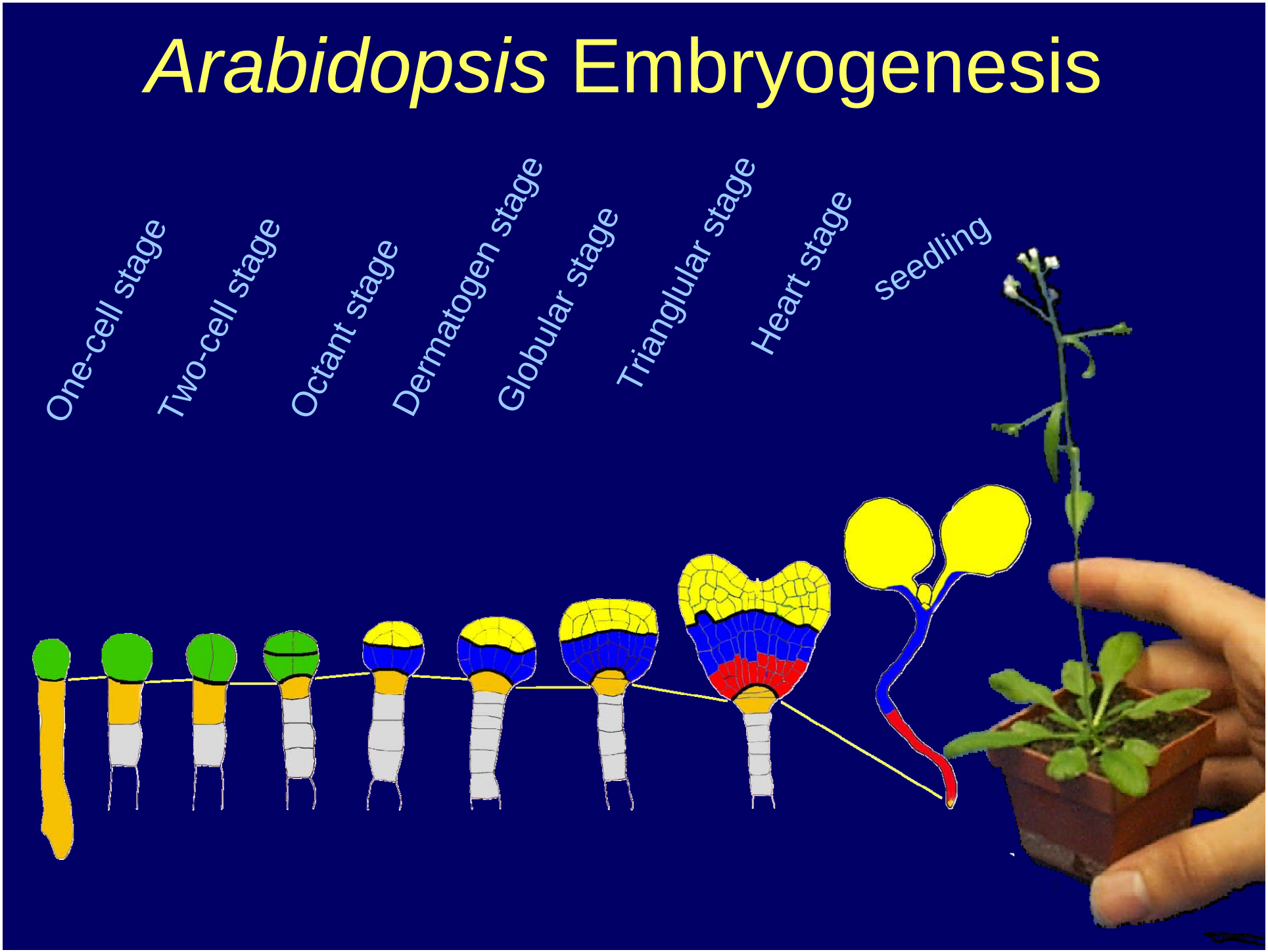
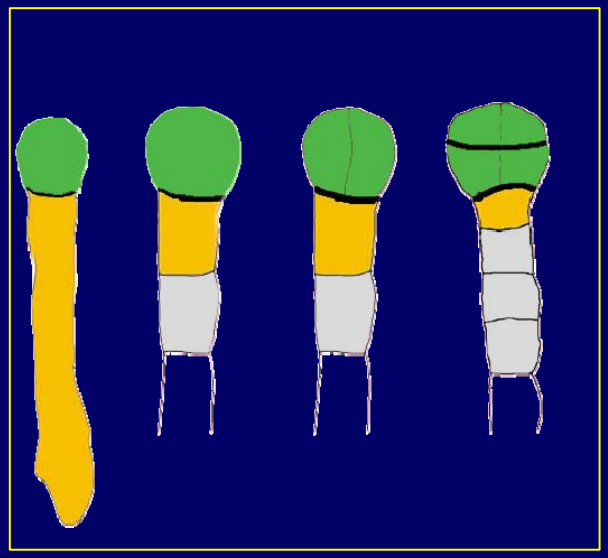
Embryogenesis

Arabidopsis Embryogenesis



Comparison of embryo development in Angiosperms

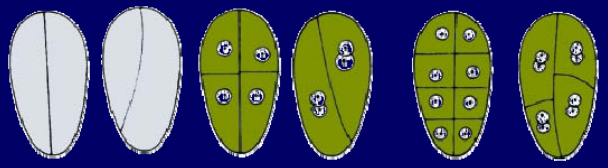


Caryophyllad

Chenopodiad

Solanad

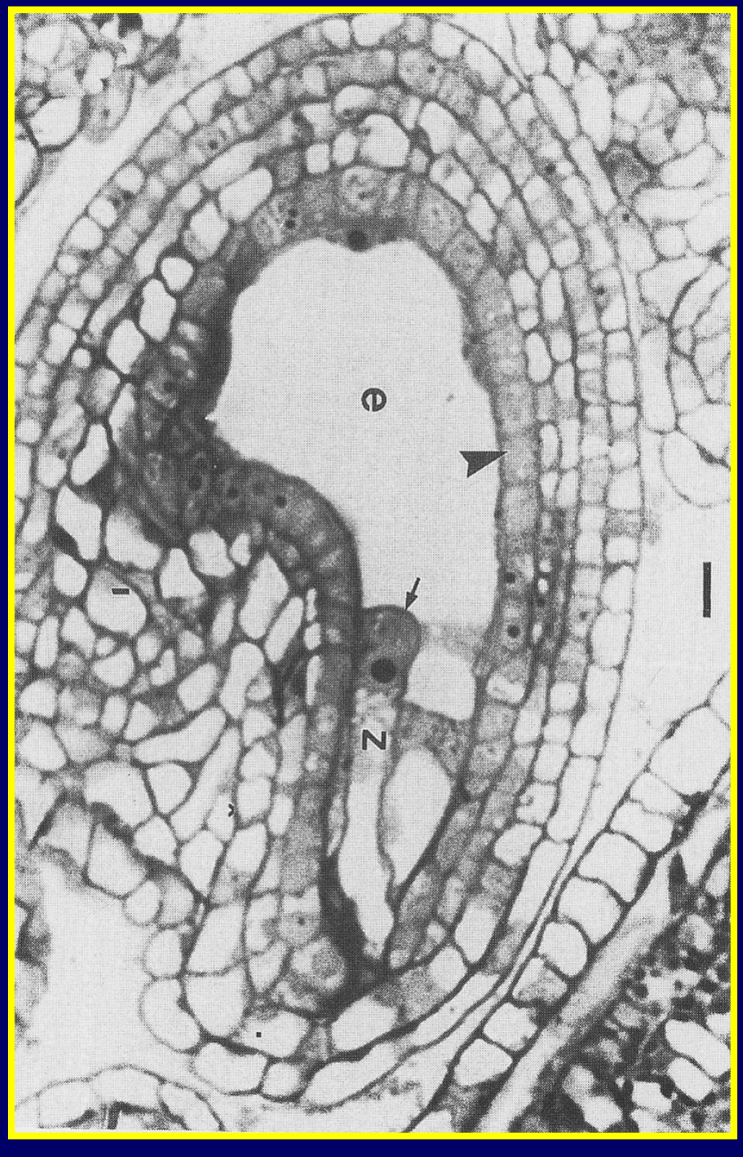
Asterad



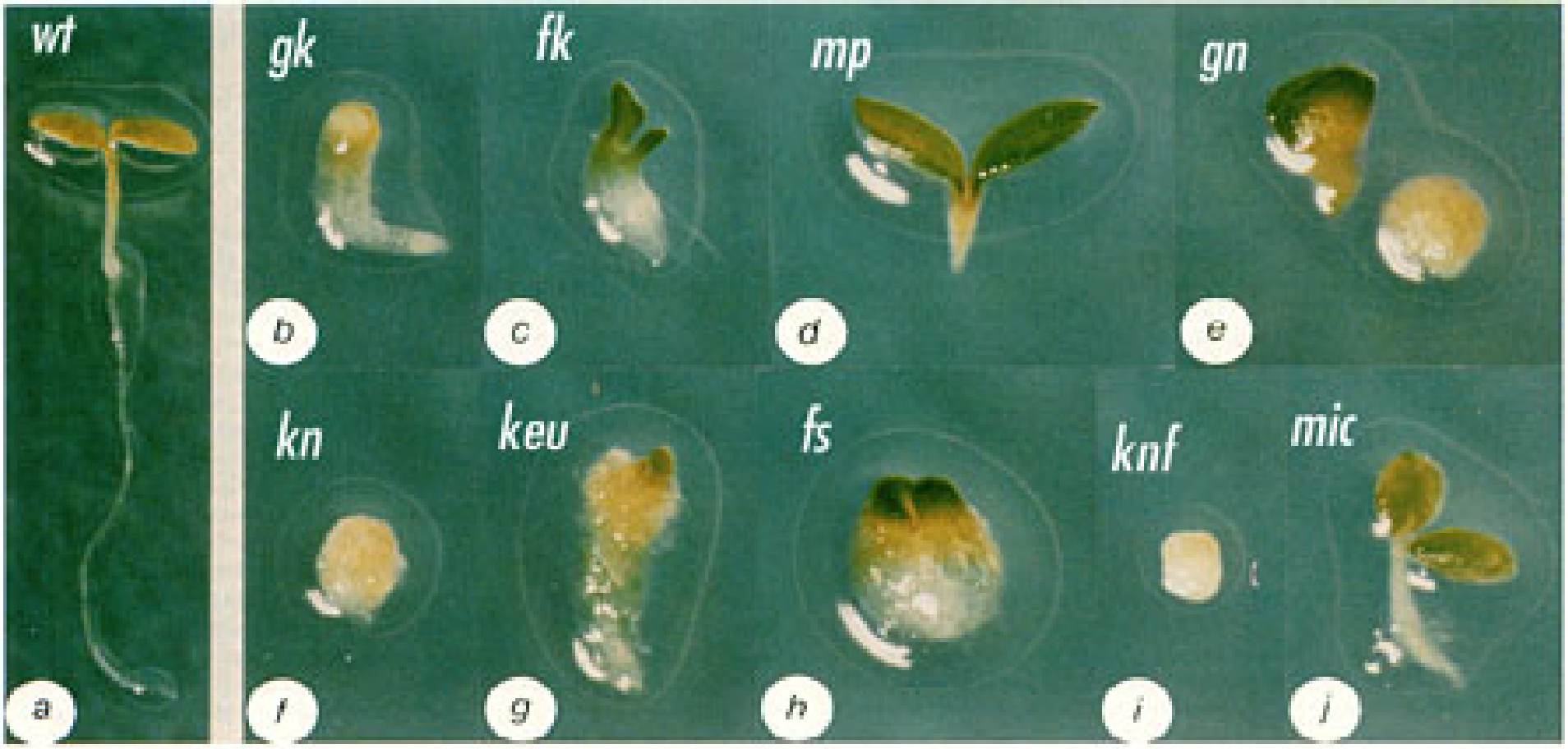
Piperad

Modified after
Johri et al. 1992

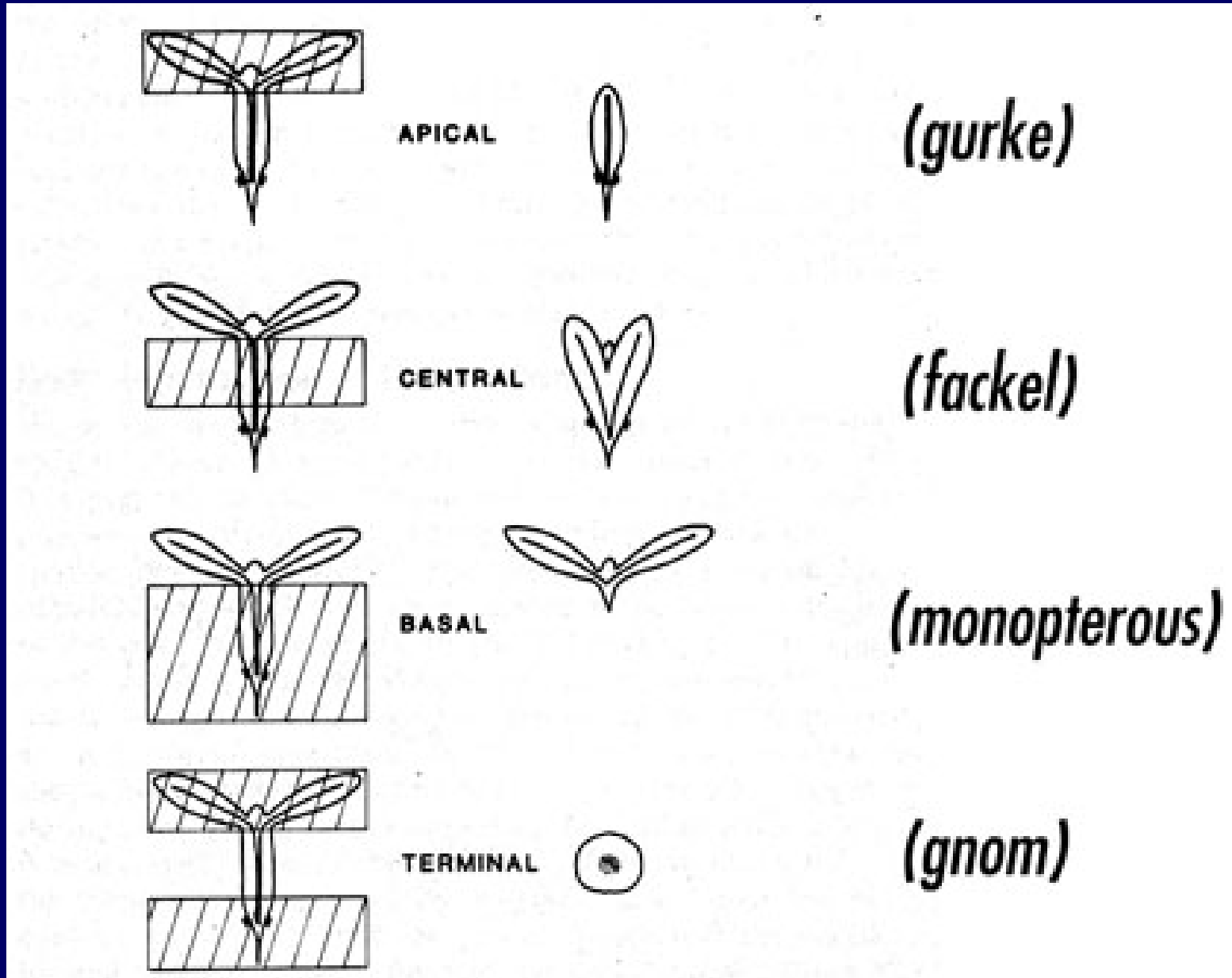
How can such a protected system be investigated experimentally?



Mutant screen at seedling level



Patterning mutant types

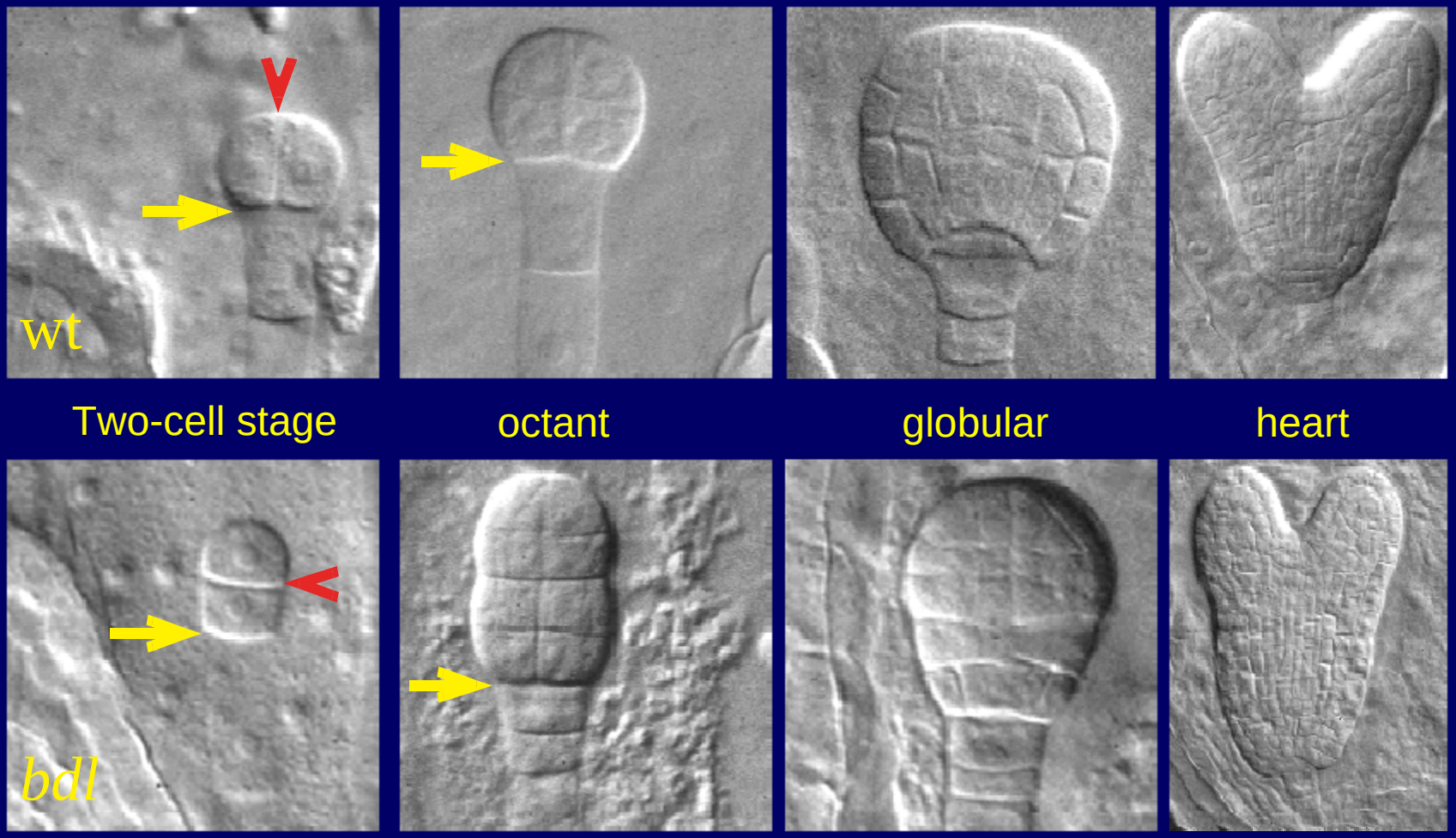


Mutations in the ***BODENLOS*** (*bdl*) and ***MONOPTEROS*** (*mp*) genes lead to very similar deletions of basal pattern elements

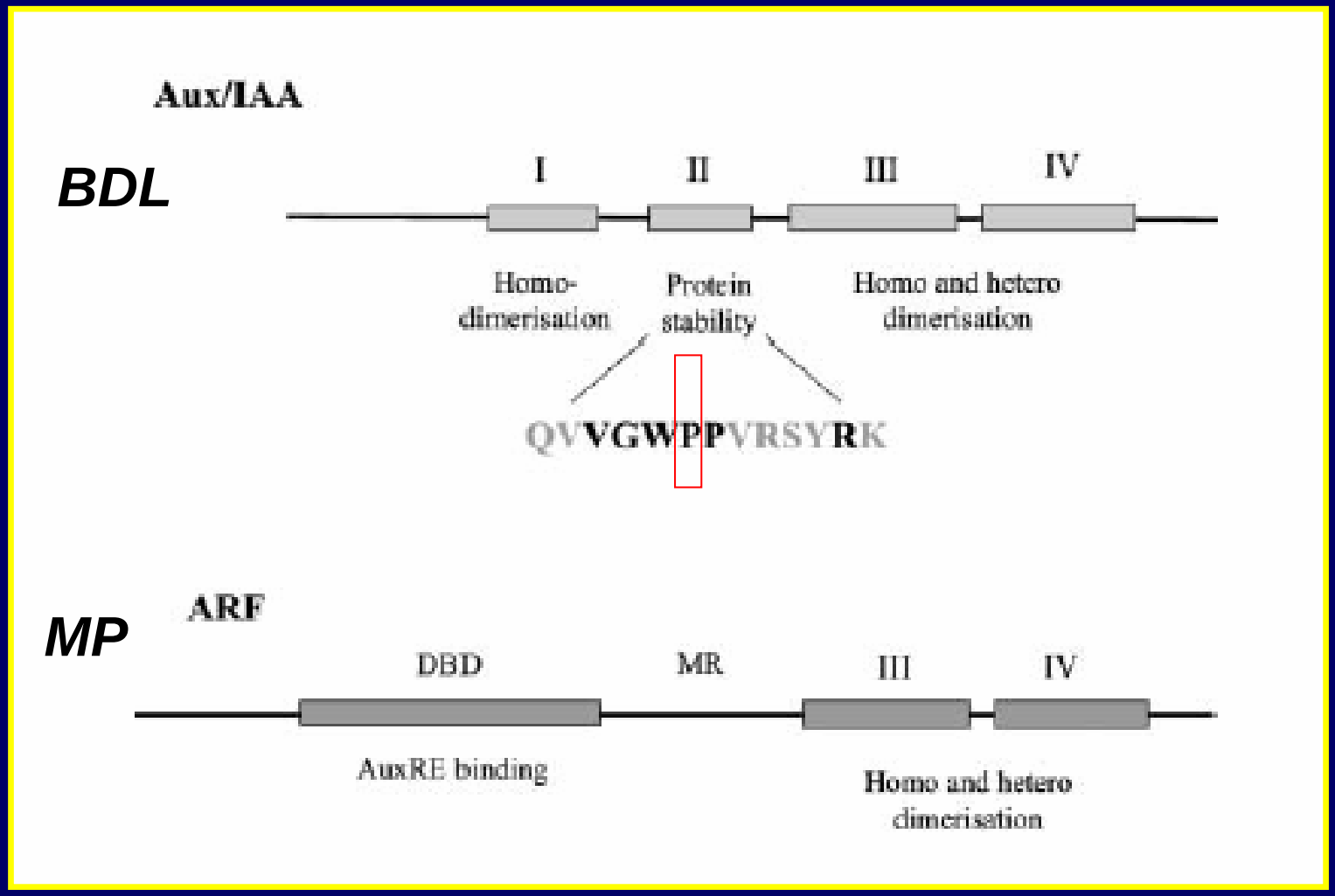
- ***mp*** seedling

- ***bdl*** seedling

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

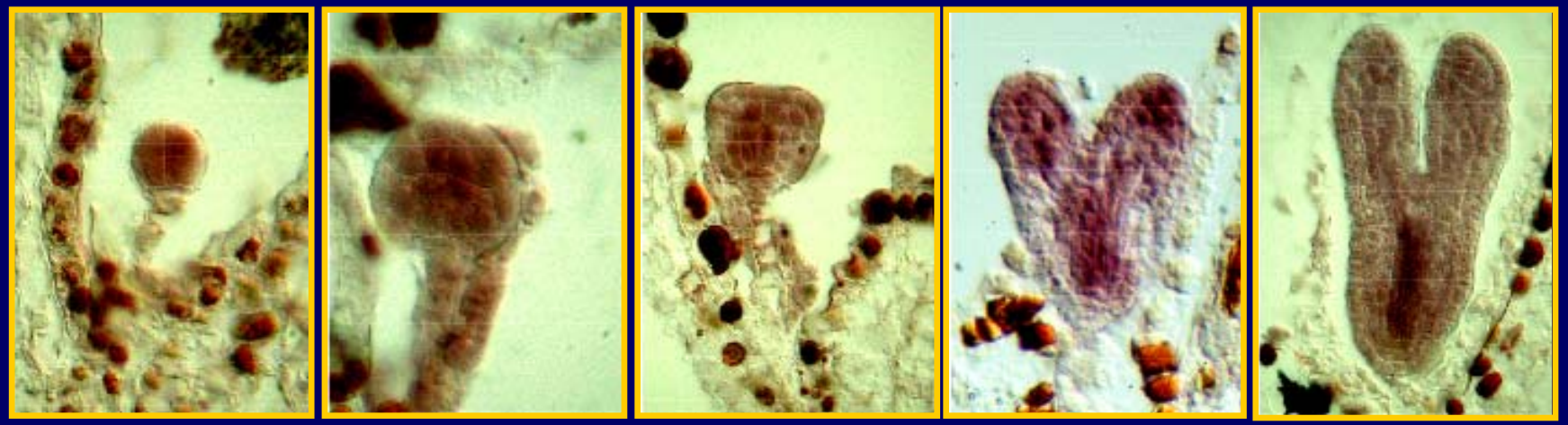


MP encodes for ARF5, an **activator** of auxin response, whereas *BDL* encodes for IAA12 the corresponding **repressor**

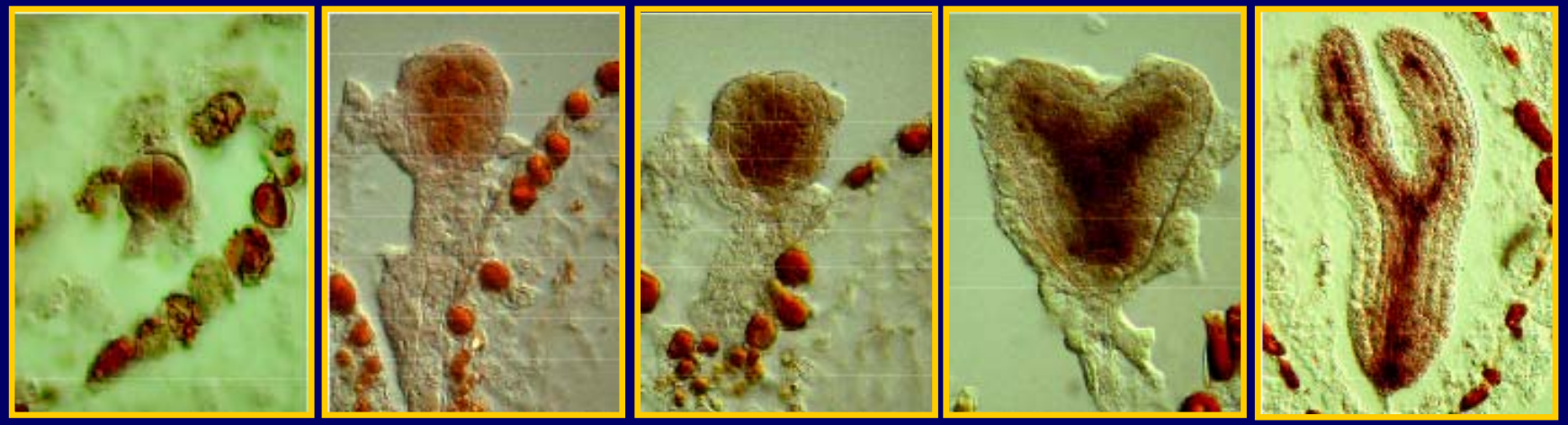


Expression patterns of BDL and MP

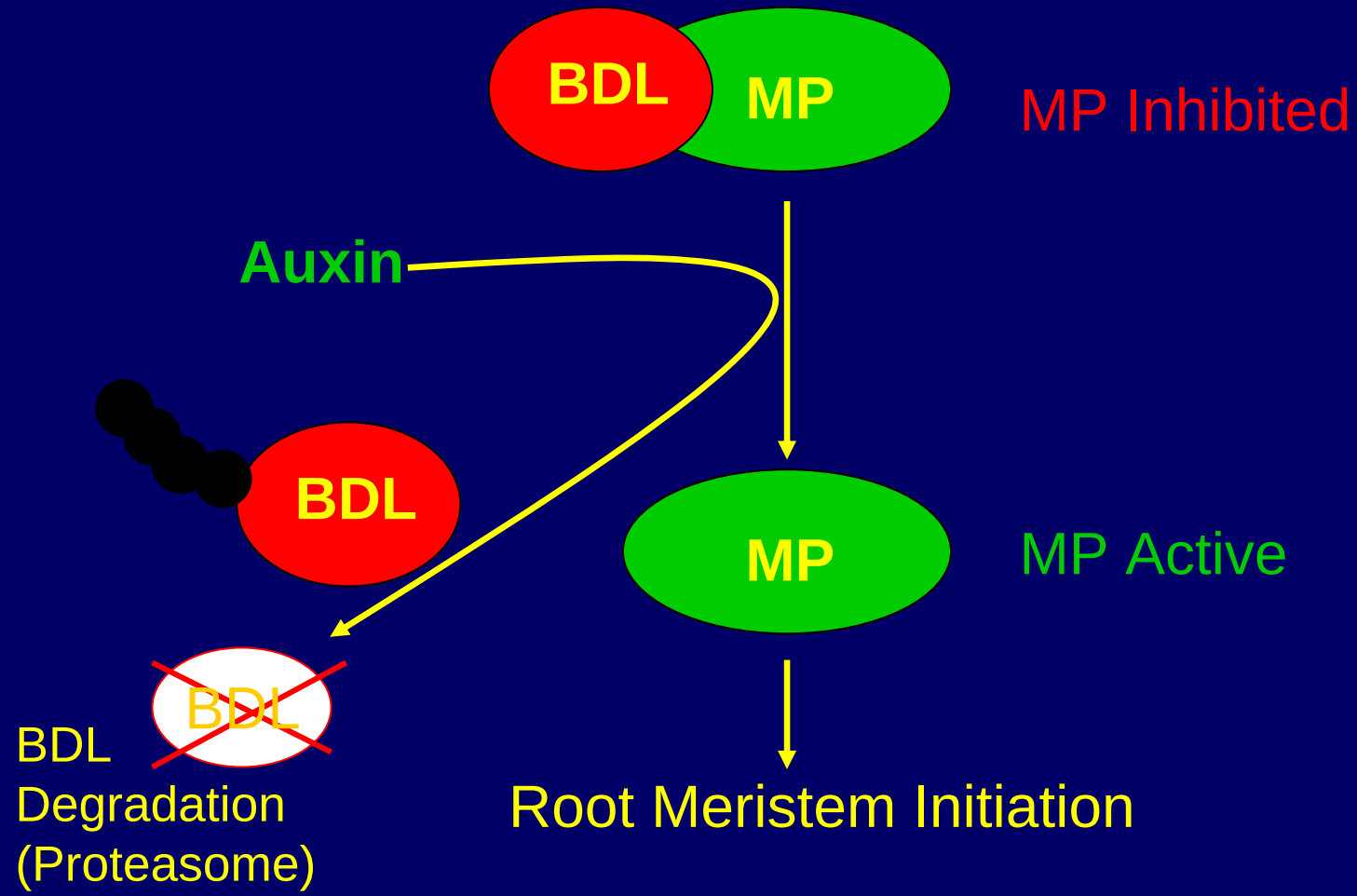
bdl



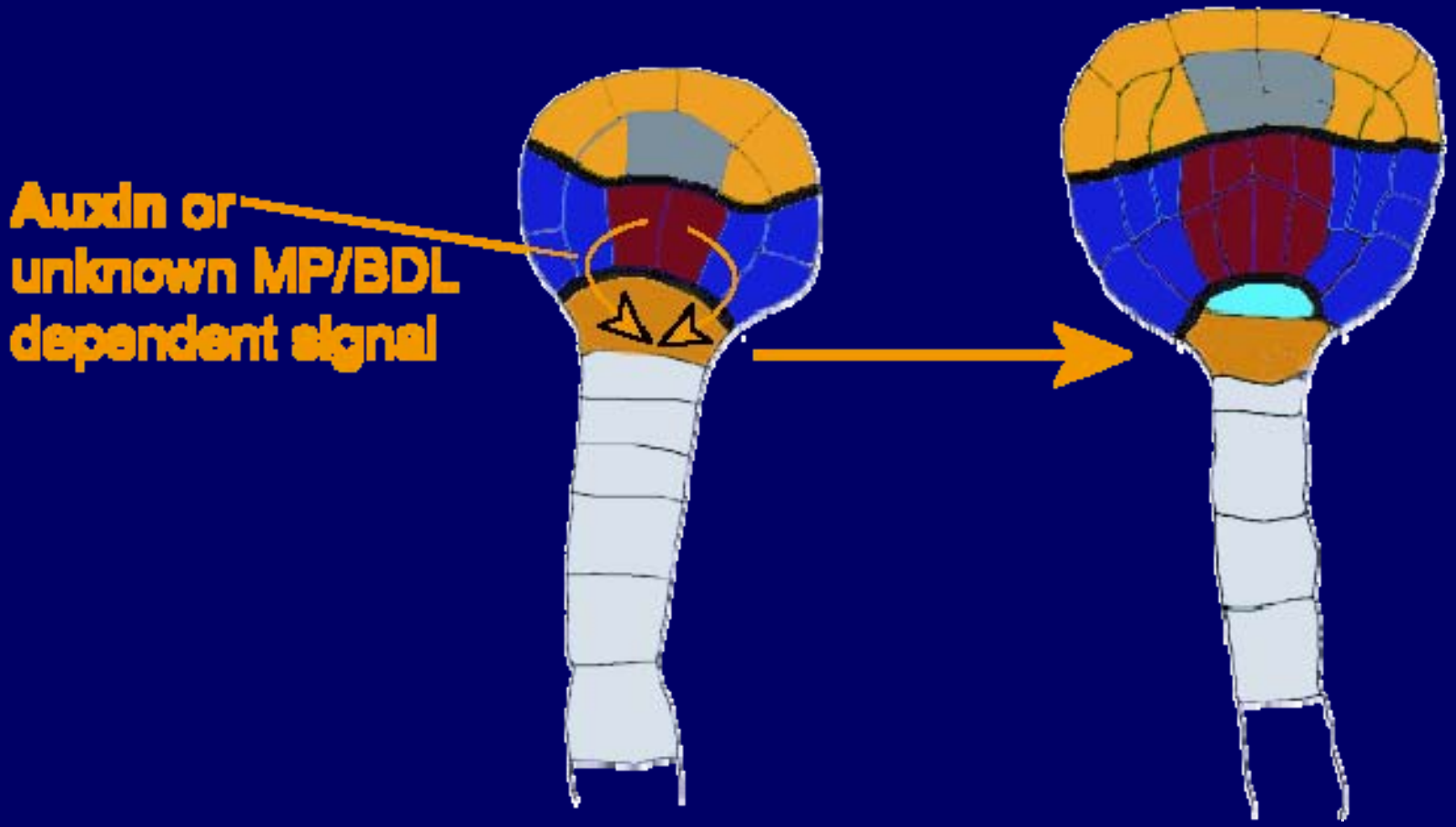
mp



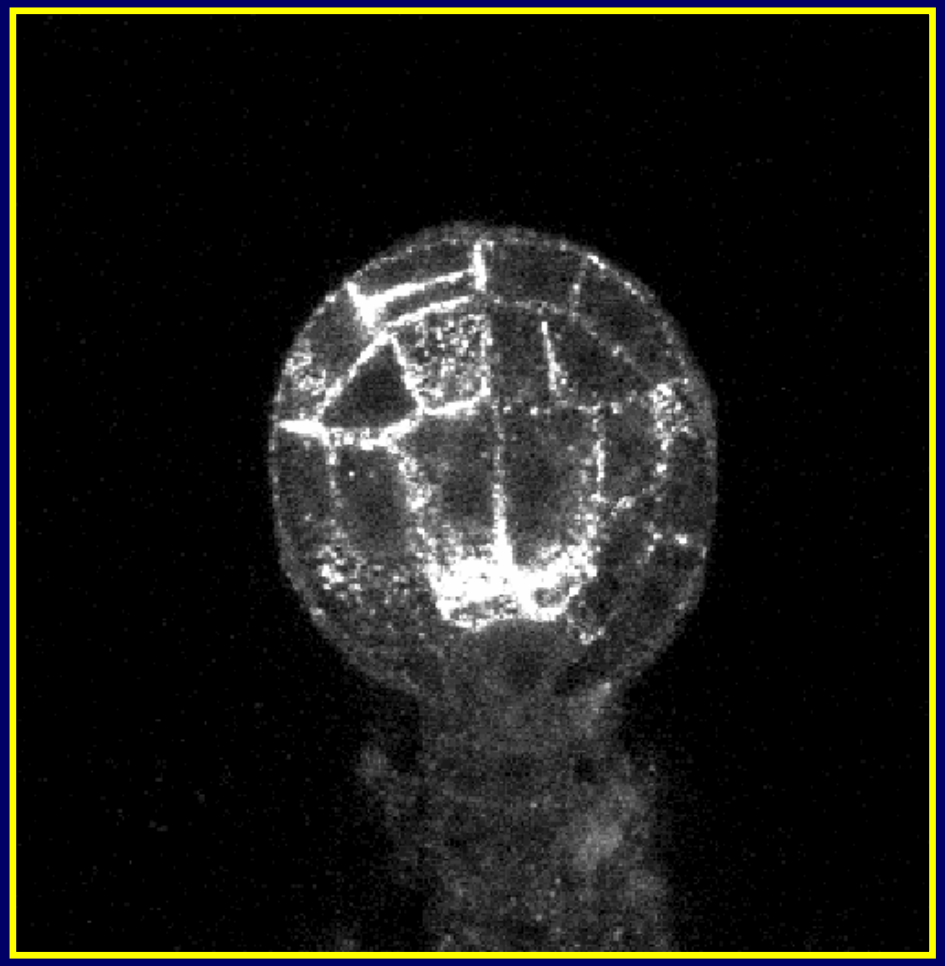
Model of BDL/MP interaction



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



PIN1 efflux carrier localisation suggests auxin flux towards the hypophysis



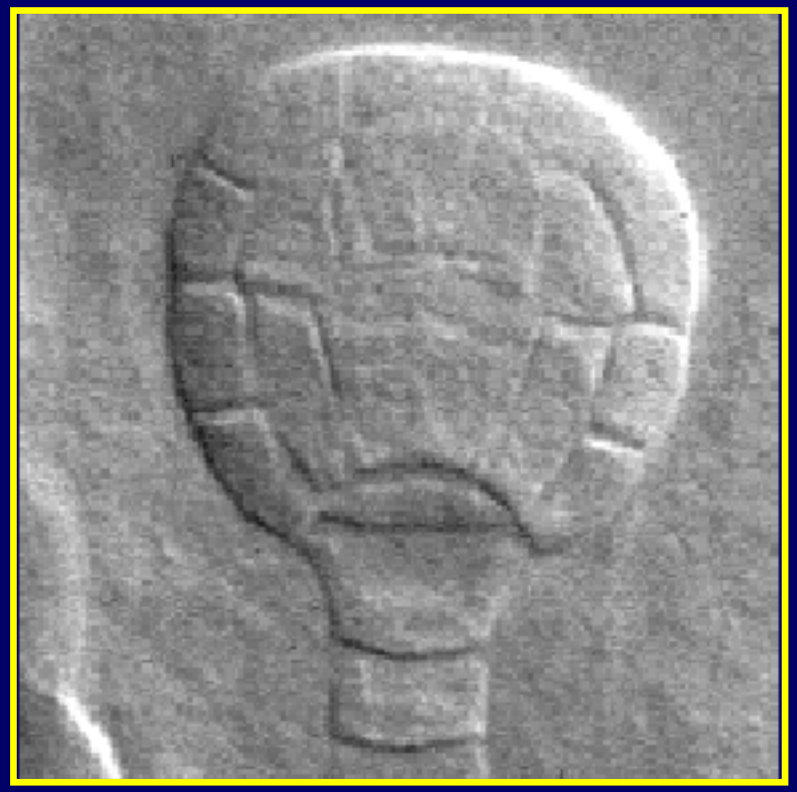
Genetic Interference with Auxin Response and Transport Disrupts Embryo Patterning

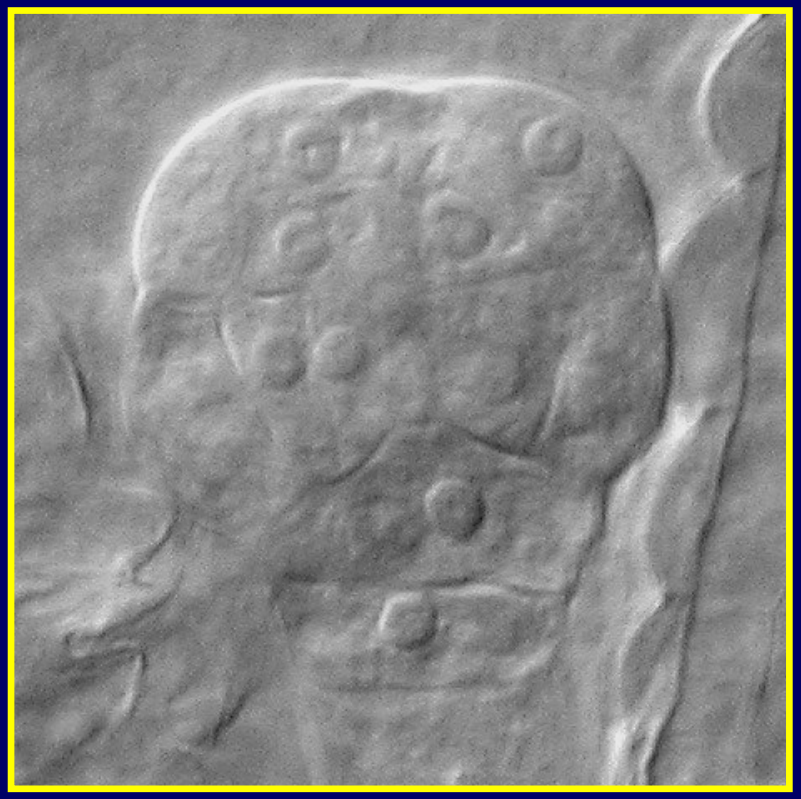
monopteros

bodenlos

gnom

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



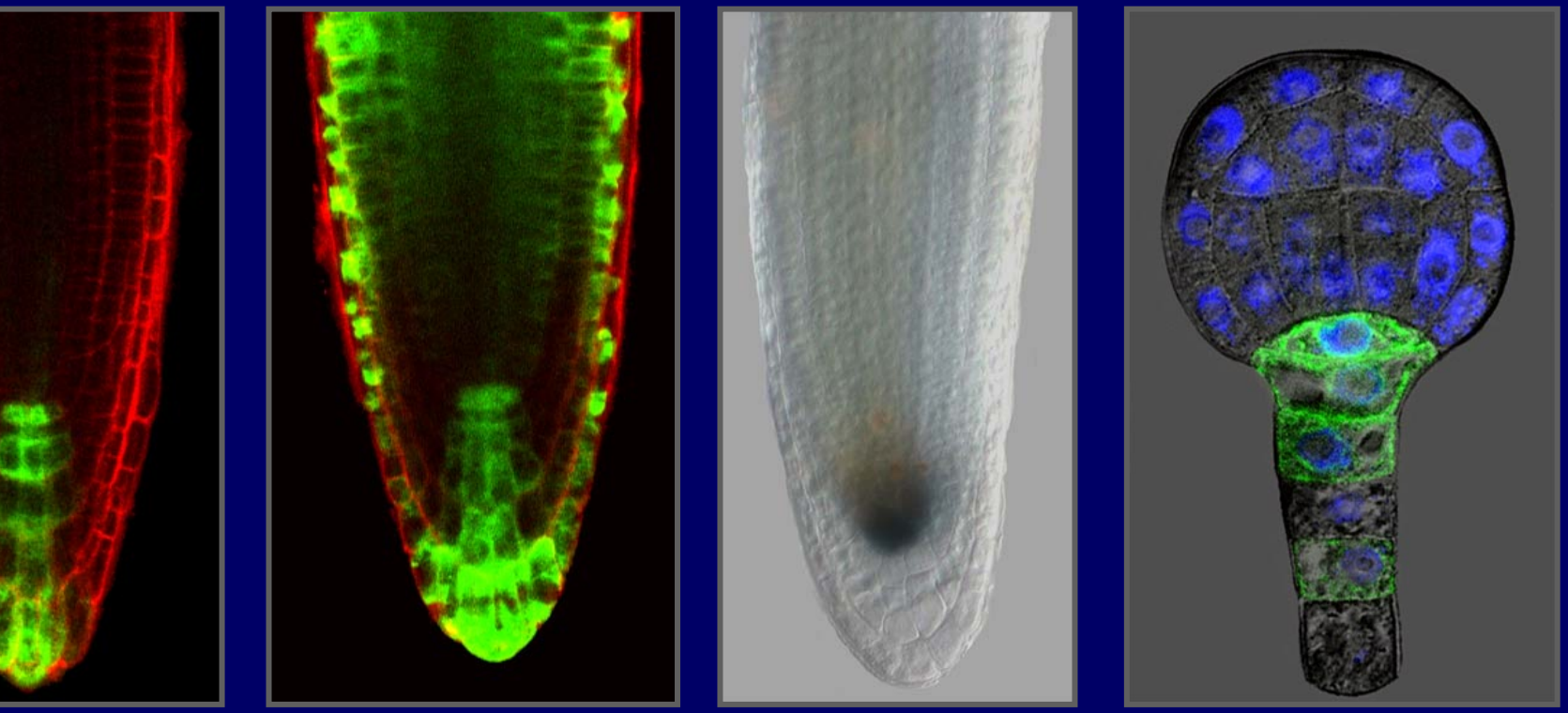


wt

gn

DR5::GFP Auxin Reporter

5´ CCTTT* TGTCTC *3´
9x inv.

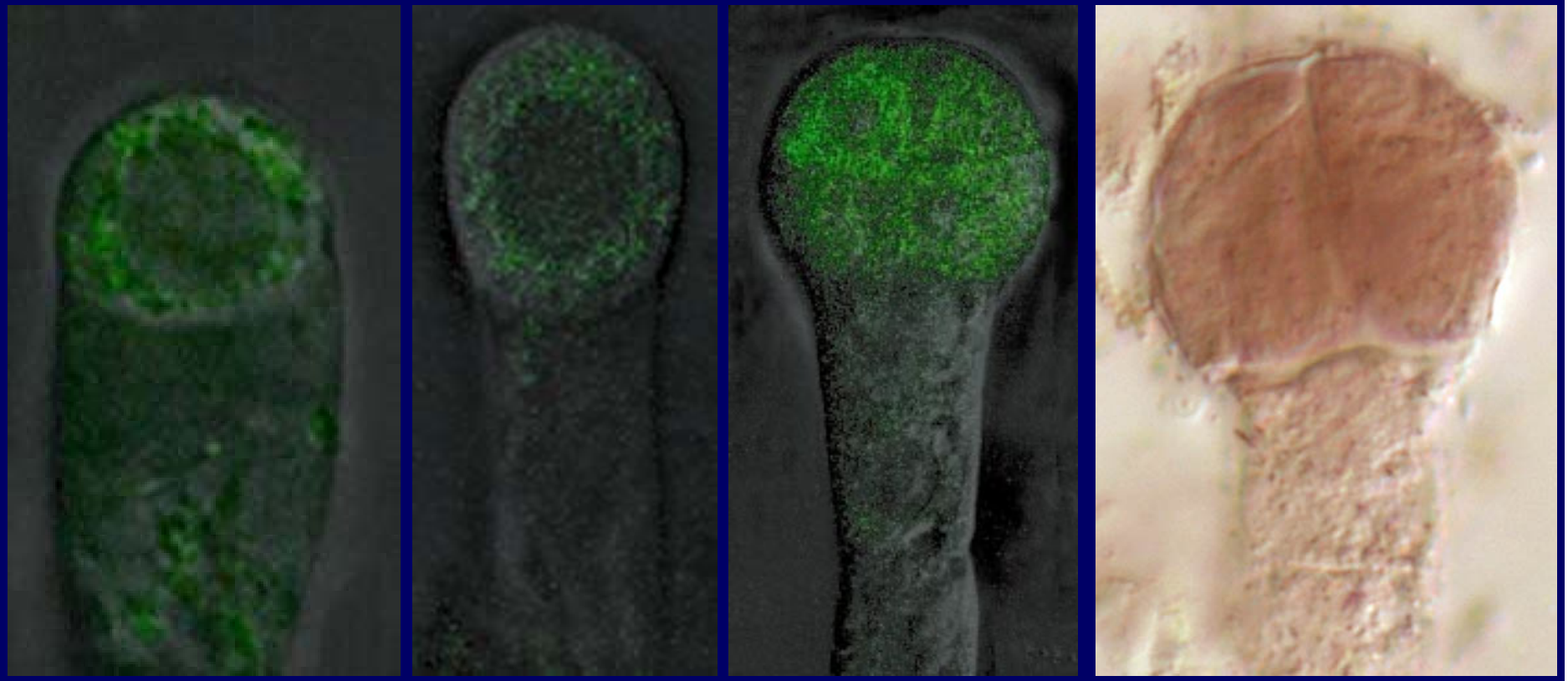


Root **Root + Auxin** **anti-IAA AB** **Embryos**

Auxin in Early Embryogenesis

DR5::GFP

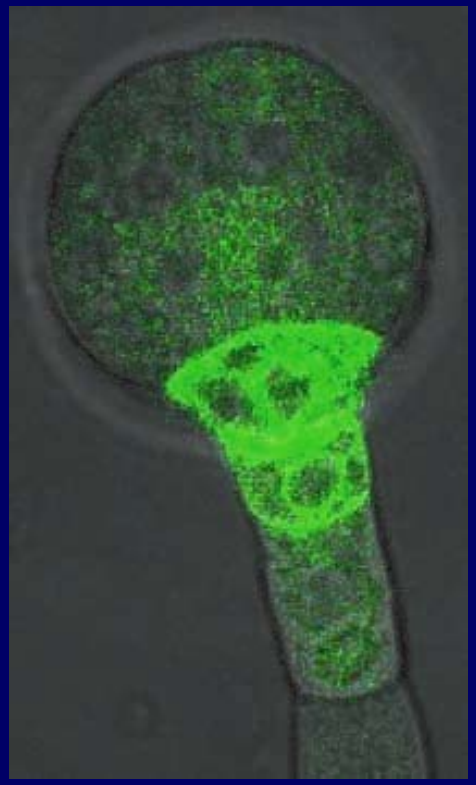
IAA localisation

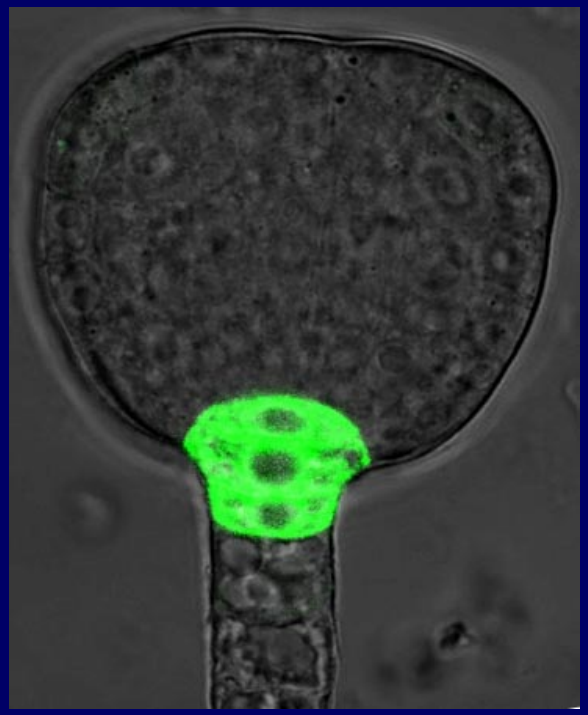


Auxin in Embryogenesis

DR5::GFP

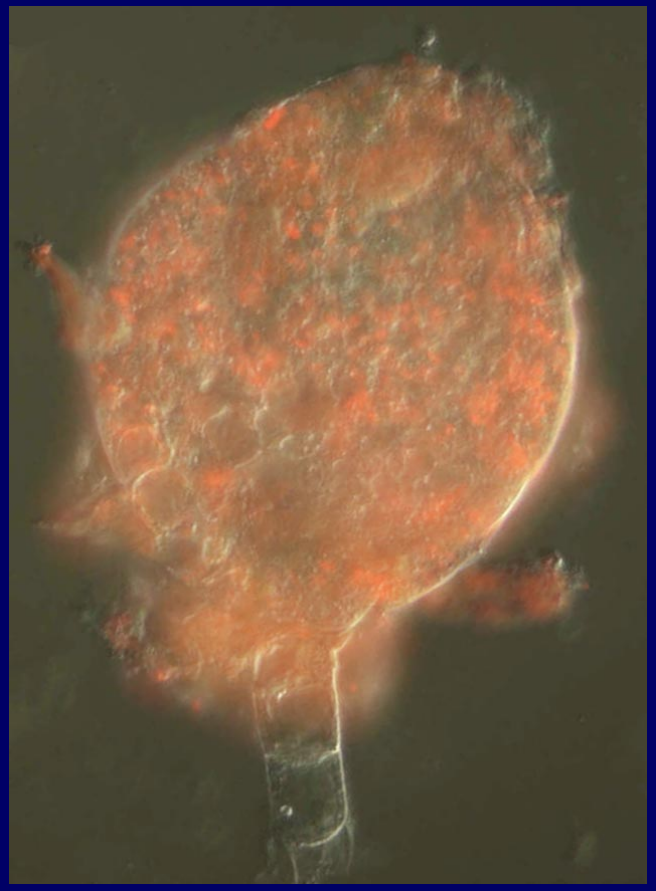
IAA localisation





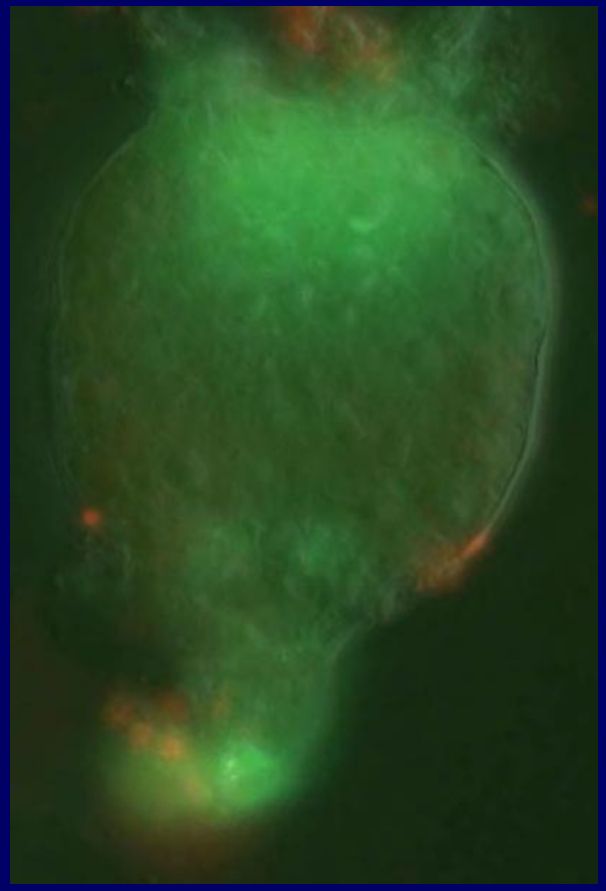
DR5::GFP in Embryo Mutants

Auxin signaling



monopteros

Auxin transport

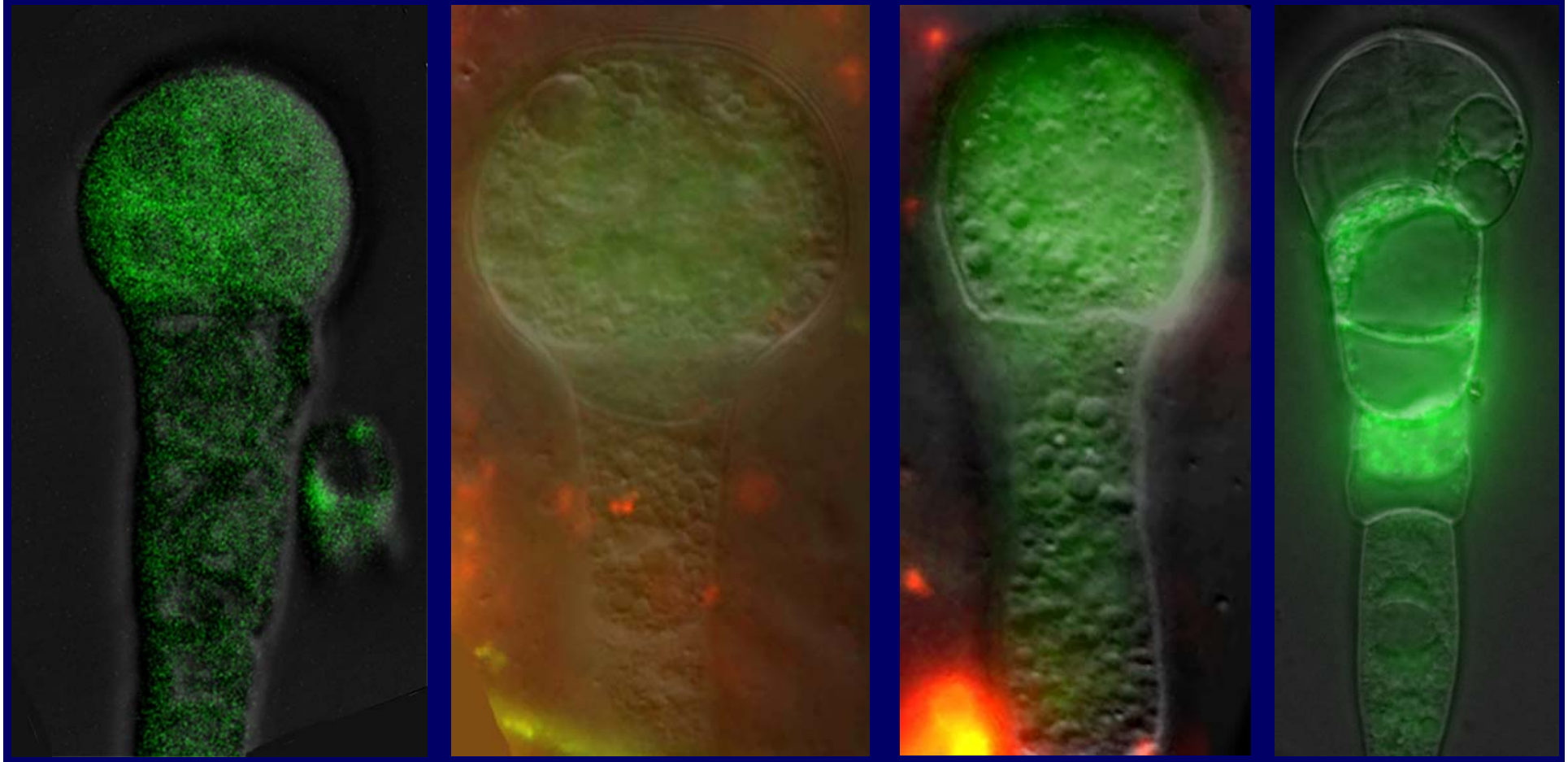


gnom

BFA treatment

DR5::GFP – in vitro Culturing

Preglobular embryos – short time treatments

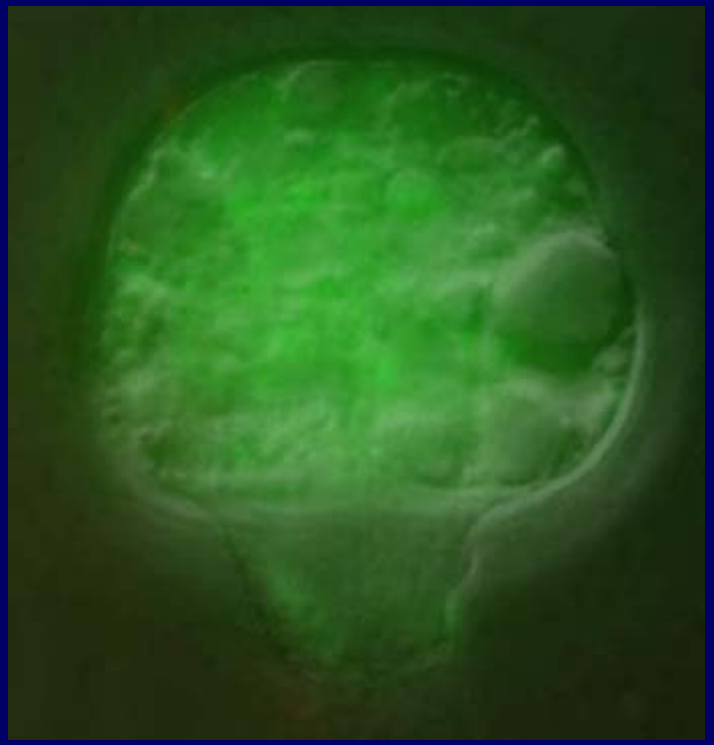


Control **0** **NAA** **NPA**

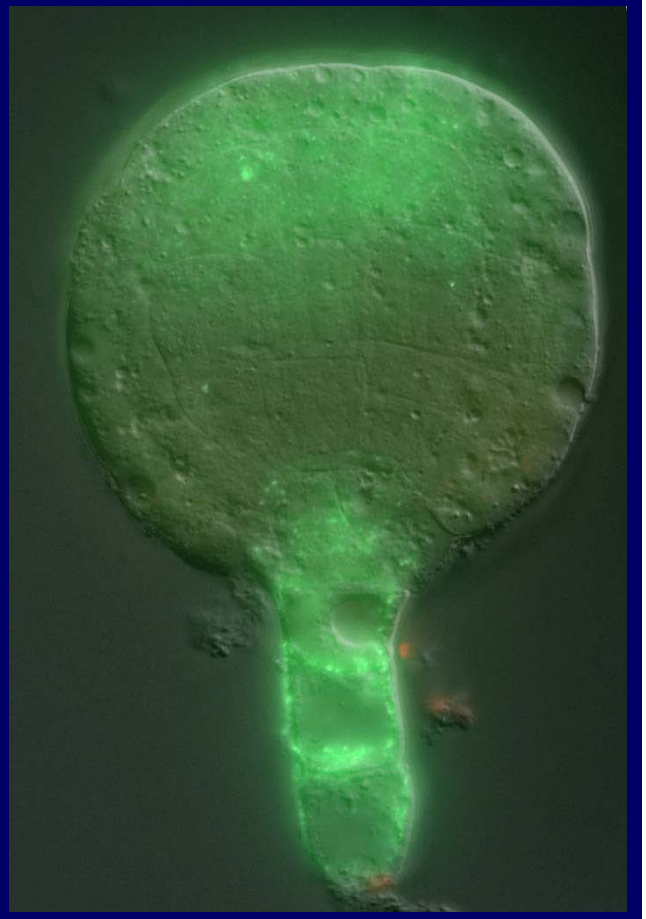
DR5::GFP – in vitro Culturing

Globular embryos – short time treatments

NAA



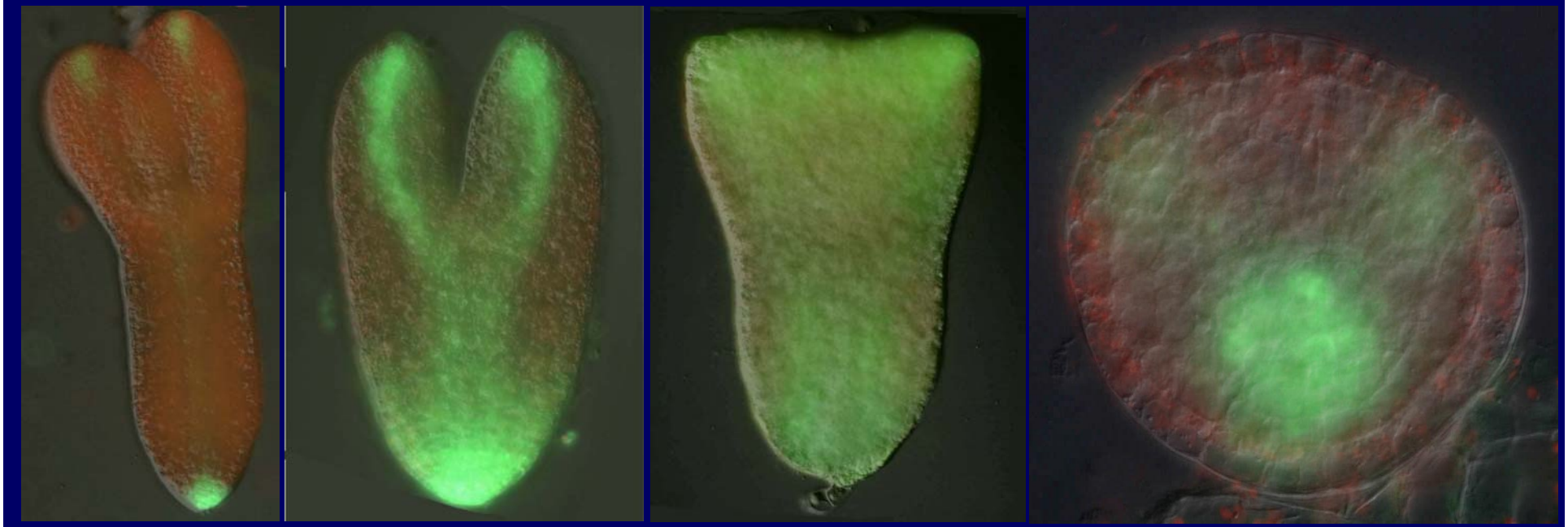
2,4D



BFA

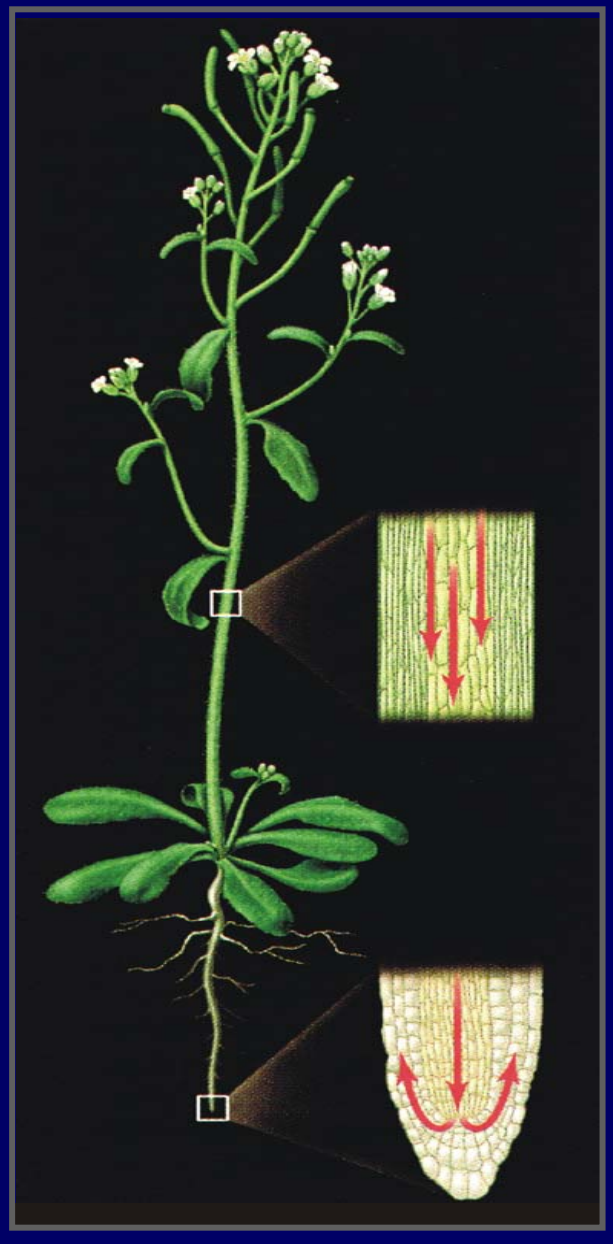
DR5::GFP – in vitro Culturing

Long time treatments

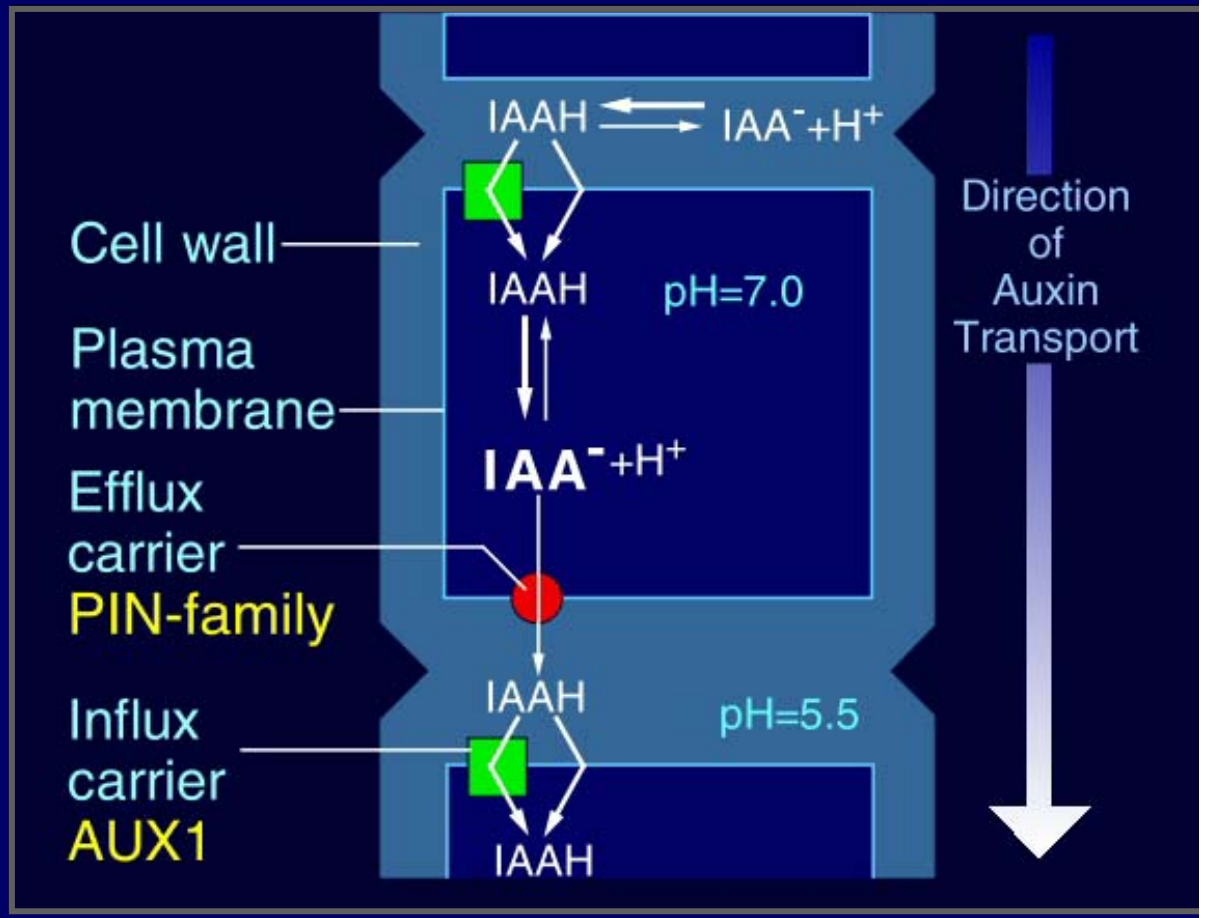


Control **NAA** **2,4D** **NPA or BFA**

Auxin Transport

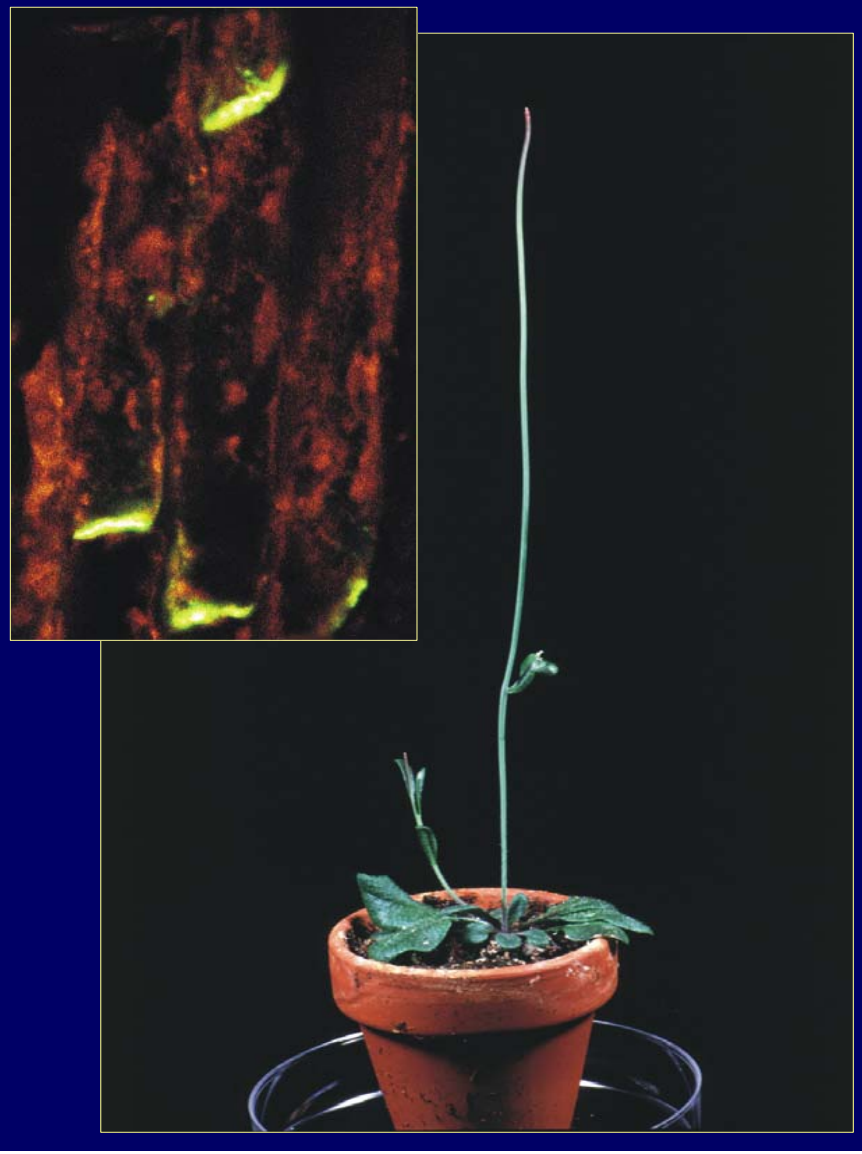


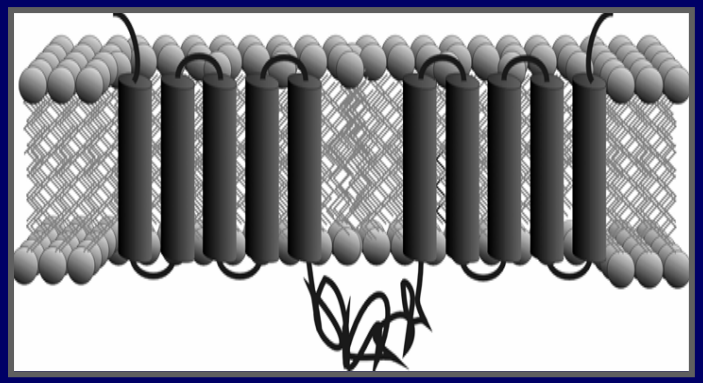
Chemiosmotic hypothesis



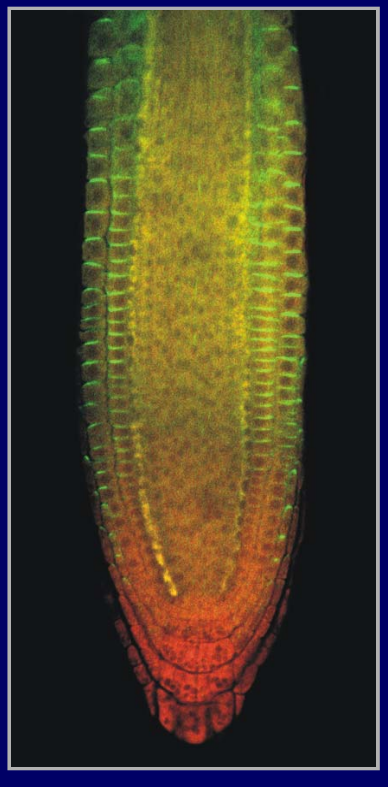
Molecular Genetics of Auxin Efflux

PIN1

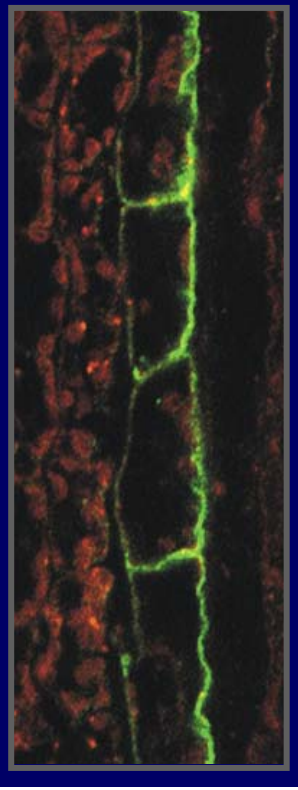




PIN2

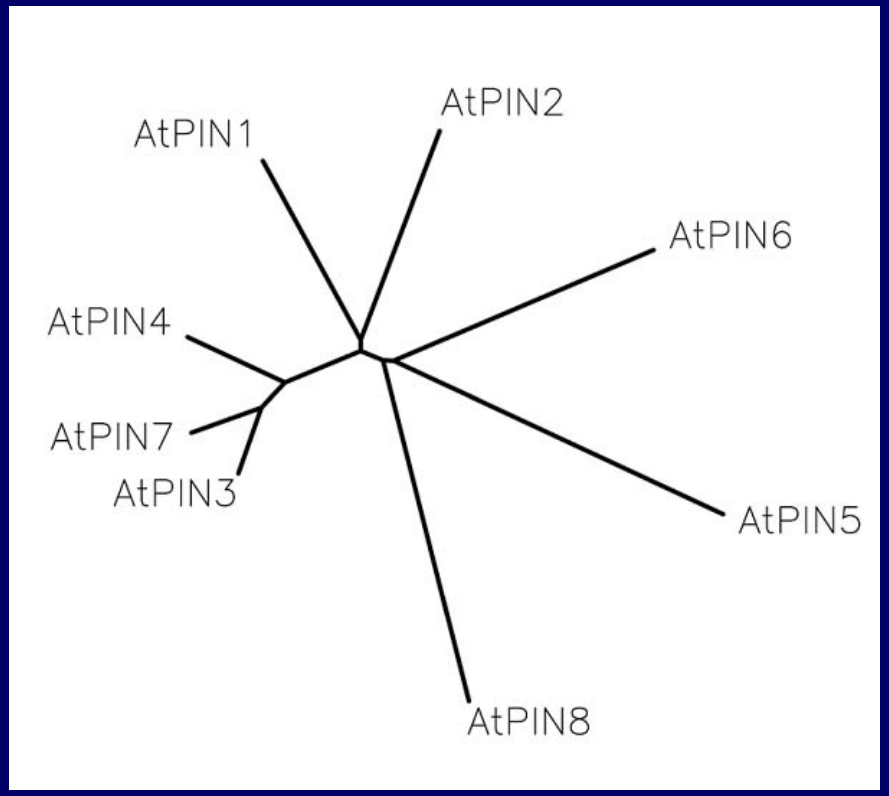


PIN3

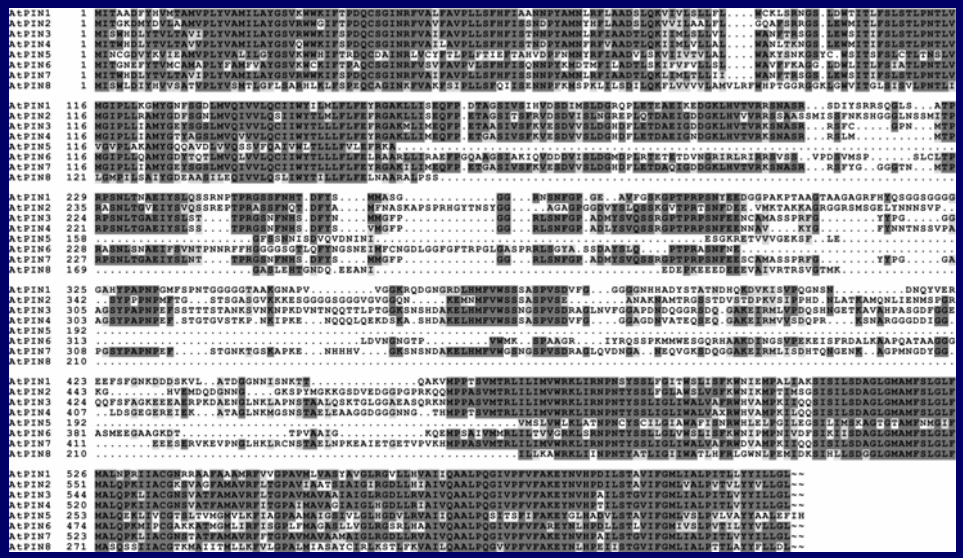


Arabidopsis PIN Protein Family

Phylogenetic tree

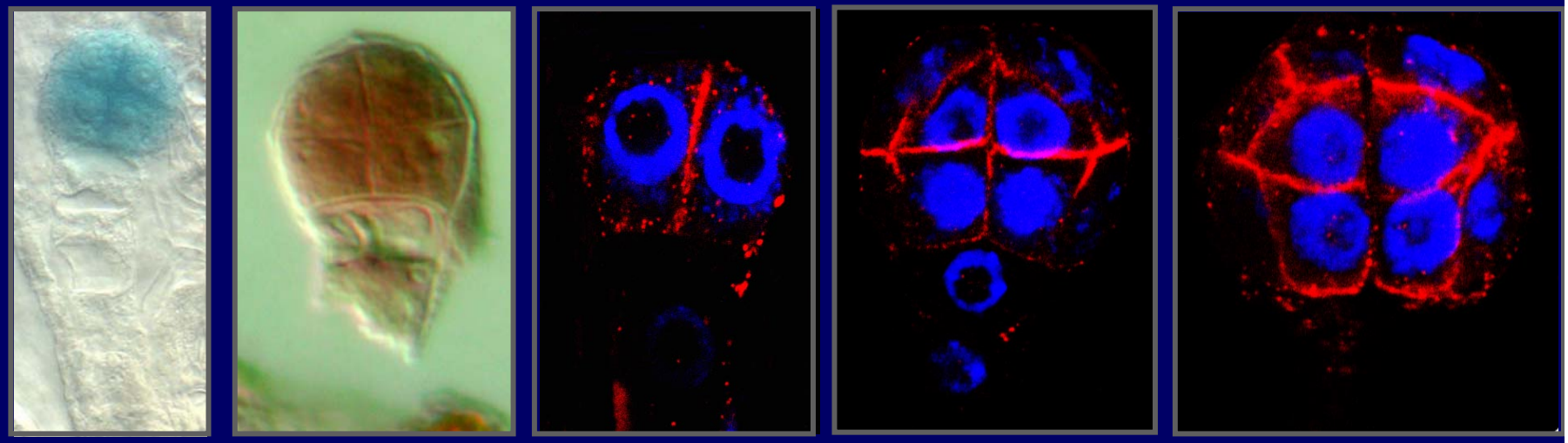


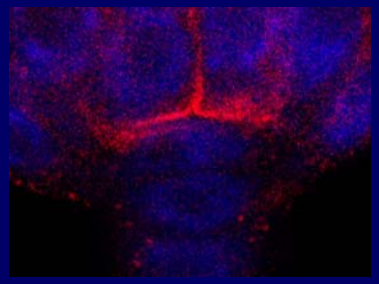
Homology of PIN proteins

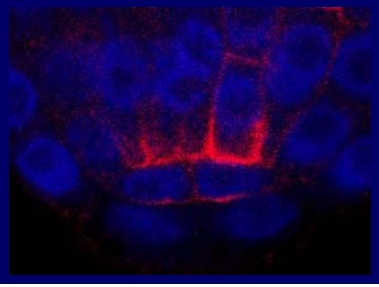


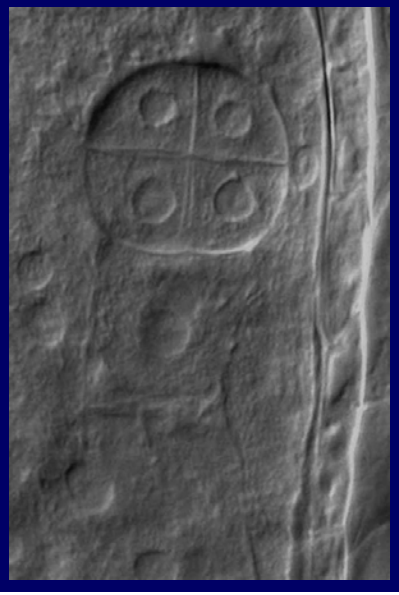
PIN1 in Early Embryogenesis

GUS mRNA Protein





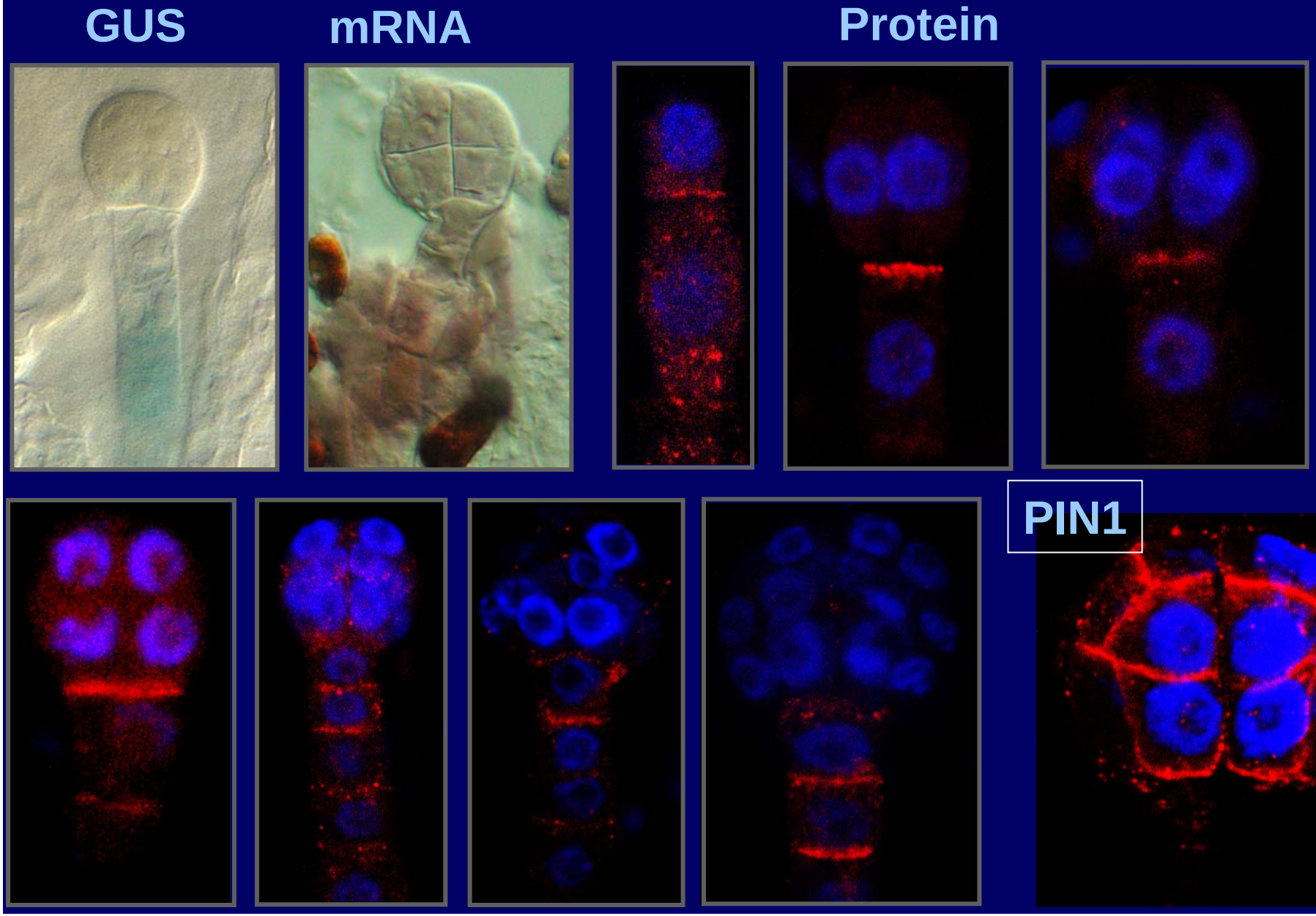




Enk
6,1%

pin1
30,4%

PIN7 in Embryogenesis



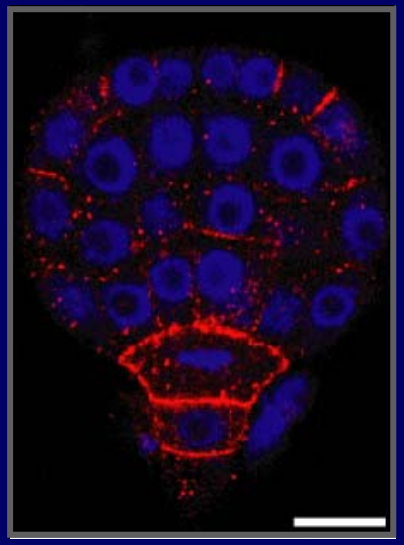
PIN4 in Embryogenesis

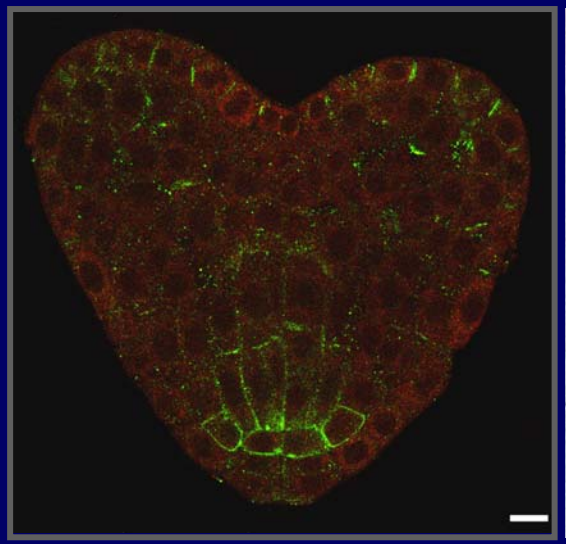
PIN4 protein

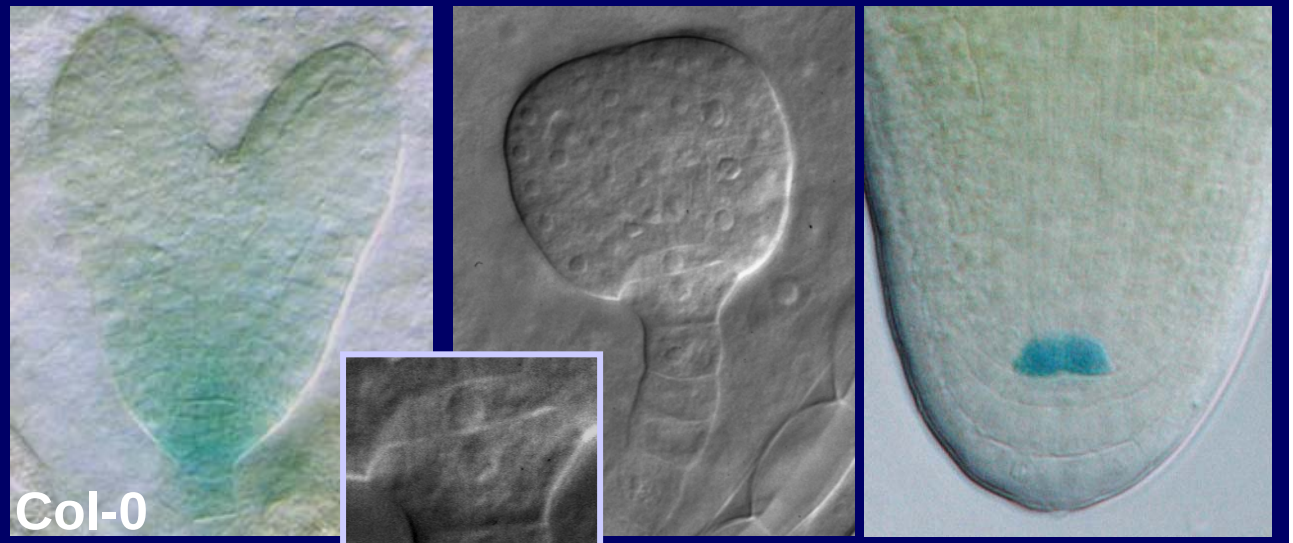
DR5

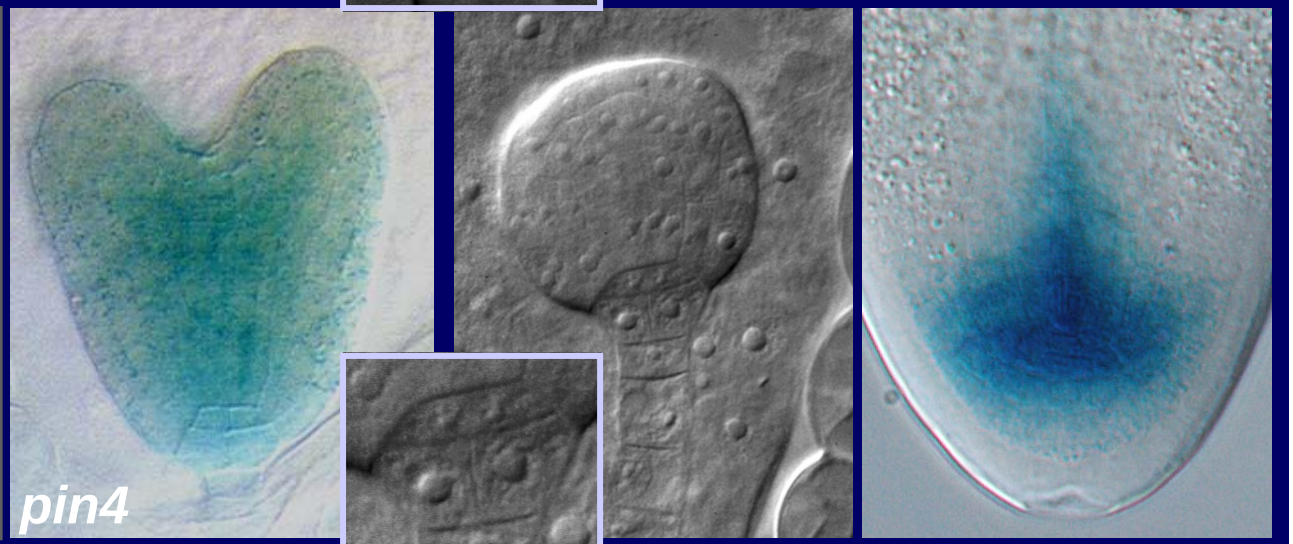
basal defects

QC marker



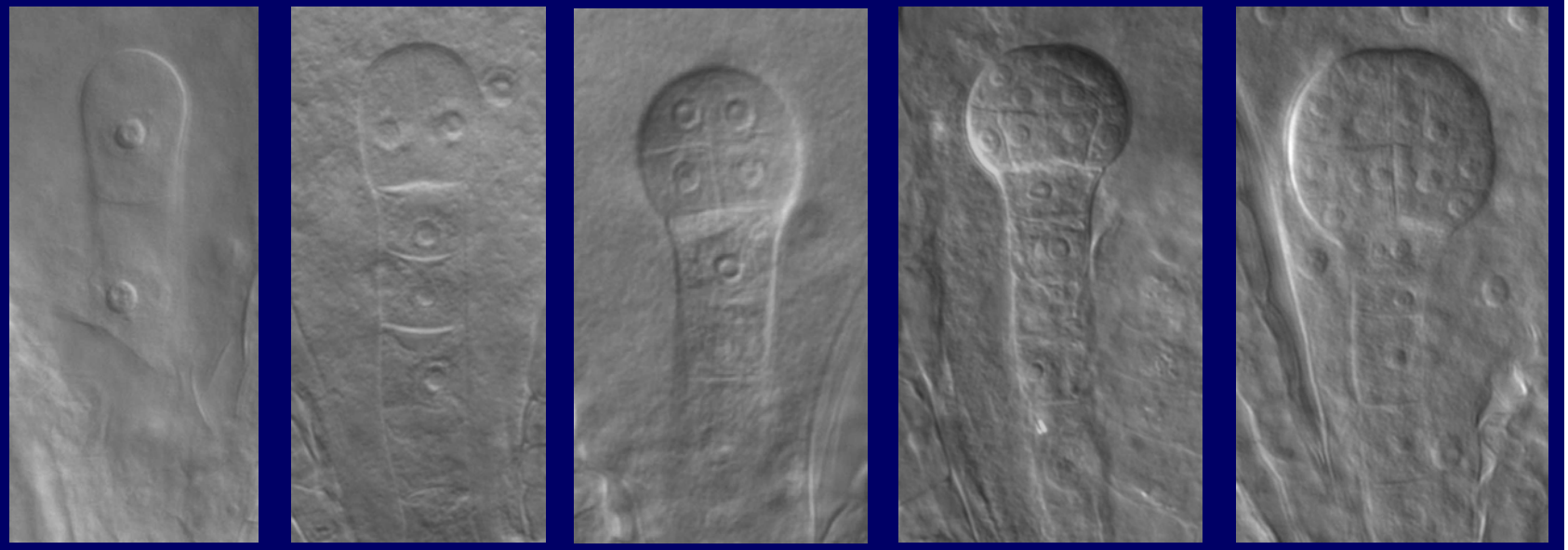




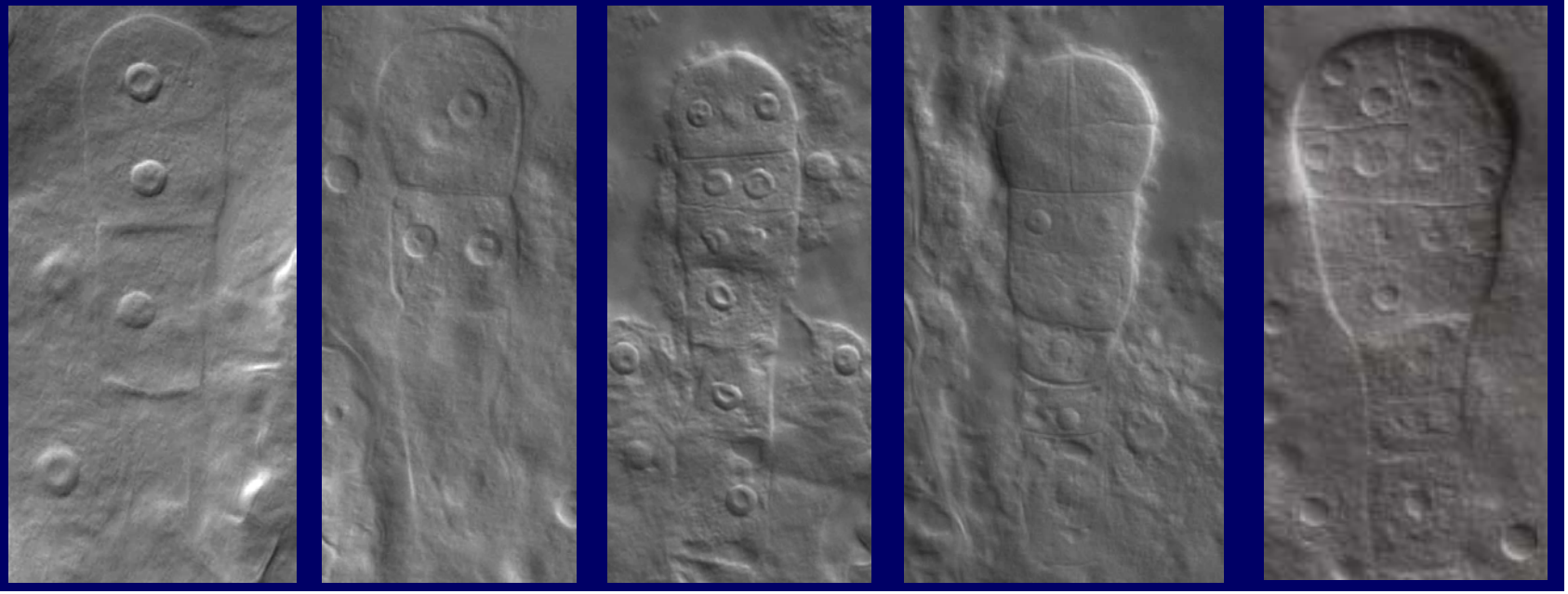


Embryo Phenotype of *pin7* Mutants

Col-0

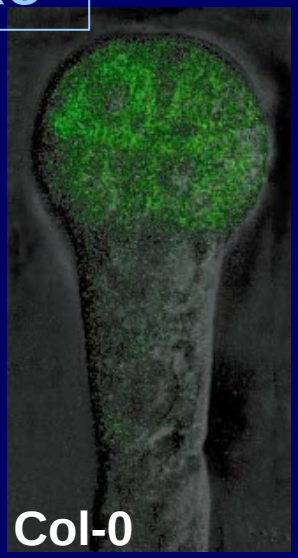


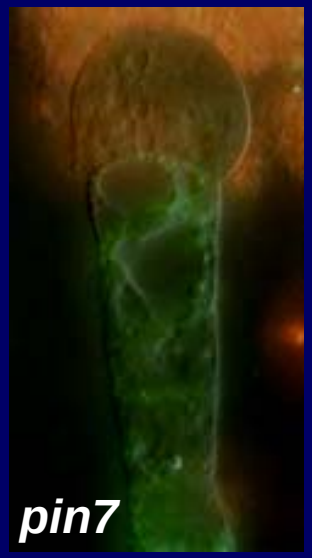
pin7



Analysis of Markers in *pin7*

DR5

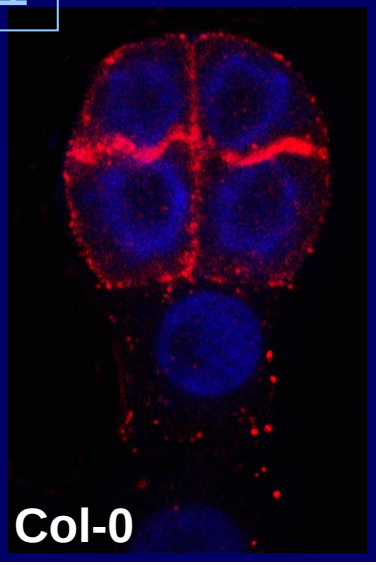


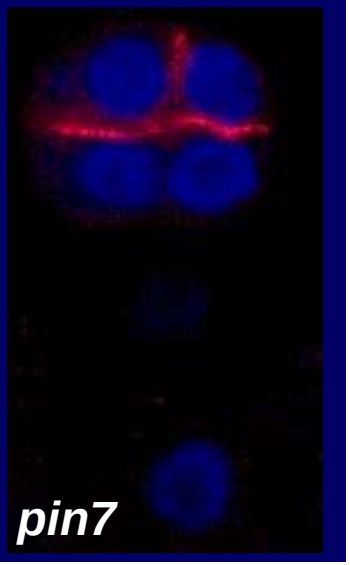


PIN7::GUS

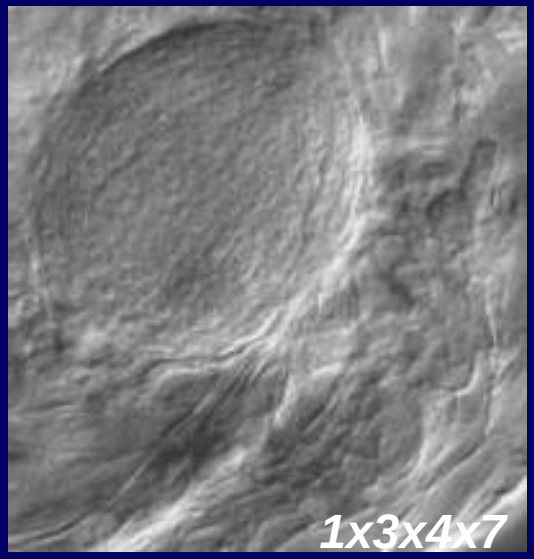
PIN1::GUS

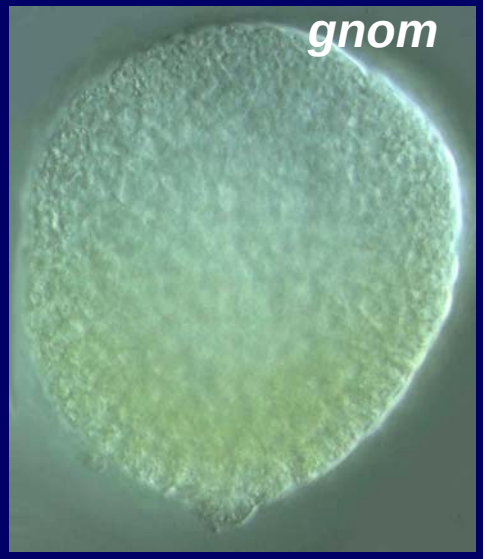
PIN1





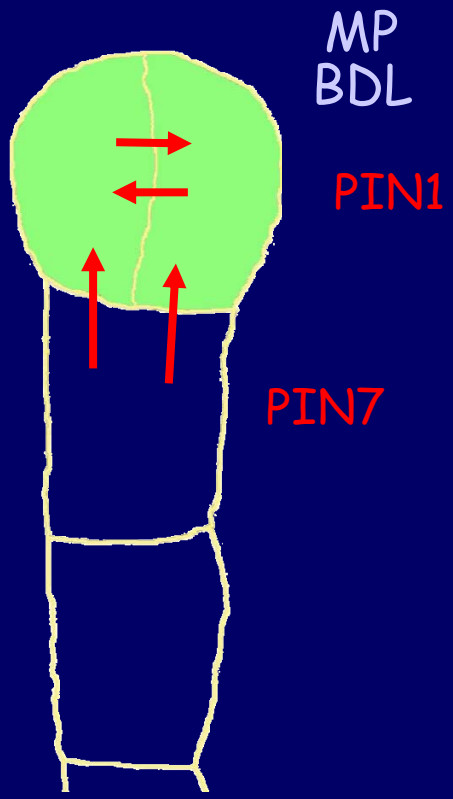
Phenotypes of *pin* Multiple Mutants





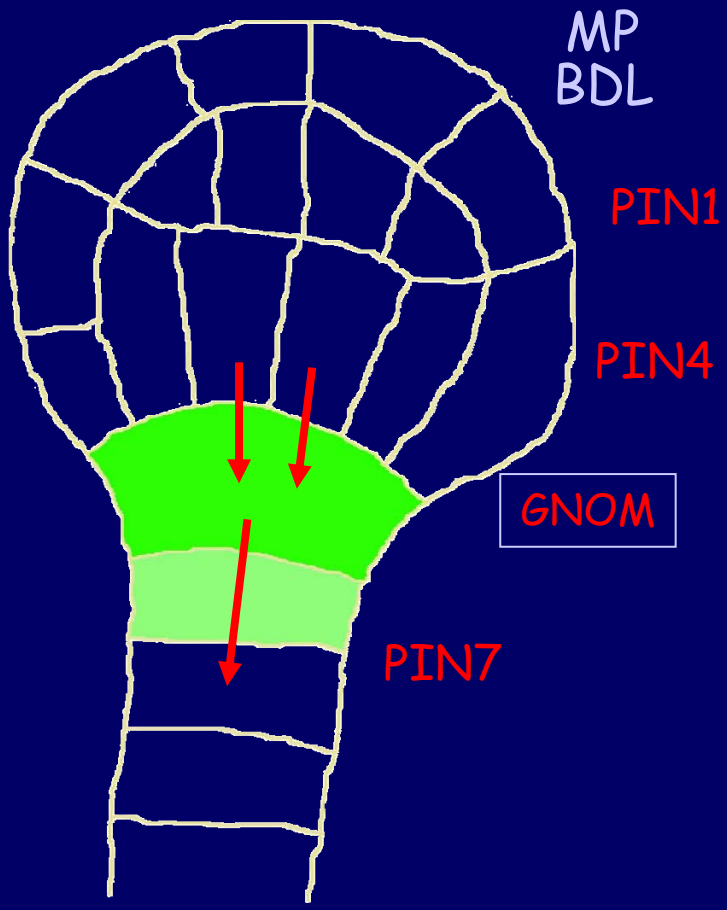
Auxin and Embryogenesis

Apical pole specification



Two-Cell

Root pole specification

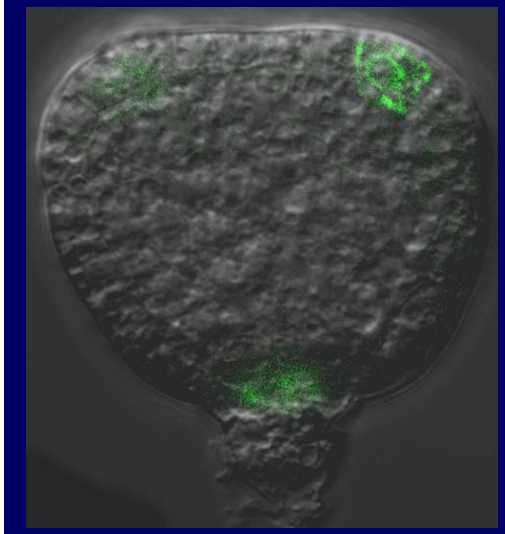


Globular

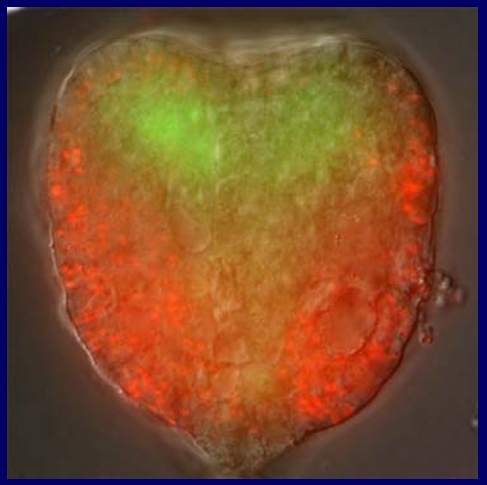
Organogenesis

Auxin in Cotyledon Formation

DR5



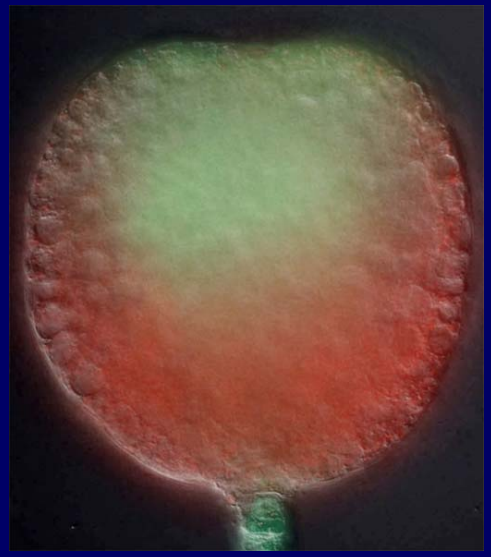
BFA



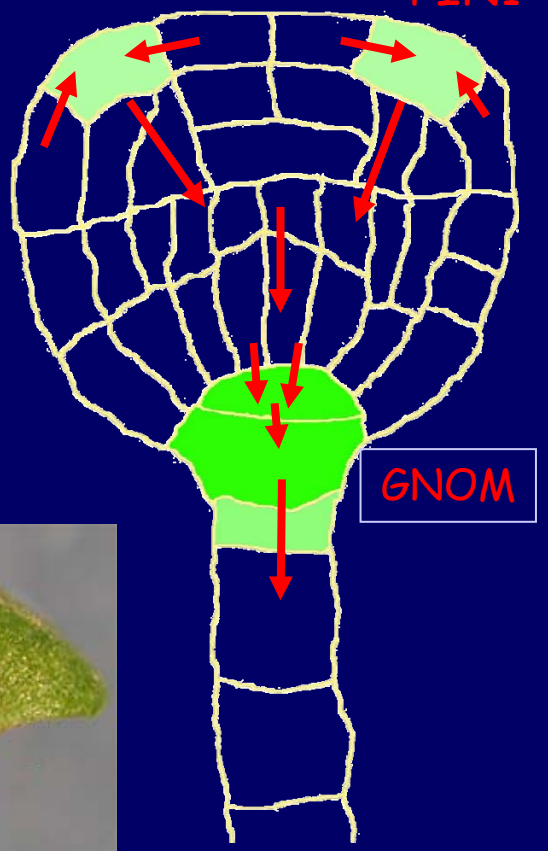
pins

IAA

gnom



pin1

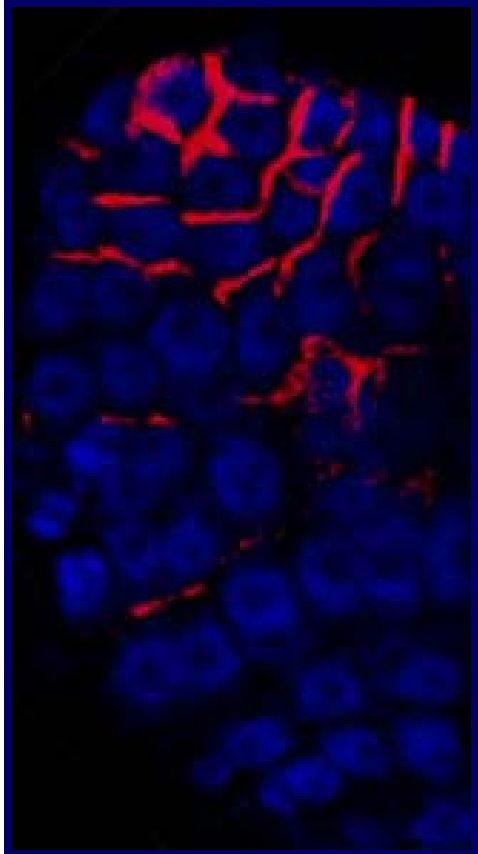


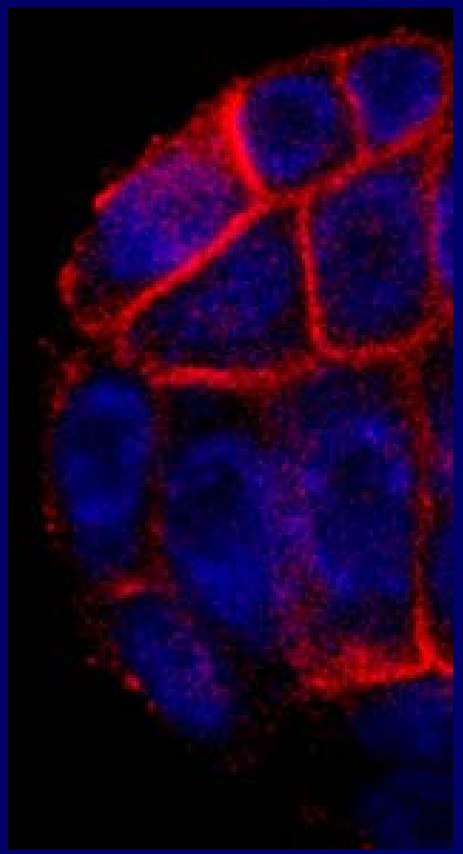
PIN1 Polarity in Cotyledon Formation

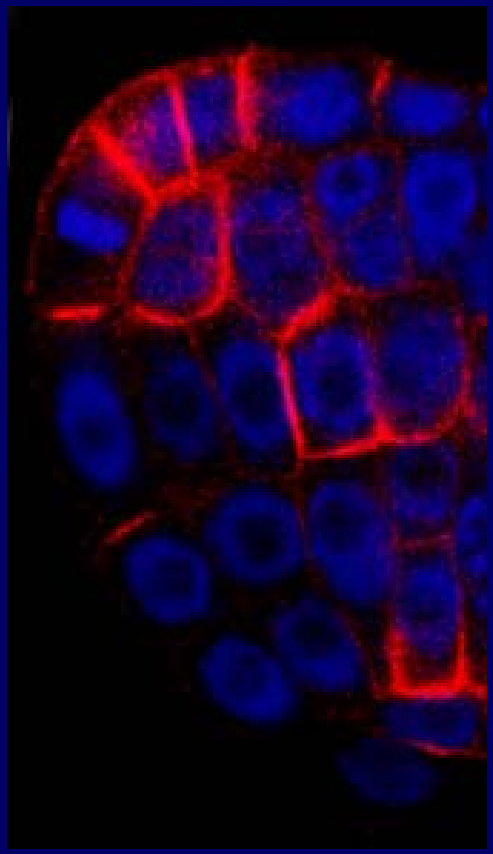
Outer layer

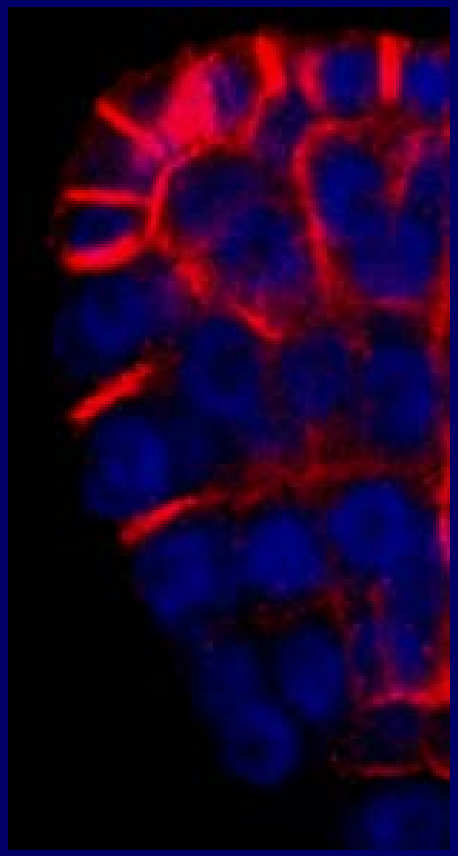
Inner layers

BFA treatment









Heart

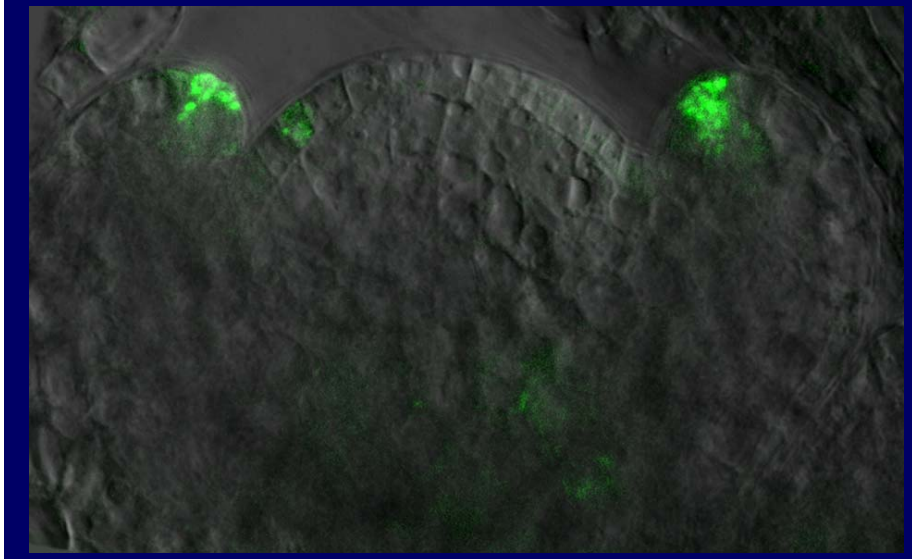
Globular

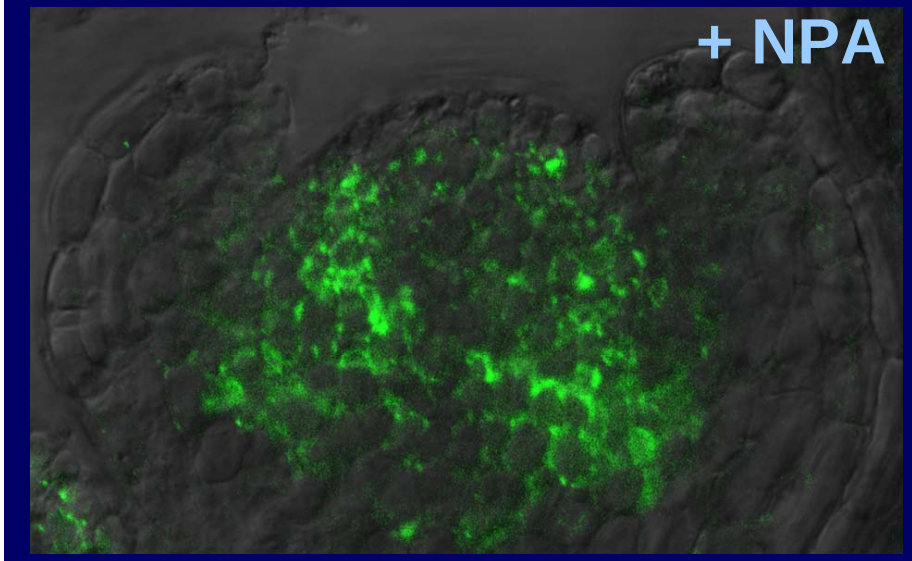
Heart

Heart

Auxin in Flower and Leave Formation

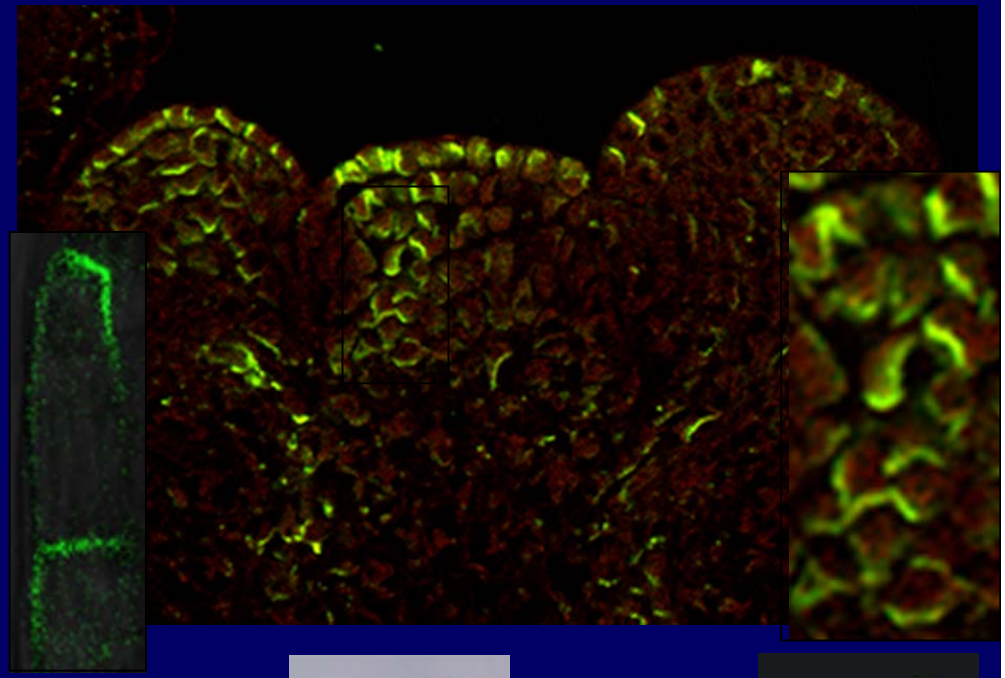
DR5rev::GFP





+ NPA

PIN1 localisation

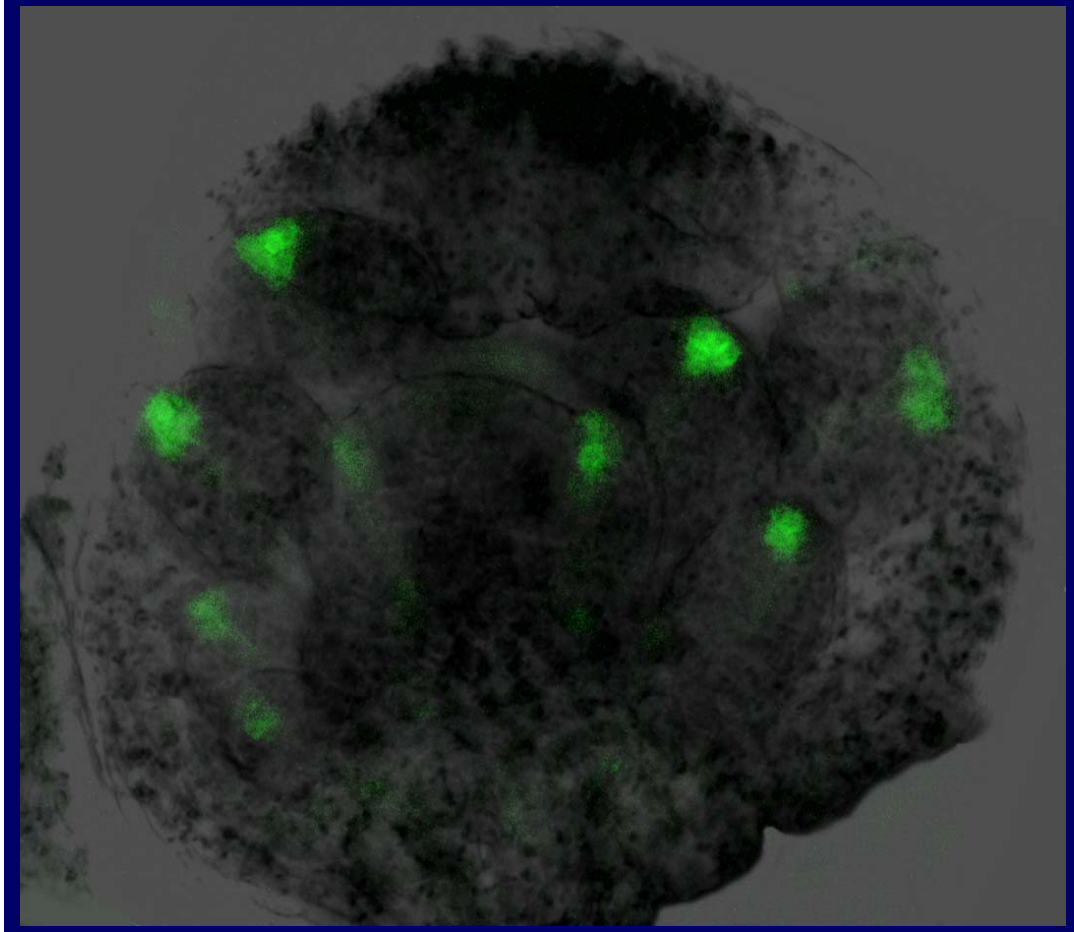


+ NPA

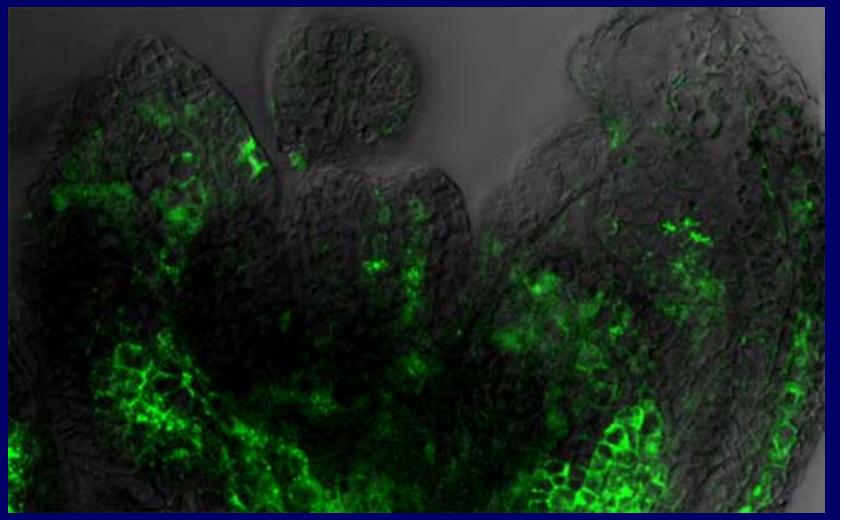
pin1

DR5 in Floral Organ Formation

DR5rev::GFP

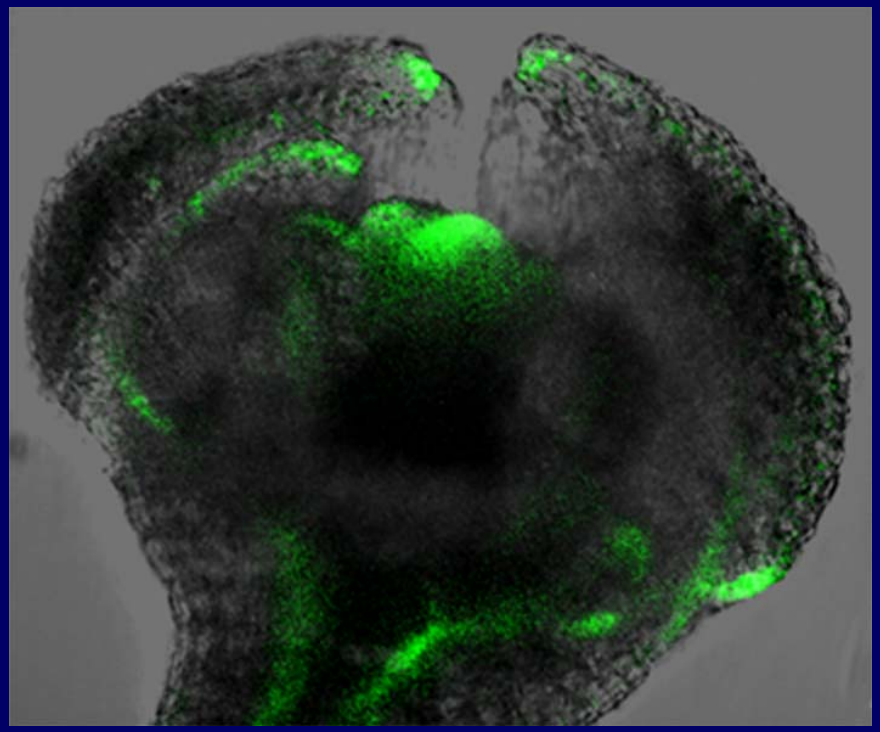


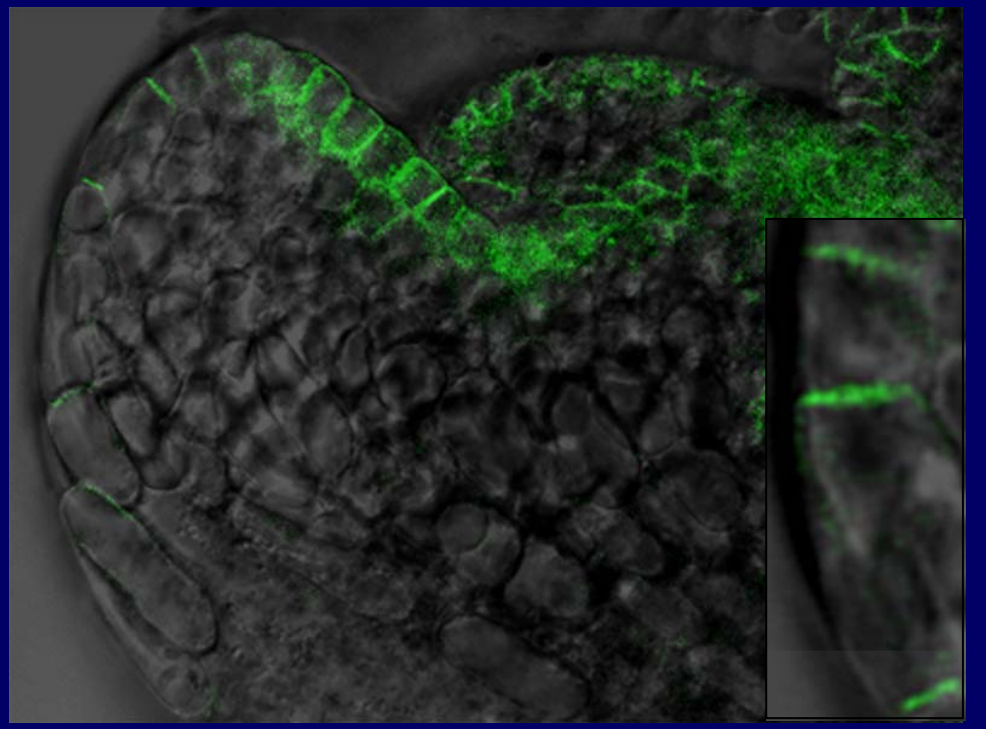
+ NPA



***pin* mutants**

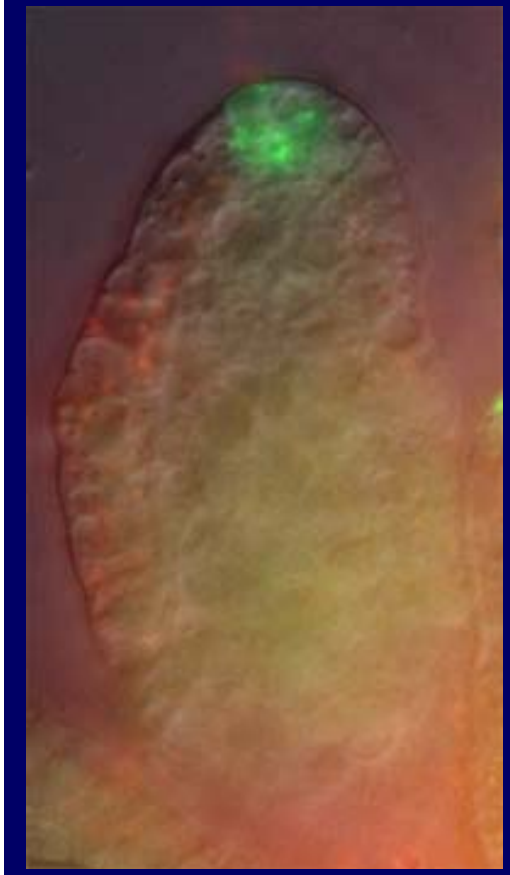
PIN1 in Floral Organ Formation





DR5 in Ovule Formation

Ovule primordium

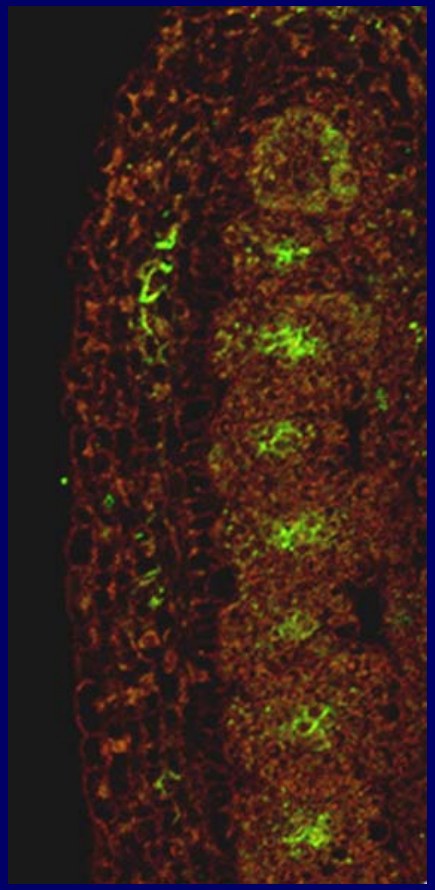


Ovule with Integuments primordia

Ovule defects in *pin1*

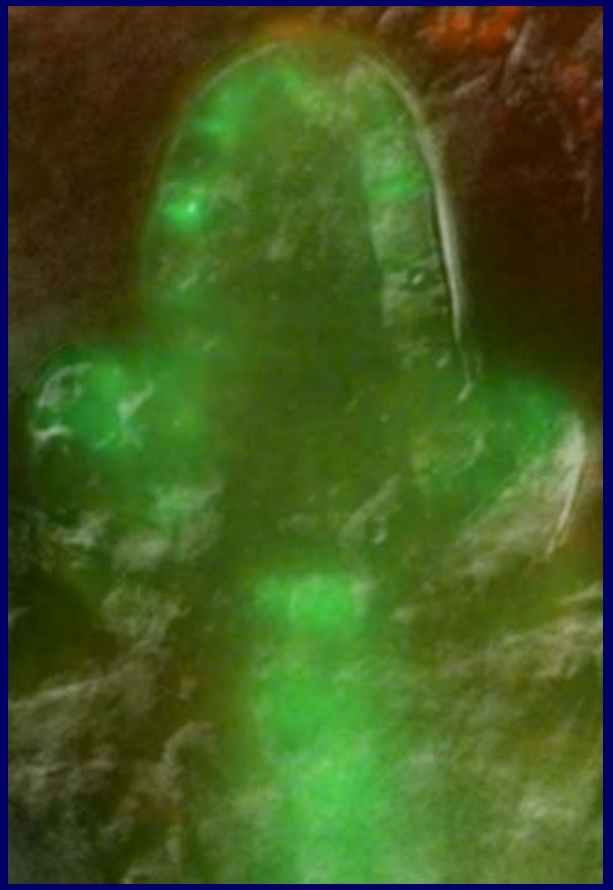
PIN1 in Ovule Formation

Gynoecium with ovule primordia

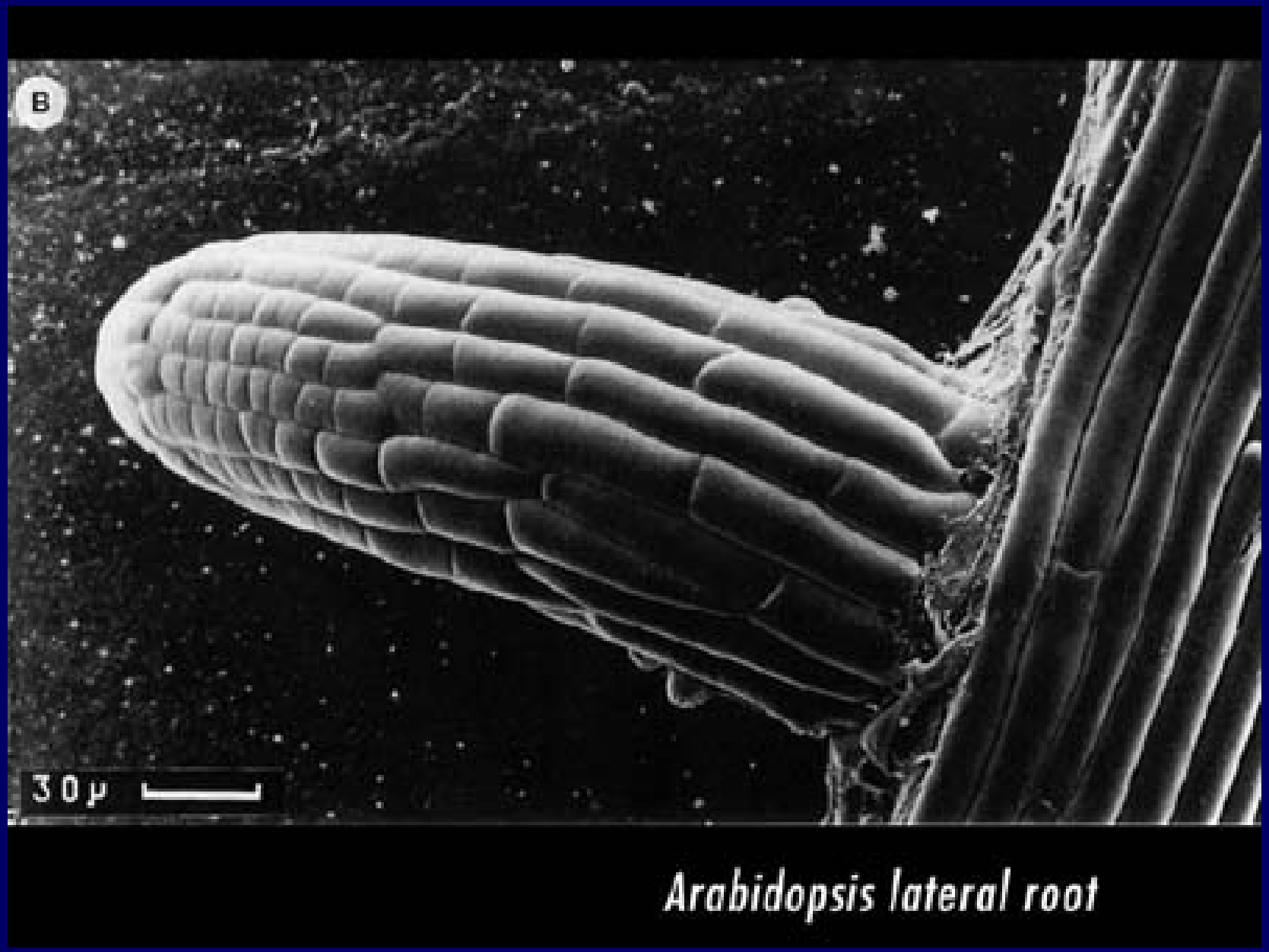


Ovule primordium

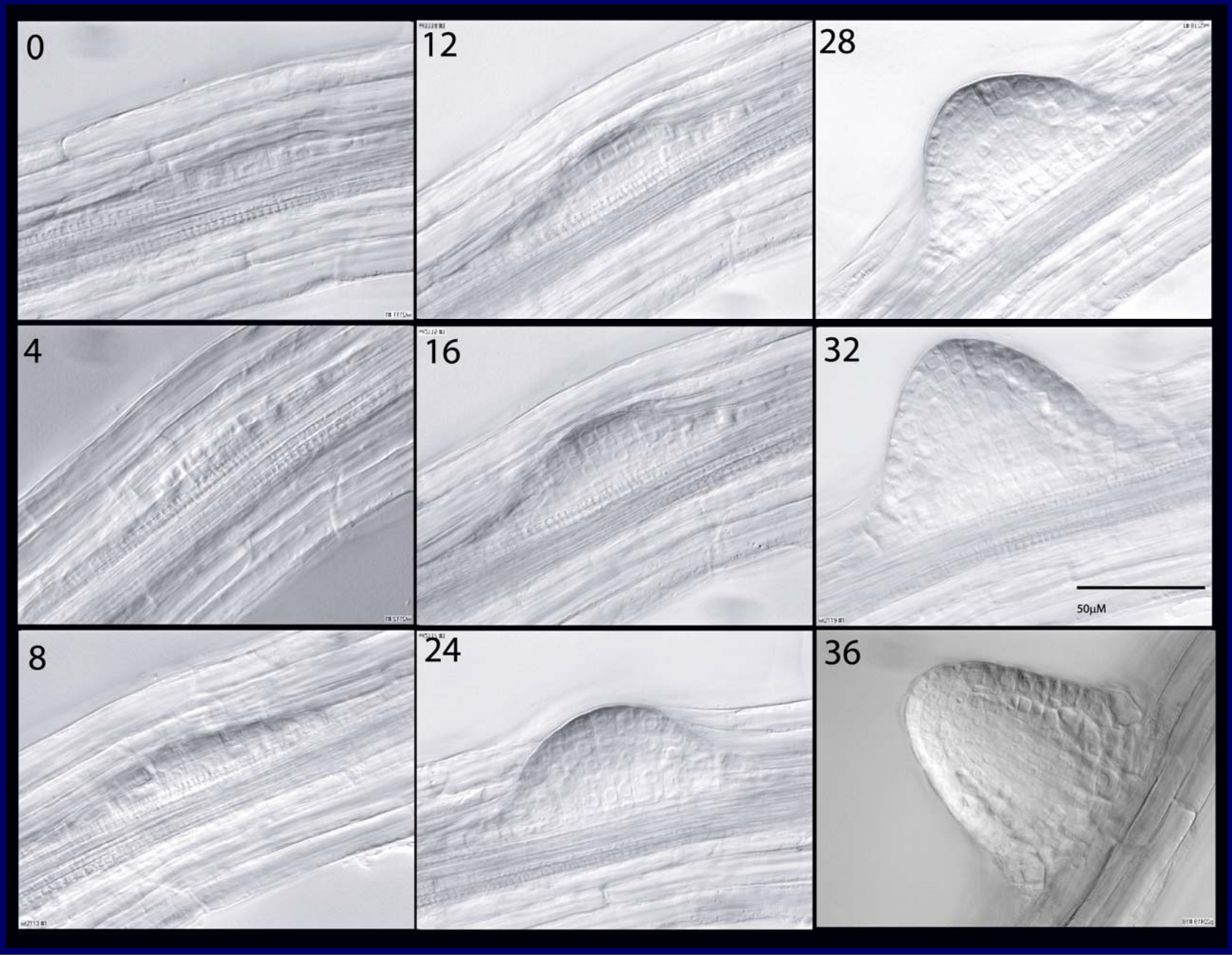
Ovule with Integuments primordia



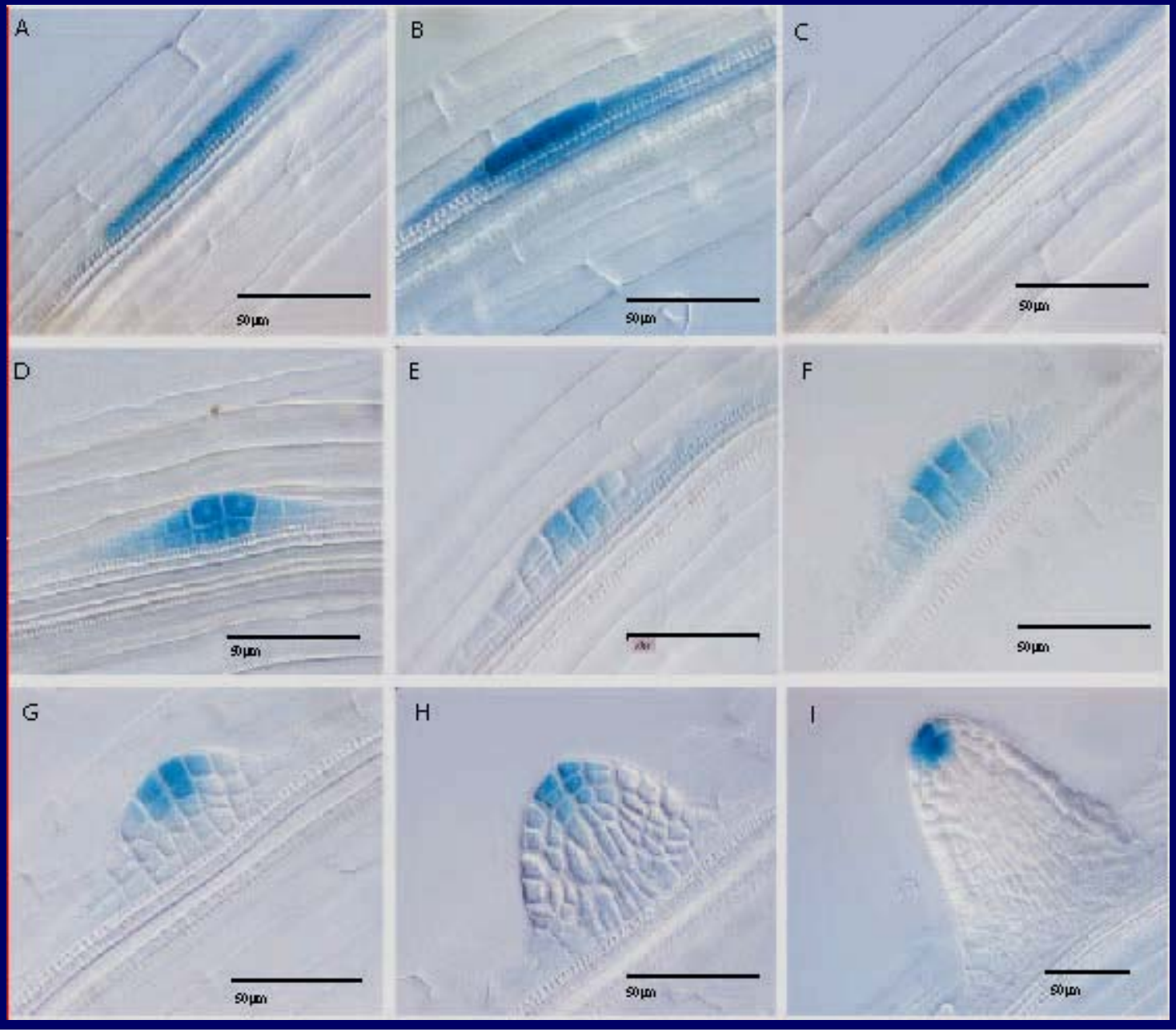
Lateral Root Development



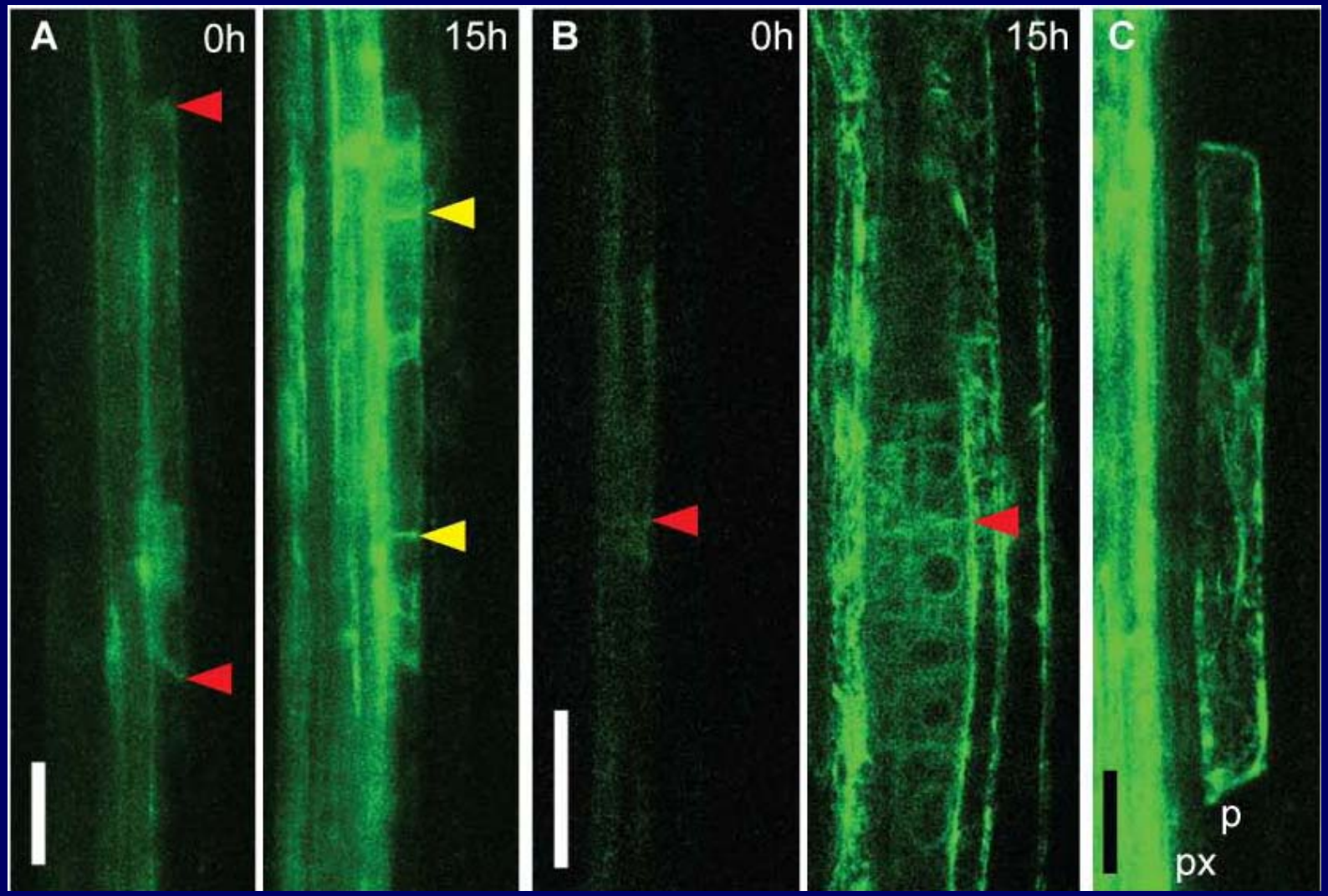
Lateral Root Development in Time



Auxin in Lateral Root Development

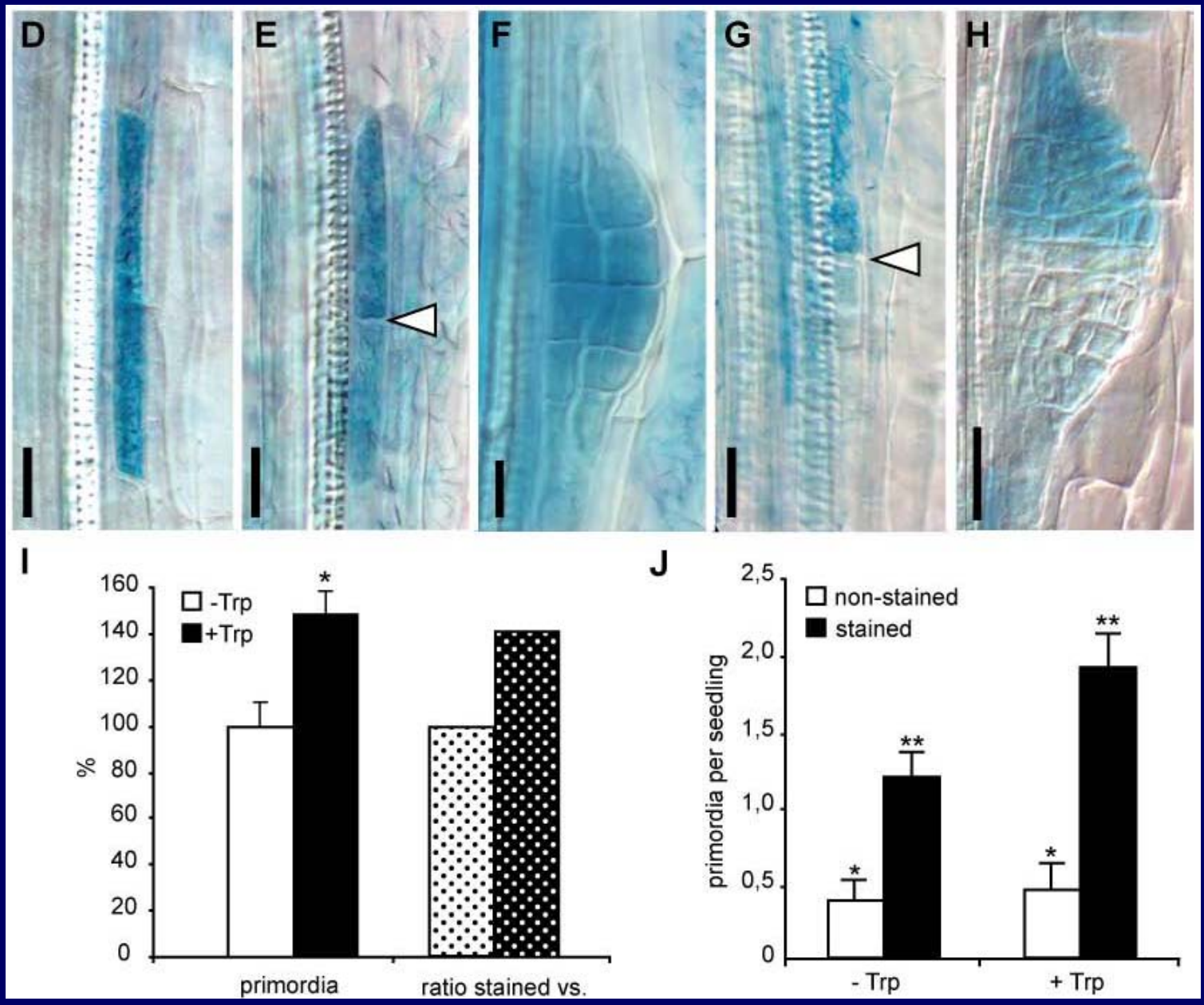


DR5 Activity Correlates with Lateral Root Initiation



unpublished

Local Auxin Production Specifies Founder Cells

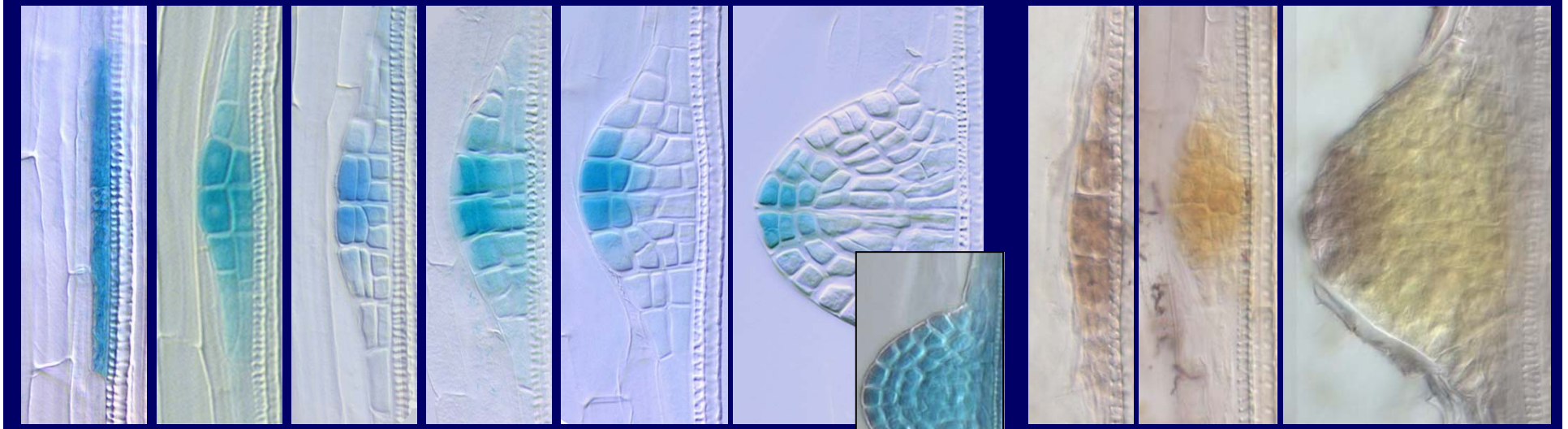


unpublished

DR5 in Lateral Root Formation

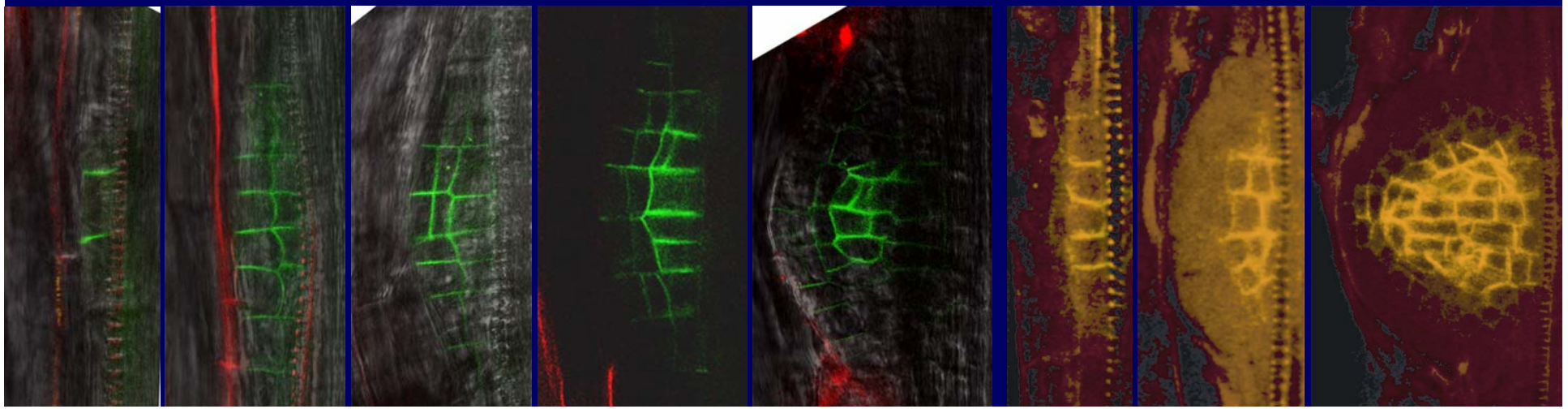
DR5rev::GUS

IAA

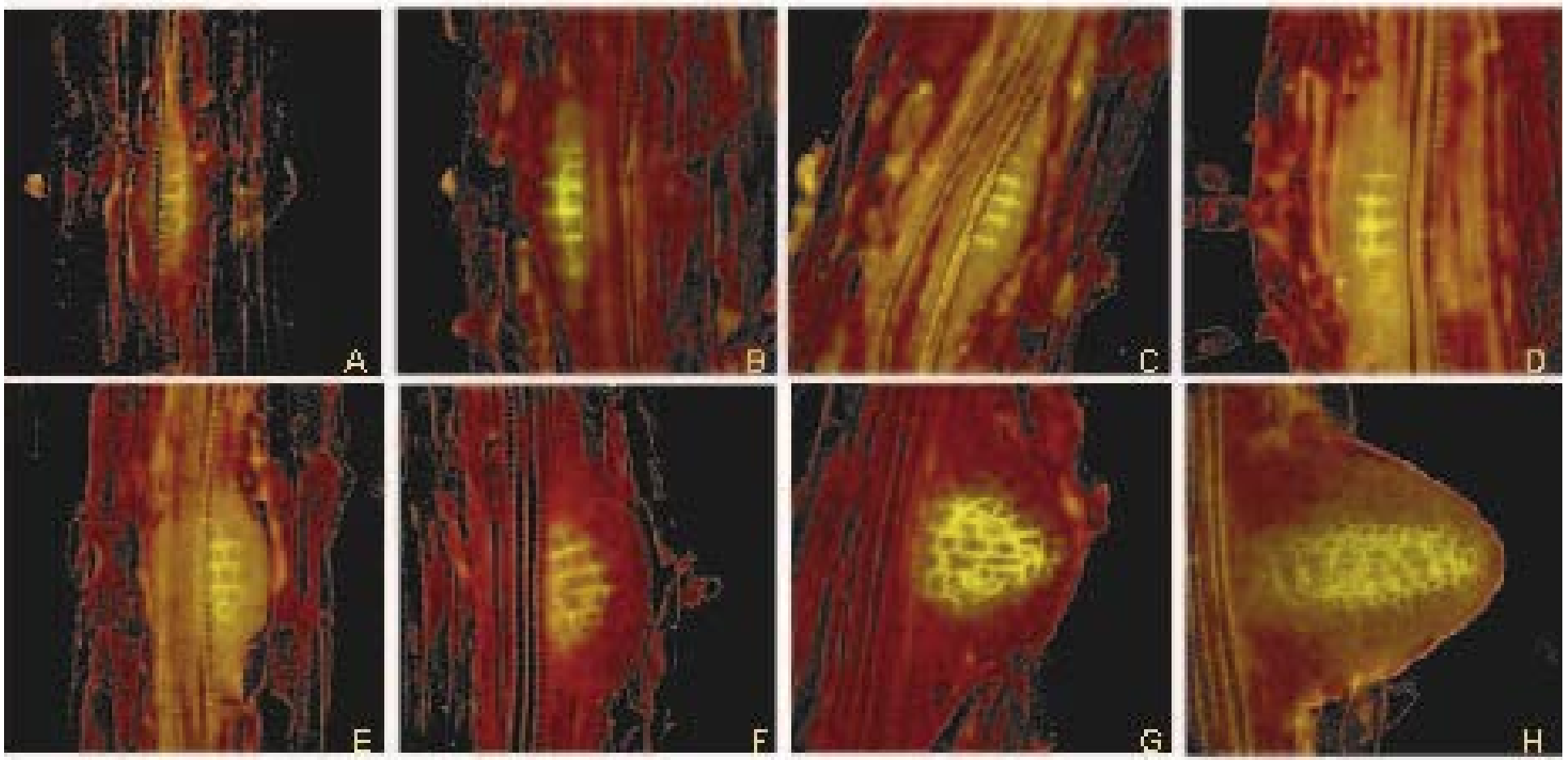


PIN1:GFP

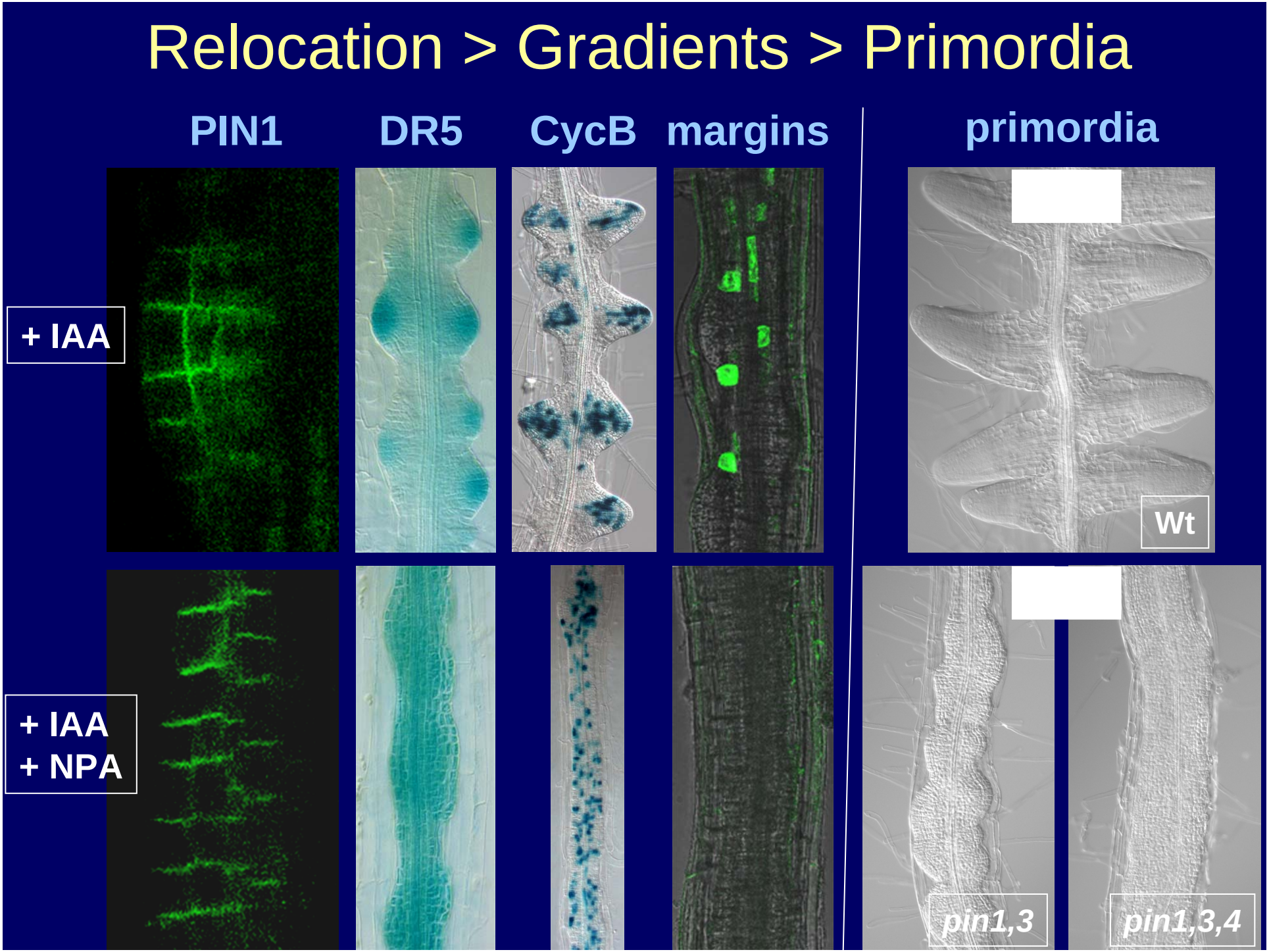
PIN1



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.

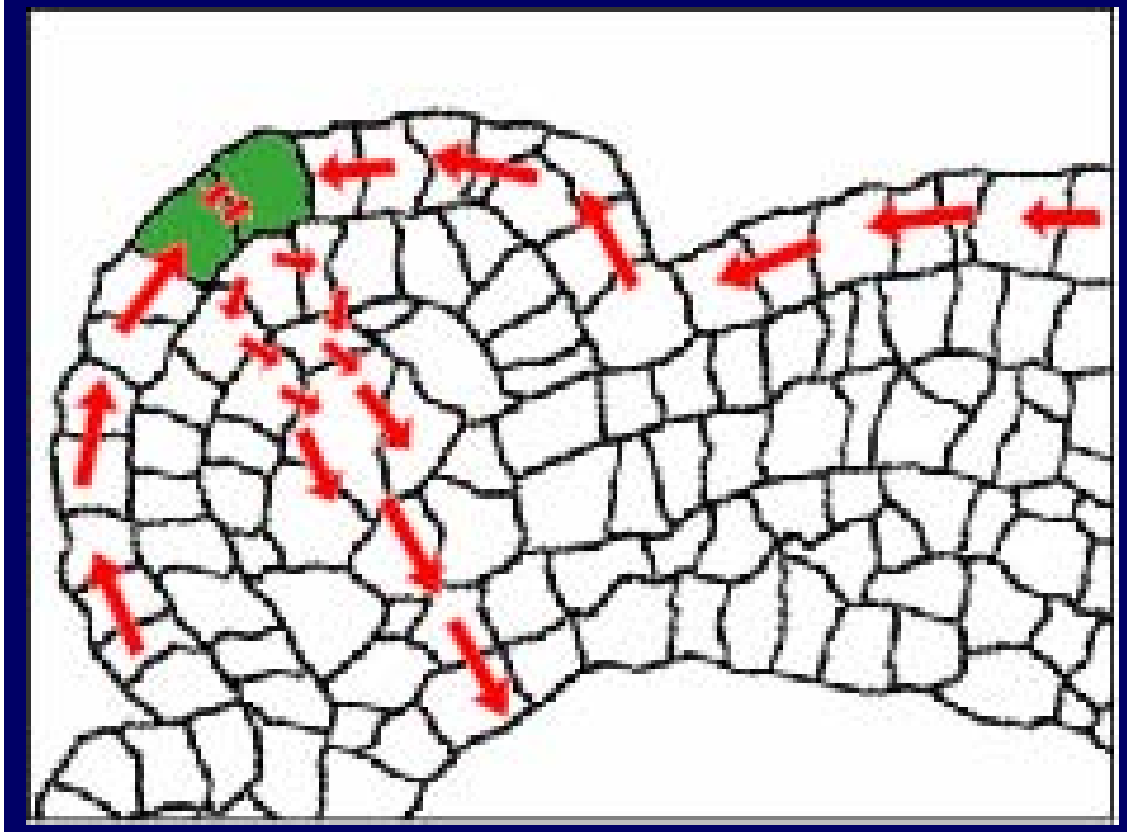
-Initiation (in pericycle) and development phases can be distinguished.

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

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

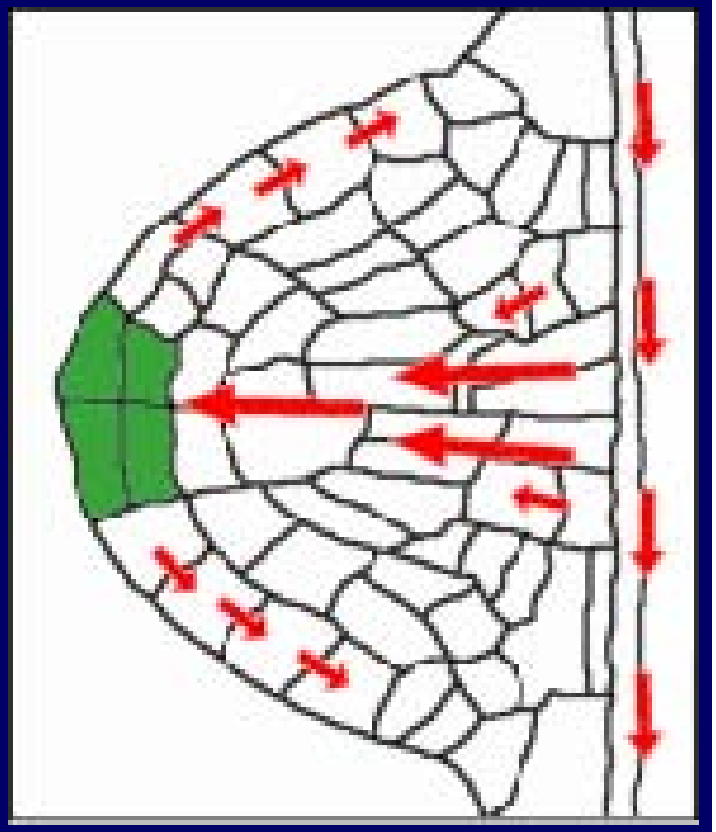
Common module for organ formation

Aerial organogenesis



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

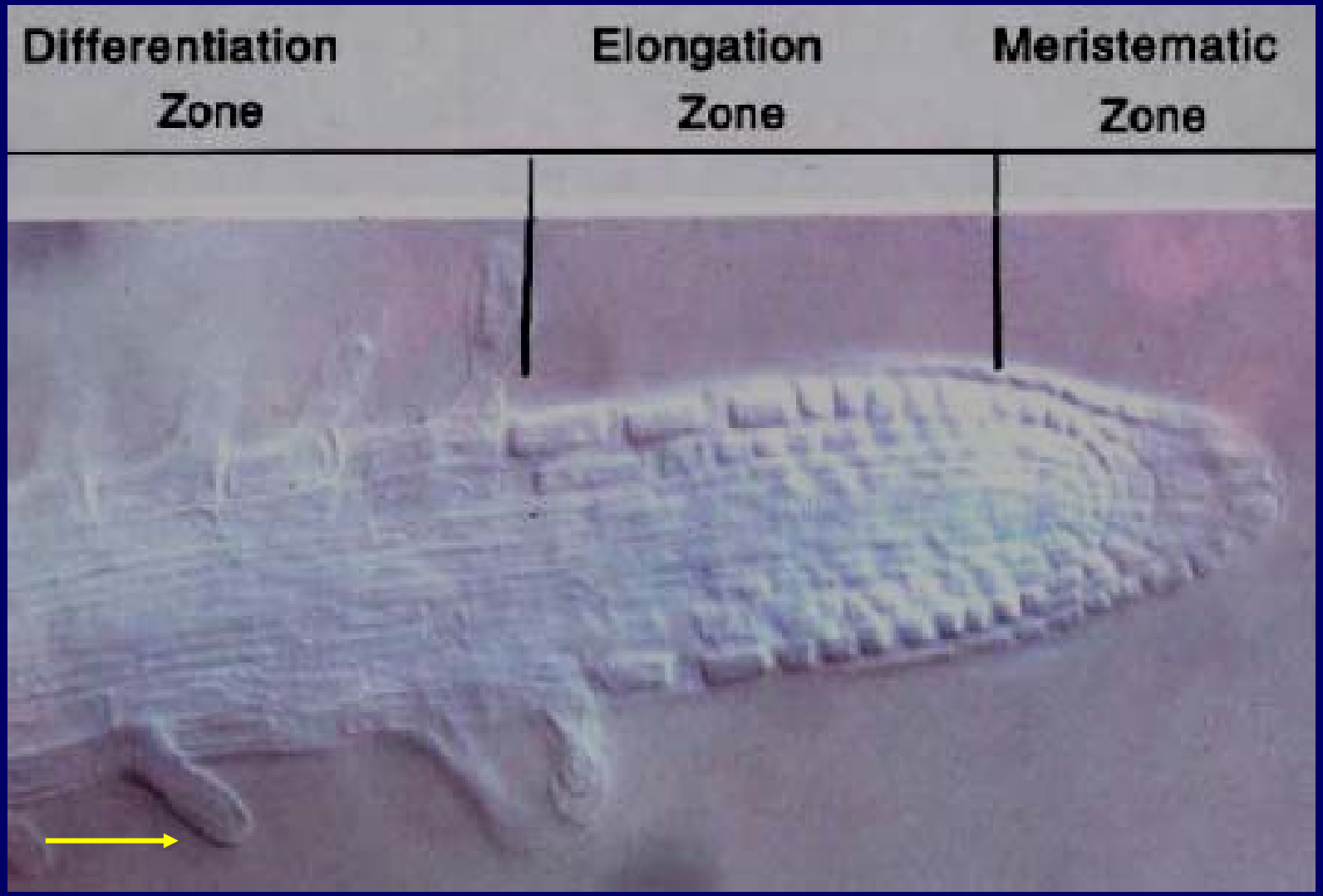
Underground organogenesis



Lateral roots

Root meristem

Parts of the Primary Root

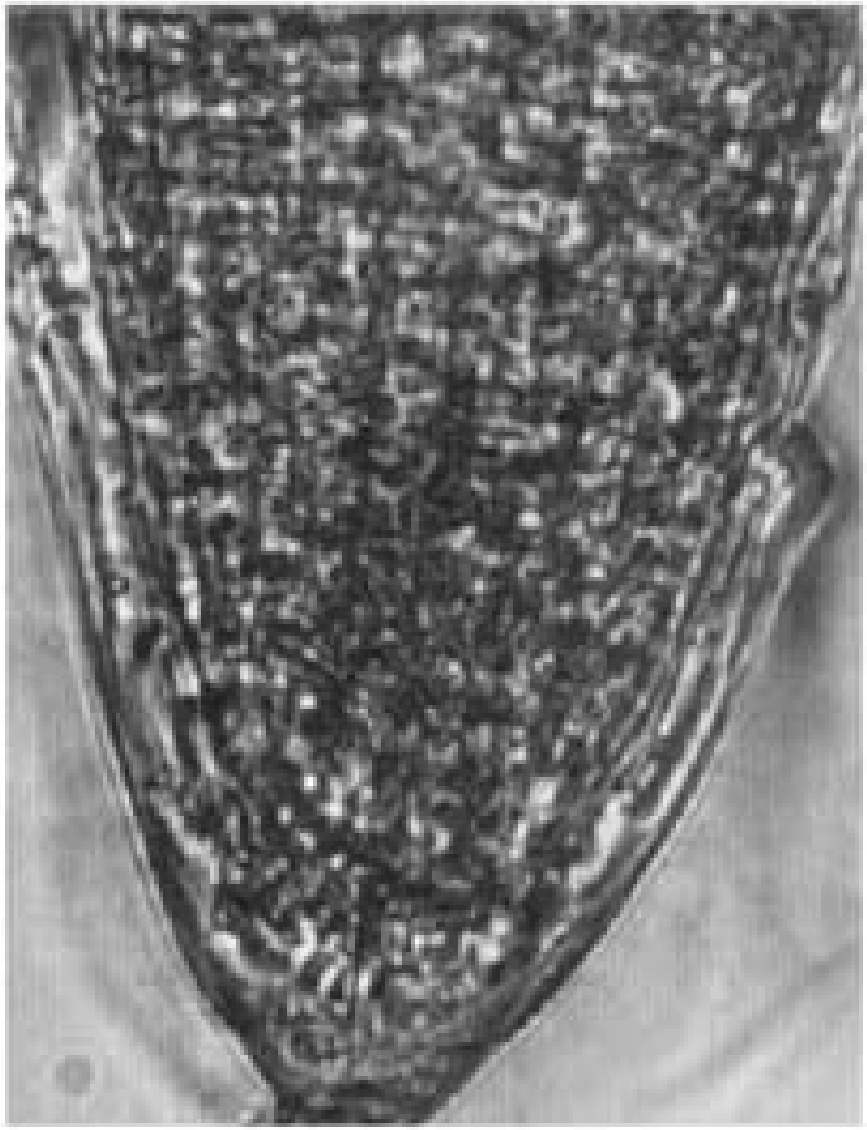


Root hair

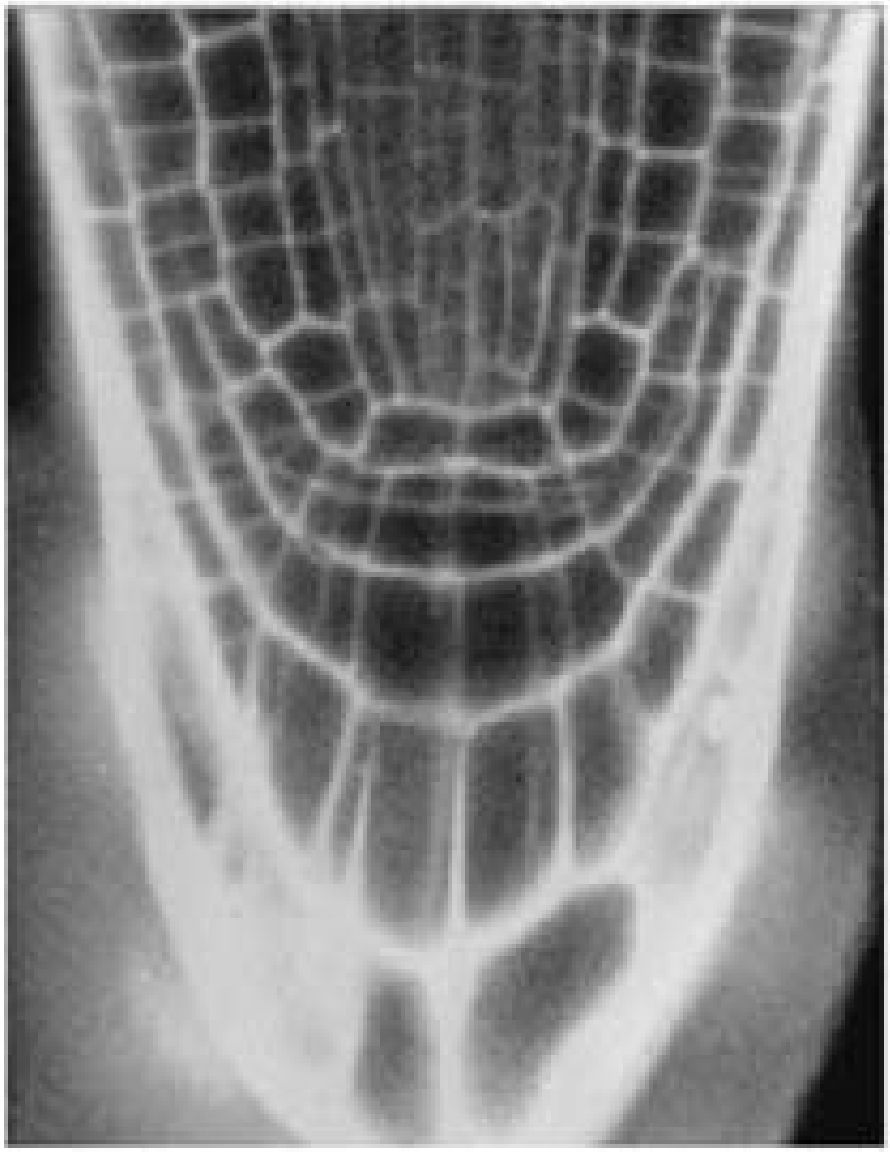
Differentiation Elongation Division

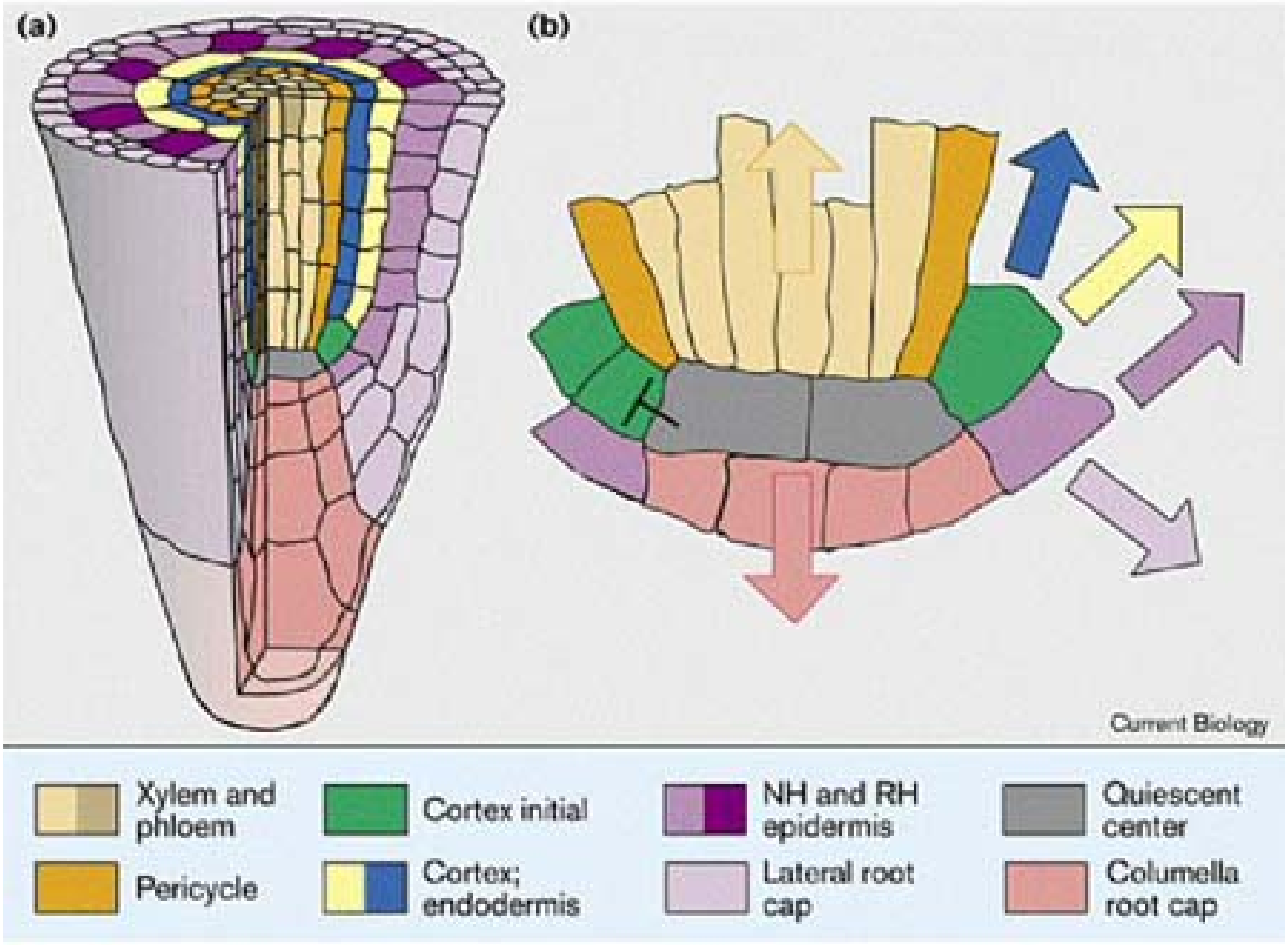
Root Meristem

Light microscopy

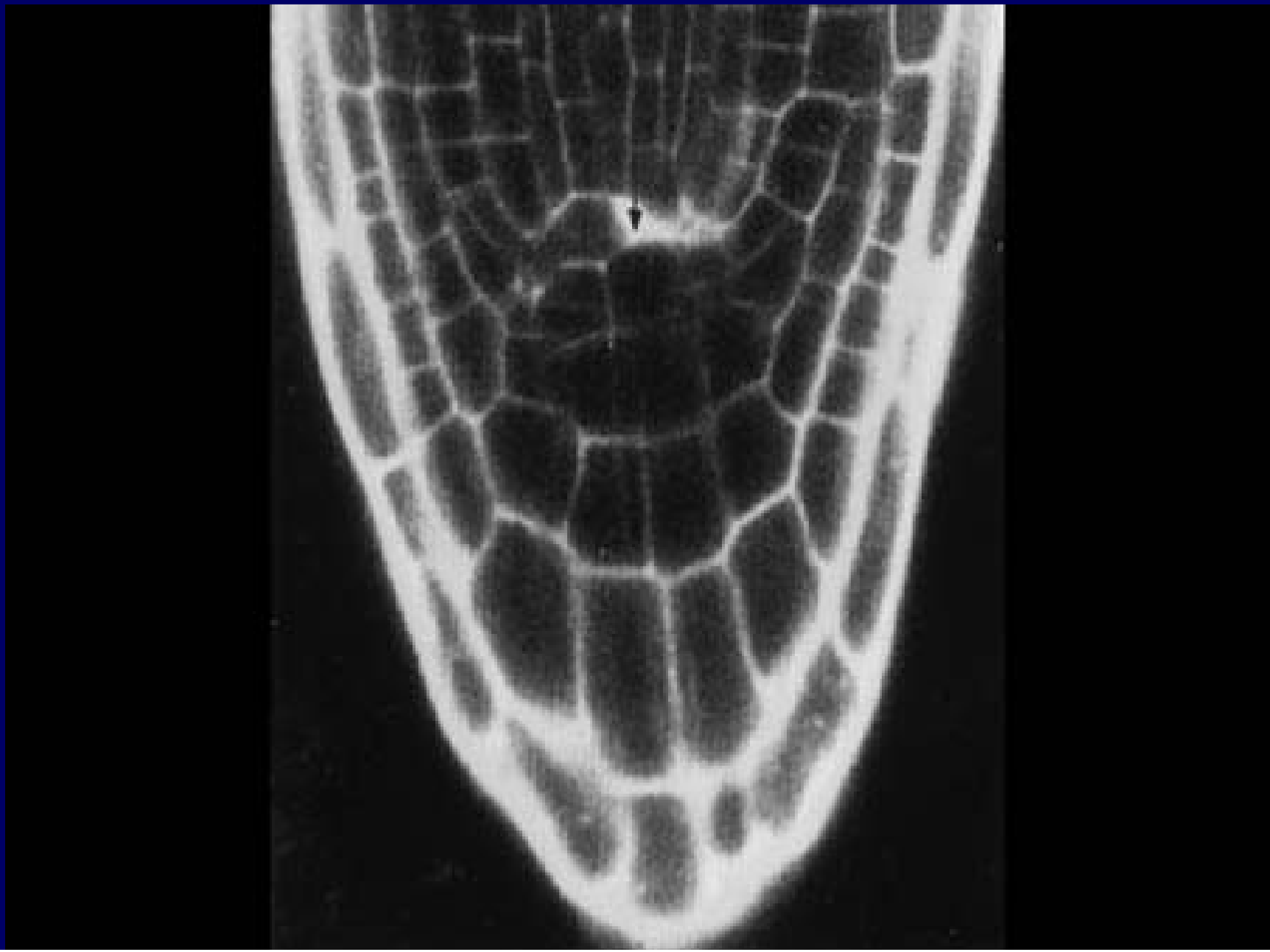


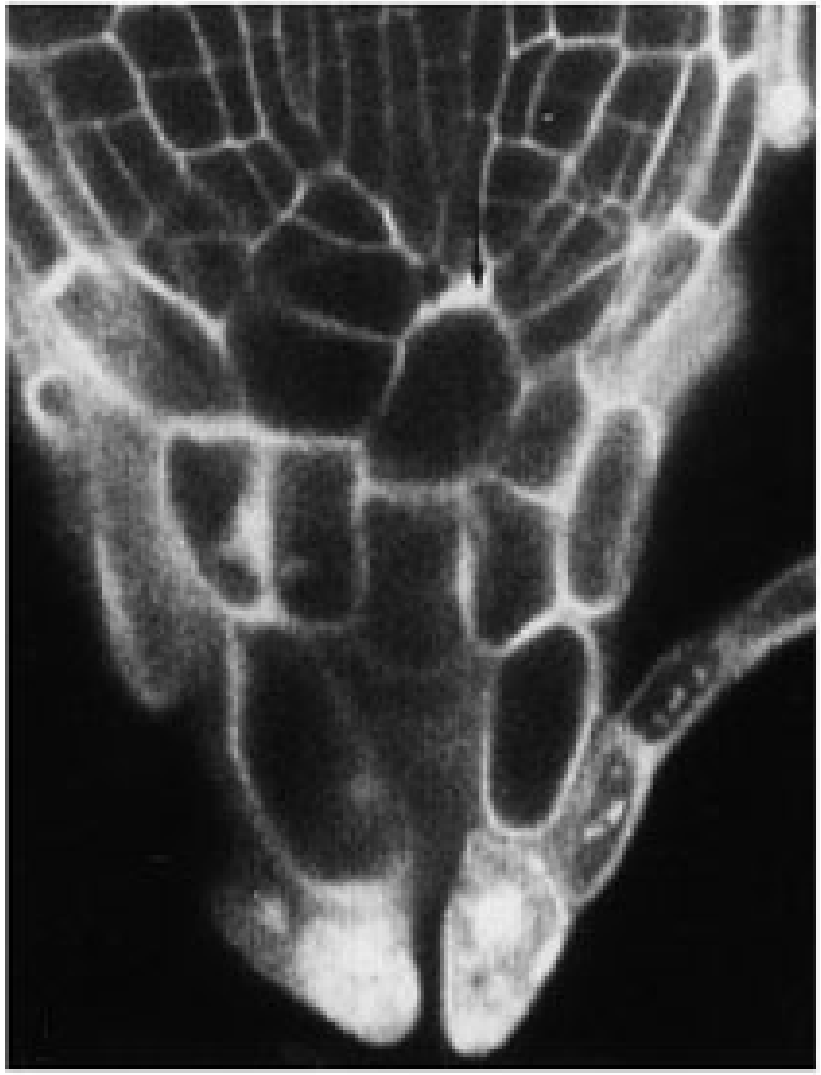
Confocal microscopy

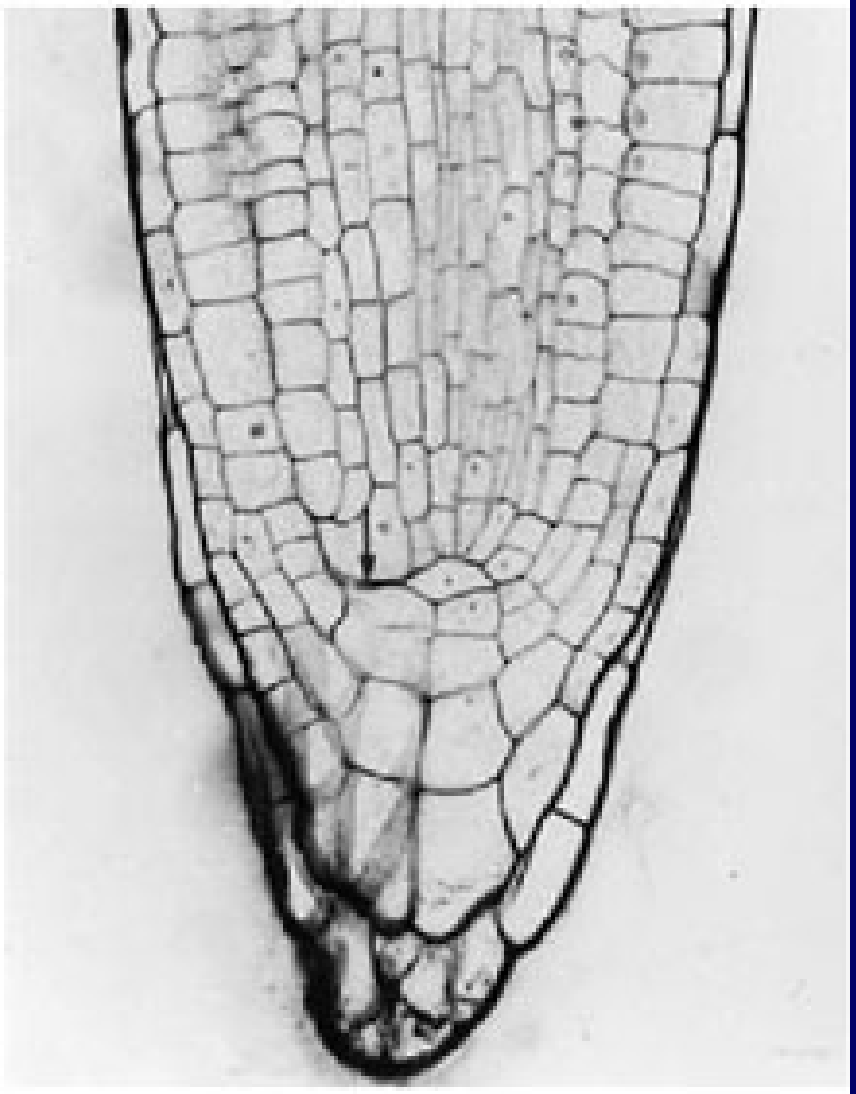



(a)
(b)
Current Biology
Xylem and phloem
Pericycle
Cortex initial
Cortex; endodermis
NH and RH epidermis
Lateral root cap
Quiescent center
Columella root cap

Laser Ablation of Single QC Cell







Laser Ablation of Quiescent Centre

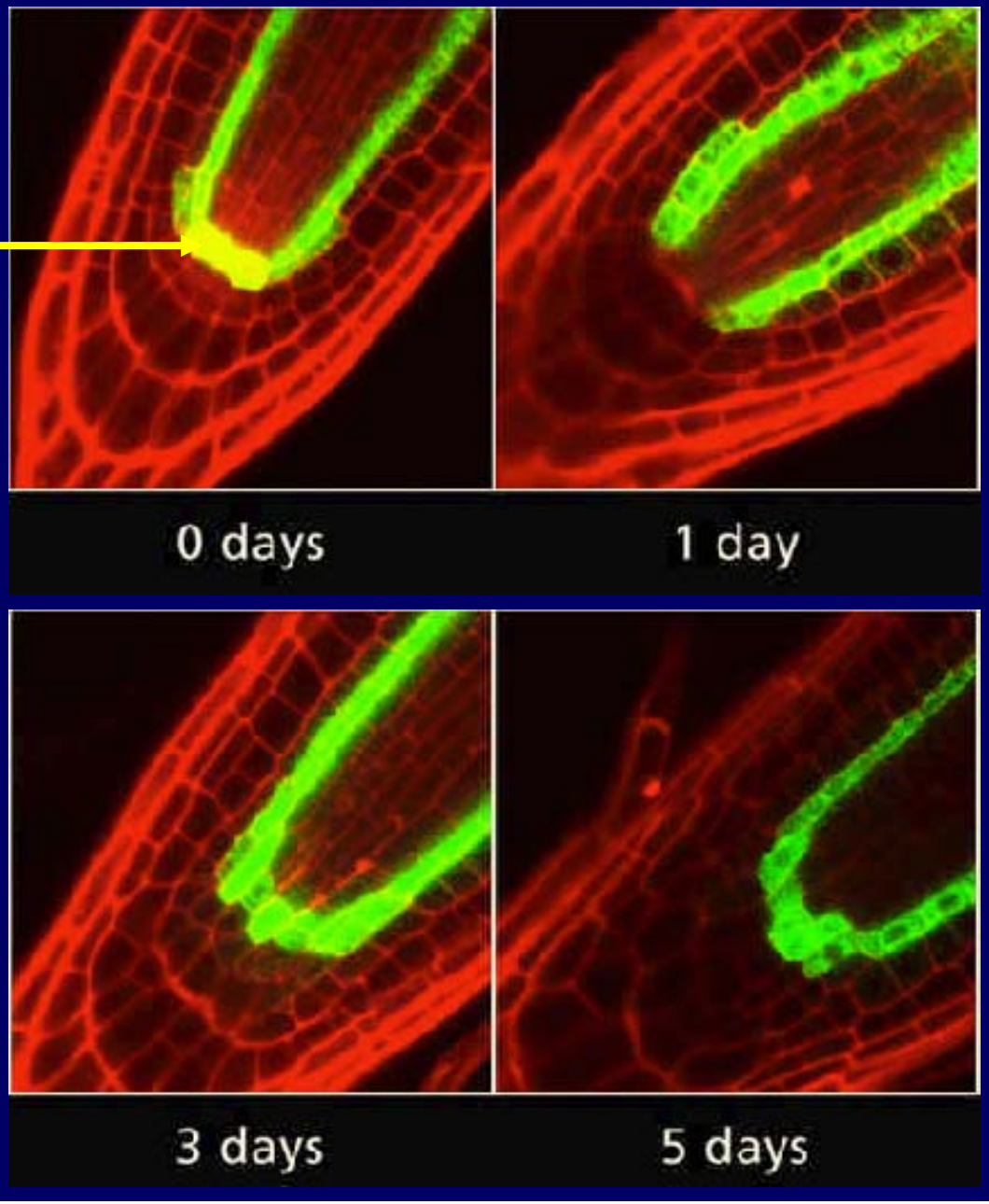
GFP

Propidium-Iodid

Regeneration

Regeneration of Quiescent Centre

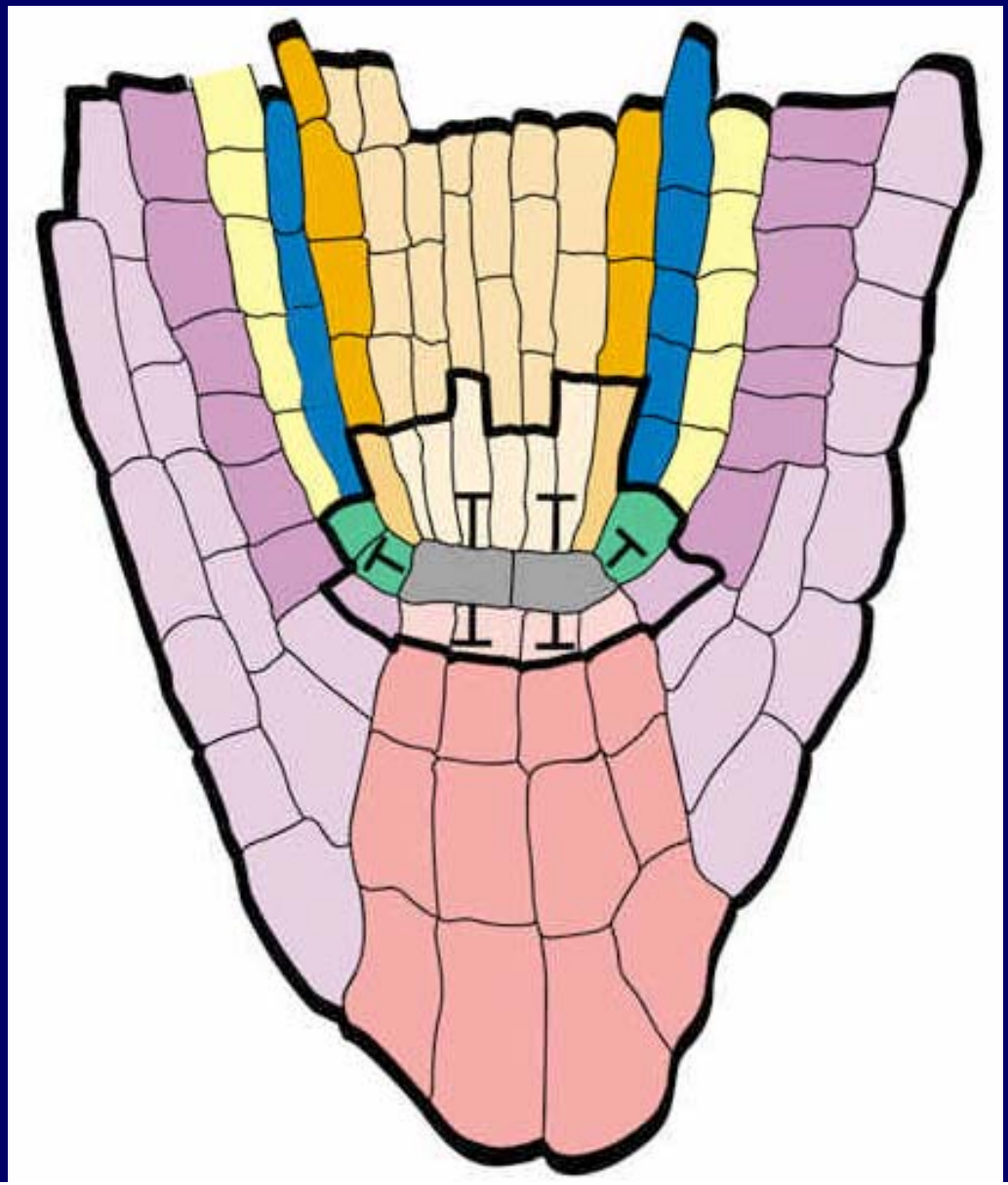
QC

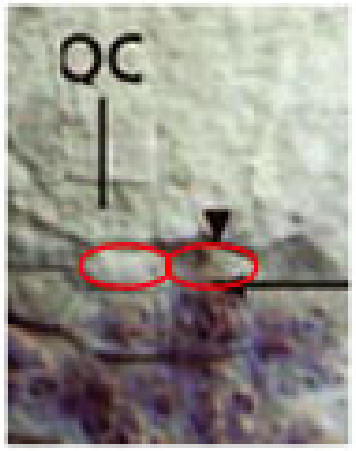


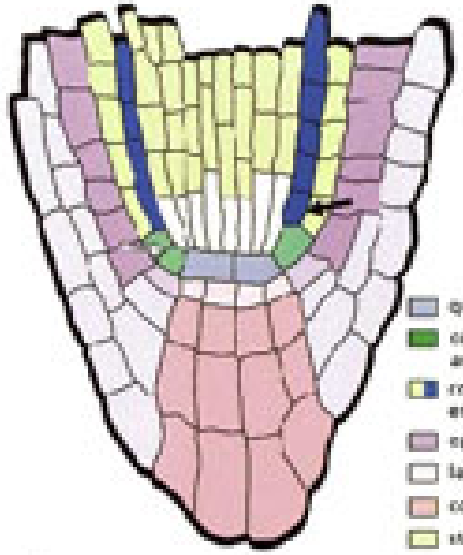
SCR::GFP

(Endodermis + QC)

Model for Role of QC in Keeping Stem Cells

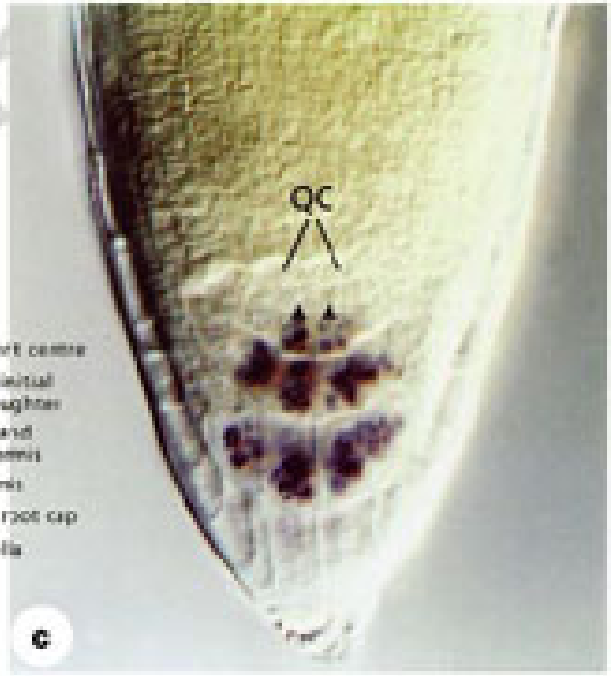






quiescent centre
cortex initial and daughter
cortex and endodermis
epidermis
lateral root cap
columella
stele

a



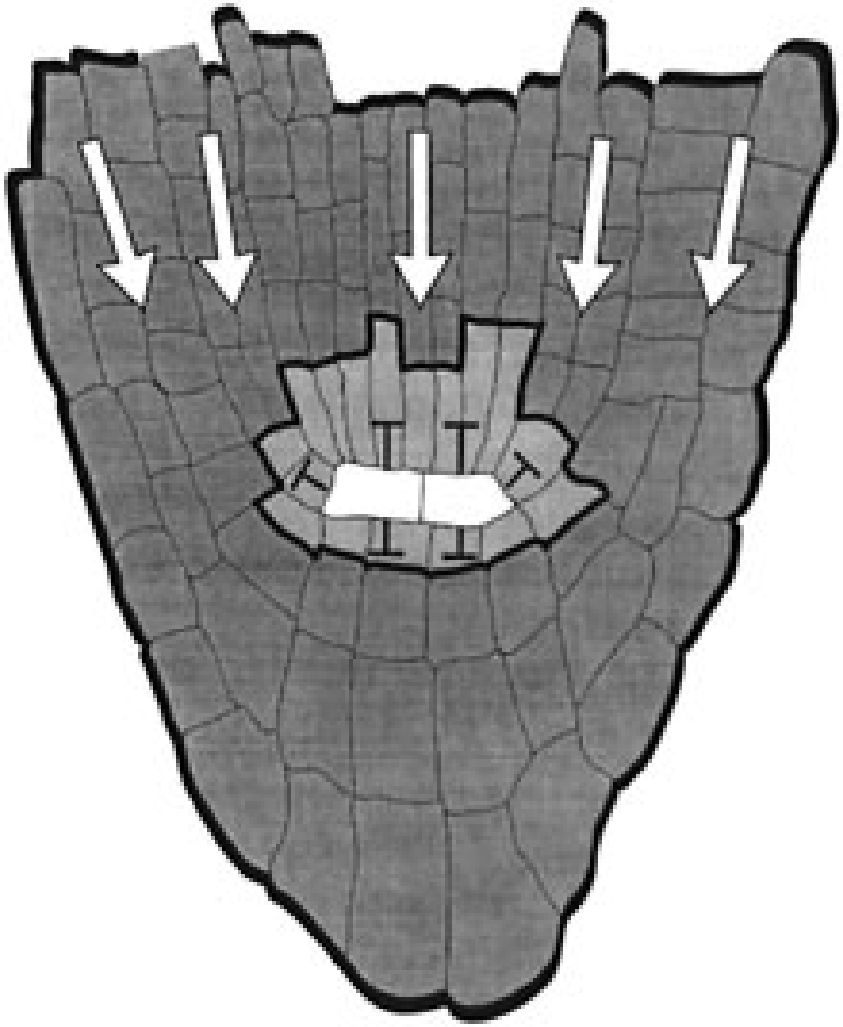


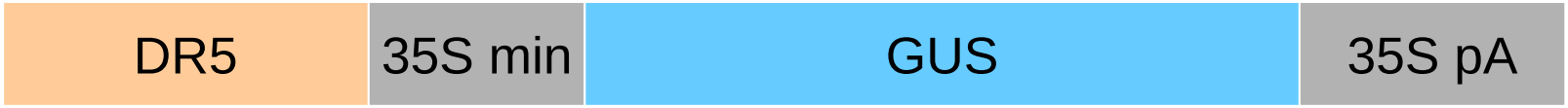
Fig. 4. A simple model representing two different regulatory signals within the root meristem. The quiescent centre inhibits differentiation of surrounding initials, whereas positional cues direct differentiation into different cell types

Indirect Visualisation of Auxin

DR5::GUS (Auxin) Response Reporter

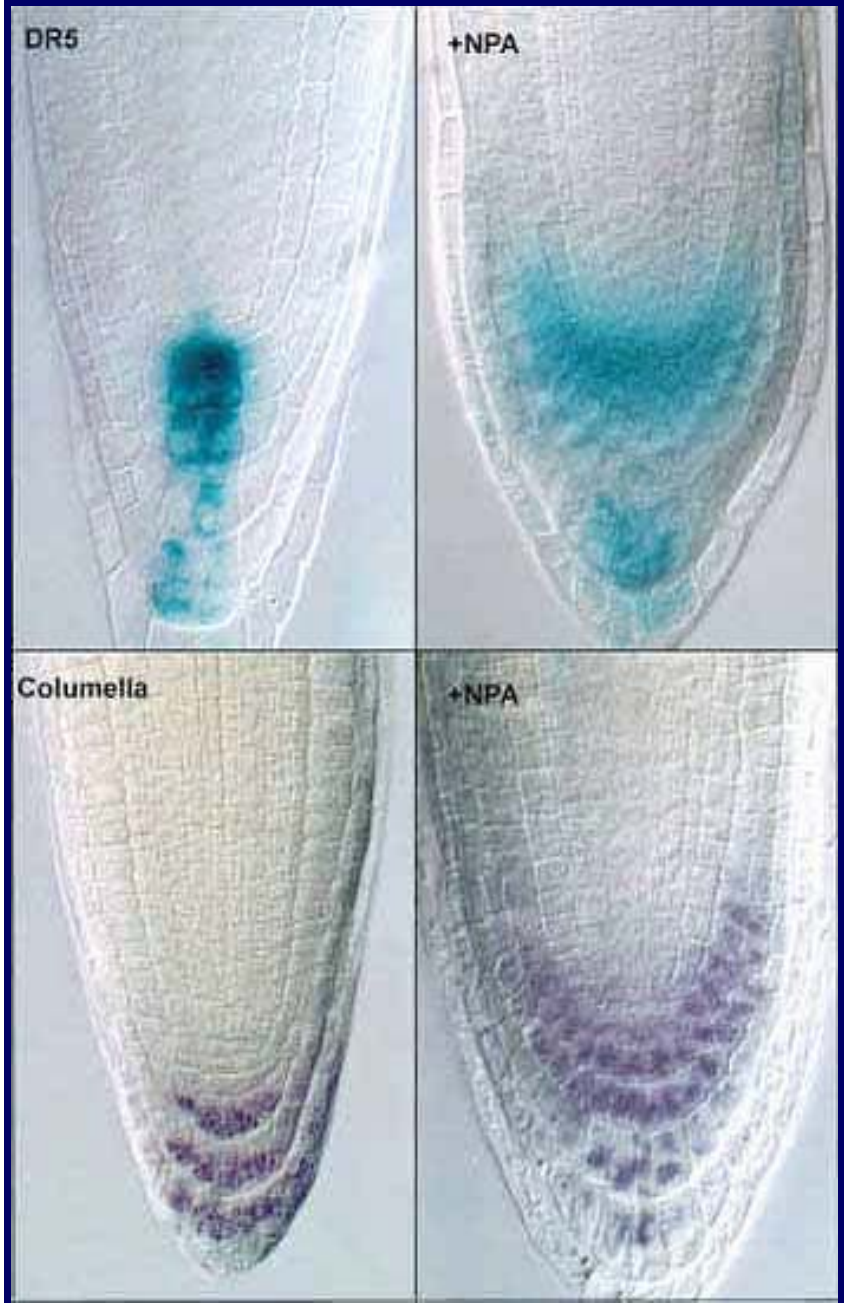
5´ CCTTT TGTCTC *3´*

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*)

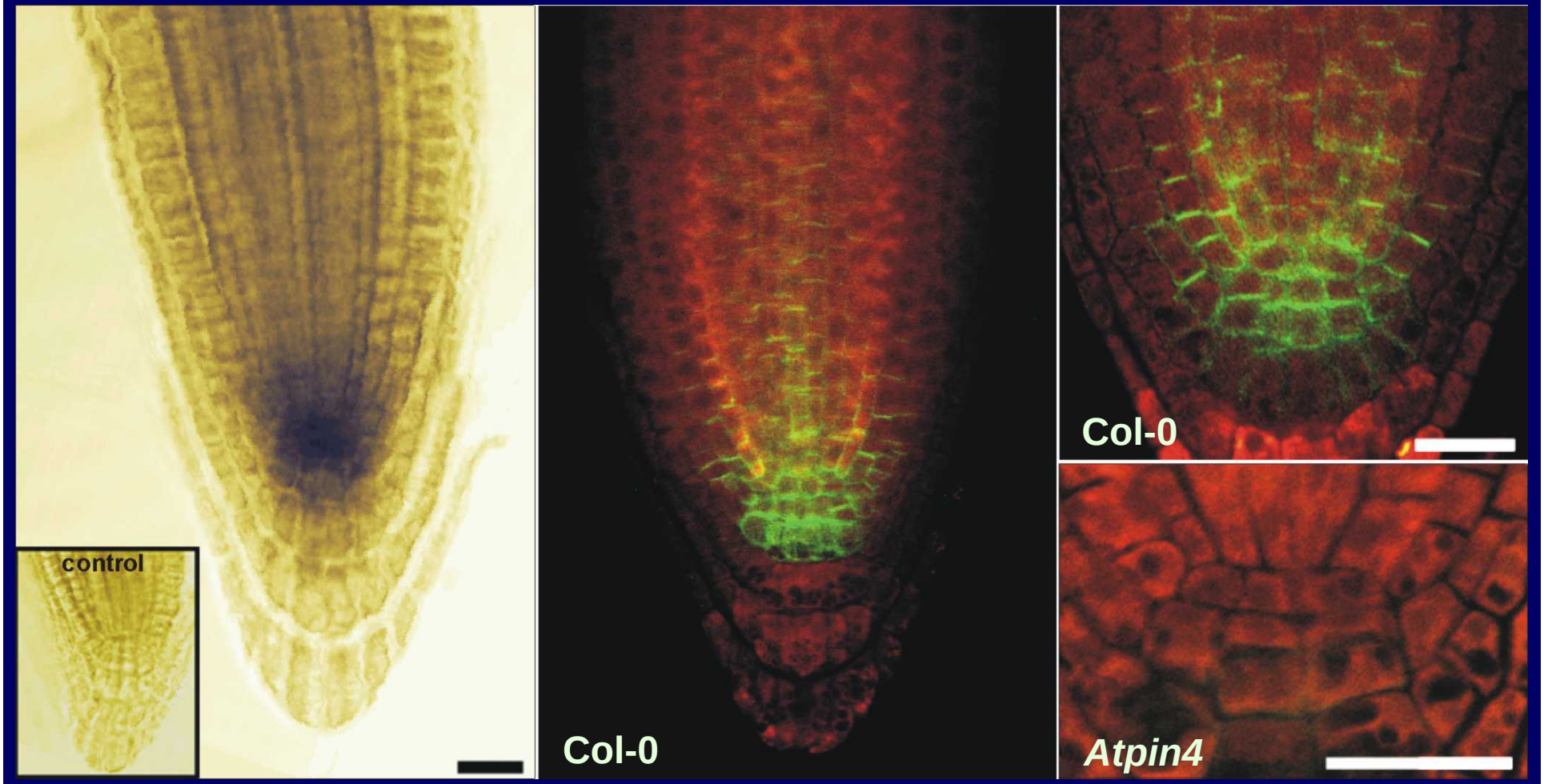
Auxin transport – *pin4*

AtPIN4 in Arabidopsis Root Tip

in situ RNA hybridisation

The AtPIN4 protein

The AtPIN4 protein



DR5 Auxin Response in Roots

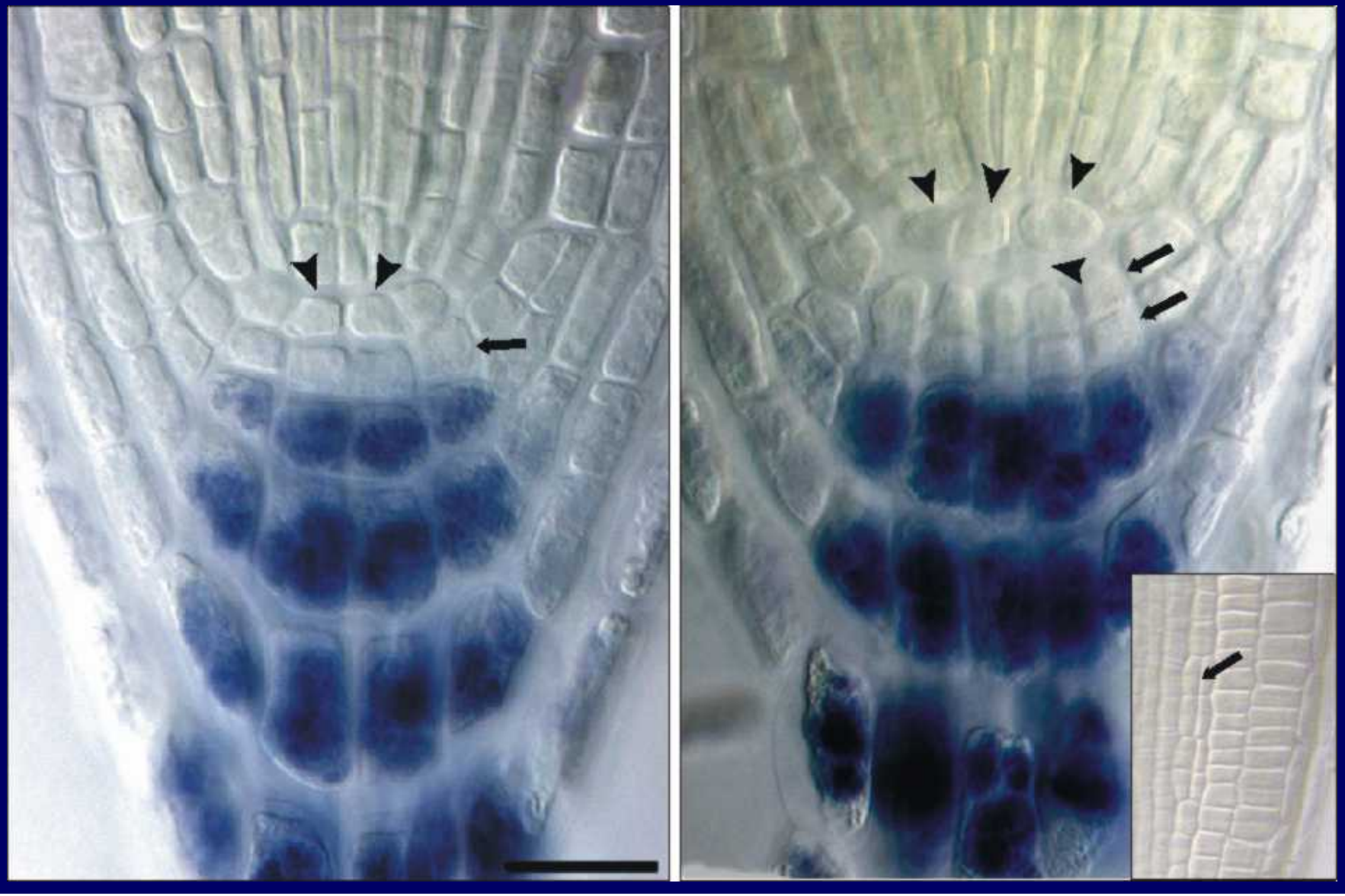
Col-0

Atpin4

Atpin4 Root Pattern (4 days)

Col-0

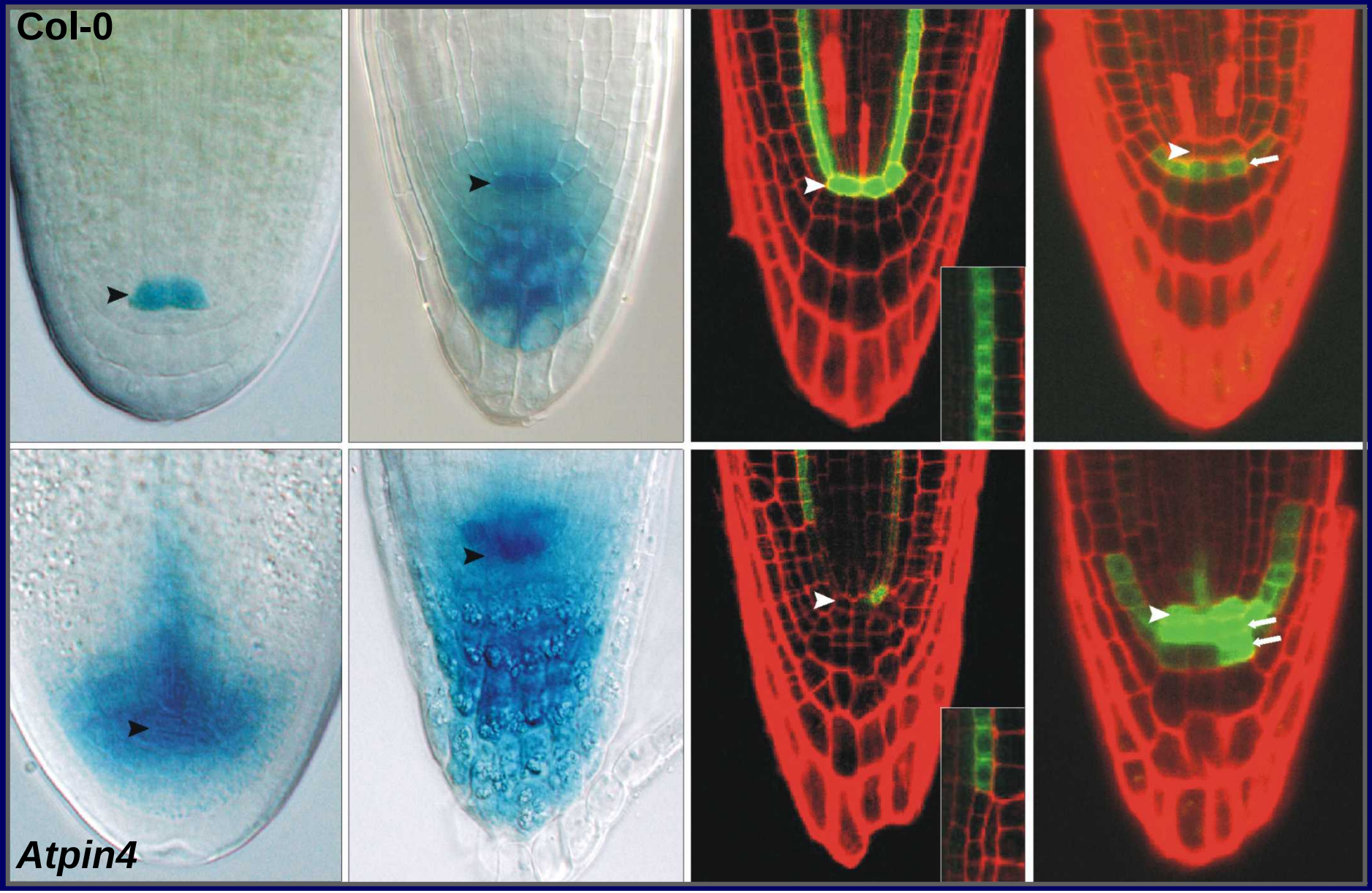
Atpin4



Atpin4 Root Pattern (10 days)

AtPIN4* antisense** ***Atpin4 ***Atpin4***

Changes in Cell Fates in *Atpin4* Mutant



Primary Root Meristem

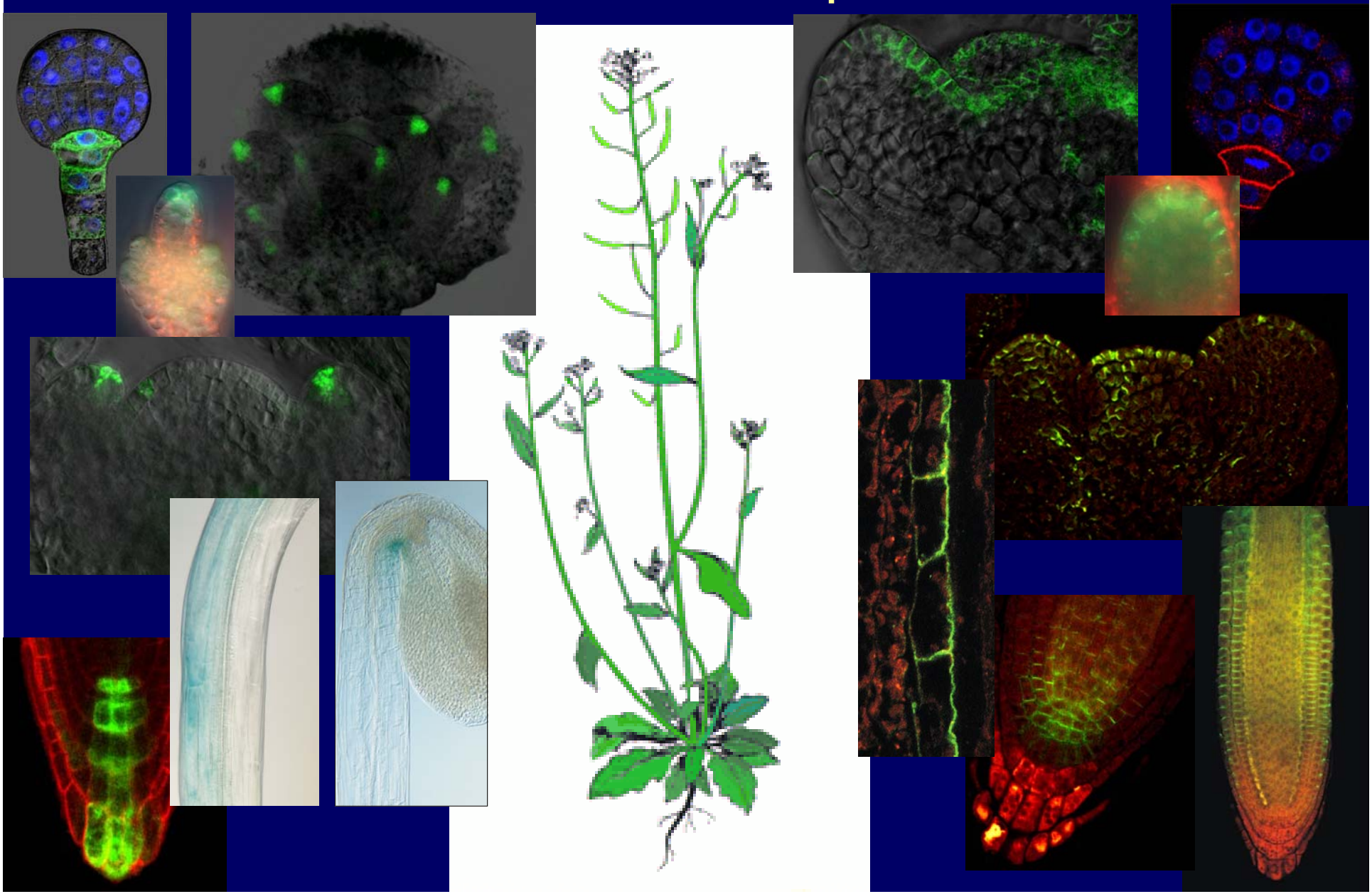
- Simple, highly invariant cell anatomy.

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

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

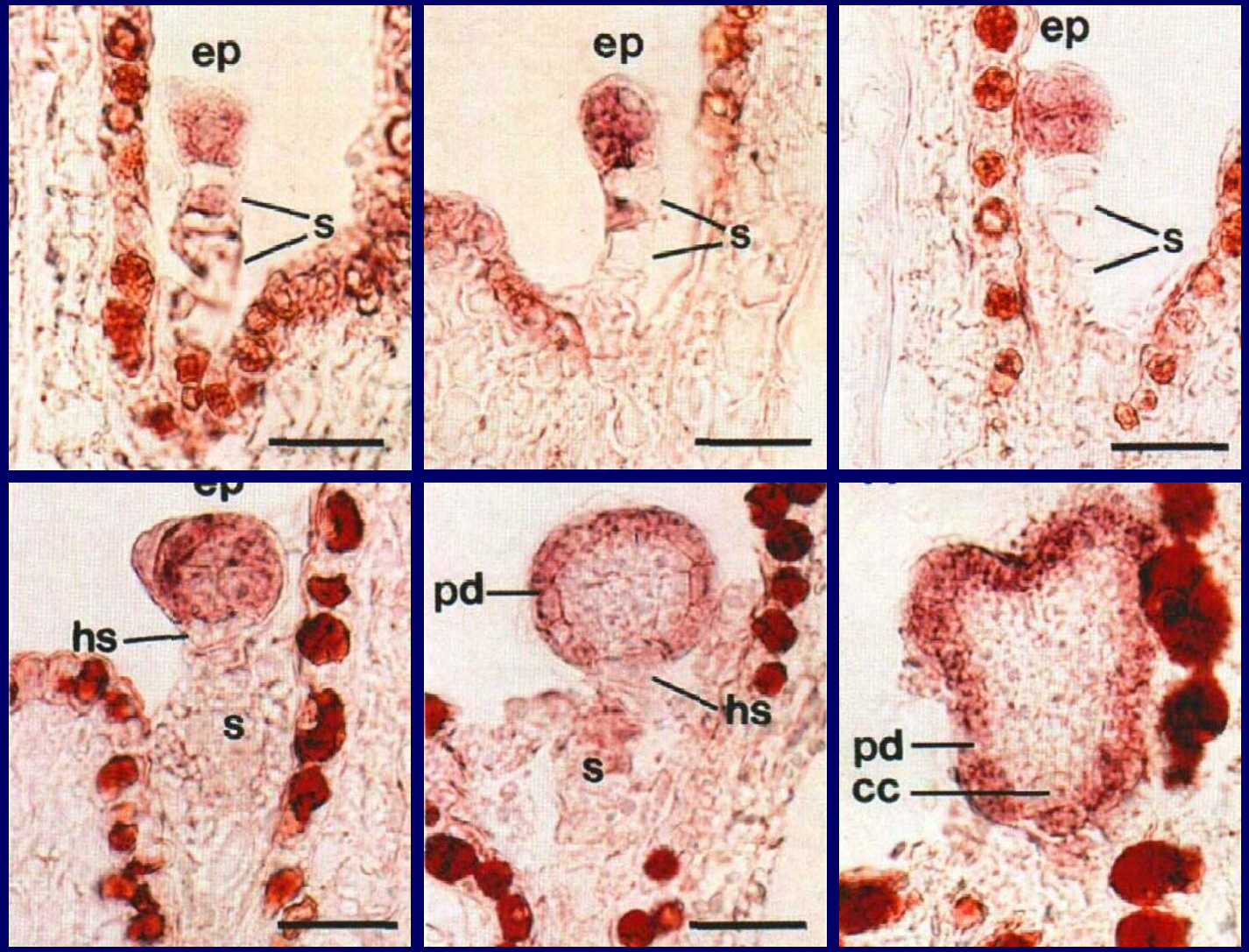
-The auxin gradients instructive for meristem patterning are maintained by polar auxin transport system.

PIN-dependent Auxin Gradients in Plant Development



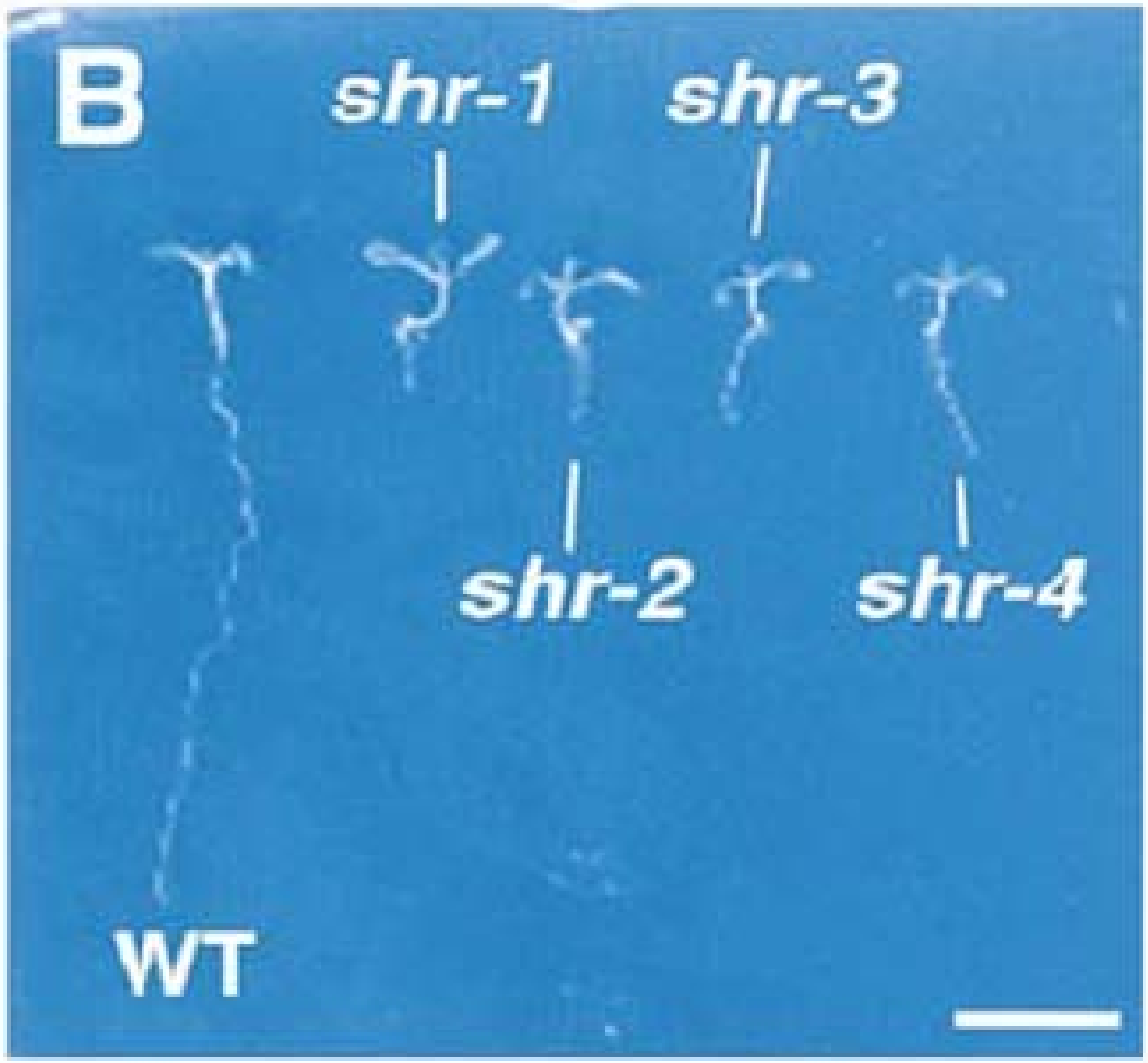
Radial Patterning of *Arabidopsis* Root

Example for radial patterning – the AtML1 gene

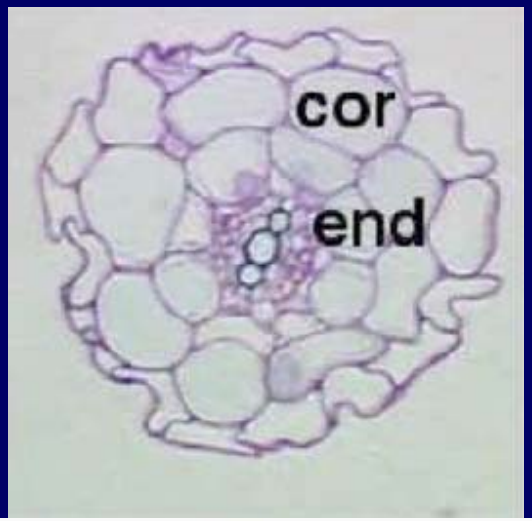


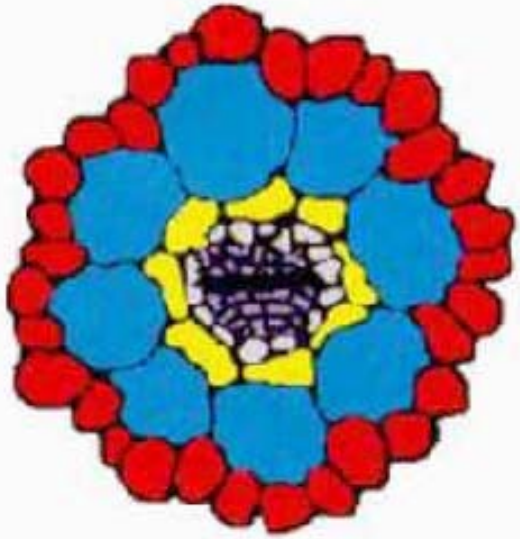
Lu P et al. Plant Cell, 1996

Short-root mutant alleles

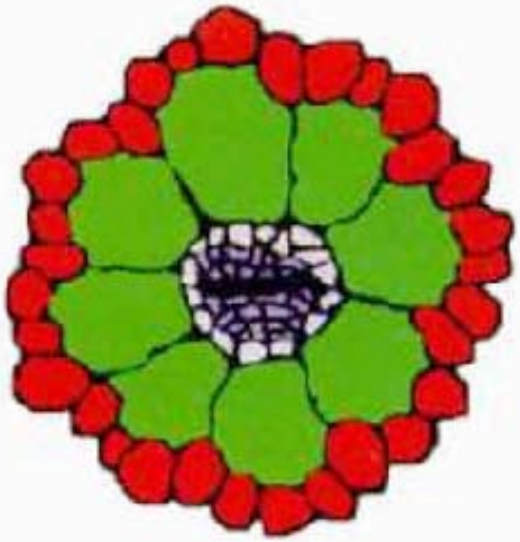


Radial Mutants with Defects in Ground Tissue

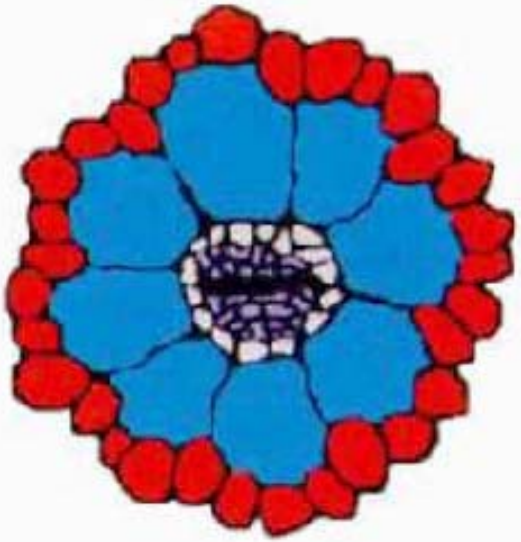




Wildtype



scarecrow



short root

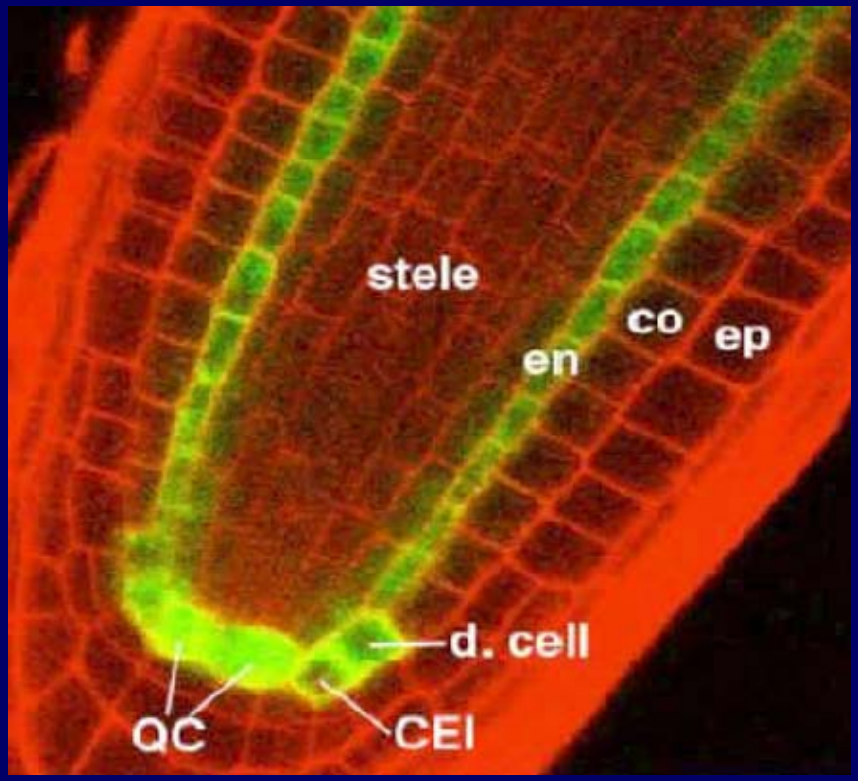
Cortex + Endodermis

Mixed (Cx+En)

Cortex

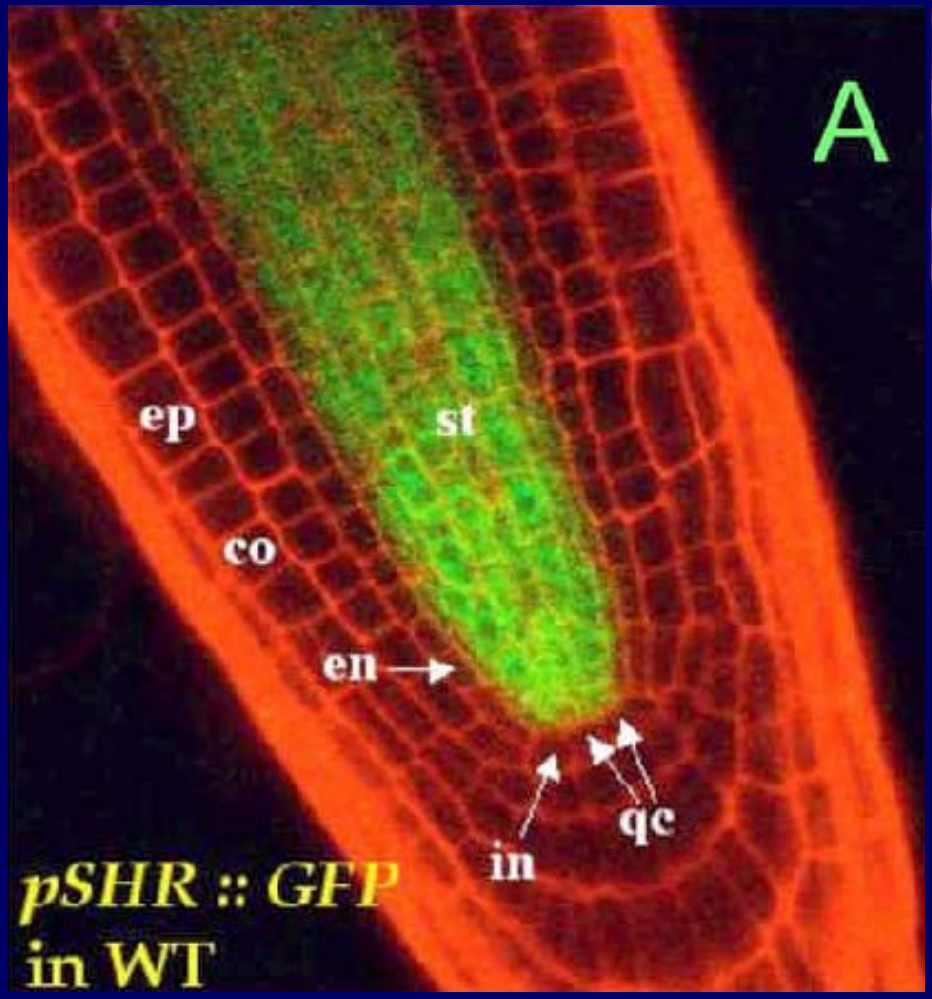
SCR Expression in Endodermis

mRNA

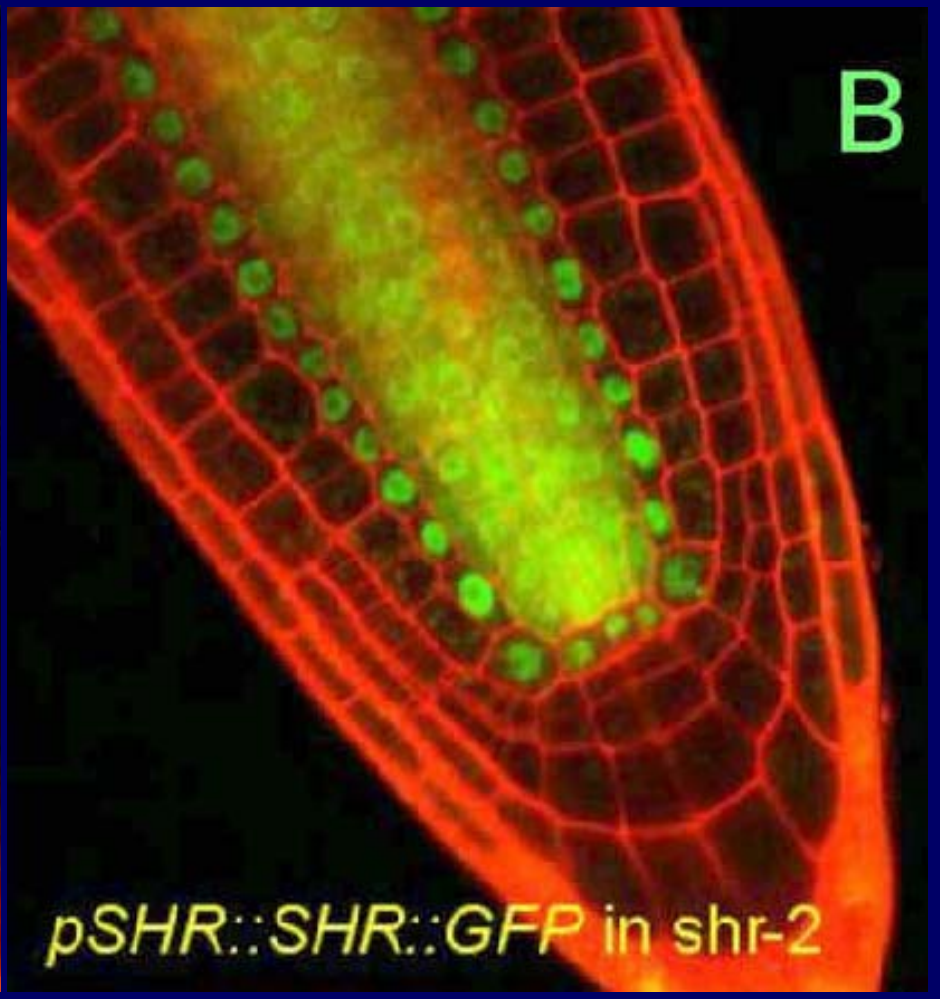


SCR::GFP

SHR Expression + Proteintransport

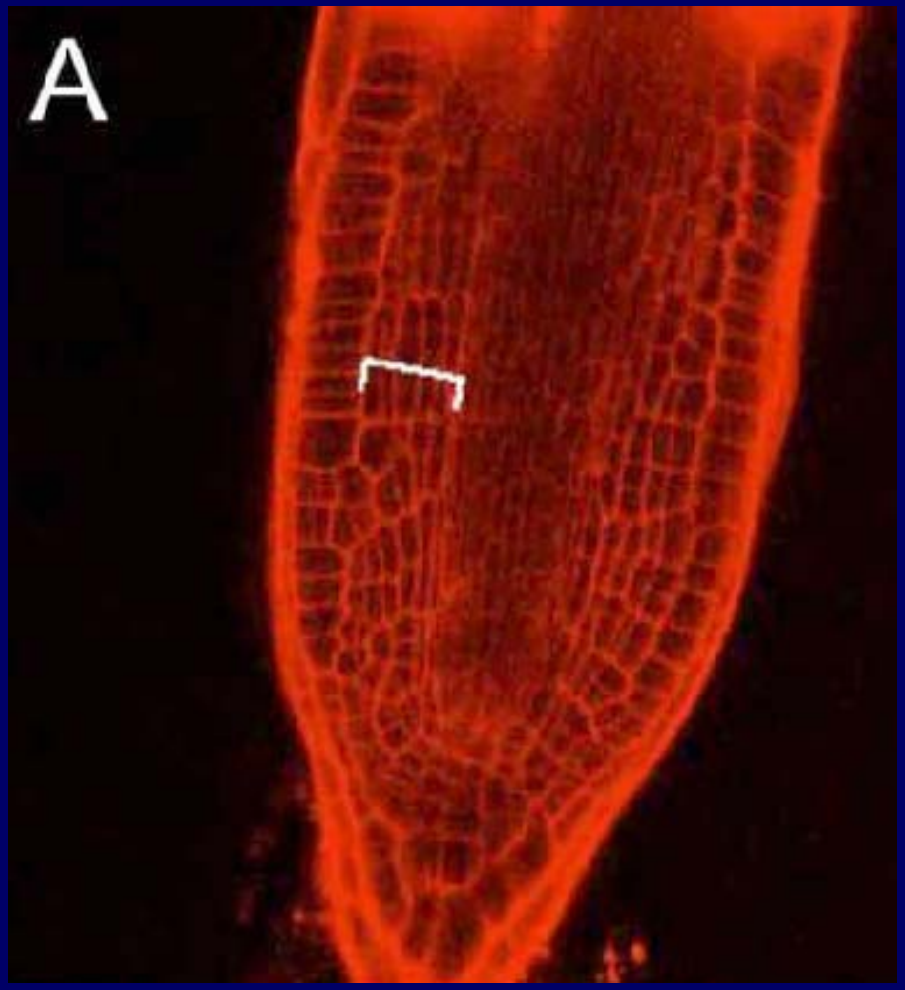


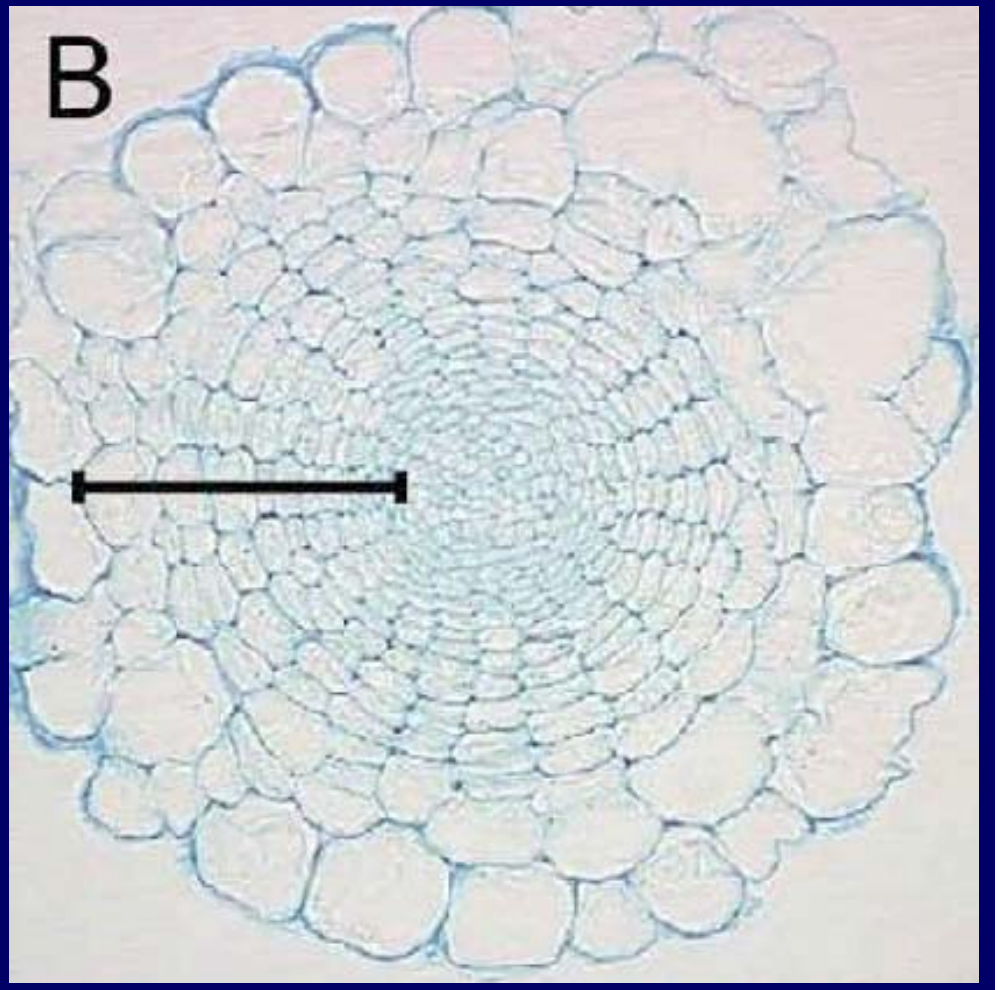
Genexpression in stele



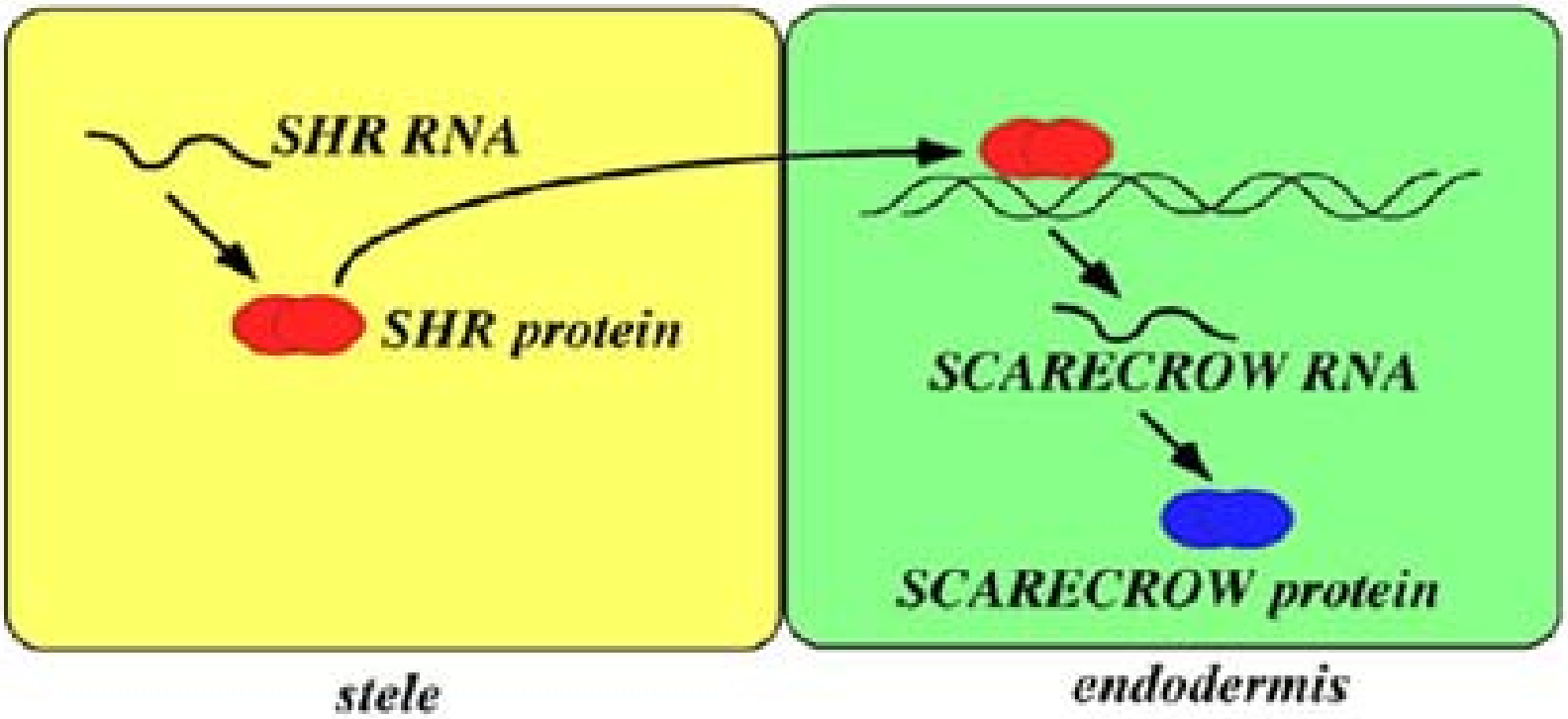
Protein in Endodermis + QC

SCR::SHR Expression: More Ground Tissue

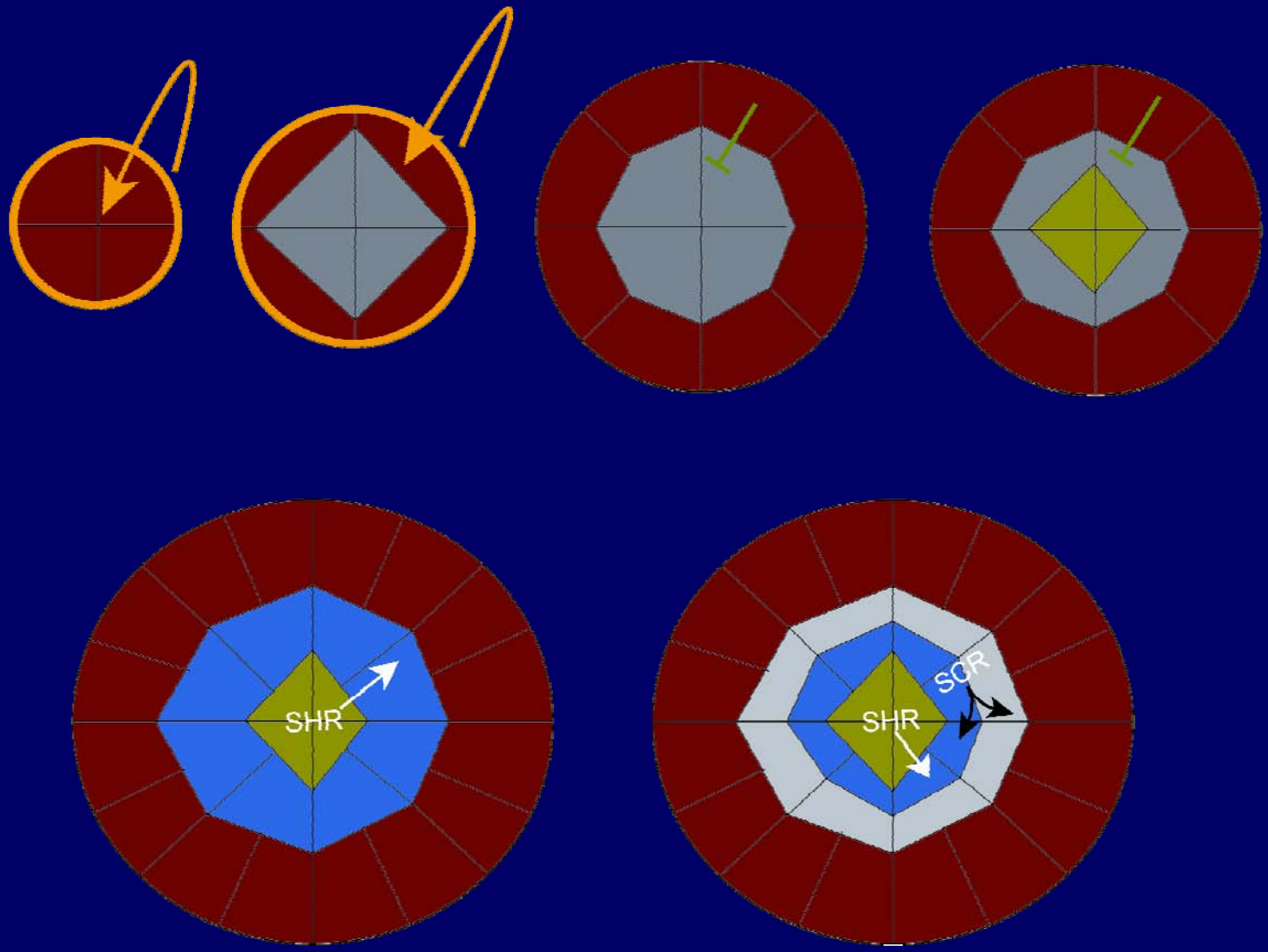




Model of SHR and SCR Action



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.