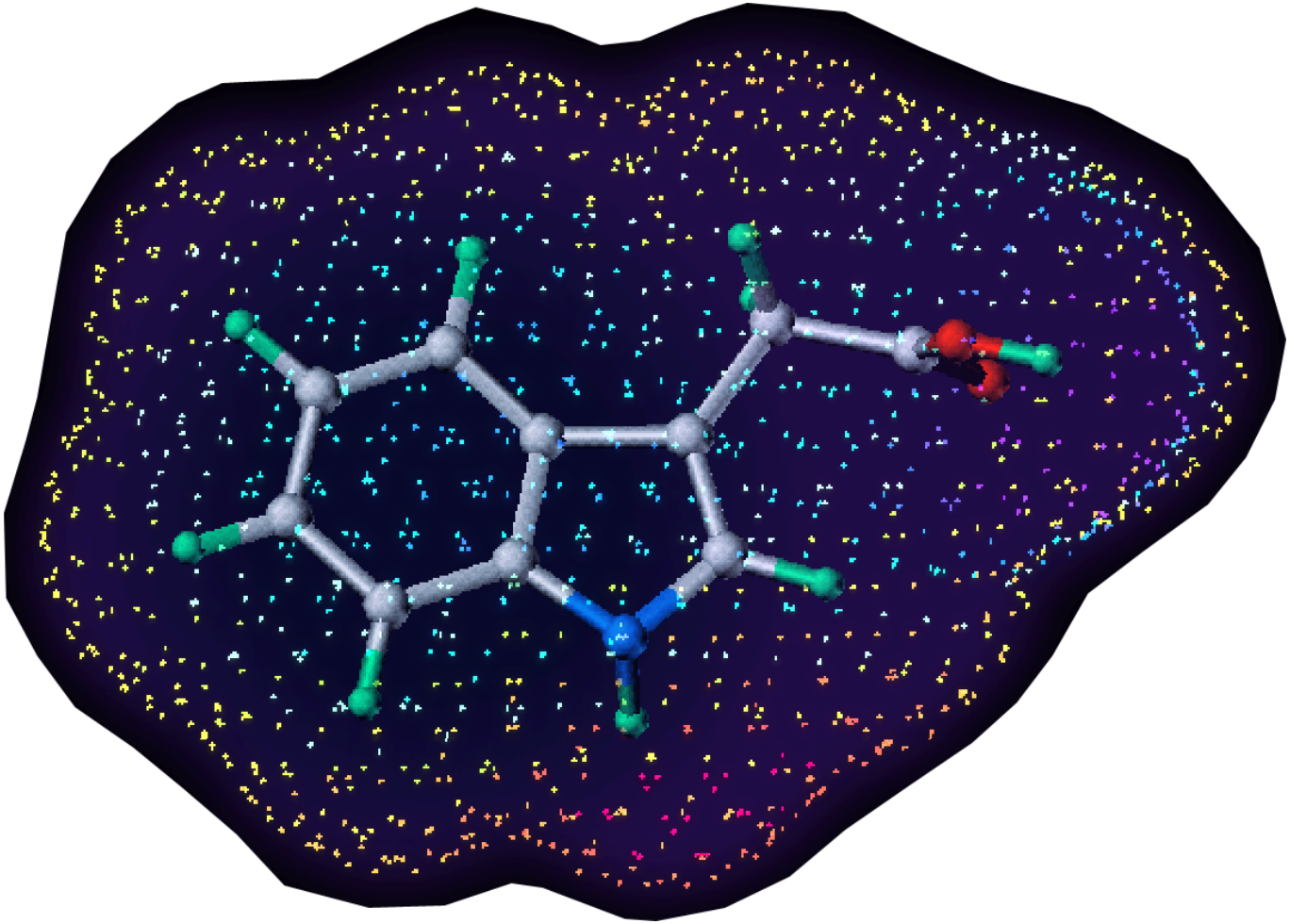
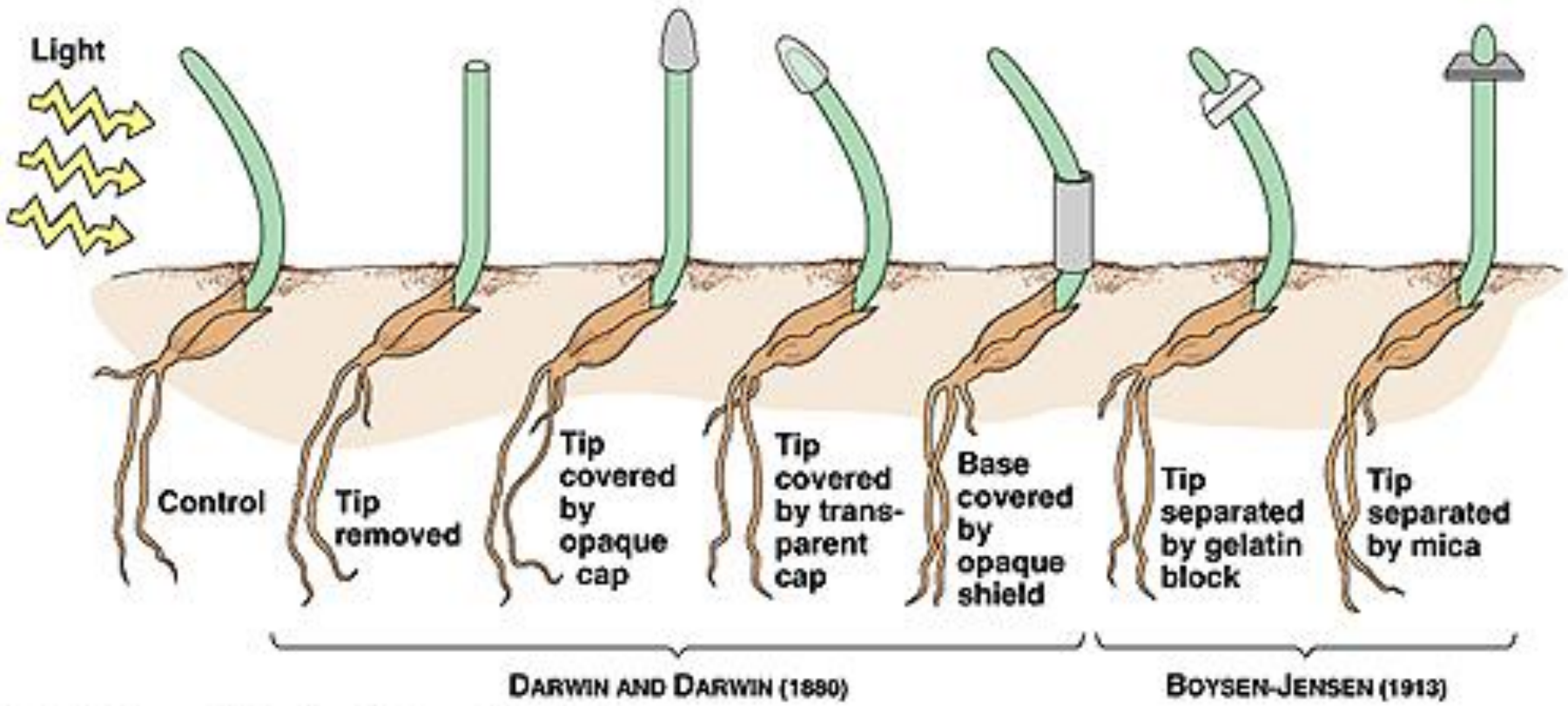
Auxin Signaling and Transport

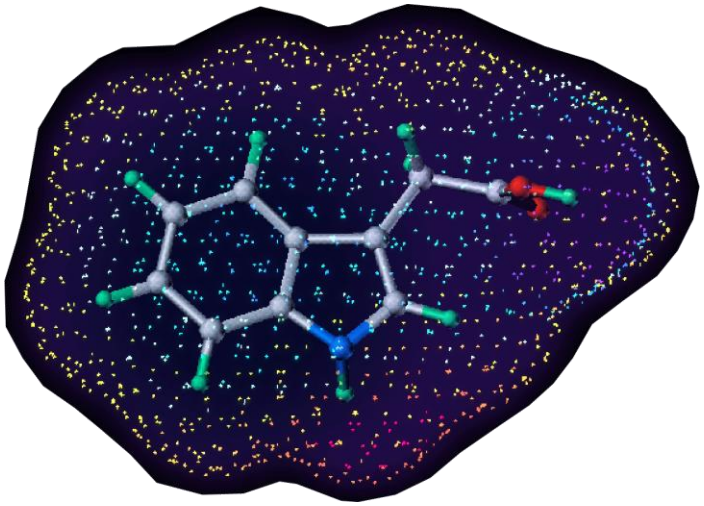


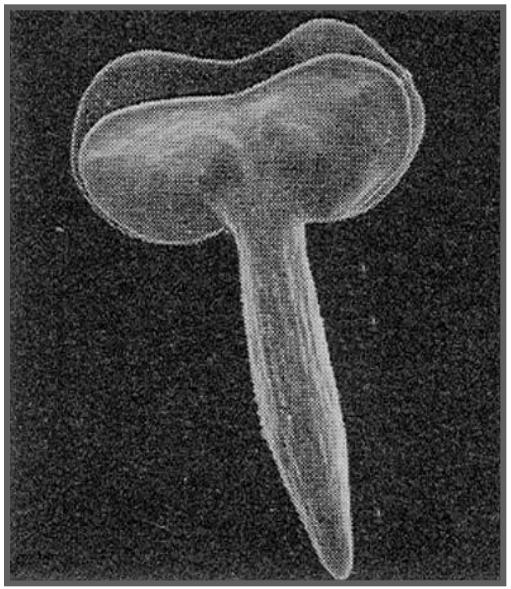
Discovery of the First Plant Signaling Molecule – Auxin and its Transport

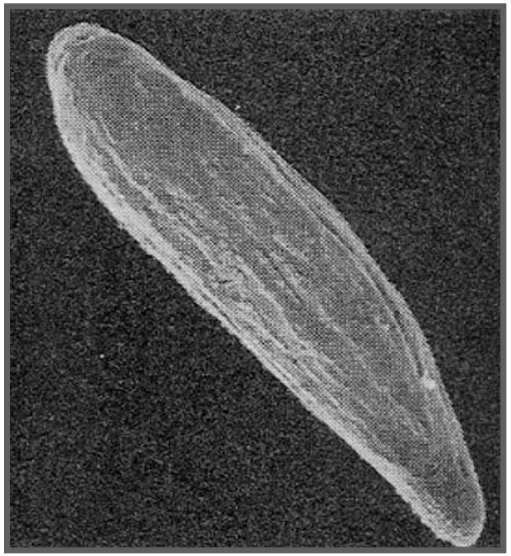


AUXIN

mediates

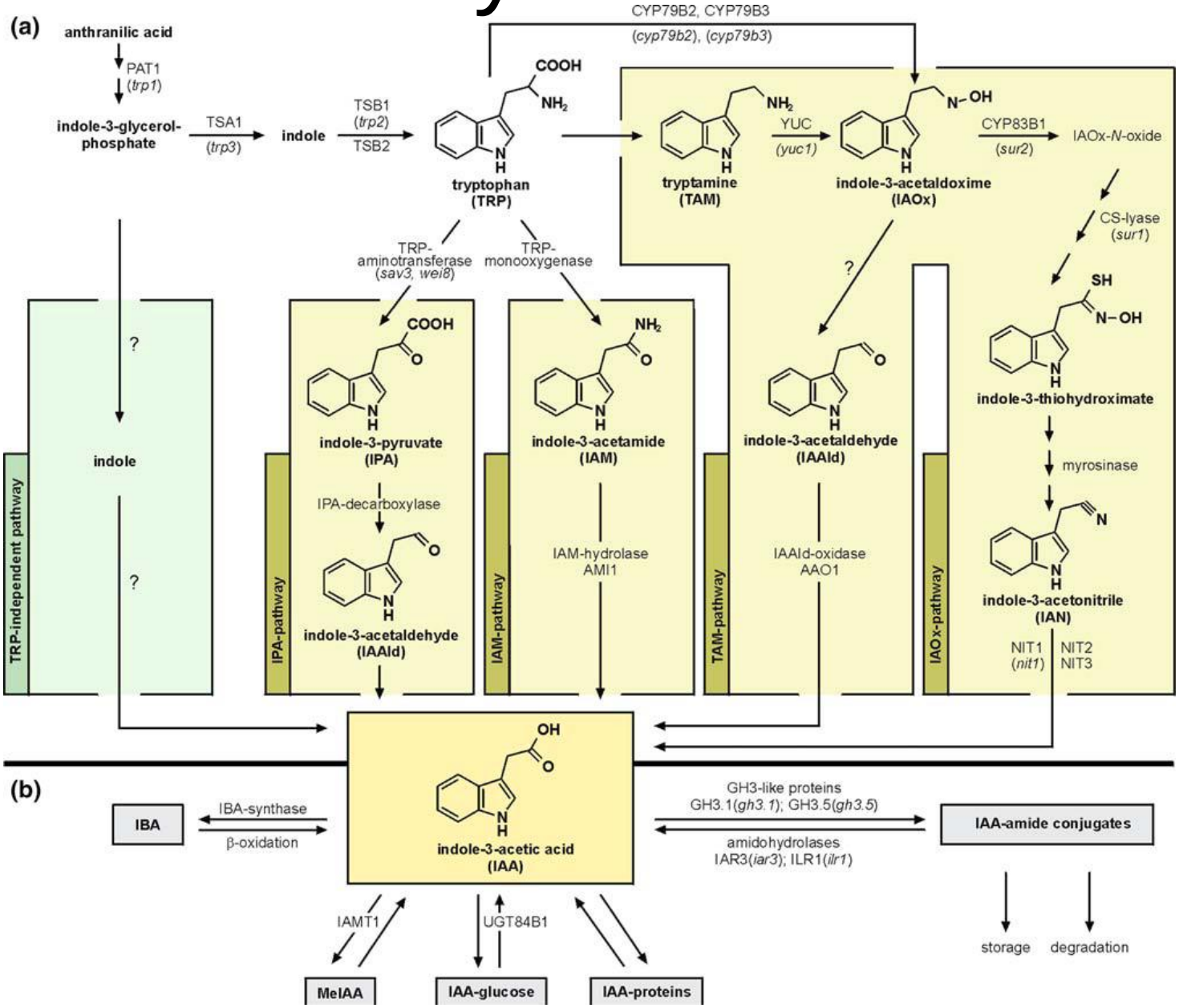




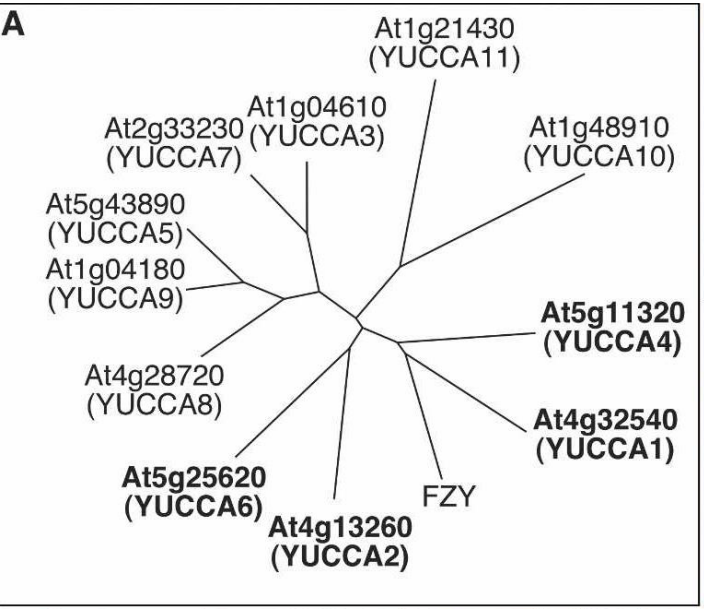


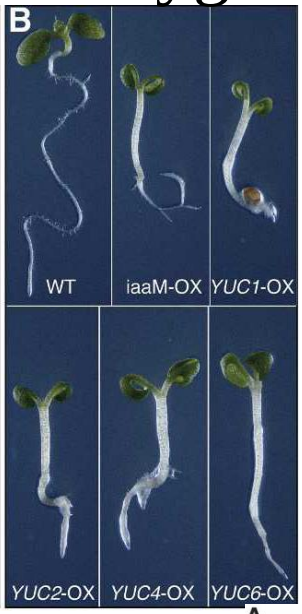
- **Embryo development**
- **Organ initiation and positioning**
- **Vascular tissue differentiation**
- **Shoot and root elongation**
- **Growth responses to light and gravity**
- **Apical hook formation**

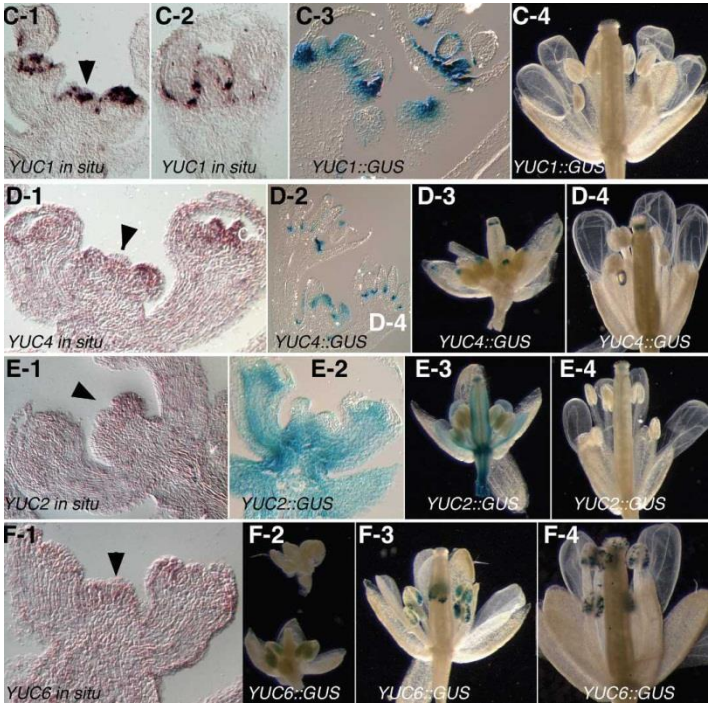
Auxin biosynthesis

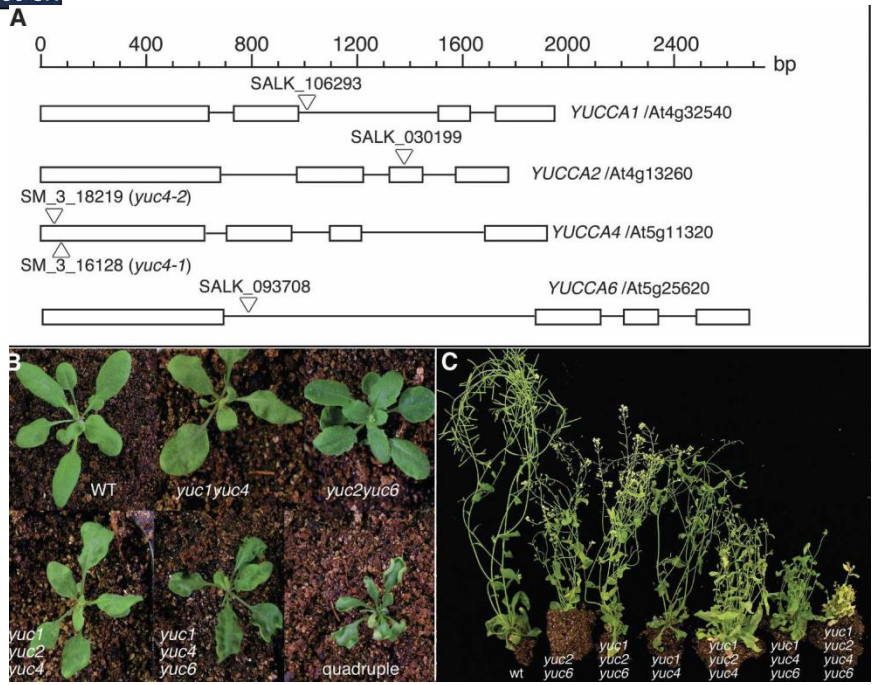


YUCCA - flavin monooxygenases, IAA biosynthesis

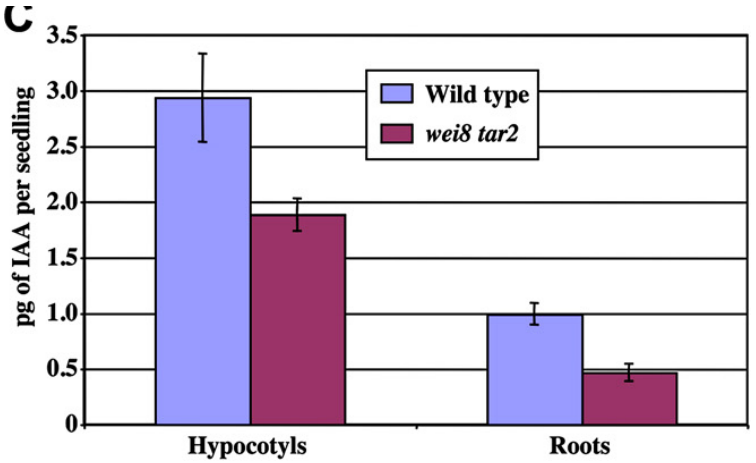


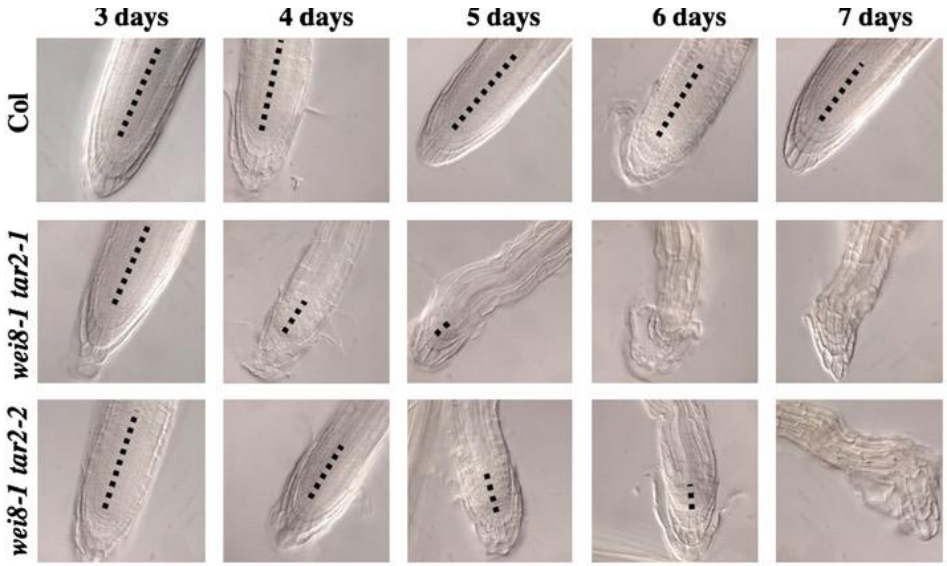


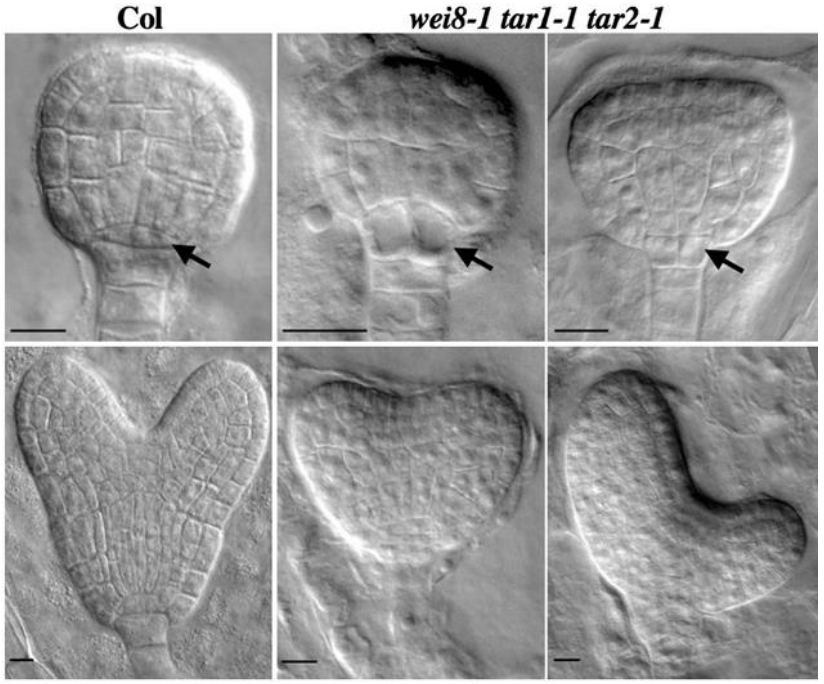




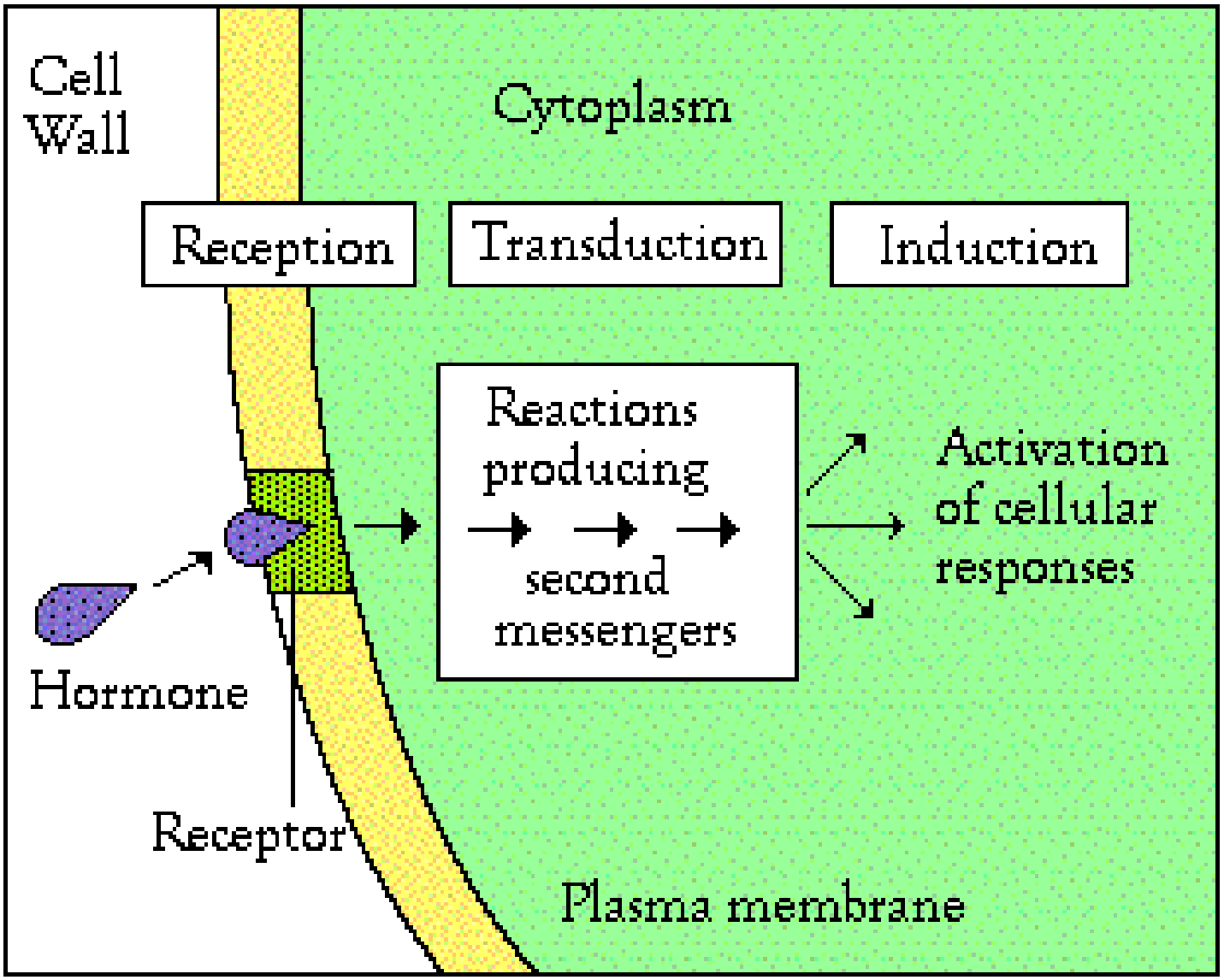
TAA1 tryptophan aminotransferase







Signal Transduction



Biochemical Approach to Identify Auxin Receptor

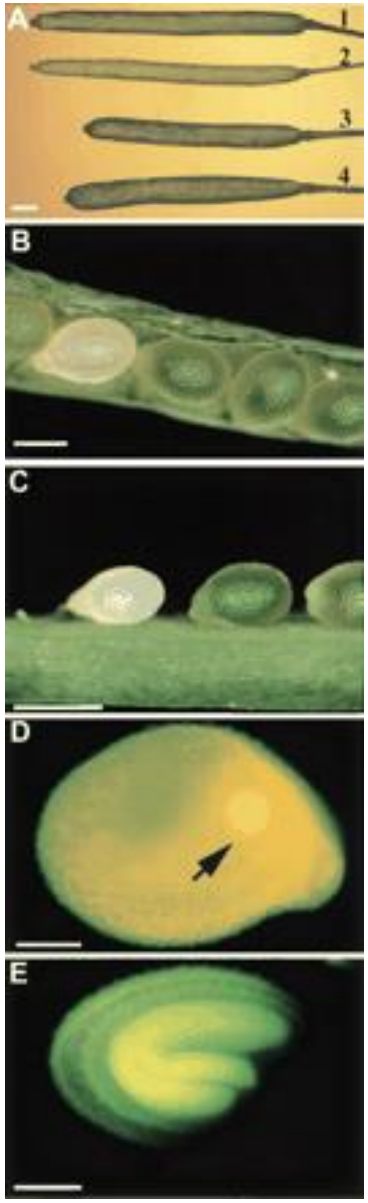
Isolation of auxin binding proteins

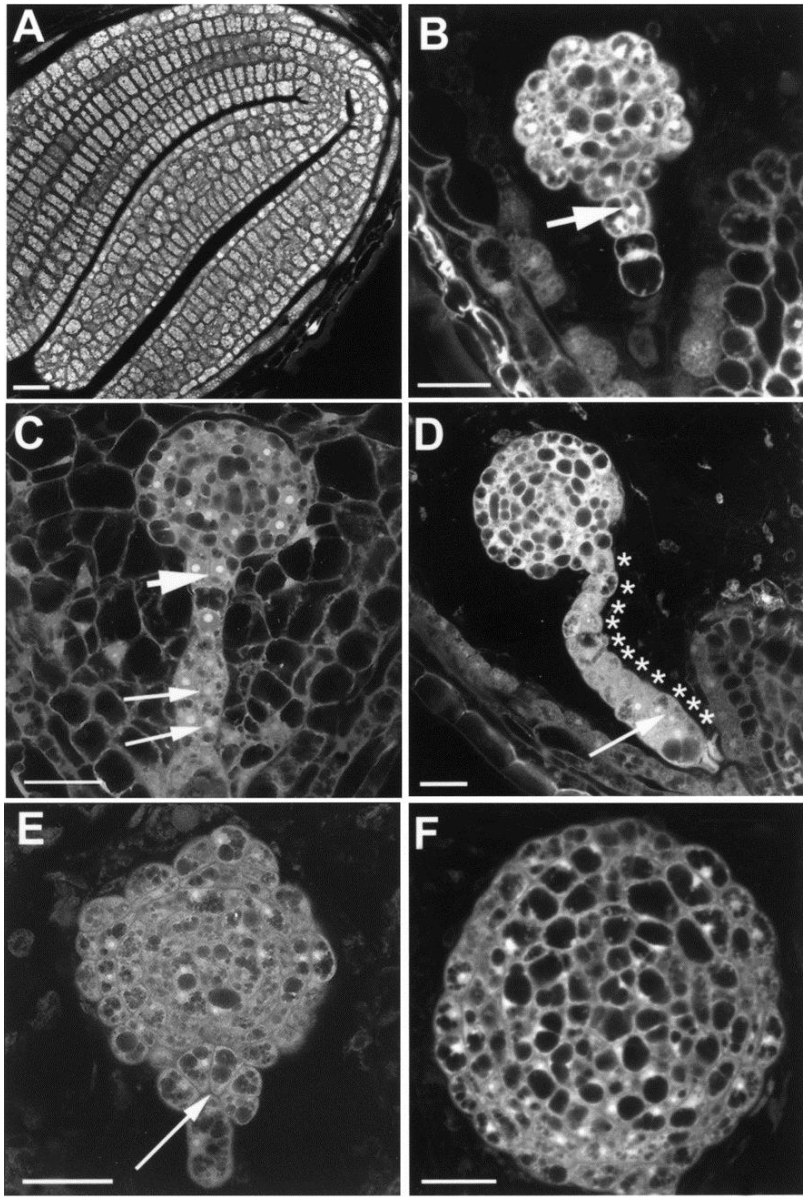
- **Azidolabeling**
- **Affinity chromatography**

Protein sequencing, cDNA screening, gene identification

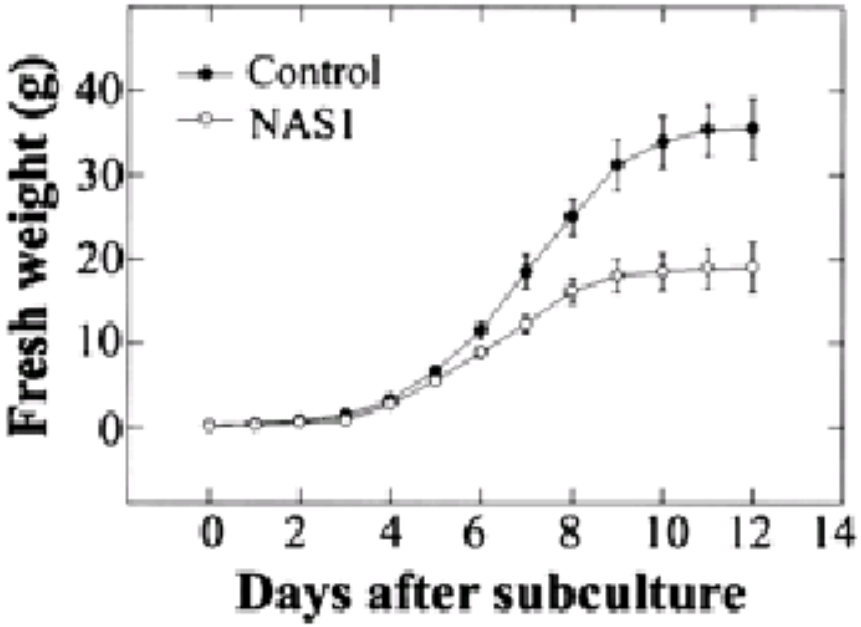
=> Auxin Binding Protein (ABP1)

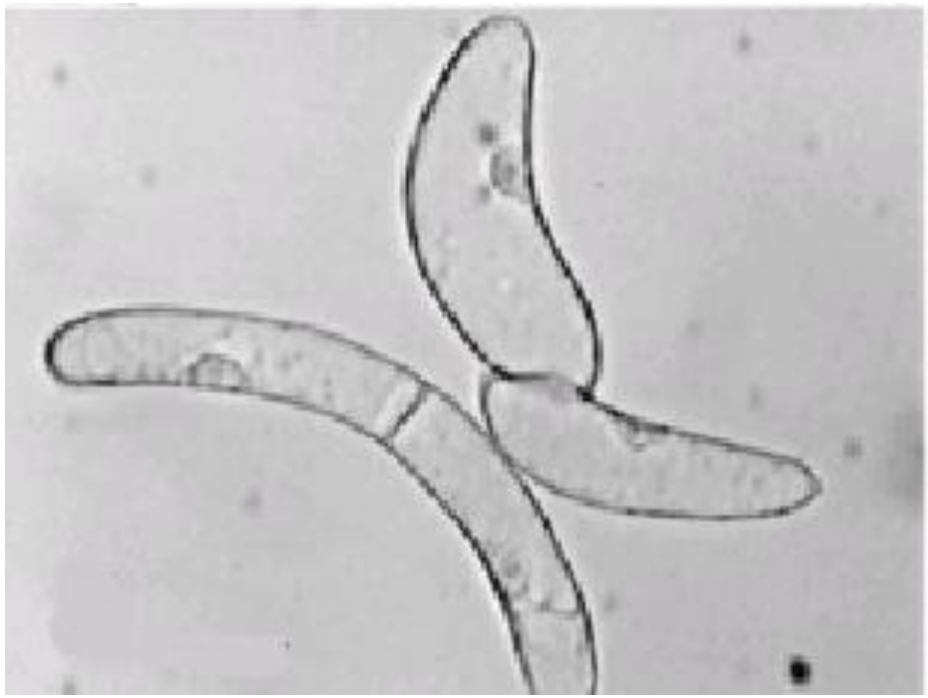
Reverse Genetic – Embryo Lethal *abp1* Mutant



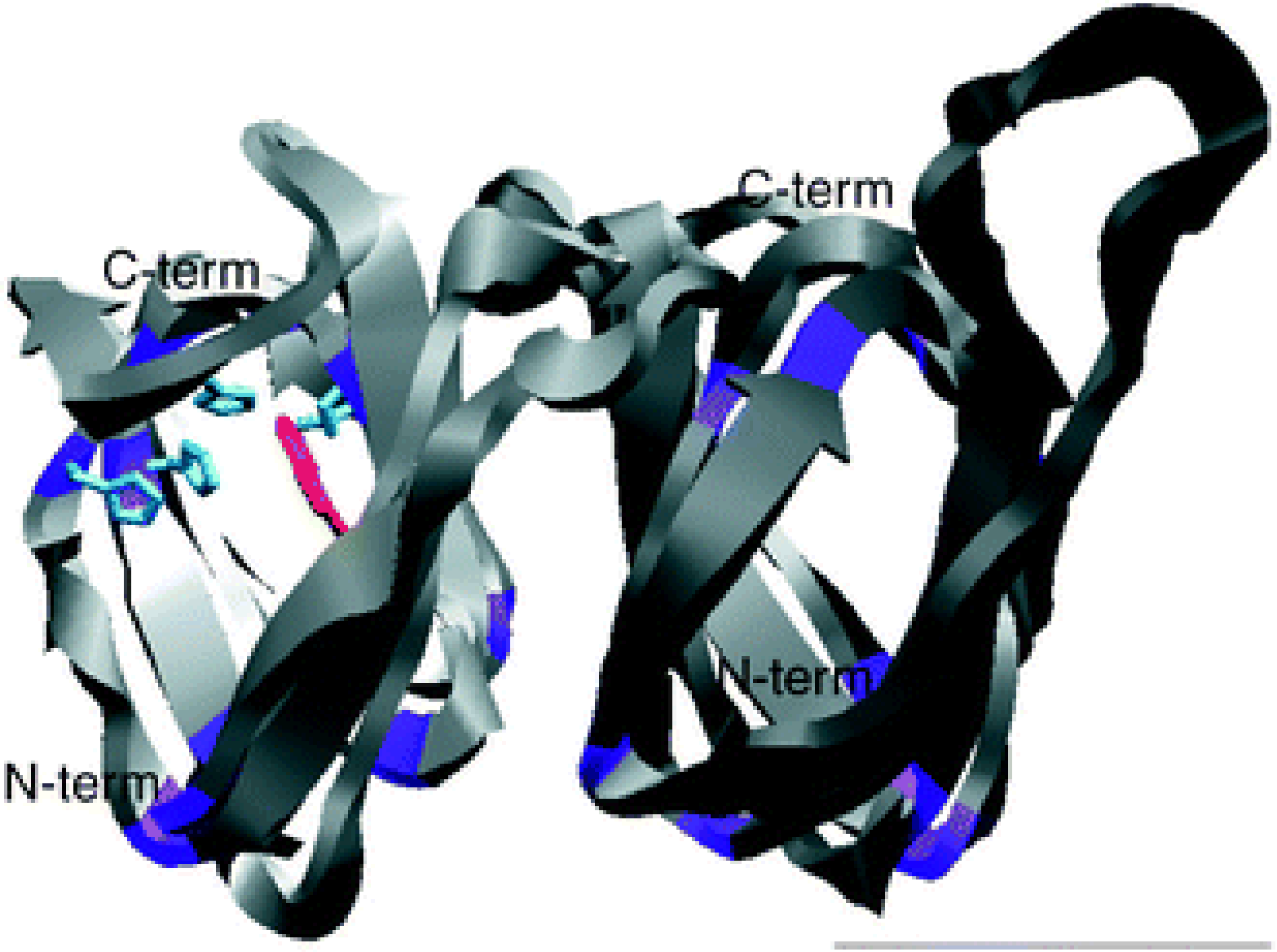


ABP1 Antisense BY-2 Cells Display Defects in Auxin Dependent Cell Elongation





ABP1 – Structure



Molecular Biology Approach to Elucidate Auxin Signaling

Does auxin regulate gene expression?

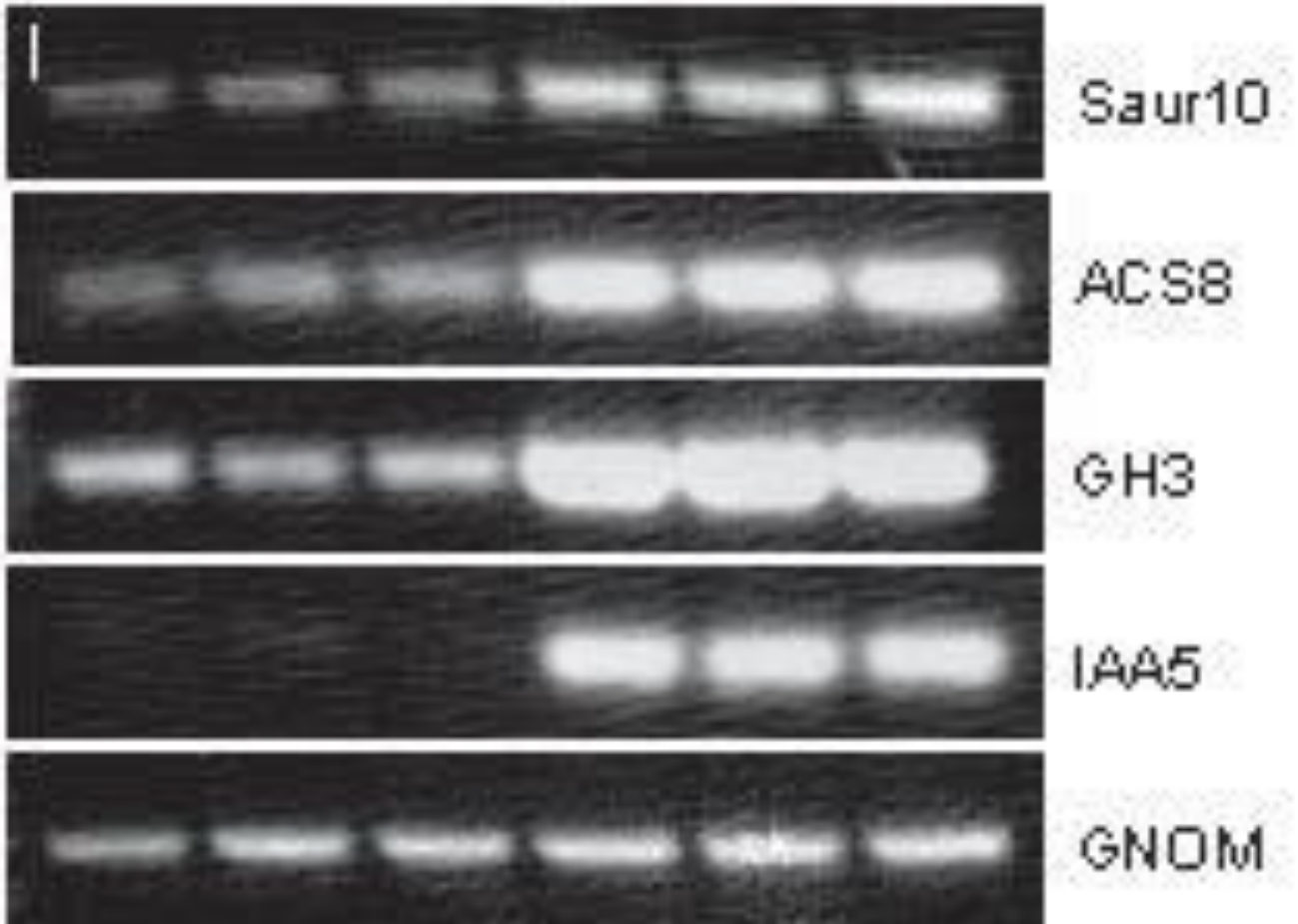
- **Rapidly upregulated mRNAs (*GH3, SAUR, AUX/IAA* genes)**

- **One hybrid screen with Auxin Response Elements => Auxin Response Factors (ARF)**

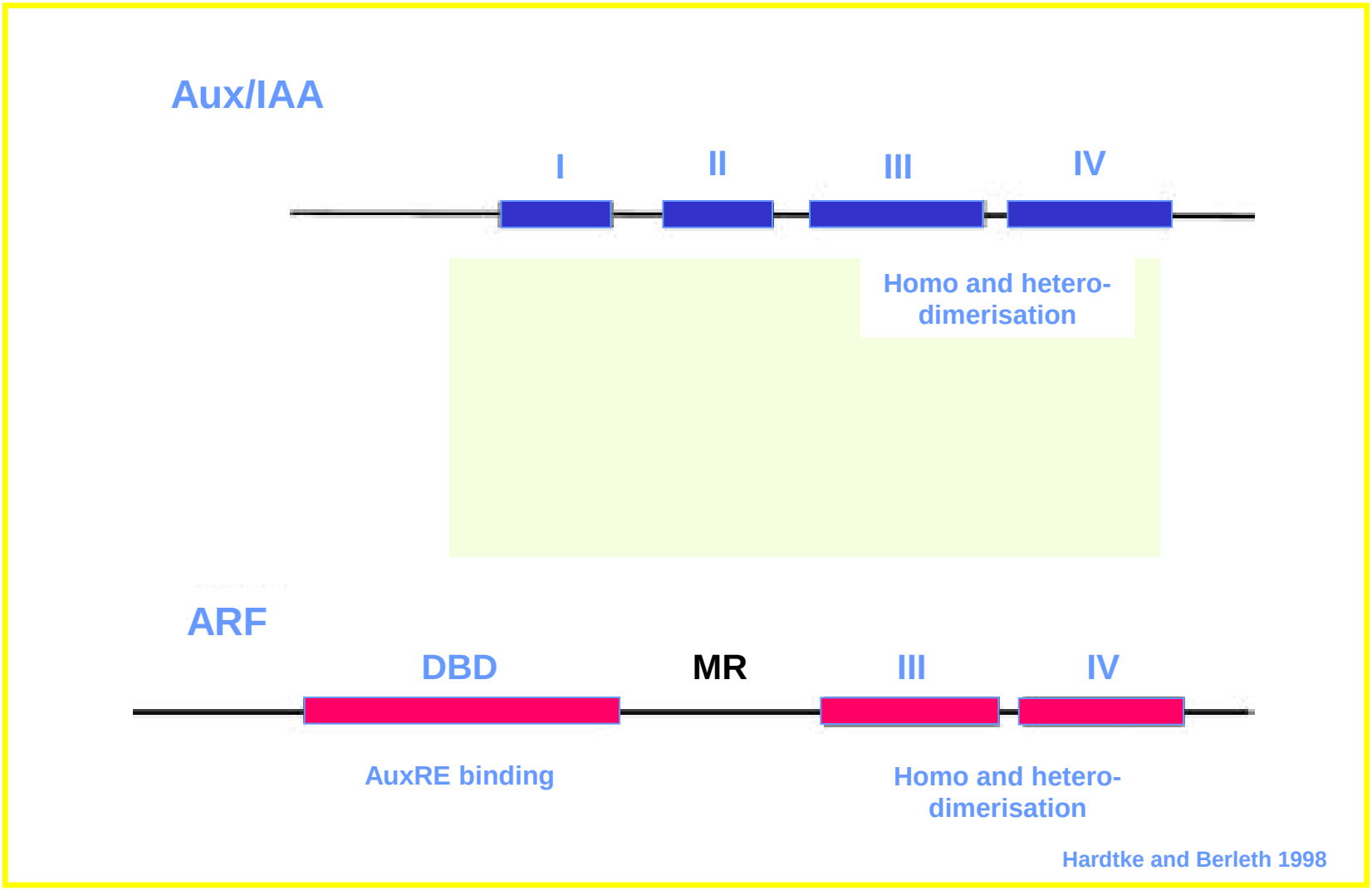
- **Two hybrid => AUX/IAAs interact with ARFs**

Molecular Biology Approach to Elucidate Auxin Signaling

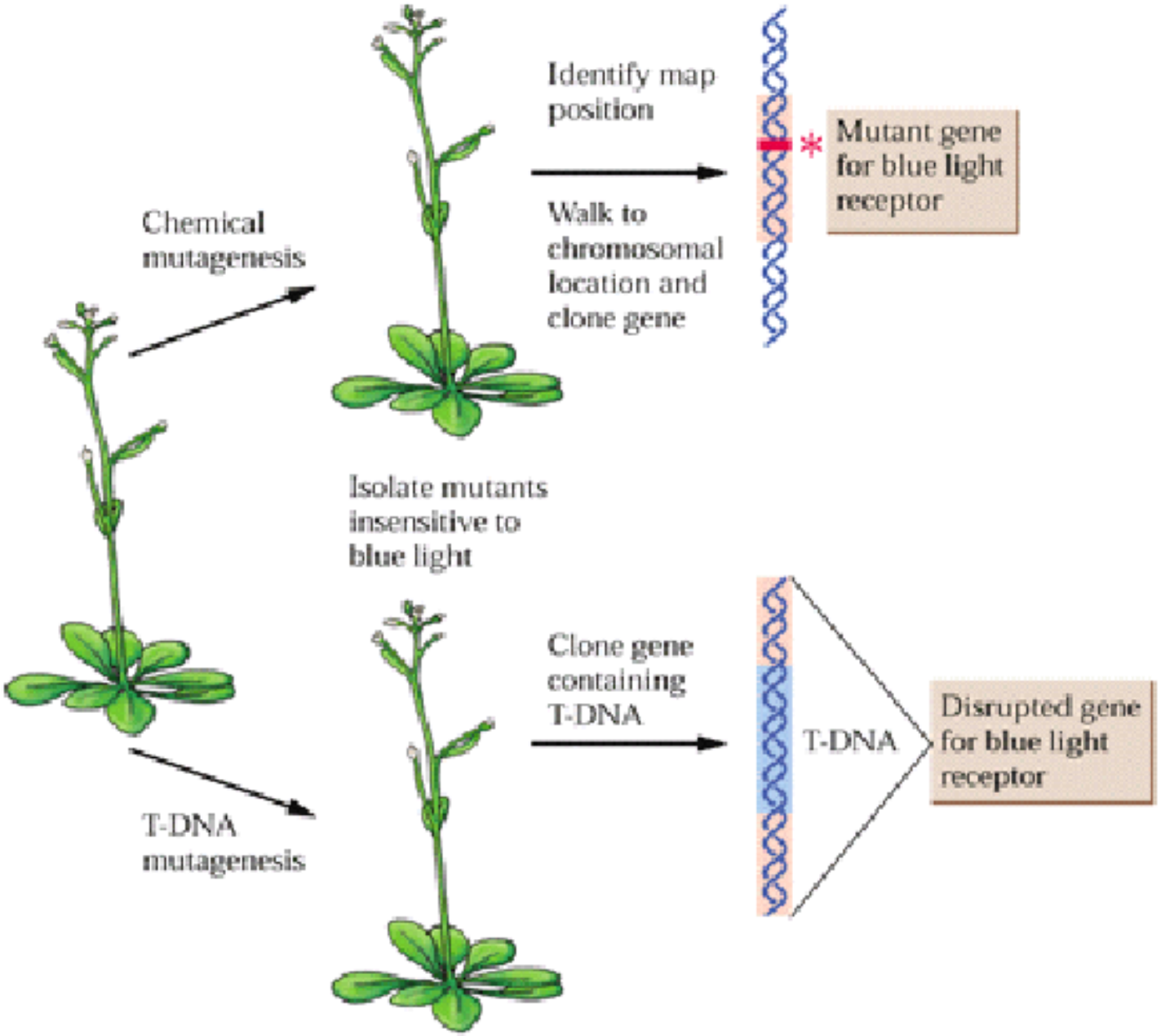
RT-PCR



ARFs auxin response factors – TF controlling, Aux/IAA expression



Forward genetics



Genetic Approach to Identify Auxin Receptor

- Auxin resistant (axr): *axr1 – axr6*

- Transport inhibitor response (tir): *tir1 – tir7*

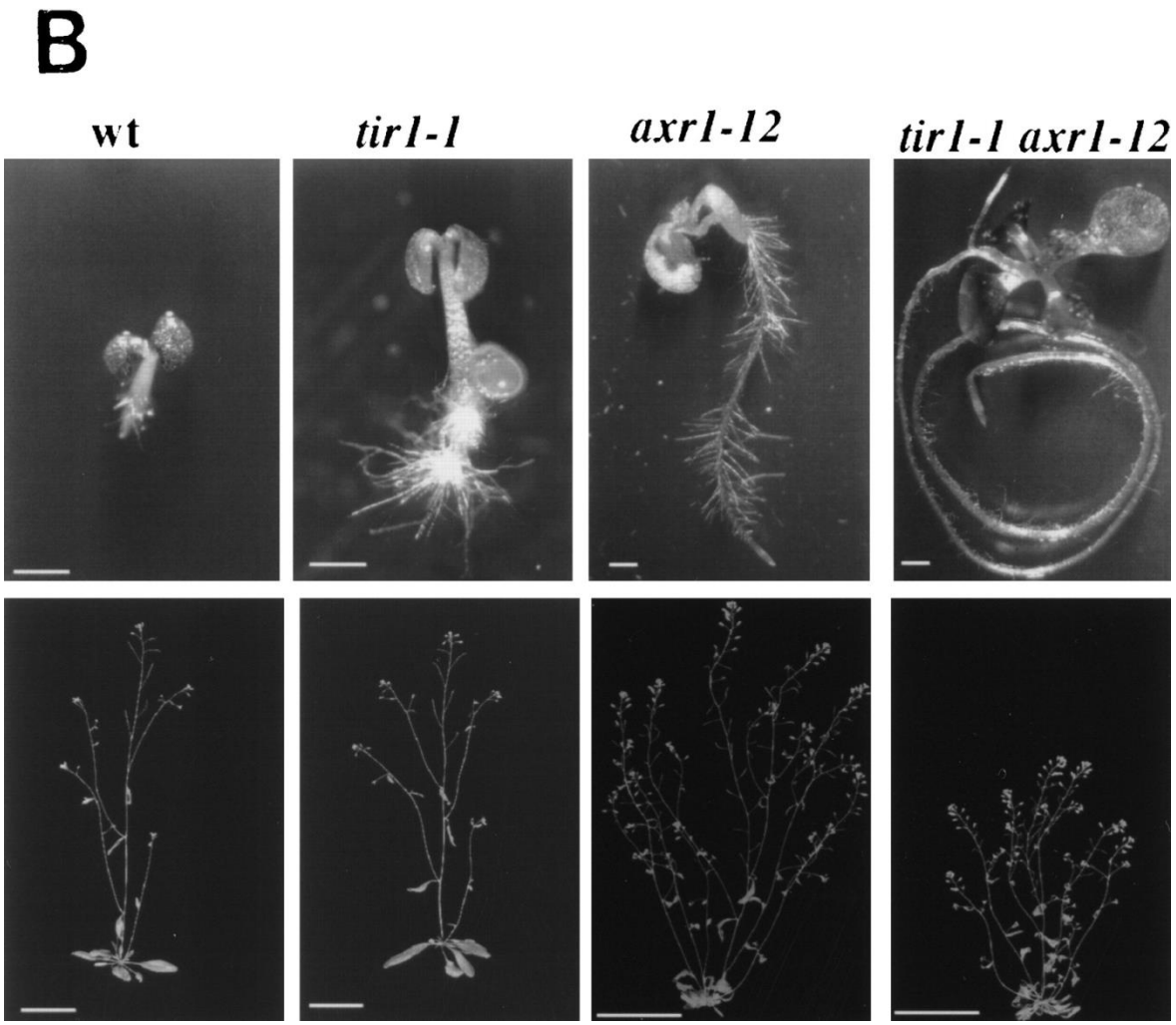
Morphological mutants (*monopteros, bodenlos,* etc.)

=> Role of regulated protein degradation and transcriptional regulation in auxin signaling

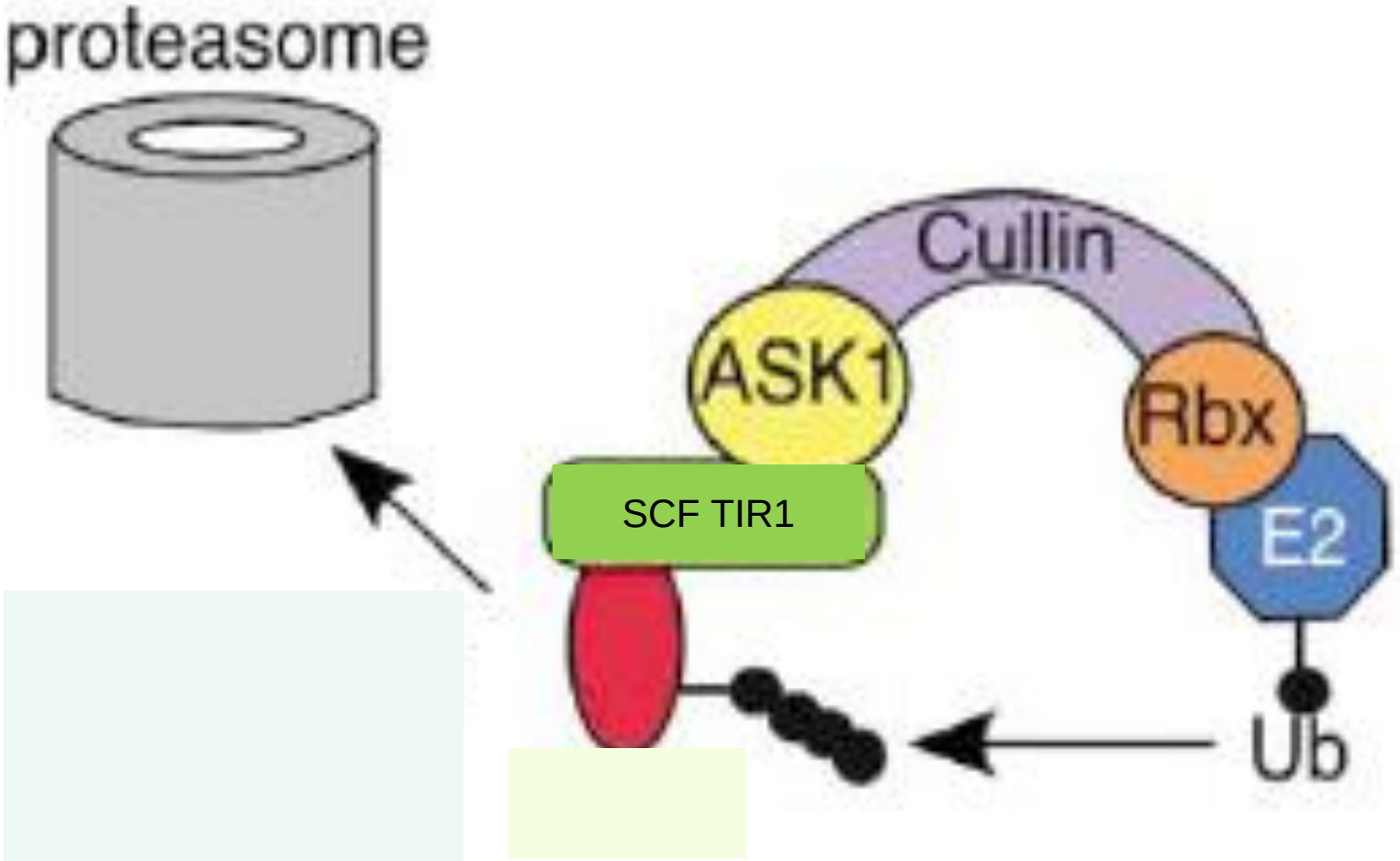
None of the identified gene looks like a receptor

Auxin resistant (axr): ***axr1 – axr6***

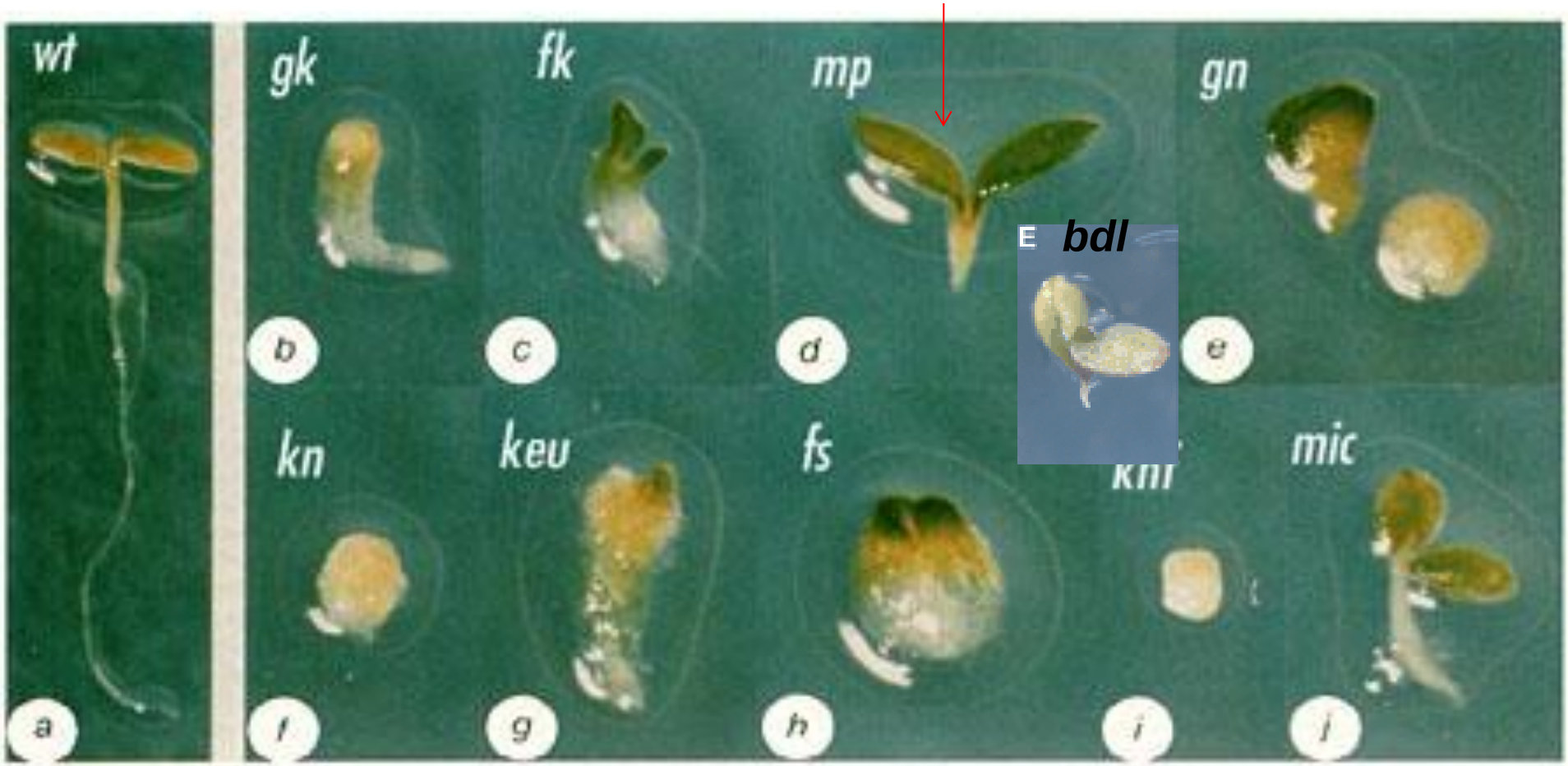
Auxin Transport inhibitor response (tir):



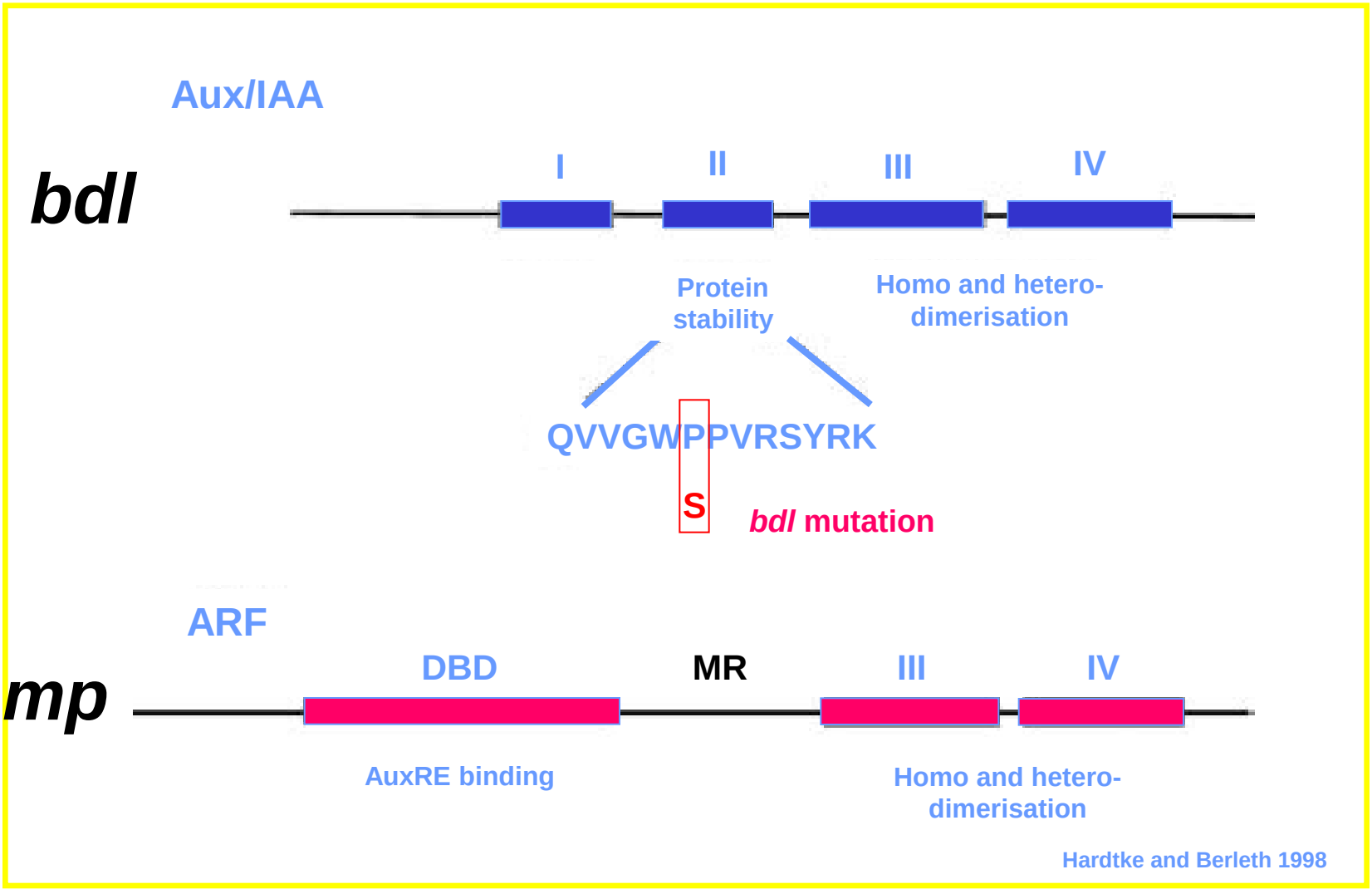
Subunits of ubiquitin ligase


proteasome
Cullin
ASK1
Rbx
SCF TIR1
E2
Ub

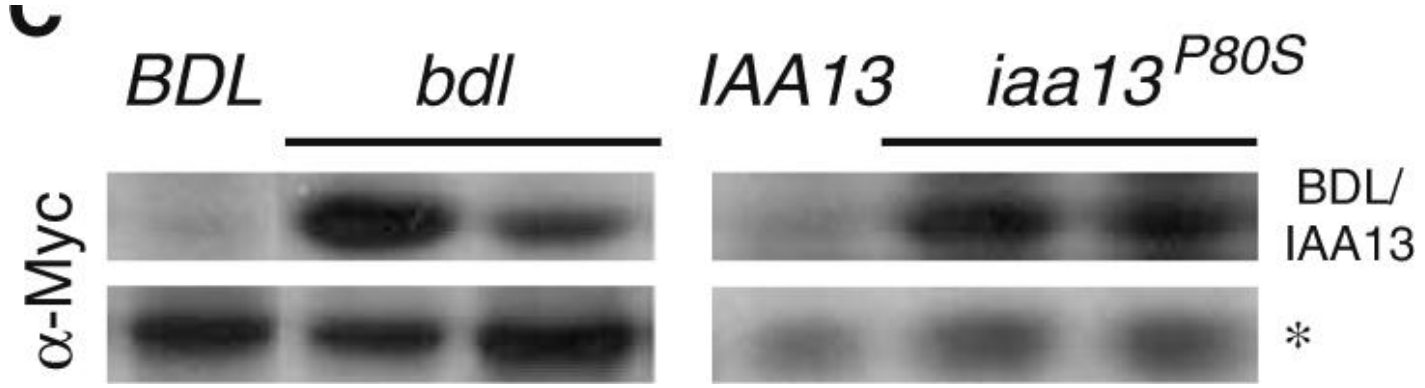
Mutant Screen at Seedling Level



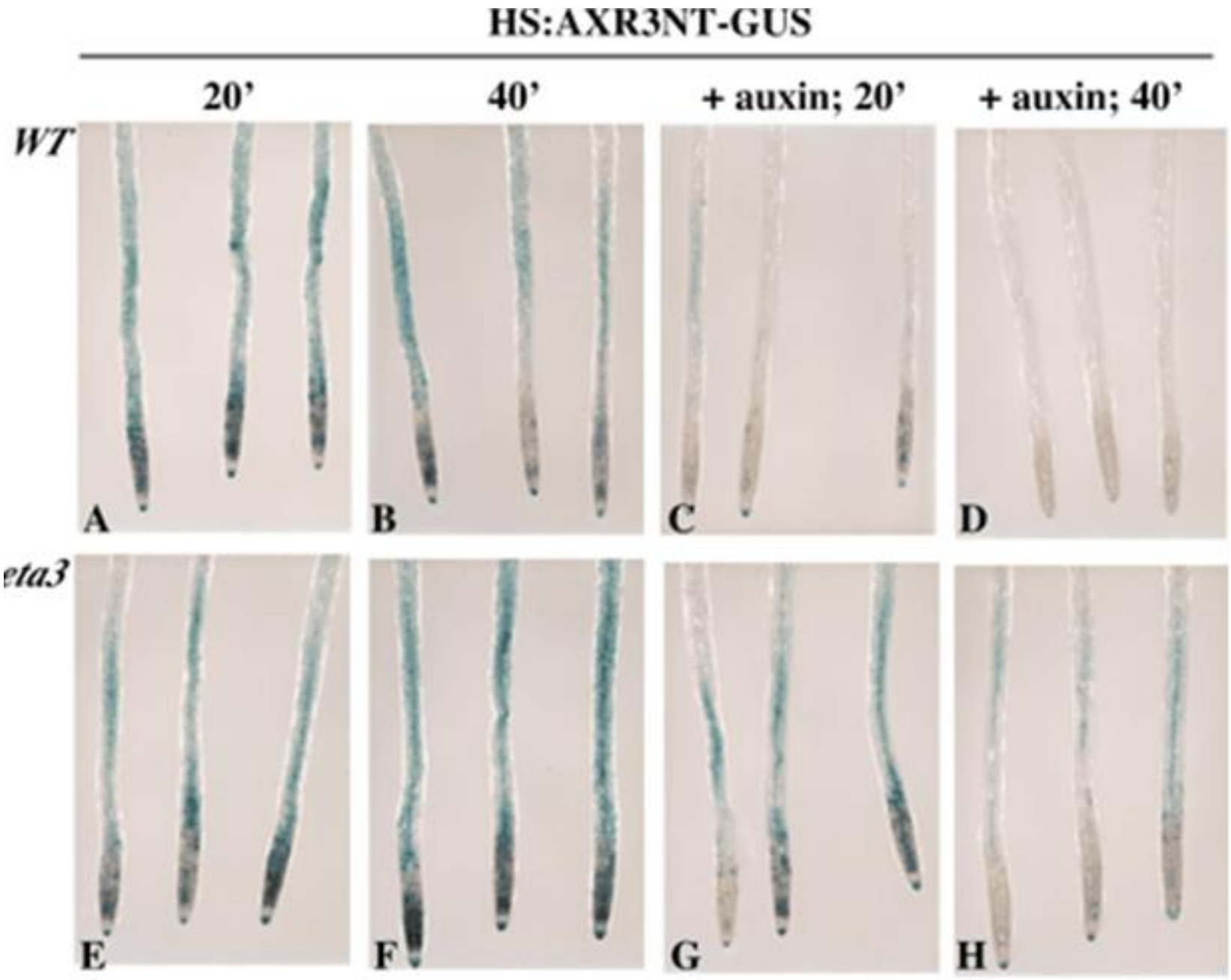
Some ARFs are **Activators**, and AUX/IAAs **Repressors** of Auxin Response



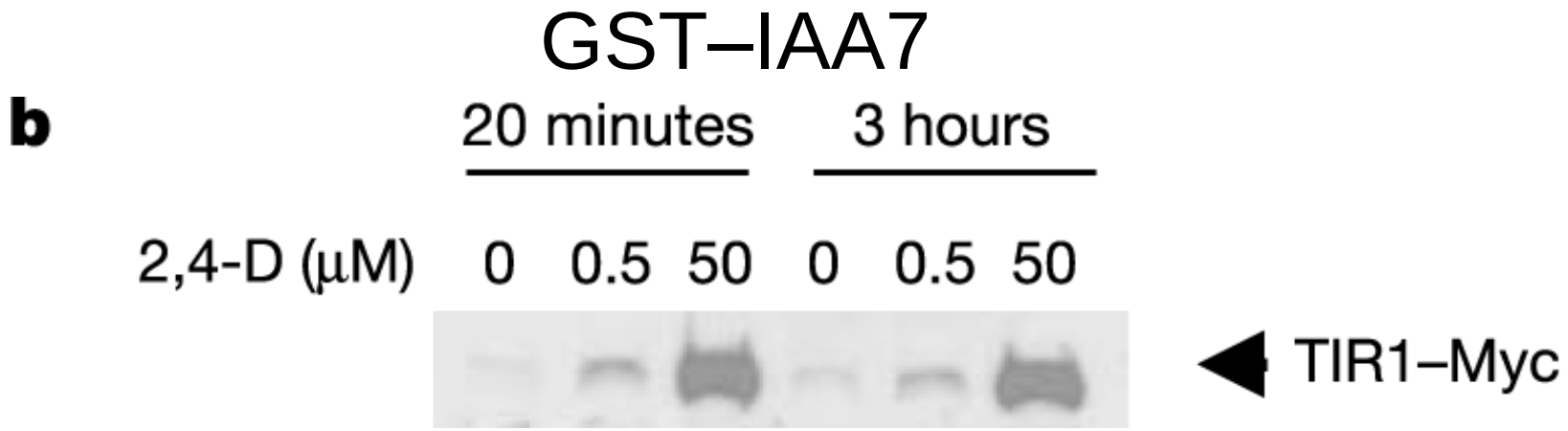
Mutation in domain II stabilizes Aux/IAAs



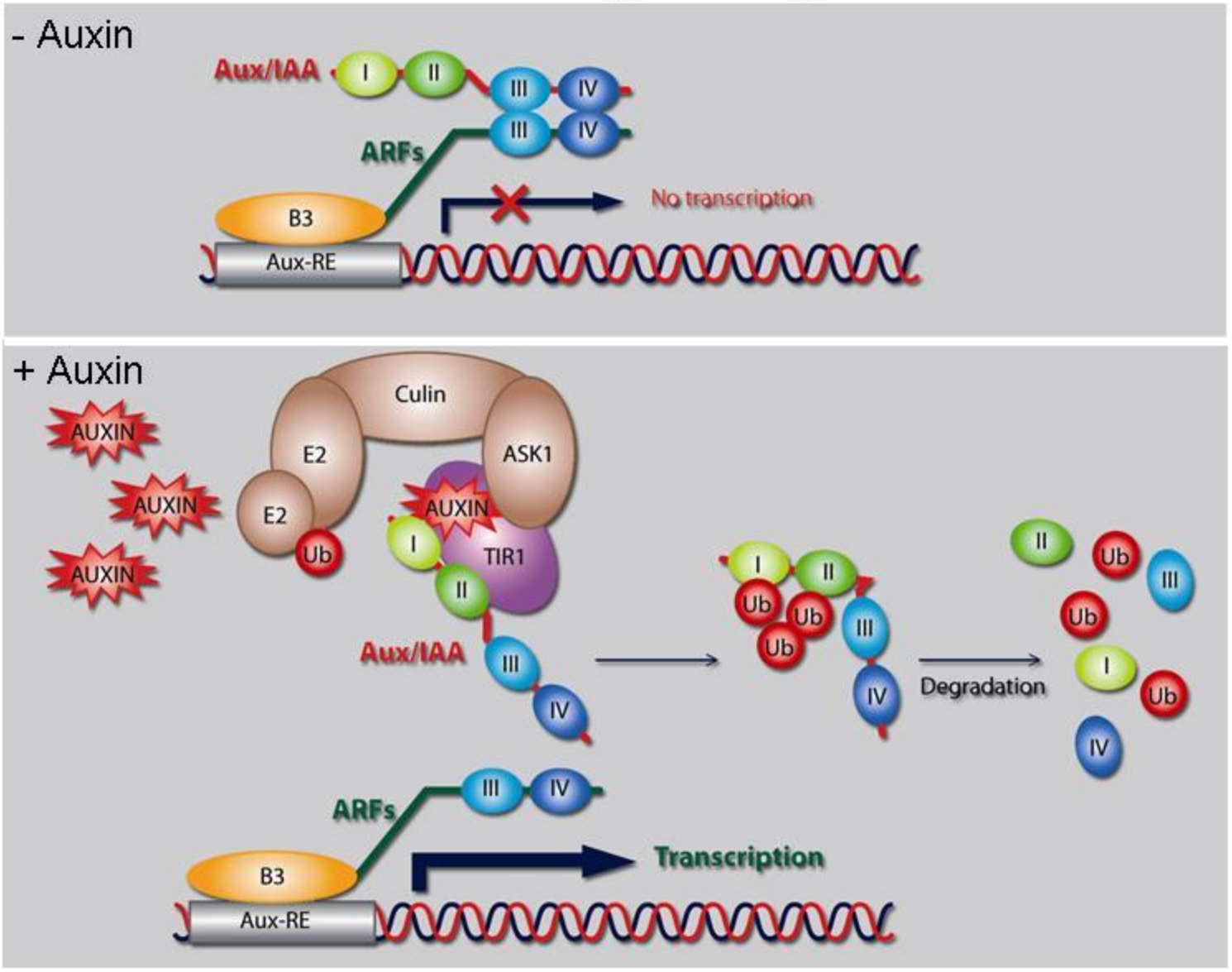
AUX/IAAs are stabilized in enhancer TIR1 mutant



Pull-down



Auxin signaling



Summary for Auxin Signaling

Biochemical approach – auxin binding protein ABP1

binds auxin, important in embryogenesis, role in endocytosis

Genetic approach – role of protein degradation (*axr1, tir1*)

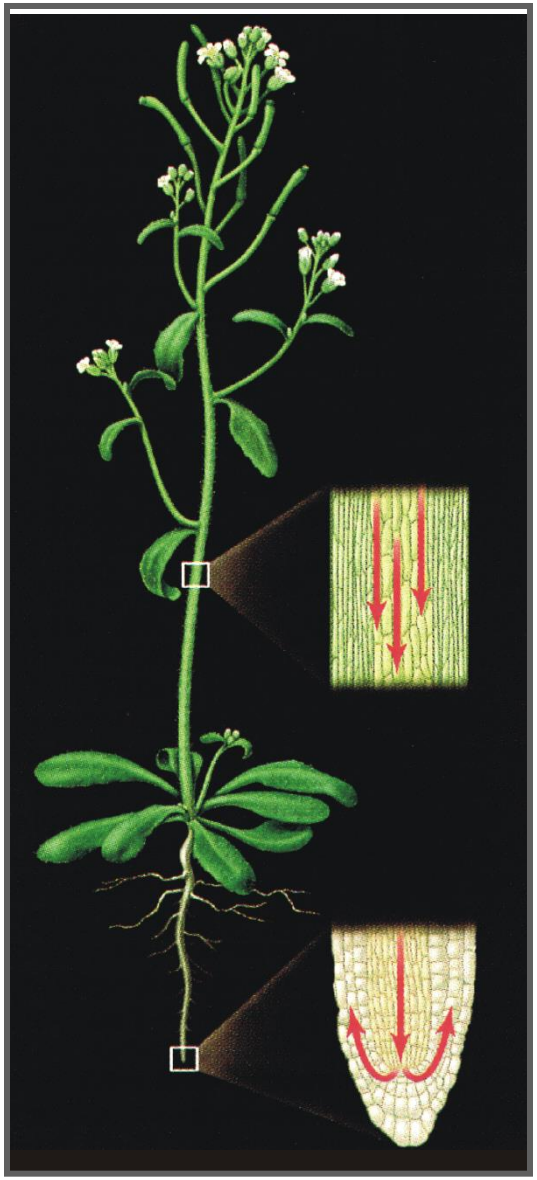
Molecular approach – auxin regulates expression

ARE in promotors of auxin regulated genes

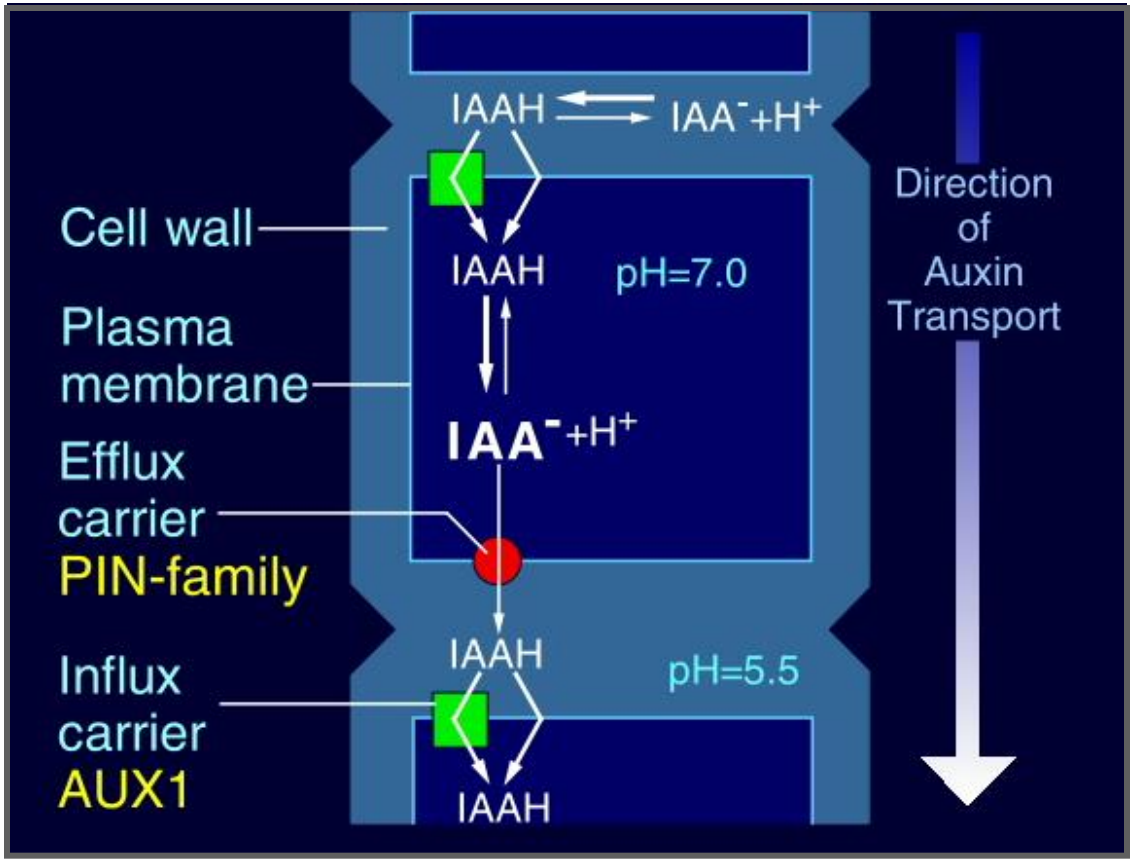
ARF transcription factors binds to ARE

AUX/IAA proteins repress ARF and are degraded upon auxin signal

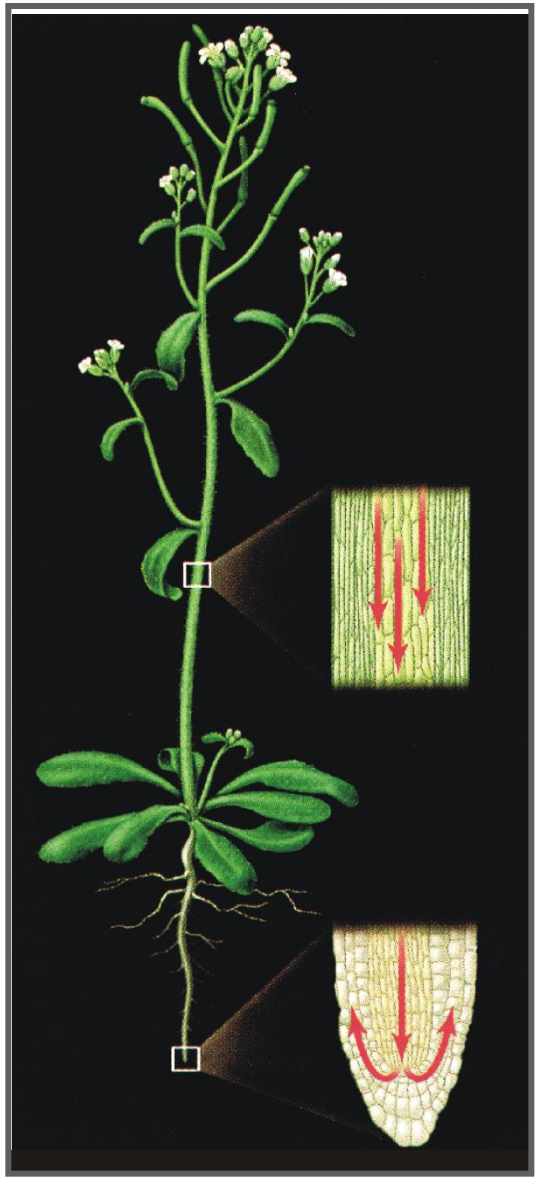
Auxin Transport



Chemiosmotic hypothesis

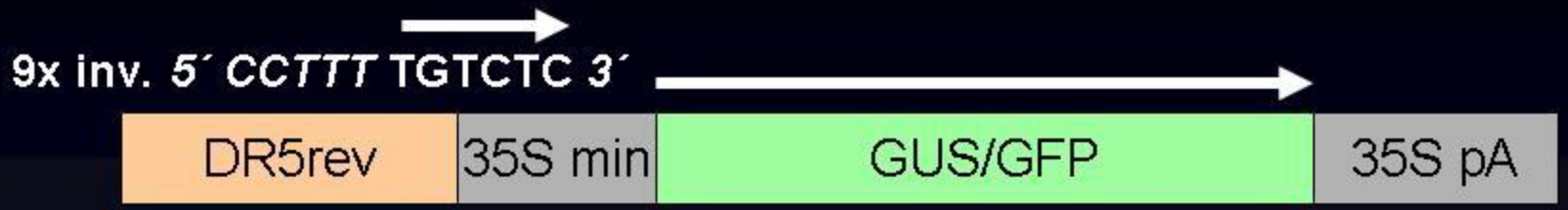


Auxin Transport



Inhibited auxin transport

DR5 Auxin Response Reporter

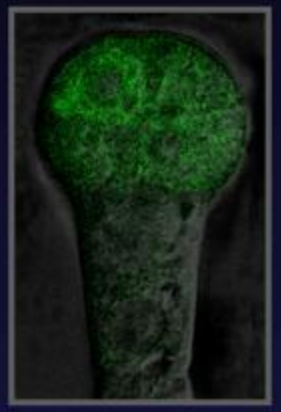


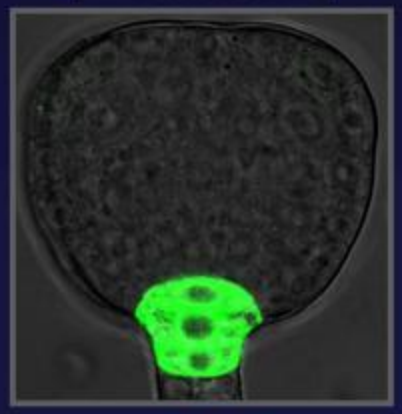
Root

Embryos

DR5

anti-IAA



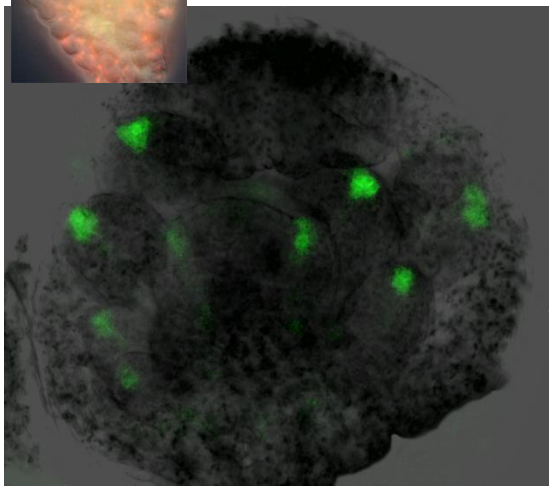


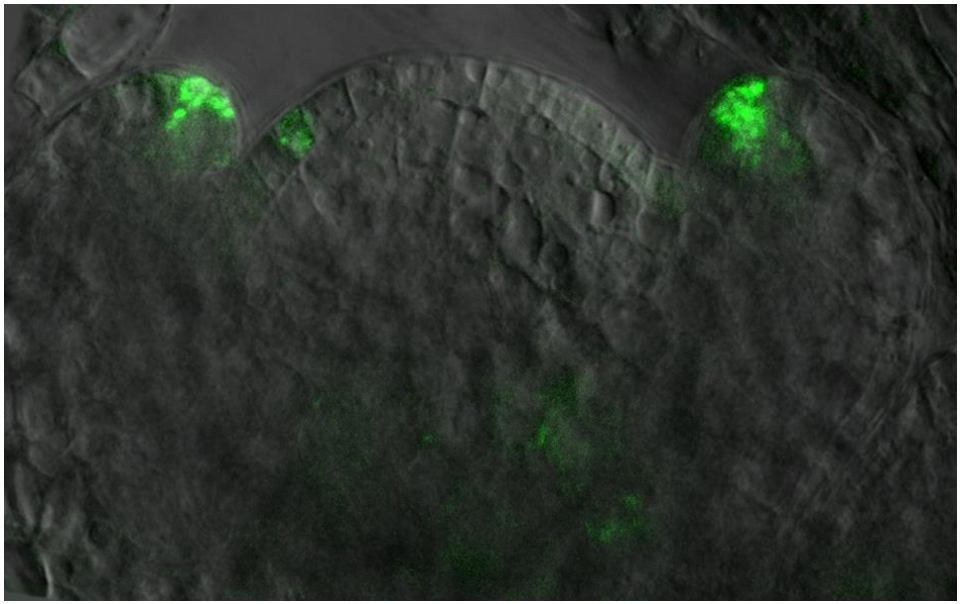
DR5

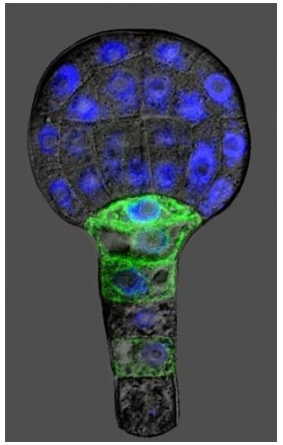
anti-IAA

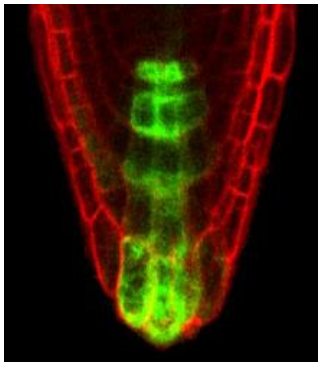
Ulmasov et al. 97; Sabatini et al. 99; Benková et al. 03; Friml et al. 03

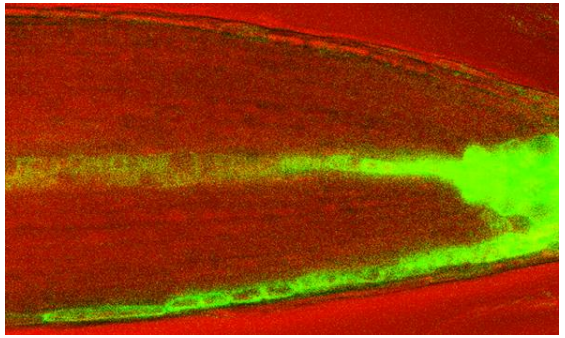
Local Auxin Gradients in Plant Development



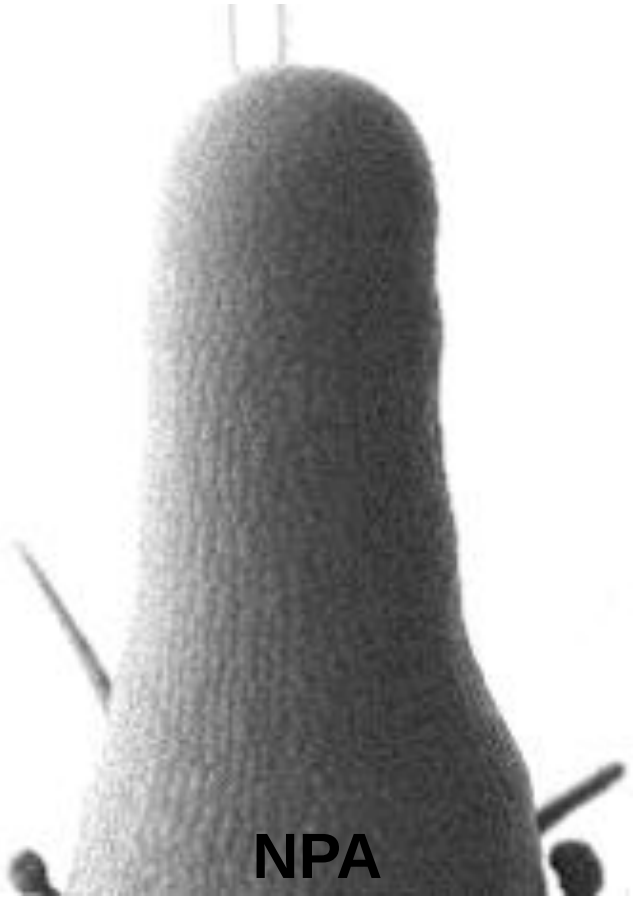


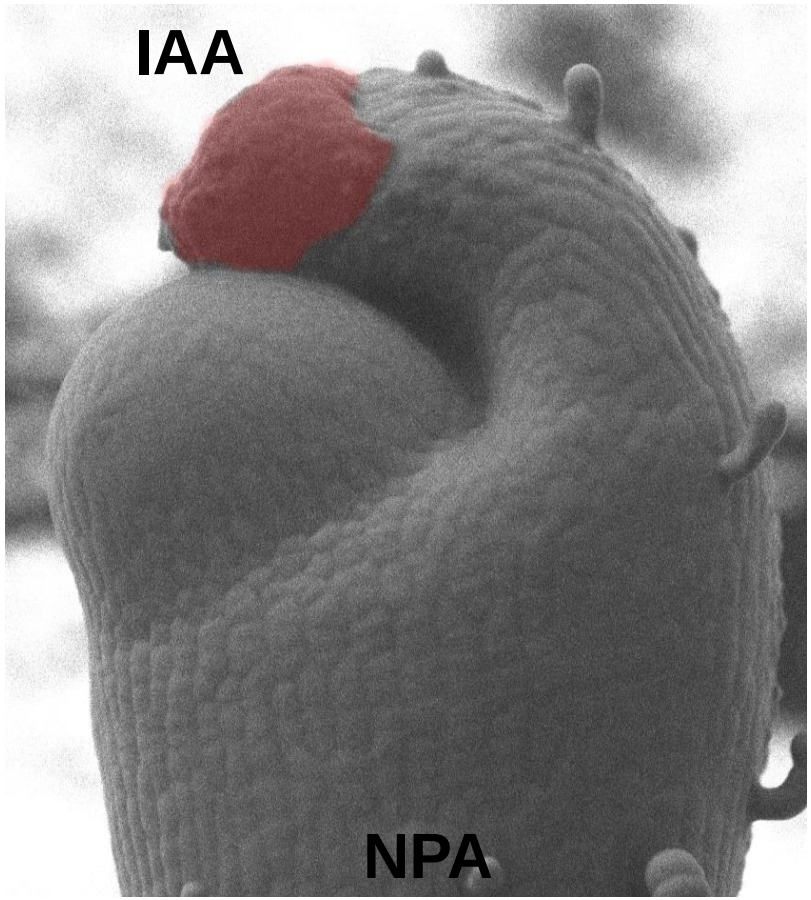






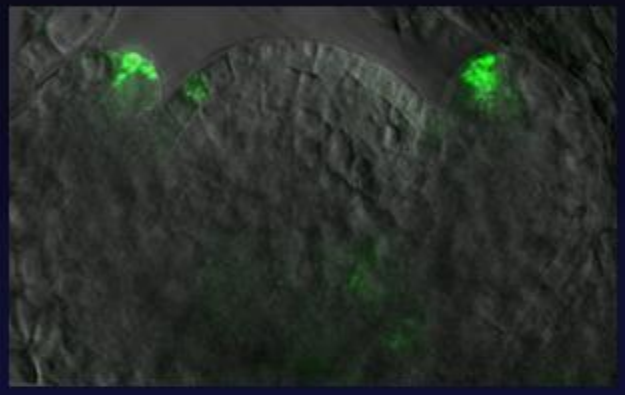
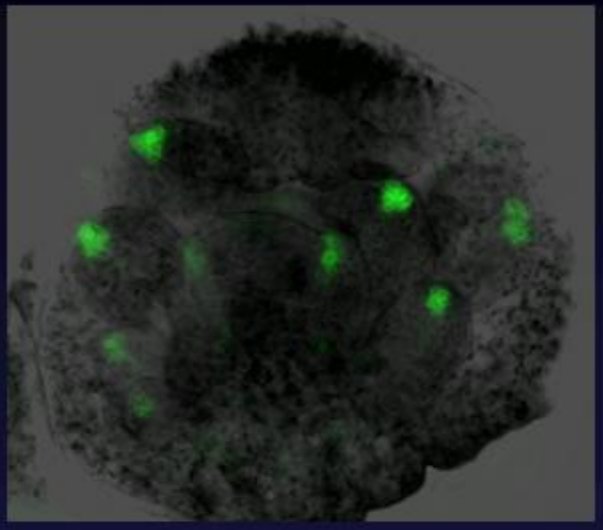
Local Application of Auxin Induces Organ Formation

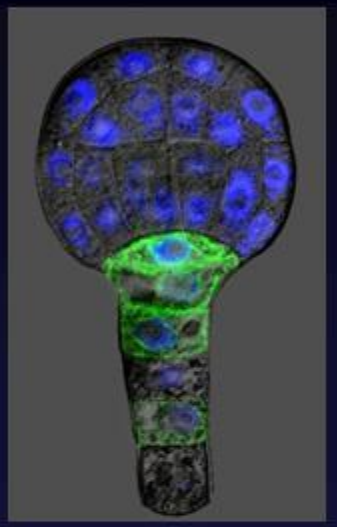
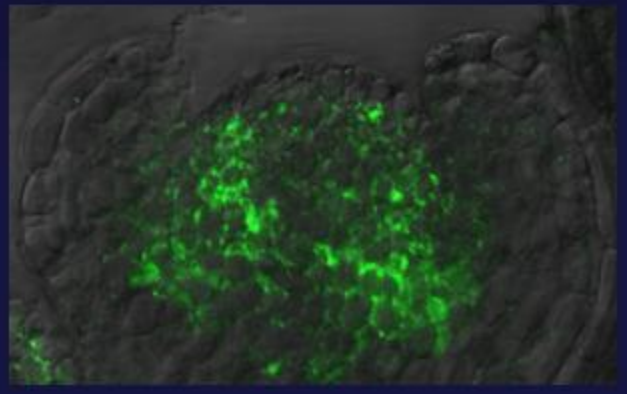
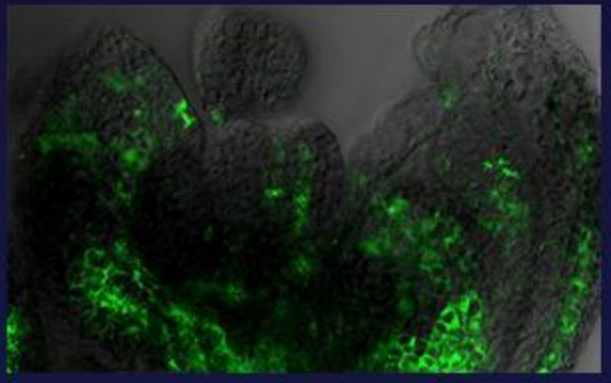


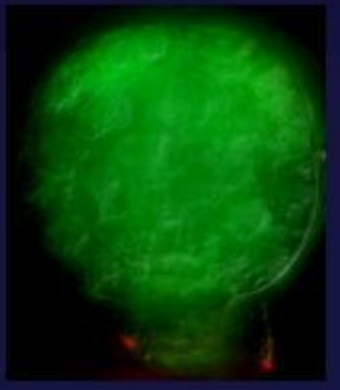


Reinhardt et al. 03

Local Auxin Gradients Require Active Polar Auxin Transport



Auxin Transport

Proteins involved in auxin transport
-PIN proteins (efflux)
-AUX1 proteins (influx)

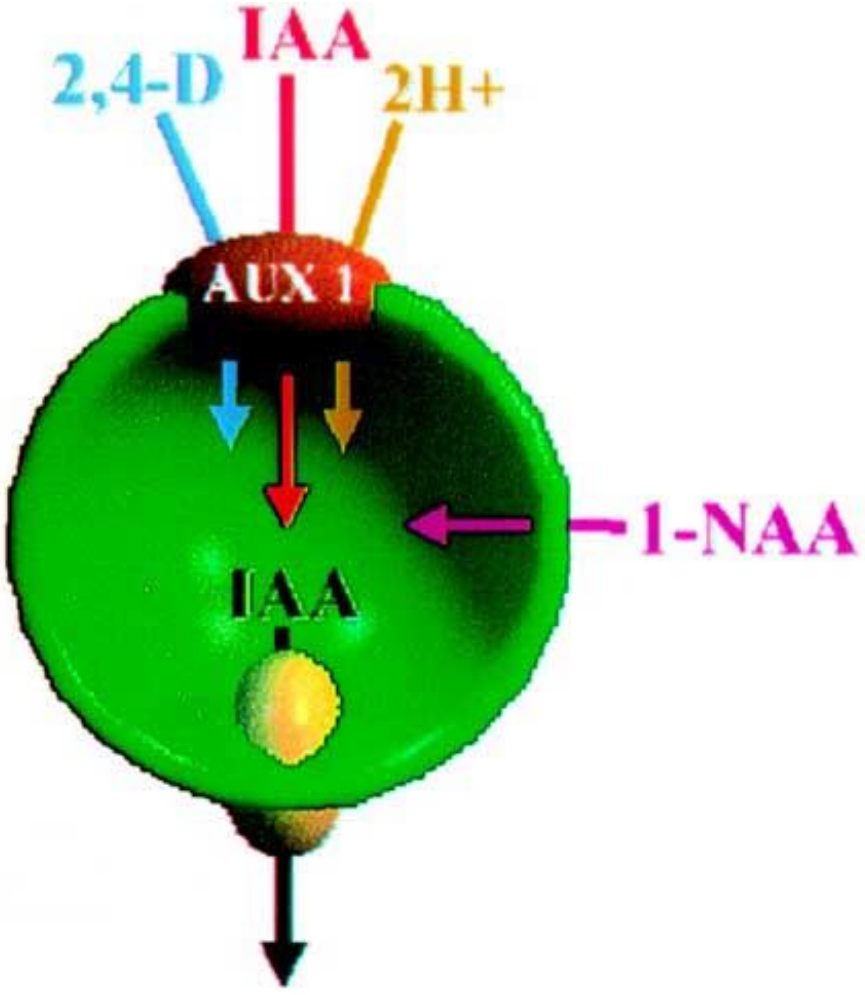
Role of GNOM dependent vesicle trafficking

PIN proteins cycling and its role

Auxin Influx

aux1 is Resistant to Auxin

Transport properties of different auxins

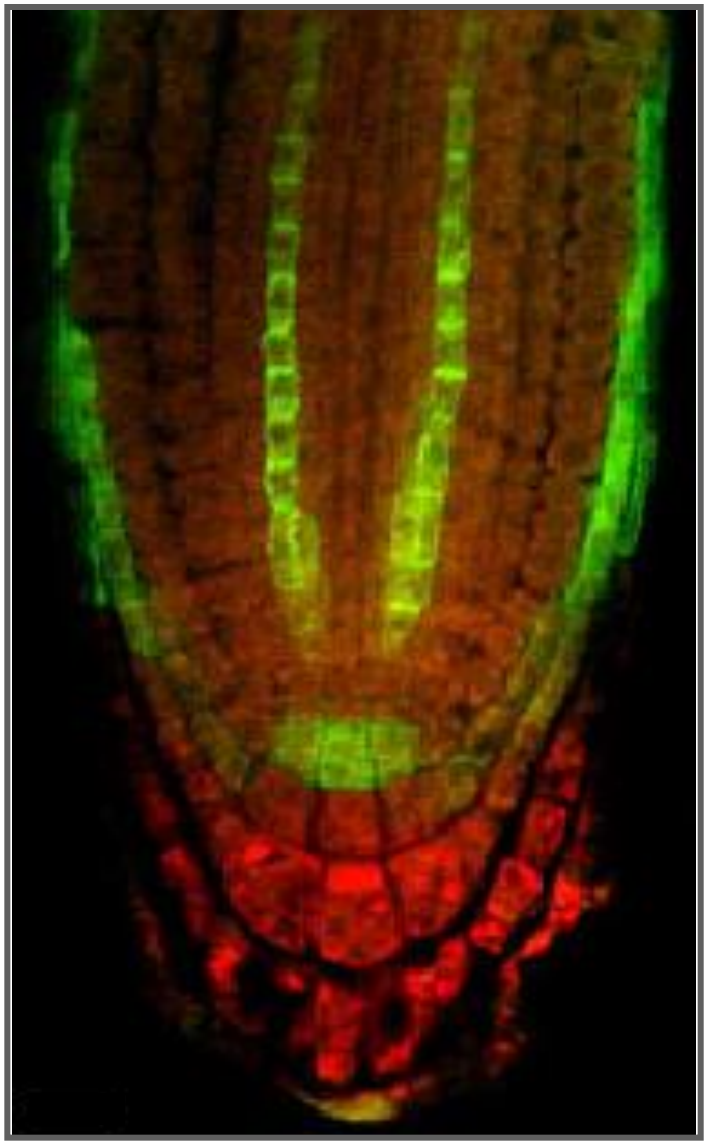


***aux1* phenotype**

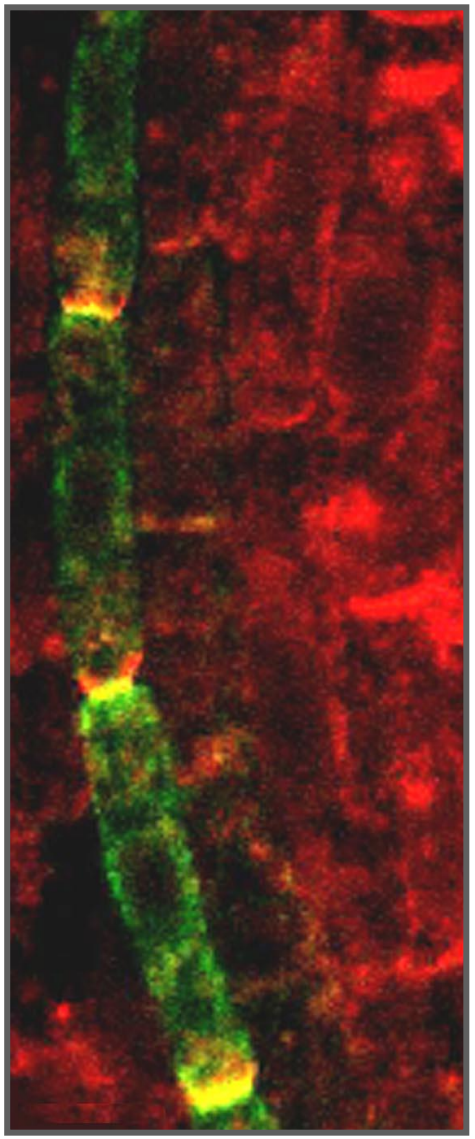
AUX1 – Expression and Localization

AUX1::GUS

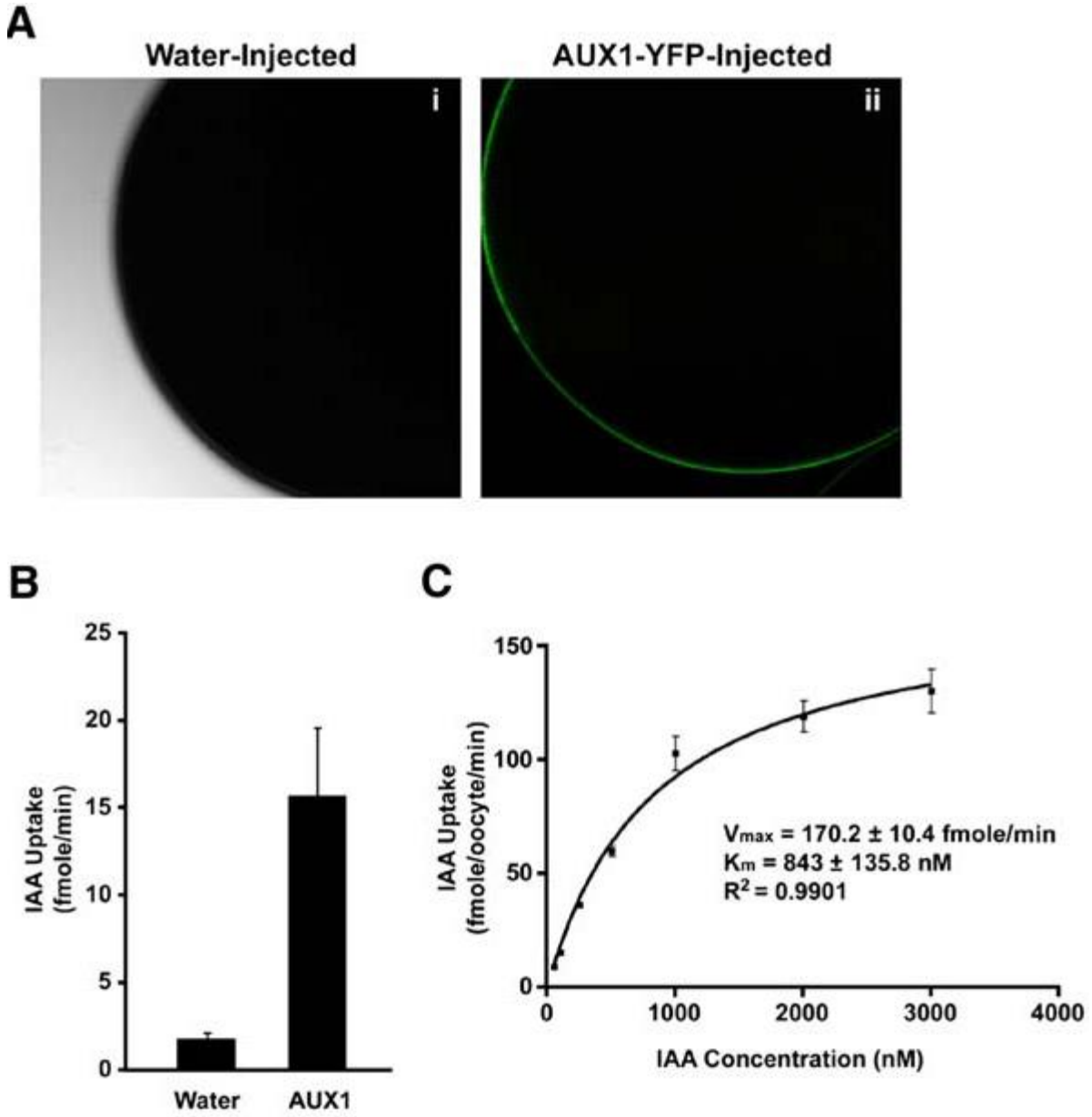
AUX1 protein



PIN1/AUX1



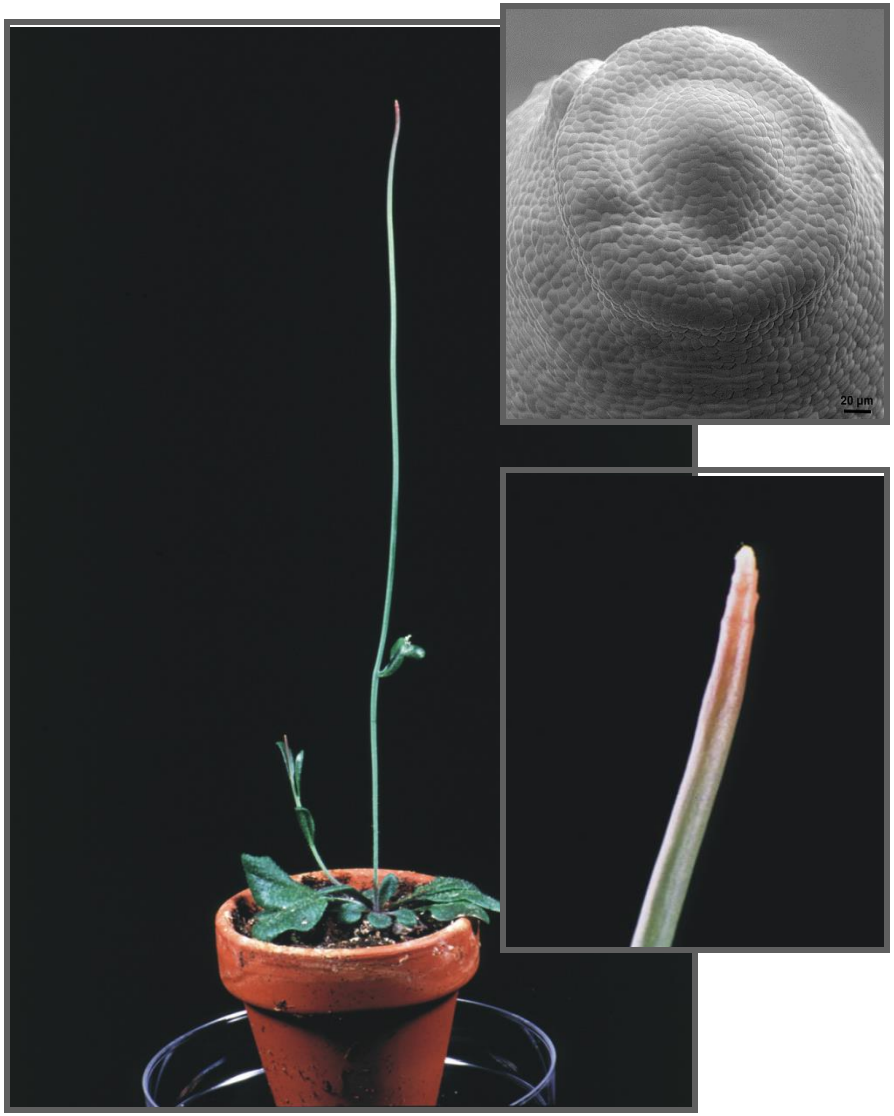
AUXI – auxin transport into Xenopus Oocytes



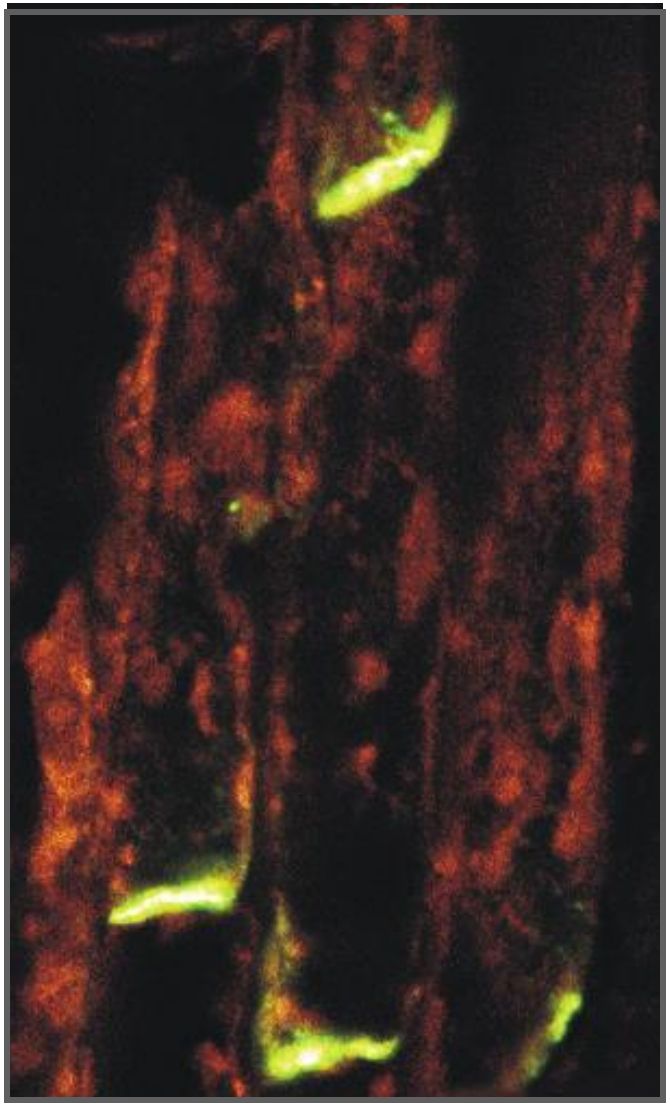
Auxin Efflux

PIN1 – the Auxin Efflux Carrier?

***pin1* mutant**

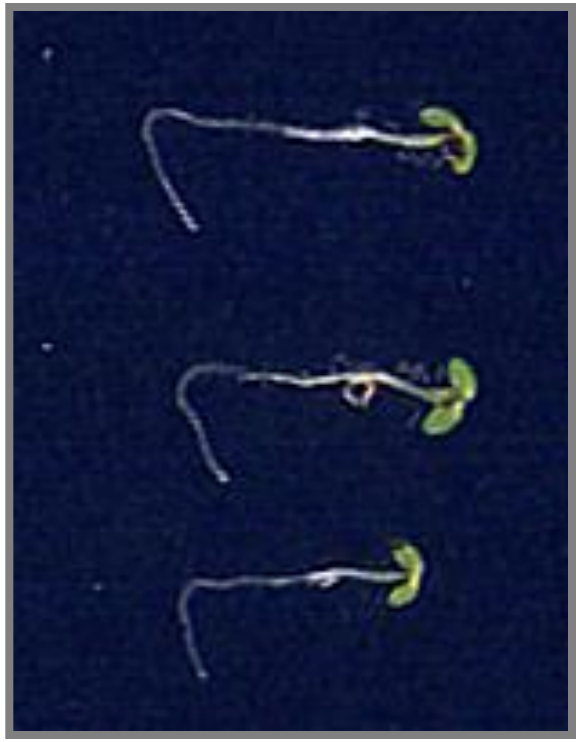


PIN1 protein

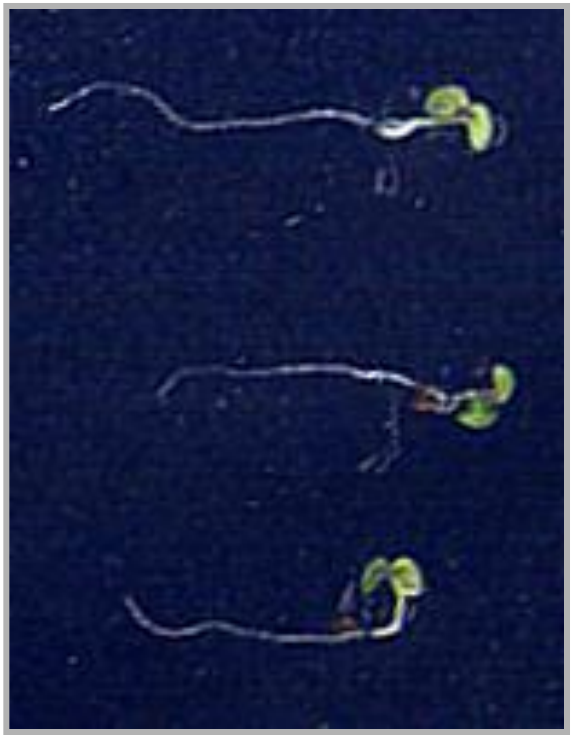


PIN2 – Root Gravitropism

Col-0



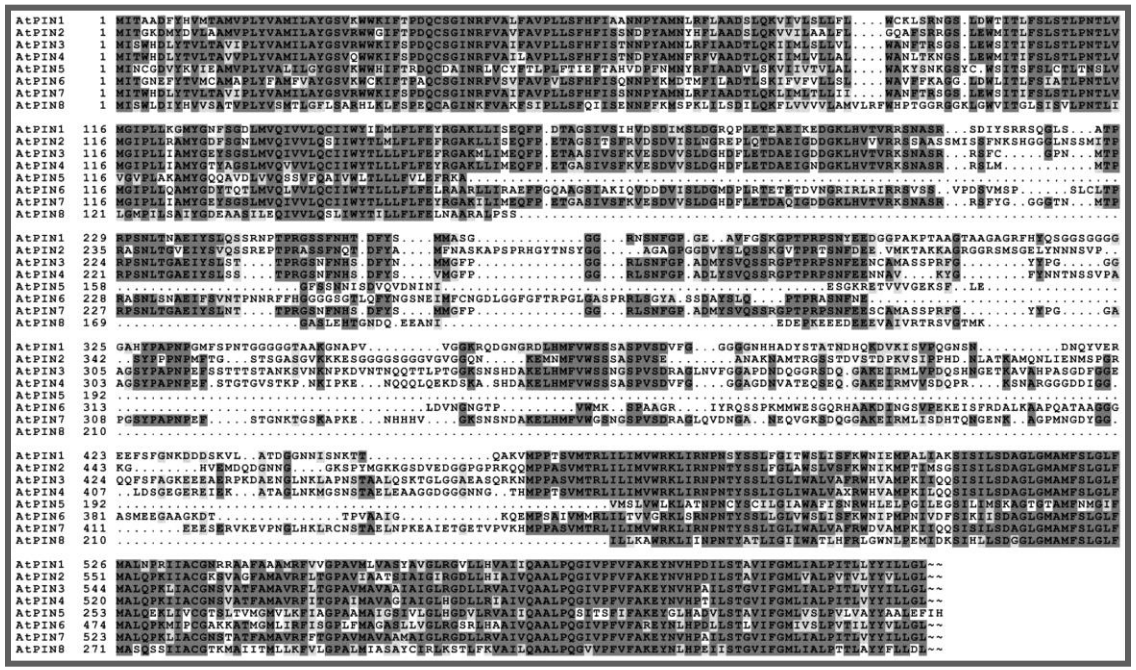
pin2



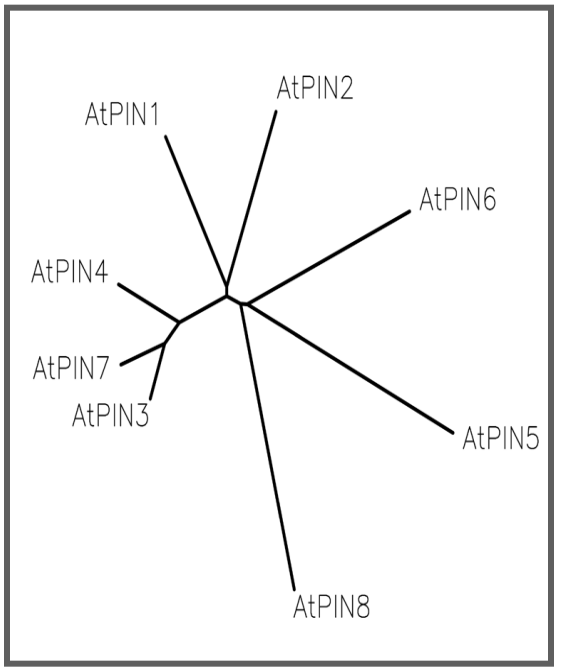
PIN2 protein

The Arabidopsis *PIN* Gene Family

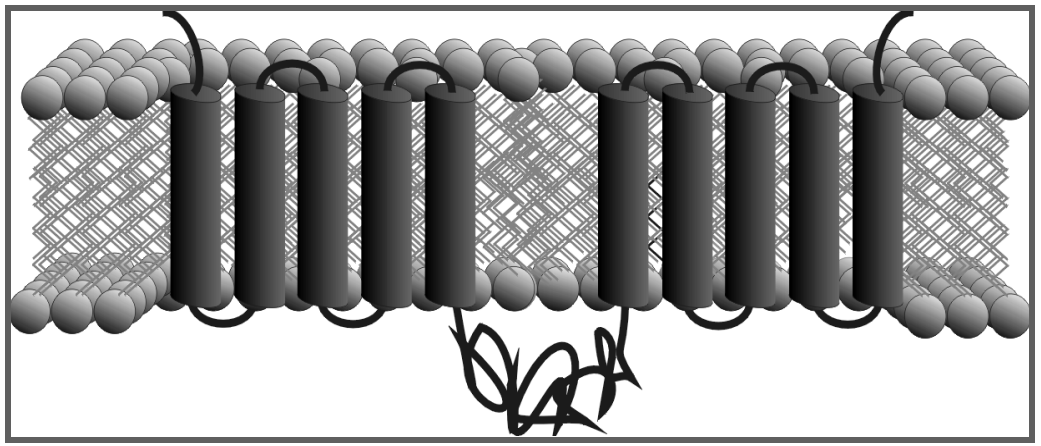
Comparison of *Arabidopsis* PIN proteins



Phylogenetic tree



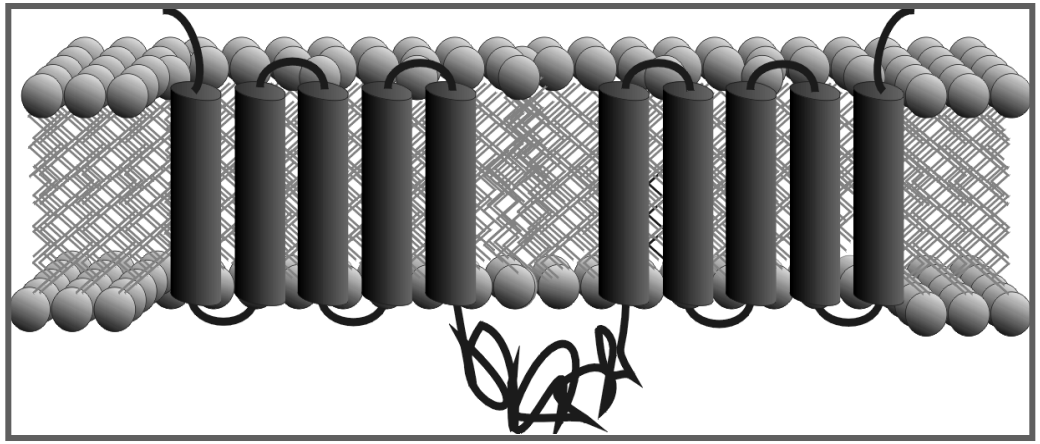
Membrane topology model



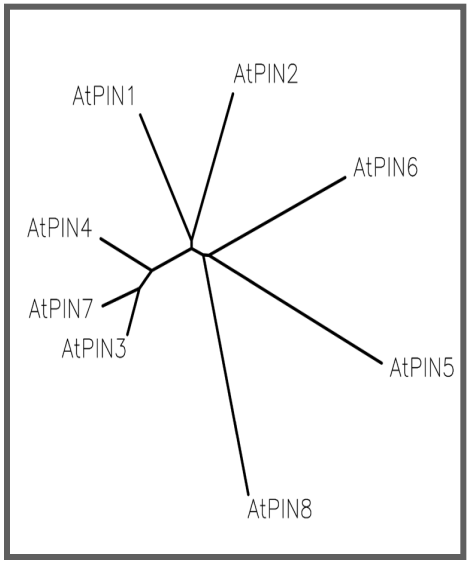
What is Molecular Role of PIN Proteins in Auxin Transport?

PINs Are Essential Components of Auxin Transport

Putative topology of PIN proteins



Phylogenetic tree



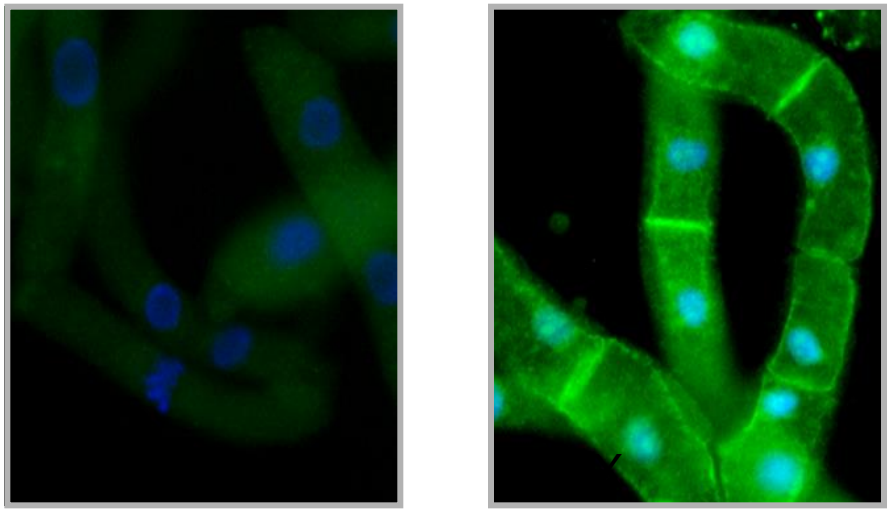
- All defects in *pin* loss-off-function mutants are in auxin transport-dependent processes and can be phenocopied by auxin transport inhibitors

- Local auxin distribution (gradients) are affected in *pins*

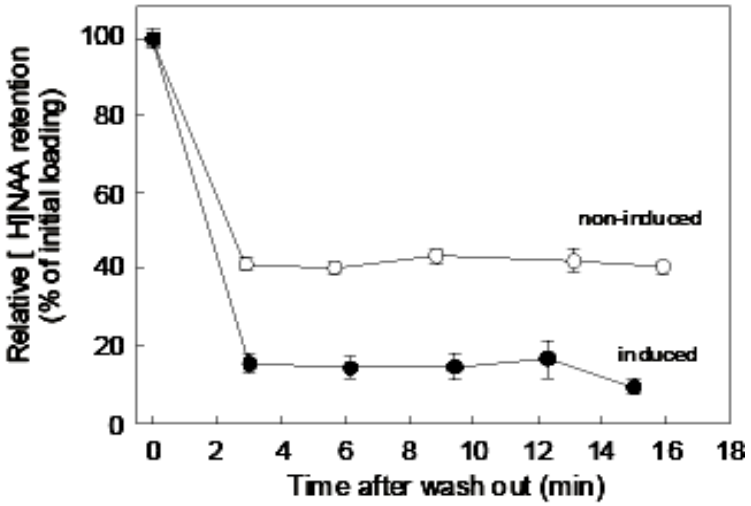
- Polar PIN localization determines direction of auxin flow

PINs Are Rate-limiting Factors in Auxin Efflux

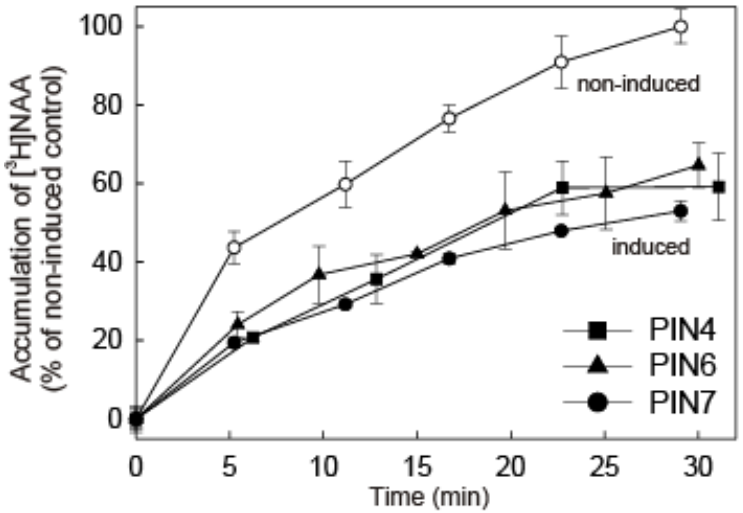
Inducible PIN1 expression



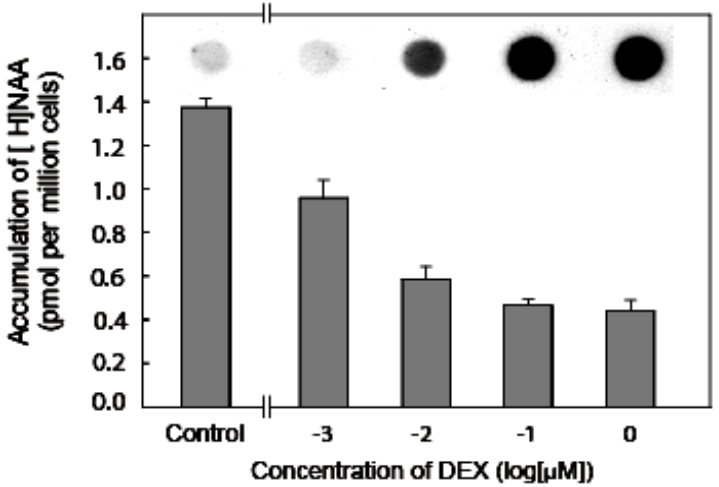
PIN-dependent auxin efflux from *GVG-PIN7* tobacco cells



NAA accumulation kinetics

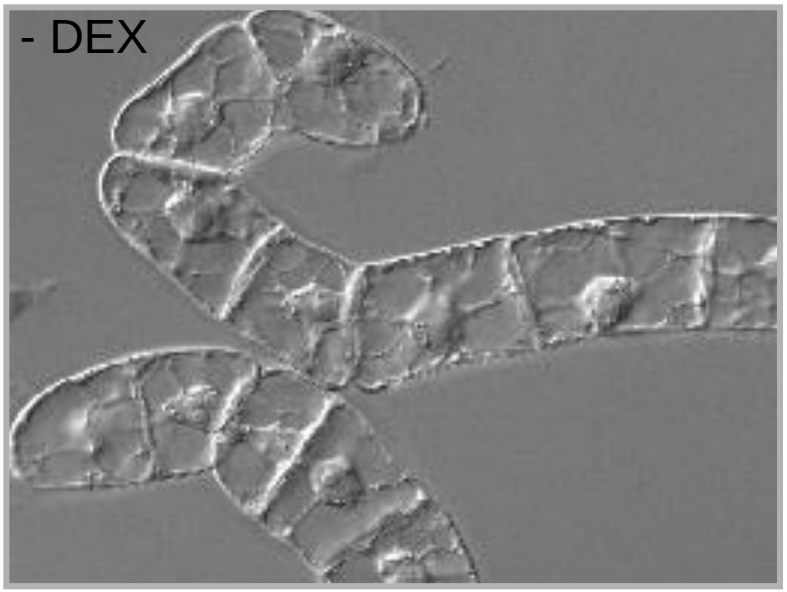


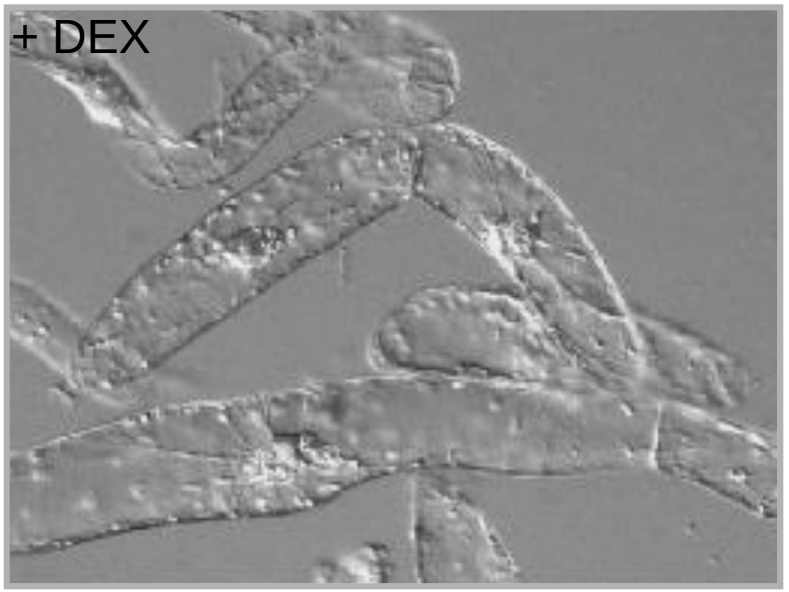
[3H]NAA accumulation in GVG-PIN7 tobacco cells in relation to DEX concentration

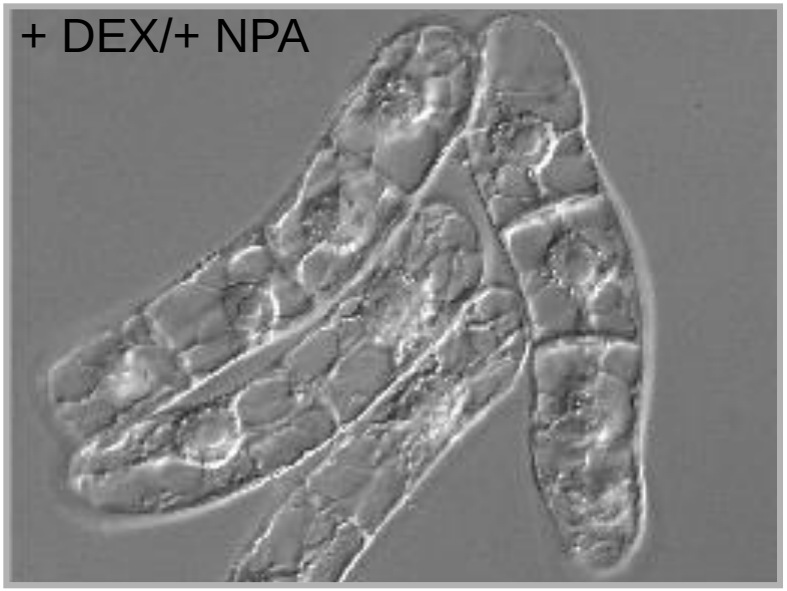


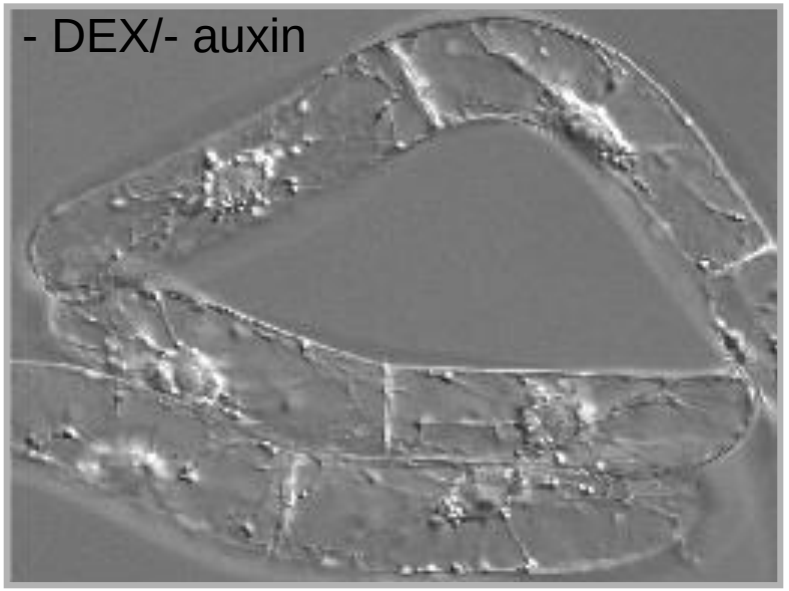
Petrasek et al., 2006

PIN-induced Phenotypes in BY-2 Cells





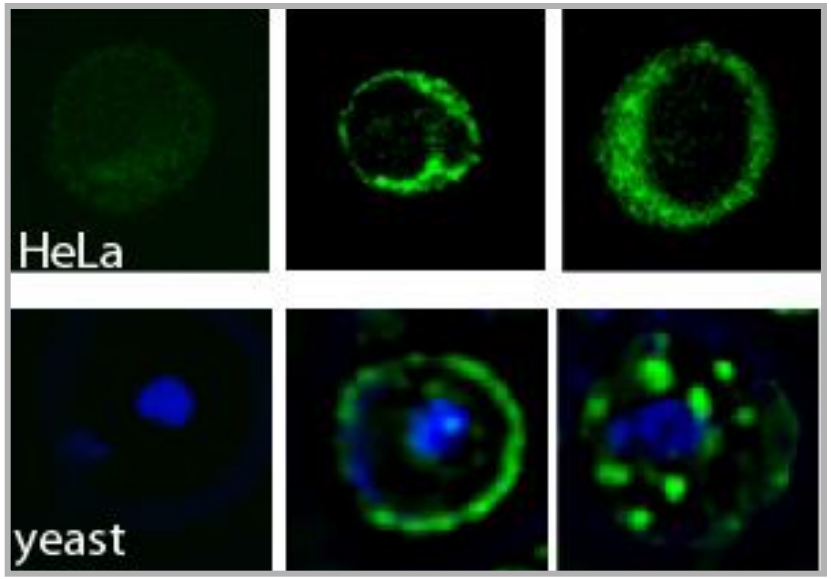




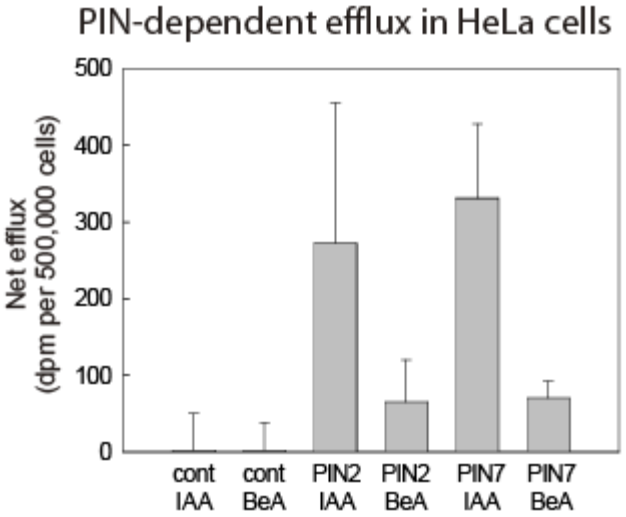
unpublished

Expression of PINs in HeLa and Yeast

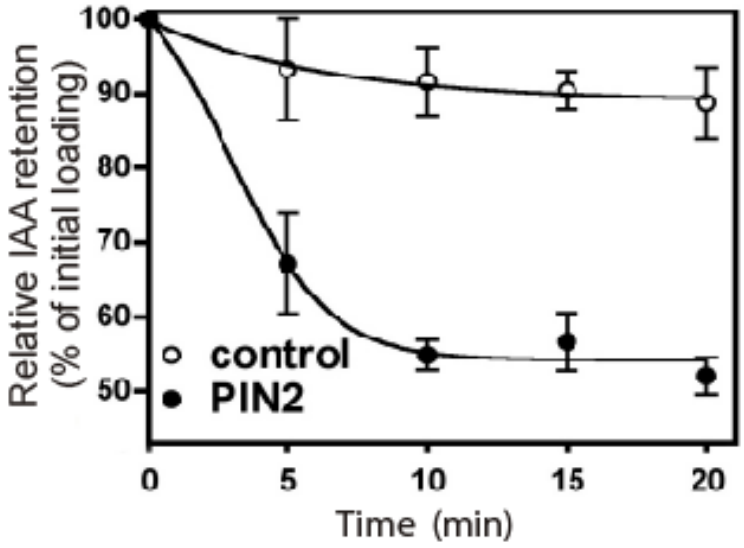
Heterologous PIN2 expression

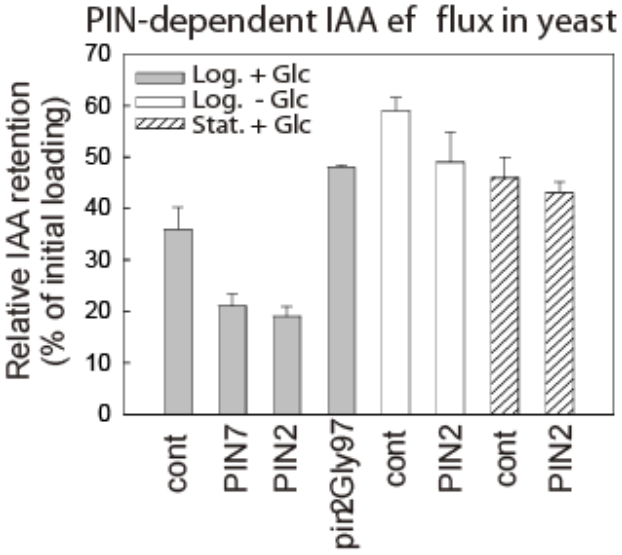


auxin efflux activity



IAA ef flux in yeast

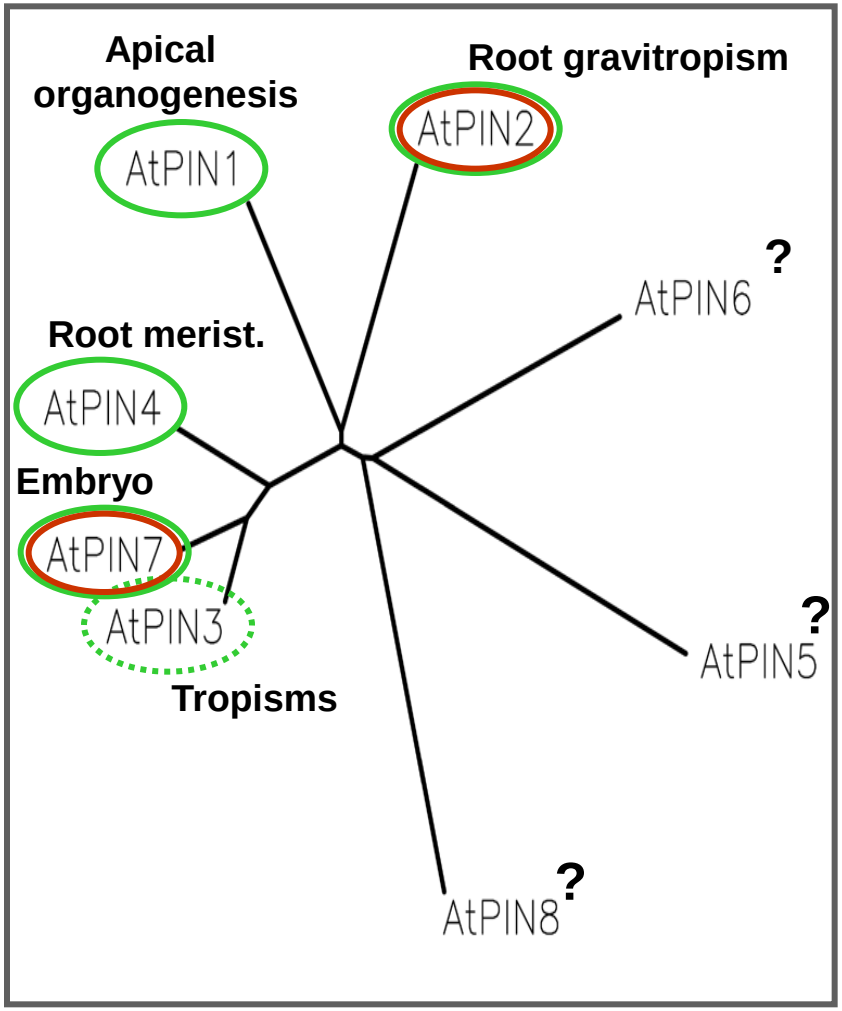




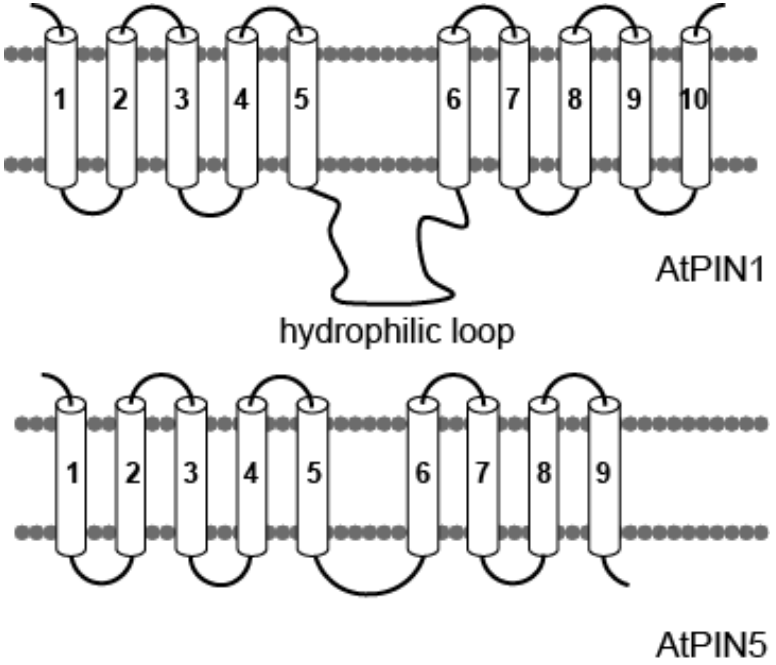
Petrasek et al., 2006

PIN gene family

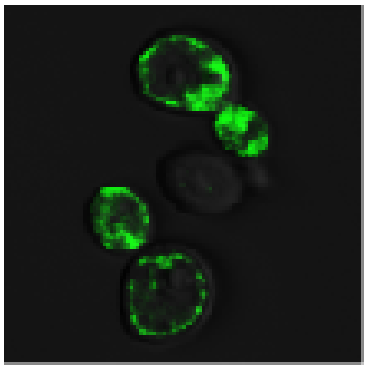
PINs in *Arabidopsis*

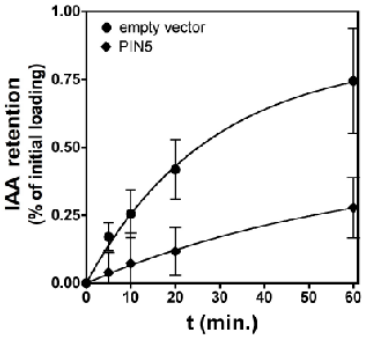


Predicted PIN Protein Topology

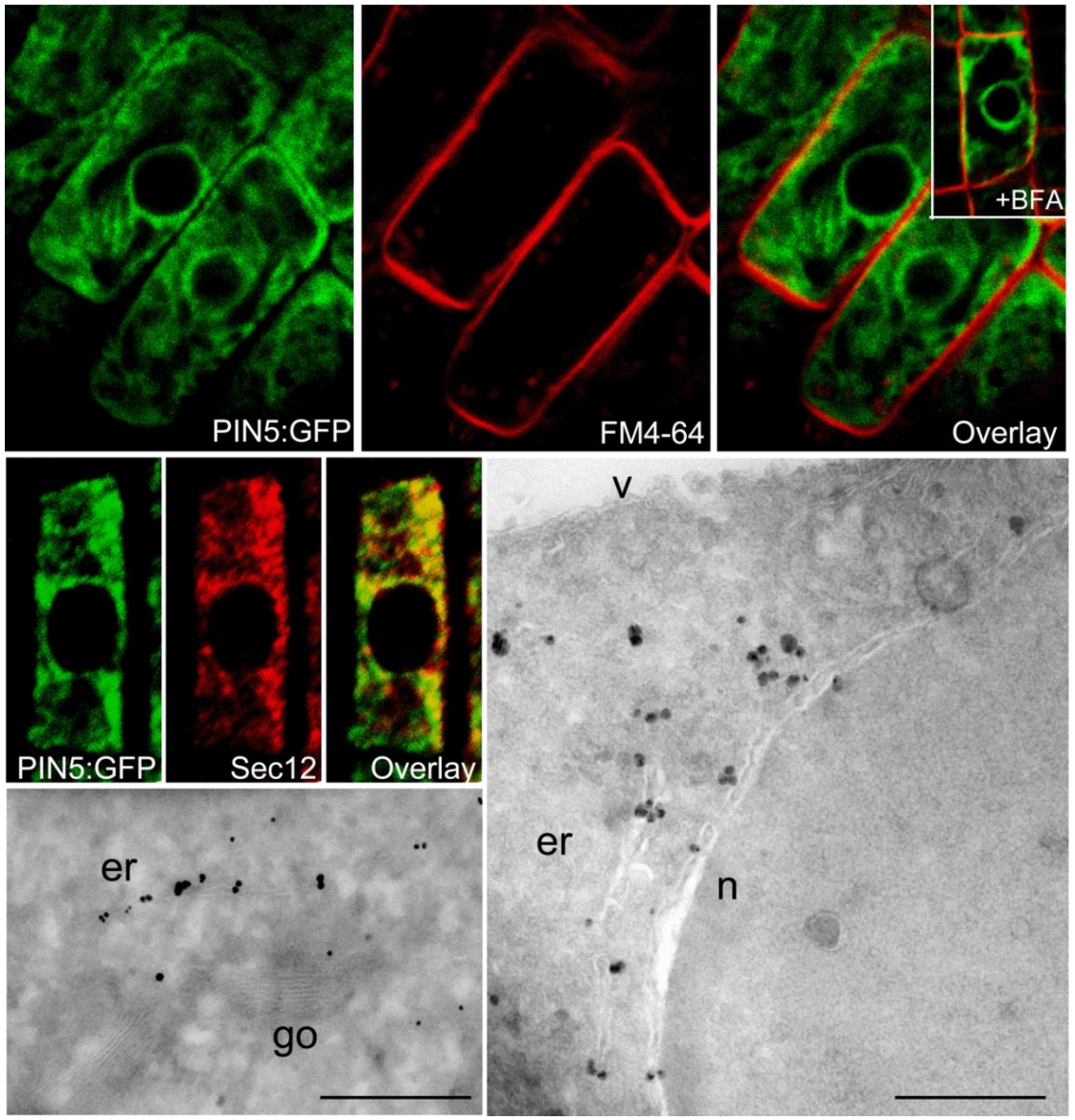


Auxin Transport in Yeast



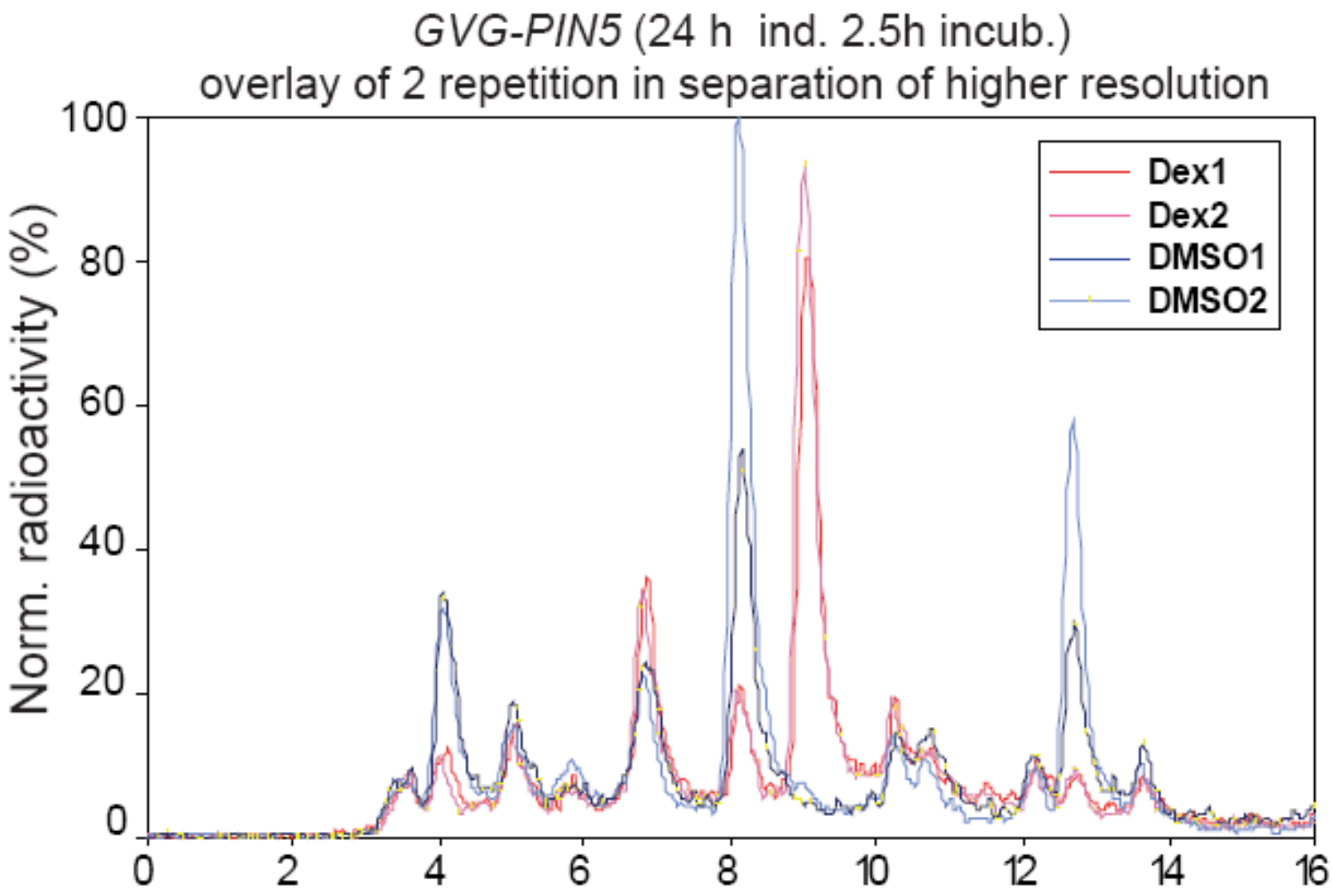


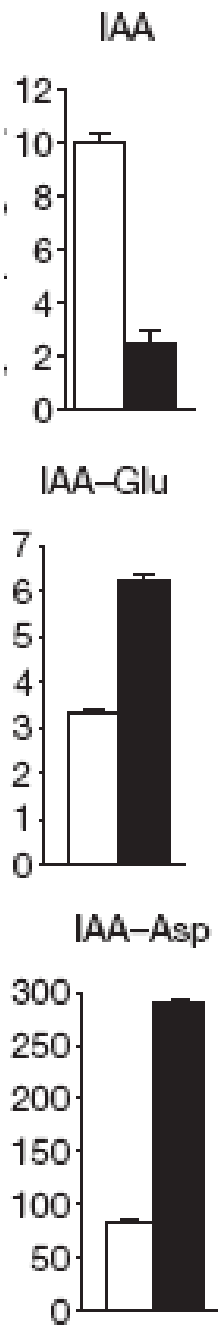
ER-based PIN5-dependent auxin transport



Mravec et al., 2009

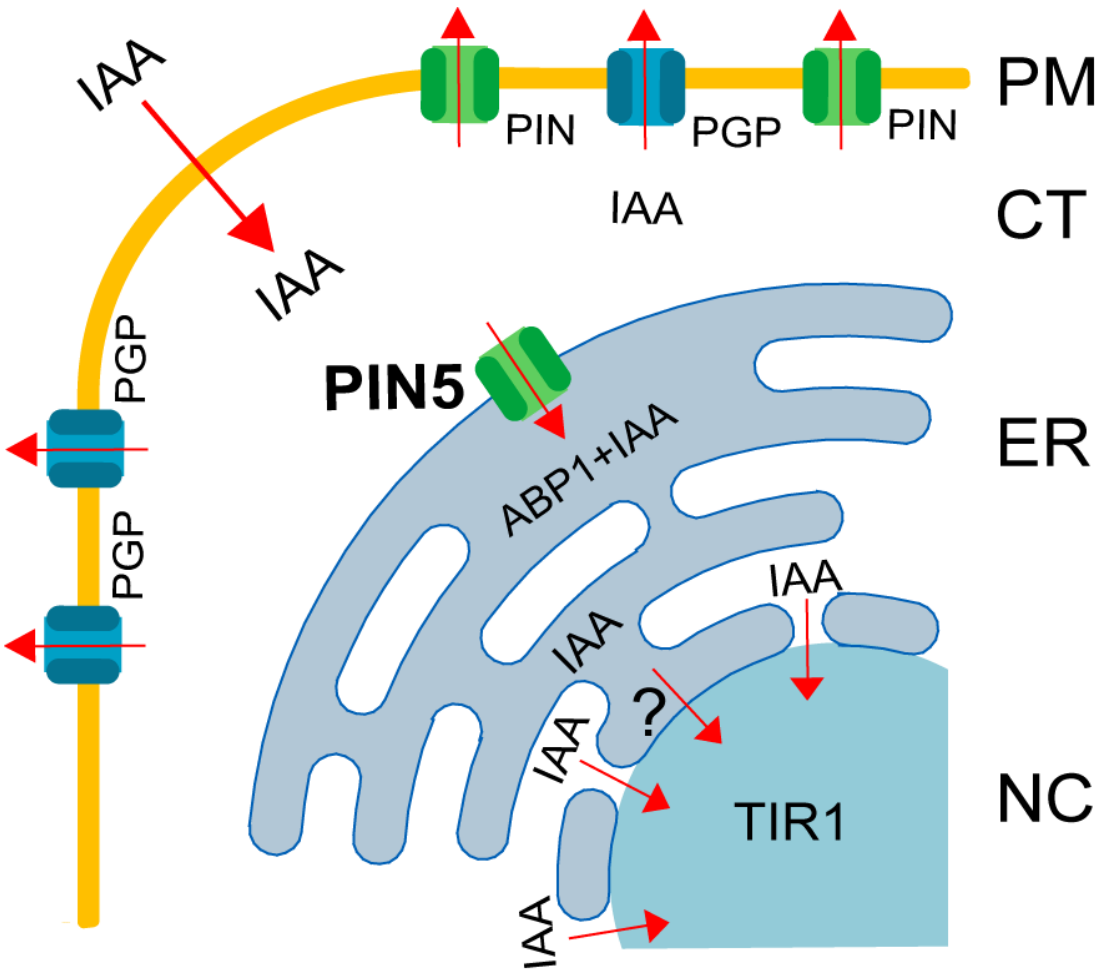
PIN5 regulates auxin metabolism



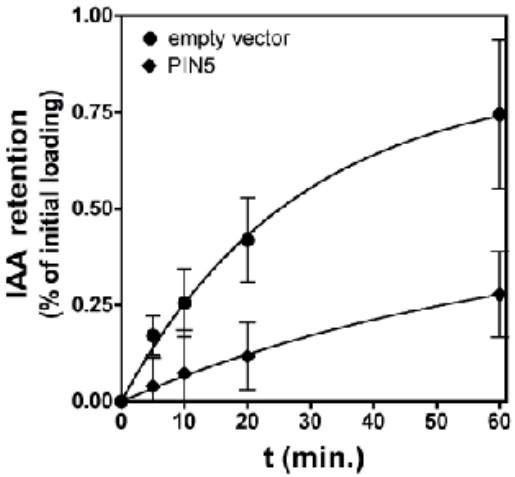


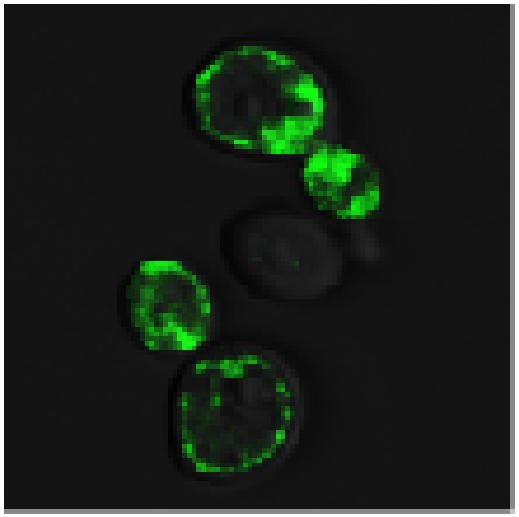
Mravec et al., 2009

PIN5-dependent auxin transport into ER



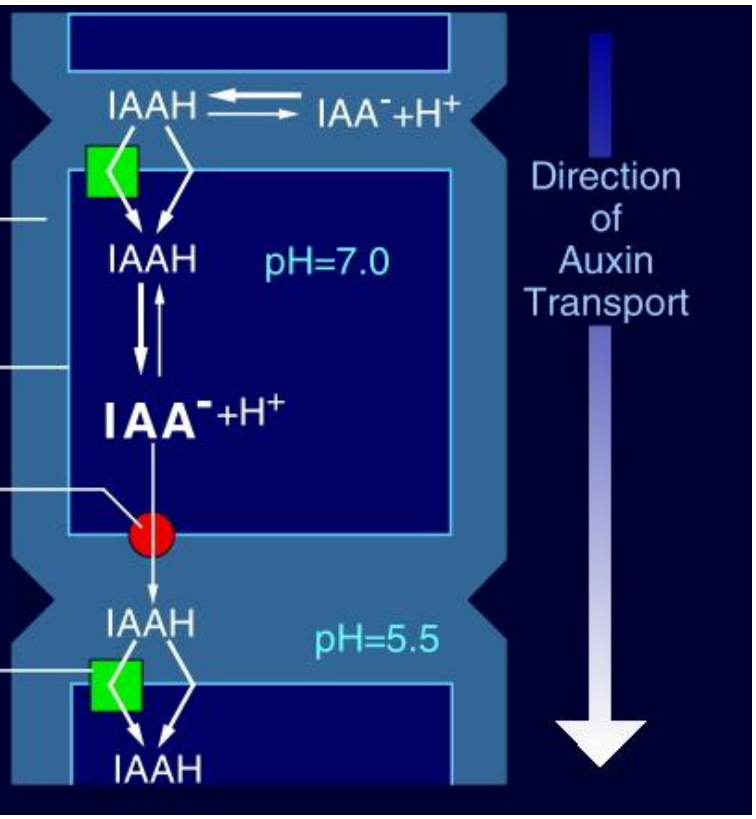
Yeast

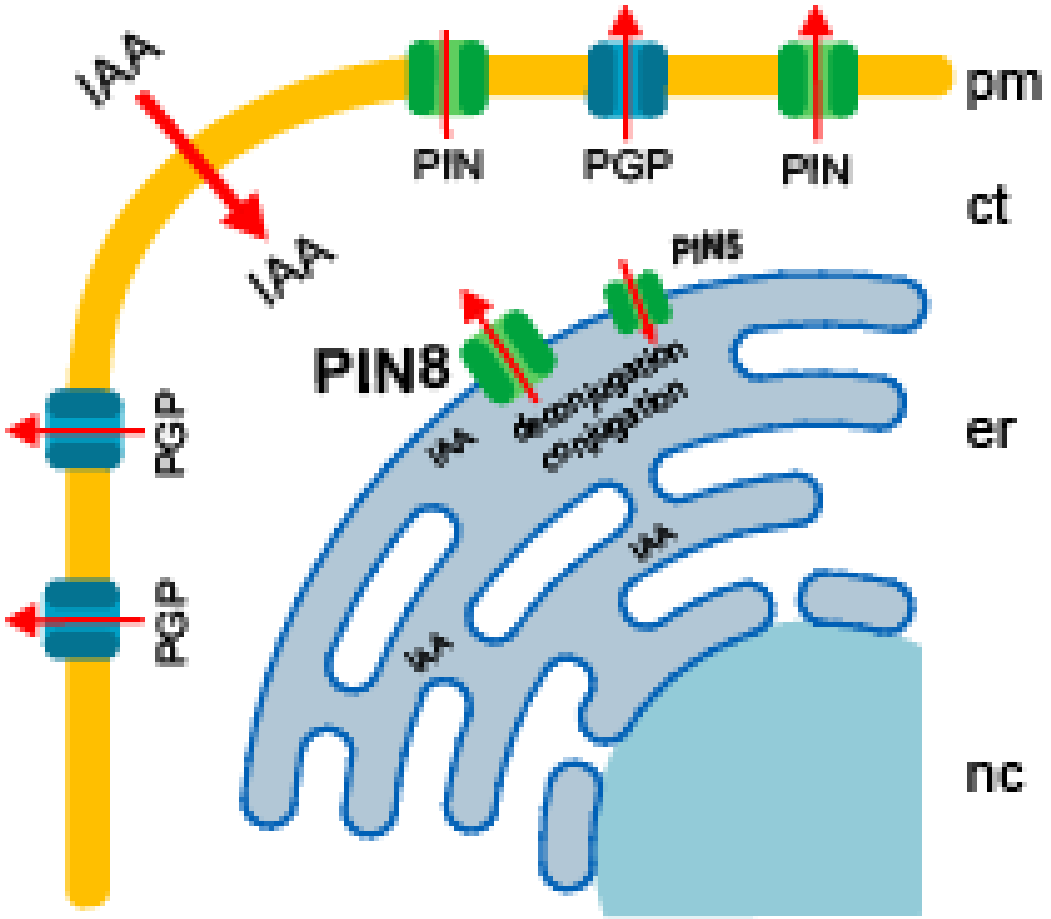




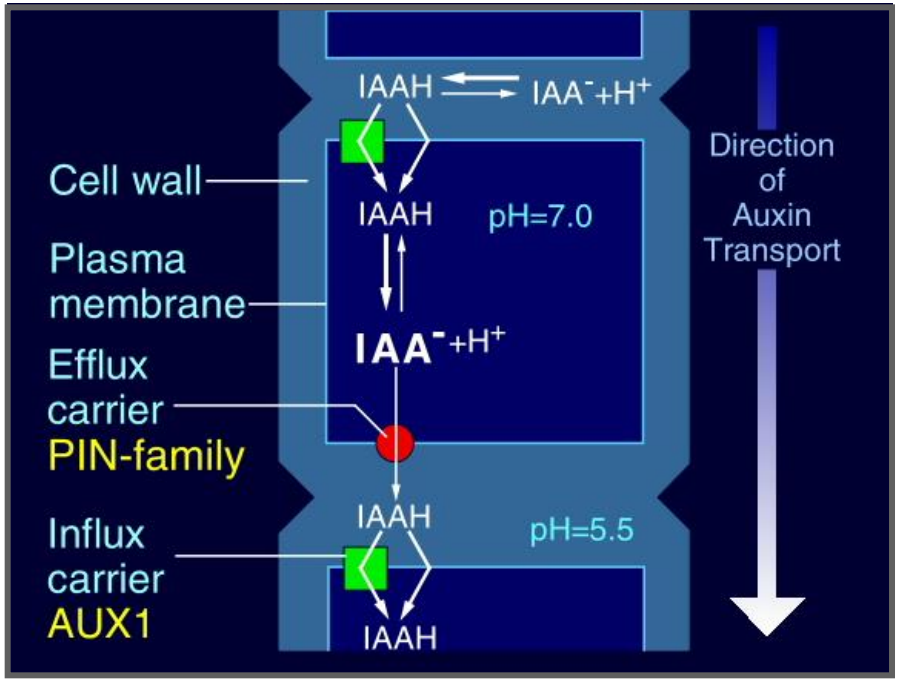
Mravec et al., 2009

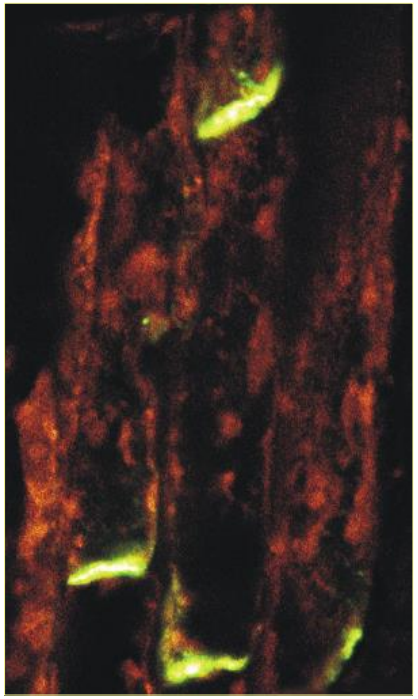
Updated model for auxin transport





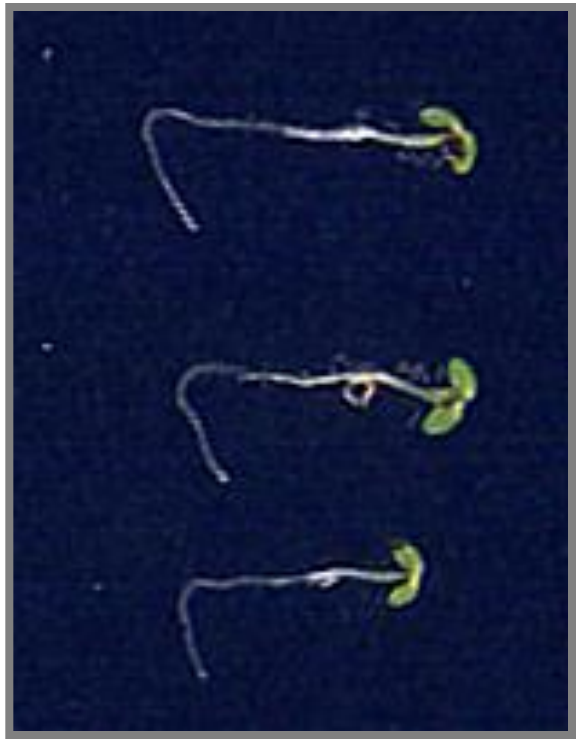
Cellular Polarity of PIN Localization and Directionality of Intercellular Auxin Flow



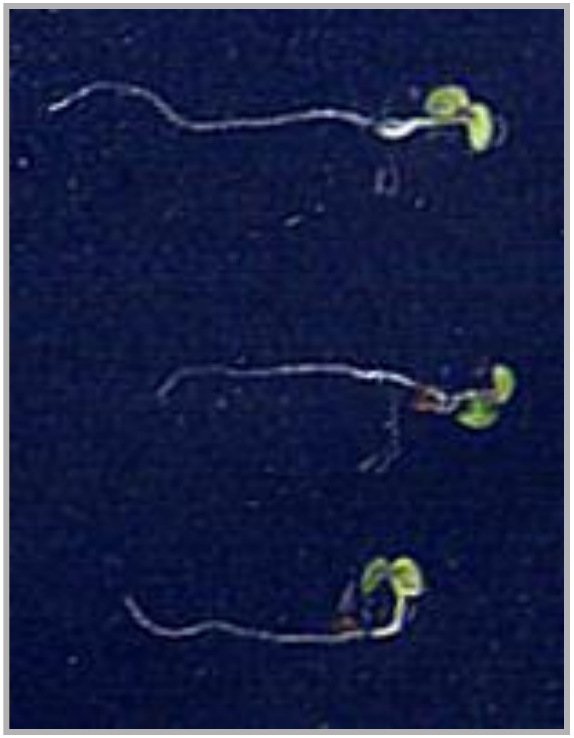


PIN2 – Root Gravitropism

Col-0

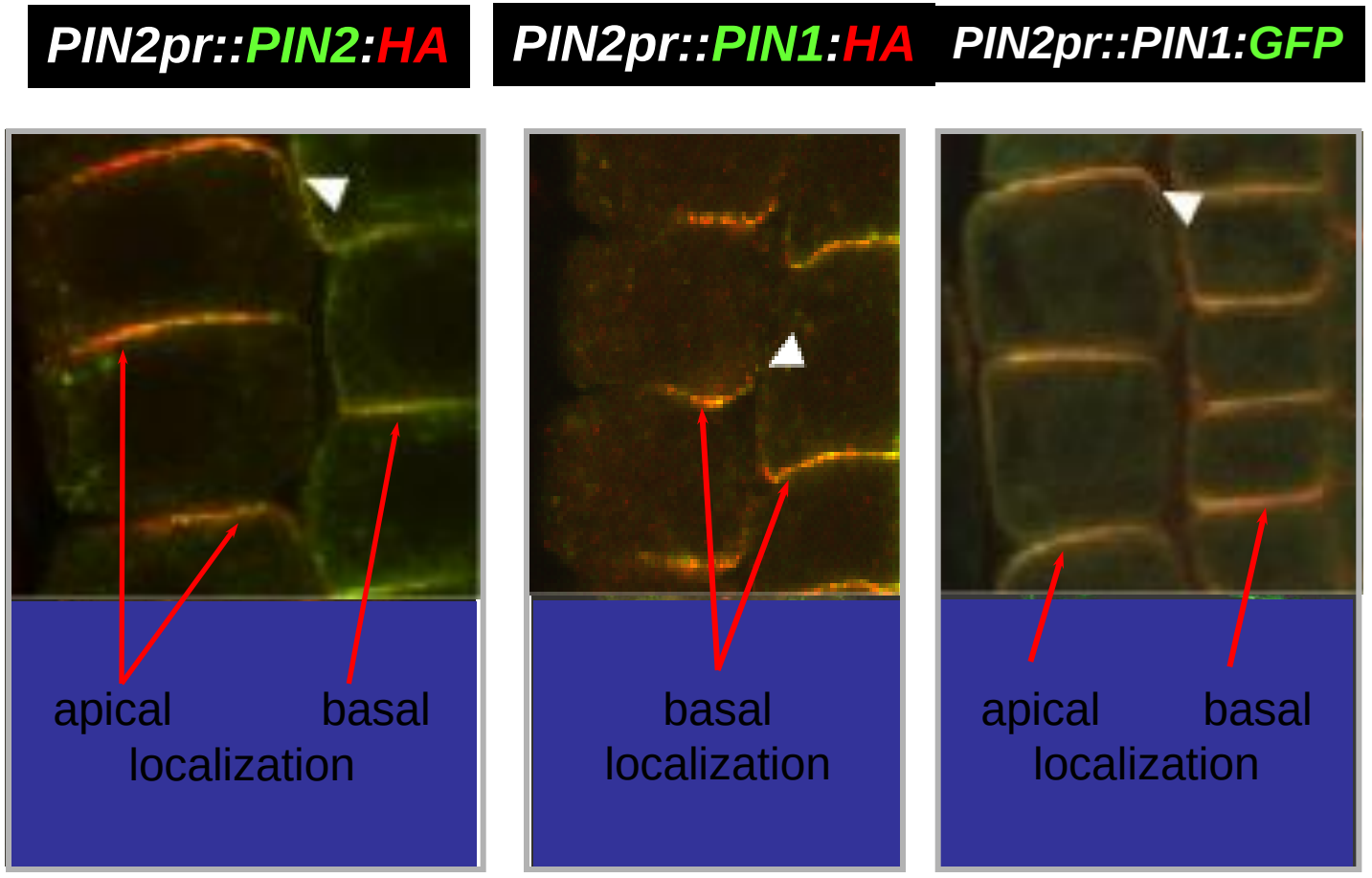


pin2



PIN2 protein

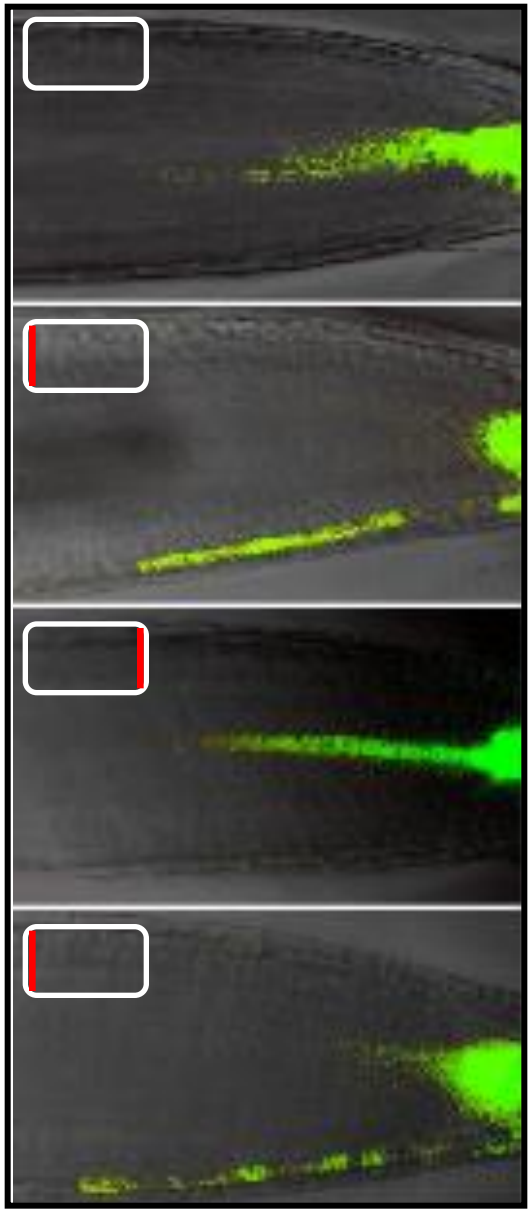
PIN-specific Signals for Polar Targeting



Wisniewska et al. 2006

PIN Polarity Determines Direction of Auxin Flow

DR5rev::GFP



pin2 (eir1, agr1)

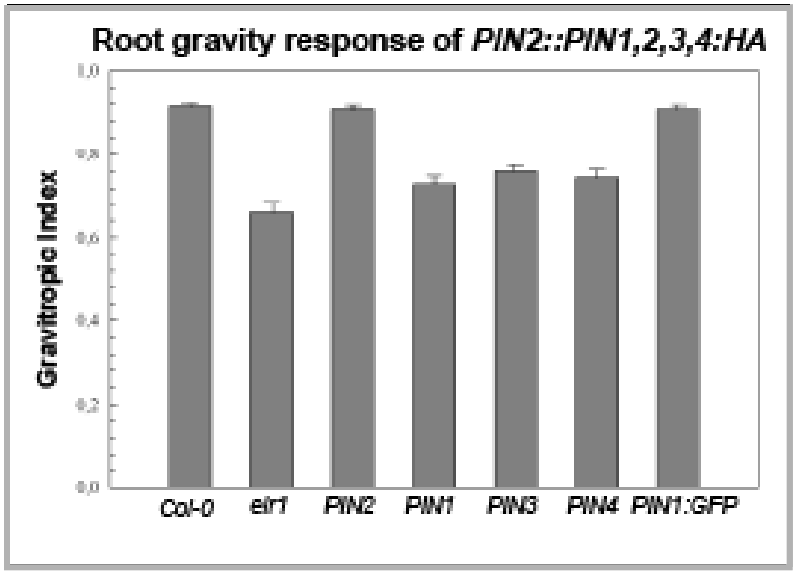
PIN2::PIN2:HA

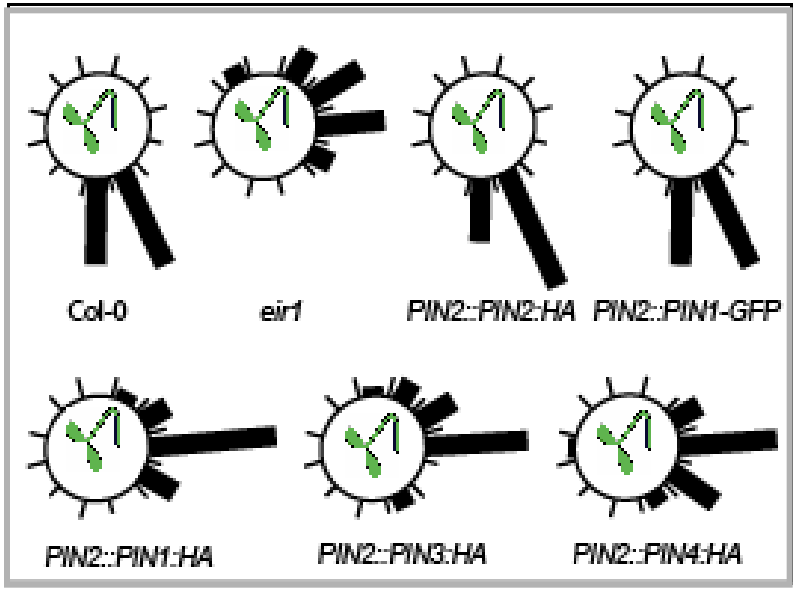
PIN2::PIN1:HA

PIN2::PIN1:GFP-2

PIN2::PIN1:GFP-3

gravitropism





Wisniewska et al. 2006

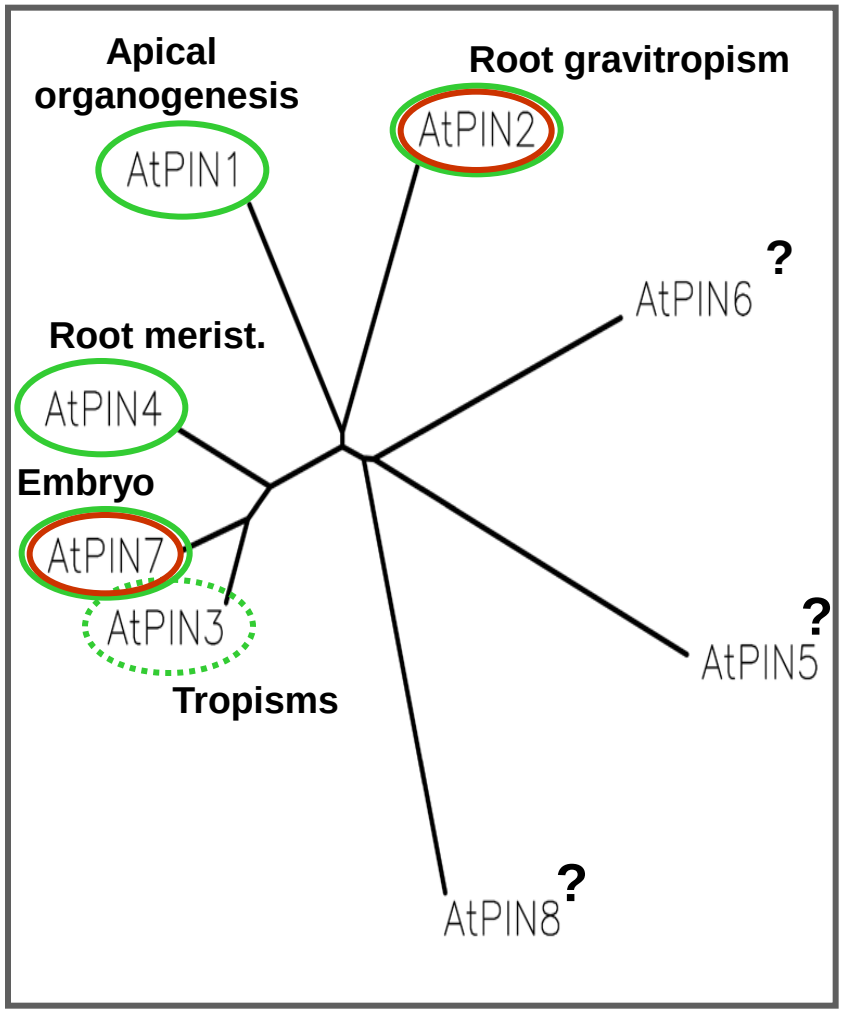
PIN proteins are rate-limiting factors in auxin efflux from cells

and

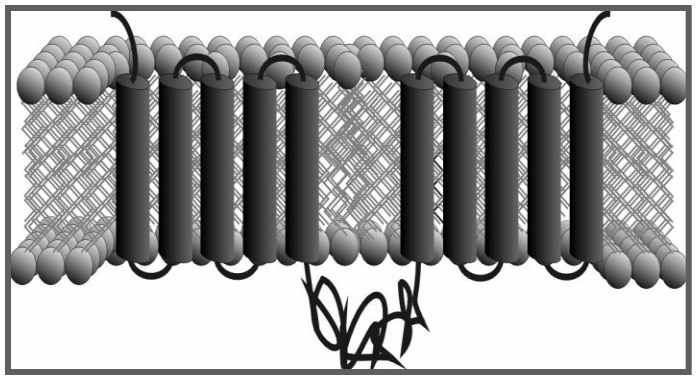
the polarity of their subcellular localization determines direction of intercellular auxin flow

PIN gene family: at PM and ER

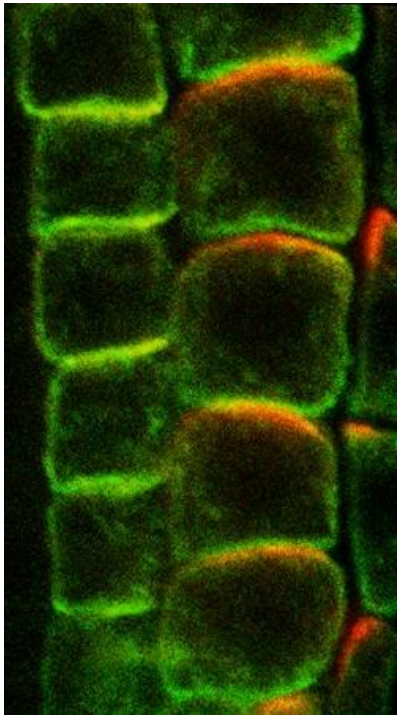
PINs in *Arabidopsis*



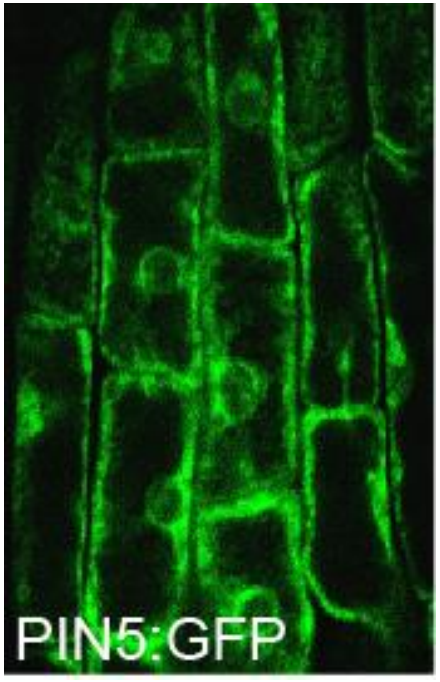
PIN Protein Topology



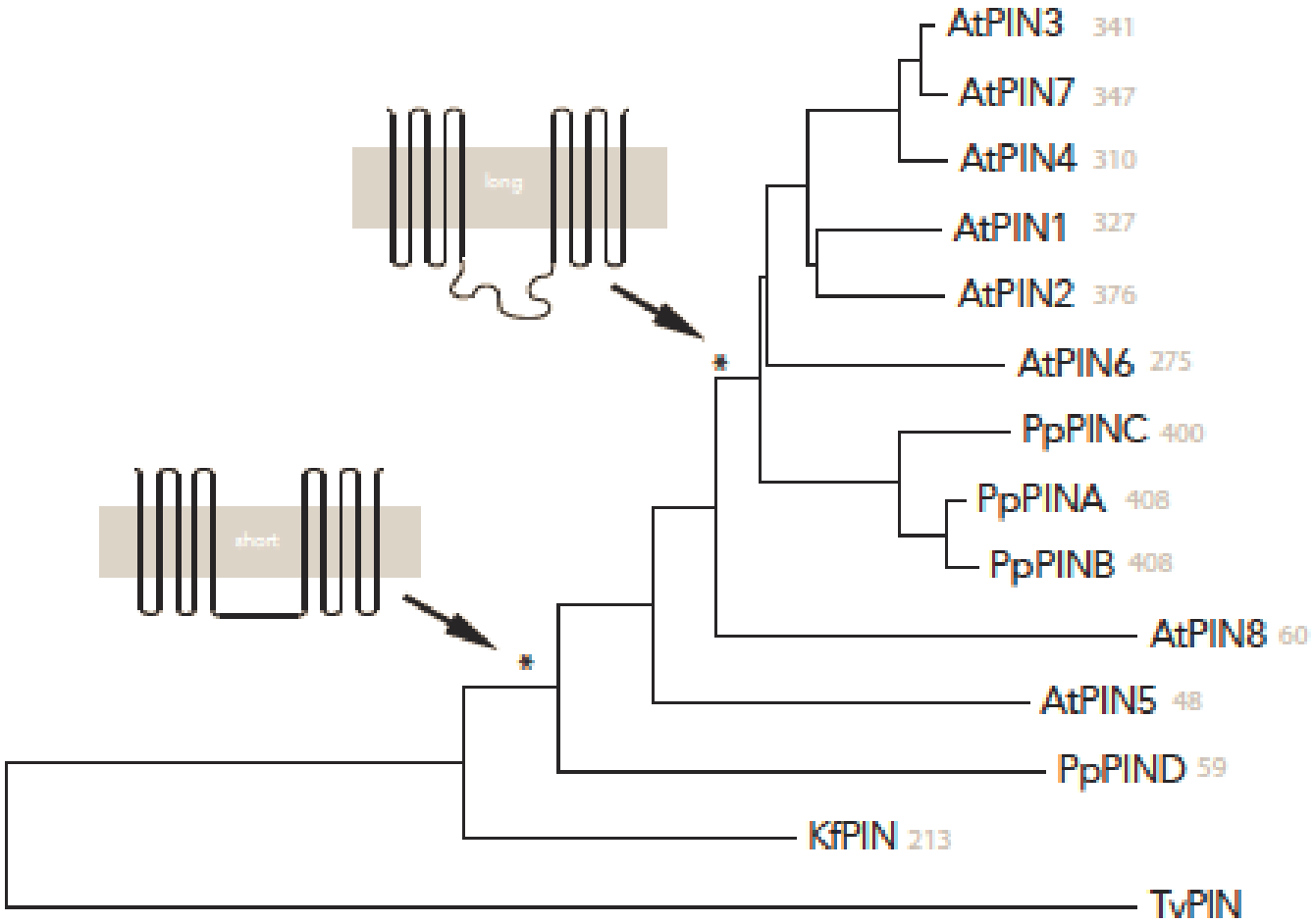
PIN1, PIN2

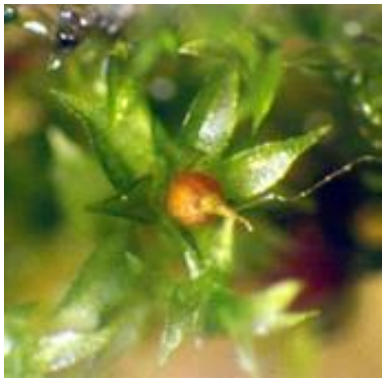


PIN5



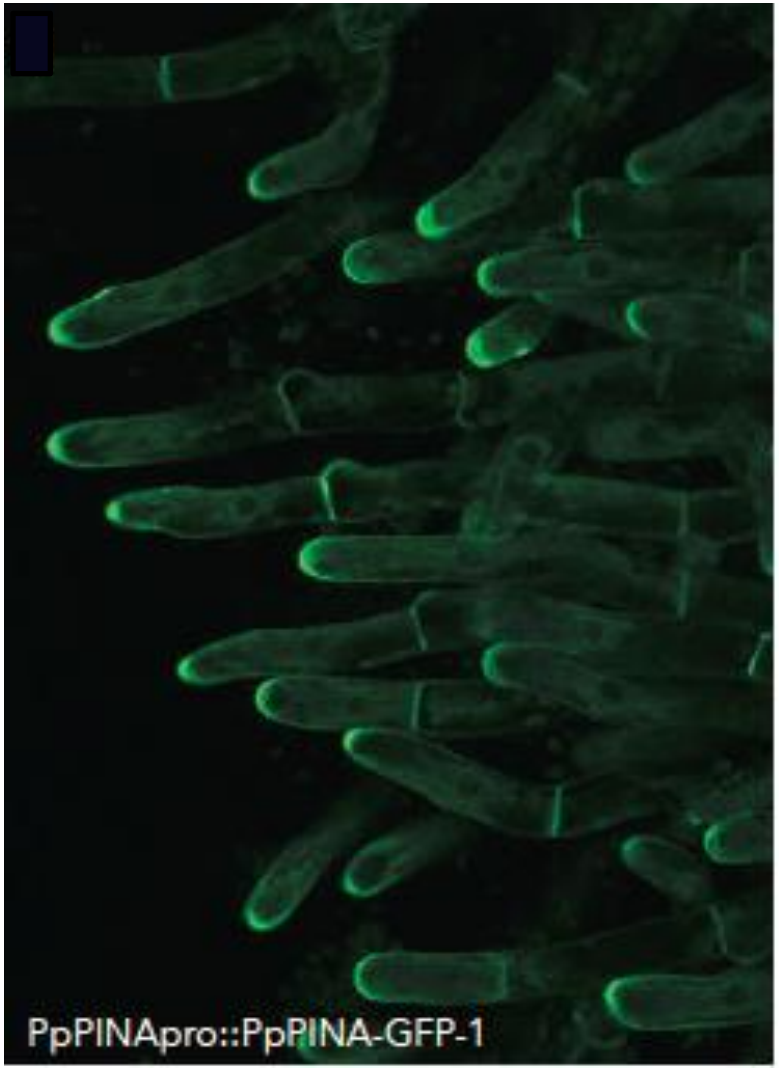
PIN Evolutionary Aspects

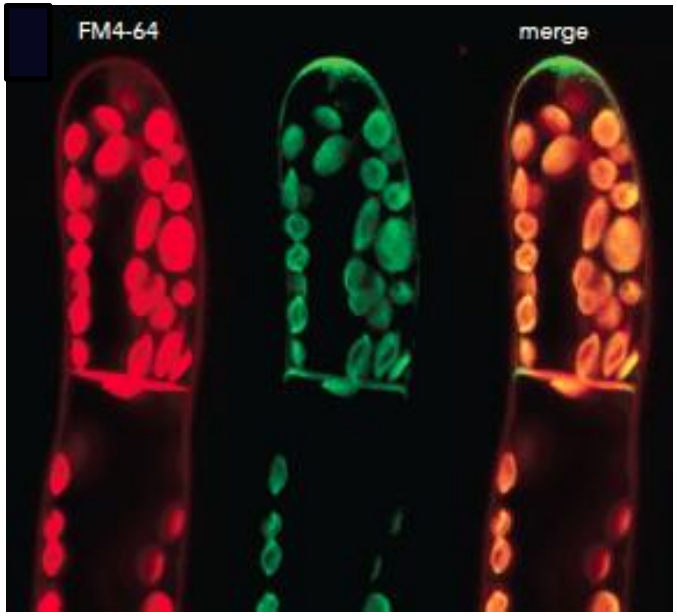




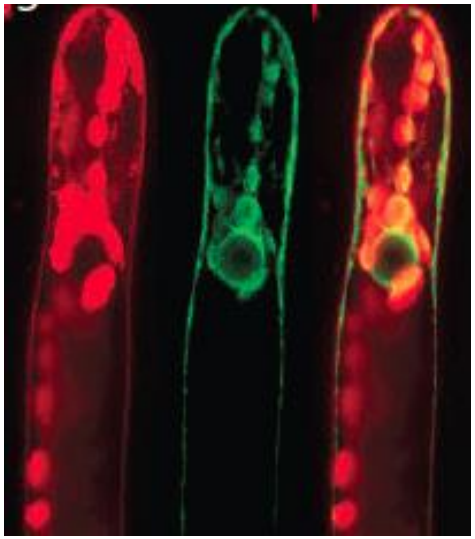
PIN localization in moss

Long PIN



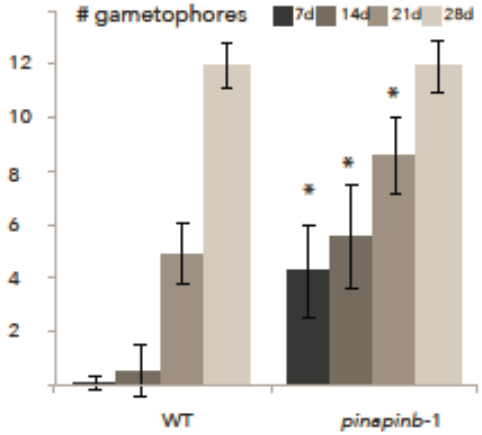


Short PIN

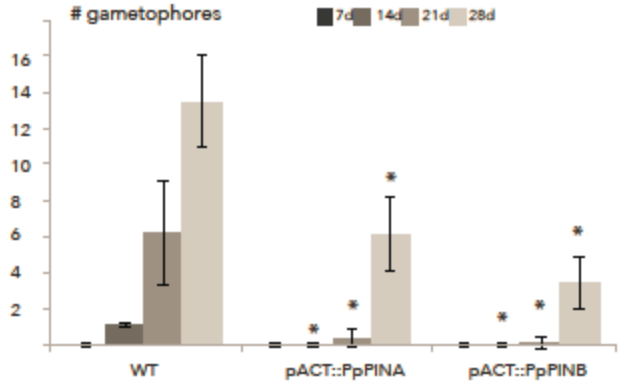


PINs and developmental transitions in moss

loss-of-function

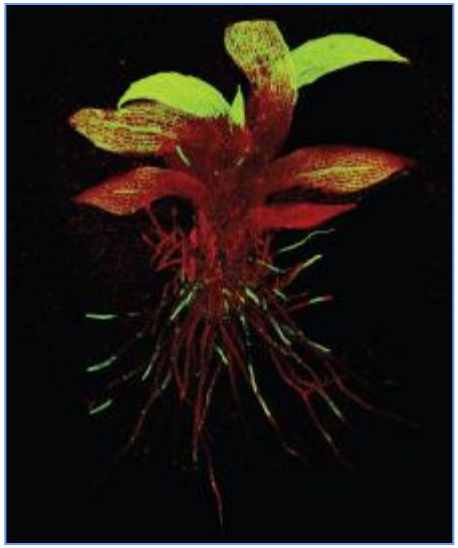


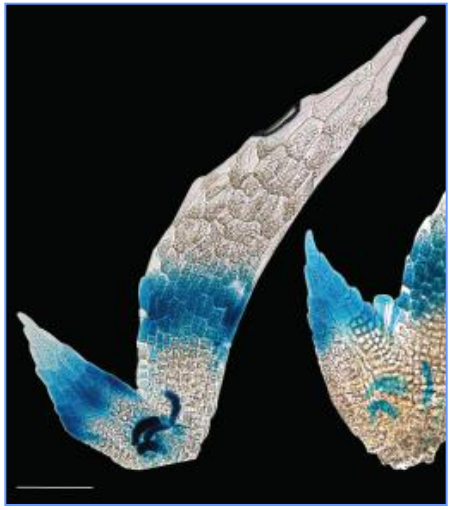
gain-of-function



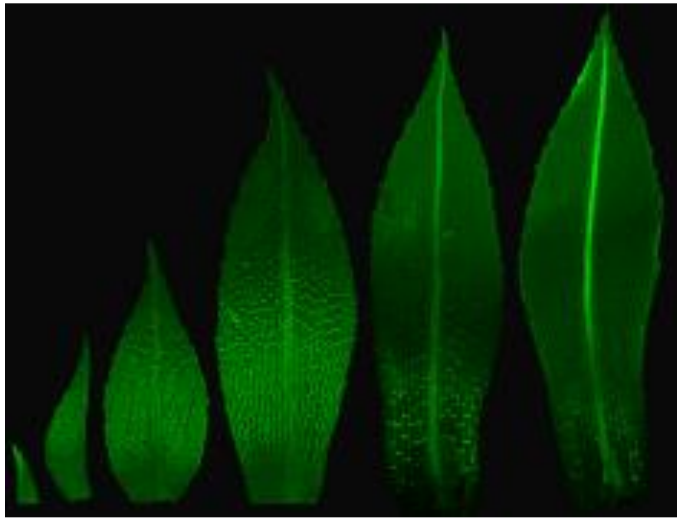
PINs in moss gametophores

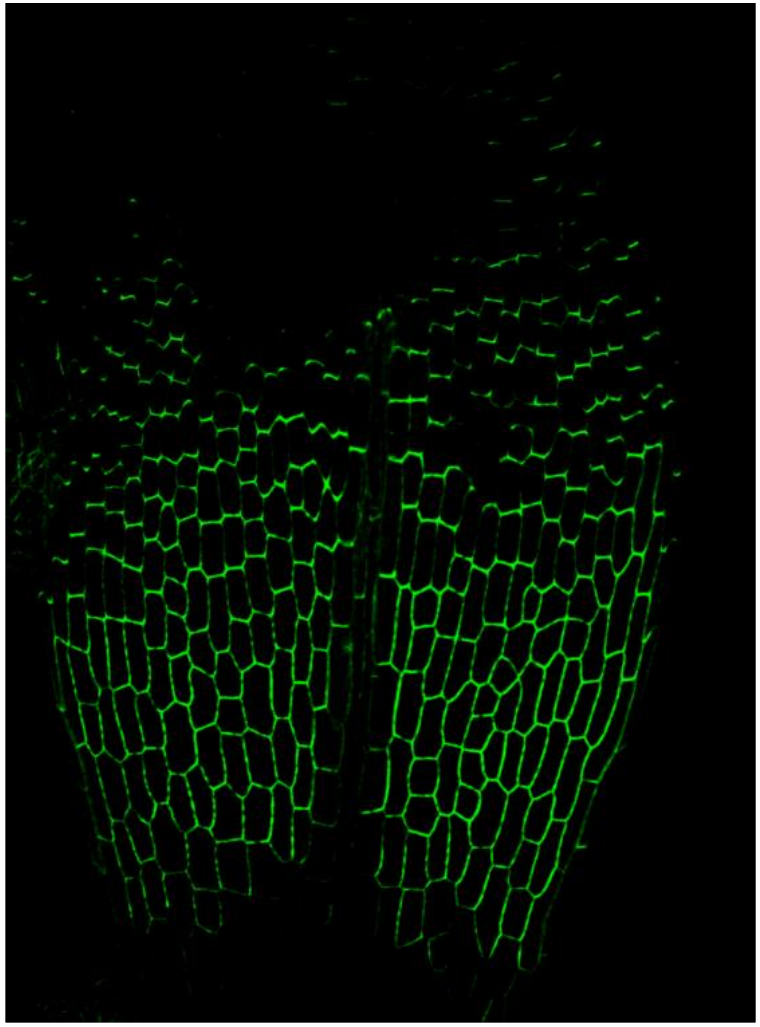
Expression



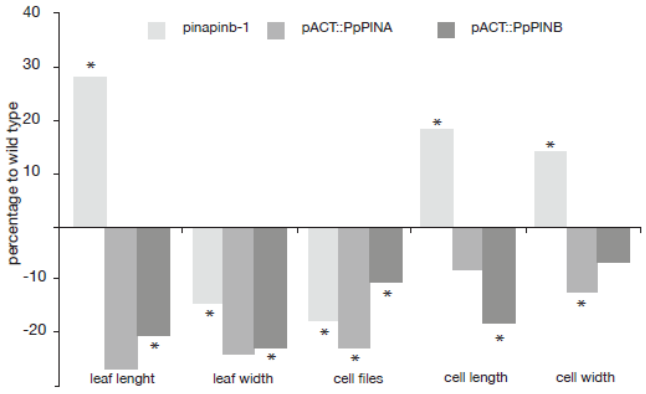


Localization





Phenotypes

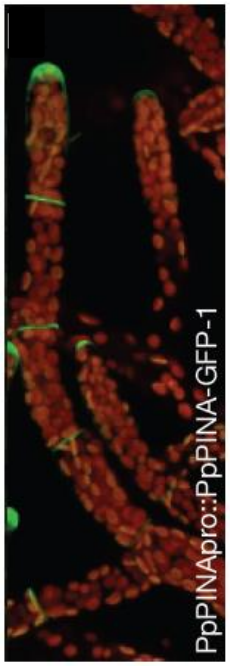


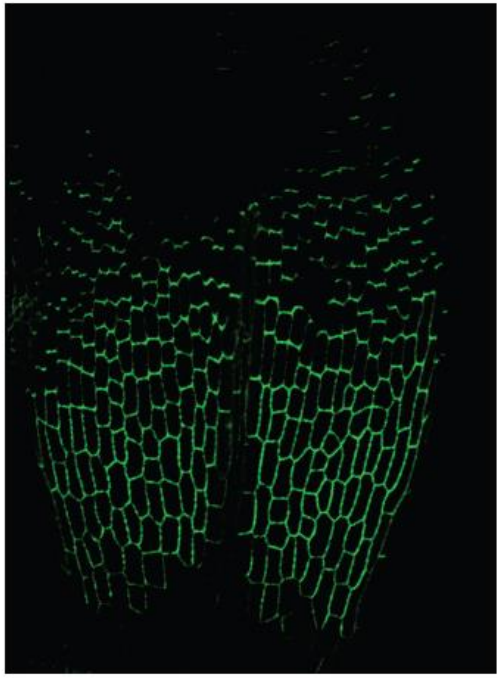
Evolution of tissue polarization

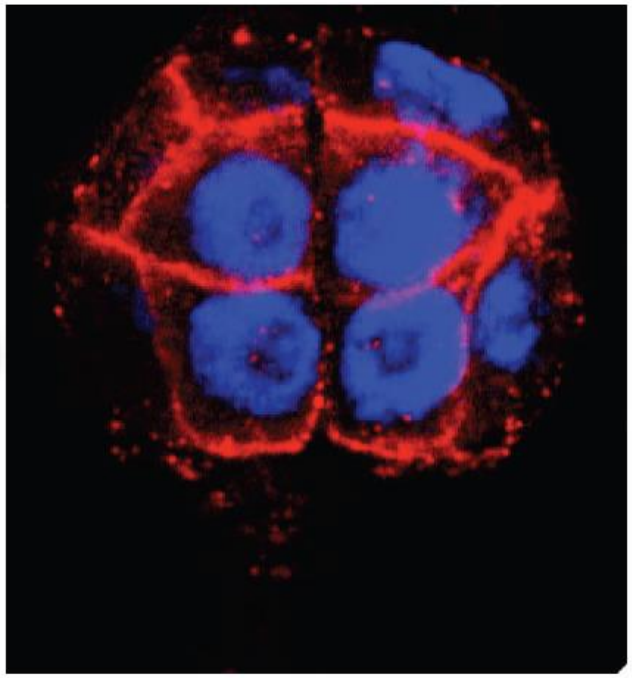
1-D filament

2-D moss leaf

3-D embryo

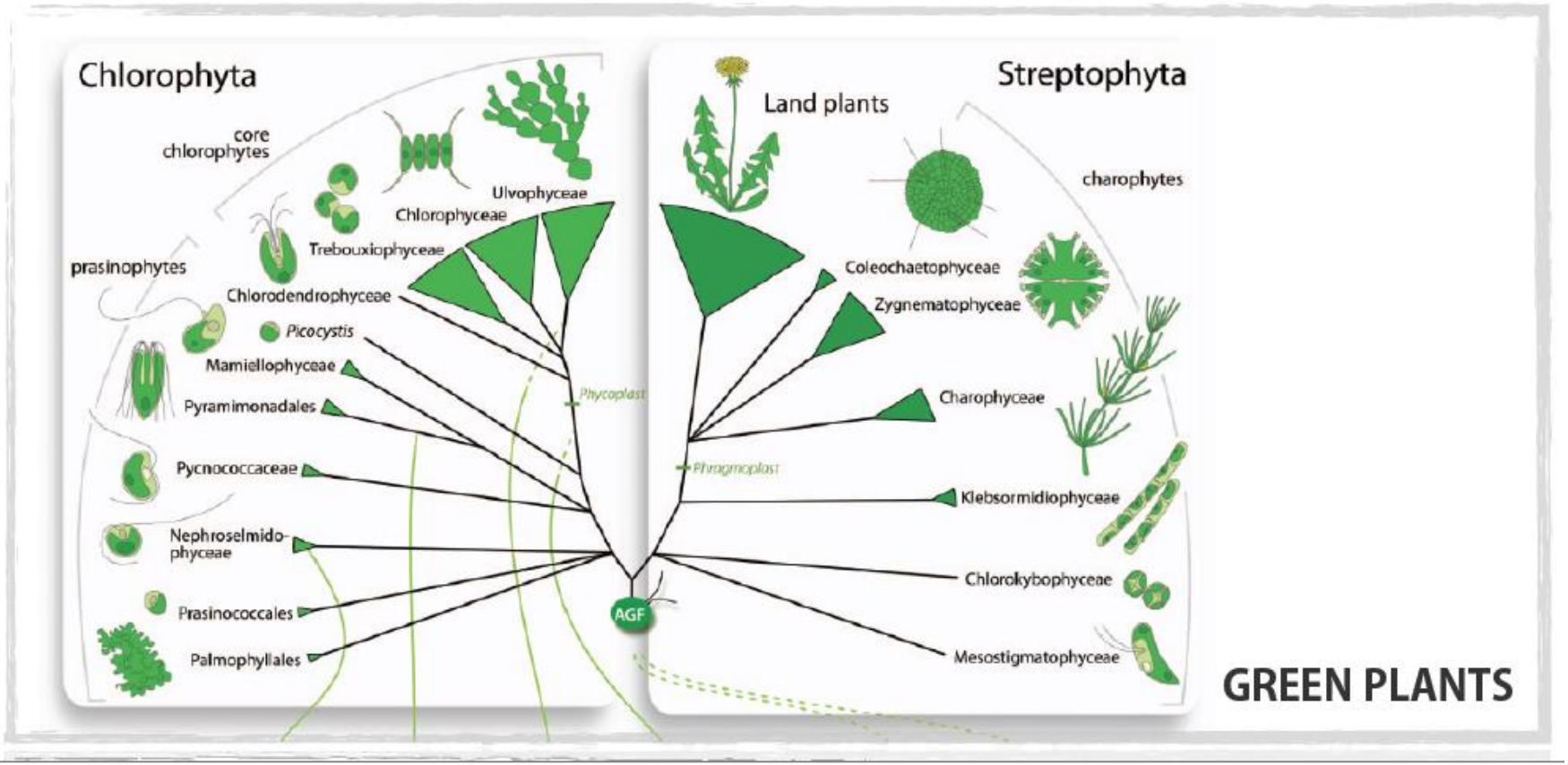






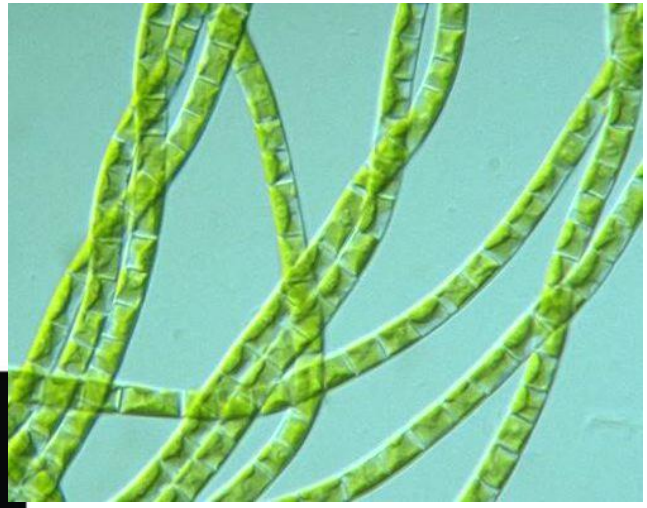
Mechanistic connection between patterning in tip growing cells
in mosses and complex multicellular tissues in advanced land plants

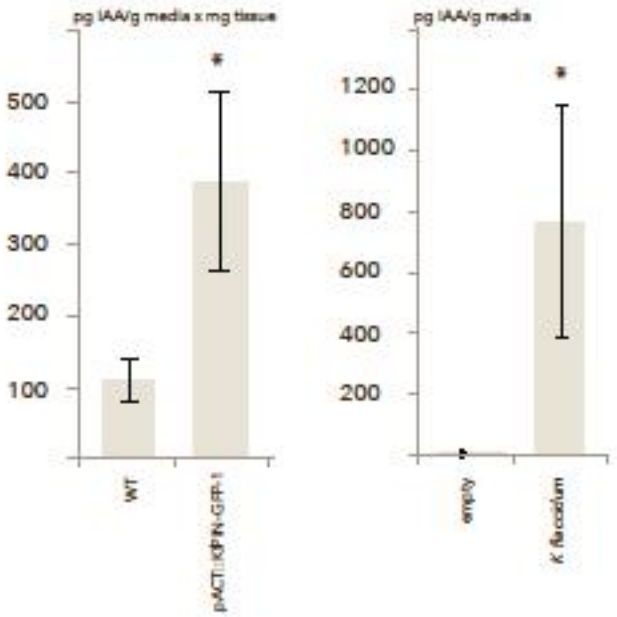
How did PINs started?

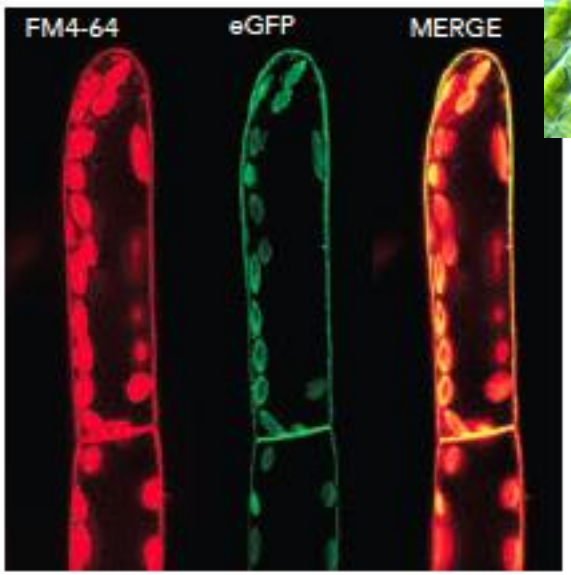


Most ancient PIN in filamentous algae

Klebsormidium



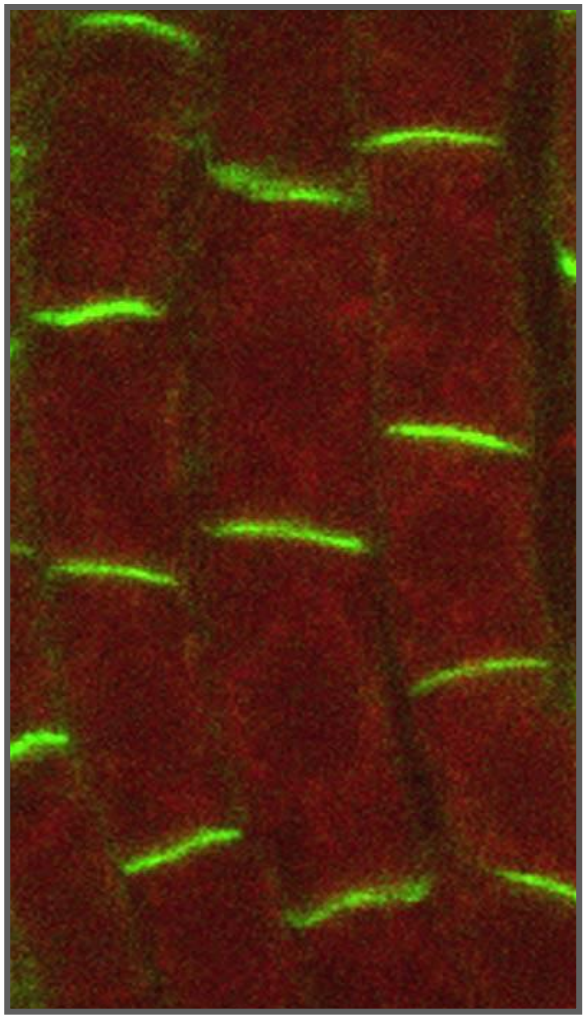




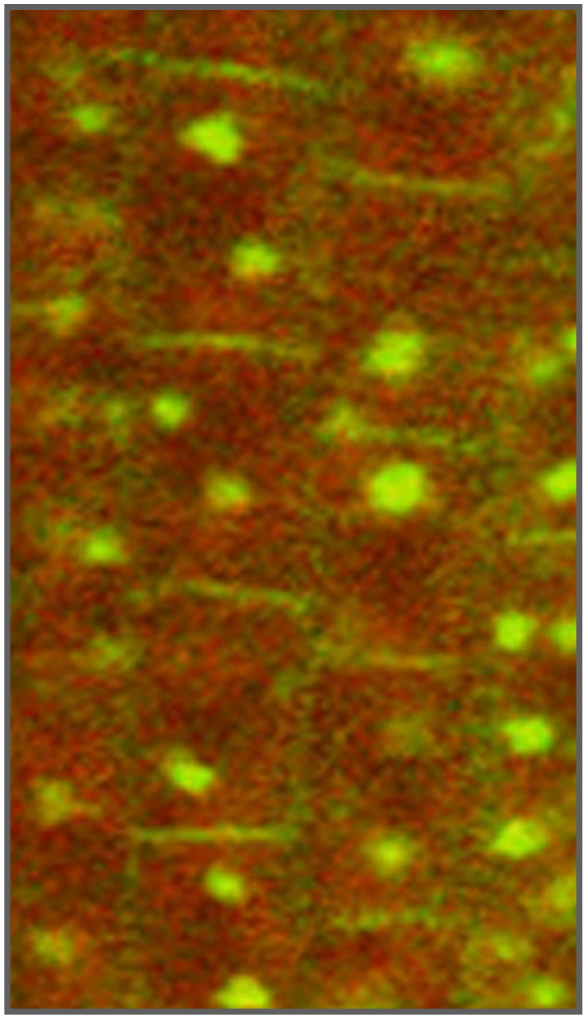
Constitutive Cycling of PINs

PIN1 Subcellular Movement

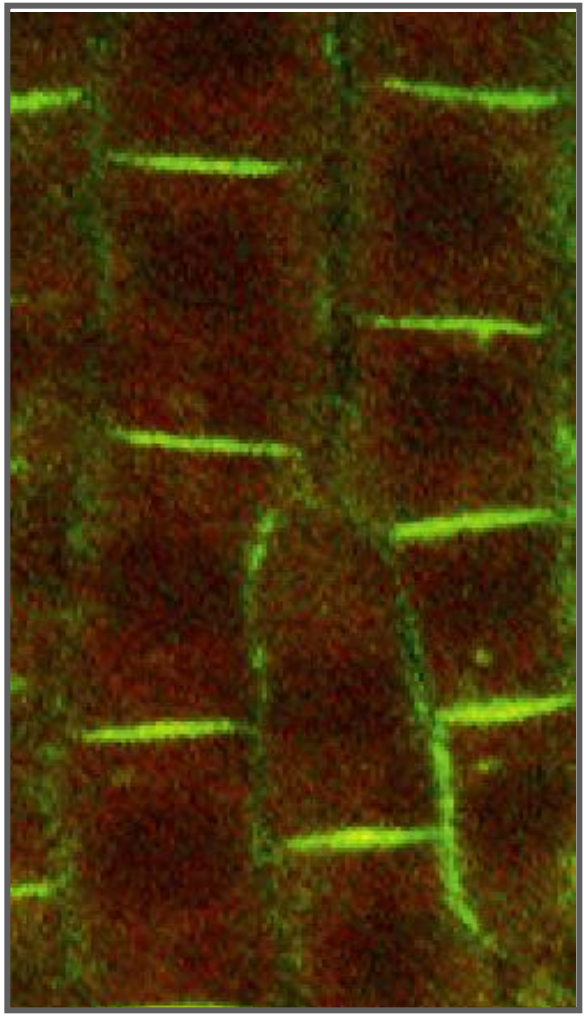
untreated



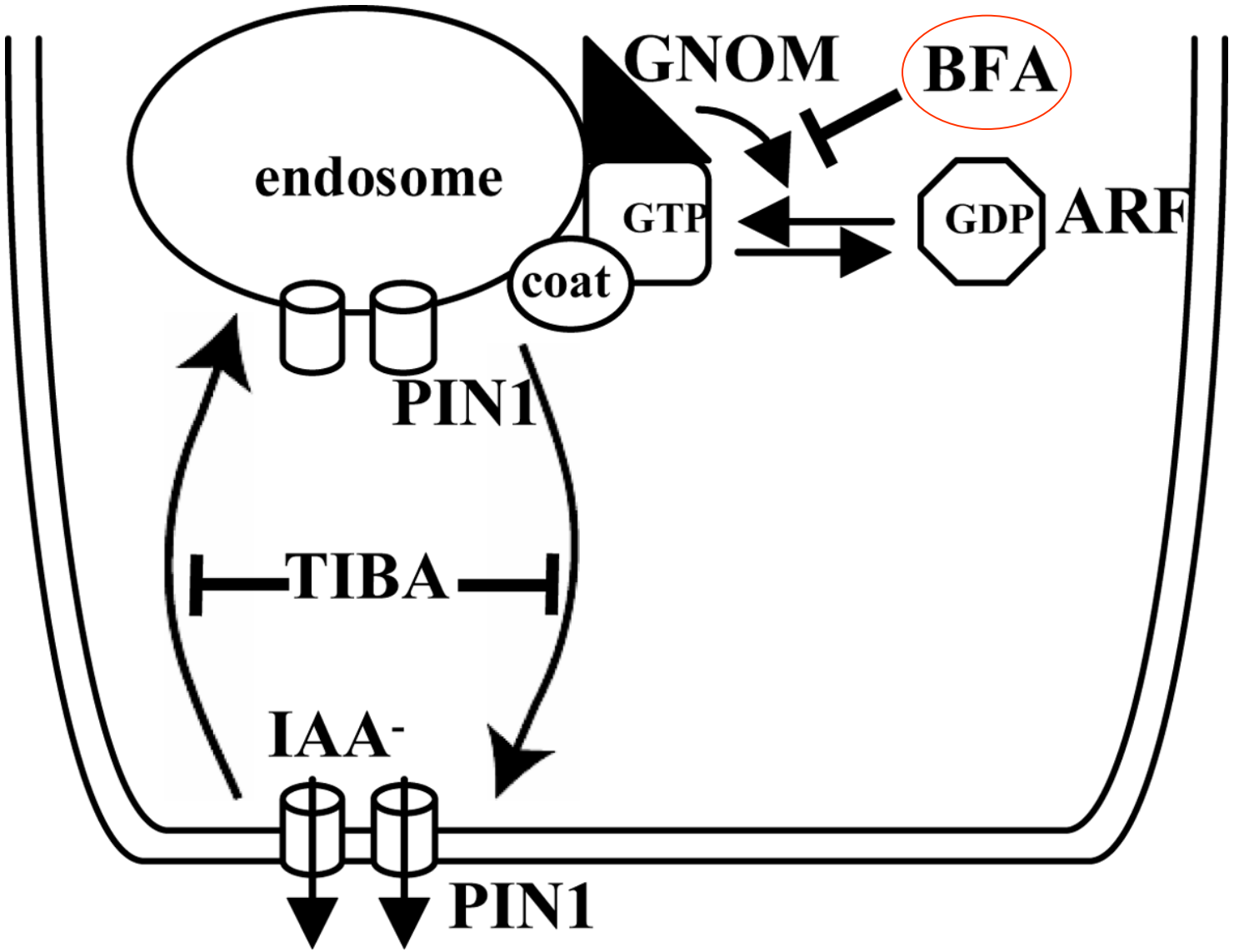
+ BFA



- BFA

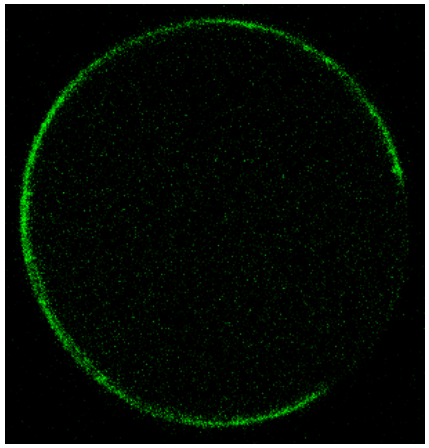


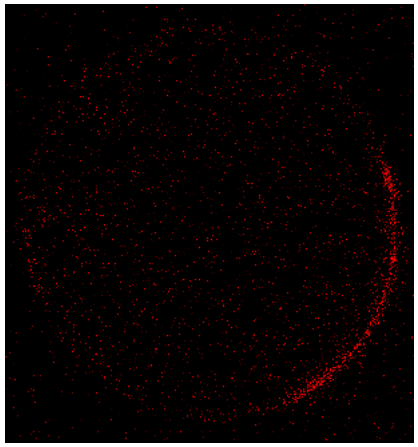
Dynamic Movement of PIN Proteins

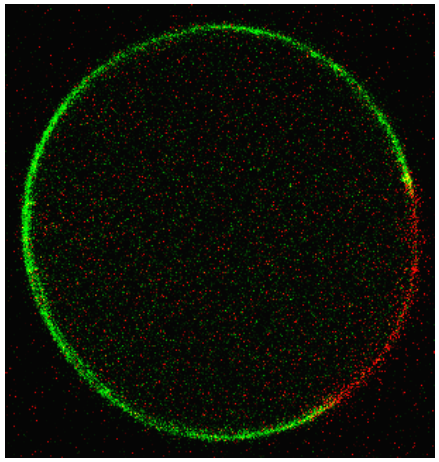


UV-activated PIN2-EosFP

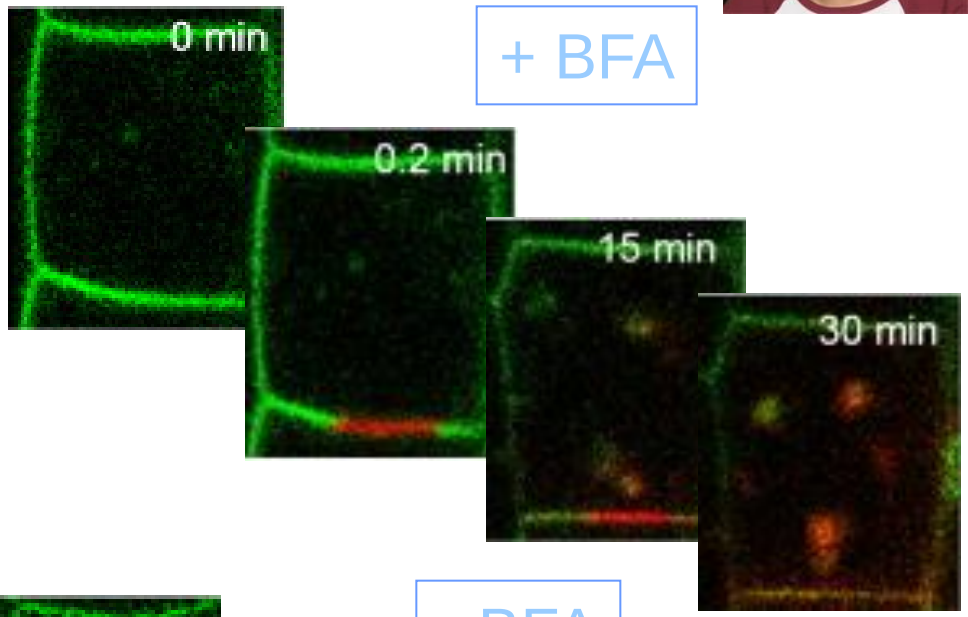
Protoplasts



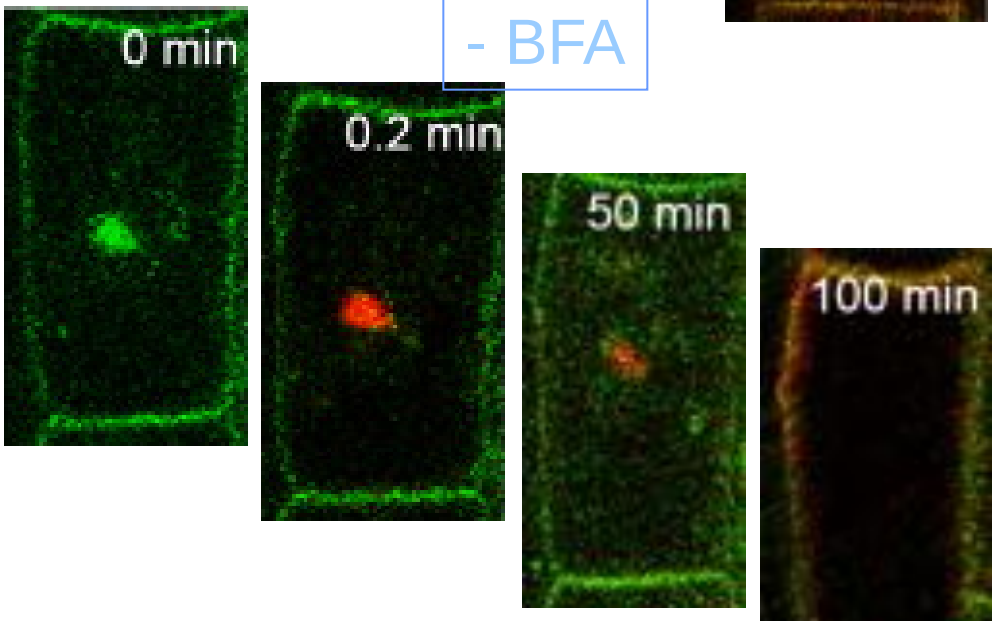




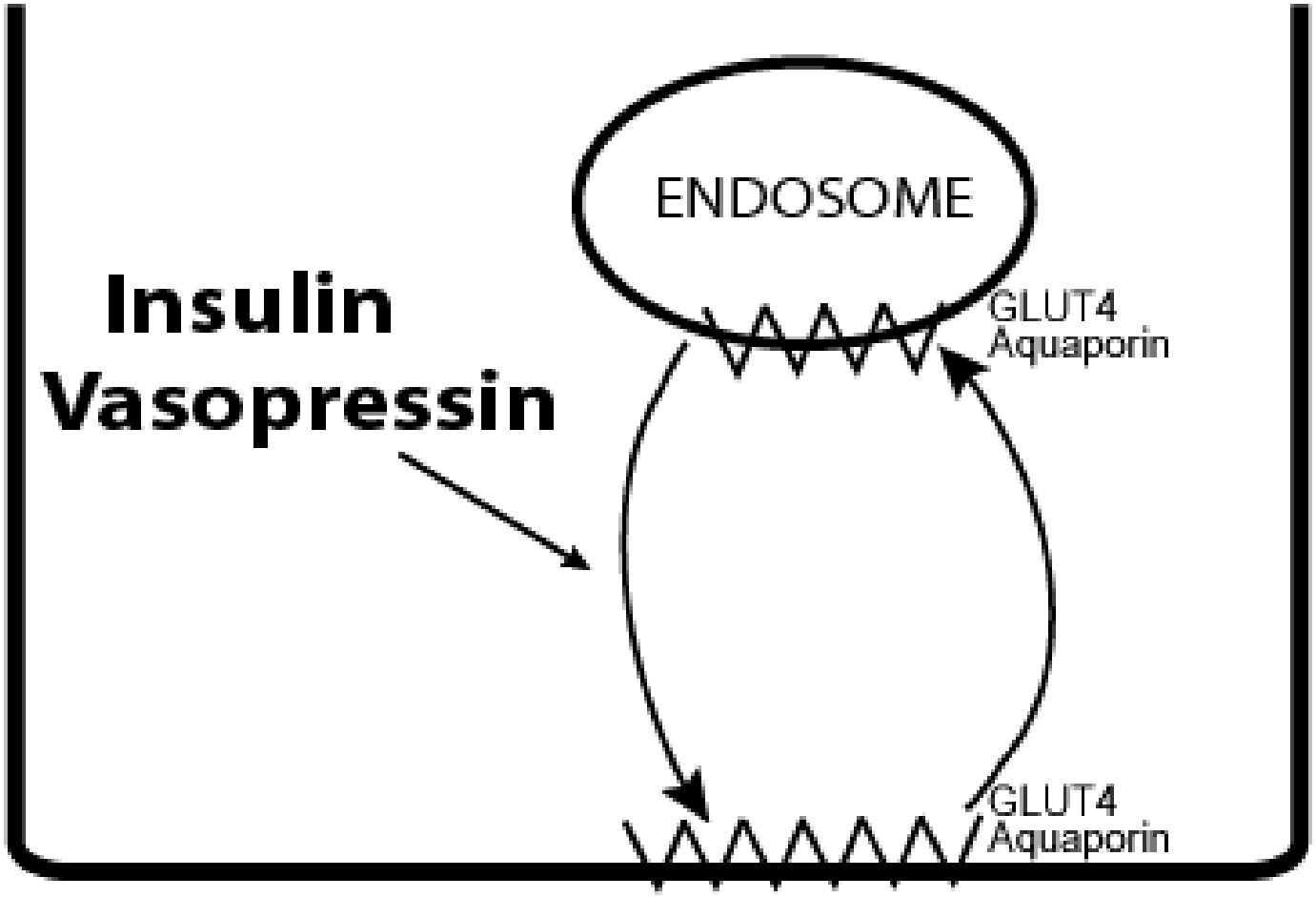
+ BFA



- BFA

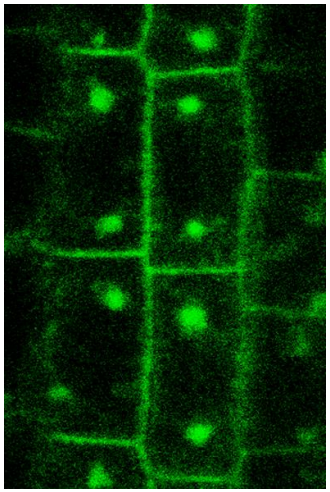


Subcellular Cycling – Means to Modulate Protein Activity?

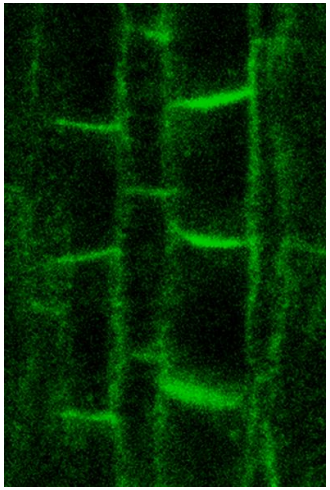


Auxin Inhibits PIN Internalization and Stimulates its Efflux

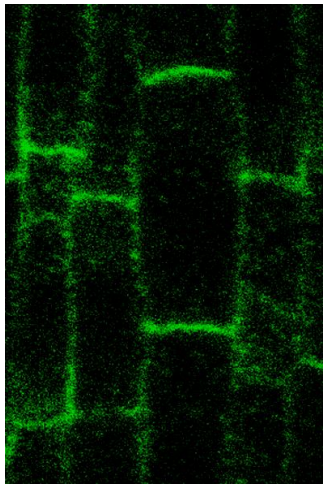
BFA



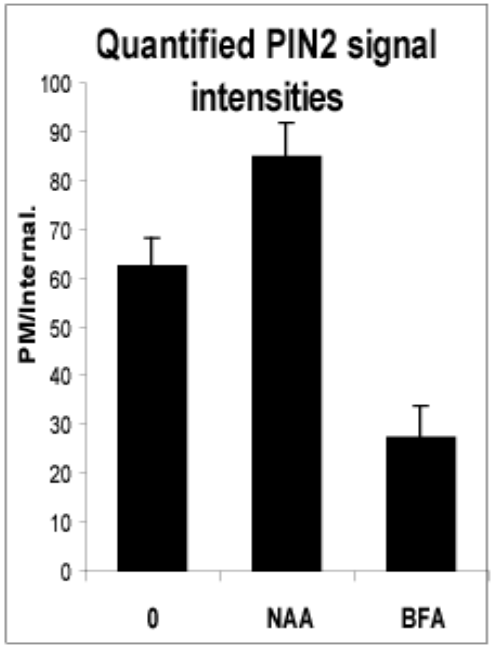
IAA/BFA



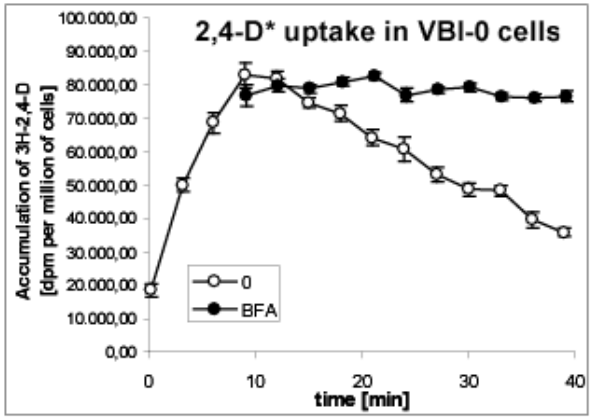
2,4-D/BFA



PIN2 at PM



Auxin efflux

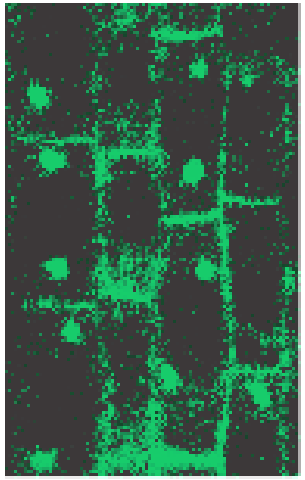


Paciorek et al., 2005

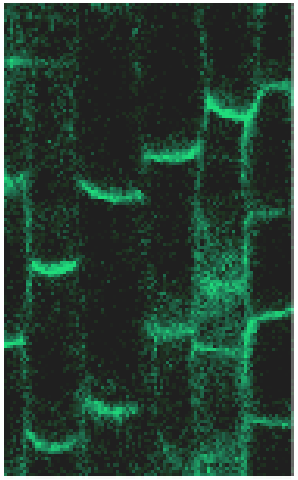
Novel Pathway of Auxin Action

PIN1

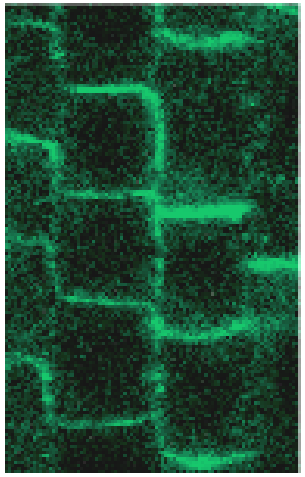
BFA



2,4-D/CHX /BFA

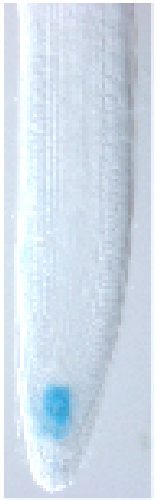


2,4-D/MG132 /BFA



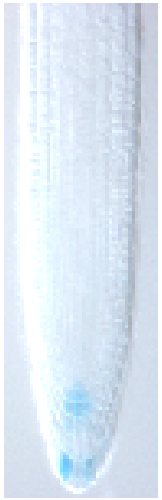
DR5

0

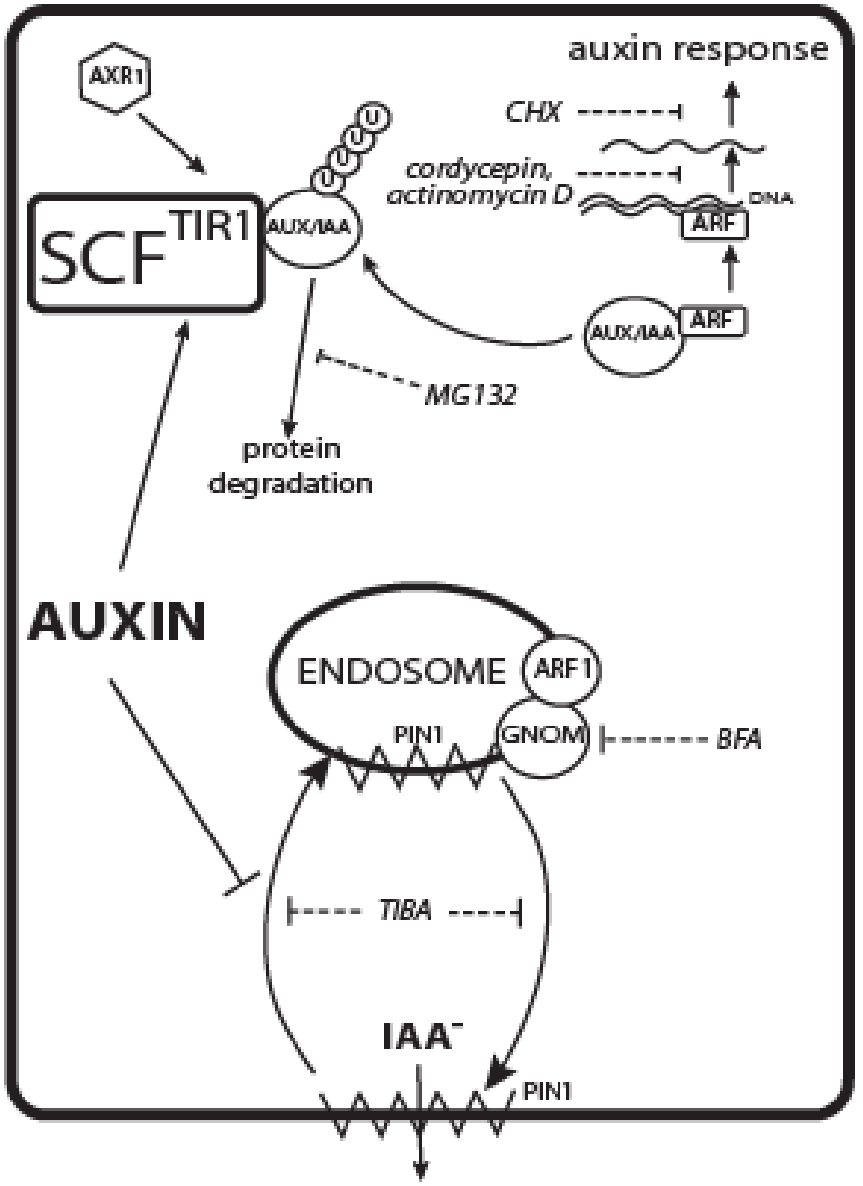


2,4-D

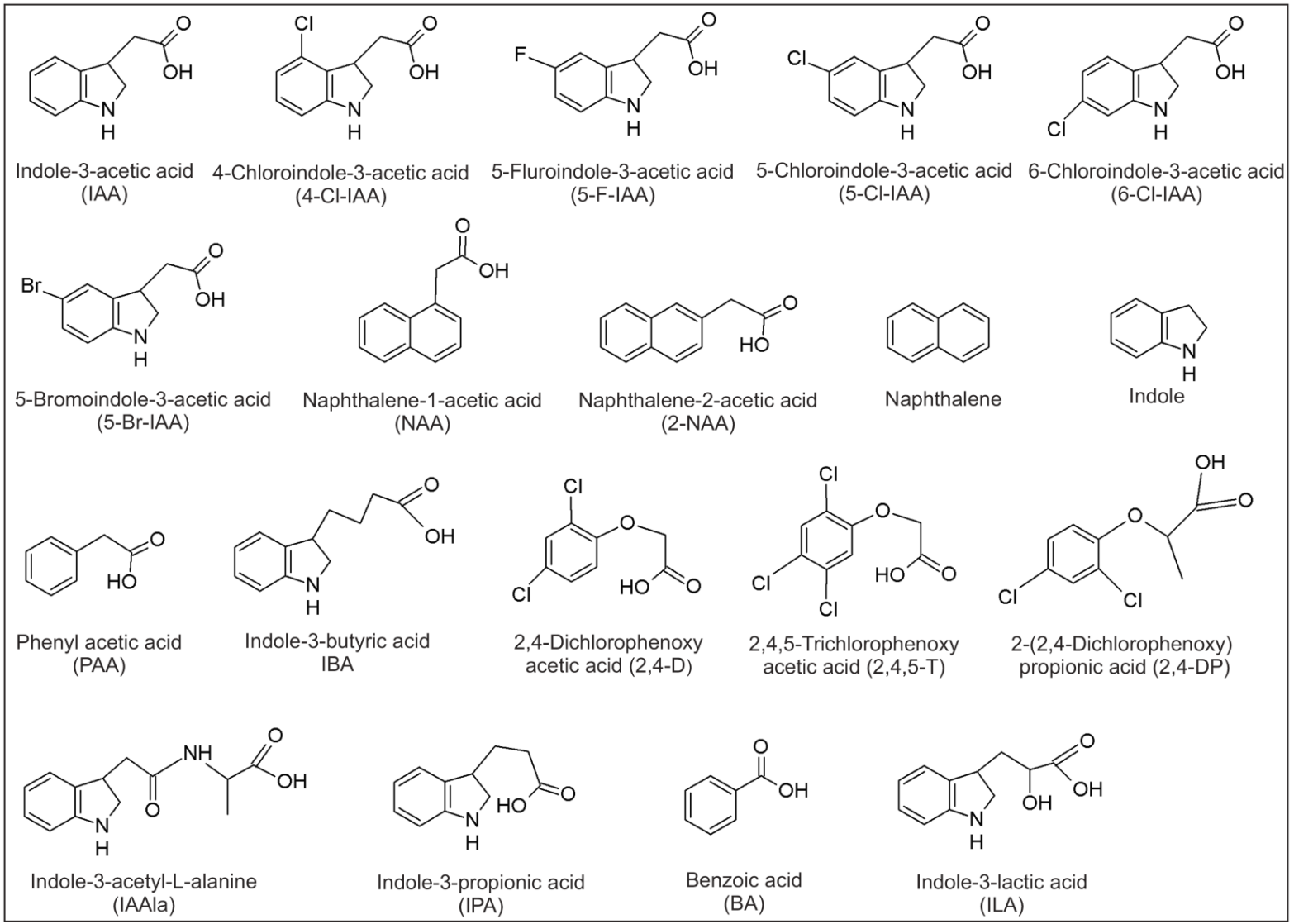
2,4-D/ CHX



2,4-D/ MG132

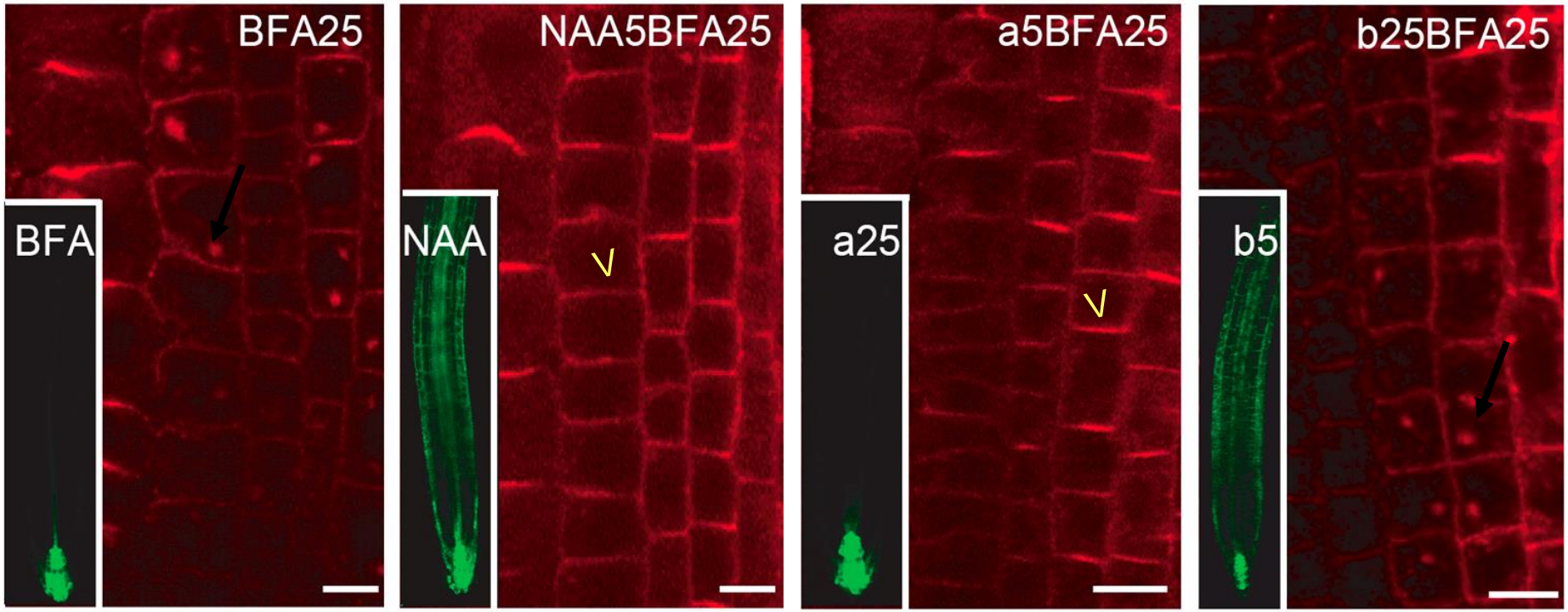


Auxin analogues: mapping the binding sites



Robert et al., 2010

Auxin-mediated regulation of transcription and endocytosis involve different binding sites



Robert et al., 2010

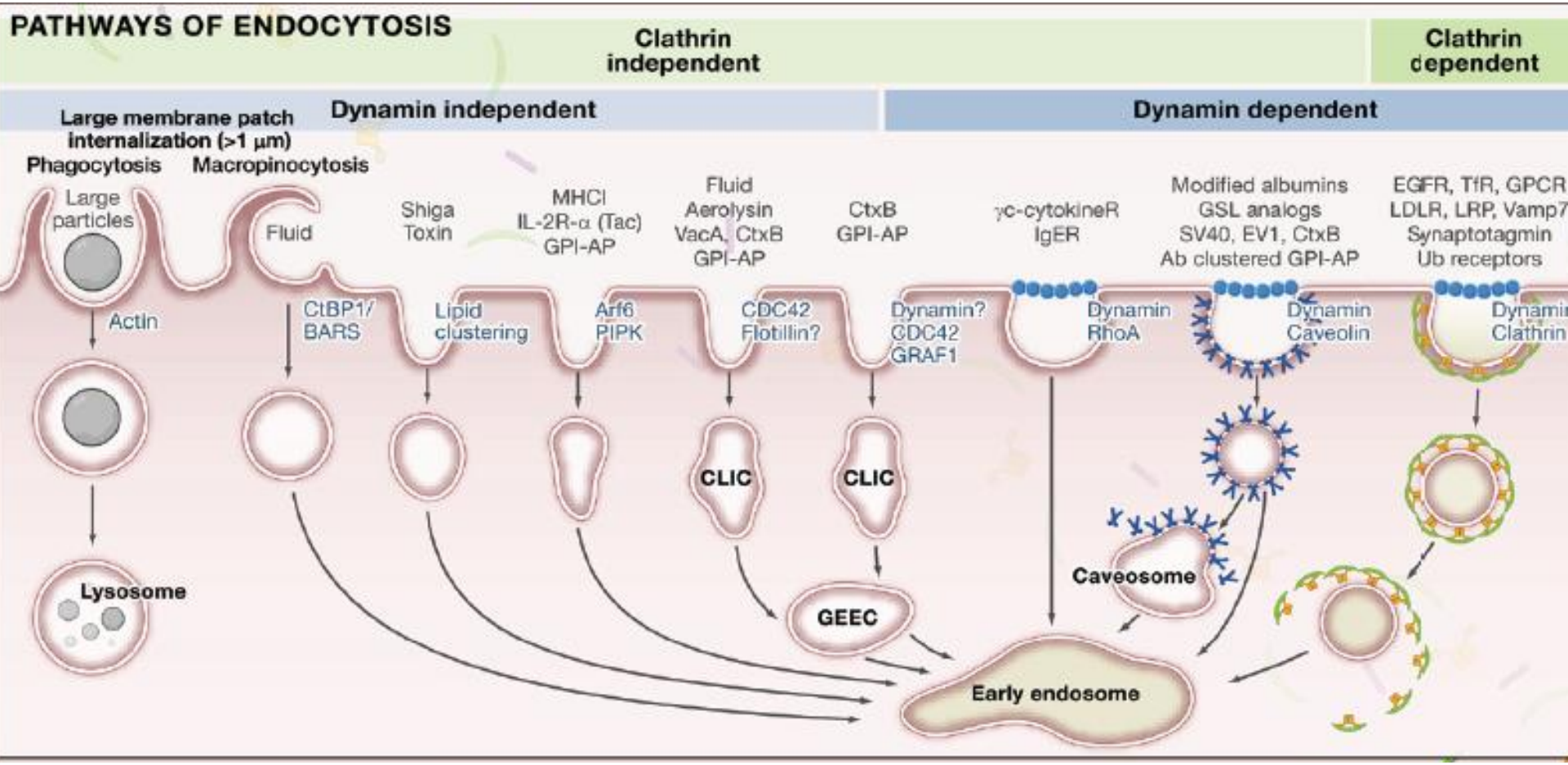
Novel Pathway for Auxin Signaling

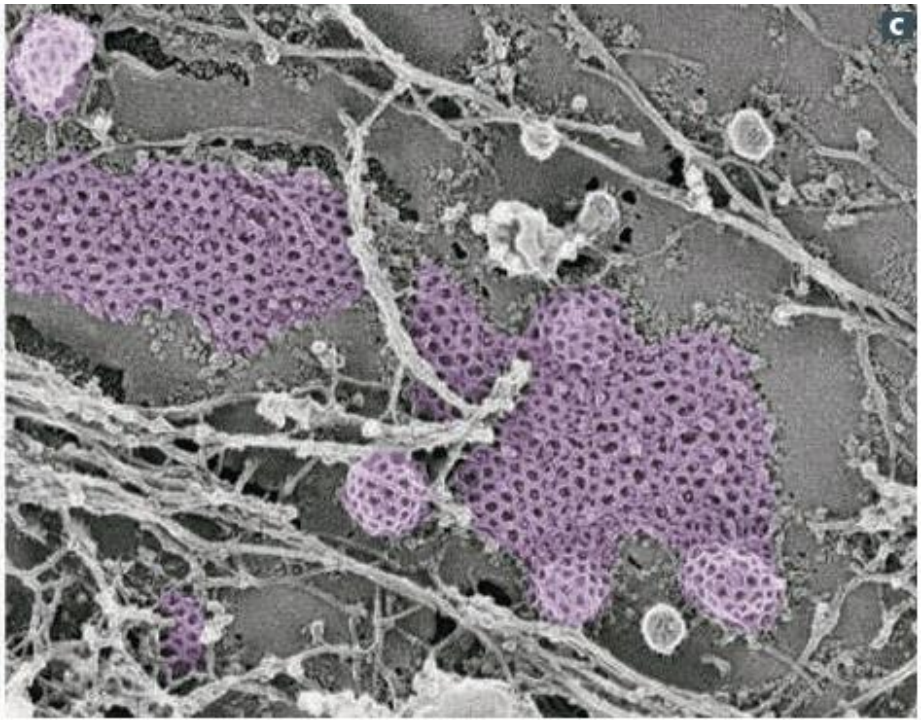
Auxin inhibits endocytosis including internalization of PIN proteins

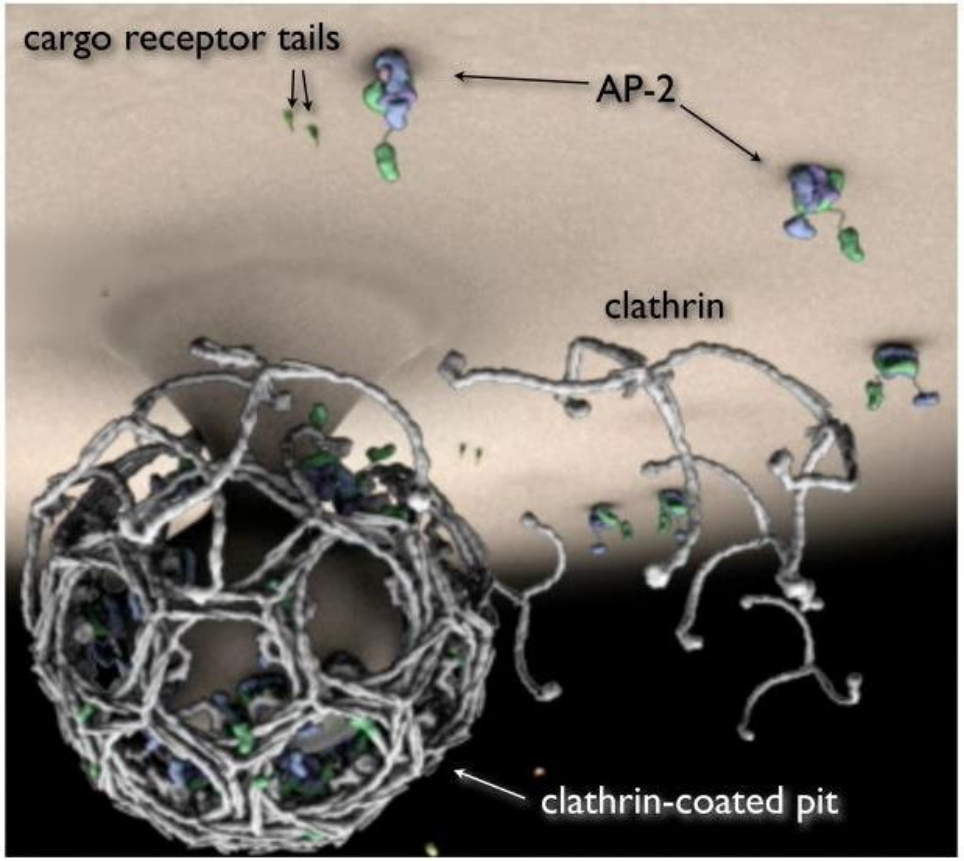
This is mechanism by which auxin stabilizes PINs at the cell surface thus stimulating auxin efflux.

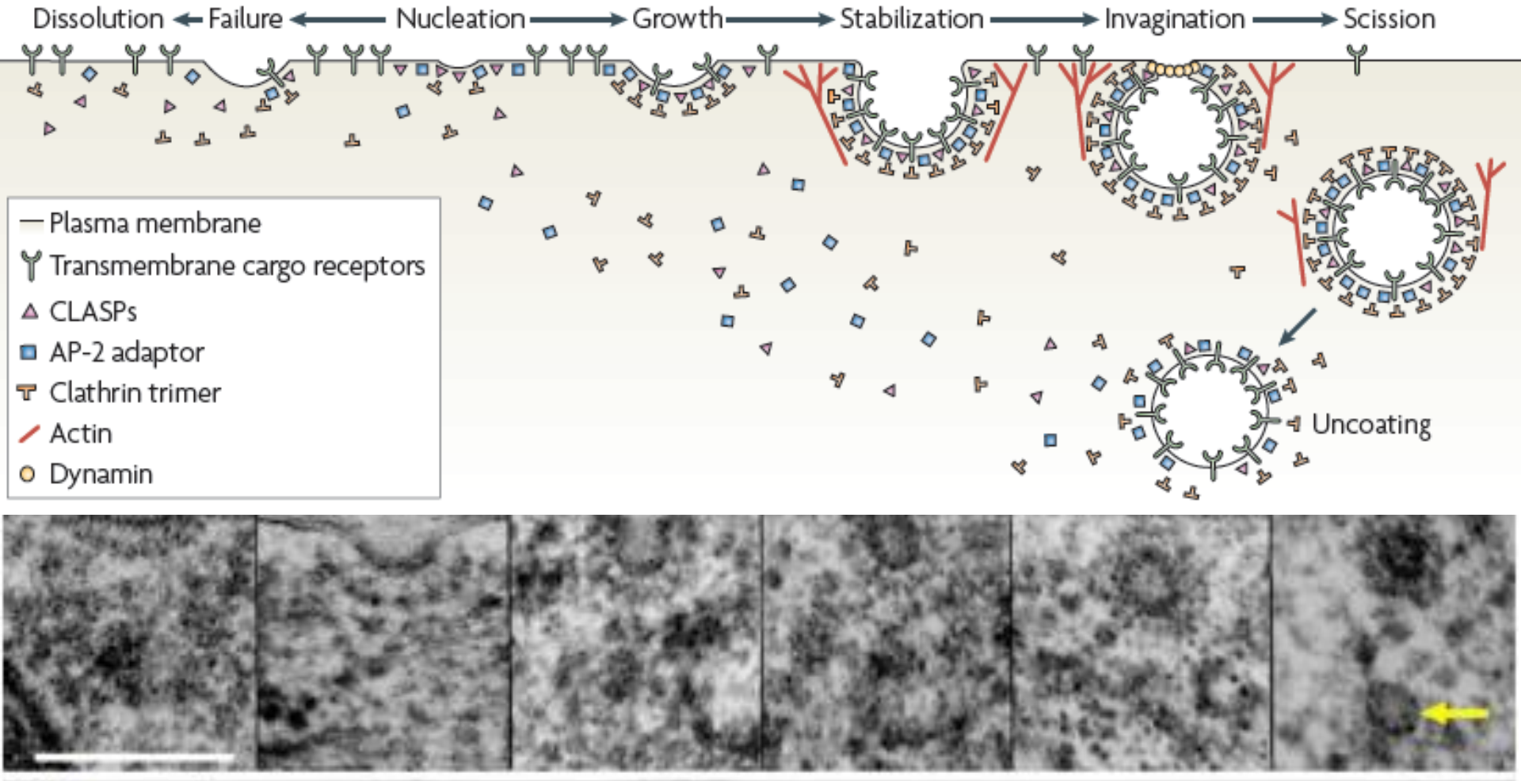
This auxin effect involves novel, genetically tractable auxin pathway

Mechanisms for endocytosis in animals

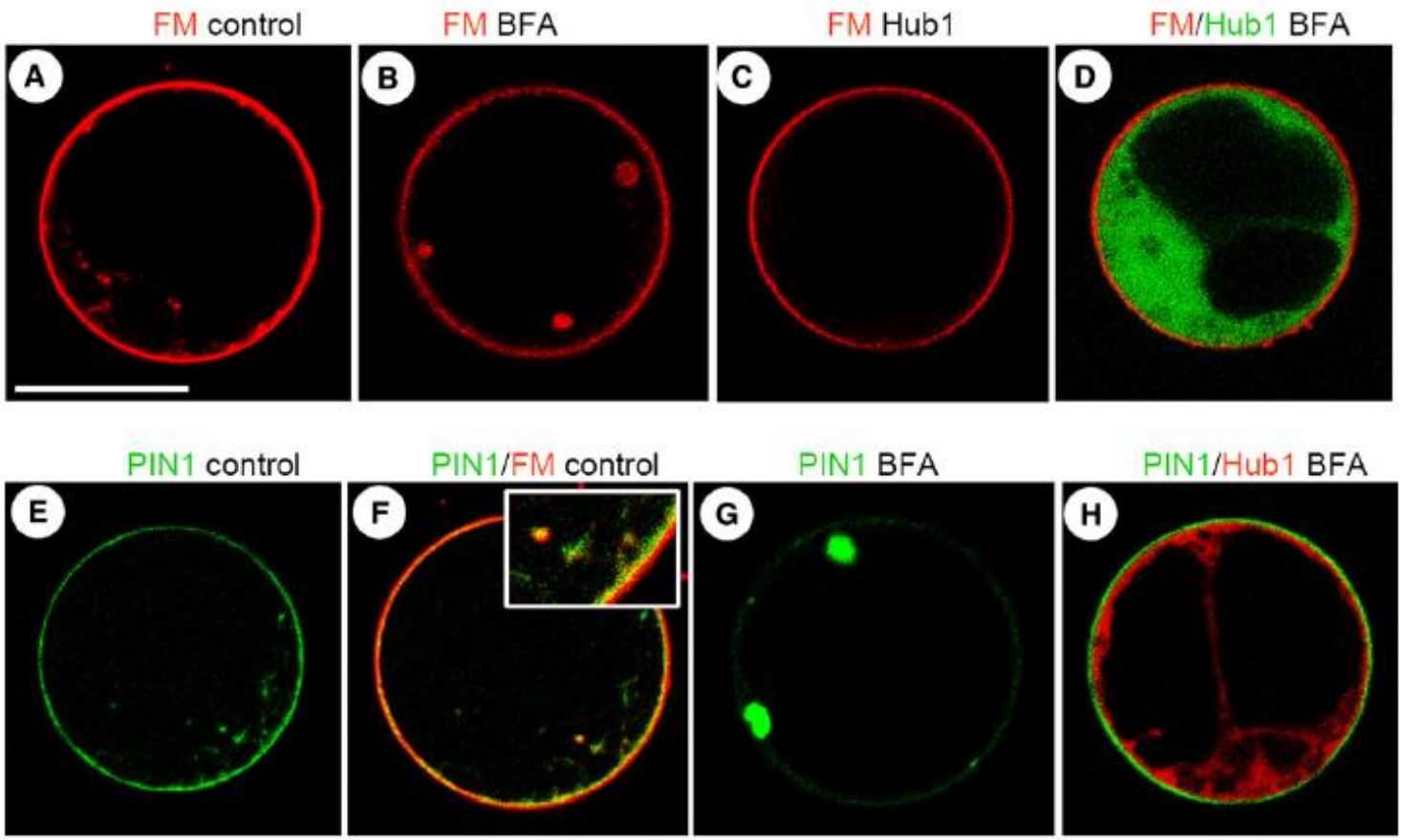



c


cargo receptor tails
AP-2
clathrin
clathrin-coated pit

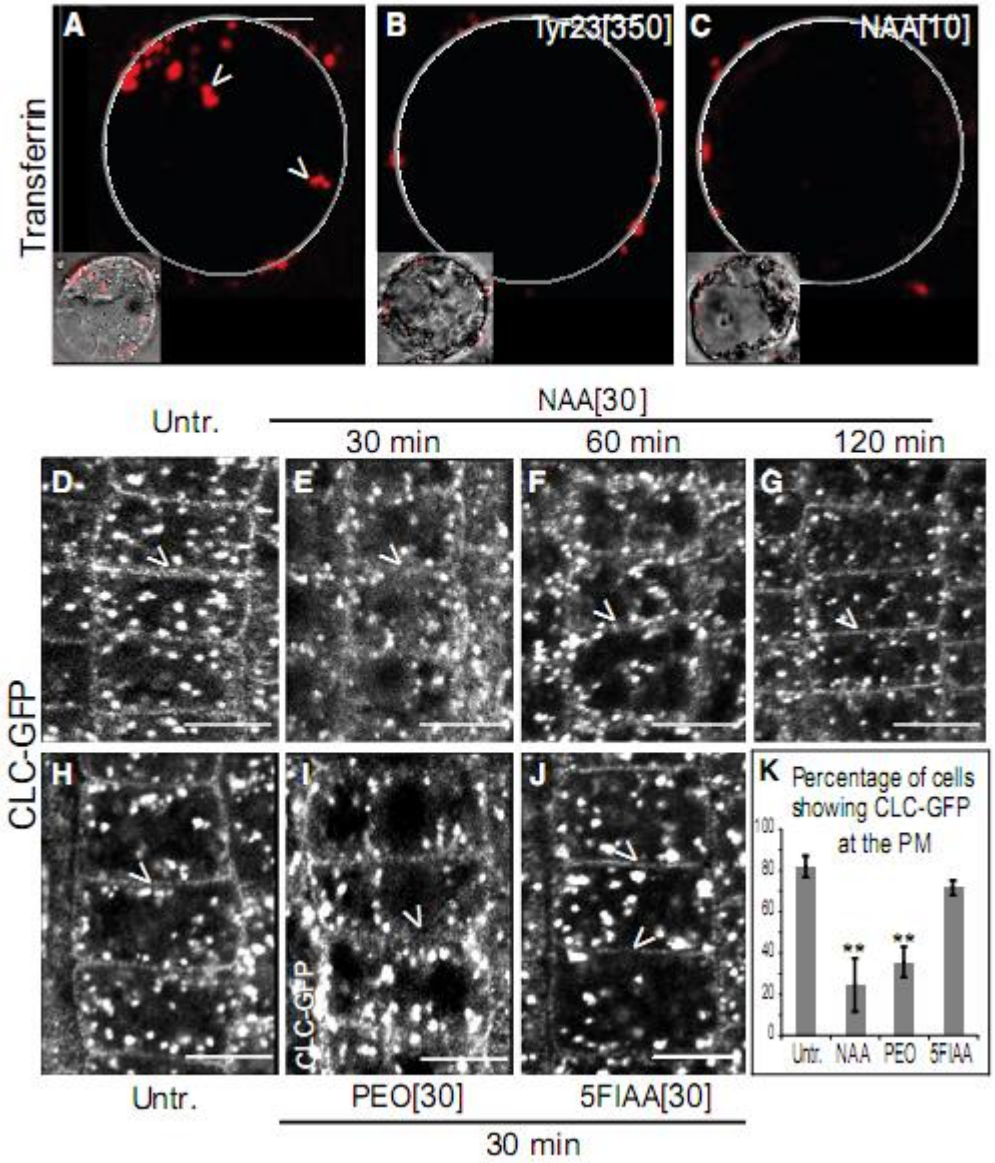

Dissolution
Failure
Nucleation
Growth
Stabilization
Invagination
Scission
Plasma membrane
Transmembrane cargo receptors
CLASPs
AP-2 adaptor
Clathrin trimer
Actin
Dynamin
Uncoating

Clathrin is required for PIN internalization in Arabidopsis protoplasts



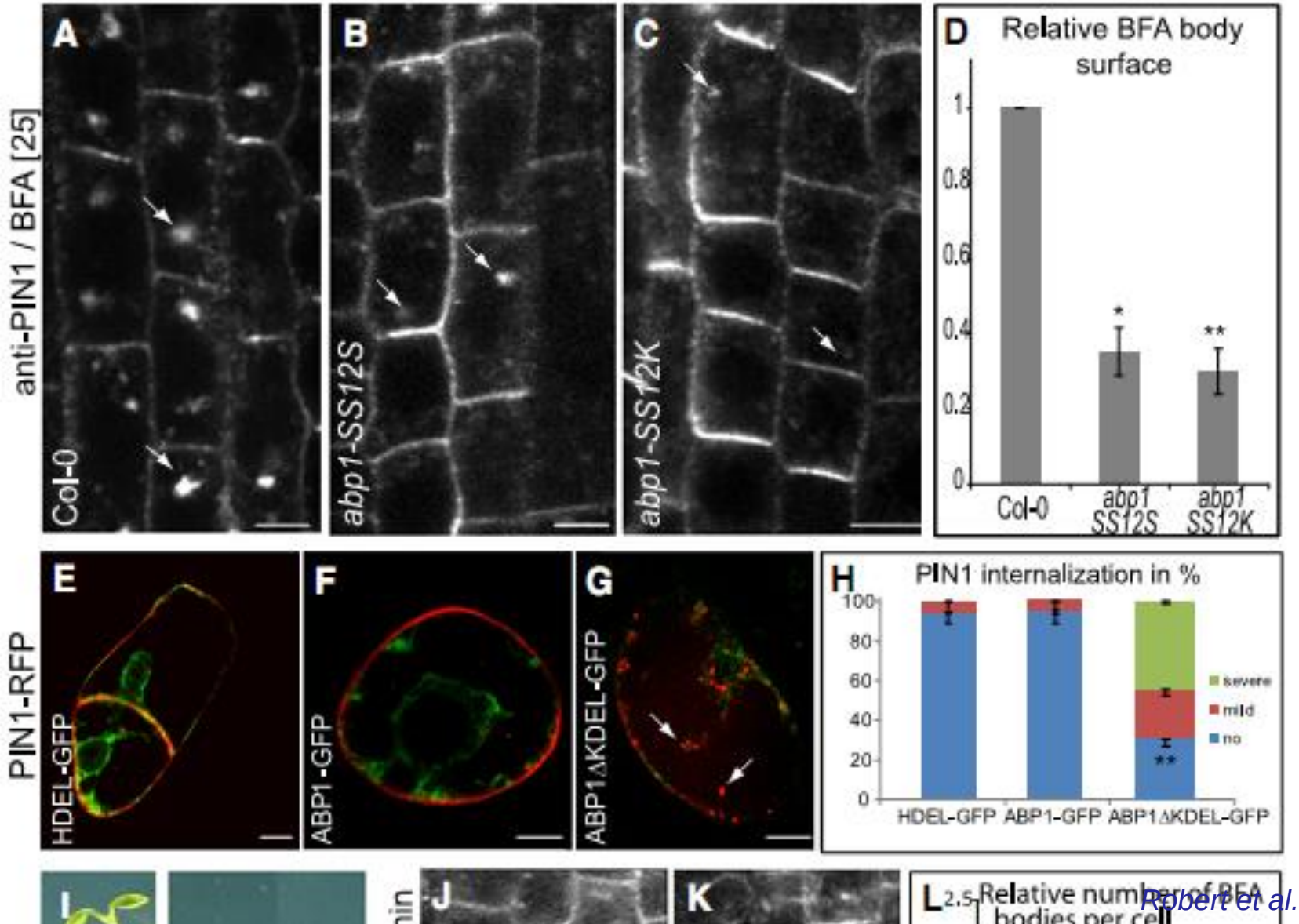
Dhonukshe et al., 2007

Auxin targets clathrin mechanism of endocytosis



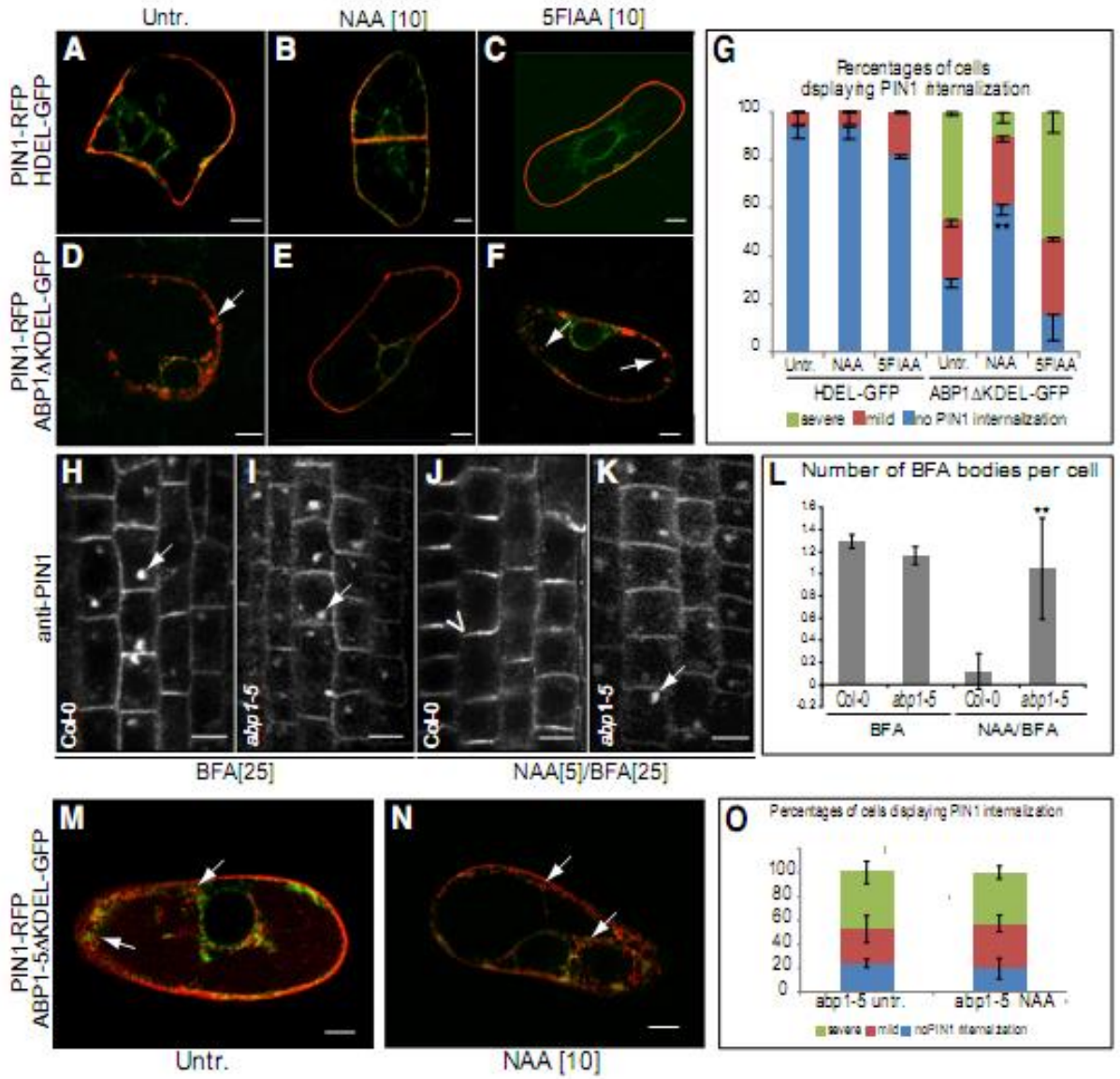
Robert et al., 2010

ABP1 positively regulates endocytosis



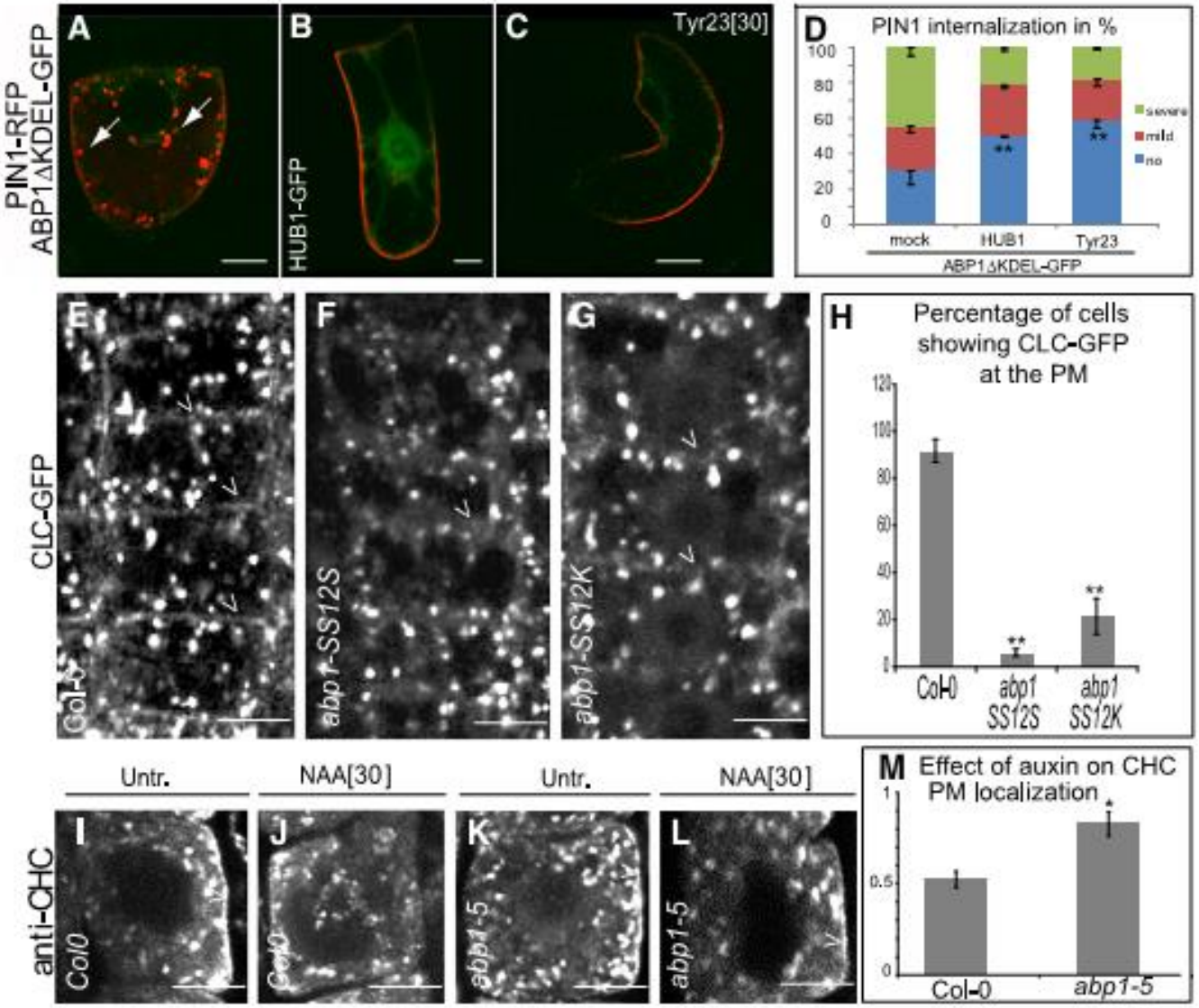
Robert et al., 2010

ABP1 mediates auxin effect on endocytosis



Robert et al., 2010

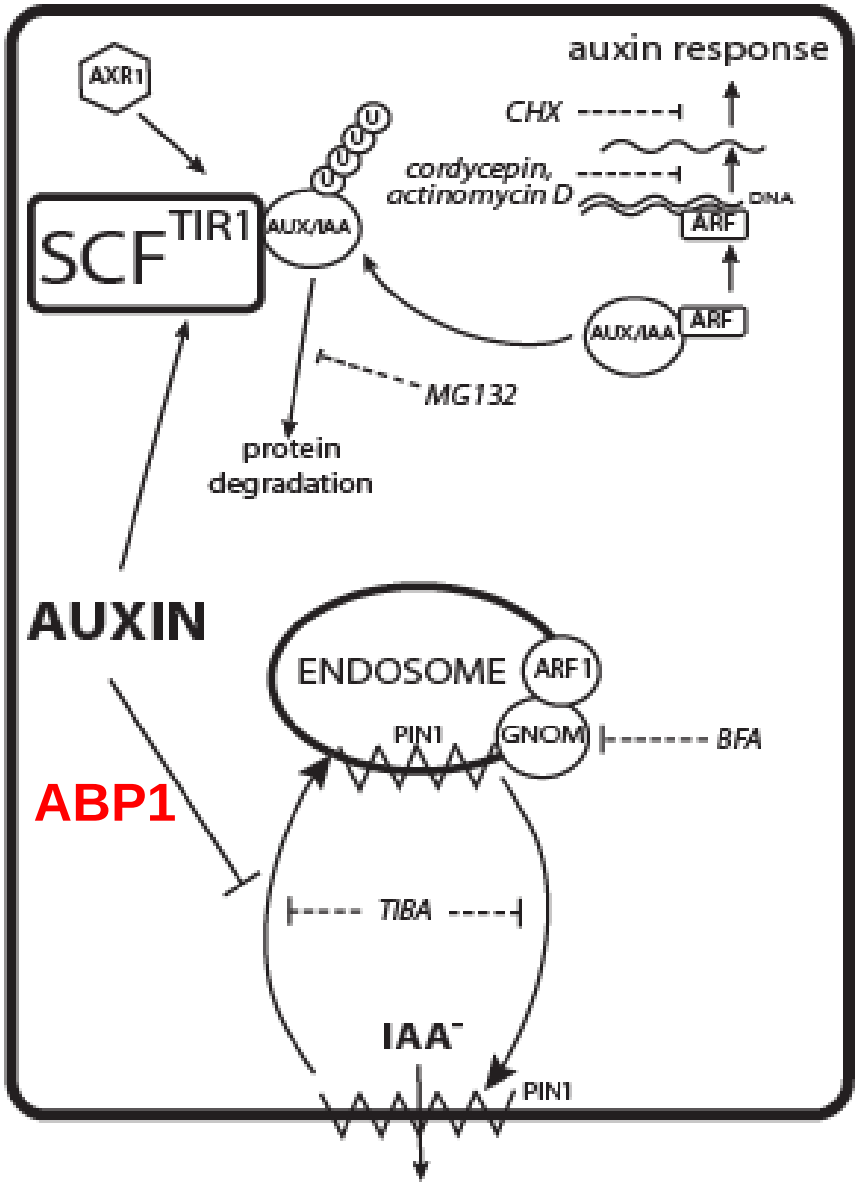
ABP1 mediates auxin effect on clathrin



Robert et al., 2010

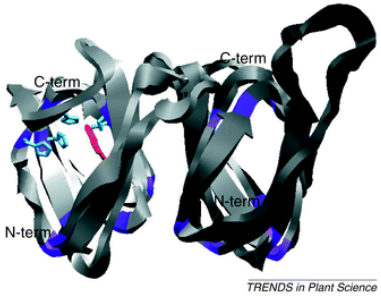
Auxin Signaling for Endocytosis

Zhenbiao Yang
Riverside



Auxin

ABP1

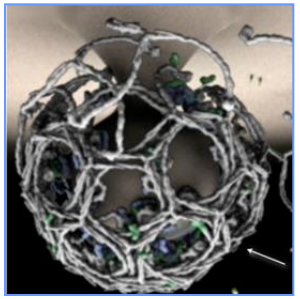


TMK

?

ROP

?

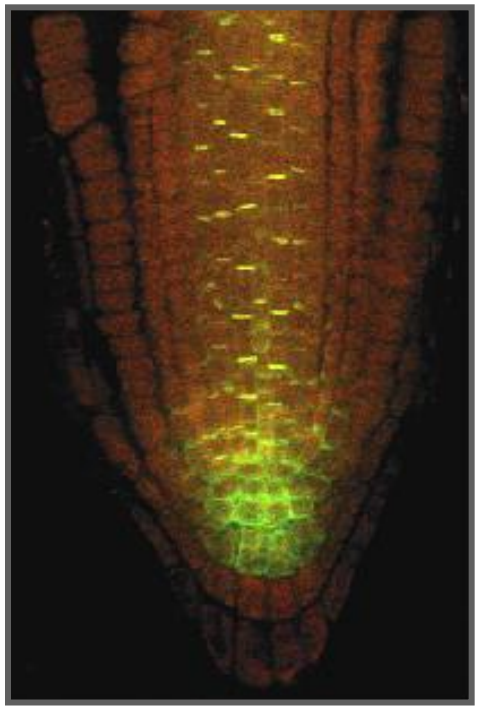


Clathrin

PIN endocytosis

Robert et al., 2010; Xu et al., 2012

Mutant Screen for Components of PIN Polarity and Cycling



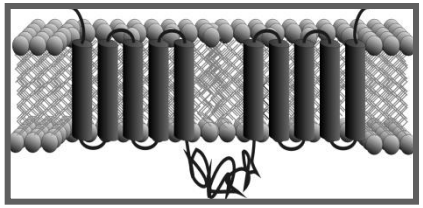
PIN:GFP

EMS mutagenesis. Screening for polarity and cycling defects.

mutant lines

intragenic

extragenic

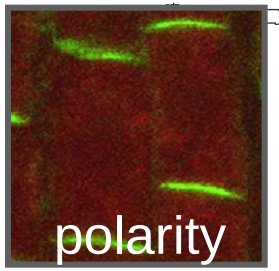


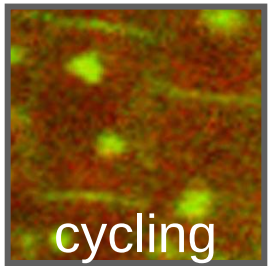
sequencing

cloning

important residues

novel genes

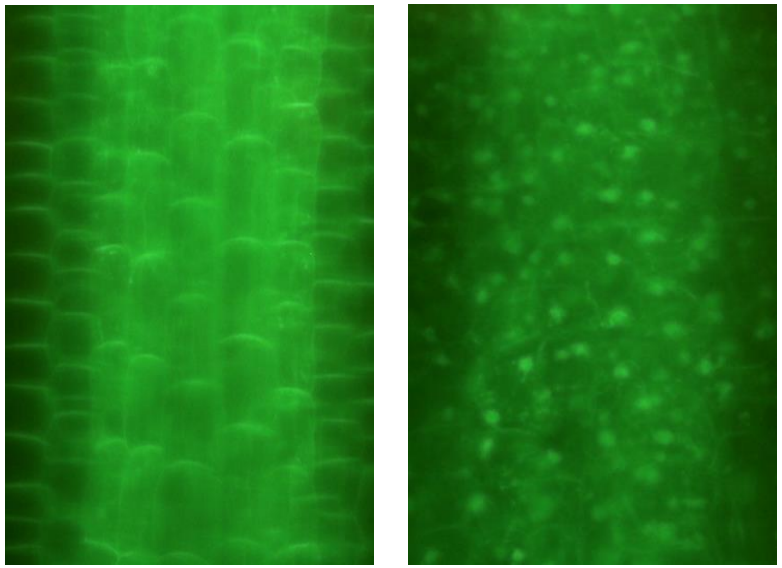




“Cell Biological” Mutant Screens in Progress:

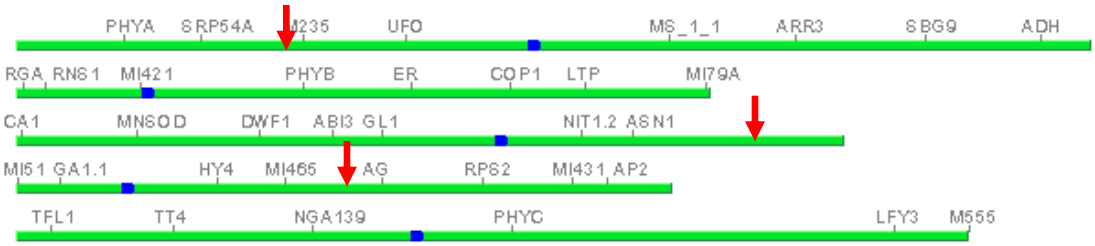
Auxin effect on endocytosis: 3 confirmed mutants

30´ NAA 30 µM/90´BFA 50 µM



wt mutant

Auxin-resistant BFA patches mutants



unpublished

Novel Pathway for Auxin Signaling

Auxin inhibits endocytosis including internalization of PIN proteins

This is mechanism by which auxin stabilizes PINs at the cell surface thus stimulating auxin efflux.

This auxin effect involves novel, genetically tractable auxin pathway