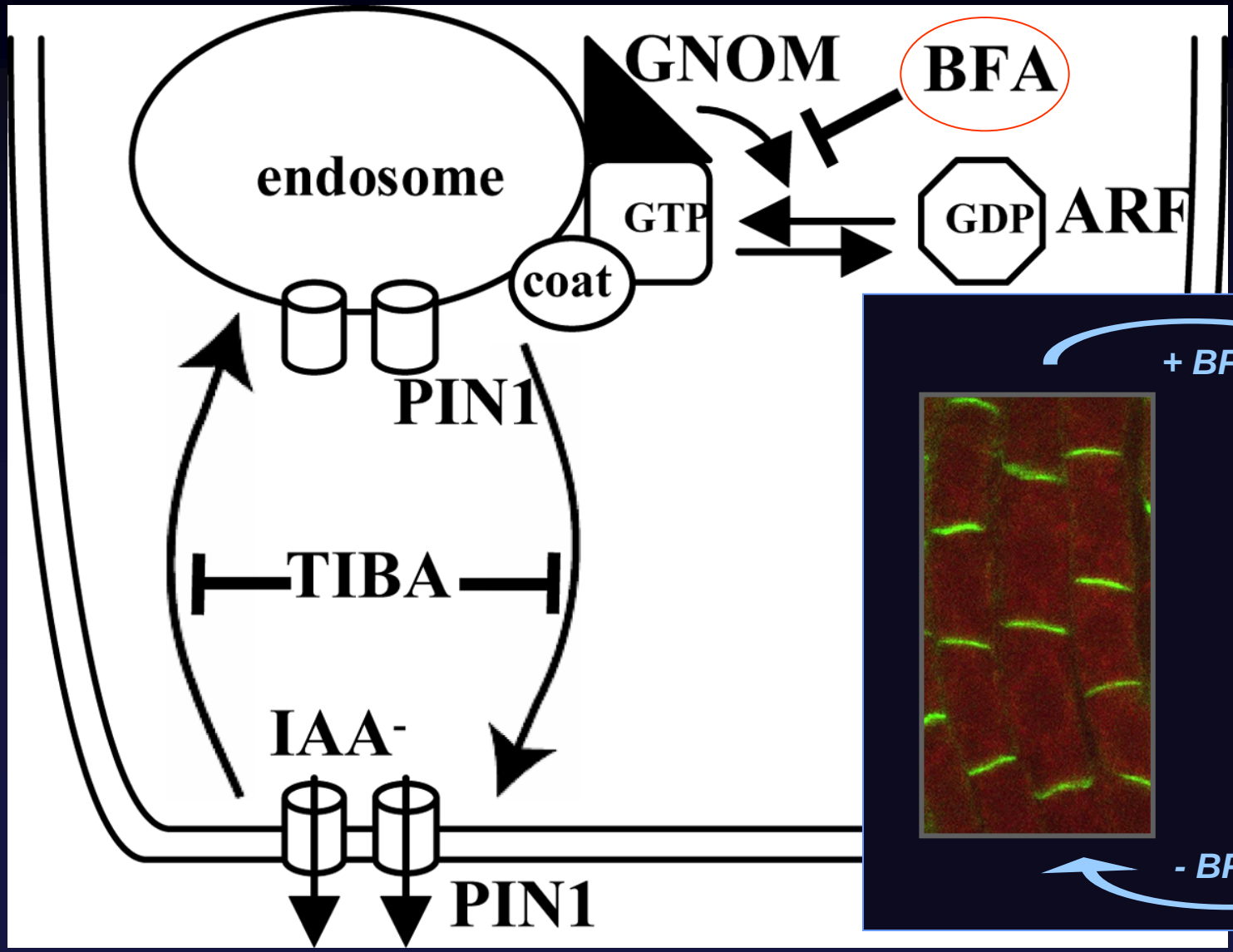
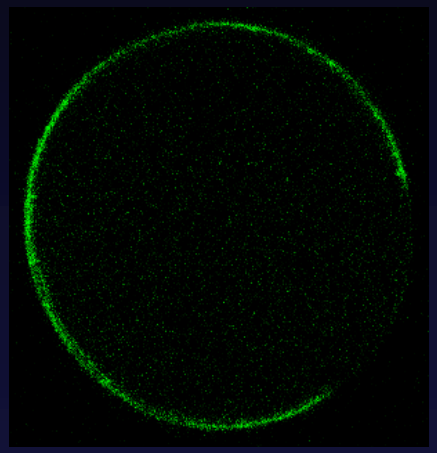
Subcellular Cycling of PIN Proteins

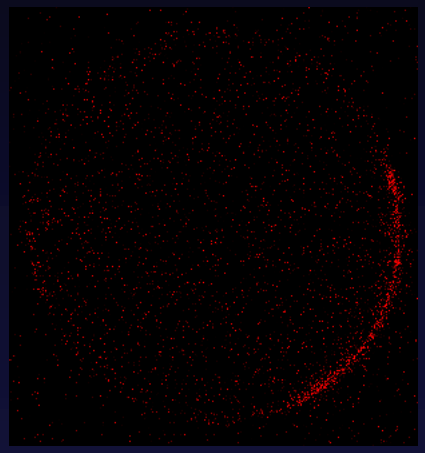


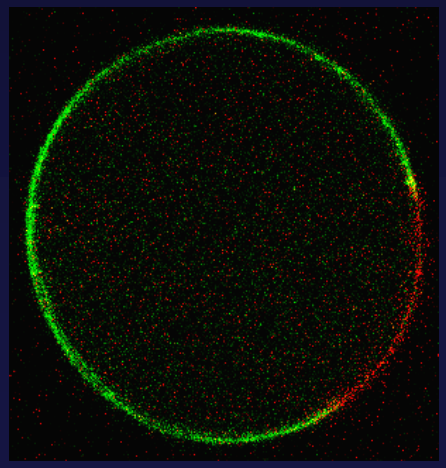
Geldner et al., 2001

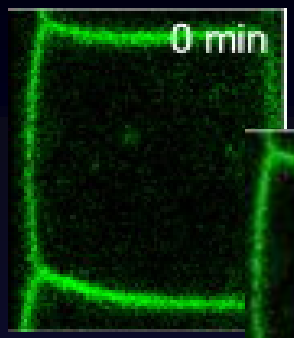
UV-activated PIN2-EosFP

Protoplasts

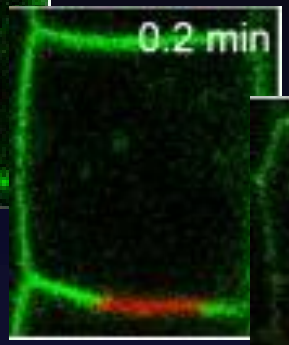


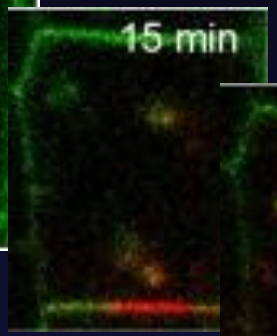


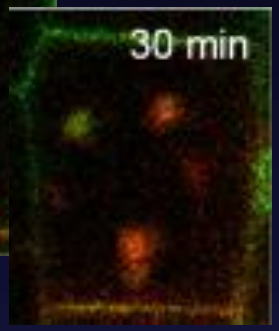


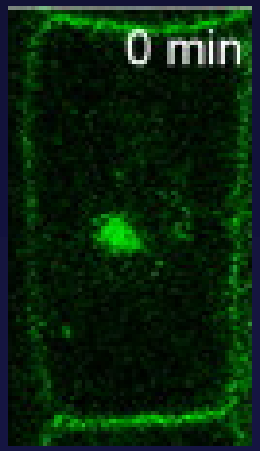


+ BFA









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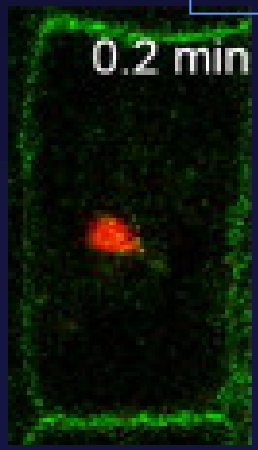
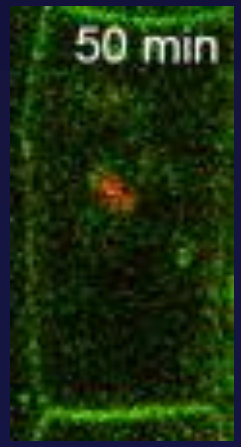
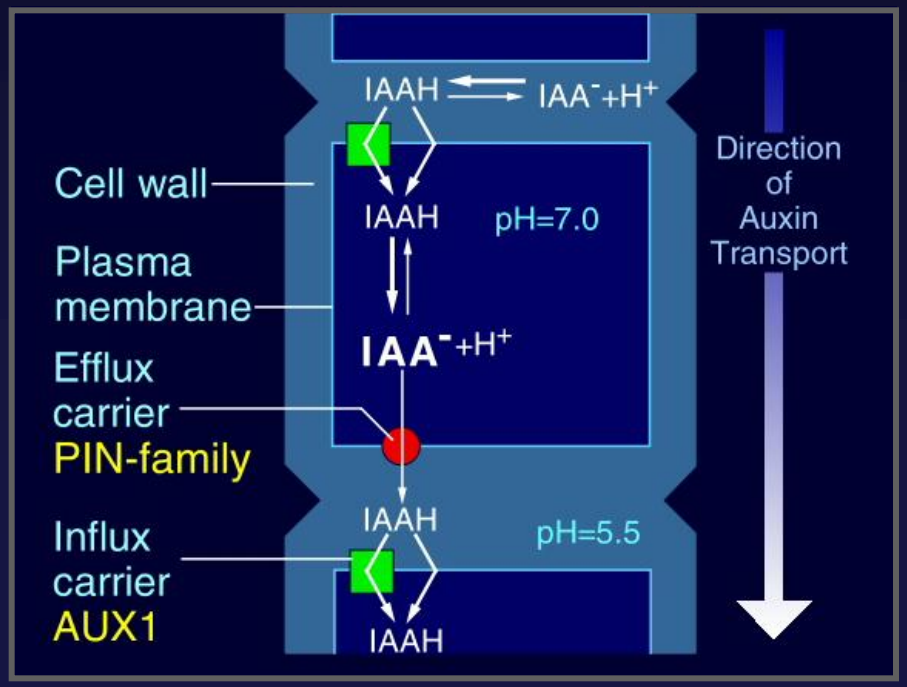
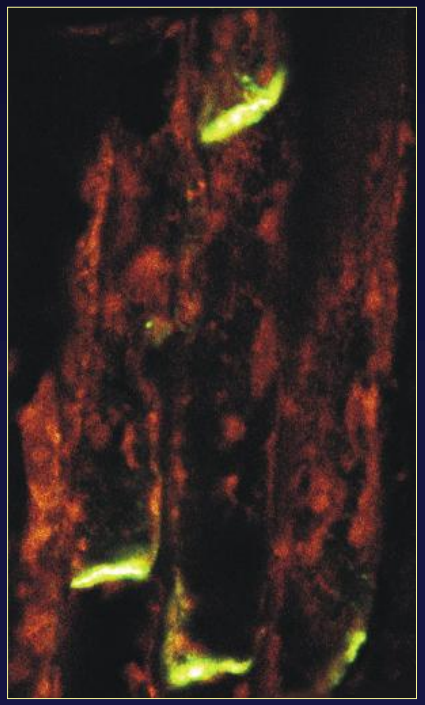




Dhonukshe et al., 2007

Cellular Polarity of PIN Localisation and Directionality of Intercellular Auxin Flow

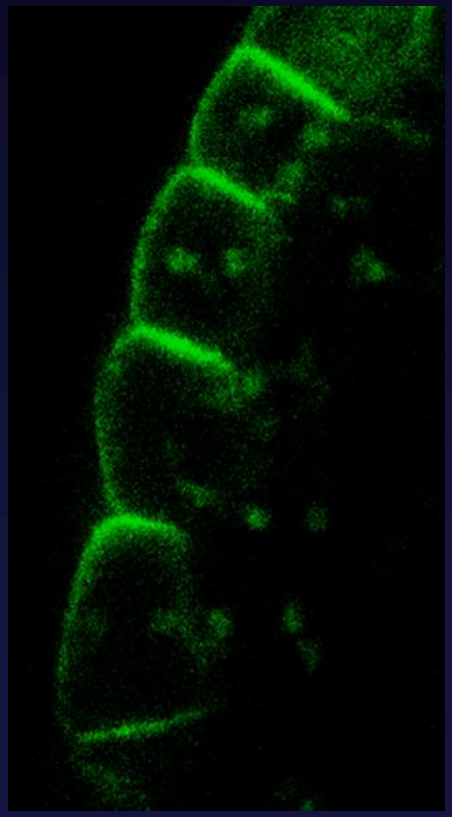




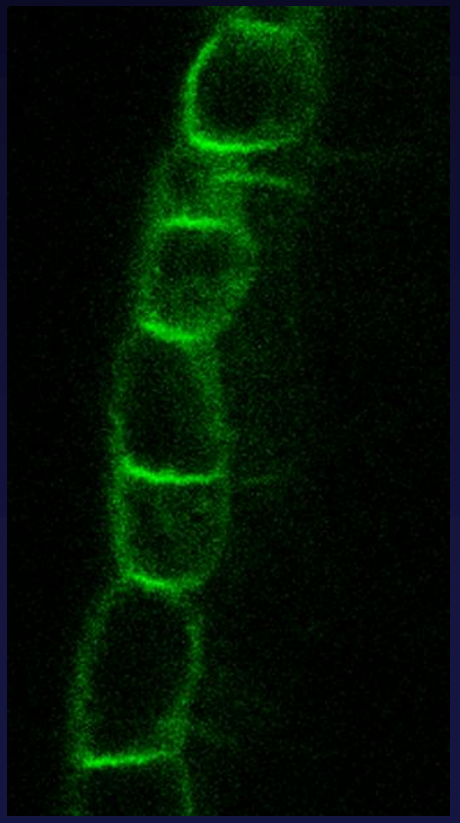
Molecular Components of PIN Polar Targeting

Ser/Thr protein kinase PINOID (PID)

Col-0

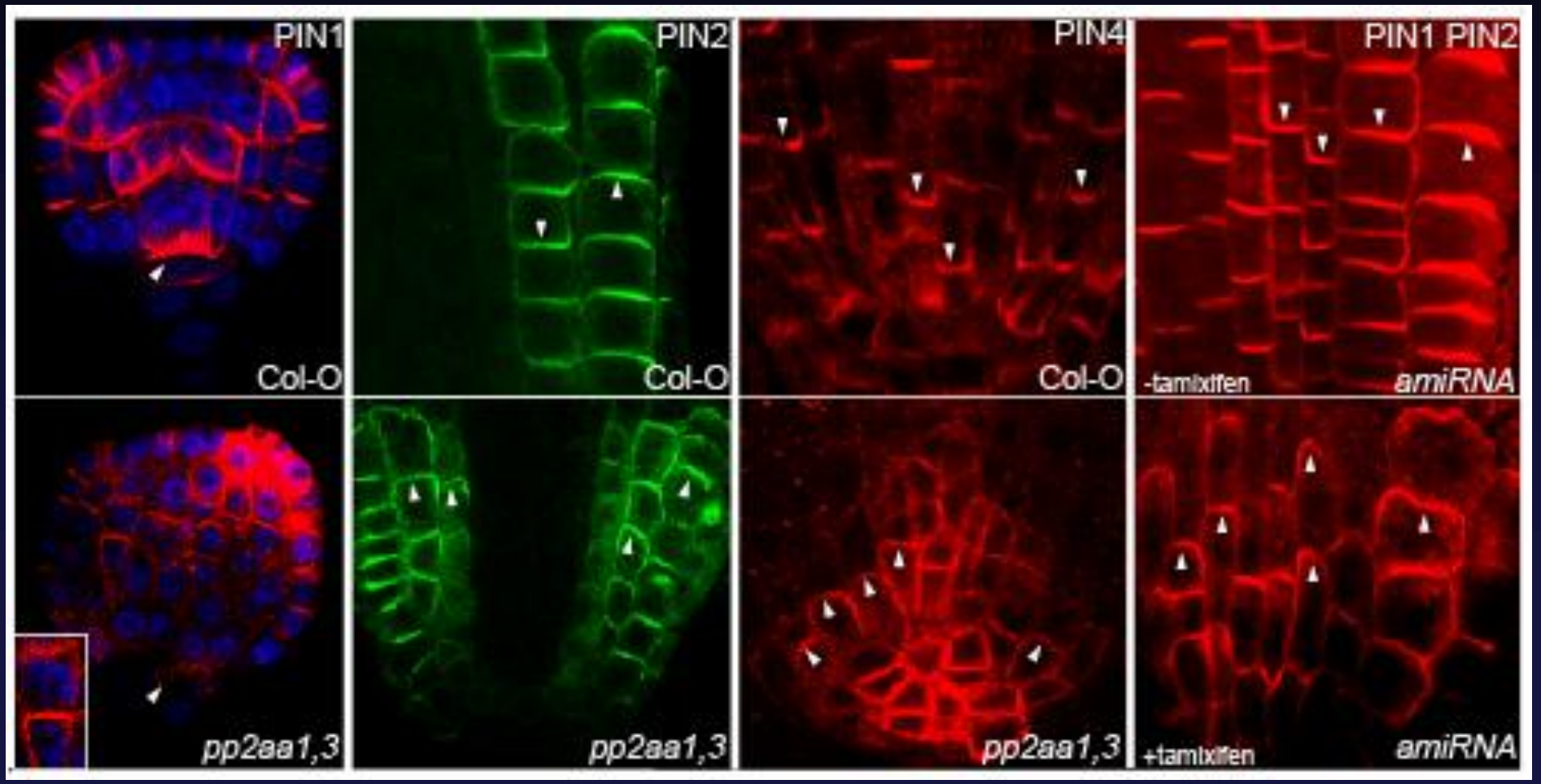


pinoid



Christensen et al., 2000; Benjamins et al., 2001; Friml et al., 200

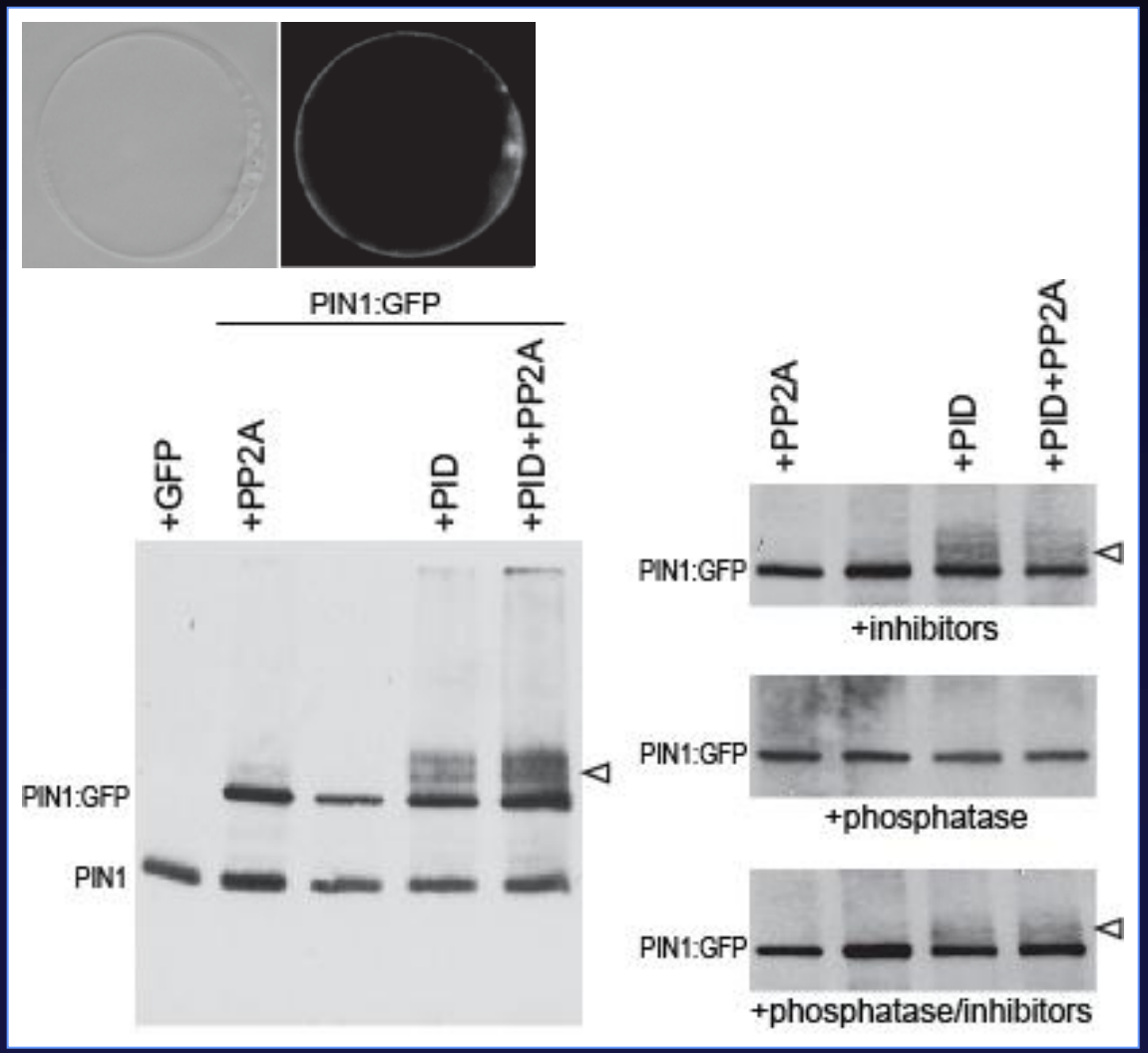
PP2A Phosphatase and PIN Apical-Basal Targeting



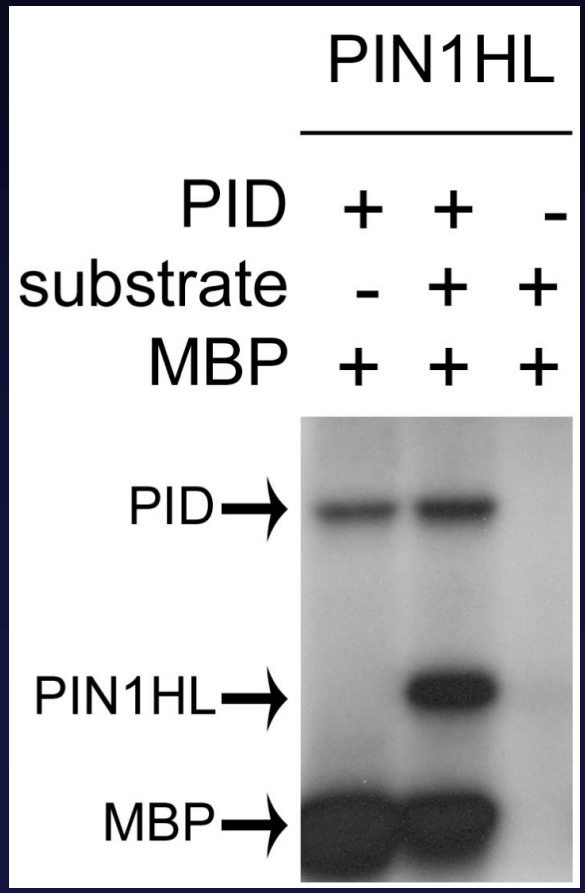
Michniewicz et al., 2007

PID Phosphorylates PINs

Phosphorylation assays in protoplast

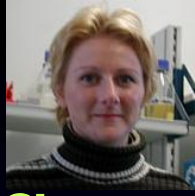


in vitro phosphorylation

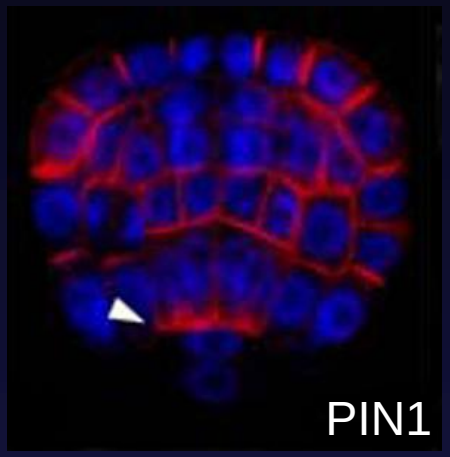


Michniewicz et al., 2007

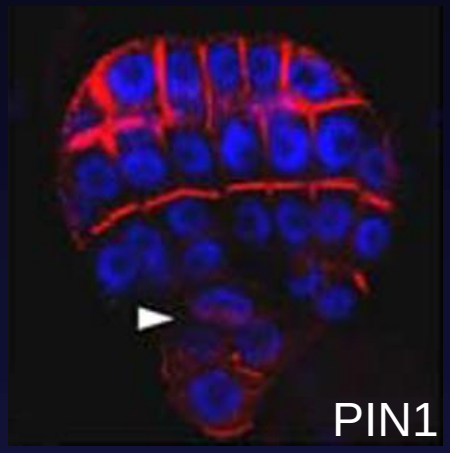
Role of PID in Controlling PIN Polarity > Auxin Flow > Patterning



Col-0

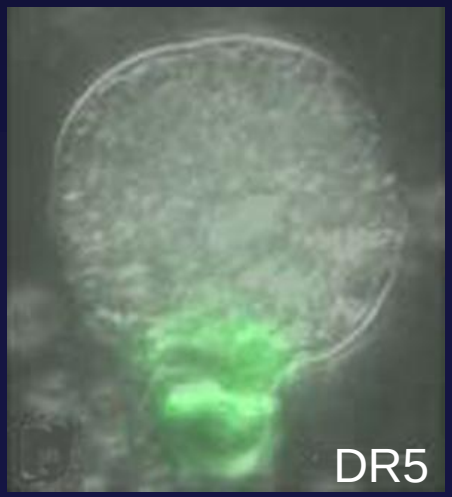


RPS5::PID



Col-0

RPS5::PID

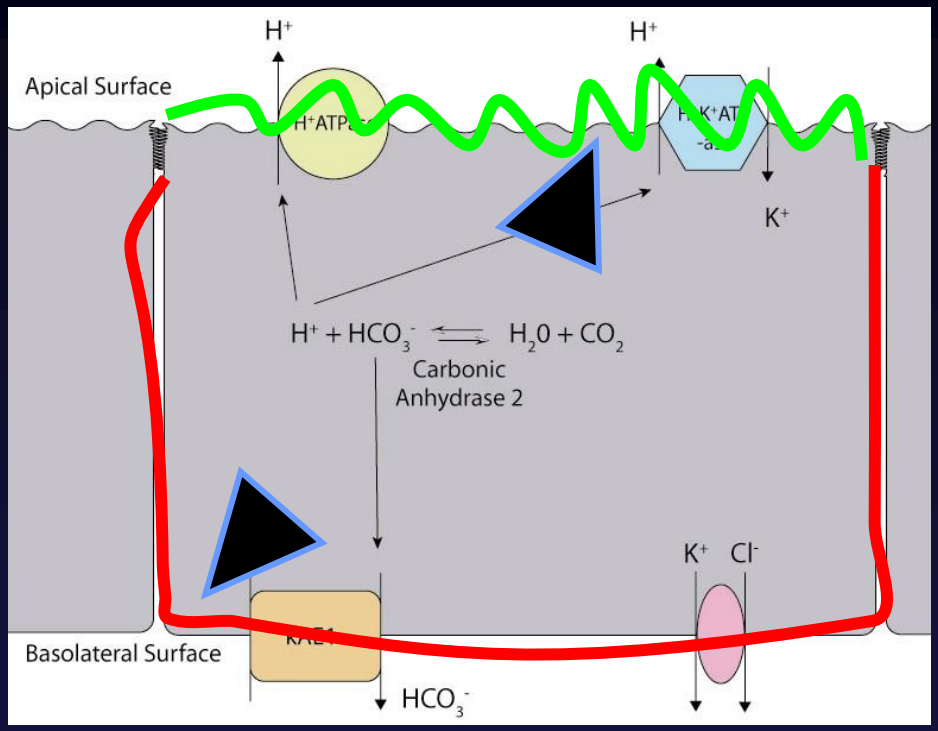


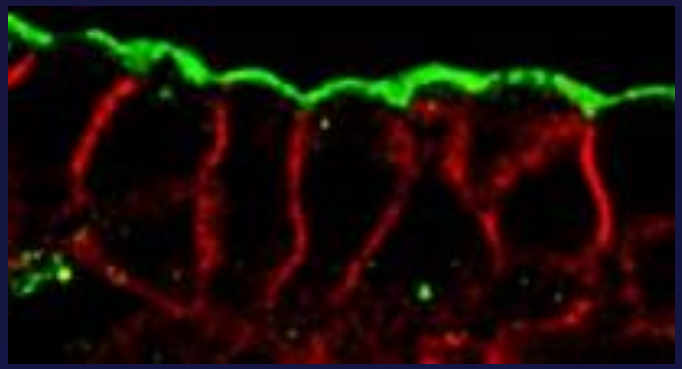
RPS5::PID seedlings

Friml et al., 2004

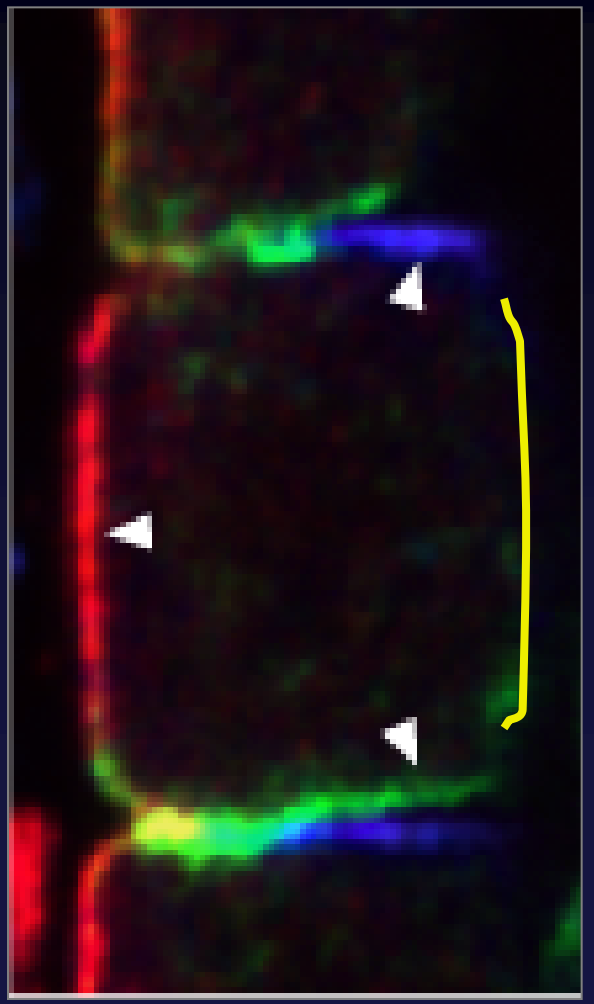
Polar delivery of proteins

Animal

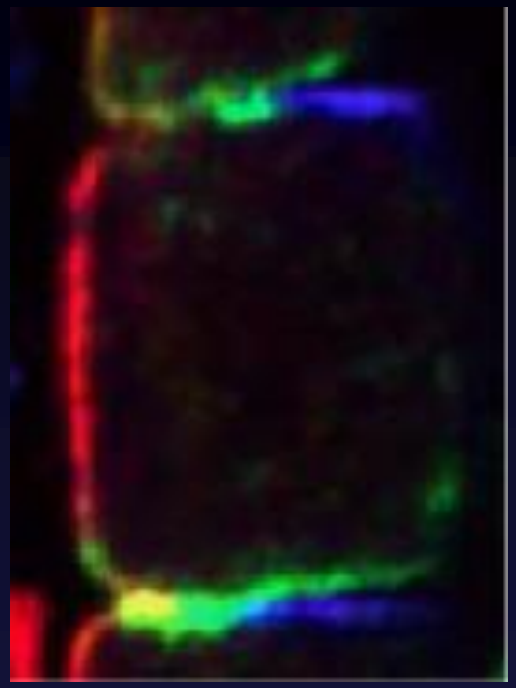


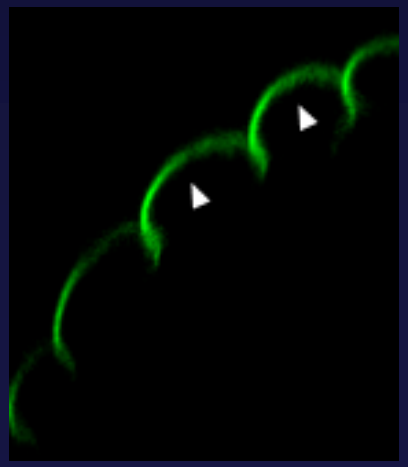


Plant

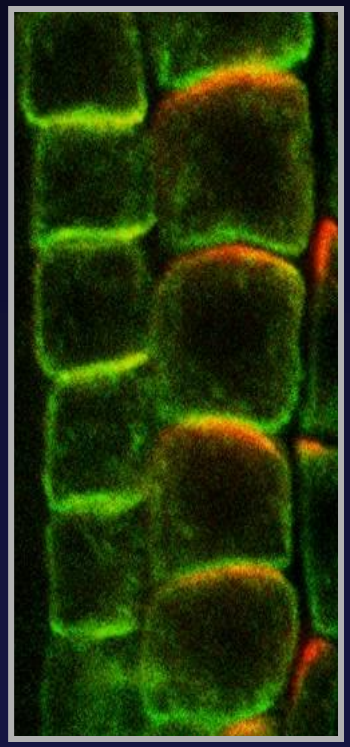


“Plant Epithelium”: root-soil interface

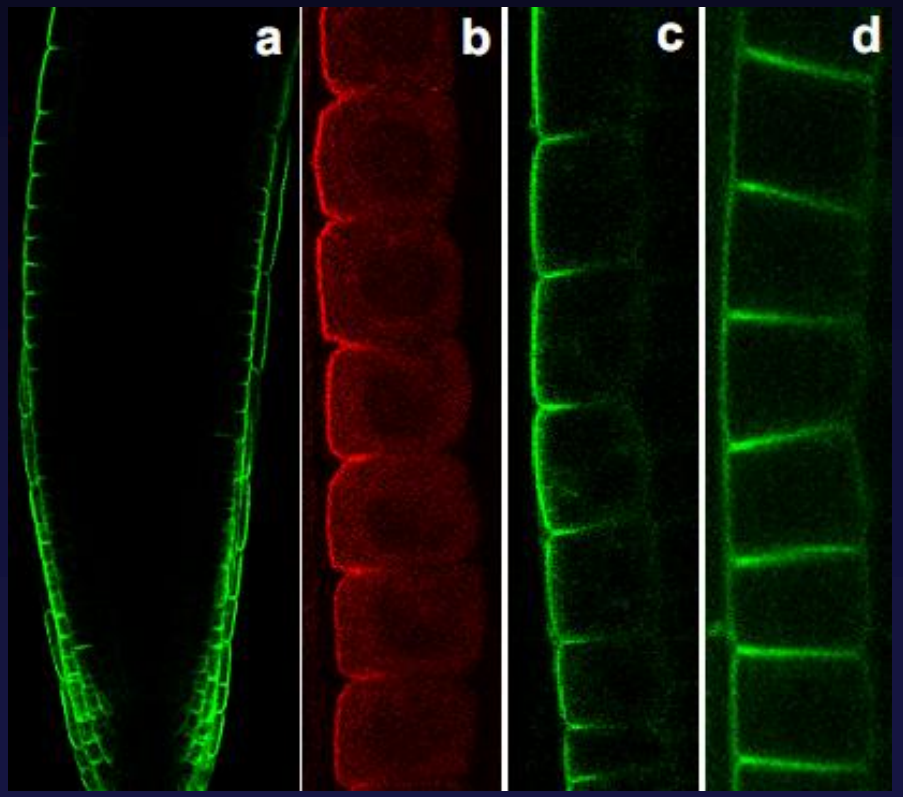




PINs

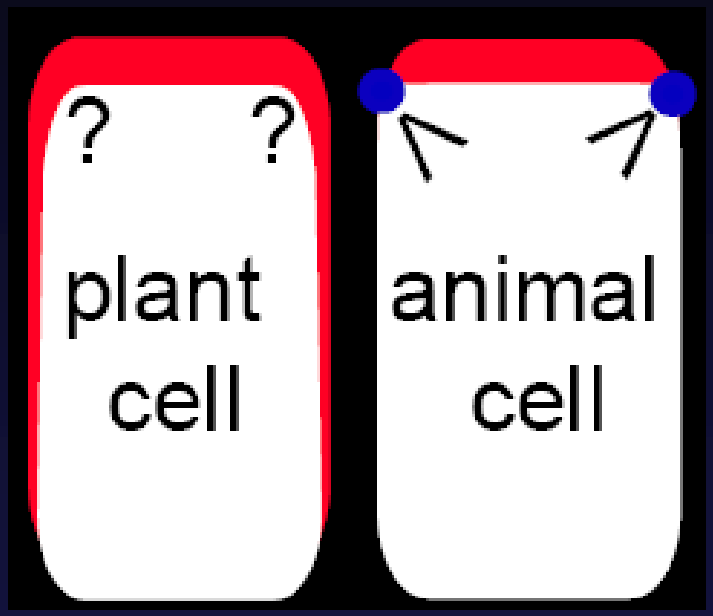


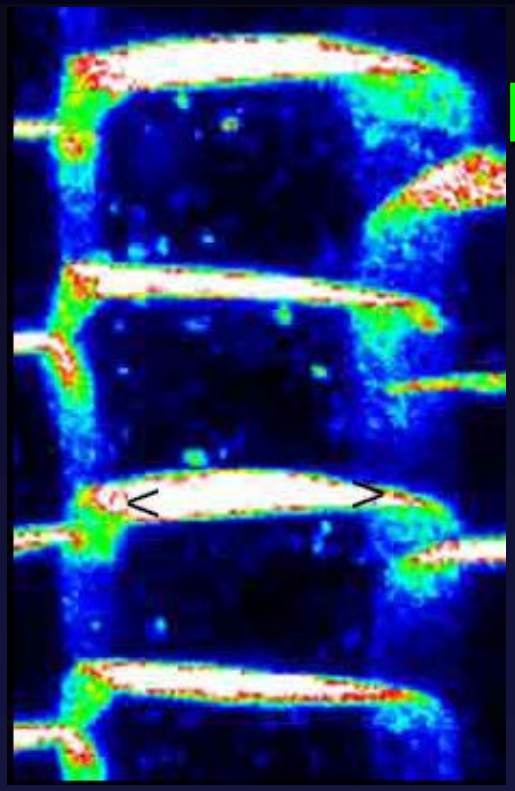
PEN3 IBA Cd B



Langowski et al., 2010

Mechanistic Insight into Polar Targeting in Plants

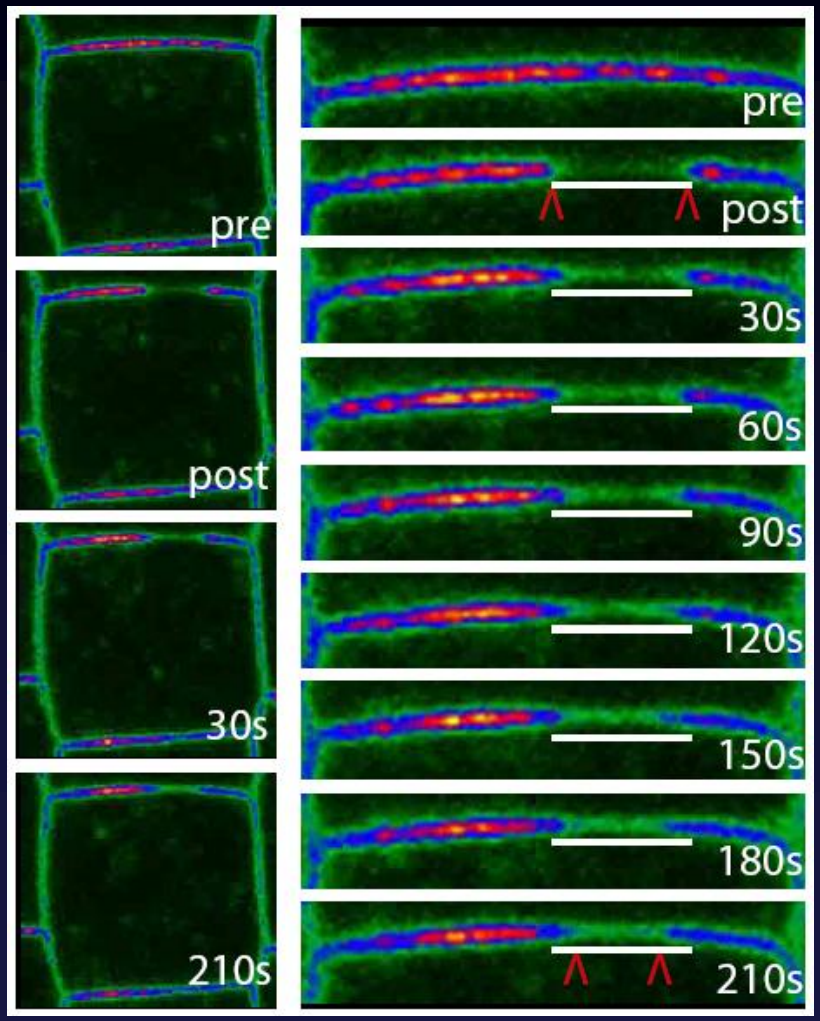




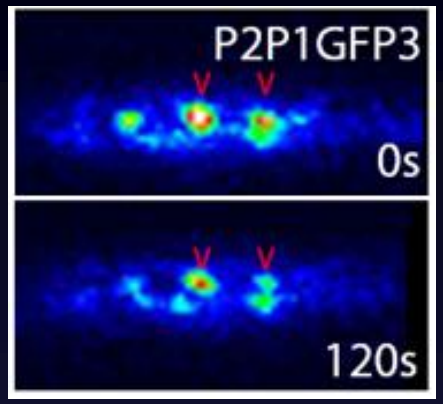
PIN2-GFP

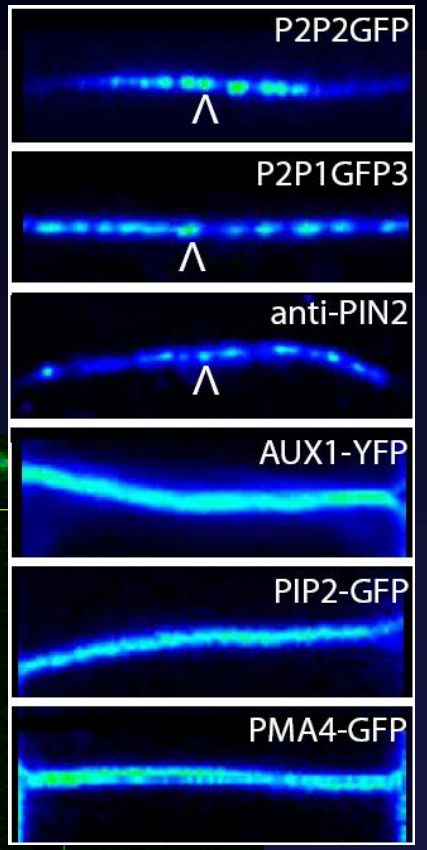
- Sterol-dependent reduced lateral diffusion
- Super polar exocytosis

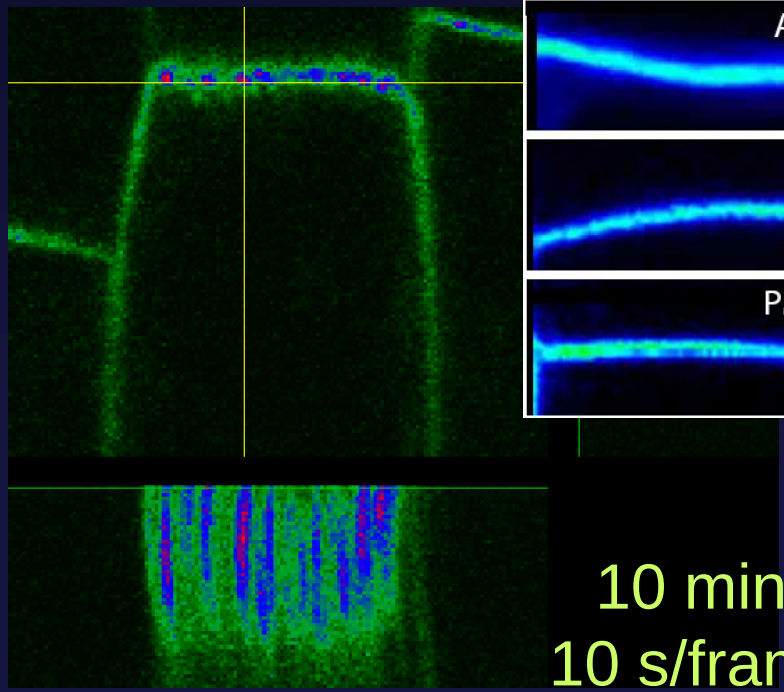
Lateral Diffusion



PIN2-GFP





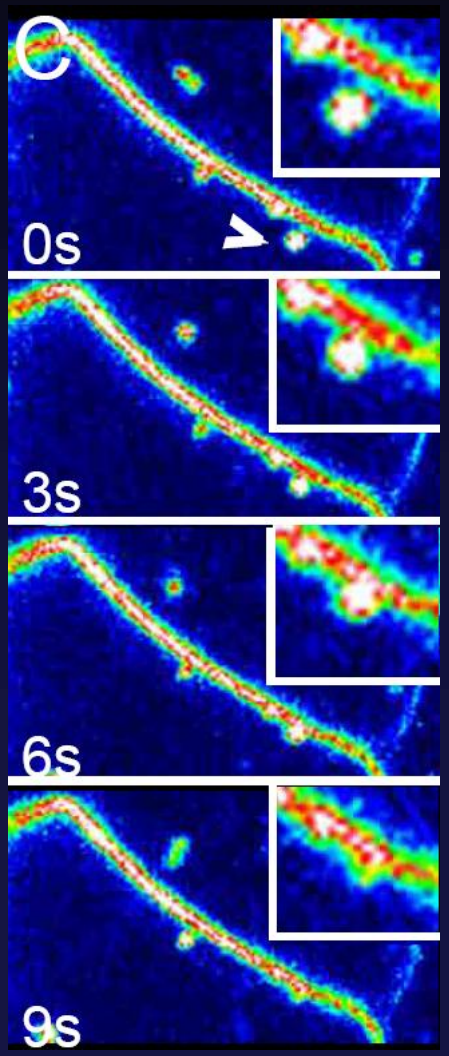


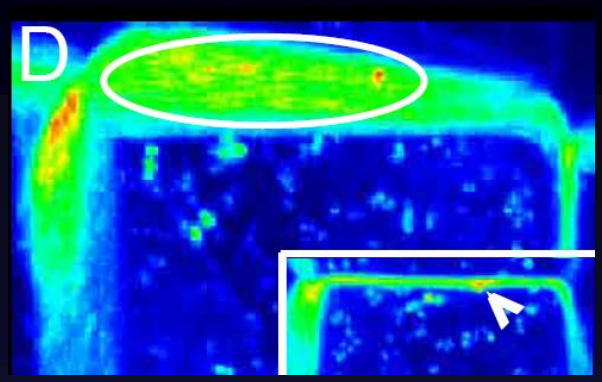
10 min
10 s/frame

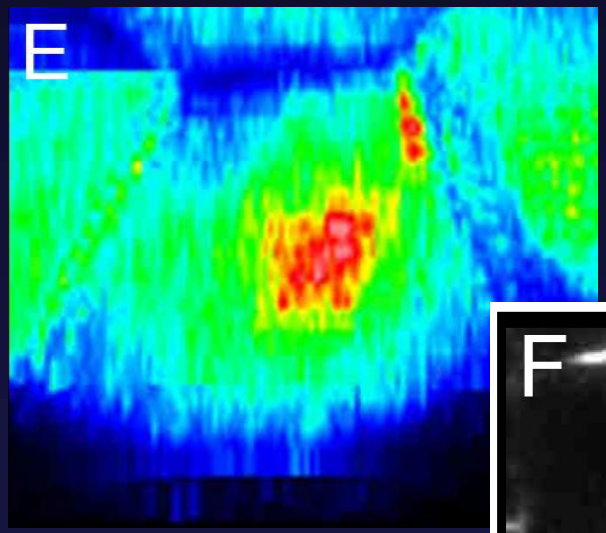
unpublished

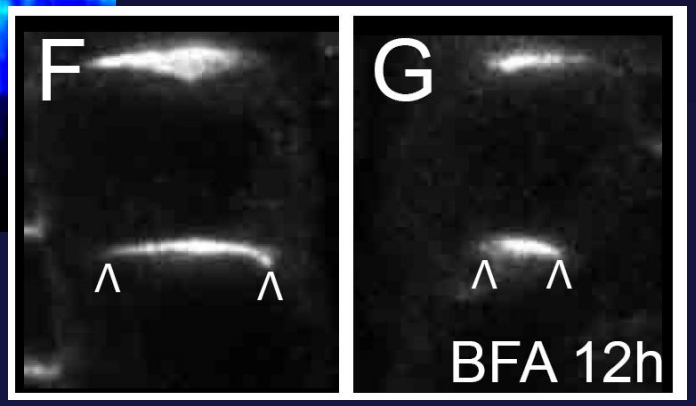
Super Polar Delivery

PIN2-GFP









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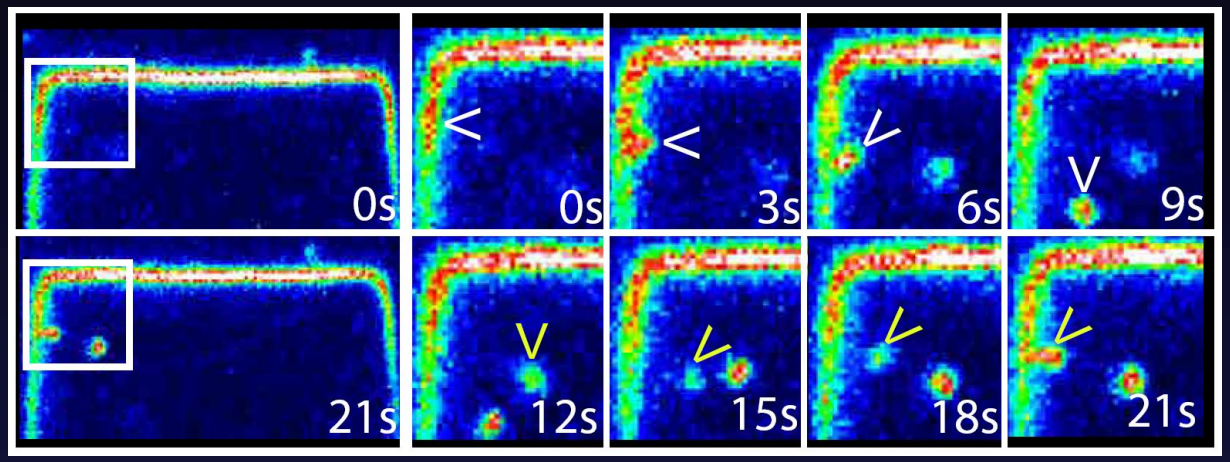
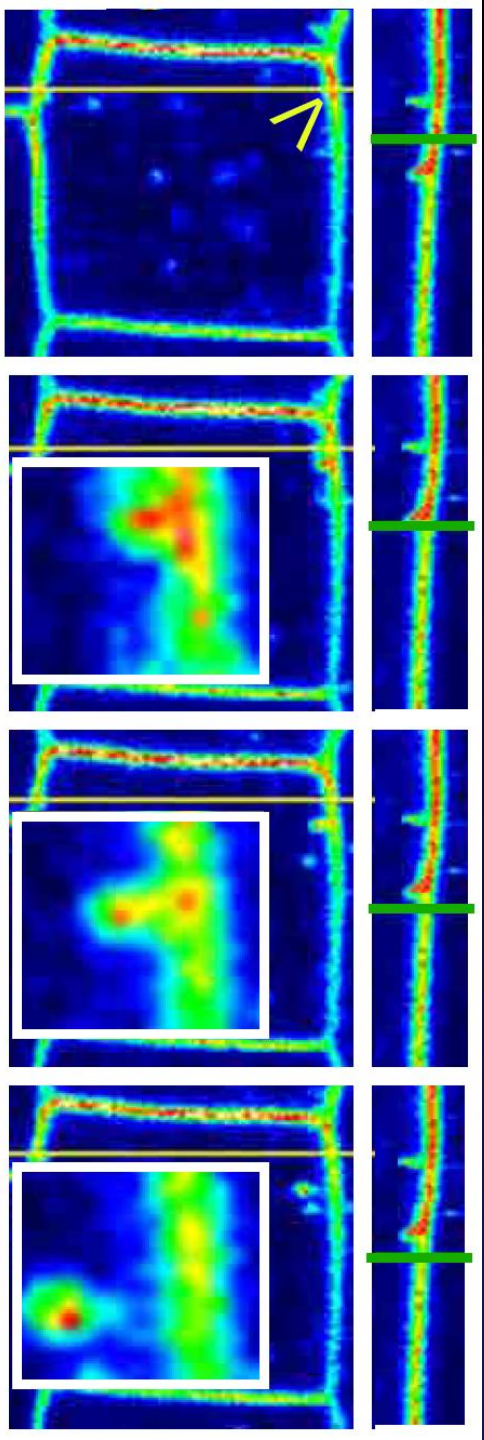
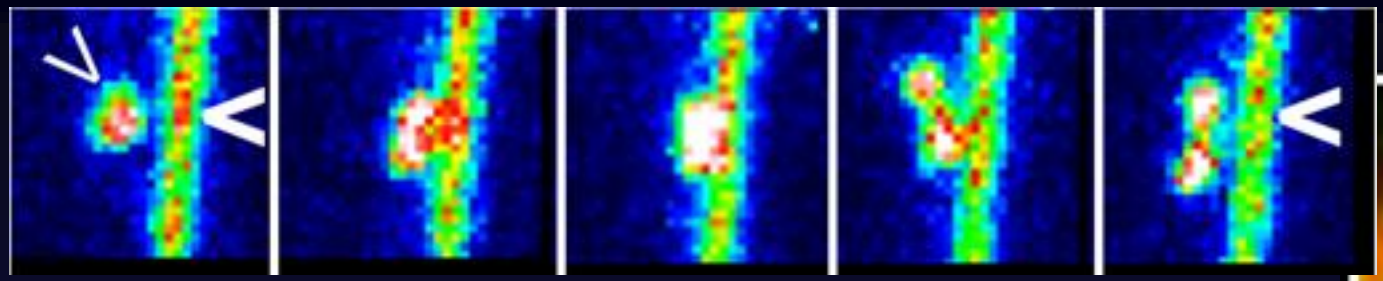
Internalisation Hot Spots

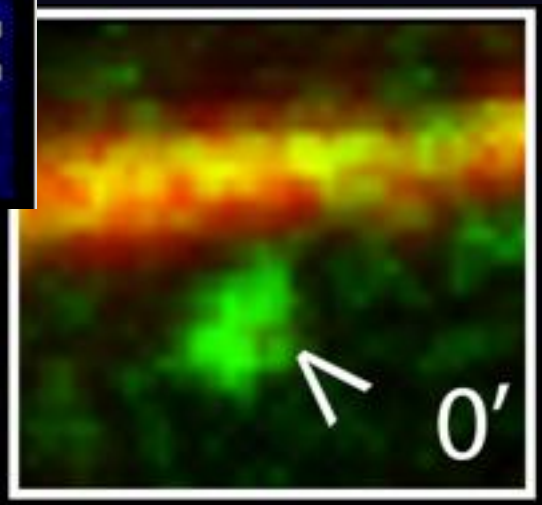


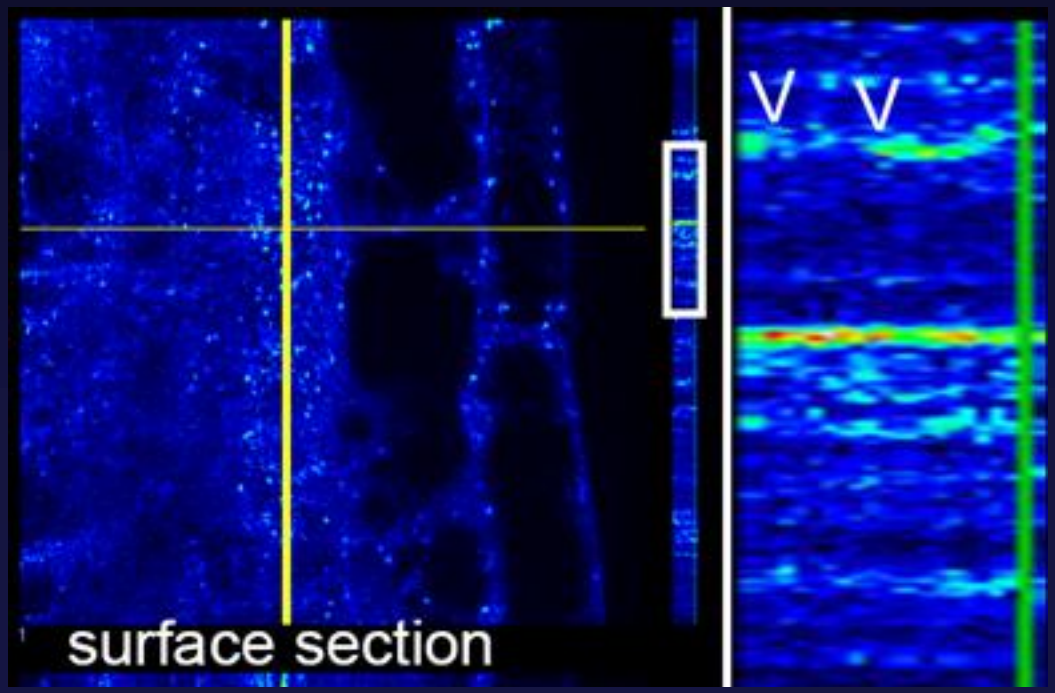


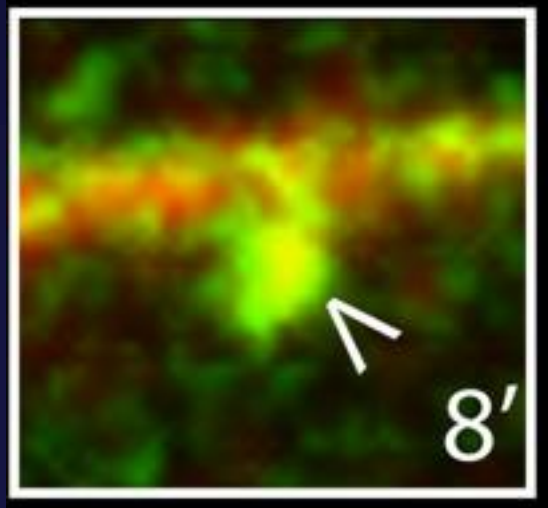
unpublished

Pick-up Service at Internalisation Hot Spots



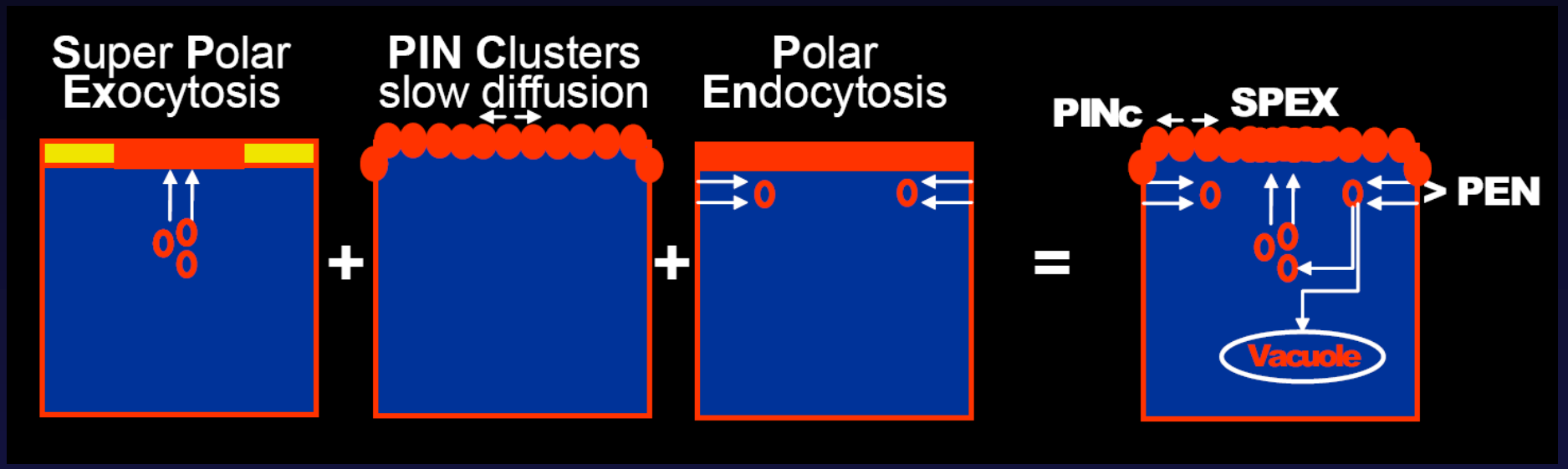






unpublished

Mechanistic insights into cell polarity in plants



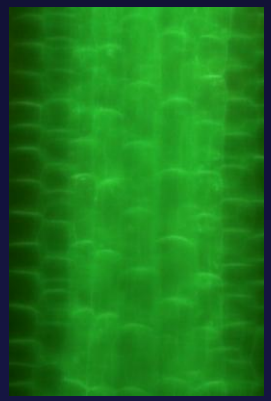
unpublished

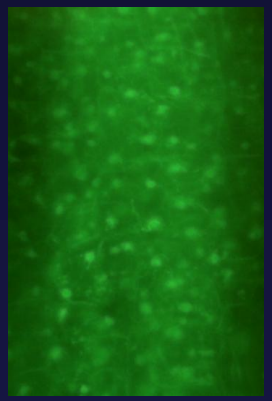
Genetic approaches:

Marker:FP

Forward Genetic Screens

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





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

EMS mutagenesis.
Epifluorescence
Screening

mutant lines

Deep sequencing

novel genes

Chemical Genetic Screens

Endocytosis
Polar Targeting

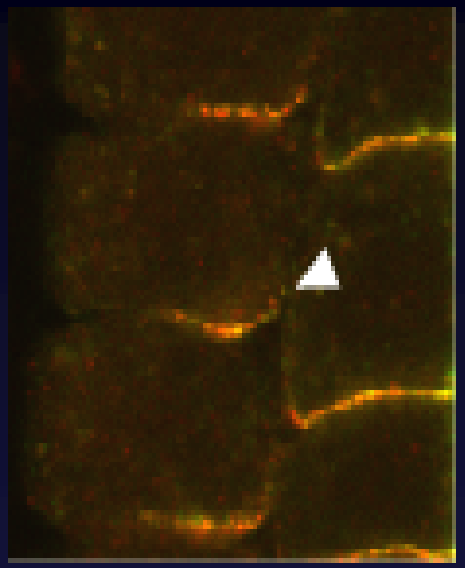
Reverse Genetics

Tanaka et al., 2009, Feraru et al., 2010; Feraru et al., 2011, unpublished

Beauty of forward genetics

Polarity screen - design

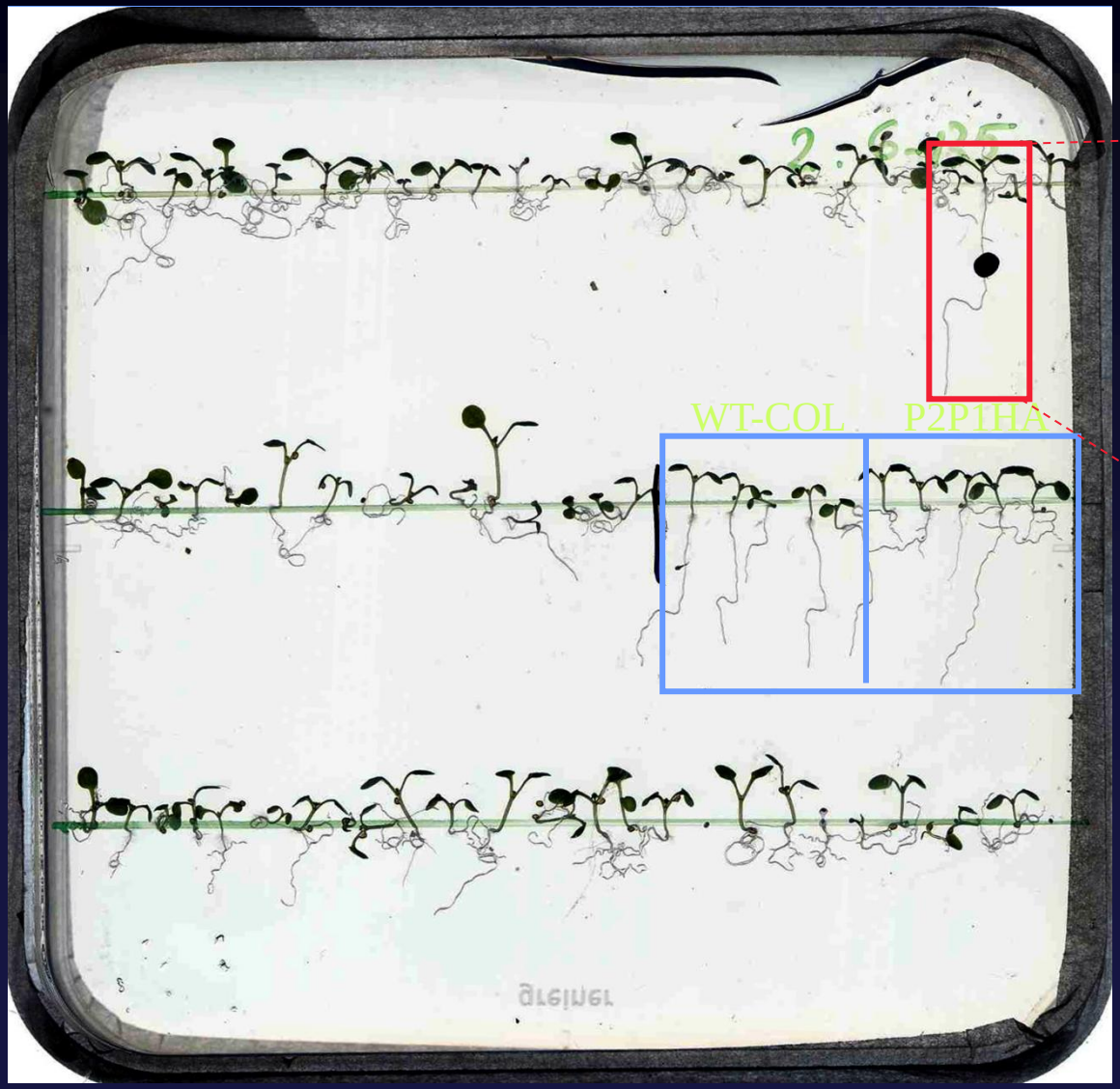
gravitropic **agravitropic** **gravitropic**

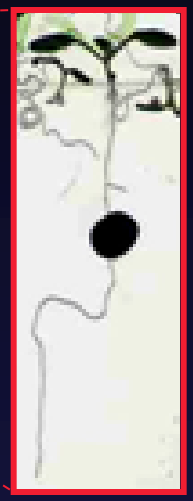


WT - epid cells **P2::P1:HA in *pin*2 - epid cells** ***mutant* - epid cells**

Feraru et al., 2011

Polarity screen



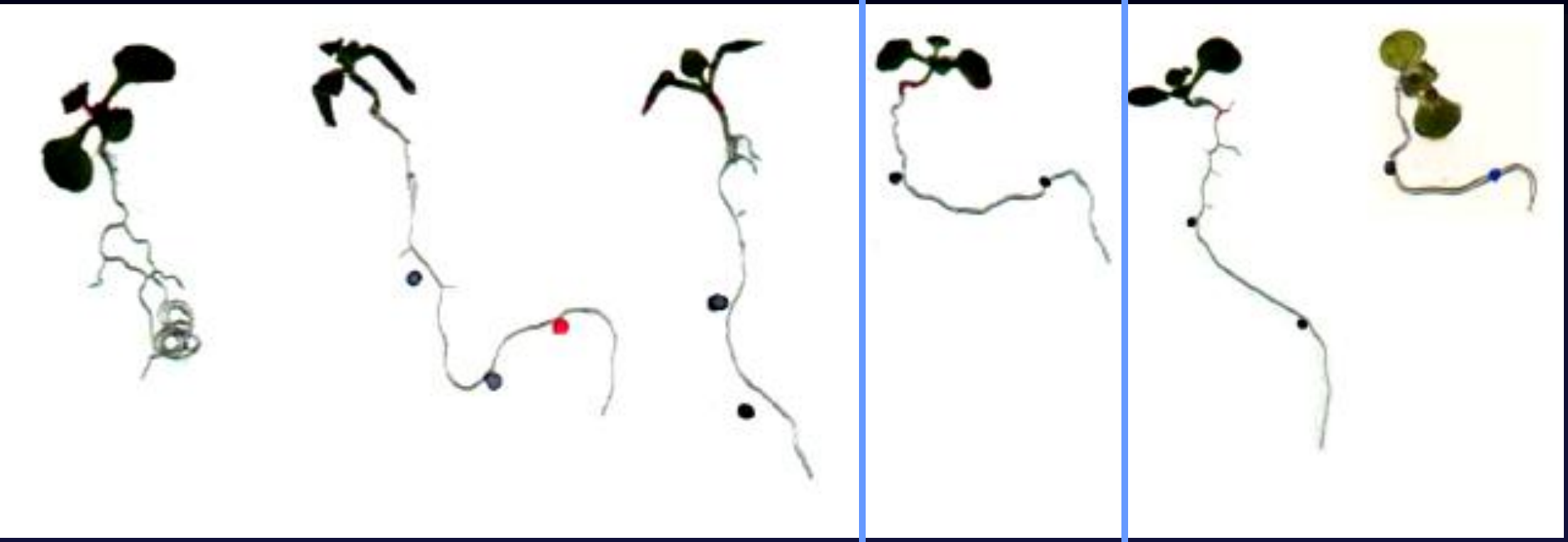


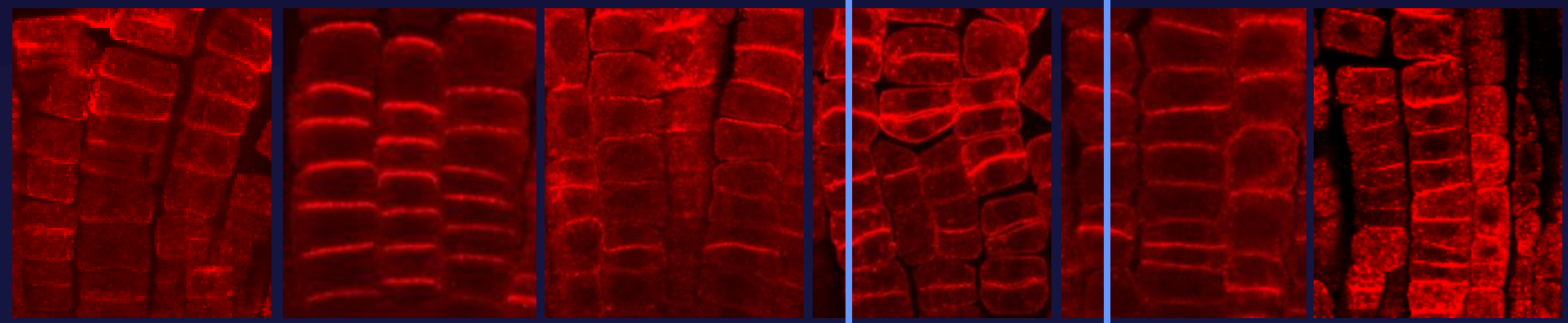
A good hit!!

Feraru et al., 2011

regulator of PIN polarity (repp)

P2::P1:HA ***repp1*** ***repp2*** ***repp3*** ***repp4*** ***repp5***

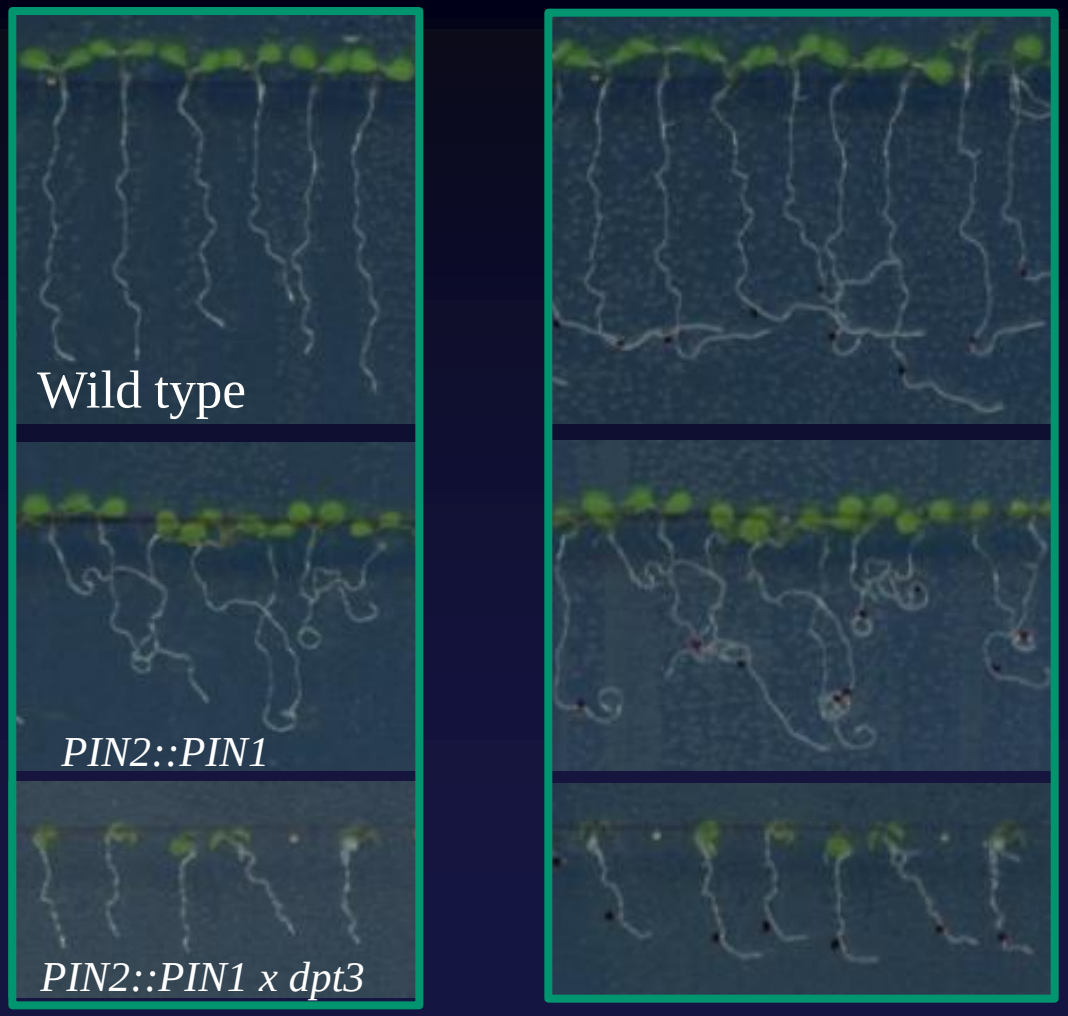




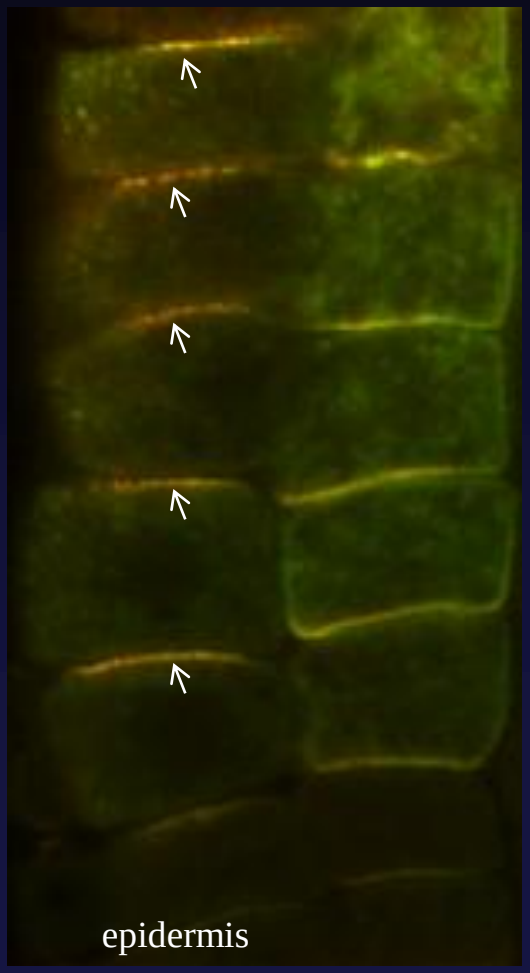
Feraru et al., 2011

repp3 rescues gravitropism and PIN polarity

Gravistimulated



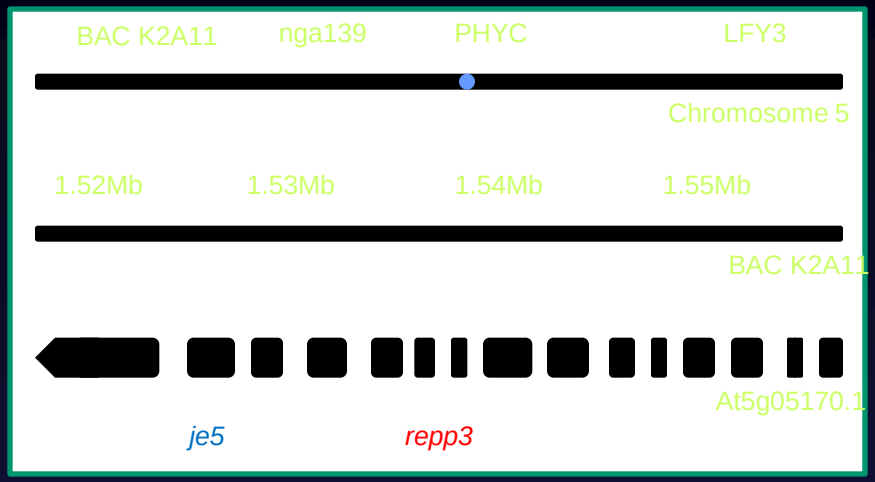
repp3 (50-60%)



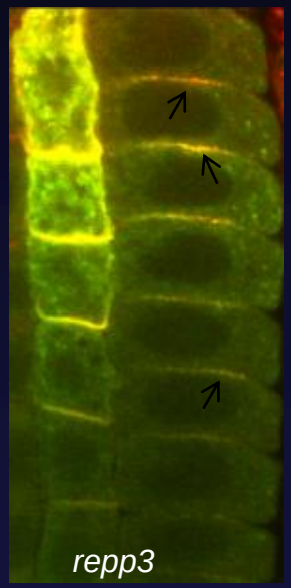
Feraru et al., 2011

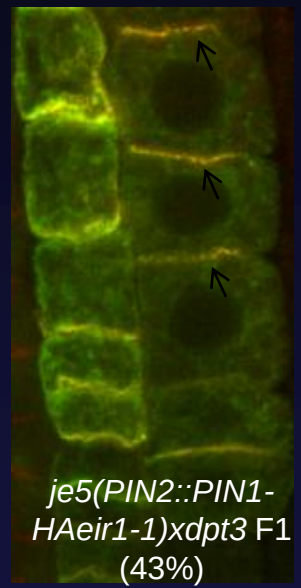
REPP3 encodes cellulose synthase 3 (CESA3/CEV1/IXR1/ELI1)

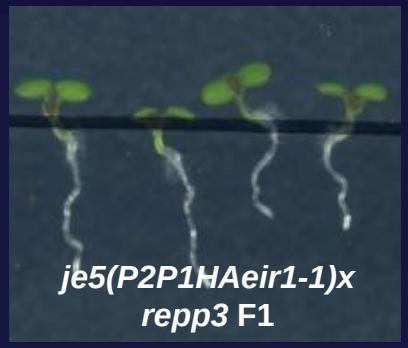
Mapping

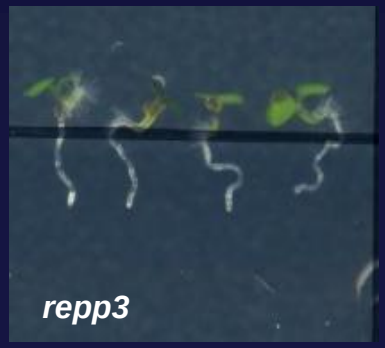


Allelic test







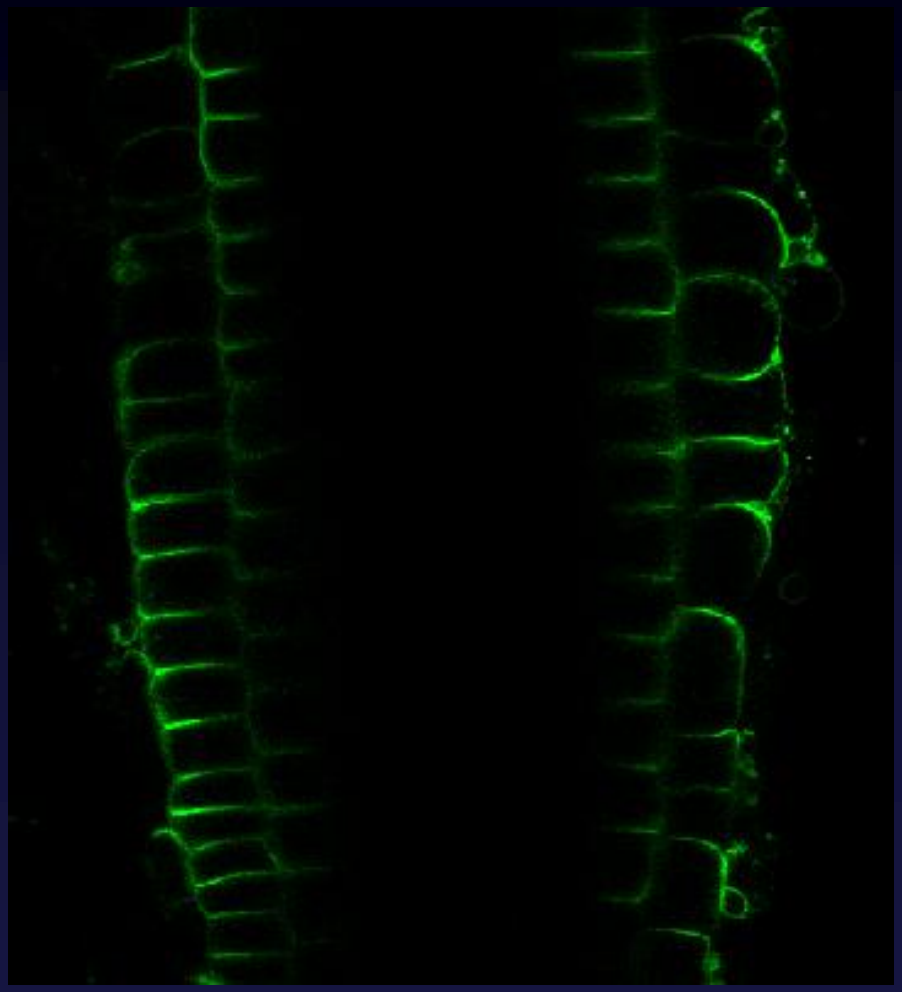


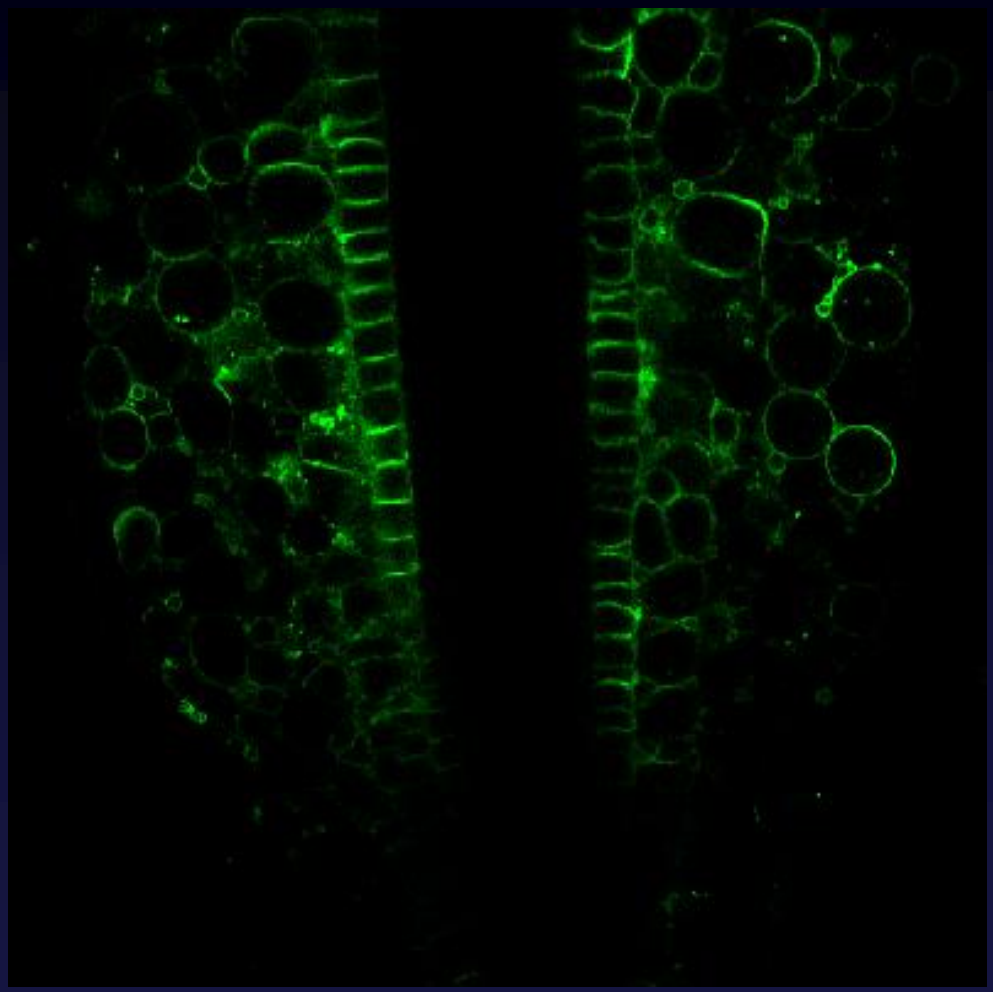
Feraru et al., 2011

Degradation of cell wall results in loss of polarity

10’ protoplasting

15’ protoplasting





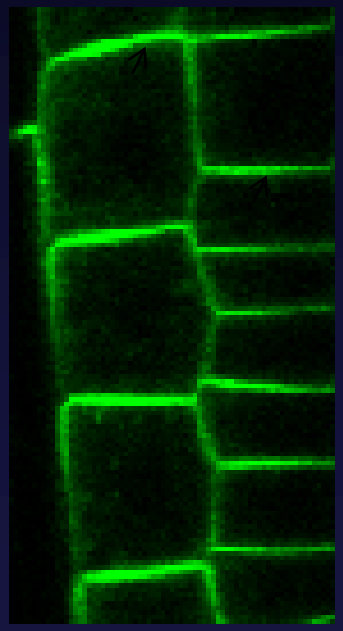
PIN2::PIN2-GFP

Feraru et al., 2011

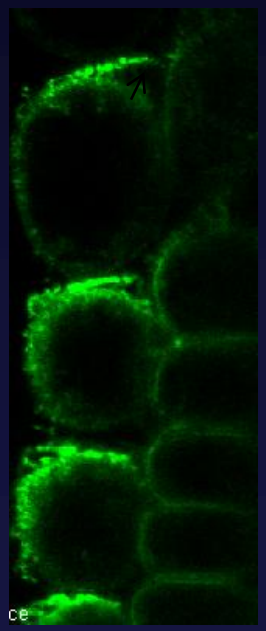
PIN proteins are attached to cell wall

Partial degradation of cell wall

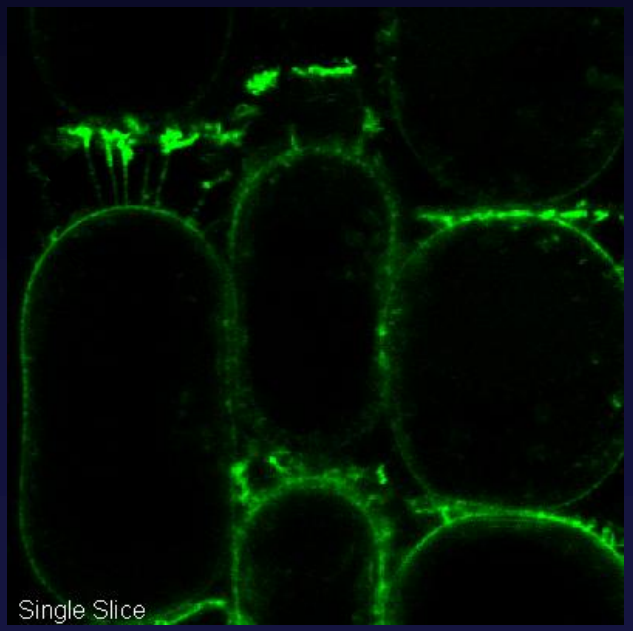
Before



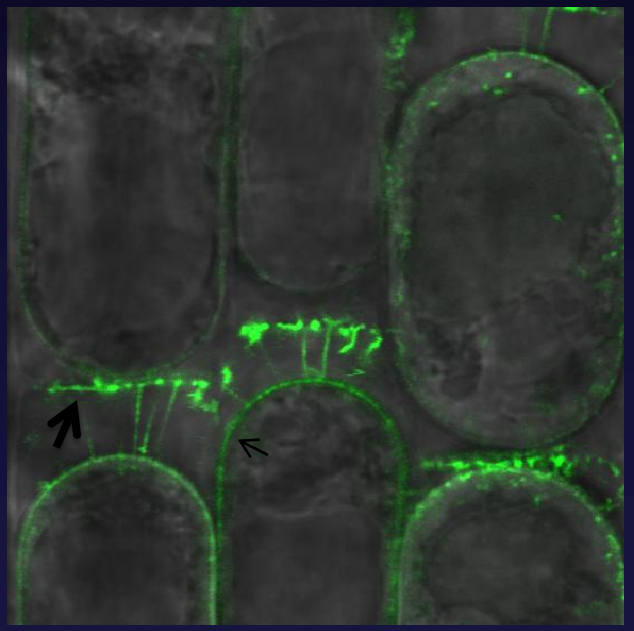
After 30 min



After 1.5 hours



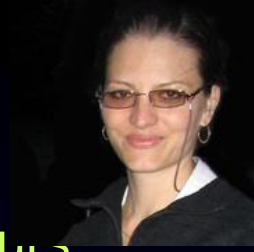
After 1.5 hours



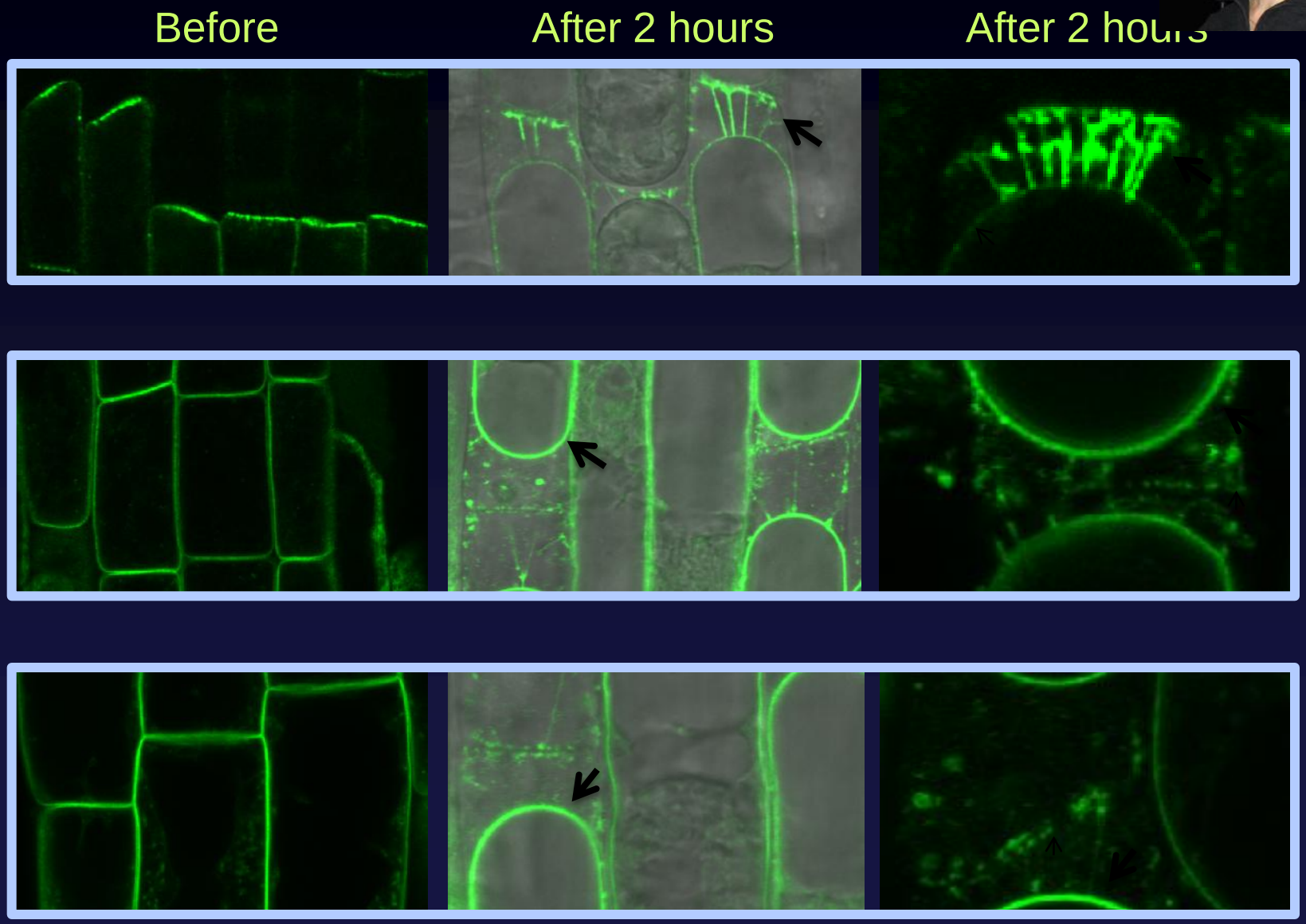
PIN2::PIN2-GFP

Feraru et al., 2011

Polar cargos are attached to cell wall



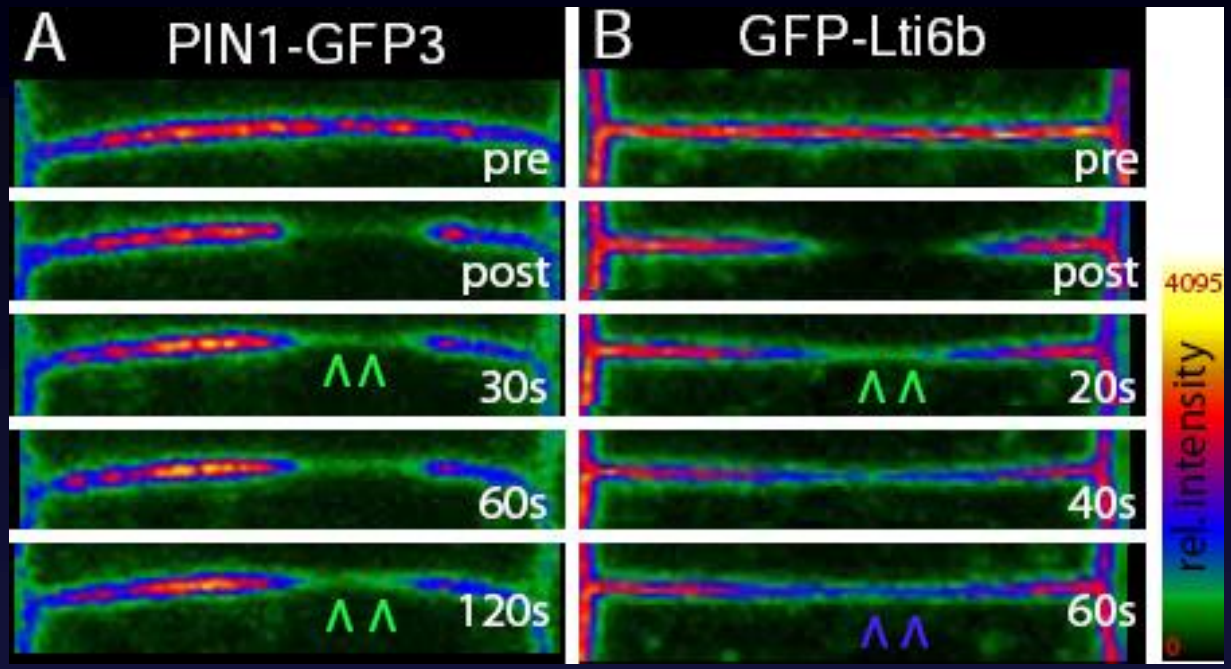
Partial degradation of cell wall



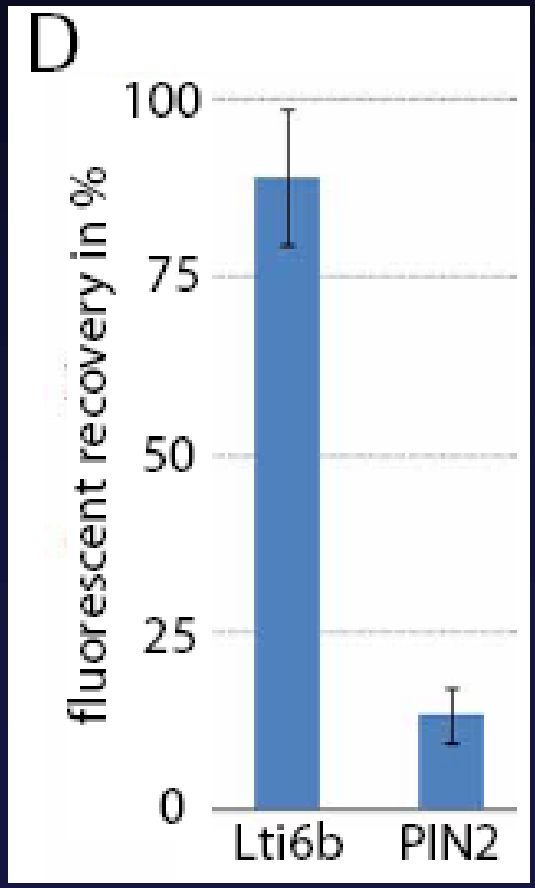
Feraru et al., 2011

PIN Proteins Display Reduced Mobility

FRAP analysis

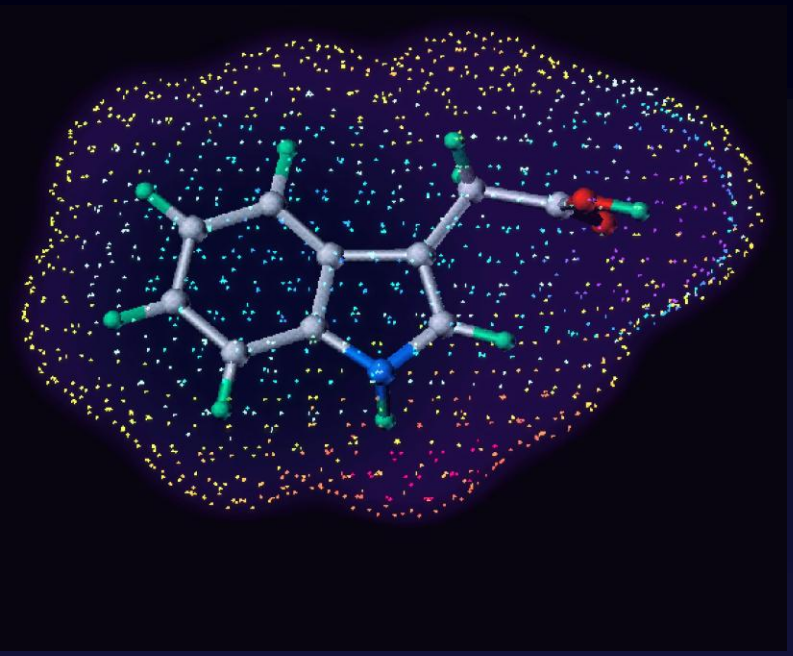


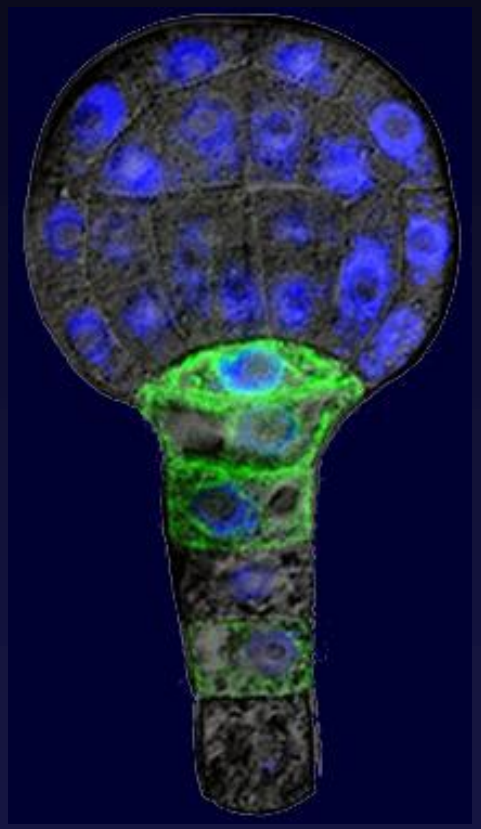
	Mean	StDev	Repetition	T test two tail
PIN2 untreated	14.1	5.3	18	
PIN2 IX treated	20.1	5.8	11	0.00545

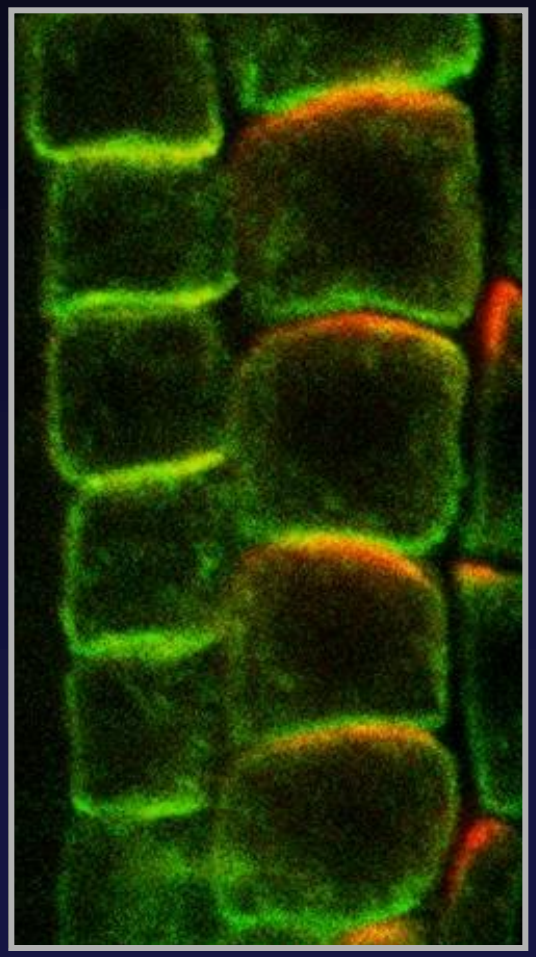


Feraru et al., 2011

Patterning in Plant Development



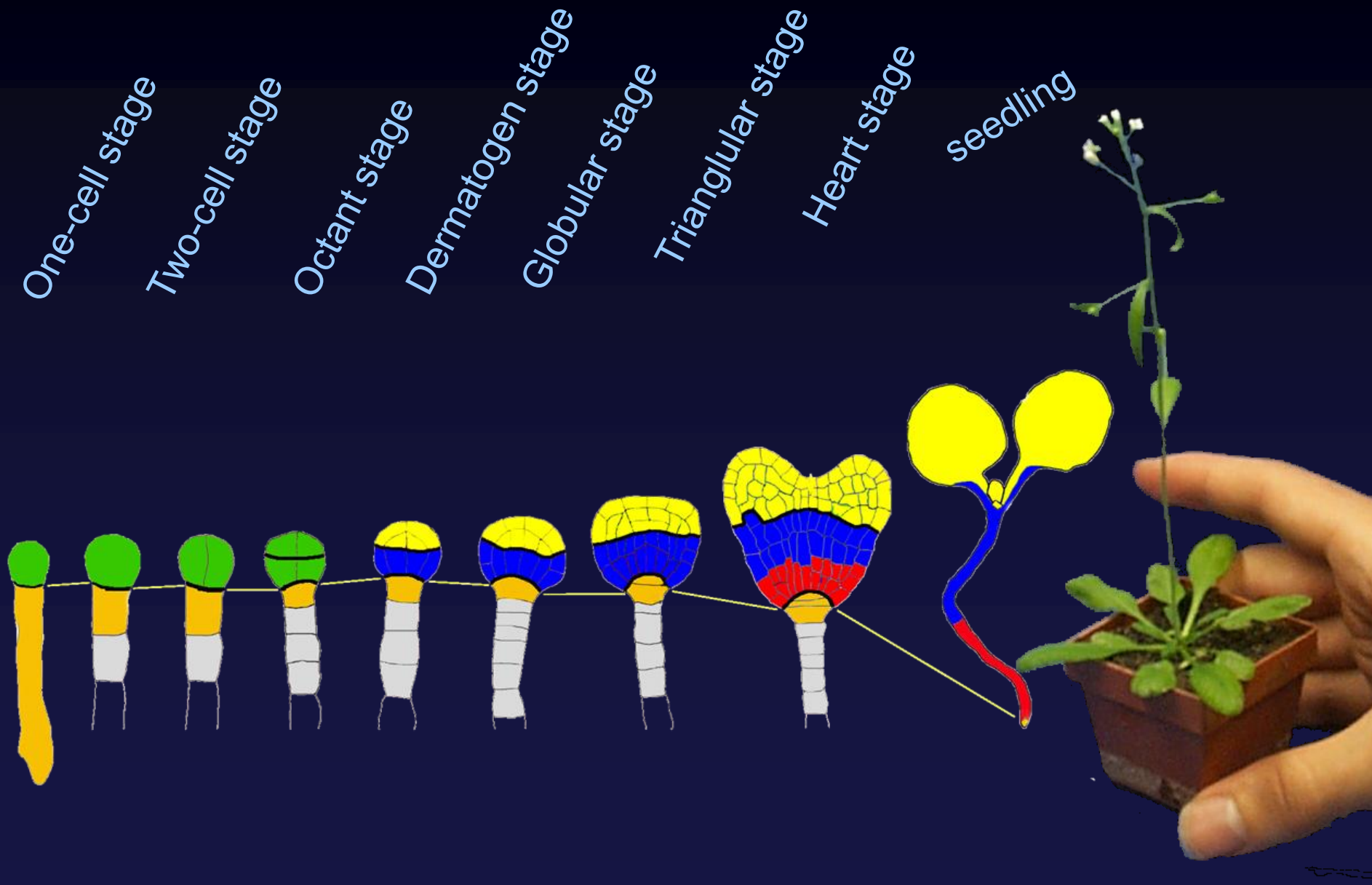




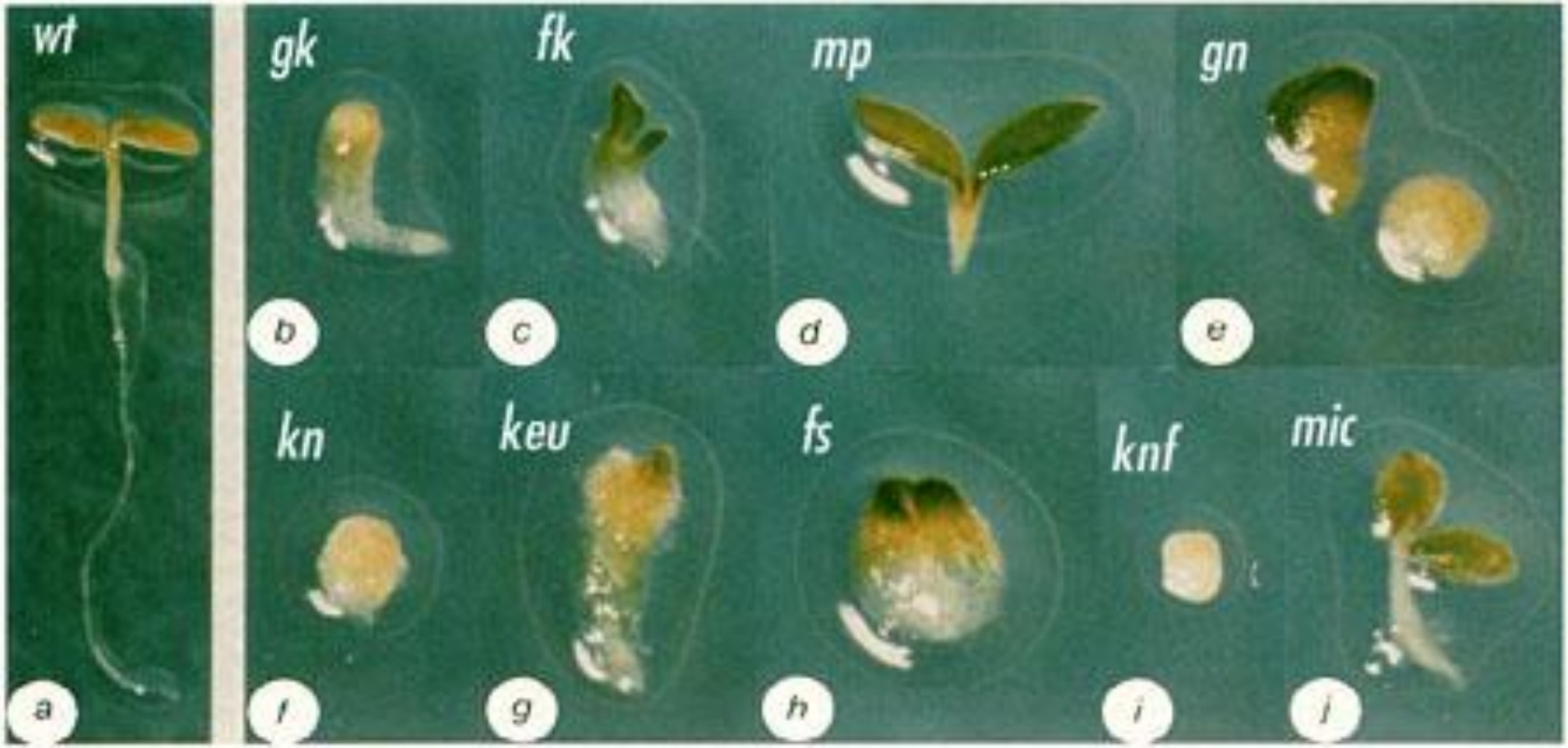
Jiří Friml,
ZMBP Tübingen

Plants and Animals Live Different Lives

Arabidopsis Embryogenesis



Mutant screen at seedling level

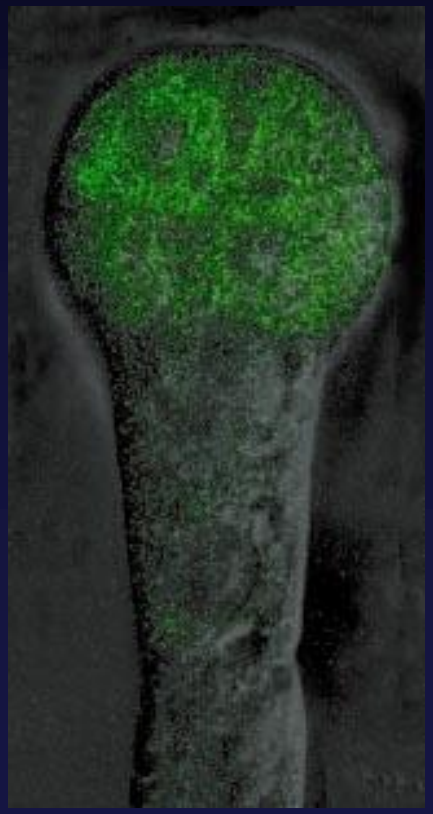


Auxin in Early Embryogenesis

DR5::GFP

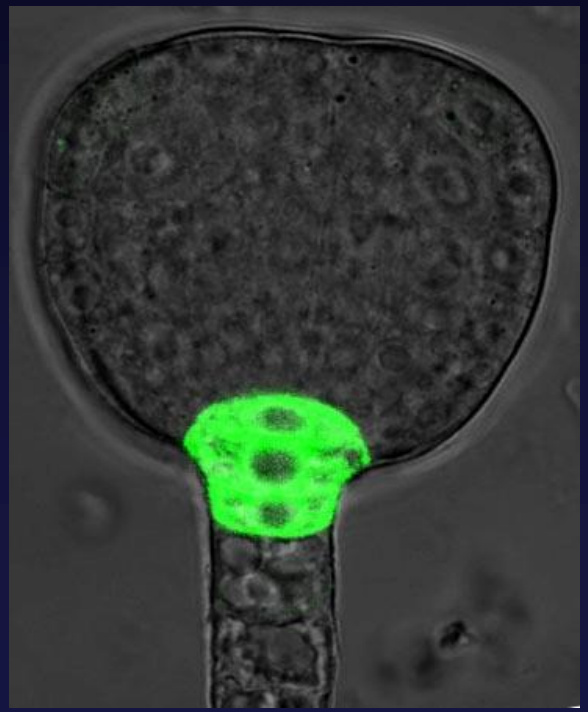
IAA localisation





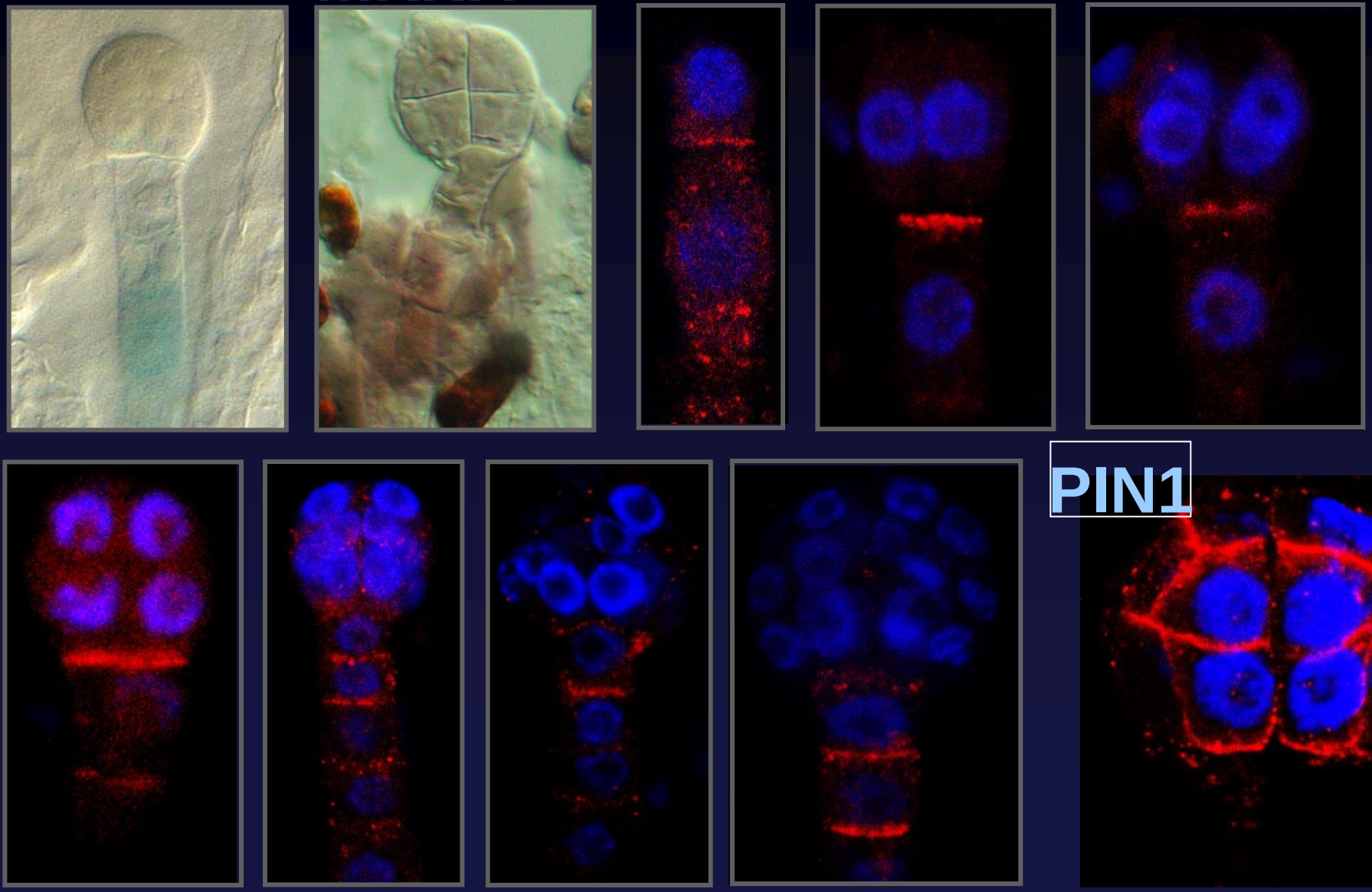

Auxin in Embryogenesis

DR5::GFP

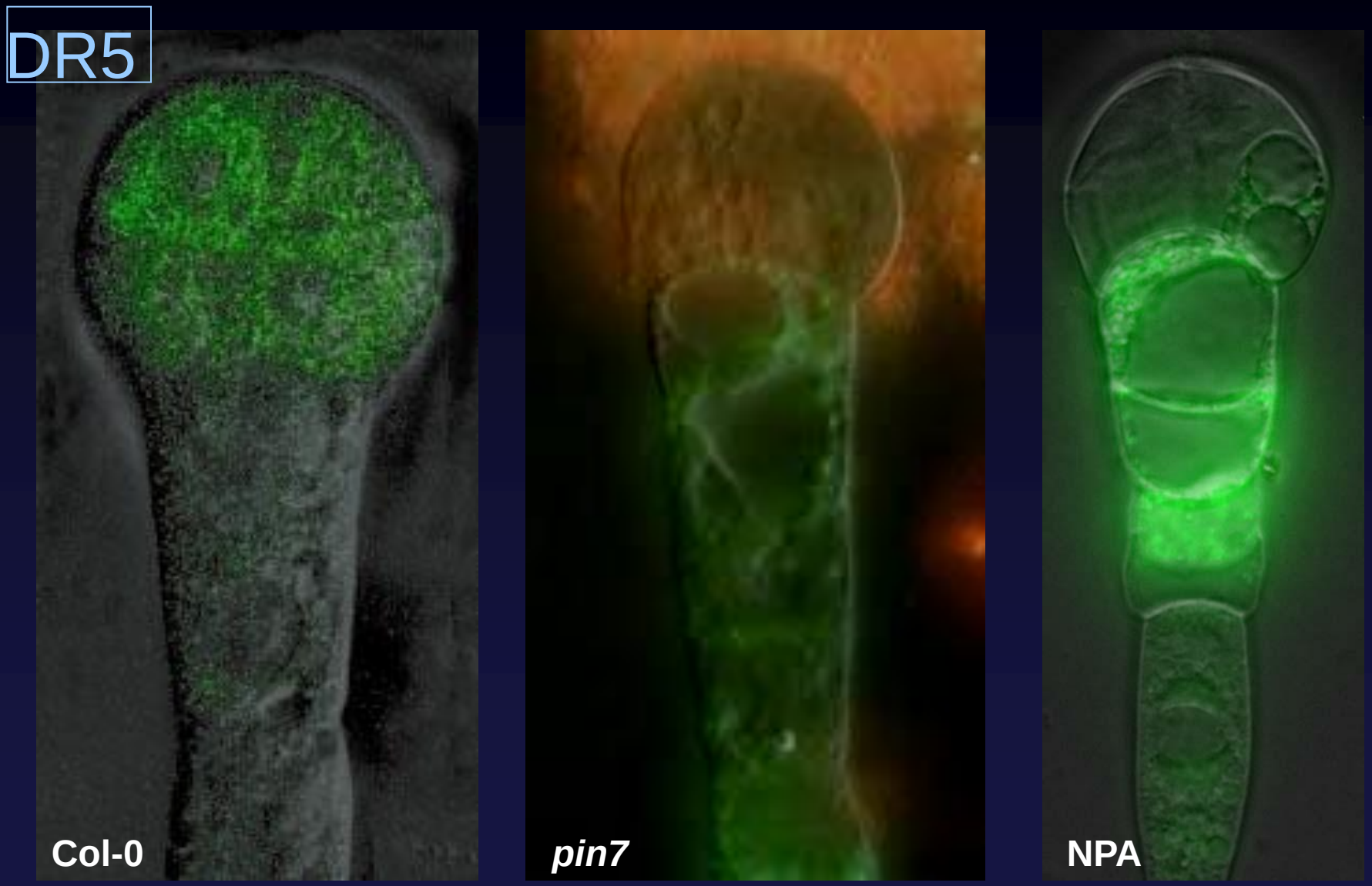


IAA localisation

PIN7 in Embryogenesis



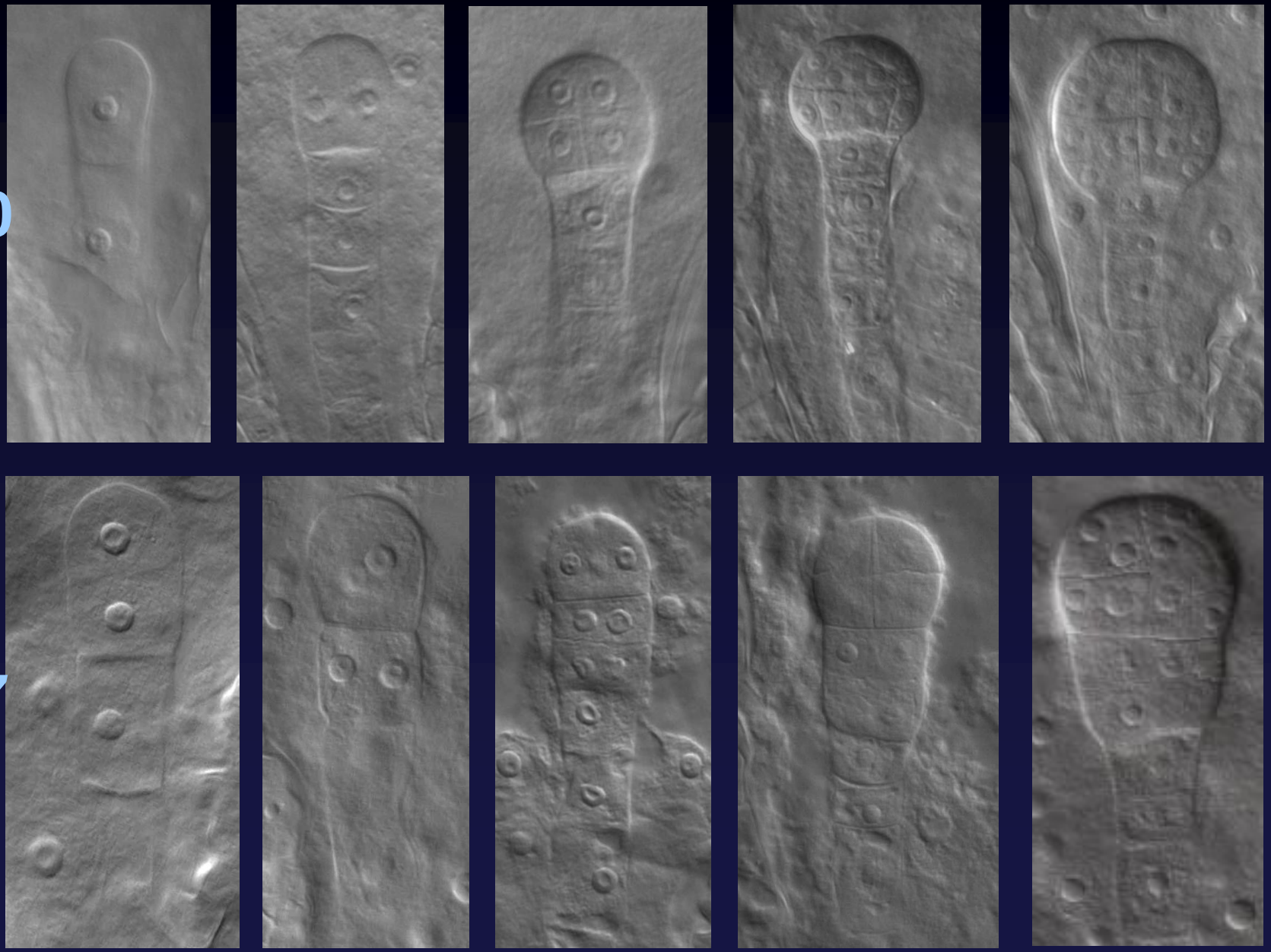
Analysis of DR5 activity in *pin7*



Embryo Phenotype of *pin7* Mutants

Col-0

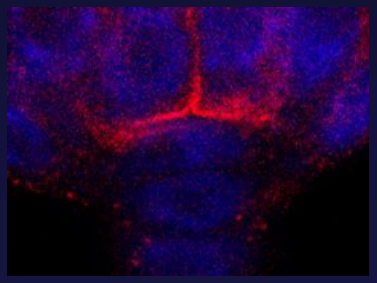
pin7

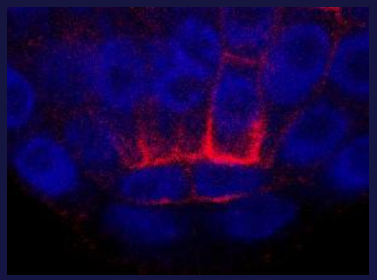


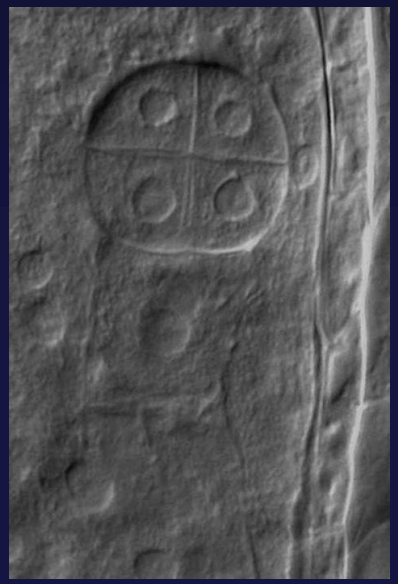
PIN1 in Early Embryogenesis

GUS mRNA

Protein







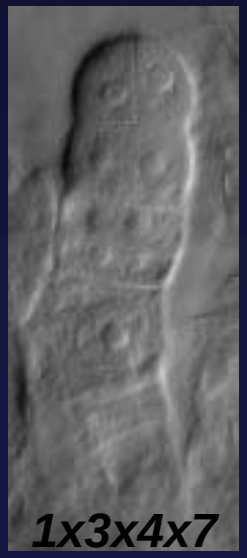
Enk

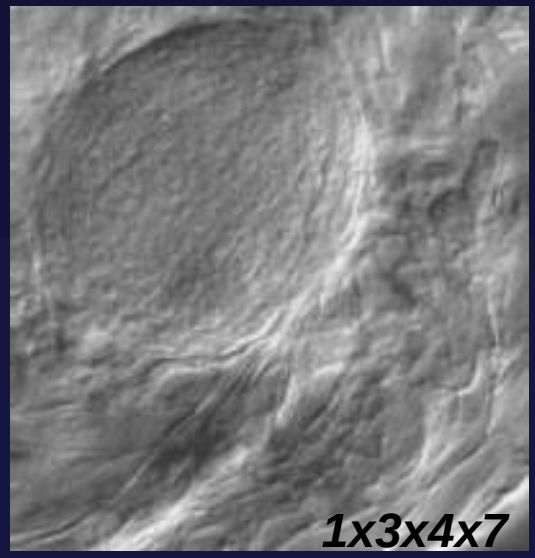
6,1%

pin1

30,4%

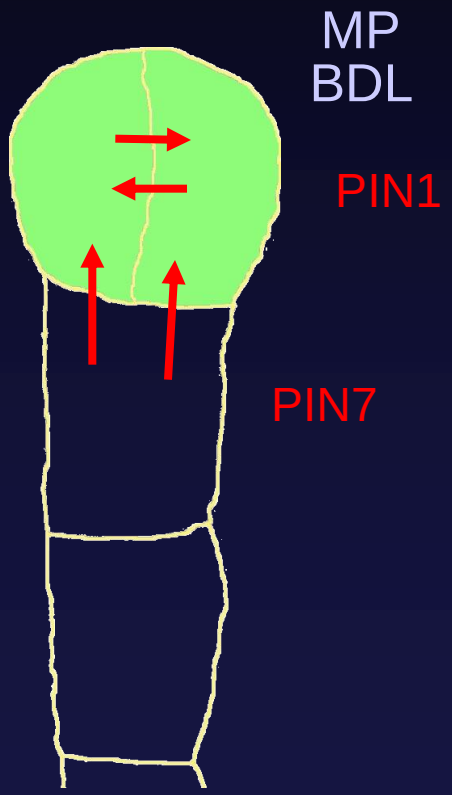
Phenotypes of *pin* Multiple Mutants





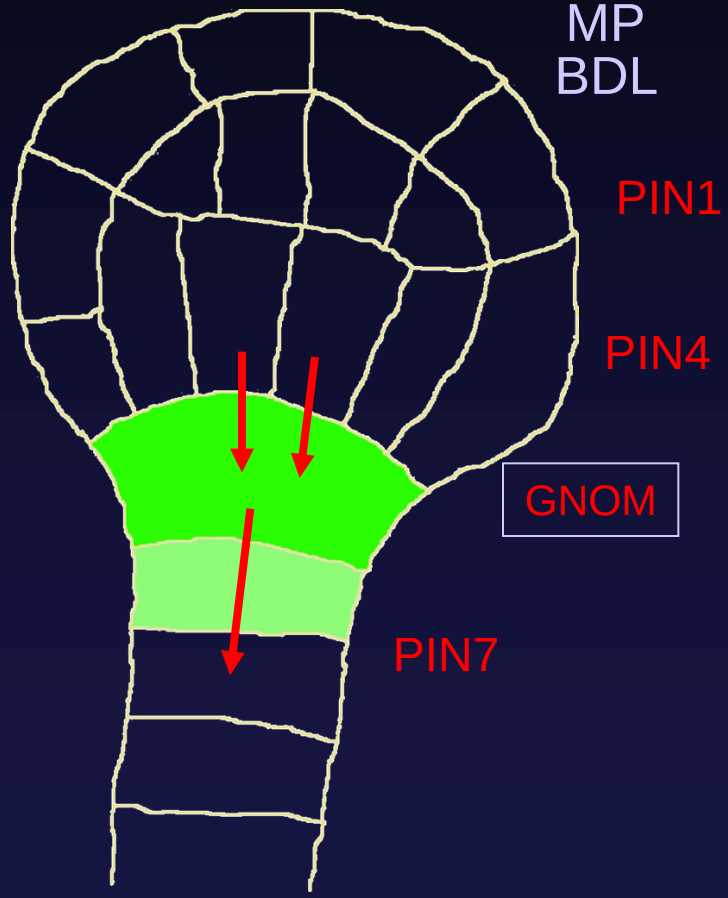
Auxin and Embryogenesis

Apical pole specification



Two-Cell

Root pole specification

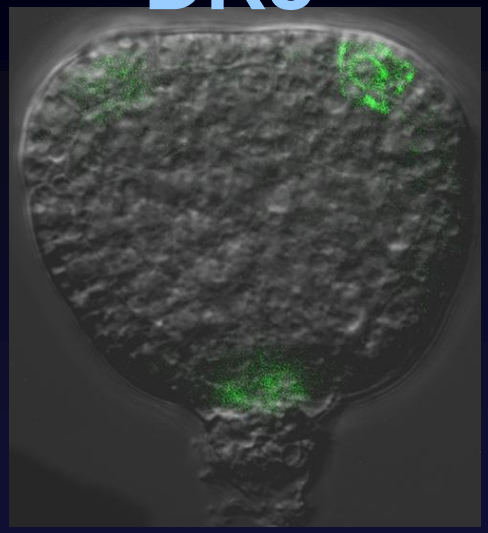


Globular

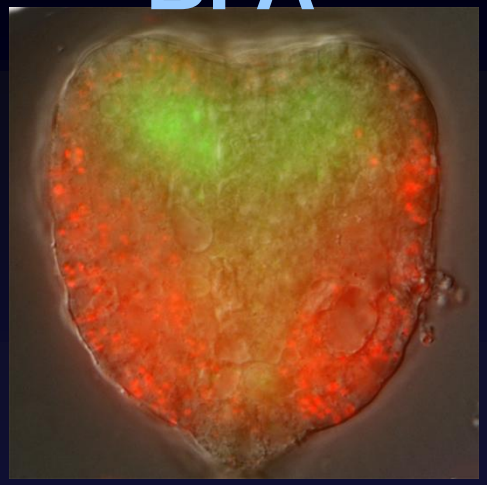
Organogenesis

Auxin in Cotyledon Formation

DR5

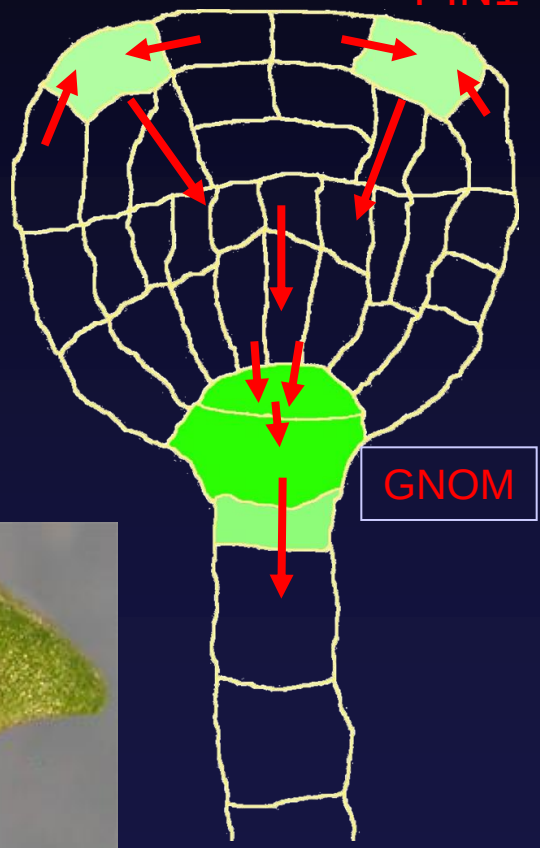


BFA



pins

MP
BDL
PIN1



IAA

gnom

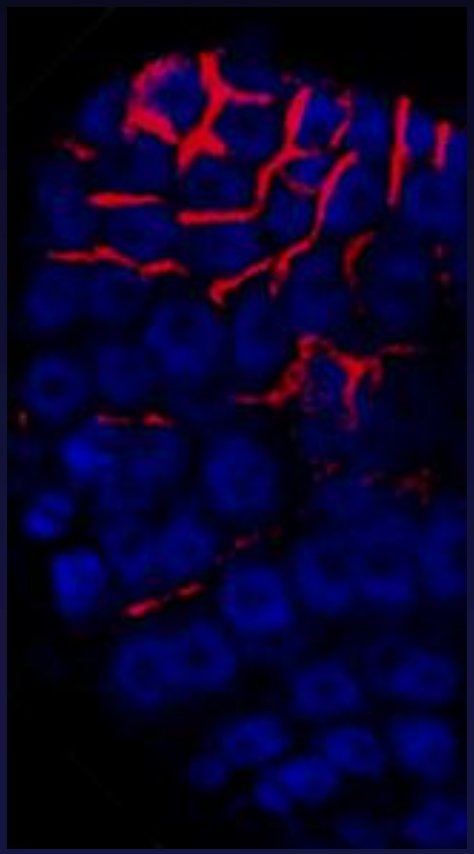
pin1

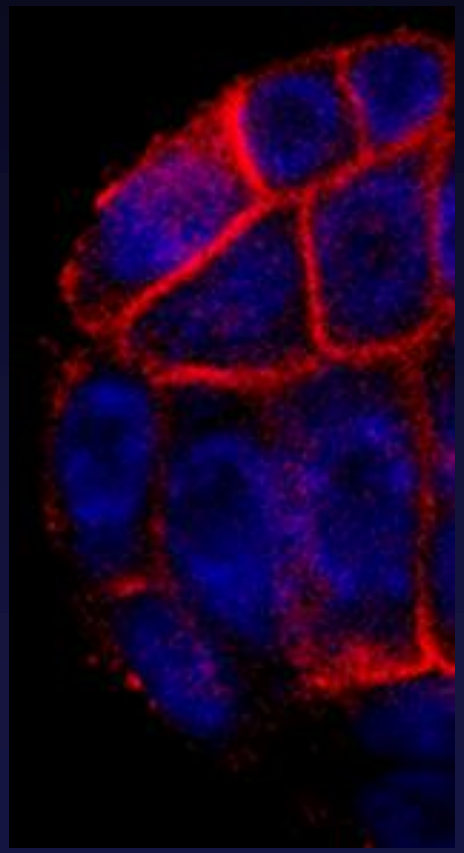
PIN1 Polarity in Cotyledon Formation

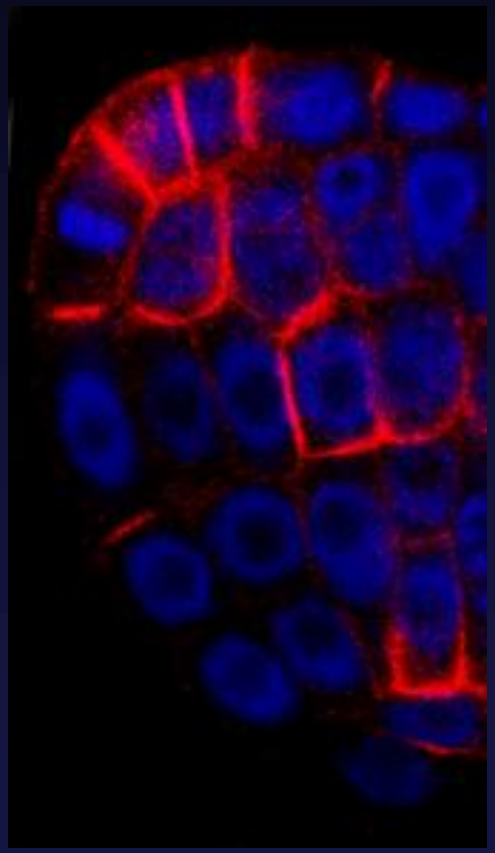
Outer layer

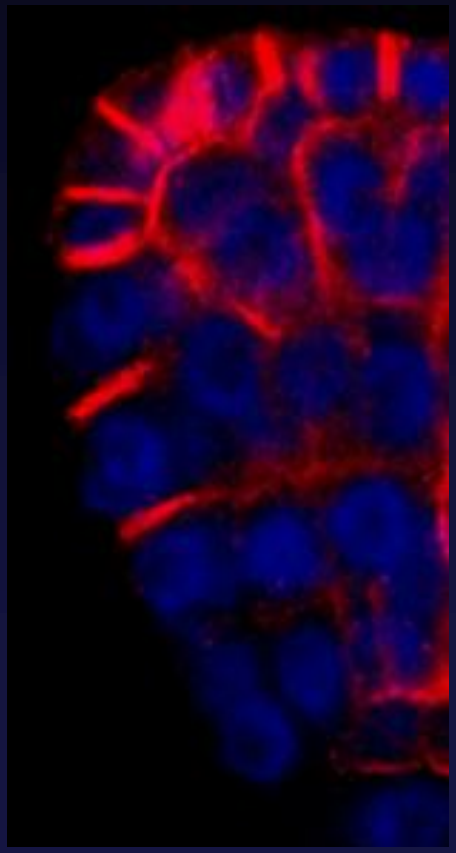
Inner layers

BFA treatmen









Heart

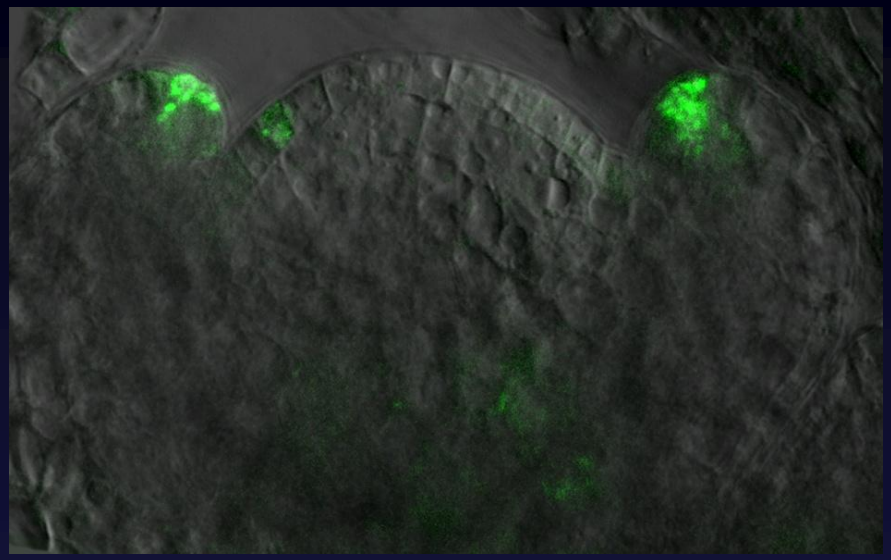
Globular

Heart

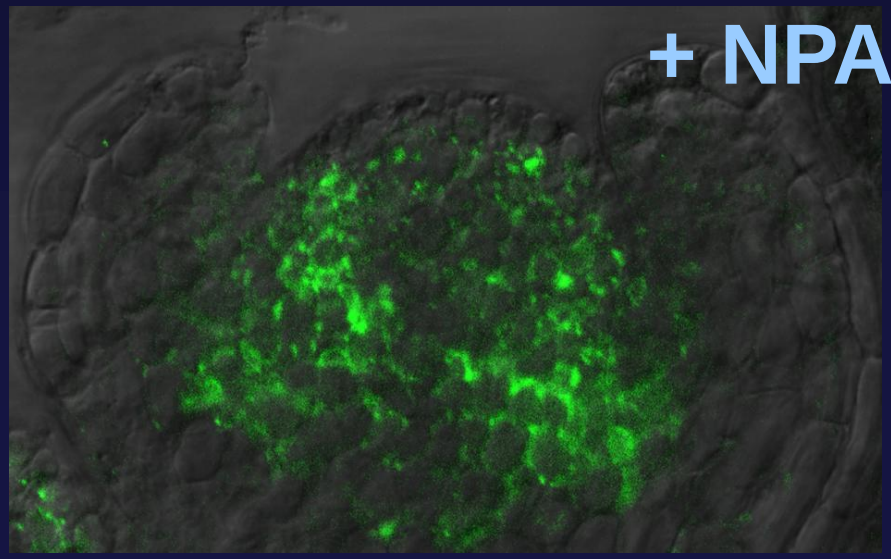
Heart

Auxin in Flower and Leave Formation

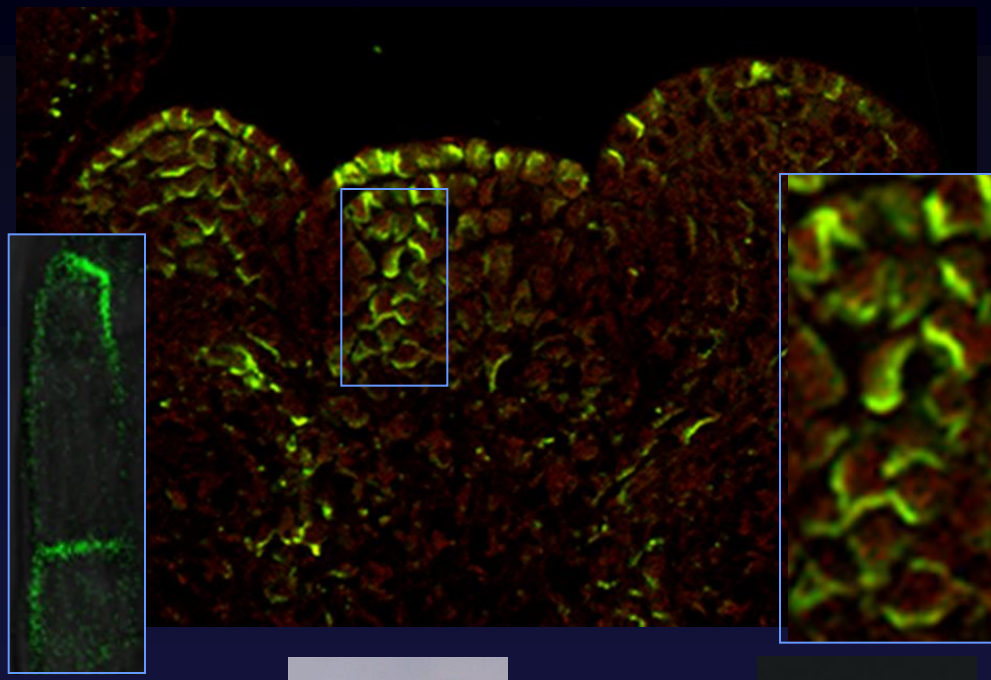
DR5rev::GFP



+ NPA



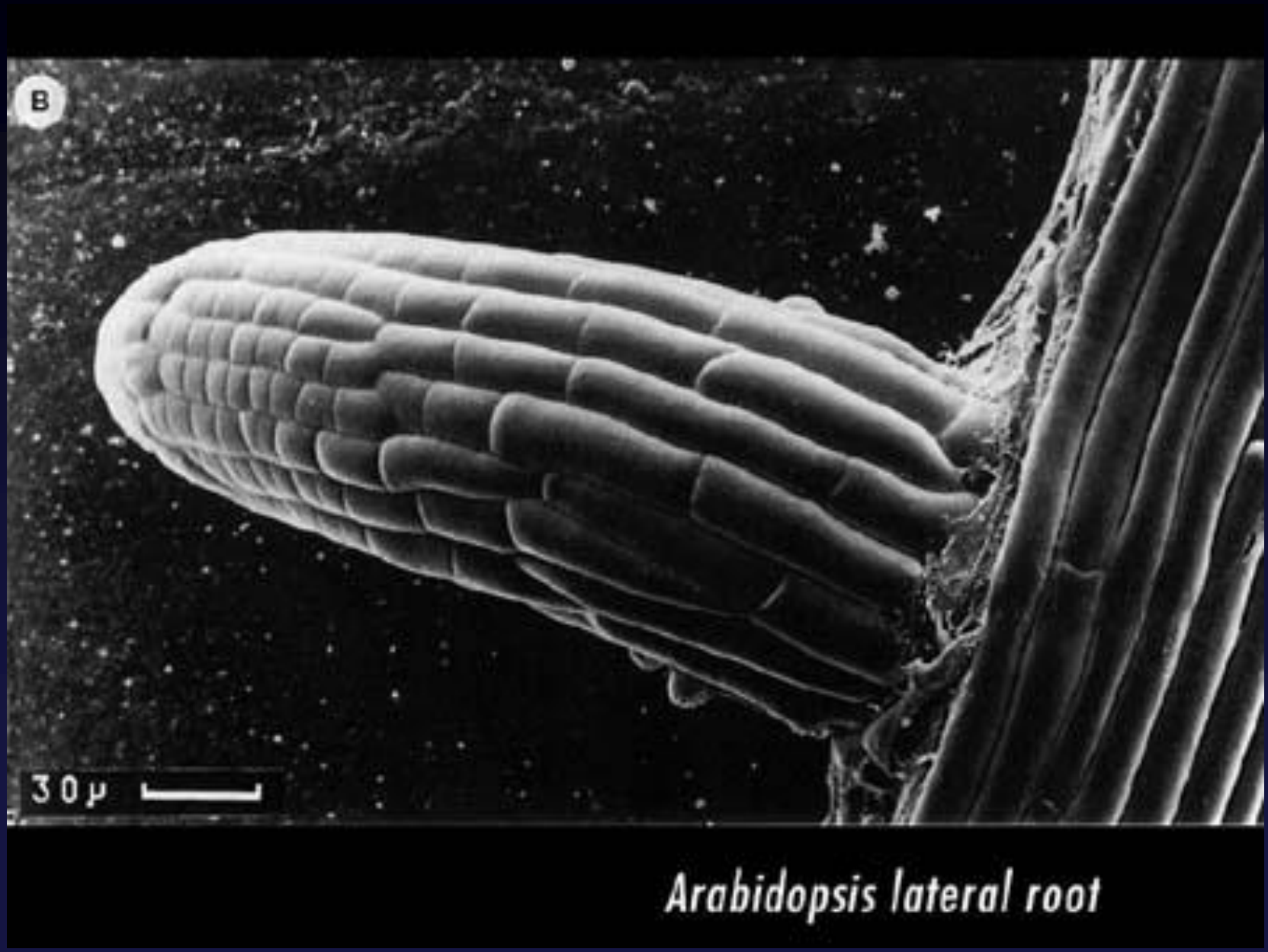
PIN1 localisation



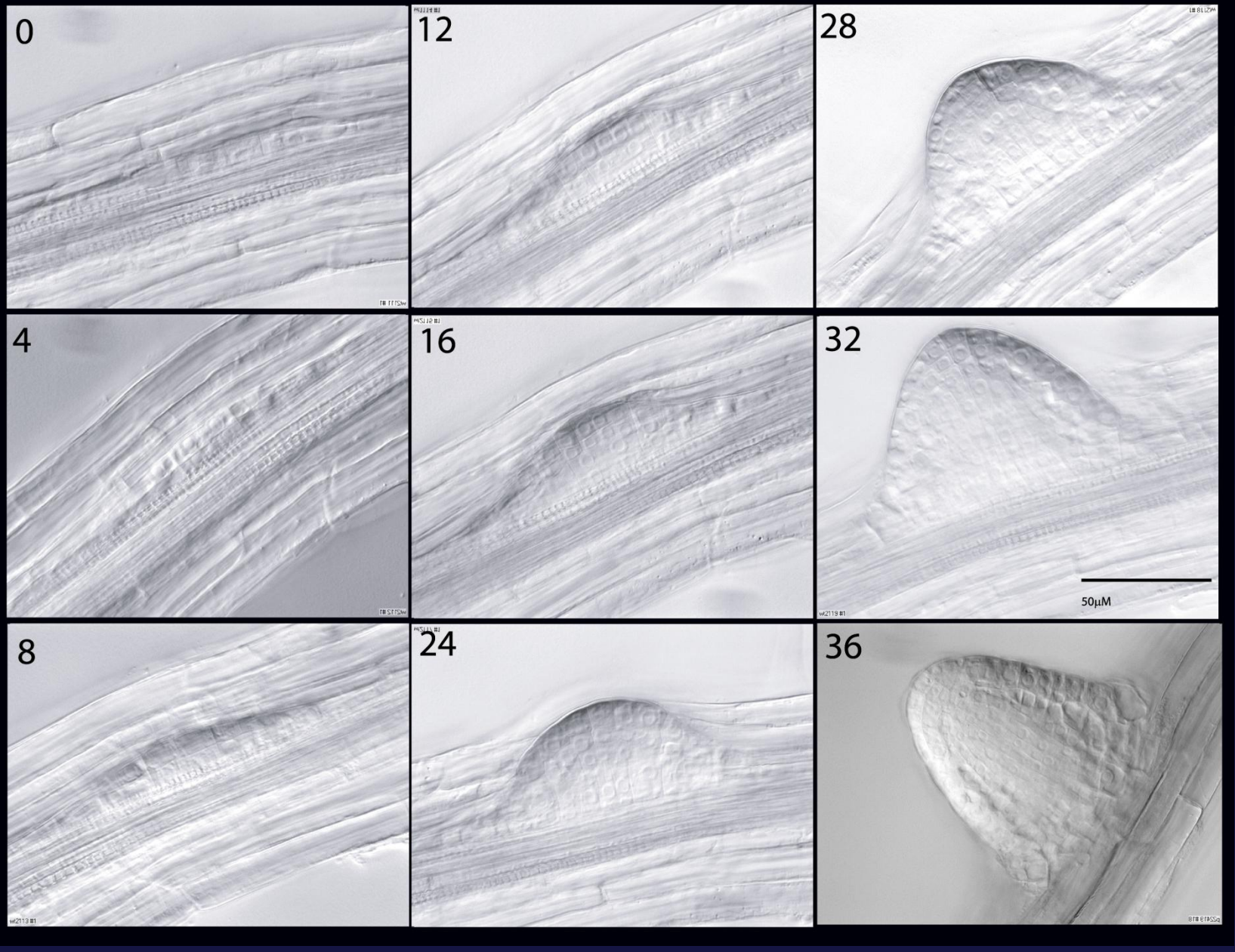
+ NPA

pin1

Lateral Root Development

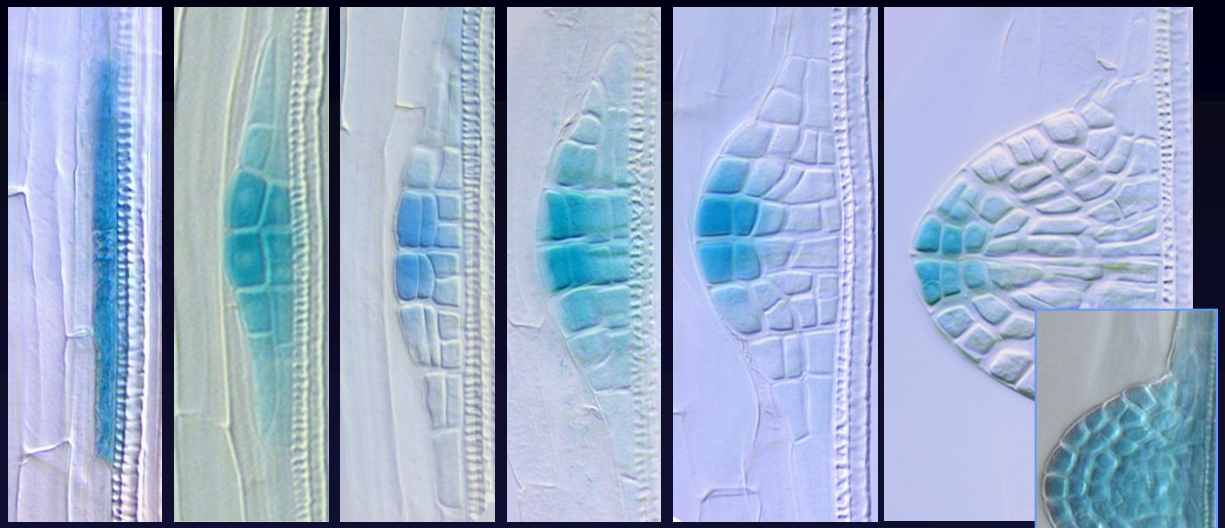


Lateral Root Development in Time



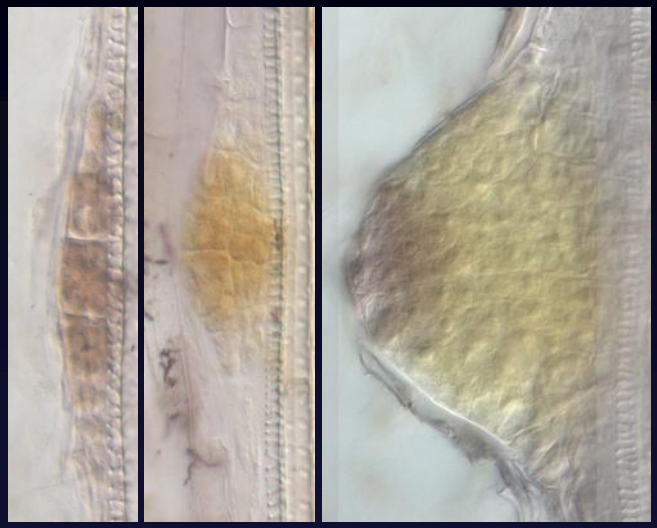
DR5 in Lateral Root Formation

DR5rev::GUS

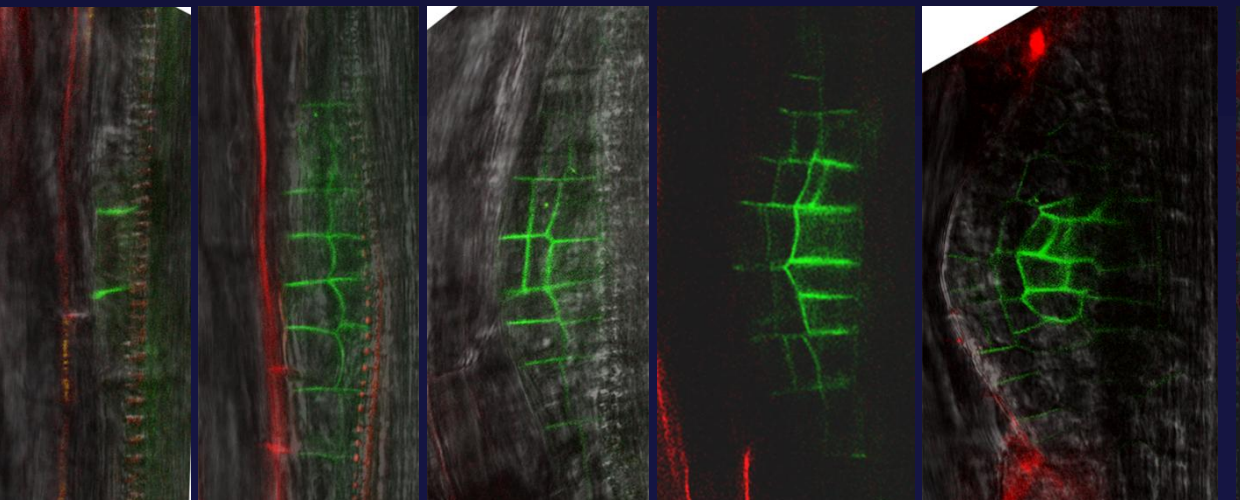


+ NPA

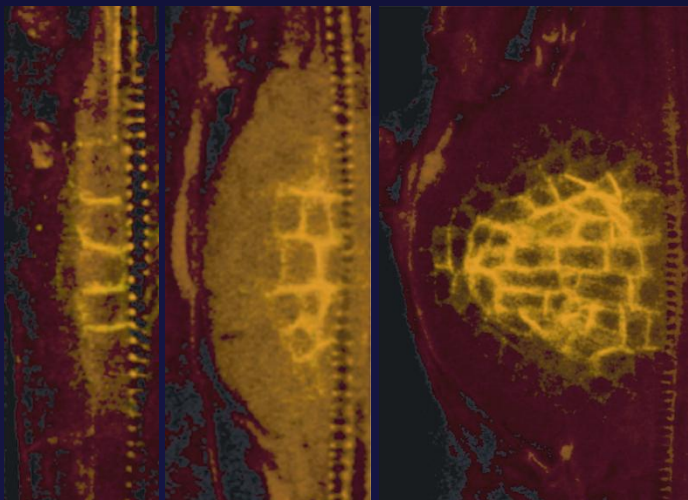
IAA



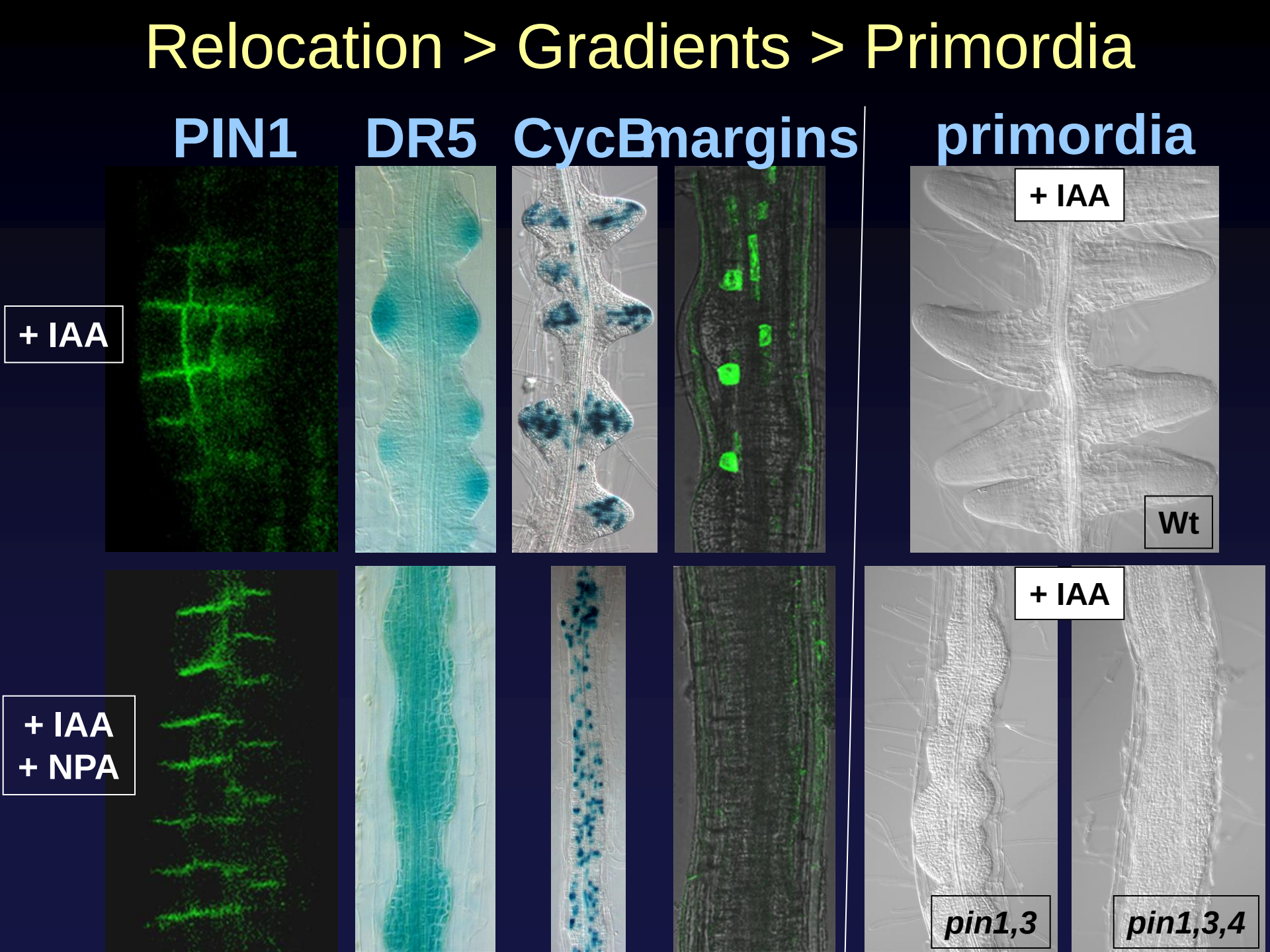
PIN1:GFP



PIN1

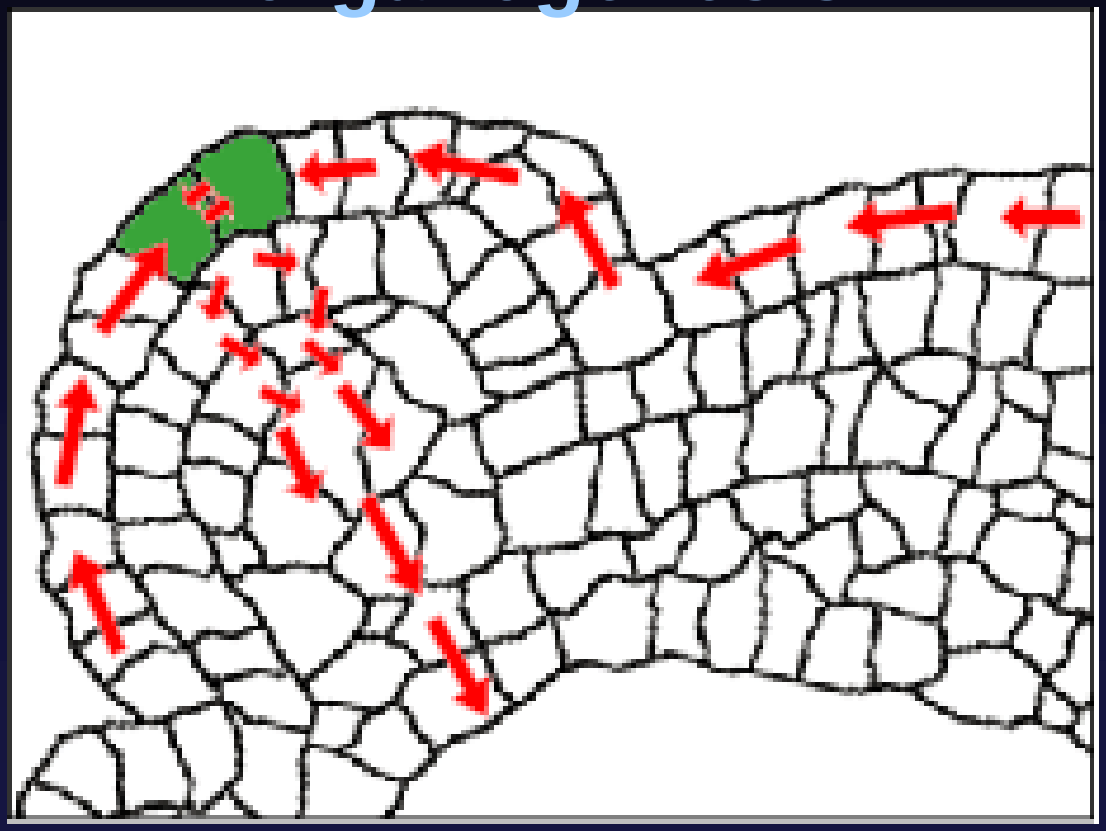


Relocation > Gradients > Primordia

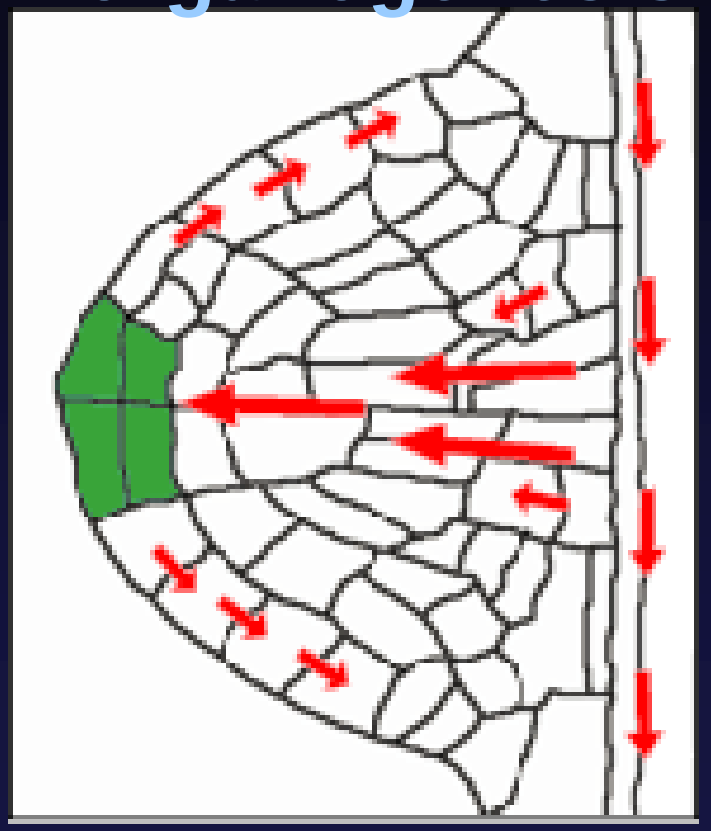


Common module for organ formation

Aerial organogenesis



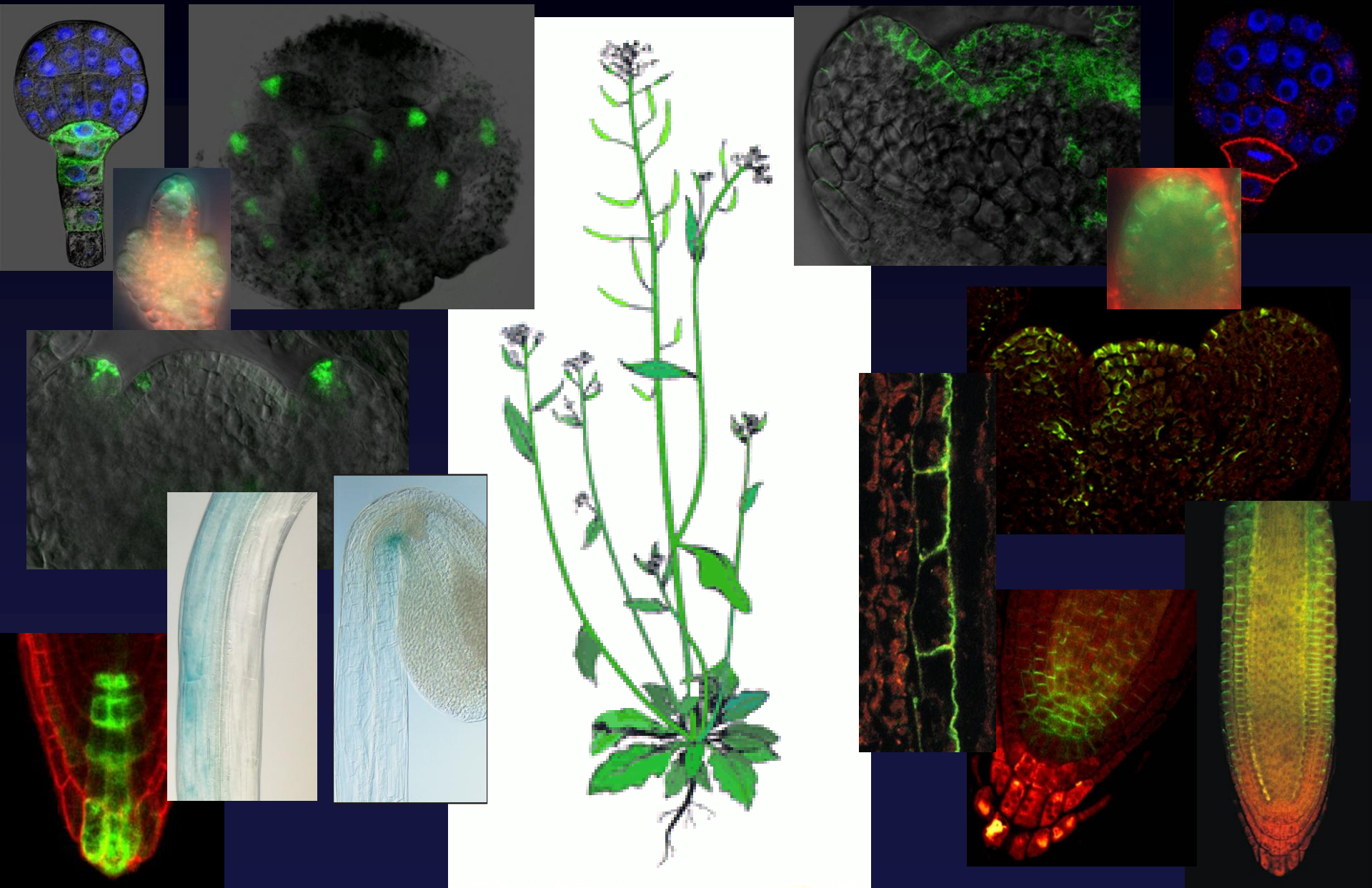
Underground organogenesis



Cotyledons, leaves, flowers, Lateral roots

al organs, ovules, integuments

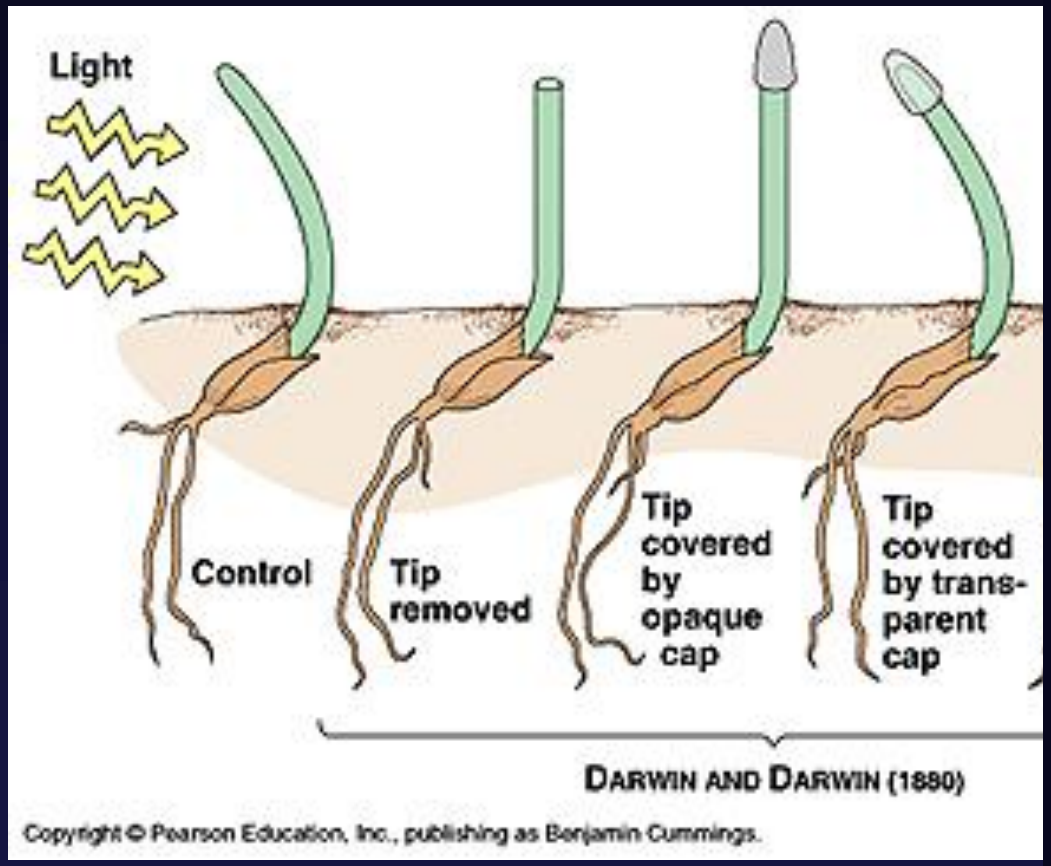
PIN-dependent Auxin Gradients in Plant Development



TROPISMS

Tropisms: „Movements“ in Plants

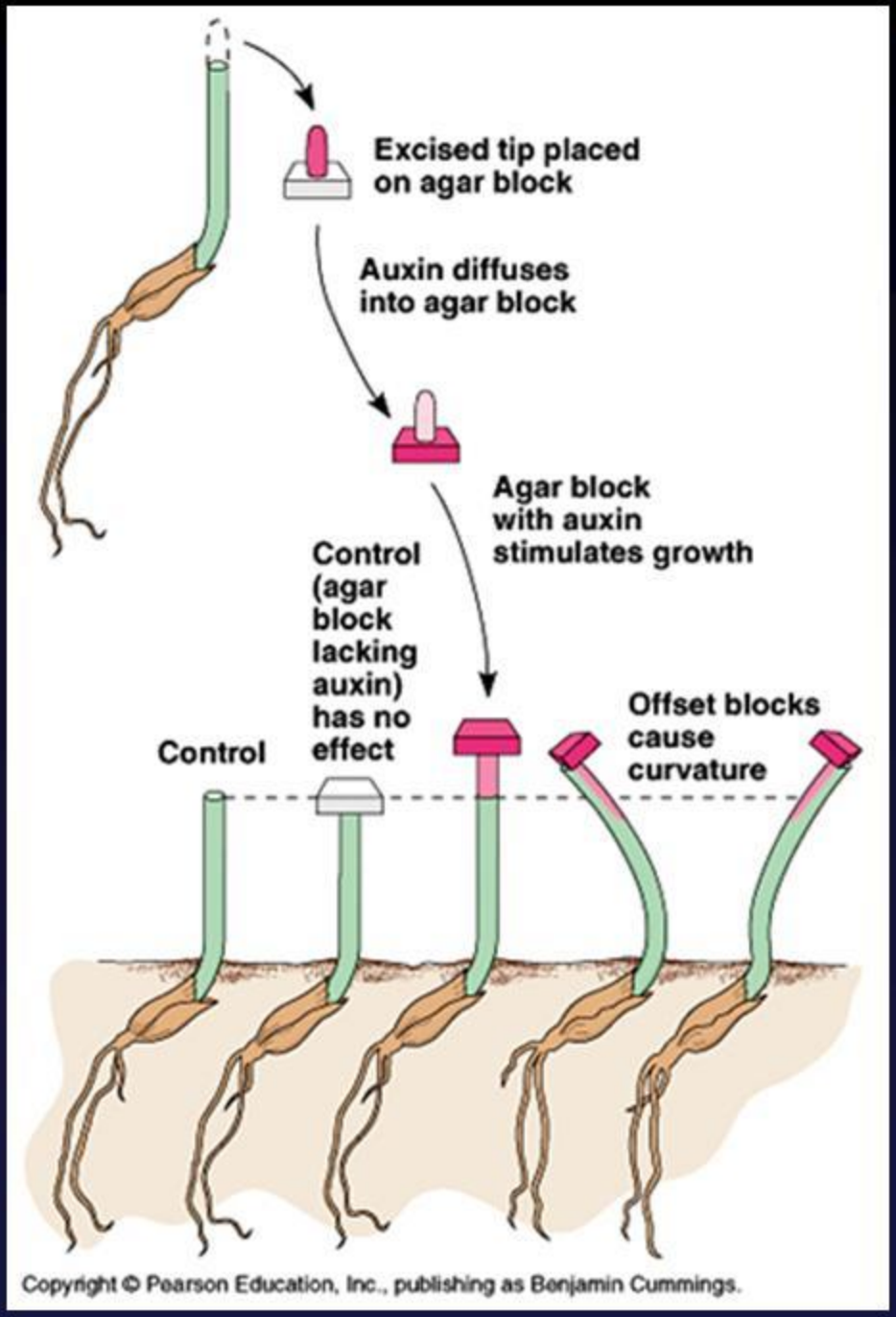
Phototropism



Gravitropism

Asymmetric Auxin Distribution Controls Directional Growth

- Tropisms

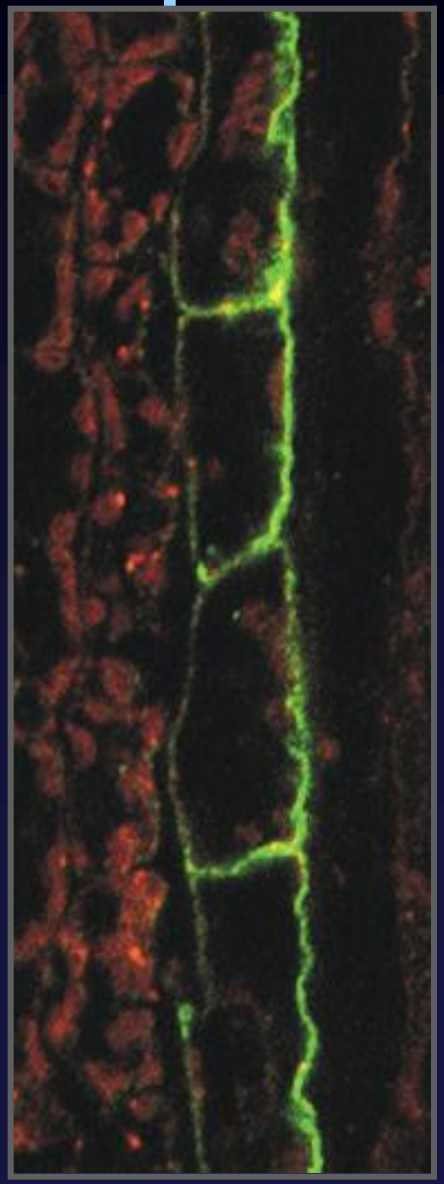


PIN3 – Lateral Auxin Transport

Auxin response

***pin3* mutant**

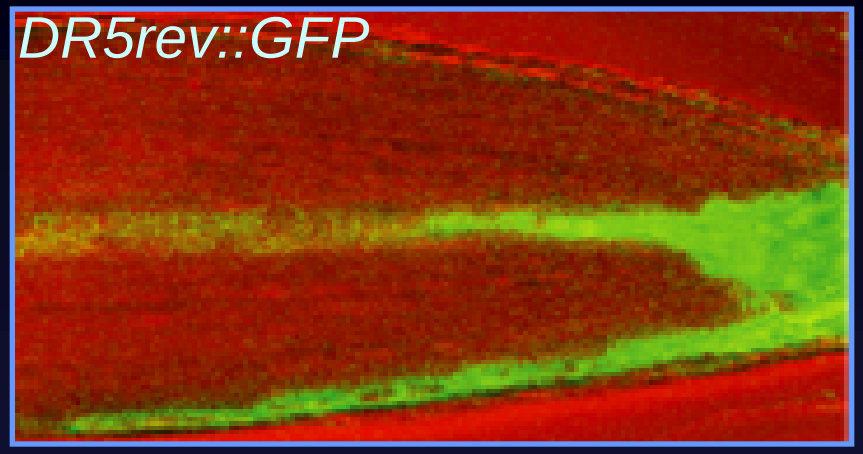
PIN3 protein



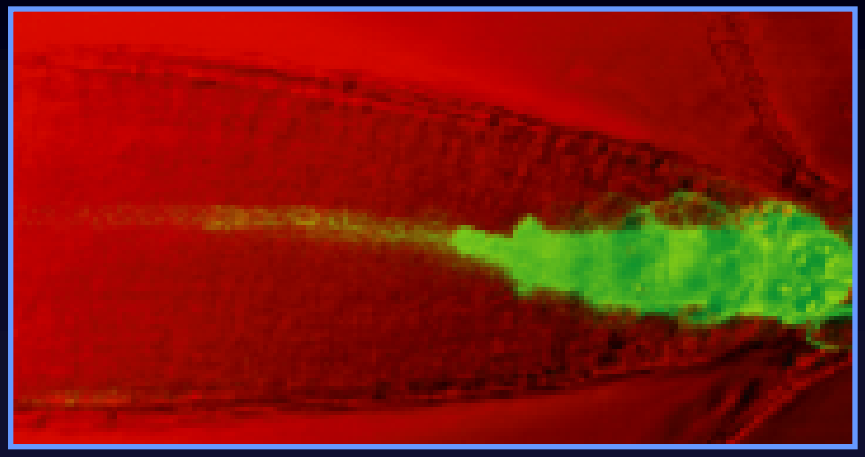
Friml et al. 2002

Root Gravitropism

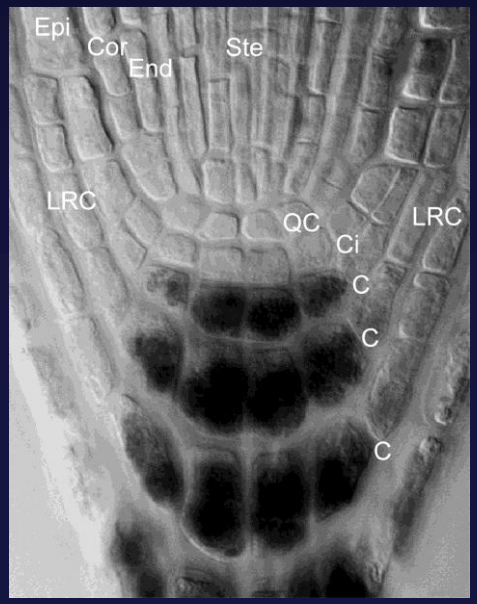
gravity stimulated



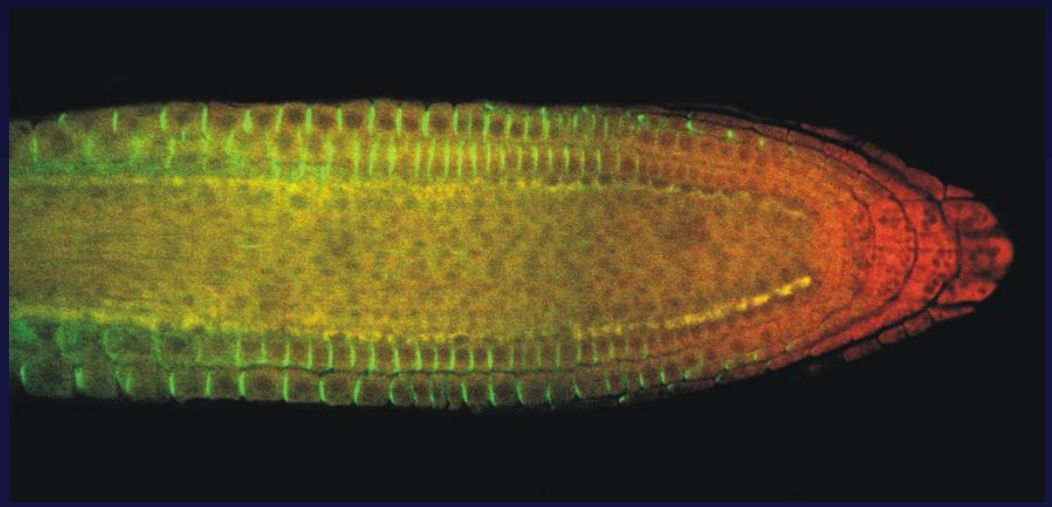
gravity + NPA



Statoliths
- gravity
perception

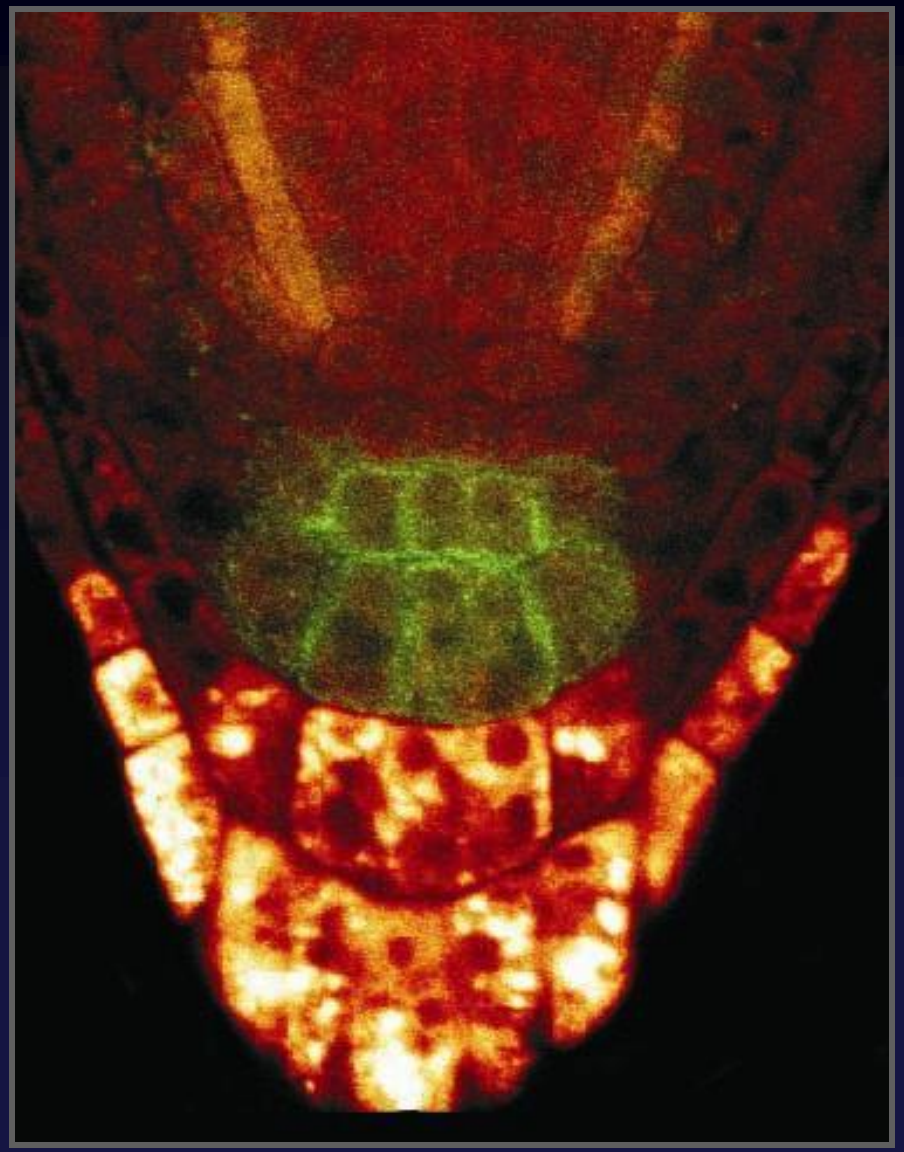


PIN2 localization

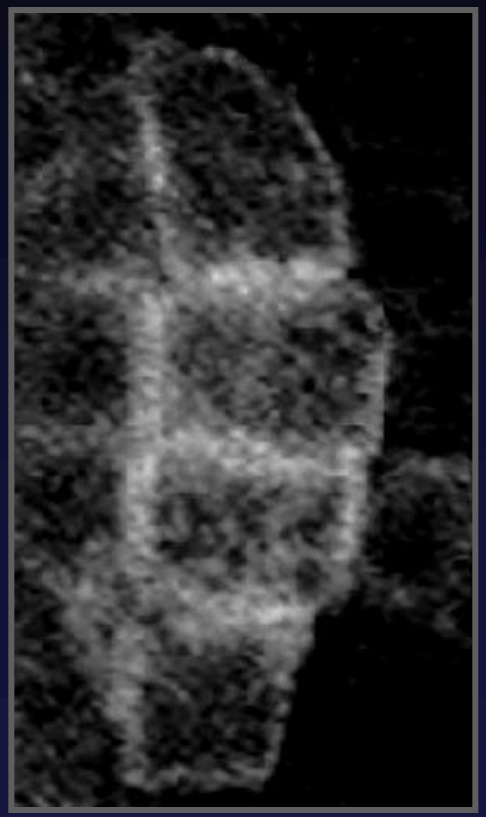


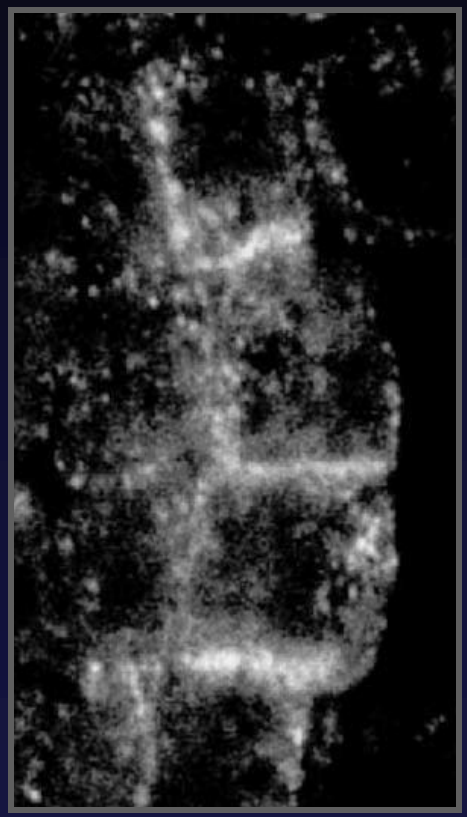
Relocation of PIN3 during Gravitropism

PIN3 in vertical root



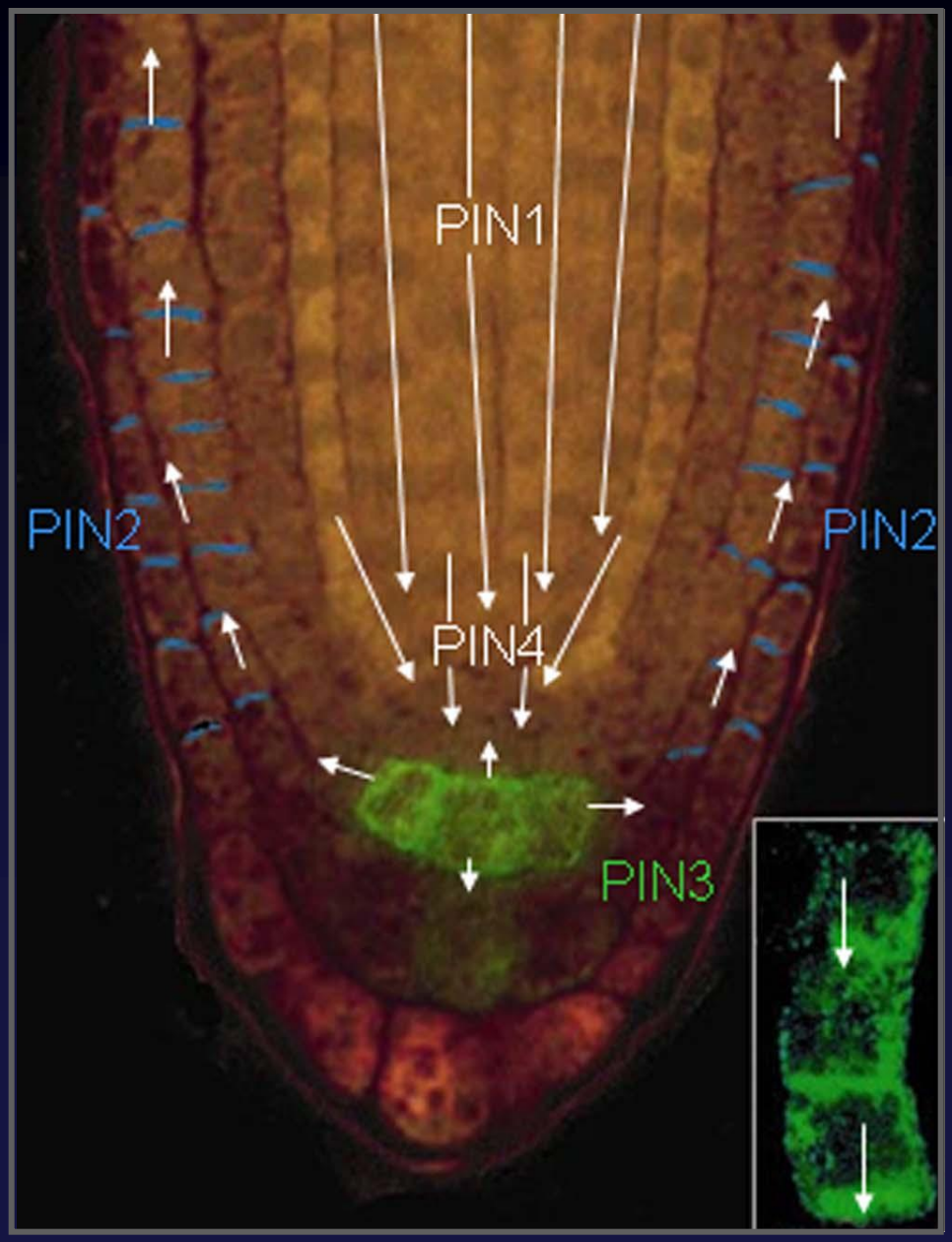
PIN3 in root on its side



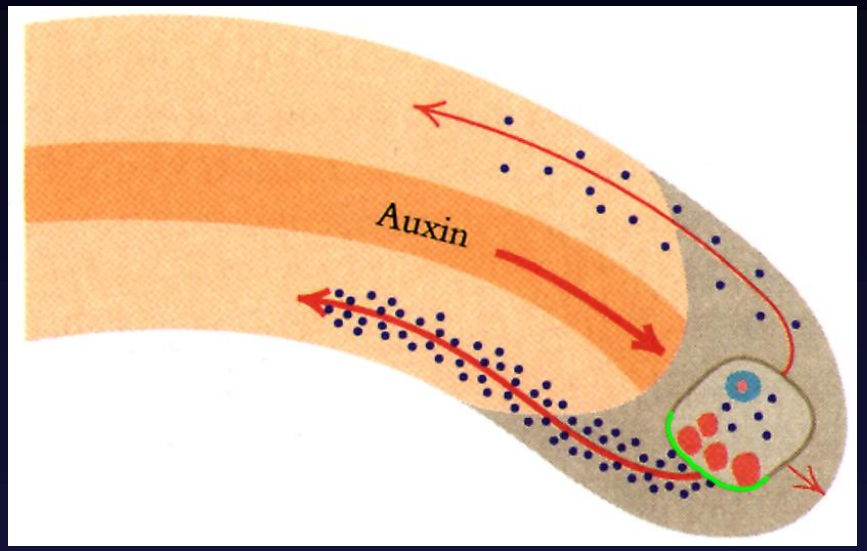


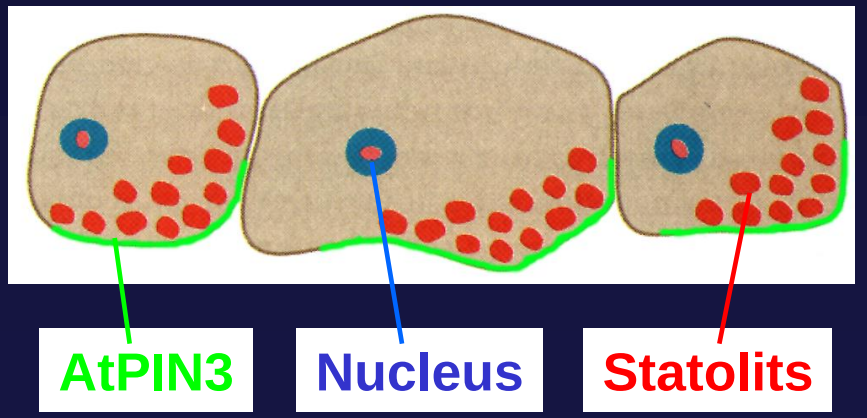
0 min **2 min**

PIN3 Polarity Switch in Gravitropic Response



Root turned on its side

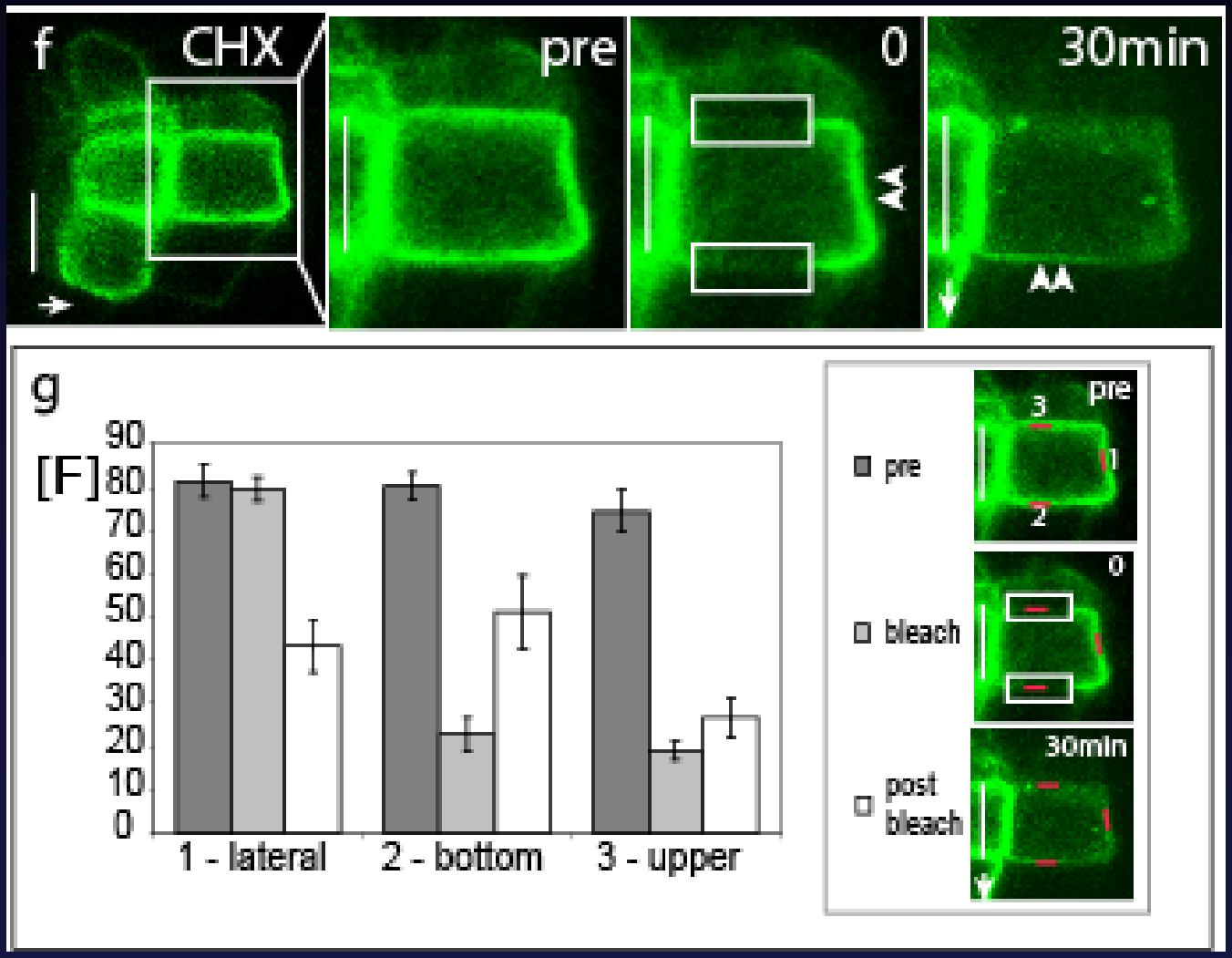




Friml et al. 02

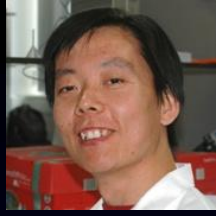
Gravity-induced PIN3 transcytosis

FRAP of PIN3-GFP



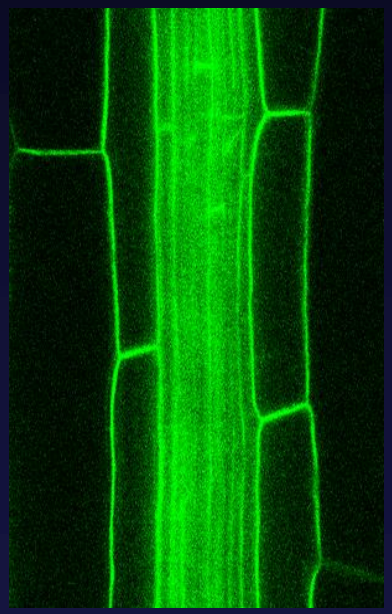
Kleine-Vehn et al., 2010

PIN3 in Phototropic Response



Auxin response

Light-dependent PIN3 relocation



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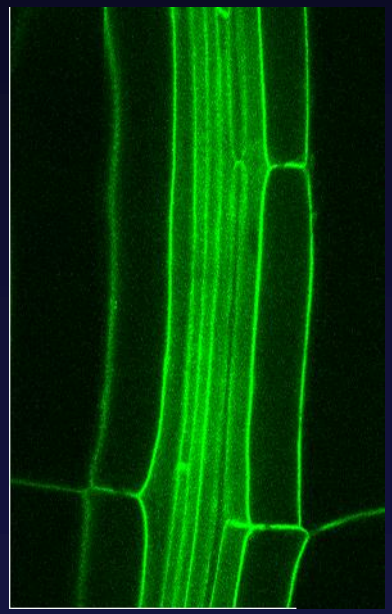
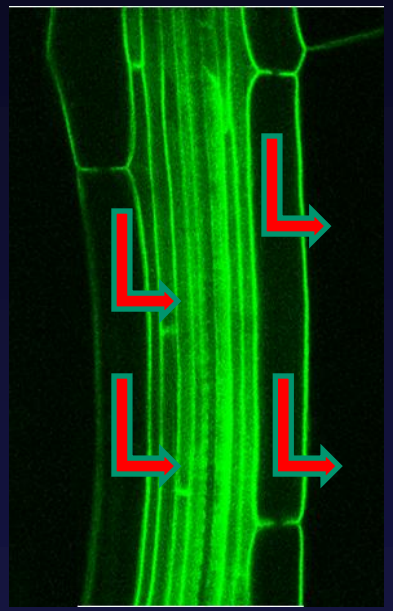


2 hours

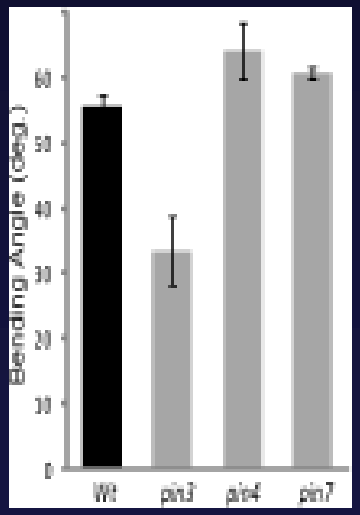


6 hours

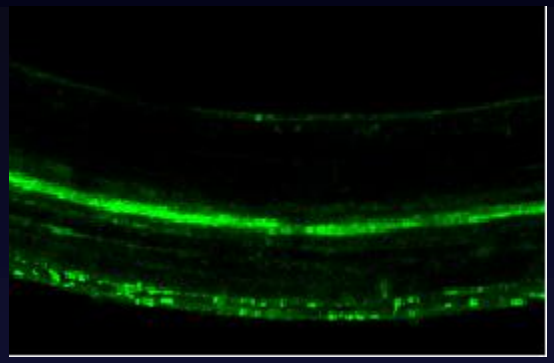
Ding et al., 2011

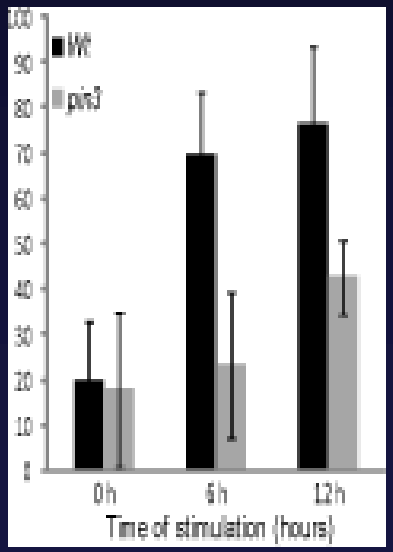
Shoot gravitropic response

Bending

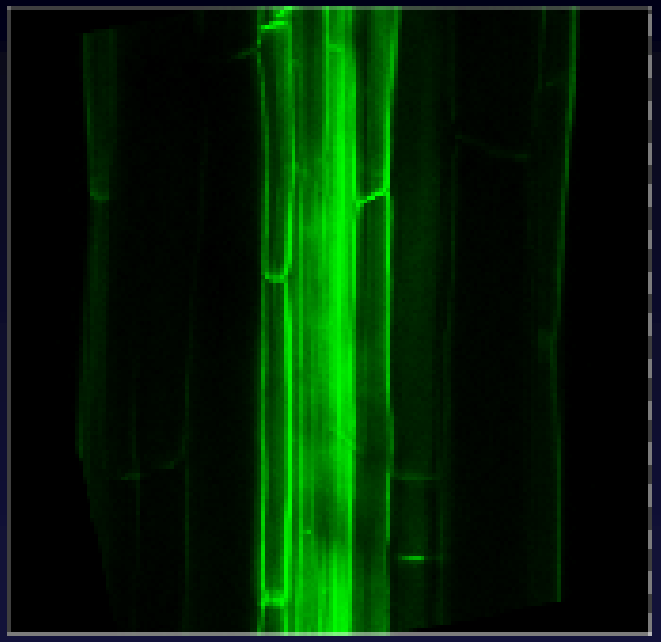


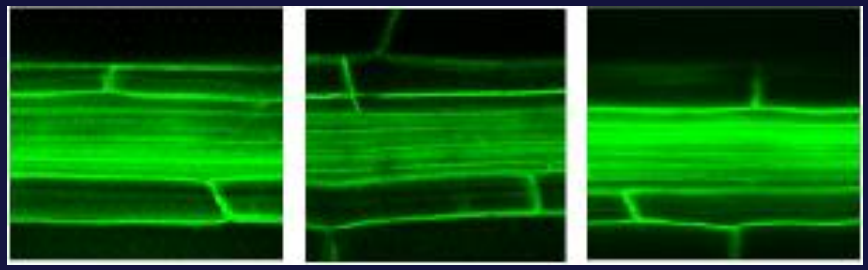
DR5 response





PIN3 polarization





unpublished

Cell-biological Determinants

Signal

s

Gravity

Friml et al. 2002
unpublished

Light

unpublished

Develop. context

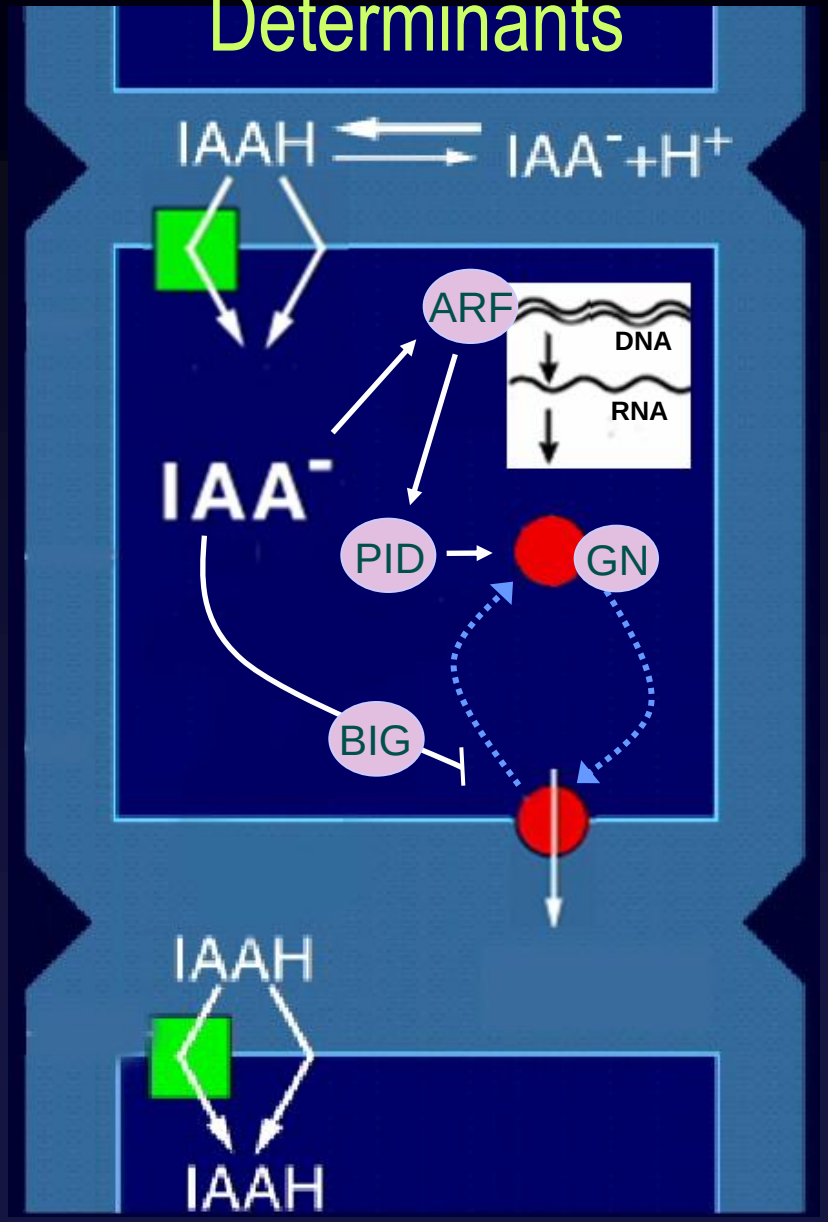
Benková et al. 2003
Friml et al. 2003
Reinhardt et al. 2003

Tissue context

Wisniewska et al., 2006

Auxin

Sauer et al., 2006
Paciorek et al., 2005
unpublished



Auxin Gradients