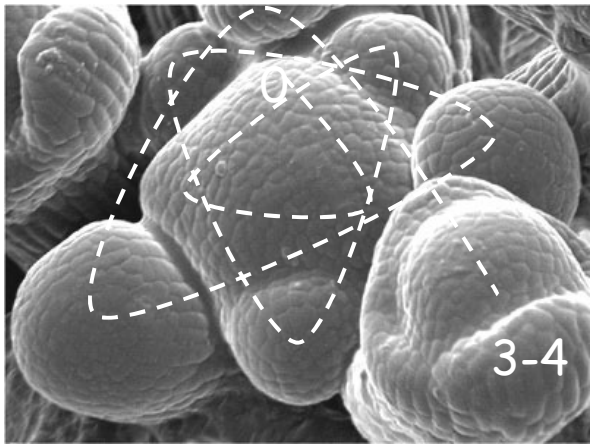
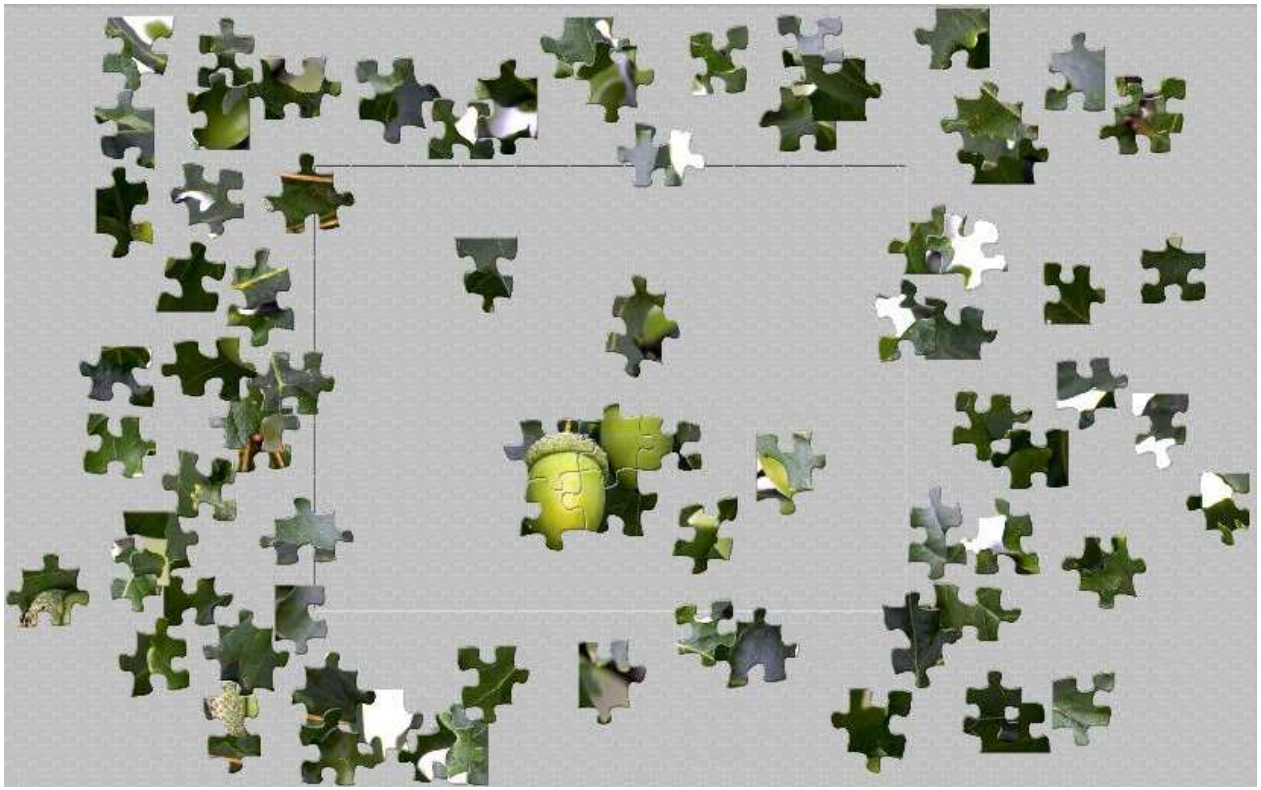
How do molecular networks control flower development?

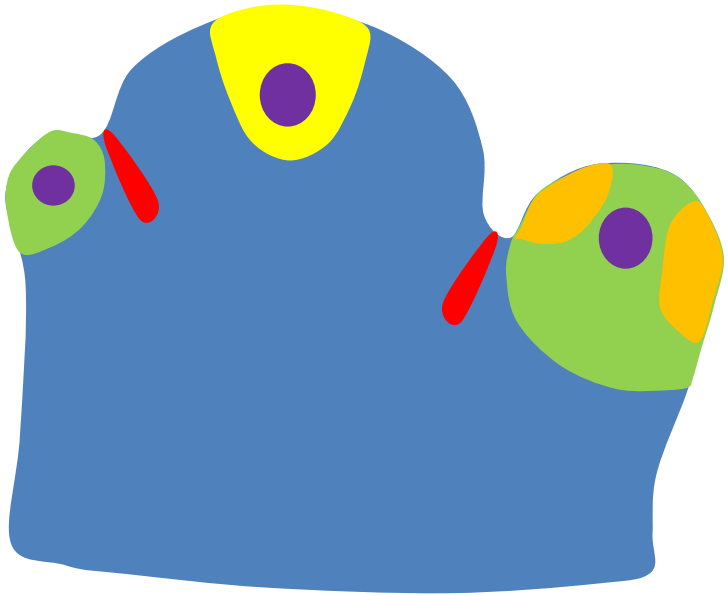


Actual knowledge on flower development?



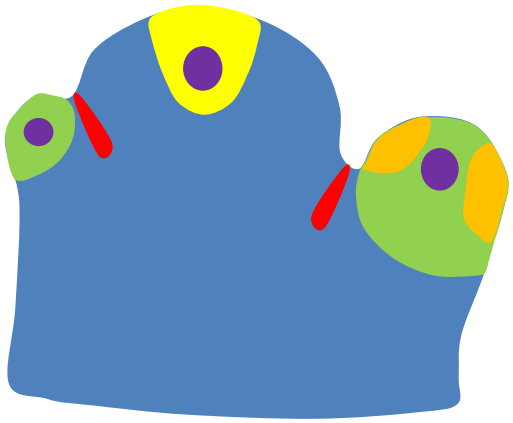
How to integrate the huge amount of heterogeneous data in a coherent manner?

Cell identities are associated with molecular steady states



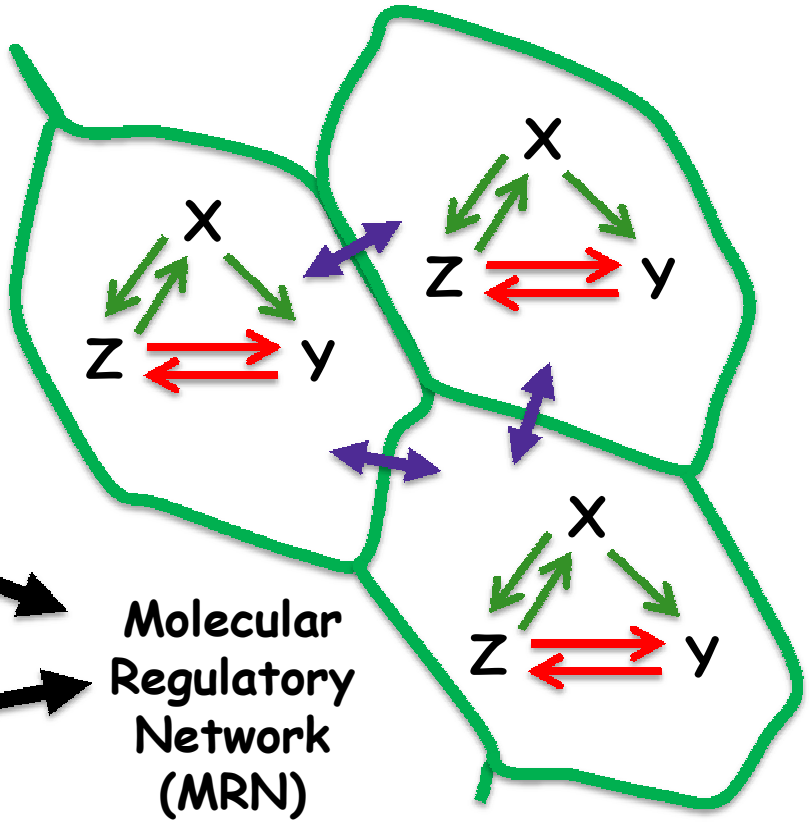
Development can be seen as a succession of molecular steady states evolving through time

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



Expression patterns
(steady states)

Long range
interactions



Molecular
Regulatory
Network
(MRN)

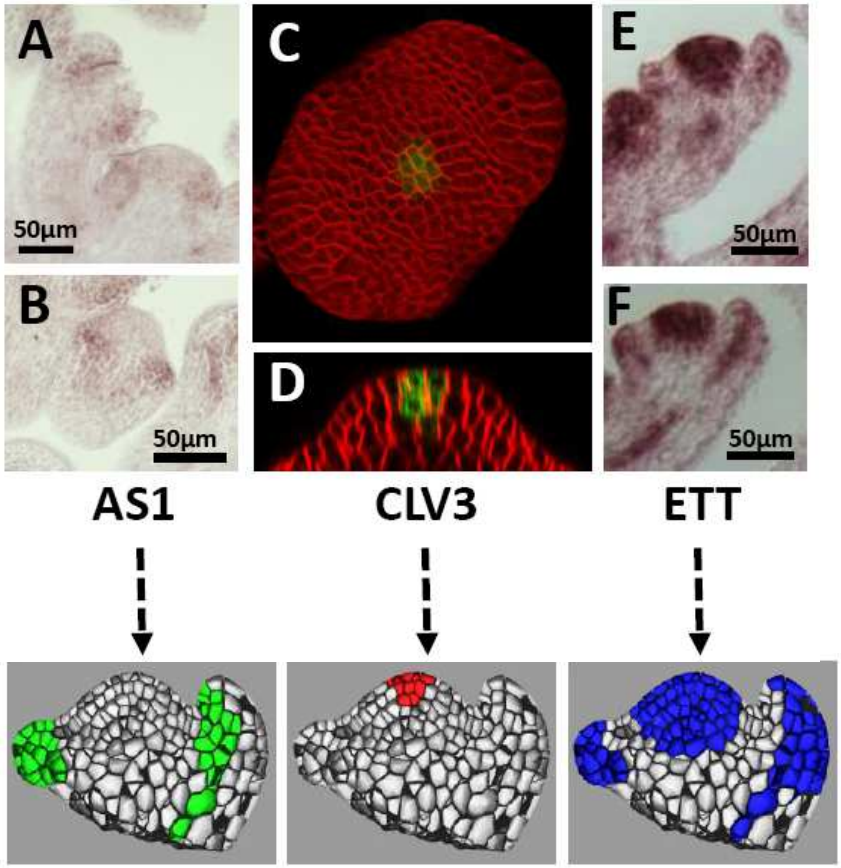
behavior

structure

1- Interaction database

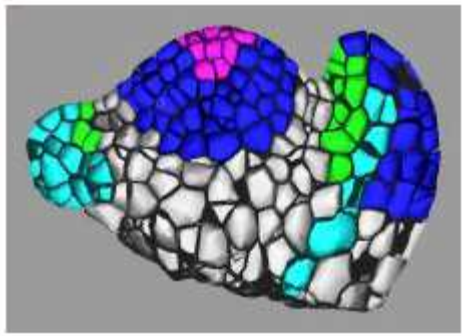
- direct molecular interactions
- induction evidence
- genetic interactions (can be direct or not)

2- Expression database > atlas > molecular states



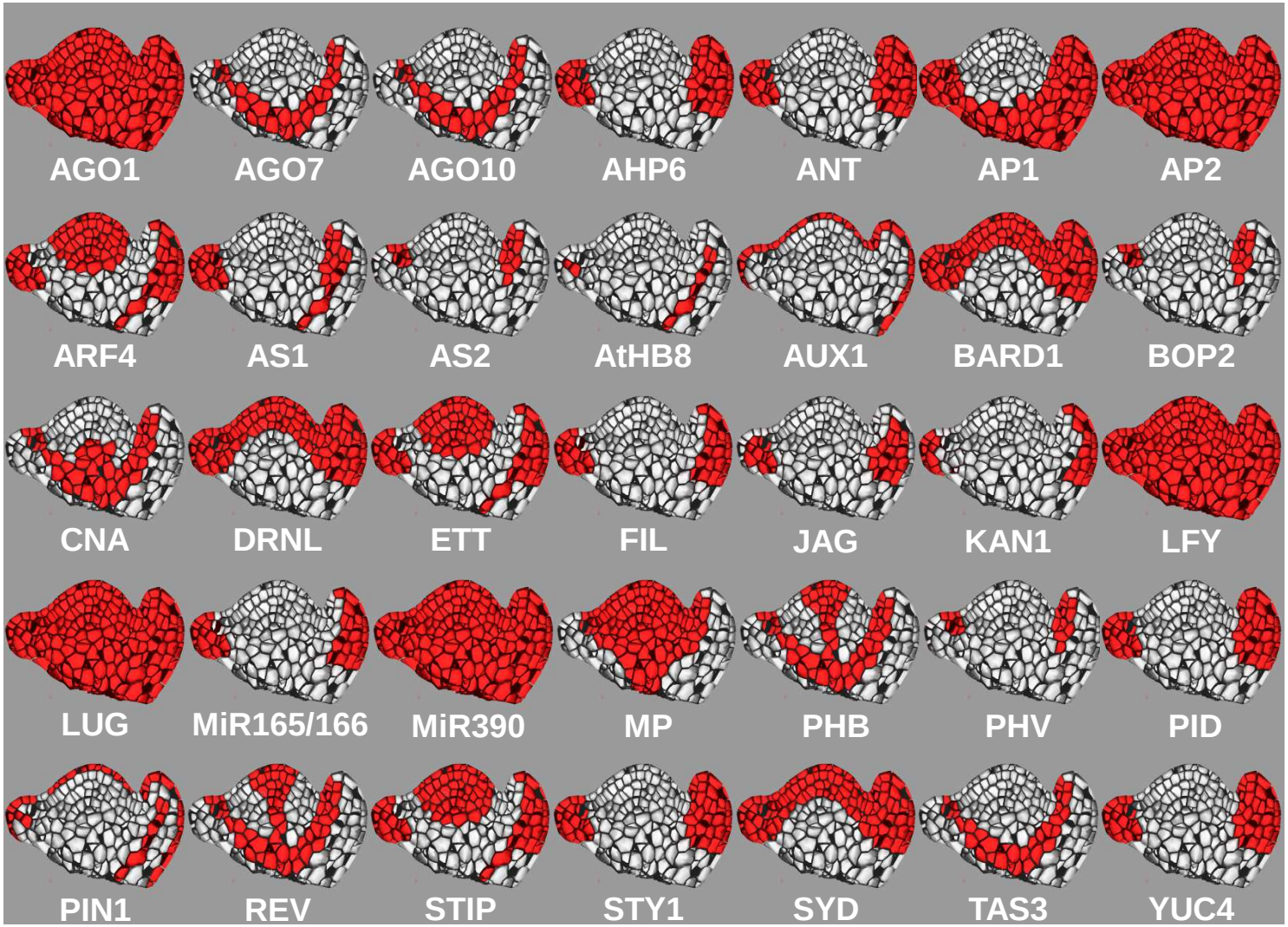
	Zone 1	Zone 2	Zone 3	Zone 4
AS1	1	0	1	0
CLV3	0	0	0	1
ETT	0	1	1	1

Steady states



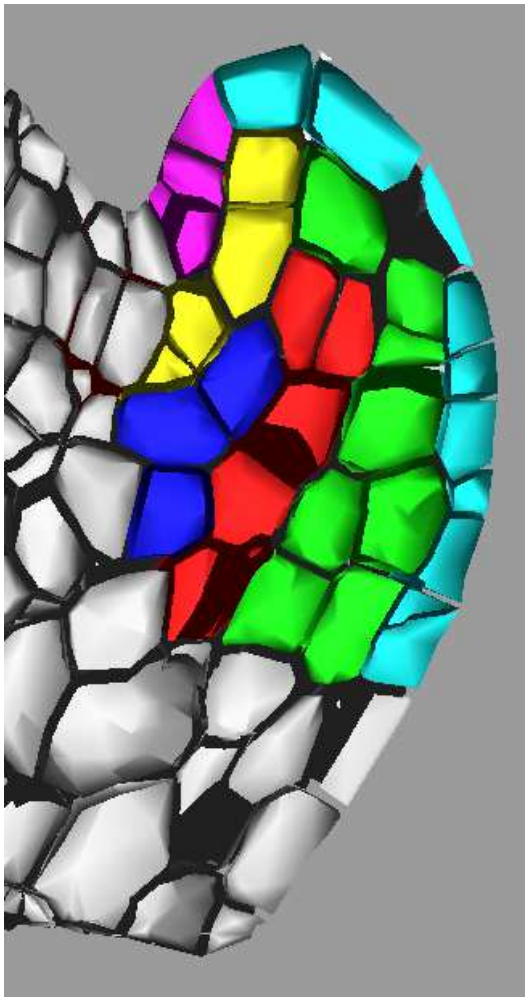
Superimposition

35 sepal genes projected (flower bud stage 3)

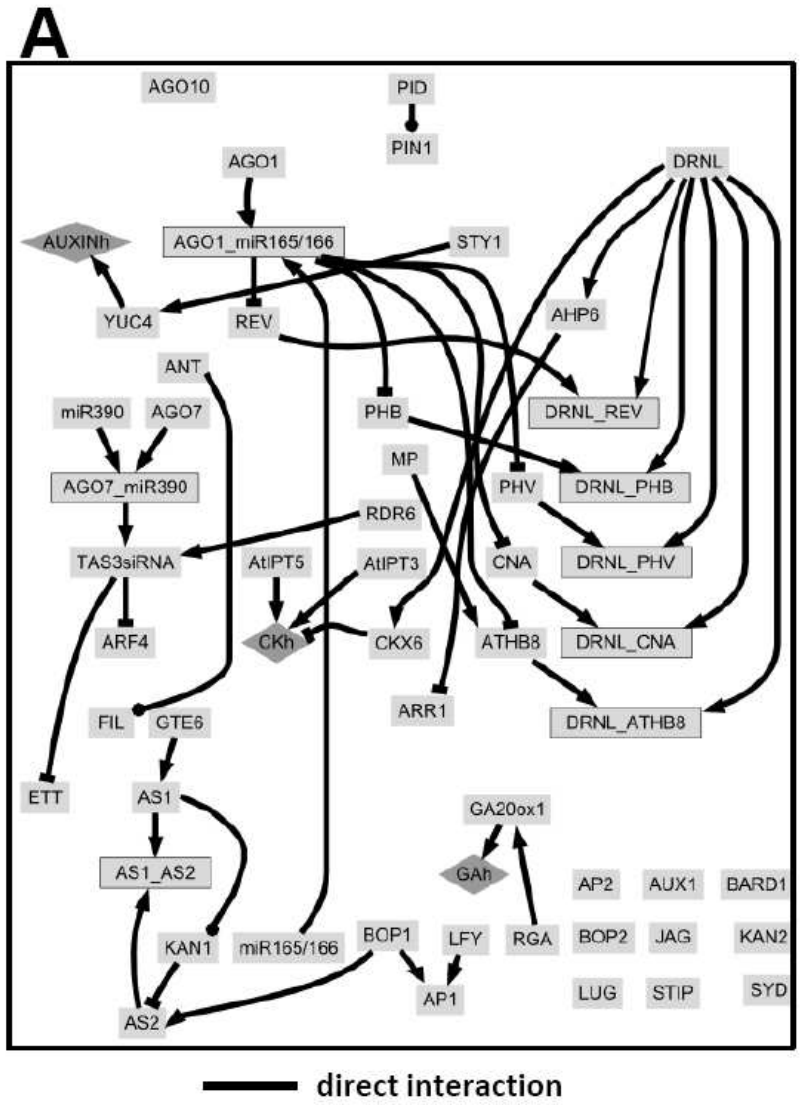


Sepal primordium : 6 molecular states with 35 elements

	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



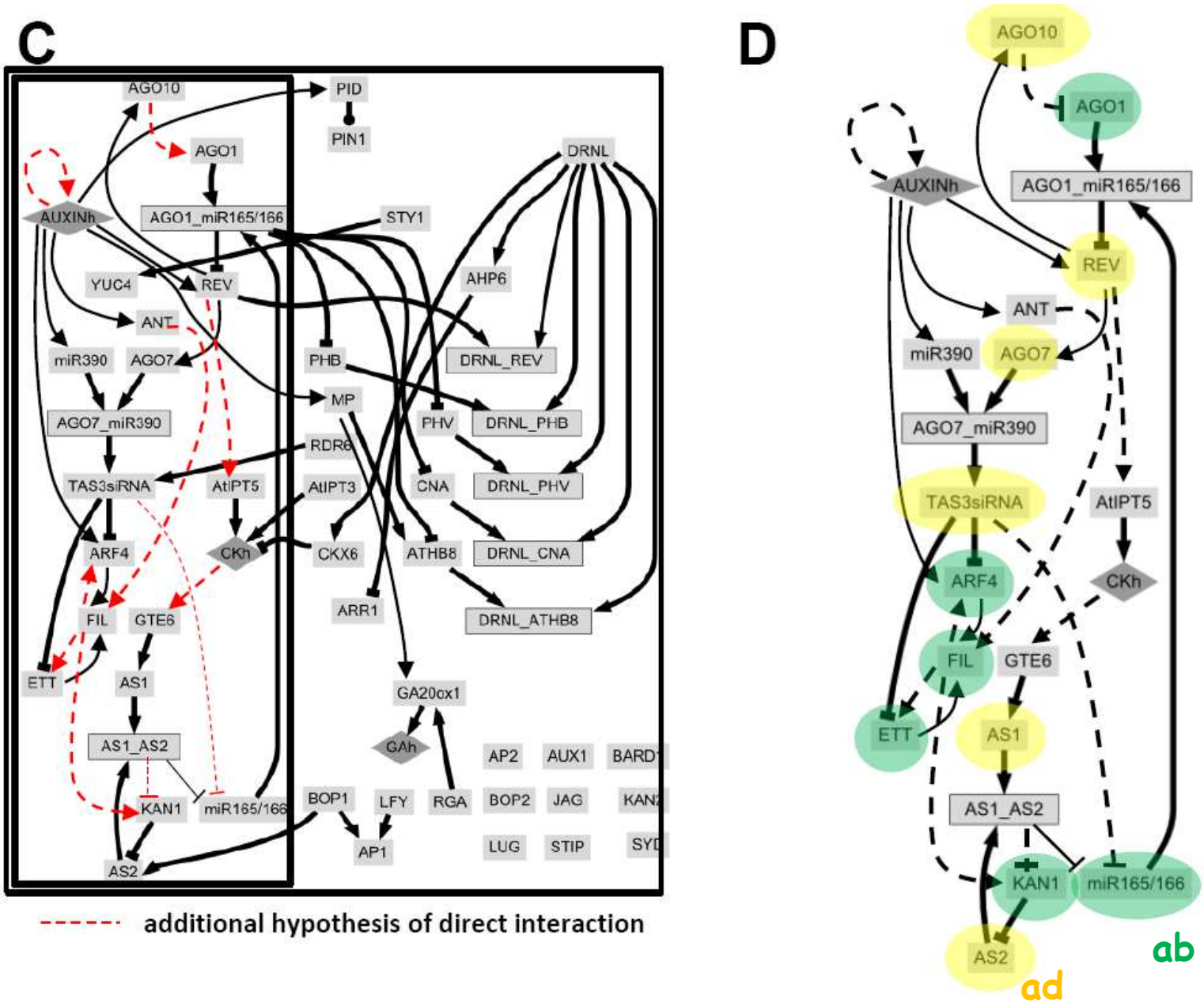
3- Candidate molecular interaction graph



Theoretical requirements

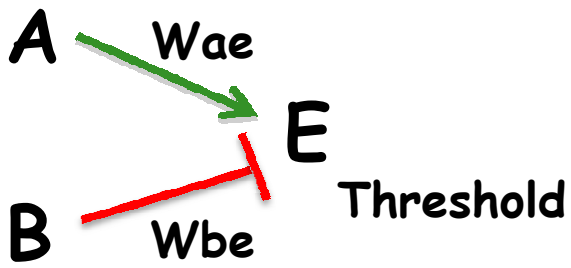
- close circuits
- inputs

3- Candidate molecular interaction graph



A mathematical model is required

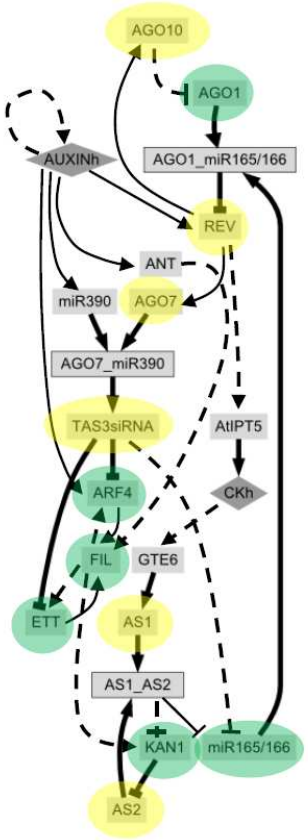
The behavior of each element is determined by its inputs



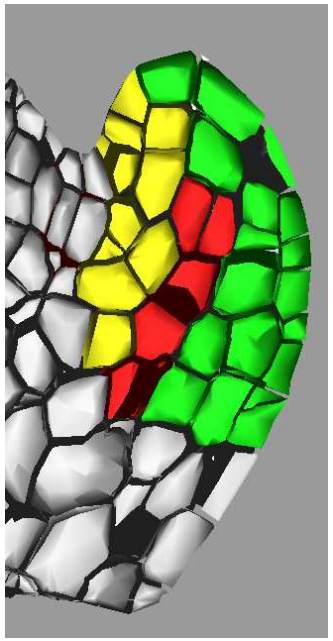
W = force of the influence of an element on another
T = threshold of activation

Different possible behavior depending on the parameter values

Expected behavior of our network

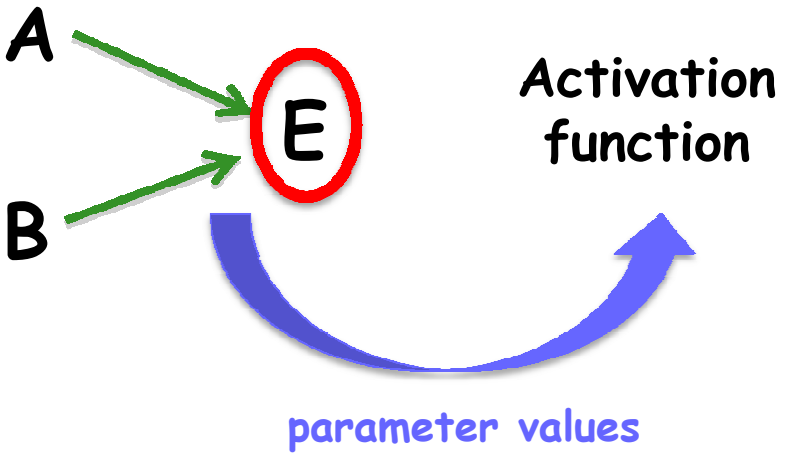


	Zone 1 Adaxial	Zone 2 Vascular	Zone 3 Abaxial
AGO1	1	1	1
AGO10	1	0	0
AGO7	1	0	0
ANT	1	1	1
ARF4	0	1	1
AS1	1	1	0
AS2	1	0	0
ETT	0	1	1
FIL	0	1	1
KAN1	0	0	1
MiR165/166	0	1	1
MiR390	1	1	1
REV	1	0	0
TAS3	1	0	0



4- Parameter values inferred from expression data > solution(s)

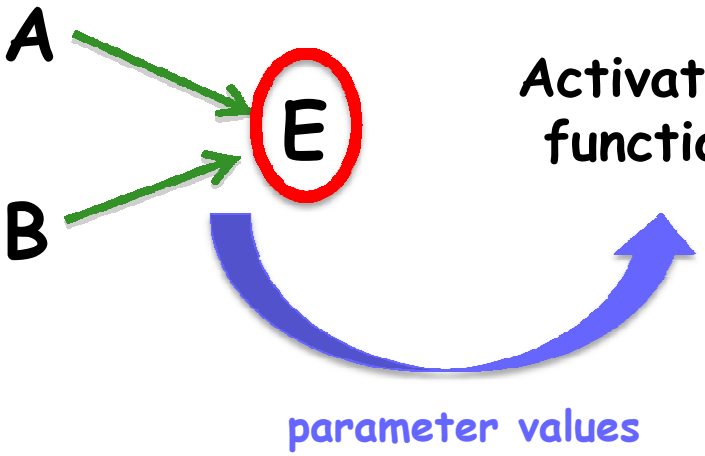
Behavior expressed as an activation function



state of the inputs		output
A	B	E
0	0	0 0 0 .....
0	1	0 1 0 .....
1	0	0 1 1 .....
1	1	1 1 1 .....

4- Parameter values inferred from expression data > solution(s)

Behavior expressed as an activation function



Activation function

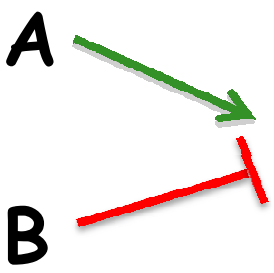
state of the inputs A	B	output E	
0	0	0 0 0	.....
0	1	0 1 0	.....
1	0	0 1 1	.....
1	1	1 1 1	.....

Activation functions are found for each element

A solution is represented by a set of activation functions

47 solutions were found

Are all interactions functional ?



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

B has no effect on E

2 solutions with
all interactions functional

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
44	2	1	8	2	8	2	2	14	2	8	1	2	2	2	192	2	4	1	2	4	2
45	2	1	8	2	8	2	2	14	2	8	1	2	2	3	192	2	4	1	2	4	2
52	2	1	8	2	8	2	2	15	2	8	1	2	2	3	128	2	4	1	2	4	2
54	2	1	8	2	8	2	2	10	2	8	1	2	2	2	192	2	4	1	2	4	2
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
62	2	1	8	2	8	2	2	15	2	8	1	2	2	3	192	2	4	1	2	5	2
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
94	2	1	8	2	8	2	2	15	2	8	1	2	2	2	168	2	4	1	2	4	2
99	2	1	8	2	8	2	2	15	2	8	1	2	2	3	128	2	5	1	2	4	2
114	2	1	8	2	8	2	2	14	2	8	1	2	2	2	128	2	4	1	2	4	2
115	2	1	8	2	8	2	2	14	2	8	1	2	2	3	128	2	4	1	2	4	2
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
135	2	1	8	2	8	2	2	12	2	8	1	2	2	3	128	2	4	1	2	4	2
140	2	1	8	2	8	2	2	15	2	8	1	2	2	3	128	2	12	1	2	4	2
161	2	1	8	2	8	2	2	12	2	8	1	2	2	2	128	2	4	1	2	4	2
165	2	1	8	2	8	2	2	8	2	8	1	2	2	2	128	2	4	1	2	4	2
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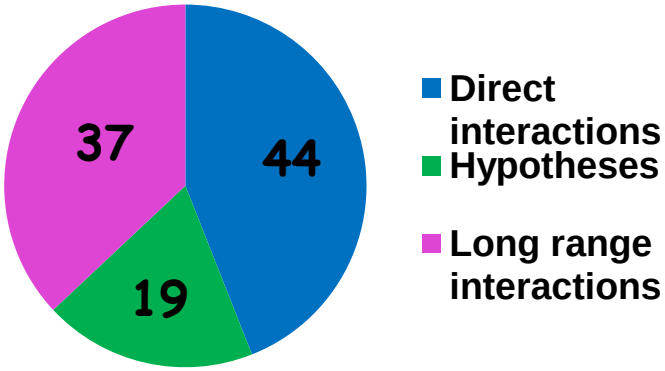
176	2	1	8	2	8	2	2	12	2	8	1	2	2	2	192	2	4	1	2	4	2
177	2	1	8	2	8	2	2	12	2	8	1	2	2	3	192	2	4	1	2	4	2
187	2	1	8	2	8	2	2	15	2	8	1	2	2	3	128	2	4	1	2	5	2
189	2	1	8	2	8	2	2	15	2	8	1	2	2	2	128	2	5	1	2	4	2
192	2	1	8	2	8	2	2	8	2	8	1	2	2	3	128	2	4	1	2	4	2
204	2	1	8	2	8	2	2	15	2	8	1	2	2	3	128	2	13	1	2	4	2
212	2	1	8	2	8	2	2	15	2	8	1	2	2	2	128	2	13	1	2	4	2
214	2	1	8	2	8	2	2	14	2	8	1	2	2	2	128	2	4	1	2	5	2
215	2	1	8	2	8	2	2	14	2	8	1	2	2	3	128	2	4	1	2	5	2
222	2	1	8	2	8	2	2	10	2	8	1	2	2	2	128	2	4	1	2	4	2
247	2	1	8	2	8	2	2	15	2	8	1	2	2	2	128	2	4	1	2	6	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
290	2	1	8	2	8	2	2	14	2	8	1	2	2	3	128	2	12	1	2	4	2
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

5- Model validation



37 genetic interactions used
to test the dynamics of the MRNs

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

Model predictions ⟷ Experimental observations

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

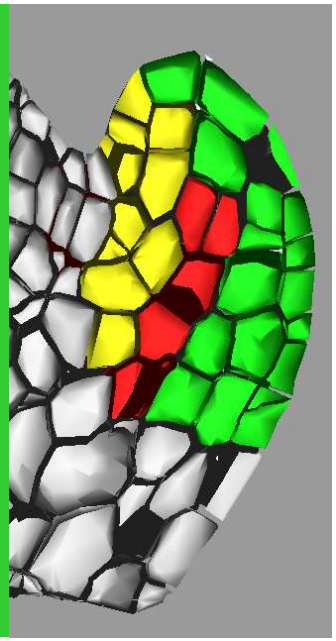
- **1- off(AGO10) → up(miR165), down(REV)**
- **2- on(AGO10) → up(REV)**
- **3- off(AGO7) → unchanged(FIL), up(ARF4,ETT)**
- **4- off(AS1) → unchanged(FIL), up(ETT)**
- **5- off(AS2) → up(ETT,FIL)**
- **6- on(AS2) → down(FIL)**
- **7- off(AS2,AGO10) → up(miR165), down(REV)**
- **8- off(AS1,AGO7) → up(FIL)**
- **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)**

5- 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



Steady states

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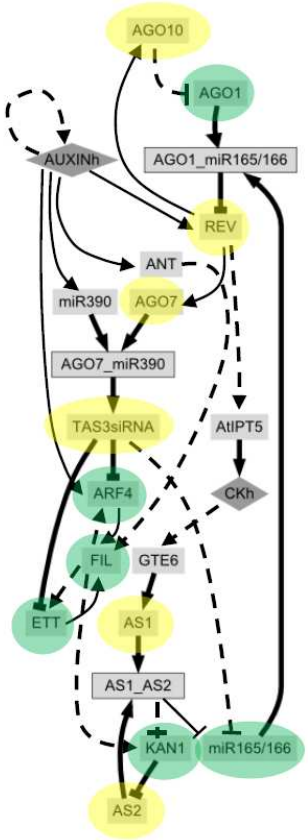
- 3- off(AGO7) → unchanged(FIL), up(ARF4,ETT)
- 4- off(AS1) → unchanged(FIL), up(ETT)
-
-
-

Long range interactions

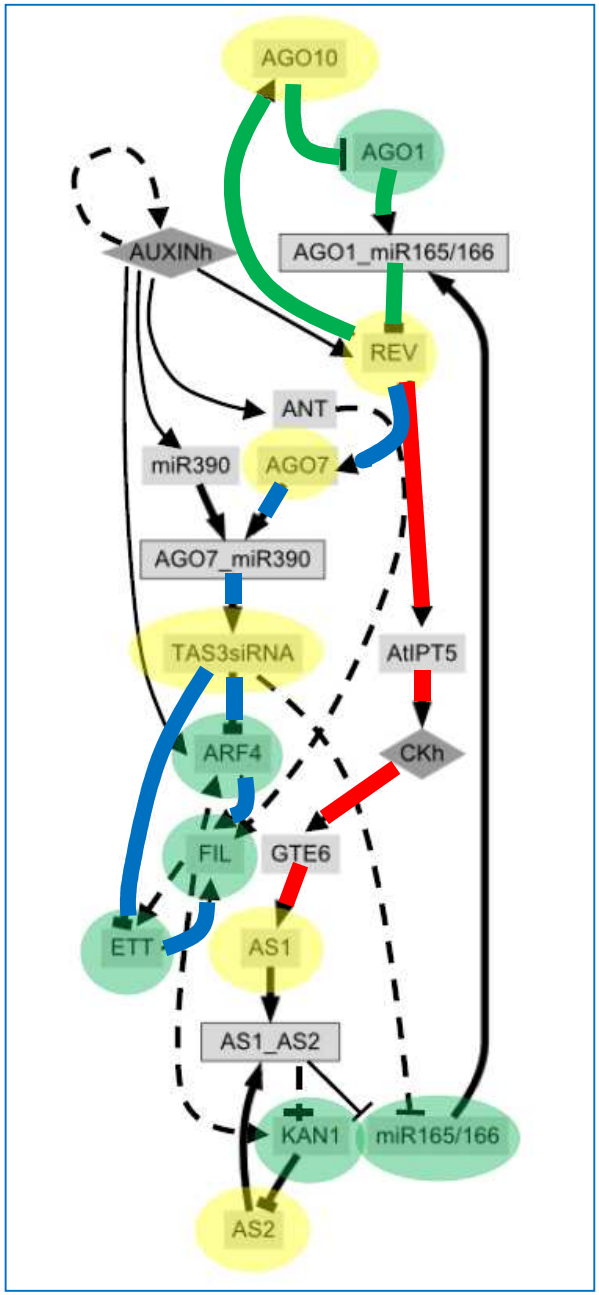
Behavior



Candidate molecular network

Structure

6- Model predictions



- The available data are mostly coherent

- Several unexpected potential pathways revealed (REV>AS1, REV>TAS3, REV>AGO1 and miR)

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

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

Conclusion

This work provided a coherent MRN model, which reproduces the abaxial and adaxial cell fates

The model revealed potential new pathways

New molecular interactions will introduce new circuits, new steady states and should explain more cell types (e.g. vasculature)

Approach which allows the extraction of biological knowledge from diverse type of data

Can be used to study developmental processes in any multicellular organism

Virtual carpel 2006-2008
Geneshape 2009-2012

Acknowlegments

Laboratoire Reproduction
et Développement des Plantes
ENS Lyon

Pradeep Das
Sandrine Paindavoine
Frédérique Rozier
Jan Traas
Françoise Monéger

Complexe Systems Institute
ENS Lyon

Camilo La Rota
Eric Boix
Michel Morvan

Virtual Plants
IRD Montpellier

Christophe Godin
Jérôme Chopard
Etienne Farcot

The Plant Cell (2011) La Rota et al.

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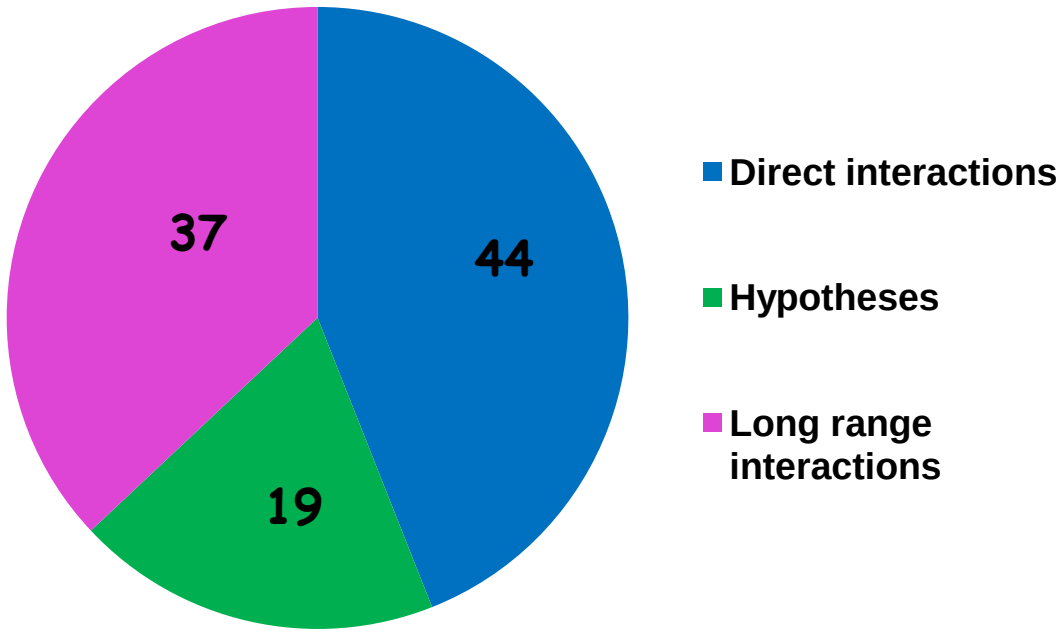
- **5- off(AS2) → up(ETT,FIL)**
- **6- on(AS2) → down(FIL)**
- **7- off(AS2,AGO10) → up(miR165), down(REV)**
- **8- off(AS1,AGO7) → up(FIL)**
- **9- off(AS2,AGO7) → up(miR165,FIL), down(REV)**
- **10- off(ANT,FIL) → down(REV)**
- **11- off(TAS3siRNA) → up(ETT,ARF4)**
- **12- off(AS1,TAS3siRNA) → down(REV), 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)**

Interactions in the sepal database



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