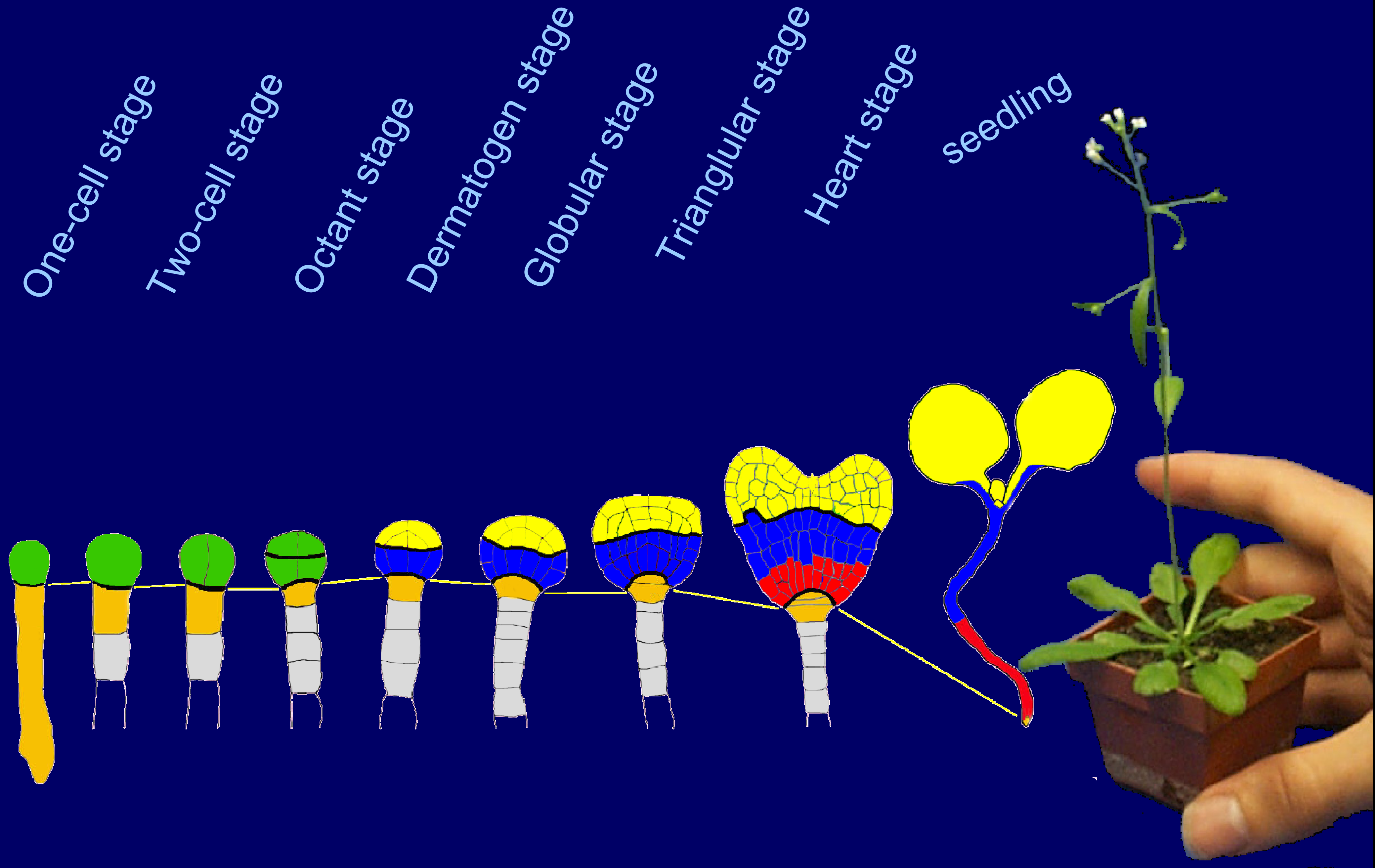
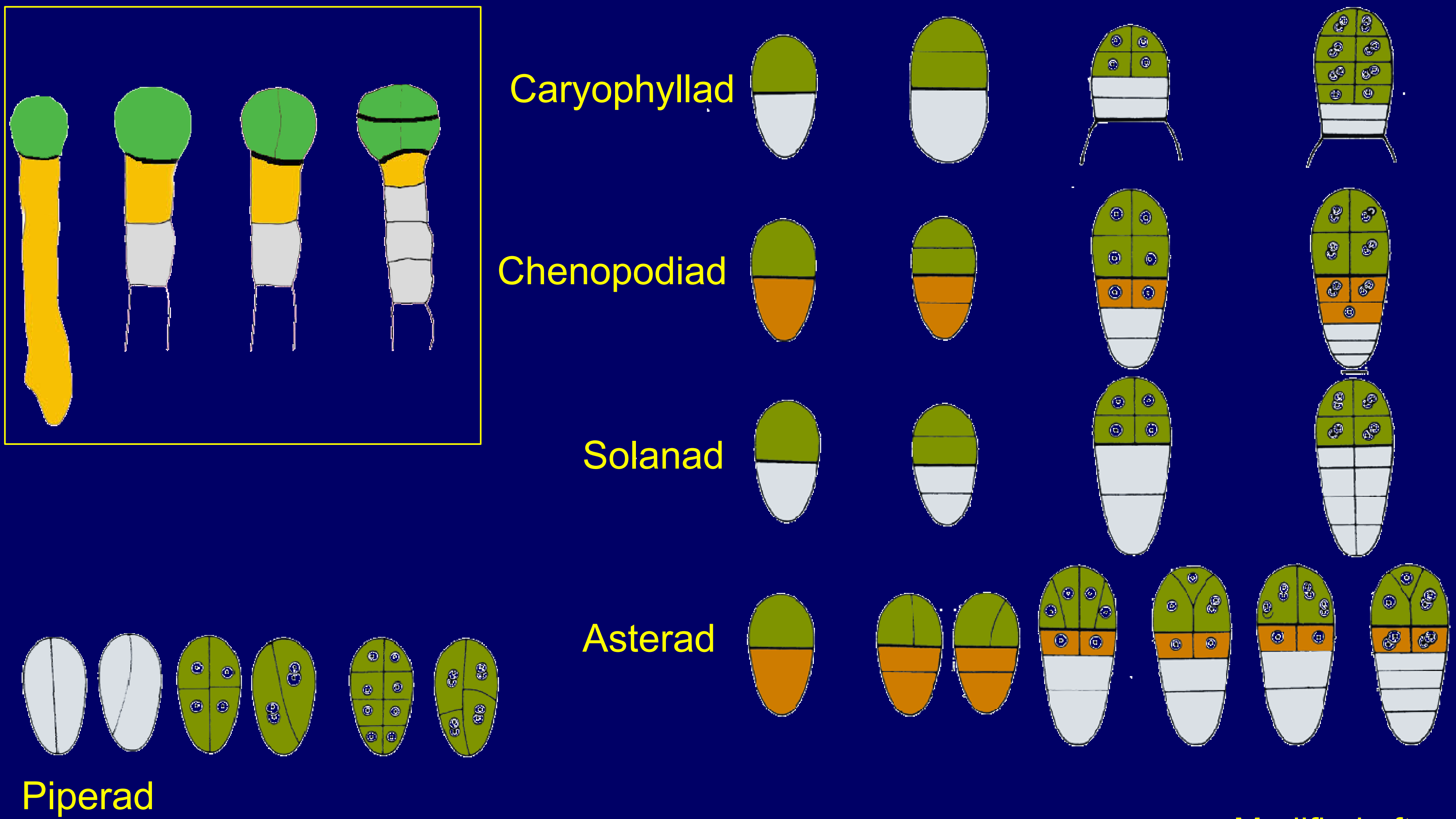
Embryogenesis

Arabidopsis Embryogenesis

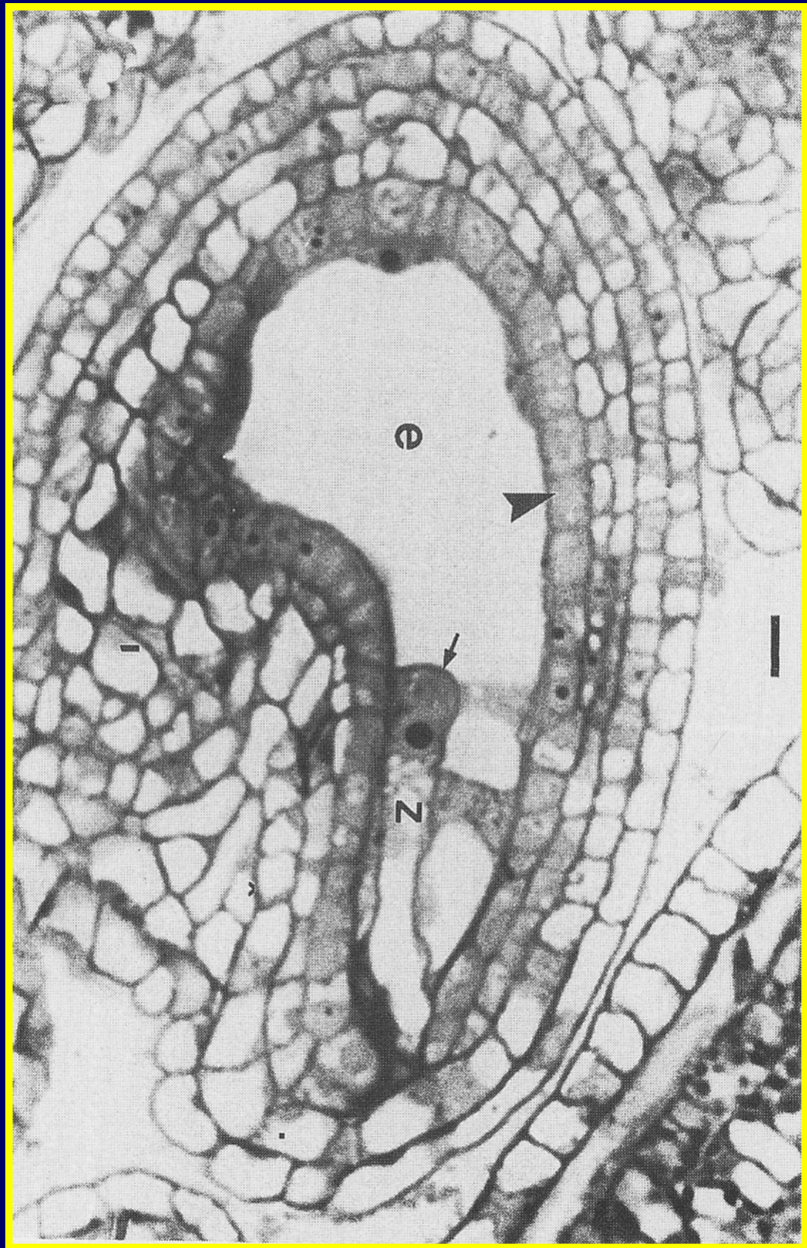


Comparison of embryo development in Angiosperms

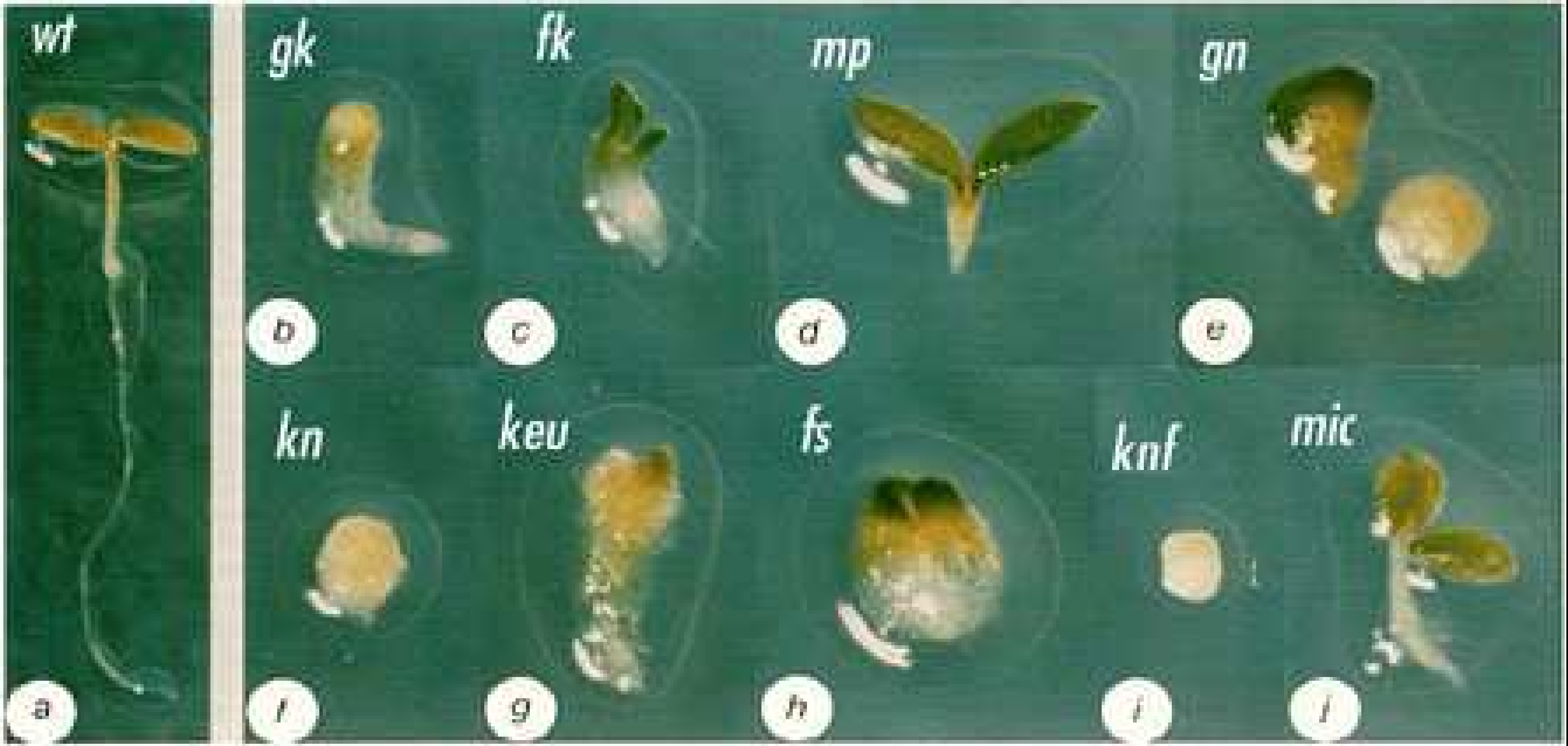


Modified after Johri et al. 1992

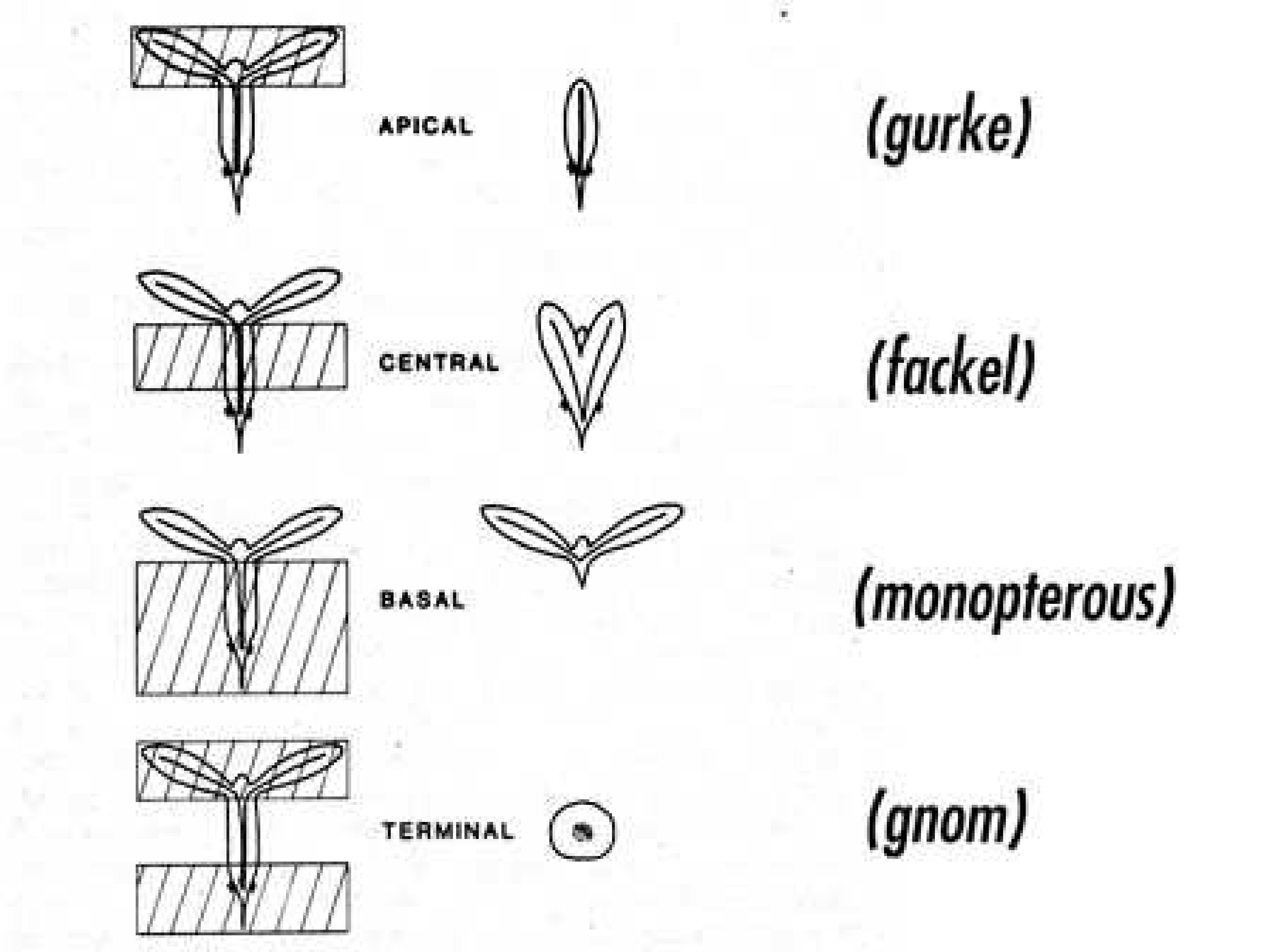
How can such a protected system be investigated experimentally?



Mutant screen at seedling level



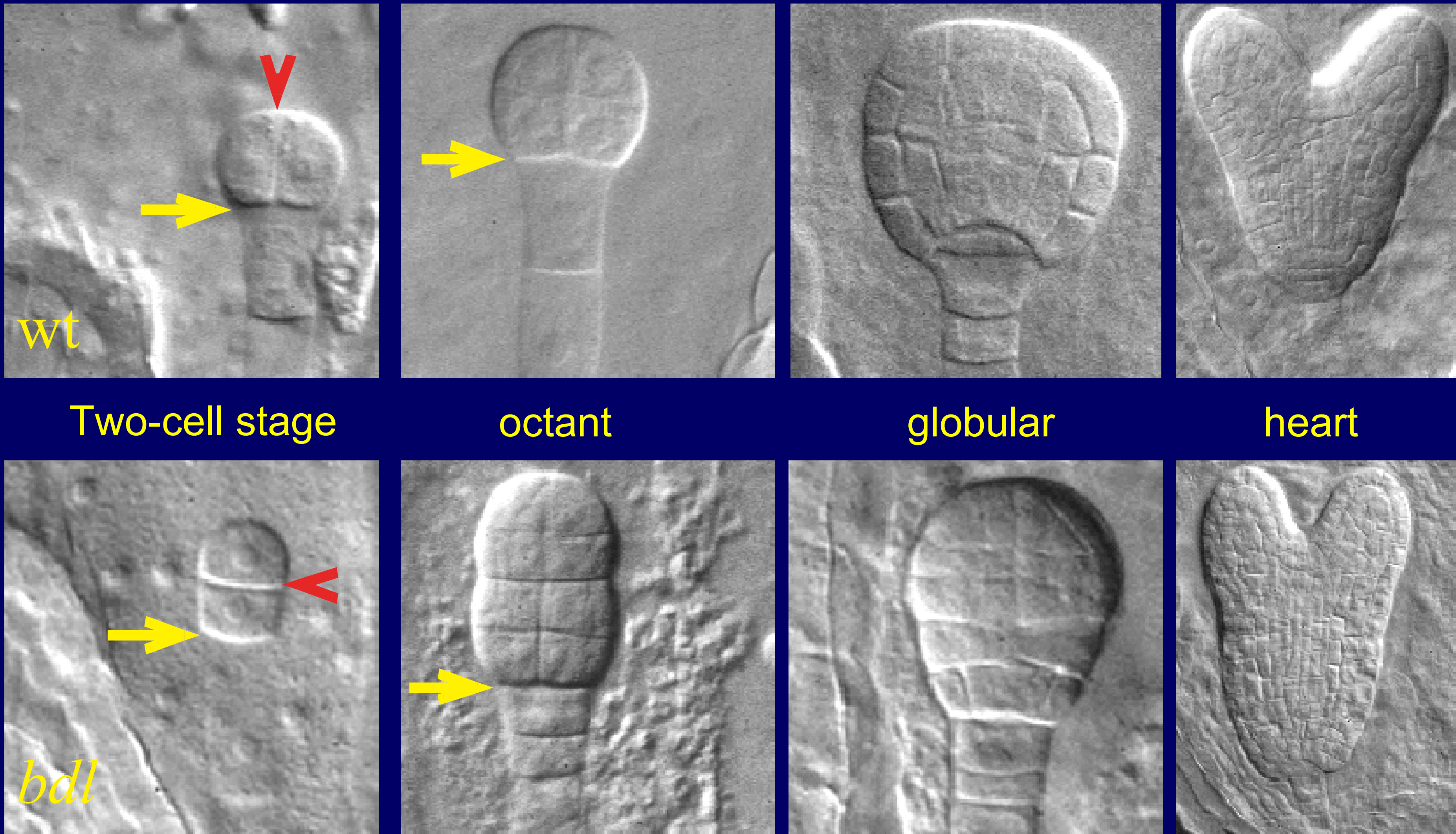
Patterning mutant types



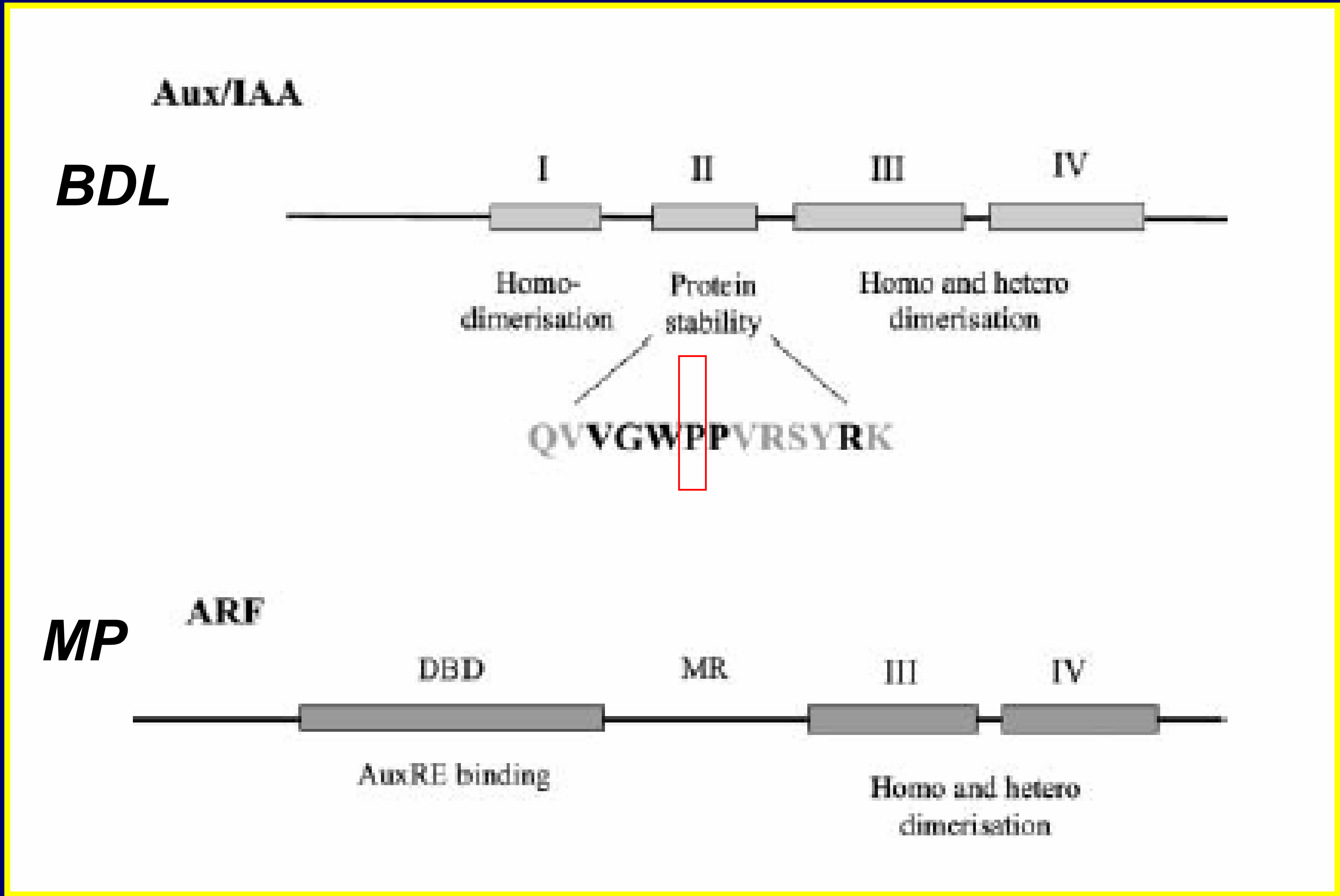
Mutations in the ***BODENLOS*** (*bdl*) and ***MONOPTEROS*** (*mp*) genes lead to very similar deletions of basal pattern elements

- ***mp*** seedling
- ***bdl*** seedling

The *bodenlos* (*bdl*) root meristem defect



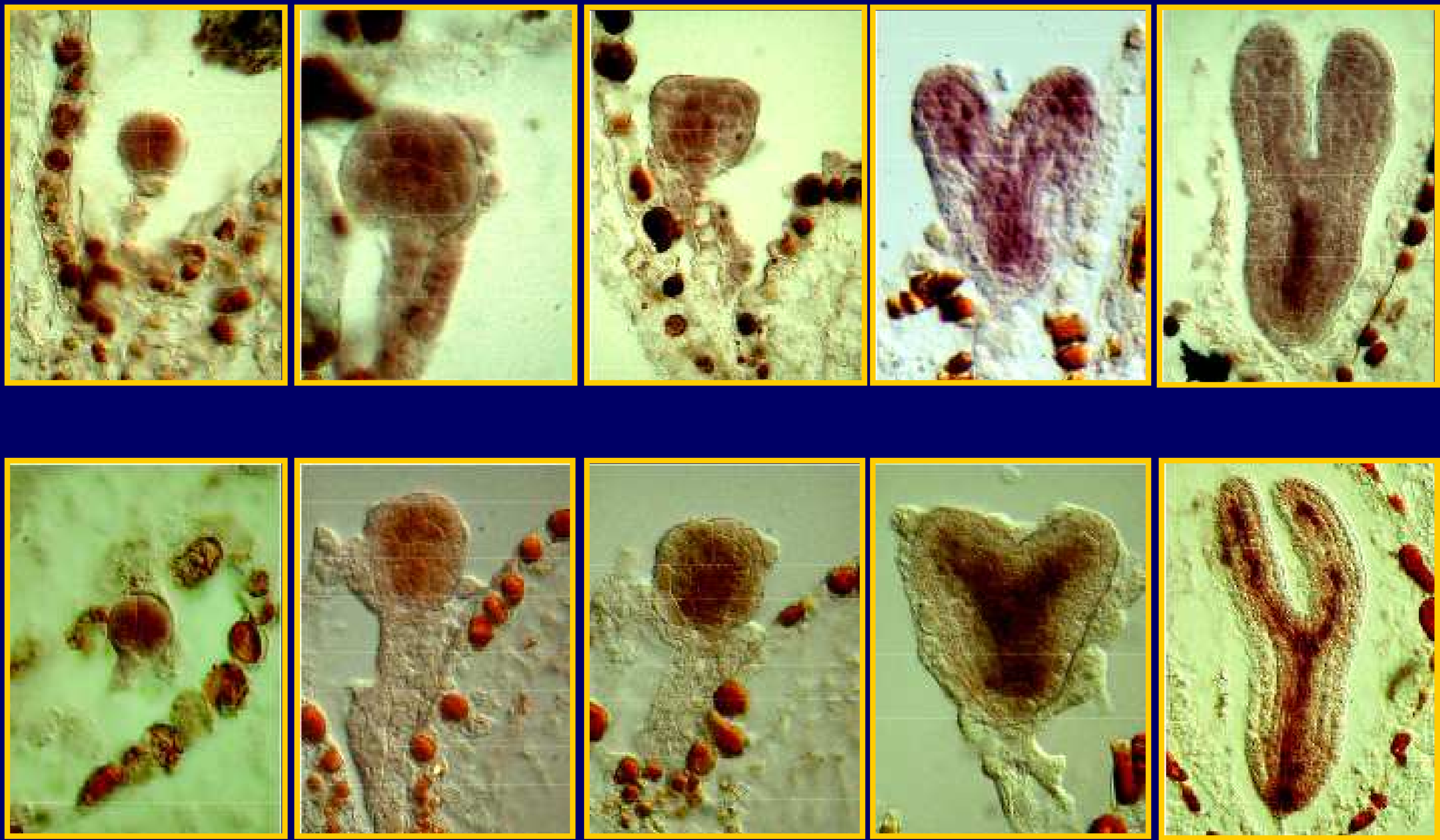
MP encodes for ARF5, an **activator** of auxin response, whereas *BDL* encodes for IAA12 the corresponding **repressor**



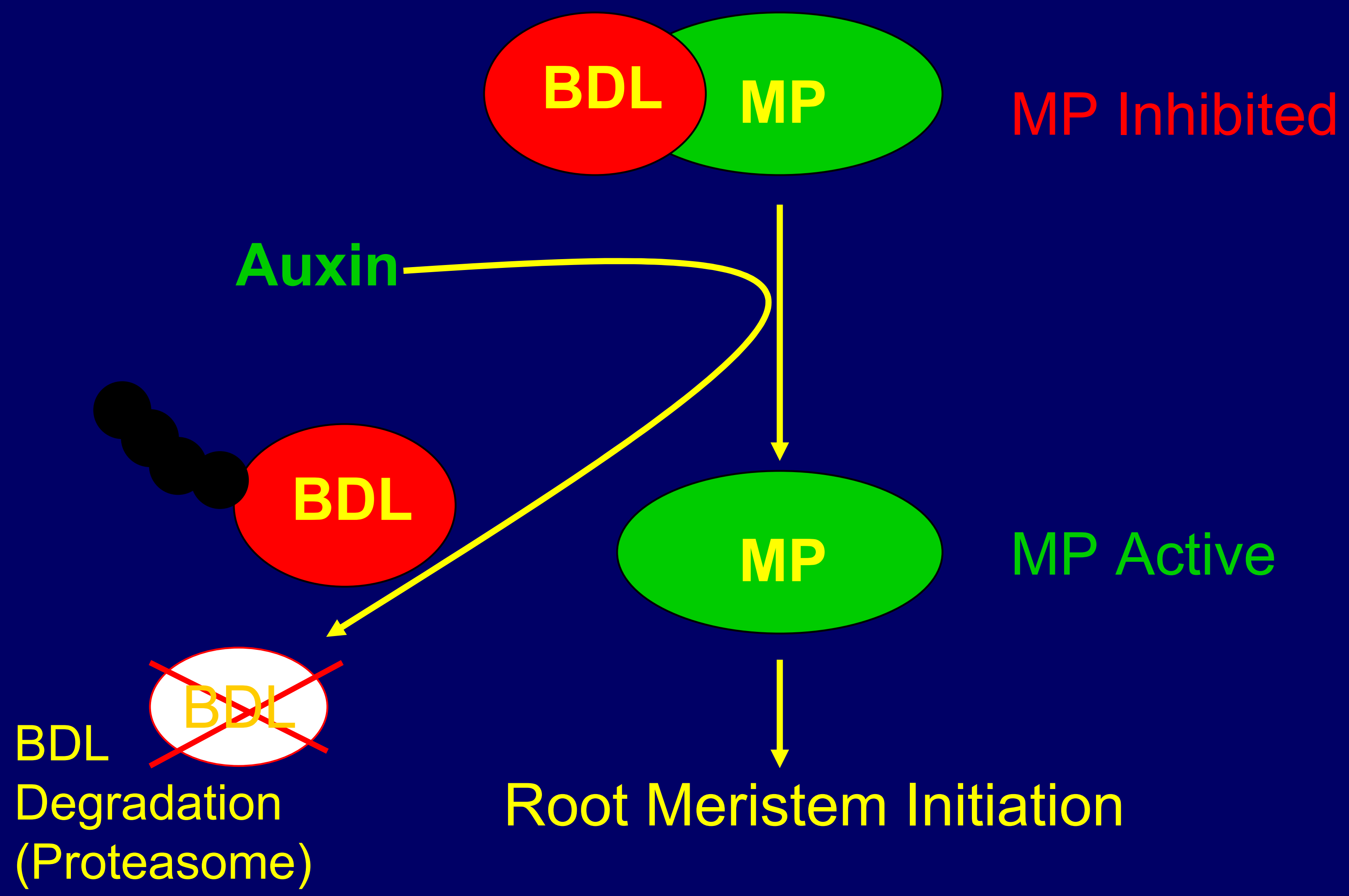
Expression patterns of BDL and MP

bdl

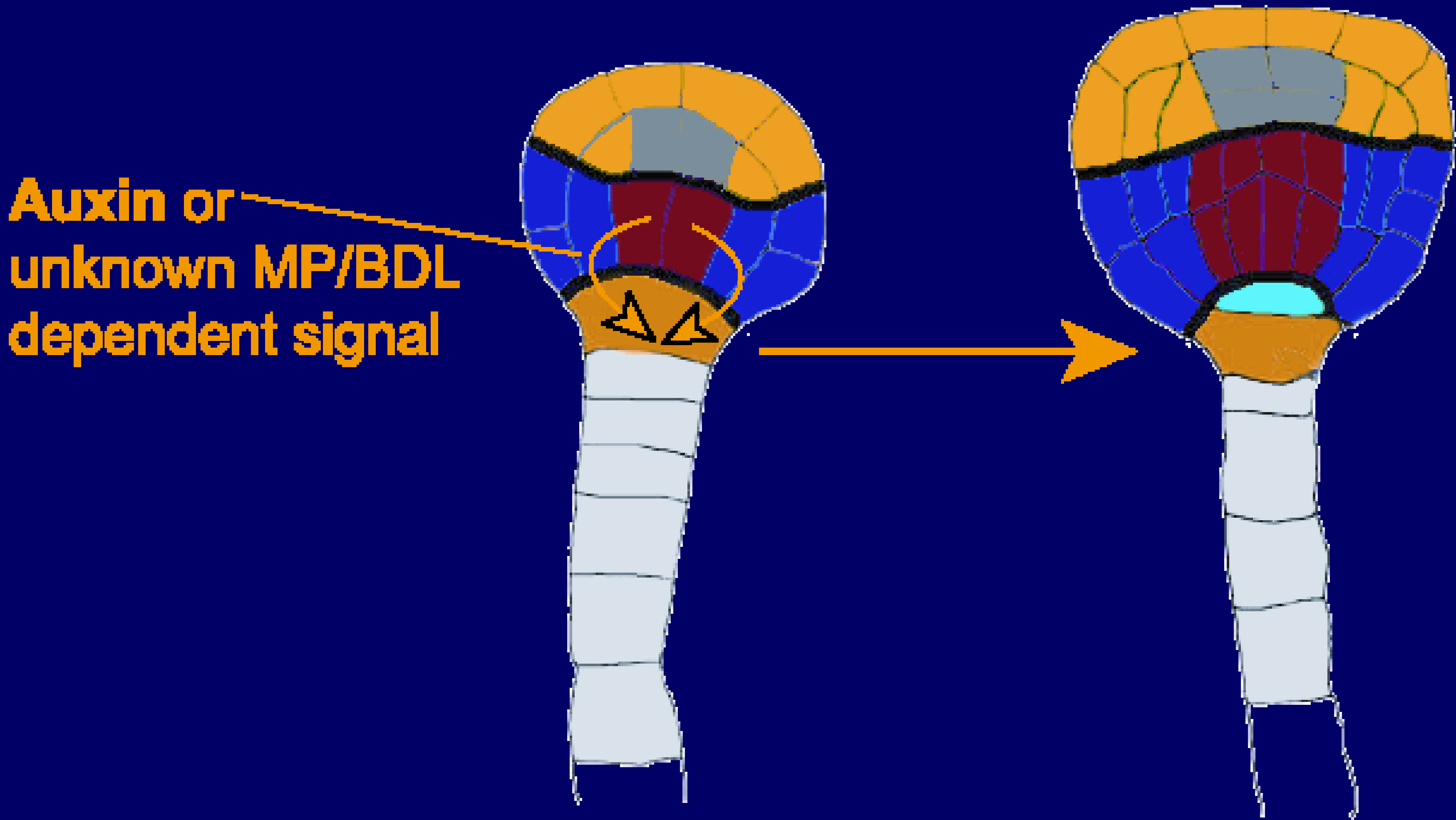
mp



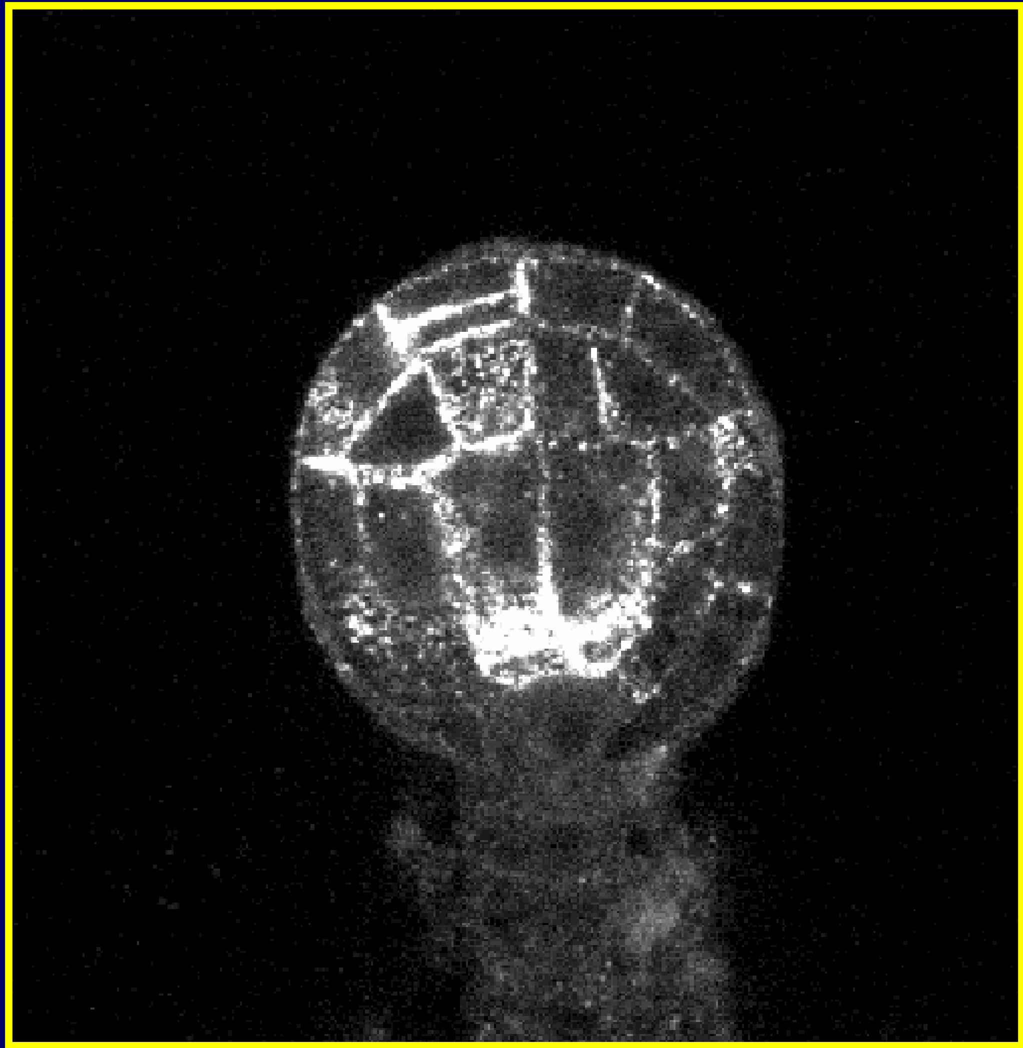
Model of BDL/MP interaction



BDL/MP act non-cell autonomously to induce hypophyseal cell fate



PIN1 efflux carrier localisation suggests auxin flux towards the hypophysis



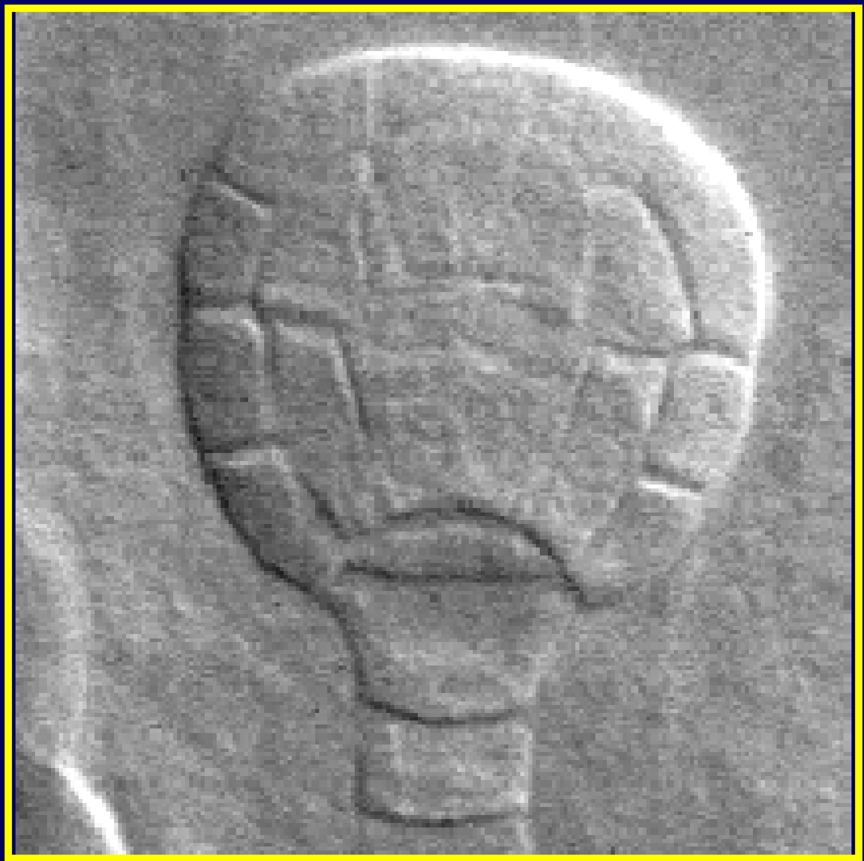
Genetic Interference with Auxin Response and Transport Disrupts Embryo Patterning

monopteros

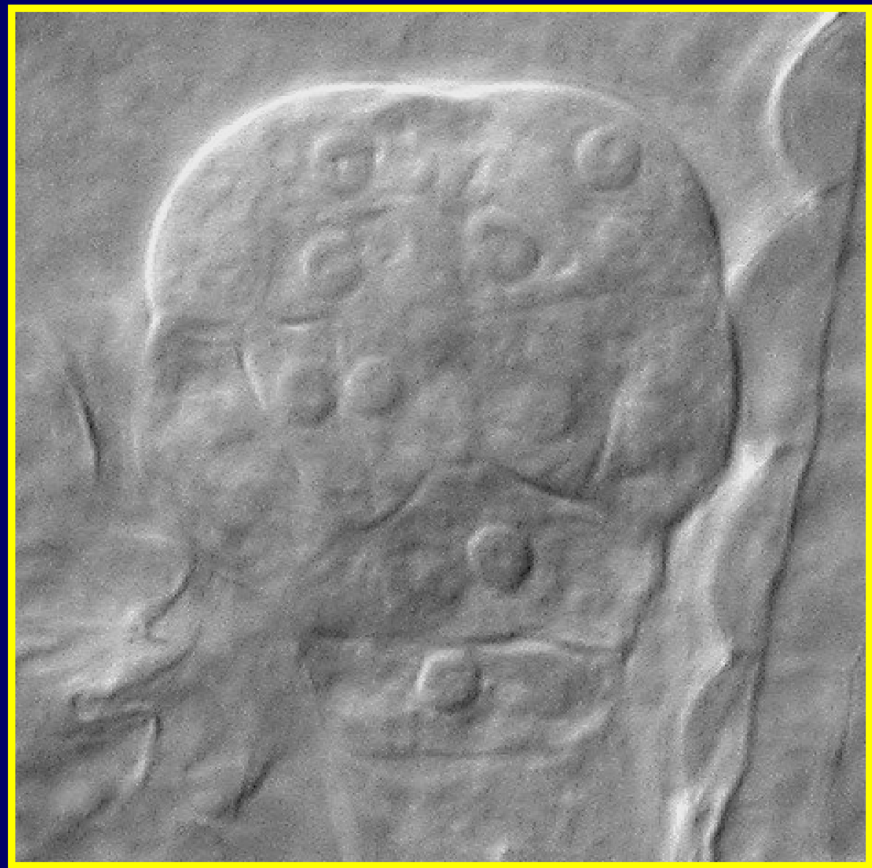
bodenlos

gnom

GNOM, a putative auxin transport mutant has similar defects in hypophyseal cell fate specification



wt



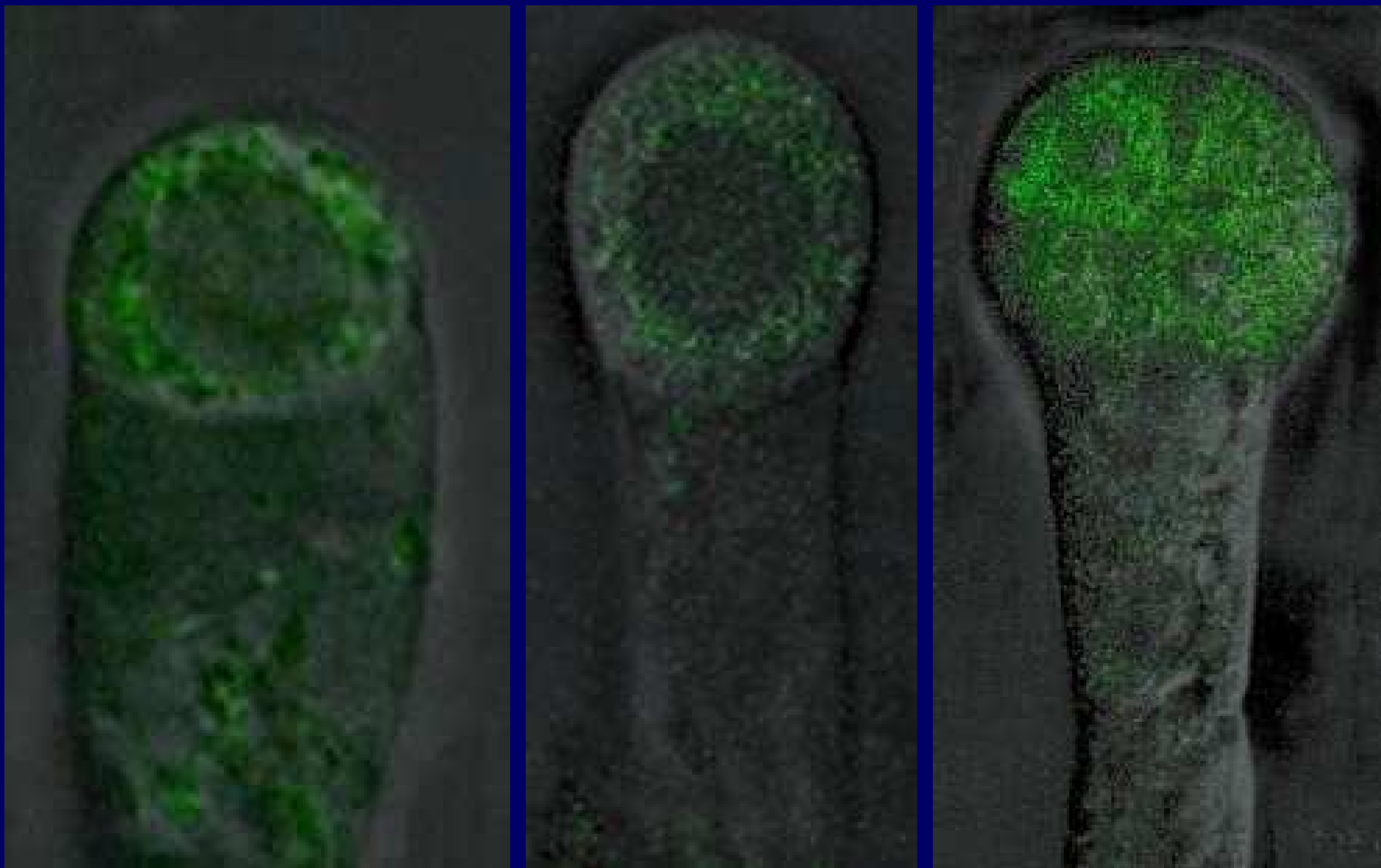
gn

DR5::GFP Auxin Reporter

Root | Root + Auxin | anti-IAA AB | Embryos

Auxin in Early Embryogenesis

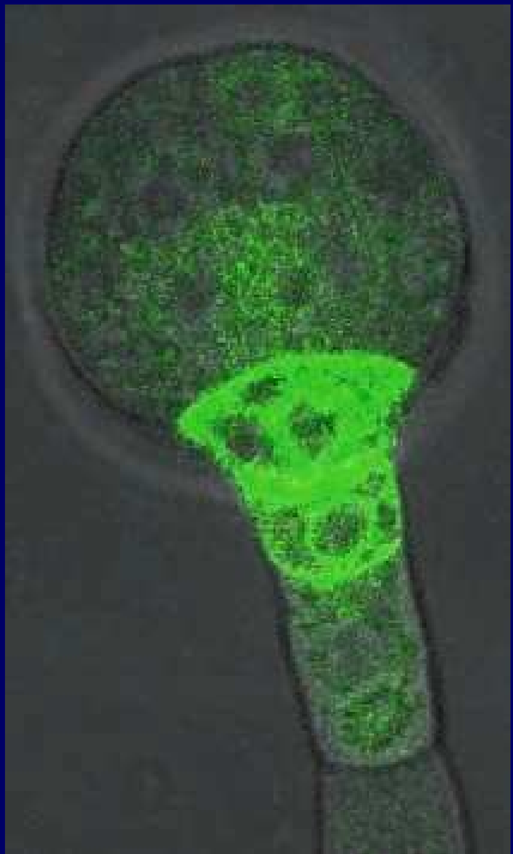
DR5::GFP

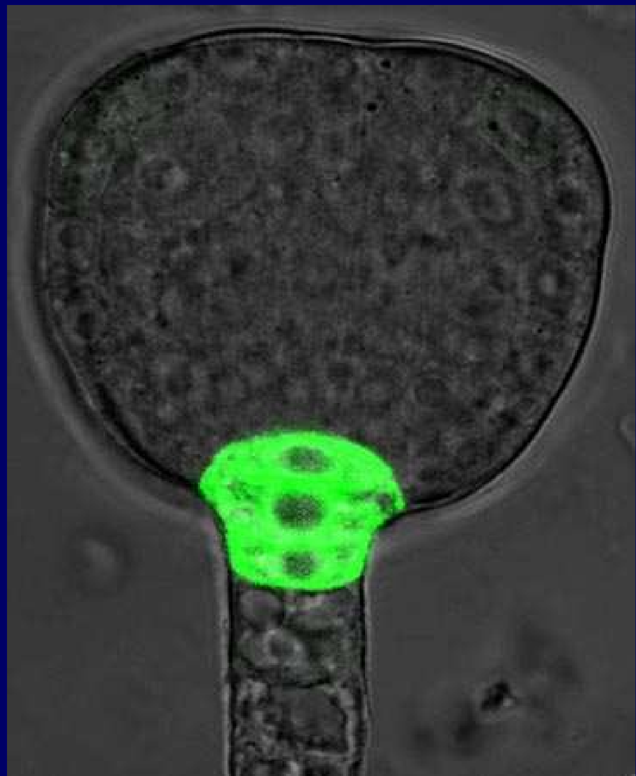


IAA localisation

Auxin in Embryogenesis

DR5::GFP





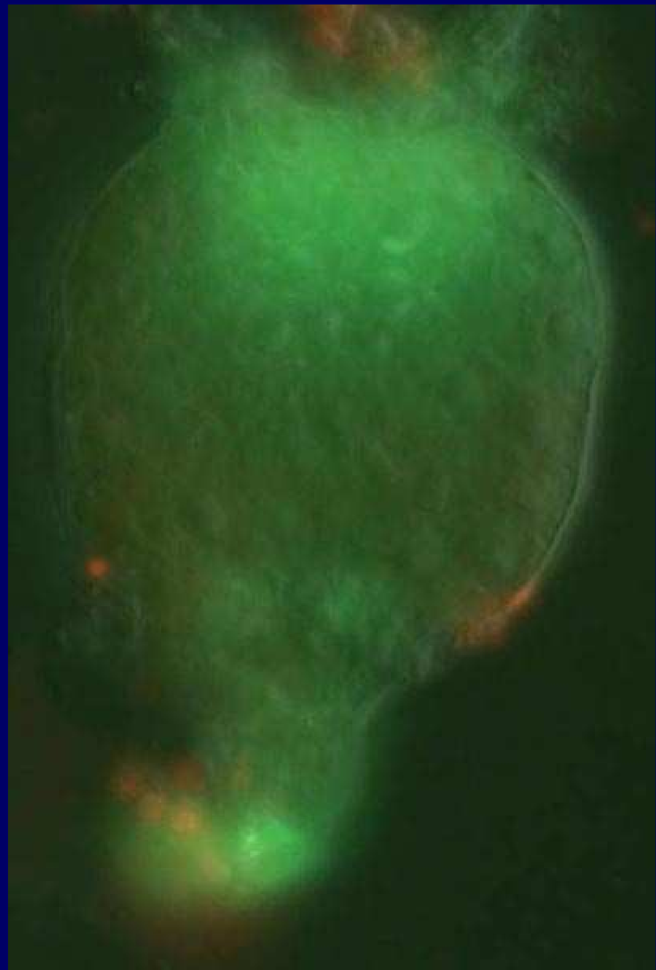
IAA localisation

DR5::GFP in Embryo Mutants

Auxin signaling

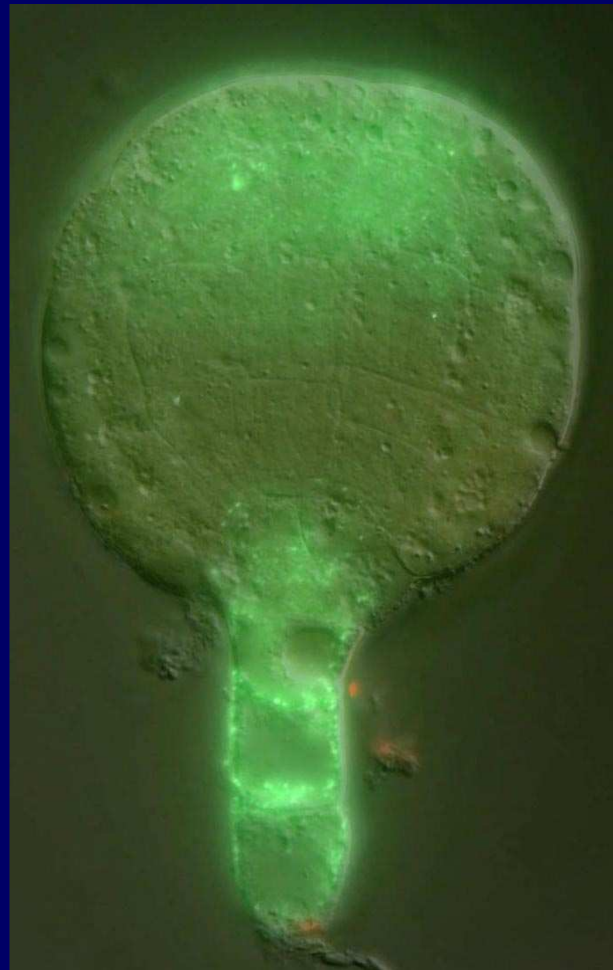
monopteros

Auxin transport



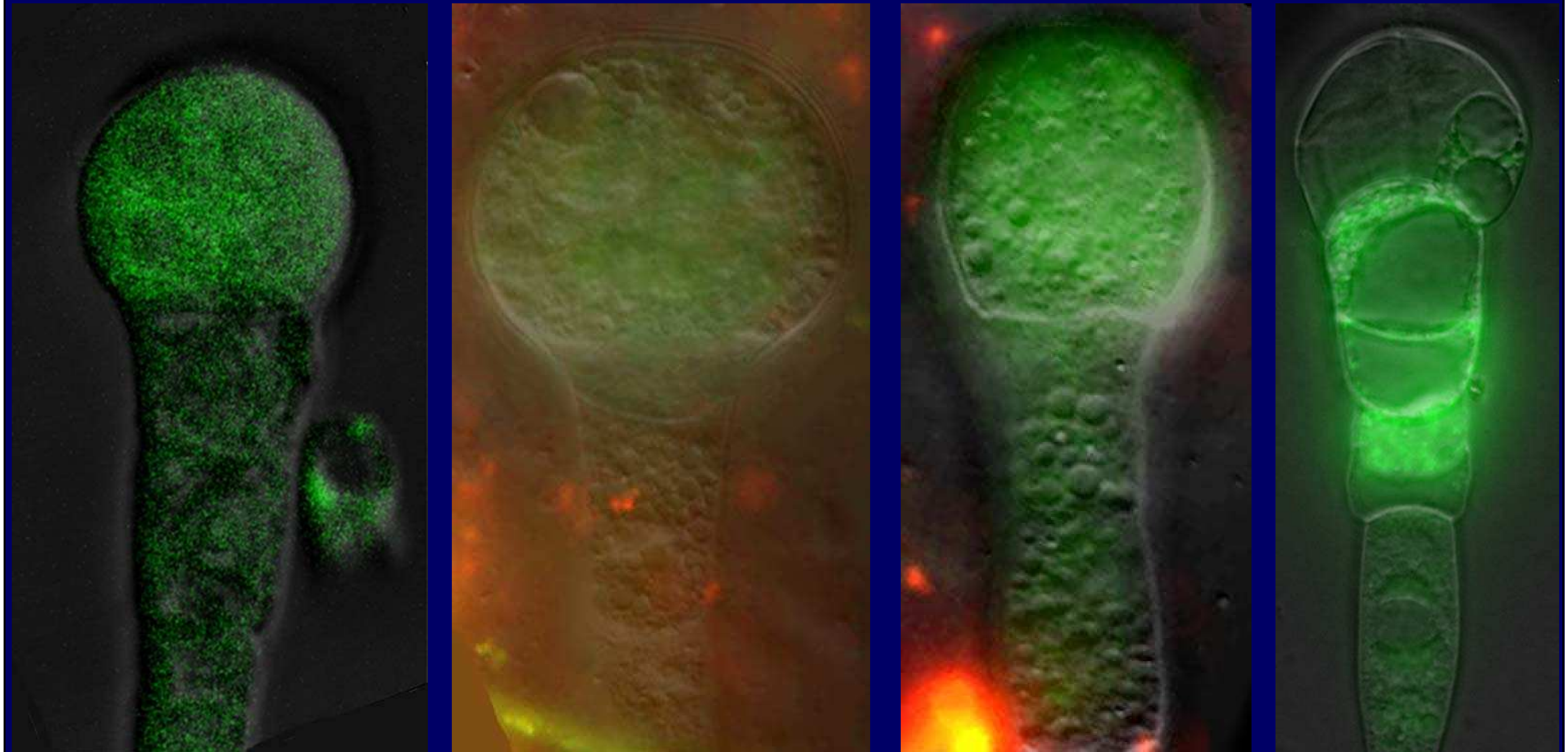
gnom

BFA treatment



DR5::GFP – in vitro Culturing

Preglobular embryos – short time treatments

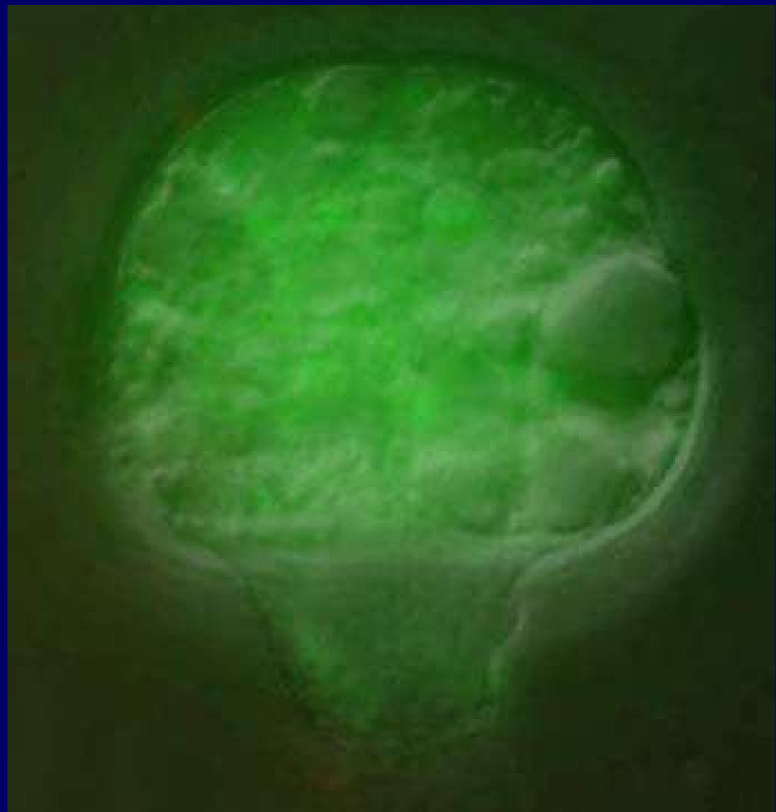


Control | **0** | **NAA** | **NPA**

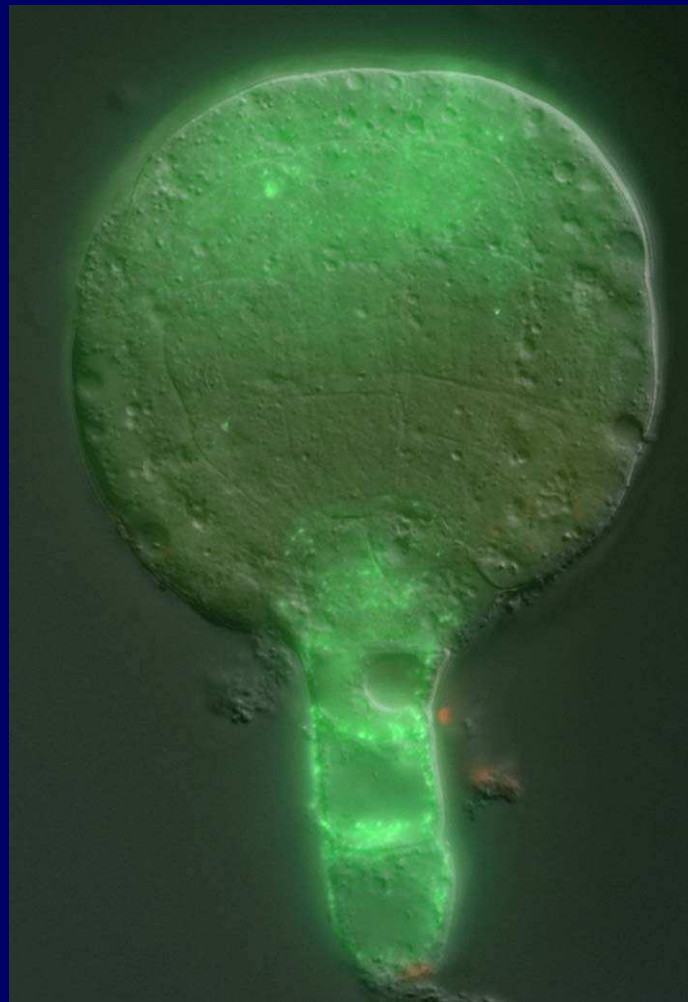
DR5::GFP – in vitro Culturing

Globular embryos – short time treatments

NAA



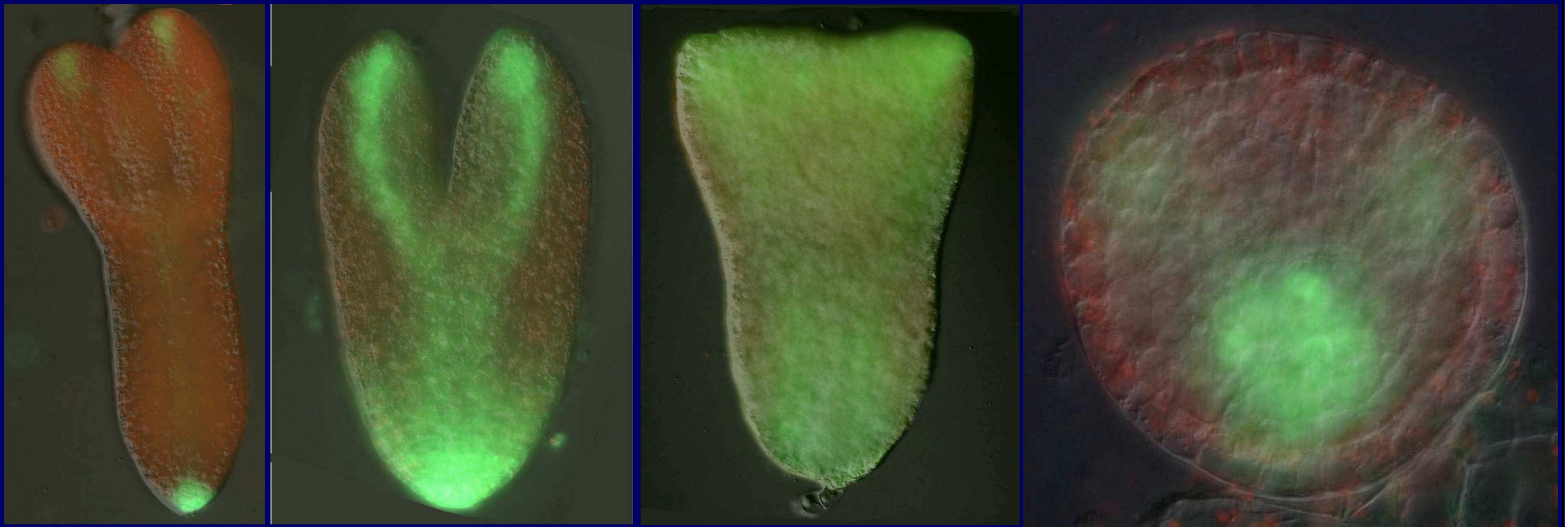
2,4D



BFA

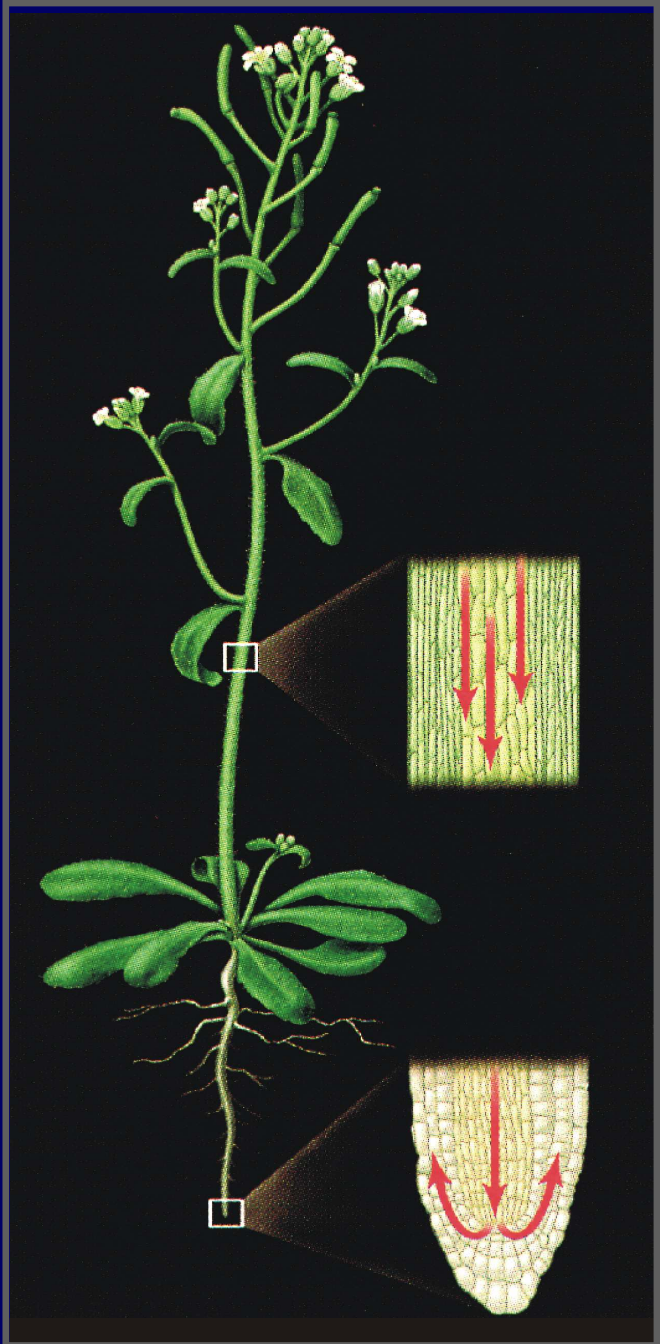
DR5::GFP – in vitro Culturing

Long time treatments

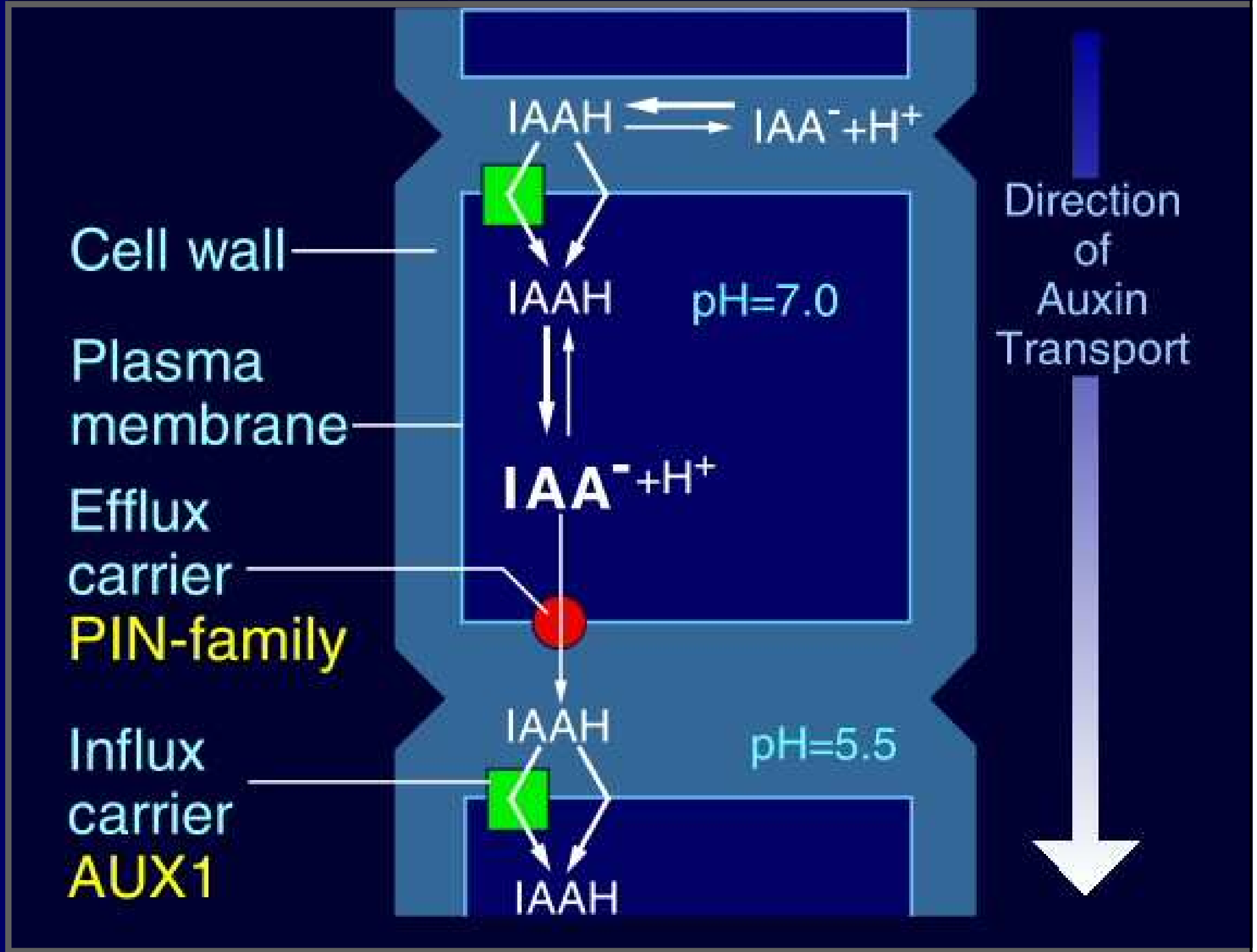


Control **NAA** **2,4D** **NPA or BFA**

Auxin Transport

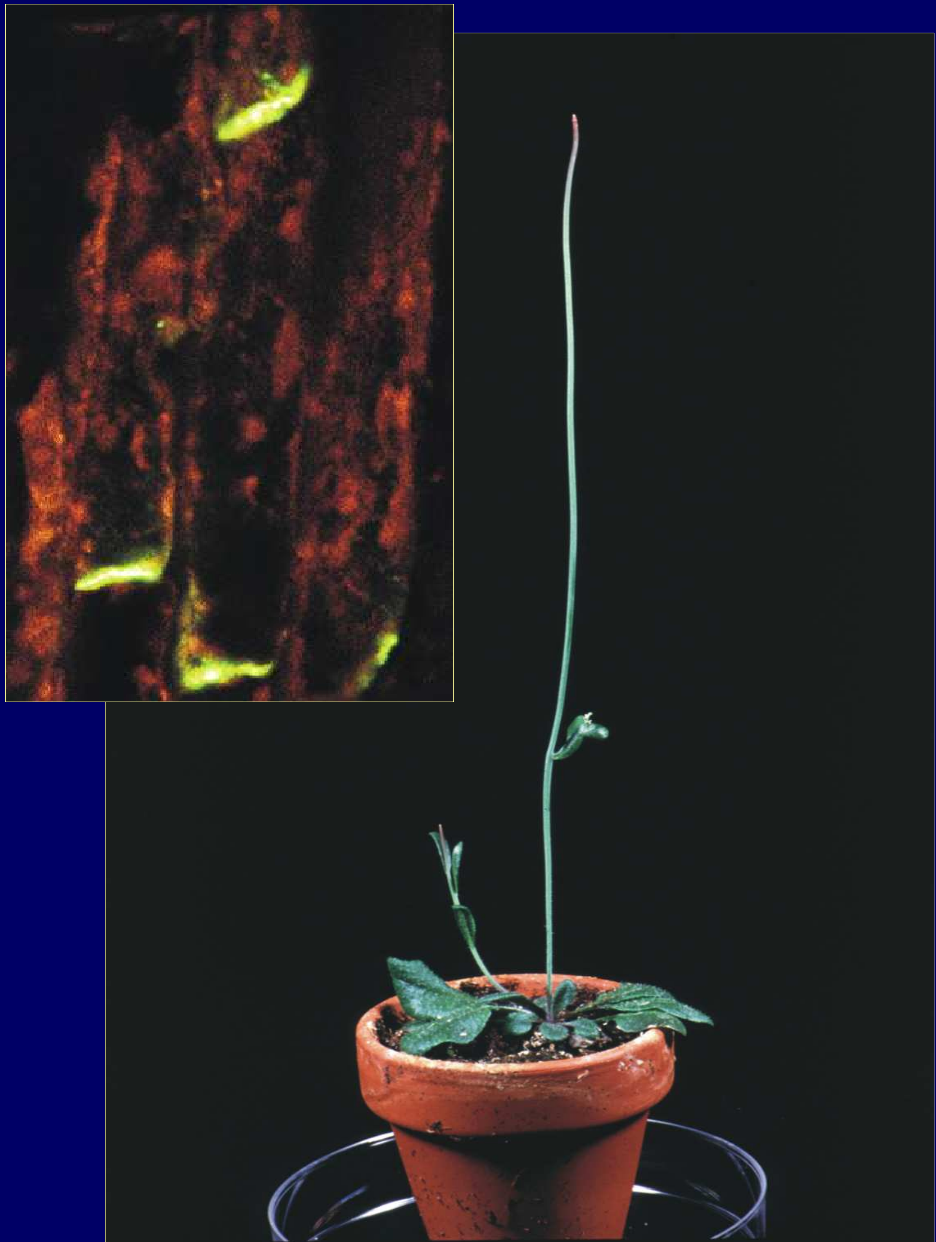


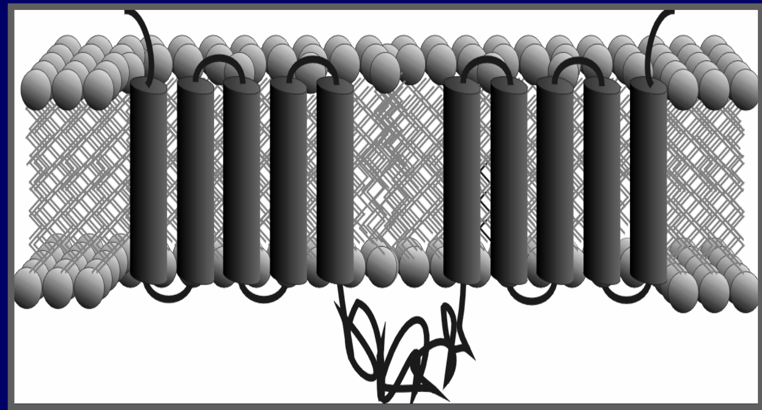
Chemiosmotic hypothesis



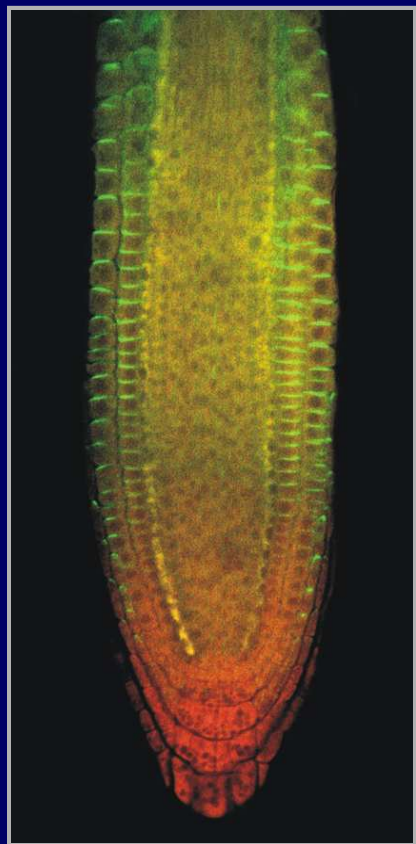
Molecular Genetics of Auxin Efflux

PIN1

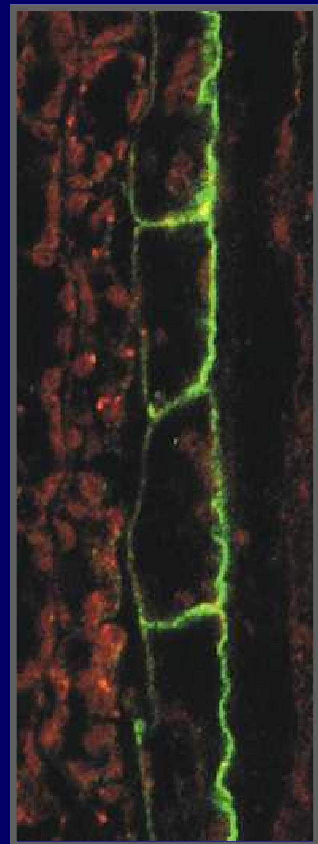




PIN2

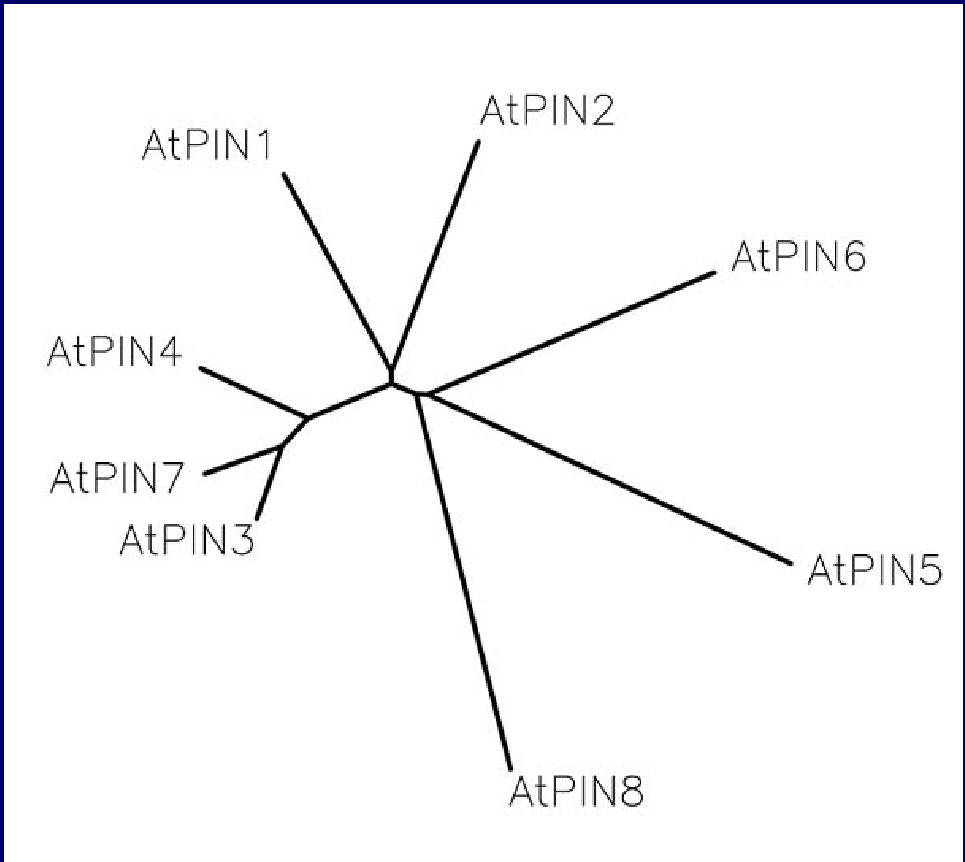


PIN3

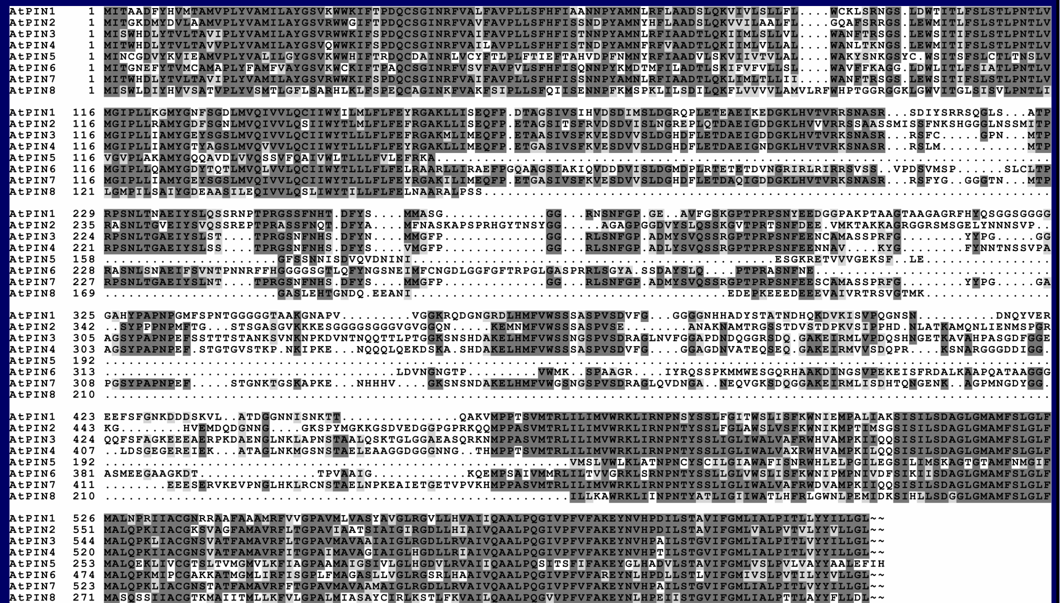


Arabidopsis PIN Protein Family

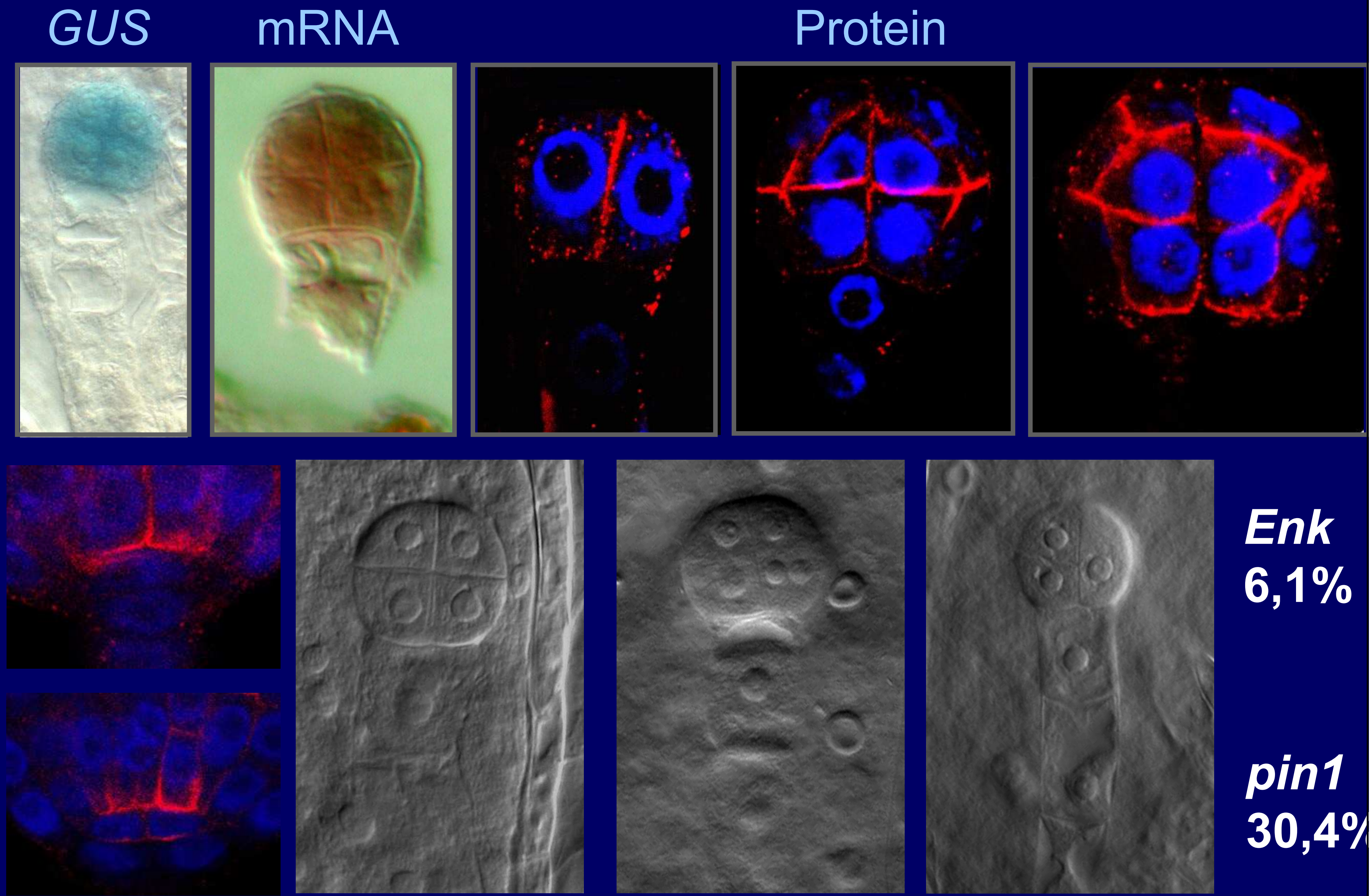
Phylogenetic tree



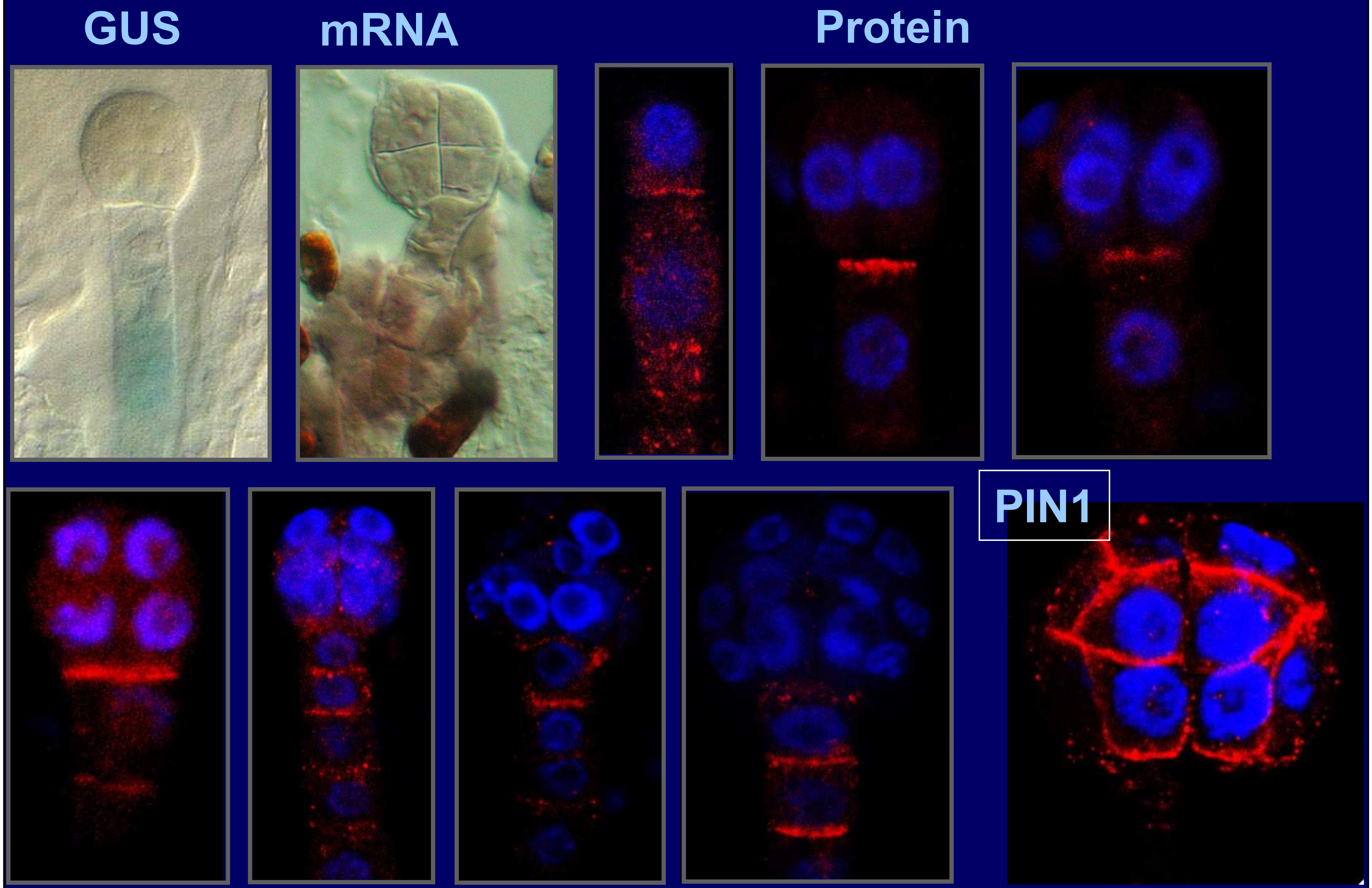
Homology of PIN proteins



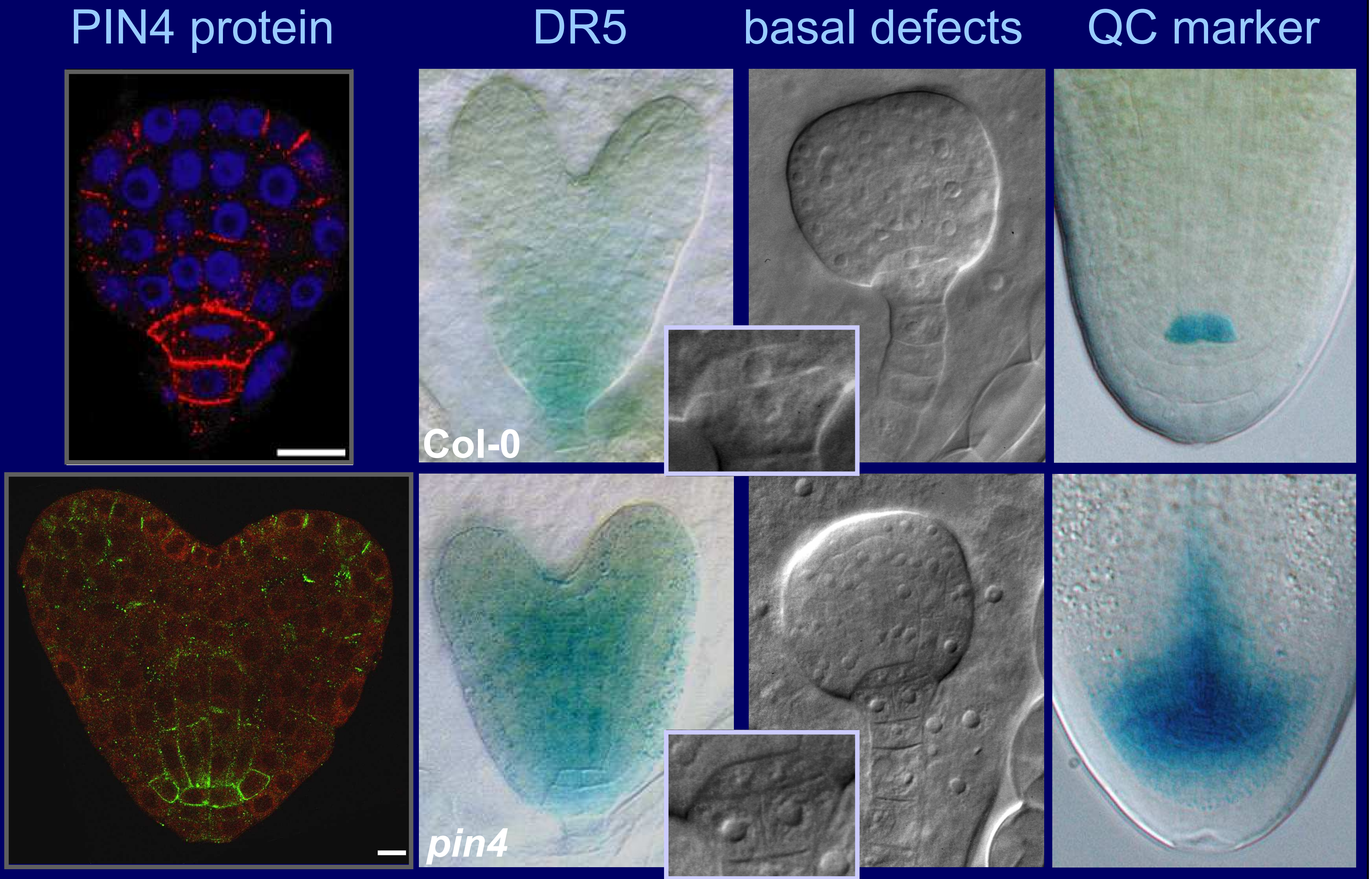
PIN1 in Early Embryogenesis



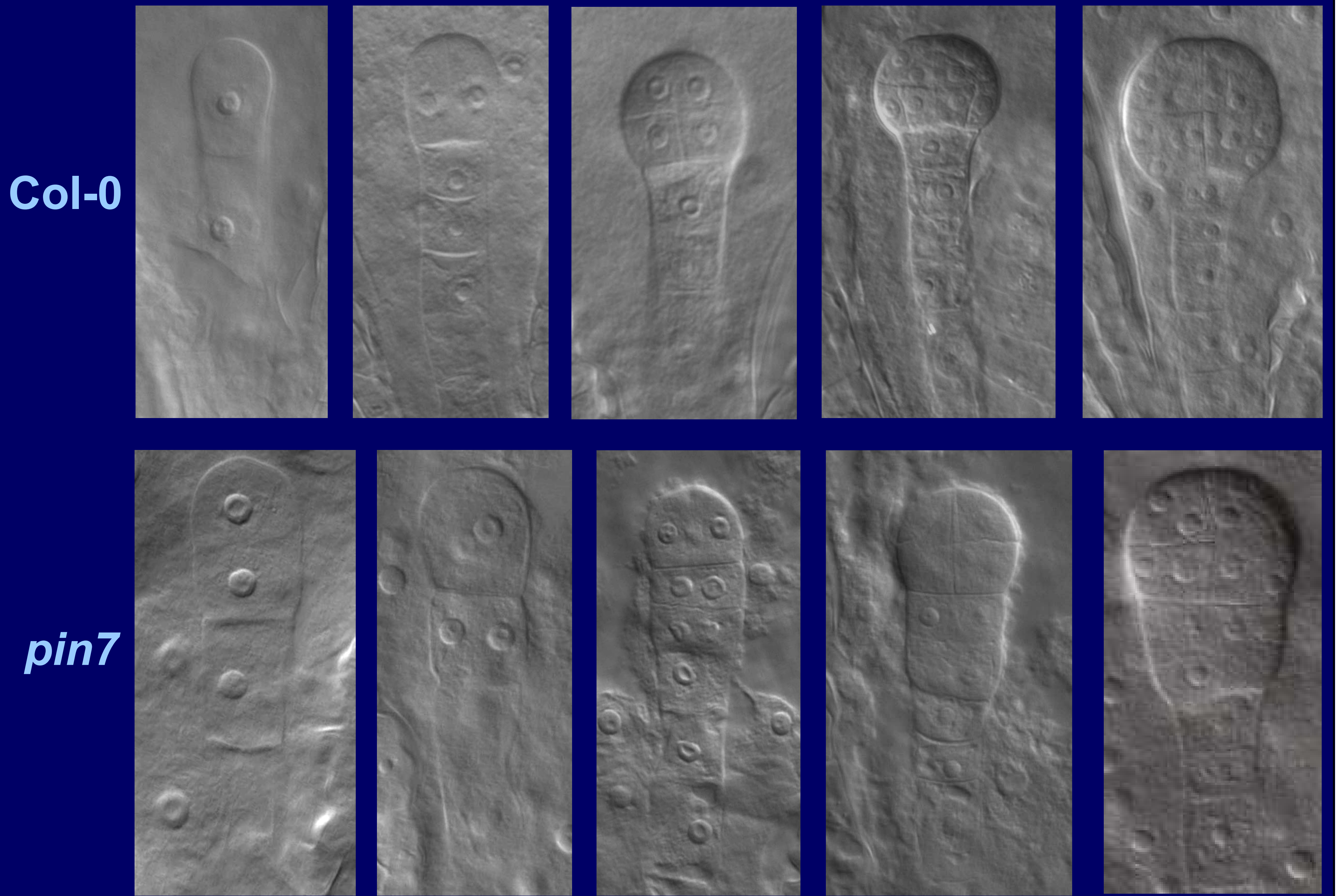
PIN7 in Embryogenesis



PIN4 in Embryogenesis

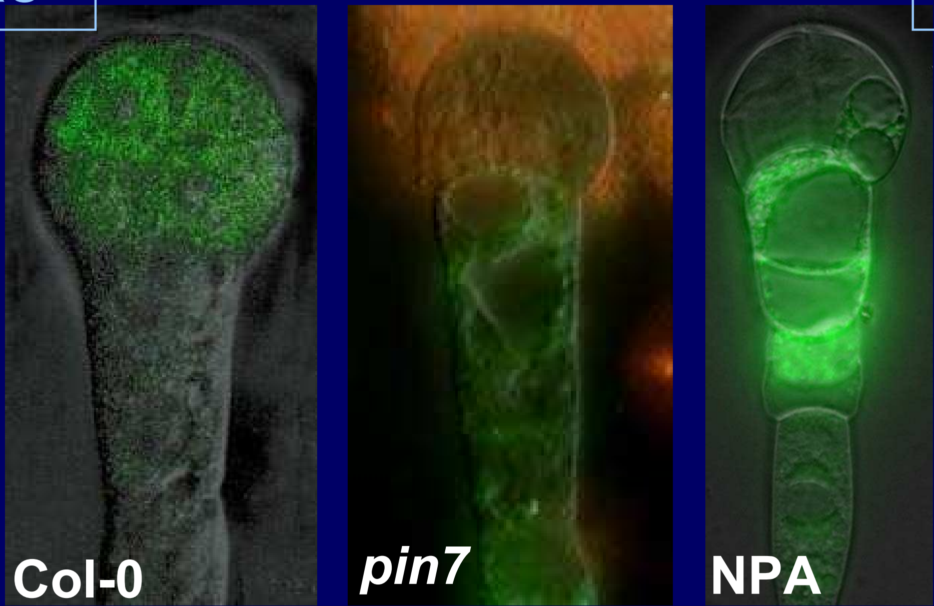


Embryo Phenotype of *pin7* Mutants

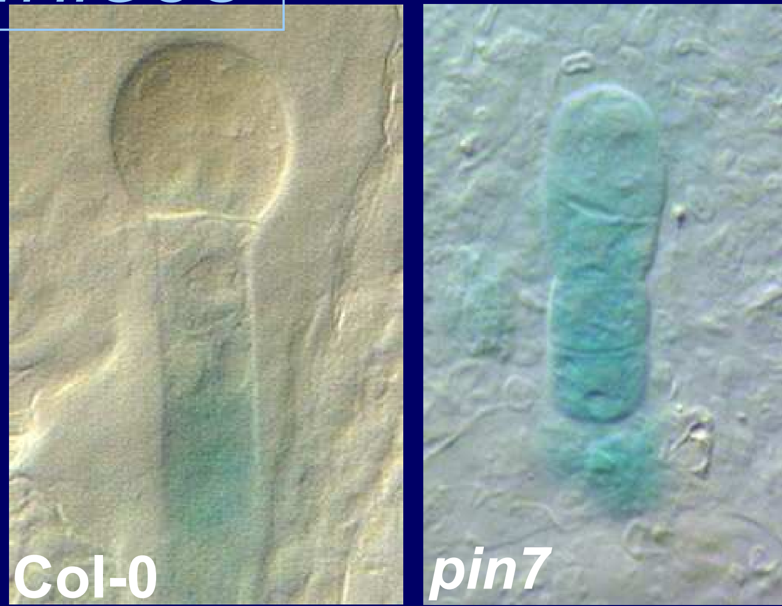


Analysis of Markers in *pin7*

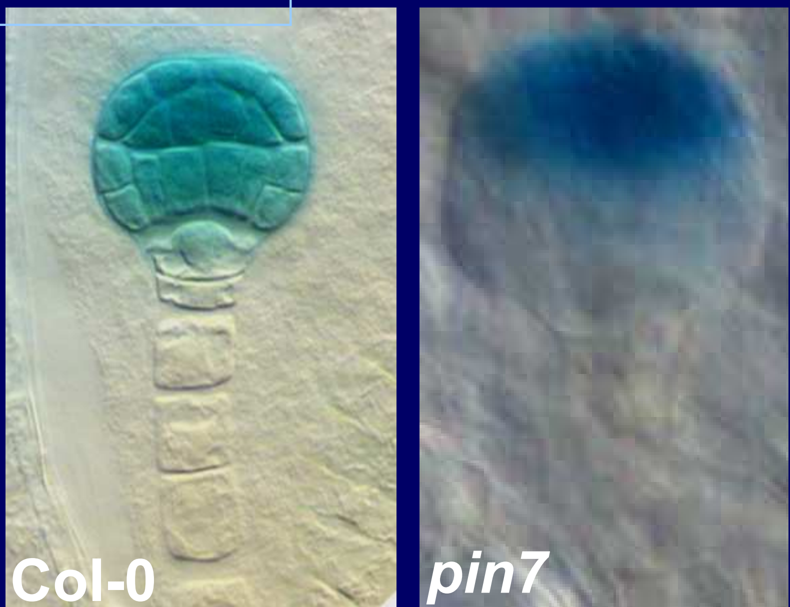
DR5



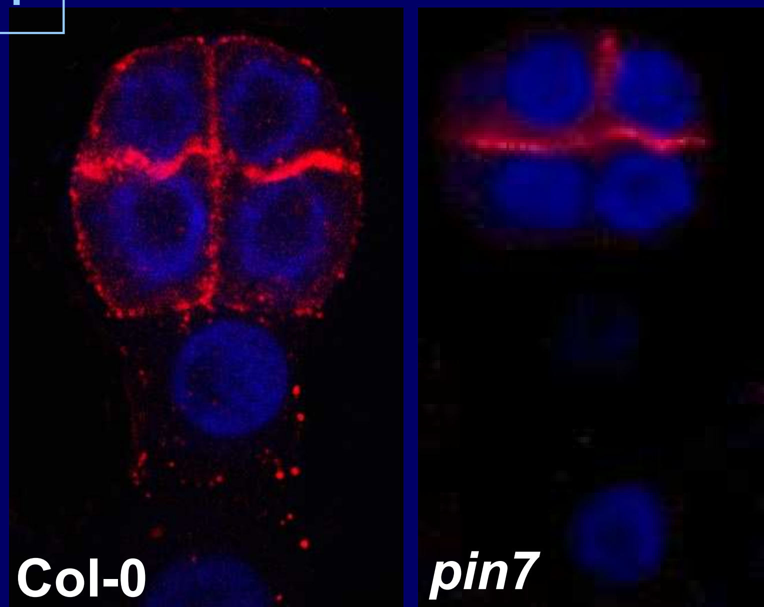
PIN7::GUS



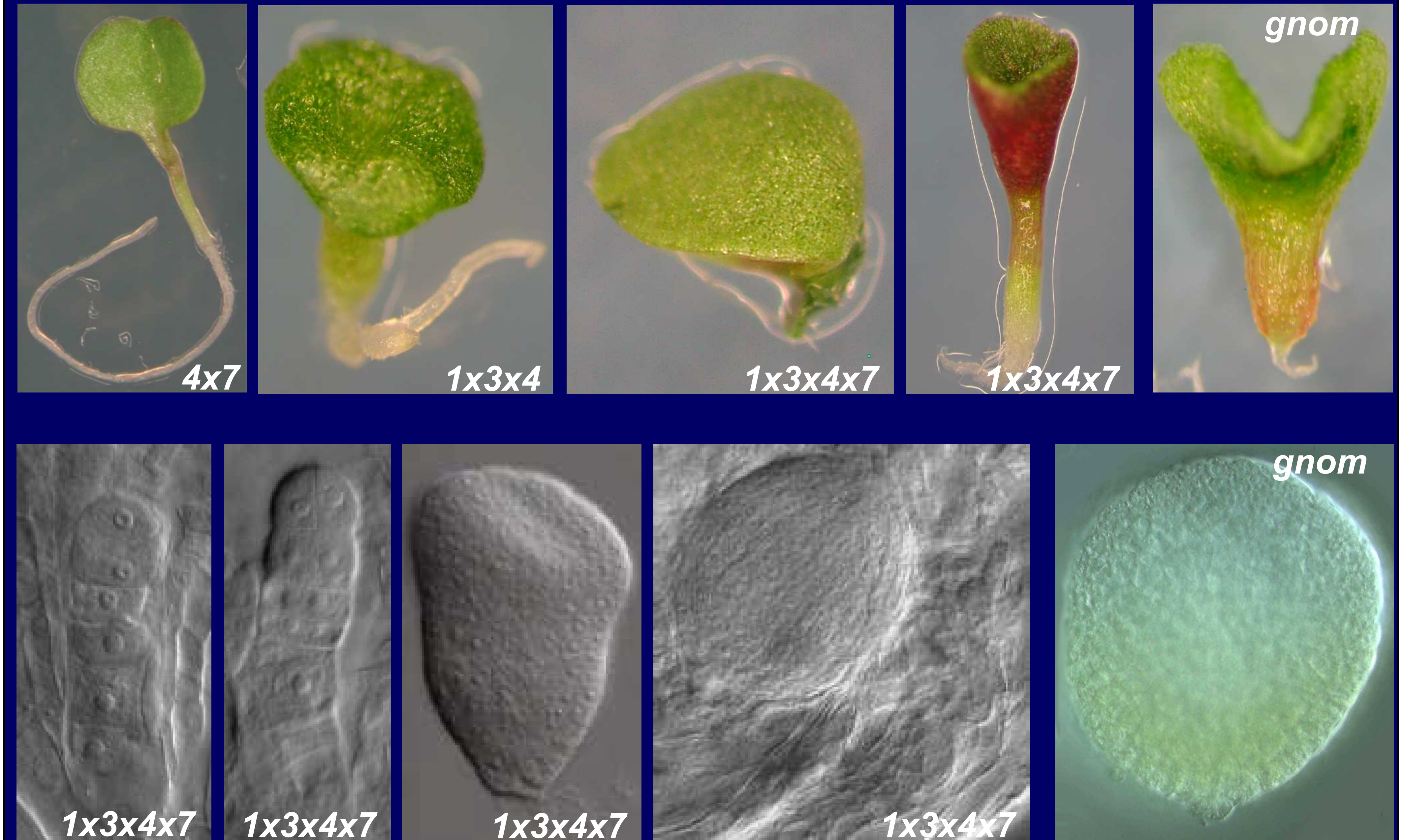
PIN1::GUS



PIN1

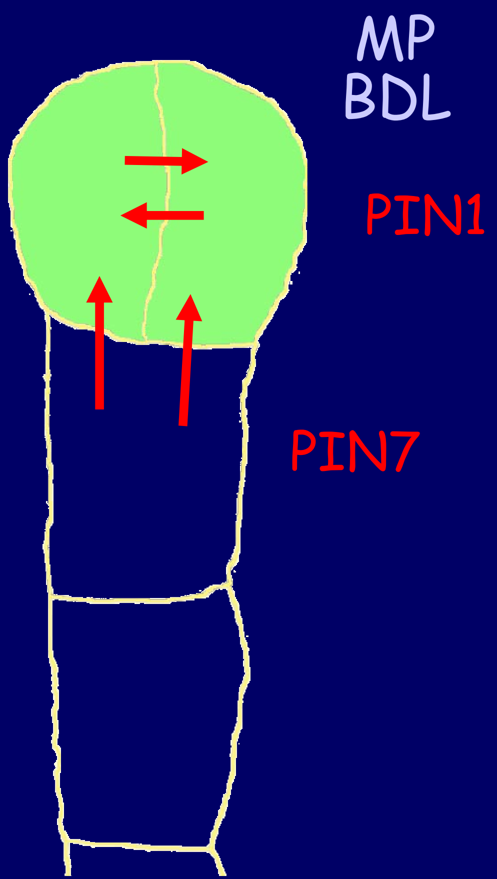


Phenotypes of *pin* Multiple Mutants



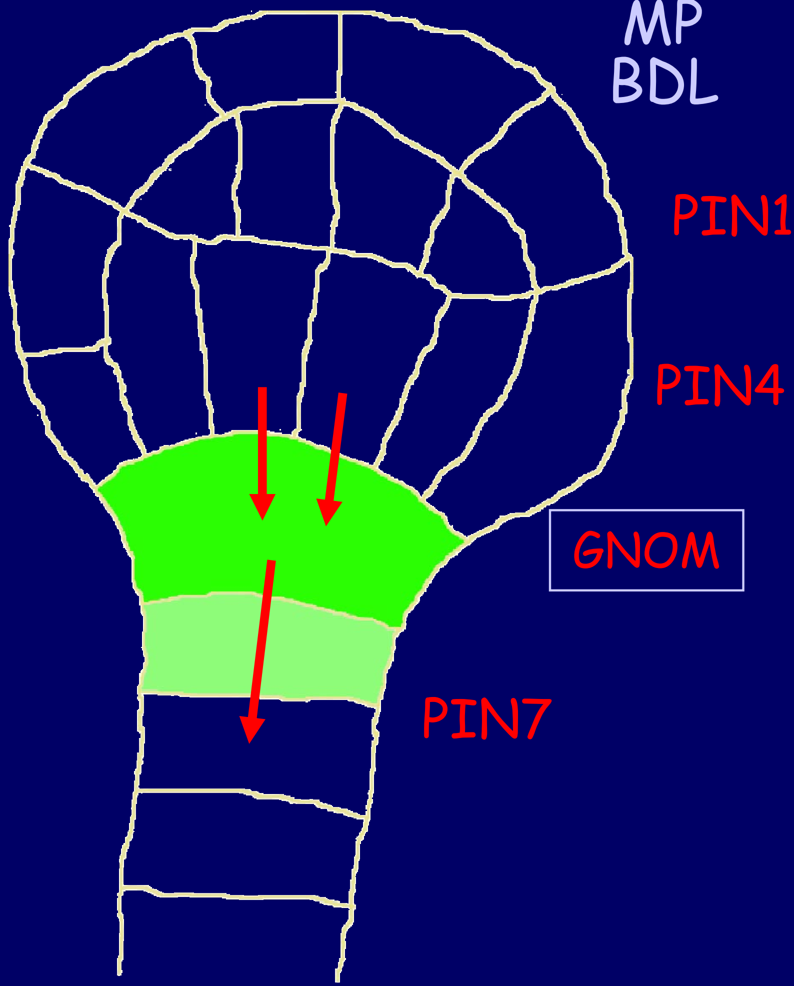
Auxin and Embryogenesis

Apical pole specification



Two-Cell

Root pole specification

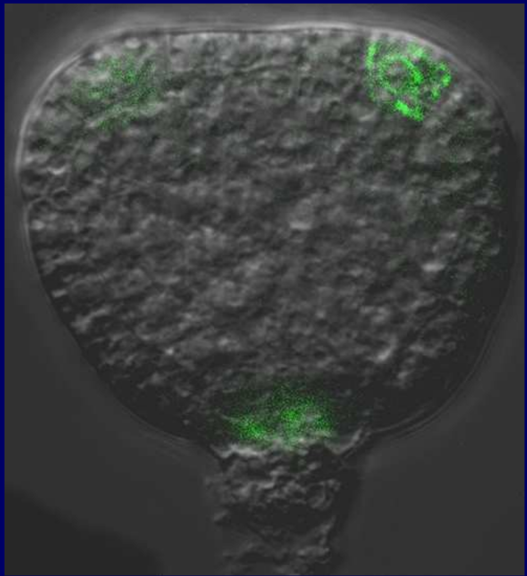


Globular

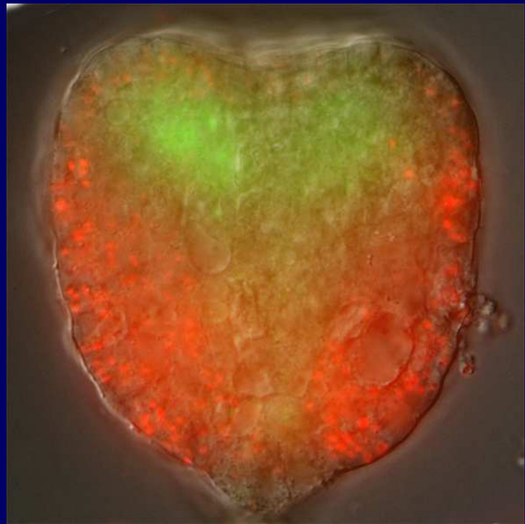
Organogenesis

Auxin in Cotyledon Formation

DR5



BFA

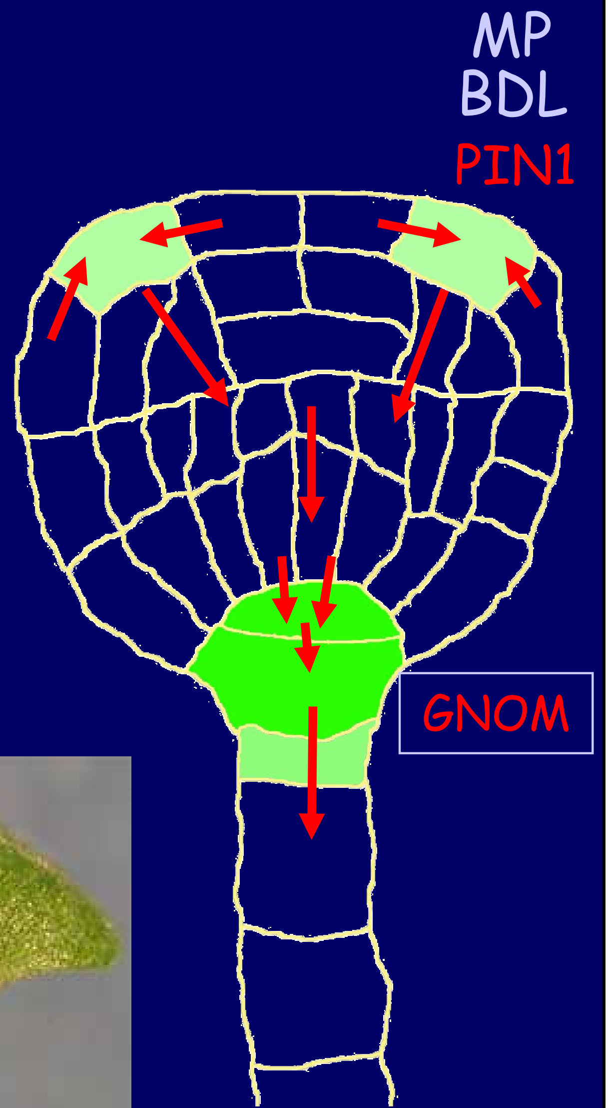


pins

gnom

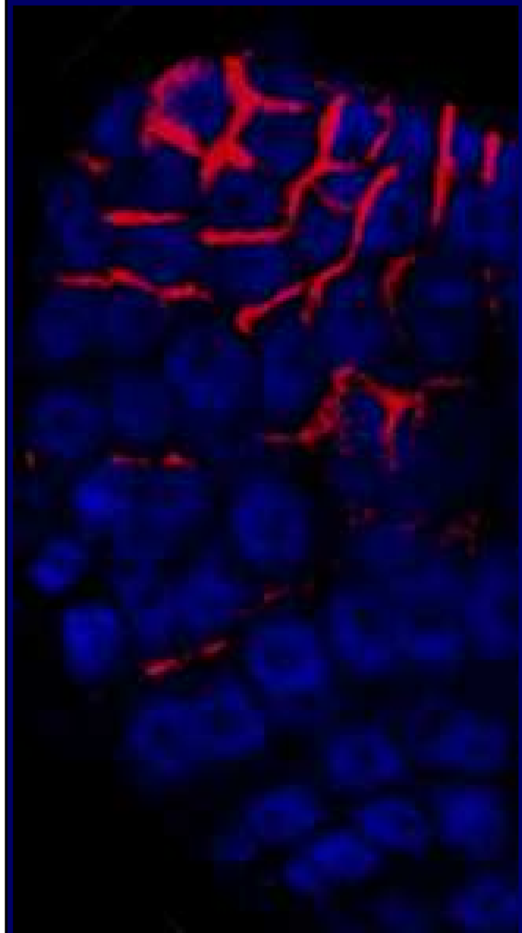
IAA

pin1

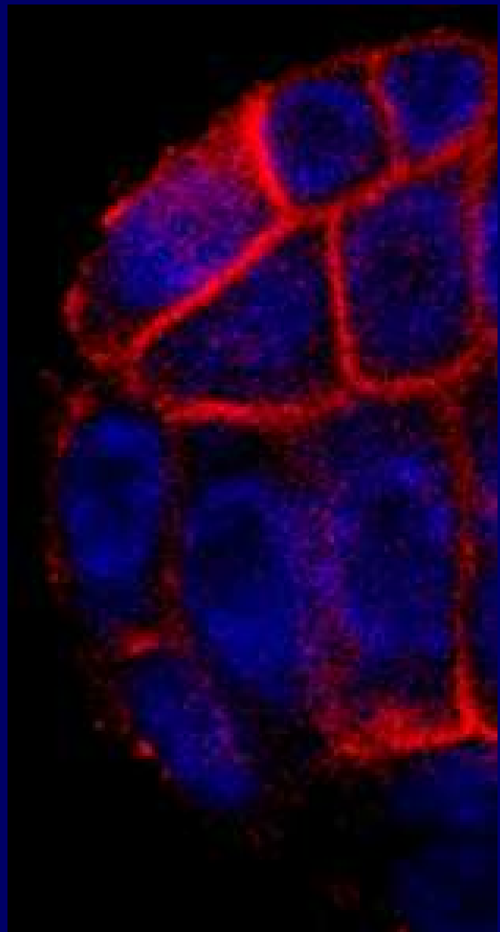


PIN1 Polarity in Cotyledon Formation

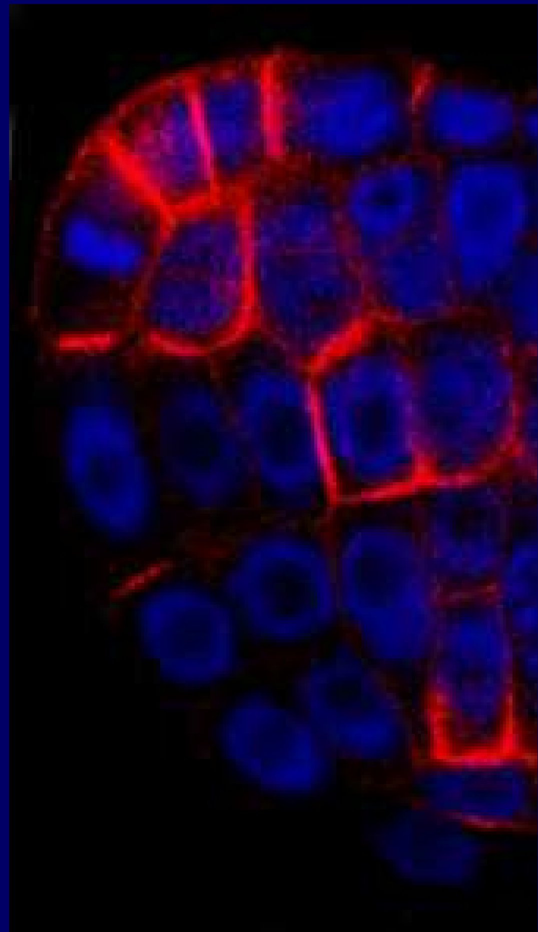
Outer layer | Inner layers | BFA treatment



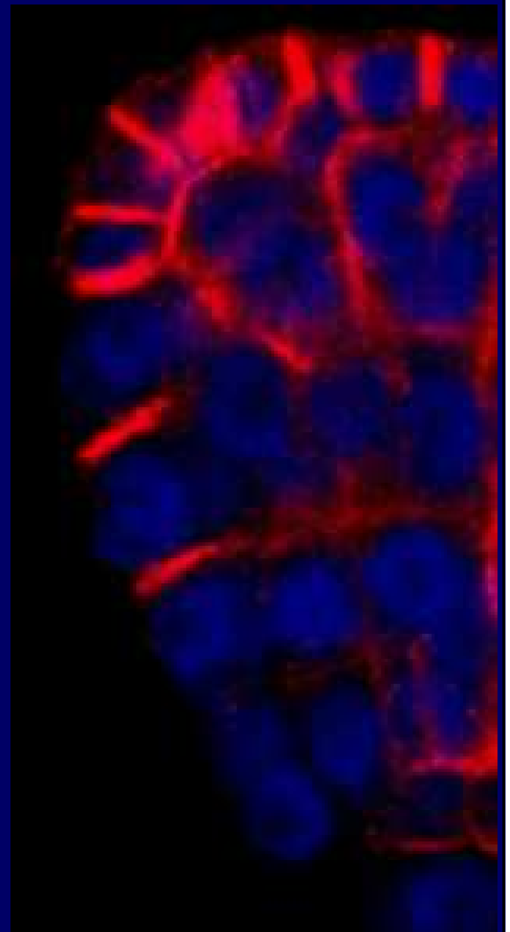
Heart



Globular



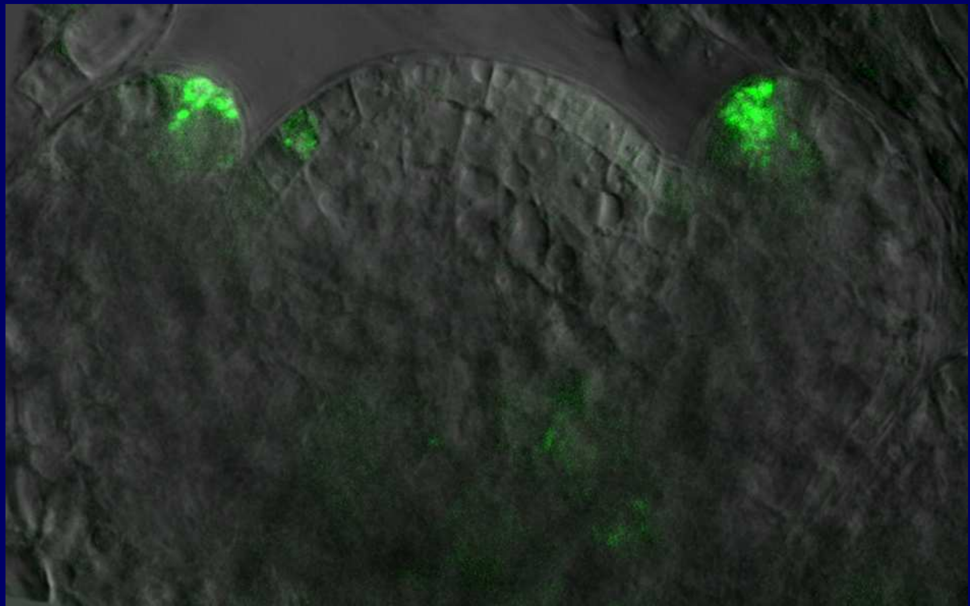
Heart

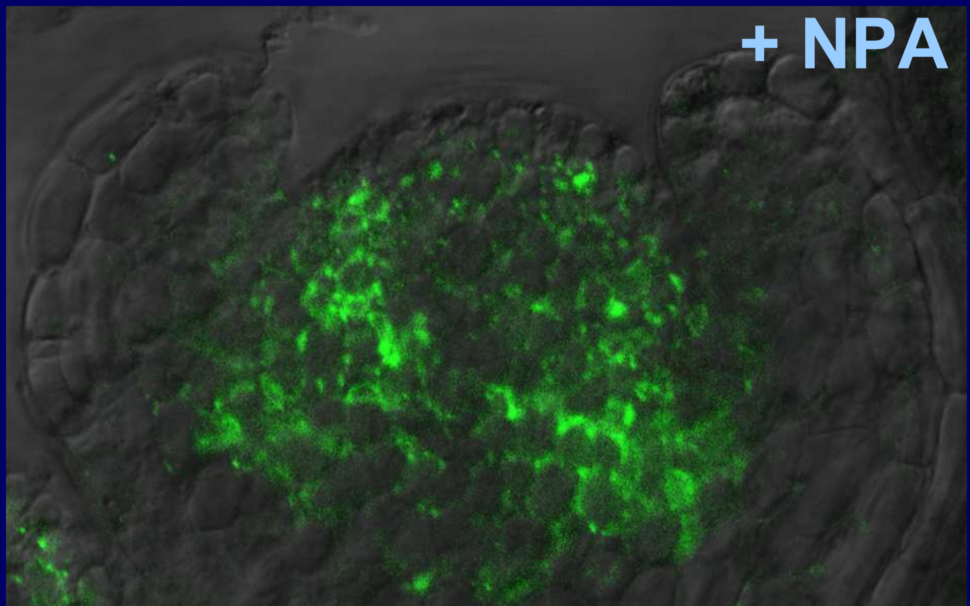


Heart

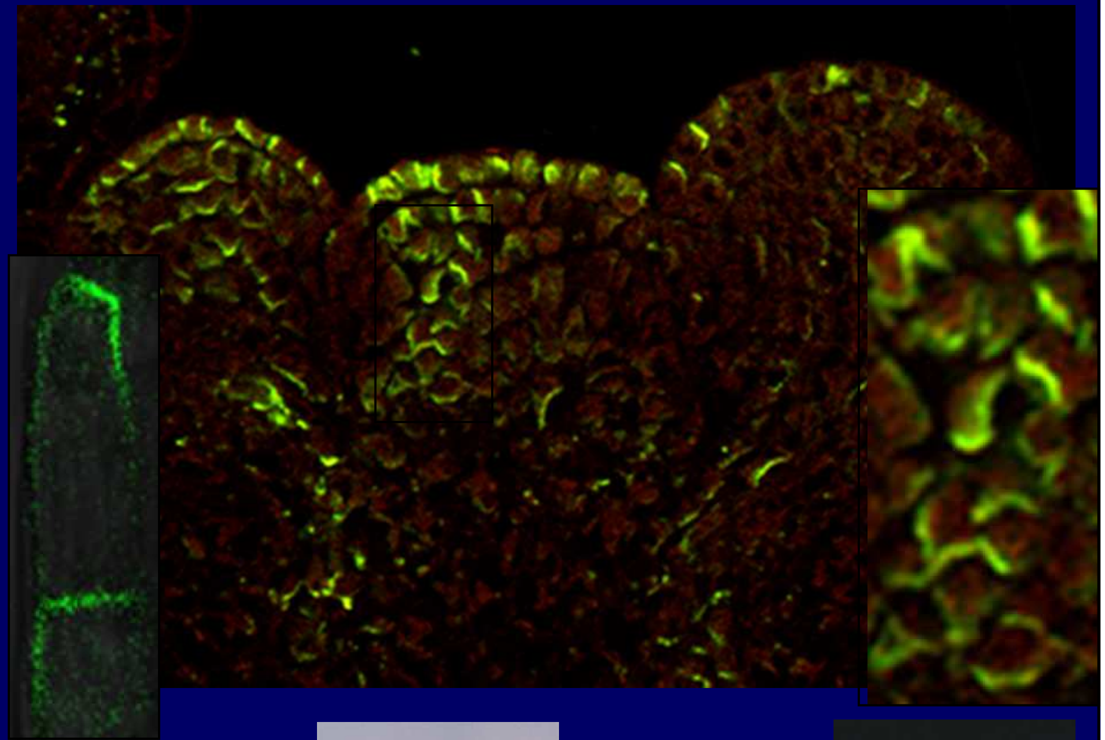
Auxin in Flower and Leave Formation

DR5rev::GFP





PIN1 localisation

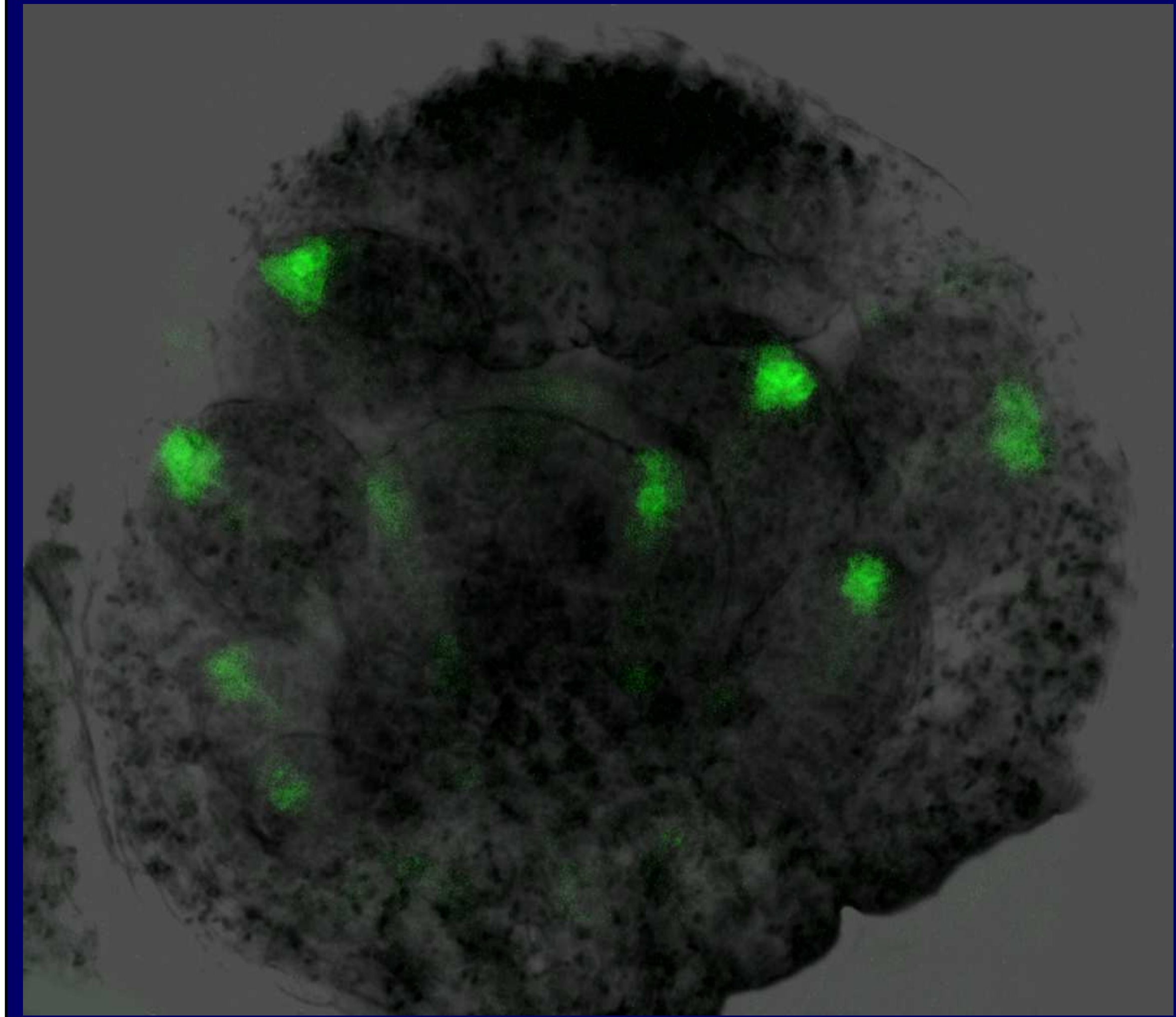


+ NPA

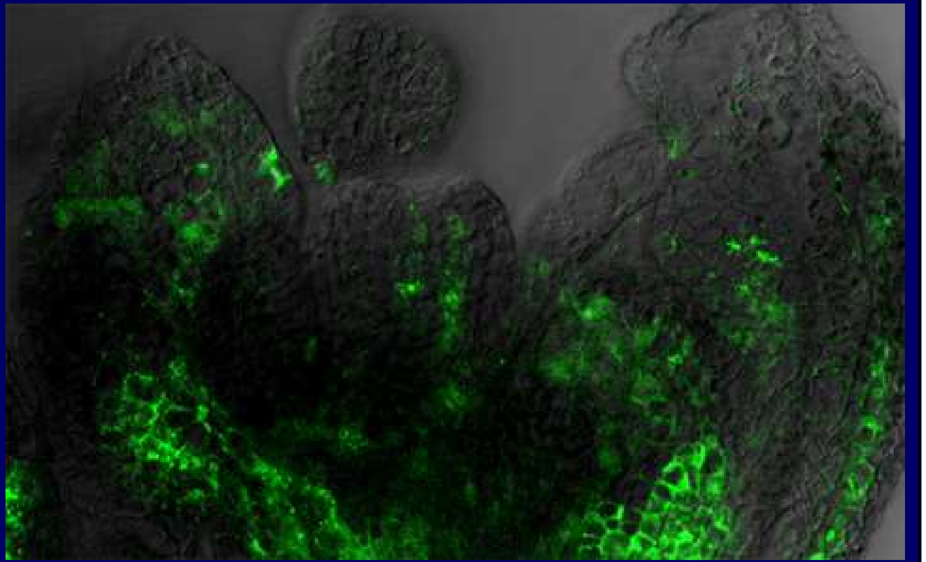
pin1

DR5 in Floral Organ Formation

DR5rev::GFP

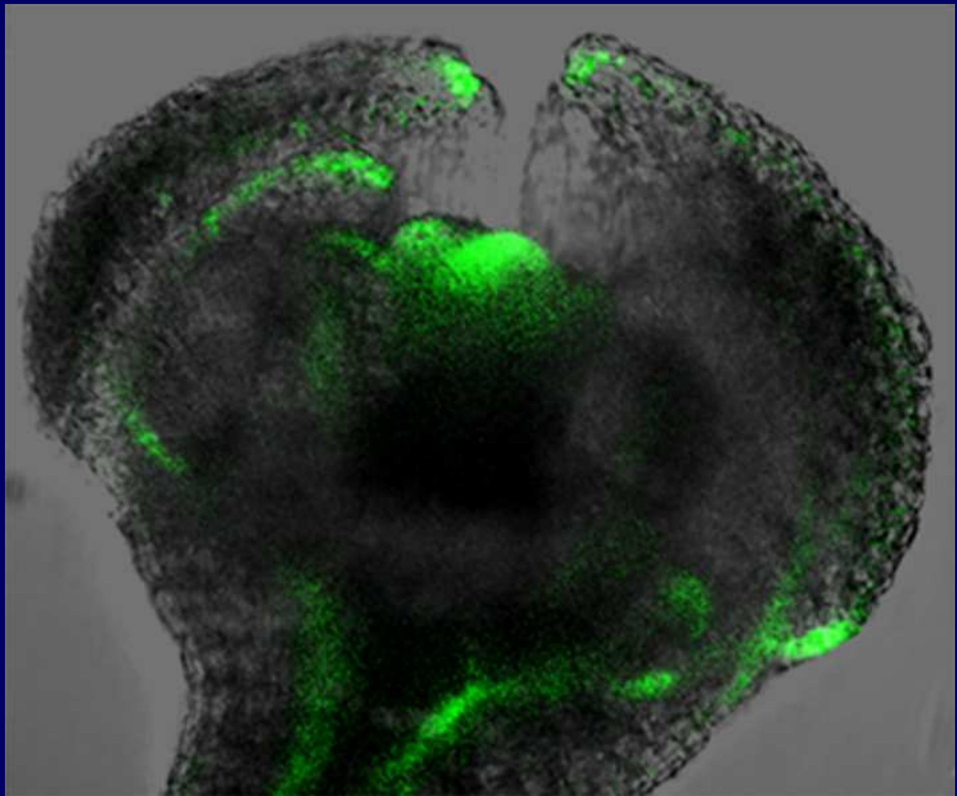


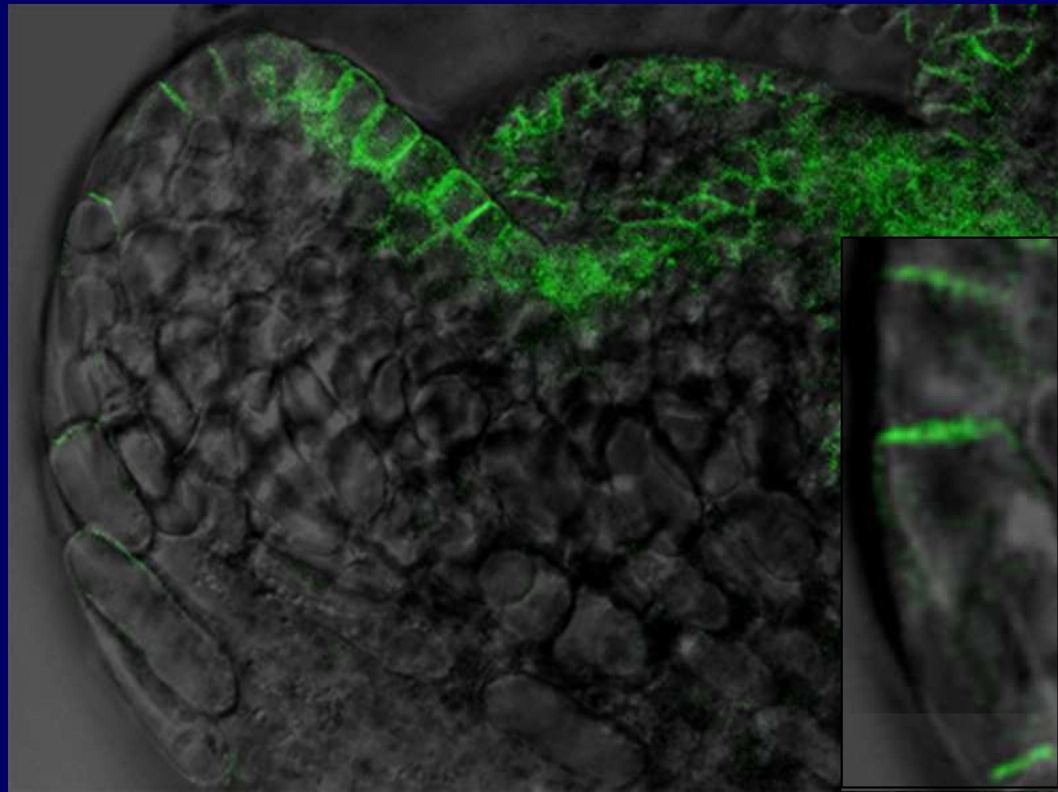
+ NPA



***pin* mutants**

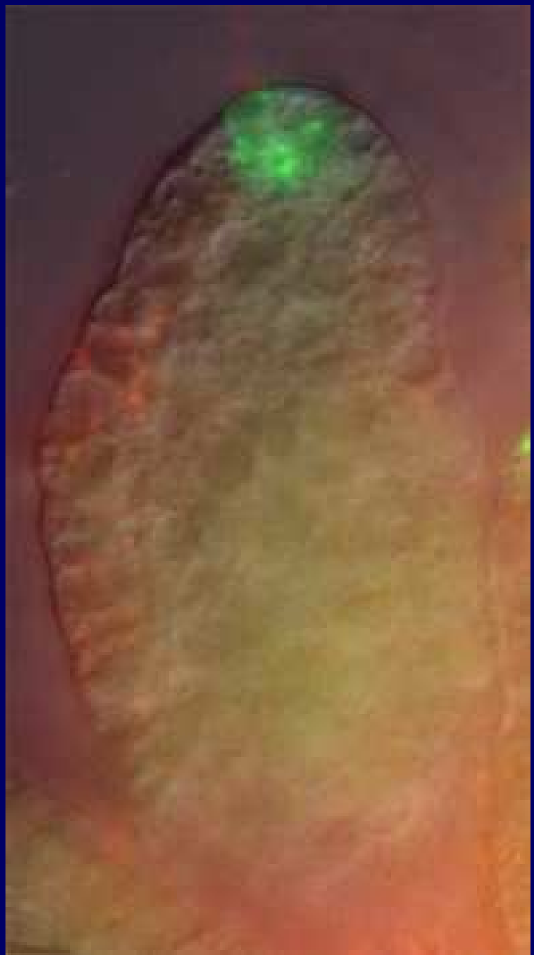
PIN1 in Floral Organ Formation





DR5 in Ovule Formation

Ovule primordium

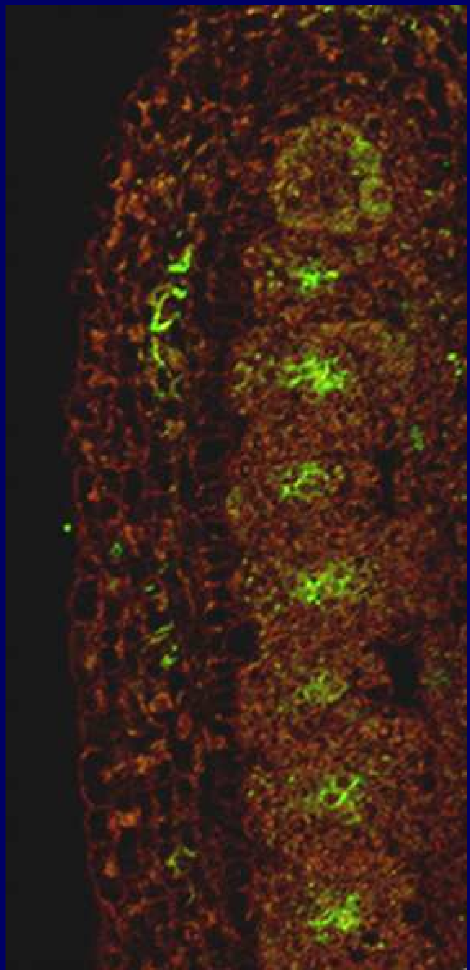


Ovule with Integuments primordia

Ovule defects in *pin1*

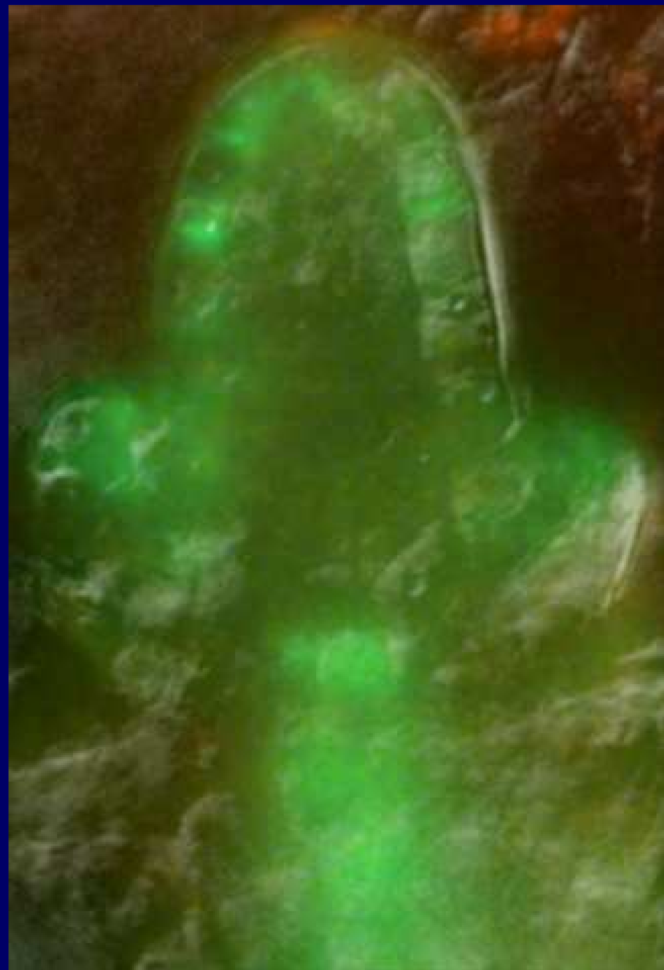
PIN1 in Ovule Formation

Gynoecium with ovule primordia

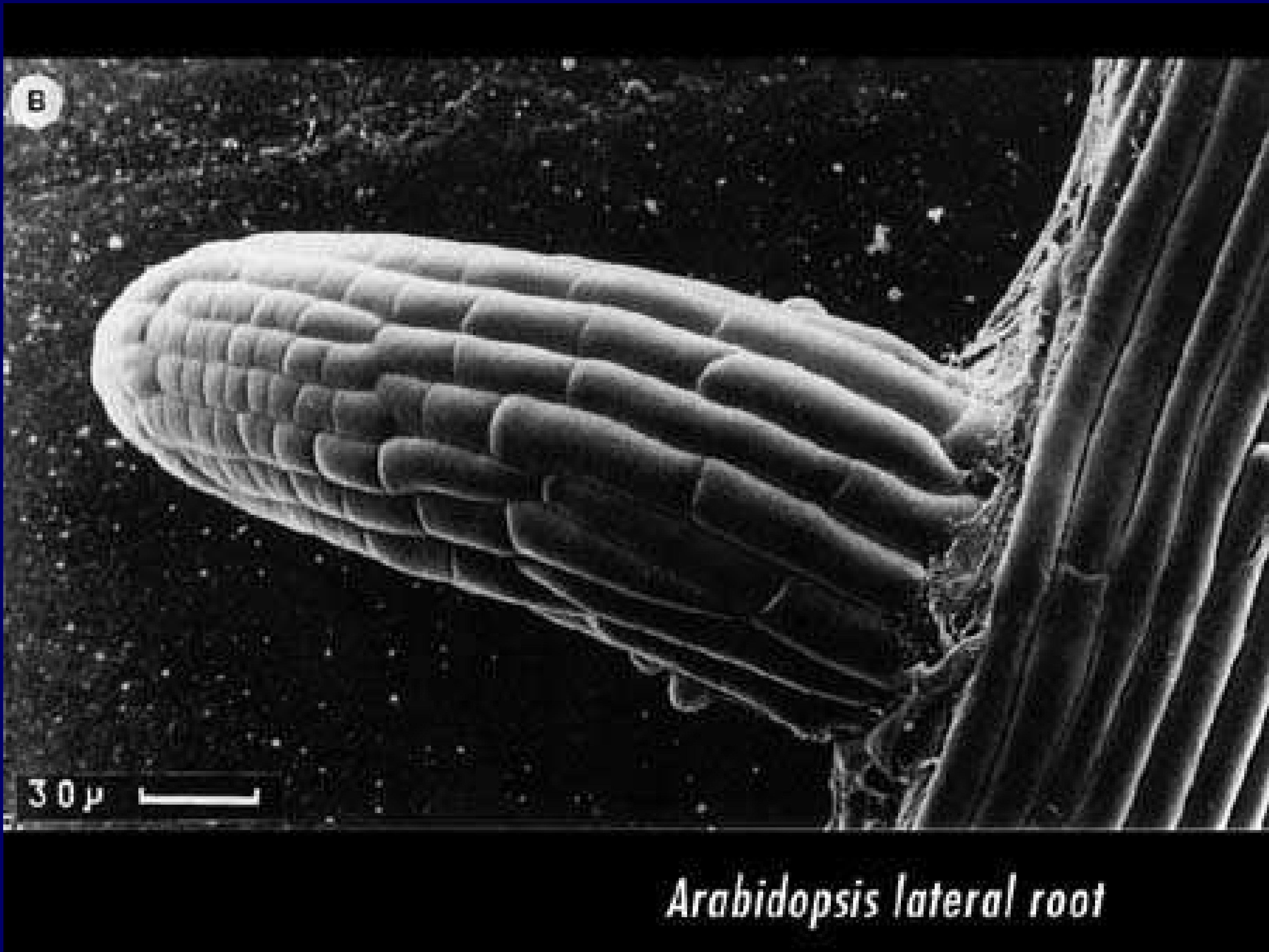


Ovule primordium

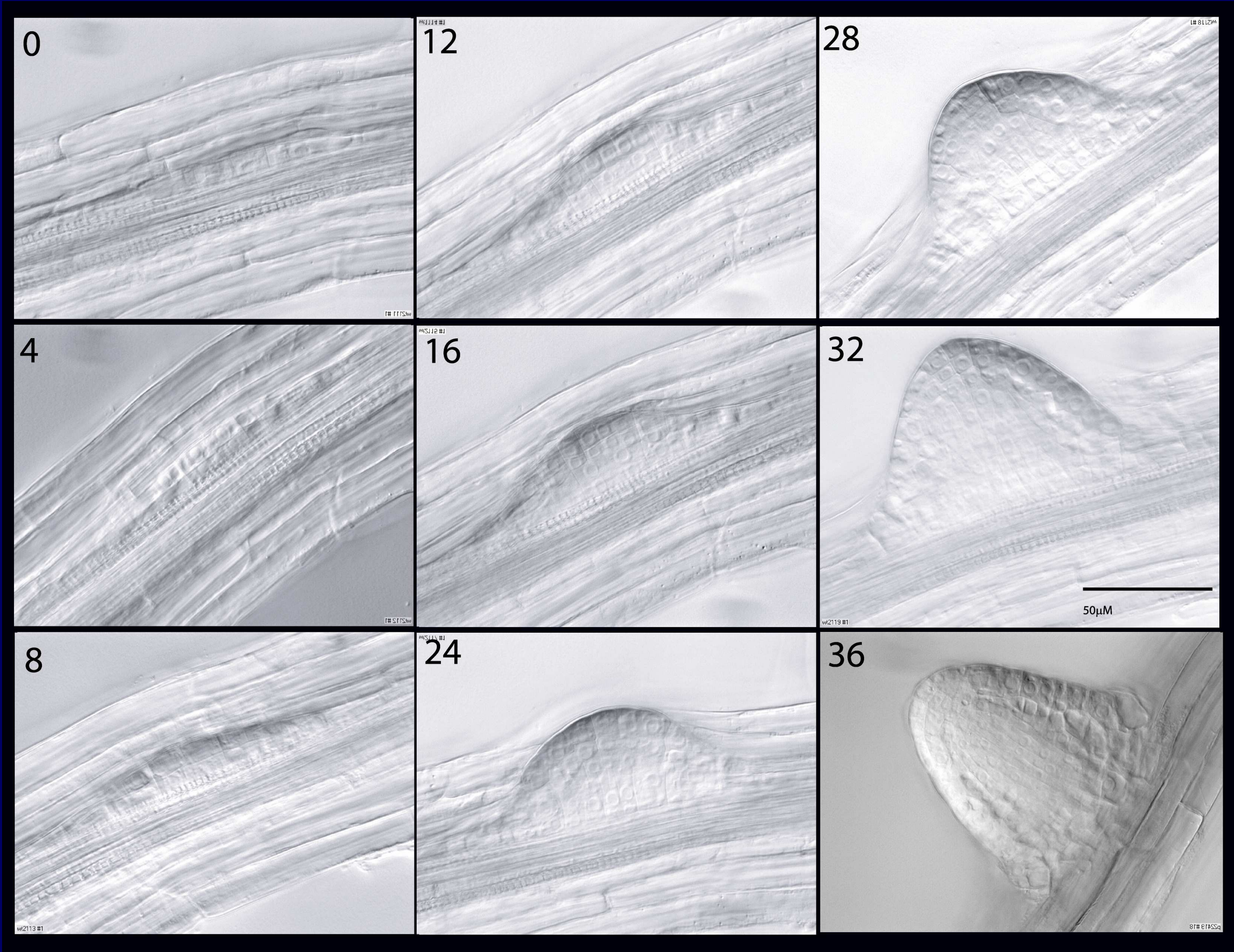
Ovule with Integuments primordia



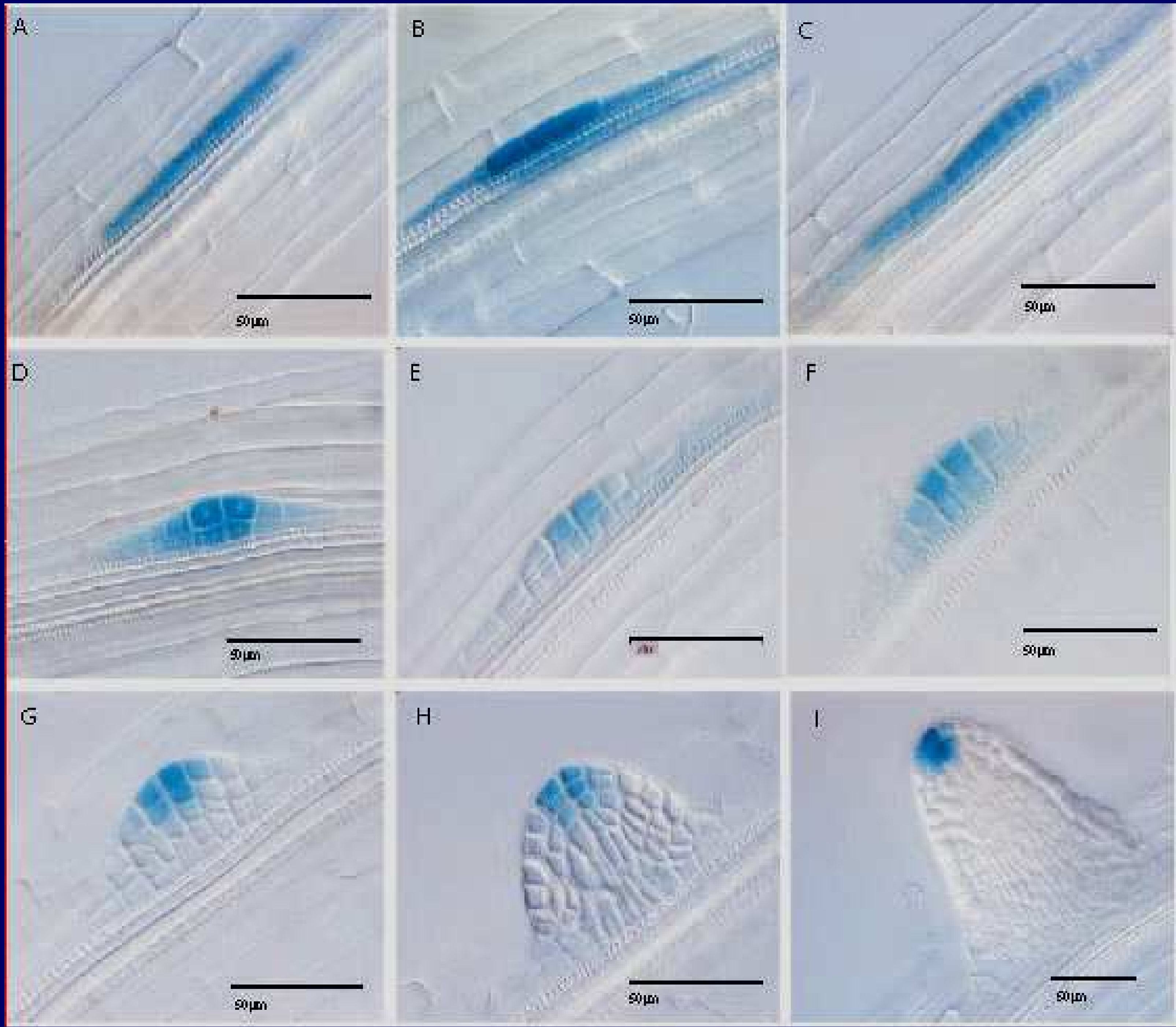
Lateral Root Development



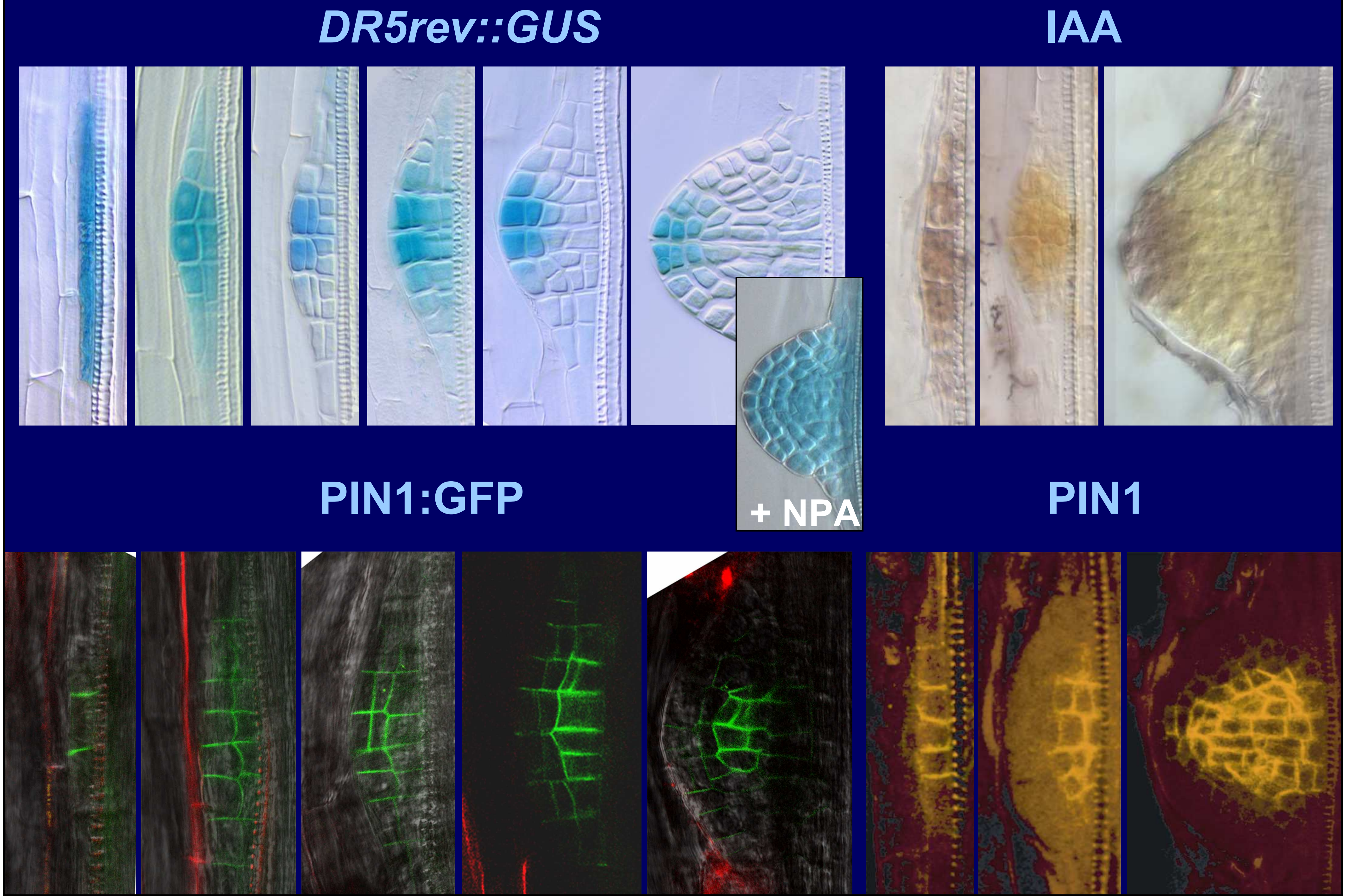
Lateral Root Development in Time



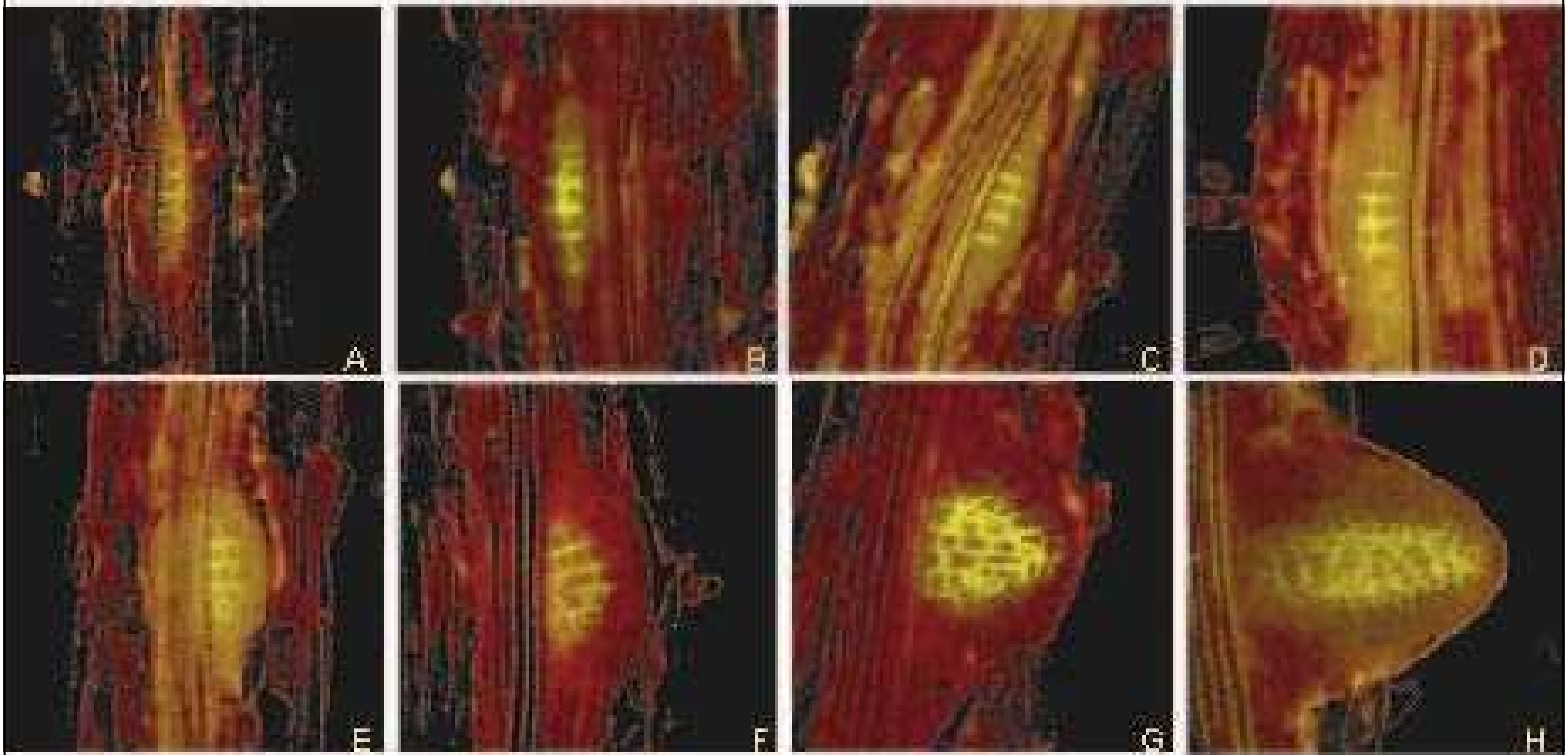
Auxin in Lateral Root Development



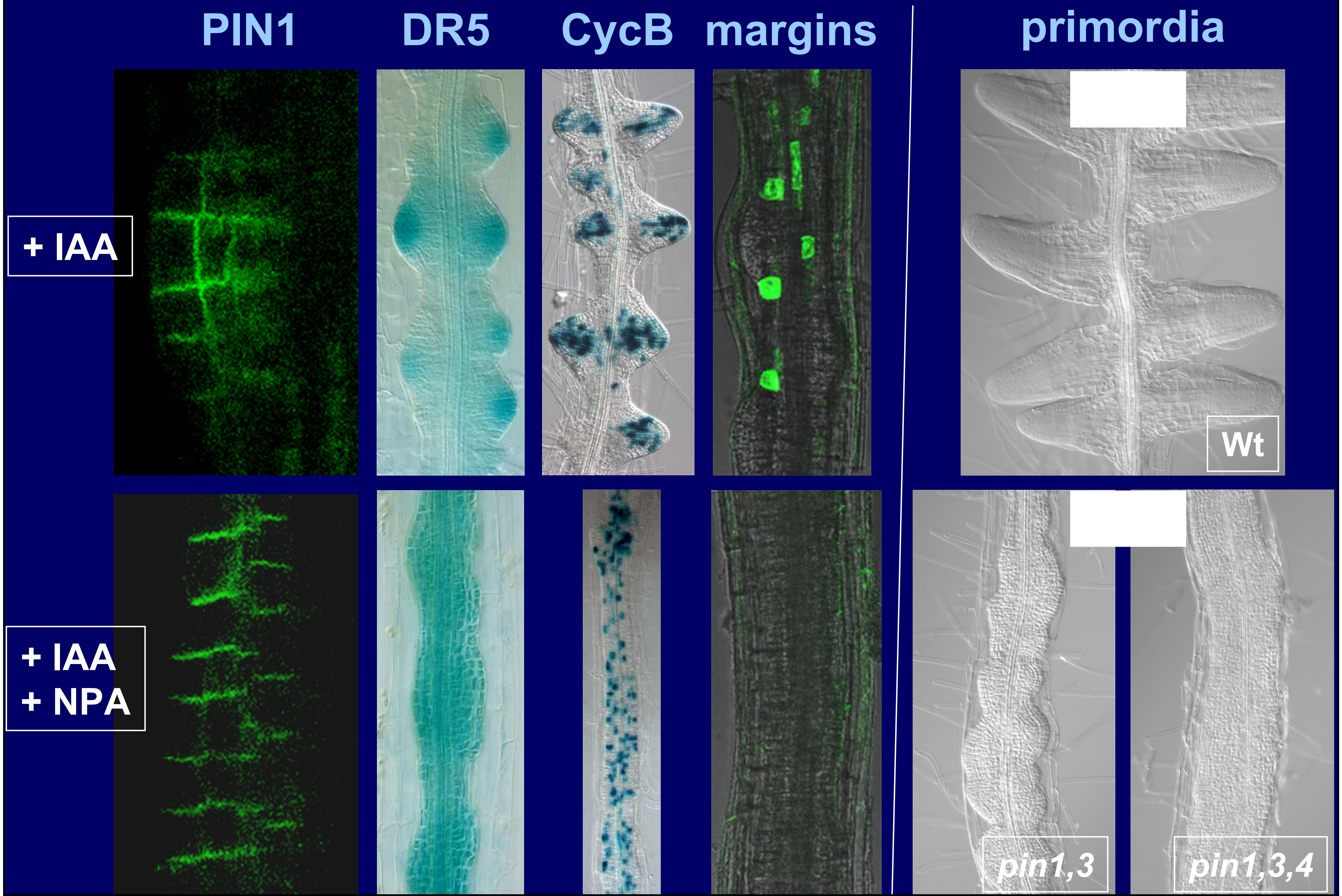
DR5 in Lateral Root Formation



PIN1 in Lateral Root Development



Relocation > Gradients > Primordia



Lateral Root Development

- -Organogenic process involving re-entry into cell cycle and coordinated cell divisions and differentiation.

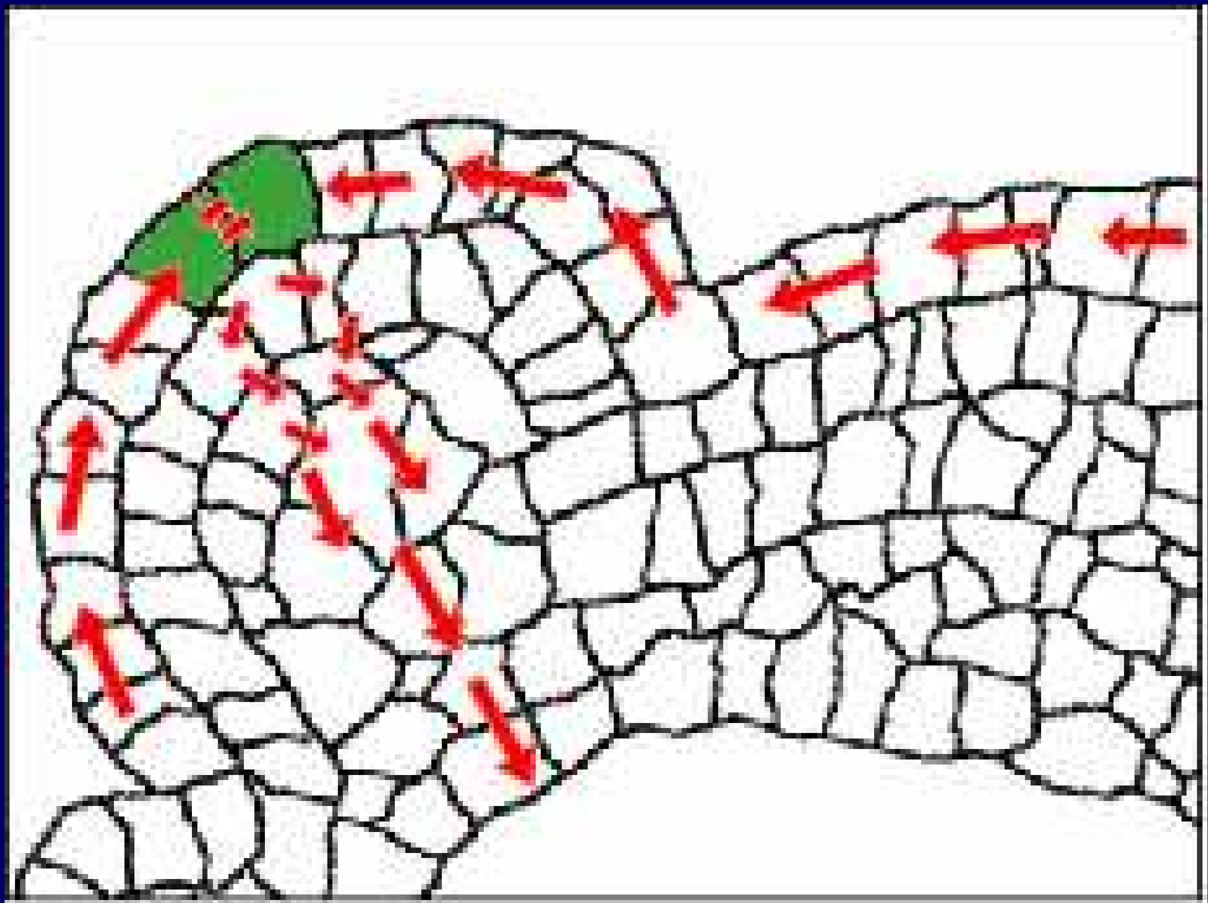
- -Initiation (in pericycle) and development phases can be distinguished.

- -Both phases require both long and short distance signaling probably by auxin and cytokinin.

- -The lateral root meristem development is mediated by auxin gradient.

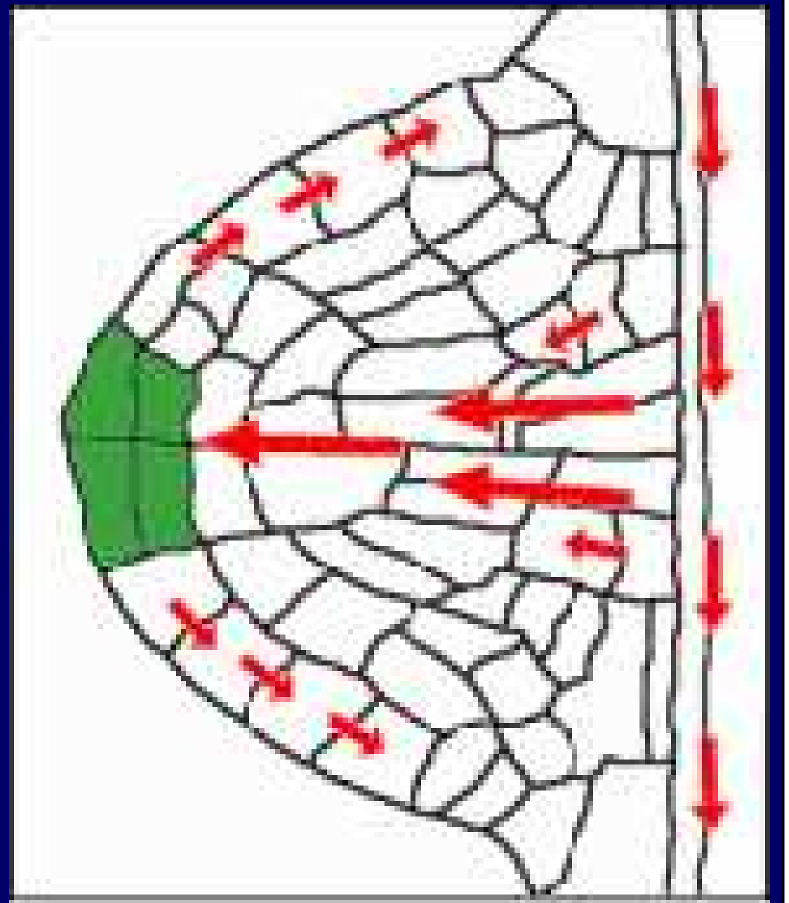
Common module for organ formation

Aerial organogenesis



Cotyledons, leaves, flowers, floral organs, ovules, integuments

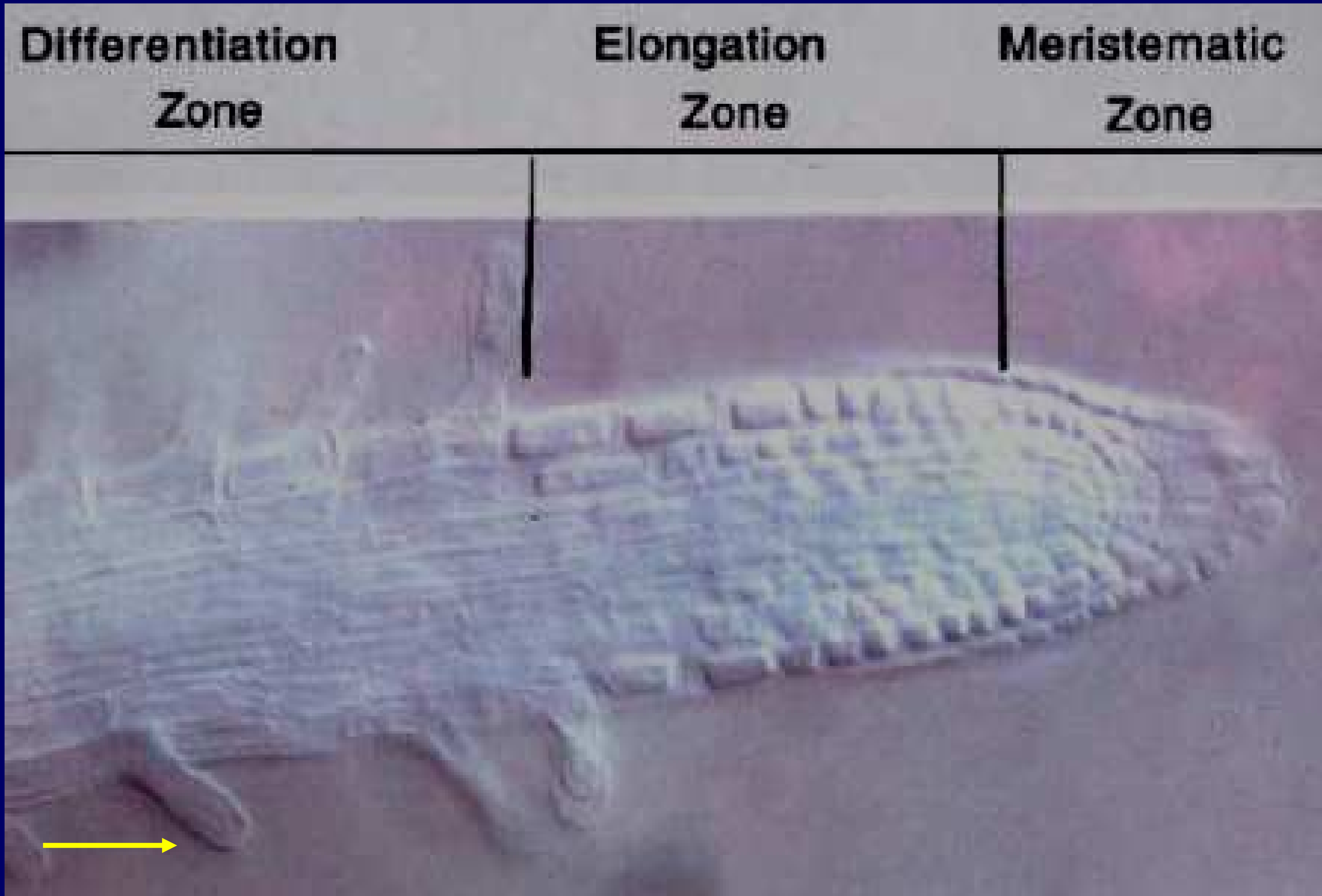
Underground organogenesis



Lateral roots

Root meristem

Parts of the Primary Root

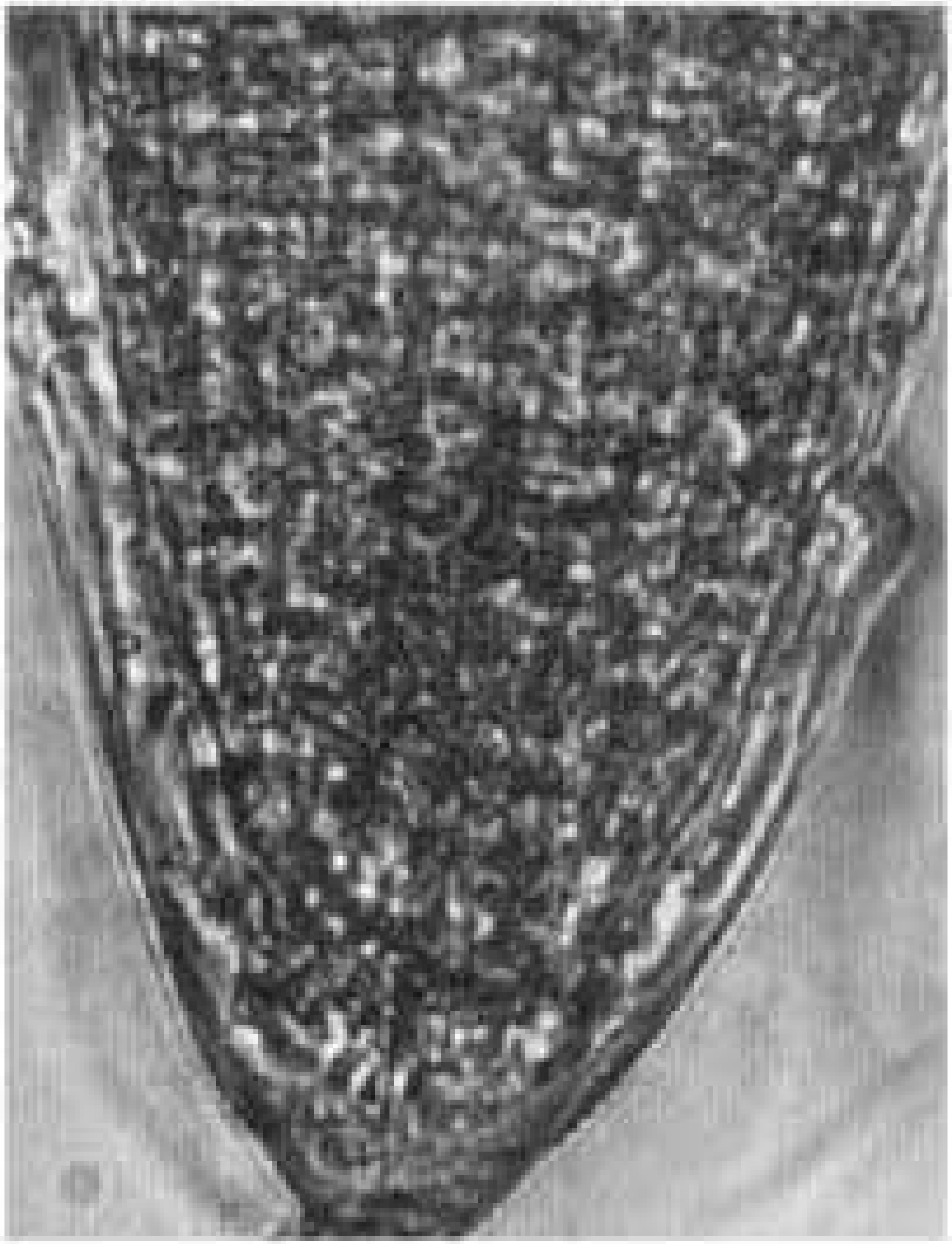


Root hair

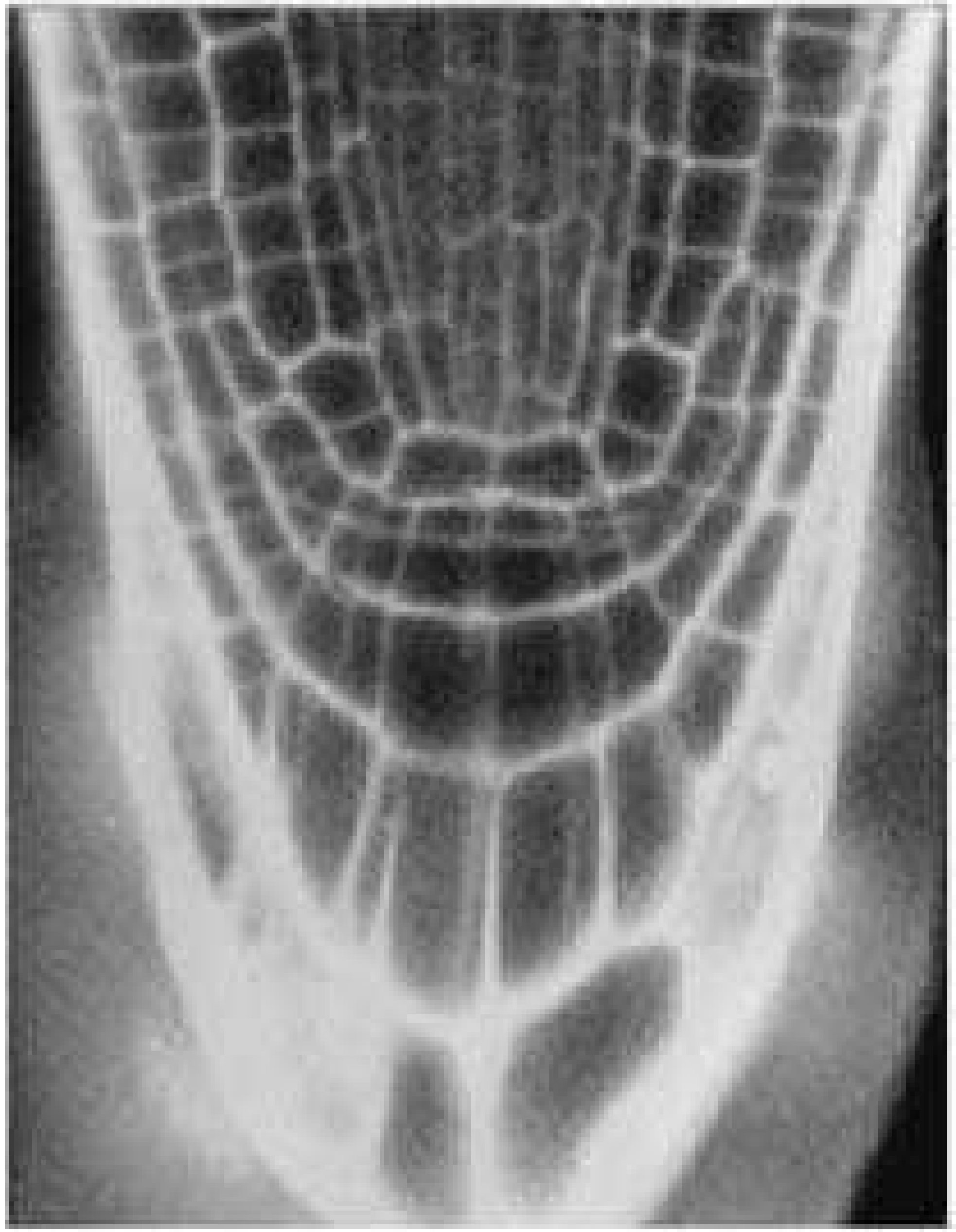
Differentiation Elongation Division

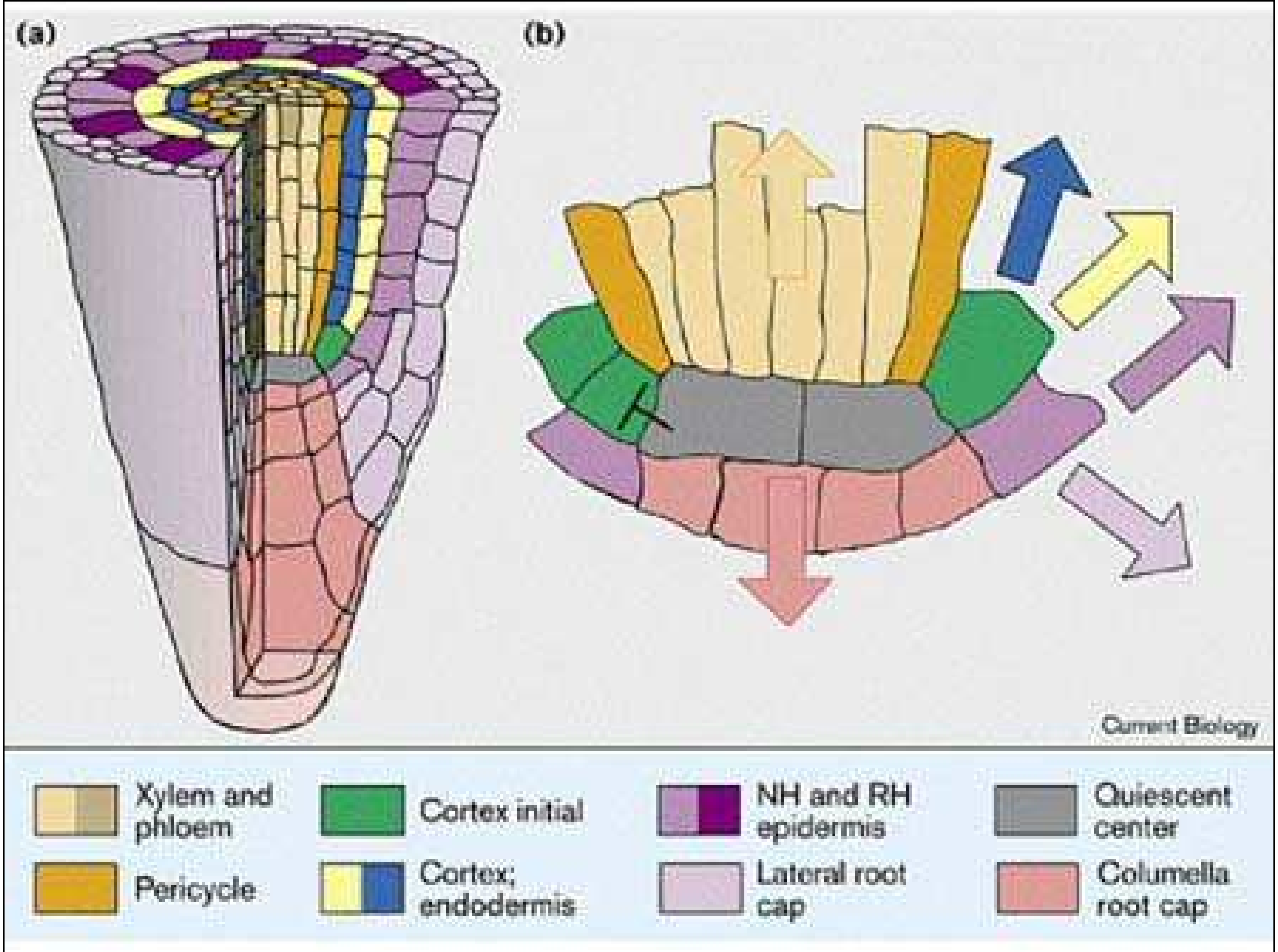
Root Meristem

Light microscopy

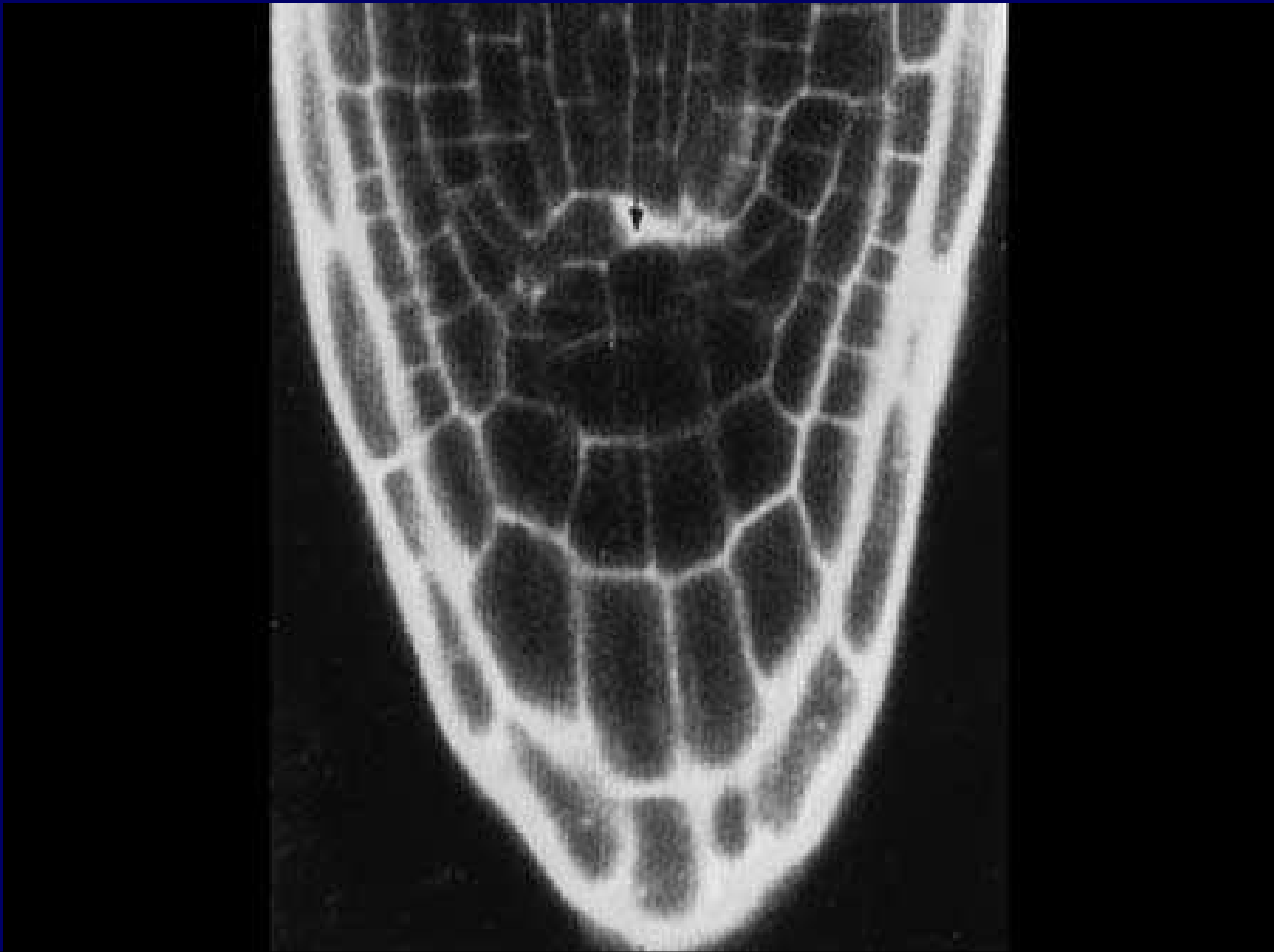


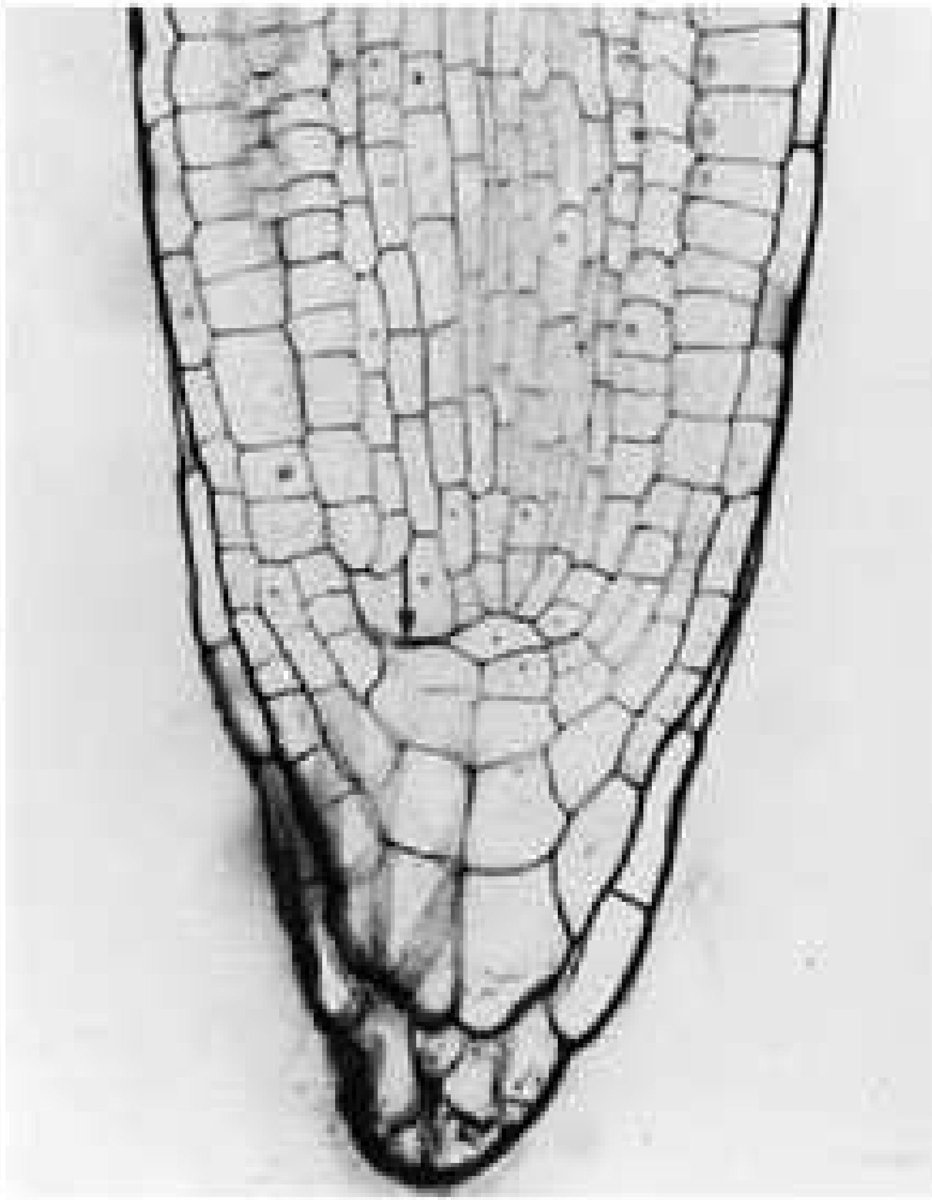
Confocal microscopy



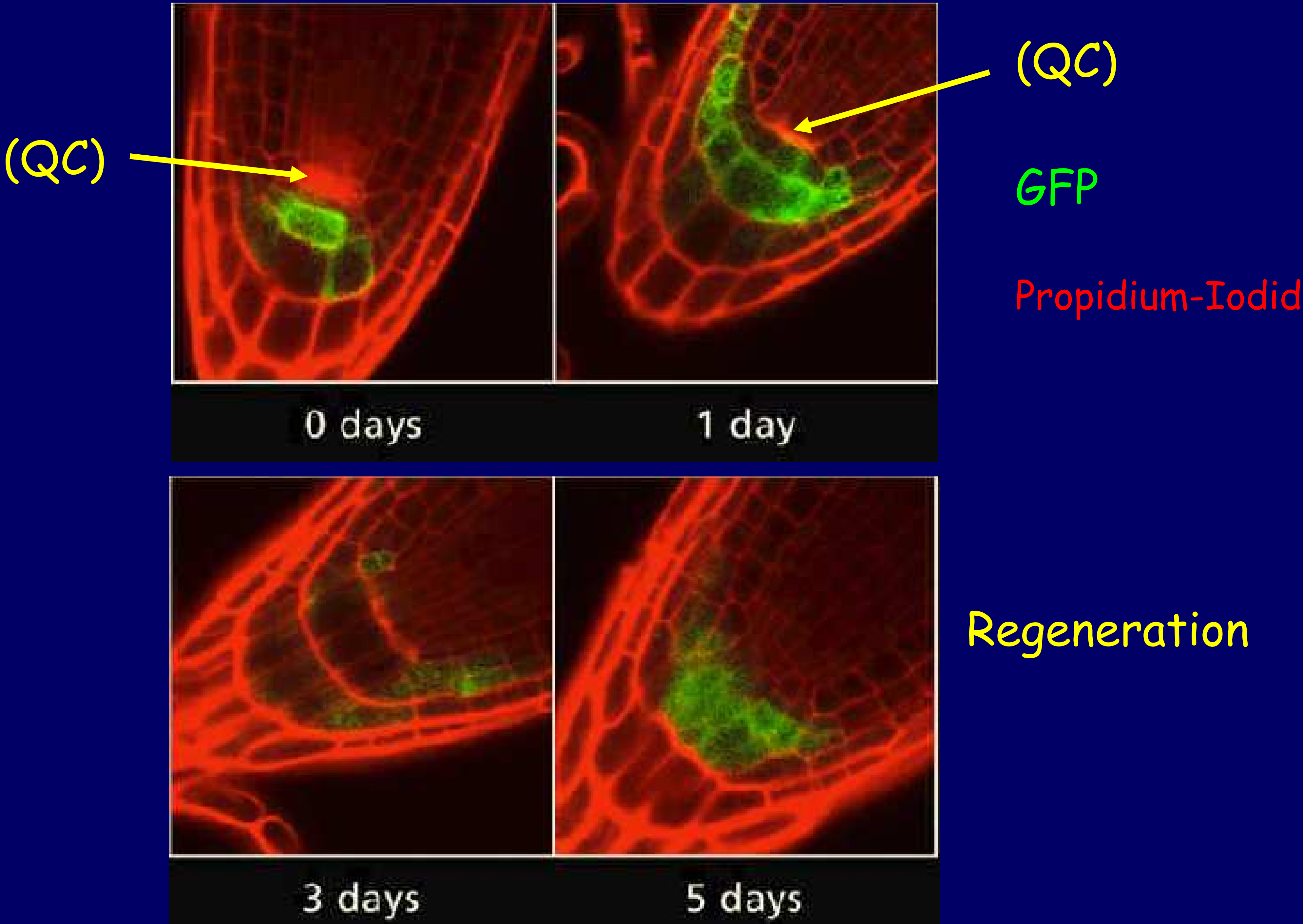

(a)
(b)
Current Biology
Xylem and phloem
Cortex initial
NH and RH epidermis
Quiescent center
Pericycle
Cortex; endodermis
Lateral root cap
Columella root cap

Laser Ablation of Single QC Cell

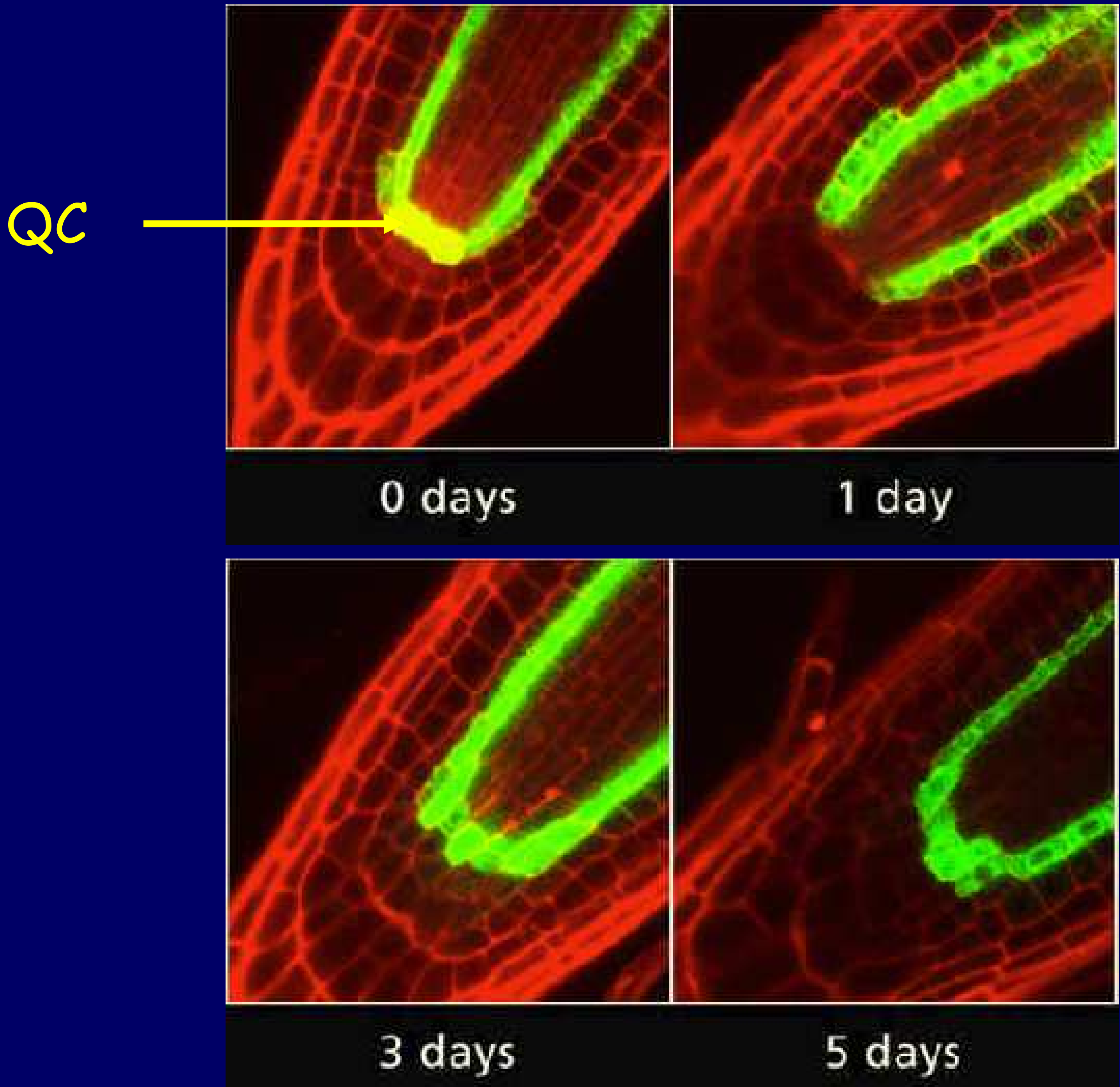


Laser Ablation of Quiescent Centre



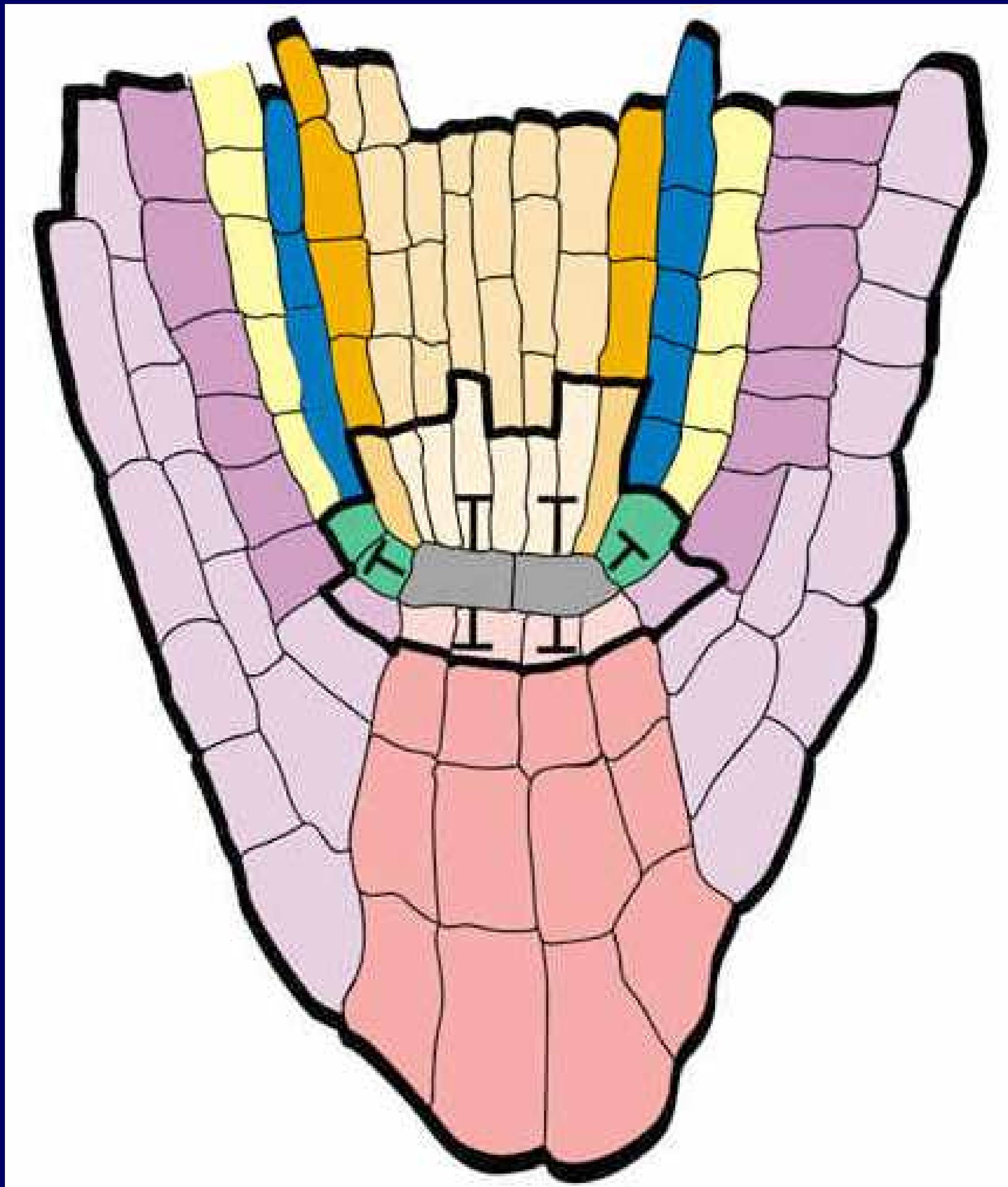
Regeneration of Quiescent Centre

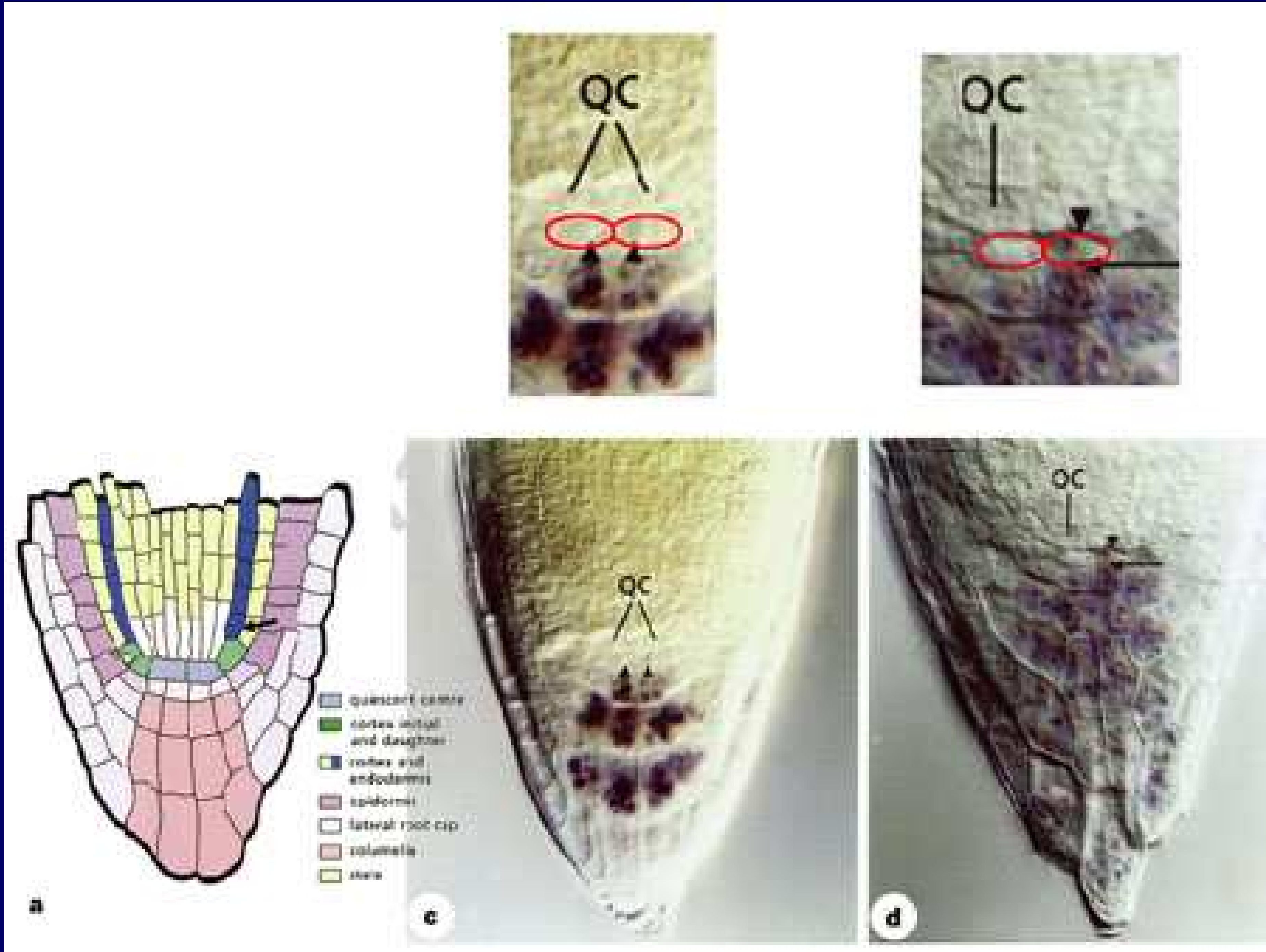


SCR::GFP

(Endodermis + QC)

Model for Role of QC in Keeping Stem Cells




QC
QC
quiescent centre
lateral root cap
columella
stele
a
QC
c
QC
d

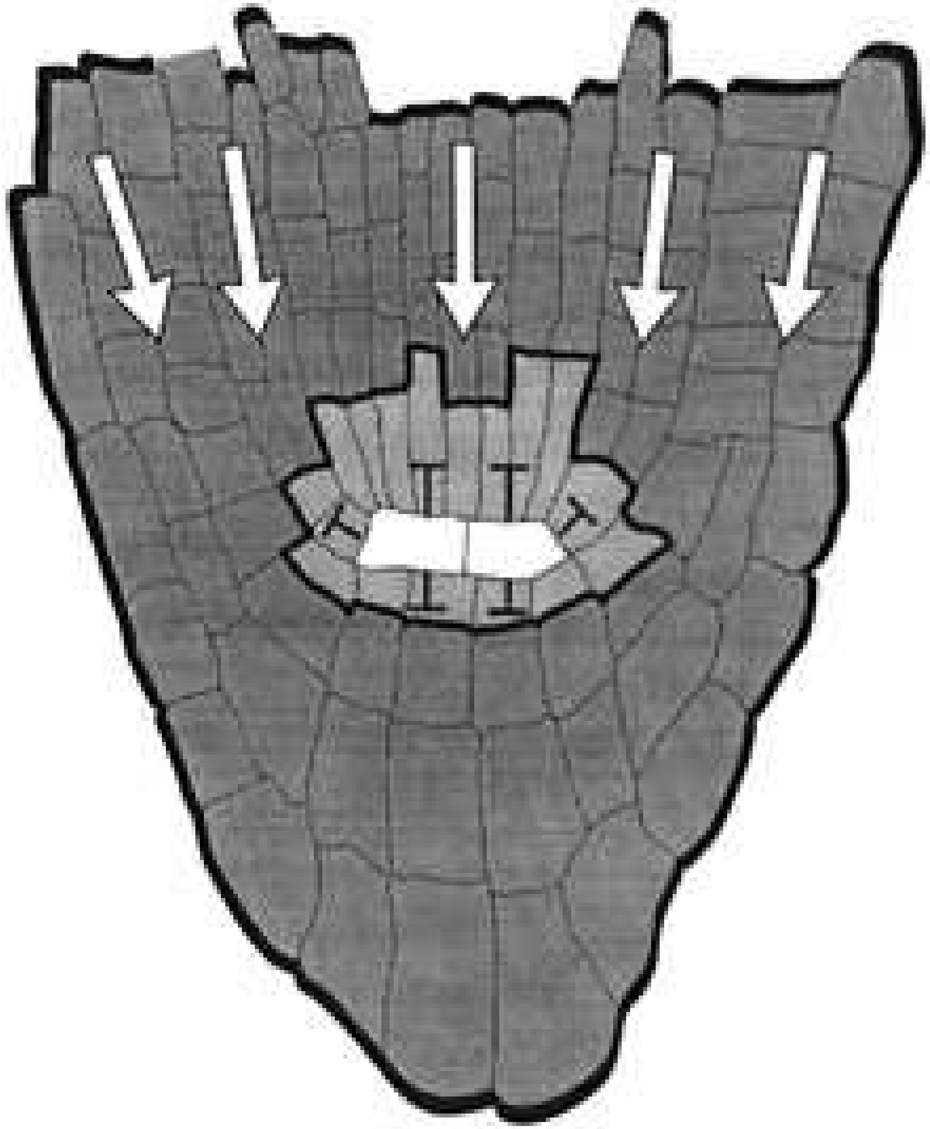


Fig. 4. A simple model representing two different regulatory signals within the root meristem. The quiescent centre inhibits differentiation of surrounding initials, whereas positional cues direct differentiation into different cell types

Indirect Visualisation of Auxin

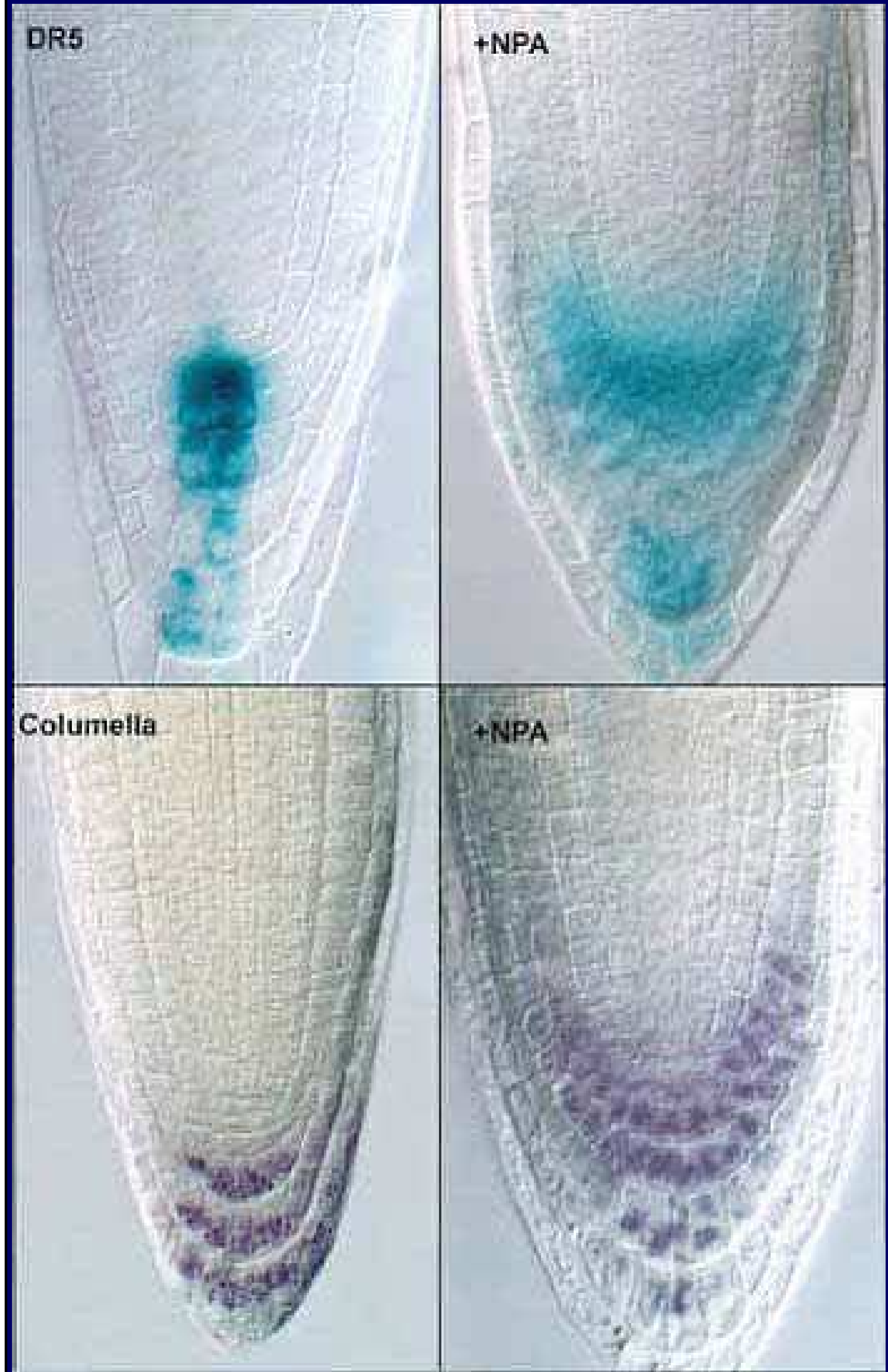
DR5::GUS (Auxin) Response Reporter

5´ CCTTT TGTCTC *3´*

9x inv.

Ulmasov *et al.*, 1997

Auxin and Root patterning



Auxin related mutants affecting root pattern

Auxin resistant – *axr1*, *axr6*

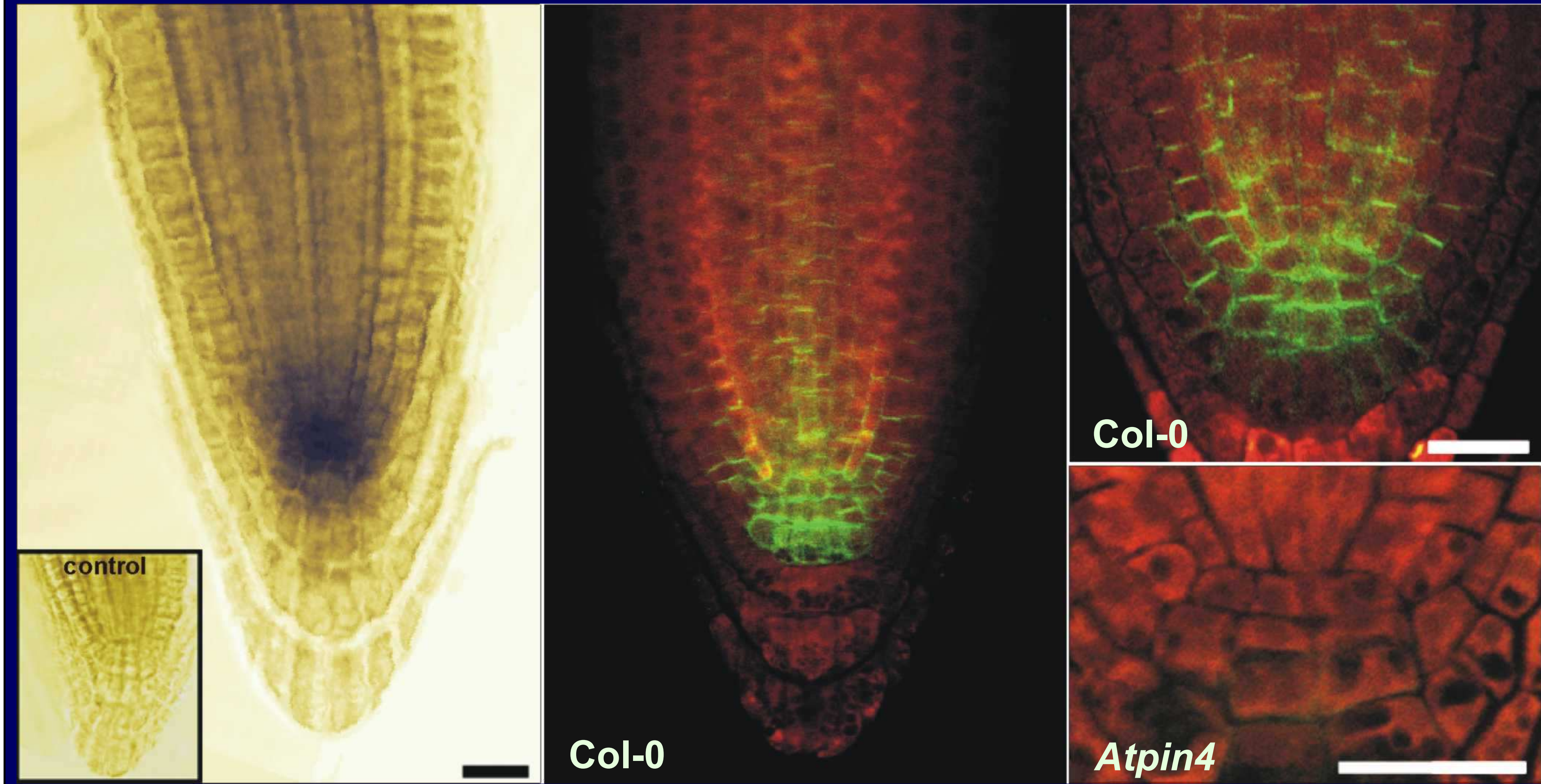
AUX/IAA – bodenlos (*bdl*)

Auxin response factors
– monopteros (*mp*)

Auxin transport – *pin4*

AtPIN4 in Arabidopsis Root Tip

***in situ* RNA hybridisation** **The AtPIN4 protein** **The AtPIN4 protein**



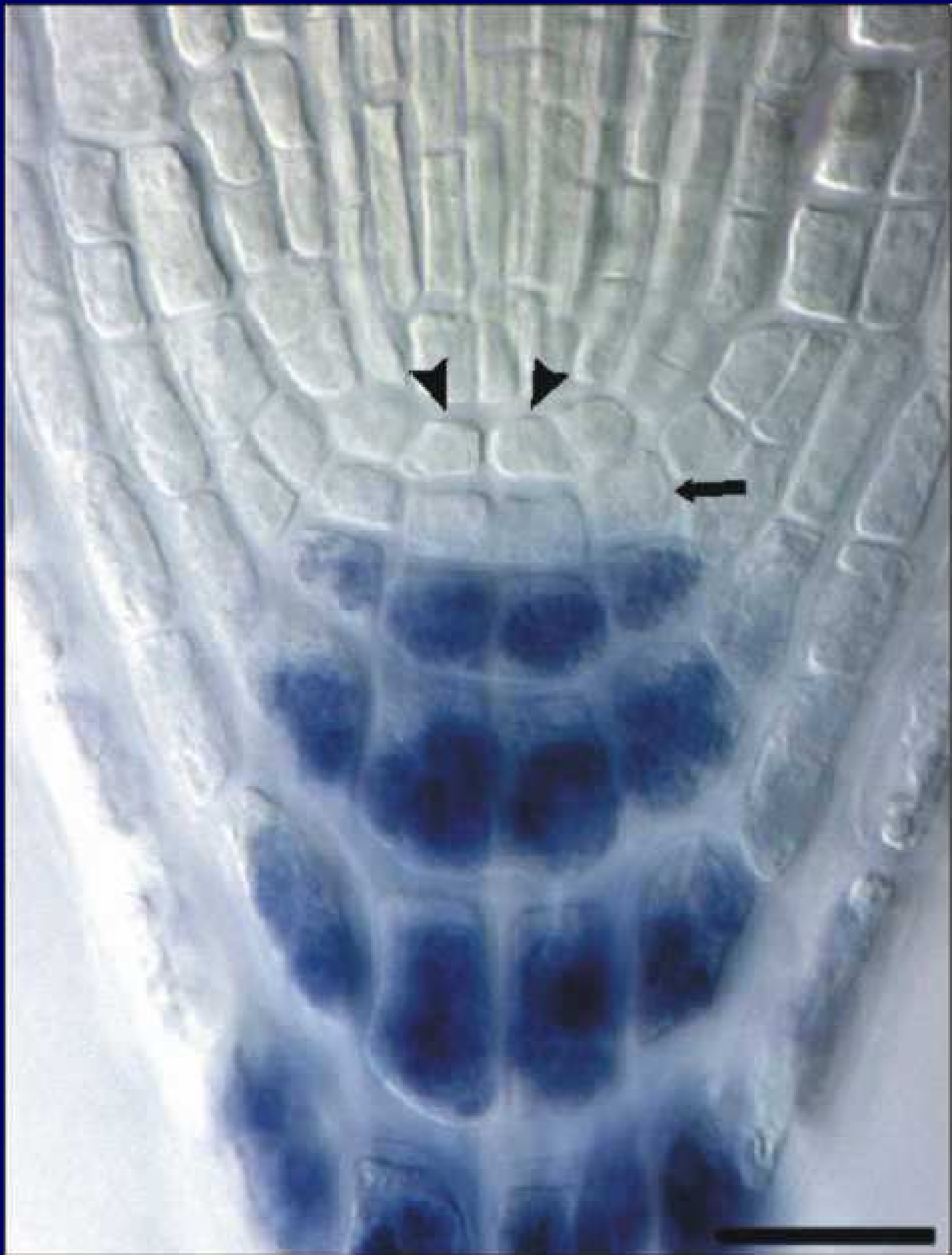
DR5 Auxin Response in Roots

Col-0

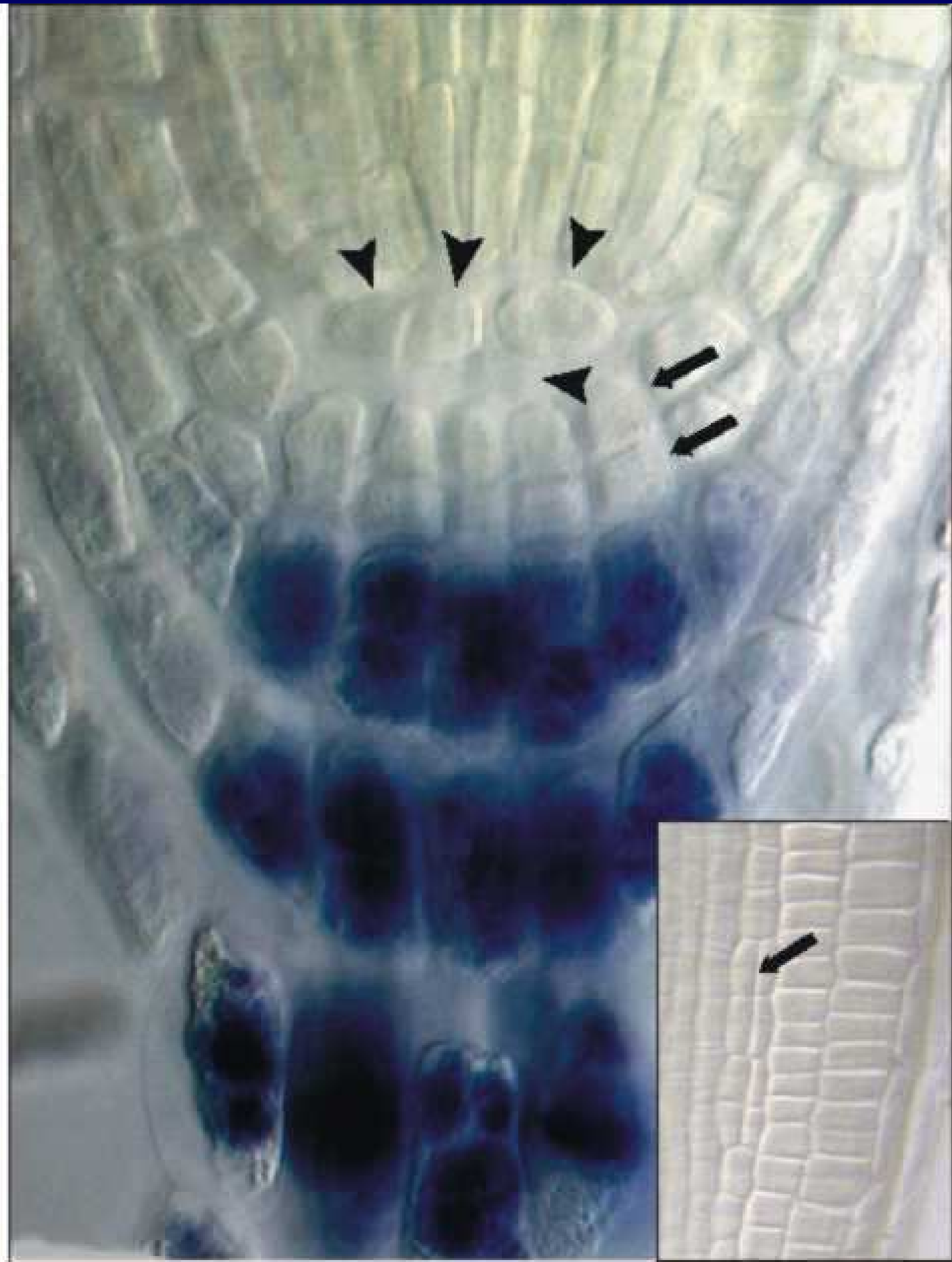
Atpin4

Atpin4 Root Pattern (4 days)

Col-0



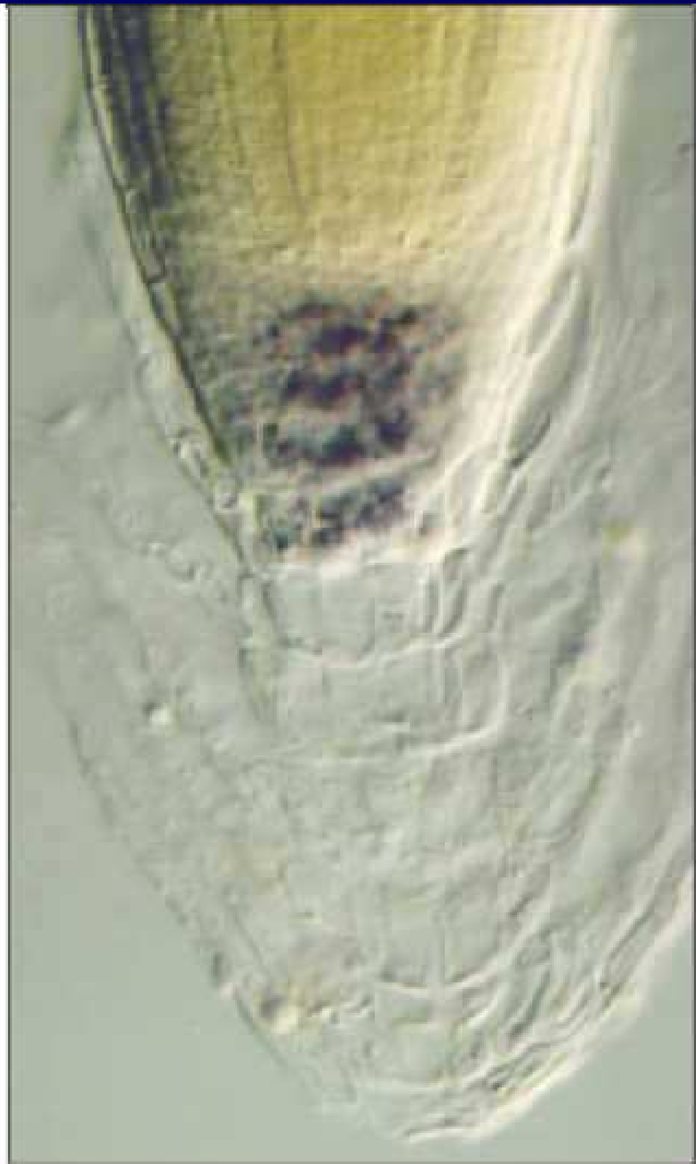
Atpin4



Atpin4 Root Pattern (10 days)

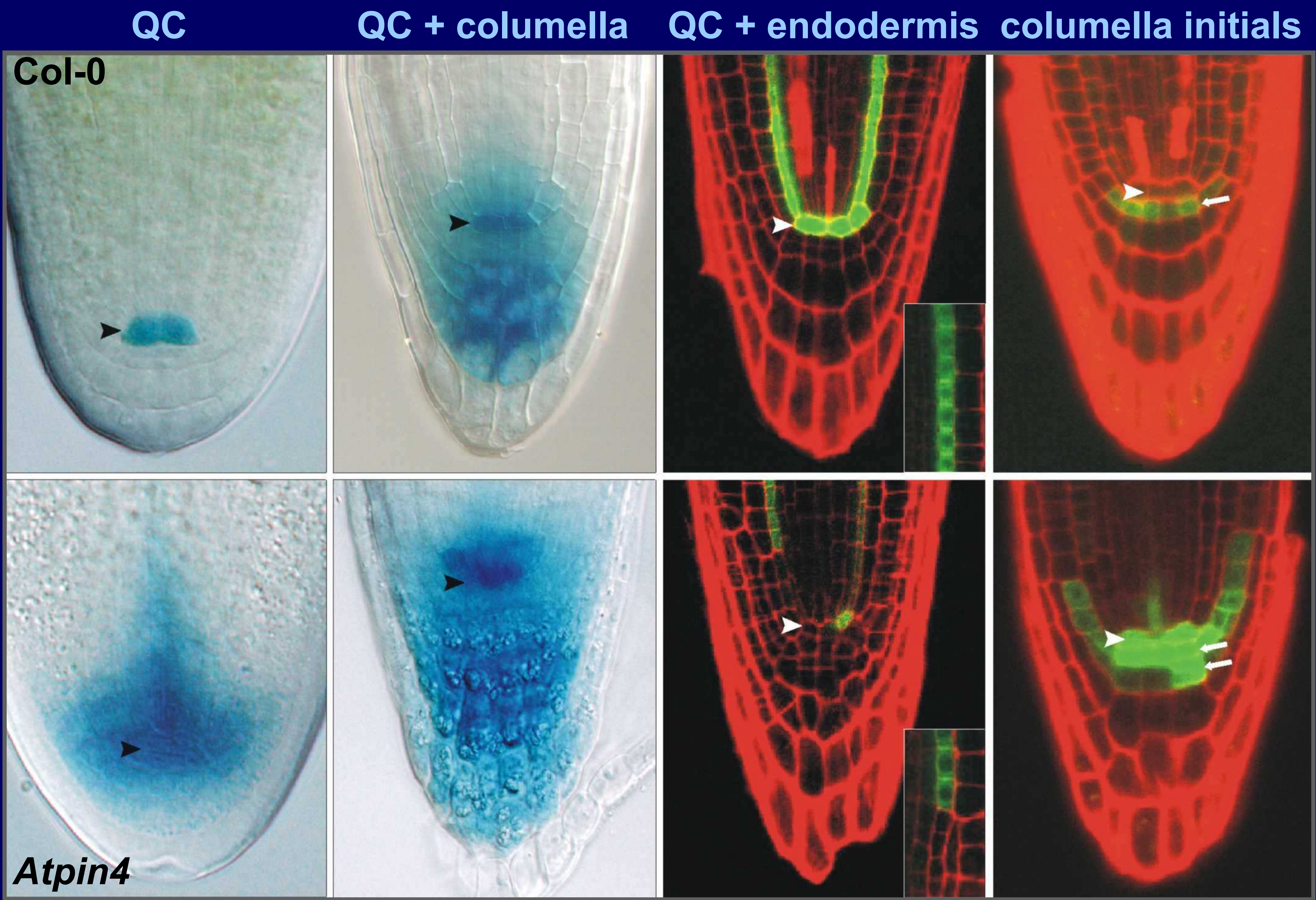
***AtPIN4* antisense**

Atpin4



Atpin4

Changes in Cell Fates in *Atpin4* Mutant



Primary Root Meristem

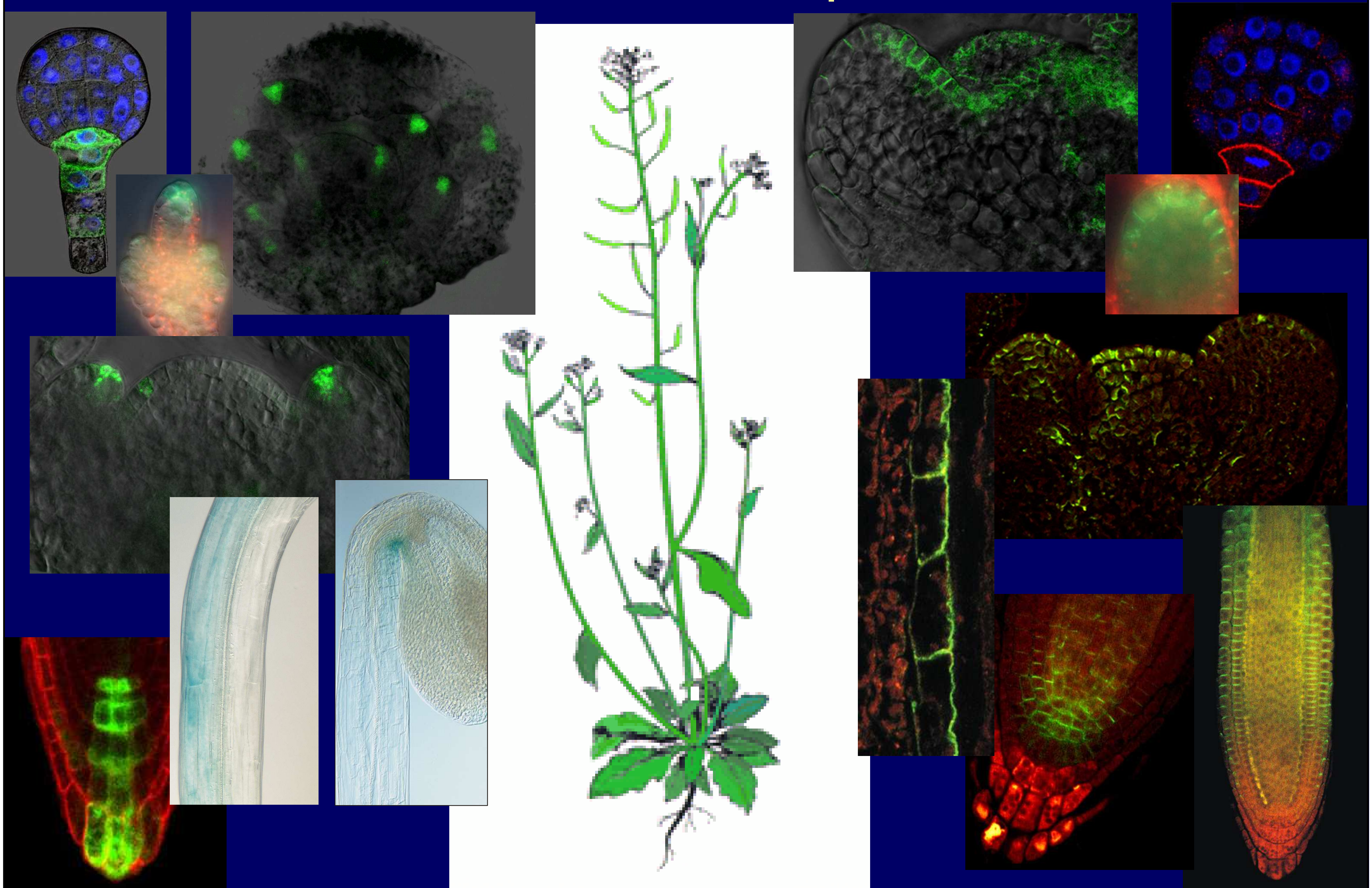
- Simple, highly invariant cell anatomy.

- The QC in the root meristem centre keeps the surrounding initials undiferentiated.

- Positional signal (probably auxin) instruct cell to differentiate into respective cell types.

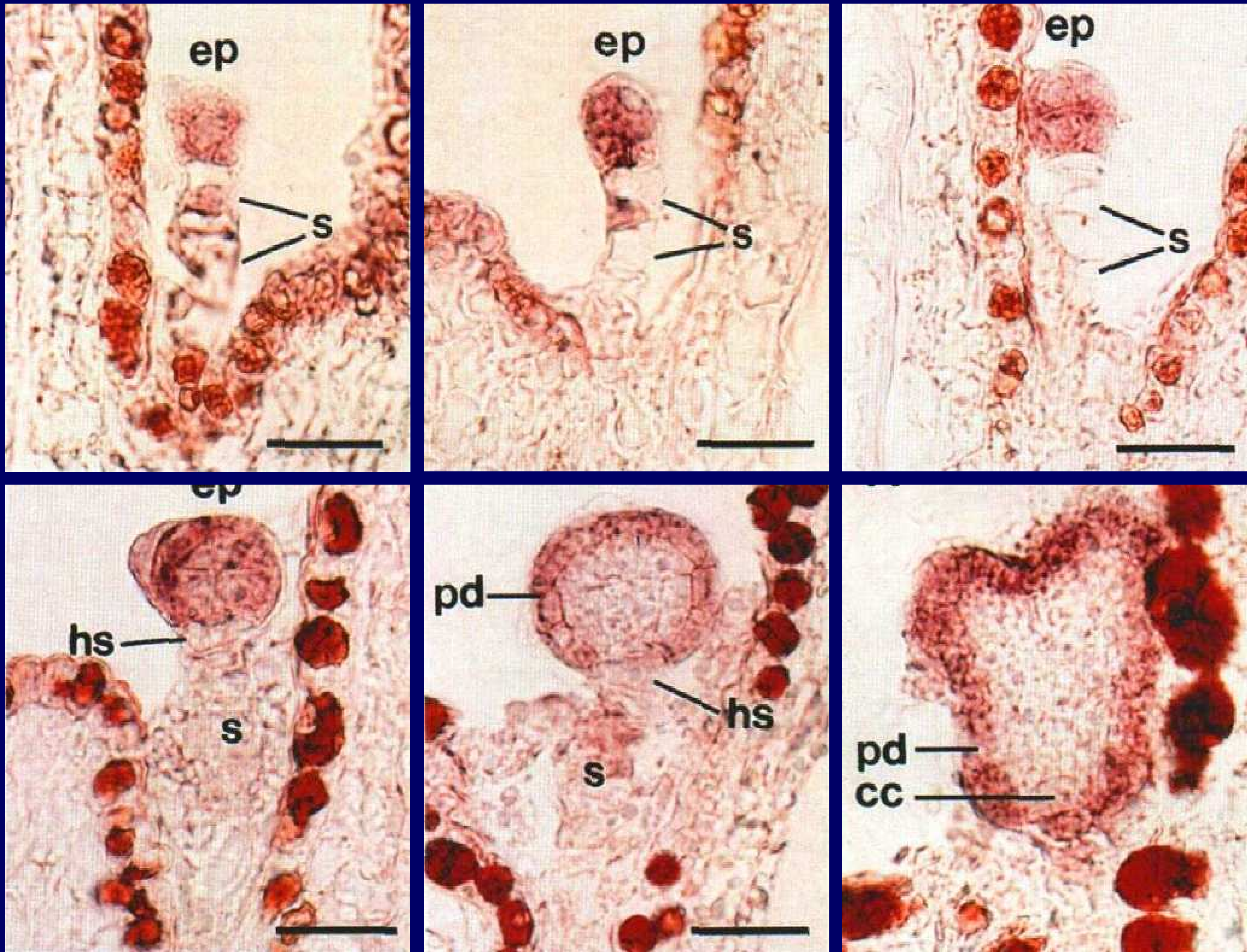
-The auxin gradients instructive for meristem patterning are maintained by polar auxin transport system.

PIN-dependent Auxin Gradients in Plant Development



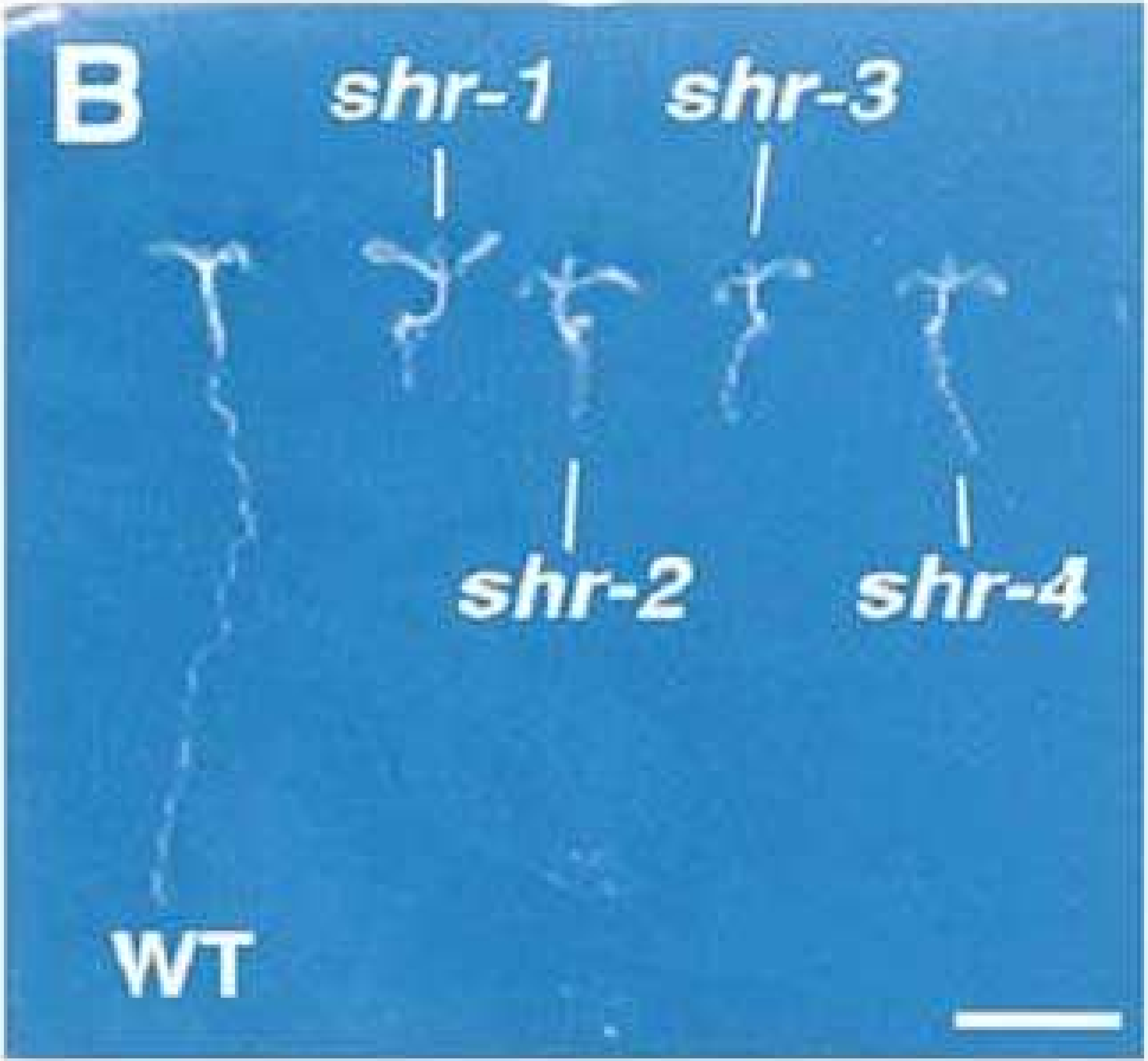
Radial Patterning of Arabidopsis Root

Example for radial patterning – the AtML1 gene

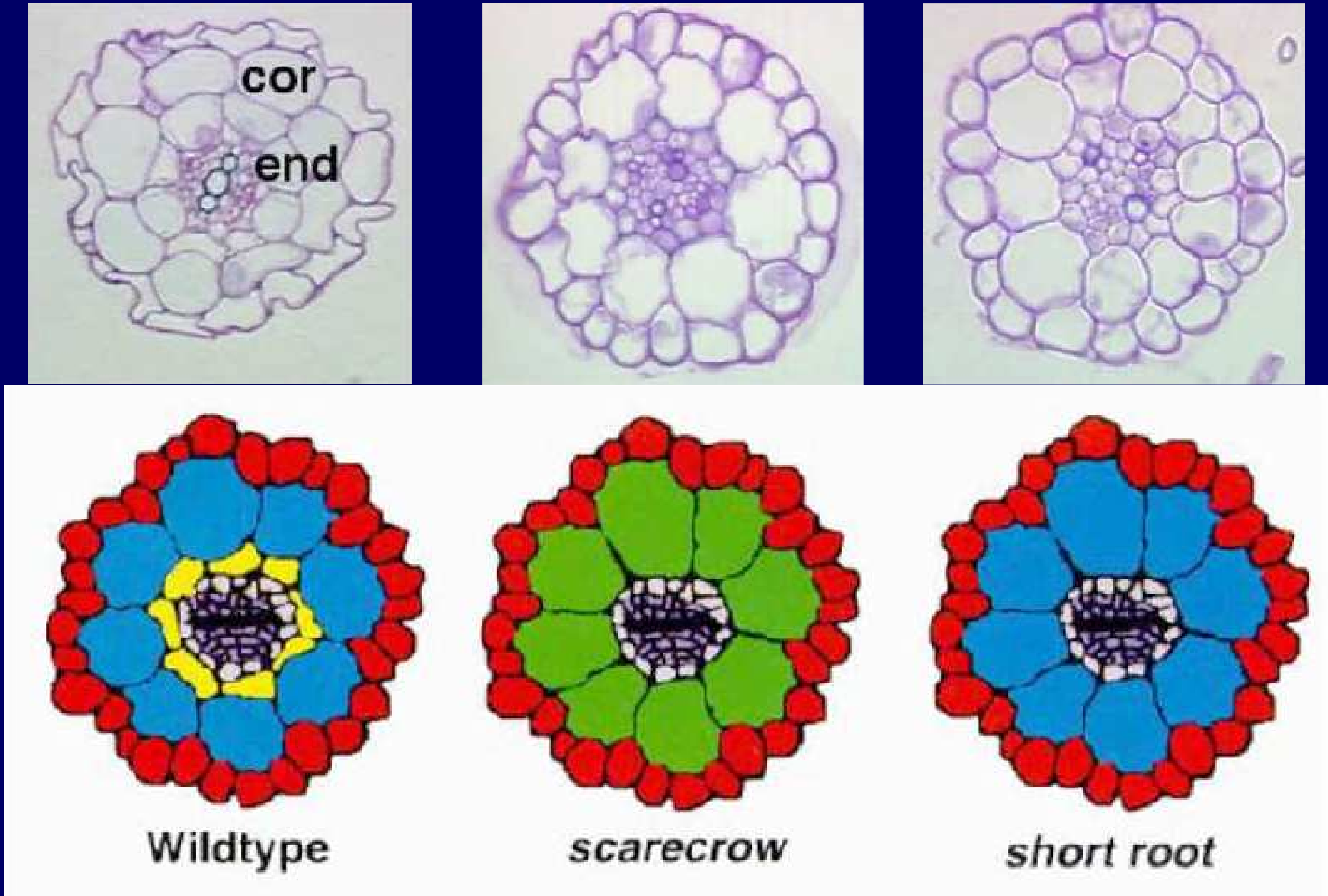


Lu P et al.
Plant Cell,
1996

Short-root mutant alleles



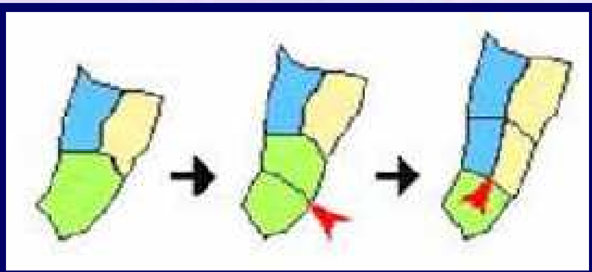
Radial Mutants with Defects in Ground Tissue

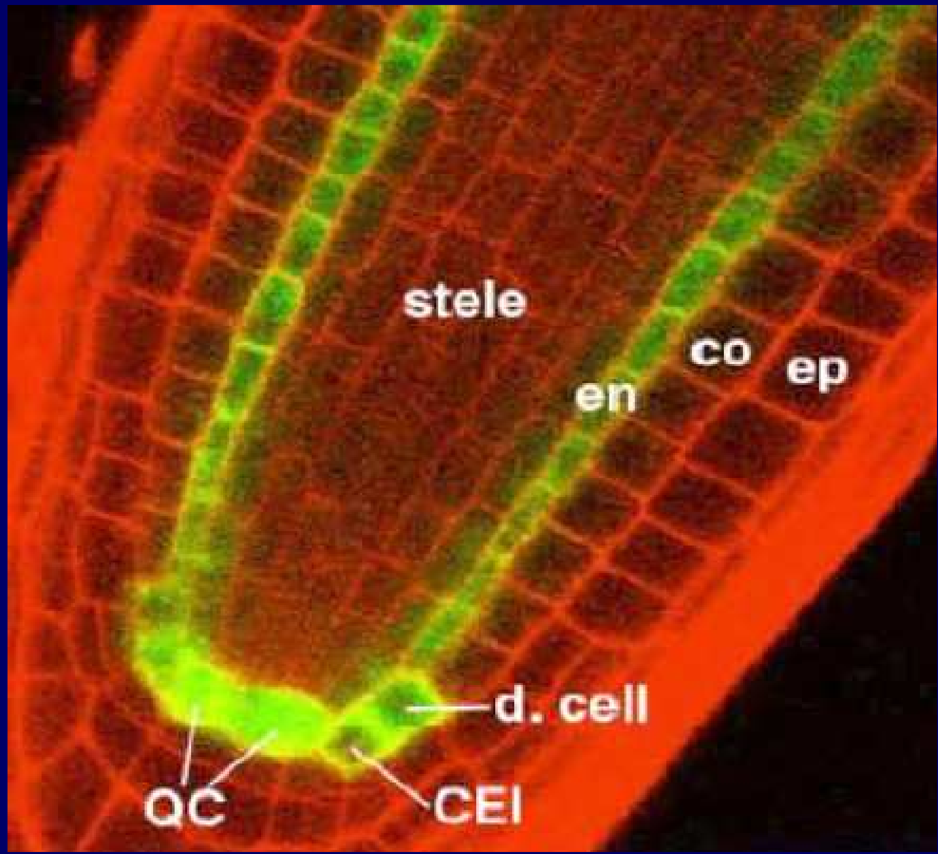


Cortex + Endodermis | Mixed (Cx+En) | Cortex

SCR Expression in Endodermis

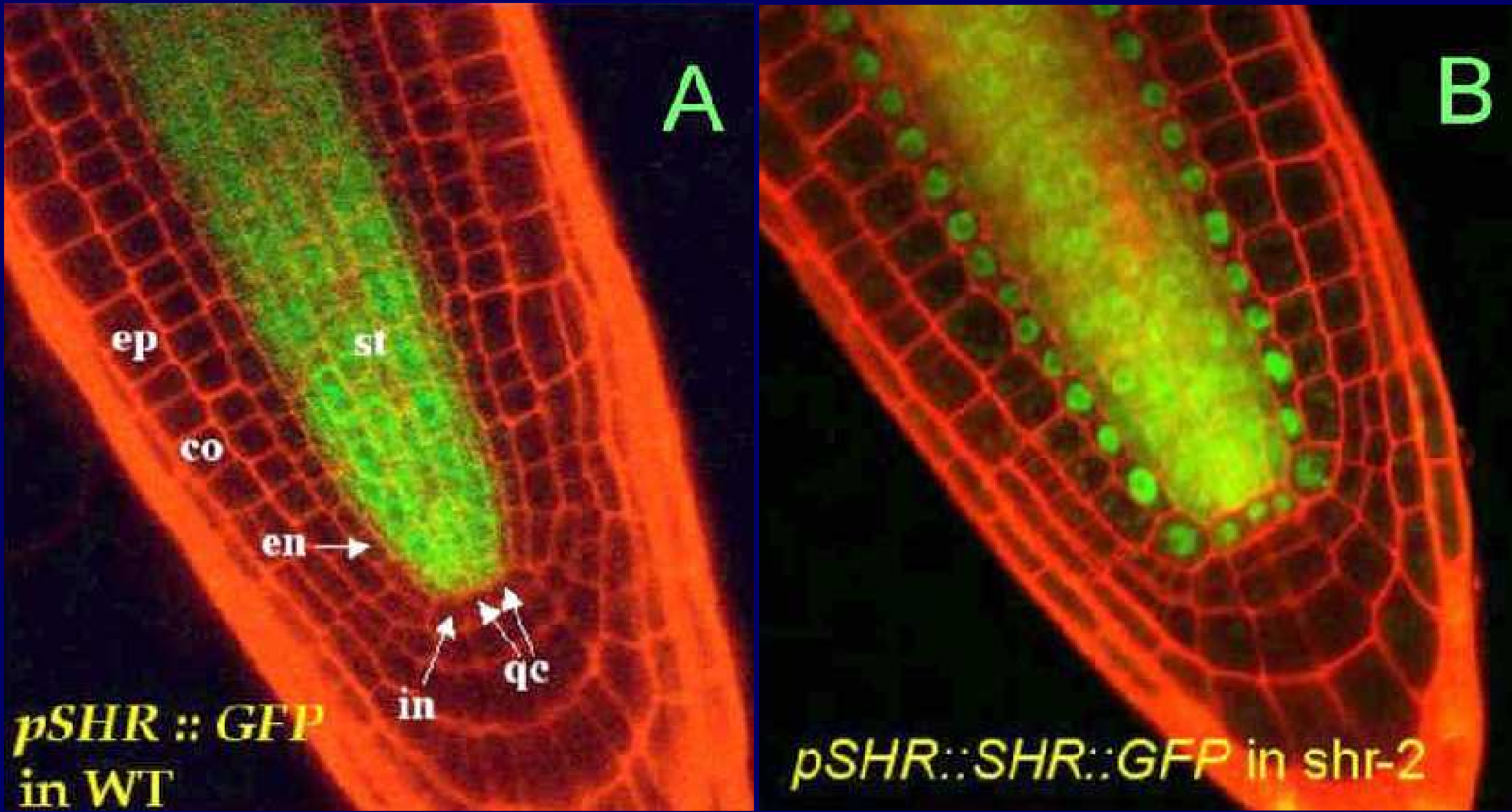
mRNA





SCR::GFP

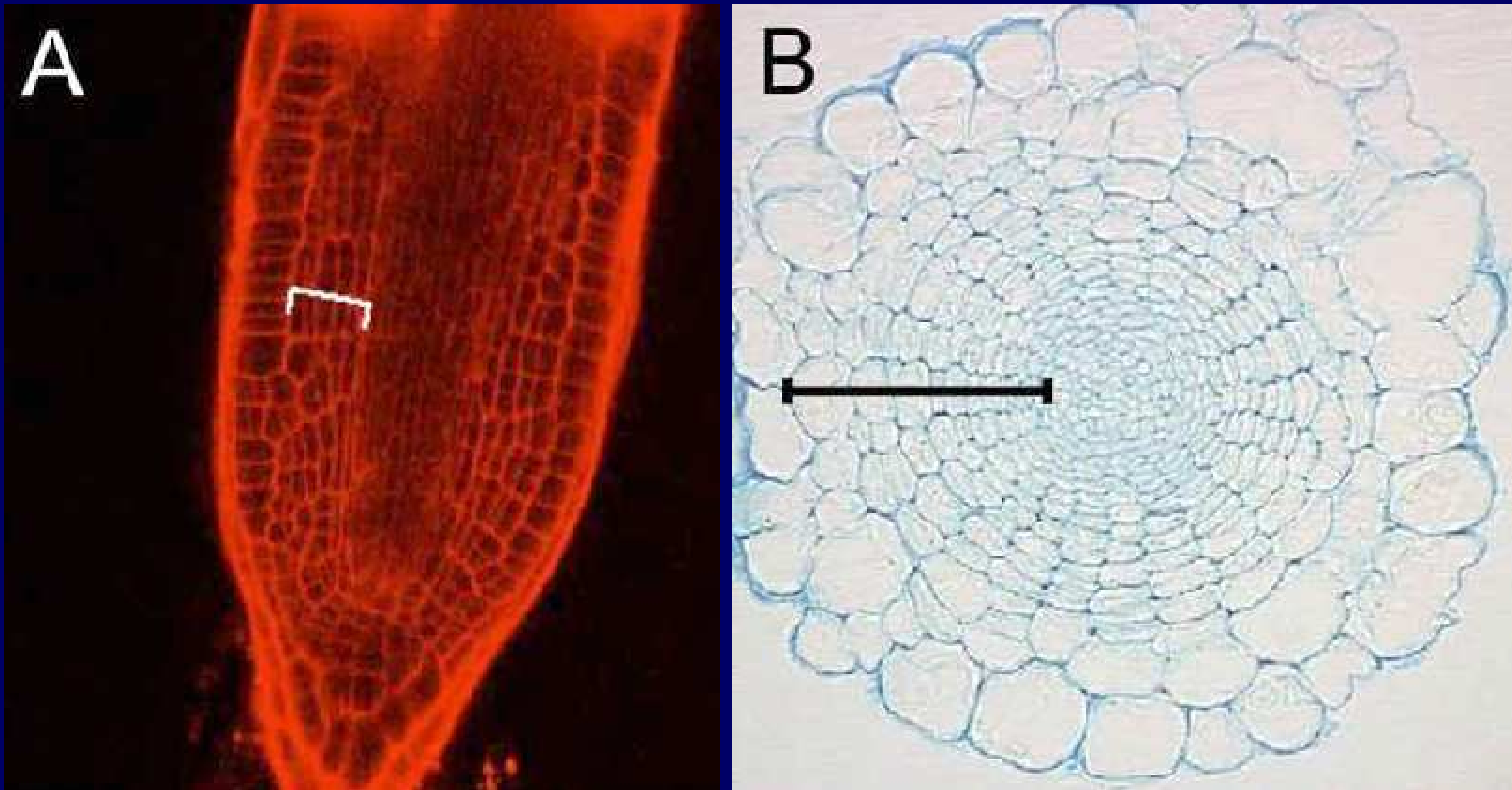
SHR Expression + Proteintransport



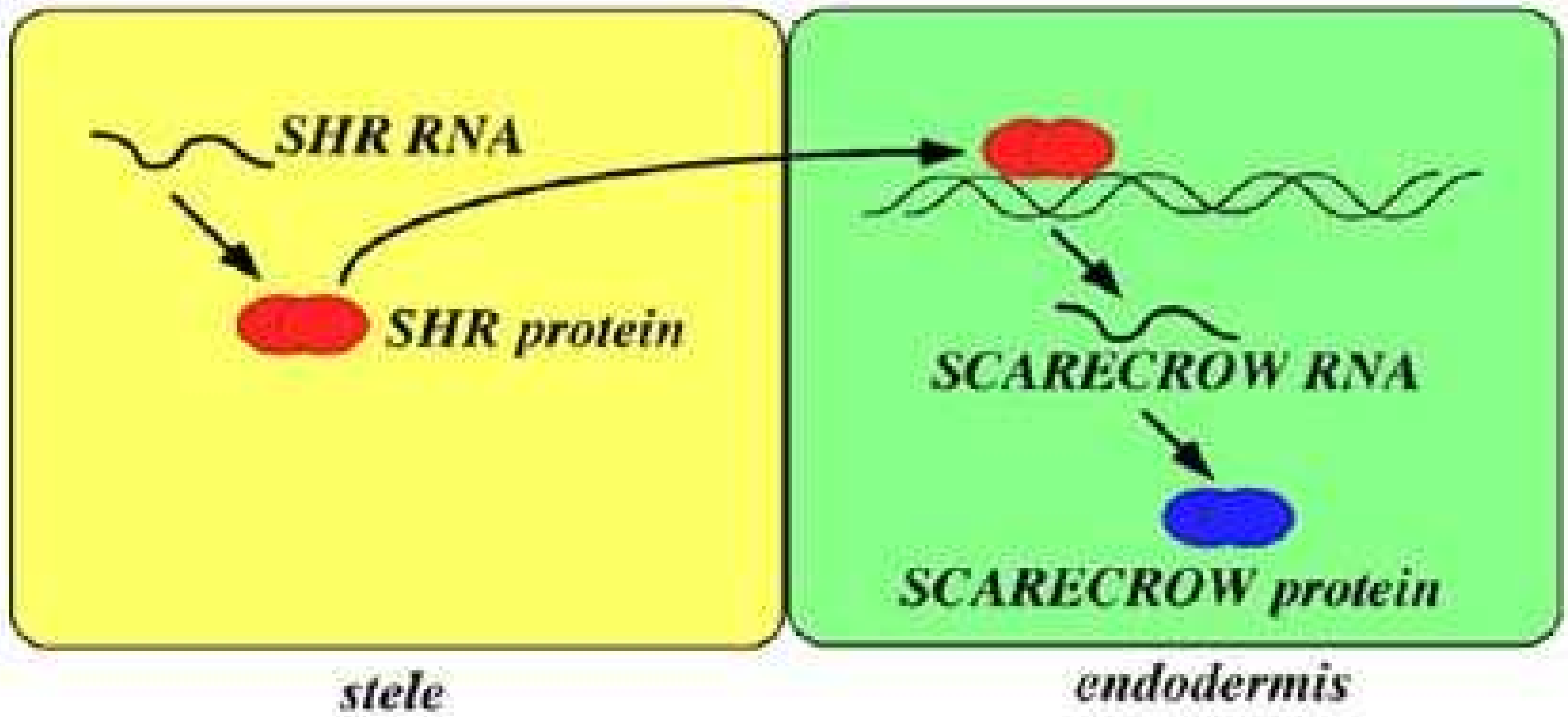
Genexpression in stele

Protein in Endodermis + QC

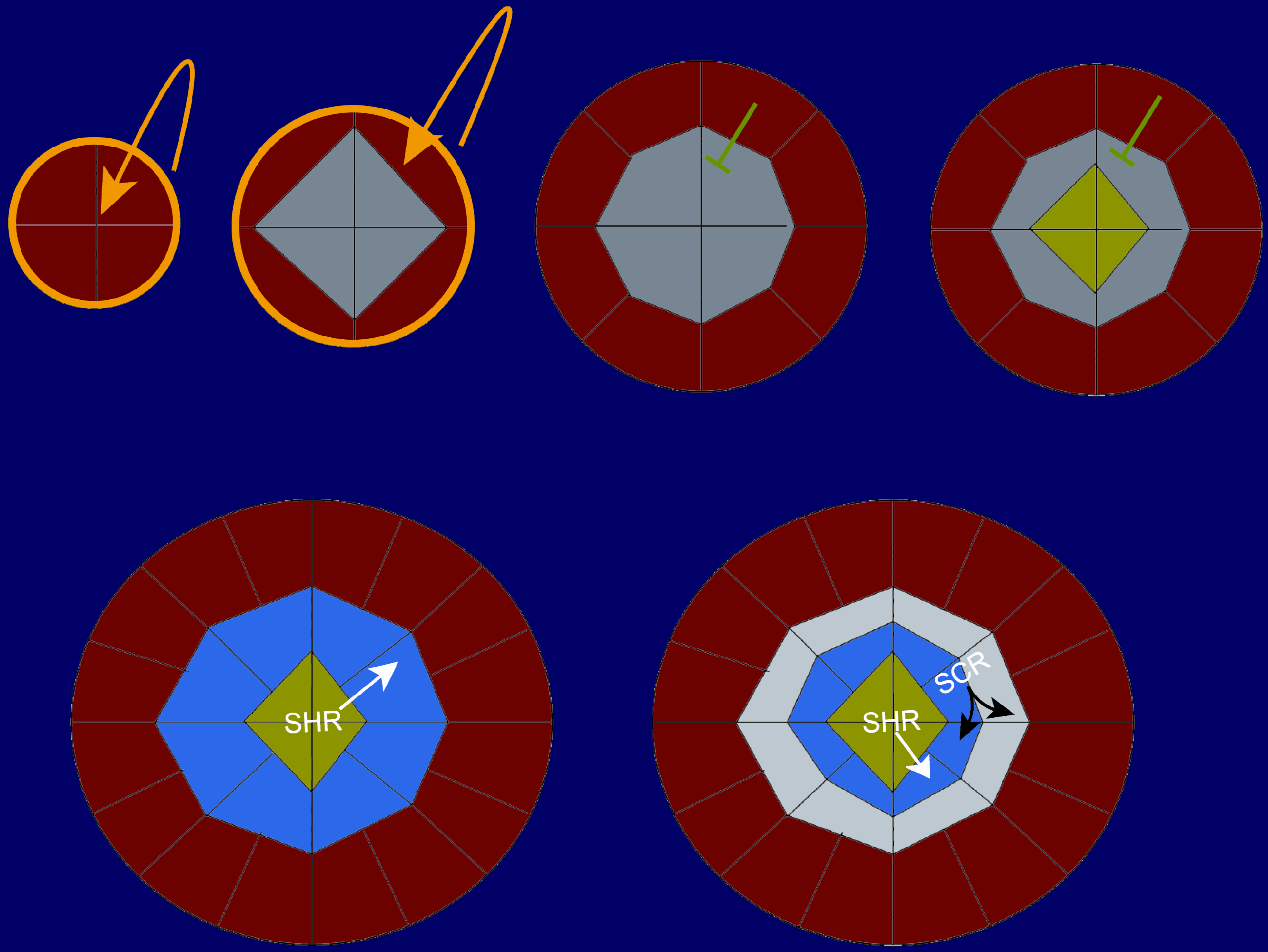
SCR::SHR Expression: More Ground Tissue



Model of SHR and SCR Action



A model of radial patterning



Root Radial Patterning

-Epidermis, cortex, endodermis, pericycle, stele cell types.

-Genetic analysis – shortroot (*shr*) and scarecrow (*scr*) mutants.

-SHR transcription factor is expressed in pericycle, moves into endodermis, activates SCR expression, which in turn properly specify endodermis.