (aa)

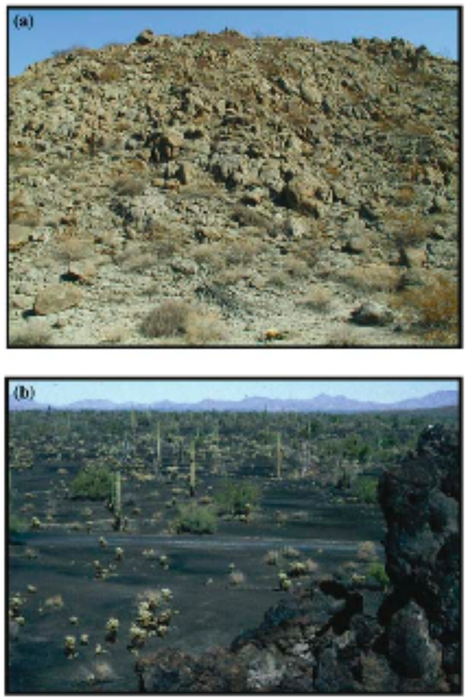
↓

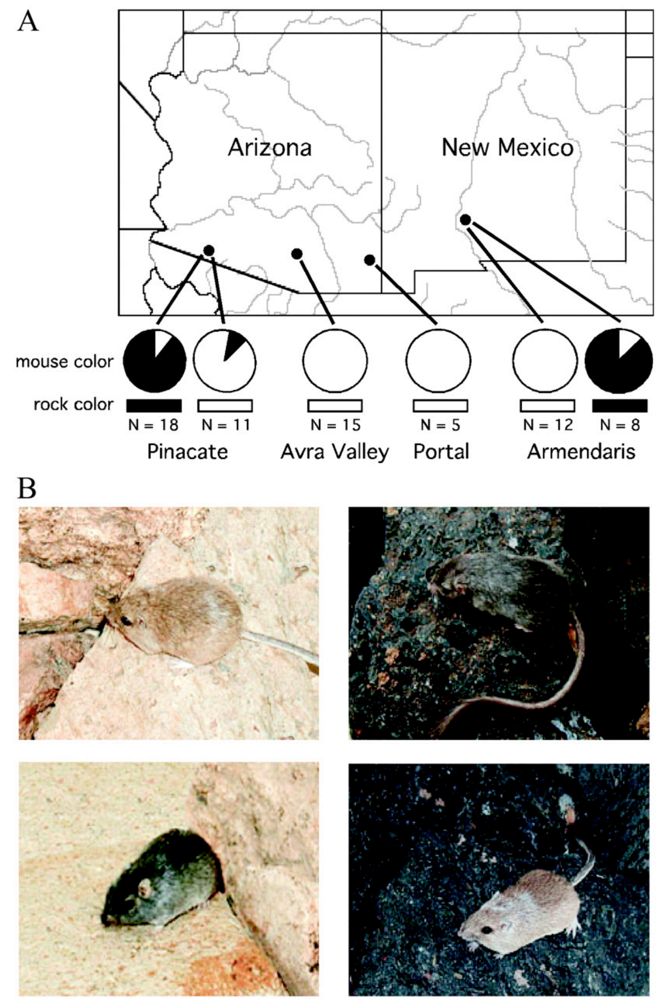
58 F1 progeny

Genotype @ Gac4174	Plate Morph: Low	Complete
Aa	2	24
aa	30	2

David Kingsley group, Stanford University

The Ingredients of Evolution: Natural Selection



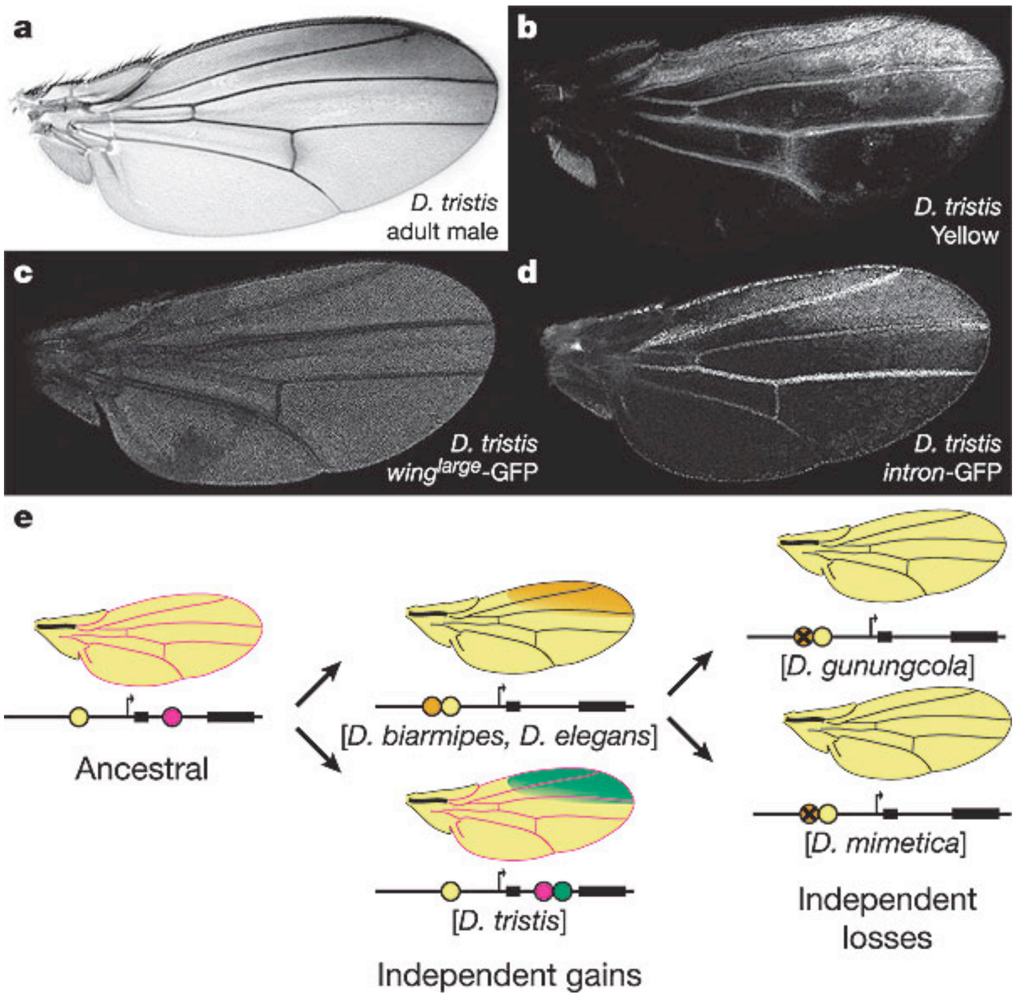


PNAS | April 29, 2003 | vol. 100 | no. 9 | 5268-5273

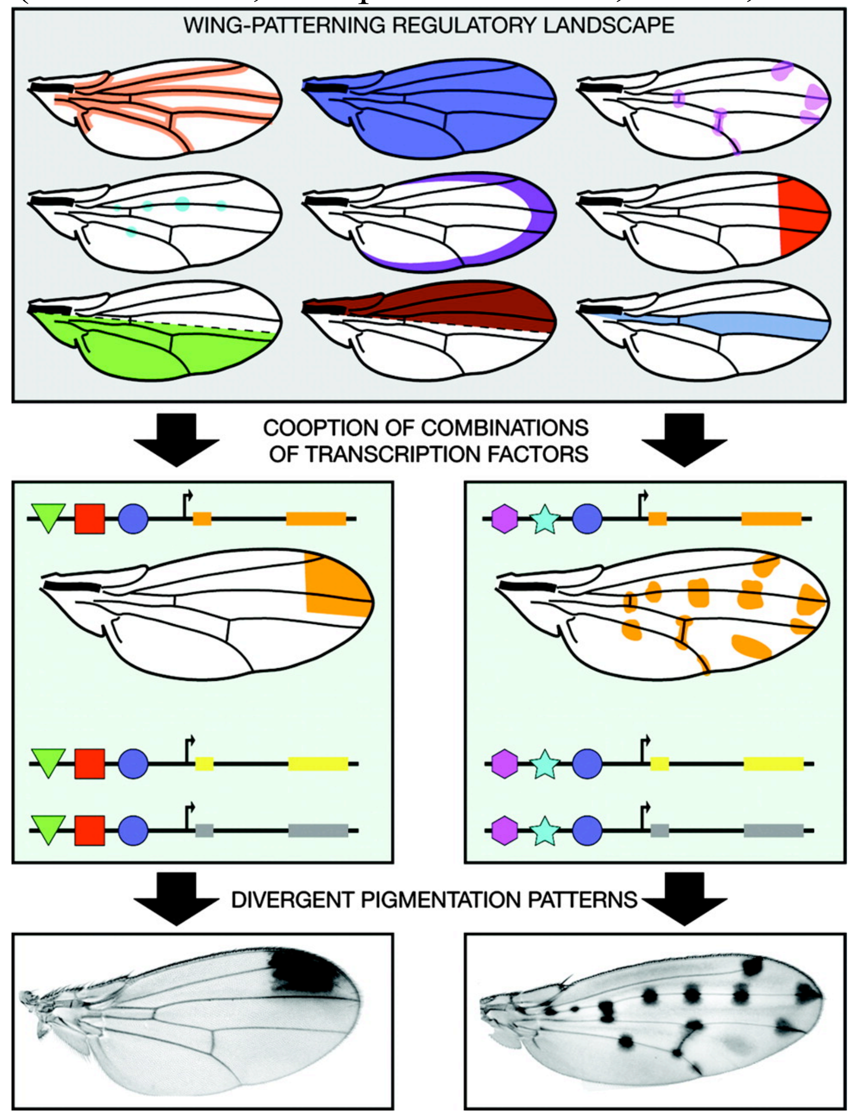
- *If the offspring have different success (``fitness"), they will yield more offspring and will drive the population in new directions.*
- *An exciting and concrete case study is that of the rock pocket mouse, some of which live on lava rock and are most often dark colored.*
- *Modest changes in a receptor protein suffice to change the color. Recurring theme!*

Chance Caught on a Wing: Decision Making and Evolution

(Prud'homme, Gompel and Carroll, et al., Nature, 2006)



(Prud'homme, Gompel and Carroll, PNAS, 2007)



DNA molecules as "documents of evolutionary history" - these sequences are a stopwatch!