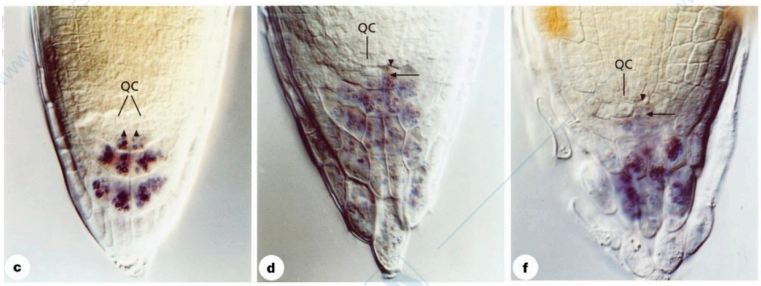
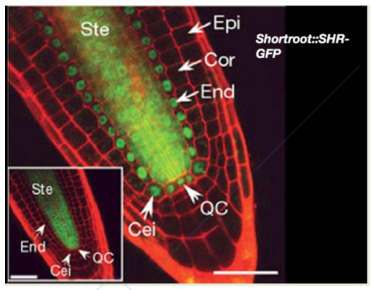
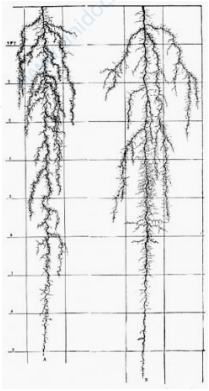
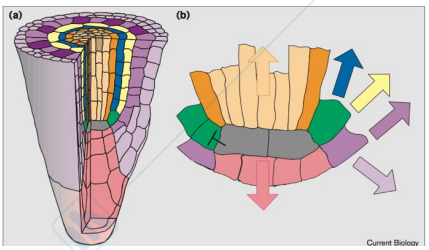


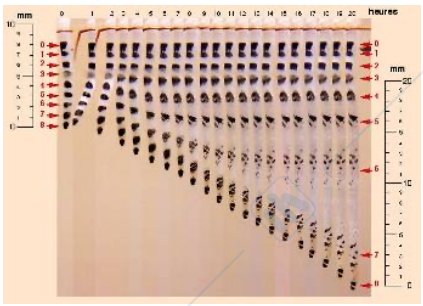


Development from meristems: Root apical meristem

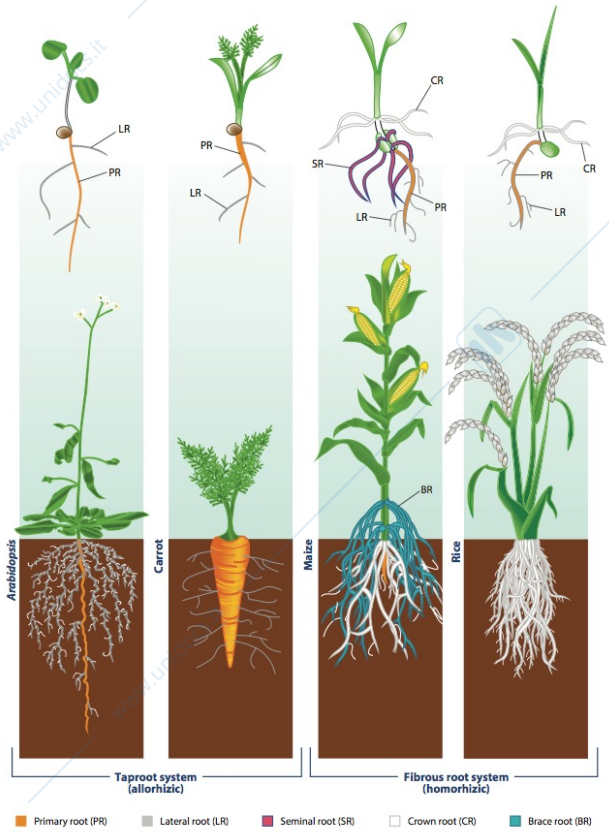
G. Tichtinsky
gabrielle.tichtinsky@univ-grenoble-alpes.fr







Root systems

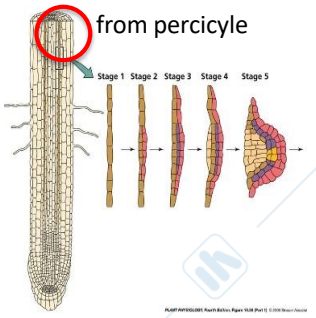


Producing roots?

PRIMARY ROOT

from the embryonic radicle

LATERAL ROOTS



+ endodermis (grasses)

ADVENTIVE ROOTS

from cells close to vascular tissues

Primary root

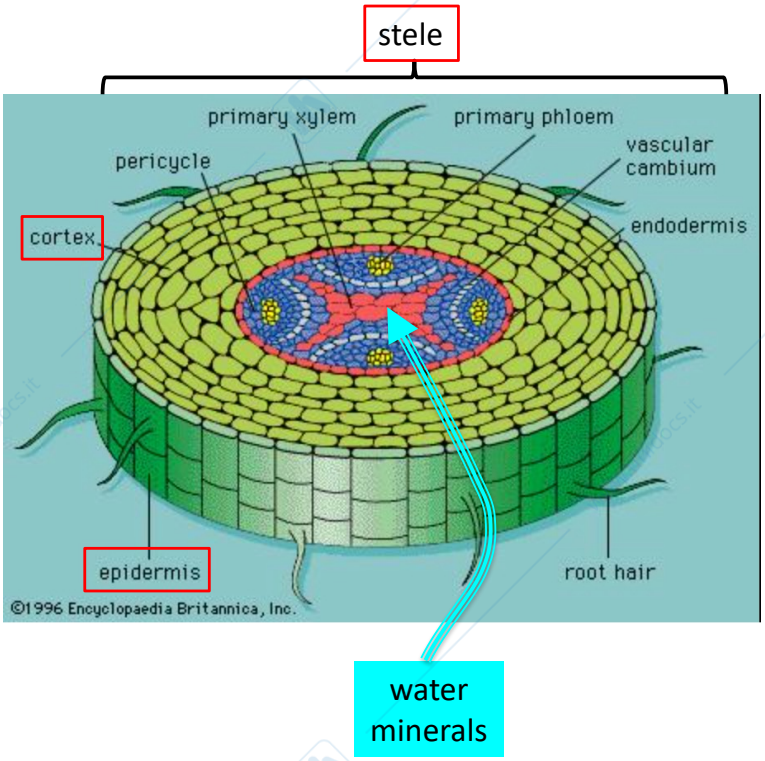
Lateral root

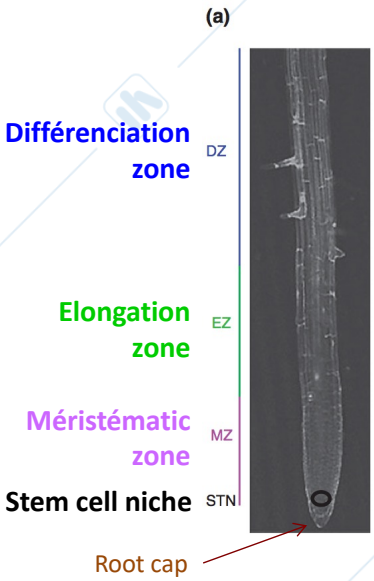
Adventitious roots

Local auxin maxima induce root production

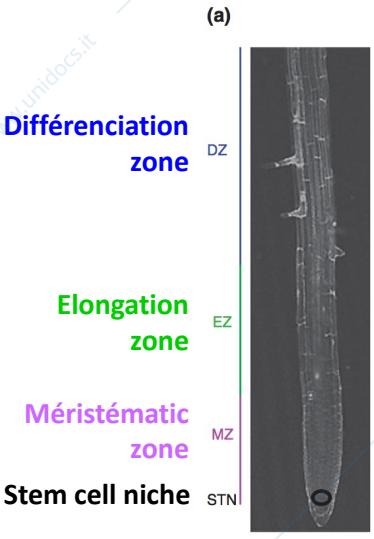
Bellini *et al.*, 2014

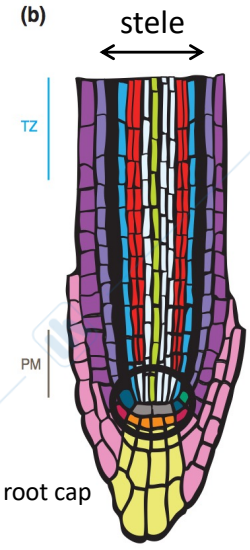
The root structure



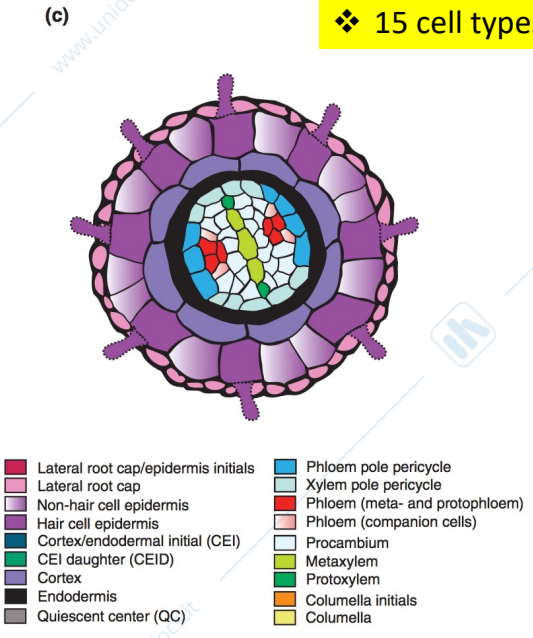


The root structure





PM : proximal meristem (division)
TZ : transition zone (cells start to elongate)



❖ 15 cell types

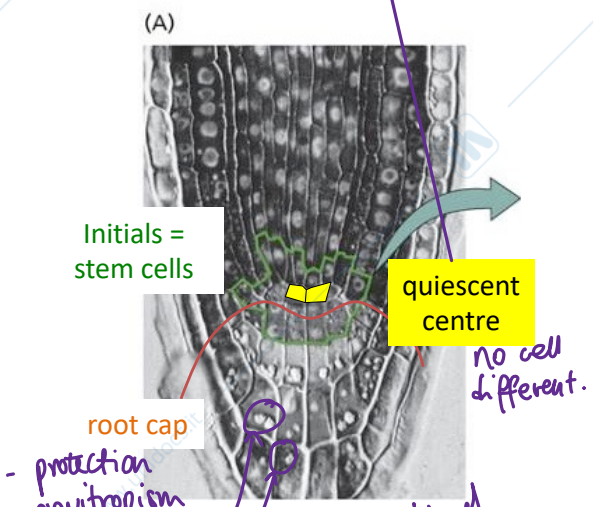
➢ Setting up a regular organization?

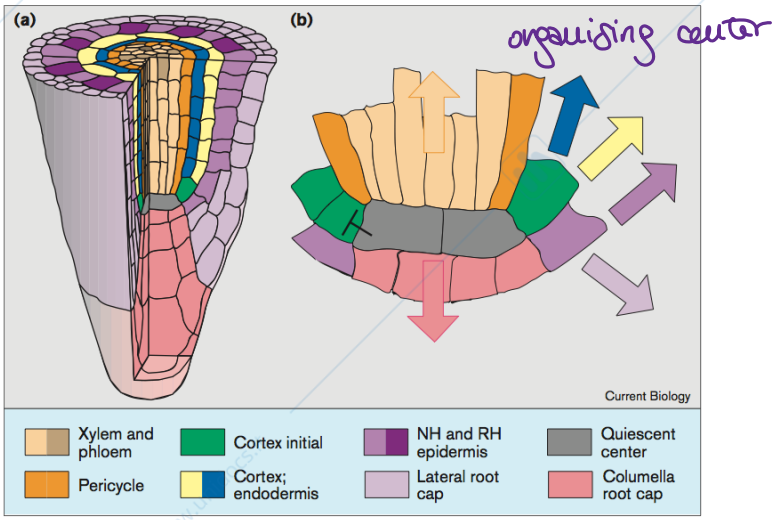
➢ Specifying cellular identities?

Cederholm *et al.*, 2012

- mitotically active cells - maintain root stem cells (no cell differentiation) + generate non-cell autonomous signals (prevent diff.)
- low proliferation rate

The root meristem





Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.
Benfey, 2000

Division planes

Dolan *et al.*, Development, 1993

fight control cell division

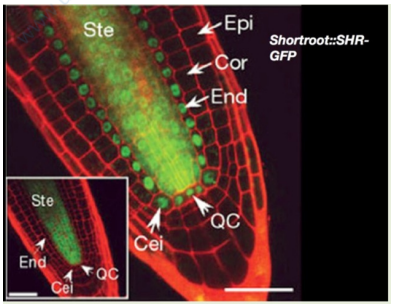
perpendicular to tip of root

30 years of Arabidopsis root as a developmental model

Tools to study root development

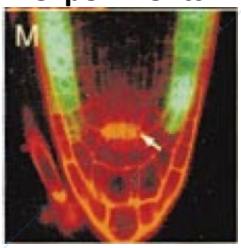
non invasive observations

Confocal microscopy



Laser ablation experiments

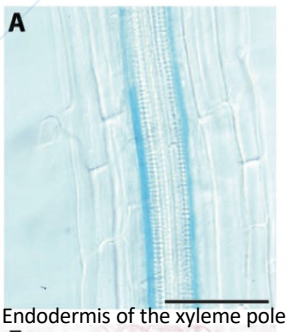
destroy specific cells



Enhancer trap lines ➢ Specific expression patterns

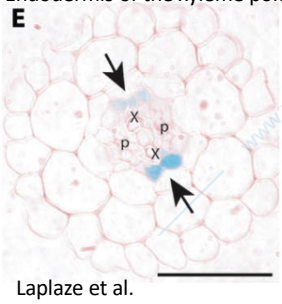
random insertion in the genome & by how it would insert before a gene will specific function

stain special cell identities
↳ where are specific cell types



Endodermis of the xyleme pole

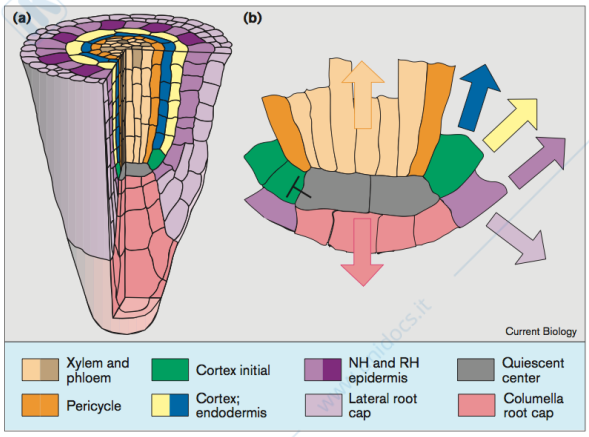
Quiescent centre



Laplaze et al.

➢ Setting up / maintaining a regular organization?

1.
Function of the quiescent centre



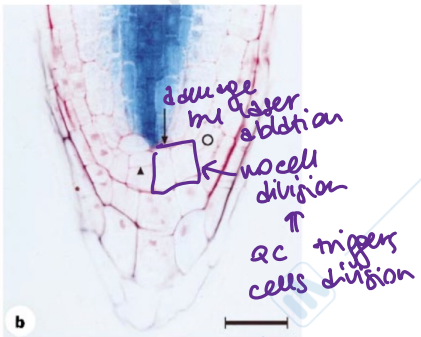
Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.

Benfey, 2000

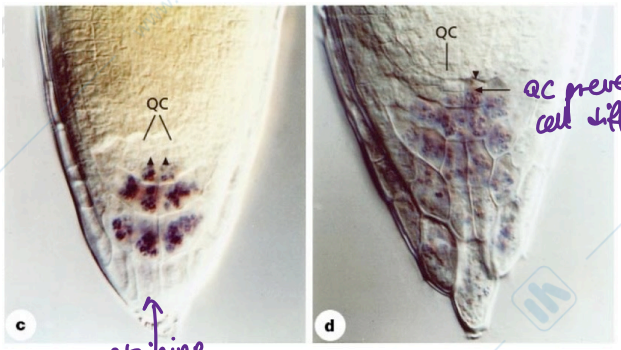
The function of the quiescent centre

Laser ablation experiments:

Elimination of one cell of the quiescent centre…



…prevents cell division



… allows differentiation

➢ **stimulation of adjacent initial cells division**
➢ **inhibition of adjacent initial cells differentiation**

give stem cell identity

The quiescent centre is an organizing centre preventing stem cell differentiation

→ non cell autonomous effect

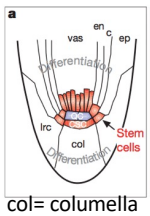
➢ Diffusion of a short range signal

Van den Berg *et al.*, Nature, 1997

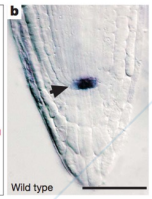
The function of the quiescent centre

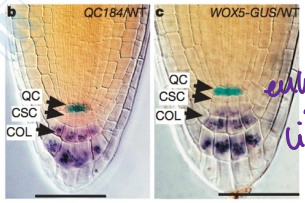
WOX5 controls columella différentiation [wus/clv]

WOX5 : Specific marker of the quiescent centre

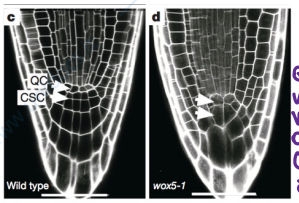


col= columella



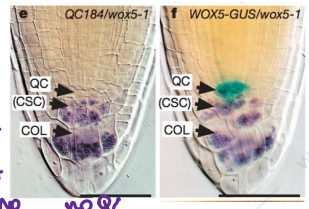


enhancer trap lines



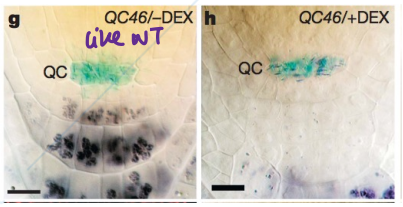
wox5 mutant

QC not well orga. & cells start dividing



no QC marker
↳ no QC

Inducible over-expression of WOX5



* GR = glucocorticoid receptor

inducible DEX system
↓
expression of wox5 35S promoter (but has to be induced bc it destroys cell so fused w/ GR
will remain in cytoplasm
↑
ligand for GR is DEX - w you add it will bind & will en in the nucleu

WOX5 prevents stem cell differentiation

- WOX5 is sufficient to **inhibit** differentiation of columella cells
- WOX5 **inhibits**, with SCR, differentiation of initial cells for the stele

Sarkar *et al.*, 2007

- WOX5 : Transcription factor of the WOX (*WUS RELATED HOMEOBOX) family*

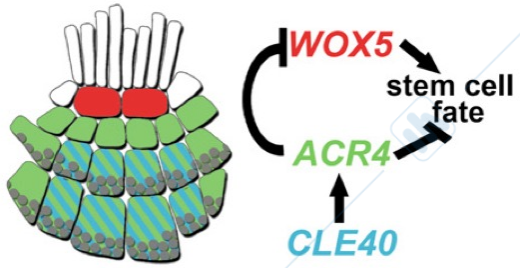
↳ in cytosol IS inactive
↳ presence of wox5 prevents cell differentiation

The function of the quiescent centre

study via reverse genetics

The quiescent centre functions through a CLV/WUS type regulation loop

H



receptor like kinase

RLK / signal peptide **signalling module** restricting WOX5 expression to the quiescent centre

- In the columella cells, CLE40/ACR4 inhibits WOX5 expression.

- **WOX5**: *WUS RELATED HOMEOBOX* transcription factor
- **CLE40** : CLE peptide (CLV3/EMBRYO SURROUNDING REGION family) ← below QC
- **ACR4** : Receptor kinase

↳ triggers cellular differentiation (see below)

Stahl *et al.*, Current Biol, 2009

The function of the quiescent centre

WOX5 is regulated *via* a RLK / signal peptide signalling module

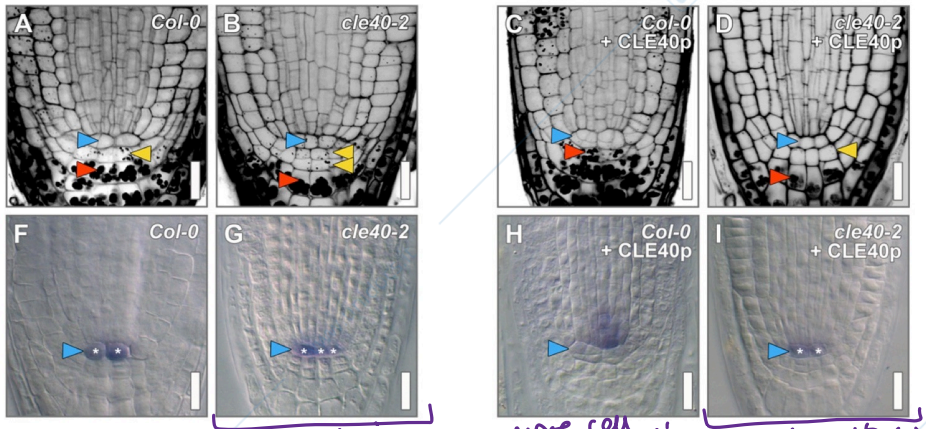
receptor like kinase

Expression of CLE40 — peptide

pCLE40:CLE40-GFP
below QC

cle40 mutant

CLE40 over-expressor



less cell differentiation

more cell differentiation

complementation (see if construct for overexpression works)

Stahl *et al.*, 2009, 2012

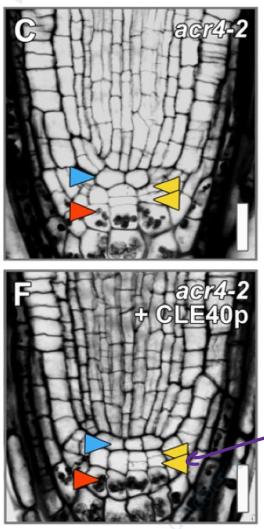
- WOX5 : Transcription factor of the WOX (*WUS RELATED HOMEOBOX) family*
- CLE40 : peptide of the CLE family (CLV3/EMBRYO SURROUNDING REGION)

ARC4a receptor for CLE40

The function of the quiescent centre

WOX5 is regulated *via* a RLK / signal peptide signalling module

Mutants ***acr4***

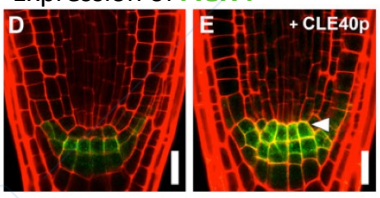


CLE 2 ACR work together

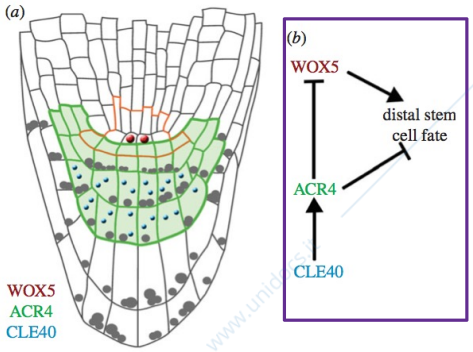
cannot be restored w/ peptide

CLE19 needs receptor for the signal

Expression of ACR4



expressed where it shouldn't be - overexpression of RLK



Stahl *et al.*, 2009, 2012

- WOX5 : Transcription factor of the WOX (*WUS RELATED HOMEOBOX) family*
- CLE40 : peptide of the CLE family (CLV3/EMBRYO SURROUNDING REGION)
- ACR4 : Receptor kinase

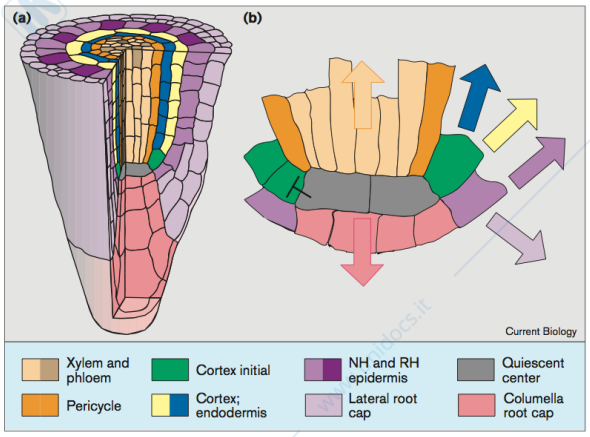
➢ Setting up / maintaining a regular organization?

1.
Function of the quiescent centre

➢ organizing centre

2.
Positional control of the root mersitem location

3.
Embryonic establishment of the root meristem



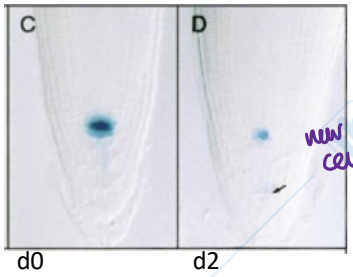
Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.

Benfey, 2000

2.
Positional control of the root meristem

Laser ablation of the quiescent centre

Quiescent centre marker line

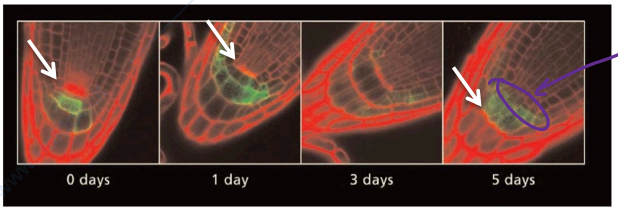


Destroy completely quiescent center

new quiescent center

- Repositioning of the stem cell niche just above the affected zone
- Relocalization of a new auxin maximum

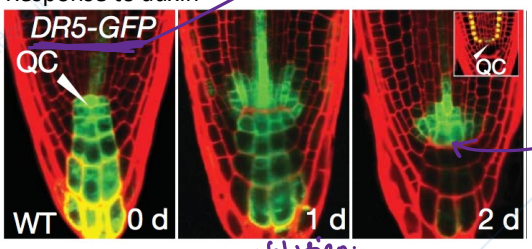
Columella marker line



new QC

Response to auxin

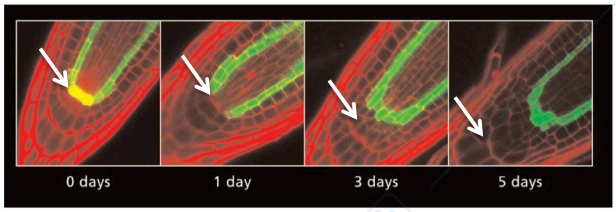
response to auxin



auxin seen where new QC is formed

laser ablation: auxin everywhere

PromSCR:GFP (endodermis and quiescent cntre marker line)



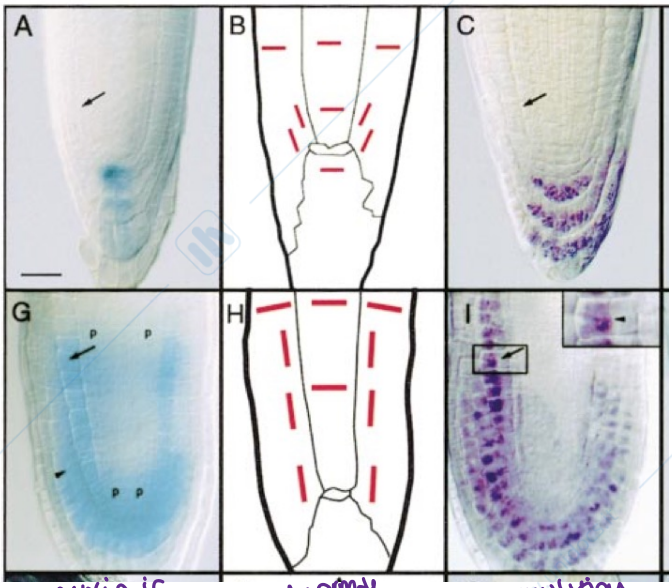
Sabatini *et* al., 1999, Grieneisen *et al.*, 2007

if destroyed, the QC can be restored by repositioning of stem cell niche

Auxin and root patterning

reporter gene

DR5 Divisions Starch



disruption of auxin fluxes

+ NPA
(polarized auxin transport inhibitor)

auxin is present everywhere

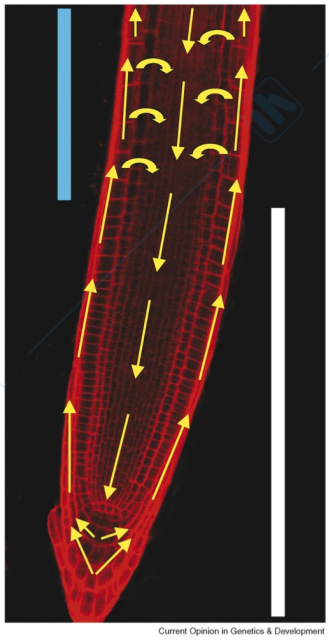
abnormal division

accumulation everywhere

auxin important for patterning

Control by auxin of the quiescent centre position

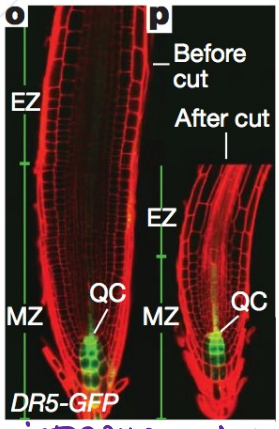
Auxin flux



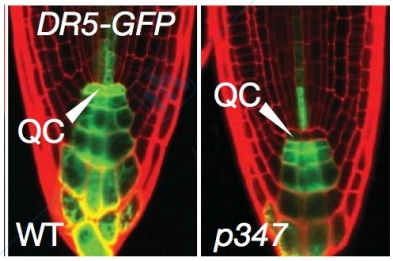
strong accumulation

auxin

cycling fluxes



Auxin flux induces an auxin maximum



pin3 pin4 pin7

Maintenance of auxin flux even w/o plant → stable fluxes in roots

explains how you can reinstate QC

✧ Polarized transport induced **auxin flux** in the root:

- Localises a very stable auxin maxiumum ;
- Induces an auxin gradient.

✧ The **auxin repartition** in the root:

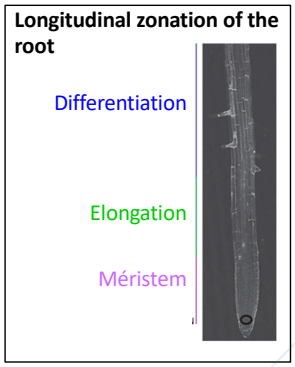
- localises the quiescent centre ;
- triggers a longitudinal zonation.

Petricka *et al.*, 2008, Grieneisen *et al.*, 2007

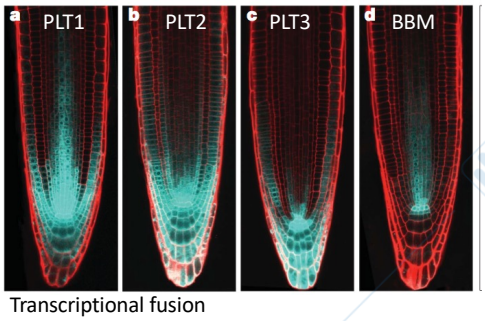
mediators

PLETHORA (PLT) regulators

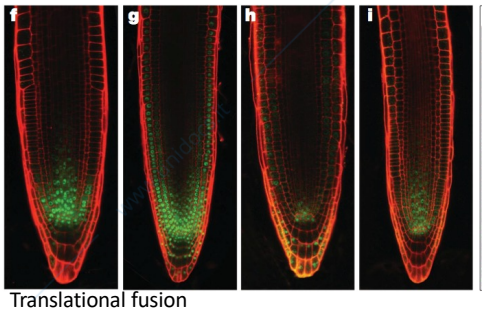
- ❖ AP2 type transcription factors
- ❖ Multigenic family



A gradient expression



Transcriptional fusion



Translational fusion

Meristematic activity depends on PLT expression levels:

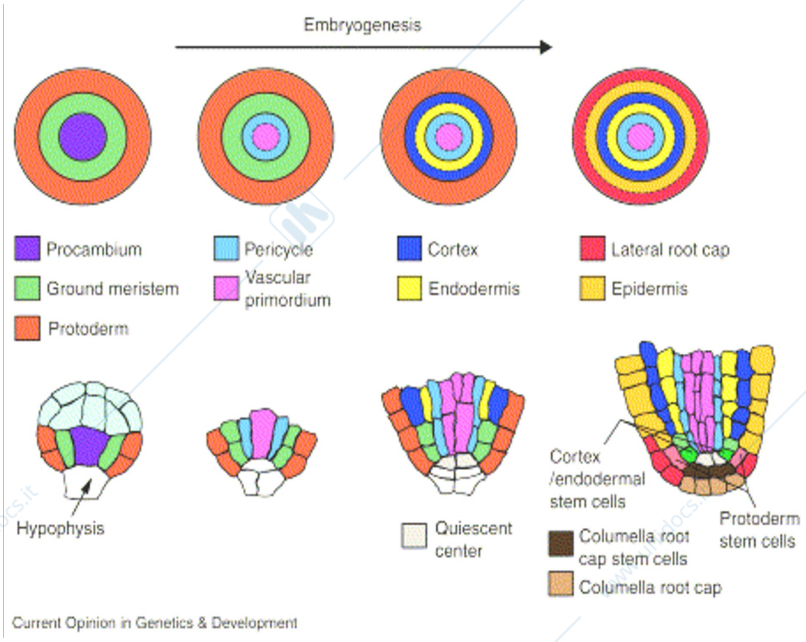
- Low level > differentiation
- Intermediate level > mitotic activity
- High level > identity and maintenance of stem cells

➢ **Control of the longitudinal zonation of the root meristematic region**

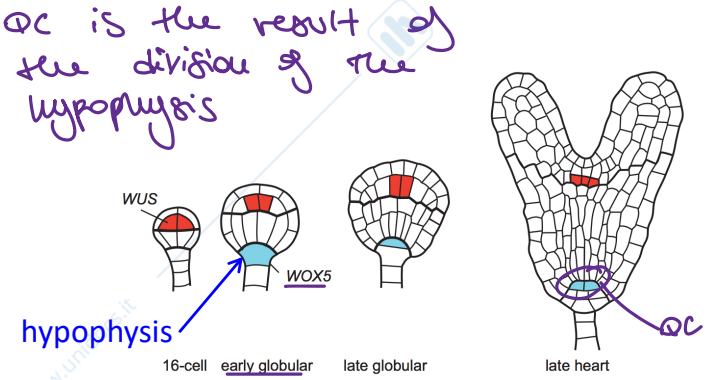
Galinha *et al.*, 2007

3.
Embryonic establishment of the root meristem

Progressive specification of root tissues in the embryo



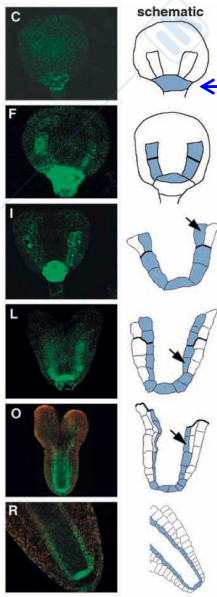
Early embryonic root markers



QC is the result of the division of the hypophysis

wox 5 expressed @ globular stage

SCR expression
(specification of the endodermis)



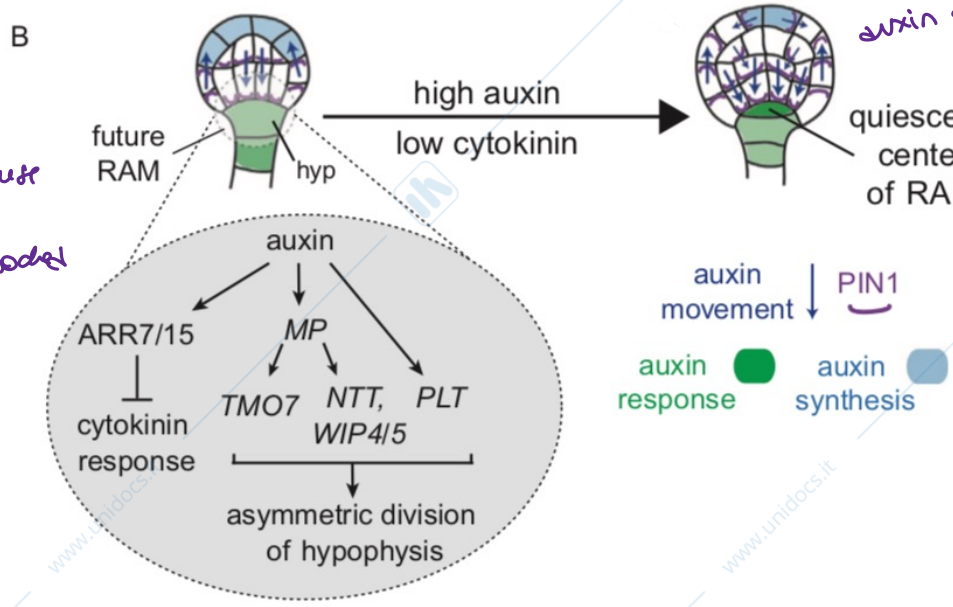
will start endodermis @ QC during embryo development

↓ established very early during development

➢ Spécification of the hypophysis?

Sarkar et al. 2007 ; Wysocka-Diller *et al.*, 2000

Auxin and root development during embryogenesis



auxin accumulation

SAM needy cytokine response
↓
so this is blocked in RAM

Armenta-Medina and Gillmor, 2019

➢ Setting up / maintaining a regular organization?

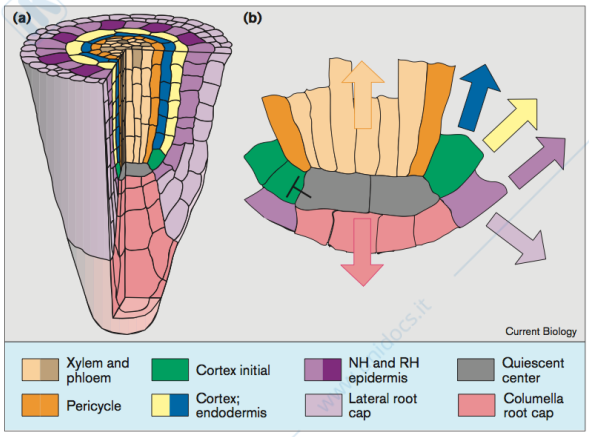
1.
Function of the quiescent centre

➢ organizing centre

2.
Positional control of the root mersitem location

3.
Embryonic establishment of the root meristem

➢ mediated by auxin fluxes



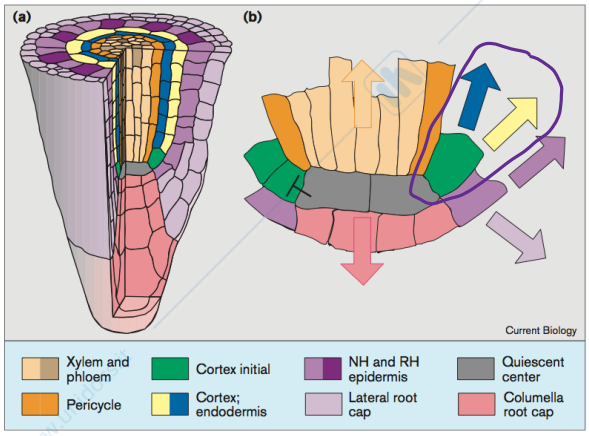
Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.

Benfey, 2000

➢ Specifying cellular identities?

4.
Cortex / Endodermis specification

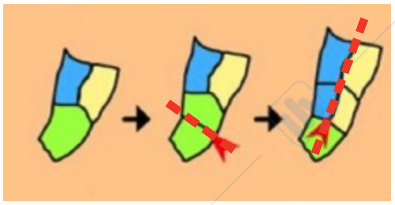
Cortex / endodermis specification



Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.

Benfey, 2000

Asymmetric cell division pattern of the cortex/endodermis initial



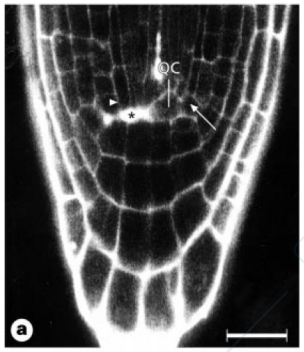
1° longitudinal
2° perpendicular (divide that cell in two)

1. Anticlinal division of the initial > regenerative division
2. Periclinal division of the initial daughter > proliferative division

Under control of the quiescent centre

QC laser ablation…

… triggers early periclinal division

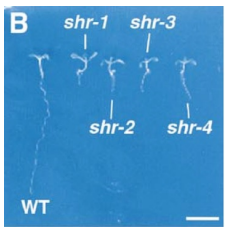


Control division of cortex initial

Cortex / endodermis specification

scarecrow and *short-root* mutants

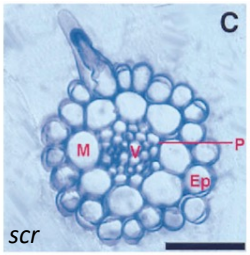
direct genetic approach

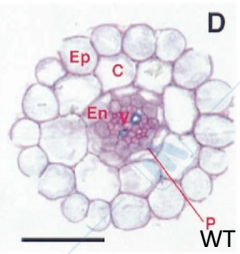


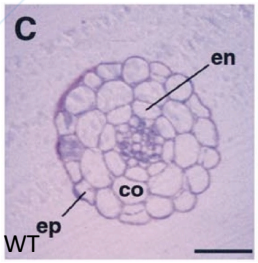
instead of cortex and endo you have one cell layer

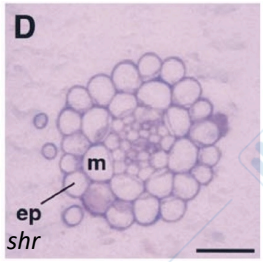
↓

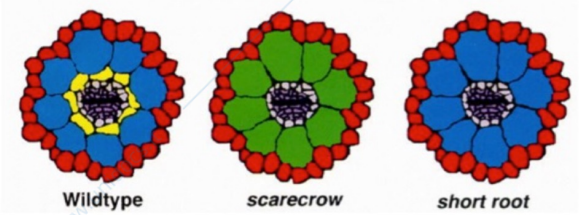
no clear differentiation











Endodermal + cortex identity

intermediate identity

Endodermal identity lost

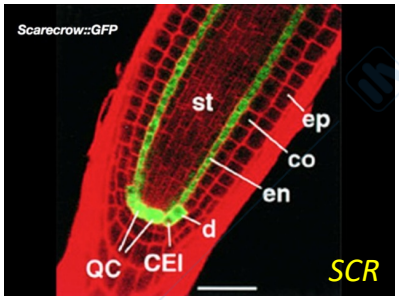
- Control of the **division** of the cortex/endodermis initial by **SCR and SHR**
- Control of **the endodermis identity by SCR**

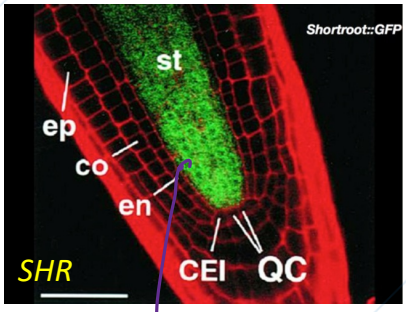
❖ **GRAS transcription factors**

Helariutta *et al.*, Cell, 2000 ; Di Laurenzio *et al.*, Cell, 1996 ; site web de J. Haselhoff

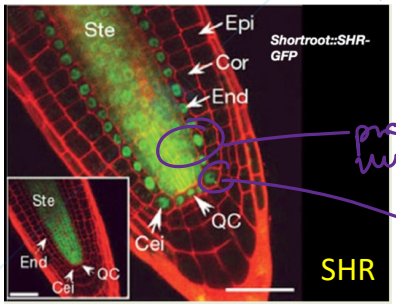
Cortex / endodermis specification

Expression of SHORT-ROOT and SCARECROW

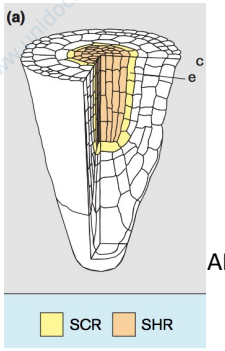




Transcriptional fusions

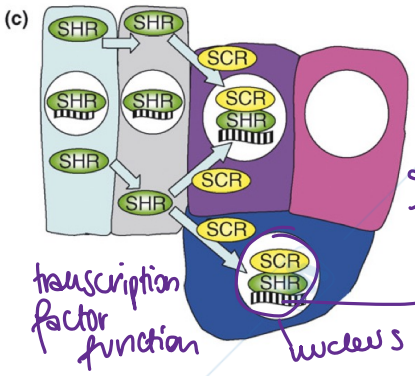


Translational fusion



ARN

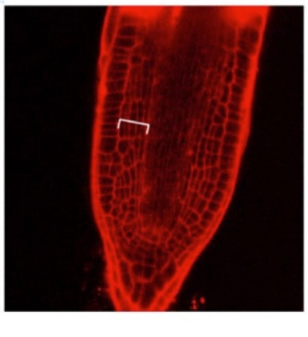
- Non cell autonomous function of SHR: migration to adjacent cells

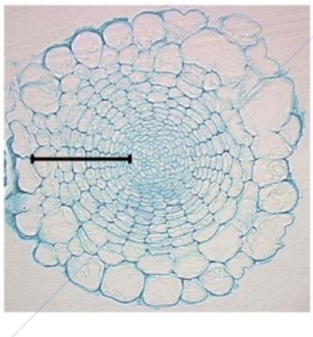


Benfey, 2000 ; site web de J. Haselhoff, Petricka *et al.*, 2008

Cortex / endodermis specification

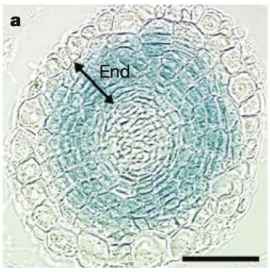
Expression of SHORT-ROOT under the control of SCR promotor

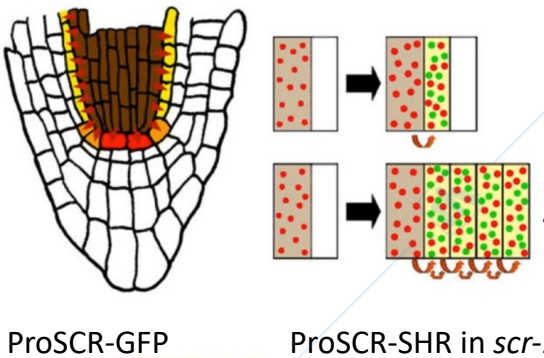




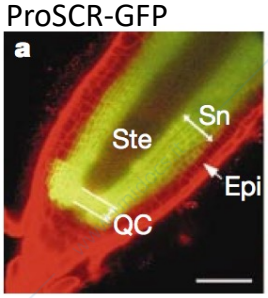
Ectopic expression of SHORTROOT triggers the production of extra layers of endodermis

Endodermis specific marker line

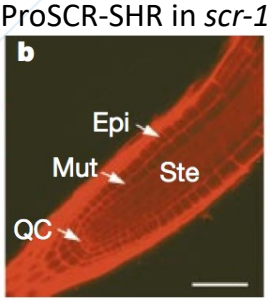




ProSCR-GFP



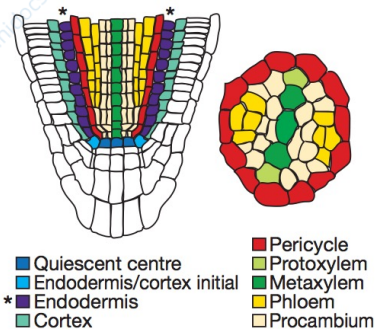
ProSCR-SHR in *scr-1*



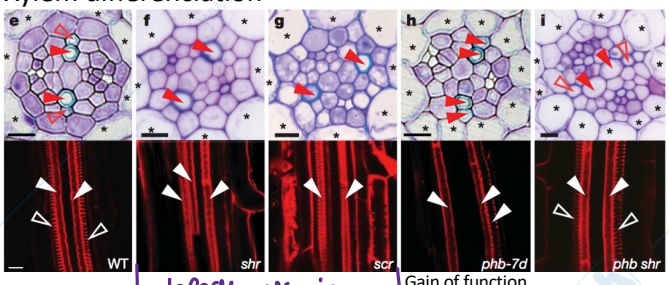
Helariutta *et al.*, Cell, 2000 ; Nakajima *et al.*, Nature, 2001

5.
Control of stele tissues specification

Role of SHR and SCR in stele tissues specification

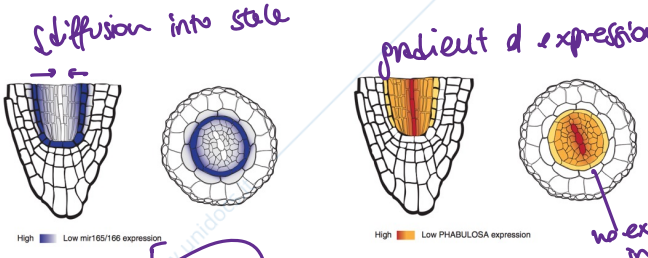


Xylem differenciation



defects also in stele

two metaxylem cells differentiating



diffusion into stele

gradient of expression

no expression in endodermis

SHR/SCR → miR165/6 ⊣ PHB

PHB → Xylem differentiation

- *SHR* et *SCR* are necessary for proper xylem differentiation
- They act through the HD-ZIP III transcription factor PHABULOSA (PHB)
- They control the localised expresion of PHB through the expression of micro RNA miR165/6 migrating into the stele

➢ Non cell autonomous effect

Carlsbecker *et al.*, Nature, 2012

➢ Setting up / maintaining a regular organization?

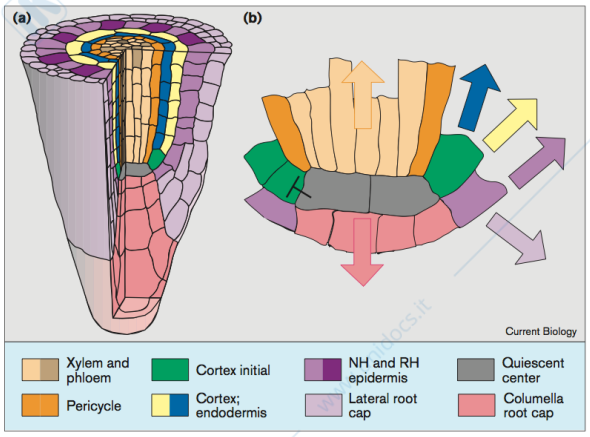
1.
Function of the quiescent centre

➢ organizing centre

2.
Positional control of the root mersitem location

3.
Embryonic establishment of the root meristem

➢ mediated by auxin fluxes



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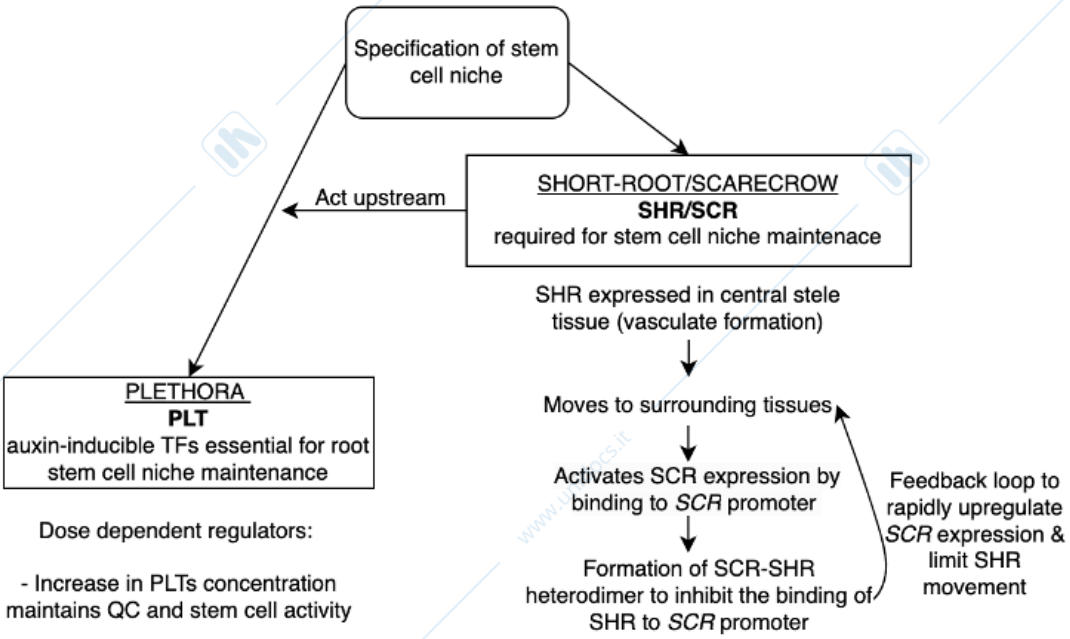
Benfey, 2000

4.
Endodermis / cortex specification

5.
Stele tissues specification

➢ Specifying cellular identities?

➢ transcription factors cascades
➢ non cell autonomous effects



Dose dependent regulators:

- Increase in PLTs concentration maintains QC and stem cell activity

- Intermediate levels of PLTs regulate division and differentiation of transit amplifying cells

- Low PLTs concentration causes the differentiation

Expression of *PLT* genes requires auxin response TFs, forms gradients that are not direct readouts (mechnism still unclear)

PLT gradient could be explained as follows:

PLT proteins in meristematic cells
When these divide they maintain PLTs from mother, but are diluted
There is also a cel-to-cell movement of PLTs

PLT gradient of expression is PIN dependent
-> control of auxin mediated root patterning

- PIN proteins restrict *PLT* transcription in basal embryo to initiate root primordium formation
- PLTs maintain transcription of *PINs* that stabilize the position of stem cell niche