

# Modeling Regulation Networks

~~for~~ **with Biologists**

Amir Rubinstein

School of Computer Science, Tel-Aviv University

Computational Genomics Advanced Seminar

January 2012

# Introduction

- Part of this work was done in 2004–2006 at the Technion, under the supervision of
  - Prof. Ron Pinter (CS)
  - Prof. Yona Kassir (biology)
- The more recent part of the research began a year ago, with Prof. Kassir and Ofir Hazan (math student, Ort Braude College)

# Introduction

We propose a computational model for regulatory networks. This model:

- is **simple** and **intuitive**
- is **qualitative** (no need for detailed quantitative data)
- most its observations were **validated *in vivo***.
- developed **with** biologists, **not for** them.

Promoted an ongoing dialogue between CS and biological approaches, bridging over gaps in terminology and concepts.

# Lecture Outline

- The biological questions
- The Boolean model
- The discrete model + results
- Some recent extensions + recent results
- Current/future work

# The Biological Questions

- The model organism at Kassir's lab is budding yeast *S.Cerevisiae*
- Pathways under study:  
meiosis and cell-cycle (mitosis)  
i.e. developmental pathways

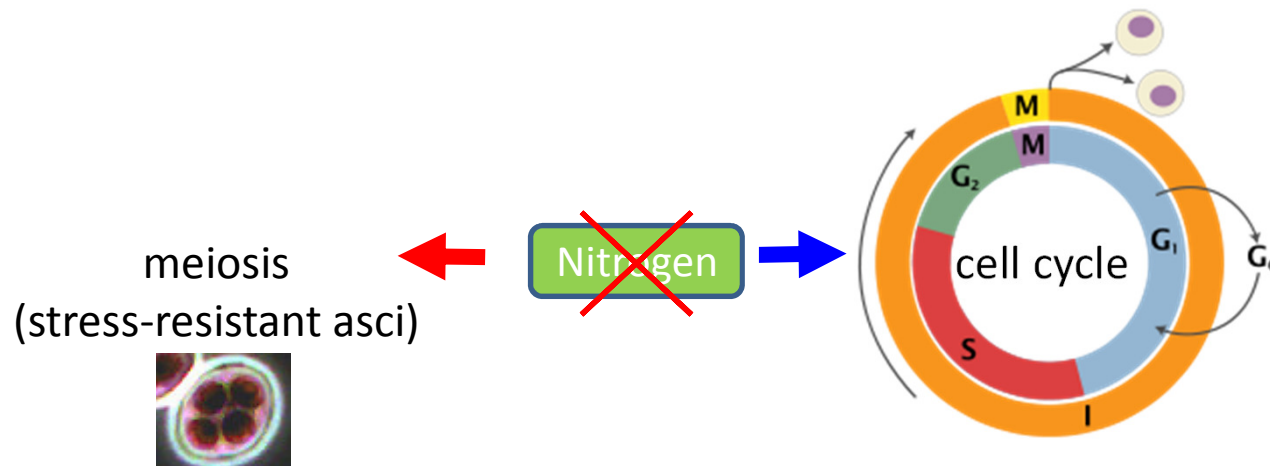
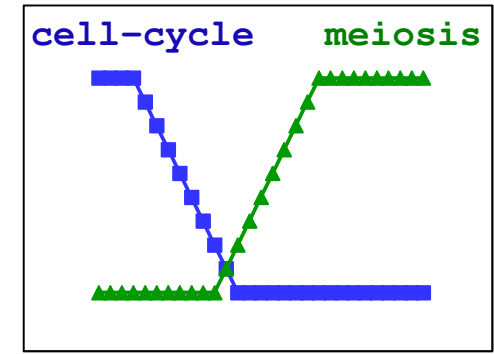


Figure taken from wikipedia

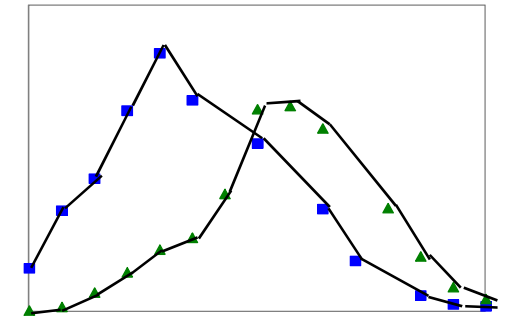
# The Biological Questions (cont'd)

- Some typical biological questions:

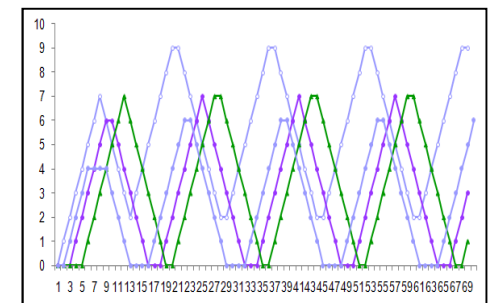
- **Bi-stability**: how do cells ensure that entry into one developmental pathway inhibits entry into the alternative?



- What mechanisms allow **transient expression** of genes?



- What mechanisms allow **oscillations**?



# The Biological Questions (cont'd)

- How is **robustness** to mutations achieved?
- How is **stability** with regards to changes in initial conditions achieved?
- When is repression simply the **lack of activation** (as opposed to active repression?)

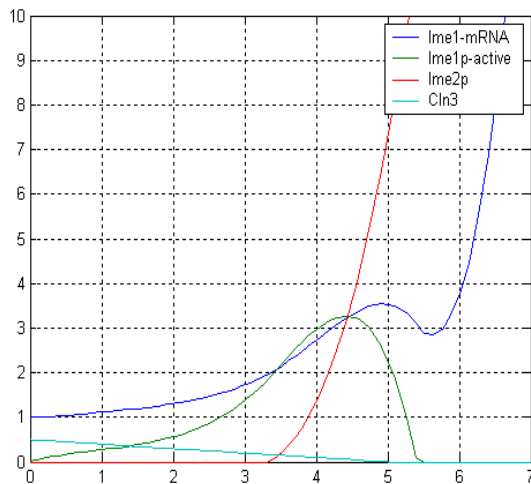
# Lecture Outline

- ✓ The biological questions
- The Boolean model
- The discrete model + results
- Some recent extensions + recent results
- Current/future work



# Which Model Fits Our Needs?

## Continuous



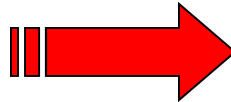
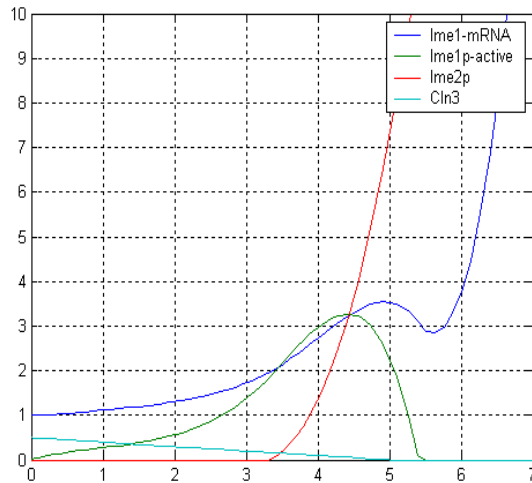
$$\begin{aligned}dIME1 &= k_{11} + \text{sign}(t, t_c) * k_{12} * Ime1 - k_{13} * IME1 \\dIme1 &= k_{21} * IME1 - k_{22} * Ime2 - k_{23} * Cln3 - k_{24} * Ime1 \\dIME2 &= k_{31} * Ime1 - k_{32} * IME2 \\dIme2 &= k_{41} * IME2 - k_{42} * Ime2 \\dCln3 &= -k_{51} * Cln3\end{aligned}$$

But we don't have the quantitative data!

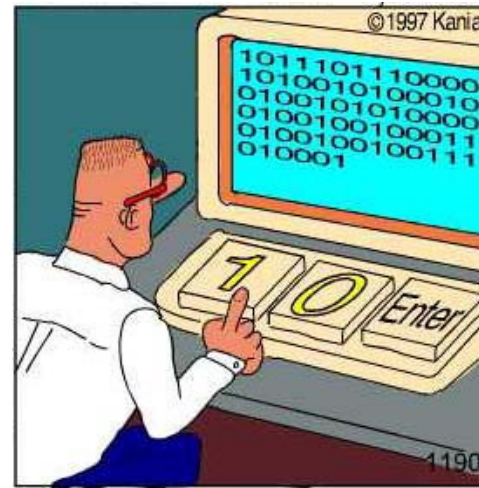
# Which Model Fits Our Needs?

- Let's start as simple as possible:

## Continuous



## Boolean



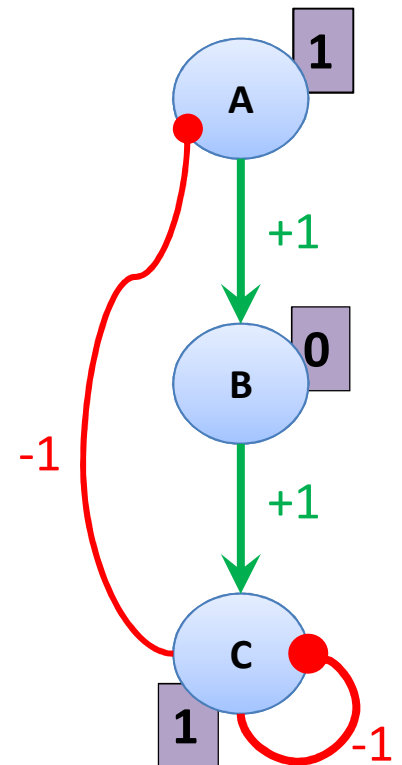
Real programmers code in binary.

F. Li, T. Long, Y. Lu, Q. Ouyang and C. Tang .  
“The yeast cell-cycle network is robustly designed”.  
PNAS 101 (14),4781-6, 2004

# The Boolean Computational Model

- Nodes: represent:
  - proteins *e.g. Ime1*
  - mRNA *IME1*
  - nutrients *N<sub>2</sub>*
  - cellular events *Meiosis*
- can assume **state 0** (no activity)  
**1** (full activity)
- biologists determine **initial states**

- Edges: regulation effects  
**weighted**  
(+ **activation**)  
(- **repression**)

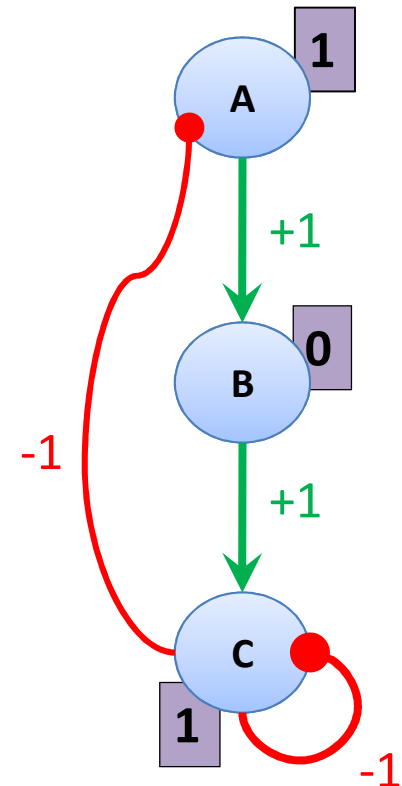


# The Boolean Computational Model

- Time is discrete (time steps = 1, 2, 3, ...)
- A transition function determines the states of nodes in the next time step in a synchronous fashion

$$s_i(t+1) = \begin{cases} 1 & \sum_j w(j,i) \cdot s_j(t) > 0 \\ 0 & \sum_j w(j,i) \cdot s_j(t) < 0 \\ s_i(t) & \text{else} \end{cases}$$

- Transition function is applied repeatedly until:  
steady state / loop



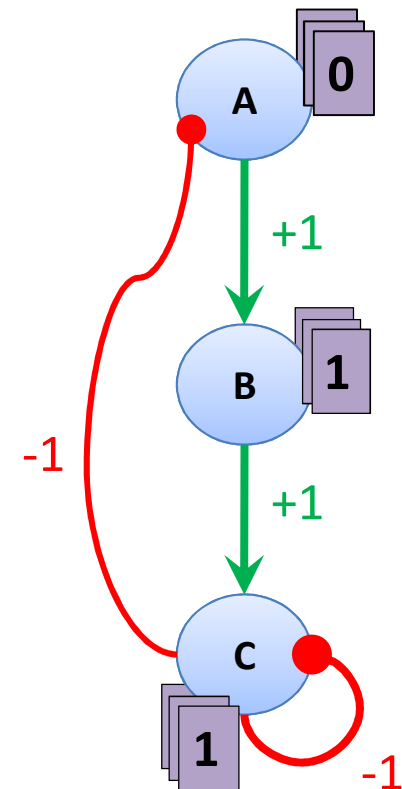
# The Boolean Computational Model

- simulation of a simulation:

	A	B	C
$t_1$	1	0	1
$t_2$	0	1	0
$t_3$	0	1	1
$t_4$	0	1	1

← Initial vector

← Final vector (steady state)



- Some examples of oscillatory network?

# Applying The Boolean Model to Yeast Meiosis

- Network for early meiosis in yeast:

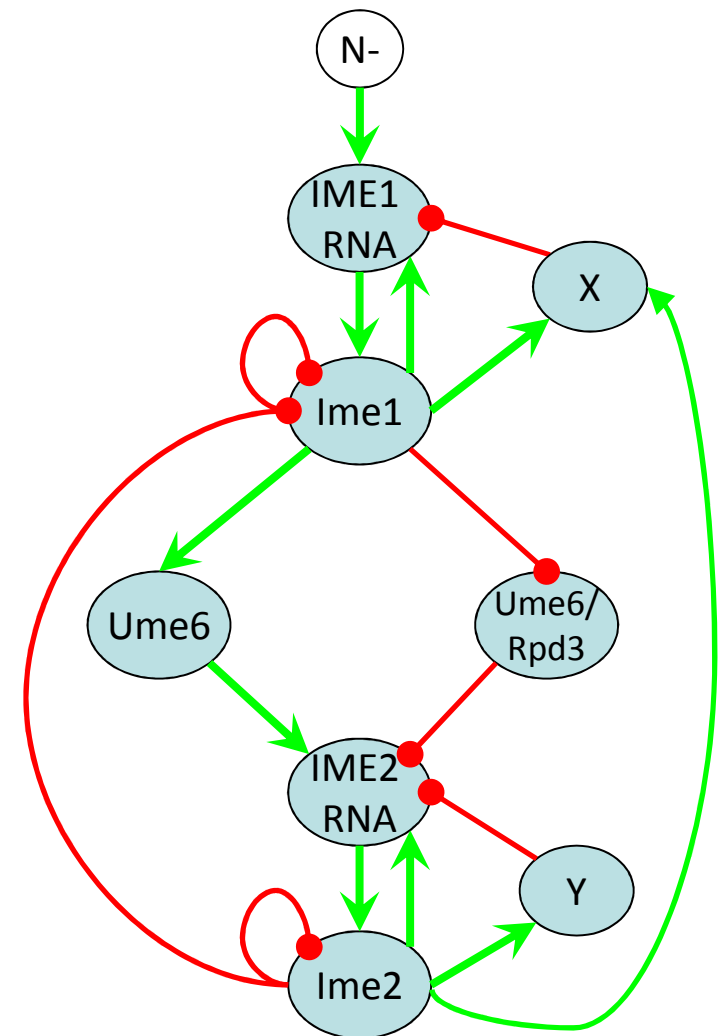
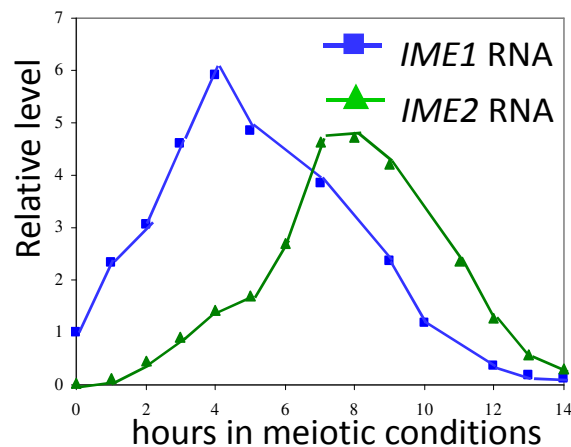
Initial states (at entry to meiosis):

(N-) = 1 (depletion of Nitrogen)

Ume6/  
Rpd3 = 1

all the rest are 0

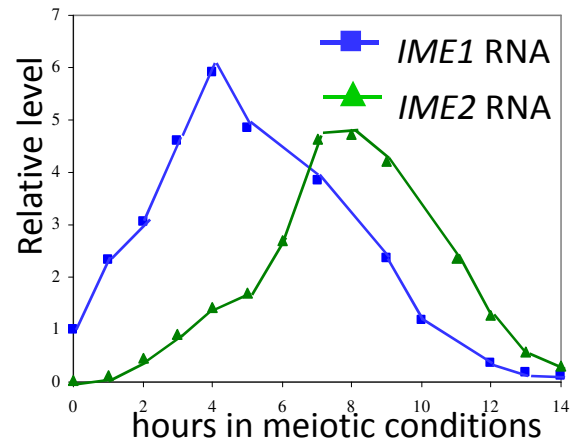
The expected behavior:



# Applying The Boolean Model to Yeast Meiosis

- Network for early meiosis in yeast:

## The expected behavior:



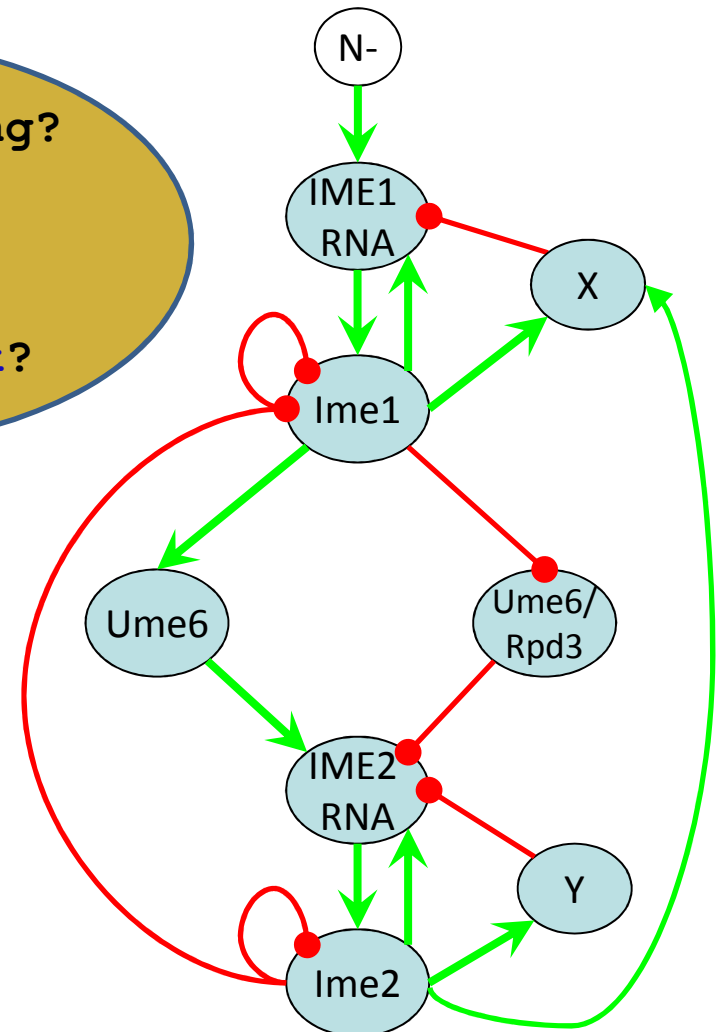
So what's wrong?

- the model?

- the network?

## Simulation:

	IME1 RNA	<i>Ime1</i>	IME2 RNA	<i>Ime2</i>	Ume6	Ume6/Rpd3	X	Y
$t_1$ :	0	0	0	0	0	1	0	0
$t_2$ :	1	0	0	0	0	1	0	0
$t_3$ :	1	1	0	0	0	1	0	0
$t_4$ :	1	1	0	0	1	0	1	0
$t_5$ :	1	1	1	0	1	0	1	0
$t_6$ :	1	1	1	1	1	0	1	0
$t_7$ :	1	0	1	1	1	0	1	1



# Extending The Boolean Model

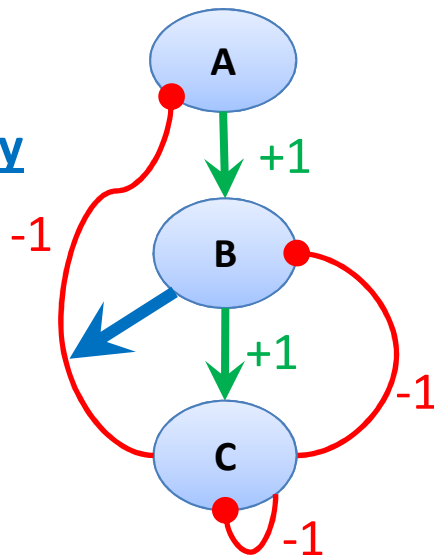
- What's wrong? The **model**? The **network**?

Maybe both...

- After long discussions with lab members, we identified an additional type of regulation: **conditional regulation**

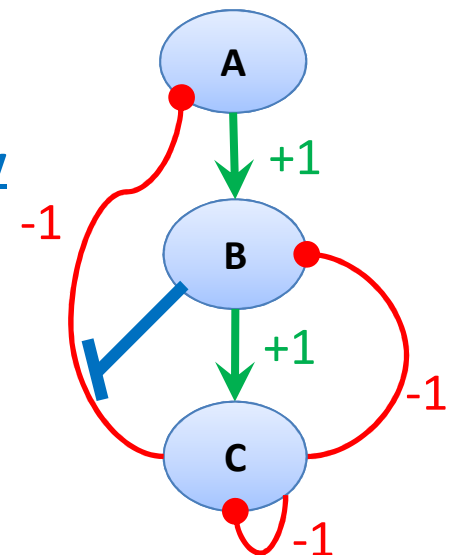
Positive dependency

C affects A only if B is active



Negative dependency

C affects A only if B is inactive

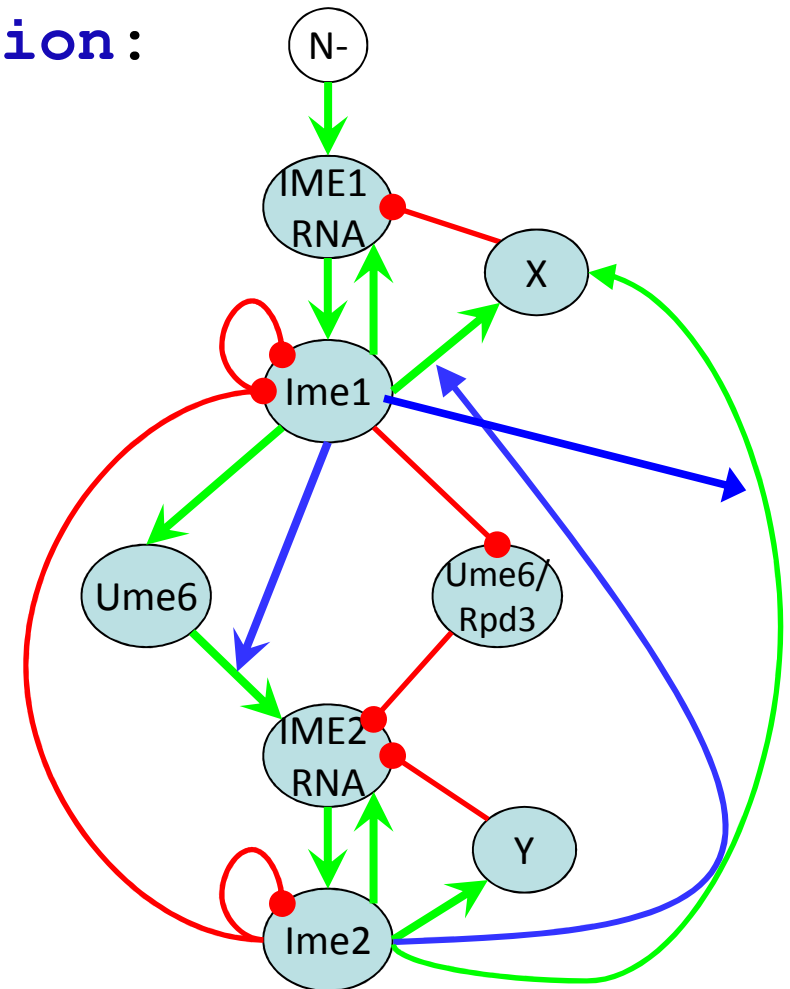




# Applying The Extended Model to Yeast Meiosis

- Adding **dependency regulation**:
- Still bad results:

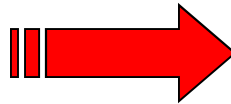
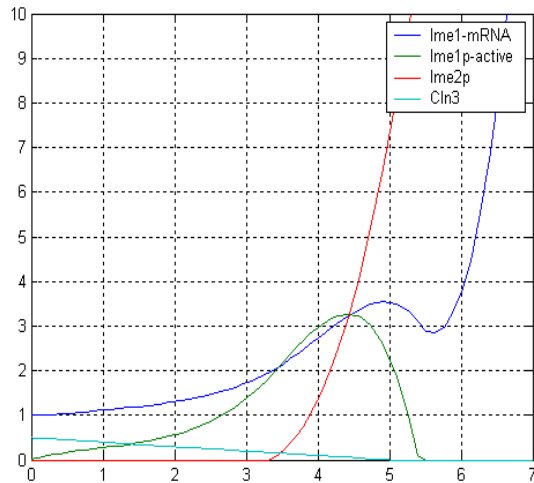
	IME1 RNA	<i>Ime1</i>	IME2 RNA	<i>Ime2</i>	X
$t_1$ :	0	0	0	0	0
$t_2$ :	1	0	0	0	0
$t_3$ :	1	1	0	0	0
$t_4$ :	1	1	0	0	1
$t_5$ :	1	1	1	0	1
$t_6$ :	1	1	1	1	1
$t_7$ :	1	0	1	1	1



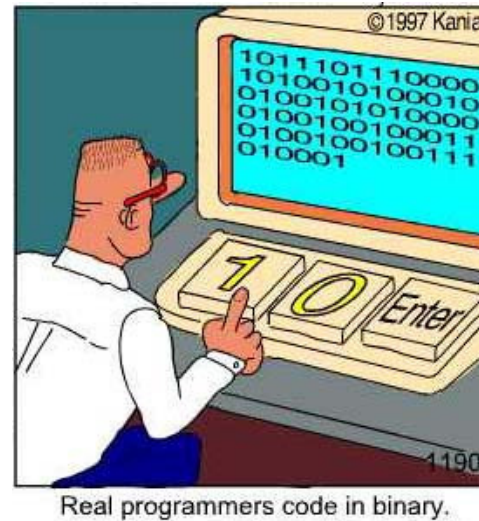
- Why doesn't IME1 go down?
- Don't want to fix it with edges weights (no data + overfitting!)

# Which Model Fits Our Needs (2)?

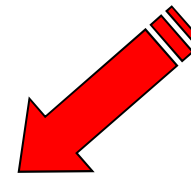
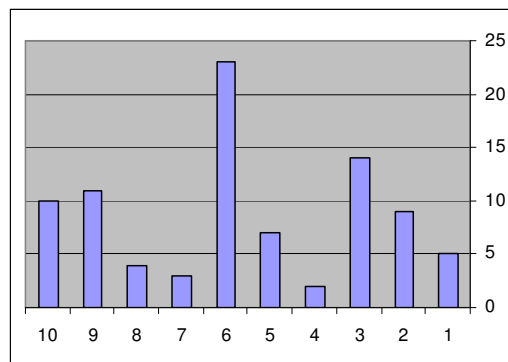
## Continuous



## Boolean



## Discrete



# Lecture Outline

- ✓ The biological questions
- ✓ The Boolean model
- The discrete model + results
- Some recent extensions + recent results
- Current/future work

# The Discrete Model

- Instead of 0/1, nodes can now assume **states** between  $0, \dots, N$  (some positive integer)
- For technical reasons we mostly used  $N=9$
- **Transition function** changes accordingly:

$$s_i(t+1) = \begin{cases} \min(N, s_i(t) + 1) & \sum_j w(j, i) \cdot s_j(t) > 0 \\ \max(0, s_i(t) - 1) & \sum_j w(j, i) \cdot s_j(t) < 0 \\ s_i(t) & \textit{else} \end{cases}$$

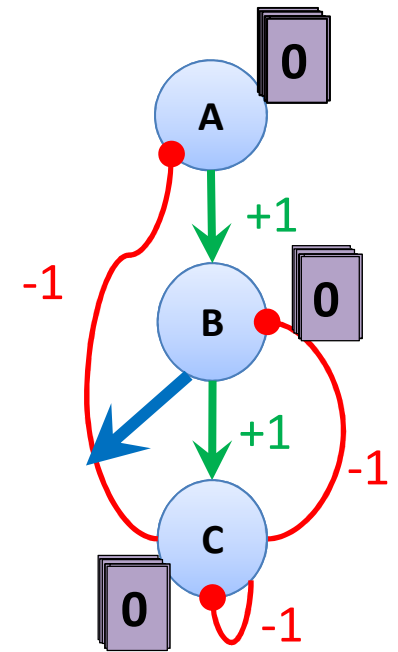
# The Discrete Model

- Simulation of simulation:

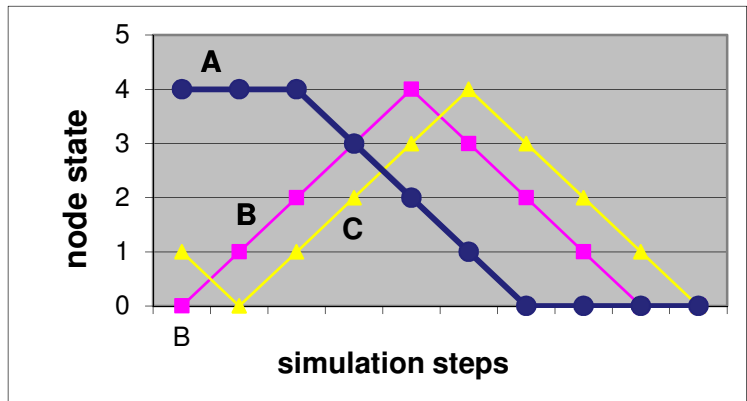
	A	B	C
$t_1$	4	0	1
$t_2$	4	1	0
$t_3$	4	2	1
$t_4$	3	3	2
$t_5$	2	4	3
$t_6$	1	3	4
$t_7$	0	2	3
$t_8$	0	1	2
$t_9$	0	0	1
$t_{10}$	0	0	0

Initial vector

Final vector (steady state)

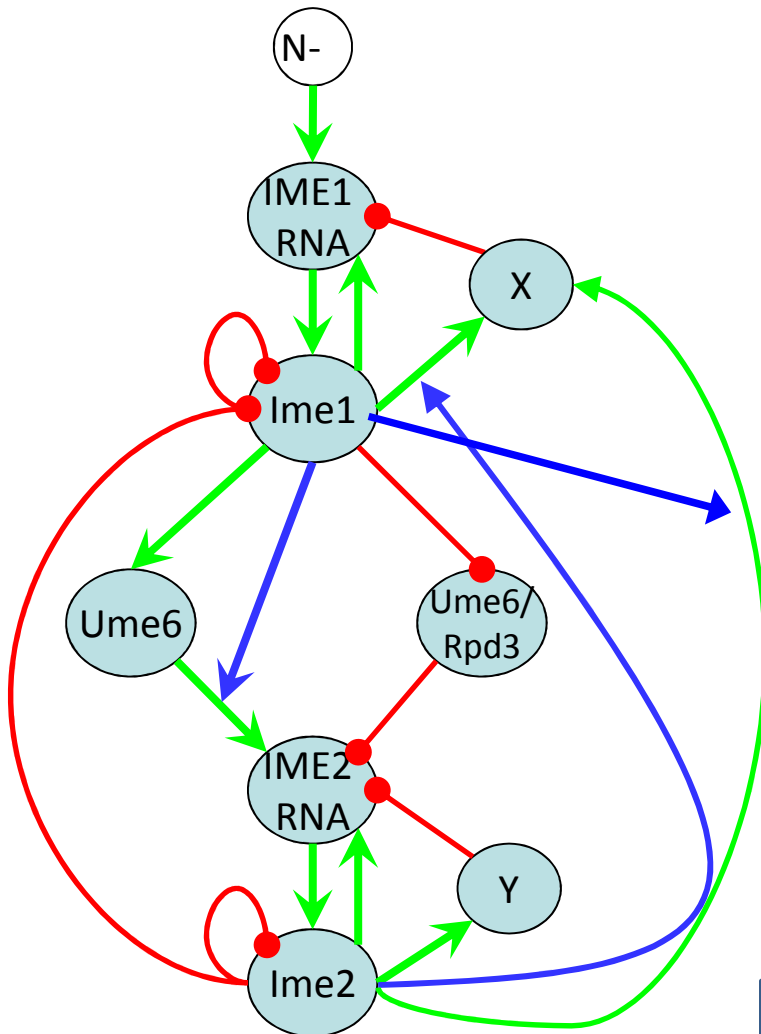


Profiles for nodes A, B, C



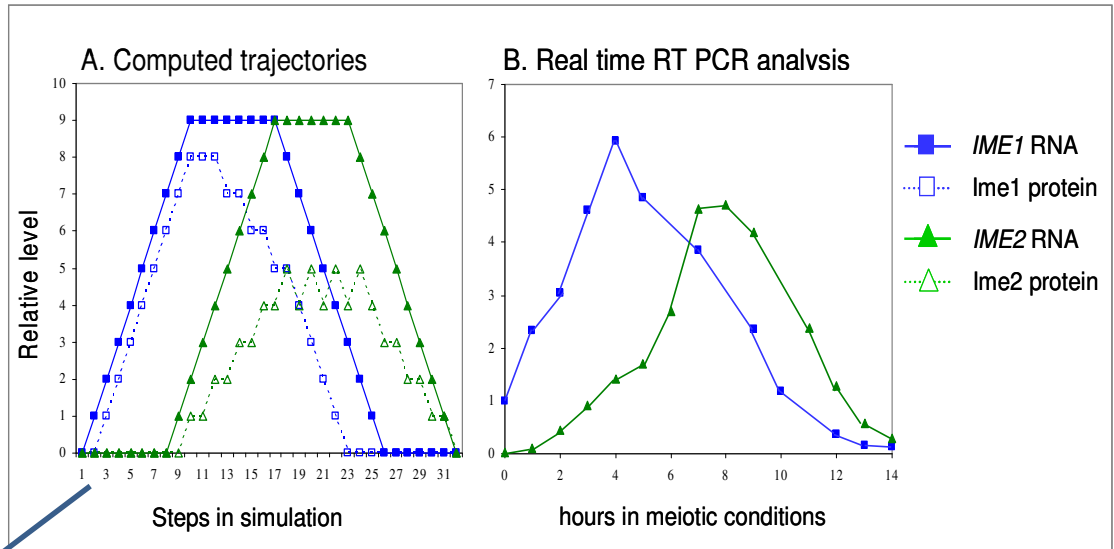
# Applying The Discrete Model to Yeast Meiosis

- $N=9$



Initial states (at entry to meiosis):

- $(N-) = 1$  (depletion of Nitrogen)
- $Ume6 = 0$        $Ume6/Rpd3 = 9$
- All the rest are 0/1 (basal level)
- We have  $2^6 = 64$  initial vectors

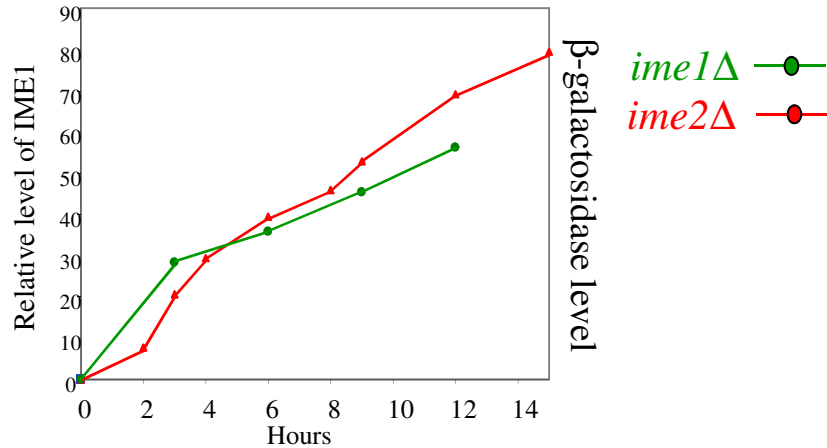


46 / 64 initial vectors  
We'll get back to this soon

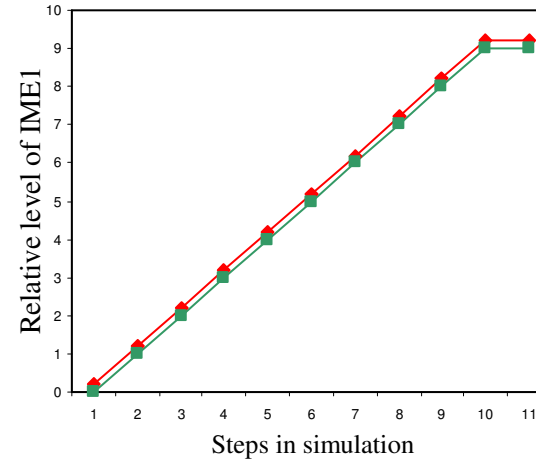
# Validation of the Discrete Model

## lab experiments

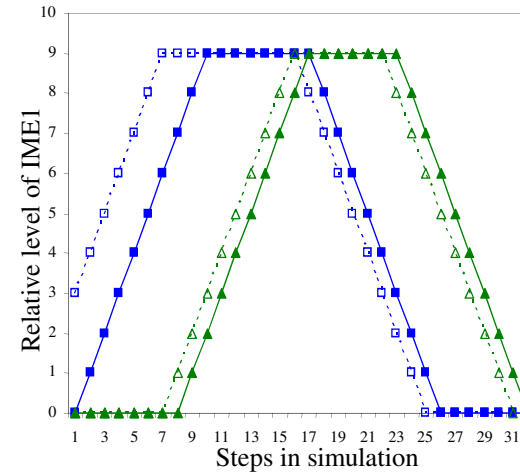
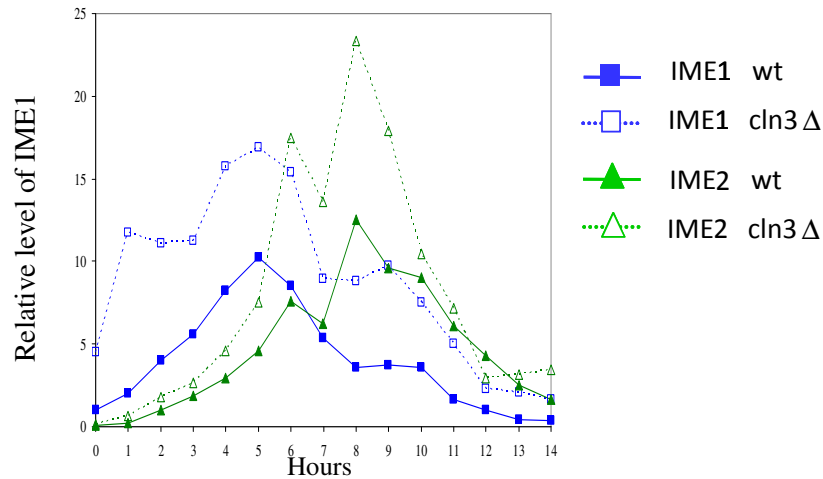
- *Ime1* or *Ime2* knockout



## simulations



- Premature expression of IME1



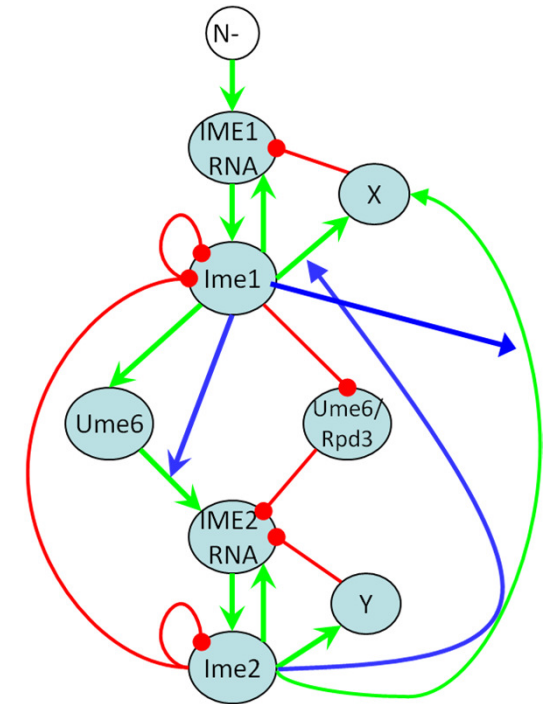
# Predictions – example 1

- What is the identity of  $X$ ?
  - We have a few candidates
  - What are the evidences?

## Evidence No.1

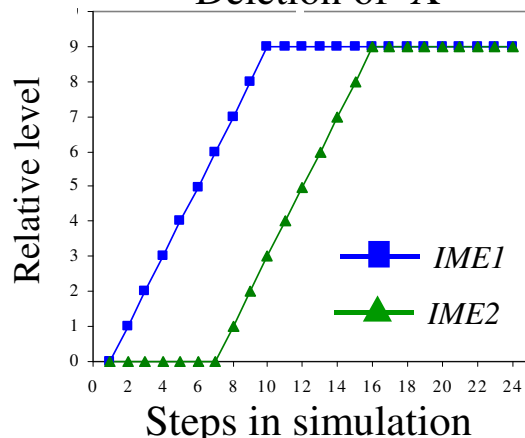
deletion of  $X$   
results in a  
non-transient  
expression

so does  
deletion in the  
lab of a gene  
called *RPD3*



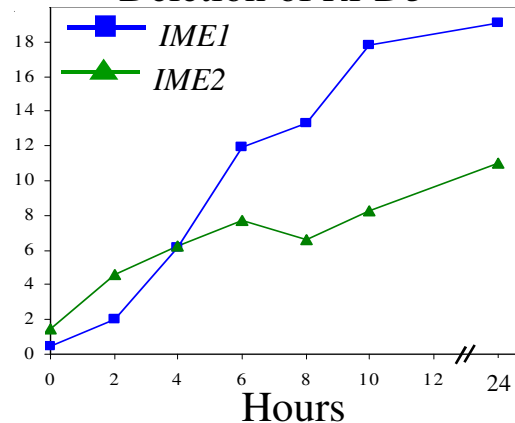
### Simulation

Deletion of  $X$



### Lab

Deletion of *RPD3*



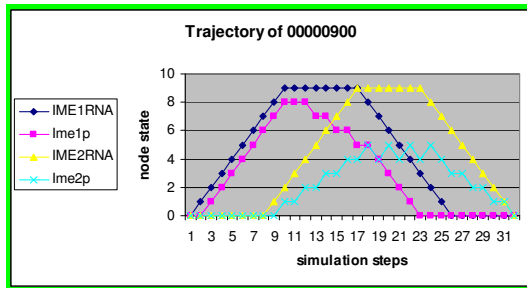


# Predictions – example 1

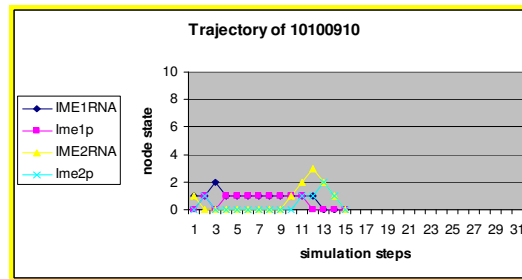
- Evidence No.2

Let's see the effect of X's initial state.

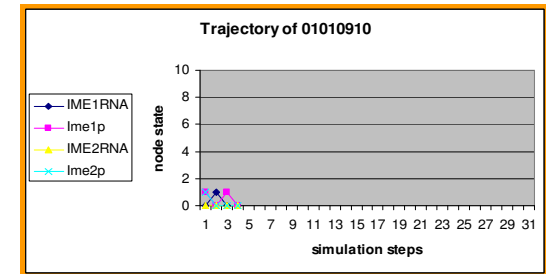
behavior	Initially X=0	Initially X=1
Total	32	32
Normal transient expression	32	14
Restrained transient expression	0	6
No transient expression	0	12




Normal



Restrained



No expression

- Basal levels of X partly shut down meiosis.
- It is known that all Rpd3 is bound as a complex  at entry to meiosis.

# Predictions – example 1

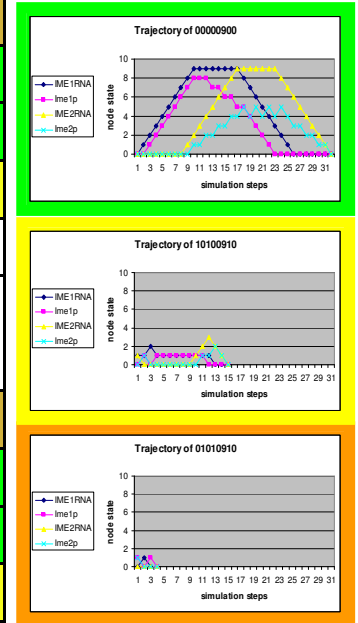
- Evidence No.3

Let's go deeper into X's effect.

We use Karnaugh-like maps.

		IME2 / Ime2						IME2 / Ime2					
		00	01	11	10			00	01	11	10		
<b>X=0, Y=0</b>		00	32	33	33	34	<b>X=1, Y=0</b>		00	1	2	2	3
IME1 /	01	31	32	32	33	IME1 /	01	31	4	4	33		
Ime1	11	30	32	32	31	Ime1	11	30	14	14	30		
	10	31	33	33	32		10	31	33	33	15		
<b>X=0, Y=1</b>		IME2/Ime2				<b>X=1, Y=1</b>		IME2/Ime2					
		00	01	11	10			00	01	11	10		
IME1 /	00	33	33	33	34	IME1 /	00	1	2	2	3		
	01	32	32	32	33		01	32	4	4	33		
Ime1	11	31	32	32	31	Ime1	11	31	14	14	30		
	10	32	33	33	32		10	32	33	33	15		

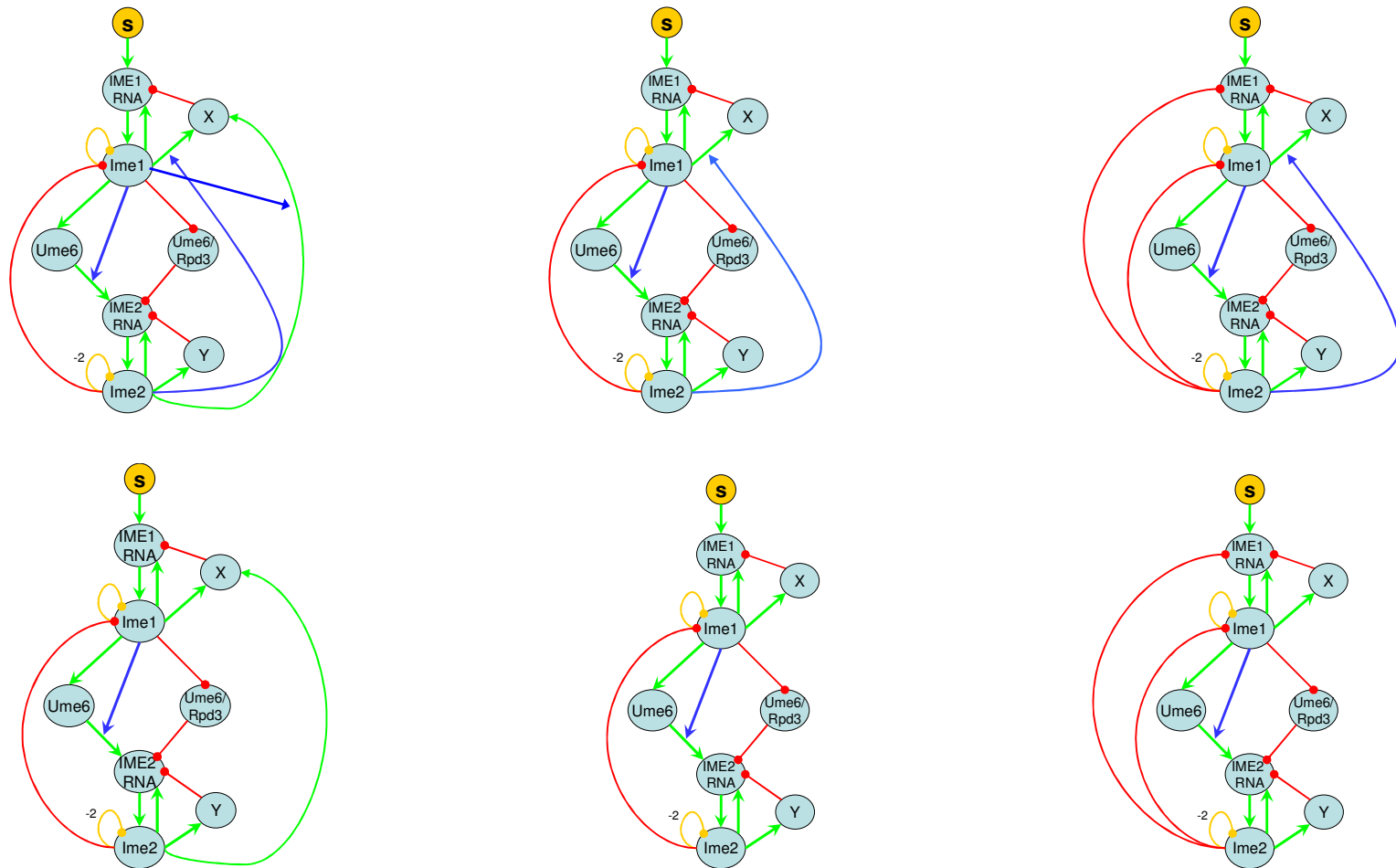
Numbers indicate length of trajectory



- Basal level of X creates sensitivity to initial levels of Ime1 and Ime2.
- This is yet to be experimented in lab, with *Rpd3*.

# Predictions – example 2

- We used the model to distinguish among several hypotheses concerning network structure



# Predictions – example 2

- Won't get into details here...
- We used similar analyses.
- Refuted some hypothesized alternative network structures

A. Karnaugh maps for Dependent Networks

		Network 0				Network 1				Network 2			
		00	01	11	10	00	01	11	10	00	01	11	10
X=0 Y=0	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	01	32	33	33	34	31	32	32	33	30	31	31	32
	11	31	32	32	31	30	32	32	31	29	31	31	30
X=0 Y=1	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	01	33	33	33	34	32	32	32	33	31	31	31	32
	11	31	32	32	31	31	32	32	31	30	31	31	30
X=1 Y=0	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	01	1	2	2	3	1	2	2	3	1	2	2	3
	11	31	4	4	33	31	4	4	33	30	2	2	3
X=1 Y=1	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	01	1	2	2	3	1	2	2	3	1	2	2	3
	11	32	4	4	33	31	4	4	33	30	4	4	29

B. Karnaugh maps for Independent Networks

		3 Network 0				4 Network 1				5 Network 2			
		00	01	11	10	00	01	11	10	00	01	11	10
X=0 Y=0	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	01	30	31	31	32	29	31	31	31	30	9	9	32
	11	11	11	11	12	11	11	11	3	11	11	11	5
X=0 Y=1	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	01	28	30	30	13	26	29	29	10	28	30	30	13
	11	29	31	31	30	28	30	30	29	29	31	31	30
X=1 Y=0	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	01	1	2	2	3	1	2	2	3	1	2	2	3
	11	4	4	4	3	4	2	2	3	4	4	4	3
X=1 Y=1	IME1	00	01	11	10	00	01	11	10	00	01	11	10
	IME2	00	01	11	10	00	01	11	10	00	01	11	10
	01	9	6	6	6	9	4	4	5	9	6	6	6
	11	10	9	9	7	10	2	2	5	10	4	4	7

# Lecture Outline

- ✓ The biological questions
- ✓ The Boolean model
- ✓ The discrete model + results
- Some recent extensions + recent results
- Current/future work

# More recent extension to the model

## Thresholds on nodes

- Biologists pointed out that some proteins are more **robust** than others.

$$s_i(t+1) = \begin{cases} \min(N, s_i(t) + 1) & \sum_j w(j, i) \cdot s_j(t) > \text{threshold}_i^+ \\ \max(0, s_i(t) - 1) & \sum_j w(j, i) \cdot s_j(t) < \text{threshold}_i^- \\ s_i(t) & \text{else} \end{cases}$$

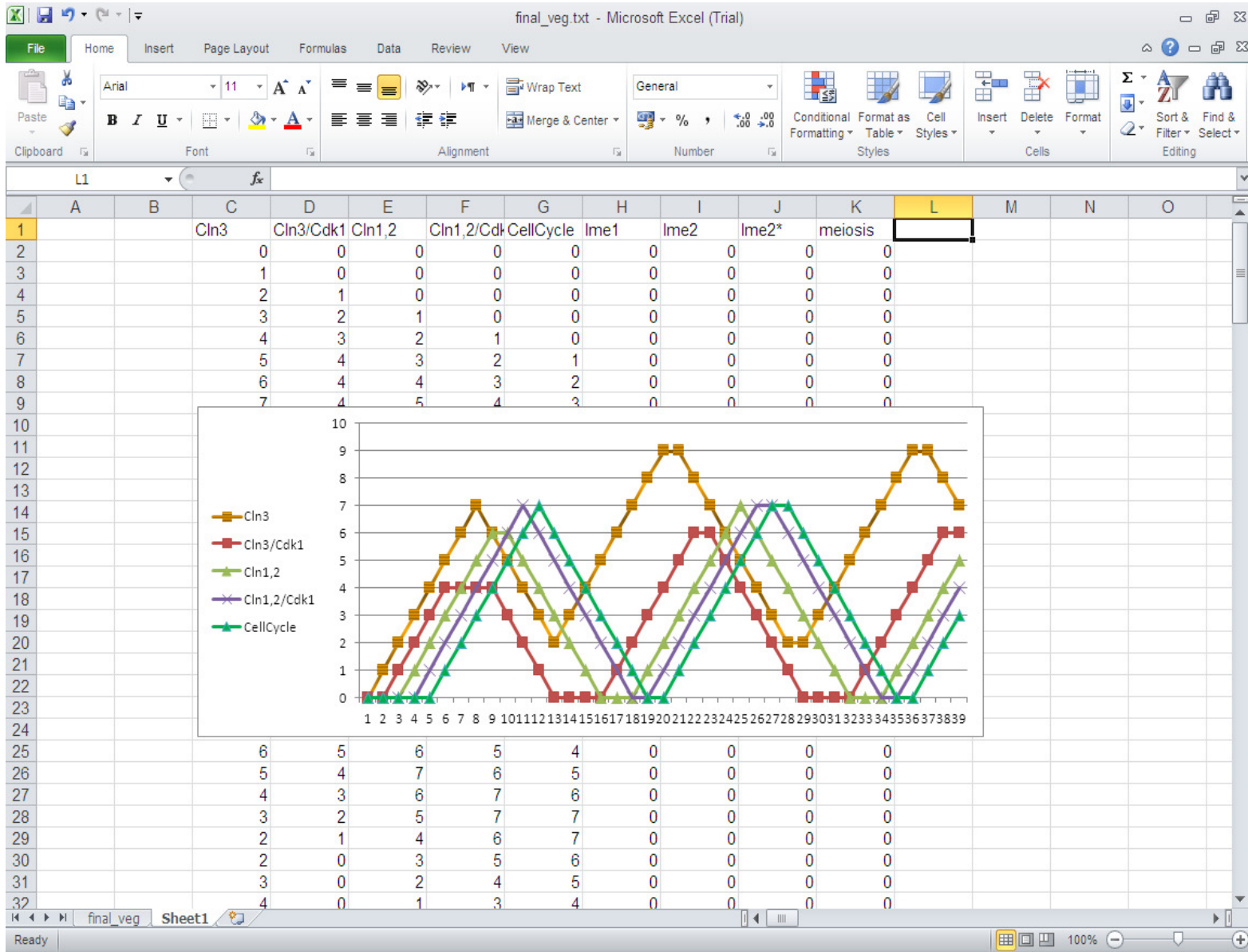
# More recent extension to the model

## Proportional transition function

- Biologists pointed out that levels of activity change **proportionally** to the strengths of regulations
- So  **$\pm 1$**  is not subtle enough
- We introduces **logarithmic-order transitions**

$$s_i(t+1) = \begin{cases} \min(N, s_i(t) + O(\log(\Sigma))) & \sum_j w(j, i) \cdot s_j(t) > threshold_i^+ \\ \max(0, s_i(t) - O(\log(\Sigma))) & \sum_j w(j, i) \cdot s_j(t) < threshold_i^- \\ s_i(t) & else \end{cases}$$

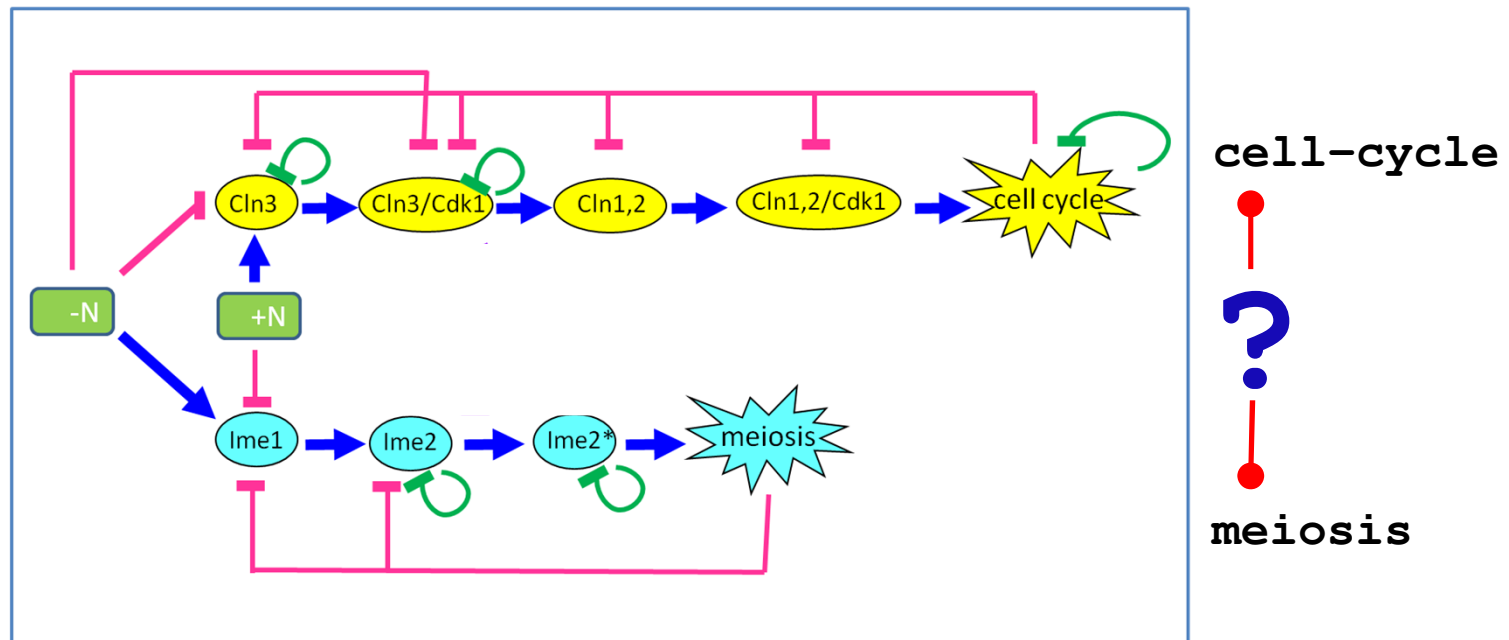
# The Tool





# Latest Results – 1 (2011)

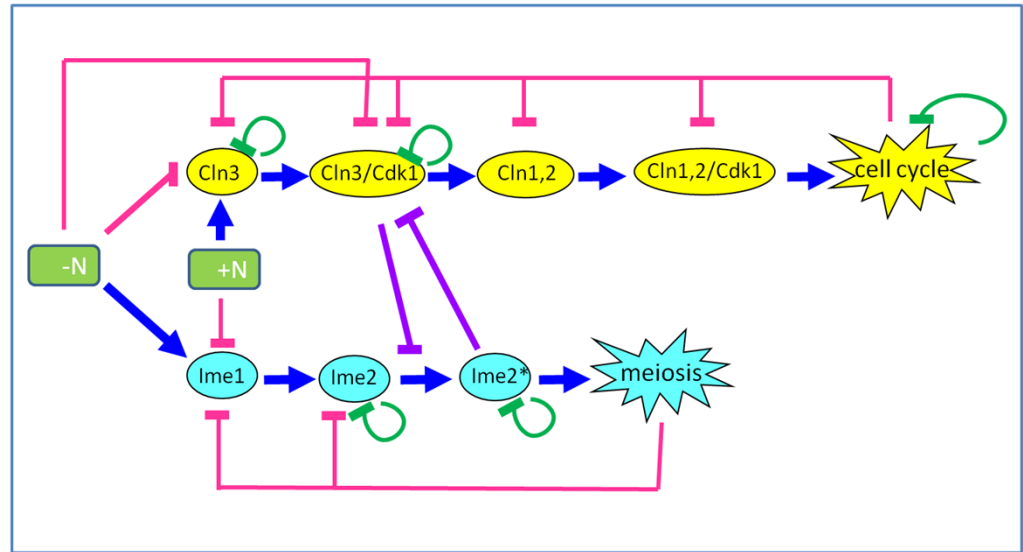
- We constructed a network that may explain the **bi-stable** switch between meiosis and cell-cycle.
- The hypothesized specific mechanism for negative effect between cell-cycle and meiosis agrees with biological data



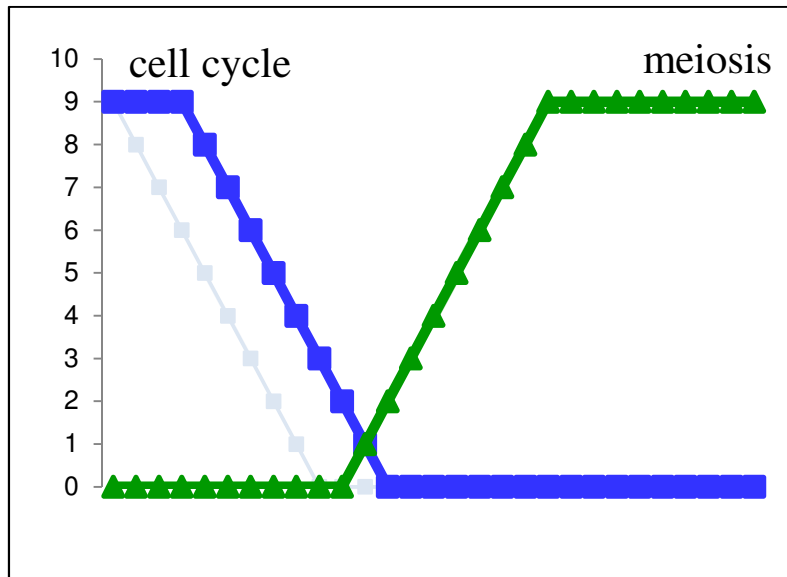
# Latest Results – 1

- Meiotic conditions  
( $-N=9$  ,  $+N=0$ )

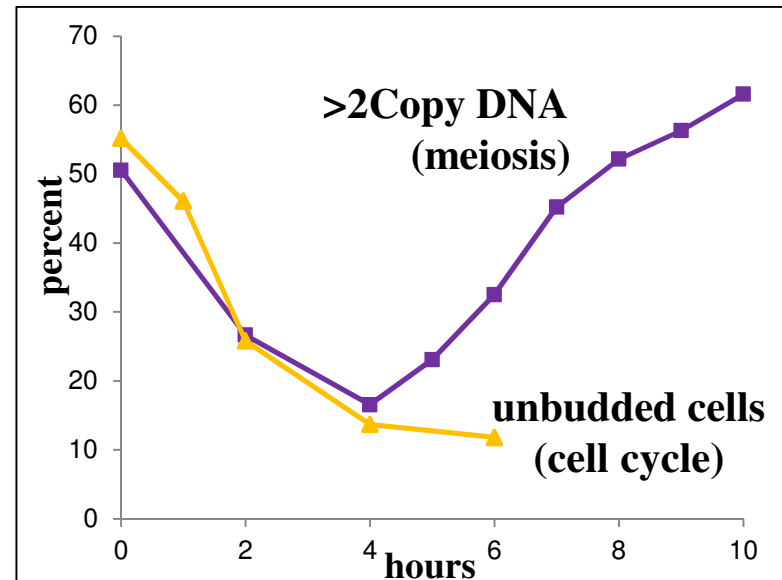
The shift from cell-cycle to meiosis is bi-stable



Simulation



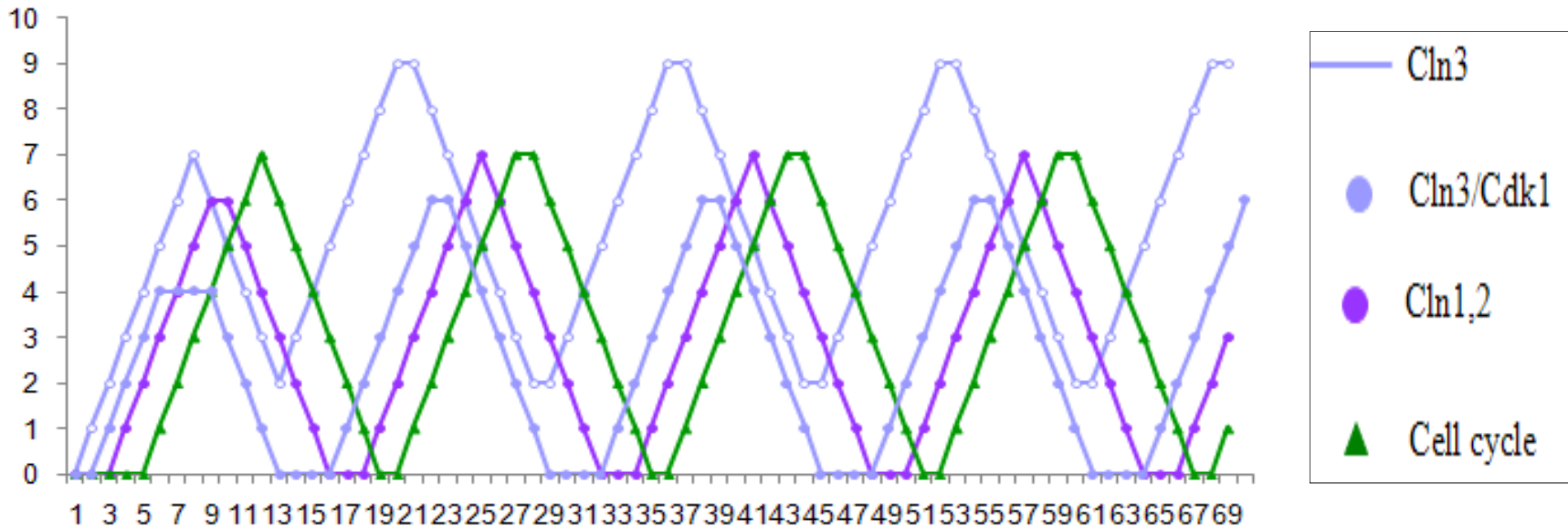
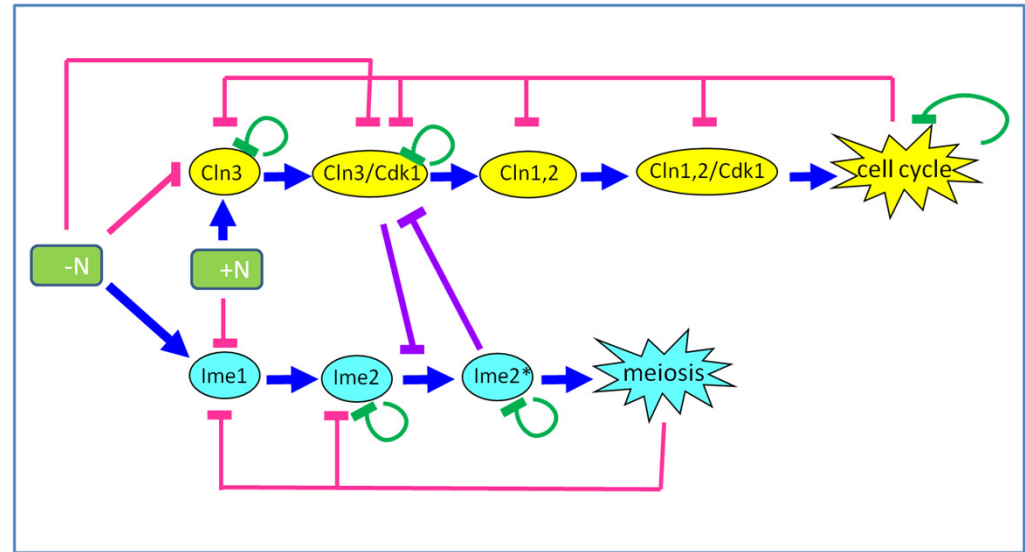
Lab



# Latest Results – 2

- Mitotic conditions  
 ( $-N=0$  ,  $+N=9$ )

Network exhibits  
cell-cycle



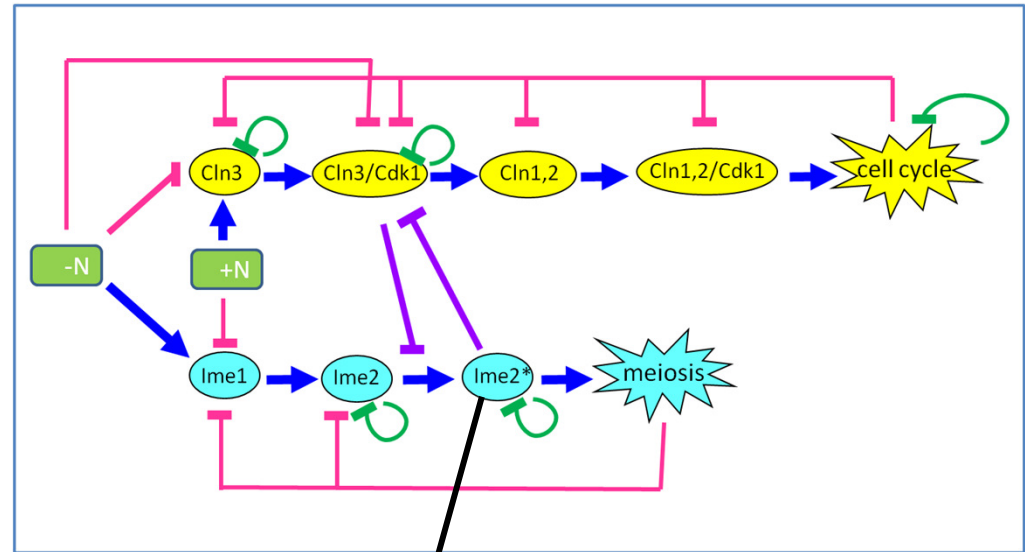
# Latest Results – 3

- Mutant

Mitotic conditions  
(-N=0 , +N=9)

**BUT**

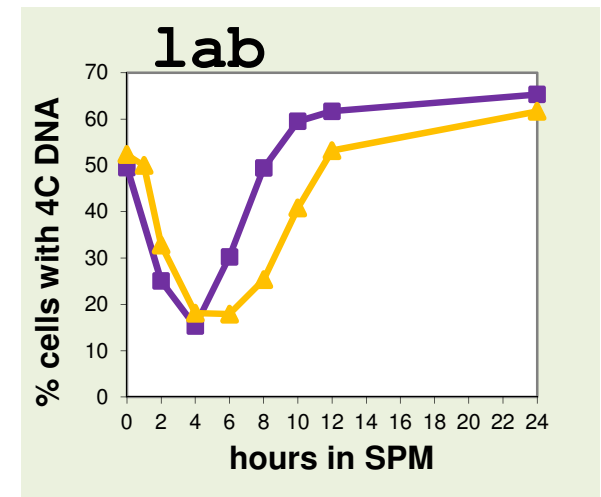
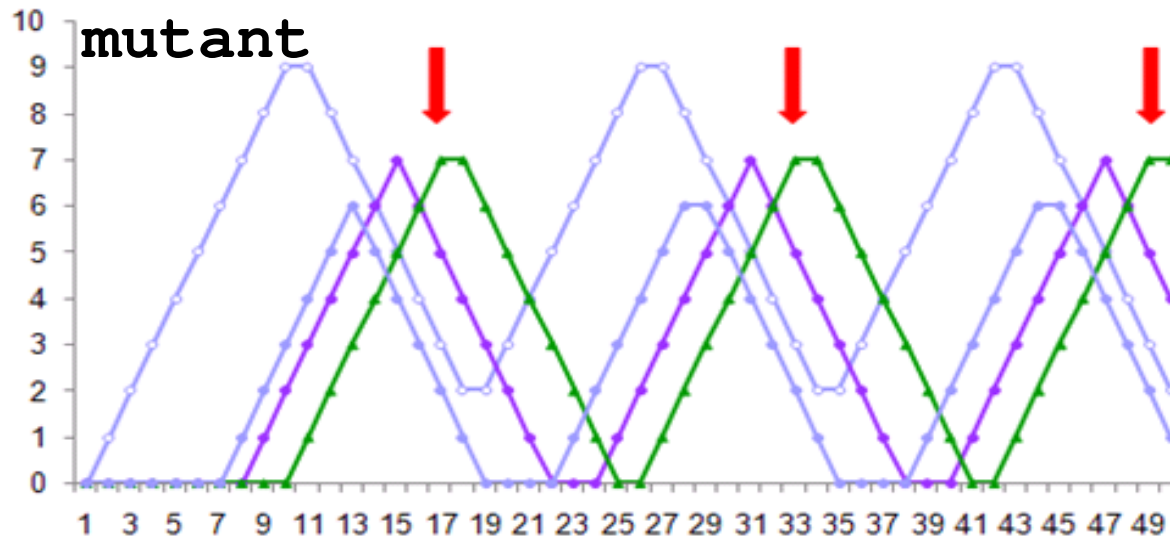
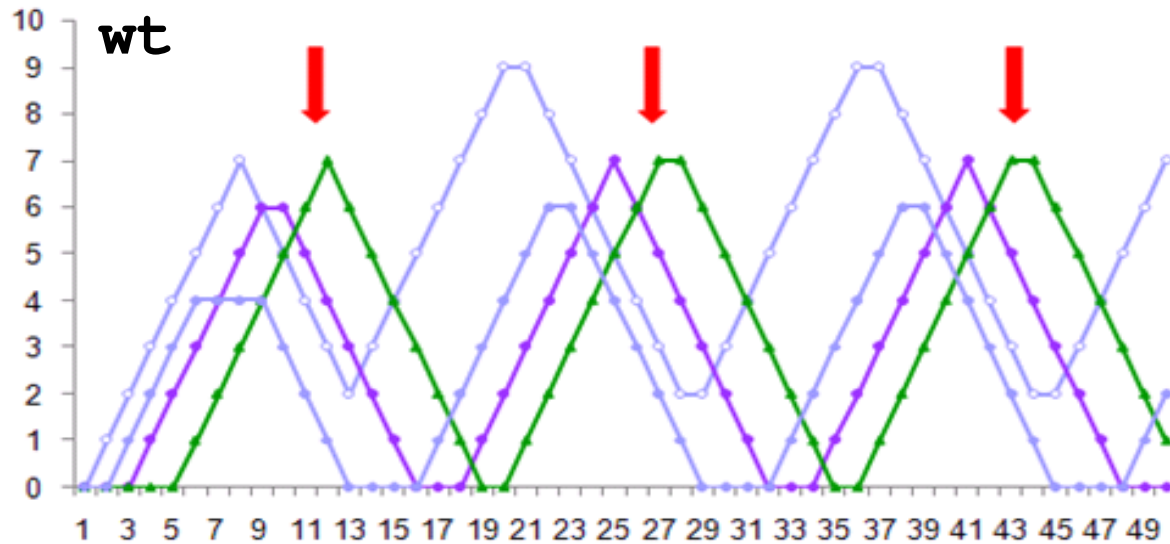
Ime2\* starts at 9



Initial state=9 (mutation)

- Result: **delay** in entry into the cell cycle,  
(simulation + lab)

# Latest Results – 3



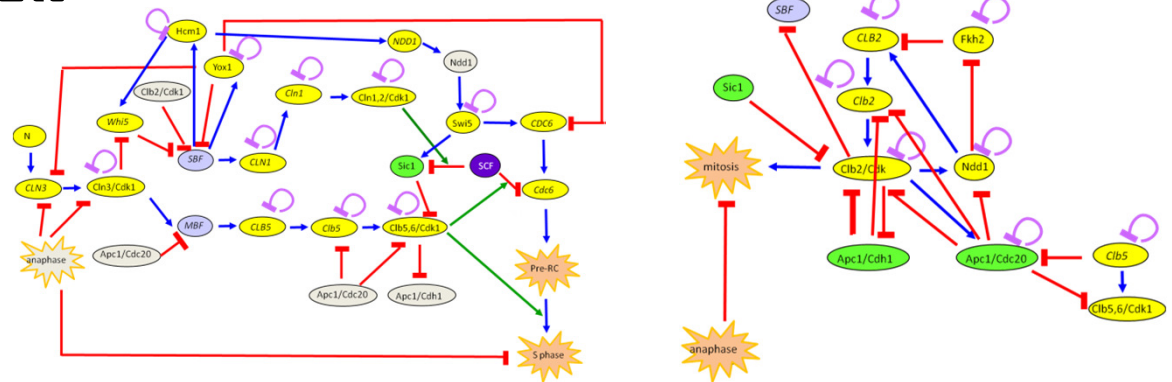
# Latest Results – 4

- These days we finish mapping the whole cell-cycle yeast network

- >50 nodes !!

- Oscillation

- Correct expression timings (according to various phases of cell-cycle: G1, S, G2, M)



# Lecture Outline

- ✓ The biological questions
- ✓ The Boolean model
- ✓ The discrete model + results
- ✓ Some recent extensions + recent results
- **Current/future work**

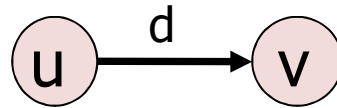
# Current and Future Work

## Networks:

- (very) New collaboration on Cop9 signalosome (w/ Prof. B.Chor and Prof. D.Chamovitz)

## The model:

- Time delay



u affects v after  $d(u,v)$  time steps

- More complex regulation effects

## GUI:

- Enhance existing tool with GUI

(with J.Hashoul and S.Nachum, Ort Braude College)



**Thank you**