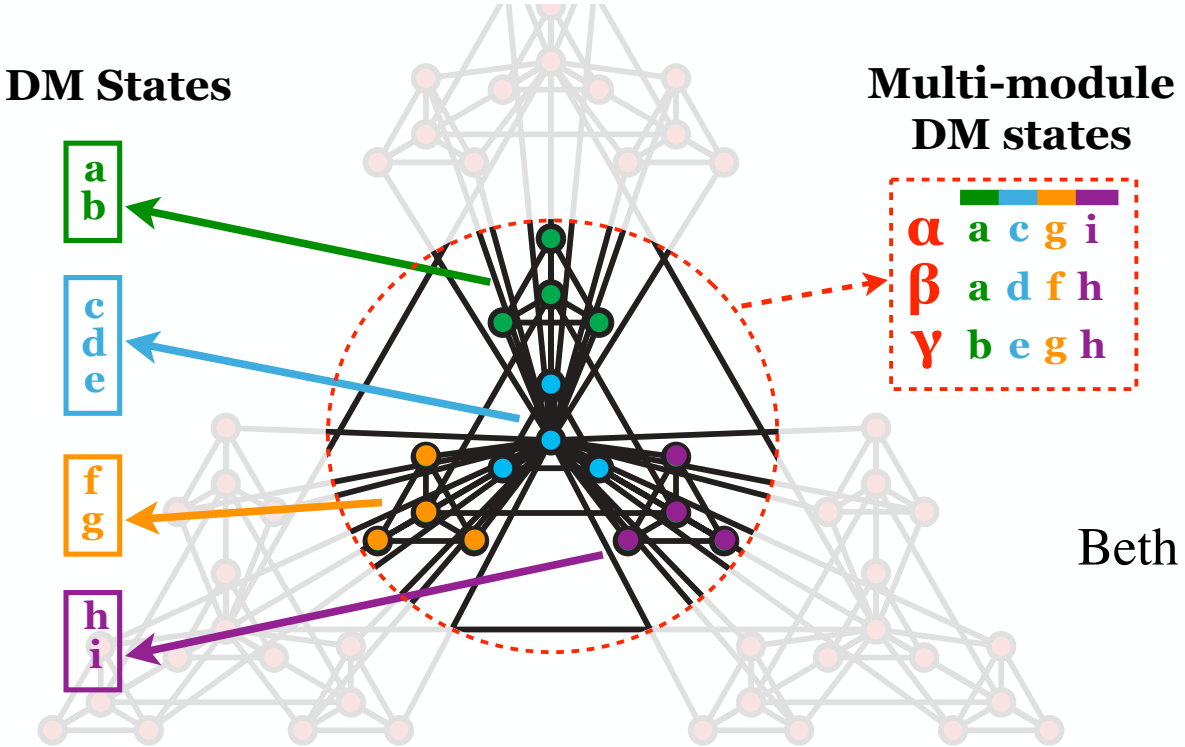
Principles of dynamical modularity in biological regulatory networks

Erzsébet Ravasz Regan

Biochemistry and Molecular Biology

THE COLLEGE OF WOOSTER



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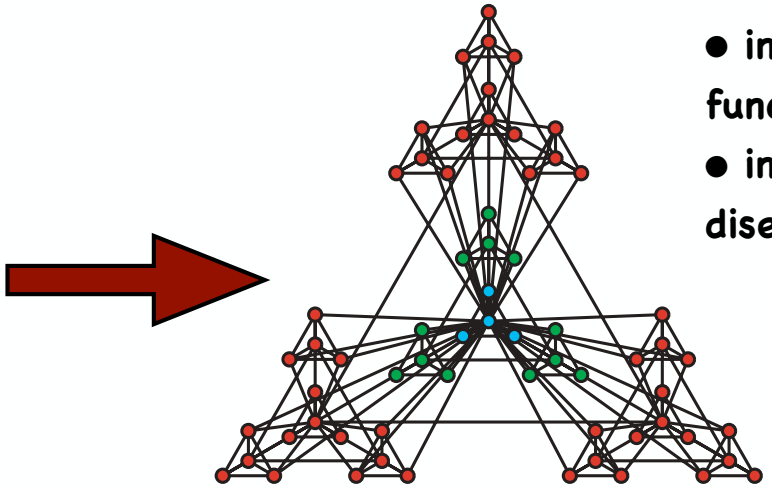
Biological networks are hierarchically modular

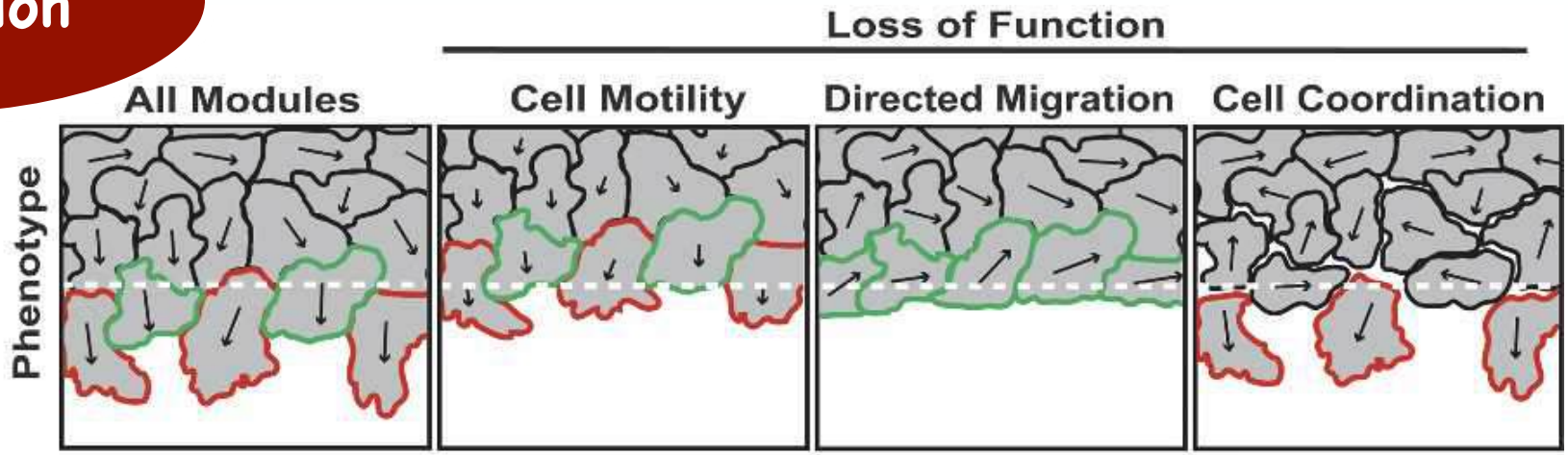
In structure



- in network connectivity (structure)
- in association between nodes and function
- in association between nodes and disease

Ravasz, E., Somera, A. L., Mongru, D. A., Oltvai, Z. N. & Barabási, A.-L. *Science* **297**, 1551–1555 (2002).

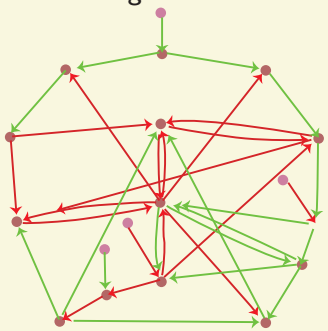
In function



Vitorino, P. & Meyer, T. *Genes & Development* **22**, 3268–3281 (2008).

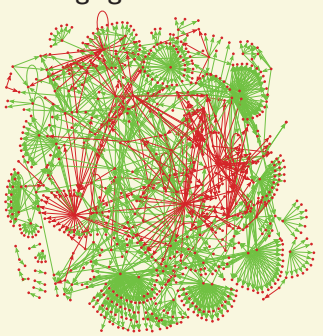
What types of regulatory networks generate MODULAR phenotype combinations?

Mid-size genetic network



phenotypes → Phenotype-combinations ?

Large genetic network



phenoptypes = stable, robust dynamical states or processes => **attractors**

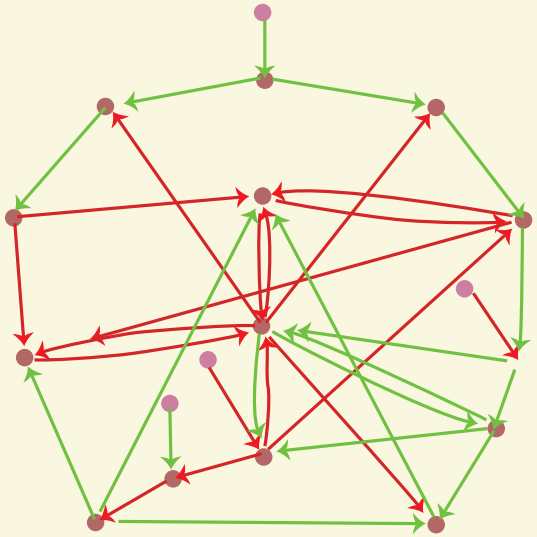
- Proliferation
- Migration
- Apoptosis
- Differentiation

- Dividing (alive) liver cell
- quiescent (alive) neuron

cell-wide phenoptypes = attractors

Problem: how do small circuits (responsible for specific phenotypes) work INSIDE large changing networks?

Mid-size genetic network



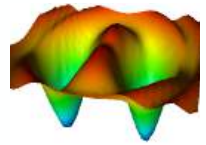
Large genetic network

General principles ?

module characteristics ?

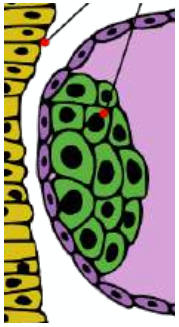
how are modules wired in ?

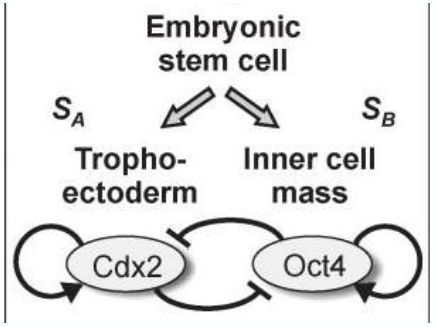
Discrete phenotypes are governed by switches



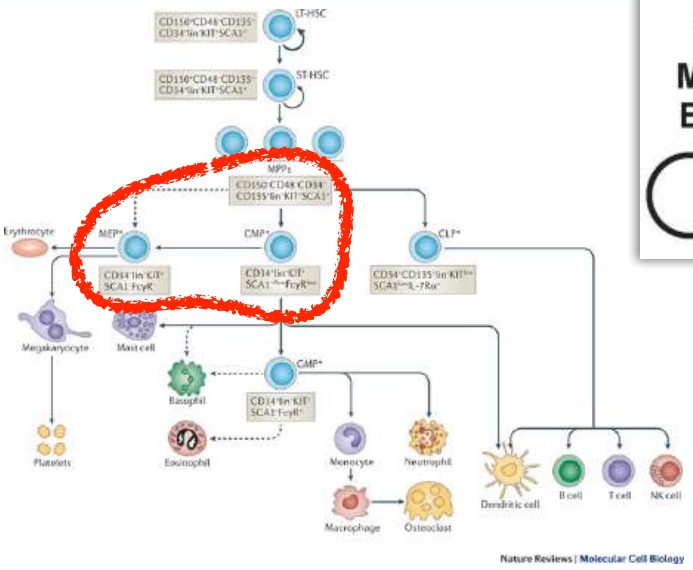
Development

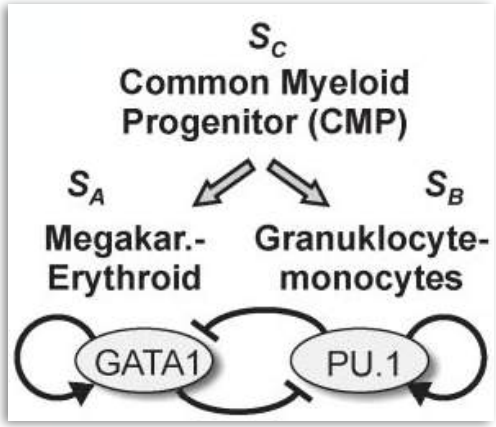
the first differentiation event



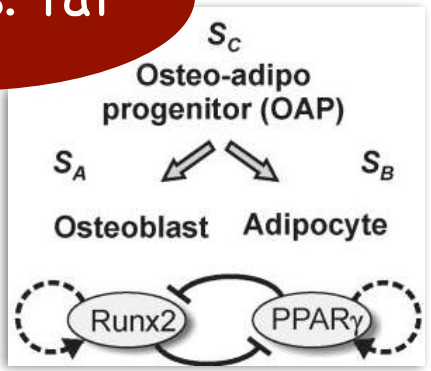


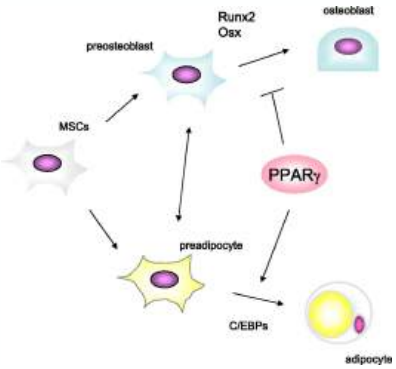
Hematopoiesis



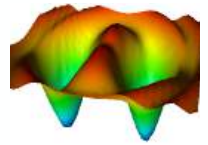


Bone vs. fat

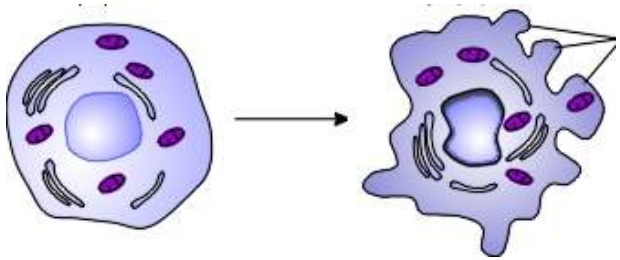




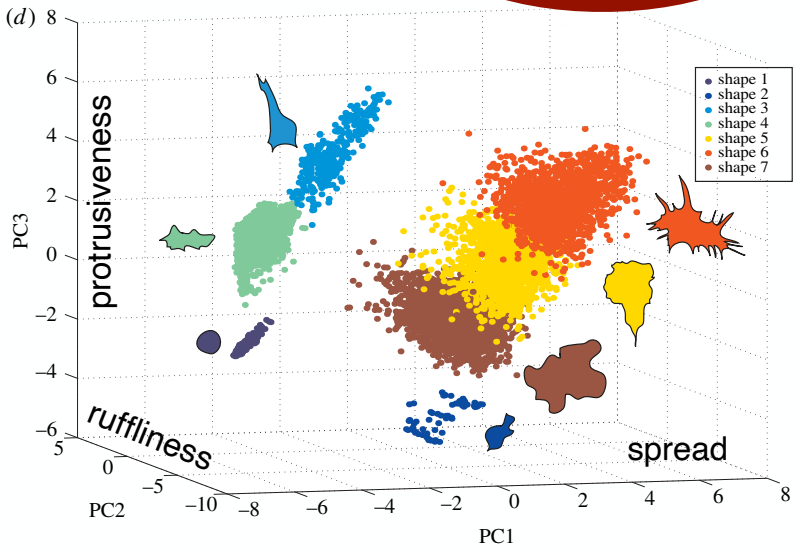
Discrete phenotypes are governed by switches

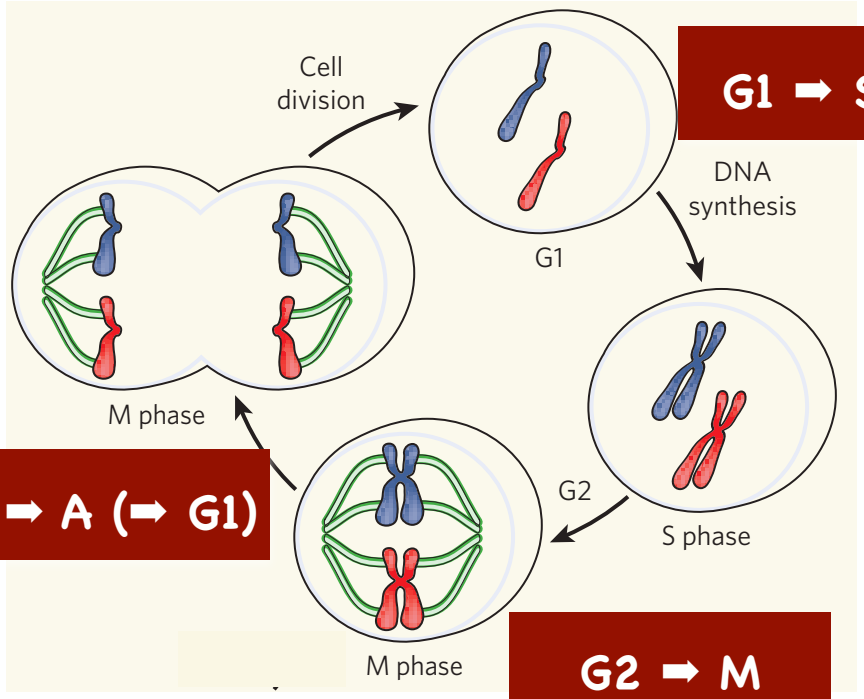


Programmed cell death



Cell shape





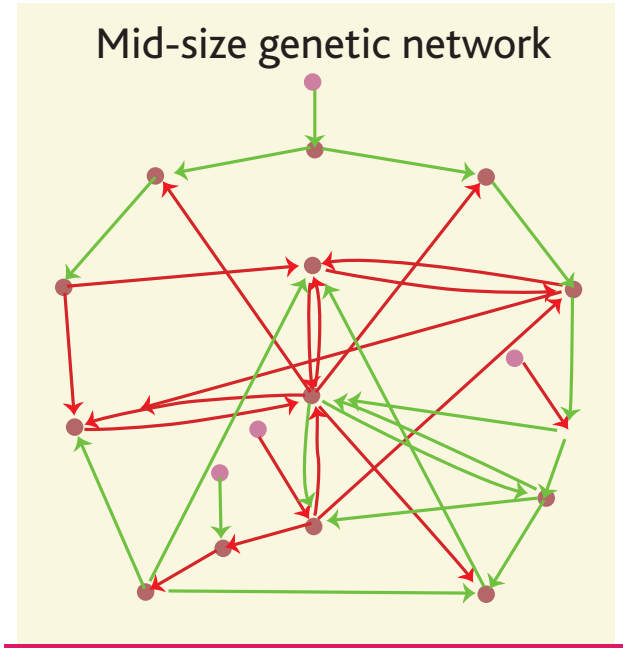
G1 ➡ S

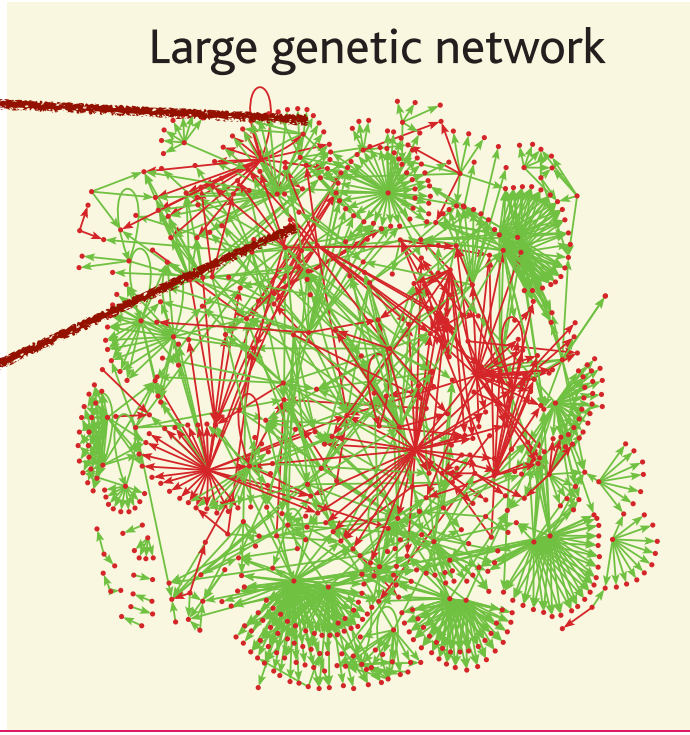
M ➡ A (➡ G1)

G2 ➡ M

G. Bosco, *Nature* **26**:1051,2010;
B. Novak et al., *Nature Cell Biology* **9**:724, 2007;
D. Madar et al., *BMC systems biology* **7**:136, 2013;
Z. Yin et al., *Nature Cell Biology* **15**:860, 2013.

Problem: how do regulatory switches (responsible for specific phenotype-choices) work INSIDE large changing networks?





Phenotype switch

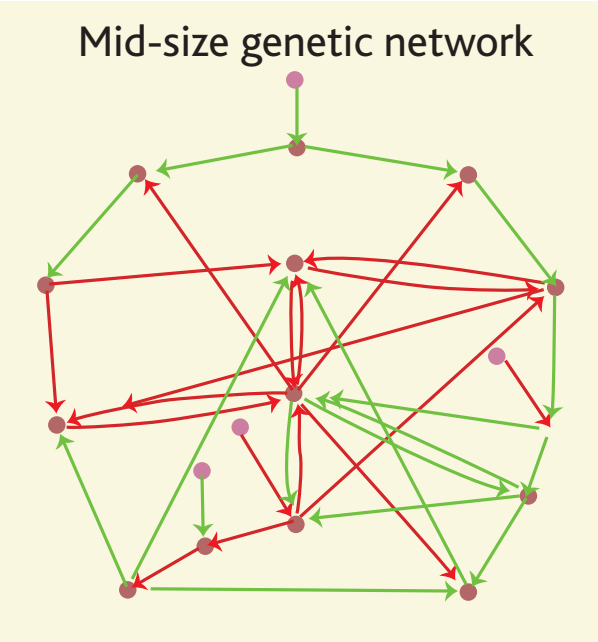
cell cycle entry vs. quiescence

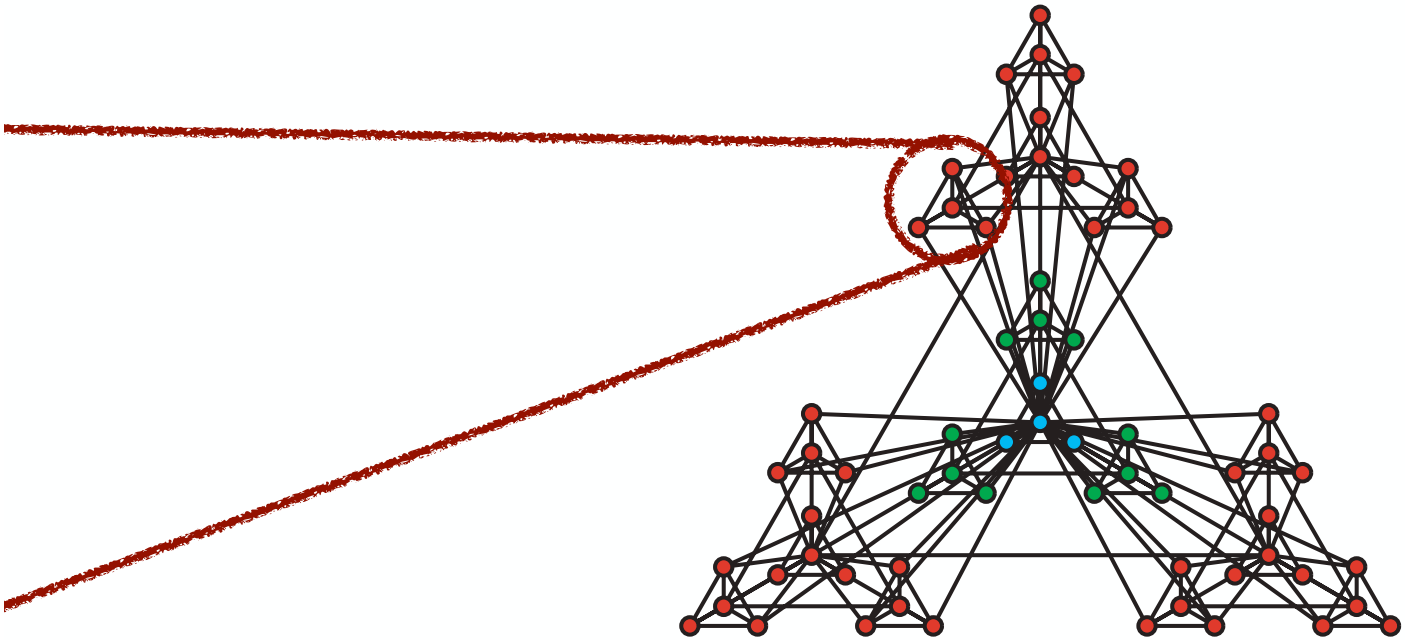
Apoptosis vs. survival

MSC vs. fat vs. bone

how do switches "talk" ?

Problem: how do regulatory switches (responsible for specific phenotype-choices) work INSIDE large modular networks?





Phenotype switch

Phenotype-combination switch ?

cell cycle entry vs. quiescence

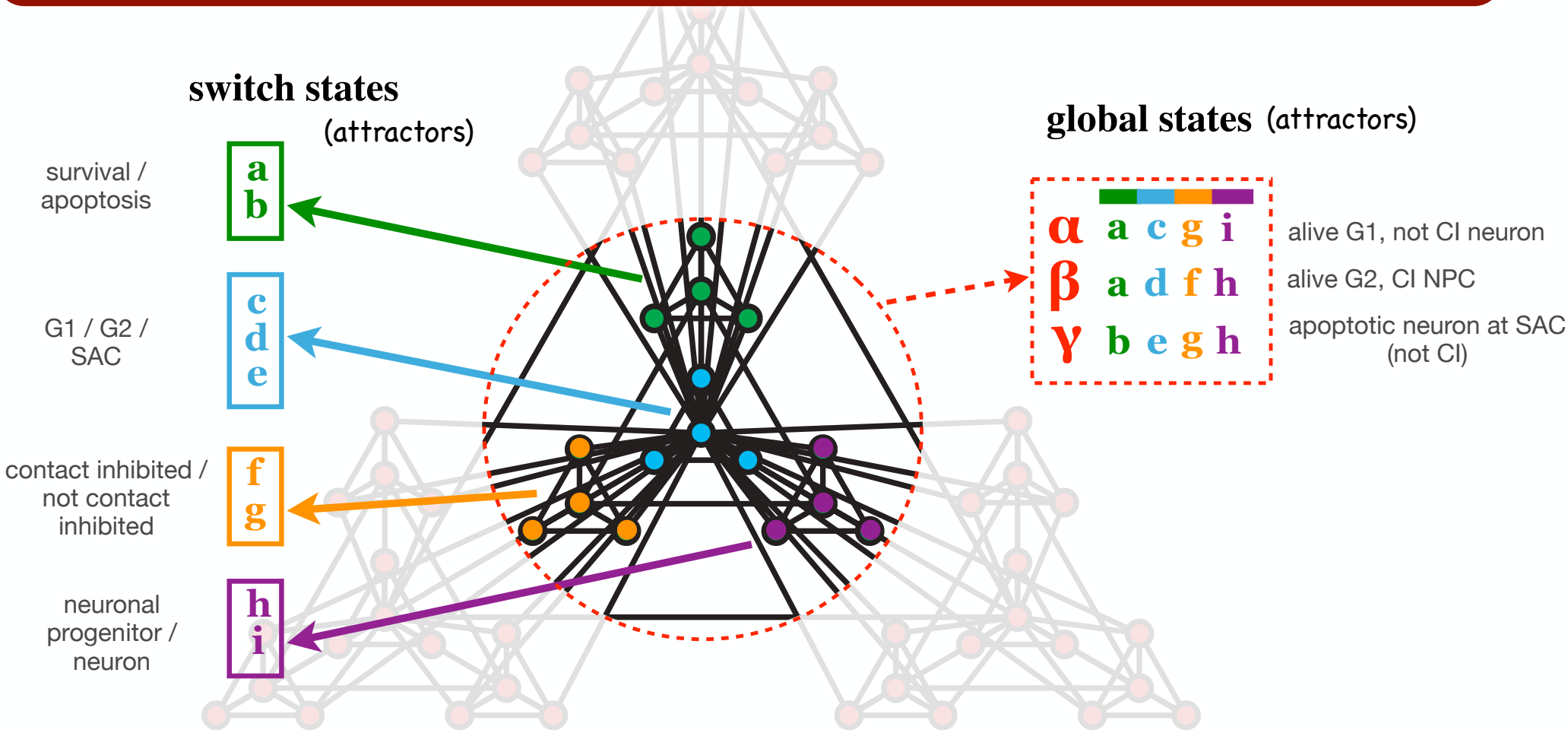
Apoptosis vs. survival

MSC vs. fat vs. bone

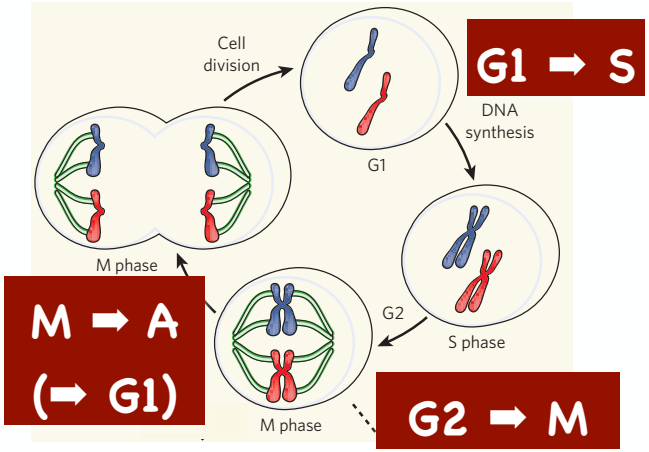
Suggests a general principle that governs how regulatory networks assemble!

Principle of dynamical modularity

Phenotypes of a multi-switch regulatory system = COMBINATIONS OF SWITCH-PHENOTYPES

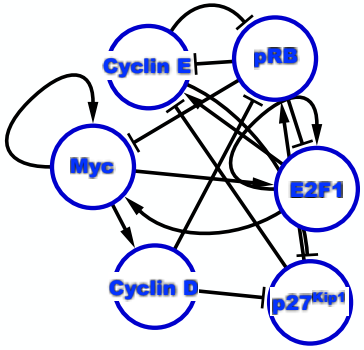


Boolean case study - the mammalian cell cycle

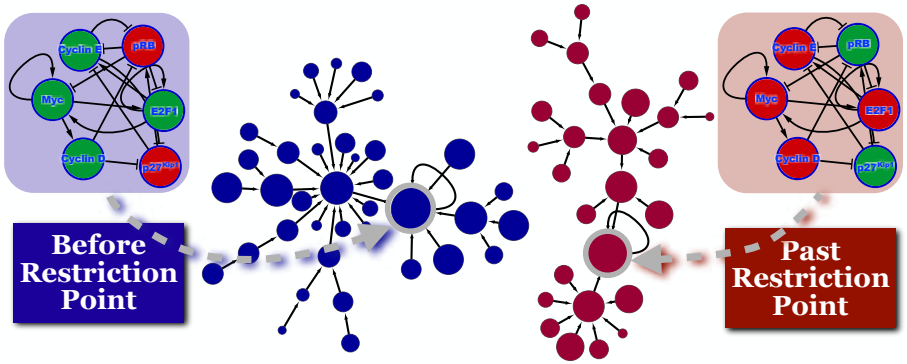


Restriction switch

- G1 -> S



network



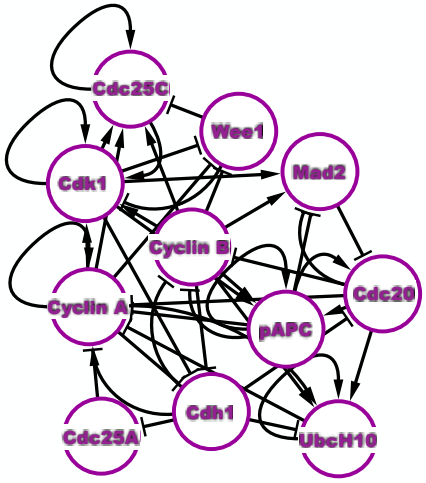
attractor 1

Boolean state transition graph

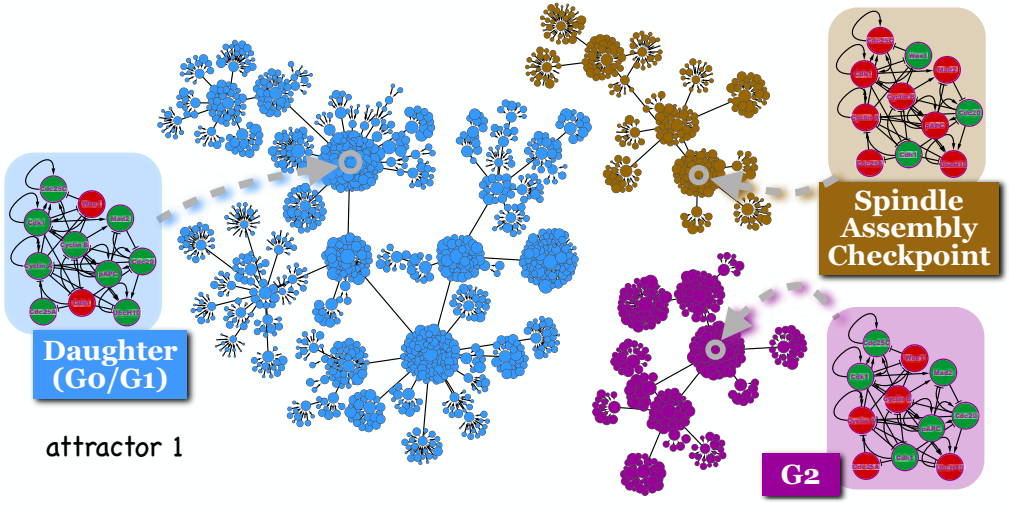
attractor 2

Phase switch

- G2 -> M
- Metaphase (SAC)



network

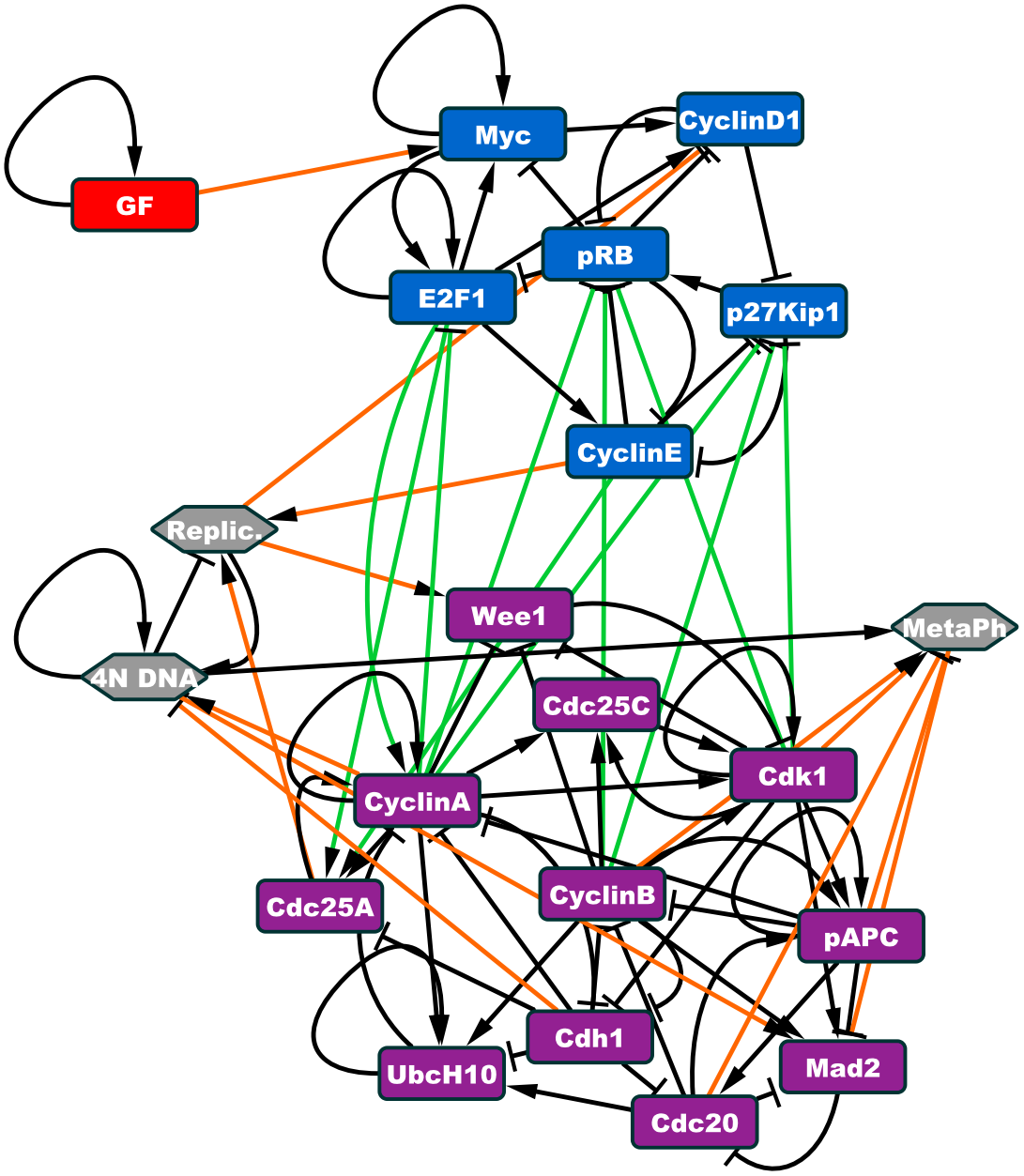


attractor 1

attractor 3

attractor 2

Two-module cell cycle model



Restriction switch

- 2 phenotypes (before/after R-point)

- **2 cell-wide phenotypes**

quiescence

- fixed-point

Cell cycle

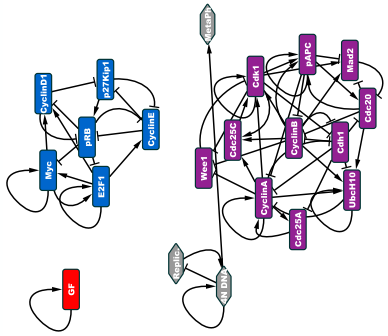
- limit cycle

Phase switch

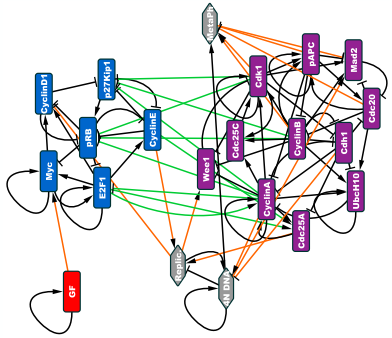
- 3 phenotypes (G1/G2/SAC)

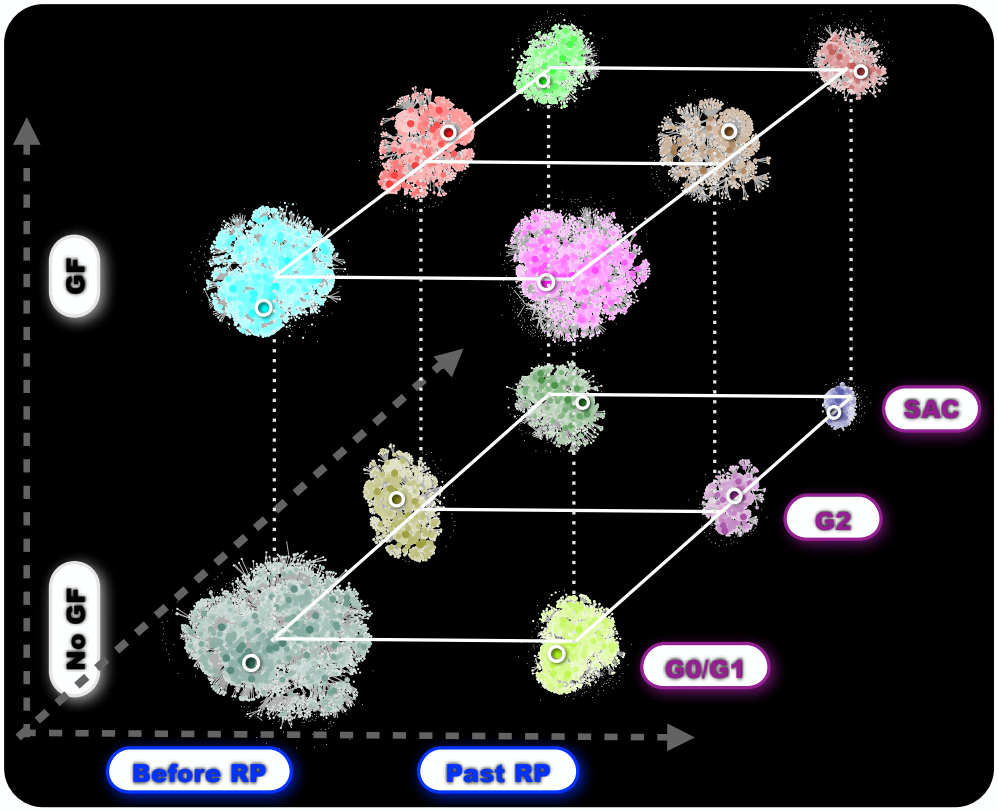
How do the cell cycle switches work together?

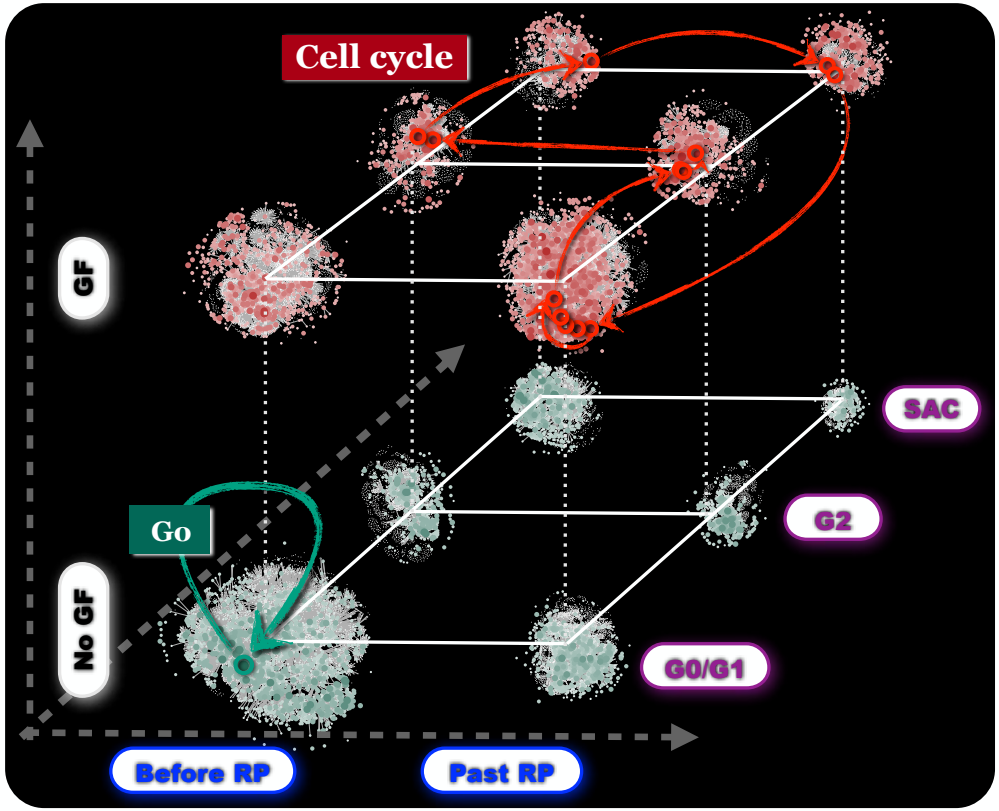
Isolated switches



Couples switches





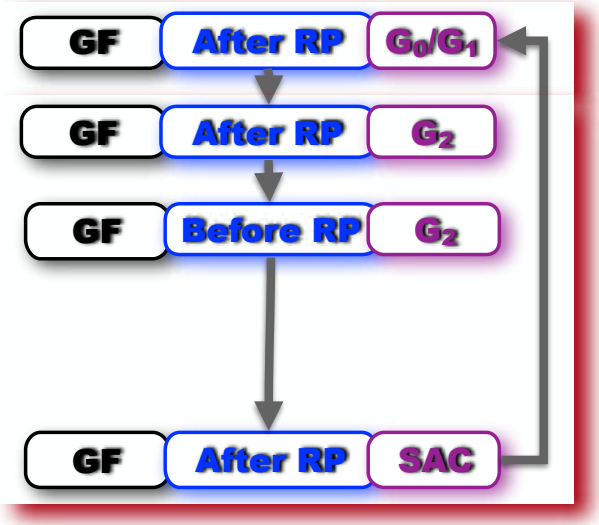


- **nodes of the 3D layout**: global network states (each state has the same position on both panels)

How do the cell cycle switches work together?

Cell cycle

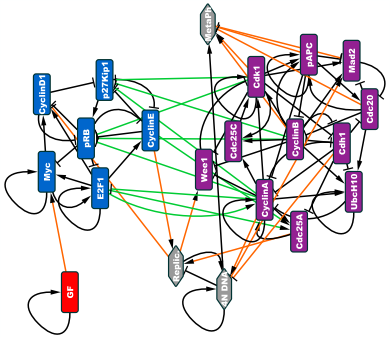
- two switches toggle each other in a global cycle

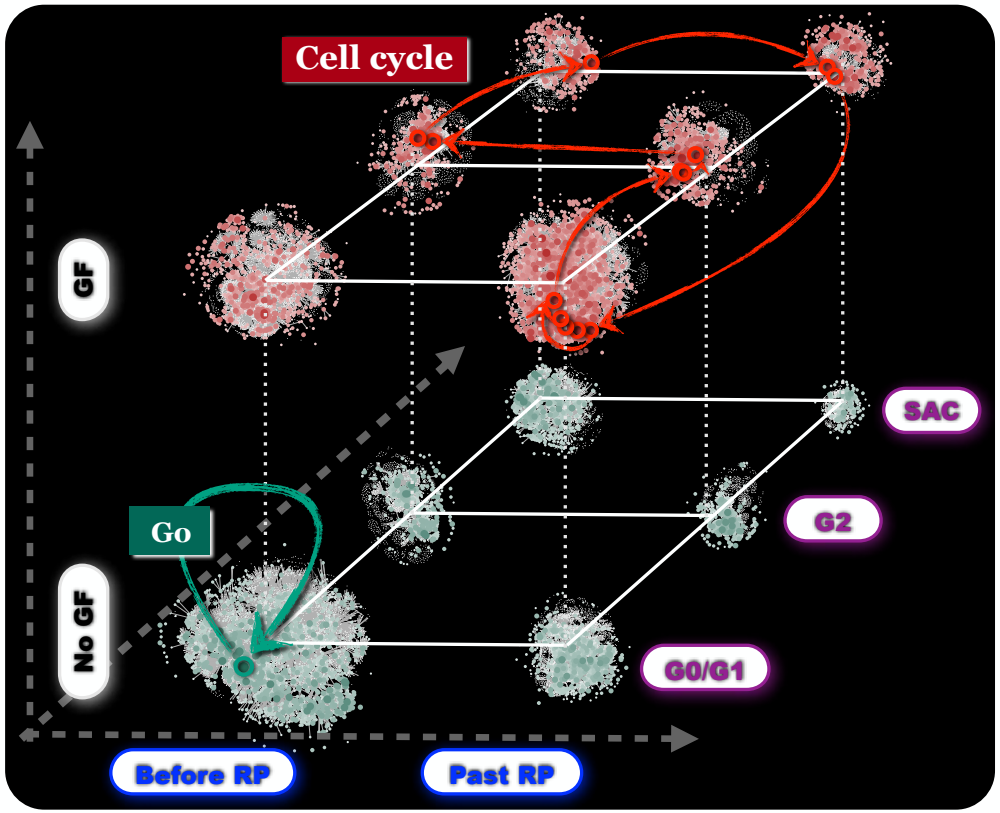


quiescence

- combination of switch-phenotypes

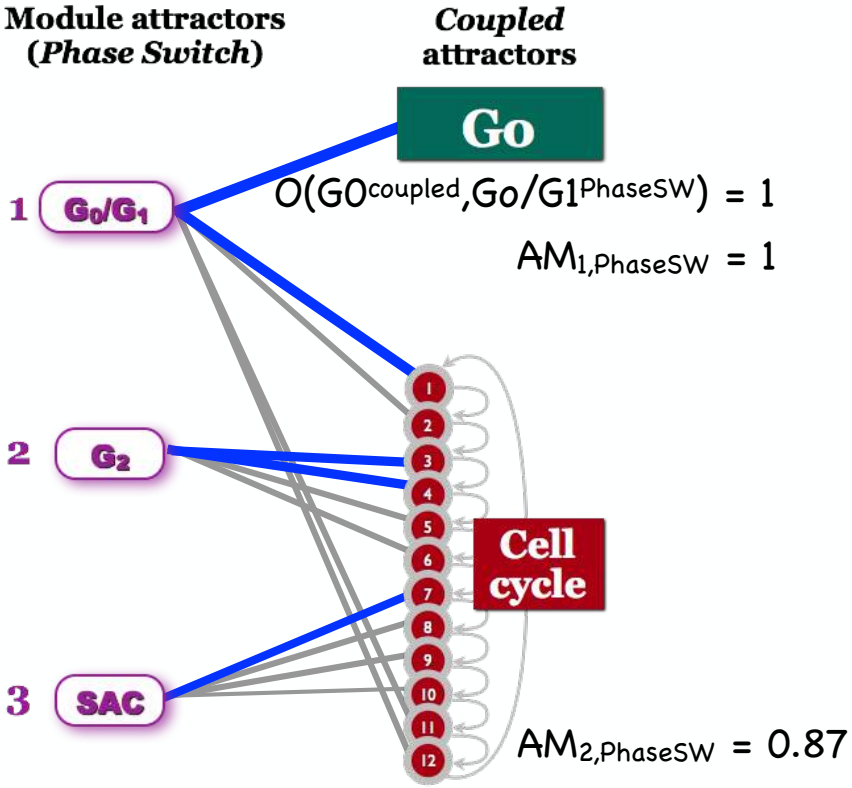
Couples switches





Can we quantify the modularity of global dynamics?

Attractor Modularity Measure (AMM)



$O(G0^{coupled}, Go/G1^{PhaseSW}) = 1$

$AM_{1,PhaseSW} = 1$

$AM_{2,PhaseSW} = 0.87$

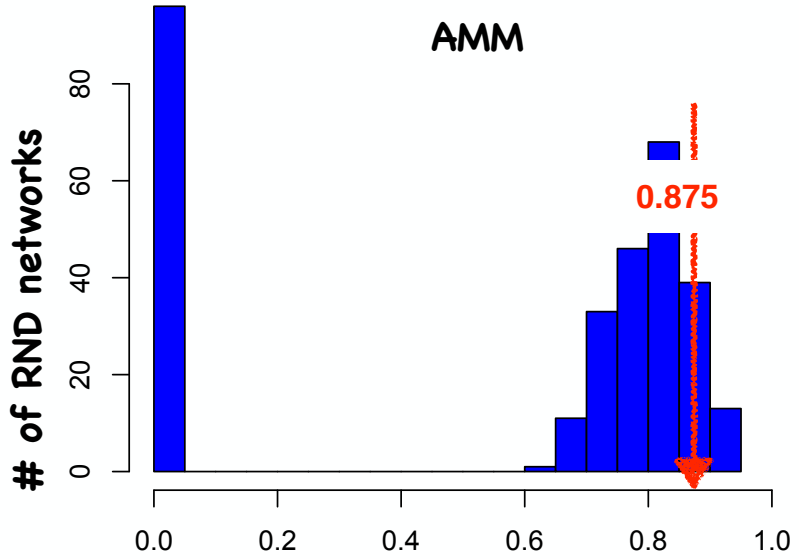
$$AM_{i,m} = 2 \cdot \prod_{O(Q_i^{\text{coupled}}, Q_j^{\text{m}})>0} \left[\max\left(O(Q_i^{\text{coupled}}, Q_j^{\text{m}}), \frac{1}{2} \right) - \frac{1}{2} \right]$$

$$AM_m = \left[\prod_i AM_{i,m} \right]^{1/q_c}$$

- for the 2-module cell cycle:

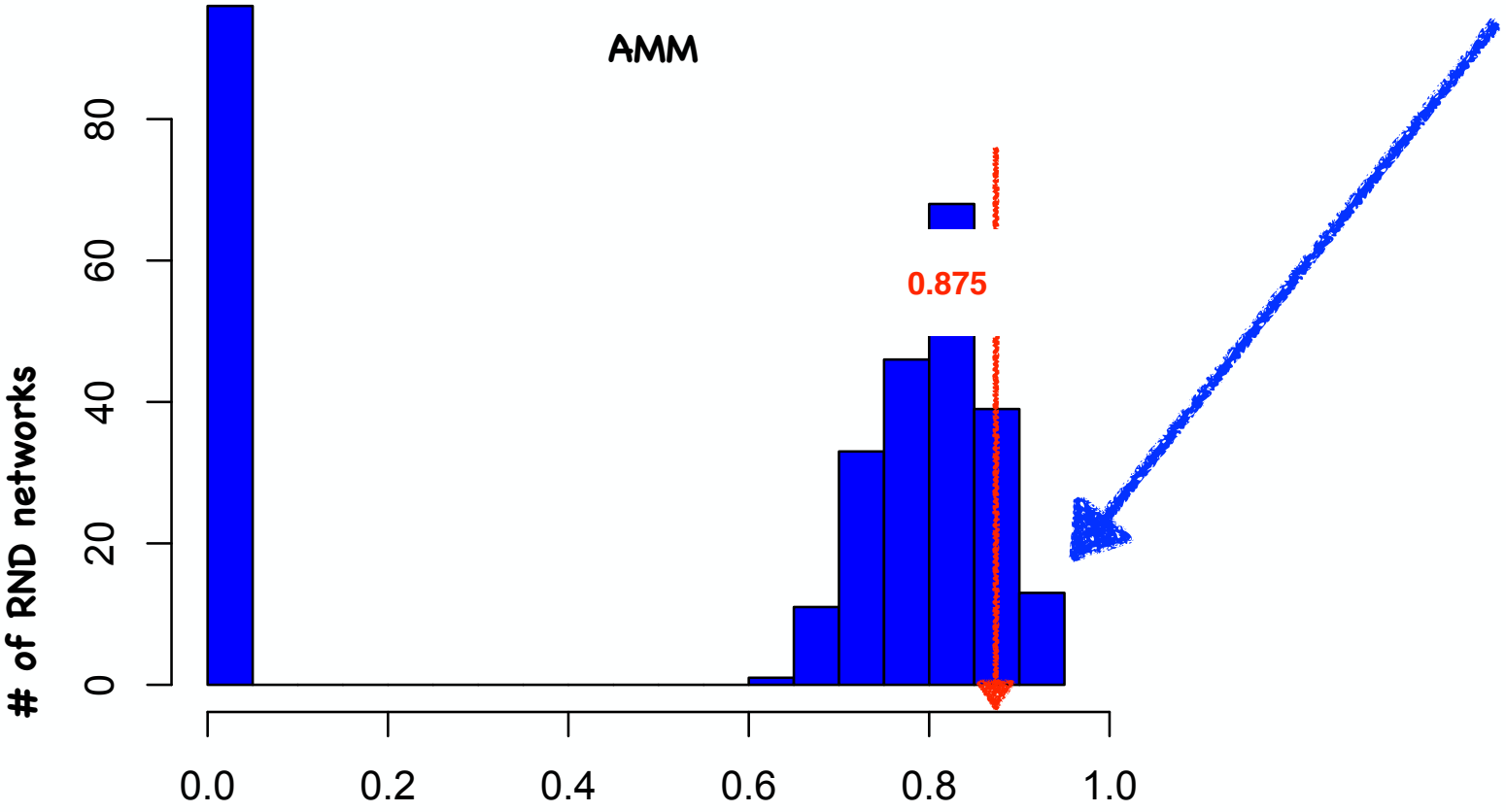
$$AMM = \left(\prod_m AM_m \right)^{1/M} = 0.875$$

- Randomized links between modules



What types of random networks do better than cell cycle?

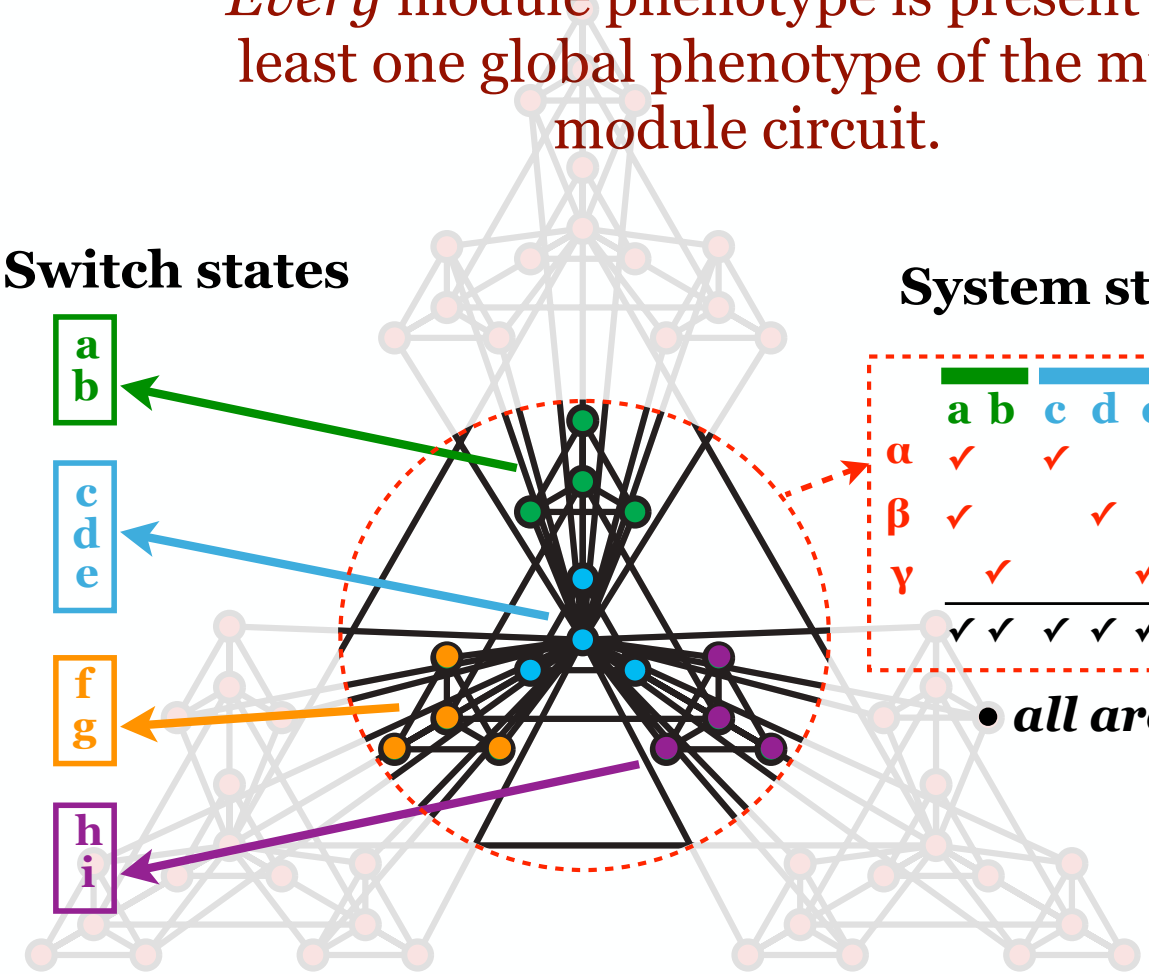
- 1 robust global state!



A biological network shouldn't "loose" a regulatory switch-phenotype!

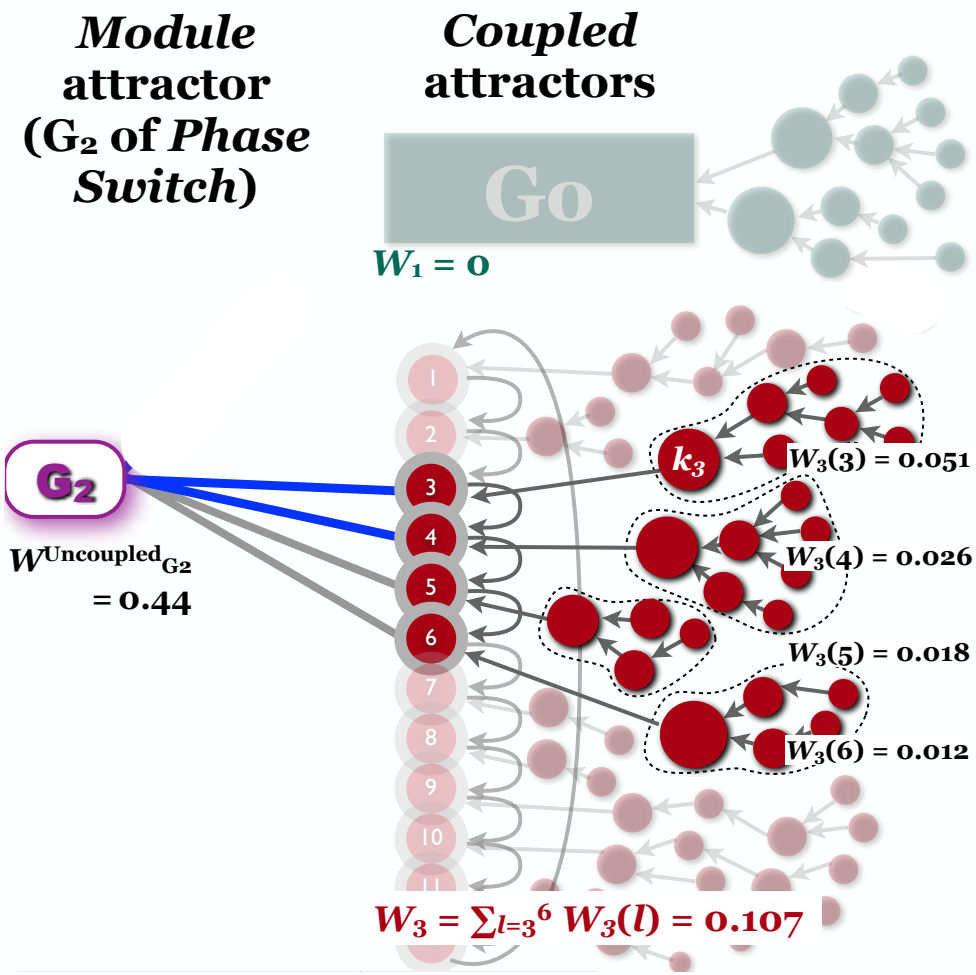
Principle of switch-phenotype relevance

Every module phenotype is present in at least one global phenotype of the multi-module circuit.



- 2*3*2*2 = 24 possible combinations
- only 3 global states

Switch Stability Measure (SSM) in Boolean models



EVERY SWITCH PHENOTYPE APPEARS IN AT LEAST 1 GLOBAL STATE

- Switch Phenotype Stability:

$$PS_{m:j} = \min(PS^{C}_{m:j} / PS^{U}_{m:j}, 1)$$

- Switch Stability for 1 switch:

$$SS_m = \left(\prod_j PS_{m:j}\right)^{1/q_m}$$

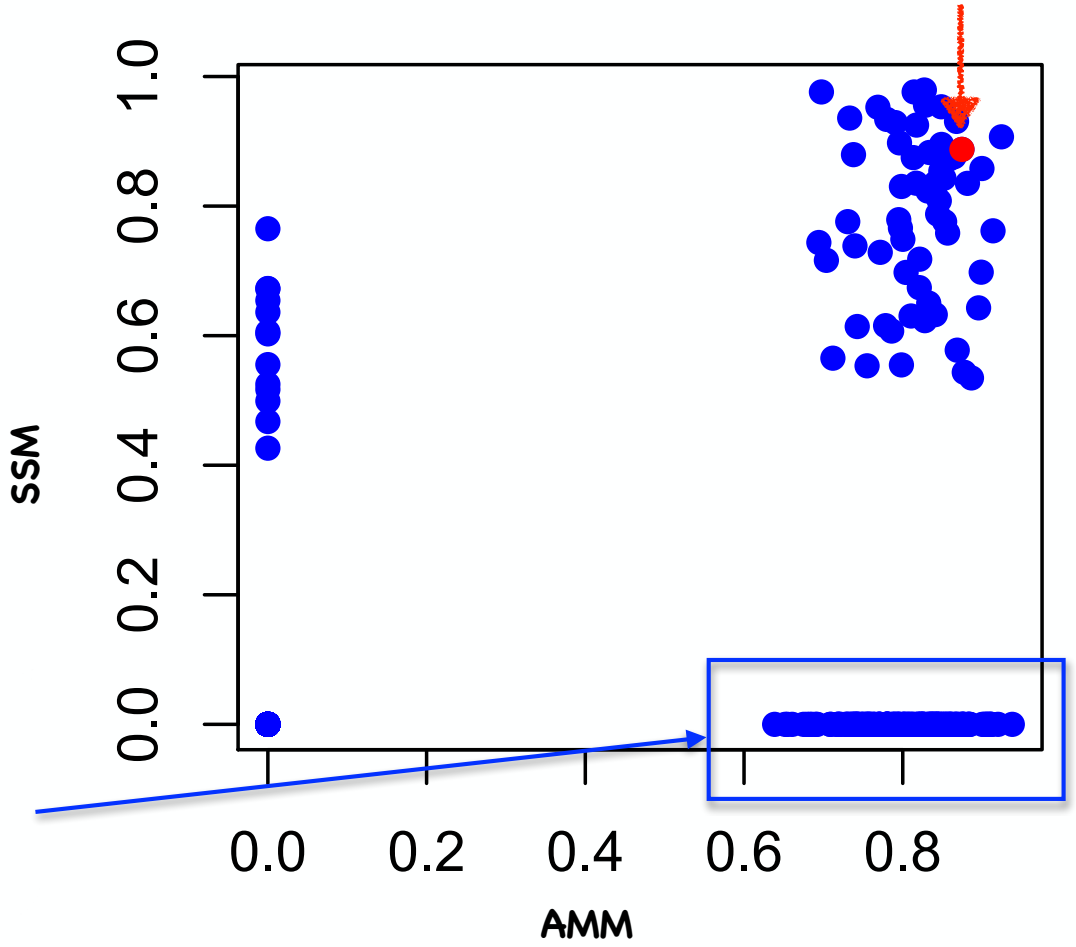
- Switch Stability Measure:

$$SSM = \left(\prod_m SS_m\right)^{1/M}$$

Quantify the relevance of switch phenotypes: Swith Stability Measure (SSM)

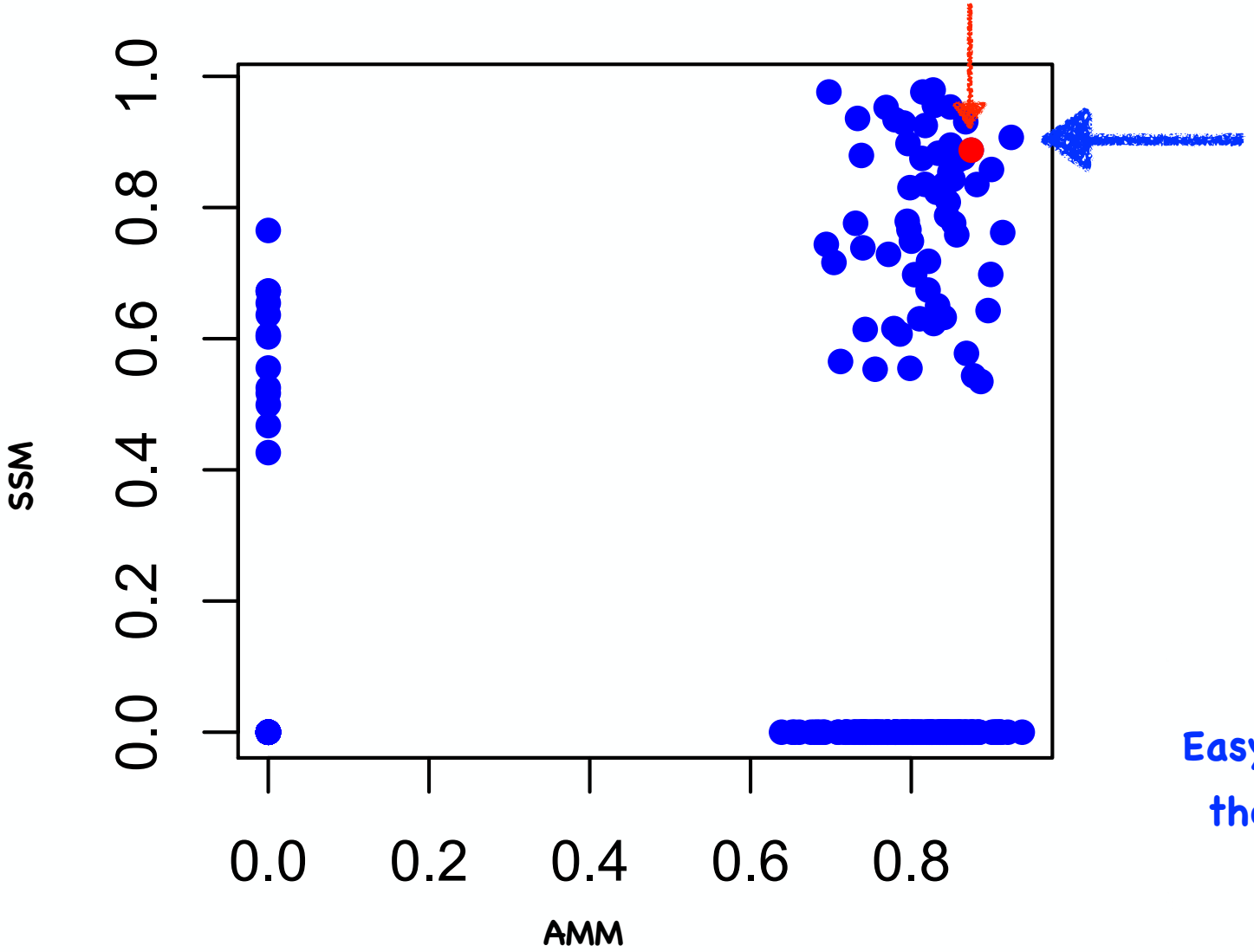
- how much time does the global dynamics spend "expressing" a switch-phenotype?

EVERY SWITCH PHENOTYPE APPEARS IN AT LEAST 1 GLOBAL STATE



Some randomized switch-phenotypes do not appear in any global phenotype

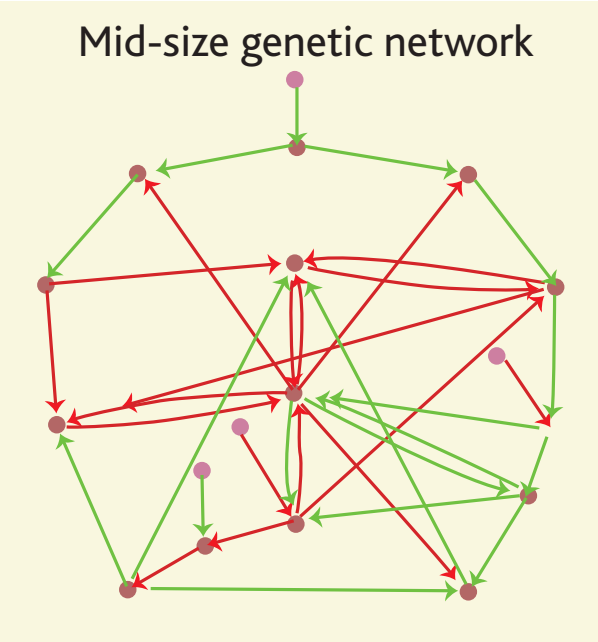
What types of random networks *still* beat the cell cycle?

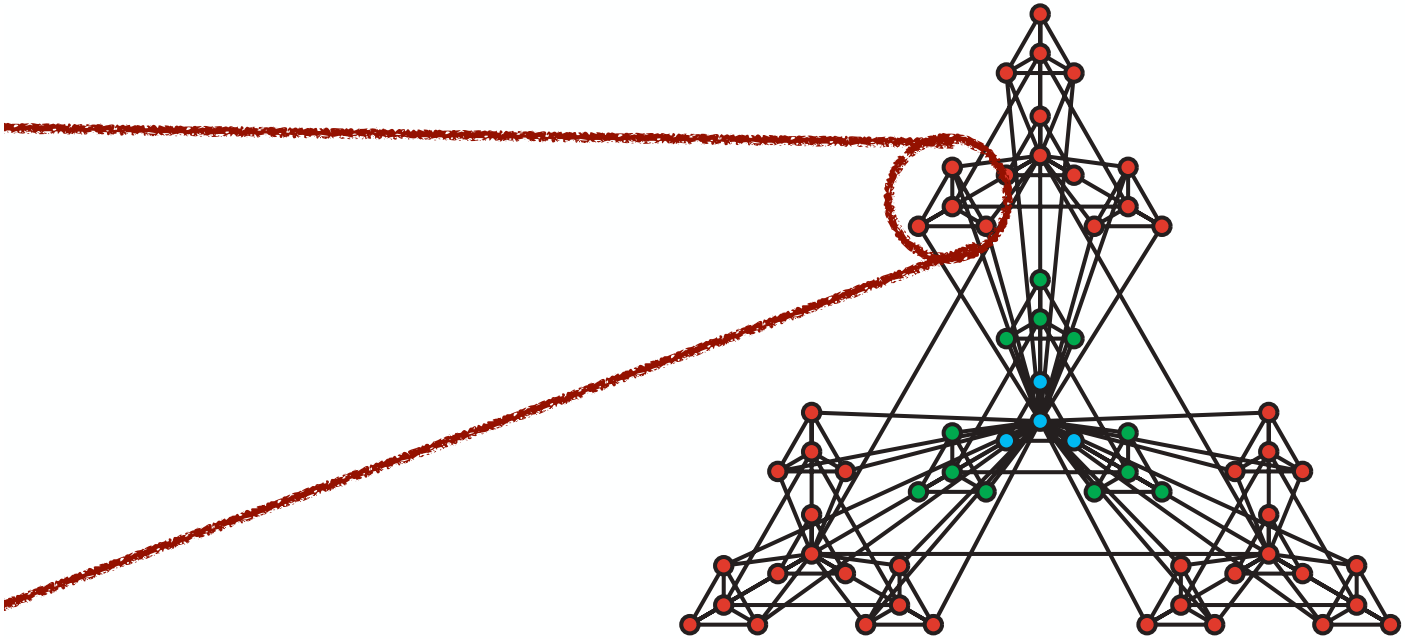


- every switch-phenotype combination is a global state

Easy to follow both rules if the switches "don't talk"!

Independence vs. coordination of module dynamics





Phenotype switch

Phenotype-combination switch

Independence:

- I. modular dynamics
- II. switch phenotype relevance

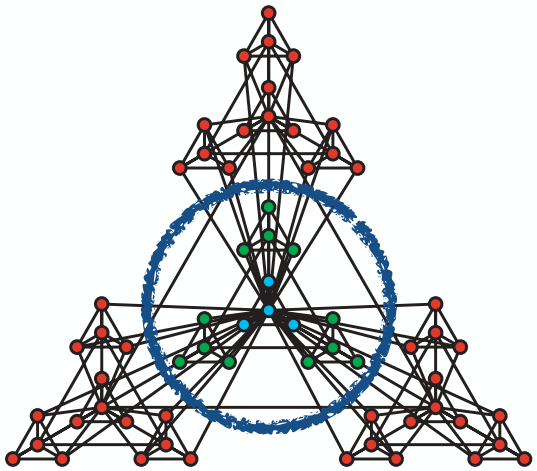
Coordination:

- III. ????

How do switches work in a hierarchy?

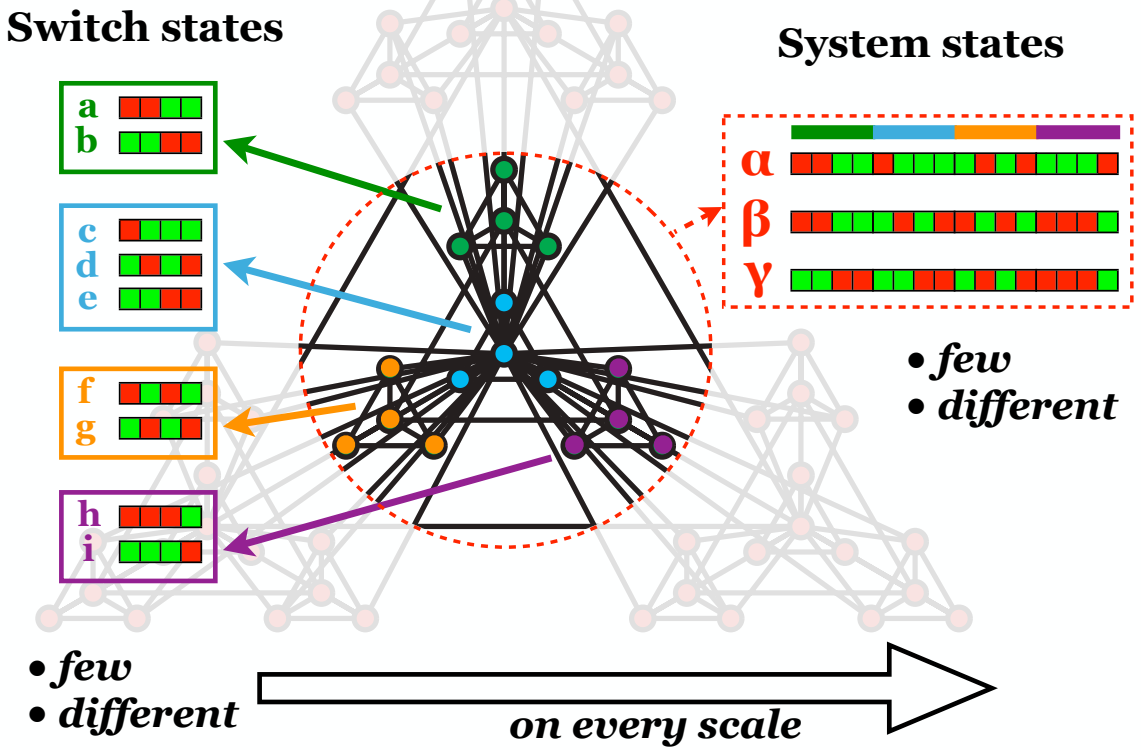
Switches that form a higher-scale module restrict each other's phenotypes

Principle of switch coodination



- tightly coupled!

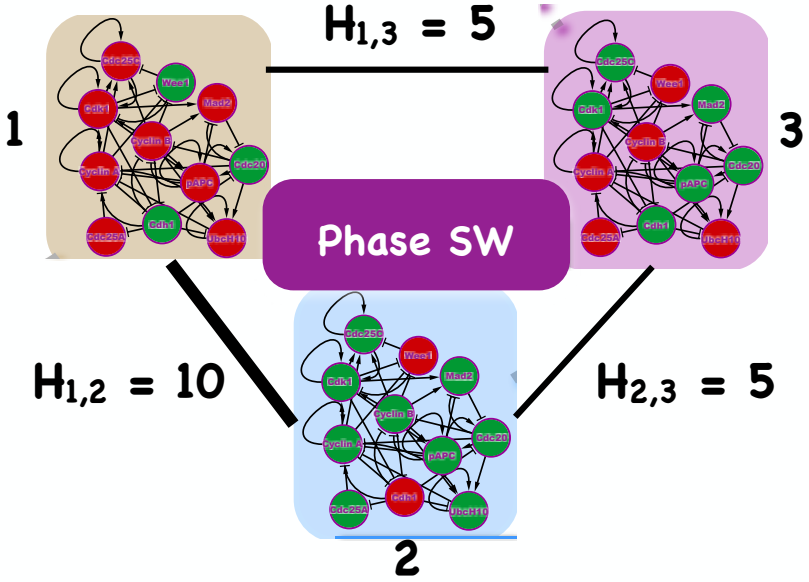
Dynamical Modules at all scales are robust switches with *minimal number* of *radically different* phenotypes.



Module Quality & Coordination (MQC) in Boolean models

MODULES AT ALL LEVELS ARE SWITCHES AMONG FEW, DISTINCT PHENOTYPES

1. How distinct are the phenotypes?



$MC_{PSW} = \langle H_{i,j} \rangle / H_{max}(3)$

2. How strong is the coupling between switches?

Module Coordination Measure

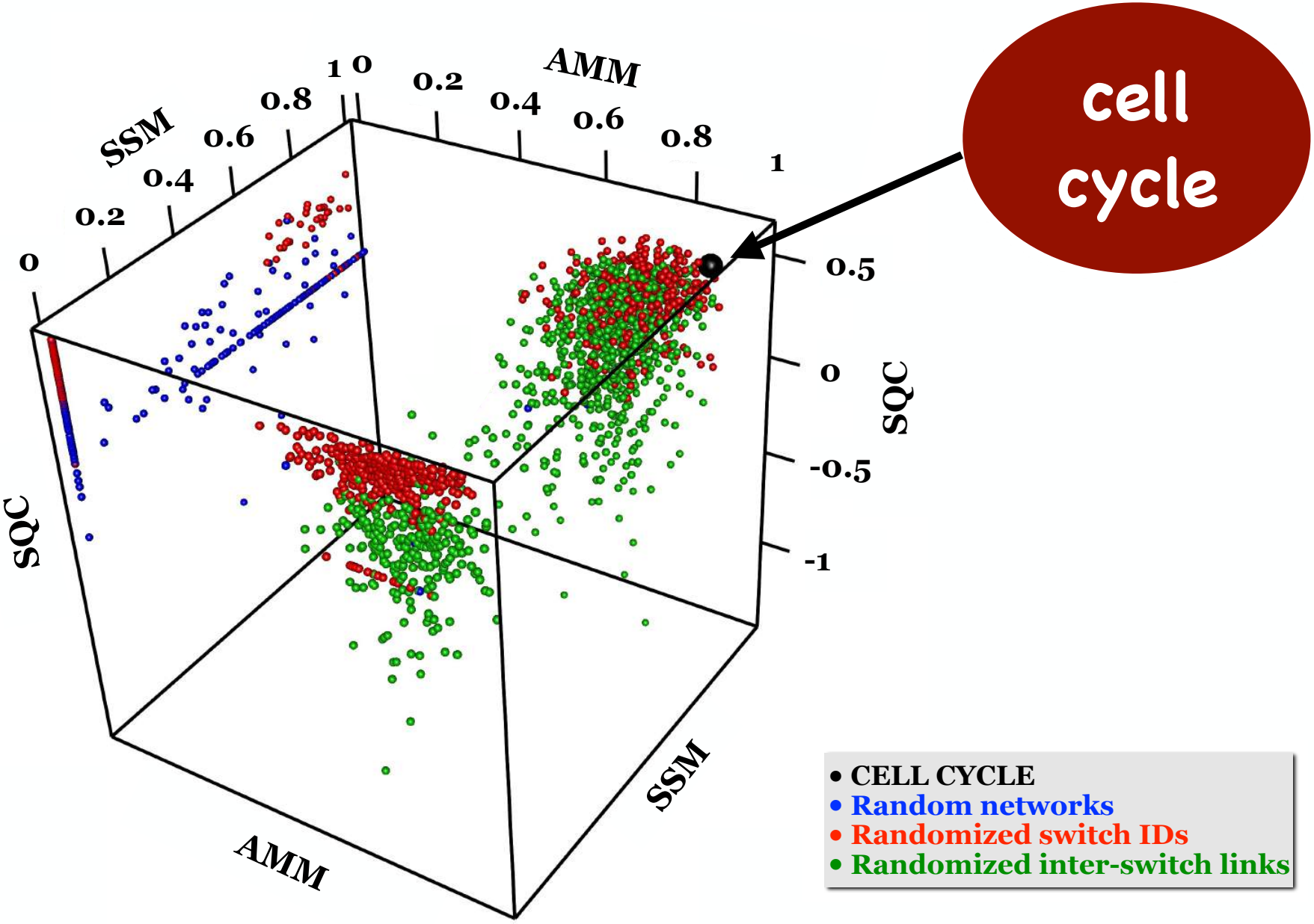
$$MCM = \left[\left(\prod_m q_m\right) - q_c\right] \Big/ \left(\prod_m q_m\right)$$

$$MQC = MQM \cdot MCM$$

Module Quality Measure

$$MQM = MQ_c \left(\prod_m MQ_m\right)^{1/M}$$

Random networks are abysmal at balancing all 3 rules

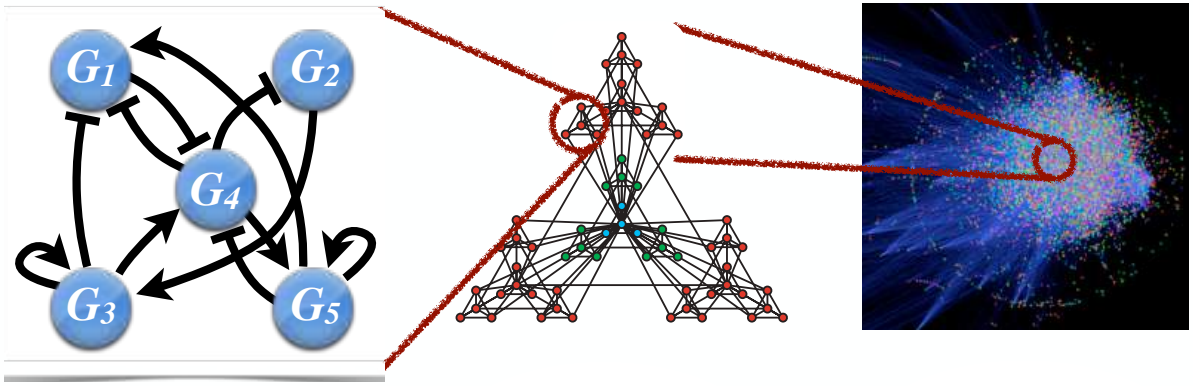


Summary of dynamical modularity in cellular regulatory networks

CORE PREMISE

Regulatory Module =
Discrete Phenotype Switch

Regulatory Network =
Hierarchy of Coupled Switches



MODULE

MODULE OF MODULES

HIERARCHY OF MODULES

I. Phenotypes of a multi-switch system are switch-phenotype combinations
II. Every switch-phenotype is present in at least one global phenotype
III. Dynamical modules at all scales are multistable switches with a small number of radically different phenotypes

Have these insights helped?

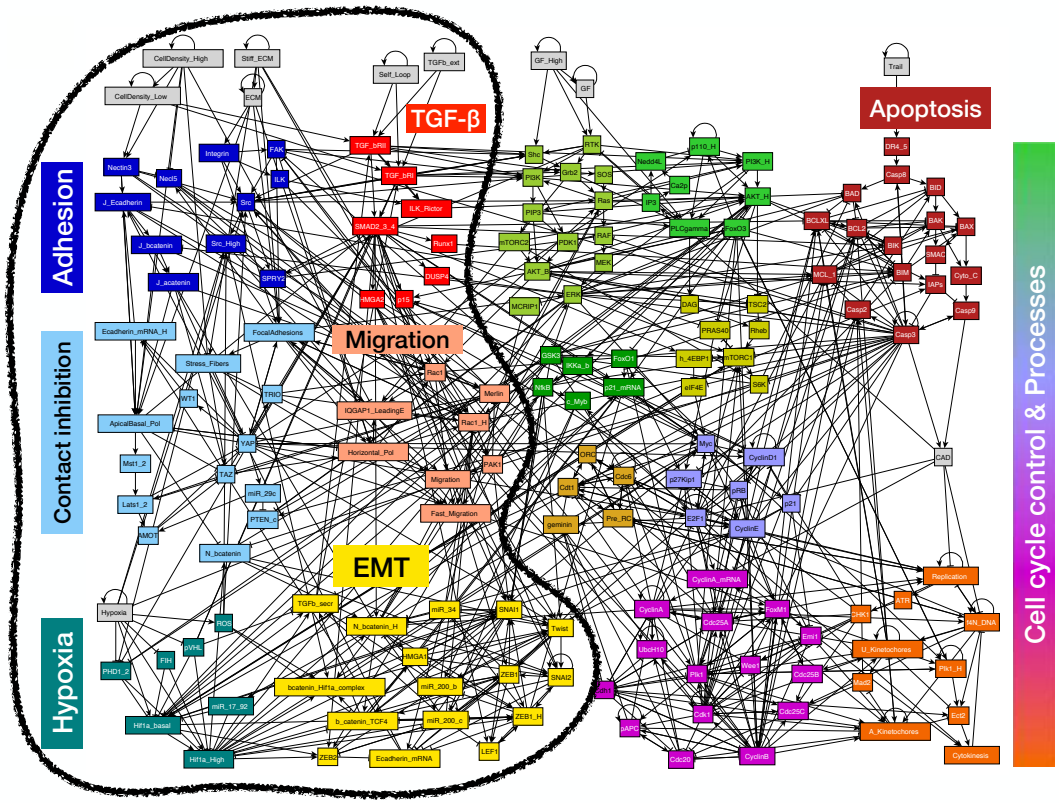
— *current work* —

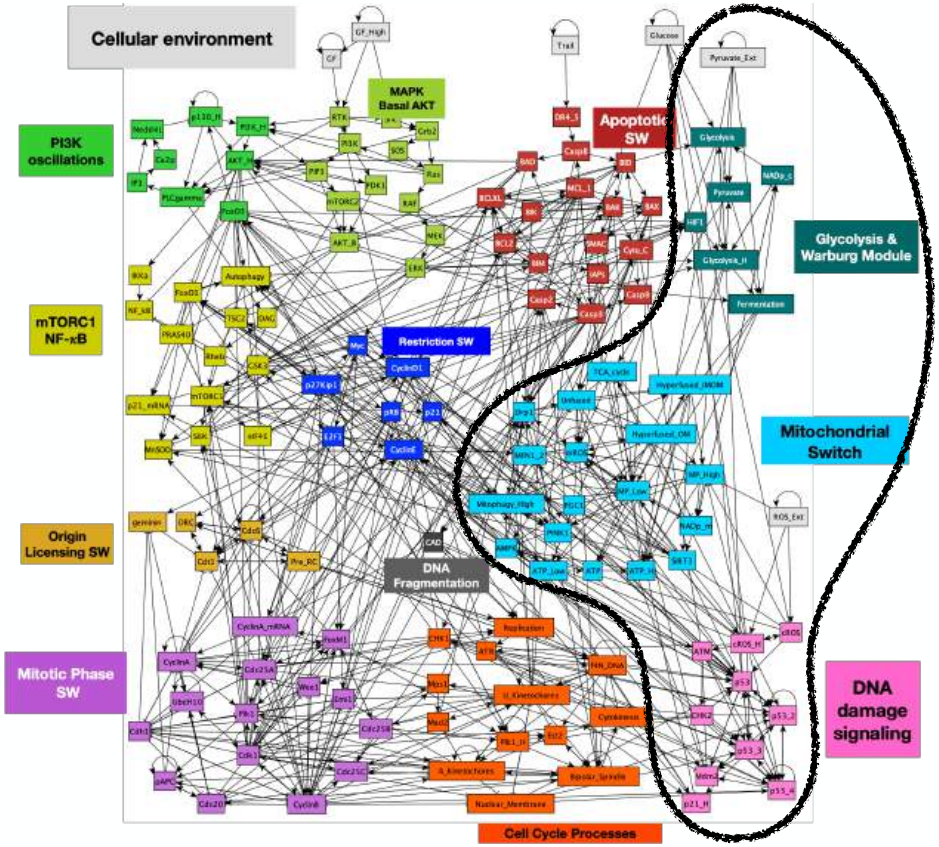
EMT model

- TFGβ
- hypoxia
- biomechanical cues (ECM, density)

MiDAS model

- ROS
- SIRT3 KO
- mito dysfunction



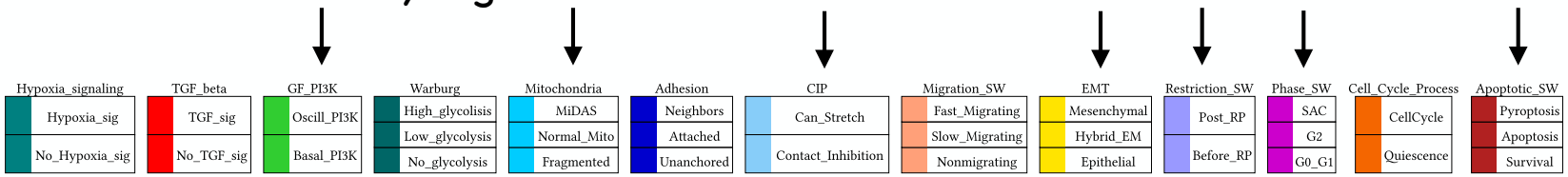


Greene et al, 2025. *PLoS Comp. Biol.*, **21**(4): e1012735.

Sizek et al, 2024. *Translational Oncology*, **49**:102084.

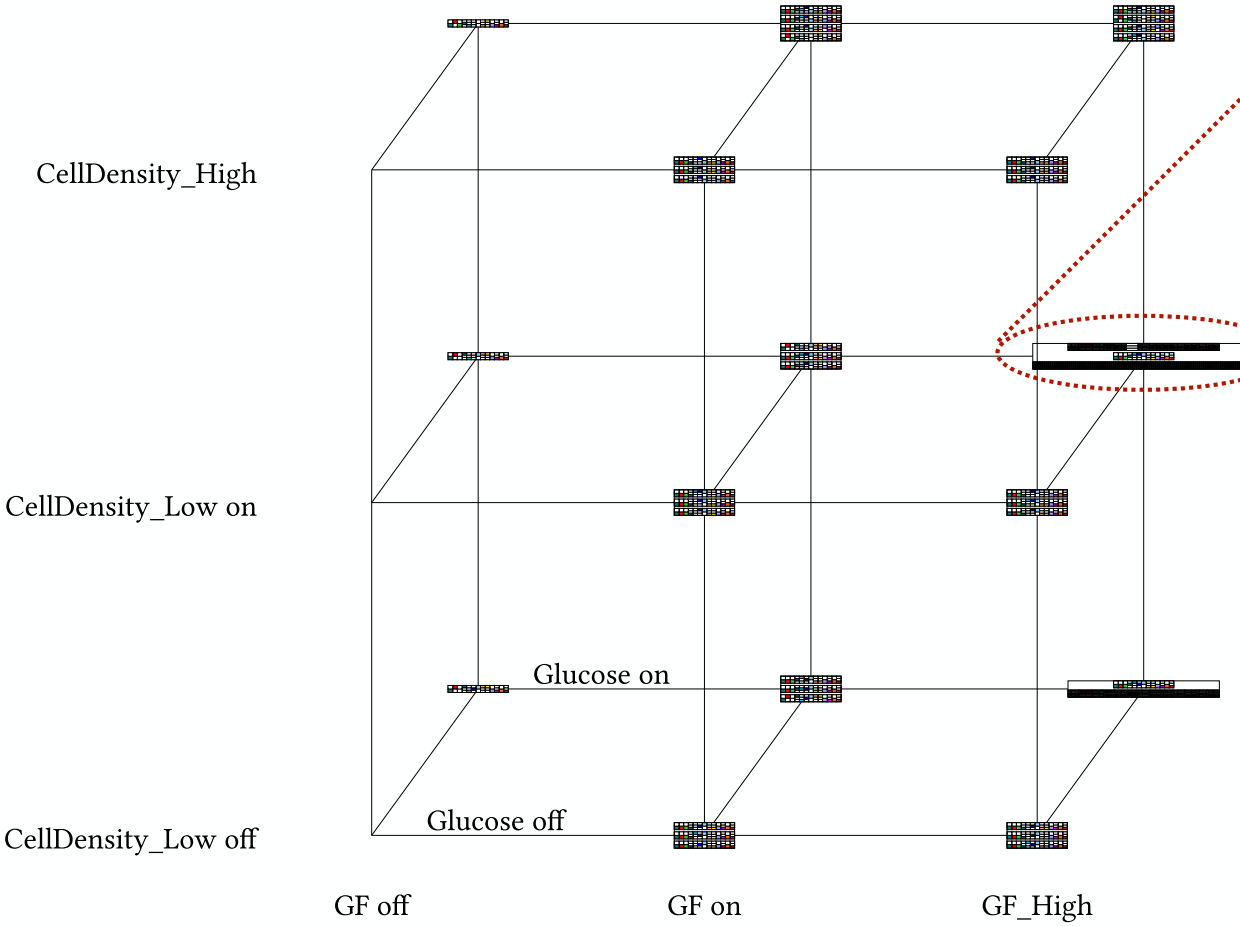
Modular analysis tools

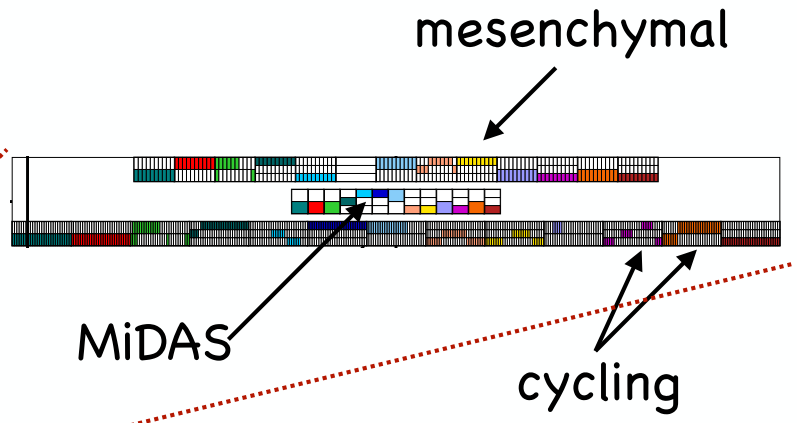
- switch attractors by signature



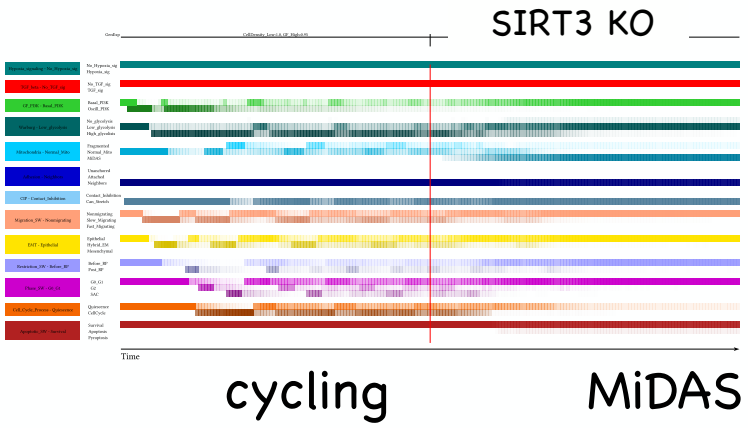
e.g.: Survival = (Casp3:0, Casp9:0, Casp1:0, GSDMD:0)

- model attractors by input signal-combination

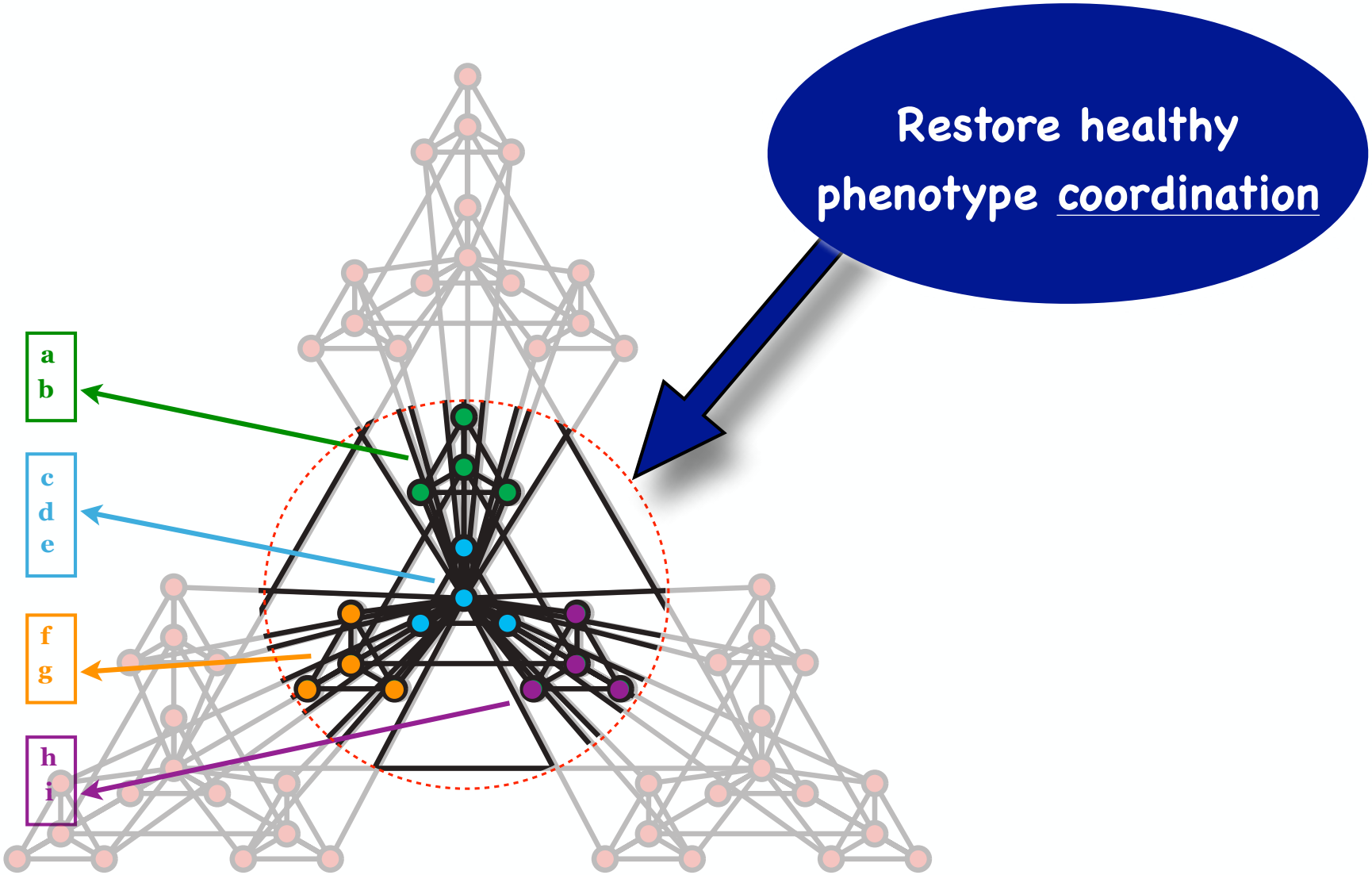




- timecourse in terms of phenotypes

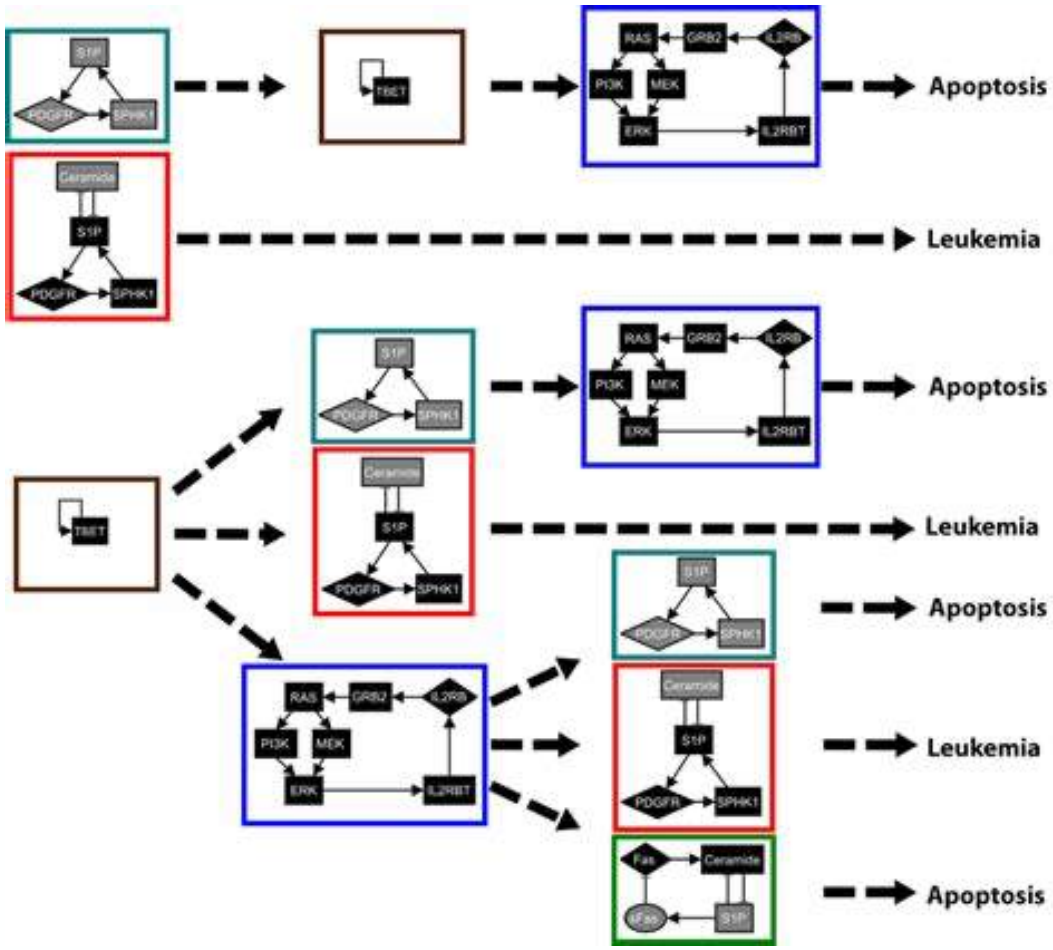


Dynamically modular outlook on restoring heatlhy cell function

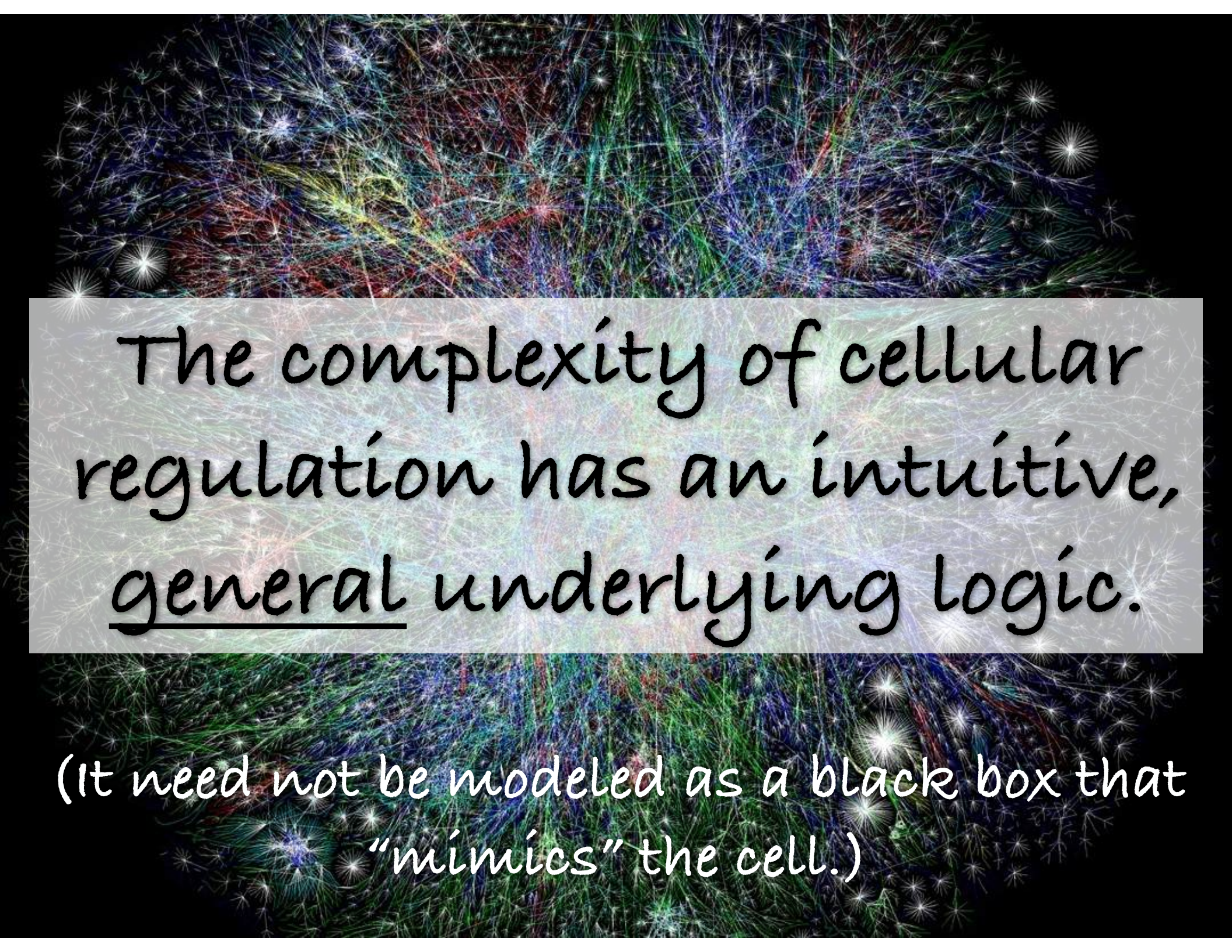


Major questions & future theory work

- how does dynamical modularity relate to **stable motifs?**



- can we use stable motifs to find regulatory modules in large networks?
- dynamical modularity measures for continuous models
- how to conceptualize **parts of a network that are NOT switches**?
  - input signaling cascades that process dynamical info but run one-way
  - "connective tissue" between switches
  - modules with an excitable but robust oscillator (e.g. p53 oscillations)


The complexity of cellular regulation has an intuitive, general underlying logic.
(It need not be modeled as a black box that "mimics" the cell.)

Thank you!

Acknowledgements

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Wooster
— all my undergraduate research students

Papers

Dynamical modularity: *https://www.nature.com/articles/srep21957*

Conditional Stable Motifs: *https://www.nature.com/articles/s41598-019-52725-1*

Mechanosensitive EMT: *https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1012735*

Mitochondrial dysfunciton -> senescence: *https://pubmed.ncbi.nlm.nih.gov/39163758/*