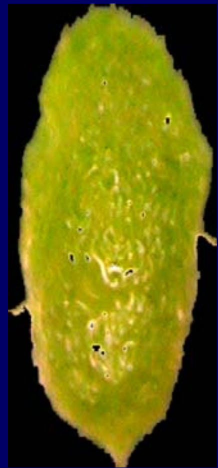
Cell Biology of Auxin Transport

gnom Phenotype is Mimicked by Alteration of Auxin Homeostasis

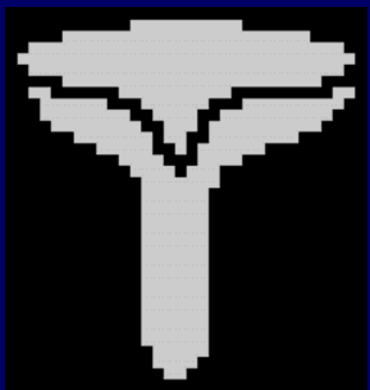


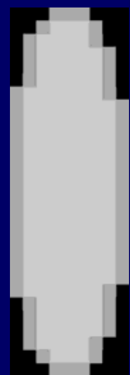
split collar cotyledon

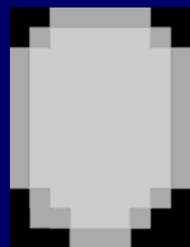
collar cotyledon

cucumber

ball/egg







data from Hadfi et al., 1998

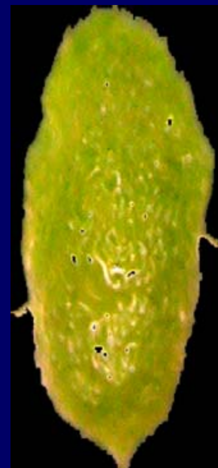
gnom embryos display variable defects in the apical-basal axis of polarity

Wild-type and *gnom* Arabidopsis seedling

split collar cotyledon

collar cotyledon

ball/egg



cucumber

GNOM Encodes a Homolog of Yeast ARF G-protein Exchange Factors

GNOM rescues gea2Δ, gea1-19 temperature sensitive yeast strains

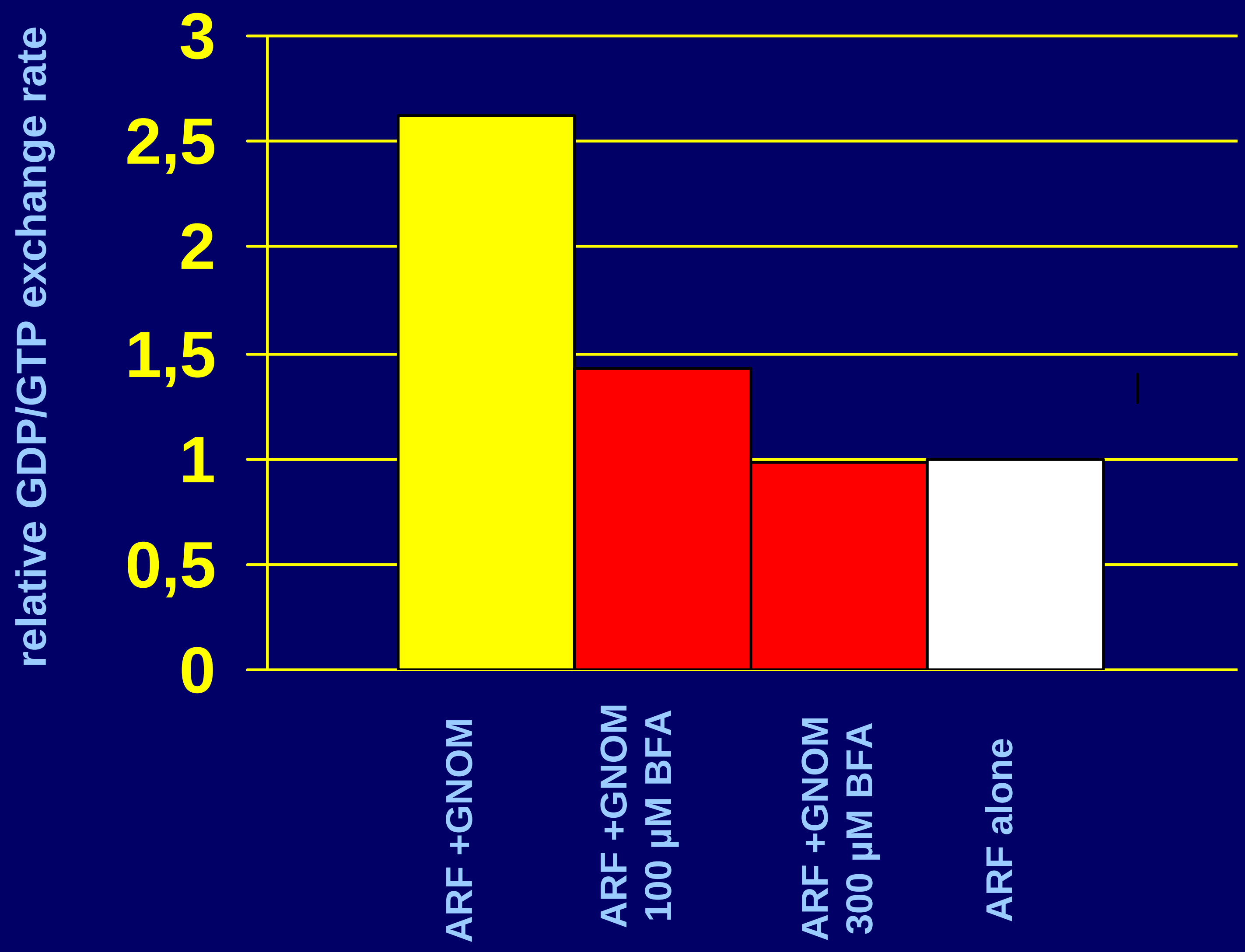
25°C

GNOM Vector

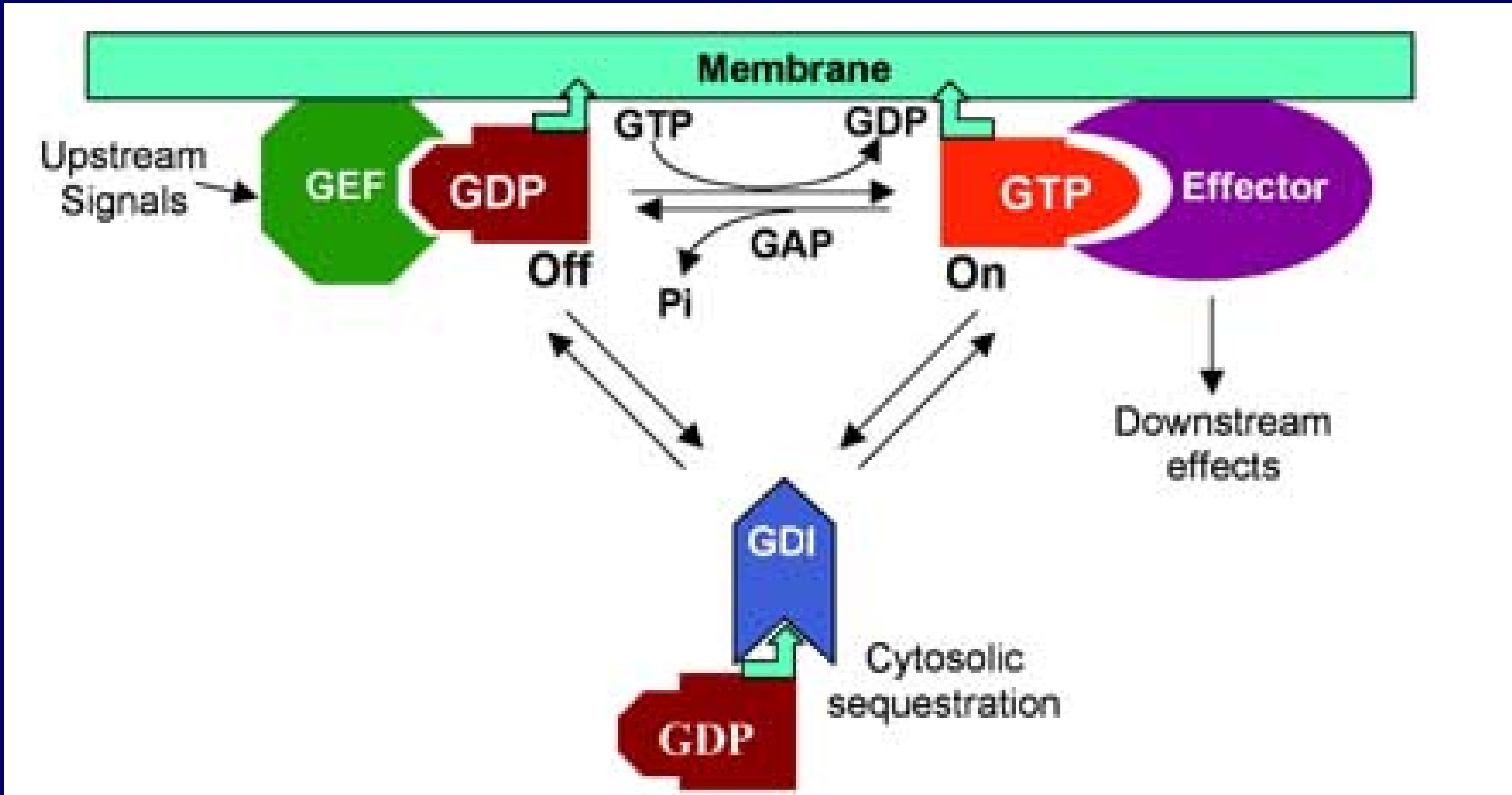
32°C

GNOM Vector

GNOM Has BFA Sensitive GDP/GTP Exchange Activity on ARF



Small GTPases – Universal Switchers



Secretory pathway

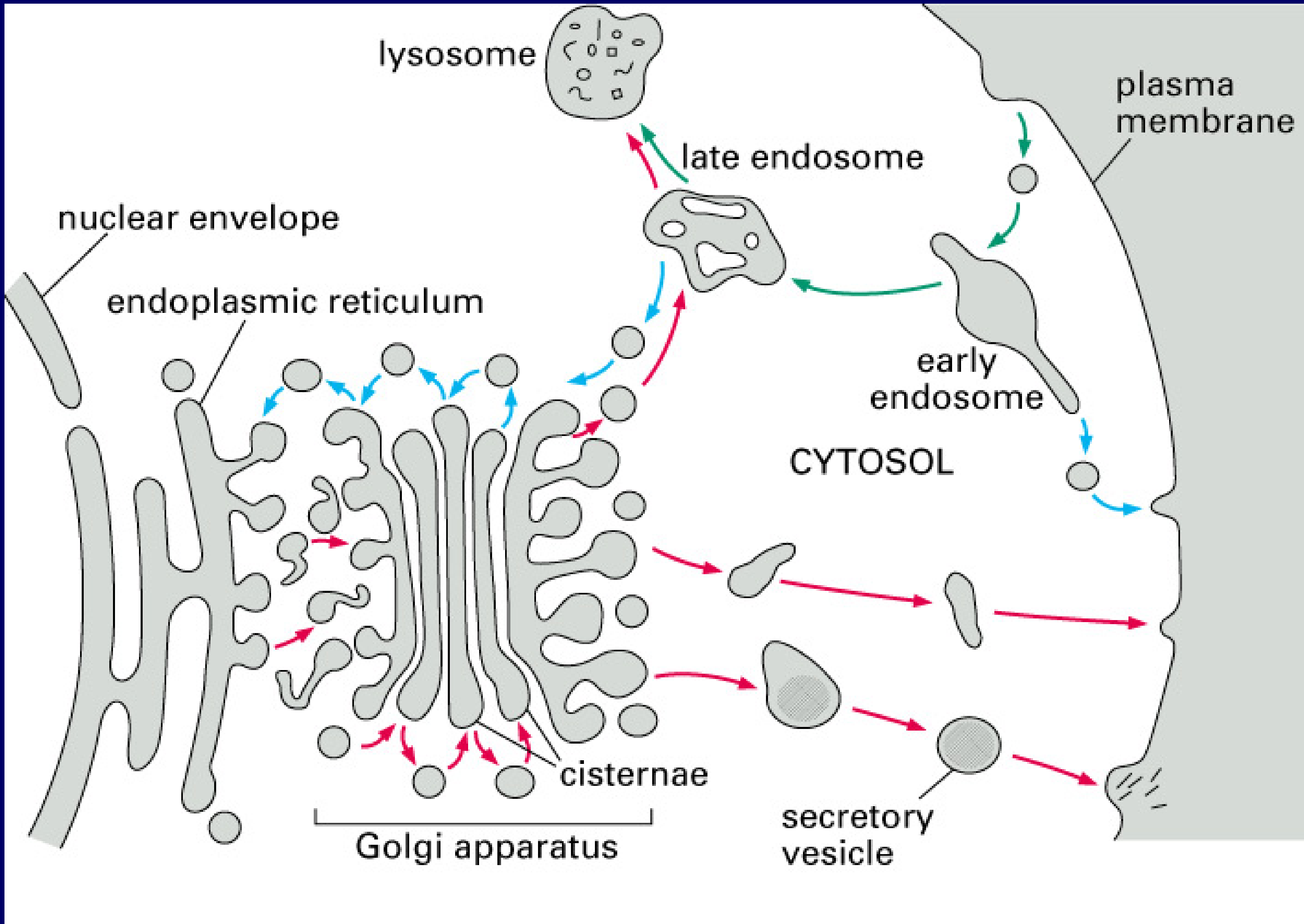
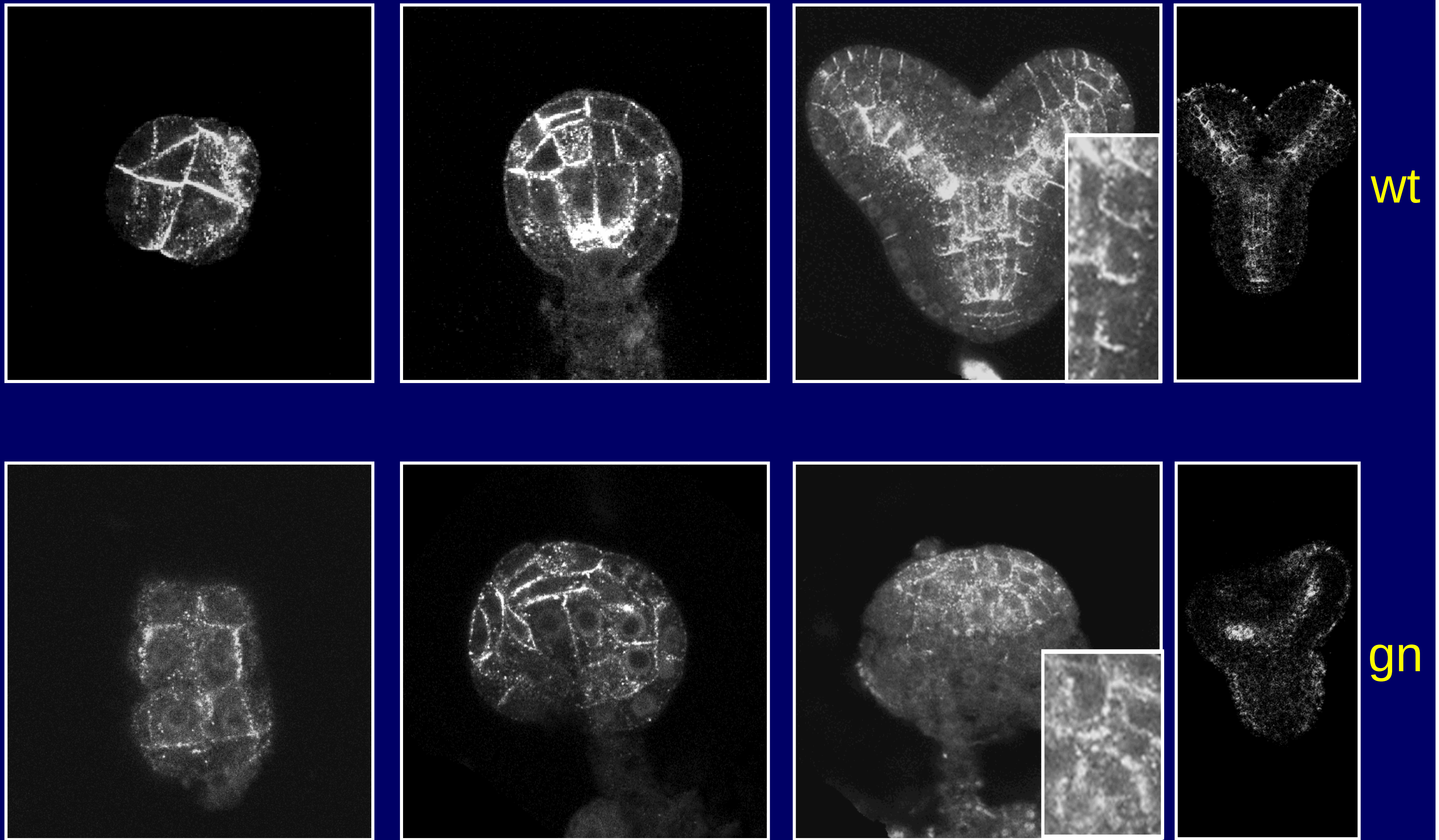


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Establishment of coordinated PIN1 polarity is disrupted in gnom embryos



PIN Proteins Cycling

BFA reveals subcellular movement of PIN1

PIN1 cycles between endosome and plasma membrane

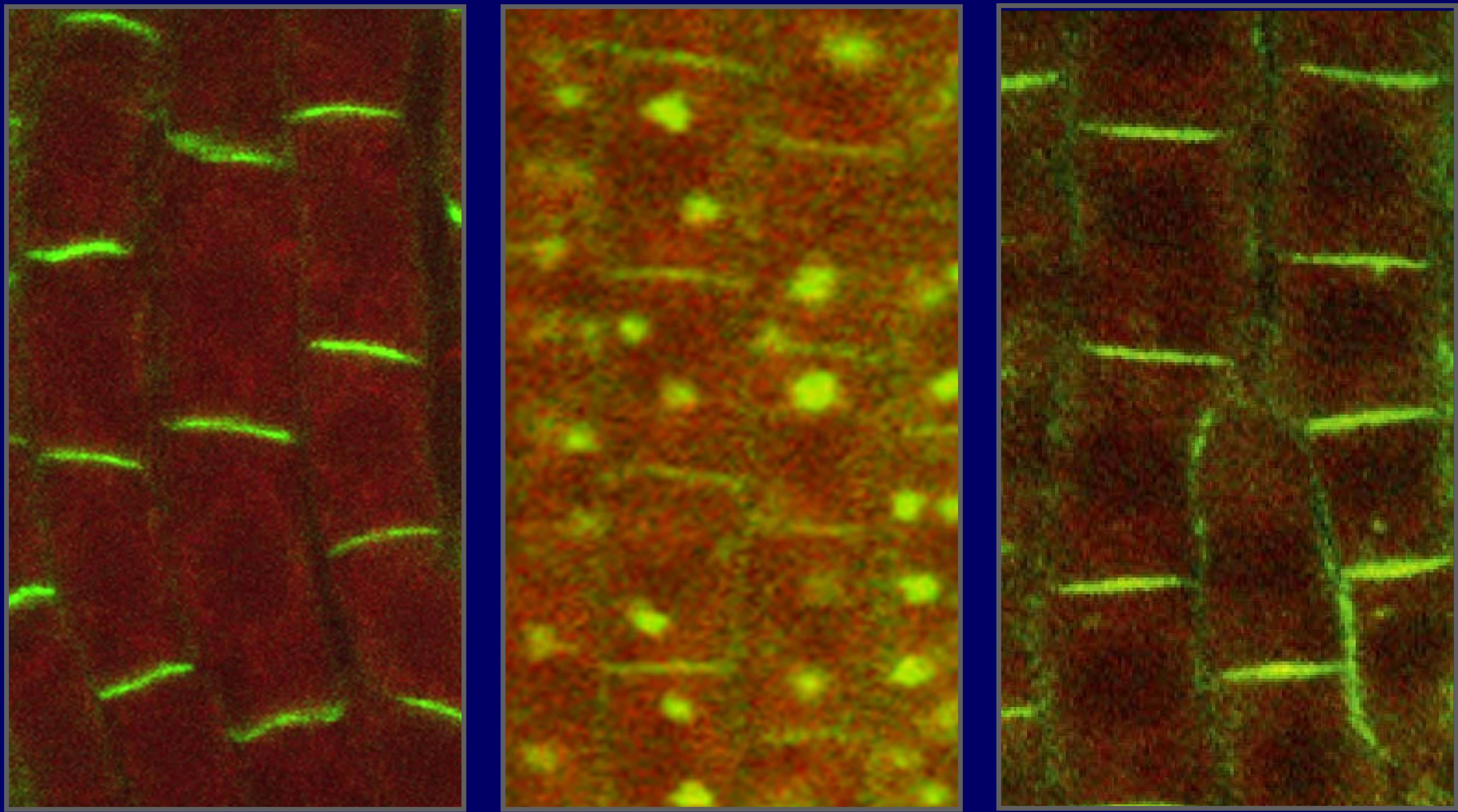
PIN1 cycling is actin but not tubulin dependent

Auxin transport inhibitors interfere with protein trafficking

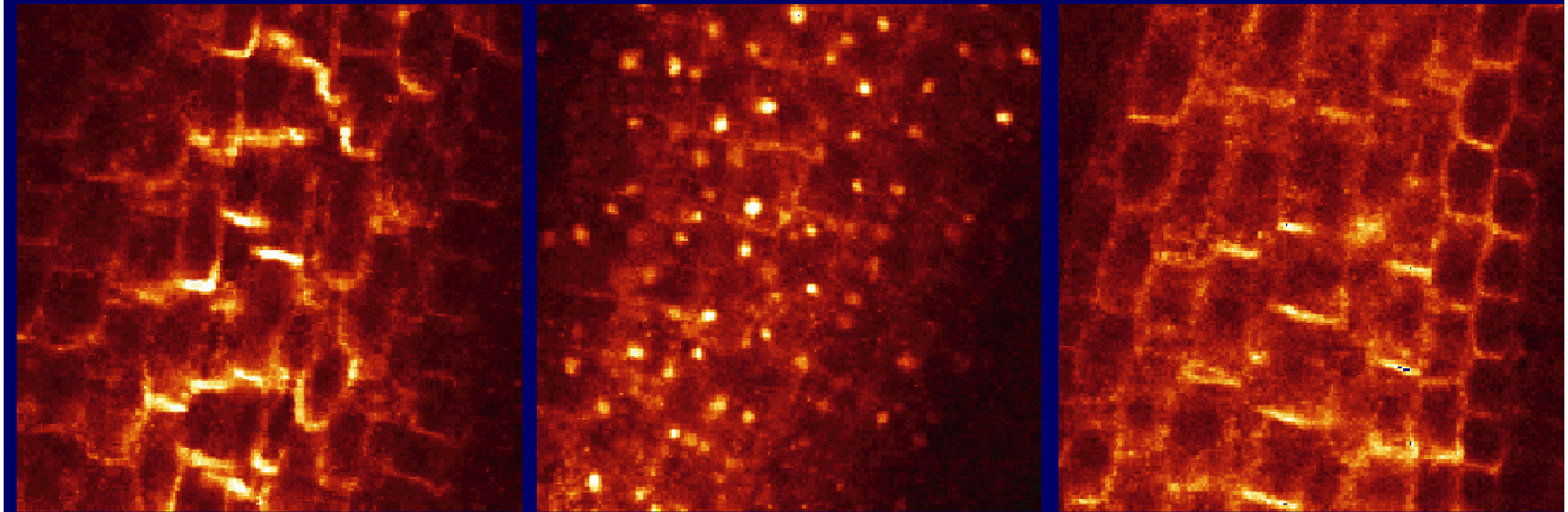
Role for cycling – root gravitropism

PIN1 Subcellular Movement

untreated | ***+ BFA*** | ***- BFA***

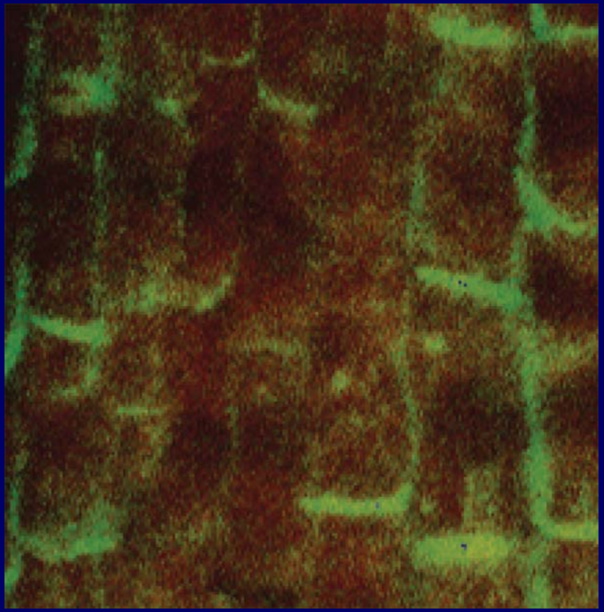


Intracellular Cycling of AtPIN1 is Independent of Newly Synthesised Protein

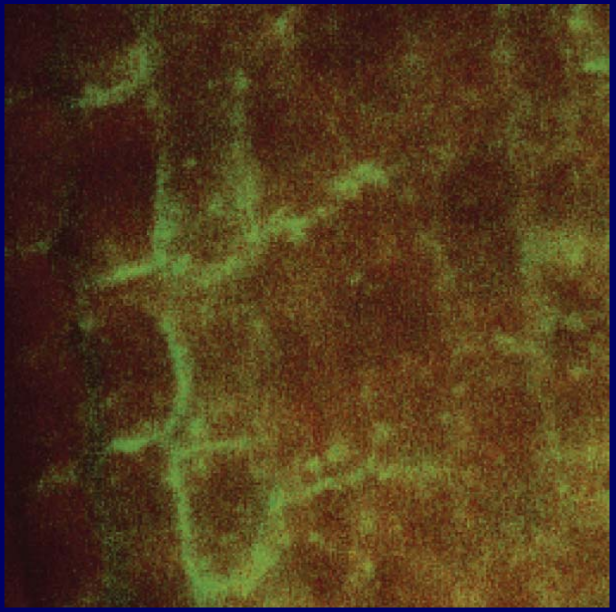


Cycloheximide   Cycloheximide + BFA   BFA + Cycloheximide

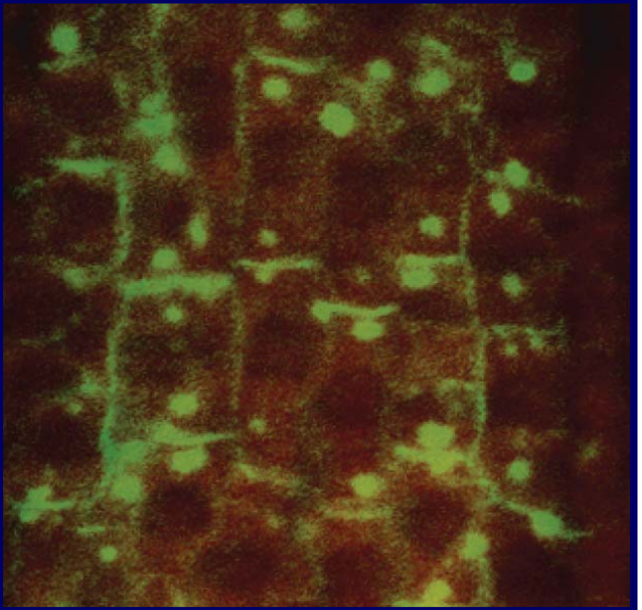
PIN1 Cycling is Actin Dependent



Cytochelasin D



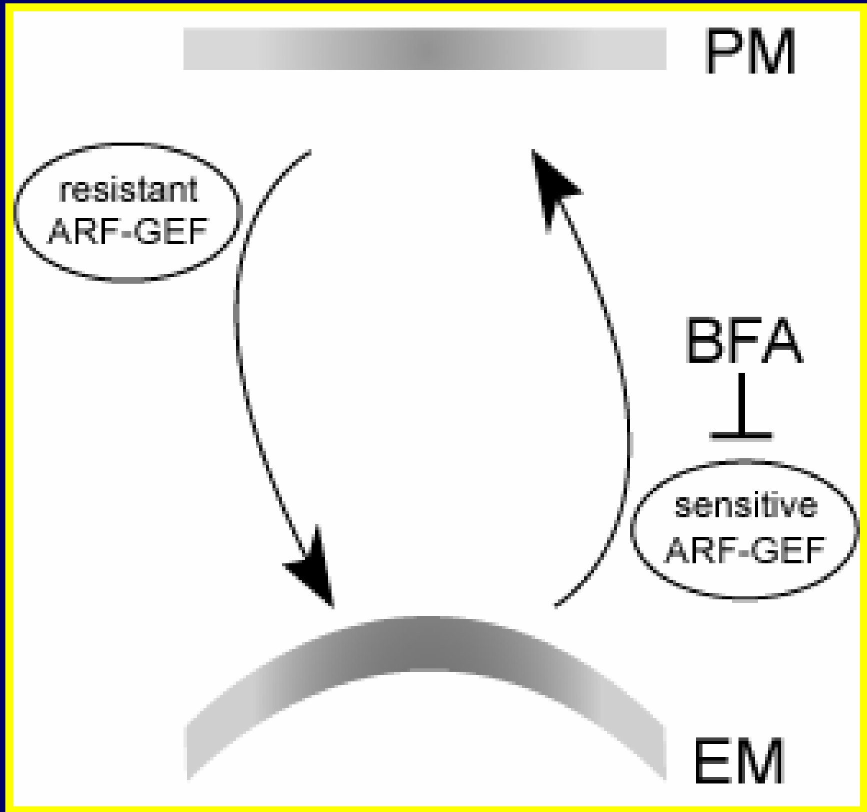
Cytochelasin D + BFA



BFA + Cytochelasin D

Hypothesis:

GNOM regulates intracellular transport of auxin efflux carriers, such as PIN1.



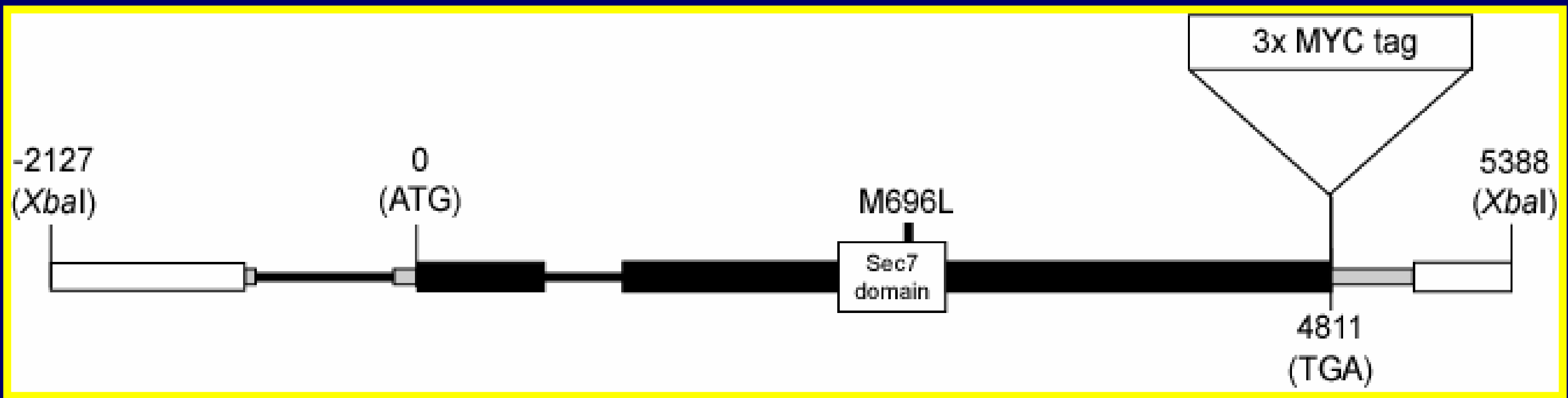
How can we show this?

Engineering BFA-resistant GNOM

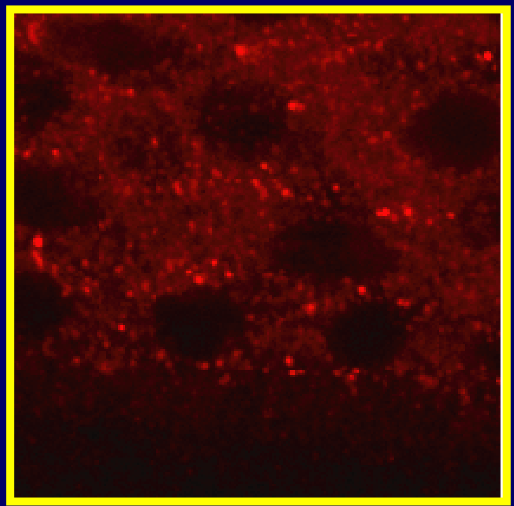
Amino Acid Residues of ARF-GEFs Conferring BFA Resistance

H. sapiens						
ARNO	VLS	FA	VI	M	LNTSLH	BFA resistant
CYTOHESIN	VLS	FA	II	M	LNTSLH	BFA resistant
GBF1	SLA	YA	VI	M	LNTDQH	BFA resistant
S. cerevisiae						
Gea1p	VLS	YS	II	M	LNTSSH	BFA sensitive
Gea1p * YS to FA	VLS	FA	II	M	LNTSSH	BFA resistant
Gea1p * M to L	VLS	YS	II	L	LNTSSH	BFA resistant
Arabidopsis						
At5g19610	VLS	YS	II	M	LNTSSH	BFA sensitive?
At5g39500	VLA	YS	II	L	LNTDQH	BFA resistant?
GNOM	ILC	YS	LI	M	LNTDQH	BFA sensitive
GNOM * YS to FA	VLS	FA	II	M	LNTSSH	BFA resistant?
GNOM * M to L	VLS	YS	II	L	LNTSSH	BFA resistant?

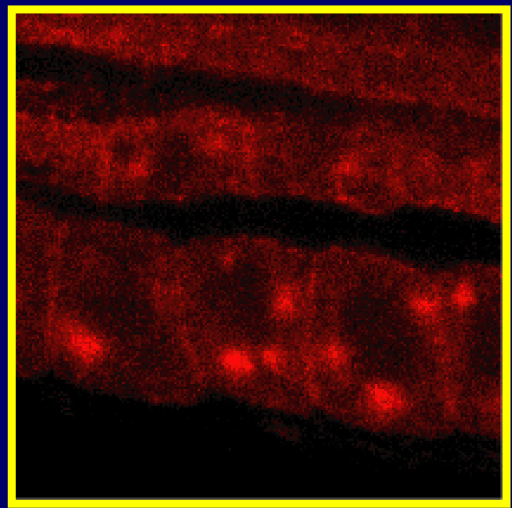
The engineered GNOM construct



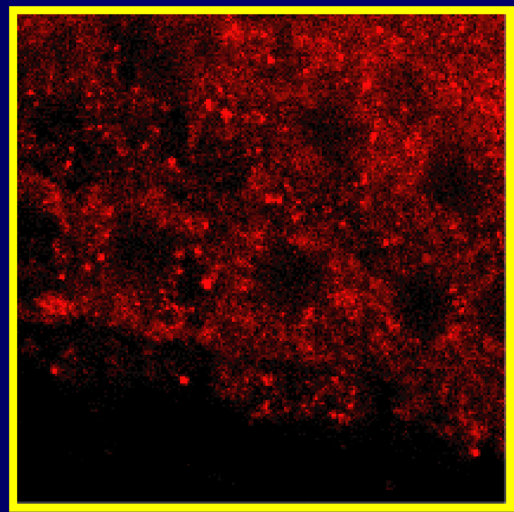
GNOM localises to the cytosol and some endomembrane compartment



control

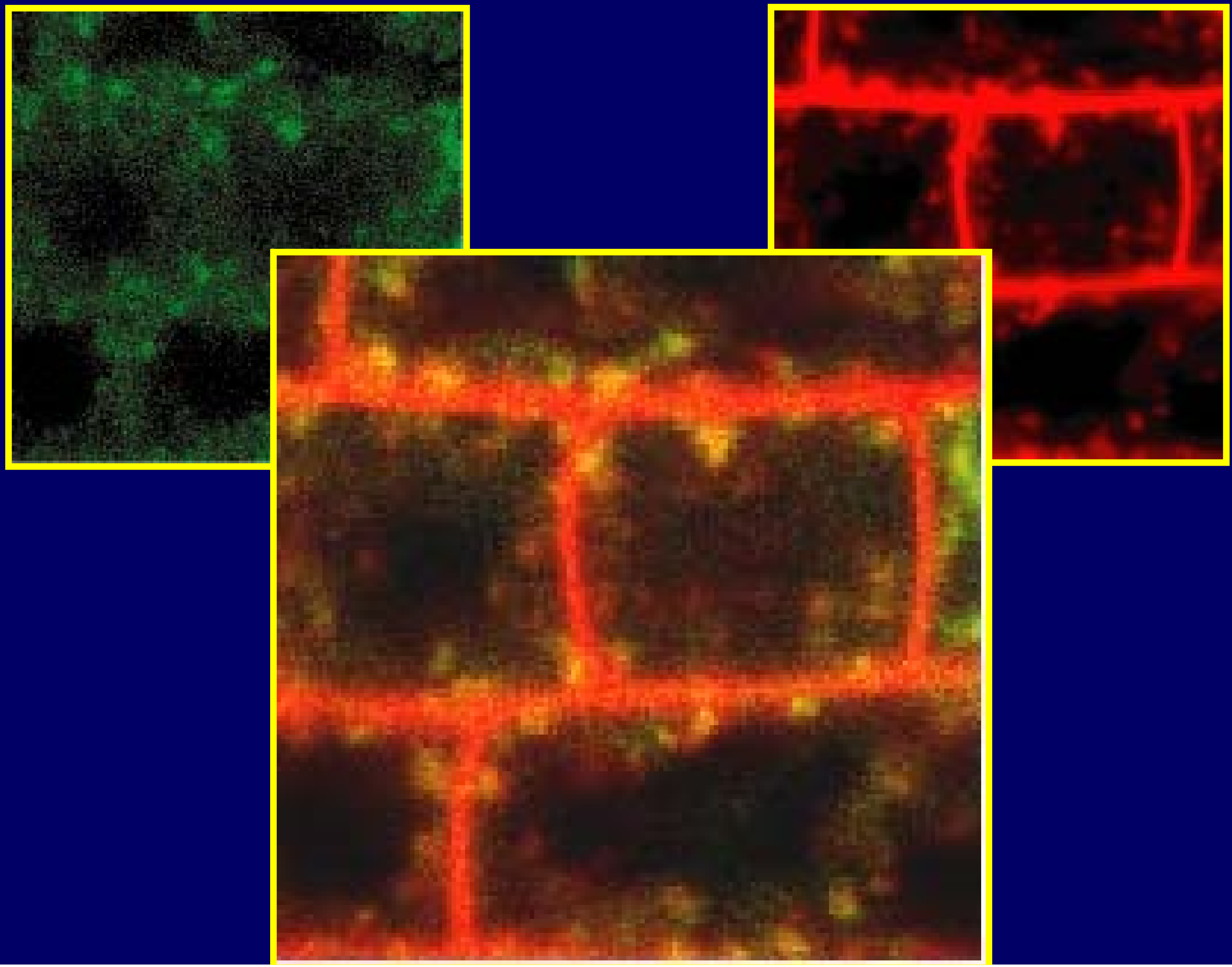


BFA, 60 min
GN^{wt}-*myc* line

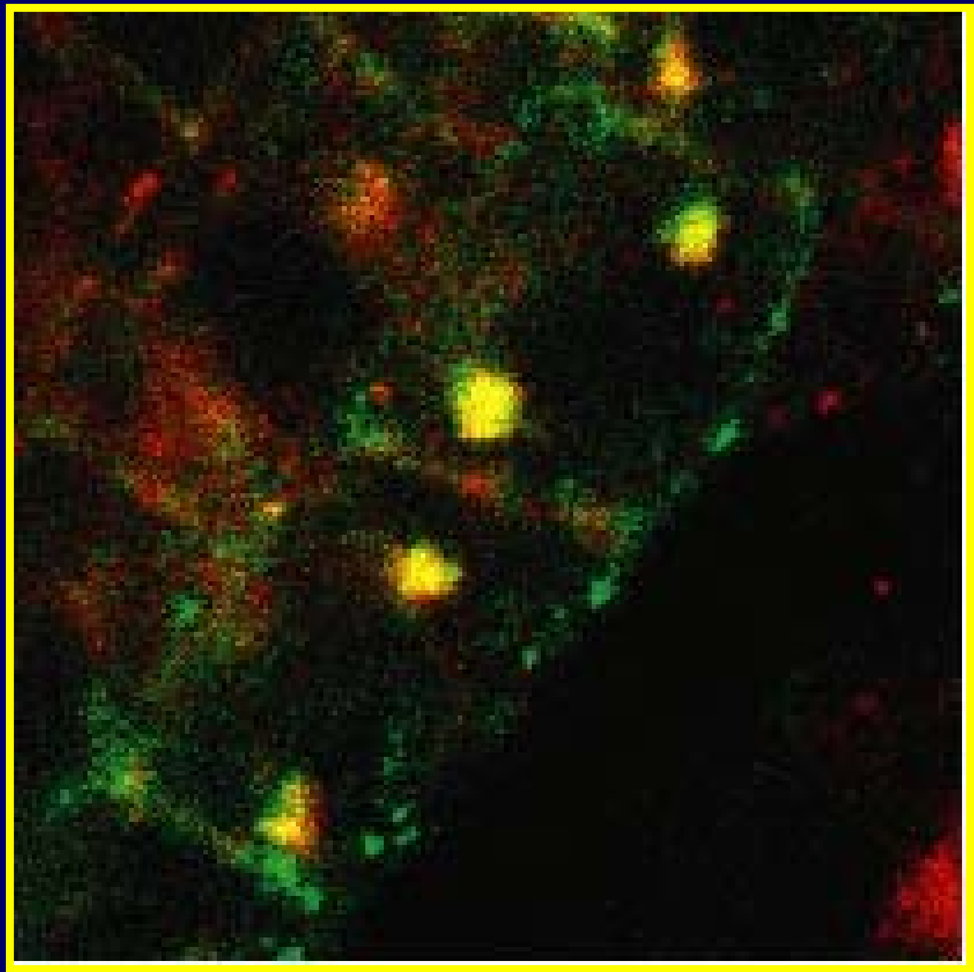


BFA, 60 min
GN^{M696L}-*myc* line

GNOM partially co-localises with FM4-64, an endocytic tracer

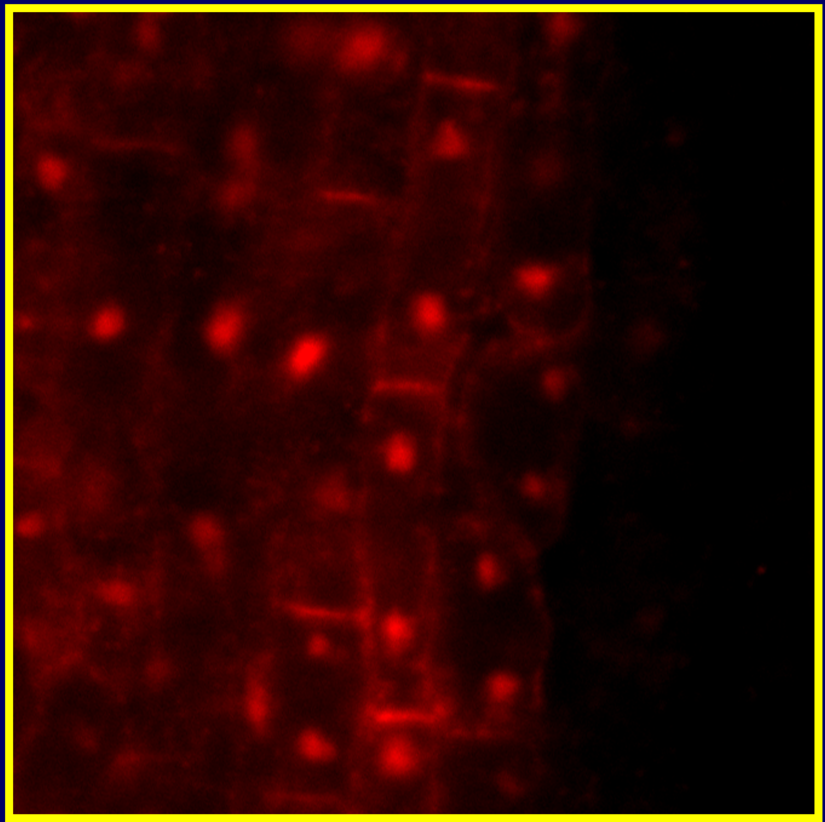


GNOM and PIN1 co-localise after BFA treatment



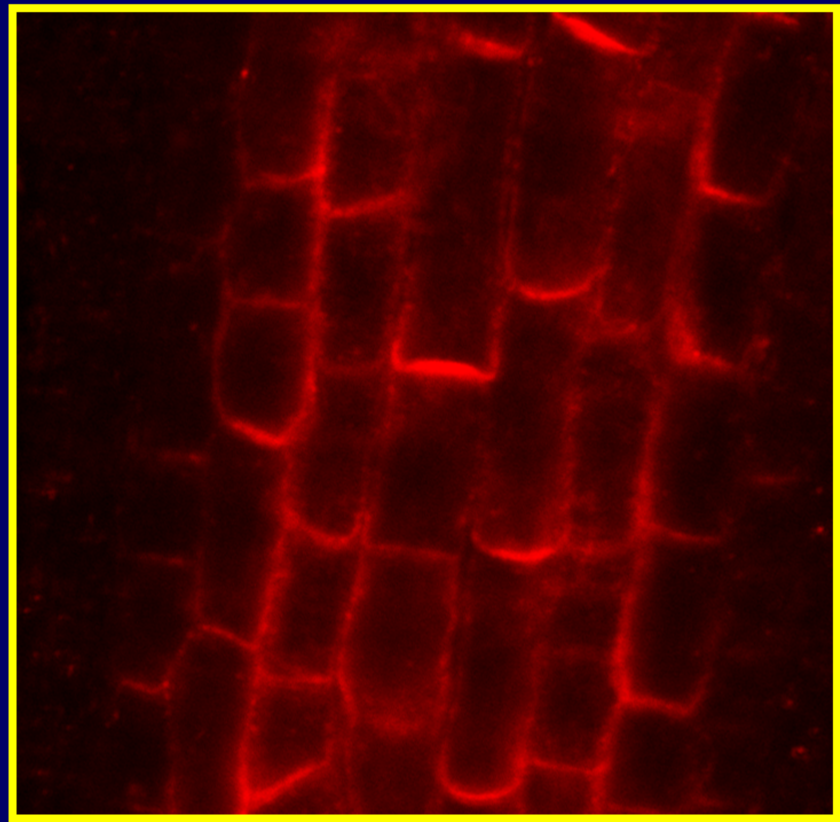
PIN1 Localisation in BFA-resistant GNOM

sensitive (control)



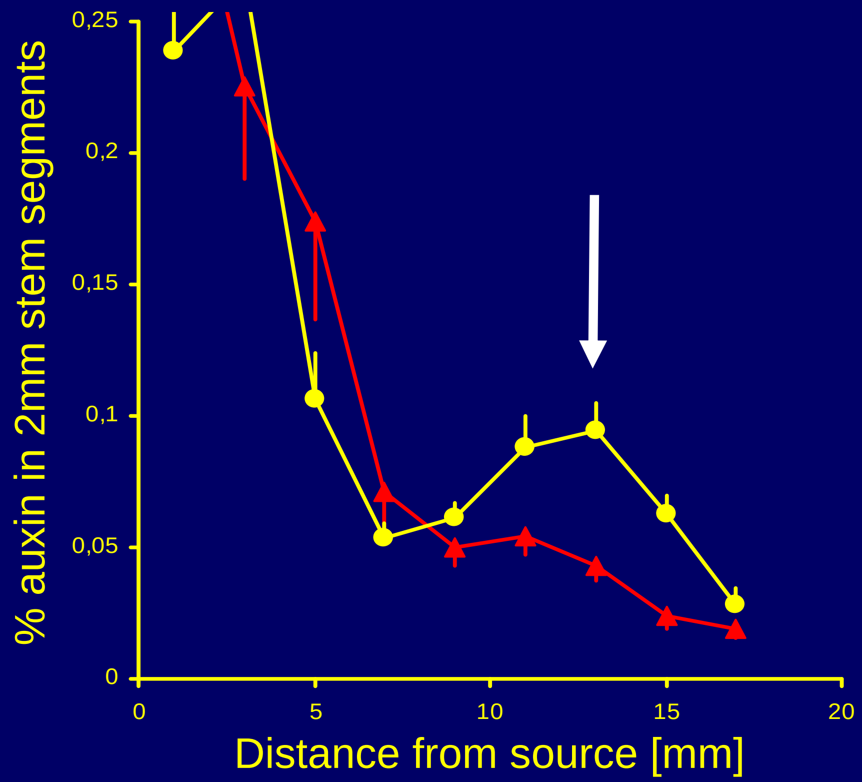
BFA 50 µM, 45 min
Col background

resistant

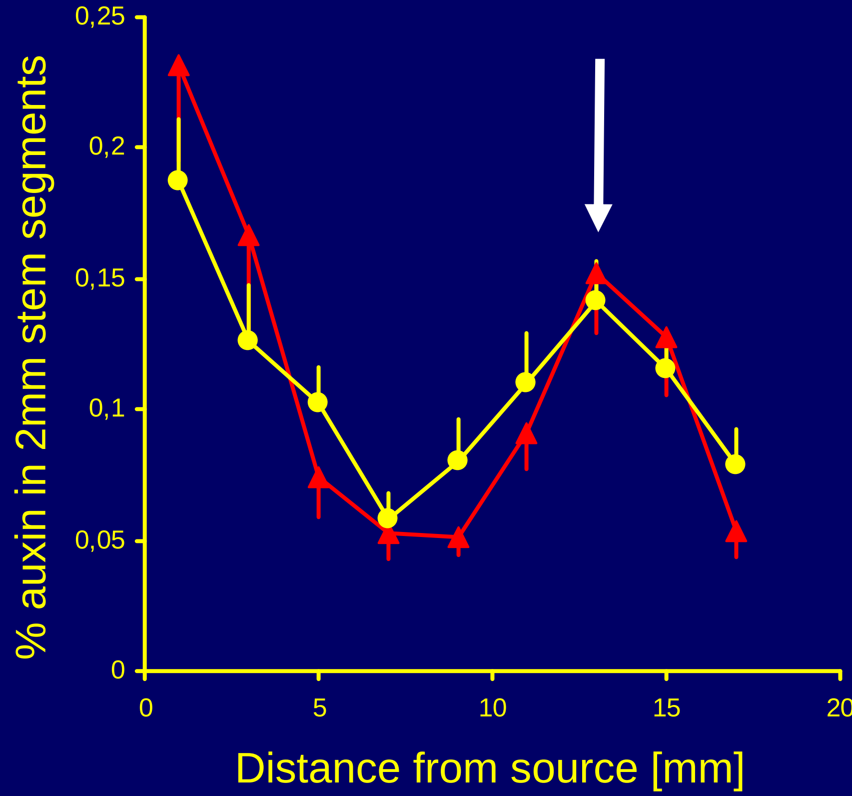


BFA 50 µM, 45 min
GNOM$^{BFA\ res.}$ transgenic line

GNOM BFA-resistant lines display BFA insensitive auxin flux

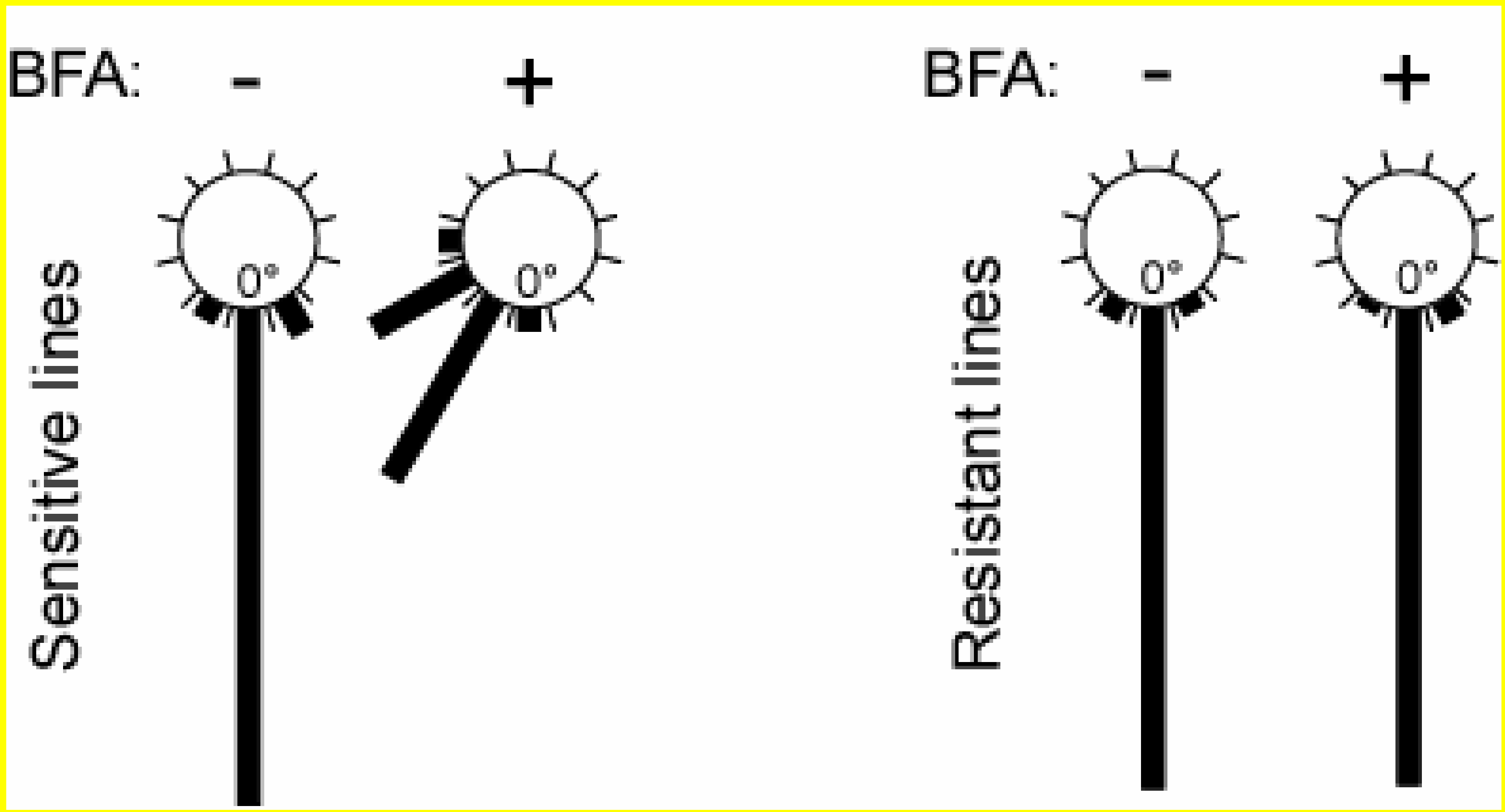


GN[wt]-myc line

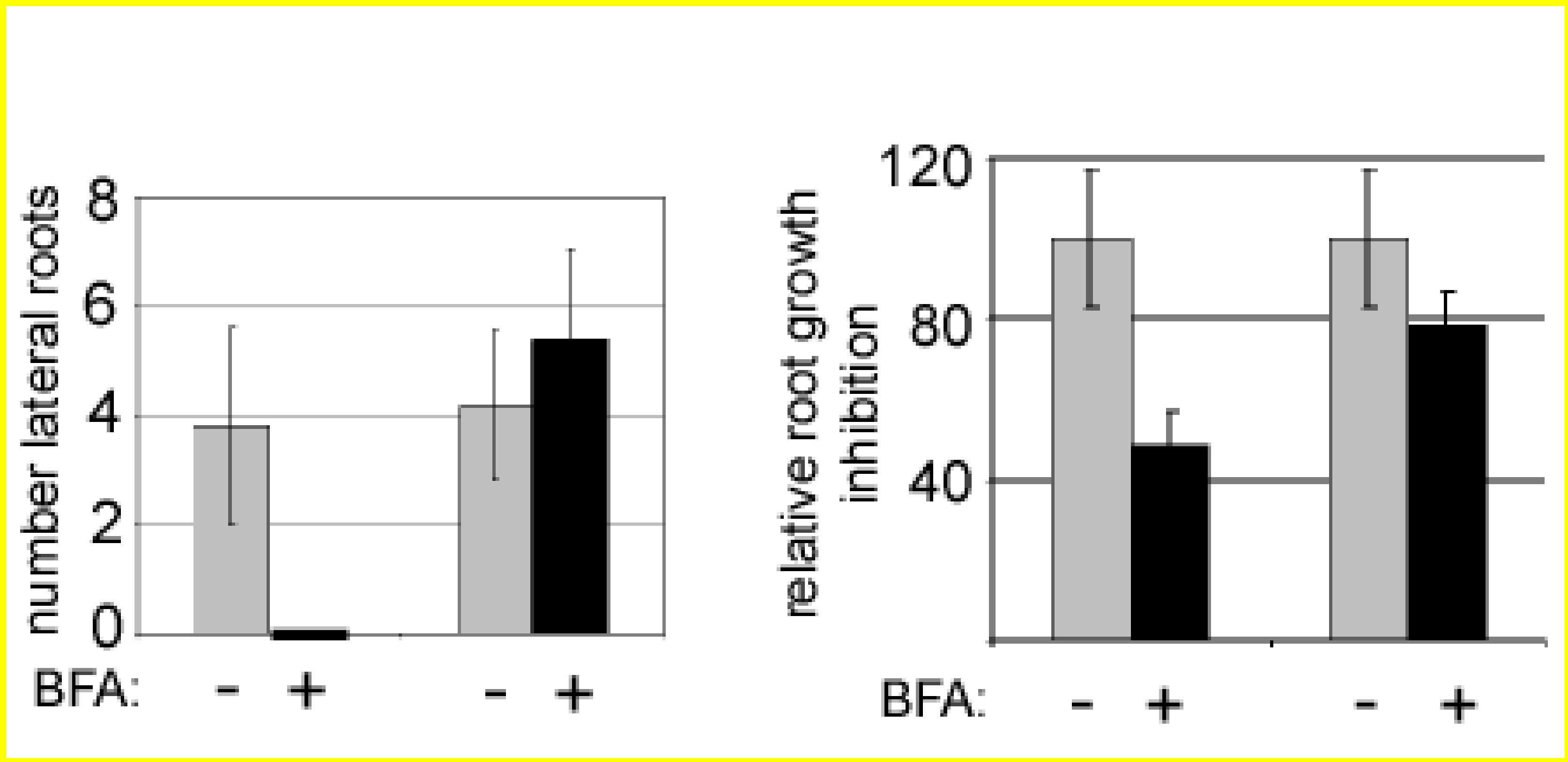


GN[M696L]-myc line

The root gravitropic response becomes BFA-resistant in *GNOM* BFA-resistant lines



Side root formation and primary root elongation also become BFA-resistant in *GNOM* BFA-resistant lines



Sensitive line *Resistant line* *Sensitive line* *Resistant line*

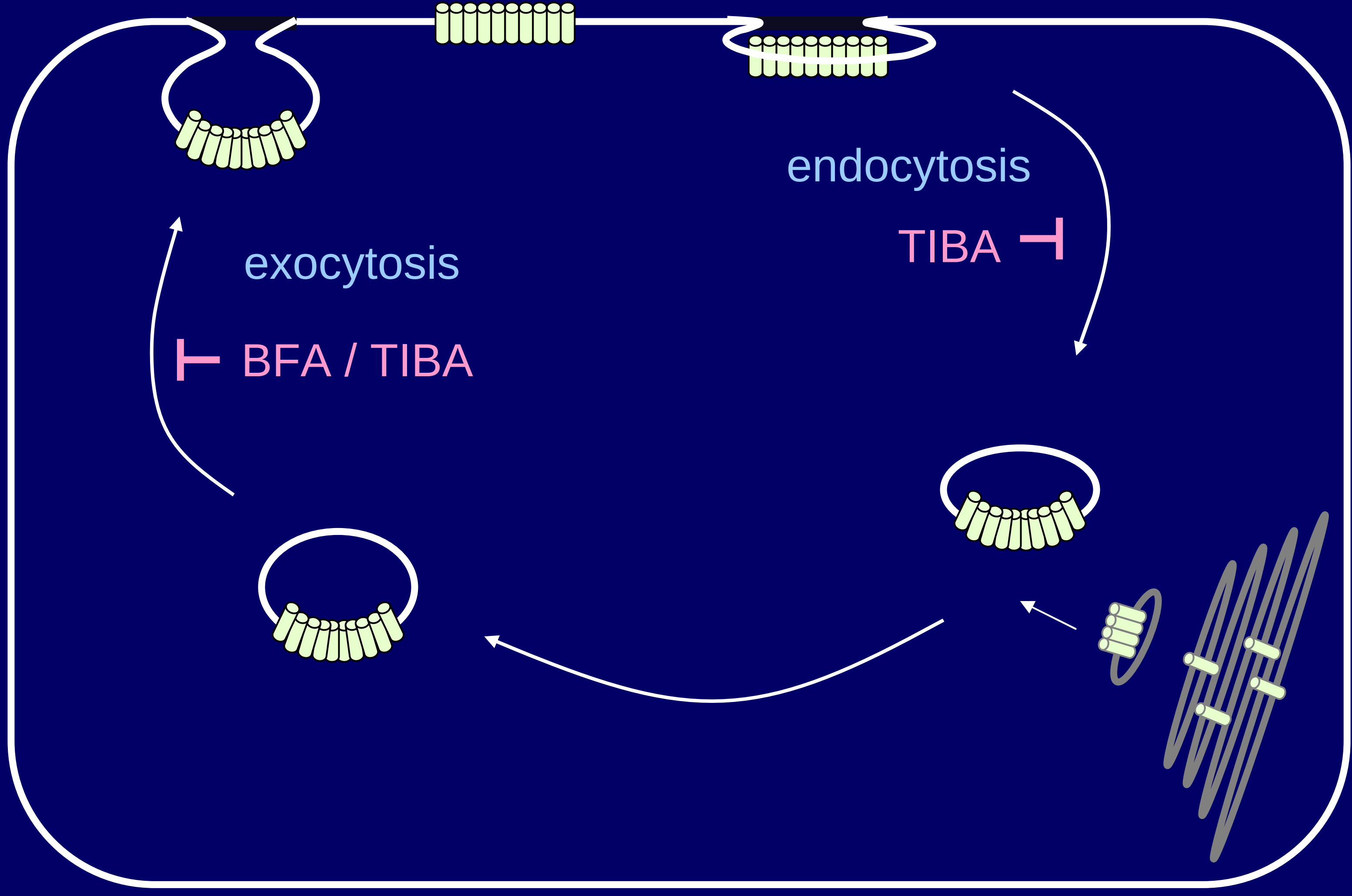
GNOM BFA-resistant lines lead to strong phenotypic BFA-resistance

BFA 5 µM

GN^{wt}*-myc* line

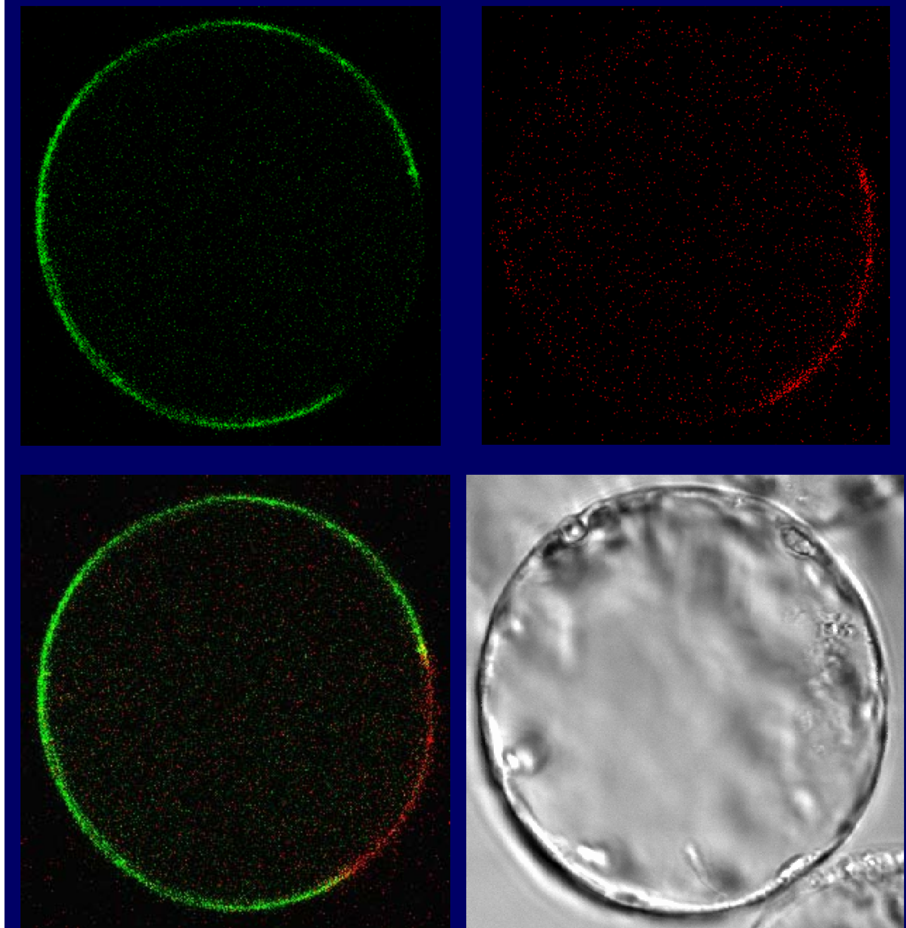
GNOM^{M696L}*-myc* line

Dynamic Movement of PIN1

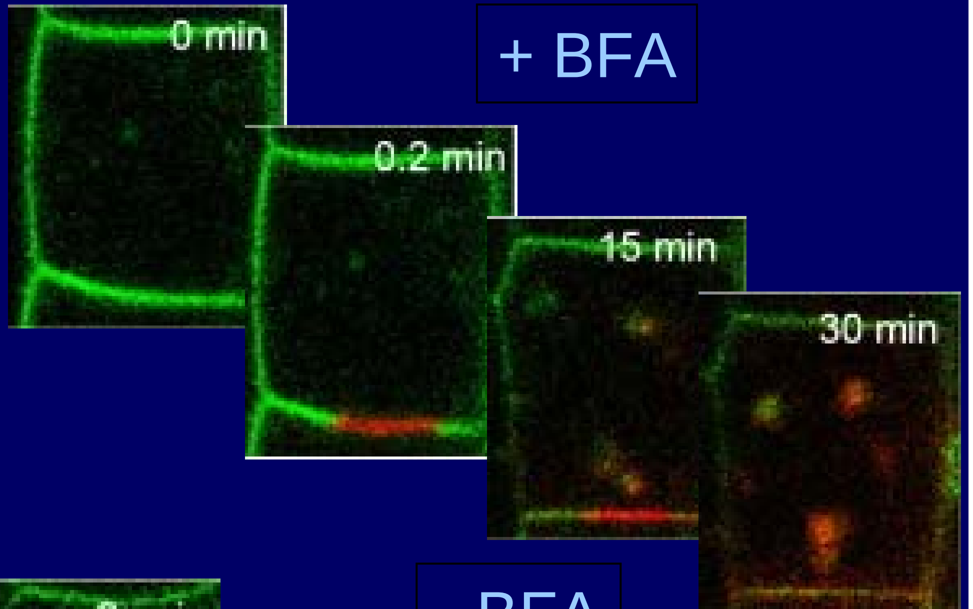


UV-activated PIN2-EosFP Reveals Constitutive PIN Endocytosis

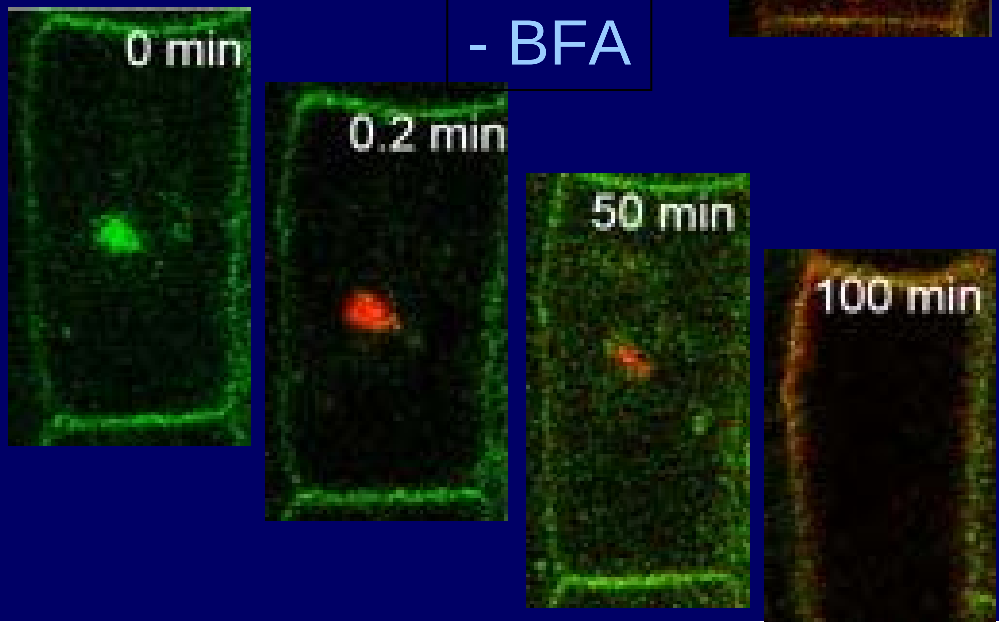
Protoplasts



+ BFA

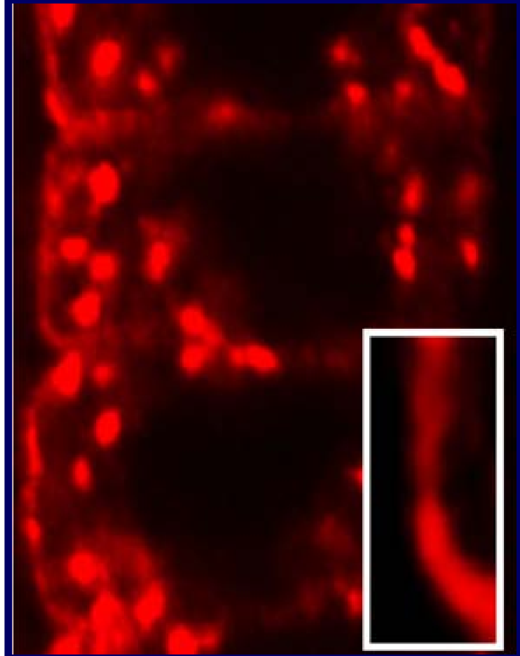


- BFA



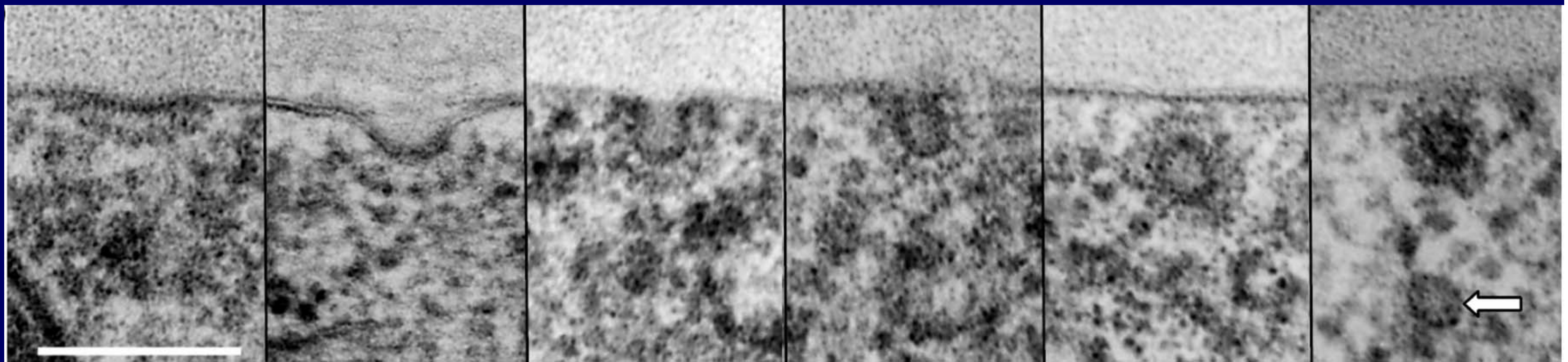
Dhonukshe et al., 2007

anti-Clathrin



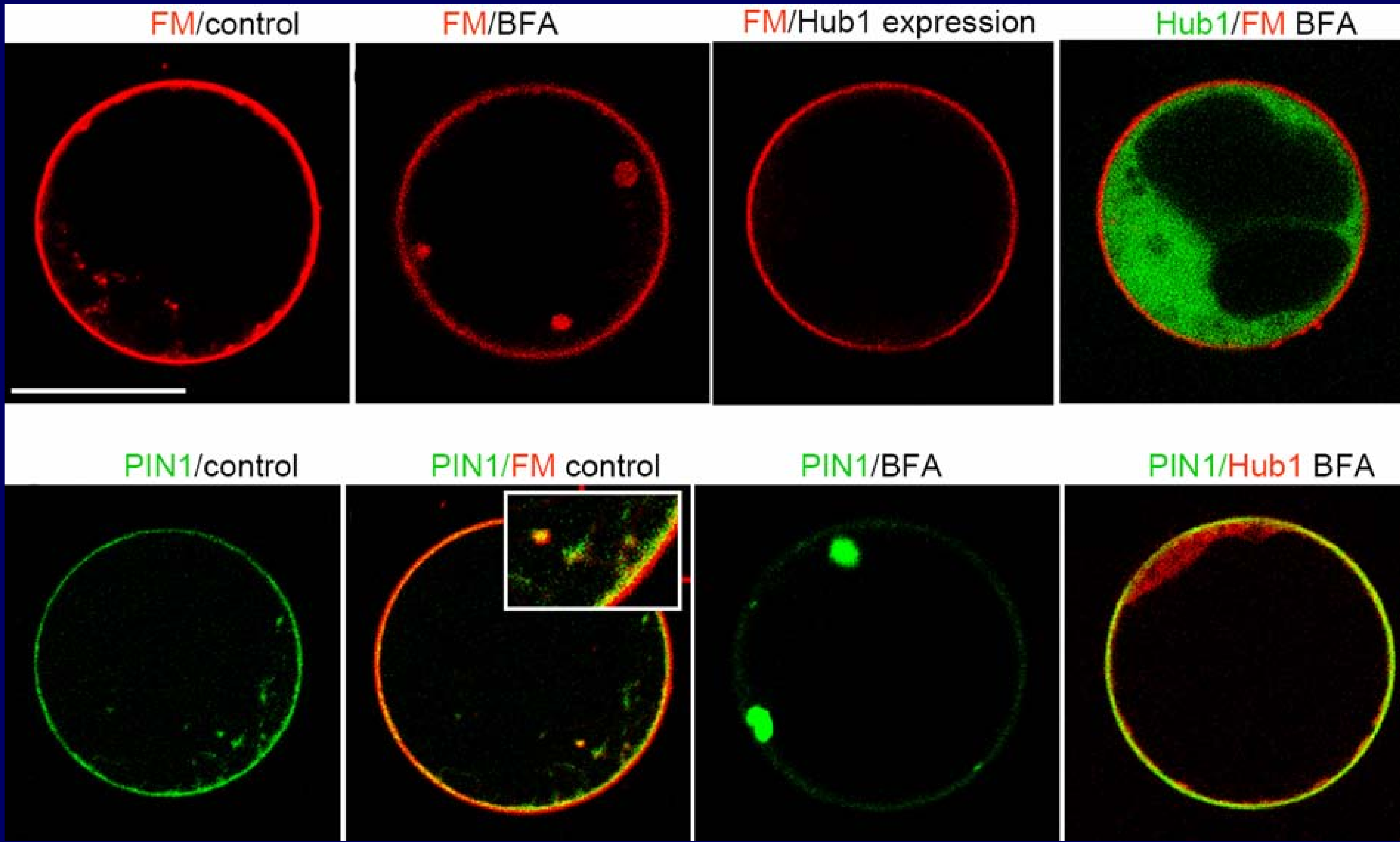
Incidence of Clathrin and Clathrin-coated Pits at Plasma Membrane of *Arabidopsis* Cells

Different Stages of Forming
Clathrin-coated Vesicles at Plasma Membrane



Dhonukshe et al., 2007

Genetic Interference with Clathrin Function Inhibits PIN Endocytosis



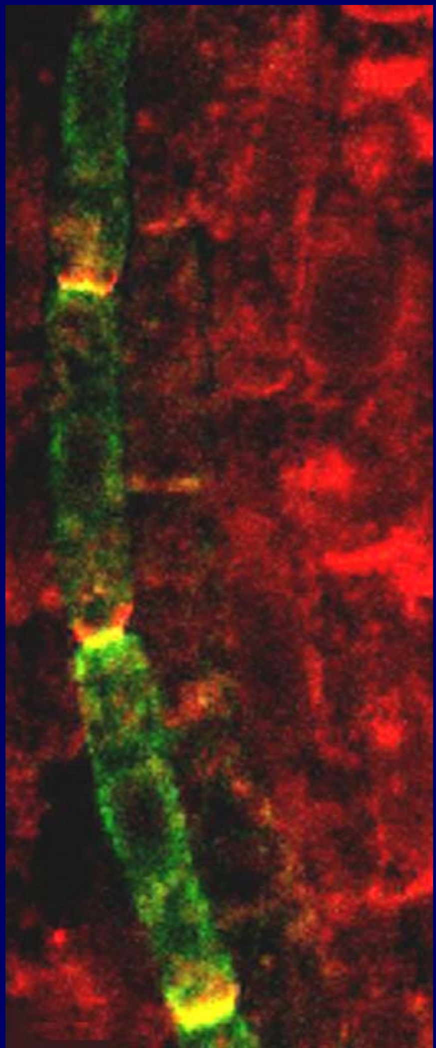
Dhonukshe et al., 2007

Molecular Components of PIN Polarity Control

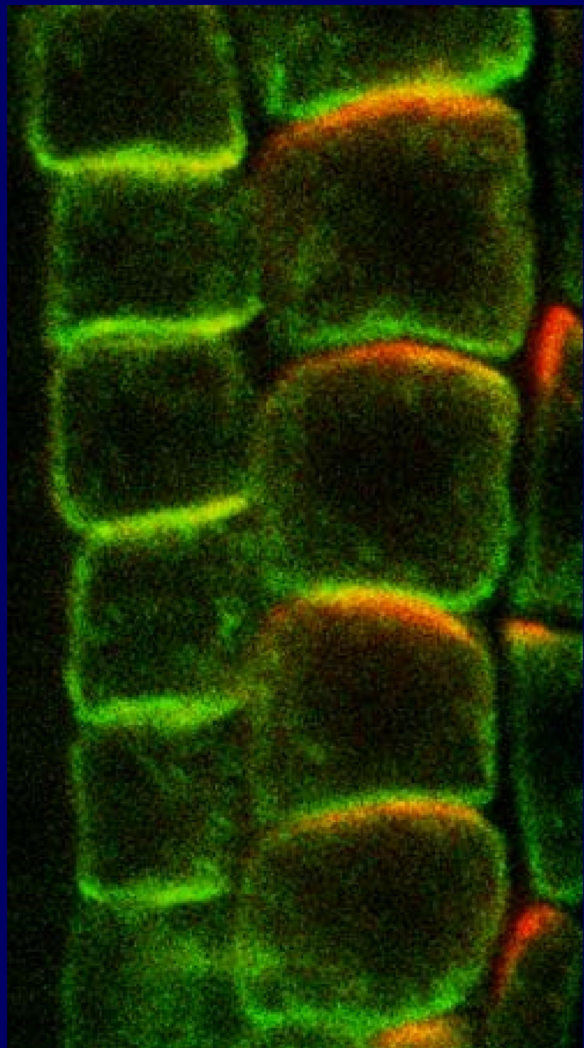
Role of PINOID protein kinase

Regulation of PIN Polarity

AUX1/PIN1



PIN1/pin1



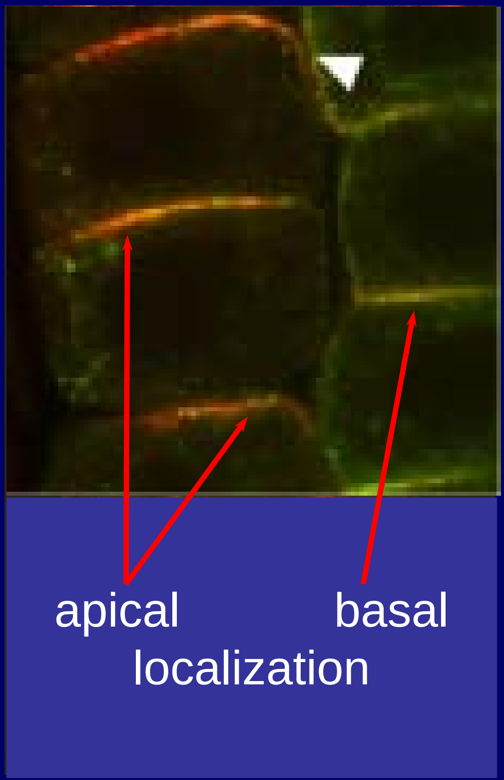
What are determinants of polarity of PIN localisation?

Does PIN polarity determines the direction of auxin flow?

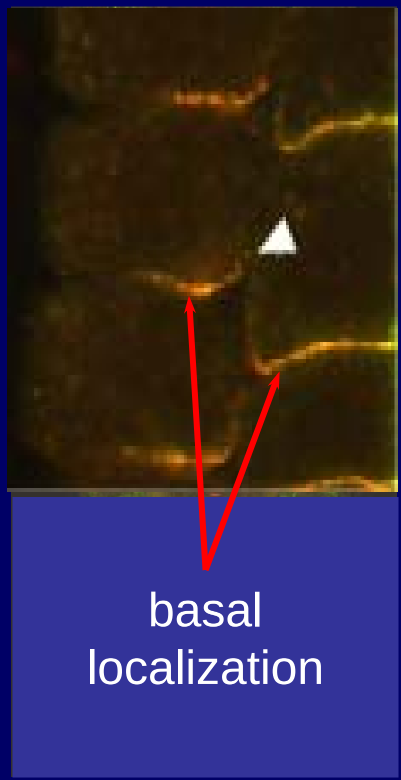
Molecular mechanism of the polar targeting pathway in plants?

PIN-specific Signals for Polarity

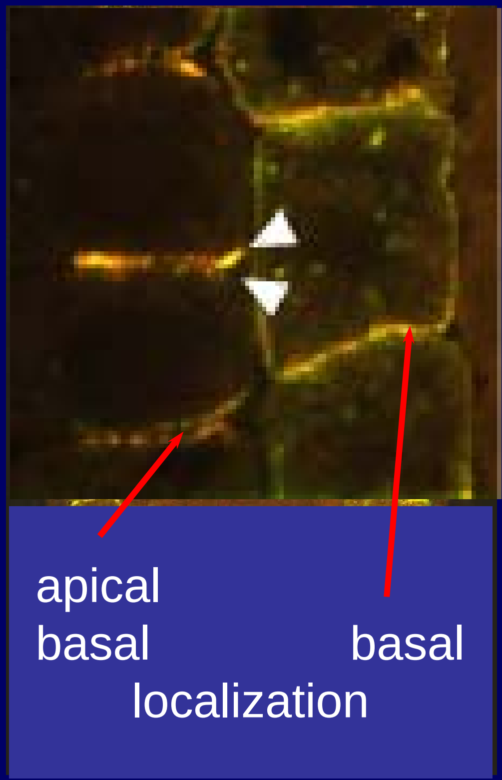
PIN2pr::PIN2:HA



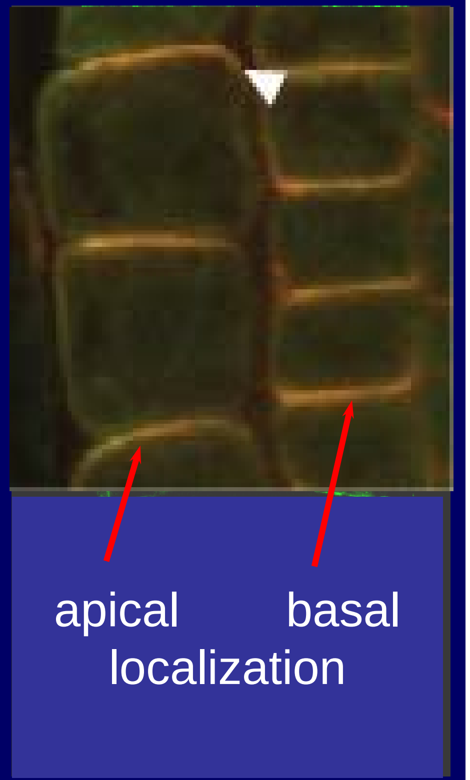
PIN2pr::PIN1:HA



PIN2pr::PIN3:HA

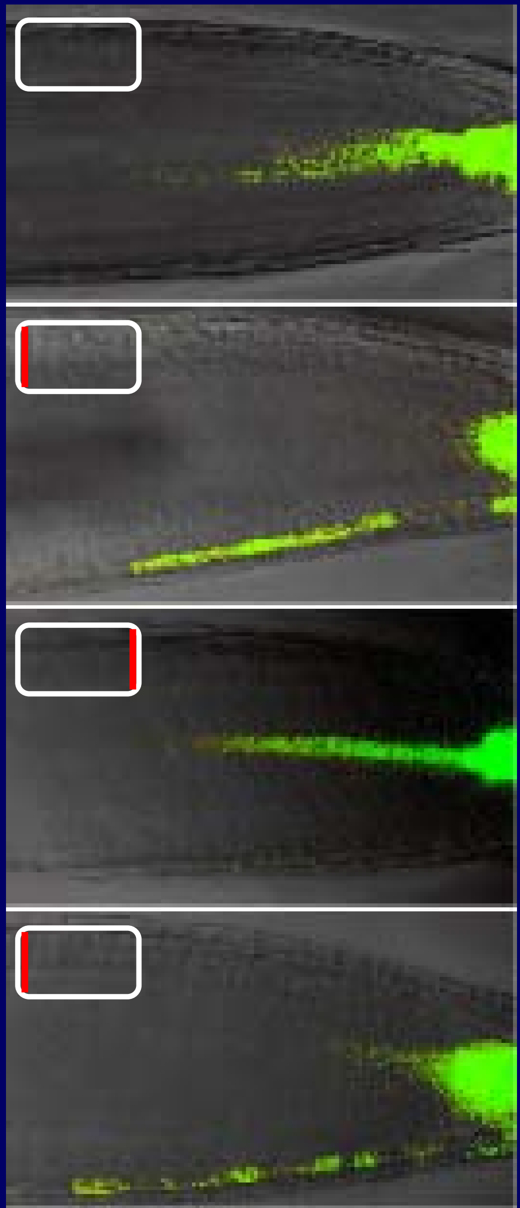


PIN2pr::PIN1:GFP



PIN Polarity Determines Direction of Auxin Flow

DR5rev::GFP



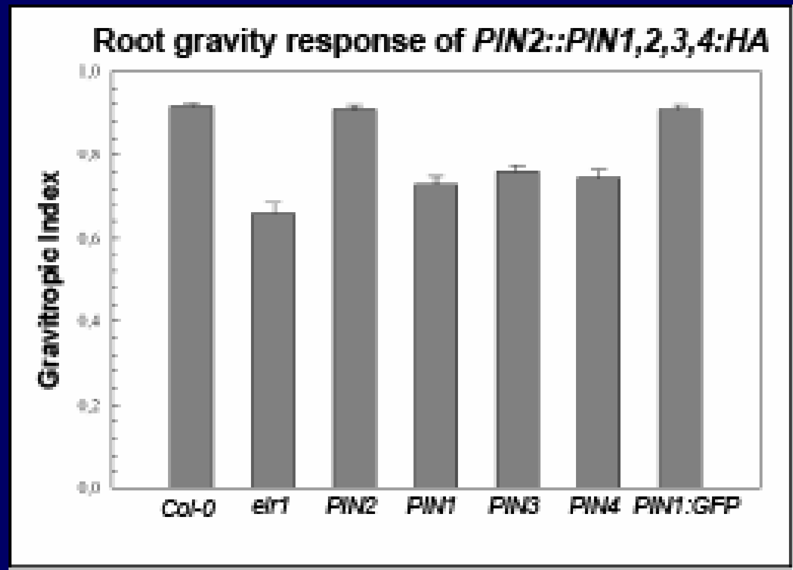
eir1

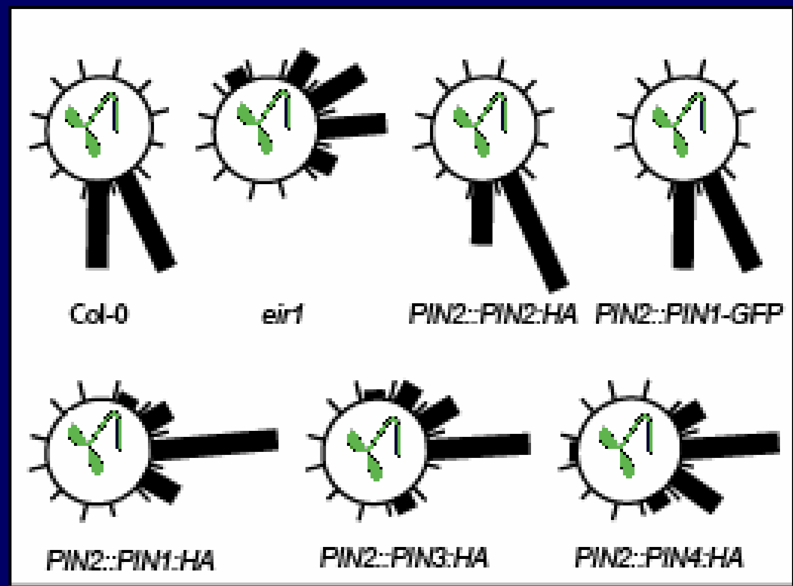
PIN2::PIN2:HA

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

PIN2::PIN1:GFP-3

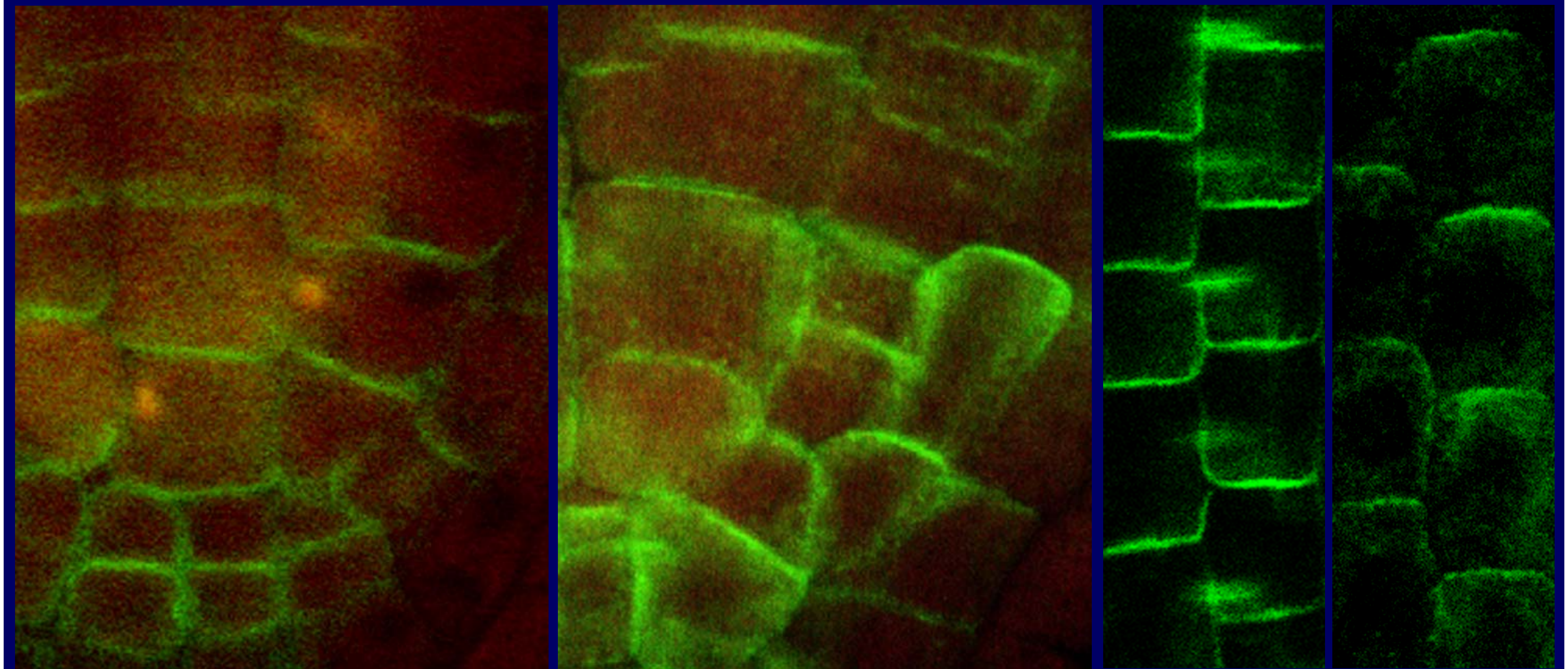
gravitropism





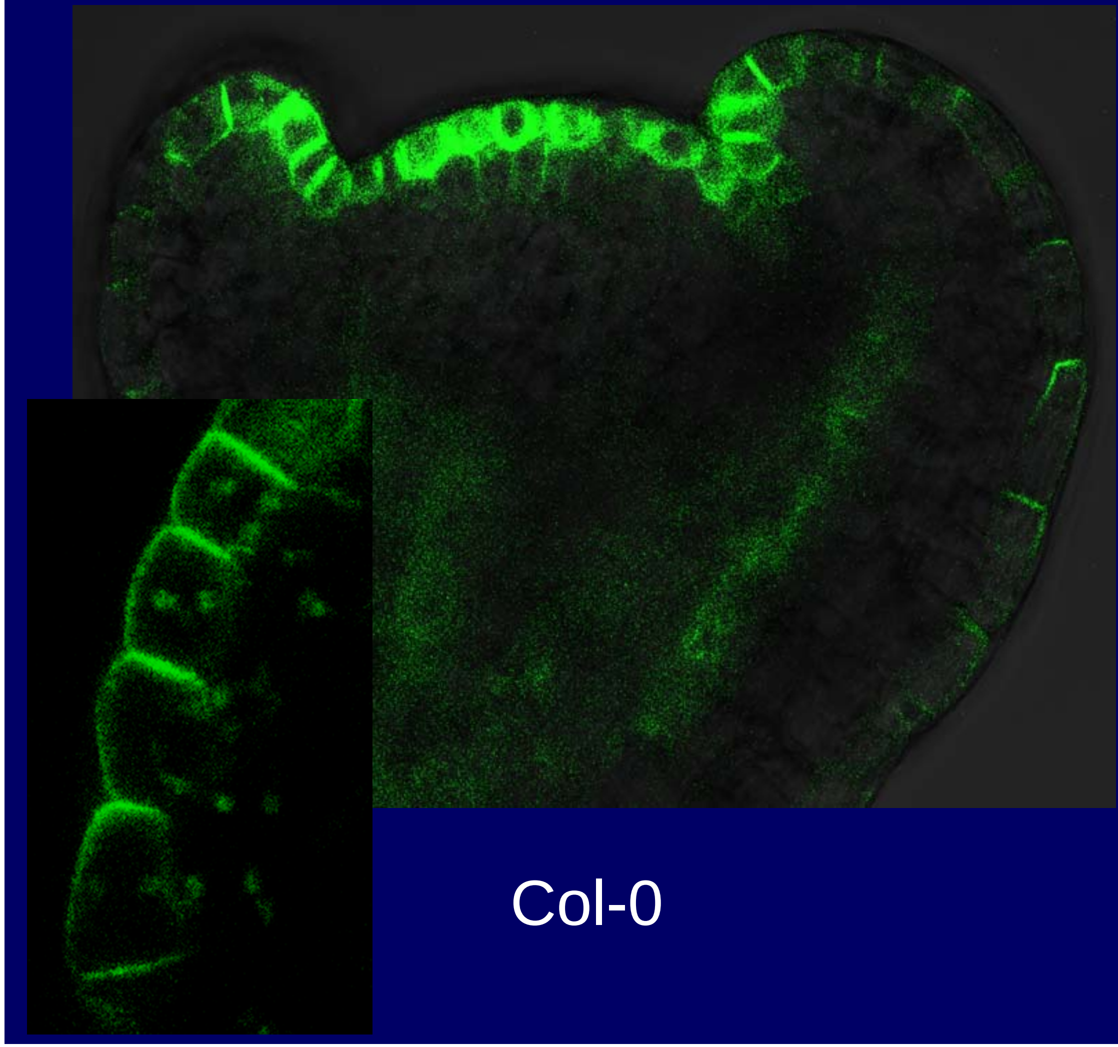
Role of PINOID Kinase in PIN Polar Targeting

Col-0 | *35S::PID* | Col-0 | inducible *PID*

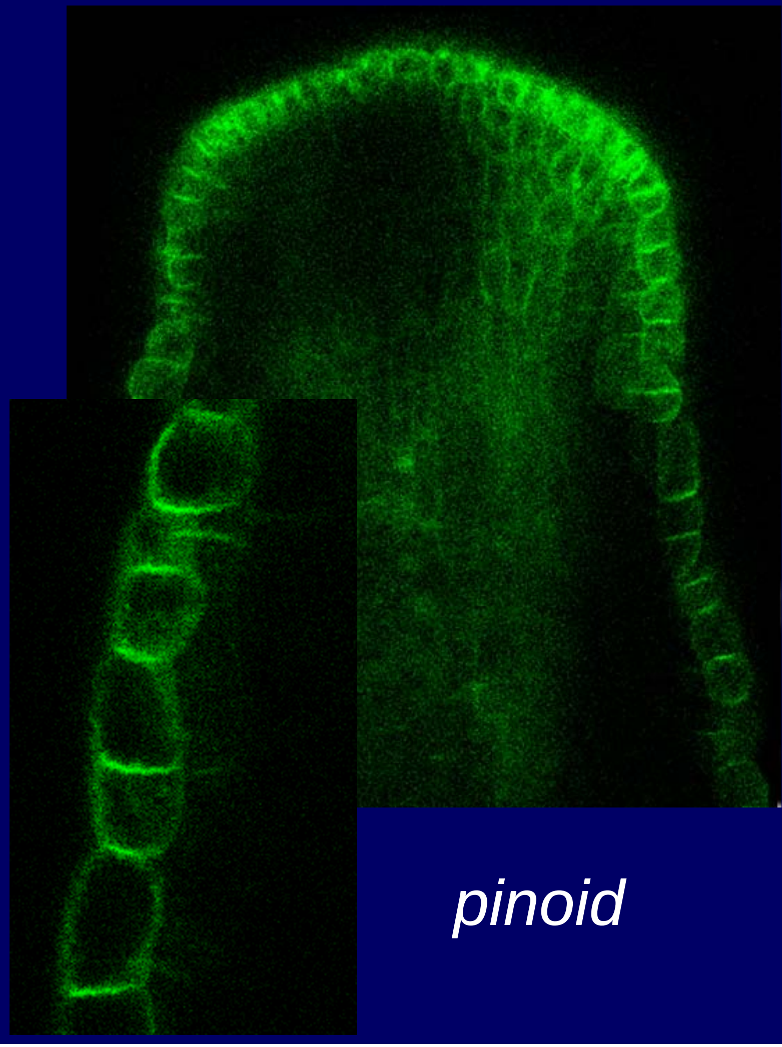


PIN4 | **PIN1**

PINOID kinase loss-of-function > basal PIN targeting

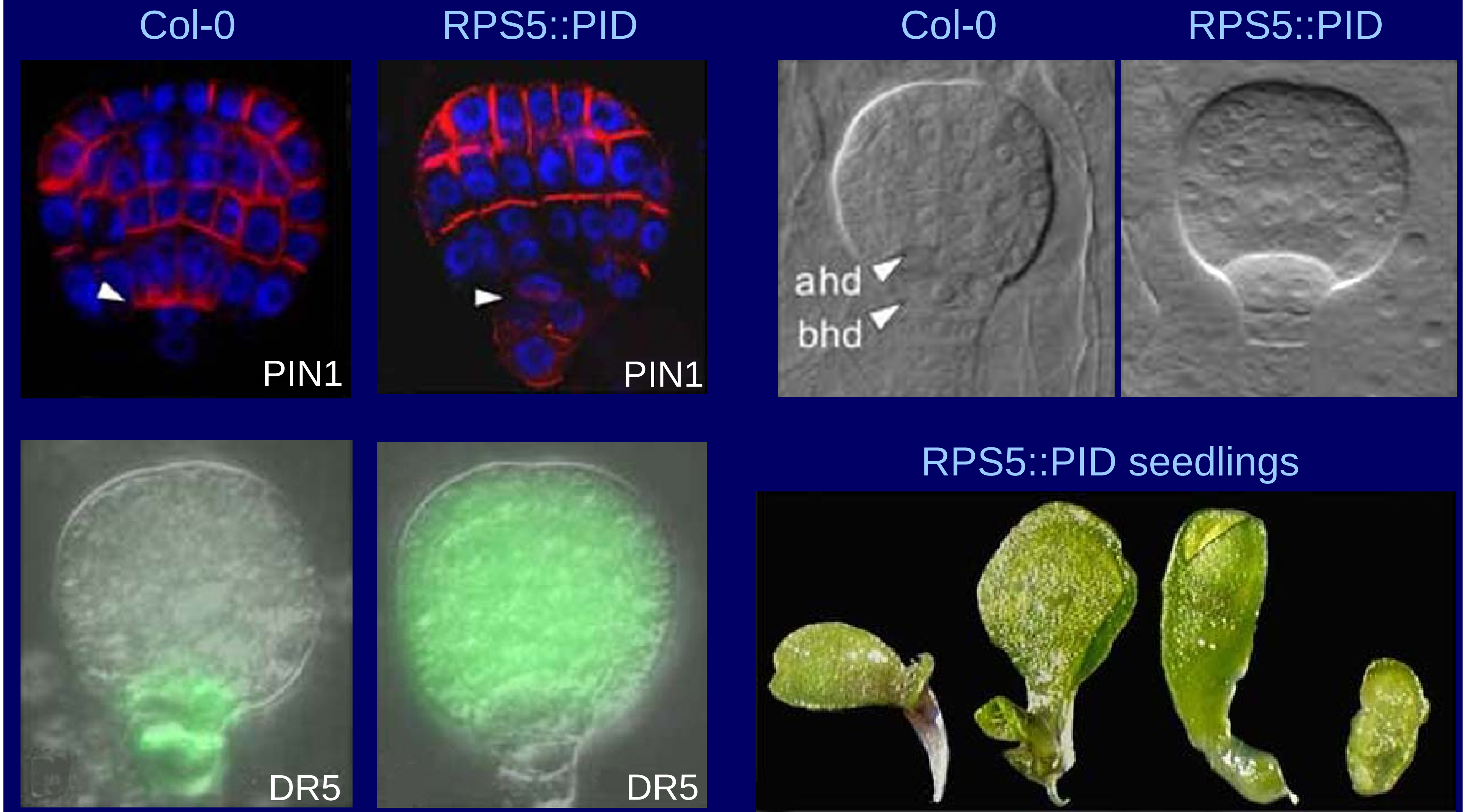


Col-0



pinoid

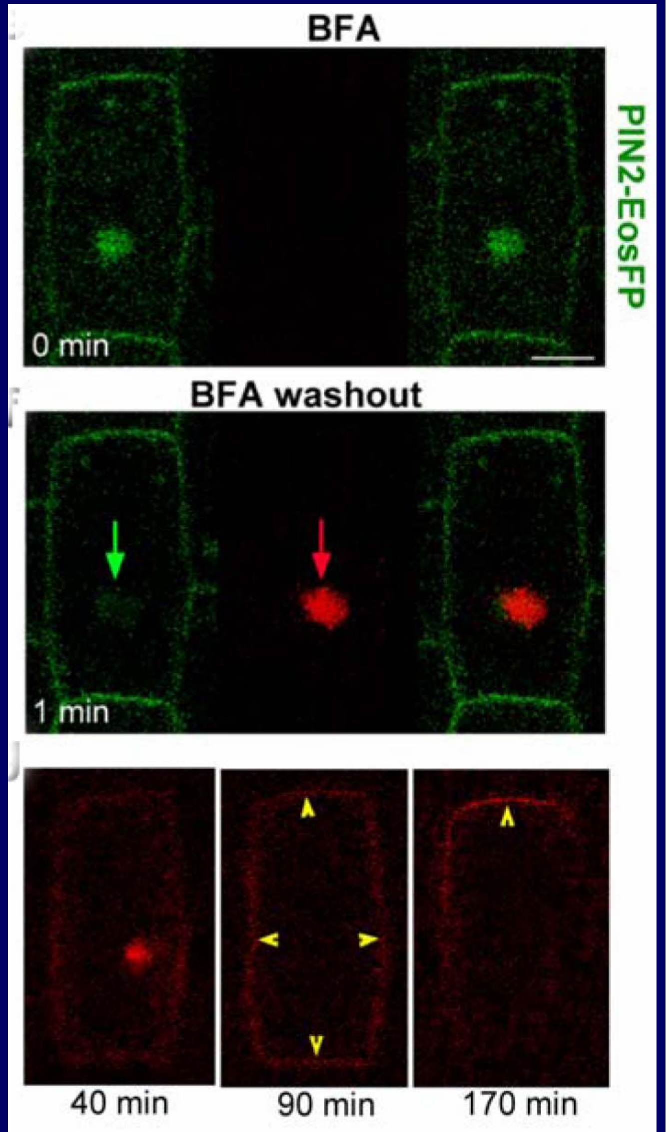
Role of PID in Controlling
PIN Polarity > Auxin Flow > Patterning



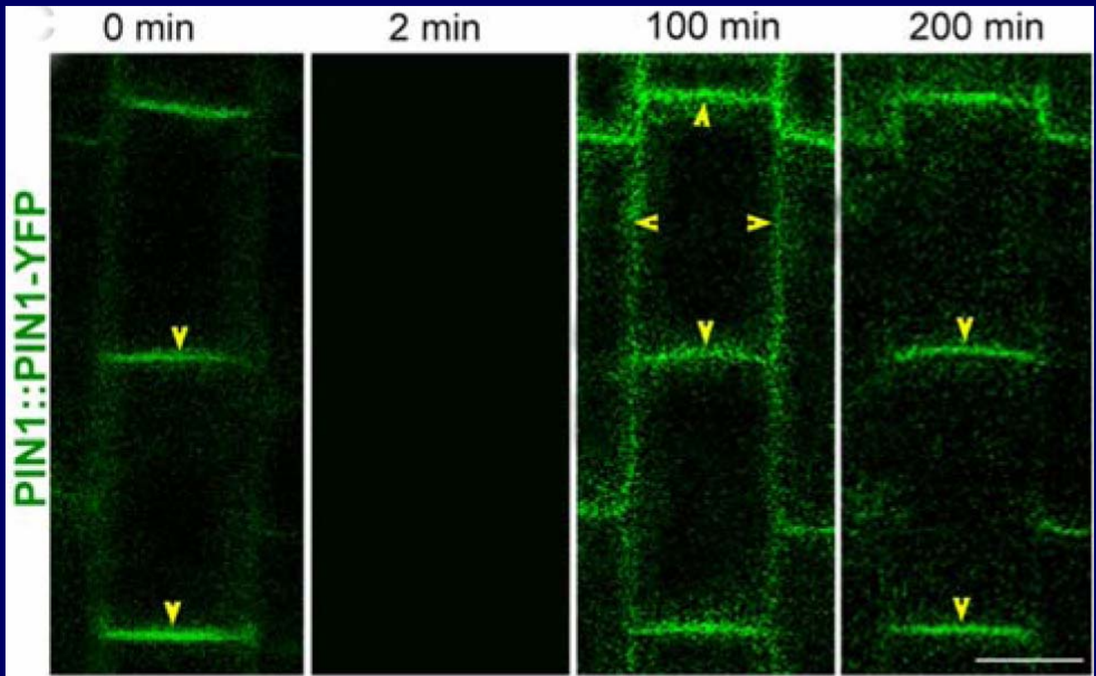
Link between constitutive recycling and cell polarity

Two Step Mechanism of PIN Polarity Establishment

BFA washout:
Non-polar recovery of PIN2



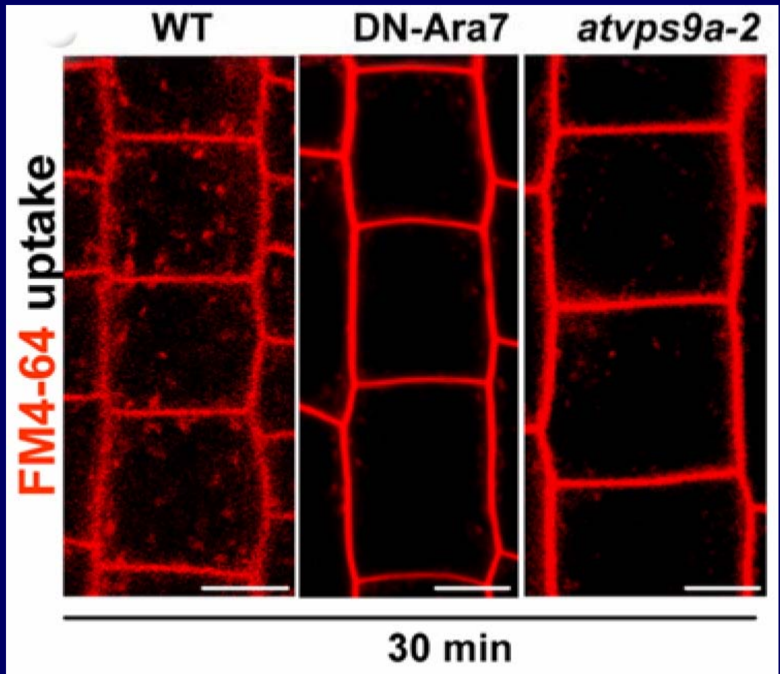
FRAP: Non-polar targeting of PIN1



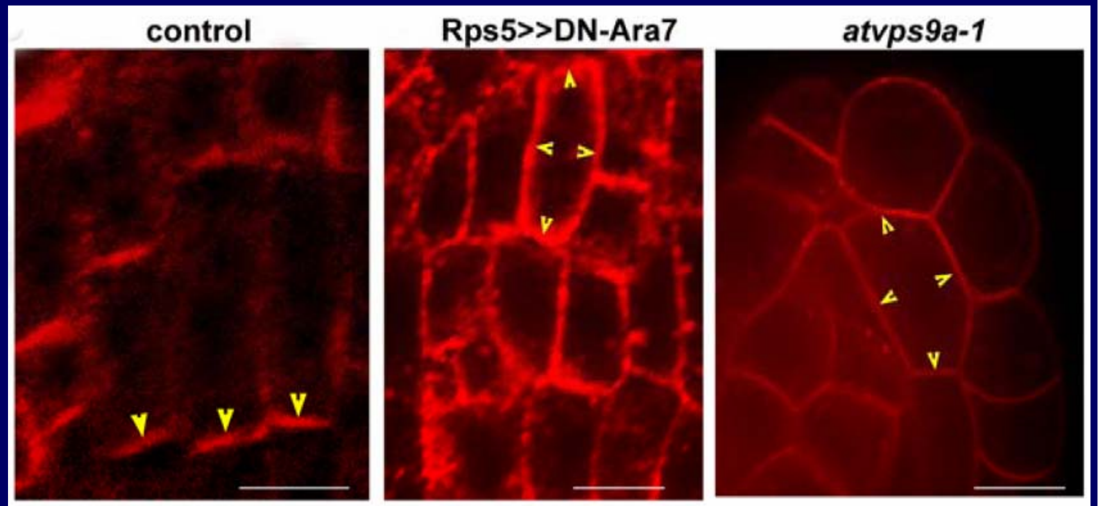
unpublished

Attenuation of Rab5 Function Inhibits Endocytosis and Leads to PIN Polarity Defects

FM4-64 uptake



PIN1 polarity in embryogenesis

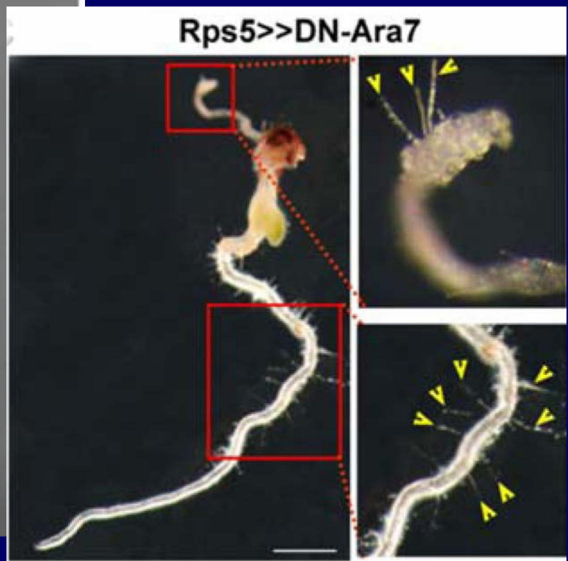


unpublished

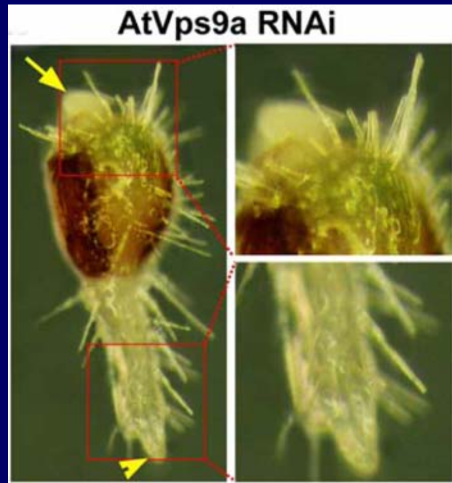
Inhibition of Endocytosis Leads to Transformation of Leaves to Roots

Rab5 dominant negatime

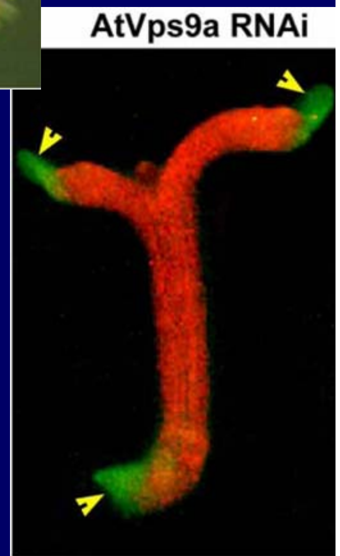
QC25



Vps9a loss-of-function



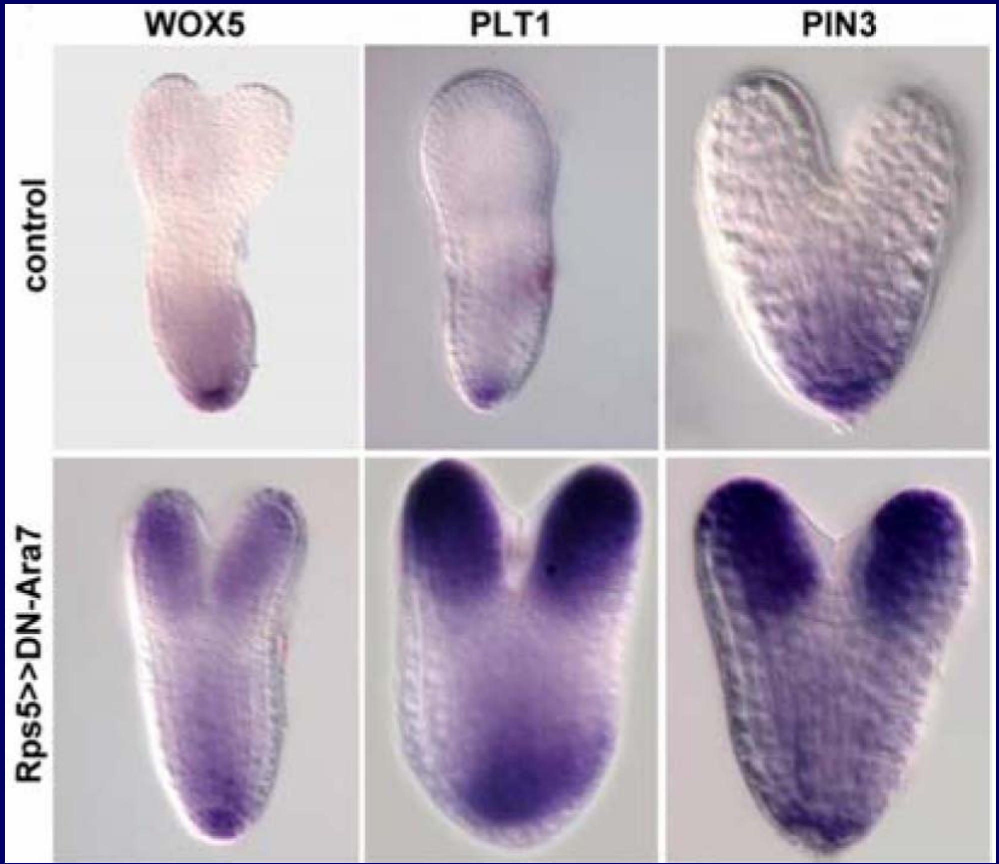
PIN2-GFP



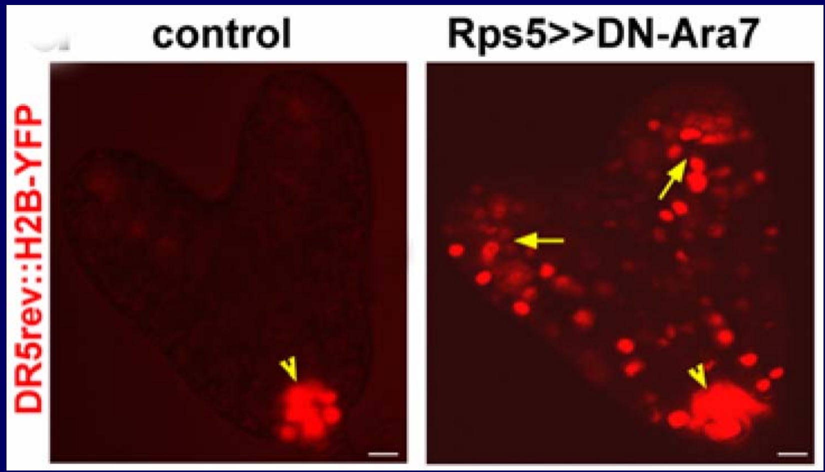
unpublished

Inhibition of Endocytosis Causes Transformation of Embryonic Leaves into Root Fates

in situ mRNA of root markers



DR5 auxin response



unpublished

TROPISMS

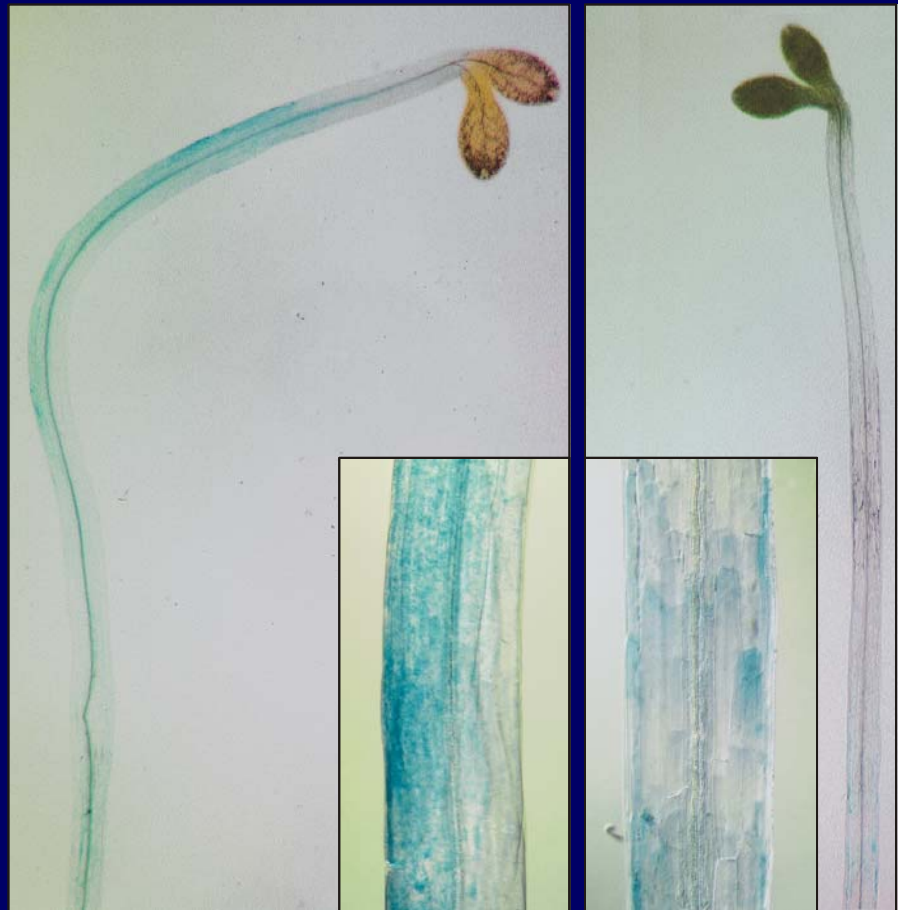
Asymmetric Auxin Distribution Underlies Tropisms

Phototropism

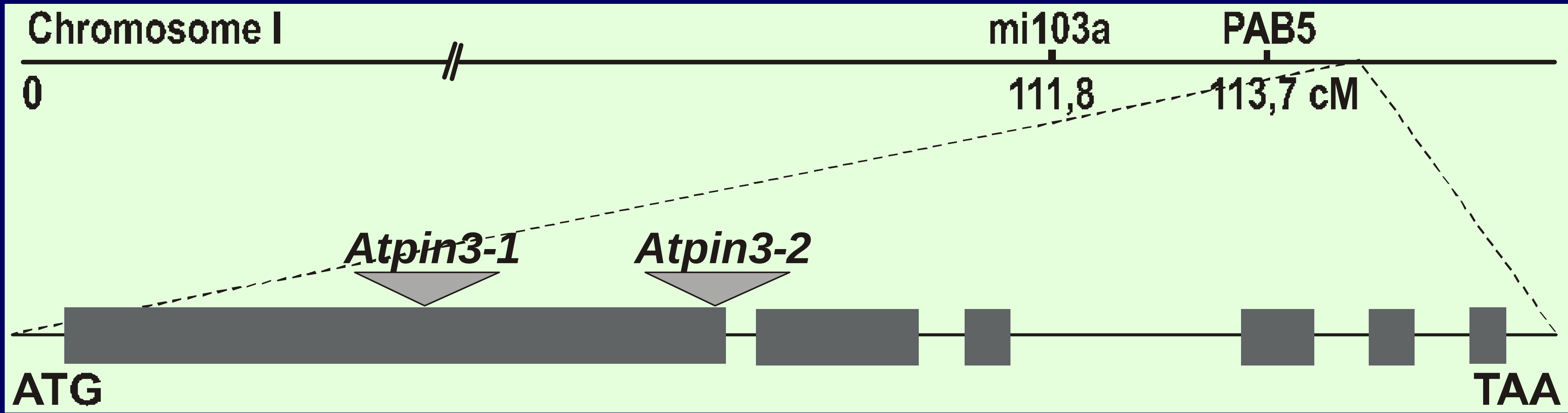
- NPA **+ NPA**

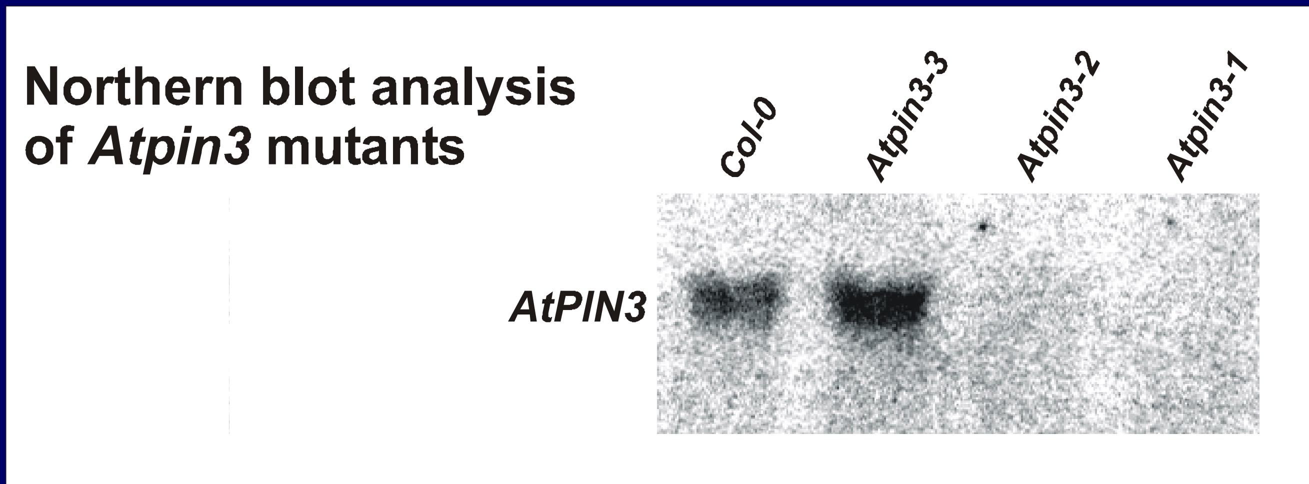
Gravitropism

- NPA **+ NPA**

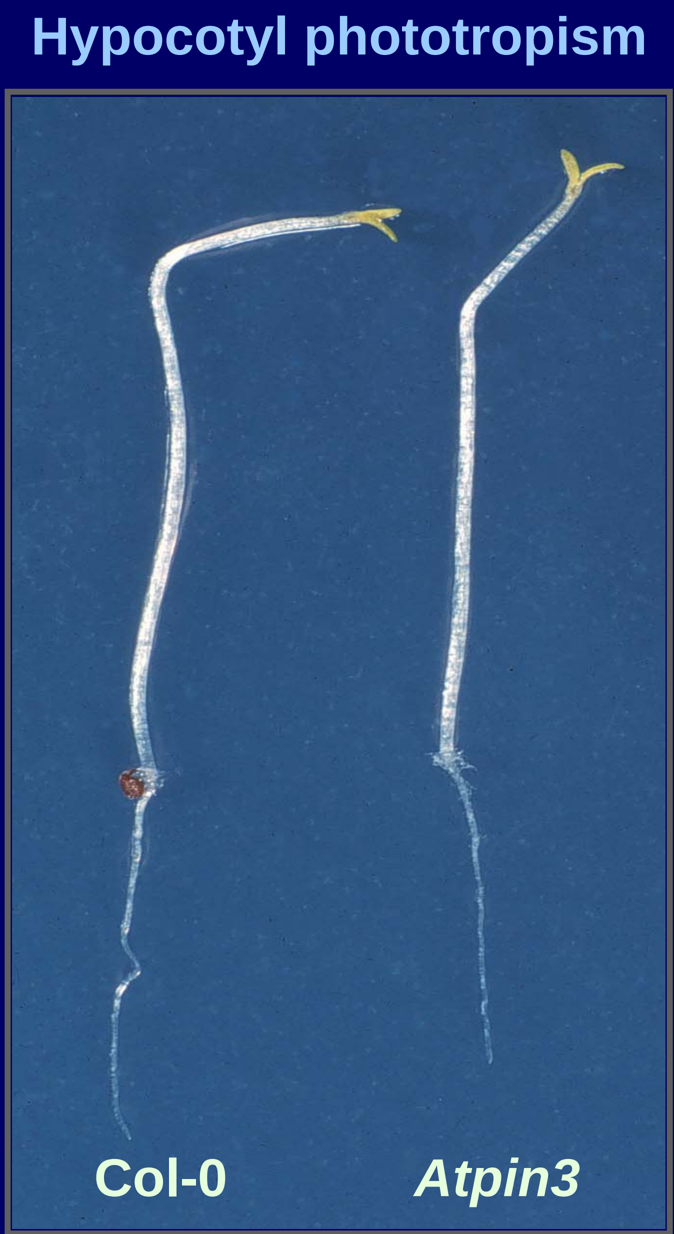


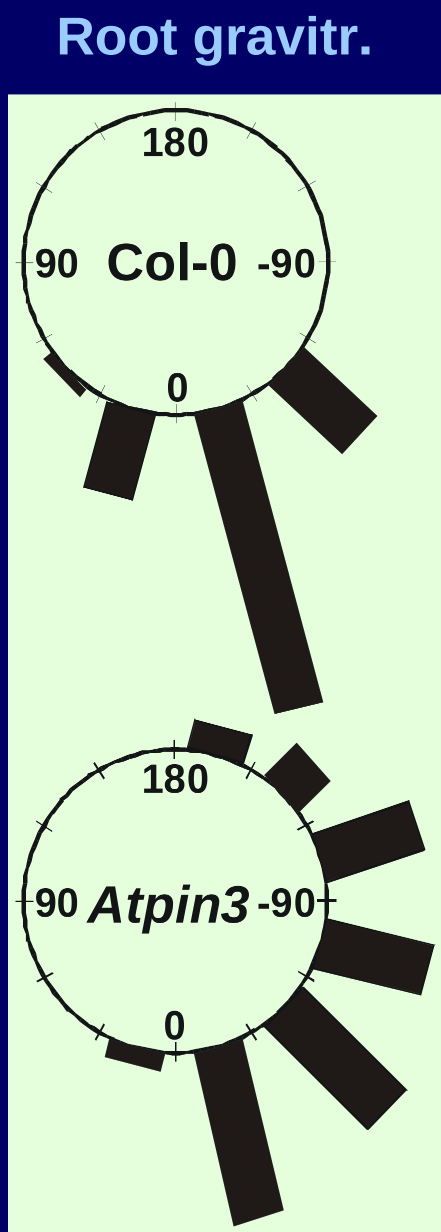
AtPIN3 Gene and *Atpin3* Knock-Out Mutants



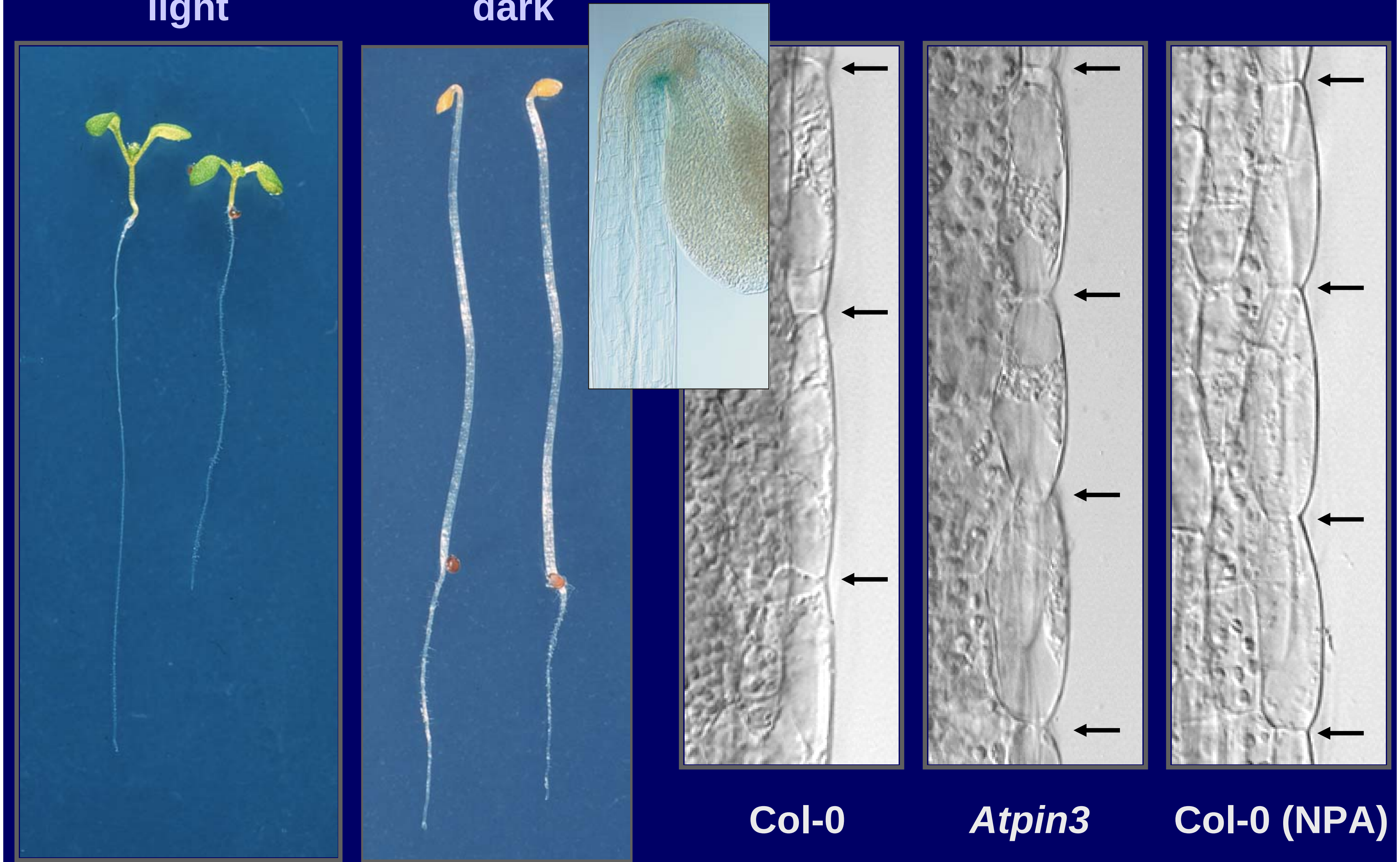


Atpin3 Tropisms





Atpin3 Hypocotyl and Apical Hook



light

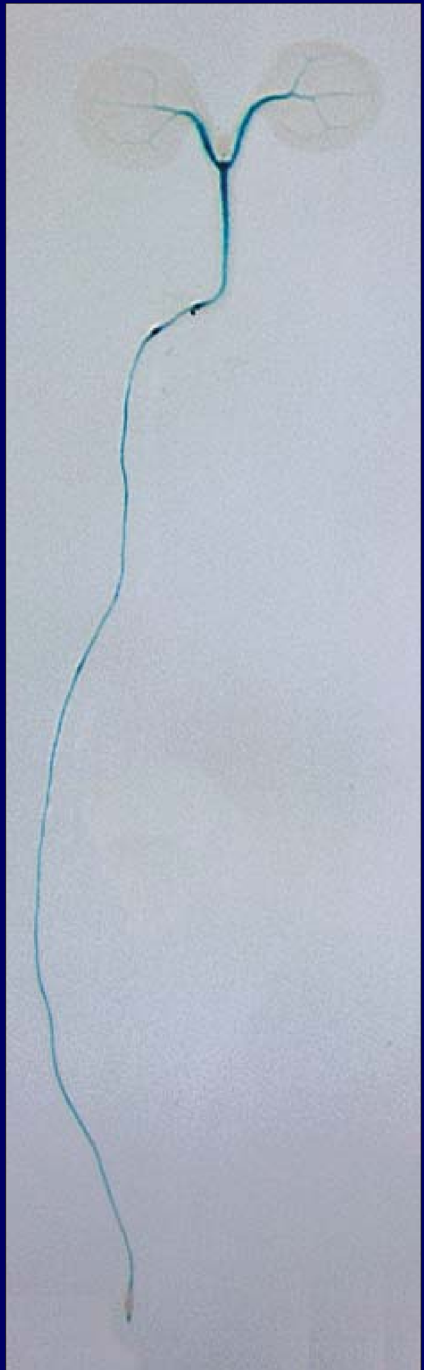
dark

DR5

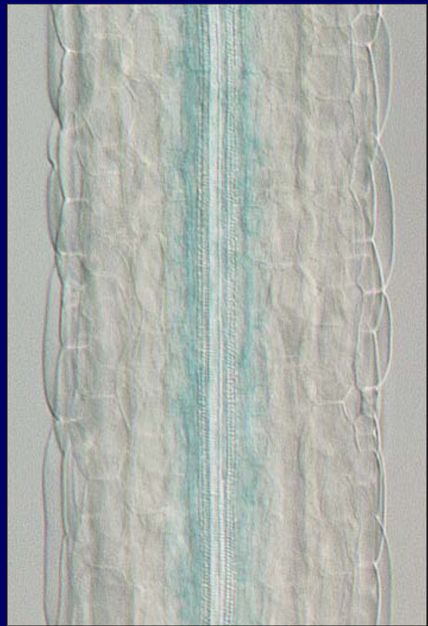
Col-0

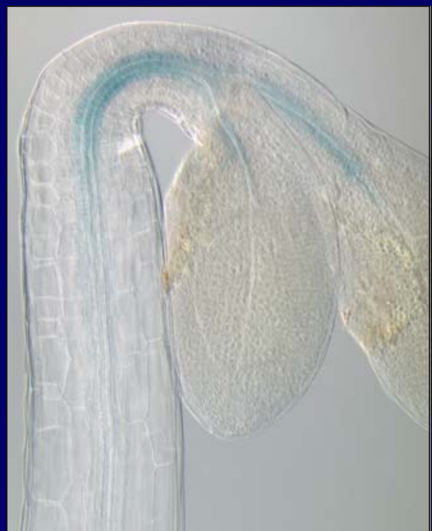
Atpin3

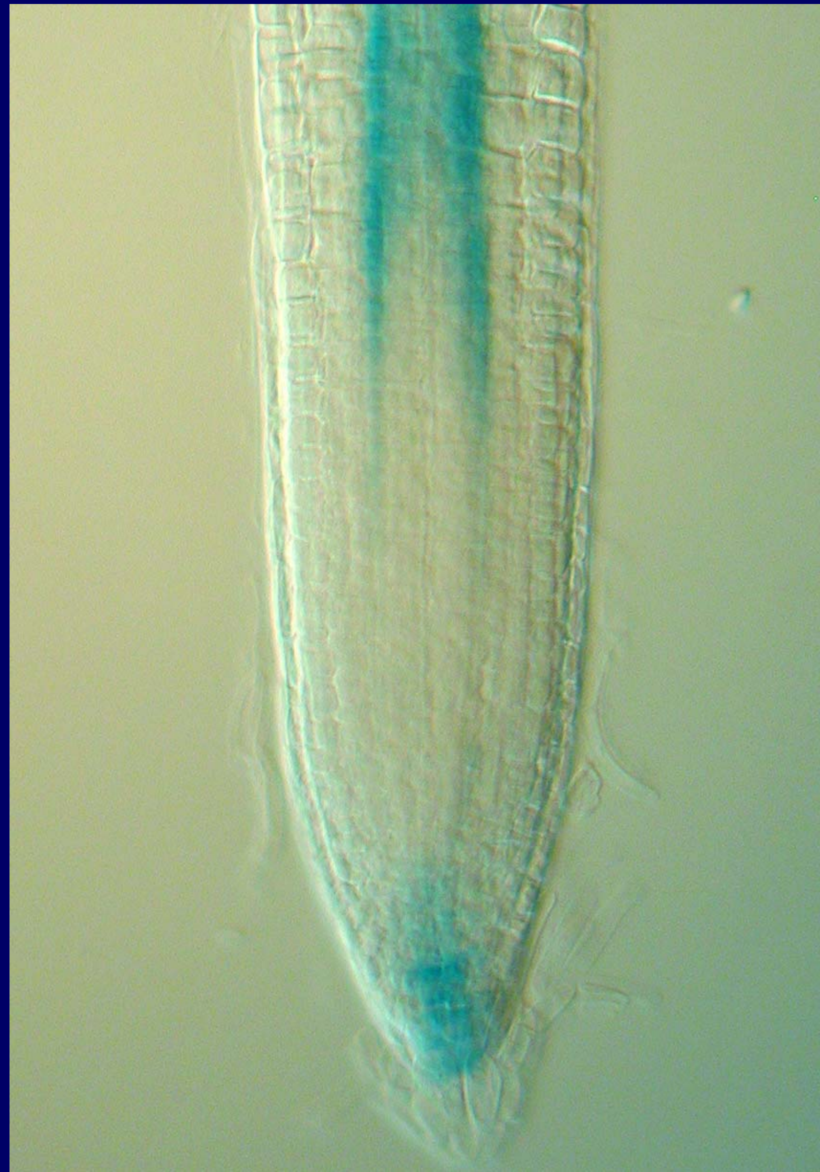
Col-0 (NPA)



AtPIN3::GUS Transgenics

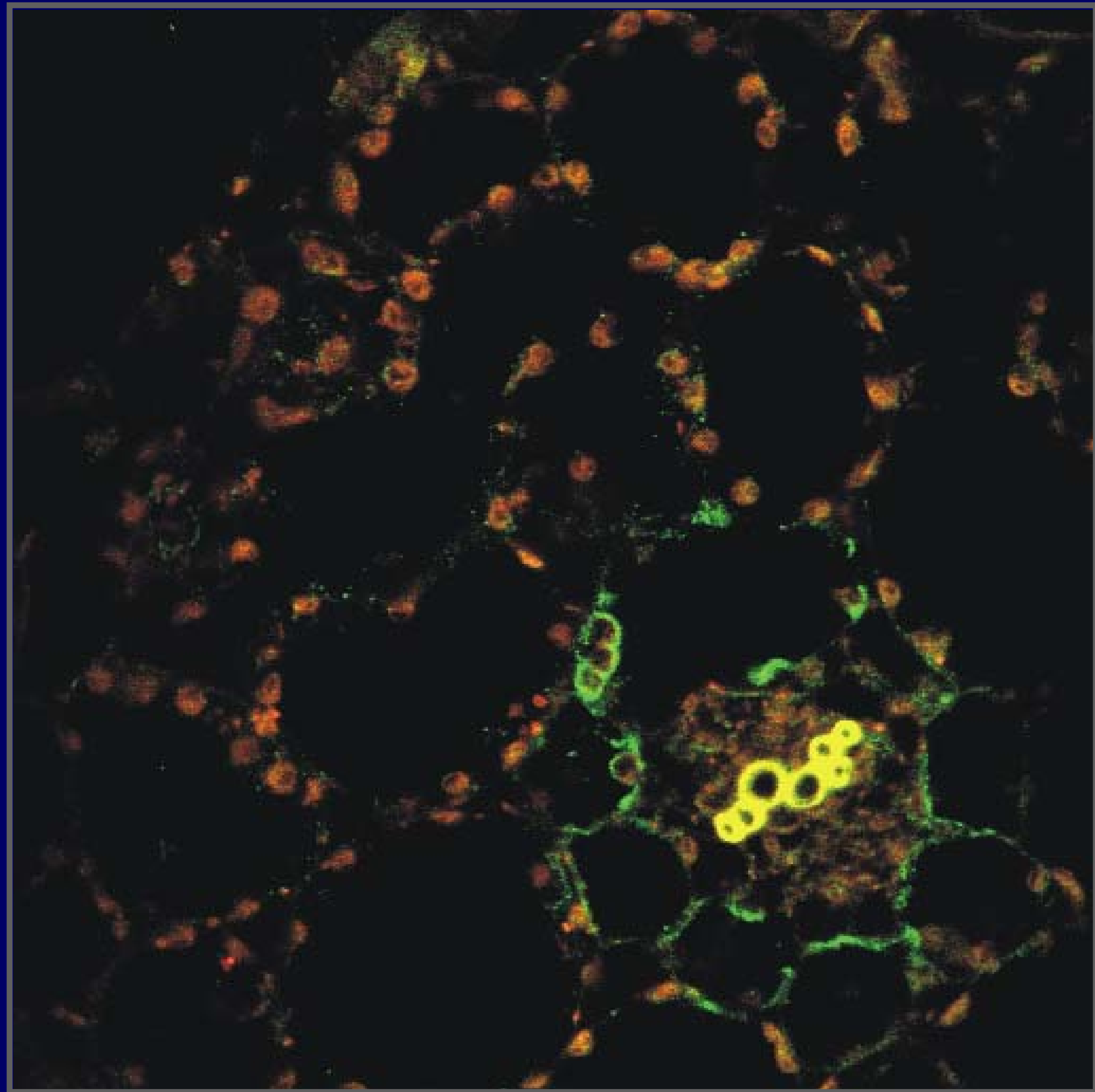




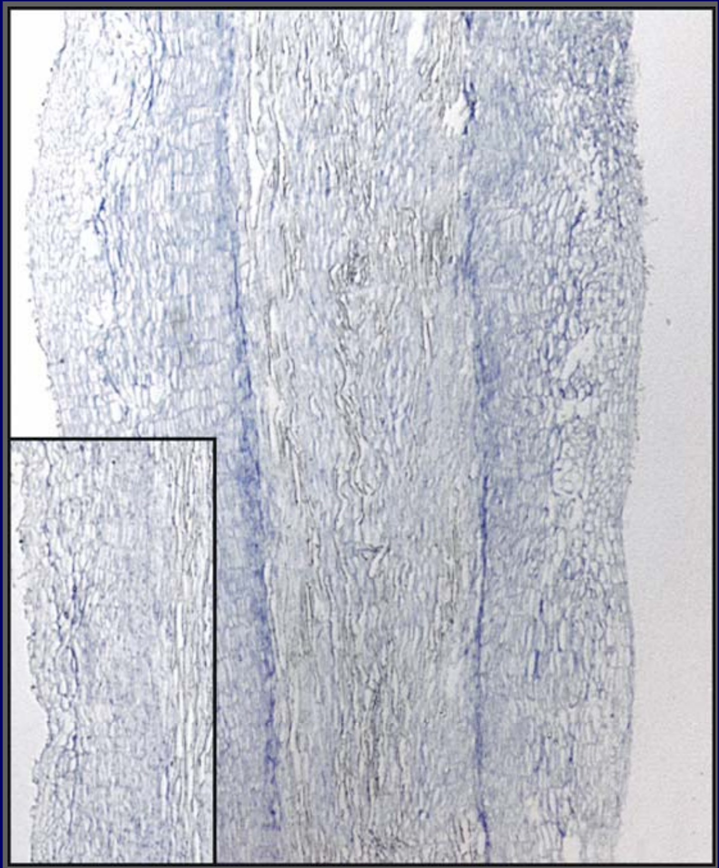


AtPIN3 in Hypocotyl

The AtPIN3 protein

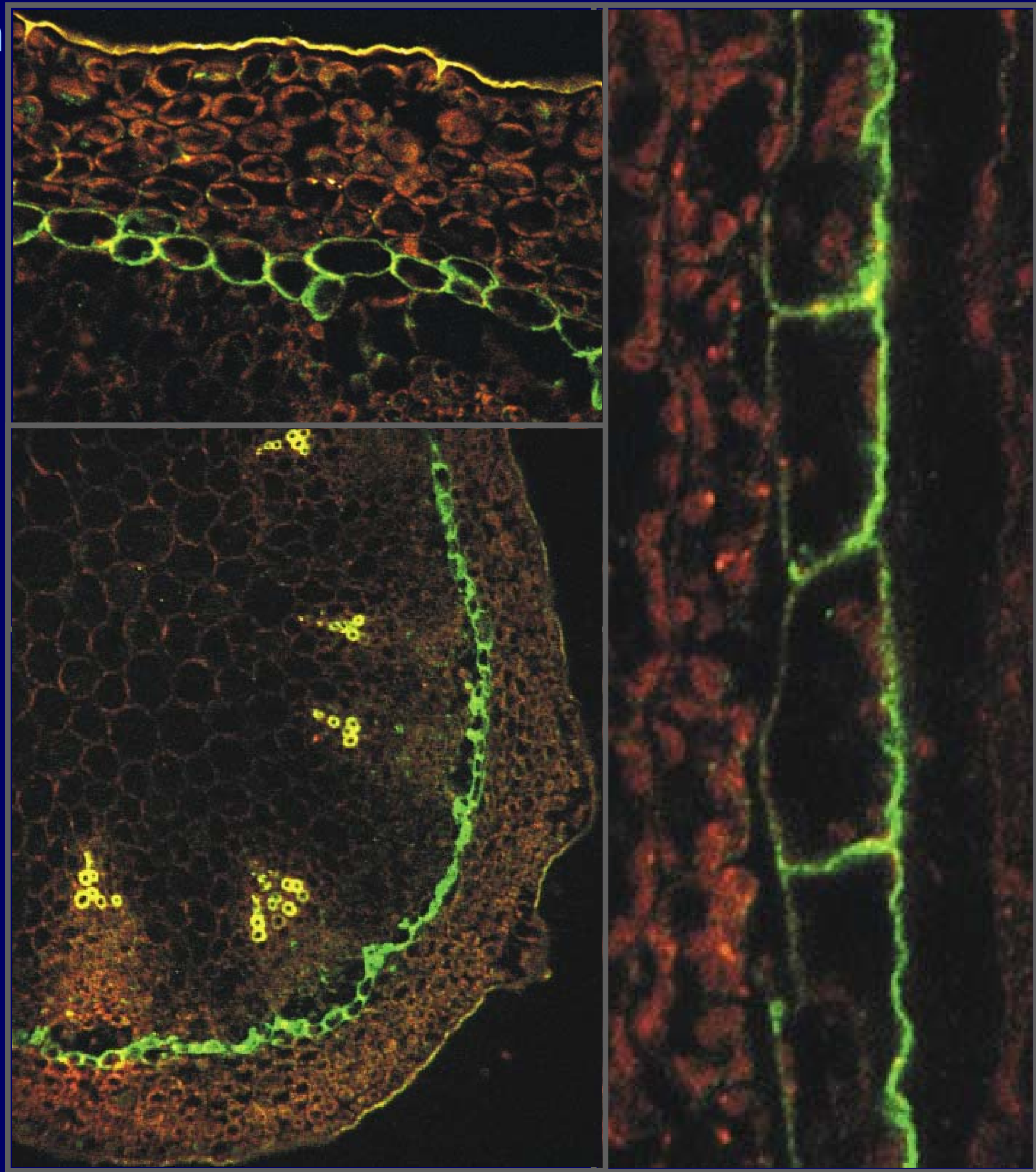


***in situ* RNA hybridisation**

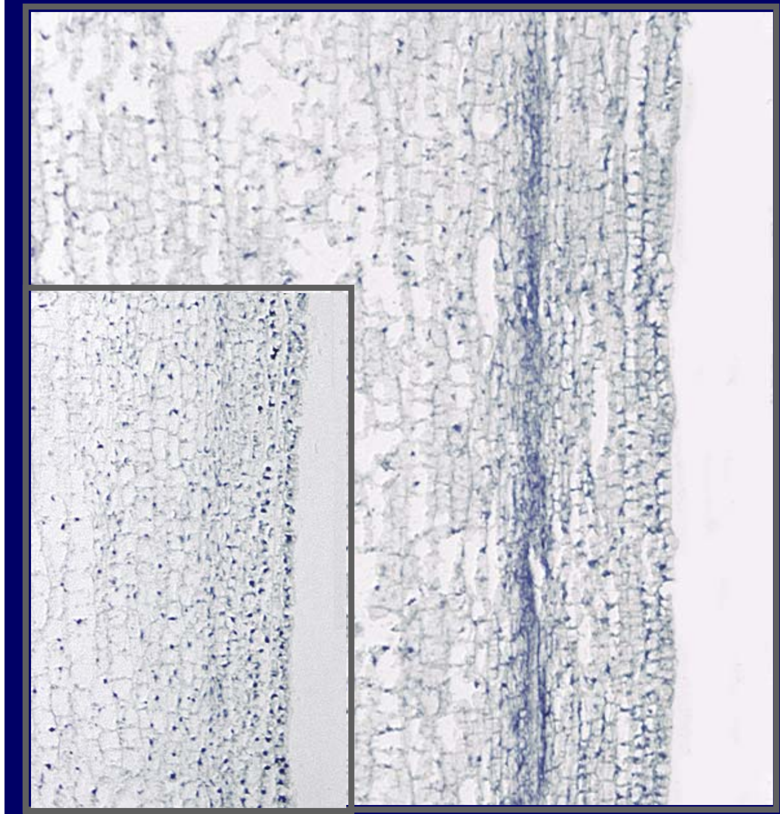


AtPIN3 in Inflorescence Axis

The AtPIN3 protein



***in situ* RNA hybridisation**

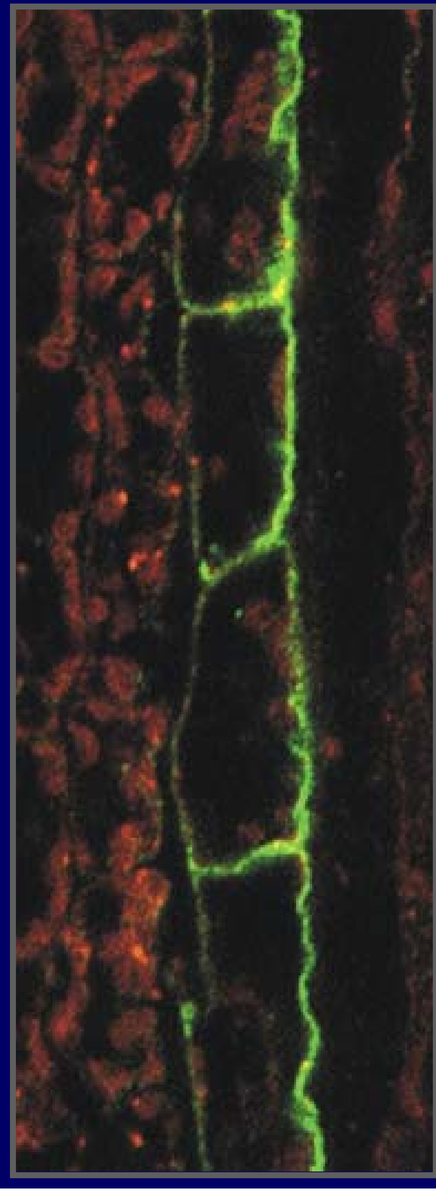


PIN3 – Lateral Auxin Transport

DR5 - phototropism

pin3 phototropism

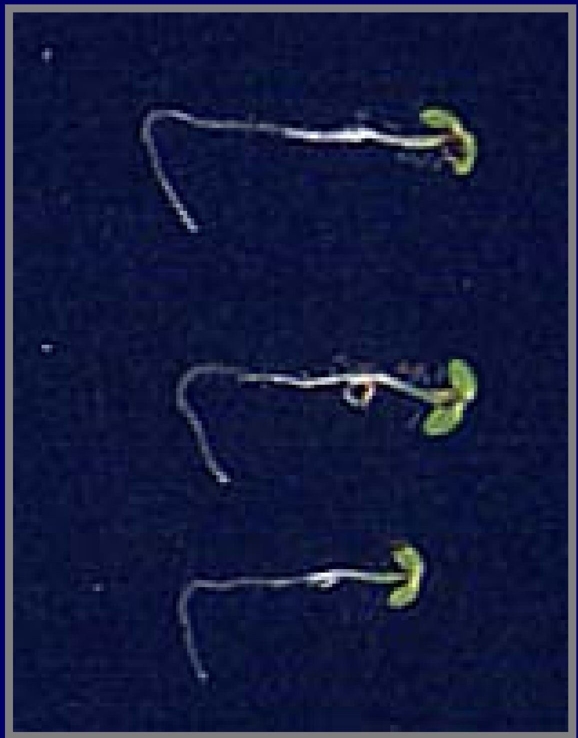
PIN3 protein



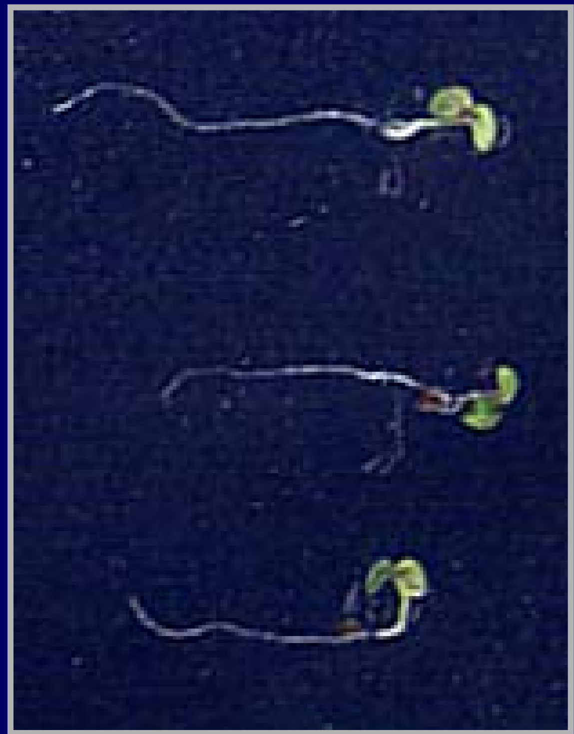
PIN2 – Root Gravitropism

PIN2 protein

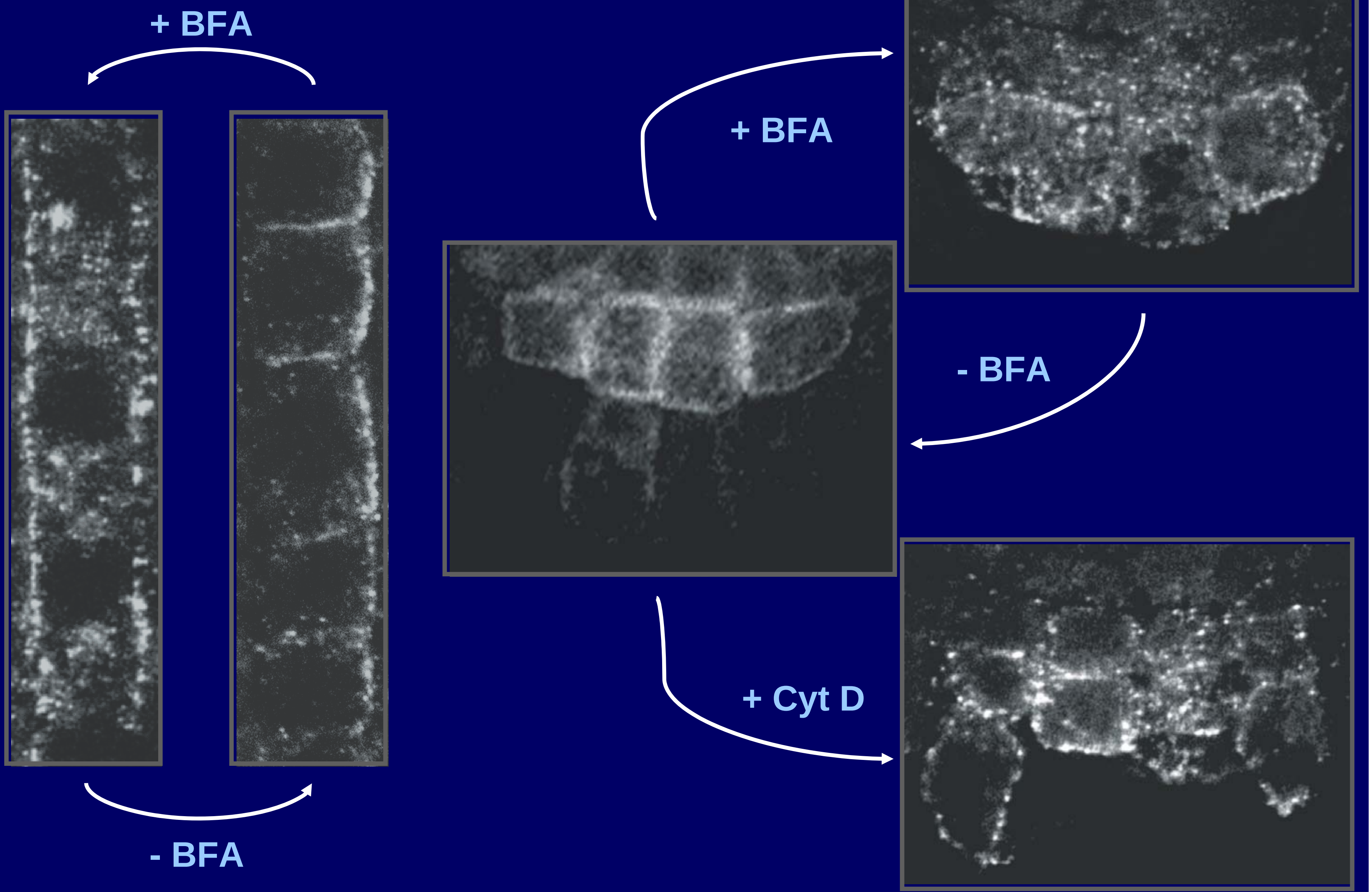
Col-0



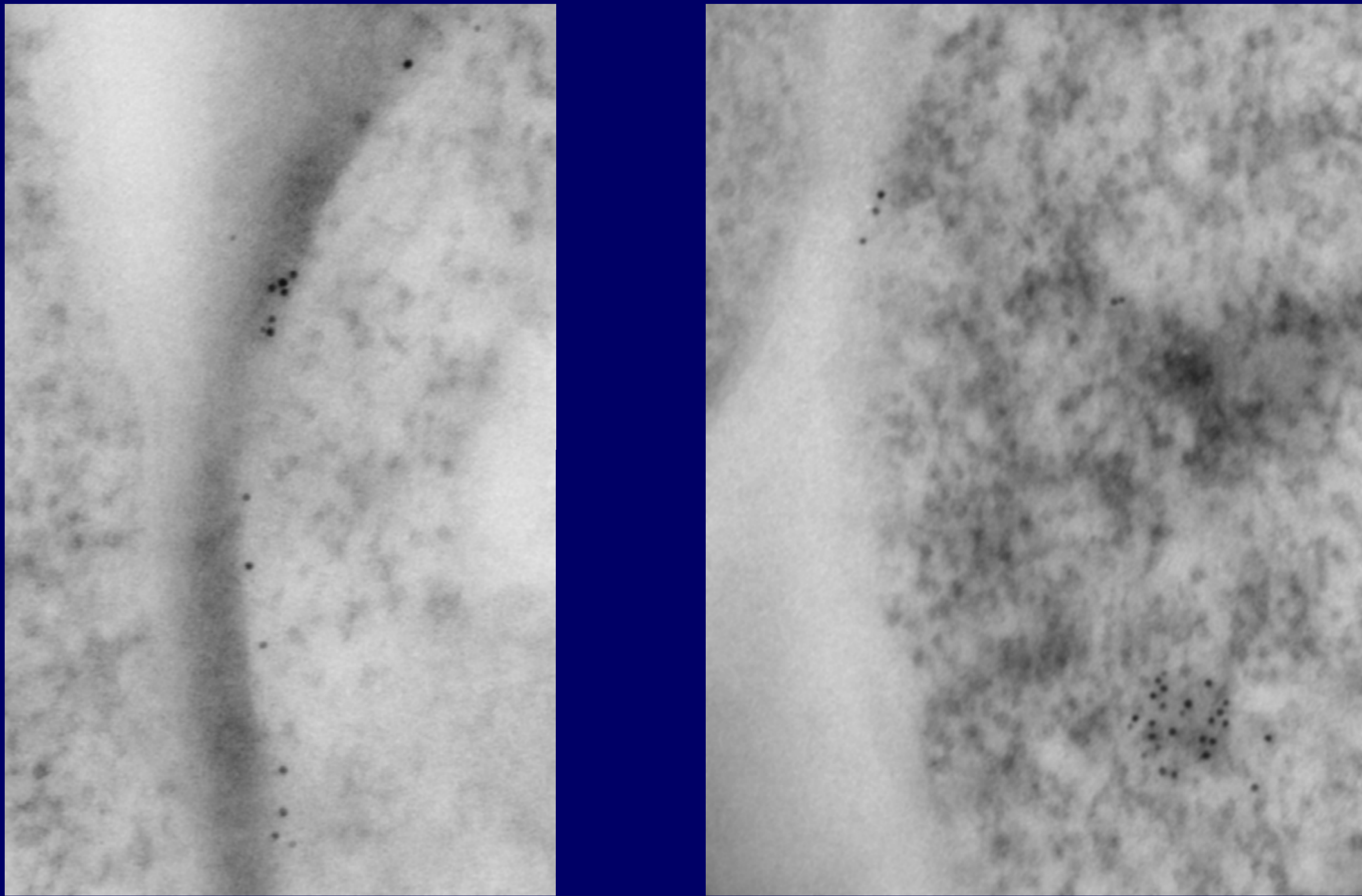
pin2



PIN3 Actin Dependent Cycling

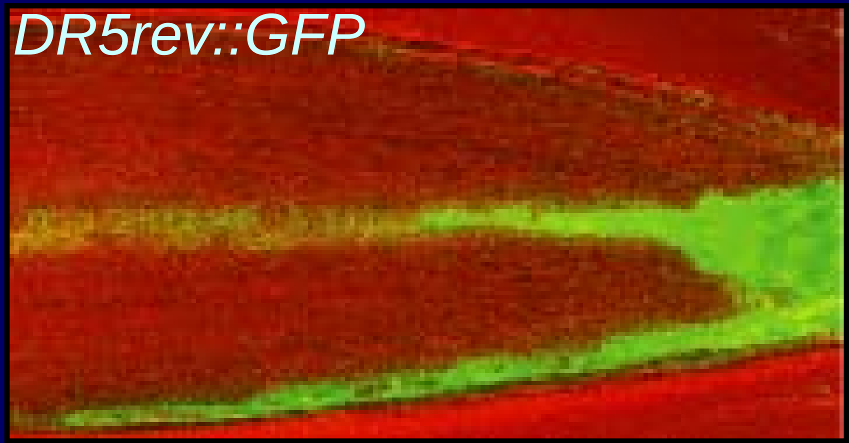


PIN3 Immunogold Electron Microscopy

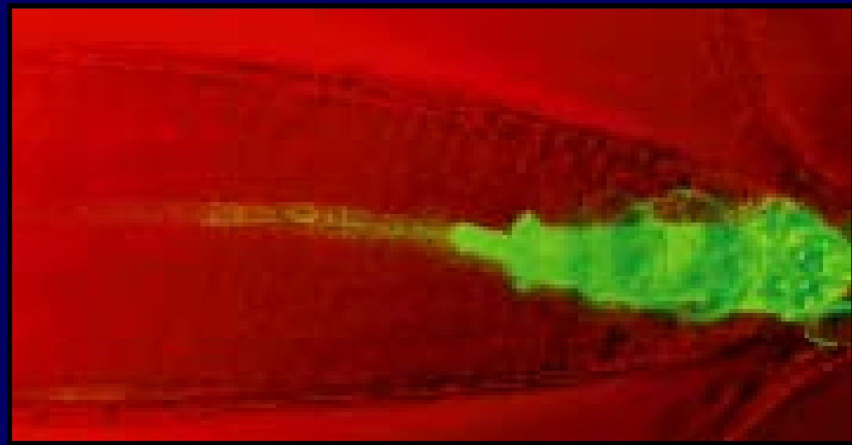


Root Gravitropism

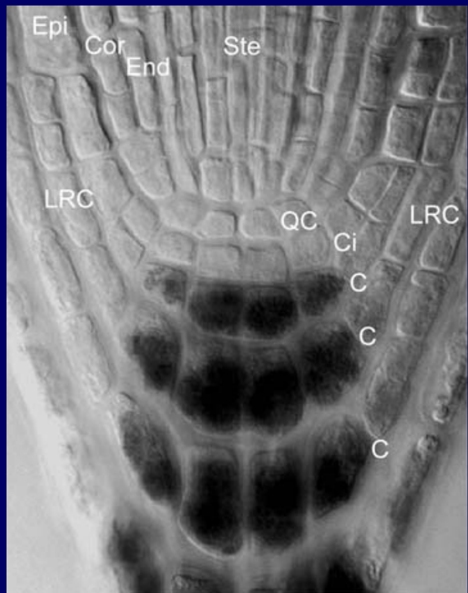
gravity stimulated



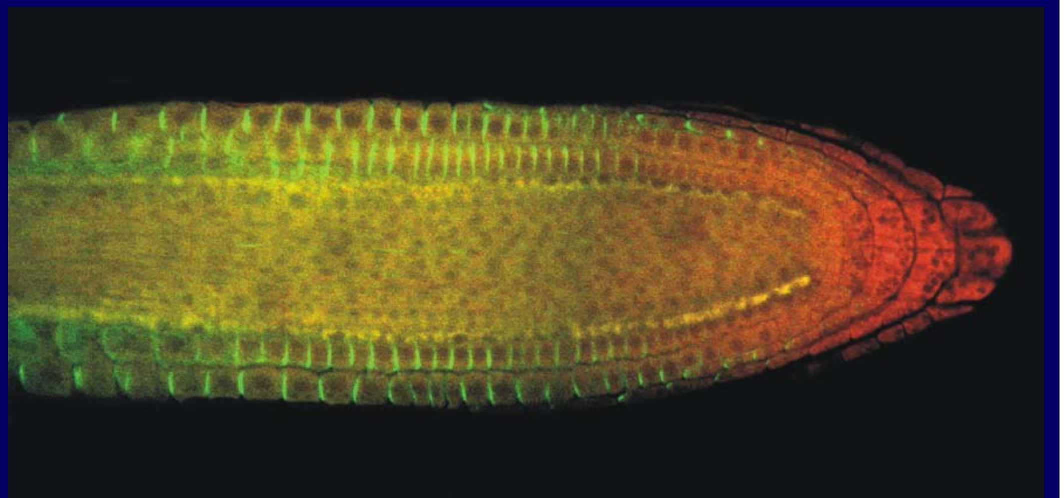
gravity + NPA



Statoliths
- gravity perception

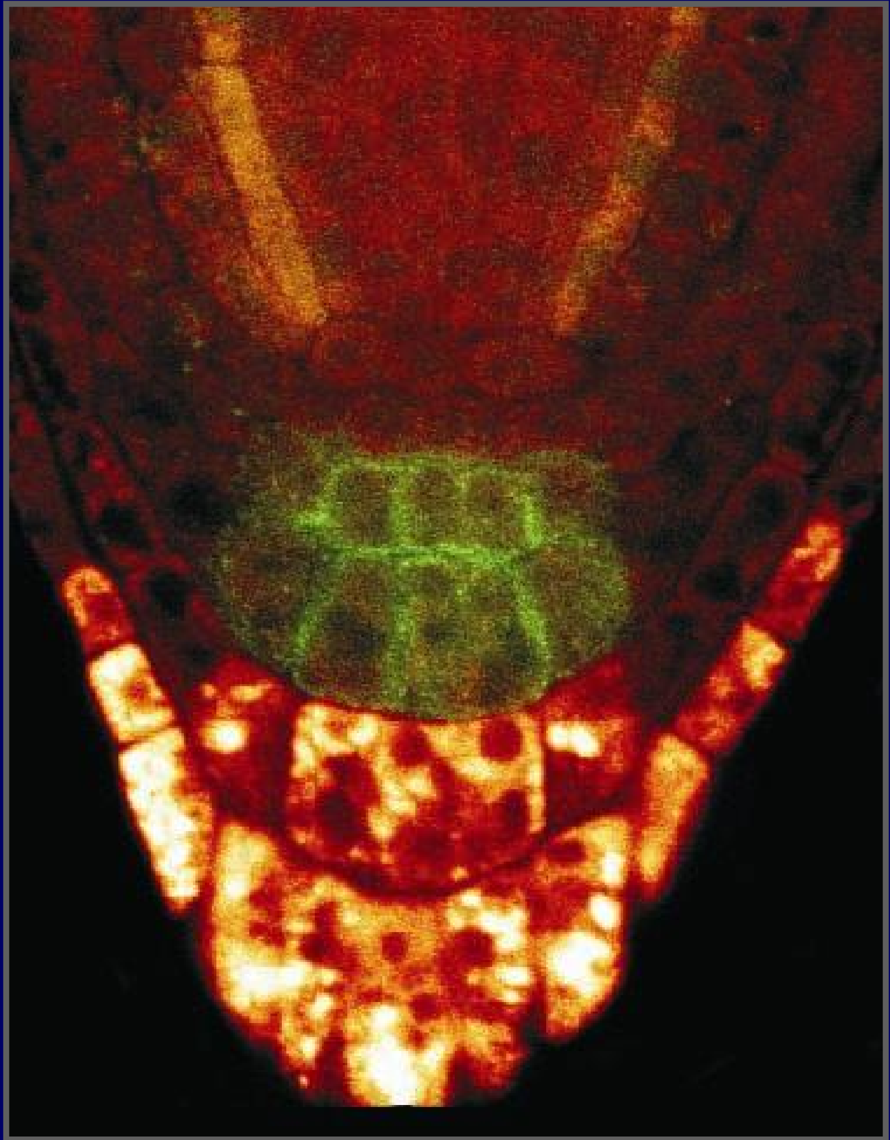


PIN2 localization

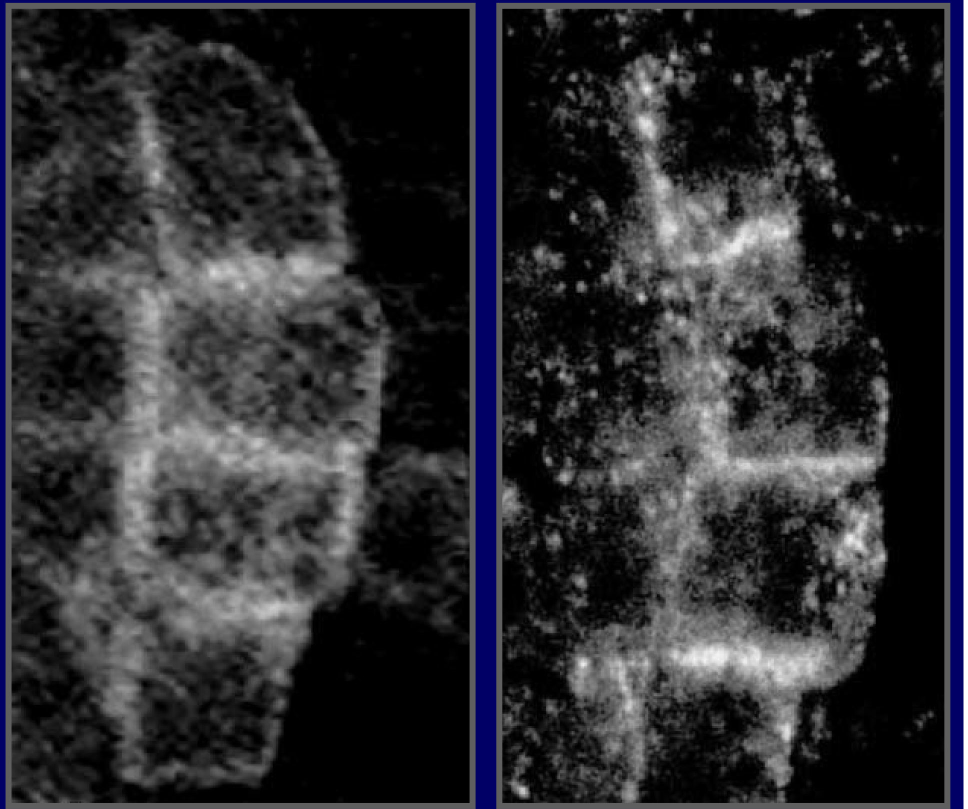


Relocation of PIN3 during Gravitropism

PIN3 in vertical root



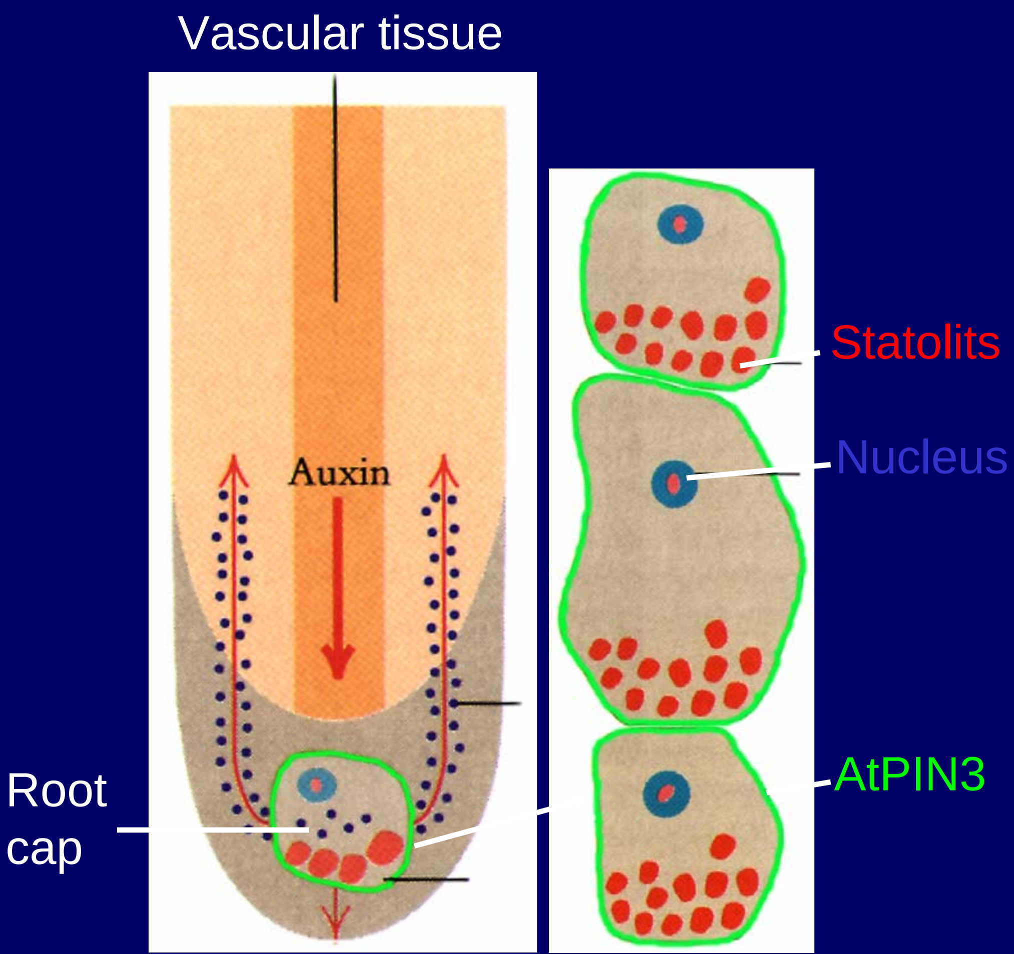
PIN3 in root on its side

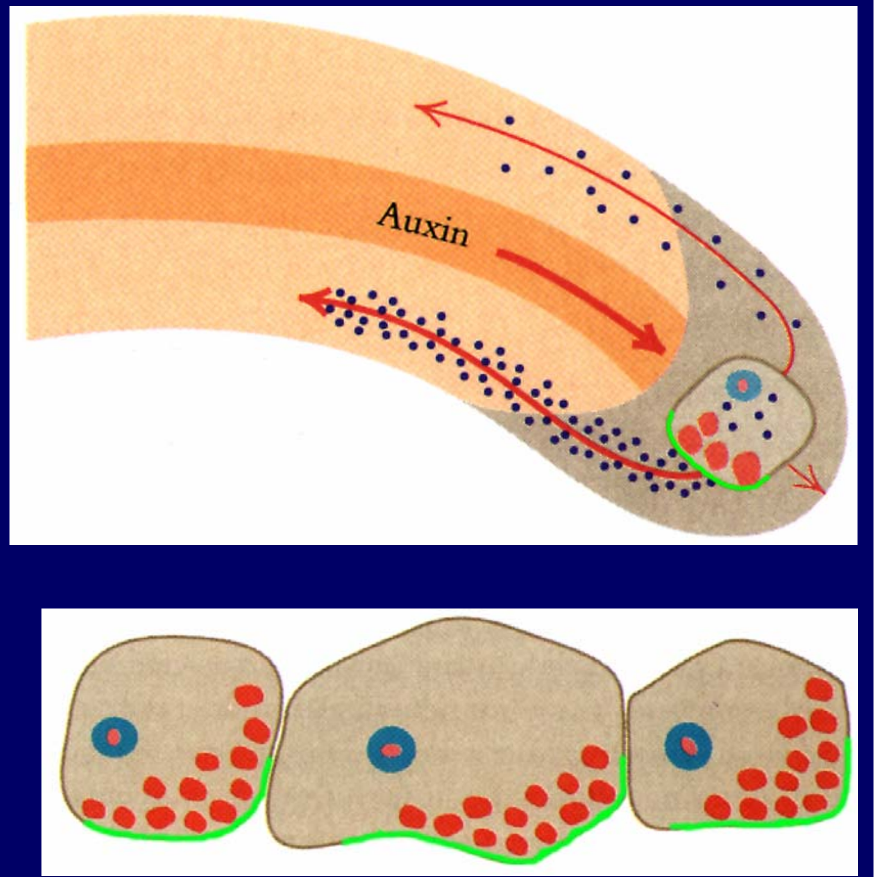


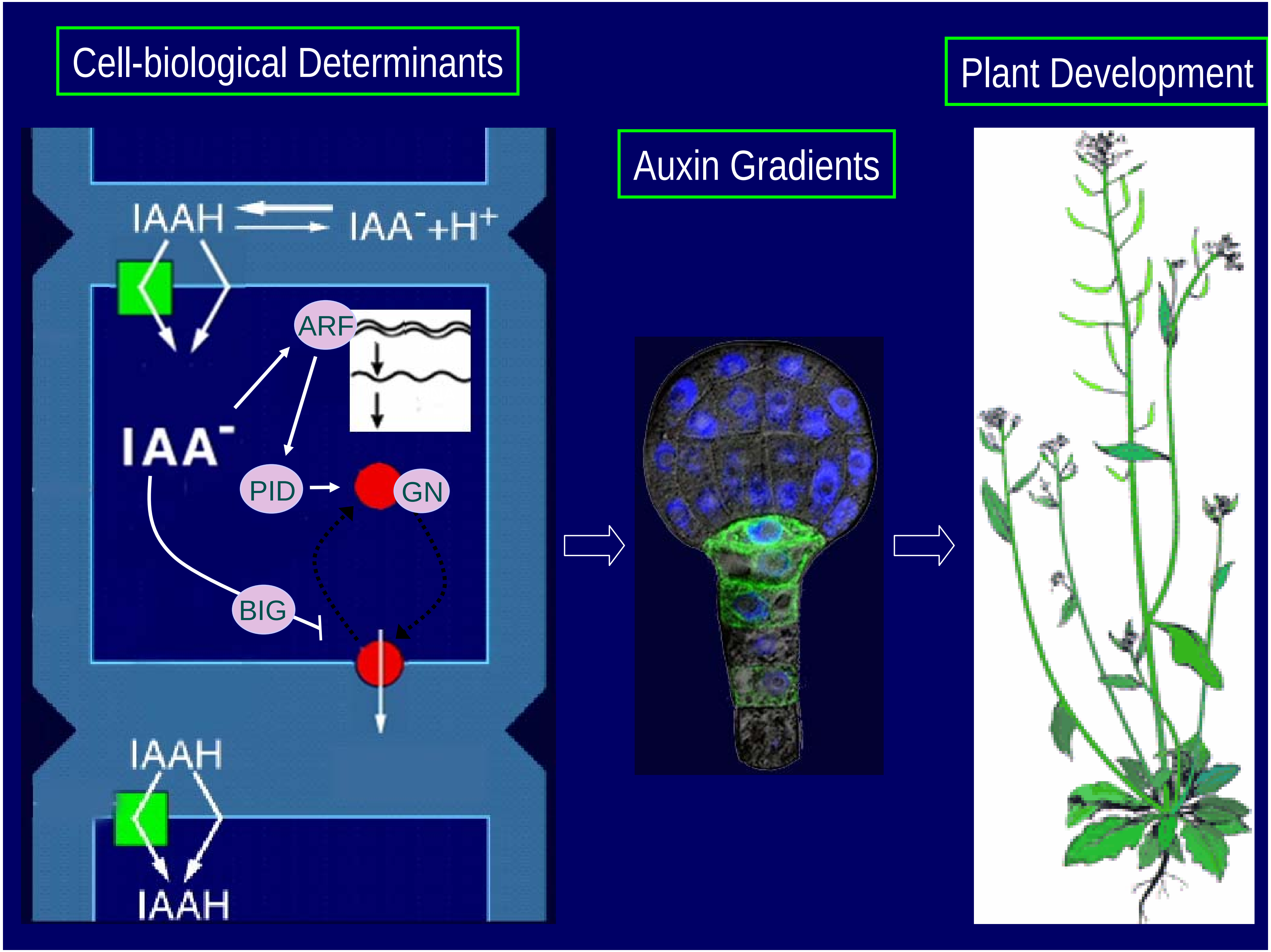
0 min **2 min**

Model for Root Gravitropism

Vertical root | **Root turned on its side**






Cell-biological Determinants
Auxin Gradients
Plant Development
IAAH
IAA⁻+H⁺
ARF
IAA⁻
PID
GN
BIG
IAAH
IAAH