GNOM and Auxin Transport

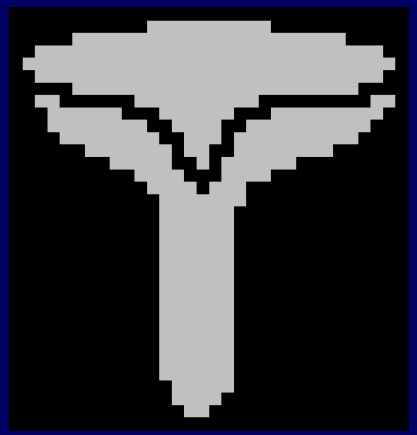
gnom Phenotype is Mimicked by Alteration of Auxin Homeostasis

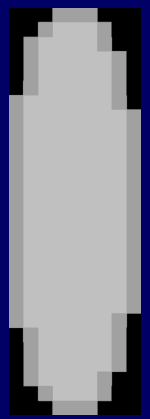
split collar cotyledon

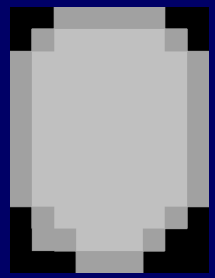
collar cotyledon

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

collar cotyledon

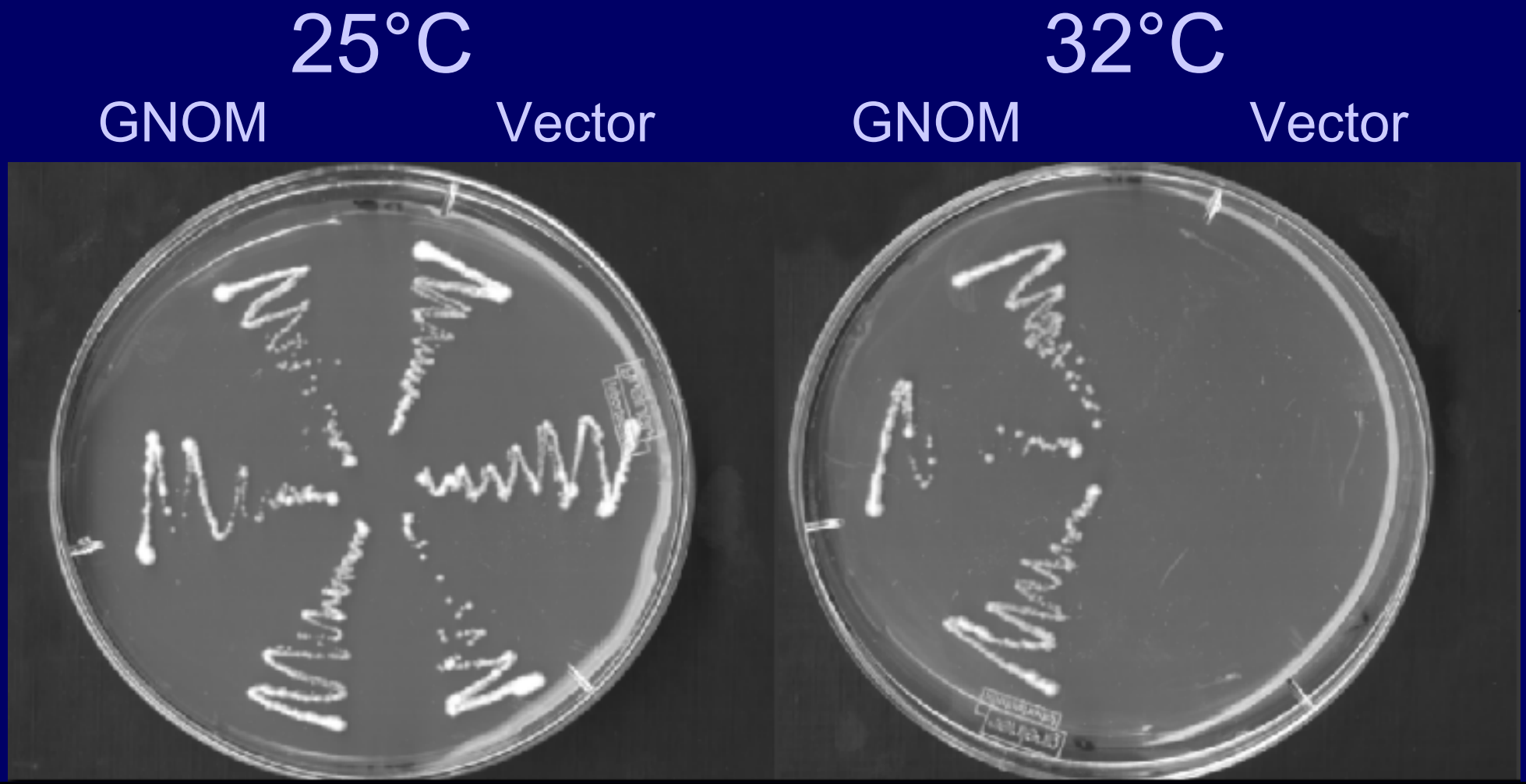
split collar cotyledon

cucumber

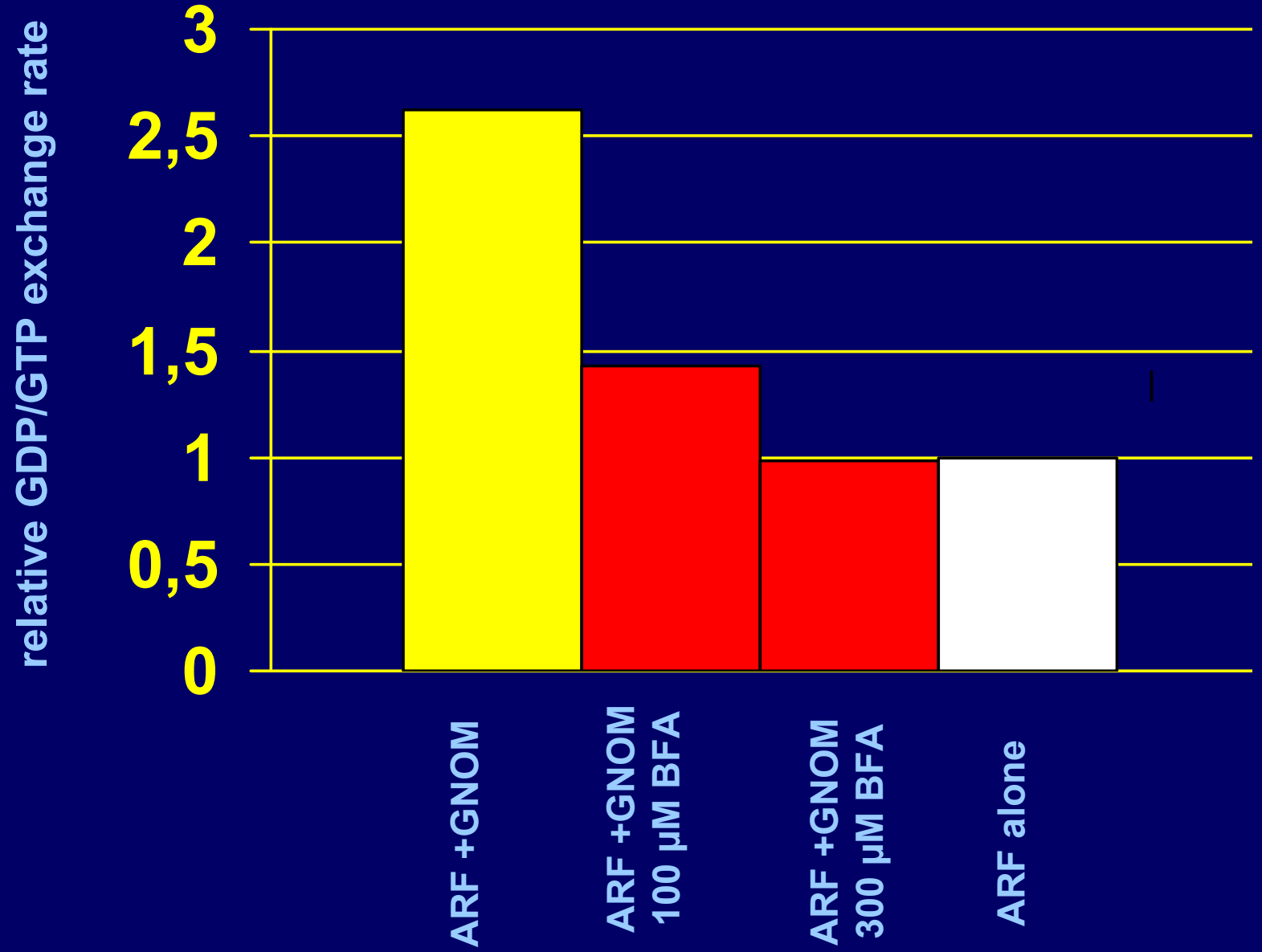
ball/egg

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

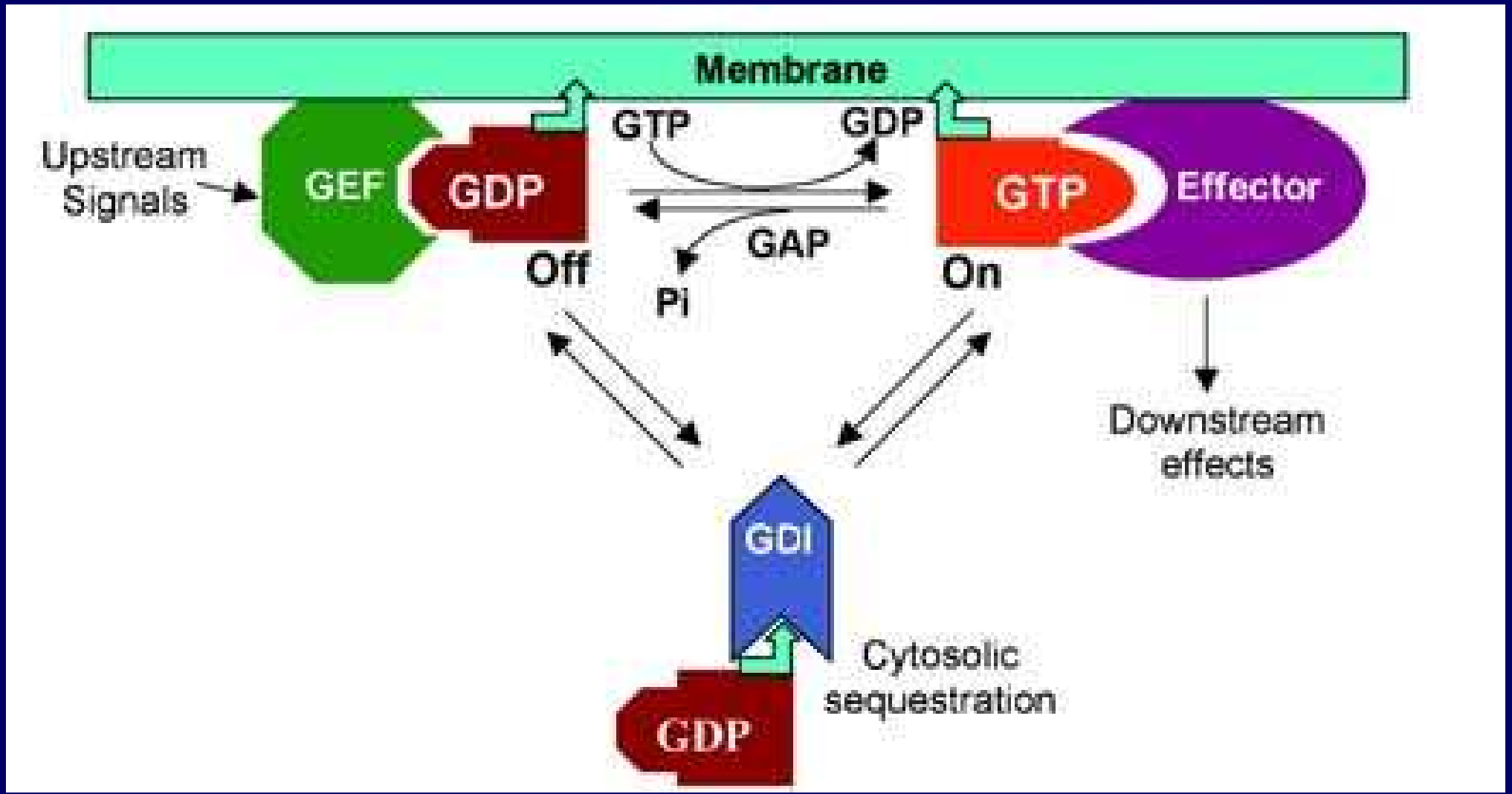
GNOM rescues gea2Δ, gea1-19 temperature sensitive yeast strains



GNOM Has BFA Sensitive GDP/GTP Exchange Activity on ARF



Small GTPases – Universal Switchers



Secretory pathway

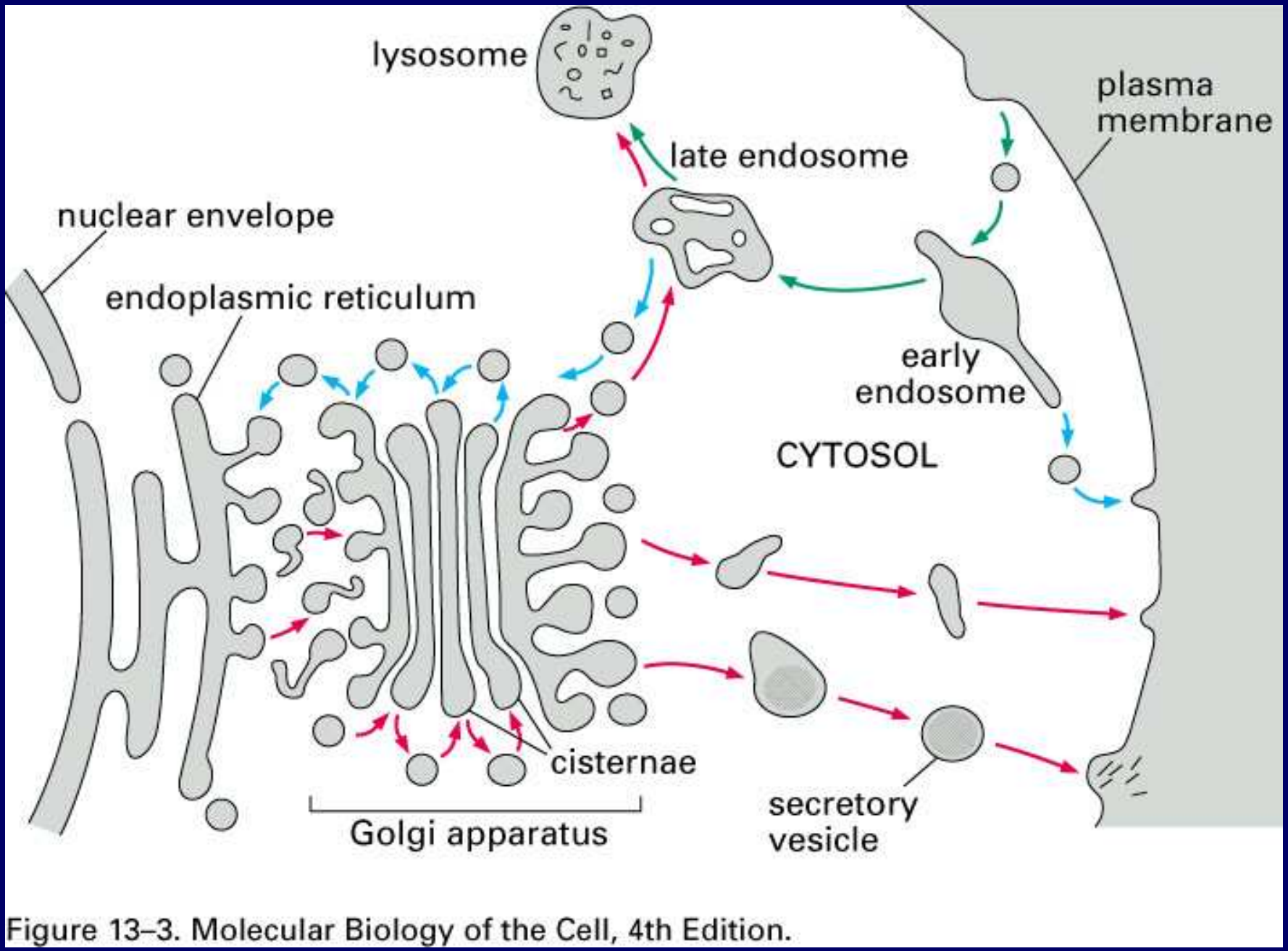
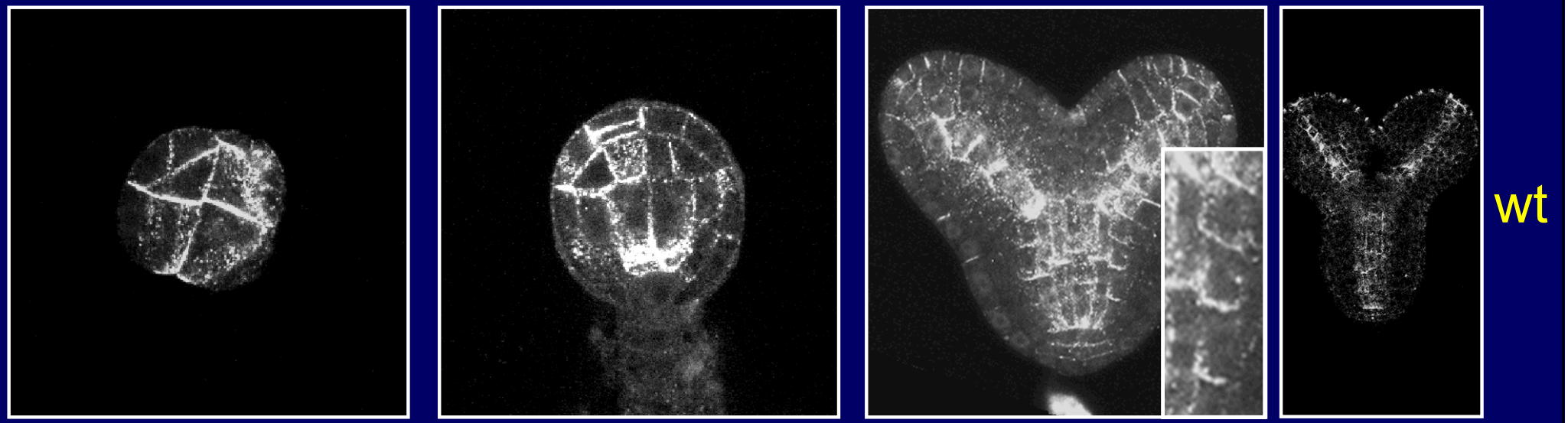
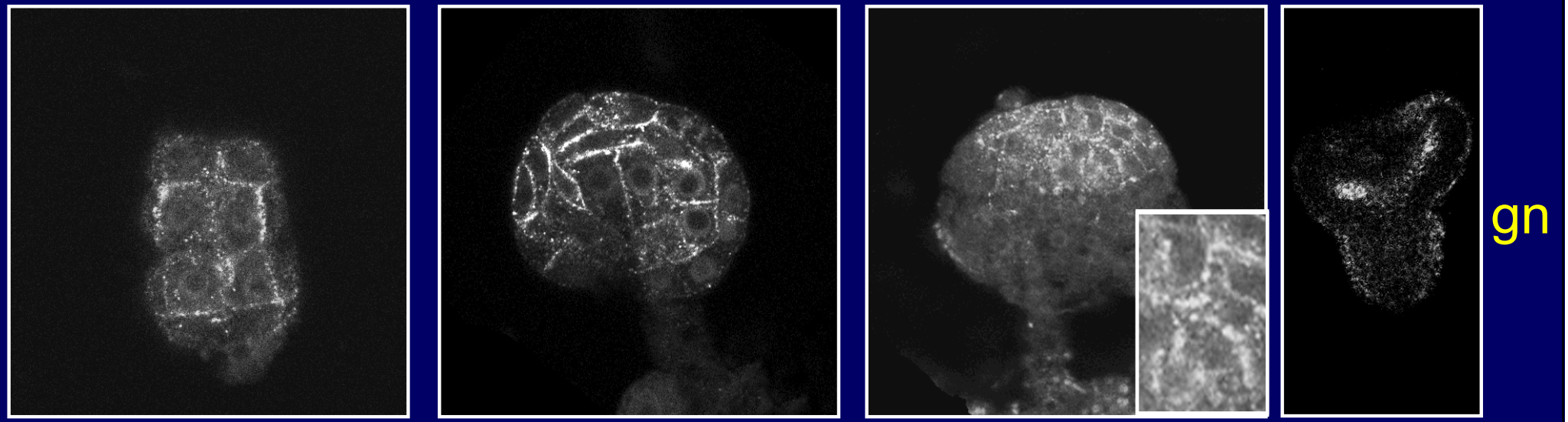


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Establishment of coordinated PIN1 polarity is disrupted in gnom embryos



wt



gn

PIN Proteins Cycling

BFA reveals subcellular movement of PIN1

PIN1 cycles between endosome and plasma membrane

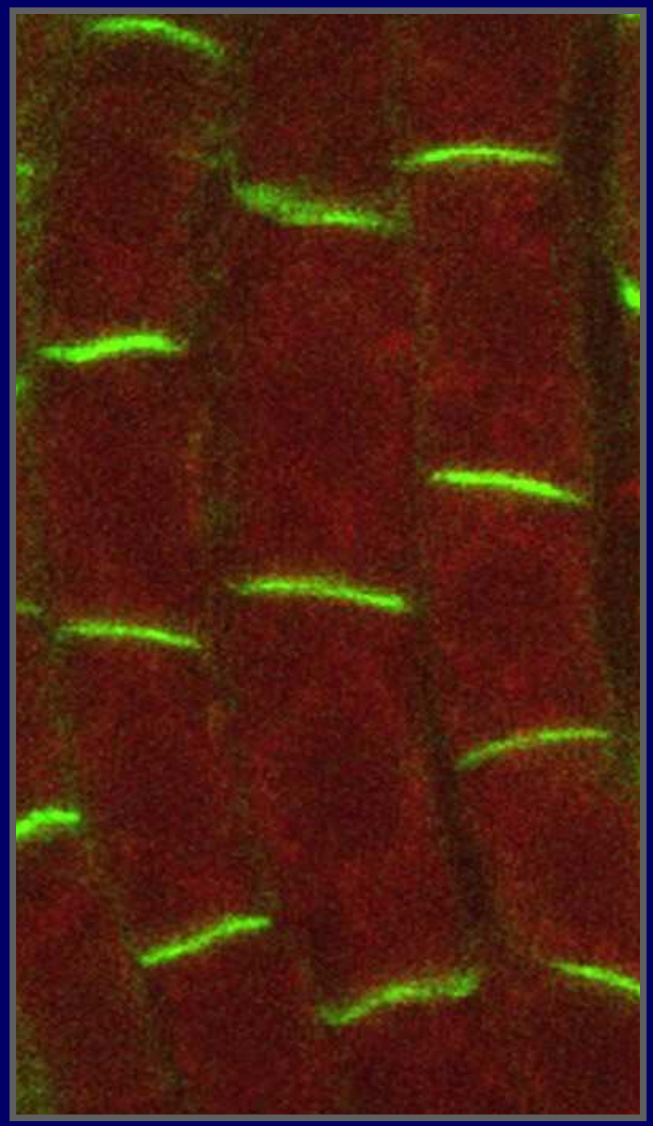
PIN1 cycling is actin but not tubulin dependent

Auxin transport inhibitors interfere with protein trafficking

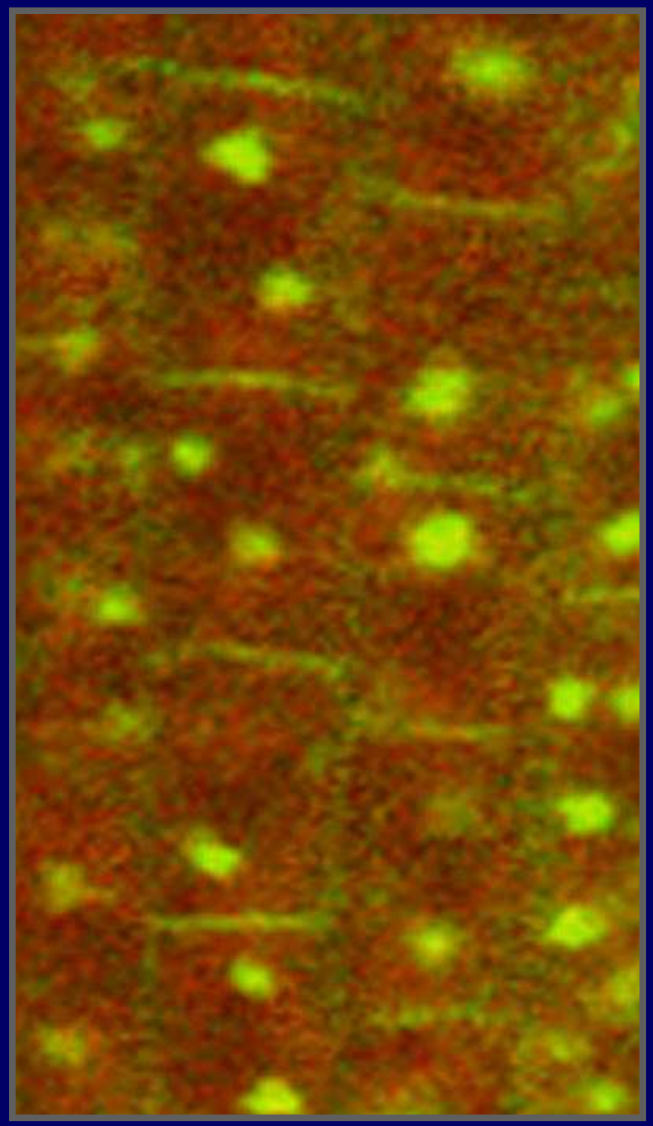
Role for cycling – root gravitropism

PIN1 Subcellular Movement

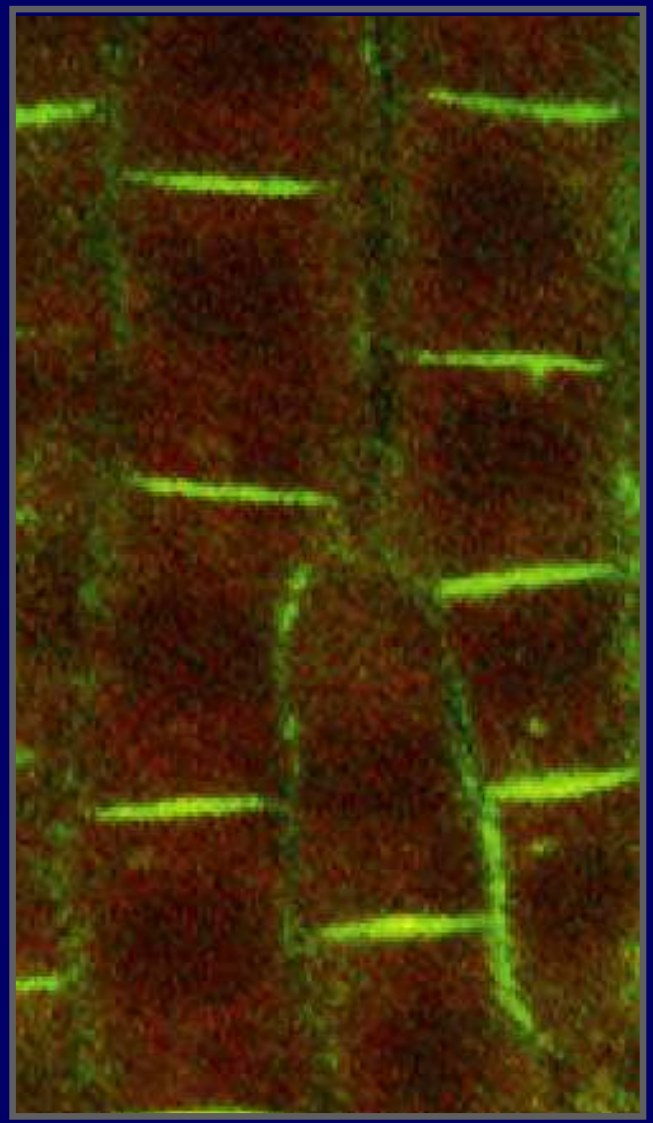
untreated



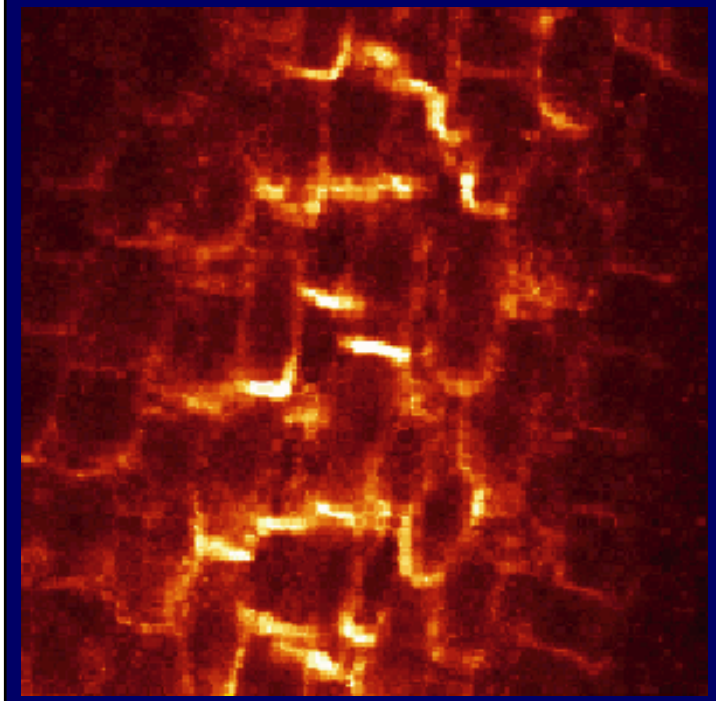
+ BFA



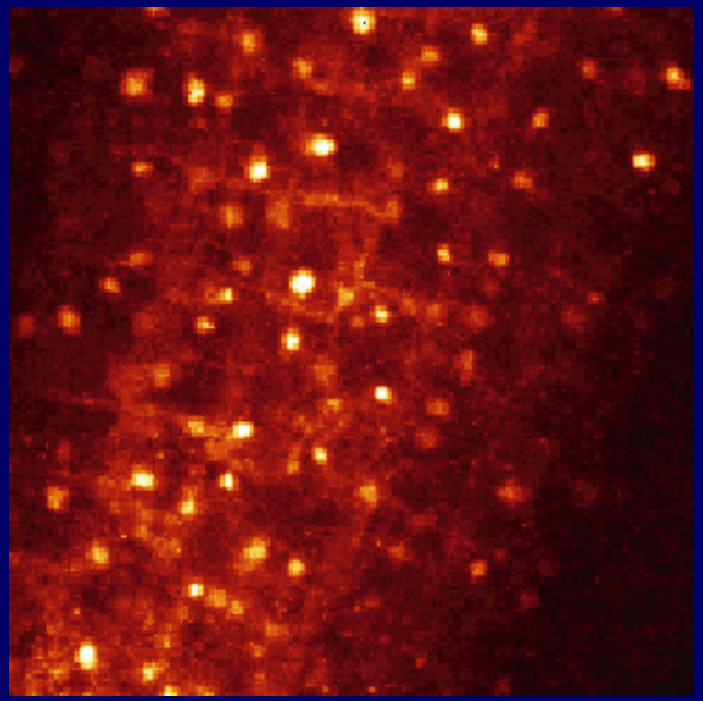
- BFA



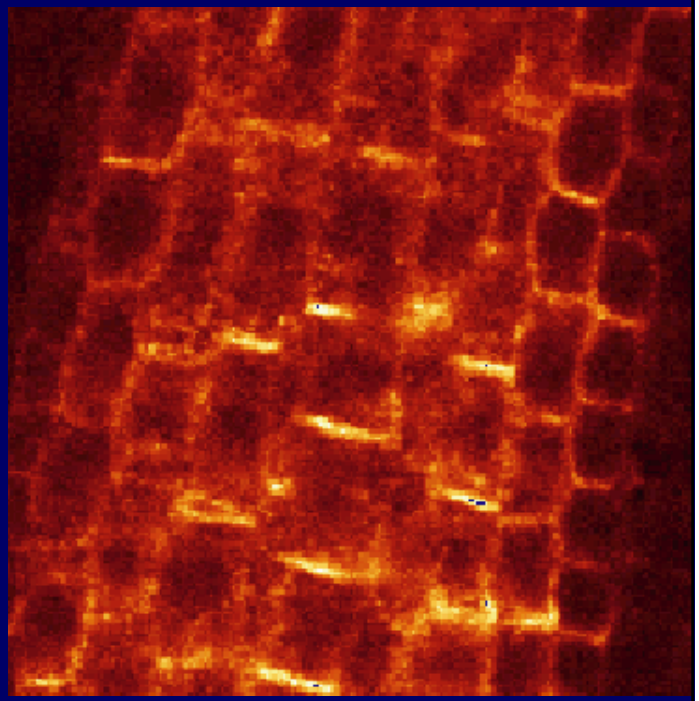
Intracellular Cycling of AtPIN1 is Independent of Newly Synthesised Protein



Cycloheximide

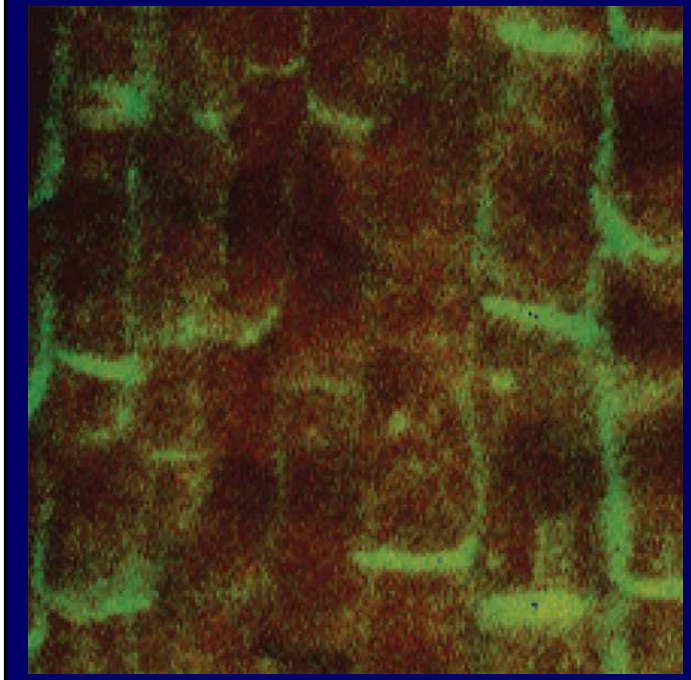


Cycloheximide + BFA

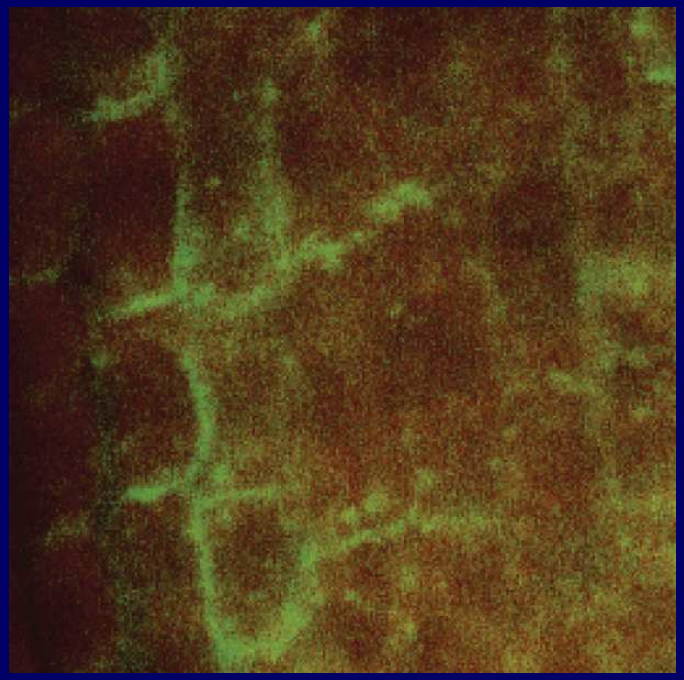


BFA + Cycloheximide

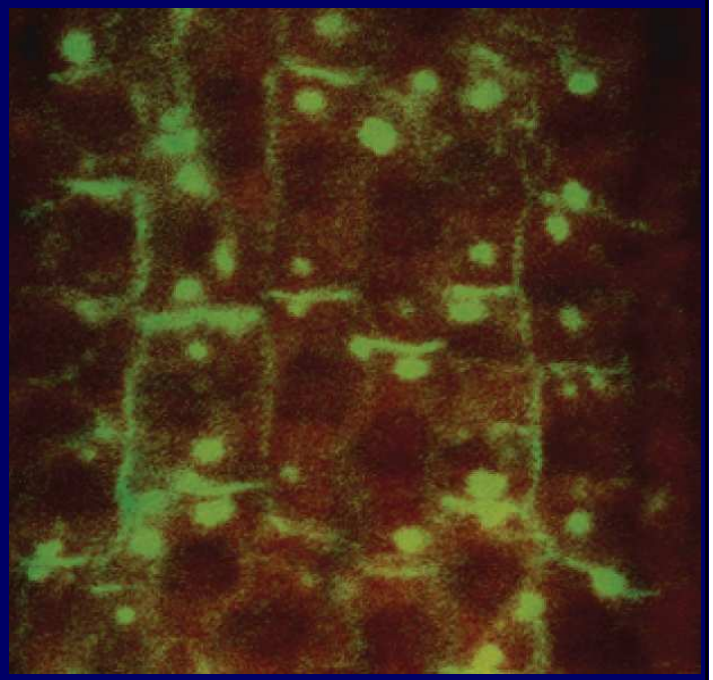
PIN1 Cycling is Actin Dependent



Cytochelasin D



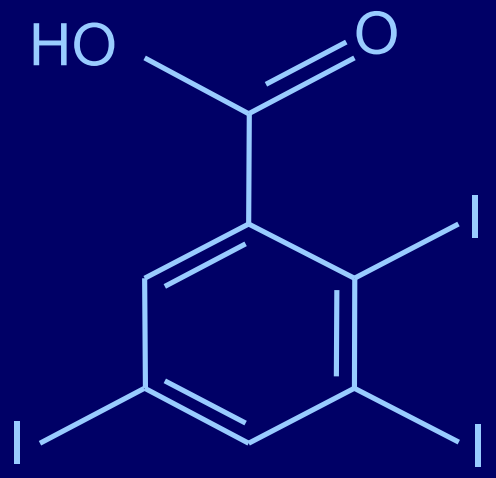
Cytochelasin D + BFA



BFA + Cytochelasin D

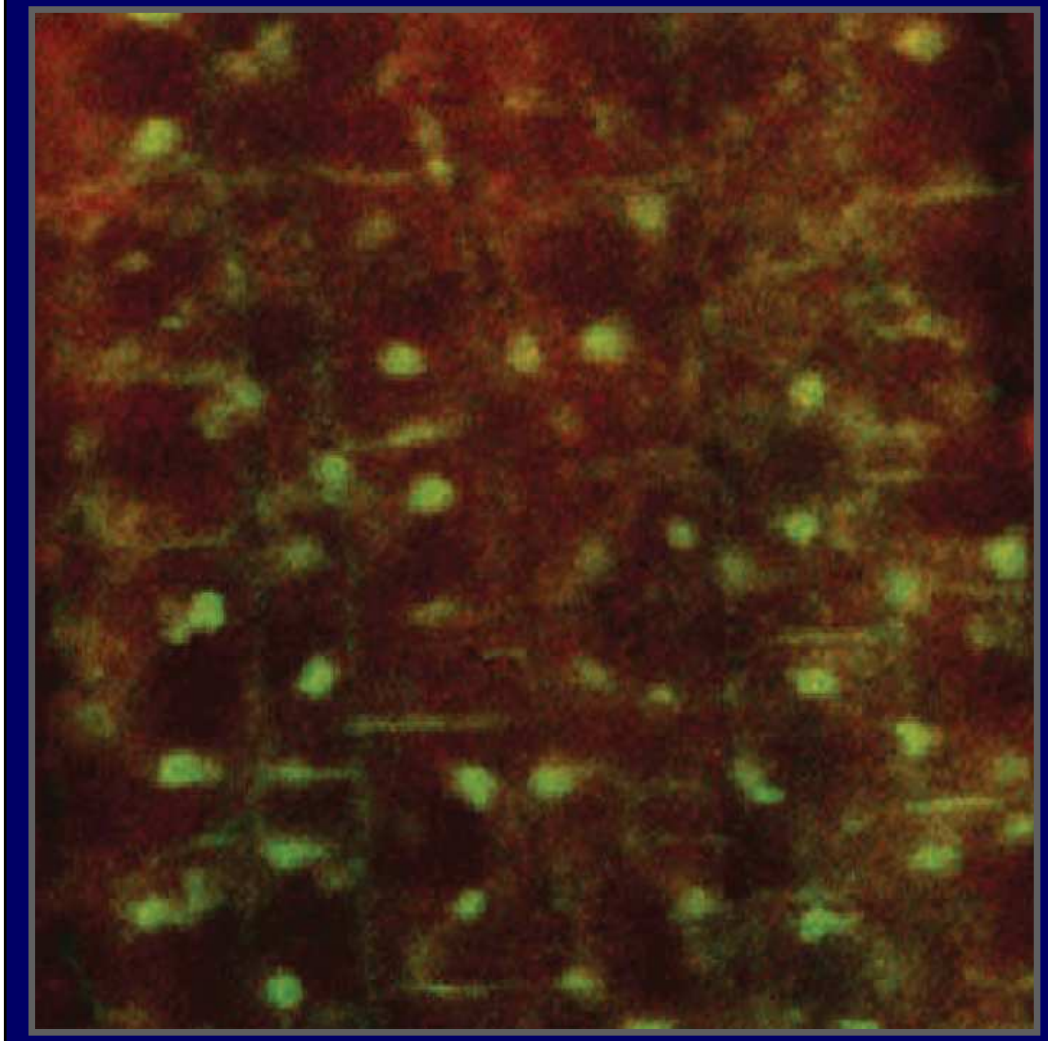
What Are the Effects of Auxin Transport Inhibitors?

NPA

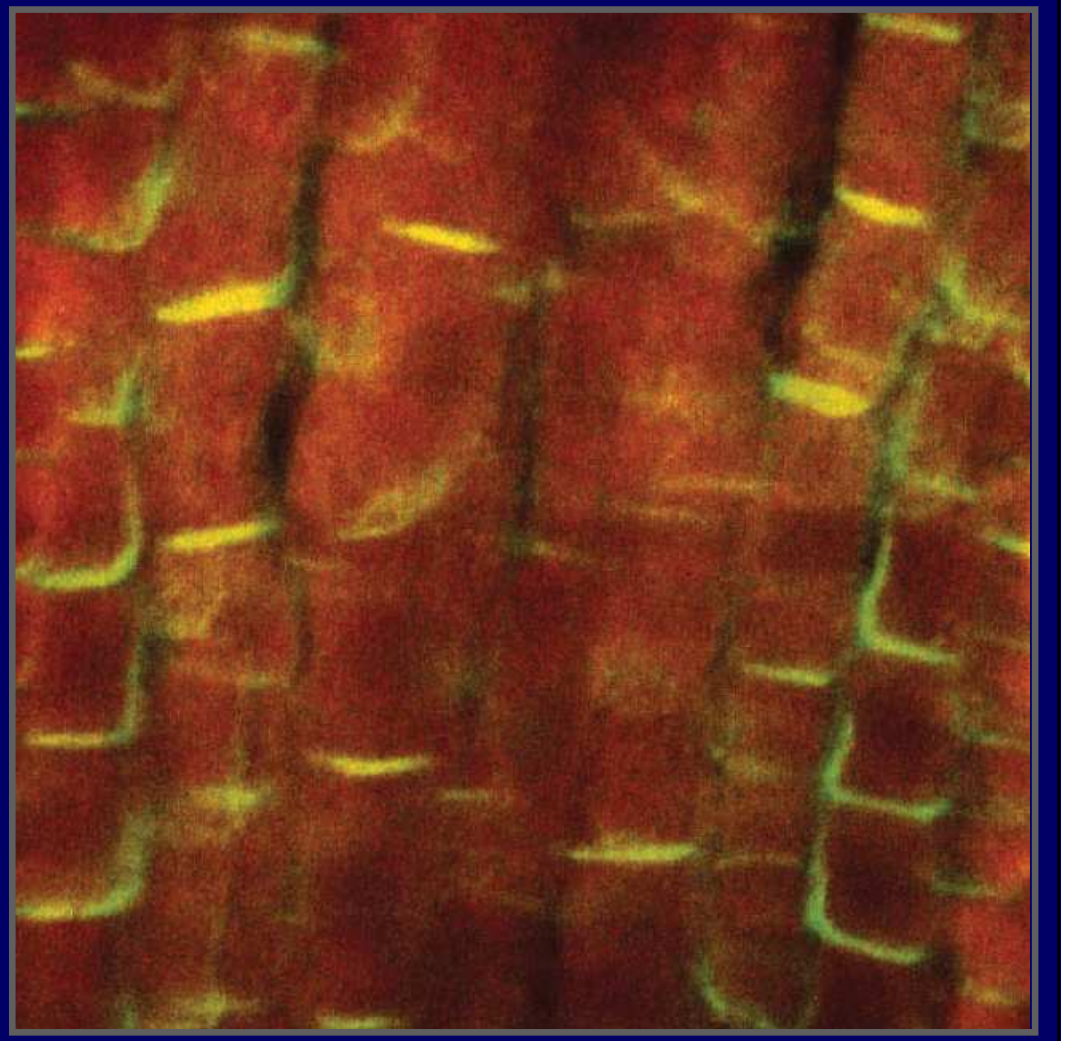


TIBA

PIN1 Exocytosis is Disrupted by Auxin Transport Inhibitors

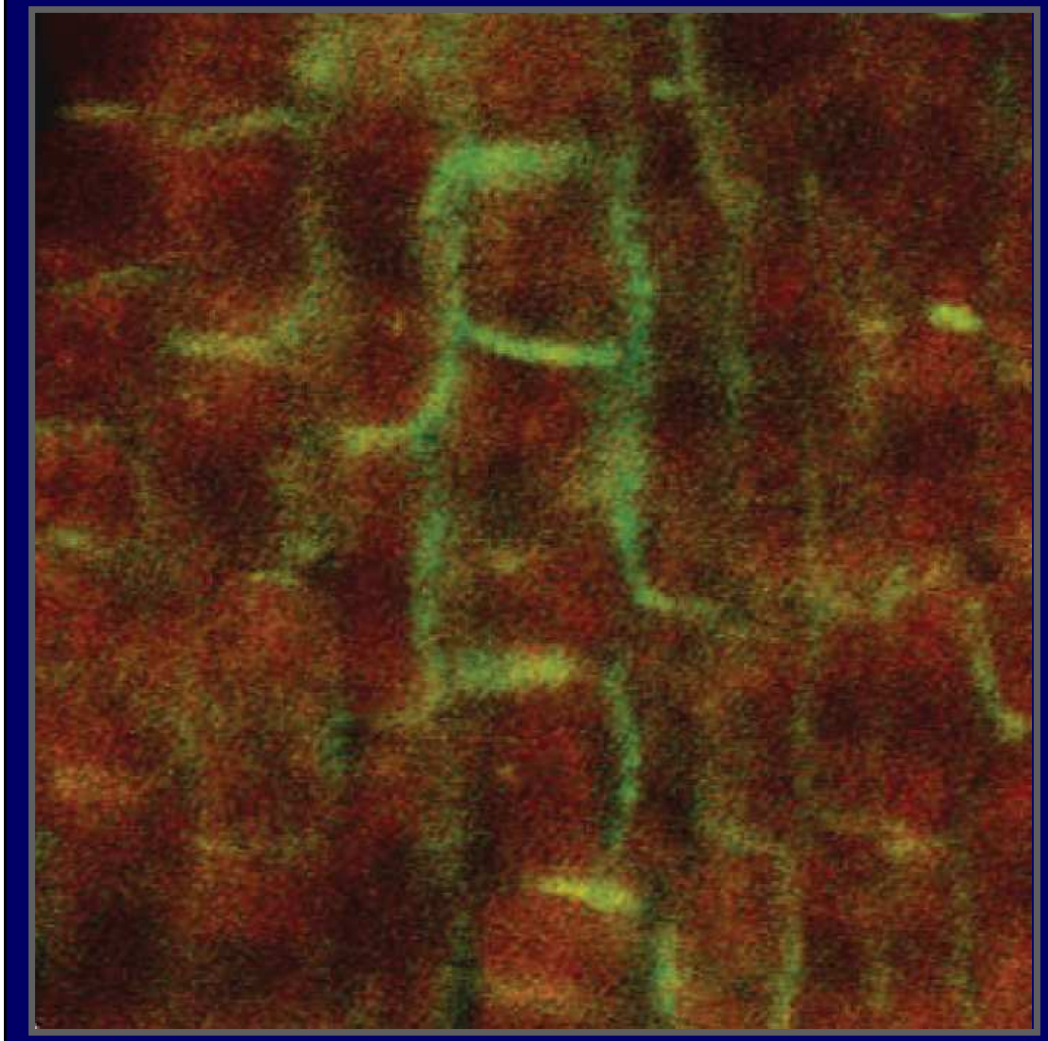


BFA + TIBA

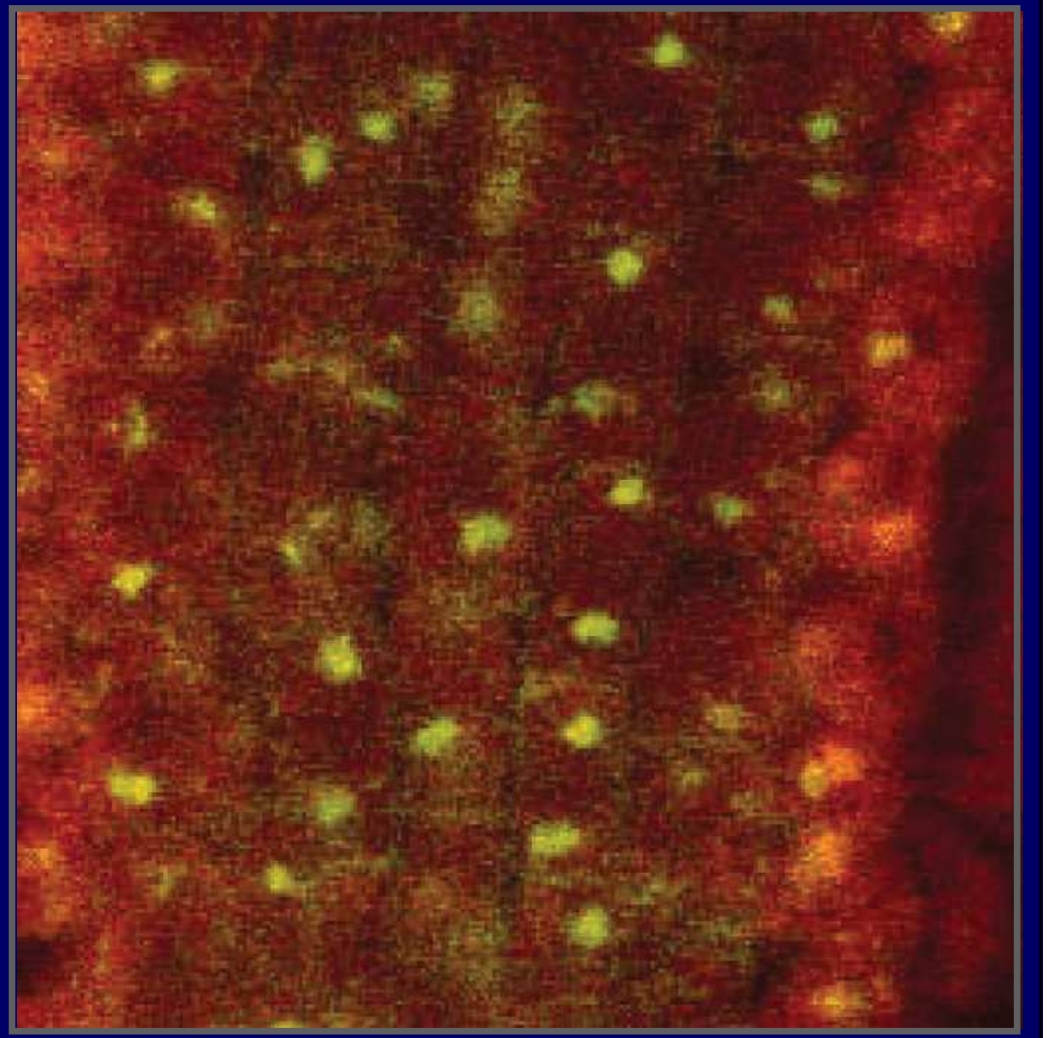


BFA + benzoic acid

PIN1 Endocytosis is Disrupted by Auxin Transport Inhibitors

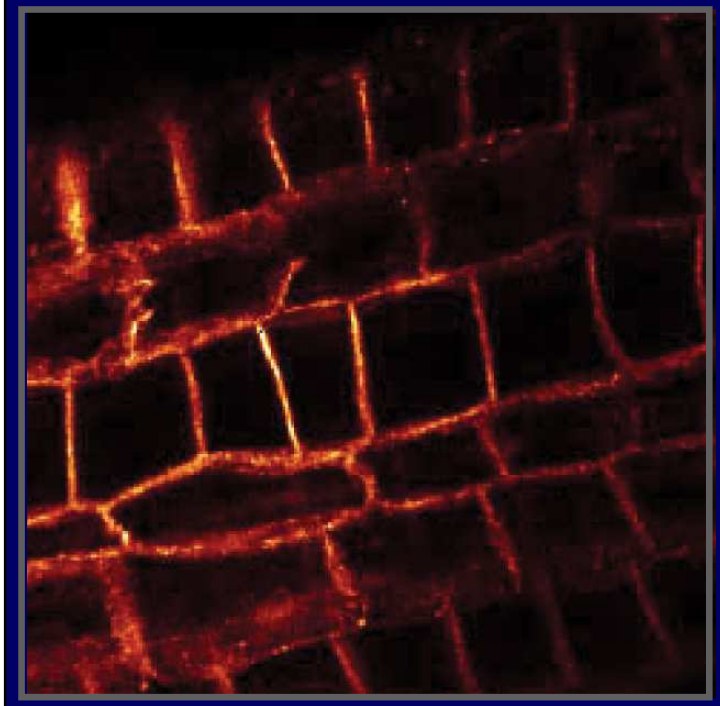


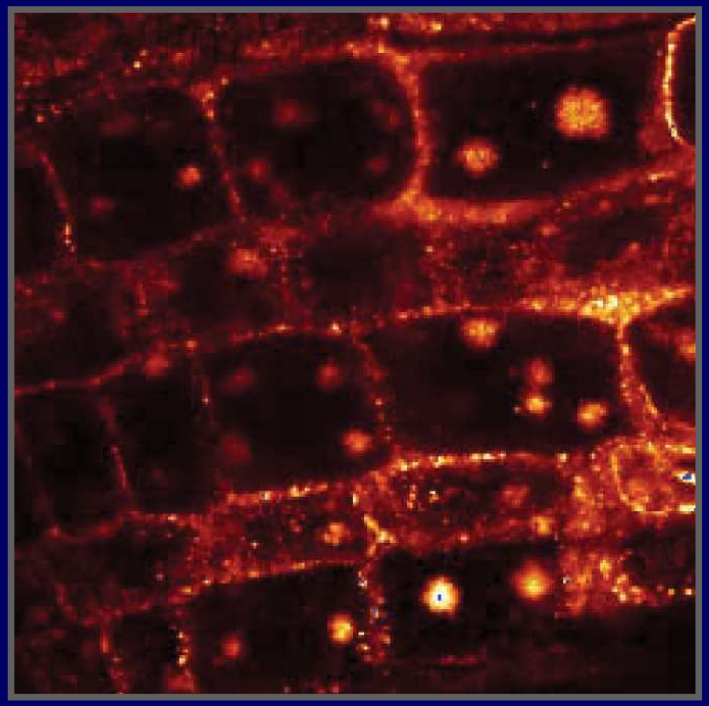
TIBA + BFA

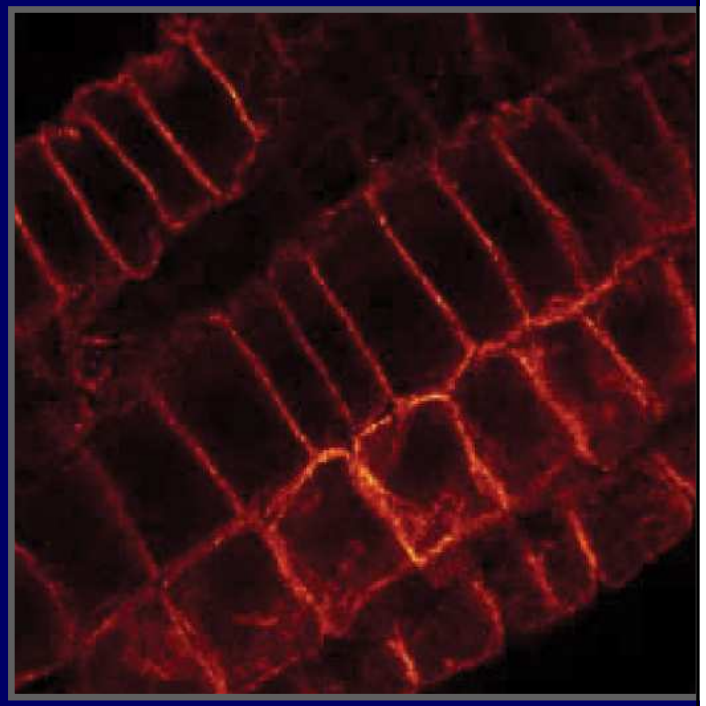


benzoic acid + BFA

Plasma Membrane ATPase Cycling is Blocked by TIBA





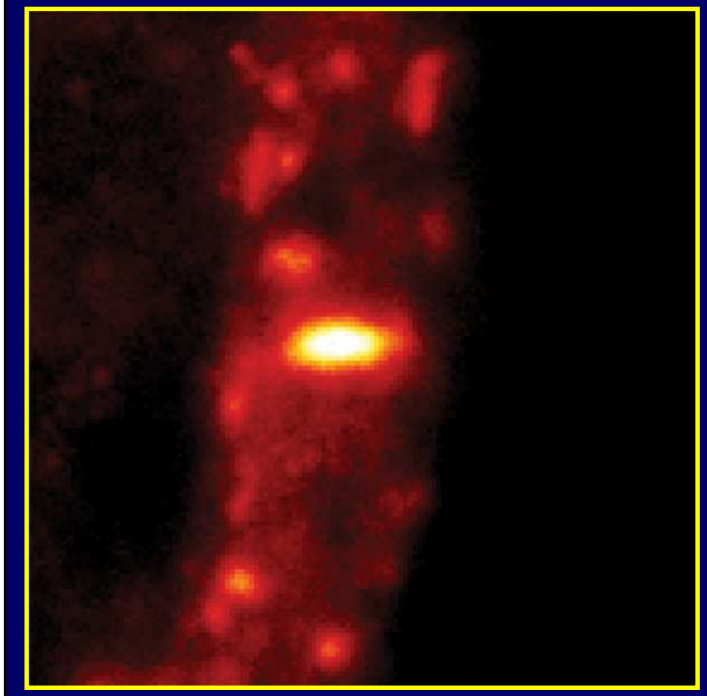


No treatment

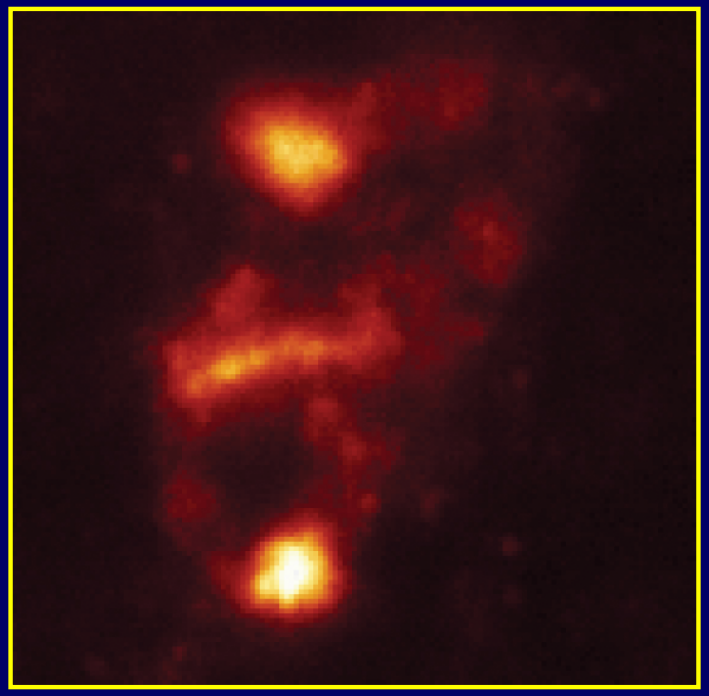
BFA

TIBA + BFA

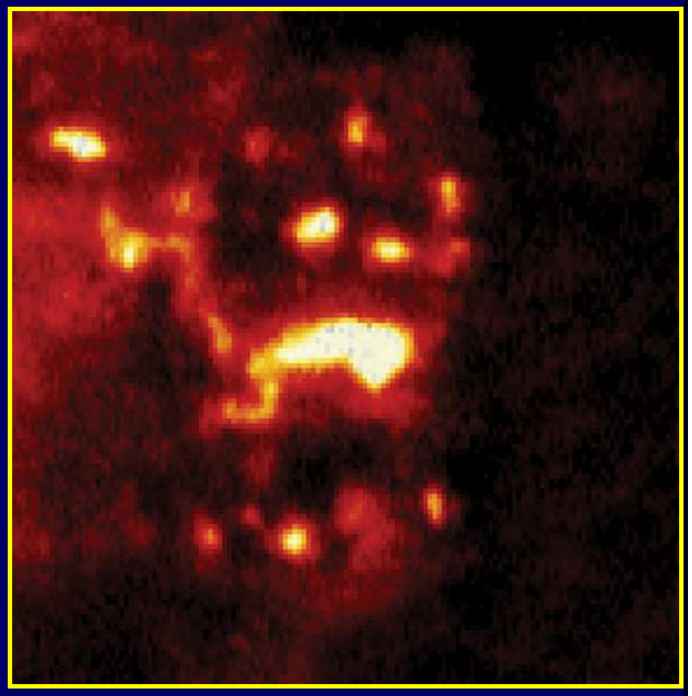
KNOLLE Syntaxin Cycling is Blocked by TIBA



No treatment



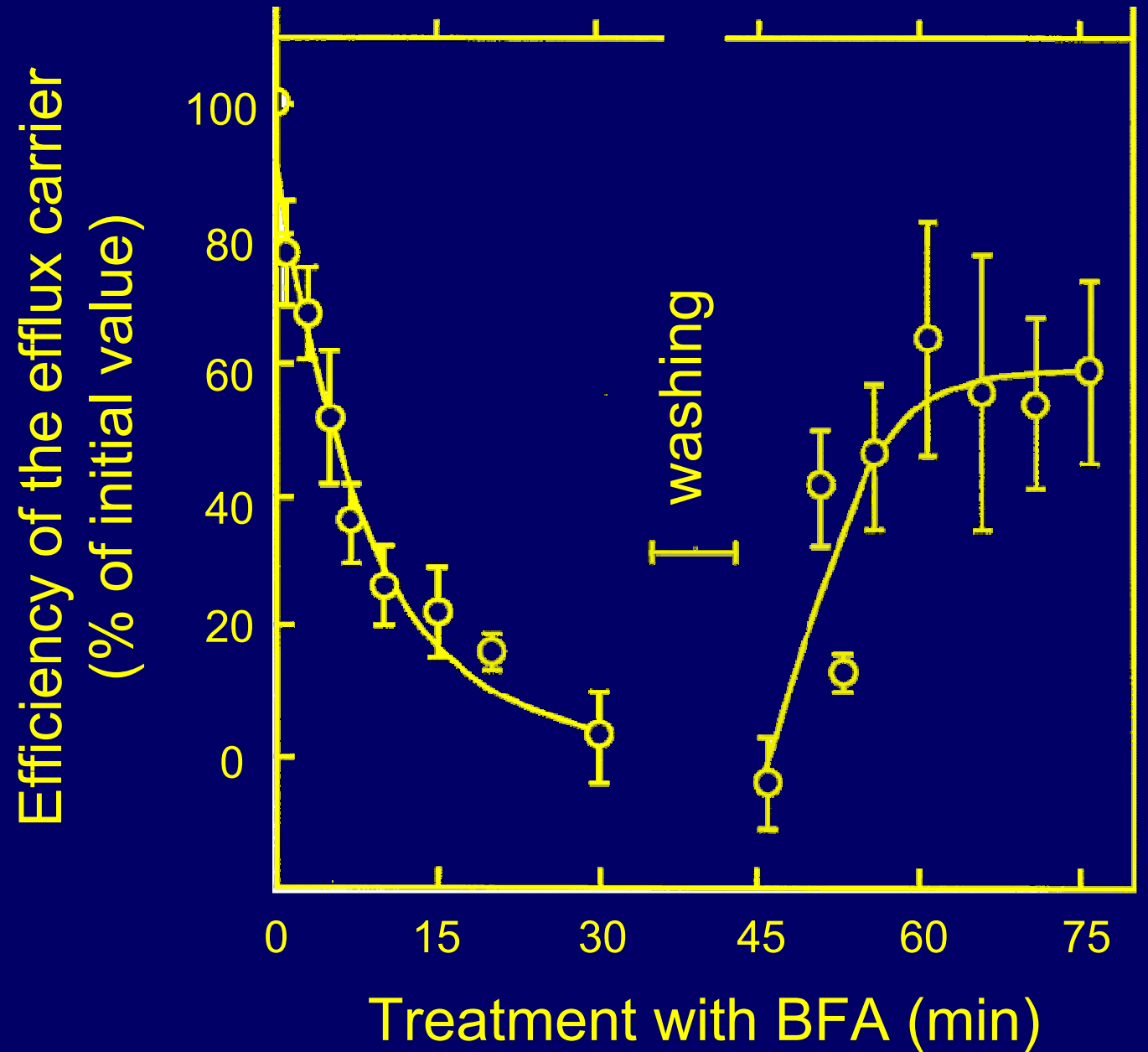
BFA



TIBA + BFA

F1

BFA rapidly inhibits auxin efflux out of cells



data from
Delbarre et al., 1998

Snímek 18

F1 weissen Hintergrund wegmachen

Florence; 17.2.2002

Applying BFA to seedlings phenocopies a number of transport inhibition phenotypes

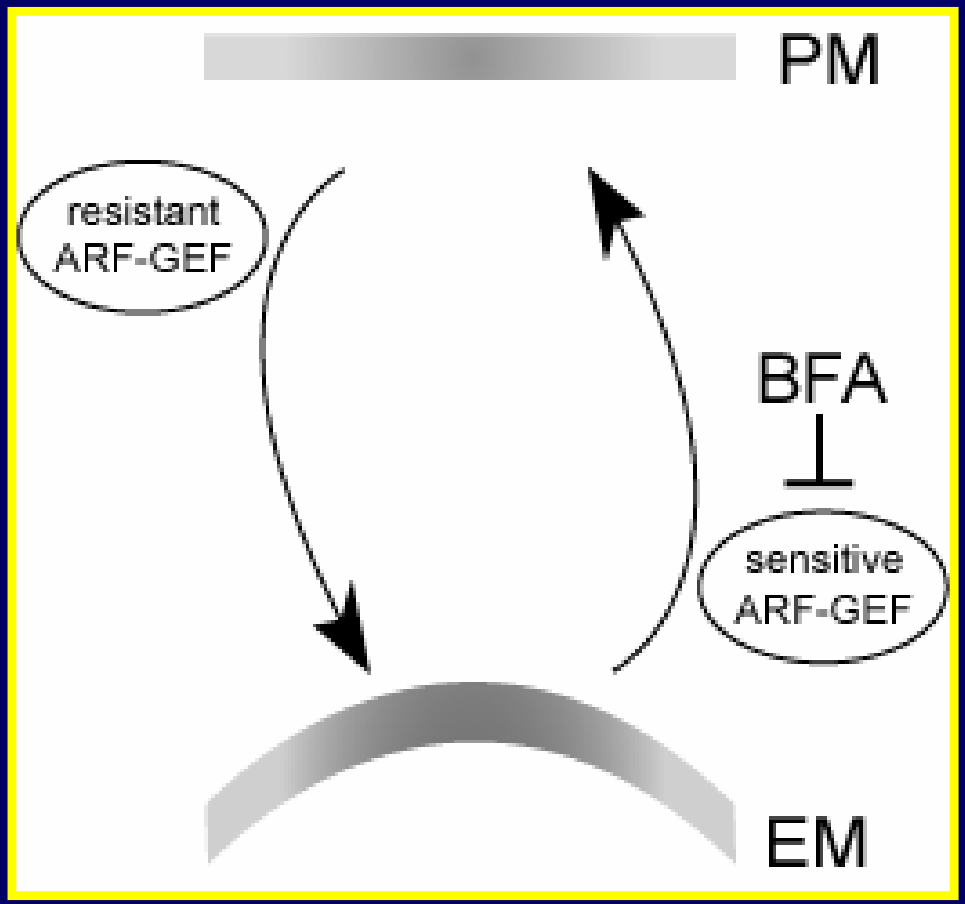
untreated

BFA 10 µM

BFA 20 µM

Hypothesis:

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



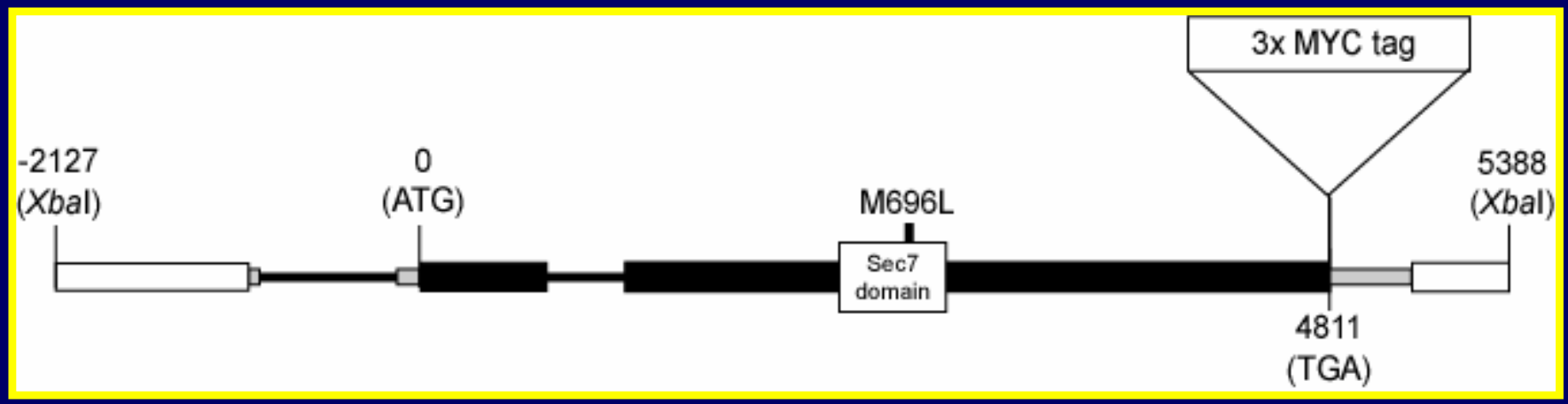
How can we show this?

Engineering BFA-resistant GNOM

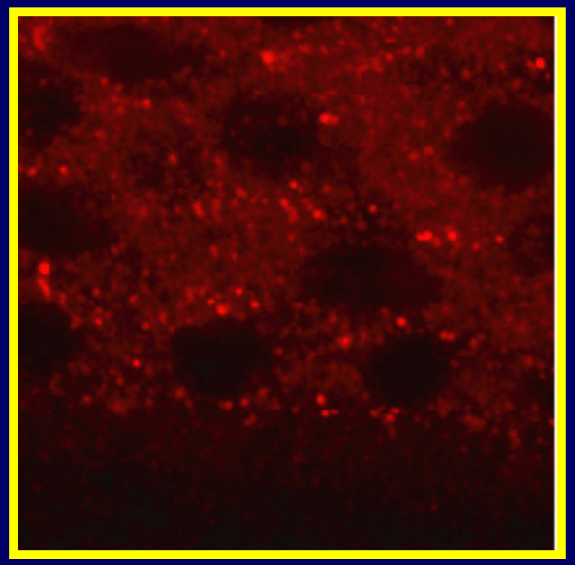
Amino Acid Residues of ARF-GEFs Conferring BFA Resistance

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?

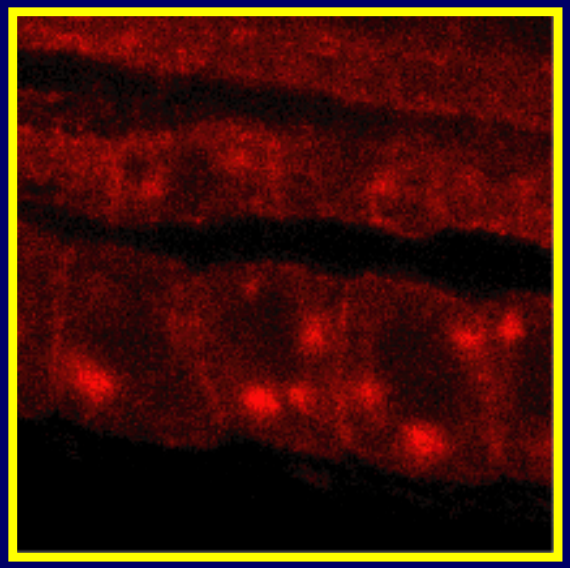
The engineered GNOM construct



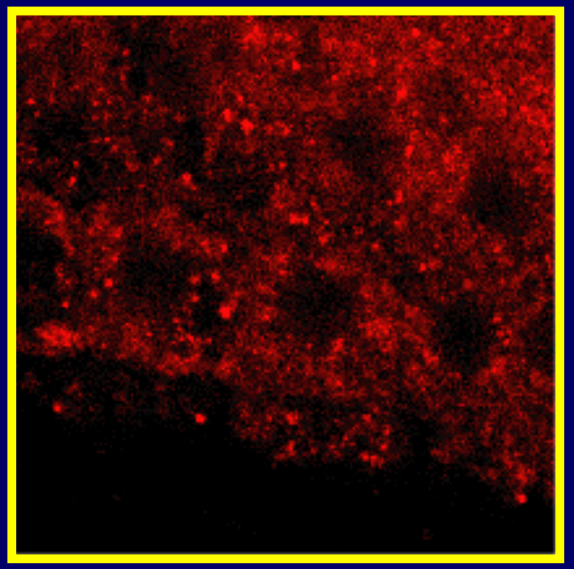
GNOM localises to the cytosol and some endomembrane compartment



control

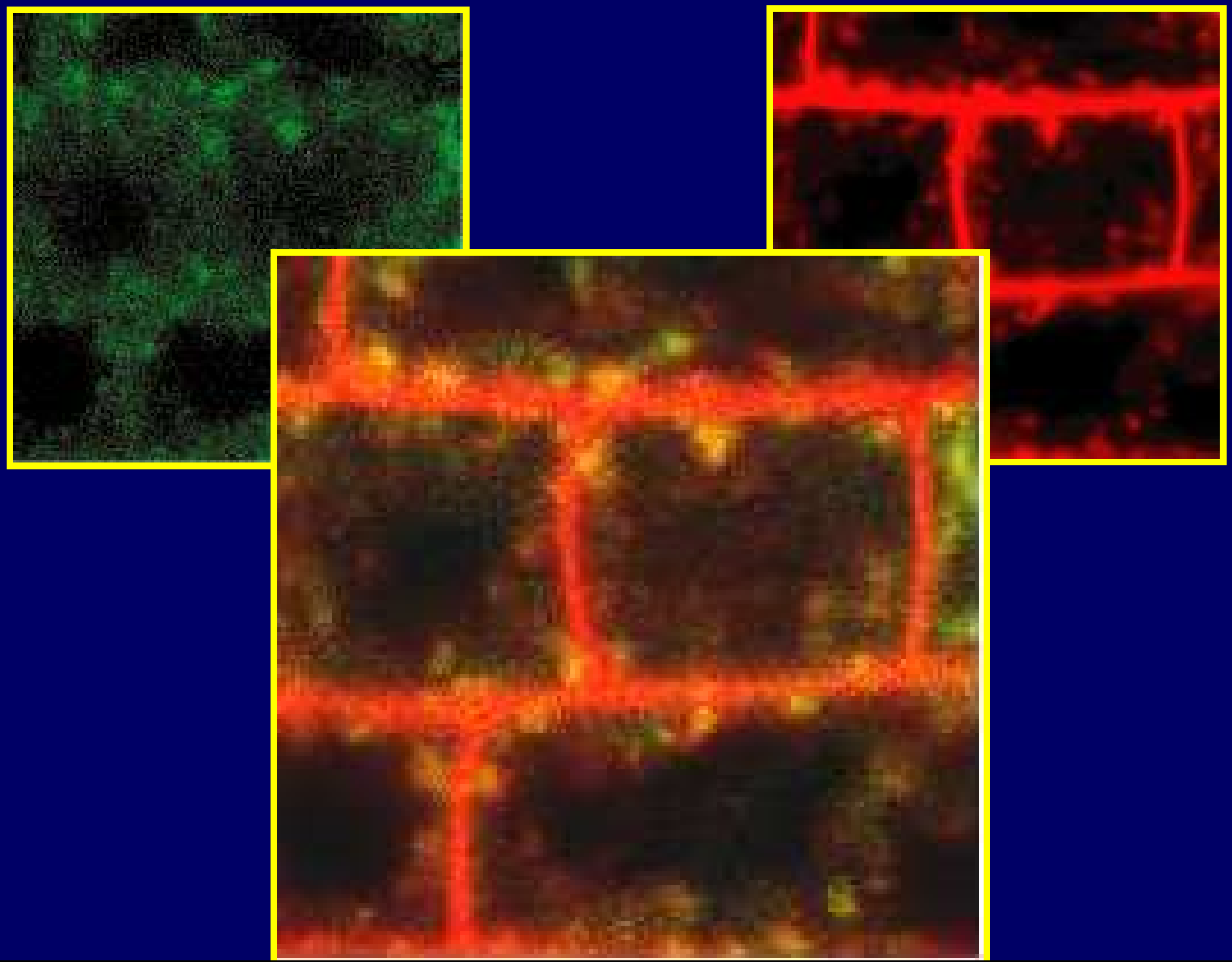


BFA, 60 min
GN^{wt}*-myc* line

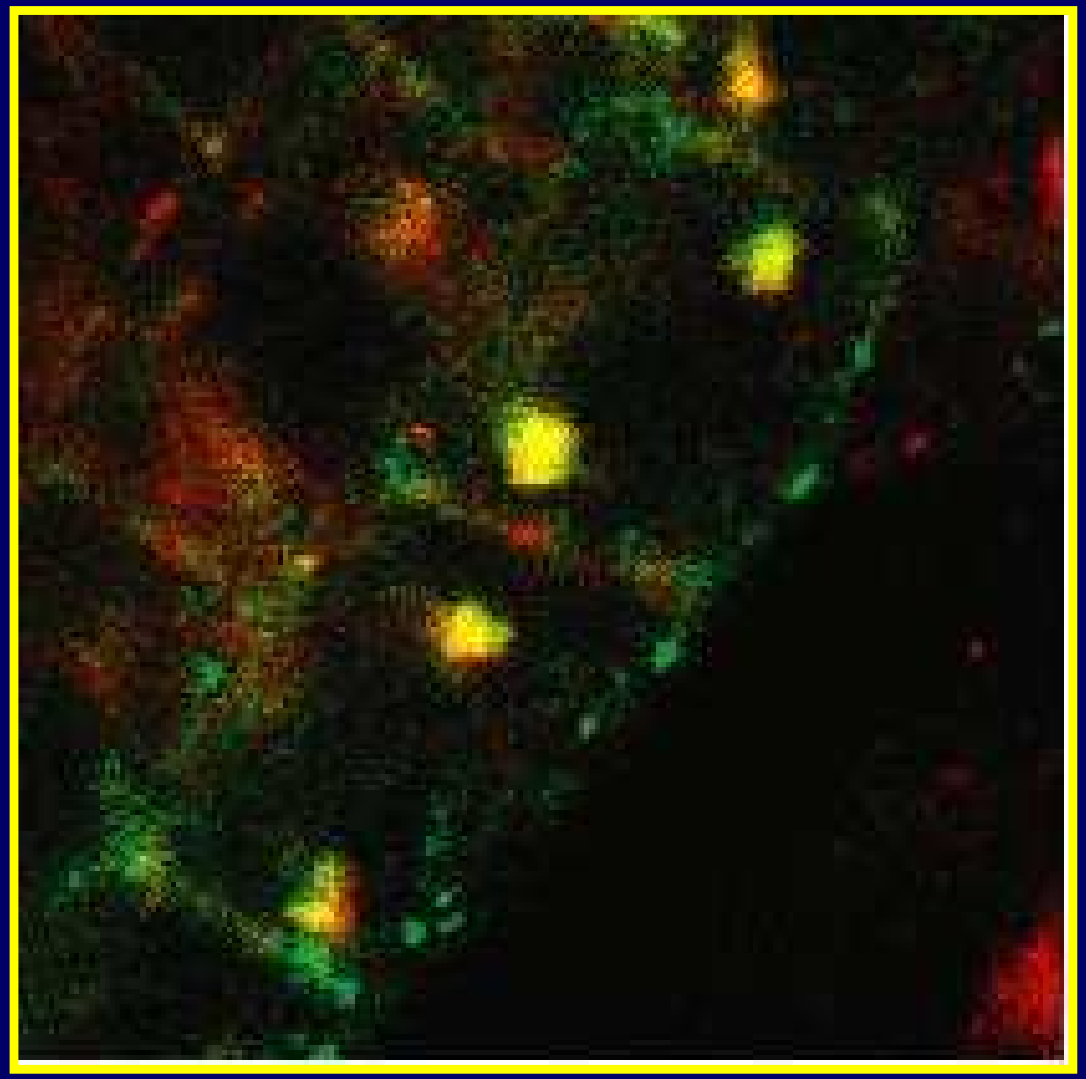


BFA, 60 min
GN^{M696L}*-myc* line

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

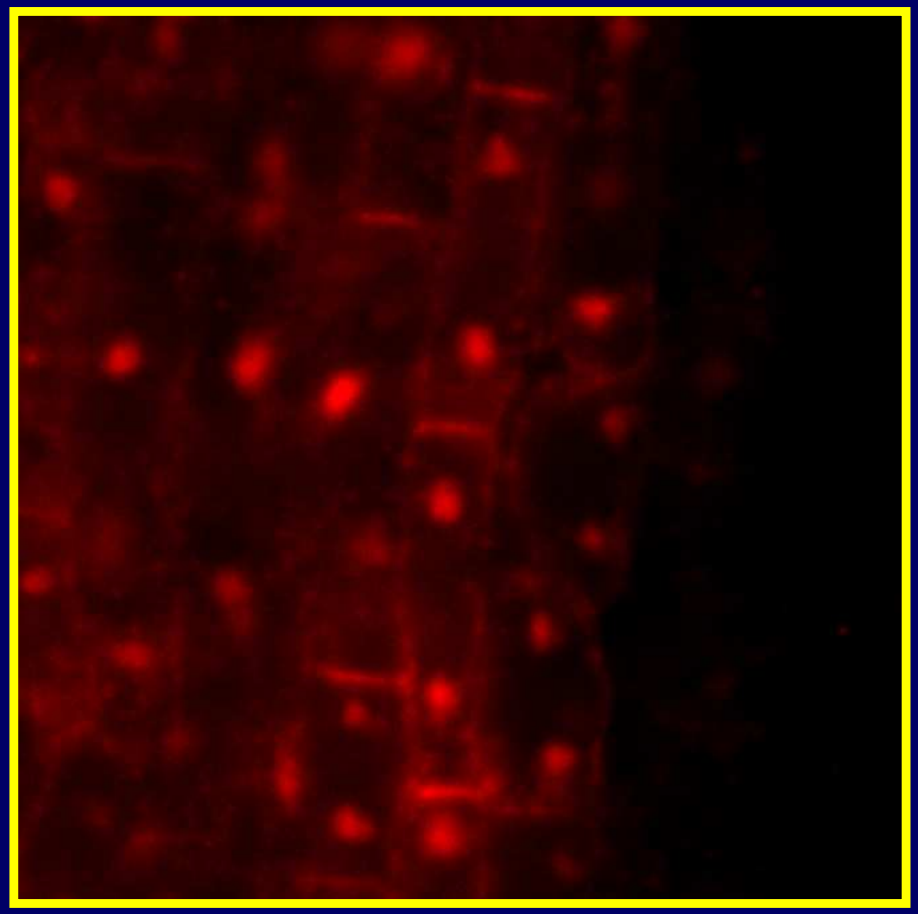


GNOM and PIN1 co-localise after BFA treatment



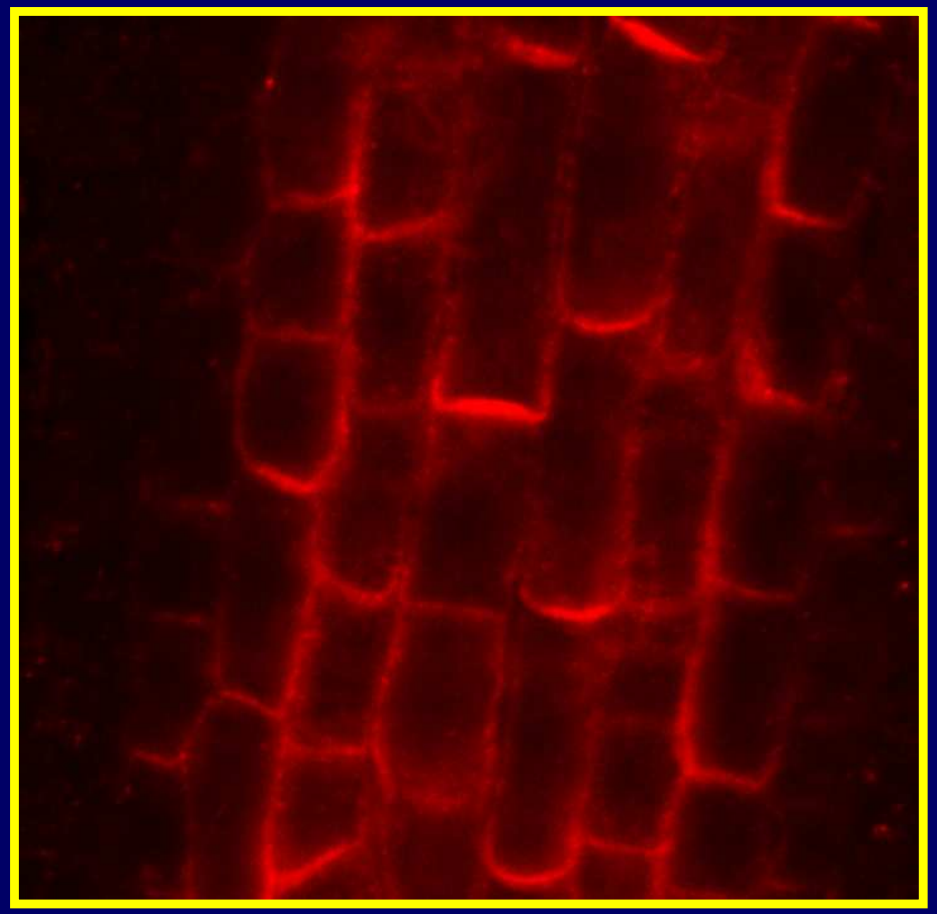
PIN1 Localisation in BFA-resistant GNOM

sensitive (control)



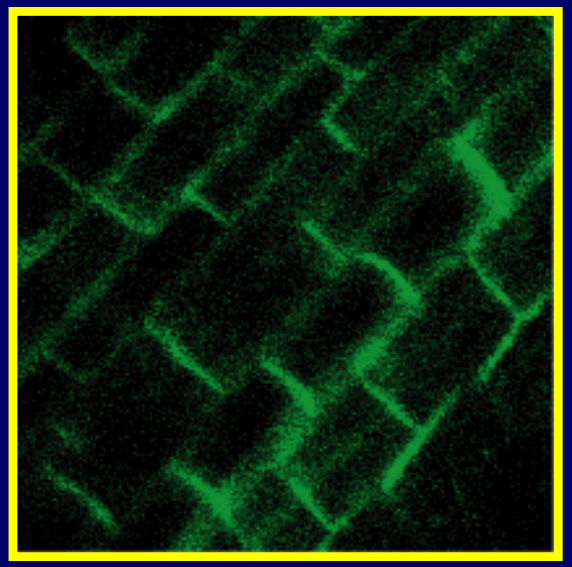
BFA 50 µM, 45 min
Col background

resistant

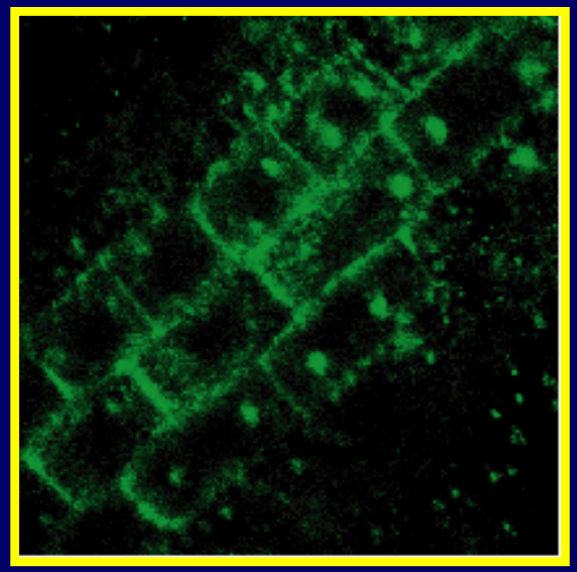


BFA 50 µM, 45 min
GNOM$^{BFA\ res.}$ transgenic line

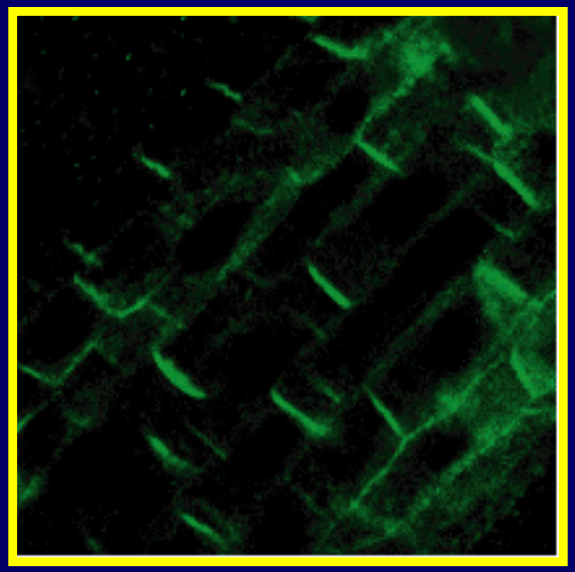
PIN localisation becomes BFA-resistant in BFA-resistant GNOM line



control

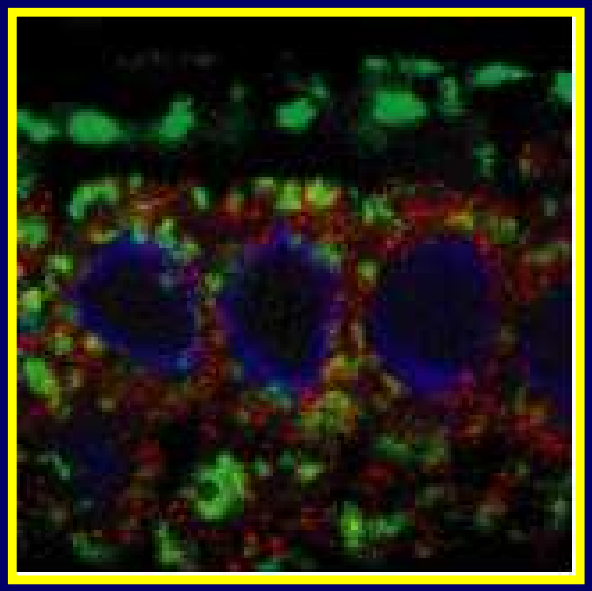


BFA, 60 min
GN^{wt}*-myc* line



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

GNOM does not co-localise with Golgi coat component γCOP



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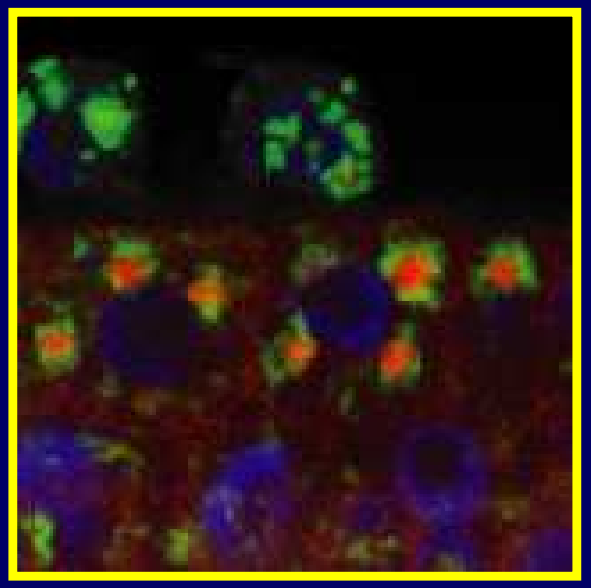
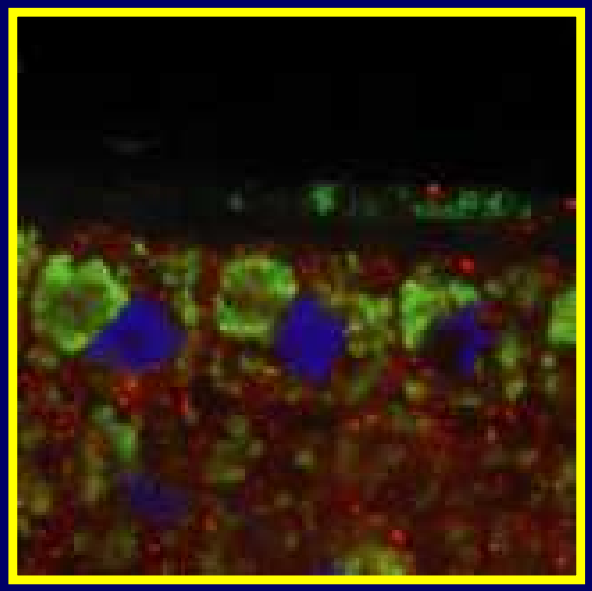
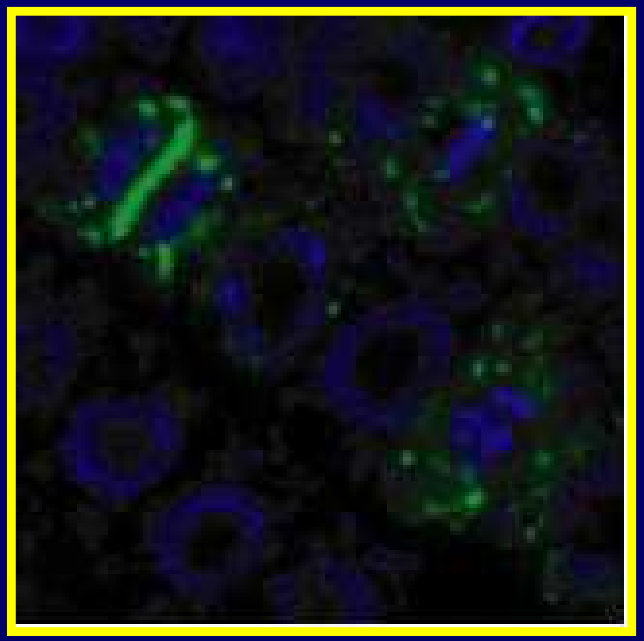


BFA, 60 min
GN^{wt}-myc line

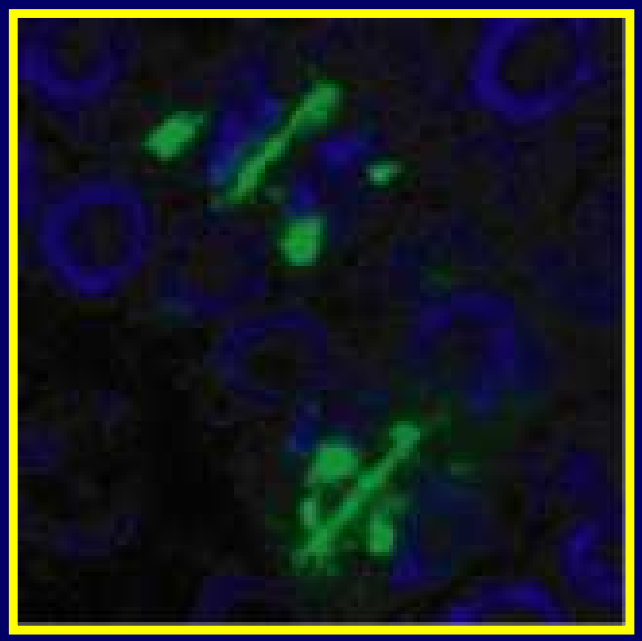


BFA, 60 min
GN^{M696L}-myc line

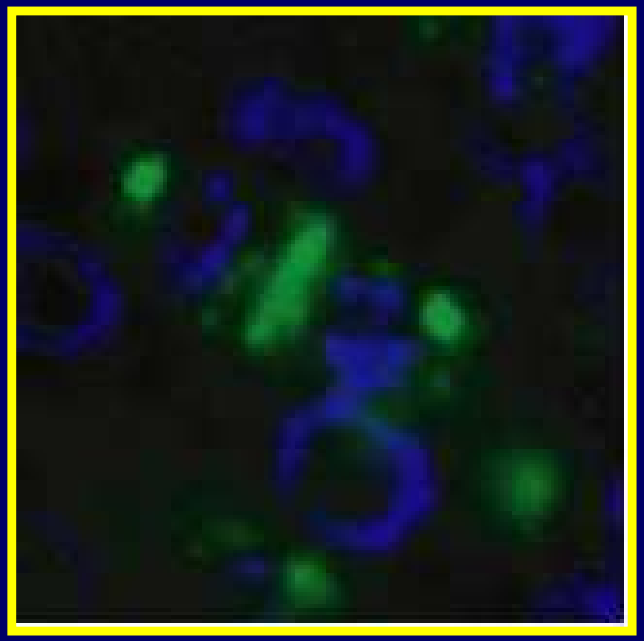
KNOLLE trafficking does not become BFA-resistant



control

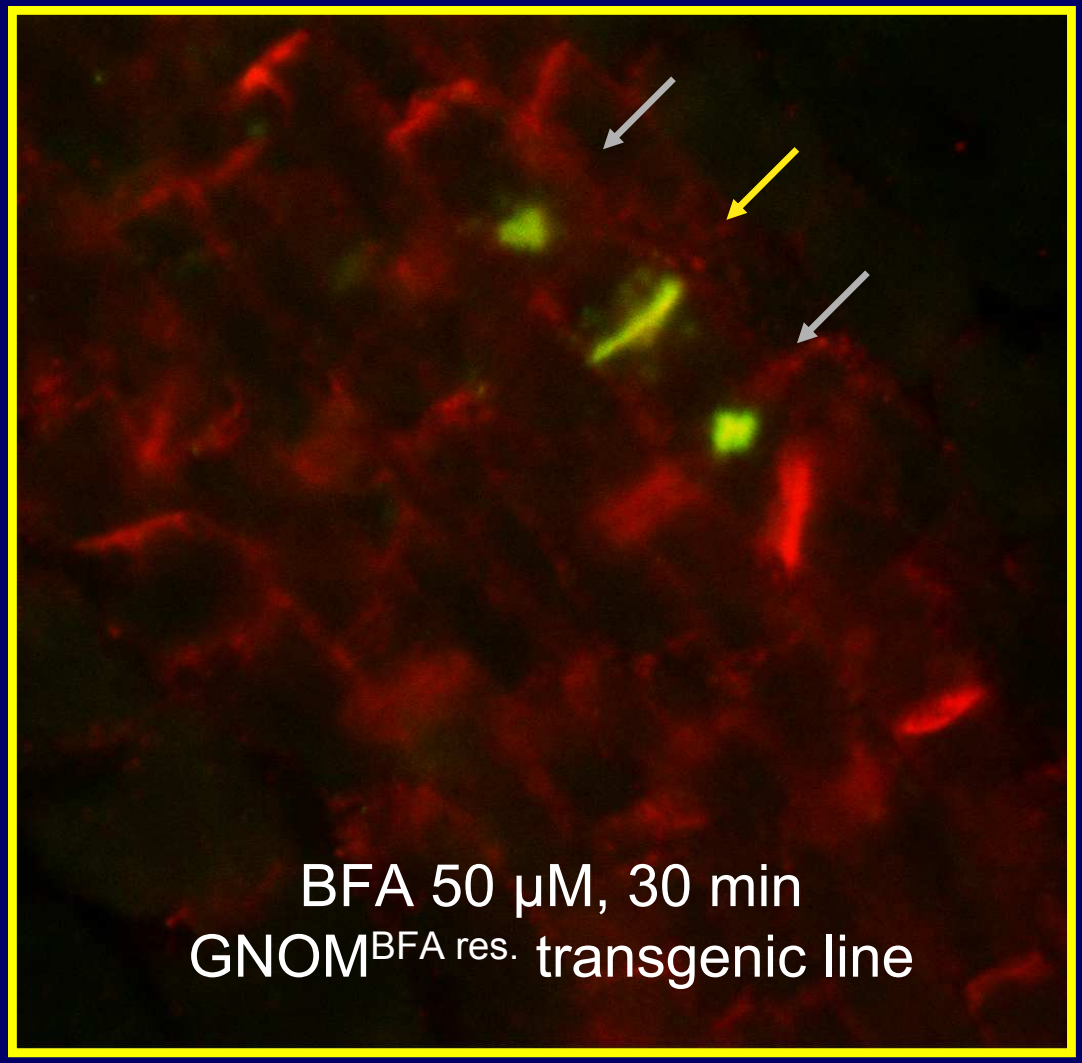


BFA, 60 min
GN^{wt}*-myc* line



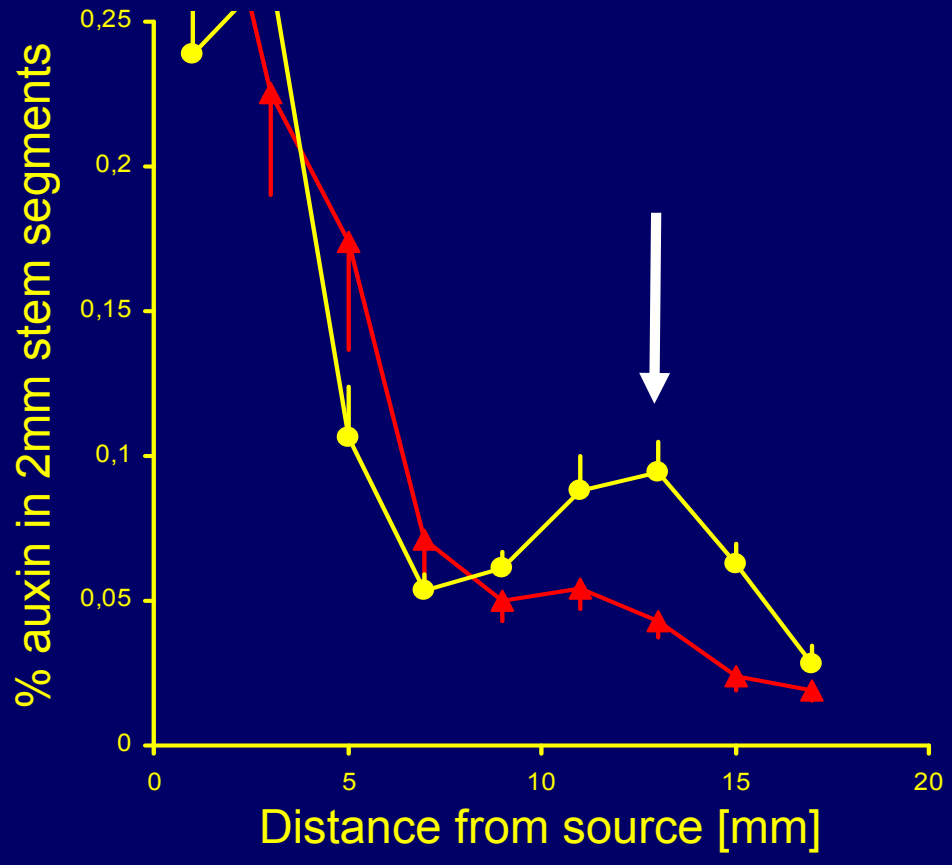
BFA, 60 min
GN^{M696L}*-myc* line

KNOLLE Accumulation in BFA Compartments in BFA-resistant GNOM

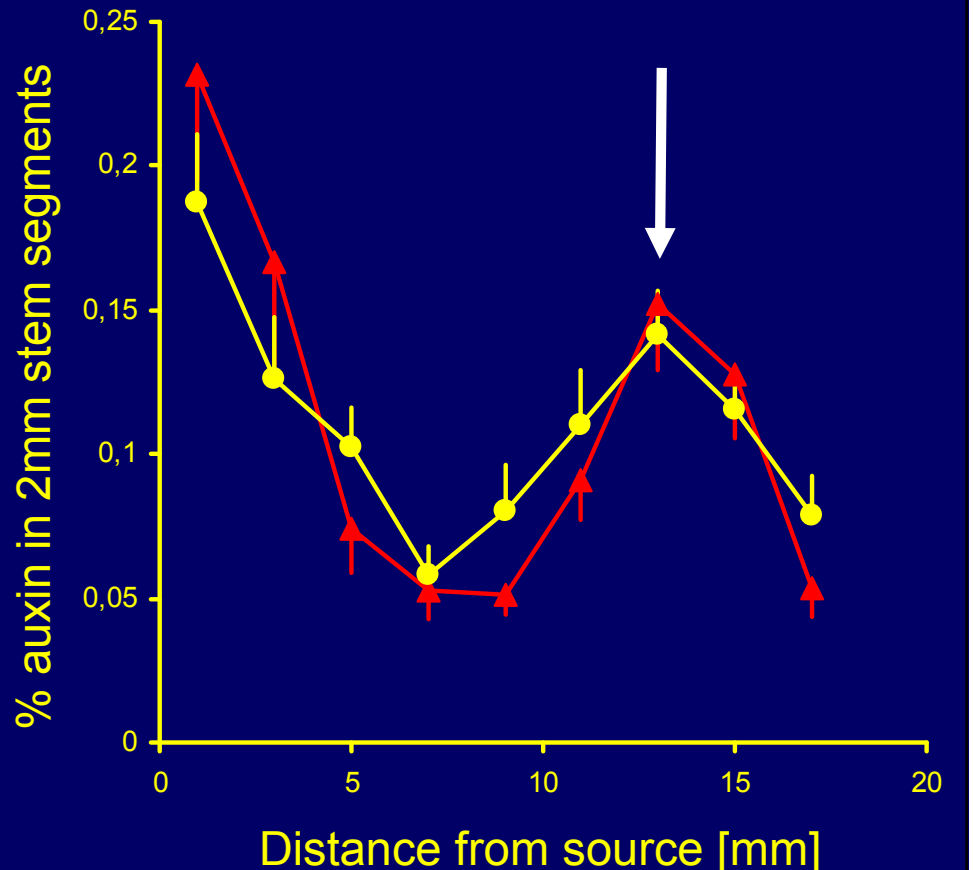


PIN1 = red KNOLLE = green PIN1 + KNOLLE = yellow

GNOM BFA-resistant lines display BFA insensitive auxin flux

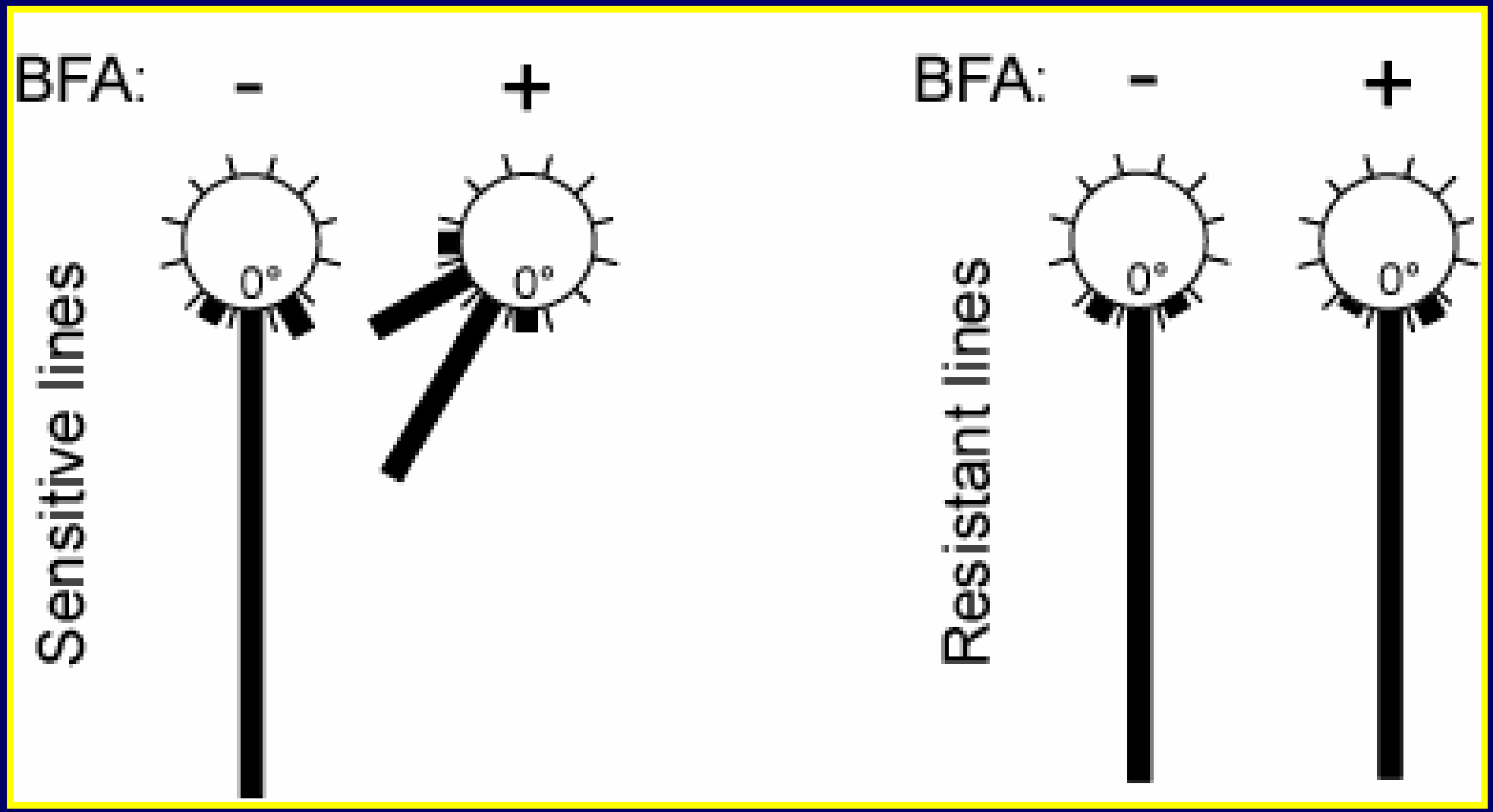


GN^{wt}*-myc* line

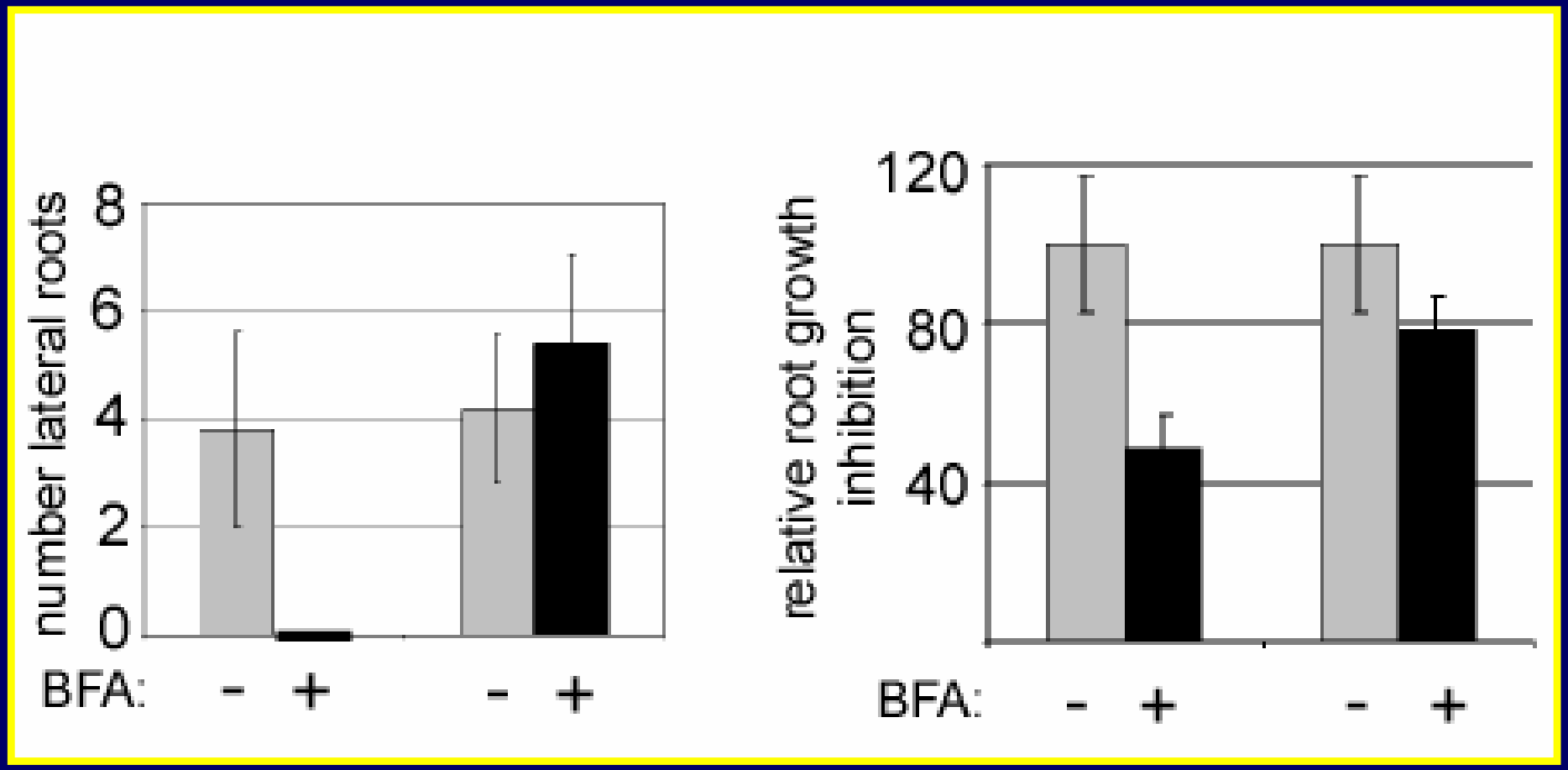


GN^{M696L}*-myc* line

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*

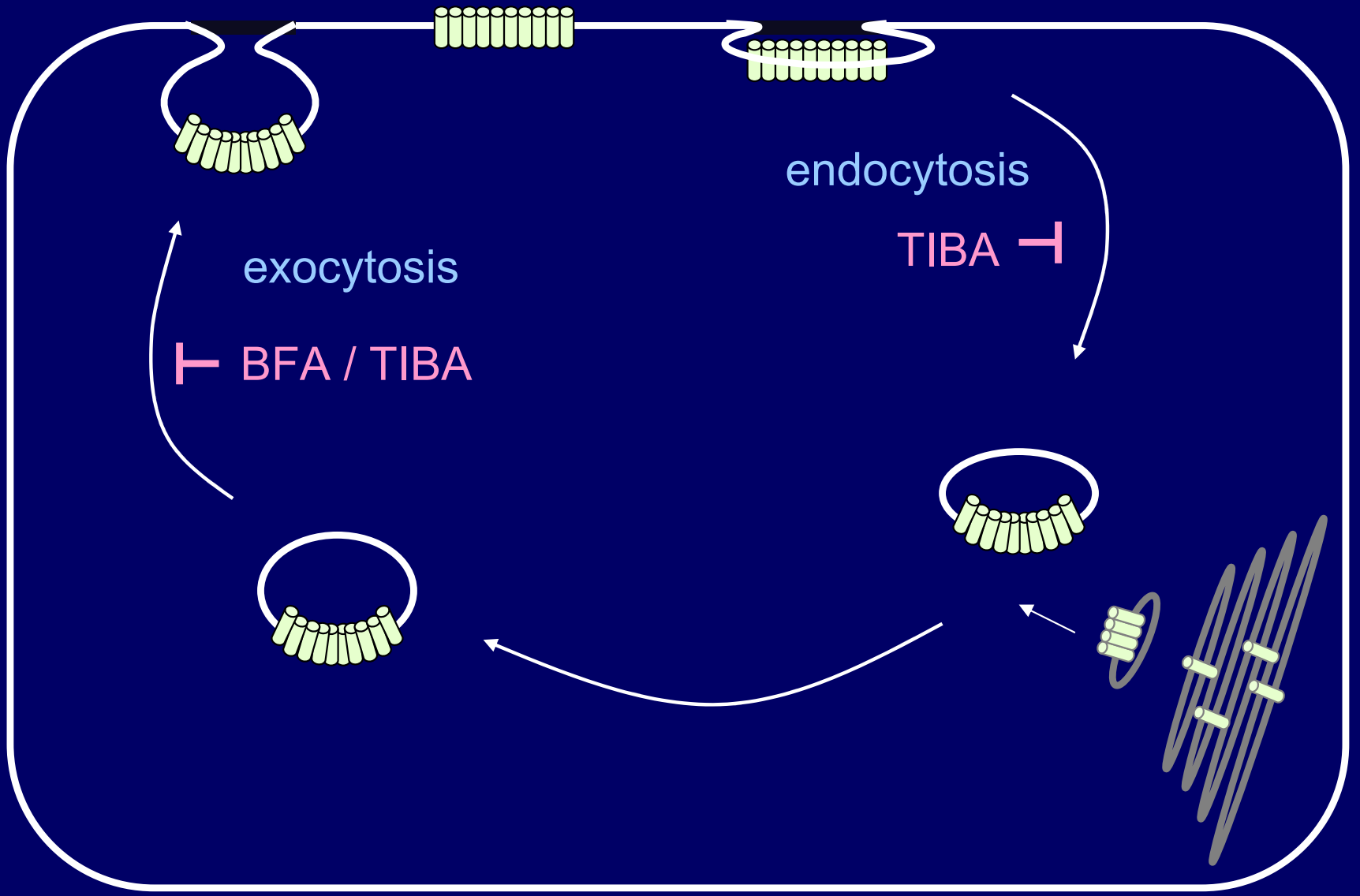
GNOM BFA-resistant lines lead to strong phenotypic BFA-resistance

BFA 5 µM

GN^{wt}*-myc* line

GNOM^{M696L}*-myc* line

Dynamic Movement of PIN1

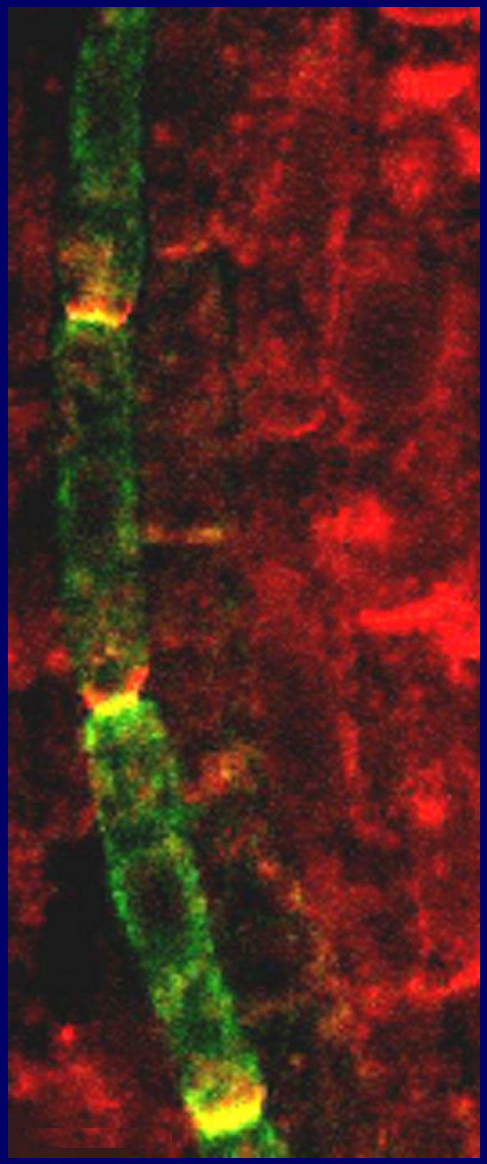


Molecular Components of PIN Polarity Control

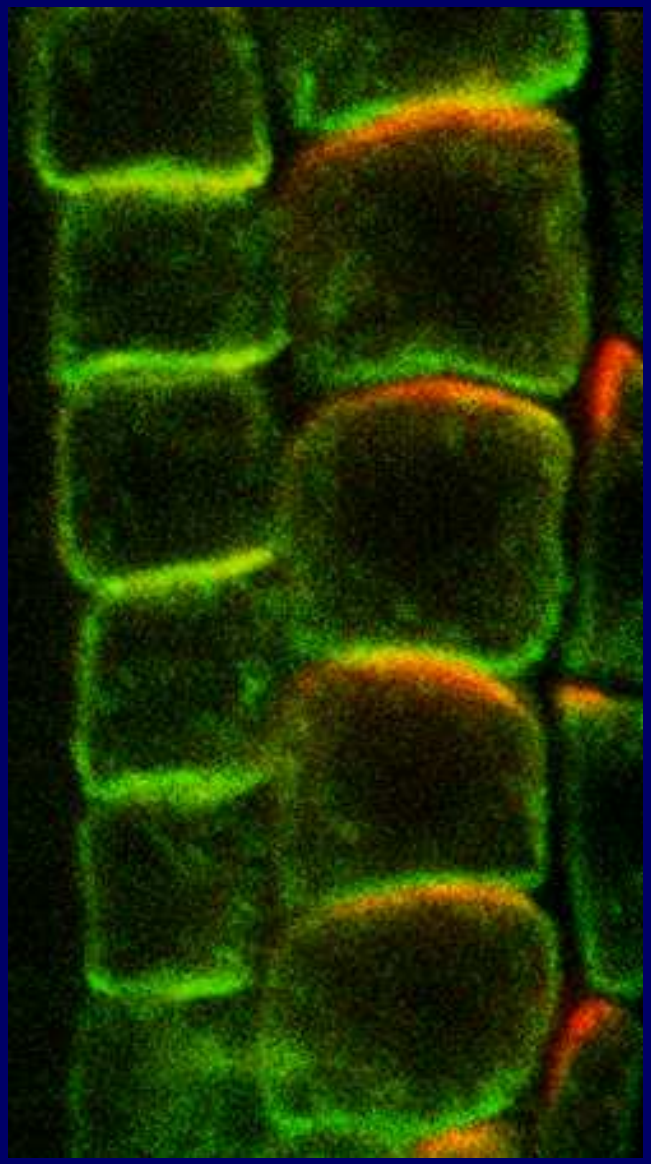
Role of PINOID protein kinase

Regulation of PIN Polarity

AUX1/PIN1



PIN1/pin1

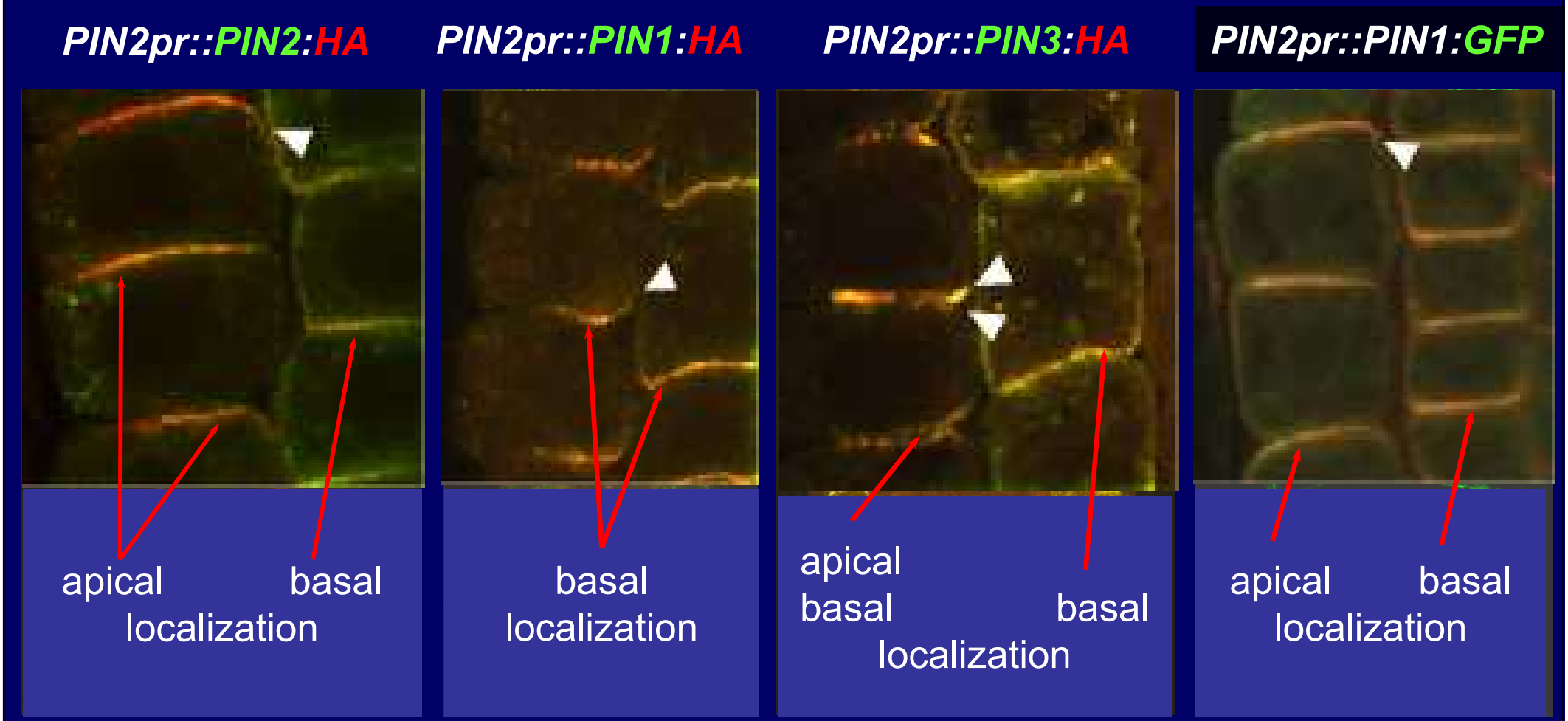


What are determinants of polarity of PIN localisation?

Does PIN polarity determines the direction of auxin flow?

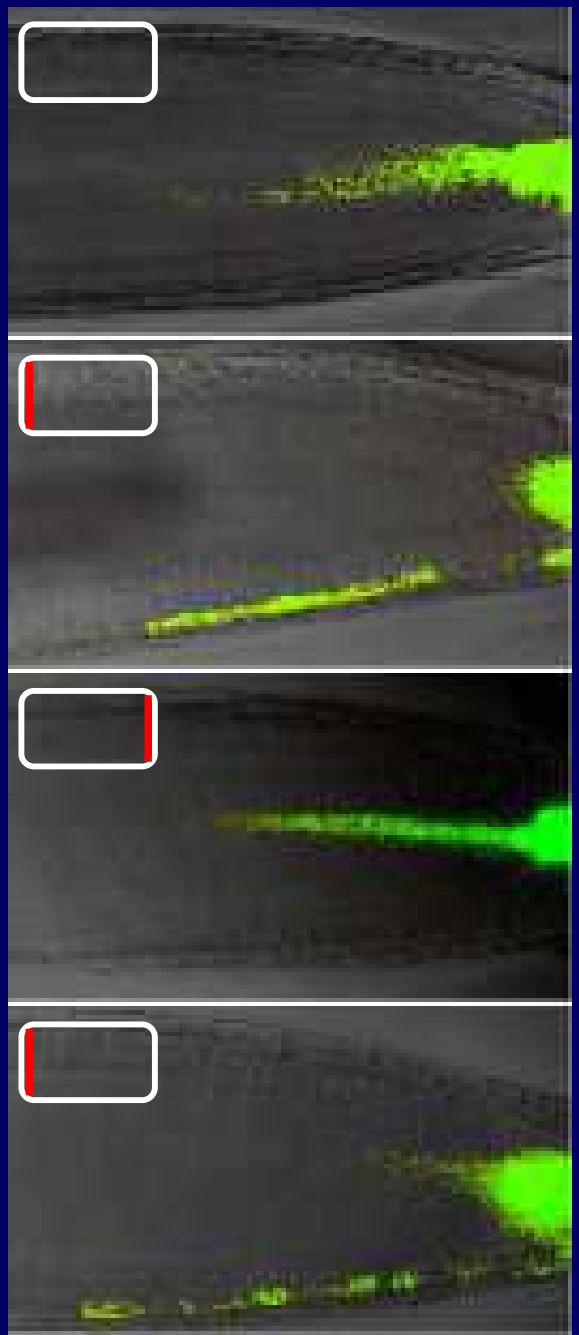
Molecular mechanism of the polar targeting pathway in plants?

PIN-specific Signals for Polarity



PIN Polarity Determines Direction of Auxin Flow

DR5rev::GFP



eir1

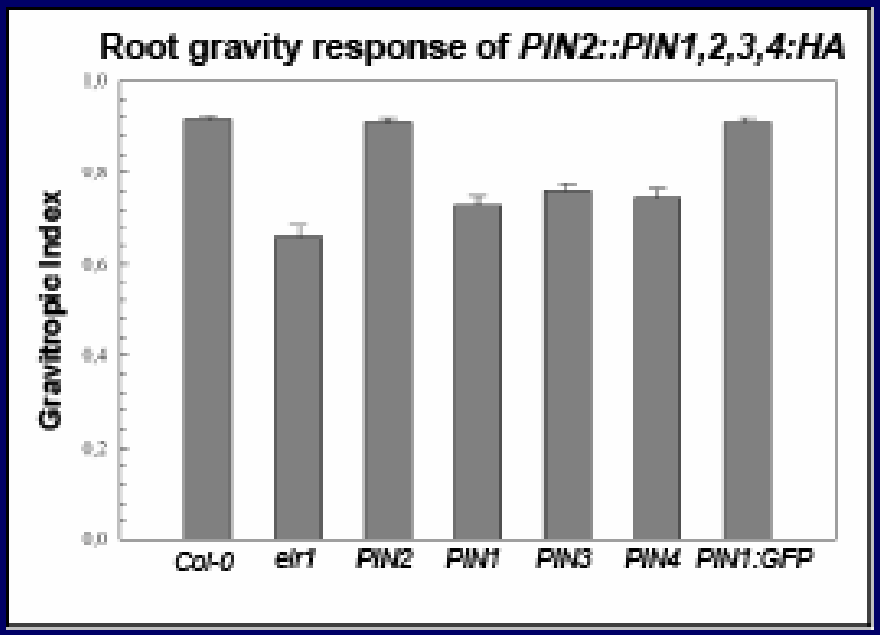
PIN2::PIN2:HA

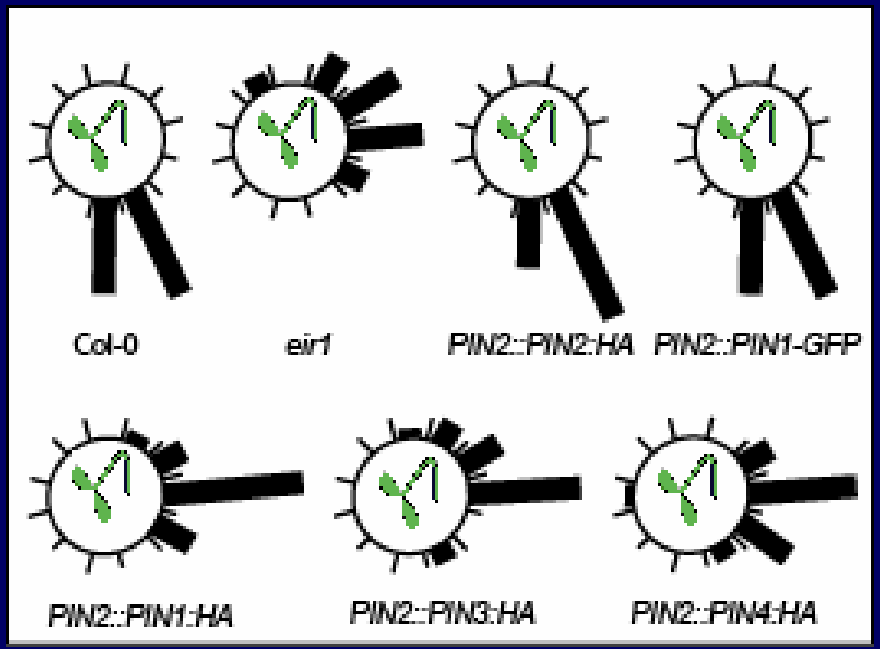
PIN2::PIN1:HA

PIN2::PIN1:GFP-2

PIN2::PIN1:GFP-3

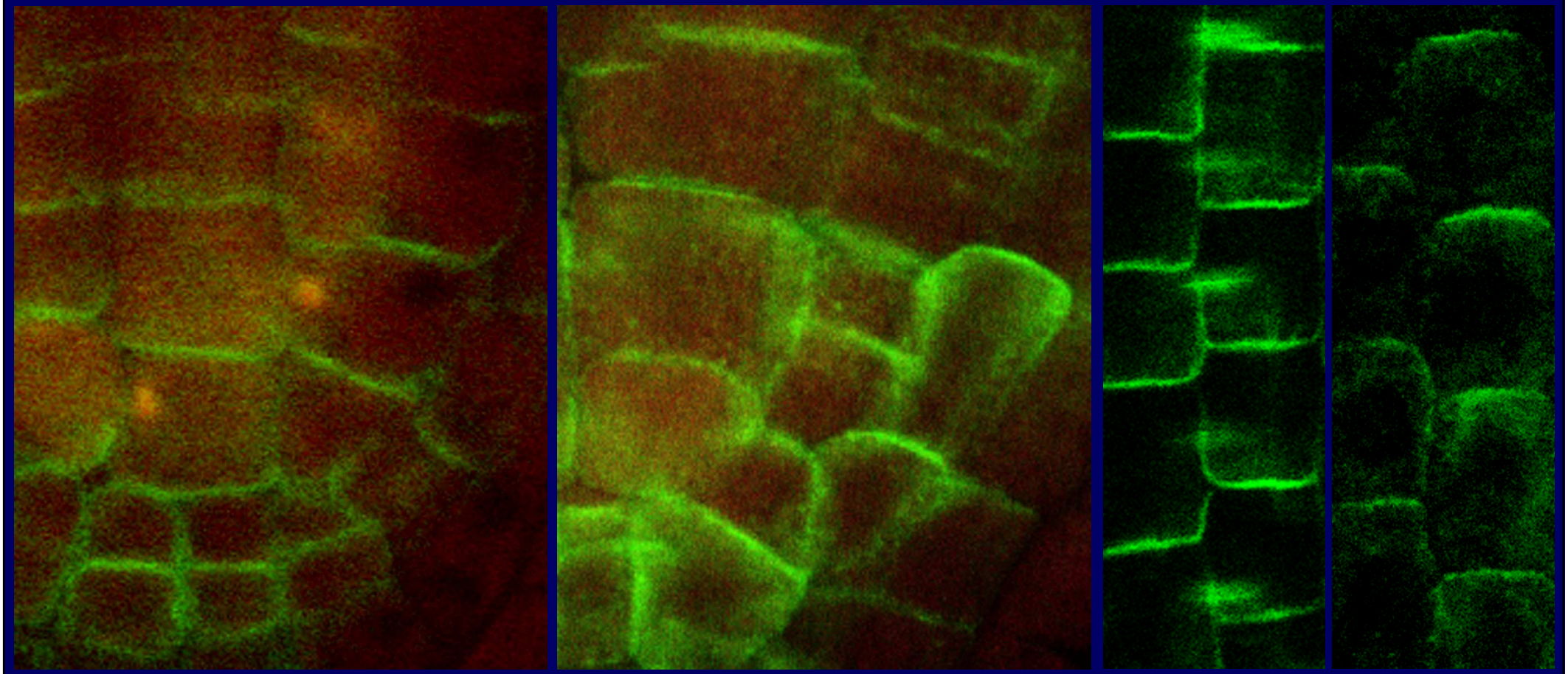
gravitropism





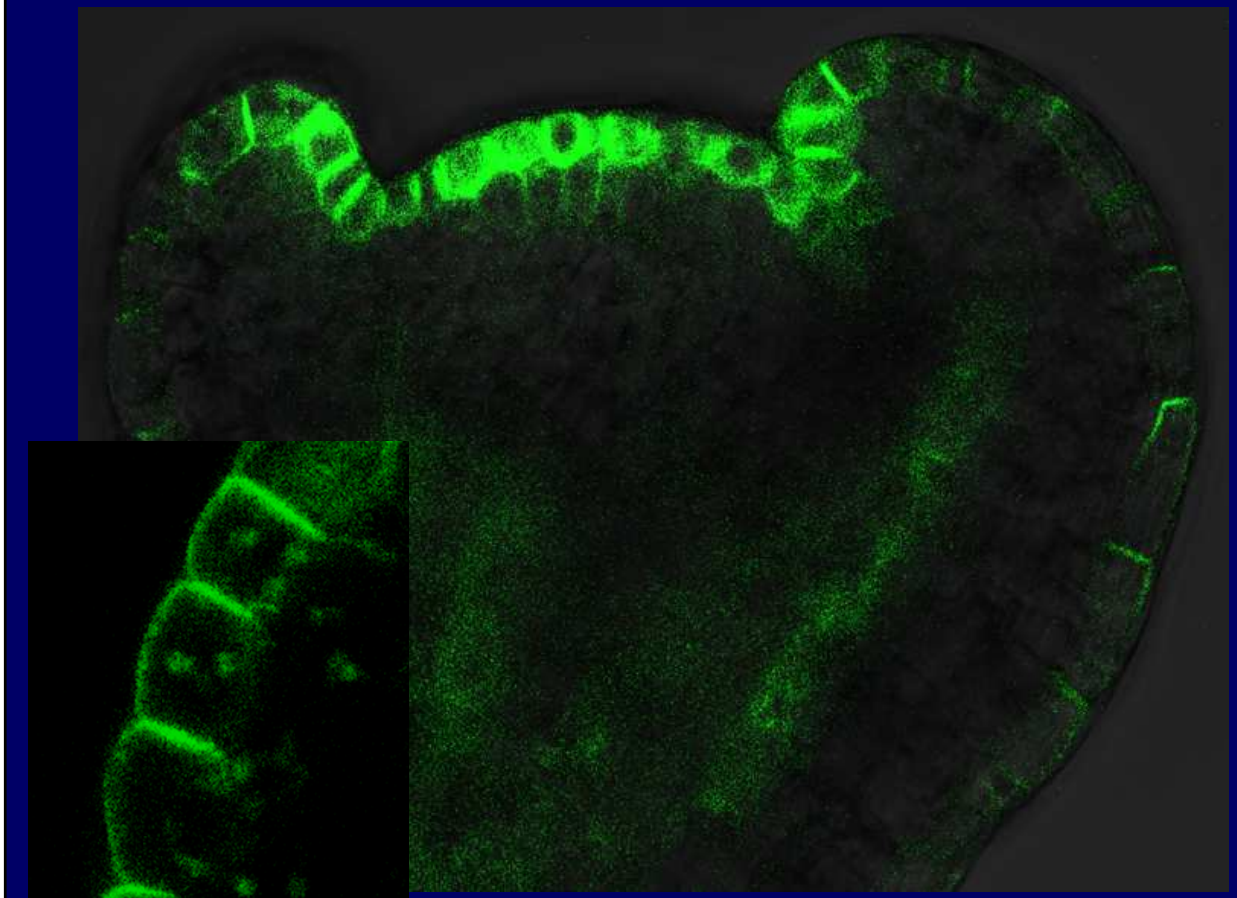
Role of PINOID Kinase in PIN Polar Targeting

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



PIN4 | **PIN1**

PINOID kinase loss-of-function > basal PIN targeting

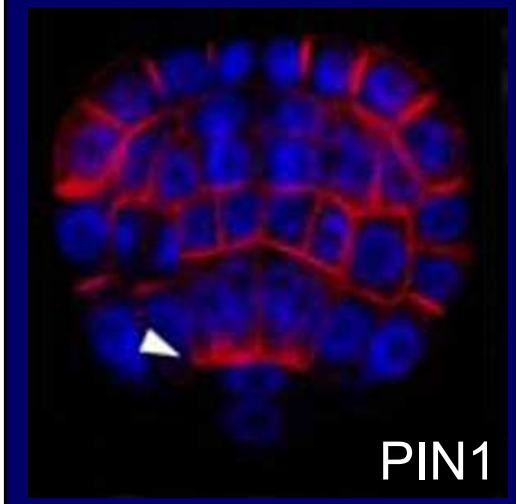


Col-0

pinoid

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

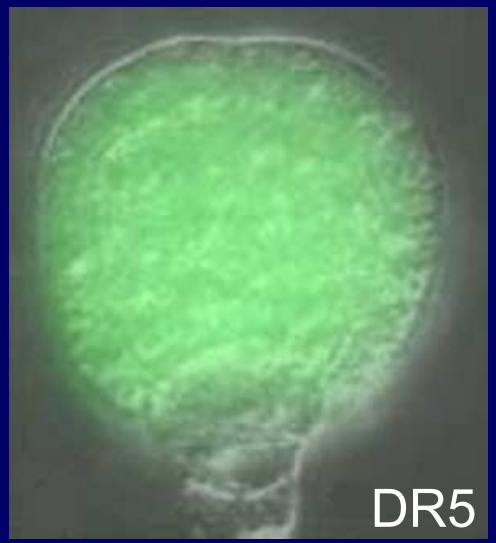
Col-0



RPS5::PID

Col-0

RPS5::PID



RPS5::PID seedlings

TROPISMS

Asymmetric Auxin Distribution Underlies Tropisms

Phototropism

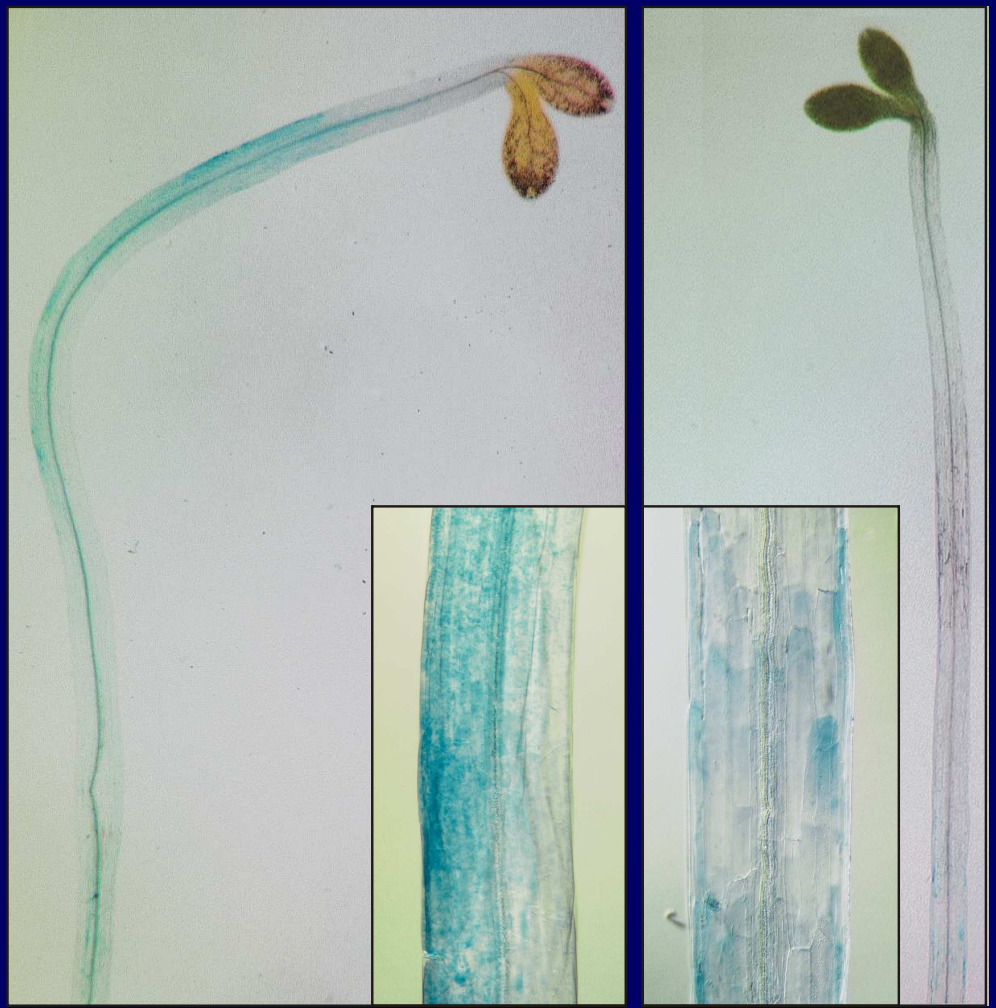
- NPA

+ NPA

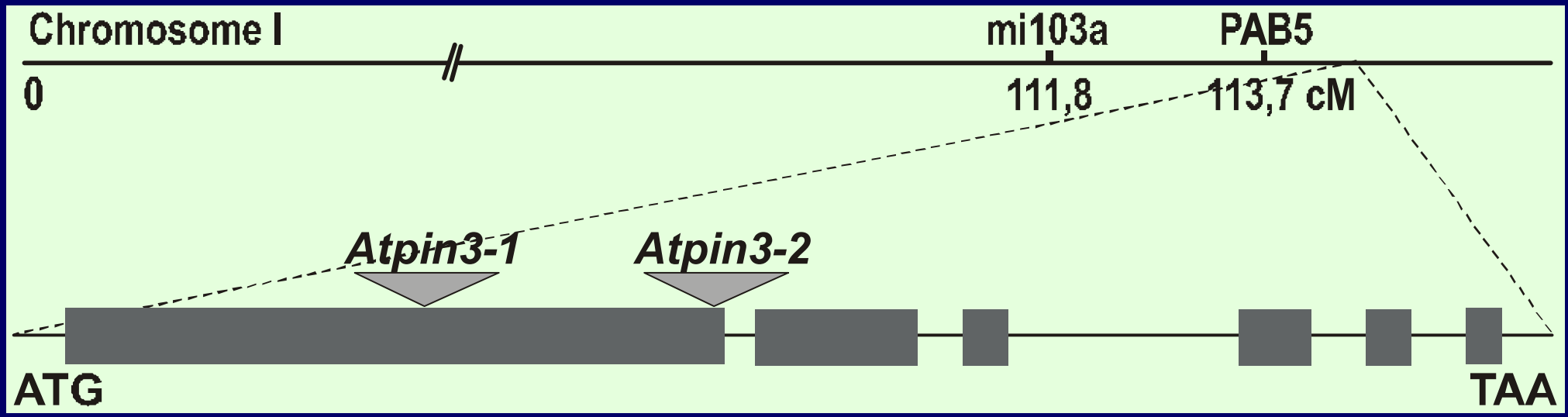
Gravitropism

- NPA

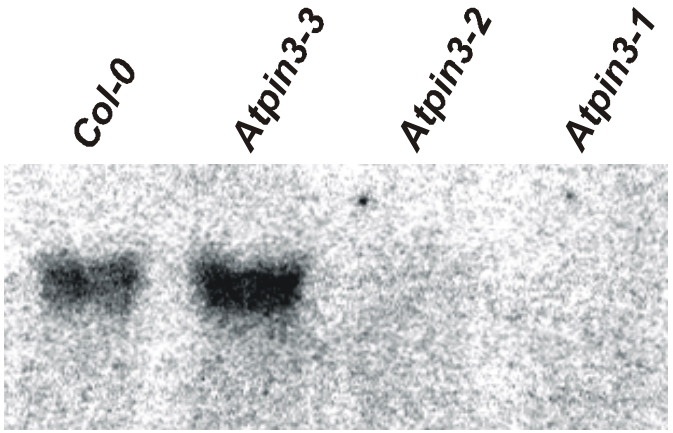
+ NPA



AtPIN3 Gene and *Atpin3* Knock-Out Mutants

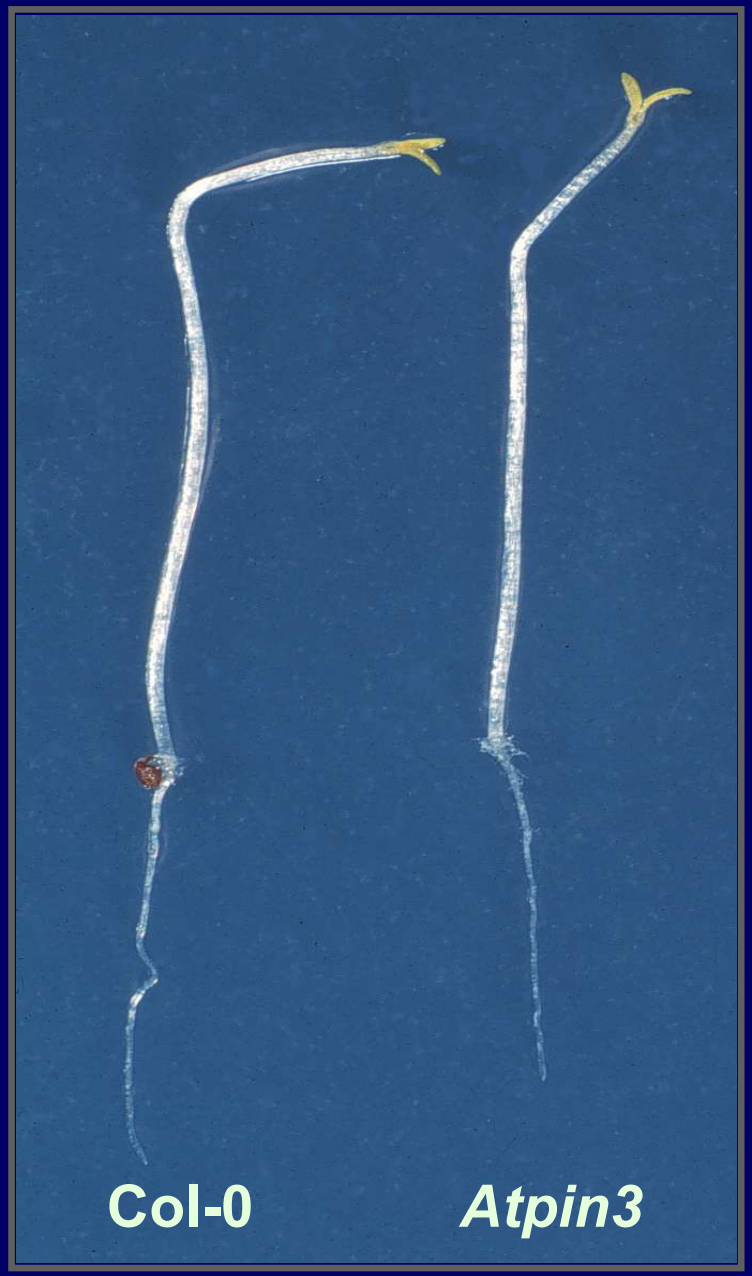


Northern blot analysis of *Atpin3* mutants



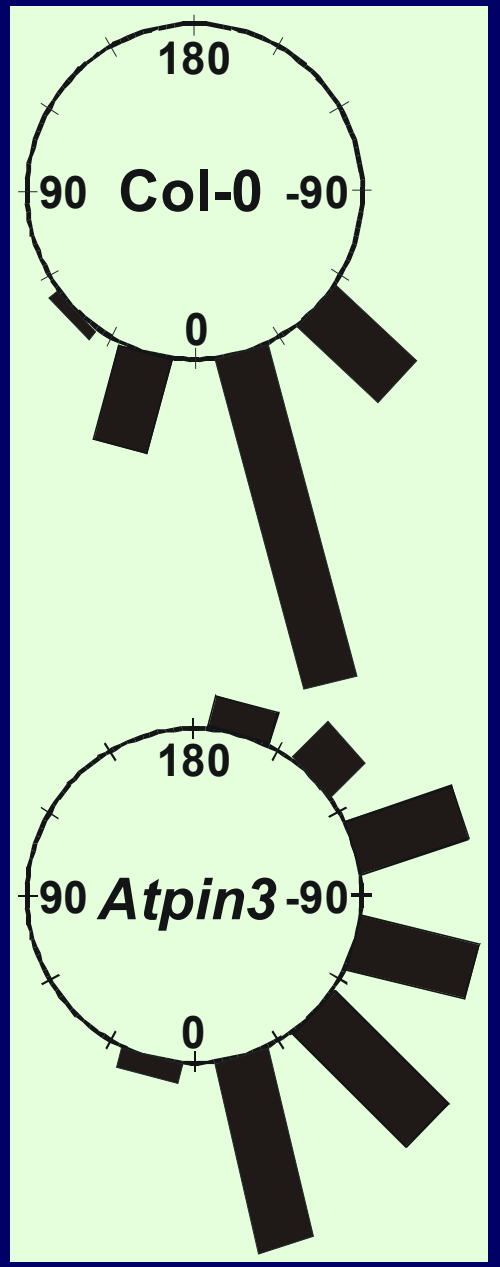
Atpin3 Tropisms

Hypocotyl phototropism

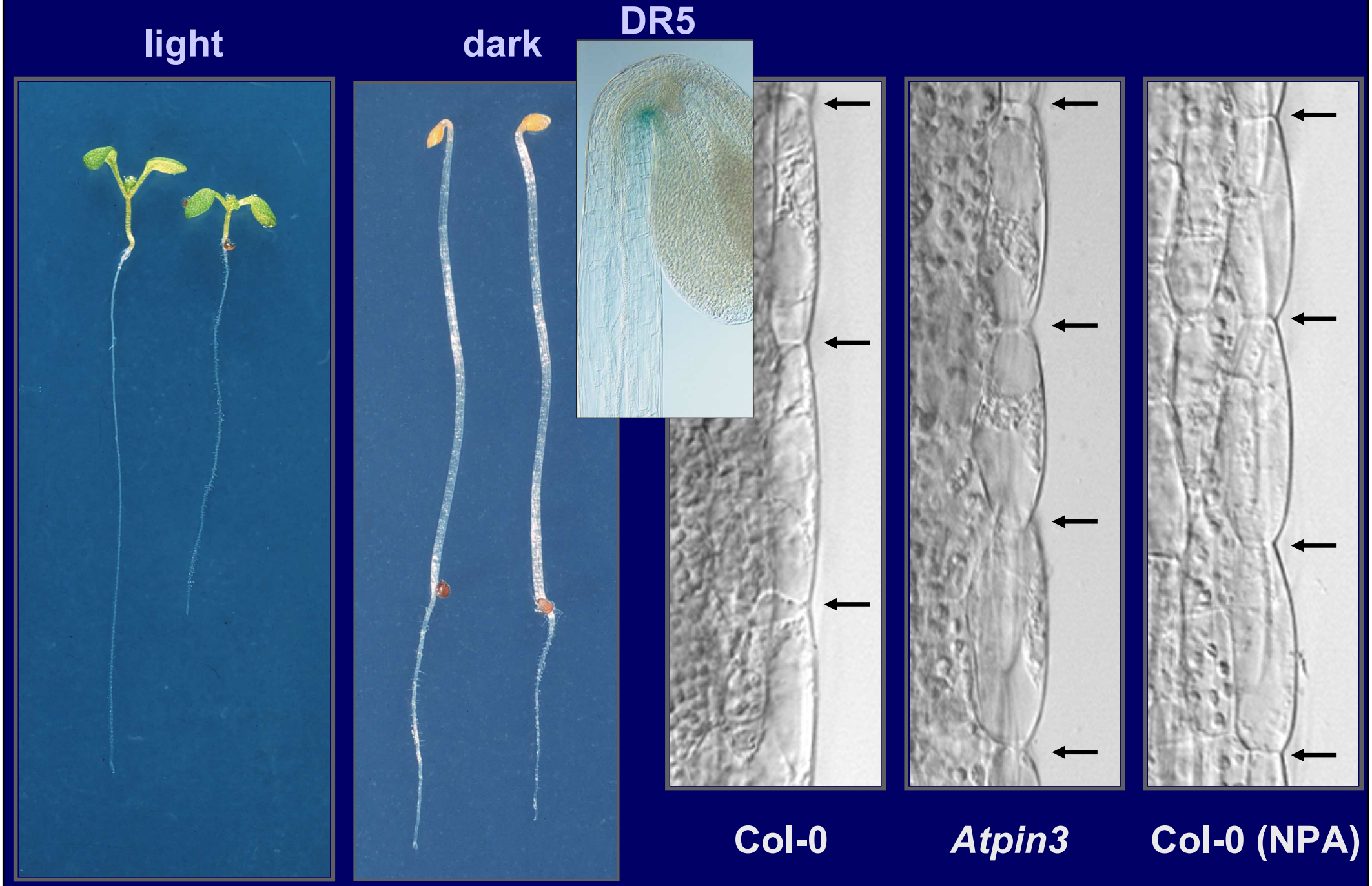


Hypocotyl gravitropism

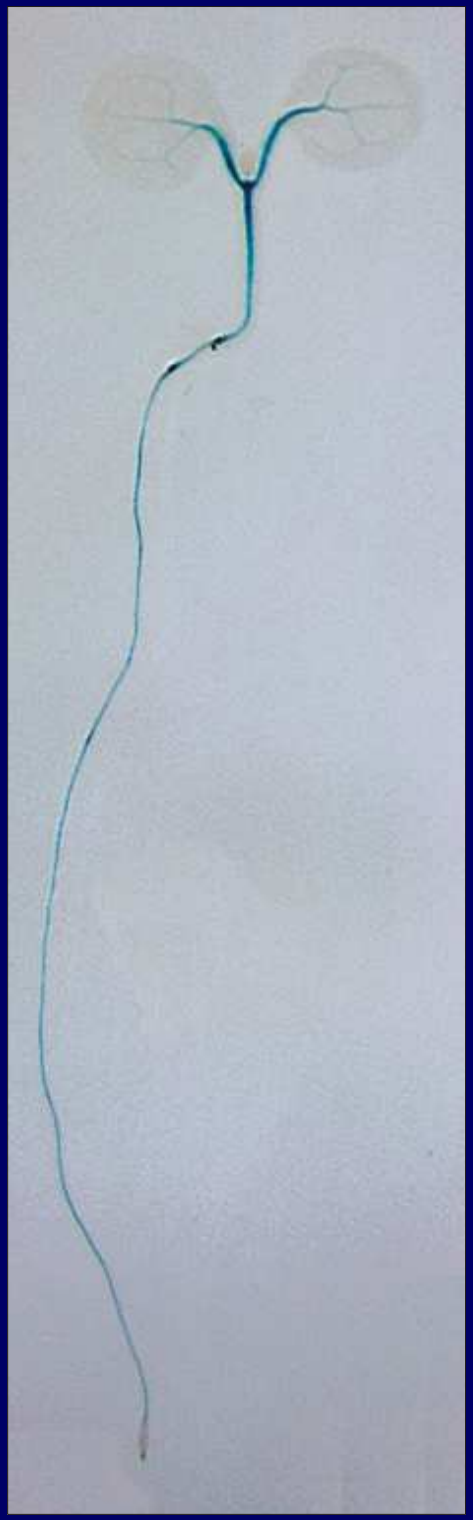
Root gravitr.

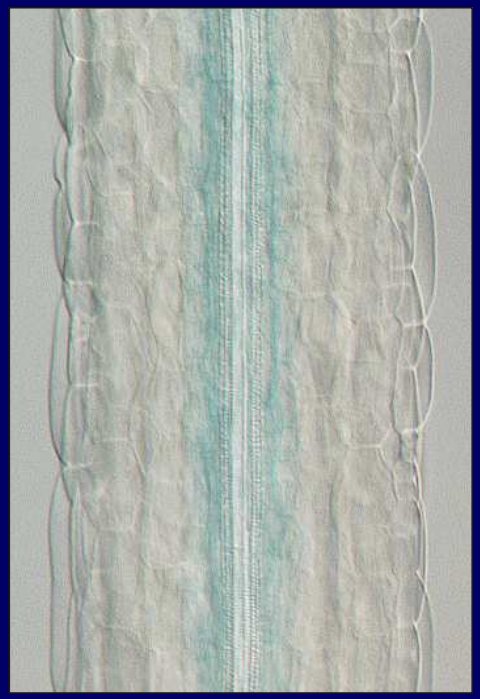


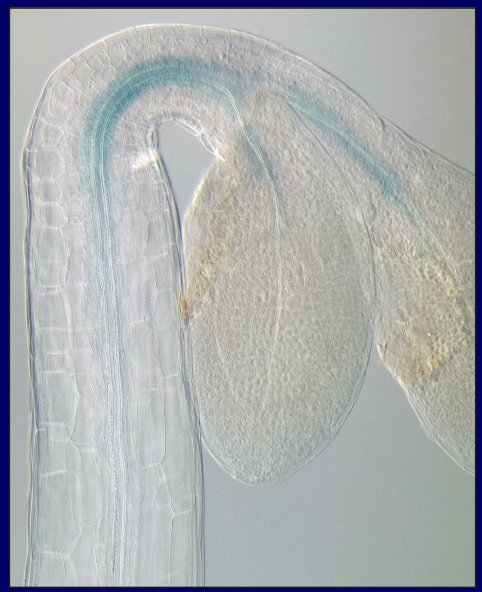
Atpin3 Hypocotyl and Apical Hook

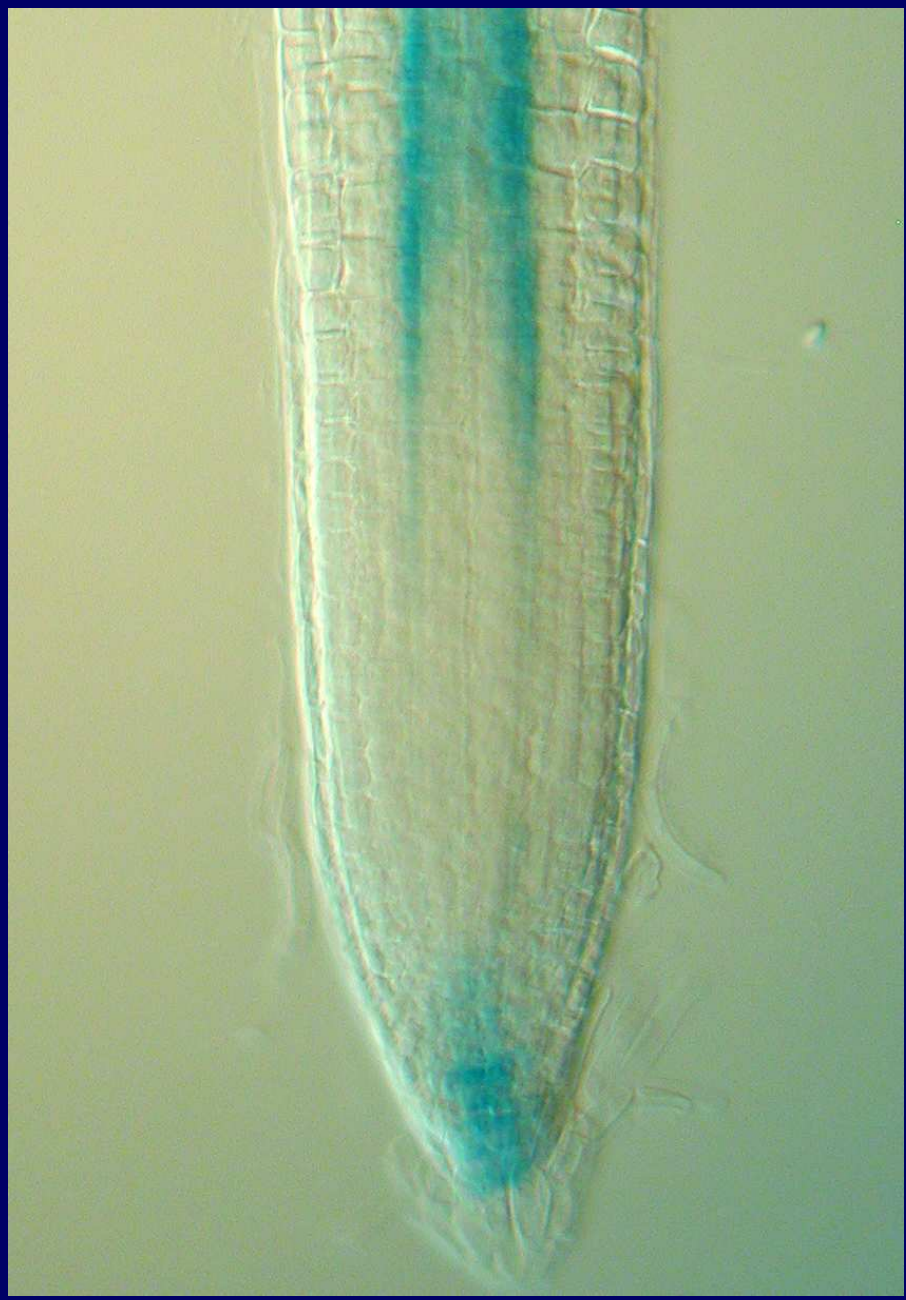


AtPIN3::GUS Transgenics



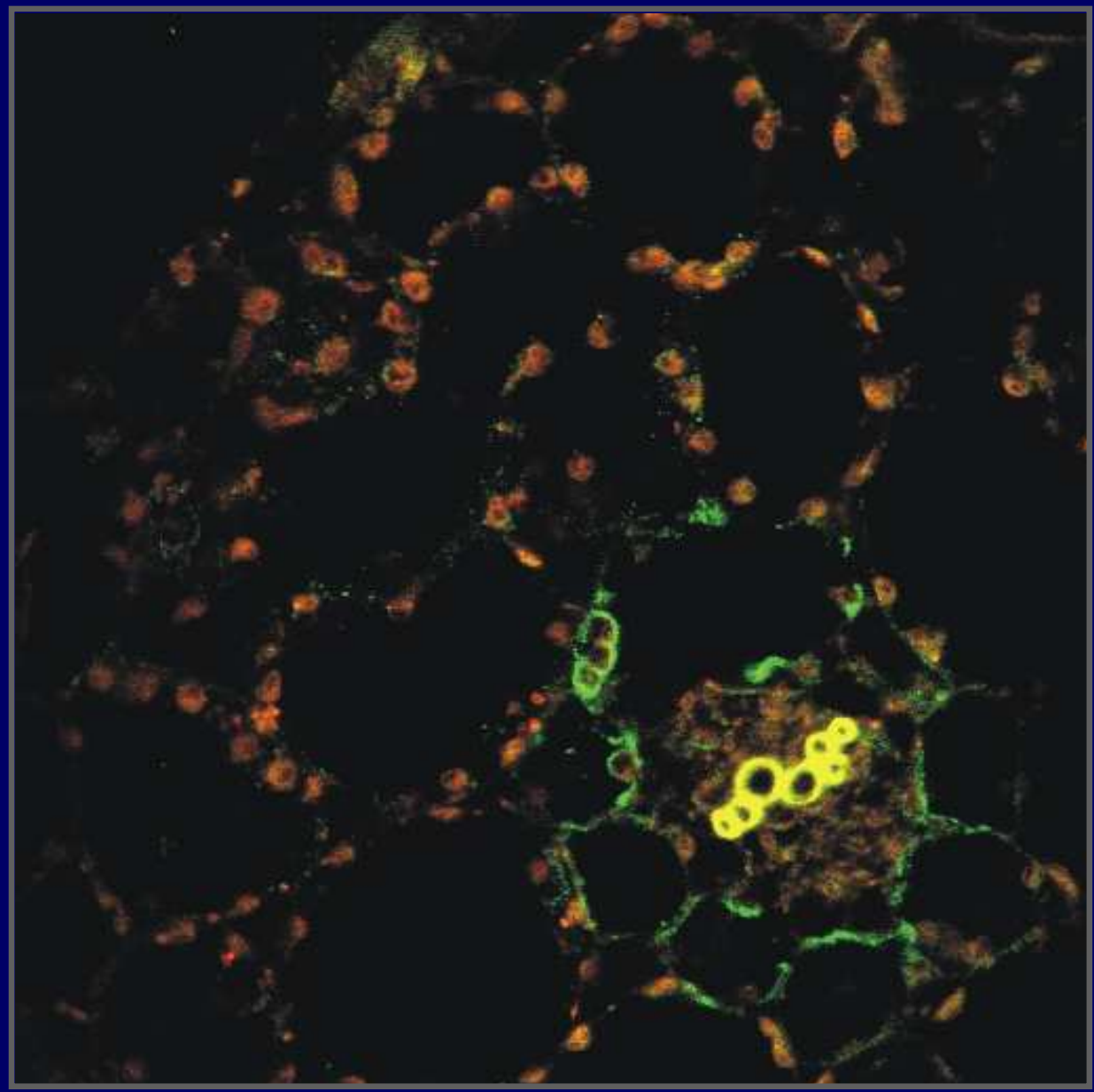




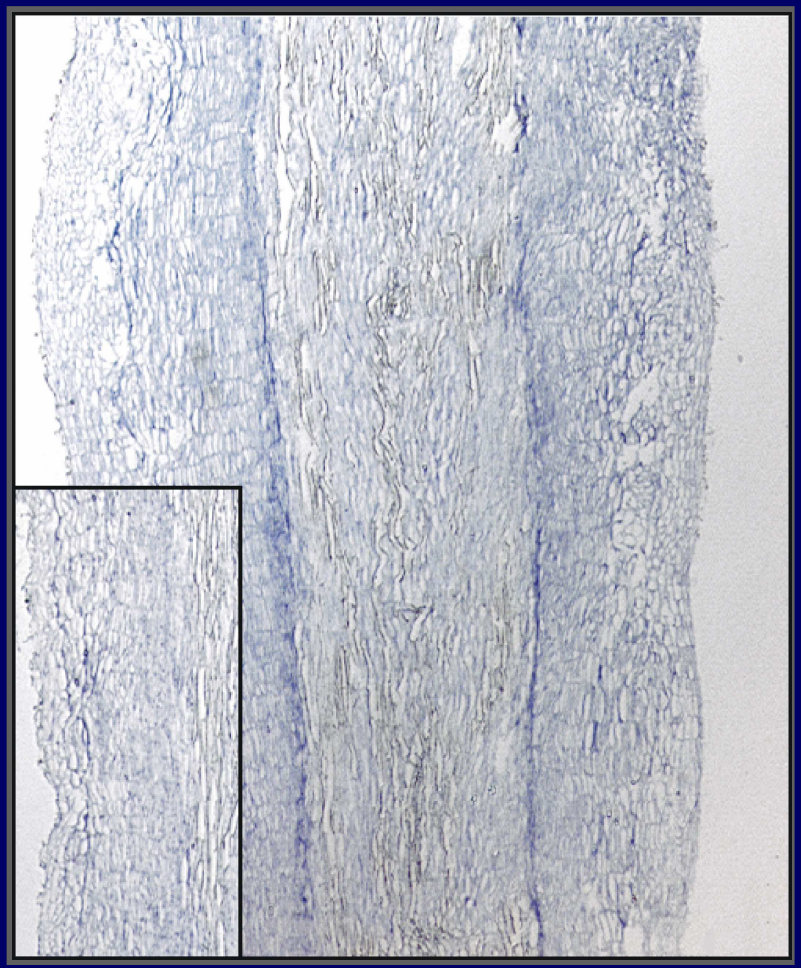


AtPIN3 in Hypocotyl

The AtPIN3 protein

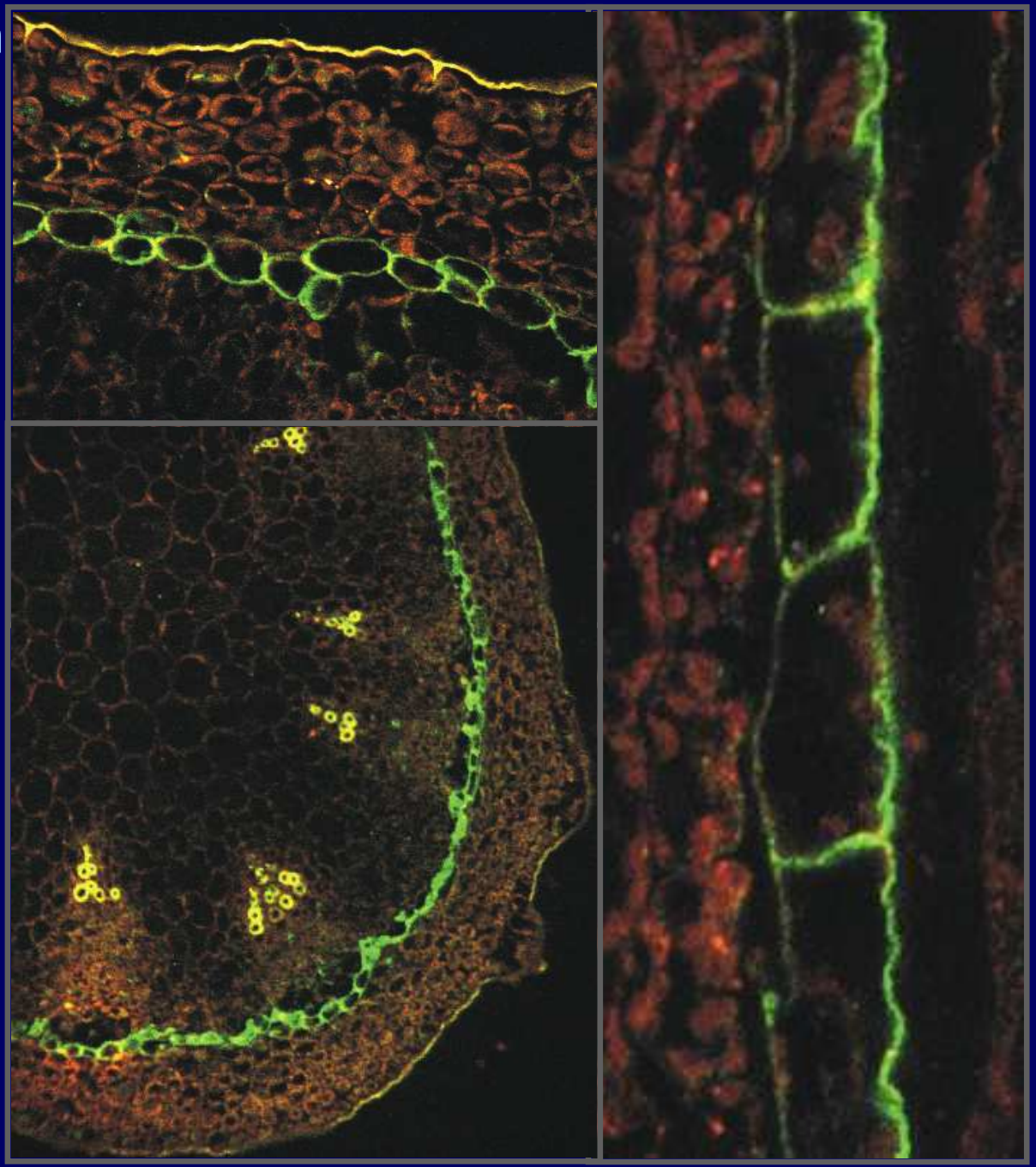


***in situ* RNA hybridisation**

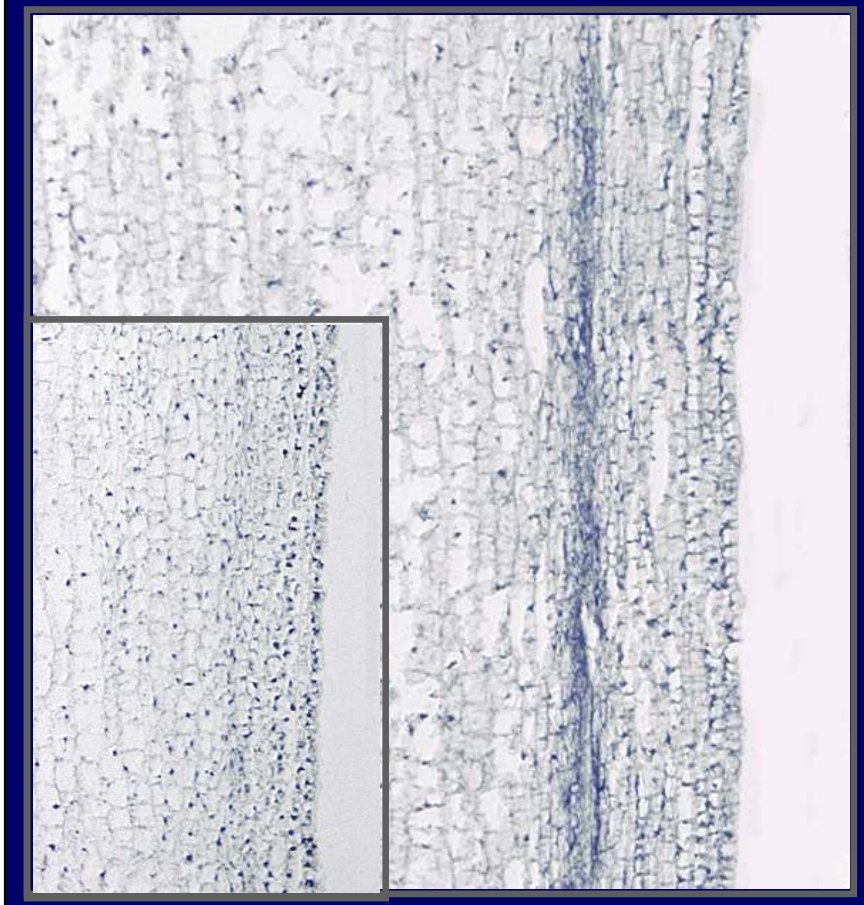


AtPIN3 in Inflorescence Axis

The AtPIN3 protein



***in situ* RNA hybridisation**

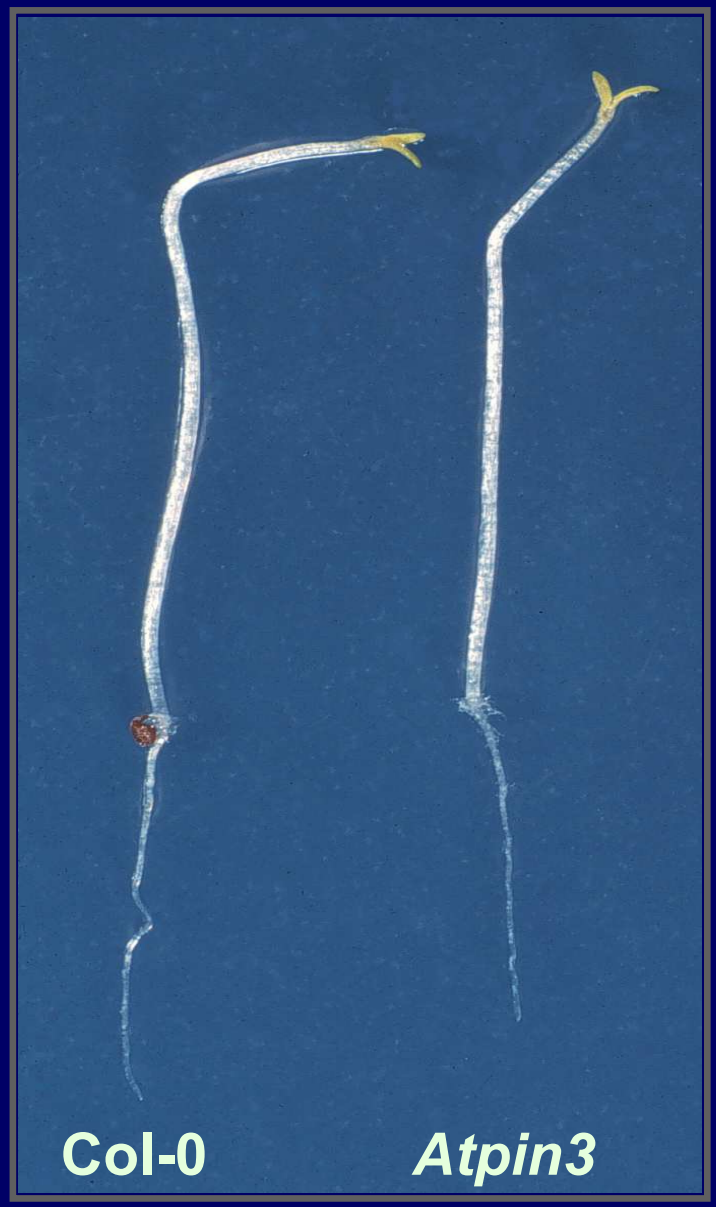


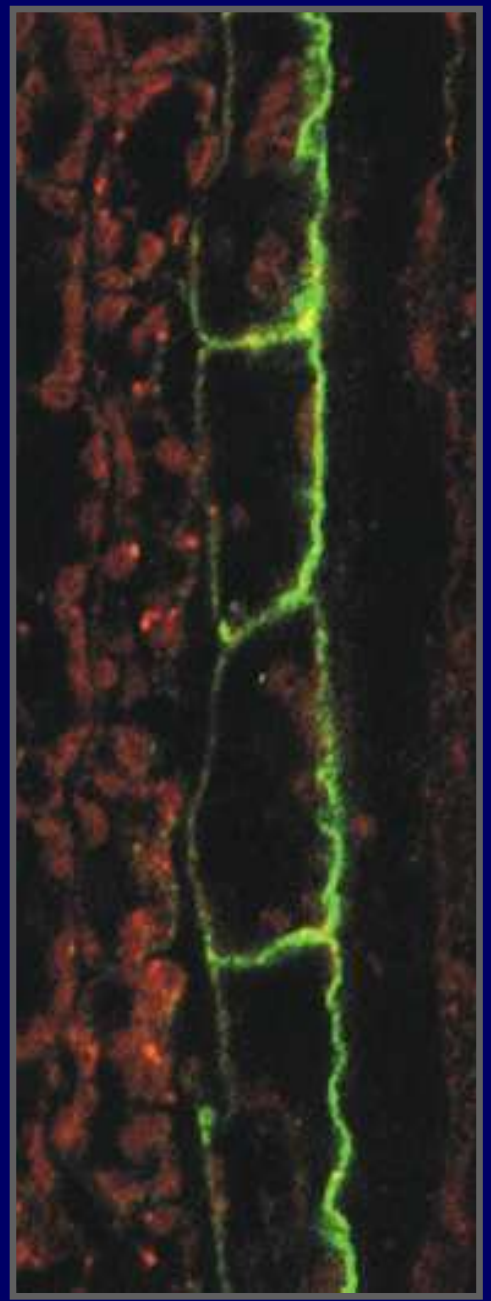
PIN3 – Lateral Auxin Transport

PIN3 protein

***DR5* - phototropism**

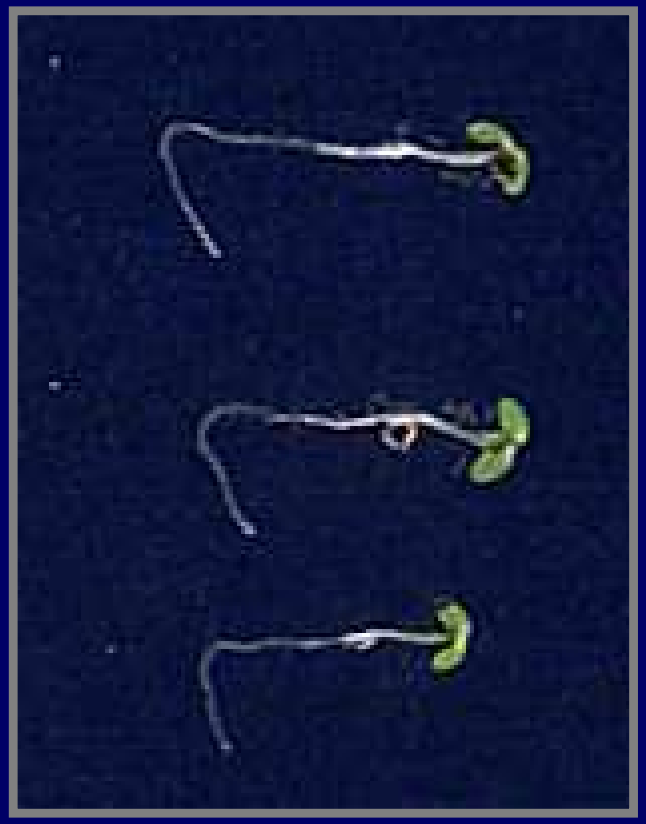
***pin3* phototropism**



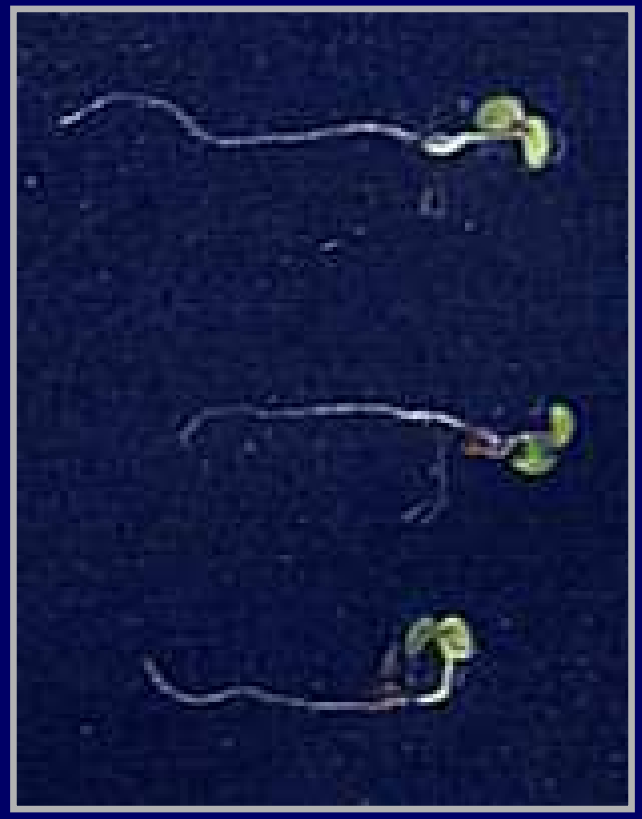


PIN2 – Root Gravitropism

Col-0

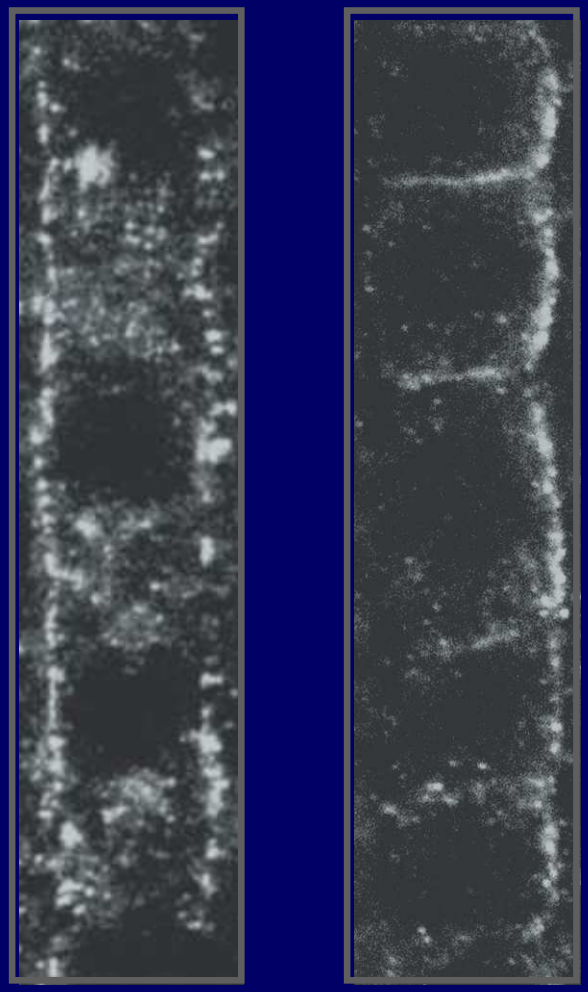


pin2



PIN2 protein

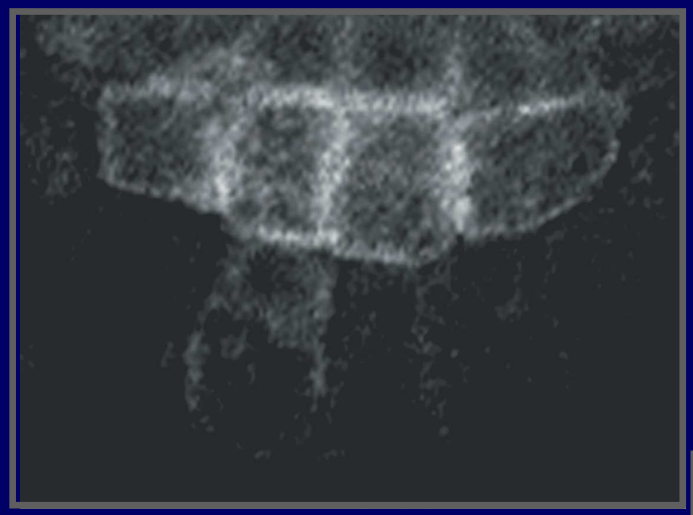
PIN3 Actin Dependent Cycling

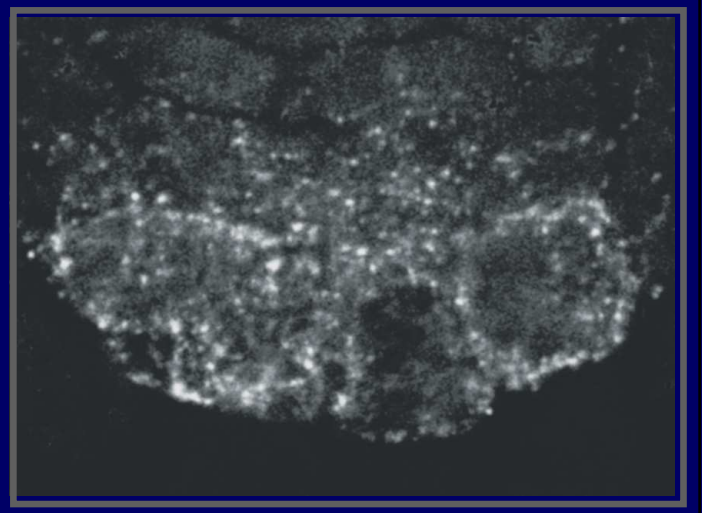


+ BFA

- BFA

+ BFA

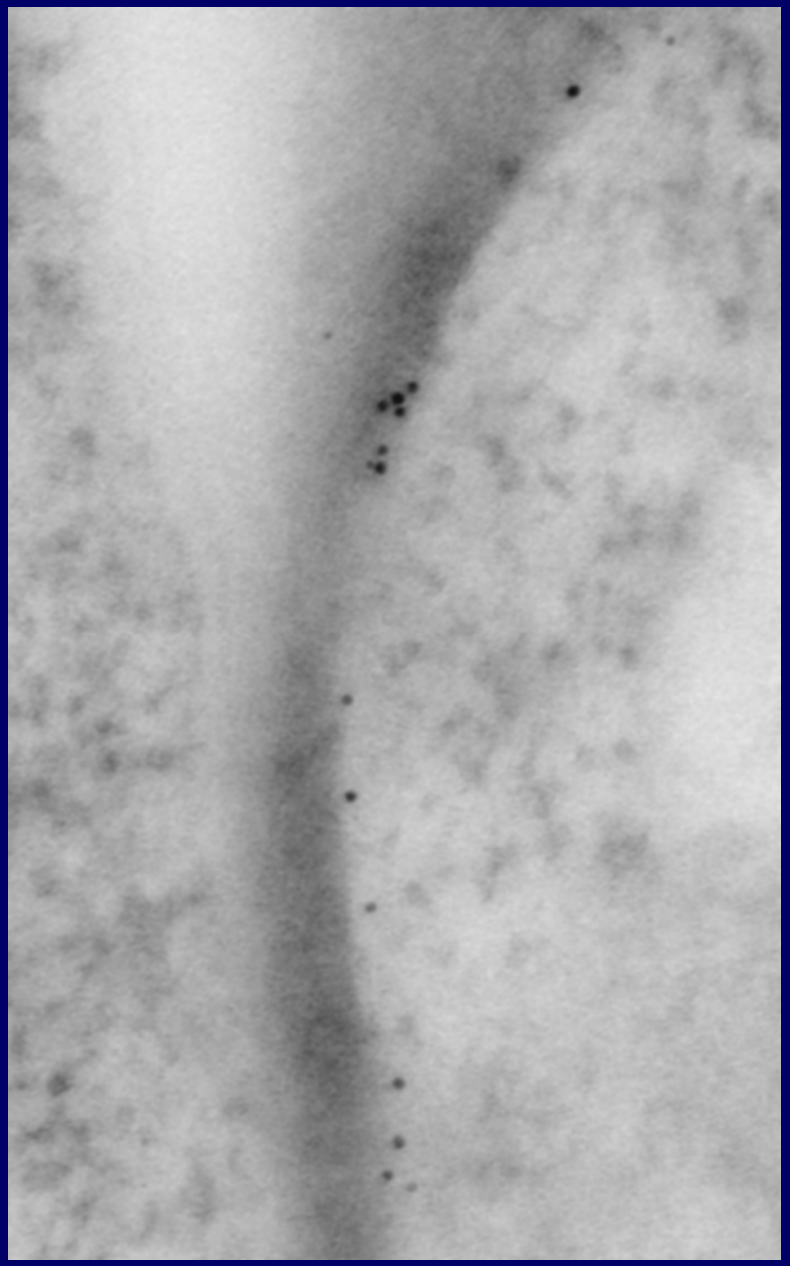


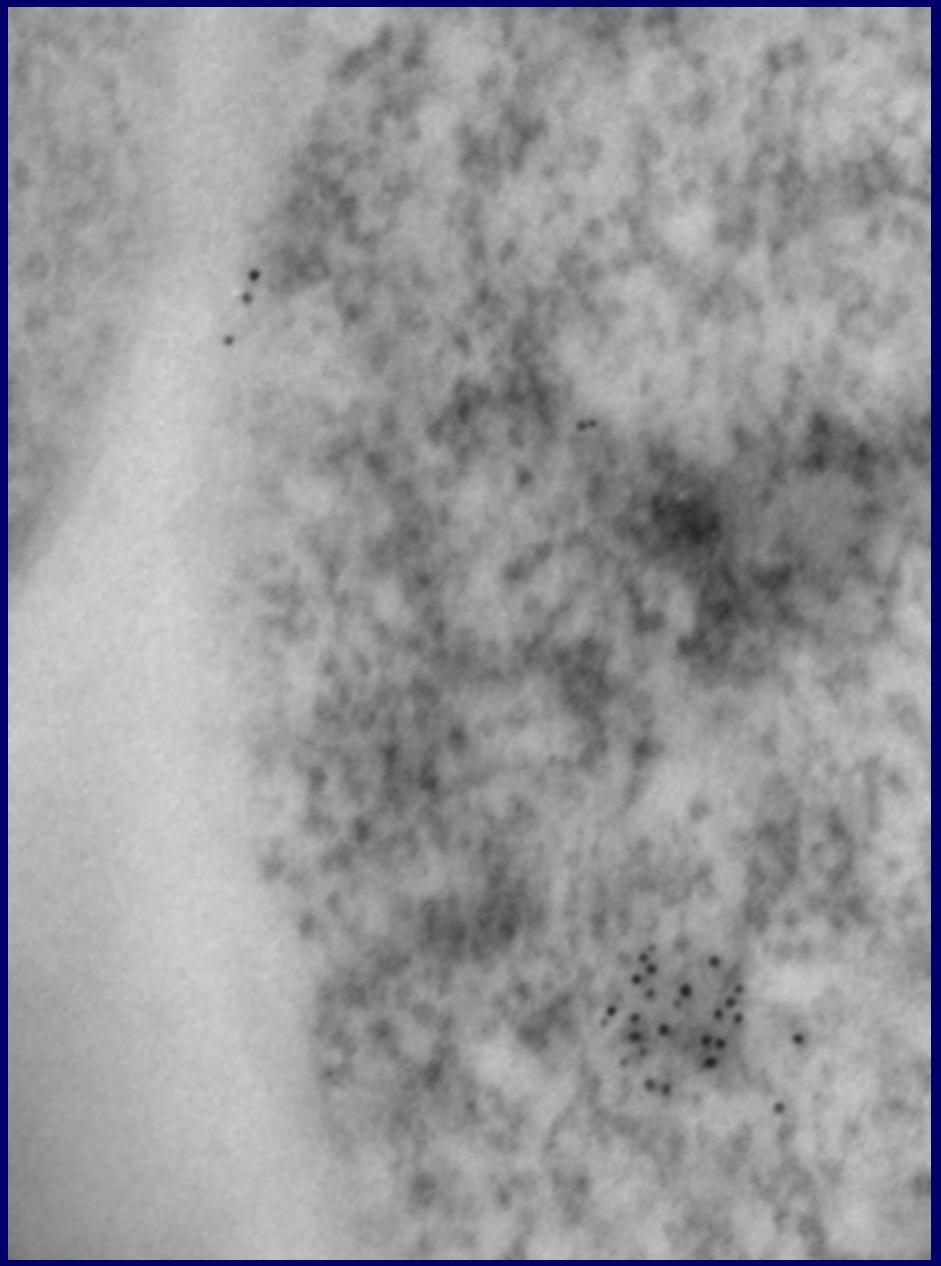


- BFA

+ Cyt D

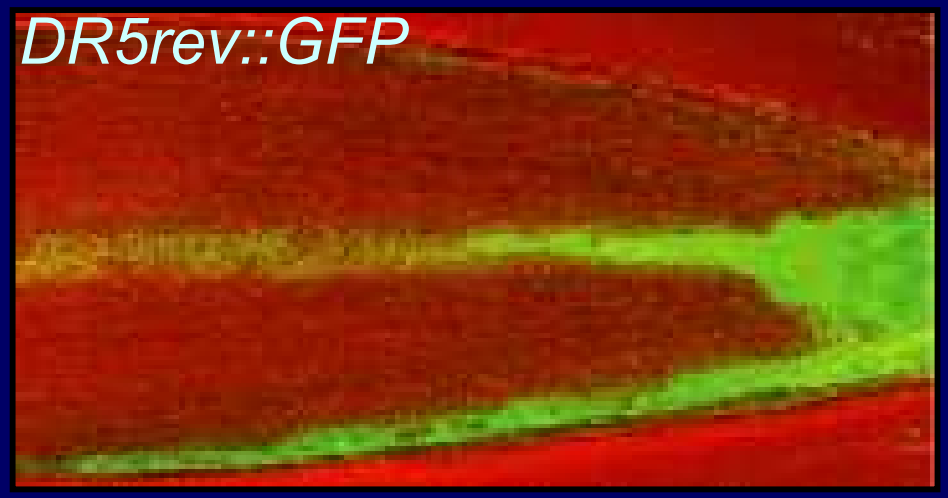
PIN3 Immunogold Electron Microscopy



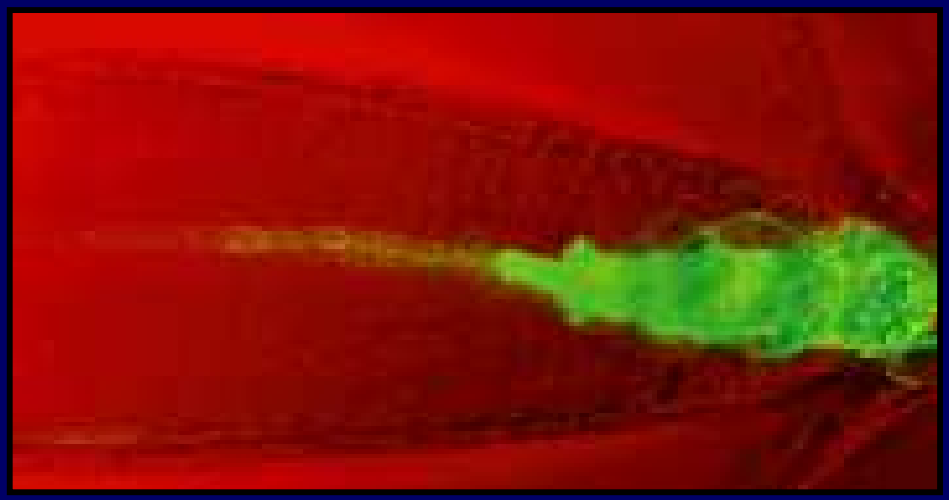


Root Gravitropism

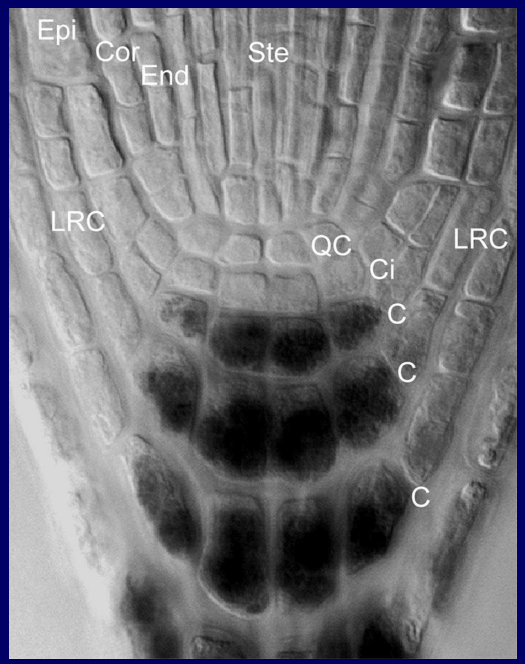
gravity stimulated



gravity + NPA



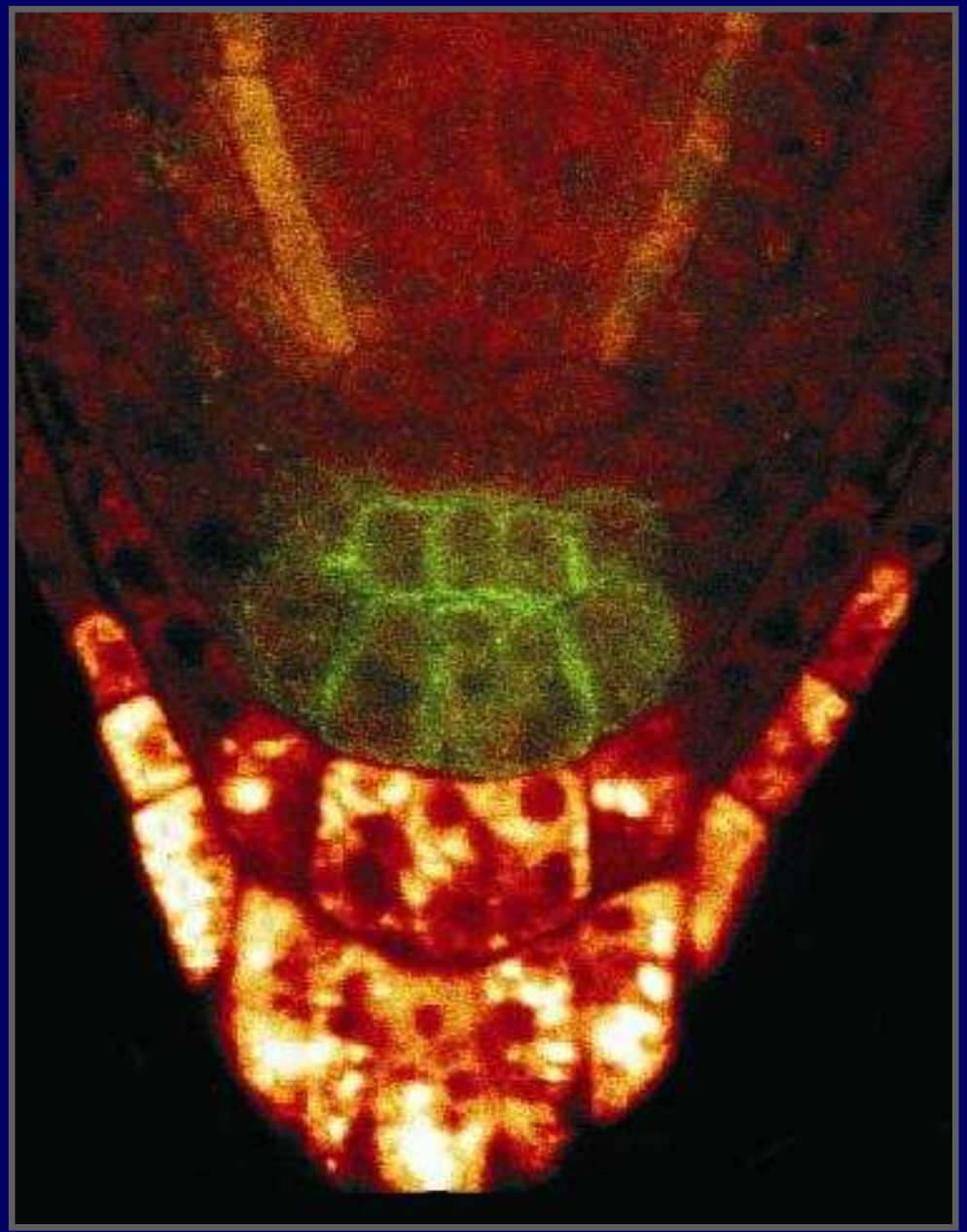
Statoliths
- gravity
perception



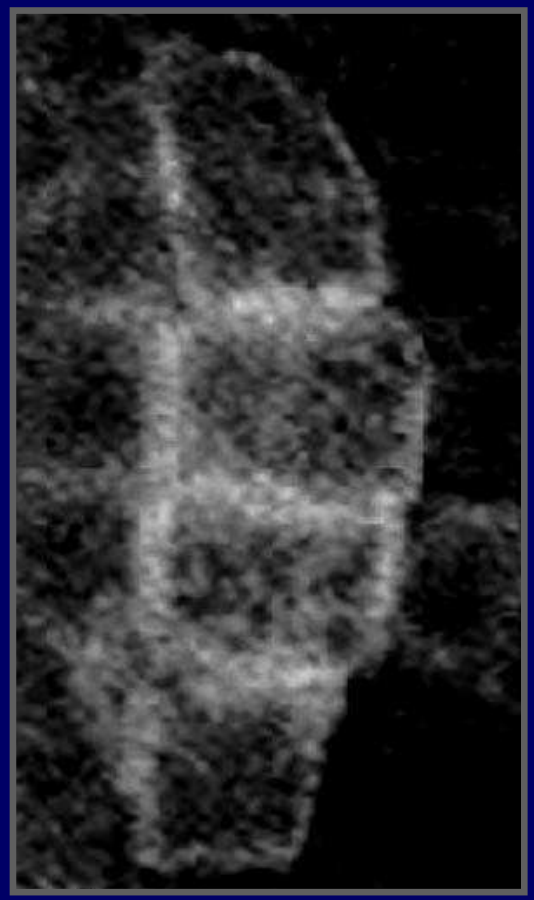
PIN2 localization

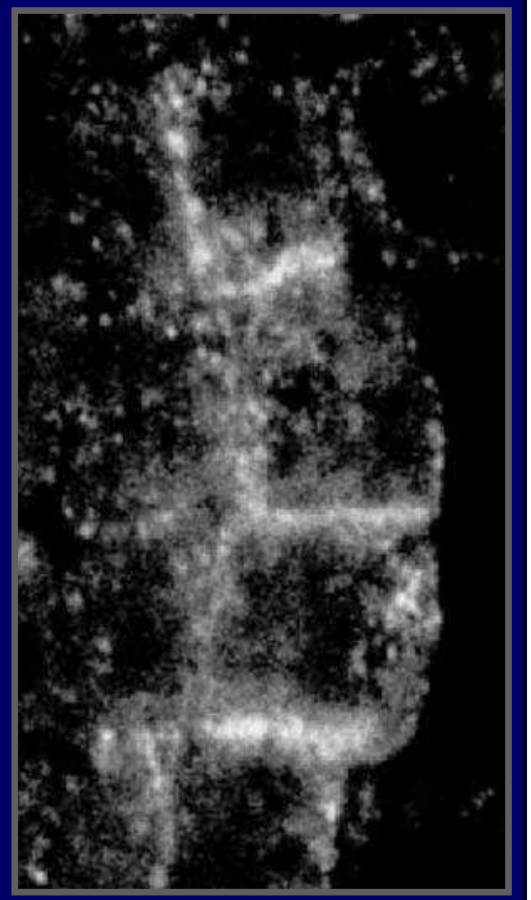
Relocation of PIN3 during Gravitropism

PIN3 in vertical root



PIN3 in root on its side

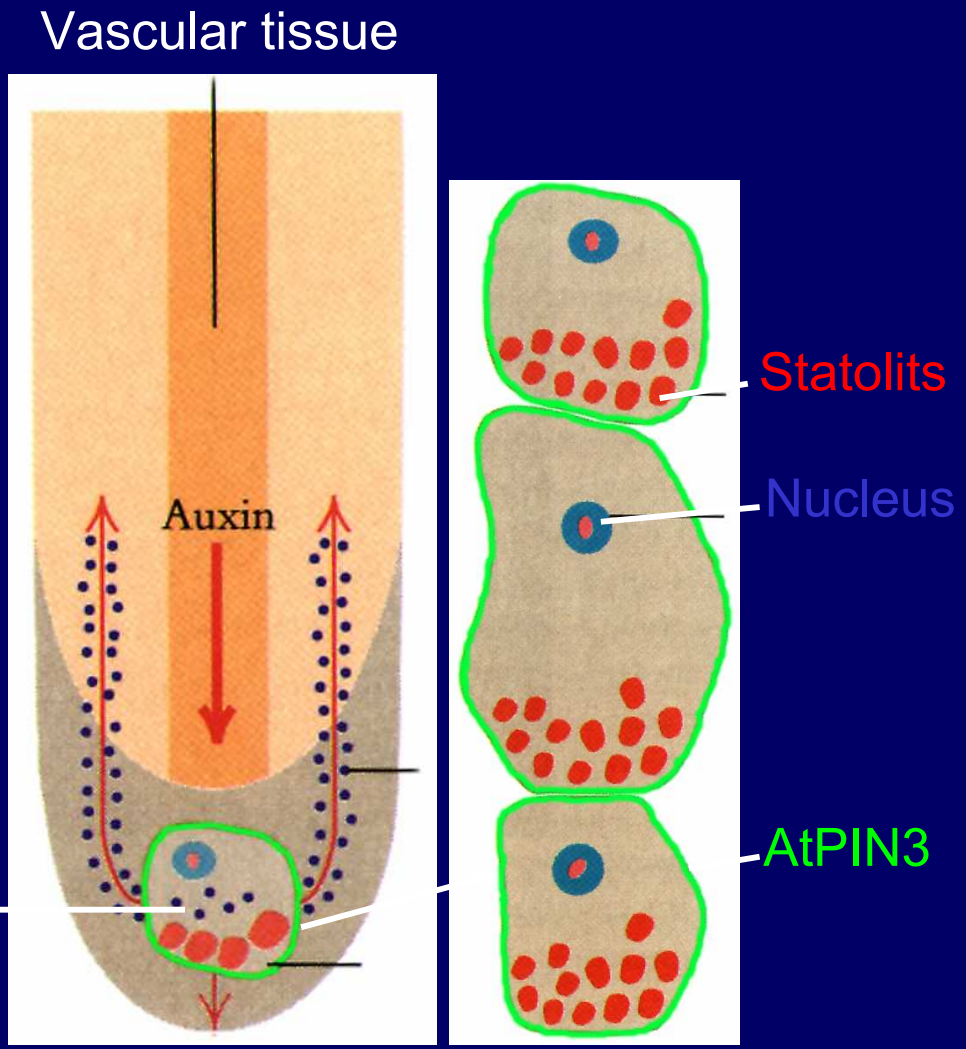




0 min **2 min**

Model for Root Gravitropism

Vertical root



Root turned on its side