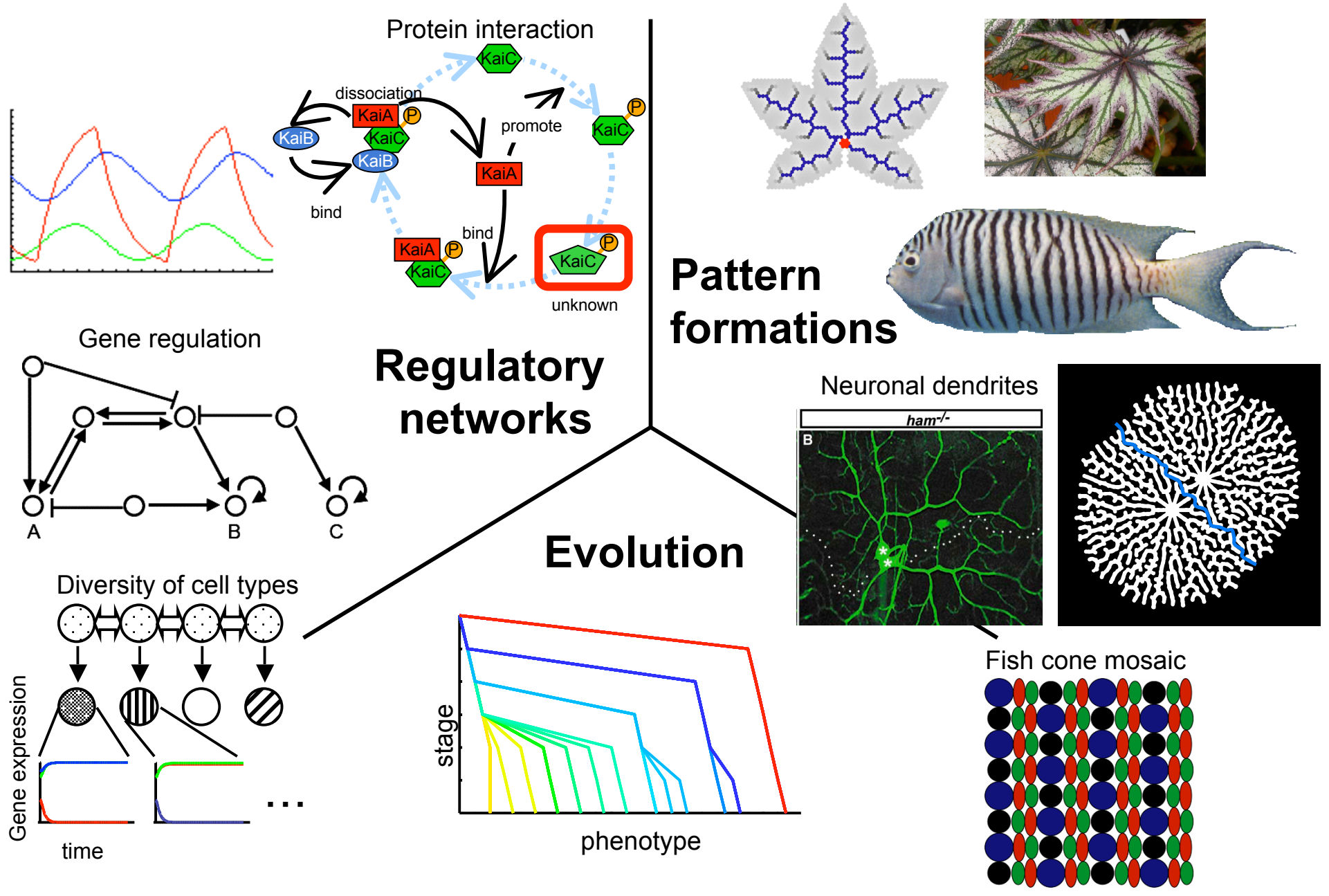# Structure of regulatory networks and dynamics of bio-molecules

Atsushi Mochizuki

Theoretical Biology Laboratory
RIKEN Advanced Science Institute/

mochi@riken.jp

Mathematical studies for biological phenomena

Protein interaction

KaiC

dissociation

KaiA P
KaiC

KaiB

bind

promote

KaiC P

KaiA

KaiA P
KaiC

bind

KaiC P

unknown

Pattern formations

Regulatory networks

Gene regulation

A   B   C

Evolution

Diversity of cell types

Gene expression

time

...

stage

phenotype

Neuronal dendrites

ham^{-/-}

B

Fish cone mosaic

Why mathematical modeling?

- Understanding genes → higher-order phenomena

- Development = spatio-temporal dynamics

- Complex interactions between many genes
  → Exceed human ability of information processing

Many biological phenomena can be understood by mathematical/computational methods.

Two policies

(1) Studying concrete biological phenomena.

    Collaborations with experimental biologists
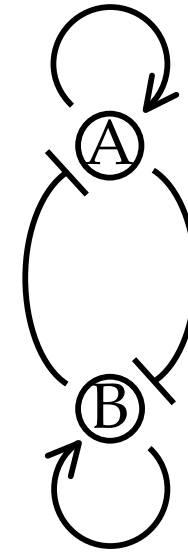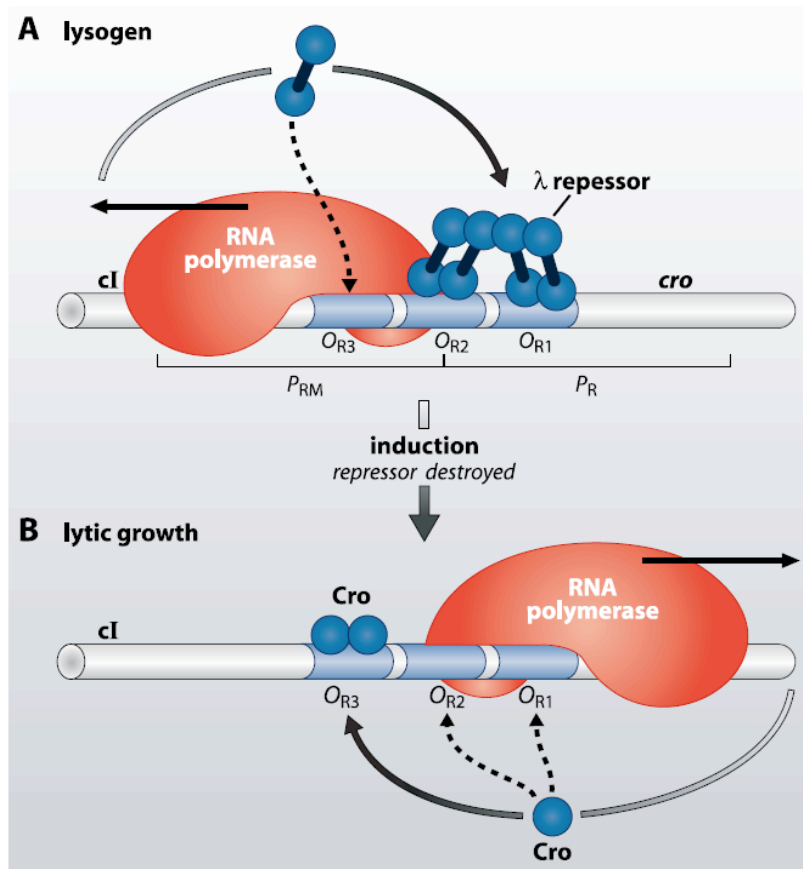
    Deriving testable predictions

    →Toward future biology that will progress by repeats of predictions and tests.

(2) Developing biology-oriented original theory.

    At present, we are just applying techniques developed in mathematical science and physics.

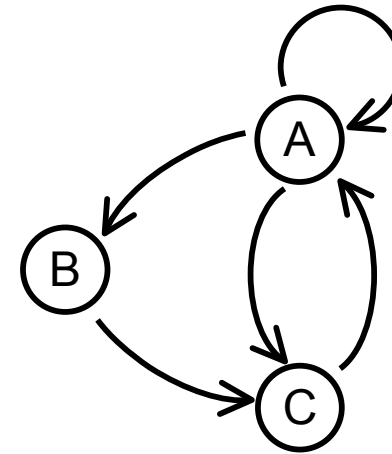# understanding functions from diagram of regulation

λphage

Two exclusive positive feedbacks
→bi-stability in gene activation
→Two phenotypes

Modeling regulatory networks by ODE system
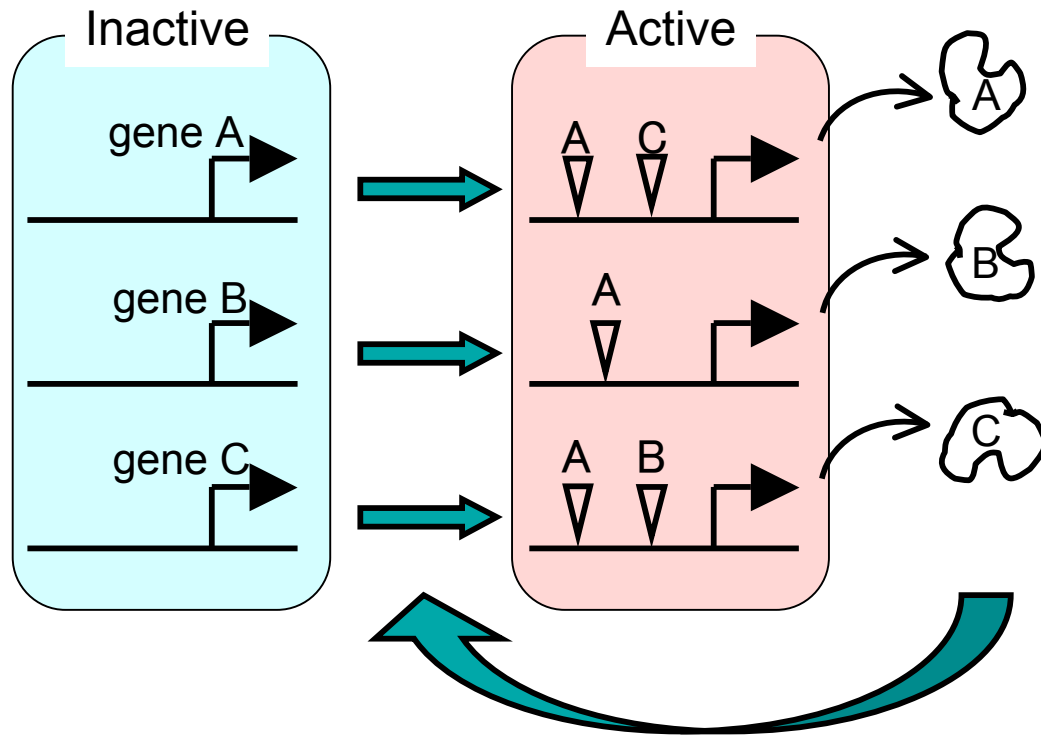
Regulatory network

Dynamics of protein concentration
(or gene activity)

$$\dot{u}_A = F_A\left(u_A, u_C\right) - du_A$$

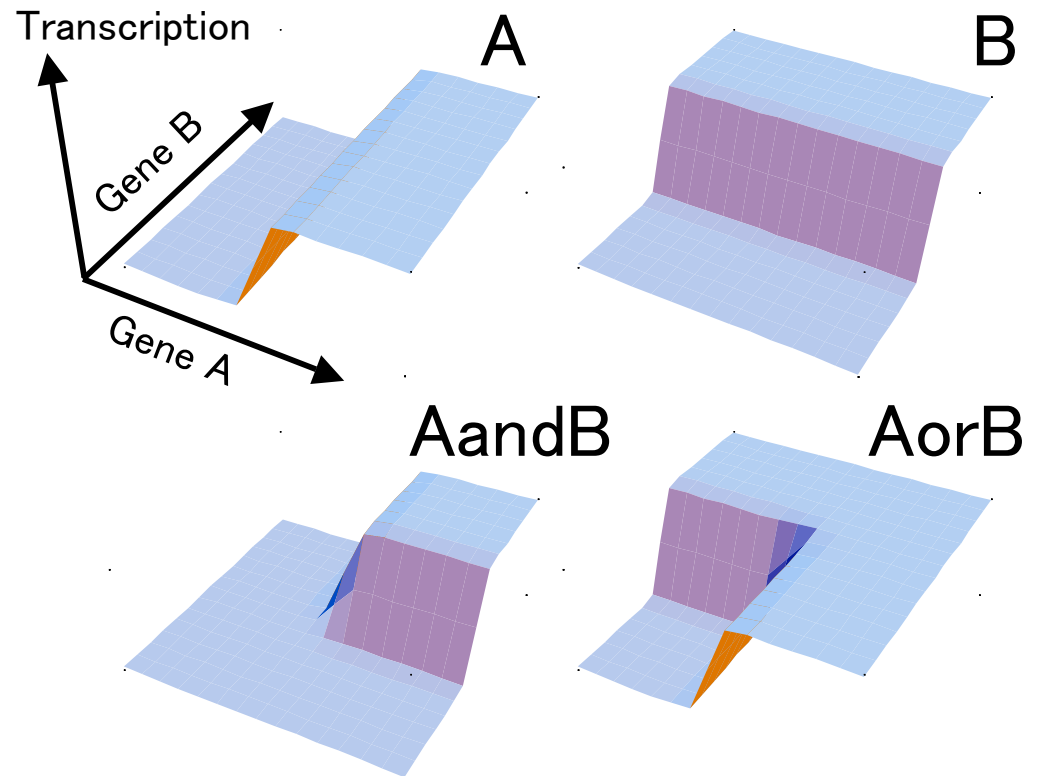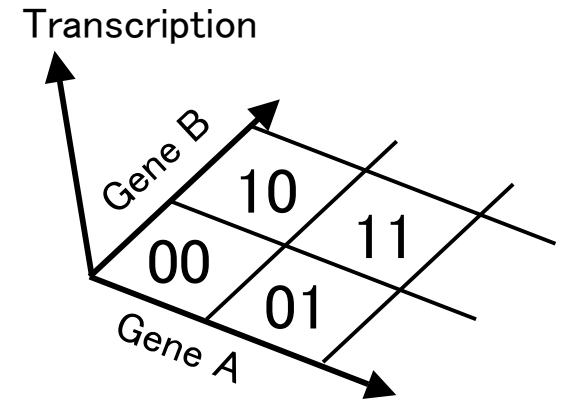$$\dot{u}_B = F_B\left(u_A\right) - du_B$$

$$\dot{u}_C = F_C\left(u_A, u_B\right) - du_C$$

Piecewise Linear Model（Boolean transcription）

A　B

C B D

D　E

......

...
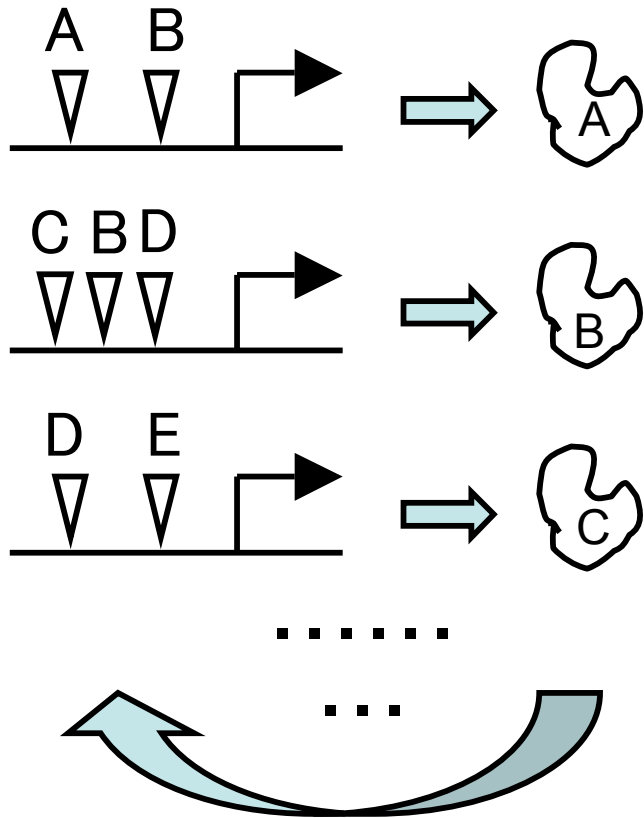
$$\dot{u}_i = F_i(\mathbf{v}) - du_i$$

$$(i = A, B, ...)$$

Transcription functions
（Examples of 2 genes）

Transcription

Gene B

Gene A

	10		11
00		01	

Transcription

Gene B

Gene A

A

B

AandB

AorB

2 genes system

Expression level

Gene B protein

Gene A protein
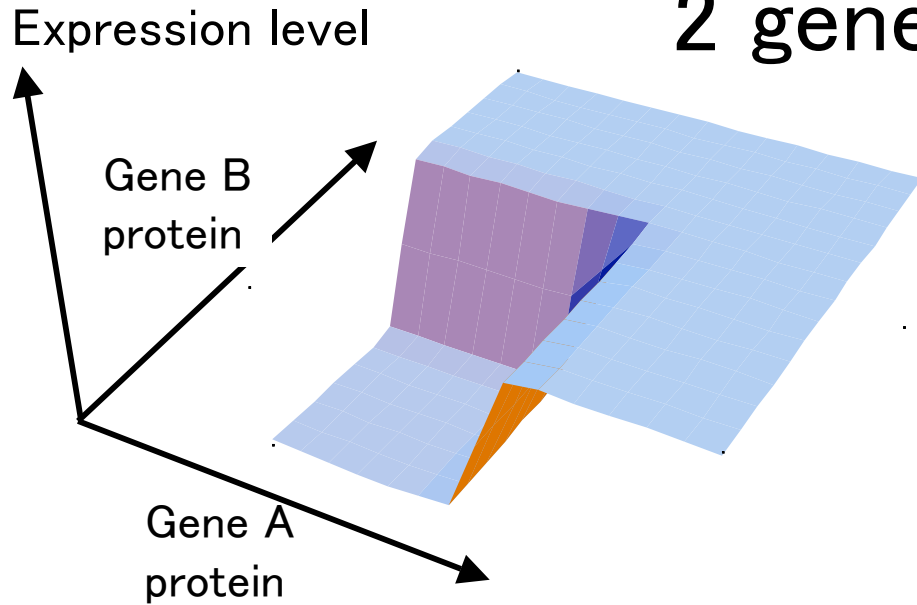
$$F_i(\mathbf{v}) = \sum_{s=00}^{11} K_{i,s}(\mathbf{v})\, reg(s,i)$$

Summation of $K_{i,s}$ with the weight $reg(s,i)$.

$K_{i,s}$ takes about 1 only in each domain.

| 00 | 01 | 10 | 11 |
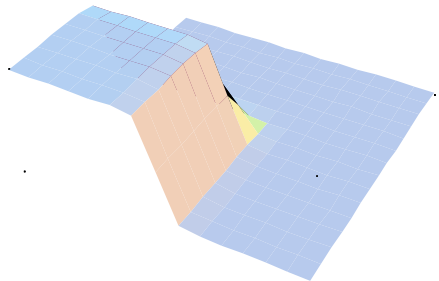
$$K_{i,00}(\mathbf{v}) = \frac{1}{1+\exp\left[\lambda_A\left(v_A - T_{iA}\right)\right]}$$
$$\times \frac{1}{1+\exp\left[\lambda_B\left(v_B - T_{iB}\right)\right]}$$
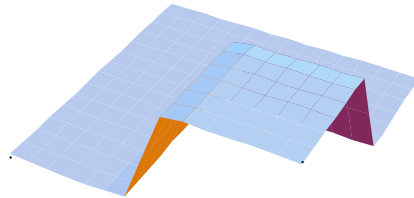
$$K_{i,01}(\mathbf{v}) = \frac{1}{1+\exp\left[-\lambda_A\left(v_A - T_{iA}\right)\right]}$$
$$\times \frac{1}{1+\exp\left[\lambda_B\left(v_B - T_{iB}\right)\right]}$$
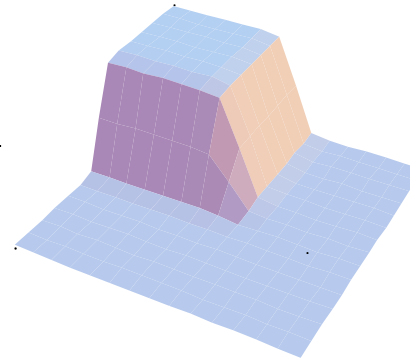
$$K_{i,10}(\mathbf{v}) = \frac{1}{1+\exp\left[\lambda_A\left(v_A - T_{iA}\right)\right]}$$
$$\times \frac{1}{1+\exp\left[-\lambda_B\left(v_B - T_{iB}\right)\right]}$$
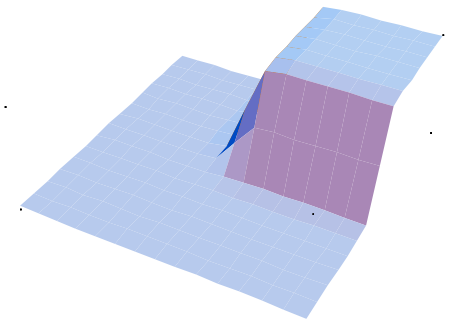
$$K_{i,11}(\mathbf{v}) = \frac{1}{1+\exp\left[-\lambda_A\left(v_A - T_{iA}\right)\right]}$$
$$\times \frac{1}{1+\exp\left[-\lambda_B\left(v_B - T_{iB}\right)\right]}$$

例えば…
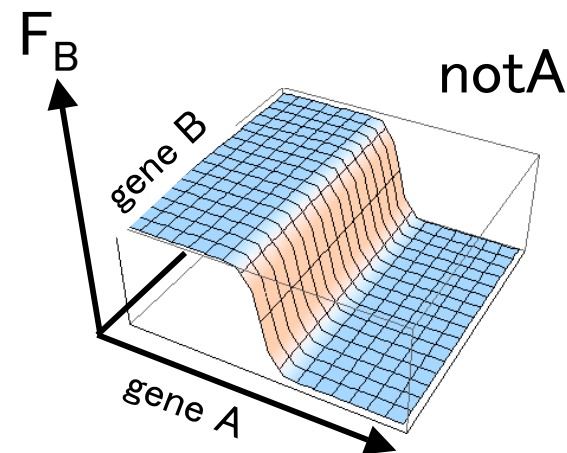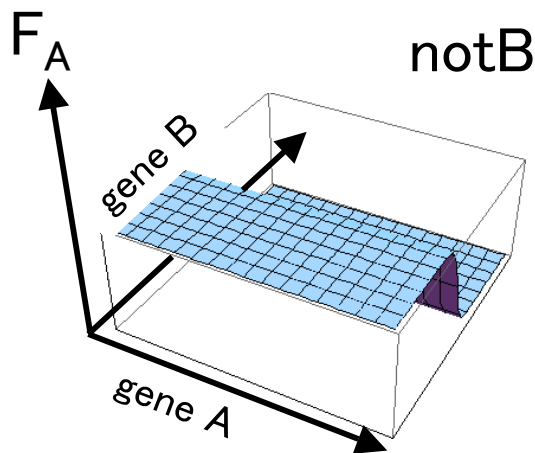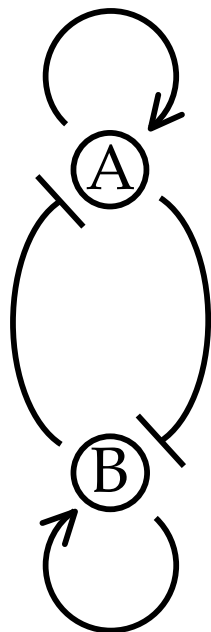
$$\dot{u}_A = F_A\left(u_A, u_B\right) - du_A$$

$$\dot{u}_B = F_B\left(u_A, u_B\right) - du_B$$
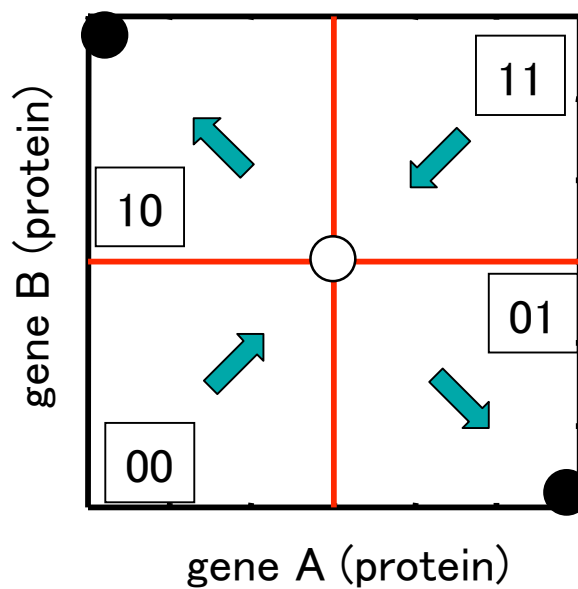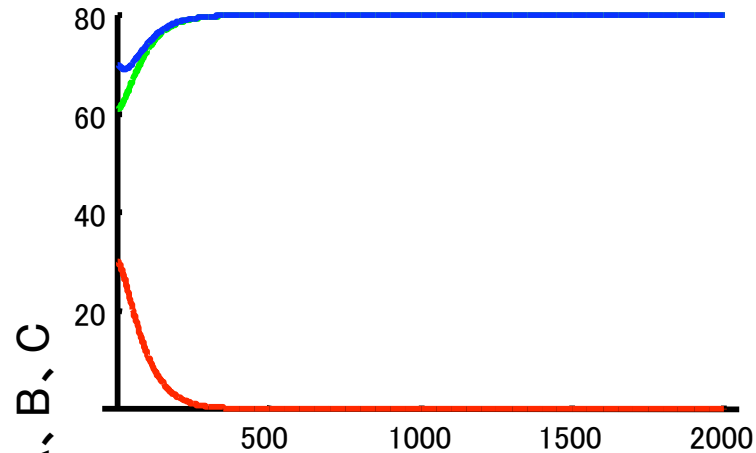
Equilibria

$$F_A\left(u_A, u_B\right) = du_A$$

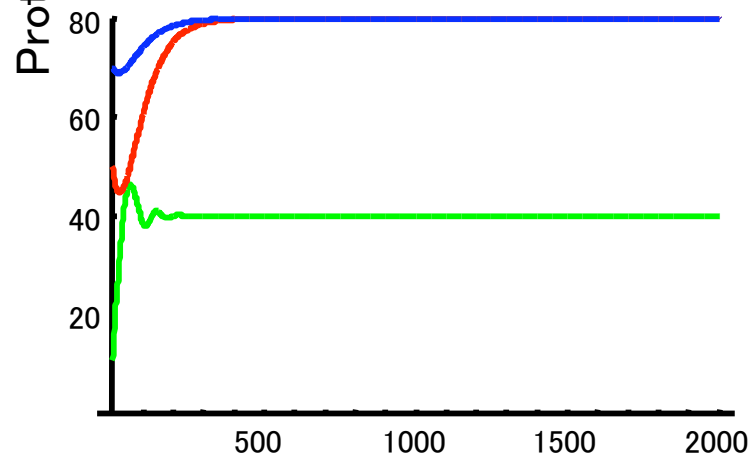$$F_B\left(u_A, u_B\right) = du_B$$

(0,1), (1,0), (0.5,0.5)

(normalized values)

Two types of steady states

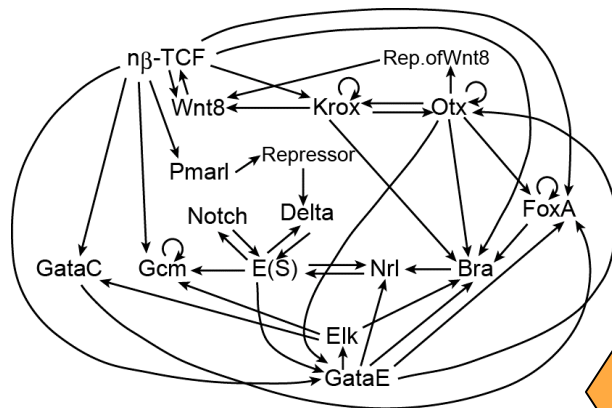Regular stationary point (RSP)
 ・Each gene is either on(1) or off(0).

Singular stationary point (SSP)
 ・Each gene is either on(1) or off(0)
  or intermediate(0.5).

Structure and dynamics

Topological structure of regulatory linkage

$$\dot{x}_{Wnt8} = f_{Wnt8}\left(n\beta TCF, Krox\right) - x_{Wnt8}$$

$$\dot{x}_{Krox} = f_{Krox}\left(n\beta TCF, Krox, Otx\right) - x_{Krox}$$

…

Regulatory links =
Argument set of each
differential equation

Dynamics of gene activities

Gene activities

Time t

Higher-order phenomena

- Cell differentiation

Cells

Differentiated cells
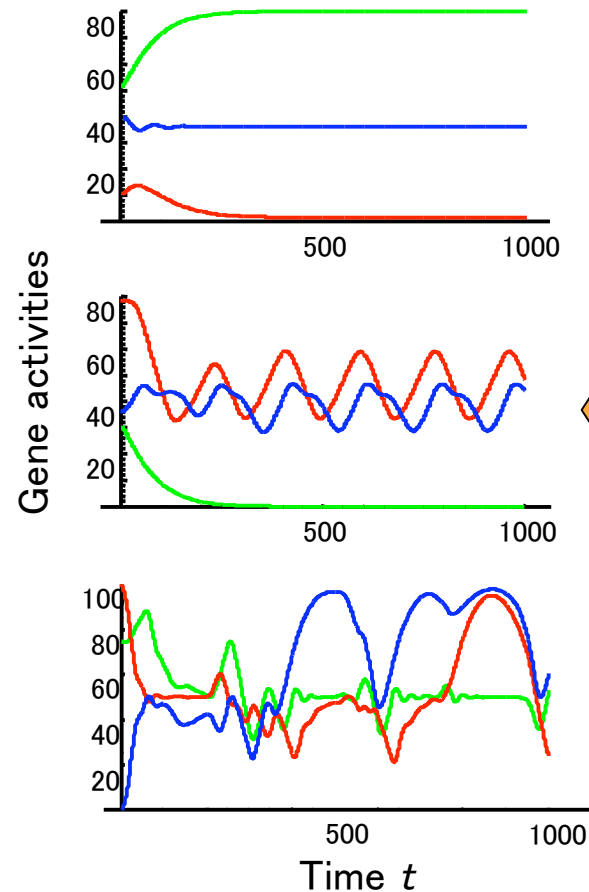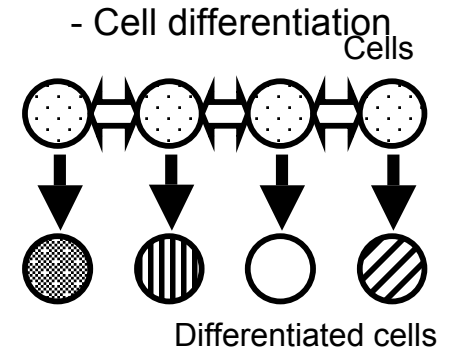
- Pattern formation
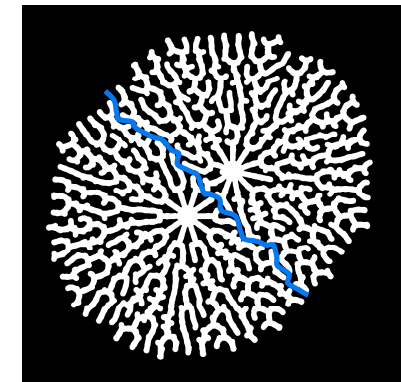
- Circadian rhythms

**Regulatory network is the origin of the complex dynamics,
but its structure also restricts the dynamics.**

What is the origin of cell diversity?

from E. Davidson et al. (2002)

· cell states ≒ gene expression patterns
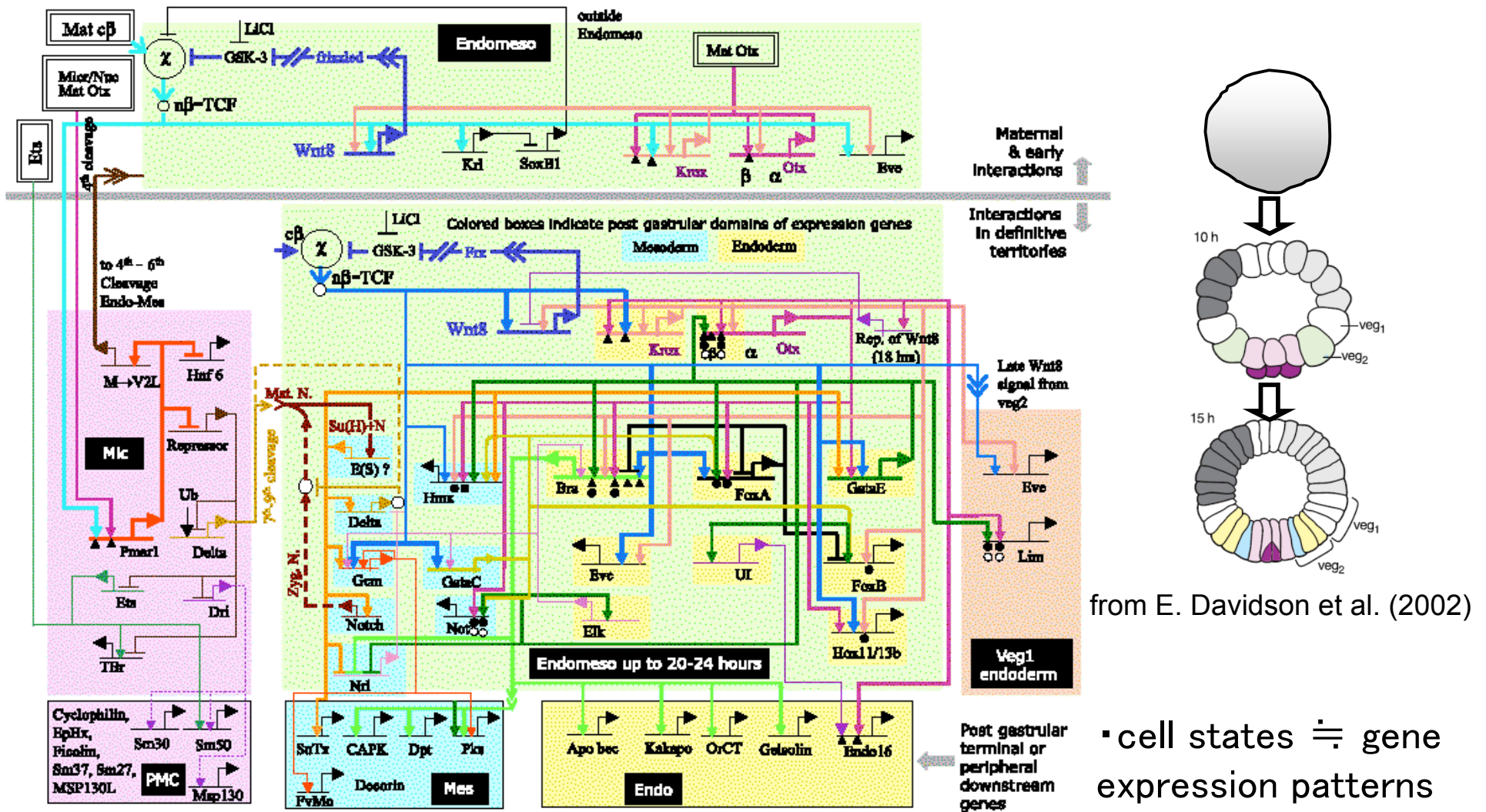
How diversities of gene expression patterns are generated from gene regulatory networks?

Topology of regulatory networks
⇔
Diversity of steady states
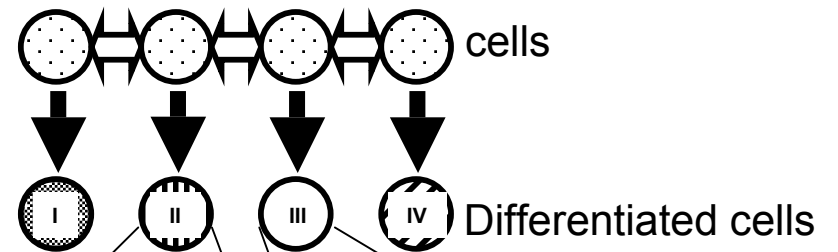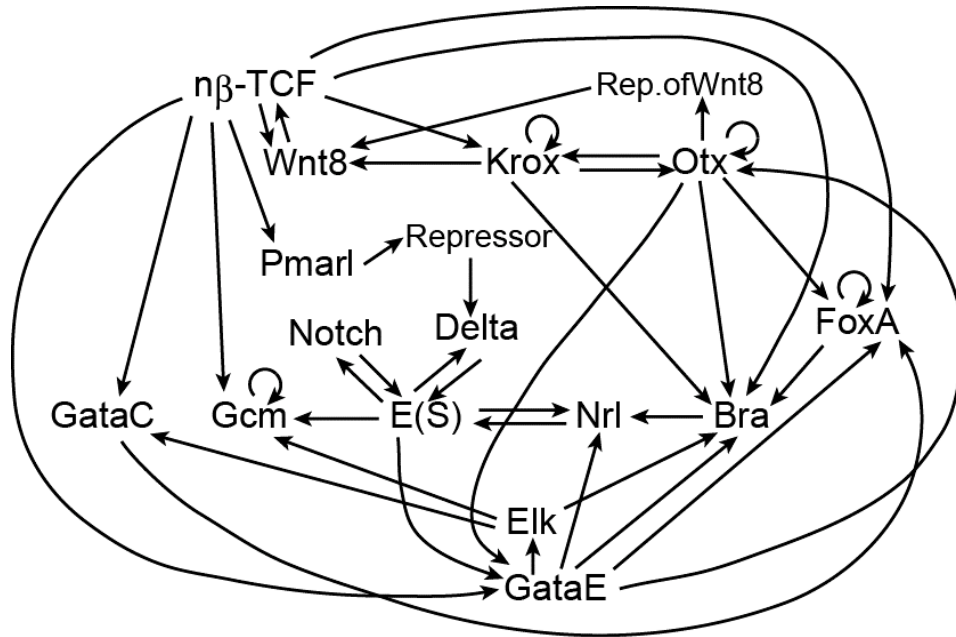
Focus on steady states

$$F_i(\mathbf{u}) = u_i$$

$$(i = A, B, ...)$$
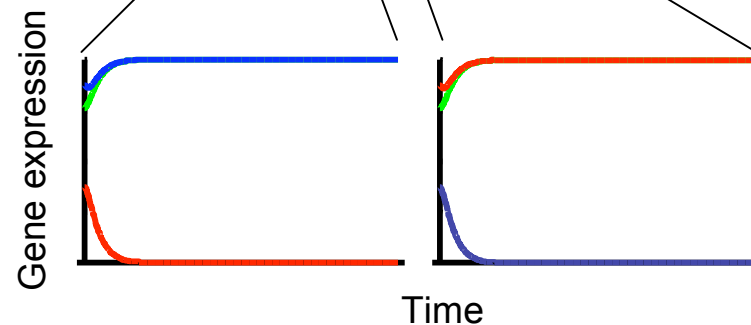
"unique input → unique output"
(1) incompatibility

any function
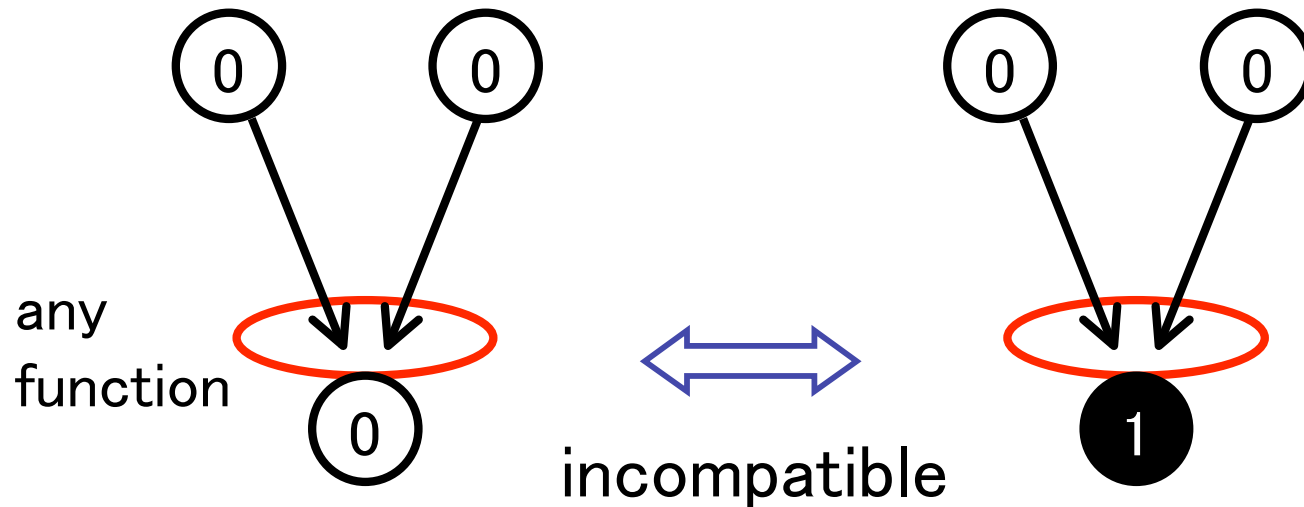
incompatible

Determine possible steady states from linkage information

Linkage -> Compatible steady states

Ex.
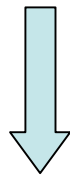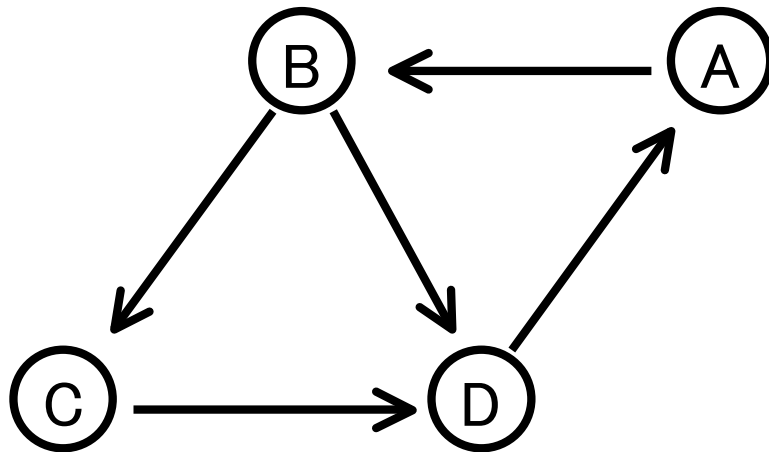
2 states
possible

| Case 1 | (0,0,0,0), (1,1,0,1) |
| Case 2 | (0,0,0,0), (1,1,1,1) |

ABCD
0000	steady state ○	○	○
0001	×	×	×
0010	×	○	○
0011	×	×	×
0100	×	×	×
0101	×	×	×
0110	×	×	×
0111	×	×	×
1000	×	×	×
1001	×	×	×
1010	×	×	×
1011	×	×	×
1100	×	×	×
1101	○	steady	×
1110	×	×	×
1111	○	×	steady

× : incompatible domain

Calculation of domains which are incompatible from (0000)

Two rules for calculating union.

$$\left(u_1,...,0,...,u_N\right)\cup\left(u_1,...,1,...,u_N\right)=\left(u_1,...,-,...,u_N\right)$$

$$\mathbf{u}=\left(u_1,u_2,...,u_N\right) \qquad u_j=\{0,1,-\}$$

$$\left(u_1,...,0,...,0,...,u_N\right)\cup\left(u_1,...,-,...,1,...,u_N\right)\supset\left(u_1,...,0,...,-,...,u_N\right)$$

Each gene determines compatible
region in N-dimensional binary space.
Taking union of them.
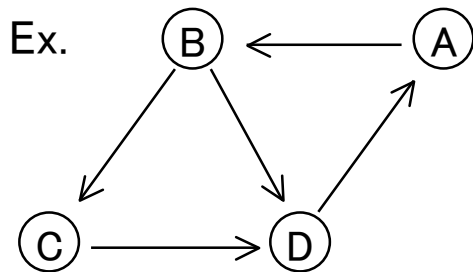
Ex.

$$\mathbf{U}^{0*}=\mathbf{U}^0\cup\left(0,0,...,0\right)$$

$$\mathbf{U}^{0*}=\begin{pmatrix} 1 & - & - & 0 \\ 0 & 1 & - & - \\ - & 0 & 1 & - \\ - & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 \end{pmatrix}$$

Ex.

$$\supseteq\begin{pmatrix} 1 & - & - & 0 \\ 0 & 1 & - & - \\ - & 0 & 1 & - \\ 0 & 0 & 0 & - \end{pmatrix}$$

$$\mathbf{U}^0=\left(1--0\right)\cup\left(01--\right)\cup\left(-01-\right)\cup\left(-001\right)$$

$$\supseteq\begin{pmatrix} 1 & - & - & 0 \\ 0 & 1 & - & - \\ 0 & 0 & - & - \end{pmatrix}$$

$$\supseteq\begin{pmatrix} 1 & - & - & 0 \\ 0 & - & - & - \end{pmatrix}$$

$$\supseteq\left(0 \quad - \quad - \quad -\right)$$
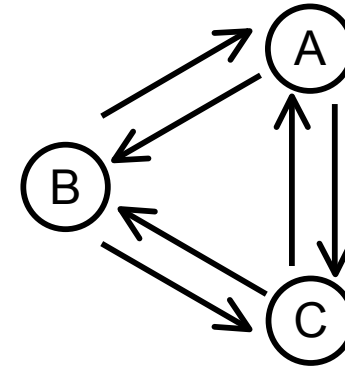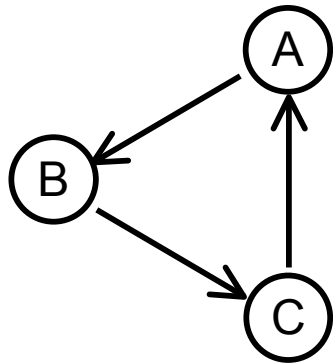
The proximity region of a steady state where there should be no other steady states.
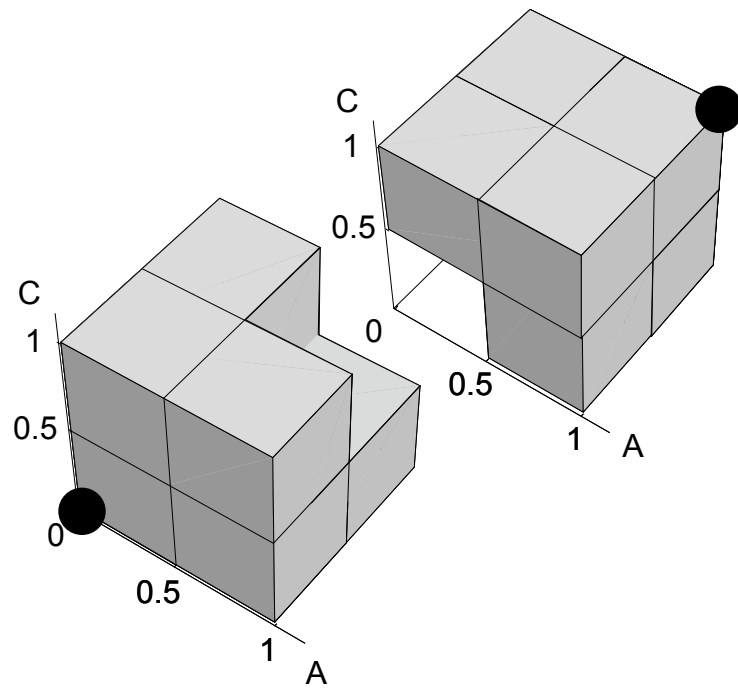
Maximally 2 steady states
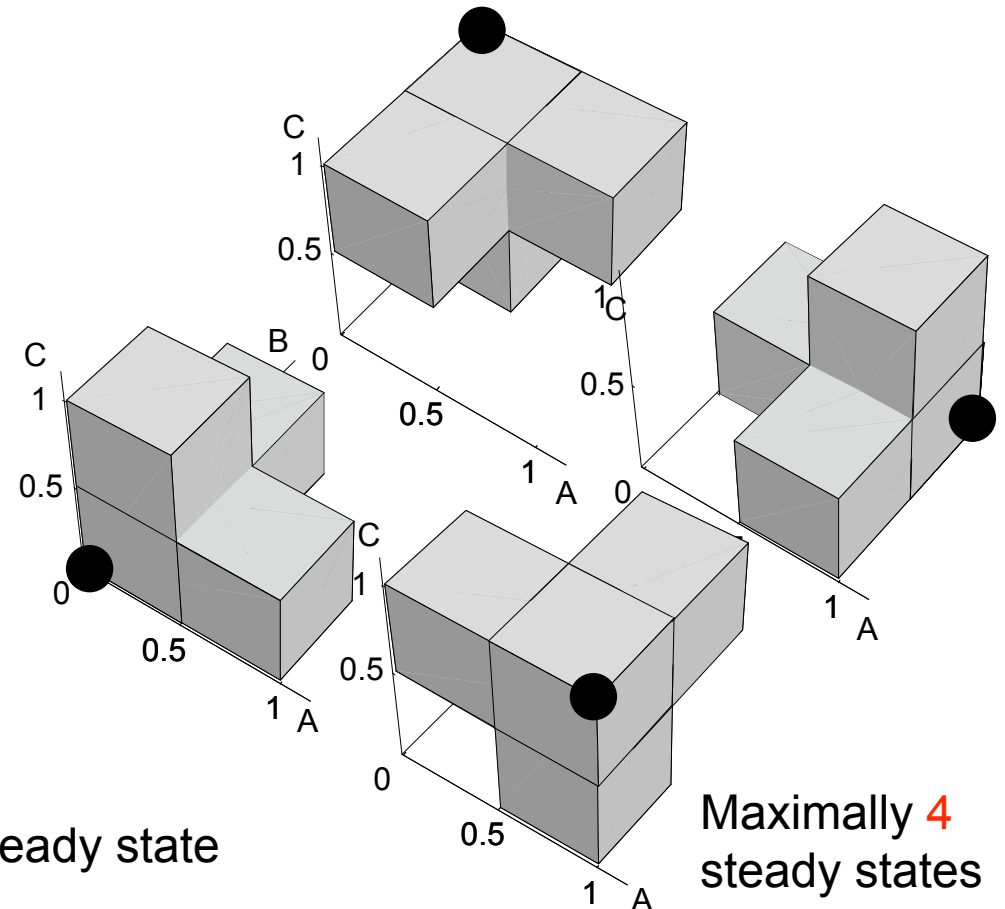
Maximally 4 steady states

● : steady state

Structure of regulatory network and maximum number of steady states
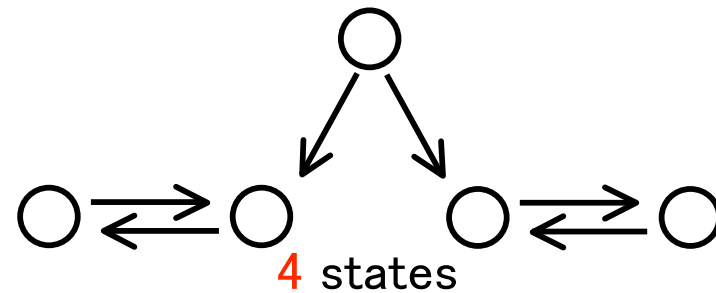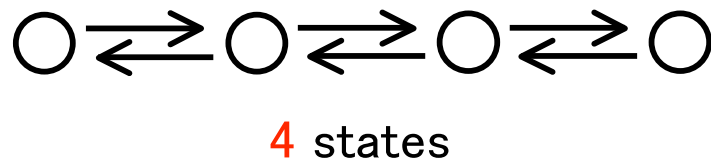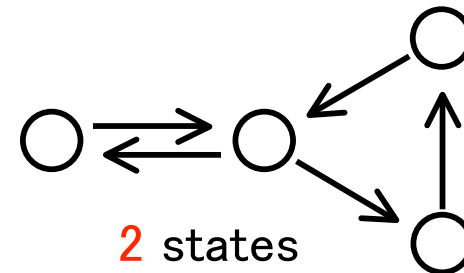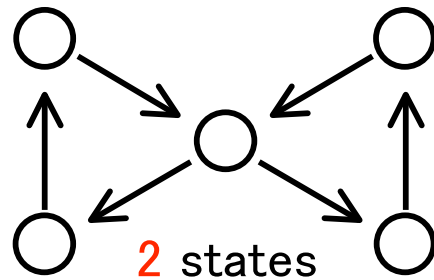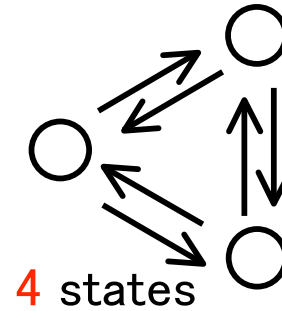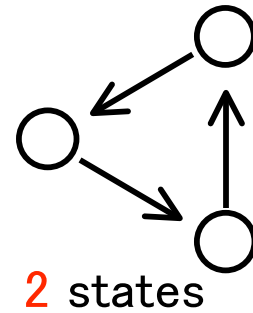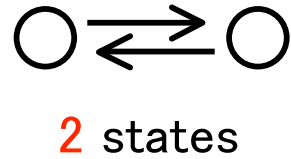
General rules of relation between structure of regulatory networks and diversity of steady states
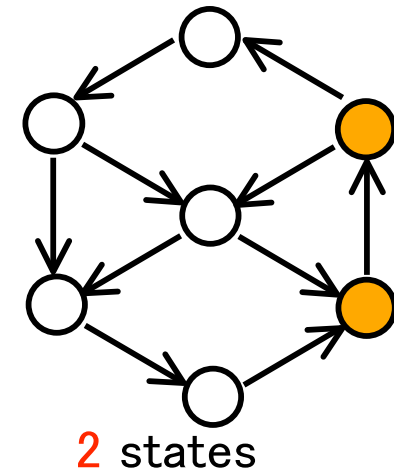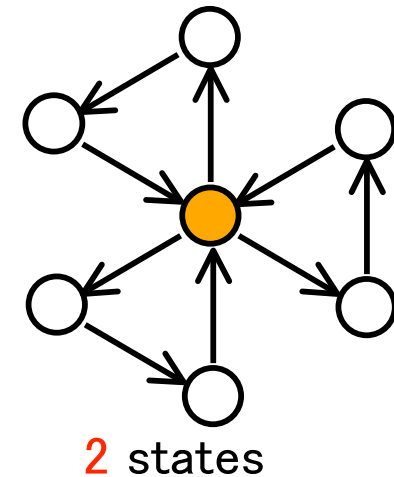
（1）A cascade does not affect diversity.
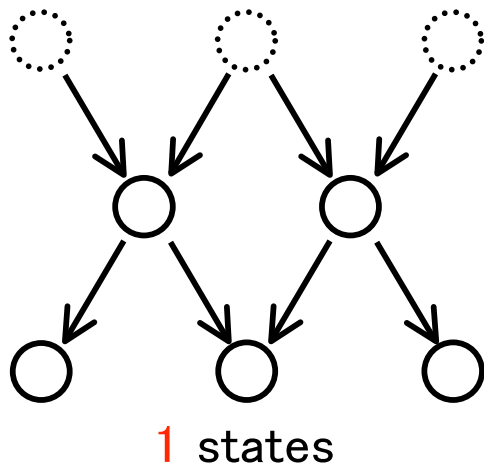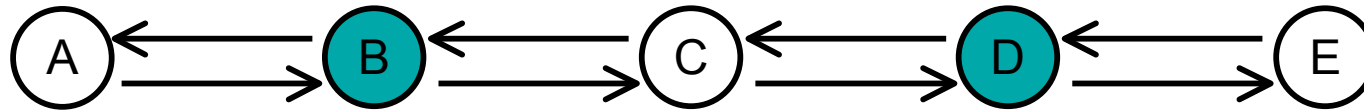
（2）A feedback loop can generate multiple states.
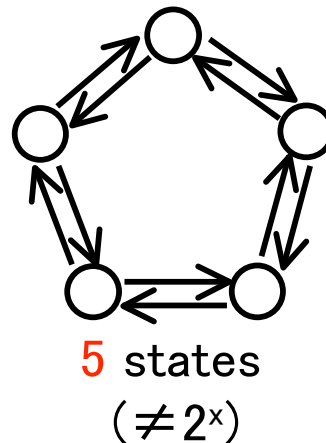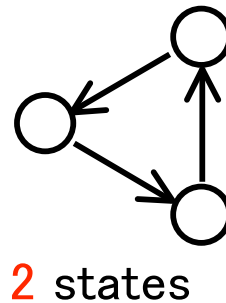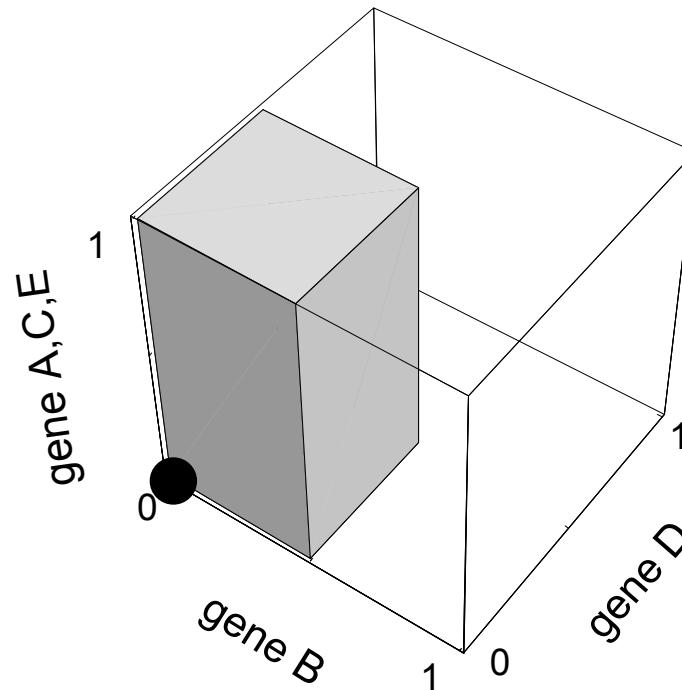
（3）Multiple loops sharing genes do not increase diversity.

1 states

2 states

5 states
（≠2ˣ）

2 states

2 states

Informative nodes
responsible for diversity of steady states

4 states

Region of no other steady states

B,D: should change activities in different steady states.
A,C,E: should be unique, if B and D are determined.

Diversity of steady states is reflected to only a part of nodes in networks.
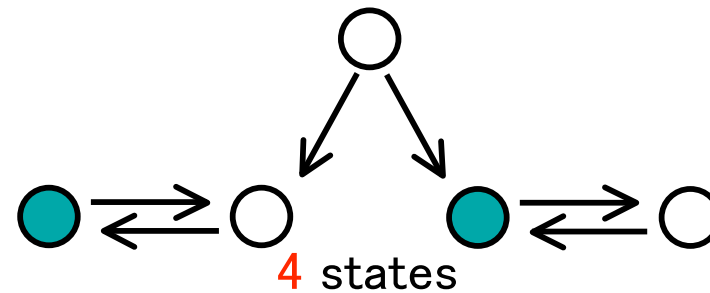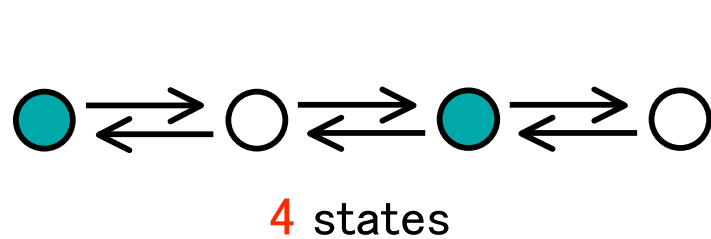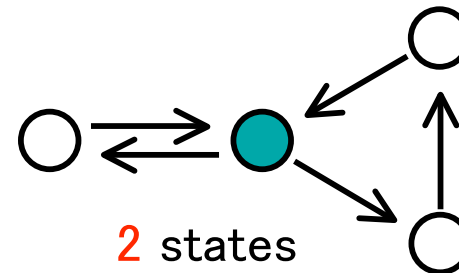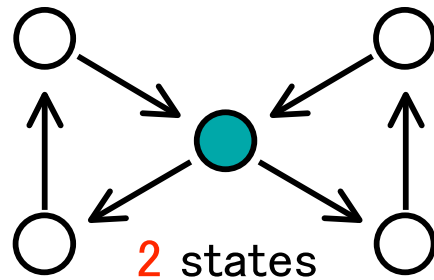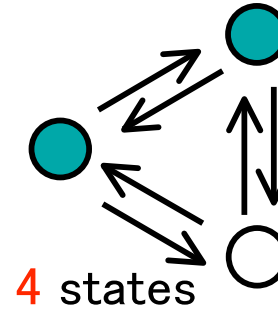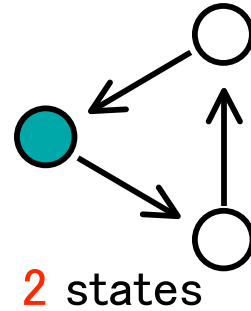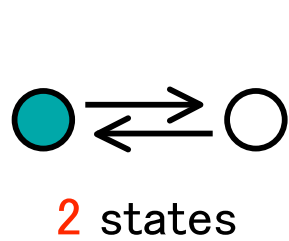
Can we say anything about cell diversity from the data?
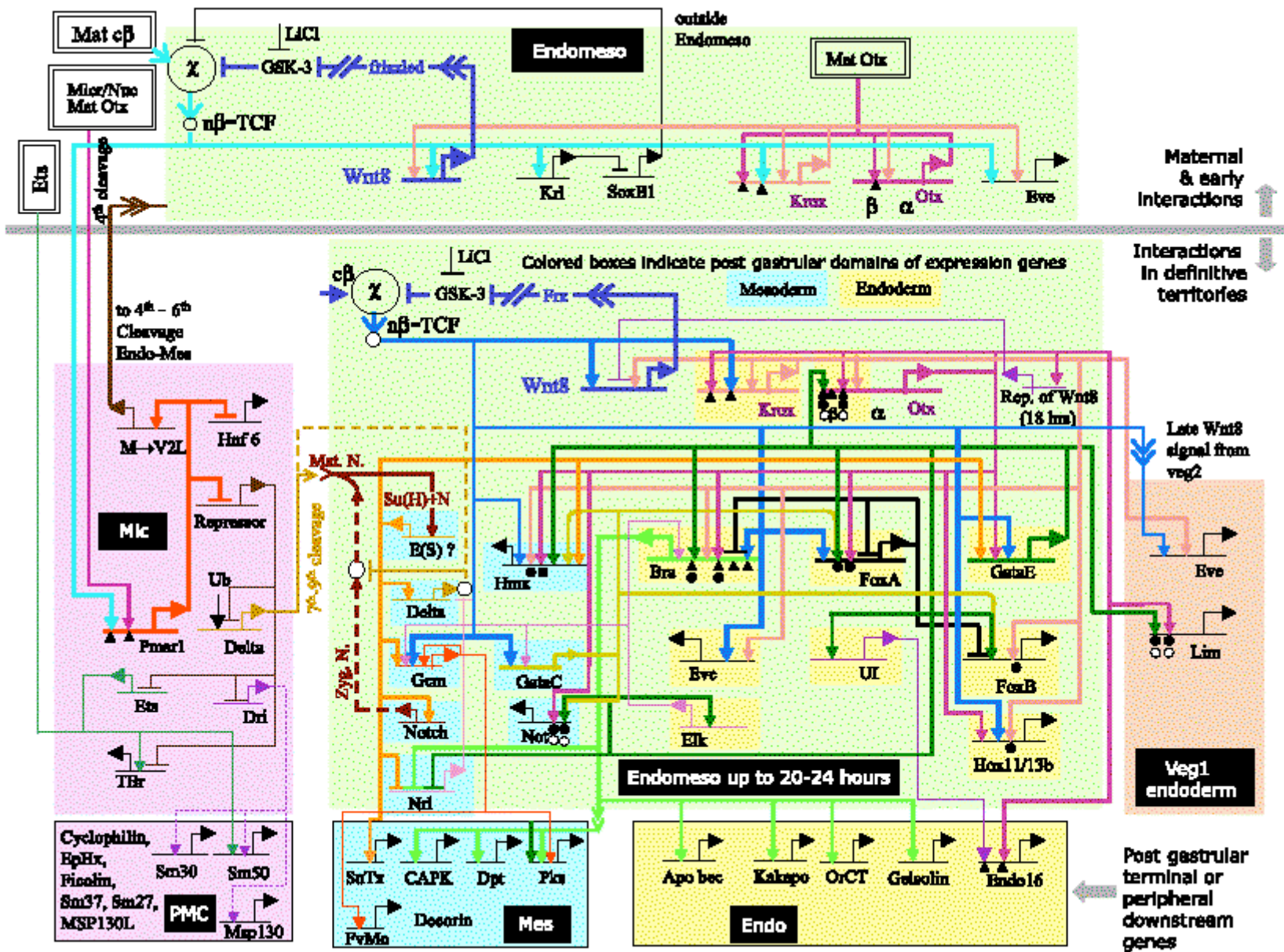
Based on perturbation analysis from E. Davidson et al. (2002)

Origin of multiple cell states
―Sea urchin―

nb-TCF Wnt8

2 states

Krox

2 states

Otx

2 states

Gcm

2 states

Notch

Delta E Nrl
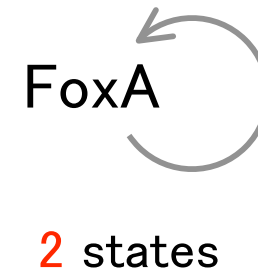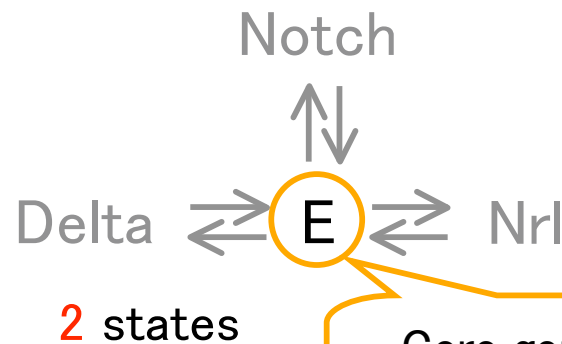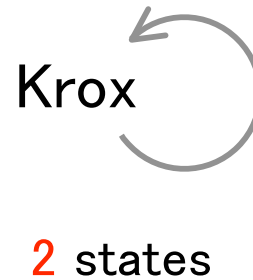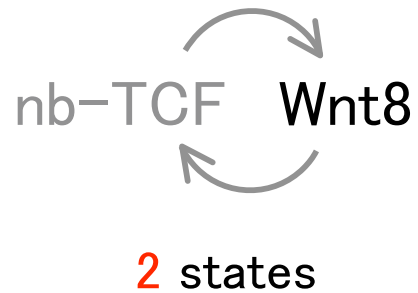
2 states

Core gene of
state-switching

FoxA

2 states

Dynamics of more than 50 genes
⇒ Steady states is reflected in only 6 genes
（>50 dimensions ⇒ 6 dimensions）

Topology of regulatory network
⇒Restriction to dynamics

1. Incompatibility
   →　Maximum number of diversity of steady states
   →　Informative nodes (reflecting states of system)
2. Independency
   →　Possible combinations of molecular activities

→ Predicting unknown regulations or expressions

Diversity＝How many?
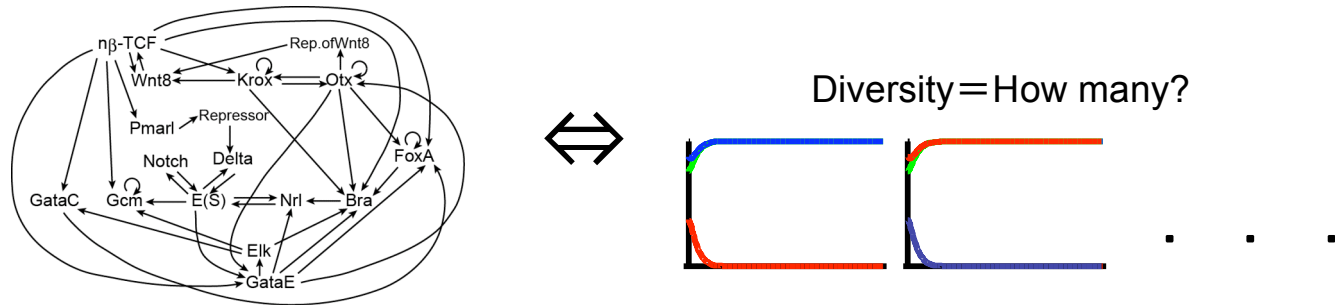
Mochizuki, A. (2008) *J. theor. Biol.* **250**, 307-321.

"unique input → unique output"
(2) independency
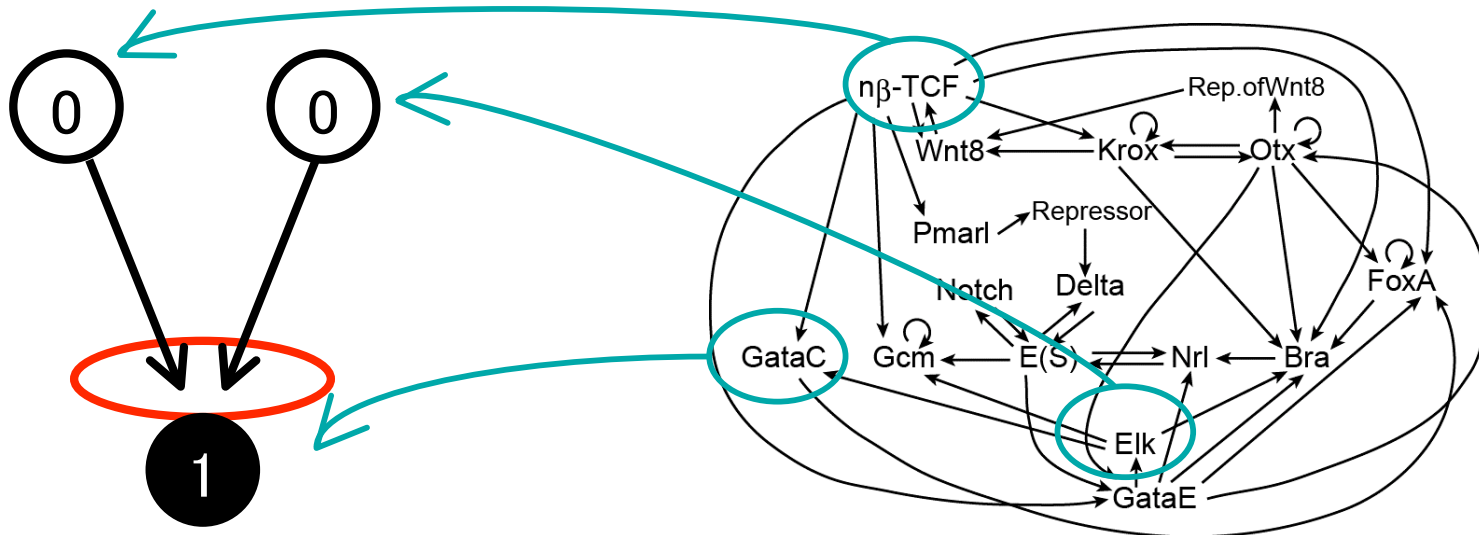
Dynamics of the focal molecule should depend
only on the controlling molecules,
should not depend on other molecules.
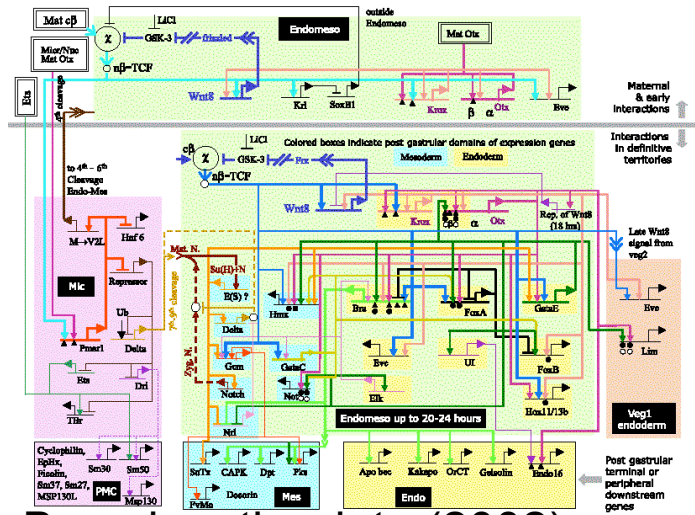
Linkage -> Possible set of steady states

Incompatibility -> maximum diversity
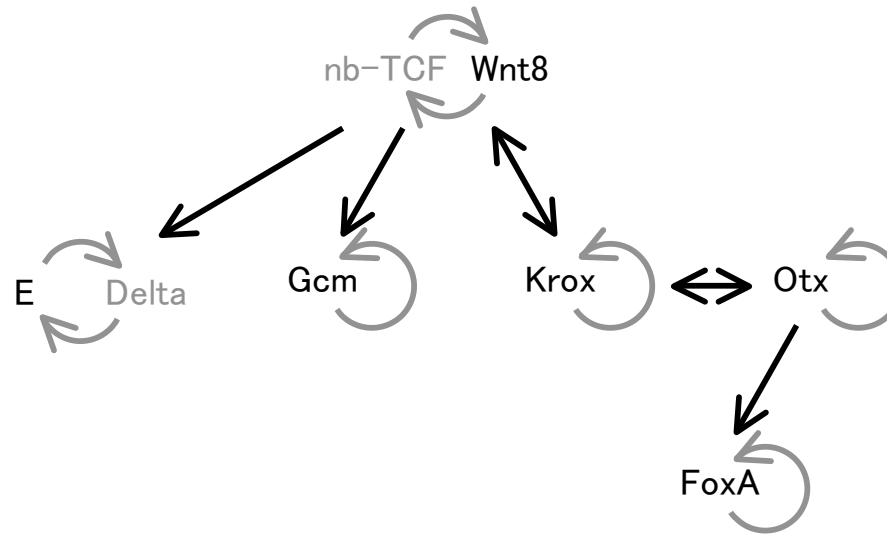# Independency -> possible combination
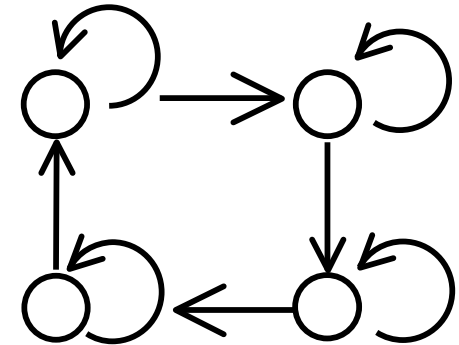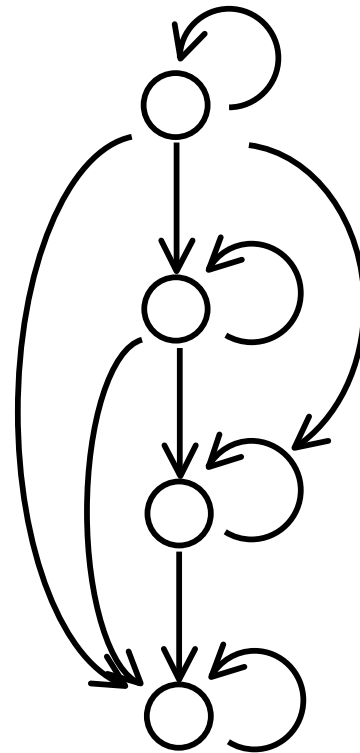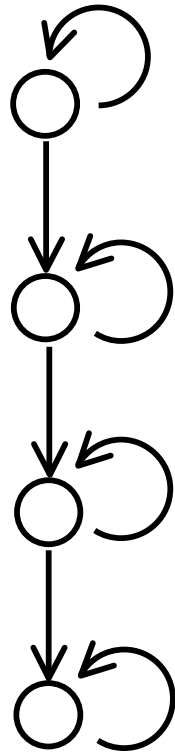
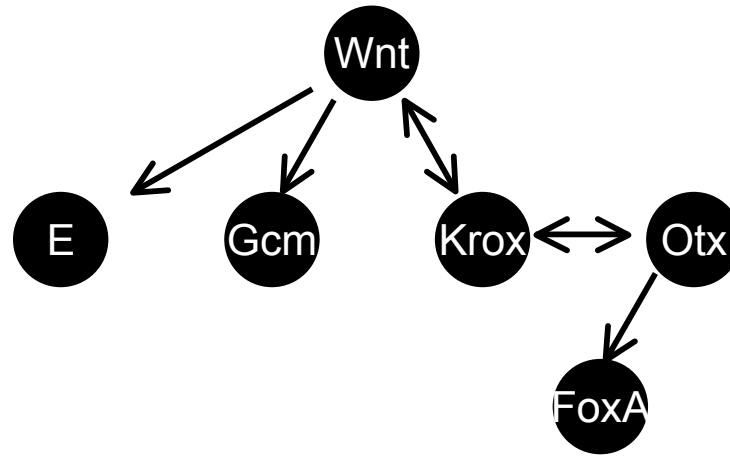

| Maximum diversity | 16 | 16 | 16 |
| Impossible number | 11,13,14,15 | — | 11,13,14,15 |

Based on the data (2002)
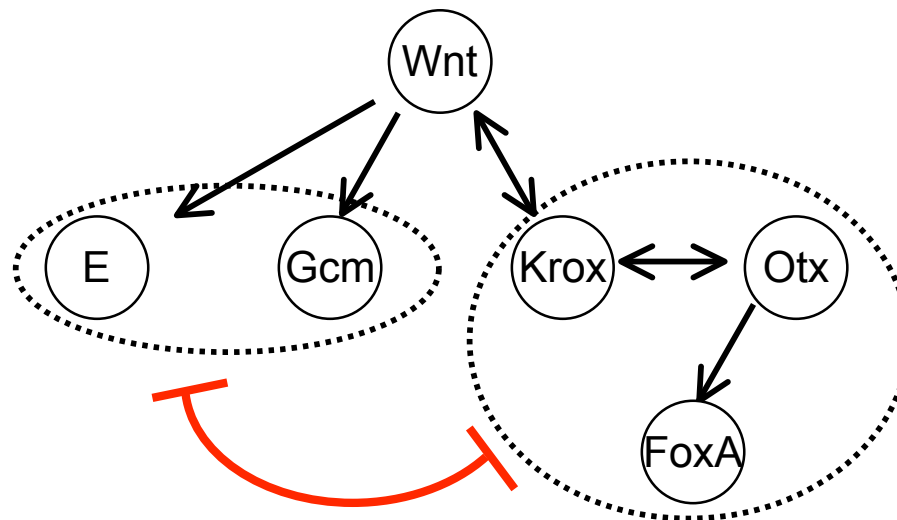
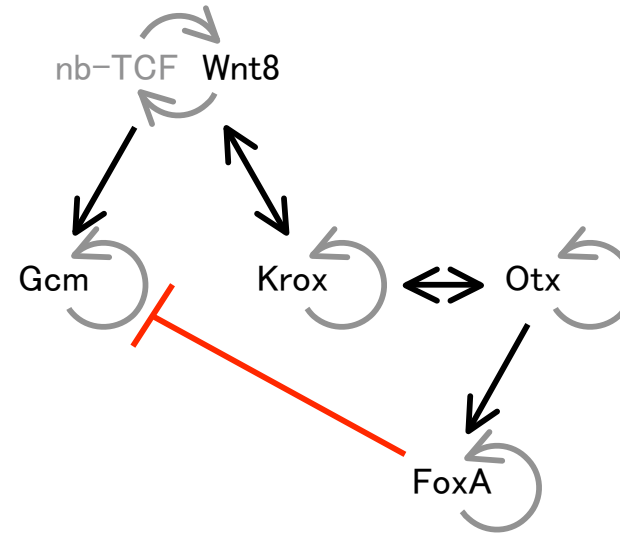Possible expression from linkage information
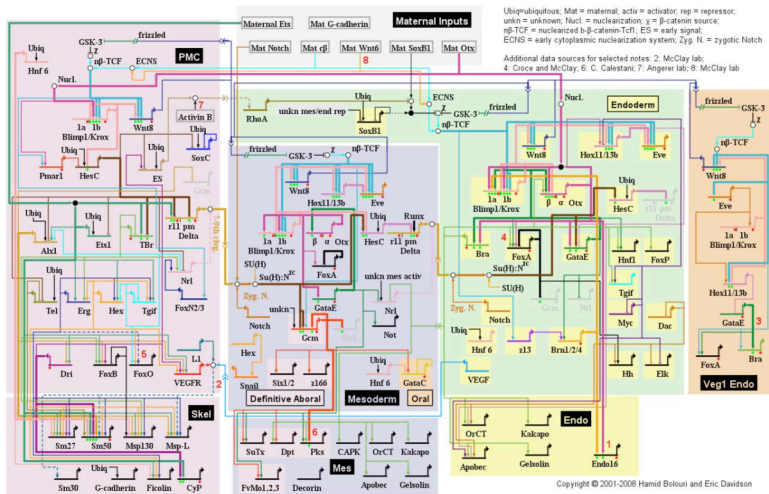
PMC

Endomesoderm

Mesoderm
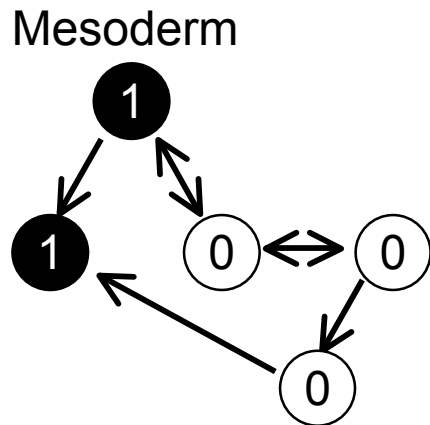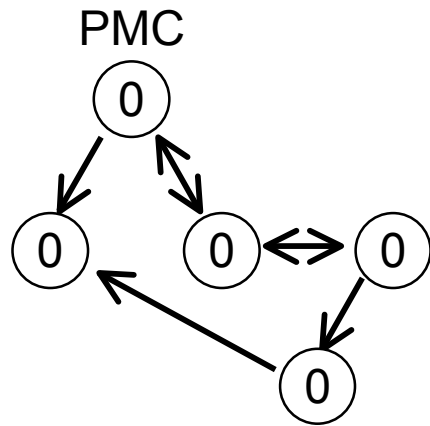
Endoderm
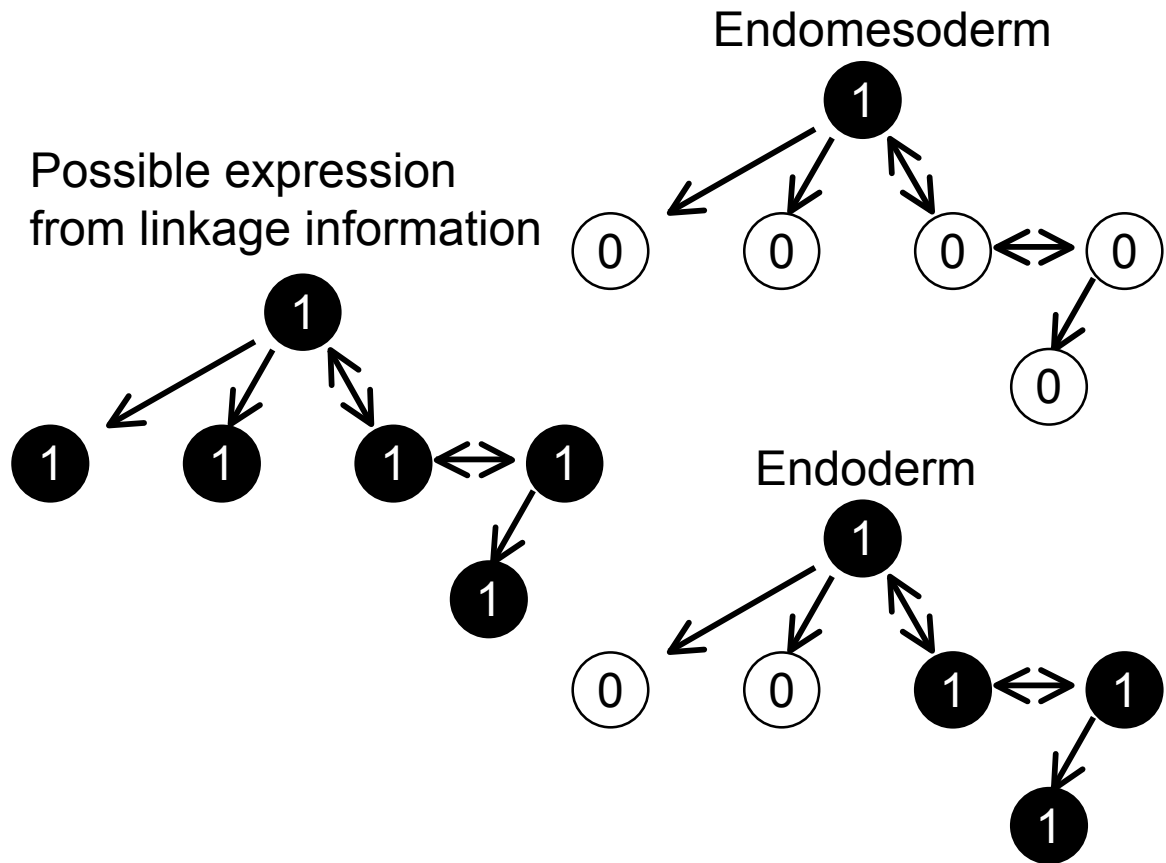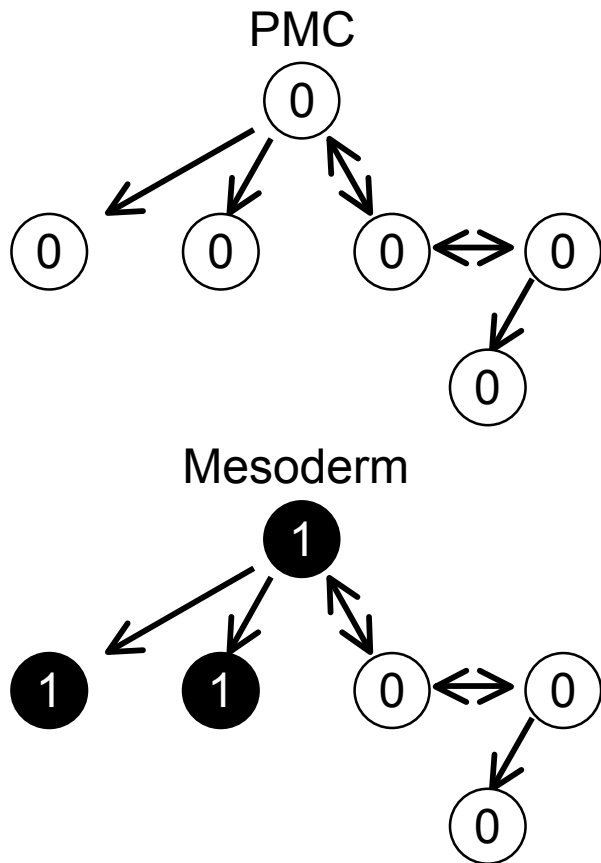
Possible expression from network structure

But, it is not observed actually

There may be unknown regulation that avoid
co-expression of (Gcm, E) and (Krox, Otx, FoxA).
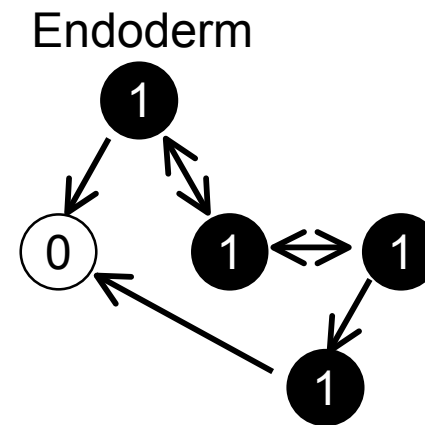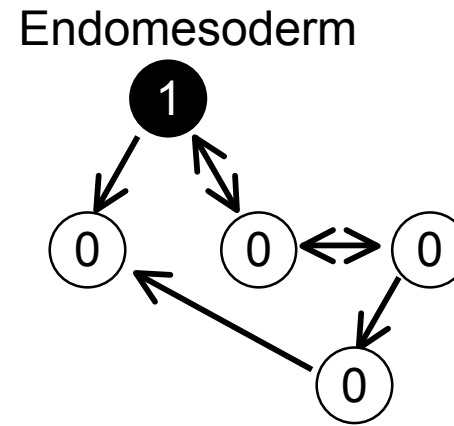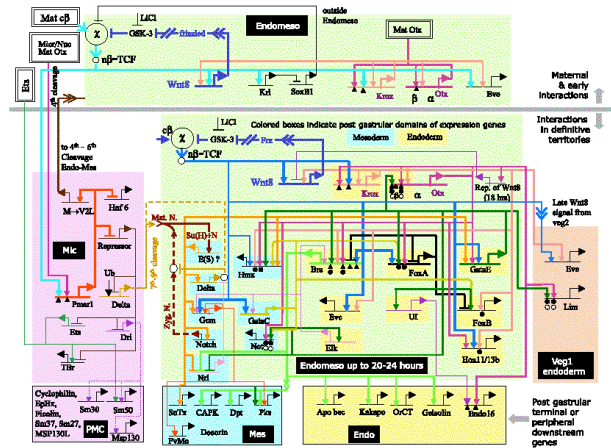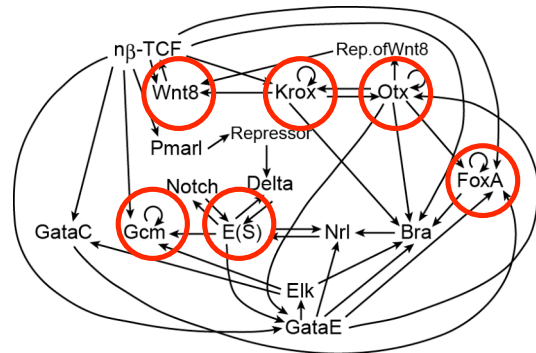
Based on the data (2008)

PMC

Endomesoderm

No extra
unknown expression

Mesoderm

Endoderm

Regulatory network

Steady state set

Prediction of unknown regulation

Reduction of network

Identification of informative molecules
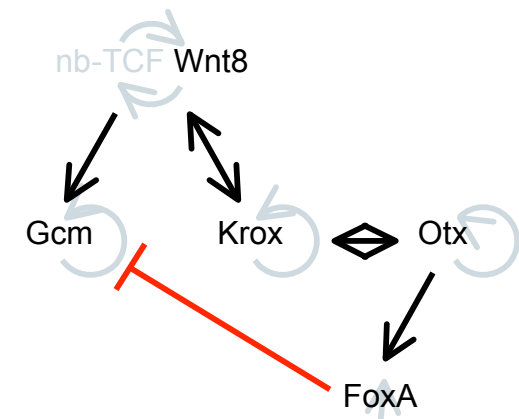
Experimental confirmation

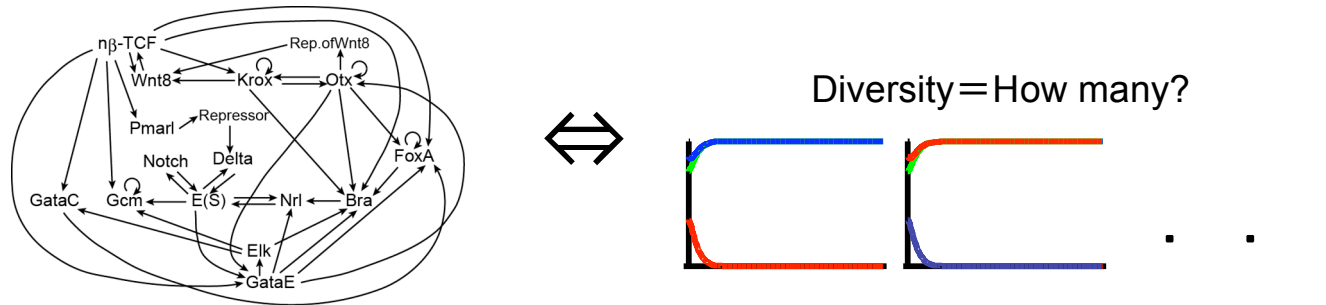# Topology of regulatory network
⇒Restriction to dynamics

1. Incompatibility
    → Maximum number of diversity of steady states
    → Informative nodes (reflecting states of system)

2. Independency
    → Possible combinations of molecular activities

→ Predicting unknown regulations or expressions

Mochizuki, A. (2008) *J. theor. Biol.* **250**, 307-321.