BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

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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 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.

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

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

(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.
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.
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
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.
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

Rate of packing: 100bp/sec

“Some assembly required”

Self-assembly

Rate of ejection: ≈ 100 - 1000bp/sec

Forceful ejection

Infection

Lysis

DNA Replication & Protein Synthesis

Capsid Assembly

DNA Packing
THE LIFE CYCLE OF BACTERIOPHAGE LAMBDA
A GENETIC SWITCH

A Genetic Switch
Third Edition
Phage Lambda Revisited

Mark Ptashne

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

A Genetic Switch, 3rd edition, 2004
© Cold Spring Harbor Laboratory Press
Chapter 3, Figure 1

A Genetic Switch, 3rd edition, 2004
© Cold Spring Harbor Laboratory Press
Chapter 3, Figure 3
THE LAMBDA SWITCH:LYSOGENIC STATE
THE LAMBDA SWITCH: LYTIC STATE

A Genetic Switch, 3rd edition, 2004
© Cold Spring Harbor Laboratory Press
Chapter 1, Figure 23

A Genetic Switch, 3rd edition, 2004
© Cold Spring Harbor Laboratory Press
Chapter 1, Figure 4

Chapter 1, Figure 24
DNA GEOGRAPHY OF THE SWITCH

A Genetic Switch, 3rd edition, 2004
© Cold Spring Harbor Laboratory Press
Chapter 2, Figure 16
Measuring fold change: the cell as a test tube

Fold-change(YFP) = [Diagram showing expression changes]

Fold-change(lacZ) = [Diagram showing expression changes]

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 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.

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.**

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 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?
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

![Diagram of counting messenger RNAs in cells]

**Haploid**
INPUT-OUTPUT CURVES: AN ANALOGY

Electronic Devices

Regulatory Devices

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

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

<table>
<thead>
<tr>
<th>Fold-change</th>
<th>Number of repressors</th>
<th>( \Delta \varepsilon_{rd} (k_B T) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>900</td>
<td>-16.2</td>
</tr>
<tr>
<td>0.048</td>
<td>3.1 \times 10^{-3}</td>
<td>-13.7</td>
</tr>
<tr>
<td>0.77</td>
<td>0.063</td>
<td>-10.5</td>
</tr>
</tbody>
</table>

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.

\[ \text{fold change} = (1 + \frac{R}{N} e^{-\beta \Delta \epsilon})^{-1} \]

(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 = \left( 1 + \frac{K}{N} e^{-\beta \Delta \varepsilon} \right)^{-1}  

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

- **A**
  - CFP (linear scale)
  - Ci-YFP (log scale)

- **B**
  - Fluorescence segregation errors and their RMS (red)
  - Splitting cell with \( N_{\text{tot}} \)
  - Parent cell
  - Daughter cells
  - \( N_1 \), \( N_2 \)
  - CFP production rate [apparent molecules, cell \(^{-1}\) min\(^{-1}\)]

- Images: Cell division and fluorescence over time.
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.
INFORMATION PROCESSING IN LIVING CELLS: BEYOND FIRST APPROXIMATIONS

Ido Golding

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ILLINOIS
Center for the Physics of Living Cells

Caltech 11/2008
SIMPLE CASE OF TURNING A GENE “ON”

Approximations used to describe the process...
**ENGINEERING BACTERIA TO REPORT ON GENE ACTIVITY**

**Gene of interest:**

- **P_{lac/ara}**
- **RFP**
- **96x MS2-bs**

**P_Lac/ara**

**IPTG, arabinose**

**RNA target**

**MS2-GFP**

**binding**

**transcription**

**translation**

**RFP protein**

(RNA-tagging protein; in excess in the cell)

Golding et al., Cell (2005)
**Measuring mRNA & Protein Numbers**

\[ \text{mRNA} \propto \text{number of bound MS2-GFPs} \]
\[ \propto \text{photon flux from localized green fluorescence} \]

\[ \text{Protein} \propto \text{number of RFPs} \]
\[ \propto \text{photon flux from whole-cell red fluorescence} \]

**Histogram of RNA copy number:**

1\textsuperscript{st} peak = inter-peak interval = 50-100 \times \text{GFP} = 1 \text{ transcript}

**Controls:**

- QPCR
- Protein levels

*Lux: Lutz & Bujard 1997*

(Thanks to: A. Raj, A. van Oudenaarden)
RNA KINETICS IN INDIVIDUAL CELLS

Distribution of burst size

Distribution of on & off times

# mRNA vs time

Distribution of on & off times

Distribution of burst size

Probability

Time (min)

Probability

Number of RNA

τ_{off}, 117 events

τ_{on}, 95 events

Δn, 95 events

mRNA

mRNA

mRNA

mRNA

0 50 100 150 200 250

0 20 40 60 80

0 0.1 0.05 0.0

0 0.4 0.3 0.2 0.1 0.0

0 2 4 6
MRNA PRODUCTION IN E. COLI
Golding et al. '05