Discrete modeling of regulatory networks

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1. Introduction

Mathematical modeling approaches

- Continuous
  - Ordinary differential equations
  - Partial differential equations
- Discrete
  - Logical networks
  - Petri nets
  - Process calculi
- Hybrid discrete/continuous
- Stochastic

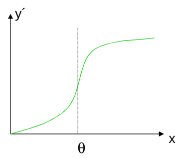
Differential vs. logical descriptions

- Differential
  - Continuous variables
  - State as a function of time
  - (Nonlinear) differential equations
- Logical
  - Discrete variables (small number of distinct levels)
  - Finitely many states, discrete switch
  - Logical equations, time delays

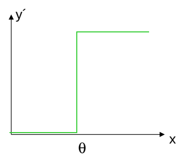
⇨ quantitative vs. qualitative modeling

Regulatory interactions

- Sigmoid function
  - x activates synthesis of y
  - threshold θ



- Approximation by a step function



- Idealization

Logical modeling

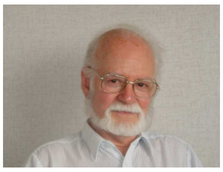
- Boolean vs. multi-valued logic
- Synchronous vs. asynchronous dynamics
- deterministic vs. non-deterministic

Early history (1960s, 1970s):

Sugita, Kauffman, Glass, Thomas, …

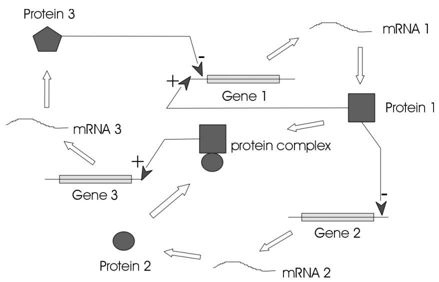
2. Kinetic logic

R. Thomas: Boolean formalization of genetic control circuits. J. Theor. Biol. 42, 565 – 583, 1973



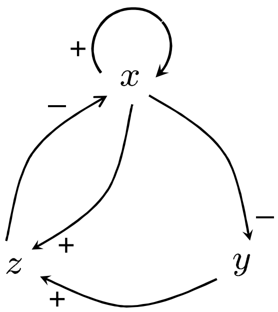
René Thomas (1928 – 2017)

Gene regulatory network



http://www.zaik.uni-koeln.de/AFS/

Interaction graph



Logical variables

- Genes X, Y, …
- Logical Variables X , Y , …
  - $X = 0$: gene X off
  - $X = 1$: gene X on
- Gene products x, y, …
- Logical variables x , y , …
  - $x = 0$: gene product x absent
  - $x = 1$: gene product x present

Logical functions

State of a gene depends on presence or absence of gene products

$$X = \Phi(x, y, z, \ldots)$$

$$Y = \Psi(x, y, z, \ldots)$$

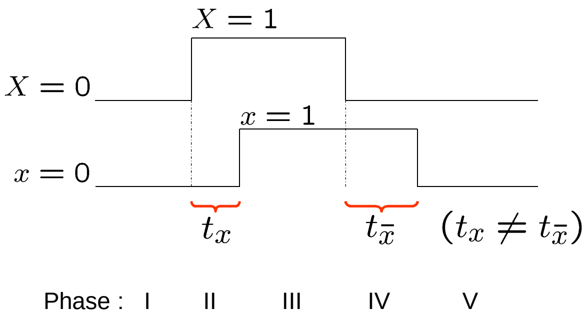
where Φ and Ψ are logical (Boolean) functions.

Examples

$$\begin{aligned} X &= \bar{z} \\ Y &= \bar{z} \wedge u \end{aligned}$$

- Gene X is on iff product z is absent
- Gene Y is on iff product z is absent and product u is present

Time delays

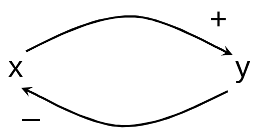


Logical network

- N = (V, F)
- V = $\{x_1, \ldots, x_n\}$ a set of n Boolean variables $x_i \in \{0,1\}$
- F = $\{\phi_1, \ldots, \phi_n\}$ a set of Boolean functions $\phi_i : \{0,1\}^n \rightarrow \{0,1\}$

Two-element negative circuit

- Suppose product x activates gene Y, and product y represses gene X.
- Interaction graph



Formalization

- N = (V,F)
- V = $\{x, y\}$
- F = $\{\Phi, \Psi\}$ with

$$X = \Phi(x, y) = \bar{y}$$
$$Y = \Psi(x, y) = x$$

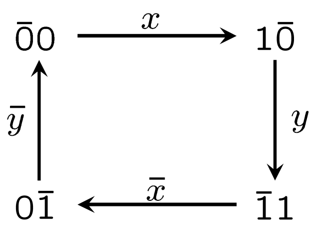
State table

x	y	X	Y
0	0	1	0
0	1	0	0
1	0	1	1
1	1	0	1

or

x	y
$\bar{0}$	0
0	$\bar{1}$
1	$\bar{0}$
$\bar{1}$	1

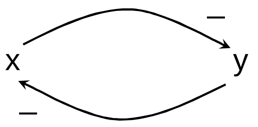
State transition graph



Periodic behavior

Two-element positive circuit

- Two genes each of which is repressed by the product of the other
- Interaction graph



Formalization

- N = (V,F)
- V = $\{x, y\}$
- F = {Φ, Ψ} with

$$X = \Phi(x,y) = \bar{y}$$
$$Y = \Psi(x,y) = \bar{x}$$

State table

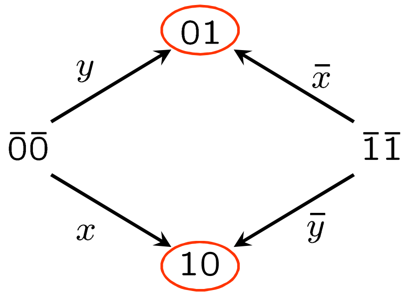
x	y	X	Y
0	0	1	1
0	1	0	1
1	0	1	0
1	1	0	0

or

x	y
$\bar{0}$	$\bar{0}$
0	1
1	0
$\bar{1}$	$\bar{1}$

Two stable states

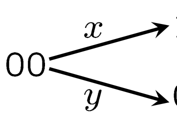
State transition graph



Multiple stable states

Remarks

- The actual transitions depend on the time delays, e.g.



if $t_x < t_y$

if $t_y < t_x$

- The transitions $00 \rightarrow 11$, with $t_x = t_y$, and $00 \leftarrow 11$, with $t_{\bar{x}} = t_{\bar{y}}$, are not included.

States

- State of the system

$$(x_1, \ldots, x_n) \in \{0,1\}^n$$

- Extended state

$$(x_1, \ldots, x_n, X_1, \ldots, X_n) \in \{0,1\}^{2n}$$

- $X_i = \Phi_i(x_1, \ldots, x_n)$: Effect of x_1, … ,x_n on the gene X_i that produces x_i.

Simplified notation (in the Boolean case)

$$(\tilde{x}_1, \ldots, \tilde{x}_n),$$

where $\tilde{x}_i$ means that we put a dash over x_i iff

$$x_i \neq \Phi_i(x_1, \ldots, x_n)$$

State transition graph

- Nodes — States

$$(x_1, \ldots, x_n) \in \{0,1\}^n$$

- Arcs — State transitions

$$(x_1, \ldots, x_{i-1}, \quad x_i, \quad x_{i+1}, \ldots, x_n)$$
$$\downarrow$$
$$(x_1, \ldots, x_{i-1}, \quad \Phi_i(x_1, \ldots, x_n), \quad x_{i+1}, \ldots, x_n),$$

if $x_i \neq \Phi_i(x_1, \ldots, x_n)$ (update only 1 variable)

⇒ non-deterministic dynamics

Stable states

A state

$$(x_1, \ldots, x_n) \in \{0,1\}^n$$

is stable

iff there is no transition to another state

$$(x'_1, \ldots, x'_n) \in \{0,1\}^n,$$

i.e.,

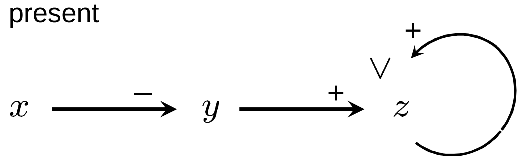
$$x_i = \Phi_i(x_1, \ldots, x_n),$$

for all i = 1,…,n.

3. Kinetic logic (II)
Selecting pathways

Three variable example

- Gene X expressed constitutively
- Gene Y expressed only in absence of product x
- Gene Z expressed if product y or z is present

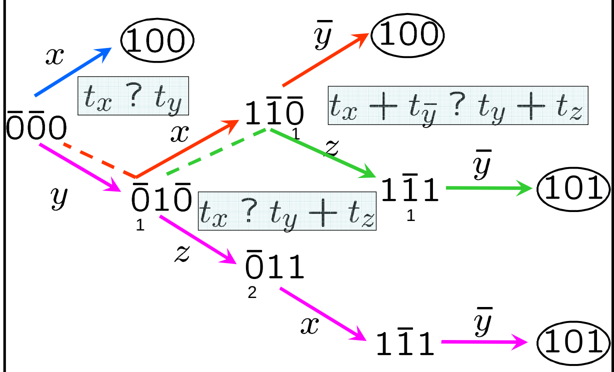


Logical description

$$X = 1$$
$$Y = \bar{x}$$
$$Z = y \vee z$$

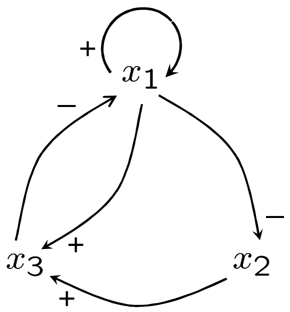
x	y	z	X	Y	Z
0	0	0	1	1	0
0	0	1	1	1	1
0	1	0	1	1	1
0	1	1	1	1	1
1	0	0	1	0	0
1	0	1	1	0	1
1	1	0	1	0	1
1	1	1	1	0	1

Pathways



4. Continuous and discrete models of gene regulation

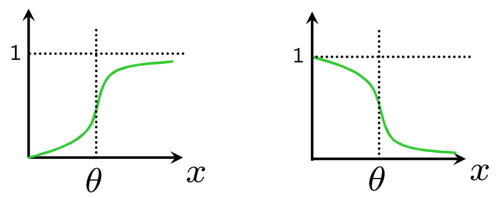
Interaction graph



Continuous model

- Real variables $x_1,\dots,x_n$:
  x_i gives the concentration of the product of gene i.
- Regulatory interaction: Activation/inhibition of x_i by x_j is effective only if x_j lies above a certain threshold θ_{ij}.

Hill functions



$$F_m^+(x,\theta) = \frac{x^m}{\theta^m + x^m}, \quad F_m^-(x,\theta) = \frac{\theta^m}{\theta^m + x^m}$$

Kinetics

- Effect of activation/inhibition of x_i by x_j described by kinetic parameter k_{ij}
- Degradation rate: k_{-i}

ODE model

For i = 1,…,n:

$$\frac{dx_i}{dt} = \sum_{j=1}^{n} k_{ij} F_m^{\alpha_{ij}}(x_j, \theta_{ij}) - k_{-i} x_i$$

where

$k_{ij} \geq 0, \alpha_{ij} \in \{+,-\}, k_{-i} > 0, m \geq 1.$

Piecewise linear ODE model

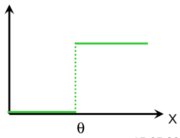
For m → ∞:

$$\frac{dx_i}{dt} = \sum_{j=1}^{n} k_{ij} F^{\alpha_{ij}}(x_j, \theta_{ij}) - k_{-i} x_i$$

where $k_{ij} \geq 0, \alpha_{ij} \in \{+,-\}, k_{-i} > 0,$

$$F^+(X,\theta) = \begin{cases} 1, & \text{if } X > \theta \\ 0, & \text{if } X < \theta \end{cases}$$

$$F^-(X,\theta) = 1 - F^+(X,\theta)$$



Discretization

Thomas 73, Snoussi 89

- Suppose gene i acts on n_i other genes
  - ⇨ n_i thresholds
    $0 < \theta^1 < \theta^2 < \ldots < \theta^{n_i}$
- Discretization operation

$$d_i : \mathbb{R}^+ \to \{0, \ldots, n_i\}$$

$$d_i(x) = \begin{cases} 0, & \text{if } 0 < x < \theta^1 \\ 1, & \text{if } \theta^1 < x < \theta^2 \\ \vdots & \\ n_i, & \text{if } \theta^{n_i} < x \end{cases}$$

Discrete model

$$X_i' = d_i\left(\sum_{j=1}^{n} K_{ij} F^{\alpha_{ij}}(X_j, \Theta_{ij})\right)$$

where

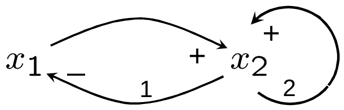
- $X_i, X_i' \in \{0, \ldots, n_i\}$ — discrete variables
- X_i' new value for X_i — discrete “derivative”
- $K_{ij} = k_{ij}/k_{-i} \geq 0$
- $\Theta_{ij} \in \{0.5, 1.5, 2.5, \ldots\}$ — discrete thresholds

Discrete dynamics

- State
  $X = (X_1, \ldots, X_n), \; X_i \in \{0, \ldots, n_i\}$
- State transitions
  $(X_1, \ldots, X_n) \longrightarrow (X_1, \ldots, X_i \pm 1, \ldots, X_n)$
  if $X_i' > X_i$ resp. $X_i' < X_i$.
- Only one variable is updated at a time.
- Several successor states are possible.
  - ⇨ Generalized kinetic logic

5. An illustrating example

Example



- $X_1 \in \{0,1\}$
- $X_2 \in \{0,1,2\}$
- Assume $\theta_{12} < \theta_{22}$, i.e., upon activation, X_2 acts first on X_1, then on itself.

Discrete and ODE model

$$\begin{aligned} X_1' &= d_1(K_{12}F^-(X_2, \Theta_{12})) \\ X_2' &= d_2(K_{21}F^+(X_1, \Theta_{21}) \\ &\quad +K_{22}F^+(X_2, \Theta_{22})) \end{aligned}$$

$$\begin{aligned} \frac{dx_1}{dt} &= k_{12}F_m^-(x_2, \theta_{12}) - k_{-1}x_1 \\ \frac{dx_2}{dt} &= k_{21}F_m^+(x_1, \theta_{21}) \\ &\quad +k_{22}F_m^+(x_2, \theta_{22}) - k_{-2}x_2 \end{aligned}$$

Logical parameters

$$\mathbf{K}_{ij} \stackrel{\text{def}}{=} d_i(K_{ij}) \in \{0, \ldots, n_i\}$$

$$\mathbf{K}_{ij+ij'} \stackrel{\text{def}}{=} d_i(K_{ij} + K_{ij'}) \in \{0, \ldots, n_i\}$$

(only finitely many possible values)

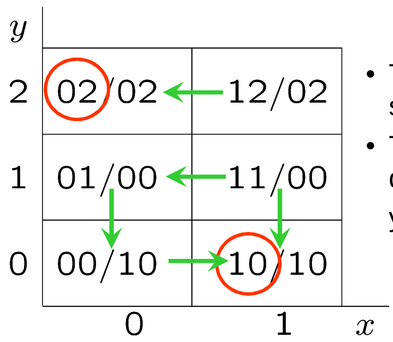
State table

X_1	X_2	X_1'	X_2'
0	0	$\mathbf{K}_{12}$	0
0	1	0	0
0	2	0	$\mathbf{K}_{22}$
1	0	$\mathbf{K}_{12}$	$\mathbf{K}_{21}$
1	1	0	$\mathbf{K}_{21}$
1	2	0	$\mathbf{K}_{21+22}$

States in phase space

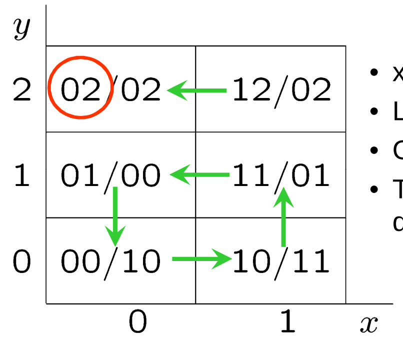
X_2		
2	$02/0\mathbf{K}_{22}$	$12/0\mathbf{K}_{21+22}$
1	$01/00$	$11/0\mathbf{K}_{21}$
0	$00/\mathbf{K}_{12}0$	$10/\mathbf{K}_{12}\mathbf{K}_{21}$
	0	1 X_1

$K_{12} = 1$, $\boldsymbol{K_{21} = 0}$, $K_{22} = 2$, $K_{21+22} = 2$



- Two stable states
- Two separate domains for $y > 1$ and $y \leq 1$

$K_{12} = 1$, $\boldsymbol{K_{21} = 1}$, $K_{22} = 2$, $K_{21+22} = 2$

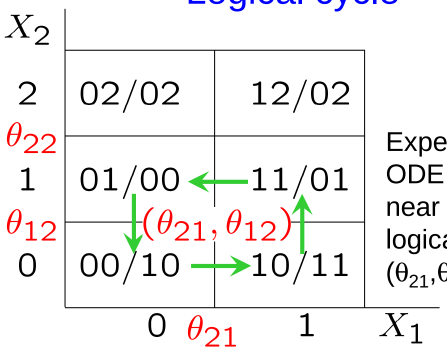


- x acts on y
- Logical cycle
- One stable state
- Two separate domains

Stable state

- Stable logical state : 02
- Corresponds to the region $0 < x_1 < \theta_{21}$ and $\theta_{22} < x_2$ in the phase plane, i.e., $F^+(x_1,\theta_{21}) = 0$, $F^+(x_2,\theta_{12}) = F^+(x_2,\theta_{22}) = 1$.
- Steady state equations
  $$\frac{dx_1}{dt} = k_{-1}x_1 = 0, \quad \frac{dx_2}{dt} = k_{22} - k_{-2}x_2 = 0$$
- Steady state
  $$x_1 = 0, \quad x_2 = k_{22}/k_{-2} = K_{22}$$

Logical cycle



Expect focus in ODE description near singular logical state $(\theta_{21},\theta_{12})$

Parameter inference

$$\mathbf{K}_{ij} = d_i(K_{ij}) = d_i(k_{ij}/k_{-i})$$

➡ logical parameters yield inequalities to be satisfied by ODE parameters

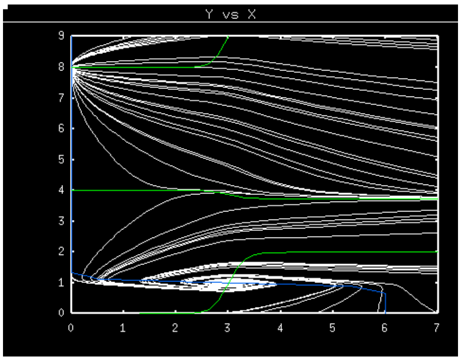
Example

- Suppose $k_{-1} = 2$, $k_{-2} = 1$
- Suppose $\theta_{12} = 1$, $\theta_{21} = 3$, $\theta_{22} = 4$
- From $\mathbf{K}_{12} = 1$, derive $K_{12} = k_{12}/k_{-1} > \theta_{21} = 3$
  Choose $K_{12} = 6$, i.e., $k_{12} = 12$
- From $\mathbf{K}_{22} = 2$, derive $K_{22} = k_{22}/k_{-2} > \theta_{22} = 4$
  Choose $K_{22} = 8$, i.e., $k_{22} = 8$
- From $\mathbf{K}_{21} = 1$, derive $1 = \theta_{21} < K_{21} < \theta_{22} = 4$,
  Choose $K_{21} = k_{21}/k_{-2} = 2$, i.e., $k_{21} = 2$.

ODE model

- Stable logical state : 02
  Expected location for steady state:
  $(0, k_{22}/k_{-2}) = (0, 8)$
- Logical cycle 00 → 10 → 11 → 01 → 00
  Expected focus near
  $(\theta_{21}, \theta_{12}) = (3, 1)$
- Separatrix close to $y = \theta_{22} = 4$

Illustration



6. Multistationarity and stable periodicity

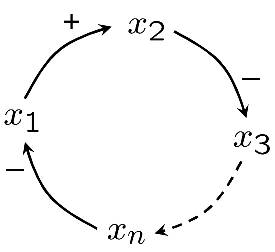
Biological properties

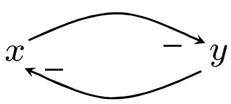
- Multistationarity (Differentiation)
- Stable periodic behavior (Homeostasis)
- Three models
  - – ODE
  - – piecewise linear ODE
  - – discrete
- What information on the ODE/PL model can be obtained from the PL/discrete one ?

Steady states and periodicity

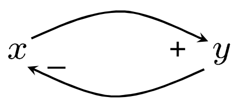
- Asymptotically stable steady states in ODE model are related to stable logical states in the discrete model (Snoussi 89).
- Stable periodicity in ODE model is related to cycles in the discrete model (Snoussi/Thomas 93).
- In a logical cycle, the equivalent of the focus in the ODE description is located at the junction of logical states, i.e., at threshold values.
- Logical description should take into account threshold values ⇨ singular logical states

Positive and negative circuits





Positive 2-circuit



Negative 2-circuit

Sign of circuit =
Product of signs of arcs

Thomas´ conjectures

Thomas´81

1. A positive circuit in the interaction graph is a necessary condition for multistationarity.
2. A negative circuit in the interaction graph is a necessary condition for stable periodic behavior.

Proofs exist in various particular cases.

Conclusion

- Modeling levels
  - ODE
  - piecewise linear ODE
  - discrete
- Biological properties
  - Multistationarity
  - Stable periodicity

Conclusion II

- Logical parameters (only finitely many combinations) ⇒ inequalities to be satisfied by kinetic parameters
- Stable logical state ⇒ asymptotically stable steady state
- Logical cycle ⇒ periodic behavior (stable/unstable focus, limit cycles)

References

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- R. Thomas and R. D´Ari: *Biological Feedback*. CRC Press, 1990
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