Boolean models for genetic regulatory networks

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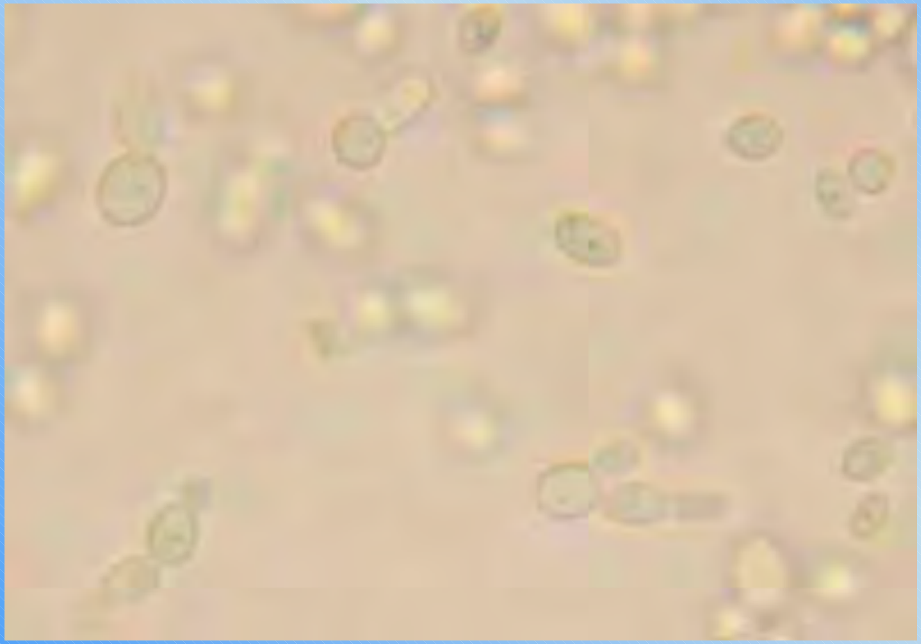
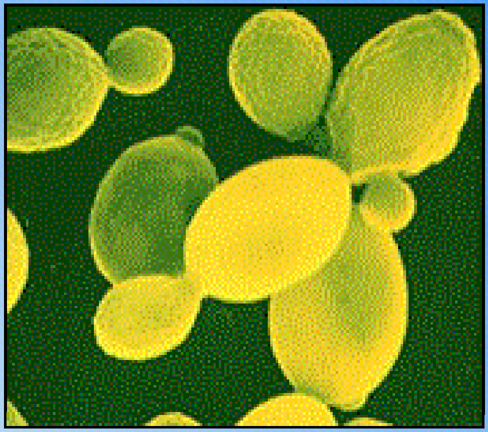
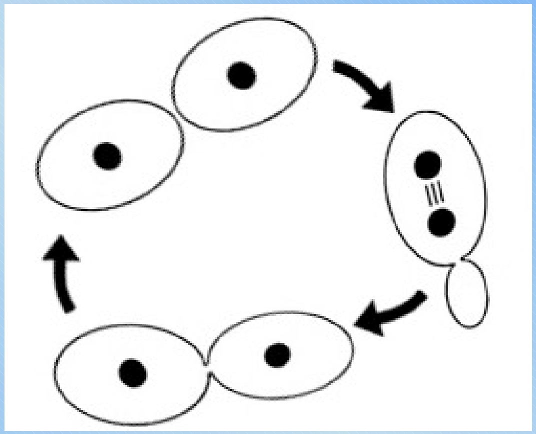
Outline

- Introduction: Cell cycles, genetic regulatory networks, and phenotypic development
- Phenotypes, mutations, and genetic variation. The canalization concept
- Representations of genetic logic: Boolean network models
- Cell type and cycle length in Kauffman network models
- Mapping of the gene strategy tables to Ising hypercubes
- Group theoretic concepts (brief review)
- Symmetry properties of strategies for combinatoric enumeration
- Preserving the phenotype through network interactions
- Summary

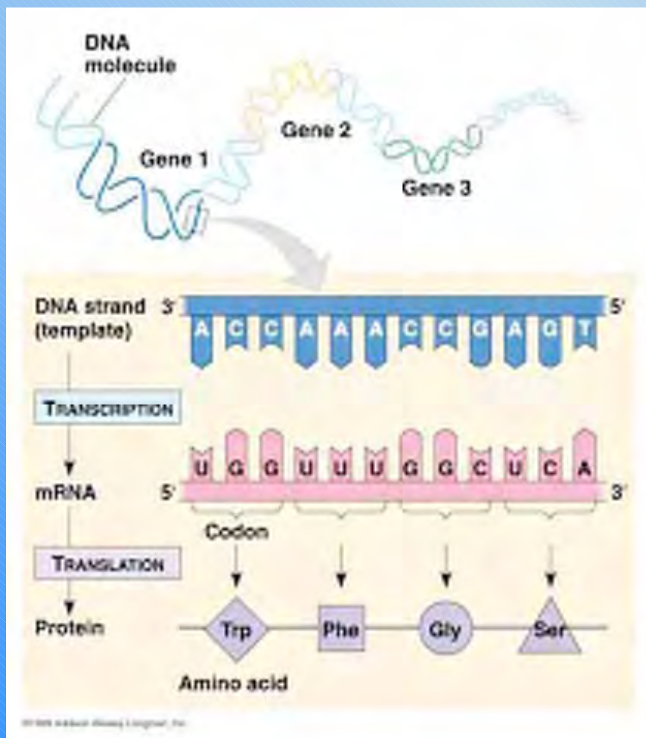
Cell cycle in *Saccharomyces cerevisiae*

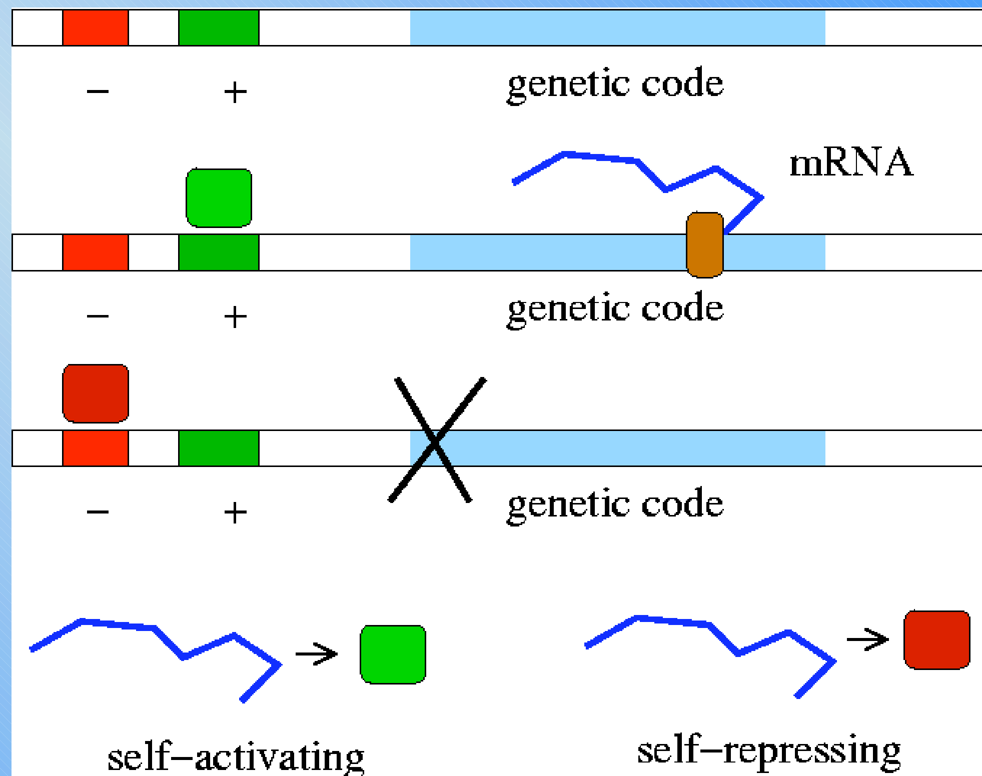






The brain of yeast





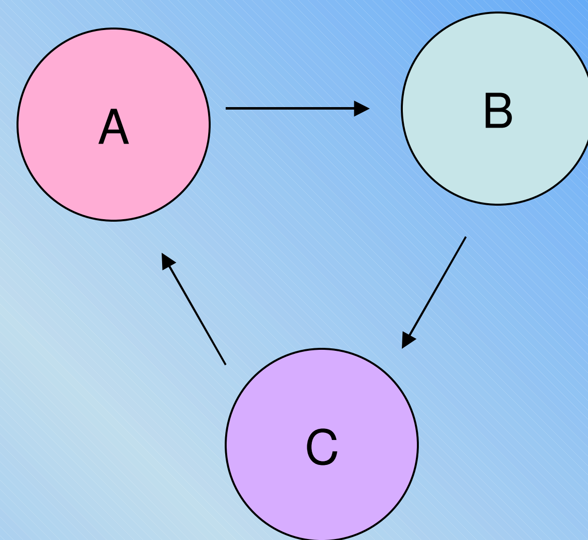
Gene-gene interactions

Gene 1
- Produces protein A
- Activated by protein C
- Suppressed by protein B

Gene 2
- Activated by protein A
- Suppressed by protein C
- Produces protein B

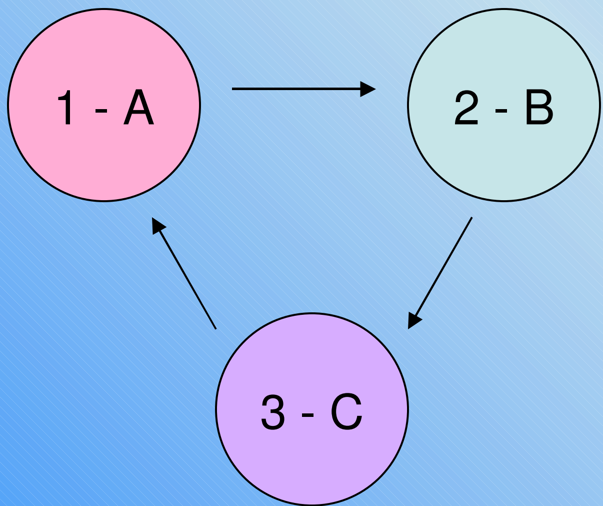
Gene 3
- Activated by protein B
- Suppressed by protein A
- Produces protein C

Current state of cell

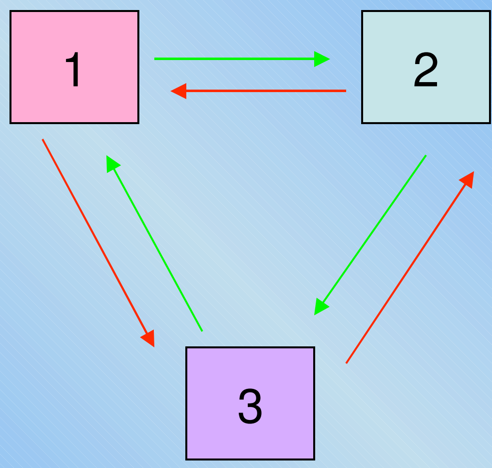


Gene-gene interaction **network**

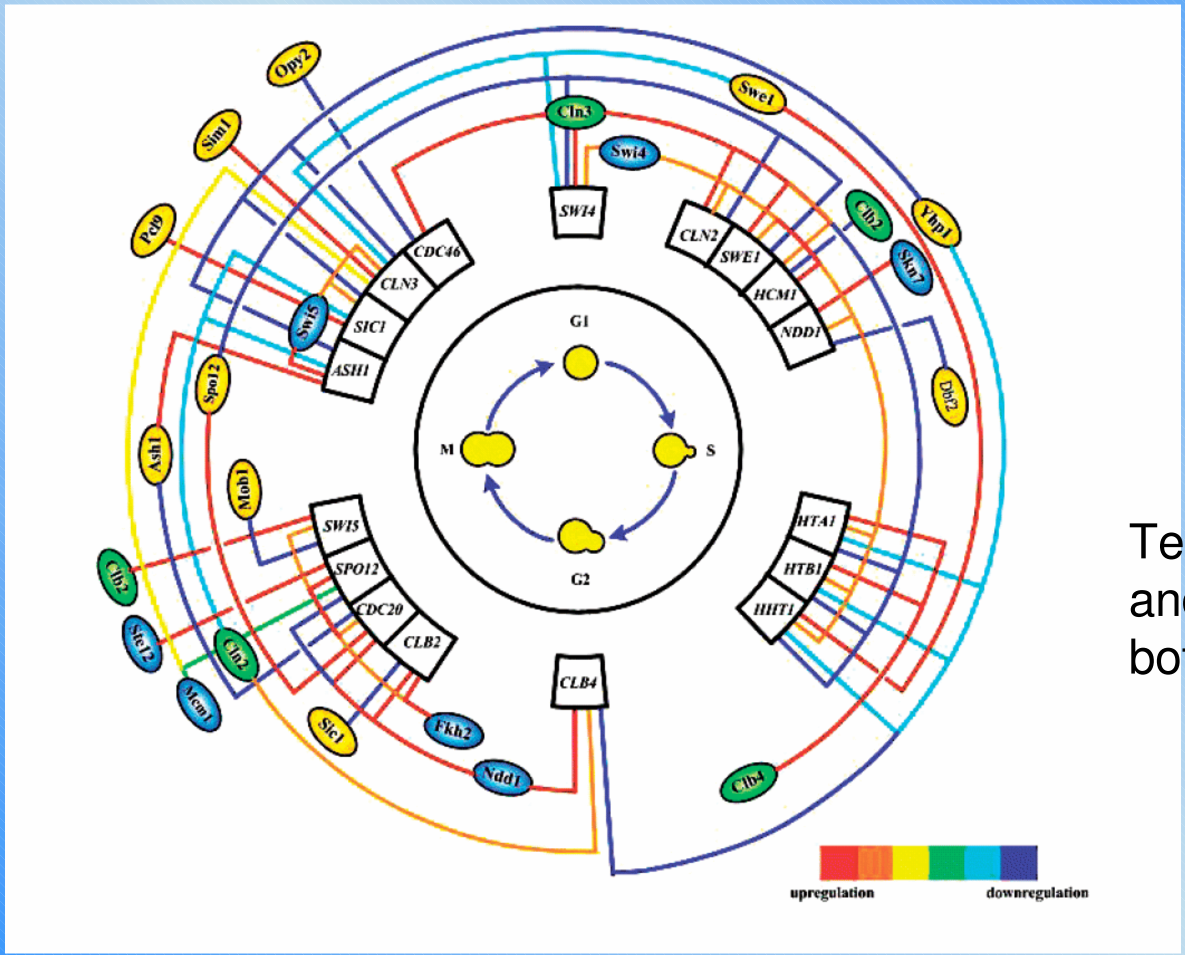
Cell **cycle** (temporal)



Interaction **network** (logical)



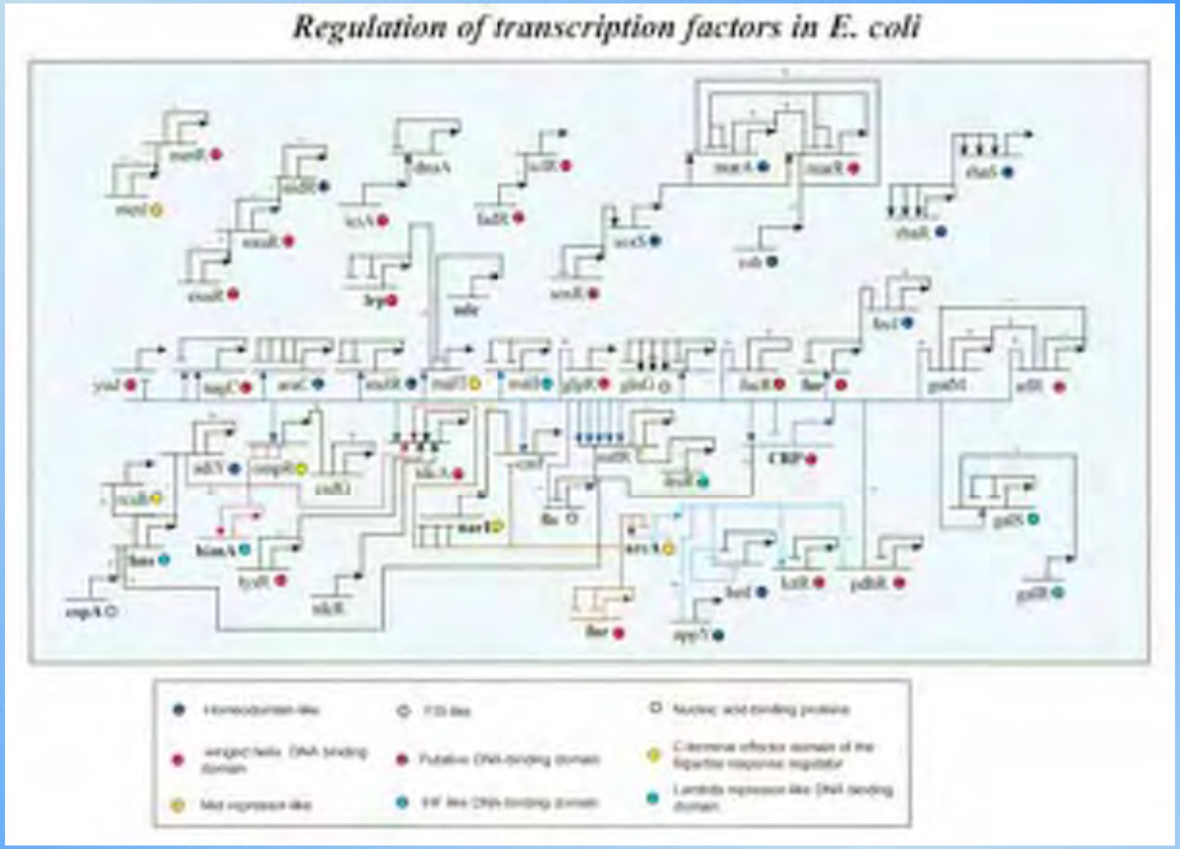
Genetic regulation of *S. cerevisiae*



Temporal states and logical network both shown

H.-C. Chen et al, Bioinformatics 20, 1914 (2004)

Gene regulation in *Escherichia coli*



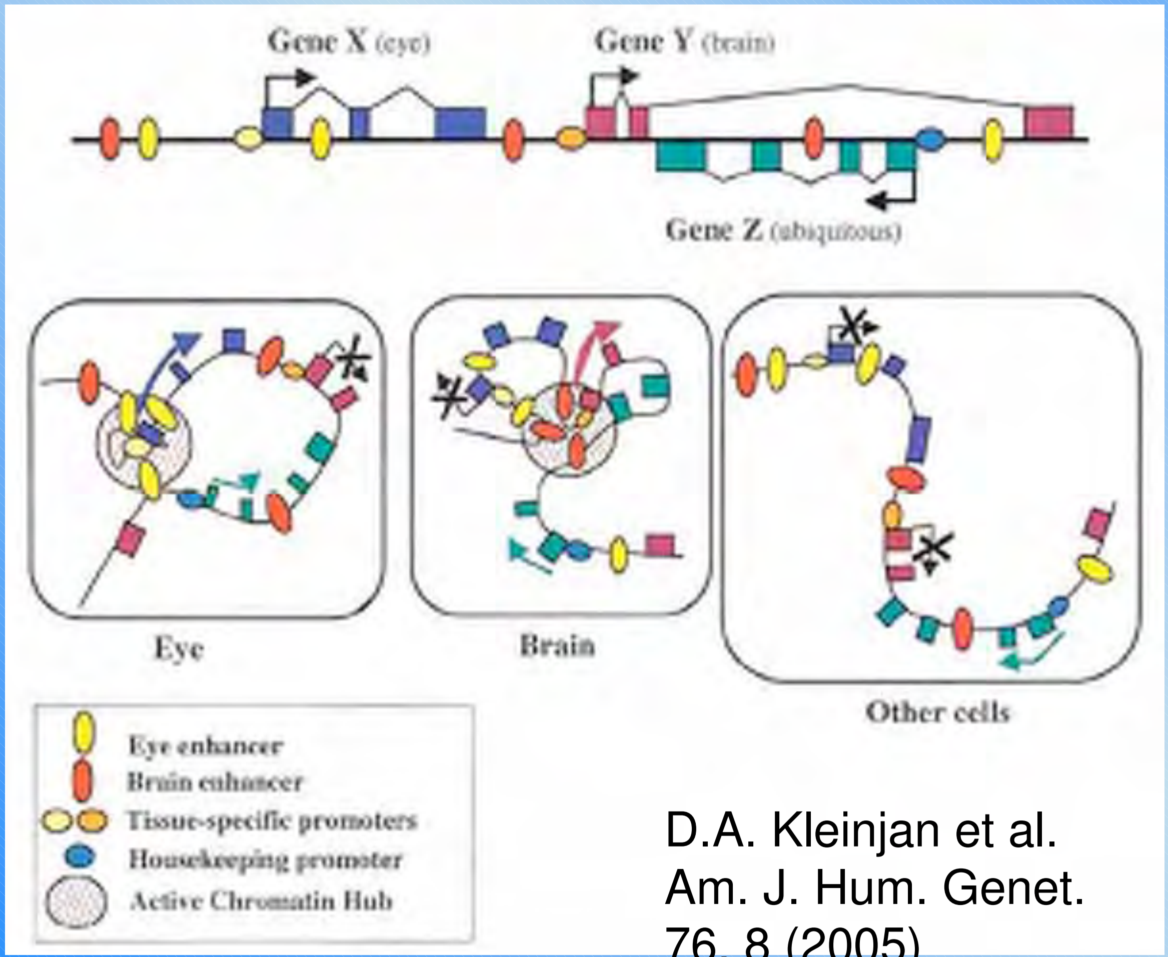
Logical network

M.M. Babu et al, Nucl. Acid Res. 31, 1234 (2003)

What about multicellular organisms?

Strongylocentrotus purpuratus
(Purple sea urchin)

Genetic logic for multiple cells



D.A. Kleinjan et al.
Am. J. Hum. Genet.
76, 8 (2005)

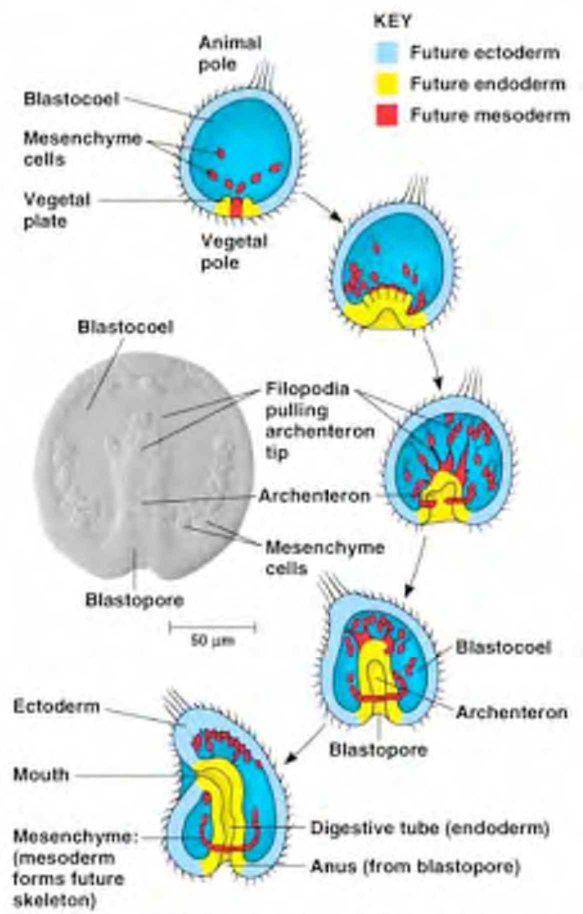
Tissue differentiation: Phenotype expression

Development of the sea urchin embryo from a mass of undifferentiated cells (blastula)

Each cell type has its own genetic regulatory cycle

The DNA must contain one possible cycle per cell type

More complex organisms = More complex DNA



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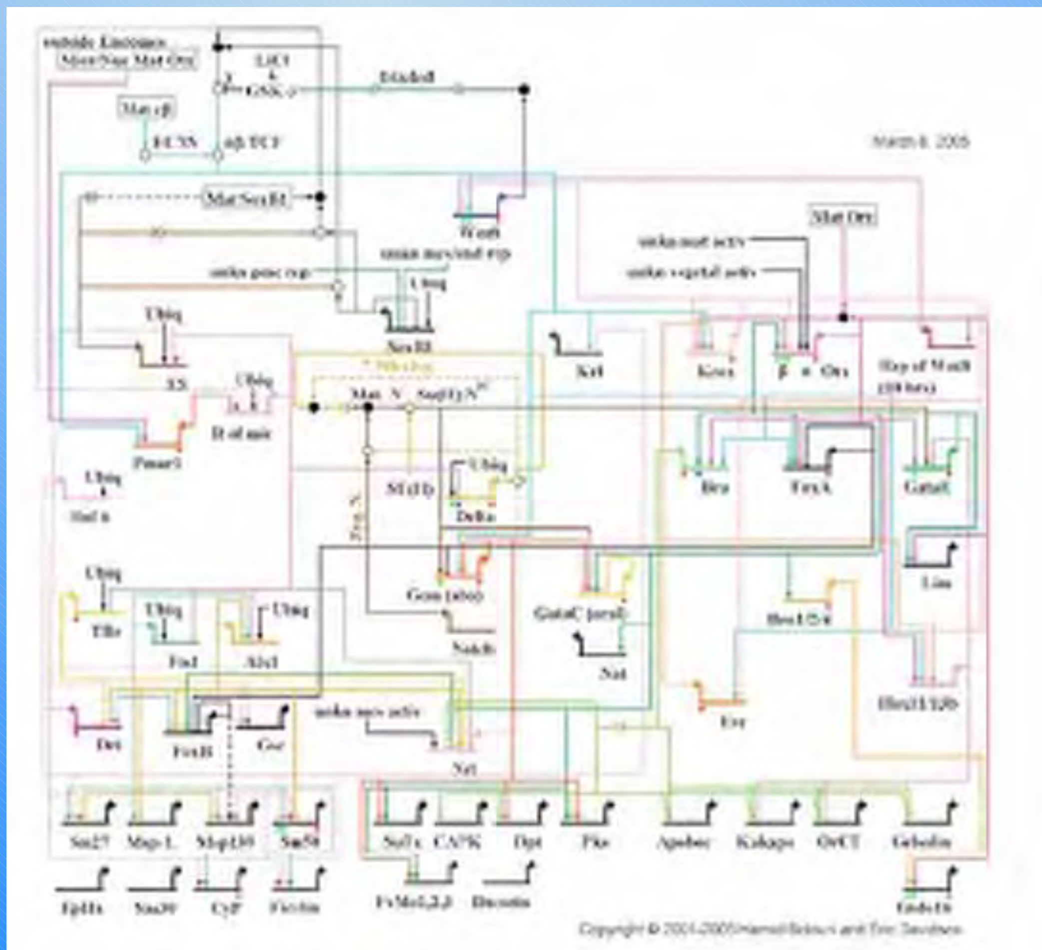
Genetic regulation during development



Endomesoderm genetic regulatory network for *S. Purpuratus*

W.J.R. Longabaugh et al, Dev. Biol. 283, 1 (2005)

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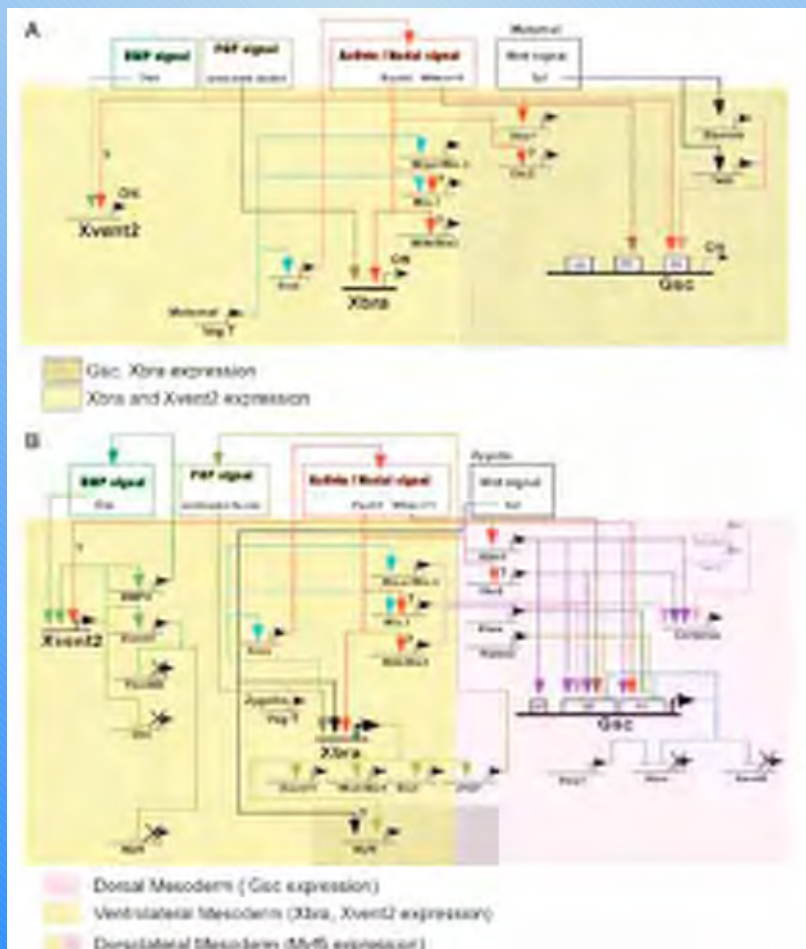
Xenopus laevis (African clawed frog)



Mesodermal genetic regulatory network
T. Koide et al, PNAS 102, 4943 (2005)

Arabadopsis thaliana

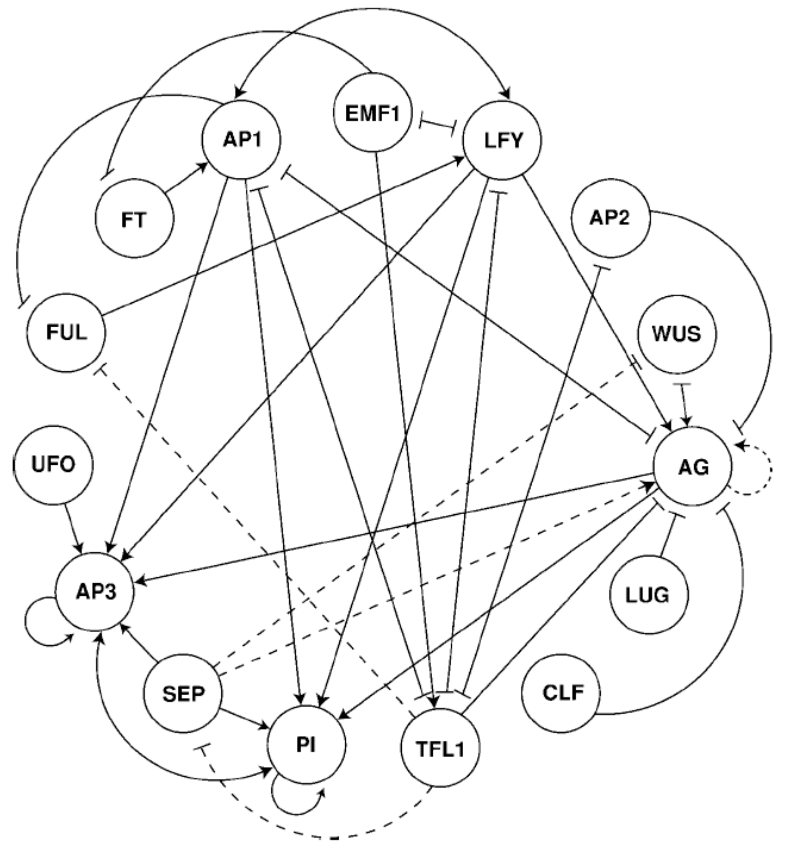
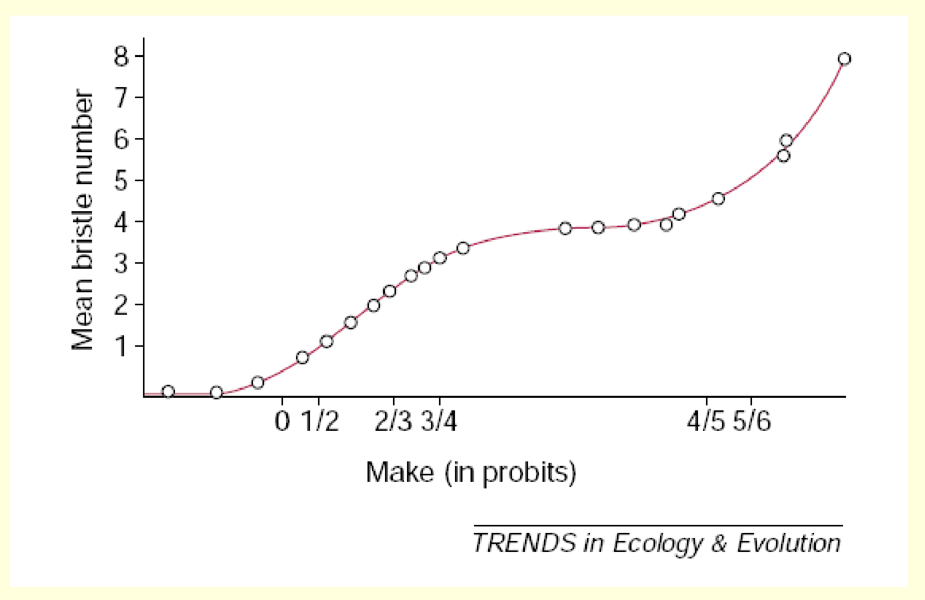


Figure 4. Gene Network Architecture for the Arabidopsis Floral Organ Fate Determination.

C. Espinosa-Soto et al, Plant Cell 16, 2923 (2004)

Resistance to phenotype mutation



Drosophila sp.

J.M. Rendel, Evolution 13, 425 (1959).
C.D. Meiklejohn, D.L. Hartl, Trends Ecol. Evol. 17, 468 (2002).

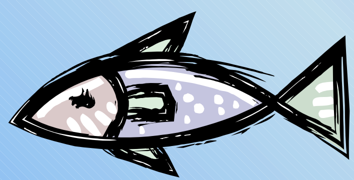
Canalization: A biologist's view

C.H. Waddington, Nature 150, 563 (1942).
"Canalization and the inheritance of acquired characters"

"Once the developmental path has been canalized, it is to be expected that many different agents, including a number of mutations available in the germplasm of the species, will be able to switch development into it. By such a series of steps, then, it is possible that an adaptive response can be fixed without waiting for the occurrence of a mutation."

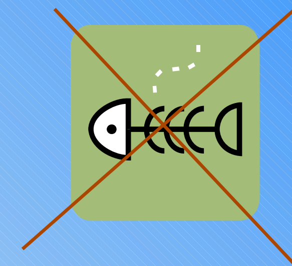
Canalization is mediated by "developmental reactions [that] are adjusted so as to bring about one definite end result regardless of minor variations in conditions during the course of the reaction."

Canalization: Allowing evolution to work offline?



Fixed phenotype

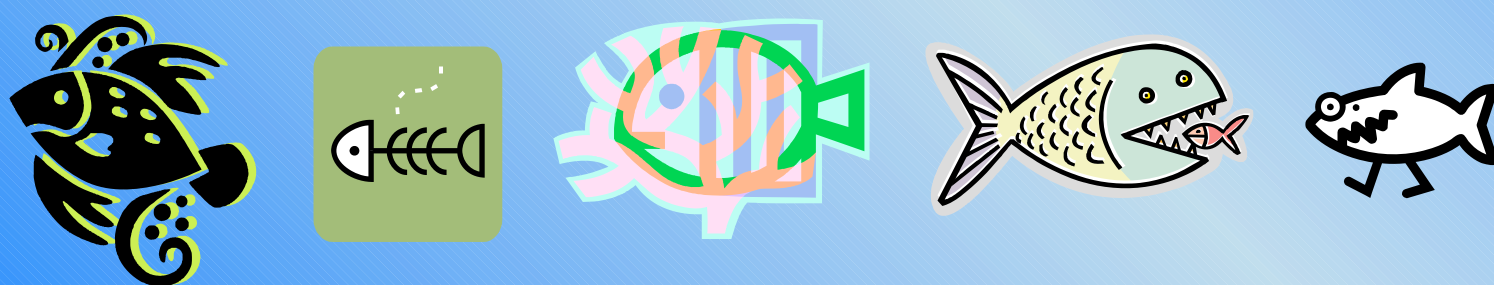
Active Inactive



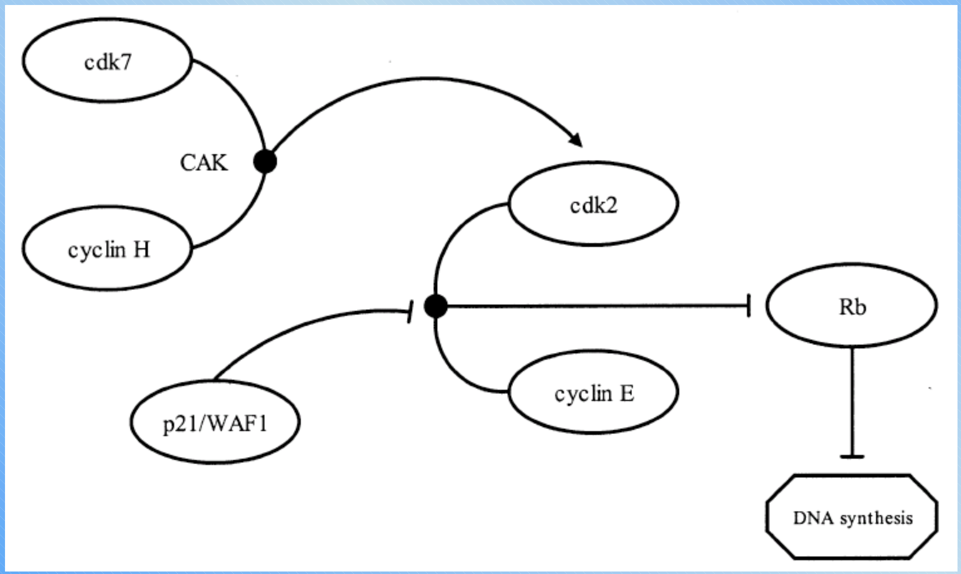
Expression of new phenotype due to changing conditions

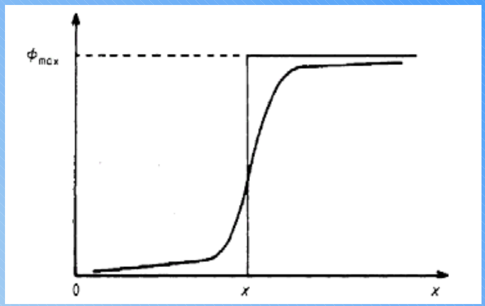
Inactive Active

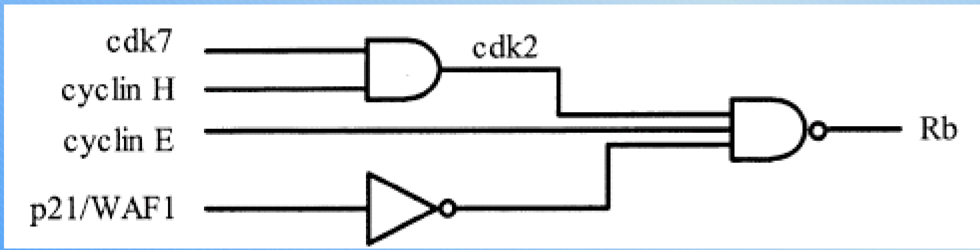
Mating display (Lethal) Camouflage Better hunting ?



Genetic interactions in the eyes of a physicist





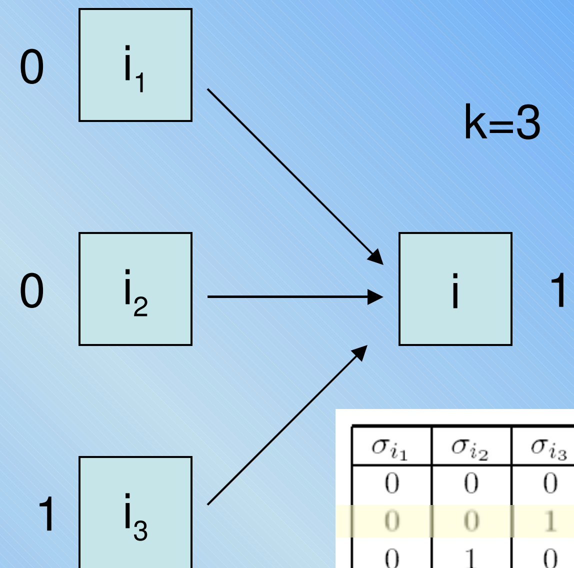


I. Shmulevich et al, Proc. IEEE 90, 1778 (2002)

Kauffman’s genetic logic model

S.A. Kauffman, “Metabolic stability and epigenesis in randomly constructed genetic nets,” J. Theor. Biol. 22, 437 (1969)

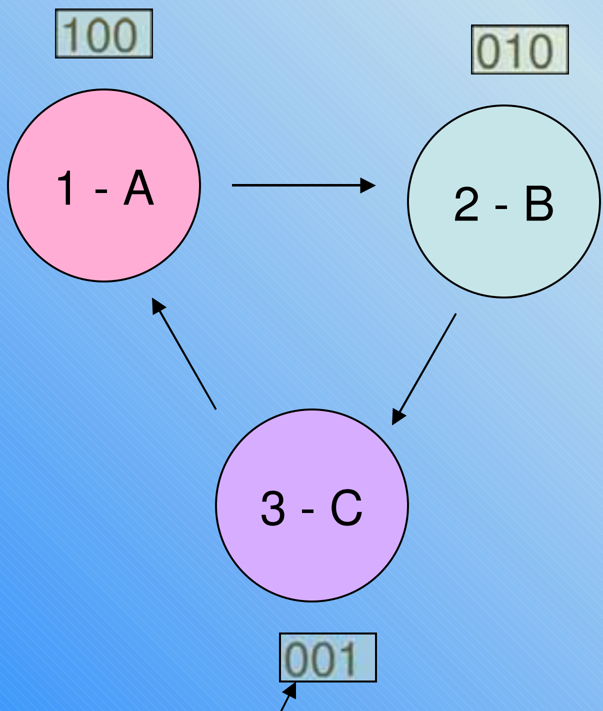
- Replace each gene with a Boolean logic element having two states, “off (0)” and “on (1)”
- Genes are randomly connected to k other input genes in a network
- The response of each gene to its k inputs is given by a randomly chosen Boolean strategy table



σ_{i_1}	σ_{i_2}	σ_{i_3}	f_i
0	0	0	0
0	0	1	1
0	1	0	0
0	1	1	1
1	0	0	0
1	0	1	1
1	1	0	0
1	1	1	0

Gene-gene interaction **network**

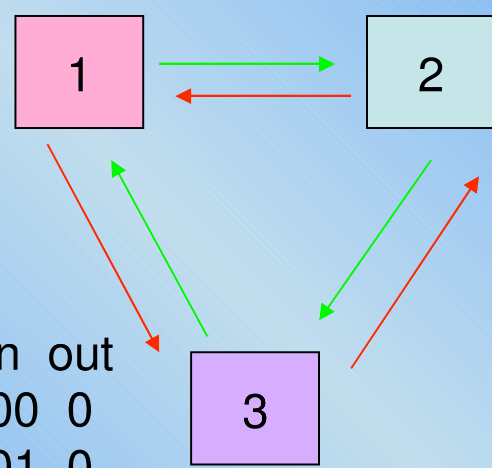
Cell **cycle** (temporal)



Current **state** of system

100 -> 010 -> 001 -> 100

Interaction **network** (logical)



in	out
00	0
01	0
10	1
11	0

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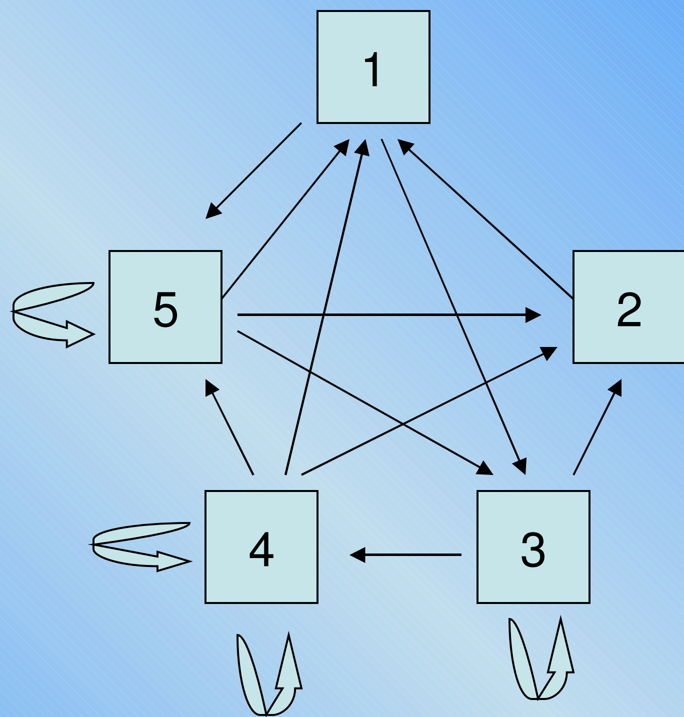
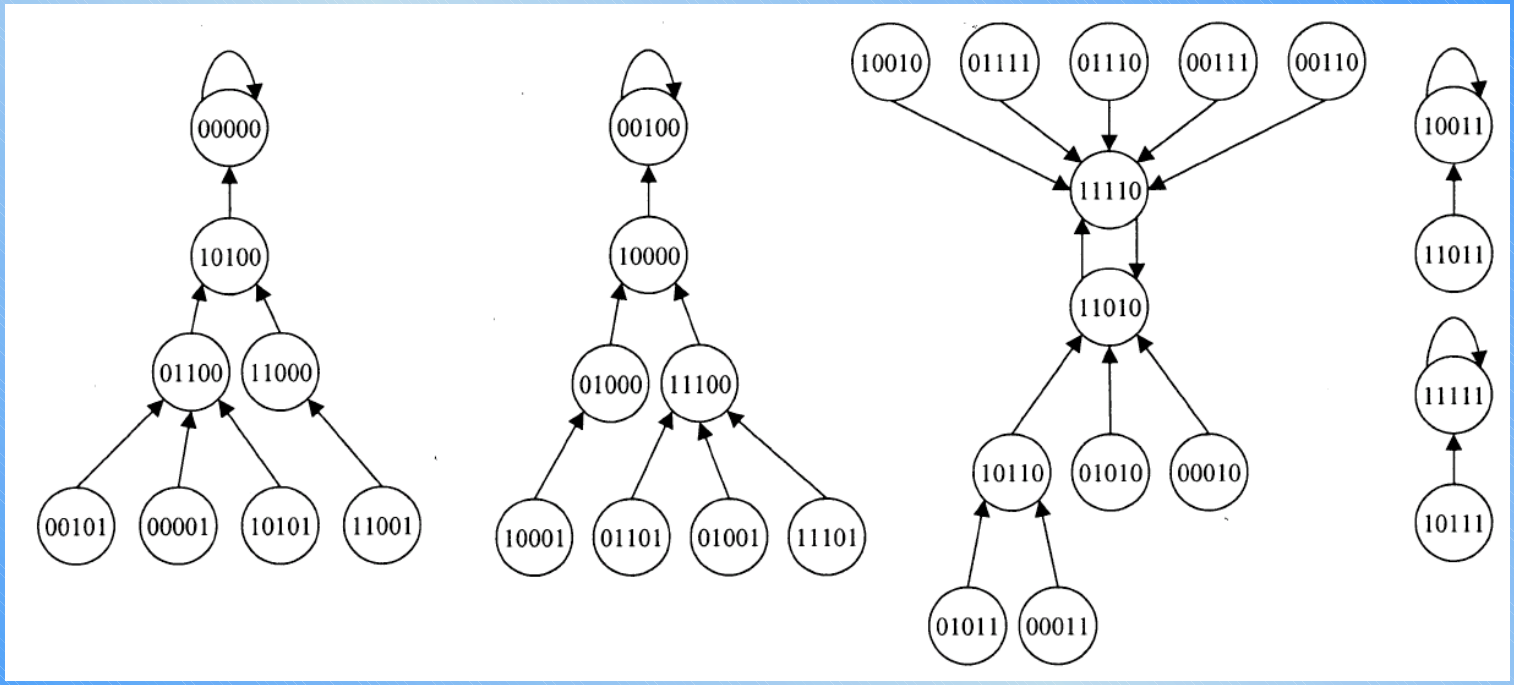
Cycles in a random Boolean network

	f_1	f_2	f_3	f_4	f_5
	0	0	0	0	0
	1	1	1	0	0
	1	1	1	0	0
	1	0	0	1	0
	0	0	1	0	0
	1	1	1	1	0
	1	1	0	1	0
	1	1	1	1	1
j_1	5	3	3	3	5
j_2	2	5	1	4	4
j_3	4	4	5	4	1

N=5, k=3



Cycles in a random Boolean network



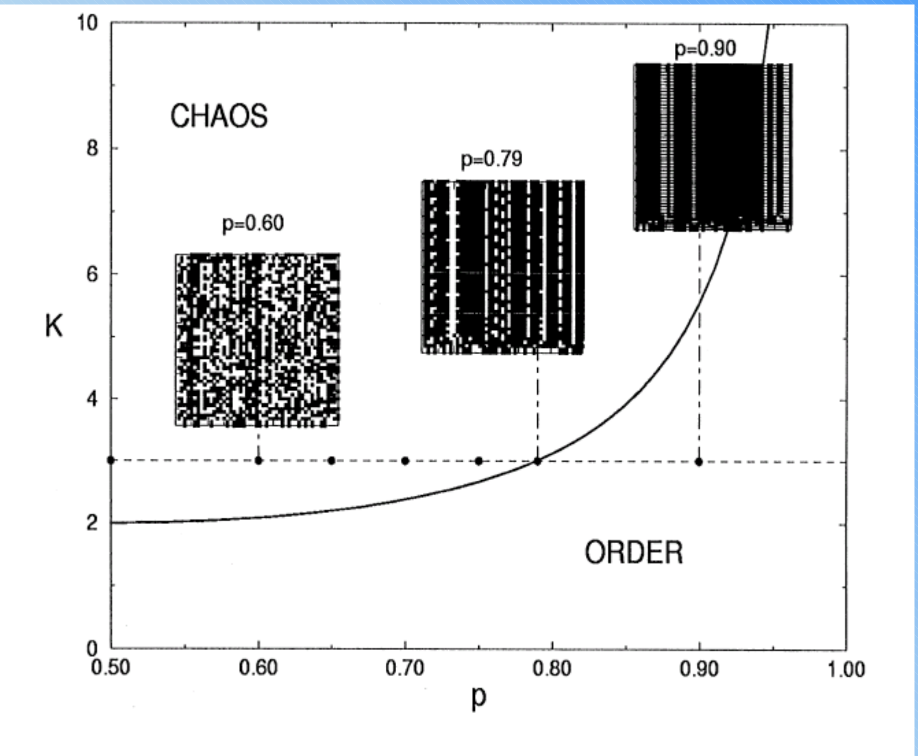
Kauffman: Each attractor corresponds to a **cell type**

I. Shmulevich et al, Proc. IEEE 90, 1778 (2002)

Internal homogeneity

p=0.5	p=0.75
00 1	00 1
01 0	01 1
10 1	10 1
11 0	11 0

p=0.75	p=1.0
00 0	00 1
01 0	01 1
10 1	10 1
11 0	11 1



$$K = \frac{1}{2p(1-p)} .$$

Avg period increases exponentially with N in chaotic regime; as a power of N in the ordered regime; intermediate at boundary.

B. Luque et al, Physica A 284, 33 (2000)

Values of k in real genetic networks: *E. coli*

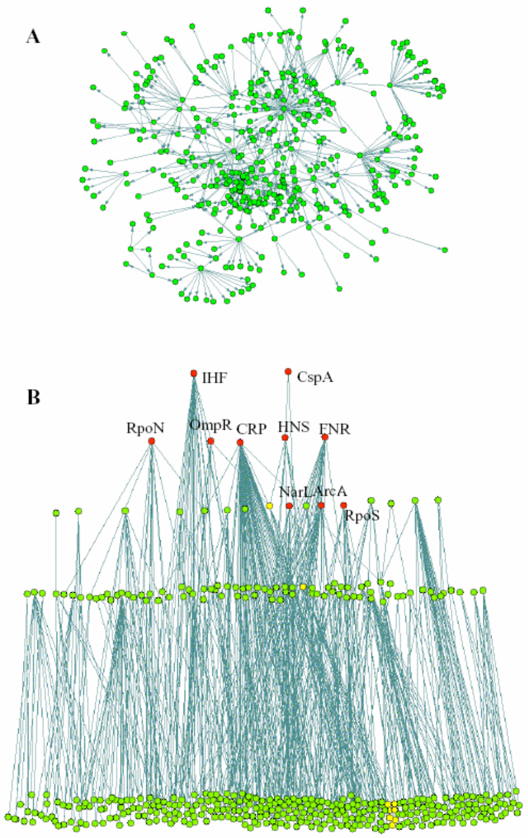


Figure 1
Hierarchical structure of *E. coli* transcriptional regulatory network. A: The original unorganized network. B: the hierarchical regulation structure in which all the regulatory links are downward. Nodes in the graph are operons. Links show the transcriptional regulatory relationships. The global regulators found in this work are shown in red. The yellow marked nodes are operons in the longest regulatory pathway related with flagella motility.

H.W. Ma et al, BMC Bioinformatics 5, 199 (2004)

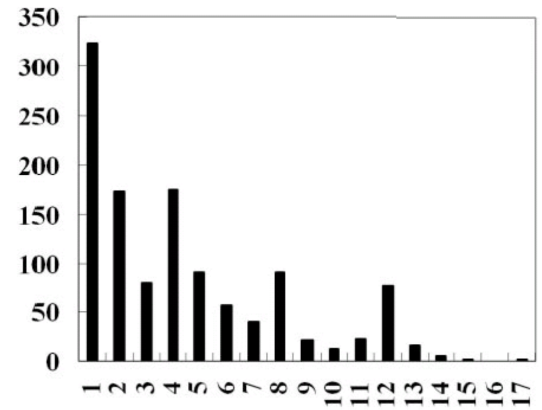


Figure 4. The distribution of number of regulators (directly or indirectly) for the genes in the *E.coli* TRN.

H.W. Ma et al, Nucl. Acids Res. 32, 6643 (2004)

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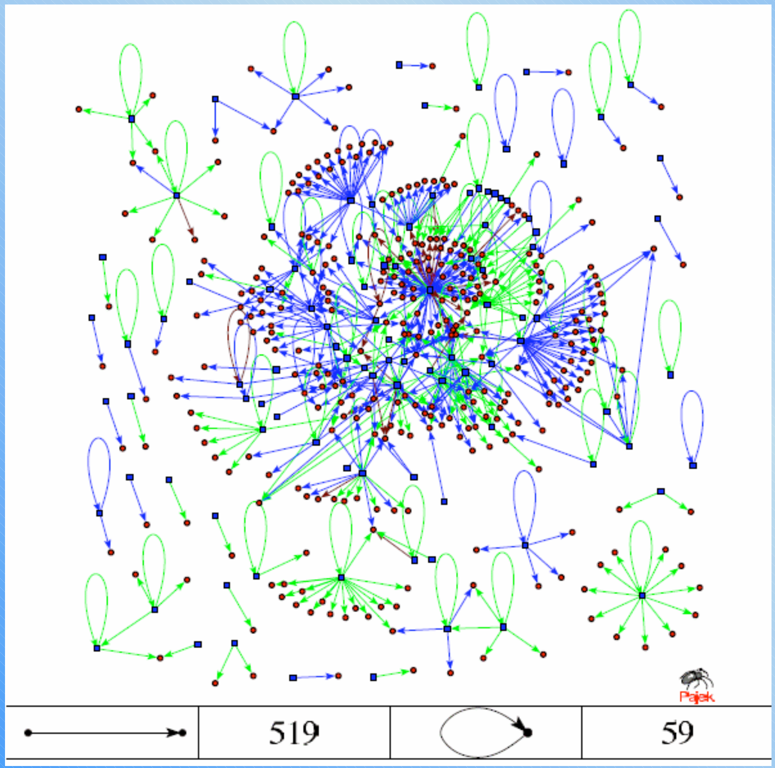
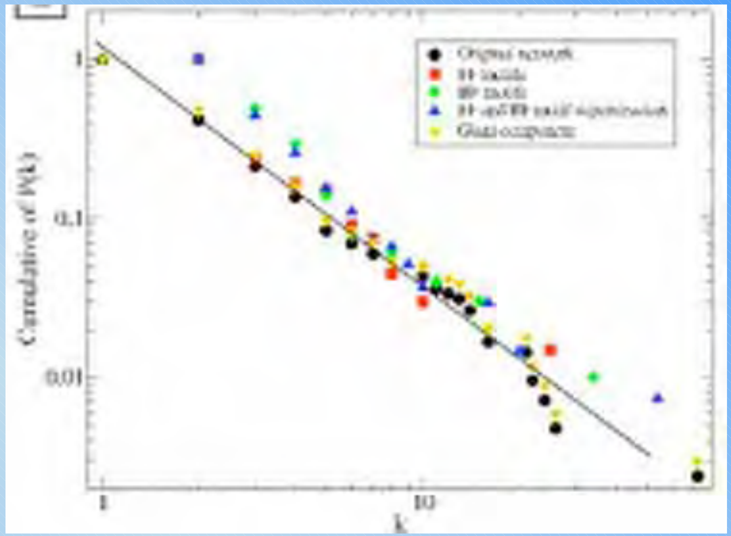
Small values of k and much autoregulation





Yeast transcription regulatory network structure
R. Dobrin et al, BMC Bioinformatics 5, 10 (2004)

Classifying strategies

Number of strategies: 2^{2^k}

k	N_s
2	2
3	16
4	256
5	65536
6	4294967296
7	18446744073709551616
8	Much larger than Avogadro's number

Due to combinatoric explosion, it is not possible to reach k=10 by direct inspection

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Classifying k=2 strategies

TABLE I. The 16 update functions for nodes with 2 inputs. The first column lists the 4 possible states of the two inputs; the other columns represent one update function each, falling into four classes.

In	$\mathcal{F}$		$\mathcal{C}_1$				$\mathcal{C}_2$								$\mathcal{R}$	
00	1	0	0	1	0	1	1	0	0	0	0	1	1	1	1	0
01	1	0	0	1	1	0	0	1	0	0	1	0	1	1	0	1
10	1	0	1	0	0	1	0	0	1	0	1	1	0	1	0	1
11	1	0	1	0	1	0	0	0	0	1	1	1	1	0	1	0

Fixed

Sensitive to only one input

(*Acts like k=1*)

Sensitive to one state of one input

Sensitive to both inputs

p=1

p=0.5

p=0.75

p=0.5

V. Kaufman et al, PRE 72, 046124 (2005)

Minority game for an evolving N-K network

•System of N nodes is initialized with fixed K. All nodes are assigned an unbiased strategy (equal number of 0s and 1s).

•Repeatedly update the network until the attractor is reached.

•For each update on the attractor, determine whether "0" or "1" is the output of the majority of the nodes.

•Assign a "point" +1 to all nodes in the majority on each update.

•The node which is in the majority most often (has the most "points") loses the game and is assigned a new randomly chosen unbiased strategy. This completes an "epoch."

•Repeat the procedure for the new network.

K.E. Bassler, C. Lee, Y. Lee, PRL 93, 038101 (2004)

Fourteen k=3 classes of strategies with equal evolutionary advantage

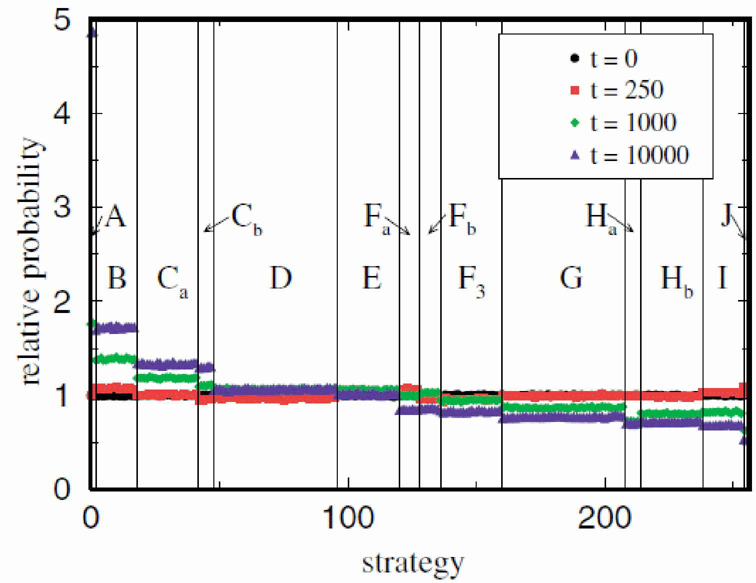

FIG. 3 (color online). Selection for canalization. The average relative probability of occurrence of the 256 different $K = 3$ Boolean strategies shown at different stages in the evolutionary process. The strategies are grouped according to the classification in Table I, and the groups are ordered with decreasing canalization. Results are the average of 10 000 different realizations.

K.E. Bassler, C. Lee, Y. Lee, PRL 93, 038101 (2004)

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Classifying k=3 strategies

K.E. Bassler, C. Lee, Y. Lee, PRL 93, 038101 (2004)

p

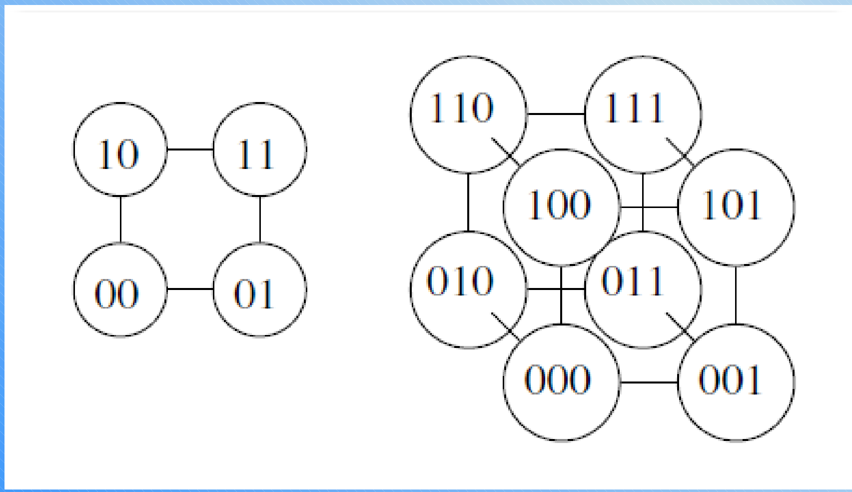
Class	A	B	C_a	C_b	D	E	F_a	F_b	F_c	G	H_a	H_b	I	J
Size	2	16	24	6	48	24	8	8	24	48	6	24	16	2
$\mathcal{P}_0$	1	0												
$\mathcal{P}_1$	1	1/2	1/3		1/6		0							
$\mathcal{P}_2$	1	3/4	2/3		7/12	1/2	1/2			5/12	1/3		1/4	0
h	1	7/8	3/4	1/2	5/8	3/4	3/4	1/2		5/8	1/2		5/8	1/2
Sym	S	N	N	A	N	N	S	A	N	N	S	N	N	A

Table 2. Classification of the 256 $K=3$ Boolean functions according to their canalization properties, internal homogeneity, and parity symmetry. (S indicates symmetric, A indicates anti-symmetric, and N indicates neither, or non-symmetric.)

Strategies in the same class have the same evolutionary advantage when the network is allowed to evolve under some rule.

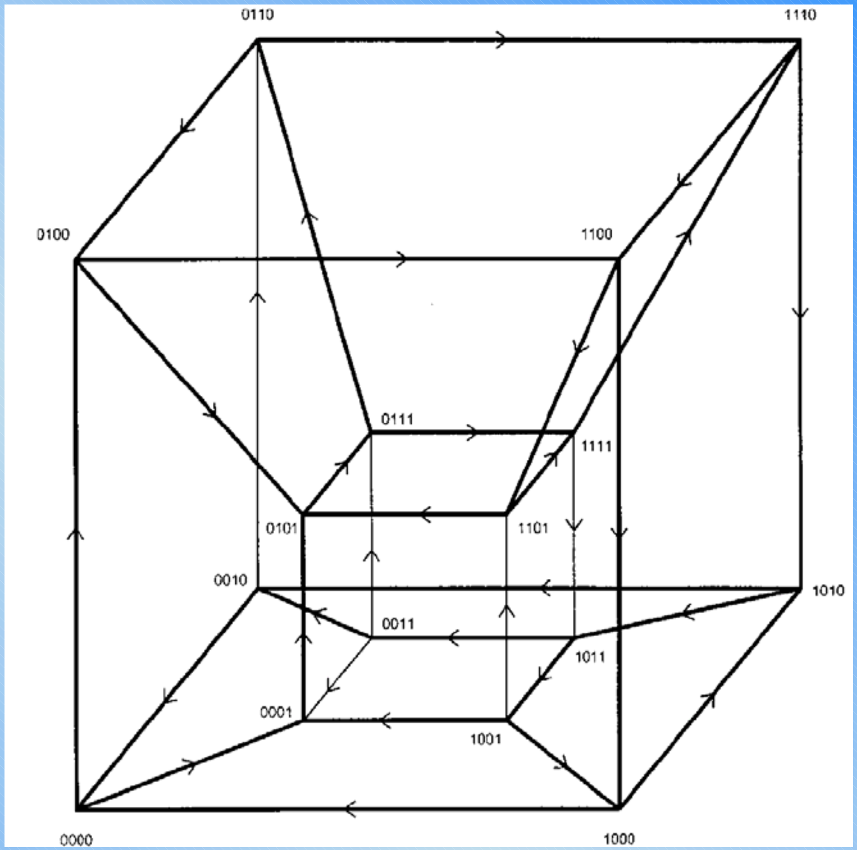
How to identify classes? What about k>3?

Map strategies to Ising k-hypercubes



k=2

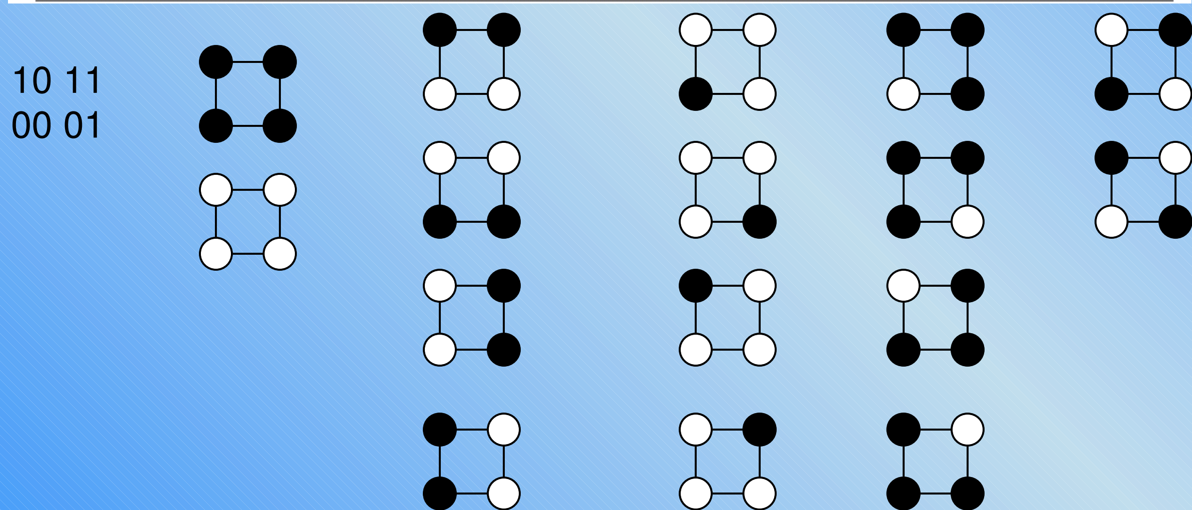
k=3



k=4

Example: k=2

00	1	0	0	1	0	1	1	0	0	0	0	1	1	1	1	0
01	1	0	0	1	1	0	0	1	0	0	1	0	1	1	0	1
10	1	0	1	0	0	1	0	0	1	0	1	1	0	1	0	1
11	1	0	1	0	1	0	0	0	0	1	1	1	1	0	1	0



Each strategy group contains all objects in a particular group orbit of the Ising hypercube symmetry group plus parity

Counting strategy classes

All strategies correspond to all states of the k-hypercube.

We can identify the total number of strategy classes by counting the number of group orbits that exist for the k-hypercube.

N_c	k	N_s
-	2	2
4	3	16
14	4	256
	5	65536
	6	4294967296
	7	18446744073709551616

Permutations and Cyclic Decomposition

Given an ordered set of elements, a permutation is a reordering of that set where each element occurs only once.

“game”	“emag”	“ameg”	“eagm”
{1,2,3,4}	{4,3,2,1}	{2,3,4,1}	{4,2,1,3}

Cyclic decomposition: Consider the permutation {4,2,1,3} of {1,2,3,4}. Repeated applications of this permutation result in a cycle:
game -> eagm -> maeg -> game

The permutation can be written in terms of cycles of elements:
(2)(143)

Basic Group Theory

Definition 1.1. A *group* $(G, \cdot)$ is a set G with a binary operation

$$\cdot : G \times G \to G,$$

and a unit $e \in G$, possessing the following properties.

(1) Unital: for $g \in G$, we have $g \cdot e = e \cdot g = g$.
(2) Associative: for $g_i \in G$, we have $(g_1 \cdot g_2) \cdot g_3 = g_1 \cdot (g_2 \cdot g_3)$.
(3) Inverses: for $g \in G$, there exists $g^{-1} \in G$ so that $g \cdot g^{-1} = g^{-1} \cdot g = e$.

A set of elements S of G is said to *generate* G if every element of G may be expressed as a product of elements of S, and inverses of elements of S. That is to say, given $g \in G$, there exists $s_i \in S$ and $\epsilon_i \in \{\pm 1\}$ so that

$$g = s_1^{\epsilon_1} \cdots s_n^{\epsilon_n}.$$

If a group G is a generated by a single element, it is said to be *cyclic*. Every element of a cyclic group G is of the form g^n for some $n \in \mathbb{Z}$.

Orbit-counting theorem

Total number of classes P_G

$$P_G(x_1, x_2, \ldots) = \frac{1}{|G|}\sum_{g\in G}|X^g|$$

$|G|$: number of symmetry operators (generators) g
X^g: the set of elements in X that are left invariant by g

Counting the number of classes for higher k:

- Identify the symmetry operators of the k-hypercube with parity
- Write these symmetry operators in terms of cycles
- Find the number of fixed points for each symmetry operator

The symmetry group for the k-hypercube is isomorphic to the hyperoctahedral group O_n with n=k, which has $n!2^n$ symmetry operations

Example: k=2

- Identify the symmetry operators of the k-hypercube with parity
- Write these symmetry operators in terms of cycles
- Find the number of fixed points for each symmetry operator

$k!2^k = 8$ for k=2

(1)(2)(3)(4) E	2^4	(1)(2)(3)(4) P	0
(1243) E	2	(1243) P	2
(3421) E	2	(3421) P	2
(14)(23) E	2^2	(14)(23) P	2^2
(12)(34) E	2^2	(12)(34) P	2^2
(13)(24) E	2^2	(13)(24) P	2^2
(14)(2)(3) E	2^3	(14)(2)(3) P	0
(23)(1)(4) E	2^3	(23)(1)(4) P	0

48/16 = 4 classes

Identifying generators by inspection is difficult for k>3

Arbitrarily high k: Cycle representation of the Zyklenzeiger group

THEOREM 7. *The cycle index of $\mathfrak{G}_n$ is given by*

$$Z_{\mathfrak{G}_n} = \frac{1}{n!\,2^n} \sum_{(j)} \frac{n!\,2^n}{\prod_{i=1}^{n} j_i!\,(2i)^{j_i}} \mathop{\times}_{i=1}^{n} \Big(\prod_{d \mid i} f_d^{\,e(d)} + \prod_{\substack{d \mid 2i \\ d \nmid i}} f_d^{\,g(d)}\Big)^{\times j_i}$$

where the sum is over all solutions of

$$\sum_{i=1}^{n} i j_i = n;$$

where

$$e(k) = \frac{1}{k} \sum_{d \mid k} 2^d \mu\left(\frac{k}{d}\right)$$

and

$$g(2k) = \frac{1}{2k} \sum_{\substack{d \mid 2k \\ d \nmid k}} 2^{d/2} \mu\left(\frac{2k}{d}\right)$$

where $\mu(a)$ is the Möbius function.

M.A. Harrison, J. SIAM 11, 806 (1963)

Example: k=2

$$x_1^4 + 3x_2^2 + 2x_1^2x_2 + 2x_4$$

x_1^4	(1)(2)(3)(4) E	2^4	(1)(2)(3)(4) P	0
x_4	(1243) E	2	(1243) P	2
x_4	(3421) E	2	(3421) P	2
x_2^2	(14)(23) E	2^2	(14)(23) P	2^2
x_2^2	(12)(34) E	2^2	(12)(34) P	2^2
x_2^2	(13)(24) E	2^2	(13)(24) P	2^2
$x_1^2x_2$	(14)(2)(3) E	2^3	(14)(2)(3) P	0
$x_1^2x_2$	(23)(1)(4) E	2^3	(23)(1)(4) P	0

For each operator without parity, the number of functions left invariant is equal to 2^{N_c}, where $N_c = \sum_{i=1}^{k} b_i$ is the total number of cycles in the operator. Parity must be treated separately; no functions are left invariant by the parity operator with any k-hypercube operator containing at least one cycle of length 1. Thus there are 2^{N_p} functions left invariant for the eight operators which include parity, where $N_p = (1 - \Theta(b_1)) \sum_{i=1}^{k} b_i$ and Θ is the Heaviside step function.

Generating polynomials through k=5

Table 3. Cycle polynomials for $k = 1$ through 5 and the number of classes P_G for each k.

k	Cycle polynomial	P_G
1	$(1/2)\left(x_1^2 + x_2\right)$	2
2	$(1/8)\left(x_1^4 + 3x_2^2 + 2x_1^2x_2 + 2x_4\right)$	4
3	$(1/48)\left(x_1^8 + 13x_2^4 + 8x_1^2x_3^2 + 8x_2x_6 + 6x_1^4x_2^2 + 12x_4^2\right)$	14
4	$(1/384)\left(x_1^{16} + 12x_1^8x_2^4 + 51x_2^8 + 12x_1^4x_2^6 + 32x_1^4x_3^4 + 48x_1^2x_2x_4^3 + 84x_4^4\right.$	
	$\left. + 96x_2^2x_6^2 + 48x_8^2\right)$	238
5	$(1/3840)\left(x_1^{32} + 384x_{10}^3x_2 + 20x_1^{16}x_2^8 + 60x_1^8x_2^{12} + 231x_2^{16} + 80x_1^8x_3^8\right.$	
	$+ 320x_{12}^2x_4^2 + 240x_1^4x_2^2x_4^6 + 240x_2^4x_4^6 + 520x_4^8 + 384x_1^2x_5^6$	
	$\left. + 160x_1^4x_2^2x_3^4x_6^2 + 720x_2^4x_6^4 + 480x_8^4\right)$	698 635

Class structure: Isomer chemistry

Substitute a term of the form A^a+B^a for each x_a

Table 4. Class structure for $k = 2$.

Class type	N_h	$\langle S_c \rangle$
A^4	1	2
$A^3 B$	1	8
$A^2 B^2$	2	3

k=2 (max 16)

Table 5. Class structure for $k = 3$.

Class type	N_h	$\langle S_c \rangle$
A^8	1	2
$A^7 B$	1	16
$A^6 B^2$	3	18.667
$A^5 B^3$	3	37.333
$A^4 B^4$	6	11.667

K=3 (max 96)

Table 6. Class structure for $k = 4$.

Class type	N_h	$\langle S_c \rangle$
A^{16}	1	2
$A^{15} B$	1	16
$A^{14} B^2$	4	60
$A^{13} B^3$	6	186.667
$A^{12} B^4$	19	191.58
$A^{11} B^5$	27	323.56
$A^{10} B^6$	50	320.32
$A^9 B^7$	56	408.57
$A^8 B^8$	74	173.9

K=4 (max 768)

$$S_c^{\max} = k!\,2^{k+1}.$$

$$\langle S_c \rangle = N_f / N_h.$$

$$N_f(m,n) = (2-\delta_{m,n})(2^k)!/(m!n!), \text{ where } m+n = 2^k,$$

NNSA

Los Alamos

Class structure for k=5

Table 7. Class structure for $k = 5$.

Class type	N_h	$\langle S_c \rangle$
A^{32}	1	2
$A^{31}B$	1	64
$A^{30}B^2$	5	198.4
$A^{29}B^3$	10	992
$A^{28}B^4$	47	1530.2
$A^{27}B^5$	131	3074.4
$A^{26}B^6$	472	3839.8
$A^{25}B^7$	1 326	5076.7
$A^{24}B^8$	3 779	5566.7
$A^{23}B^9$	9 013	6224.1
$A^{22}B^{10}$	19 963	6463.2
$A^{21}B^{11}$	38 073	6777.7
$A^{20}B^{12}$	65 664	6877.2
$A^{19}B^{13}$	98 804	7031.6
$A^{18}B^{14}$	133 576	7058.7
$A^{17}B^{15}$	158 658	7131.3
$A^{16}B^{16}$	169 112	3554.3

K=5 (7680)

Characteristic polynomials

Do two randomly chosen strategies belong to the same class?

Each class has a unique characteristic polynomial.

Construct the adjacency matrix: $A_{ij}=1$ if link, 0 if no link
Place the strategy on the diagonal
Find the determinant

$$\begin{matrix} a & 1 & 1 & 0 \\ 1 & b & 0 & 1 \\ 1 & 0 & c & 1 \\ 0 & 1 & 1 & d \end{matrix}$$

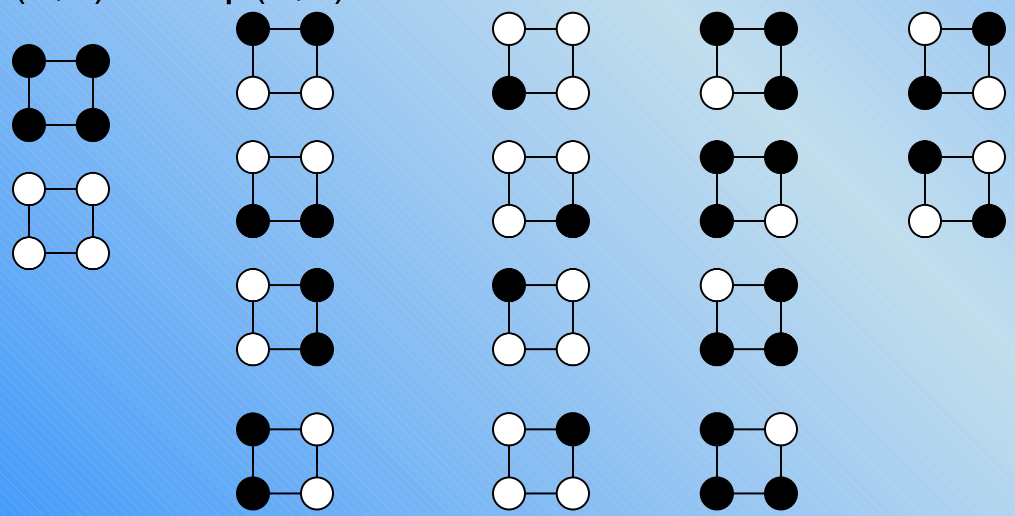
Det: $-ab-ac-bd-cd-abcd$

$-4A^2+A^4$
$-4AB+A^2B^2$
$-A^2-2AB-B^2+A^2B^2$
$-2AB-2B^2+A^2B^2$

Geometric strategy classification

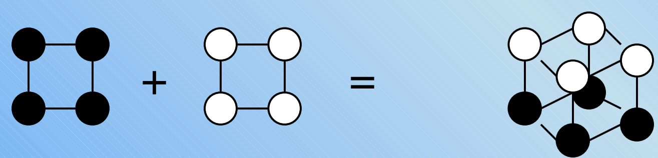
We classify a given strategy depending on the number of edges, faces, and higher dimensional objects having all entries the same, p(d,k), d<=k. This represents varying degrees of canalization.

h=1	h=0.5	h=0.75	h=0.5
p(1,2)=4	p(1,2)=2	p(1,2)=2	p(1,2)=0
p(2,2)=1	p(2,2)=0	p(2,2)=0	p(2,2)=0



Recursion relations

We can think of a k+1 strategy as being assembled out of two k strategies



00 1
01 1
10 1
11 1

p(1,2)=4
p(2,2)=1

00 0
01 0
10 0
11 0

p(1,2)=4
p(2,2)=1

000 1
001 1
010 1
011 1
100 0
101 0
110 0
111 0

p(1,3)=8
p(2,3)=2
p(3,3)=0

$$h(k+1) = \sum_{i=1}^{2} h_i(k)$$

$$p(d,k+1) \geq \sum_{i=1}^{2} p_i(d,k)$$

$$N_d(k) = \frac{2^{k-d}k!}{(k-d)!d!}$$

$$N_d(k+1) \geq p(d,k+1) \geq \sum_{i=1}^{2} p_i(d,k)$$

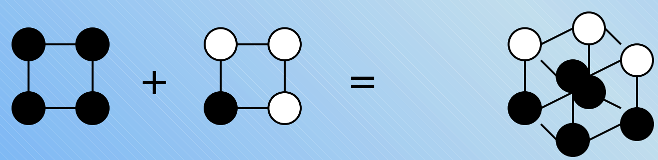
$$\frac{N_d(k)}{N_d(k-1)} = \frac{2k}{k-d}$$

Recursion relations

Improving on the maximum bound

$$p(d,k+1) \geq \sum_{i=1}^{2} p_i(d,k)$$

$$p(d,k+1) \leq \sum_{i=1}^{2} p_i(d,k) + \min\left(p_1(d,k), p_2(d,k)\right)$$

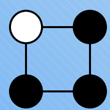
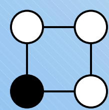


00 1	00 1	000 1
01 1	01 0	001 1
10 1	10 0	010 1
11 1	11 0	011 1
		100 1
		101 0
		110 0
		111 0
p(1,2)=4	p(1,2)=2	p(1,3)=7
p(2,2)=1	p(2,2)=0	p(2,3)=1
		p(3,3)=0

6 <= p(1,3) <=8

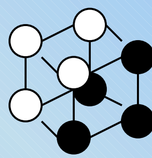
Lower symmetry cases

4 <= p(1,3) <=6

 +  =

00 1	00 1
01 1	01 0
10 0	10 0
11 1	11 0

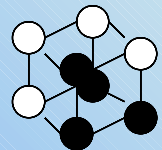
p(1,2)=2	p(1,2)=2
p(2,2)=0	p(2,2)=0



+ all possible rotations

p(1,3)=6
p(2,3)=0
p(3,3)=0
symmetric

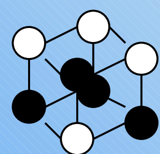
OR



+ all possible rotations

p(1,3)=6
p(2,3)=0
p(3,3)=0
asymmetric

OR



+ all possible rotations

p(1,3)=5
p(2,3)=0
p(3,3)=0

Bounding canalization

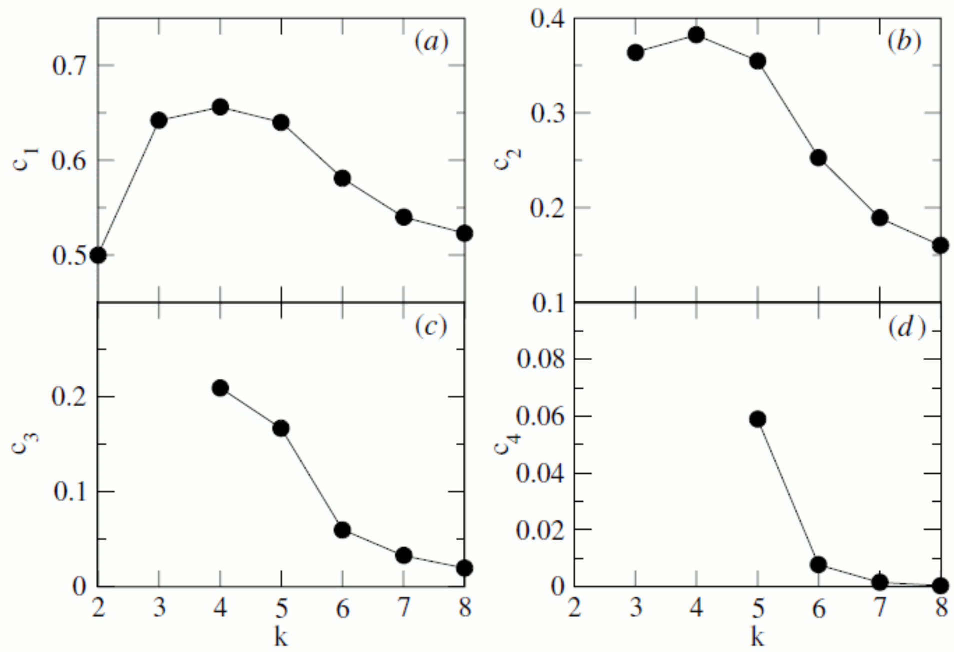


Figure 4. Average fraction c_d of homogeneous d-dimensional sides in randomly selected Boolean functions versus k for (a) $d = 1$, (b) $d = 2$, (c) $d = 3$ and (d) $d = 4$.

Although the fraction of fully canalizing functions drops rapidly with k, the fraction of partially canalizing functions remains large.

Effective k may be lower than actual k

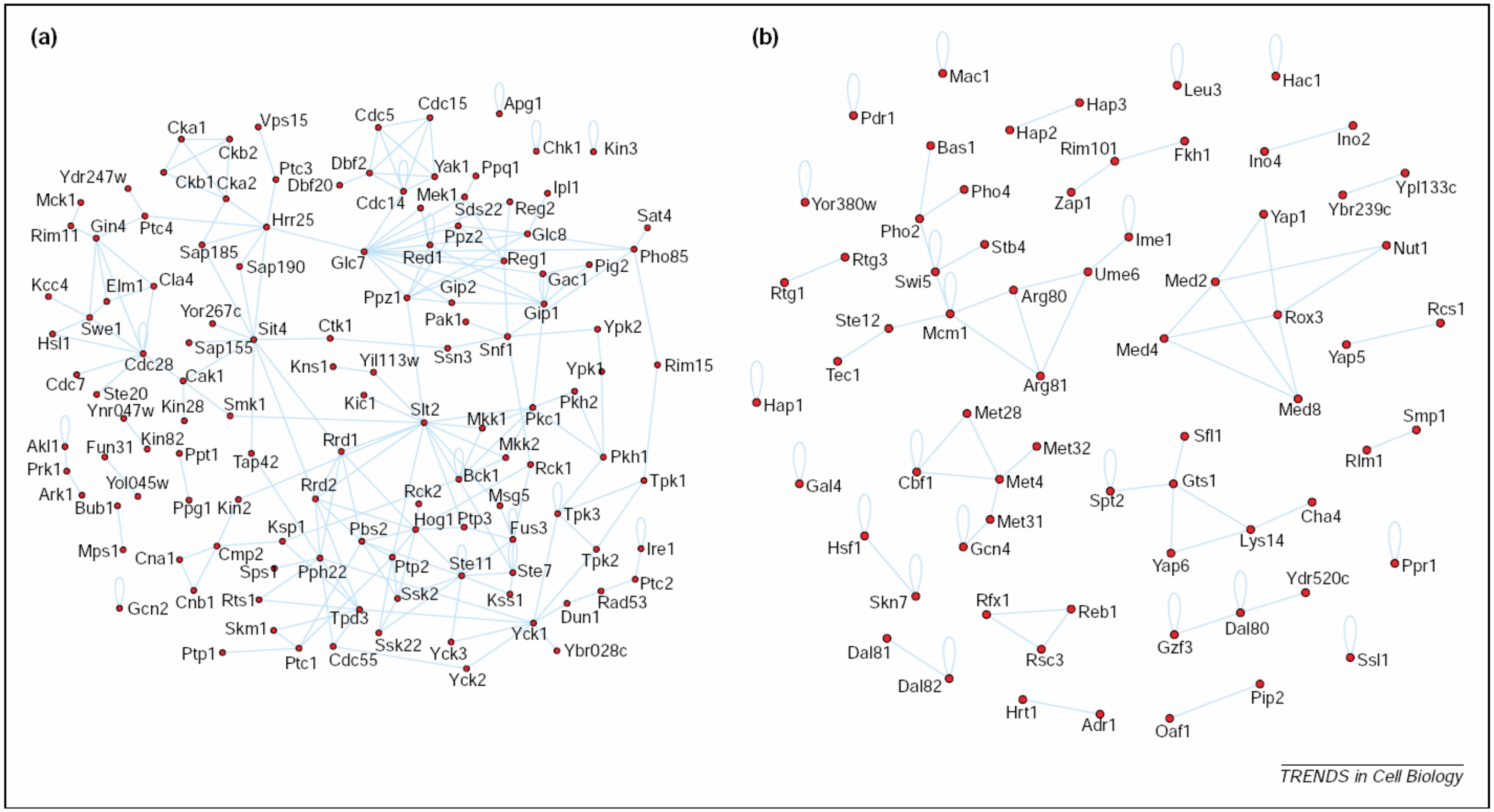


Fig. 5. A large network of protein–protein interactions among kinases and phosphatases in yeast. (a) Kinases and phosphatases are very well connected in a large protein–protein interaction network. (b) Transcription factors, a functional class similar in size to the kinase/phosphatase class, are not. This is an example of an unanticipated result that is completely unobtainable without genome-wide studies. Loops indicate self-interactions.

G.D. Bader et al, Trends Cell Biol. 13, 344 (2003)

Summary

- Boolean network models can be used to represent genetic regulatory networks.
- The number of possible strategies grows rapidly with connection degree k; the number of network states grows rapidly with network size N.
- Nodes which respond to only a fraction of their inputs have an effectively reduced k, which reduces the available phase space.
- Mapping of the gene strategy tables to Ising hypercubes allows us to use symmetry properties to enumerate strategy classes.
- By assembling k+1 strategies out of k strategies recursively, we can put bounds on the amount of canalization present in the k+1 strategies.
- A significant fraction of strategies are at least partially canalized, reducing the complexity and cycle length of the logical network.