Journées NetBIO - 14/10/2019

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

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#### Context : Root System

#### Development

## Root Arcl itecture

Nutrition

Anchorage

Interaction with microorganisms

#### Context : Secondary organogenesis



... to a mature

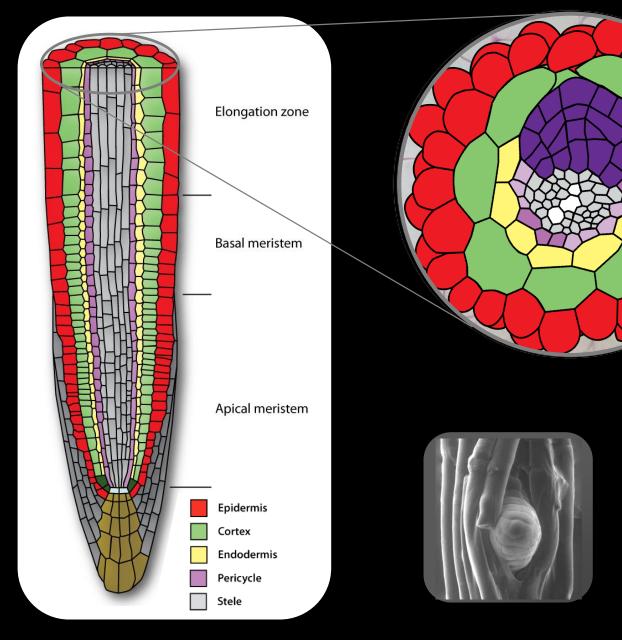
root system ...

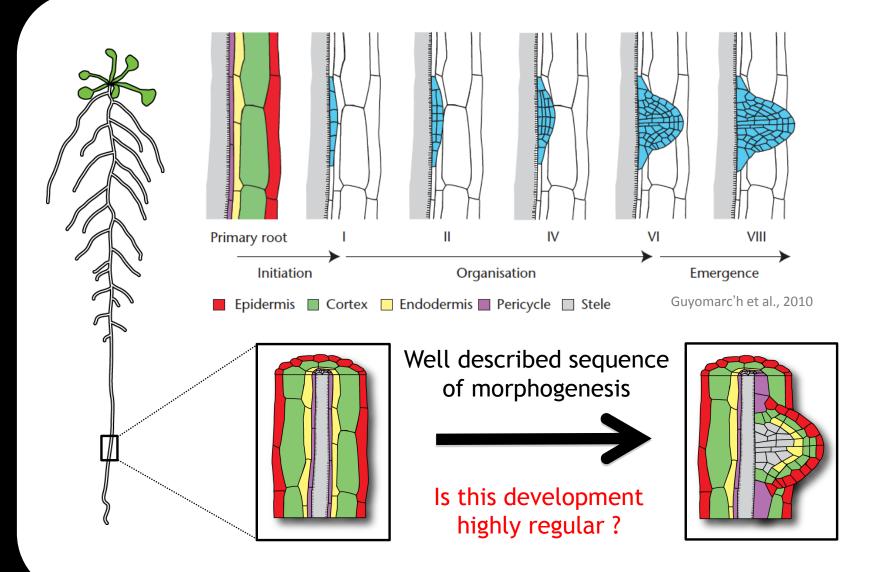
... with a complex architecture resulting from interactions between genetics and environment.

In Arabidopsis, LR initiation occurs in the pericycle

And LR develop through the tissues to finally emerge



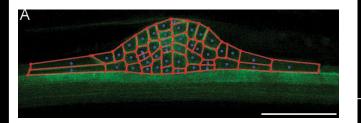


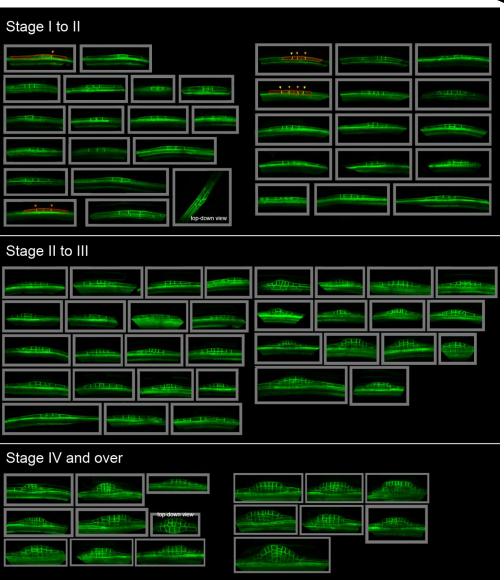


#### (Lucas et al. 2013)

#### Context : Lateral root organogenesis

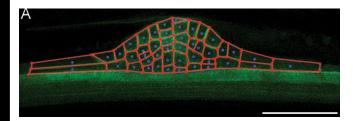
#### Let's have a look at lots of LRP

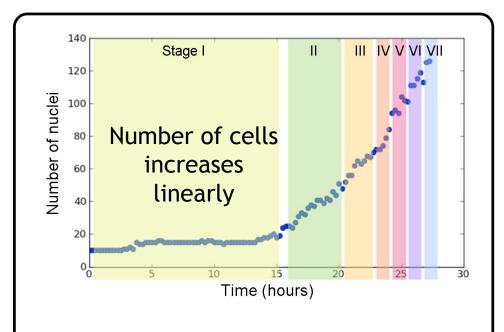




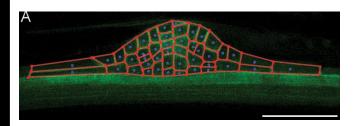
(Lucas et al. 2013)

#### Let's have a look at lots of LRP



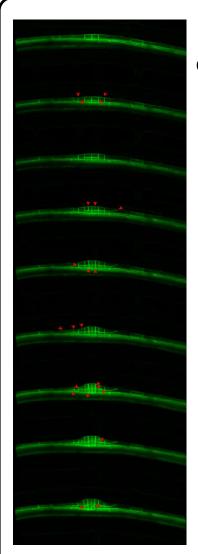


#### Let's have a look at lots of LRP

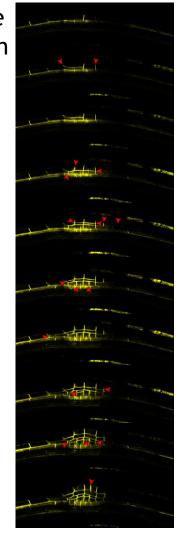


LRP patterning is not stereotypical

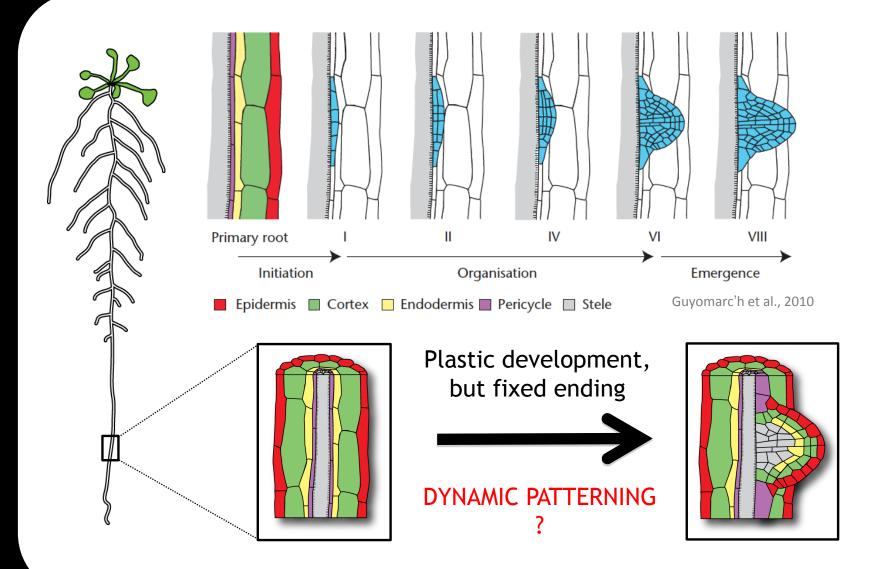
# Multiple ways of building a LRP

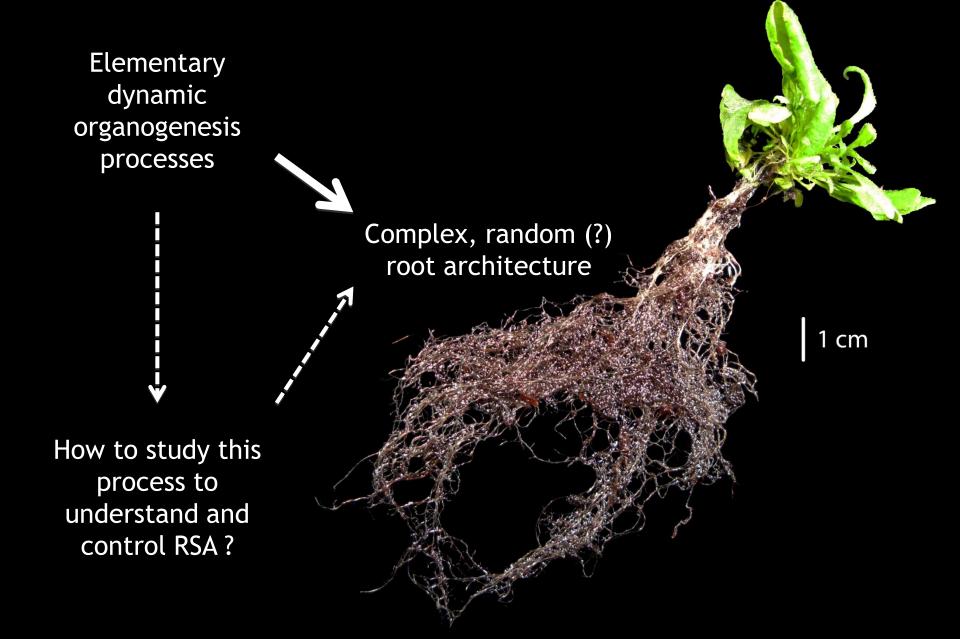


Sequence of division events actually varies between LRP



(Lucas et al. 2013)

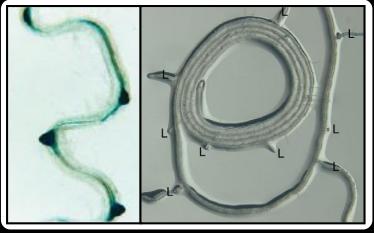




#### Modulation of lateral root initiation

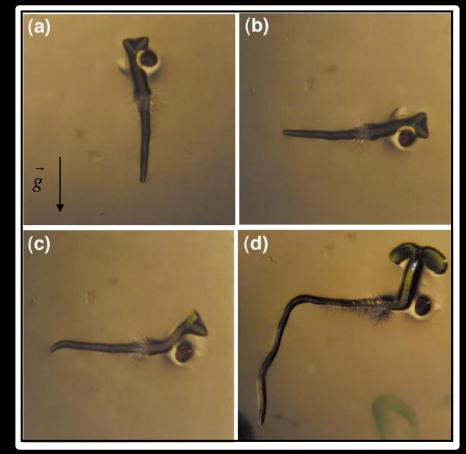
## LRP initiation correlated with root bending

#### Gravitropism induces root bending



(De Smet et al. 2007)

Can new lateral roots be induced using gravistimulation ?



(Rosen, 1999)

#### Modulation of lateral root initiation

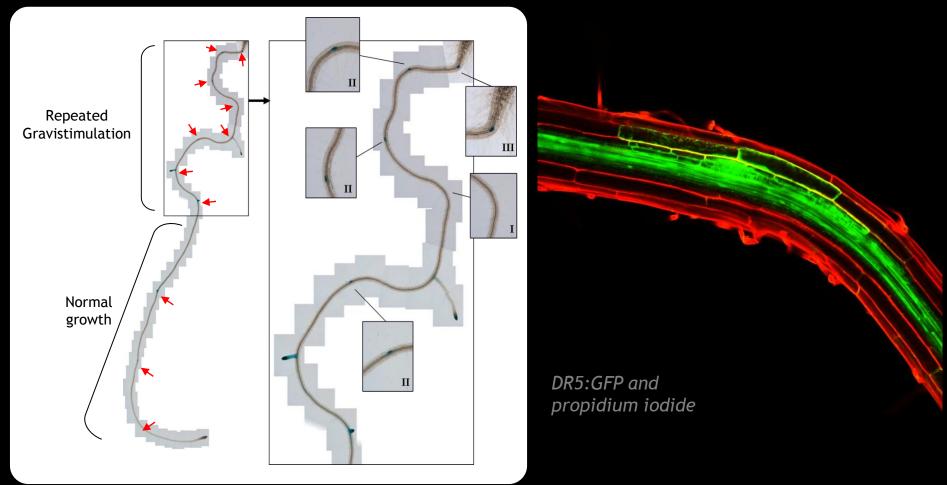


(Lucas et al. 2008)

### Induction of rhizogenesis by gravistimulation

## Gravistimulation induces initiation...

## ... within a tightly controlled spatio-temporal window

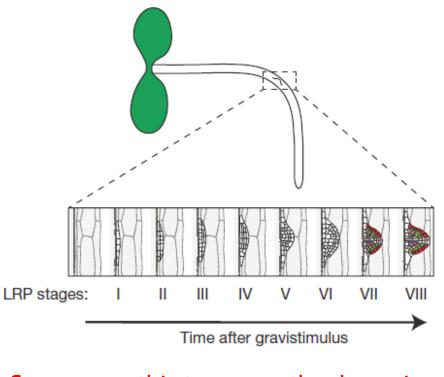


(Lucas et al. 2008)

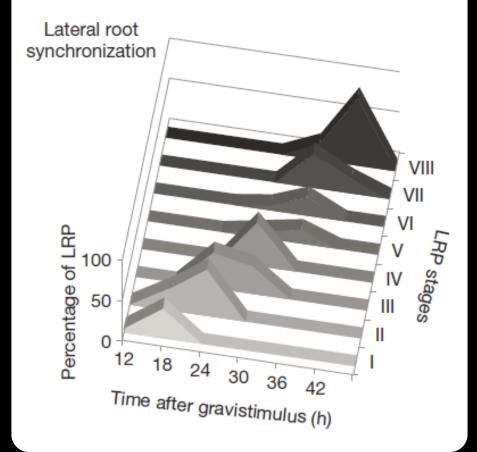
## Control of rhizogenesis by gravistimulation

## Gravistimulation induces initiation...

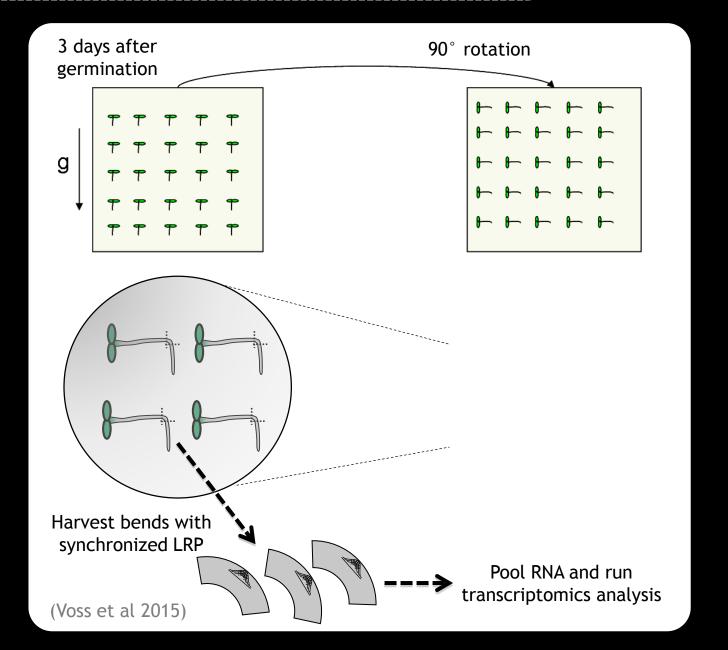
## ... within a tightly controlled spatio-temporal window



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



<sup>(</sup>Péret et al. 2012)





300 to 400 bends per timepoint

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

4 replicates

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

(Voss et al 2015)

Illustration of some transcription factors expression profiles from the database

12.00

10.00

8.00

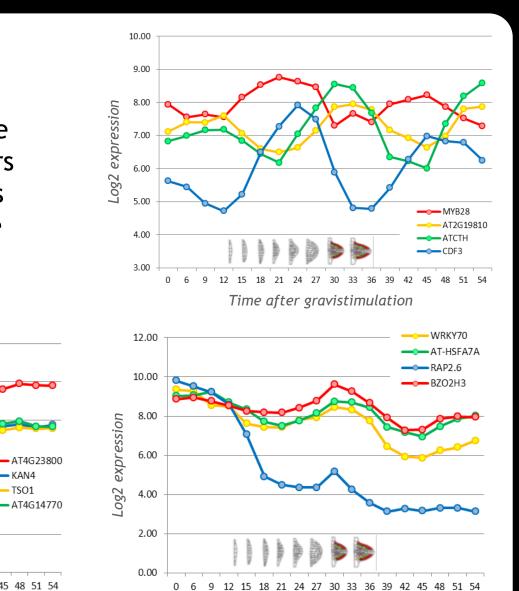
6.00

4.00

2.00

0.00

Log2 expression



0 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 Time after gravistimulation

KAN4 TSO1



#### Expression database exploitation

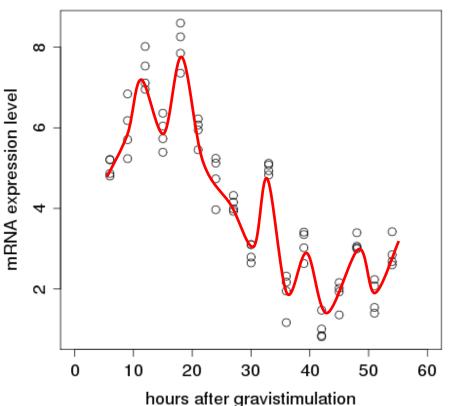
Extracting information from the LR dataset ?

### V

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

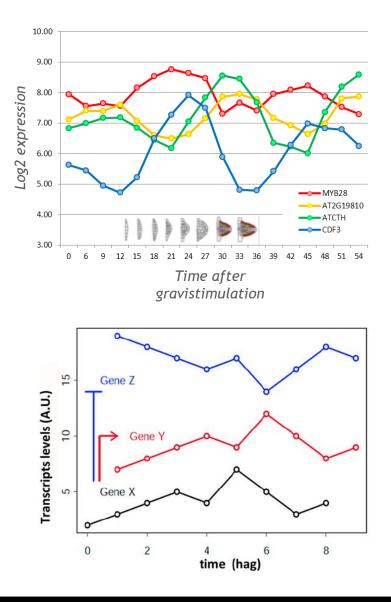
• Implemented in R (CRAN)

• Runs on expression profiles extracted from the LR dataset (or any other transcriptomic kinetics dataset)

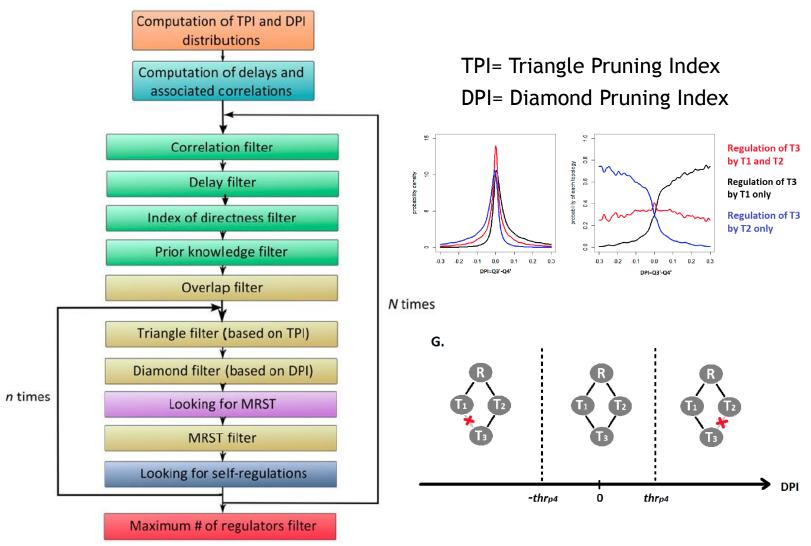
• Looks for non-combinatorial linear interactions

• 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



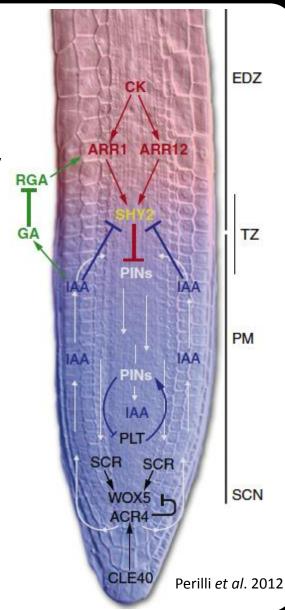
Lavenus et al., Plant Cell 2015

#### Using TDCor on the LR dataset

Selection of genes involved in

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

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

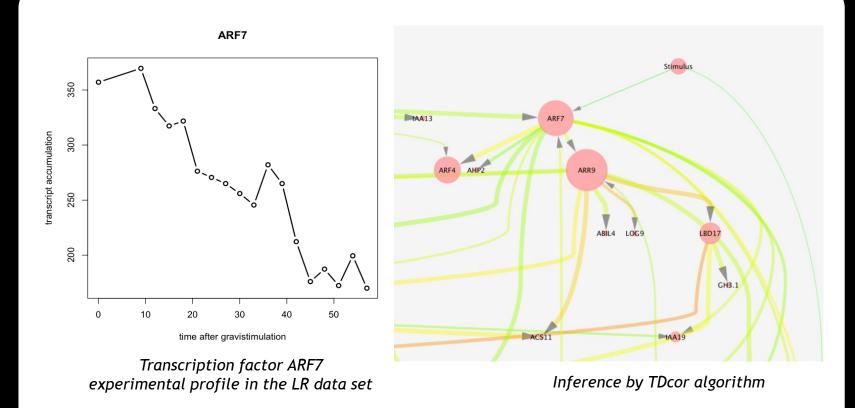
- Selected a list of 261 genes
- Not only transcription factors
- A "prior" data is given to each gene, based on the litterature, to indicate if transcriptional regulation activity has been reported or not
- This "prior" information helps the inference procedure by authorizing or not the algorithm to 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	АНКЗ	0
AT1G27450	APT1	0
AT1G28130	GH3.17	0
AT1G30330	ARF6	1
AT1G30490	PHV	2
AT1021220		2

#### Using TDCor on the LR dataset

ARF8	CRF8	APTS	AXR4	GH3.5	CYCB1.1	ABIL4	GATA23	СРС	ARF4	МР	CLV1	AHP1	ARR4	WRKY43
LOG6	ANT	PGP4	FEZ	PINA	PLT7	PHV	ARR15	CDKB2.1	MYB41	BRII	RGF8	CRF1	АРТ4	PT5
ARF16	AGL21	ARR6	CRF12	CNA	PES	HAT2	AtMYB93	CYCAZ.4	ROW1	AGL14	B3	HDG1	ARR11	ADK1b
YUCE	SLR	ARR14	BOP2	BGLU11	GH3:6	EXPA17	CLF	ARR16	YUC2	APT2	[ IAA11	ARR10	ATML1	ICR1
PLT5	WRI3	PIN7	ARF17	СКХЗ	АНК5	ACS11	WER	CLV1.like2	EXPA4	АНК2	CKX6	BIG.ASA1	MAIL1	LBD17
	ARF19	IAA29	100	ввм	LBD4	MEL4	LOG2	ACR4	НАМЗ	WOX13	GH3.17	AT3G11280	APT1	SHR
PINE	SHP1	EXPA20	ADK2	CYCD2.1	CYCD6.1	CKX4	AHP6	AFB1	AFB2	ARR17	MELT	EGL3	HAM1	IAA2
JKD	IPTZ	LBD18	ARR3	AHP3	ZF3	LBD33	NAP	AHKS	d2FC	GL2	PUB4	HAM2	AHP2	AUX1
СОІІ	ABI8	AFBB	KMD2	KCSZ	ARR12	GH3.8	U-box	РНВ	WOE.AHK4	AHK1	PID2	KMD1	тмот	WOX14
MAIN	ARR7	CLV1.like3	IPTZ	KAN4	MAB4	PLT3	IAA19	CYCD1.1	JAZ1	ARF7	Τιι	IPHPT1	JUC8	LOG 5
Stimulus	ADK	TAR2	MEL2	ARF1	CYCD3.1	MYB52	HAT22	ARR2	ARF18	ARR9	IPT6	RBR1	тмо5	LOG3
МҮВ56	BDL	LAX3	IAA18	тмоб	CRF2	PIN3	CUC3	KAN1	KRP2	PLT2	TAA1	IAA5	CLV1.like4	LOG7
EZFA	EXPA14	CYP735A2	BAK1	CLE2	PID	IAA1	WAG1	LOG4	YUC3	ARF6	CK.N.GT	CRF6	РІ	IAA28
LOG	CRF11	RGF7	REV	AT5G06270	CRF3	APT5	ARR8	DPA	CH3 1	ARR23	CYP735A1	HDG12	ARF9	LOG9
AT1G04880	MYB53	IPT3	CLV2	SCR	WAD2	IAA1B	ARF2	ETO1	TIR1	PLT1	Ск	CRF5	ADK1	AtWIP4
LBD29	U.box	LRP1	SHY2	🗧 рисні 🍃	ARR5	CLV1.like1	LBD16	IPT9	PINI	WKRY56	PGP19	CRF10	CKX1	GCN5
YUCS	SMB	MGP	PKL	bZIP8	LHW	RGF4								
	-												Су	toscap

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 ?



ARF7 is predicted to occupy a upstream position in the network

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

Index of directness

(ID)

Stimulatory interaction Inhibitory interaction

1.5

2.5

indirect interactions

2.0

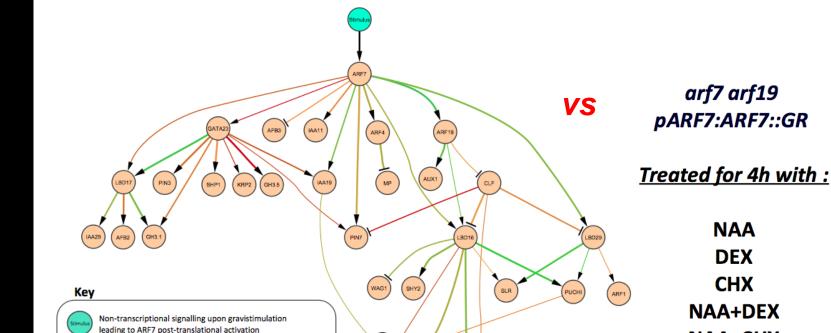
3.0

High bootstrap Low bootstrap

1.0

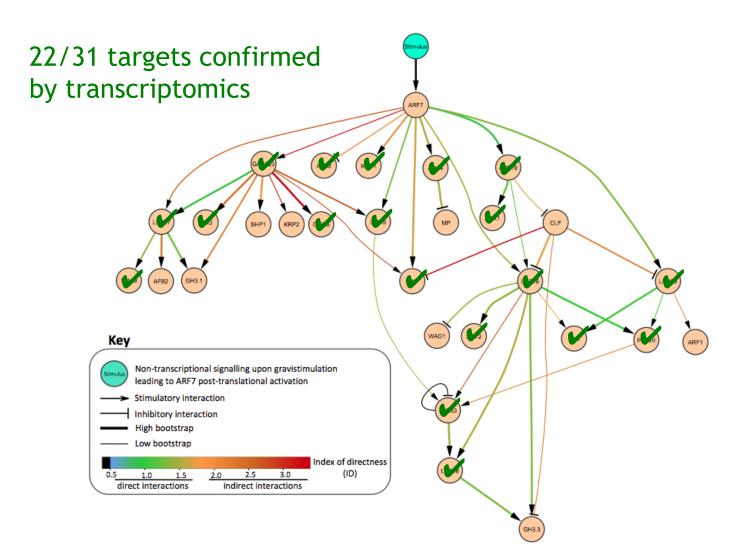
direct interactions

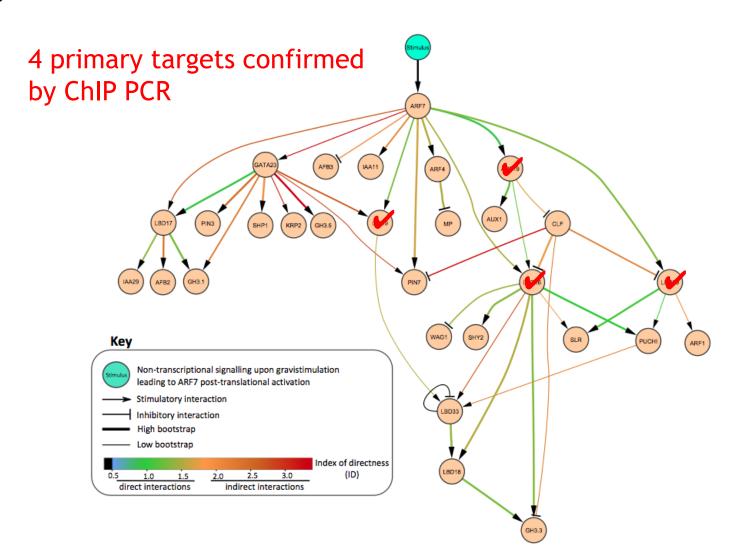
0.5



LBD18

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



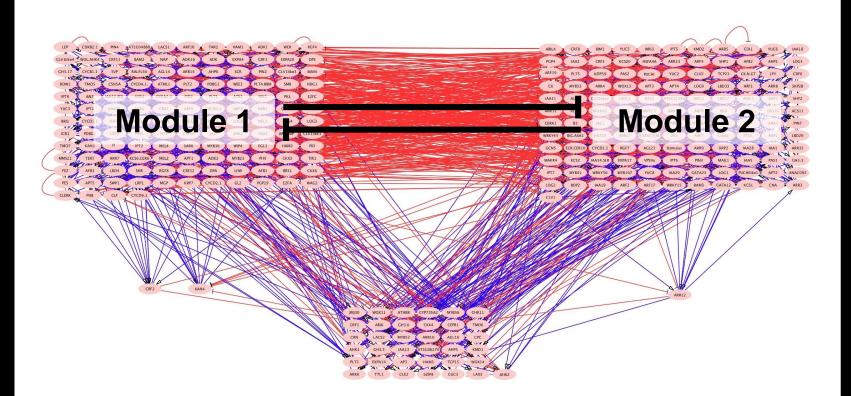


#### Moving forward with the network

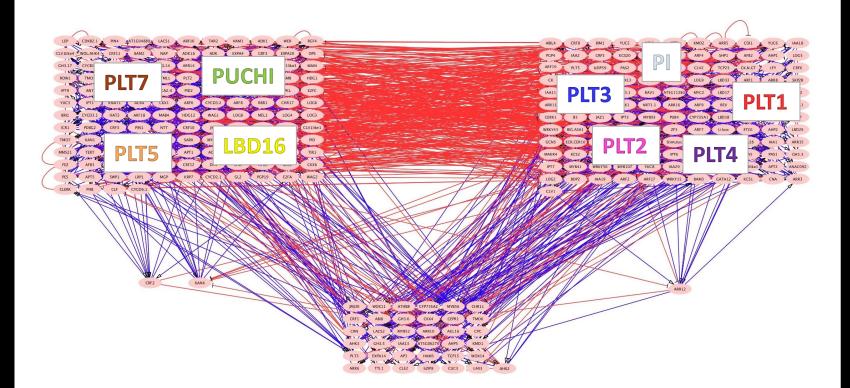
ARF8	CRF8	APTA	AXR4	GH3.5	CYCB1.1	ABIL4	GATA23	СРС	ARF4	МР	CLV1	AHP1	ARR4	WRKY43
LOG6	ANT	PGP4	FEZ	PIN	PLT7	РНУ	ARR15	CDKB2.1	MYB41	BRI1	RGF8	CRF1	АРТ4	рть
ARF16	AGL21	ARR6	CRF12	CNA	PES	HAT2	AtMYB93	CYCA2.4	ROW1	AGL14	вз	HDG1	ARR11	ADK1b
YUCE	SLR	ARR14	BOP2	BGLU11	CH3:6	EXPA17	CLF	ARR16	YUC2	APT2	IAA11	ARR10	ATML1	CR1
PLT5	WRI3	PIN7	ARF17	СКХЗ	АНК5	ACS11	WER	CLV1.like2	EXPA4	AHK2	dkx6	BIG ASA1	MAIL1	LBD17
NTT	ARF19	IAA29		BBM	LBD4	MEL4	LOG2	ACR4	НАМЗ	WOX13	Gң3.17 /	AT3G11280	APT1	SHR
PINE	SHP1	EXPA20	ADK2	CYCD2.1	CYCD6.1	CKX4	AHP6	AFB1	AFB2	ARR17	MELT	EGL3	HAM1	IAA2
JKD	IPTZ	LBD18	ARR3	AHP3	ZF3	LBD33	NAP	АНКЗ	12FC	GL2	PUB4	HAM2	AHP2	AUX1
СОІТ	ABI8	AFBB	KMD2	ксяр	ARR12	Снз з	U-box	РНВ	WOE.AHK4	AHK1	PID2	KMD1	тмот	WOX14
MAIN	ARR7	CLV1.like3	IPT	KAN4	MAB4	PLT3	IAA19	CYCD1.1	JAZ1	ARF7	1 тп1	PPT1	HUCS	LOGS
Stimulus	ADK	TAR2	MEL2	ARF1	CYCD3.1	MYB52	HAT22	ARR2	AKF18	ARR9	ІРТ6	RBR1	тмо5	LOC3
МУВ56	BDL	LAX3	IAA18	тмоб	CRF2	PIN3	CUC3	KAN1	KRP2	PLT2	TAA1	IAA5	CLV1.like4	LOG7
EZFA	EXPA14	СҮР735А2	BAK1	CLE2	PID	IAA1	WAG1	LOC4	YUC3	ARF6	CK.N.GT	CRF6	РІ	IAA28
LOGS	CRF11	RGF7	REV	AT5C06270	CRF3	APT5	ARR8	DPA	СН31	ARR23	CYP735A1	HDG12	ARF9	LOG9
AT1G04880	MYB53	IPT3	CLV2	SCR	WAO2	IAA1B	ARF2	ETO1	TIR1	PLT1	Ск	CRF5	ADK1	AtWIP4
LBD29	U.box	LRP1	SHY2	🗧 рисні 🎐	ARR5	CLV1.like1	LBD16	IPT9	PIN1	WKRY56	PCP19	CRF10	CKX1	GCN5
YUCS	SMB	MGP	PKL	bZIP8	LHW	RGF4								
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Having validated our inference approach, we went forward with the network exploration

#### Topology of the LR GRN

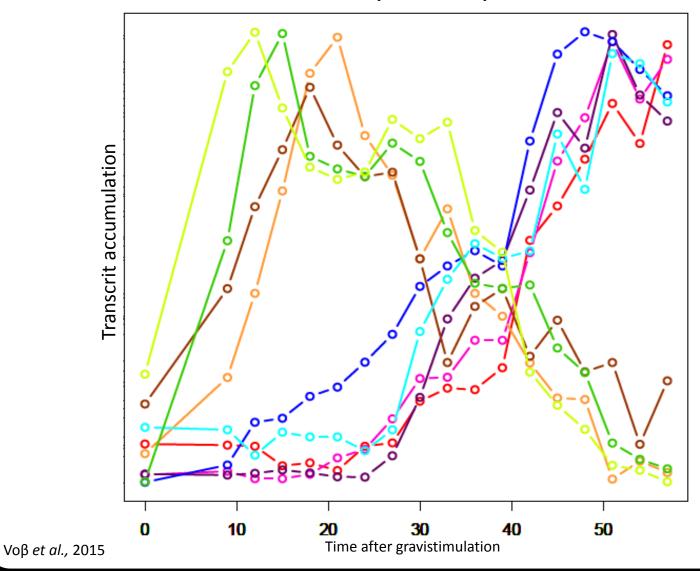


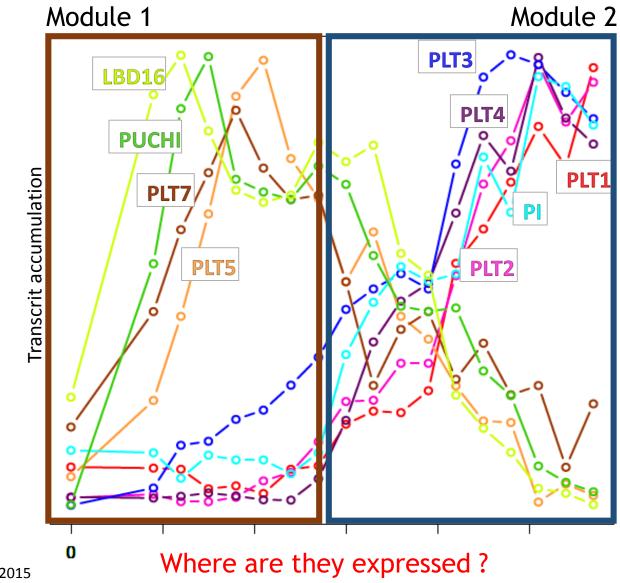
Expert (i.e. by hand) analysis of the network structure revealed a modular organisation.



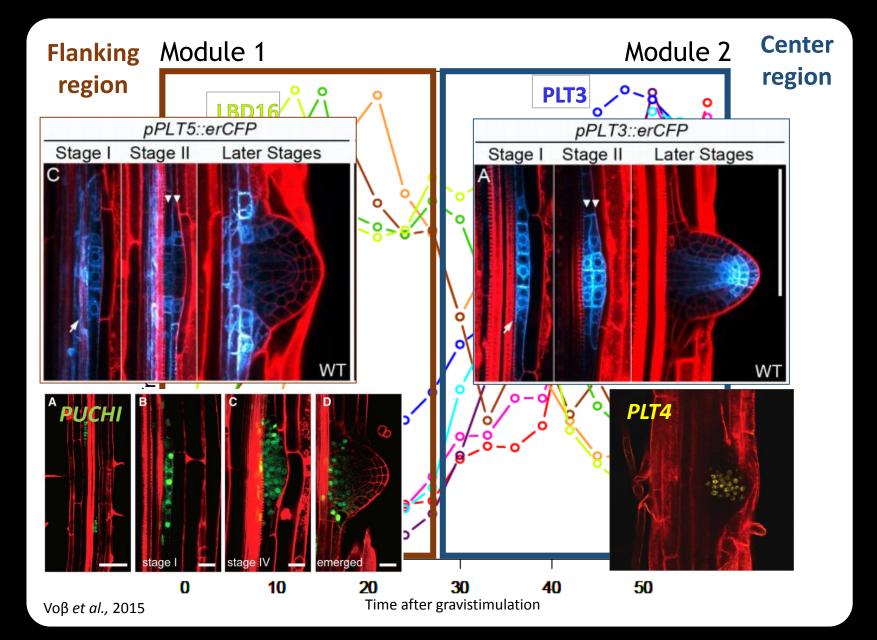
Having a look at some of the genes in those two modules...

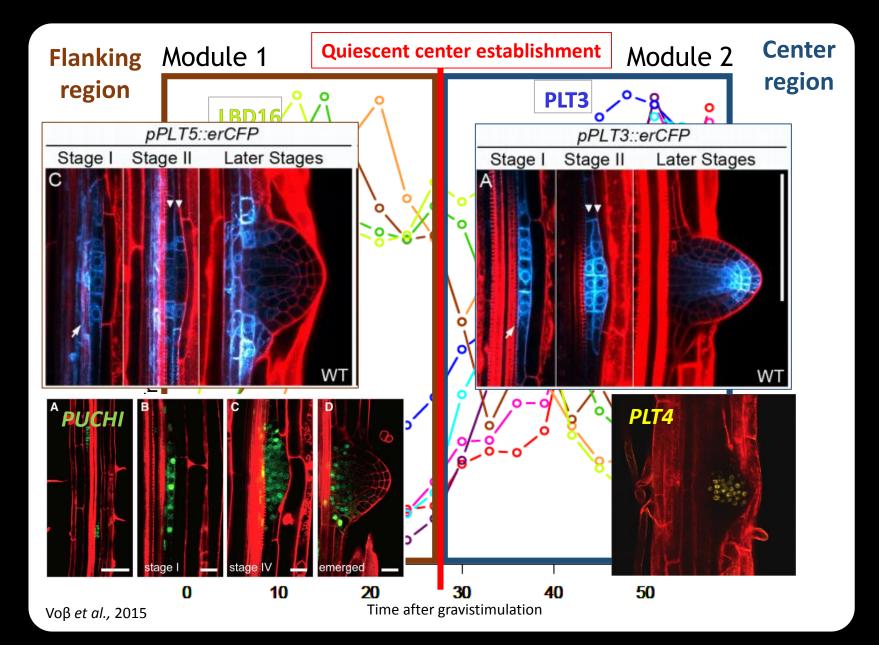
What are their expression profile like?

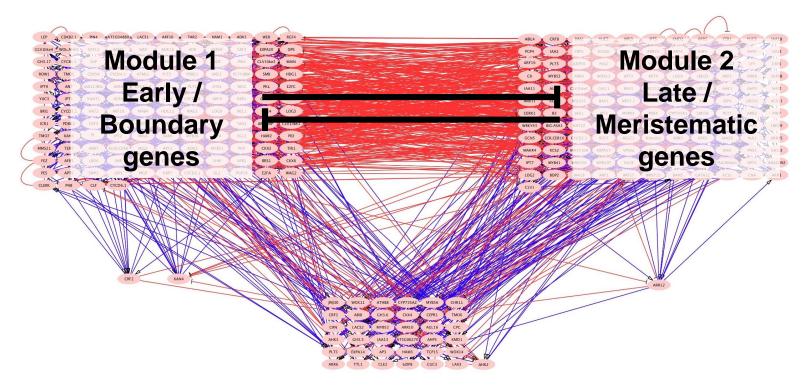




Voβ *et al.,* 2015





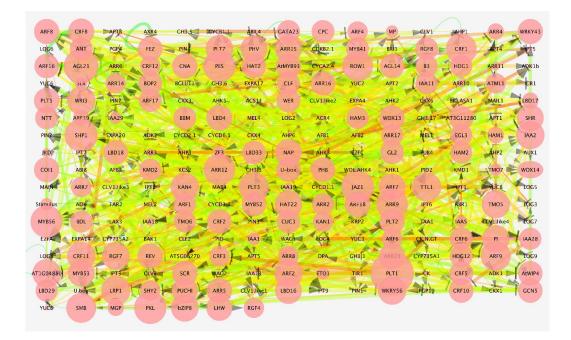


There appears to be biological meaning behing this modular topology

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.

#### Modeling GRN dynamics - PANTHEON

# PANTHEON

A Python -based Generic Boolean Network simulator

Based on Boolean formalism

Automatically model largescale genes network

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

#### Modeling GRN dynamics - PANTHEON

Initialize Network	
Import network	<u></u>
Gene List File	
	Browse
Network Structure File	
	Browse
Gene network Automated Initiation Algorithm (GAIA)	'- -1
Number of nodes	
Number of interactions	
Run mode	
Batch Computation Mode O Visual Network Mode	
Import Cancel	8 Batch Mode
	Input : Lis

<u>GUI</u> : no need to code to simulate your gene network behavior

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

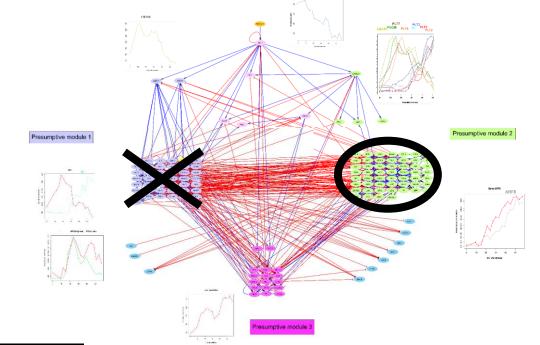
<u>Tools included :</u> in silico mutants study with a click among other things

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

h Mode		PROVIDENTS - Prove Reserve	
Input : List of genes		Input : Interactions in the network	Predict System Fates
AFB1		['ANT', '-1', 'IAA29']	Model type logical -
AFB2		['ANT', '-1', 'YUC2']	Boundary conditions transient
AFB3		['ANT', '1', 'CKX6']	Boundary conditions transient -
AHP6	- 11 <b>-</b>	['ANT', '1', 'CUC3']	Genes initial states random
ANT	Police	['ANT', '1', 'CYCD3.1'] Nouvelle	Number of initial states : O All possible states O Given number of states
ARF1		['ARF1', '-1', 'ARF1']	Number of Initial states : O All possible states O Given number of states
ARF16	Case st	[ARF1', '-1', 'EXPA14'] Chapadhees	KO mutation type none
ARF17 ARF18	_	['ARF1', '-1', 'GL2']	List of KO genes
ARF18 ARF19		['ARF1', '-1', 'PIN2'] ['ARF1', '-1', 'SCR']	
ARF2	Mast in	[ARF1, -1, SOR]	OA mutation type none
ARF4	(early e	['ARF16', '-1', 'GATA23']	List of OA genes
ARF6	repress	['ARF16', '-1', 'IAA11']	
ARF7		['ARF16', '-1', 'IAA29']	Run PYTHONIS
ARF8	L	['ARF16', '-1', 'LBD17']	
ARF9	LINE ST	['ARF16', '-1', 'PIN3']	Gene selverk Automated histoin Aporthin (GAA)
AT3G11280	6	['ARF17', '-1', 'AFB3']	Automatically Research Genes Of Significance——
ATML1		['ARF17', '-1', 'AXR4']	Number of iterations 1
AUX1		['ARF17', '-1', 'CDKB2.1']	Choose the models to run through ARGOS :
AXR4 BAK1		['ARF17', '-1', 'CKX6'] ['ARF17', '-1', 'CRF3']	
BBM	G	[ARF17, -1, CUC3]	logical  transient
3DL		['ARF17', '-1', 'EXPA20']	algebraic constant
BIG.ASA1	2	['ARF17', '-1', 'PHV']	
3RI1	(1) S	['ARF17', '-1', 'PLT3']	
CDKB2.1	Webster 1	['ARF17', '1', 'BAK1']	Run ARGOS
CKX6		['ARF17', '1', 'IAA2']	- Ruit ARGOS
DLF		['ARF18', '-1', 'ARF7']	
CLV1		['ARF18', '-1', 'IAA28']	
CLV2	uter des co	['ARF18', '-1', 'IAA5']	Challenge Fates
CNA CRF1		['ARF18', '-1', 'LBD17']	Placeholder - WIP algo
CRF2		['ARF19', '-1', 'CLF'] ['ARF19', '1', 'AUX1']	Current Fate :
CRF3		[ARF19', 1', ADA1]	Targeted Fate :
CUC3	-	['ARF2', '-1', 'AHP6']	Targeled Fale .
			Run APOLLO
ract core network :	Run HEI	PHAISTOS	
ick to files import	Exit		

### 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

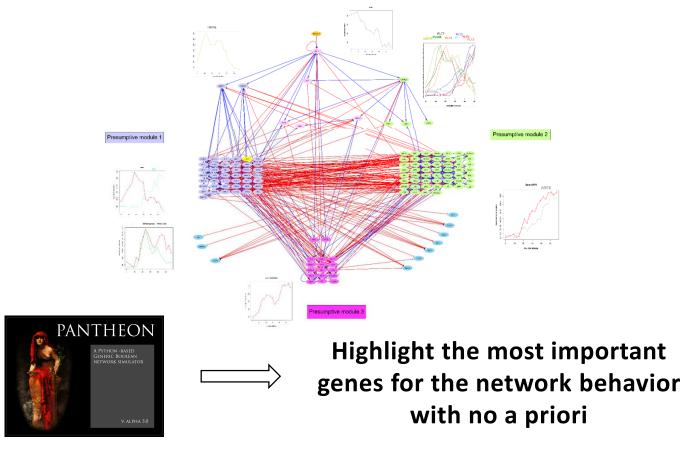




Prediction of majority stable state corresponding to meristematic state (genes from module 2 active, genes from module 1 inactive)

#### 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)

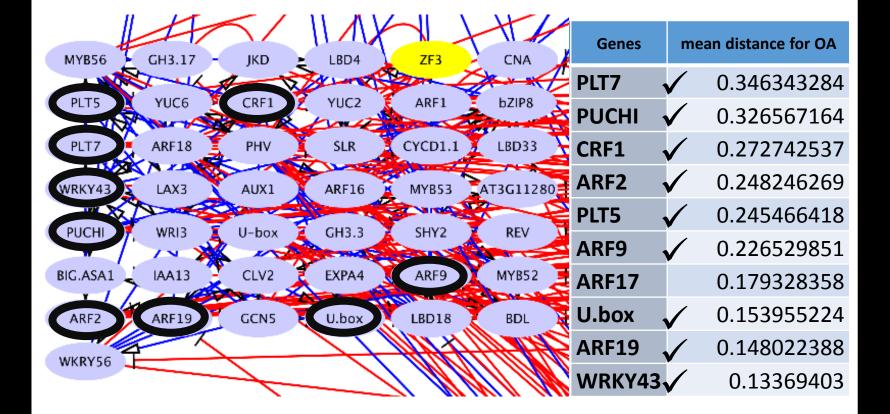


ARGOS Module - Mass *in-silico* mutagenesis and computation of a score of impact on network behavior for each gene

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
РНВ	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

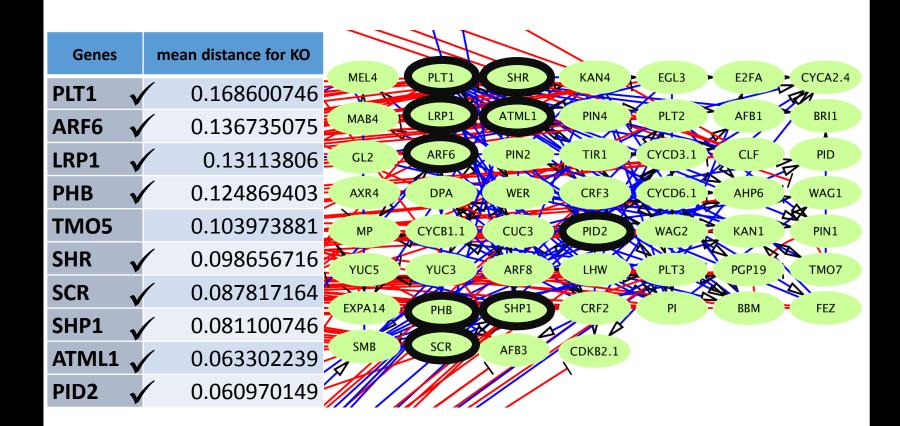
TOP10 predicted as most significant genes when KO or OA

#### PANTHEON - the LR dataset case study



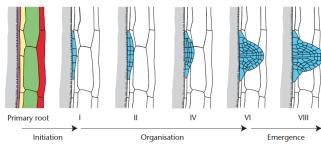
Most impactful genes when OA are in module 1 (early genes which we need to be repressed later on)

#### PANTHEON - the LR dataset case study



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

### LRP morphogenesis - In summary



📕 Epidermis 🔲 Cortex 🔲 Endodermis 🔲 Pericycle 🔲 Stele

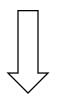
Time after gravistimulation



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

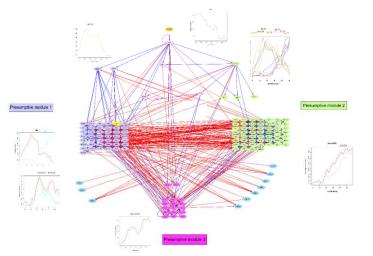


Creation of the LRP database covering the full development of the organ



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 a 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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