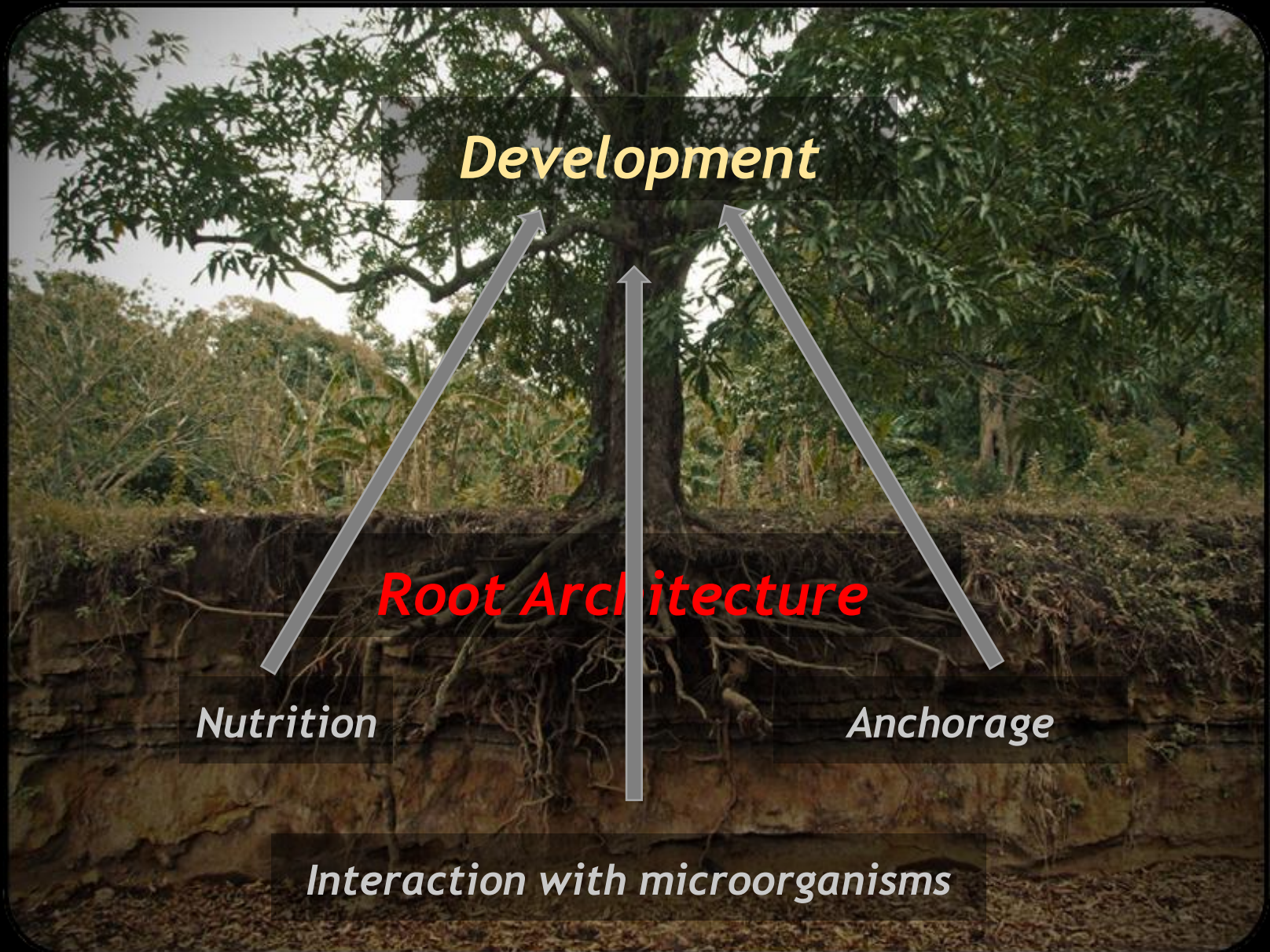


**Dynamics of Genes Regulatory Network
Governing *de novo*
Lateral Root Primordium Development
in *Arabidopsis thaliana***

Mikaël Lucas - CR IRD
Kevin Bellande - Post-doctorant IRD

Context : Root System



Context : Secondary organogenesis

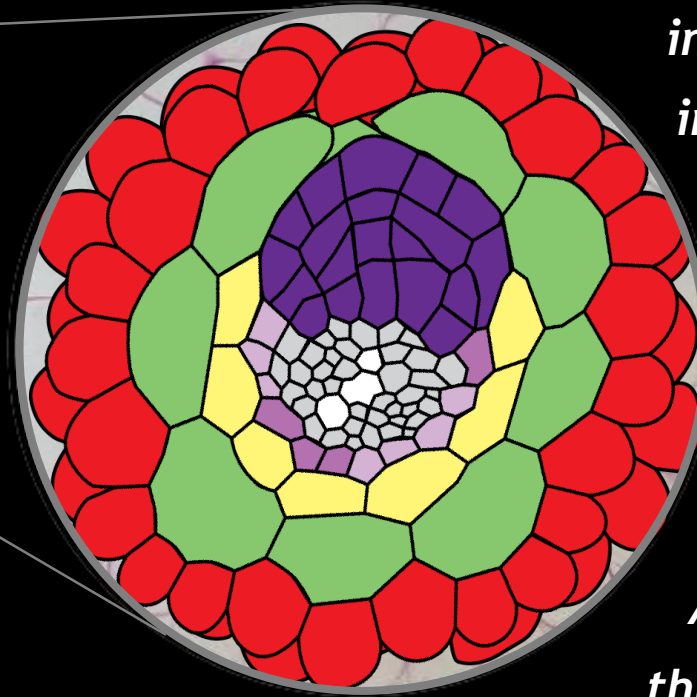
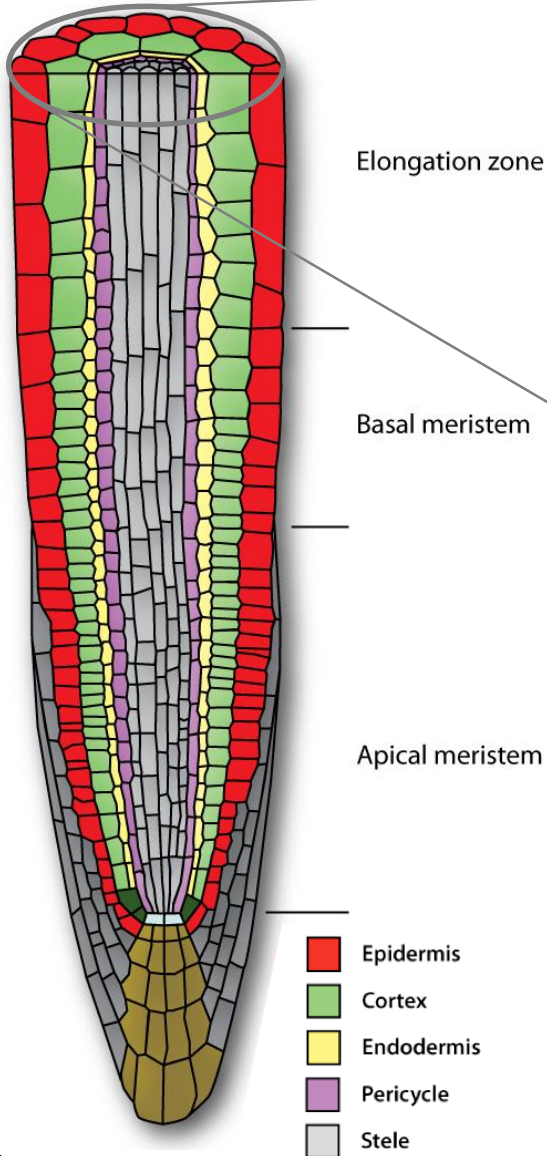


... to a mature root system ...

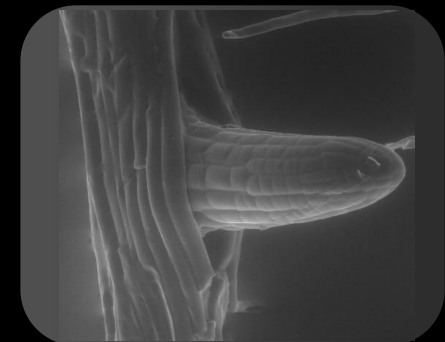
... with a complex architecture resulting from interactions between genetics and environment.

Context : Lateral root organogenesis

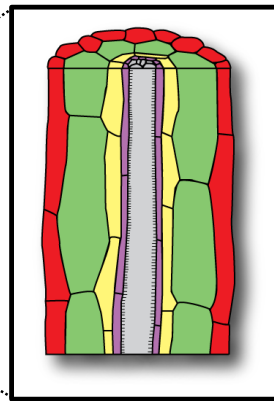
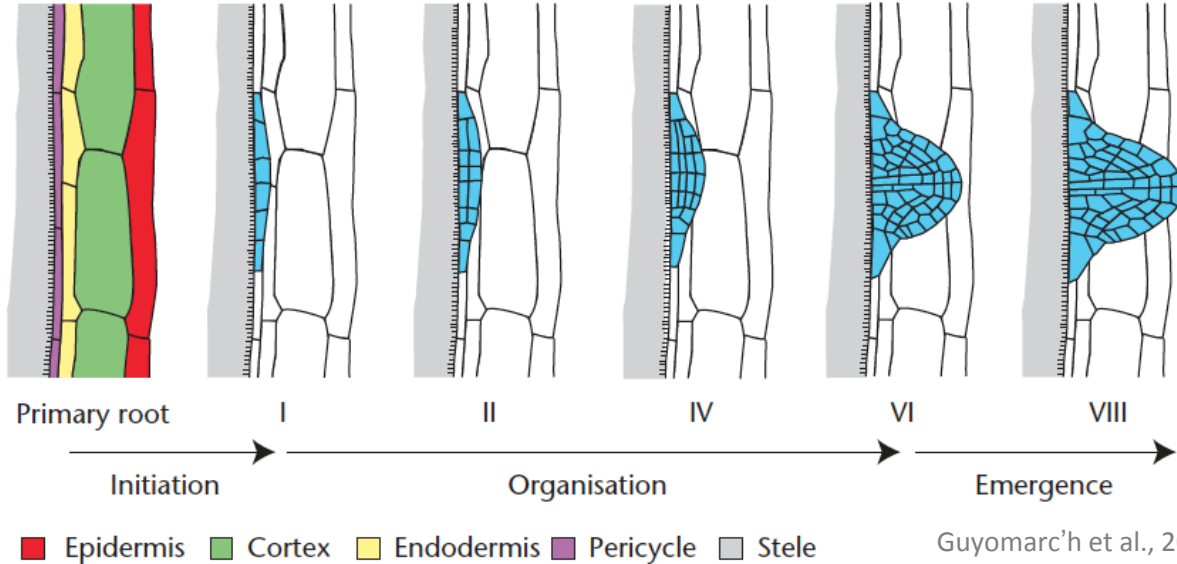
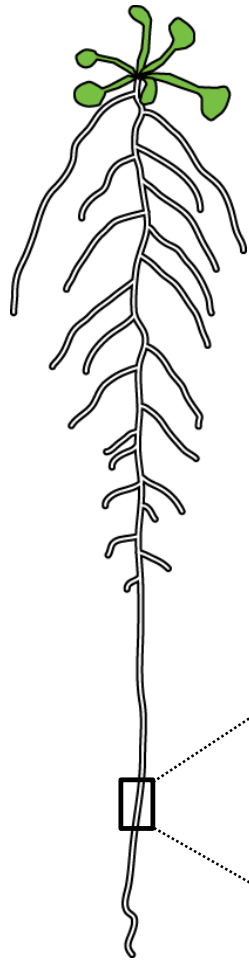
In Arabidopsis, LR initiation occurs in the pericycle



And LR develop through the tissues to finally emerge



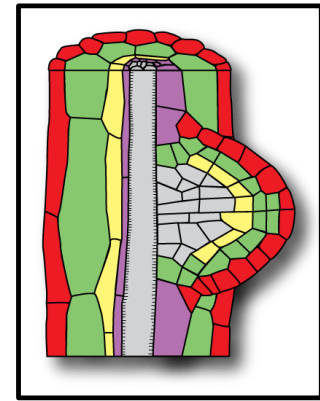
Context : Lateral root organogenesis



Well described sequence
of morphogenesis

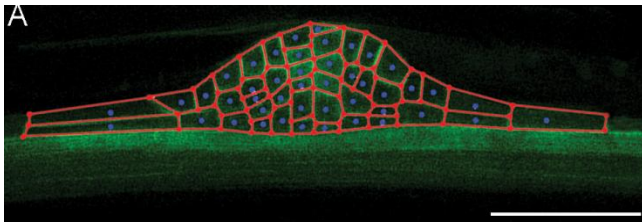


Is this development
highly regular ?

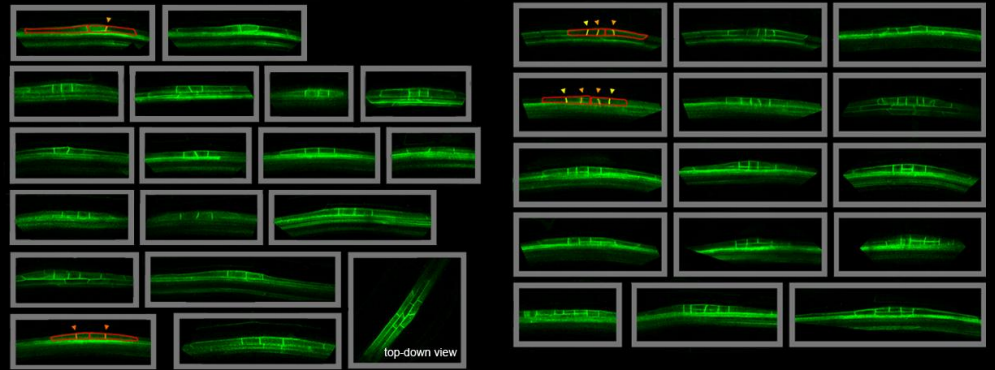


Context : Lateral root organogenesis

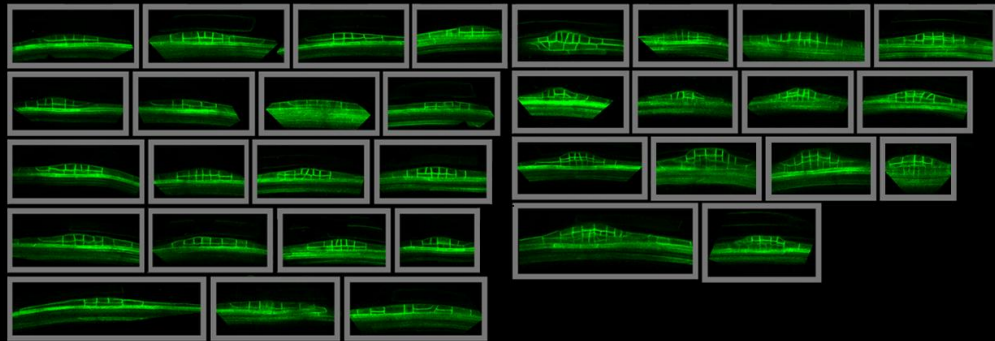
Let's have a look
at lots of LRP



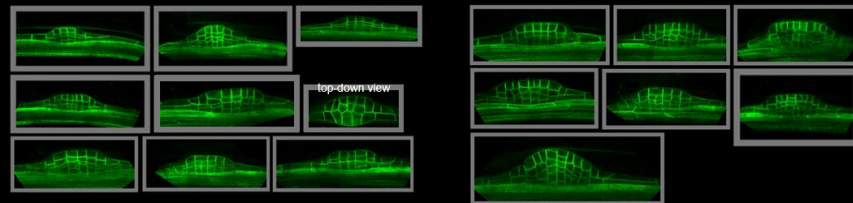
Stage I to II



Stage II to III

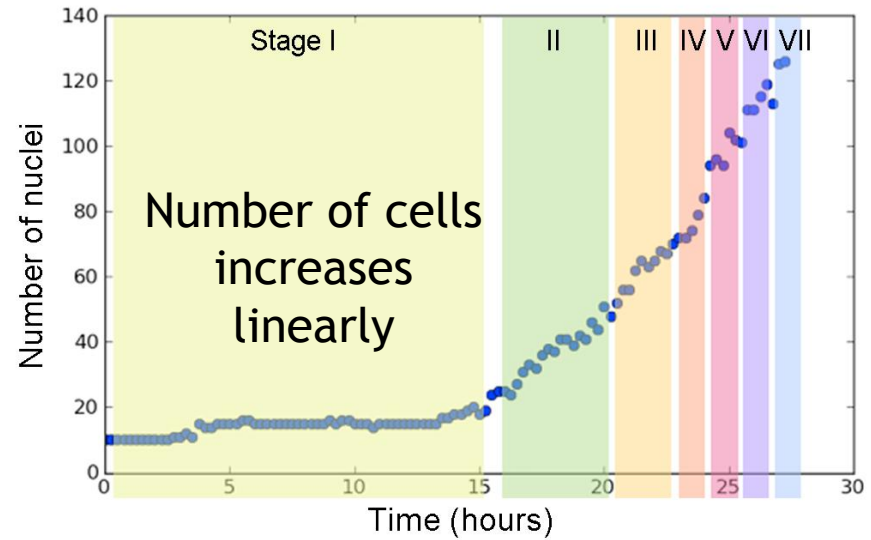
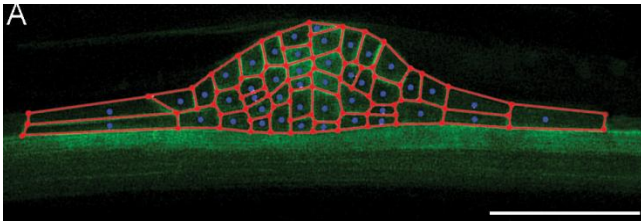


Stage IV and over



Context : Lateral root organogenesis

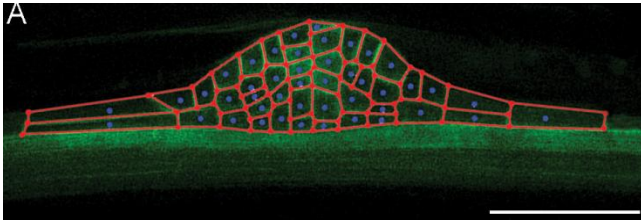
Let's have a look
at lots of LRP



(Lucas et al. 2013)

Context : Lateral root organogenesis

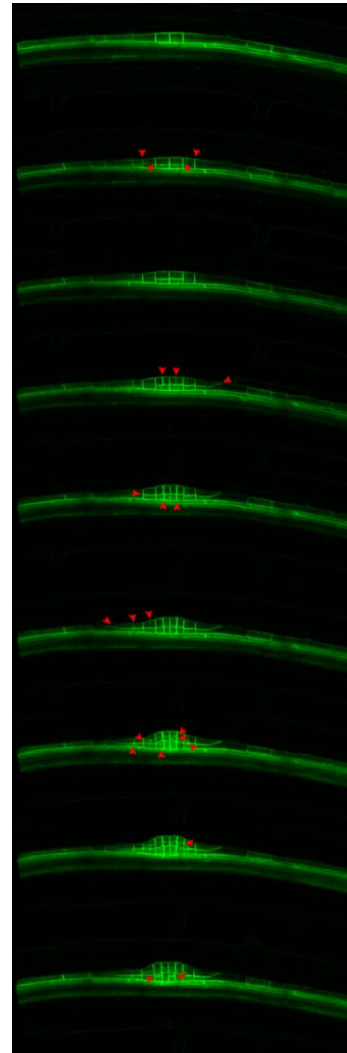
Let's have a look
at lots of LRP



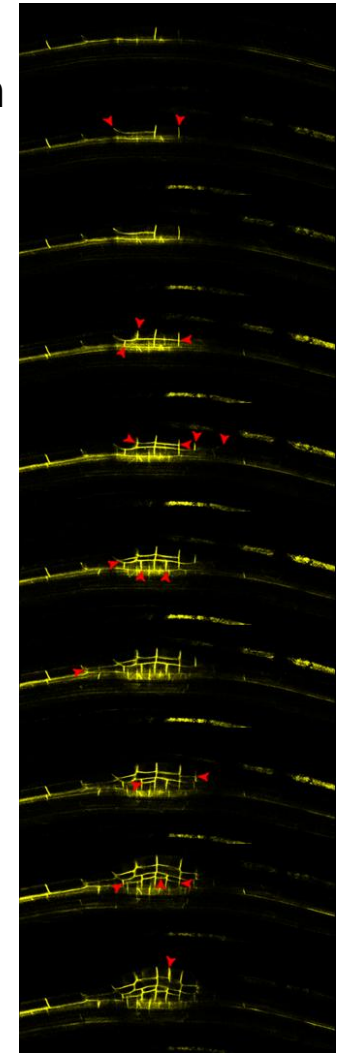
LRP patterning is
not stereotypical

Multiple ways of
building a LRP

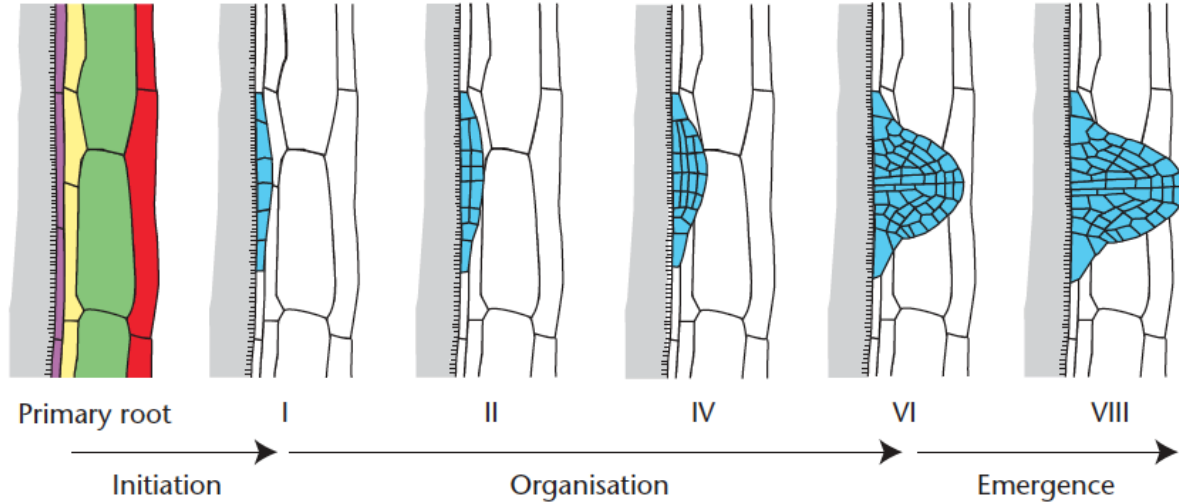
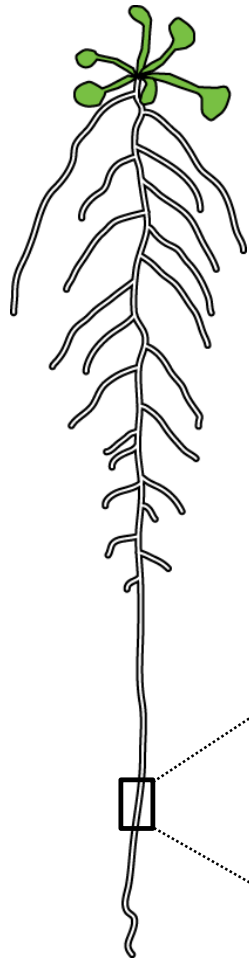
(Lucas et al. 2013)



Sequence
of division
events
actually
varies
between
LRP

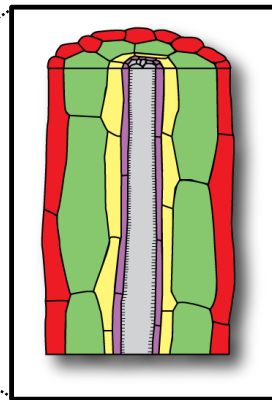


Context : Lateral root organogenesis



■ Epidermis
 ■ Cortex
 ■ Endodermis
 ■ Pericycle
 ■ Stele

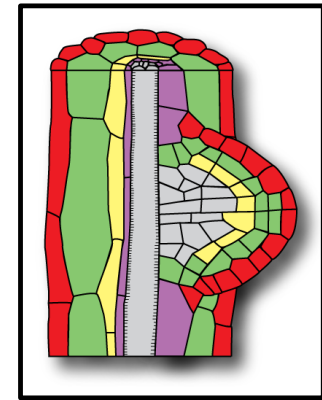
Guyomarc'h et al., 2010



Plastic development,
but fixed ending



DYNAMIC PATTERNING
?

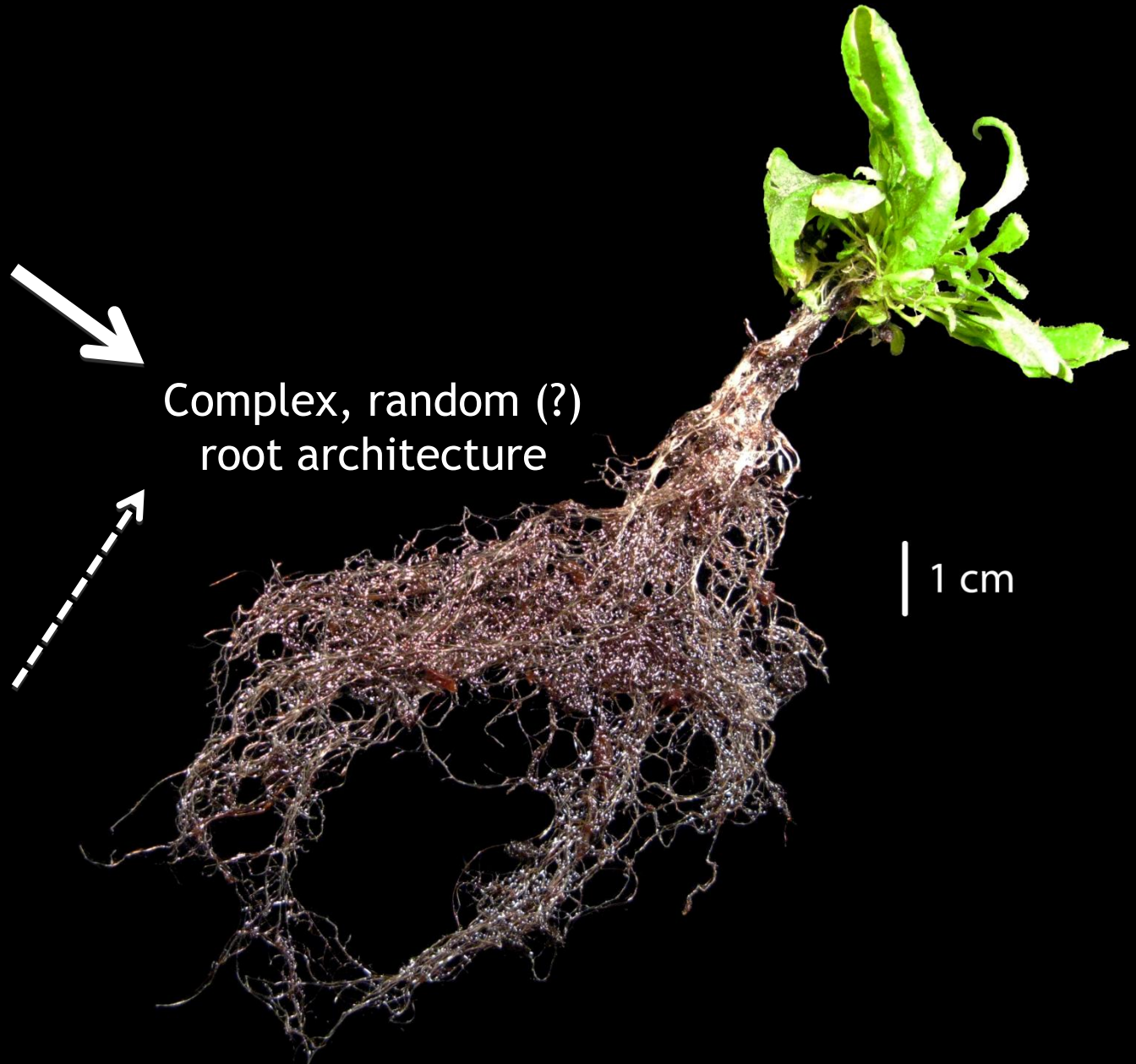


Context : Lateral root organogenesis

Elementary
dynamic
organogenesis
processes

Complex, random (?)
root architecture

How to study this
process to
understand and
control RSA ?



Modulation of lateral root initiation

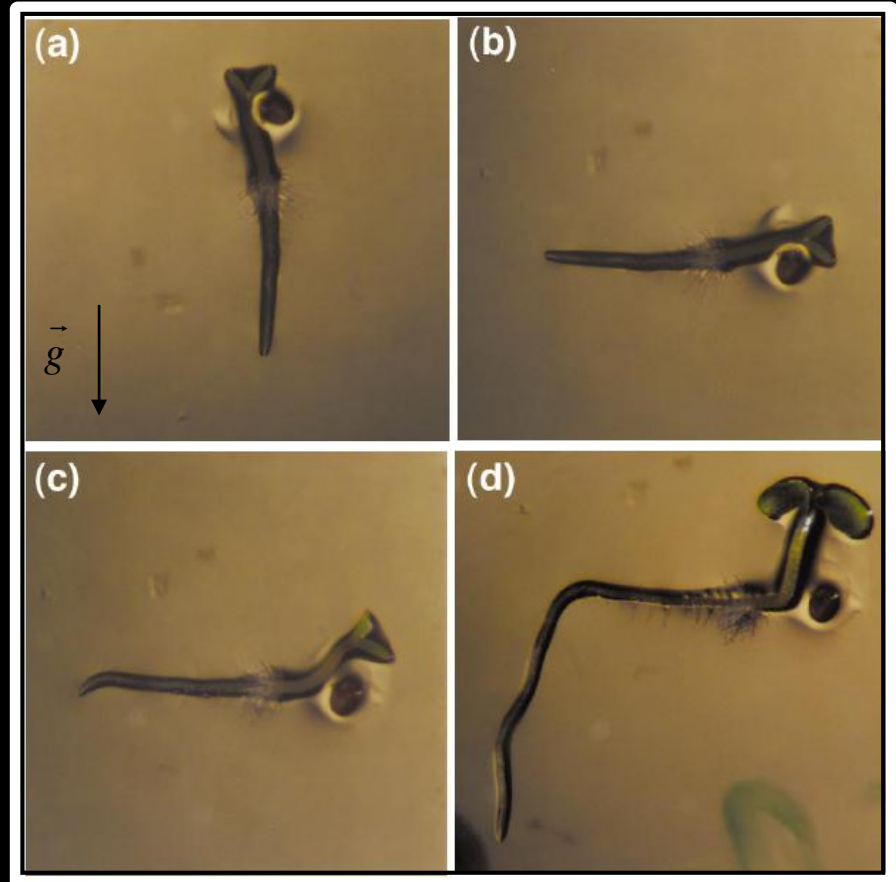
LRP initiation correlated with root bending



(De Smet et al. 2007)

Can new lateral roots be induced using gravistimulation?

Gravitropism induces root bending



(Rosen, 1999)

Modulation of lateral root initiation

Control



TBR:

1 hour

3 hours

6 hours

12 hours

24 hours



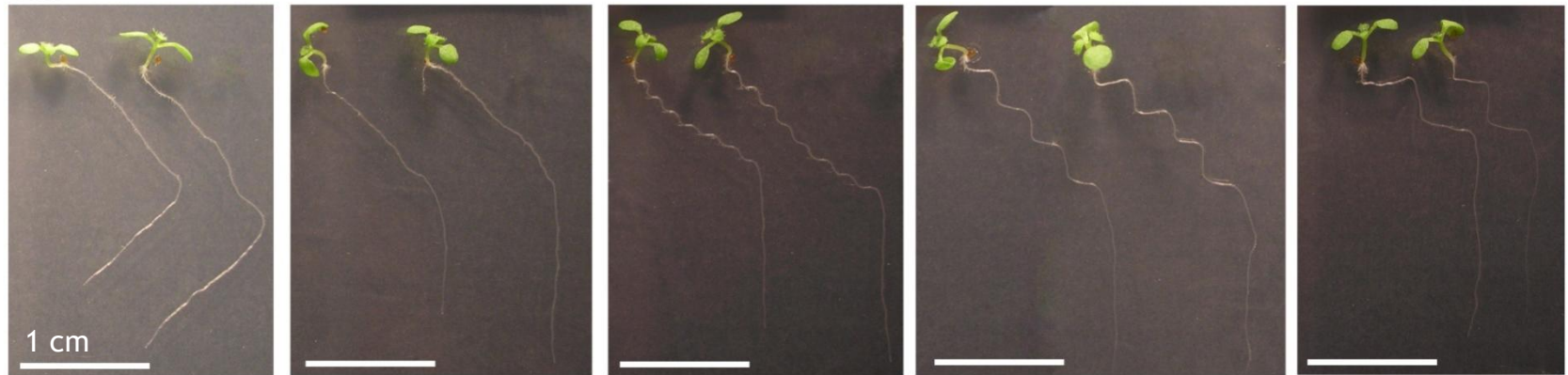
TBR: 1 hour

3 hours

6 hours

12 hours

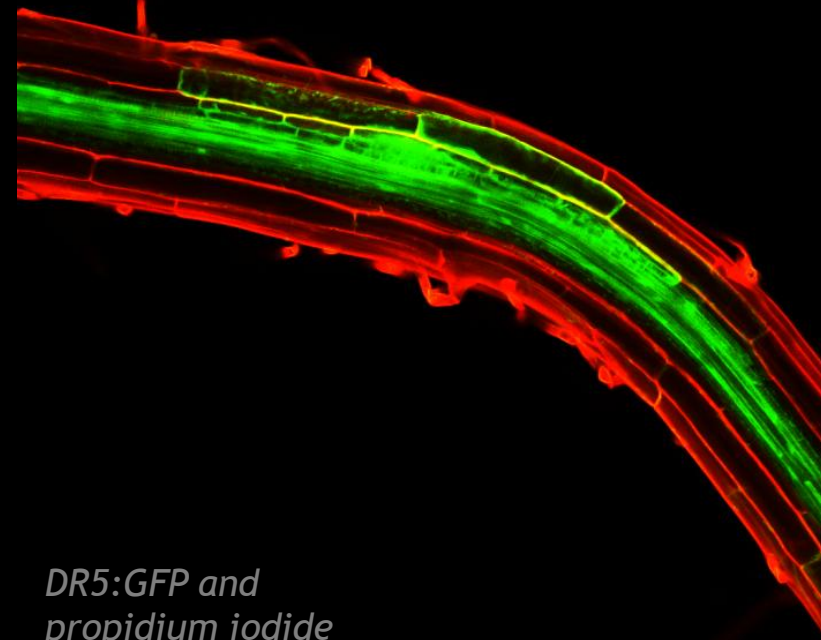
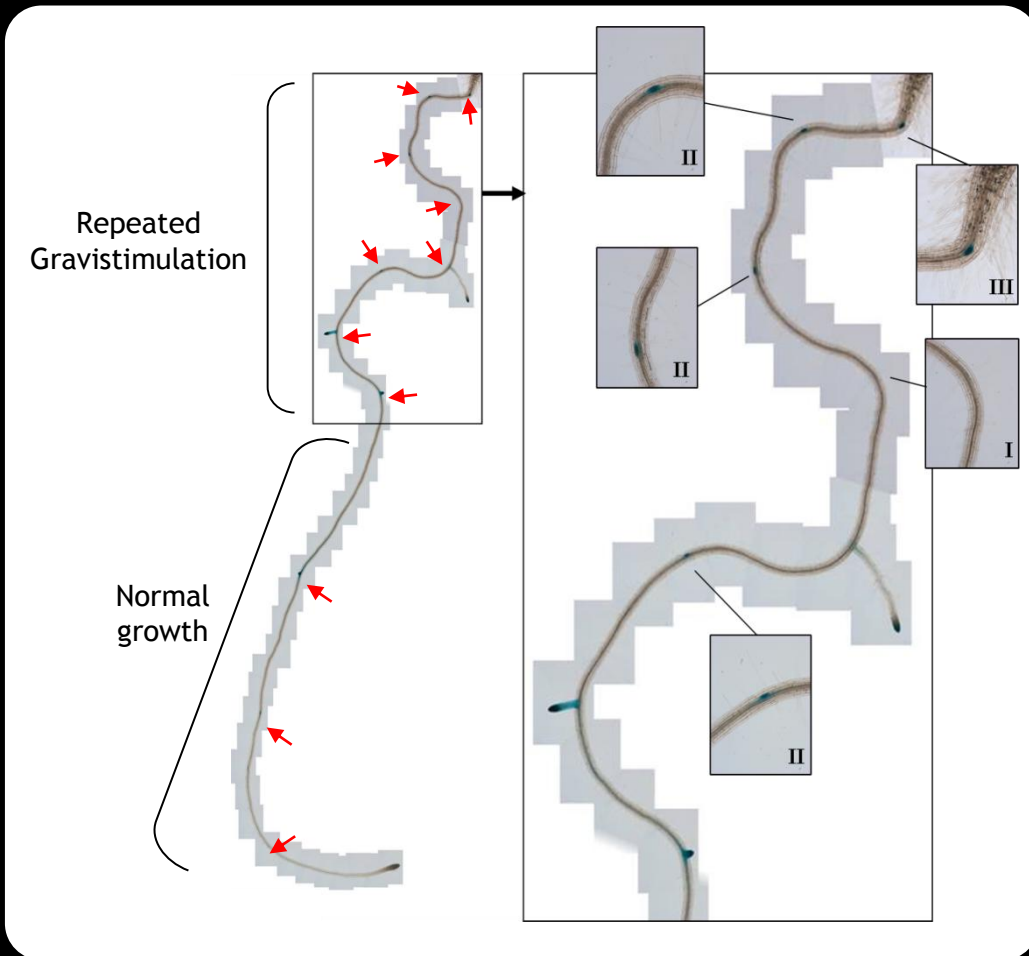
24 hours



Induction of rhizogenesis by gravistimulation

Gravistimulation induces initiation...

... within a tightly controlled spatio-temporal window



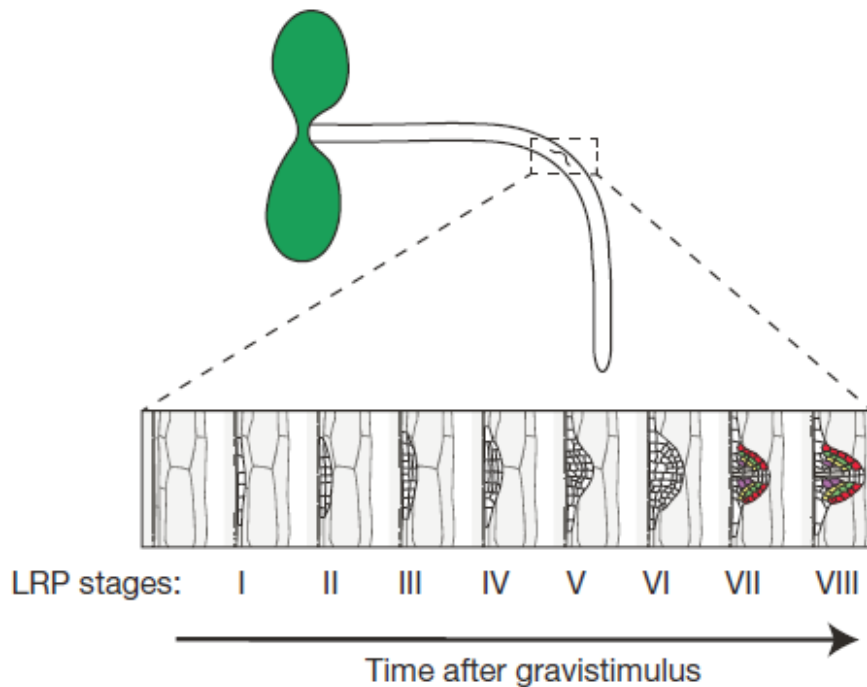
DR5:GFP and propidium iodide

(Lucas et al. 2008)

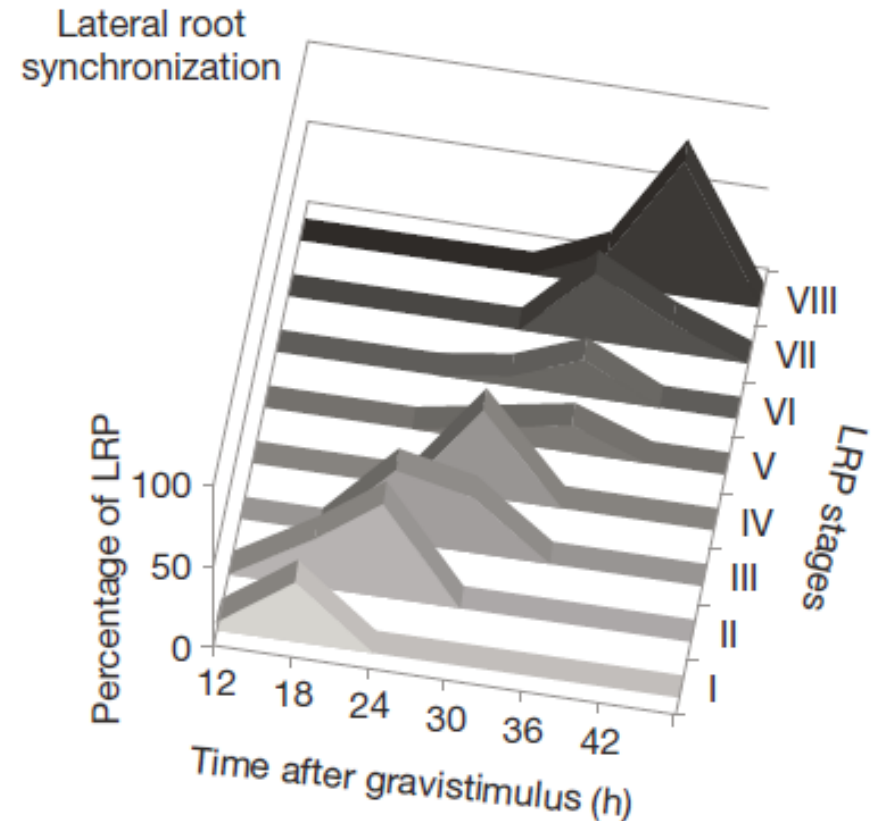
Control of rhizogenesis by gravistimulation

Gravistimulation induces initiation...

... within a tightly controlled spatio-temporal window



Can we use this to access the dynamics of LR morphogenesis regulation ?



Transcriptomics of LRP development

3 days after germination

90° rotation



Harvest bends with synchronized LRP

(Voss et al 2015)

Pool RNA and run transcriptomics analysis

Transcriptomics of LRP development



(Voss et al 2015)

Transcriptomics of LRP development



300 to 400 beads per timepoint

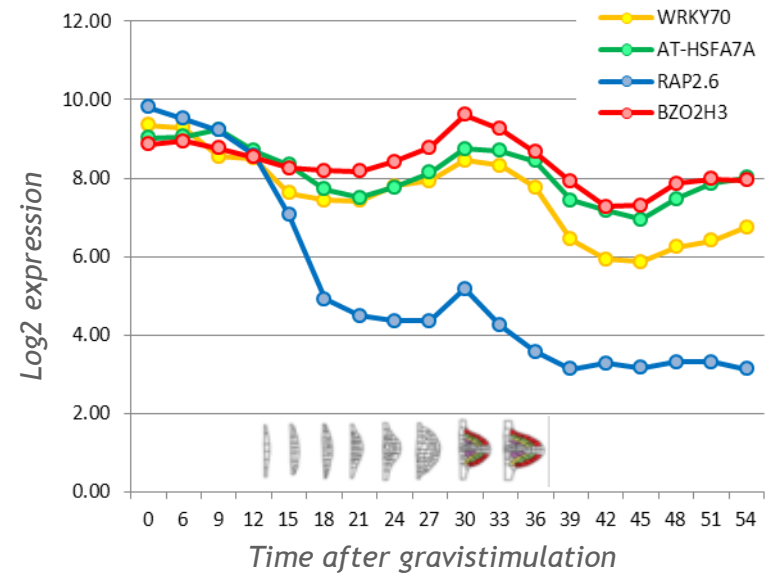
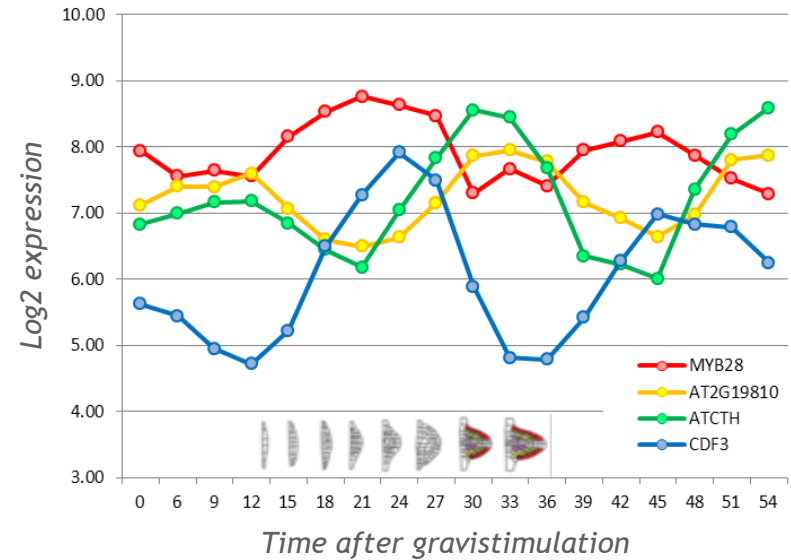
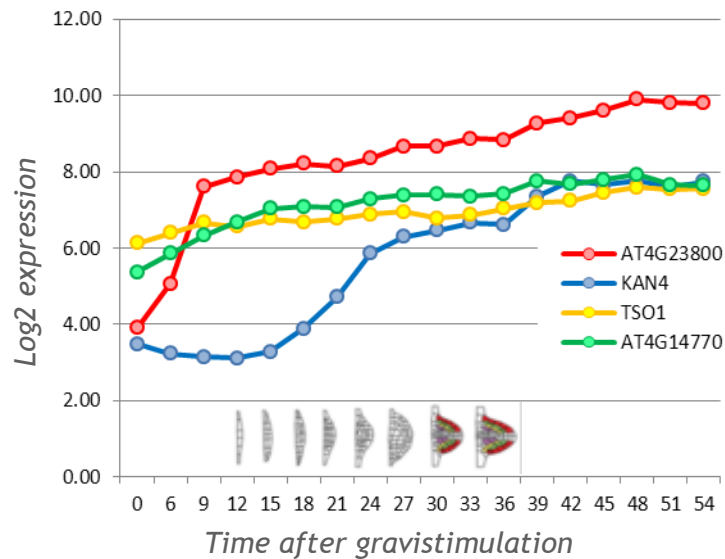
*18 timepoints : 3 hours apart,
from before initiation to after emergence*

4 replicates

Database of ~8500 differentially
expressed genes (inc. 700 TFs)

Transcriptomics of LRP development

Illustration of some transcription factors expression profiles from the database



Expression database exploitation

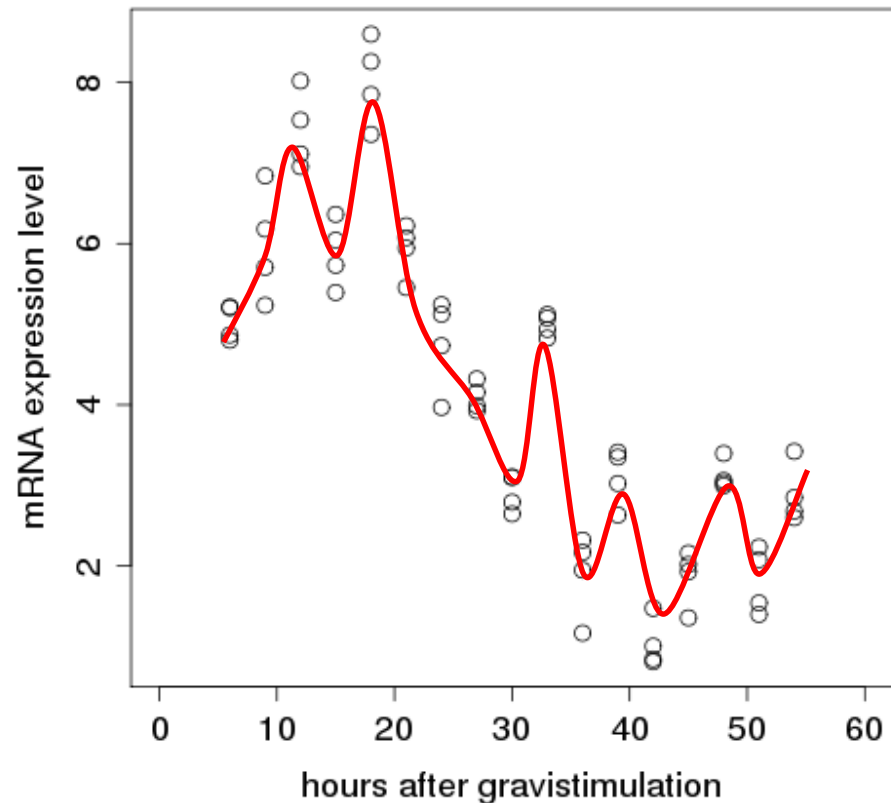
Extracting information
from the LR dataset ?



Use statistical
network inference
methods

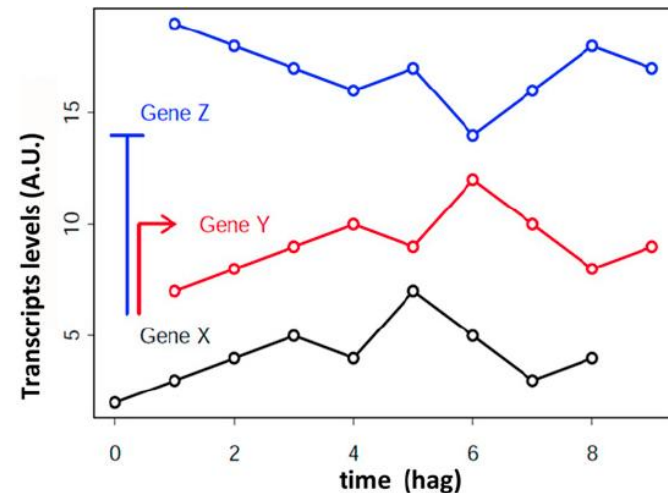
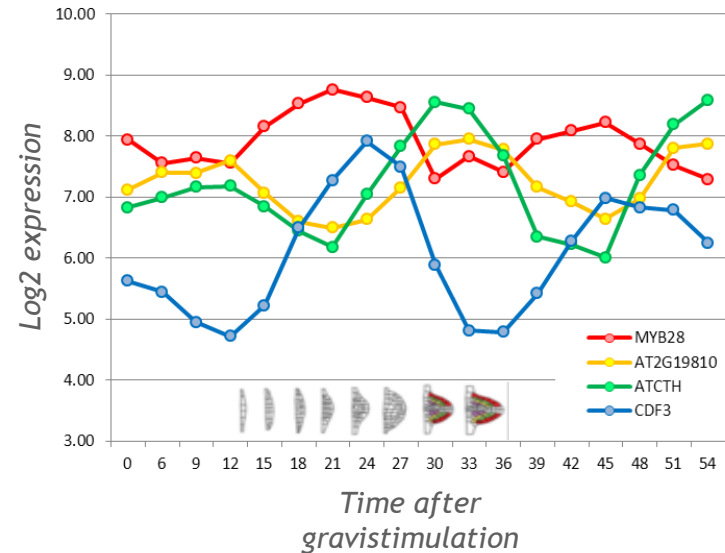
Developed a new
algorithm in our lab
(J. Lavenus thesis)

A typical time profile for mRNA level in lateral root primordia
following gravistimulation

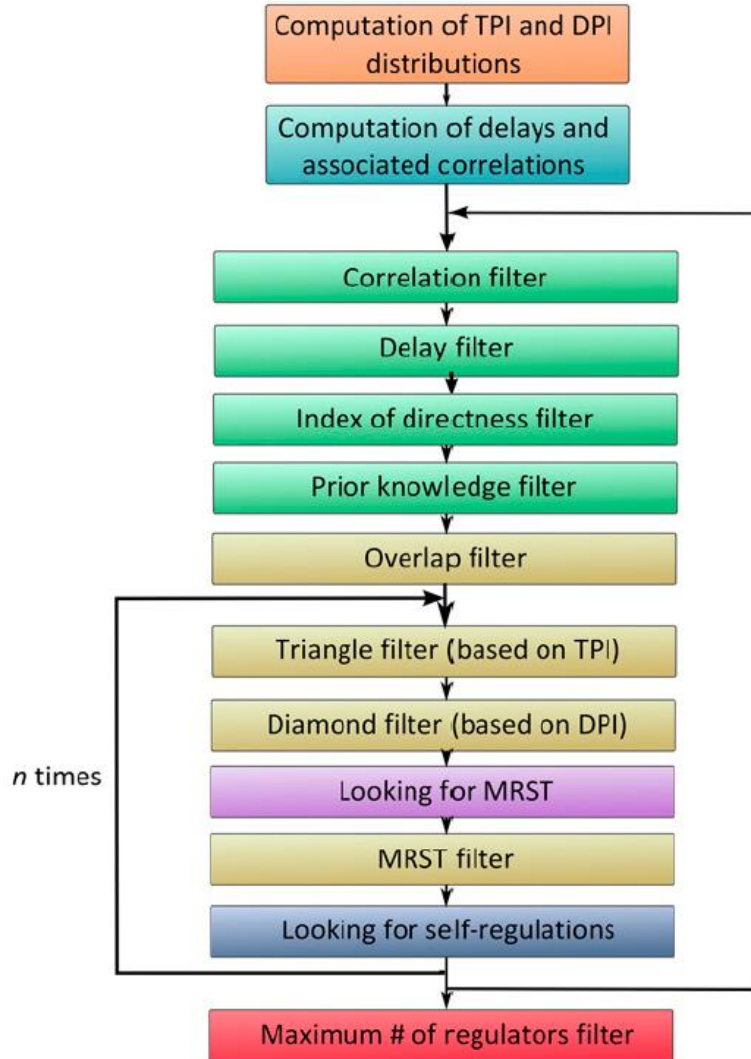


Time Delay Correlation - TDCor

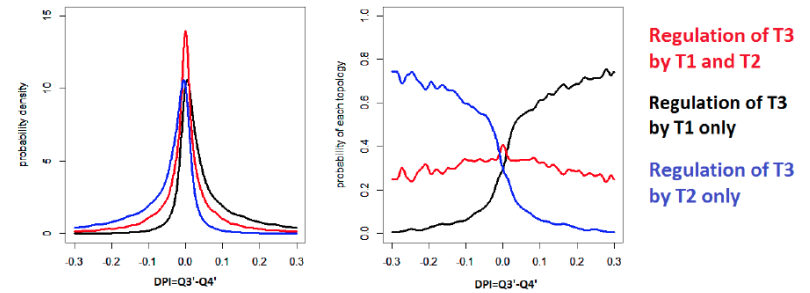
- Implemented in R (CRAN)
- Runs on expression profiles extracted from the LR dataset (or any other transcriptomic kinetics dataset)
- Looks for non-combinatorial linear interactions
- Uses Pearson's correlation with time delay computation to produce a preliminary network
- Uses bootstrap and statistical filters to eliminate false positive and refine the network topology



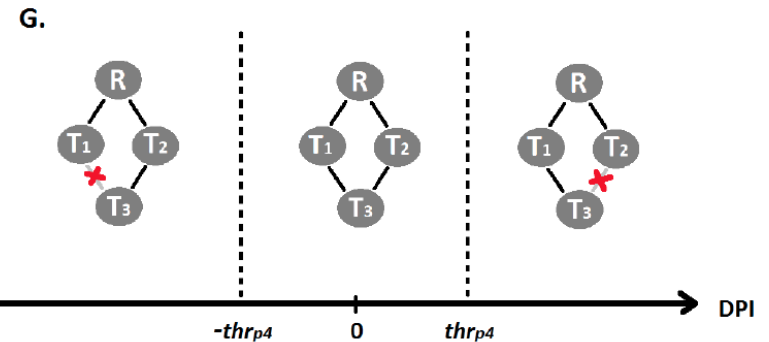
TDCor - data treatment pipeline



TPI= Triangle Pruning Index
 DPI= Diamond Pruning Index



N times

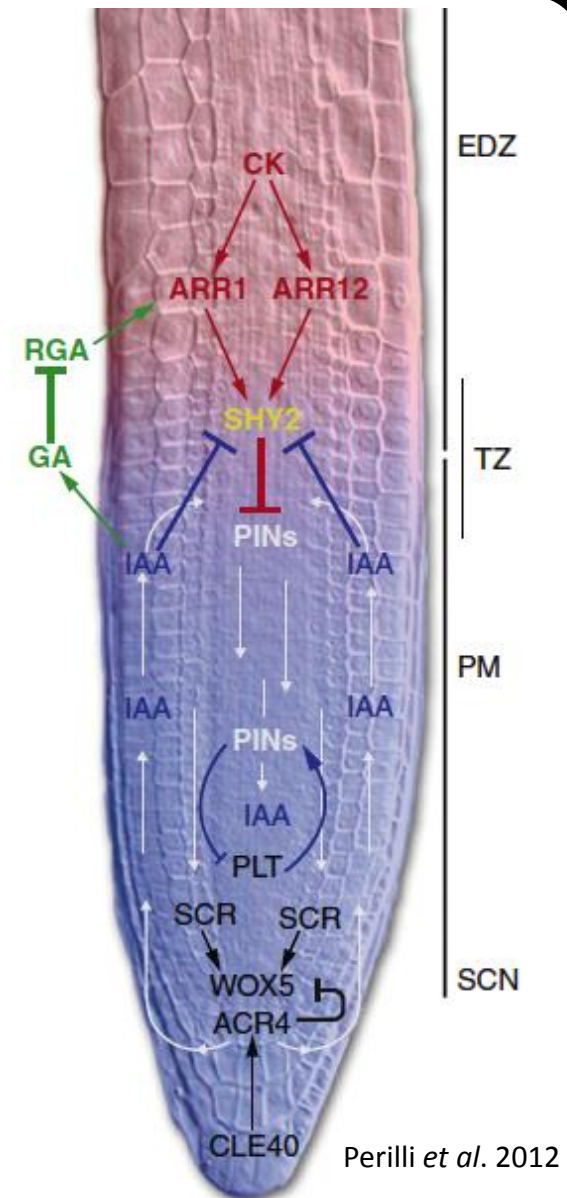


Using TDCor on the LR dataset

Selection of genes involved in

- lateral root formation
- root meristem organization and activity
- hormonal transduction
- cell division
- cell differentiation

Possibility to include any other gene present on the Affymetrix chip (*e.g.* selected because of interesting features of its expression profile ...)

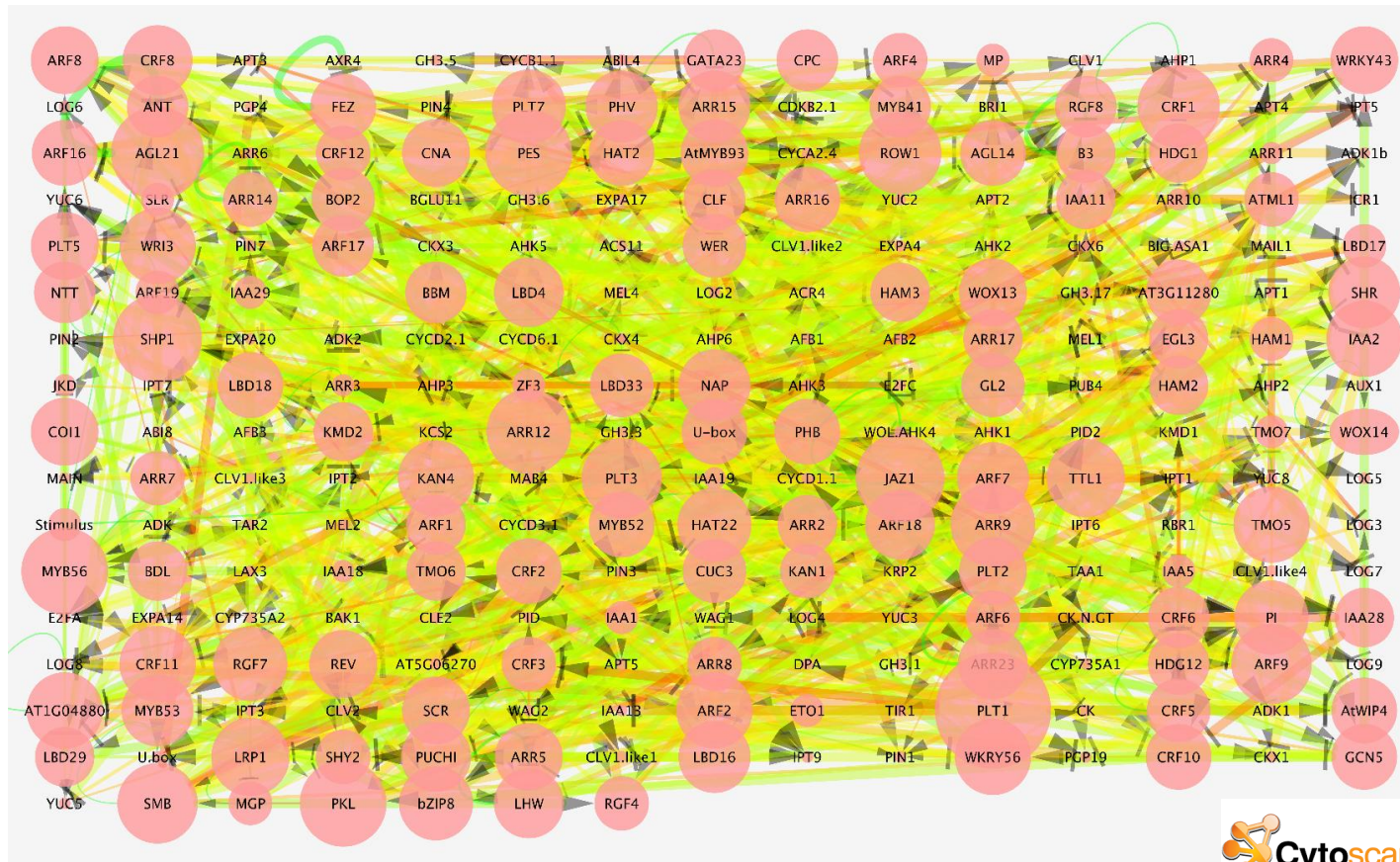


Using TDCor on the LR dataset

- Selected a list of 261 genes
- Not only transcription factors
- A “prior” data is given to each gene, based on the literature, to indicate if transcriptional regulation activity has been reported or not
- This “prior” information helps the inference procedure by authorizing or not the algorithm to draw outward edges from the node. However indicating a prior is not compulsory (prior = 2)

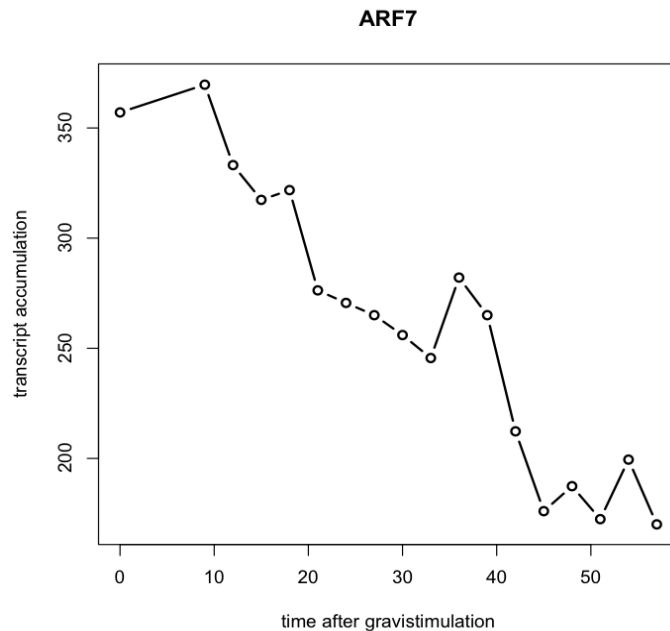
Gene	Name	Prior
AT1G02850	BGLU11	0
AT1G03430	AHP5	0
AT1G03840	MGP	2
AT1G04220	KCS2	0
AT1G04240	SHY2	-1
AT1G04550	BDL	-1
AT1G04610	YUC3	0
AT1G04880	AT1G04880	2
AT1G10470	ARR4	-1
AT1G12820	AFB3	0
AT1G15580	IAA5	-1
AT1G15670	KMD2	-1
AT1G16060	WRI3	2
AT1G17950	MYB52	2
AT1G19050	ARR7	-1
AT1G19180	JAZ1	2
AT1G19220	ARF19	2
AT1G19850	MP	1
AT1G20700	WOX14	2
AT1G23080	PIN7	0
AT1G25410	IPT6	0
AT1G25470	CRF12	1
AT1G26680	B3	2
AT1G26870	FEZ	2
AT1G27320	AHK3	0
AT1G27450	APT1	0
AT1G28130	GH3.17	0
AT1G30330	ARF6	1
AT1G30490	PHV	2
AT1G31220	IPD4	2

Using TDCor on the LR dataset

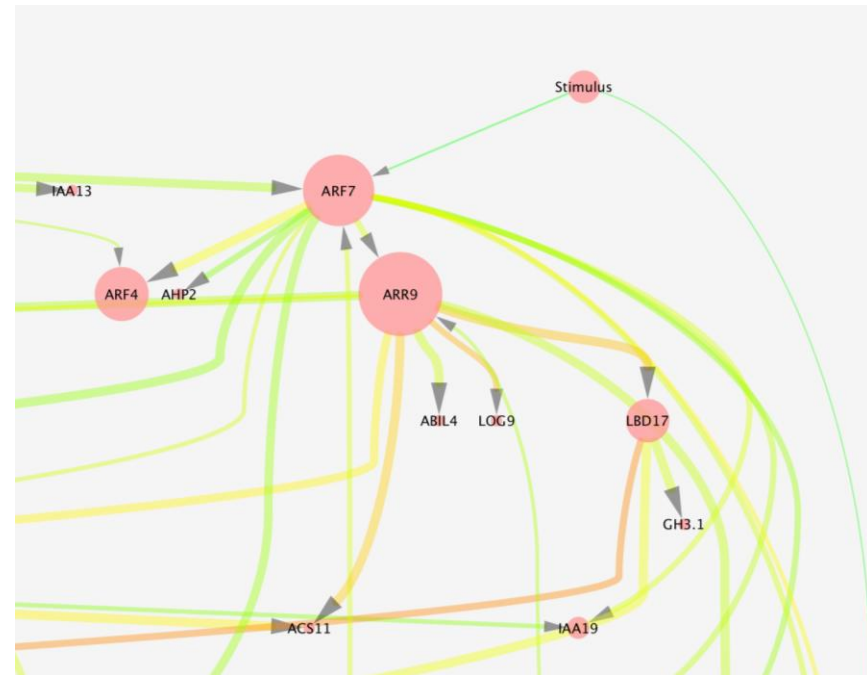


Generated a full network (~3h computation on standard PC)
With indices of confidence and directness for each interaction.
But are we confident in the predictions ?

Validating the network - the ARF7 case



*Transcription factor ARF7
experimental profile in the LR data set*

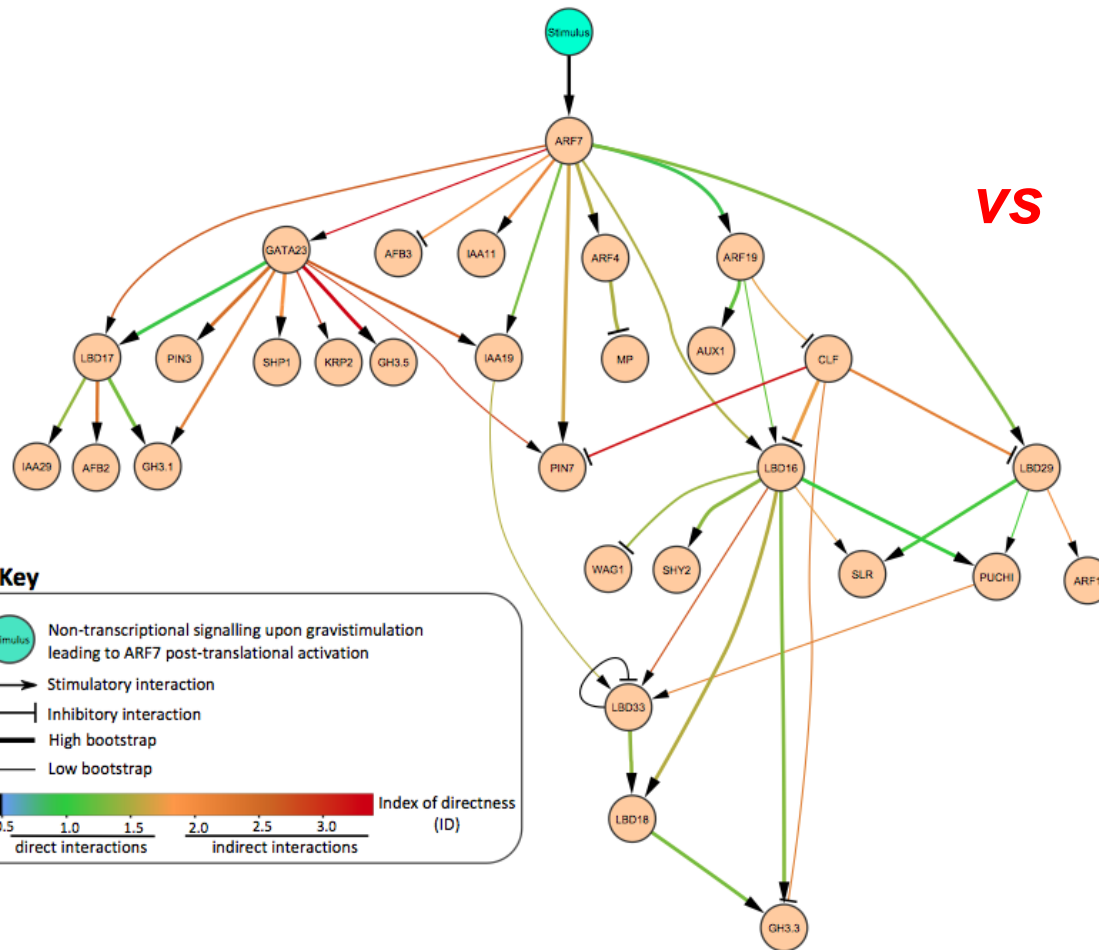


Inference by TDCor algorithm

ARF7 is predicted to occupy a upstream position in the network

ARF7 is predicted to positively regulate a LOB/PUCHI genetic module

Validating the network - the ARF7 case



VS

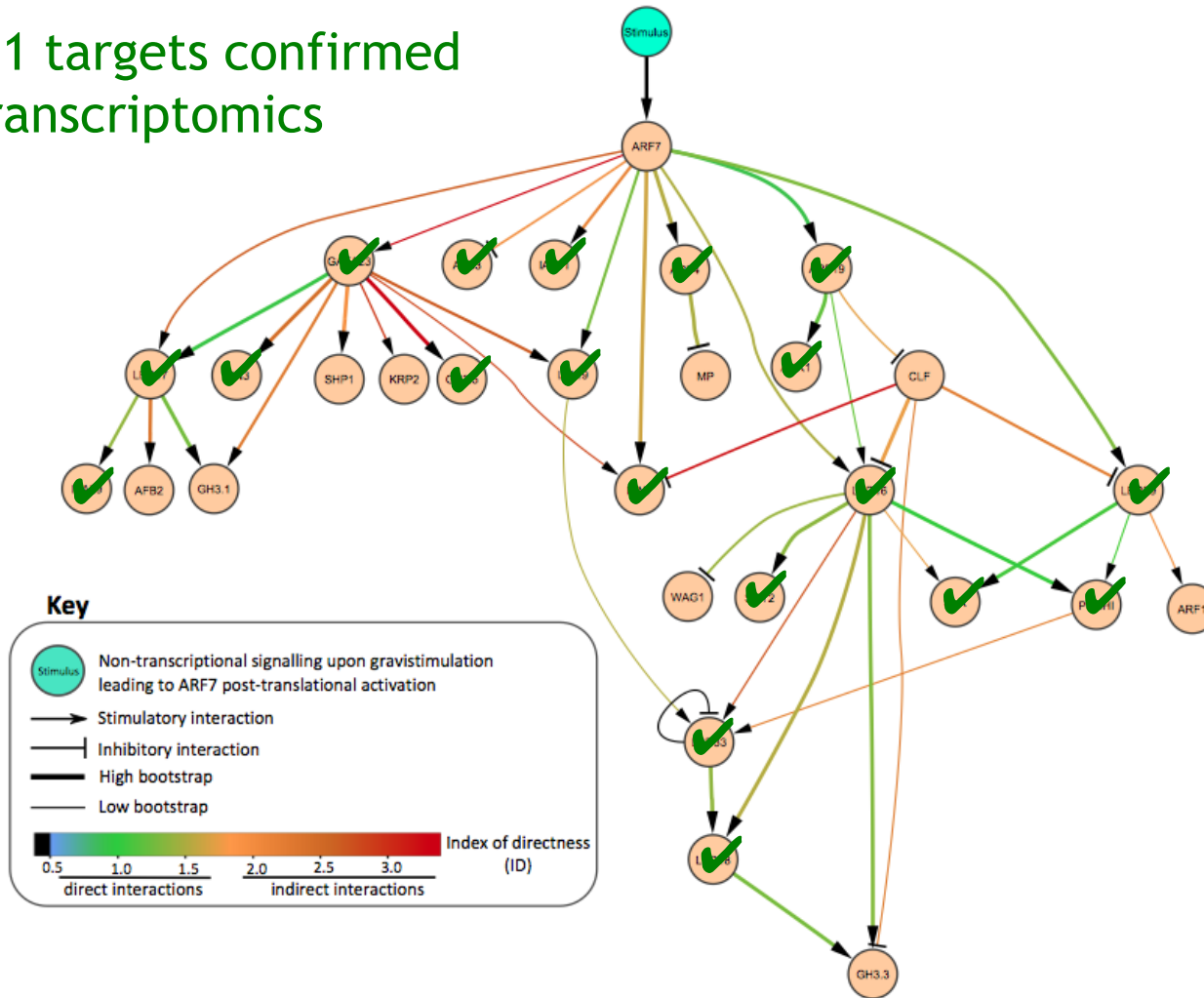
arf7 arf19
pARF7:ARF7::GR

Treated for 4h with :

NAA
DEX
CHX
NAA+DEX
NAA+CHX
DEX+CHX
NAA+DEX+CHX

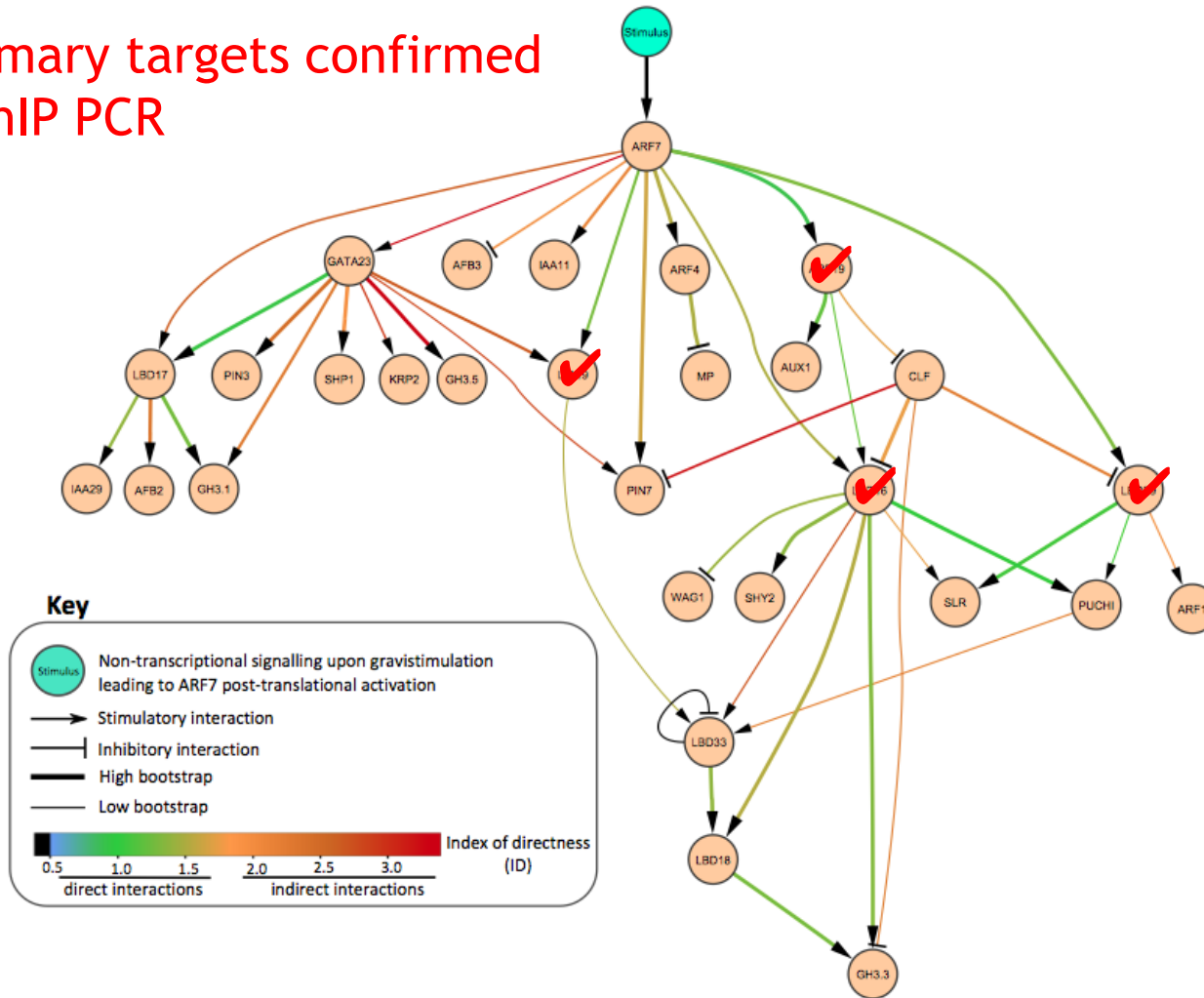
Validating the network - the ARF7 case

22/31 targets confirmed
by transcriptomics

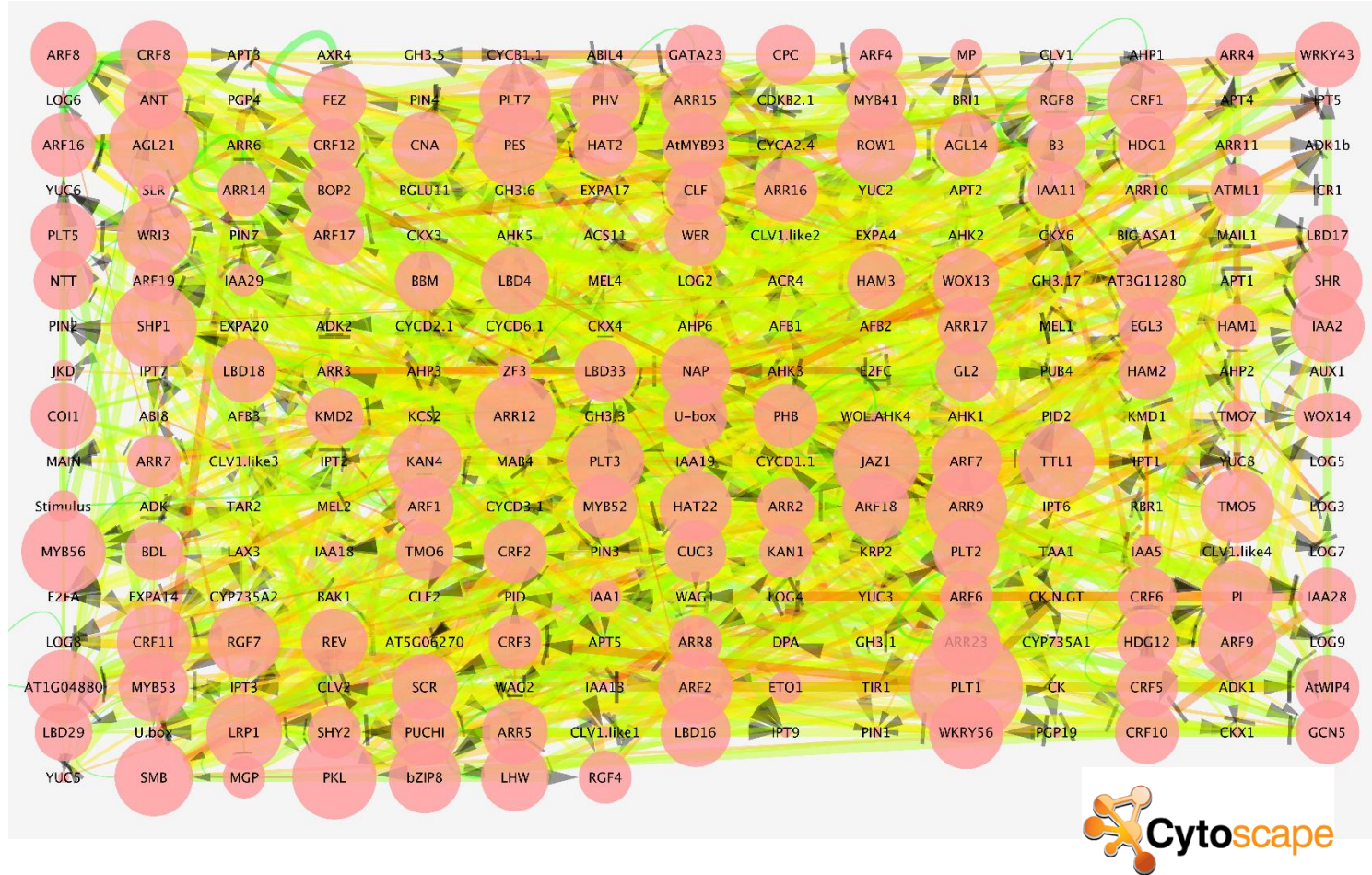


Validating the network - the ARF7 case

4 primary targets confirmed by CHIP PCR

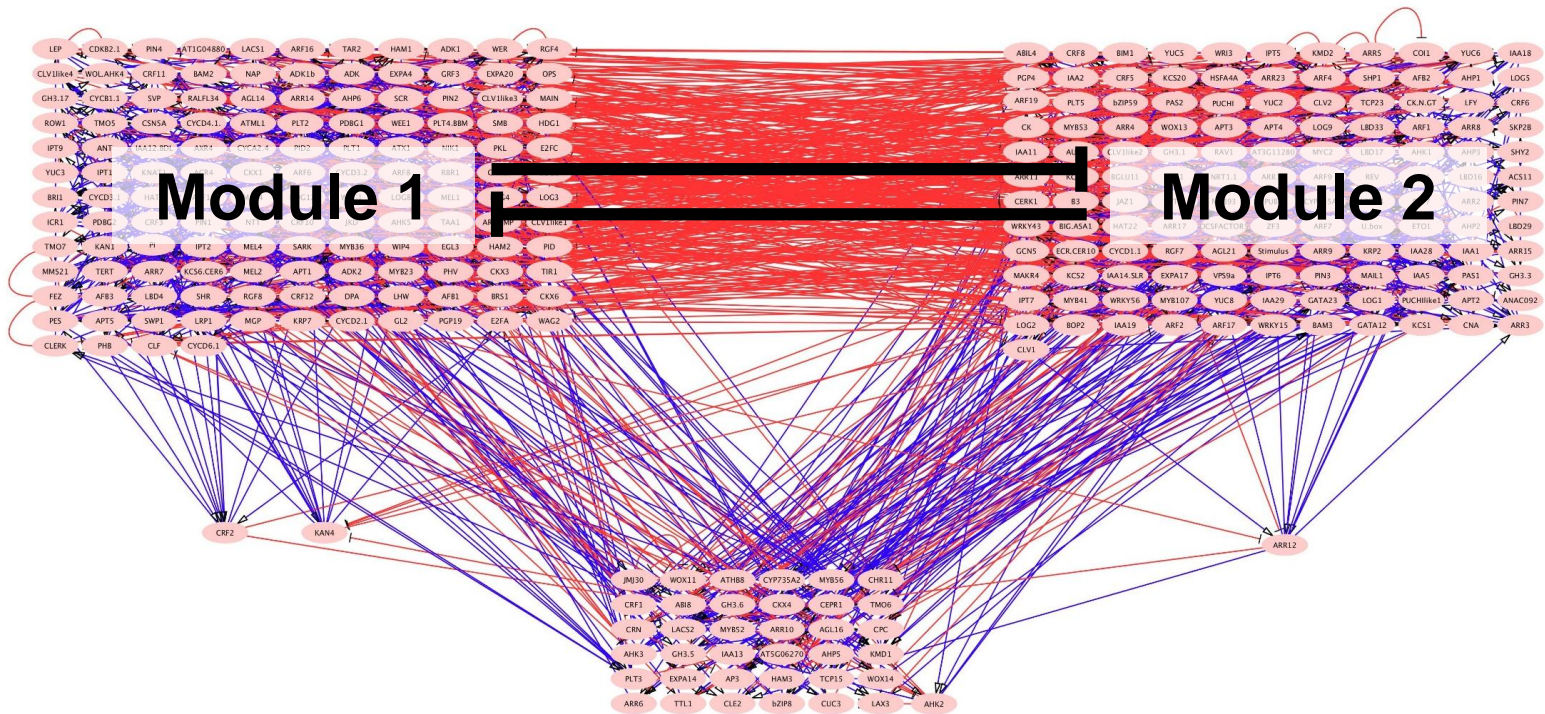


Moving forward with the network



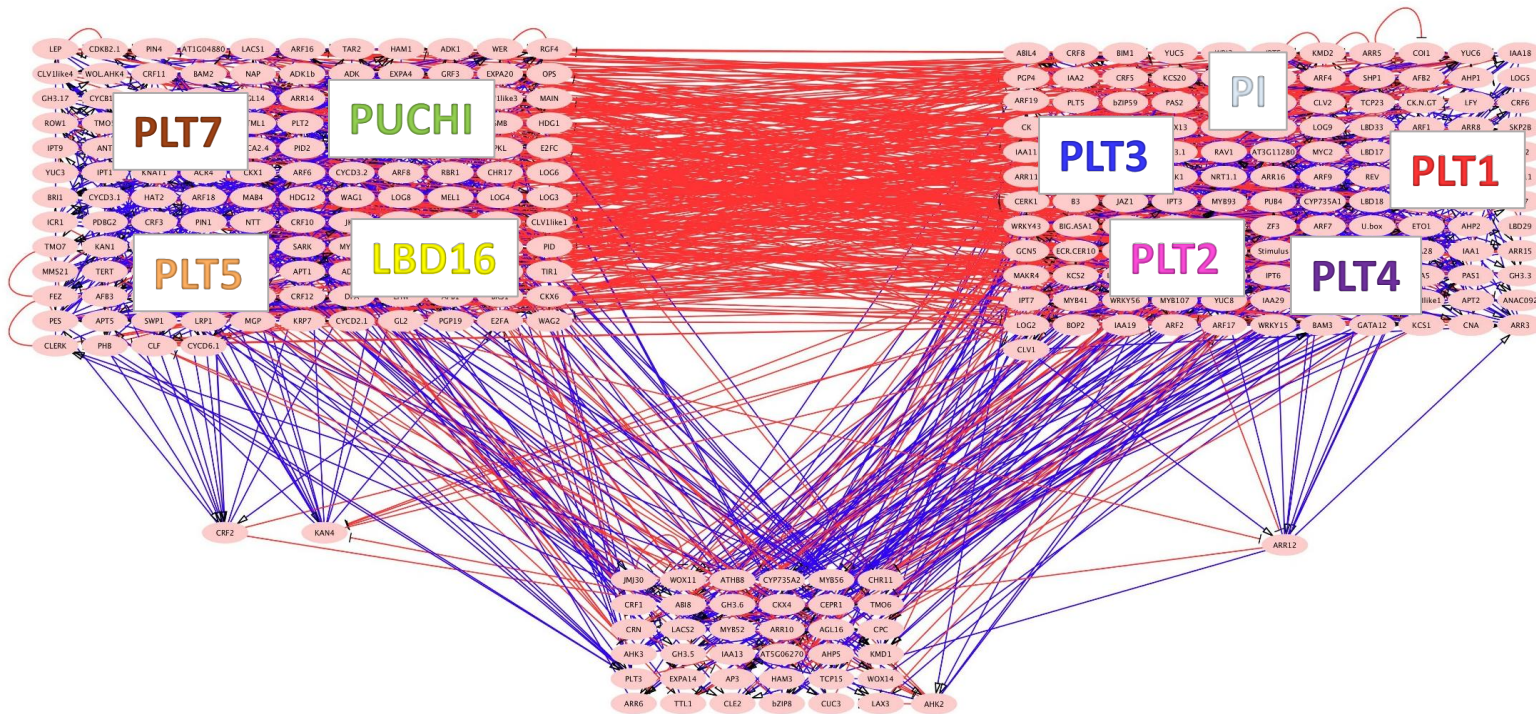
Having validated our inference approach, we went forward with the network exploration

Topology of the LR GRN



Expert (i.e. by hand) analysis of the network structure revealed a modular organisation.

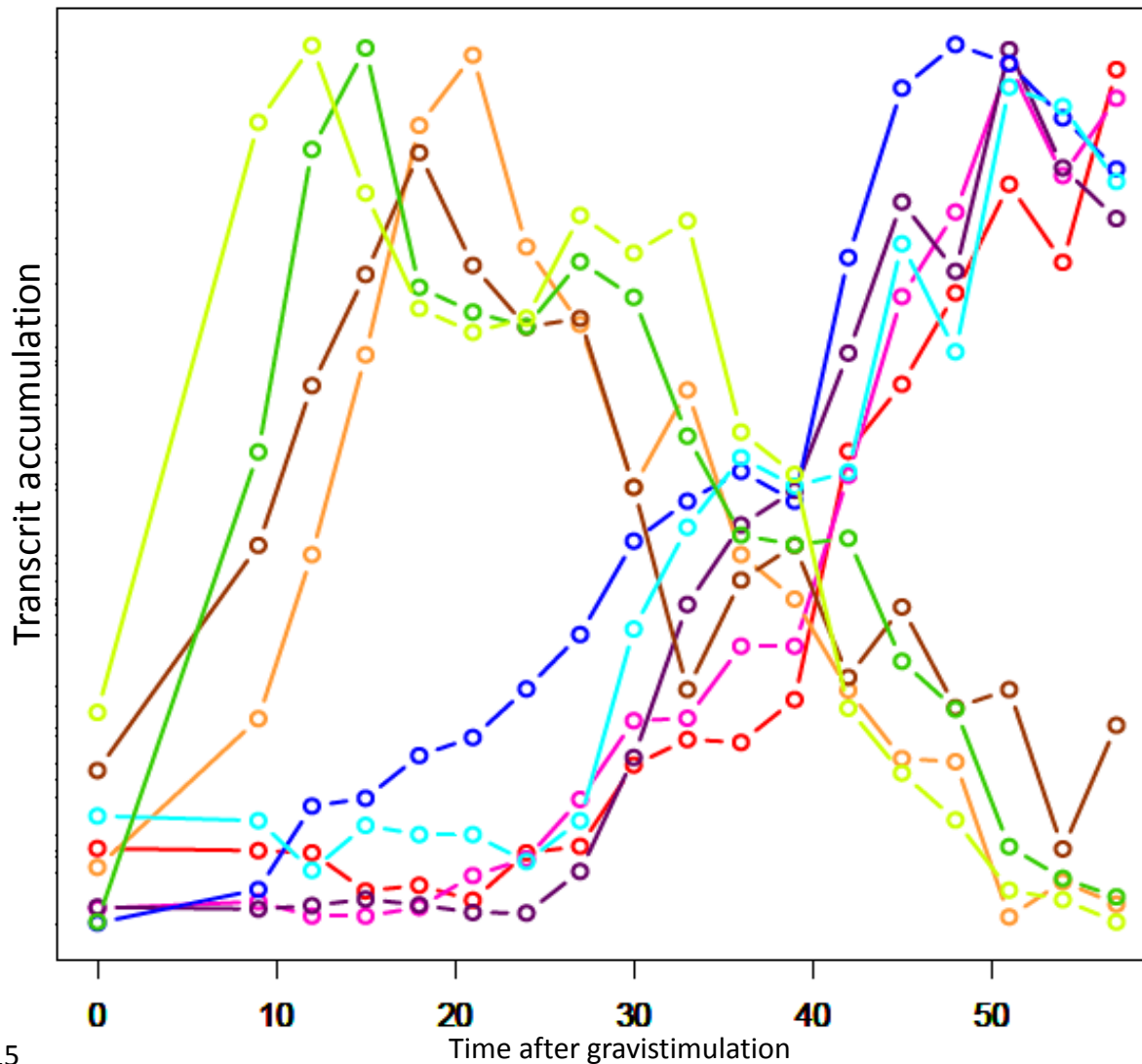
Topology of the LR GRN - biological meaning ?



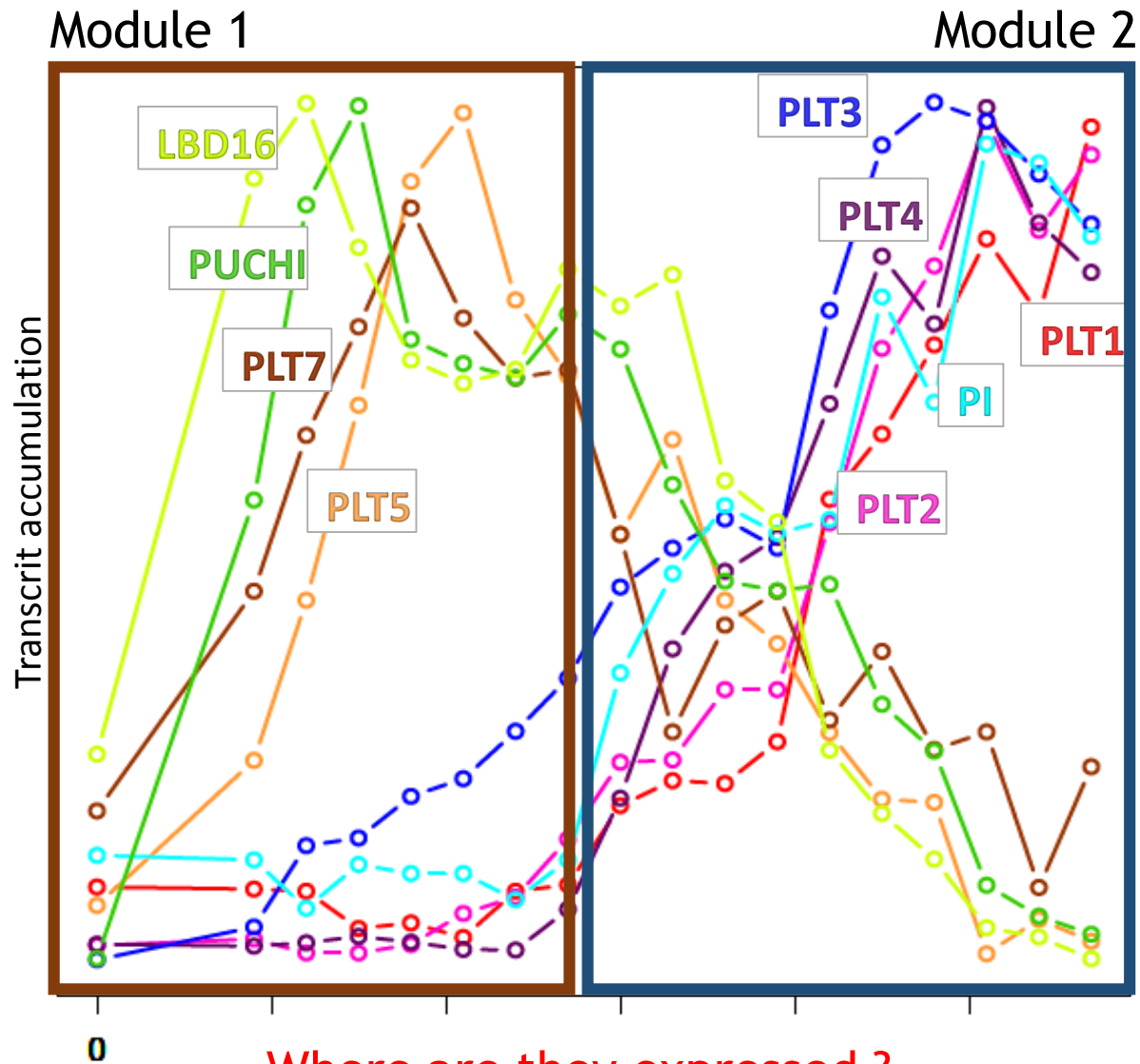
Having a look at some of the genes in those two modules...

Topology of the LR GRN - biological meaning ?

What are their expression profile like ?

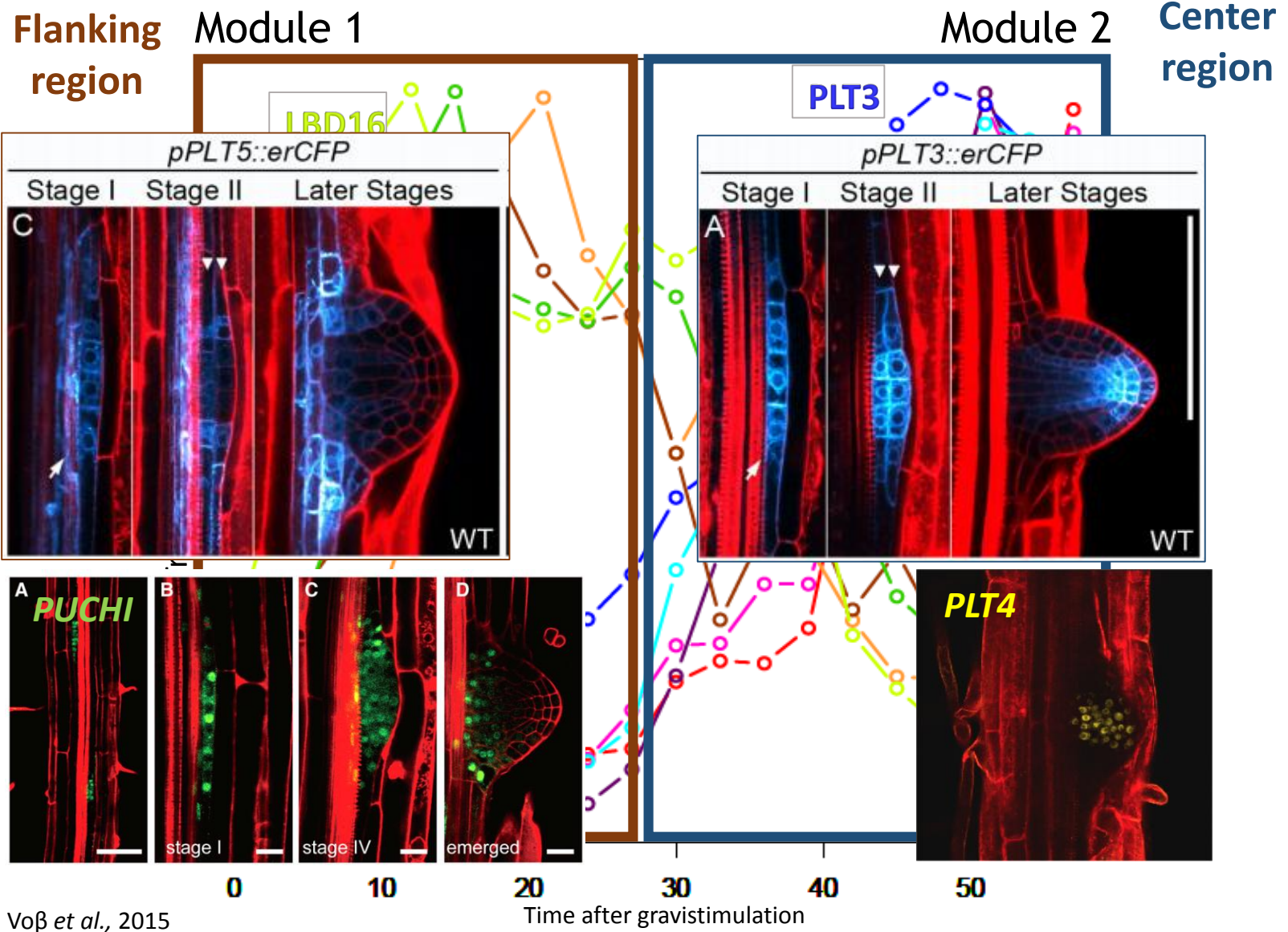


Topology of the LR GRN - biological meaning ?



Where are they expressed ?

Topology of the LR GRN - biological meaning ?



Topology of the LR GRN - biological meaning ?

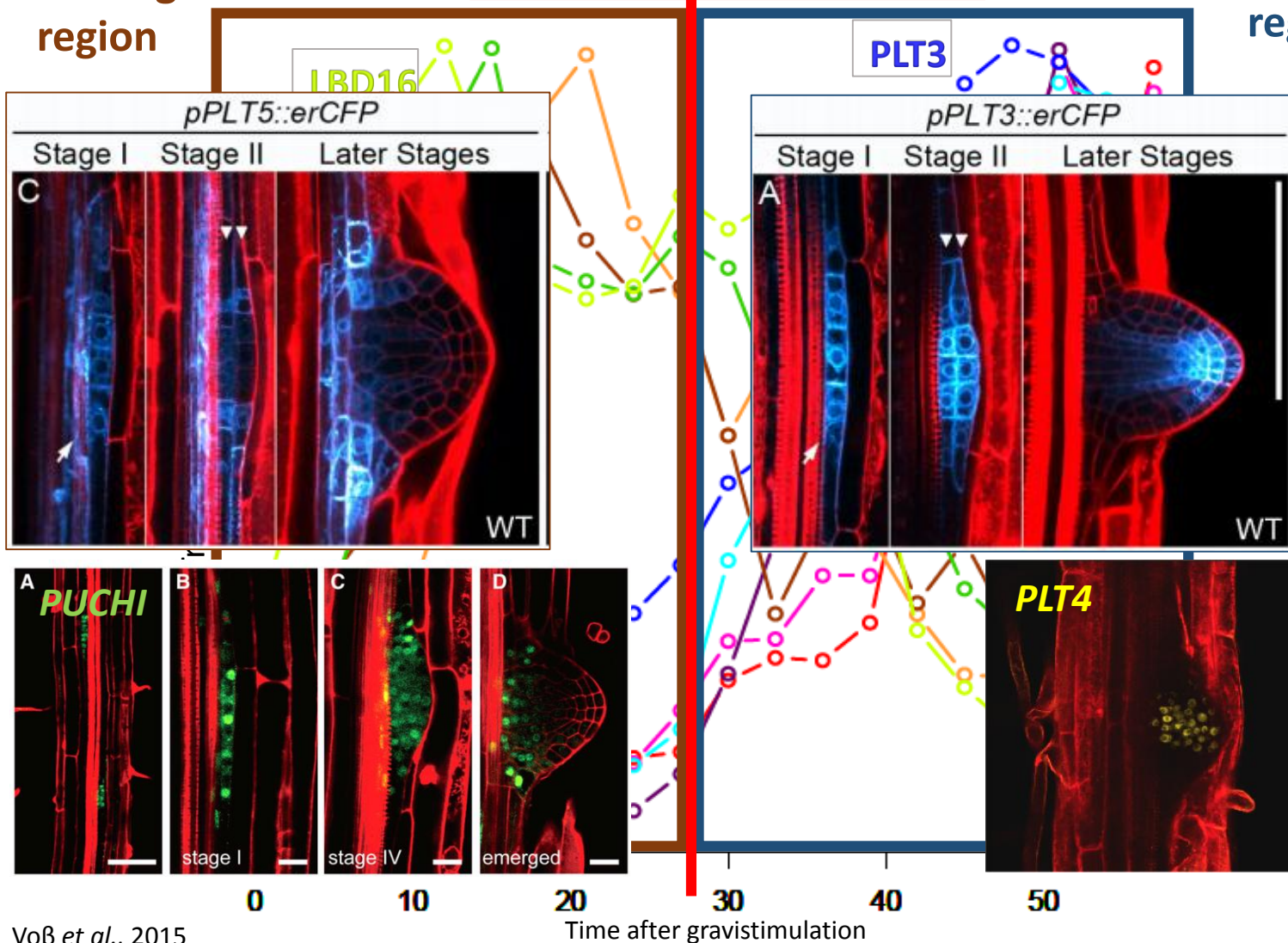
Flanking region

Module 1

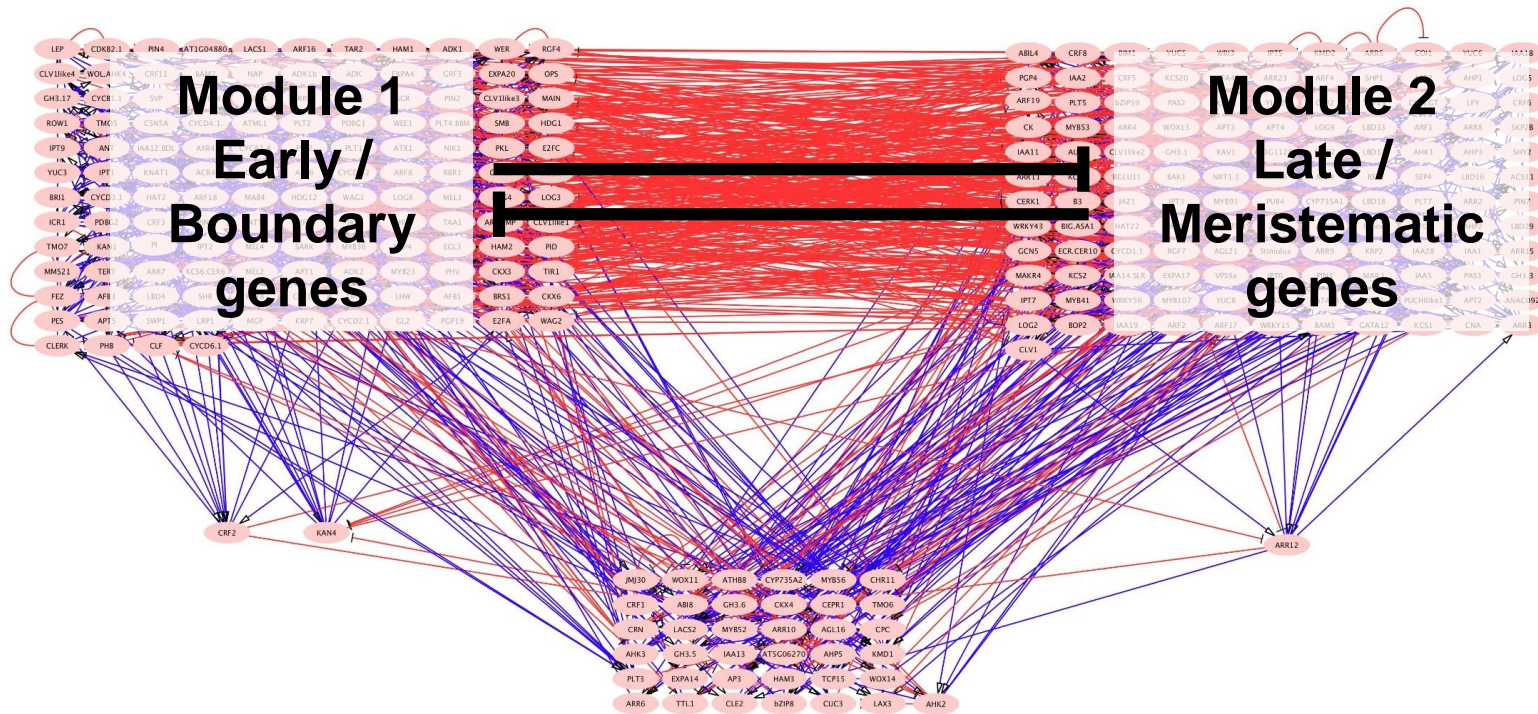
Quiescent center establishment

Module 2

Center region



Topology of the LR GRN - biological meaning ?

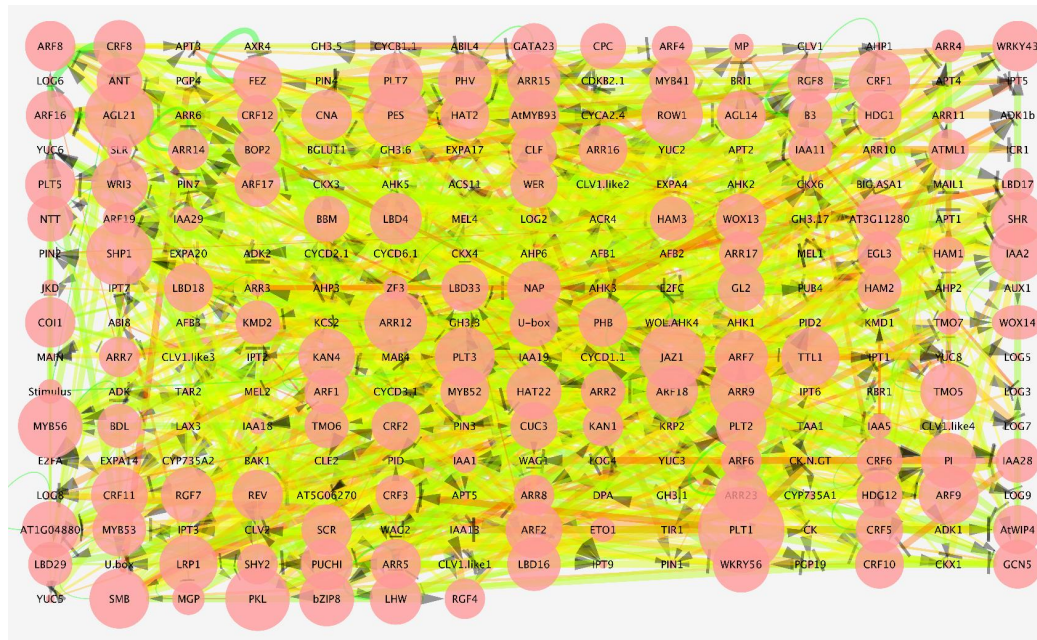


There appears to be biological meaning
behind this modular topology

Can we investigate the dynamics of this patterning event
(establishment of QC / definition of boundary) ?

Modeling GRN dynamics - PANTHEON

We wanted to investigate the precise dynamics of our GRN.



With several hundred of genes and interactions to consider and no already available solution to simulate such a system easily, we opted to develop our own software.

PANTHEON

A PYTHON -BASED
GENERIC BOOLEAN
NETWORK SIMULATOR

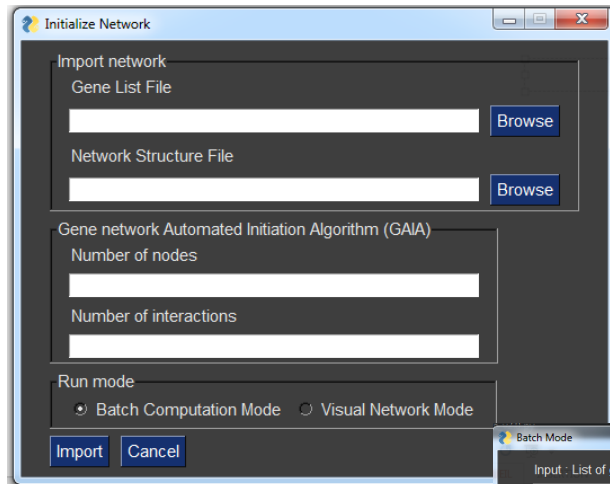
Based on Boolean formalism

**Automatically model large-
scale genes network**

**Designed to work from
simple network description
(list of genes and
interactions)**



Modeling GRN dynamics - PANTHEON

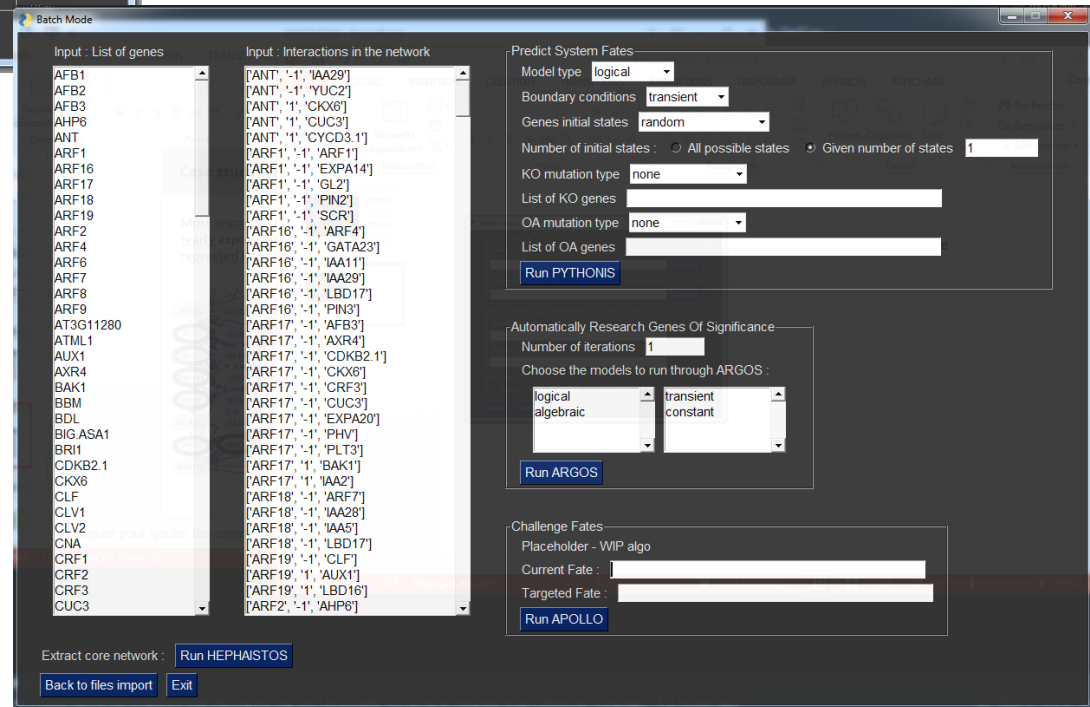


GUI : no need to code to simulate your gene network behavior

Import your network or generate a random one / Export simulation results as csv files

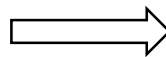
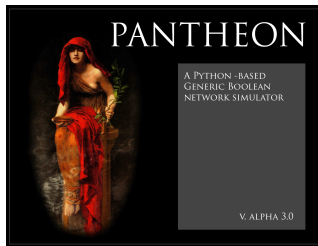
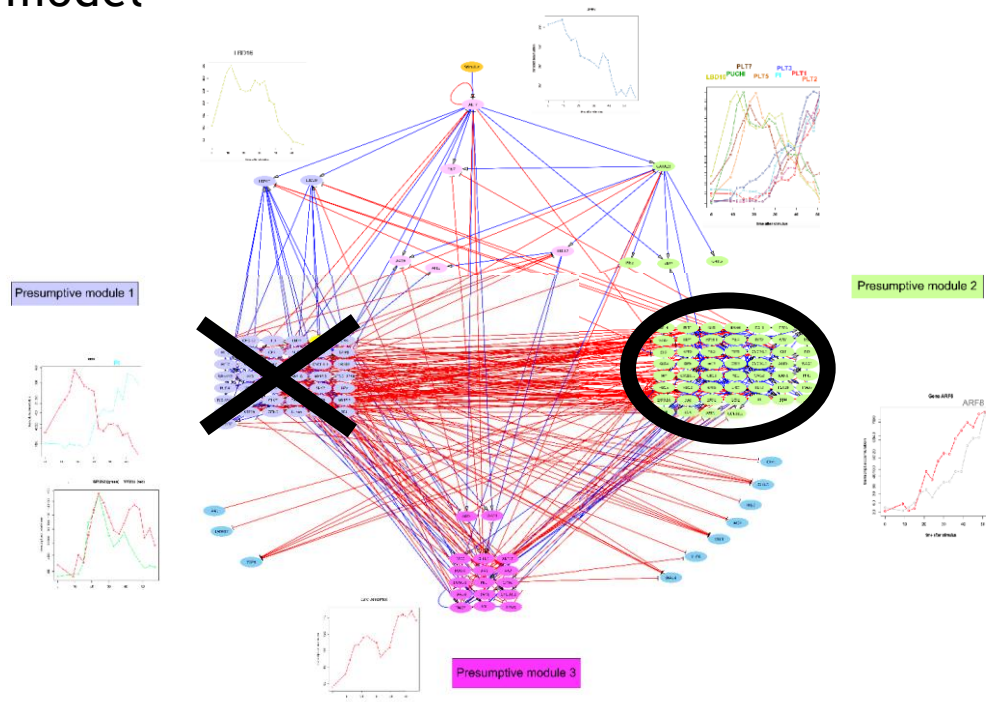
Tools included :
in silico mutants study with a click among other things

Modular structure :
base library of regulation models can be extended at will with your own



PANTHEON - the LR dataset case study

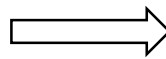
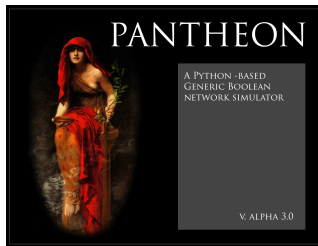
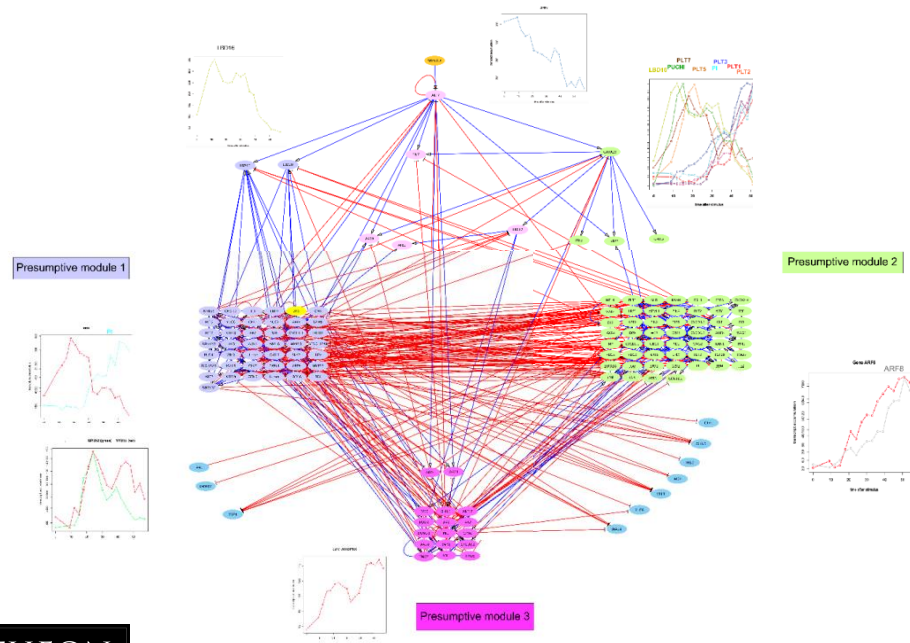
Working on a subset of 134 genes / 495 interactions, full simulation of the network behavior using pure logical or algebraic model



**Prediction of majority stable state
corresponding to meristematic state
(genes from module 2 active,
genes from module 1 inactive)**

PANTHEON - the LR dataset case study

ARGOS Module - Mass *in-silico* mutagenesis and computation of a score of impact on network behavior for each gene (mean hammond distance between wild-type and mutants stable states for all model and mutation combinations)



Highlight the most important genes for the network behavior with no a priori

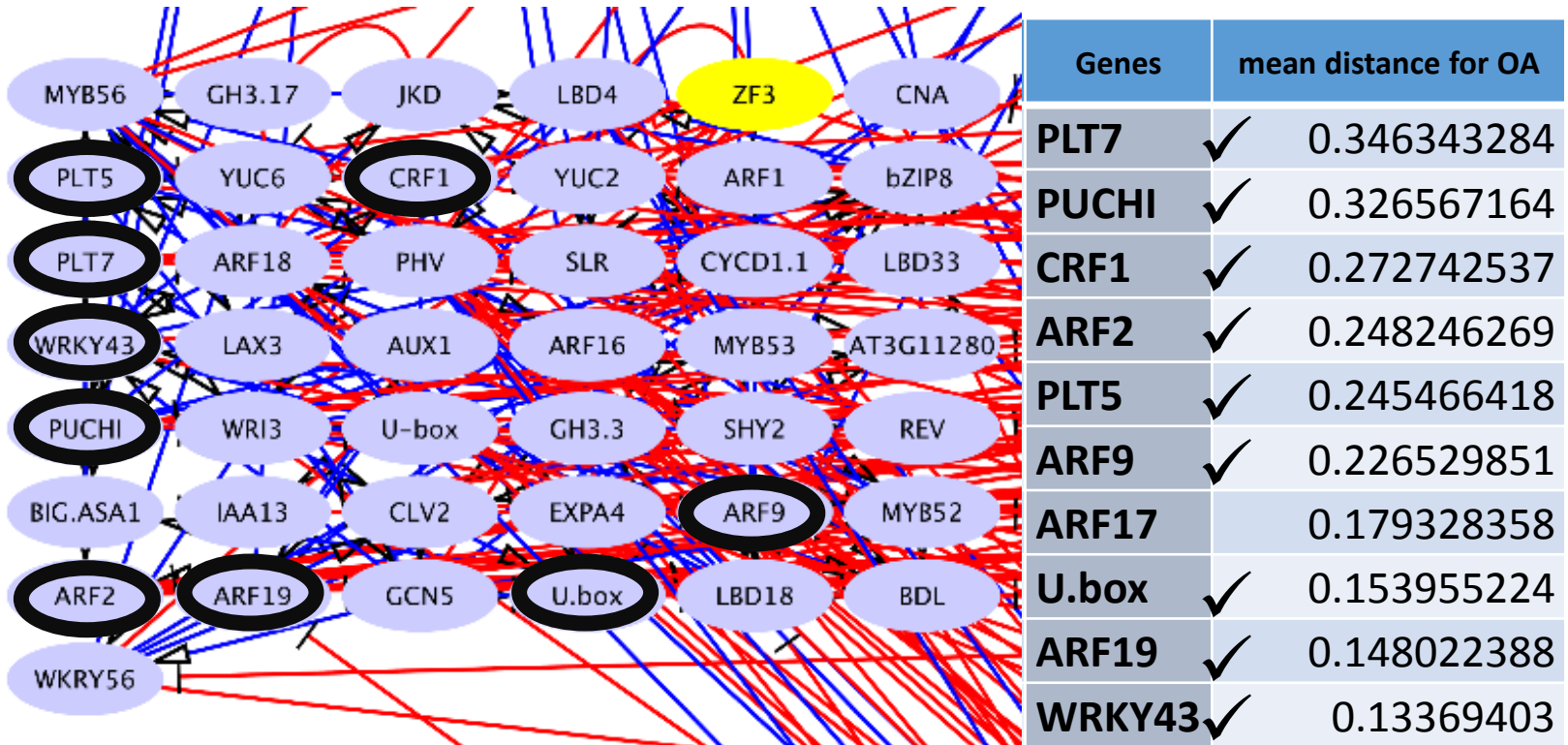
PANTHEON - the LR dataset case study

ARGOS Module - Mass *in-silico* mutagenesis and computation of a score of impact on network behavior for each gene

Genes	mean distance for KO		Genes	mean distance for OA
PLT1	0.168600746		PLT7	0.346343284
ARF6	0.136735075		PUCHI	0.326567164
LRP1	0.13113806		CRF1	0.272742537
PHB	0.124869403		ARF2	0.248246269
TMO5	0.103973881		PLT5	0.245466418
SHR	0.098656716		ARF9	0.226529851
SCR	0.087817164		ARF17	0.179328358
SHP1	0.081100746		U.box	0.153955224
ATML1	0.063302239		ARF19	0.148022388
PID2	0.060970149		WRKY43	0.13369403

TOP10 predicted as most significant genes when KO or OA

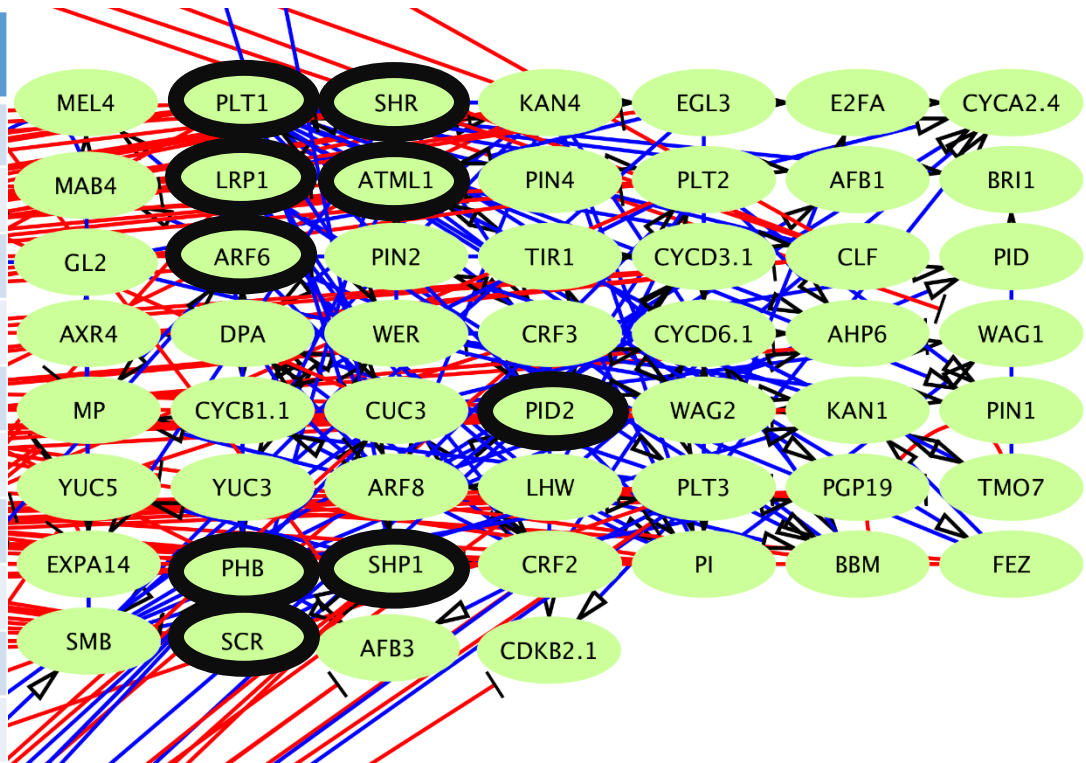
PANTHEON - the LR dataset case study



Most impactful genes when OA are in module 1
(early genes which we need to be repressed later on)

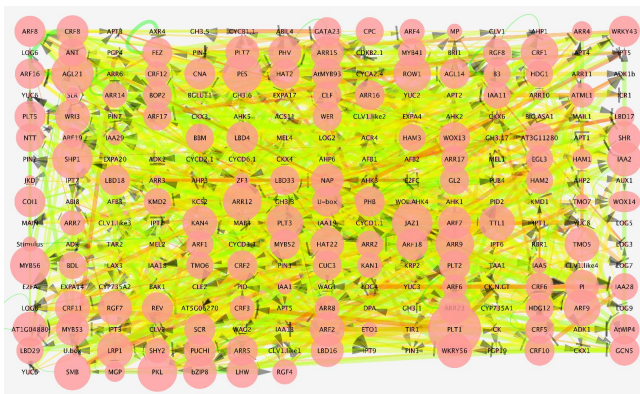
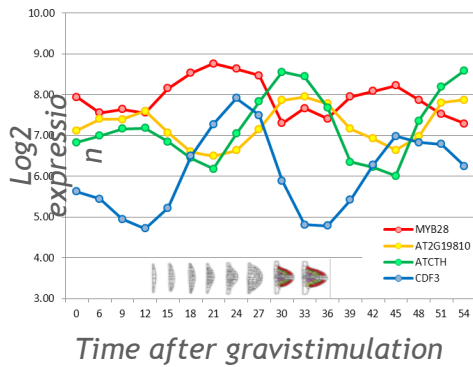
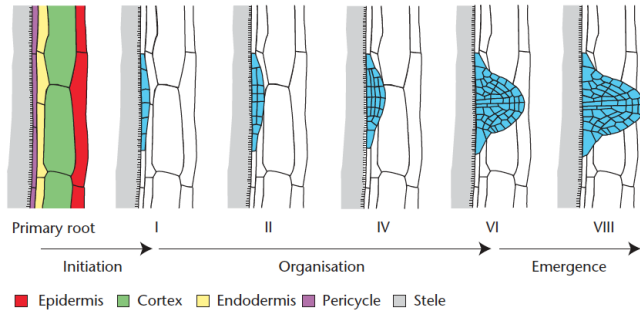
PANTHEON - the LR dataset case study

Genes		mean distance for KO
PLT1	✓	0.168600746
ARF6	✓	0.136735075
LRP1	✓	0.13113806
PHB	✓	0.124869403
TMO5		0.103973881
SHR	✓	0.098656716
SCR	✓	0.087817164
SHP1	✓	0.081100746
ATML1	✓	0.063302239
PID2	✓	0.060970149



Most impactful genes when KO are in module 2
(late genes which we need to be expressed for LRP development)

LRP morphogenesis - In summary



Arabidopsis LR as an excellent model system of organogenesis : simple, controllable, accessible

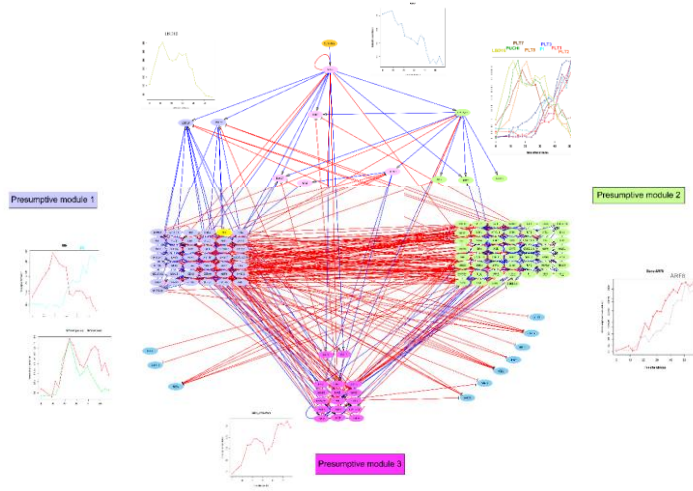


Creation of the LRP database covering the full development of the organ



Creation of the TDCor algorithm and inference of the LRP development GRN

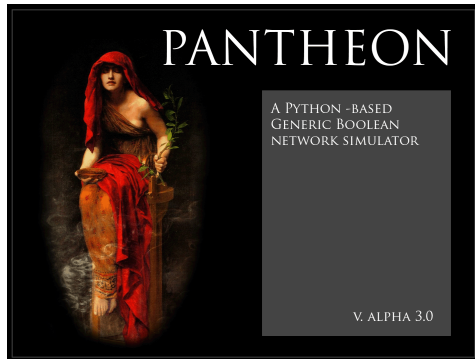
LRP morphogenesis - In summary



Topological analysis revealed a modular structure tied to biological function and a possible bifurcation switch between flank/organizing center identities



Creation of an automated Boolean modeling software which predicted that the topology of the GRN was enough to generate a meristematic identity and was able to retrieve modular organization with no *a priori*



Once now, back to biology to confirm the prediction of the model (i.e. working on generating and characterizing mutants...)

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