

Bioinformatics 3

# V8 – Gene Regulation

Tue, Nov 15, 2011

# Recently in PLoS Comp. Biol.

OPEN  ACCESS Freely available online

PLOS COMPUTATIONAL BIOLOGY

## Structural Properties of the *Caenorhabditis elegans* Neuronal Network

Lav R. Varshney<sup>1</sup>, Beth L. Chen<sup>2</sup>, Eric Paniagua<sup>3</sup>, David H. Hall<sup>4</sup>, Dmitri B. Chklovskii<sup>5\*</sup>

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Reconstruction and classification of the worm's neuronal network

*PLoS Comput. Biol.* **7** (2011) e1001066

**"Network" => What can we apply???**

# Excursion: *C. elegans*



Small worm:  $L = 1 \text{ mm}$ ,  $\varnothing \approx 65 \mu\text{m}$   
lives in the soil, eats bacteria

Consists of 959 cells, 302 nerve cells,  
all worms are "identical"

Completely sequenced in 1998 (first  
multicellular organism)

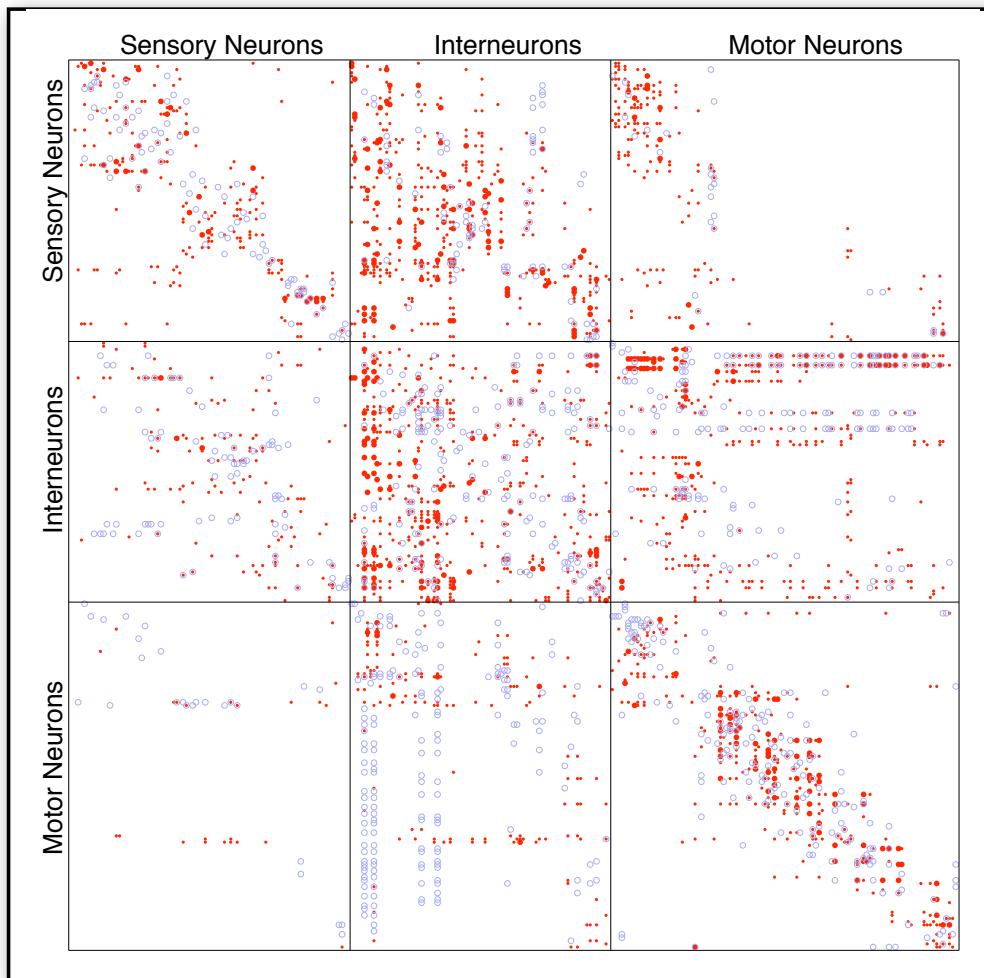
Very simple handling, transparent

=> One of the **prototype organisms**

Database "everything" about the worm:  
[www.wormbase.org](http://www.wormbase.org)



# Adjacency Matrix



Two types of connections between neurons:

- **gap junctions**  
=> electric contacts  
=> undirected
- **chemical synapses**  
=> neurotransmitters  
=> directed

Observations:

- three groups of neurons (clustering)
- gap junction entries are symmetric, chemical synapses not (directionality)

# Some Statistics

TABLE S1  
CONNECTED COMPONENTS OF THE GAP JUNCTION NETWORK. NOTE THE SINGLE GIANT COMPONENT AND THE LARGE NUMBER OF DISCONNECTED/ISOLATED NEURONS.

**Giant Component (248 neurons)**

ADAL/R	ALNL	AVG	DD01-05	PDA	PVR	RIVL/R	SABVL/R	URYVLR
ADEL/R	AQR	AVHL/R	DVA	PDB	PVT	RMDDL/R	SDQL/R	VA01-12
ADFL/R	AS01-11	AVJL/R	DVB	PDEL/R	PVWL/R	RMDL/R	SIADL/R	VB01-11
ADLL/R	ASGL/R	AVKL/R	DVC	PHAL/R	RIBL/R	RMDVL/R	SIAVL/R	VC01-05
AFDL/R	ASHL/R	AVL	FLPL/R	PHBL/R	RICL/R	RMED	SIBDL/R	VD01-10,13
AIAL/R	ASIL/R	AVM	IL1DL/R	PHVL/R	RID	RMEL/R	SIBVL/R	
AIBL/R	ASKL/R	AWAL/R	IL1L/R	PLML/R	RIFL/R	RMEV	SMBDL/R	
AIML	AUAL/R	AWBL/R	IL1VL/R	PQR	RIGL/R	RMFL	SMBVL/R	
AINL/R	AVAL/R	BAGL/R	IL2L/R	PVCL/R	RIH	RMGL/R	SMDDL/R	
AIYL/R	AVBL/R	CEPDL/R	LUAL/R	PVM	RIML/R	RMHL/R	SMDVL/R	
AIZL/R	AVDL/R	CEPVL/R	OLLL/R	PVNL	RIPL/R	SAADL/R	URBL/R	
ALA	AVEL/R	DA01-09	OLQDL/R	PVPL/R	RIR	SAAVL/R	URXL/R	
ALML/R	AVFL/R	DB01-07	OLQVL/R	PVQL/R	RIS	SABD	URYDL/R	

**First Small Component (2 neurons)**

ASJL/R

**Second Small Component (3 neurons)**

HSNL/R PVNR

**Neurons with no gap junctions (26 neurons)**

AIMR	ASEL/R	BDUL/R	IL2DL/R	PLNL/R	RIAL/R	URADL/R	VD1I-12
ALNR	AWCL/R	DD06	IL2VL/R	PVDL/R	RMFR	URAVL/R	

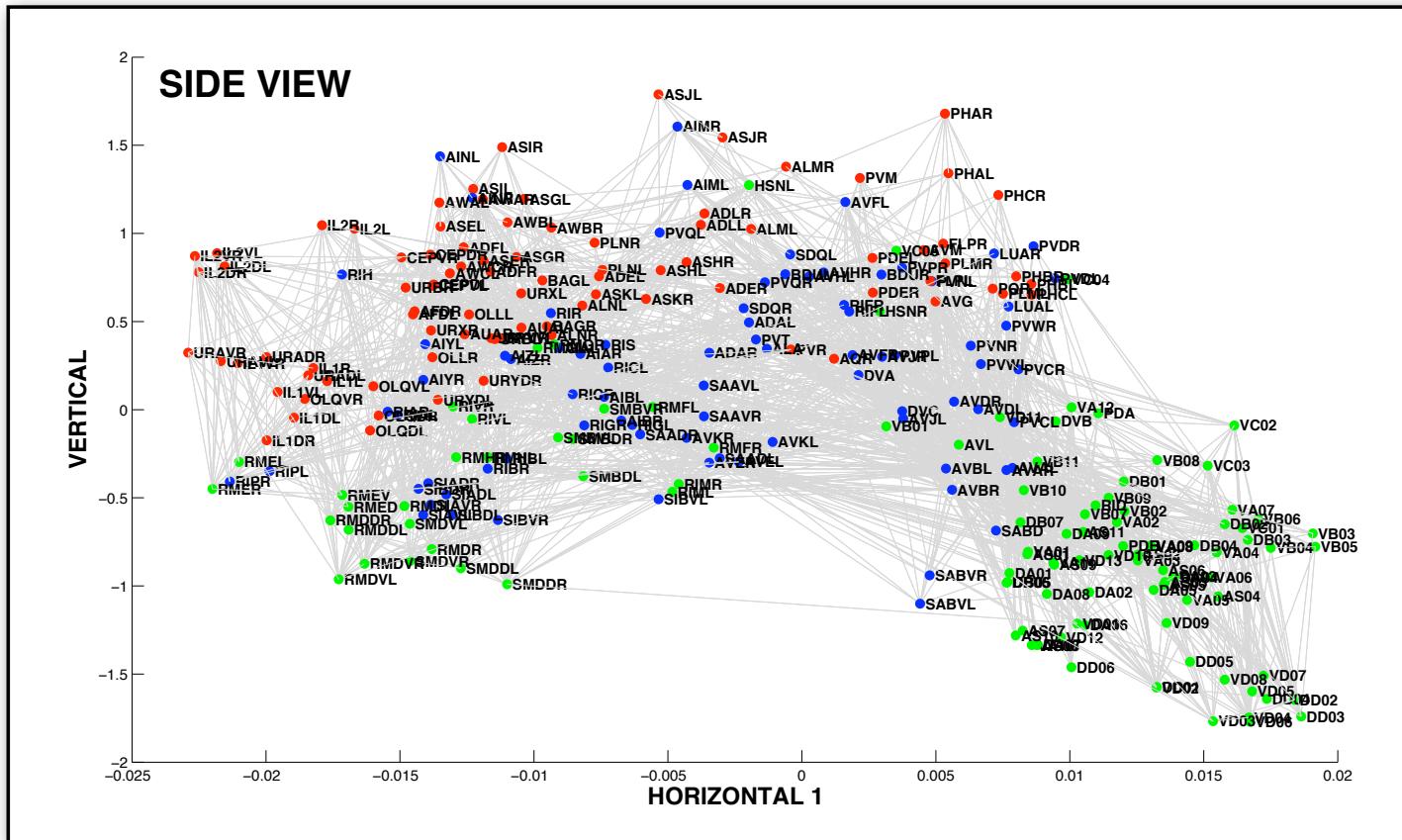
TABLE S2

(A) NUMBER OF GAP JUNCTION CONTACTS BETWEEN DIFFERENT NEURON CATEGORIES. (B) PERCENT OF GAP JUNCTIONS ON NEURONS OF THE ROW CATEGORY THAT CONNECT TO NEURONS OF THE COLUMN CATEGORY.

A	Sensory	Inter-	Motor
Sensory	108	119	26
Inter-	119	368	342
Motor	26	342	324

B	Sensory	Inter-	Motor
Sensory	42.7%	47.0%	10.3%
Inter-	14.4%	44.4%	41.3%
Motor	3.8%	49.4%	46.8%

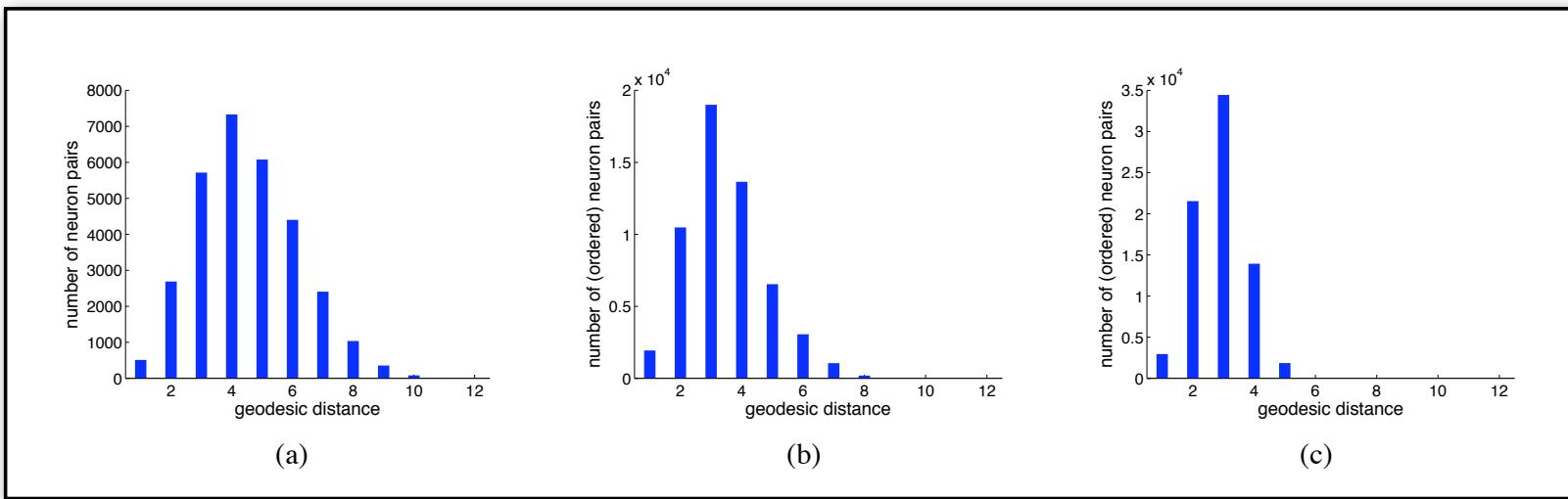
# Information Flow



Network arranged so that information flow is (mostly) top => bottom  
sensory neurons      interneurons      motorneurons

# Network Size

Geodesic distance (shortest path) distributions of giant component of...



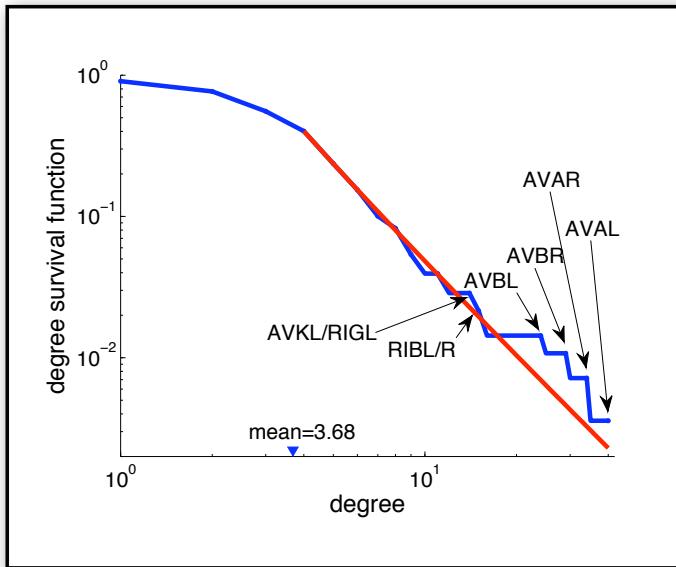
(electric)  
gap junctions

(chemical)  
synapses

combined  
network

=> a worm is a small animal :-)

# Degree Distribution

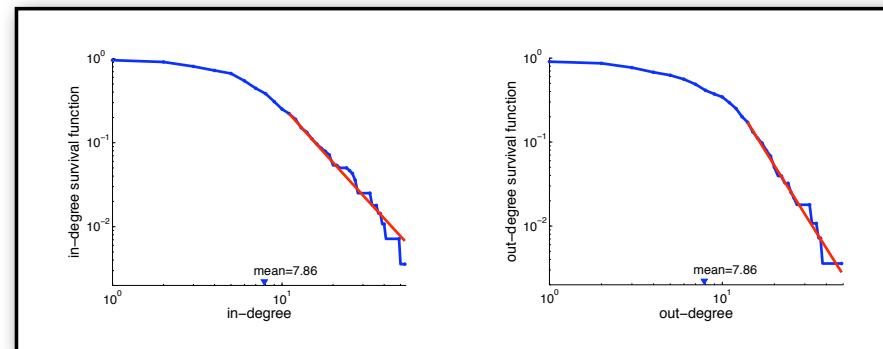


Plot of the "survival function" of  $P(k)$   
( $1 - \text{cumulative } P(k)$ )  
for the (electric) gap junctions

$$P(d) = \sum_{k=d}^{\infty} p(k)$$

Power law for  $P(k)$  with  $\gamma = 3.14$  ( $\approx \pi?$ )

In/out degrees of the  
chemical synapses  
=> fit with  $\gamma = 3.17 / 4.22$   
(but clearly not SF!)



# Some More Statistics

TABLE S3

COMPARISON OF CLUSTERING COEFFICIENT AND CHARACTERISTIC PATH LENGTH OF THE GIANT COMPONENT OF THE *C. elegans* GAP JUNCTION NETWORK AND SEVERAL OTHER NETWORKS THAT HAVE BEEN CLASSIFIED AS SMALL WORLD NETWORKS. THE CLUSTERING COEFFICIENT OF AN EQUIVALENT ERDÖS-RÉNYI RANDOM NETWORK IS INDICATED IN PARENTHESES. THIS IS CALCULATED USING THE WATTS AND STROGATZ APPROXIMATIONS TO  $L$  AND  $C$  BY FINDING

$$C_r \approx \frac{1}{N} \exp\left(\frac{\ln(N)}{L}\right).$$

Network	$N$	$C$ ( $C_r$ )	$L$
Giant component of gap junction network	248	0.21 (0.014)	4.52
Analog electronic circuit [100]	329	0.34 (0.019)	3.17
Class dependency graph of Java computer language [101]	1376	0.06 (0.002)	6.39
Film Actors [13]	225226	0.79 (0.00013)	3.65
Power Grid [13]	4941	0.080 (0.00032)	18.7

Much higher  
clustering than ER

TABLE S4

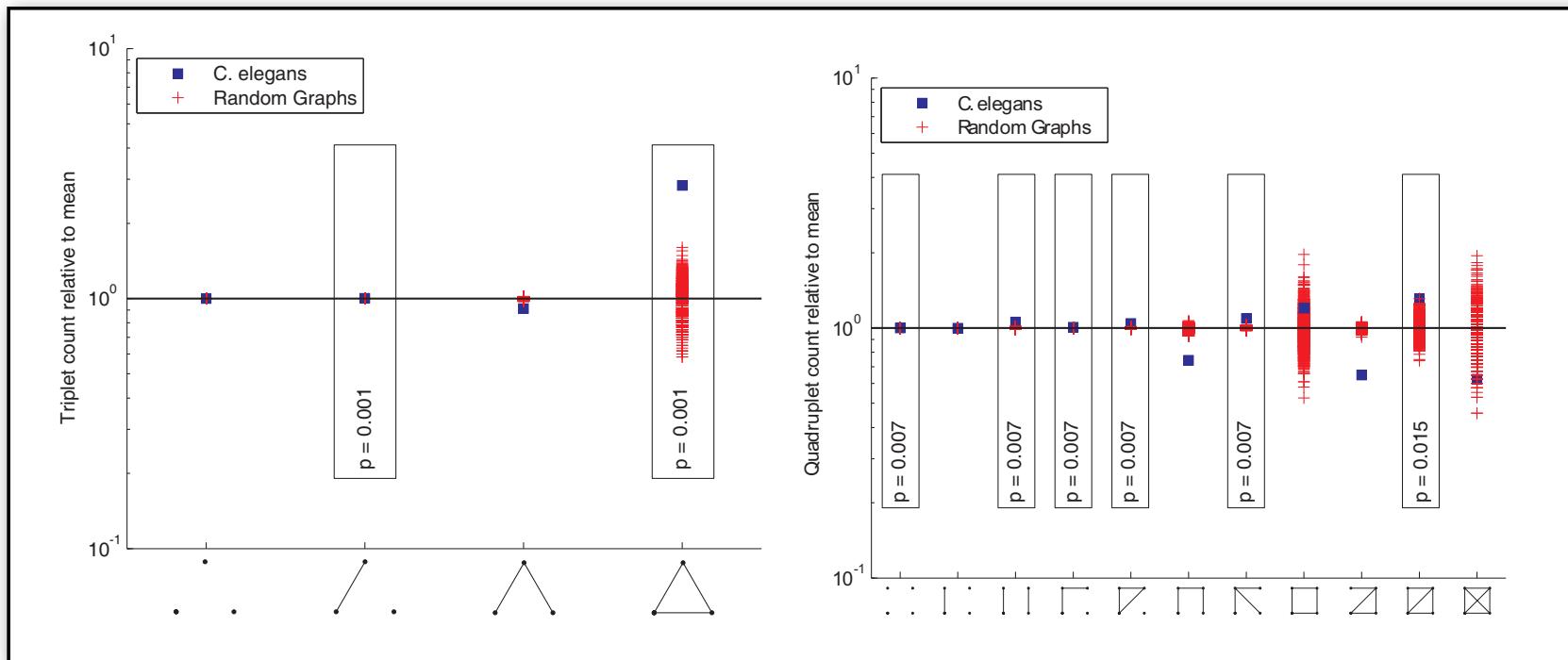
(A) NUMBER OF CHEMICAL SYNAPSE CONTACTS FROM ROW CATEGORY TO COLUMN CATEGORY. (B) PERCENT OF SYNAPSES IN ROW CATEGORY THAT SYNAPSE TO COLUMN CATEGORY.

A	Sensory	Inter-	Motor
Sensory	474	1434	353
Inter-	208	1359	929
Motor	30	275	1332

B	Sensory	Inter-	Motor
Sensory	21.0%	63.4%	15.6%
Inter-	8.3%	54.5%	37.2%
Motor	1.8%	16.8%	81.4%

# Network Motifs

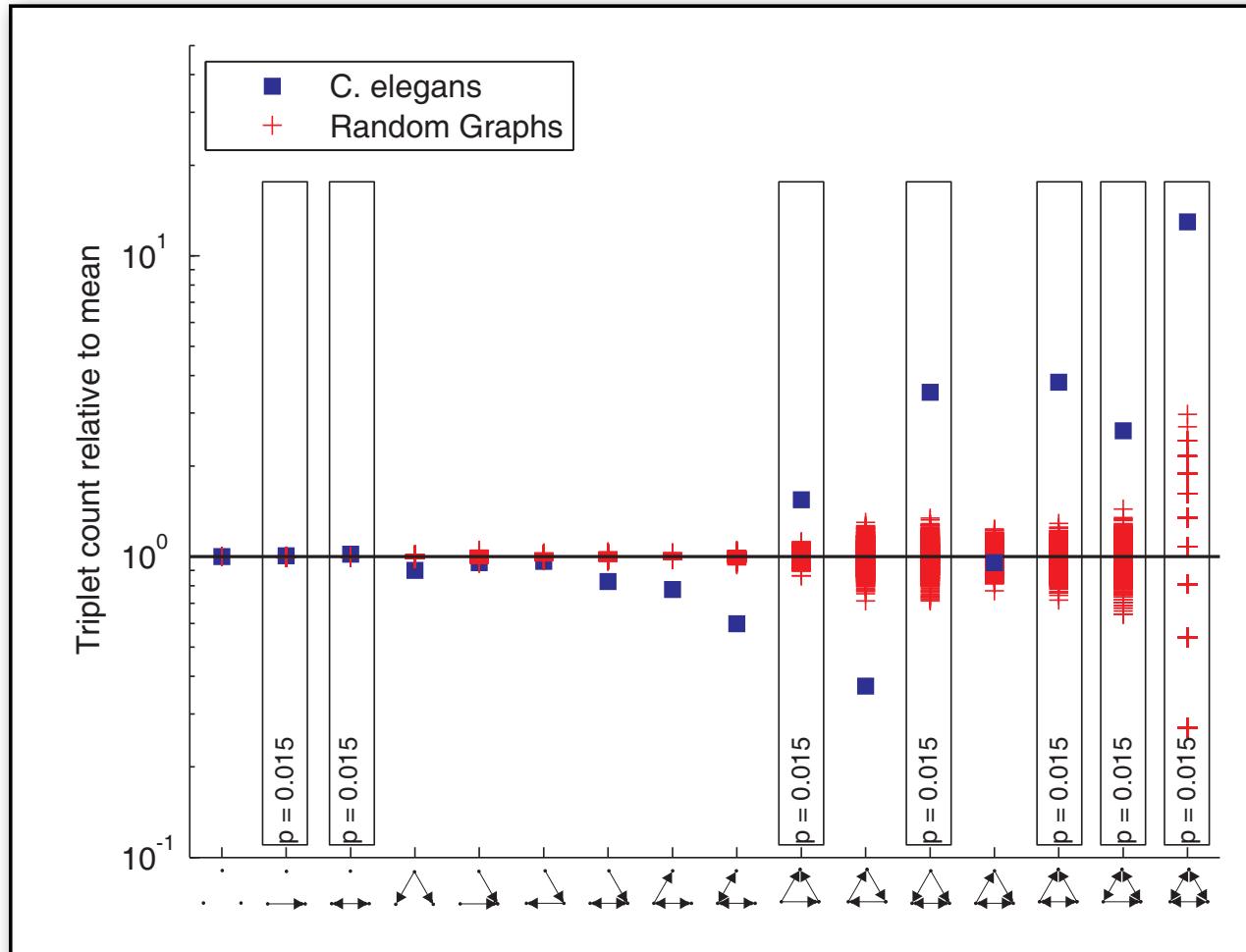
Motif counts of the electric gap junction network relative to random network



=> symmetric structures are overrepresented

=> clearly not a random network

# Motifs II



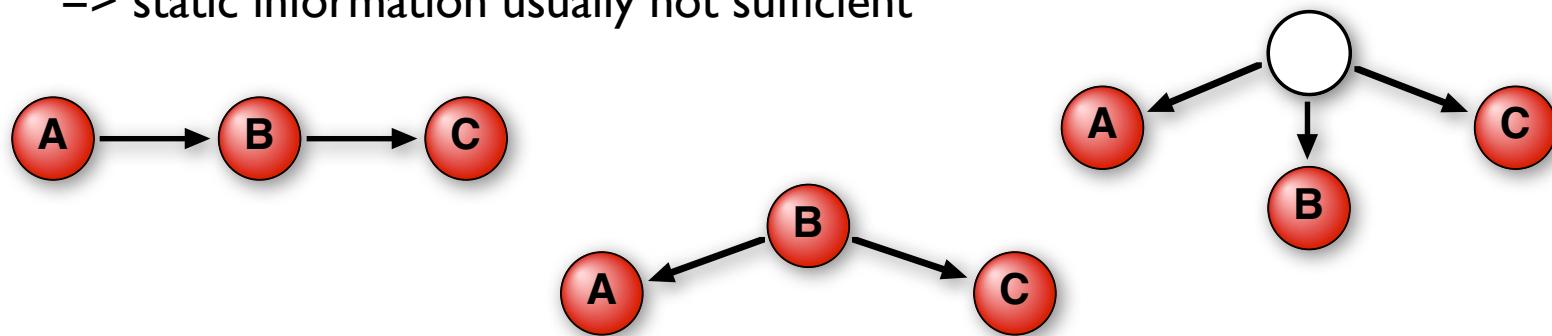
Similar picture for the chemical synapses: not random

# Network Reconstruction

Experimental data: DNA microarray => expression profiles

**Clustering** => genes that are **regulated simultaneously**  
=> Cause and action??? Are all genes known???

Three different networks that lead to the same expression profiles  
=> **combinatorial explosion** of number of compatible networks  
=> static information usually not sufficient



Some formalism may help  
=> **Bayesian networks** (formalized conditional probabilities)  
but usually too many candidates...

# Network Motifs

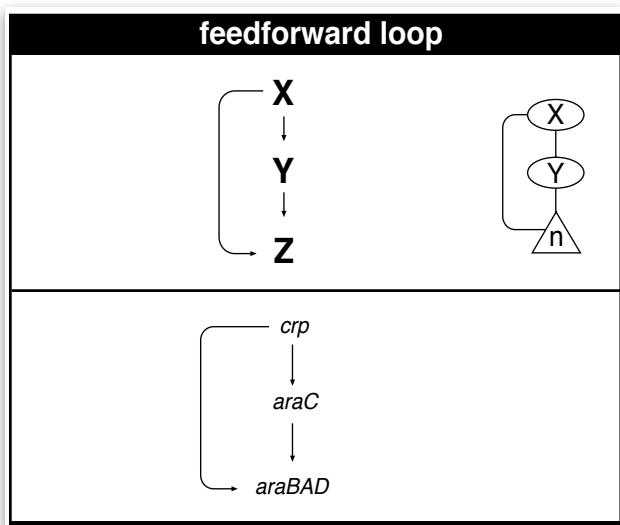
## **Network motifs in the transcriptional regulation network of *Escherichia coli***

Shai S. Shen-Orr<sup>1</sup>, Ron Milo<sup>2</sup>, Shmoolik Mangan<sup>1</sup> & Uri Alon<sup>1,2</sup>

*Nature Genetics* **31** (2002) 64

RegulonDB + their own hand-curated findings  
=> break down network into motifs  
=> statistical significance of the motifs?  
=> behavior of the motifs <=> location in the network?

# Motif I: Feed-Forward-Loop



X = general transcription factor  
Y = specific transcription factor  
Z = effector operon(s)

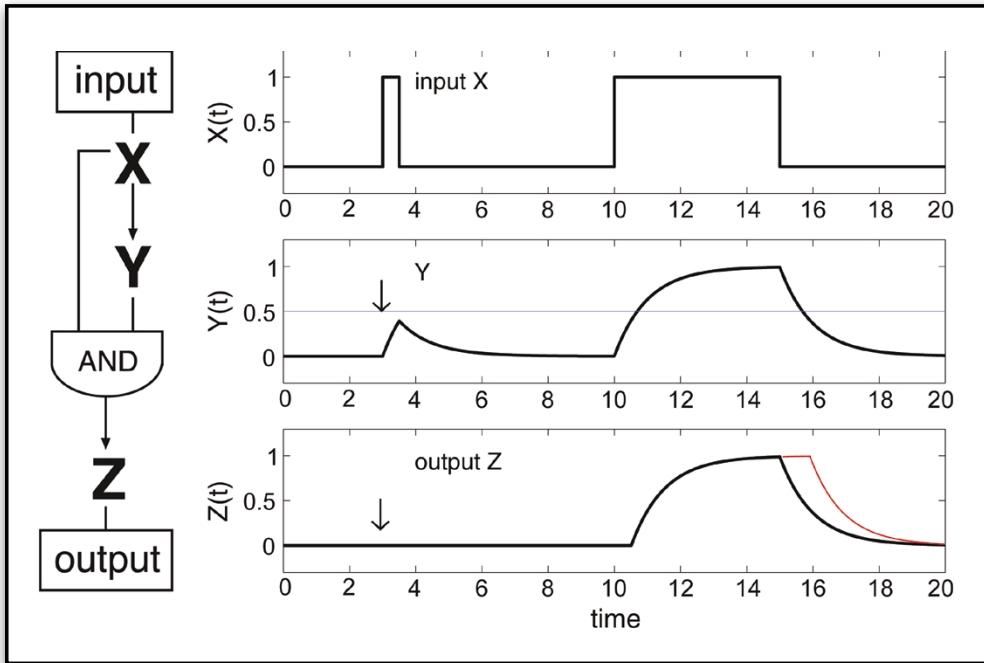
Why not direct regulation without Y?

X and Y **together** regulate Z:

"**coherent**", if X and Y have the **same** effect on Z (activation vs. repression), otherwise "incoherent"

85% of the FFL in E coli are coherent

# FFL dynamics



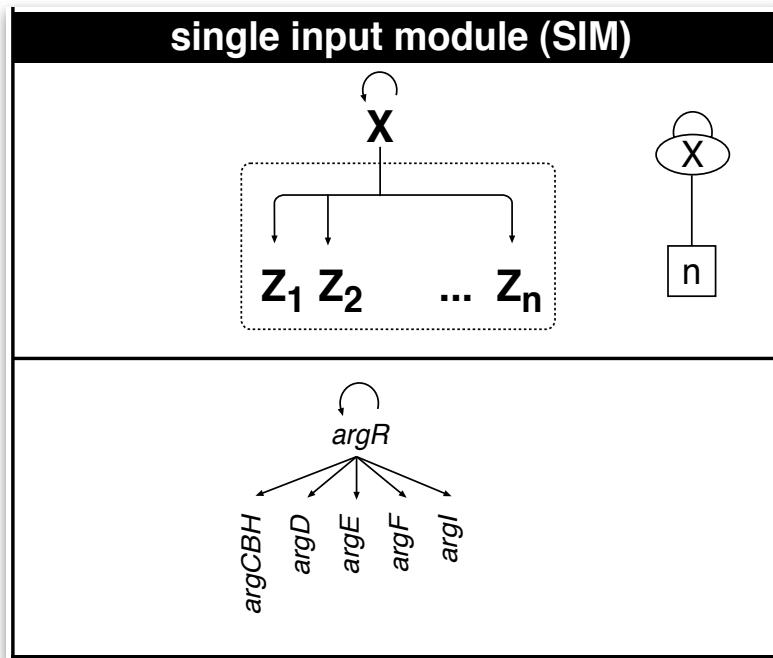
In a coherent FFL:  
**X and Y activate Z**

- Dynamics:
- input activates X
  - X activates Y (delay)
  - (X && Y) activates Z

Delay between X and Y => signal must persist longer than delay  
=> reject transient signal, react only to **persistent** signals  
=> fast shutdown

Helps with **decisions** based on **fluctuating signals**

# Motif 2: Single-Input-Module

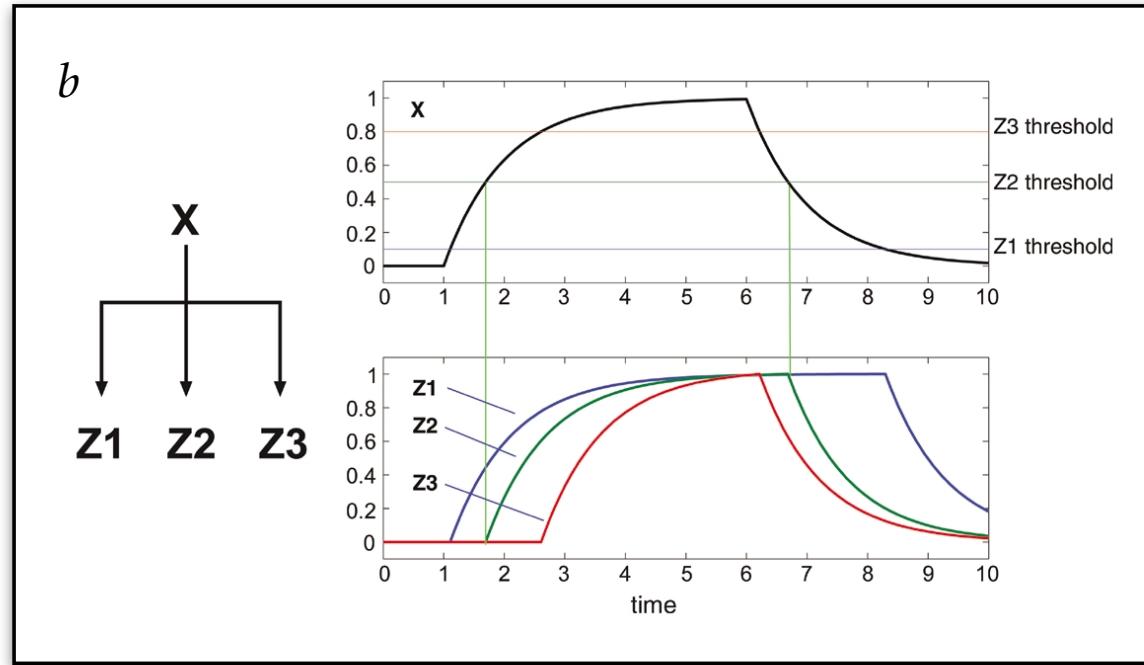


Set of operons controlled by a single transcription factor

- same sign
- no additional regulation
- control usually autoregulatory (70% vs. 50% overall)

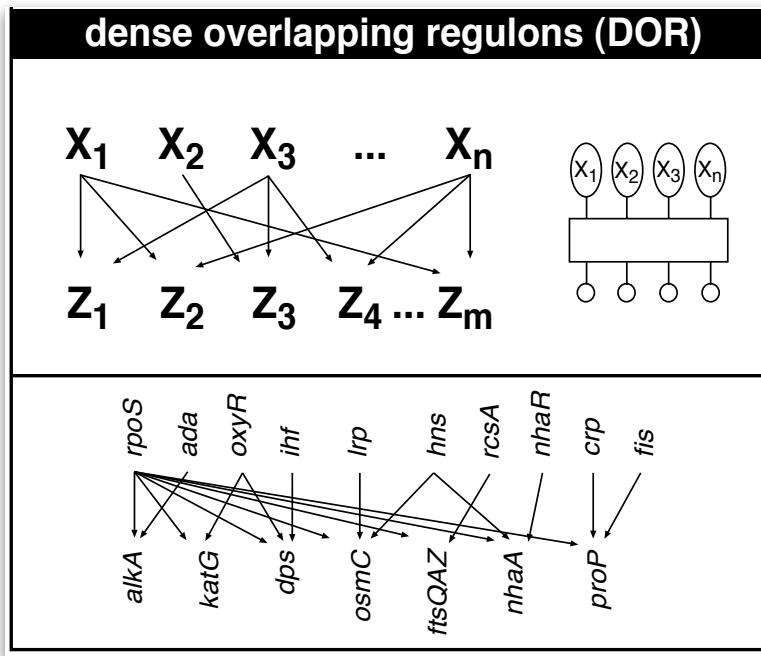
Mainly found in genes that code for **parts** of a protein **complex** or metabolic **pathway**  
=> relative stoichiometries

# SIM-Dynamics



With different thresholds for each regulated operon:  
=> first gene that is activated is the last that is deactivated  
=> well defined temporal ordering (e.g. flagella synthesis) + stoichiometries

# Motif 3: Dense Overlapping Regulon



Dense layer between groups of transcription factors and operons  
=> much denser than network average ( $\approx$  community)

Usually each operon is regulated by a different combination of TFs.

Main "**computational**" units of the regulation system

Sometimes: same set of TFs for group of operons => "multiple input module"

# Motif Statistics

**Table 1 • Statistics of occurrence of various structures in the real and randomized networks**

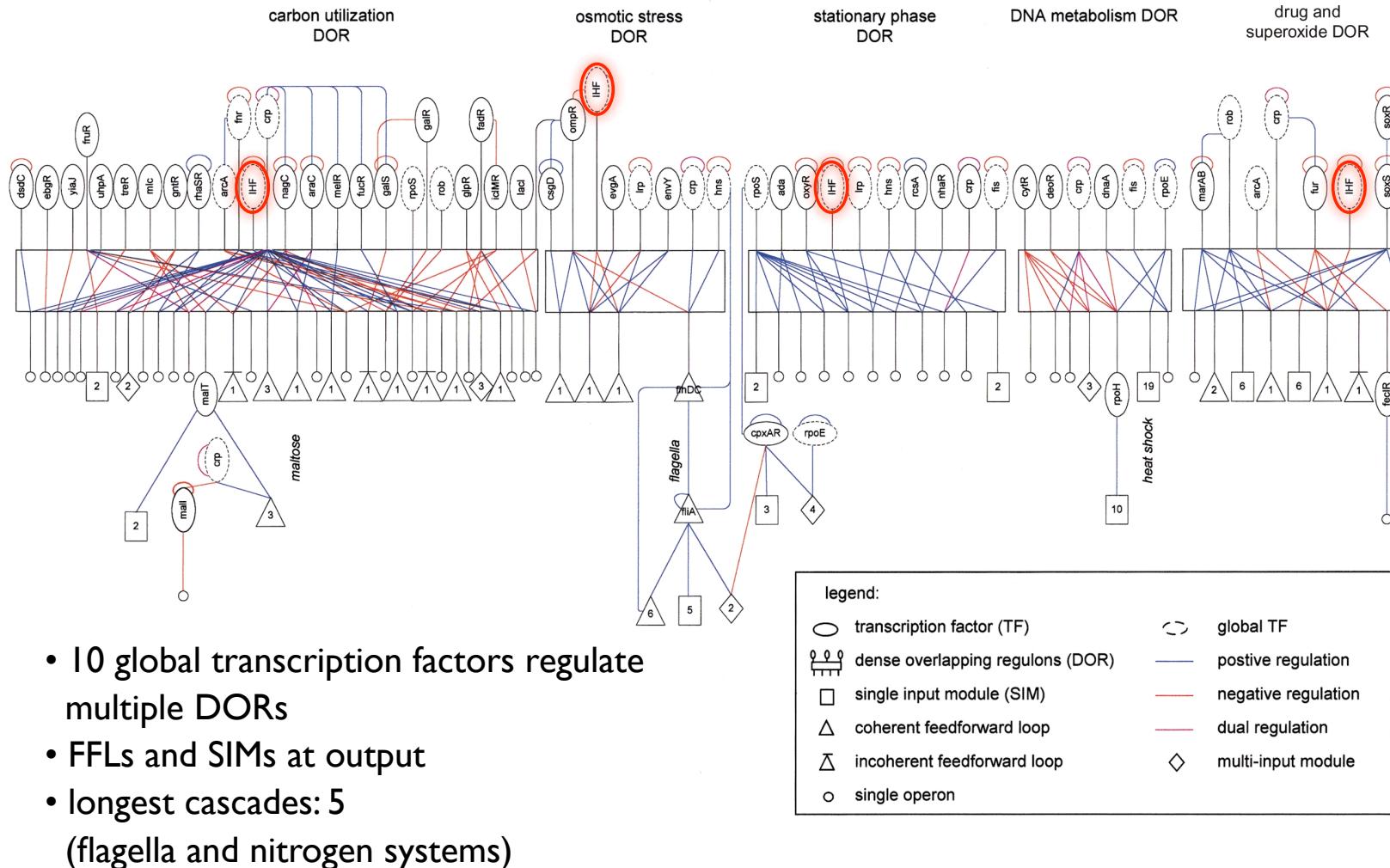
Structure	Appearances in real network	Appearances in randomized network (mean $\pm$ s.d.)	P value
Coherent feedforward loop	34	4.4 $\pm$ 3	P < 0.001
Incoherent feedforward loop	6	2.5 $\pm$ 2	P ~ 0.03
Operons controlled by SIM (>13 operons)	68	28 $\pm$ 7	P < 0.01
Pairs of operons regulated by same two transcription factors	203	57 $\pm$ 14	P < 0.001
Nodes that participate in cycles*	0	0.18 $\pm$ 0.6	P ~ 0.8

\*Cycles include all loops greater than size 1 (autoregulation). P value for cycles is the probability of networks with no loops.

All motifs are highly **overrepresented** compared to randomized networks

No cycles (X => Y => Z => X), but this is not statistically significant

# Network with Motifs



- 10 global transcription factors regulate multiple DORs
- FFLs and SIMs at output
- longest cascades: 5  
(flagella and nitrogen systems)

# Motif-Dynamics

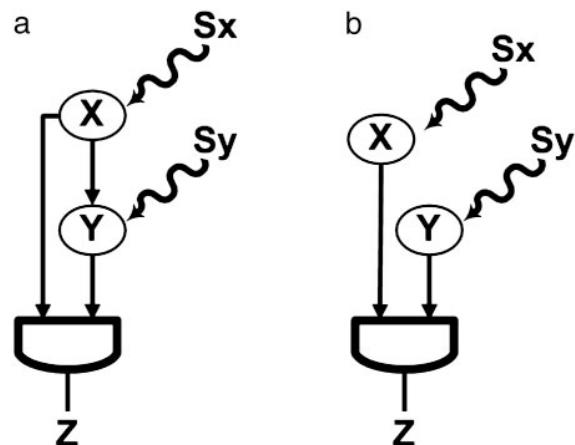
## Structure and function of the feed-forward loop network motif

S. Mangan and U. Alon<sup>†</sup>

Departments of Molecular Cell Biology and Physics of Complex Systems, Weizmann Institute of Science, Rehovot 76100, Israel

Edited by Arnold J. Levine, Institute for Advanced Study, Princeton, NJ, and approved August 25, 2003 (received for review June 22, 2003)

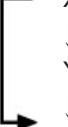
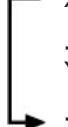
PNAS 100 (2003) 11980

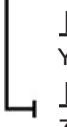


Compare dynamics of response Z to stimuli Sx and Sy for FFL (a) vs simple system (b).

# Coherent and Incoherent FFLs

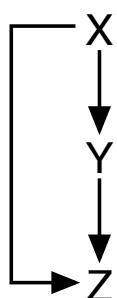
(in)coherent:  $X \Rightarrow Z$  has (opposite)same sign as  $X \Rightarrow Y \Rightarrow Z$

Species	Coherent type 1		Coherent type 2		Coherent type 3		Coherent type 4	
	Structure	Abundance	Structure	Abundance	Structure	Abundance	Structure	Abundance
<i>E. coli</i>		28		2		4		1
<i>S. cerevisiae</i>		26		5		0		0
from interaction occurrences:	8		2		2		2	

Species	Incoherent type 1		Incoherent type 2		Incoherent type 3		Incoherent type 4	
	Structure	Abundance	Structure	Abundance	Structure	Abundance	Structure	Abundance
<i>E. coli</i>		5		0		1		1
<i>S. cerevisiae</i>		21		3		1		0
from interaction occurrences:	4		1		4		4	

In *E. coli*: 2/3 are activator, 1/3 repressor interactions  
 => relative abundances **not explained** by interaction occurrences

# Logic Response



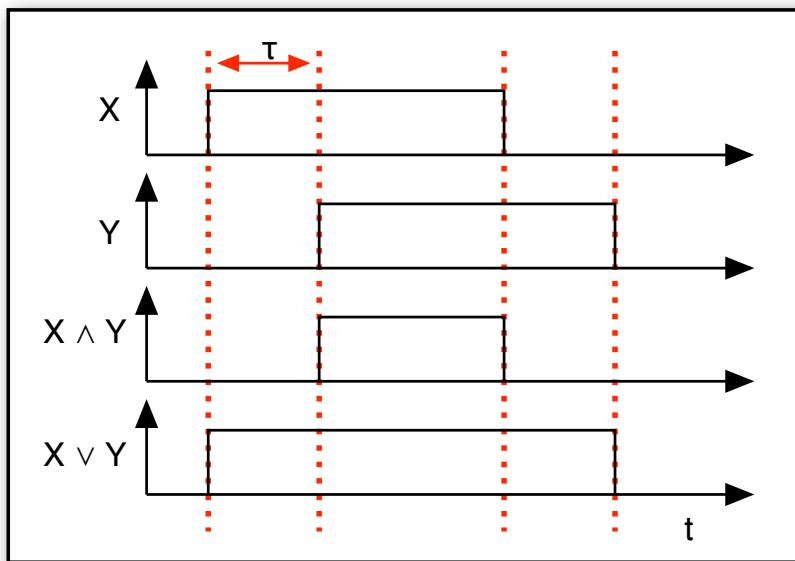
...X and Y are on  
=> AND type

"Complex of TFx and TFy"

Z goes on when ...

...X or Y is on  
=> OR type

"TFx or TFy alone suffices"



=> same steady state response  
Z is on when X is on

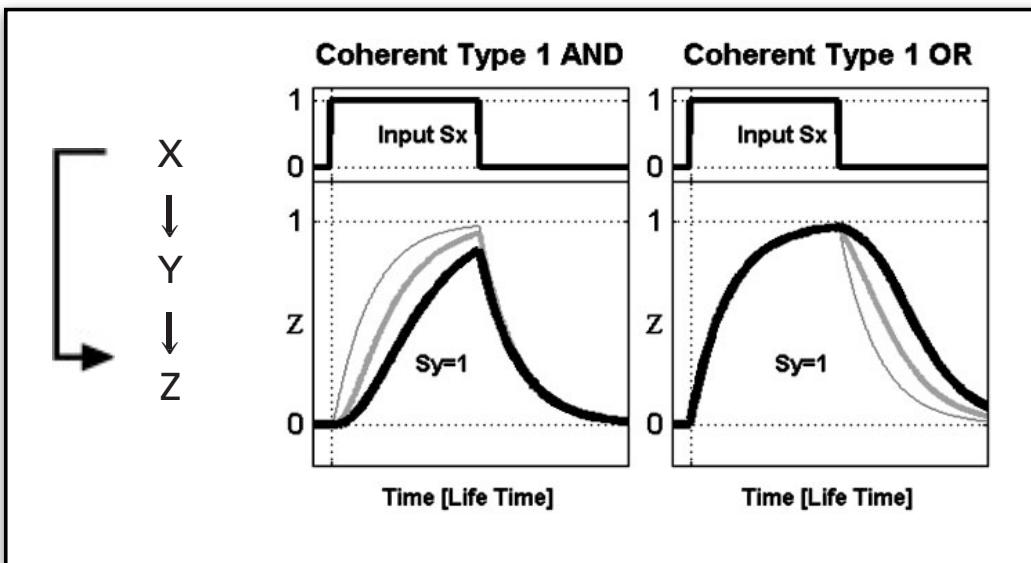
=> different dynamic responses  
due to delay  $X \Rightarrow Y$

# Dynamics

Model with differential equations:

$$dY/dt = B_y + \beta_y f(X^*, K_{xy}) - \alpha_y Y$$

$$dZ/dt = B_z + \beta_z G(X^*, K_{xz}, Y^*, K_{yz}) - \alpha_z Z .$$



thick and medium lines:  
coherent FFL type I  
(different strengths  $Y \Rightarrow Z$ )  
thin line: simple system

AND: **delayed**  
response to Sx-on

OR: **delayed**  
response to Sx-off

=> Handle **fluctuating signals** (on- or off-fluctuations)

# Fast Responses

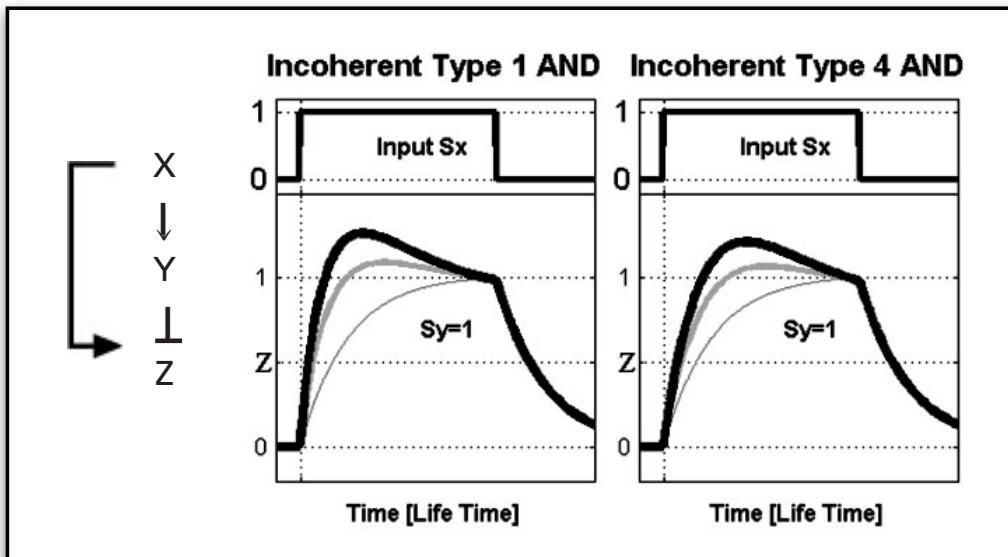
Scenario: we want a fast response of the protein level

- gene regulation on the minutes scale
- protein lifetimes  $O(h)$

At **steady state**: protein production = protein degradation

=> degradation determines  $T_{1/2}$  for given stationary protein level

=> for fast response: faster degradation or negative regulation of production



On the genes:  
no autoregulation for  
protein-coding genes  
=> incoherent FFL for  
**upstream regulation**

# All Behavioral Patterns

Table 1. Structure and function of the coherent FFL types, with AND- and OR- gates at the Z promoter

Species	Coherent type 1		Coherent type 2		Coherent type 3		Coherent type 4	
	Structure	Abundance	Structure	Abundance	Structure	Abundance	Structure	Abundance
<i>E. coli</i>		28		2		4		1
<i>S. cerevisiae</i>		26		5		0		0
Z Logic →	AND	OR	AND	OR	AND	OR	AND	OR
Steady-state Z(Sx,Sy)	$S_x \wedge S_y$	$S_x$	$\bar{S}_x \wedge S_y$	$\bar{S}_x$	$\bar{S}_x$	$\bar{S}_x \wedge \bar{S}_y$	$S_x$	$S_x \vee \bar{S}_y$
Response delay	Delay	—	—	Delay	—	—	Delay	Delay
Sx on step	Delay	—	Delay	—	Delay	—	—	—
Sx off step	—	Delay	Delay	—	Delay	Delay	—	—
Inverted out	No	No	Yes	Yes	Yes	Yes	No	No

Coherent FFL types and their abundance in transcription databases of *E. coli* and *S. cerevisiae* (6, 11). Z(Sx,Sy): Steady-state Z expression of coherent FFLs for the four combinations of Sx and Sy on and off levels ( $\wedge, \vee, \neg$  represent AND, OR, NOT). Response: Response delay of coherent FFLs to on and off  $S_x$  steps in the presence of  $S_y$ . —, not delayed. Inverted out means that Z goes off in response to  $S_x$  on step.

Table 2. Structure and function of the incoherent FFL types, with AND-gates at the Z promoter

Species	Incoherent type 1		Incoherent type 2		Incoherent type 3		Incoherent type 4	
	Structure	Abundance	Structure	Abundance	Structure	Abundance	Structure	Abundance
<i>E. coli</i>		5		0		1		1
<i>S. cerevisiae</i>		21		3		1		0
Z logic →	AND		AND		AND		AND	
Steady-state Z(Sx,Sy)	$S_x \wedge \bar{S}_y$		$\bar{S}_x \wedge \bar{S}_y$		0		0	
Pulse								
Sx on step	Weak		—		—		Strong	
Sx off step	—		Weak		Strong		—	
Sy effect	Destroy		Destroy		Enable		Enable	
Response acceleration								
Sx on step	Accelerate		—		—		Accelerate	
Sx off step	—		Accelerate		Accelerate		—	

Incoherent FFL types and their abundance in transcription databases (6, 11). Z(Sx,Sy): Steady-state Z expression of incoherent FFL with no basal level of Y ( $\vee, \neg$  represent AND, NOT). Pulse: Response to steps of Sx, in the presence of Sy, in FFLs with no basal activity. Sy effect on pulse: Enable, no pulse is created when Sy is off; Destroy, Z output is a low pulse when Sy is on, but is high and steady when Sy is off (Fig. 3). Response acceleration: Acceleration of response of and steady-state values of incoherent FFL with basal activity to on and off steps in the presence of Sy. —, not accelerated.

# Summary

## Today:

- Gene regulation networks have **hierarchies**:  
=> global "cell states" with specific expression levels
- Network **motifs**: FFLs, SIMs, DORs are overrepresented  
=> different functions, different temporal behavior

## Next lecture:

- Simple dynamic modelling of transcription networks  
=> Boolean networks, Petri nets