From gene expression du genetic network

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My name is Françoise Monéger

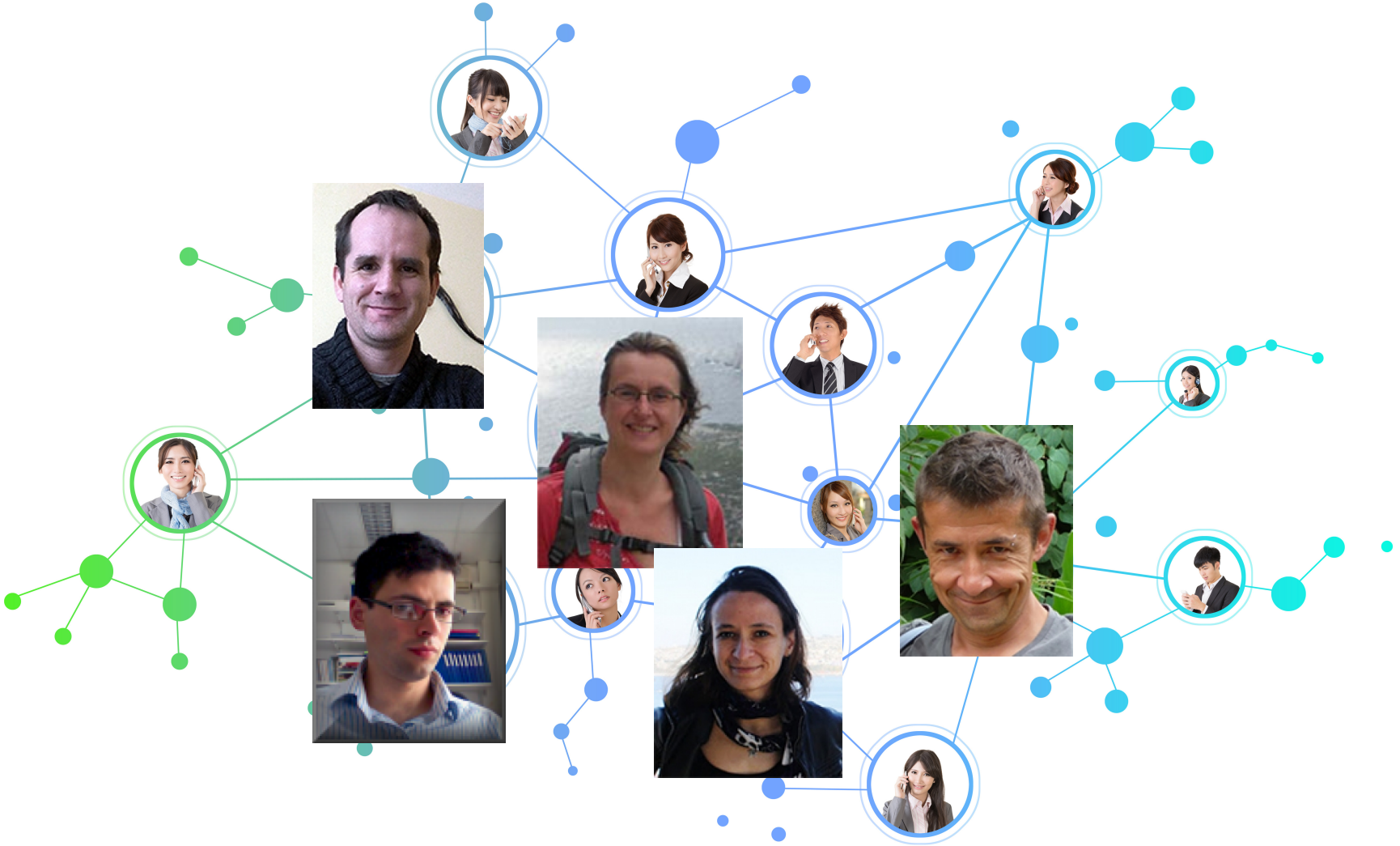
I work at Ecole Normale Supérieure de Lyon in the Laboratory Plant Reproduction and development

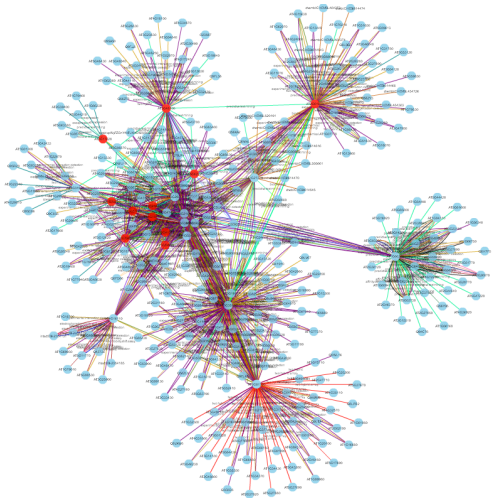
I am good at developing molecular approaches to understand flower development, in particular identifying targets of transcription factors controlling flower development

I am interested in investigating how modeling of gene regulatory networks can help biologists to increase their knowledge of their favorite biological system

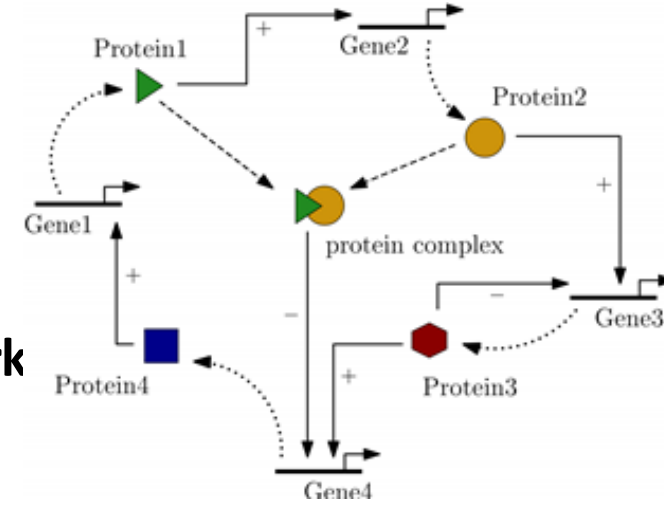
I come mainly for sharing my experience of gene regulatory networks, learn from others who have a different approach of these networks, in particular with statistical tools

Networks are everywhere!



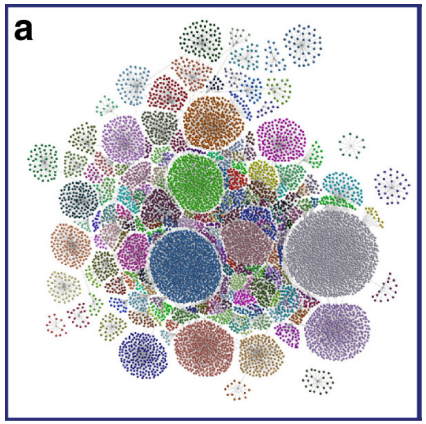


Biological networks



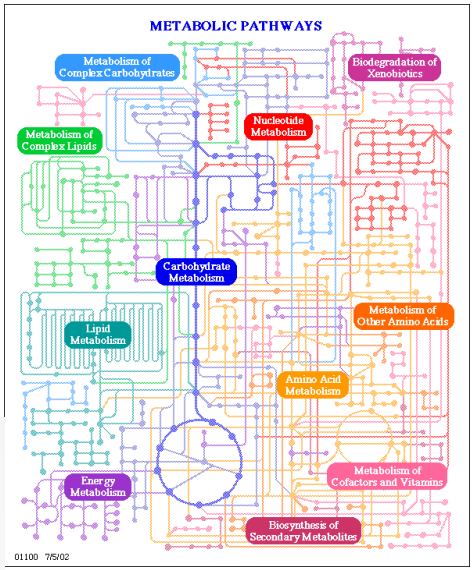
Protein-protein interaction network

Gene regulatory networks

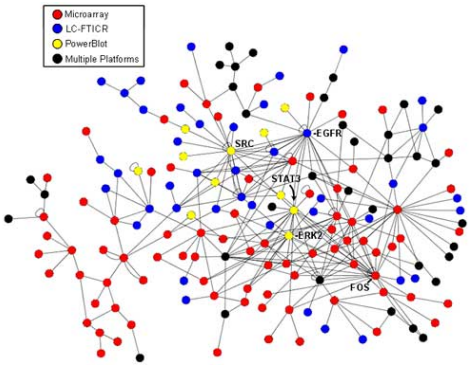


Gene co-expression networks

Metabolic networks



Signaling networks



Neuronal networks

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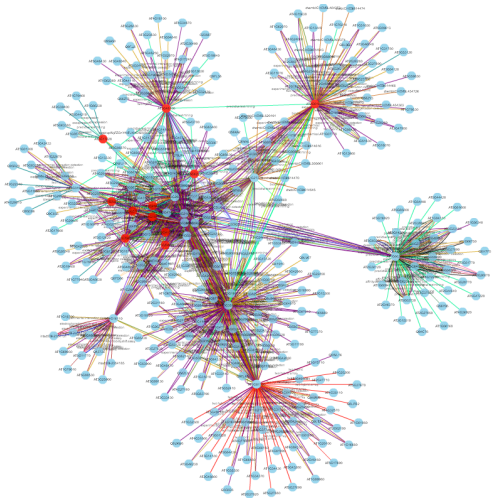
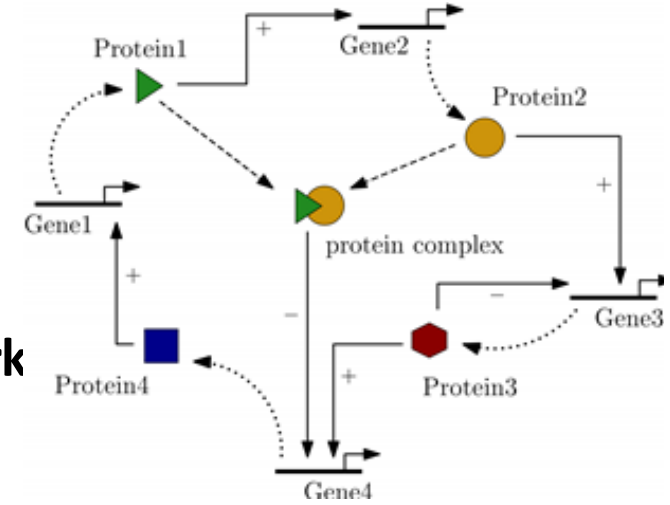
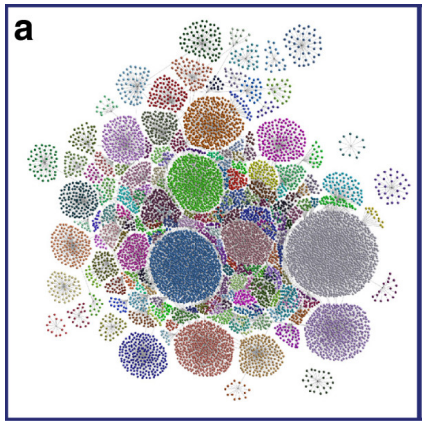


Biological networks



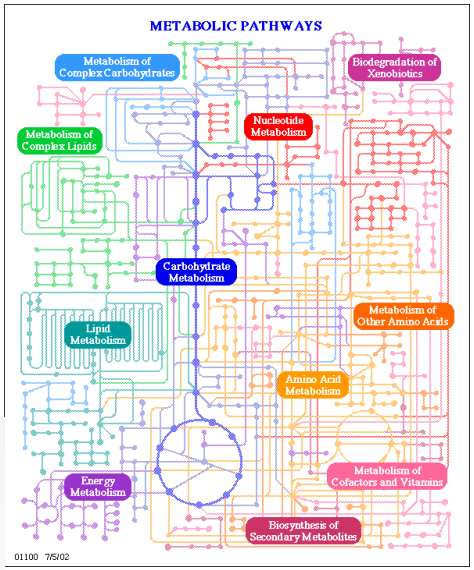
Protein-protein interaction network

Gene regulatory networks



Gene co-expression networks

Metabolic networks



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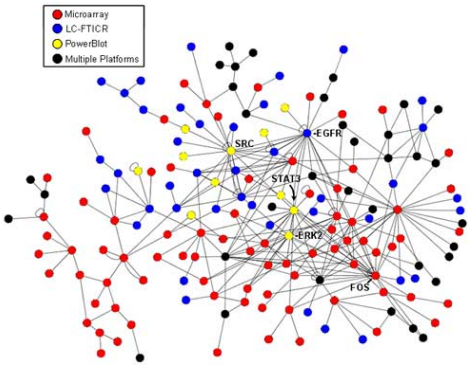

Neuronal networks

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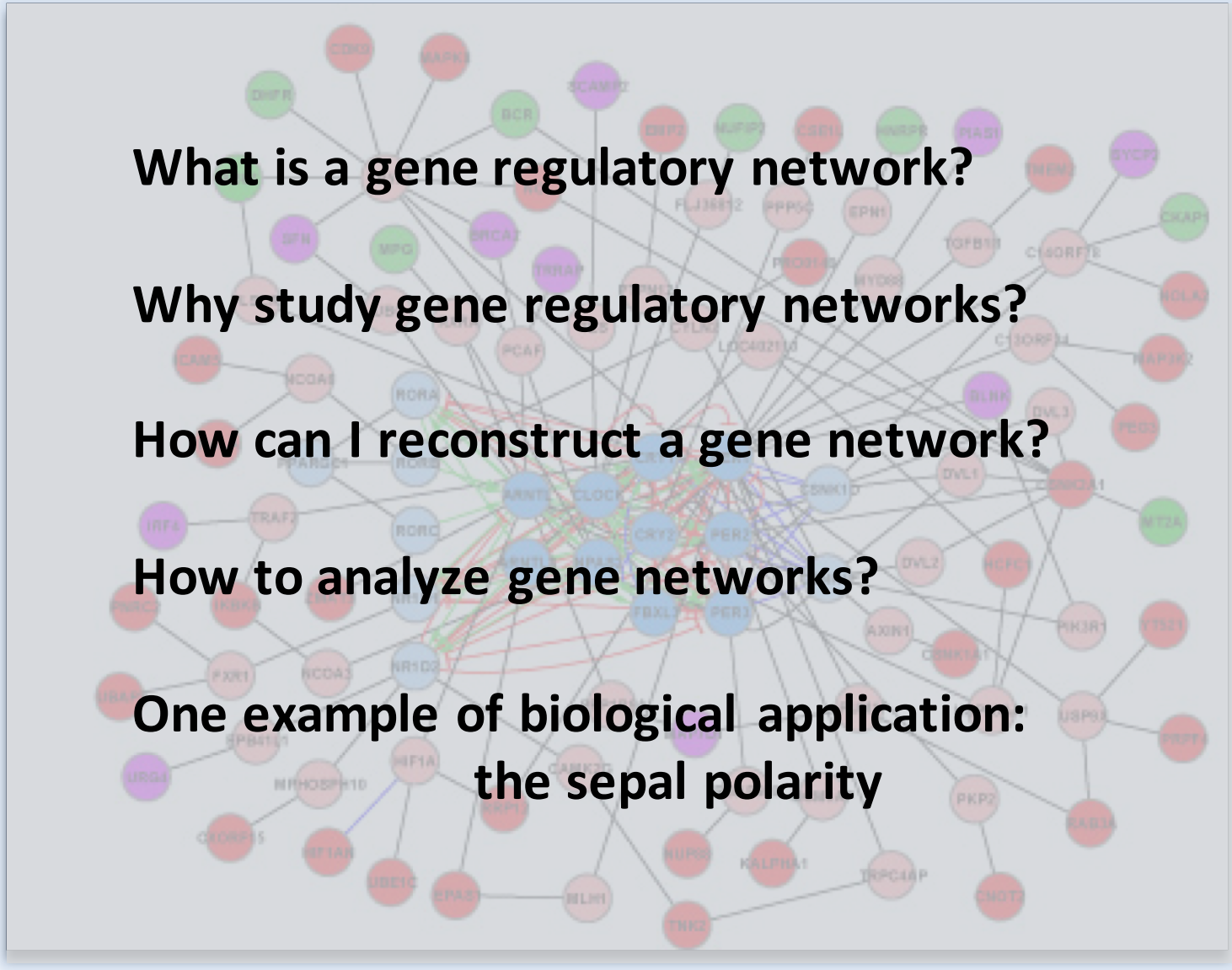
If one of these questions is of interest to you, stay with us !!



What is a gene regulatory network?

Why study gene regulatory networks?

How can I reconstruct a gene network?

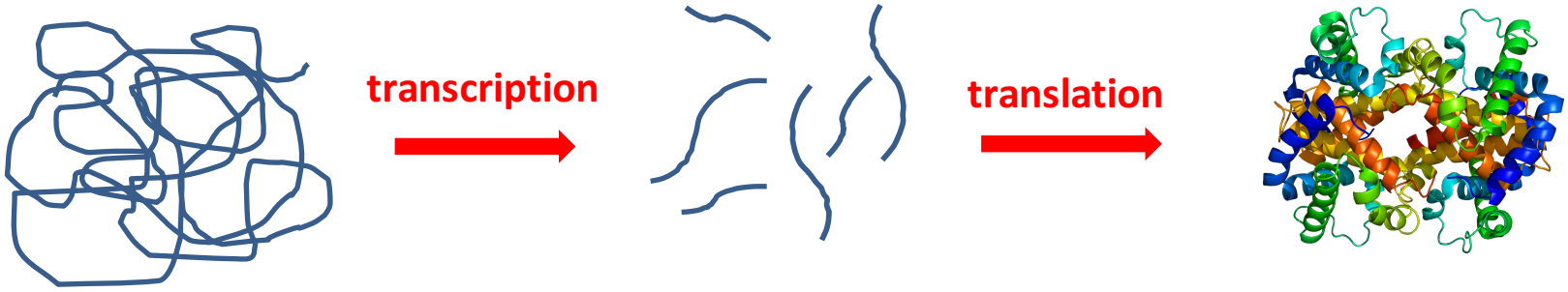
How to analyze gene networks?

One example of biological application: the sepal polarity

1- What is a gene regulatory network?

A **gene (or genetic) regulatory network (GRN)** is a collection of molecular regulators that interact with each other and with other substances in the cell to govern the expression levels of mRNA and proteins. These play a central role in morphogenesis, the creation of body structures, which in turn is central to evolutionary developmental biology (evo-devo).

Data to construct gene regulatory networks?



DNA
(genes)

RNA
(transcripts)

Proteins

Identified by sequencing

Identified by RT-PCR, RNA-SEQ, micro-array, *in situ* hybridization

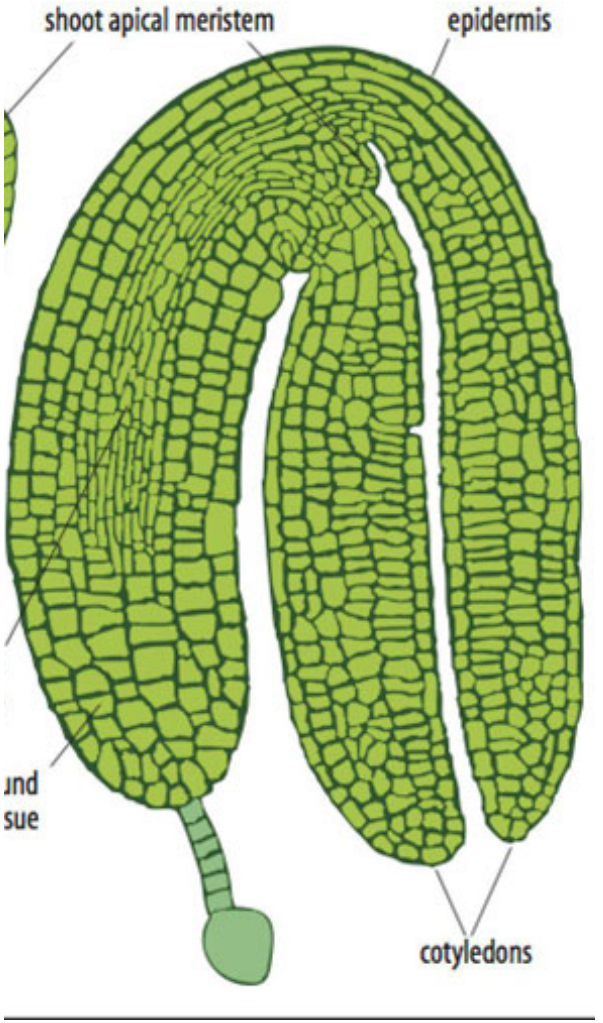
Identified by immunodetection, mass spectrometry, immunolocalization

Data to construct gene regulatory networks?

RNA-SEQ, microarrays, RT-PCR
require RNA extraction

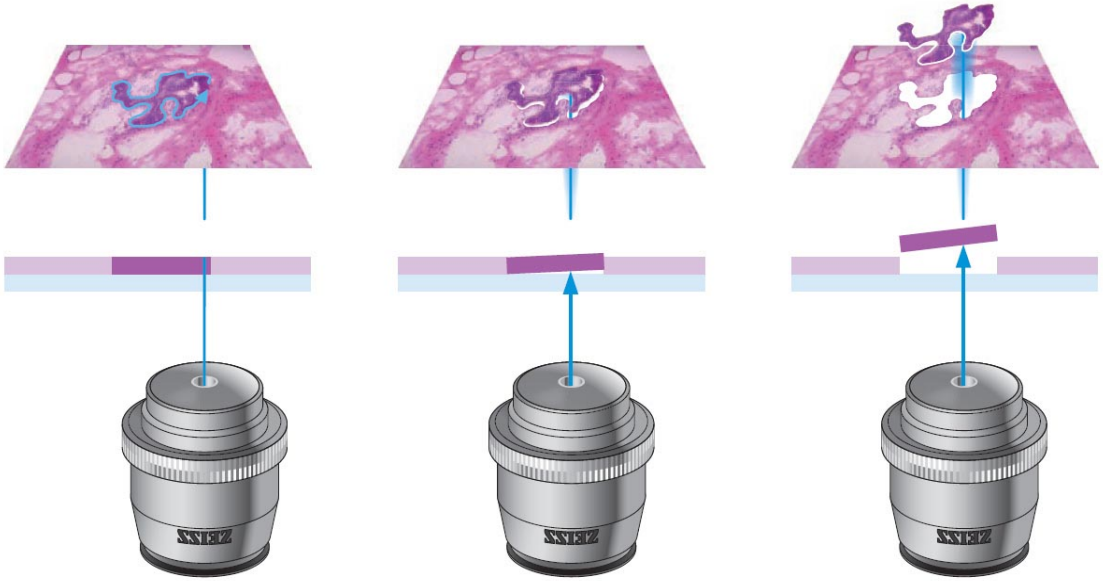
Immunodetection and mass spectrometry
require protein extraction

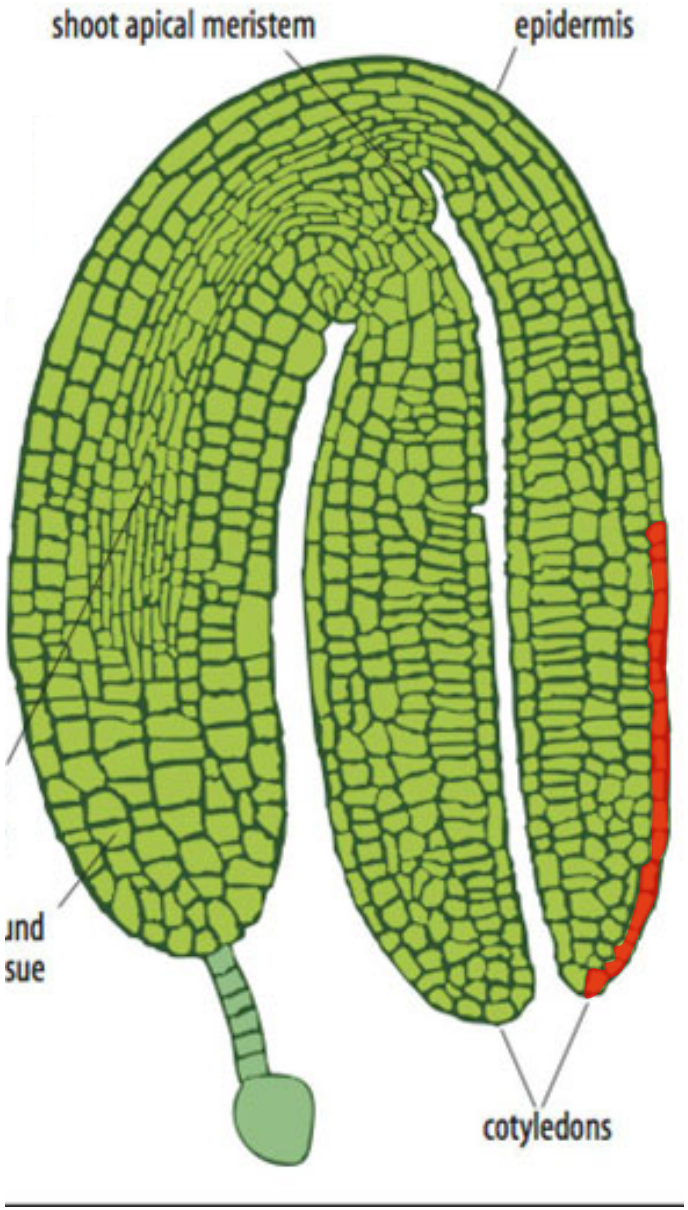
Cells with different transcriptome and proteome
will be mixted together



Data to construct gene regulatory networks?

Possible improvement
by laser microdissection
(LCM)





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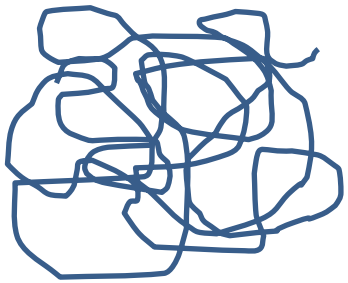
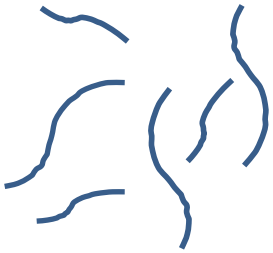
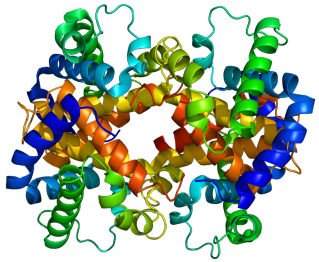


transcription



translation



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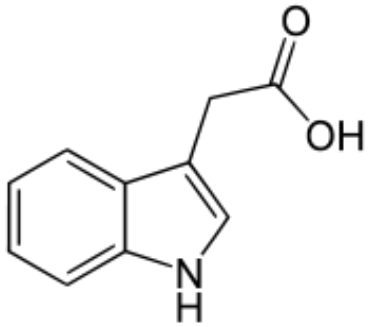
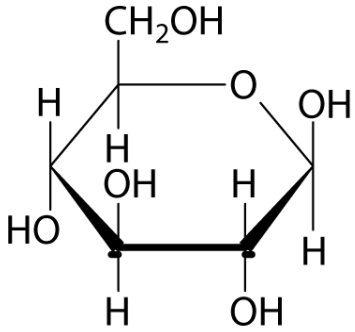
Proteins



Hormones

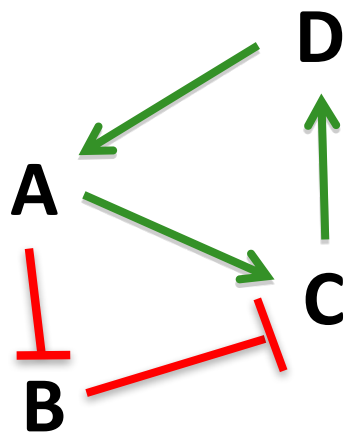


Sugars

Metabolites, Ca^{++}, pathogens, stress…

How to represent gene regulatory network?

Gene regulatory networks are commonly represented as graphs of interactions: the nodes are connected by interactions.

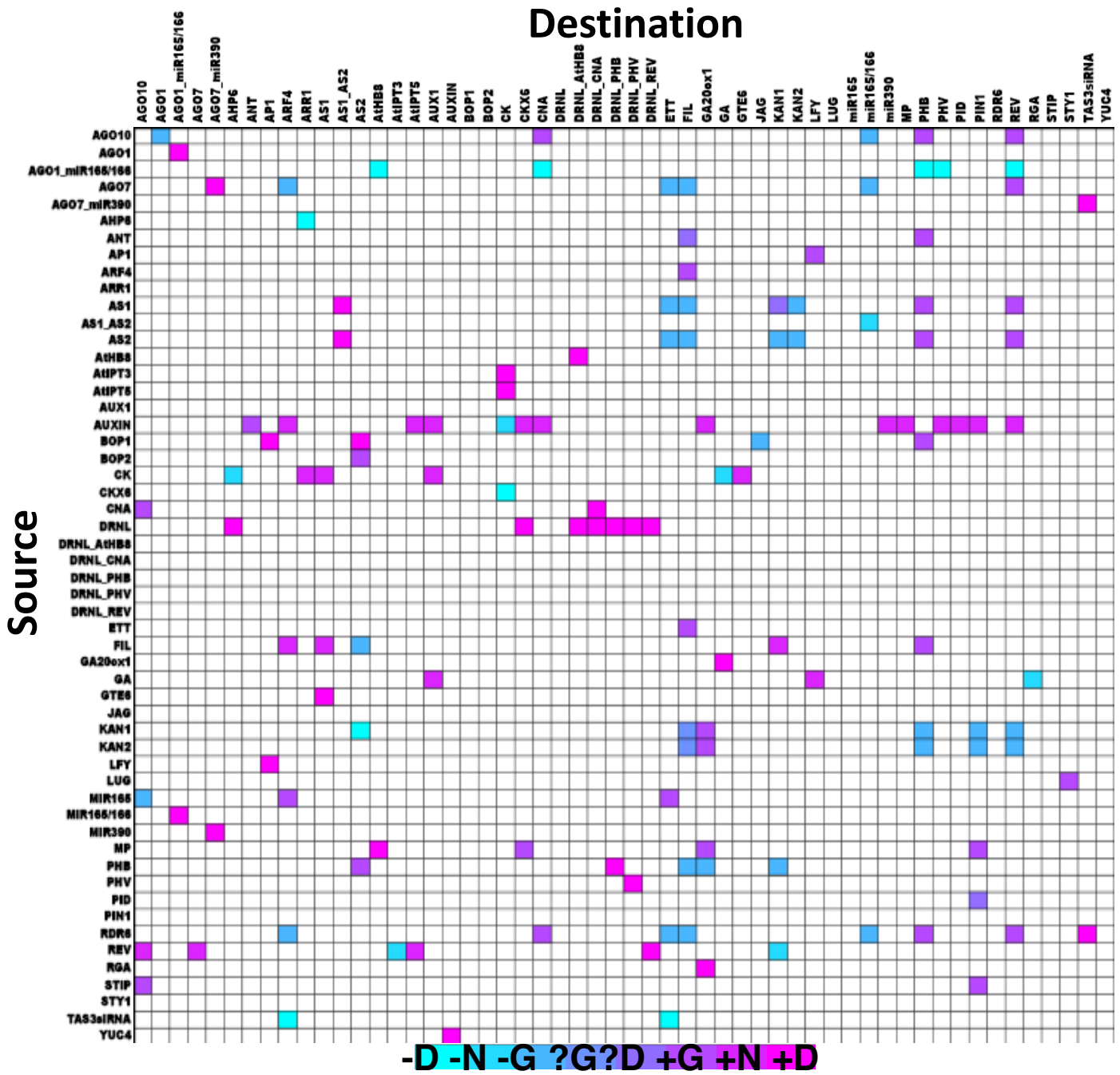


A node can be any molecule but often represents DNA, RNA, protein or complexe of them

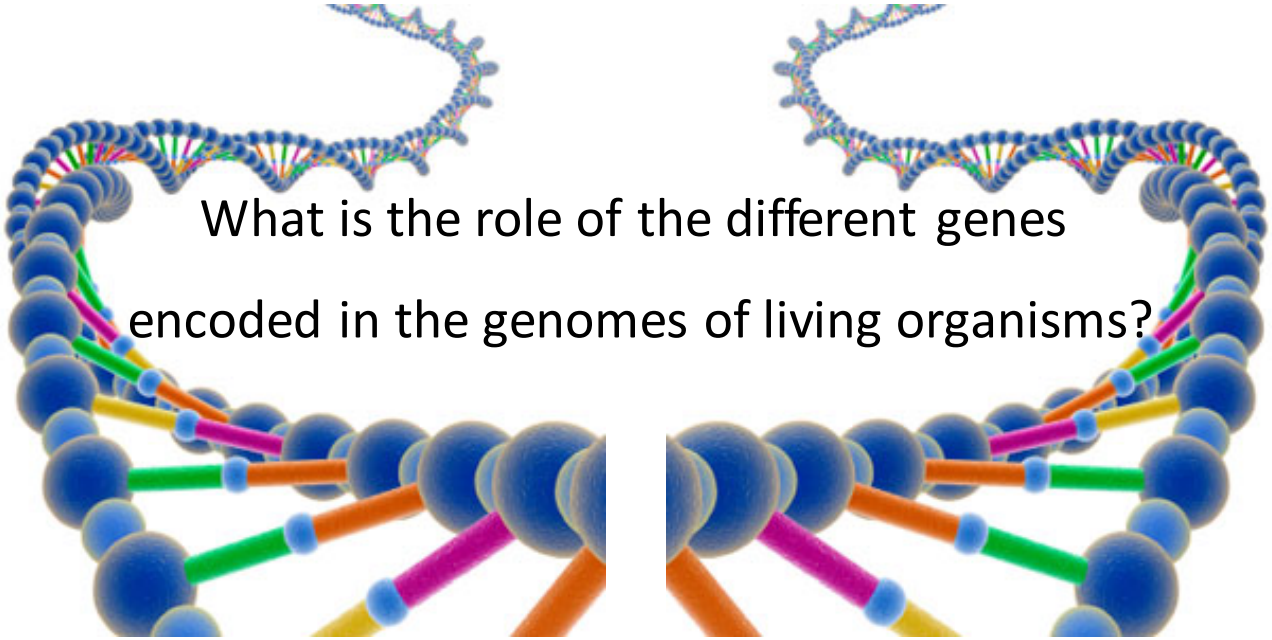
The interactions can be direct or indirect

The interactions are oriented > circuit

How to represent gene regulatory network?



2- Why study gene regulatory networks?



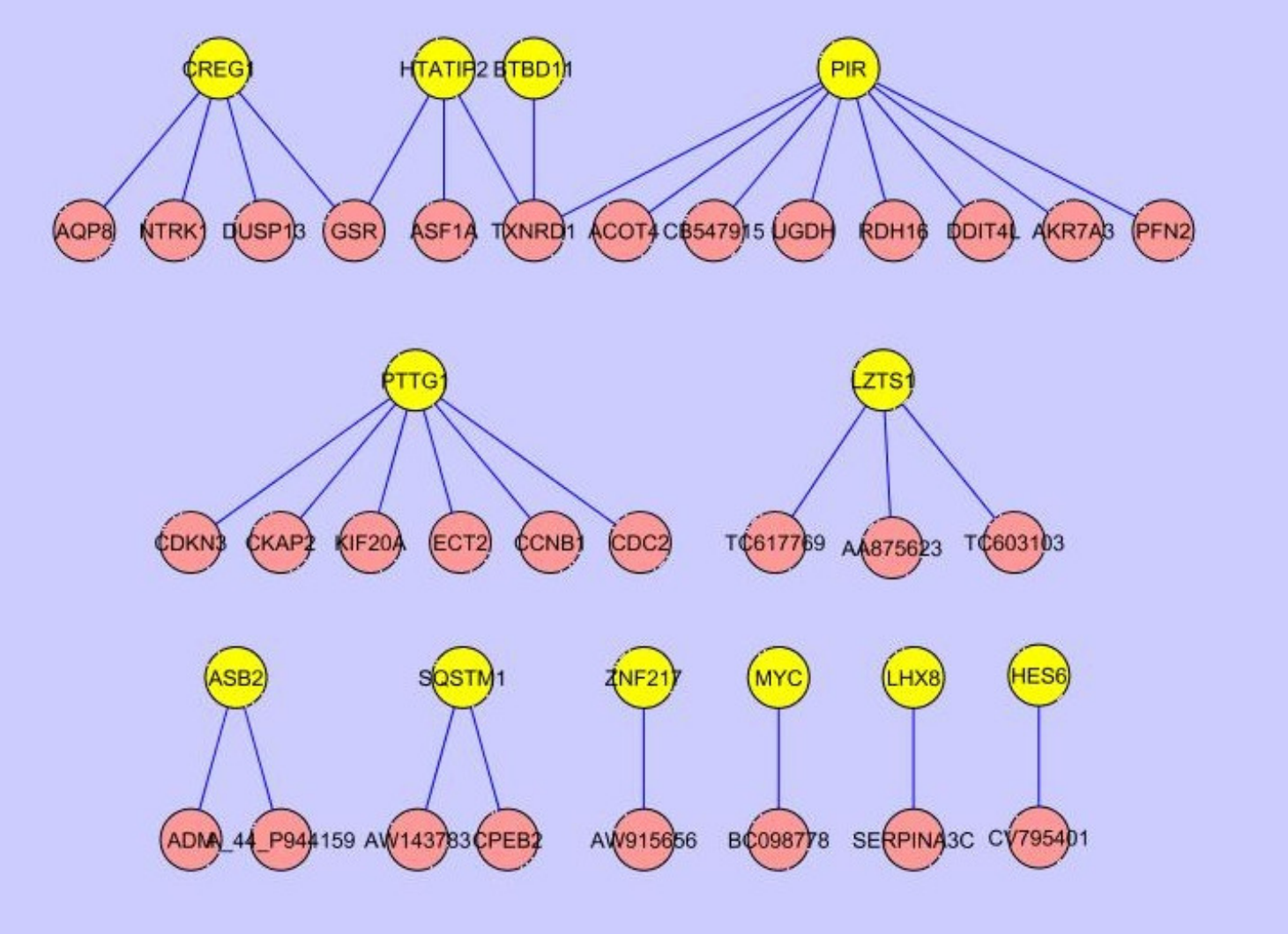
To answer this question, biologists have a robust method:

they inactivate a given gene and see what is does

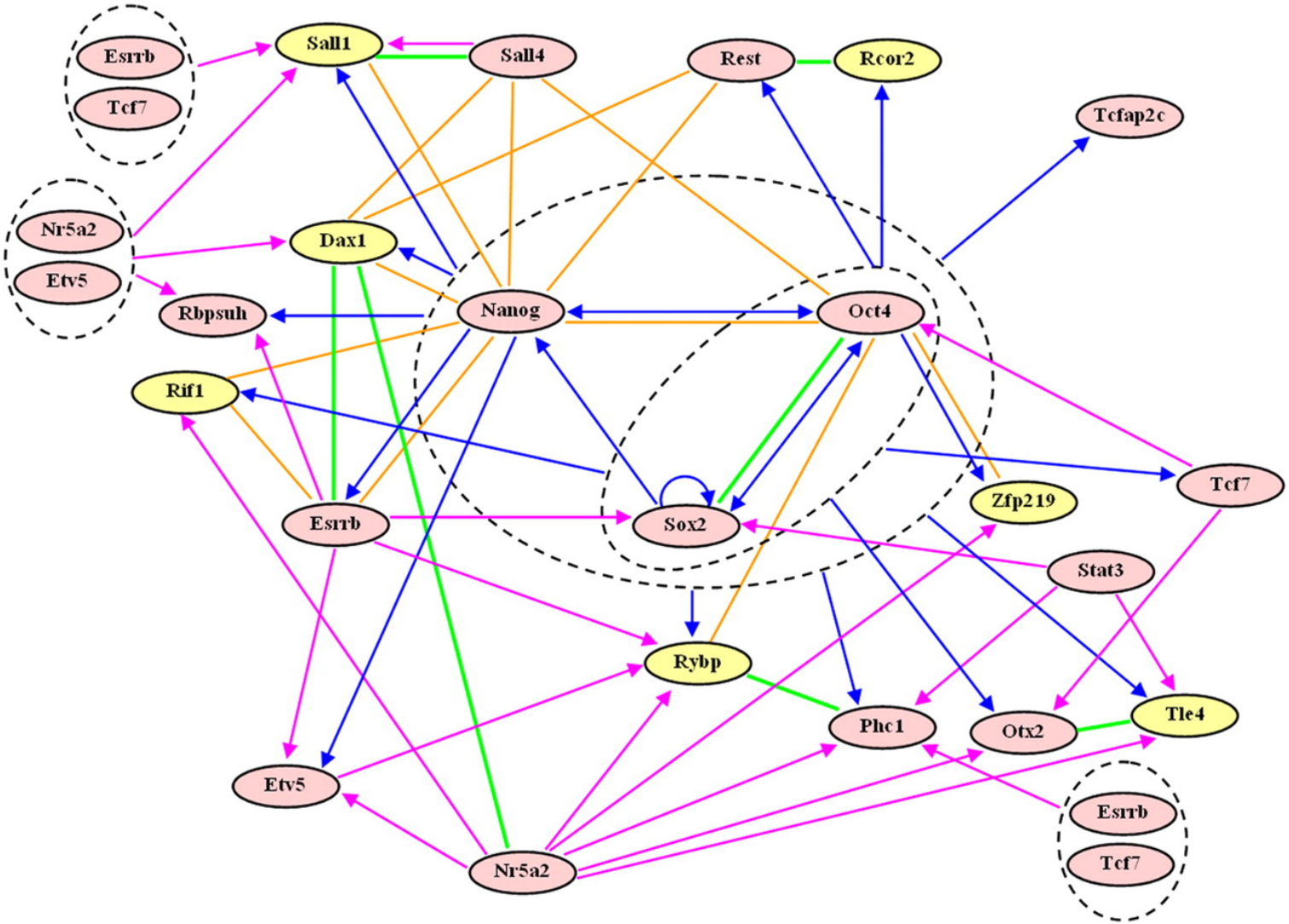
Genetic approaches widely used since the 80's

These approaches give an impression of verticality:

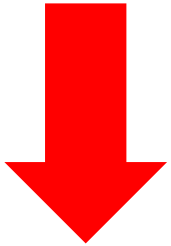
1 gene / 1 phenotype



However, biological processes are controlled by gene regulatory networks rather than isolated genes



Living cells are self-organising systems



This self-organization is encoded in the properties of the gene regulatory networks:

- **Each element is doing something on another**
- **Each element is itself regulated by another**
- **Presence of closed regulatory circuits**

Is it clear what is a gene regulatory network ?

Why it is important to study them ?

3- How can I reconstruct a gene network?

3- How can I reconstruct a gene network?

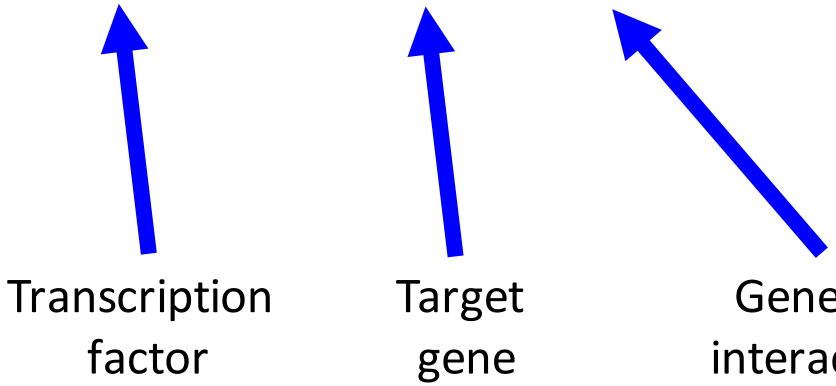
Many different ways !!

Reconstruction of a gene network: the different steps

- Define a given process to study
- List the regulatory elements known to participate
- List the interactions linked to these elements

Reconstruction of a gene network: the different steps

Source	Destination	Action	Experimental evidence	Reference
ARF4p	FILa	+G	RT-PCR analyses showed that FILAMENTOUS FLOWER (FIL) is up-regulated in plants over-expressing ARF4, while it is repressed in arf4-2 mutant	Yoon et al, 2009



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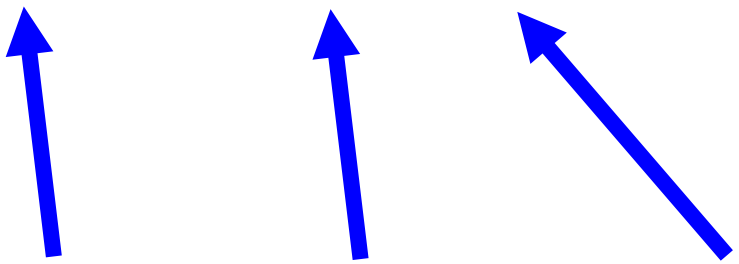

Inactive Histidine protein kinase

Target Transcription factor

Direct interaction

Reconstruction of a gene network: the different steps

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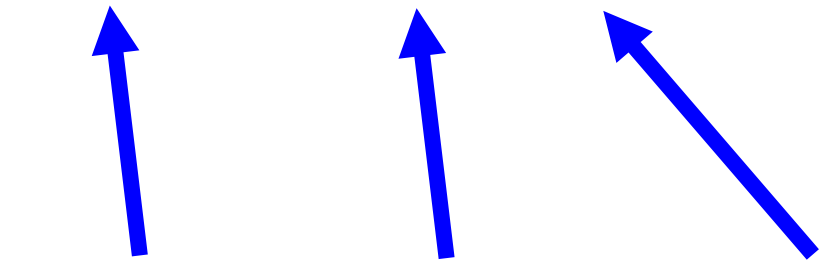

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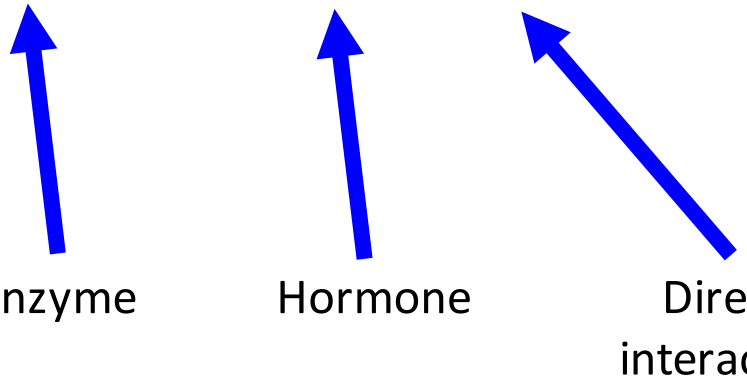

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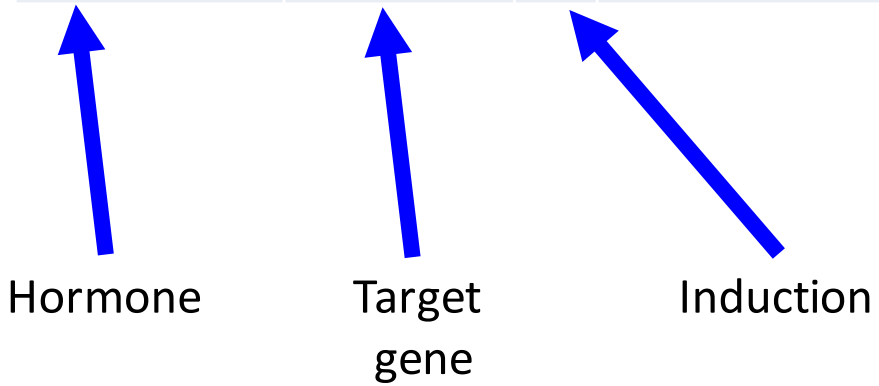
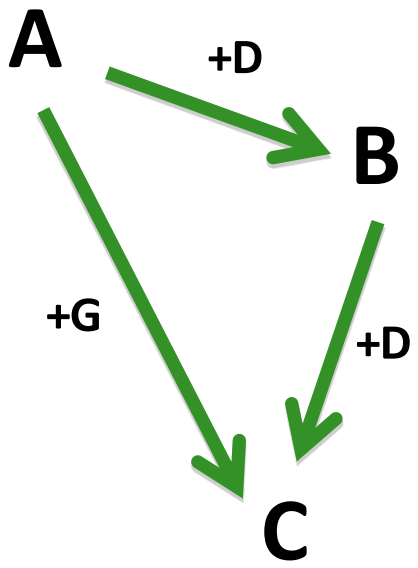
AUXINh	CKX6a	+N	CKX6 expression is induced by auxin (microarray data, 1, 2)	1- Sawa et al, 2002 2- Overvoorde et al, 2005



Consider the nature of the interaction: why?

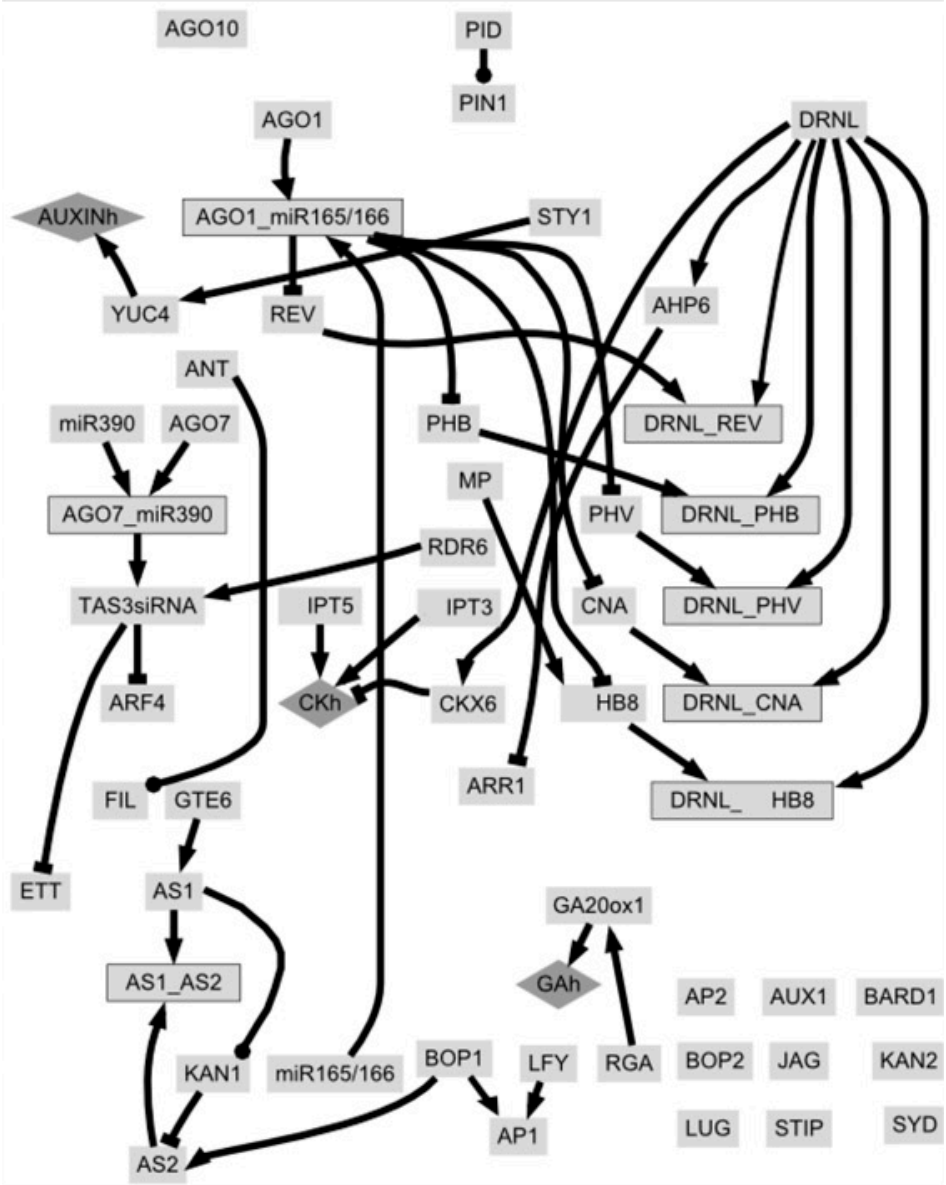


Risk of redundancy if you mix direct and indirect interactions

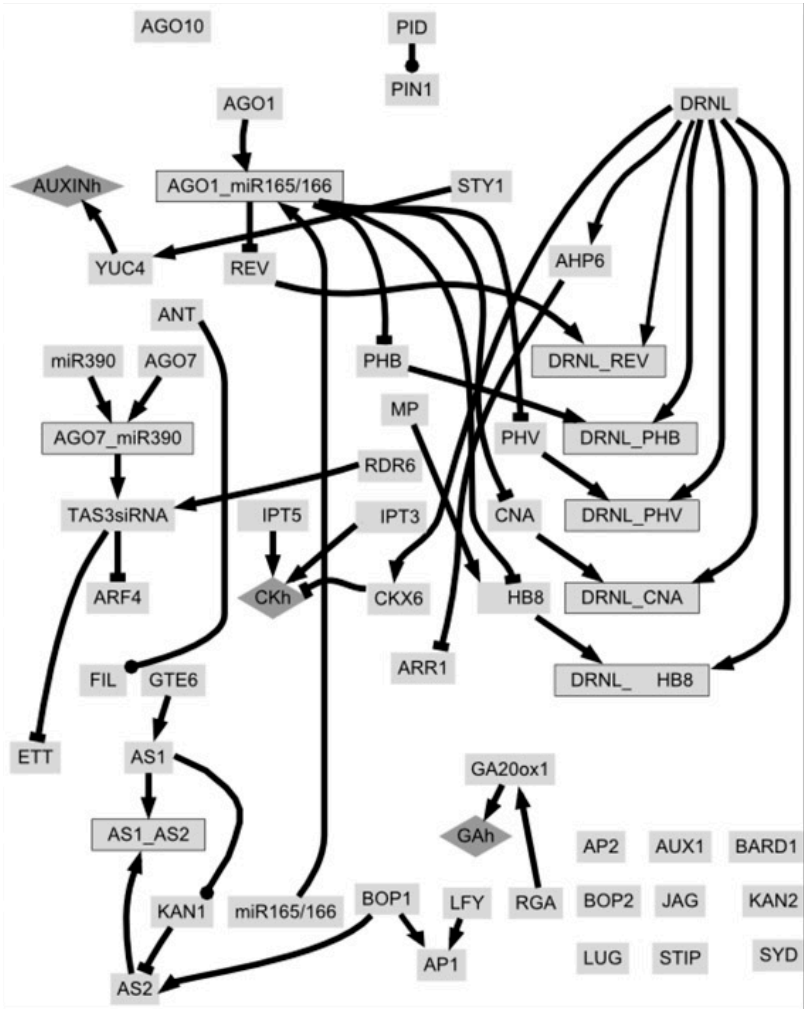
Evaluate the reliability of experimental data

- Accumulation of different technics to support the interaction
- Contradicting data between different labs?

From this list, you can draw a graph



It appears that information is lacking



Some elements have no input

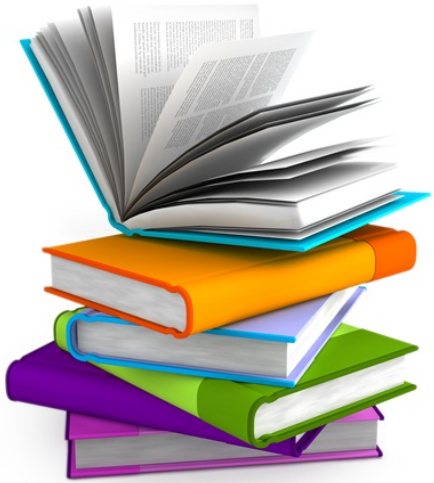
Some elements have no output

There is no closed circuits

A biological network must have all these properties!

Solution 1

Try to fill the gaps > back to the litterature or to the bench



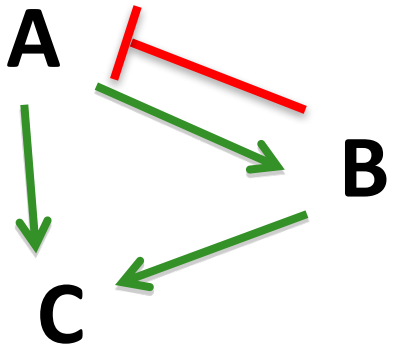
Solution 2

focuse on a subset of interactions

for which requirements are fullfilled

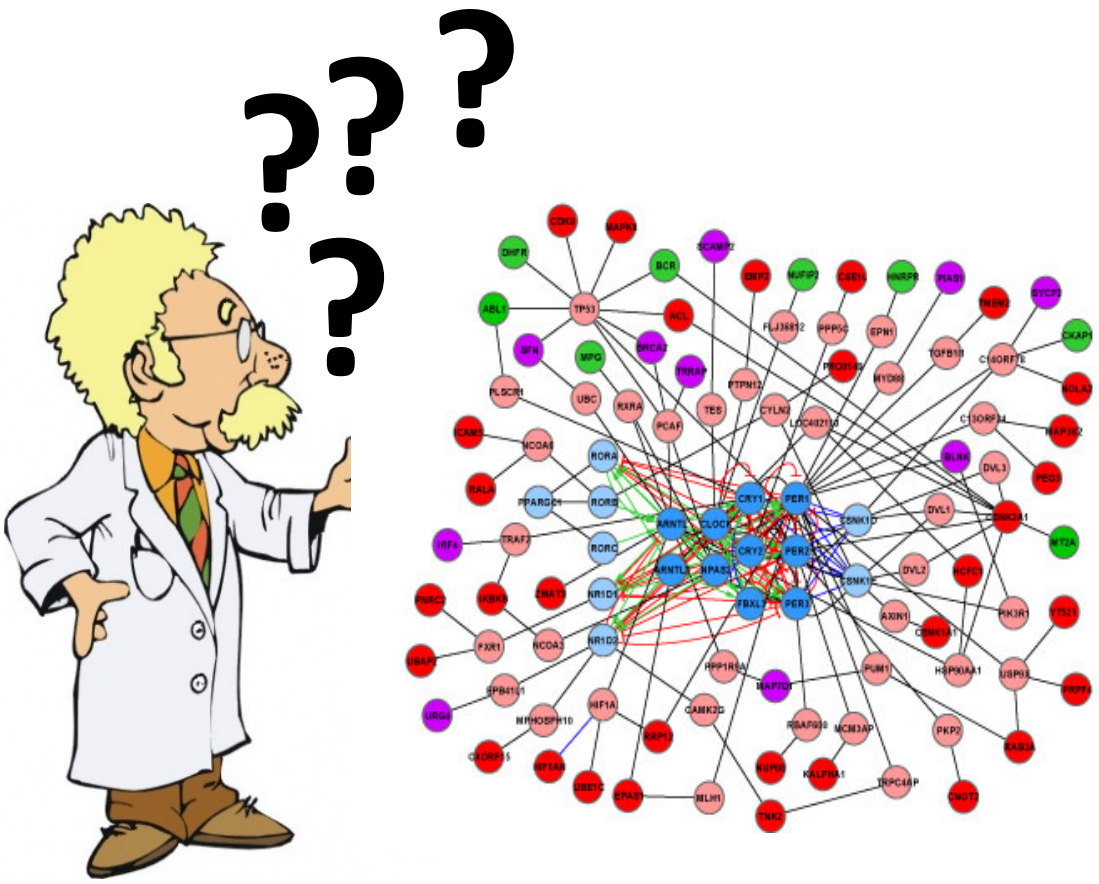
- inputs for all the elements
- closed circuits

Once the minimal properties are fullfilled



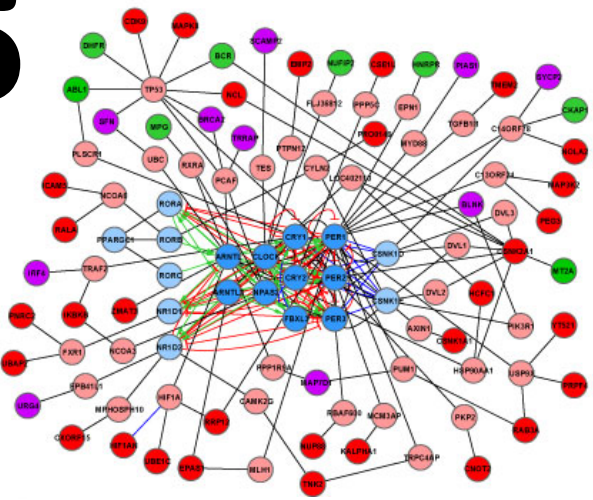
Modeling can reveal the behaviors of your network

4- How to analyze gene networks?



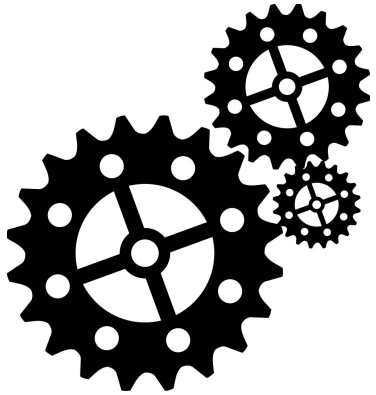
Biologist

Main wrong preconceived idea



Biologist

Mathematician



A model in itself is useless

It is by building the model that you learn how your system works!

It’s like coffee again !!!

Many methods exist to model gene regulatory networks

Quantitative models

Require informations we usually don't have
(half life for RNA and proteins, concentrations…)

Heavy in terms of computing time

Can only compute small networks

Require technical skills (differential equations)

Boolean network

Definition: A **Boolean network** consists of a discrete set of Boolean variables each of which has a Boolean function (possibly different for each variable) assigned to it which takes inputs from a subset of those variables and output that determines the state of the variable it is assigned to.

Boolean network

Drawbacks: the nodes can only be ON or OFF (1 or 0)

Advantages:
- easy to use
- can be applied to big networks
- compatible with most biological data which are often not quantitative

How does it work? It uses logical tables

input output

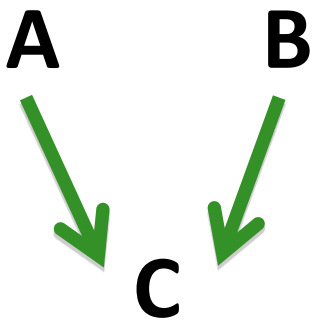
A	B
0	0
1	1

Activation

A	B
0	1
1	0

Repression

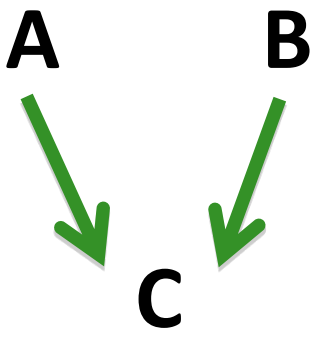
A	B	C
0	0	0
0	1	1
1	0	1
1	1	1



Redundant activation

How does it work? It uses logical tables

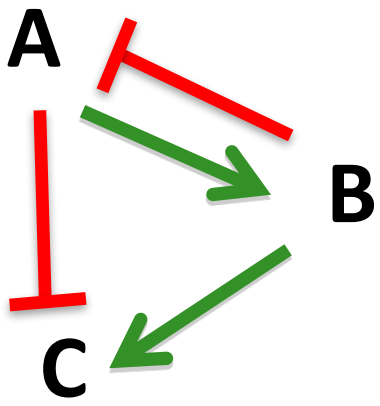
A	B	C
0	0	0
0	1	0
1	0	0
1	1	1



Cooperative activation

(protein dimer)

Logical tables

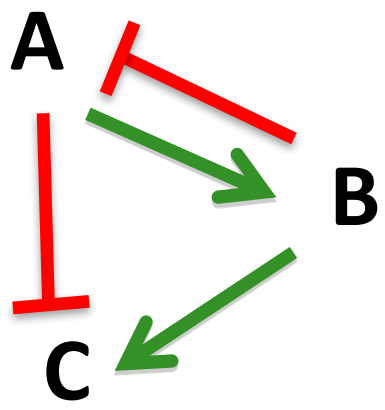


A	B
0	0
1	1

B	A
0	1
1	0

A	B	C
0	0	0
0	1	1
1	0	0
1	1	0

Logical tables and steady states



A	B
0	0
1	1

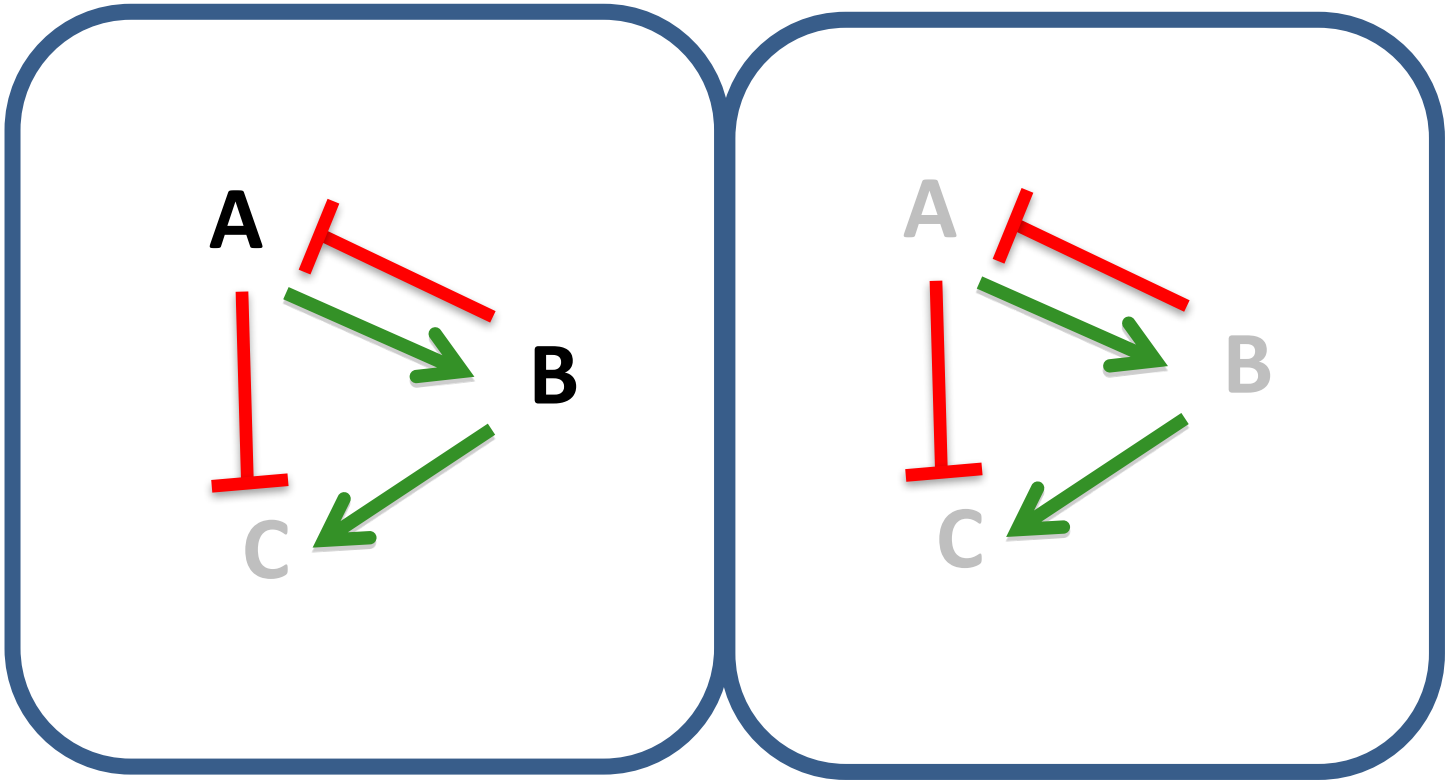
B	A
0	1
1	0

Step 1: A switched ON
Step 2: B switched ON by A
Step 3: A switched OFF by B
and C switched ON by B
Step 4: B switched OFF by lack of A
Step 5: C switched OFF by lack of B

A	B	C
0	0	0
0	1	1
1	0	0
1	1	0

Stable state with all elements OFF unless A is ON > cycle

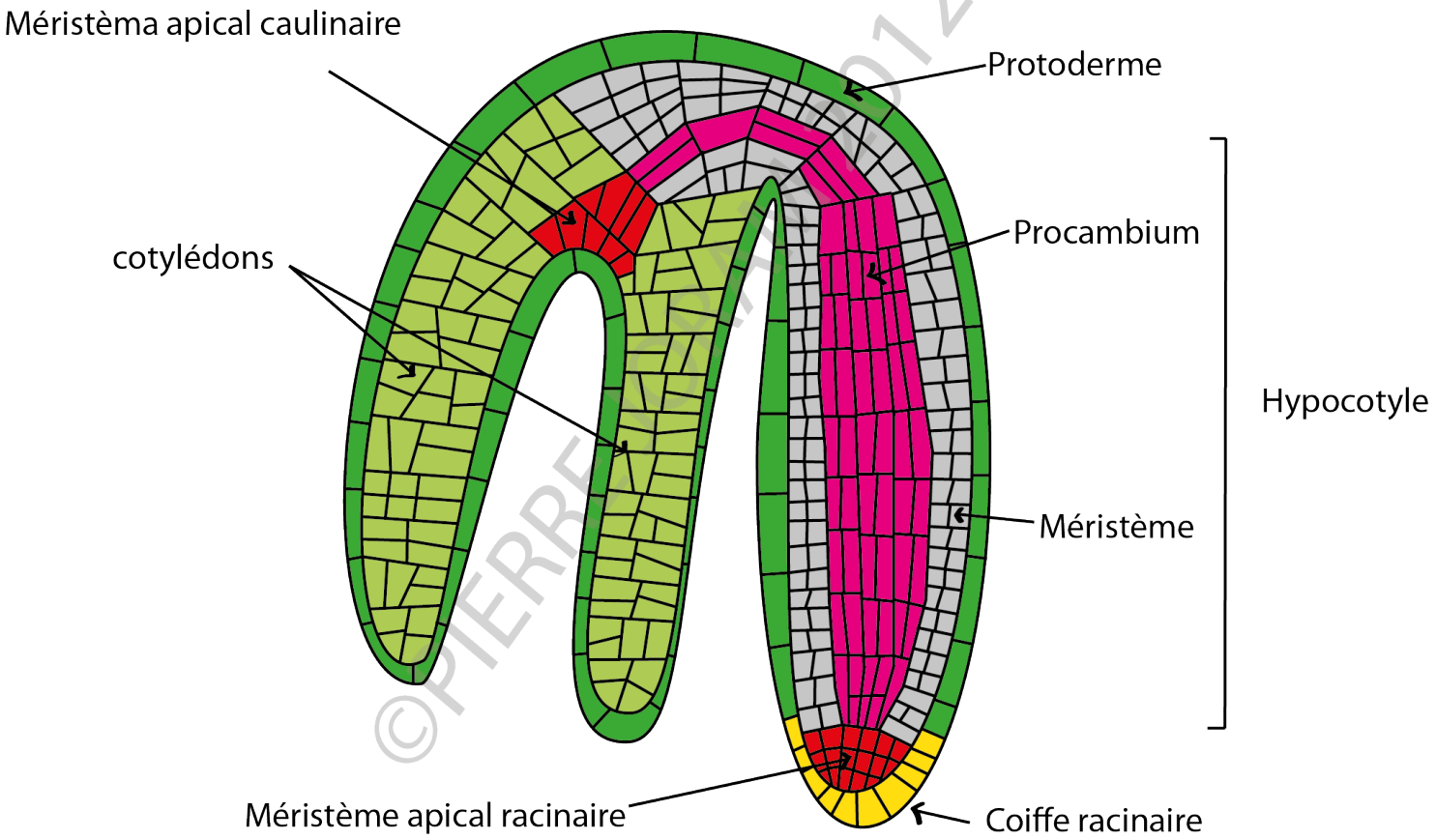
The interaction network is the same in all the cells but the state can be different



Communication between cells affect the state of the network

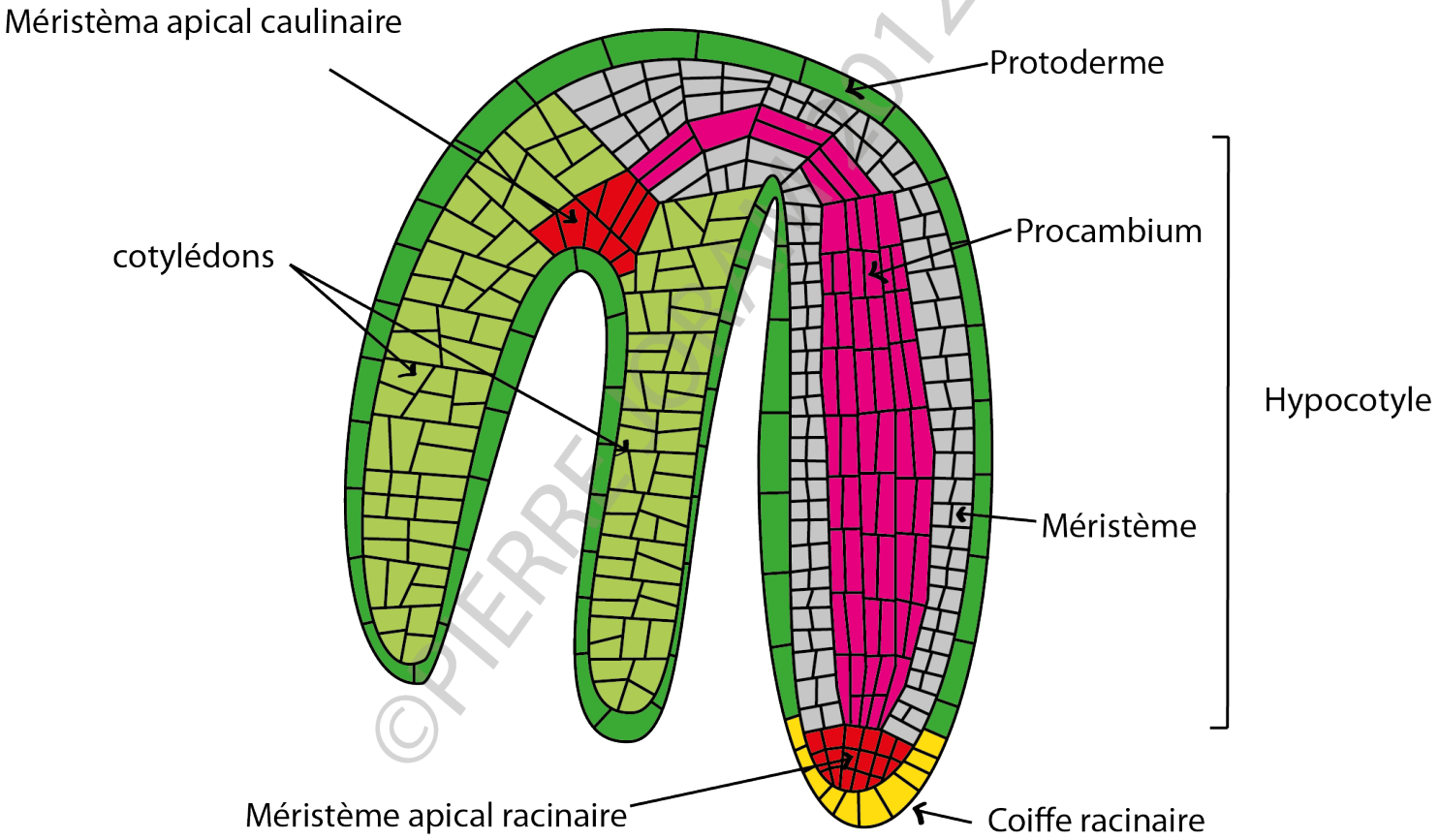
Examples?

Back to biology and steady states



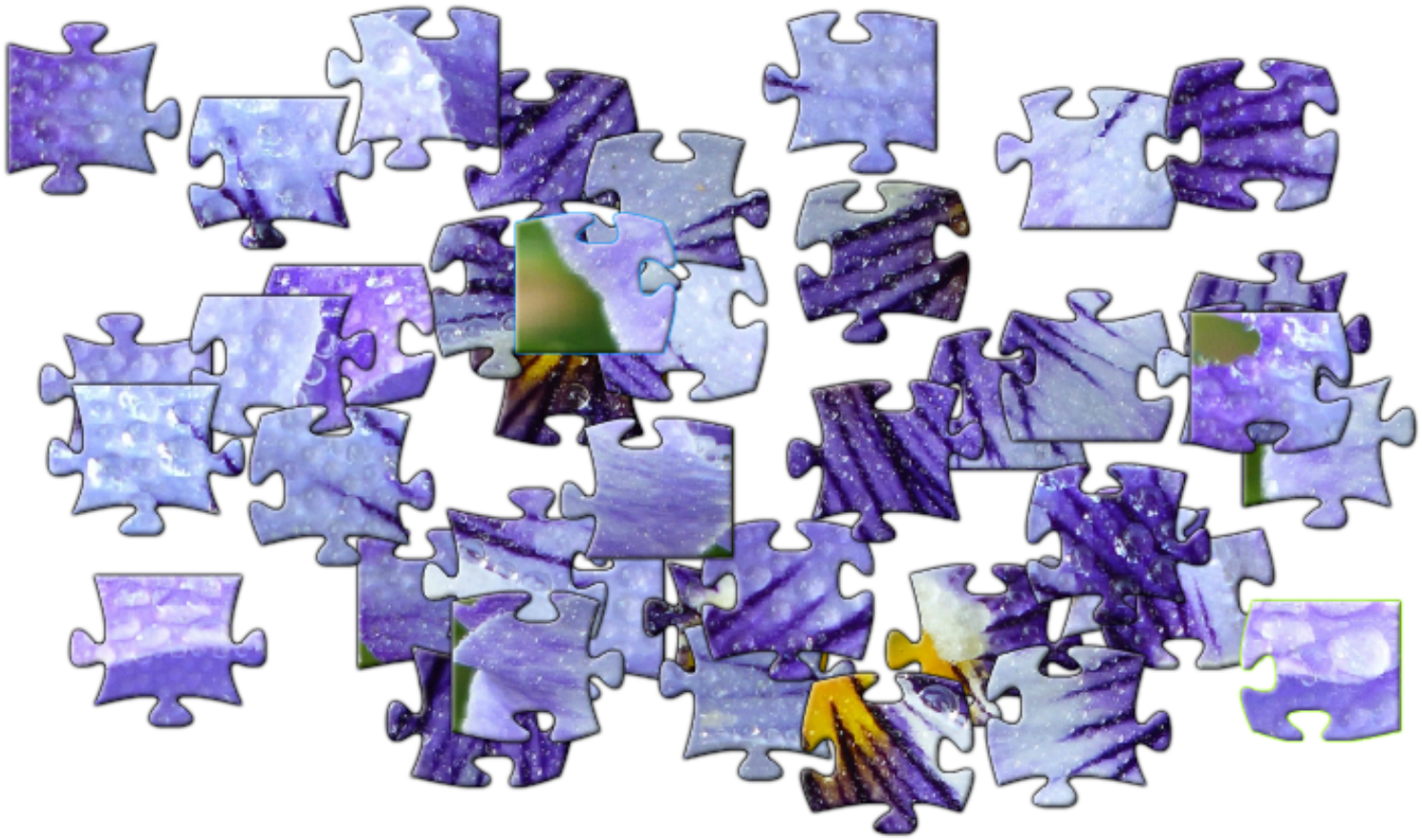
The steady states correspond to the molecular state of a cells

Development can be seen as a succession of molecular steady states evolving through time autonomously



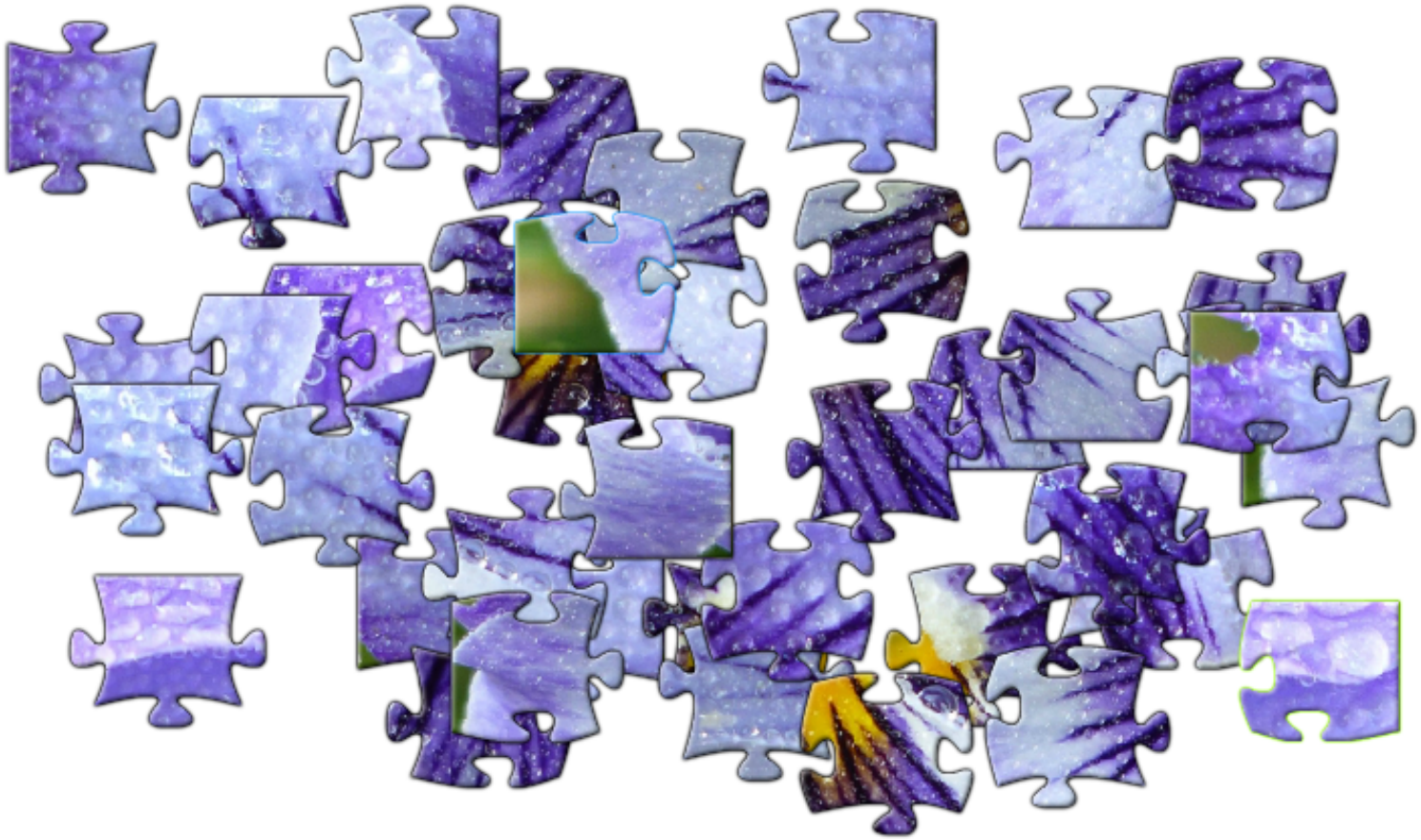
Time for break

One example of biological application: the sepal polarity

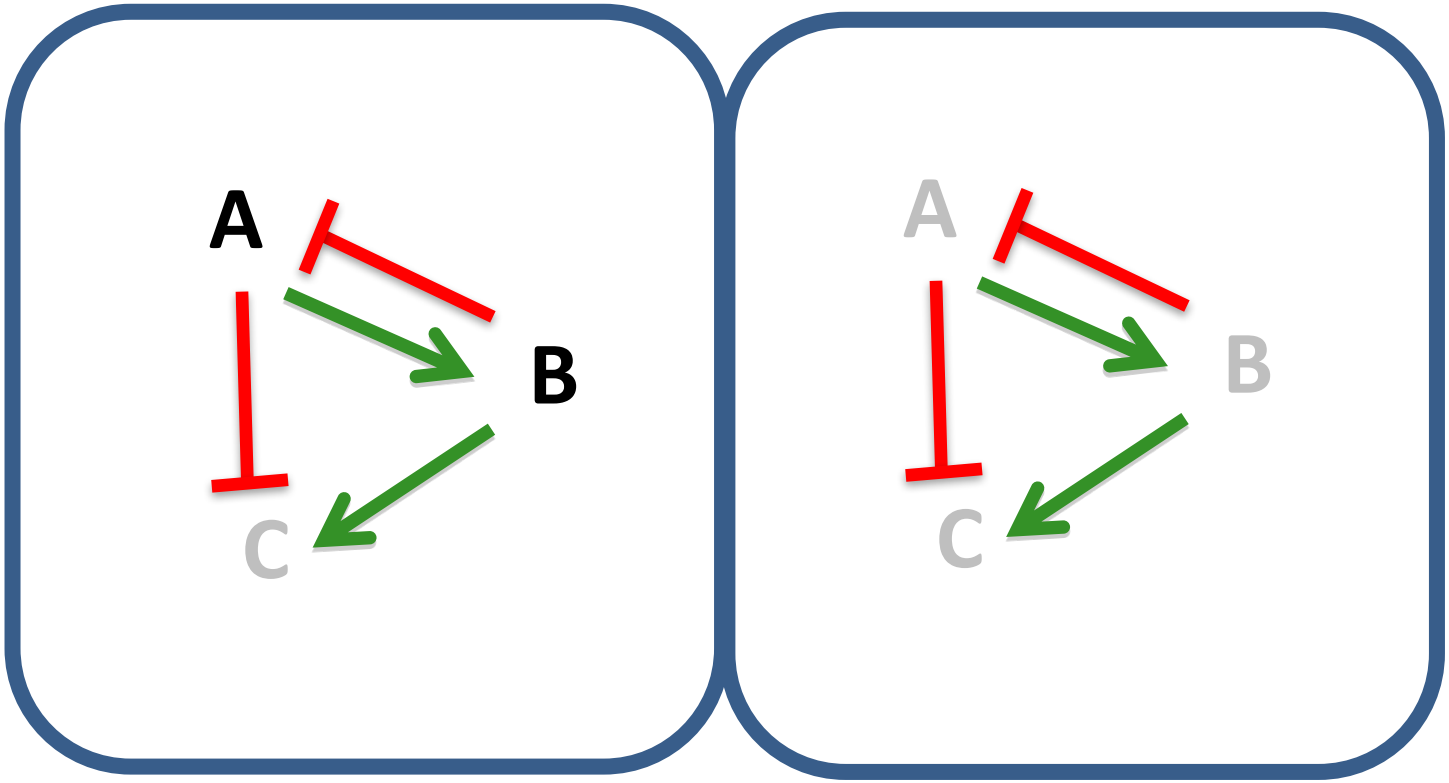


Increasing the number of pieces does not necessarily increase our knowledge

How to integrate heterogeneous data in a coherent manner?



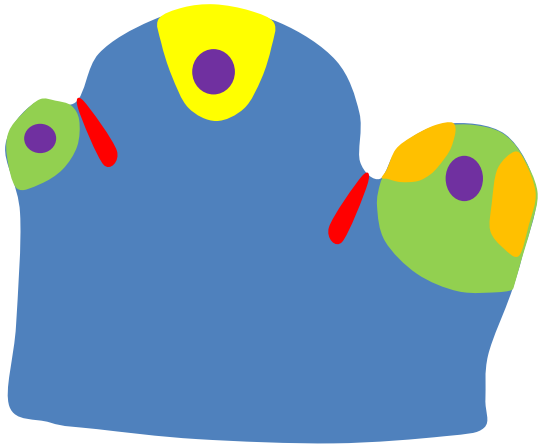
The interaction network is the same in all the cells but the state can be different



Communication between cells affect the state of the network

How to construct a network which is coherent with expression patterns and long range interactions?

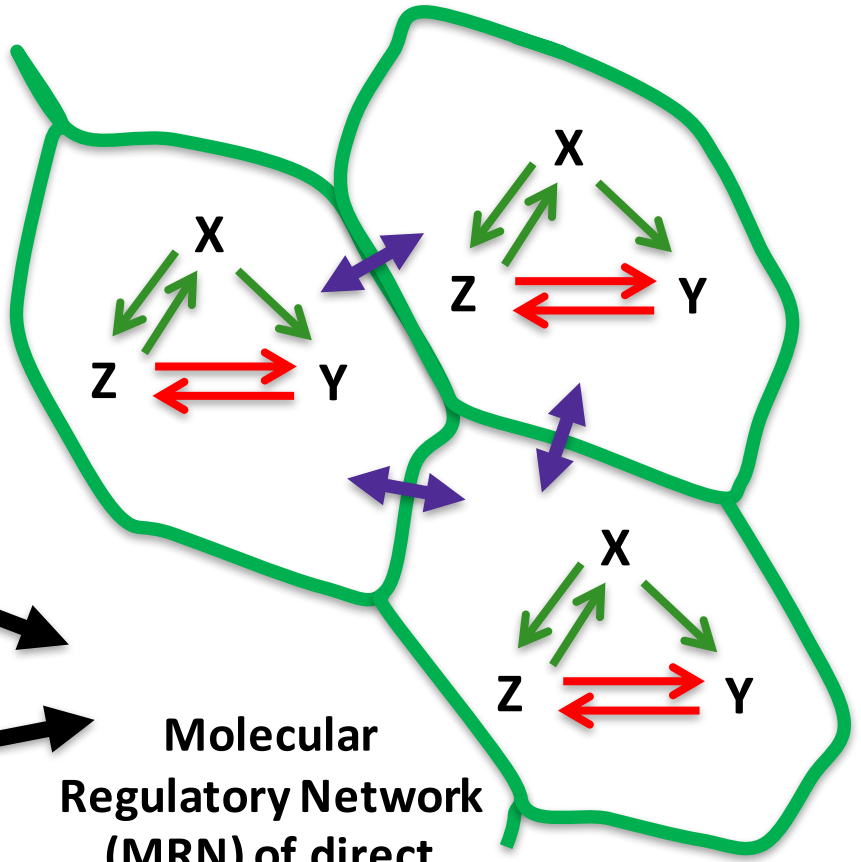
behavior



Expression patterns
(steady states)

Long range
interactions

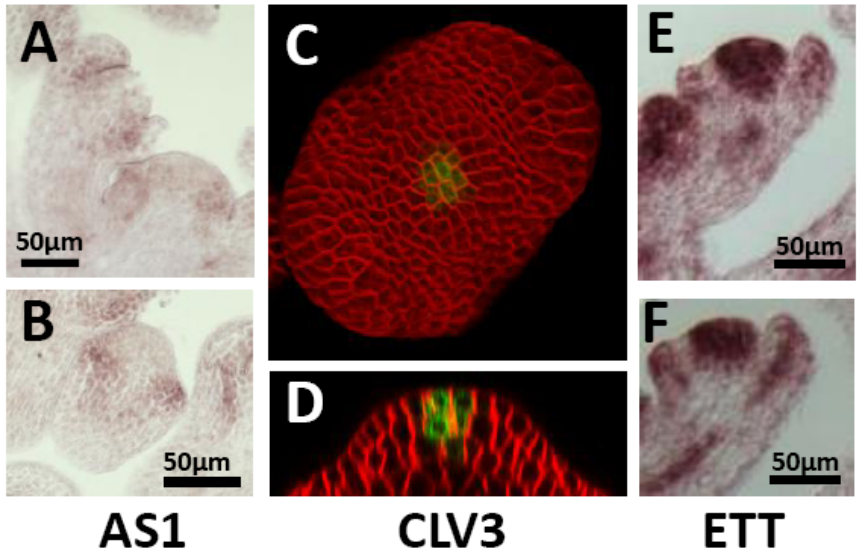
structure



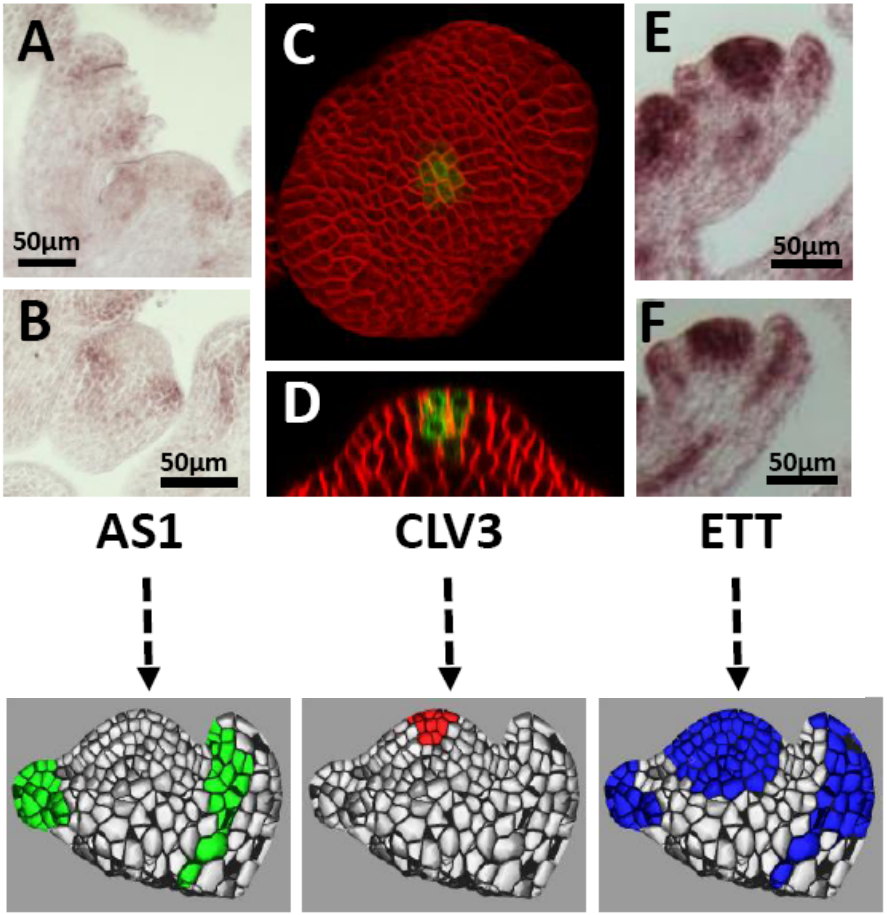
Molecular
Regulatory Network
(MRN) of direct
interactions

ex: Z and Y are not expected
to be co-expressed

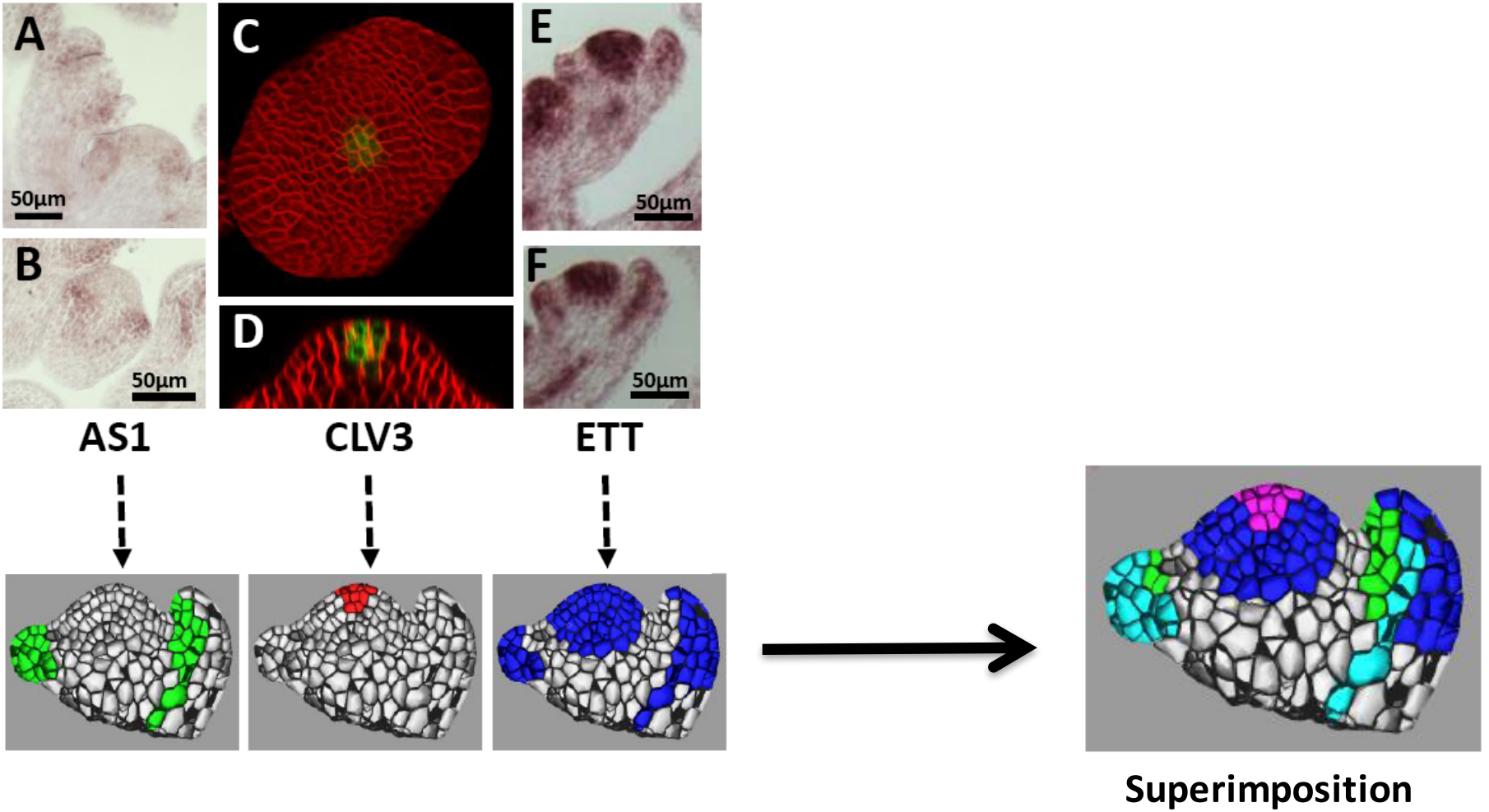
Expression database > atlas > molecular states



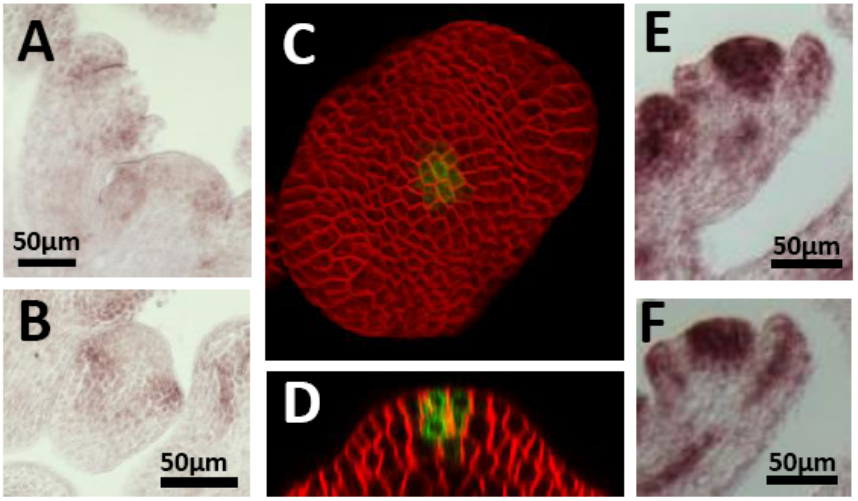
Expression database > atlas > molecular states



Expression database > atlas > molecular states

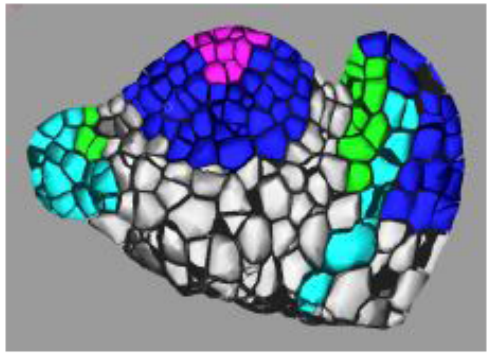


Expression database > atlas > molecular states



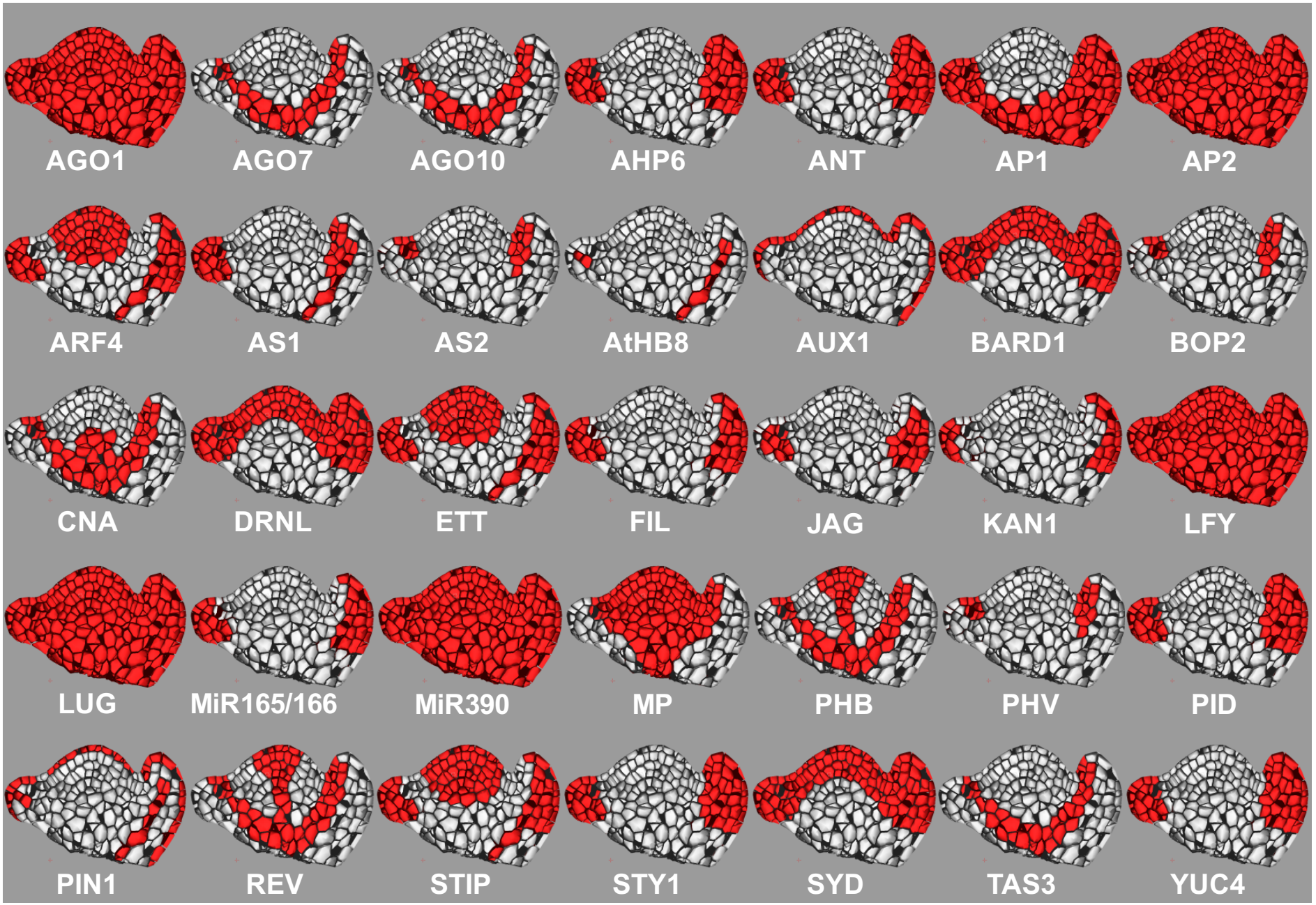
	Zone 1	Zone 2	Zone 3	Zone 4
AS1	1	0	1	0
CLV3	0	0	0	1
ETT	0	1	1	1

Steady states



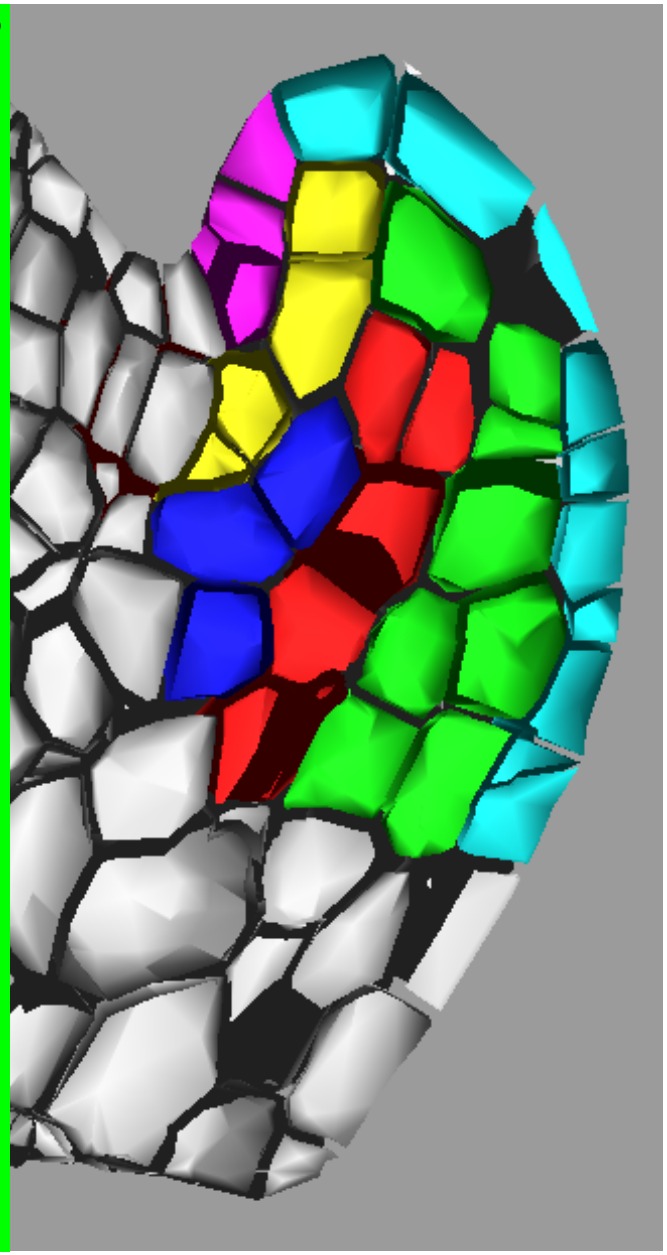
Superimposition

35 genes projected

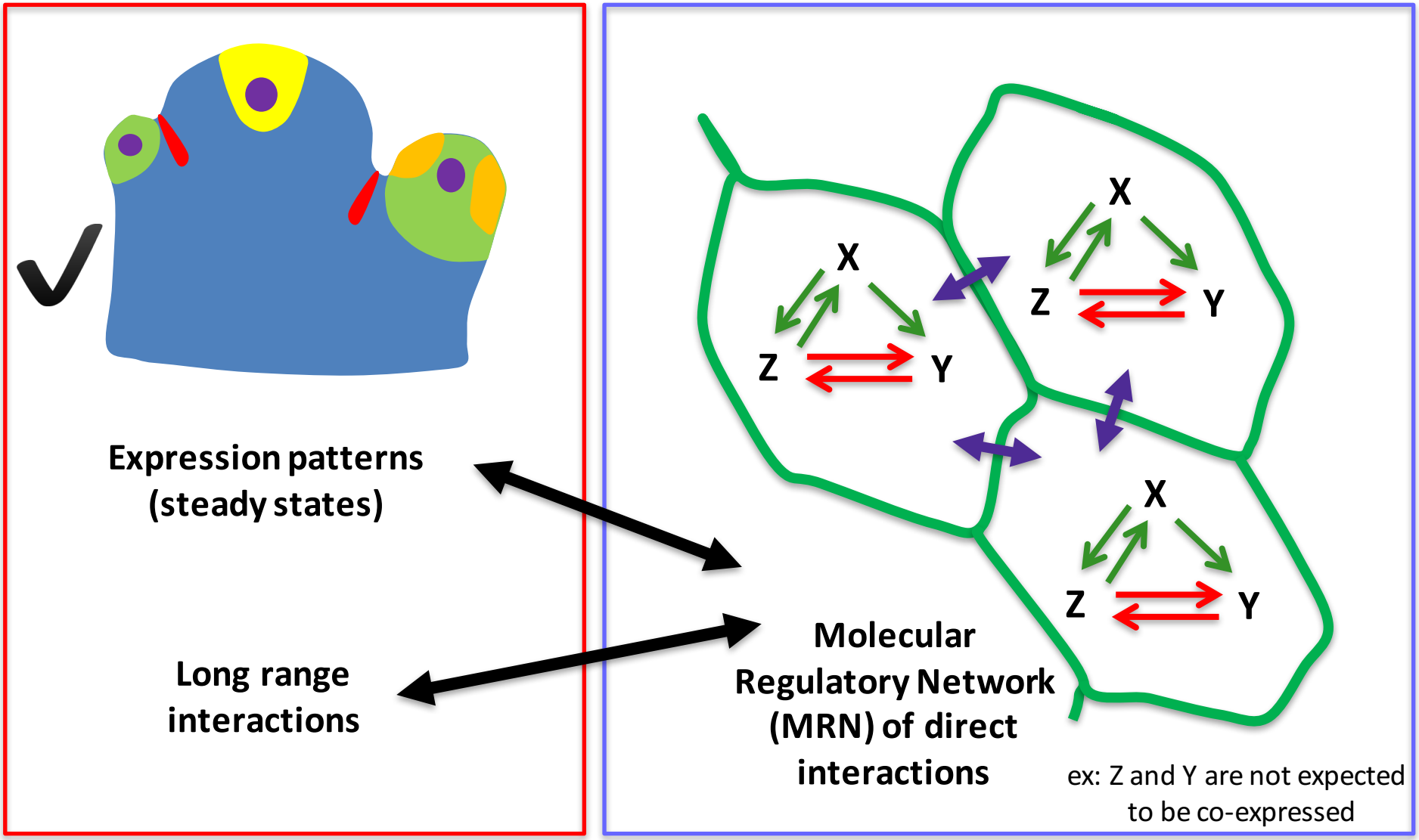


6 molecular states with 35 elements

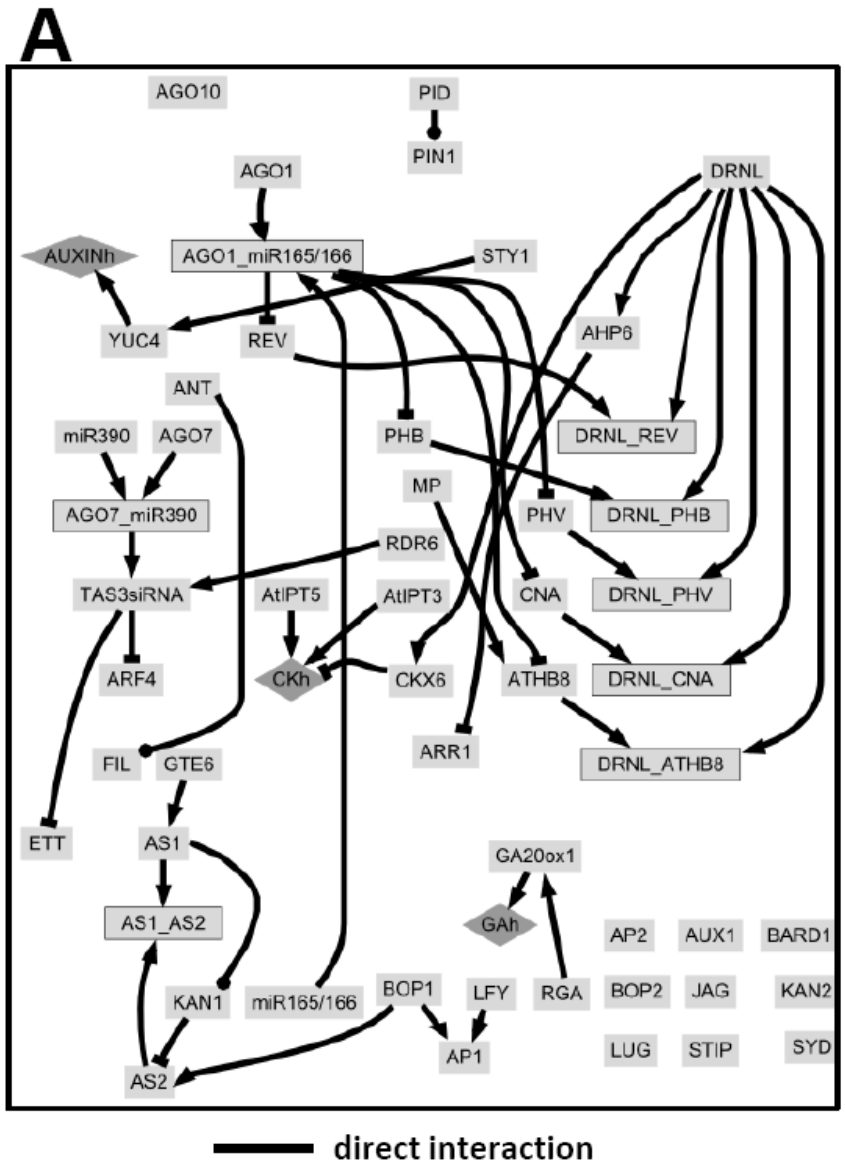
	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6
AGO1	1	1	1	1	1	1
AGO7	1	1	1	0	0	0
AGO10	1	1	1	0	0	0
AHP6	1	1	1	1	1	1
ANT	1	1	1	1	1	1
AP1	1	1	1	1	1	1
AP2	1	1	1	1	1	1
ARF4	0	0	0	1	1	1
AS1	1	1	1	1	0	0
AS2	1	1	0	0	0	0
AtHB8	0	0	0	1	0	0
AUX1	1	0	0	0	1	0
BARD1	1	1	1	1	1	1
BOP2	1	1	0	0	0	0
CNA	1	1	1	0	0	0
DRNL	1	1	1	1	1	1
ETT	0	0	0	1	1	1
FIL	0	0	0	1	1	1
JAG	0	0	1	1	0	1
KAN1	0	0	0	0	1	1
LFY	1	1	1	1	1	1
LUG	1	1	1	1	1	1
MiR165/166	0	0	0	1	1	1
MiR390	1	1	1	1	1	1
MP	1	1	1	0	0	0
PHB	1	1	1	0	0	0
PHV	1	1	0	0	0	0
PID	1	1	1	1	1	1
PIN1	1	0	0	1	1	0
REV	1	1	1	0	0	0
STIP	0	0	0	1	1	1
STY1	1	1	1	1	1	1
SYD	1	1	1	1	1	1
TAS3	1	1	1	0	0	0
YUC4	1	1	1	1	1	1



Objective: construct a network which is coherent with expression patterns and long range interactions



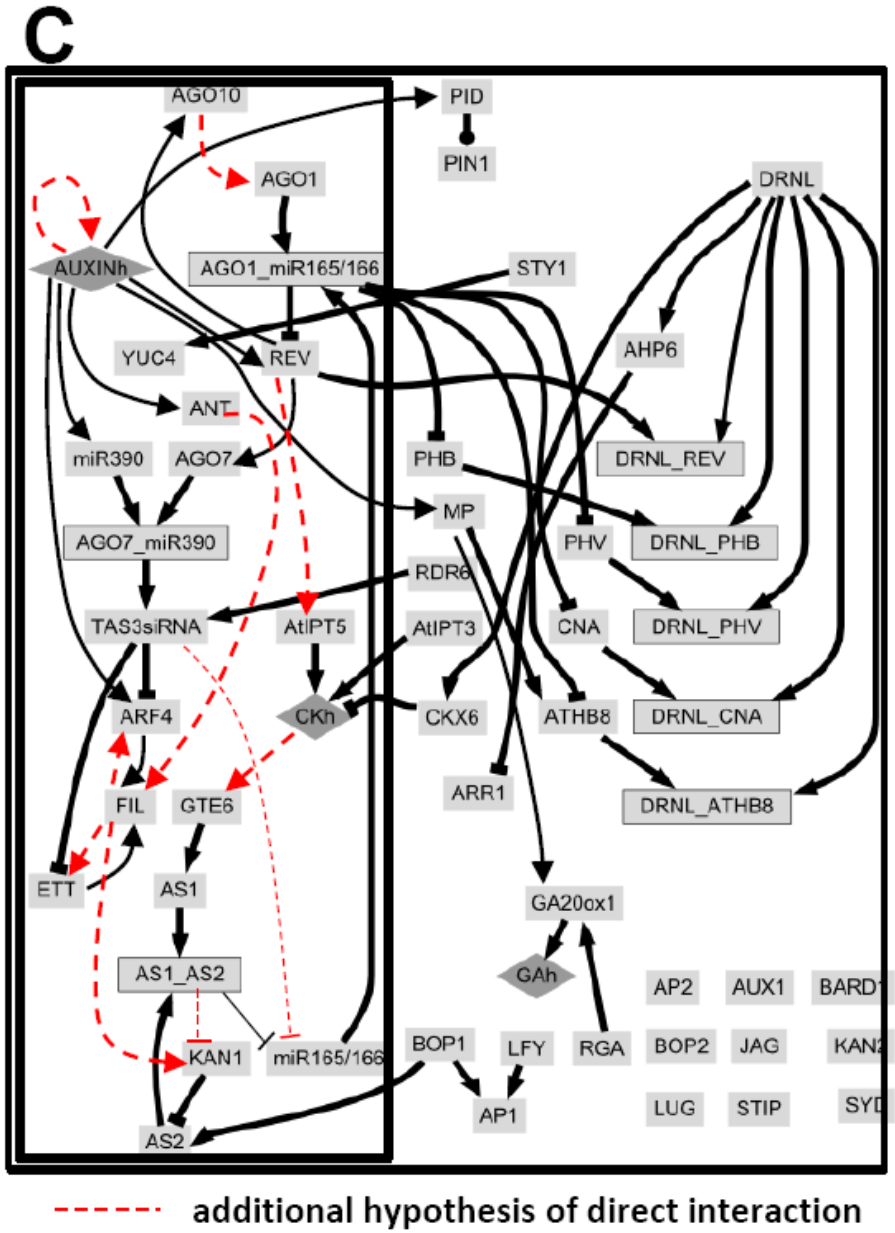
Candidate molecular interaction graph (using direct interactions)



Theoretical requirements

- close circuits
- inputs for each element

We can make hypotheses to fulfill requirements: how?

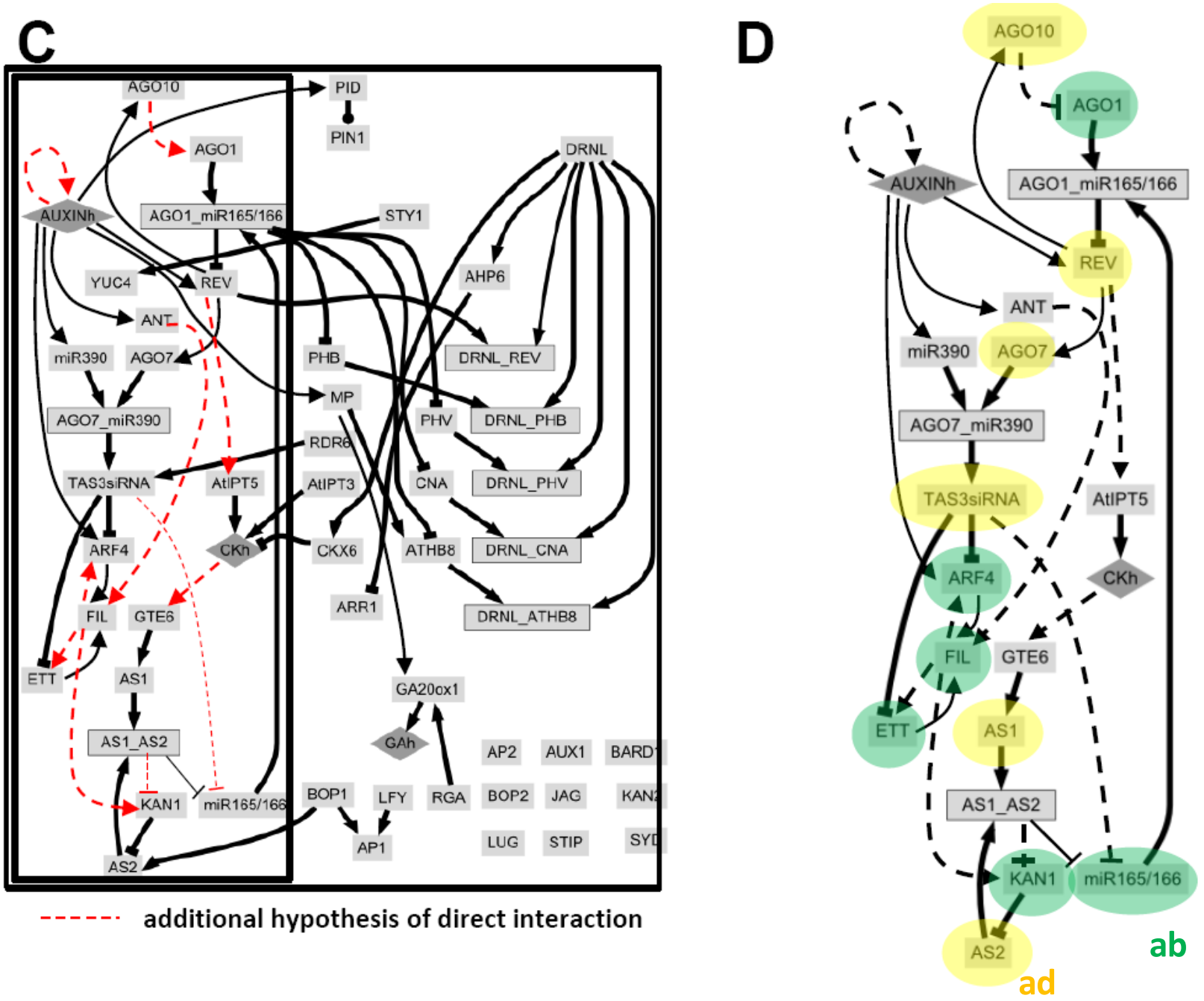


Example:
Genetic evidence for a TF activating gene X

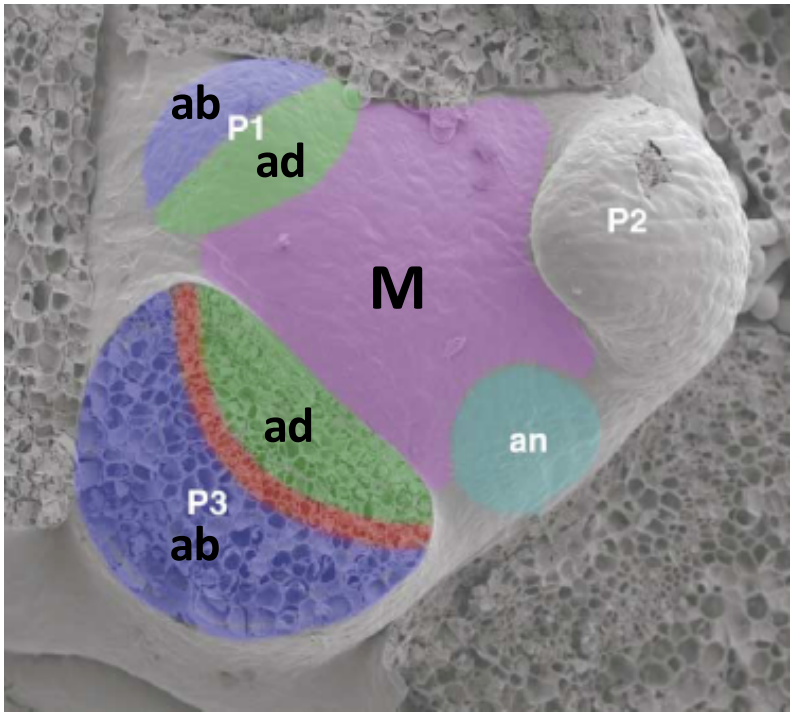
Does the promoter of gene X contain predicted binding site for the TF?

If yes, hypothesis of direct interaction

Candidate molecular interaction graph

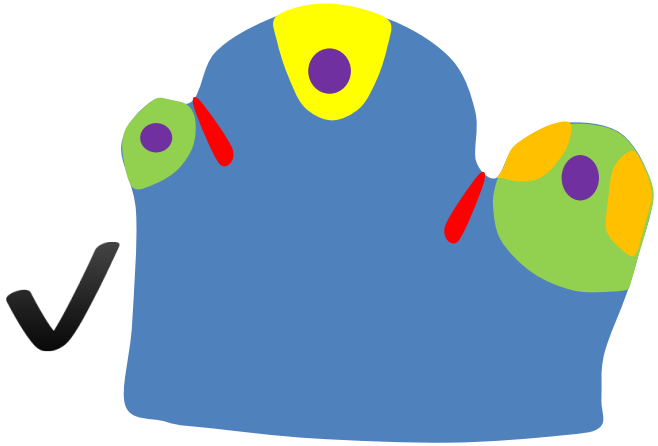


Positional information



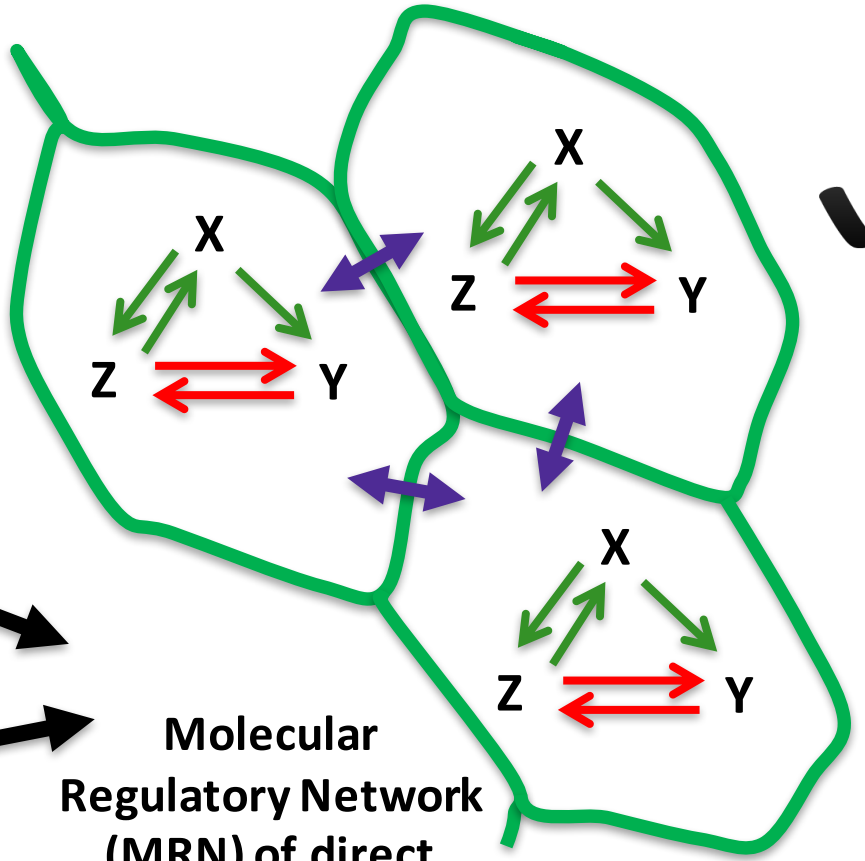
The adaxial side is closer to the meristem (adjacent) compared to the abaxial side

Objective: construct a network which is coherent with expression patterns and long range interactions



Expression patterns
(steady states)

Long range
interactions



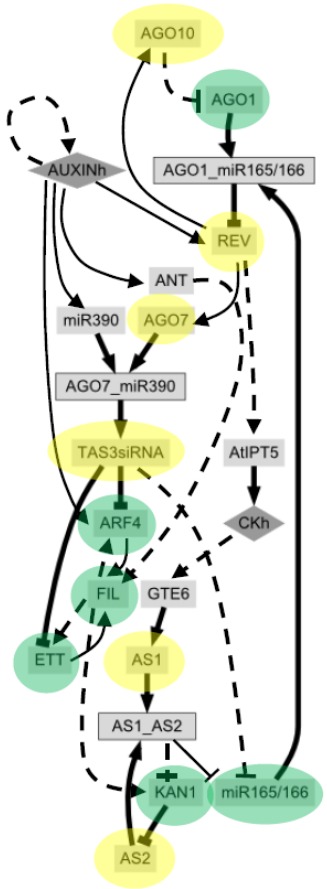
Molecular
Regulatory Network
(MRN) of direct
interactions

ex: Z and Y are not expected
to be co-expressed

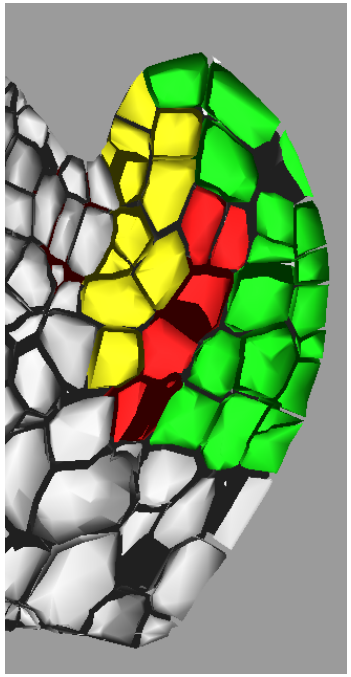
behavior

structure

Expected behavior of our network

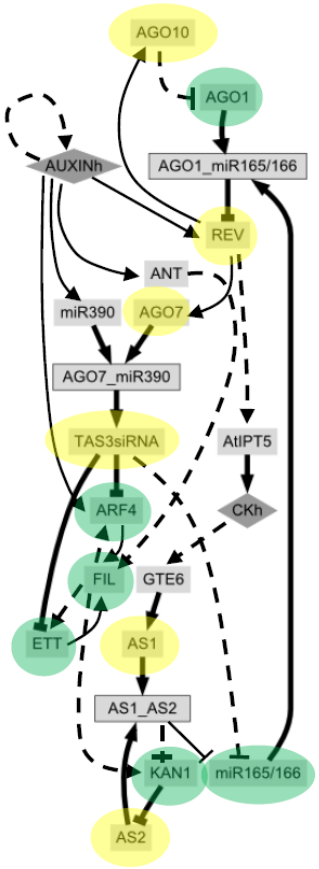


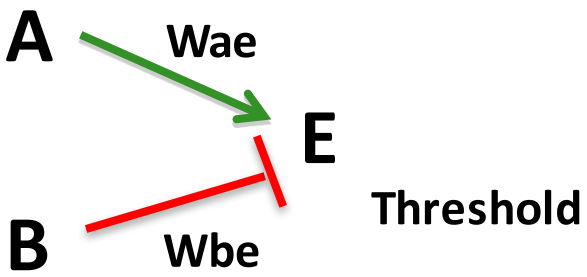
	Zone 1 Adaxial	Zone 2 Vascular	Zone 3 Abaxial
AGO1	1	1	1
AGO10	1	0	0
AGO7	1	0	0
ANT	1	1	1
ARF4	0	1	1
AS1	1	1	0
AS2	1	0	0
ETT	0	1	1
FIL	0	1	1
KAN1	0	0	1
MiR165/166	0	1	1
MiR390	1	1	1
REV	1	0	0
TAS3	1	0	0



A mathematical model is required

The behavior of each element is determined by its inputs

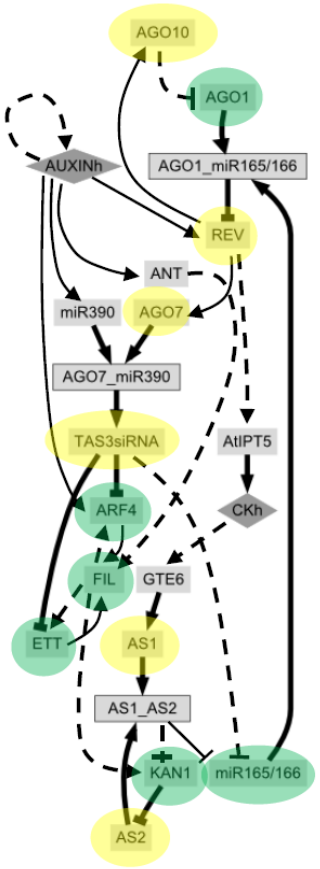


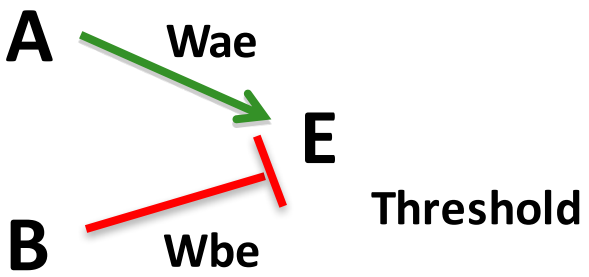


W = force of the influence of an element on another
T = threshold of activation

A mathematical model is required

The behavior of each element is determined by its inputs





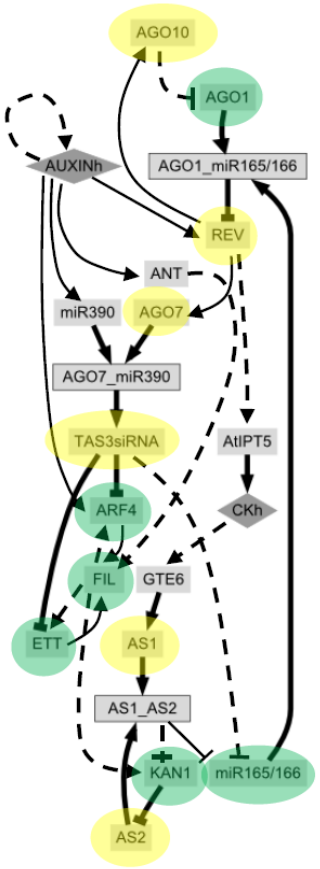
A	B	E
0	0	0
1	0	1
0	1	0
1	1	0

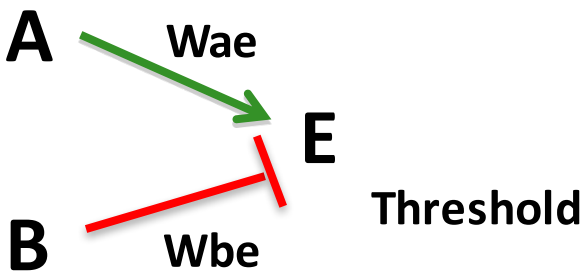
W = force of the influence of an element on another
T = threshold of activation

A	B	E
0	0	0
1	0	1
0	1	0
1	1	1

A mathematical model is required

The behavior of each element is determined by its inputs





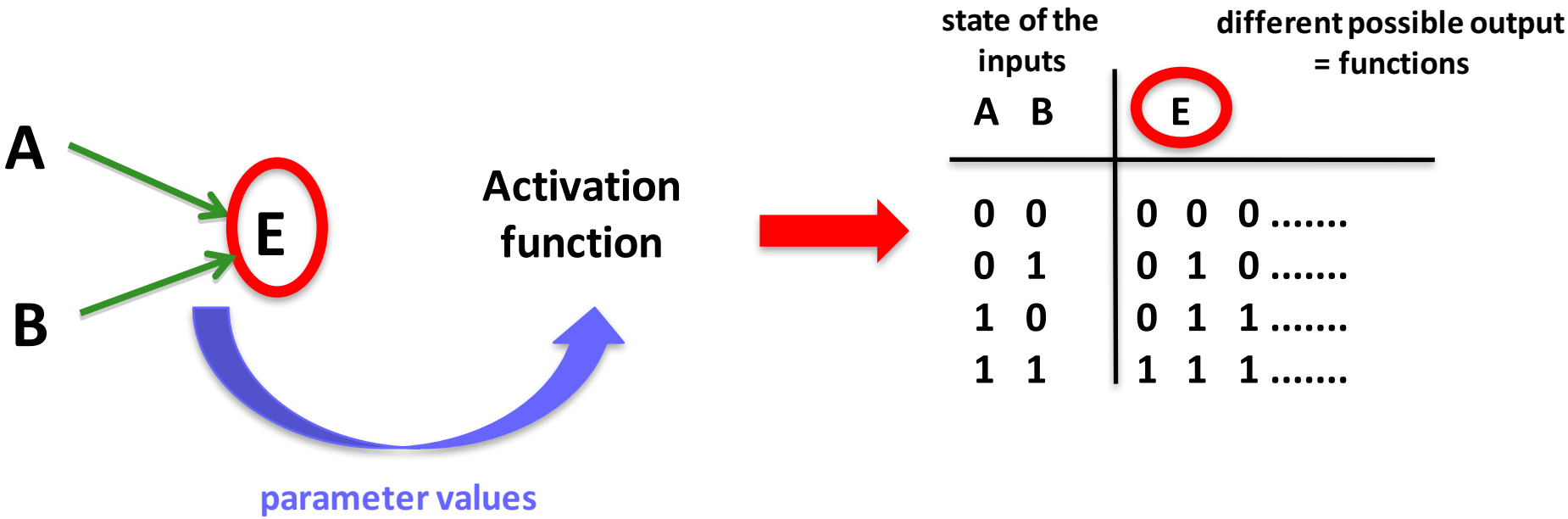
A	B	E
0	0	0
1	0	1
0	1	0
1	1	0

W = force of the influence of an element on another
T = threshold of activation

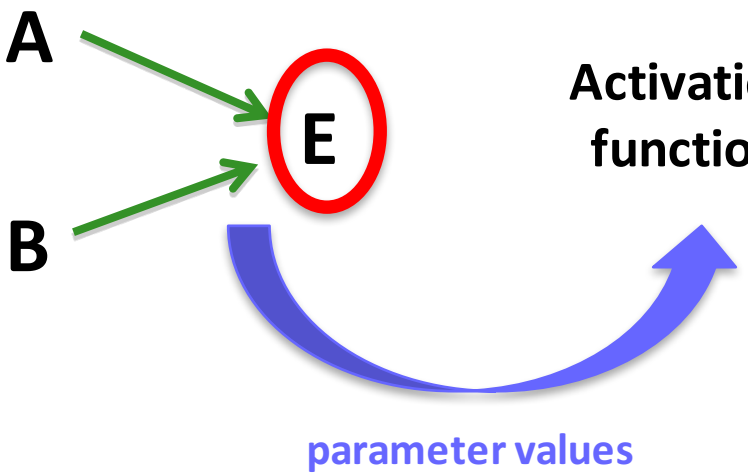
A	B	E
0	0	0
1	0	1
0	1	0
1	1	1

Different possible behavior depending on the parameter values

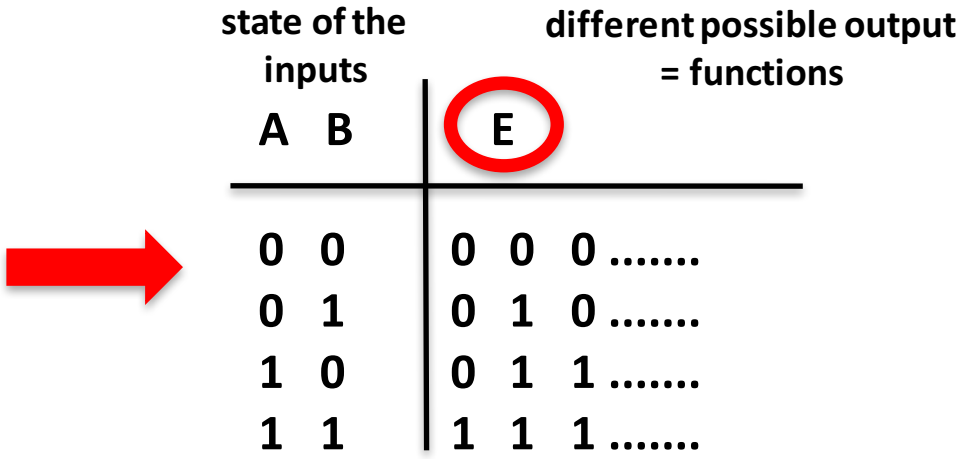
Behavior expressed as an activation function



Behavior expressed as an activation function



Activation function

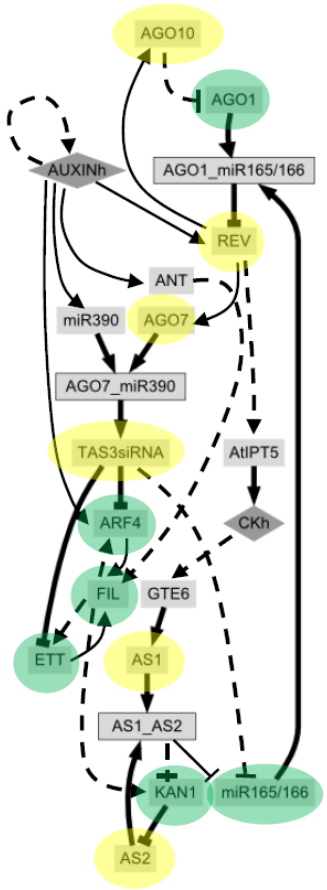


state of the inputs		different possible output = functions		
A	B	E		
0	0	0	0	0 .....
0	1	0	1	0 .....
1	0	0	1	1 .....
1	1	1	1	1 .....

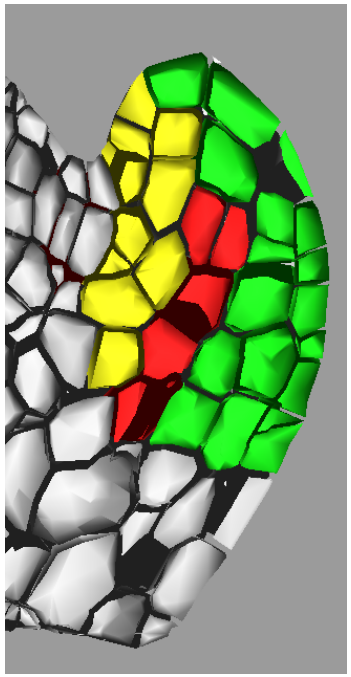
Activation functions are found for each element

A solution is represented by a set of activation functions

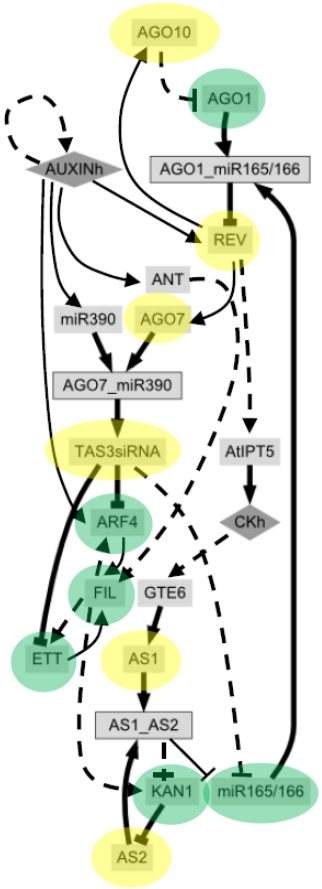
Parameter values inferred from expression data > solution(s)



	Zone 1 Adaxial	Zone 2 Vascular	Zone 3 Abaxial
AGO1	1	1	1
AGO10	1	0	0
AGO7	1	0	0
ANT	1	1	1
ARF4	0	1	1
AS1	1	1	0
AS2	1	0	0
ETT	0	1	1
FIL	0	1	1
KAN1	0	0	1
MiR165/166	0	1	1
MiR390	1	1	1
REV	1	0	0
TAS3	1	0	0



47 solutions were found



Solutions	AGO10	AGO1	AGO1_miR165	AGO7	AGO7_MIR390	ANT	AtIPT5	ARF4	AS1	AS1_AS2	AS2	AUXIN	CK	ETT	FIL	GTE6	KAN1	miR165	MiR390	REV	TAS3siRNA
20	2	1	8	2	8	2	2	15	2	8	1	2	2	2	224	2	4	1	2	4	2
34	2	1	8	2	8	2	2	15	2	8	1	2	2	3	160	2	4	1	2	4	2
35	2	1	8	2	8	2	2	15	2	8	1	2	2	3	192	2	4	1	2	4	2
39	2	1	8	2	8	2	2	15	2	8	1	2	2	2	192	2	4	1	2	4	2
41	2	1	8	2	8	2	2	15	2	8	1	2	2	3	192	2	12	1	2	4	2
42	2	1	8	2	8	2	2	15	2	8	1	2	2	3	192	2	5	1	2	4	2
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45	2	1	8	2	8	2	2	14	2	8	1	2	2	3	192	2	4	1	2	4	2
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247	2	1	8	2	8	2	2	15	2	8	1	2	2	2	128	2	4	1	2	5	2
276	2	1	8	2	8	2	2	12	2	8	1	2	2	2	128	2	5	1	2	4	2
277	2	1	8	2	8	2	2	12	2	8	1	2	2	3	128	2	5	1	2	4	2
278	2	1	8	2	8	2	2	14	2	8	1	2	2	3	128	2	5	1	2	4	2
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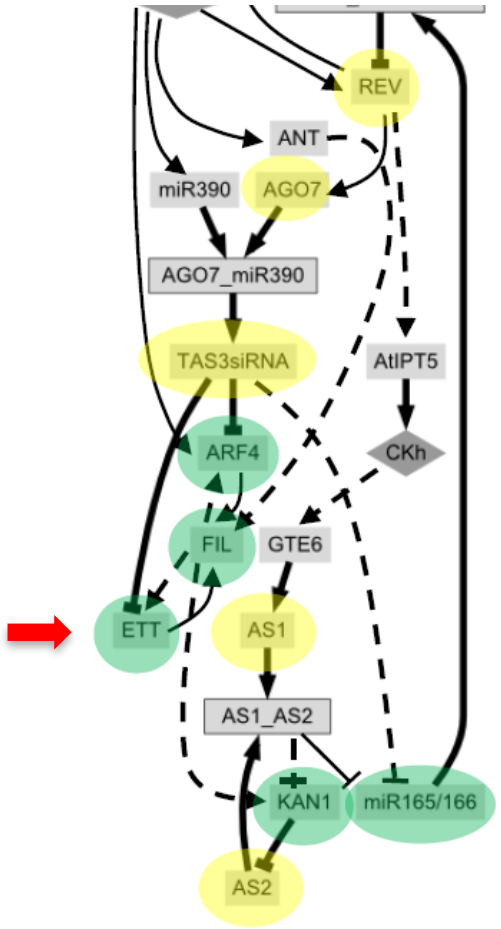
315	2	1	8	2	8	2	2	14	2	8	1	2	2	2	240	2	4	1	2	4	2
316	2	1	8	2	8	2	2	14	2	8	1	2	2	2	160	2	4	1	2	4	2
317	2	1	8	2	8	2	2	14	2	8	1	2	2	3	160	2	4	1	2	4	2
320	2	1	8	2	8	2	2	14	2	8	1	2	2	2	128	2	5	1	2	4	2

47 solutions were found

ETT

Inputs		Functions	
FIL	TAS3	f_2	f_3
0	0	0	1
1	0	1	1
0	1	0	0
1	1	0	0



Solutions	AGO10	AGO1	AGO1_miR165	AGO7	AGO7_MIR390	ANT	AtIPT5	ARF4	AS1	AS1_AS2	AS2	AUXIN	CK	ETT	FIL	GTE6	KAN1	miR165	MiR390	REV	TAS3siRNA
20	2	1	8	2	8	2	2	15	2	8	1	2	2	2	224	2	4	1	2	4	2
34	2	1	8	2	8	2	2	15	2	8	1	2	2	3	160	2	4	1	2	4	2
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39	2	1	8	2	8	2	2	15	2	8	1	2	2	2	192	2	4	1	2	4	2
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55	2	1	8	2	8	2	2	10	2	8	1	2	2	3	192	2	4	1	2	4	2
57	2	1	8	2	8	2	2	15	2	8	1	2	2	3	224	2	4	1	2	4	2
61	2	1	8	2	8	2	2	15	2	8	1	2	2	3	168	2	4	1	2	4	2
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66	2	1	8	2	8	2	2	15	2	8	1	2	2	2	160	2	4	1	2	4	2
68	2	1	8	2	8	2	2	15	2	8	1	2	2	2	128	2	4	1	2	4	2
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114	2	1	8	2	8	2	2	14	2	8	1	2	2	2	128	2	4	1	2	4	2
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116	2	1	8	2	8	2	2	15	2	8	1	2	2	3	136	2	4	1	2	4	2
127	2	1	8	2	8	2	2	15	2	8	1	2	2	2	128	2	12	1	2	4	2
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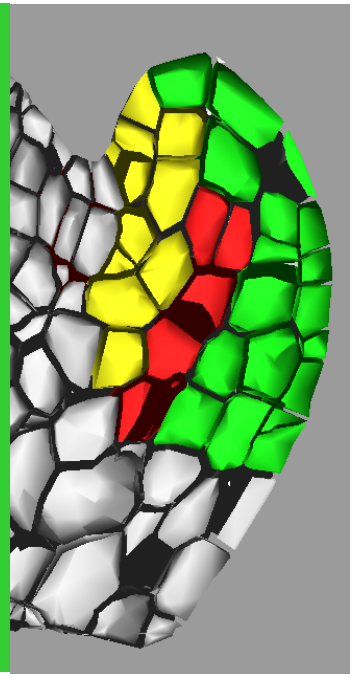
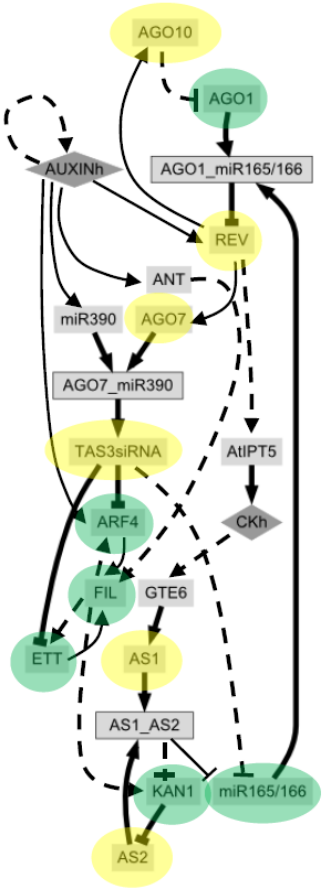
317	2	1	8	2	8	2	2	14	2	8	1	2	2	3	160	2	4	1	2	4	2
320	2	1	8	2	8	2	2	14	2	8	1	2	2	2	128	2	5	1	2	4	2

	Zone 1 Adaxial	Zone 2 Vascular	Zone 3 Abaxial
AGO1	1	1	1
AGO10	1	0	0
AGO7	1	0	0
ANT	1	1	1
ARF4	0	1	1
AS1	1	1	0
AS2	1	0	0
ETT	0	1	1
FIL	0	1	1
KAN1	0	0	1
MiR165/166	0	1	1
MiR390	1	1	1
REV	1	0	0
TAS3	1	0	0



Steady states

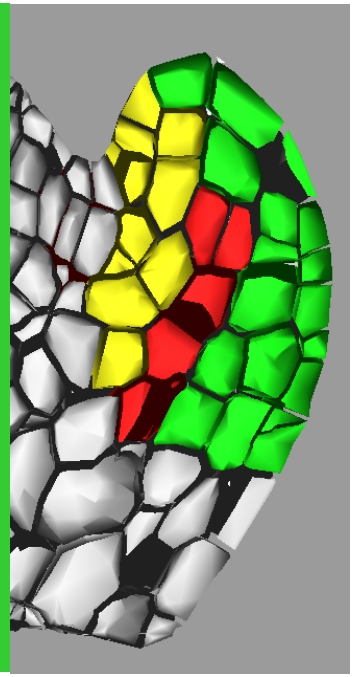
47 models can explain the data



Candidate molecular network

Structure

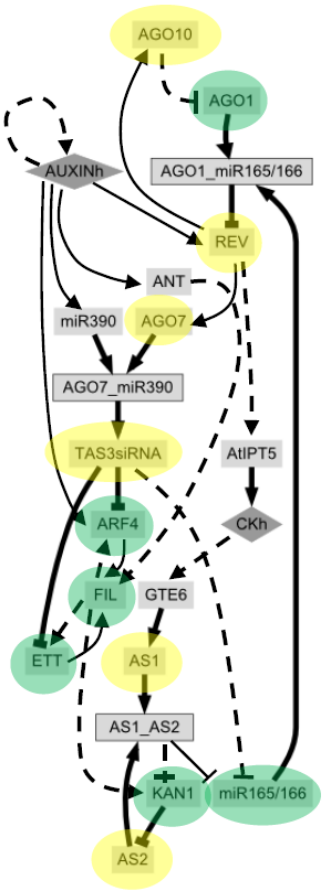
	Zone 1 Adaxial	Zone 2 Vascular	Zone 3 Abaxial
AGO1	1	1	1
AGO10	1	0	0
AGO7	1	0	0
ANT	1	1	1
ARF4	0	1	1
AS1	1	1	0
AS2	1	0	0
ETT	0	1	1
FIL	0	1	1
KAN1	0	0	1
MiR165/166	0	1	1
MiR390	1	1	1
REV	1	0	0
TAS3	1	0	0



Steady states

Predictions of these models

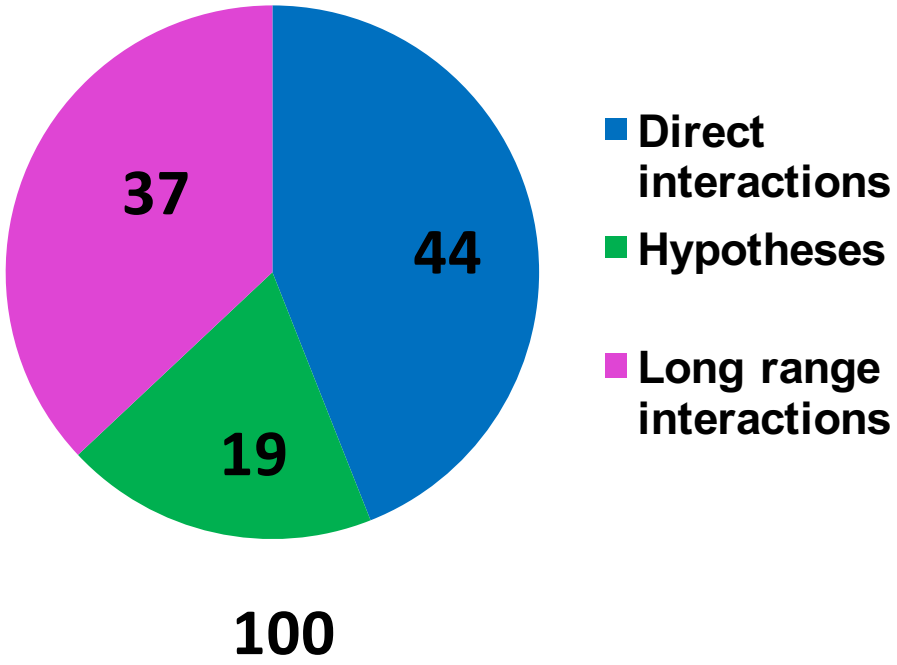
Long range interactions



Candidate molecular network

Structure

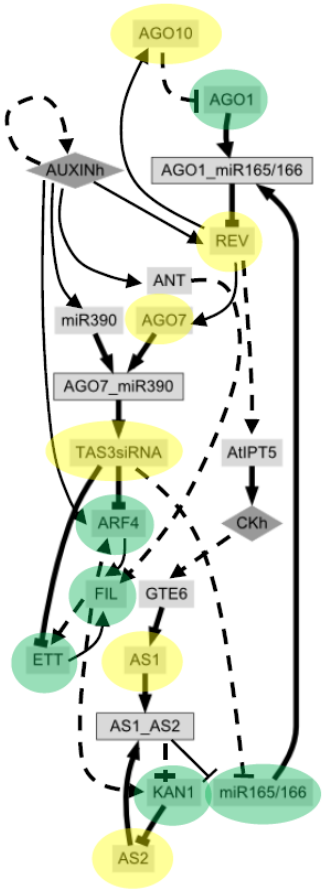
Model validation



100

37 genetic (indirect) interactions can be used to test the dynamics of the networks

Gain and loss-of-function mutations tested = 37 genetic interactions



Candidate molecular network

- 1- off(AGO10) → up(miR165), down(REV)
- 2- on(AGO10) → up(REV)
- 3- off(AGO7) → unchanged(FIL), up(ARF4,ETT)
- 4- off(AS1) → unchanged(FIL), up(ETT)
- 5- off(AS2) → up(ETT,FIL)
- 6- on(AS2) → down(FIL)
- 7- off(AS2,AGO10) → up(miR165), down(REV)
- 8- off(AS1,AGO7) → up(FIL)
- 9- off(AS2,AGO7) → up(miR165,FIL), down(REV)
- 10- off(ANT,FIL) → down(REV)
- 11- off(TAS3siRNA) → up(ETT,ARF4)
- 12- off(AS1,TAS3siRNA) → down(REV), up(miR165)
- 13- off(AS2,TAS3siRNA) → down(REV), up(miR165,FIL)
- 14- on(AUXIN) → down(CK), up(AtIPT5)
- 15- off(KAN1) → up(REV)
- 16- on(KAN1) → down(REV)
- 17- on(miR165) → down(AGO10), up(ETT,ARF4)
- 18- on(REV) → down (FIL, KAN1), up(AS2)
- 19- on(CK) → up(AS1)
- 20- on(FIL) → down(AS2)

Model validation

For each of the 47 solutions, we run simulations of gain and loss of function

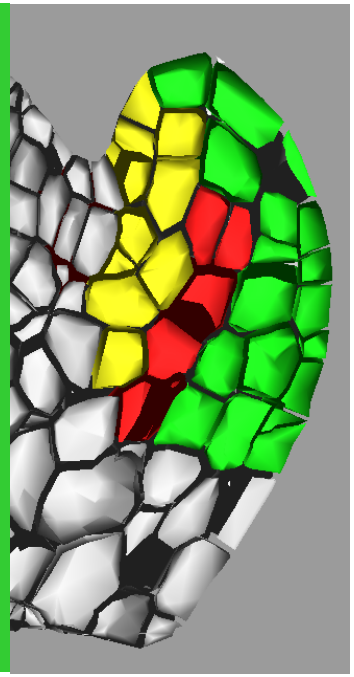
Model predictions **Experimental observations**

Model validation

35 (out of 37) indirect interactions predicted by the model were supported by experimental observations

The 47 solutions were all equally good

	Zone 1 Adaxial	Zone 2 Vascular	Zone 3 Abaxial
AGO1	1	1	1
AGO10	1	0	0
AGO7	1	0	0
ANT	1	1	1
ARF4	0	1	1
AS1	1	1	0
AS2	1	0	0
ETT	0	1	1
FIL	0	1	1
KAN1	0	0	1
MiR165/166	0	1	1
MiR390	1	1	1
REV	1	0	0
TAS3	1	0	0



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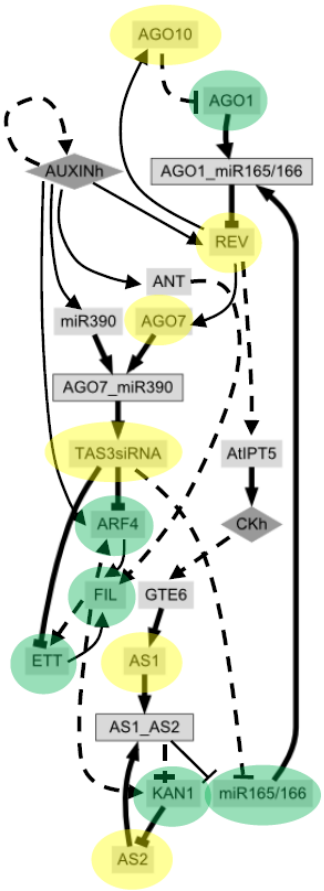
- 3- off(AGO7) → unchanged(FIL), up(ARF4,ETT)
- 4- off(AS1) → unchanged(FIL), up(ETT)
-
-
-

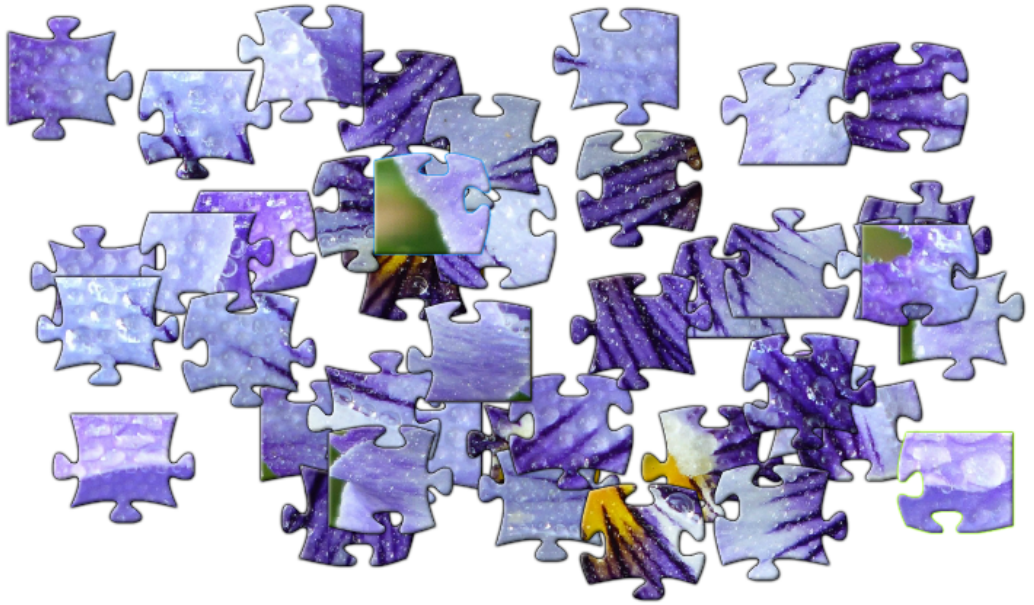
Long range interactions

Behavior

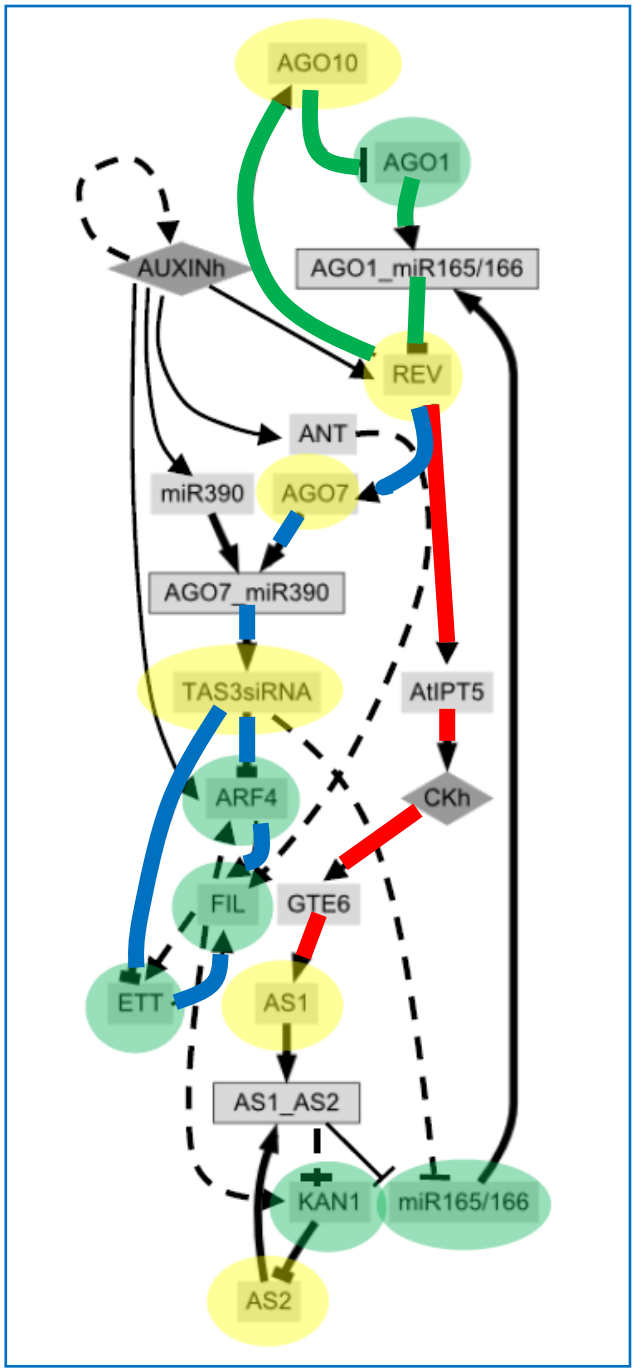


Candidate molecular network

Structure



What have we learned?

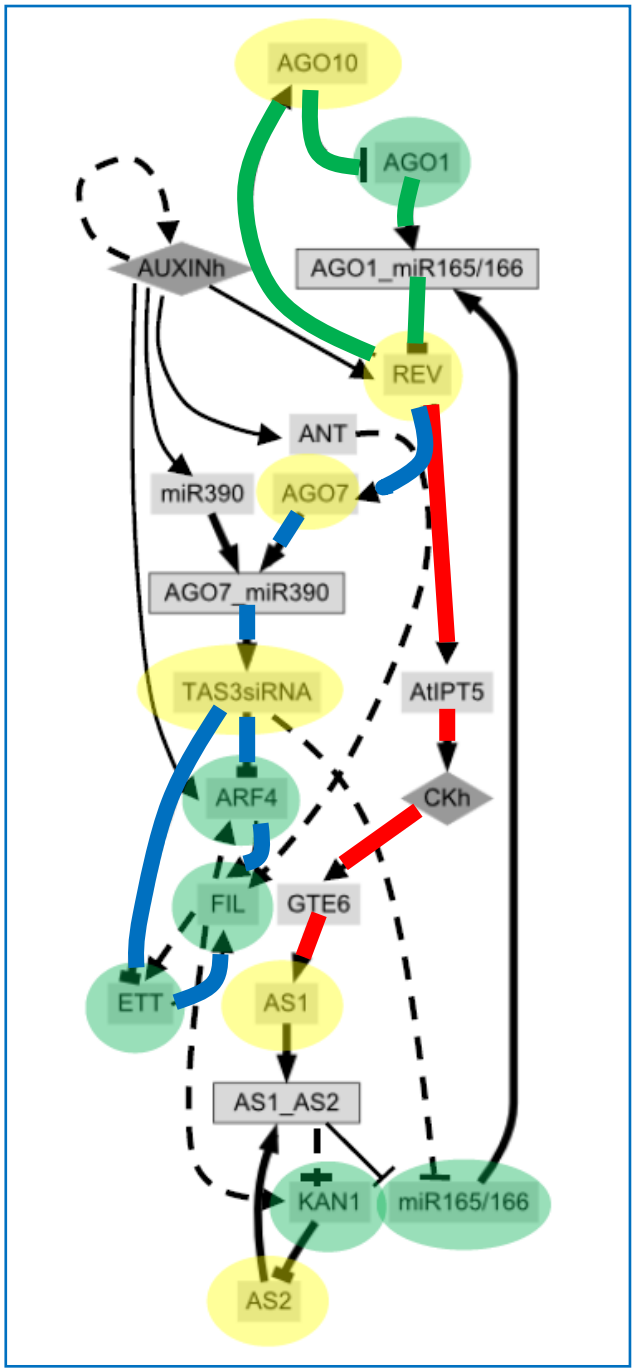


- **Several unexpected potential pathways revealed**

(REV>AS1, REV>TAS3, REV>AGO1 and miR)

La Rota et al. (2011) The Plant Cell

What have we learned?



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- **several predictions for activation functions of the elements (e.g. FIL requires all inputs active)**

La Rota et al. (2011) The Plant Cell

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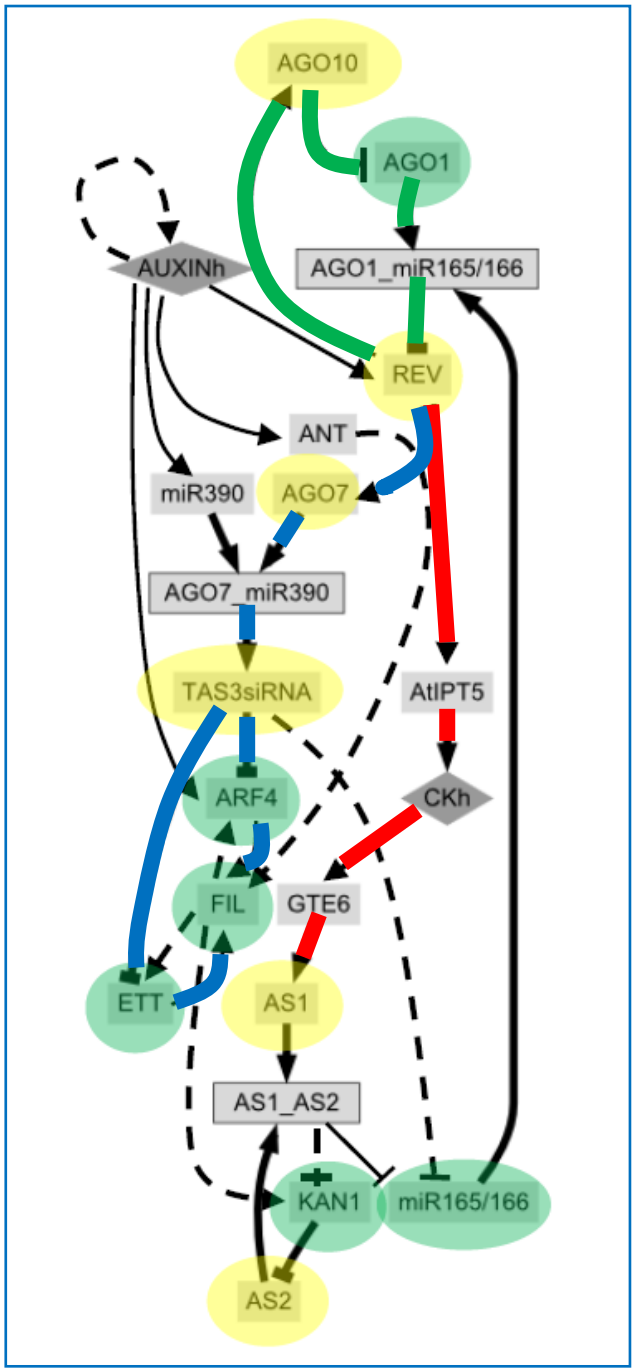

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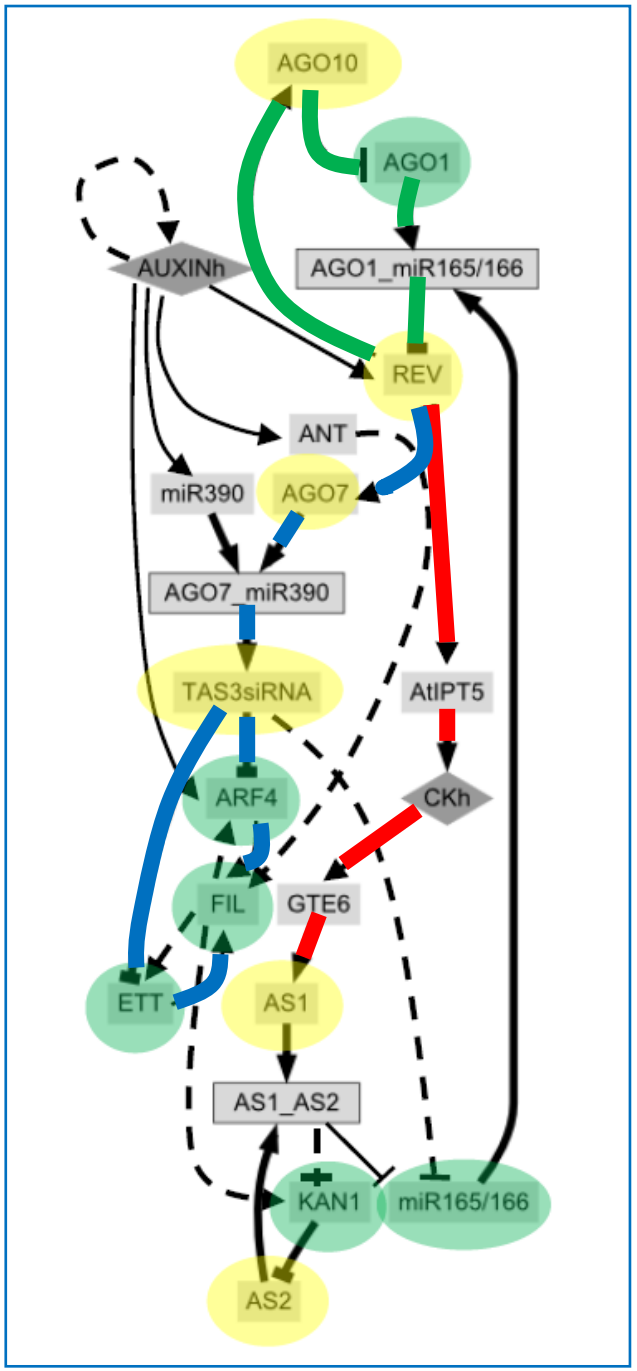
- all assumptions and hypotheses are predictions that can be tested experimentally

La Rota et al. (2011) The Plant Cell

What have we learned?



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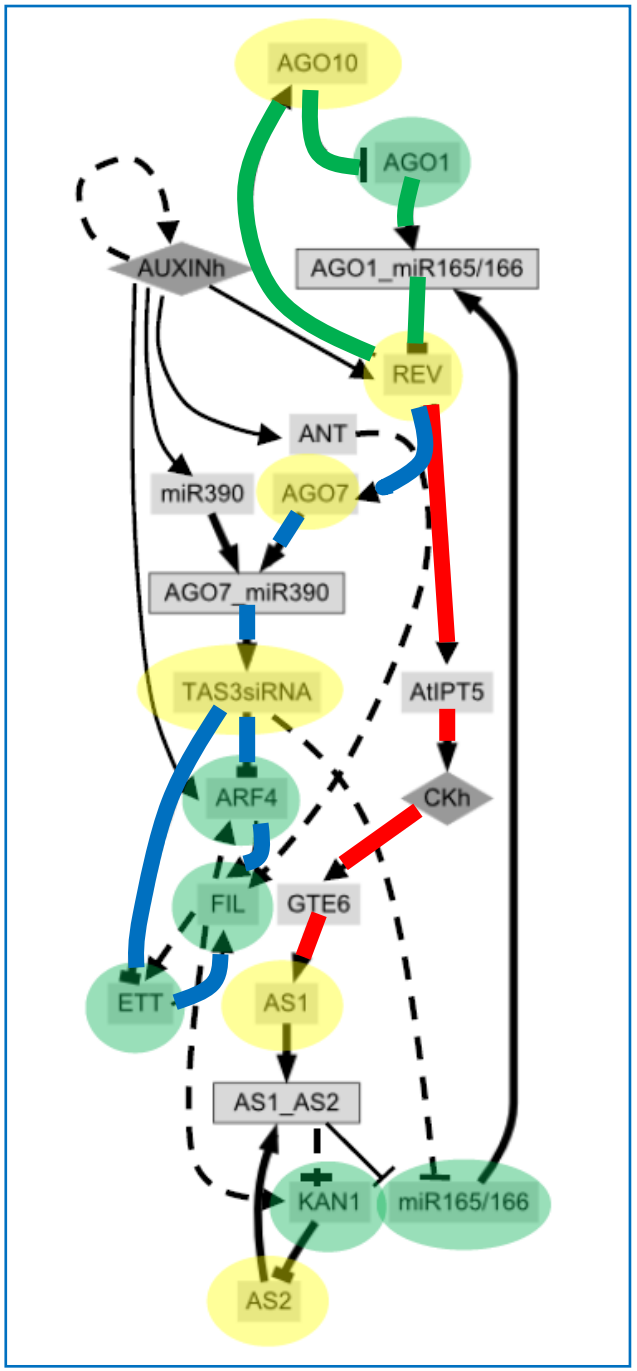
La Rota et al. (2011) The Plant Cell

What have we learned?



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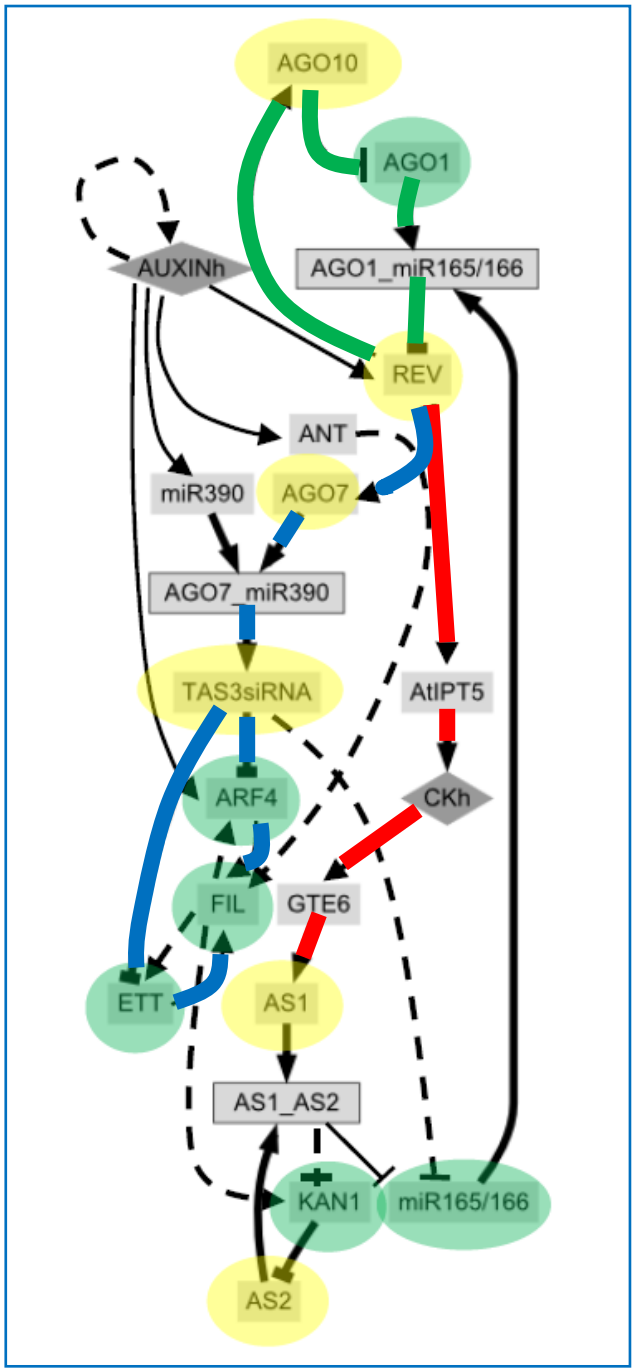
- Only based on published data !

La Rota et al. (2011) The Plant Cell

What have we learned?



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- several predictions for activation functions of the elements
(e.g. FIL requires all inputs active)

- all assumptions and hypotheses are predictions that can be tested experimentally

- The available data are mostly coherent > this network probably account for adaxial and abaxial identities

- Only based on published data !

- Can be implemented as new interactions are described

La Rota et al. (2011) The Plant Cell

Take home message

Genes

?

Development of living organisms

Take home message

Genes

Development of living organisms

Gene networks

Tissue behavior

molecular steady states

cell behavior

THE END

ARF4

Inputs			Functions				
AUX	FIL	TAS3	f_8	f_{10}	f_{12}	f_{14}	f_{15}
0	0	0	0	0	0	0	1
1	0	0	0	1	0	1	1
0	1	0	0	0	1	1	1
1	1	0	1	1	1	1	1
0	0	1	0	0	0	0	0
1	0	1	0	0	0	0	0
0	1	1	0	0	0	0	0
1	1	1	0	0	0	0	0

ETT

Inputs		Functions	
FIL	TAS3	f_2	f_3
0	0	0	1
1	0	1	1
0	1	0	0
1	1	0	0

FIL

Inputs			Functions						
ANT	ARF4	ETT	f_{128}	f_{136}	f_{160}	f_{168}	f_{192}	f_{224}	f_{240}
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0
1	1	0	0	1	0	1	0	0	0
0	0	1	0	0	0	0	0	0	1
1	0	1	0	0	1	1	0	1	1
0	1	1	0	0	0	0	1	1	1
1	1	1	1	1	1	1	1	1	1

KAN1

Inputs		Functions			
AS1_AS2	FIL	f_4	f_5	f_{12}	f_{13}
0	0	0	1	0	1
1	0	0	0	0	0
0	1	1	1	1	1
1	1	0	0	1	1

MiR165/166

Inputs		Functions
AS1_AS2	TAS3	f_1
0	0	1
1	0	0
0	1	0
1	1	0

REV

Inputs		Functions	
AGO1_miR165/166	AUXh	f_4	f_5
0	0	0	1
1	0	0	0
0	1	1	1
1	1	0	0