2011 q-bio Summer School:
Stochastic Gene Regulation

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Stochastic Biochemistry: Theme Overview


Origins of Stochasticity:
1) Small molecular copy numbers

- Proteins build cellular structures, pass cellular information and regulate cellular activities. Variable copy numbers (~0-100,000/cell).

- mRNA transfer instructions for creating specific proteins. Low copy numbers (~0-100/cell).

- DNA contains all of the genetic instructions. Extremely low copy numbers (~0-5/cell).

The Central Dogma of Molecular Biology
Origins of Stochasticity:

2) Spatial fluctuations of cellular constituents.

Thermal fluctuations will lead to randomness in times between reactions.
Origins of Stochasticity:
3) Competition of different events.

Different reactions will lead to different consequences.

Which ever molecule wins the race will define the reaction.
Origins of Stochasticity:
4) Extrinsic fluctuations.

Changes in temperature, nutrients, radiation, chemicals, pressure, etc...

Fluctuations of upstream genes, intercellular signals.
Intrinsic versus Extrinsic Noise

• Variability is present and can be measured


• Inserted two reporters on the chromosome (cfp, yfp)
• Each was controlled by the same promoter
• Expression of cfp shown in green, yfp in red
Stochastic Effects Lead to Phenotypical Differences

Fingerprints of identical twins

Cc, the first cloned cat and her genetic mother, Rainbow


Slide Contributed by Mustafa Khammash
Stochastic Phenomena:
1) Signal Amplification (or damping).

\[ \phi \xrightarrow{k_{\alpha}} I \xrightarrow{k_p} P \xrightarrow{1} \phi \]
\[ \phi \xrightarrow{k_s} S \]
\[ \phi \xrightarrow{k_d} S \]


- Stochastic mean value different from deterministic steady state
- Noise *enhances* signal!
Stochastic Phenomena:
2) Noise Induced Oscillations

Circadian rhythm

- Oscillations disappear from deterministic model after a small reduction in deg. of repressor
- (Coherence resonance) Regularity of noise induced oscillations can be manipulated by tuning the level of noise [El-Samad, Khammash]

Vilar, Kueh, Barkai, Leibler, PNAS 2002

El-Samad, Khammash
Stochastic Phenomena:
3) Stochastic Switching

Same chemical environment.
Same genetic code.

Random reactions can lead to vastly different results!

Harmless phenotype.

Highly infectious phenotype.

Munsky, Trinh, Hernday, Khammash, Low, *under preparation*, 2011
The Importance of Single Cell Analyses

For these systems, we need single cell analyses to answer:

★ What will happen?
★ How frequently?
★ Why does it happen?
★ Under what conditions?

★ What advantages does it provide?
★ How can we prevent it?
★ How can we cause it?
Same genetic code.

Highly infectious phenotype.

Harmless phenotype.

Random reactions can lead to vastly different results!

Genetic manipulations make it easy to see changes under the microscope.

Munsky, Trinh, Hernday, Khammash, Low, *under preparation*, 2010
Stochastic Biochemistry: Theme Overview


Advances in single cell research.

- Automated tools
- Statistical methods
- Model Fitting and Validation
- Bio-Control
- Synthetic Design
Automated tools

Flow Cytometry and fluorescence activated cell sorting

Single molecule Fluorescence in situ Hybridization (FISH)
Raj, Nature Methods 2007

Time lapse fluorescence microscopy
Cagatay et al, Cell 2009

Fluorescence microscopy,
Muzzey et al, Cell 2009
Advances in single cell research.
Statistical methods

Population of mRNA’s

Single molecule Fluorescence in situ Hybridization (FISH)

Time lapse fluorescence microscopy Cagatay et al, Cell 2009

Flow Cytometry
Advances in single cell research.

- Automated tools
- Statistical methods
- Model Fitting and Validation
- Bio-Control
- Synthetic Design
Model Fitting and Validation

Population of mRNA's

Probability Densities at Different Times after Induction

Different Control Signals

IPTG OUT → IPTG IN

lacI Promoter

lacI lac GFP

Probability Densities

Total Fluorescence: GFP + Background (Arbitrary Units)
Advances in single cell research.

- Automated tools
- Statistical methods
- Model Fitting and Validation
- Synthetic Design
- Bio-Control

New experiments with controllable biological inputs.
Genetic Toggle Switch, Kobayashi et al., 2004

Light sensing Bacteria, Voigt Lab, 2005

Cagatay et al, Cell 2009
Advances in single cell research.

- Automated tools
- Statistical methods
- Model Fitting and Validation
- Bio-Control
- Synthetic Design

- New experiments with controllable biological inputs
- Optimized design of synthetic biological mechanisms
- Techniques to fit data and validate quantitative models
- Statistical methods to represent biological data
Bio-Control

- **Tetracycline**
  - **tet**
- **Arabinose**
  - **Pbad**
  - **lrp**
- **Lrp**
- **Dam**
- **lacI促进器**
  - **lac**
  - **papI**

**Graphs:**
- **Switch Probability**
  - **Dam (nM)**
  - **Lrp (nM)**
  - **PapI (nM)**
Stochastic Biochemistry: Lecture Plan

1) Theoretical Techniques
(Munsky, Synitsyn, ten Wolde)

2) Experimental Techniques
(Werner, Hong-Geller, Neuert)
Lecture Plan:
1) Theoretical Techniques

- Today and Tomorrow--Brian Munsky (LANL - CNLS)
  - Modeling of stochastic effects in systems biology.
- Friday, July 29--Nikolai Synitsyn (LANL - T4)
  - Moment Generating Function approach to analyzing biochemical stochasticity
- Tuesday, August 9--Pieter Ren ten Wolde (AMOLF)
  - Spatio-Temporal Correlations in Biochemical Systems
- Wednesday, August 10--Ilya Nemenman (Emory)
  - Signal processing in biochemical networks (Tutorial session at conference)
- Wednesday, August 10--Brian Munsky and Gregor Neuert (MIT)
  - Identifying signal-activated GRN’s by integrating single cell measurements and stochastic analyses (tutorial as conference).
Lecture Plan:
2) Experimental Techniques

- Wednesday, August 3--Elizabeth Hong-Geller (LANL - B7)
  - Molecular tools for the analysis of Gene Regulation
- Wednesday, August 3--Jim Werner (LANL - CINT)
  - Fluorescence Correlation Spectroscopy (FCS) and 3 Dimensional Single-Molecule Tracking
- Wednesday, August 4--Gregor Neuert (MIT)
  - Integrating single cell data and stochastic models.
Lecture 1: Modeling of stochastic gene regulation (Part 1).
On the menu...

• Today (Part 1)
  ‣ Solutions for Simple Stochastic Processes (Transcription)
  ‣ Importance of Population Size
  ‣ Stochastic Chemical Kinetics
  ‣ Moment Computations for Linear Propensities
  ‣ Moment Closures for Non-Linear Propensities

• Tuesday (9:00-10:45) (Part 2)
  ‣ Monte Carlo Simulation Techniques
    * Gillespie (SSA), Tau leaping, Chemical Langevin (SDEs), Slow Scale SSA.
  ‣ Density Computations with Finite State Projection Techniques
  ‣ Switch and Trajectory Analyses
  ‣ Examples and software
The Central Dogma of Molecular Biology

• mRNA transfer instructions for the creation of specific proteins.

• Proteins assemble to build cellular structures, pass cellular information and regulate cellular activities.

• DNA contains all of the genetic instructions.
The Central Dogma of Molecular Biology

Deterministic model

\[
\frac{d[\text{mRNA}]}{dt} = -\gamma_r[\text{mRNA}] + k_r
\]

\[
\frac{d[\text{protein}]}{dt} = -\gamma_p[\text{protein}] + k_p[\text{mRNA}]
\]

Stochastic model

- Probability a single mRNA is transcribed in time \( dt \) is \( k_r dt \).
- Probability a single mRNA is degraded in time \( dt \) is \( (#\text{mRNA}) \cdot \gamma_r dt \)
Intrinsic Variability in Gene Expression

- Noise propagates through the network
- Its amount depends on:
  - Number of molecules
  - Stoichiometry
  - Regulation
  - ...
- Sometimes it is suppressed; other times it is exploited
- Deterministic models are not adequate

Source of variability at cellular level:

- Small # of molecules
- Random events

“Intrinsic noise”

Slide Contributed by Mustafa Khammash