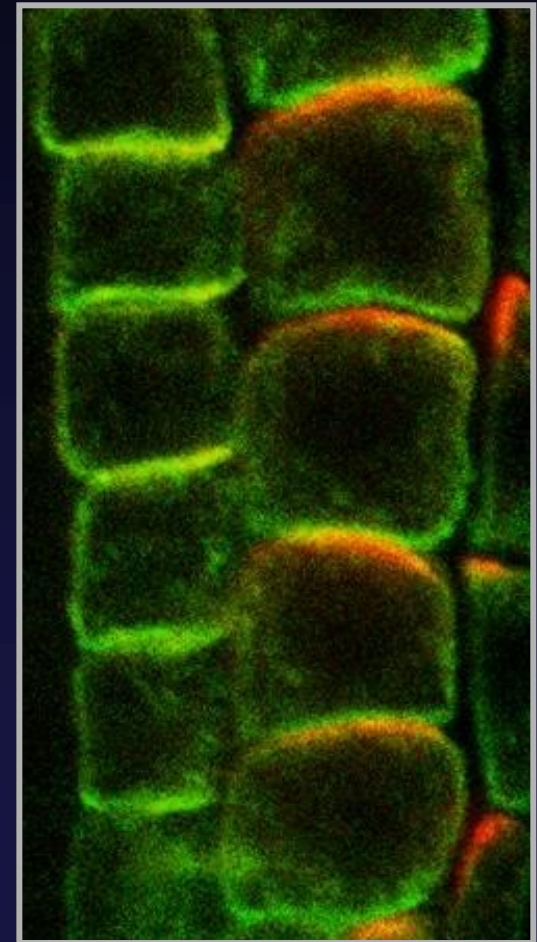
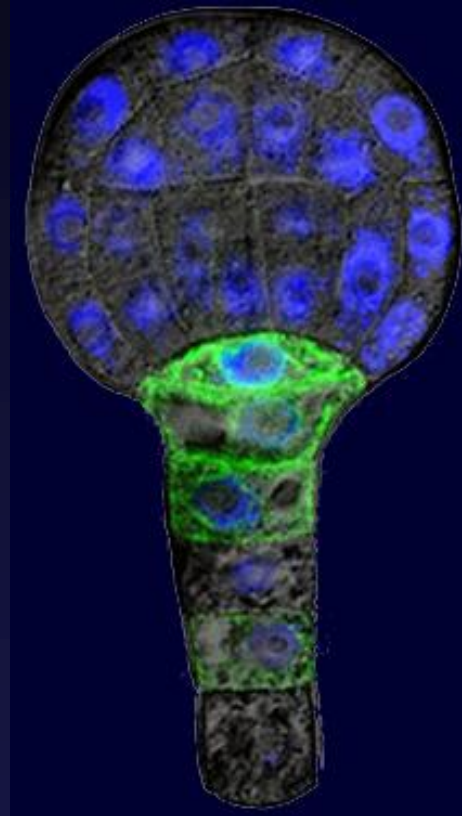
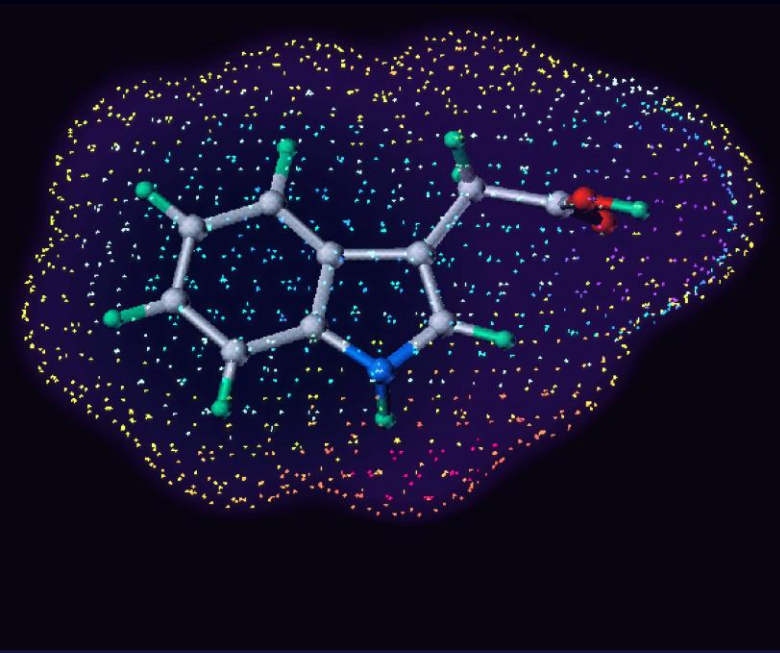


Patterning in Plant Development



*Jiří Friml,
ZMBP Tübingen*

Plants
and
Animals
Live
Different
Lives



Arabidopsis Embryogenesis

One-cell stage

Two-cell stage

Octant stage

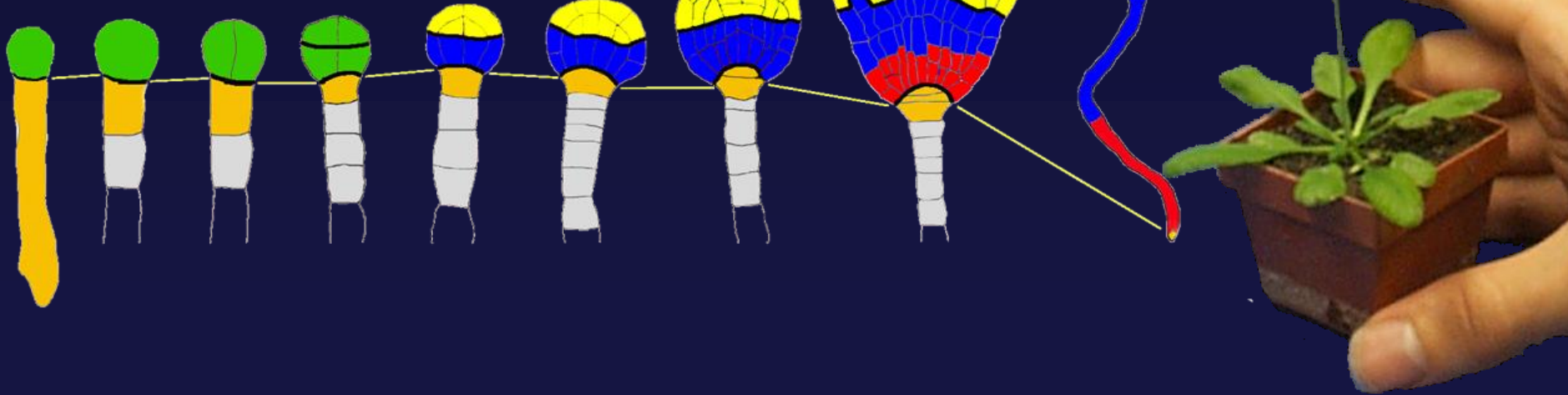
Dermatogen stage

Globular stage

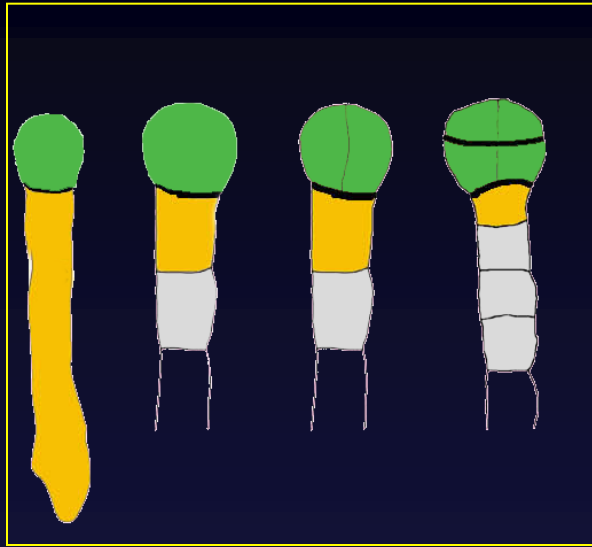
Trianglular stage

Heart stage

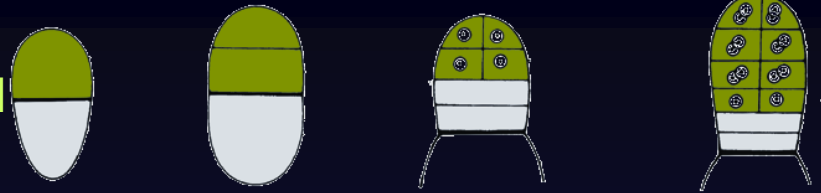
seedling



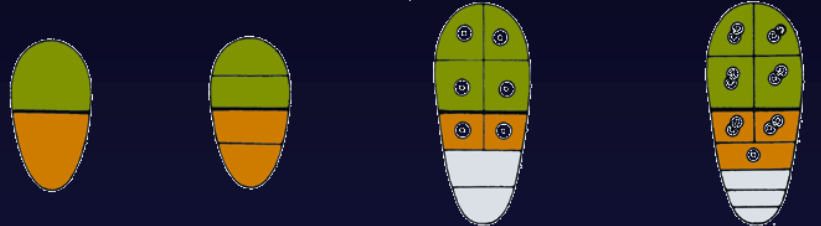
Comparison of embryo development in Angiosperms



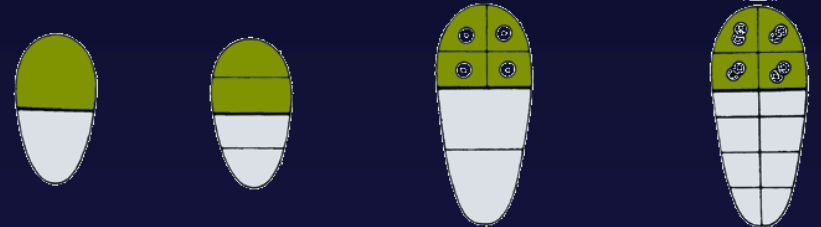
Caryophyllad



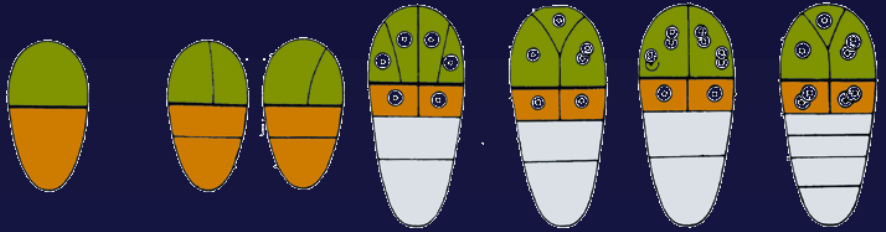
Chenopodiad



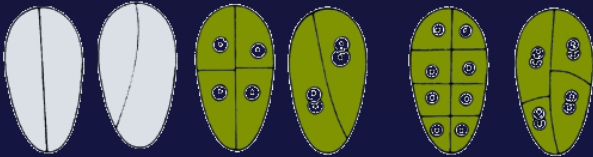
Solanad



Asterad



Piperad

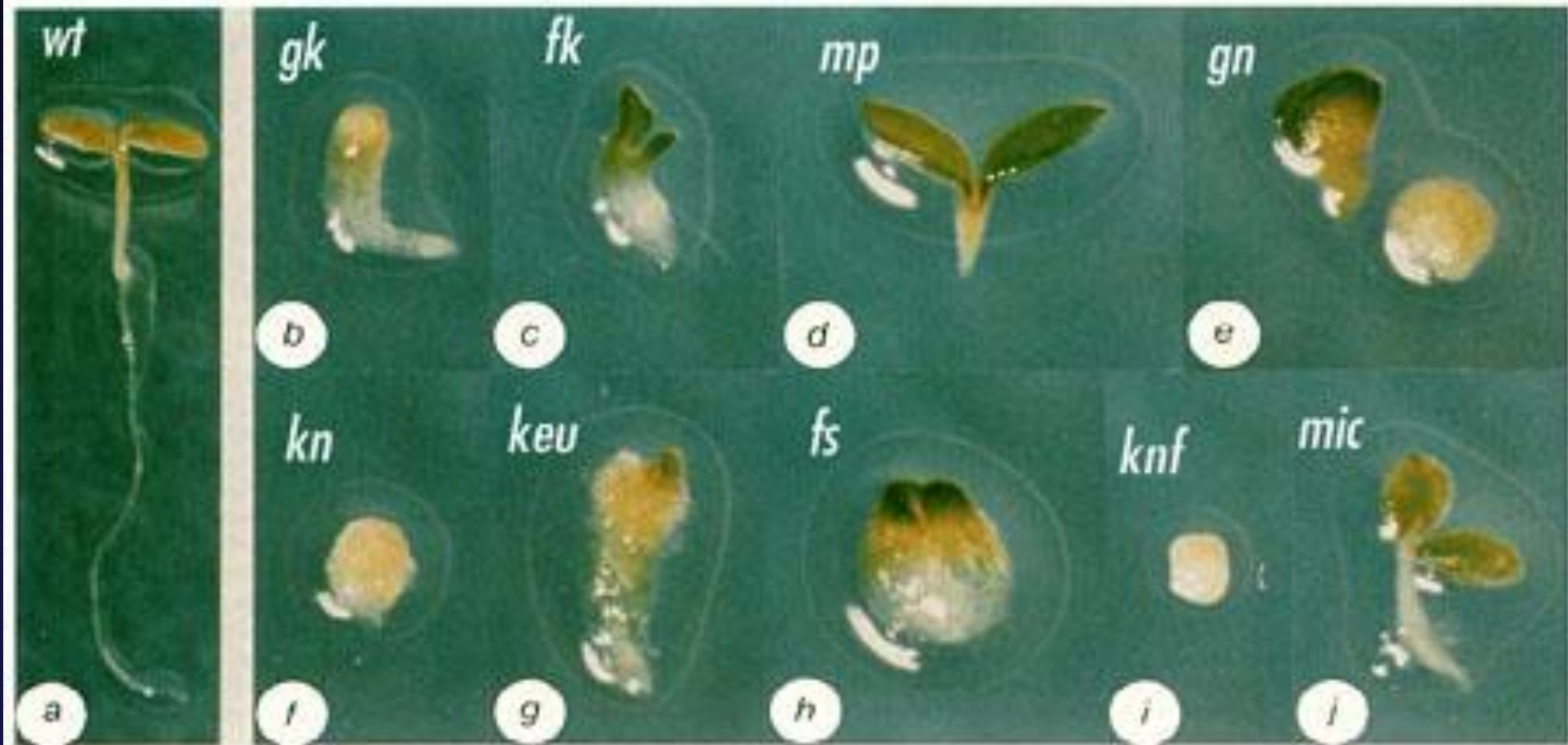


Modified after
Johri et al. 1992

How can such a protected system be investigated experimentally?



Mutant screen at seedling level



Genetic Interference with Auxin Response and Transport Disrupts Embryo Patterning



monopteros

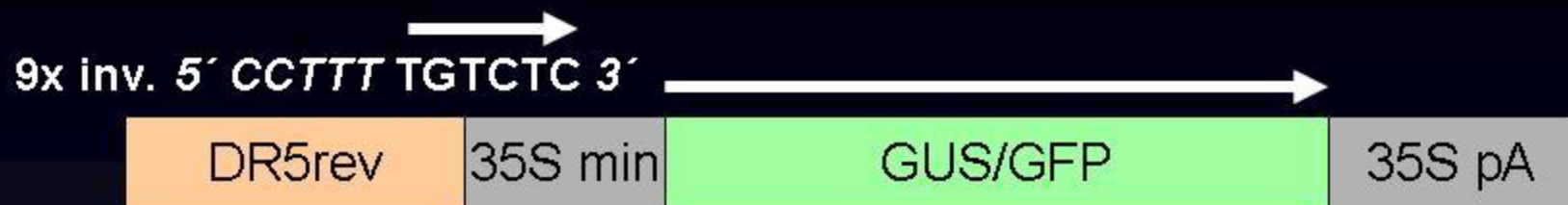


bodenl



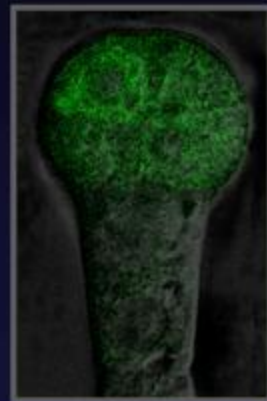
gno

DR5 Auxin Response Reporter



Root

Embryos



DR5

anti-IAA

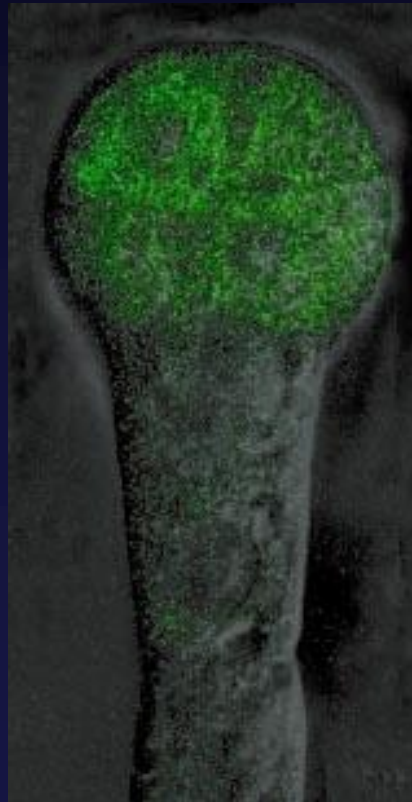
DR5

anti-IAA

Auxin in Early Embryogenesis

DR5::GFP

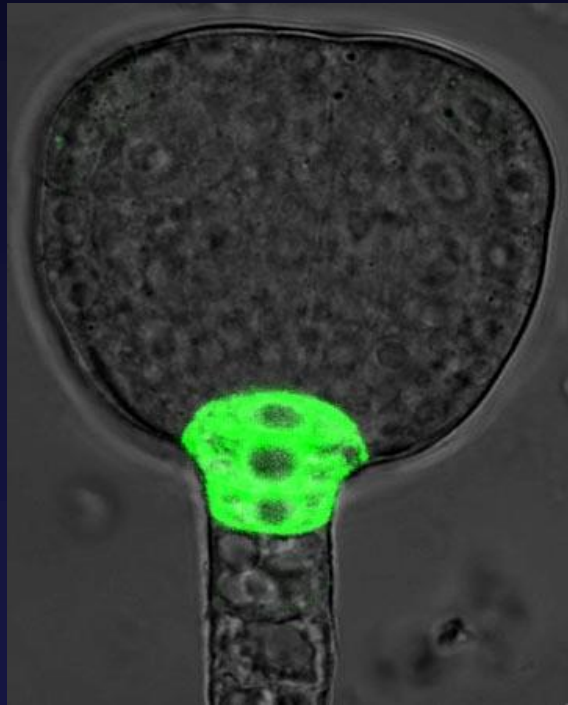
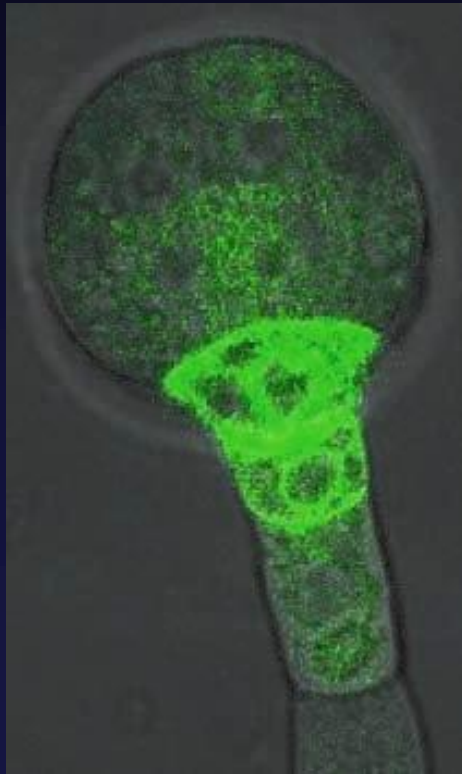
IAA
localisation



Auxin in Embryogenesis

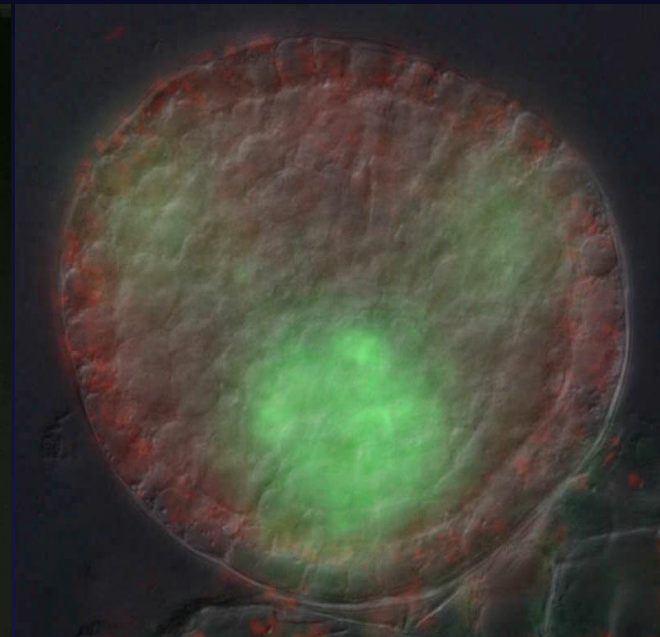
DR5::GFP

IAA localisation



DR5::GFP – *in vitro* Culturing

Long time treatments



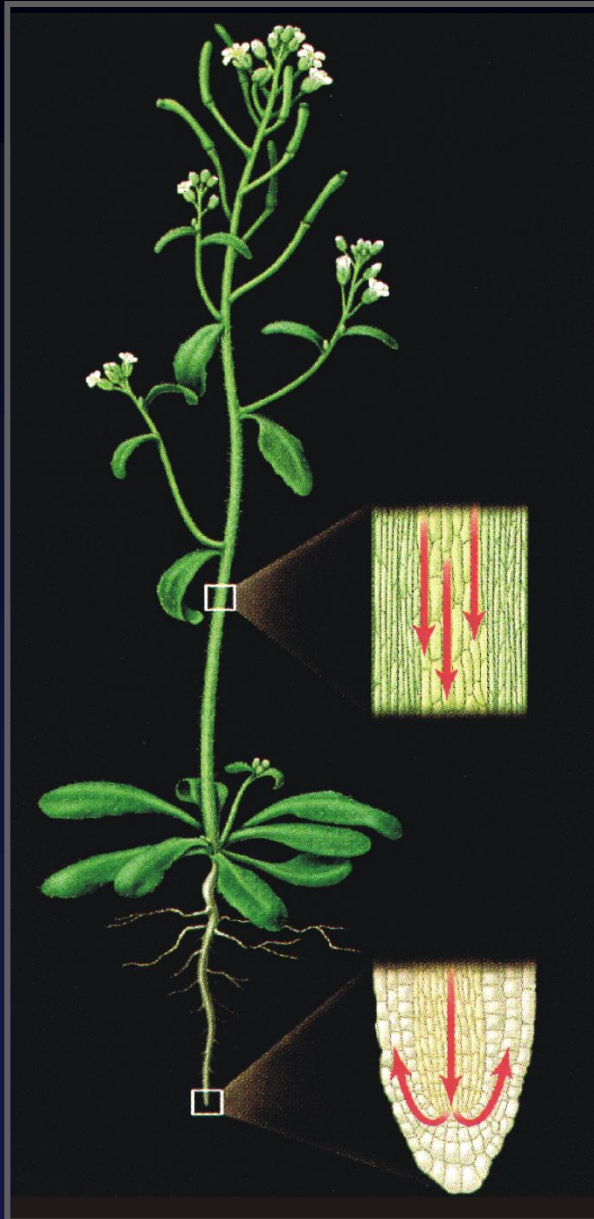
Control

NAA

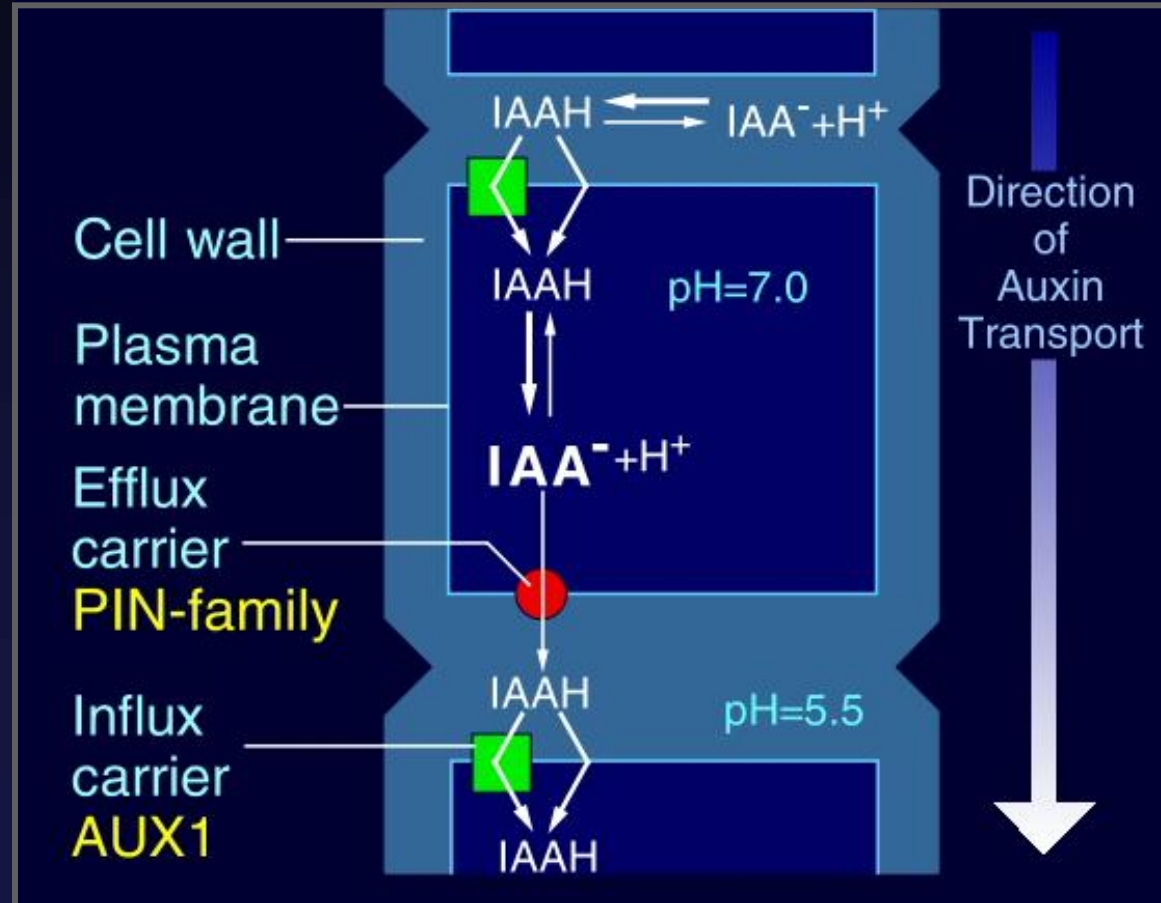
2,4D

NPA or BFA

Auxin Transport

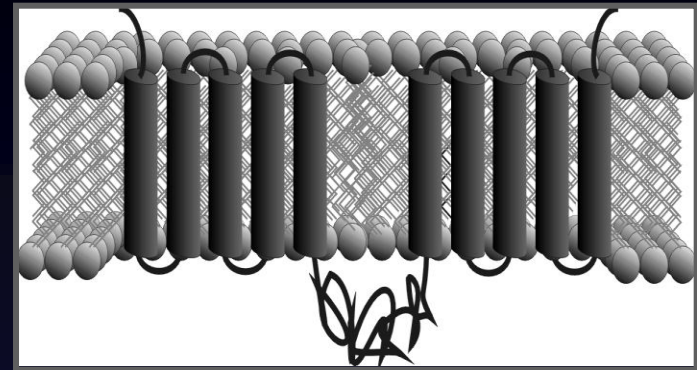
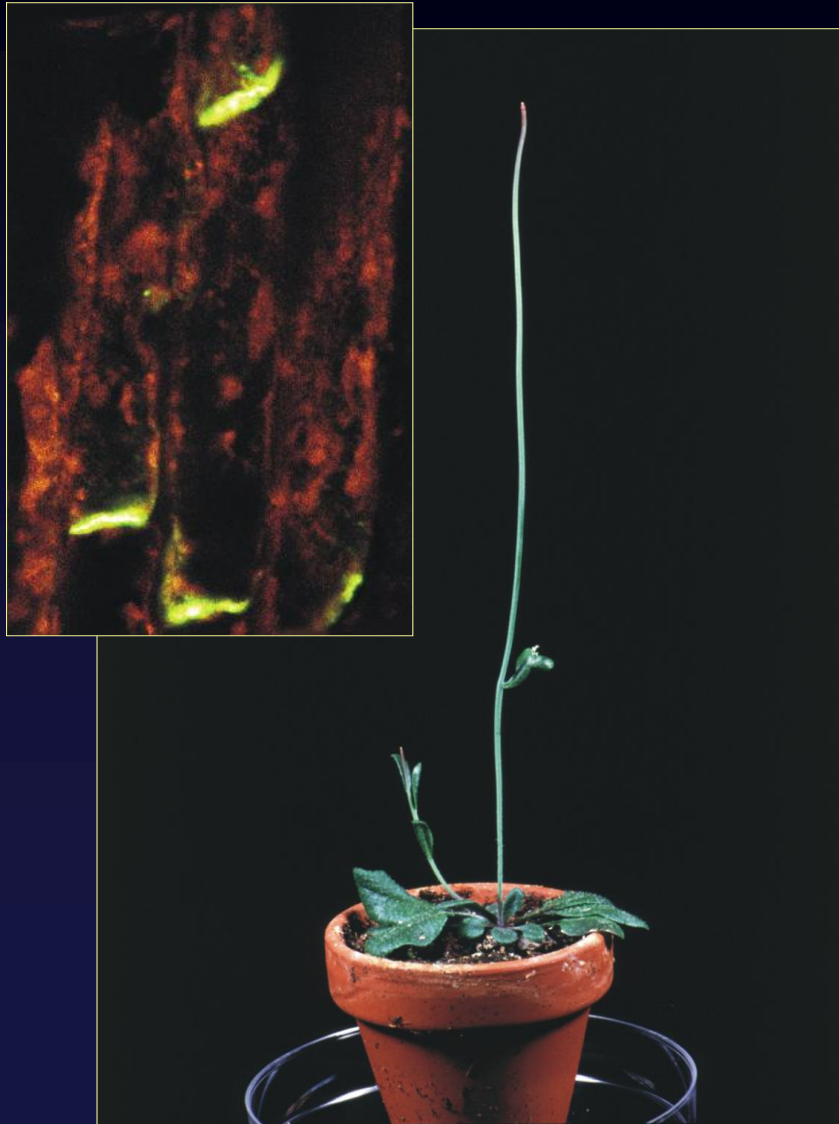


Chemiosmotic hypothesis

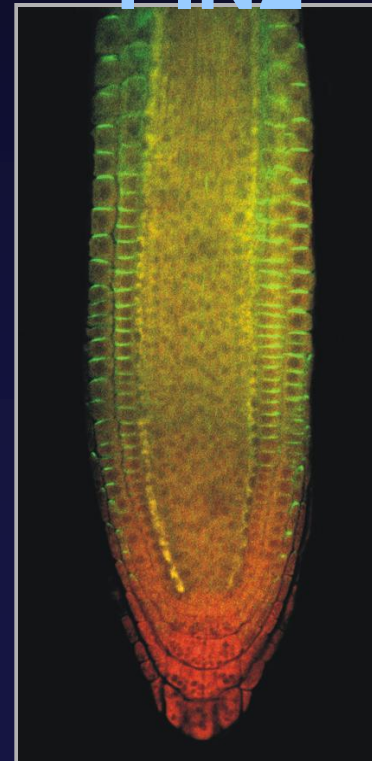


Molecular Genetics of Auxin Efflux

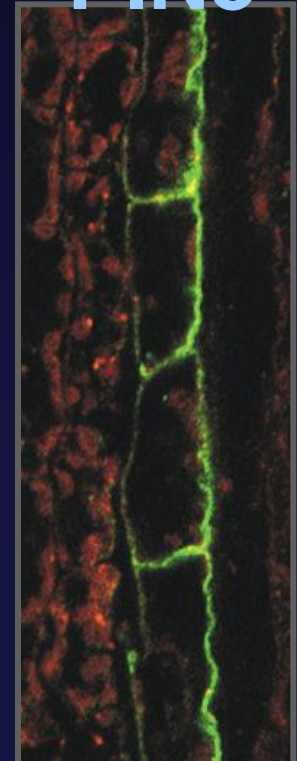
PIN1



PIN2



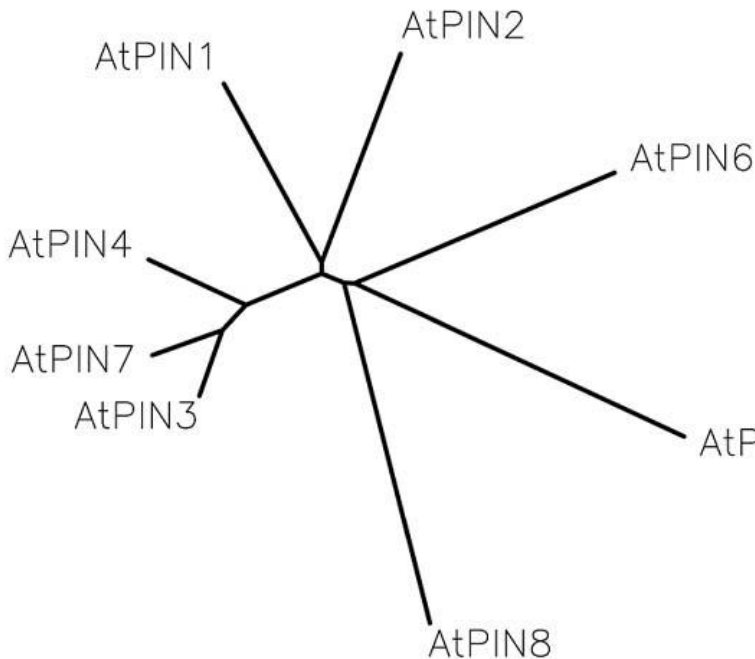
PIN3



Arabidopsis PIN Protein Family

Phylogenetic tree

Homology of PIN proteins



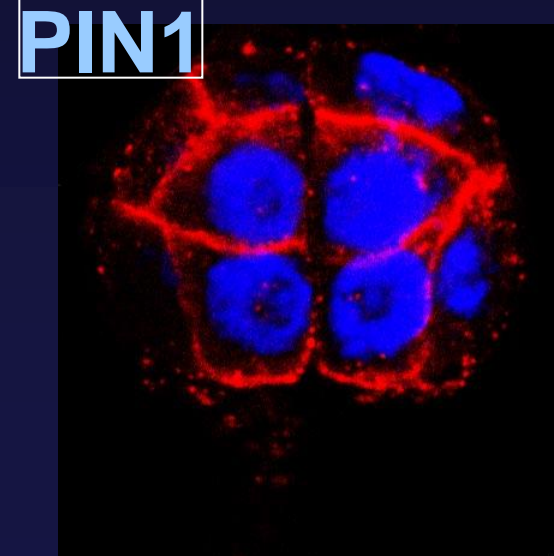
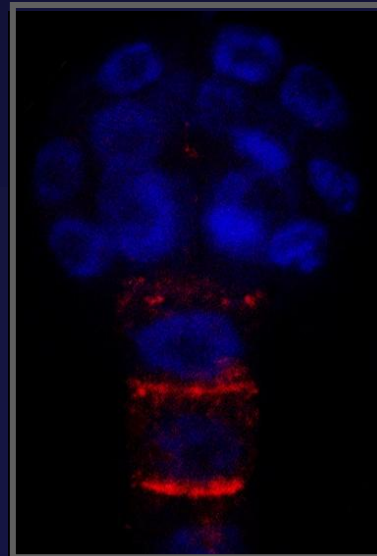
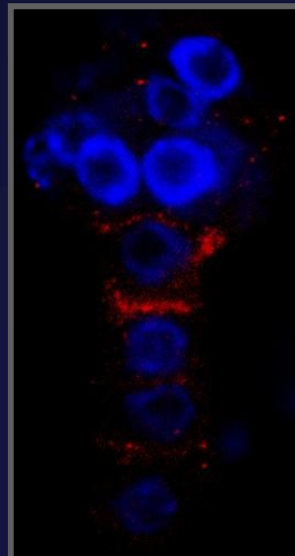
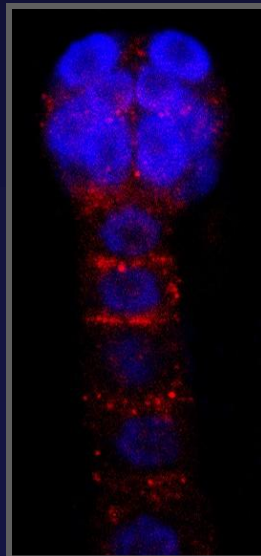
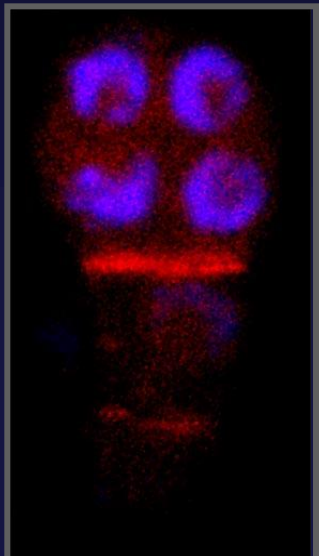
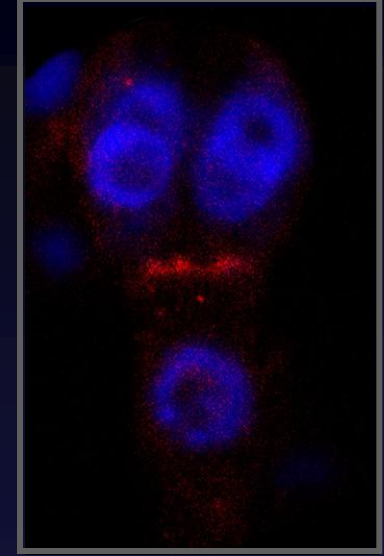
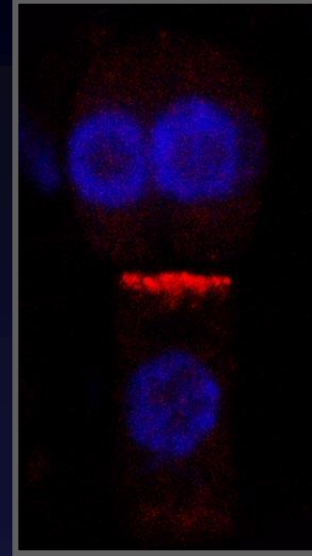
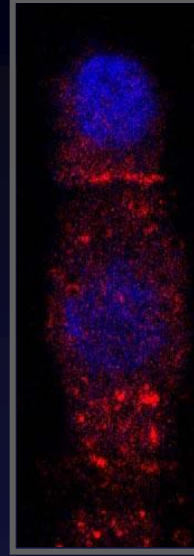
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AtPIN2 1 NIQGDMDVFAAMVPLVAMILAYGSVKNWRIPTDQCQSGINRFVALFAVLLSFHFSAAHNPYAMHLELAAGSLQVYVSEHFF...QQAASRRGS LEWMISELSTLPLNTLV
AtPIN3 1 MESHLDLTVLVAVPLVAMILAYGSVKNWRIPTDQCQSGINRFVALFAVLLSFHFSAAHNPYAMHLELAAGSLQVYVSEHFF...WAFKTKMSG LEWMISELSTLPLNTLV
AtPIN4 1 MENCGBVVKIEAMVPLVVALLIGVSKVKNWRIPTDQCQDANRLLCYELSEHFFIFETAVDVFNNHMYETAAVQSHVILVVAL...WAKYENKSGYC MDSISSELSTLPLNTLV
AtPIN6 1 MITGNHFTVMCAHRLTANFVAYGVSKWRIPTDQCQSGINRFVALFAVLLSFHFSAAHNPYAMHLELAAGSLQVYVSEHFF...HAFVFKAGL IOWLLESLTALPLNTLV
AtPIN7 1 MSHLDLTVLVAVPLVAMILAYGSVKNWRIPTDQCQSGINRFVALFAVLLSFHFSAAHNPYAMHLELAAGSLQVYVSEHFF...WAFKTKMSG LEWMISELSTLPLNTLV
AtPIN8 1 MESHLDLTVLVAVPLVAMILAYGSVKNWRIPTDQCQSGINRFVALFAVLLSFHFSAAHNPYAMHLELAAGSLQVYVSEHFF...WAKYENKSGYC MDSISSELSTLPLNTLV
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AtPIN2 235 RANLHTEVETIYVQSSREPEHRAISPHC...DEFA...MFAASAPSPRHGTYSYGG...KQAGHGGQVYVCEKSRVPEHTEHDEHE...VMKAKKARAGGGRMSDGLTNNVFP...
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AtPIN4 221 RPNELTGAISYEQS...ERGSFPHS...DEFS...MMGFF...GG...LNENFGI...ADMYVQSSRDEPTEHPCNMASSPFE...YEPG...GG
AtPIN6 228 RANLHNSRDEPTEHPCNMASSPFE...ERGSFPHS...DEFS...MMGFF...GG...LNENFGI...ADMYVQSSRDEPTEHPCNMASSPFE...YEPG...GG
AtPIN7 227 RPNELTGAISYEQS...ERGSFPHS...DEFS...MMGFF...GG...LNENFGI...ADMYVQSSRDEPTEHPCNMASSPFE...YEPG...GG
AtPIN8 169 RANLHNSRDEPTEHPCNMASSPFE...ERGSFPHS...DEFS...MMGFF...GG...LNENFGI...ADMYVQSSRDEPTEHPCNMASSPFE...YEPG...GG
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AtPIN2 342 RYFPEHFWGC...STGASGVRKESGGGGGGVGVGCN...EENWVSSASFPVSVH...ANAKRMTADYSTANDHGDVVKVSGOQSN...DHOQVFR
AtPIN3 305 AGSYPAPNPE...STGTGVSTPK...NRIPE...NQQLQKQDGA...SHDA...ELHMFVSSASFPVSVH...GAGQVATEVSDQPR...GNRAGGGDDIG...
AtPIN4 303 AGSYPAPNPE...STGTGVSTPK...NRIPE...NQQLQKQDGA...SHDA...ELHMFVSSASFPVSVH...GAGQVATEVSDQPR...GNRAGGGDDIG...
AtPIN5 192 ...VMSLWELKATFNPCYSCIGLIMAFINRHELEGLGELGSLMKEKSTAMENMGI
AtPIN6 313 ...LYRQSPKMMHSGQRHAKMDNGS...EKEKISFRDLKAPATAAGSG
AtPIN7 308 PGEFAPNPE...STONKQSAPE...NHHV...VMSLWELKATFNPCYSCIGLIMAFINRHELEGLGELGSLMKEKSTAMENMGI
AtPIN8 210 ...EELKAKRKL...HNTKATGSL...MATH...LGNL...EKEKISFRDLKAPATAAGSG
AtPIN1 423 RFPSPKNDKSKVL...ATDGMNINKTE...QAKVFF...VHNRLLIMVWRKLIINPQSSSE...EIT...SELESKKNIEHNA...EAKISILSDAGLGMAMFSLGI
AtPIN2 443 KG...HVNDDQDNN...GKSPYMGKGSVDVGGGPPKQCPASVMTLLIMVWRKLIINPQSSSE...EIT...SELESKKNIEHNA...EAKISILSDAGLGMAMFSLGI
AtPIN3 424 QPSPFAGKEEERPKDANRNLKLIAPNS...ALQSKTGLGGASQRKNHFPASVMTLLIMVWRKLIINPQSSSE...EIT...SELESKKNIEHNA...EAKISILSDAGLGMAMFSLGI
AtPIN4 407 LDDGEHEIK...ATAENKMSG...LEAAGDDGGNG...WHEP...EMERLLIMVWRKLIINPQSSSE...EIT...SELESKKNIEHNA...EAKISILSDAGLGMAMFSLGI
AtPIN5 192 ...VMSLWELKATFNPCYSCIGLIMAFINRHELEGLGELGSLMKEKSTAMENMGI
AtPIN6 381 ASNEGAAGKDT...TPVAIG...KQHEH...VYMRLLITVYVWRKLIINPQSSSE...EIT...SELESKKNIEHNA...EAKISILSDAGLGMAMFSLGI
AtPIN7 411 ...EES...KVKVFW...MKKCS...LNPKEA...YKQV...VH...EELKAKRKL...HNTKATGSL...MATH...LGNL...EKEKISFRDLKAPATAAGSG
AtPIN8 210 ...EELKAKRKL...HNTKATGSL...MATH...LGNL...EKEKISFRDLKAPATAAGSG
AtPIN1 526 MADNRIACGRRAAFANREVVQAVNLASYVGLRQVLEHVAIQALPQCIVFFVFAKENVHEDLSAVIPGMLALP...EITVLLIGL
AtPIN2 551 MALQRLIACGRVQFAMAVRELPQAVYAT...IAIGRGLLEHIAVQALPQCIVFFVFAKENVHEDLSAVIPGMLALP...EITVLLIGL
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AtPIN4 500 MALQRLIACGRVATFAMAVRELPQAVYAT...IAIGRGLLEHIAVQALPQCIVFFVFAKENVHEDLSAVIPGMLALP...EITVLLIGL
AtPIN5 253 MALQRLVQST...ELVYVGLK...IAGPMA...HIG...VLE...H...V...L...I...D...A...L...P...Q...C...I...V...F...F...A...K...E...N...V...H...E...D...L...S...A...V...I...P...G...M...L...A...L...P...E...I...T...V...L...L...I...G...L
AtPIN6 474 MALQRLVQST...ELVYVGLK...IAGPMA...HIG...VLE...H...V...L...I...D...A...L...P...Q...C...I...V...F...F...A...K...E...N...V...H...E...D...L...S...A...V...I...P...G...M...L...A...L...P...E...I...T...V...L...L...I...G...L
AtPIN7 523 MALQRLIACGRVATFAMAVRELPQAVYAT...IAIGRGLLEHIAVQALPQCIVFFVFAKENVHEDLSAVIPGMLALP...EITVLLIGL
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PIN7 in Embryogenesis

GUS

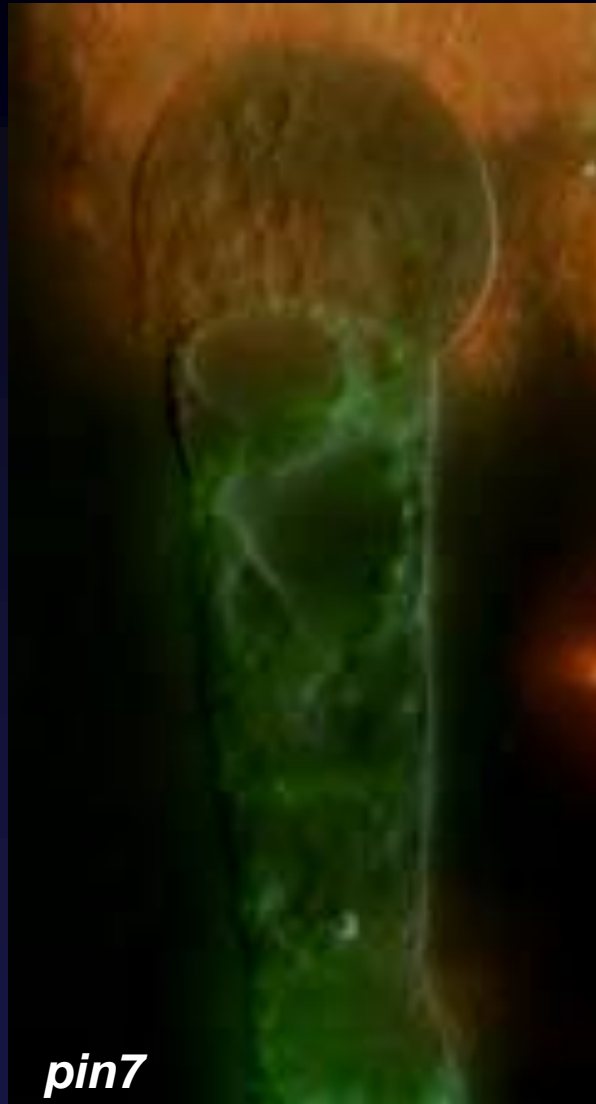
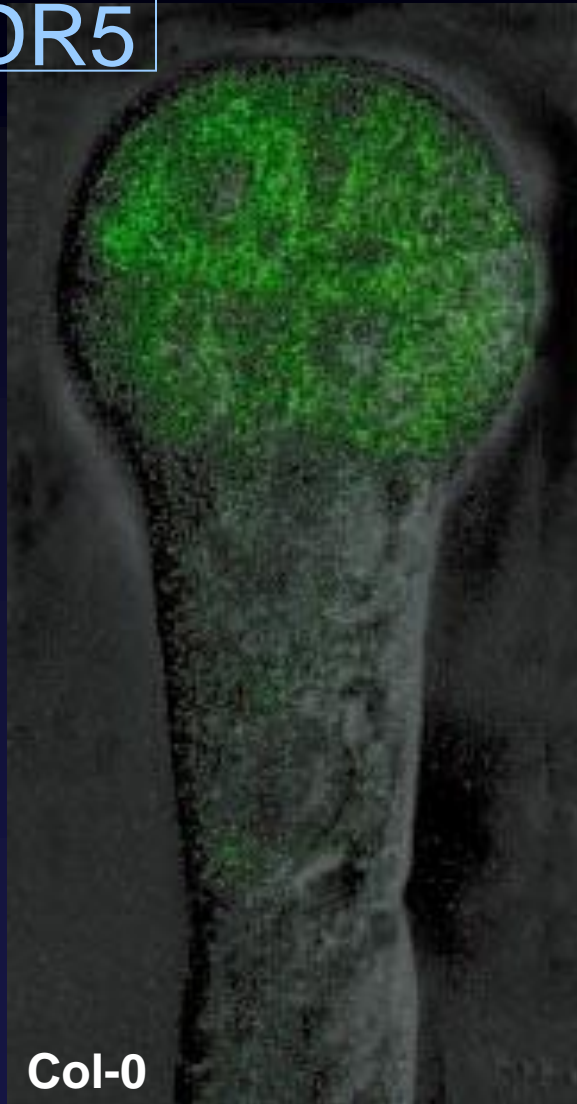
mRNA

Protein



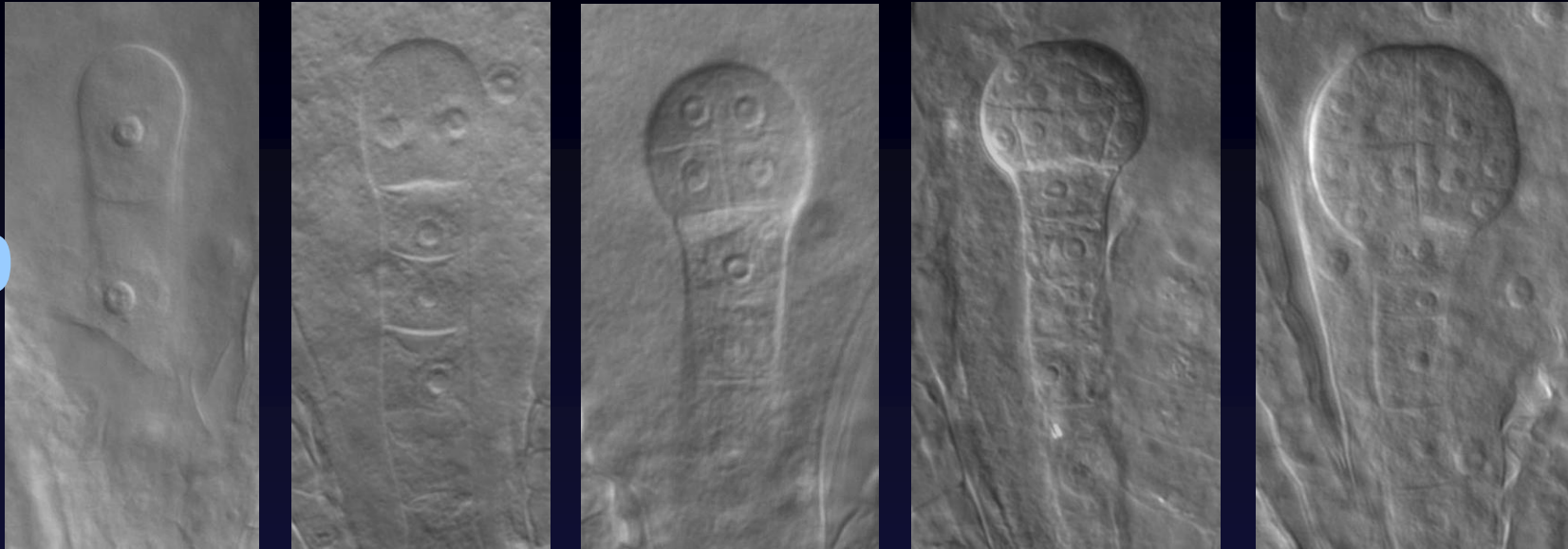
Analysis of DR5 activity in *pin7*

DR5



Embryo Phenotype of *pin7* Mutants

Col-0



pin7



PIN1 in Early Embryogenesis

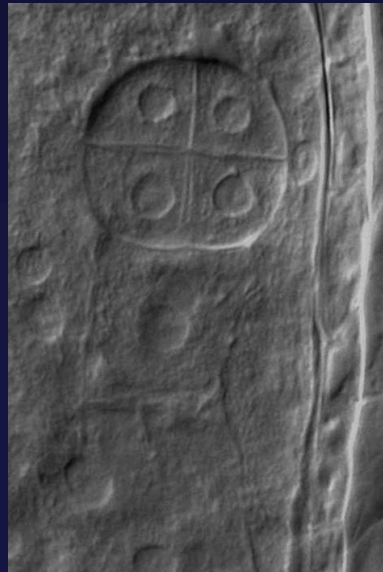
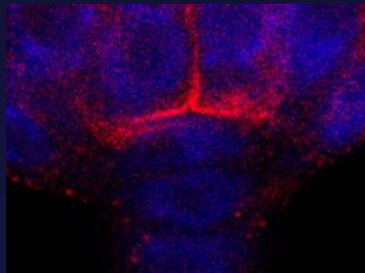
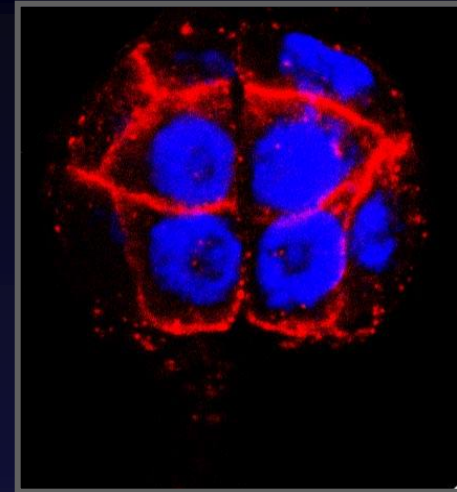
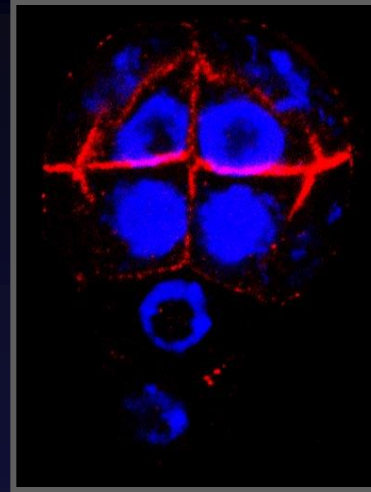
GUS



mRNA



Protein

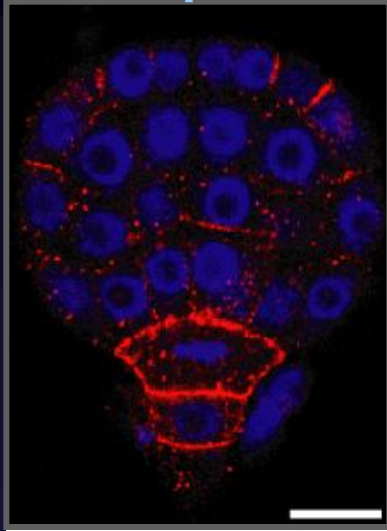


Enk
6,1%

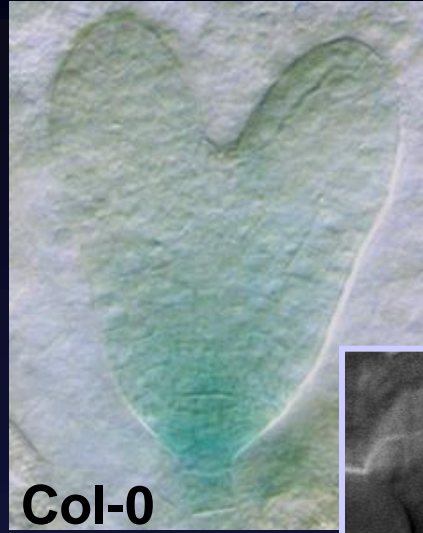
pin
30,4%

PIN4 in Embryogenesis

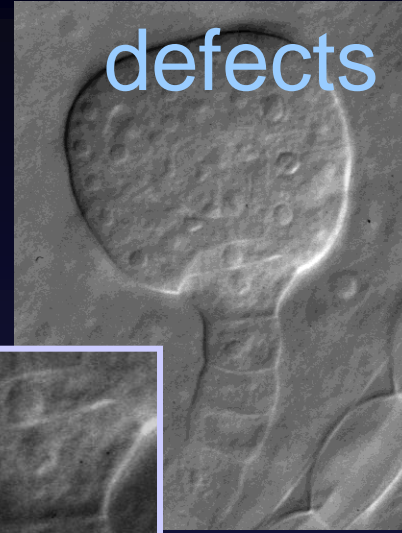
PIN4 protein



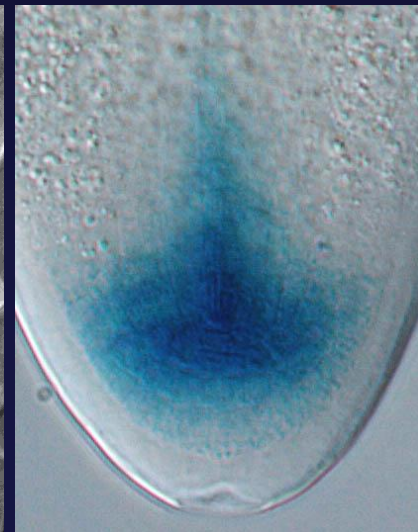
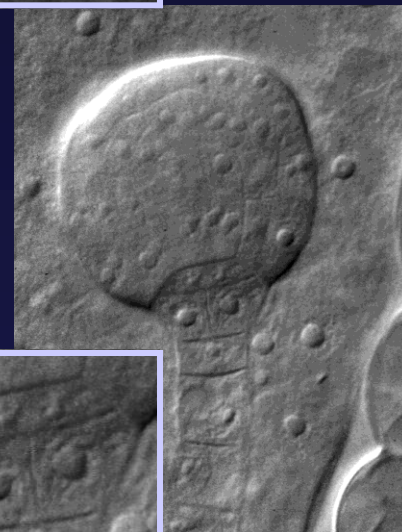
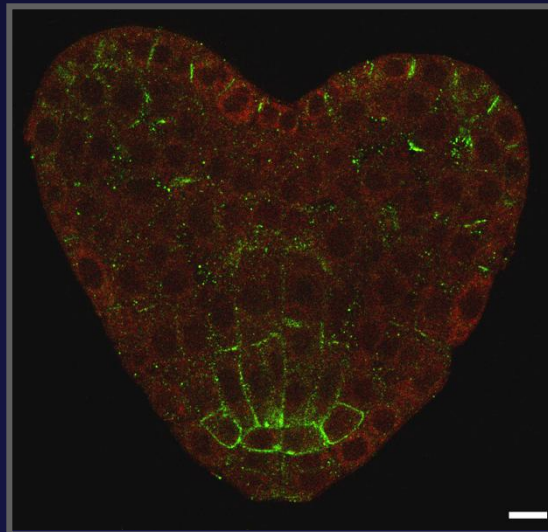
DR5



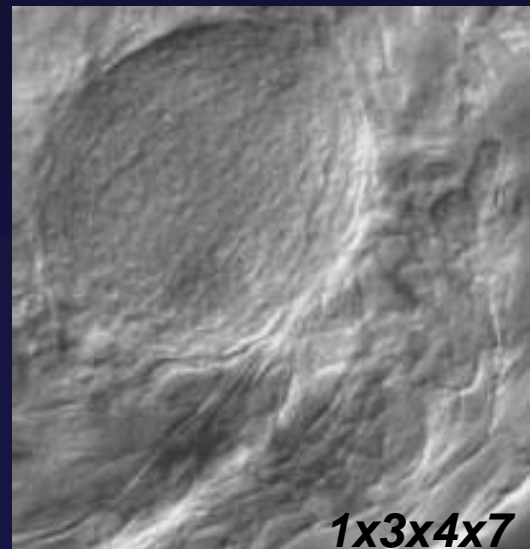
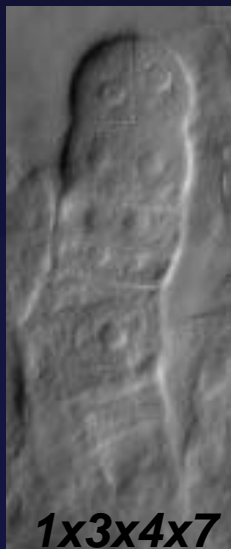
basal defects



QC marker

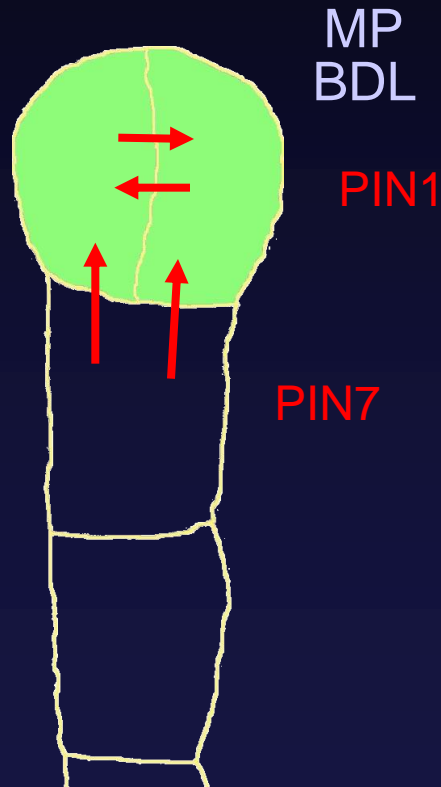


Phenotypes of *pin* Multiple Mutants



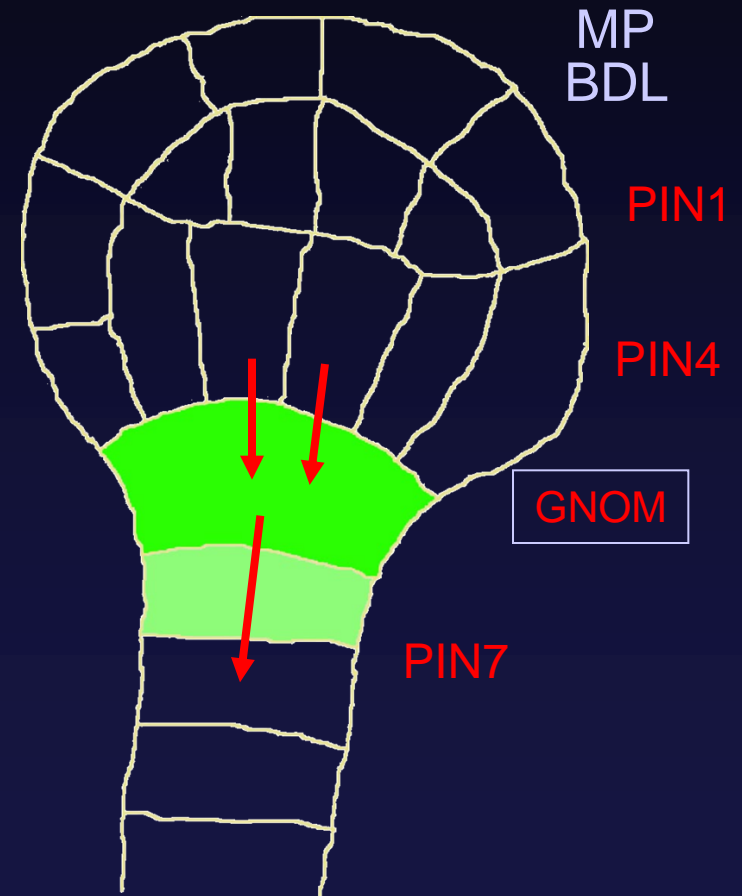
Auxin and Embryogenesis

Apical pole
specification



Two-Cell

Root pole
specification

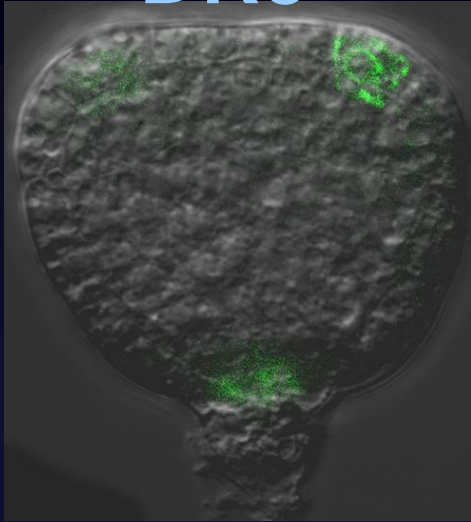


Globular

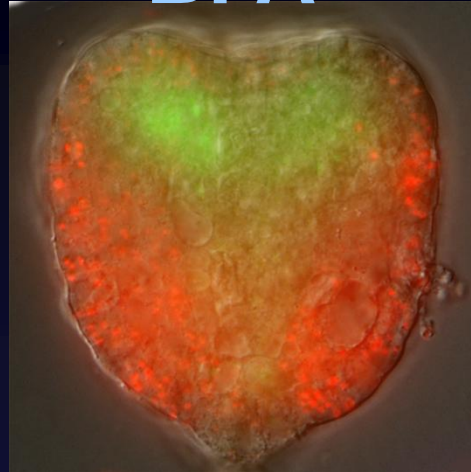
Organogenesis

Auxin in Cotyledon Formation

DR5



BFA



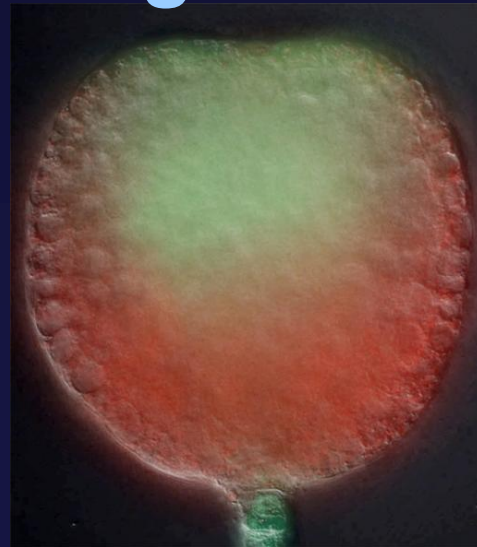
pins



IAA



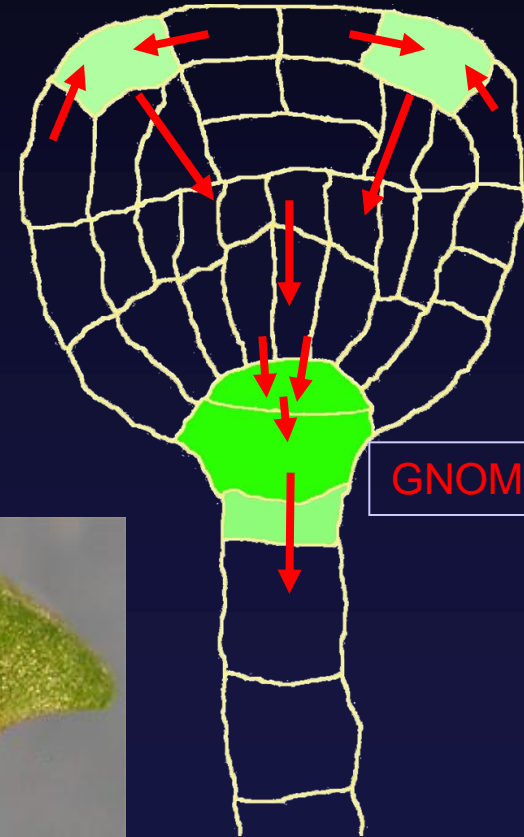
gnom



pin1



MP
BDL
PIN1



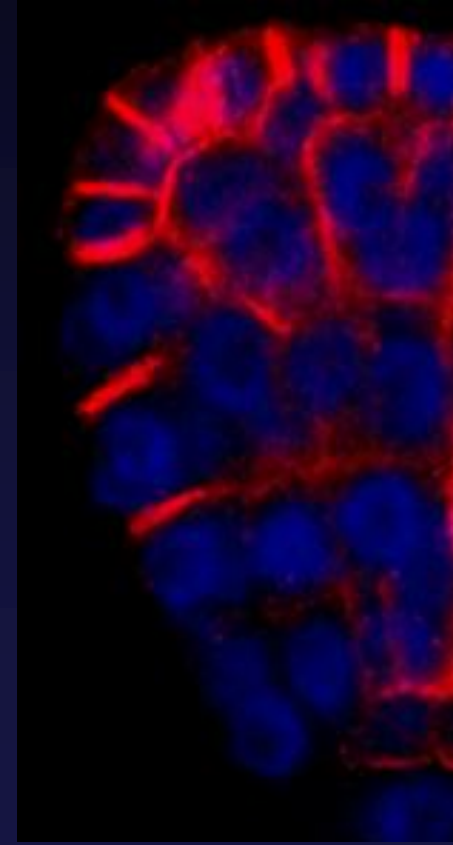
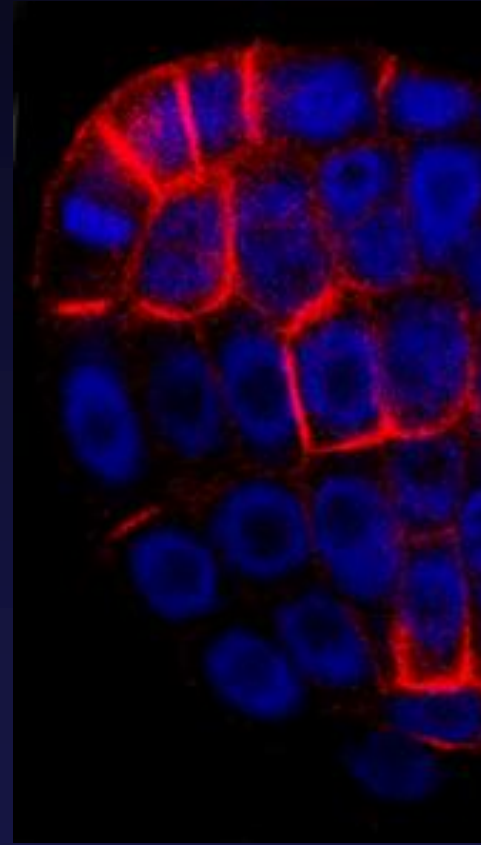
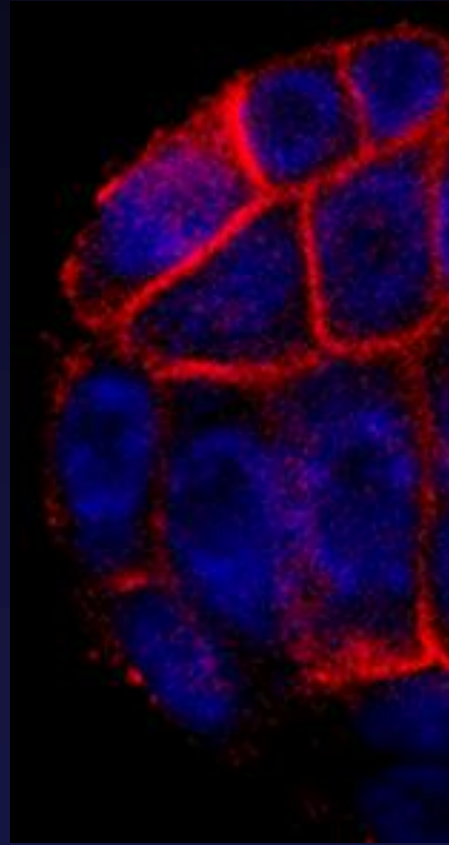
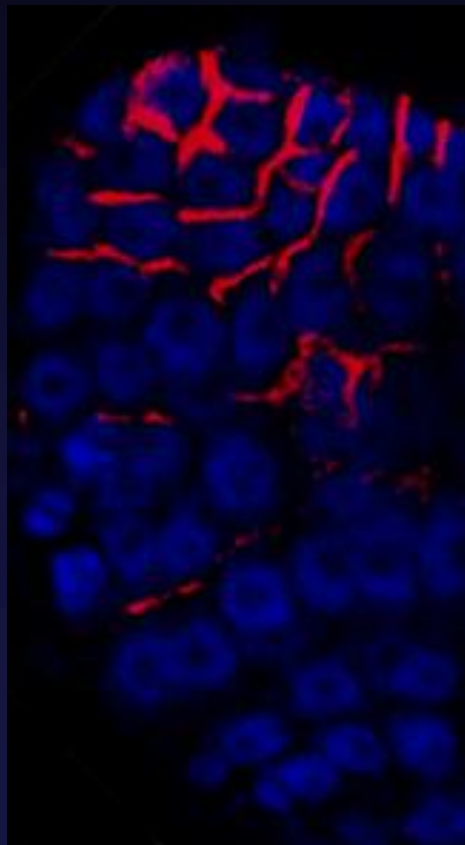
GNOM

PIN1 Polarity in Cotyledon Formation

Outer layer

Inner layers

BFA treatment



Heart

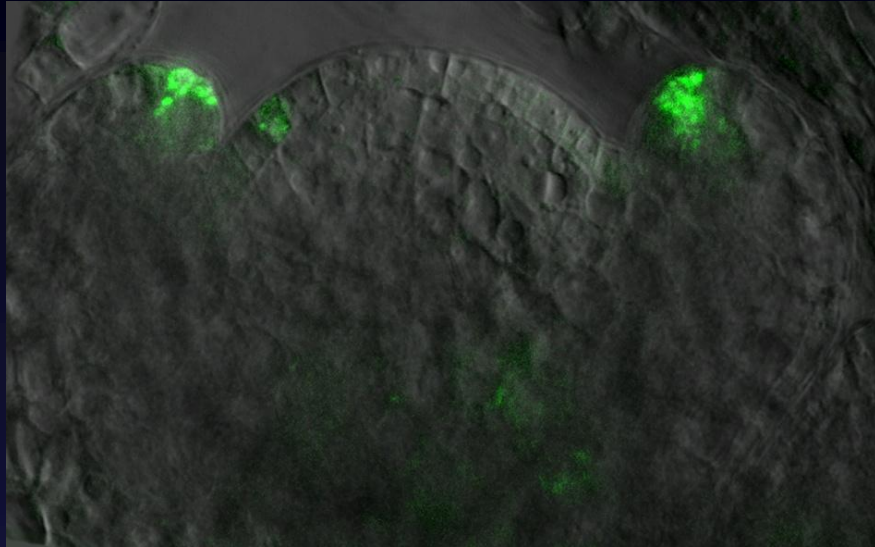
Globular

Heart

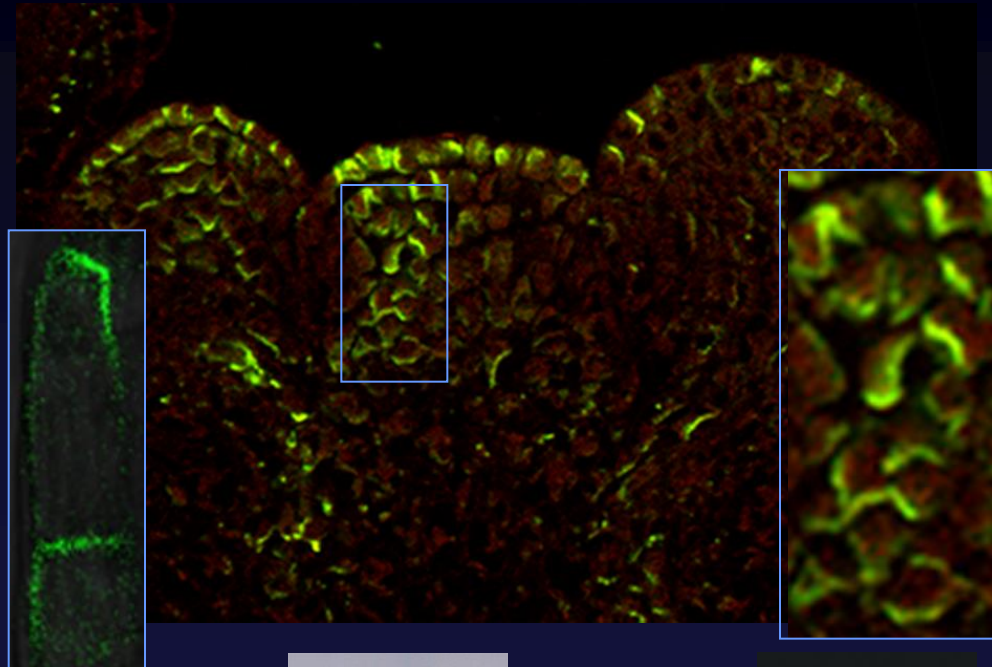
Heart

Auxin in Flower and Leave Formation

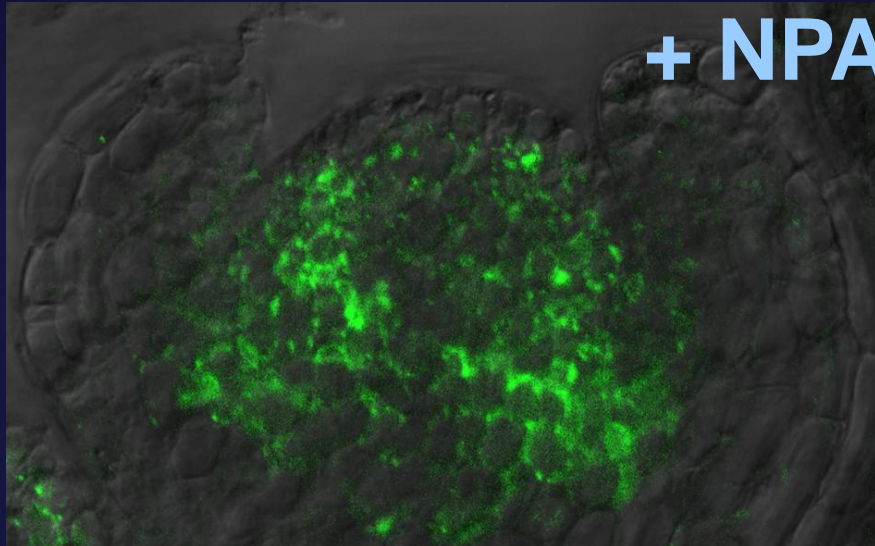
DR5rev::GFP



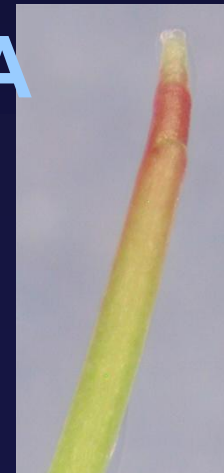
PIN1 localisation



+ NPA



+ NPA



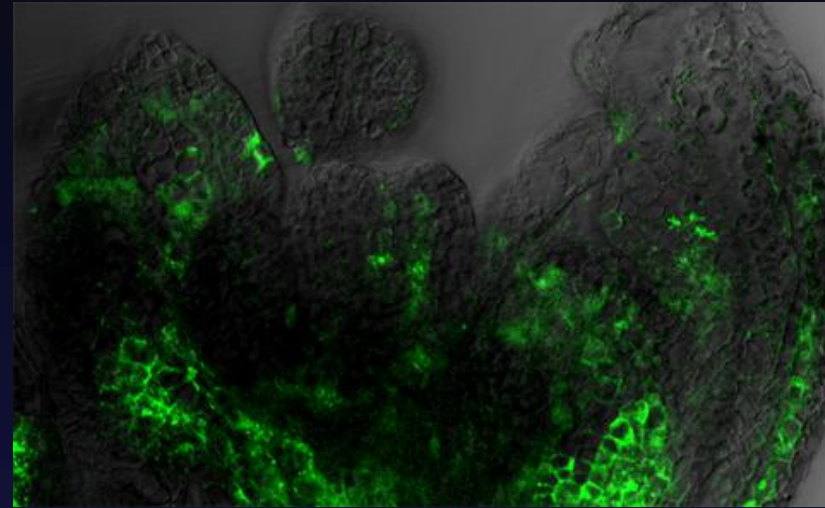
pin1



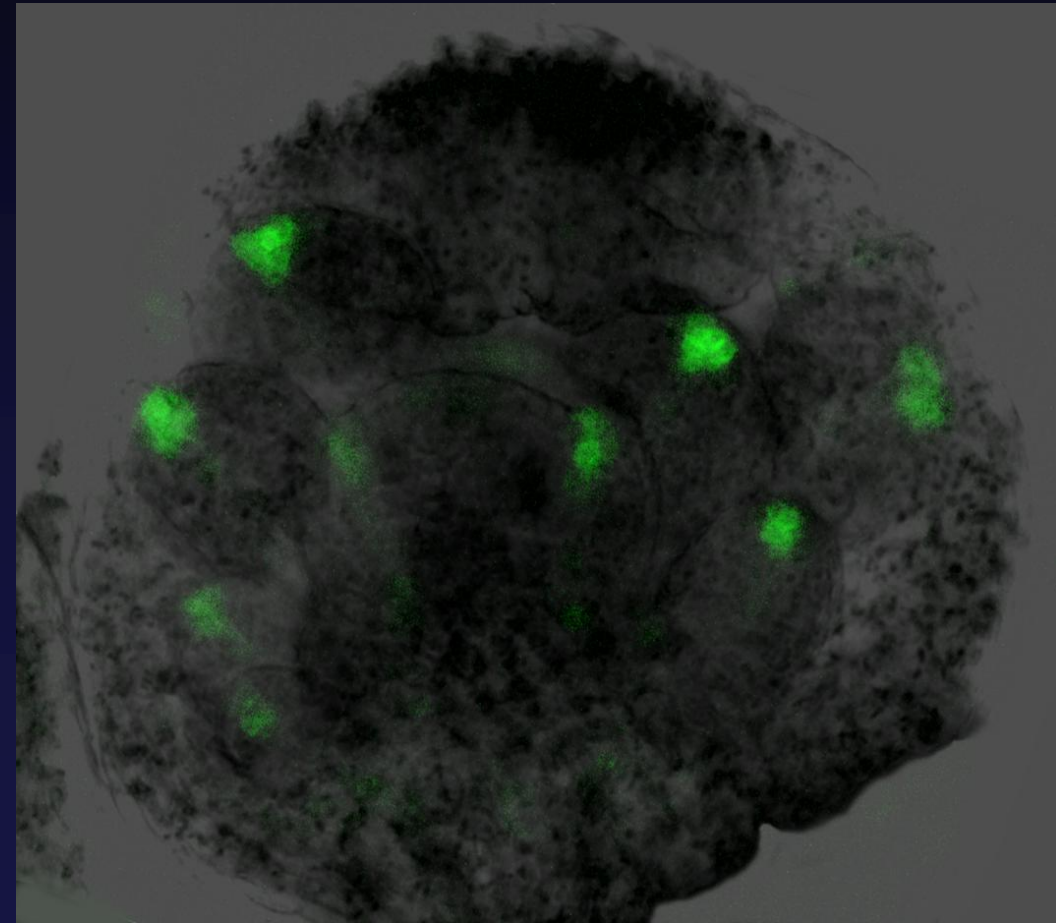
DR5 in Floral Organ Formation

DR5rev::GFP

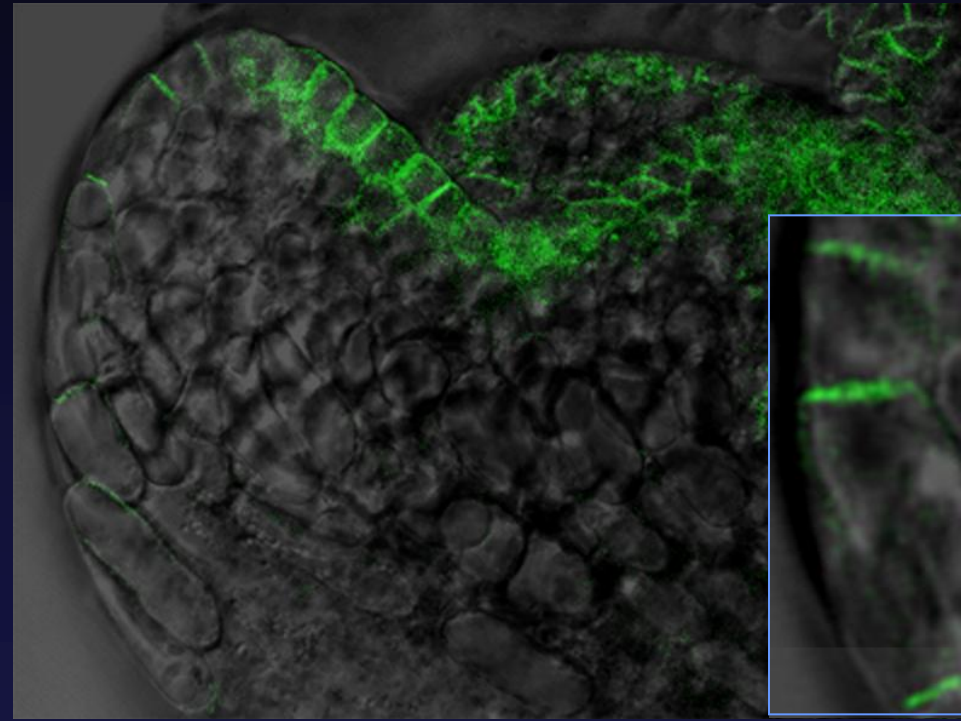
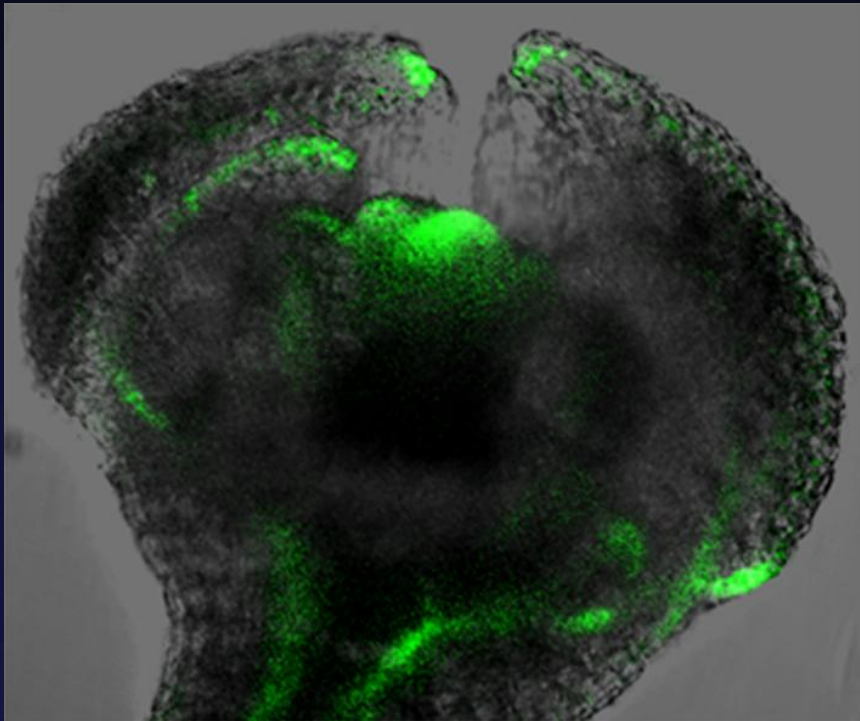
+ NPA



pin mutants

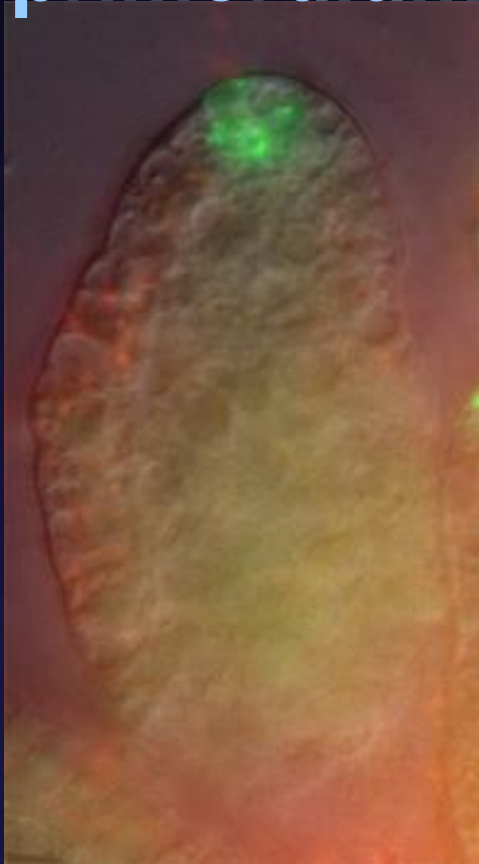


PIN1 in Floral Organ Formation



DR5 in Ovule Formation

Ovule
primordium



Ovule with
Integuments
primordia

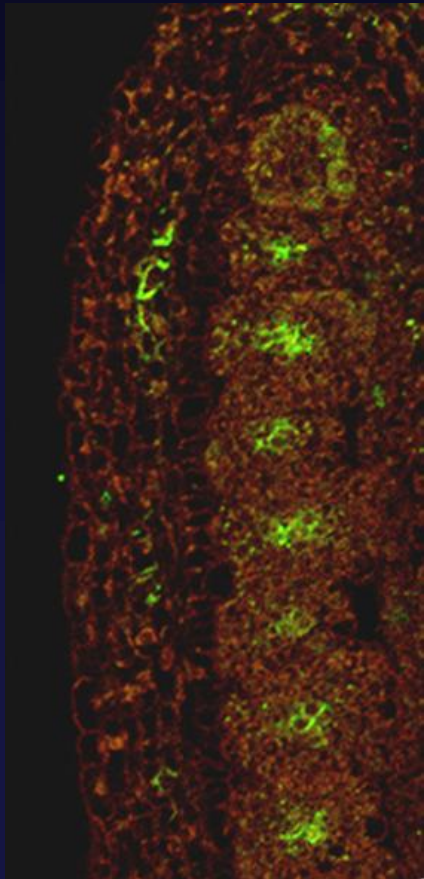


Ovule defects
in *pin1*

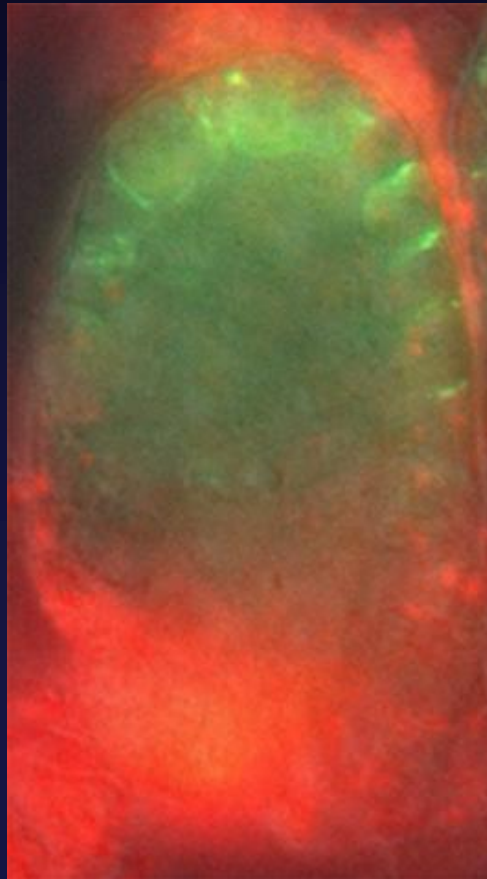


PIN1 in Ovule Formation

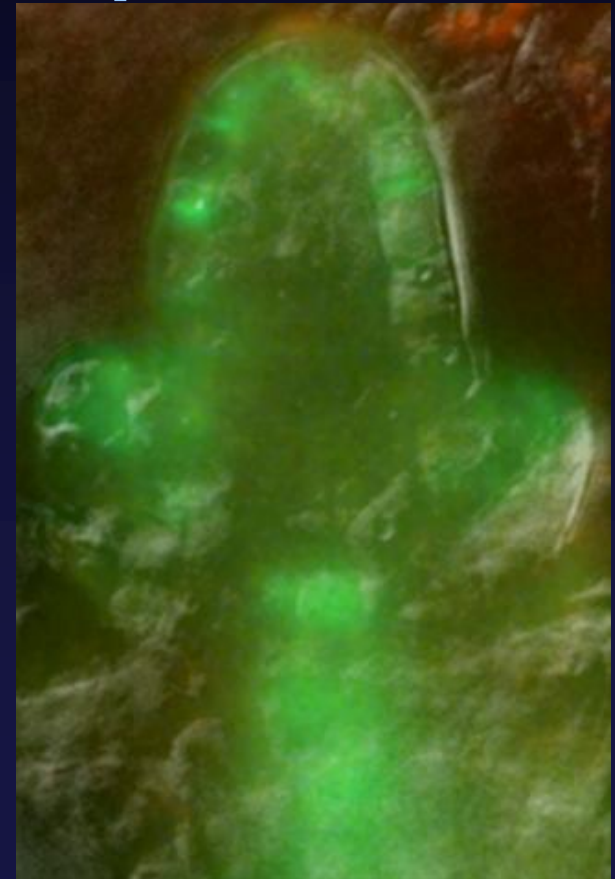
**Gynoecium
with ovule primordia**



**Ovule
primordium**



**Ovule with
Integuments
primordia**

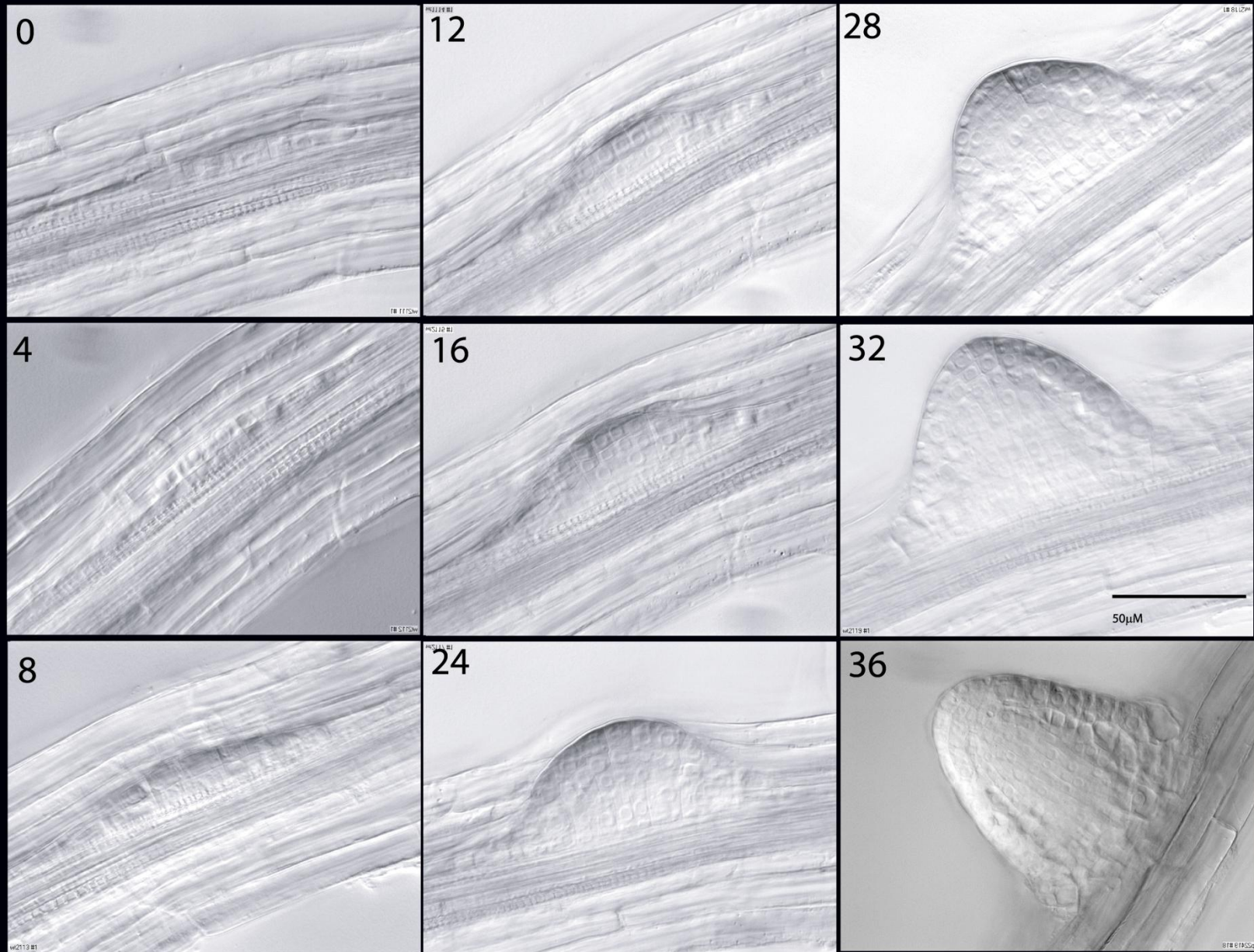


Lateral Root Development



Arabidopsis lateral root

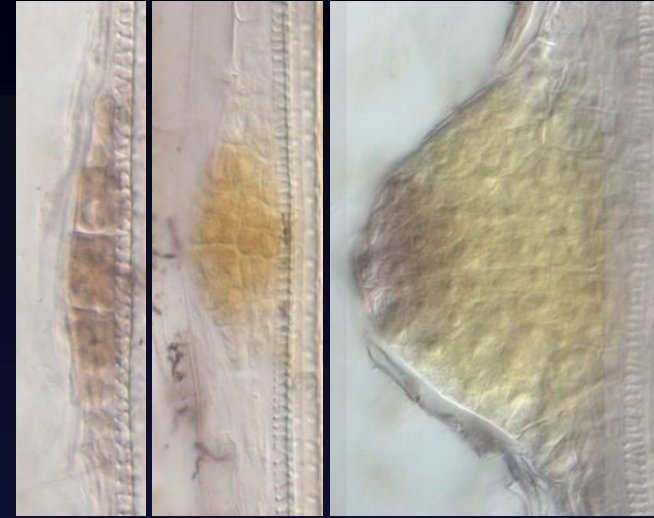
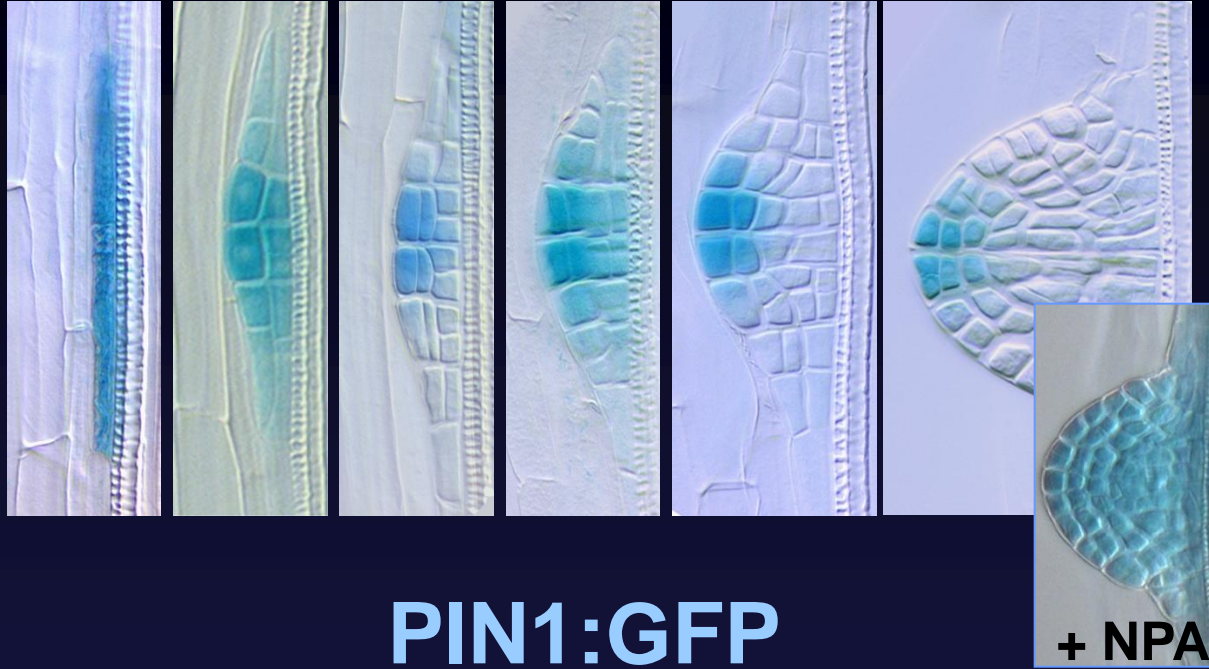
Lateral Root Development in Time



DR5 in Lateral Root Formation

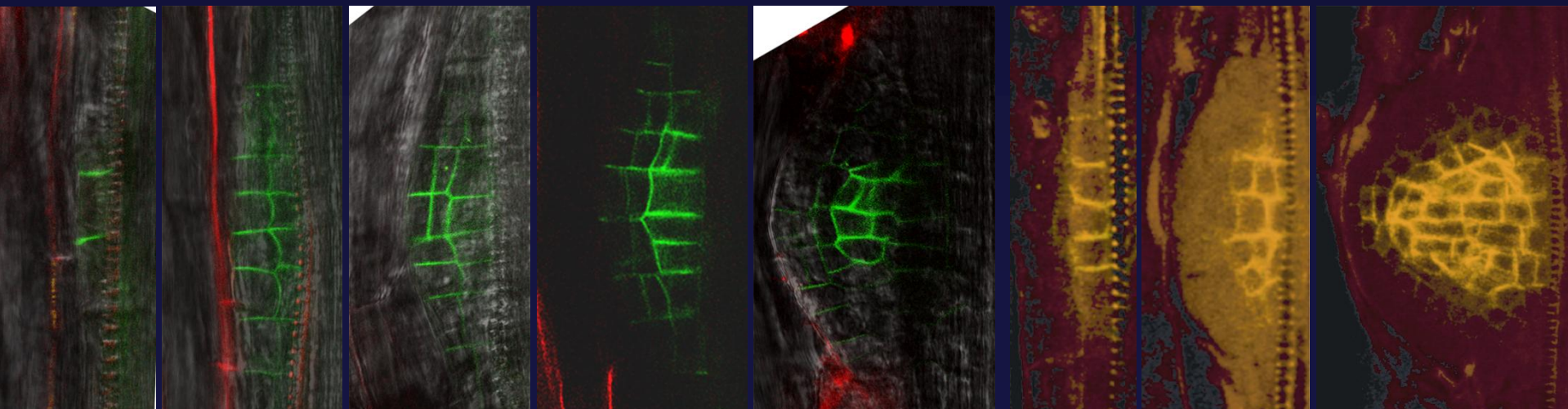
DR5rev::GUS

IAA



PIN1:GFP

PIN1



Relocation > Gradients > Primordia

PIN1

DR5

CycBmargins

primordia

+ IAA

+ IAA

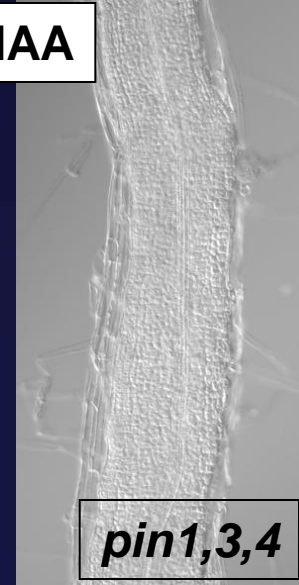
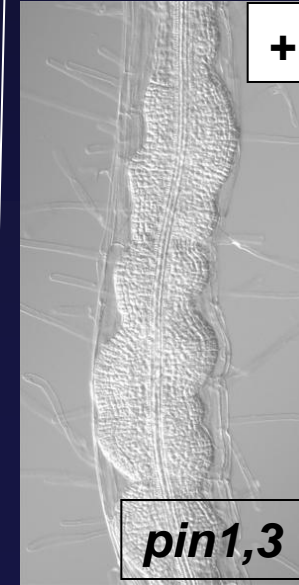
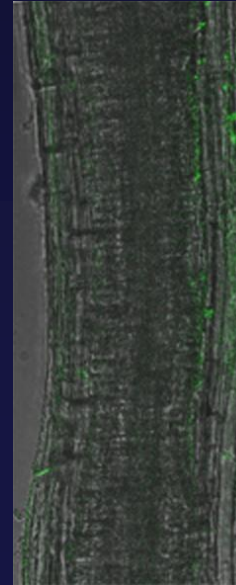
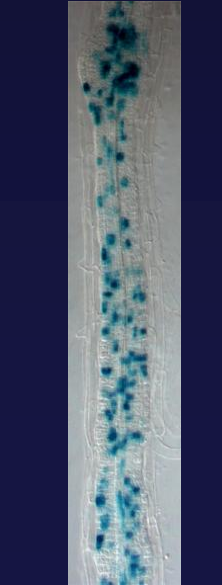
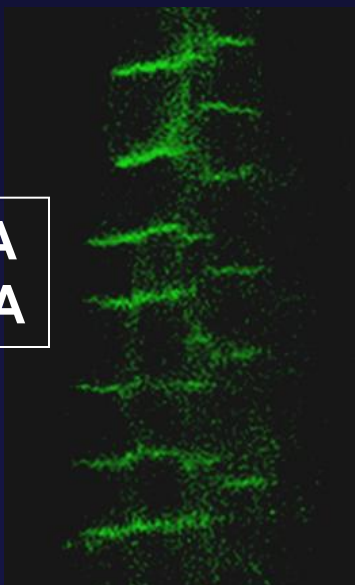
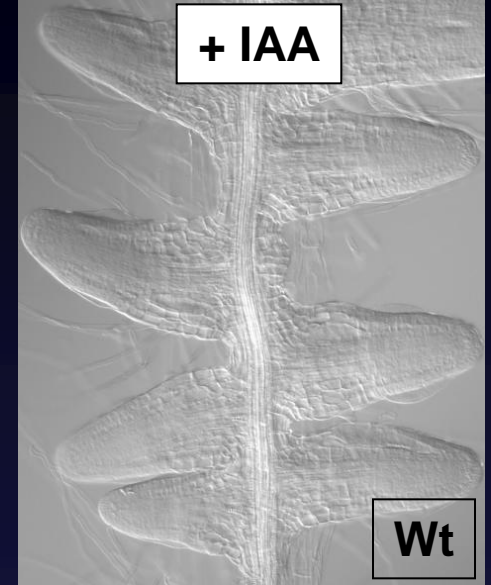
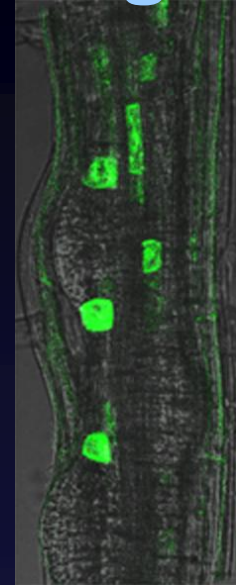
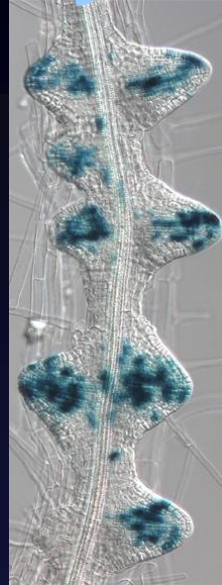
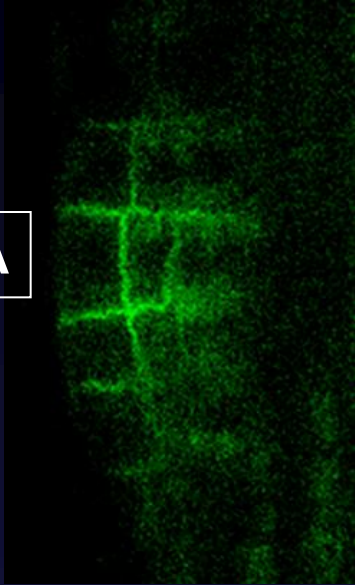
Wt

**+ IAA
+ NPA**

+ IAA

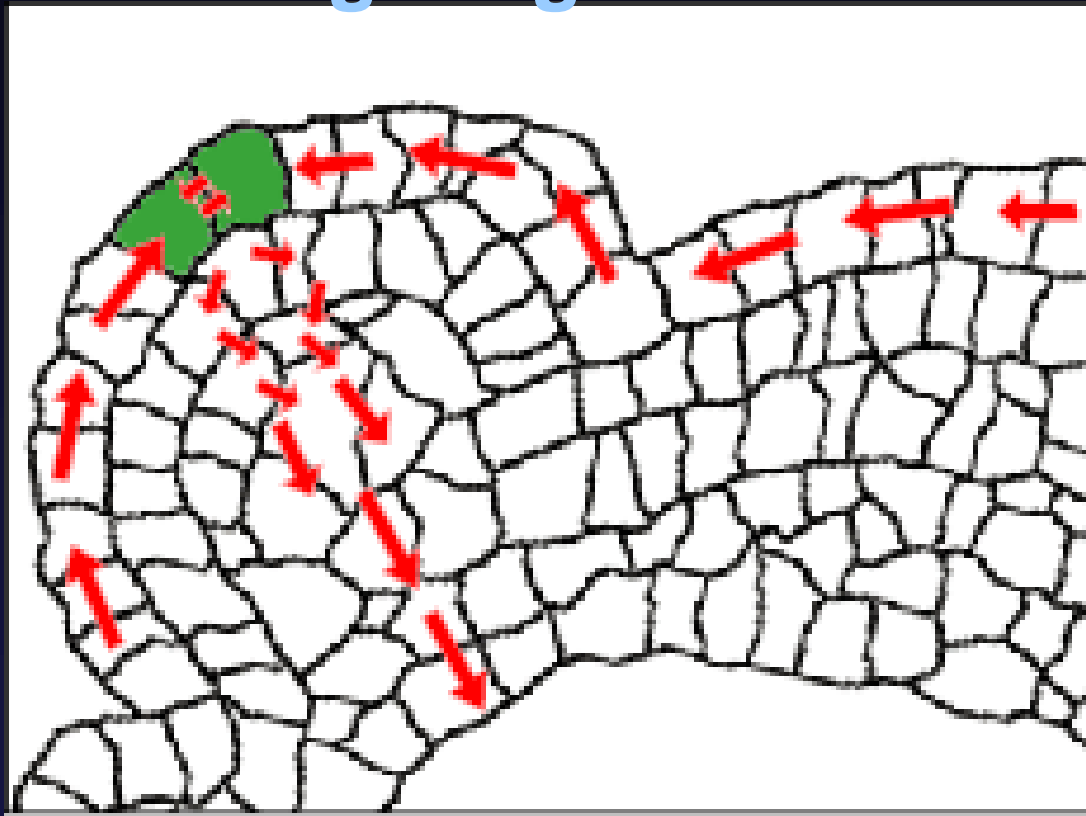
pin1,3

pin1,3,4

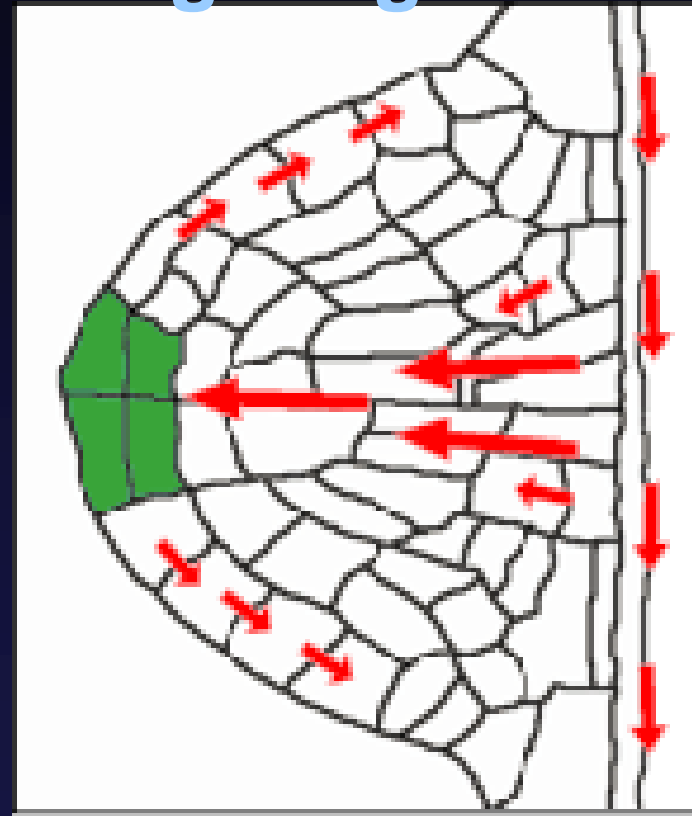


Common module for organ formation

Aerial organogenesis

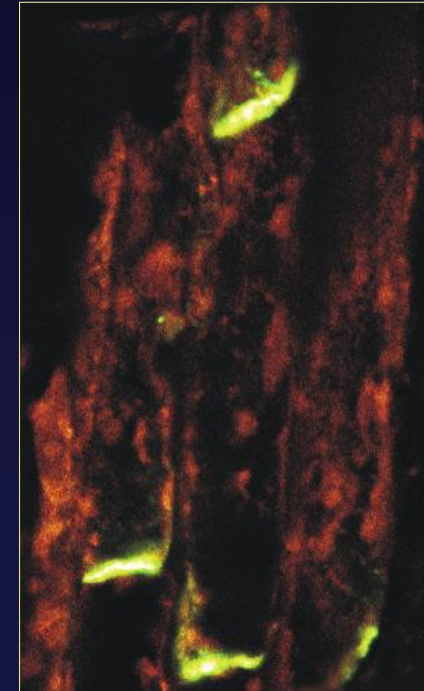
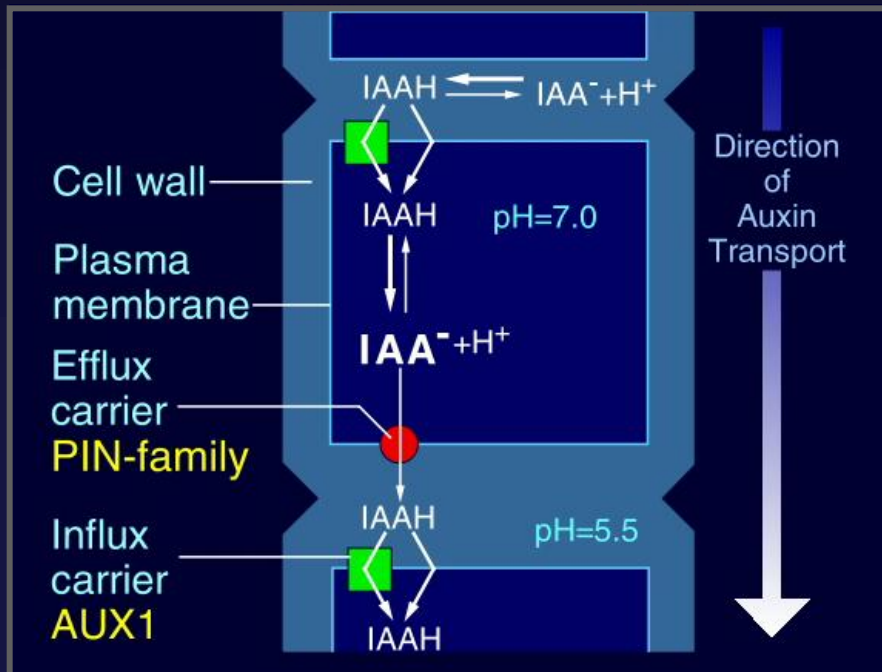


Underground organogenesis



Cotyledons, leaves, flowers, Lateral roots
axillary organs, ovules, integuments

Cellular Polarity of PIN Localisation and Directionality of Intercellular Auxin Flow



PIN-specific Signals for Polar Targeting

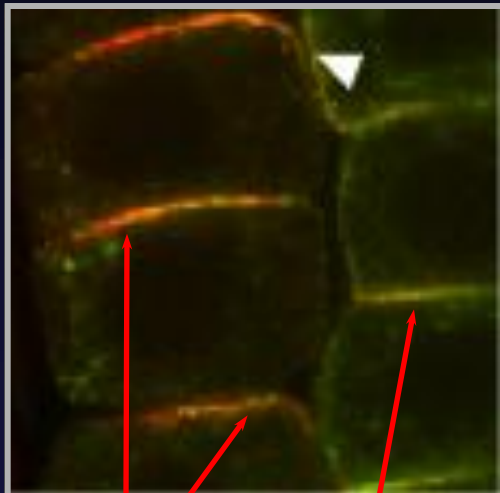


PIN2pr::PIN2:HA

PIN2pr::PIN1:HA

PIN2pr::PIN1:GFP

PIN1/PIN1:GFP



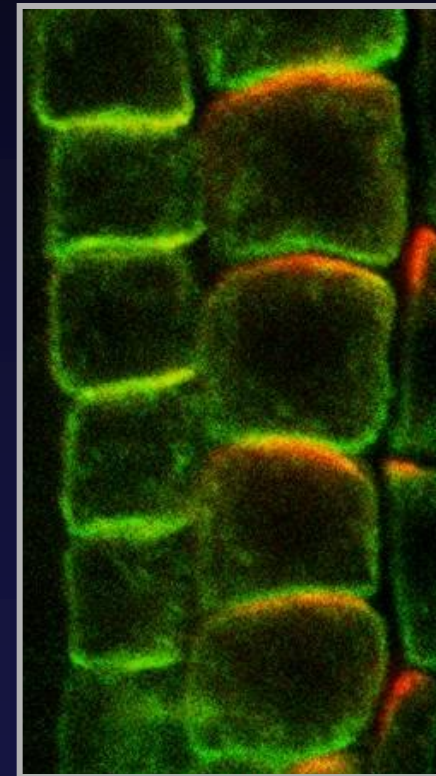
apical
basal
localization



basal
localization



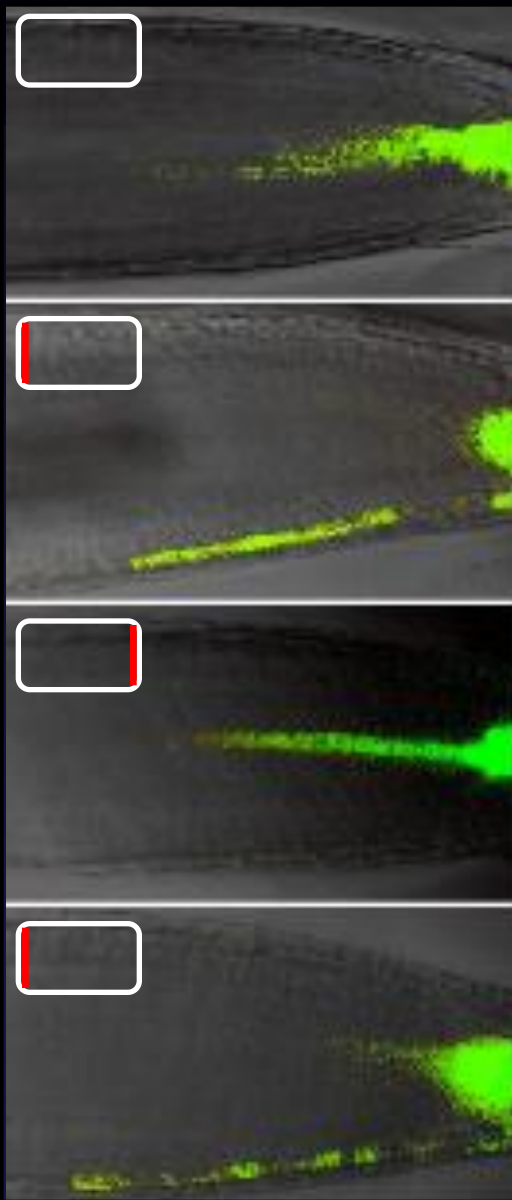
apical
basal
localization



PIN Polarity Determines Direction of Auxin Flow



DR5rev::GFP



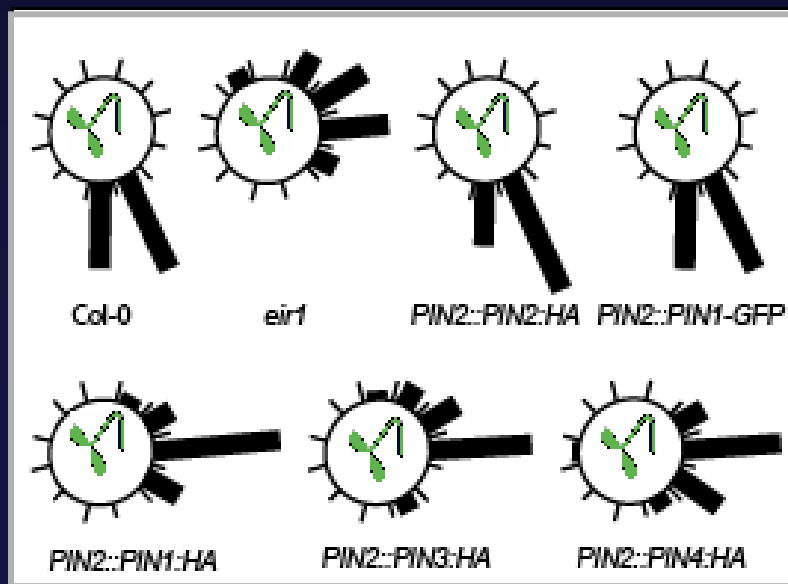
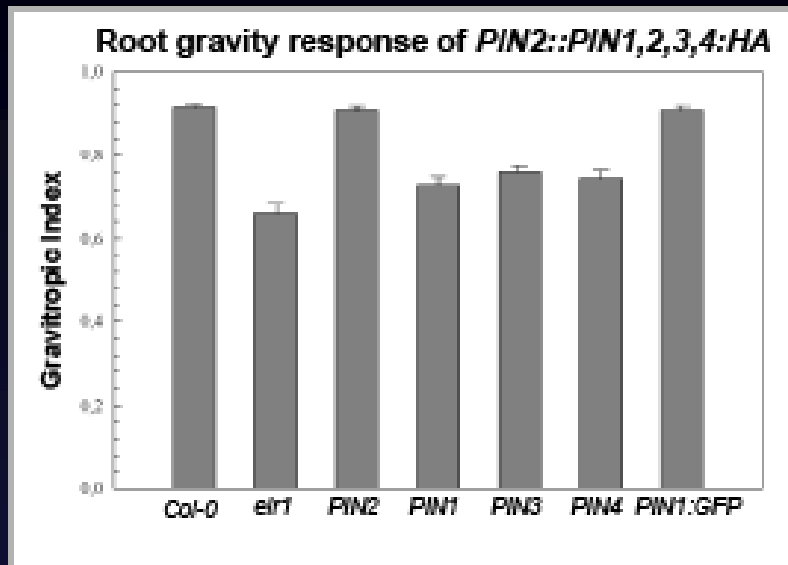
pin2 (eir1, agr1)

PIN2::PIN2:HA

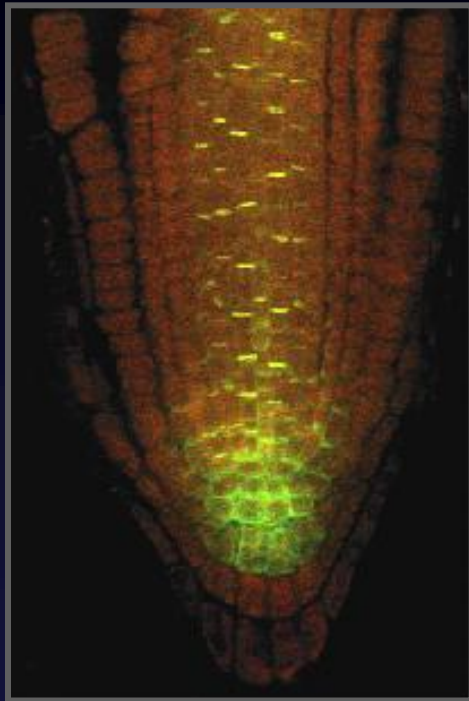
PIN2::PIN1:HA
PIN2::PIN1:GFP-2

PIN2::PIN1:GFP-3

gravitropism



Mutant Screen for Components of Polarity and Recycling



PIN:GFP

EMS mutagenesis.
Screening for
polarity and cycling
defects.



mutant lines

intragenic

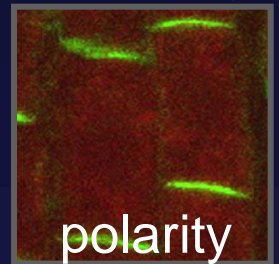
extragenic

sequencing

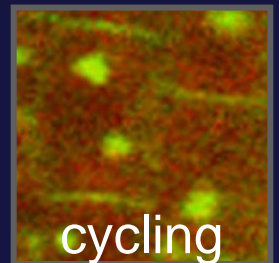
cloning

**important
residues**

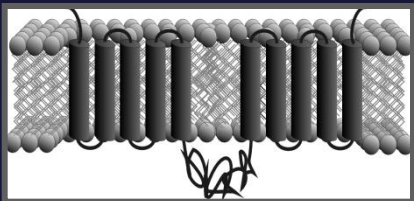
novel genes



polarity



cycling



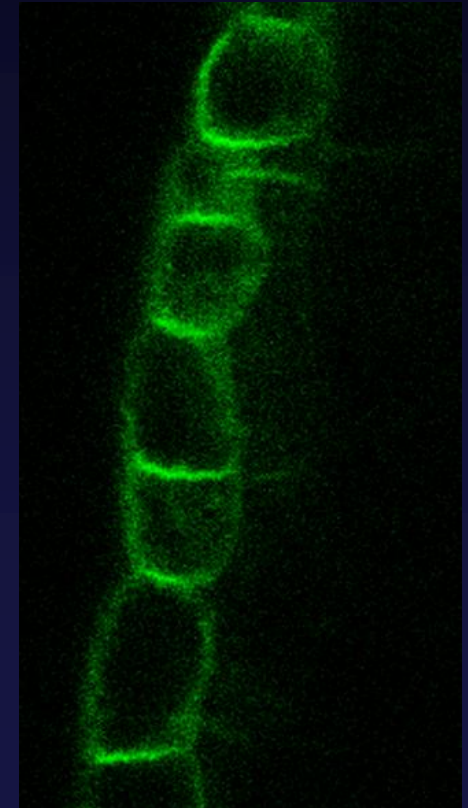
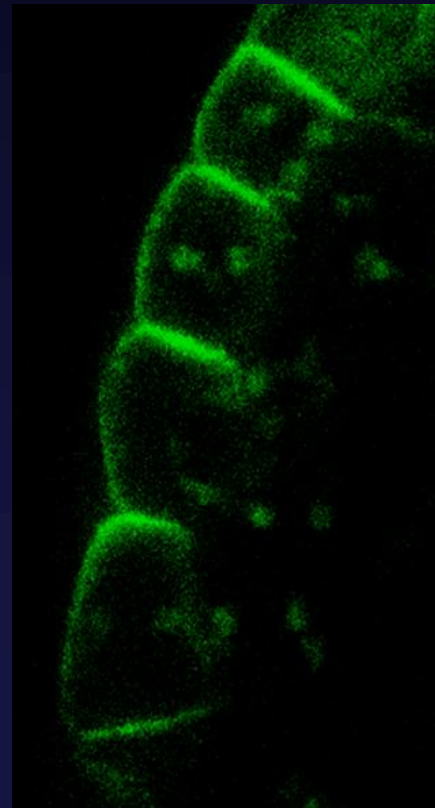
Molecular Components of PIN Polar Targeting

Ser/Thr protein kinase PINOID (PID)

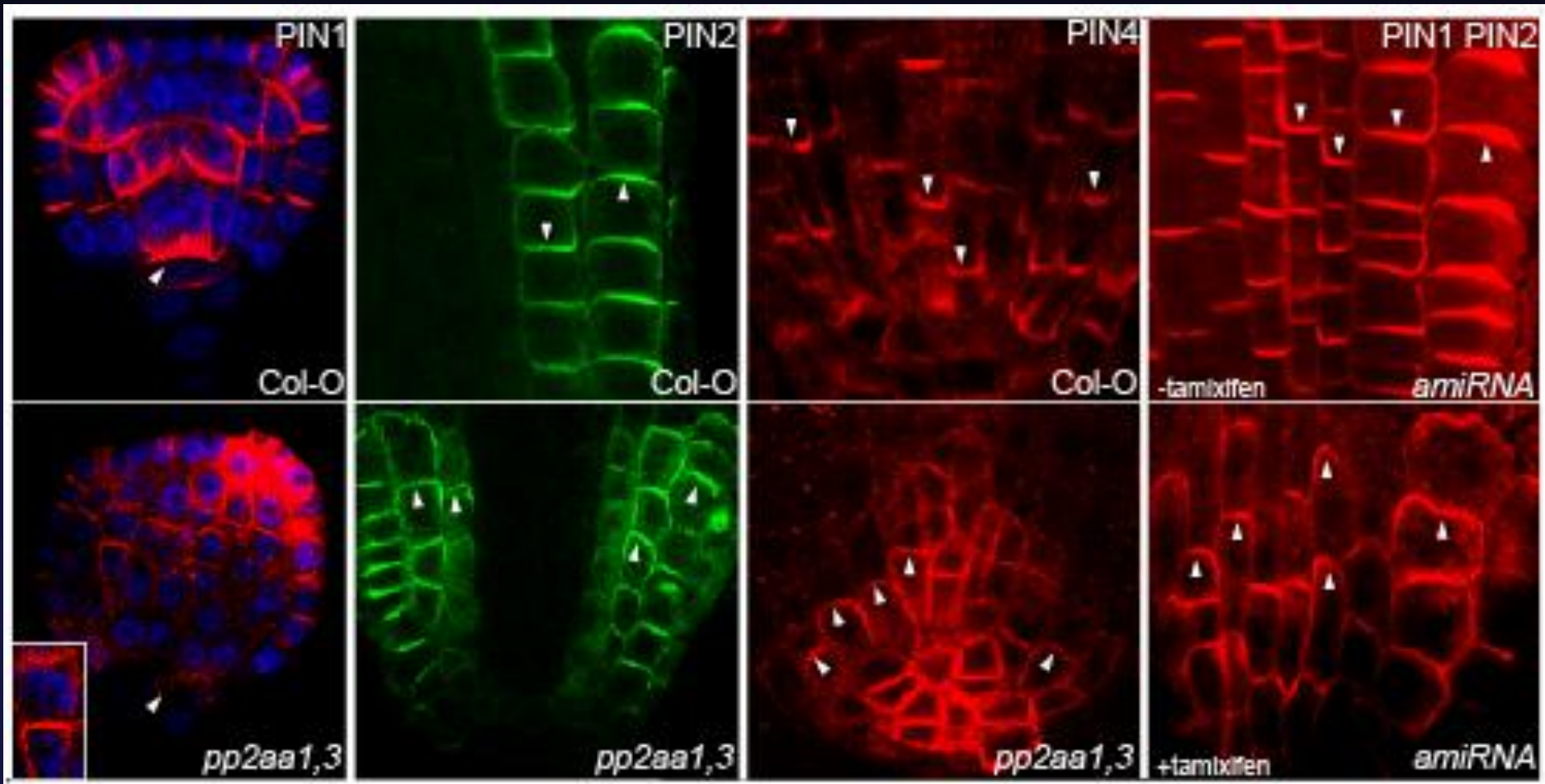


Col-0

pinoid



PP2A Phosphatase and PIN Apical-Basal Targeting

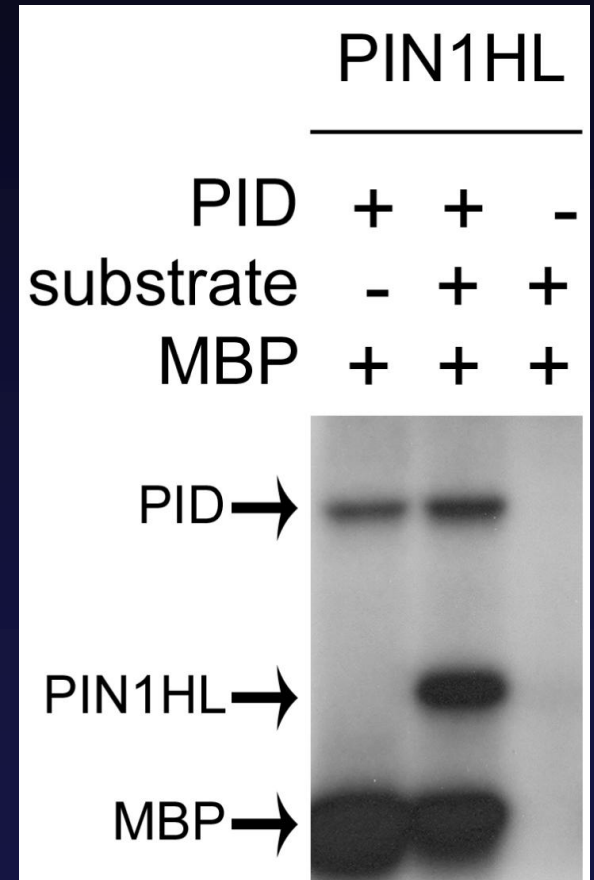
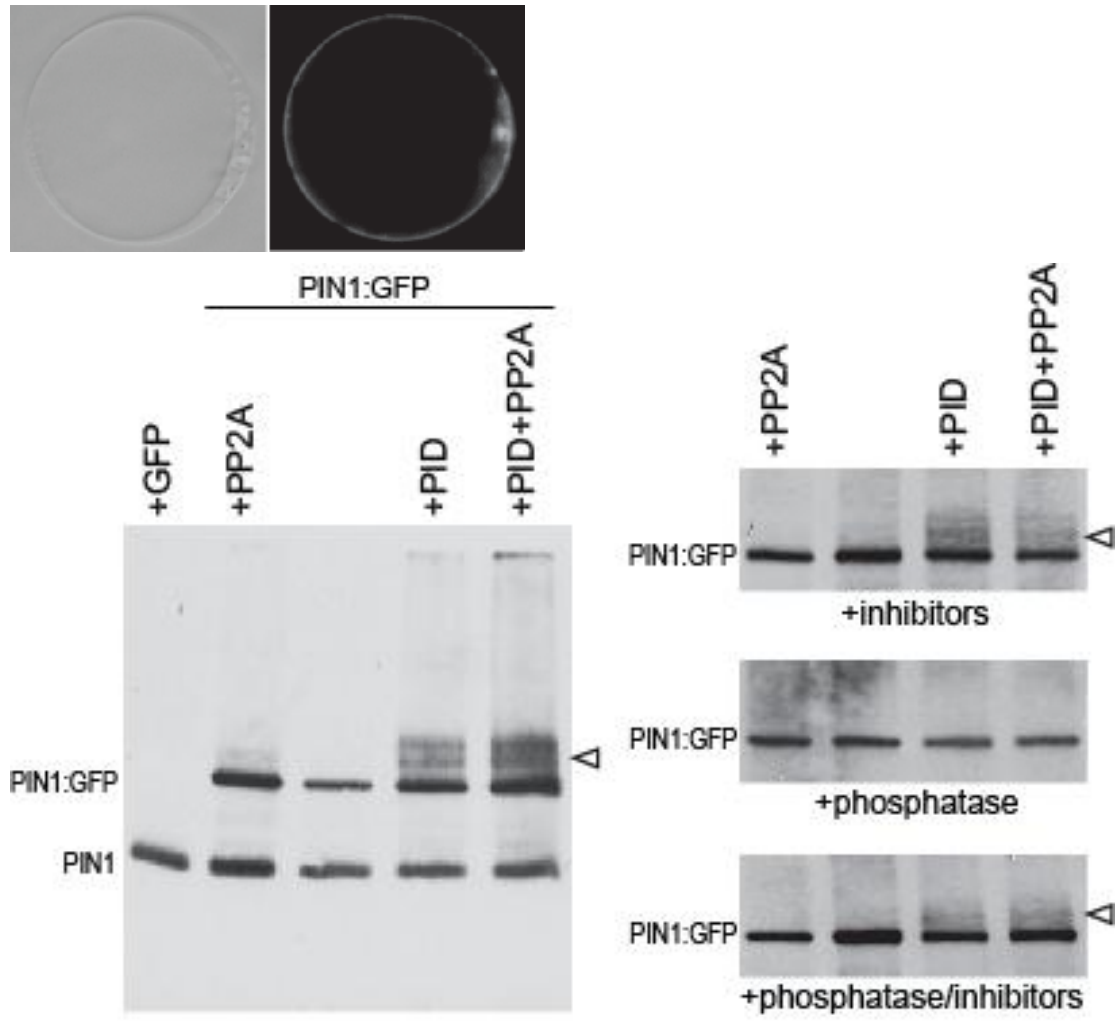


PID Phosphorylates PINs



Phosphorylation assays in protoplast

in vitro phosphorylation





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

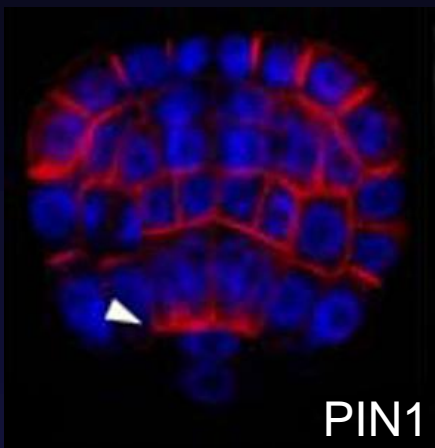


Col-0

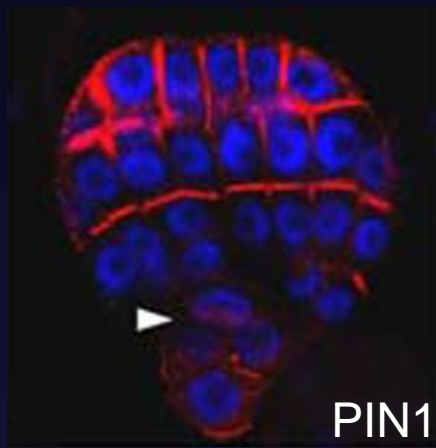
RPS5::PID

Col-0

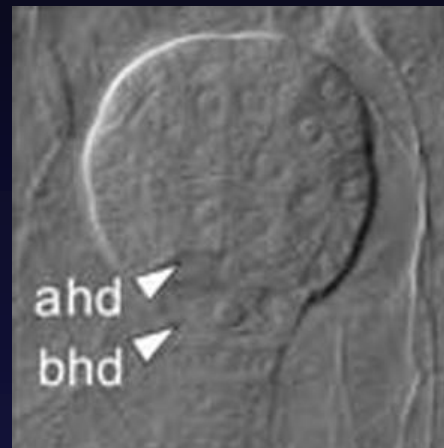
RPS5::PID



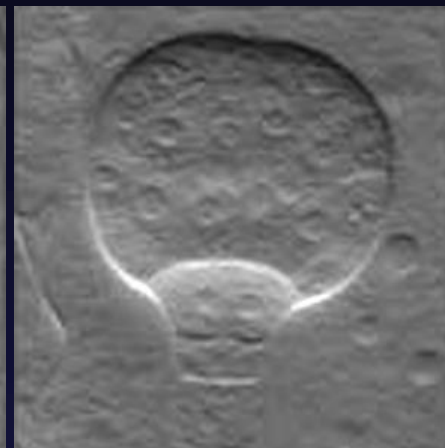
PIN1



PIN1



ahd
bhd



RPS5::PID seedlings



DR5

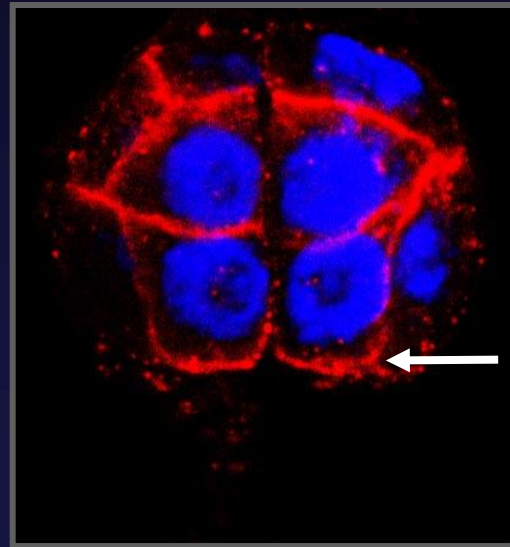
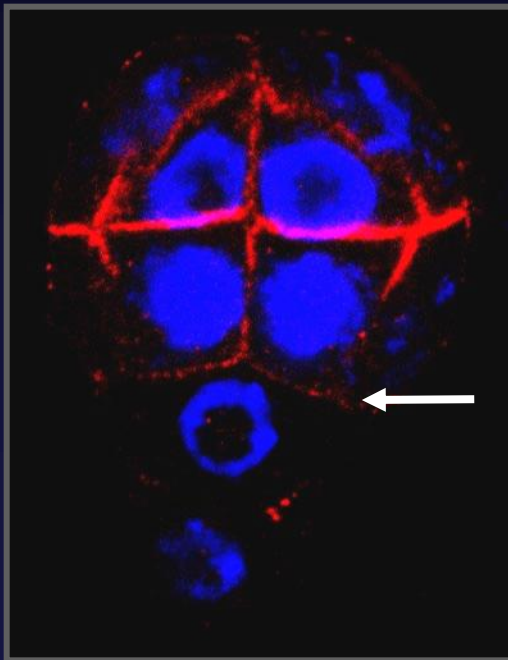


DR5

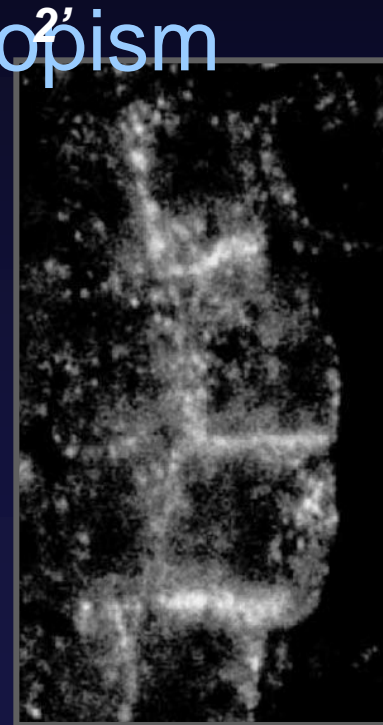
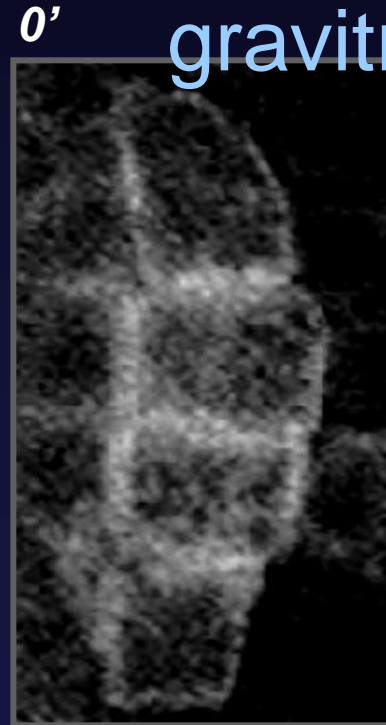


Rapid Changes in PIN Polarity for Redirection of Auxin Flow

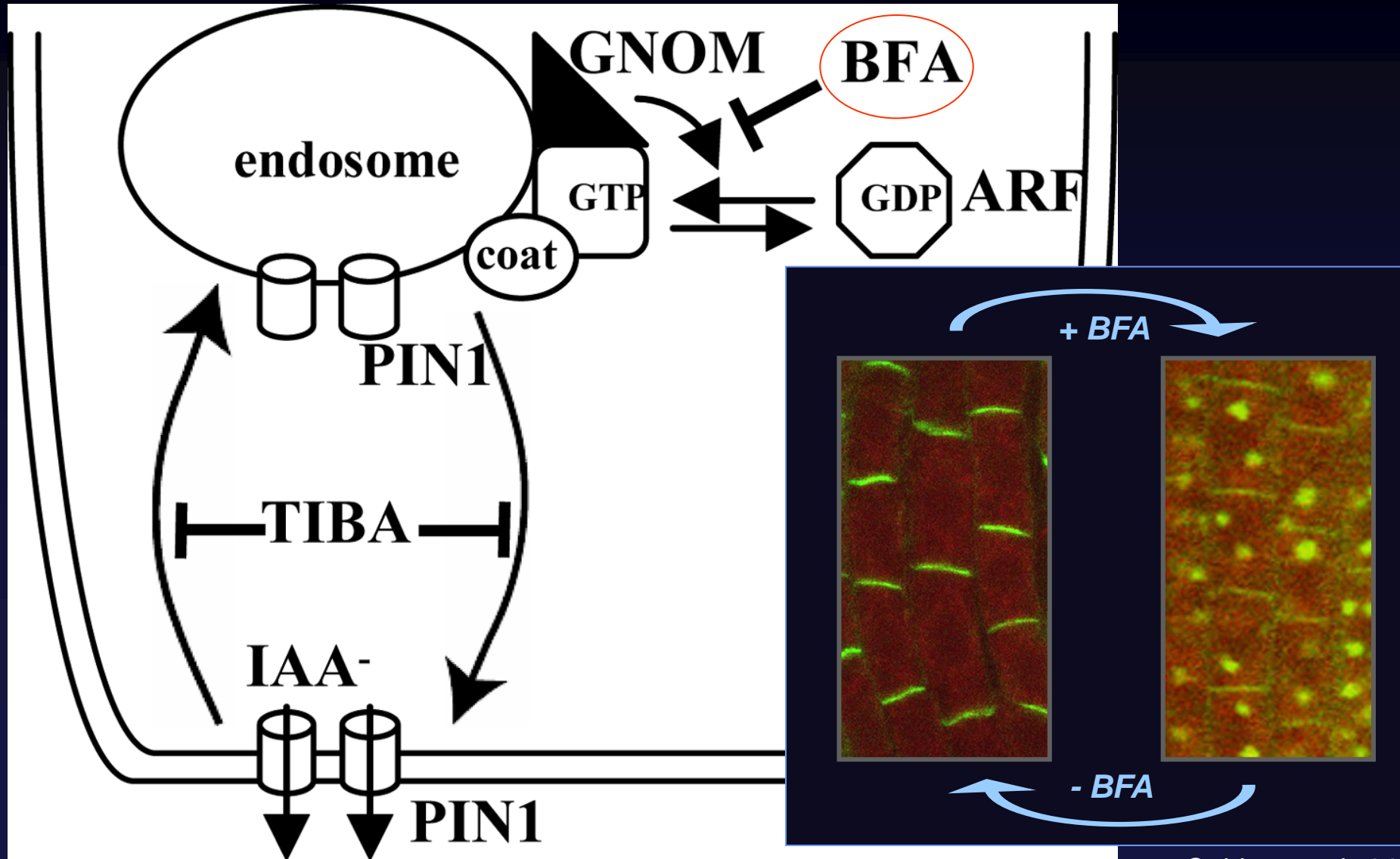
PIN1 in
embryogenesis



PIN3 for
gravitropism



Subcellular Cycling of PIN Proteins

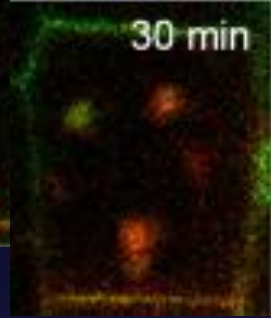
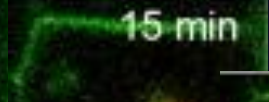
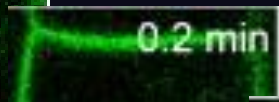


UV-activated PIN2-EosFP

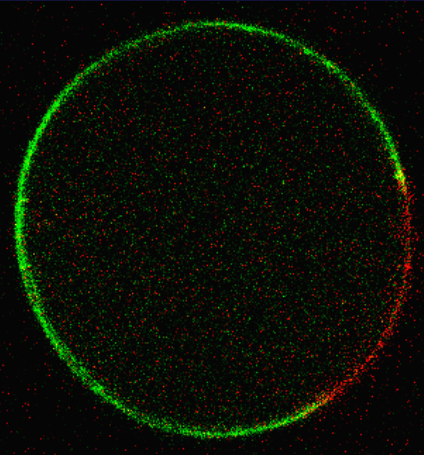
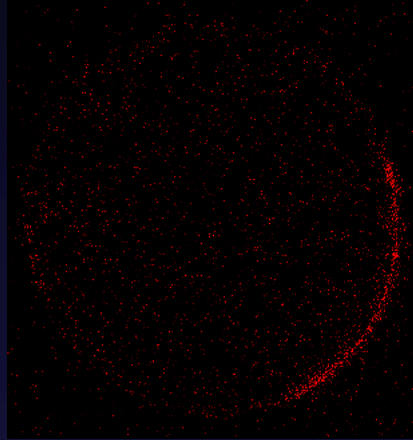
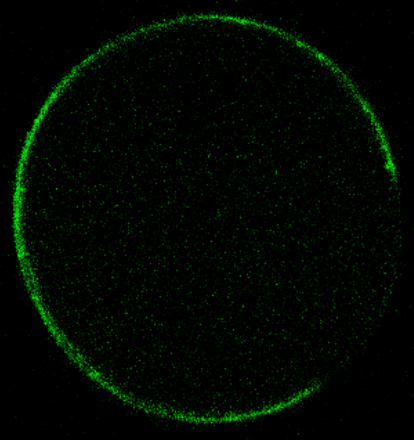
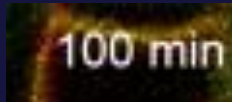
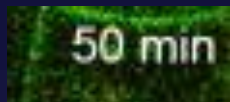


Protoplasts

+ BFA

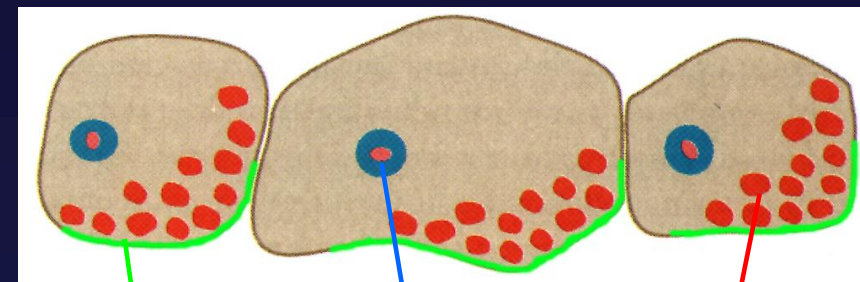
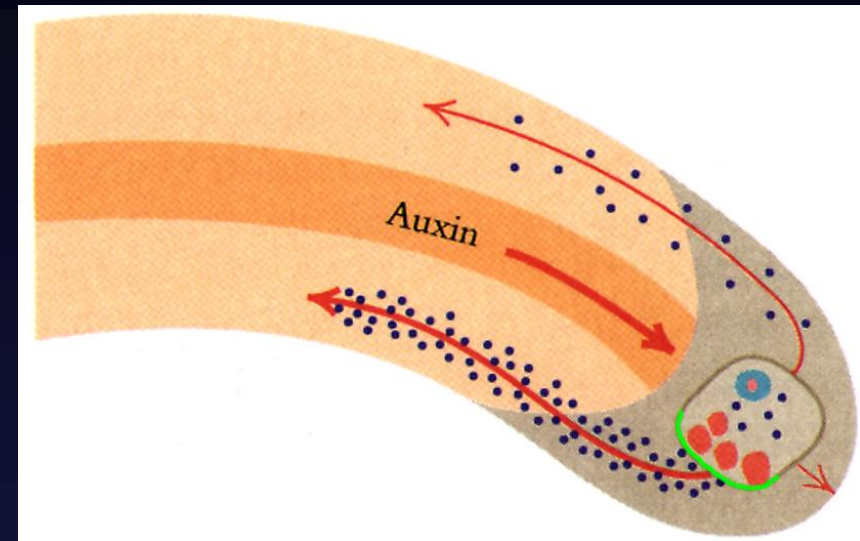
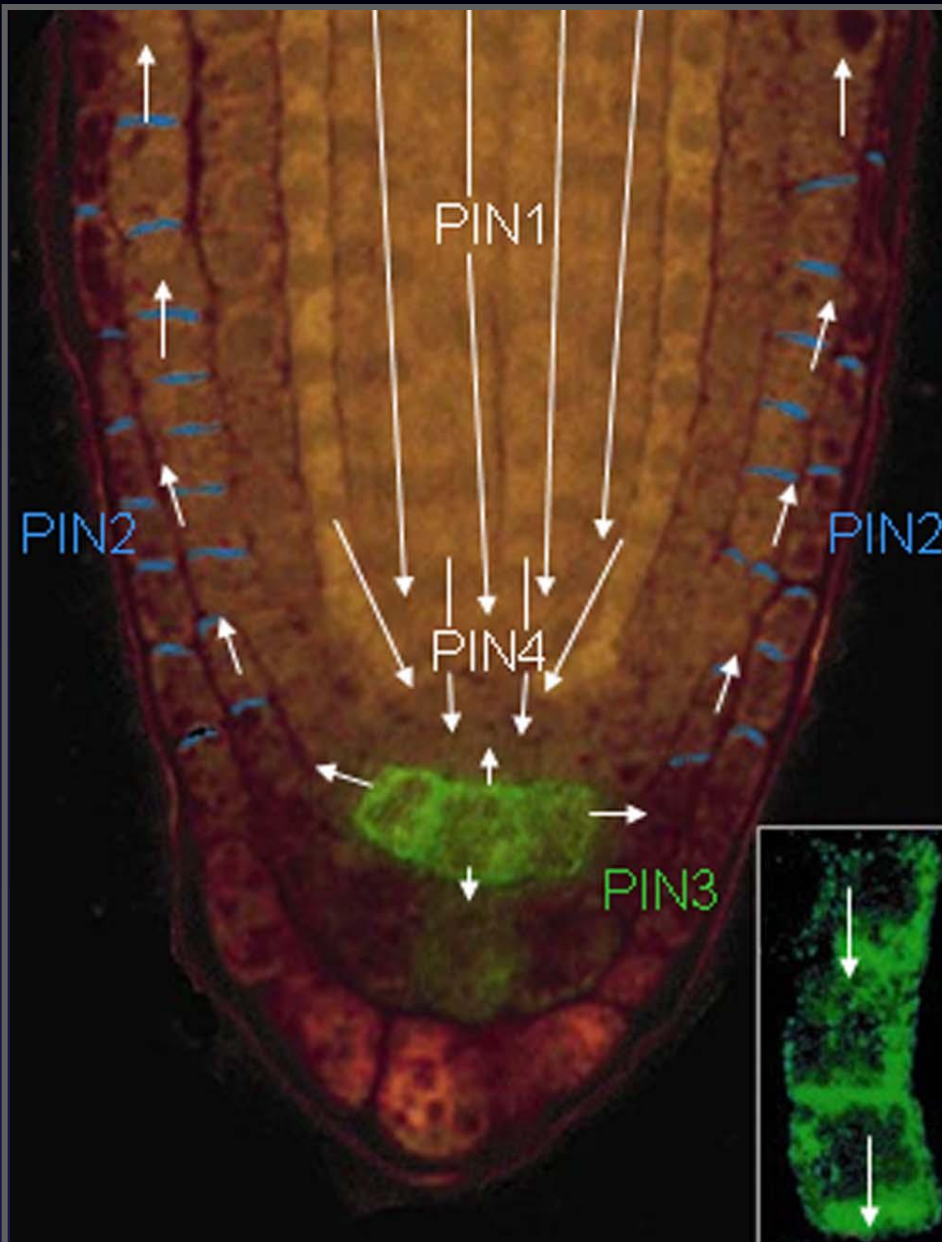


- BFA



PIN3 Polarity Switch in Gravitropic Response

Root turned on its side

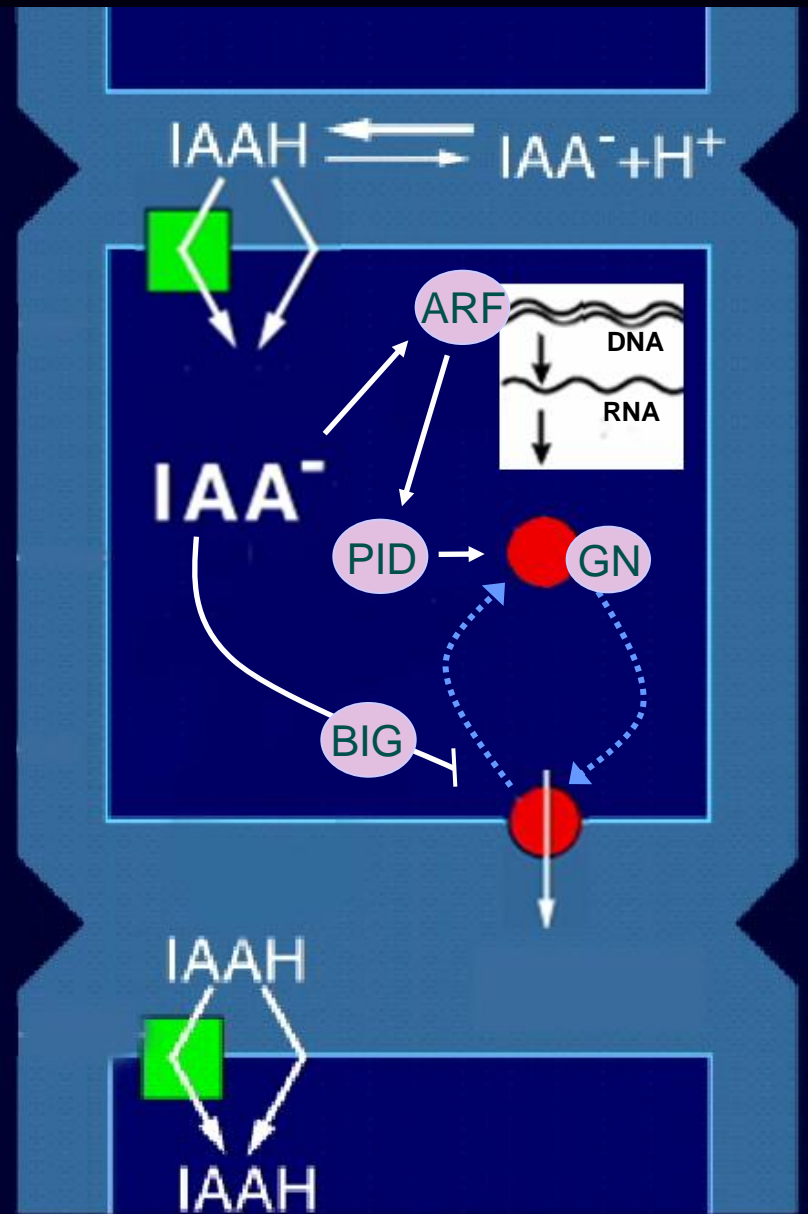


AtPIN3

Nucleus

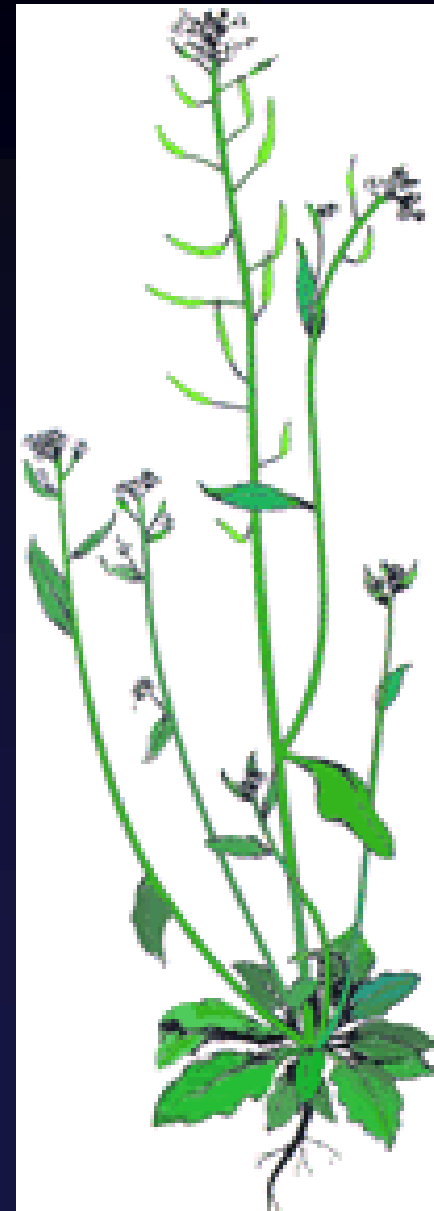
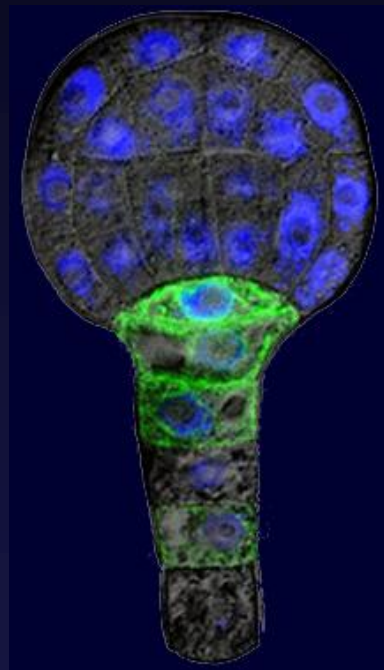
Statolites

Cell-biological Determinants



Plant Development

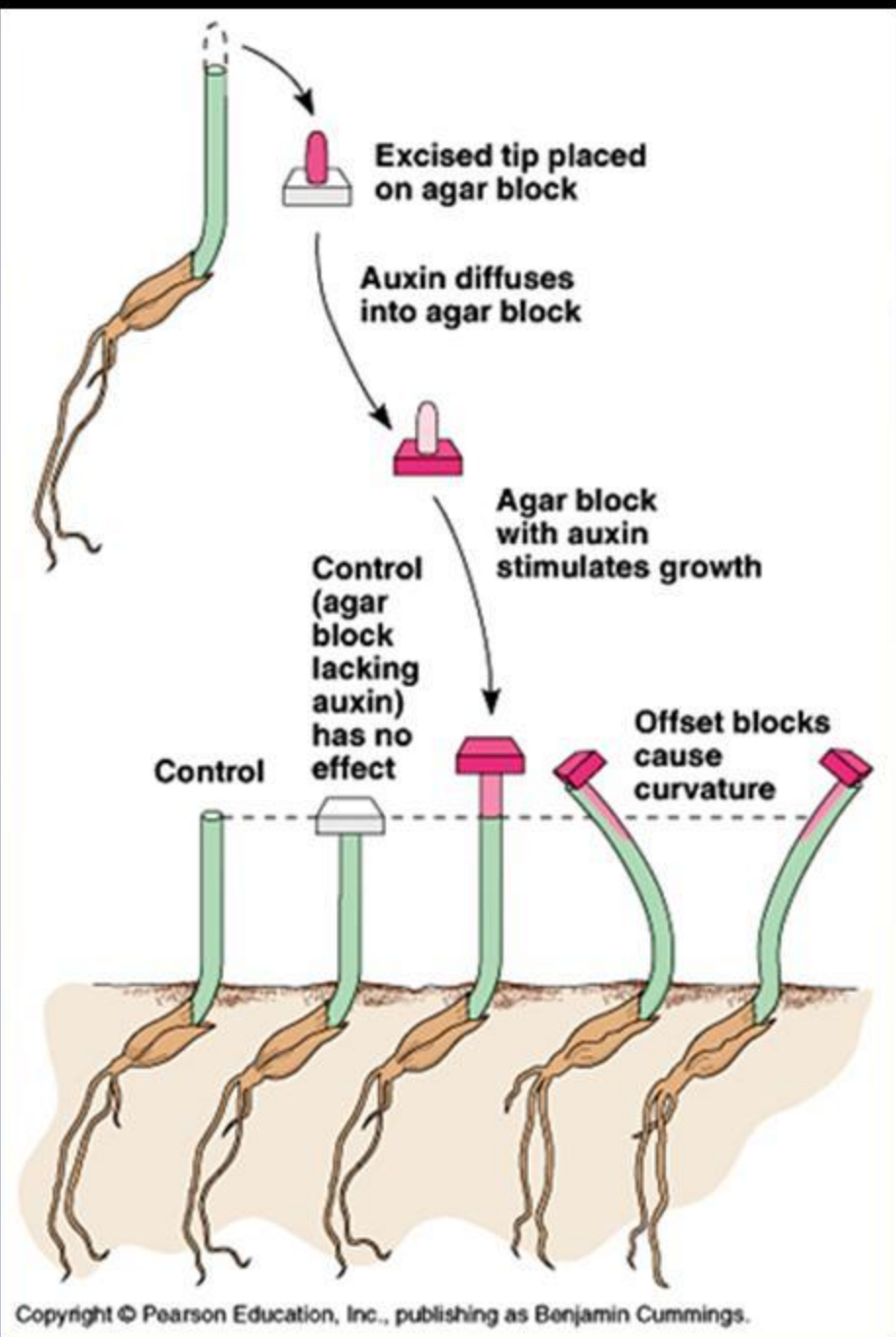
Auxin Gradients



TROPISMS

Asymmetric Auxin Distribution Controls Directional Growth

- Tropisms



Asymmetric Auxin Distribution Underlies Tropisms

Phototropism

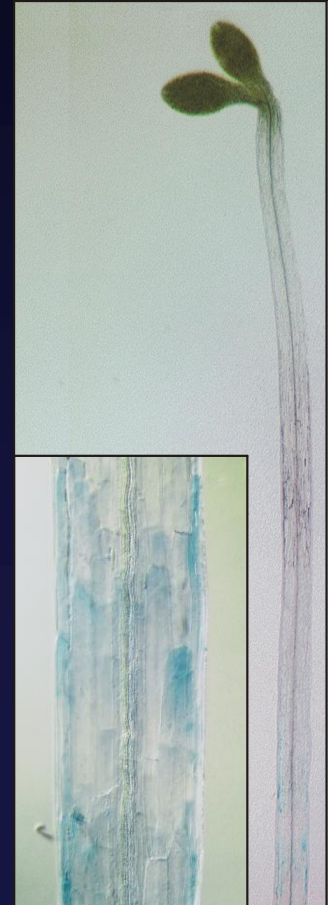
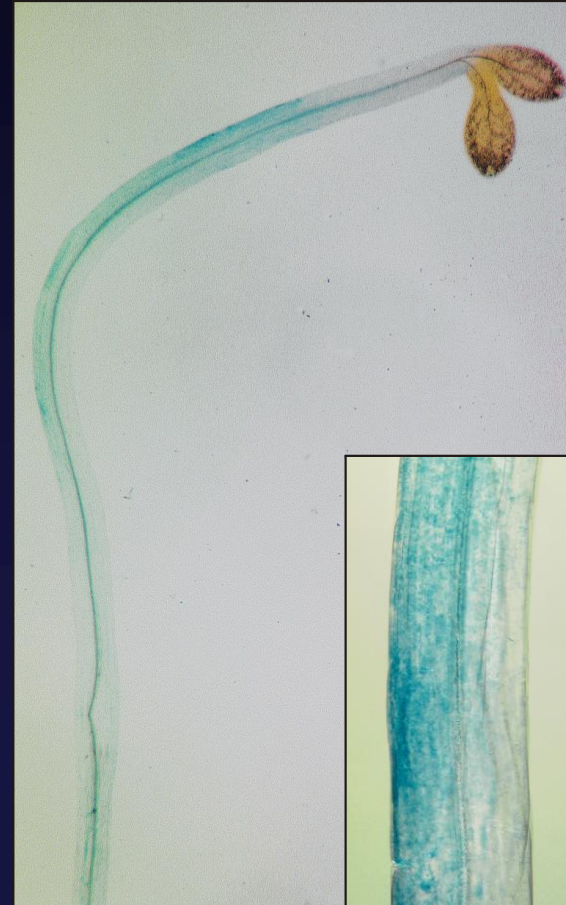
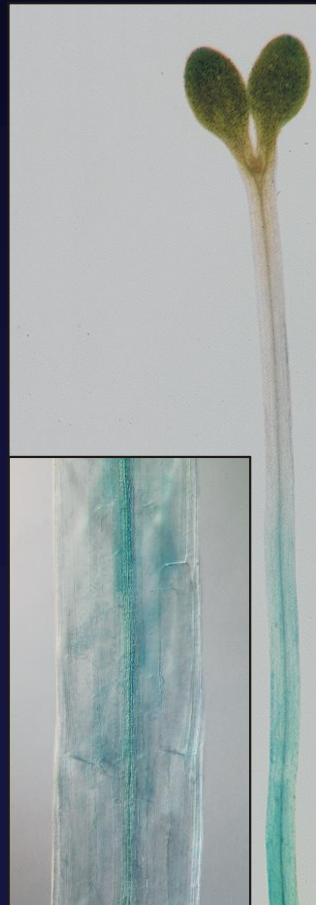
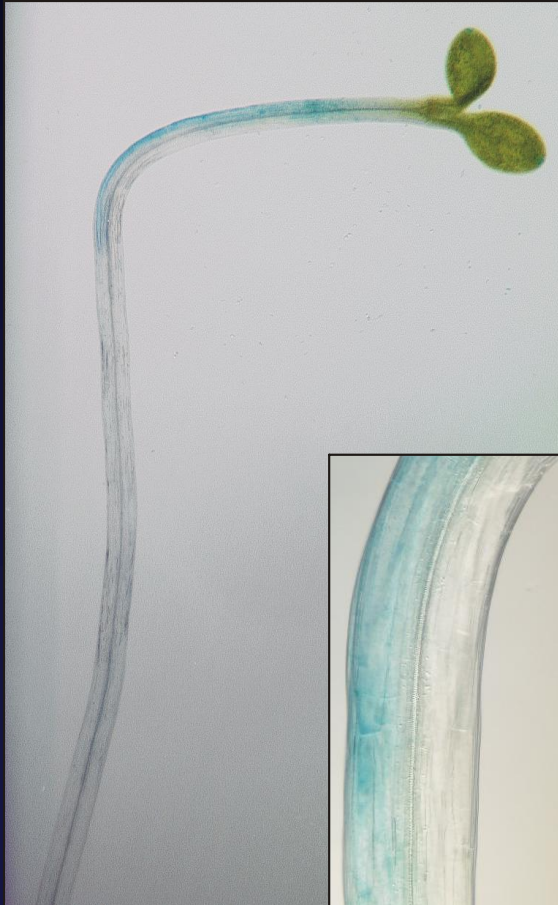
Gravitropism

- NPA

+ NPA

- NPA

+ NPA



pin3 is Defective in Tropisms

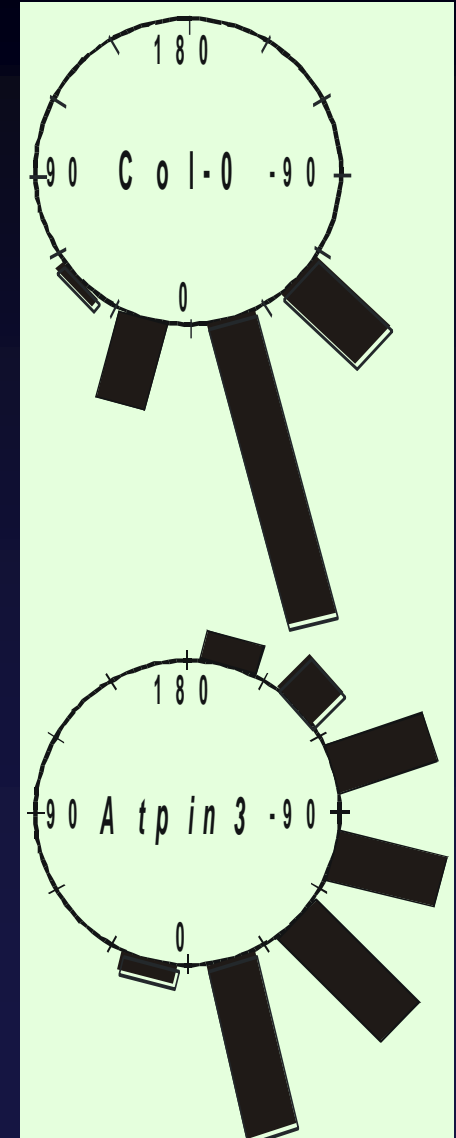
Hypocotyl phototropism



Hypocotyl gravitropism



Root gravitr.

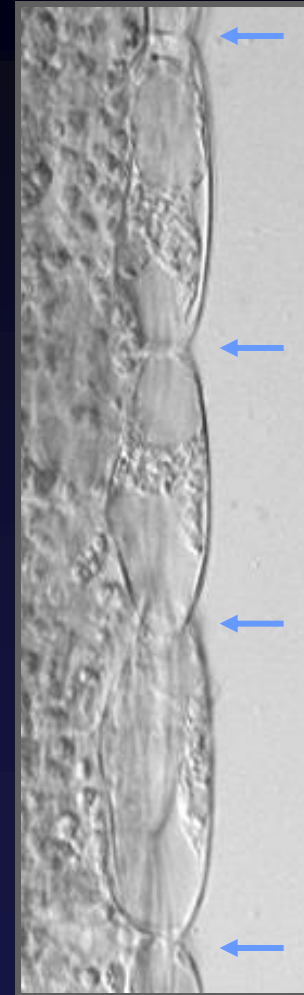
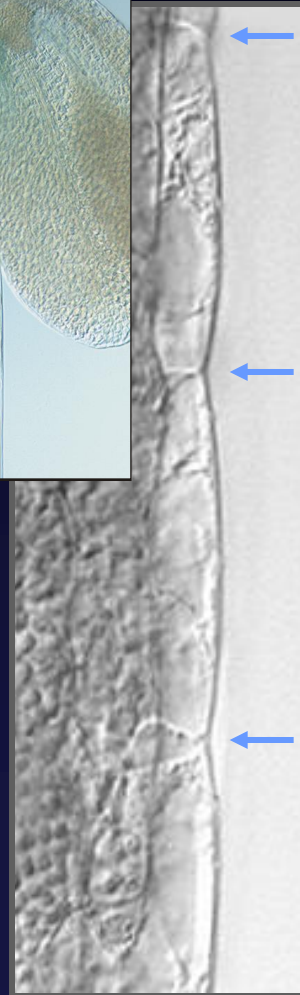
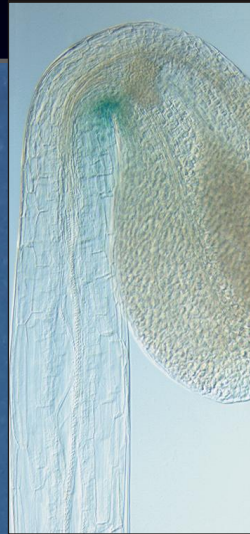
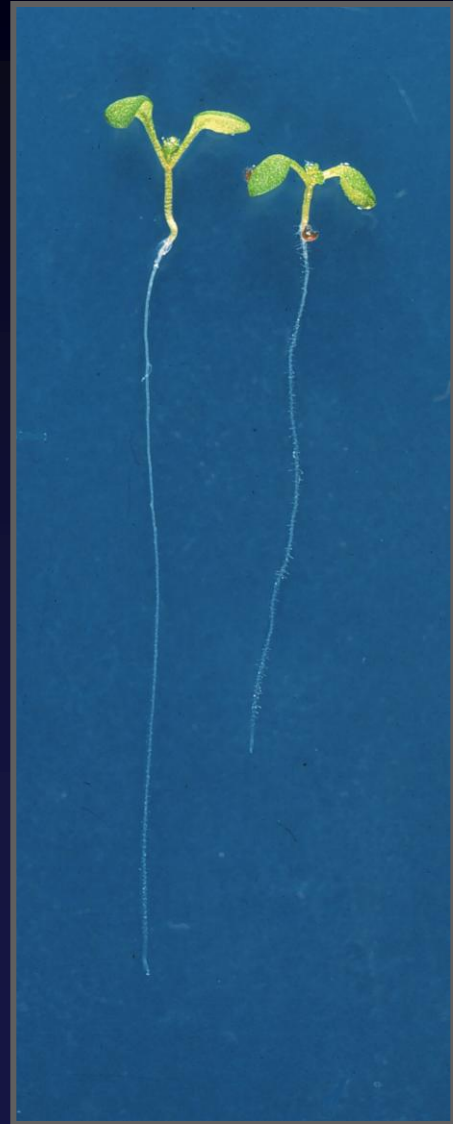


pin3 Hypocotyl and Apical Hook Phenotypes

light

dark

DR5



Col-0

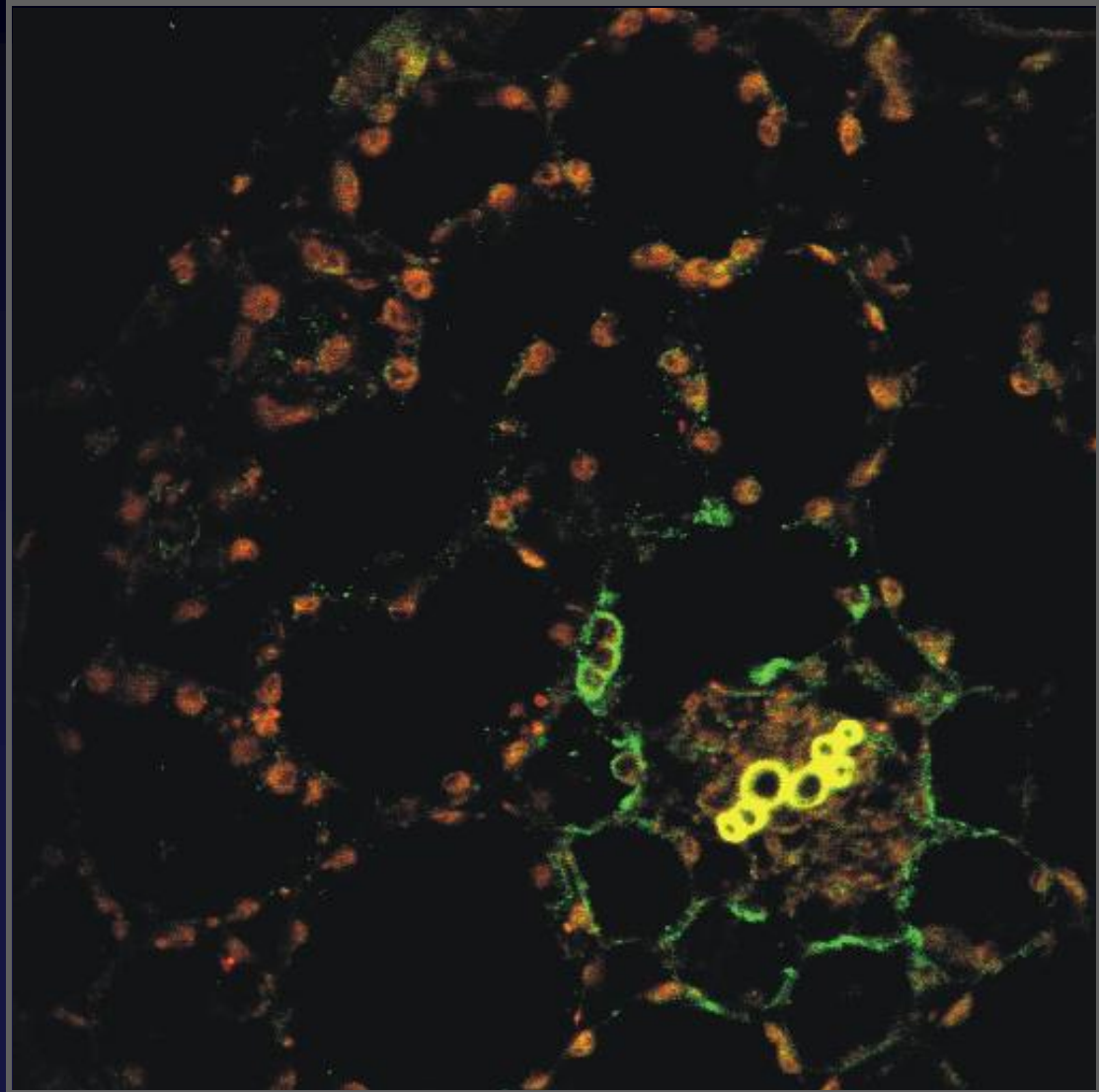
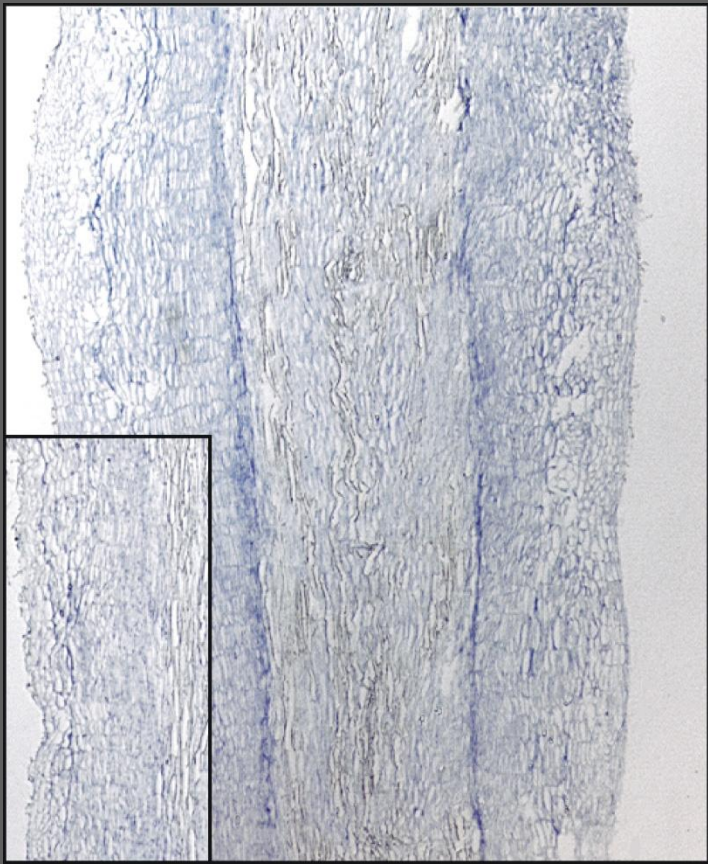
Atpin3

Col-0 (NPA)

PIN3 in Hypocotyl

PIN3 protein

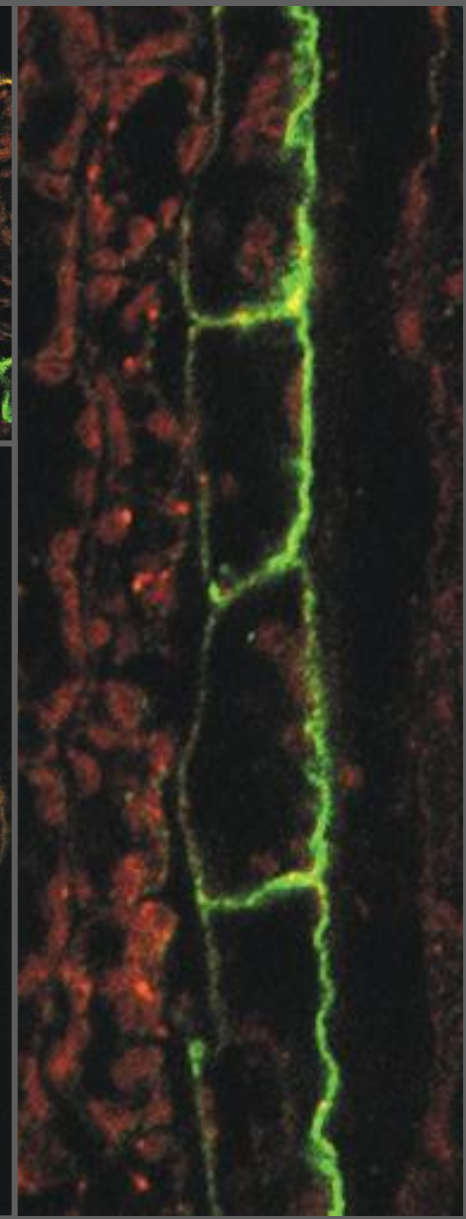
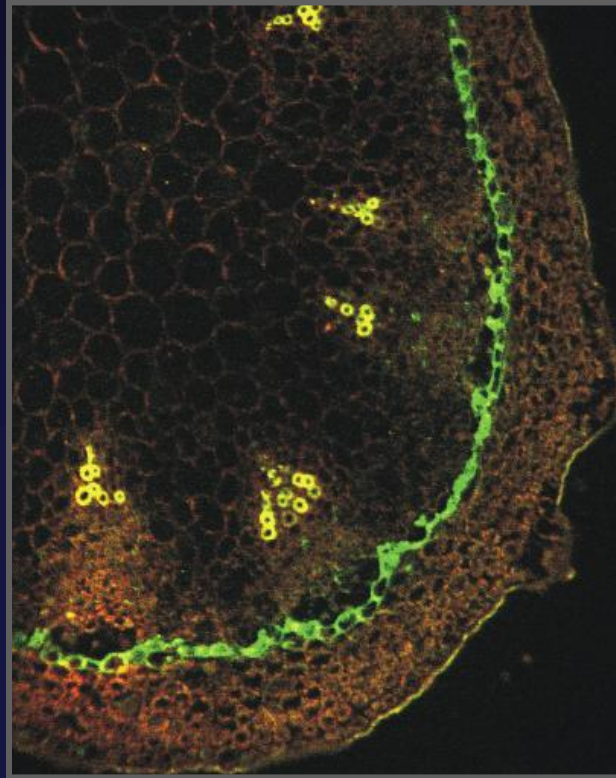
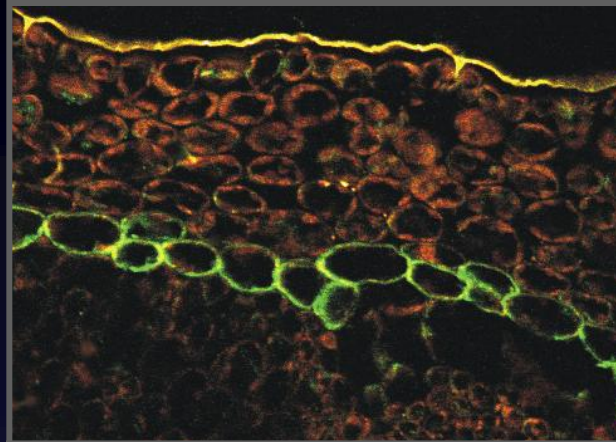
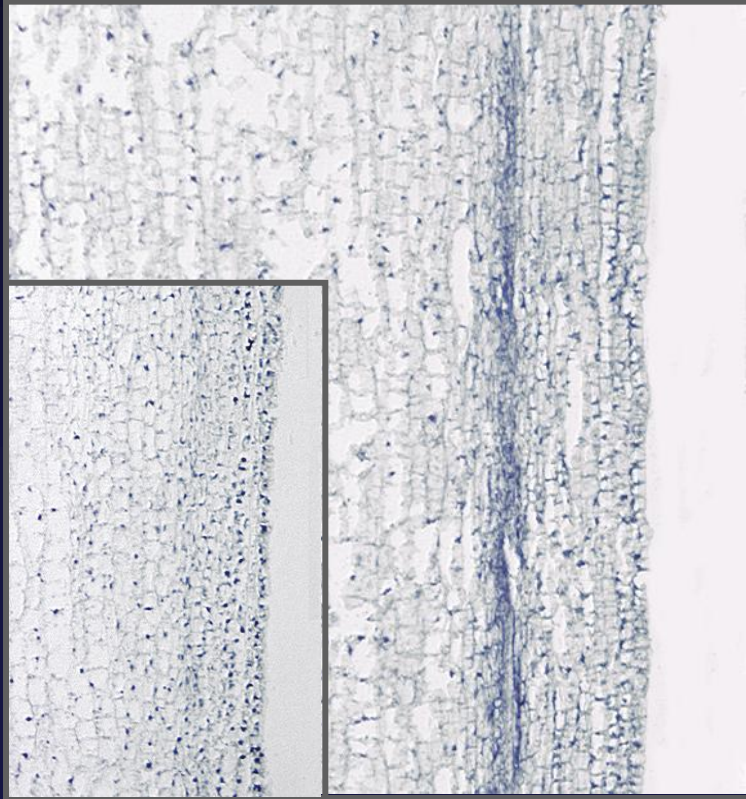
in situ RNA hybridization



PIN3 in Inflorescence Axis

PIN3 protein

in situ RNA hybridization

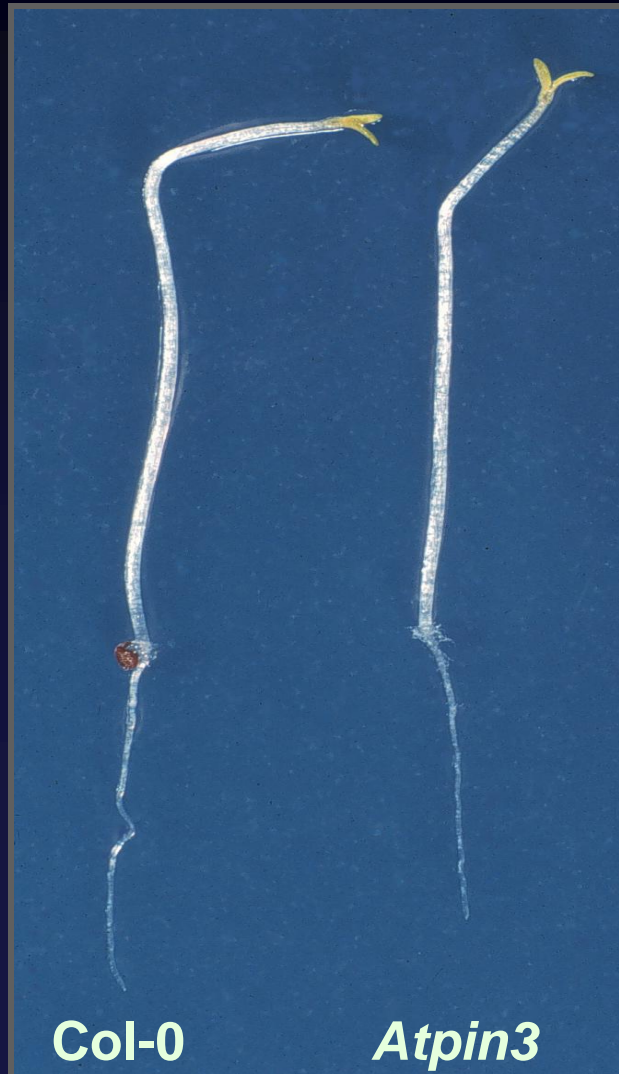


PIN3 – Lateral Auxin Transport

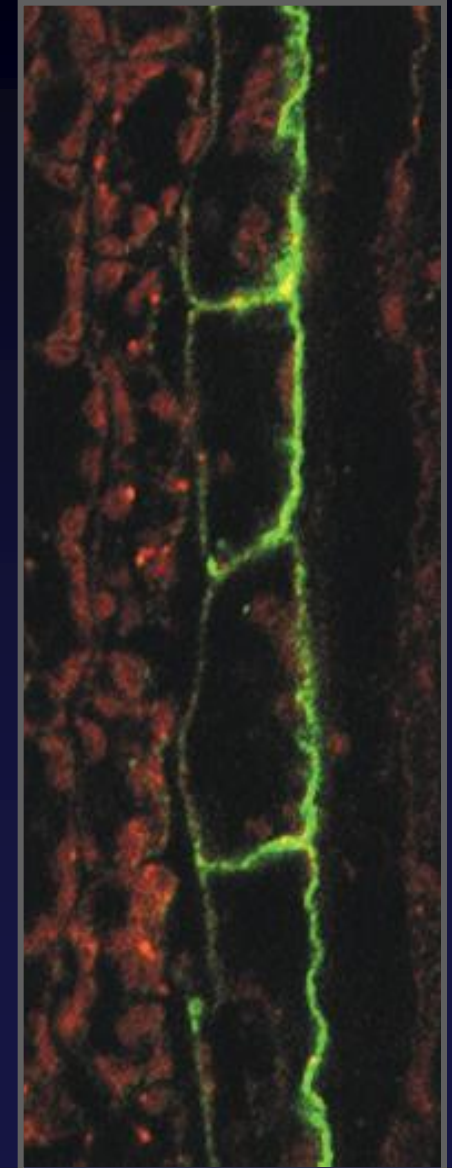
DR5 - phototropism



pin3 phototropism



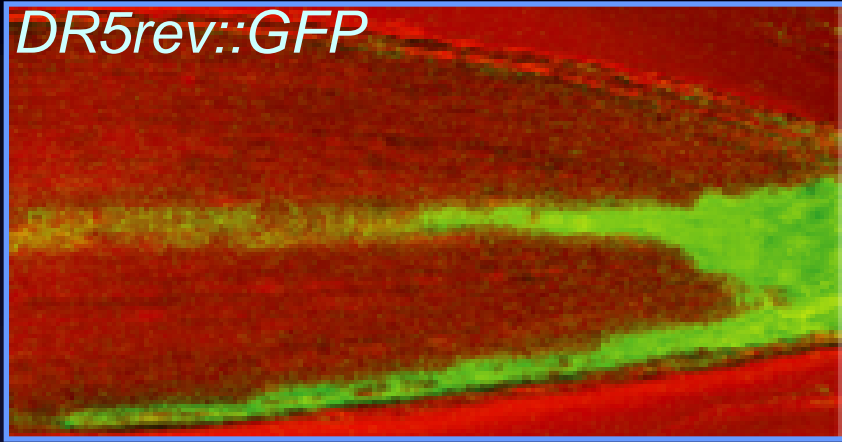
PIN3 protein



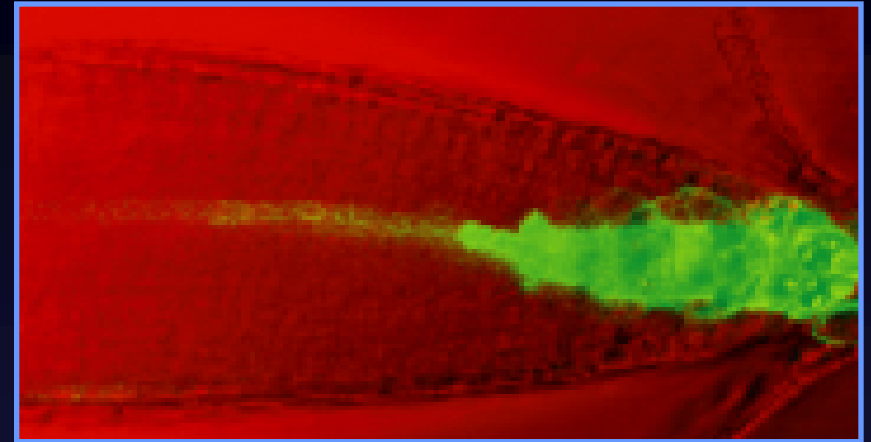
Root Gravotropism

gravity stimulated

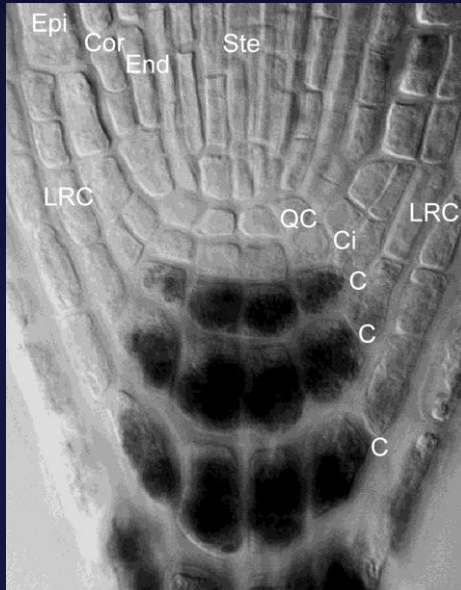
DR5rev::GFP



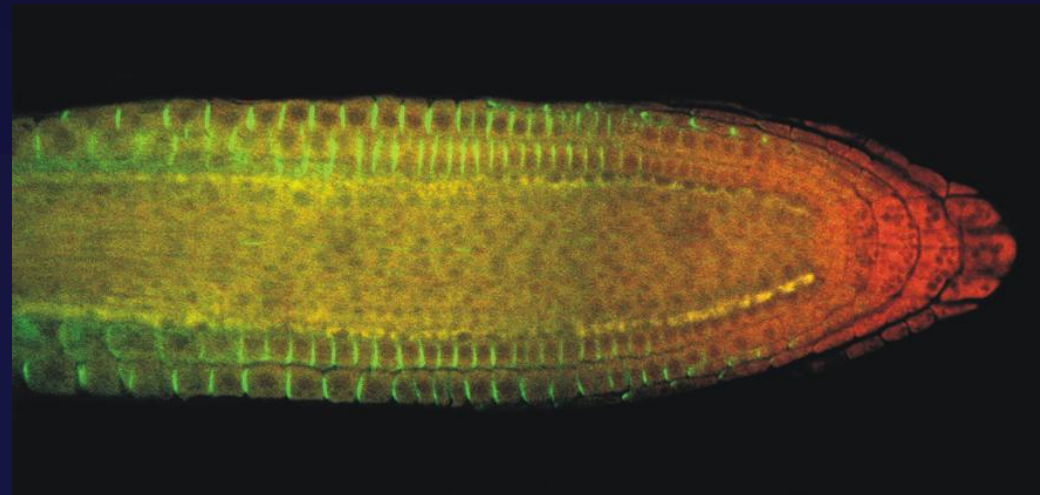
gravity + NPA



Statoliths
- gravity
perception

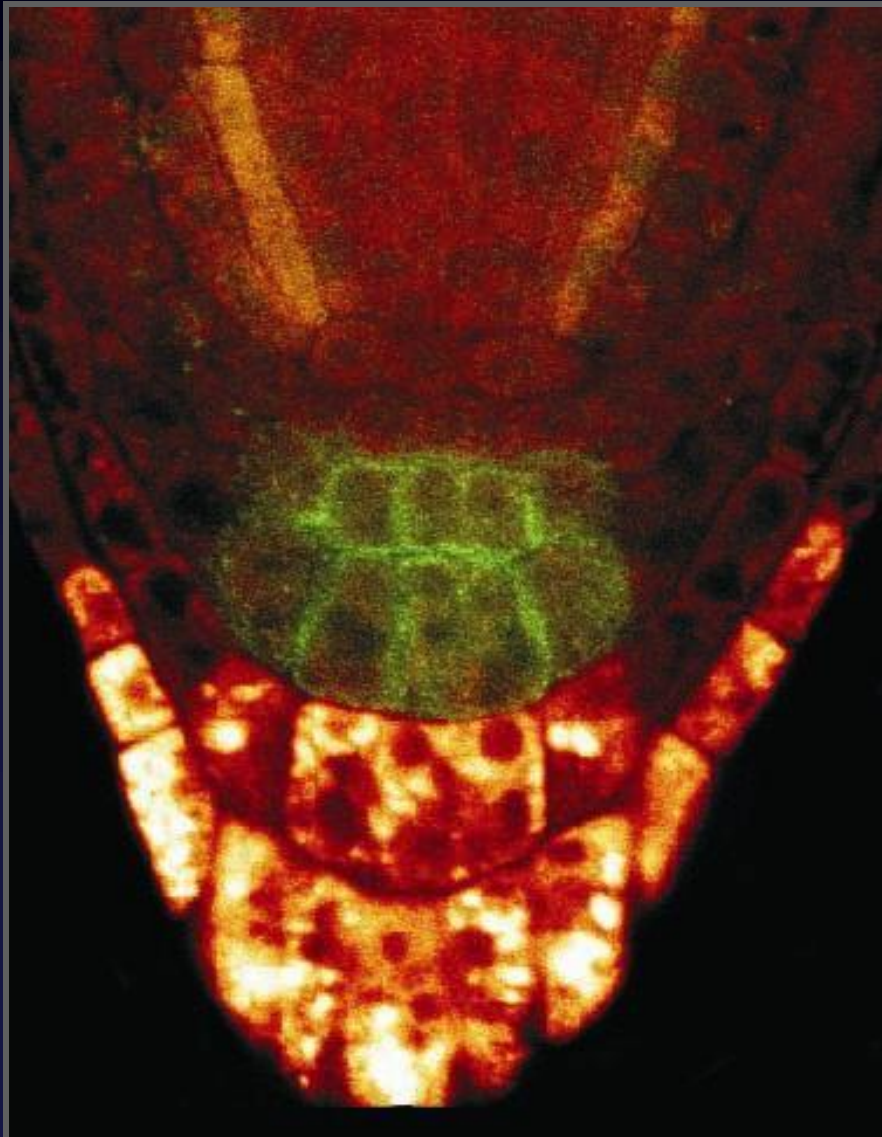


PIN2 localization

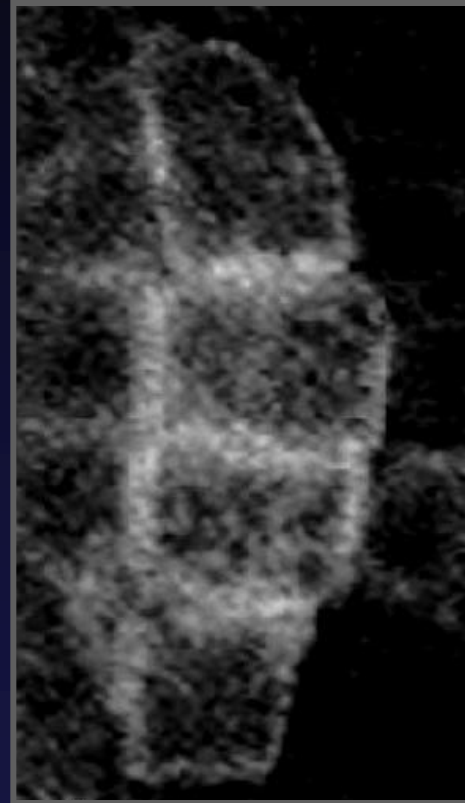


Relocation of PIN3 during Gravitropism

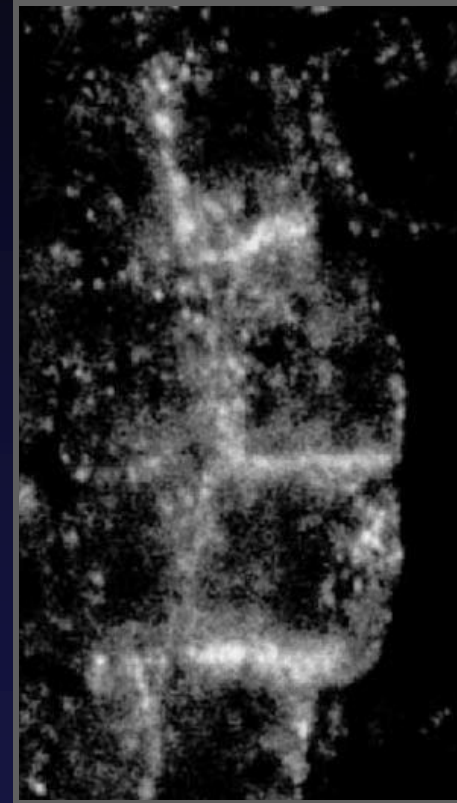
PIN3 in vertical root



PIN3 in root on its side



0 min



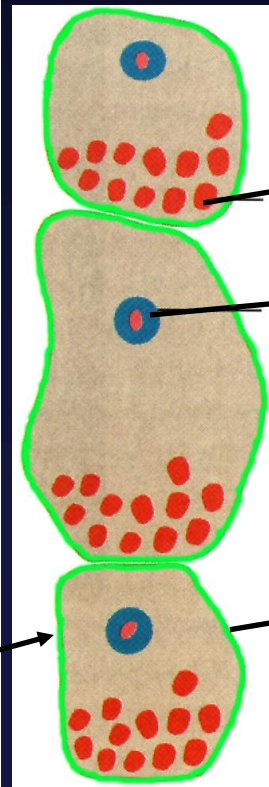
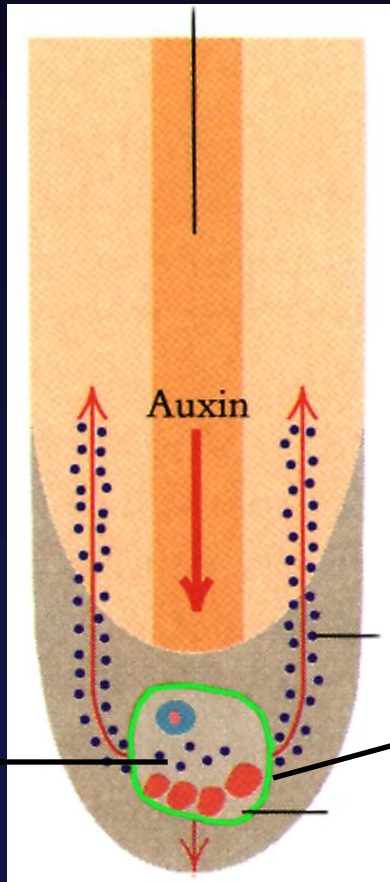
2 min

Model for Root Gravitropism

Vertical root

Root turned on its side

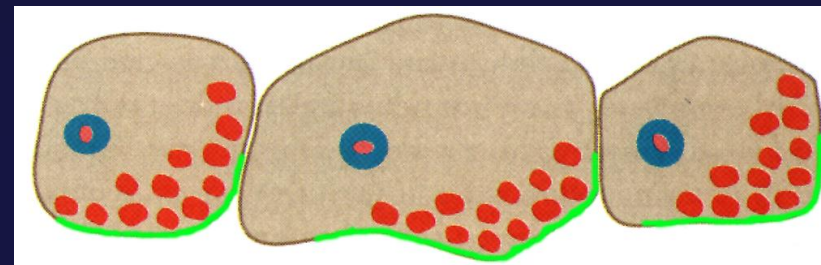
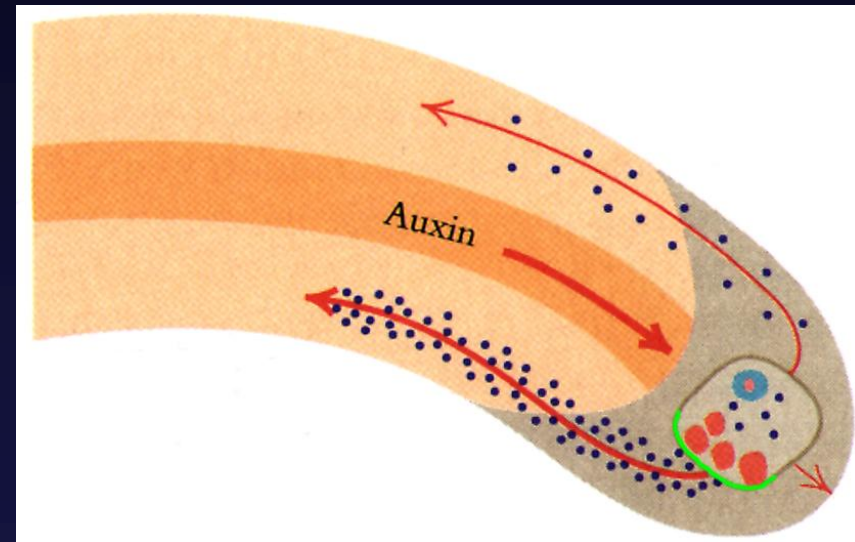
Vascular tissue



Statolits

Nucleus

AtPIN3



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

PIN-dependent Auxin Gradients in Plant Development

