Chapter 6: Regulatory Networks

6.3 Boolean Network Models

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Overview

- Boolean network models
- Sample applications
- Kaufmann's theory of evolution
- Learning (reverse engineering) Boolean nets

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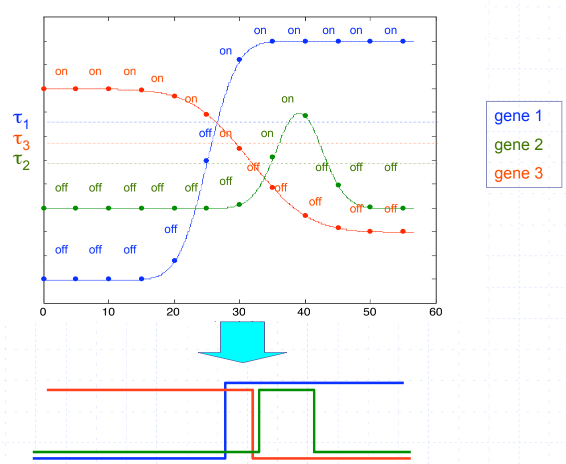
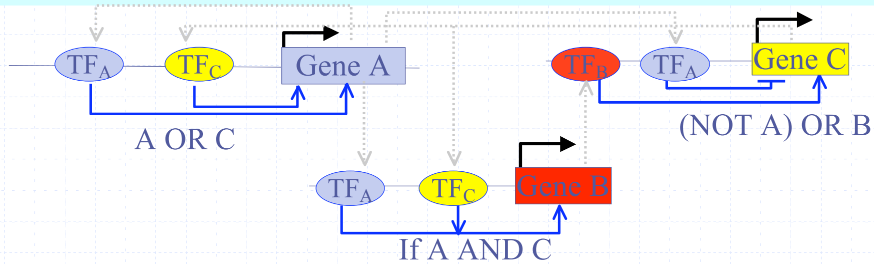
Intro To Boolean Networks

Example: Thresholding Gene Expression

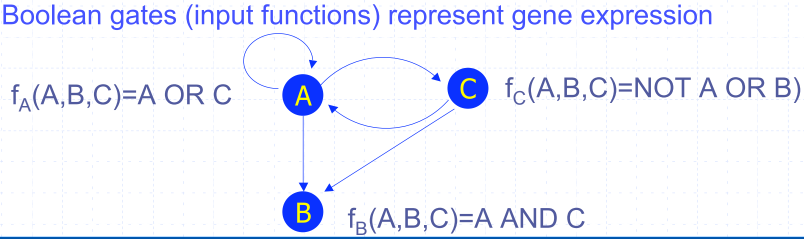
- Boolean model: discretize expressions to on/off model



Boolean Network Model



- A Boolean Network Model:
  - Nodes represent transcription factors
  - Edges represent regulatory input
  - Boolean gates (input functions) represent gene expression



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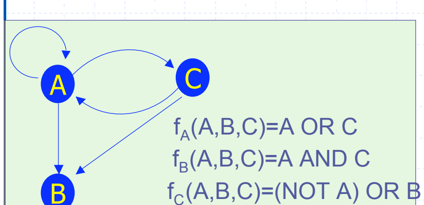
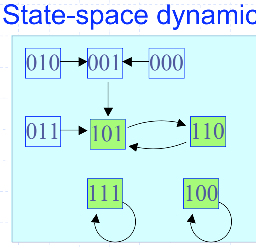
Dynamics

- Network State: X=(A,B,C…) is a Boolean vector
- State evolution: X(t+1)=f(X(t))=(f_A(X(t)),f_B(X(t)), ….)
  - E.g., X(t+1)=(A OR C, A AND C, (NOT A) OR B)
  - (0,1,1)=>(1,0,1)
- This is discrete time synchronous dynamics
  - State transitions occur through concurrent gates firings

X(t)	X(t+1)
000	001
001	101
010	001
011	101
100	100
101	110
110	101
111	111

State-space dynamics





Cycle

Attractors

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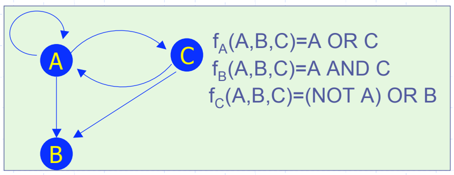
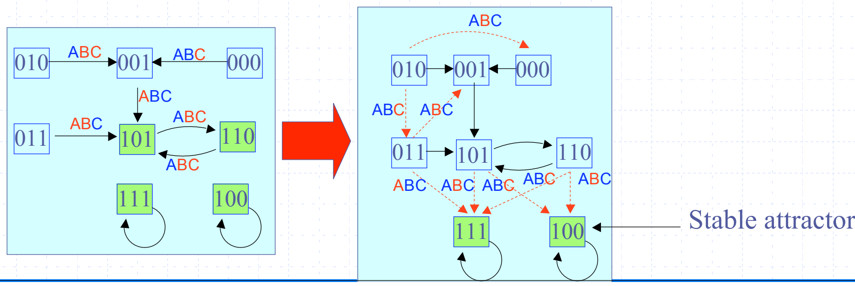
Noisy (Stochastic) Dynamics

- If gene-gates "fire" randomly
- The network becomes asynchronous
- The dynamics landscape changes

X(t)	X(t+δ)
000	001
001	101
010	001,000,011
011	101,111,001
100	100
101	110, 111,100
110	101,111,100
111	111





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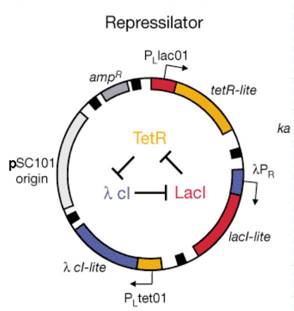
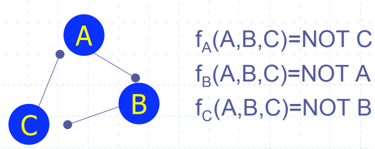
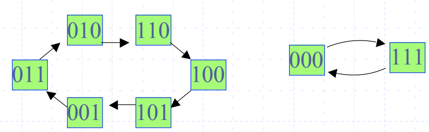
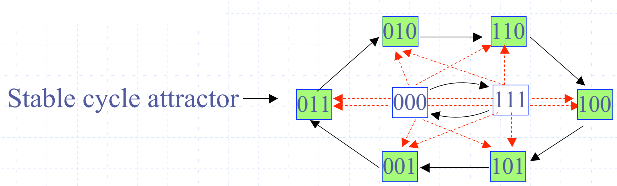
Example: Boolean Repressilator

- Repressilator has three repressors in a loop:









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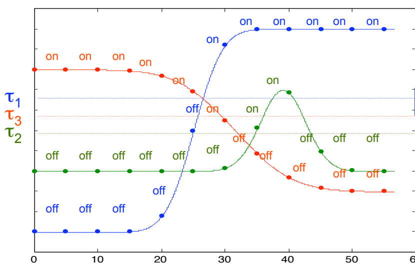
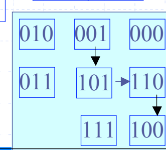
More Generally

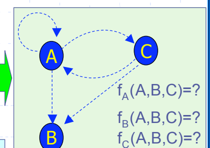
- Boolean Network:
  - A digraph G=<V,E>; nodes = genes, edges=regulation
  - For each node assign a Boolean function over ingress neighbors
- Attractors & cycles describe dynamics of expression
- Learning (reverse engineering; identification):
  - Extract Boolean network model from expression levels



Time	State
1	001
2	101
3	110
4	100

001	101
101	110
110	100

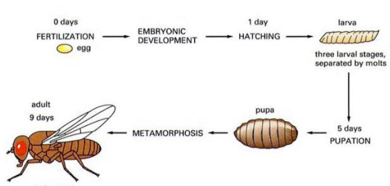


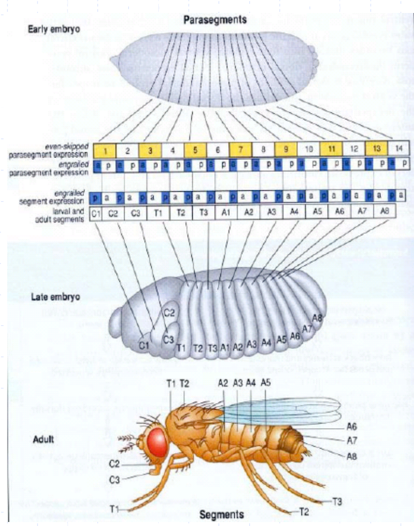


Example: Regulation of Drosophila Patterns

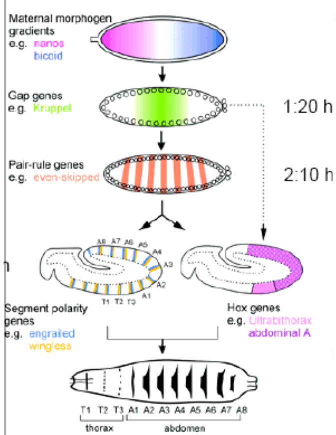
R. Albert & H.G., Othmer; Journal of Theoretical Biology 223 (2003)

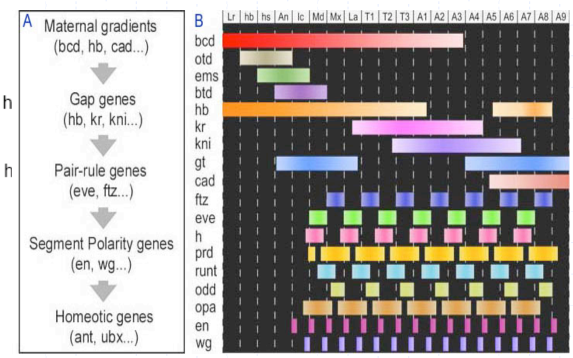
- Fundamental question: how do gene's regulate spatial patterns?





Segmentation Is Regulated By A Cascade





- Genes are activated in precise temporal order
- Use regulatory interaction to coordinate development functions

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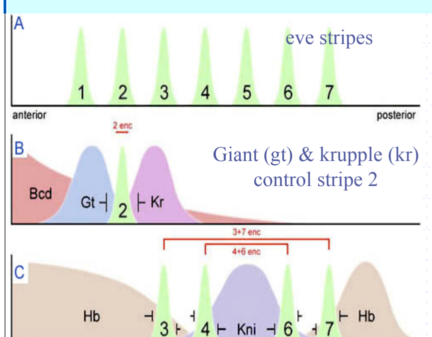
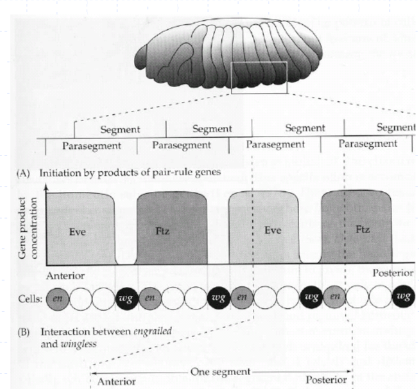
Segmentation Is Regulated By A Cascade



knirps (kni) & hunchback (hb) control stripes 3-7



- Pair rule genes initiate stripes
  - eve, ftz..
- Segment polarity genes control anterior/posterior structure
  - Engrailed, wingless

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A Boolean Network Model

R. Albert & H.G., Othmer; Journal of Theoretical Biology 223 (2003)

$$en_i^{t+1} = (WG_{i-1}^t \text{ or } WG_{i+1}^t) \text{ and not } SLP_i^t$$

$$hh_i^{t+1} = EN_i^t \text{ and not } CIR_i^t$$

$$ptc_i^{t+1} = CIA_i^t \text{ and not } EN_i^t \text{ and not } CIR_i^t$$

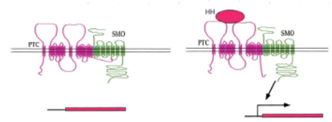
$$ci_i^{t+1} = \text{not } EN_i^t$$

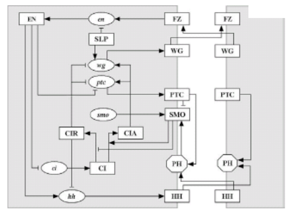
$$EN_i^{t+1} = en_i^t$$

$$WG_i^{t+1} = wg_i^t$$

$$CI_i^{t+1} = ci_i^t$$

$$HH_i^{t+1} = hh_i^t$$





$$PH_i^t = PTC_i^t \text{ and } (HH_{i-1}^t \text{ or } HH_{i+1}^t)$$

$$SMO_i^t = \text{not } PTC_i^t \text{ or } HH_{i-1}^t \text{ or } HH_{i+1}^t$$

$$wg_i^{t+1} = (CIA_i^t \text{ and } SLP_i^t \text{ and not } CIR_i^t) \text{ or } [wg_i^t \text{ and } (CIA_i^t \text{ or } SLP_i^t) \text{ and not } CIR_i^t]$$

Either of the activators can counter mRNA decay.

$$PTC_i^{t+1} = ptc_i^t \text{ or } (PTC_i^t \text{ and not } HH_{i-1}^t \text{ and not } HH_{i+1}^t)$$

Free PTC does not decay .

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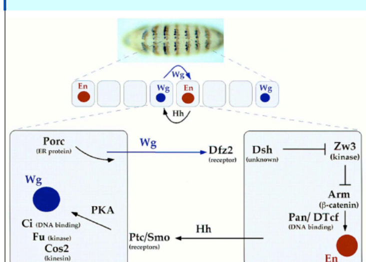
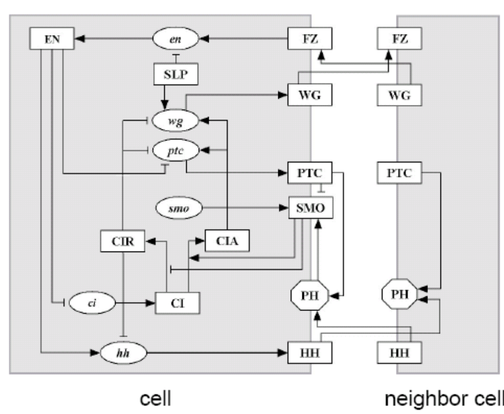
Coordinated Regulation



Cadigan, Nusse, Genes & Development 11, 3286 (1997)



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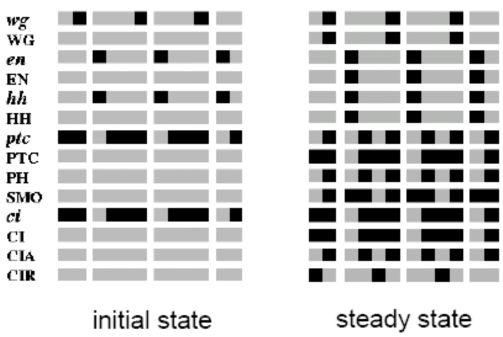
The Steady State (Attractor)

The model reproduces the wild type steady state



Agrees with the wild type pattern of the segment polarity genes.

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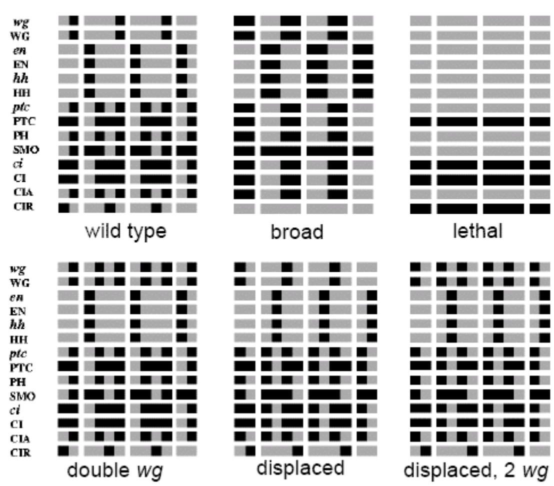
Compute Attractor Expression

Possible stable patterns



The latter states have very small probability.

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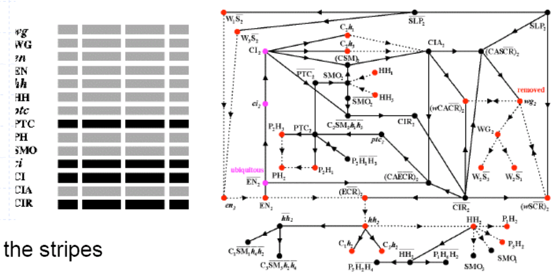
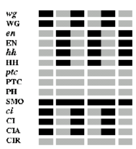
Mutations (Perturbations)

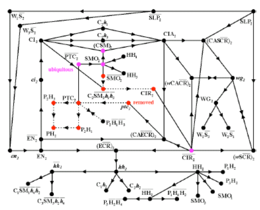
wg, *en* or *hh* mutations are lethal



ptc mutation broadens the stripes



final state



The *wg*, *en* and *hh* stripes broaden, regardless of initial state.

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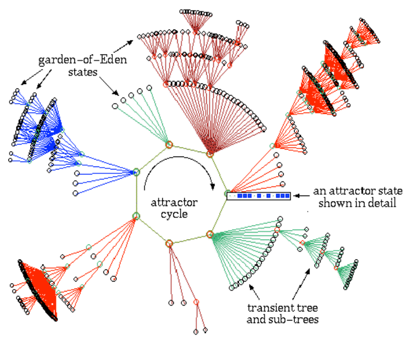
Kauffman’s Model

Kauffman’s Model [60’s, 93]

- Study Boolean networks to describe evolution
- BN: a graph of “genes” each with a random Boolean function
  - N=# of nodes; k=connectivity
- BN traverses trajectories over the hypercube $[0,1]^n$
- Converges to best fit response to random inputs

- Trajectories: series of state transitions
- Attractors: repeating trajectories
- Basin of Attraction: all states leading to an attractor

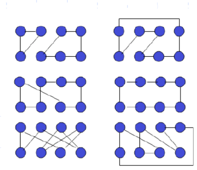


One attractor basin for a BN n=13, k=3. The cycle is of size 7

Evolution of Boolean Networks

- Nature evolves an ensemble of networks
  - Mutations change connectivity/gene-transition-function
- Genes select best-fitness transition functions
- What happens if k is large (e.g., k=N-1)?
  - X(t+1) is uncorrelated with X(t)
  - The number of attractors is very small; cycles are huge with period of some $2^{0.5N}$
  - Most genes would be oscillating
  - Network is very sensitive to small perturbations
- Need to keep k small
  - K=1, too small; gene’s do not interact
  - K=2; large number of attractors ~$N^{0.5}$; avg cycle $N^{0.5}$

An ensemble of N=8, k=2 Networks

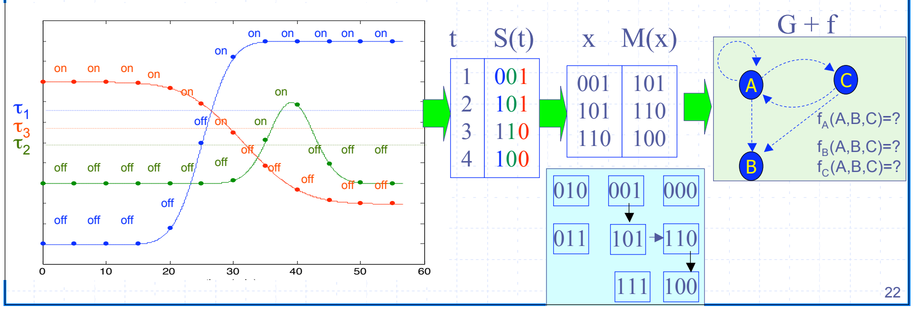


Learning Boolean Nets

(“Reverse Engineering” “Identification”)

The Challenge

- Discretization: Given expression profiles vector X(t)
  - Set expression thresholds τ (how?)
  - Extract a time-state map S(t); compute state transitions map M(x)
- Learning:
  - Given: state transition map M
  - Compute: a Boolean vector function f such that M(x)=f(x)



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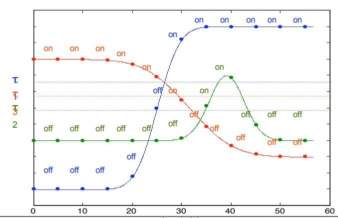
Akutsu Algorithm (99)

- Brute force search for f
- Fix k, and consider networks of max degree k
  - For each gene i, and for each subset of k ingress genes find all functions f_i that are compatible over this ingress set for all {S(r)}
  - i.e., $S'_i(r)=f_i(S'(r-1))$ where S' is the restriction of S to the ingress set
  - For k fixed: $O(k2^{2^k}n^{k+1}m)$; if k is not fixed, learning is NP complete.
- Notes
  - Works for small k…does not handle noise…
  - Later improvements handle noise



t	S(t)
1	001
2	101
3	110
4	100

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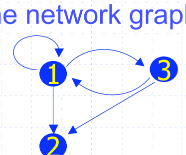
Suppose the Network Graph is Known

- Given M and G, computing f is simple:
  - The truth table for $f_i(X)$ obtains by projecting M
  - The network graph G guides the projections

G
+
M



Input state			Output state		
X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

X_1	X_3	f_1
0	0	0
0	1	1
1	0	1
1	1	1

$f_1(X) = X_1 \text{ OR } X_3$

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How Do We Find The Network Graph ?

- An intuitive approach:
  - $f(X_1,X_2,X_3)$ depends on X_1, iff $f(1,X_2,X_3) \neq f(0,X_2,X_3)$ for some (X_2,X_3)
  - We call such values $<X_1,X_2,X_3,f>$ "dependency"

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

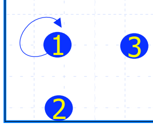
f_1 depends on X_1

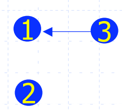
X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

f_1 is independent of X_2

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

f_1 depends on X_3





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An Intuitive Algorithm

- Repeat for all X_i and f_k :
  - Scan M to find a dependency of f_k on X_i; if found then add an X_i=>f_k edge to G
  - Else (no dependency found) then f_k is independent of X_i

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

f_1 depends on X_1

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

f_1 depends on X_3

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

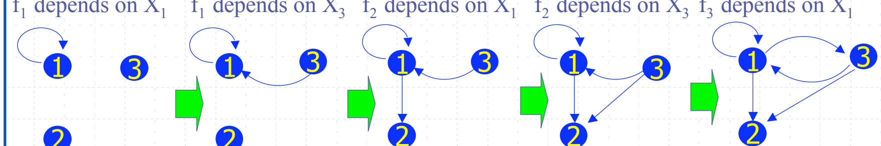
f_2 depends on X_1

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

f_2 depends on X_3

X_1	X_2	X_3	f_1	f_2	f_3
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

f_3 depends on X_1



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REVEAL (98 Liang)

- Compute network graph from mutual information measure
- Base theory:
  - Let <X,Y> be an <input,output> stream
  - Consider H(Y), the entropy of Y, and M(X,Y), the mutual information of X and Y
  - If M(Y,X)=H(Y) then X determines Y uniquely
- $H(X) = - \sum p_i \log(p_i)$
  - p_i is the probability that a random element of data stream X is i
- M(X, Y)= H(X) + H(Y)- H(X,Y)

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REVEAL Algorithm

- Step 1: compute state transition <input,output> table

Input stream			Output stream		
A_{i-1}	B_{i-1}	C_{i-1}	A_i	B_i	C_i
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

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Step 2a: Compute Entropies

Input stream value			Output stream value		
A_{i-1}	B_{i-1}	C_{i-1}	A_i	B_i	C_i
0	0	0	0	0	1
0	0	1	1	0	1
0	1	0	0	0	1
0	1	1	1	0	1
1	0	0	1	0	0
1	0	1	1	1	0
1	1	0	1	0	0
1	1	1	1	1	0

$P(A_i=0)=2/8=0.25$
$P(A_i=1)=6/8=0.75$

$H(A_i) = -((0.25)\log(0.25) + (0.75)\log(0.75))$
$= 0.81$

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Step 2a: Compute Entropies

note: $\lim_{x^+\to 0} x^x=1$, therefore in the left-hand limit, $(0)\log(0) = 0$.

$H(A_i) = -((0.25)\log(0.25) + (0.75)\log(0.75)) = 0.81$

$H(B_i) = -((0.75)\log(0.75) + (0.25)\log(0.25)) = 0.81$

$H(C_i) = -((0.5)\log(0.5) + (0.5)\log(0.5)) = 1$

$H(A_{i-1}) = H(B_{i-1}) = H(C_{i-1}) = -((0.5)\log(0.5) + (0.5)\log(0.5)) = 1$

$H(A_{i-1}, C_{i-1}) = -((0.25)\log(0.25) +(0.25)\log(0.25)+(0.25)\log(0.25)+(0.25)\log(0.25)) = 2$

$H(C_i, A_{i-1}) =-((0.5)\log(0.5) + (0.5)\log(0.5) = 1$

$H(A_i, A_{i-1}, C_{i-1})=-((0.25)\log(0.25)+(0.25)\log(0.25)+ (0.25)\log(0.25)+(0.25)\log(0.25))=2$

$H(B_i, A_{i-1}, C_{i-1})=-((0.25)\log(0.25)+(0.25)\log(0.25)+(0.25)\log(0.25)+(0.25)\log(0.25))= 2$

……

……

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Step 2b: Compute Network

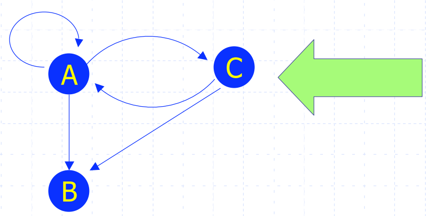
- First compute mutual information

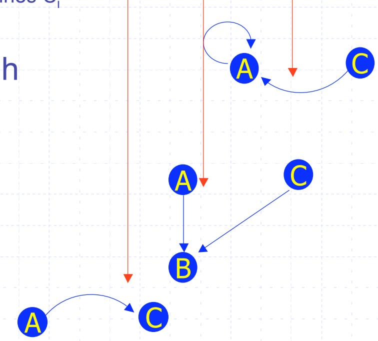
(I) $M(A_i, [A_{i-1}, C_{i-1}]) = H(A_i) + H(A_{i-1}, C_{i-1}) - H(A_i, A_{i-1}, C_{i-1}) = 0.81 + 2 - 2 = 0.81$
$= H(A_i)$, therefore A_{i-1} and C_{i-1} determine A_i

(II) $M(B_i, [A_{i-1}, C_{i-1}]) = H(B_i) + H(A_{i-1}, C_{i-1}) - H(B_i, A_{i-1}, C_{i-1}) = 0.81 + 2 - 2 = 0.81$
$= H(B_i)$, therefore A_{i-1} and C_{i-1} determine B_i

(III) $M(C_i, A_{i-1}) = H(C_i) + H(A_{i-1}) - H(C_i, A_{i-1}) = 1 + 1 - 1 = 1$
$= H(C_i)$, therefore A_{i-1} determines C_i

- Use this to determine network graph





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Step 3: Compute Boolean Functions

- Consider only network dependencies

A_{i-1}	C_{i-1}	A_i
0	0	0
0	1	1
1	0	1
1	1	1

$A_i = A_{i-1}$ OR C_{i-1}

A_{i-1}	C_{i-1}	B_i
0	0	0
0	1	0
1	0	0
1	1	1

$B_i = A_{i-1}$ AND C_{i-1}

A_{i-1}	C_i
0	1
1	0

$C_i =$ NOT A_{i-1}

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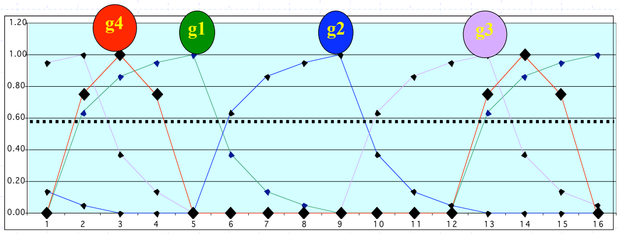
Example

- Consider the following expression scenario of 4 genes:



g_1	g_2	g_3	g_4	f_1	f_2	f_3	f_4
0	0	1	0	1	0	1	1
1	0	1	1	1	0	0	1
1	0	0	1	1	0	0	0
1	0	0	0	0	1	0	0
0	1	0	0	0	0	1	0

- A threshold at 0.6 yields: 0010→1011→1001→1000→0100→0010
  - Note: the intermediate state 0000 of the transition 1000=>0000=>0100 is ignored
- The transition map is partial
  - Can admit multiple Boolean net models
  - Exercise: find 2 distinct Boolean net models
- Exercise: use REVEAL to compute a network model

Computing A Partial Dependency Graph

g_1	g_2	g_3	g_4	f_1	f_2	f_3	f_4
0	0	1	0	1	0	1	1
1	0	1	1	1	0	0	1
1	0	0	1	1	0	0	0
1	0	0	0	0	1	0	0
0	1	0	0	0	0	1	0

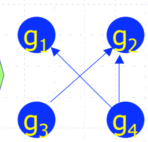
f_1 depends on g_4

g_1	g_2	g_3	g_4	f_1	f_2	f_3	f_4
0	0	1	0	1	0	1	1
1	0	1	1	1	0	0	1
1	0	0	1	1	0	0	0
1	0	0	0	0	1	0	0
0	1	0	0	0	0	1	0

f_2 depends on g_4

g_1	g_2	g_3	g_4	f_1	f_2	f_3	f_4
0	0	1	0	1	0	1	1
1	0	1	1	1	0	0	1
1	0	0	1	1	0	0	0
1	0	0	0	0	1	0	0
0	1	0	0	0	0	1	0

f_4 depends on g_3



g_1	g_2	g_3	g_4	f_1	f_2	f_3	f_4
0	0	1	0	1	0	1	1
1	0	1	1	1	0	0	1
1	0	0	1	1	0	0	0
1	0	0	0	0	1	0	0
0	1	0	0	0	0	1	0

g_1	g_2	g_3	g_4	f_1
0	0	1	0	1
1	0	1	1	1
1	0	0	1	1
1	0	0	0	0
0	1	0	0	0

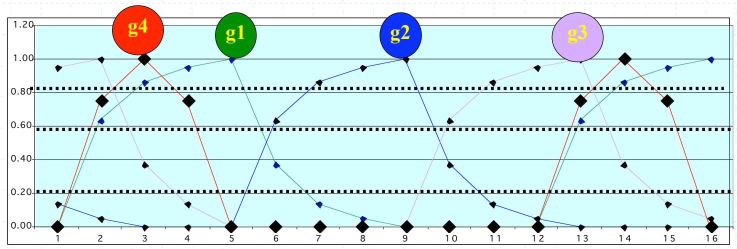
$f_1 = g_3 \vee g_4$

$f_1 = (\neg g_1 \vee g_4) \wedge (\neg g_2)$

Dependency means that g_4 must appear in any expression of f_1;
For a partial map f_1 may require other genes without depending on them.

Sensitivity Considerations & Noisy Maps

- Consider again the 4 genes example



- Different thresholds yield different Boolean dynamics:
  - Tr1/2/3/4=0.6: 0010→1011→1001→1000→0100→0010…
  - Tr1/2/3=0.2, Tr4=0.8: 0010 →1010→1011→1000→1100→0100 →01100→0010
  - Tr1/2/3/4=0.8: 0010 →0011→1000→0000→0100→0000→0010… (non-deterministic)

Research Questions

- Extend the intuitive algorithm to handle partial noisy maps
- Extend REVEAL to handle partial noisy maps
- ?Probabilistic Boolean net models?
  - ?Max likelihood training…EM…?
- ?SVM based models… Boolean kernel machines…?

Final Notes

How Good Are Boolean Models?

Advantages

- Provide good qualitative interpretation of regulation
- Particularly important for switching behaviors
  - Phage lysis...sporulation…Drosophila patterns..
  - Such systems are “robust” wrt exact expression values
- Useful connection with evolutionary behaviors

Disadvantages

- Boolean abstraction is poor fit to real expression data
- Cannot model important features:
  - Amplification of a signal; subtraction and addition of signals
  - Handling smoothly varying environmental parameter (e.g. temperature, nutrients)
  - Temporal performance behavior (e.g. cell cycle period)
  - Negative feedback control (Boolean model oscillates vs. stabilize)

A Variety of Regulatory Network Models

- Finite-field models: $X(t+1)=p(X)$
  - p is a polynomial over finite field
  - Generalizes the Boolean model
- Differential equations models: describe $dX/dt=f(X)$
  - f describes non-linear control of change by neighbors
- Linear model: $X(t+1)=W X+ B$
  - W is a weight matrix; linear approximation near steady state
- Neural network models: $x_i(t)=\sigma(WX_{Neighbors(i)} +B)$
  - Sigmoid non-linearity can be trained through gradient algorithm
  - Comes with a learning algorithm
- Bayesian network models…

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