Design Principles of Genetic Regulatory Networks

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Contents

- Gene regulation
- Genetic regulatory networks
- Genetic regulatory circuits
- Patterns
- Design principles
- Mathematical models
- Controlled mathematical comparisons
- Structure-function relations

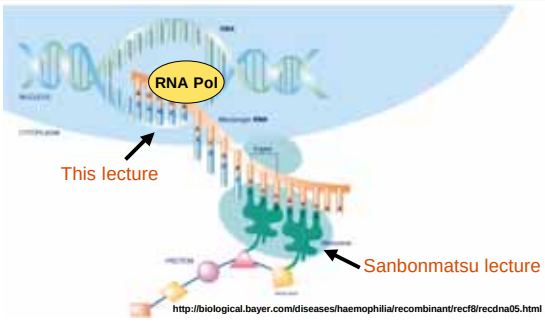
Gene Regulation

Cellular control of protein expression

DNA → mRNA → Proteins

Controls cell capabilities (e.g., metabolism)
Determines cell fate
Impact on human health
- Developmental disorders
- Cancer

Gene Expression



http://biological.bayer.com/diseases/haemophilia/recombinant/recf8/recdna05.html

An *E. coli* Cell is ~1% RNA

1/4 of amount of DNA by weight

TABLE 1 Composition of an average *E. coli* B/r cell[a]

Components	% Total dry wt[b]	Amt (g, 10^{-13}) per cell[c]	Mol wt	Molecules per cell	No. of different kinds of molecules[d]
Protein	55.0	156	4.0×10^4	2,350,000	1,850
RNA	20.5	58			
23S rRNA		31.0	1.0×10^6	18,700	1
16S rRNA		15.3	5.0×10^5	18,700	1
5S rRNA		1.2	3.9×10^4	18,700	1
tRNA		8.2	2.5×10^4	198,000	60
mRNA		2.3	1.0×10^6	1,380	600
DNA	3.1	8.8	2.5×10^9	2.1	1
Lipid	9.1	25.9	705	22,000,000	
Lipopolysaccharide	3.4	9.7	4,070	1,430,000	1
Peptidoglycan	2.5	7.1	(9041)n	1	1
Glycogen	2.5	7.1	1.0×10^6	4,300	1
Polyamines	0.4	1.1			
Putrescine		0.83	88	5,600,000	1
Spermidine		0.27	145	1,100,000	1
Metabolites, cofactors, ions	3.5	9.9			800+

[a] Calculated for an average cell in a population of *E. coli* B/r in balanced growth at 37°C in aerobic glucose minimal medium with a mass doubling time of 40 min. The cell is defined by dividing the total biomass, or the amount of any of its measured components, by the total number of cells in the population. This average cell, therefore, is approximately 68% through its division cycle (see reference 11 for the function describing the distribution of cell ages in an asynchronous, steady-state population) and, assuming that increase in cell mass is exponential, is approximately 33% larger than when it was born. This table is modified from data in Table 1 of reference 10.

[b] Relative amounts of the major components based on information in references 3, 12, and 16 and on unpublished experiments of the author (F.C.N.; see text). In some cases, data from strains other than B/r, from growth conditions other than the reference one, or from both had to be used (see references concerning glycogen [3], polyamine [9], and lipid [16]).

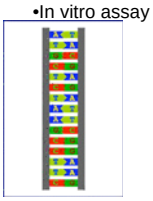
[c] Based on measurements of the total dry mass and the number of cells measured in portions of a reference culture (unpublished observations). The wet weight is calculated from the assumption that 70% of *E. coli* protoplasm is water. The total dry weight per cell is 2.8×10^{-13} g; the water content (assuming that 70% of the cell is water [3]) is 6.7×10^{-13} g; the total weight of one cell is 9.5×10^{-13} g.

[d] Based on the following information sources: protein, examination of two-dimensional polyacrylamide gels (unpublished observations); stable RNA, chapter 88; mRNA, assuming three genes per average transcriptional unit; lipid, an indeterminant number of species because of the variety of fatty acids associated with the four major types of phospholipids exclusive of lipopolysaccharide (i.e., 76% phosphatidylethanolamine, 20% phosphatidylglycerol, and small amounts of cardiolipin and an unidentified species) (1, 12); and metabolites, cofactors, and ions, roughly estimated as described in Table 3 of reference 10.

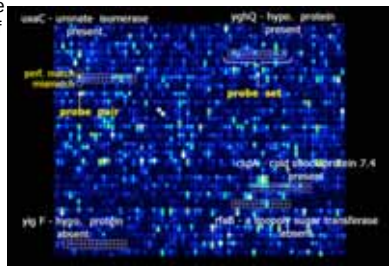
Neidhardt, Chapter 3

High-throughput Gene Expression Assays

- Microarrays
  - Genome-wide
  - Thousands of genes
  - Imprecise
  - In vitro assay

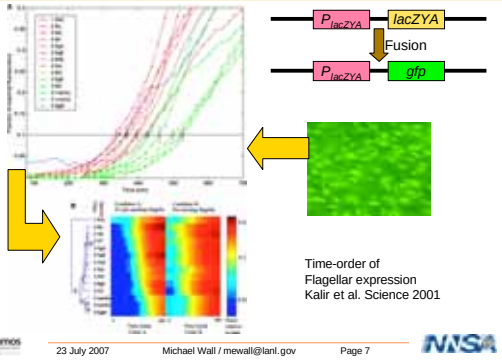


http://www.tokyo-med.ac.jp/genet/tests/dd.gif



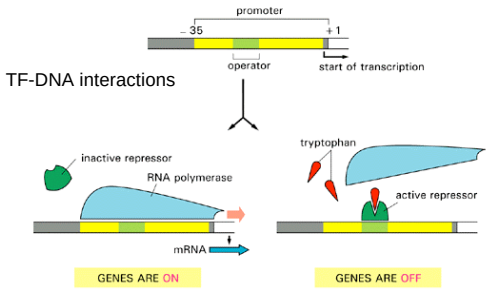
http://www.leelab.org/researchint/dnachip.jpg

Reporter Assays



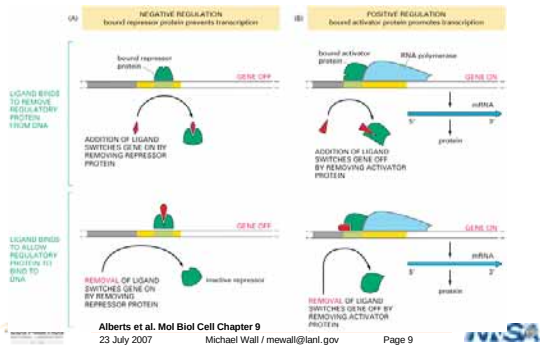
Time-order of
Flagellar expression
Kalir et al. Science 2001

Mechanisms of Gene Regulation



Alberts et al. 3d ed. Mol Biol Cell Chapter 9

Mode of Control



Alberts et al. Mol Biol Cell Chapter 9

Mechanisms of Gene Regulation
Eukaryotes

- Transcription factors bind to upstream regulatory regions of DNA and communicate the "regulatory logic" to the RNA polymerase
- Chromosome reorganization
- DNA confined to nuclear envelope

http://elmo.shore.ctc.edu/biotech/recombdna/genelecture/eukupstream.gif

Gene Regulation
Themes in q-bio Summer School Session

- Protein levels are regulated by control of transcription
  - Determine mechanisms of transcription and transcriptional control
    - Mike Wall poster
  - Determine mechanisms of translation and translational control
    - Kevin Sanbonmatsu
- Genetic regulatory circuits adjust gene expression in response to specific environmental signals
  - Identify genetic regulatory circuits
    - Chris Wiggins
- The overall genetic regulatory response depends on the detailed structure of a gene circuit
  - Study structure-function relations
    - Cynthia Olson Reichhardt, Mike Wall
- Gene-circuit performance may depend on gene-circuit design
  - Perform mathematically controlled comparisons
    - Mike Wall
- Does evolution select for designs that optimize performance?
  - Elucidate design principles
    - Mike Wall

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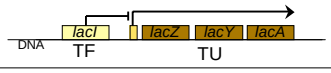
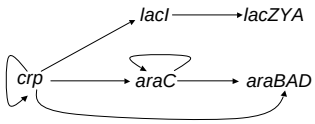
Genetic Regulatory Networks

Genetic regulatory interaction

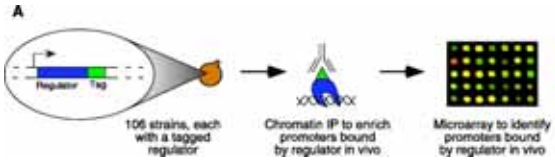


Genetic regulatory network



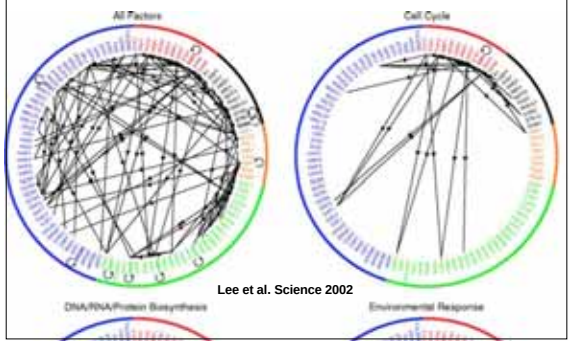
(Wiggins, Reichhardt lectures)

Genetic Regulatory Interactions
Genome-Wide Location Analysis



Lee et al. Science 2002

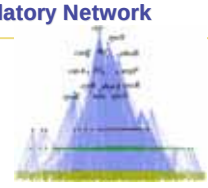
Yeast Genetic Regulatory Network



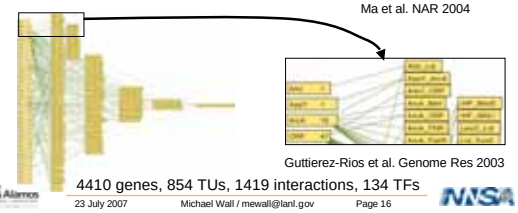
Lee et al. Science 2002

Escherichia coli Genetic Regulatory Network

RegulonDB, EcoCyc



Ma et al. NAR 2004



Guttierez-Rios et al. Genome Res 2003

4410 genes, 854 TUs, 1419 interactions, 134 TFs

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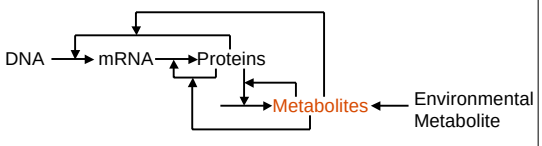
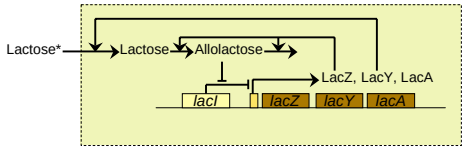
Signal-Dependent Gene Regulation
Genetic Regulatory Circuits



Understanding the functions of genetic regulatory networks will require knowledge of signal interactions

Example: *lac* in E. coli

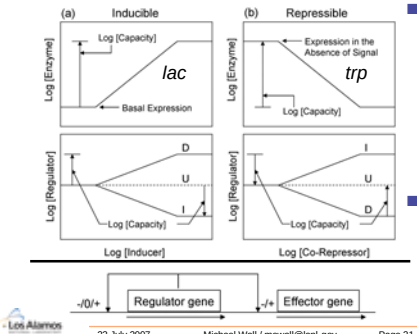


- Inducible catabolic
- Environmental signal is lactose
- *lac* signal molecule is allolactose
- Gratuitous inducers
  - IPTG
  - TMG

Elementary Gene Circuits

- Elementary gene circuits involve just one transcription factor (TF), e.g., LacI
- The TF may regulate its own expression
- TF activity is modulated by a signal
- In bacteria, many circuits fall into two classes
  - Inducible catabolic
    - Produce catabolic enzymes when a key substrate is abundant
    - *lac*
  - Repressible biosynthetic
    - Produce anabolic enzymes when an end product is scarce
    - *trp*

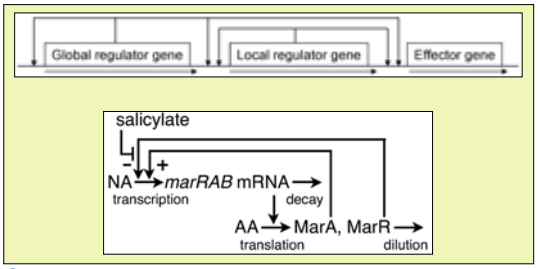
Classes of Elementary Gene Circuits



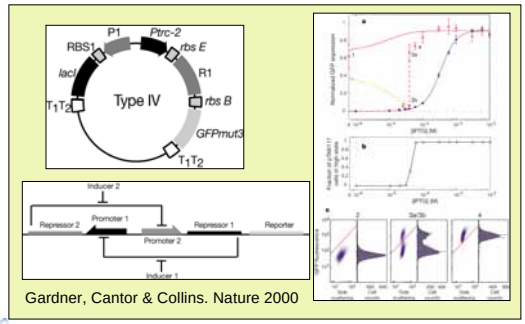
- Function
  - Inducible vs. repressible
  - Logarithmic Gain
  - Expression Capacity
  - Coupling Type (DUI)
- Design
  - Activator vs. Repressor Control
  - Signal-TF Interaction

Binary Gene Circuits

- Two TFs
- Not to be confused with boolean circuit

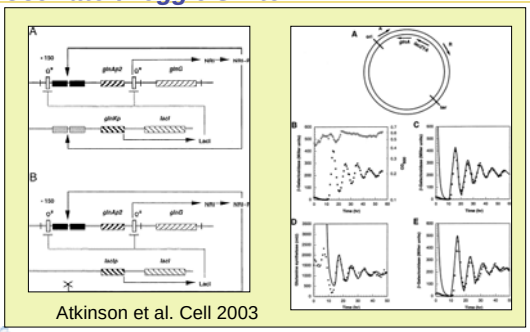


Synthetic Gene Circuits
Toggle Switch



Gardner, Cantor & Collins. Nature 2000

Synthetic Gene Circuits
Oscillator/Toggle Switch



Atkinson et al. Cell 2003

Engineered Gene Circuits
Practical Applications

- Bioremediation
  - Active biological containment (ABC) circuit (Ramos 2001)
    - Pm::asd / (Pm::lacI, xylS, lac::gef) system in a Dasd strain of P. putida
- Biosensor technology
  - Toxin-induced fluorescent circuit (Bechor 2002)
    - fabA::lux fusion plasmid in E. coli
- Gene therapy
  - Cancer-specific viral circuit (Ramachandra 2001)
    - Engineered virus that is repressed at normal p53 levels
- Metabolic engineering
  - Environment-sensitive metabolic circuit (Farmer & Liao 2000)
    - ACP-induced lycoprene production in E. coli using a modified Ntr regulon

Studies of Gene-Circuit Design

- Understand mechanisms
  - Develop models to study structure-function relations
- Understand the relation between design and performance
  - Perform mathematically controlled comparisons
- Demonstrate understanding through practical applications
  - "Tinker" with Natural and synthetic systems
- Discover and explain patterns in Natural designs
  - Characterize, document, and analyze features of Natural systems

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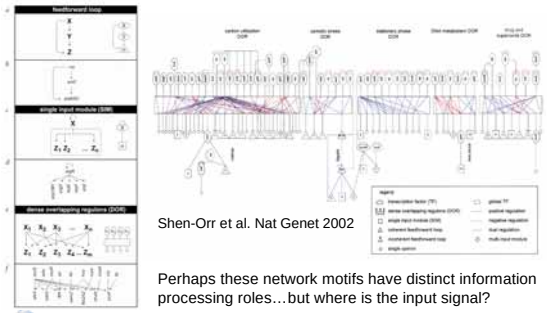
Patterns in Gene Regulation
Network Motifs in E. coli



Shen-Orr et al. Nat Genet 2002

Perhaps these network motifs have distinct information processing roles…but where is the input signal?

Examples of
E. coli FFLs

Project

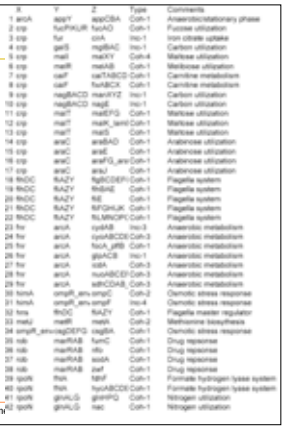
(Mangan & Alon, 2003)

Results of counting FFL types:

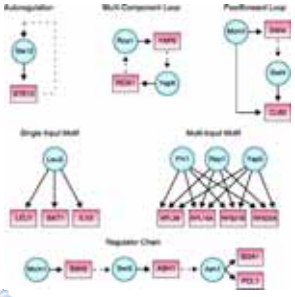
	types: [1,2,3,4]			
Coherent	28	2	4	1
Incoherent	5	0	1	1

http://www.weizmann.ac.il/mcb/UriAlon/

Also see
http://ecotfs.lanl.gov/FFLs.html



Patterns in Gene Regulation
Network Motifs in Yeast



Lee et al Science 2002

Patterns in Gene Regulation
Mode of Control

- Inducible systems
  - Repressor control
    - When substrate is seldom abundant
  - Activator control
    - When substrate is often abundant
- Repressible systems
  - Repressor control
    - When end product is often abundant
  - Activator control
    - When end product is seldom abundant

Savageau PNAS 1977

Patterns in Gene Regulation
Mode of Control, cont'd

	(-) at Regulator TU			(+) at Regulator TU			TF does not control Regulator expression
	I	U	D	I	U	D	
Inducible (+)	4[b]	3[c]	4[d]	0	0	5[e]	4[f]
Inducible (–)	0	0	9[g]	0	0	0	4[h]
Repressible (+)	0	3[i]	0	0	0	0	2[j]
Repressible (–)	0	1[k]	9[l]	0	0	0	1[m]

Wall, Hlavacek & Savageau. Nat Rev Genet 2004
http://ecotfs.lanl.gov

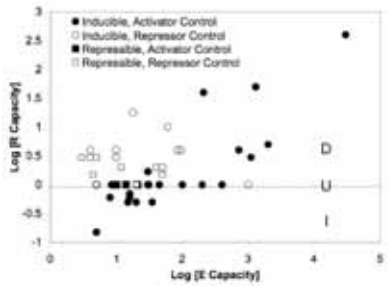
Summary of Patterns in EcoTFs

Project

- Negative Self-Regulation
  - Preference observed in natural systems (33/49)
- Positive Self-regulation
  - All Inducible (+), D
    - Different functions and performance criteria
- Inverse coupling is not found among repressible systems
- Direct coupling is preferred for systems under repressor control

http://ecotfs.lanl.gov

Patterns in Gene Regulation
Coupling of Gene Expression



Wall, Hlavacek, & Savageau. JMB 2003

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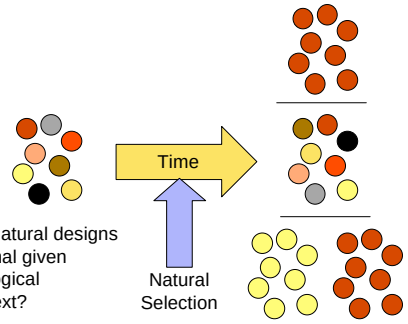
Understanding Patterns in Natural Systems
Search for Design Principles



Are natural designs optimal given ecological context?

Demand Theory

X $\xrightarrow{+/-}$ Y

+ indicates positive mode of control, or activator control
- Indicates negative mode of control, or repressor control

Are there natural preferences for mode of control?

Demand Theory

Demand Theory, cont'd

Regulator $\xrightarrow{+/-}$ Enzyme

Demand Theory:

If enzyme is in high demand, expect positive control

If enzyme is in low demand, expect negative control

Example of a design principle

Demand Theory, cont'd

High Demand

Regulator $\xrightarrow{+}$ Enzyme — **Loss of enzyme High Penalty**

Regulator $\xrightarrow{-}$ Enzyme — **Overexpression Low penalty**

Any observed preference should be for positive control

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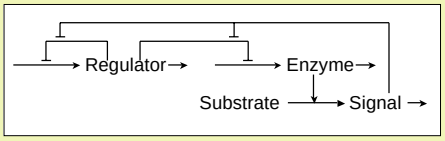
Models of Genetic Regulatory Circuits

Given knowledge of gene-circuit structure, what is gene-circuit function?



Does the level of enzyme go up or down with signal?

Need mathematical models

THE CONTROL OF THE FORMATION OF SPECIFIC PROTEINS IN BACTERIA AND IN ANIMAL CELLS

By Leo Szilard*

THE ENRICO FERMI INSTITUTE FOR NUCLEAR STUDIES, THE UNIVERSITY OF CHICAGO

Communicated January 19, 1960

Szilard, PNAS 1960

Models of Gene Regulation
Coupled ODEs

Boolean: Cynthia Reichhardt Olson
Stochastic: Other session

Transcription rate

$$dX/dt = V^{(+)} - V^{(-)}$$

First-order decay/dilution

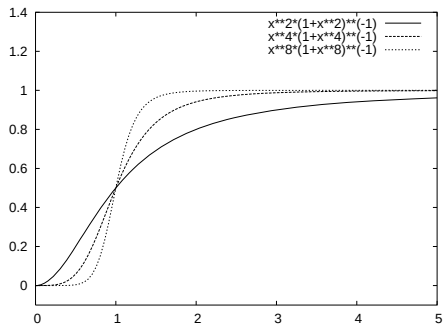
$$V^{(-)} = bX$$

Hill-function regulation model

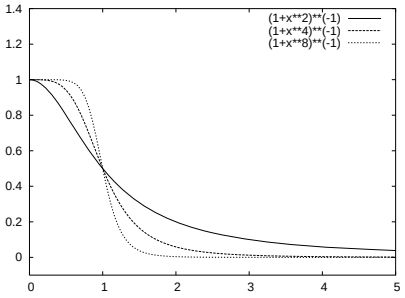
$$V^{(+)} = a\left(1 + R^n\right)^{-1}$$

$n < 0$ activation
$n > 0$ repression

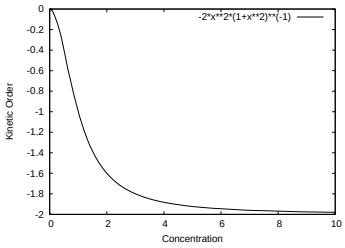
Model of Activation



Model of Repression



Power-Law Approximation



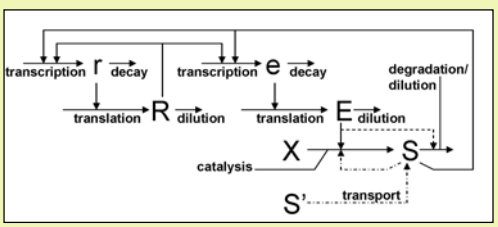
$$dX_i/dt = \alpha_i \prod_j X_j^{g_{ij}} - \beta_i \prod_j X_j^{h_{ij}}$$

$$g_{ij} = \frac{\partial \ln V_i^{(+)}}{\partial \ln X_j}; h_{ij} = \frac{\partial \ln V_i^{(-)}}{\partial \ln X_j}$$

$$\alpha_i = \frac{V_i^{(+)}}{\prod_j X_j^{g_{ij}}}; \beta_i = \frac{V_i^{(-)}}{\prod_j X_j^{h_{ij}}}$$

$$\frac{\partial}{\partial \ln x} \ln a\left(1 + x^n\right)^{-1} = -nx^n\left(1 + x^n\right)^{-1}$$

Elementary Gene Circuit Model



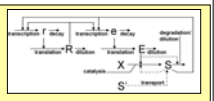
Dashes = inducible-catabolic gene circuit
Dot-dashes = repressible-biosynthetic gene circuit

HIavacek & Savageau. JMB 1996; Wall, HIavacek & Savageau. JMB 2003

Elementary Gene Circuit
Power-Law Mathematical Model

$$dX_1/dt = \alpha_1 X_6^{g_{16}} X_3^{g_{13}} X_5^{g_{15}} - \beta_1 X_1^{h_{11}}$$
$$dX_2/dt = \alpha_2 X_7^{g_{27}} X_1^{g_{21}} - \beta_2 X_2^{h_{22}}$$
$$dX_3/dt = \alpha_3 X_8^{g_{38}} X_9^{g_{39}} X_2^{g_{32}} X_3^{g_{33}} - \beta_3 X_2^{h_{32}} X_3^{h_{33}}$$
$$dX_4/dt = \alpha_4 X_6^{g_{46}} X_3^{g_{43}} X_5^{g_{45}} - \beta_4 X_4^{h_{44}}$$
$$dX_5/dt = \alpha_5 X_7^{g_{57}} X_4^{g_{54}} - \beta_5 X_5^{h_{55}}$$

X_1 = Enzyme mRNA
X_2 = Enzyme
X_3 = Intracellular signal
X_4 = TF mRNA
X_5 = TF
X_6 = nucleic acid pool
X_7 = amino acid pool
X_8 = Signal precursor
X_9 = Extracellular signal



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- Structure-function relations

Controlled Mathematical Comparisons

- Compare performance criteria for alternative systems that carry out the same function (e.g. repressible systems)
- Internal Equivalence
  - Maintain identical parameter values for all processes but those being considered as alternative designs
    - All processes but transcription of regulator and enzyme are equivalent
- External Equivalence
  - Ensure that systems being compared have the same overall biological function
    - Steady-state effector gain is the same for alternative systems

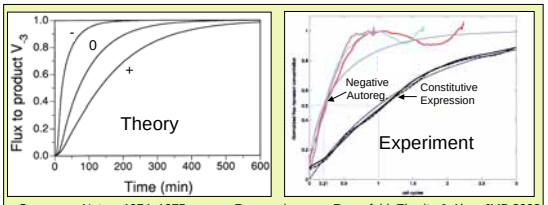
Savageau 1976 "Biochemical Systems Theory"

Performance Criteria for Elementary Gene Circuits

- Stability
  - Defined for stable systems as the ability of the steady-state enzyme level to remain stable, even when model parameter values may vary
  - Linearize equations about the steady-state solution, determine regions of parameter space with negative eigenvalues
  - Measure a distance in parameter space between a stable system and the nearest unstable system
- Steady-state robustness
  - Ability of the steady-state enzyme level to be maintained when model parameter values vary
  - Parameter sensitivity analysis
- Temporal responsiveness
  - Ability of the system to equilibrate quickly after a change in signal
  - Solve for system dynamics and calculate rise-times, decay-times, and settling times
- Information processing performance?
  - Mutual information
  - Predictive information

Project?

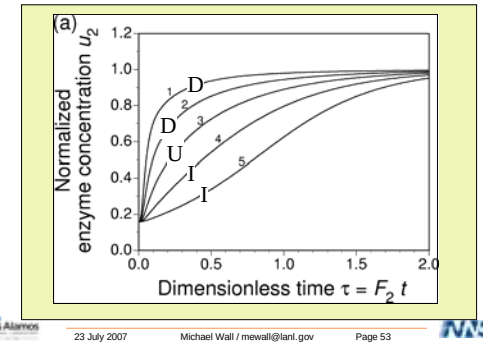
TF Negative Self-Regulation Increases Performance



Savageau. Nature 1974, 1975
Hlavacek & Savageau. JMB 1995, 1996, 1997
Wall, Hlavacek & Savageau. JMB 2003

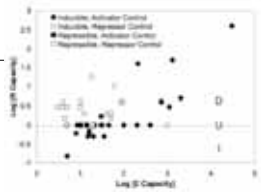
Responsiveness: Rosenfeld, Elowitz & Alon. JMB 2002
Stability: Becskei & Serrano. Nature 2000
Robustness: Little et al. EMBO J 1999

Temporal Responsiveness Changes with Coupling Type



Prediction of Optimal Coupling Types

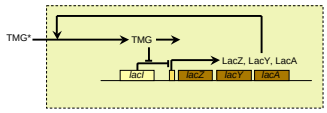
Effector TU	Low Gain	Intermediate Gain	High Gain
Inducible (+)	*Inverse*	*Uncoupled*	*Direct*
Inducible (-)	*Direct*	*Uncoupled*	*Inverse*
Repressible (+)	*Inverse*	*Direct*	*Direct*
Repressible (-)	*Direct*	*Uncoupled*	*Inverse*

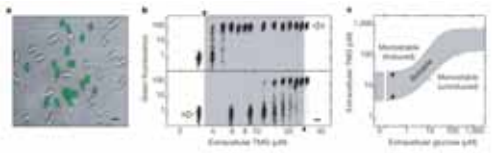


Contents

- Gene regulation
- Genetic regulatory networks
- Genetic regulatory circuits
- Patterns
- Design principles
- Mathematical models
- Controlled mathematical comparisons
- Structure-function relations

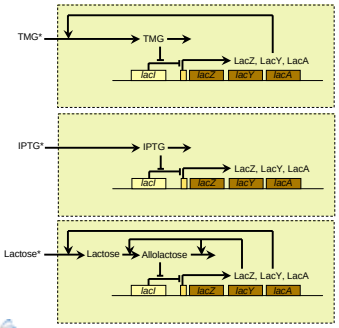
Example: Bistability in *lac*





Ozbudak et al. Nature 2004

Importance of Biological Context

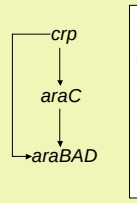


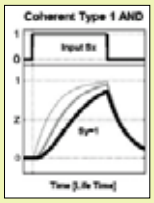
Project

- Is lac bistable in response to lactose?
- Novick "& Weiner, PNAS 1957
  - *lac* is bistable…
  - …in response to TMG!

Structure-Function Relations *Feed-Forward Loop*

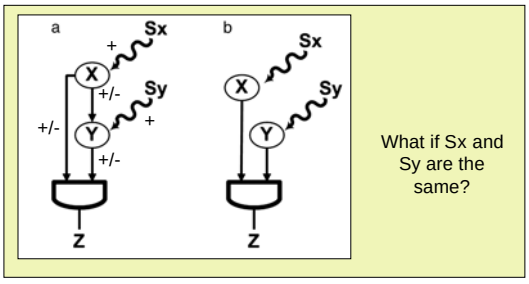
A network motif in the *E. coli* genetic regulatory network





Shen-Orr et al Nat Genet 2002
Mangan & Alon. PNAS 2003

Alternative Signal Interactions in FFLs



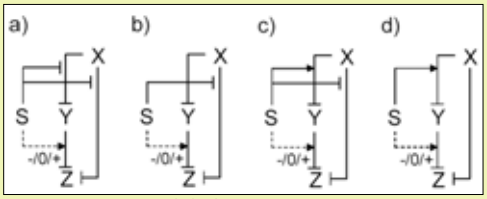
What if Sx and Sy are the same?

Mangan, S. and Alon, U. (2003) Proc. Natl. Acad. Sci. USA 100, 11980-11985

PNAS

Type 2 Incoherent FFL

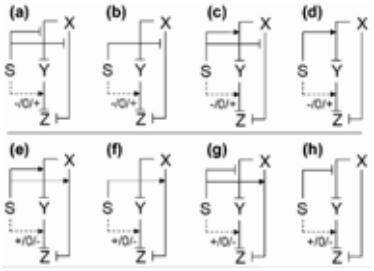
- Previous prediction of function: Repressible, Sign-sensitive accelerator



GntR/IdnR ExuR/UxuR | GalR/GalS *Coincidence circuit or Elementary circuit* | ? | *Two-step cascade*

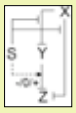
Analysis of an Exhaustive Set of Type 2 iFFLs

- a) (-,-,*)
  - *gntRKU-idnDOTR-gntKU*
  - *exuR-uxuR-uxuAB*
- b) (0,-,*)
  - *galR-galS-galETKM*



Symmetry between top & bottom systems

Mathematical Model of the Type 2 iFFL



$$H_{ij}(S_{ij}x_j/K_{ij}) = \left[1+\left(S_{ij}x_j/K_{ij}\right)^{n_{ij}}\right]^{-1}$$

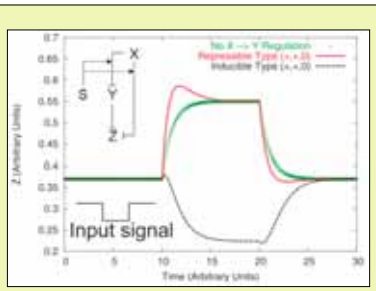
$$dY/dt = B_Y + \alpha_Y H^r_{YX}\left(S_{YX}X/K_{YX}\right) - \beta_Y Y$$

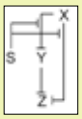
$$dZ/dt = B_Z + \alpha_Z H^r_{ZX}\left(S_{ZX}X/K_{ZX}\right)H^r_{ZY}\left(S_{ZY}Y/K_{ZY}\right) - \beta_Z Z$$

$$\beta_Y Y_\infty = B_Y + \alpha_Y H^r_{YX}\left(S_{YX}X/K_{YX}\right)$$

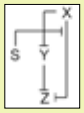
$$\beta_Z Z_\infty = B_Z + \alpha_Z H^r_{ZX}\left(S_{ZX}X/K_{ZX}\right)H^r_{ZY}\left(S_{ZY}Y_\infty/K_{ZY}\right)$$

Functions of the (+,+,0) Type 2 iFFL





Vs.



Performance of the (+,+,0) Type 2 iFFL

- De-repression rise-time
  - Time required for the level of Z to come within 5% of its steady-state value
- De-repression settling-time
  - Time beyond which the level of Z never strays farther than 5% from its steady-state value
- Repression decay-time
- Repression settling-time

Equivalence Conditions for Comparisons

$$B_Z + \alpha_Z R_{Z,\min} = B'_Z + \alpha'_Z R'_{Z,\min}$$

$$B_Z + \alpha_Z R_{Z,\max} = B'_Z + \alpha'_Z R'_{Z,\max}$$

$$\alpha_Z = \alpha'_Z\left(\frac{R'_{Z,\max} - R'_{Z,\min}}{R_{Z,\max} - R_{Z,\min}}\right)$$

$$B_Z = B'_Z + \alpha'_Z R'_{Z,\min} - \alpha_Z R_{Z,\min}$$

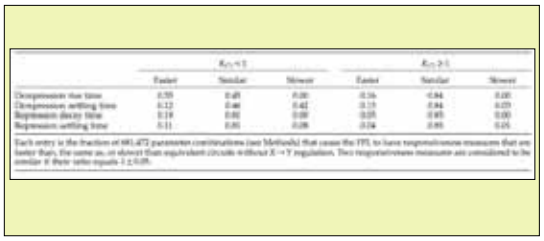
Equivalent steady-state Z at low and high signal

Parameter Sampling

- Three Hill coefficients n_{ij}: sample integers in range [1,8]
- Three thresholds K_{ij}: sample 11 log-randomly in range [0.01,100]
- 681,472 combinations in total
- Reference system:

  $B_X = B_Y = 0.1$; $\alpha_X = \alpha_Y = 0.9$; $\beta_X = \beta_Y = \beta_Z = 1$, $K_{YX} = 1$, $K_{ZX} = 1$, $K_{ZY} = 1$; $n_{YX} = n_{ZX} = |n_{ZY}| = 2$, $X = 1$

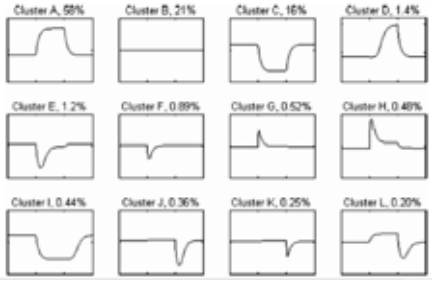
Summary of (+,+,0) Type 2 *i*FFL Temporal Responsiveness



- Derepression is either faster (rise) or slower (settle)
- Repression is faster

Clustering of Functional Responses

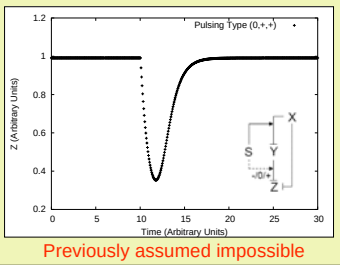
Project



Distribution of Functional Responses

Effect of Signal on TF Activities			% of Time Courses in Clusters A-L												
ZX	YX	ZY	A	B	C	D	E	F	G	H	I	J	K	L	Entropy
-	-	-	85	5	0	0	0	0	0	0	0	4	3	2	0.89
-	-	**0**	46	22	20	0	0	0	3	3	5	0	0	0	2.04
-	-	**+**	38	9	47	0	0	0	3	3	0	0	0	0	1.70
-	**0**	-	93	7	0	0	0	0	0	0	0	0	0	0	0.37
-	**0**	**0**	69	31	0	0	0	0	0	0	0	0	0	0	0.90
-	**0**	**+**	49	11	40	0	0	0	0	0	0	0	0	0	1.38
-	**+**	-	95	5	0	0	0	0	0	0	0	0	0	0	0.27
-	**+**	**0**	71	21	0	8	0	0	0	0	0	0	0	0	1.11
-	**+**	**+**	43	9	36	4	4	4	0	0	0	0	0	0	1.95
0	**+**	-	66	34	0	0	0	0	0	0	0	0	0	0	0.92
0	**+**	**0**	38	57	0	5	0	0	0	0	0	0	0	0	1.22
0	**+**	**+**	0	37	47	0	10	6	0	0	0	0	0	0	1.62
	All		58	21	16	1	1	1	1	0	0	0	0	0	1.76

Two-Step Cascade *Pulse Generator*



Previously assumed impossible

Implications for Studies of Genetic Regulatory Networks

- The functions of binary gene circuits depend on signal interactions as well as genetic regulatory interactions
  - Inducible vs repressible
  - Intermediate steady-state response
  - Acceleration or delay of dynamics
  - Overshoot, undershoot, or pulsing
- Genome-wide characterization of genetic regulatory networks will require knowledge of both signal interactions and genetic regulatory interactions (EcoTFs)

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  - Judith Cohn
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  - Ilya Nemenman
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- National Institutes of Health