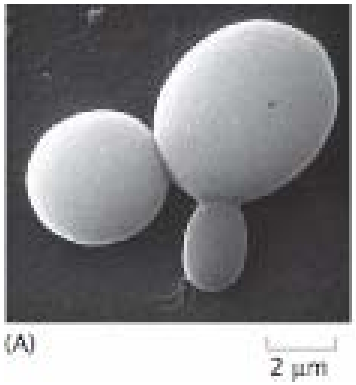
BE/APh161 – Physical Biology of the Cell

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

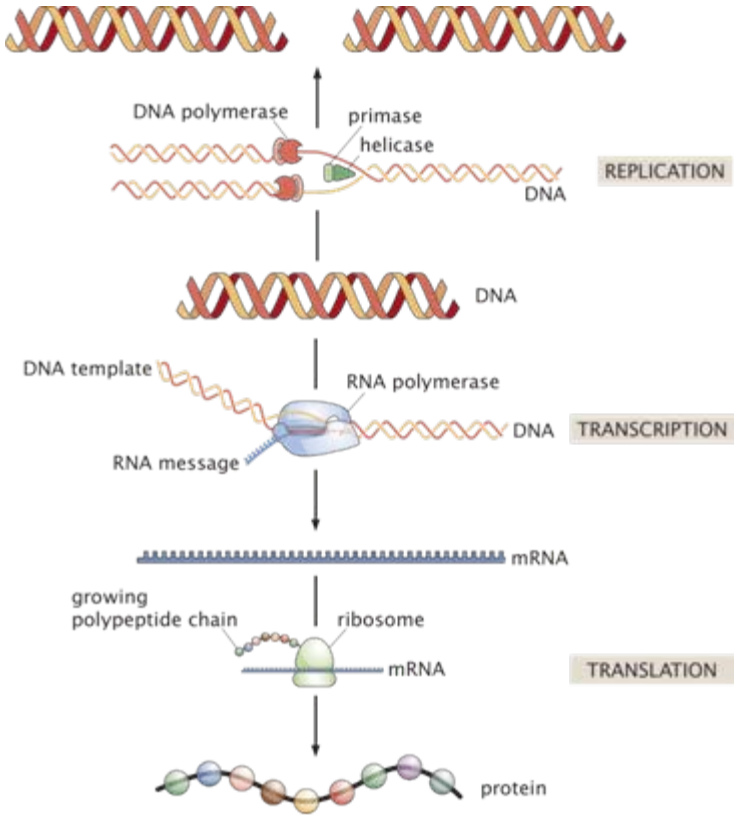


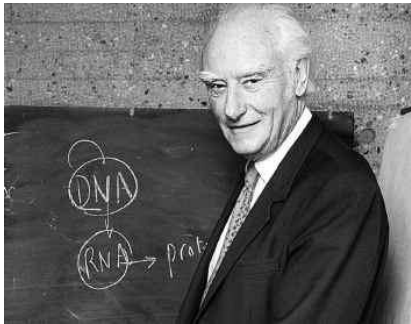
Fruit fly

- *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.***

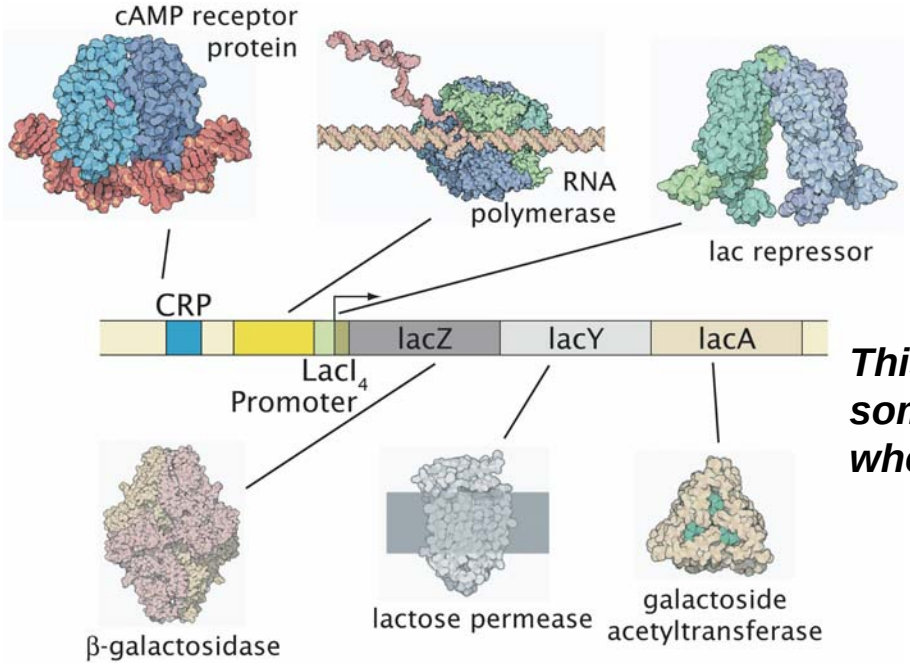




Now we have the background to tackle the question we started with: how do cells make decisions?

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.

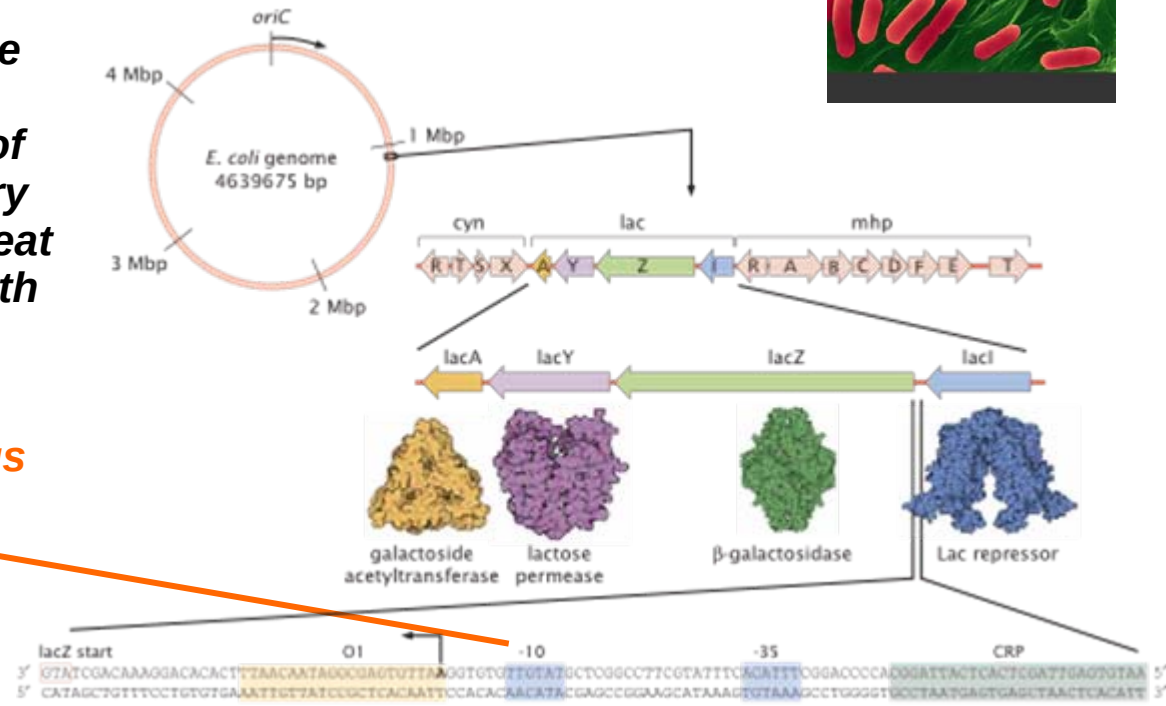


This lecture: how we found out, some beautiful examples, where we stand now.

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

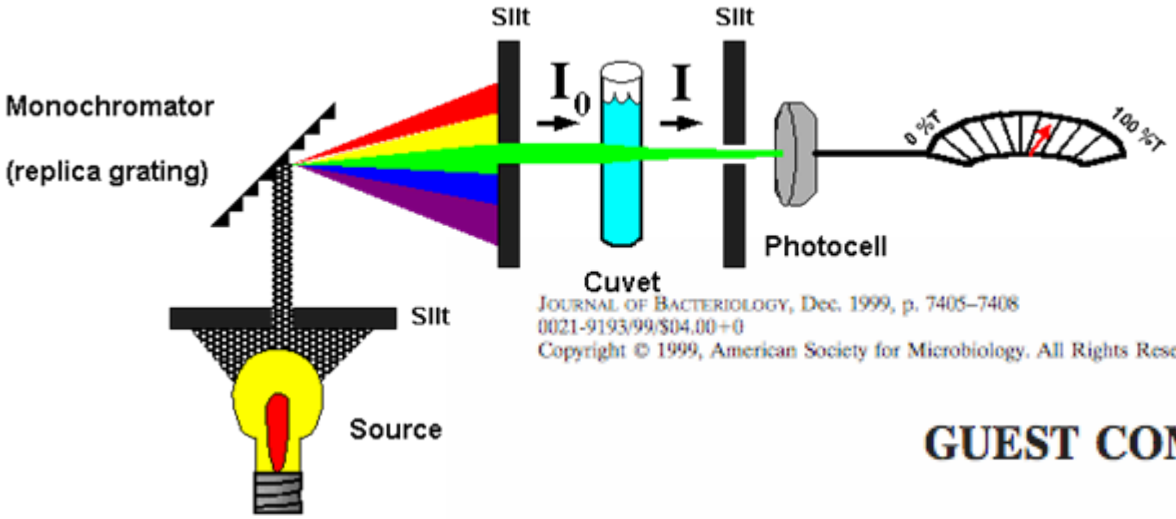
Not All DNA Codes for Proteins

- ***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

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*

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

- ***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.***

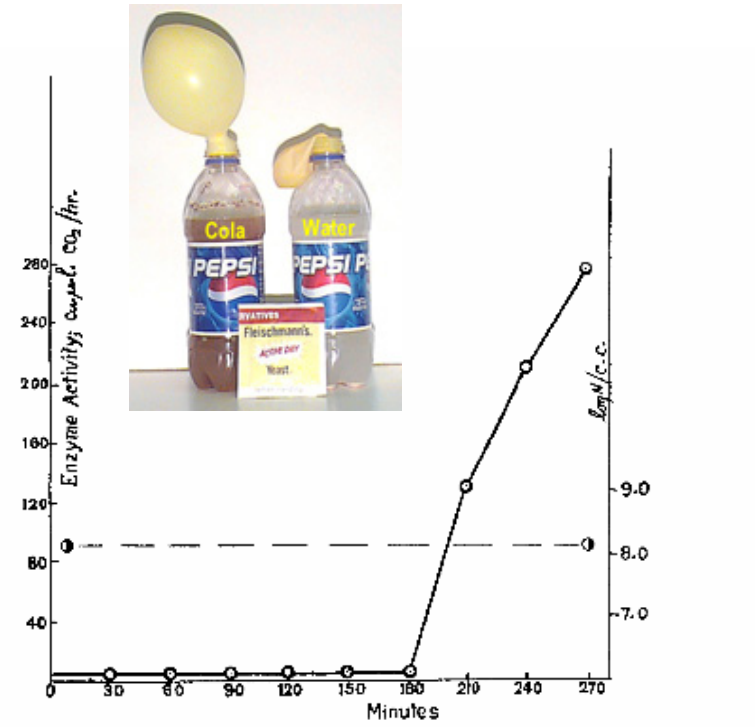
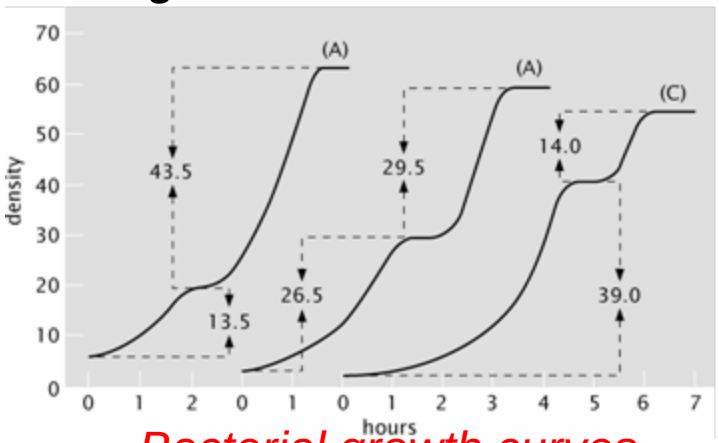


Figure 6. Adaptation to D-galactose without cell multiplication by a strain of *S. cerevisiae*: an experiment of Spiegelman and his colleagues carried out in 1943. Cells grown on D-glucose were washed in 67 mM KH_2PO_4, then resuspended in phosphate under nitrogen and D-galactose added. Carbon dioxide 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

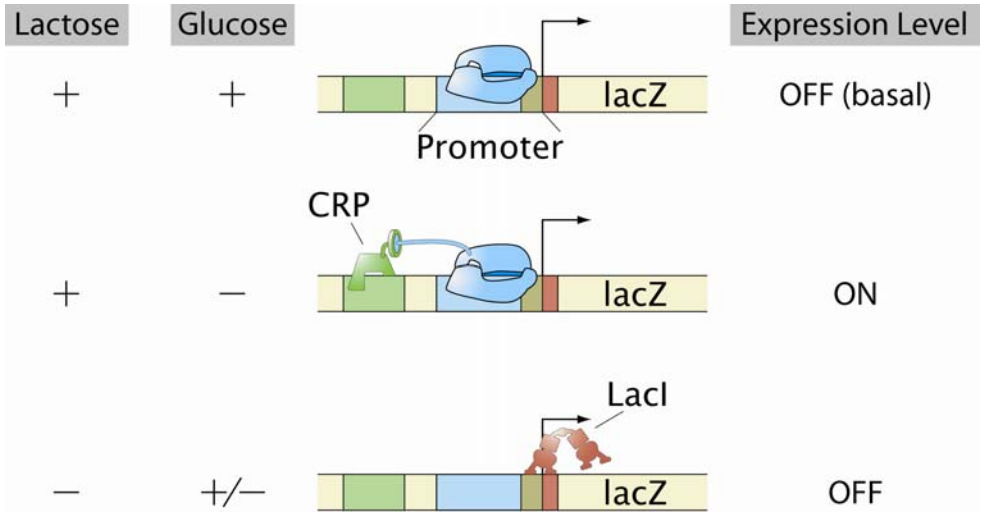
(Spiegelman et al., PNAS, 1944)

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.***

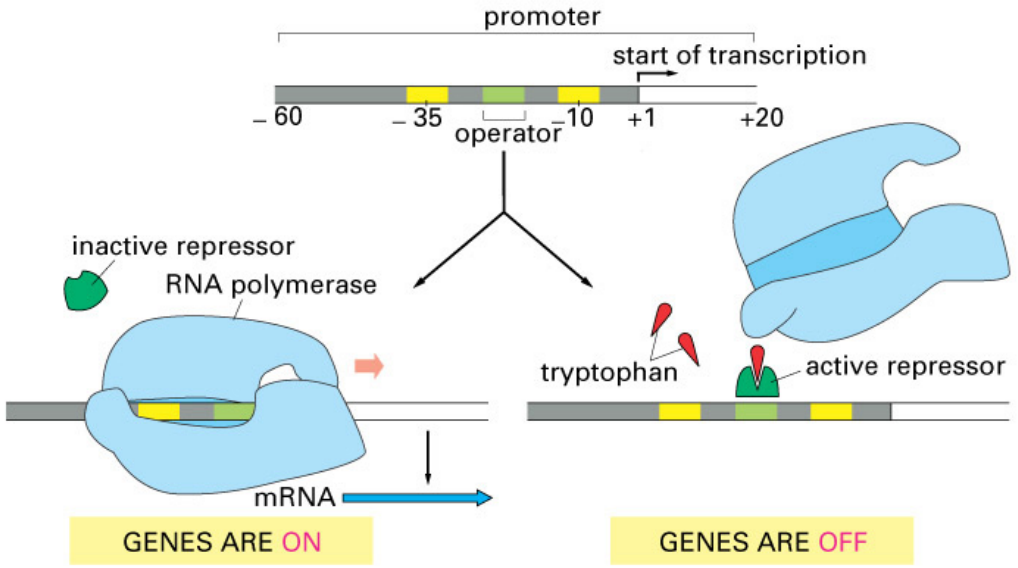
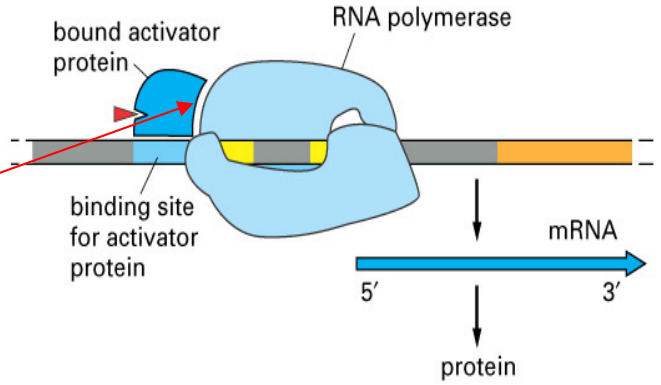


Figure 8-7 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Activators: The Cartoon

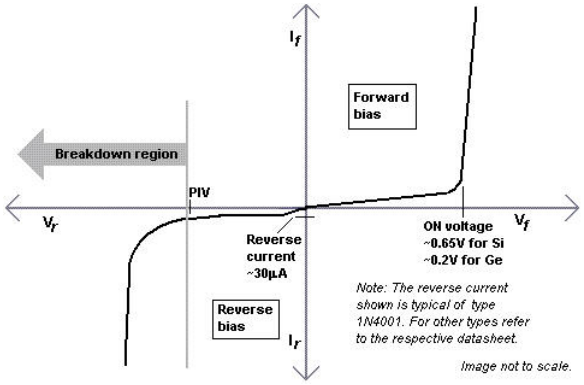
- ***Activator molecules enhance the action of RNA polymerase.***
- ***Activators can be under the control of other molecules (i.e. inducers) that dictate when activator is bound and not.***
- ***Activators "RECRUIT" the polymerase.***

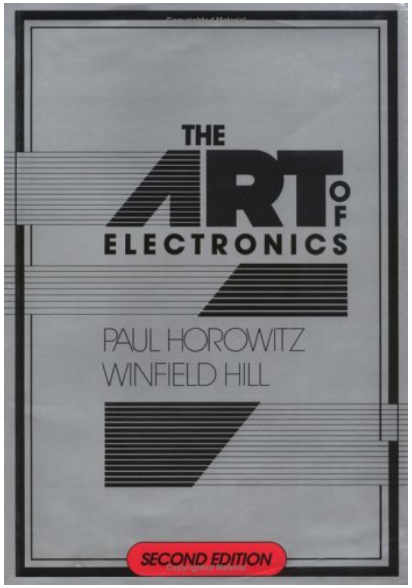


Adhesive interaction between RNAP and activator

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

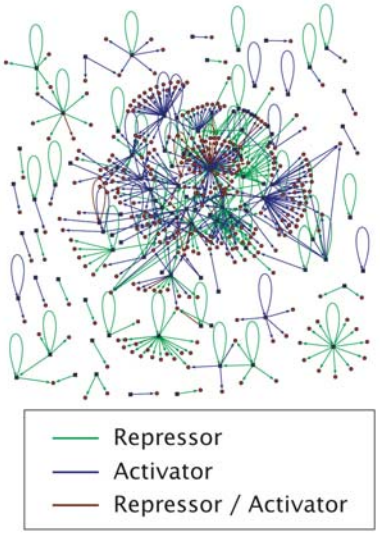
My Question: Hopeful or hopeless analogies?





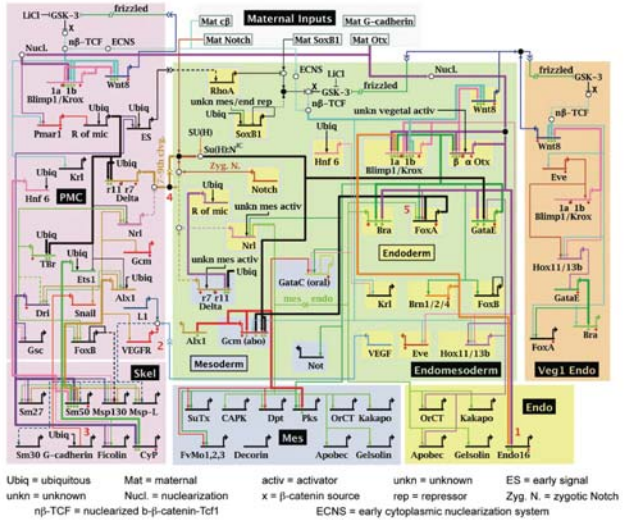
- ***Question: How well can we characterize the transfer function of regulatory networks?***
- ***Are these analogies with circuits useful and fruitful, or do they obscure a fundamental difference of kind that will make the precise characterization of input-output relations too difficult?***
- ***Goal: Derive governing equations and test them.***

E. coli
Regulatory network



Dobrin *et al.* (2004)

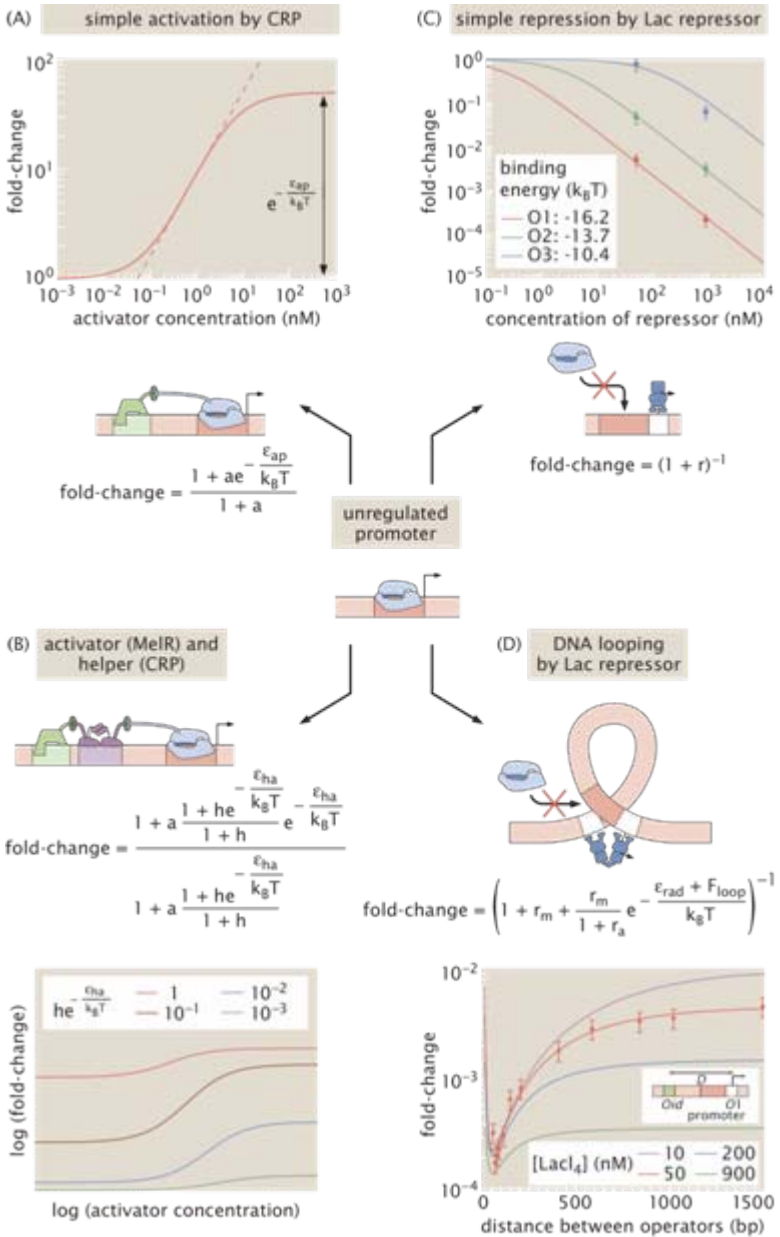
Sea urchin
Endomesoderm specification



Eric Davidson

Computing How Cells Decide: the fold Change

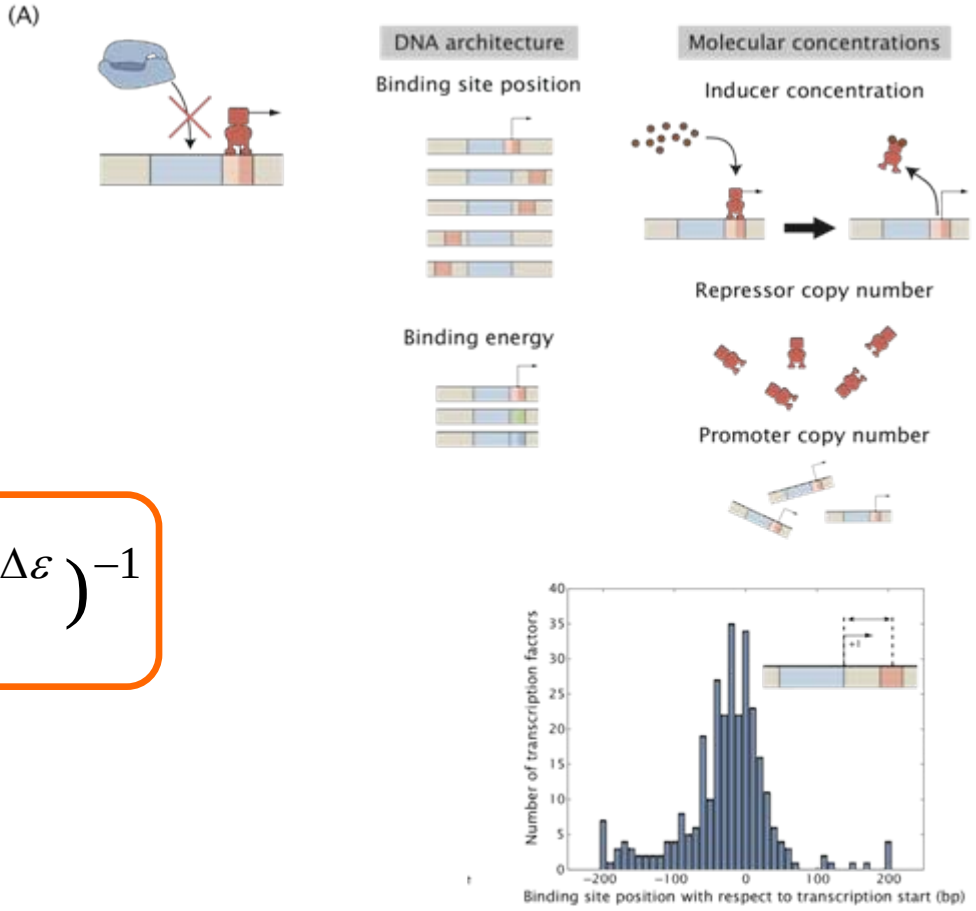
- ***The level of gene expression is described by a function that depends upon parameters such as the number of repressors and activators.***
- ***Key point: Systematic variation of parameters and examine the biological outcome. We are interested in the "fold-change" when parameters are tuned.***
- ***The equations lead to dangerous predictions (i.e. no wiggle room) for a wide variety of regulatory architectures.***



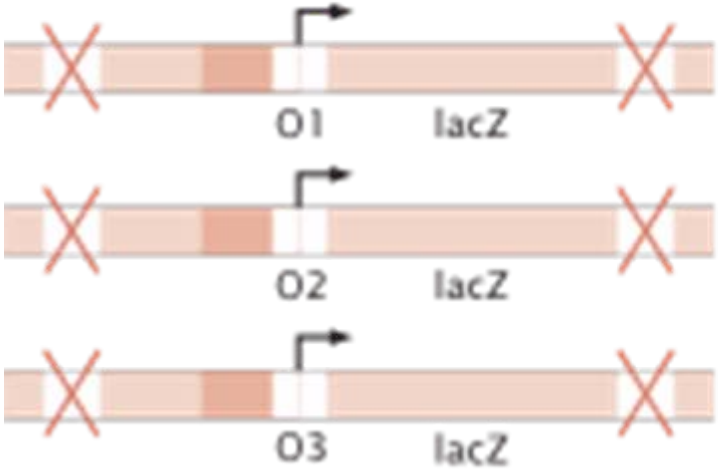
Dialing in transcriptional response: How does fold-change vary with these parameters

- ***We are interested in finding "knobs" that we can dial in both as theorists and as experimentalists.***
- ***These knobs should elicit different biological responses.***

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



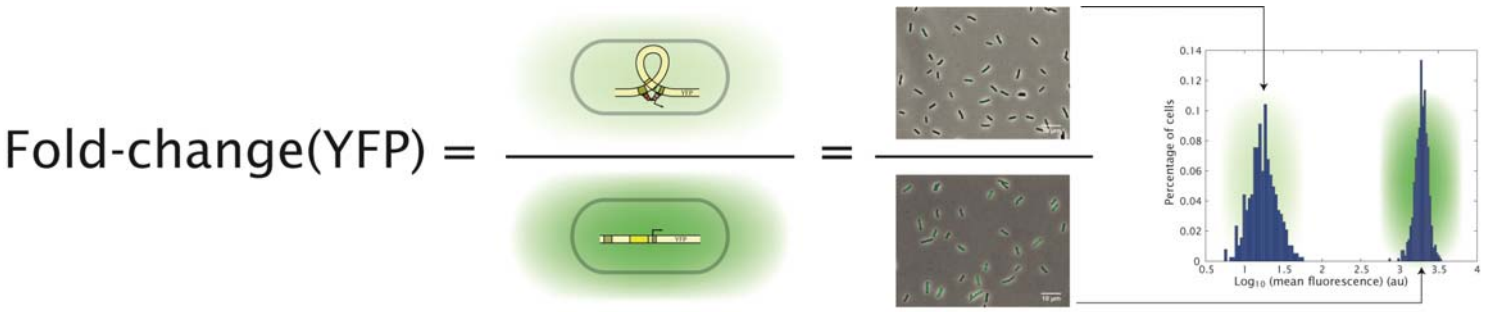
Classic experiment on simple repression

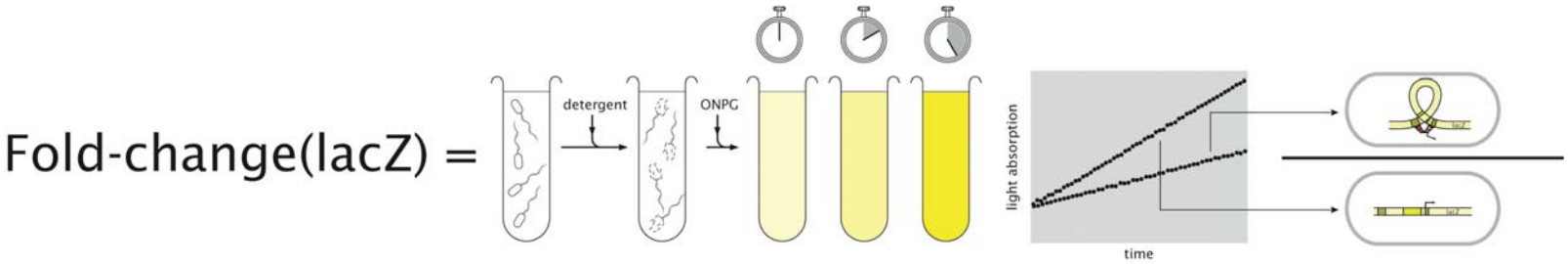


repression		
50	number of repressors	900
200		4700
21		320
1.3		16

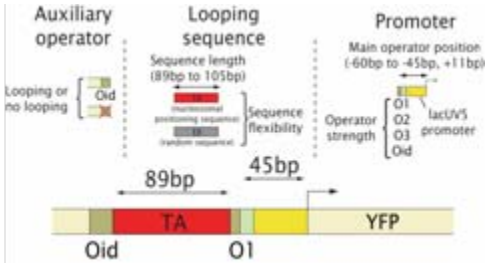
Oehler et al., 1994

Measuring fold change: The Cell as a test tube





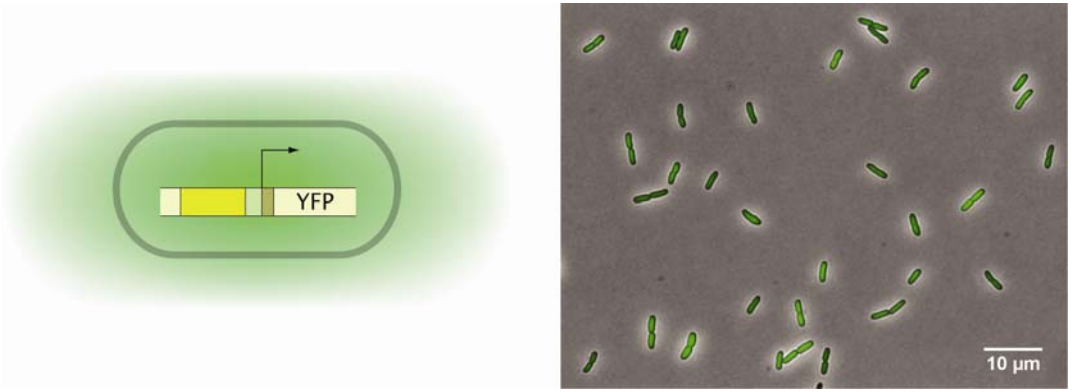
- ***Install the architecture of interest in the cell and then "read out" the state of the DNA and its battery of attendant proteins using gene expression.***



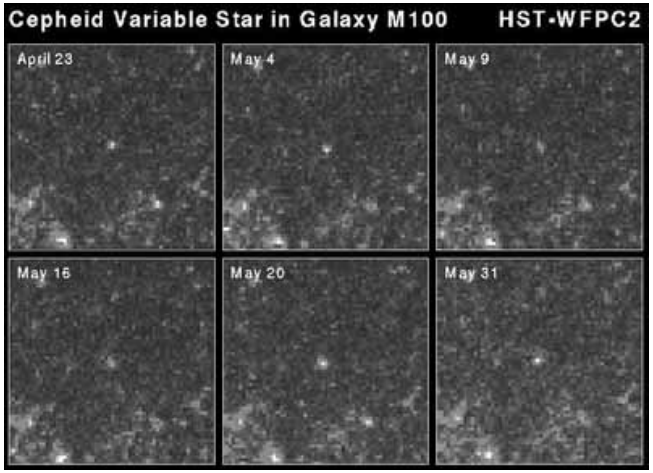
Enzymatic Assay (beta-Gal Assay)

- ***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.***

A Standard Candle for Gene Expression



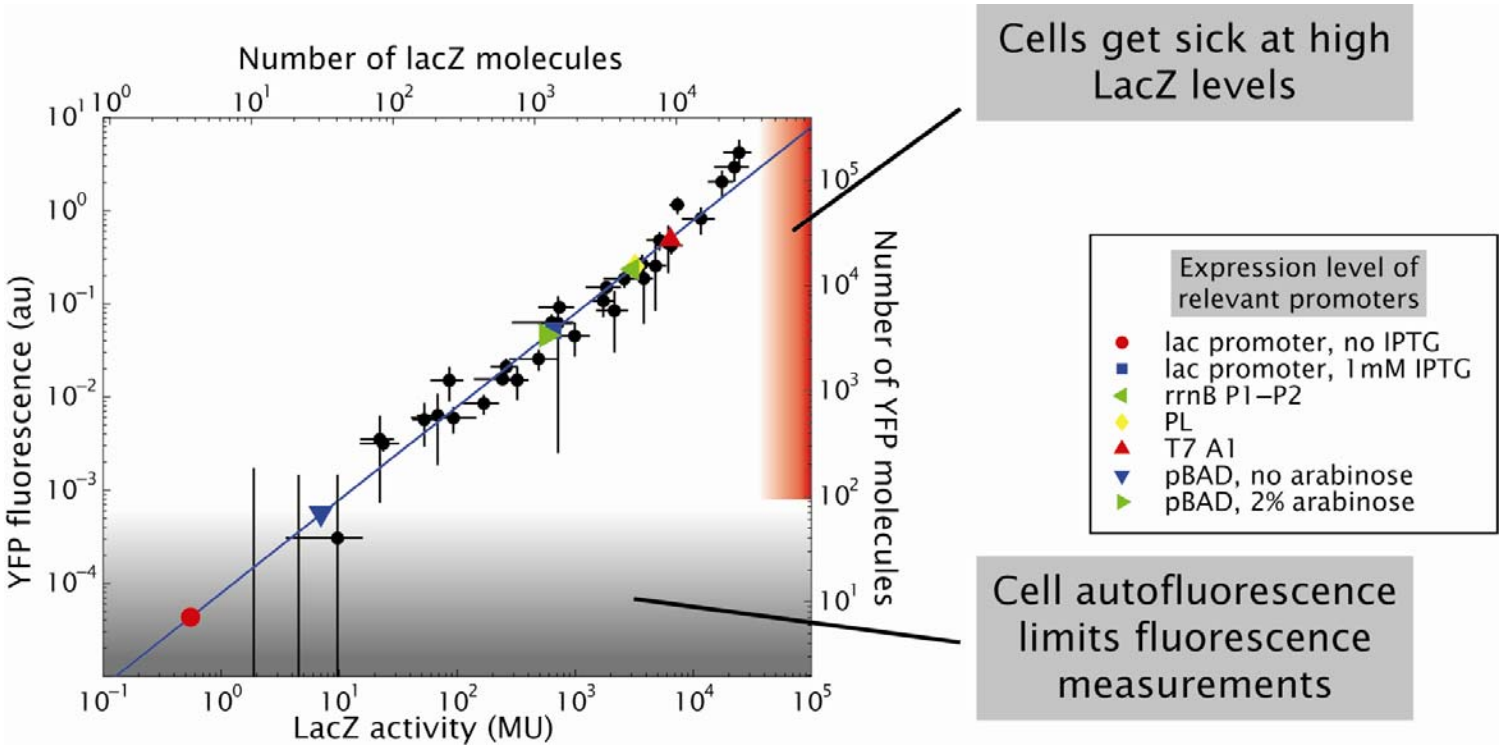
Cepheids variables



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?***

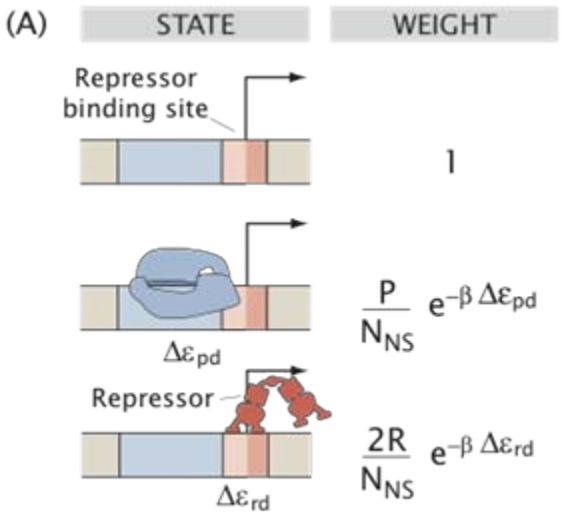
Does the Measurement Depend Upon the Technique Used?

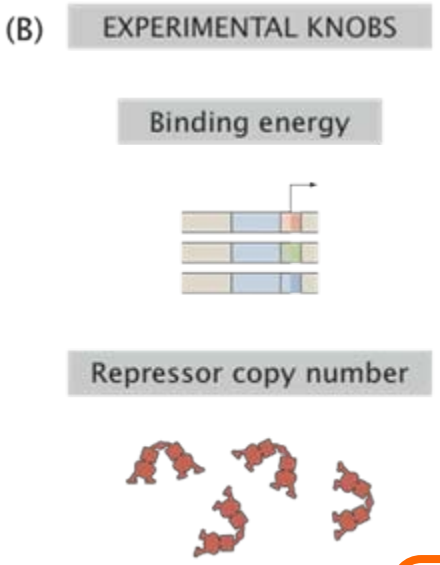


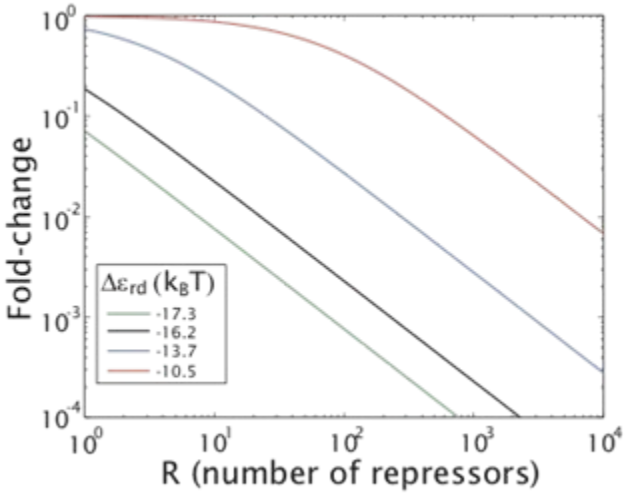
- ***One of our concerns in adopting the quantitative mindset was to see to what extent all measurements on these systems are in agreement.***
- ***But, there are many different readouts of gene expression.***
- ***With this result in hand, we turn to exploring how the expression changes in a case where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

Computing Probability of Promoter Occupancy: An Example

- ***In this case, we consider the competition between repressor and RNA polymerase for the promoter. (see Bintu et al., Vilar and Leibler)***
- ***For the simple repression motif, there is a simple expression for the fold change.***

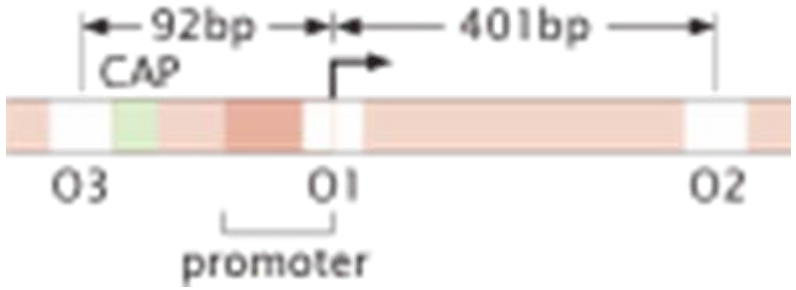


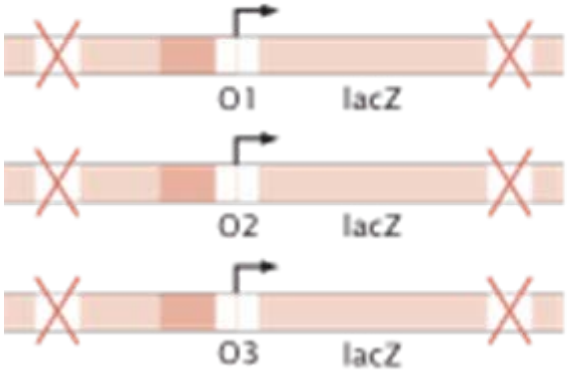




$$fold\ change = (1 + \frac{R}{N} e^{-\beta \Delta\varepsilon})^{-1}$$

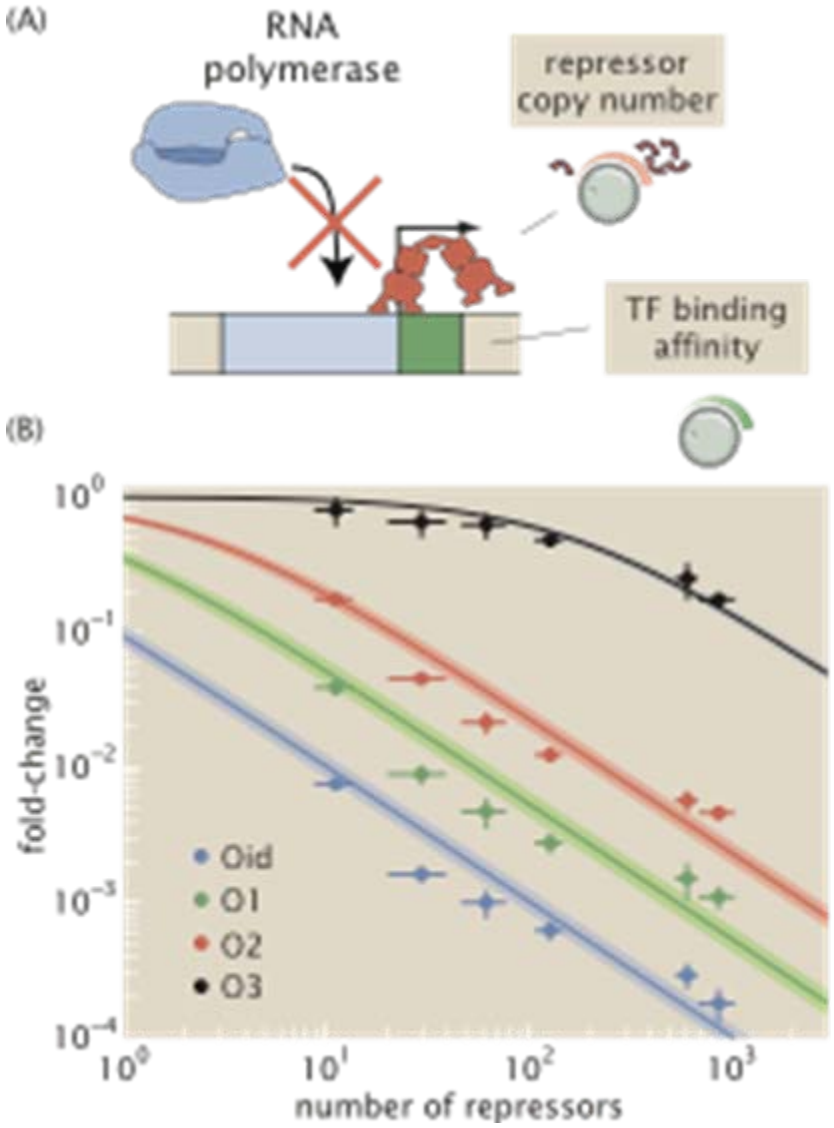
Simple repression constructs and data





repression		
50	number of repressors	900
200		4700
21		320
1.3		16

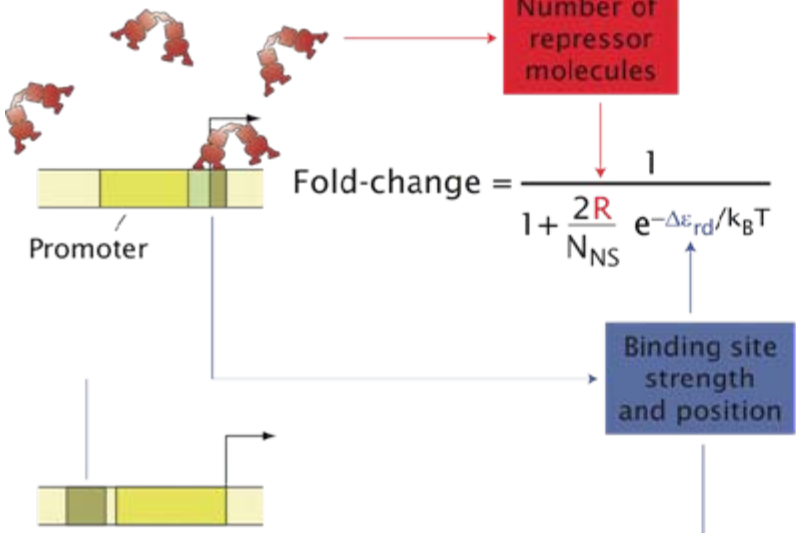
Simple repression data

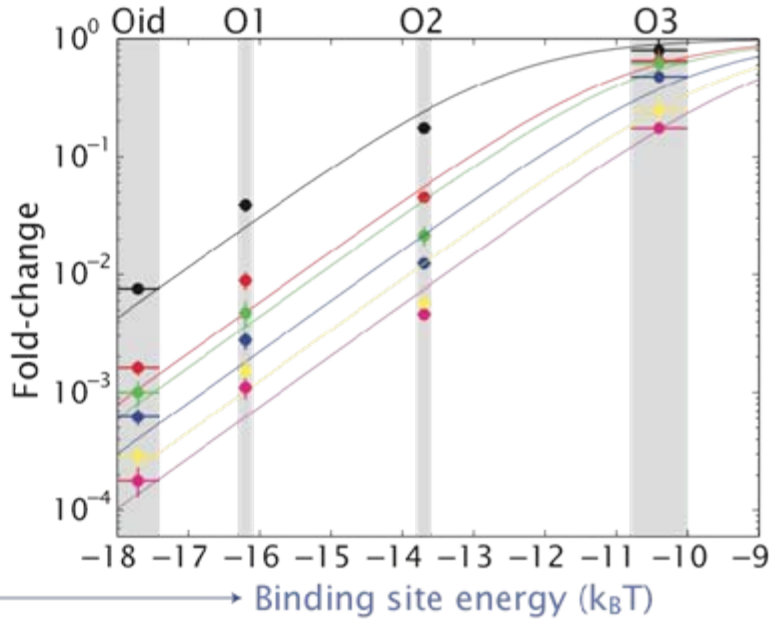


Parameter-free predictions of the repressor number: Taking the census by thinking

- ***Using the in-vivo binding energies determined from the Oehler et al. experiment and the measured fold-changes, we can unequivocally determine the number of repressors that are doing the repressing (at least as far as the statistical mechanics model is concerned).***

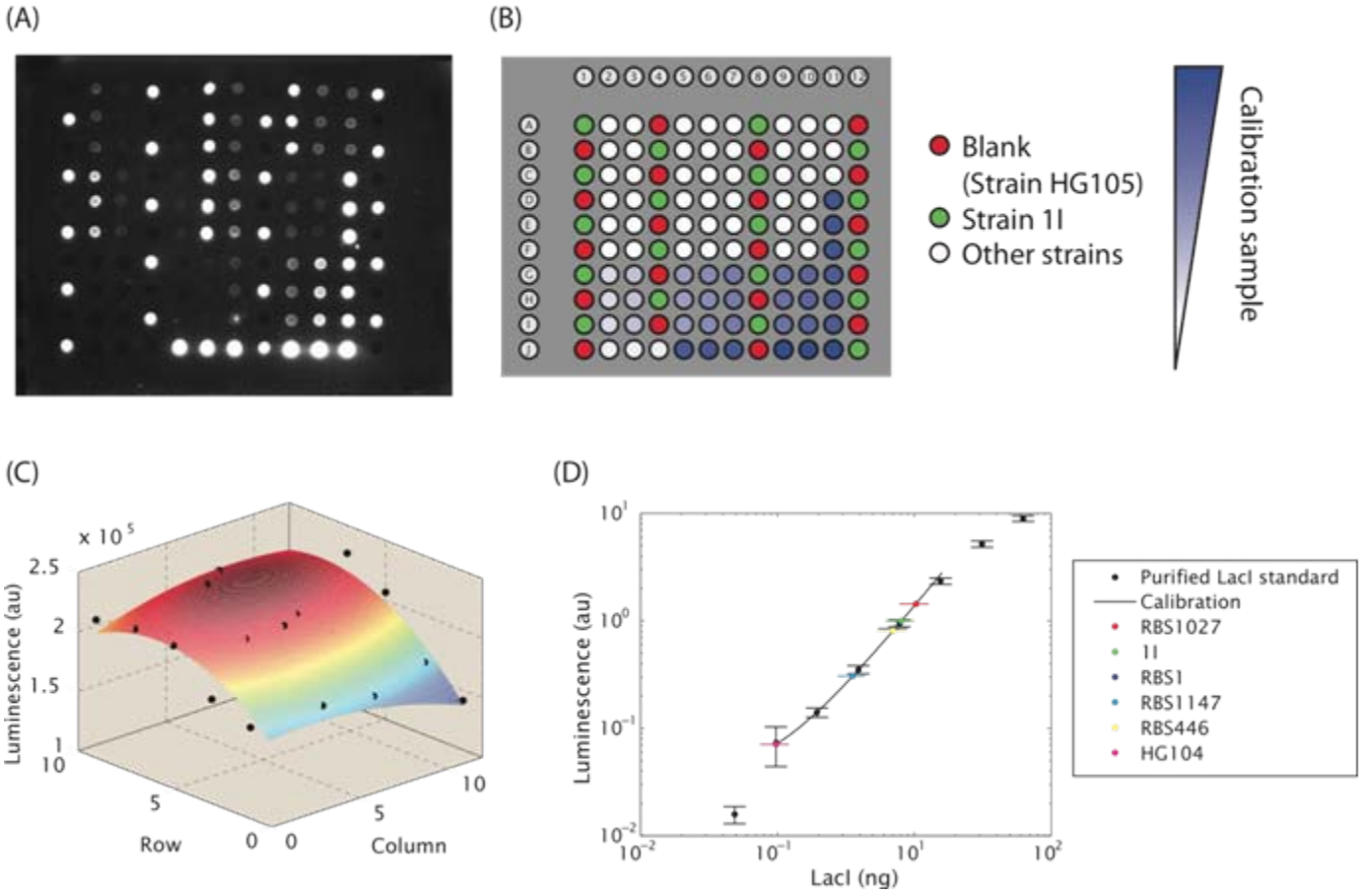
Strain	Prediction (repressors/cell)
HG104	9 ± 1
RBS1147	48 ± 8
RBS446	60 ± 20
RBS1027	130 ± 40
RBS1	220 ± 70
11	400 ± 100





Learning to count all over again

- ***We have used our model to take the repressor census, now we need to find out if that census is correct.***
- ***Concept: break open the cells, paint their contents onto a surface, quantify the number of molecules by decorating them (using antibodies) with luminescent probes.***



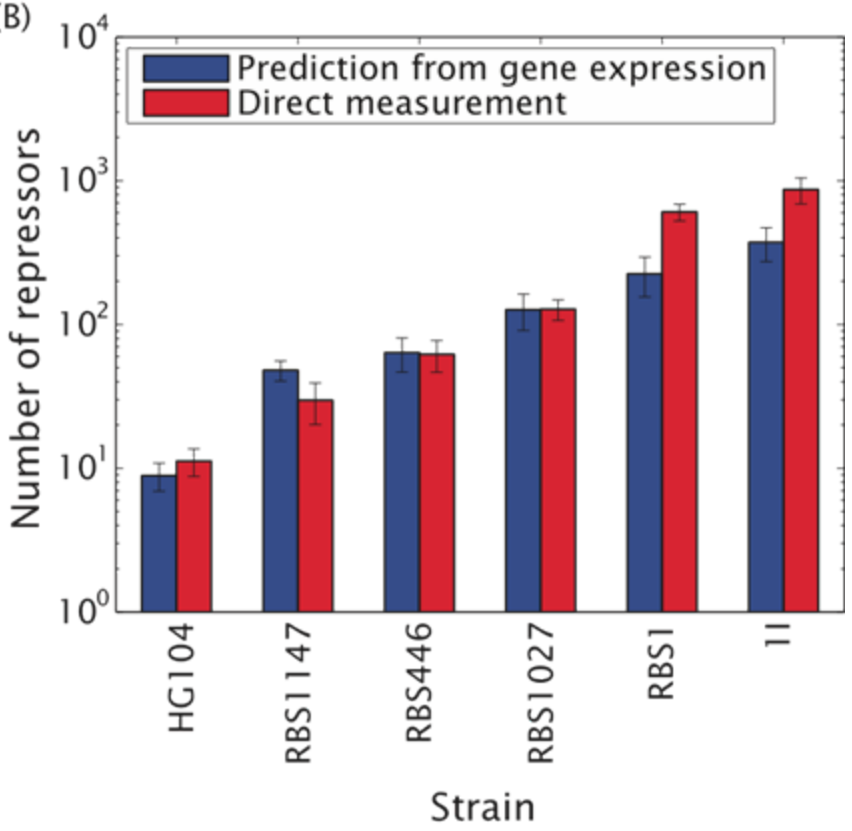
The outcome

- ***The model appears to be a viable quantitative description of the regulatory architecture.***
- ***In the strongly repressed limit (i.e. very little expression, large number of repressors), the model seems to systematically underestimate the number of repressors.***

(A)

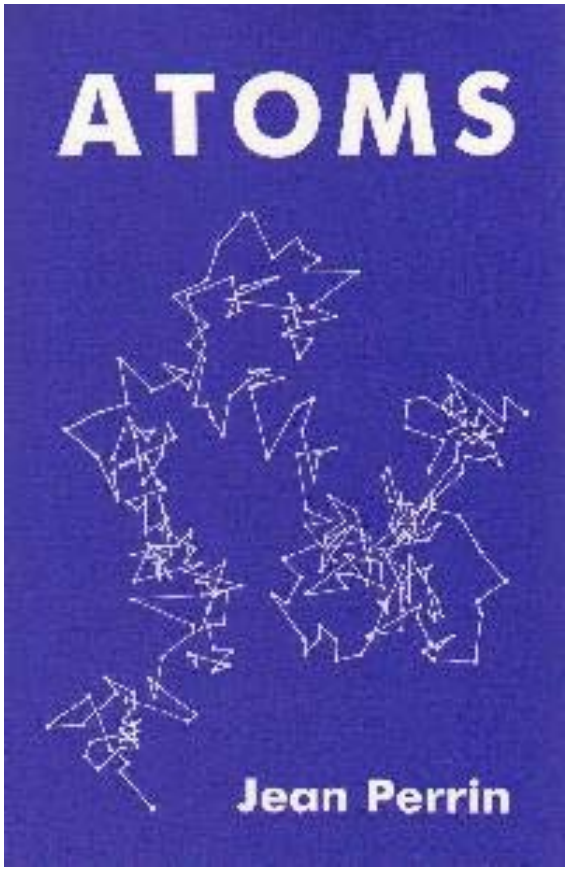
Strain	Direct measurement (repressors/cell)
HG104	11 ± 2
RBS1147	30 ± 10
RBS446	62 ± 15
RBS1027	130 ± 20
RBS1	610 ± 80
11	870 ± 170

(B)



Coming at Problems Many Ways: Perrin, Avogadro and Fluctuations

- ***Intriguing tradition of using fluctuation-based methods to shed light on the system of interest. After this table, Perrin says: "the existence of the molecule is given a probability bordering on certainty."***
- ***Perhaps boring (not to me) but necessary: do different approaches to the same problem agree?***



CONCLUSIONS.

120.—THE AGREEMENT BETWEEN THE VARIOUS DETERMINATIONS.—In concluding this study, a review of various phenomena that have yielded values for the molecular magnitude enables us to draw up the following table :—

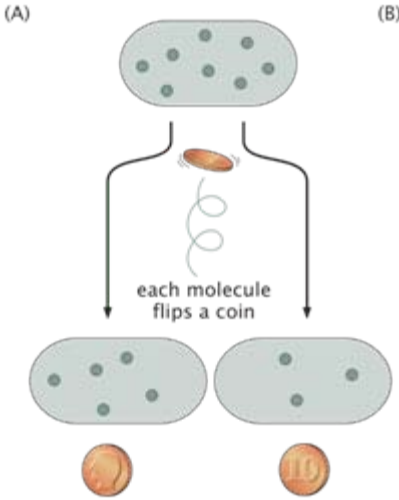
Phenomena observed.[1]		$\frac{N}{10^{22}}$.
Viscosity of gases (kinetic theory)		62 (?)
Vertical distribution in dilute emulsions		68
Vertical distribution in concentrated emulsions		60
Brownian movement	*Displacements*	64
	Rotations	65
	Diffusion	69
Density fluctuation in concentrated emulsions		60
Critical opalescence		75
Blueness of the sky		65
Diffusion of light in argon		69
Black body spectrum		61
Charge as microscopic particles		61 (?)
Radioactivity	*Projected charges*	62
	Helium produced	66
	Radium lost	64
	Energy radiated	60

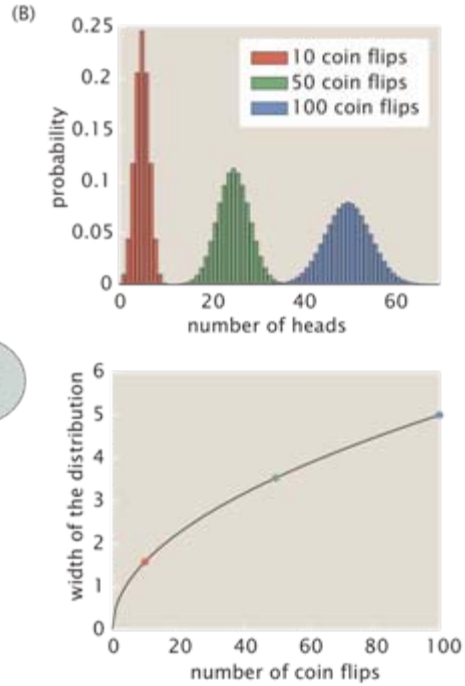
Our wonder is aroused at the very remarkable agreement found between values derived from the consideration of such widely different phenomena. Seeing that not only is the same magnitude obtained by each method when the

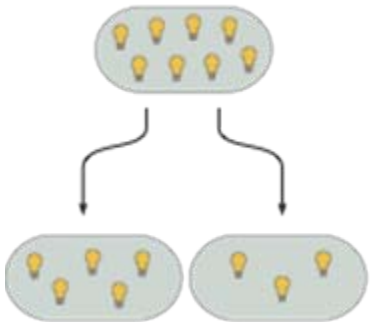
[1] Methods by which it may be hoped, in the future, to obtain results of great precision are given in italics.

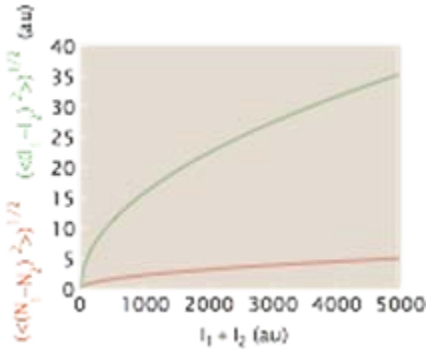
Cells do vegas

- ***If the partitioning is random, then the statistics will be like those resulting from coin flips.***
- ***Indeed, one of the main points of my whole talk is the way in which again and again there are biological secrets hidden in distributions.***
- ***Cleverly, the fluctuations can be used to establish the standard candle!***







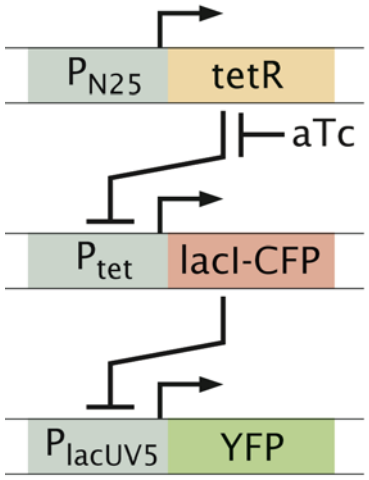


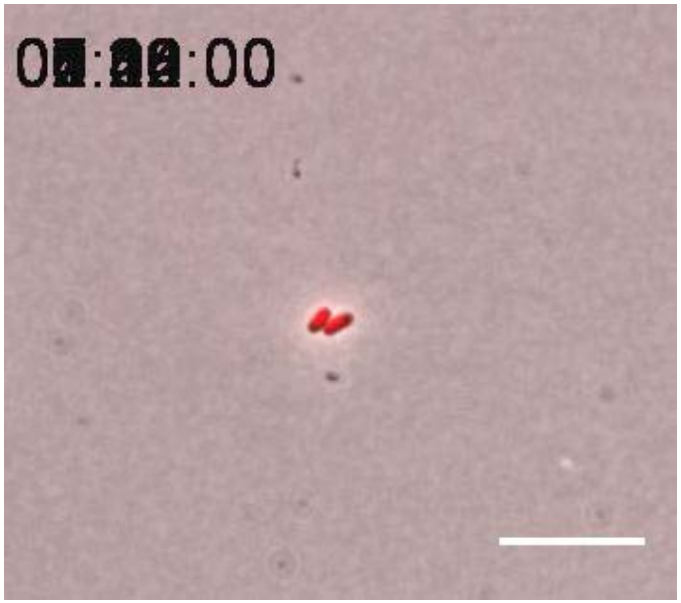
$$\sqrt{< (I_1 - I_2)^2 >} = \sqrt{\alpha I_{tot}}$$

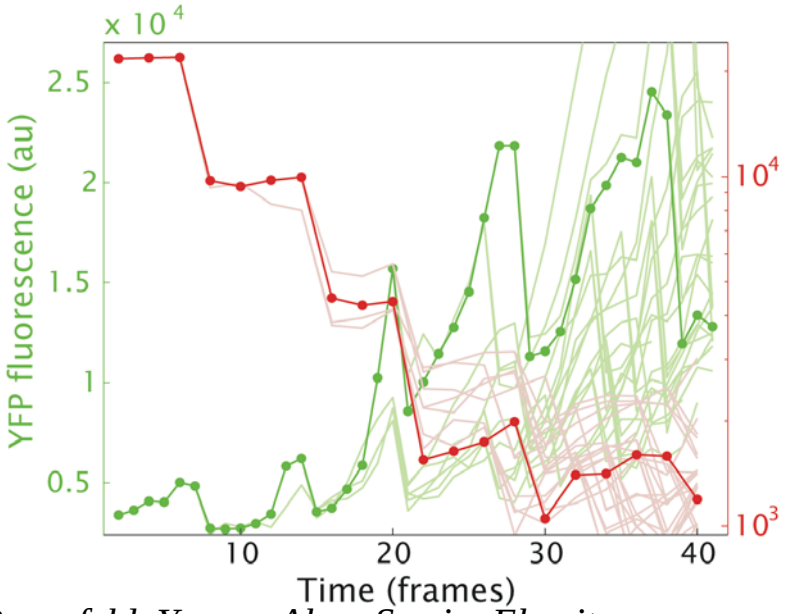
Using Drunks to Count Proteins and Measure Expression

- ***Asymmetry in partitioning of proteins during cell division gives a way to determine the calibration factor relating fluorescence and repressor count. This suggested a cool new way to count and to measure the whole fold-change function.***

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



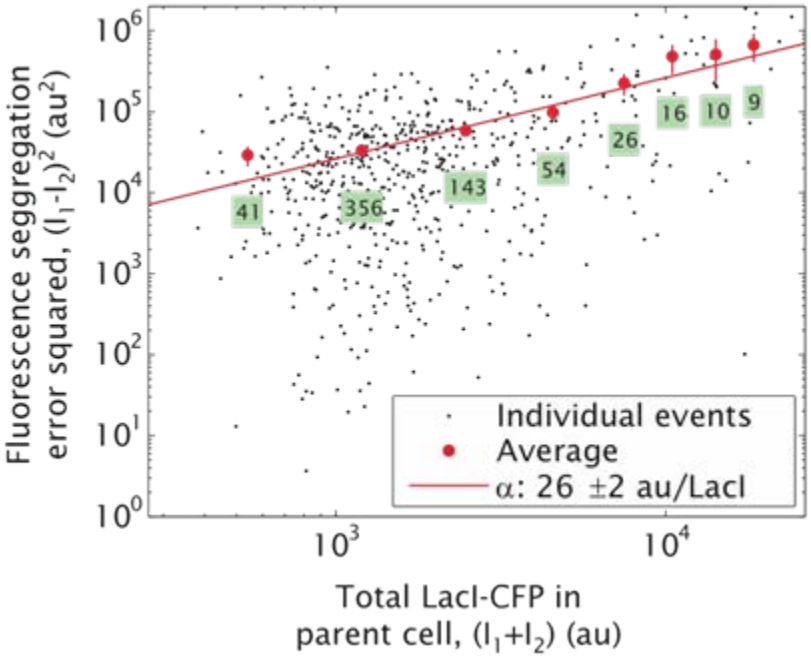


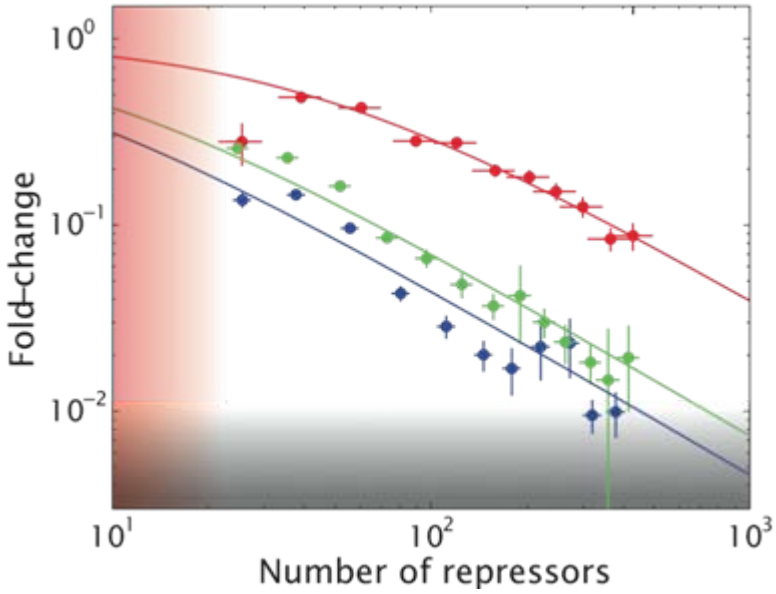


(Rosenfeld, Young, Alon, Swain, Elowitz, Science, 2005)

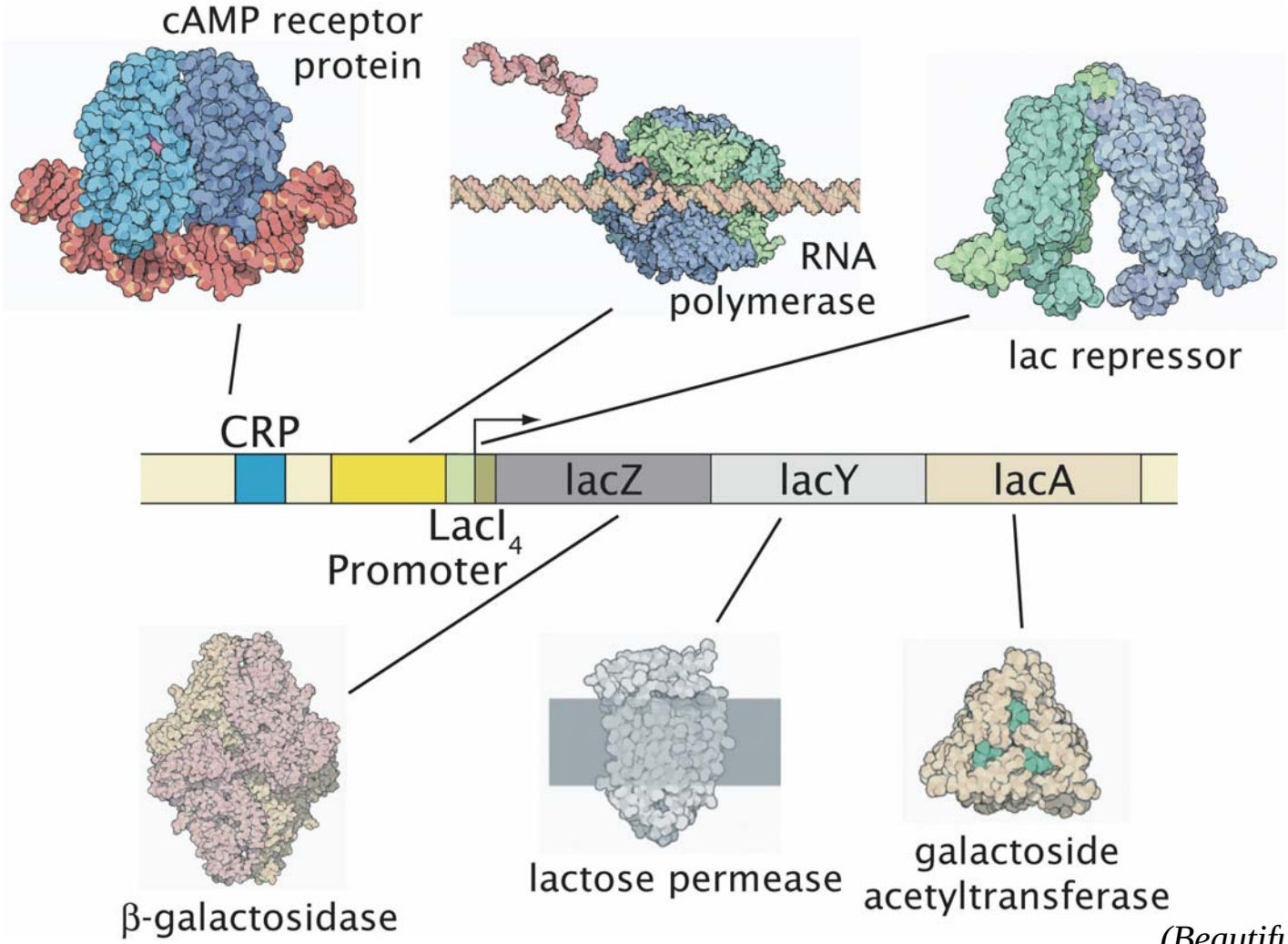
The calibration and the fold change

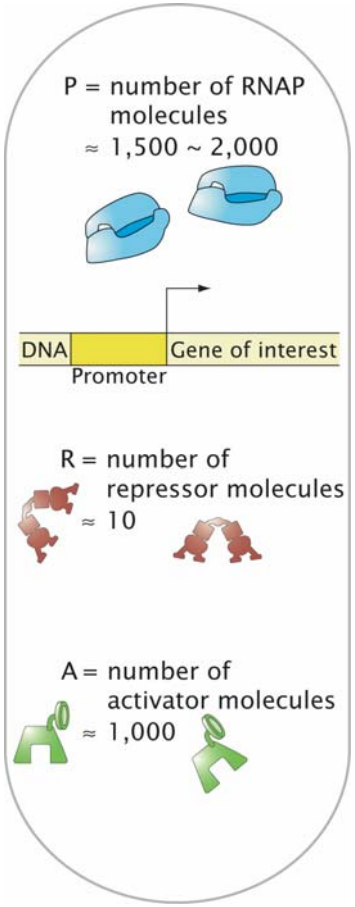
- ***Recall, the calibration factor allows us to eliminate the unwanted "arbitrary units" that fluorescence is usually reported in and to replace it with explicit molecular counts.***
- ***The theory predicts a particular functional form for the fold-change for different choices of the binding strength. Think back to the electronic circuit analogy – this is the analog of the IV curve for the regulatory circuit.***





Lac Operon: The Single Molecule Census



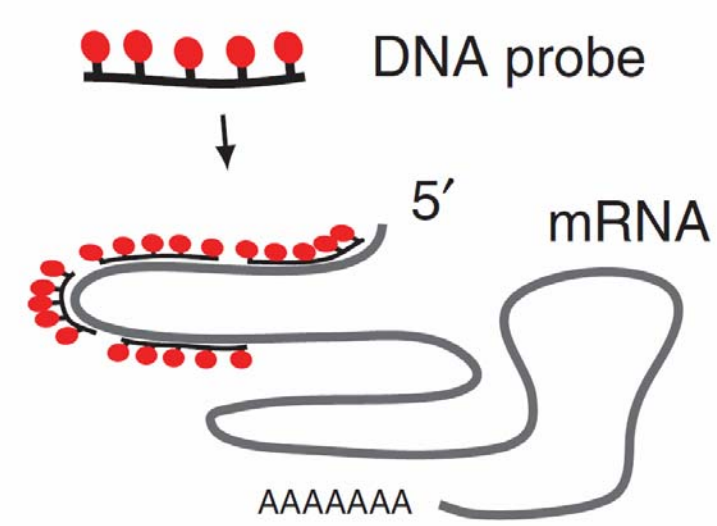


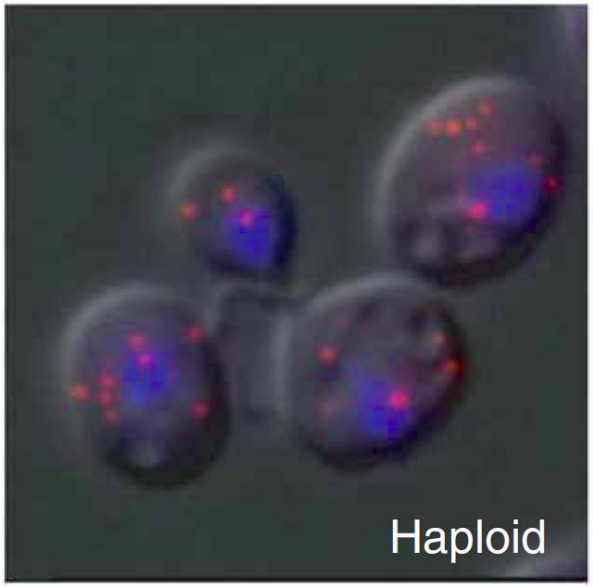
(Beautiful work of David Goodsell)

Counting messenger RNAs in cells

- *Fixed cells*

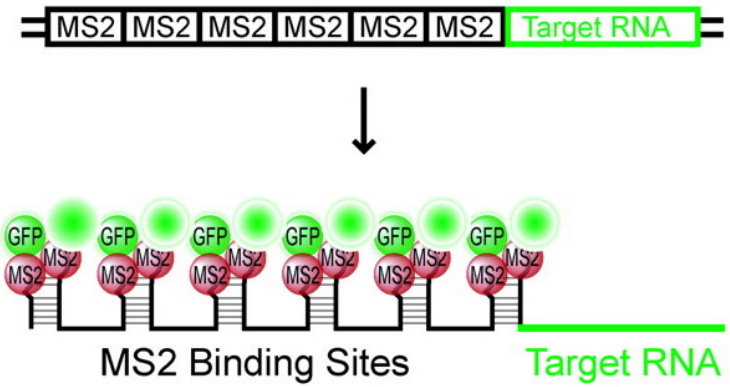
  Zenklusen et al. '08

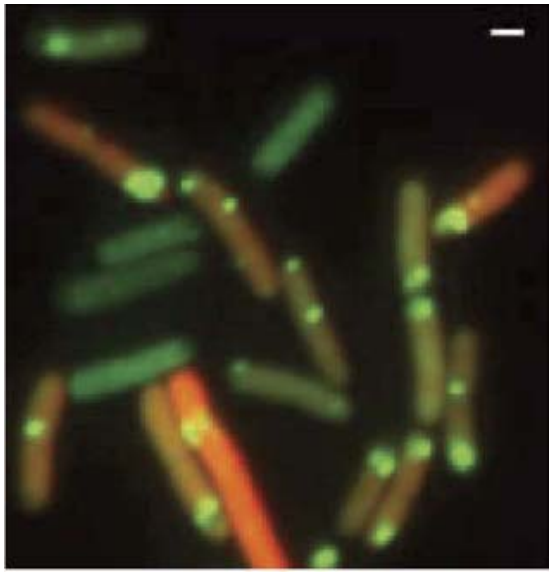




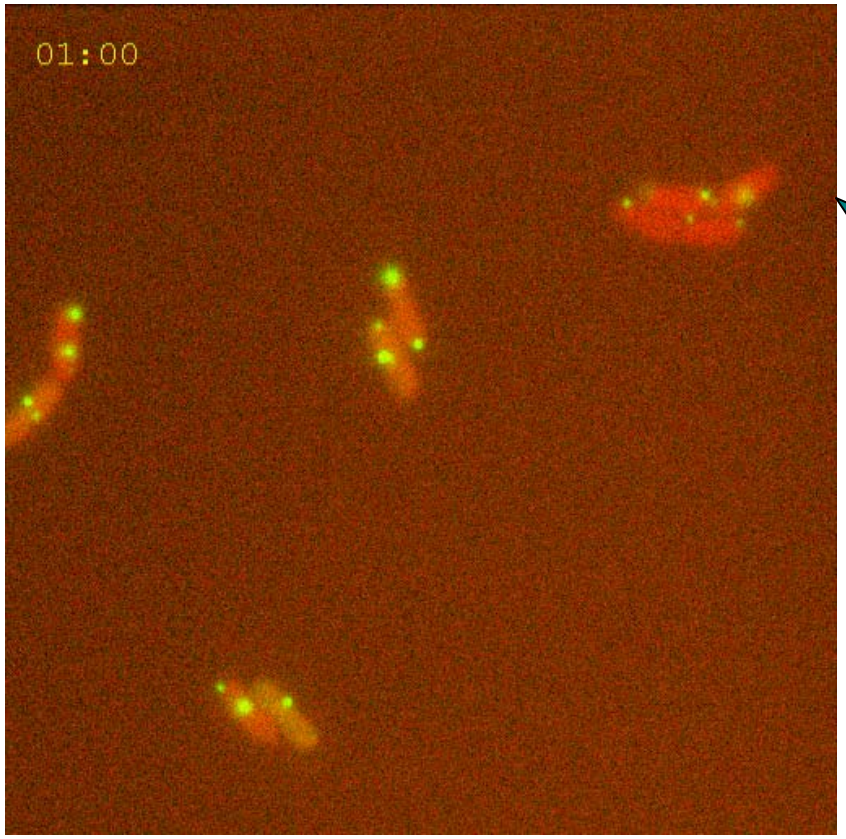
- *Live cells*

  Golding et al. '05

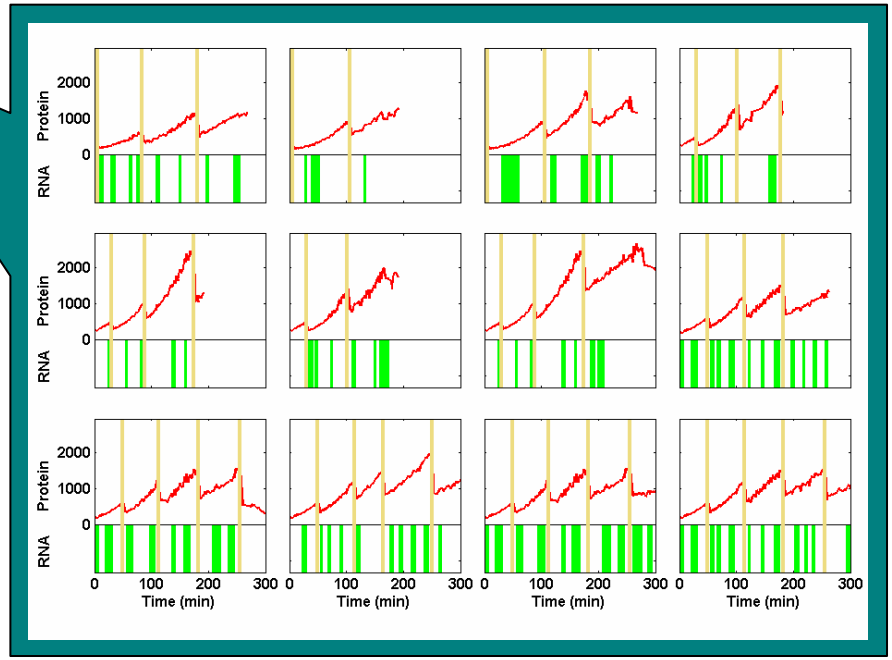




Information Processing in Living Cells: Beyond First Approximations



Ido Golding



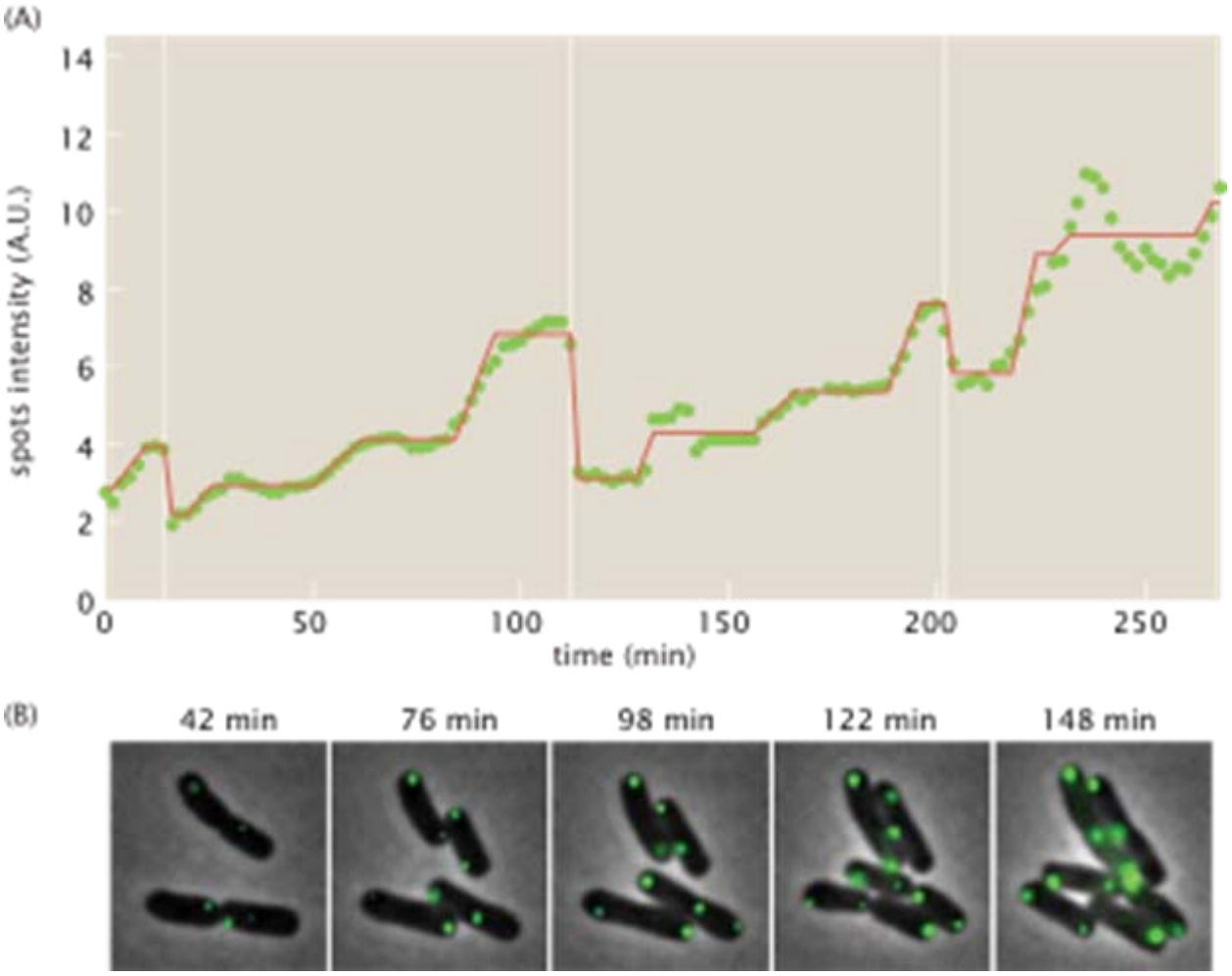
Department of Physics

ILLINOIS

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

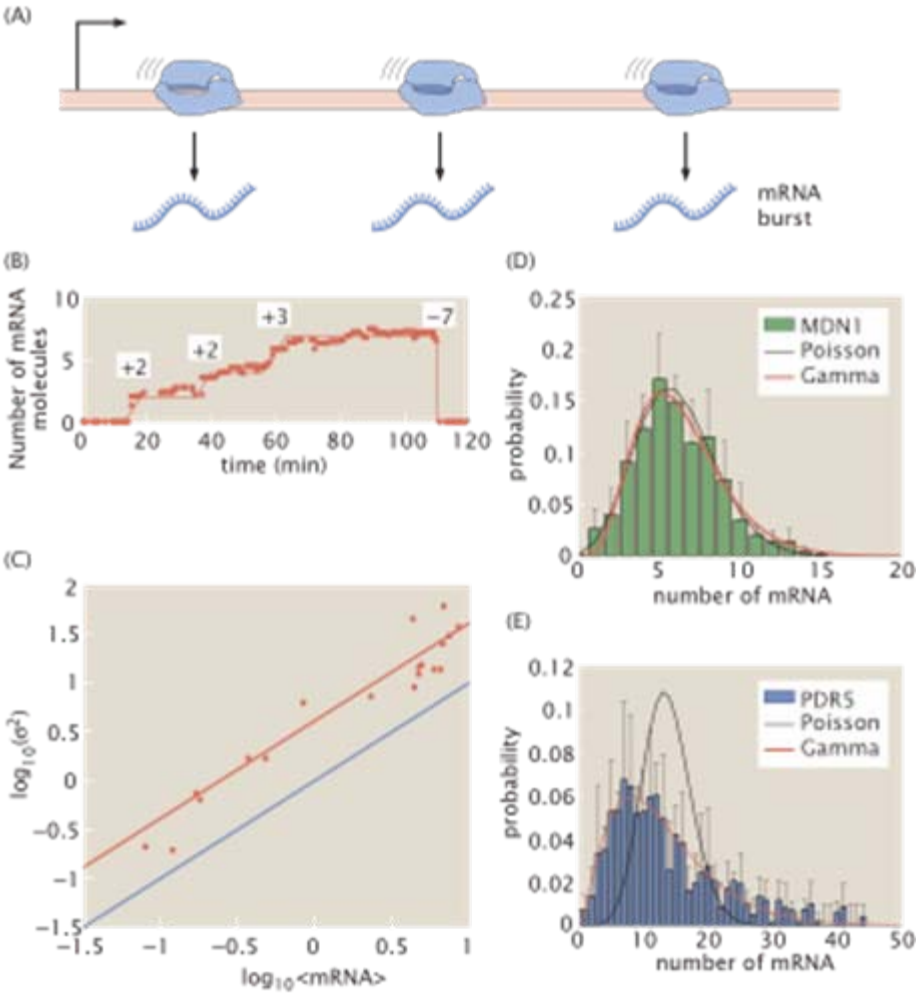
Center for the Physics of Living Cells

Exploring the mrna distribution



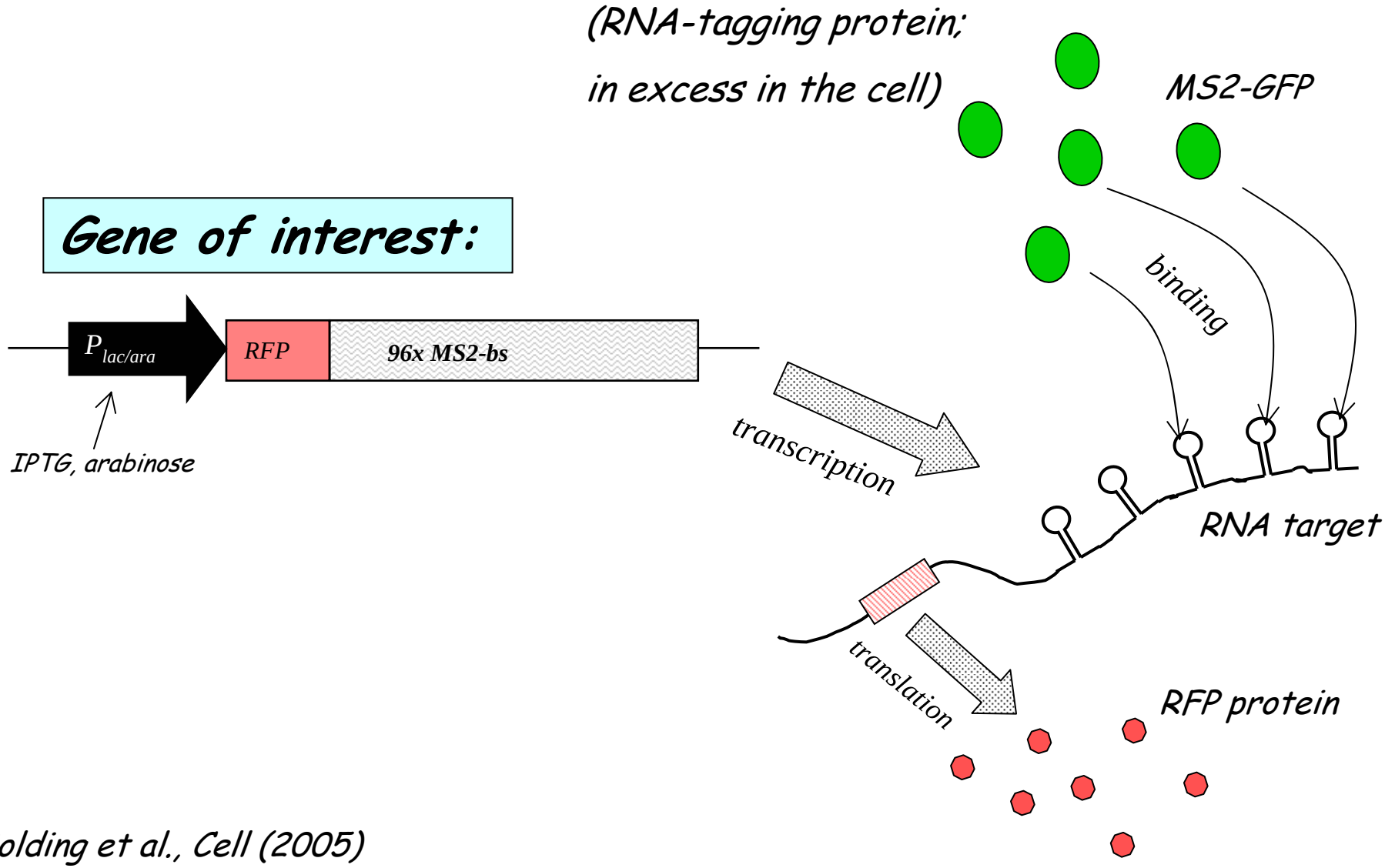
Courtesy of Ido Golding

Exploring the mrna distribution



Oehler et al., 1994

Engineering bacteria to report on gene activity



Golding et al., Cell (2005)

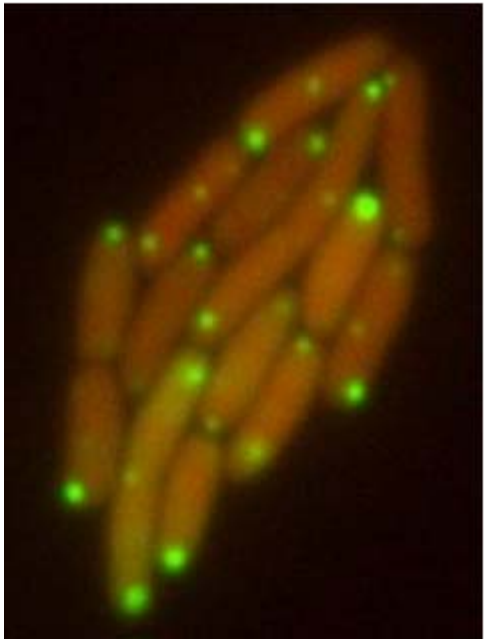
Measuring mRNA & protein numbers

mRNA ∝ *number of bound MS2-GFPs*

∝ *photon flux from localized green fluorescence*

Protein ∝ *number of RFPs*

∝ *photon flux from whole-cell red fluorescence*



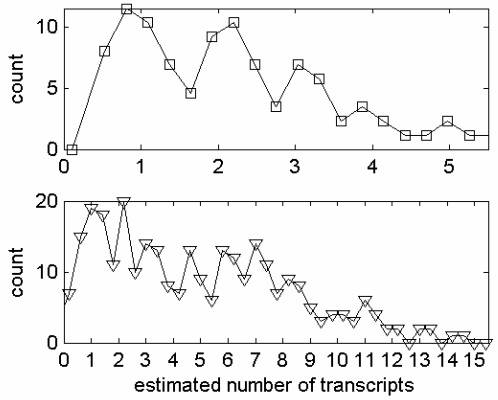
Histogram of RNA copy number:

1st peak =

inter-peak interval ≈

50-100 X GFP =

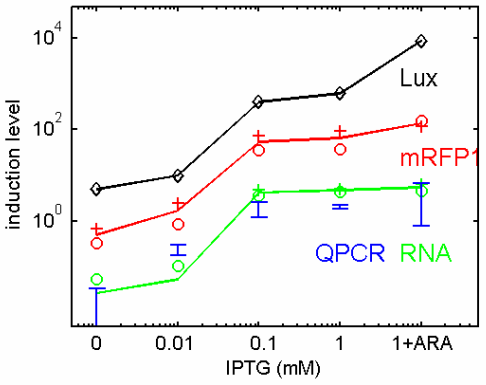
1 transcript



Controls:

QPCR

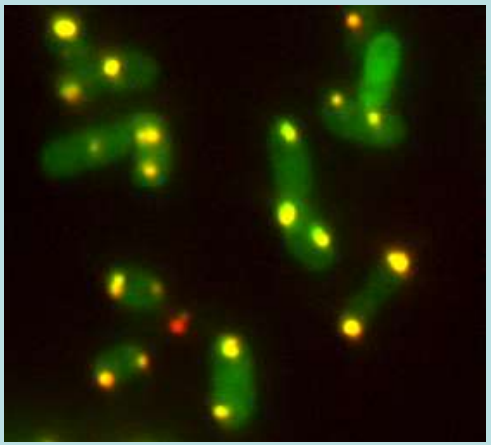
Protein levels



Lux: Lutz & Bujard 1997

Controls:

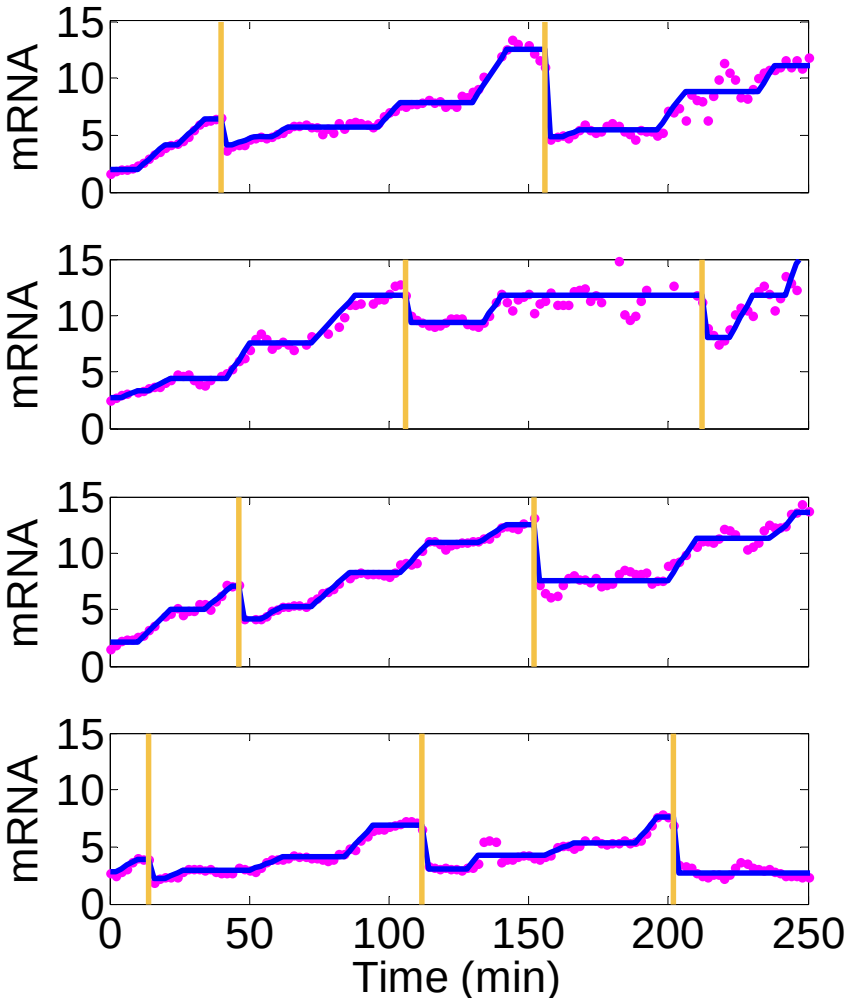
FISH



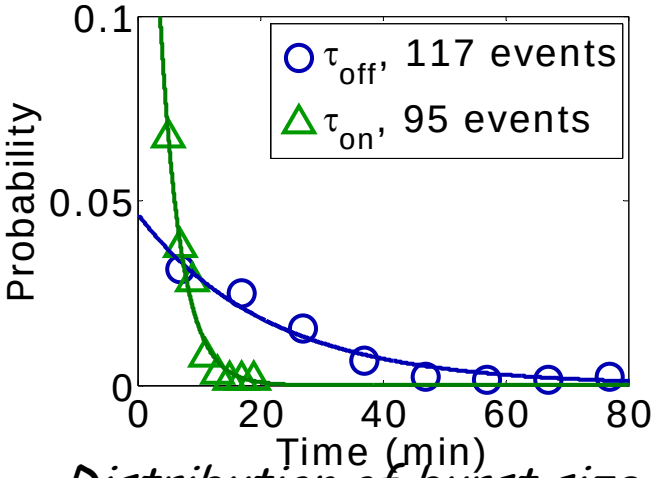
(Thanks to: A. Raj, A. van Oudenaarden)

RNA kinetics in individual cells

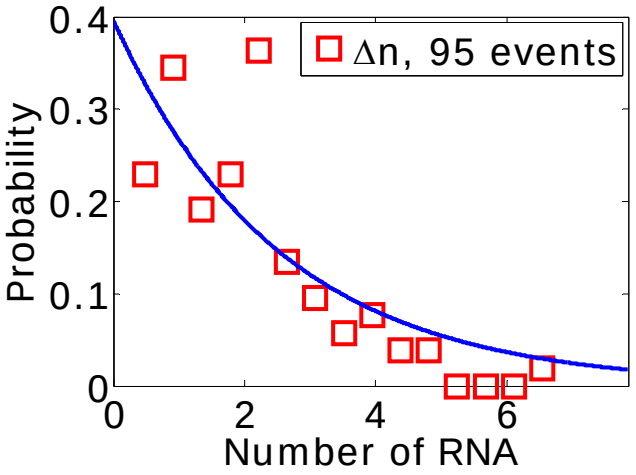
 # mRNA vs time



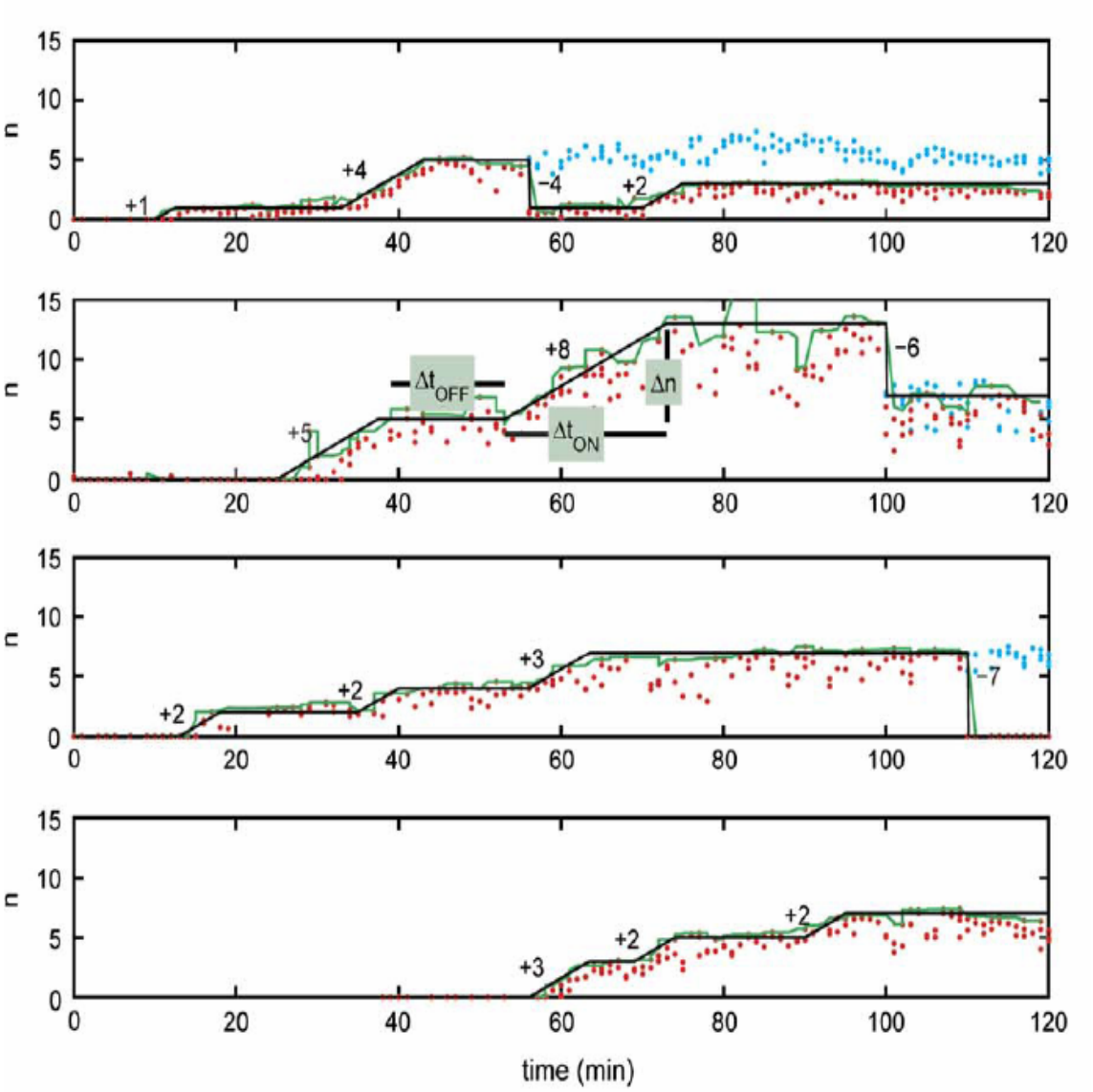
Distribution of on & off times

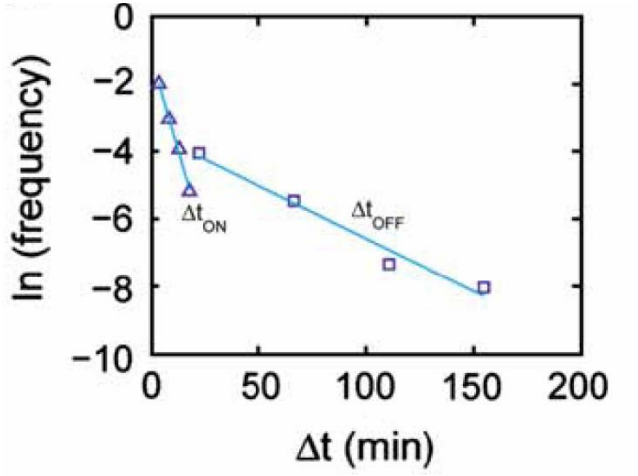


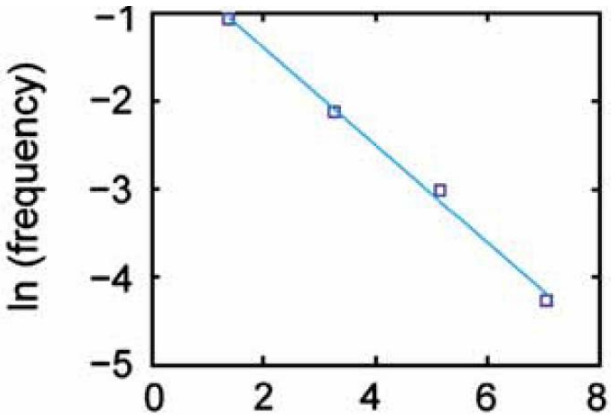
Distribution of burst size



mRNA production occurs in bursts



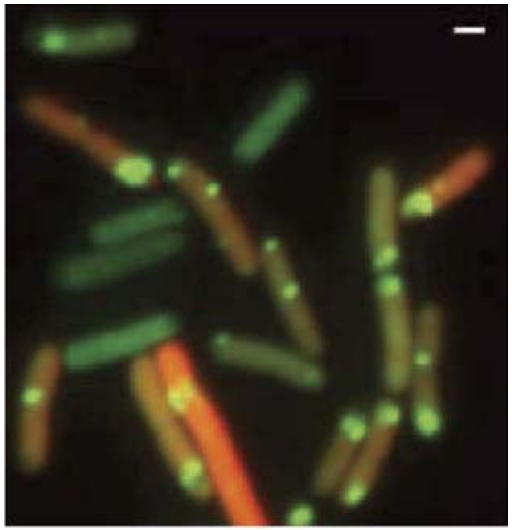


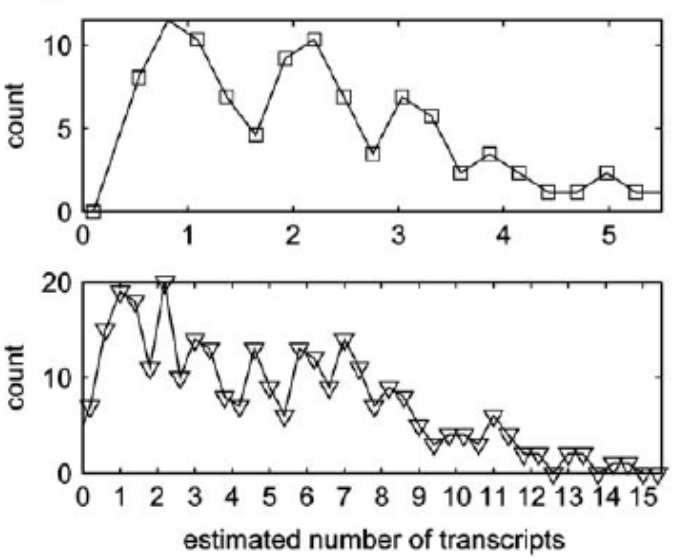


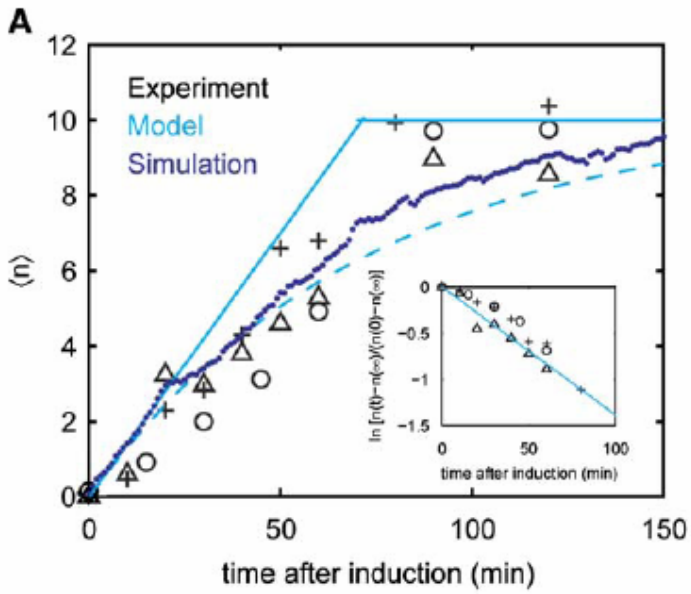
What is the origin of transcriptional bursts?

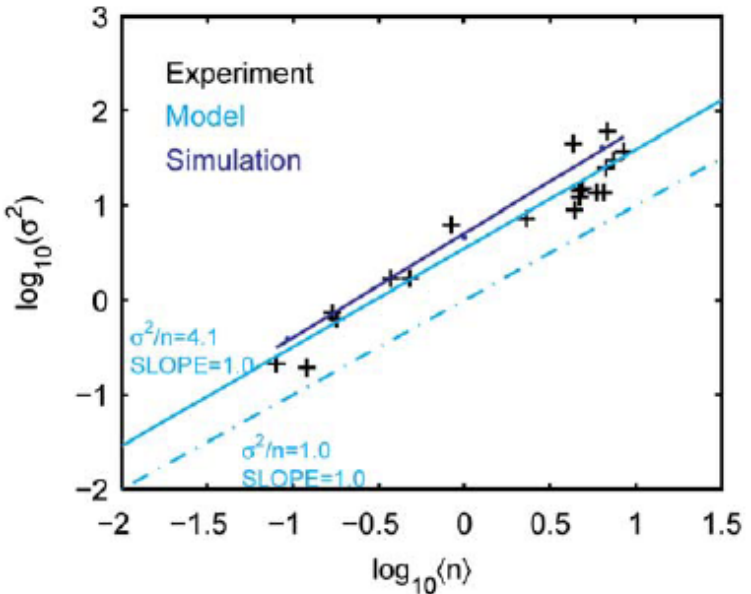
mRNA production in *E. coli*

Golding et al. '05

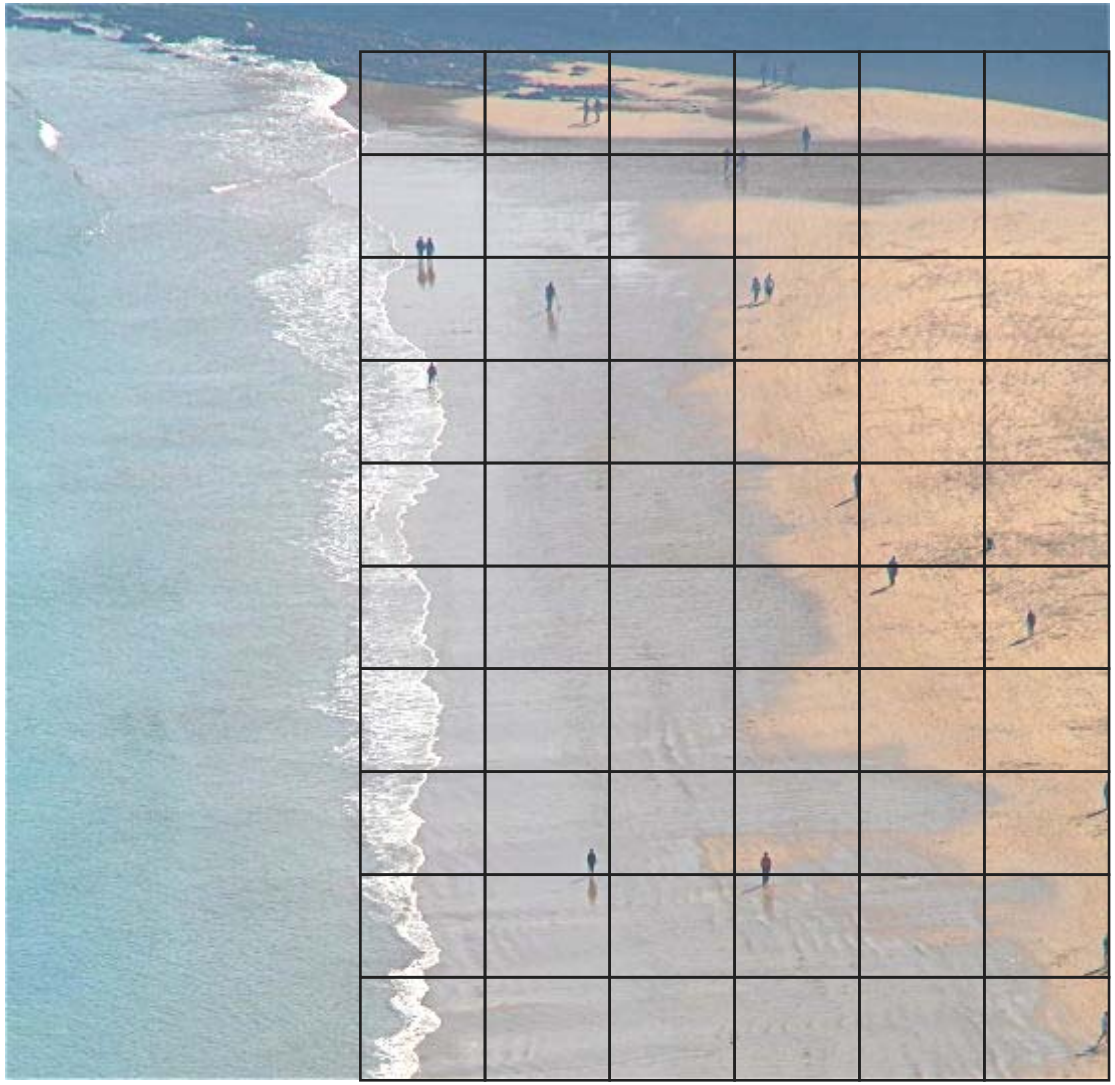








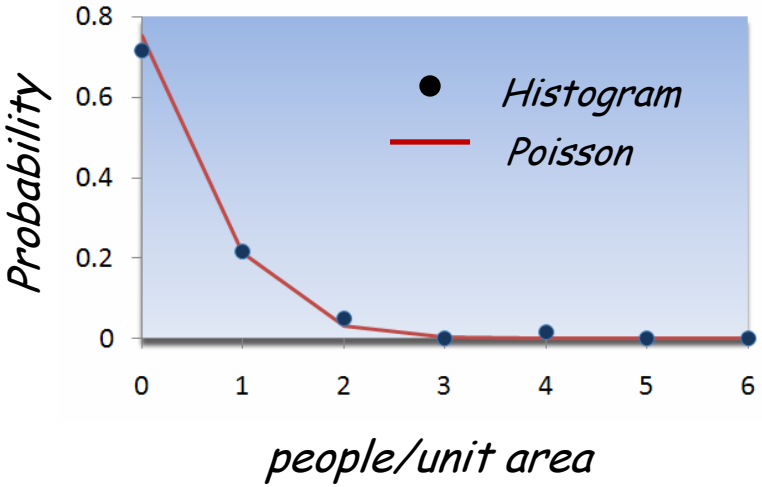
The Poisson distribution



What is the distribution of people per square?

$$P(m) = \frac{e^{-\mu}\mu^{m}}{m!}$$

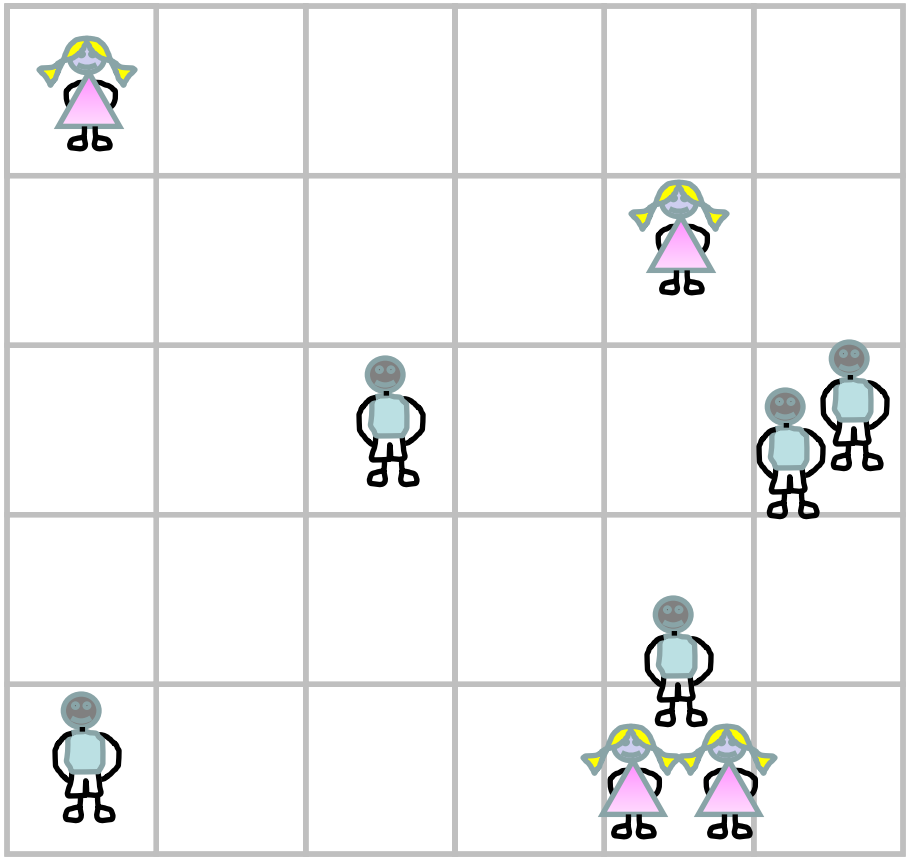
$$\mu = \frac{23 people}{60 squares} = 0.36$$



(thanks to Al Sanchez and Jane Kondev)

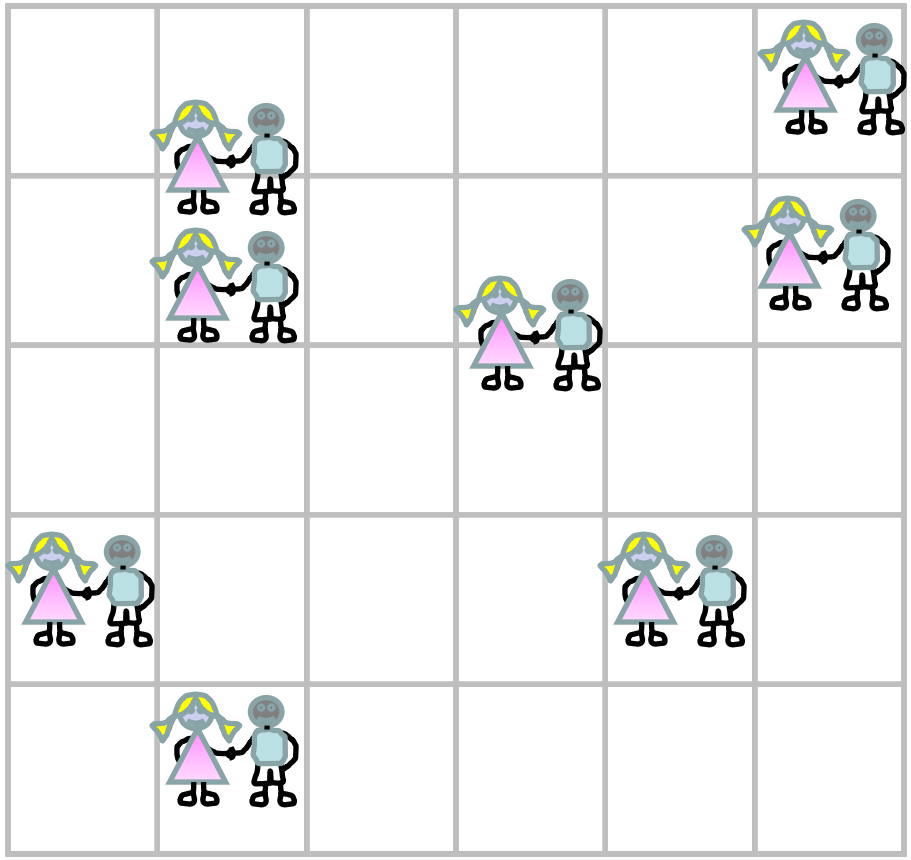
Interactions can change the distribution

Independent singles: Poisson distribution of people



$$\mathrm{var}(P) = \langle P \rangle$$

Valentine's Day: Poisson distribution of couples



$$\mathrm{var}(P) = 2 \times \langle P \rangle$$