

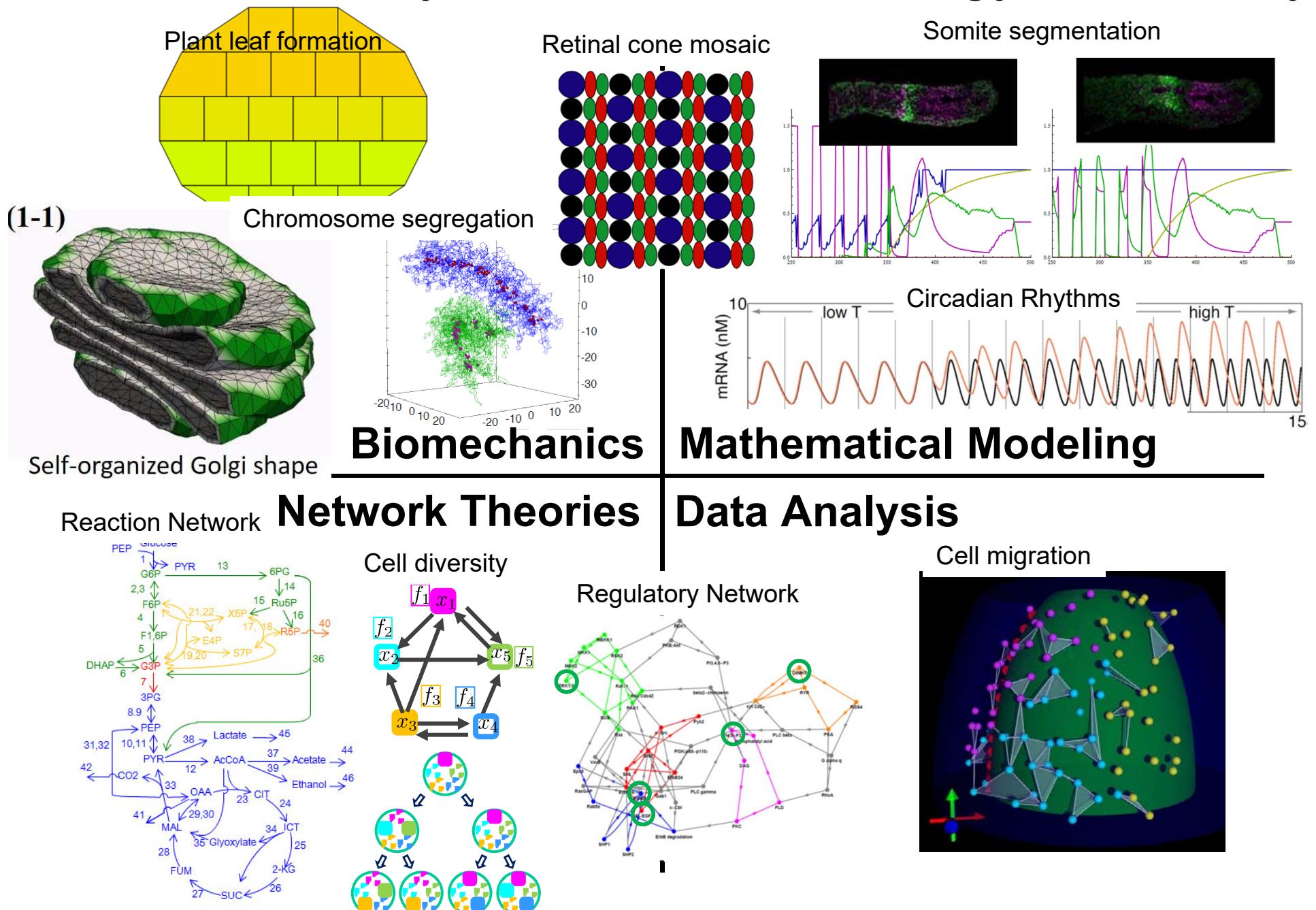
Controlling cell fate specification system based on network structure

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Research activity of Theoretical Biology Laboratory



One topic for one person

Biomechanics

Plant leaf formation

Chromosome segregation

Self-organized Golgi shape

Retinal cone mosaic

Somite segmentation

Circadian Rhythms

Mathematical Modeling

mRNA (nM)

Network Theories

Reaction Network

Cell diversity

Regulatory Network

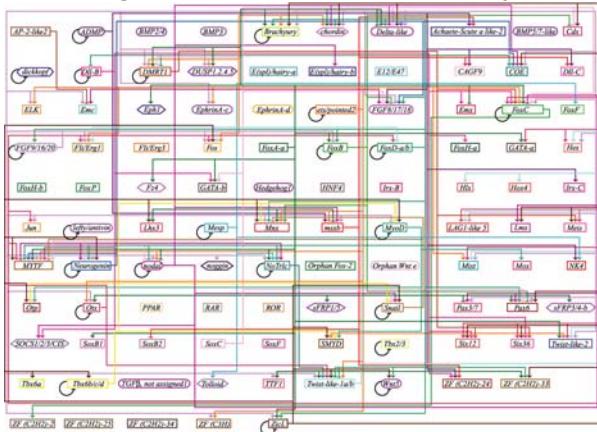
Data Analysis

Cell migration

Studying biological network systems based on theories to connect structure and dynamics

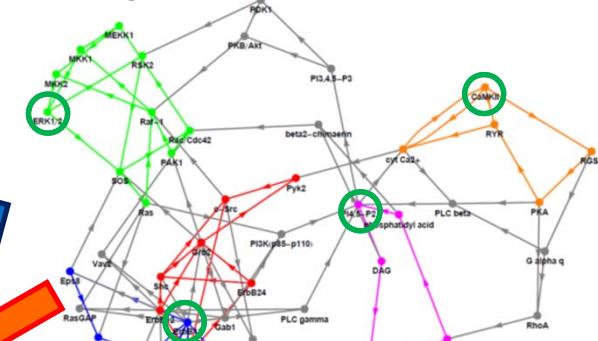
A: Gene regulatory network

Sato group, Sato lab. in Kyoto Univ.



B: Signal transduction

Hiroshima group, Sako lab. in RIKEN



Structural theories

- Linkage logic

$$\tilde{x}_j(t) - x_j(t) \xrightarrow{t \rightarrow \infty} 0 \quad \text{for all } j \in J$$

$$\text{implies } \tilde{\mathbf{x}}(t) - \mathbf{x}(t) \xrightarrow{t \rightarrow \infty} 0$$

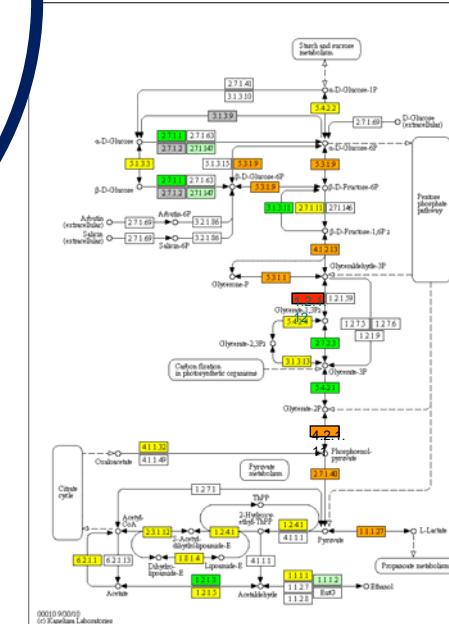
- Reaction networks

$$S \left[\begin{pmatrix} \delta w_j \\ \vdots \\ \delta w_m \end{pmatrix} + \left(\frac{\partial w_j}{\partial x_m} \right) \begin{pmatrix} \delta x_m \\ \vdots \\ \delta x_m \end{pmatrix} \right] = 0$$

CREST Program, JST
“Bio-Dynamics”

C: Carbon metabolism

Shirane group,
Nakayama lab. in Kyushu Univ.



Structural Theories

Linkage Logic

Regulatory Network

Structure -> Key molecules

Structural Sensitivity analysis

Chemical Reaction Network

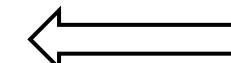
Structure -> Response to enzyme

Structural Bifurcation Analysis

Chemical Reaction Network

Structure -> Bifurcation properties

Network
information



Experiment

- Network analysis
- Measuring
- Perturbation



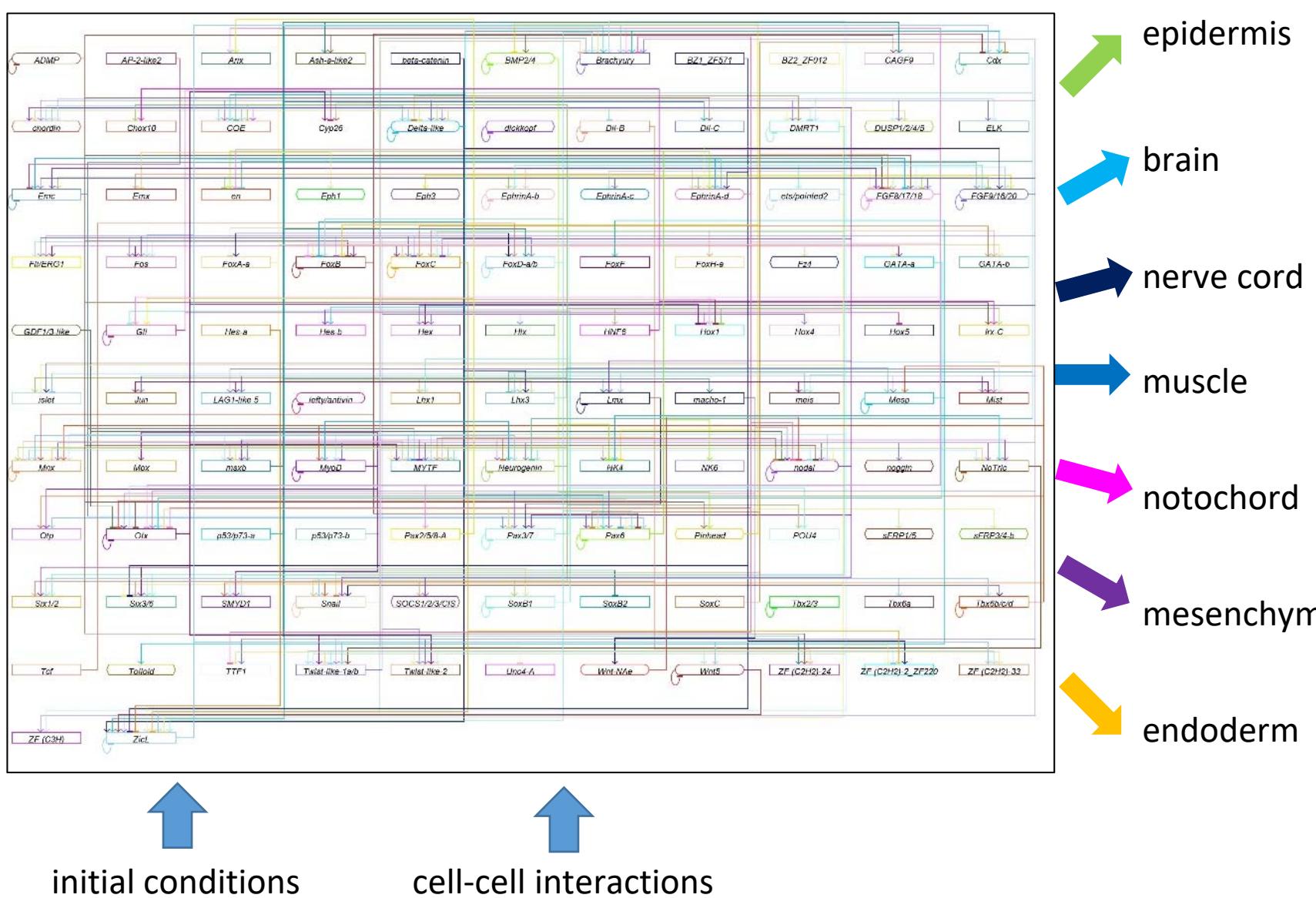
Prediction
without
assuming details

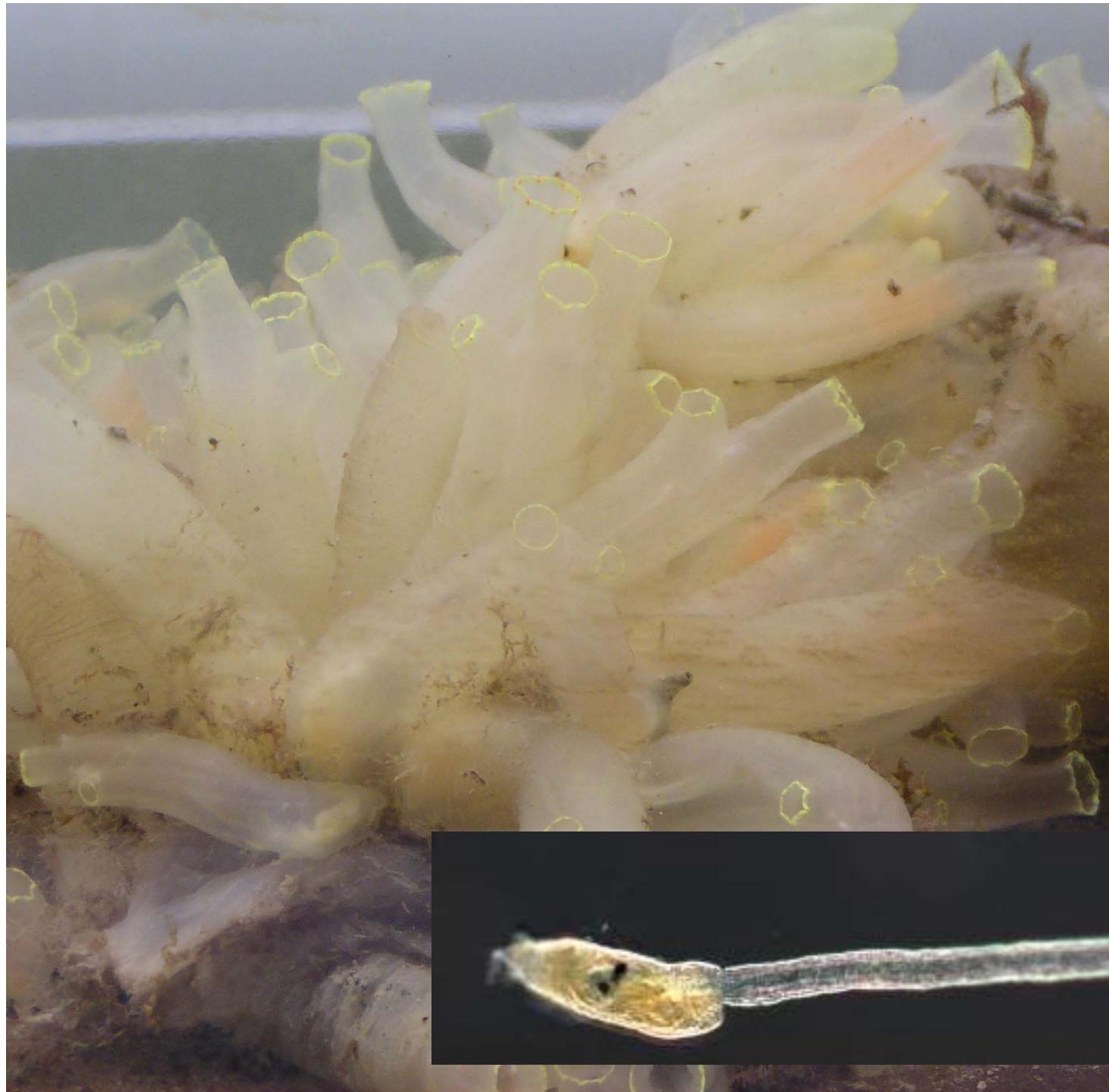
In biology, information of network are very rich,
but qualitative details of dynamics are very poor.

Structural theories use **information of networks only**.

gene regulatory network specifying cell fates

By Lab. of Dr. Yutaka Sato (Kyoto U.)



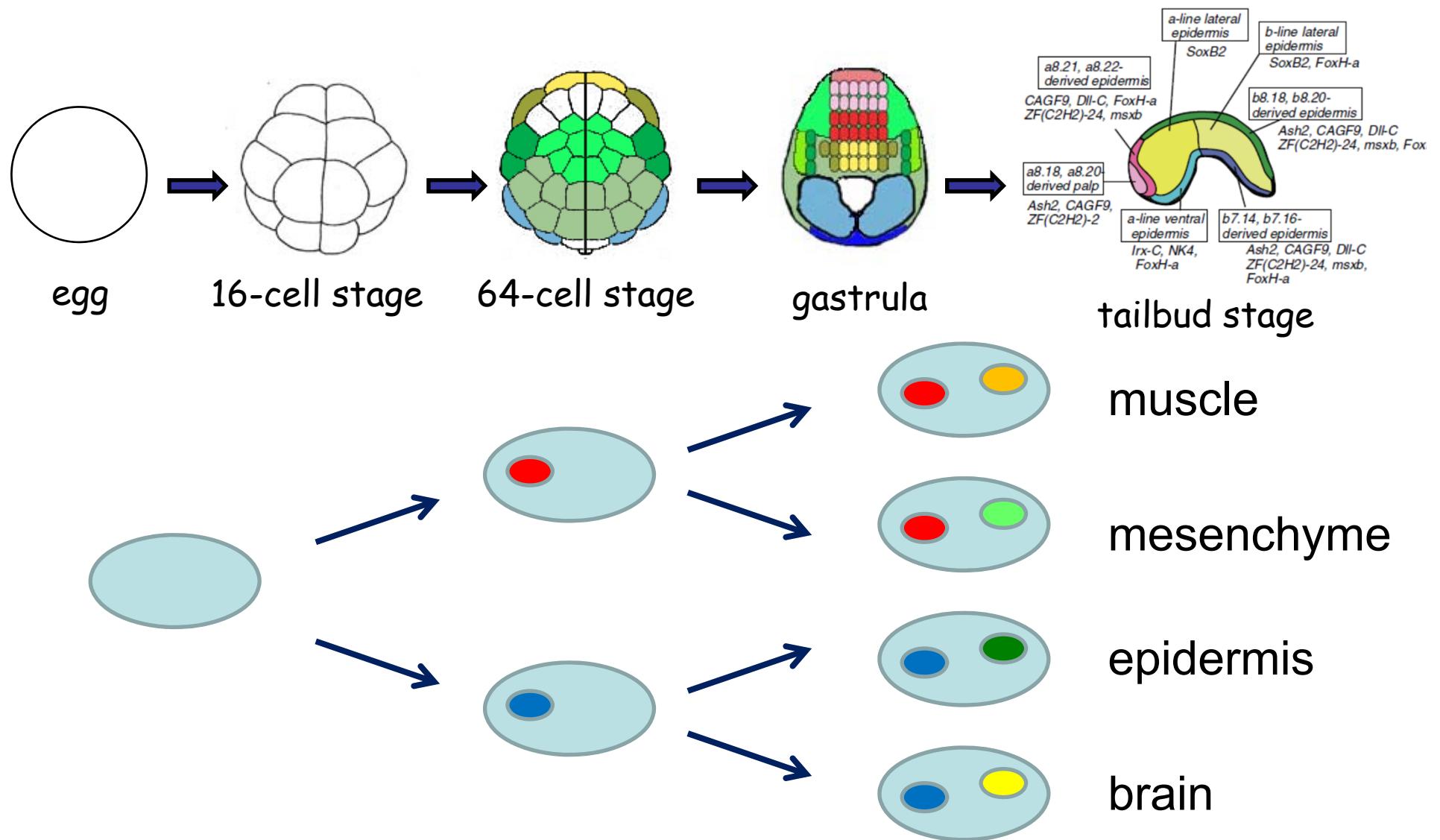


Hoya
a sea squirt
an ascidian

a chordate animal



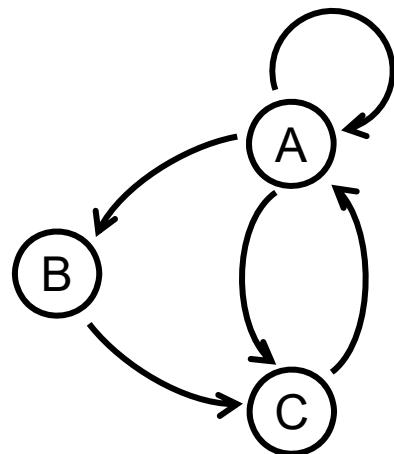
Cell differentiation and gene activities



Gene expression patterns reflect cell types.

Diversity of cell states = Multiple steady states

Regulatory network

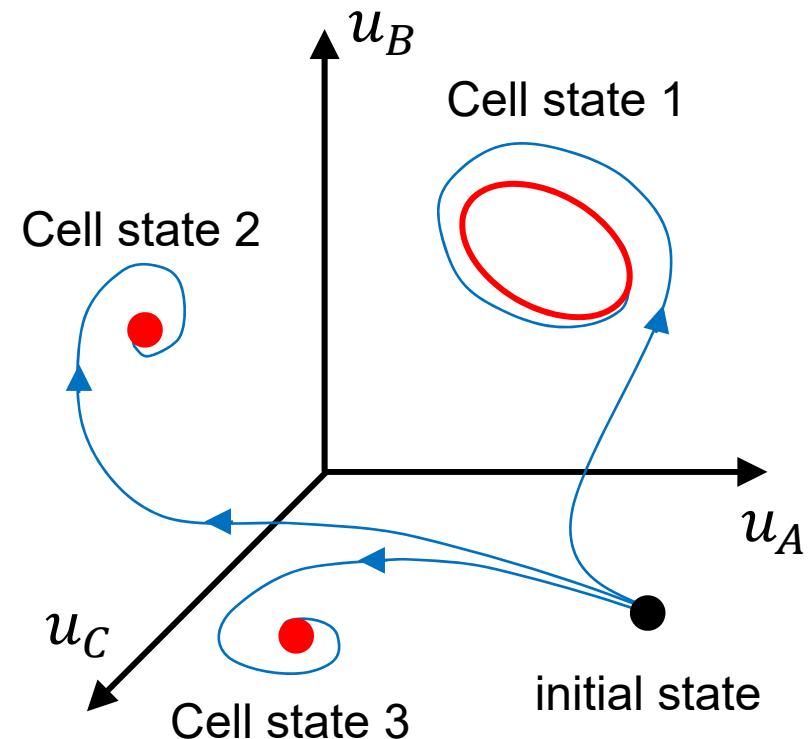


Dynamics of gene activity

$$\frac{dx_A}{dt} = F_A(x_A; \boxed{x_A, x_C})$$

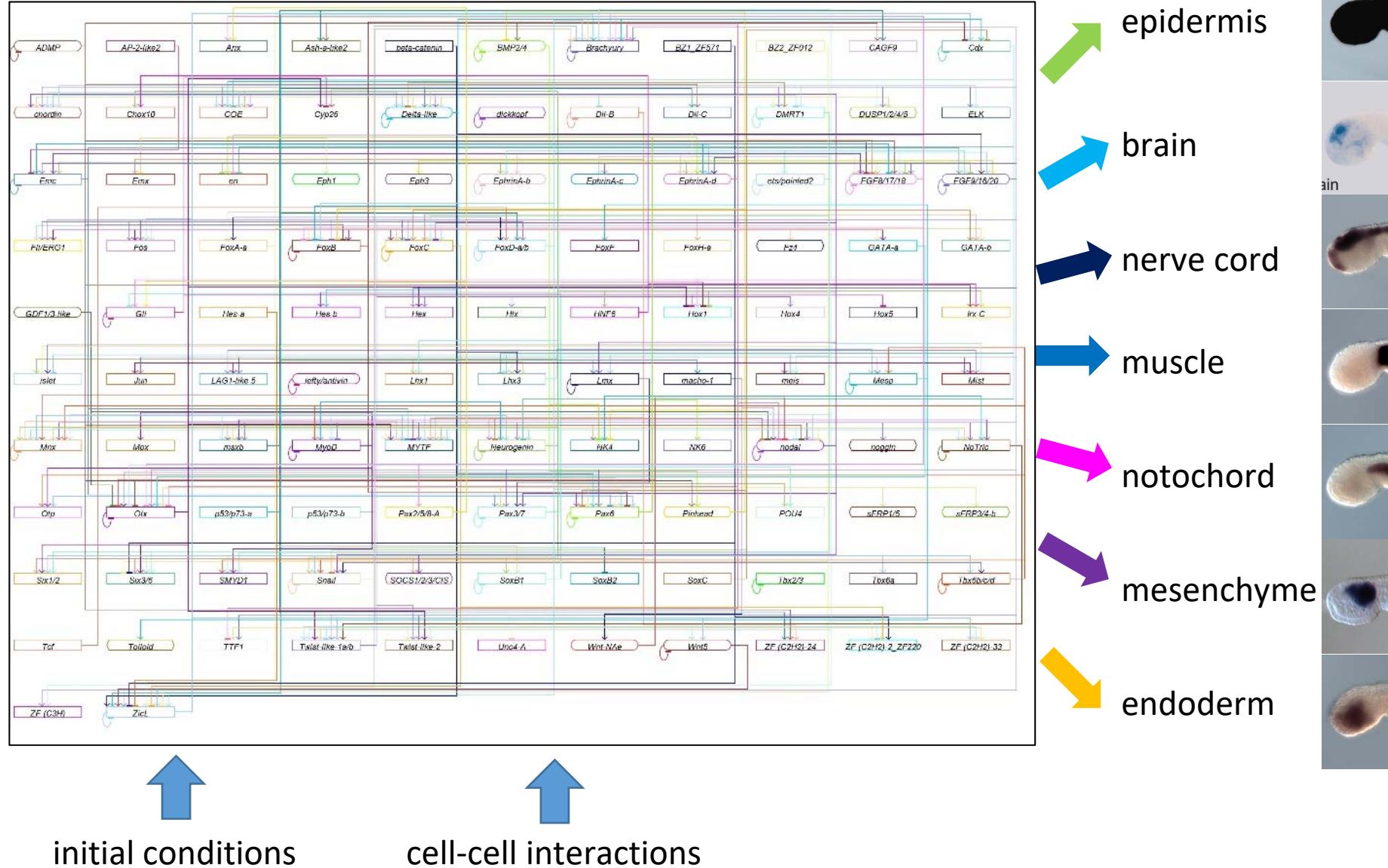
$$\frac{dx_B}{dt} = F_B(x_B; \boxed{x_A})$$

$$\frac{dx_C}{dt} = F_C(x_C; \boxed{x_A, x_B})$$



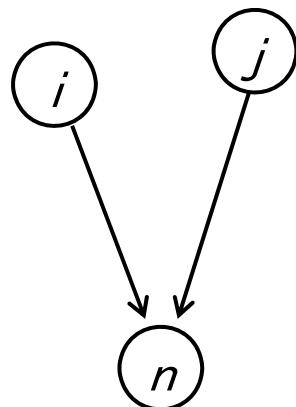
Occurrence of multi-stability depends on choices of functions and parameters.

- truly functional as expected?
- possibly incomplete?



Structural Theory(1) Linkage Logic

Regulatory Network = Dependence between variables
= Info. of argument set of ODE



$$\frac{dx_n}{dt} = F_n(x_n; x_i, x_j)$$

nodes: biomolecules
arrows: influences,
not specify functions

Treatment of argument set

$$\dot{x}_n = F_n(x_n, x_{I_n})$$

I_n : Input set of n
tails of edges pointing n

$n \in I_n$ iff gene n activates itself.

$$+\circlearrowleft \Leftrightarrow n \in I_n$$

Decay condition

$$\partial_1 F_n(x_n, x_{I_n}) < 0$$

(including self repression)

⇒ Determine important nodes (genes, or proteins)

Mathematical Concepts

Feedback Vertex Set I

$I(\subseteq V)$ is a feedback vertex set
if, and only if, $\Gamma \setminus I$ is cycle free.

Determining nodes J

Subset of variables by which all possible attractors are identified.

$$\begin{aligned}\tilde{x}_J(t) - x_J(t) &\xrightarrow[t \rightarrow \infty]{} 0 \\ \Downarrow \\ \tilde{x}(t) - x(t) &\xrightarrow[t \rightarrow \infty]{} 0\end{aligned}$$

Mathematical Proof

Feedback Vertex Set I
of the network

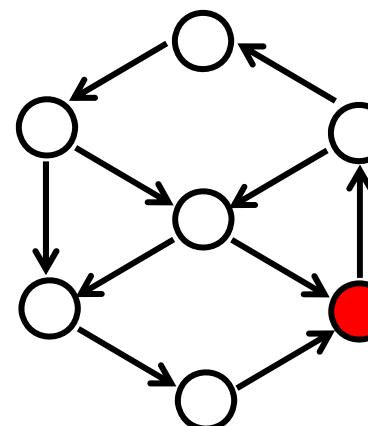
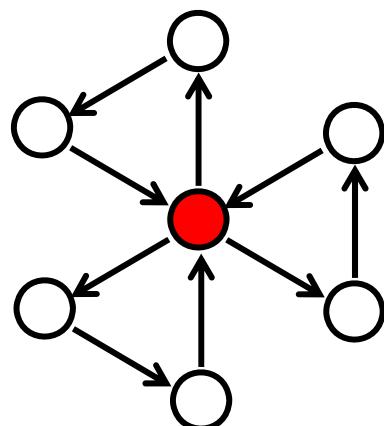
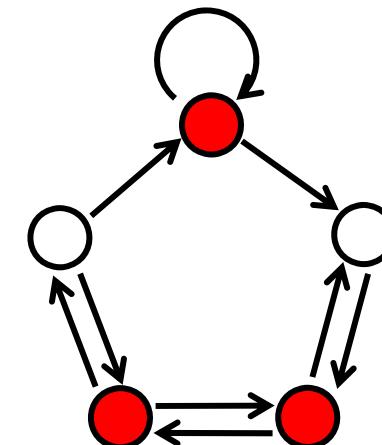
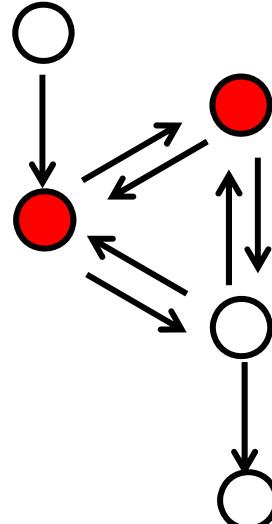
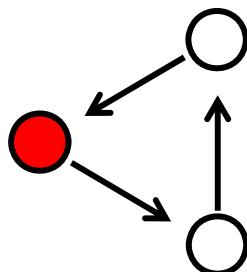
Determining nodes J of
dynamics on the network
for any functions.

Fiedler, B., Mochizuki, A. et al. *J. Dyn. Differ. Eqns.* (2013) **25**, 563-604.
Mochizuki, A., Fiedler, B. et al. *J. Theor. Biol.* (2013) **335**, 130-146

Feedback vertex set

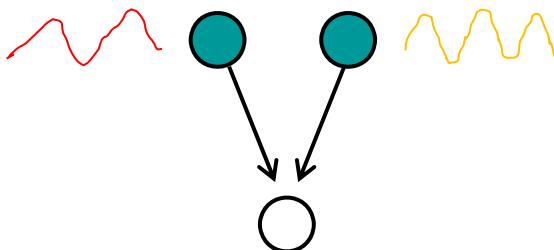
(a subset of nodes whose removal leaves a graph without cycles)

- (1) by observing FVS, all the possible attractors of the system are identifiable.
- (2) by controlling FVS, the whole system is controllable.

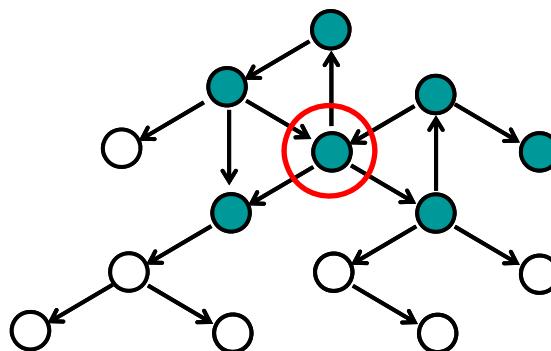


Intuitive explanation

1. If dynamics of upward is given,
dynamics of downward is determined uniquely.



2. Dynamics of appropriately selected sets determine dynamics of whole network.



3. How can we minimize the set, on which dynamics are given?
⇒ Feedback Vertex Set!

Control aspect of Feedback Vertex Set

FVS =

Determining nodes J

$$\tilde{x}_j(t) - x_j(t) \xrightarrow[t \rightarrow \infty]{} 0 \quad \text{for all } j \in J \subseteq V$$

implies

$$\tilde{\mathbf{x}}(t) - \mathbf{x}(t) \xrightarrow[t \rightarrow \infty]{} 0$$

Observing long-term dynamics
on Feedback Vertex Set

\Rightarrow

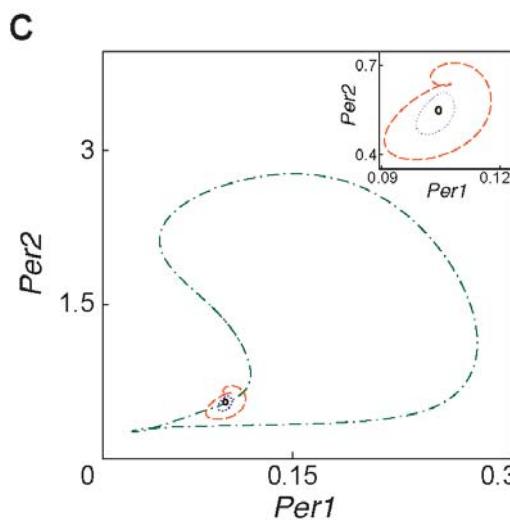
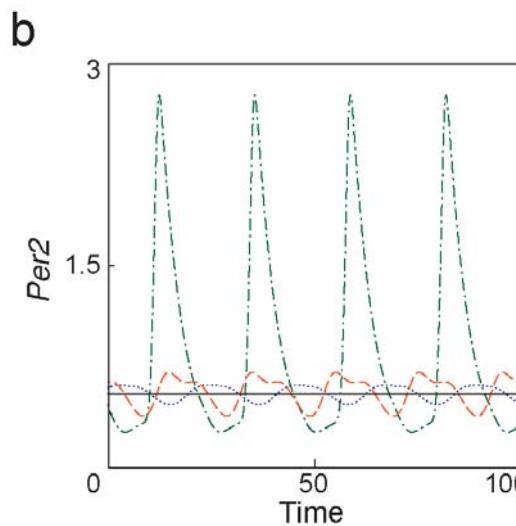
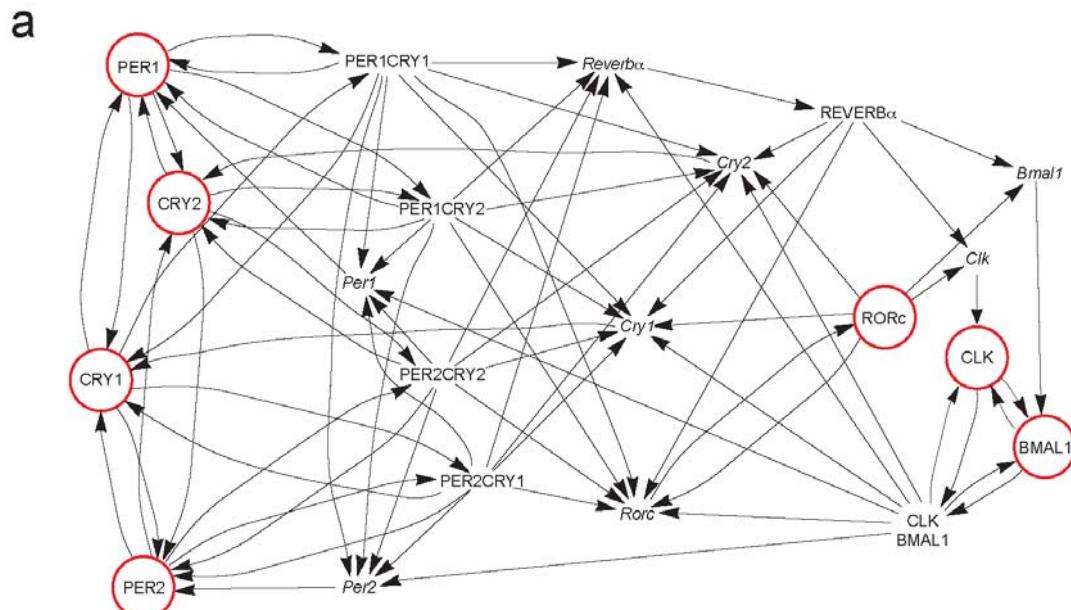
Observing attractors of total system.

Controlling Feedback Vertex Set
to converge to one of attractors

\Rightarrow

Control of total system.

Controlling a dynamical system



- A system for mammalian circadian rhythms
- **7 FVs among 21 variables**

Modified from Mirsky et al., 2009

4 solutions

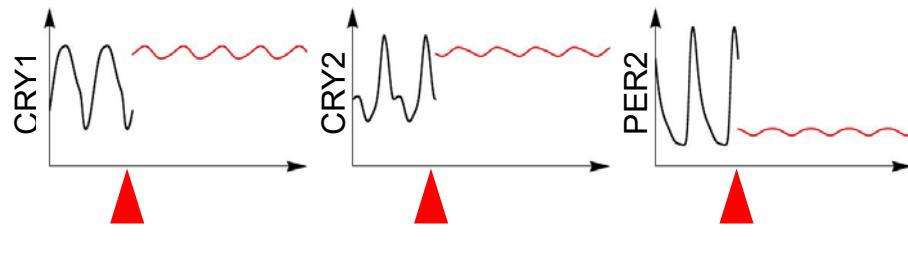
- 2 stable oscillations (**P1**、**P2**)
- 1 unstable oscillation (**UP**)
- 1 unstable stationary point (**USS**)

Controlling a dynamical system

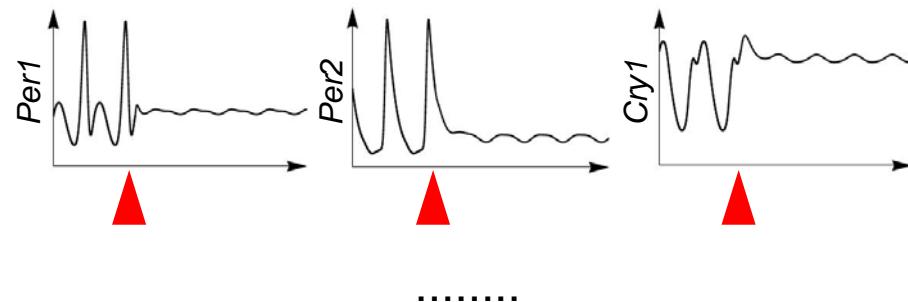
- 7 FVs among 21 variables

Whole system can be controlled by controlling FVS.

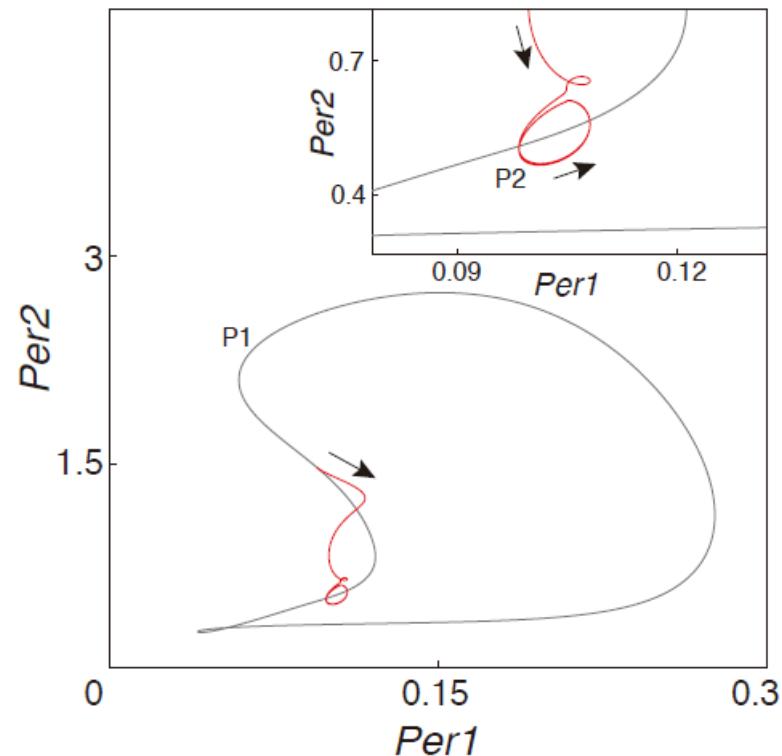
7 in FVS \leq prescribe



Other 14 \leq ODE



P1 \rightarrow P2



(1) Prepare time series of **FVS** on the solution, P1, P2, UP, USS.

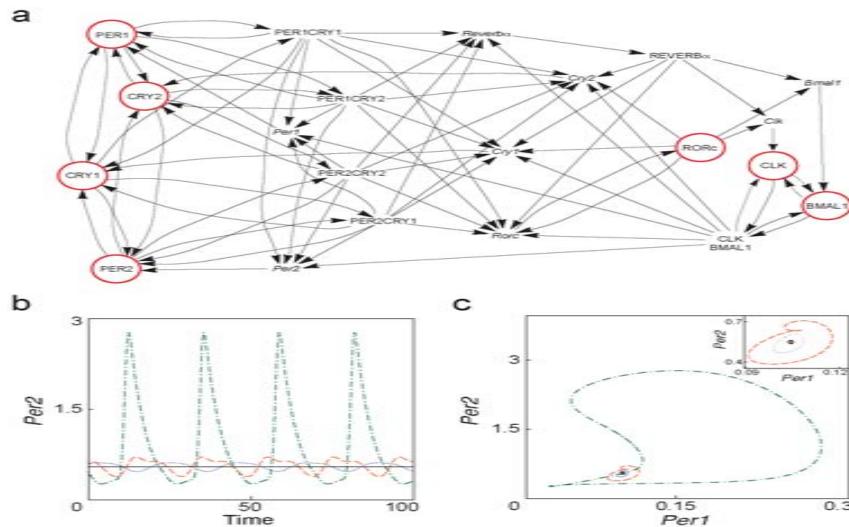
(2-1) Prescribe **FVS**, to follow the value on the solution.

(2-2) The remaining variables, **nonFVS**, are calculated by remaining ODEs.

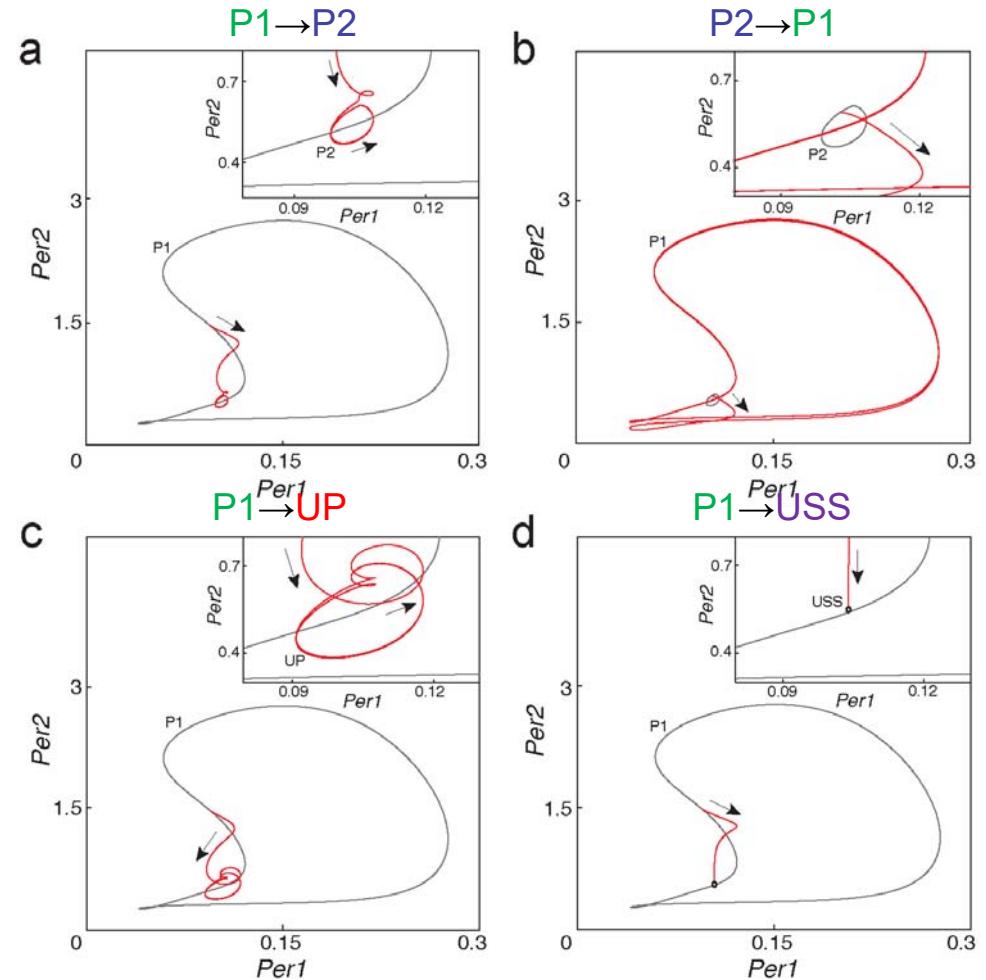
Controlling a dynamical system

Whole system can be controlled by controlling FVS.

- 7 FVs among 21 variables



FVS <= prescribe
Others <= ODE



UP, USS; unstable in original system with 21 variable, but **globally stable** in reduced system with 14 variables.

Maximum Matching

$$\begin{aligned}\dot{x}(t) &= Ax(t) + Bu(t) \\ y &= Cx\end{aligned}$$

Linear

Steering

$$u(t) \quad 0 \leq t \leq T$$

$u(t)$ has to be determined for each model.

Observing nodes \neq Controlling nodes

Any points on the linear space

FVS Control

$$\begin{aligned}\dot{x}_n &= F_n(x_n, x_{I_n}) \\ \partial_1 F_n &< 0\end{aligned}$$

Non-linear

Prescribing FVS to Target

$$x_I^*(\tau)$$

Only target trajectory on FVS is required (Observation base).

Observing nodes = Controlling nodes
(Observed data are used directly for control)

Switching between attractors

For details, See

Mochizuki, A., Fiedler, B. et al. *J. Theor. Biol.* (2013) 335, 130-146

Dynamics of complex systems

\Leftrightarrow

Structure of regulatory network

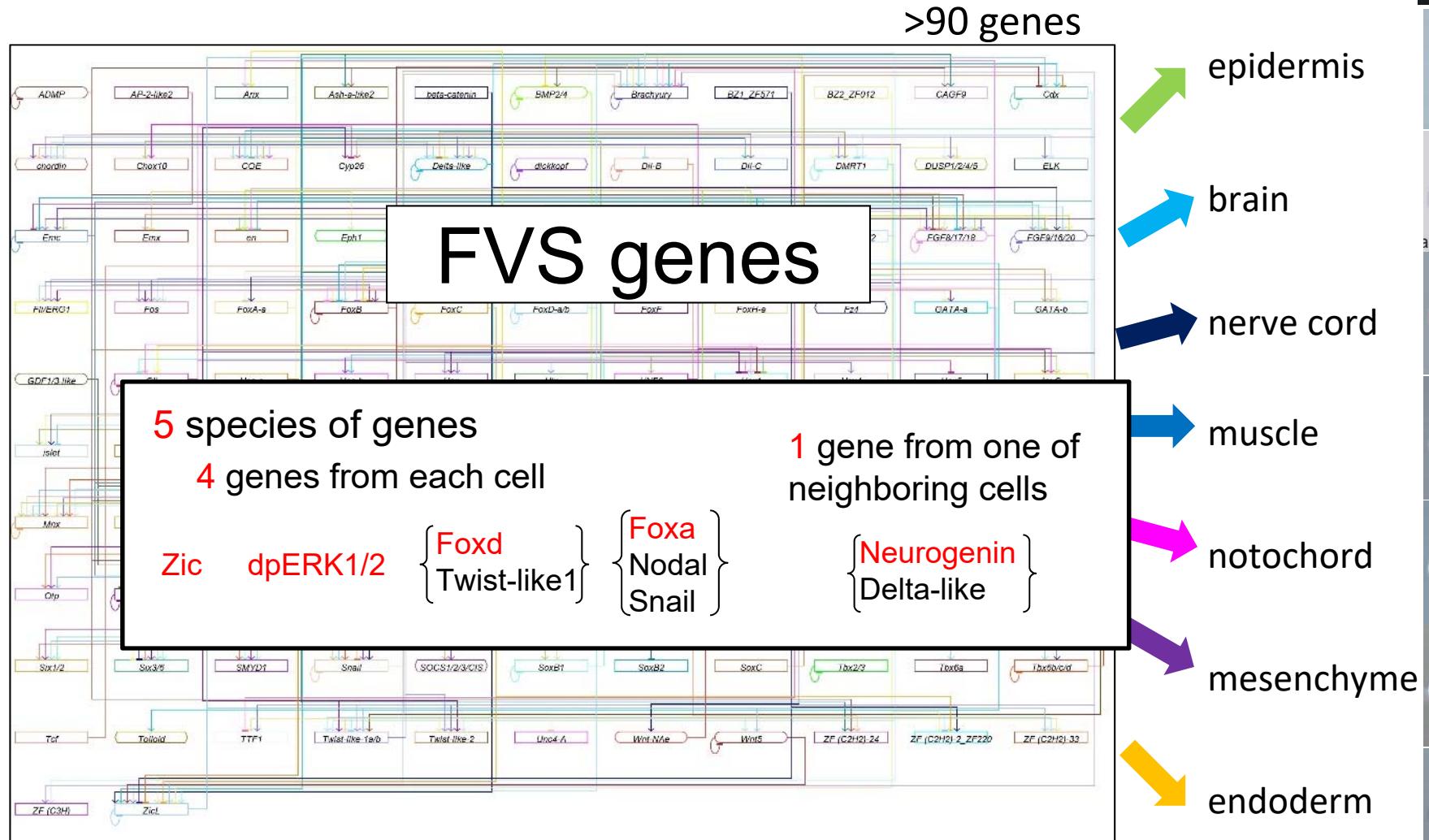
Feedback vertex set (determined from networks)

- (1) minimal sufficient set of variables
to detect all of the possible dynamical behaviors
without depending on nonlinear functions.

- (2) minimal sufficient set of variables
to control whole system without depending on functions.

Fiedler, B., Mochizuki, A. *et al.* *J. Dyn. Differ. Eqns.* (2013) **25**, 563-604.
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Analysis of gene regulatory network

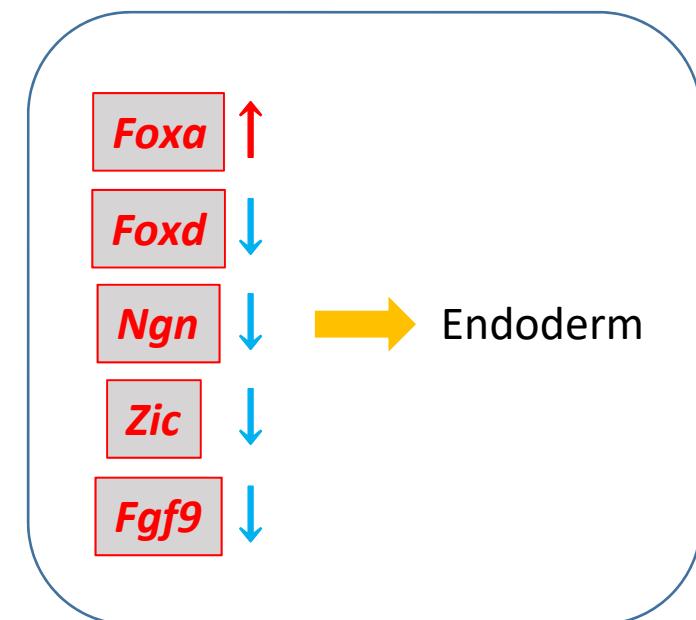
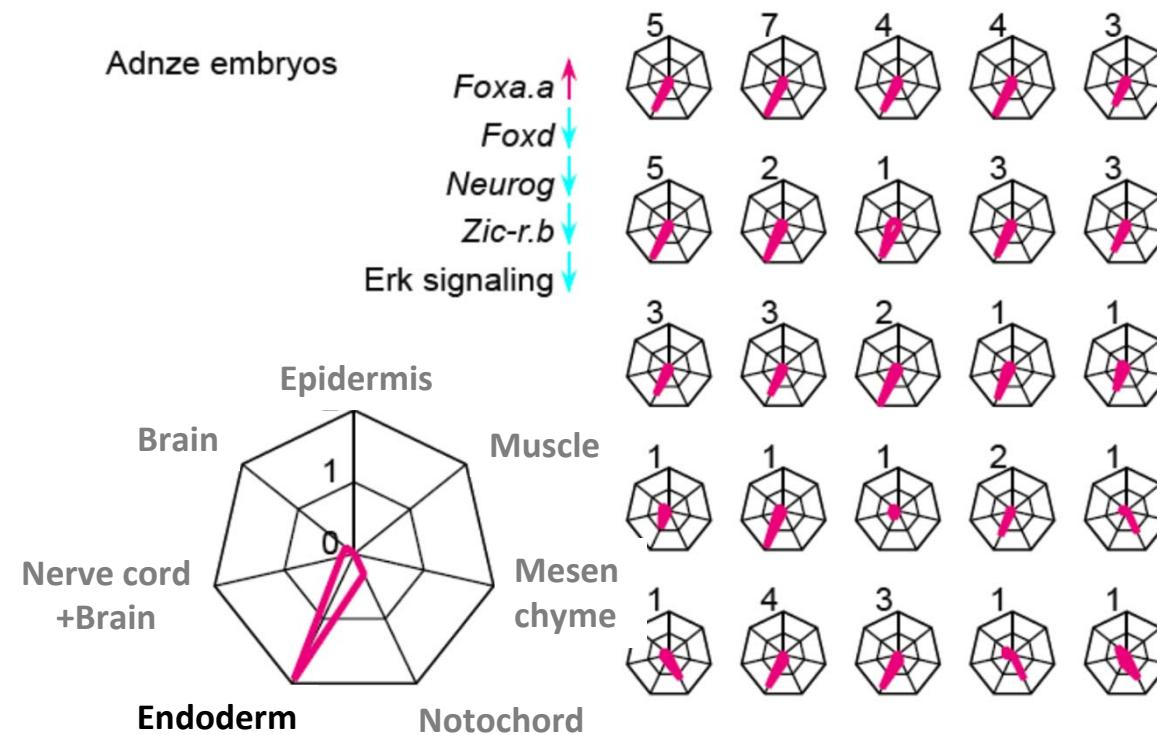
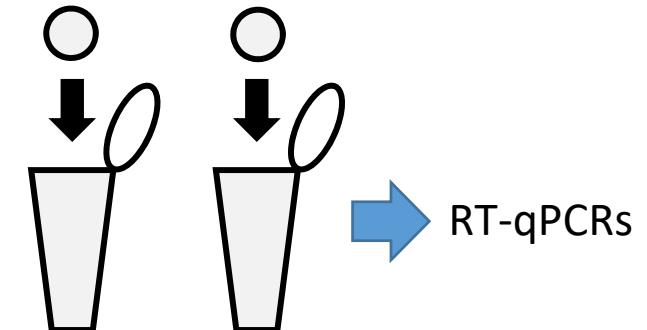
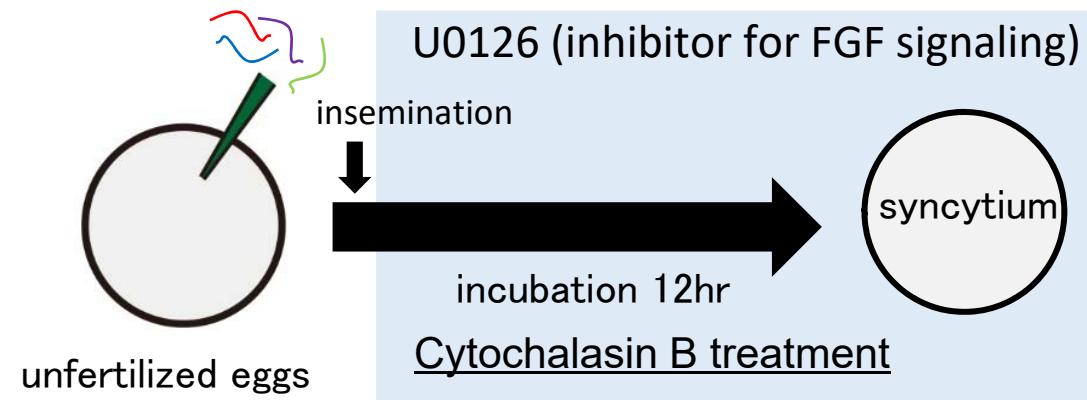


Thm: Whole system can be controlled by controlling FVS genes only.

- Verify whether cell fate can be controlled by changing expression of the five genes.

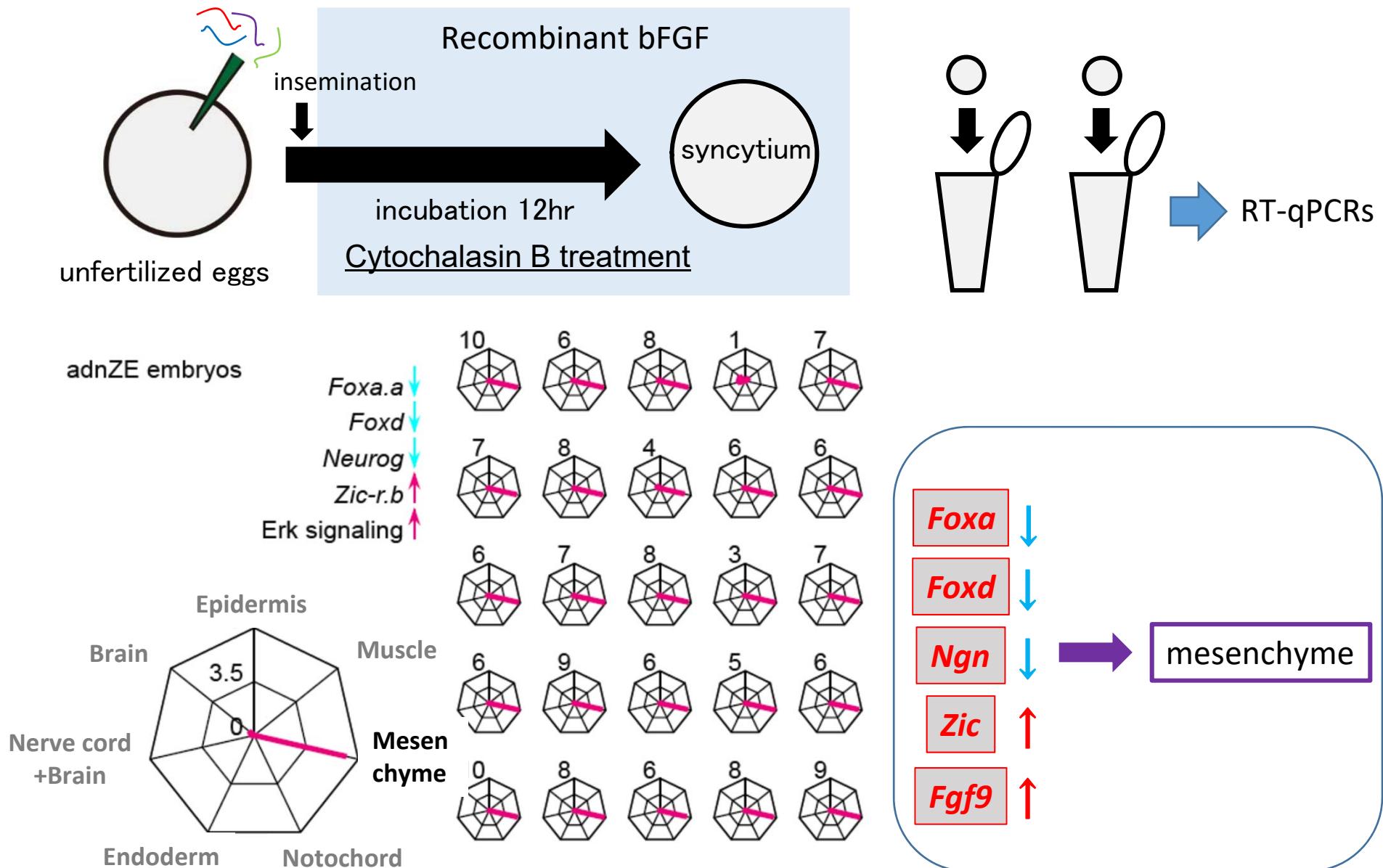
Morpholino antisense nucleotides against *Foxd*, *Ngn*, and *Zic* + mRNA for *Foxa*

Collaboration with Lab. of Dr. Yutaka Sato (Kyoto U.)



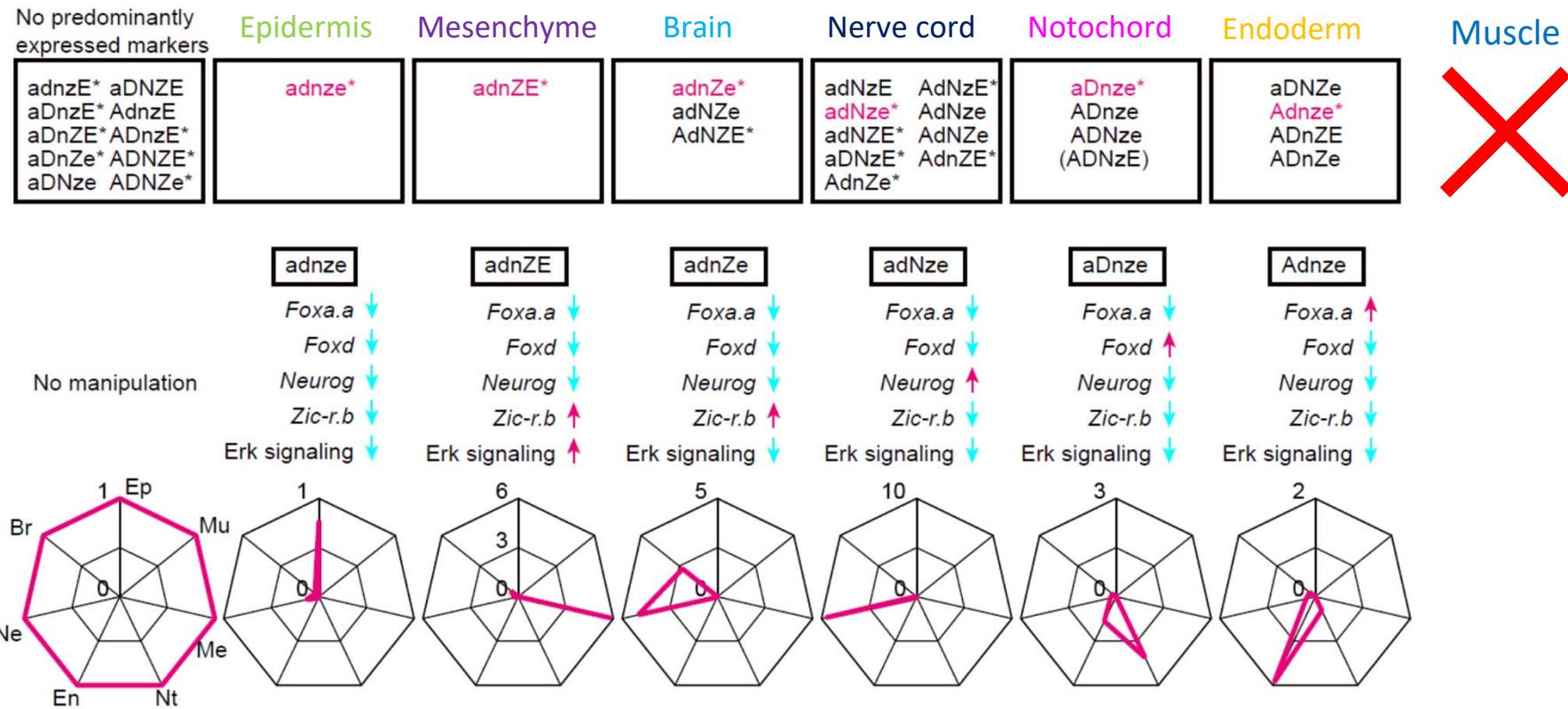
-Manipulation of the FVS genes in Single-cell system-

Morpholino antisense nucleotides against *Foxa*, *Foxd*, and *Ngn* + mRNA for *Zic*



-Manipulation of the FVS genes in Single-cell system-

Summary of the results



Almost all cell types except for muscle are reproduced by manipulation of the five FVS molecules.

Unknown regulatory links related to determination of muscles?

Structural Theory (1): Linkage Logic

Structure \Leftrightarrow Dynamics

Feedback vertex set (determined from networks)

- (1) minimal sufficient set of variables
 - to detect all of the possible dynamical behaviors without depending on nonlinear functions.
- (2) minimal sufficient set of variables
 - to control whole system without depending on functions.

Experimental verification using cell-fate determination system.

- 6 states among 7 were successfully reproduced.
- The network is almost complete, but incomplete.

“Theoretical tool” to examine network structures.

- Fiedler, B., Mochizuki, A. et al. *J. Dyn. Differ. Eqns.* (2013) **25**, 563-604.
Mochizuki, A., Fiedler, B. et al. *J. Theor. Biol.* (2013) **335**, 130-146
Kobayashi, Maeda, Tokuoka, Mochizuki, Satou. *iScience* (2018)

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