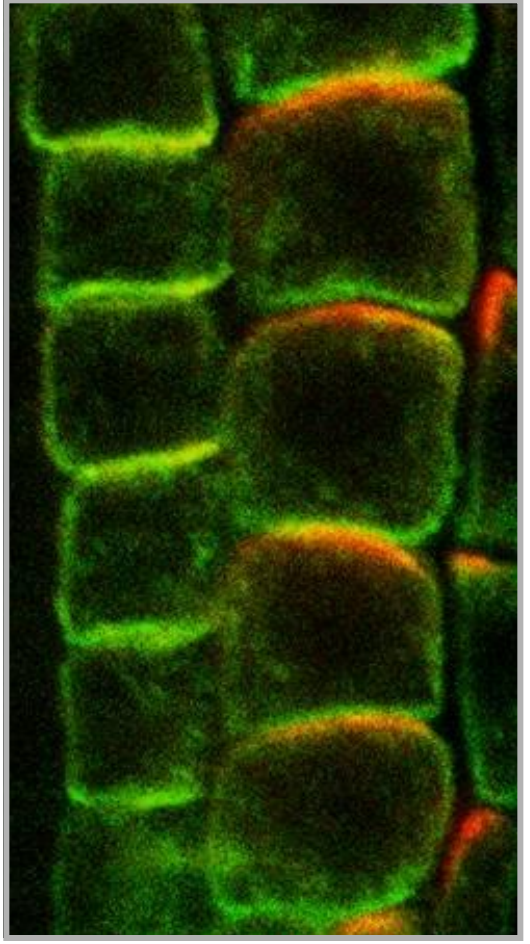
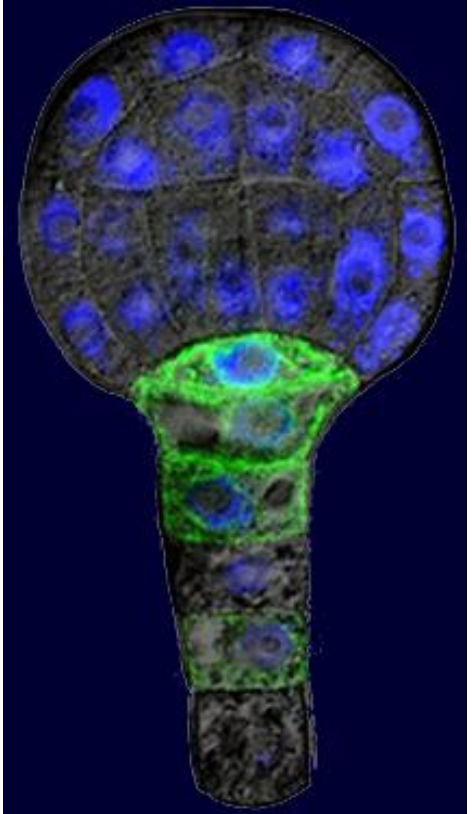
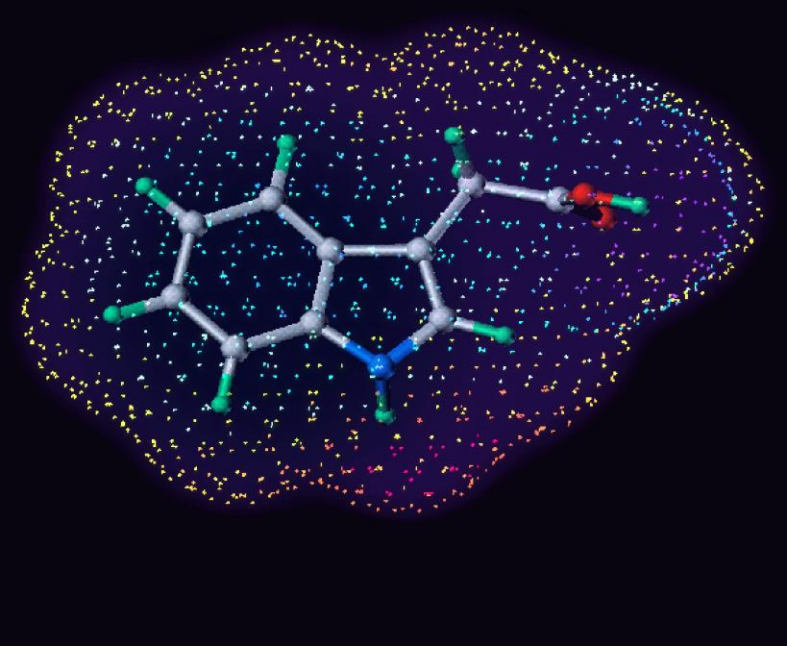


Patterning in Plant Development

Role of Auxin



Plants
and
Animals
Live
Different
Lives



Arabidopsis Embryogenesis

One-cell stage

Two-cell stage

Octant stage

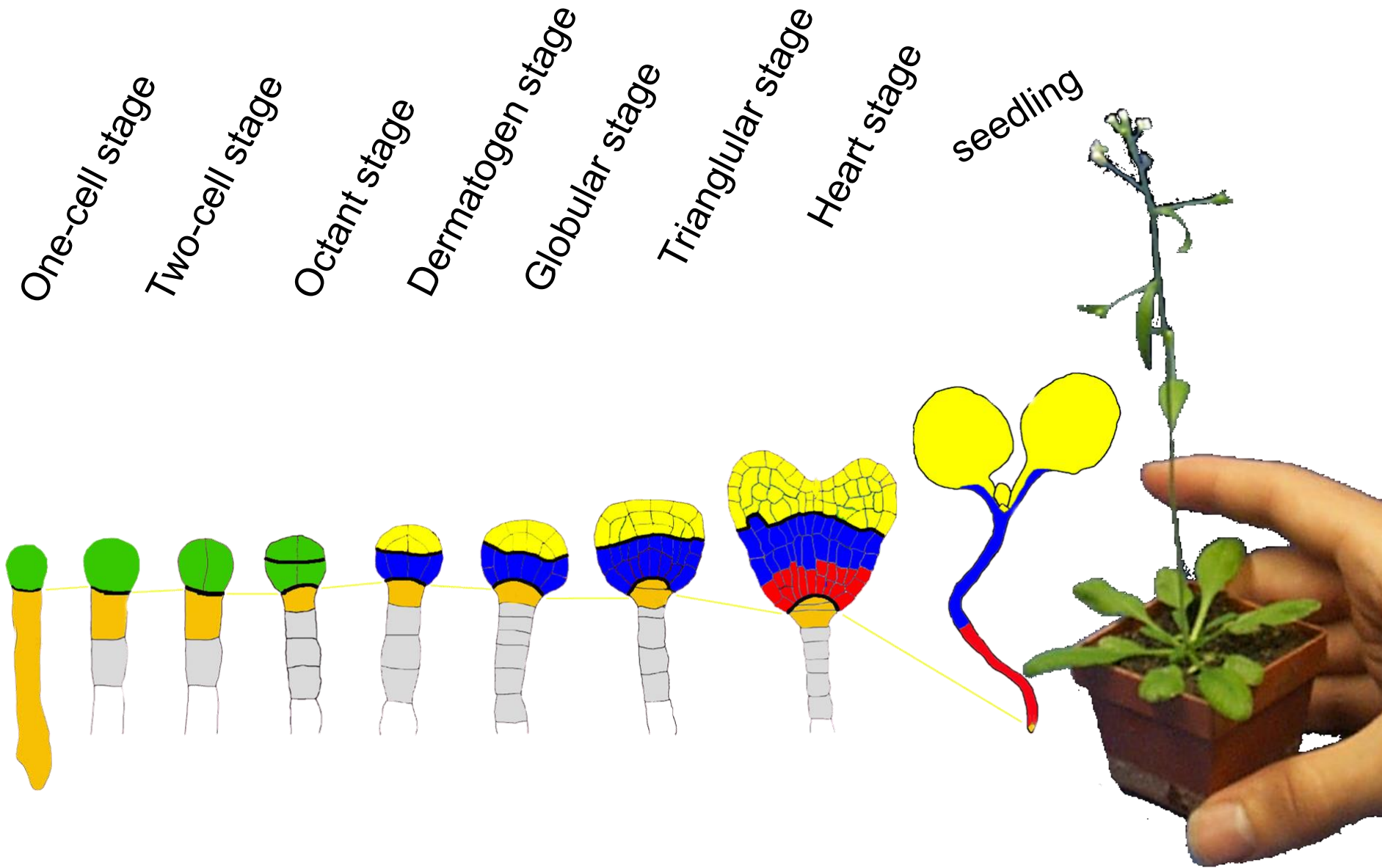
Dermatogen stage

Globular stage

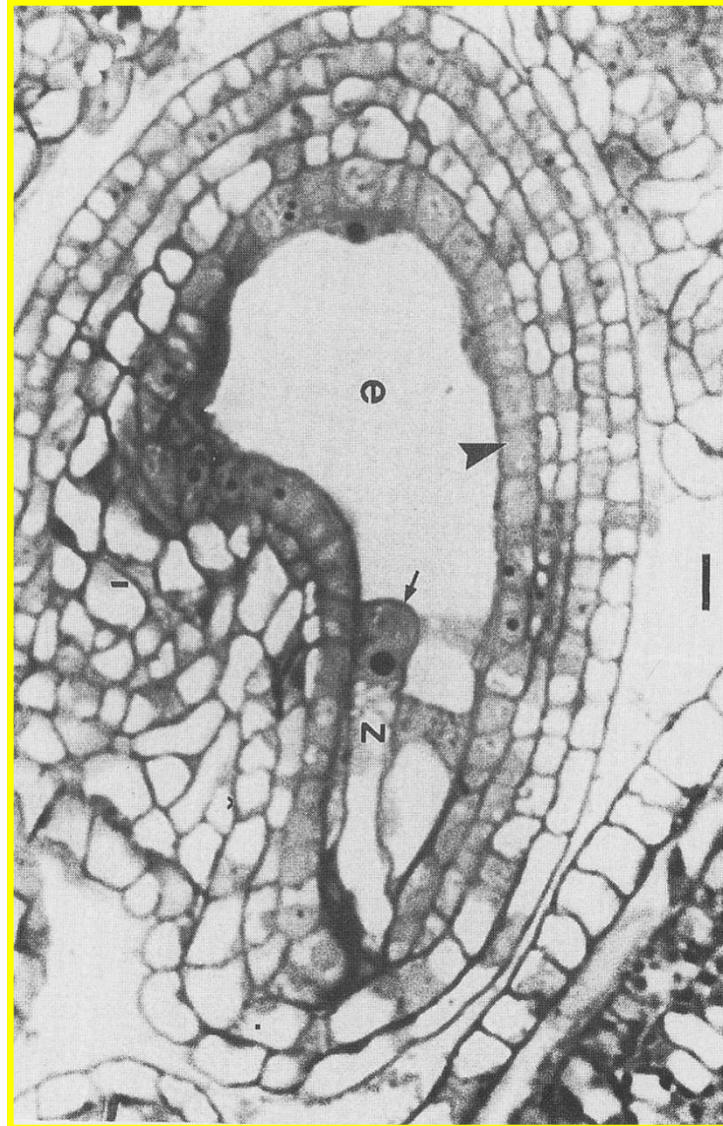
Triangular stage

Heart stage

seedling



How can such a protected system be investigated experimentally?



Genetic Interference with Auxin Response and Transport Disrupts Embryo Patterning



monopteros

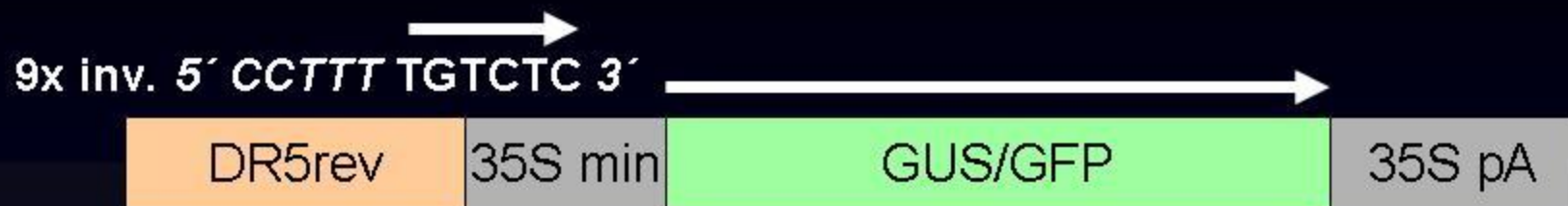


bodenlos



gnom

DR5 Auxin Response Reporter



Root

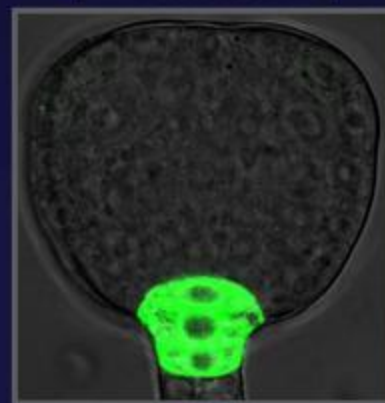
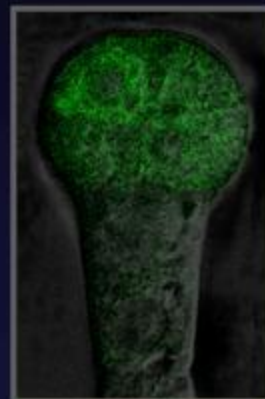
Embryos



DR5



anti-IAA



DR5

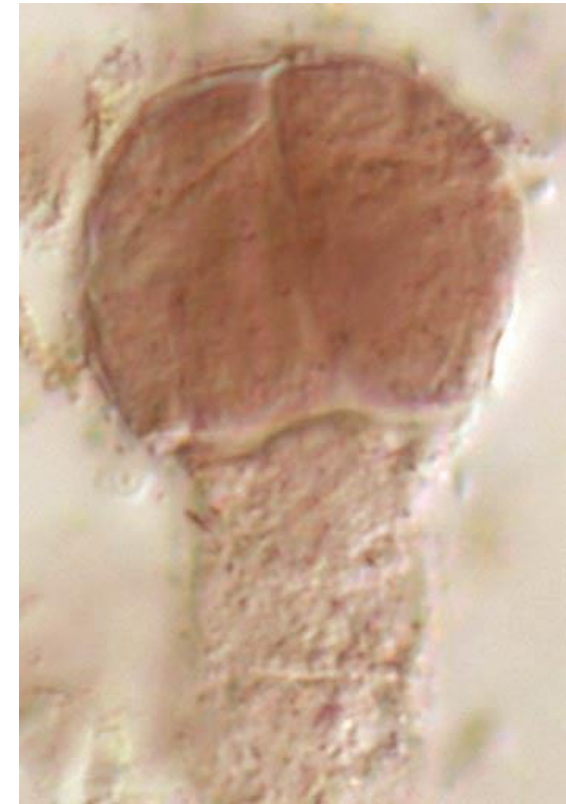
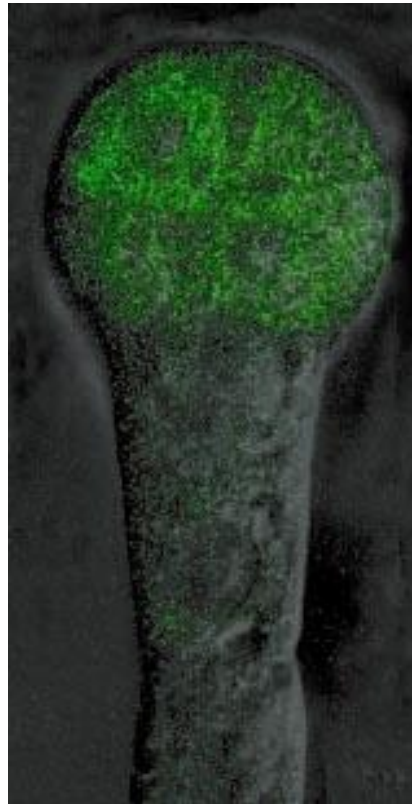


anti-IAA

Auxin in Early Embryogenesis

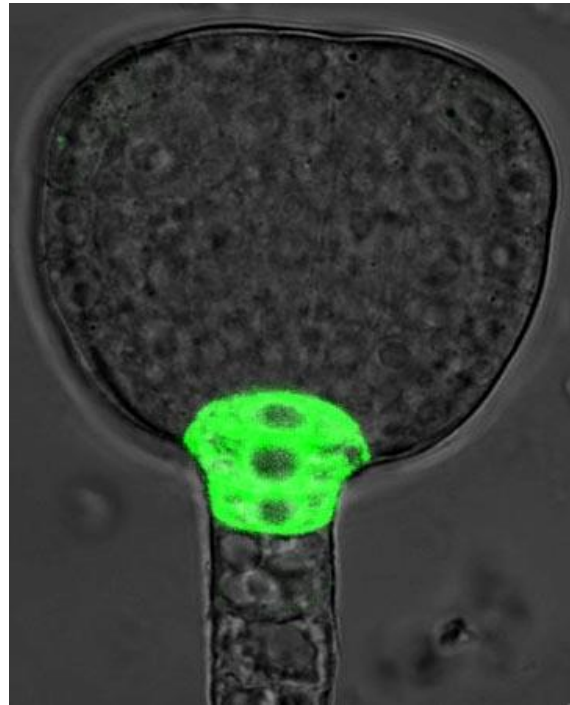
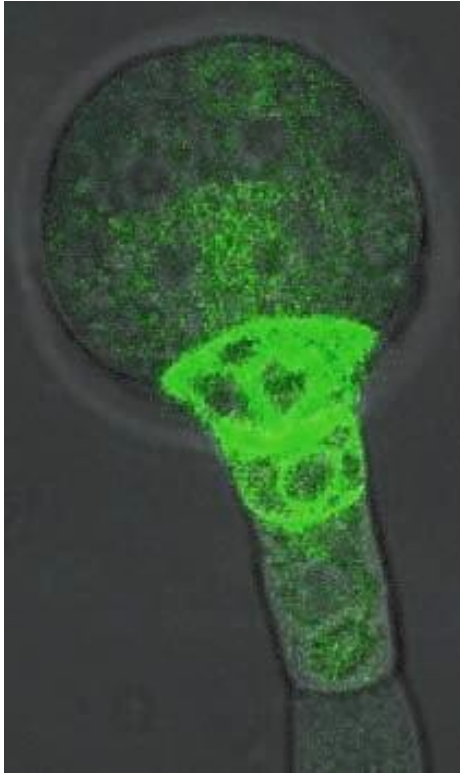
DR5::GFP

**IAA
localisation**



Auxin in Embryogenesis

DR5::GFP

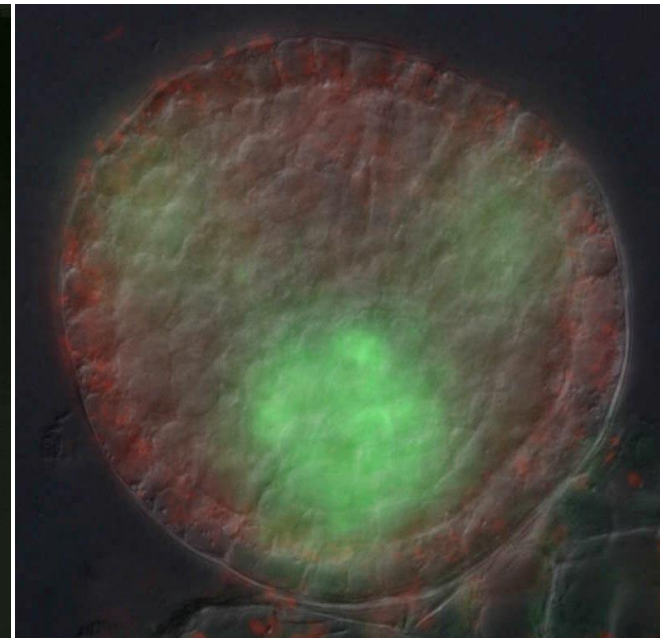
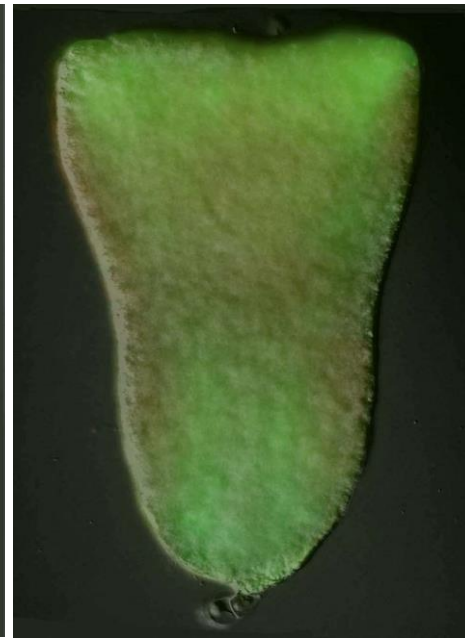


IAA localisation



DR5::GFP – *in vitro* Culturing

Long time treatments



Control

NAA

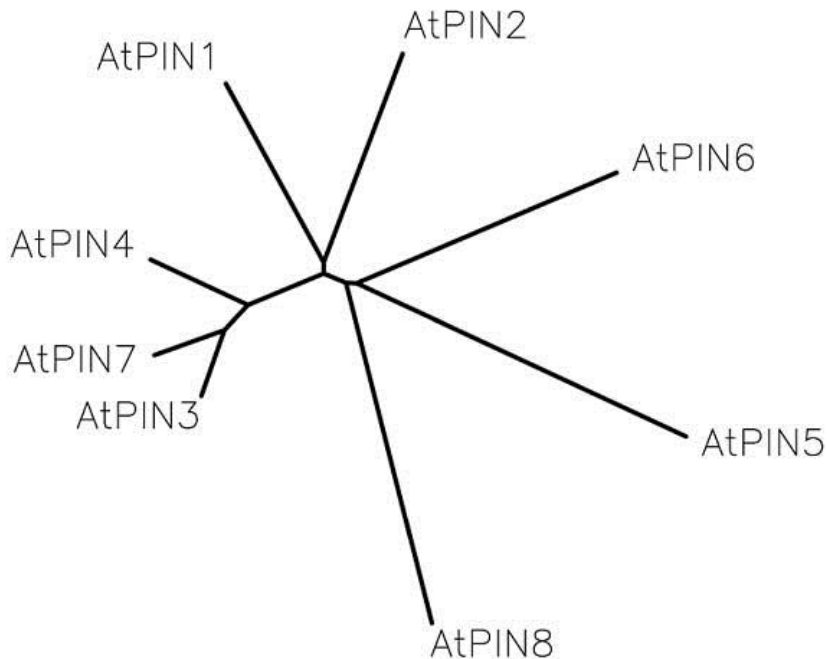
2,4D

NPA or BFA

Arabidopsis PIN Protein Family

Phylogenetic tree

Homology of PIN proteins



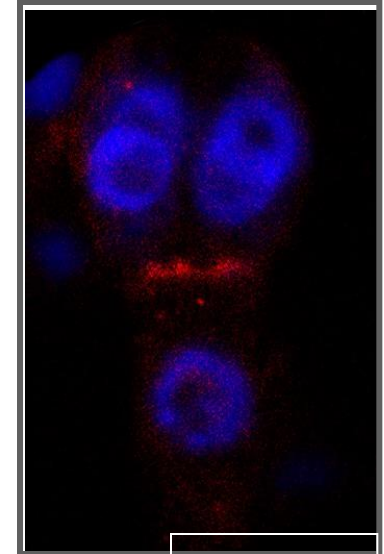
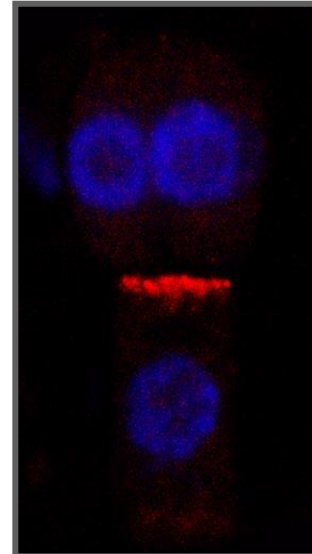
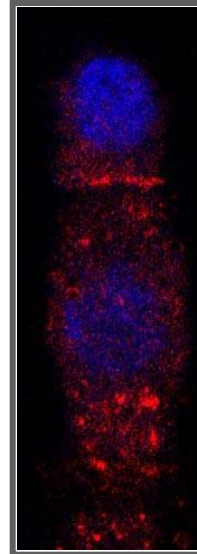
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AtPIN3 1 MESHDLTYLVVPLVYVAMILAYGSKVHWKLFDDQCSGIRRFVAVFVLLSFFHFAANHYAMHLEEARAGSQVYVVESEFF...WAFNSFSS LEMMIFLGLSTLPTLTV
AtPIN4 1 KTEHHELVYVYVPLVYVAMILAYGSKVHWKLFDDQCSGIRRFVAVFVLLSFFHFAANHYAMHLEEARAGSQVYVVESEFF...KALNTNSES LEMMIFLGLSTLPTLTV
AtPIN5 1 MENCQGVKIEAMVPLVYVAMILAYGSKVHWKLFDDQCSGIRRFVAVFVLLSFFHFAANHYAMHLEEARAGSQVYVVESEFF...WAKYENKSGYV MDSISFLCITNLSIV
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AtPIN1 229 RPNMLNKAISYEQSSRNPFPRGSPNHS DEFS...MMASG...GG...RNFNFGI...GE...AMFGKSTFPSPSHVEDGGPAKPTAAATAAGARFHQSGSGGGG
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AtPIN5 158 RPNMLNKAISYEQSSRNPFPRGSPNHS DEFS...MMASG...GG...RNFNFGI...GE...AMFGKSTFPSPSHVEDGGPAKPTAAATAAGARFHQSGSGGGG
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AtPIN5 192 ...
AtPIN6 313 ...LDVNGTPT...VMMK...RFAAGR...ITROSSPKMHWSSGGQRHARDNGSSEKKEISFRDALKAPATAASGG
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AtPIN4 407 LDDGEGEKEIK...ATAGENKMSNS...LEAARGGGGGNG...THMFTFEMERLILMHWKLSINPNTYSSEGLAWISVSKNIMKMTMSGSIISLDAGLGMAMFSLGLF
AtPIN5 192 ...VMSLVKATNPNCSYICILIAMFIRNHLKLEGLGEGSLEKMKSTAMENMGI
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PIN7 in Embryogenesis

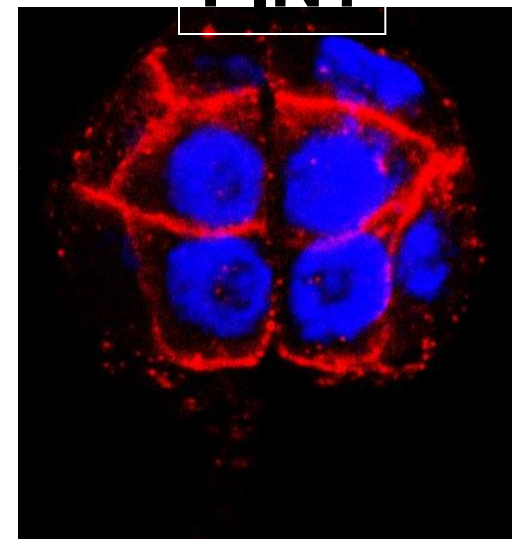
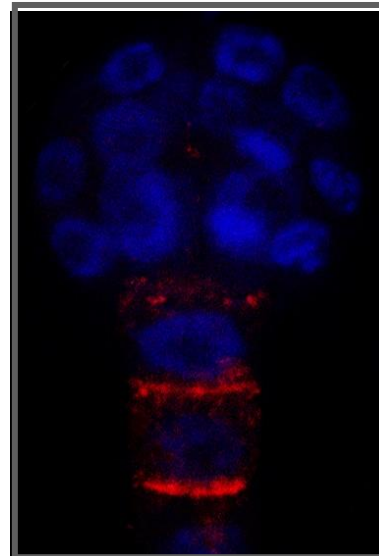
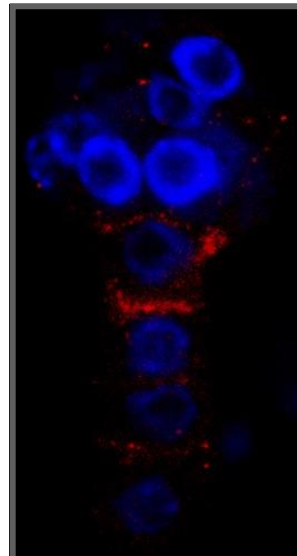
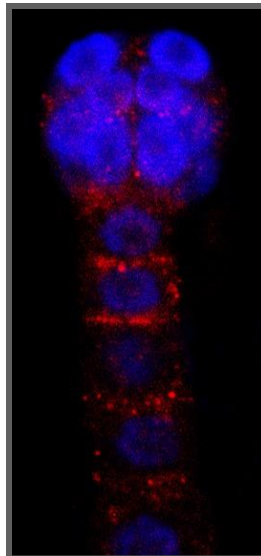
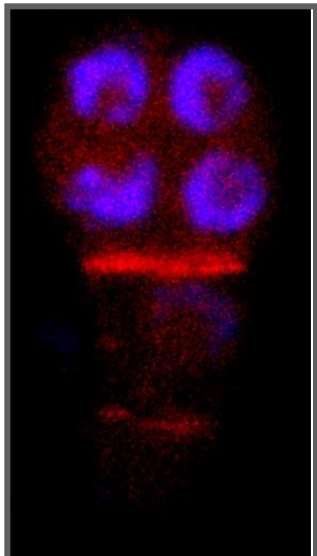
GUS

mRNA

Protein

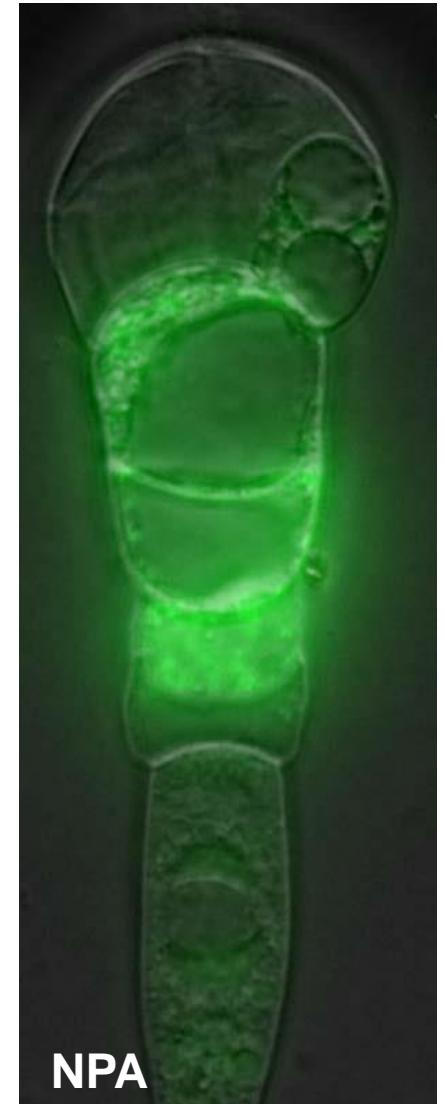
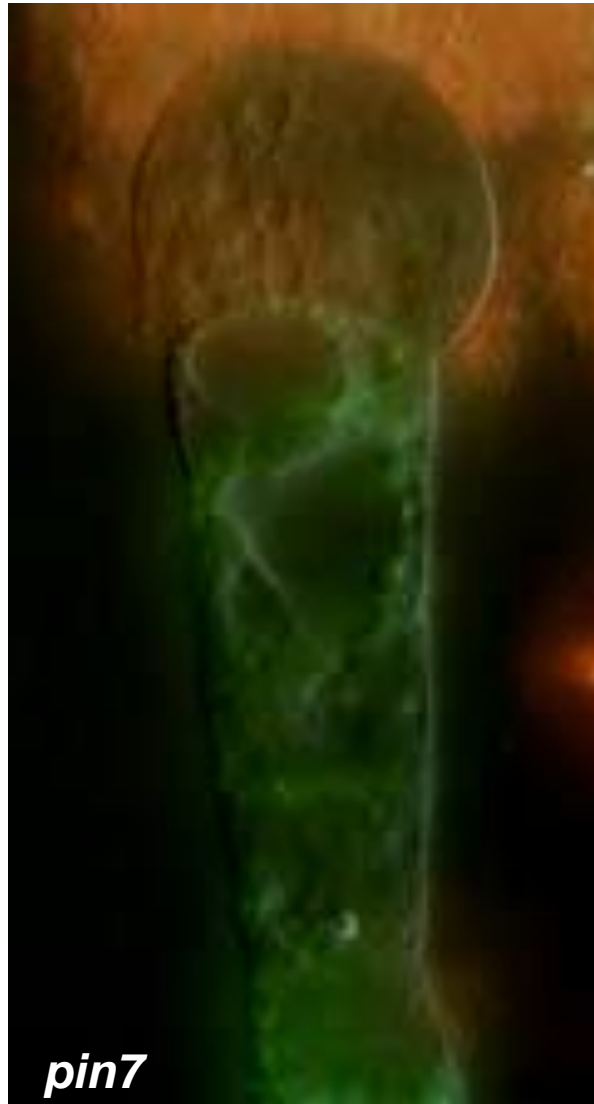
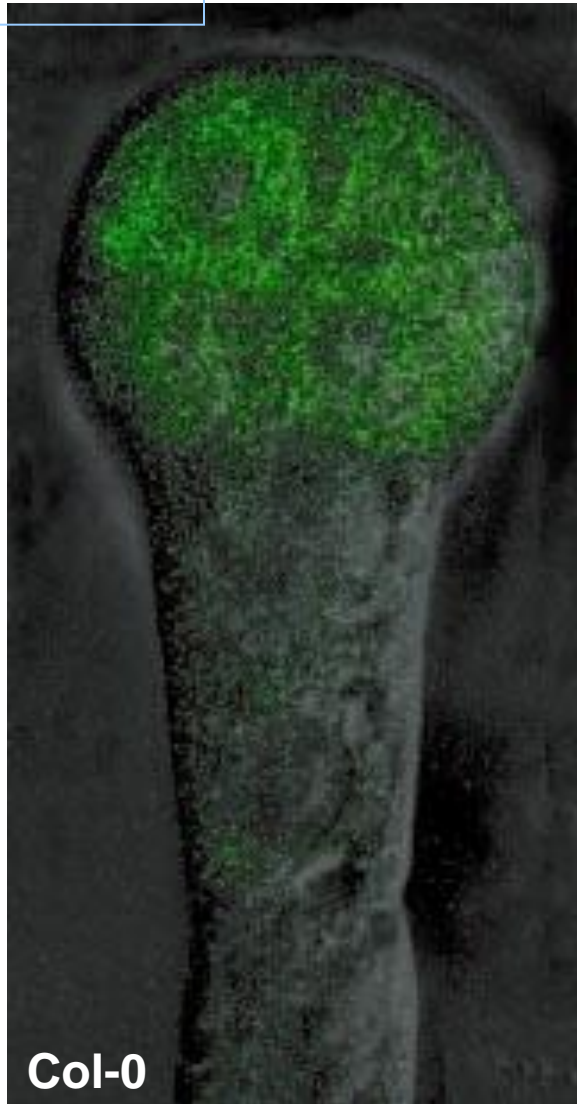


PIN1



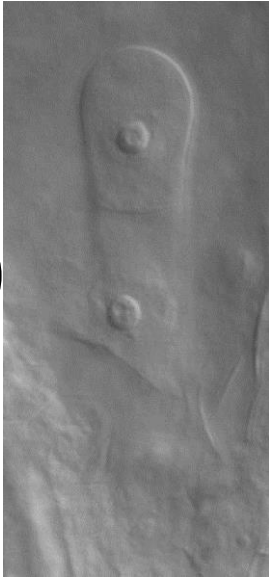
Analysis of DR5 activity in *pin7*

DR5

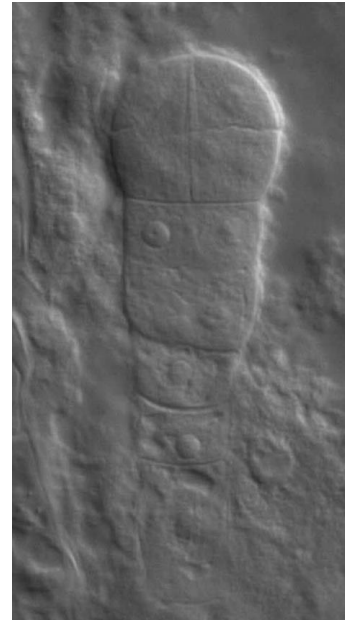
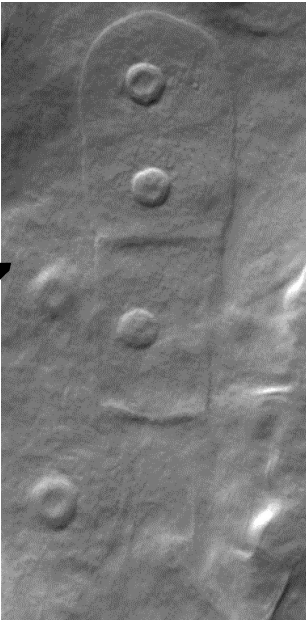


Embryo Phenotype of *pin7* Mutants

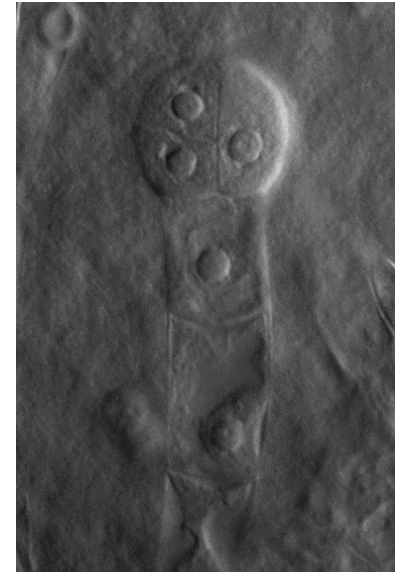
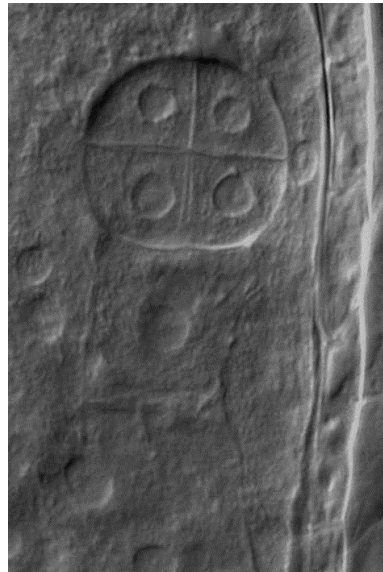
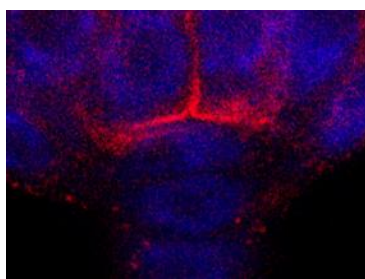
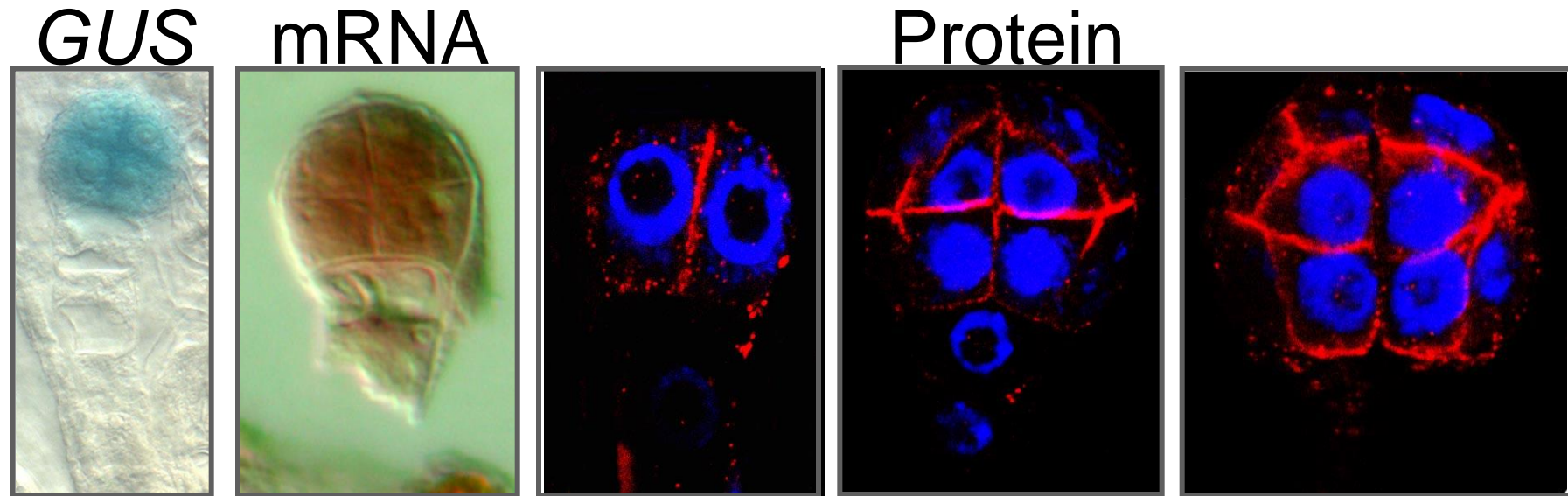
Col-0



pin7



PIN1 in Early Embryogenesis

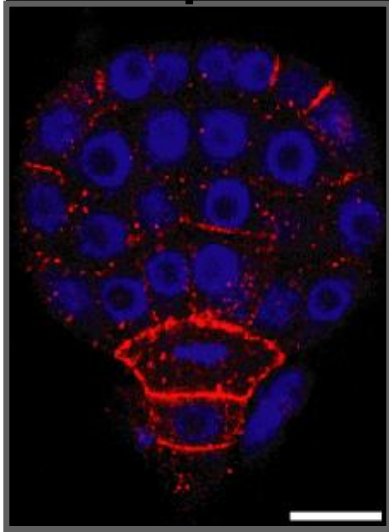


Enk
6,1%

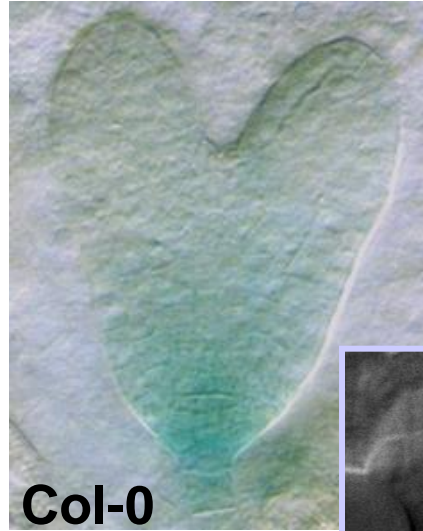
pin
30,4%

PIN4 in Embryogenesis

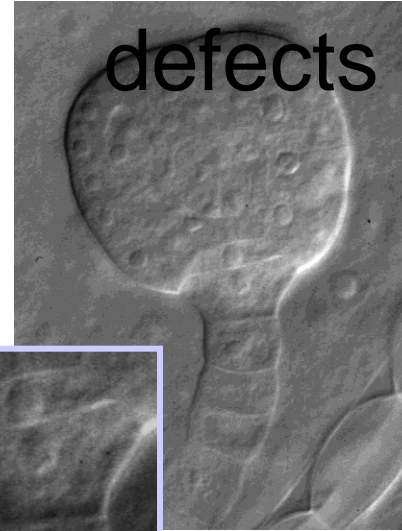
PIN4 protein



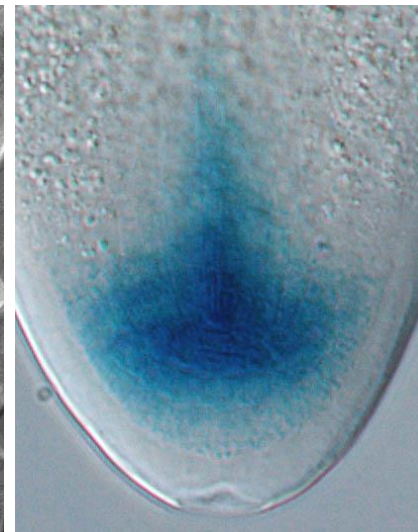
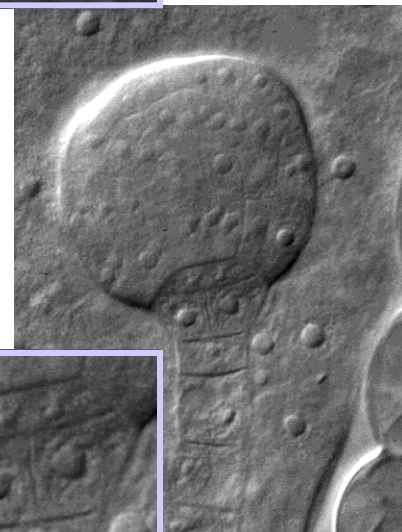
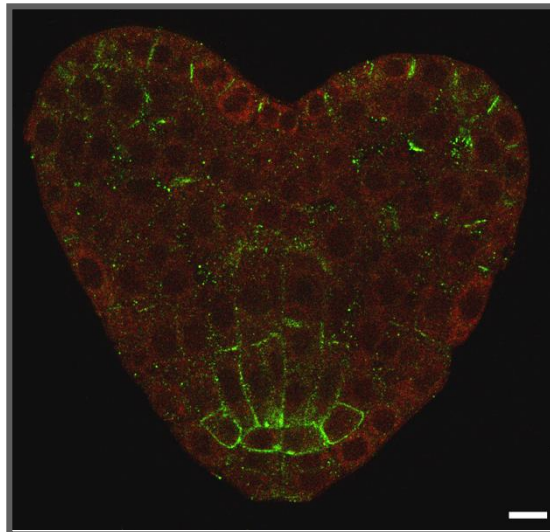
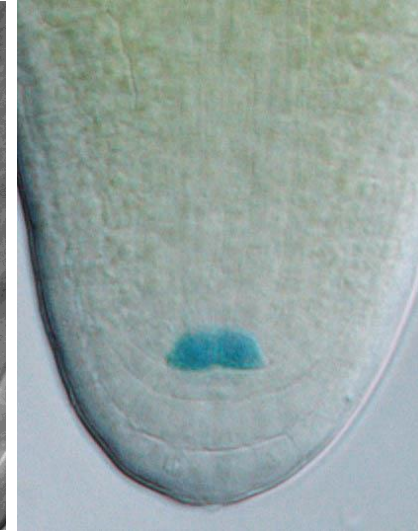
DR5



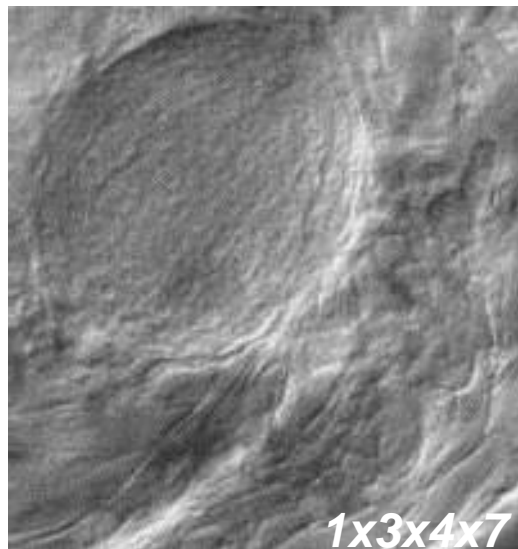
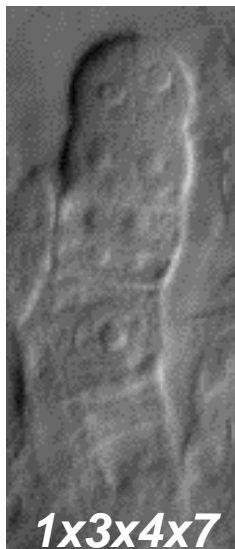
basal defects



QC marker

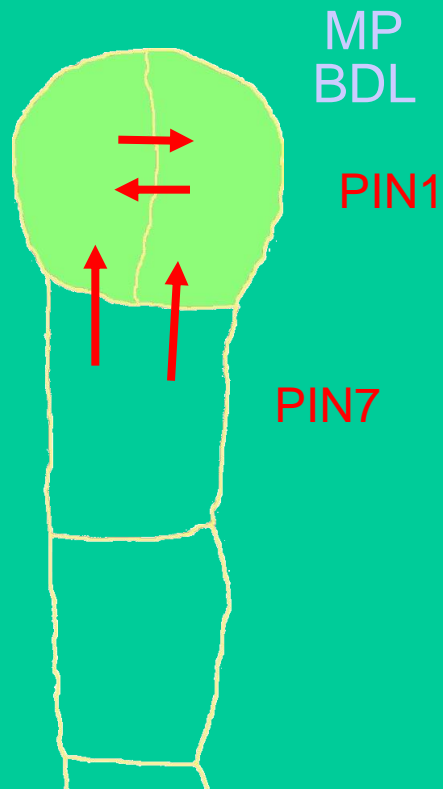


Phenotypes of *pin* Multiple Mutants



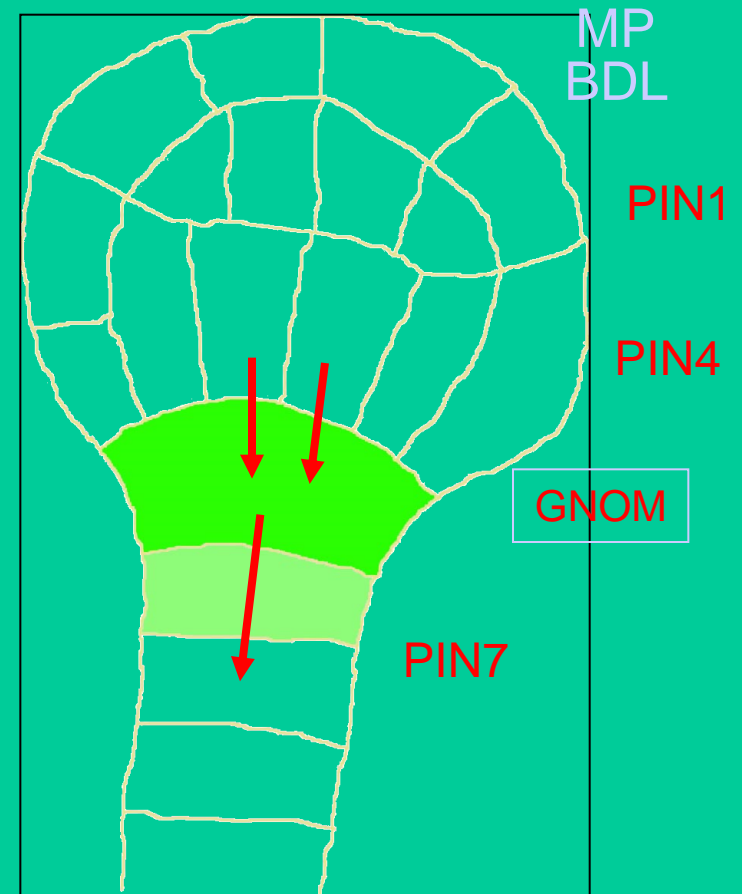
Auxin and Embryogenesis

Apical pole
specification



Two-Cell

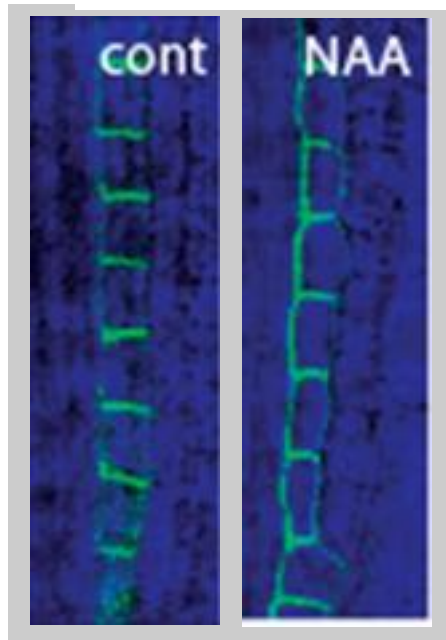
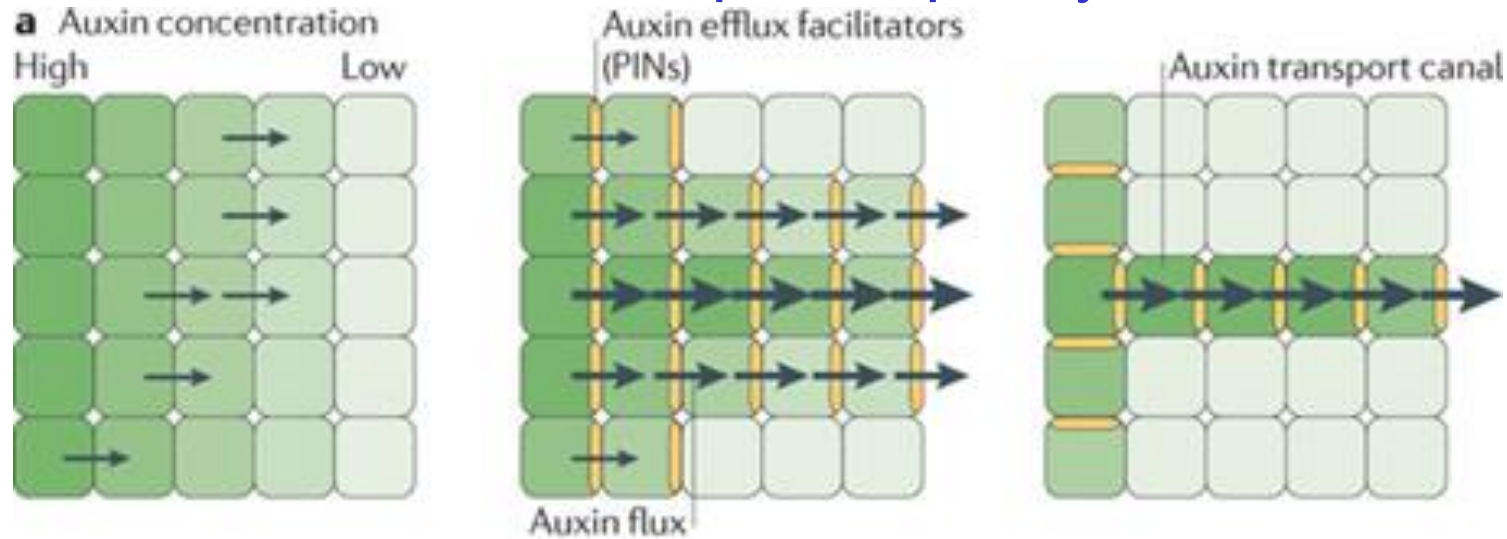
Root pole
specification



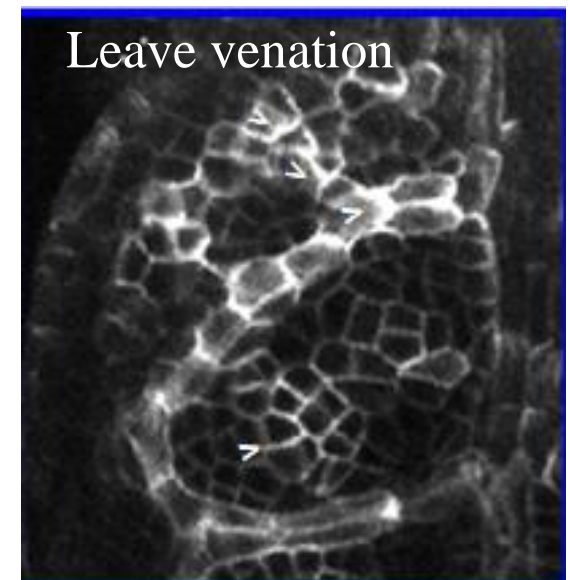
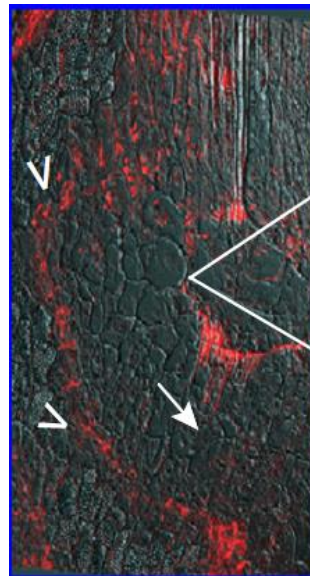
Globular

Canalization hypothesis of auxin flow

Feed-back on transport capacity and directionality



Vascular
tissue
regeneration

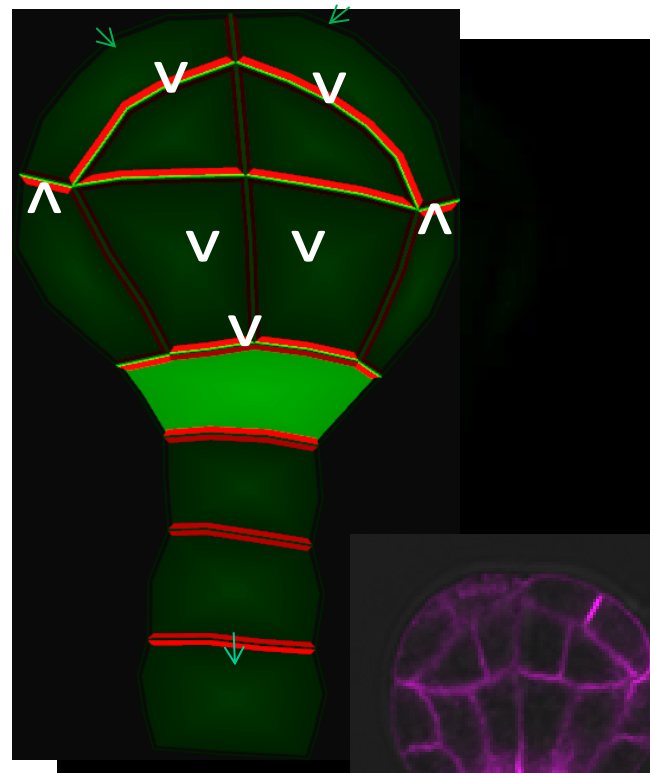
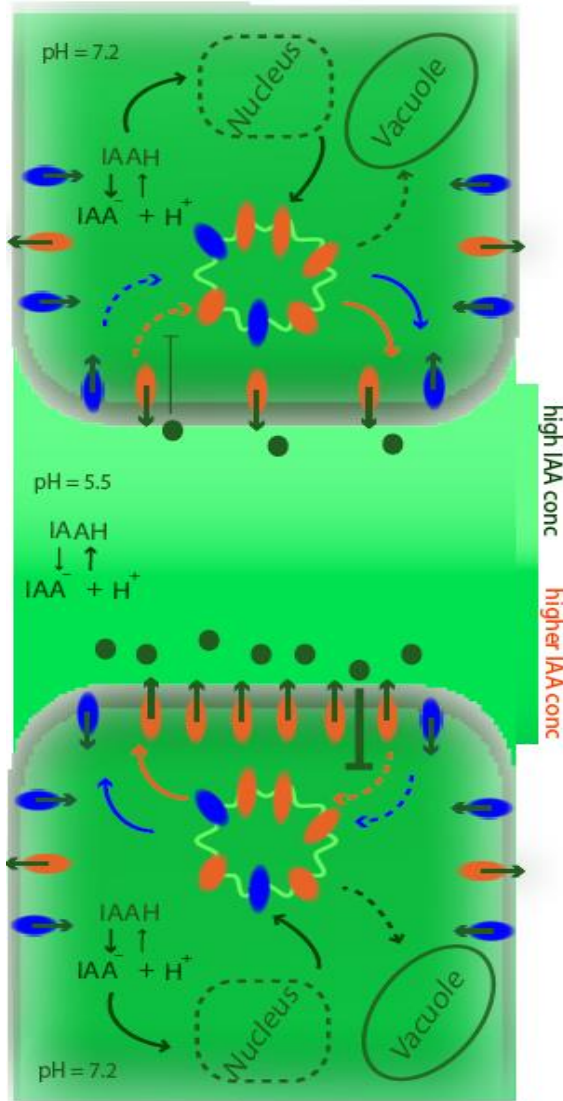


Modeling of Embryo Patterning

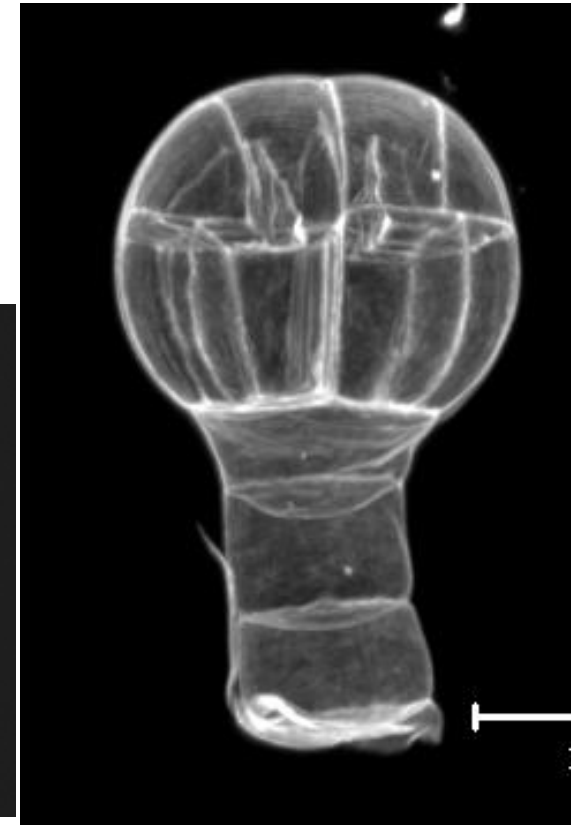


Cellular parameters

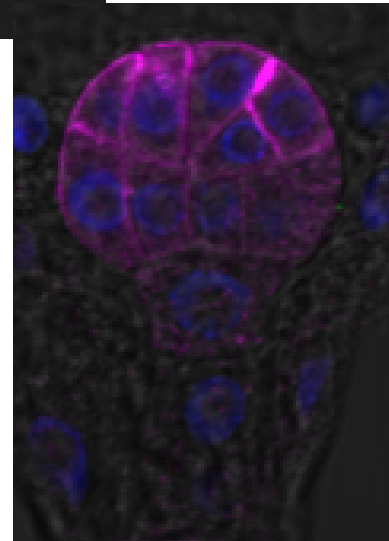
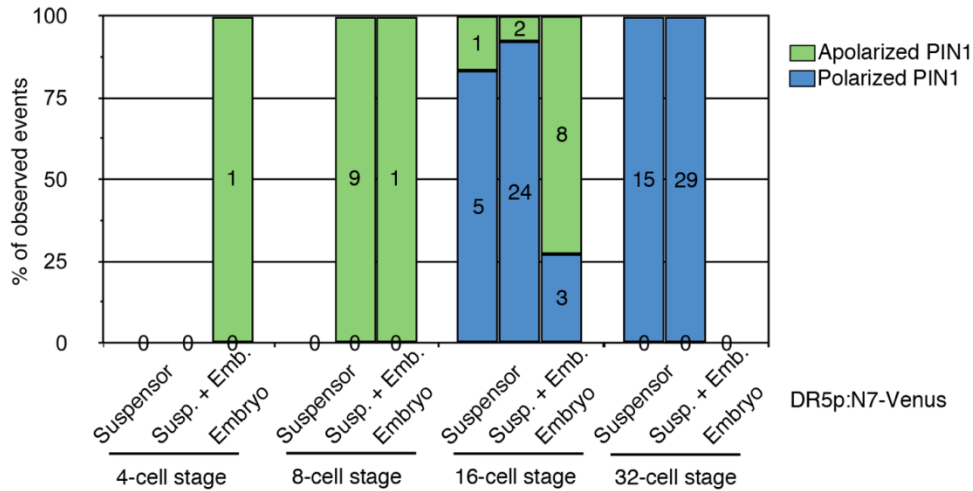
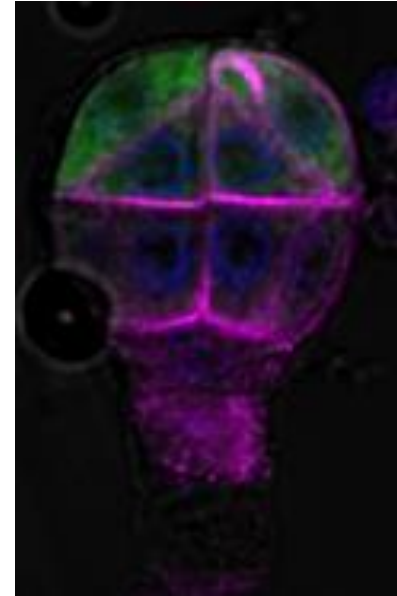
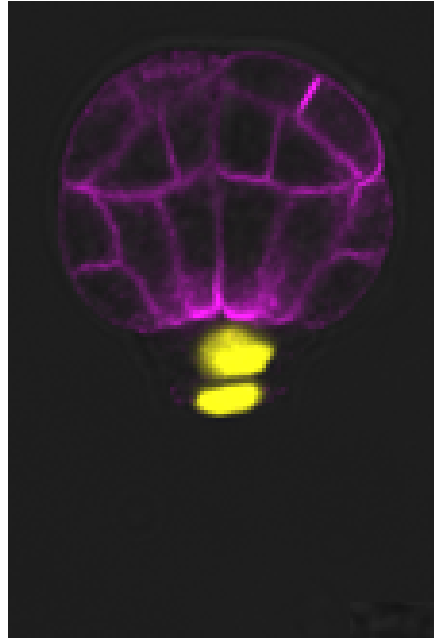
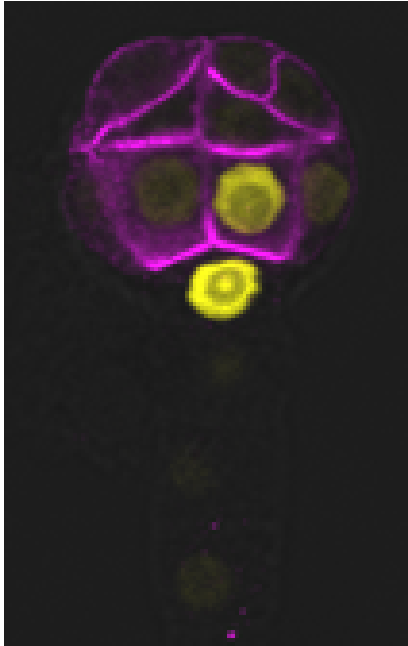
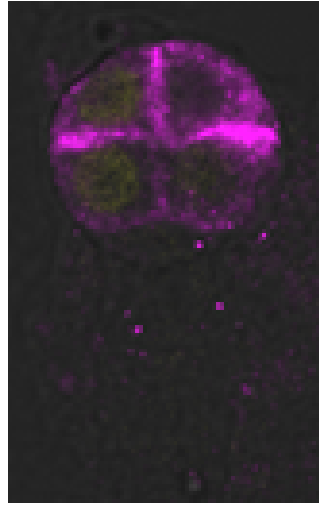
2D model



3D template



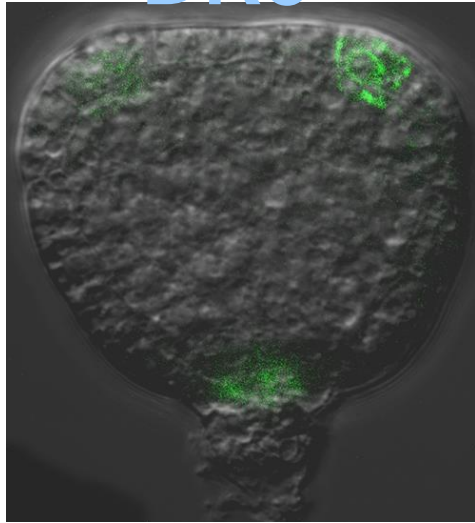
Feed-back regulation of PIN polarization by local auxin biosynthesis



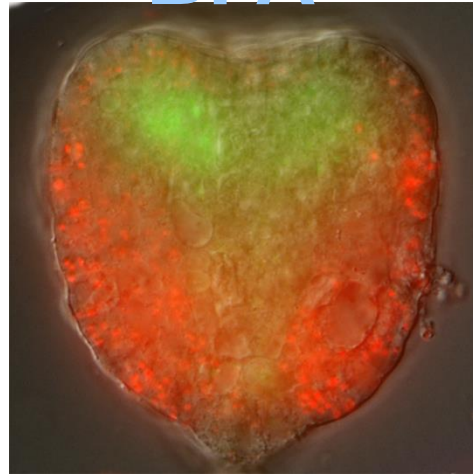
Organogenesis

Auxin in Cotyledon Formation

DR5



BFA



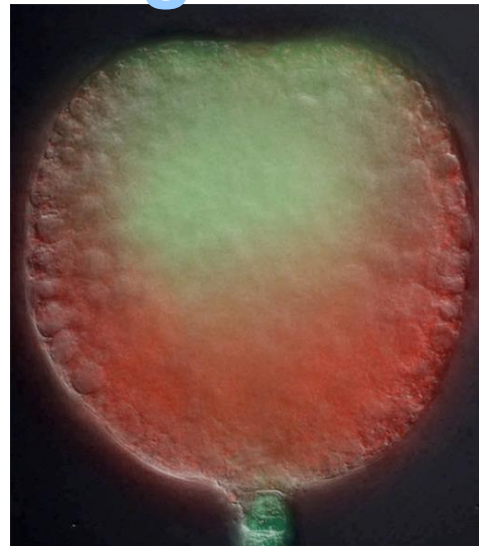
pins



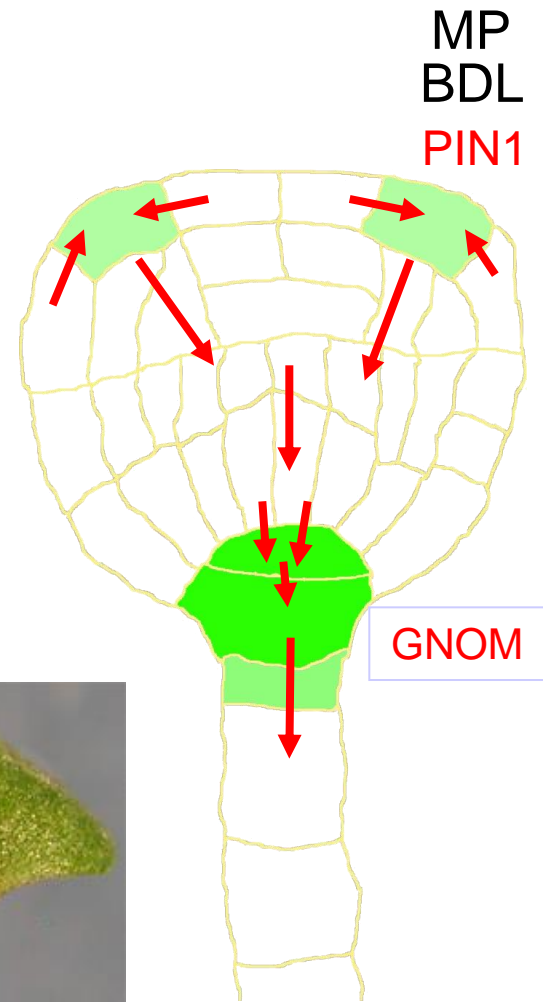
IAA



gnom



pin1



MP
BDL
PIN1

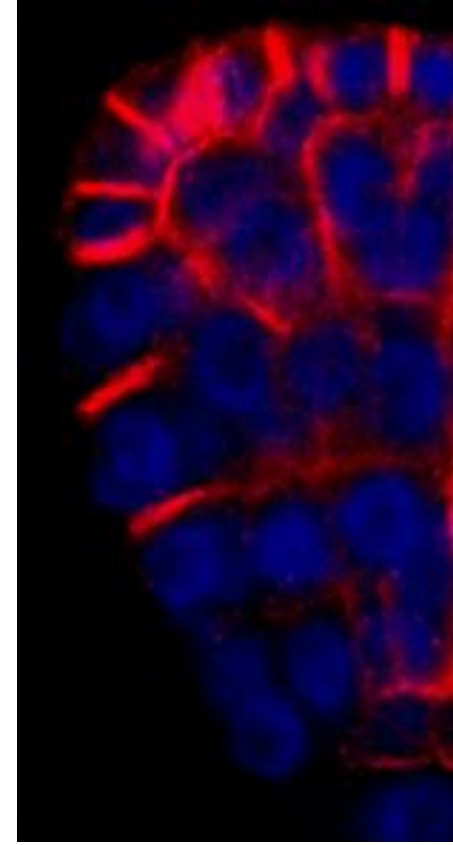
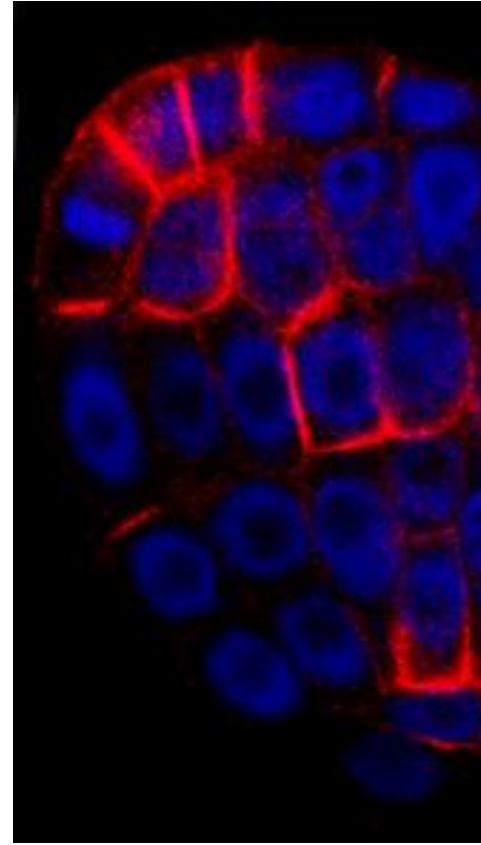
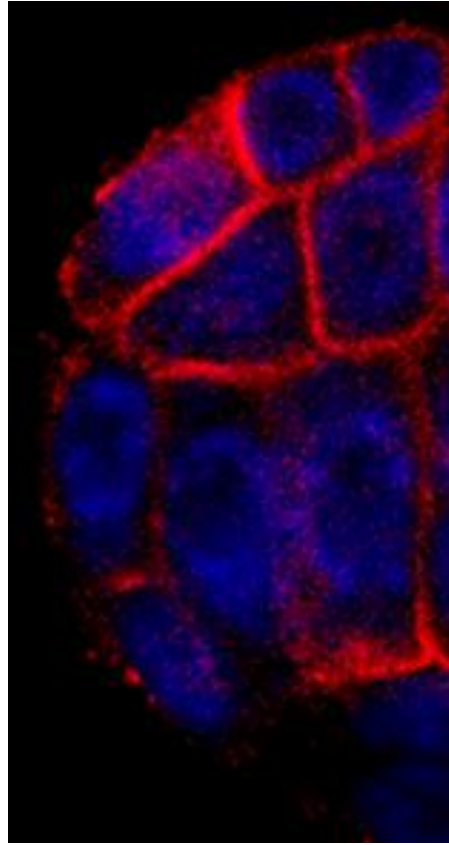
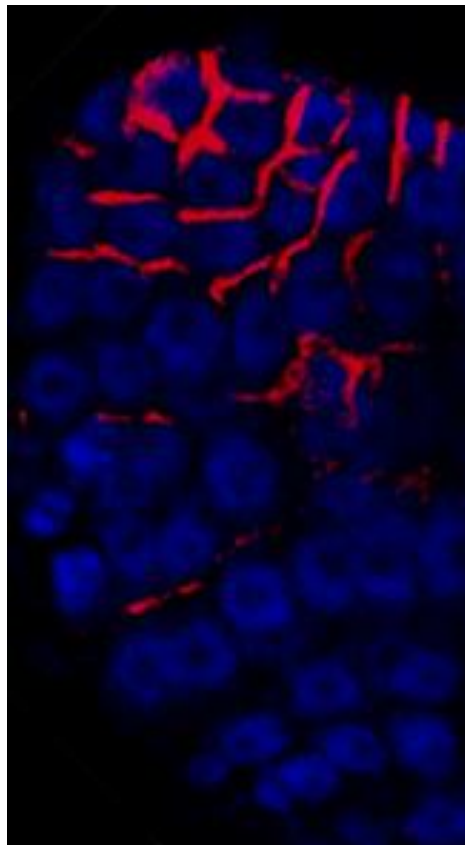
GNOM

PIN1 Polarity in Cotyledon Formation

Outer layer

Inner layers

BFA treatment



Heart

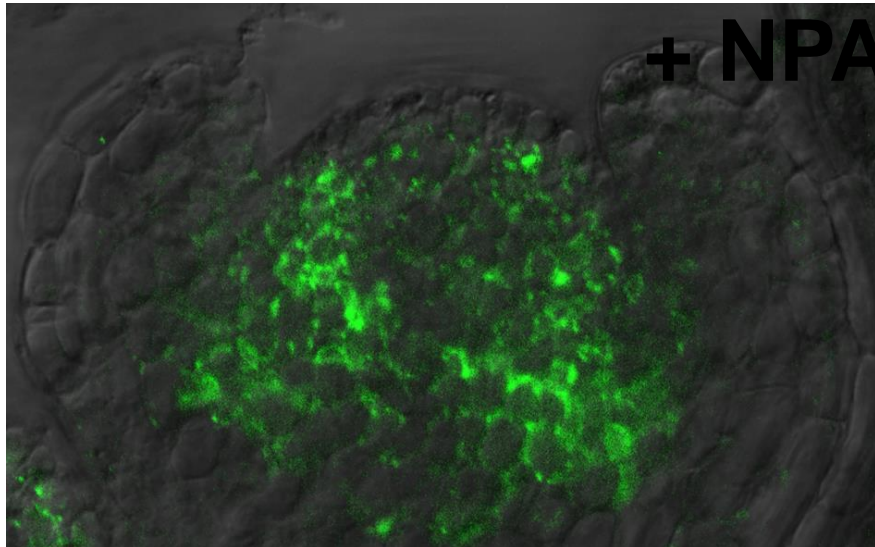
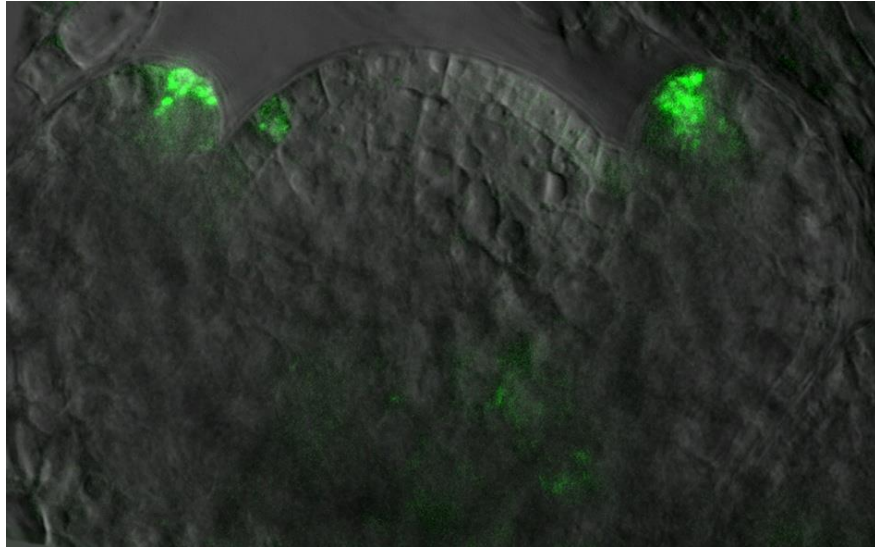
Globular

Heart

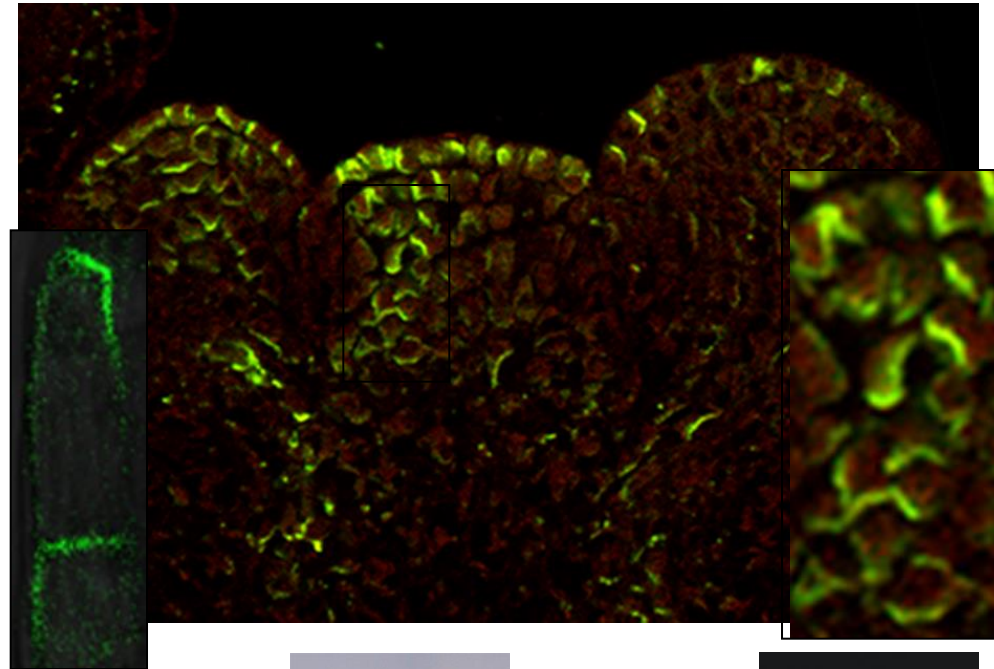
Heart

Auxin in Flower and Leave Formation

DR5rev::GFP



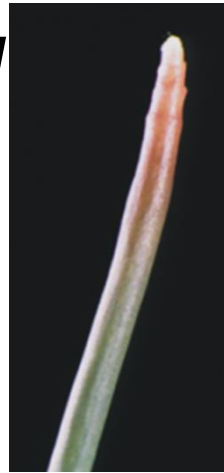
PIN1 localisation



+ NPA



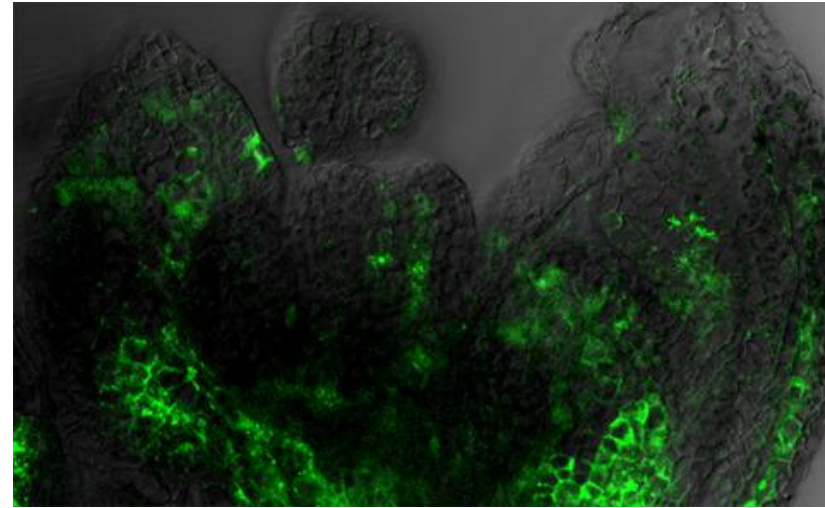
pin1



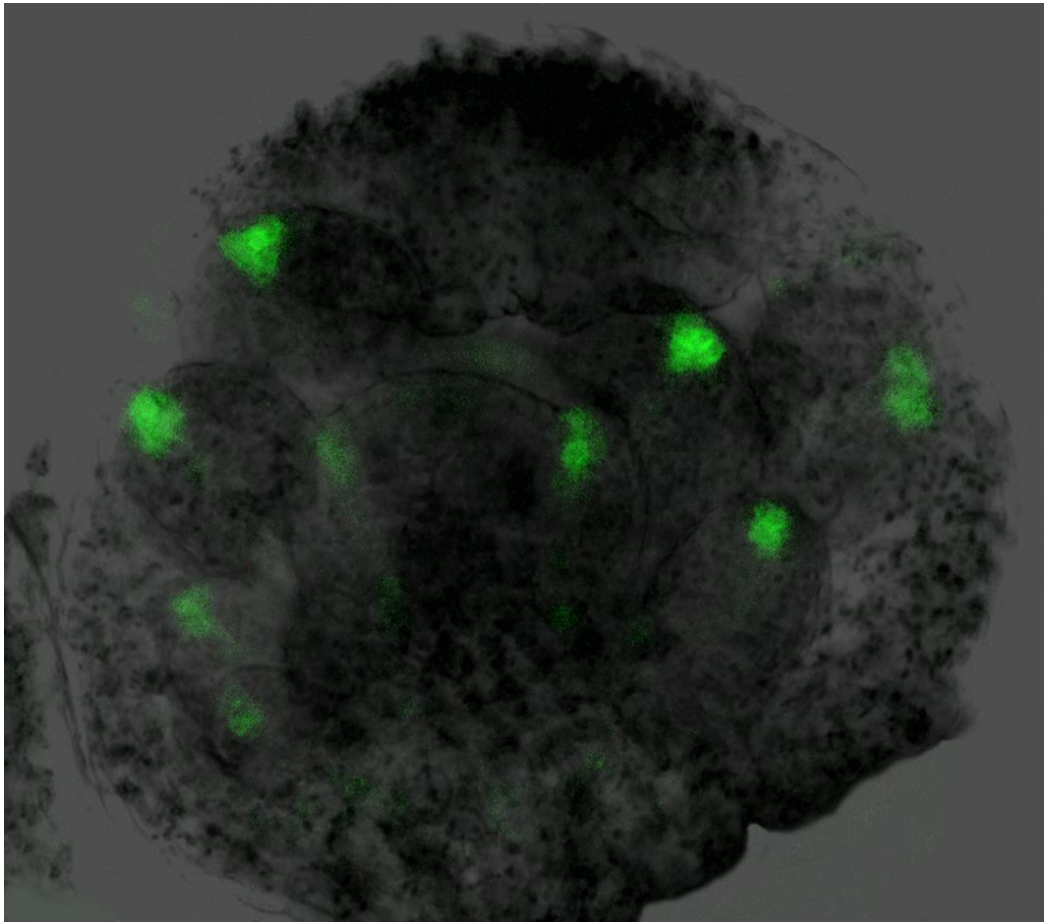
DR5 in Floral Organ Formation

DR5rev::GFP

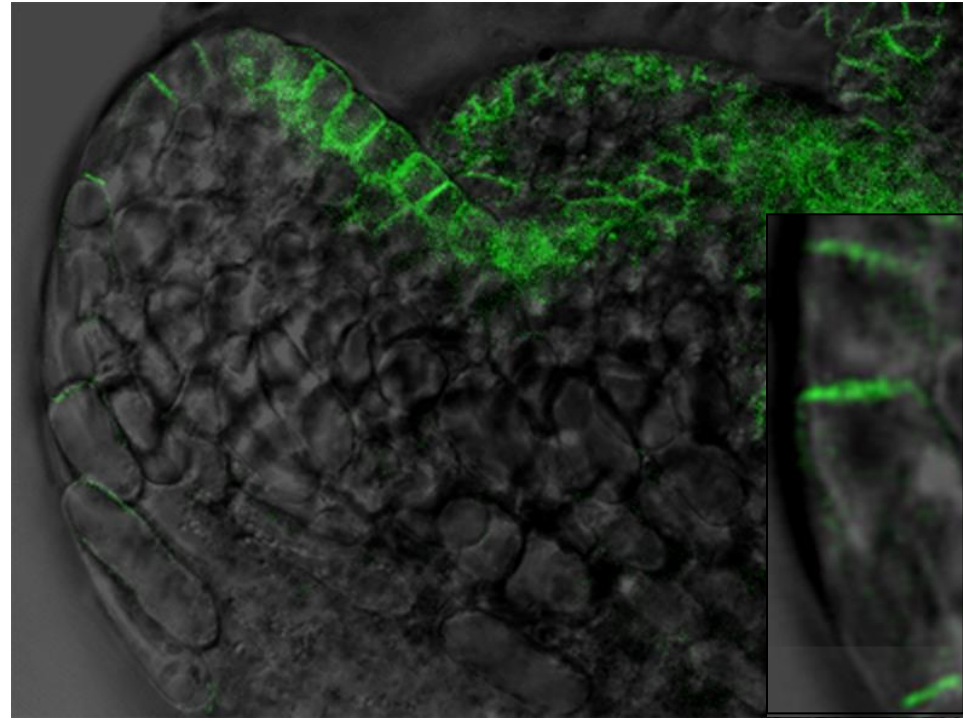
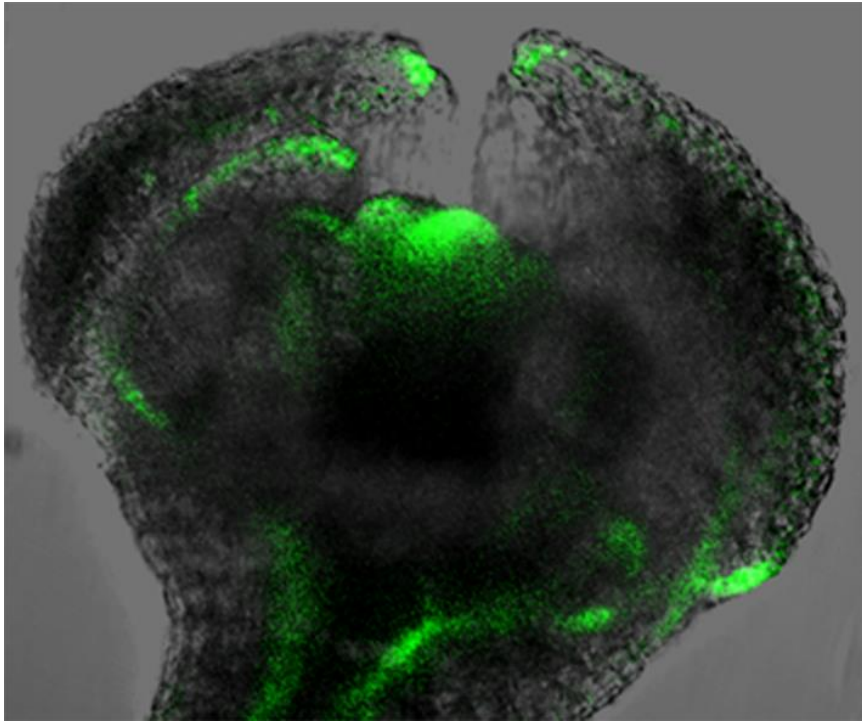
+ NPA



***pin* mutants**

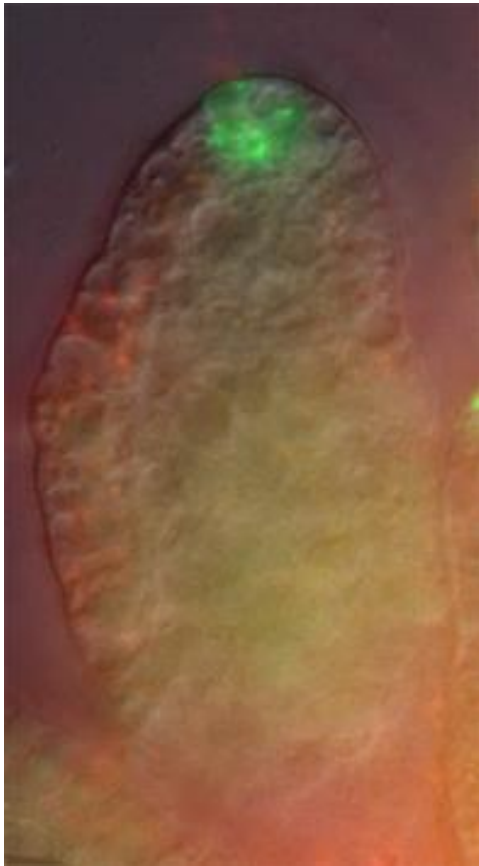


PIN1 in Floral Organ Formation



DR5 in Ovule Formation

Ovule
primordium



Ovule with
Integuments
primordia

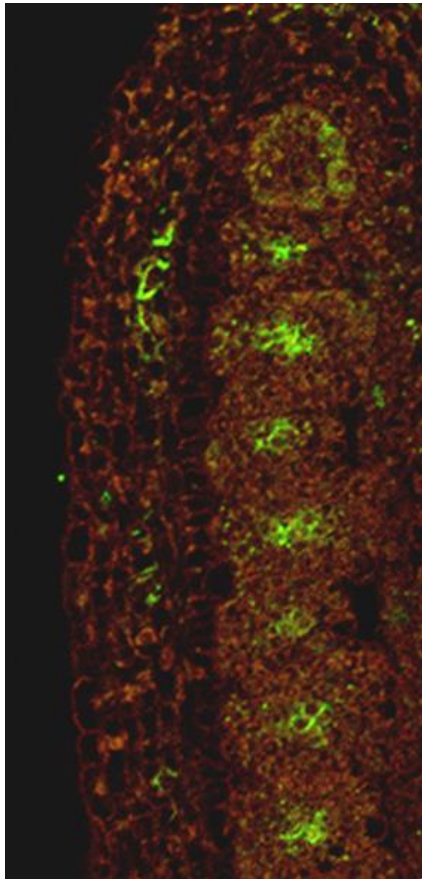


Ovule defects
in *pin1*

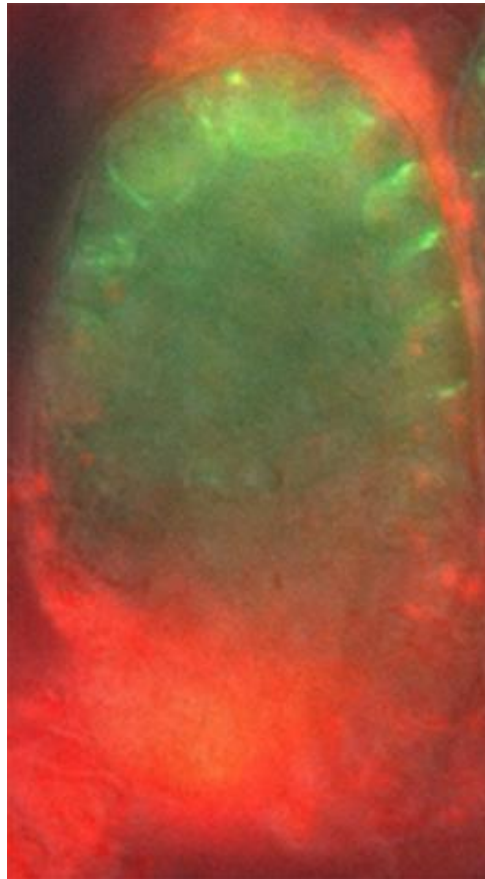


PIN1 in Ovule Formation

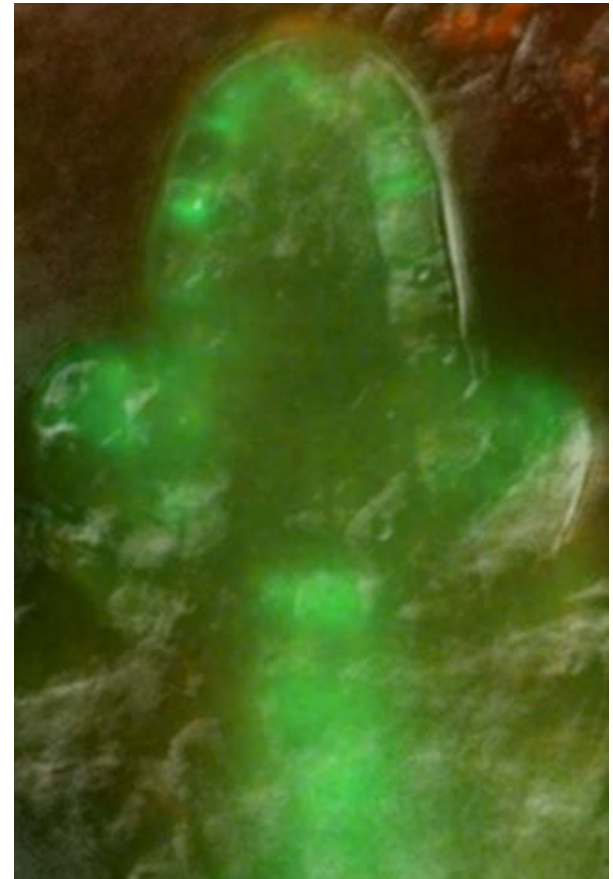
Gynoecium
with ovule primordia



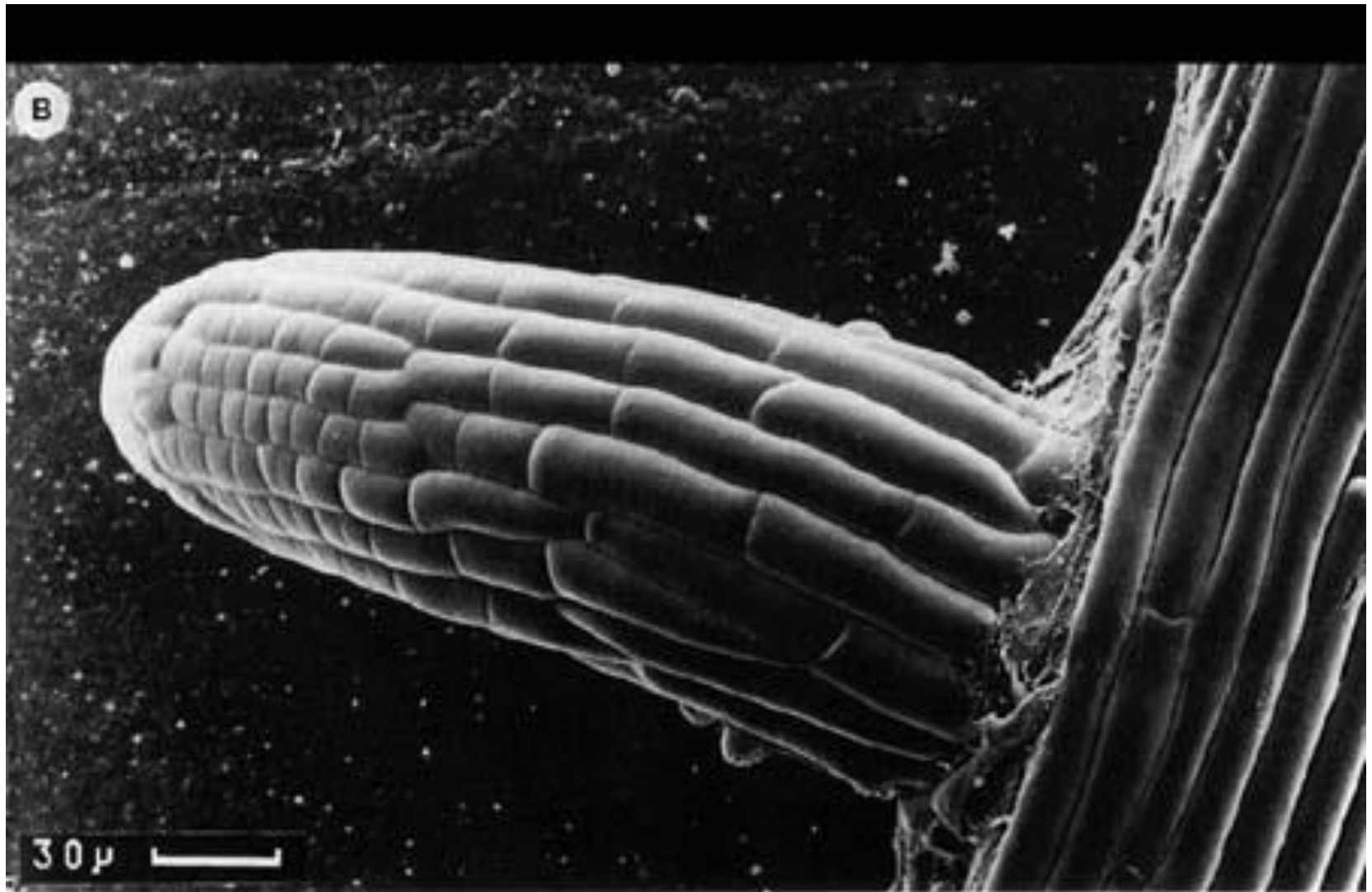
Ovule
primordium



Ovule with
Integuments
primordia

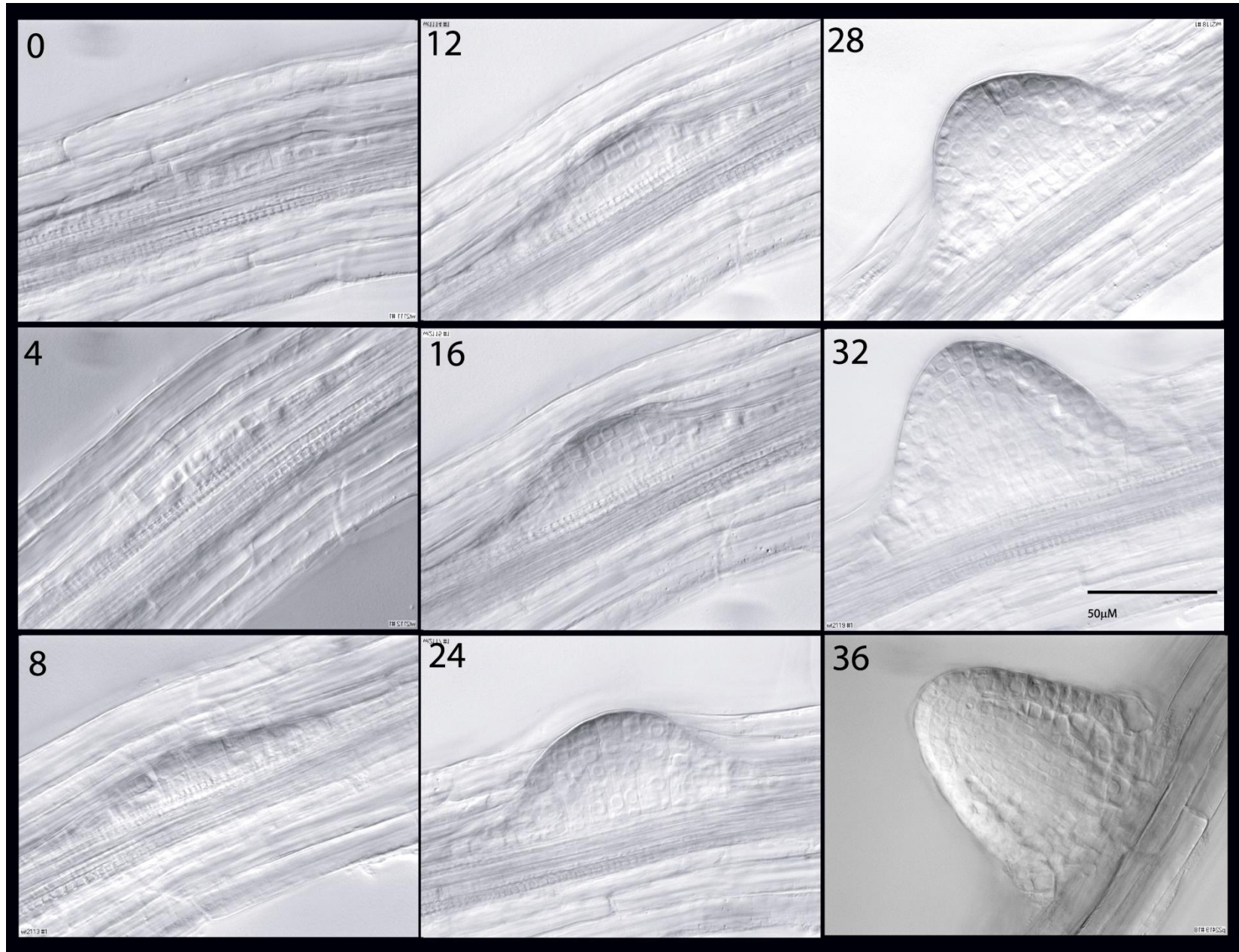


Lateral Root Development



Arabidopsis lateral root

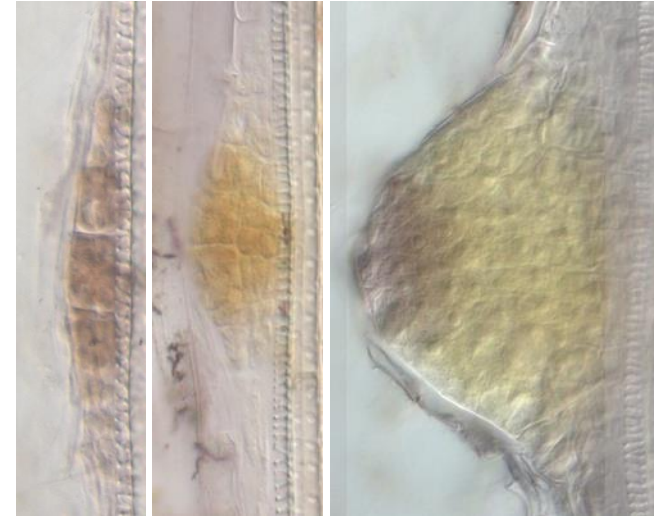
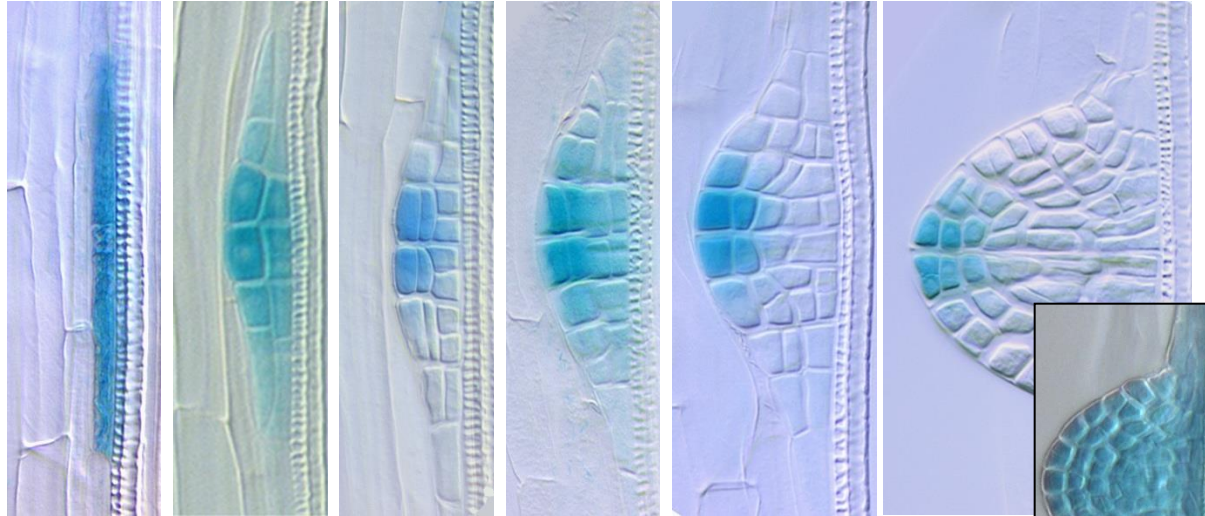
Lateral Root Development in Time



DR5 in Lateral Root Formation

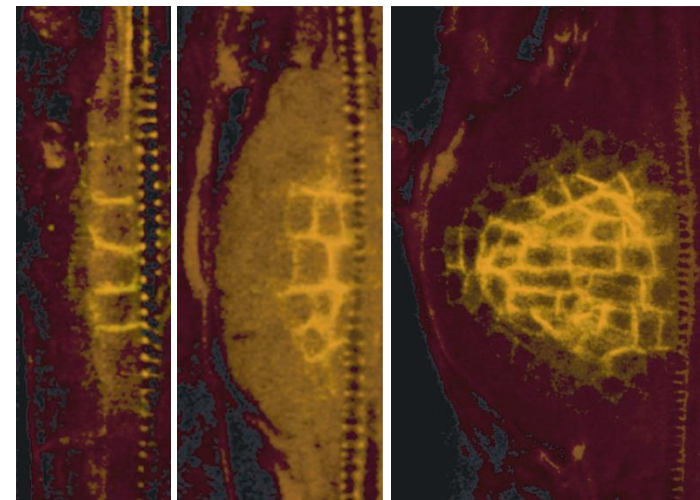
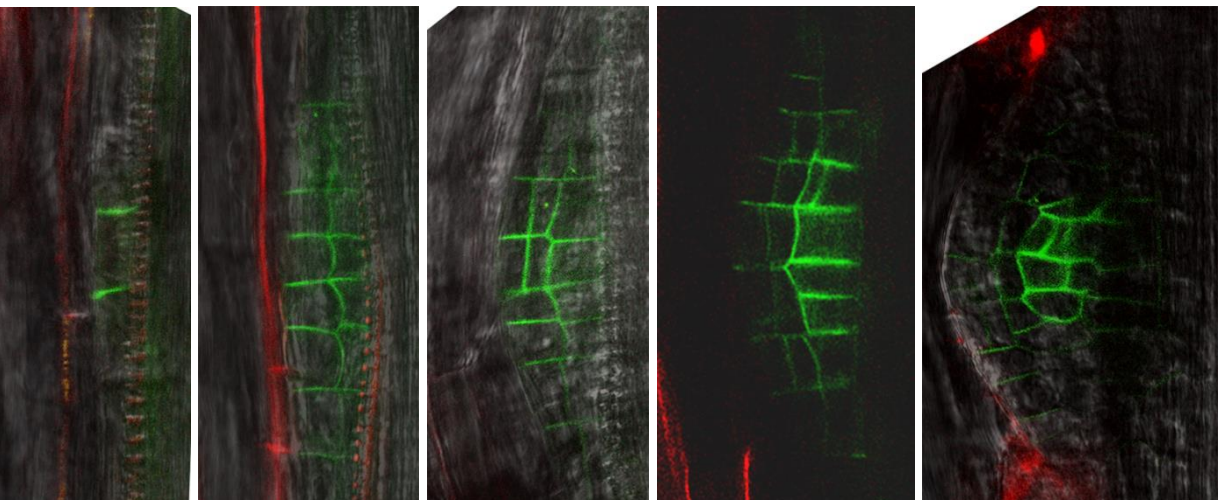
DR5rev::GUS

IAA



PIN1:GFP

PIN1



Relocation > Gradients > Primordia

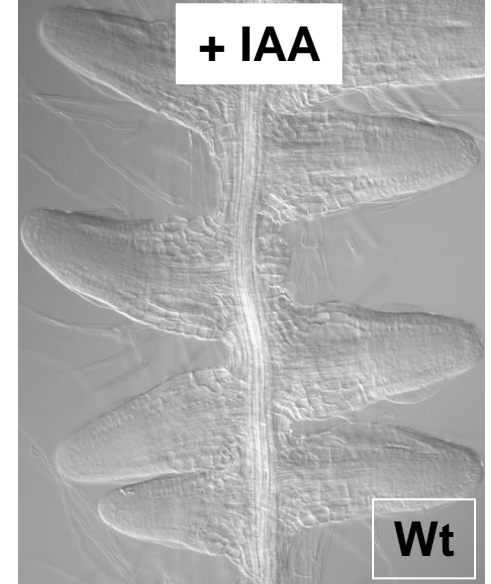
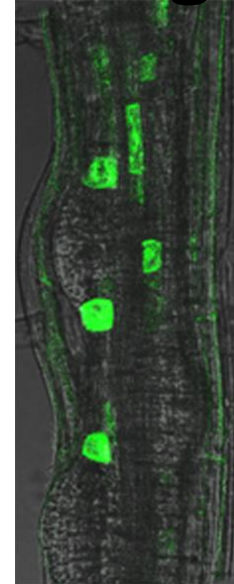
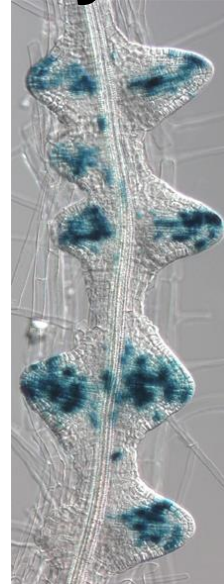
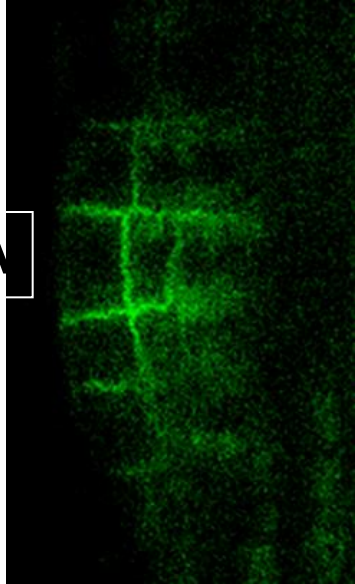
PIN1

DR5

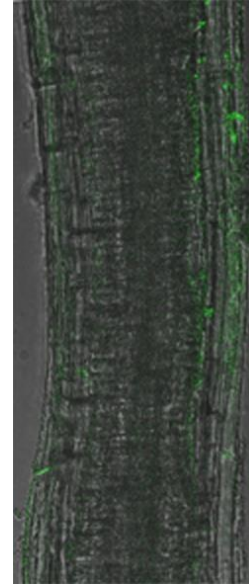
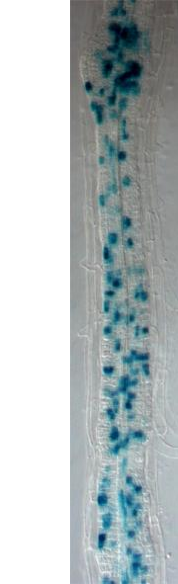
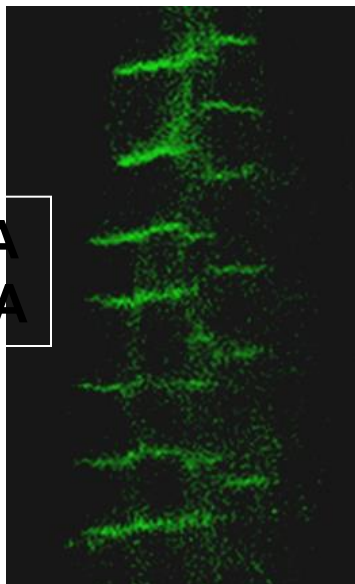
CycB margins

primordia

+ IAA

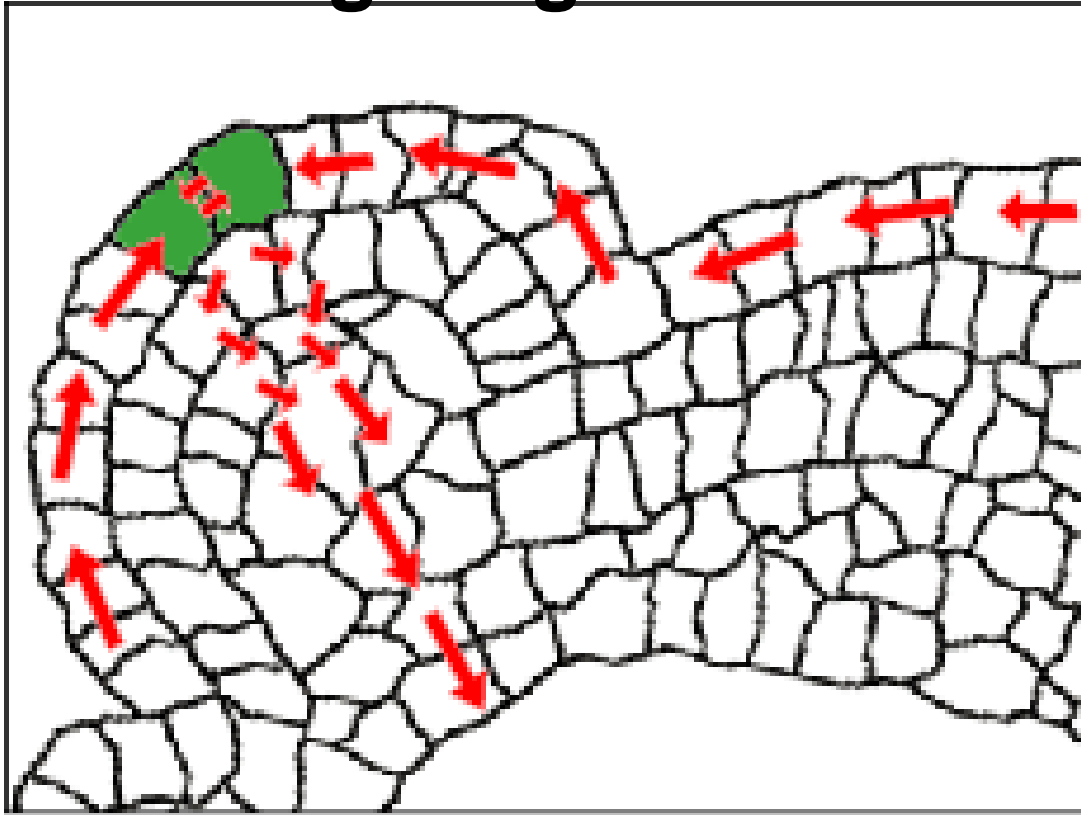


+ IAA
+ NPA

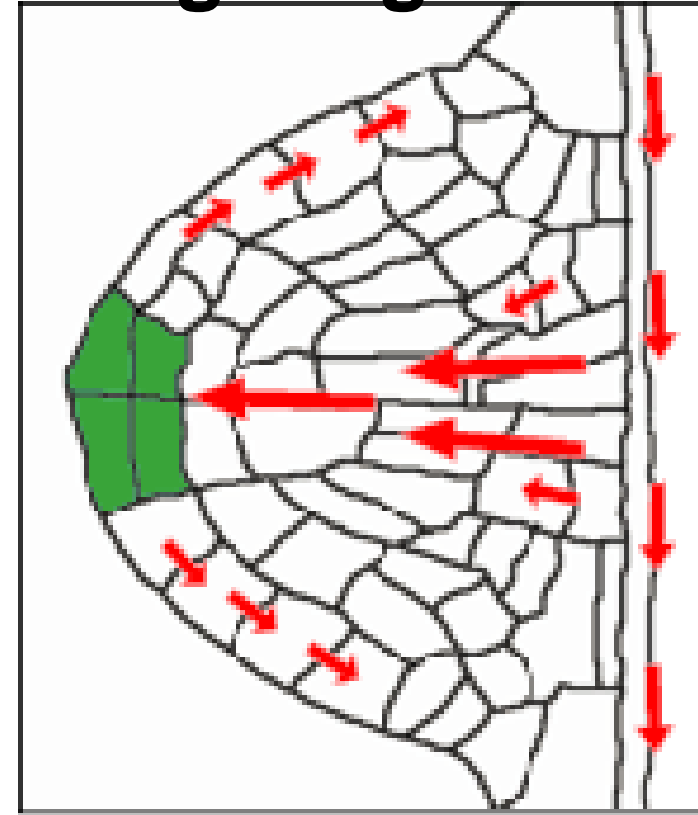


Common module for organ formation

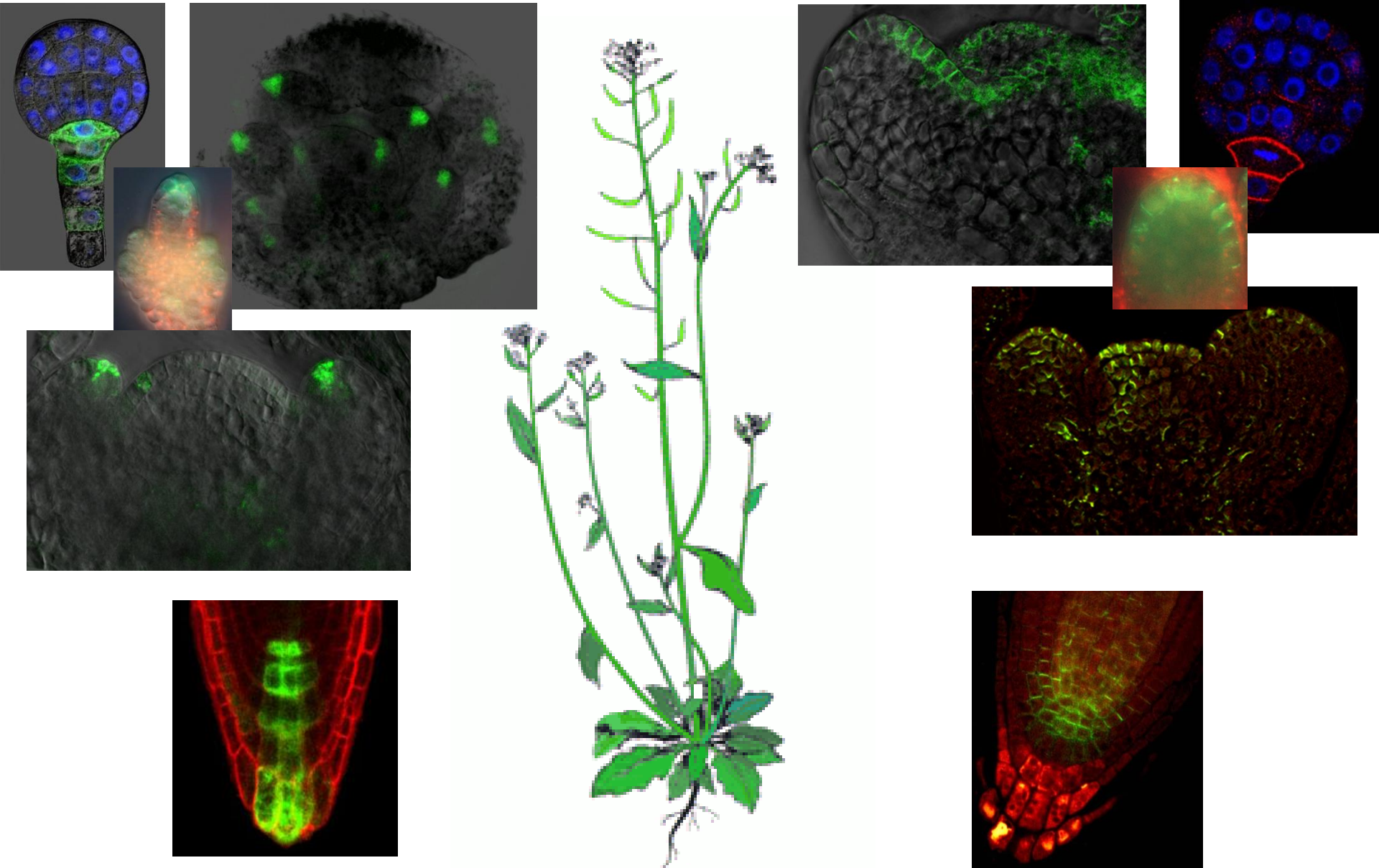
Aerial organogenesis



Underground organogenesis



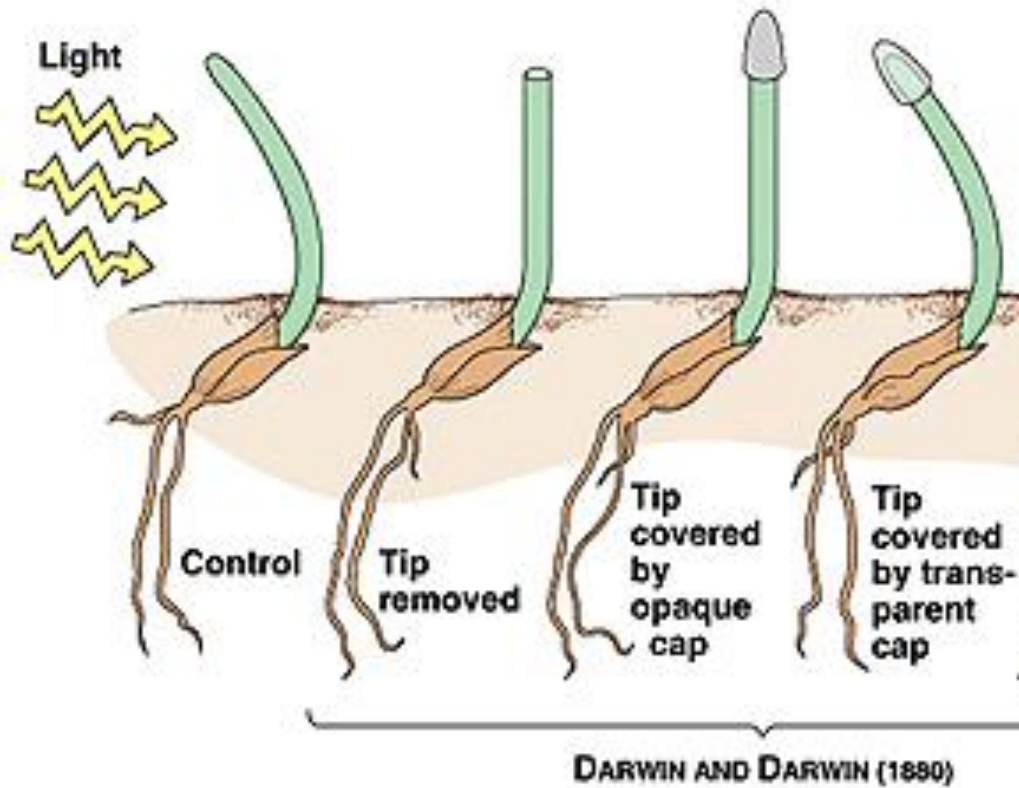
PIN-dependent Auxin Gradients in Plant Development



TROPISMS role for auxin

Tropisms: „Movements“ in Plants

Phototropism



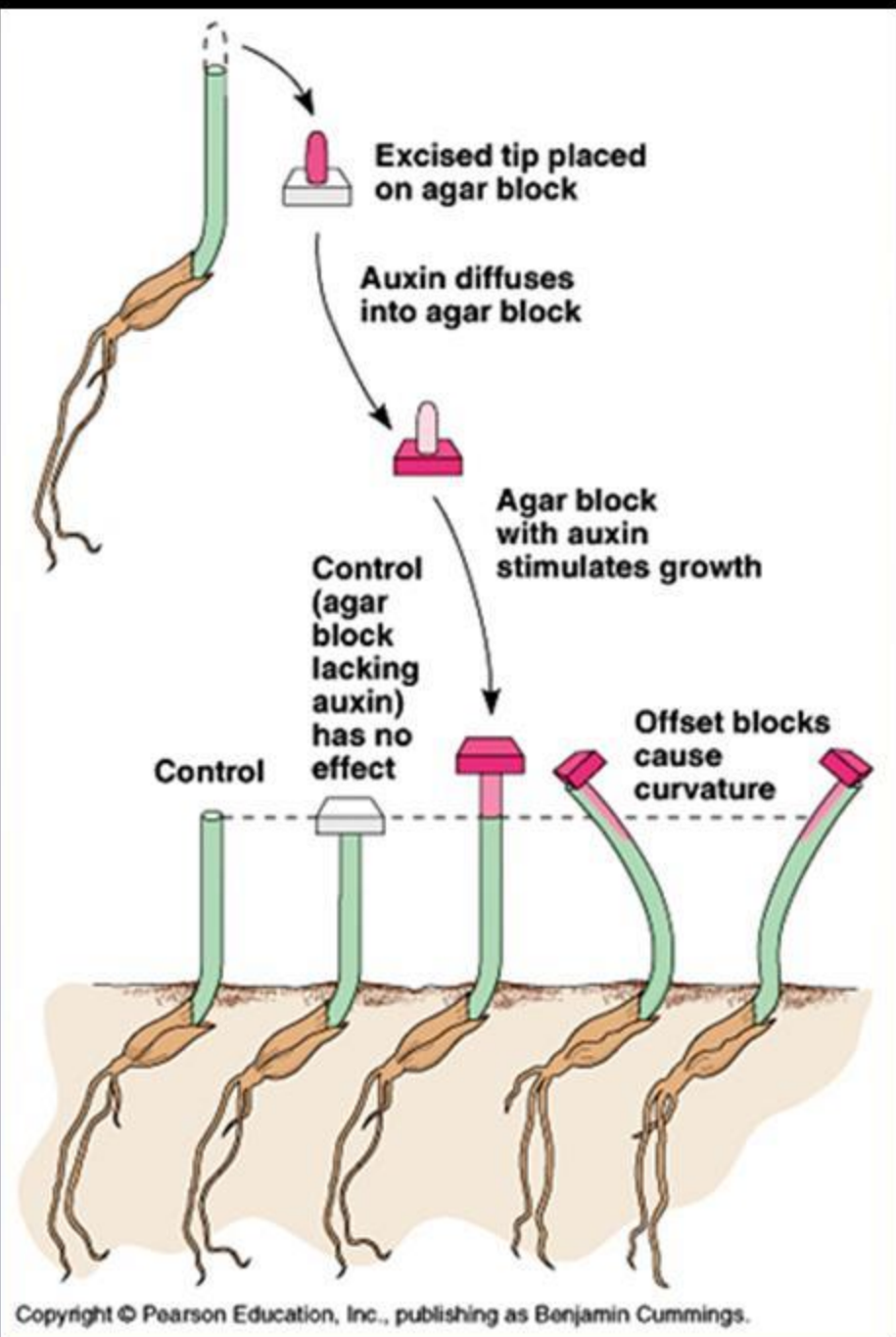
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Gravitropism



Asymmetric Auxin Distribution Controls Directional Growth

- Tropisms



Asymmetric Auxin Distribution Underlies Tropisms

Phototropism

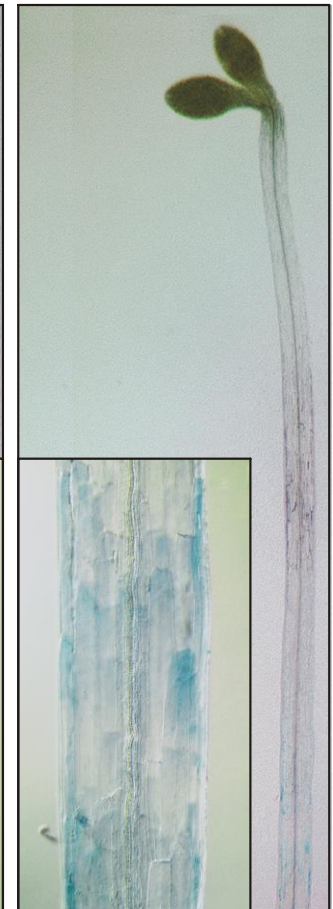
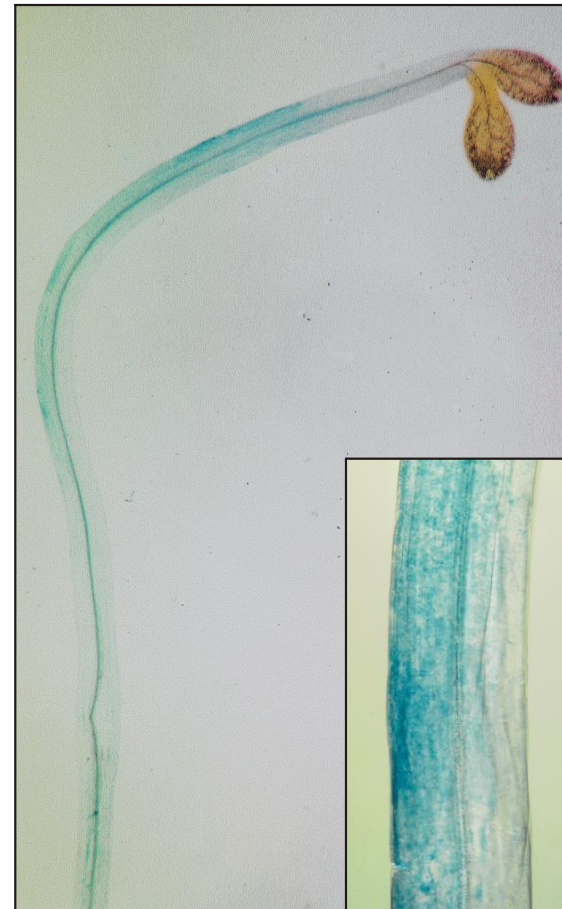
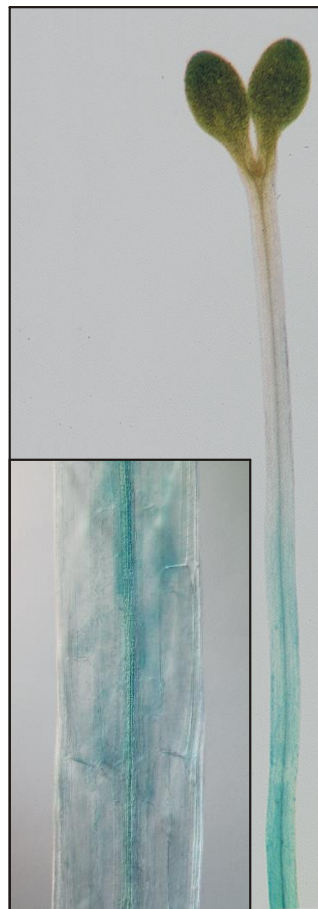
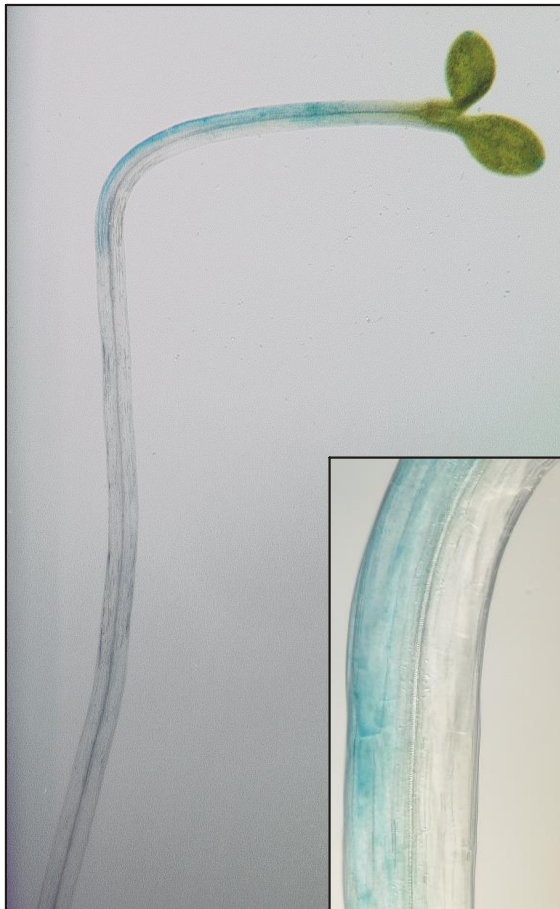
Gravitropism

- NPA

+ NPA

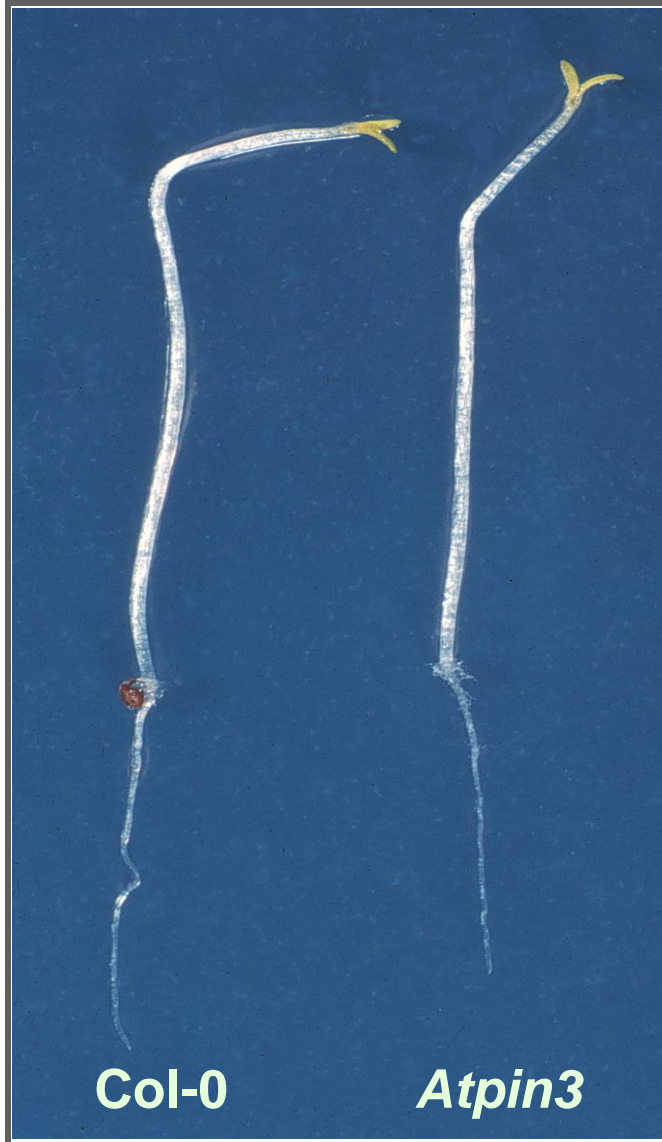
- NPA

+ NPA



pin3 is Defective in Tropisms

Hypocotyl phototropism



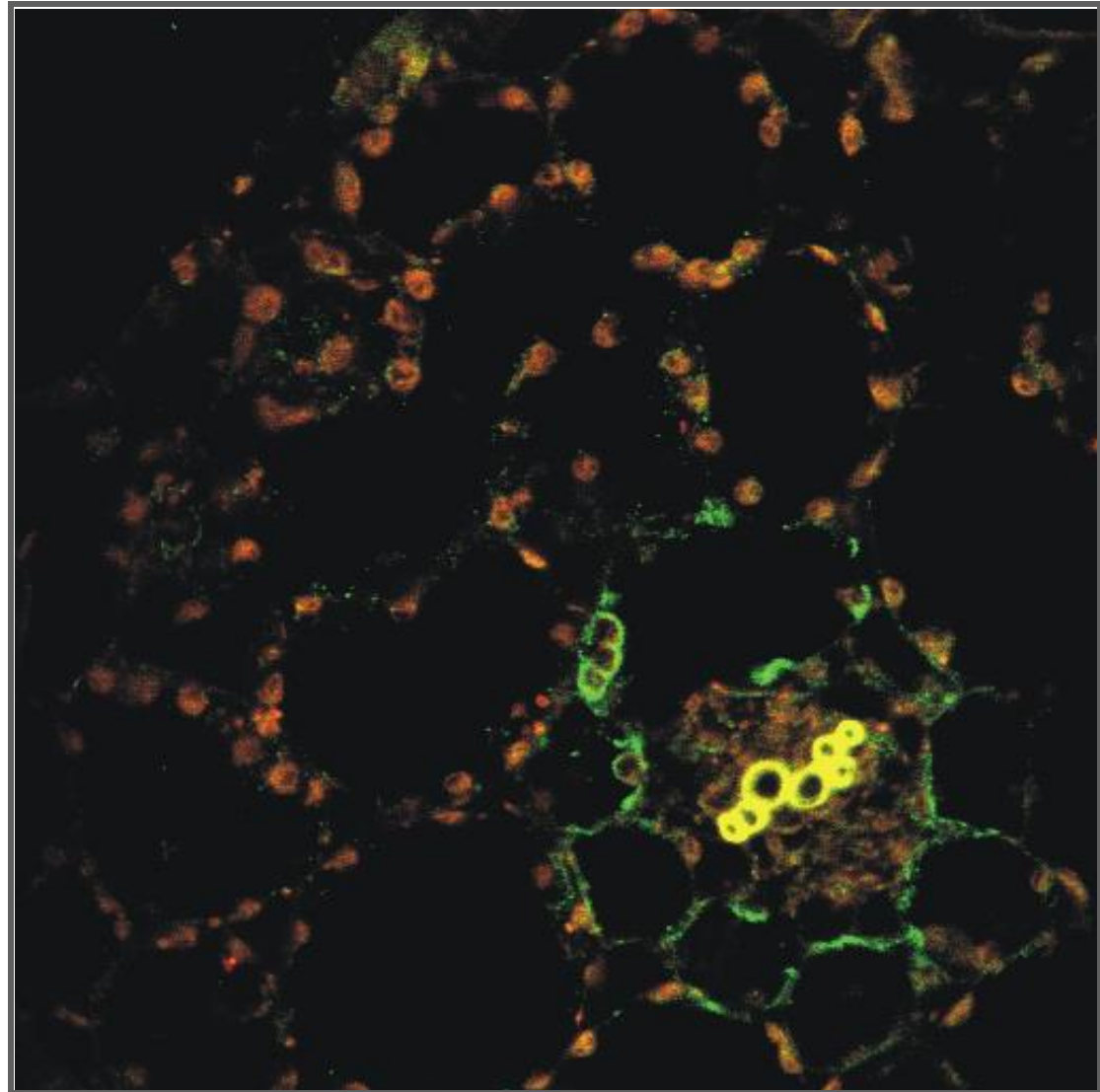
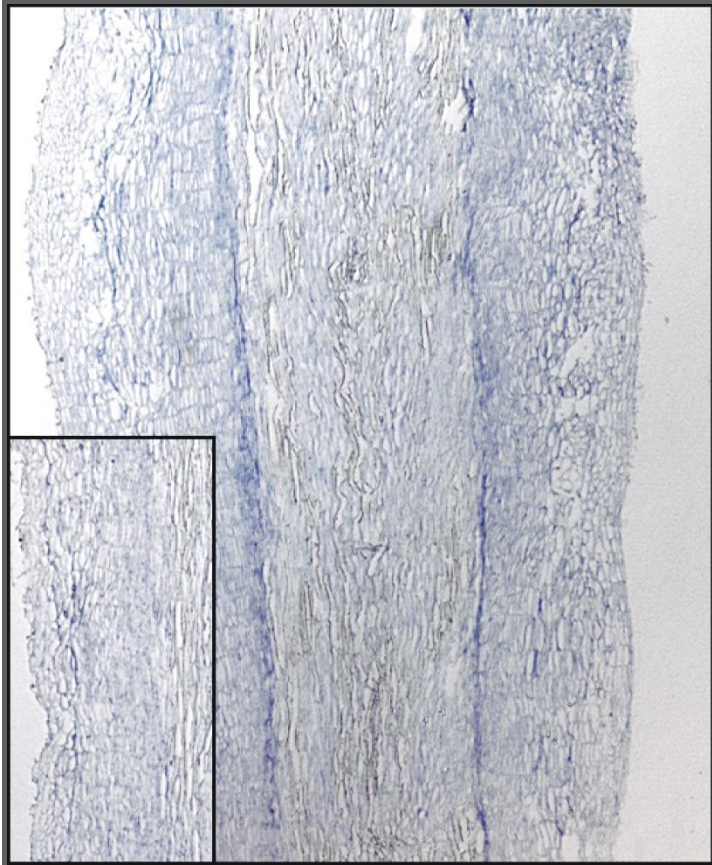
Hypocotyl gravitropism



PIN3 in Hypocotyl

PIN3 protein

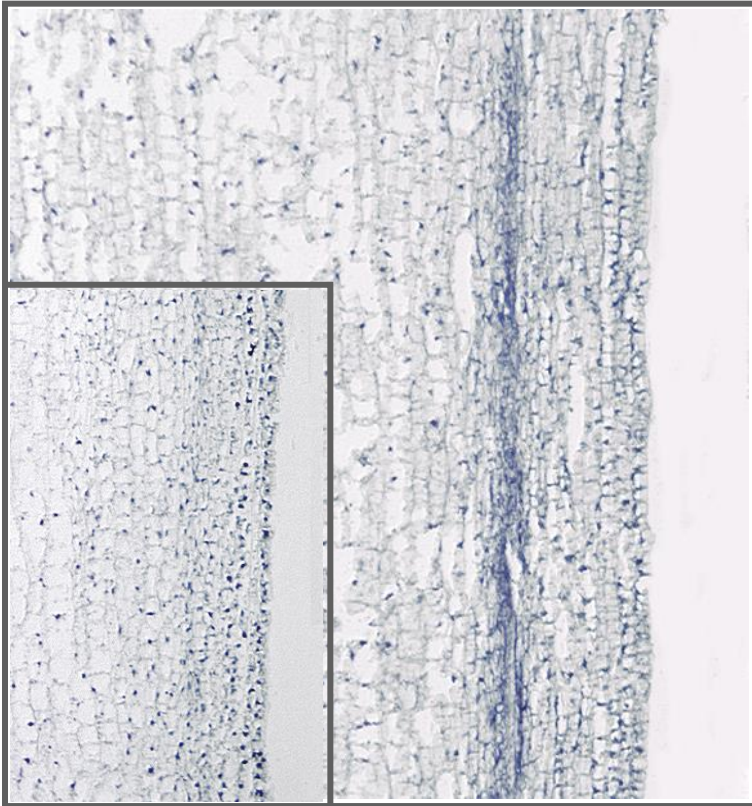
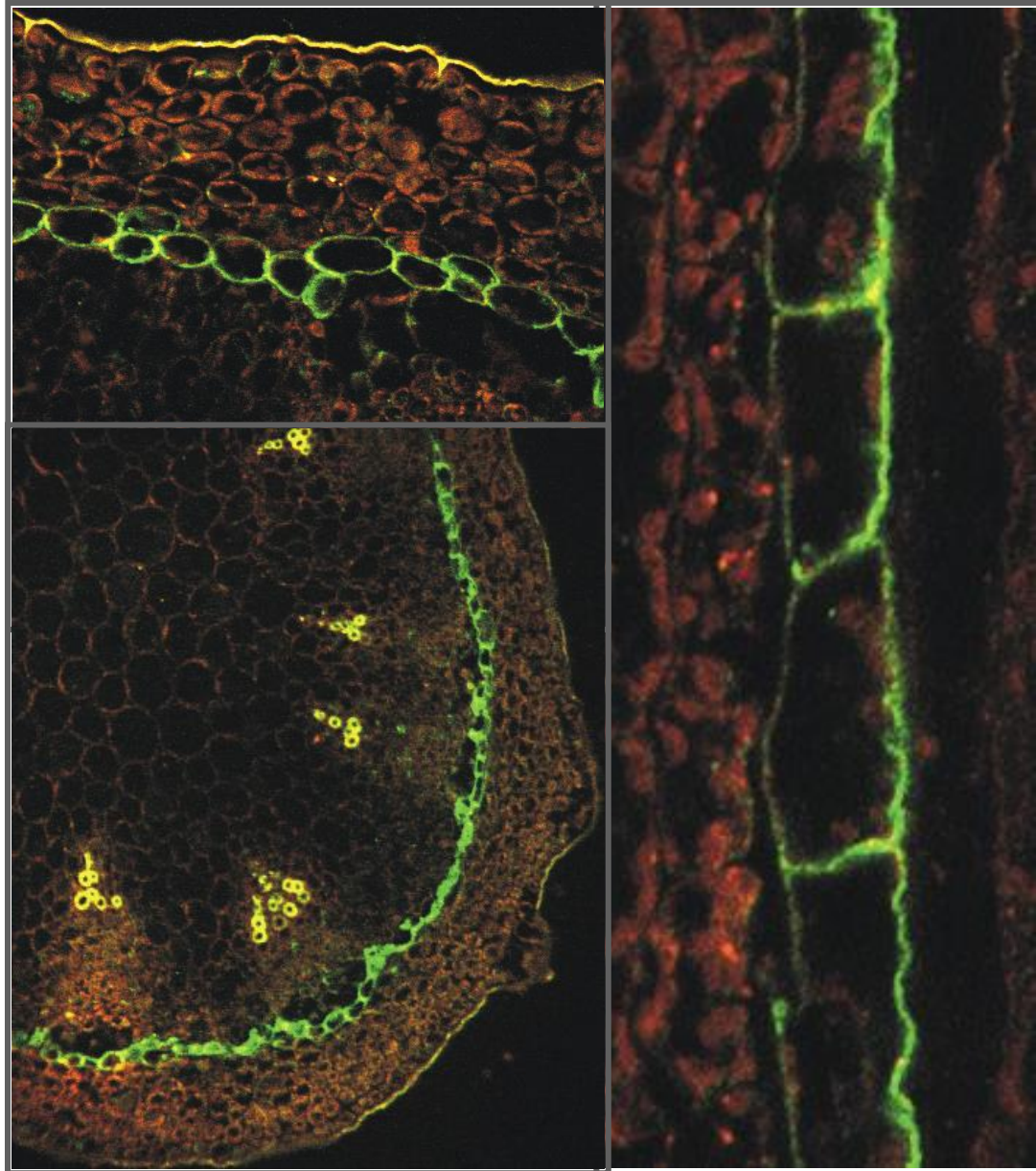
in situ RNA hybridization



PIN3 in Inflorescence Axis

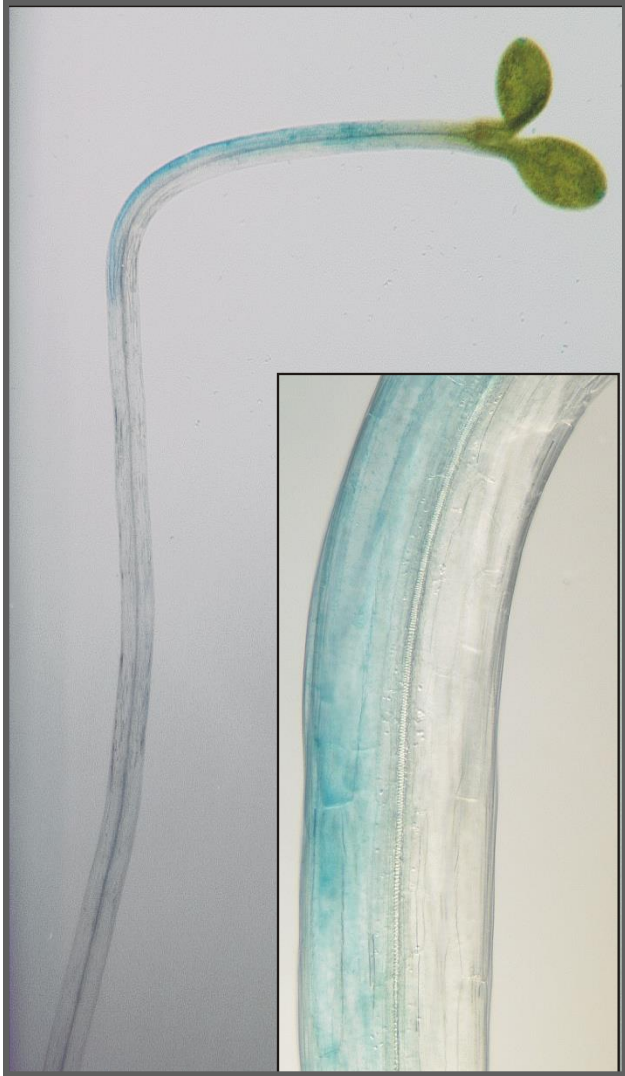
PIN3 protein

in situ RNA hybridization

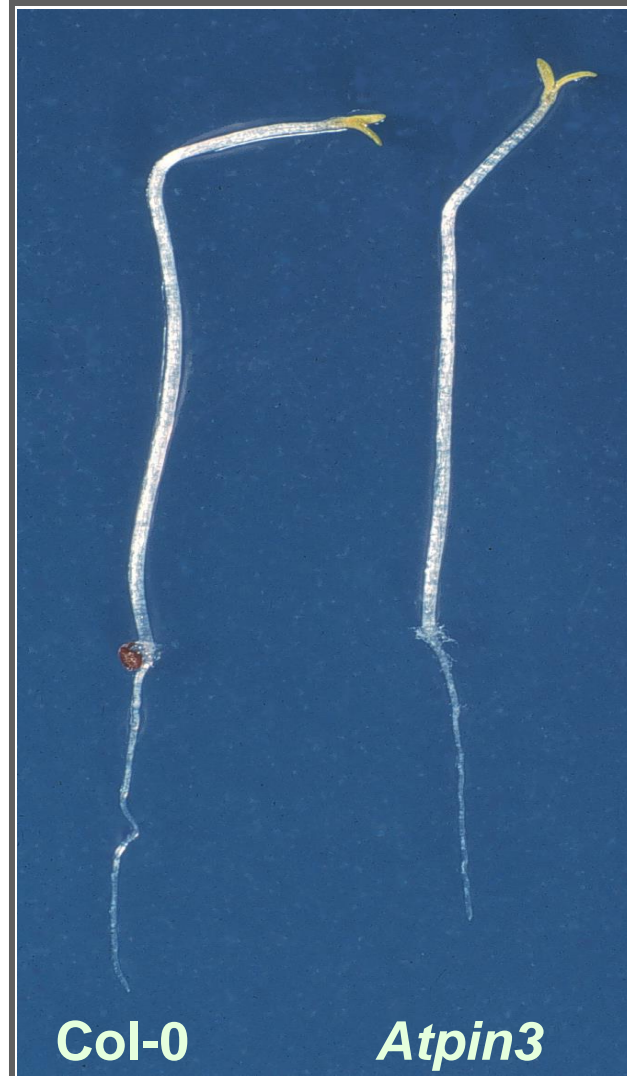


PIN3 – Lateral Auxin Transport

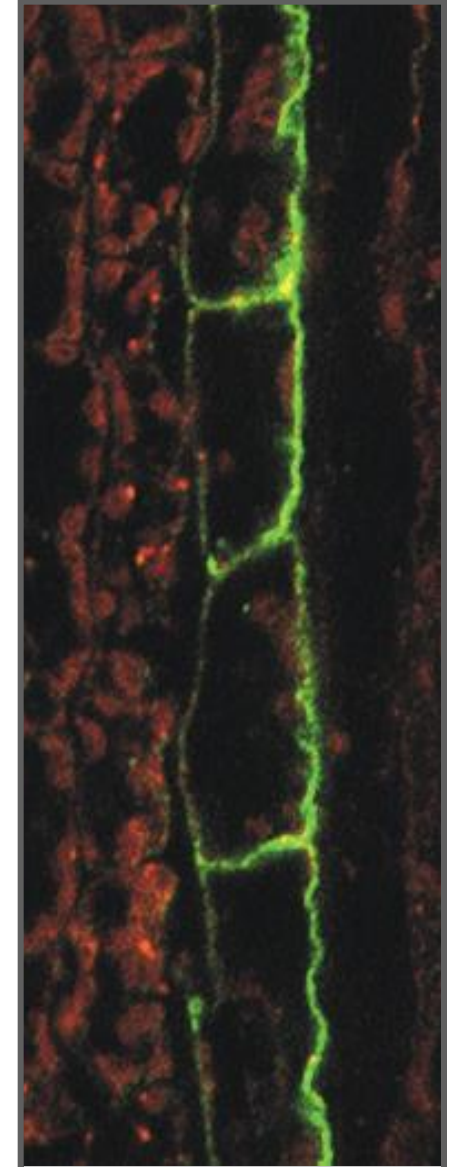
***DR5* - phototropism**



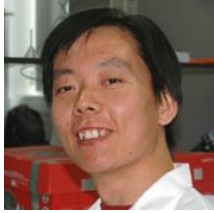
***pin3* phototropism**



PIN3 protein

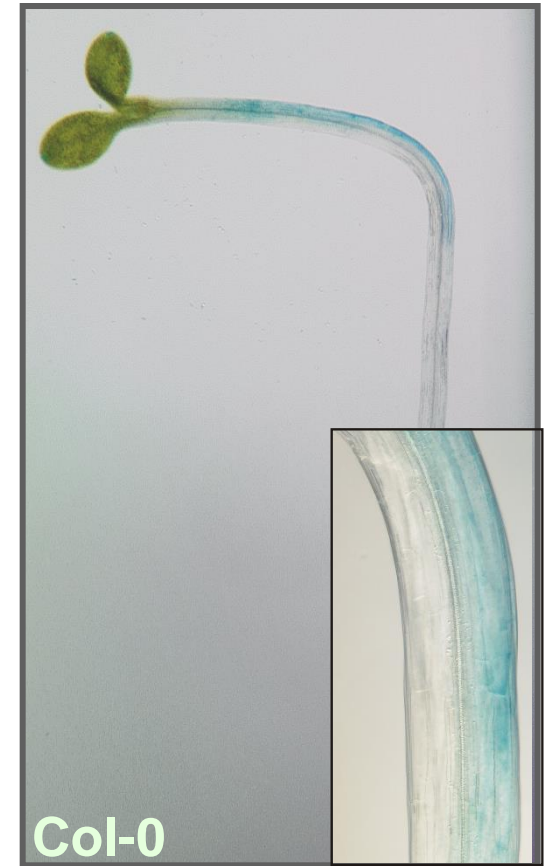
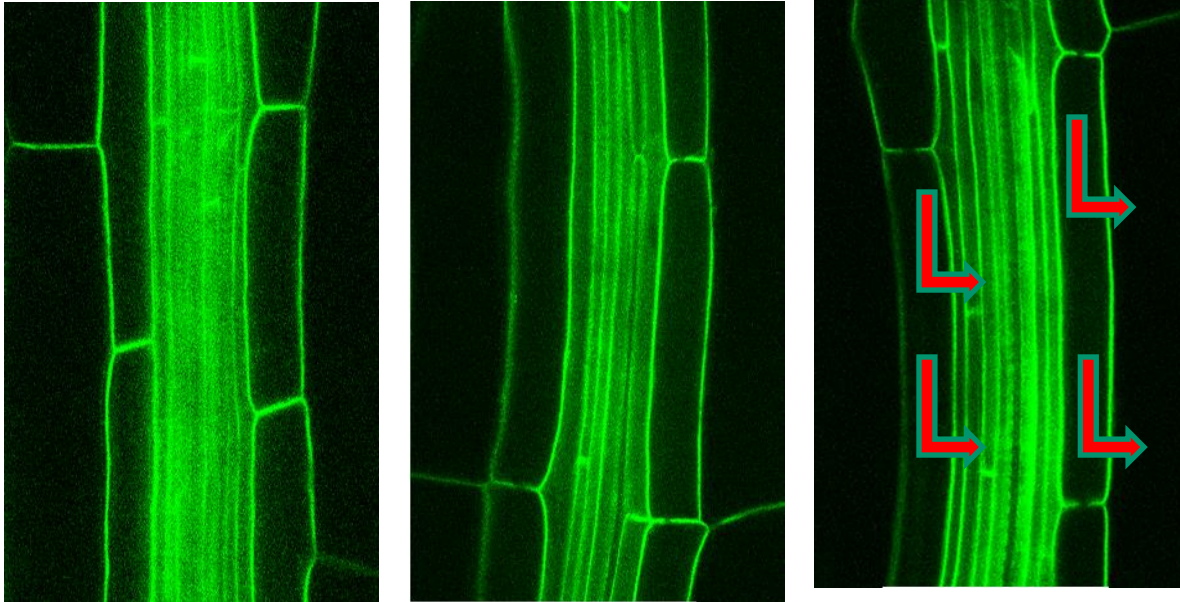


PIN3 in Phototropic Response



Auxin response

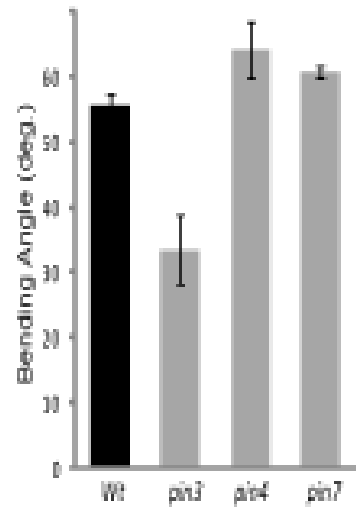
Light-dependent PIN3 relocation



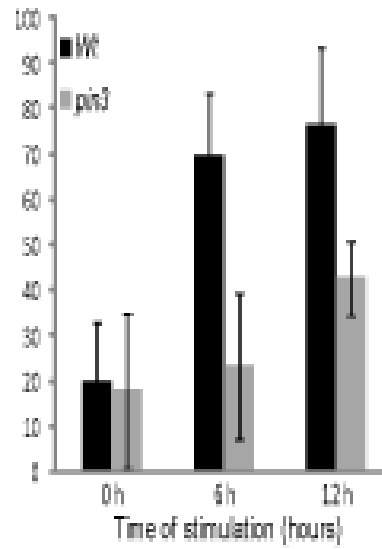
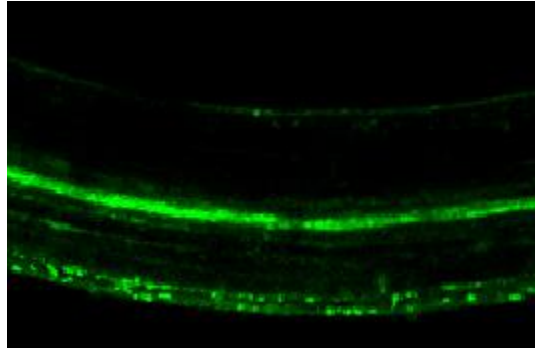
Col-0

Shoot gravitropic response

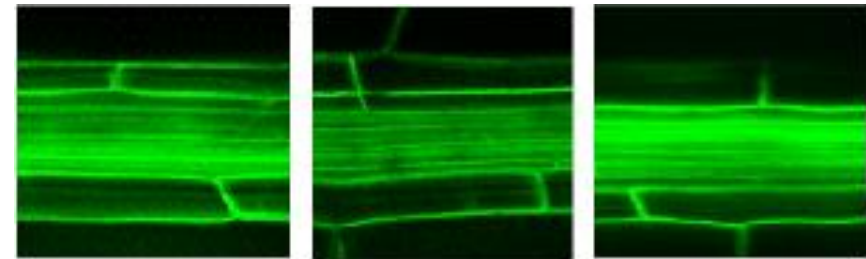
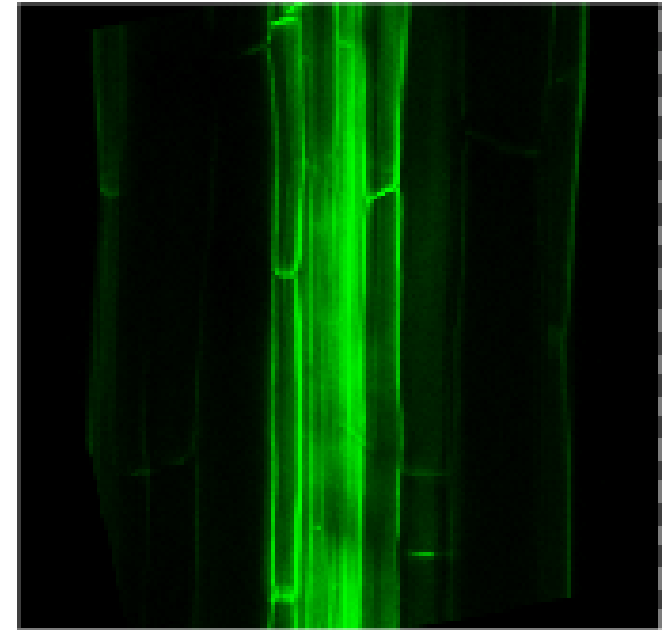
Bending



DR5 response



PIN3 polarization

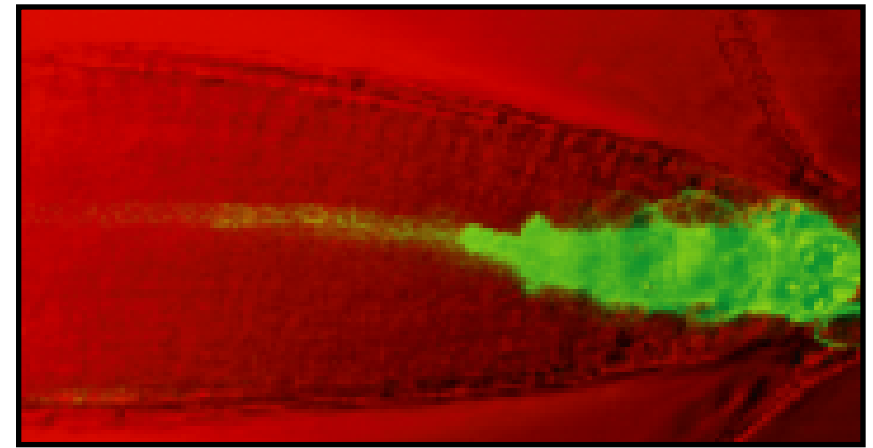


Root Gravotropism

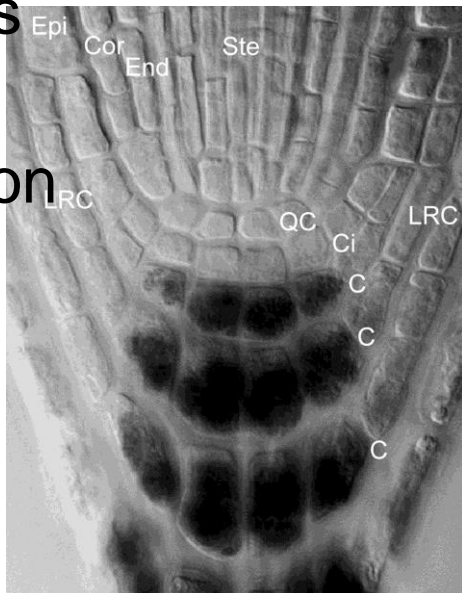
gravity stimulated



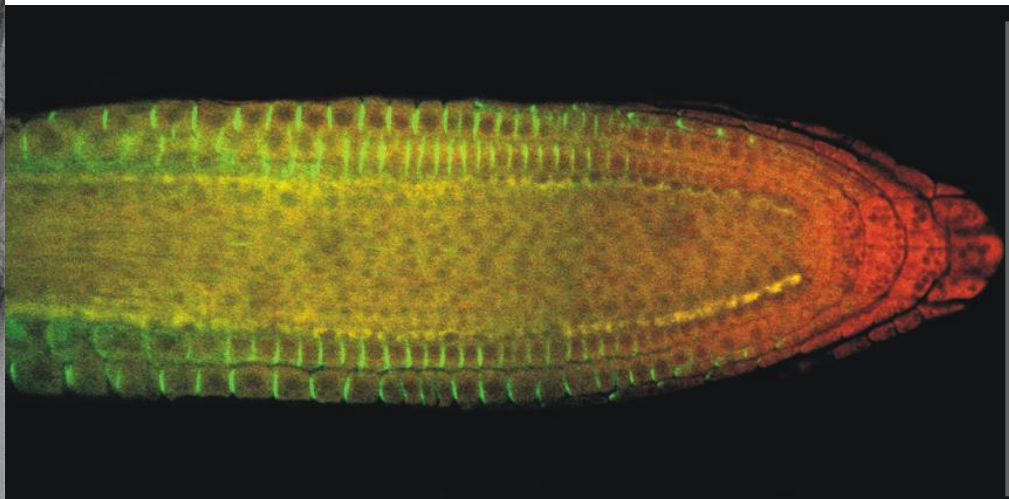
gravity + NPA



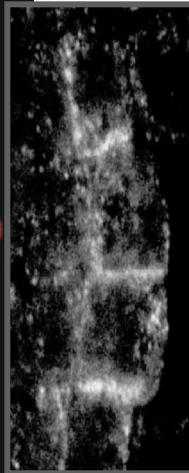
Statoliths
- gravity
perception



PIN2 localization

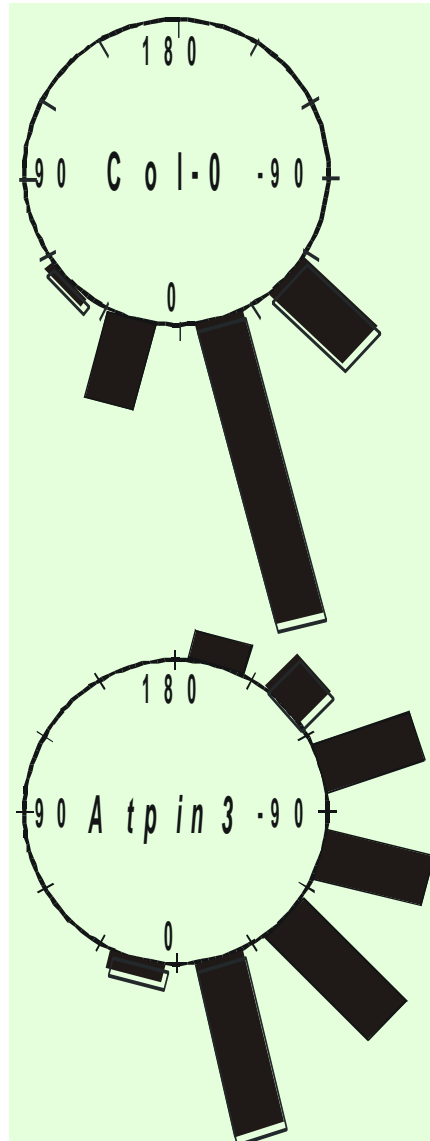


PIN3



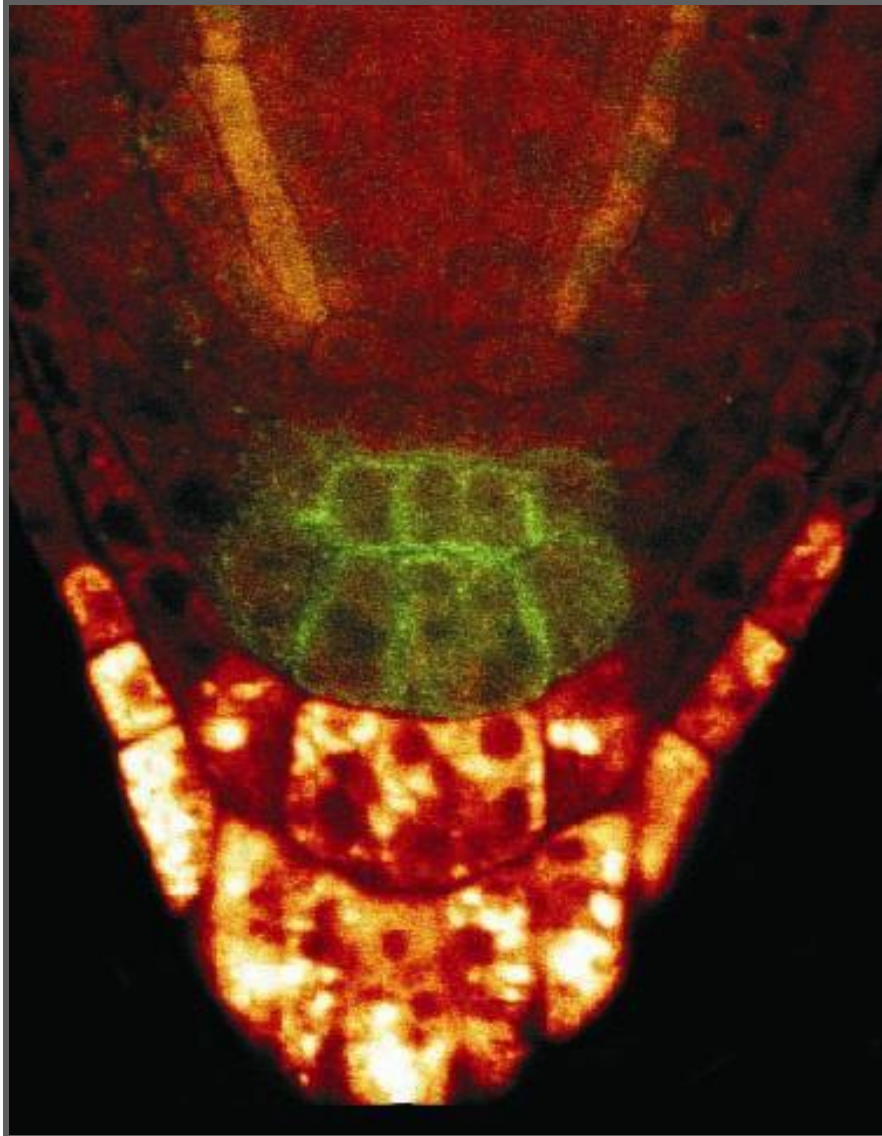
pin3 is Defective in gravitropisms

Root gravitr.

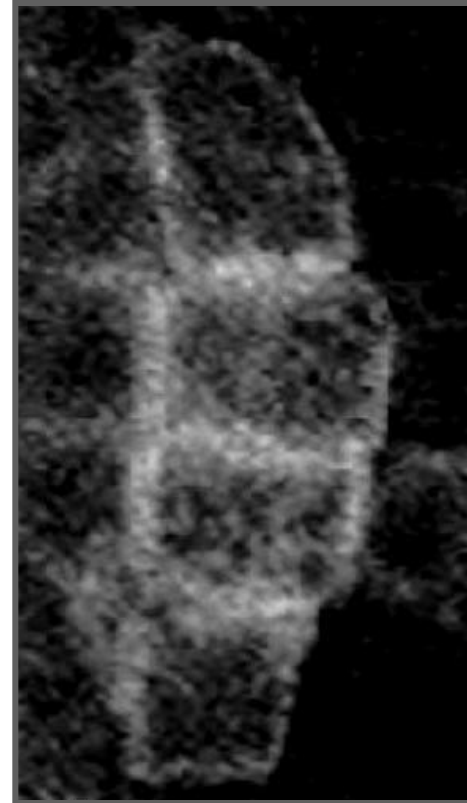


Rapid Relocation of PIN3 during Gravitropism

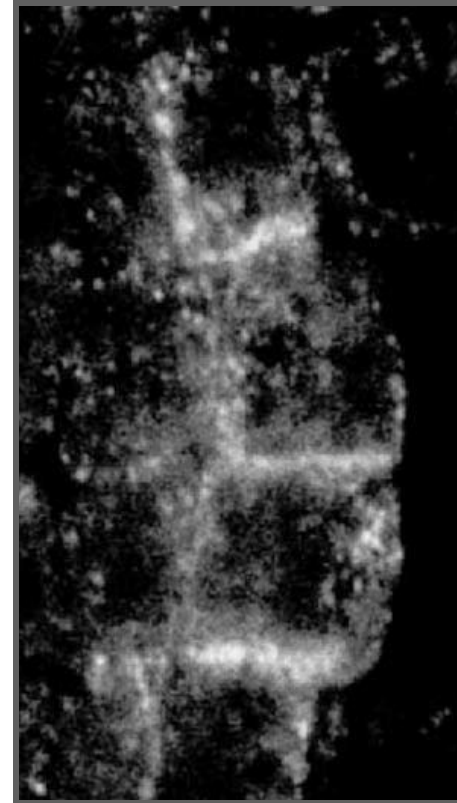
PIN3 in vertical root



PIN3 in root on its side



0 min

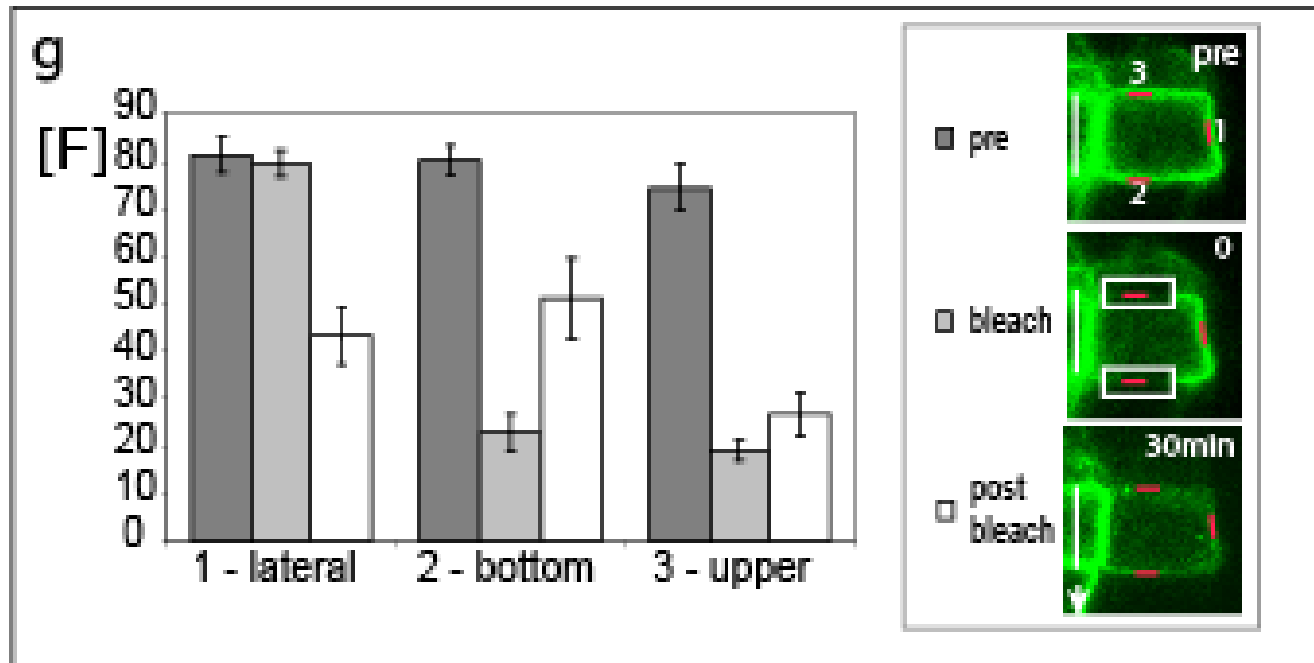
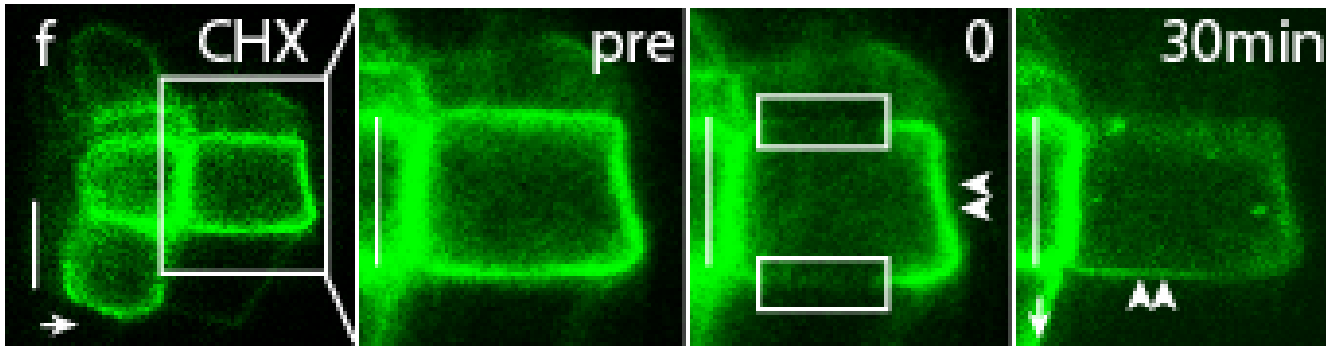


2 min

Gravity-induced PIN3 transcytosis

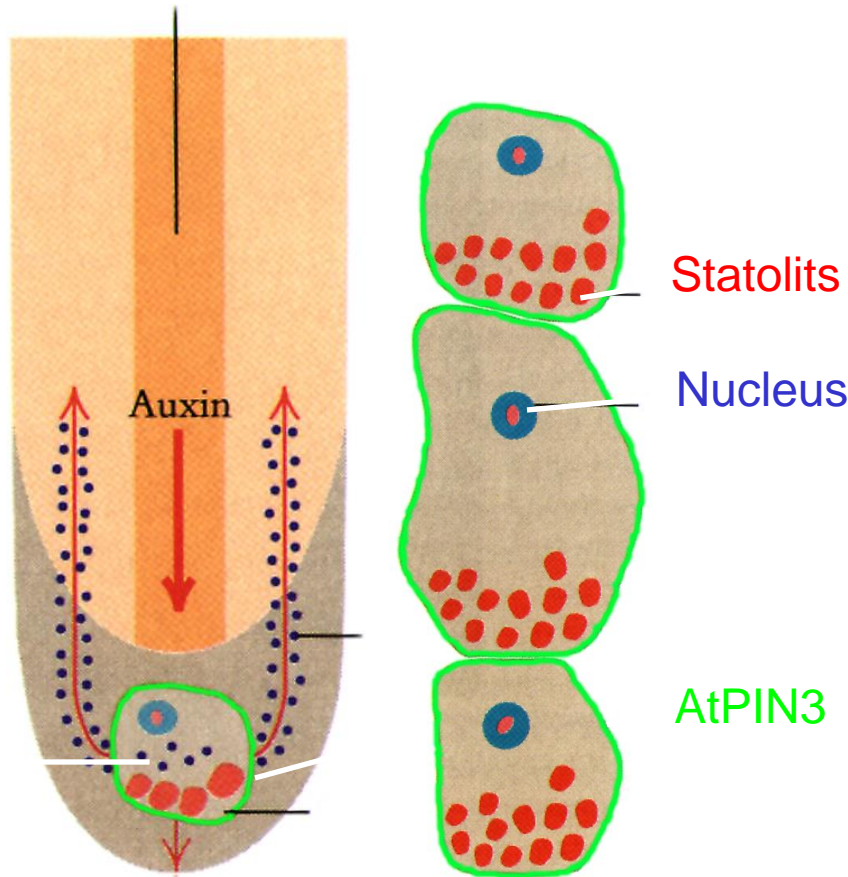


FRAP of PIN3-GFP

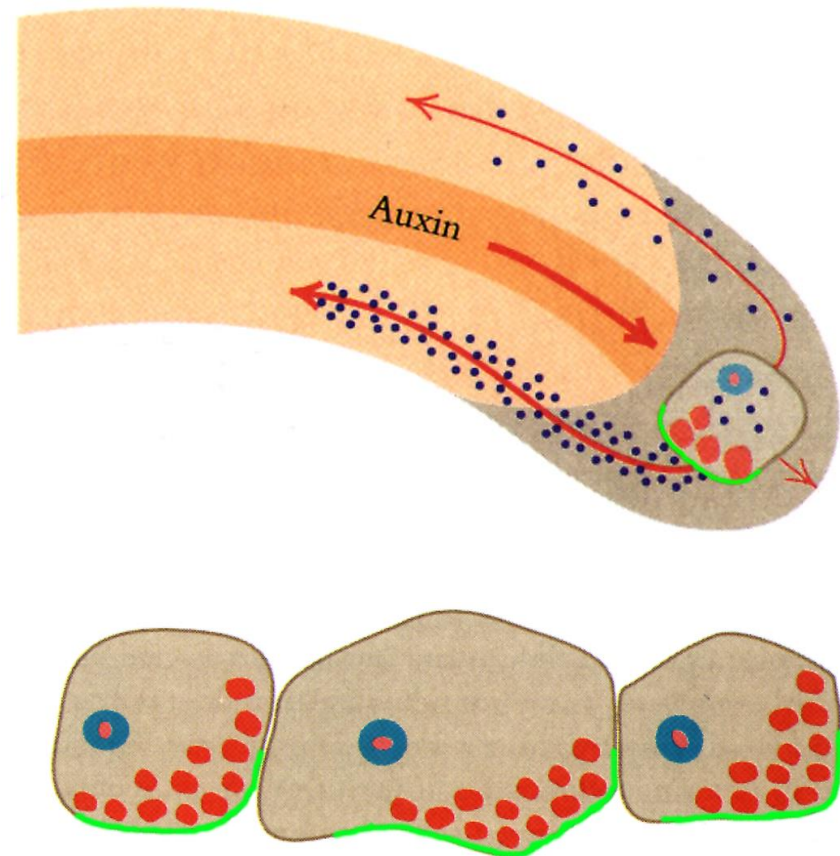


Model for Root Gravotropism

Vertical root



Root turned on its side



Role of Auxin Distribution in Tropisms

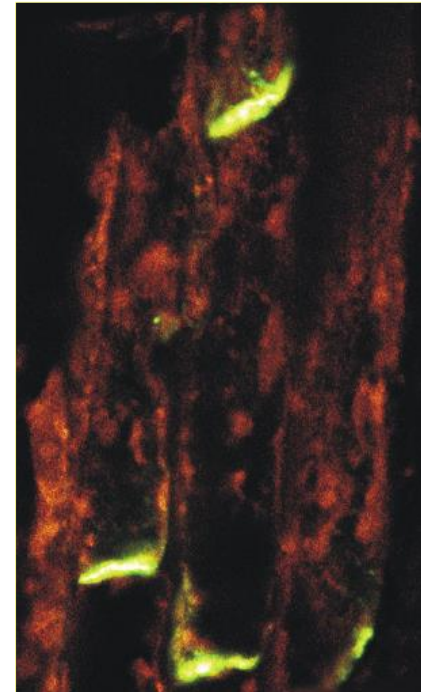
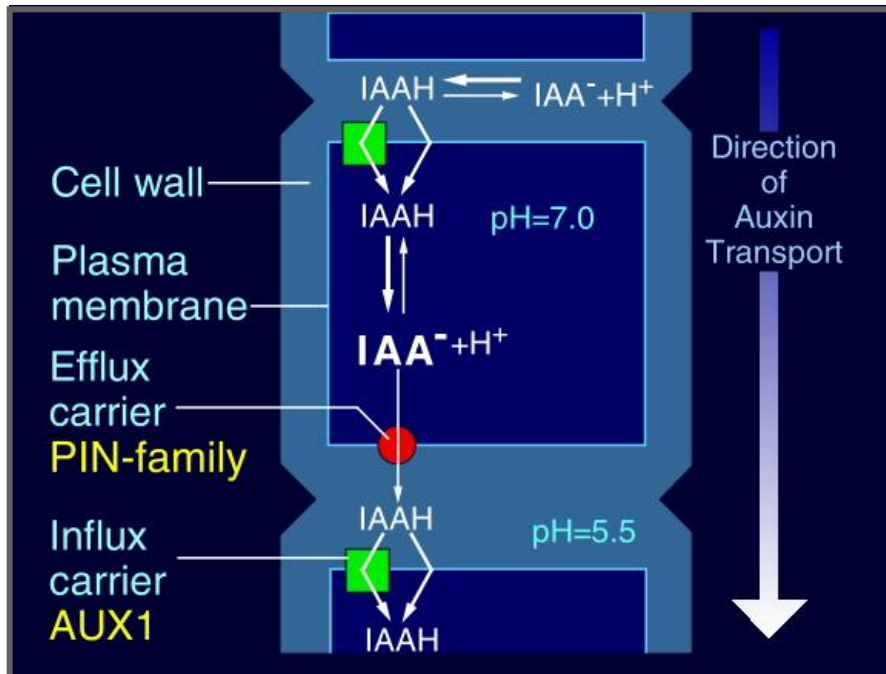
Differential distribution of auxin underlies tropisms

PIN-dependent auxin transport acts upstream of auxin distribution

External signals can be translated into redirection of auxin flow by rapid changes of PIN polarity

Downstream auxin signaling decides about stimulation or inhibition of growth and thus about positive or negative tropism

Cellular Polarity of PIN Localisation and Directionality of Intercellular Auxin Flow



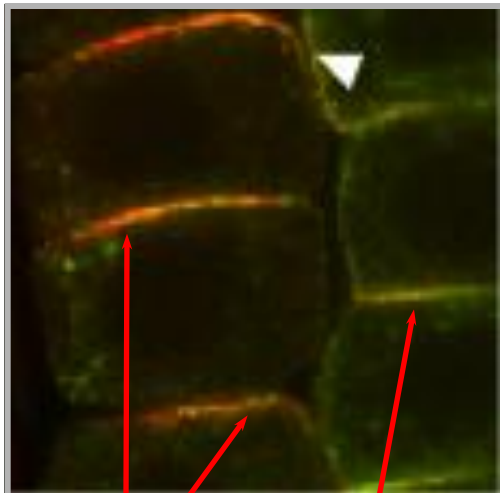
PIN-specific Signals for Polar Targeting



PIN2pr::PIN2:HA

PIN2pr::PIN1:HA

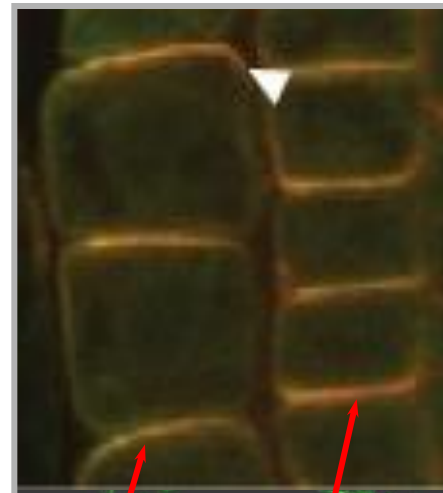
PIN2pr::PIN1:GFP



apical
basal
localization



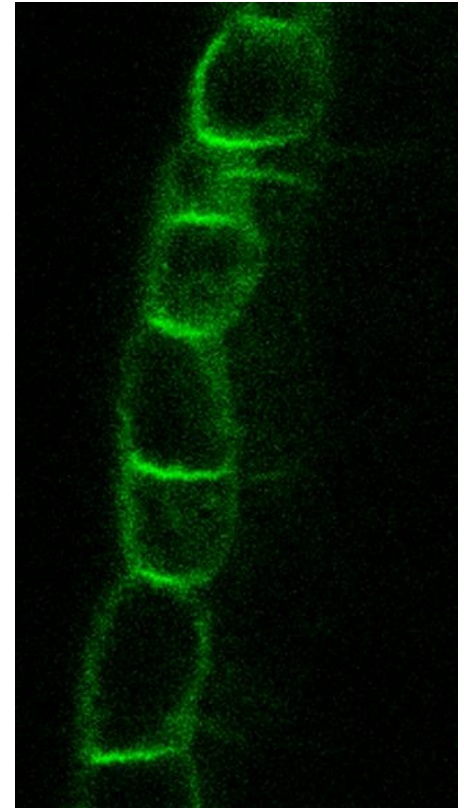
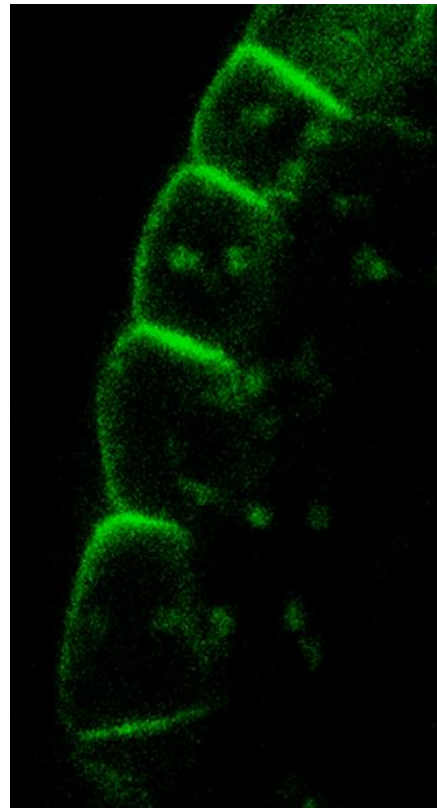
basal
localization



apical
basal
localization

Molecular Components of PIN Polar Targeting

Ser/Thr protein kinase PINOID (PID)



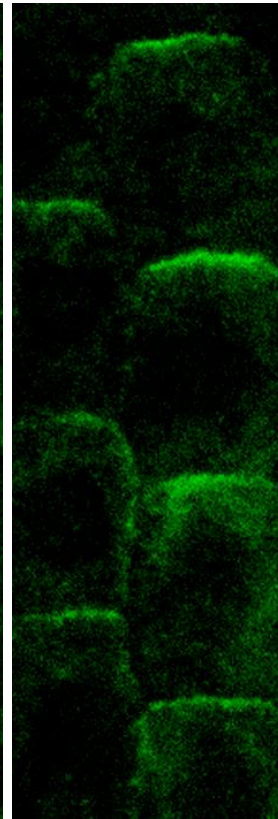
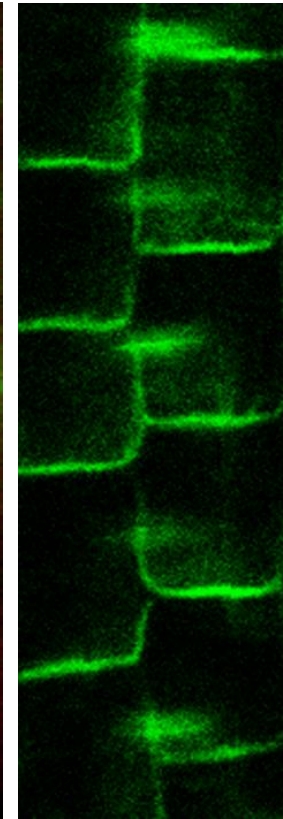
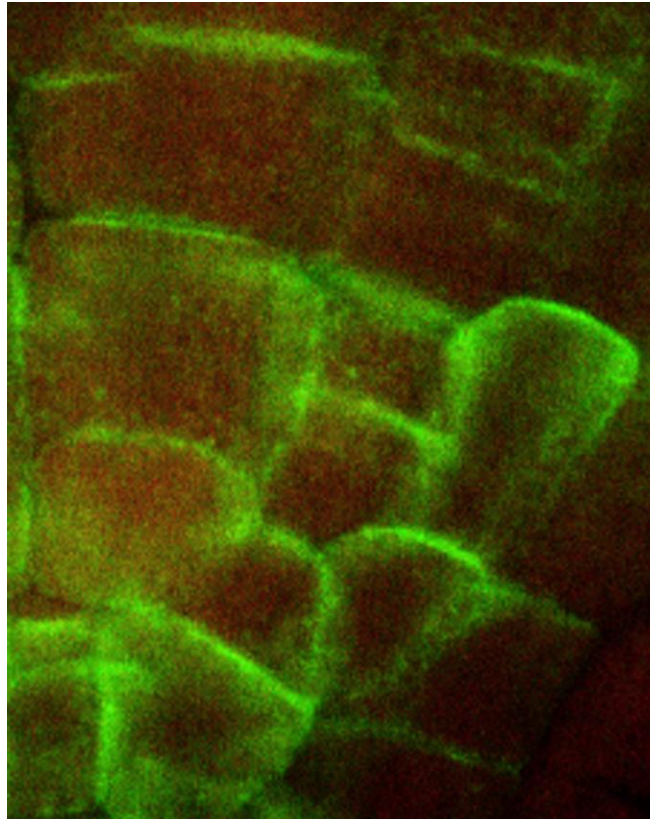
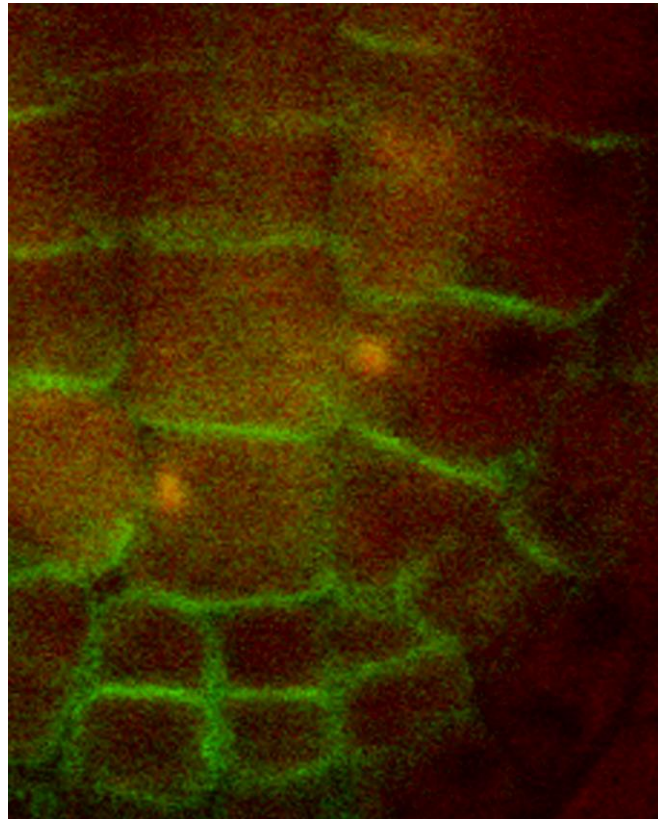
Role of PINOID Kinase in PIN Polar Targeting

Col-0

35S::PID

Col-0

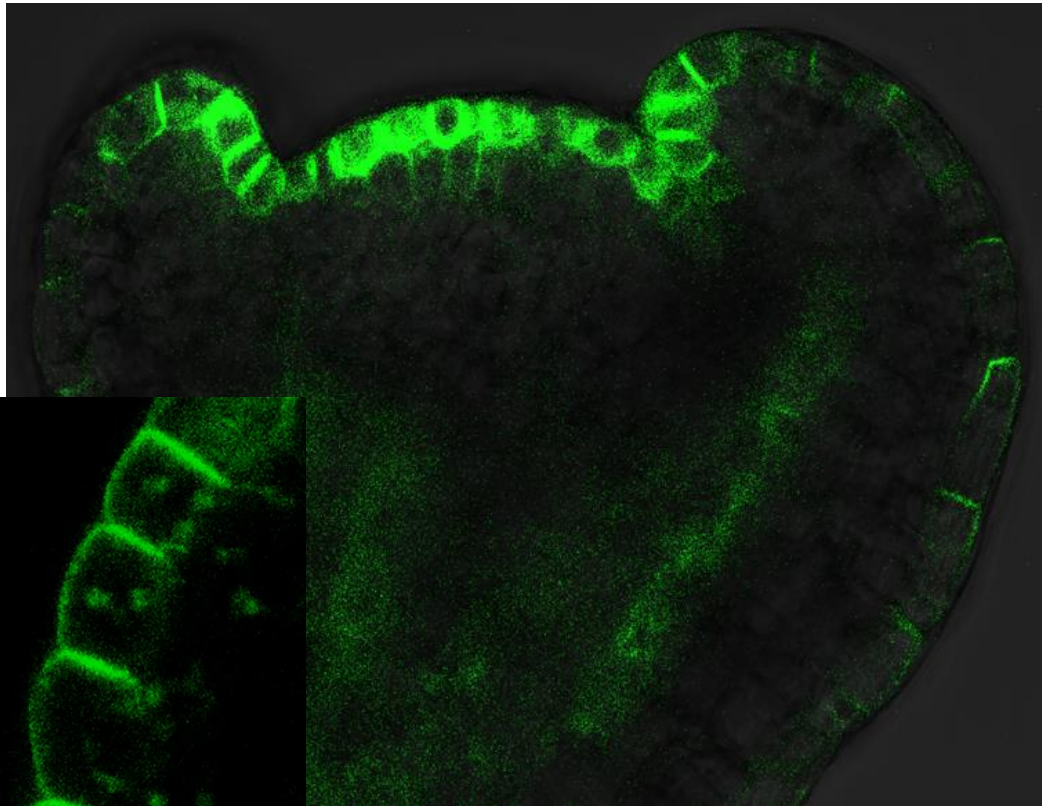
inducible
PID



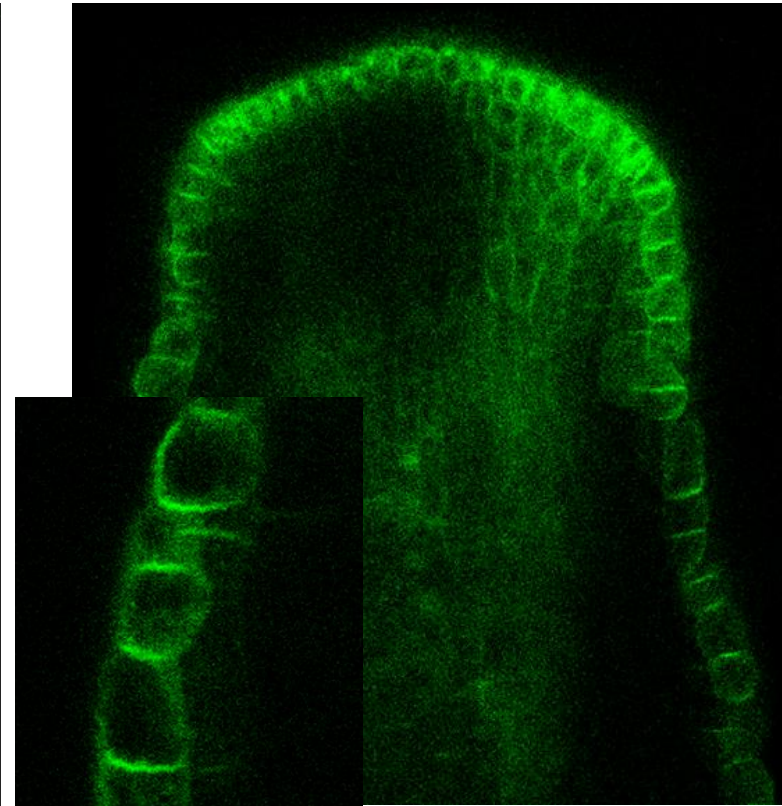
PIN4

PIN1

PINOID kinase loss-of-function > basal PIN targeting

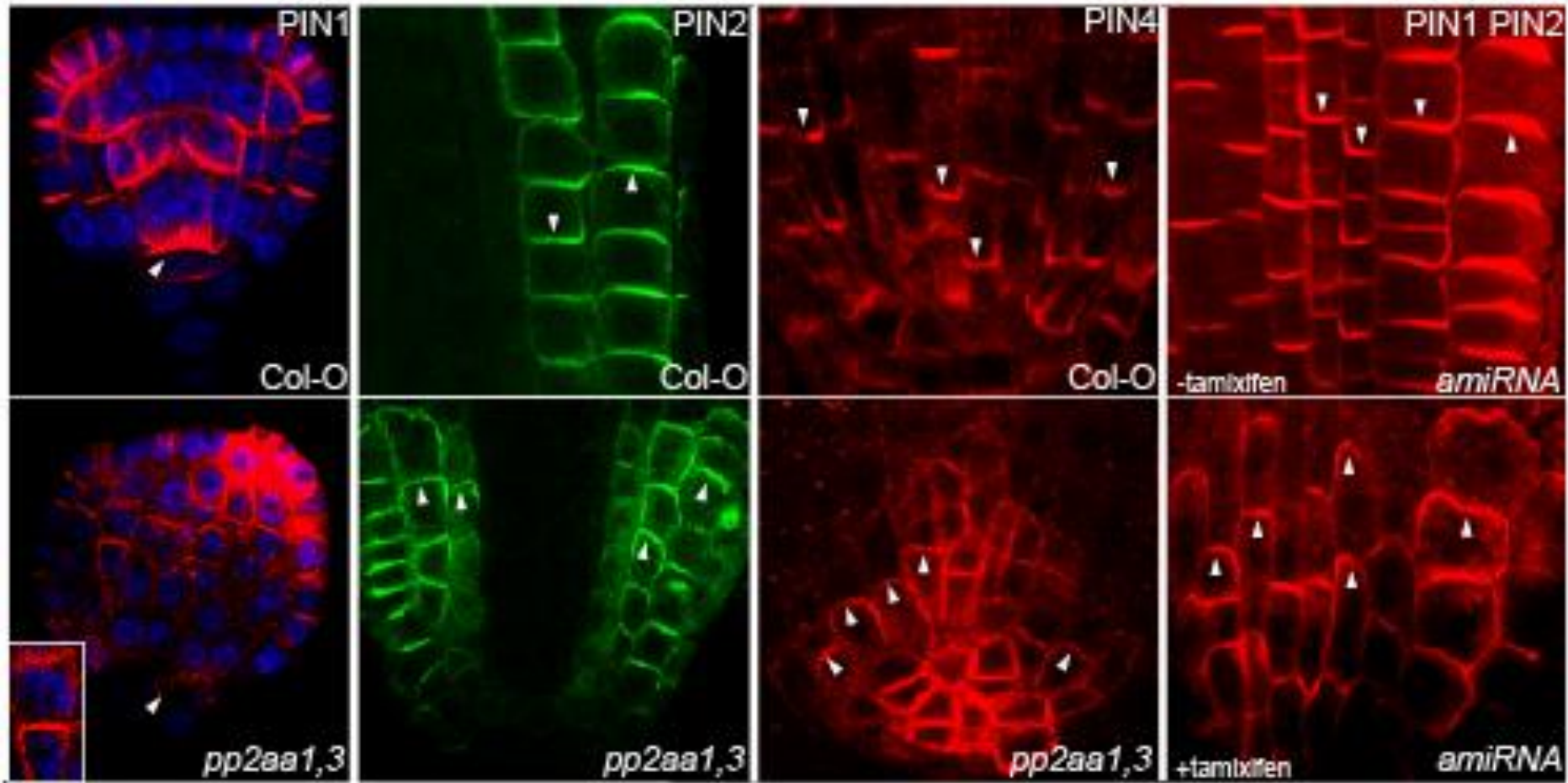


Col-0



pinoid

PP2A Phosphatase and PIN Apical-Basal Targeting

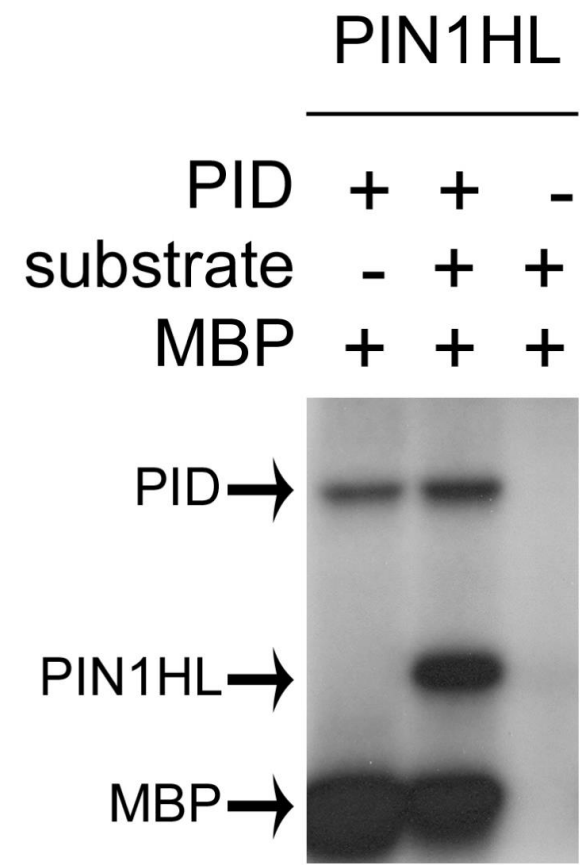
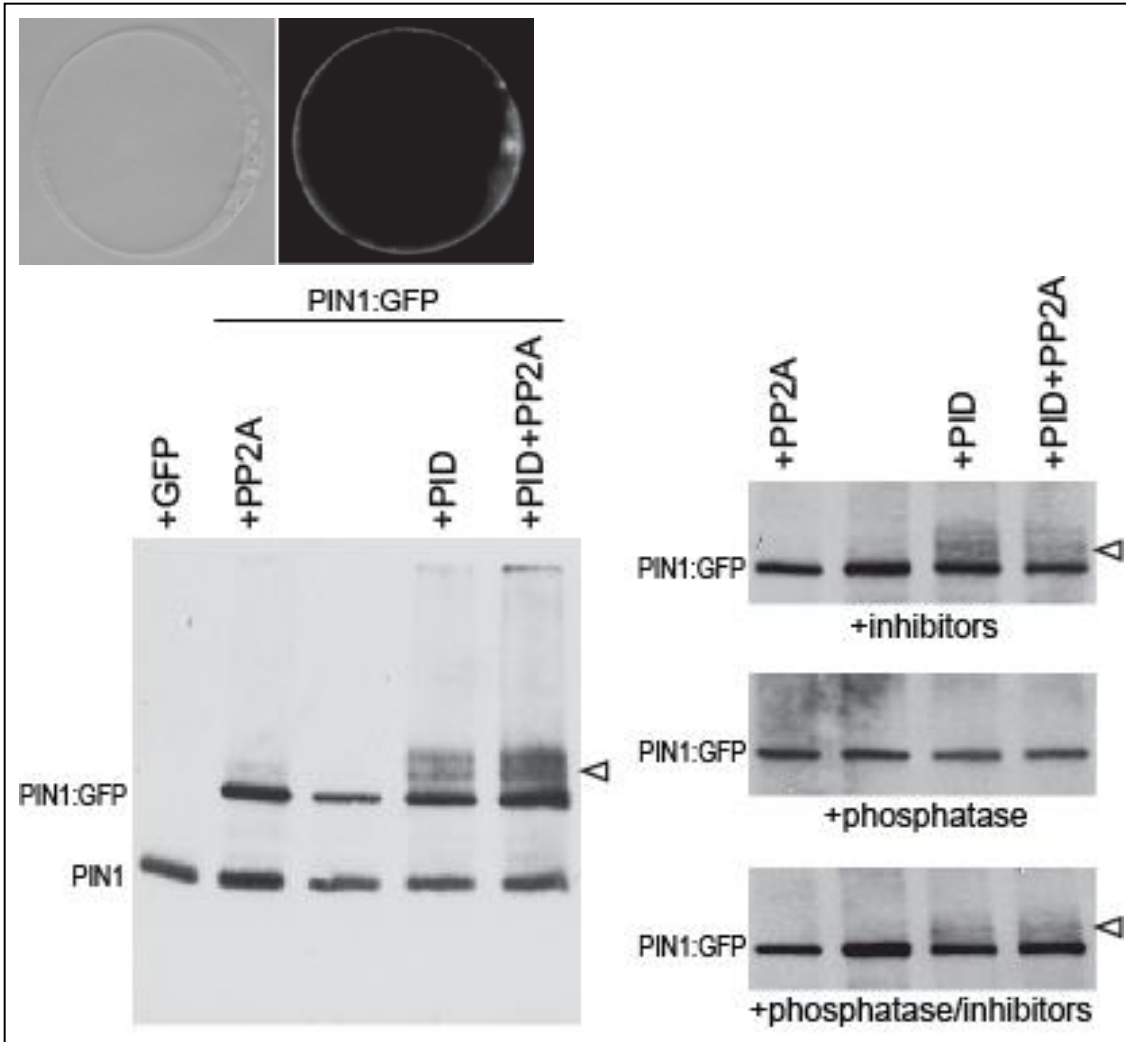


PID Phosphorylates PINs



Phosphorylation assays in protoplast

in vitro phosphorylation

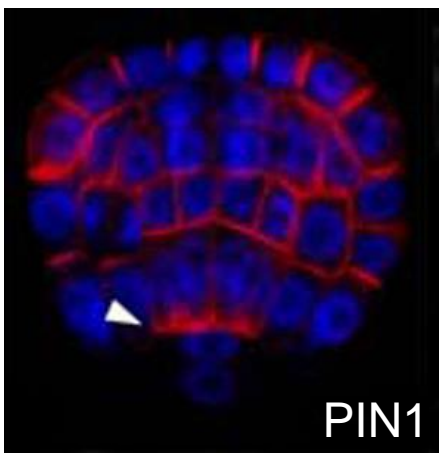




Role of PID in Controlling PIN Polarity > Auxin Flow > Patterning

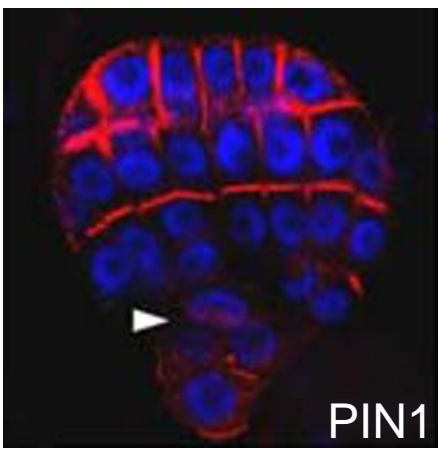


Col-0



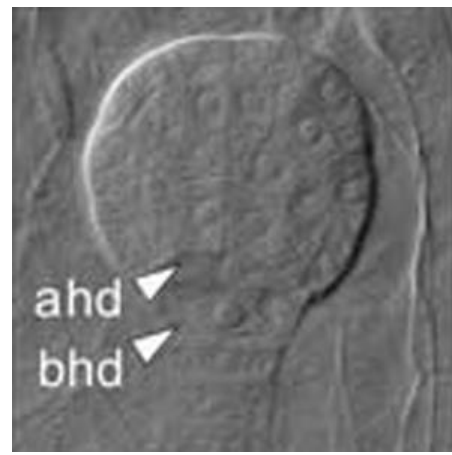
PIN1

RPS5::PID



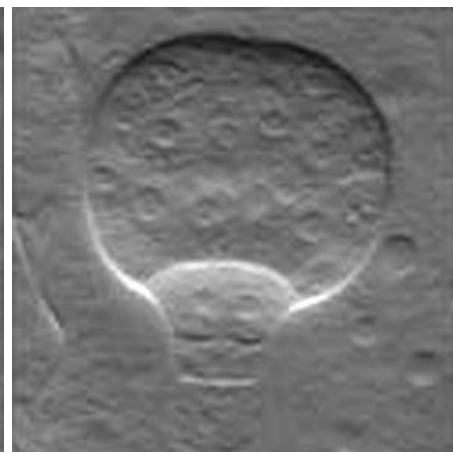
PIN1

Col-0

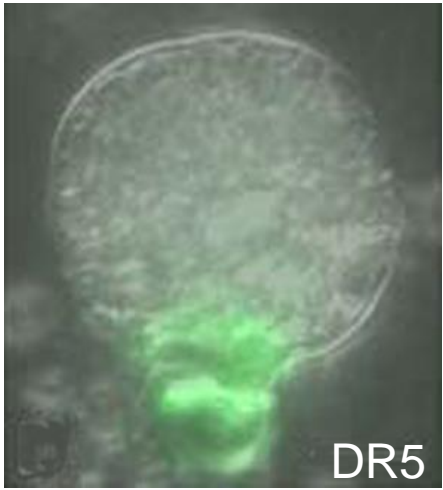


ahd
bhd

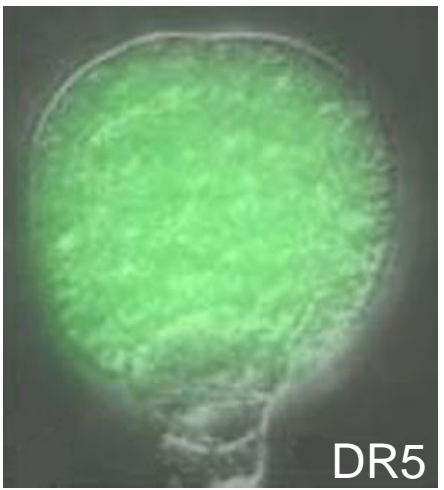
RPS5::PID



RPS5::PID seedlings



DR5



DR5



Cell-biological Determinants

Signals

Gravity

Friml et al. 2002
Kleine-Vehn et al., 2010
Rakusova et al., 2011

Light

Friml et al., 2002
Ding et al., 2011

Develop. context

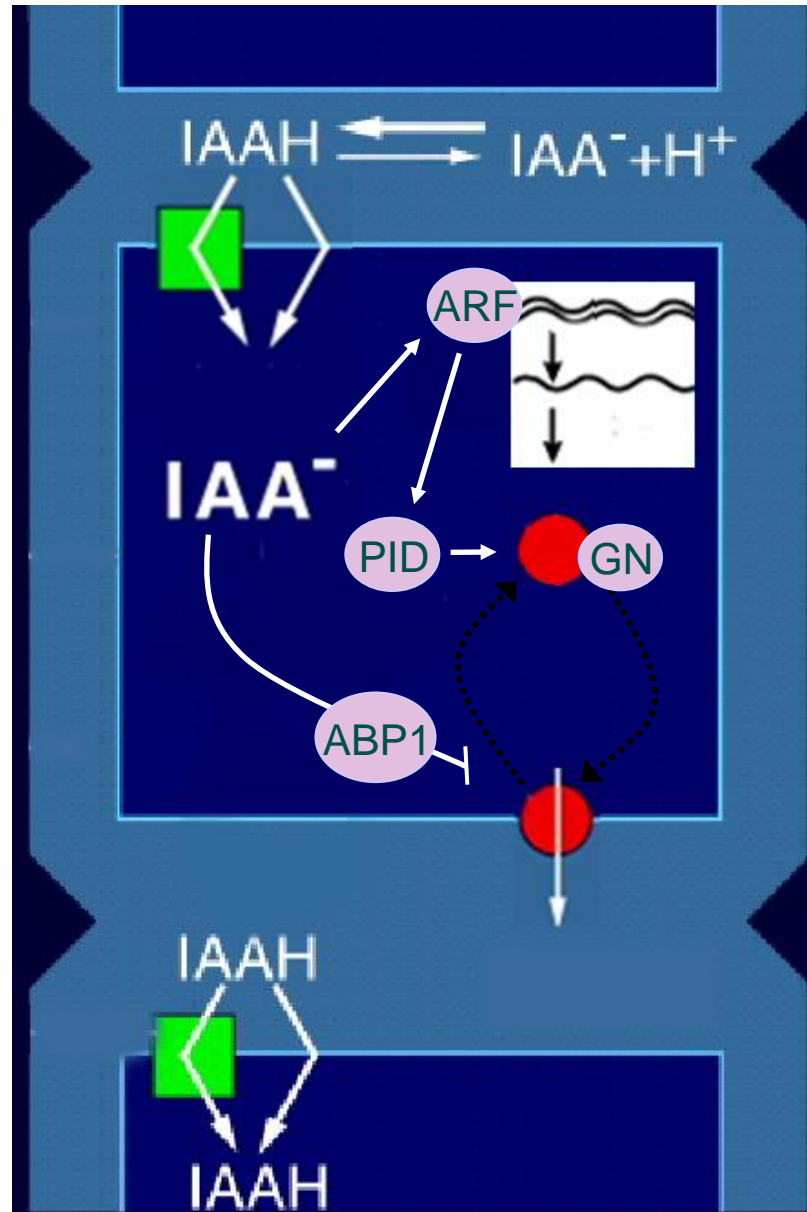
Benková et al. 2003
Friml et al. 2003
Reinhardt et al. 2003

Tissue context

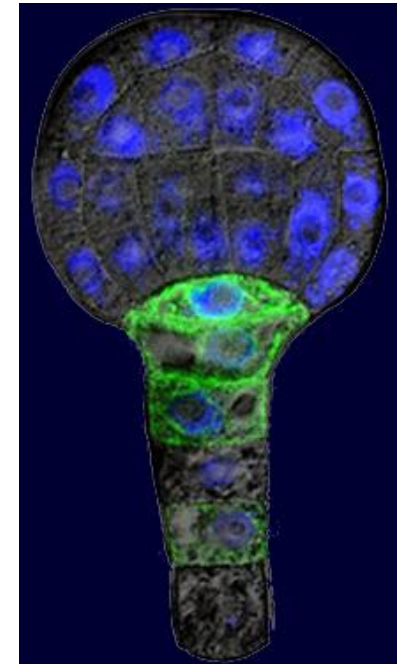
Wisniewska et al., 2006

Feed-back

Sauer et al., 2006
Paciorek et al., 2005
Robert et al., 2010
Wabnik et al., 2010

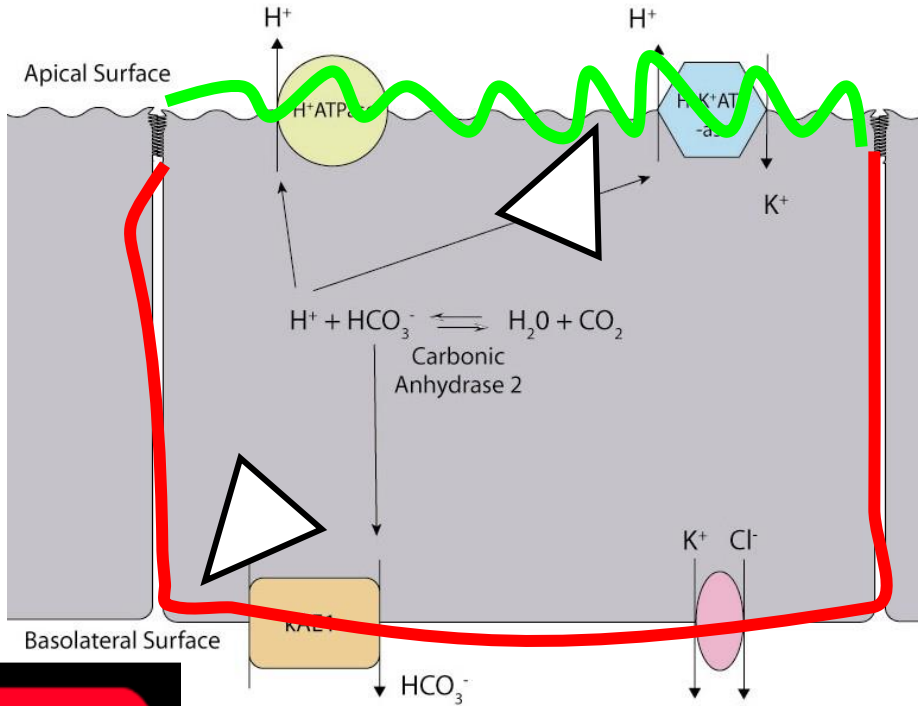


Auxin Gradients

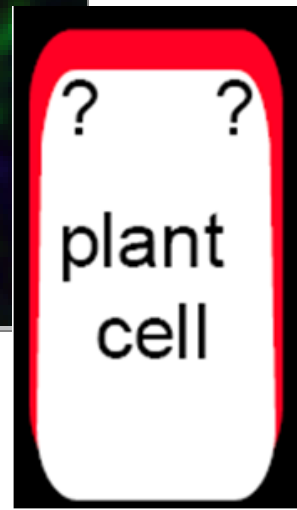
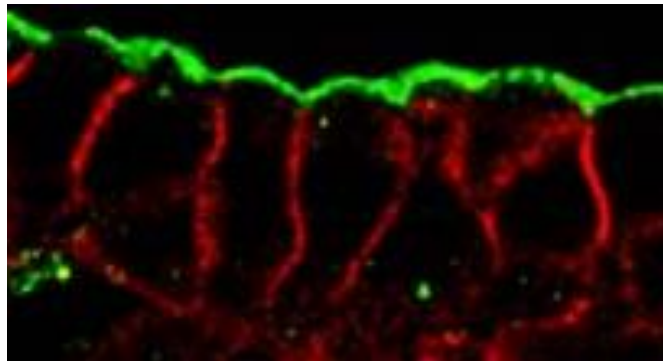
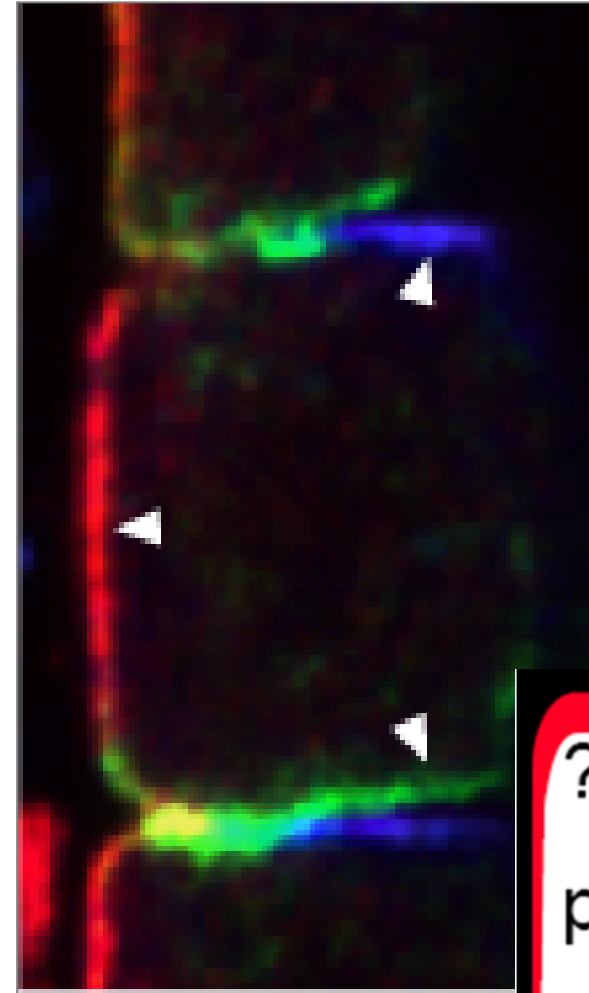


Polar Delivery in Plants

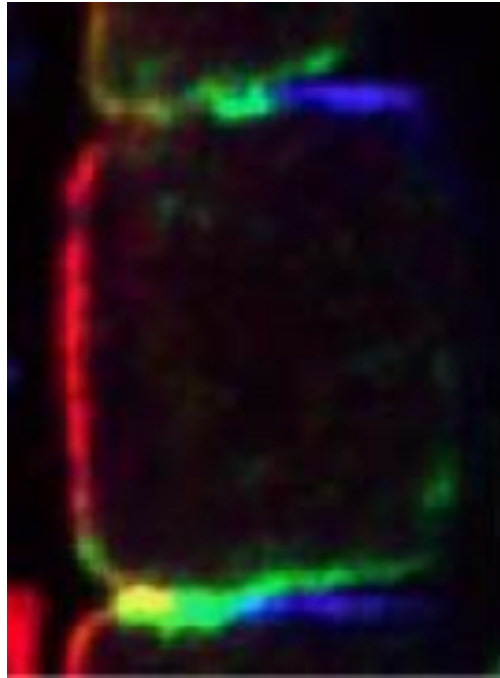
Animal



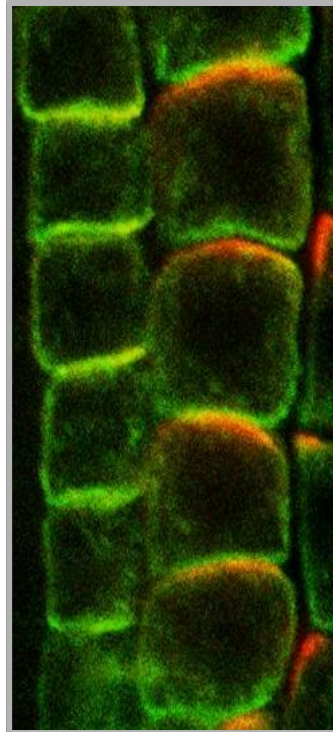
Plant



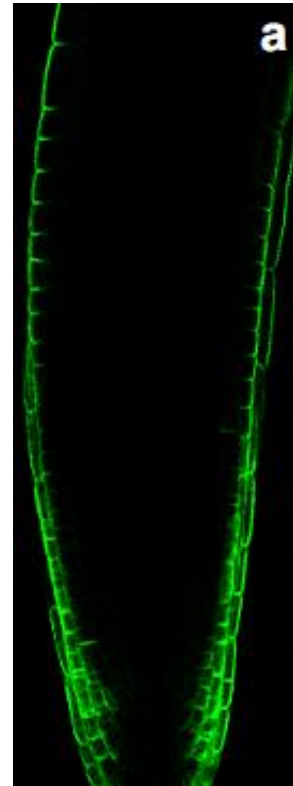
“Plant Epithelium”: Root-Soil Interface Endodermis



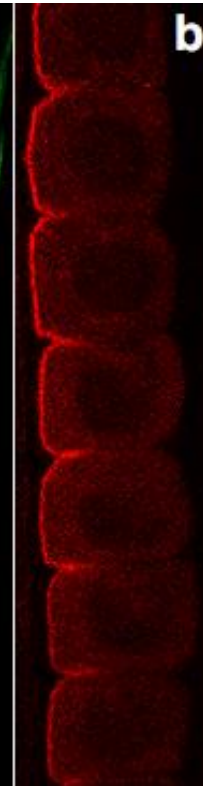
PINs



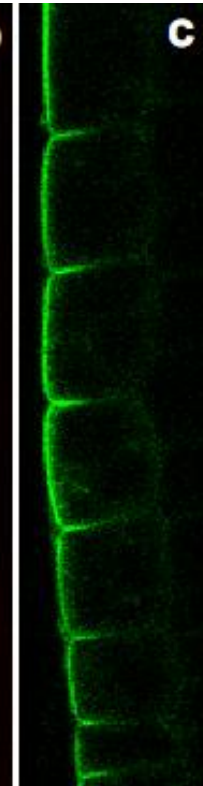
PEN3



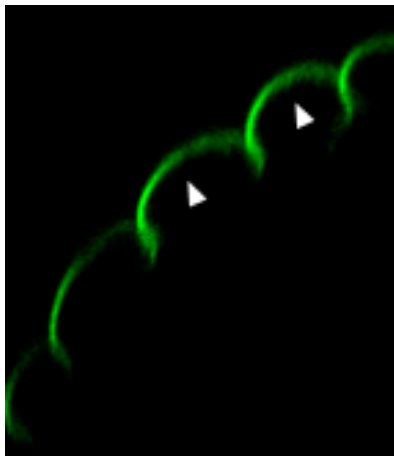
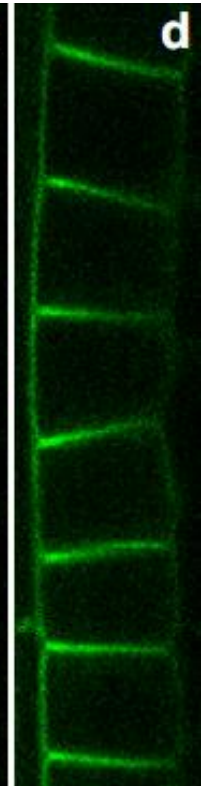
IBA



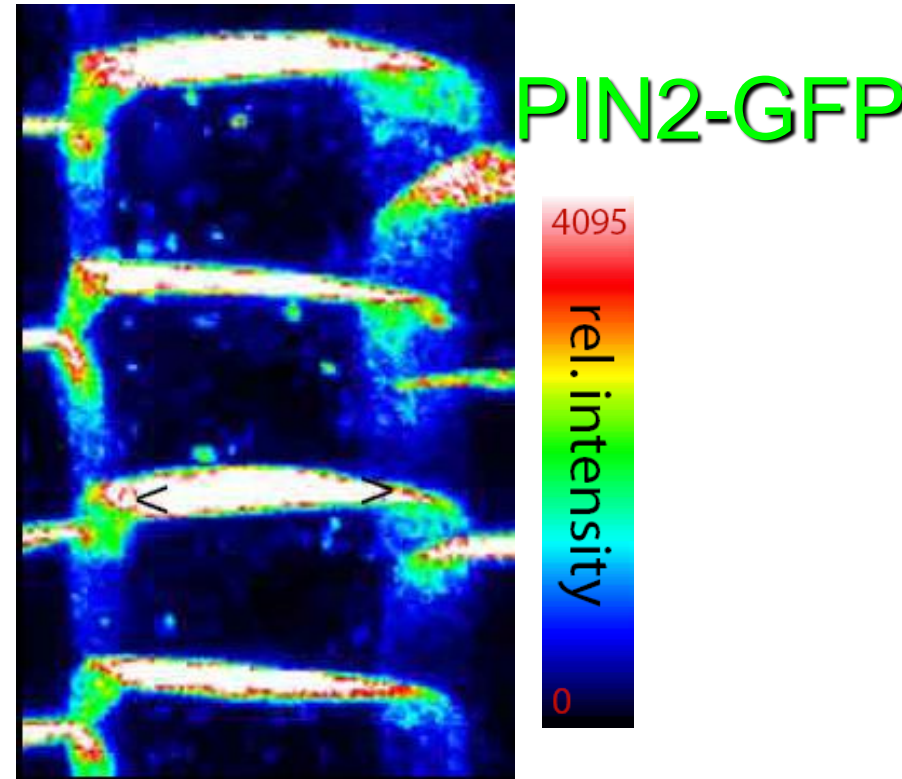
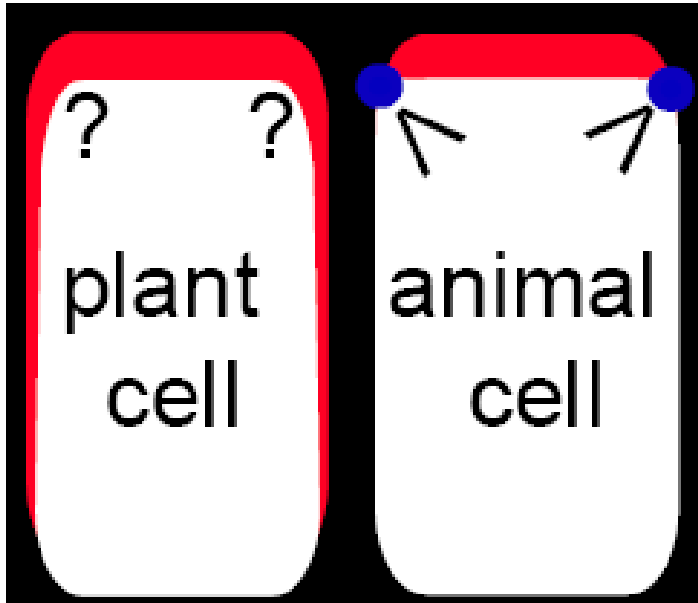
Cd



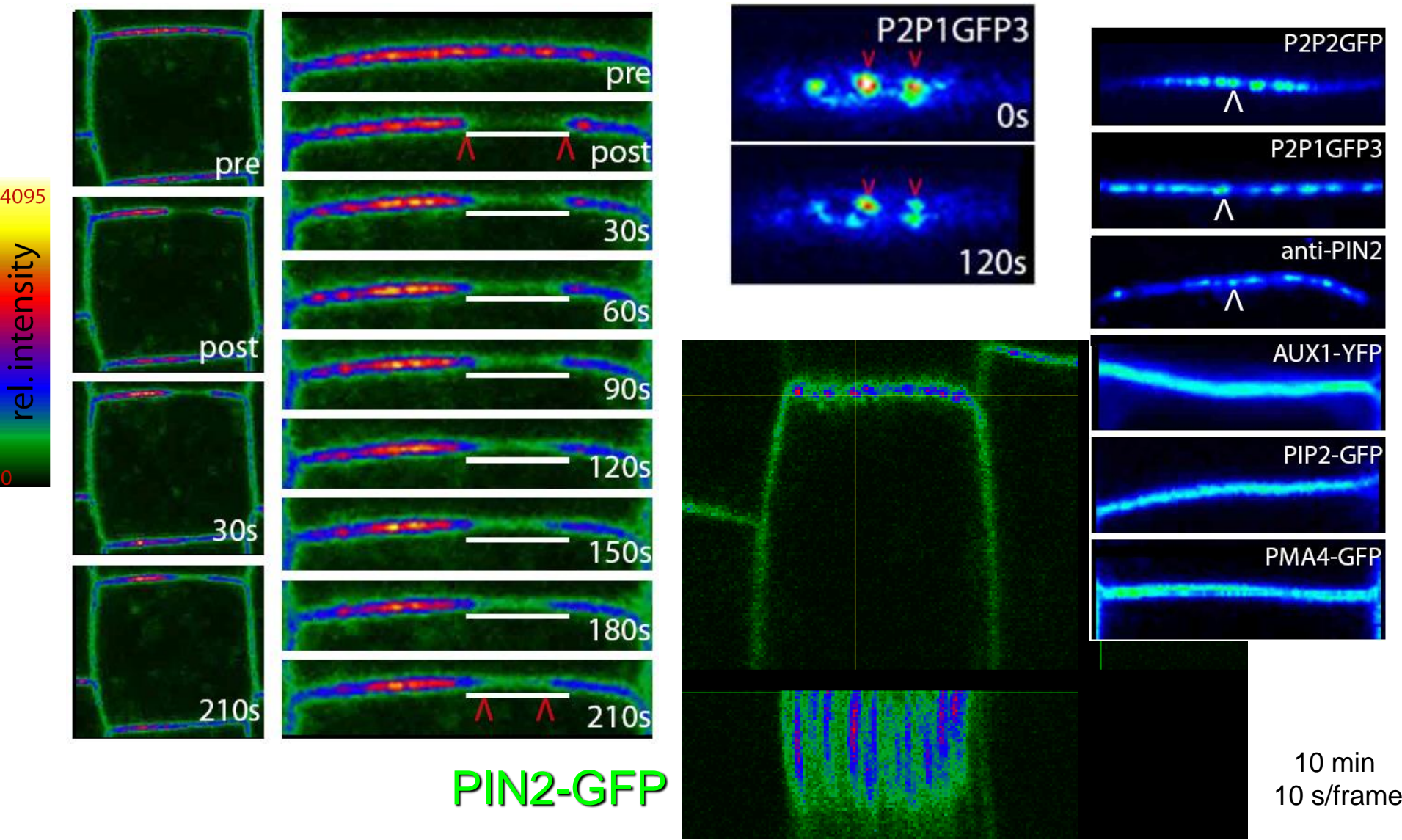
B



Mechanistic Insight into Polar Targeting in Plants

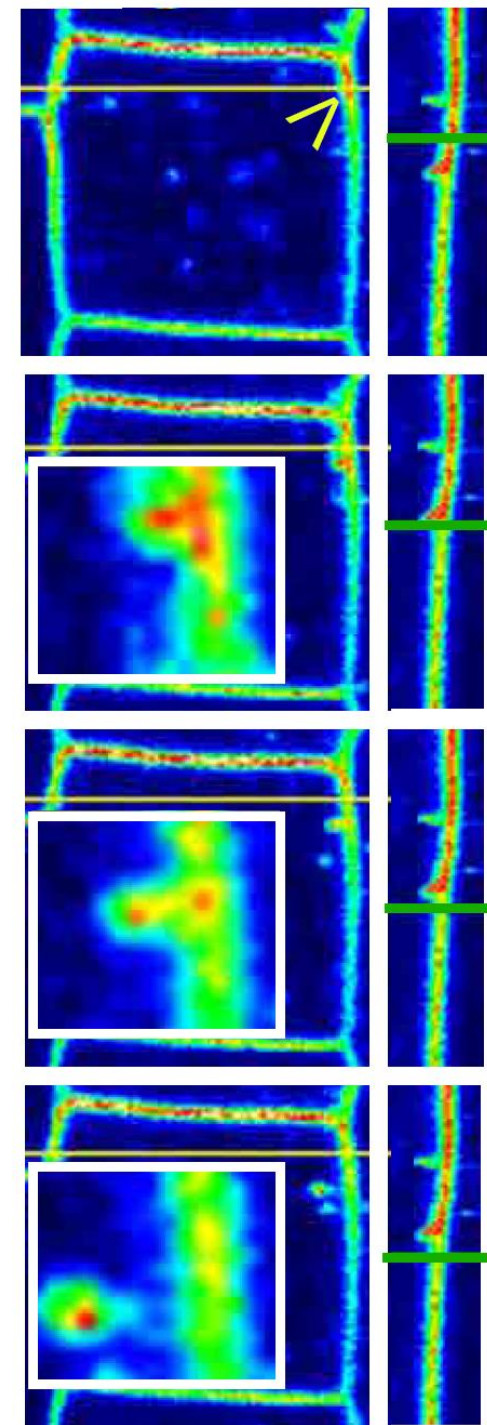
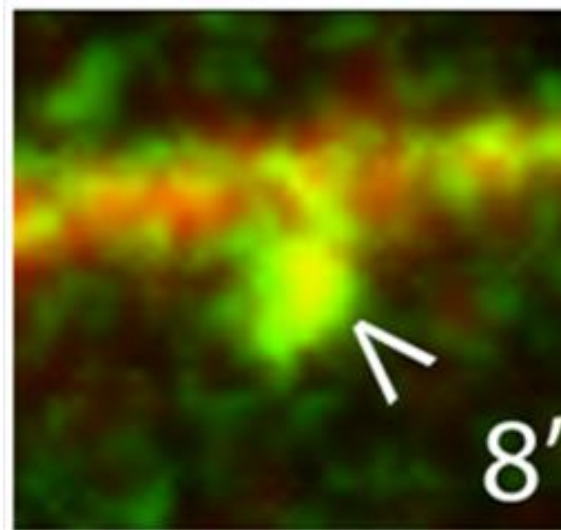
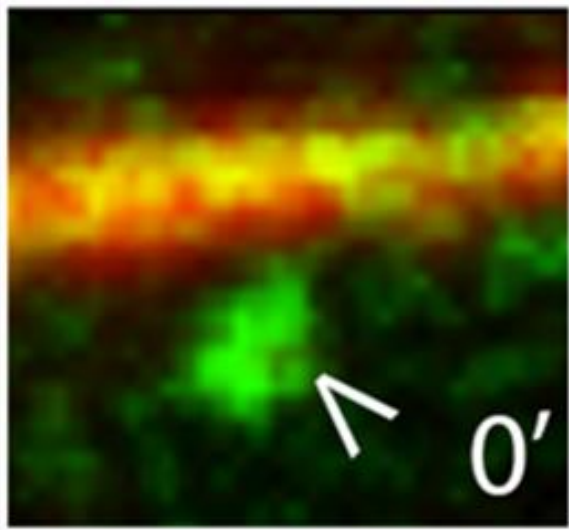
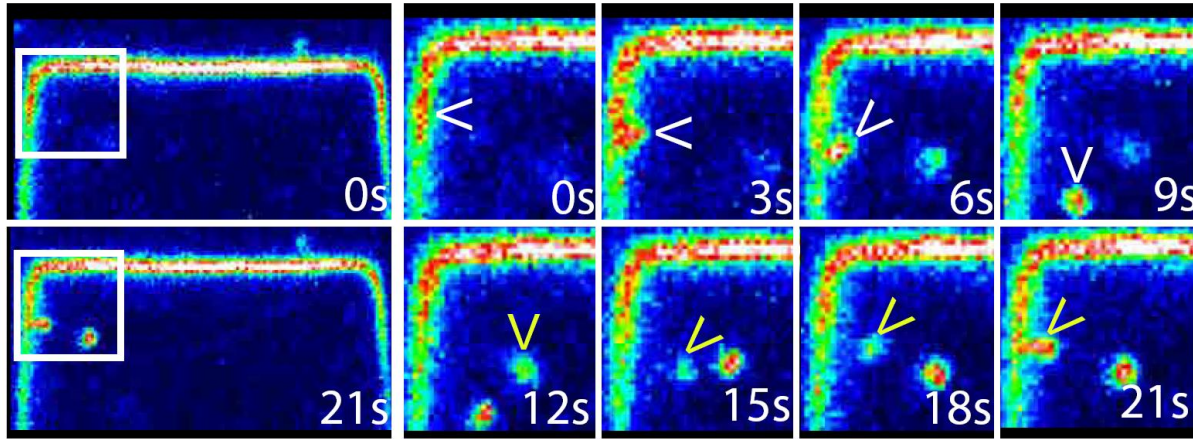


Lateral Diffusion and PIN Clusters



Internalisation „Hot Spots“

4095
rel. intensity
0

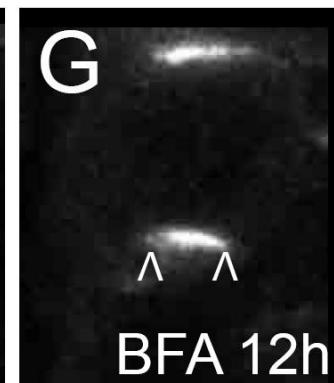
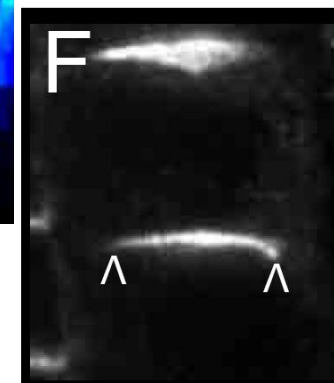
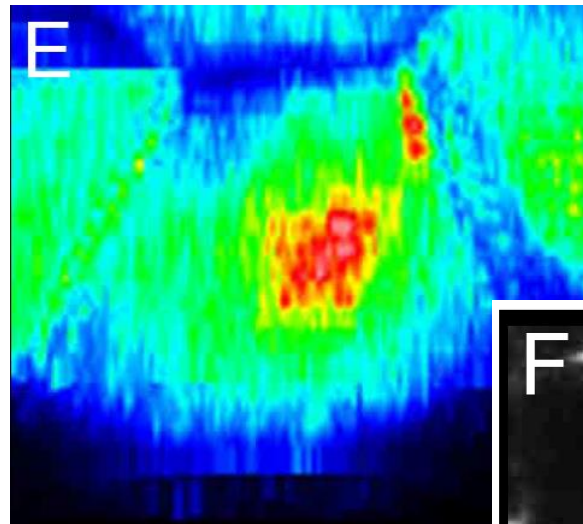
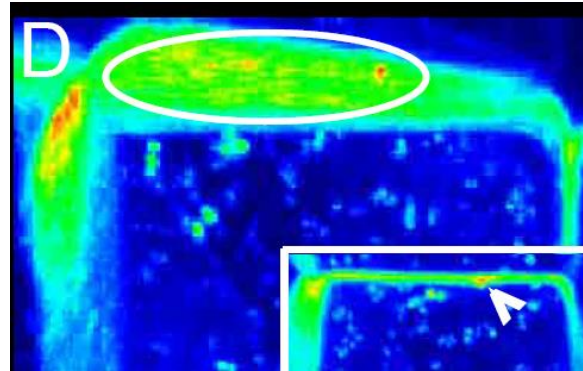
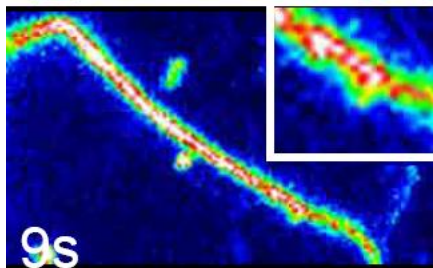
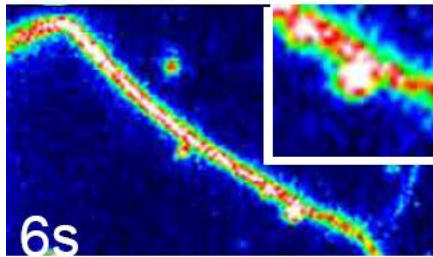
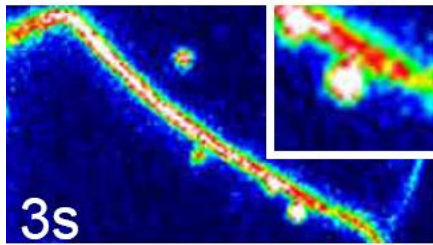
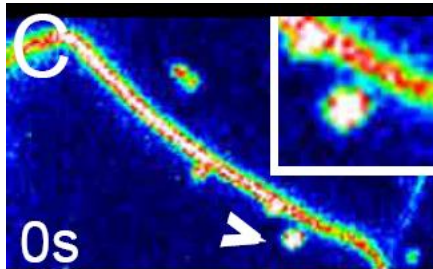


Super Polar Delivery

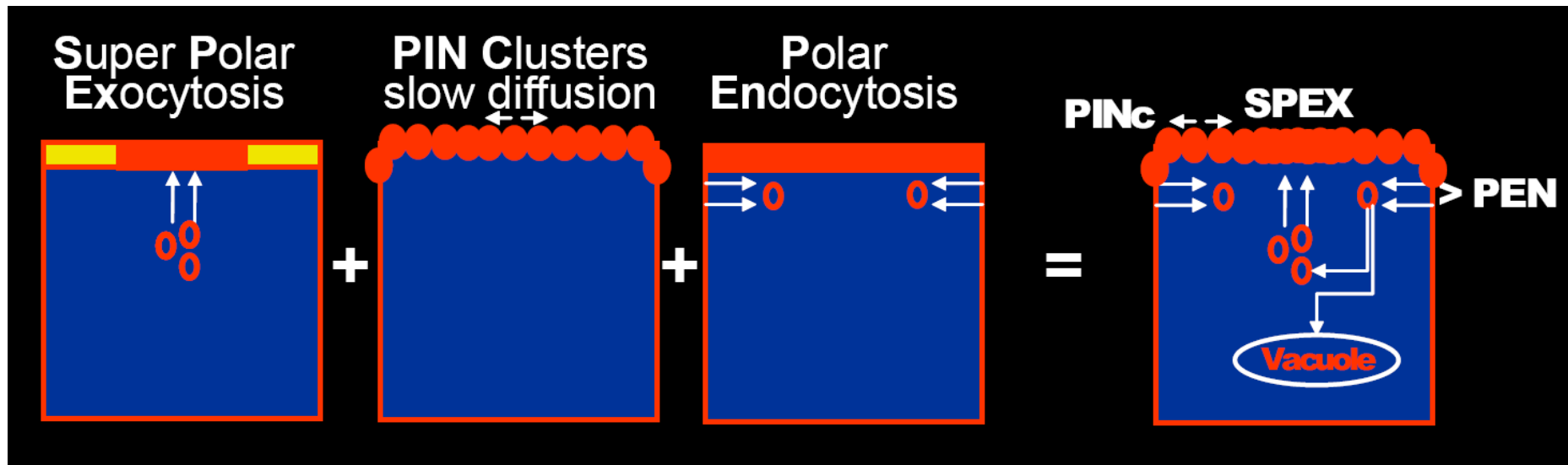


PIN2-GFP

4095
rel. intensity
0



Cell Polarity Maintenance

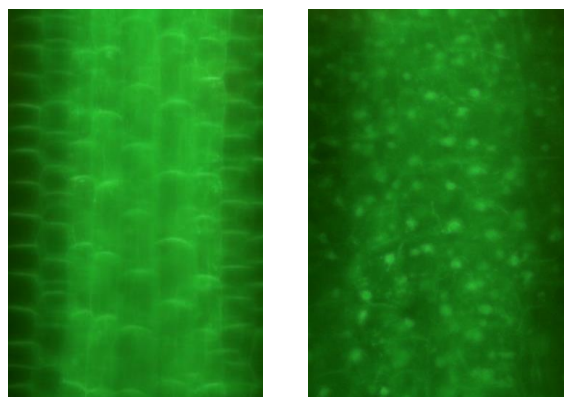


Genetic approaches:

Marker: GFP

Forward Genetic Screens

Endocytosis	<i>ben</i> ...5
Exocytosis	<i>bex</i> ...8
Vacuolar Function	<i>deg</i> ...3
Apical/Basal Targeting	<i>dpt</i> ...4
Outer Polar Targeting	<i>dol</i> ...2
Auxin – Endocytosis	<i>eon</i> ...6



EMS mutagenesis.
Epifluorescence
Screening

mutant lines

Deep sequencing

novel genes

Chemical Genetic Screens

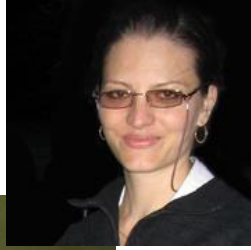
Endocytosis
Polar Targeting

Reverse Genetics

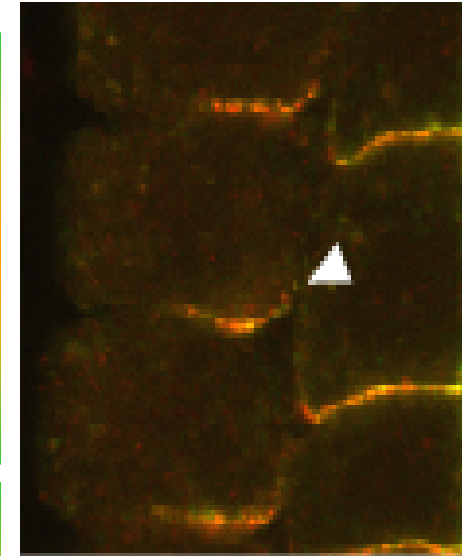
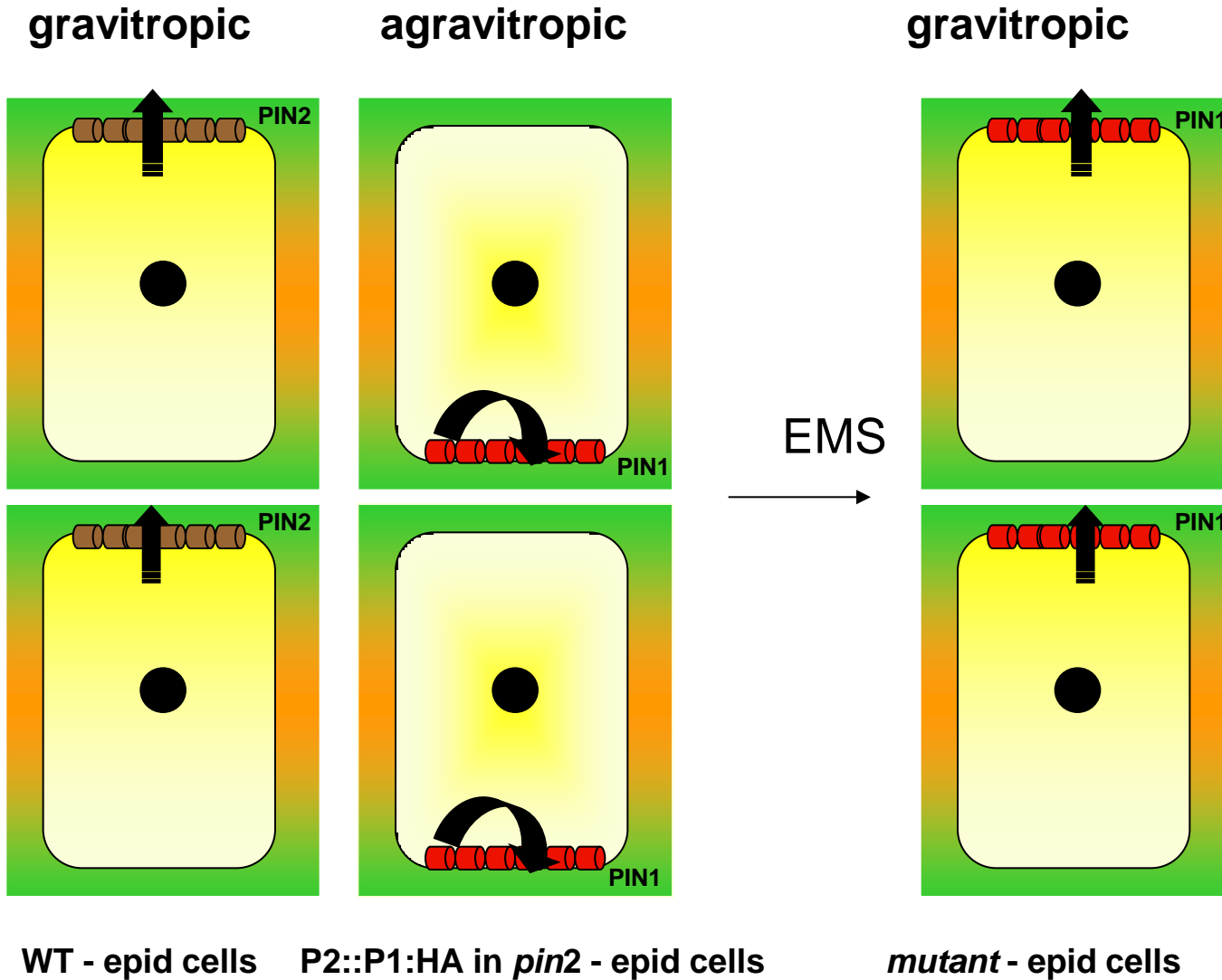


So far mapped in the lab: **11** mutants

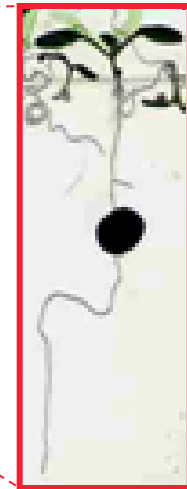
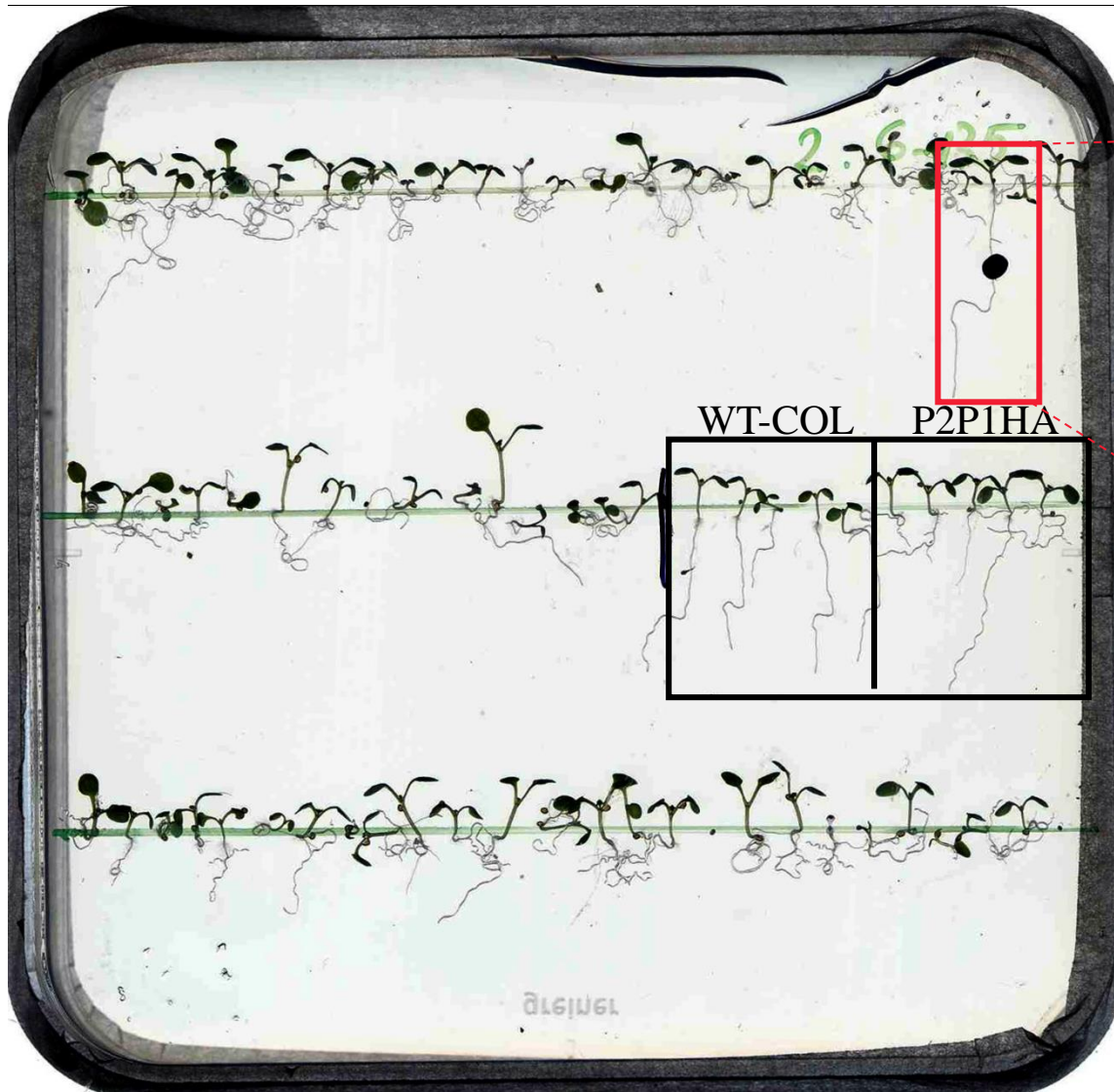
Beauty of forward genetics



Polarity screen - design



Polarity screen



A
good
hit!!

regulator of PIN polarity (repp)



P2::P1:HA

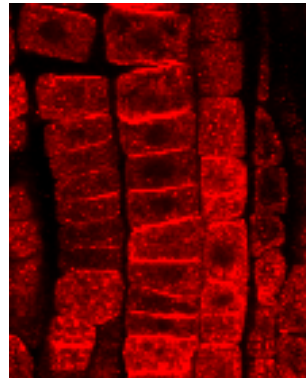
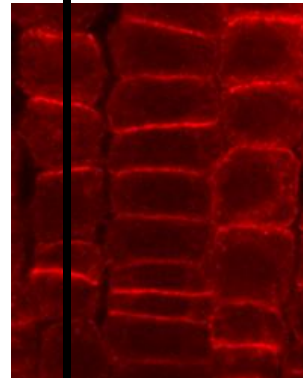
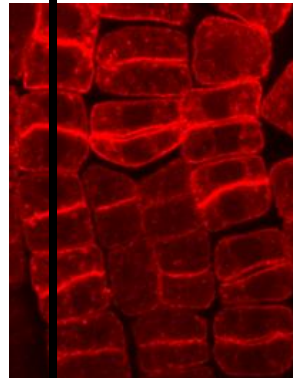
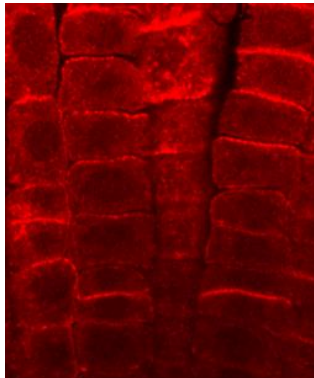
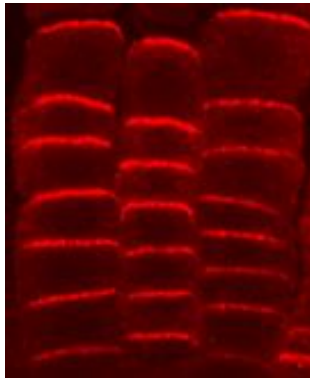
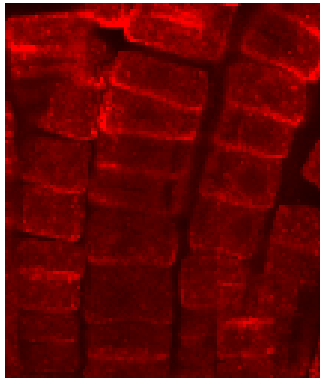
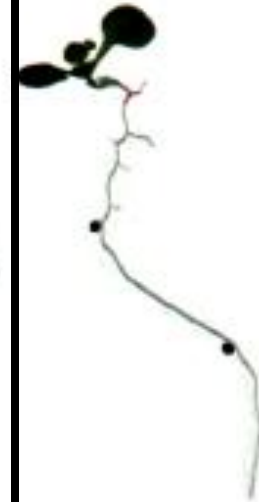
repp1

repp2

repp3

repp4

repp5

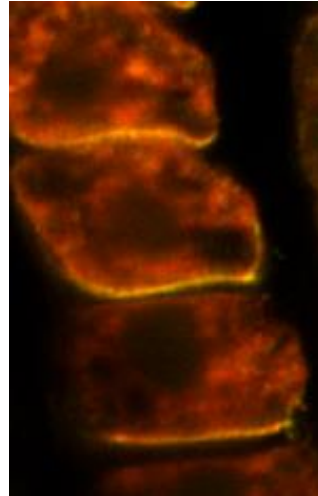
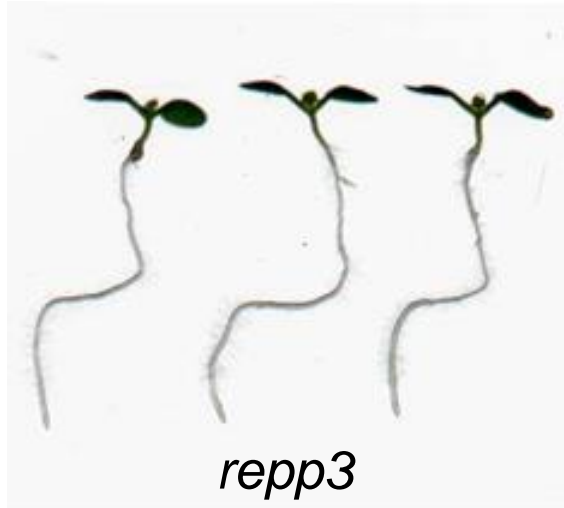


REPP3 Encodes Cellulose Synthase 3

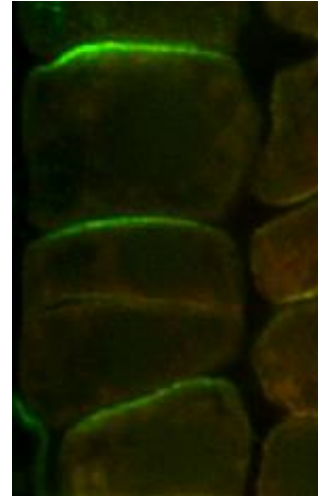


Gravitropic growth

Polarity change



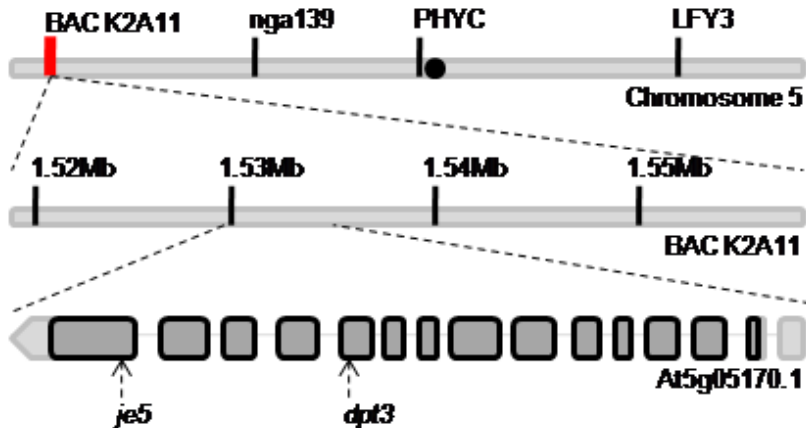
PIN1:HA



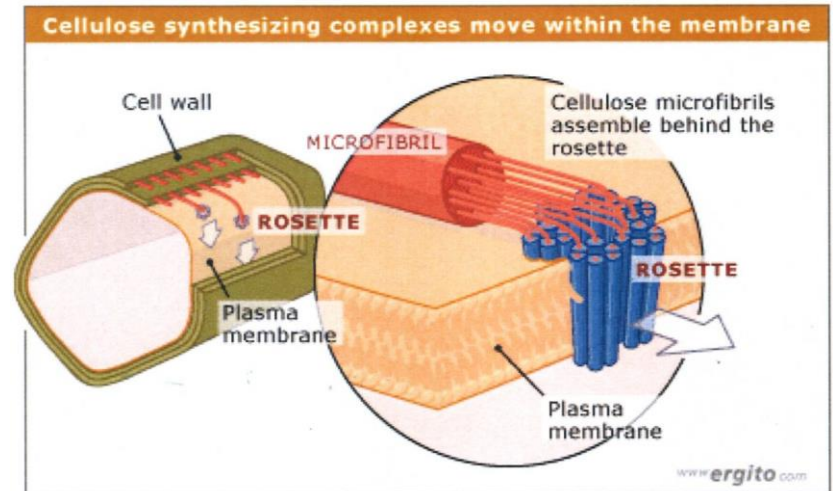
repp3



Mapping



Extracellular matrix: Cell wall

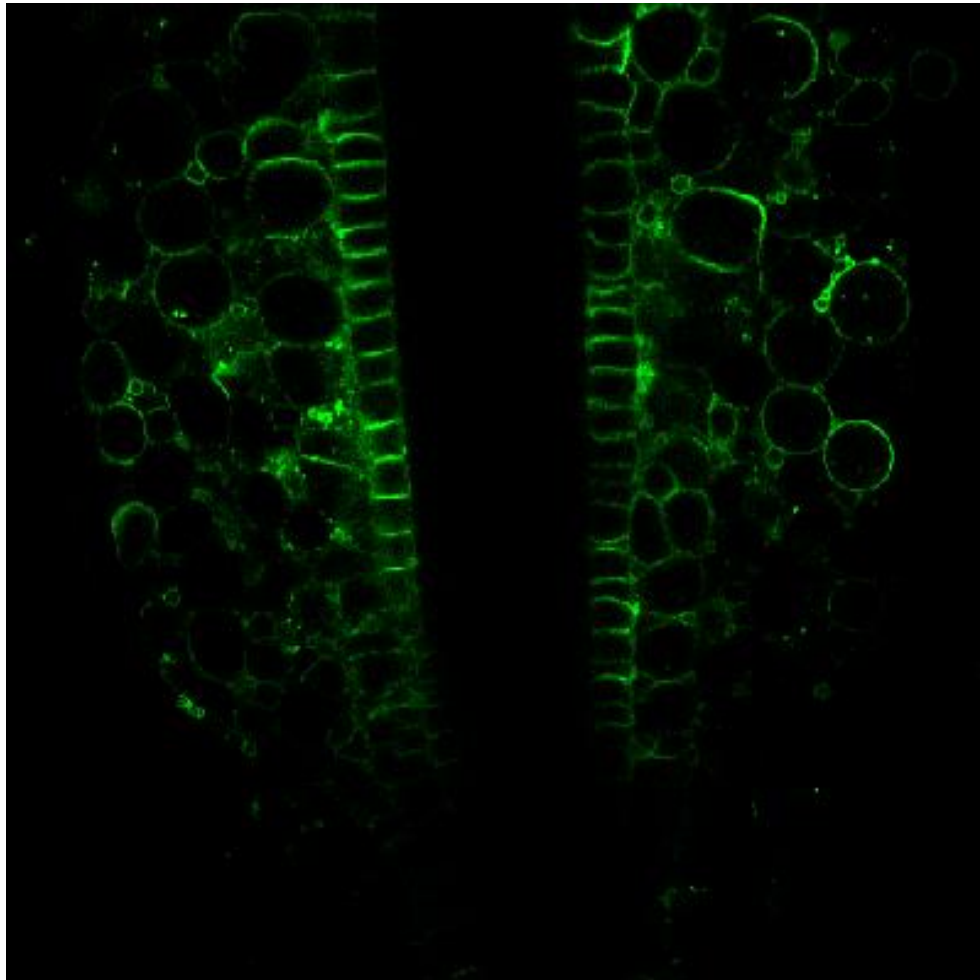
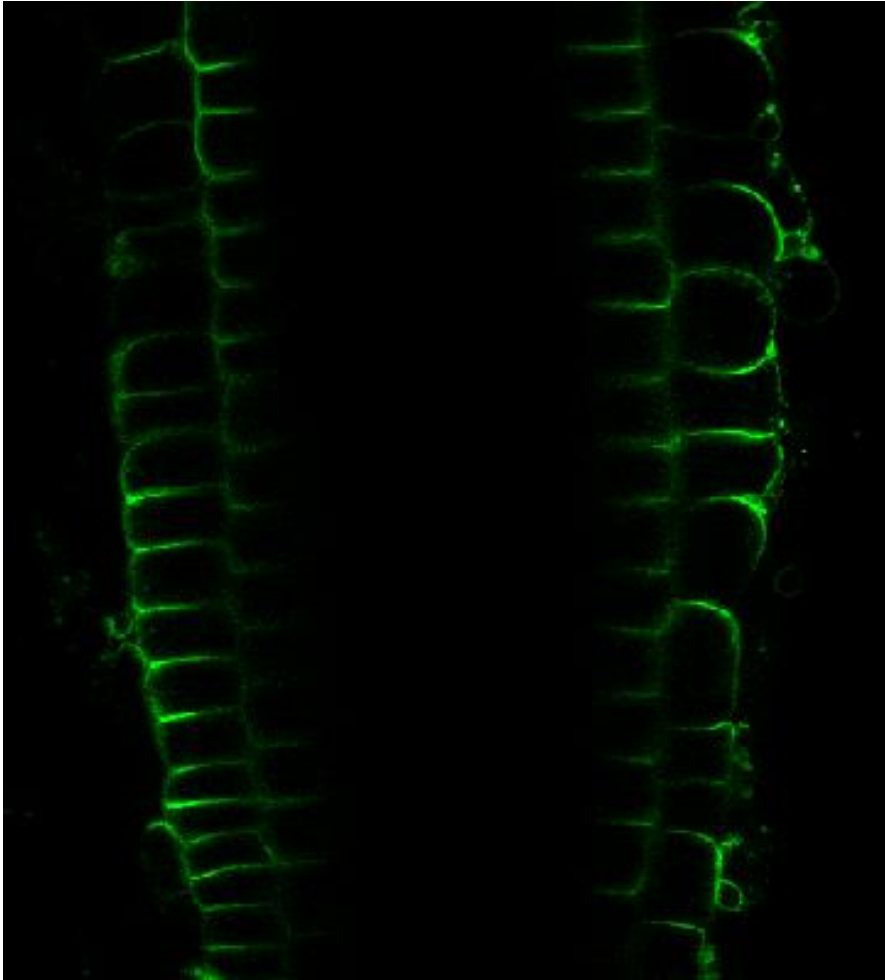


Degradation of cell wall results in loss of polarity



10' protoplasting

15' protoplasting



PIN2::PIN2-GFP

PIN proteins are attached to cell wall



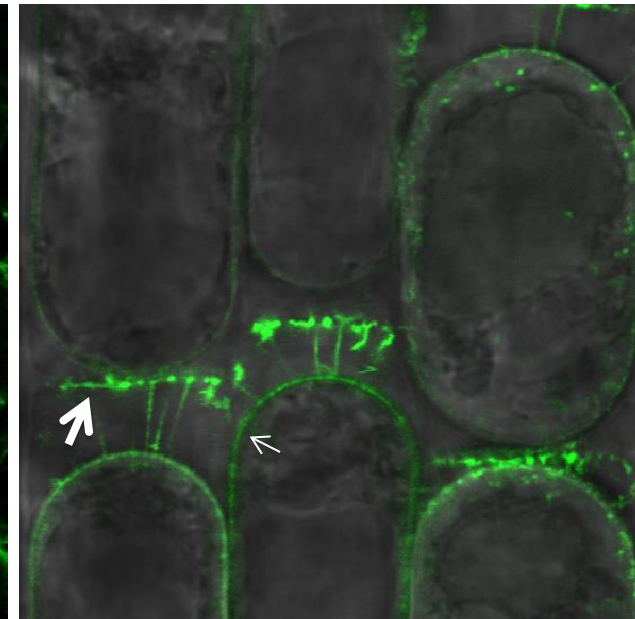
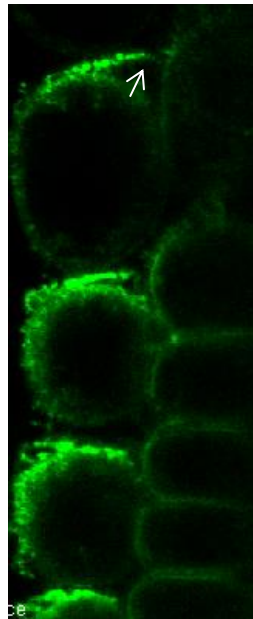
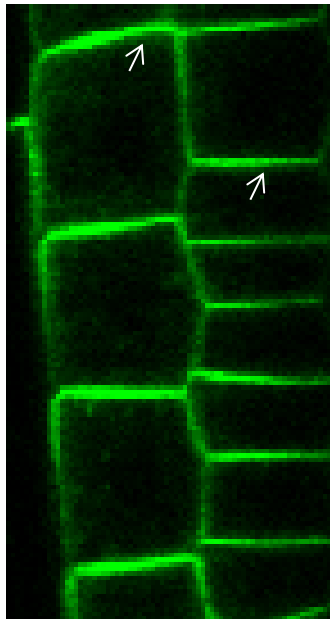
Partial degradation of cell wall

Before

After 30 min

After 1.5 hours

After 1.5 hours

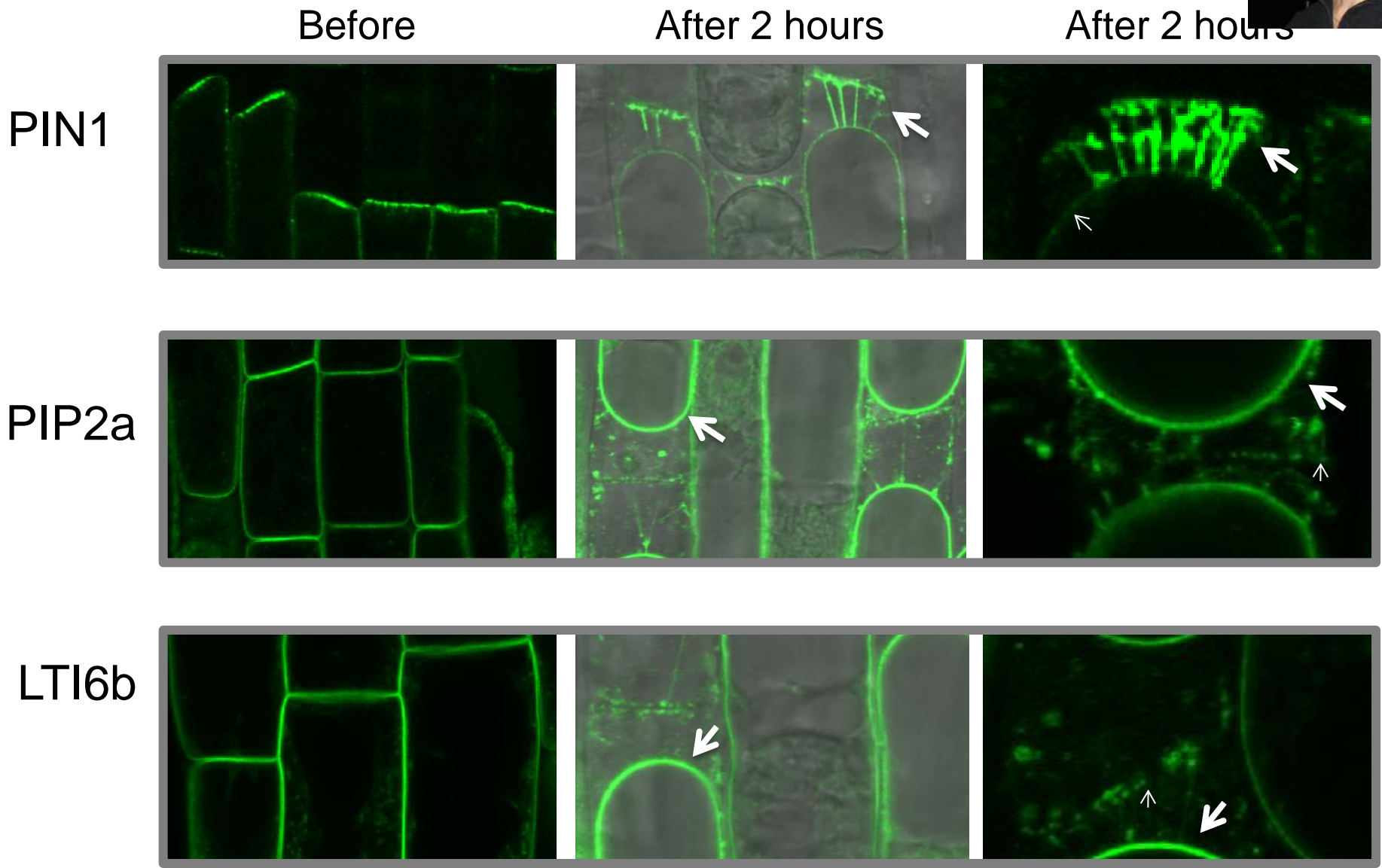


PIN2::PIN2-GFP

Polar cargos are attached to cell wall

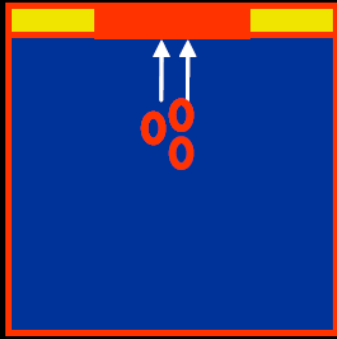


Partial degradation of cell wall

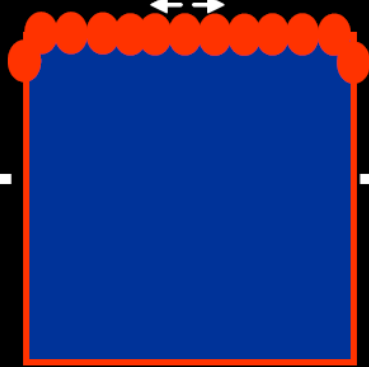


Cell Polarity Maintenance

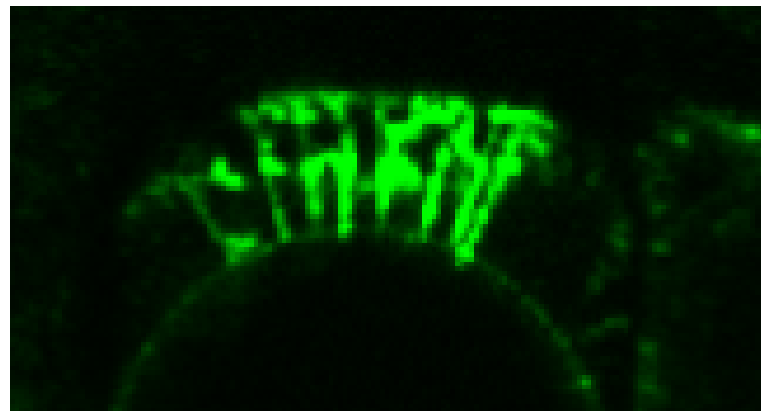
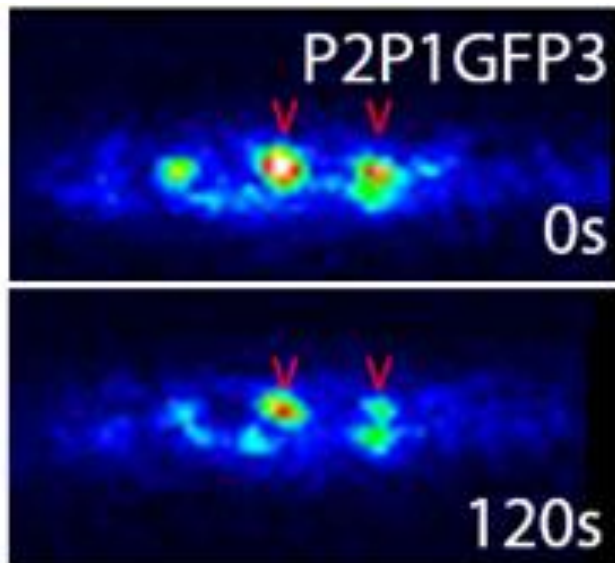
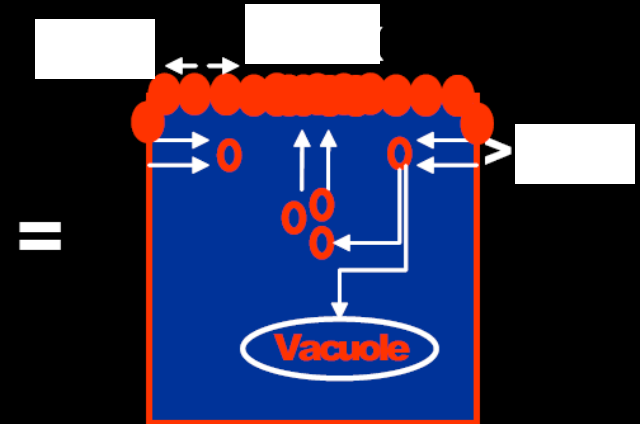
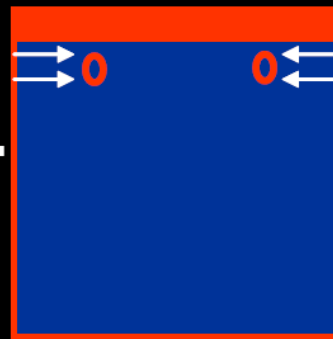
Super Polar Exocytosis



PIN Clusters slow diffusion



Polar Endocytosis



Cell-biological Determinants

Signals

Gravity

Friml et al. 2002
Kleine-Vehn et al., 2010
Rakusova et al., 2011

Light

Friml et al., 2002
Ding et al., 2011

Develop. context

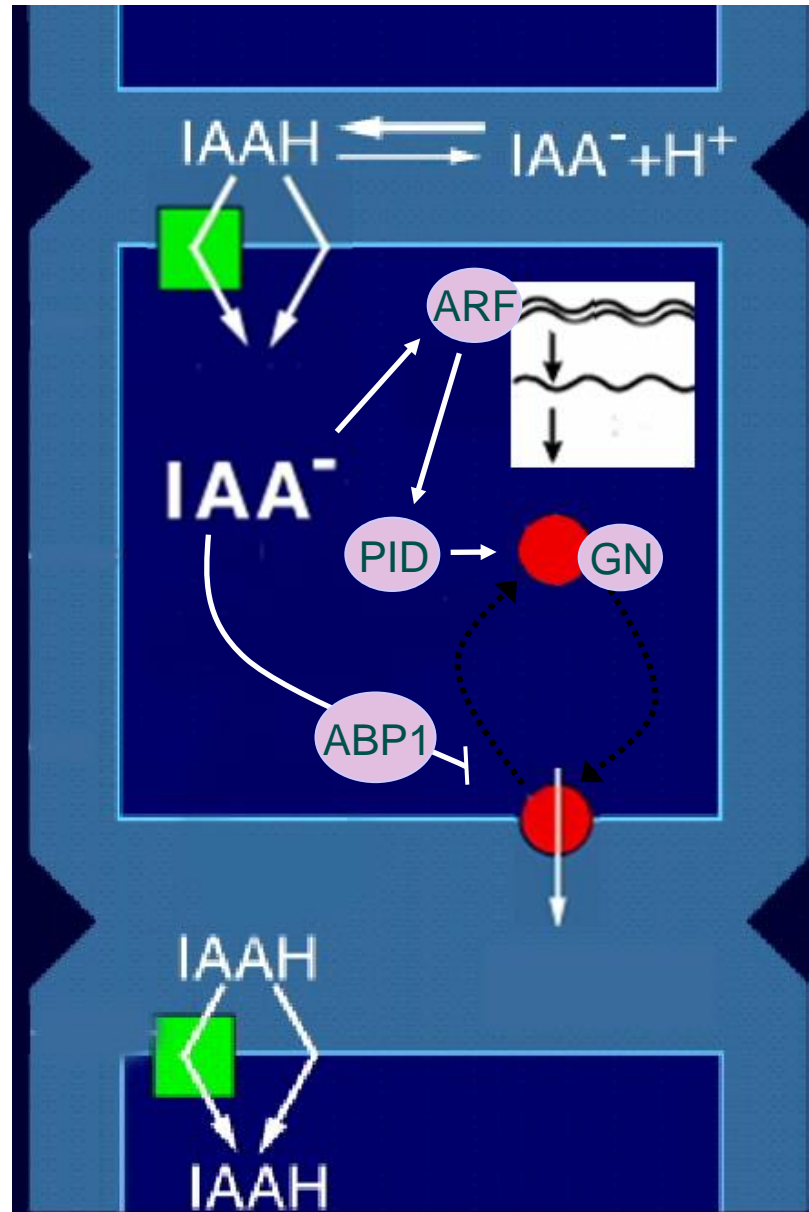
Benková et al. 2003
Friml et al. 2003
Reinhardt et al. 2003

Tissue context

Wisniewska et al., 2006

Feed-back

Sauer et al., 2006
Paciorek et al., 2005
Robert et al., 2010
Wabnik et al., 2010



Auxin Gradients

