Gene Regulation: Lecture 1
July 27, 2009

Michael A. Savageau
Ulam Scholar
Center for Nonlinear Studies, LANL
Distinguished Professor
University of California, Davis

Function, Design and Evolution of Gene Circuitry

"The problems faced by pre- and post-genomic genetics are ... much the same -- they all involve bridging the chasm between genotype and phenotype."

-- Sydney Brenner, *Science* 287:2173-4 (2000)

Function of Gene Circuitry

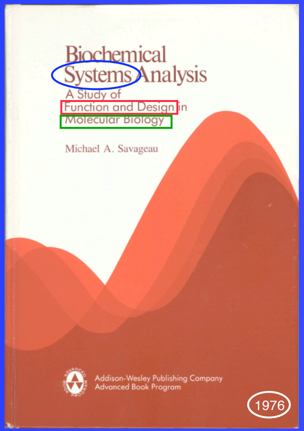
- **Superficial answer**
  - **Genotype determined by the information encoded in the DNA sequence**
  - **Phenotype by the context-dependent expression of the genome**
  - **Circuitry interprets context and orchestrates expression**
- **Deeper answer**
  - **Hierarchy of mechanisms**
  - **Diversity of design issues**
  - **Accident and rule**

Are There Rules Governing Patterns of Gene Regulation?

- “No, there are no rules! Anything is possible. There is only what exists to be discovered and history.”
- “Of course there are rules, and it is the business of science to discover them!”

My Long-Term Research Program

- **Development of quantitative methods for organizationally complex systems**
- **Aim of understanding**
  - Function
  - Design
  - Evolution
- **Focus**
  - Biochemical networks
  - Gene circuitry



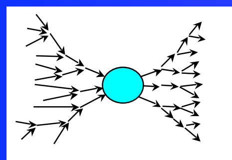
Two Modeling Strategies

- **Specific system**
  - Identify a specific system of interest
  - Assemble available information and formulate a model
  - Estimate parameter values and simulate known behaviors
  - Successful outcome
    - Mimic real system
    - Predict additional behaviors
- **Class of systems**
  - Identify class with many members
  - Abstract essential characteristics and formulate a model
  - Symbolic analysis and statistical sampling
  - Successful outcome
    - Understand the basis for nearly universal designs
    - Discover rules for distinguishing alternative designs

Search for Biological Design Principles

- Emphasize large classes of molecular circuitry with a specific function
  - E.g., inducible catabolic gene circuitry in bacteria
  - ~100 members
  - Many tests of any general prediction
- Rigorous, well-controlled, quantitative comparisons
  - Analytical
  - Computational
  - Statistical
- Goals
  - Understand the basis for nearly universal designs
  - Discover rules for distinguishing alternative designs

Metabolic Organization

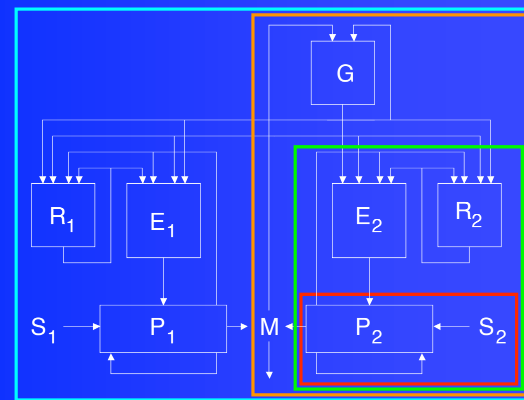


- Largely unidirectional
  - Converging catabolic inputs
  - Diverging anabolic outputs
  - Amphibolic network of intermediates

B. Davis (1961)

- Deployment of resources
  - Rebuilding the physical network every 30 minutes
  - Limited specificity
  - Dynamic connectivity
    - Adaptation
    - Evolution
- Best-studied regulatory systems on the periphery
  - Highly modular
  - Highly regulated
  - Sparsely connected
  - Examples
    - Inducible catabolic
    - Repressible biosynthetic

Modularity & Hierarchical Control



Neidhardt & Savageau, *E. coli and Salmonella* (1996)

Outline

- **Function characterized mathematically**
  - **Canonical nonlinear framework**
  - **Mathematically controlled comparisons**
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

Interlaced Levels of Description for a Chemical Reaction

Time/Number Scale: Small ↑ … ↓ Large

QM wave function	**Discrete/Stochastic**
Potential energy function	**Continuous/Deterministic**
Probability distribution function	**Discrete/Stochastic**
Rate law function	**Continuous/Deterministic**
Boolean function	**Discrete/Deterministic**

Power-Law Formalism

$$\frac{dX_i}{dt} = \sum_{k=1}^{r} \alpha_{ik} \prod_{j=1}^{n} X_j^{g_{ijk}} - \sum_{k=1}^{r} \beta_{ik} \prod_{j=1}^{n} X_j^{h_{ijk}}$$

Canonical from Four Different Perspectives

- **Fundamental**
- **Local**
- **Piece-wise**
- **Recast**

Savageau, *Chaos* **11**: 142 (2001)

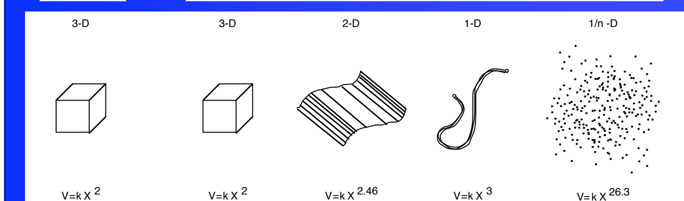
Fundamental Representation: Non-Integer Kinetic Orders

Theoretical	Scaling theory	Ovchinnikov and Zeldovich, 1978 Kang and Redner, 1984 Galfi and Racz, 1988 Bramson and Lebowitz, 1988
Computational	Monte Carlo simulations	Kopelman, 1986 Newhouse & Kopelman, 1988 Jiang and Ebner, 1990
Experimental	*in vitro* measurements	Kopelman, 1986 Koo and Kopelman, 1991

Degree of Dimensional Restriction Affects Kinetic Order

X + X → Y

Traditional	Fractal			
3-D	3-D	2-D	1-D	1/n -D



Chemical Implications of Fractal Kinetics

- Equilibrium behavior
  - Generation of thresholds for molecular recognition
  - Amplification of signals that are supra-threshold
  - Equilibrium is a function of total concentration
- Microscopic reversibility (or detailed balance)
  - Generalization of the traditional principles
  - New constraints imposed on the set of fractal kinetic orders
  - Altered distribution of reactants in complex chemical equilibria

Minton, *Biophys. J.* **63**:1090 (1992)
Savageau, *J. Mol. Recognition* **6**:149 (1993)
Savageau, *BioSystems* **47**:9 (1998)

Local Representation

Arbitrary Functional Relationship	$v = v(X_1, X_2, \ldots, X_n)$
Logarithmic Transformation	$\ln v = f(\ln X_1, \ln X_2, \ldots, \ln X_n)$
Taylor Series Approximation	$\ln v = \ln v_0 + \sum_{j=1}^{n} \left(\frac{\partial \ln v}{\partial \ln X_j}\right)_0 (\ln X_j - \ln X_{j0})$
Exponential Transformation	$v = \alpha \prod_{j=1}^{n} X_j^{g_j}$

Savageau, *Cur. Top. Cell. Reg.* **6**: 63 (1972)

Local S-System Representation within the Power-Law Formalism

Steady-state analysis reduces to linear algebra

$$\frac{dX_i}{dt} = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}} \qquad i = 1, \cdots, n$$

$$\beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}} = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}}$$

$$\sum_{j=1}^{n+m} (g_{ij} - h_{ij}) \ln X_j = \ln(\beta_i / \alpha_i)$$

$$[A]\, y] = b]$$

$$\begin{aligned} y]_d &= -[M][A]_i\, y]_i + [M]\, b] \\ &= [L]\, y]_i + [M]\, b] \end{aligned}$$

- Signal transduction (Logarithmic gains, L)
- System robustness (Parameter sensitivities, S=M)

Savageau, *JTB* 25:370 (1969)
Savageau, *Cur. Top. Cell. Reg.* **6**: 63 (1972)

Local S-System Representation within the Power-Law Formalism

Local dynamics is a linear function of the exponents

$$\left.\frac{du}{dt}\right] = \underline{F}\,[A]u]$$

$$u_i = y_i - y_{i0} = (X_i - X_{i0}) / X_{i0}$$

$$\begin{aligned} F_i &= \alpha_i X_{10}^{g_{i1}} X_{20}^{g_{i2}} \cdots X_{i0}^{(g_{ii}-1)} \cdots X_{n+m,0}^{g_{i,n+m}} \\ &= V_{+i0} / X_{i0} \\ &= \textit{pseudo} - \textit{first} - \textit{order rate constant} \end{aligned}$$

$$a_{ij} = g_{ij} - h_{ij}$$

Near local dynamics

$$\frac{dX_i}{dt} = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}} \qquad i = 1, \cdots, n$$

- Nodes
- Foci
- Saddle points

- Thresholds
- Limit cycles
- Synchronization
- Subharmonics
- Chaos

Savageau, *J. Mol. Evolution* 5:199 (1975)

Advantages of Local S-System Representation

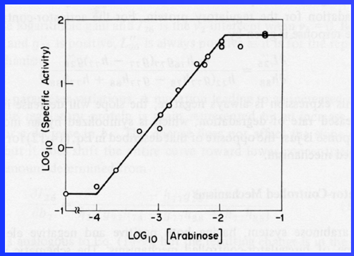
- Existence of a steady state
  - Determinant of dependent kinetic orders non-zero
- Explicit solution for the steady state
  - Kinetic orders
  - Rate constants
- Gains and parameter sensitivities
  - Kinetic orders
- Local stability conditions
  - Kinetic orders
  - Turnover numbers
- Many successful applications to homeostatic systems

Savageau, *J. Mol. Evolution* 5:199 (1975)

Piece-Wise Power-Law Representation

Savageau, *Biochemical Systems Analysis* (1976)

Induction Characteristic of the Arabinose Operon of *E. coli*



Steady-state induction characteristic for the arabinose operon. Cultures of *E. coli* B/r (strain BH13) lacking arabinose permease and ribulokinase activities were grown at 37^{0}C in the presence of various concentrations of the inducer arabinose for about six generations. Each point represents the average specific activity of arabinose isomerase from at least three independent experiments. (After M.E. Doyle, C. Brown, R.W. Hogg, and R.B. Helling, 1972, *J.Bacteriol.* **110**, 56.)

Recast Representation

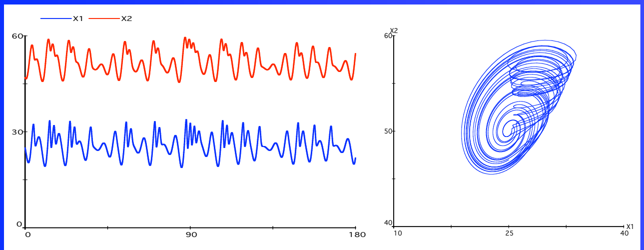
$$dx/dt = 0.343 - (y + 17.15)e^{-x} \qquad x(0) = 3.85$$
$$dy/dt = e^{-x} - (50 + z) \qquad y(0) = 7.16$$
$$dz/dt = 1.82 + (y - 9.75)z \qquad z(0) = 7.98$$

$$x_1 = e^x \quad x_2 = y + 17.15 \quad x_3 x_4 = 50 + z$$

$$dx_1/dt = 0.343x_1 - x_2 \qquad x_1(0) = 46.87$$
$$dx_2/dt = x_1 - x_3 x_4 \qquad x_2(0) = 24.31$$
$$dx_3/dt = 1346.82x_4^{-1} - 50x_2 x_4^{-1} \qquad x_3(0) = 57.98$$
$$dx_4/dt = x_2 x_4 - 26.9x_4 \qquad x_4(0) = 1$$

Savageau & Voit, *Math. Biosci.* **87**:83 (1987)

Global Accuracy of Recast Representations



Screw-type chaos

Methodology Implications of the Canonical Power-Law Formalism

- Fundamental representation
  - Reference for detailed kinetic descriptions
  - Generalization of mass-action kinetics
- Local representation
  - Regular mathematical structure
  - Reasonable degree of local accuracy
- Piece-wise representation
  - Regular mathematical structure
  - Reasonable degree of global accuracy
- Recast representation
  - Globally equivalent
  - Converts implicit equations into explicit equations
  - Efficient solver for ODEs and algebraic equations

Irvine & Savageau, *SIAM J. Numerical Anal.* **27**:704 (1990)
Mueller, Burns & Savageau, *Appl. Math. Comput.* **90**:167 (1998)

Importance of Comparisons

- **Why is there something and not nothing?**
- **Why is there something and not something else?**
- **Comparison is central to biology**
  - **Experimental investigation**
  - **Evolution**
  - **Optimization**
- **Mathematically controlled comparison**

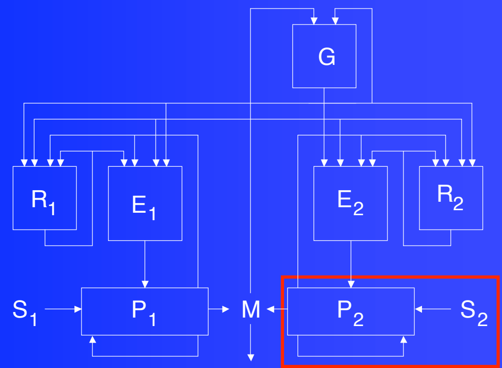
Mathematically Controlled Comparison

- **Two designs are represented in a canonical nonlinear formalism**
- **Differences are restricted to a single specific process**
- **One design is chosen as the reference**
- **Internal equivalence is maintained**
- **External equivalence is imposed**
- **The systems are characterized by rigorous mathematical and computer analysis**
- **Comparisons are made on the basis of quantitative criteria for functional effectiveness**

Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- **Design principles**
  - **Cross-talk in signal transduction**
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

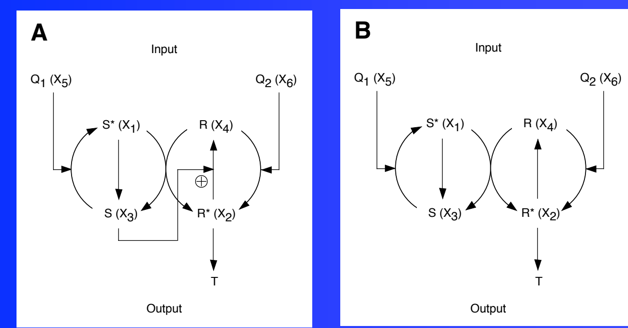
Modularity & Hierarchical Control



Neidhardt & Savageau, *E. coli and Salmonella* (1996)

Alternative Designs for Sensors of Two-Component Systems

Bifunctional sensor — Monofunctional sensor



Equations

Bifunctional sensor

$$dX_1 / dt = \alpha_{10} X_1^{g_{11}} X_5^{g_{15}} X_7^{g_{17}} - \beta_{10} X_1^{h_{11}} X_2^{h_{12}} X_8^{h_{18}}$$

$$dX_2 / dt = \alpha_{20} X_1^{g_{21}} X_2^{g_{22}} X_6^{g_{26}} X_8^{g_{28}} - \beta_{20} X_1^{h_{21}} X_2^{h_{22}} X_7^{h_{27}}$$

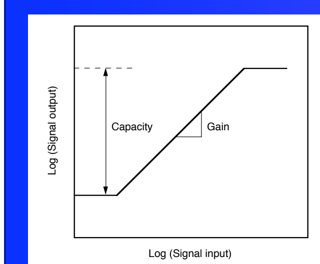
Monofunctional sensor

$$dX_1 / dt = \alpha_{10} X_1^{g_{11}} X_5^{g_{15}} X_7^{g_{17}} - \beta_{10} X_1^{h_{11}} X_2^{h_{12}} X_8^{h_{18}}$$

$$dX_2 / dt = \alpha_{20} X_1^{g_{21}} X_2^{g_{22}} X_6^{g_{26}} X_8^{g_{28}} - \beta'_{20} X_2^{h'_{22}}$$

Constraints for External Equivalence

Signal amplification



Unique parameters

$$h'_{22} = \frac{g_{15}g_{21}h_{22} + g_{26}h_{11}h_{22} - g_{15}g_{22}h_{21} - g_{26}h_{12}h_{21} - g_{11}g_{26}h_{22}}{g_{15}g_{21} + g_{26}h_{11} - g_{11}g_{26} - g_{15}h_{21}}$$

$$\log \beta'_{20} = \frac{\begin{pmatrix} g_{15}h_{21} \log \alpha_{20} - (g_{15}g_{21} - g_{26}(g_{11} - h_{11})) \log \beta_{20} \\ + g_{26}h_{21} \log(\beta_{10} / \alpha_{10}) + g_{15}g_{26}h_{21} \log(X_6 / X_5) \\ + (g_{26}(h_{27}(g_{11} - h_{11})) - g_{15}g_{21}h_{27} - g_{17}g_{26}h_{21}) \log X_7 \\ + h_{21}(g_{15}g_{28} + g_{26}h_{18}) \log X_8 \end{pmatrix}}{g_{26}(g_{11} - h_{11}) + g_{15}(h_{21} - g_{21})}$$

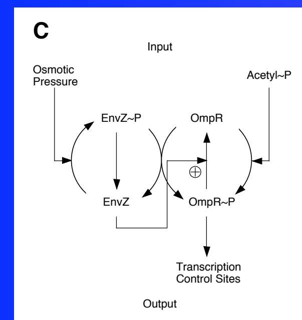
Biological Predictions

- **Bifunctional sensors**
  - **Amplify primary signals**
  - **Attenuate secondary signals**
  - **Suppress noisy cross-talk**
- **Monofunctional sensors**
  - **Attenuate primary signals**
  - **Amplify secondary signals**
  - **Integrate functional cross-talk**

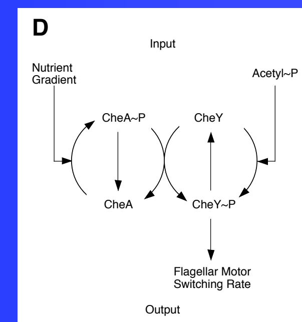
Alves & Savageau, *Mol. Microbiol.* **48**:25 (2003)
Groban et al., *J. Mol. Biol.* **390**:380 (2009)

Alternative Designs for Sensors of Two-Component Systems

Noise Suppression



Signal Integration



Alternative Designs for Sensors of Two-Component Systems

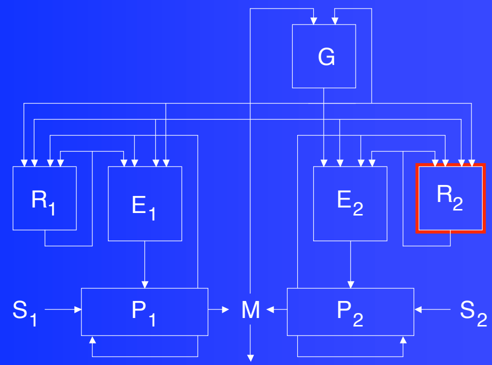
Bifunctional sensor

Monofunctional sensor

Outline

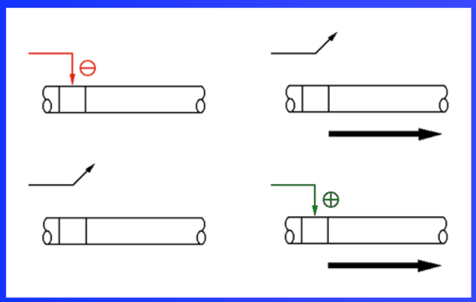
- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- **Design principles**
  - Cross-talk in signal transduction
  - **Molecular mode of gene control**
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

Modularity & Hierarchical Control

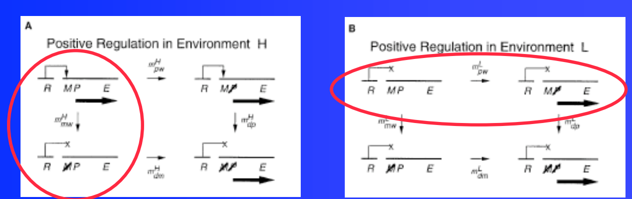


Neidhardt & Savageau, *E. coli and Salmonella* (1996)

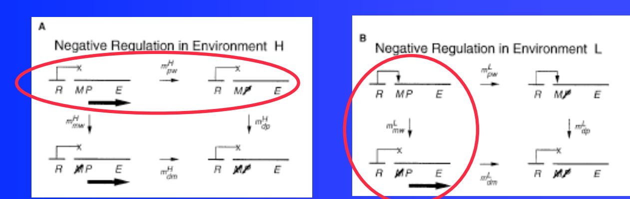
Dual Modes of Gene Control



Selection and Drift: Positive Mode



Selection and Drift: Negative Mode



Demand Theory of Gene Control

- A positive mode of control is predicted when there is a *high* demand for expression of a gene
- A negative mode of control is predicted when there is a *low* demand for expression of a gene

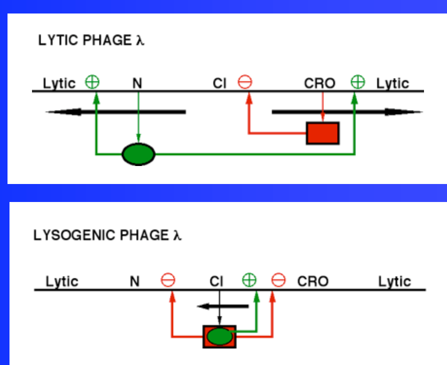
M. Savageau, *PNAS* 71:2453 (1974)

Molecular Mode of Gene Control

- Experimental evidence
  - Single demand functions >100
  - Logical coupling of functions ~ 20
  - Differentiated cell-specific functions ~ 6

M. Savageau, *PNAS* 74:5647 (1977)

Molecular Mode of Gene Control



M. Savageau, *PNAS* 80:1411 (1983)

Duality of Control in Other Biological Domains

- Metabolic pathways
  - Activator molecules
  - Inhibitor molecules
- Immune networks
  - Helper-T cells
  - Suppressor-T cells
- Neural circuits
  - Excitatory synapses
  - Inhibitory synapses

Negative Mode of Control

The Story of the Fisherman and the Genie

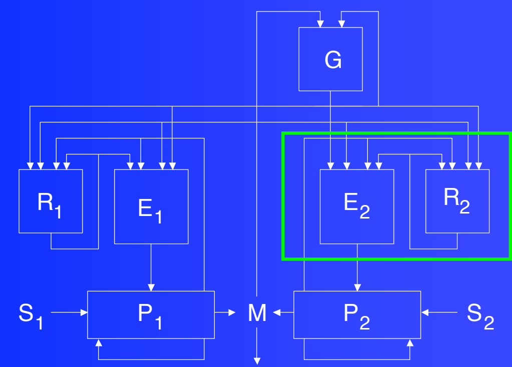
Positive Mode of Control

The Story of Aladdin; or, the Wonderful Lamp

Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- **Design principles**
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - **Coupling of elementary gene circuits**
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

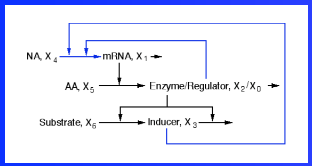
Modularity & Hierarchical Control



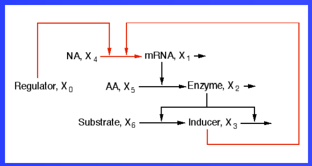
Neidhardt & Savageau, *E. coli and Salmonella* (1996)

Two Extreme Forms of Coupling Gene Expression

Perfect Coupling

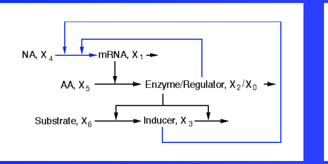


Complete Uncoupling



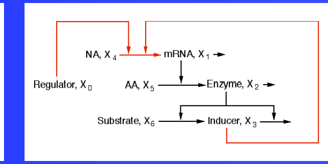
Equations

Perfect Coupling



$$\frac{dX_1}{dt} = \alpha_{1B} - \beta_1 X_1 \qquad X_3 < X_{3L}$$
$$\frac{dX_1}{dt} = \alpha_1{}^p X_2{}^{g_{12}{}^p} X_3{}^{g_{13}{}^p} - \beta_1 X_1 \qquad X_{3L} < X_3 < X_{3H}$$
$$\frac{dX_1}{dt} = \alpha_{1M} - \beta_1 X_1 \qquad X_{3H} < X_3$$
$$\frac{dX_2}{dt} = \alpha_2 X_1 - \beta_2 X_2$$
$$\frac{dX_3}{dt} = \alpha_3 X_2{}^{g_{32}} X_4{}^{g_{34}} - \beta_3 X_2{}^{h_{32}} X_3{}^{h_{33}}$$

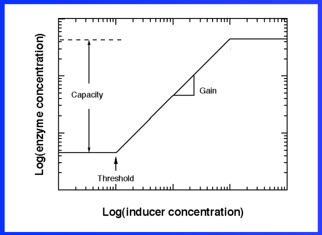
Complete Uncoupling



$$\frac{dX_1}{dt} = \alpha_{1B} - \beta_1 X_1 \qquad X_3 < X_{3L}$$
$$\frac{dX_1}{dt} = \alpha_1{}^u X_3{}^{g_{13}{}^u} - \beta_1 X_1 \qquad X_{3L} < X_3 < X_{3H}$$
$$\frac{dX_1}{dt} = \alpha_{1M} - \beta_1 X_1 \qquad X_{3H} < X_3$$
$$\frac{dX_2}{dt} = \alpha_2 X_1 - \beta_2 X_2$$
$$\frac{dX_3}{dt} = \alpha_3 X_2{}^{g_{32}} X_4{}^{g_{34}} - \beta_3 X_2{}^{h_{32}} X_3{}^{h_{33}}$$

Constraints for External Equivalence

Induction characteristic



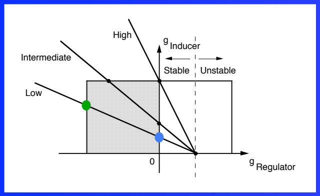
Unique parameters

$$\alpha_1{}^u = \beta_1 \left[\left(\frac{\alpha_1{}^p}{\beta_1} \right) \left(\frac{\alpha_2}{\beta_2} \right)^{g_{12}{}^p / h_{22}} \right]^{\frac{h_{11} h_{22}}{h_{11} h_{22} - g_{12}{}^p g_{21}}}$$

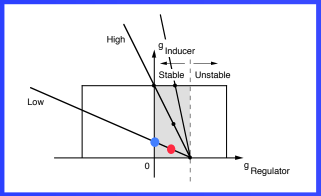
$$g_{13}{}^u = g_{13}{}^p \frac{h_{11} h_{22}}{h_{11} h_{22} - g_{12}{}^p g_{21}}$$

Design Space

Repressor control



Activator control



Line of equivalence

$$g_{13} = \frac{h_{11} h_{22} h_{33} L_{24}}{g_{21} g_{34}} - \frac{h_{33} L_{24}}{g_{34}} g_{12}$$

Example of Analytical Comparison

Robustness measured by parameter sensitivities

Parameter sensitivities defined as $S(V_i, p_j) = \dfrac{\partial V_i}{\partial p_j}\dfrac{p_j}{V_i}$

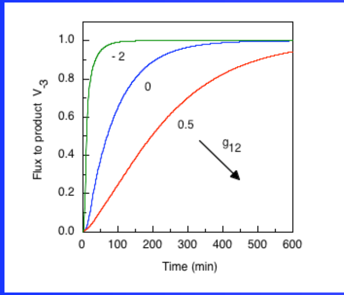
External equivalence implies $g_{13}{}^{u} = g_{13}{}^{p}\dfrac{h_{11}h_{22}}{h_{11}h_{22} - g_{12}{}^{p}g_{21}}$

Ratio for comparison $\dfrac{S(V_3, \beta_2)^p}{S(V_3, \beta_2)^u} = \dfrac{h_{11}h_{22}}{h_{11}h_{22} - g_{12}{}^{p}g_{21}} < 1 \text{ for } g_{12}{}^{p} < 0$

Conclusion: Perfectly coupled circuit with repressor control is more robust than the equivalent completely uncoupled circuit

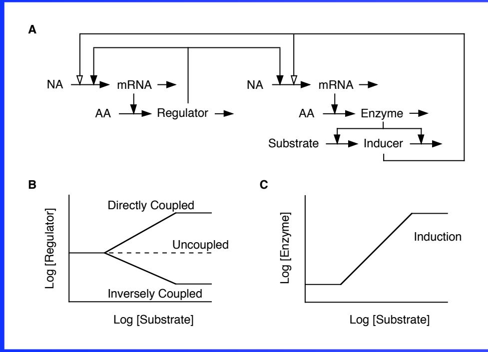
Savageau, *Nature* **229**: 542 (1971)
Becskei & Serrano, *Nature* **405**: 590 (2000)

Example of a Computational Comparison: Response Time



Savageau, *Nature* **252**: 546 (1974)
Rosenfeld, *et al.*, *J. Mol. Biol.* **323**: 785 (2002)

Coupling of Gene Expression in Elementary Circuits

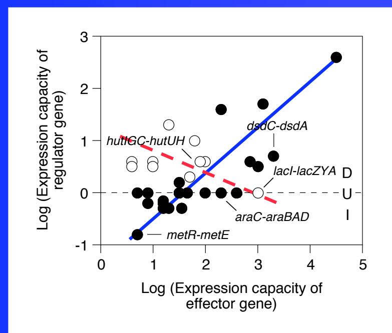


Predicted Coupling of Gene Expression in Elementary Circuits

Mode	Capacity	Predicted coupling
Positive	Small	Inverse & uncoupled
Positive	Large	Direct coupled
Negative	Small	Direct coupled
Negative	Large	Inverse & uncoupled

Hlavacek & Savageau, *J. Mol. Biol.* **248**: 739 (1995)
Hlavacek & Savageau, *J. Mol. Biol.* **266**: 538 (1997)

Experimental Evidence for Coupling of Gene Expression in Elementary Circuits



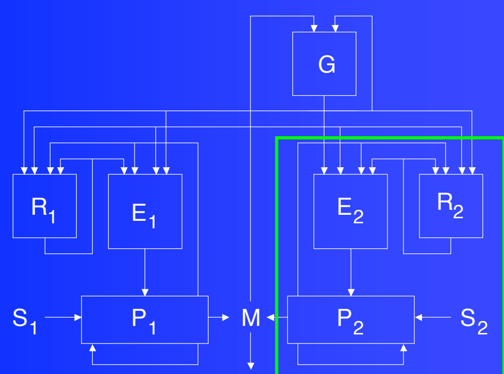
Hlavacek & Savageau, *J. Mol. Biol.* **255**: 121 (1996)

Wall, *et al.*, *Nature Rev. Genetics* **5**: 34 (2004)

Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- **Design principles**
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - **Connectivity in gene circuits**
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

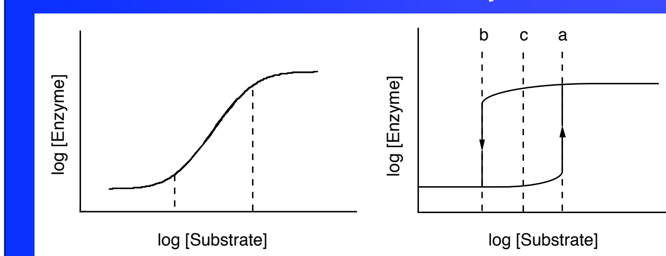
Modularity & Hierarchical Control



Neidhardt & Savageau, *E. coli and Salmonella* (1996)

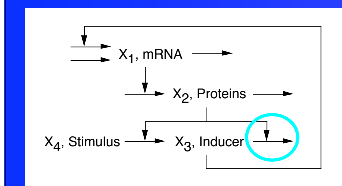
Connectivity and Switching

Graded   Hysteretic

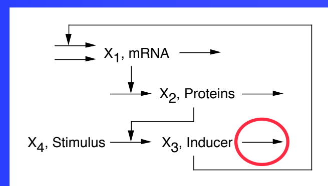


Inducible Gene Circuits

Graded

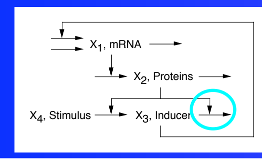


Hysteretic



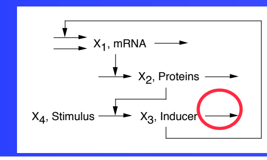
Equations

Graded



$$\frac{dX_1}{dt} = \alpha_{1B} - \beta_1 X_1 \qquad X_3 < X_{3L}$$
$$\frac{dX_1}{dt} = \alpha_1 X_2^{g_{12}} X_3^{g_{13}} - \beta_1 X_1 \qquad X_{3L} < X_3 < X_{3H}$$
$$\frac{dX_1}{dt} = \alpha_{1M} - \beta_1 X_1 \qquad X_{3H} < X_3$$
$$\frac{dX_2}{dt} = \alpha_2 X_1 - \beta_2 X_2$$
$$\frac{dX_3}{dt} = \alpha_3 X_2^{g_{32}} X_4^{g_{34}} - \beta_3^S X_2^{h_{32}^S} X_3^{h_{33}^S}$$

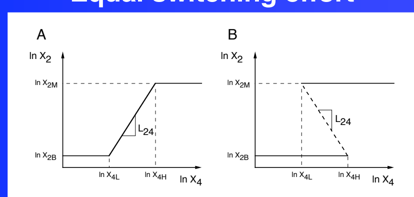
Hysteretic



$$\frac{dX_1}{dt} = \alpha_{1B} - \beta_1 X_1 \qquad X_3 < X_{3L}$$
$$\frac{dX_1}{dt} = \alpha_1 X_2^{g_{12}} X_3^{g_{13}} - \beta_1 X_1 \qquad X_{3L} < X_3 < X_{3H}$$
$$\frac{dX_1}{dt} = \alpha_{1M} - \beta_1 X_1 \qquad X_{3H} < X_3$$
$$\frac{dX_2}{dt} = \alpha_2 X_1 - \beta_2 X_2$$
$$\frac{dX_3}{dt} = \alpha_3 X_2^{g_{32}} X_4^{g_{34}} - \beta_3^D X_3^{h_{33}^D}$$

Constraints for External Equivalence

Equal switching effort



Unique parameters

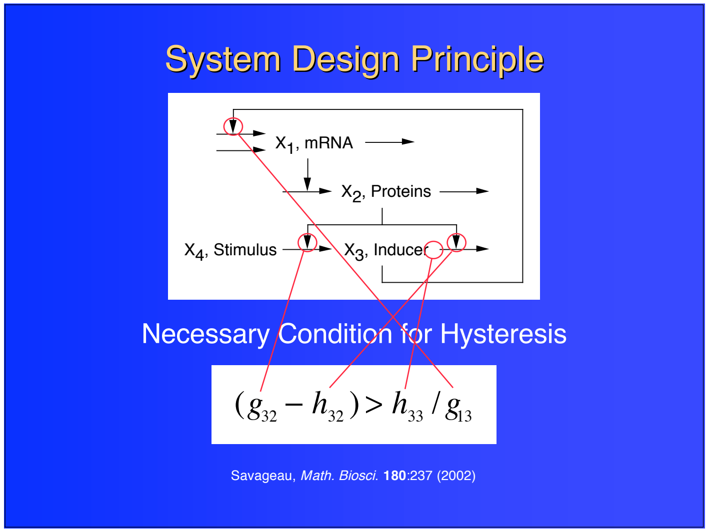
$$h_{33}^D = \left[g_{21}g_{13}\left(2g_{32} - h_{32}^S\right) / \left(h_{11} - g_{12}g_{21}\right)\right] - h_{33}^S$$

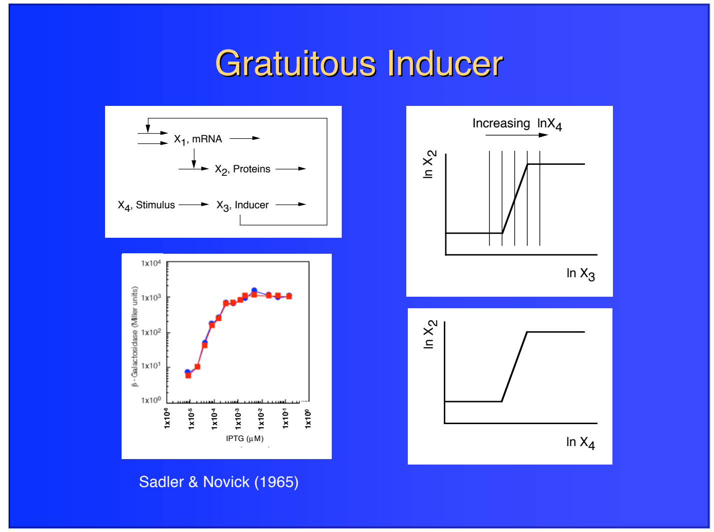
$$\beta_3^D = \beta_3^S \left[\left(\frac{\sqrt{\alpha_{1B}\alpha_{1M}}}{\beta_1}\right)\left(\frac{\alpha_2}{\beta_2}\right)^{\frac{h_{11}}{g_{21}}}\right]^{\left(\frac{2g_{21}g_{34}}{h_{11}L_{24}^S}\right)} \left[\left(\frac{\alpha_1}{\beta_1}\right)\left(\frac{\alpha_2}{\beta_2}\right)^{\frac{h_{11}}{g_{21}}}\right]^{\left[\frac{g_{21}\left(2g_{32}-h_{32}^S\right)}{h_{11}-g_{12}g_{21}} - \frac{2h_{33}^S}{g_{13}}\right]}$$

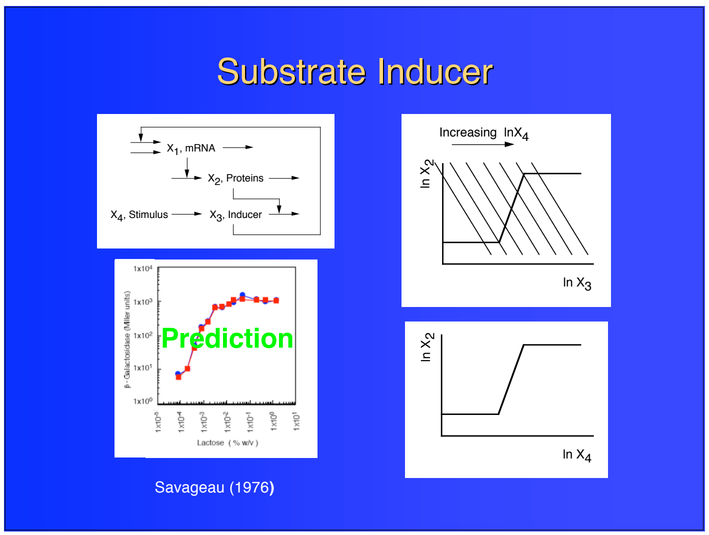
Biological Predictions

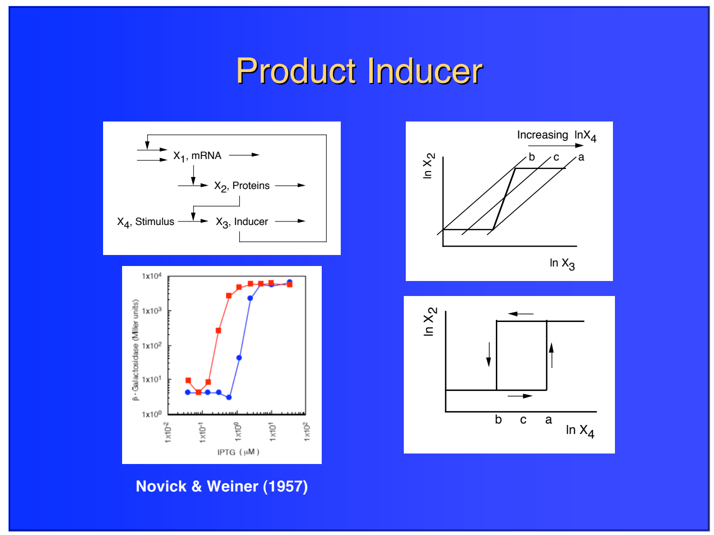
- **Graded switches for homeostatic regulation during responses to environmental change**
  - **Faster switching times**
  - **More robust switching times**
  - **More robust thresholds**
  - **Natural inducers occupy intermediate positions in the inducible circuit**
- **Hysteretic switches for irreversible commitment during differentiation**
  - **Slow response filters out fast events**
  - **Hysteresis improves signal to noise ratio**
  - **Less robust thresholds mean more evolvable**
  - **Natural inducers occupy product positions in the inducible circuit**

Savageau, *Math. Biosci.* **180**:237 (2002)

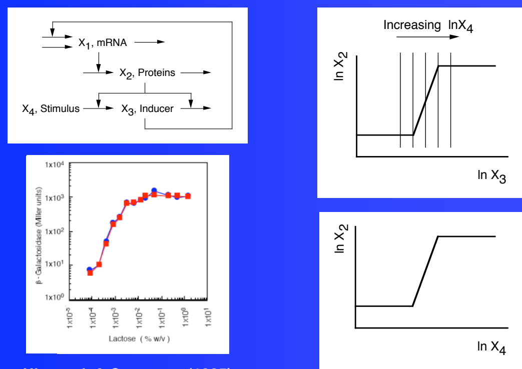

System Design Principle
X1, mRNA
X2, Proteins
X4, Stimulus
X3, Inducer
Necessary Condition for Hysteresis
$(g_{32} - h_{32}) > h_{33} / g_{13}$
Savageau, Math. Biosci. 180:237 (2002)


Gratuitous Inducer
X1, mRNA
X2, Proteins
X4, Stimulus
X3, Inducer
IPTG (μM)
Increasing lnX4
ln X2
ln X3
ln X2
ln X4
Sadler & Novick (1965)


Substrate Inducer
X1, mRNA
X2, Proteins
X4, Stimulus
X3, Inducer
Prediction
Lactose (% w/v)
Increasing lnX4
ln X2
ln X3
ln X2
ln X4
Savageau (1976)


Product Inducer
X1, mRNA
X2, Proteins
X4, Stimulus
X3, Inducer
IPTG (μM)
Increasing lnX4
b
c
a
ln X2
ln X3
ln X2
b
c
a
ln X4
Novick & Weiner (1957)

Intermediate Inducer

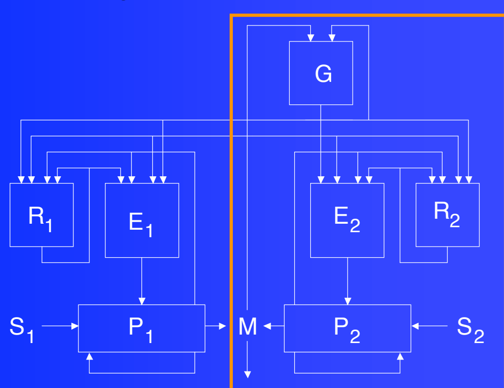


Hlavacek & Savageau (1995)

Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- **Evolution**
  - **Life cycle, Yeng-Yang, and realizability**
  - Logic, multiple demands, and timing
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

Modularity & Hierarchical Control



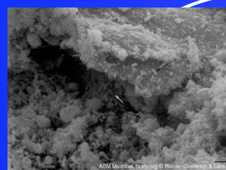
Neidhardt & Savageau, *E. coli and Salmonella* (1996)

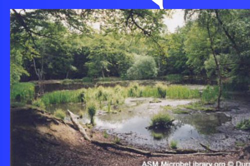
The Organism: *Escherichia coli*

- **Robust**
- **Versatile**
- **Efficient**
- **Responsive**

The Environments: In Here and Out There

Colon type





Aquatic type

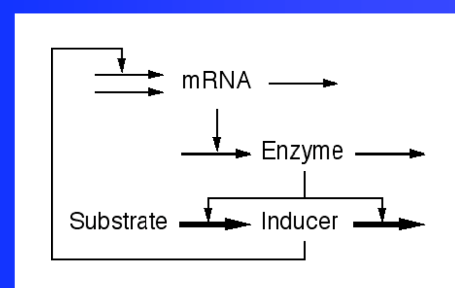
Requirements for the Elucidation of System Design Principles

- Canonical nonlinear representation
- Constraints that reduce the space of meaningful comparison
- Methods for extracting implications implicit in the system equations
- Quantitative criteria for judging functional effectiveness

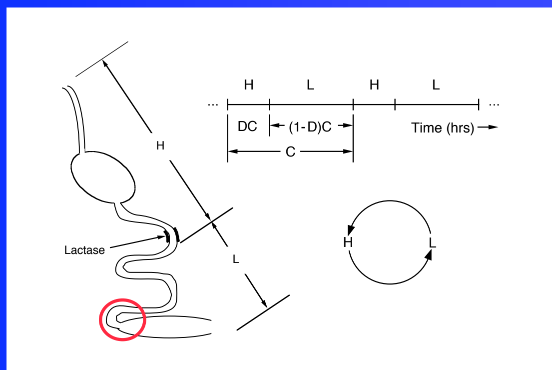
Lac operon of *E. coli*

- *Lac* circuitry
- Life cycle and demand for expression
- Mutation
- Population dynamics
- Mathematical analysis
- Quantify rules of demand theory
- Predictions relating genotype and phenotype

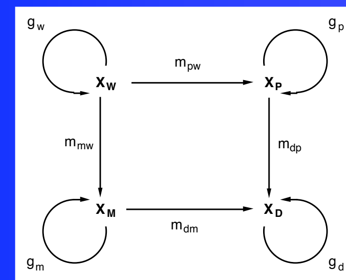
Lac Circuitry



Life Cycle of *Escherichia coli*



Population Dynamics



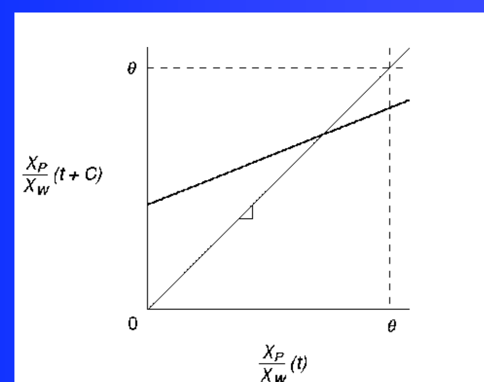
$$dX_w / dt = [1-(m_{pw}+m_{mw})]g_w X_w$$
$$dX_p / dt = [m_{pw} g_w]X_w + [(1-m_{dp})g_p]X_p$$
$$dX_m / dt = [m_{mw} g_w]X_w + [(1-m_{dm})g_m]X_m$$
$$dX_d / dt = [m_{dm} g_m]X_m + [m_{dp} g_p]X_p + [g_d]X_d$$

Recursive Equations For The Mutant Fraction Of The Population

$$\frac{X_p(t+C)}{X_w(t+C)} = \left\{\begin{array}{l} [\alpha^L_{pw} / (\alpha^L_{ww} - \alpha^L_{pp})] \\ \times\{1-\exp[(\alpha^L_{pp}-\alpha^L_{ww})(1-D)C]\} \\ +[\alpha^H_{pw} / (\alpha^H_{ww} - \alpha^H_{pp})] \\ \times\{1-\exp[(\alpha^H_{pp}-\alpha^H_{ww})DC]\} \\ \times\exp[(\alpha^L_{pp}-\alpha^L_{ww})(1-D)C] \end{array}\right\} + \left\{\begin{array}{l} \exp[(\alpha^H_{pp}-\alpha^H_{ww})DC] \\ \times\exp[(\alpha^L_{pp}-\alpha^L_{ww})(1-D)C] \end{array}\right\} \frac{X_p(t)}{X_w(t)}$$

$$\frac{X_p(t+C)}{X_w(t+C)} = \{Intercept\} + \{slope\}\frac{X_p(t)}{X_w(t)}$$

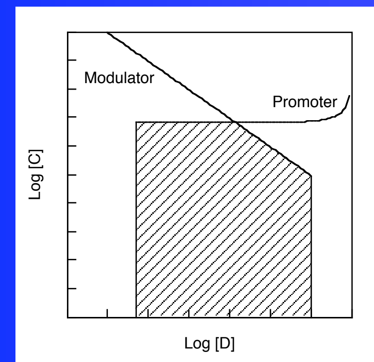
Graphical Solution



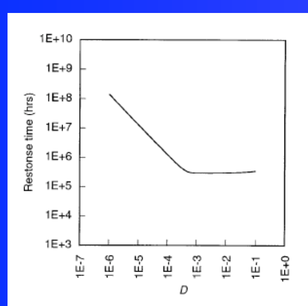
Steady State

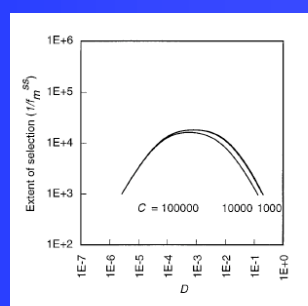
$$X_p/X_w = \{[\alpha_{pw}{}^{L}/(\alpha_{ww}{}^{L}-\alpha_{pp}{}^{L})]\{1-\exp[(\alpha_{pp}{}^{L}-\alpha_{ww}{}^{L})(1-D)C]\} + [\alpha_{pw}{}^{H}/(\alpha_{ww}{}^{H}-\alpha_{pp}{}^{H})]\{1-\exp[(\alpha_{pp}{}^{H}-\alpha_{ww}{}^{H})DC]\}^{*} \exp[(\alpha_{pp}{}^{L}-\alpha_{ww}{}^{L})(1-D)C]\} / \{1-\exp[(\alpha_{pp}{}^{H}-\alpha_{ww}{}^{H})DC + (\alpha_{pp}{}^{L}-\alpha_{ww}{}^{L})(1-D)C]\}$$

Region of Realizability

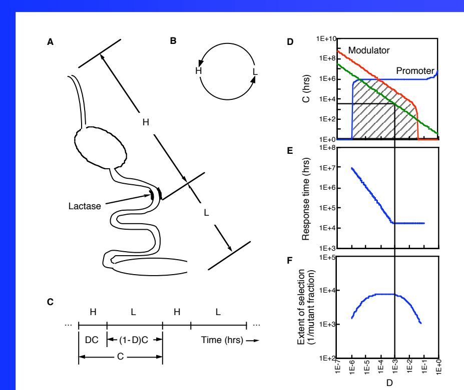


Rate & Extent of Selection





Maintenance of the Regulatory System



Predictions

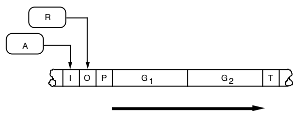
- **Cycling without colonization ≈ 26 hours**
- **Colonization without cycling ≈ 66 years**
- **Rate of re-colonization ≈ 4 months**
- **Evolutionary response time ≈ 3 years**

Savageau, *Genetics* **149**: 1677 (1998)

Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- **Evolution**
  - Life cycle, Yeng-Yang, and realizability
  - **Logic, multiple demands, and timing**
- Global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

Logic of *Lac* Control



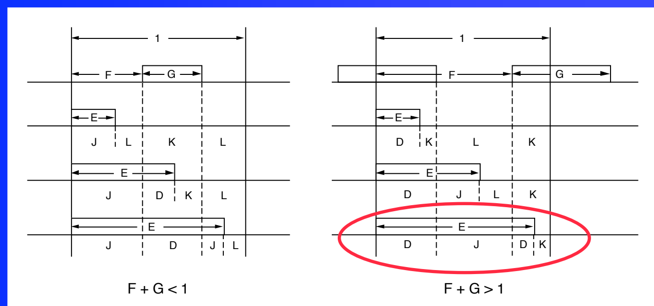
Four Possible States

Regulator		Expression	State
Activator	Repressor		
+	+	-	J
+	-	+	D
-	+	-	L
-	-	-	K

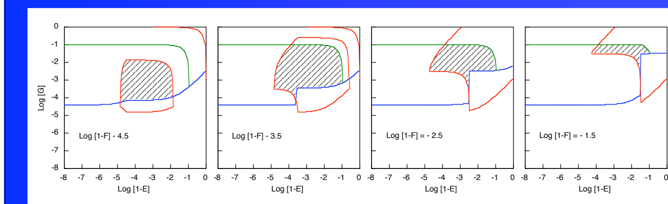
Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Switching of inducible gene circuits
  - Coupling of elementary gene circuits
- **Evolution**
  - Life cycle and molecular logic
  - **Realizability and selection**
- Global understanding of gene circuitry
  - Molecular players
  - Integrated function

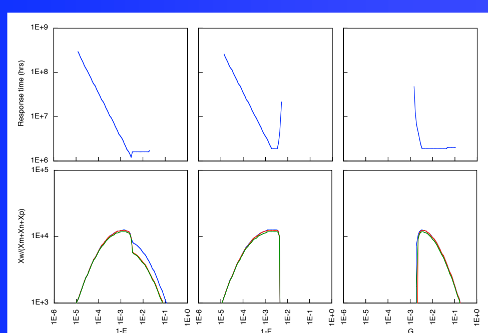
Six Possible Patterns of Expression



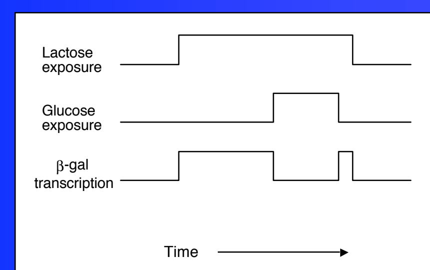
Region of Realizability



Rate & Extent of Selection



Expression in Time and Space



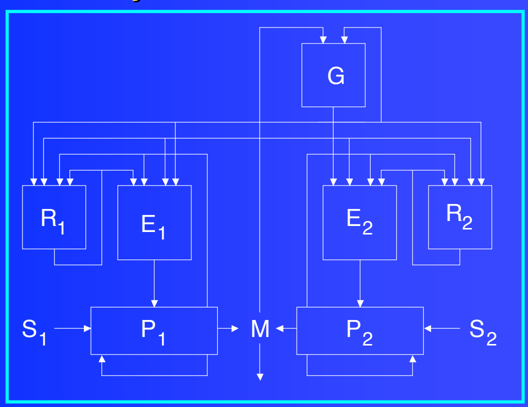
"There is one important piece of information that is almost totally missing: the sequence information that specifies when and where and for how long a gene is turned on or off."

-- Sydney Brenner, *Science* 287:2173 (2000)

Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- **Global understanding of gene circuitry**
  - Molecular players
  - Relevant interactions
  - Integrated function

Modularity & Hierarchical Control



Neidhardt & Savageau, *E. coli and Salmonella* (1996)

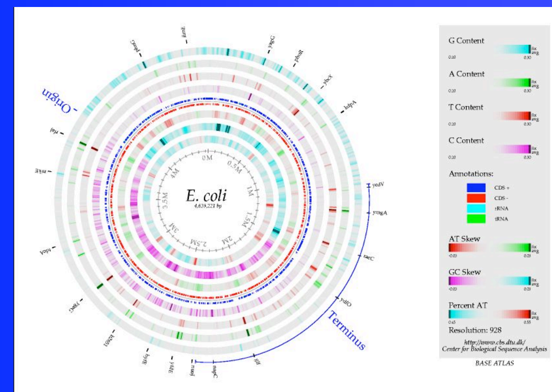
Progressive Global Understanding

Focus	Astronomy	Gene Circuitry
Data	Brahe's measurements	Genome sequence
Patterns	Kepler's laws	Transcription factor linkages
Function	Newton's theory	Systems theory

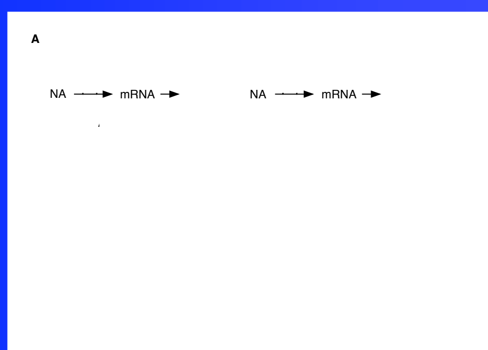
Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- **Global understanding of gene circuitry**
  - **Molecular players**
  - Relevant interactions
  - Integrated function

Complete Genome Sequence



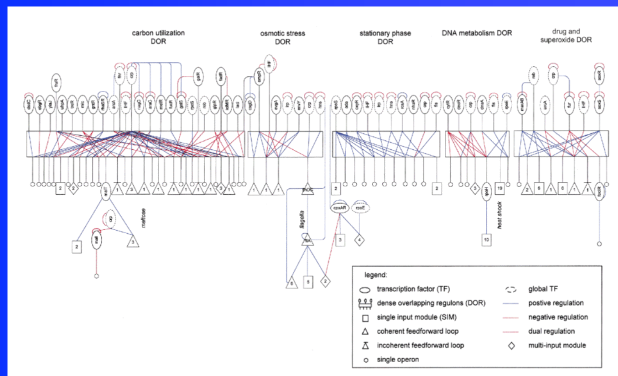
Twp Genes And Their Transcripts



Outline

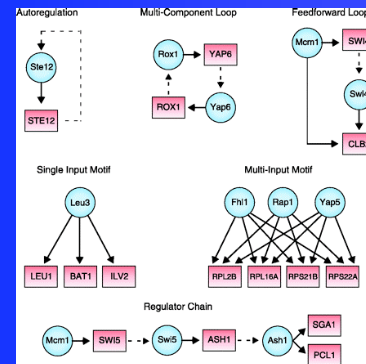
- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- **Global understanding of gene circuitry**
  - Molecular players
  - **Relevant interactions**
  - Integrated function

Transcription Network of *E. coli*



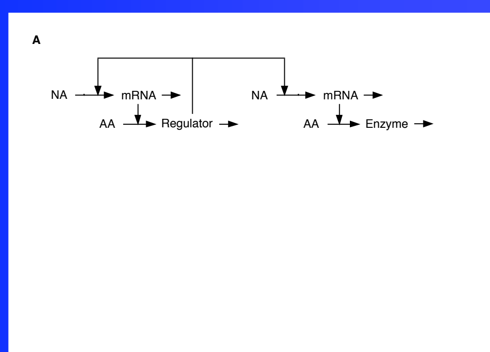
Shai, S. et al., *Nature Genetics* **31**: 64 (2002)

Transcription Factor Motifs

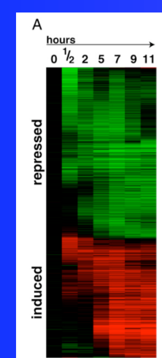


Lee, T.I., et al., *Science* **298**: 799 (2002)

Linkage of Elementary Gene Circuits



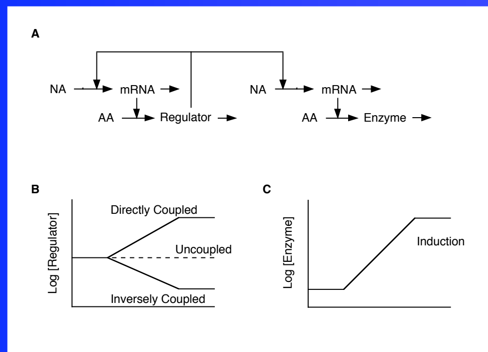
Microarray Experiment



- **Clustering**
- **Correlations**

Chu, et al. (1998)

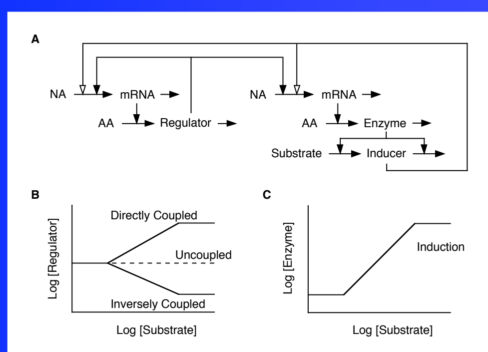
Coupling of Gene Expression in Elementary Circuits



Outline

- Function characterized mathematically
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Design principles
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- Evolution
  - Life cycle, Yeng-Yang, and realizability
  - Logic, multiple demands, and timing
- **Global understanding of gene circuitry**
  - Molecular players
  - Relevant interactions
  - **Integrated function**

Design Principles for Gene Circuitry



Summary

- Quantitative characterization of *system function*
  - Canonical nonlinear framework
  - Mathematically controlled comparisons
- Examples of *design principles*
  - Cross-talk in signal transduction
  - Molecular mode of gene control
  - Coupling of elementary gene circuits
  - Connectivity in gene circuits
- *Evolution* as constrained optimization
  - Constraints in design space
  - Selection based on system function and life cycle
- *Challenges* to global understanding of gene circuitry
  - Molecular players
  - Relevant interactions
  - Integrated function

Acknowledgements

- Eberhard Voit
- Douglas Irvine
- William Hlavacek
- Michael Wall
- Rui Alves

- NSF, NIH, ONR, DOE