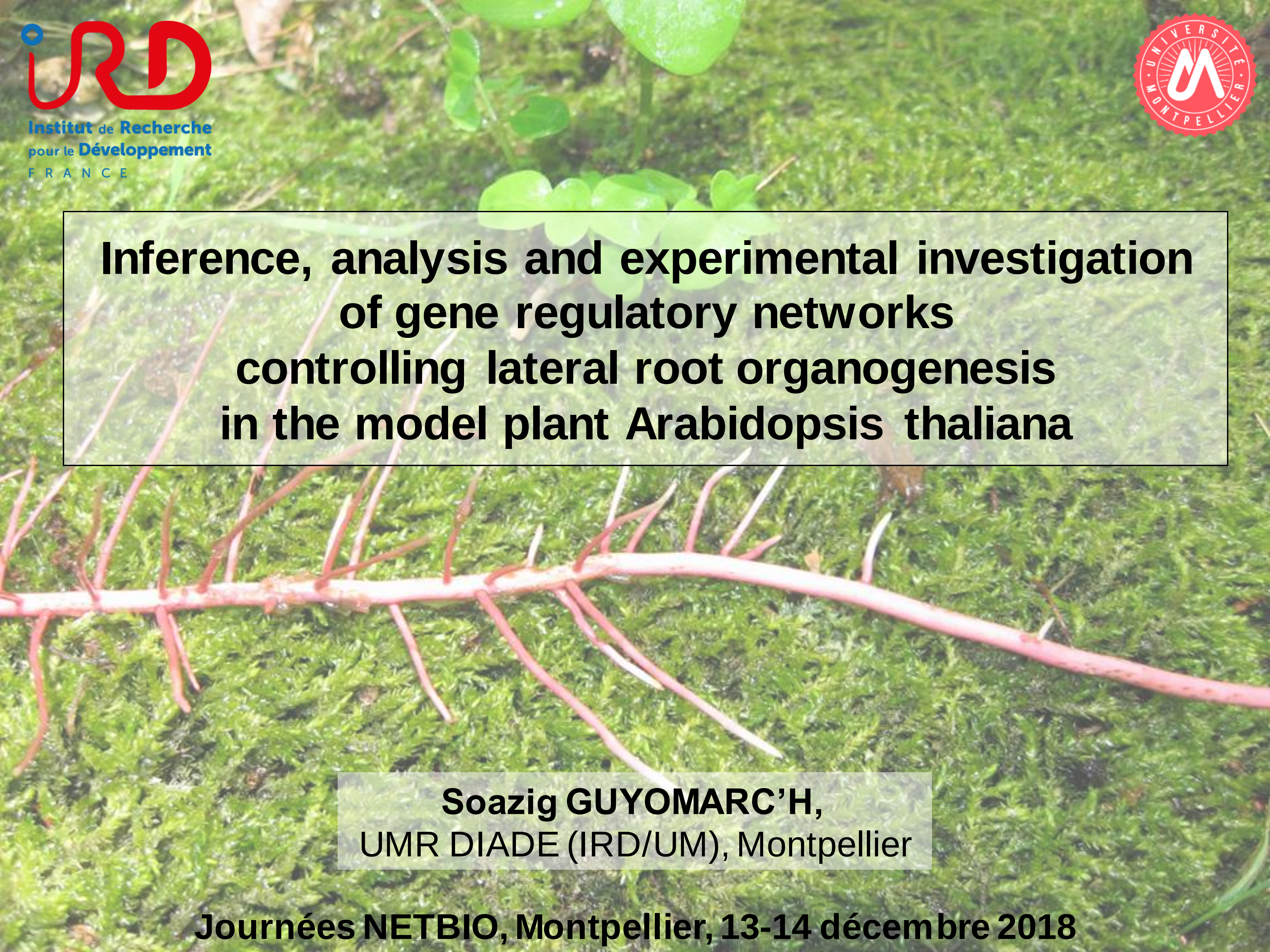

Institut de Recherche pour le Développement
FRANCE

Inference, analysis and experimental investigation of gene regulatory networks controlling lateral root organogenesis in the model plant Arabidopsis thaliana

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UMR DIADE (IRD/UM), Montpellier

Journées NETBIO, Montpellier, 13-14 décembre 2018

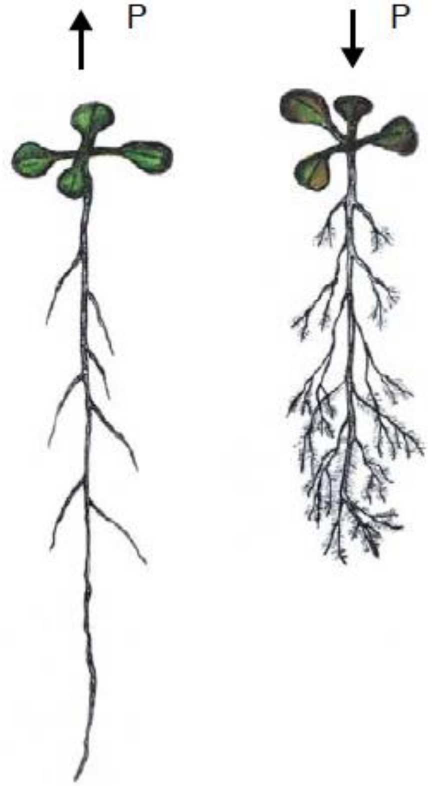
Root systems fulfills important agronomic functions

Excavated root system of an Eastern Gamagrass plant. Keith Weller, Ag Research Magazine, USDA Agricultural Research Service

- anchorage
- nutrition
- synthesis and storage
- interactions with microorganisms

Plasticity of root system development

- Balance between efficient soil exploration, water and ions capture, and carbon and energy cost.
- Integrated regulation by local and systemic signals
- Regulation of root growth, tropism, and branching



Lopez-Bucio et al., 2003

Plasticity of root system development

- Balance between efficient soil exploration, water and ions capture, and carbon and energy cost.
- Integrated regulation by local and systemic signals
- Regulation of root growth, tropism, and branching
- Modulation of:

  * Primary root growth

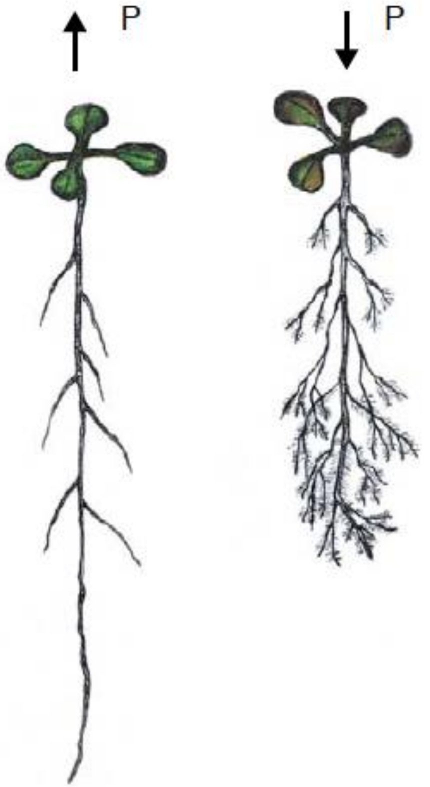
  (*root apical meristem*)

  * Lateral root development

  (*lateral root primordia*)

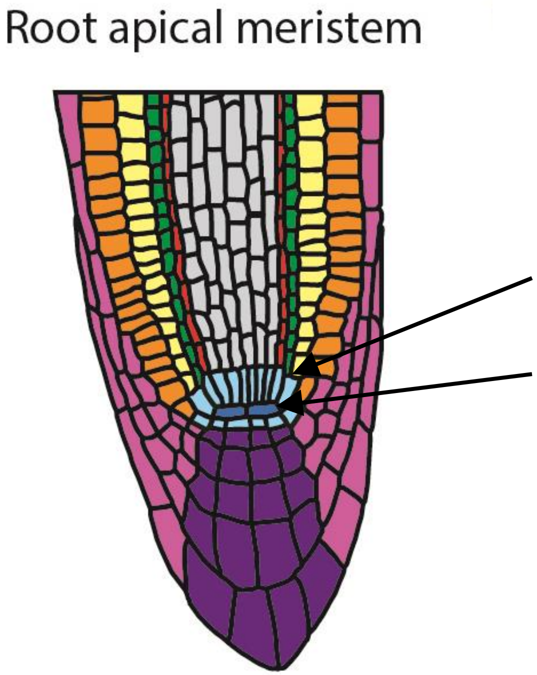
  * Adventitious root formation

  (e.g. crown roots in cereals)



Lopez-Bucio et al., 2003

The root apical meristem generates root primary tissues

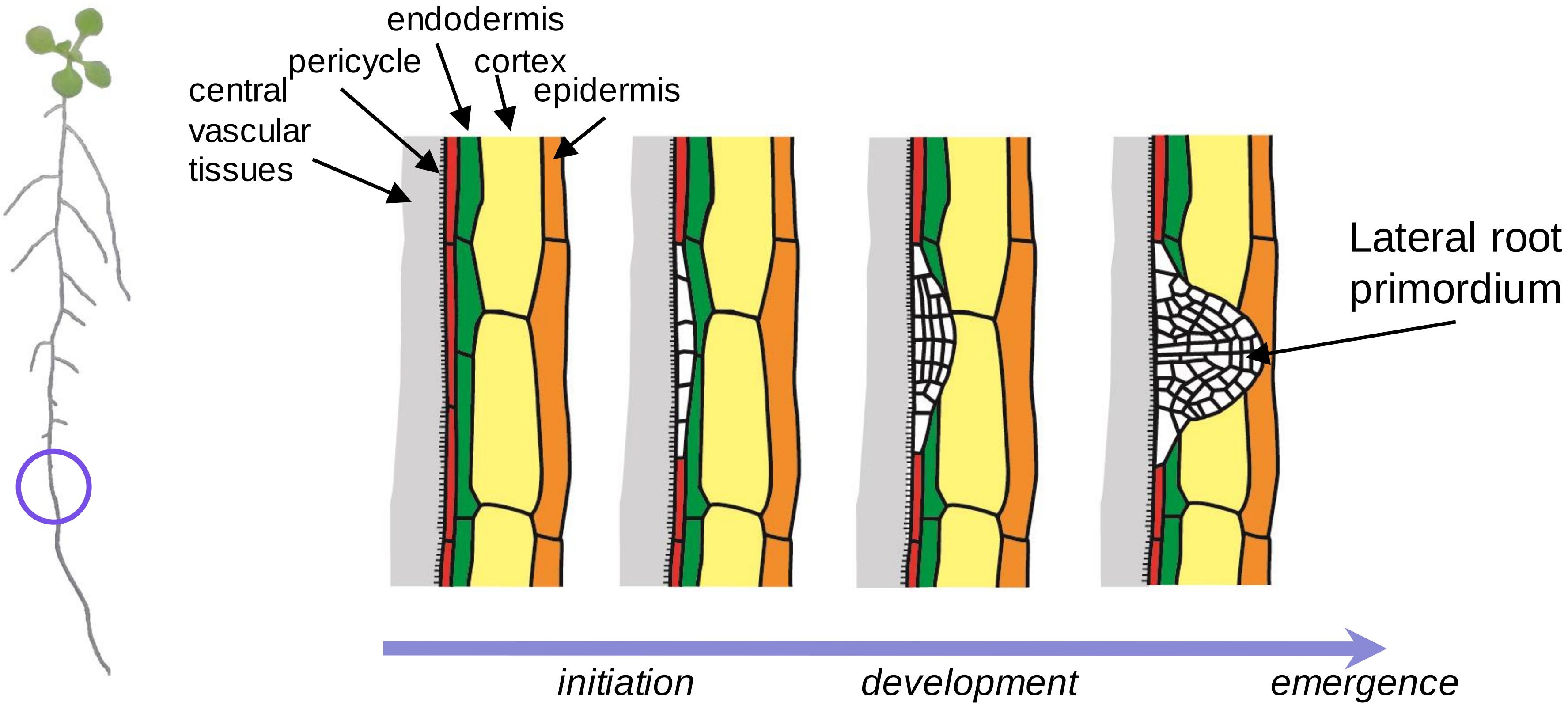


Stem cell niche

Organising Center (termed “Quiescent Center”)

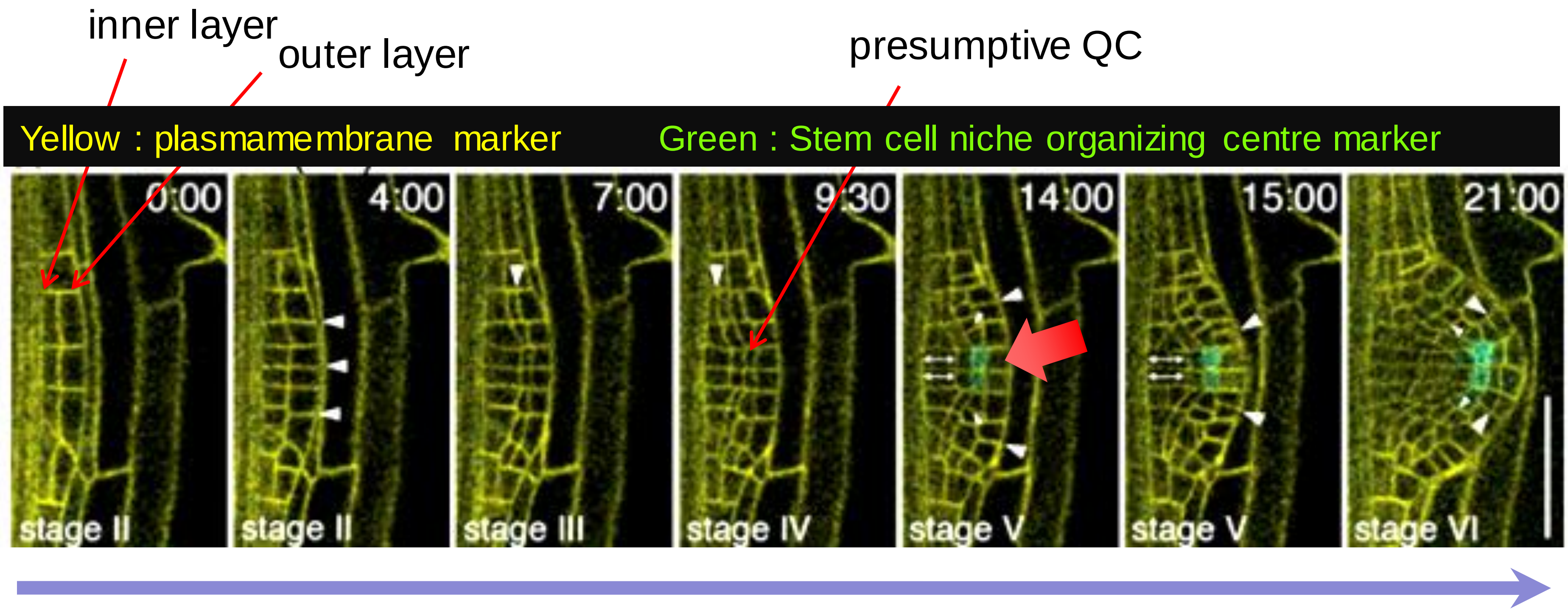
- Primary anatomical organization of roots is stereotyped.
- Root meristem organized around a central stem cell niche
- *Arabidopsis thaliana* as a simple plant model.

Root branching consists in organogenesis of a lateral root primordium…



- Selected pericycle cells resume cell division ⇒ organogenesis
- New axis of growth, distinction flank cells/ central cells…. : patterning
- *De novo* organisation of a root meristem

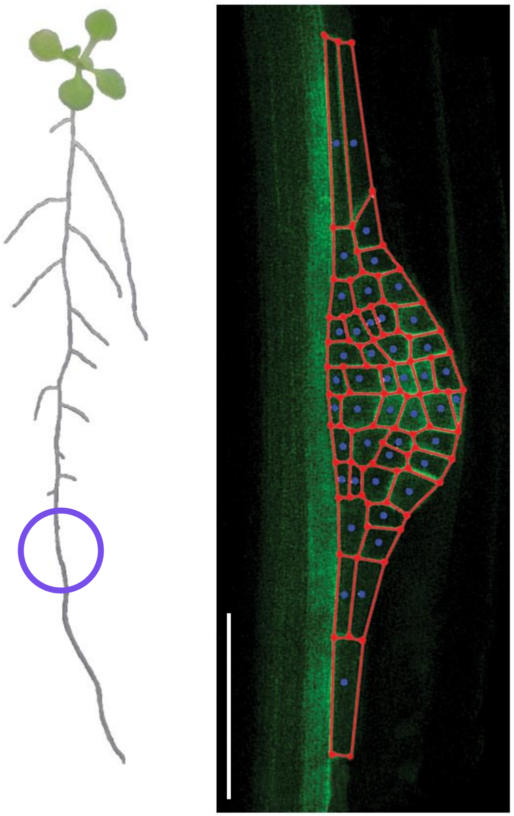
… which progressively organises into a new root apical meristem

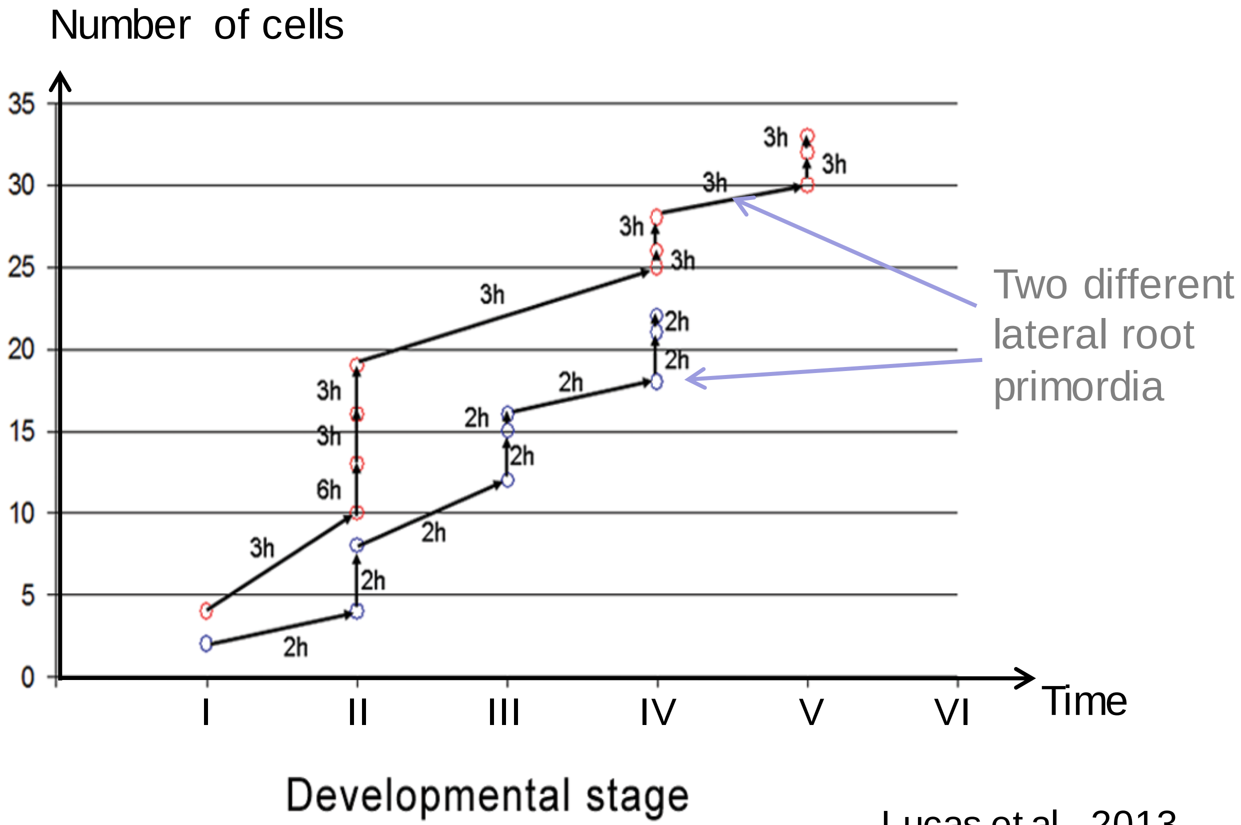


Goh et al., 2016

- Dynamic LRP functional patterning:
  - inner layer or outer layer specific-gene expression as early as stage II
  - expression of root stem cell niche marker genes at stage IV/V

Cellular organization of the LRP is not fixed





Two different lateral root primordia

Lucas et al., 2013

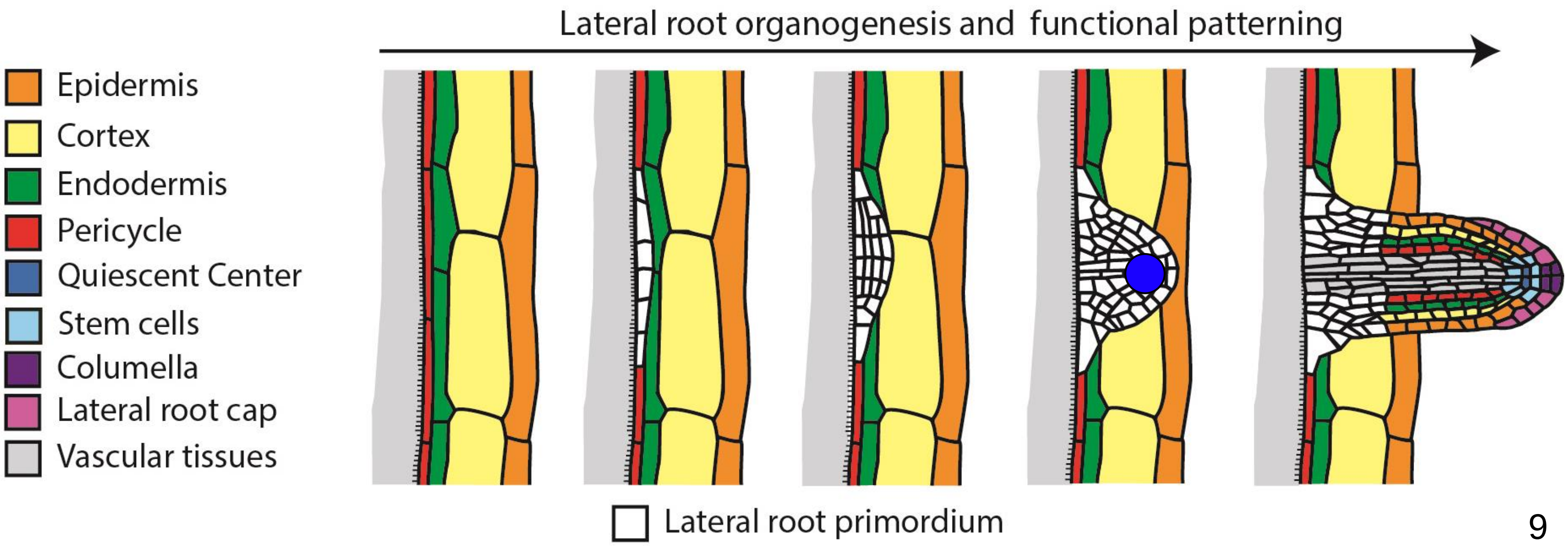
- ➢ Frequencies and locations of cell divisions are not stereotyped
- ➢ Still, the outcome is the organization of a functional root apical meristem.
- ➢ Misoriented cell division planes do not preclude functional LR formation.

What mechanisms control the functional patterning of the lateral root primordium ?

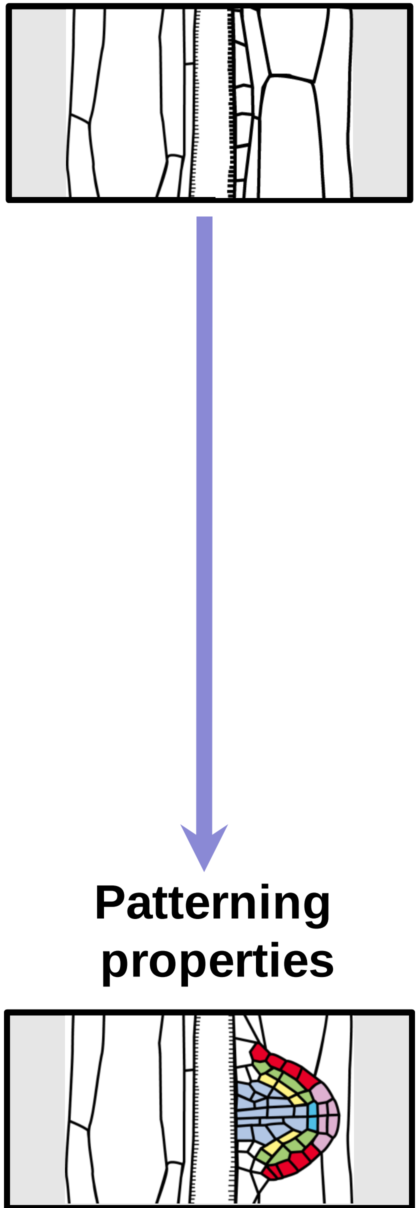
➢ Despite conserved landmarks, LRP development does not rely on a strictly controlled cell division pattern (Lucas et al., 2013; Van Damme et al., 2011)

➢ What are the mechanisms controlling cell fate and functional patterning in the developing lateral root primordium?

Focus on gene network patterning properties

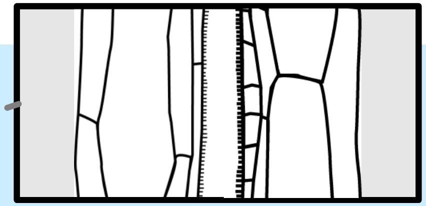


A systems biology approach of the gene regulatory network operating during LRP development

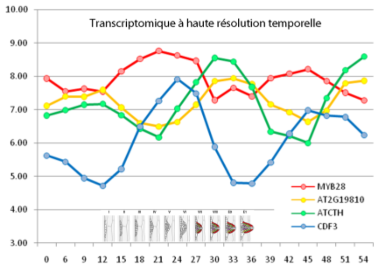


Patterning properties

A systems biology approach of the gene regulatory network operating during LRP development

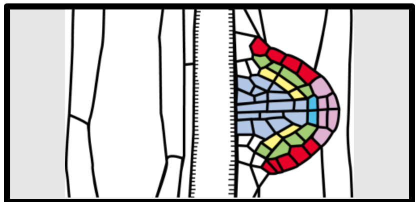


Time course transcriptomics



Lucas et al., 2008
Voss et al., 2015

Patterning properties



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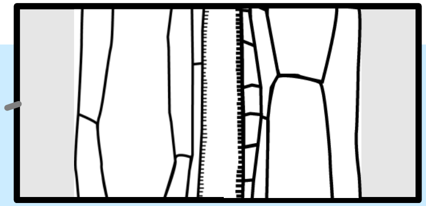
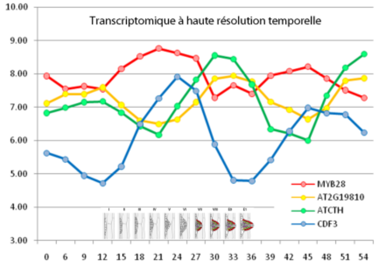


Time-course transcriptomics



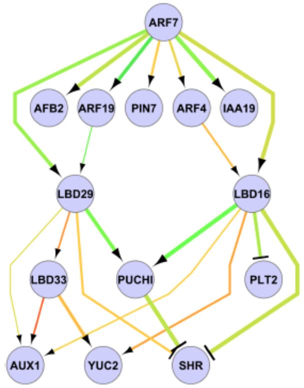
Gene network inference

$$\overline{Y}(t) \approx a.\overline{X}(t-\mu)+b$$

$$Q1 = \mathrm{cor}_{\mathcal{P}}(R^{1..18}, T_2^{1..18})$$

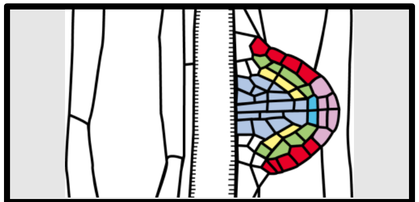
Lavenus et al., 2015

Gene network

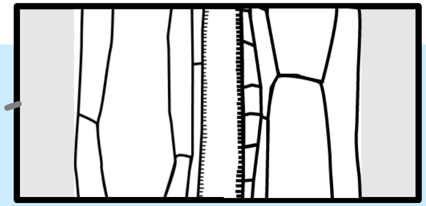


250 genes
>1000 interactions

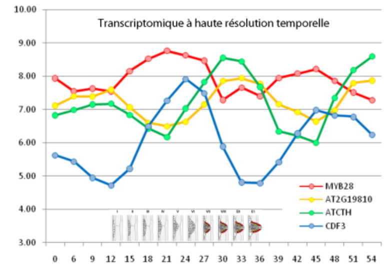
Patterning properties



A systems biology approach of the gene regulatory network operating during LRP development



Time course transcriptomics



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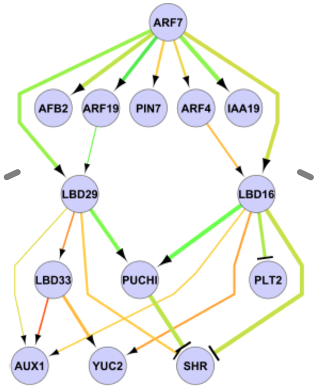
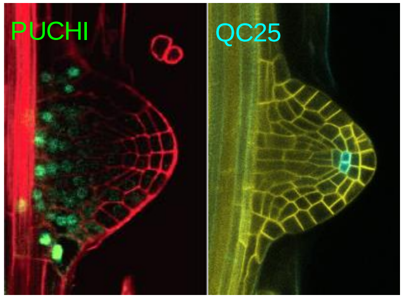
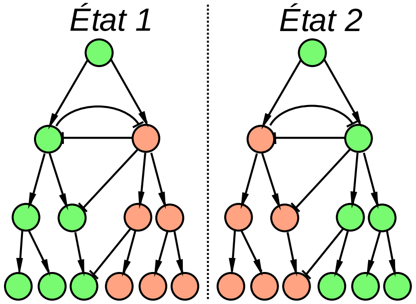


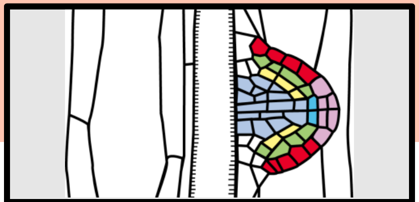
Spatialization



Formal analysis



Patterning properties

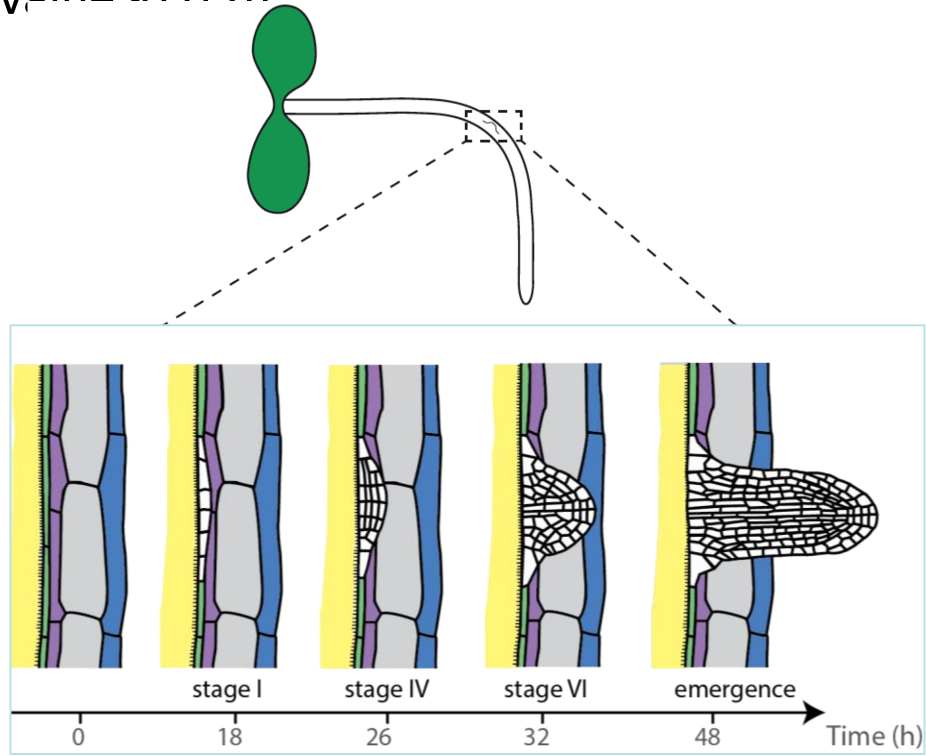


What is the global genetic system controlling lateral root formation ?

Inference of the gene network operating during LRP development

Inference of the gene regulatory network involved in lateral root primordium development

- First step : an ATH1 Affymetrix microarray-based transcriptomic dataset of lateral root formation, 54h long with 3h-time step, using synchronized gravistimulated root bends (8 000 genes differentially expressed (t-test, q value>0.05))



Lucas et al., 2008; Péret et al., 2012; Lavenus et al., 2015

Inference of the gene regulatory network involved in lateral root primordium development

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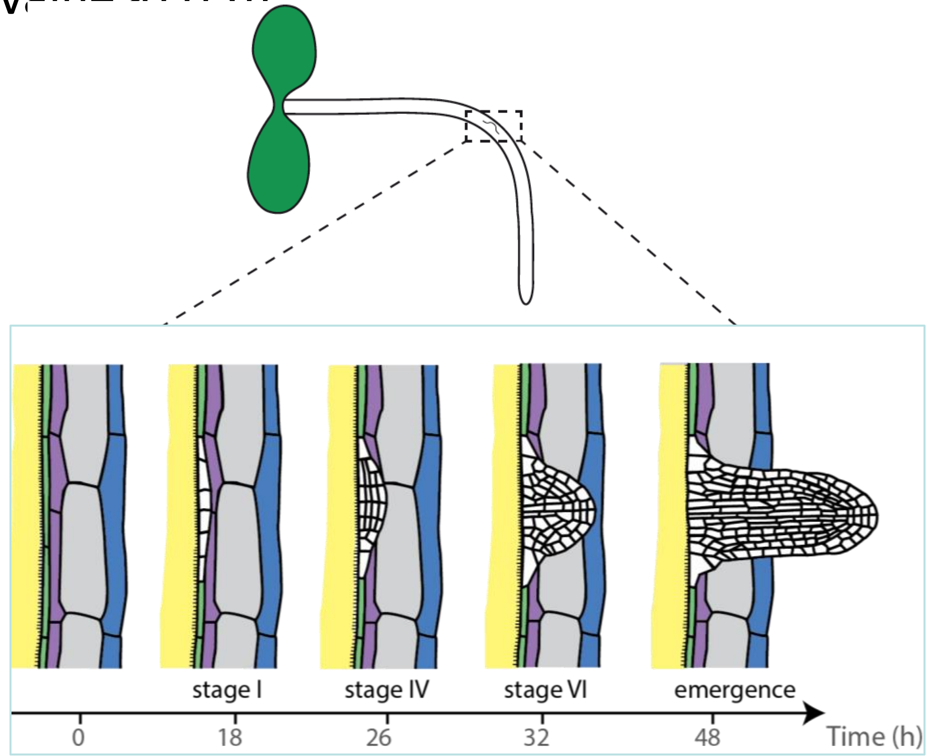
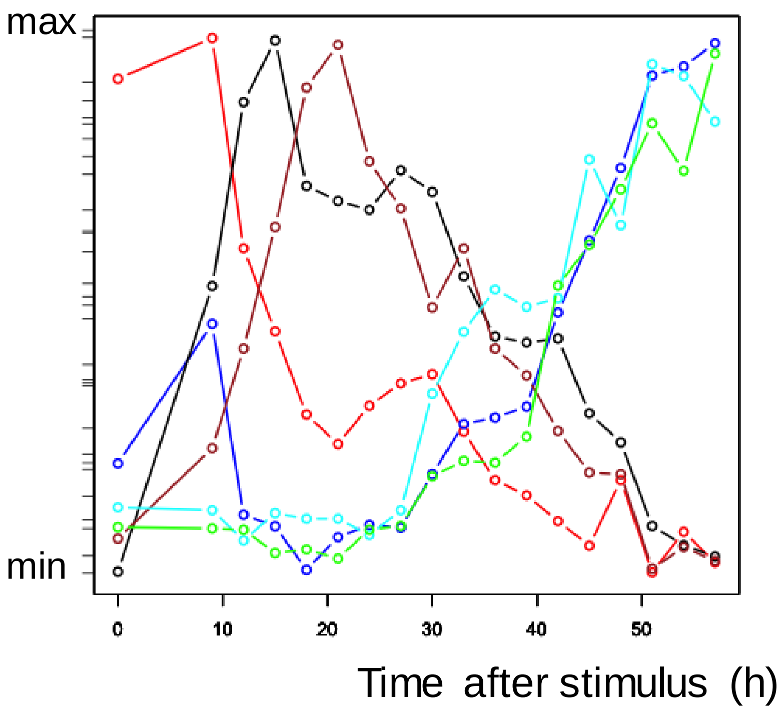
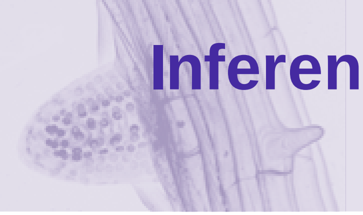


Transcript accumulation of gene of interest



Lucas et al., 2008; Péret et al., 2012; Lavenus et al., 2015



Inference of the gene regulatory network involved in lateral root primordium development

- Second step : gene network inference based on this transcriptomic dataset and focused on a core list of genes of interest (TDCor algorithm, Lavenus et al., 2015)

Search for expression profiles correlated *with delay*

⇒ **Correlation with delay could be due to expression of gene X being regulated by the product of gene Y**

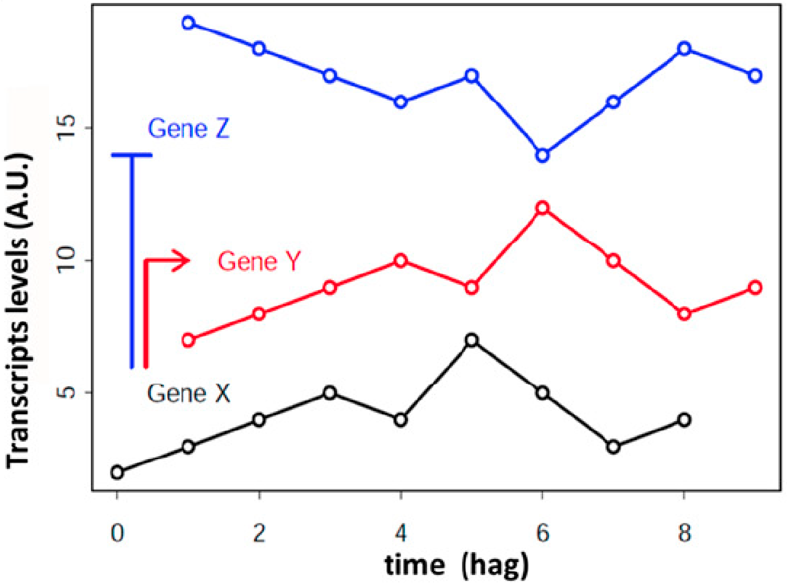
Computation of quality indexes whose probability distribution depends on topology

⇒ **Prediction of the most probable topology out of multiple co-regulated genes**

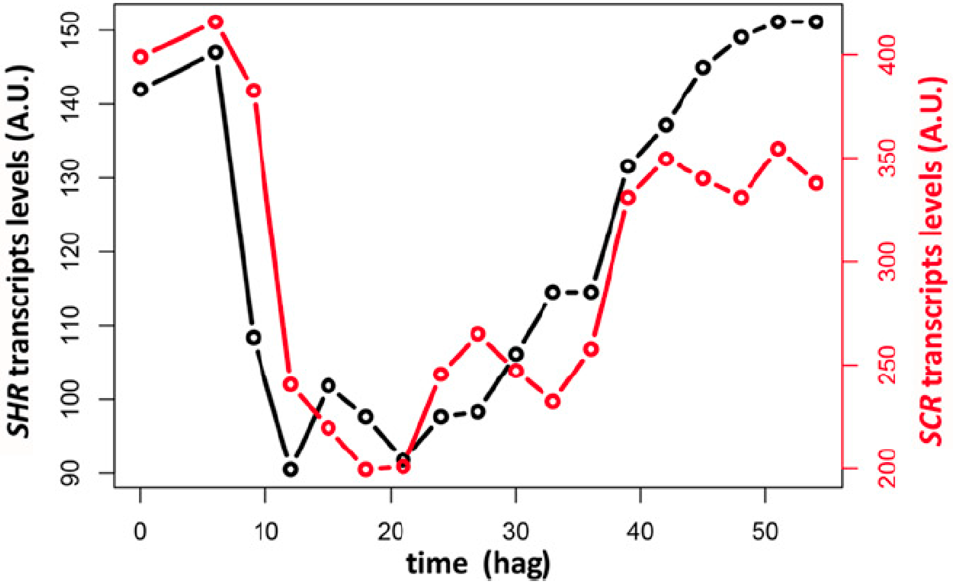
Lavenus et al., Plant Cell 2015

Linear correlation between two profiles could be due to genetic regulation

Gene expression (mRNA, a. u.)



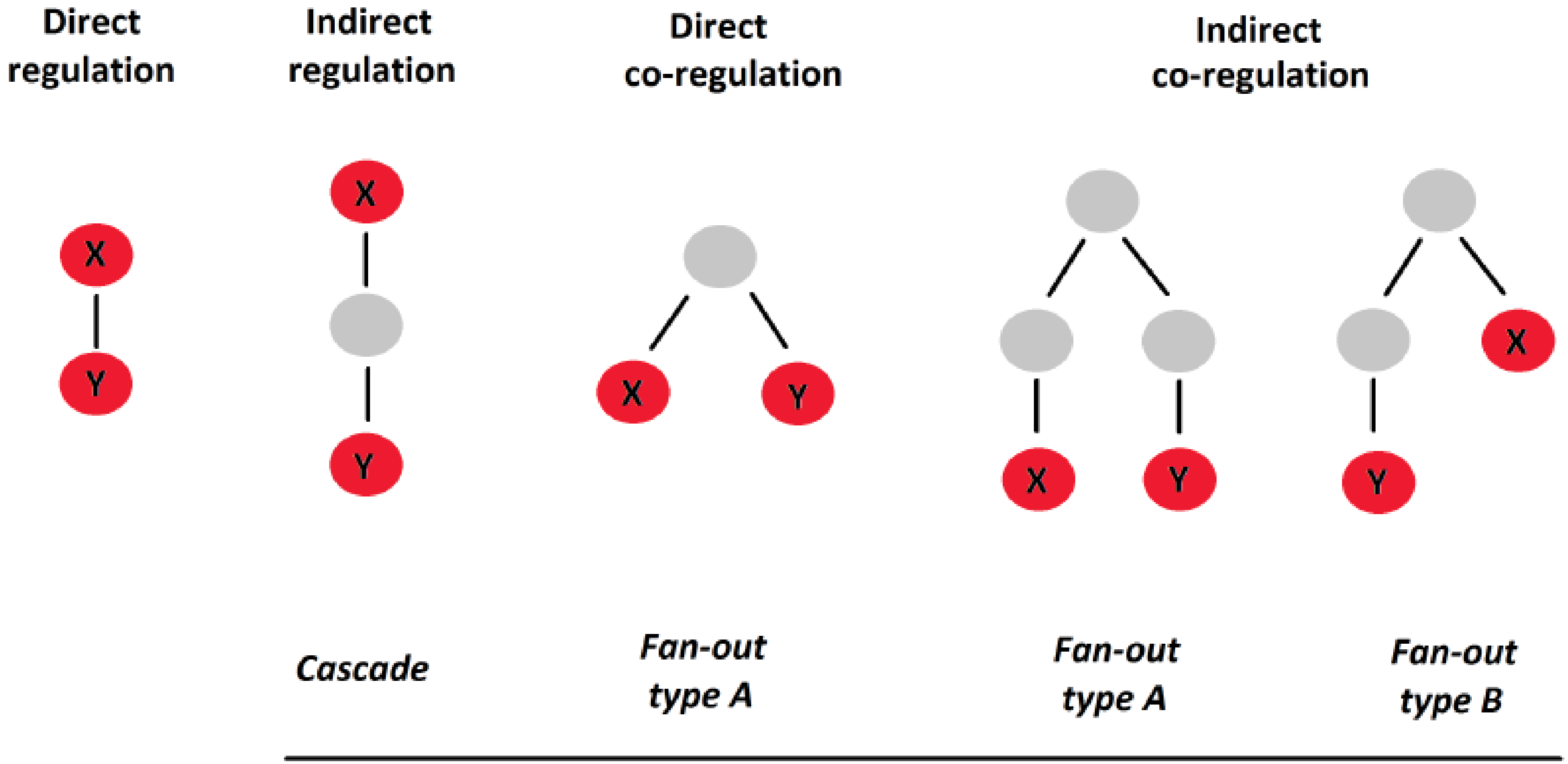
Gene expression (mRNA, a. u.)



- Calculation of Pearson's correlation coefficient between the two profiles, one shifted in time compared to the other
- Calculation of time delay for highest Pearson's correlation coefficient

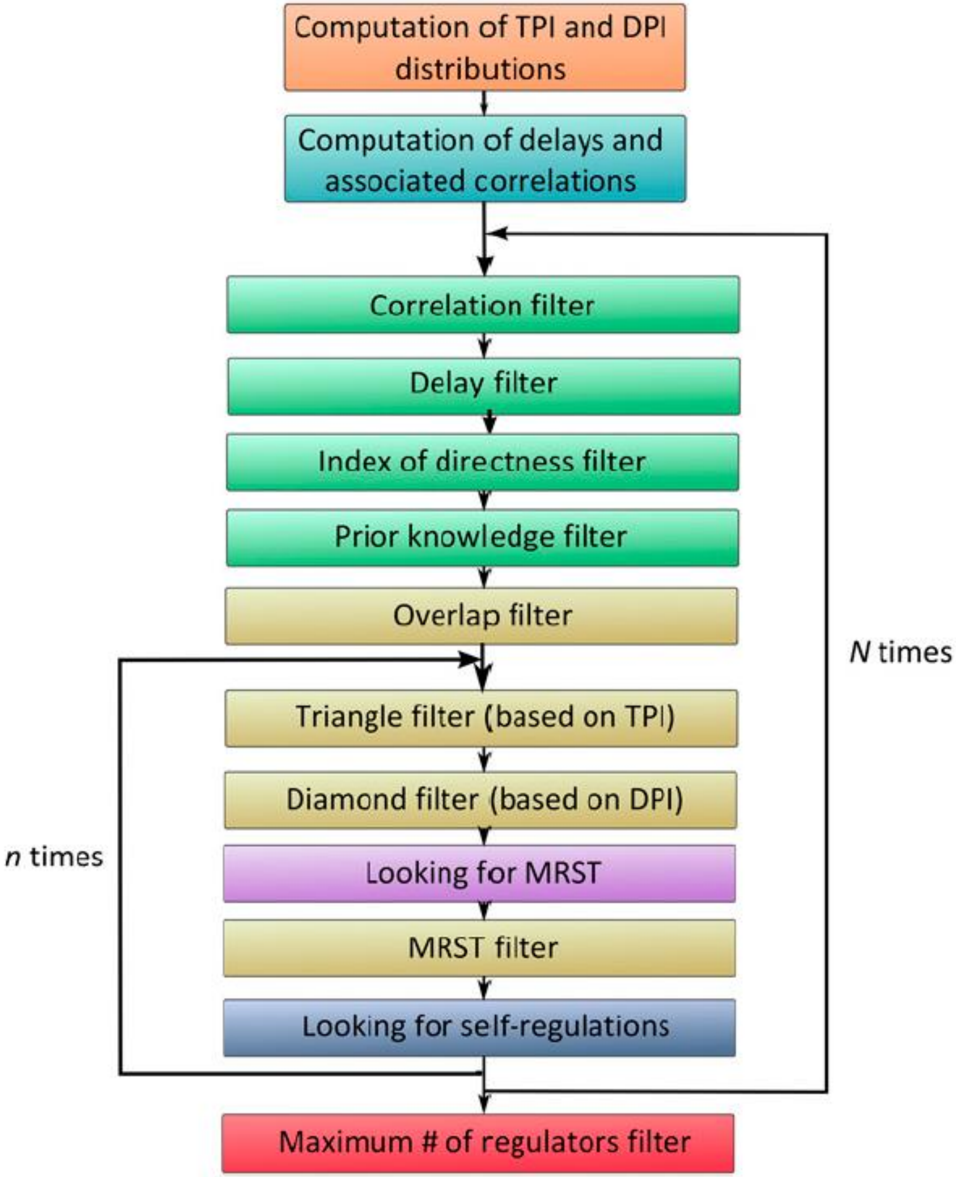
Lavenus et al., Plant Cell 2015

Several network topologies could explain profile correlation



Lavenus et al., Plant Cell 2015

Statistical filters allow selecting most probable topologies



Computation of TPI and DPI distributions

Computation of delays and associated correlations

Correlation filter

Delay filter

Index of directness filter

Prior knowledge filter

Overlap filter

Triangle filter (based on TPI)

Diamond filter (based on DPI)

Looking for MRST

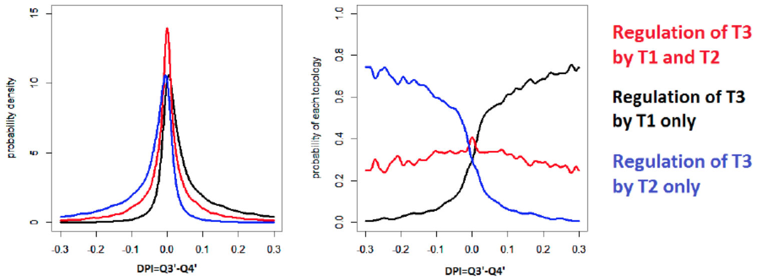
MRST filter

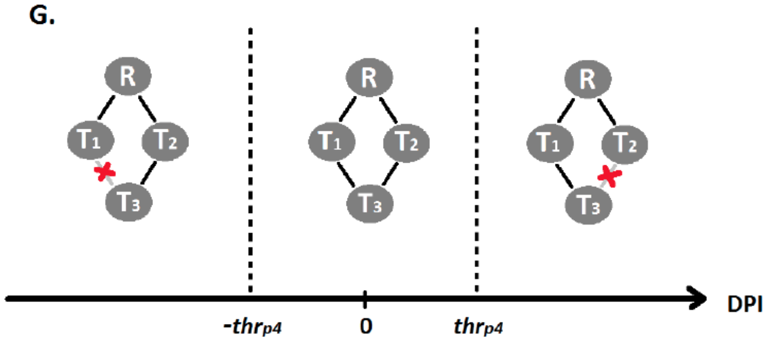
Looking for self-regulations

Maximum # of regulators filter

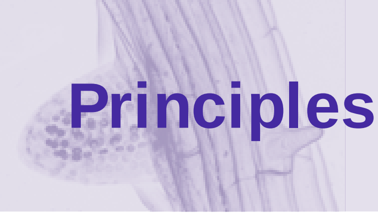
TPI= Triangle Pruning Index

DPI= Diamond Pruning Index



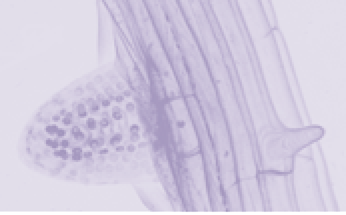


Lavenus et al., Plant Cell 2015



Principles of the gene network inference algorithm TDCor

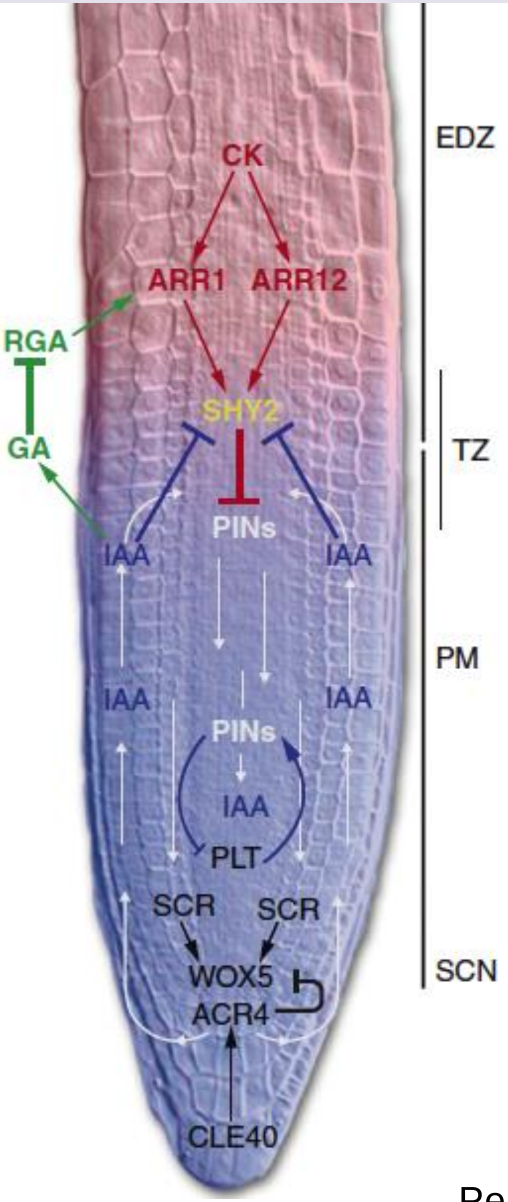
- The TDCor algorithm (Lavenus et al. 2015)
  - Runs on expression profiles extracted from the LR dataset (or any other transcriptomic kinetics dataset)
  - Looks for non-combinatorial linear interactions
  - Assumes consistent regulation relationship throughout the sample
  - Uses Pearson’s correlation to produce a raw preliminary network
  - Uses various filters to eliminate false positive and refine the network topology



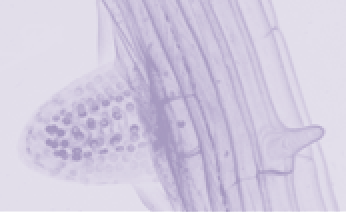
Selection of genes of interest

- 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 …)

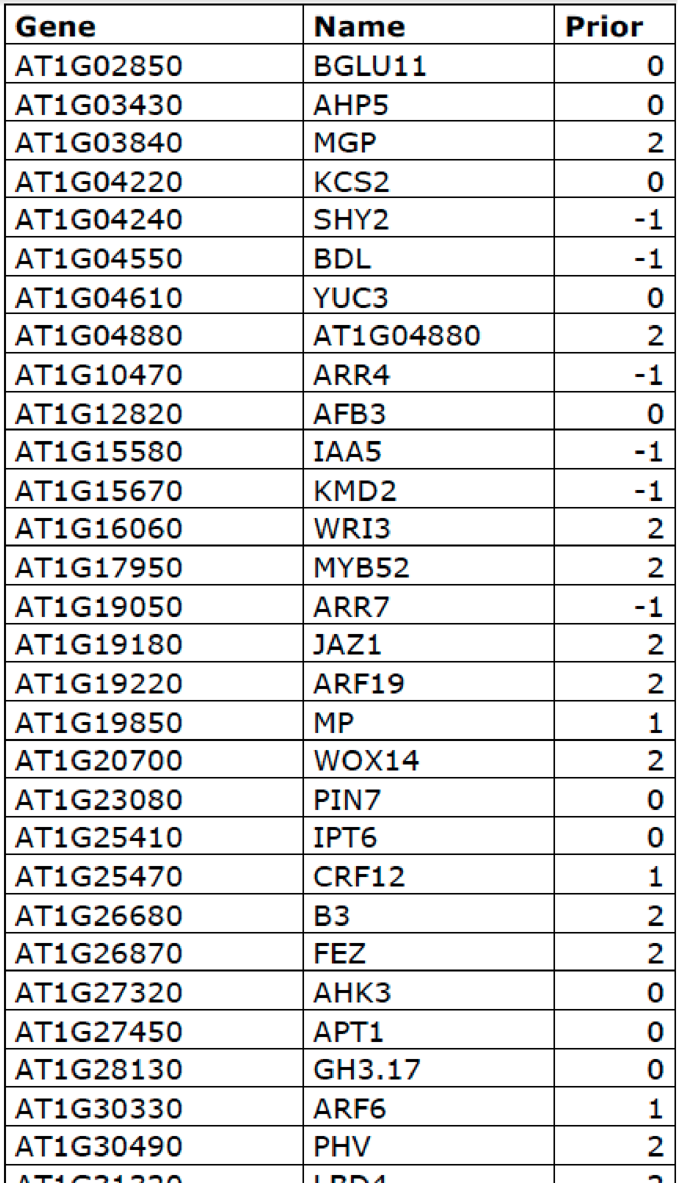


Perilli et al. 2012



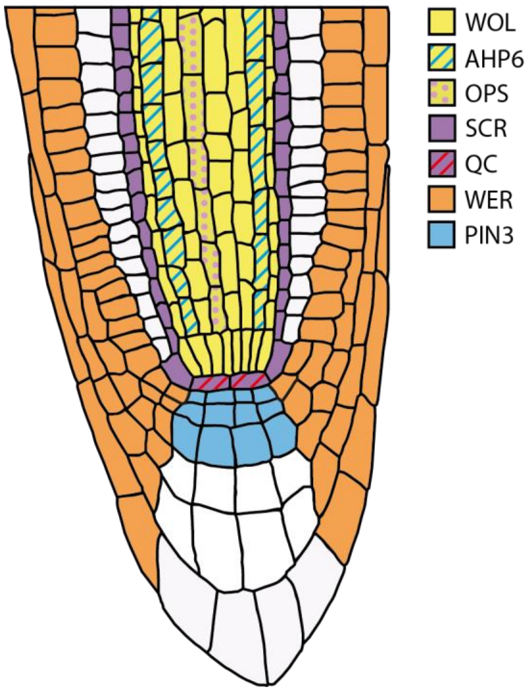
Selection of genes of interest

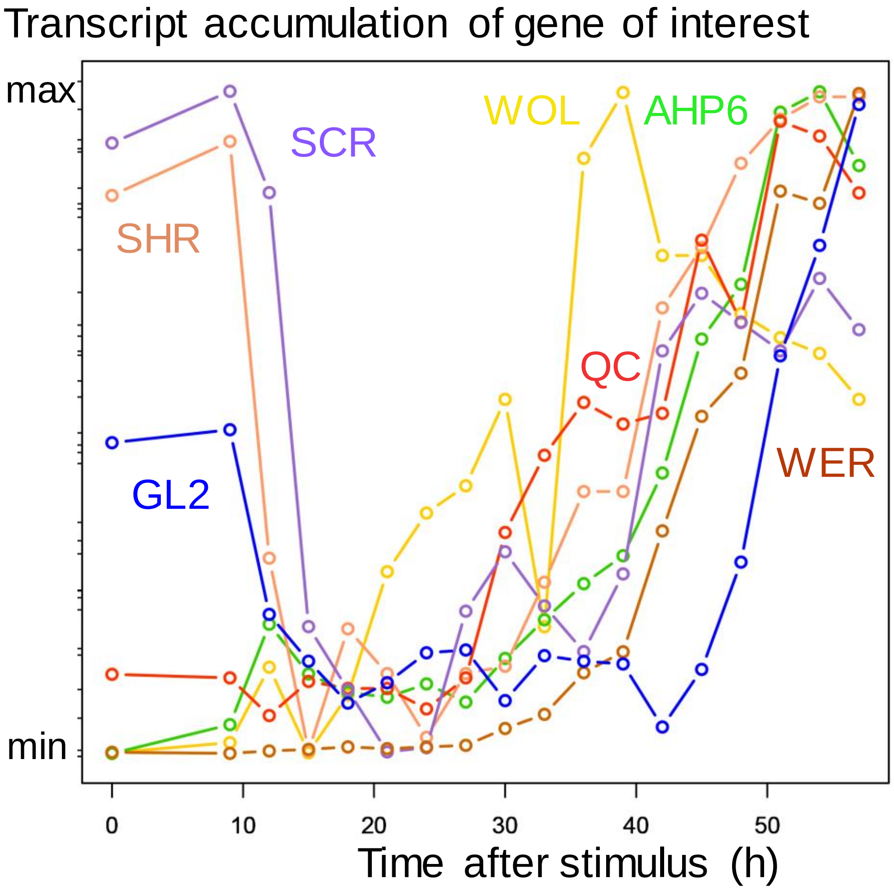
- A list of 261 genes
- Not only transcription factors
- A “prior” data is given to each gene, based on the litterature, to indicate if transcriptional regulation activity has been reported or not
- This “prior” information helps the inference procedure by authorizing or not the algorithm to draw outward 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	AHK3	0
AT1G27450	APT1	0
AT1G28130	GH3.17	0
AT1G30330	ARF6	1
AT1G30490	PHV	2

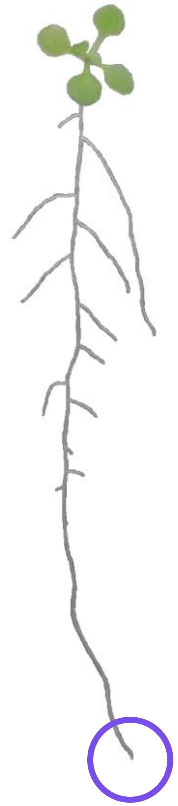
A number of marker genes for root meristem cell identities

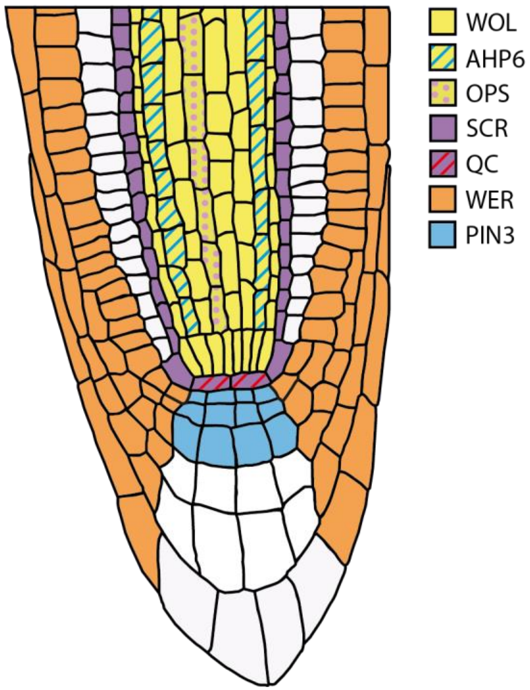


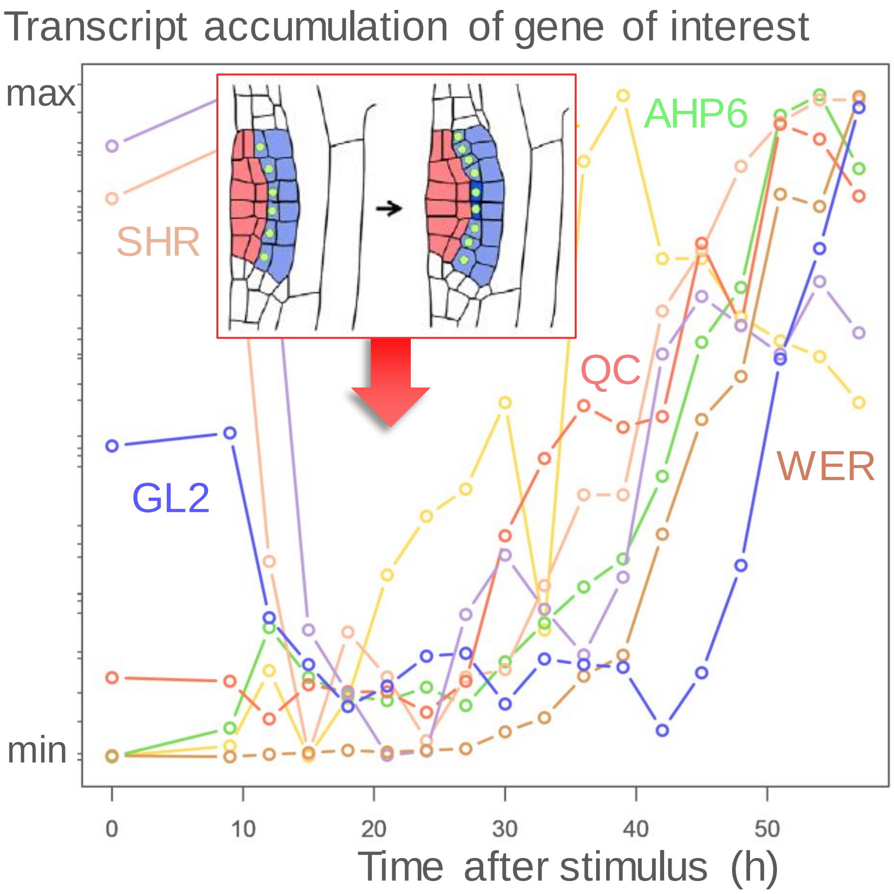


- Meristem genes are differentially expressed in the LR formation dataset

A number of marker genes for root meristem cell identities

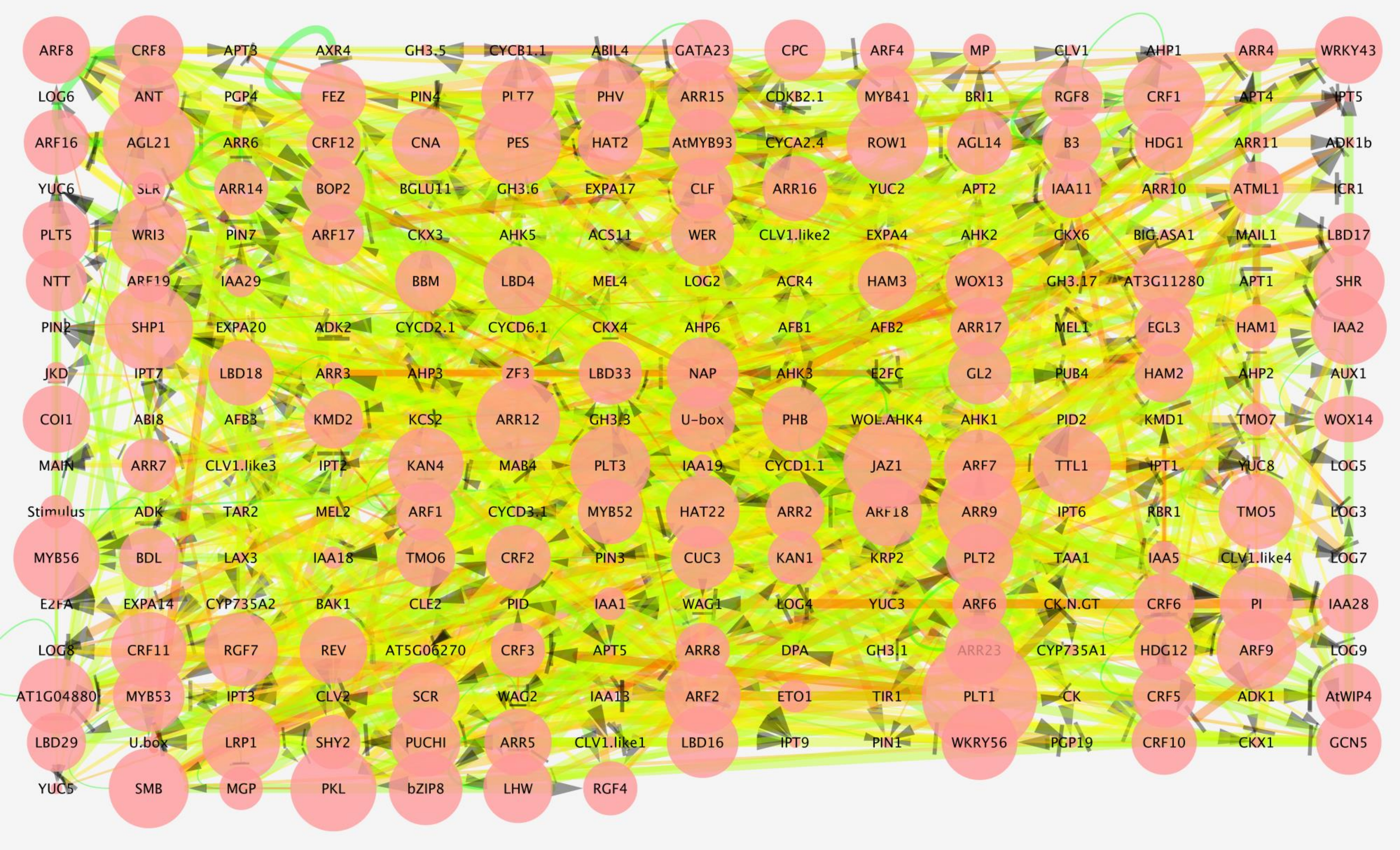






- ➢ Meristem genes are differentially expressed in the LR formation dataset
- ➢ Onset of meristem genes induction coincides with a major transition in lateral root primordium formation

TDCor output from the 261-gene list : a 247-gene network



Node diameter = outdegree (number of targets), edge width = interaction bootstrap, edge color=estimated index of directness

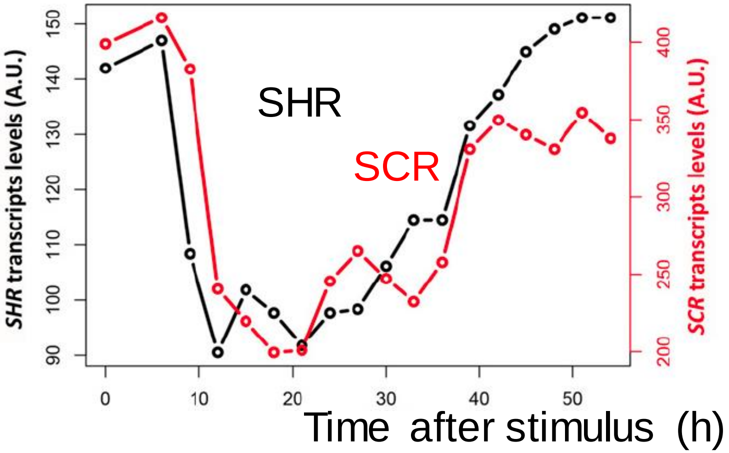
Based on the 261 gene list and the LR transcriptomic dataset, TDCor predicts a 247-gene regulatory network

- Based on the 261 gene list and the LR transcriptomic dataset, TDCor predicts a 247-gene regulatory network containing 1069 predicted interactions

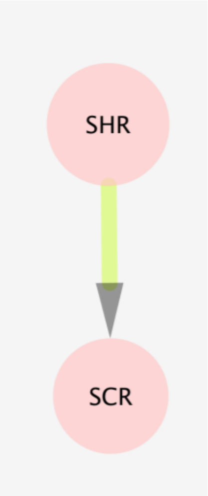
- Genes that do no appear in the network are **either not present** on the Affymetrix microarray, **or not differentially expressed** in the LR transcriptomic dataset, or not linked by any predicted interaction

- Predicted edges can correspond to a **direct or indirect** regulation relationship

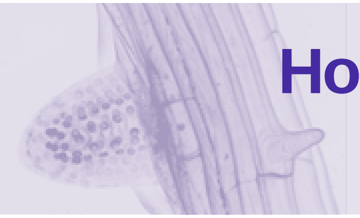
Transcript accumulation



SHR and SCR experimental profile in the LR dataset



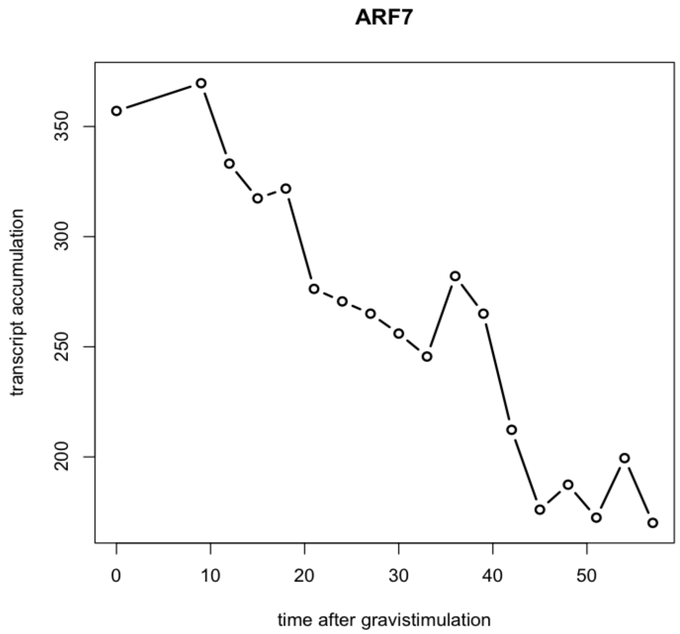
Inference by TDcor algorithm



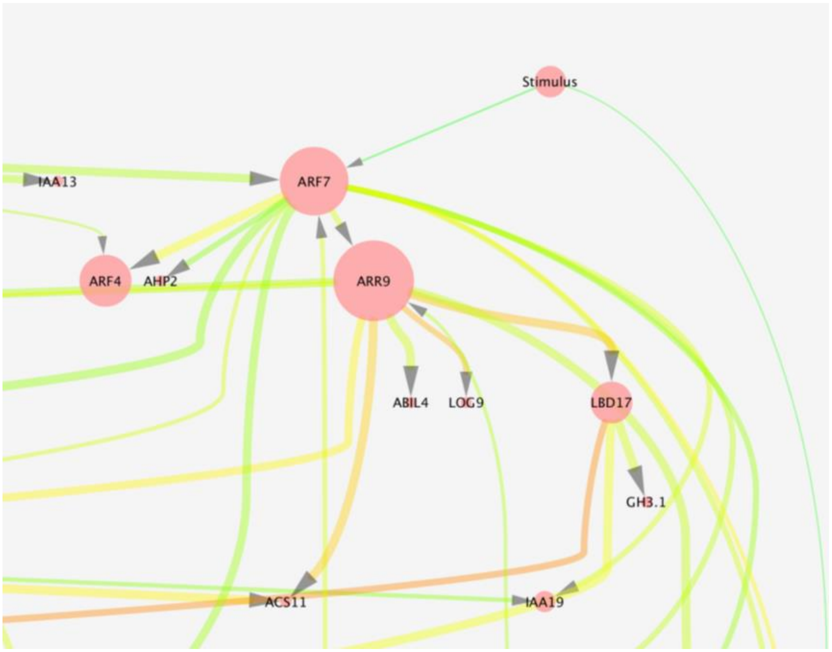
How reliable is this predicted topology of the LR formation network ?

Confrontation of ARF7 predicted targets to experimental data

What confidence to give to this prediction ?
The case of the ARF7 transcription factor



ARF7 experimental profile in the LR data set transcriptomic profile



Inference by TDcor algorithm

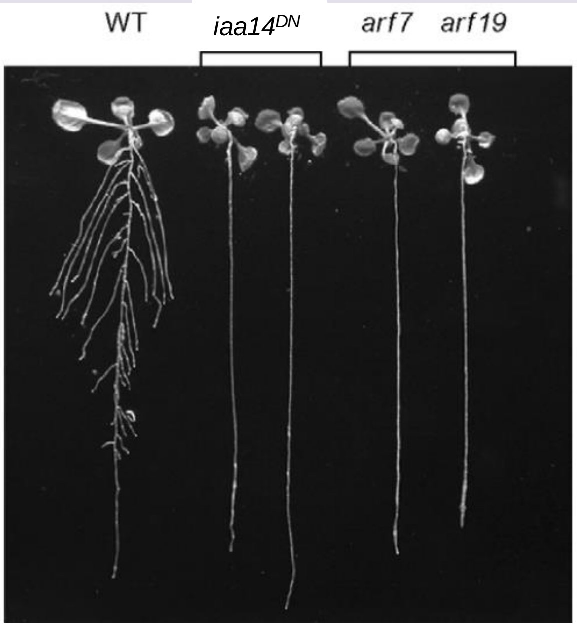
- ARF7 is predicted to occupy a upstream position in the network
- ARF7 is predicted to positively regulate a LOB/PUCHI genetic module

Lavenus et al., 2013

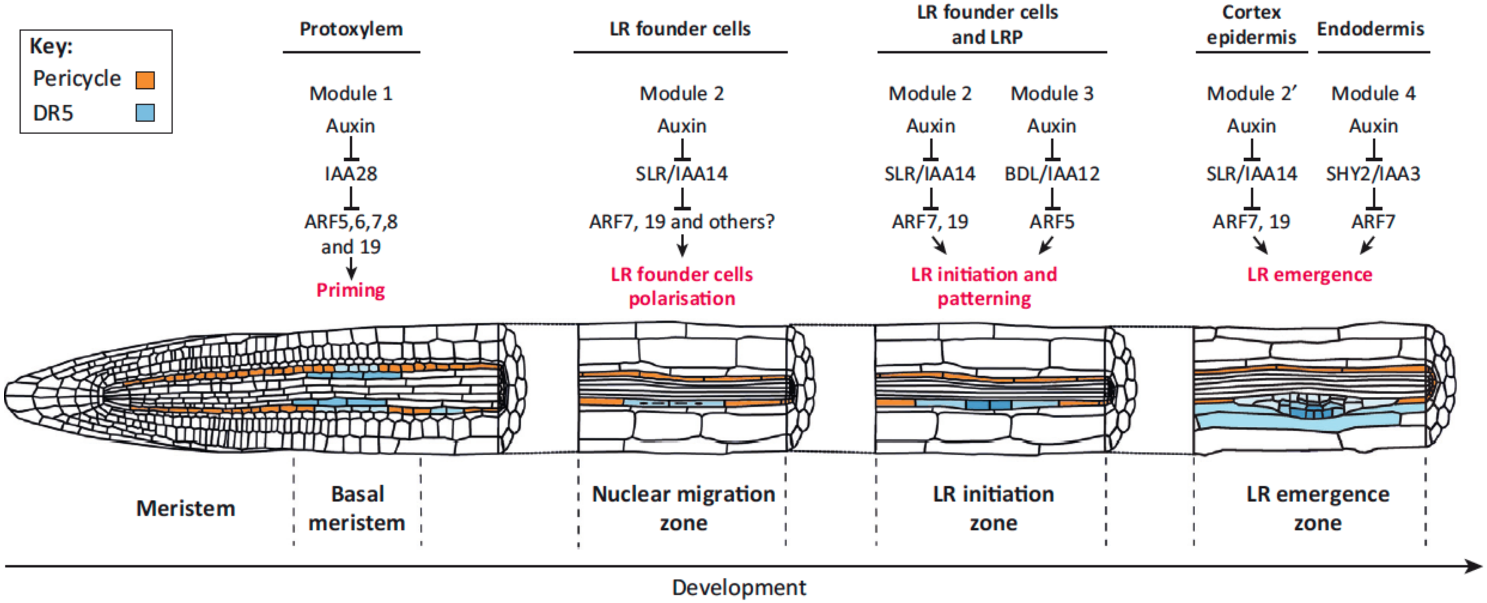
Prediction of an ARF7/ARF19 -dependent module

- This prediction of an important ARF7/ARF19-dependent module is consistent with experimental data :

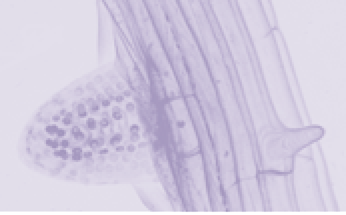
arf7 arf19 double mutation blocks lateral root formation in Arabidopsis.



Fukaki et al., 2007

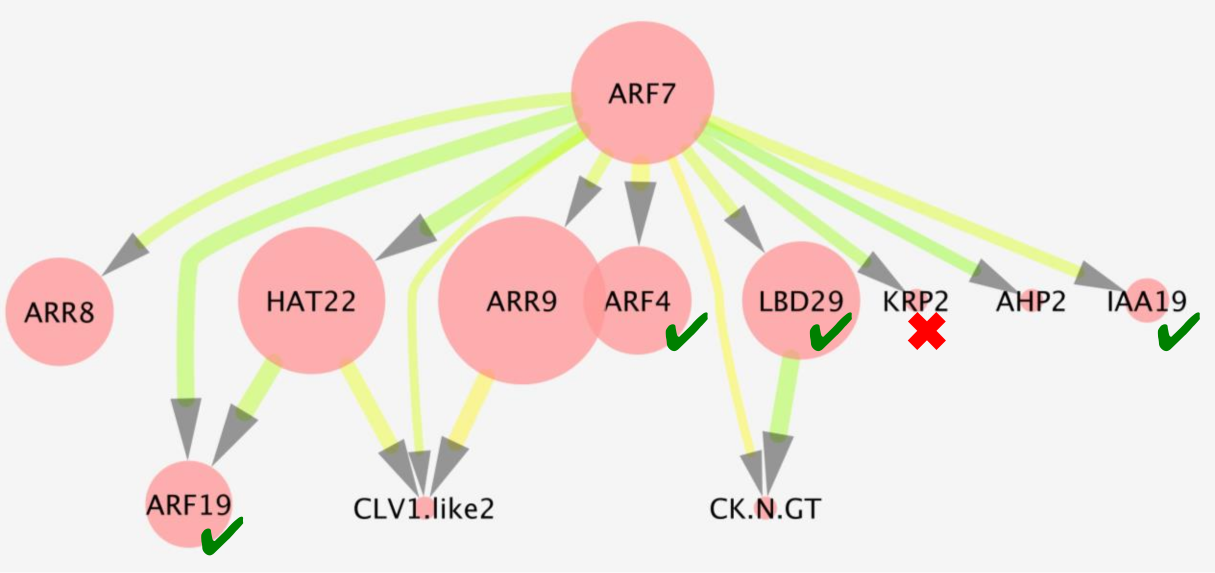


Lavenus et al., TIPS 2003
Based on experimental data

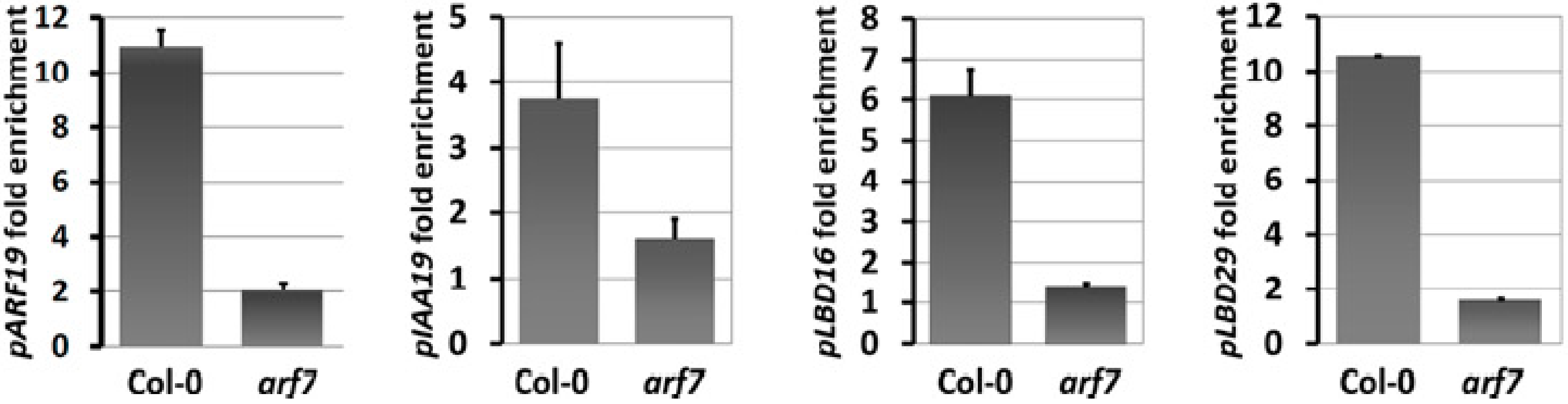


Prediction of ARF7 targets

- Out of 11 1st-order predicted targets of ARF7, 4 are confirmed by qRT-PCR and/or CHIP and 1 is rejected (KRP2)



ARF7 ChIP qPCR

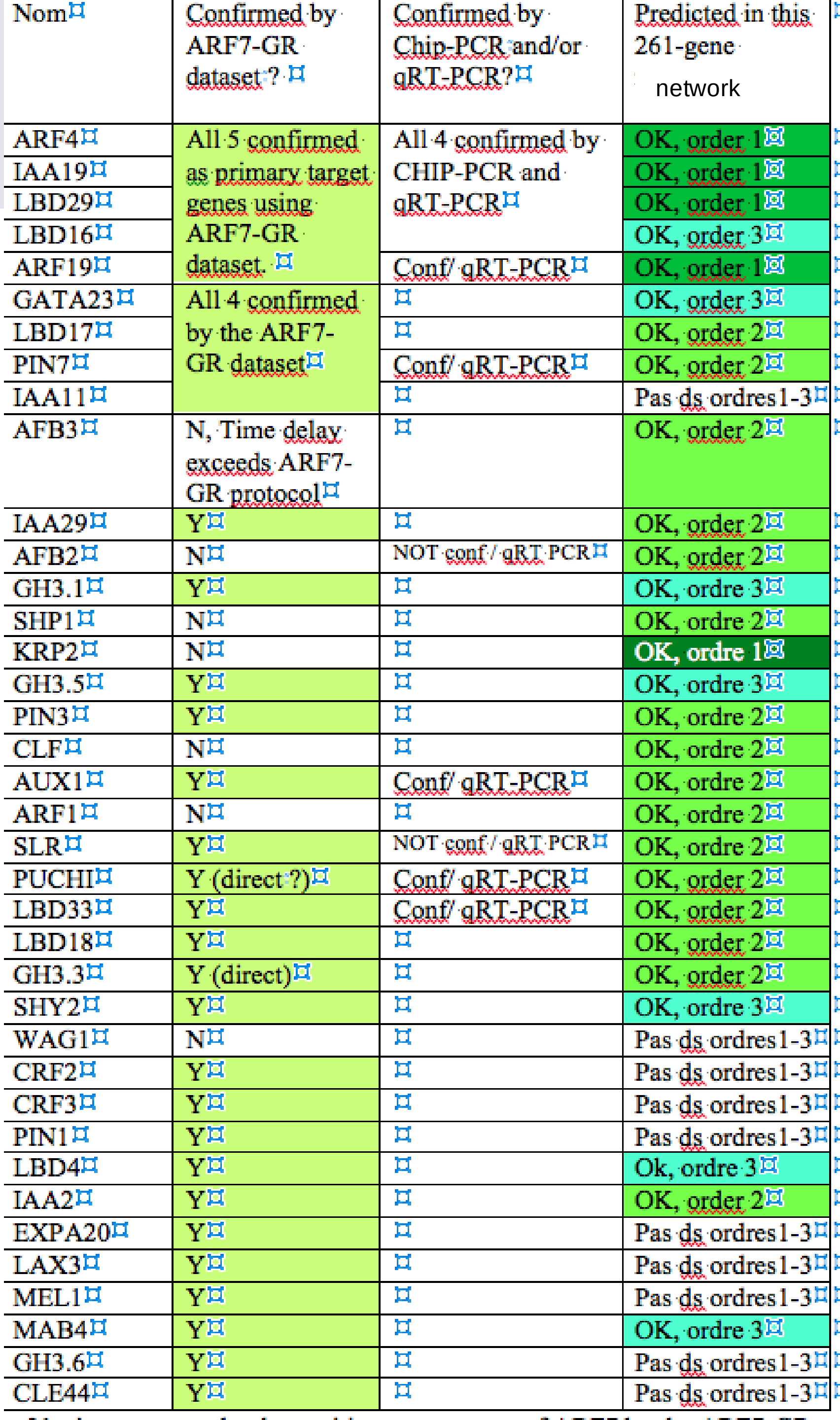


Lavenus et al., 2015

Prediction of ARF7 targets

- Experimental dataset about ARF7 targets: inducible complementation of the *arf7 arf19* mutant by a ARF7-GR construct, (H. Fukaki's kab, Kobe)

- Out of the 31 genes found to be positive target genes of ARF7 in an experimental transcriptomic dataset), 22 (71%) are predicted to be located 1, 2 or 3 edges downstream of ARF7 in the 261-gene network

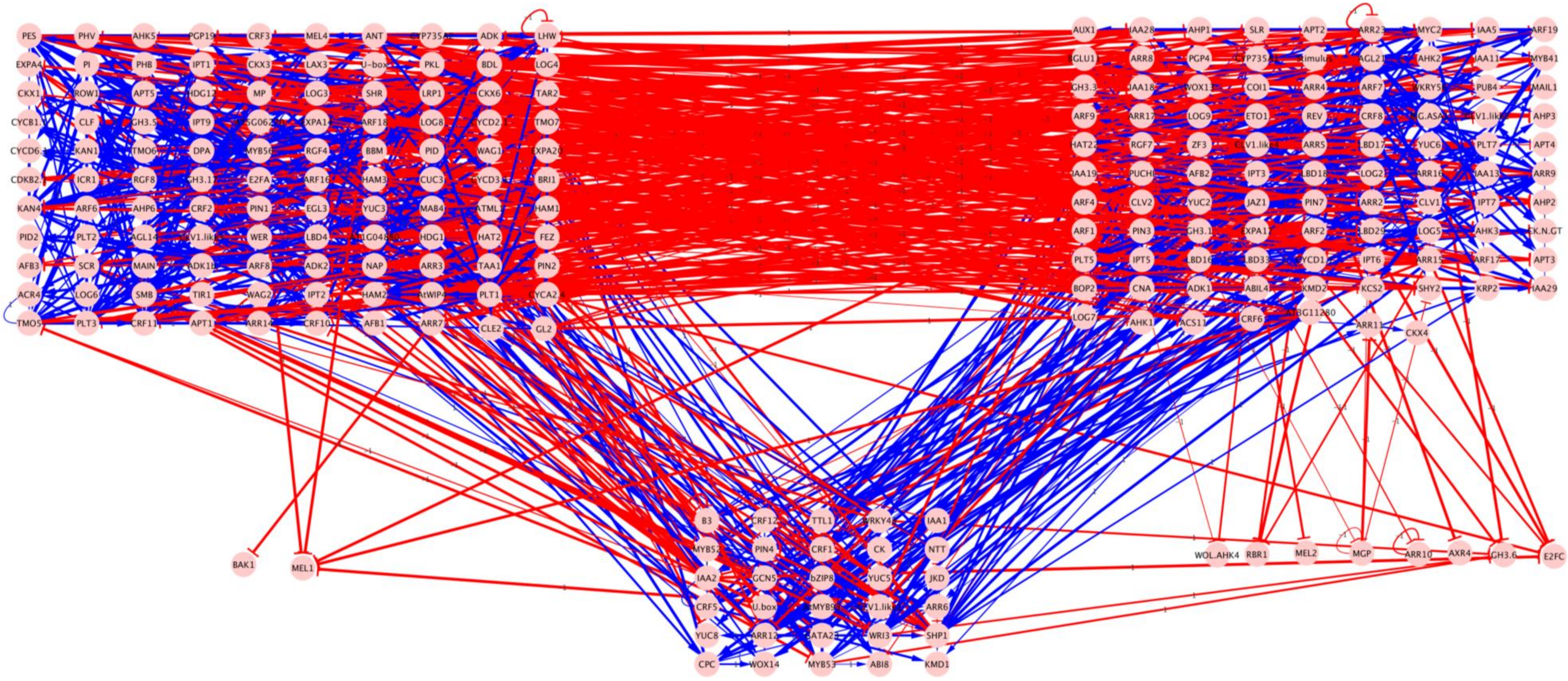


Nom	Confirmed by ARF7-GR dataset ?	Confirmed by Chip-PCR and/or qRT-PCR?	Predicted in this 261-gene network
ARF4	All 5 confirmed as primary target genes using ARF7-GR dataset.	All 4 confirmed by CHIP-PCR and qRT-PCR	OK, order 1
IAA19			OK, order 1
LBD29			OK, order 1
LBD16			OK, order 3
ARF19		Conf/ qRT-PCR	OK, order 1
GATA23	All 4 confirmed by the ARF7-GR dataset		OK, order 3
LBD17			OK, order 2
PIN7		Conf/ qRT-PCR	OK, order 2
IAA11			Pas ds ordres1-3
AFB3	N, Time delay exceeds ARF7-GR protocol		OK, order 2
IAA29	Y		OK, order 2
AFB2	N	NOT conf / qRT PCR	OK, order 2
GH3.1	Y		OK, ordre 3
SHP1	N		OK, ordre 2
KRP2	N		OK, ordre 1
GH3.5	Y		OK, ordre 3
PIN3	Y		OK, ordre 2
CLF	N		OK, ordre 2
AUX1	Y	Conf/ qRT-PCR	OK, ordre 2
ARF1	N		OK, ordre 2
SLR	Y	NOT conf / qRT PCR	OK, ordre 2
PUCHI	Y (direct ?)	Conf/ qRT-PCR	OK, order 2
LBD33	Y	Conf/ qRT-PCR	OK, order 2
LBD18	Y		OK, order 2
GH3.3	Y (direct)		OK, order 2
SHY2	Y		OK, ordre 3
WAG1	N		Pas ds ordres1-3
CRF2	Y		Pas ds ordres1-3
CRF3	Y		Pas ds ordres1-3
PIN1	Y		Pas ds ordres1-3
LBD4	Y		Ok, ordre 3
IAA2	Y		OK, order 2
EXPA20	Y		Pas ds ordres1-3
LAX3	Y		Pas ds ordres1-3
MEL1	Y		Pas ds ordres1-3
MAB4	Y		OK, ordre 3
GH3.6	Y		Pas ds ordres1-3
CLE44	Y		Pas ds ordres1-3

What are the general properties of the network ?

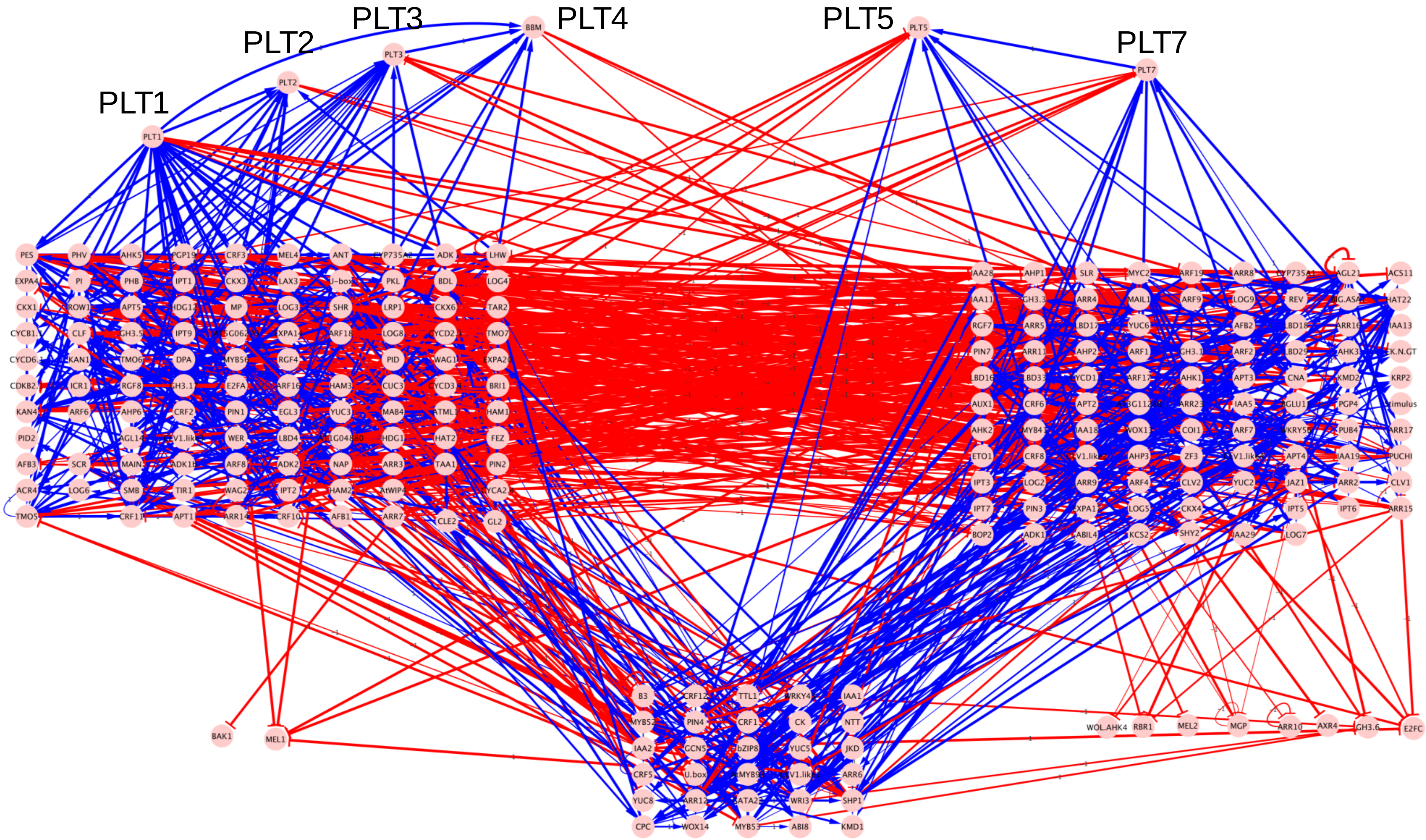
Analysis of topological and dynamic properties of the network

Genes are organised into three main groups



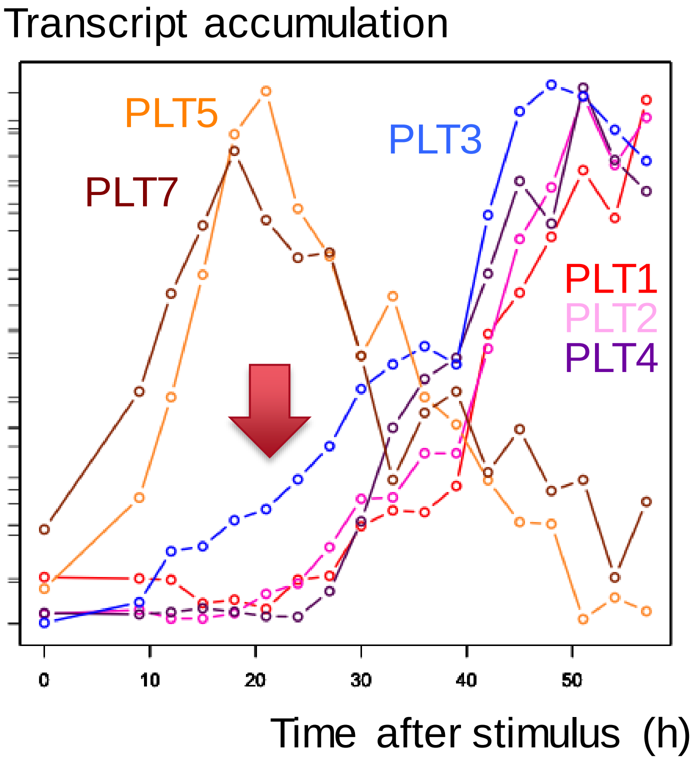
Red edge = inhibition; Blue edge = stimulation

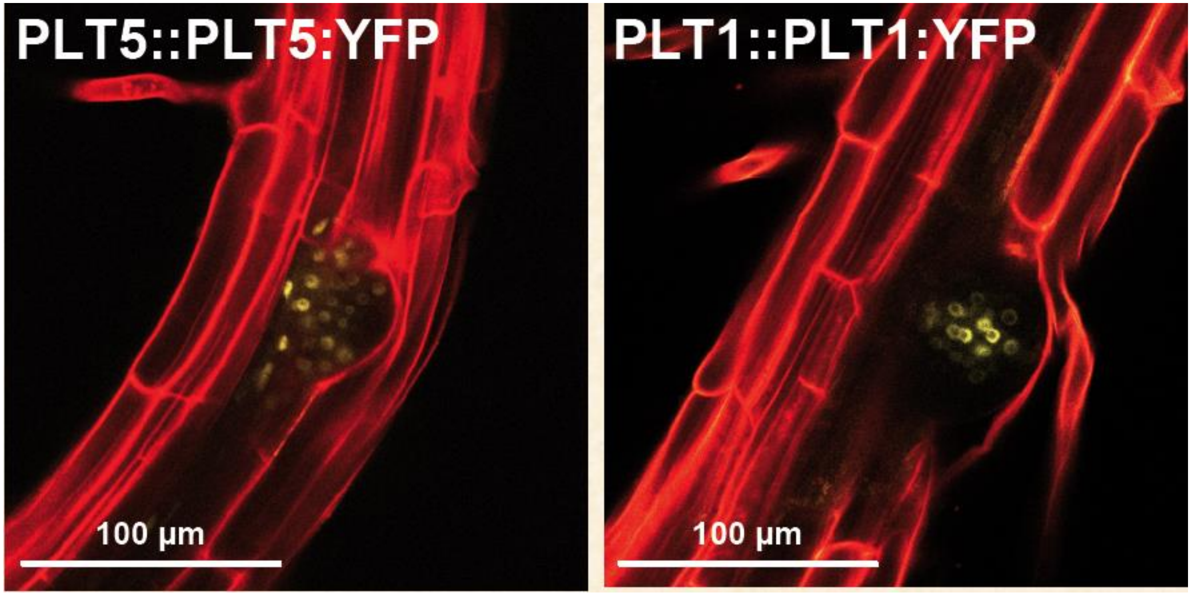
Genes are organised into three main groups: example of PLETHORA family transcription factors



Red edge = inhibition; Blue edge = stimulation

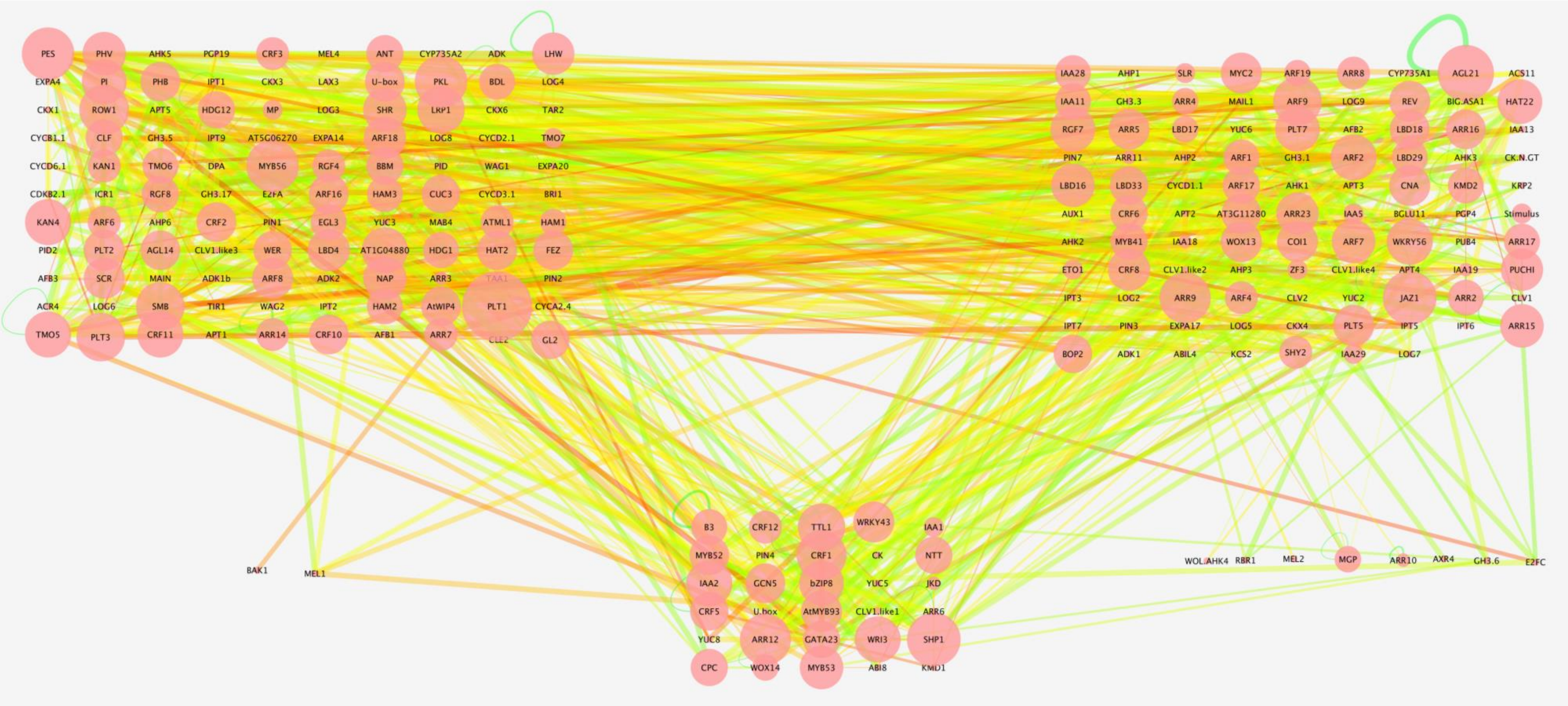
This is consistent with both temporal and spatial patterning in gene expression pattern



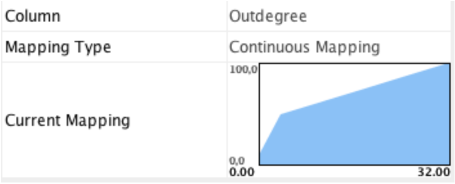


➢ The general topology of the network suggests a toggle-switch mechanism controlling a spatio temporal gene expression pattern in relation with root meristem establishment

Genes are organised into three main groups

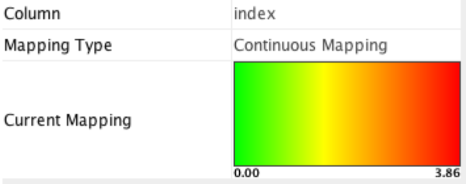


Node size



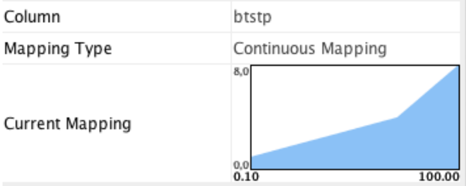
Column	Outdegree
Mapping Type	Continuous Mapping
Current Mapping	

Edge color



Column	index
Mapping Type	Continuous Mapping
Current Mapping	

Edge width

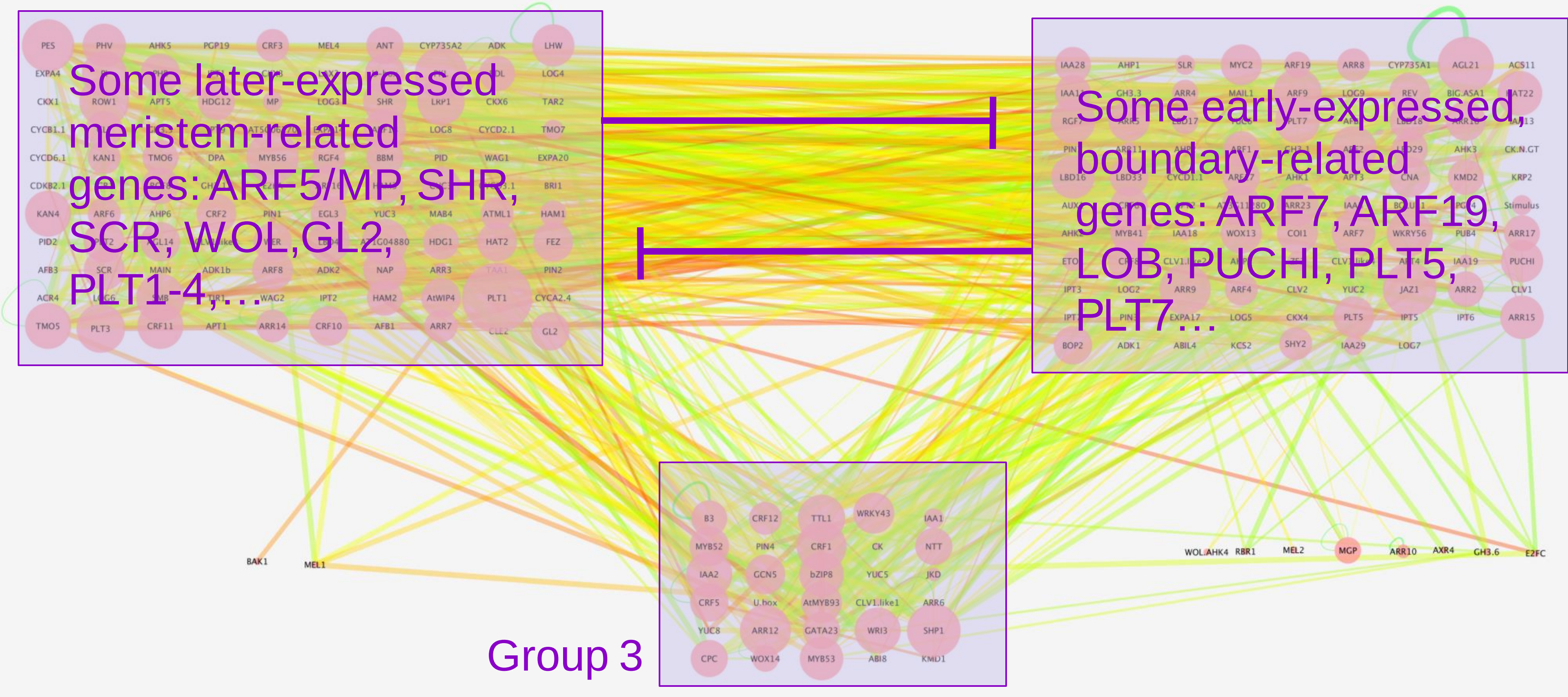


Column	btstp
Mapping Type	Continuous Mapping
Current Mapping	

Genes are organised into three main groups

Group 2

Group 1

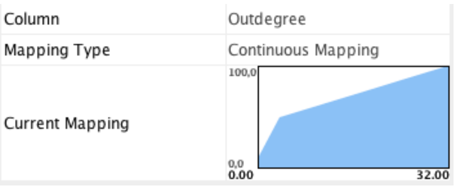


Some later-expressed meristem-related genes: ARF5/MP, SHR, SCR, WOL,GL2, PLT1-4,…

Some early-expressed, boundary-related genes: ARF7, ARF19, LOB, PUCHI, PLT5, PLT7…

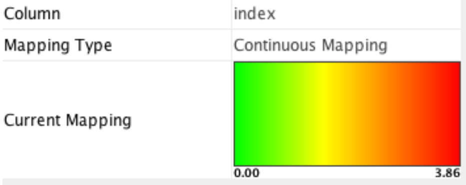
Group 3

Node size



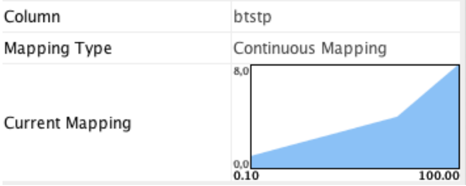
Column	Outdegree
Mapping Type	Continuous Mapping
Current Mapping	

Edge color



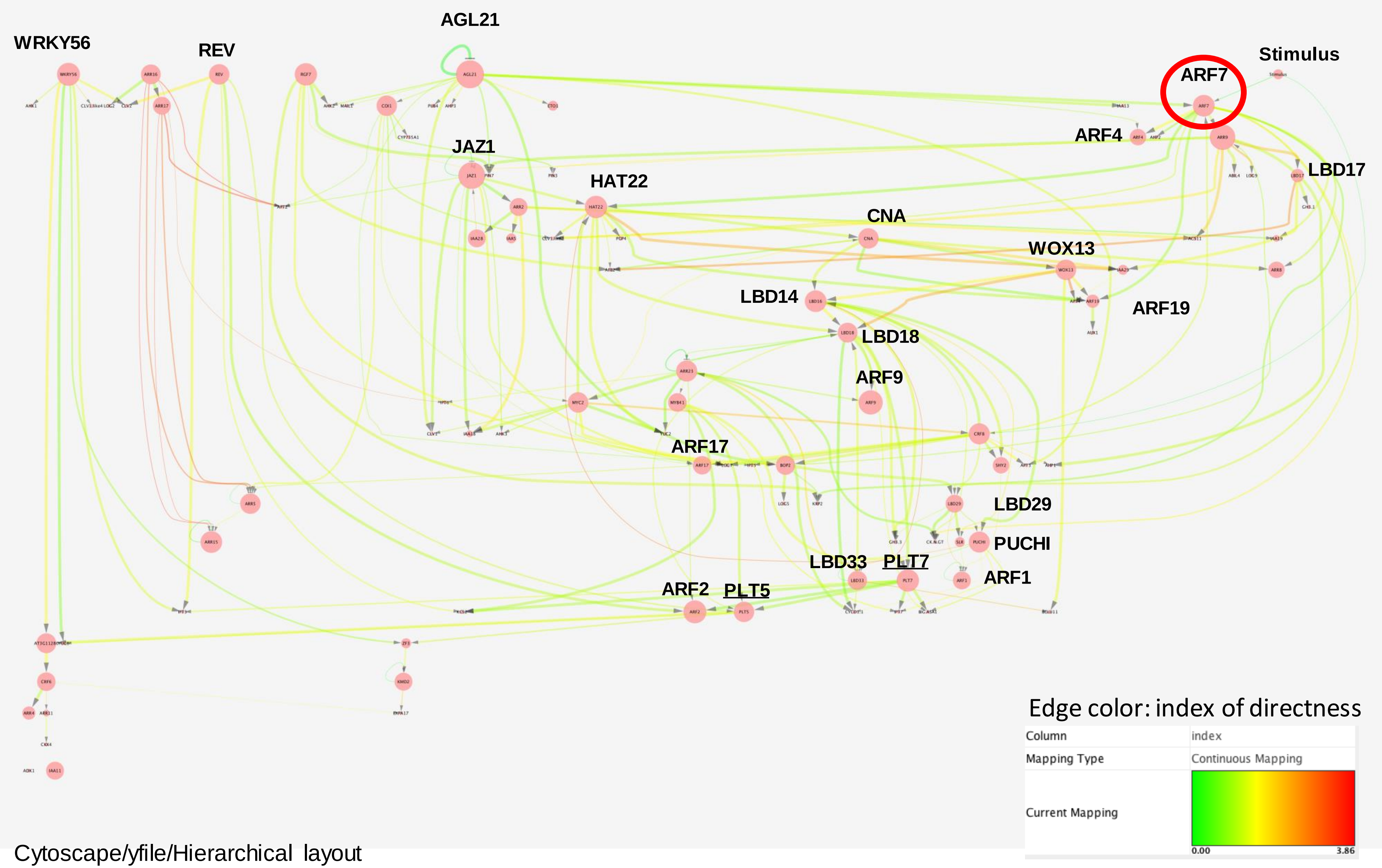
Column	index
Mapping Type	Continuous Mapping
Current Mapping	

Edge width



Column	btstp
Mapping Type	Continuous Mapping
Current Mapping	

Group 1: Prediction of an ARF7-dependent module controlling early stimulus response and boundary genes

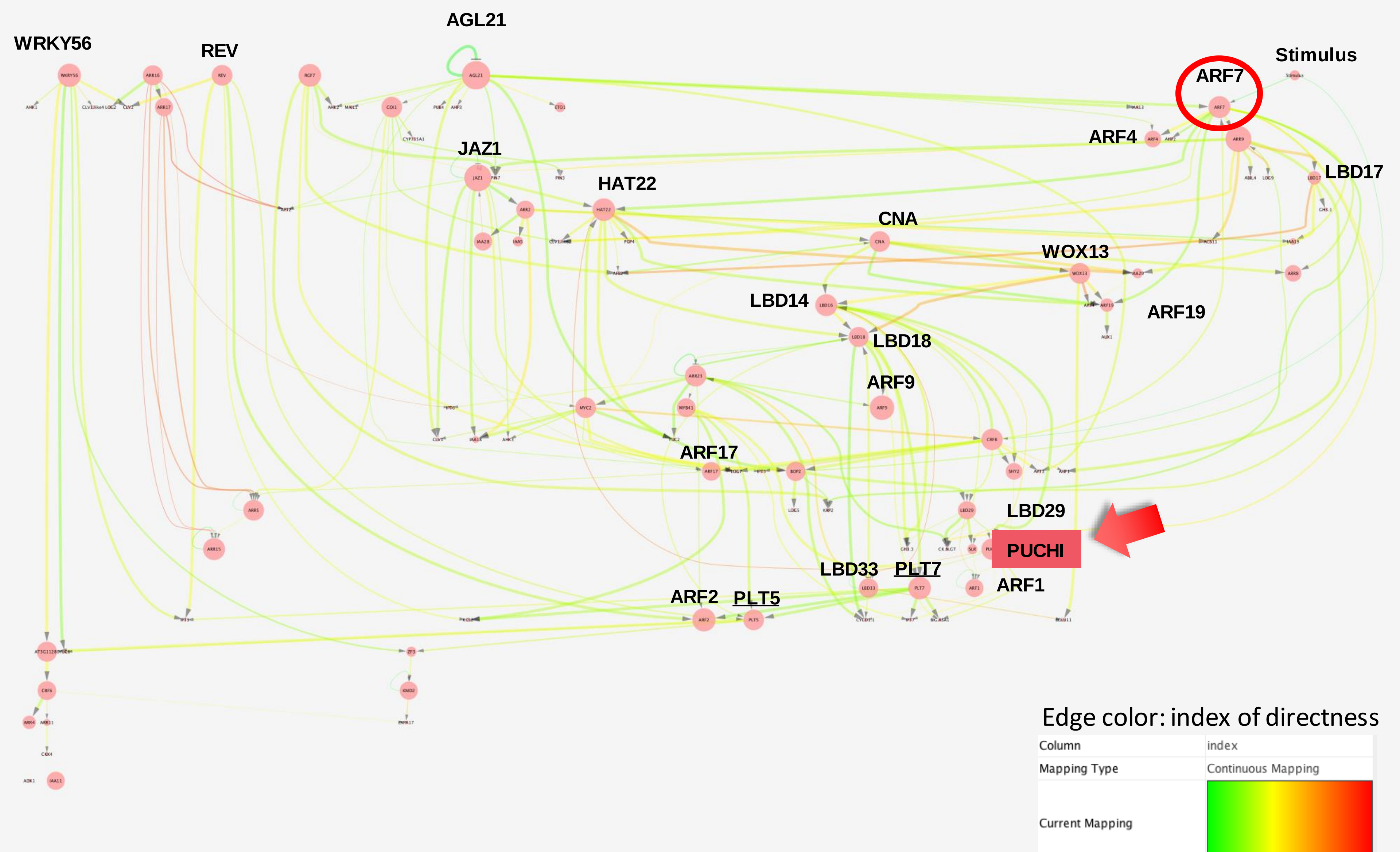


Edge color: index of directness

Column	index
Mapping Type	Continuous Mapping
Current Mapping	0.00 3.86

Cytoscape/yfile/Hierarchical layout

Group 1: Prediction of an ARF7-dependent module controlling early stimulus response and boundary genes

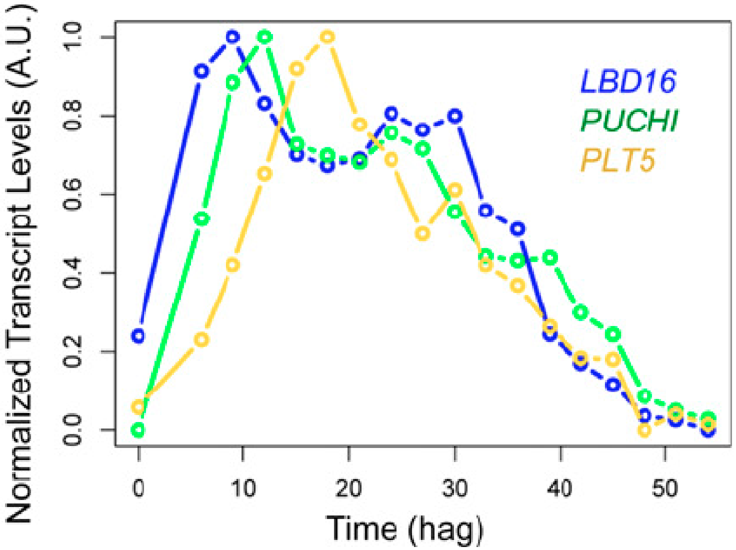


Edge color: index of directness

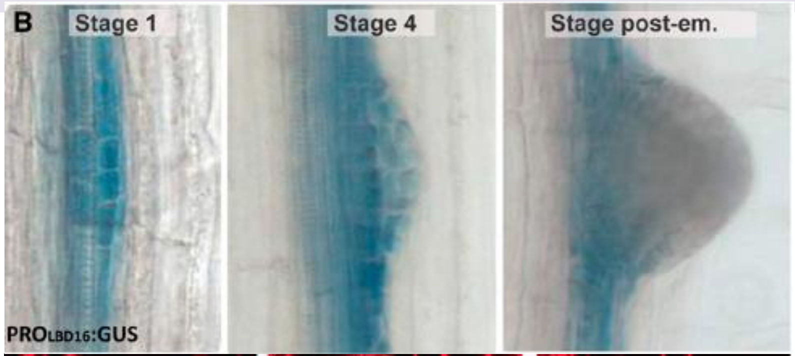
Column	index
Mapping Type	Continuous Mapping
Current Mapping	0.00 🟩🟨🟥 3.86

Cytoscape/yfile/Hierarchical layout

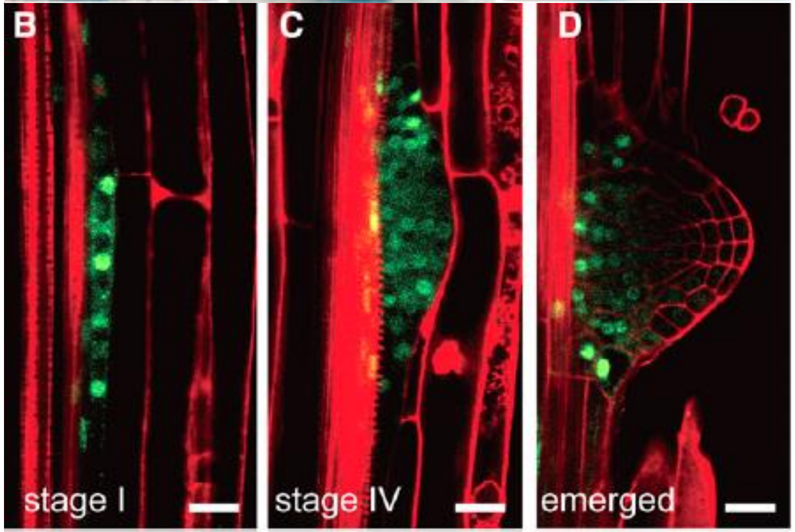
Activation pattern of the ARF7 module in the primordium



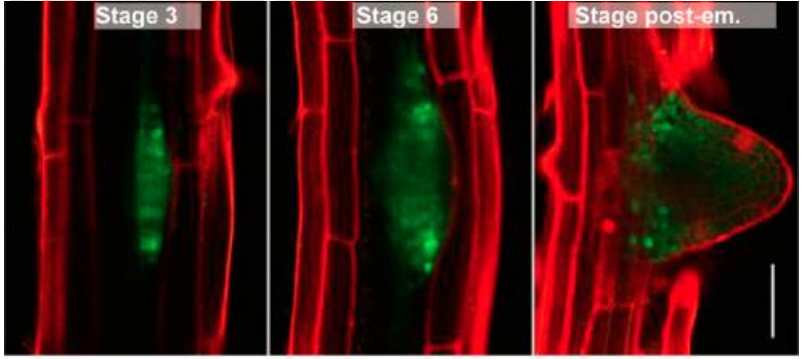
LBD16



PUCHI

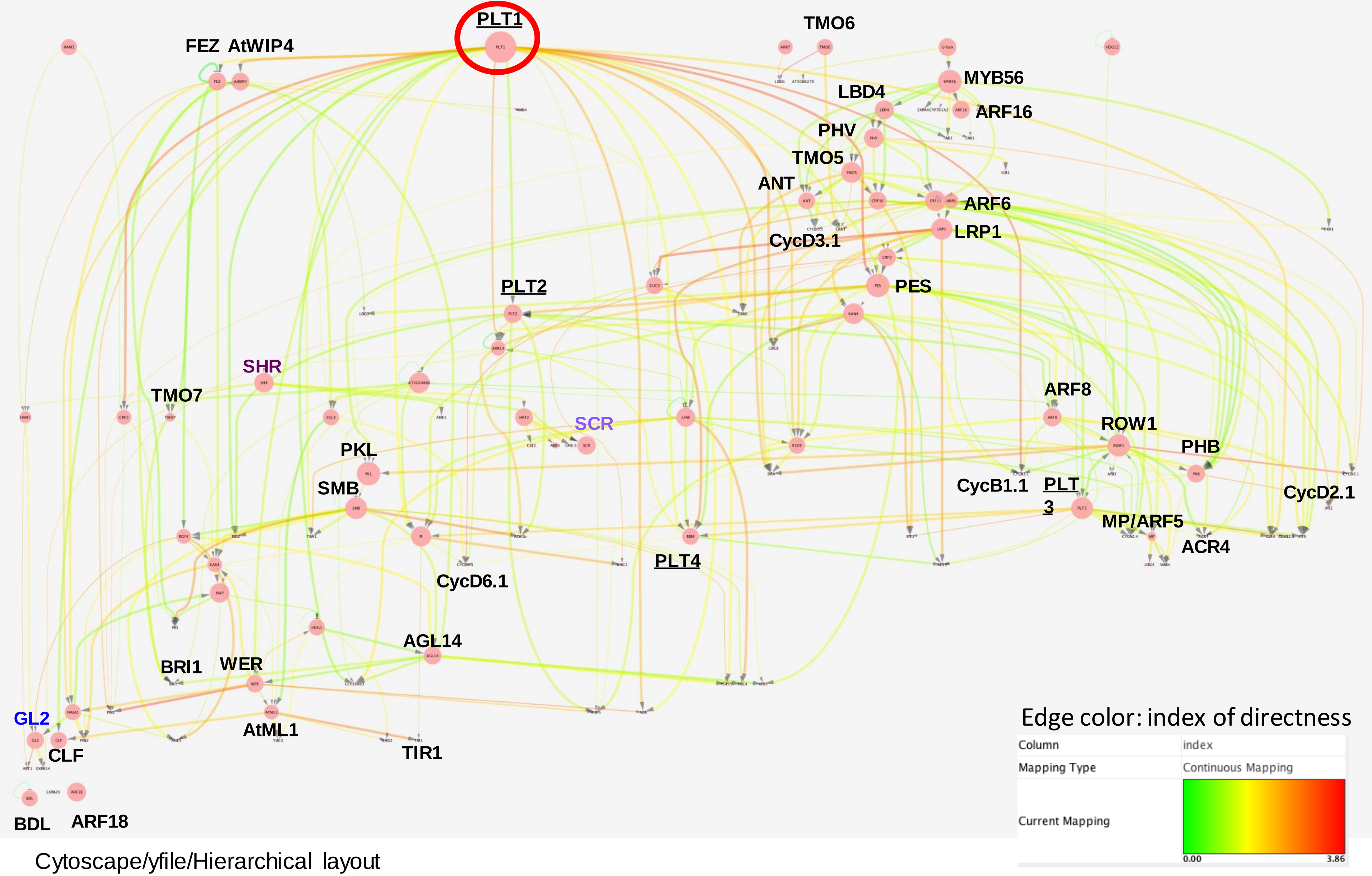


PLT5



Lavenus et al., 2015
Hirota et al., 2007

Group 2 : prediction of a PLT1-dependent module : PLT1 as a hub regulating multiple meristem genes



Edge color: index of directness

Column index

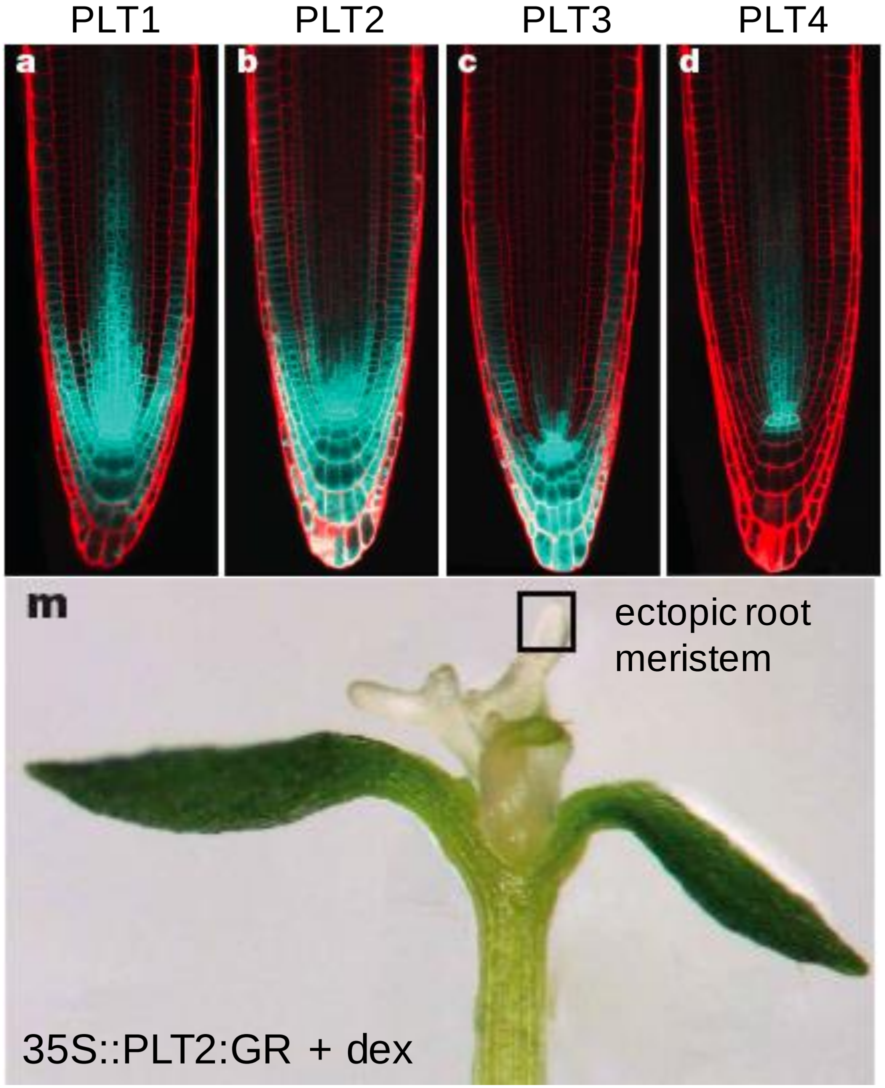
Mapping Type Continuous Mapping

Current Mapping

0.00 3.86

Cytoscape/yfile/Hierarchical layout

PLT1-4 are known master regulators of root meristem development



PLT1 PLT2 PLT3 PLT4

ectopic root meristem

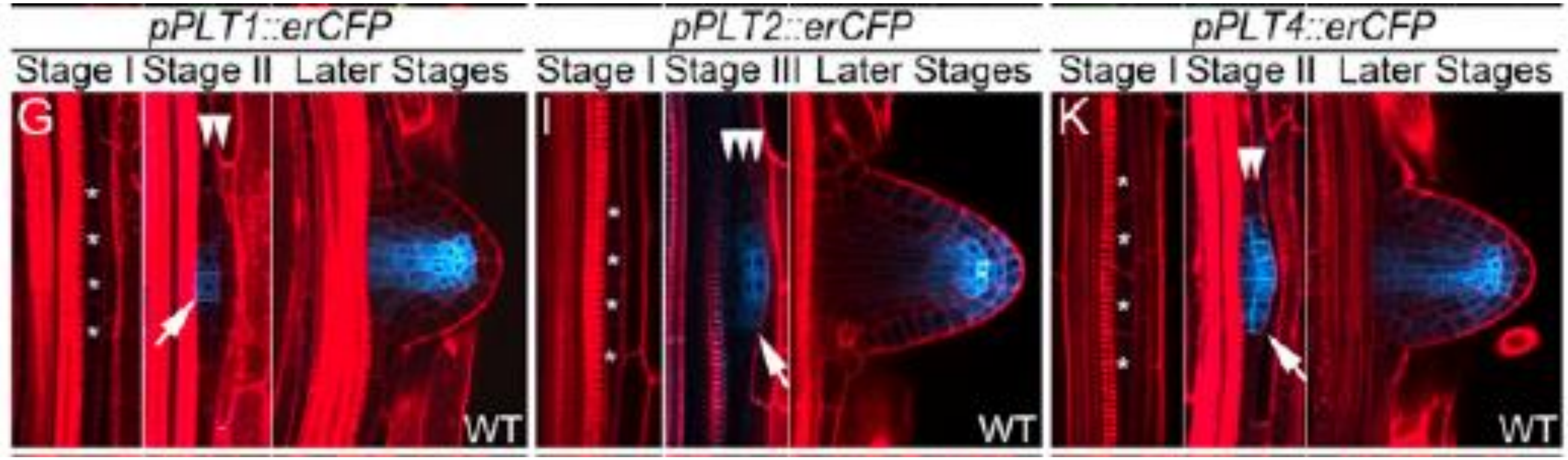
35S::PLT2:GR + dex

PLT1-4 genes are auxin inducible and highly expressed in the root meristem

PLT2 overexpression in the shoot apex converts the shoot apical meristem into a root apical meristem

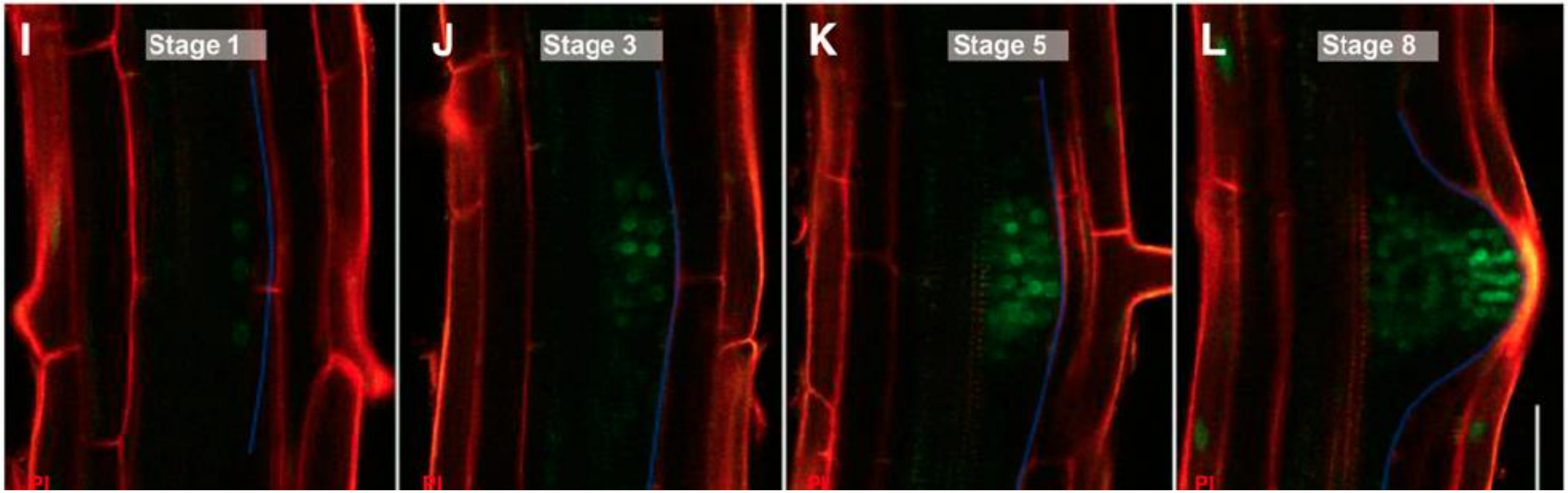
Galinha et al., 2007

Activation pattern of the ARF5 subnetwork in the LRP



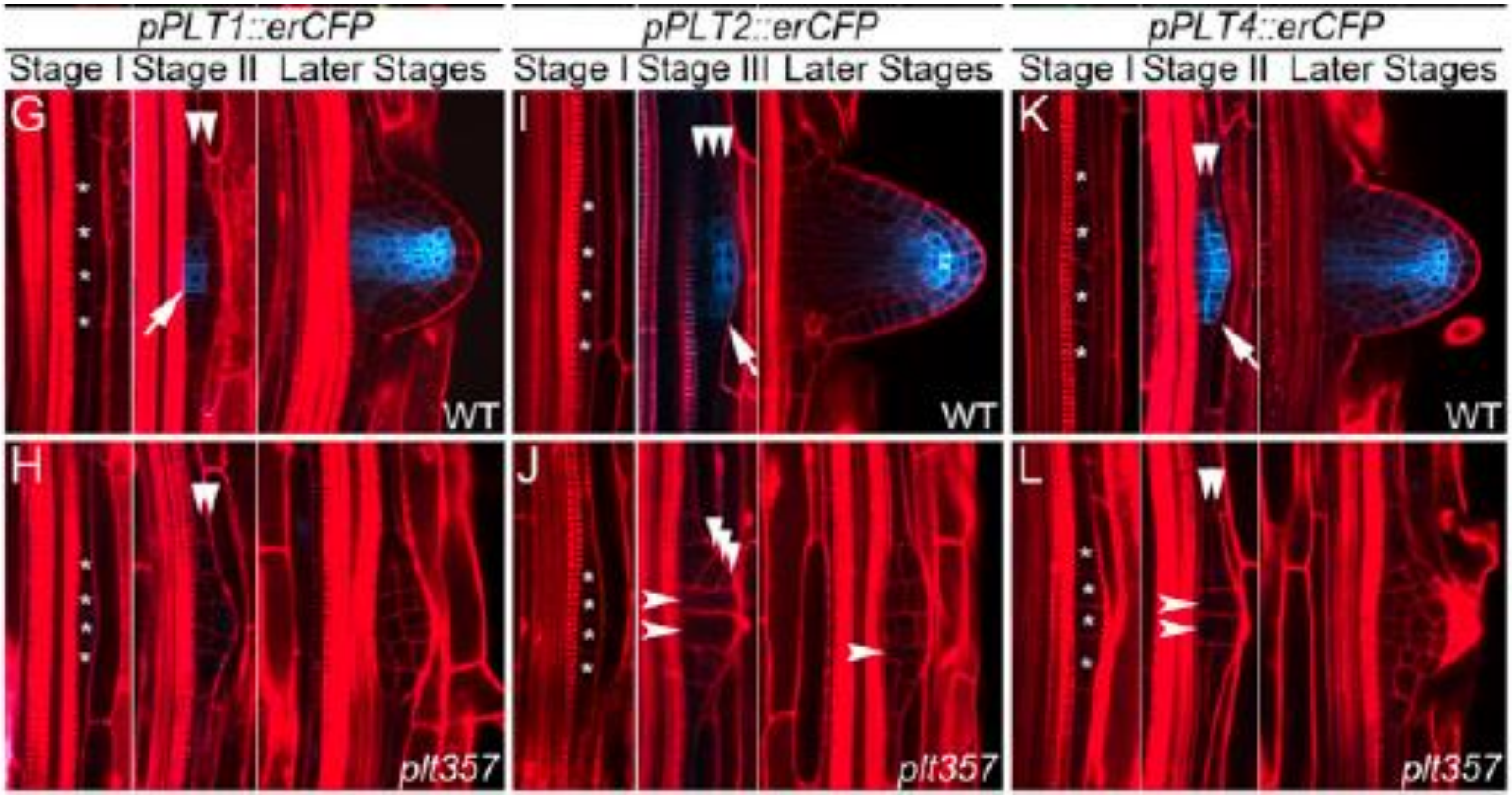
Du & Scheres, 2017

pMP::MP:GFP



Lavenus et al., 2015

Experimental data suggests a role of PLT5 and PLT7 (in group 1) and PLT3 (in group 2) in switching on *PLT1,2,4*



Du & Scheres, 2017

- Onset of meristematic *PLT1*, *PLT2* and *PLT4* gene expression is impaired in *plt3,5,7* triple mutant

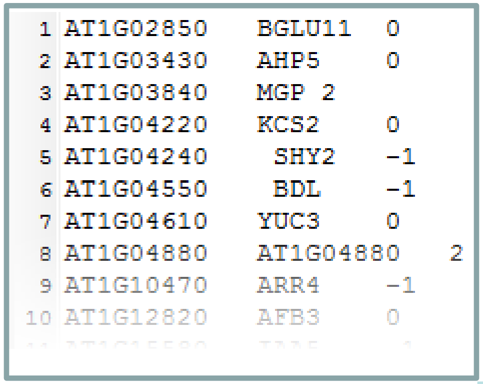
What are the general properties of the network ?

Analysis of topological and *dynamic* properties of the network

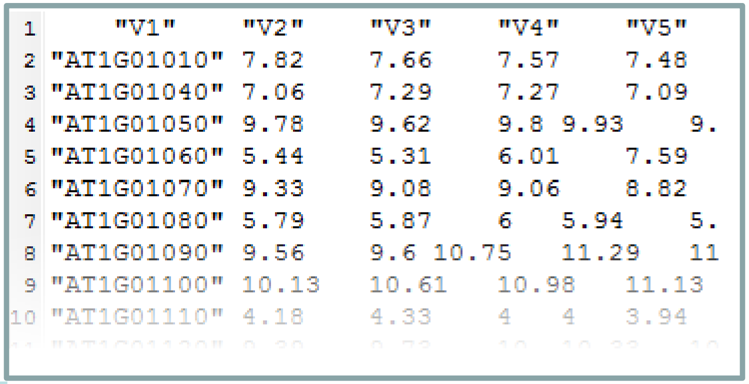
Modeling dynamic properties of the inferred network

- Aim : to model dynamically the state flow of the gene network in order to identify gene regulatory cascades, master regulators, attractor states, bifurcation behaviours…

TDCore inputs

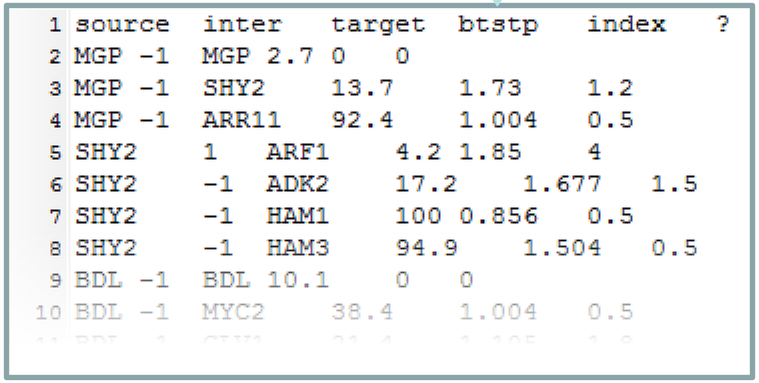


```
AT1G02850    BGLU11   0
AT1G03430    AHP5     0
AT1G03840    MGP 2
AT1G04220    KCS2     0
AT1G04240     SHY2    -1
AT1G04550     BDL     -1
AT1G04610    YUC3     0
AT1G04880    AT1G04880    2
AT1G10470    ARR4     -1
AT1G12820    AFB3     0
```

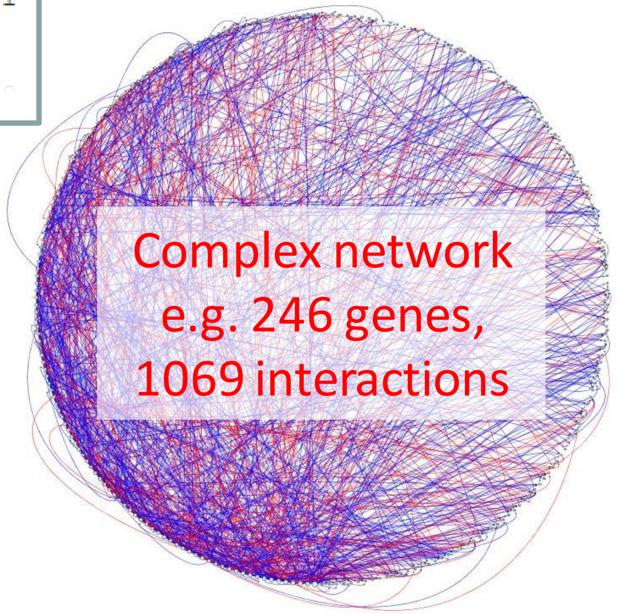


```
     "V1"      "V2"      "V3"      "V4"      "V5"
"AT1G01010" 7.82      7.66      7.57      7.48
"AT1G01040" 7.06      7.29      7.27      7.09
"AT1G01050" 9.78      9.62      9.8 9.93      9.
"AT1G01060" 5.44      5.31      6.01      7.59
"AT1G01070" 9.33      9.08      9.06      8.82
"AT1G01080" 5.79      5.87      6    5.94      5.
"AT1G01090" 9.56      9.6 10.75    11.29     11
"AT1G01100" 10.13     10.61     10.98     11.13
"AT1G01110" 4.18      4.33      4    4    3.94
```

TDCor outputs



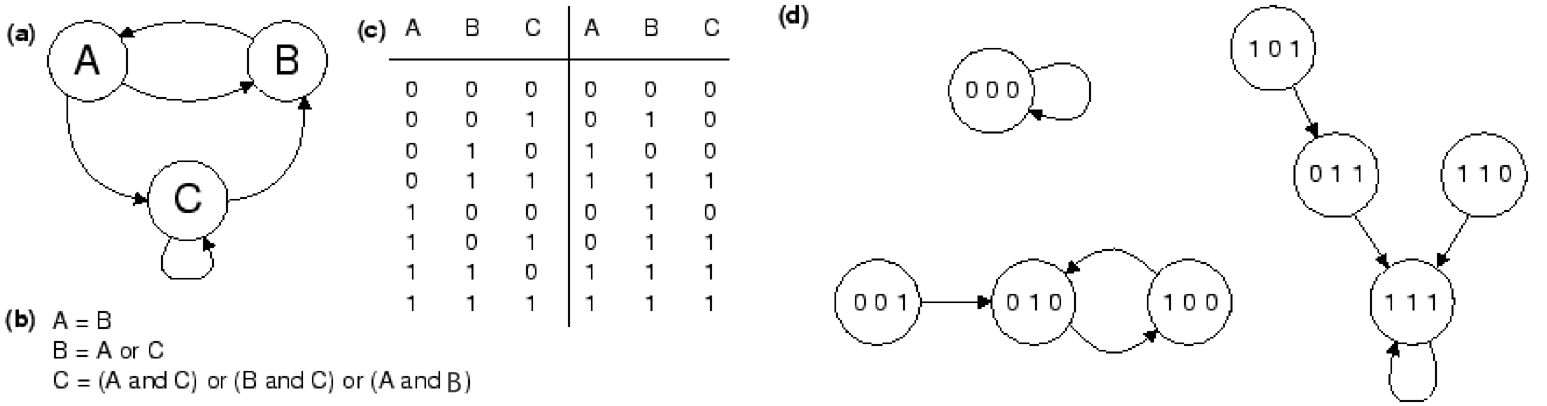
```
source  inter   target  btstp   index   ?
MGP -1  MGP 2.7 0   0
MGP -1  SHY2    13.7    1.73    1.2
MGP -1  ARR11   92.4    1.004   0.5
SHY2    1   ARF1    4.2 1.85    4
SHY2    -1  ADK2    17.2    1.677   1.5
SHY2    -1  HAM1    100 0.856   0.5
SHY2    -1  HAM3    94.9    1.504   0.5
BDL -1  BDL 10.1    0   0
BDL -1  MYC2    38.4    1.004   0.5
```



Complex network
e.g. 246 genes,
1069 interactions

Modeling dynamic properties of the inferred network

- Strategy: to use boolean modeling of the network



(b) A = B
B = A or C
C = (A and C) or (B and C) or (A and B)

(c)

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

Modeling dynamic properties of the inferred network

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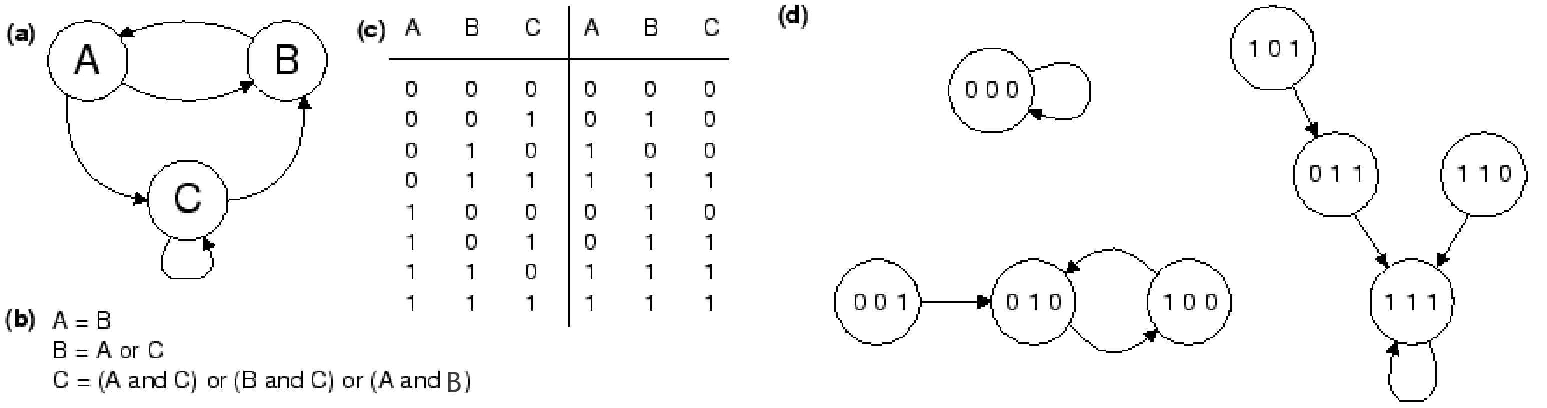

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A	B	C	A	B	C
0	0	0	0	0	0
0	0	1	0	1	0
0	1	0	1	0	0
0	1	1	1	1	1
1	0	0	0	1	0
1	0	1	0	1	1
1	1	0	1	1	1
1	1	1	1	1	1

- However most available boolean models require explicit specification of network and of each interaction rules (BooleanNet, NetDS, NetworkToolkitExtended, BooleSim, SimBoolNet, Atalia, …)
  - → Impractical for massive network modelling
  - → Currently developing implementation of automated boolean modelling for large scale networks

A new simulation algorithm – PYTHONIS

(PYTHon-based bOolean Network generIc Solver)

- Uses any given predicted topology of a GRN (e.g. 246 genes, 1069 interactions)
- Boolean modeling as a simplification (various formats)

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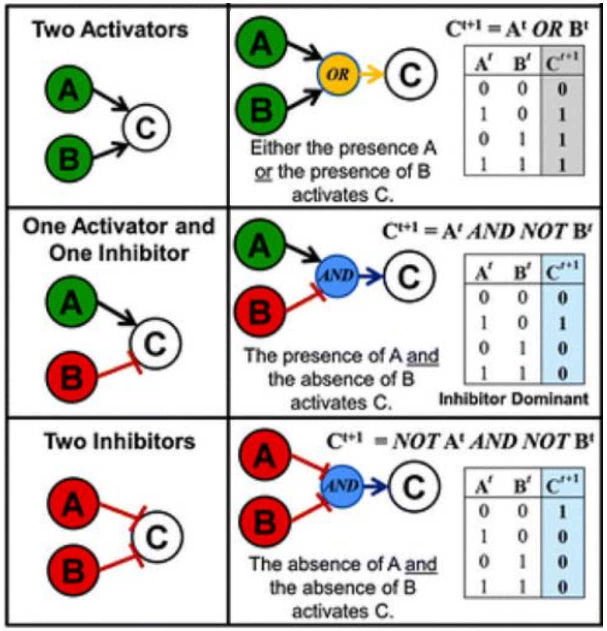
- Boolean modeling as a simplification (various formats)
- Strong biological assumptions
  - *Strong upregulators*
  - *Strong downregulators*
  - *No lazy genes*



$C^{t+1} = A^t \mathit{OR}\ B^t$

A^t	B^t	C^{t+1}
0	0	0
1	0	1
0	1	1
1	1	1

Either the presence A or the presence of B activates C.

$C^{t+1} = A^t \mathit{AND\ NOT}\ B^t$

A^t	B^t	C^{t+1}
0	0	0
1	0	1
0	1	0
1	1	0

The presence of A and the absence of B activates C.

Inhibitor Dominant

$C^{t+1} = \mathit{NOT}\ A^t \mathit{AND\ NOT}\ B^t$

A^t	B^t	C^{t+1}
0	0	1
1	0	0
0	1	0
1	1	0

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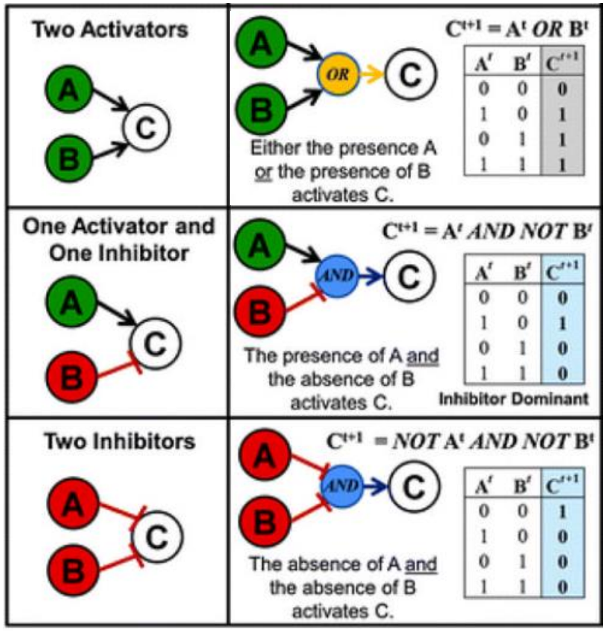

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1	0	1
0	1	1
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A^t	B^t	C^{t+1}
0	0	0
1	0	1
0	1	0
1	1	0

$C^{t+1} = \textit{NOT } A^t \textit{ AND NOT } B^t$

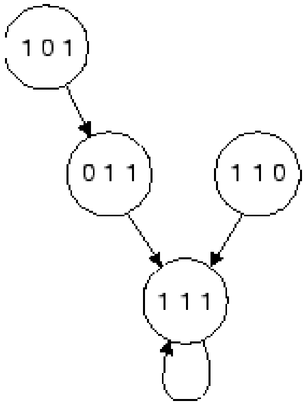
The absence of A and the absence of B activates C.

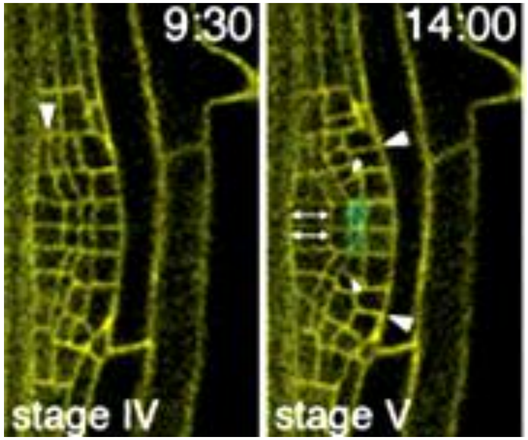
A^t	B^t	C^{t+1}
0	0	1
1	0	0
0	1	0
1	1	0

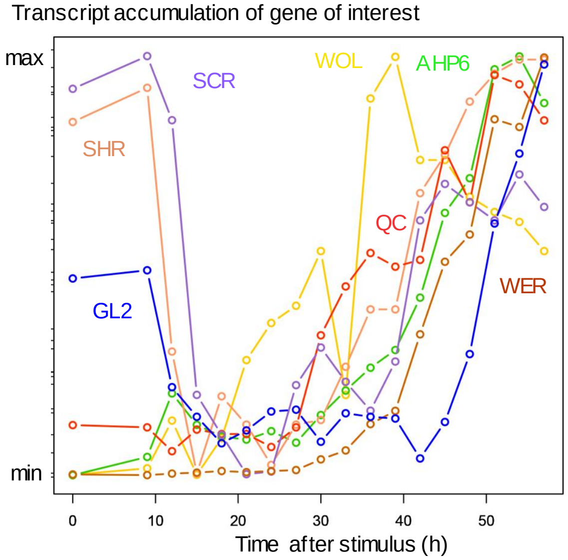
- Automated generation and solving of boolean model for entire network

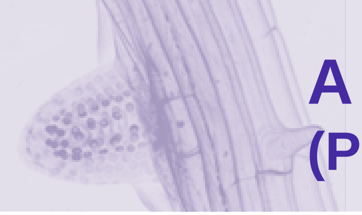
A new simulation algorithm – PYTHONIS
(PYTHon-based bOolean Network generIc Solver)

- For any random or given initial state,
  - ○ state flow (deterministic)
  - ○ final states (can be stable states or loops)
  - ○ basins of attraction
  - ○ Hamming distance between basins of attractions
  - ○ => identification of nodes important for cell fate bifurcation?









A new simulation algorithm – PYTHONIS

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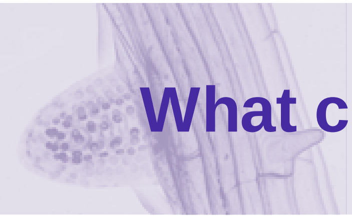
- For any random or given initial state,
  - state flow (deterministic)
  - final states (can be stable states or loops)
  - basins of attraction
  - Hamming distance between basins of attractions
  - => identification of nodes important for cell fate bifurcation?

- Simulation of the impact of knock-out (always 0) or gain-of-function (always 1) mutations
  - impact on state flow and final states
  - Hamming distance between « mutant » and « wild type » final states
  - => assessing the significance of each node in state flow

- Currently being validated against known regulatory networks

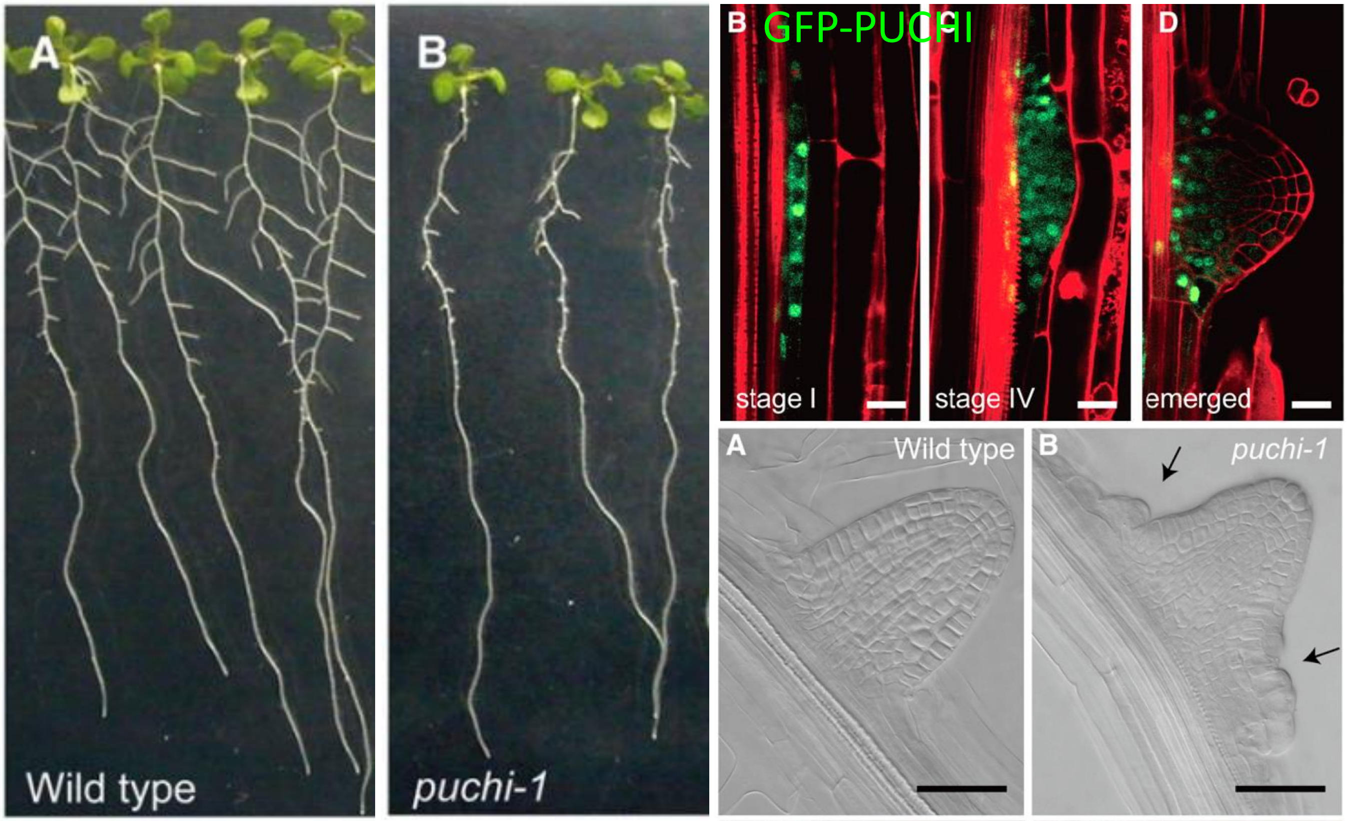


What can this GRN tell us about lateral root formation ?

Experimental validation of predicted regulatory modules

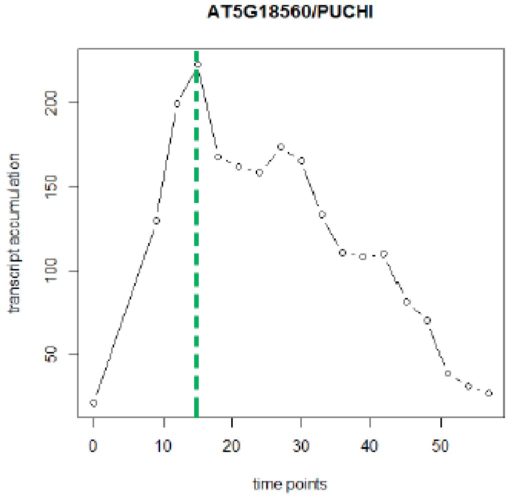
PUCHI is a regulator of lateral root development

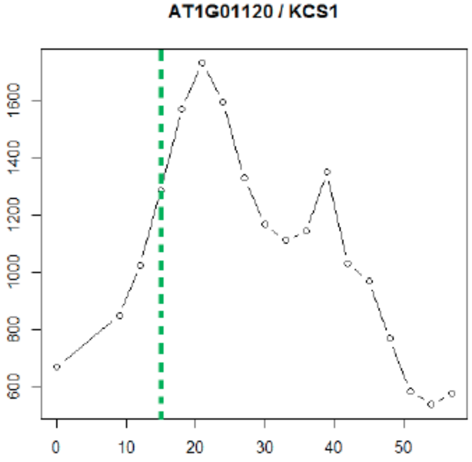
- PUCHI is an AP2/EREBP transcription factors expressed in lateral root primordia
- Induced by the root-promoting hormone auxin, but no described PUCHI targets
- PUCHI loss of function affects lateral root primordium development

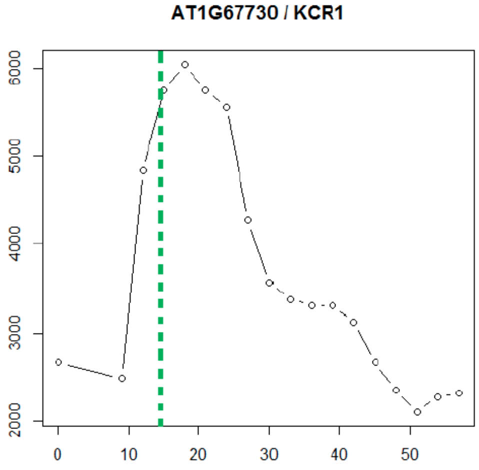


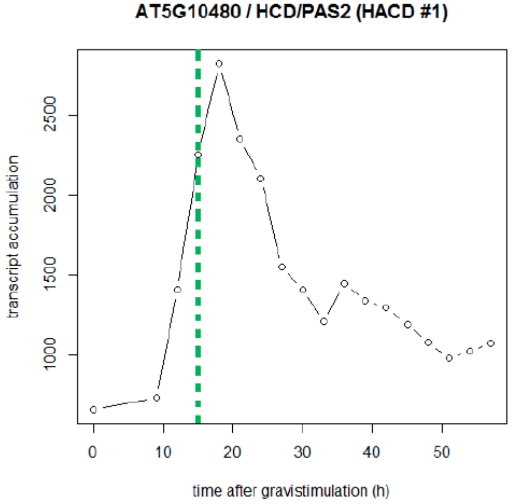
Hirota et al., 2007

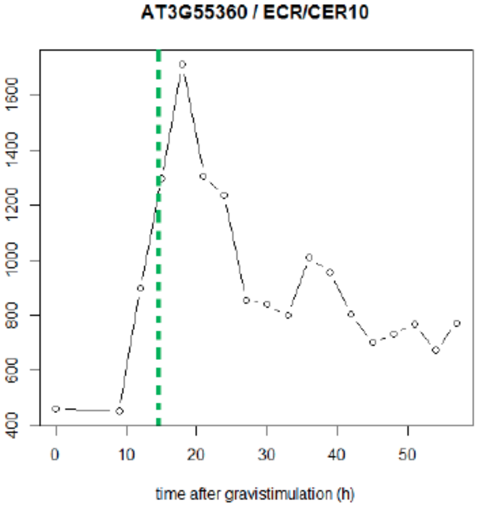
A systems biology approach to identify PUCHI targets


AT5G18560/PUCHI


AT1G01120 / KCS1

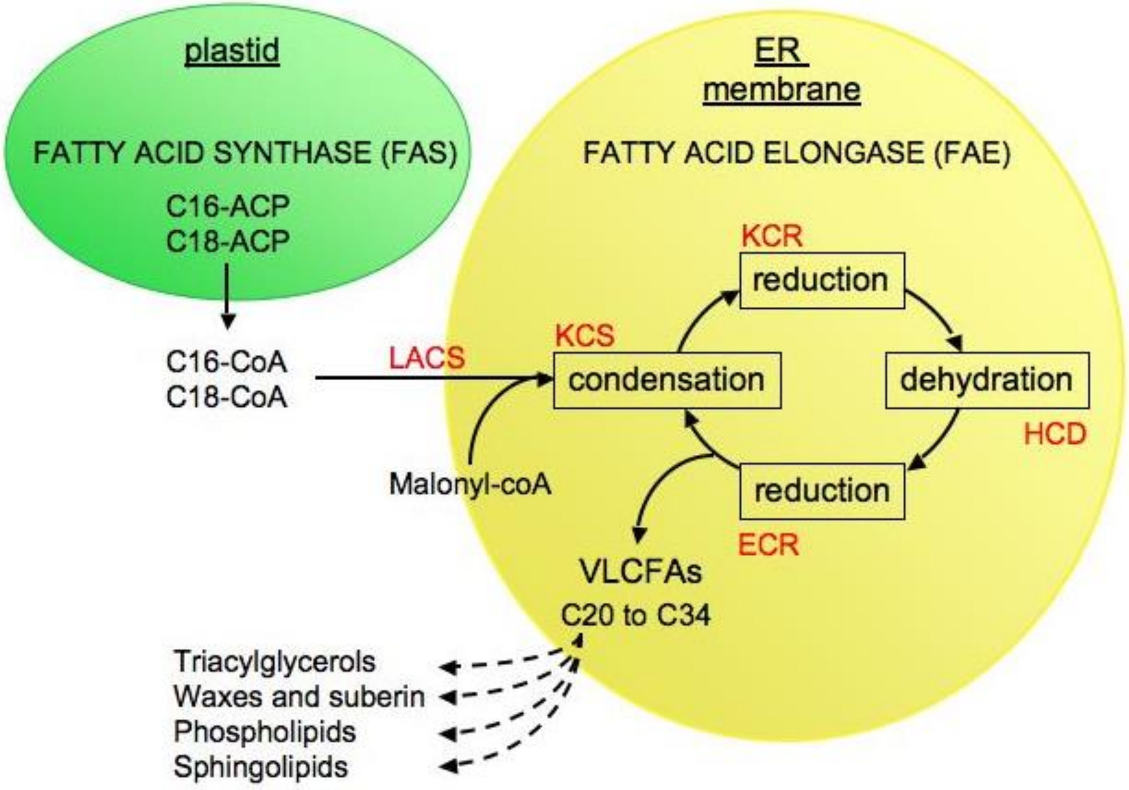

AT1G67730 / KCR1

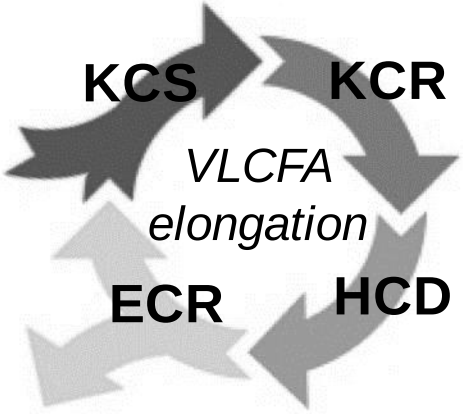

AT5G10480 / HCD/PAS2 (HACD #1)


AT3G55360 / ECR/CER10

- Among 480 genes whose expression profile is correlated with that of PUCHI during lateral root development, gene function in Very Long Chain Fatty Acids biosynthesis pathway is over represented (p-value< 0,05)

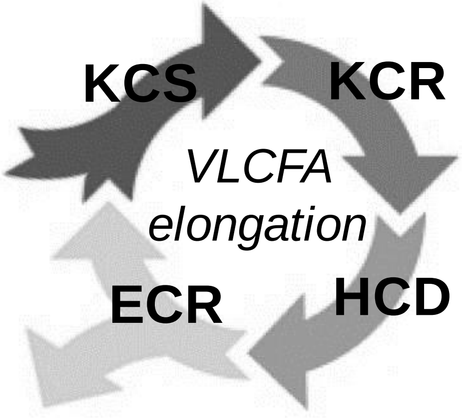
VLCFA biosynthetic pathway and function



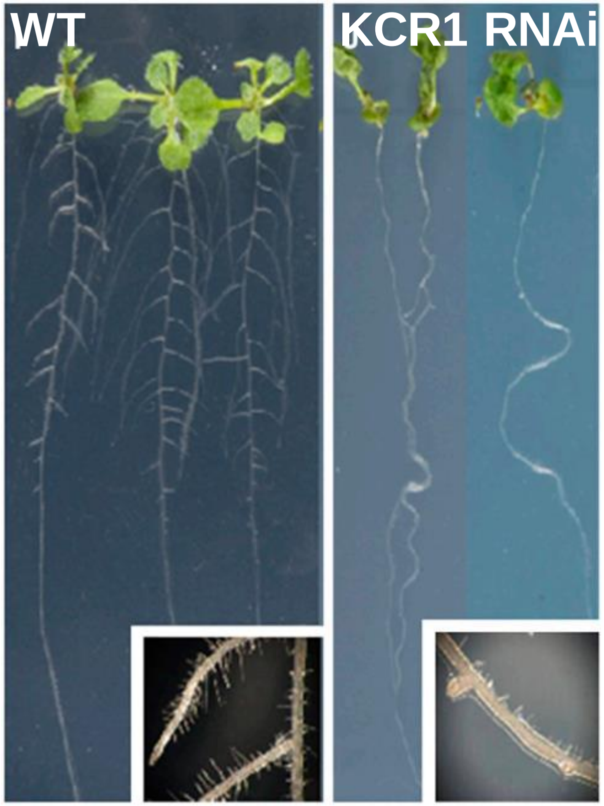


- Very Long Chain Fatty Acids are synthesized in the ER membrane and incorporated into membrane lipids, storage lipids and apoplast polymeres
- Multiple roles in membrane dynamics, cell polarity, cytokinesis, suberin and wax deposition, embryonic and post-embryonic development

VLCFA biosynthetic mutations affect plant development

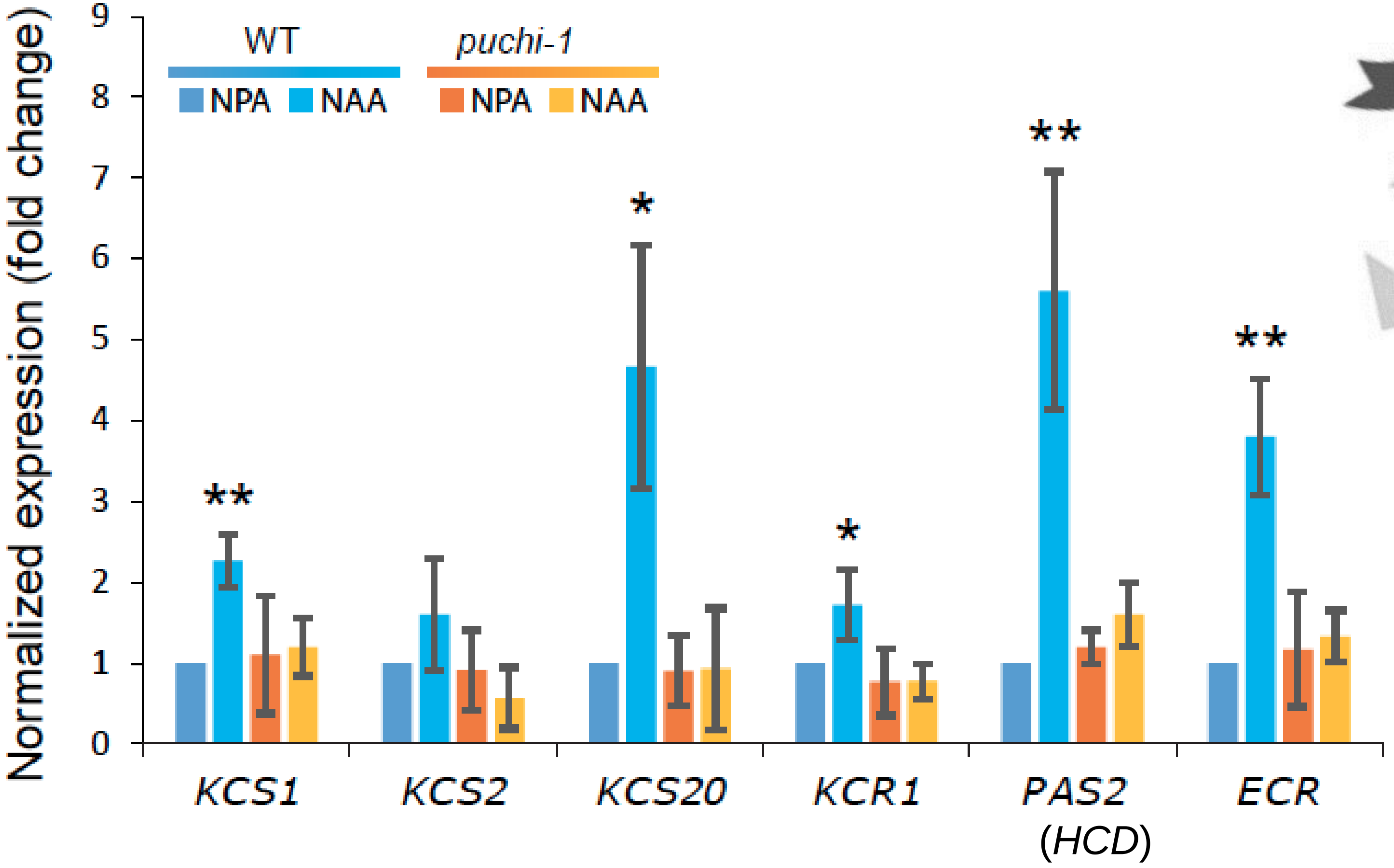


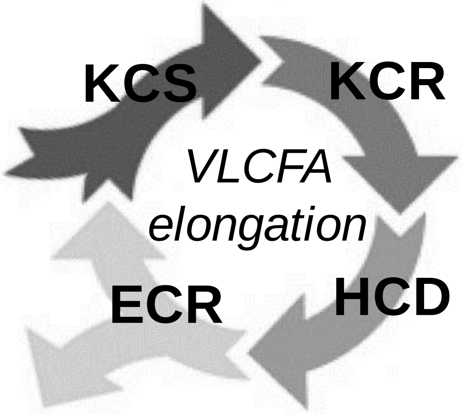
Loss of function mutation in
PAS1 (a FAE complex chaperonne)
Roudier et al., 2010



KCR1 RNAi
Beaudoin et al., 2009

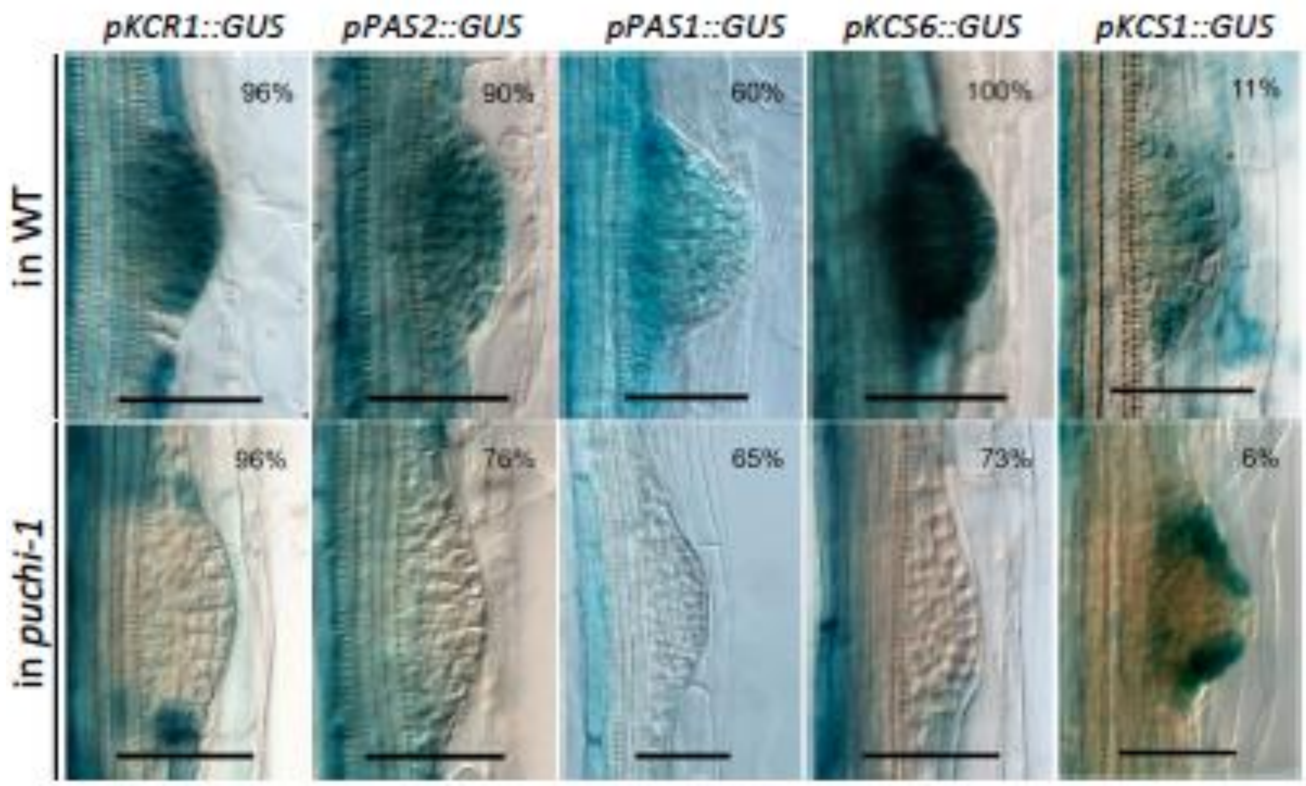
PUCHI upregulates VLCFA biosynthetic enzymes expression

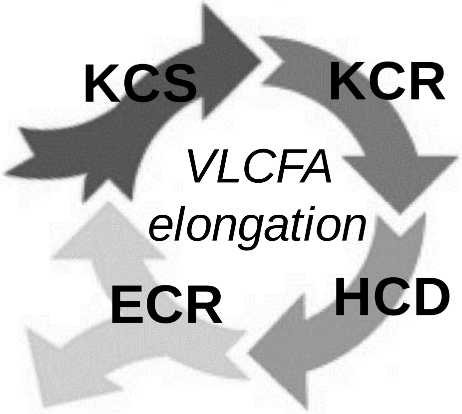




- During lateral root development (induced by NPA/NAA treatment) VLCFA biosynthetic enzymes expression are upregulated in a PUCHI-dependent manner

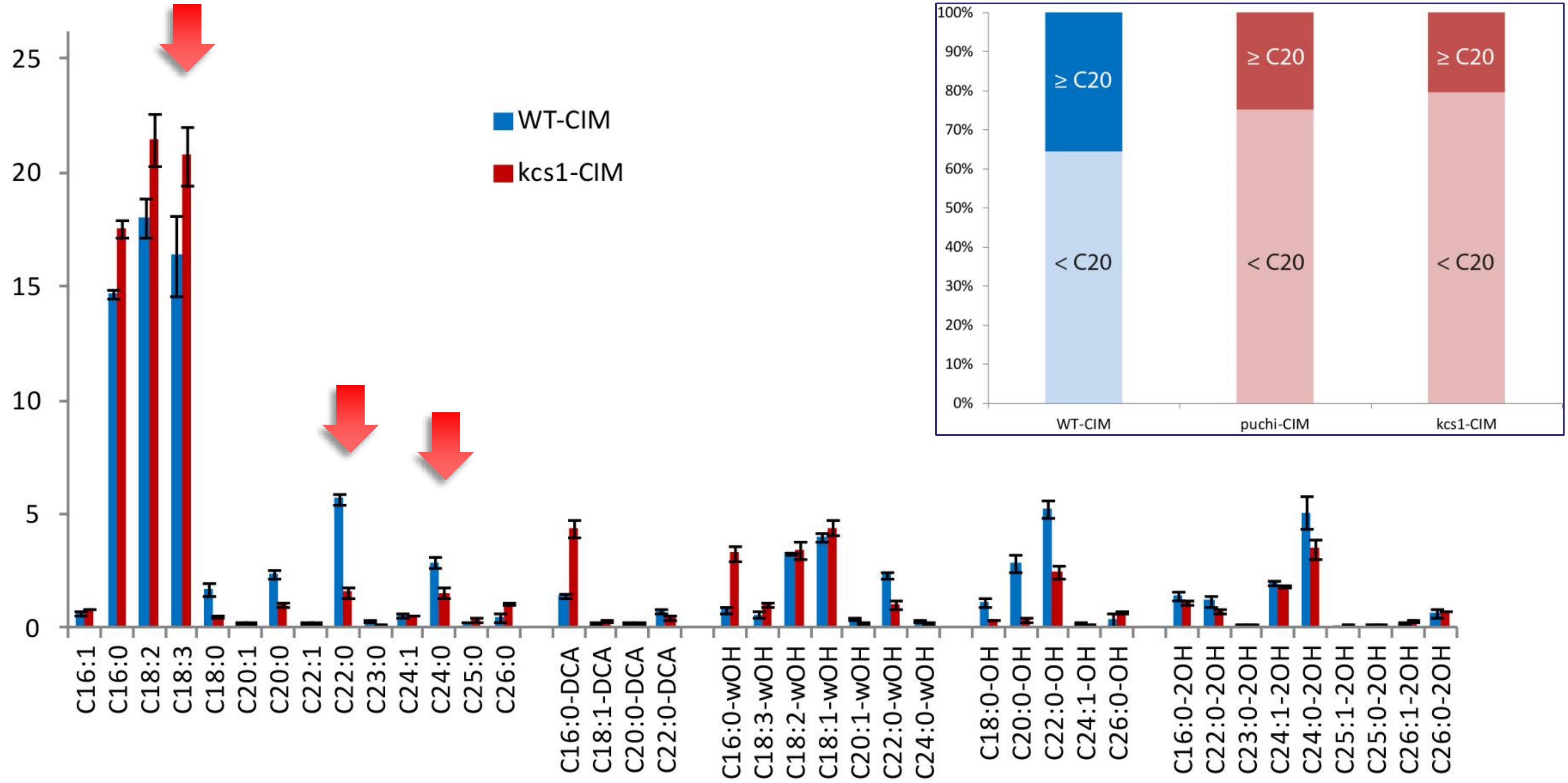
VLCFA biosynthetic enzymes expression in LRP is dependent on PUCHI




KCS
KCR
VLCFA elongation
ECR
HCD

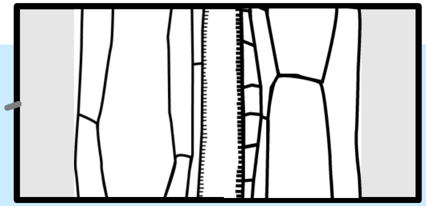
- Expression of VLCFA biosynthetic enzymes in wild-type developing lateral root primordia is dependent on PUCHI

PUCHI mutation impairs VLCFA contents in auxin-stimulated roots

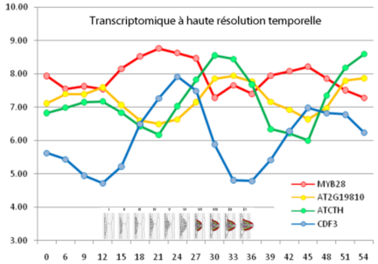


- ➢ Lipidomic profiling of roots grown of callus-induction medium showed a reduction in very long-chain fatty acid contents in *puchi* compared to WT.

A systems biology approach of the gene regulatory network operating during LRP development



Time course transcriptomics

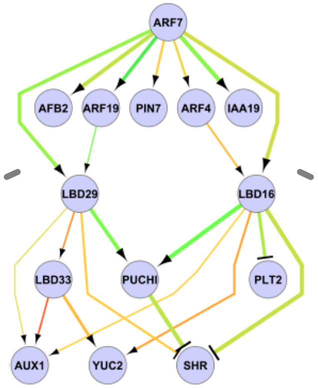


Gene network inference

$$\overline{Y}(t) \approx a.\overline{X}(t-\mu)+b$$

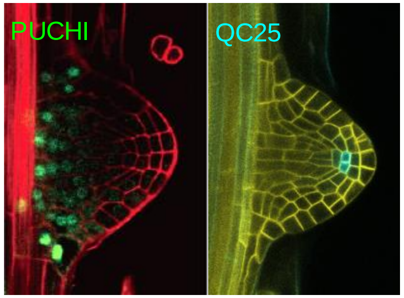
$$Q1 = \mathrm{cor}_{\mathcal{P}}(R^{1..18}, T_2^{1..18})$$

Gene network

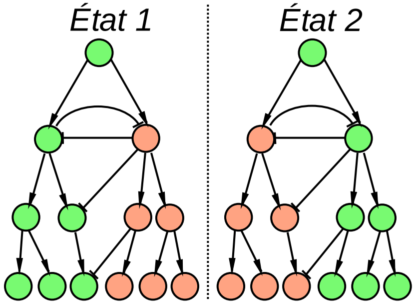


Network exploration and validation

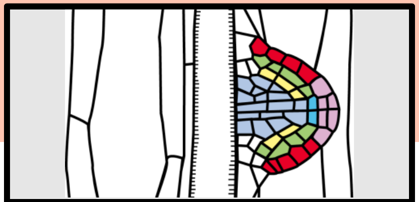
Spatialization



Formal analysis



Patterning properties



Gene regulatory networks for root branching

Arabidopsis - Rice

- Systems biology analysis of gene regulatory networks involved in root branching and patterning properties
- Functional analyses of master regulators

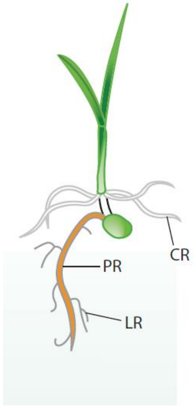
Rice - Pearl millet - Sorghum

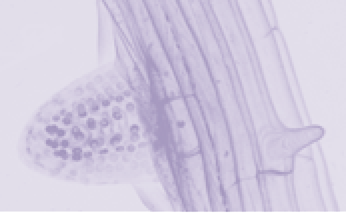
- Root architecture phenotyping of highly diverse lines
- Identification of regulatory loci and functional analysis

<ins>Gene regulatory networks with time and space information</ins>

Modeling mechanisms for root branching

Identifying gene subnetworks and master regulator genes for root-targeted plant breeding strategies





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* Univ. Kobe, lab. of Pr. H. Fukaki
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* ENS Lyon, lab of T. Vernoux
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* Equipe DAR, UMR AGAP, (CIRAD),
Montpellier

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Laurent Laplaze

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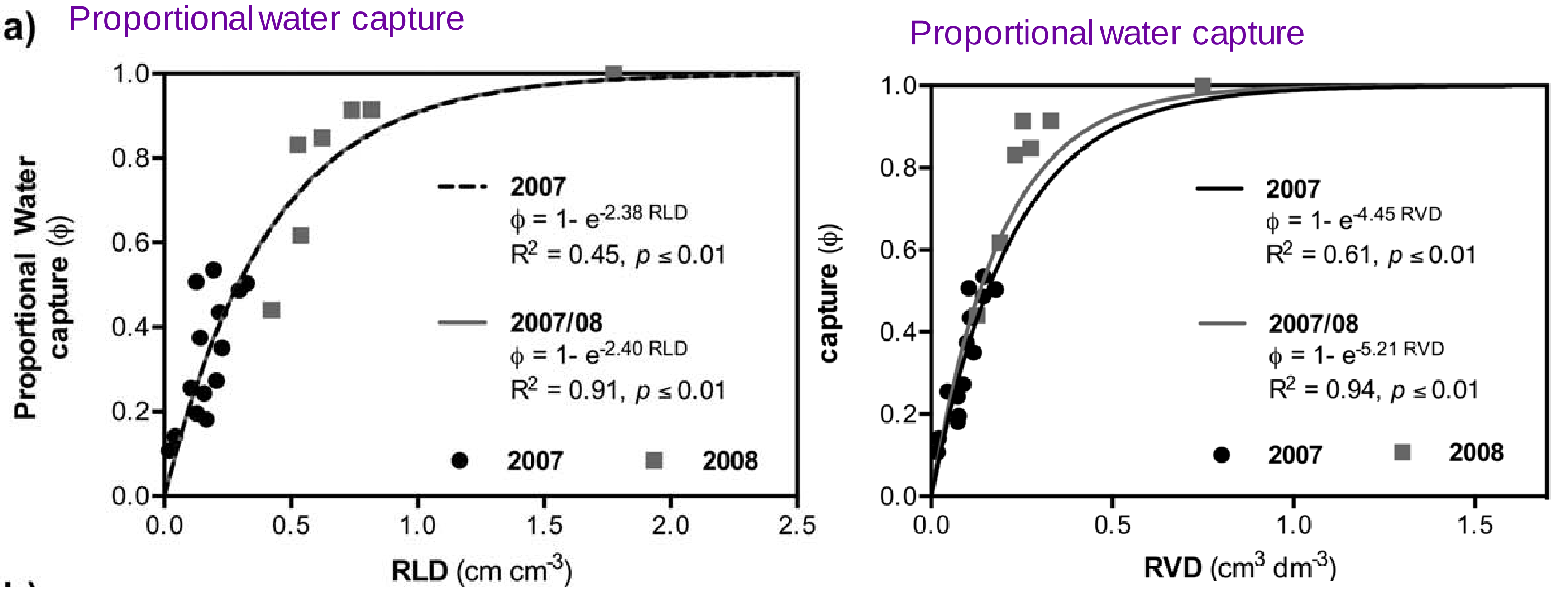
Julien Lavenus

Root system architecture influences plant nutrition



Proportional water capture

Proportional water capture

Root length average per cm^3 of soil

Root volume average per cm^3 of soil

Carvalho et al., 2013

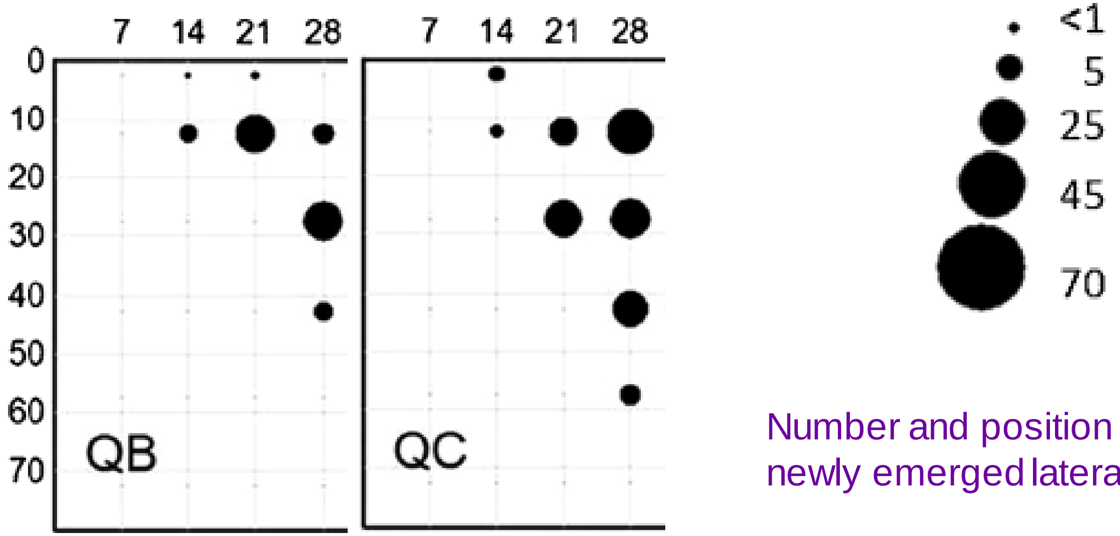
- Relationship between hydromineral nutrition (proportion of available water that is captured, φ) and root length density (RLD, cm/dm3) or root volume density (RVD, cm3/dm3) in the soil for barley (non irrigated)

Root system architecture is an important trait for plant adaptation and selection

Grown in rhizotrons in the same conditions

Quinoa from **dry** habitat

Quinoa from **wet** habitat



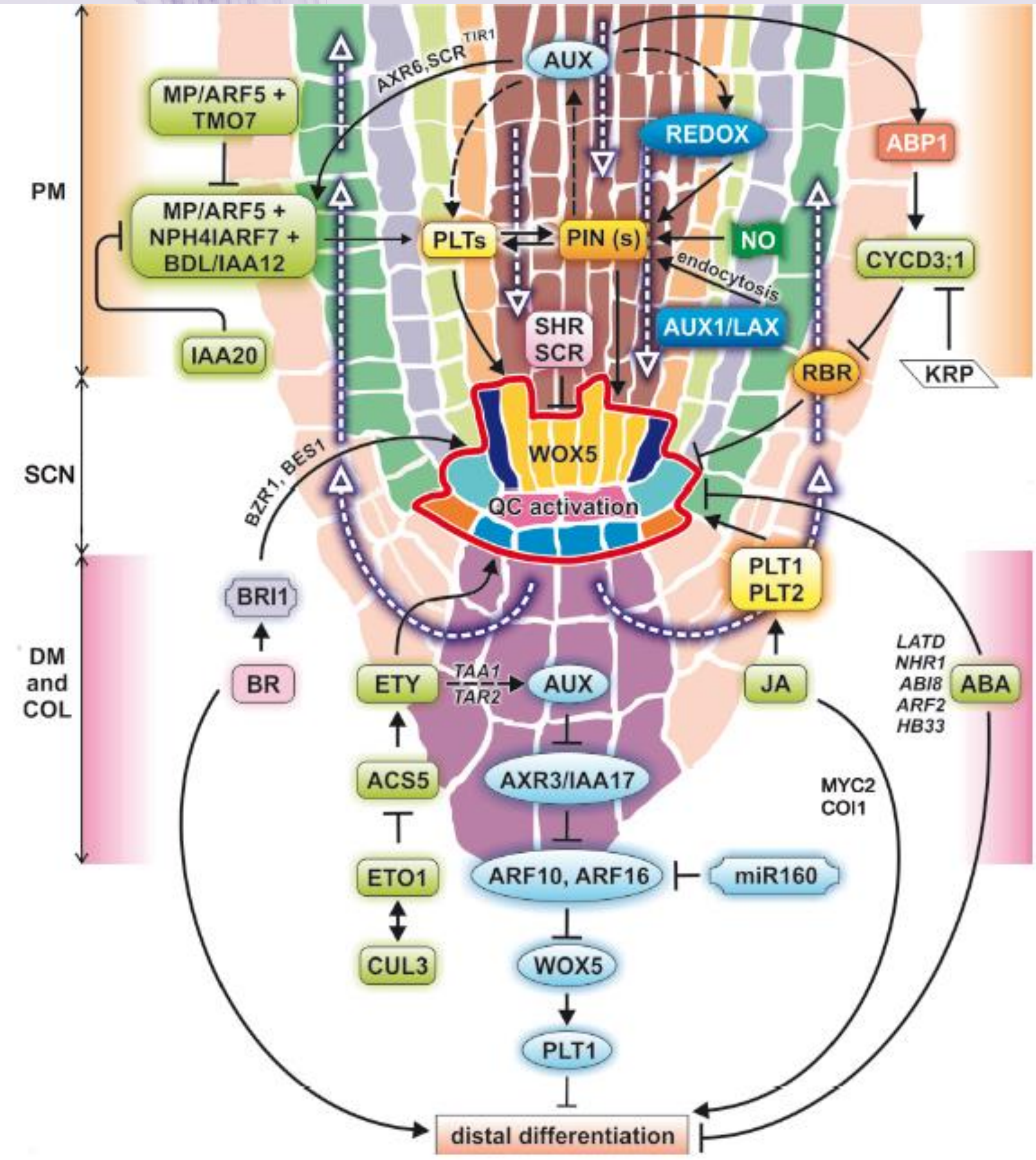
Depth (cm)

Number and position of newly emerged lateral roots

Alvares-Flores et al., 2014

- Exploration of different soil layers
- Balance between efficient soil exploration, water and ions capture, and carbon and energy cost.

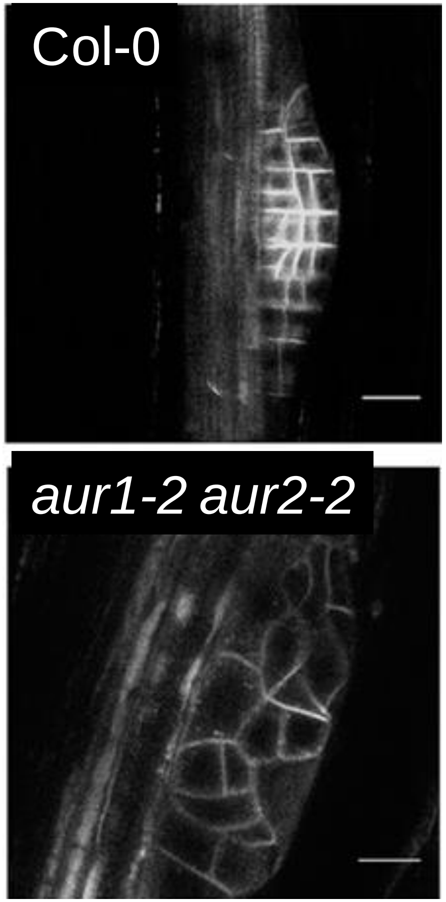
A wealth of genetic information is available on Arabidopsis root meristem regulation

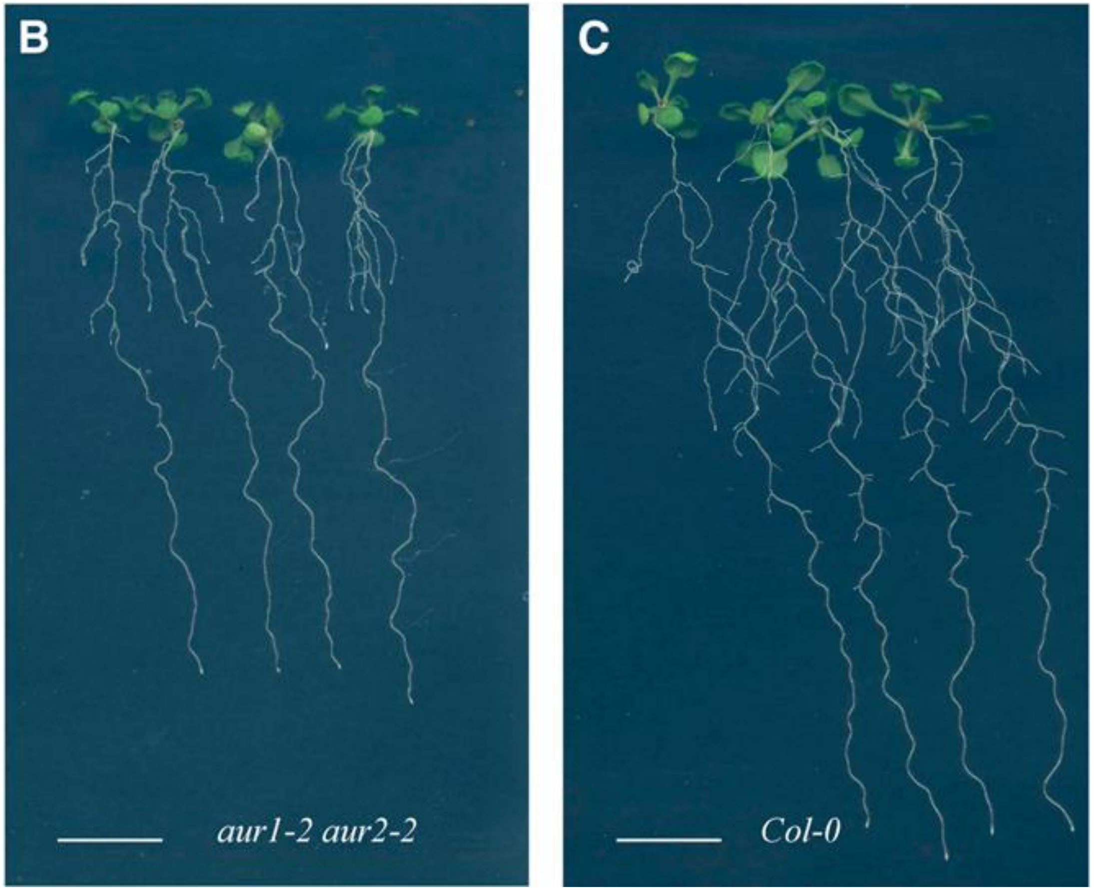


- ➢ Transcription factors such as WOX5, PLT, SHR, SCR
- ➢ Hormones and transducers
- ➢ Mobile peptides
- ➢ Cell-cycle regulators
- ➢ Chromatin modifiers

Lee et al. 2013

Functional organization of the LRP does not rely on a stereotyped cell division pattern

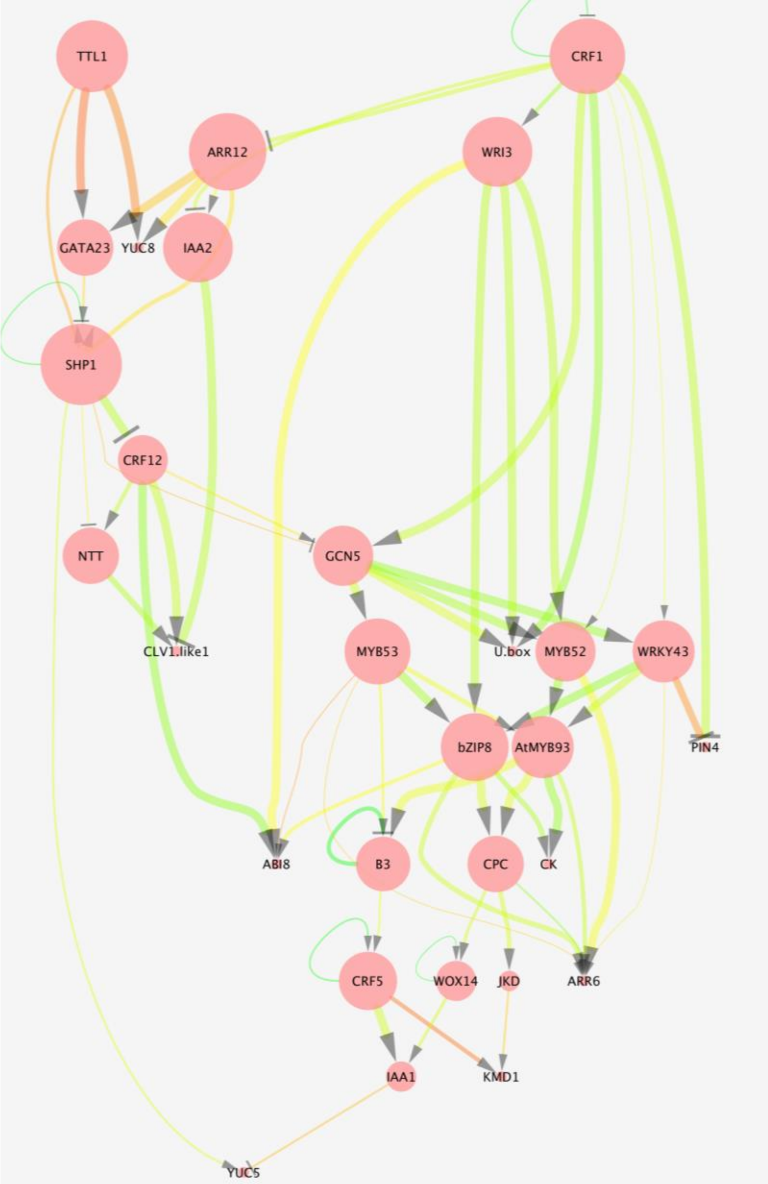




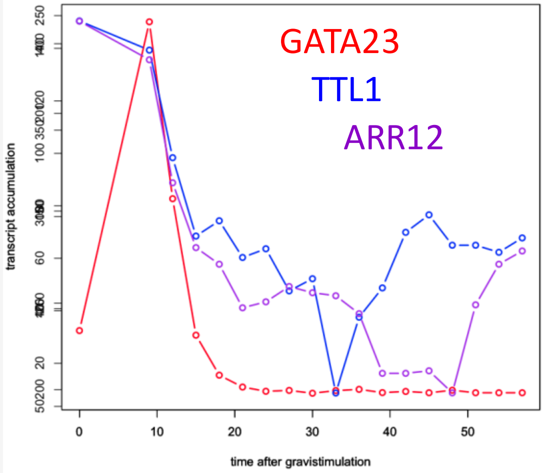
Vandamme et al., 2011

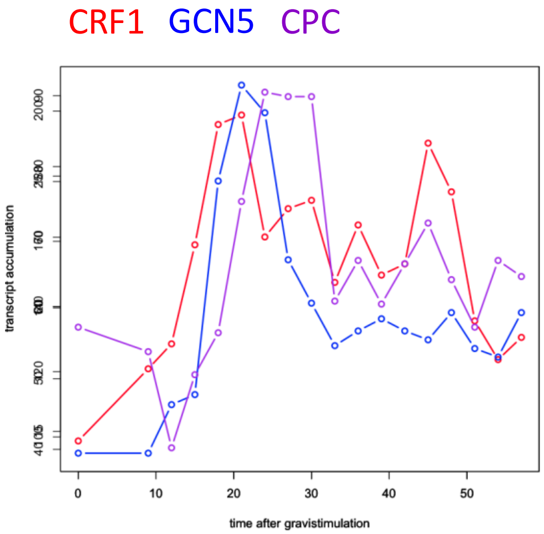
- *aurora* mutants, defective in the regulation of cell division orientation, are able to form functional lateral roots

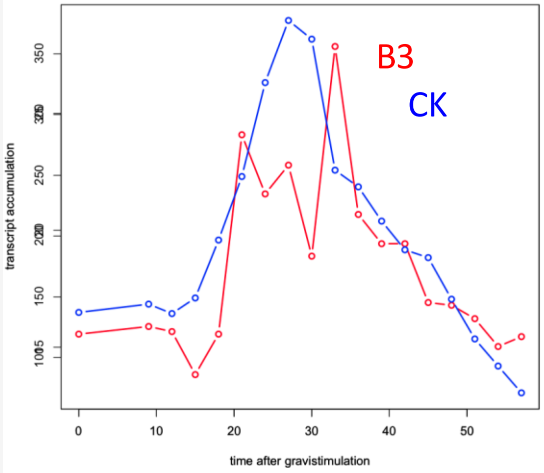
Group 3 : GATA23, CPC… a complex picture



Cytoscape/yfile/Hierarchical layout

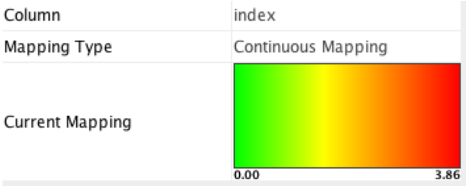






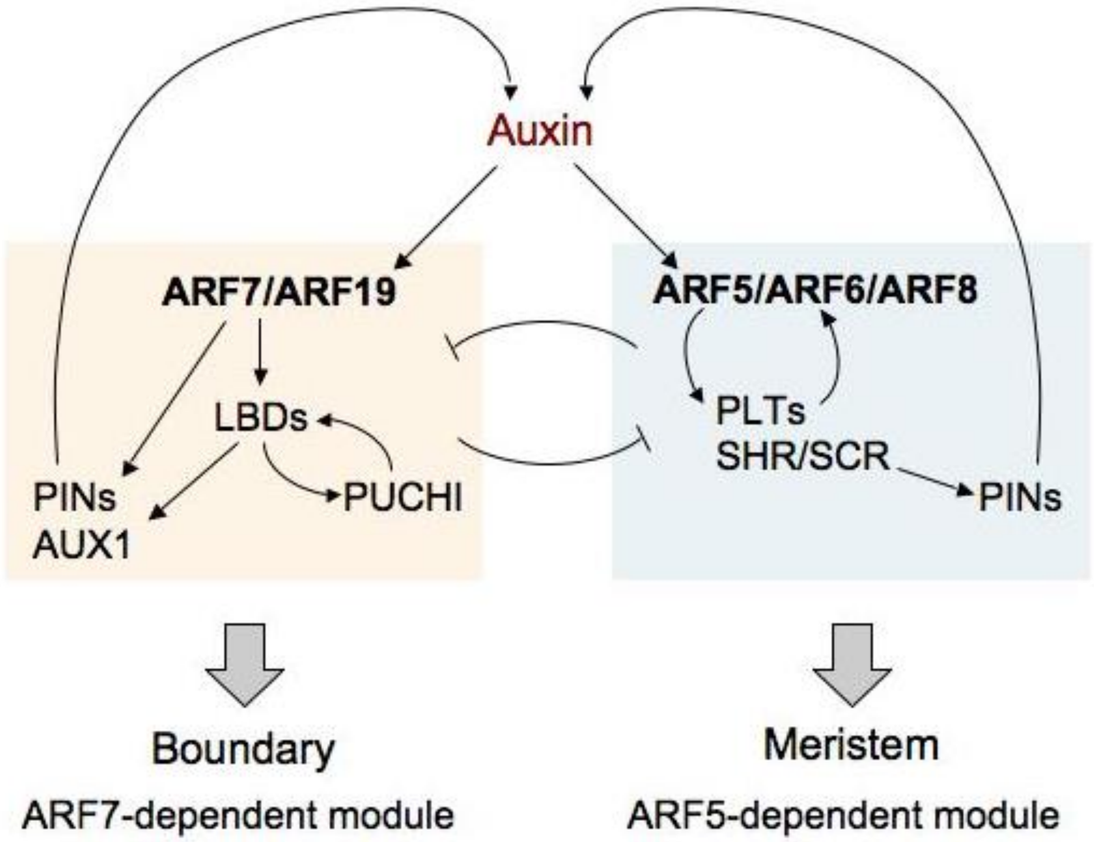
- ➢ A complex expression profile

Edge color: index of directness



Column	index
Mapping Type	Continuous Mapping
Current Mapping	0.00 … 3.86

The LR network is predicted to organize into two subnetworks with distinct crosstalk with auxin


Auxin

ARF7/ARF19

LBDs

PUCHI

PINs
AUX1

ARF5/ARF6/ARF8

PLTs
SHR/SCR

PINs

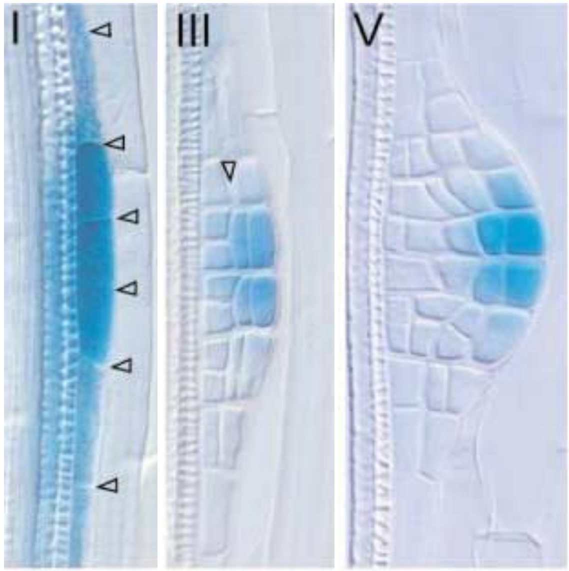
Boundary

ARF7-dependent module

Meristem

ARF5-dependent module

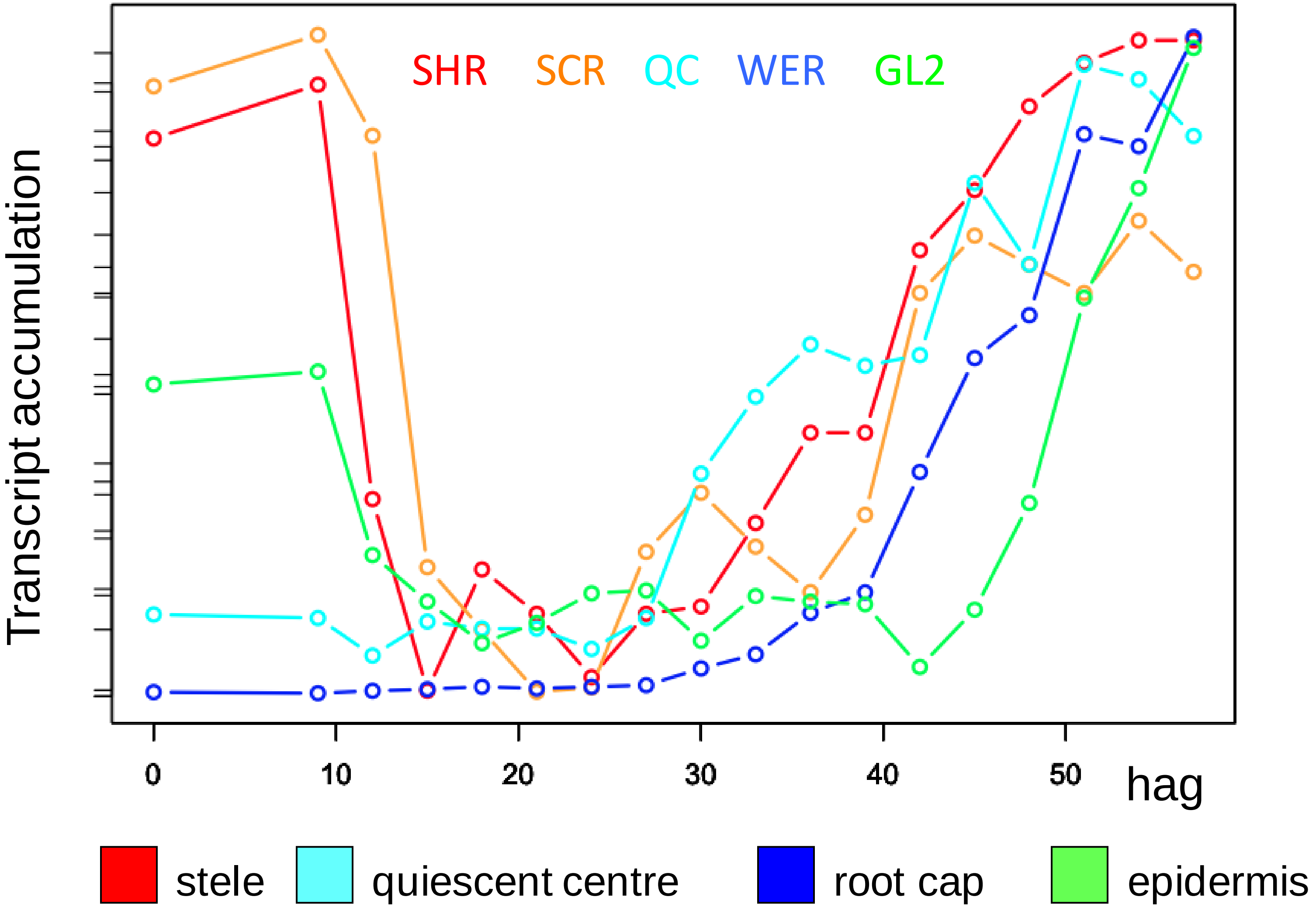
Auxin signaling (DR5::GUS)



Benkova et al., 2003

- Distinct crosstalks of each module with auxin distribution and signaling may contribute to progressive patterning of the lateral root primordium.

Use of marker genes to monitor LRP functional patterning



Use of marker genes to monitor LRP functional patterning

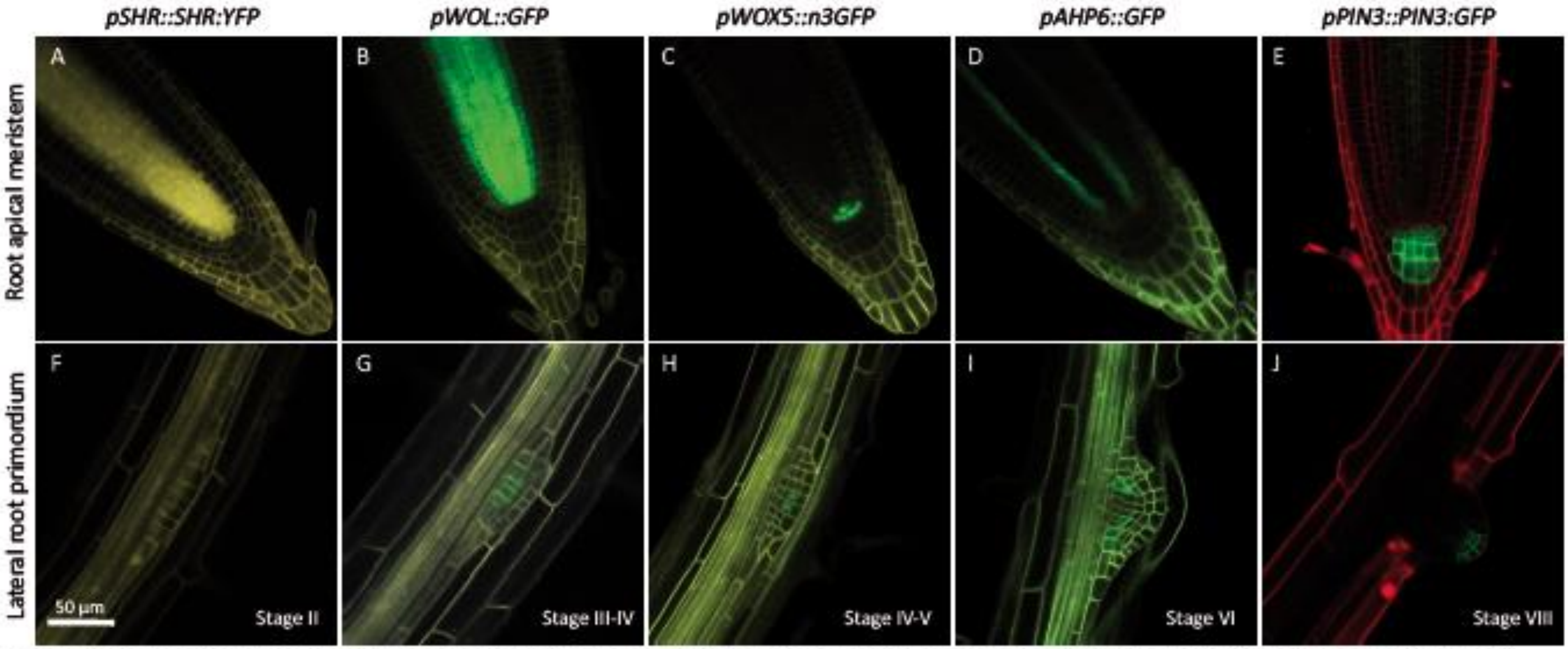
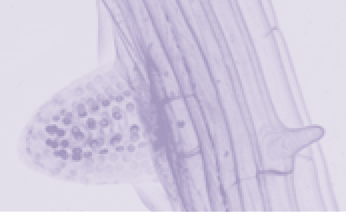
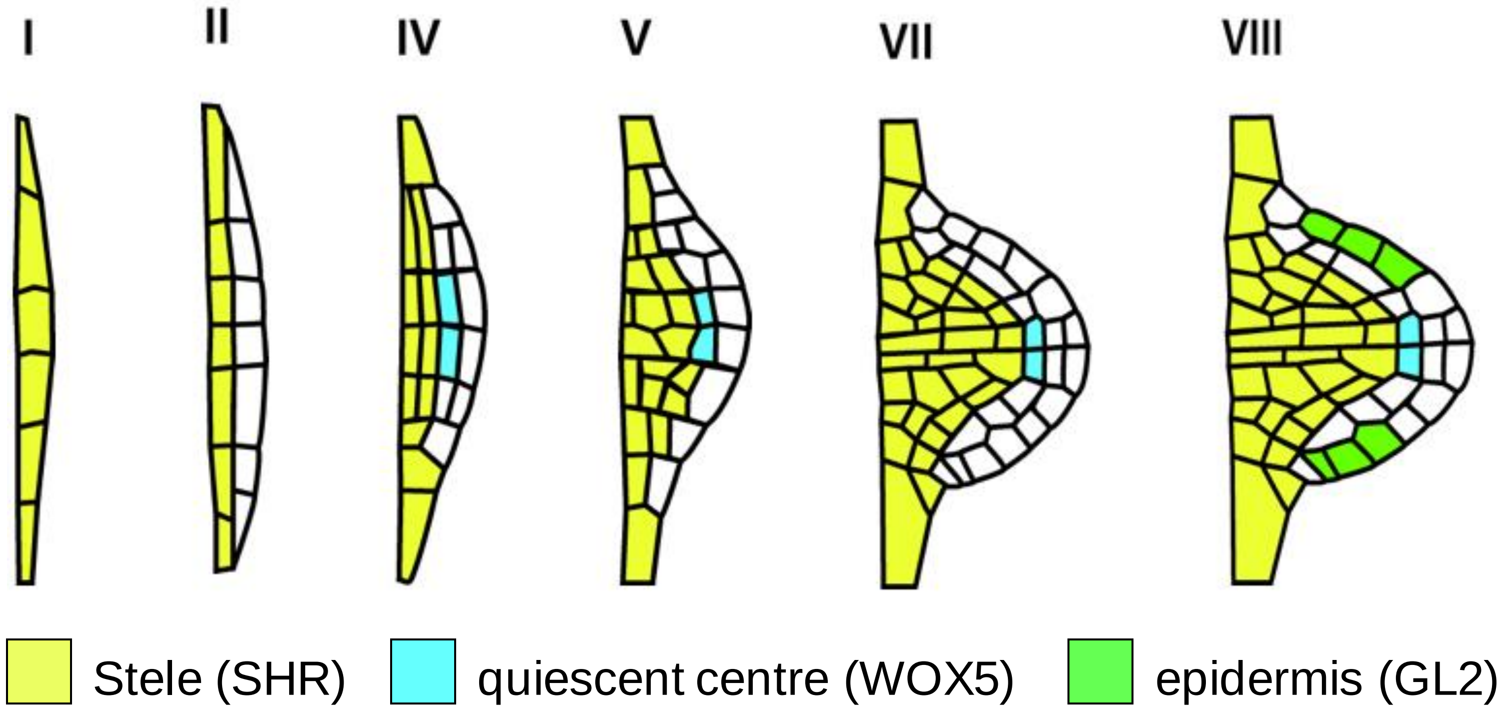


Figure 4. Expression pattern of selected reporter constructs during lateral root formation. Plasma membranes are labelled with WAVE131-YFP (A-D, F-I) or propidium iodide (E,J).



Gradual functional patterning of the LRP



- Critical changes in gene expression occur at stage I-II transition and stage IV (meristem formation phase), and early after emergence (expression of epidermis and root cap markers)

The LR transcriptomic dataset and the gene network inference: new tools to decipher the genetic regulation of lateral root development

➢ TDCor algorithm predicts candidate upstream regulators or downstream targets for a gene of interest

Analysis of the candidate subnetwork controlling stem cell niche establishment:

- Regulators of meristem formation ?

The LR transcriptomic dataset and the gene network inference: new tools to decipher the genetic regulation of lateral root development

➢ TDCor algorithm predicts candidate upstream regulators or downstream targets for a gene of interest

Analysis of the candidate subnetwork controlling stem cell niche establishment:

- Regulators of meristem formation ?

Identification of previously unknown function for a transcription factor:

- coordinated regulation of a whole metabolic pathway during LRP development

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- TDCor algorithm predicts candidate upstream regulators or downstream targets for a gene of interest

- TDCor algorithm predicts the topology of the network, allowing to identify putative hubs and master regulators in lateral root development despite functional redundancy

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- The inferred topology of the network can be subjected to *in silico* (and *in vivo*) analysis in order to identify the properties of this network that may control the functional patterning of the developing root primordium

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- Importantly, this algorithm (in R, available on the CRAN website) can be run for the analysis of any transcriptomic time-course series, provided suficient number of time points (> 10) and short time steps (ca 3h).