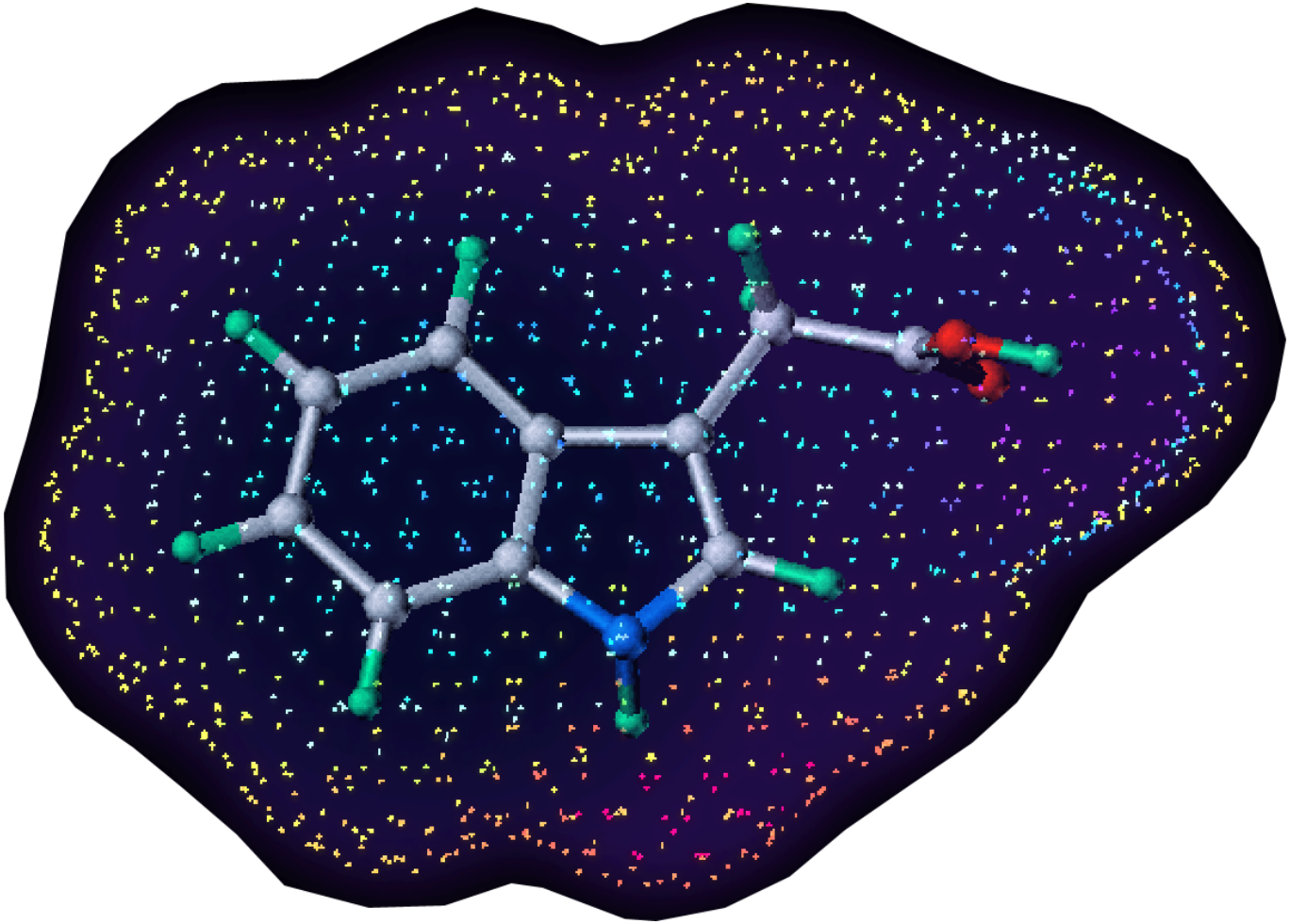
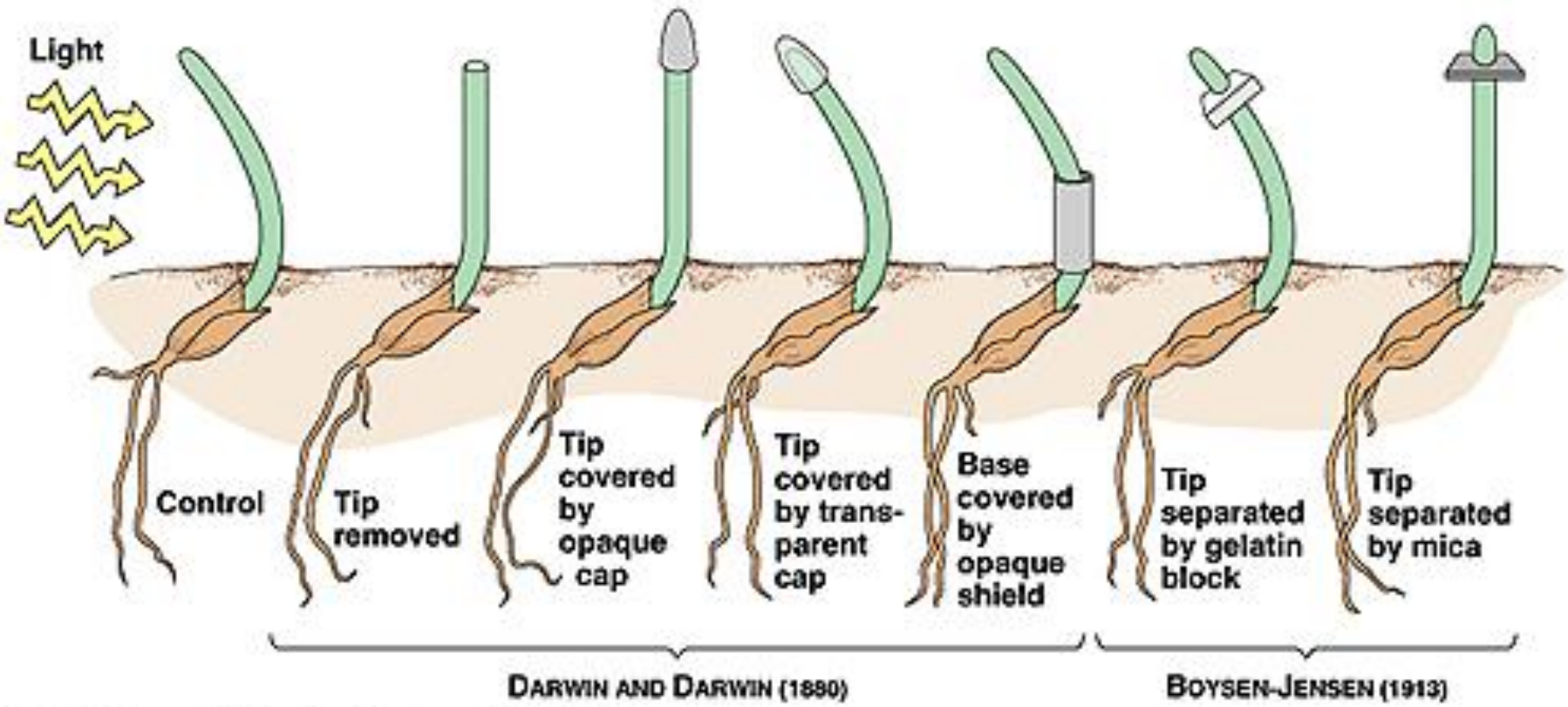
Auxin Signaling and Transport

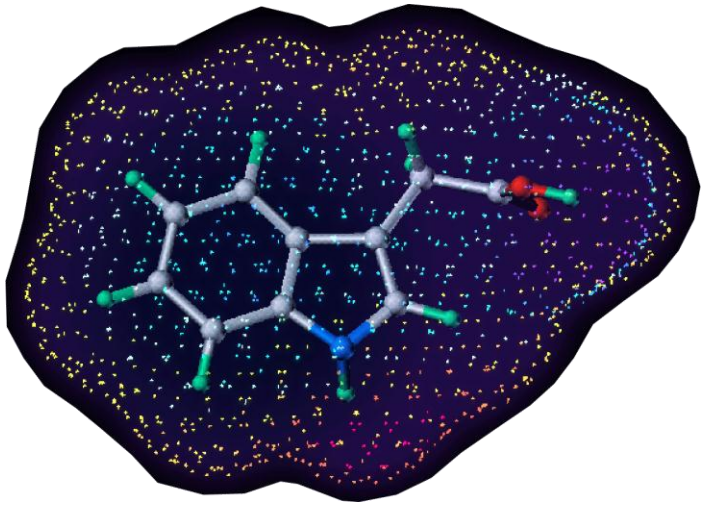


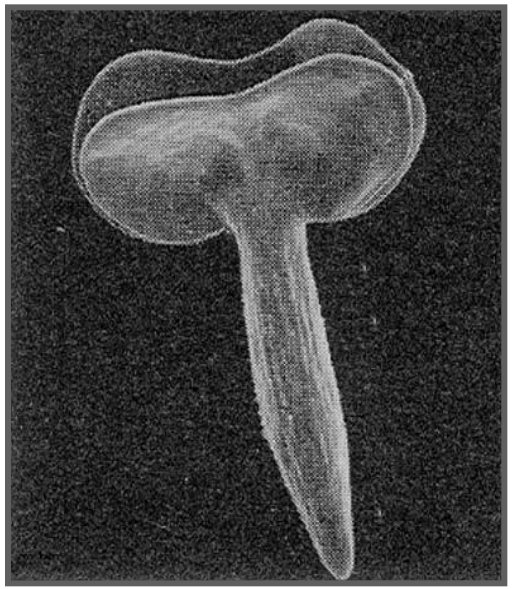
Discovery of the First Plant Signaling Molecule – Auxin and its Transport

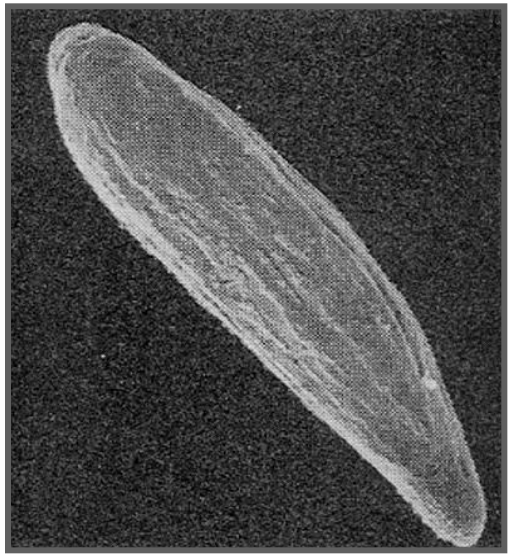


AUXIN

mediates

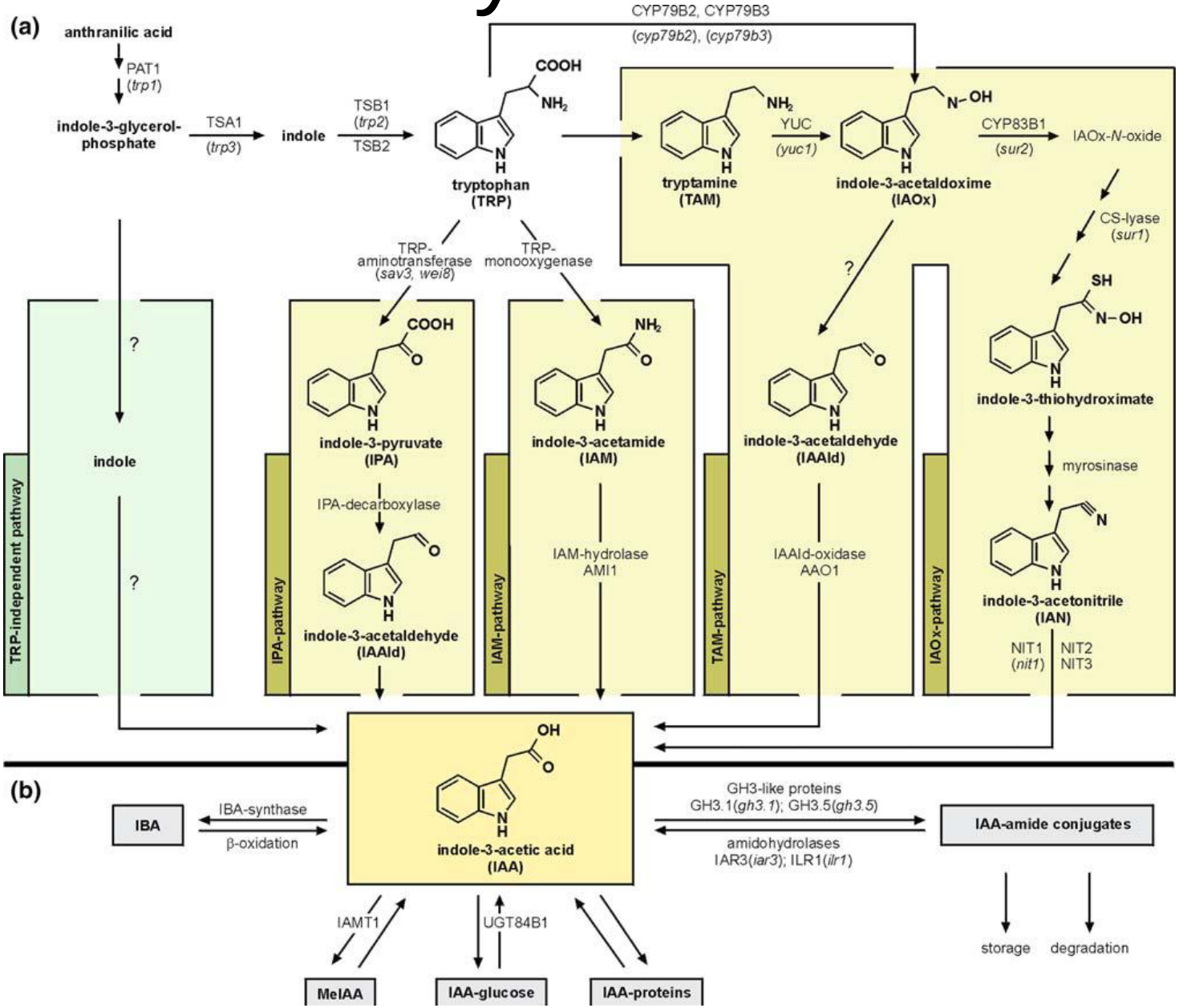




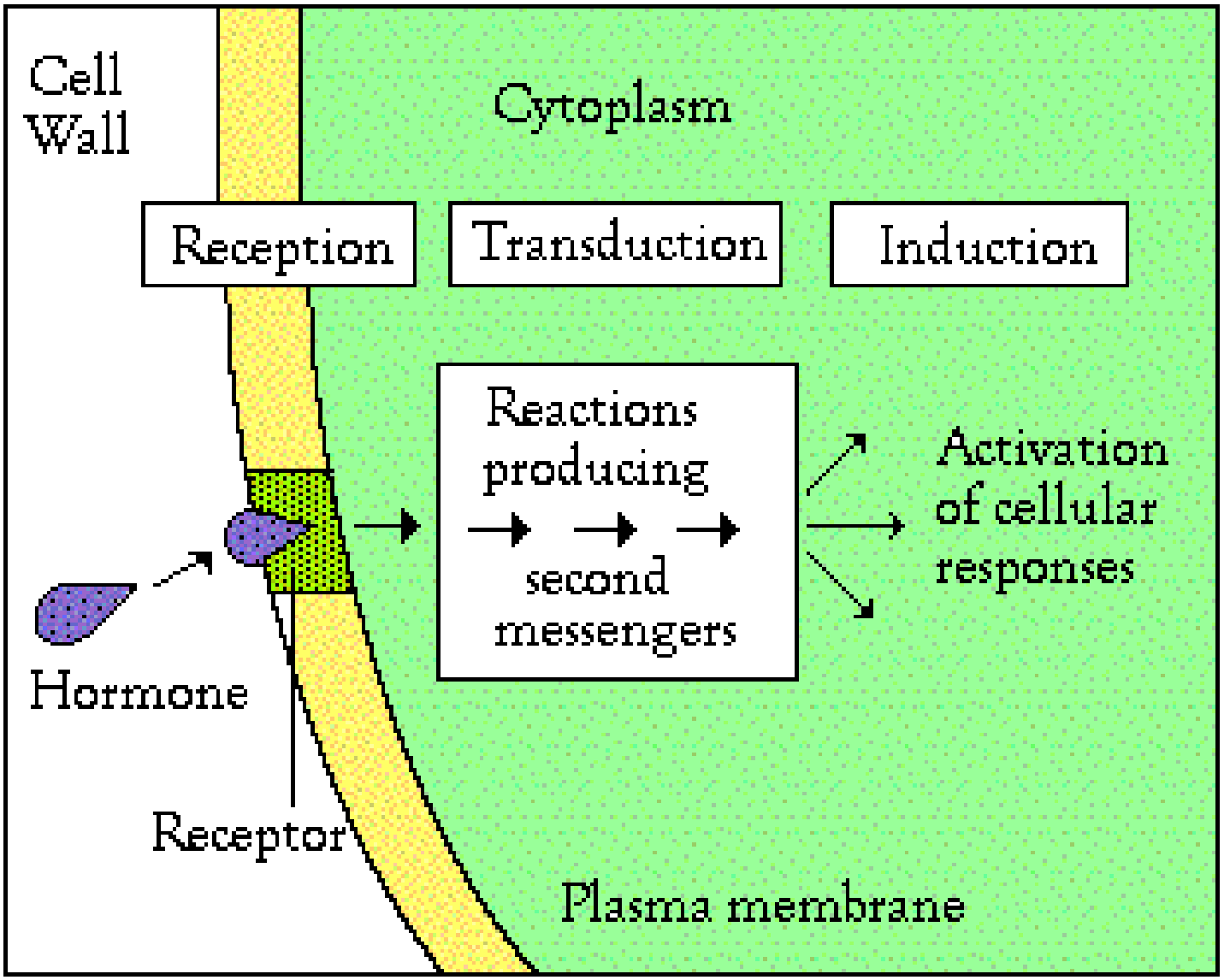


- **Embryo development**
- **Organ initiation and positioning**
- **Vascular tissue differentiation**
- **Shoot and root elongation**
- **Growth responses to light and gravity**
- **Apical hook formation**

Auxin biosynthesis



Signal Transduction



Biochemical Approach to Identify Auxin Receptor

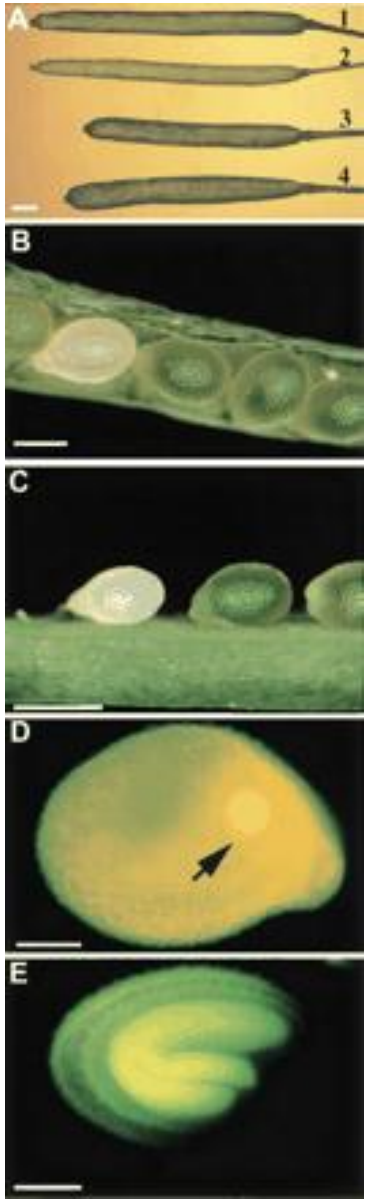
Isolation of auxin binding proteins

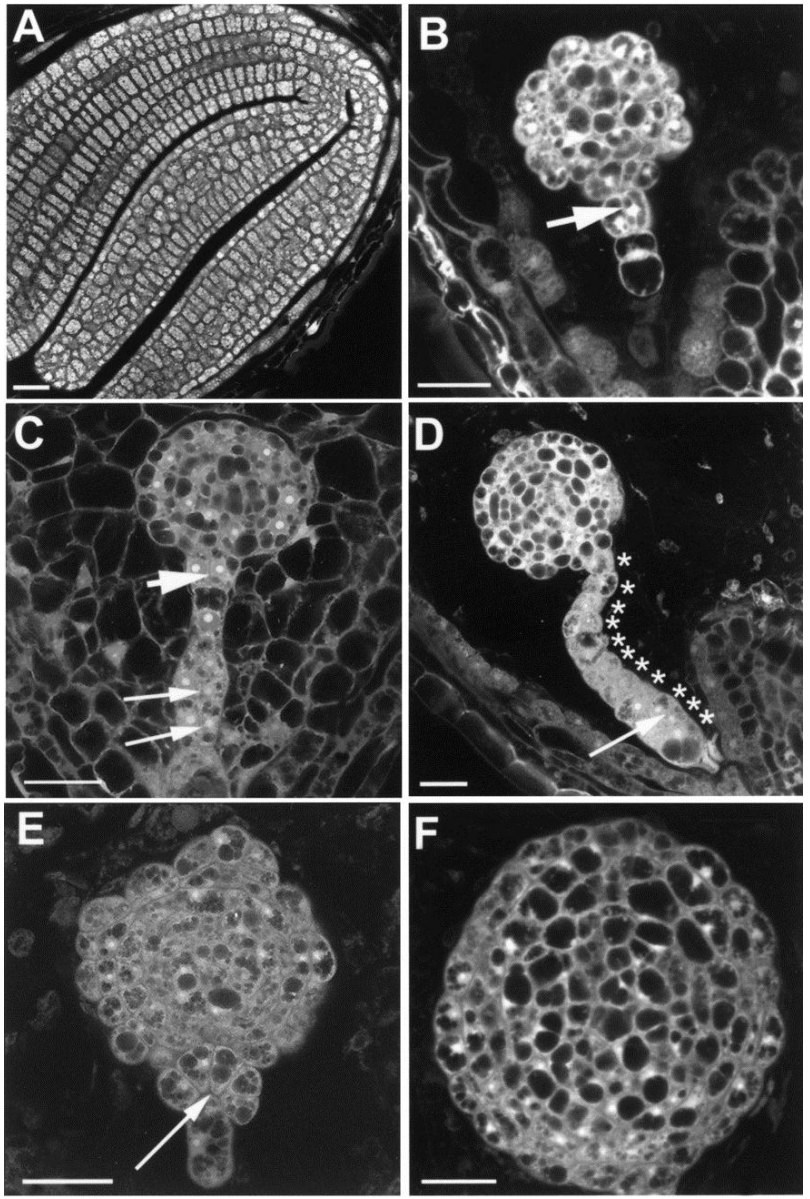
- **Azidolabeling**
- **Affinity chromatography**

Protein sequencing, cDNA screening, gene identification

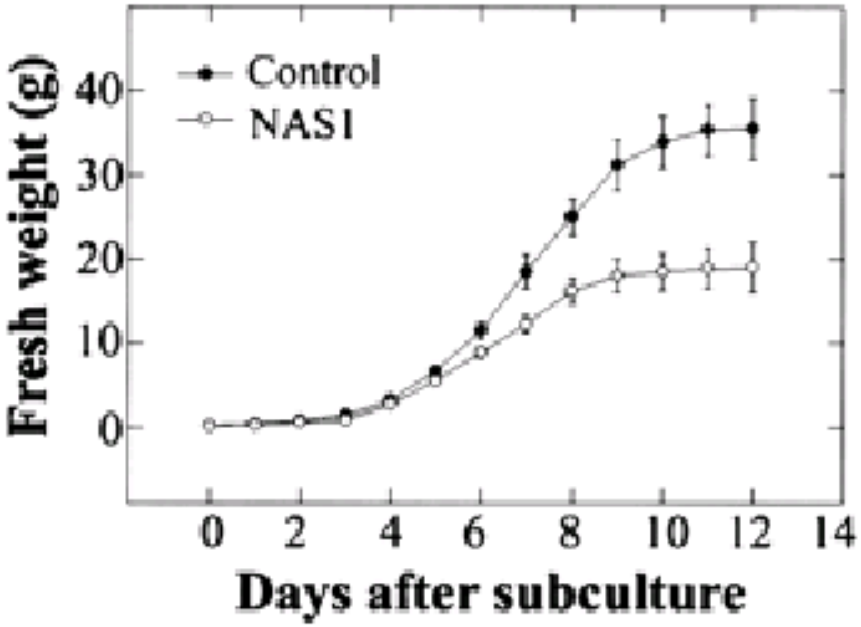
=> Auxin Binding Protein (ABP1)

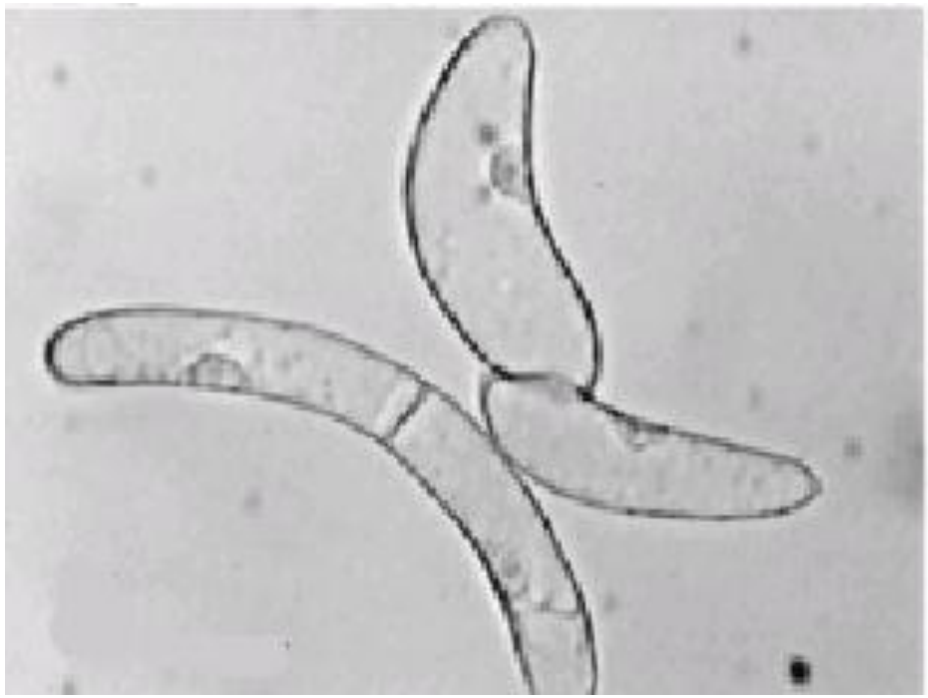
Reverse Genetic – Embryo Lethal *abp1* Mutant



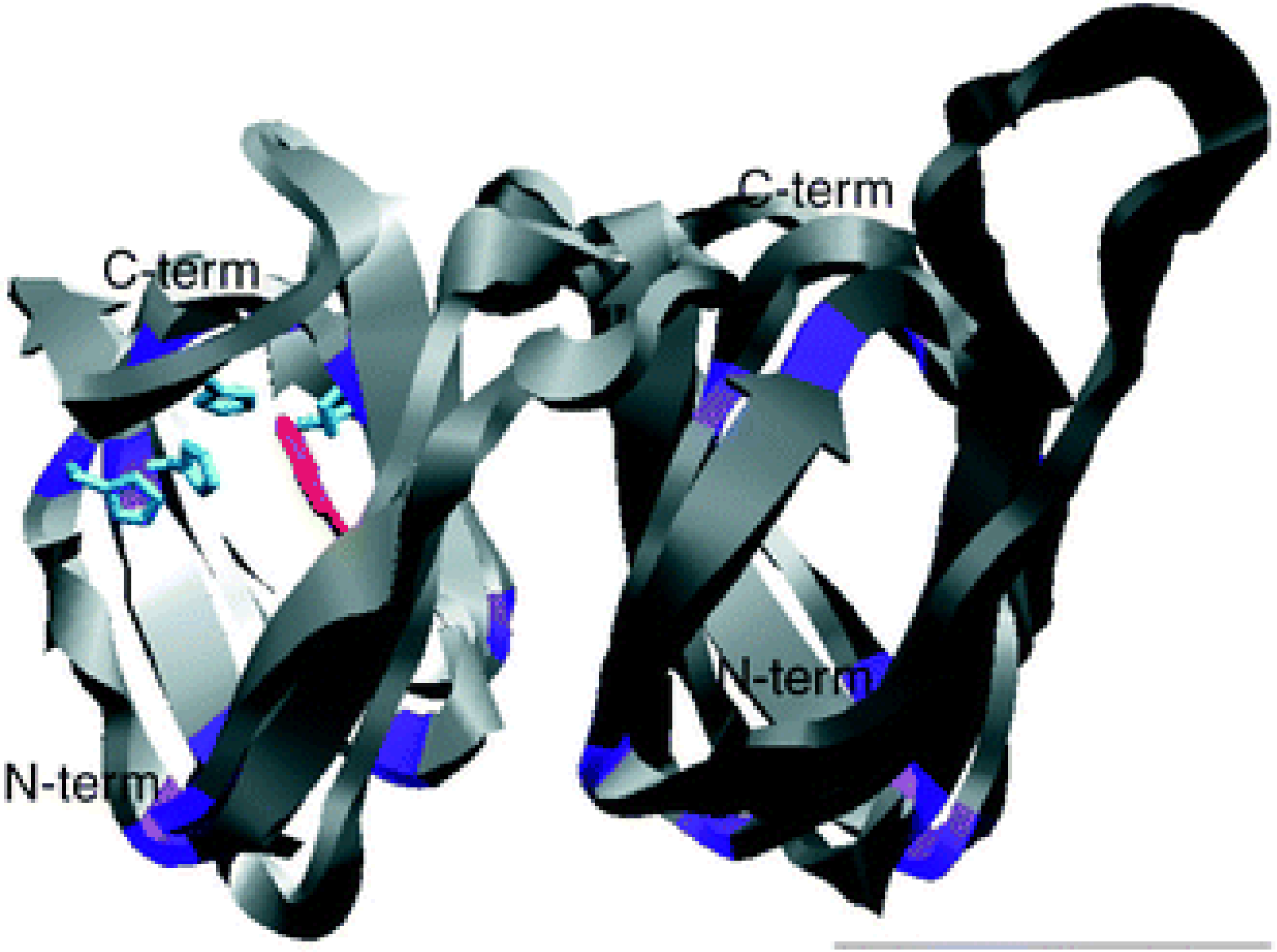


ABP1 Antisense BY-2 Cells Display Defects in Auxin Dependent Cell Elongation

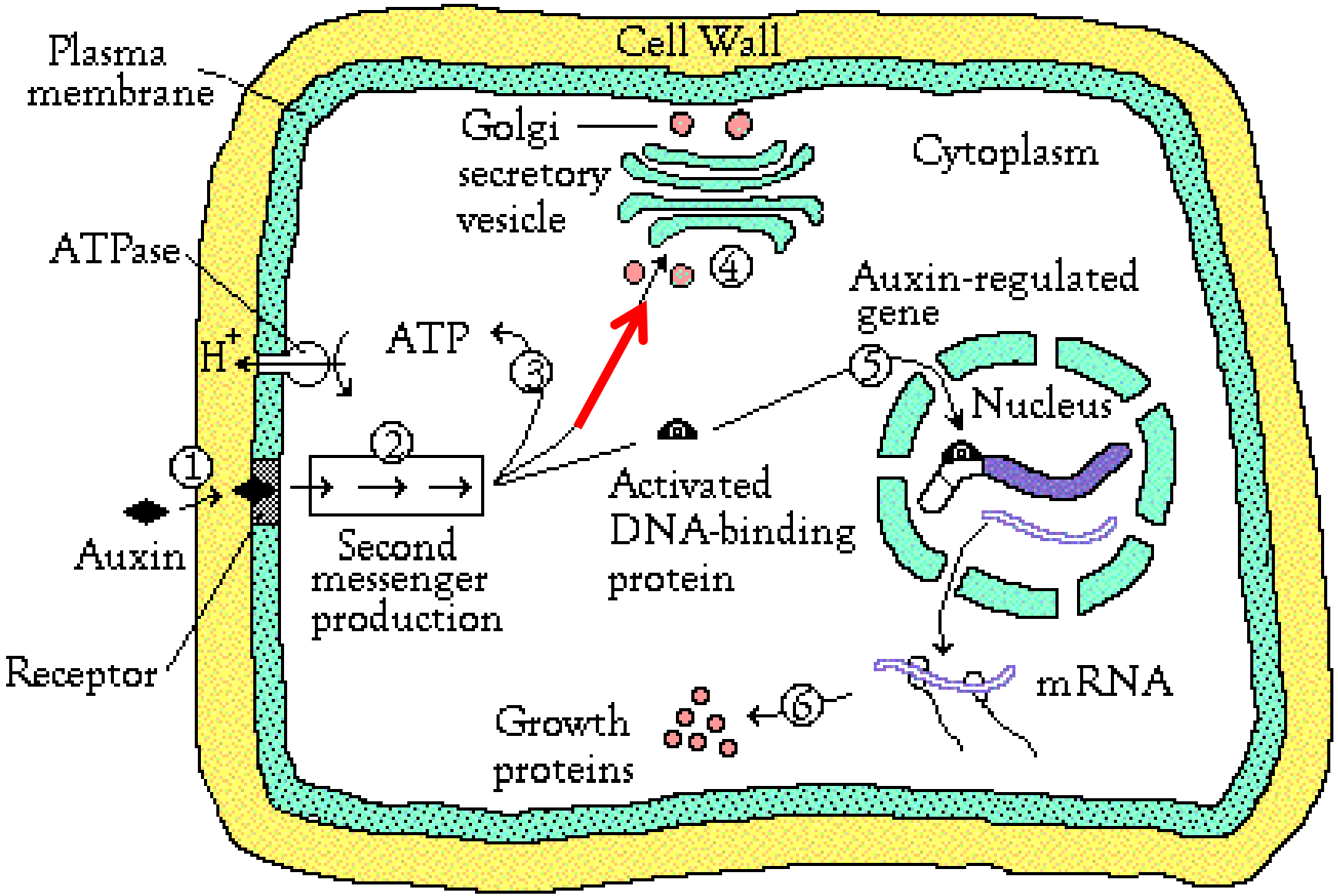




ABP1 – Structure



Optimistic Model for ABP1 Action



Molecular Biology Approach to Elucidate Auxin Signaling

Does auxin regulate gene expression?

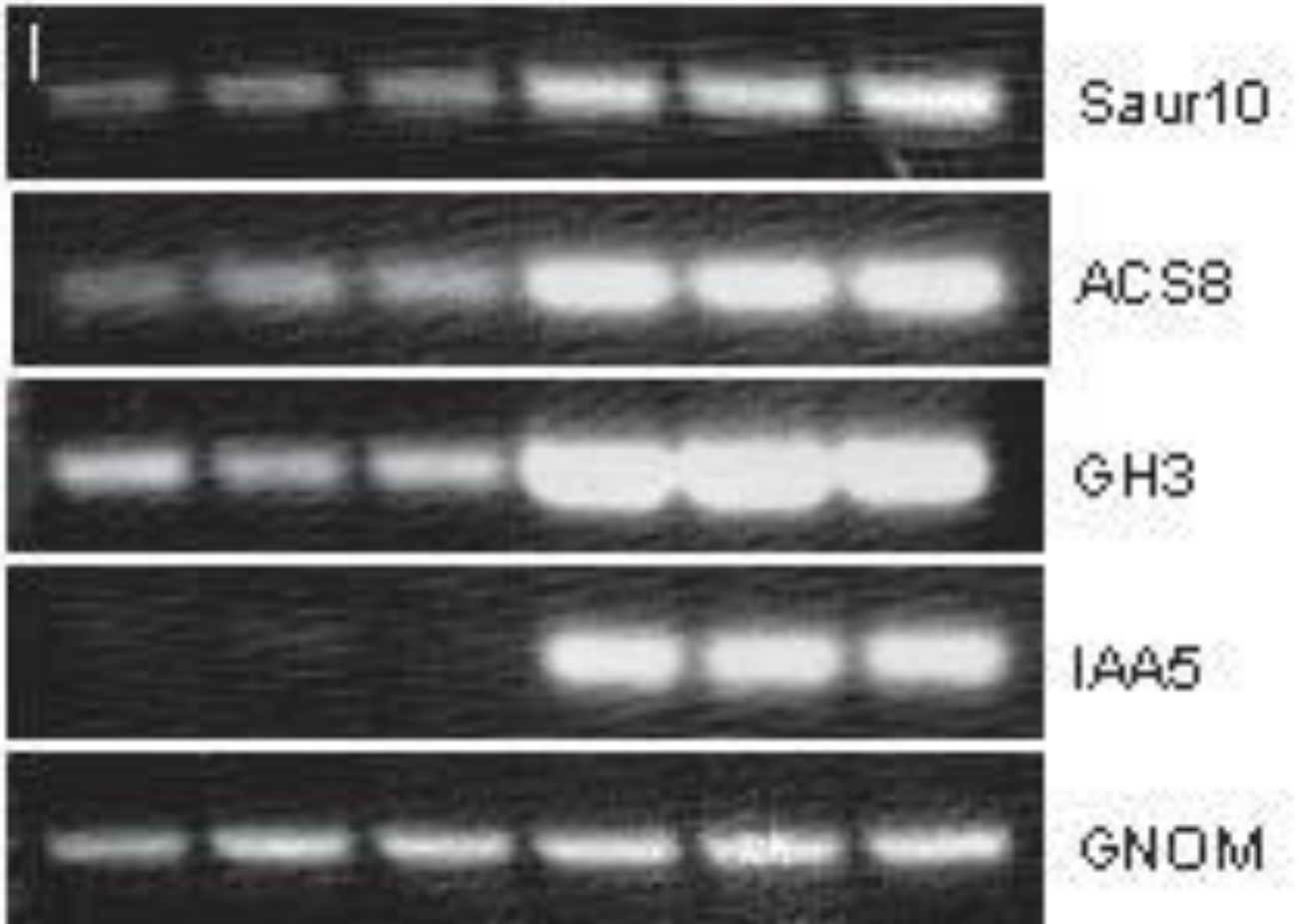
- **Rapidly upregulated mRNAs (*GH3, SAUR, AUX/IAA* genes)**

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

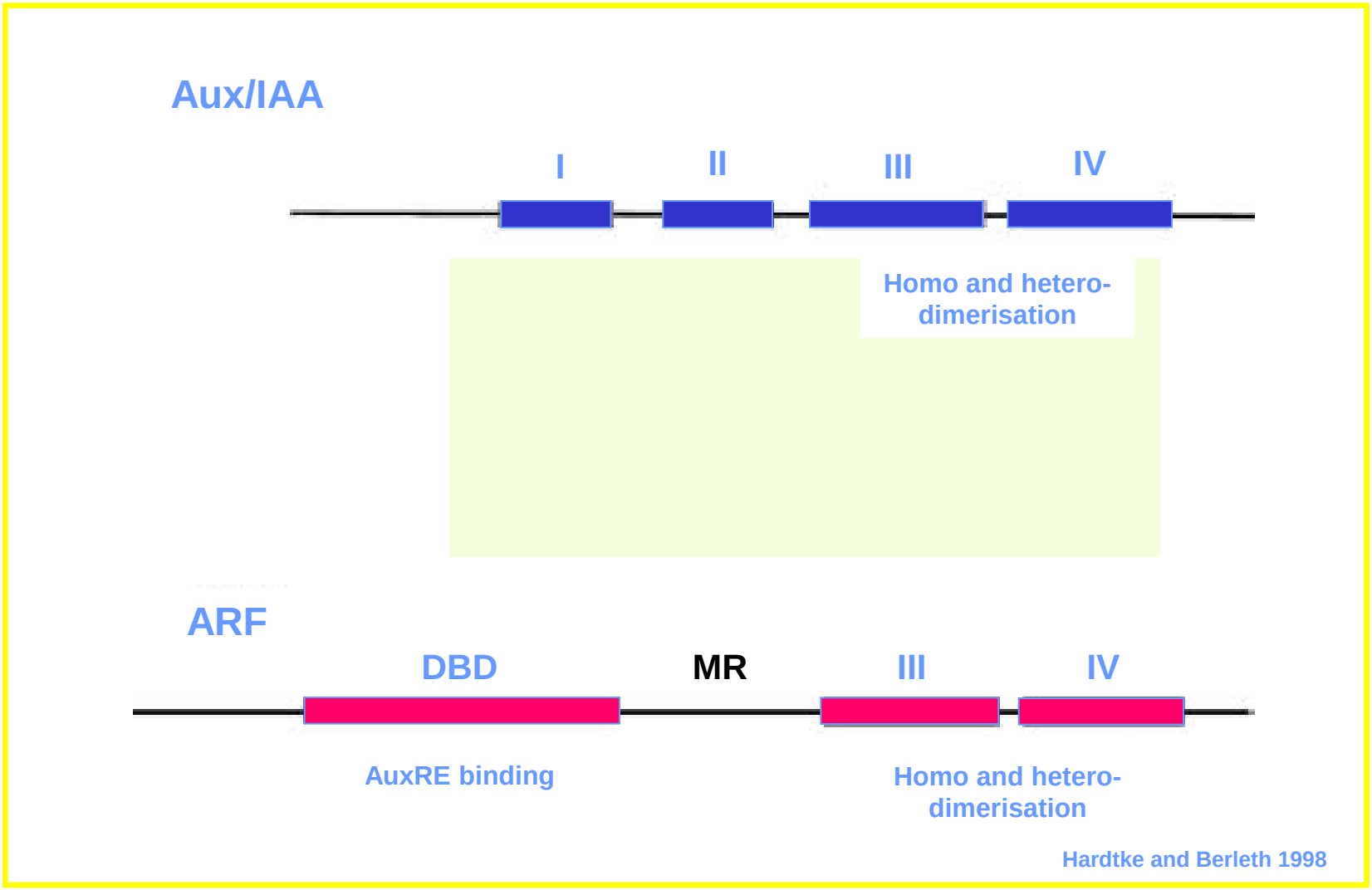
- **Two hybrid => AUX/IAAs interact with ARFs**

Molecular Biology Approach to Elucidate Auxin Signaling

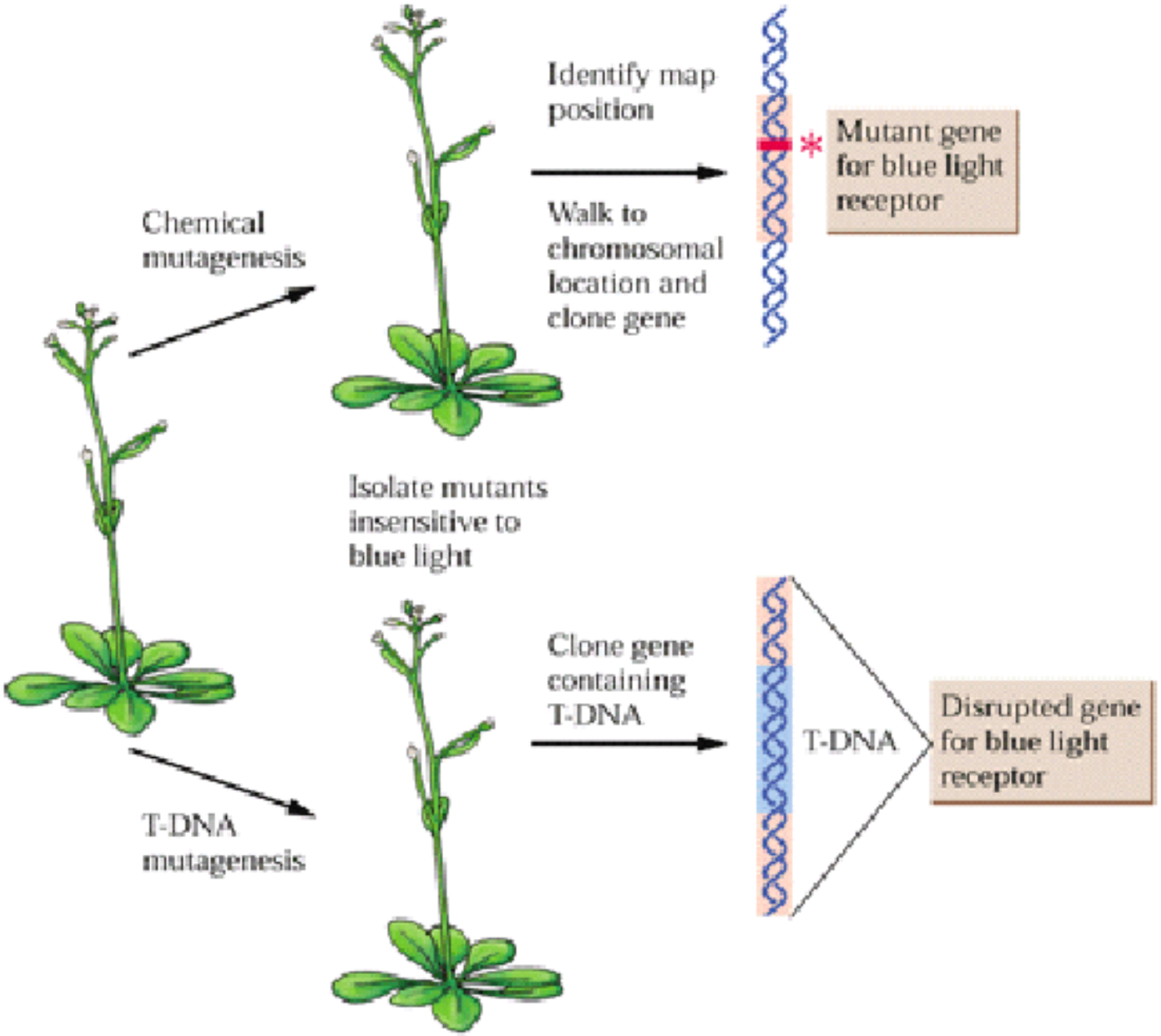
RT-PCR



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*

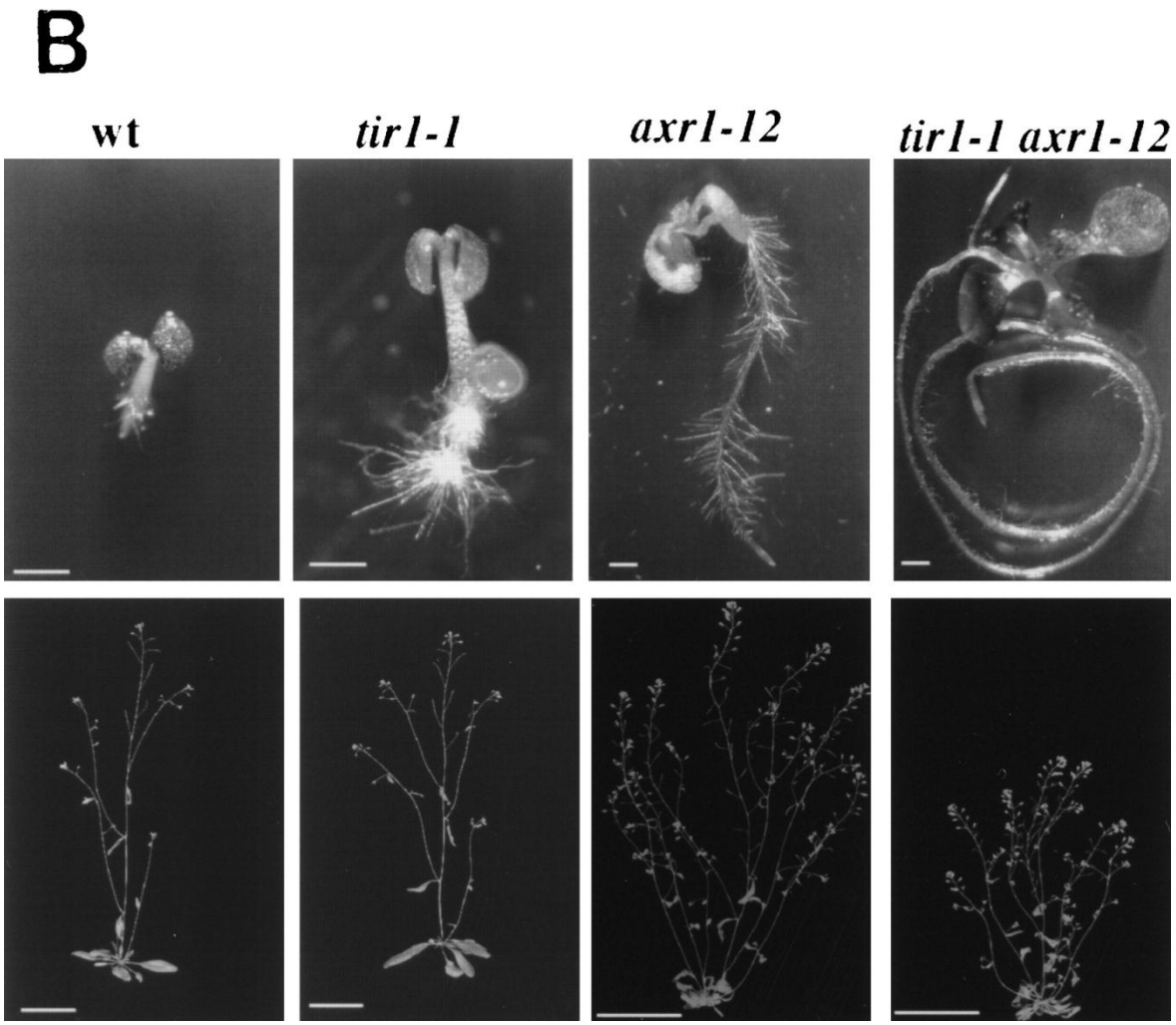
Morphological mutants (*monopteros, bodenlos,* etc.)

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

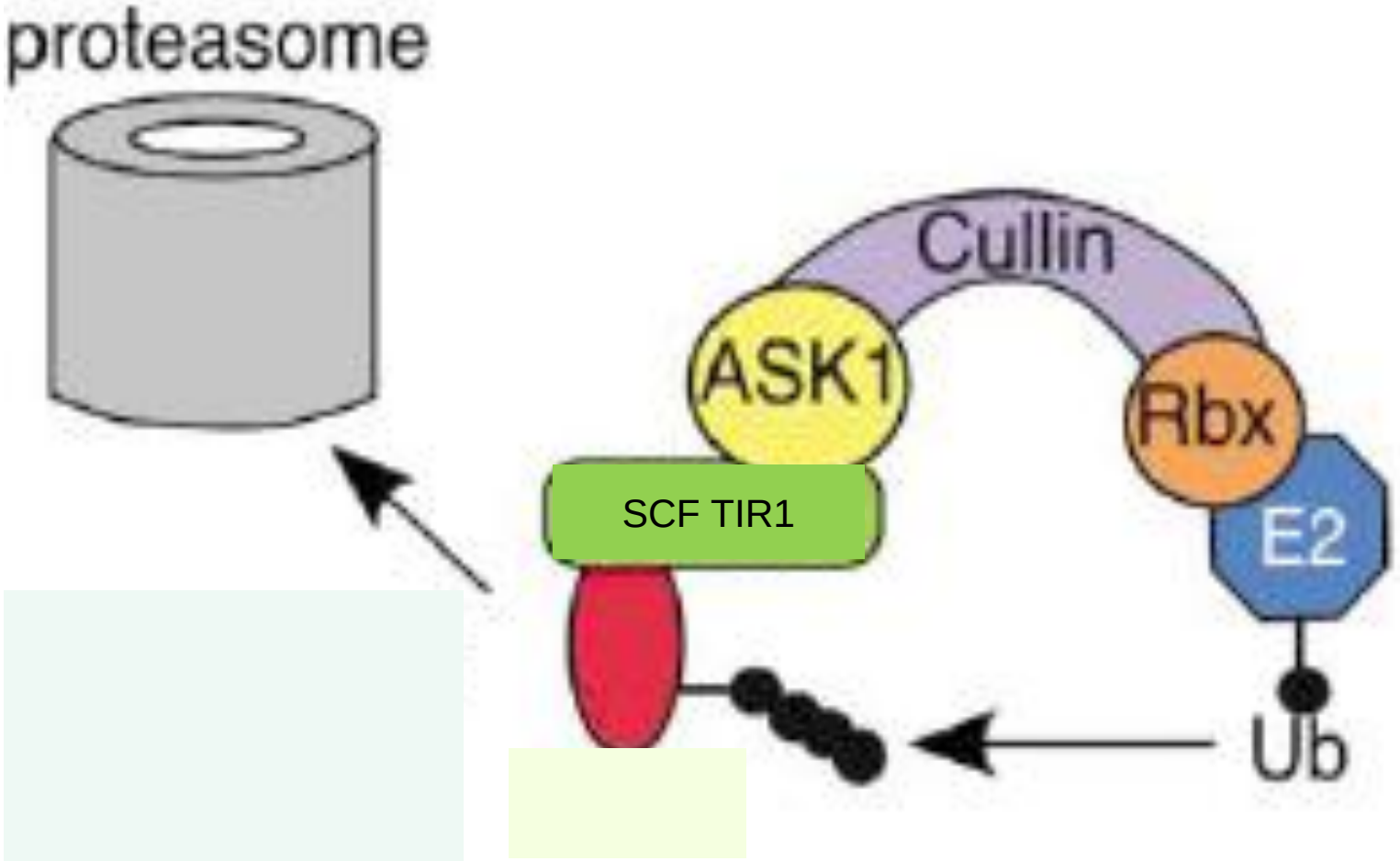
None of the identified gene looks like a receptor

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

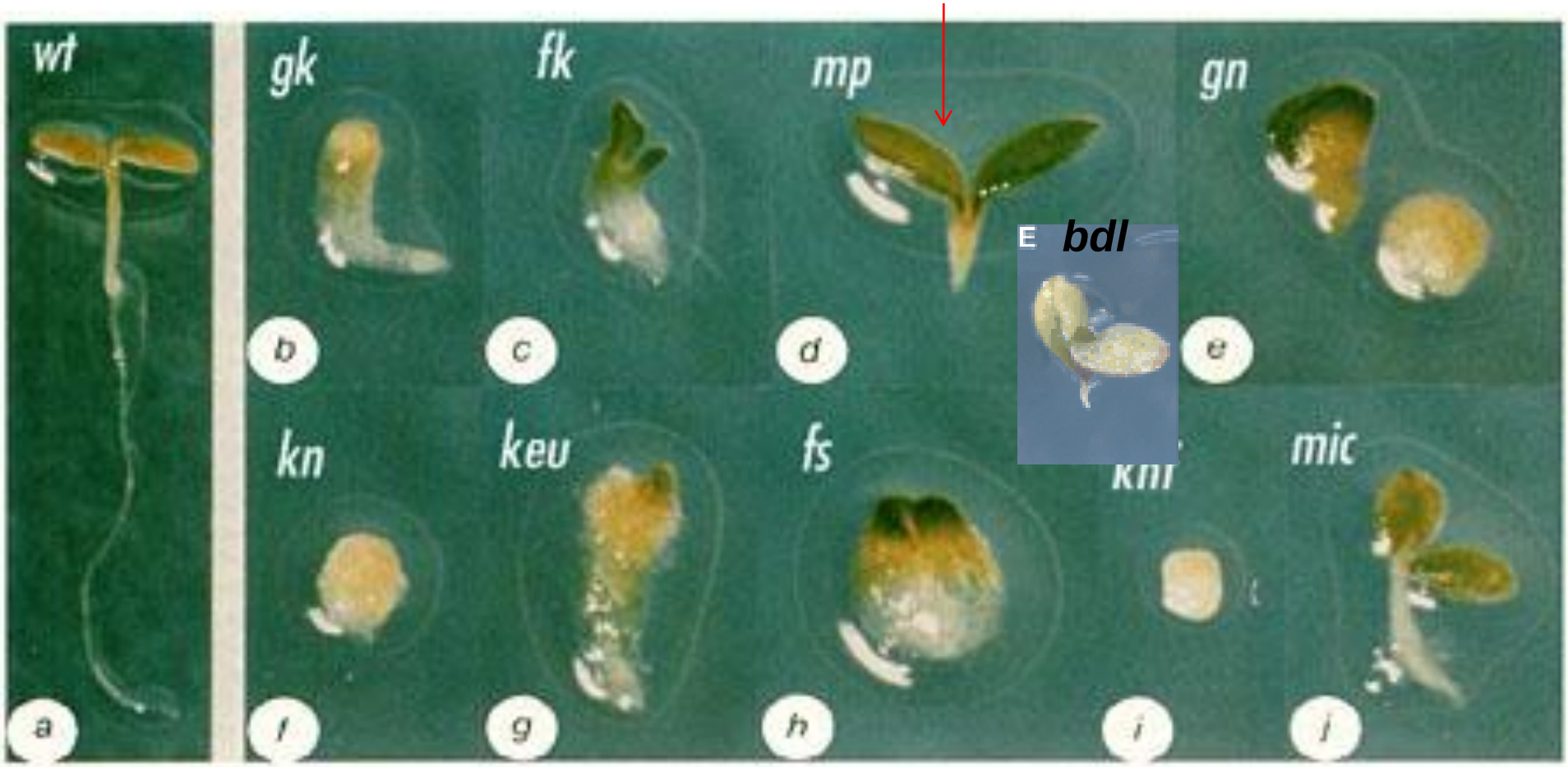
Auxin Transport inhibitor response (tir):



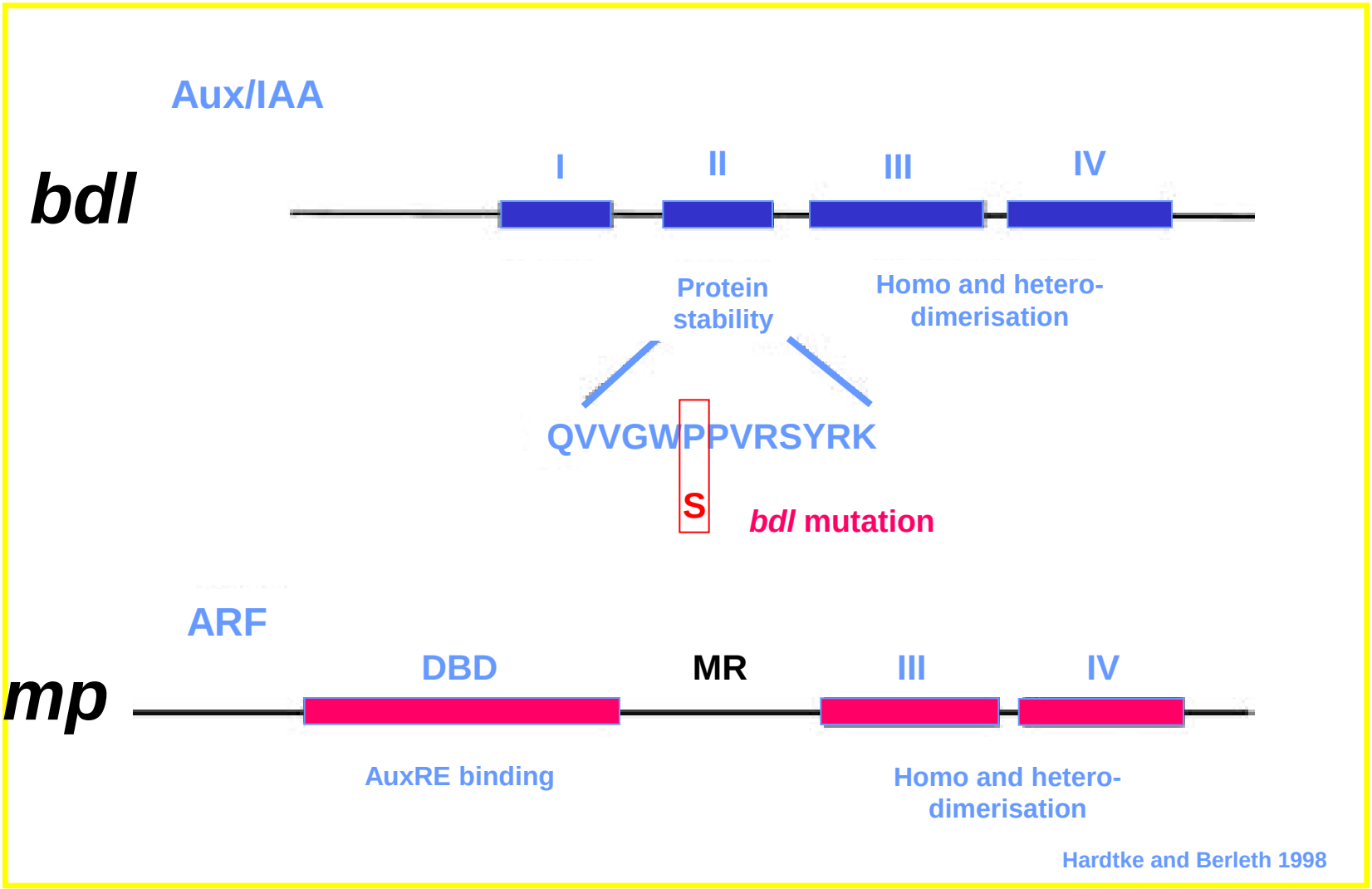
Subunits of ubiquitin ligase

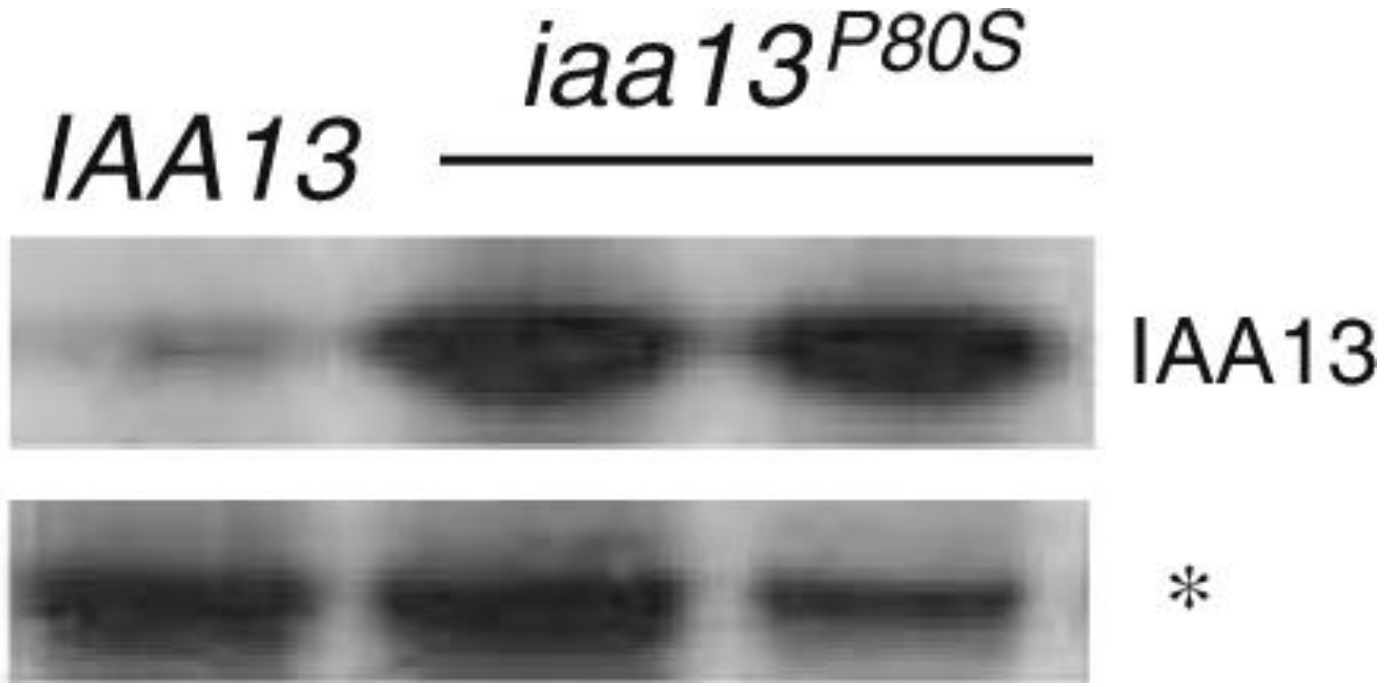

proteasome
Cullin
ASK1
Rbx
SCF TIR1
E2
Ub

Mutant Screen at Seedling Level

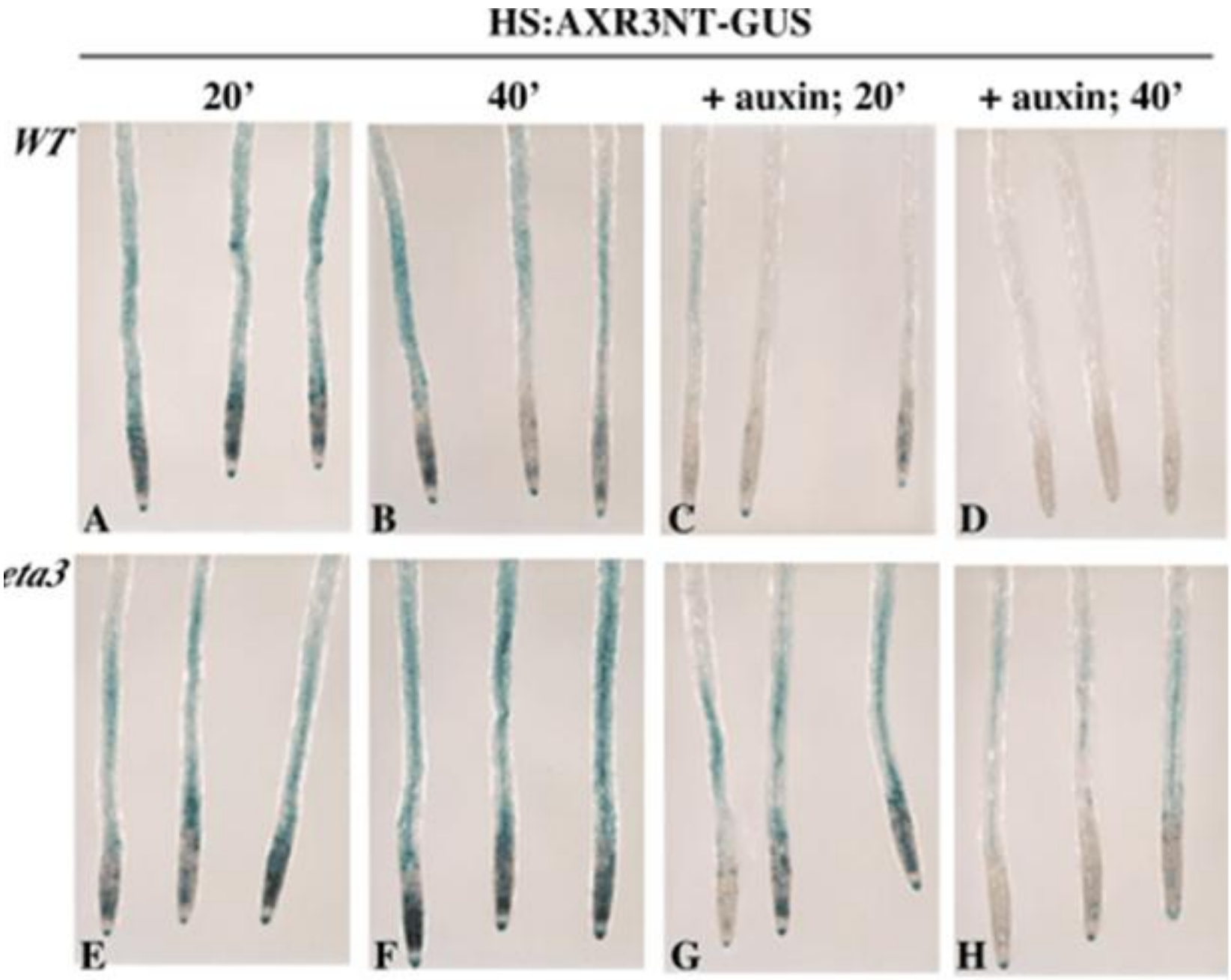


Some ARFs are **Activators**, and AUX/IAAs **Repressors** of Auxin Response

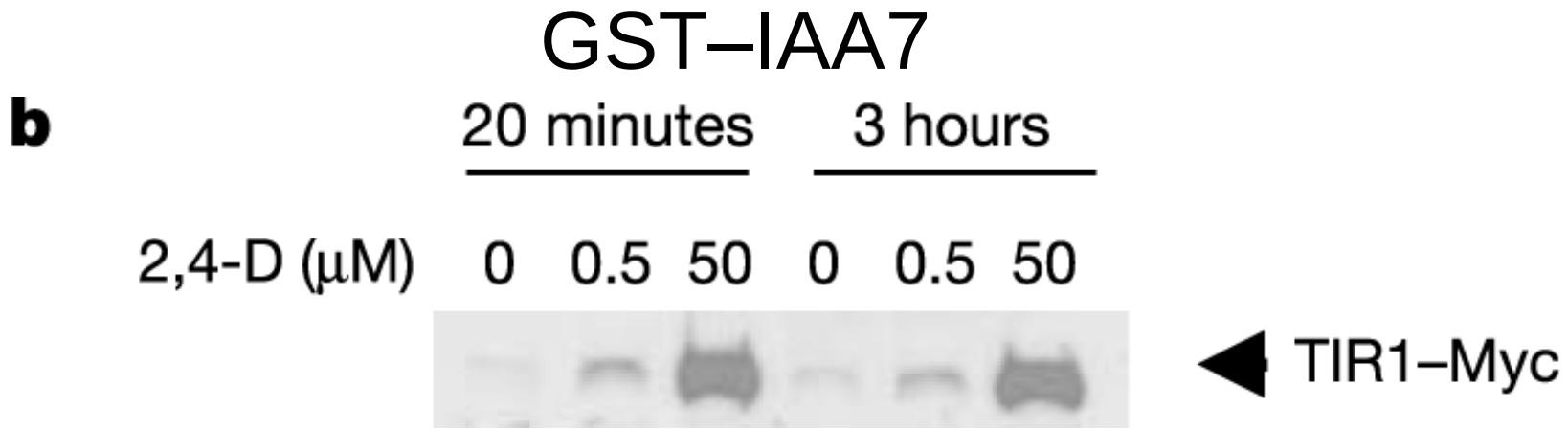



IAA13
iaa13P80S
IAA13
*

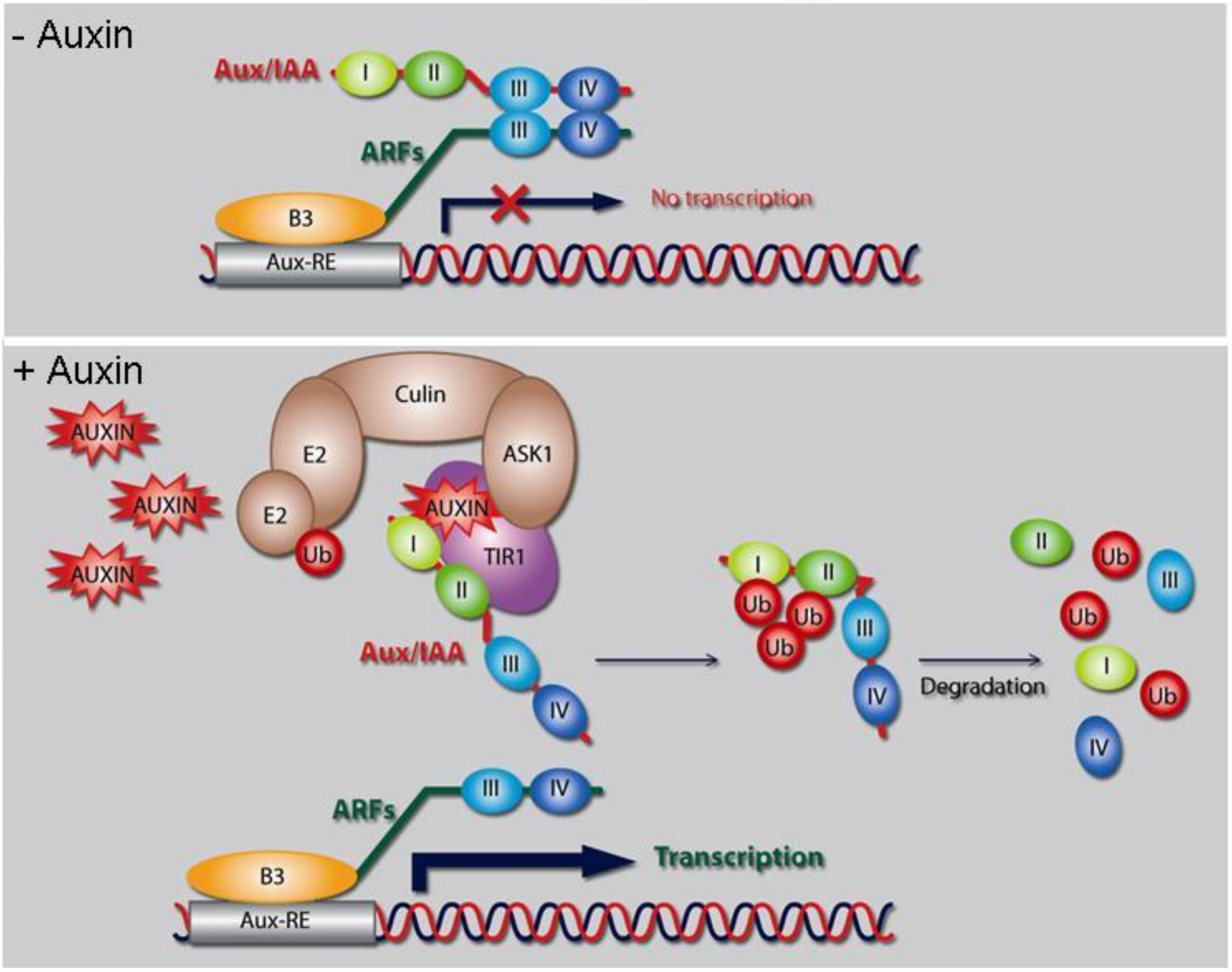
AUX/IAAs are stabilized in enhancer TIR1 mutant



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*)

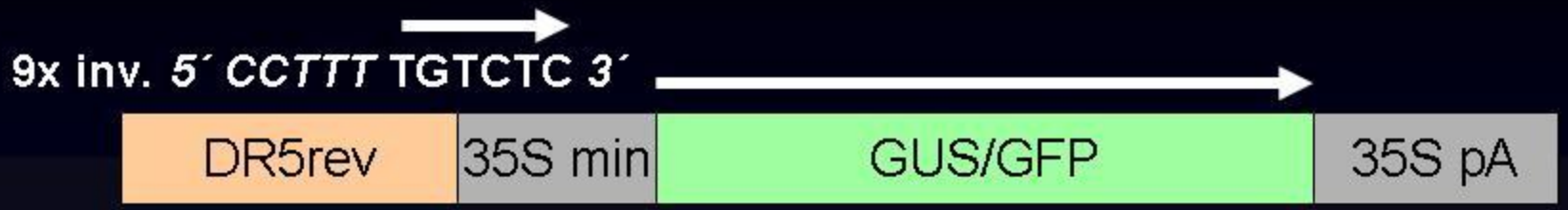
Molecular approach – auxin regulates expression

ARE in promotors of auxin regulated genes

ARF transcription factors binds to ARE

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

DR5 Auxin Response Reporter

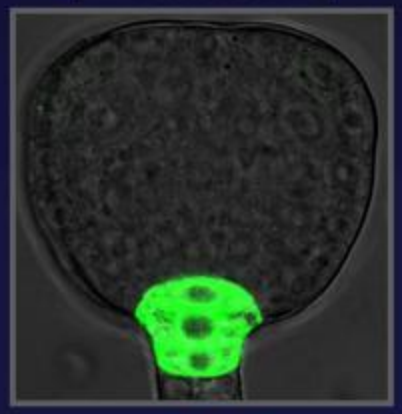


Root

Embryos

DR5

anti-IAA

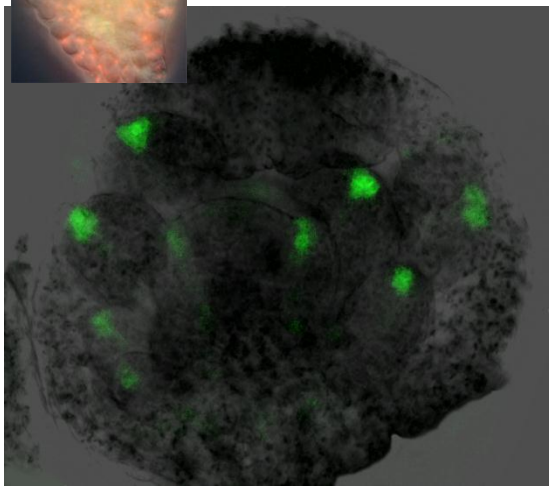


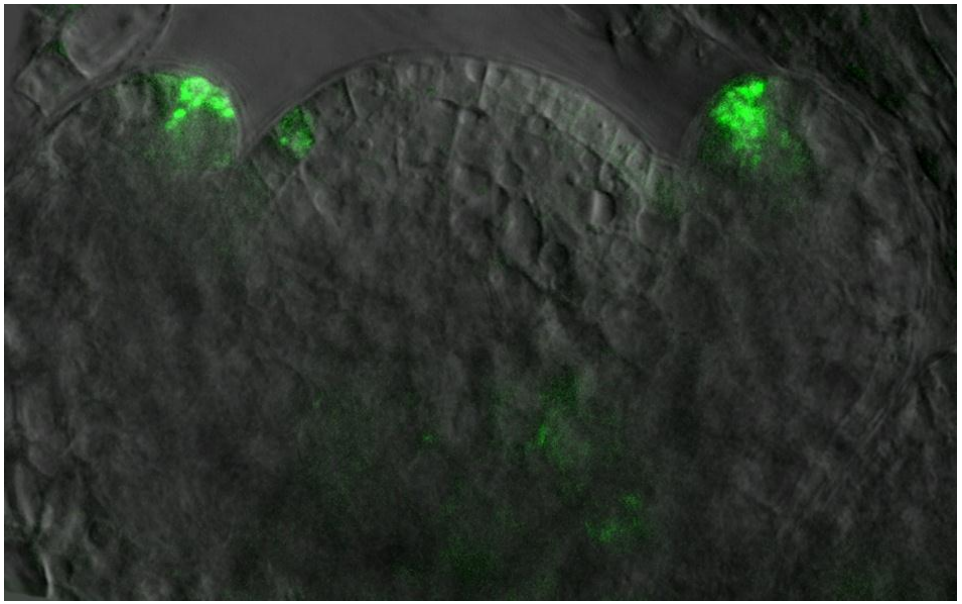
DR5

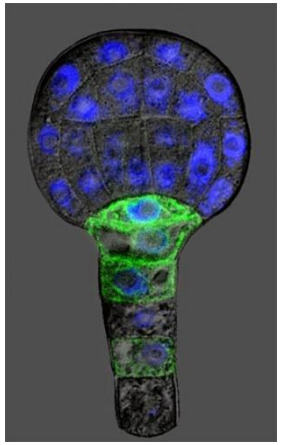
anti-IAA

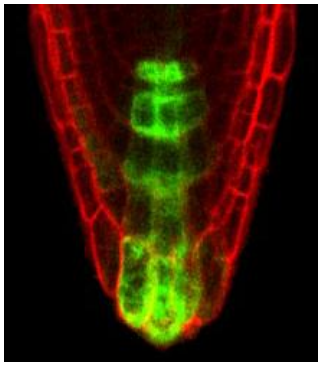
Ulmasov et al. 97; Sabatini et al. 99; Benková et al. 03; Friml et al. 03

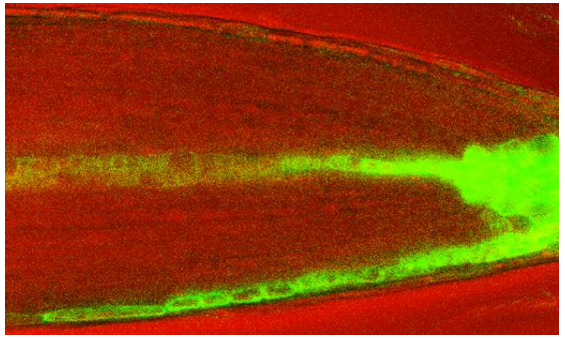
Local Auxin Gradients in Plant Development



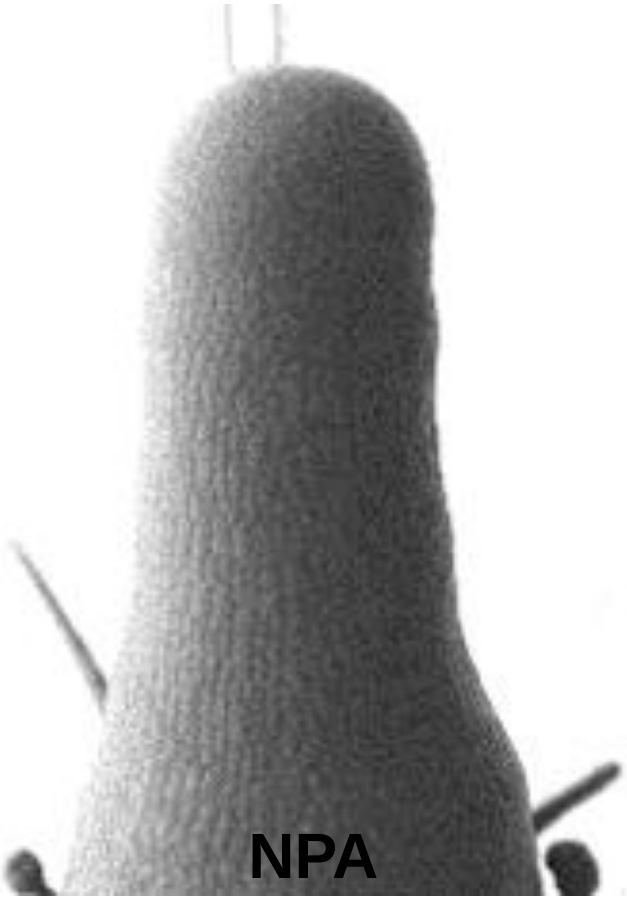


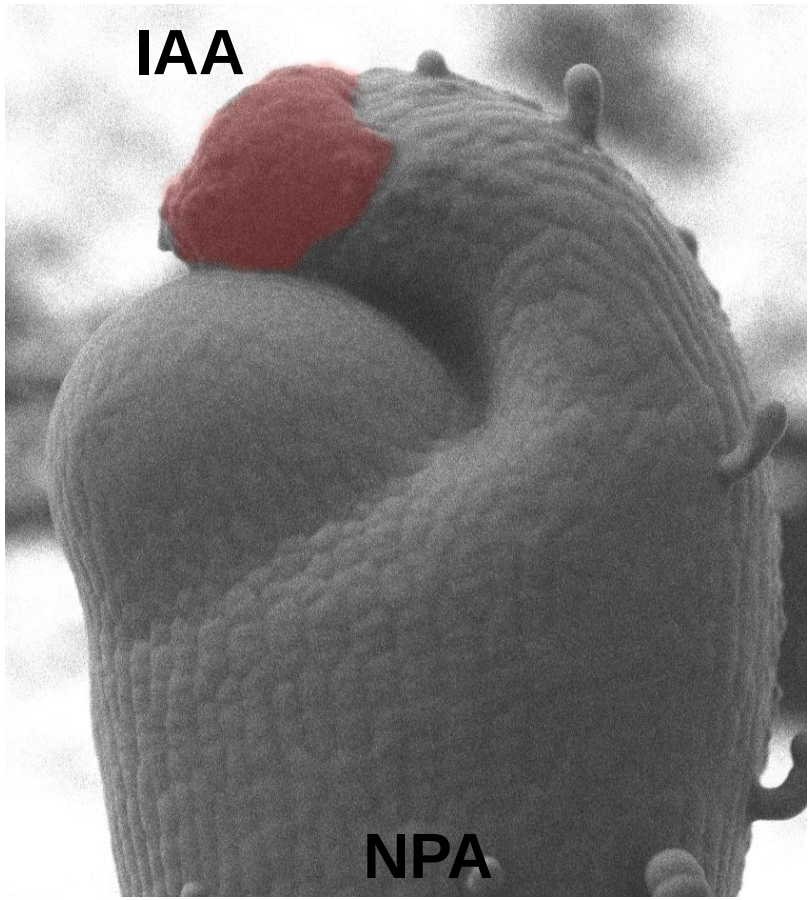






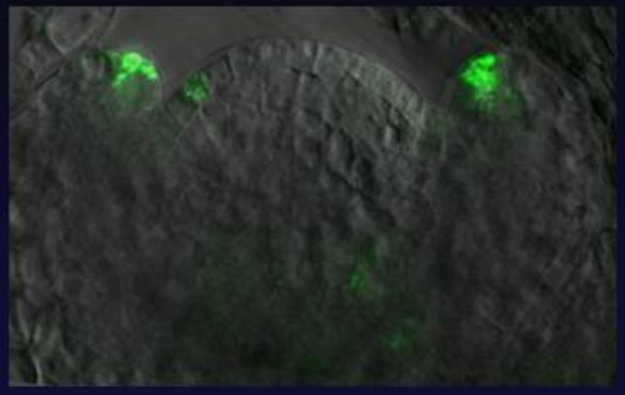
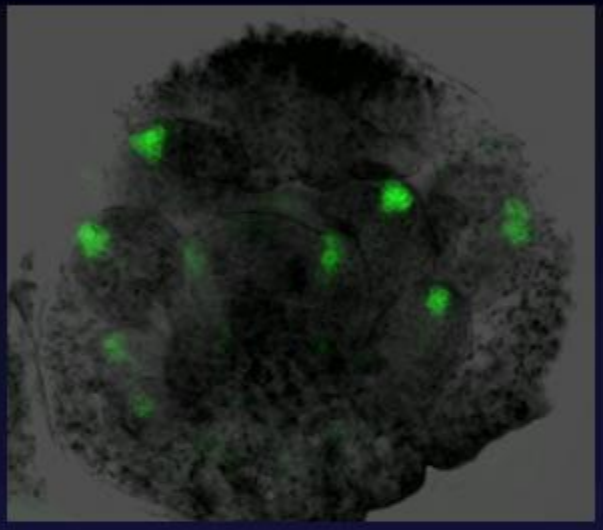
Local Application of Auxin Induces Organ Formation

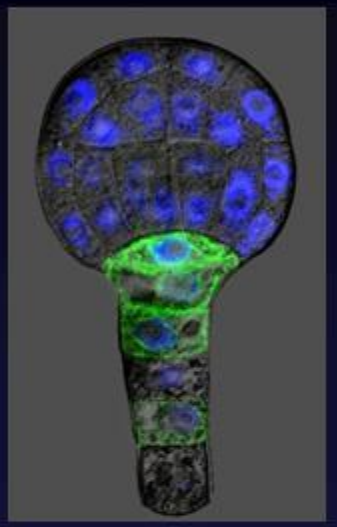
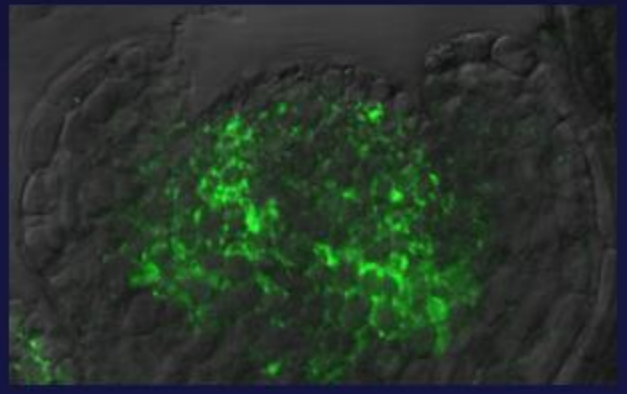
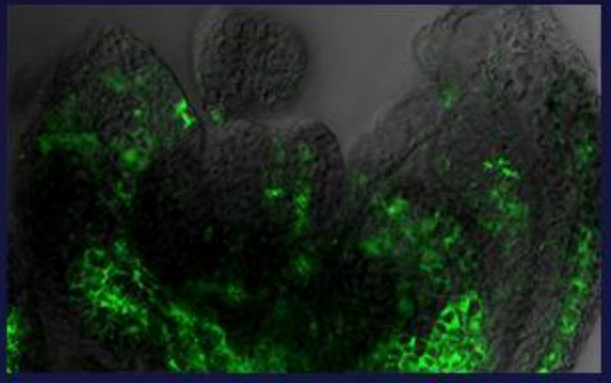


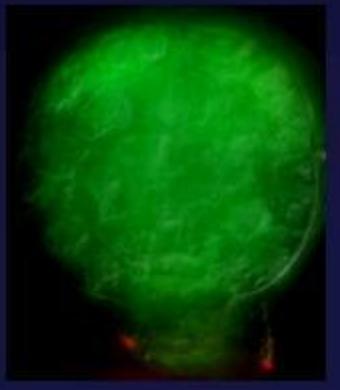


Reinhardt et al. 03

Local Auxin Gradients Require Active Polar Auxin Transport



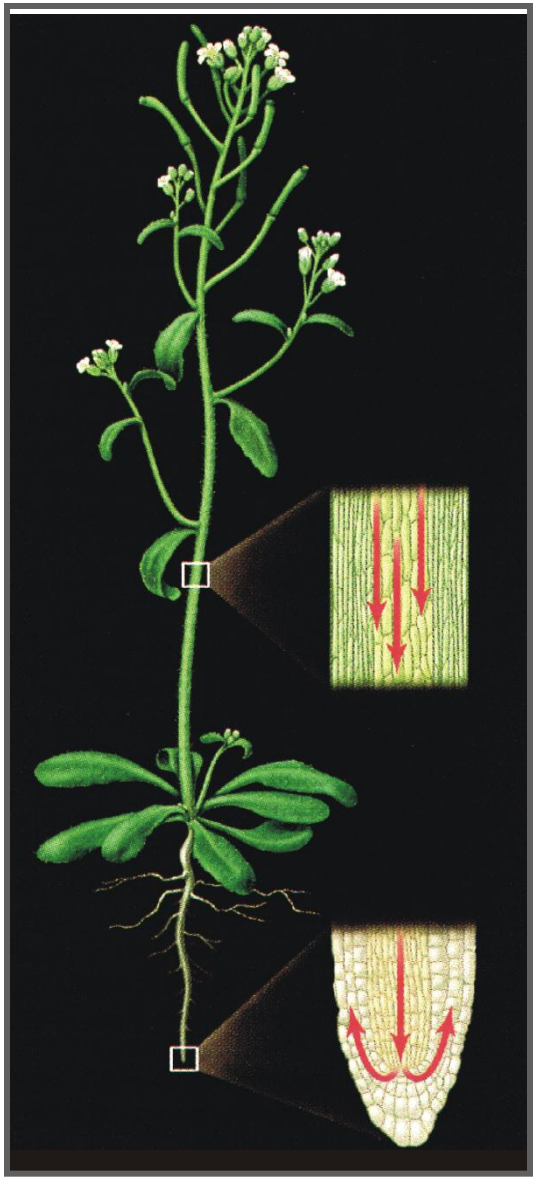
Auxin Transport

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

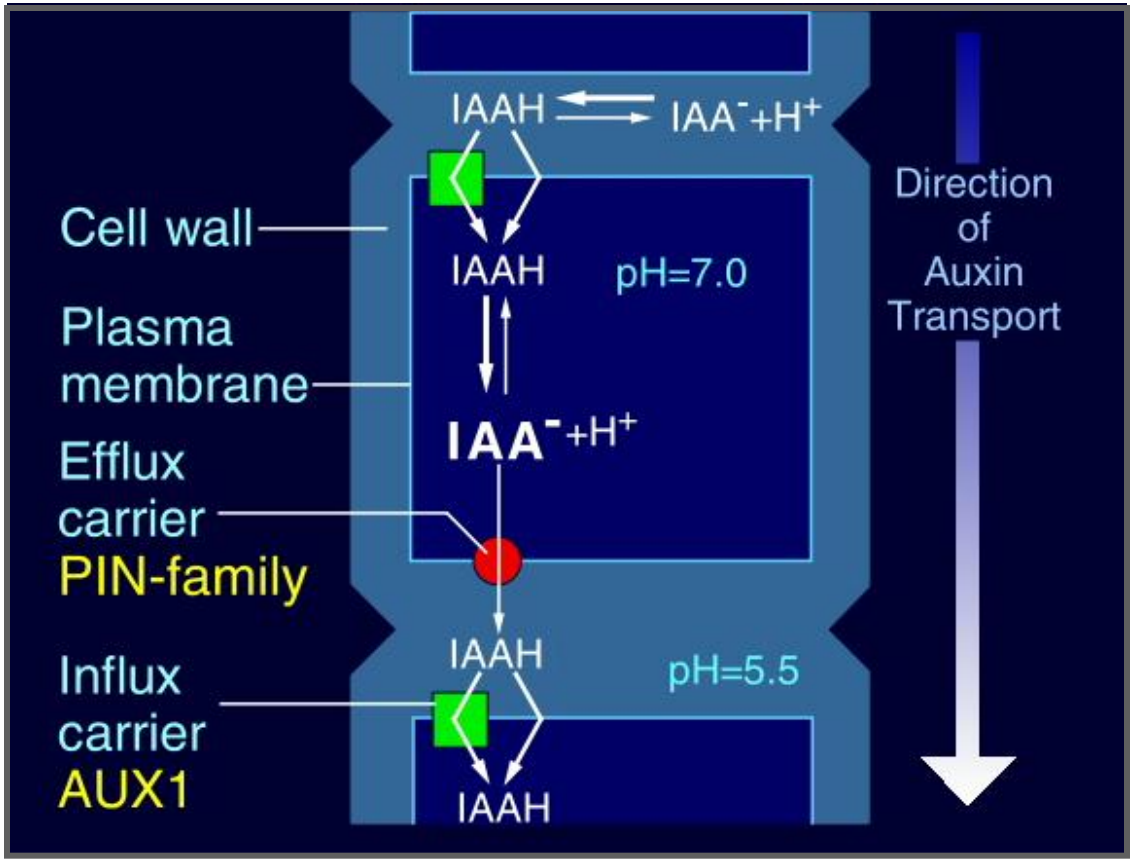
Role of GNOM dependent vesicle trafficking

PIN proteins cycling and its role

Physiology of Auxin Transport



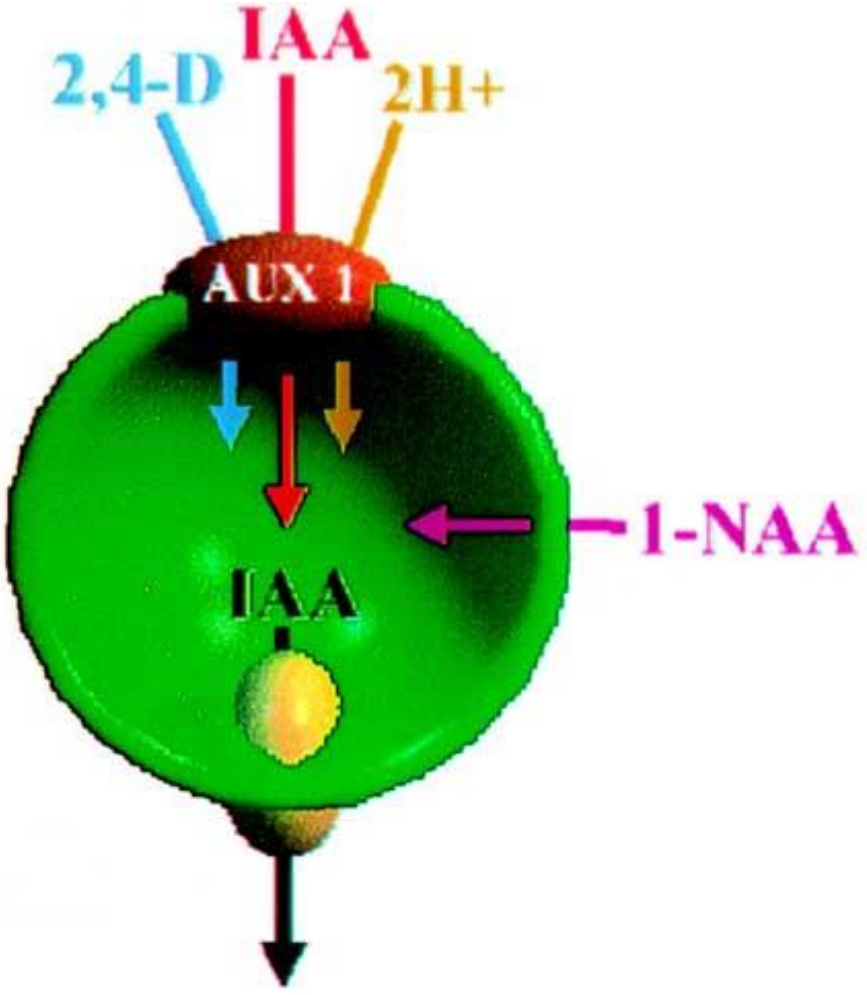
Chemiosmotic hypothesis



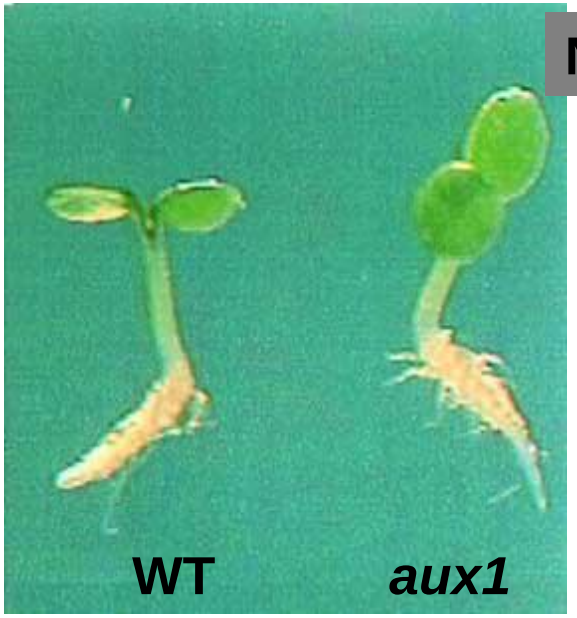
Auxin Influx

aux1 is Resistant to Auxin

Transport properties of different auxins



***aux1* phenotype**



NAA Rescues *aux1* Phenotype

- NAA

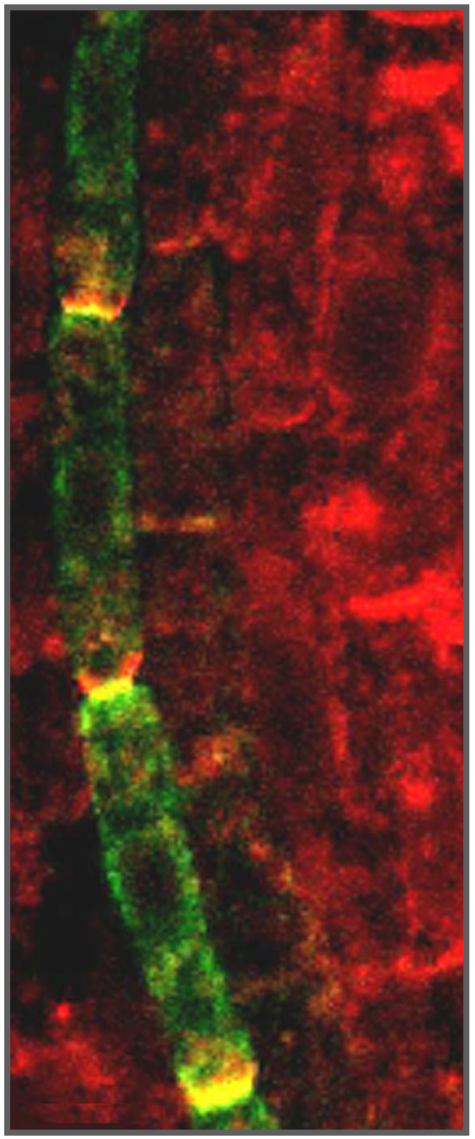
+ NAA

AUX1 – Expression and Localization

AUX1::GUS

AUX1 protein

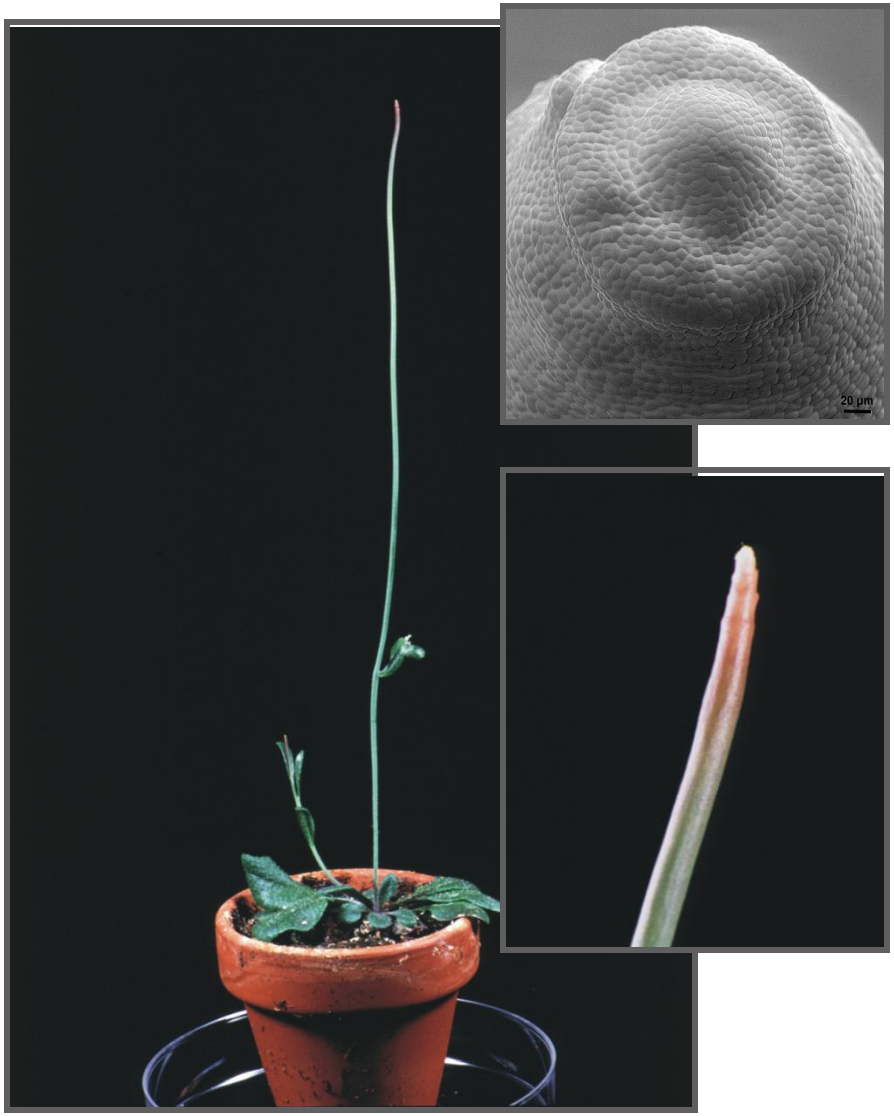
PIN1/AUX1



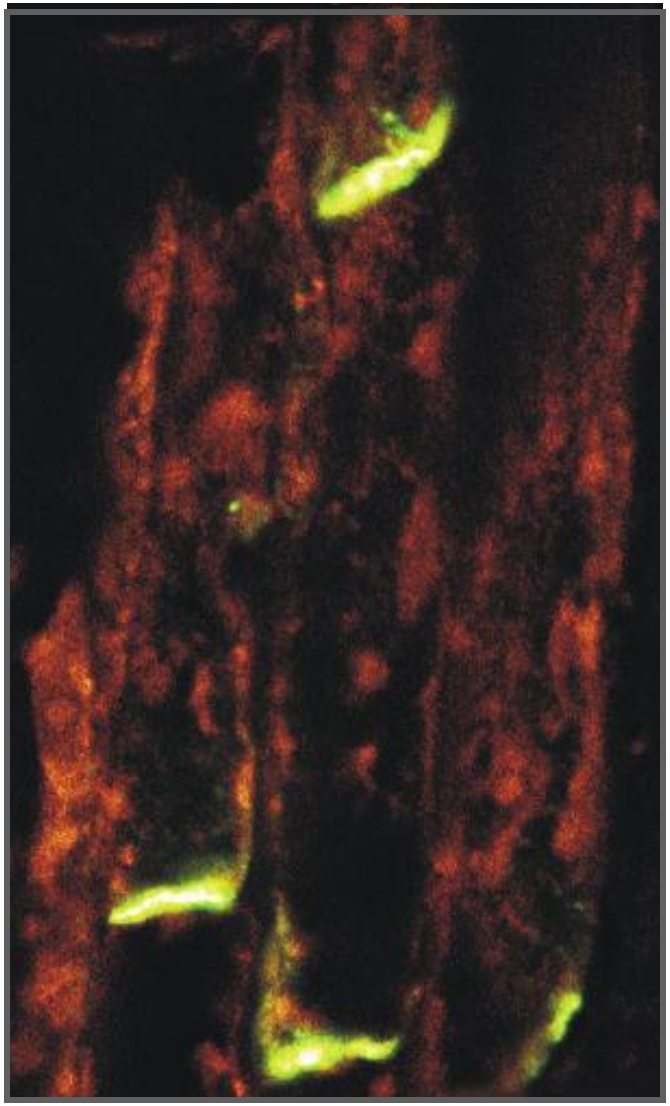
Auxin Efflux

PIN1 – the Auxin Efflux Carrier?

***pin1* mutant**

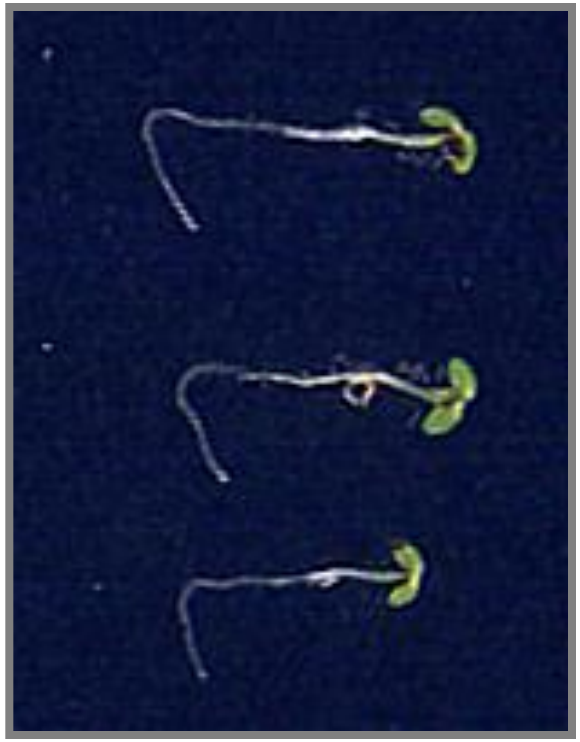


PIN1 protein

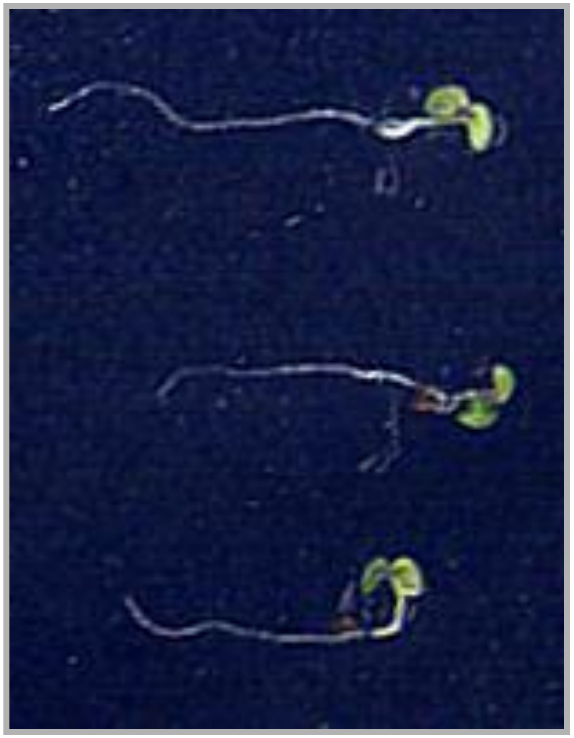


PIN2 – Root Gravitropism

Col-0



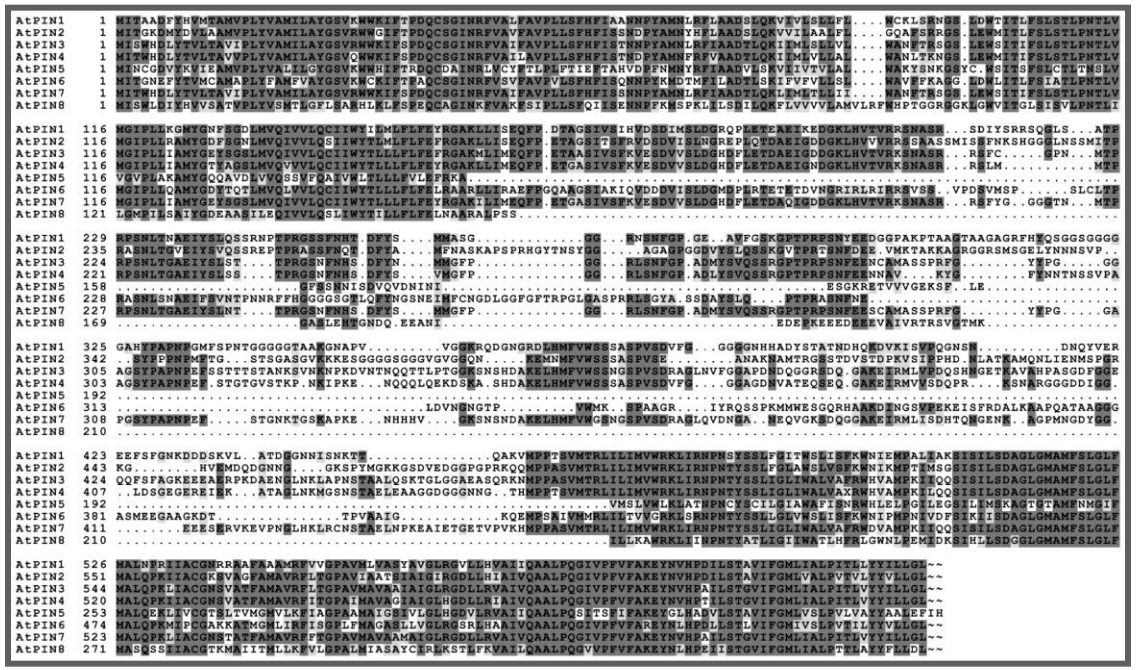
pin2



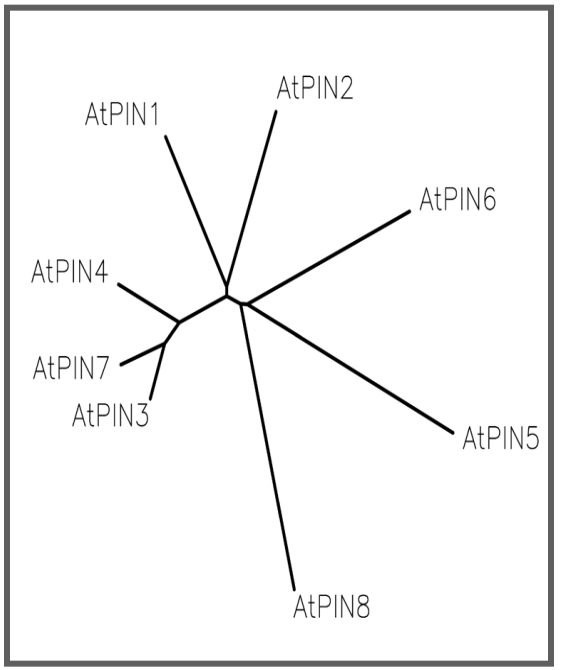
PIN2 protein

The Arabidopsis *PIN* Gene Family

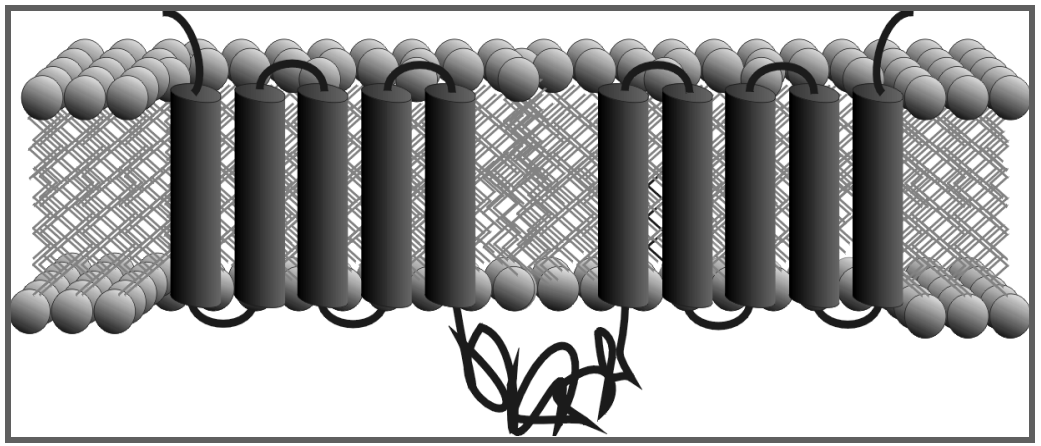
Comparison of *Arabidopsis* PIN proteins



Phylogenetic tree



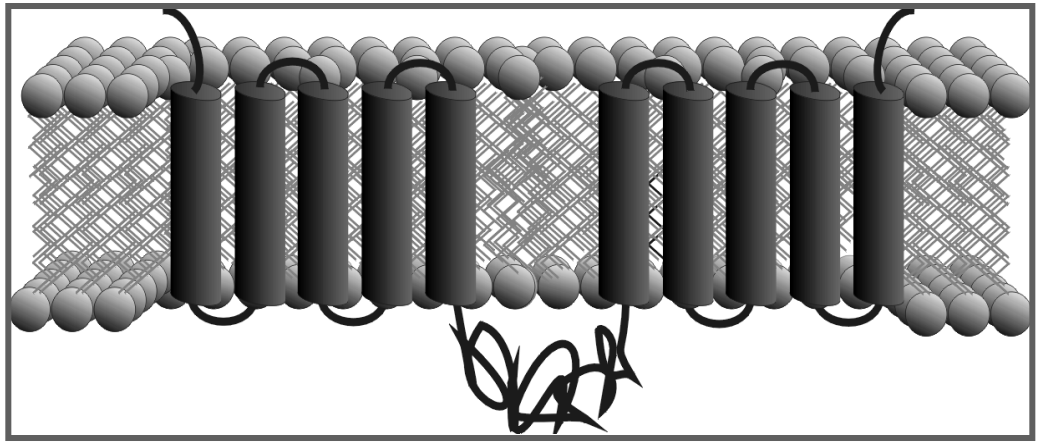
Membrane topology model



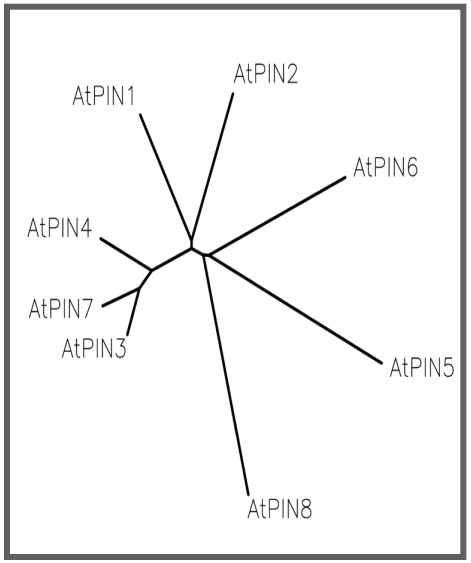
What is Molecular Role of PIN Proteins in Auxin Transport?

PINs Are Essential Components of Auxin Transport

Putative topology of PIN proteins



Phylogenetic tree



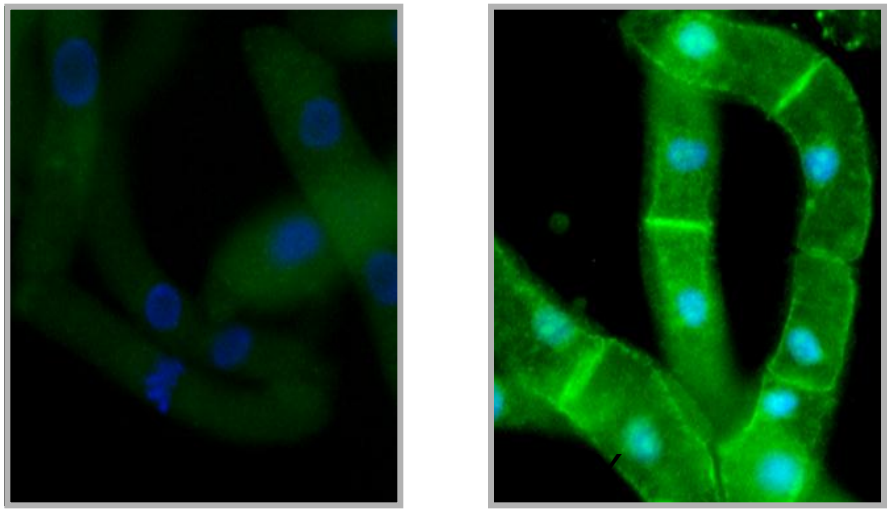
- All defects in *pin* loss-off-function mutants are in auxin transport-dependent processes and can be phenocopied by auxin transport inhibitors

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

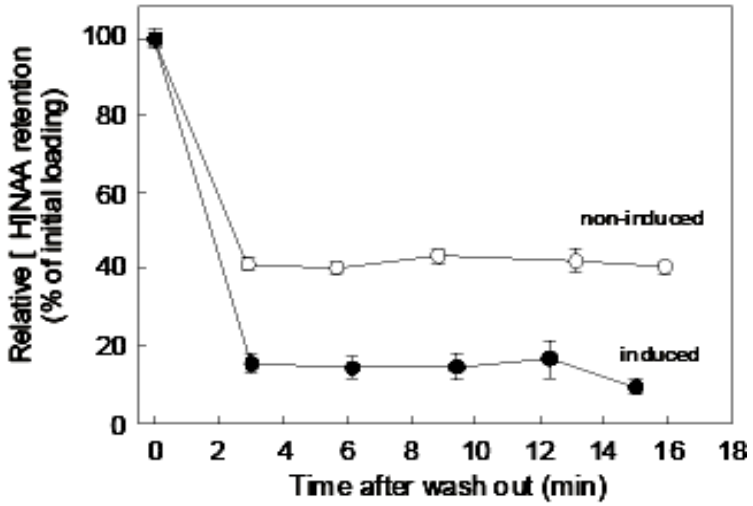
- Polar PIN localization determines direction of auxin flow

PINs Are Rate-limiting Factors in Auxin Efflux

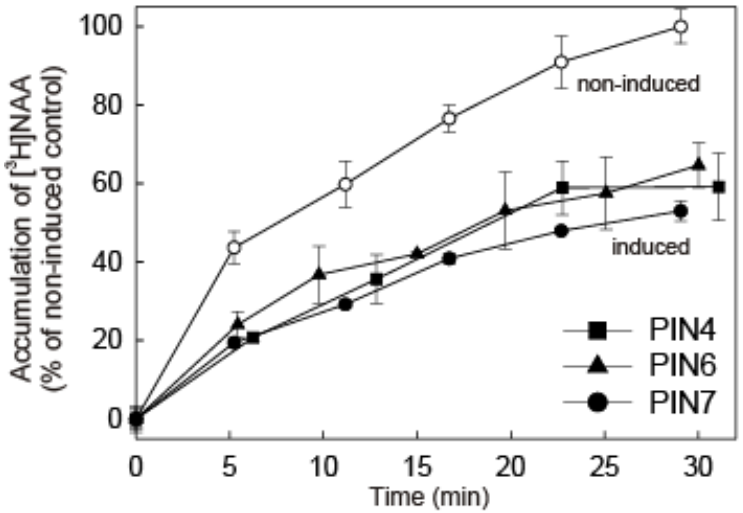
Inducible PIN1 expression



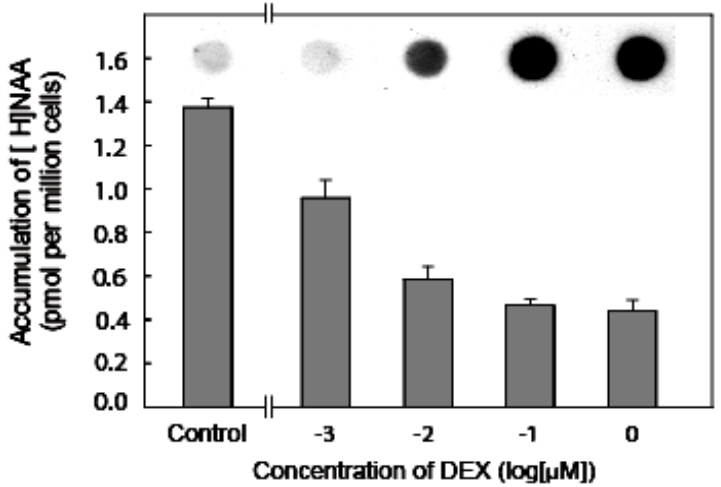
PIN-dependent auxin efflux from *GVG-PIN7* tobacco cells



NAA accumulation kinetics

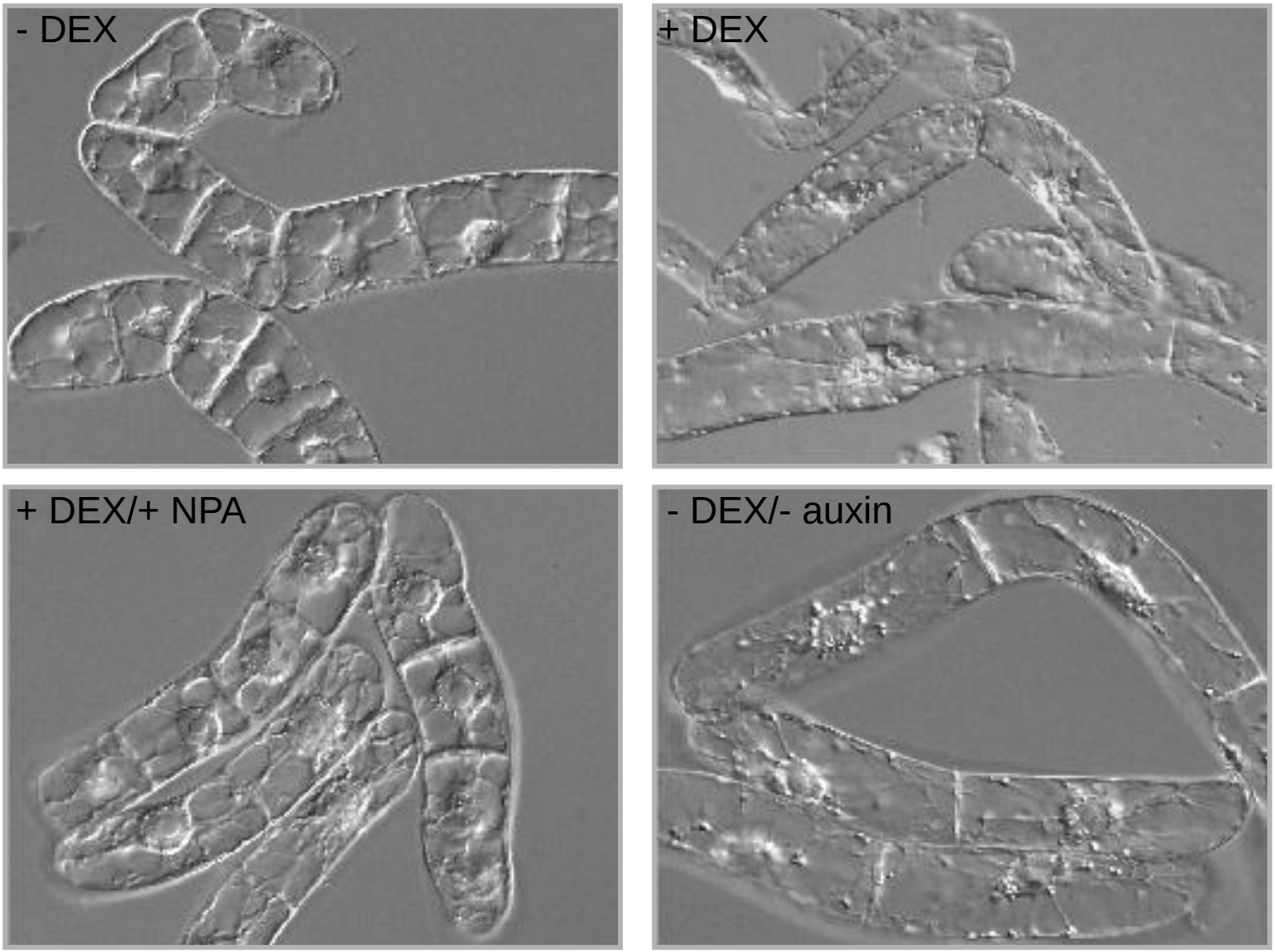


[3H]NAA accumulation in GVG-PIN7 tobacco cells in relation to DEX concentration



Petrasek et al., 2006

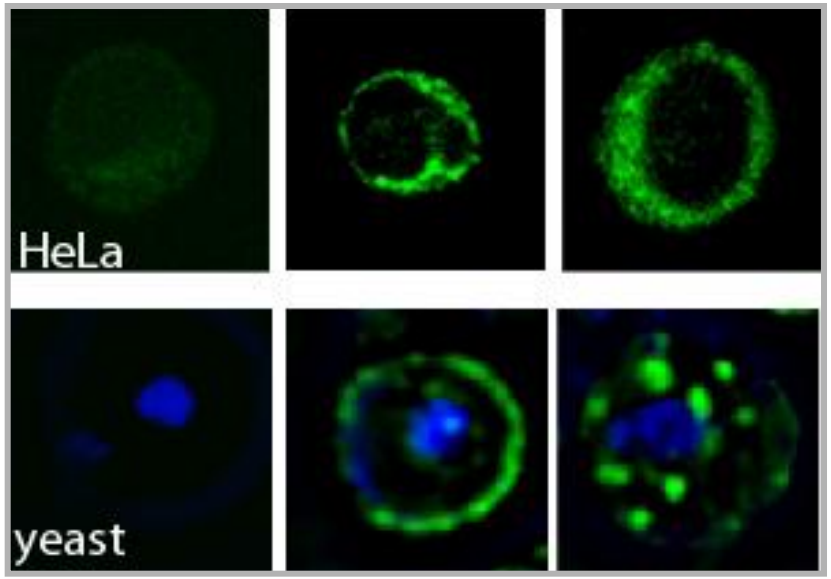
PIN-induced Phenotypes in BY-2 Cells



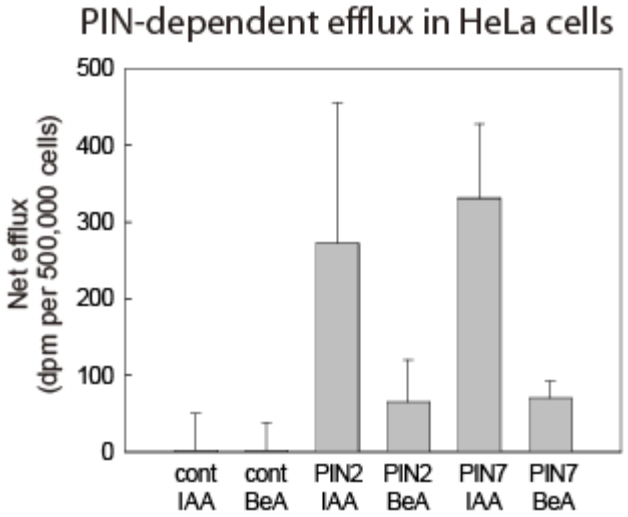
unpublished

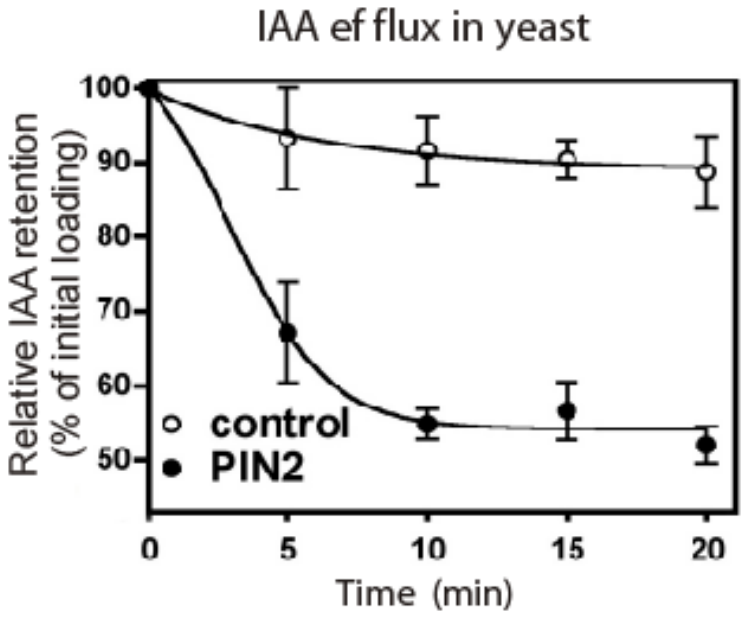
Expression of PINs in HeLa and Yeast

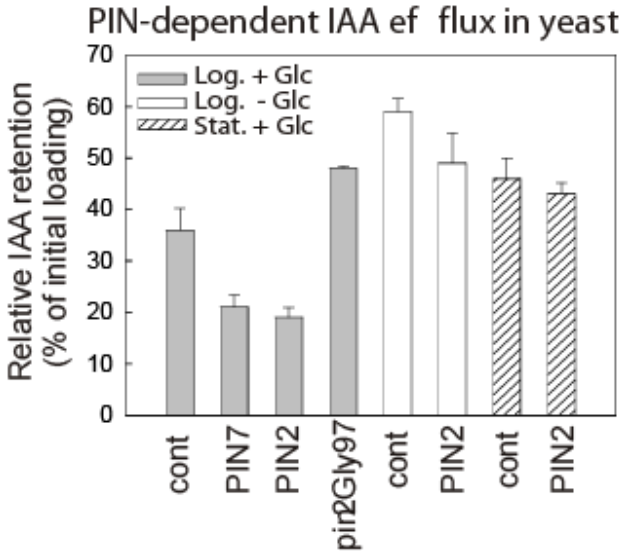
Heterologous PIN2 expression



auxin efflux activity



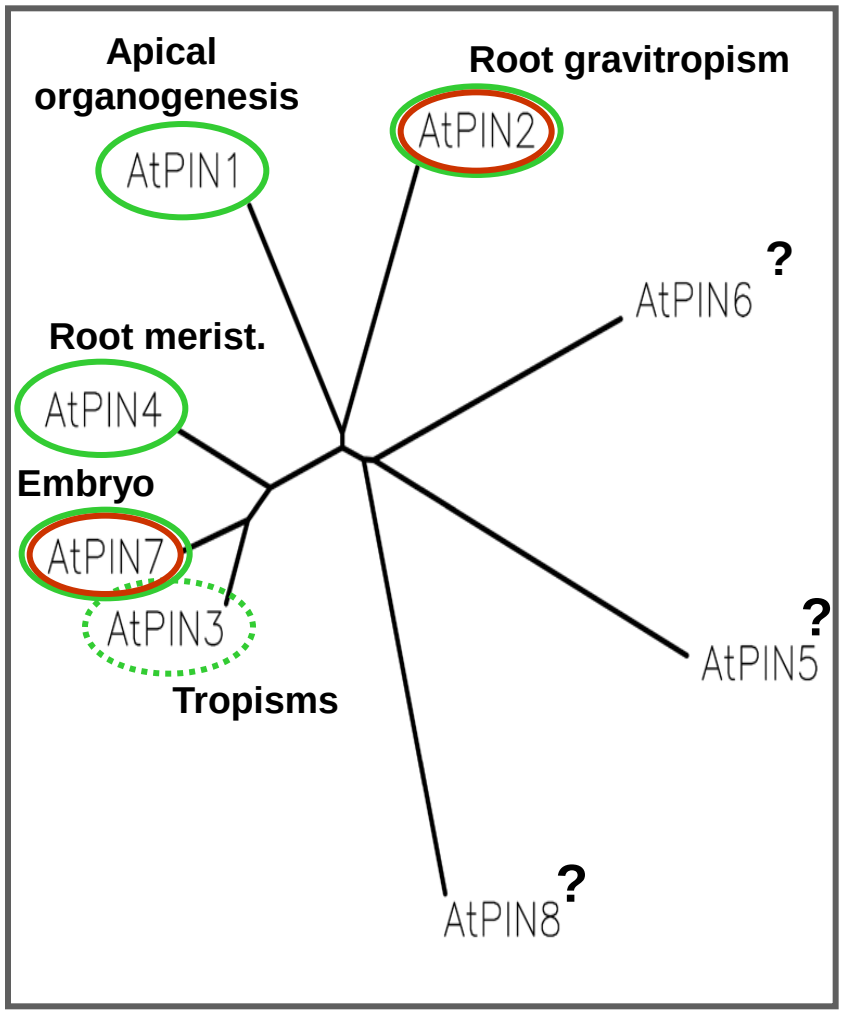




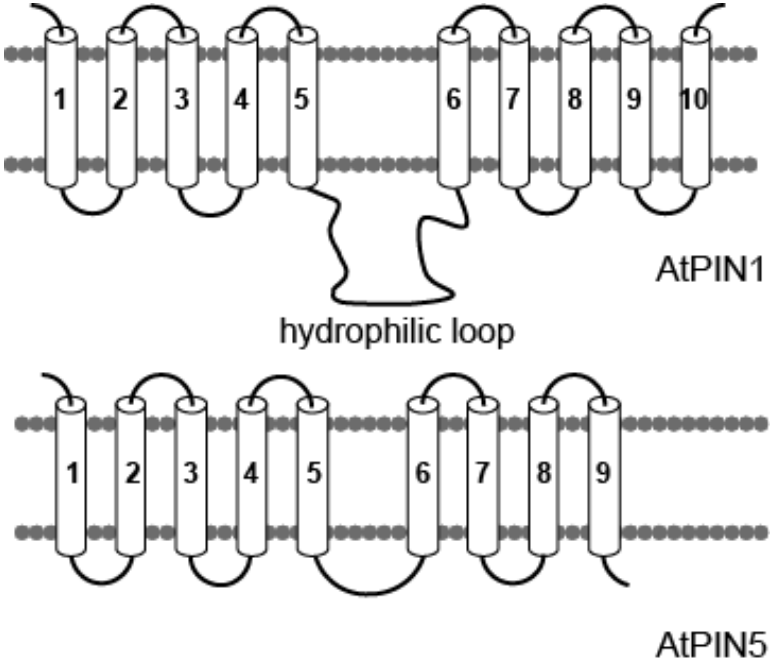
Petrasek et al., 2006

PIN gene family

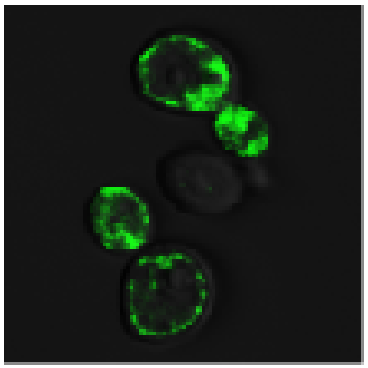
PINs in *Arabidopsis*

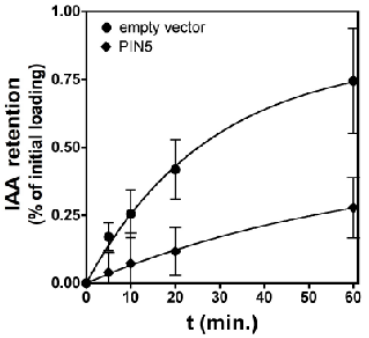


Predicted PIN Protein Topology

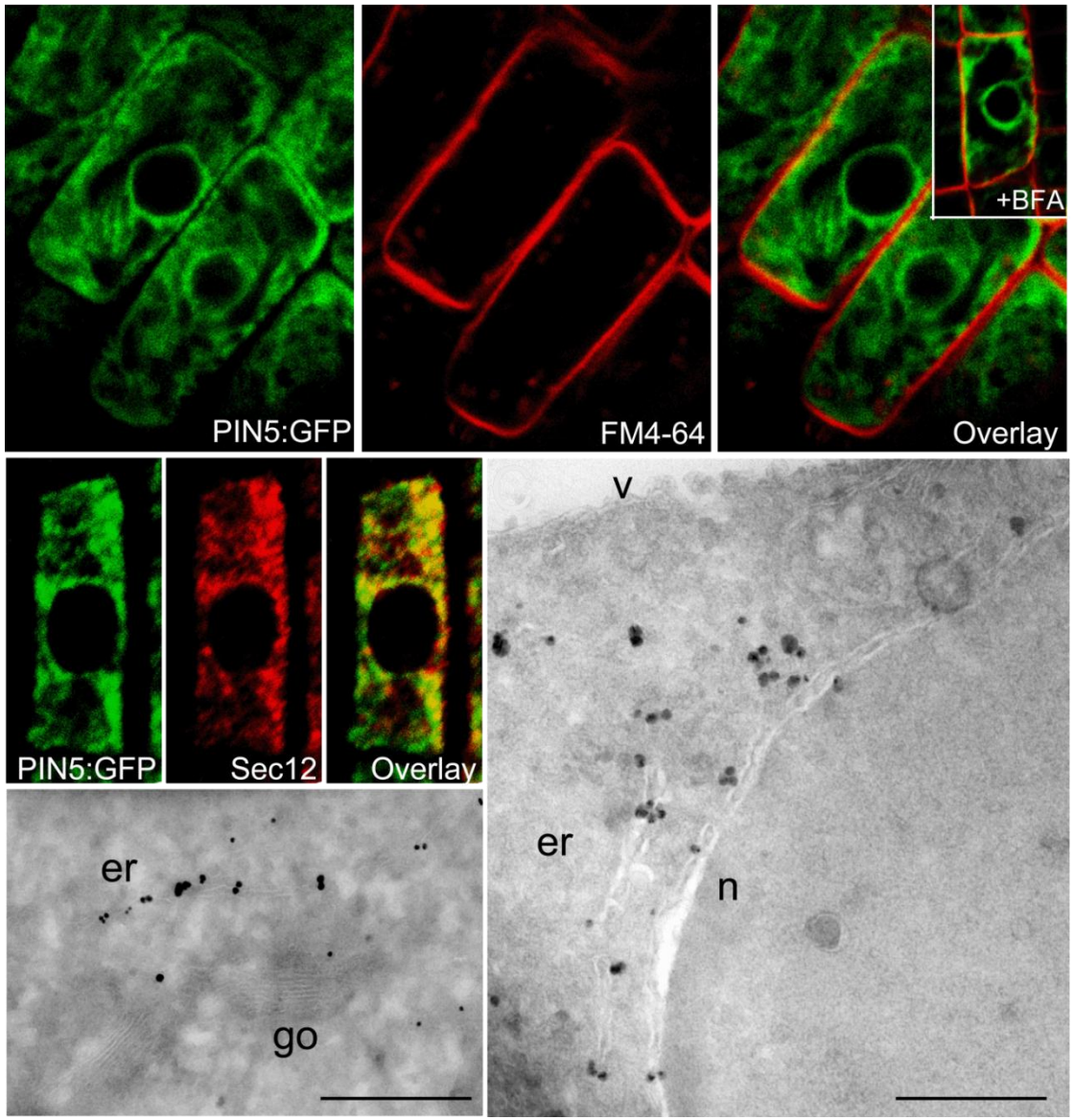


Auxin Transport in Yeast



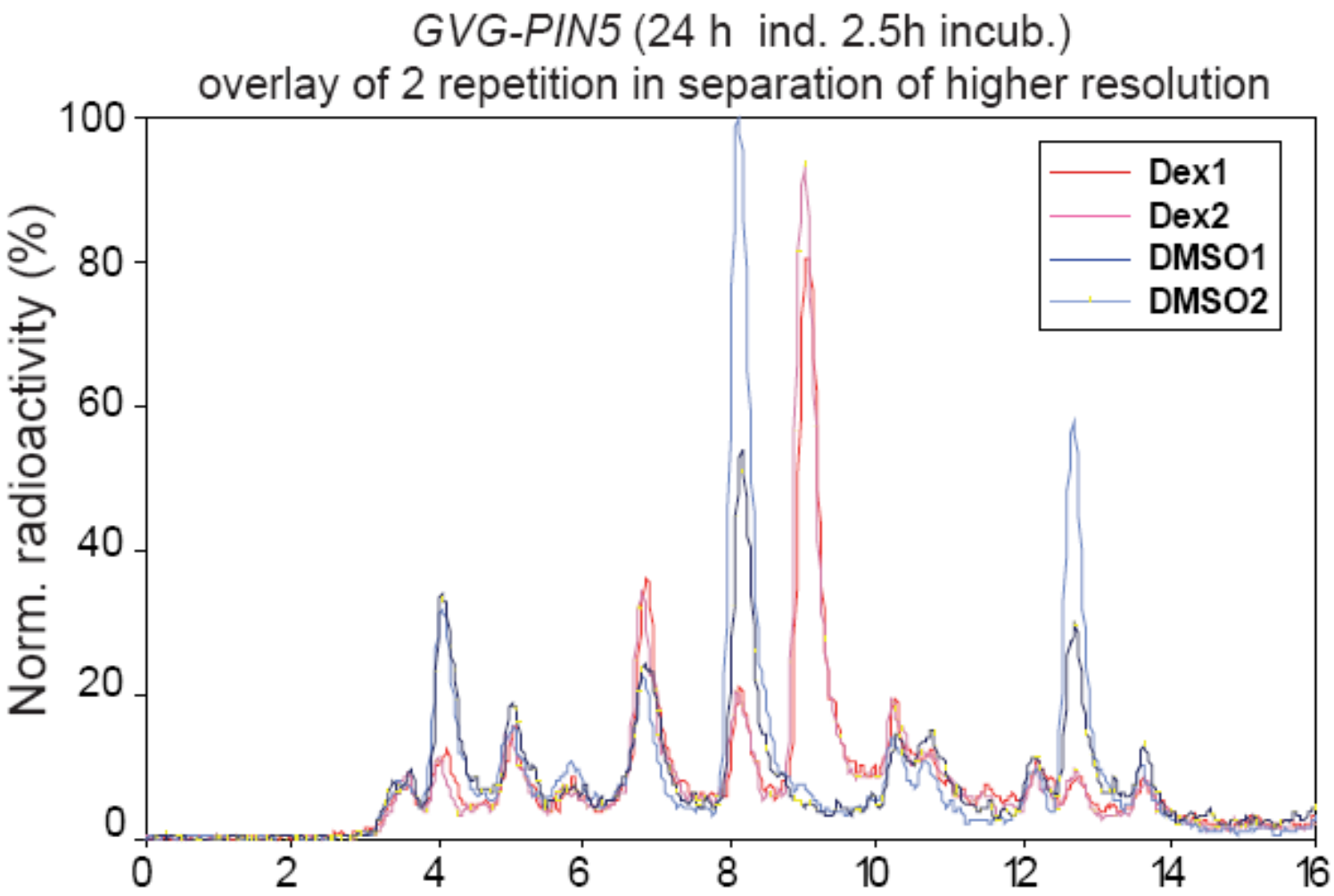


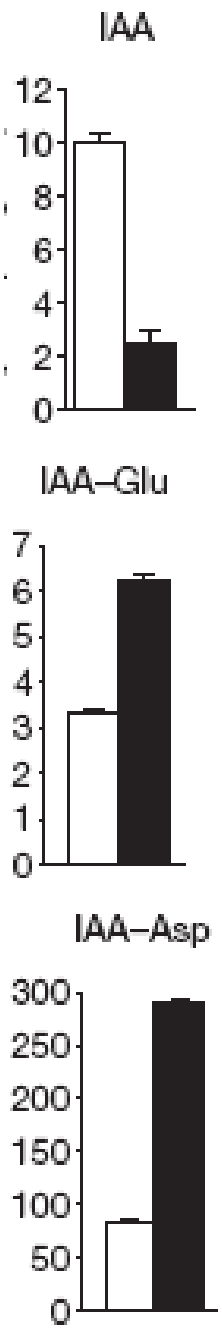
ER-based PIN5-dependent auxin transport



Mravec et al., 2009

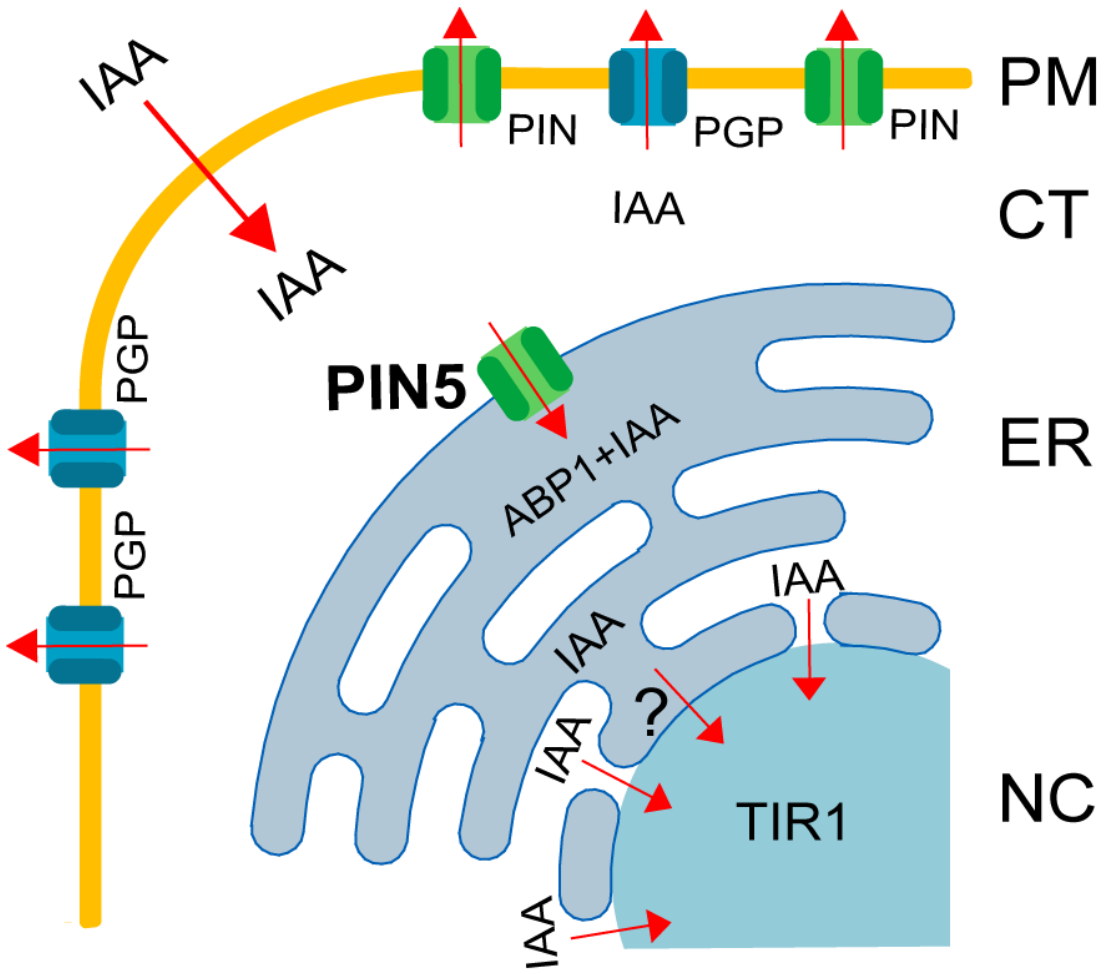
PIN5 regulates auxin metabolism



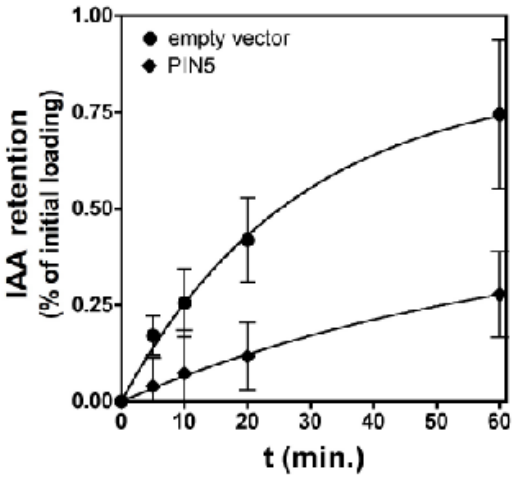


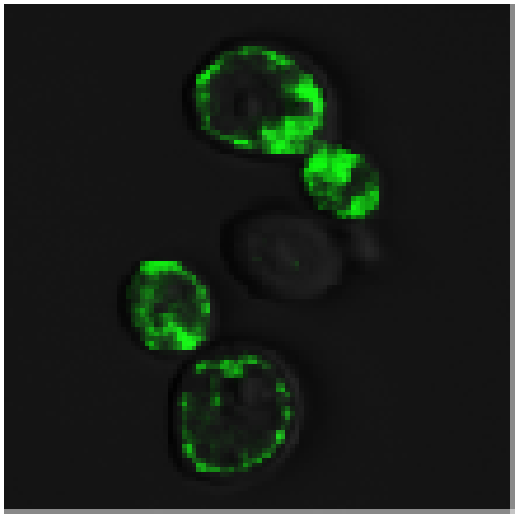
Mravec et al., 2009

PIN5-dependent auxin transport into ER



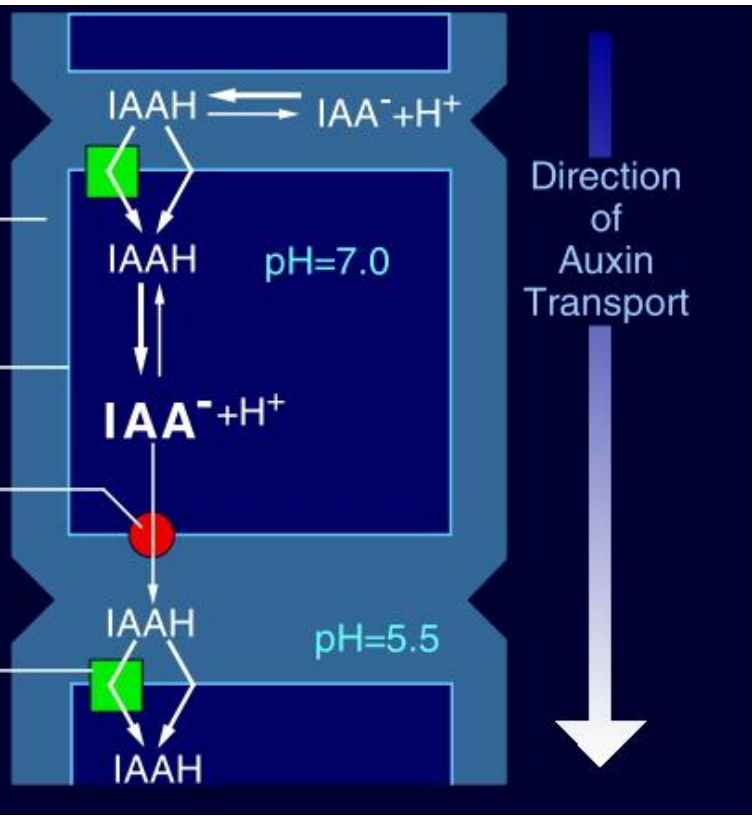
Yeast

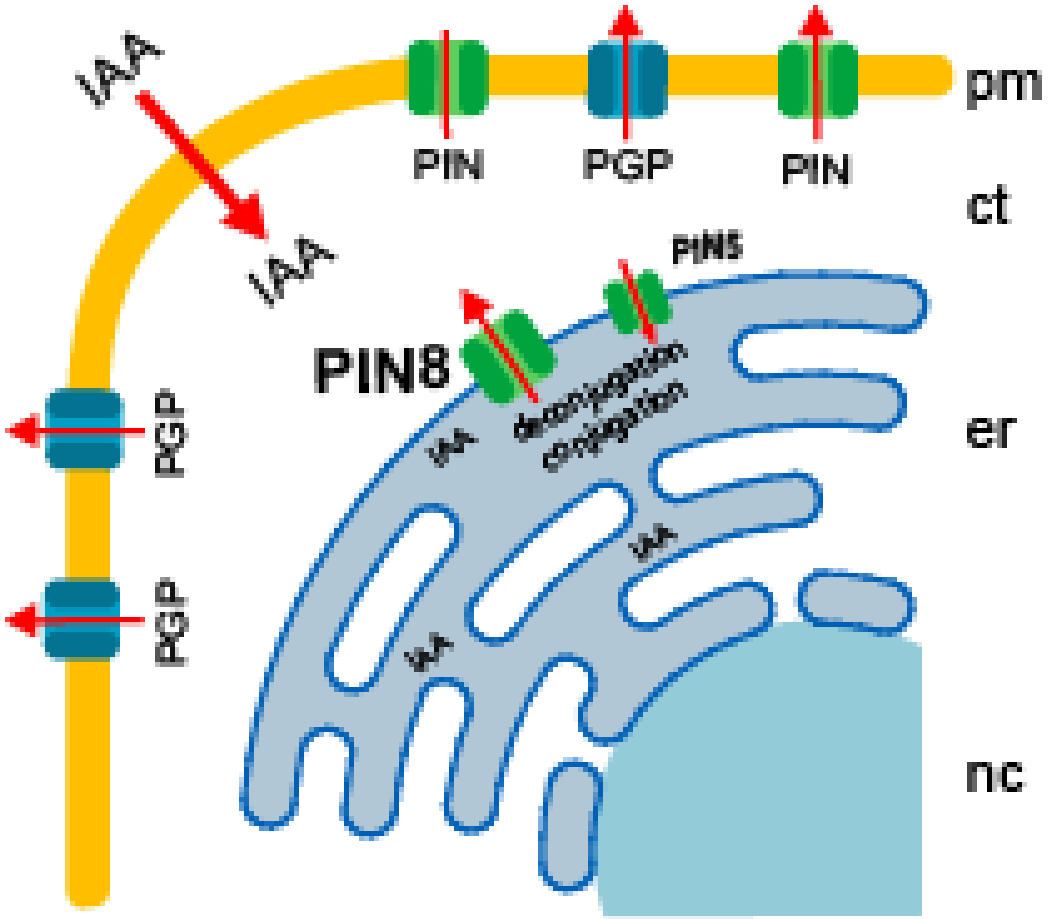




Mravec et al., 2009

Updated model for auxin transport





-Interested in novel players in auxin homeostasis

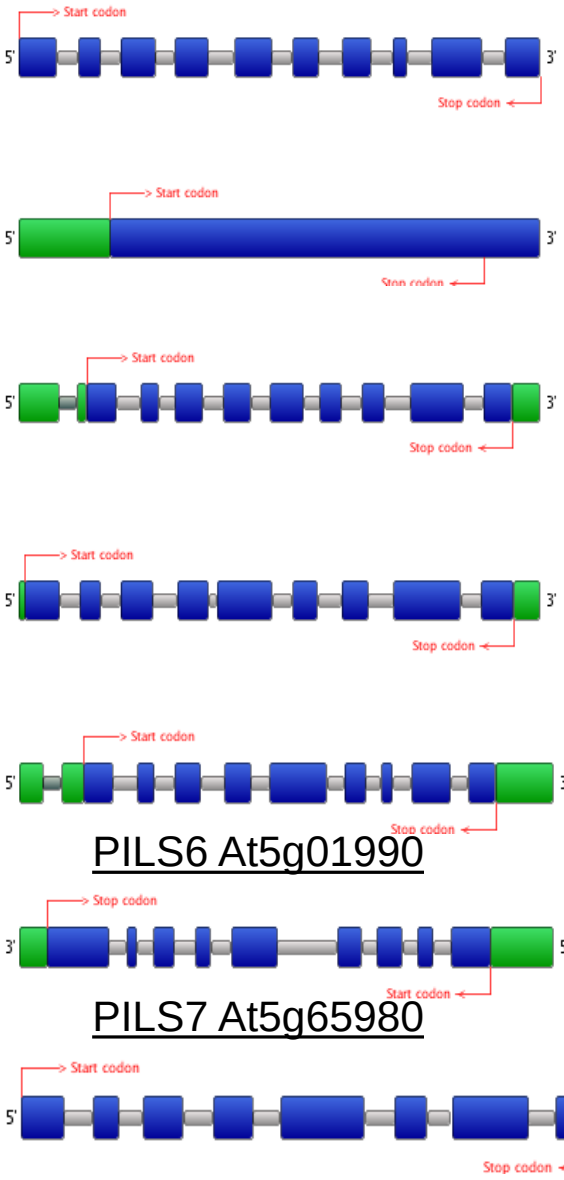
- Search for proteins with PIN like protein organisation

- SMART tool from EMBL

(→finds proteins with topology of interest)

Atg-code	gene name
At1g20925	*PILS1*
At1g71090	*PILS2*
At1g76520	*PILS3*
At1g76530	*PILS4*
At2g17500	*PILS5*
At5g01990	*PILS6*
At5g65980	*PILS7*

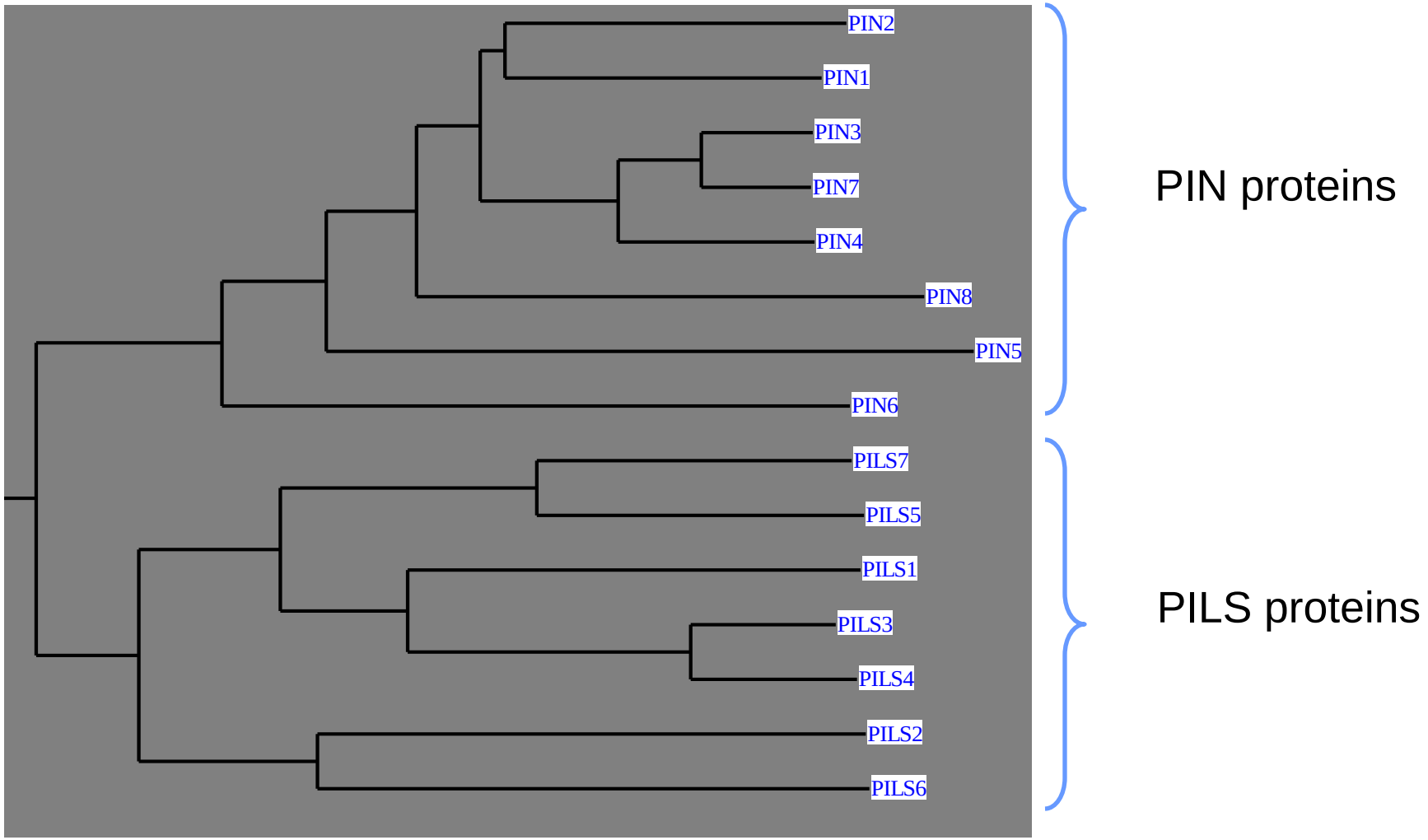
The PILS (PIN-likes) gene family



Aim of the project: Initial characterisation of the PILS family members

PINs vs PILS: Protein sequences

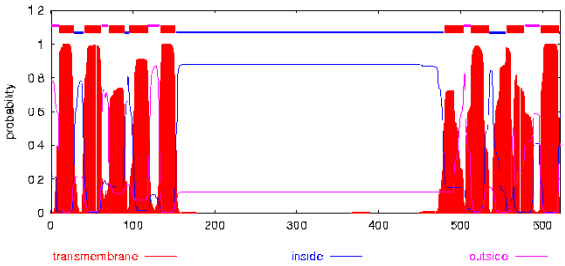
Limited AA sequence similarities reveal that PINs and PILS form two distinct protein families

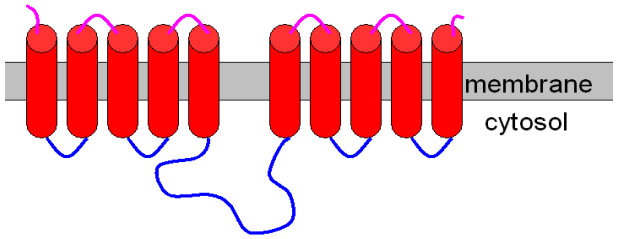


PINs vs PILS: Topology

-PILS proteins share the same predicted protein topology as the PINs

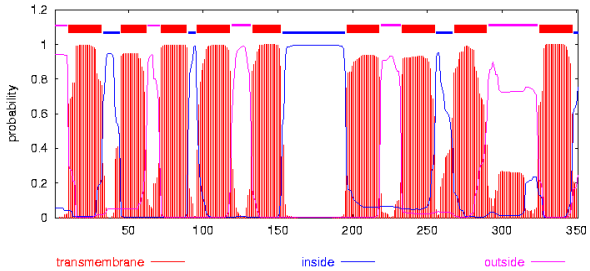
-The short central hydrophylic loop make the PILS more related to the Class I PINs

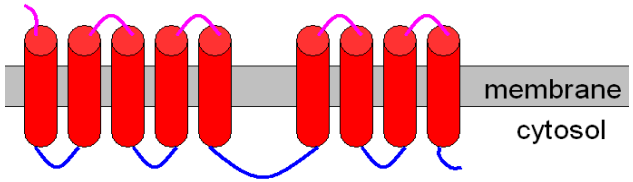




PIN1 (ClassII PINs):

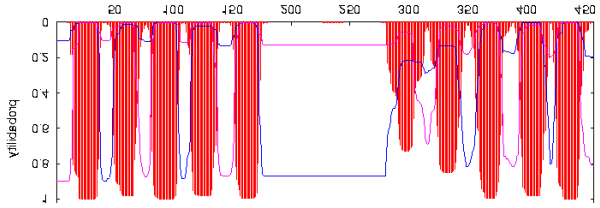
- Long hydrophylic loop

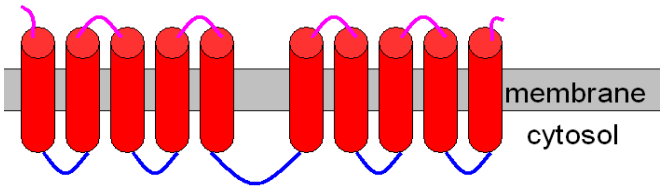




PIN8 (ClassI PINs):

- Short hydrophylic loop



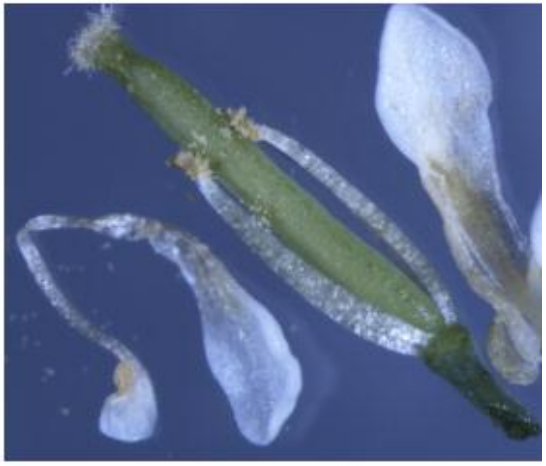


PILS2

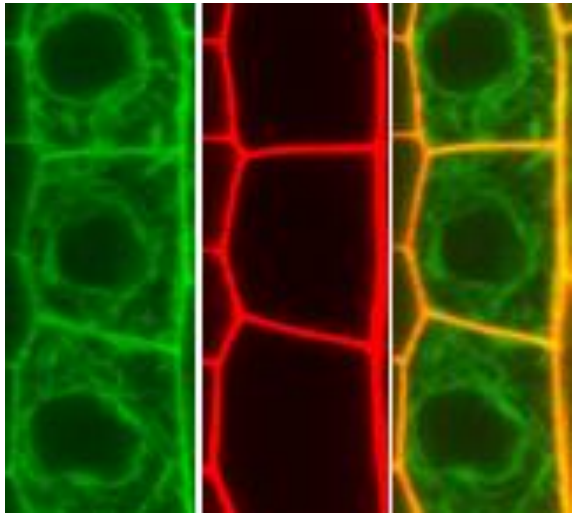
- Short hydrophylic loop

PILS genes in Arabidopsis

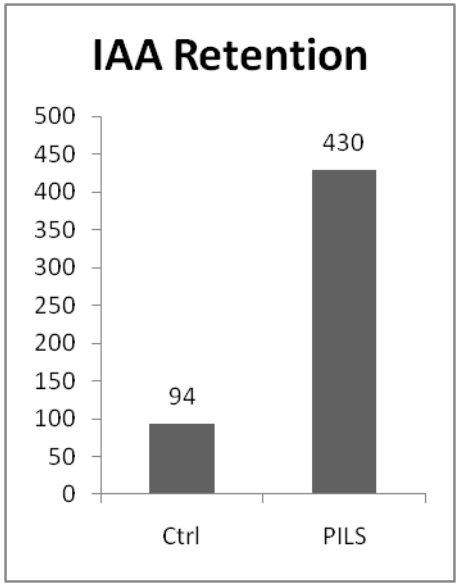
Phenotypes of *pils* mutants



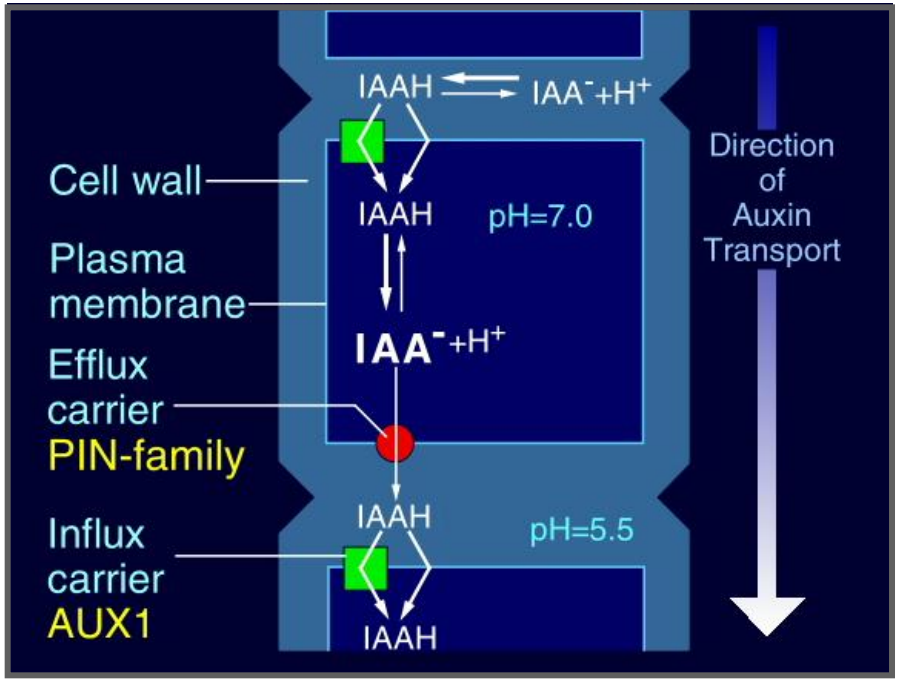
ER Localization

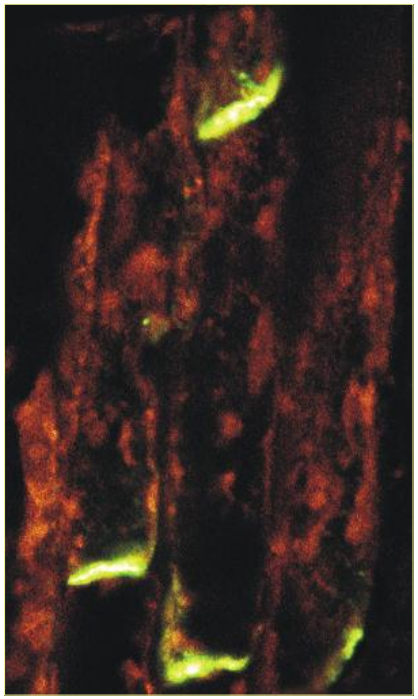


Yeast: IAA transport



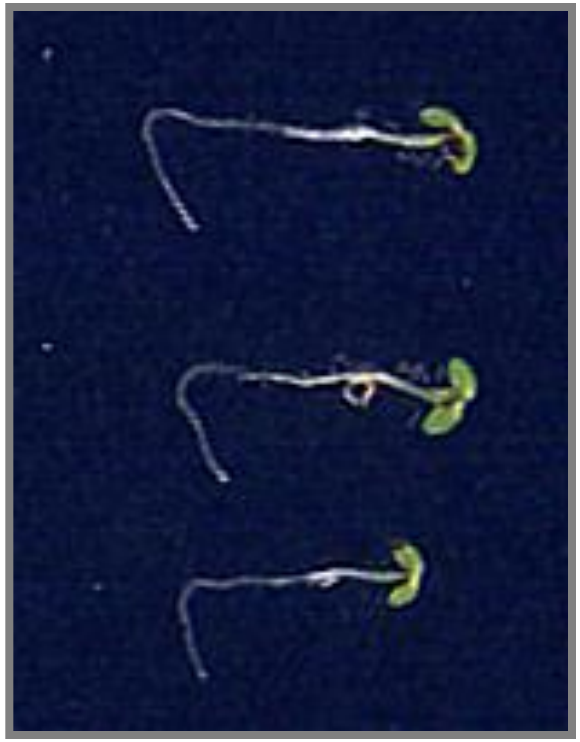
Cellular Polarity of PIN Localization and Directionality of Intercellular Auxin Flow



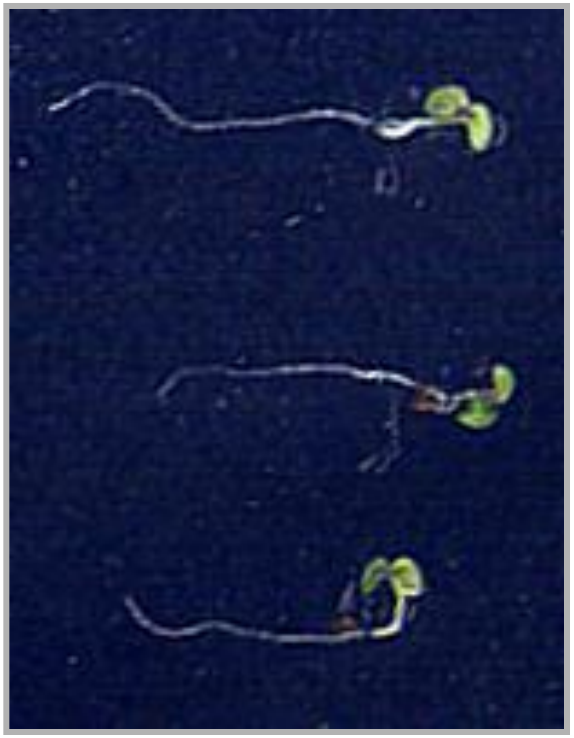


PIN2 – Root Gravitropism

Col-0

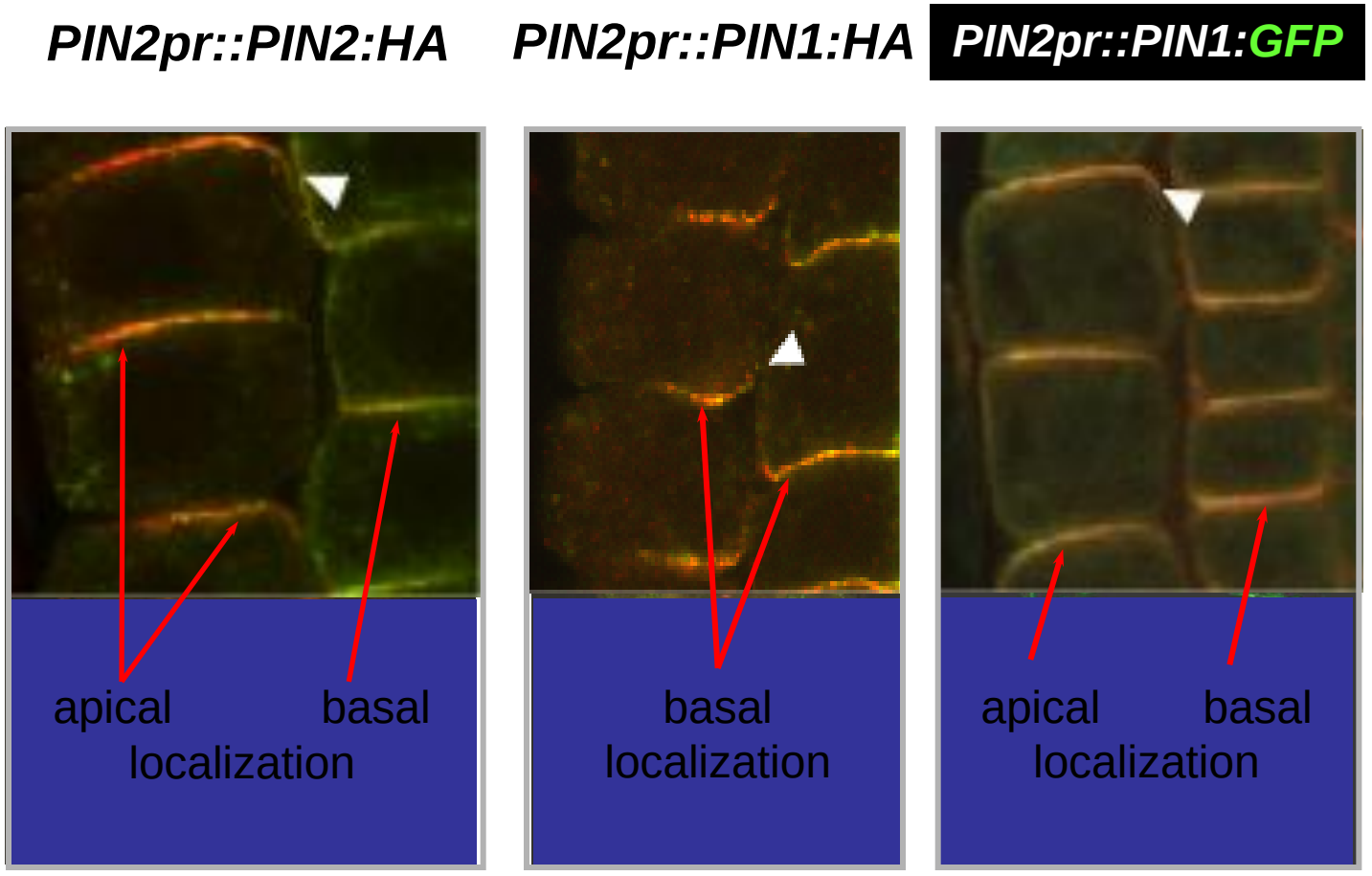


pin2



PIN2 protein

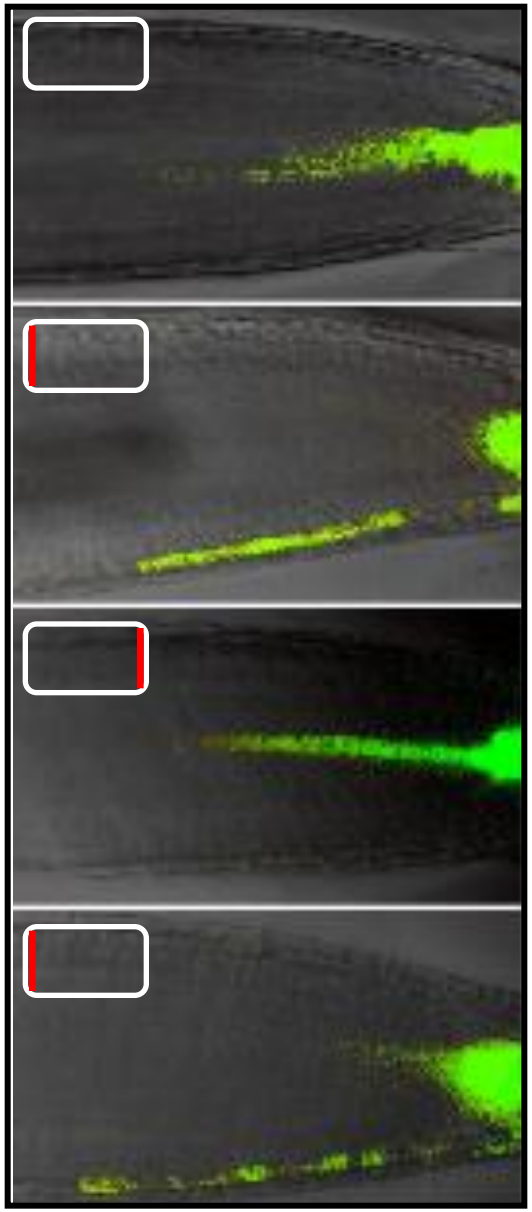
PIN-specific Signals for Polar Targeting



Wisniewska et al. 2006

PIN Polarity Determines Direction of Auxin Flow

DR5rev::GFP



pin2 (eir1, agr1)

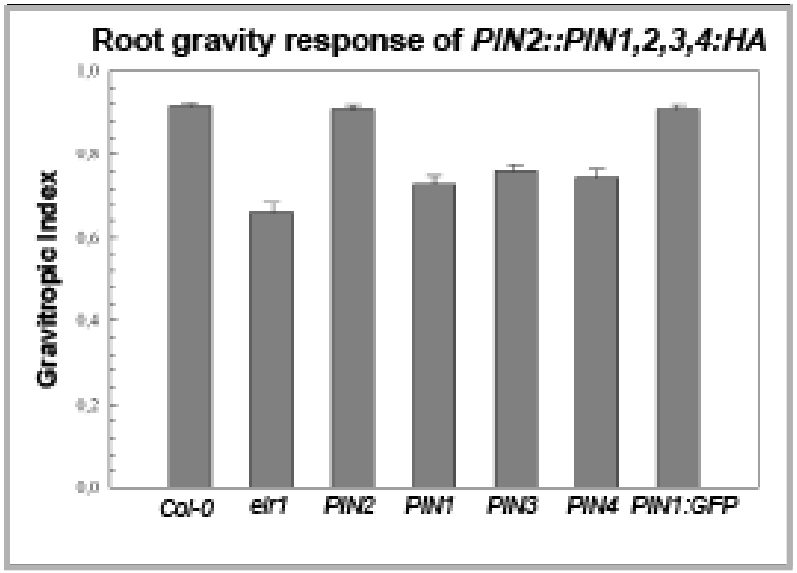
PIN2::PIN2:HA

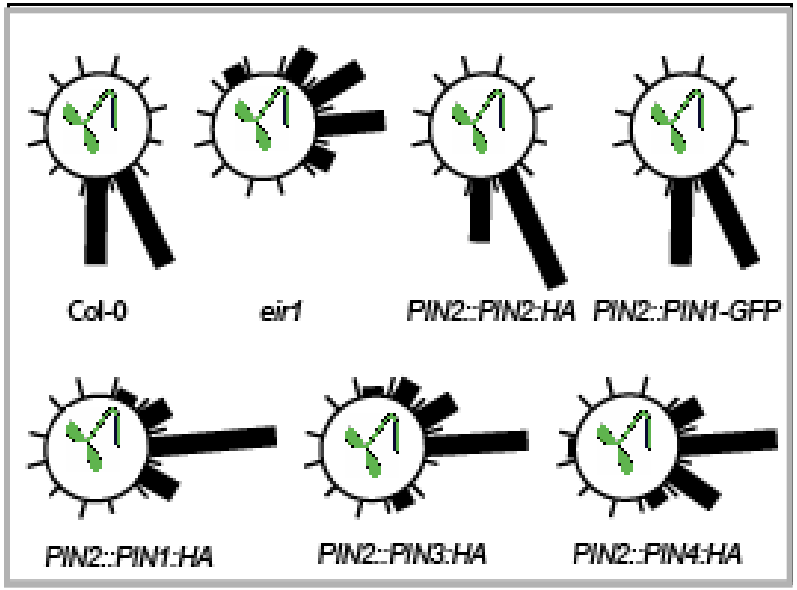
PIN2::PIN1:HA

PIN2::PIN1:GFP-2

PIN2::PIN1:GFP-3

gravitropism





Wisniewska et al. 2006

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

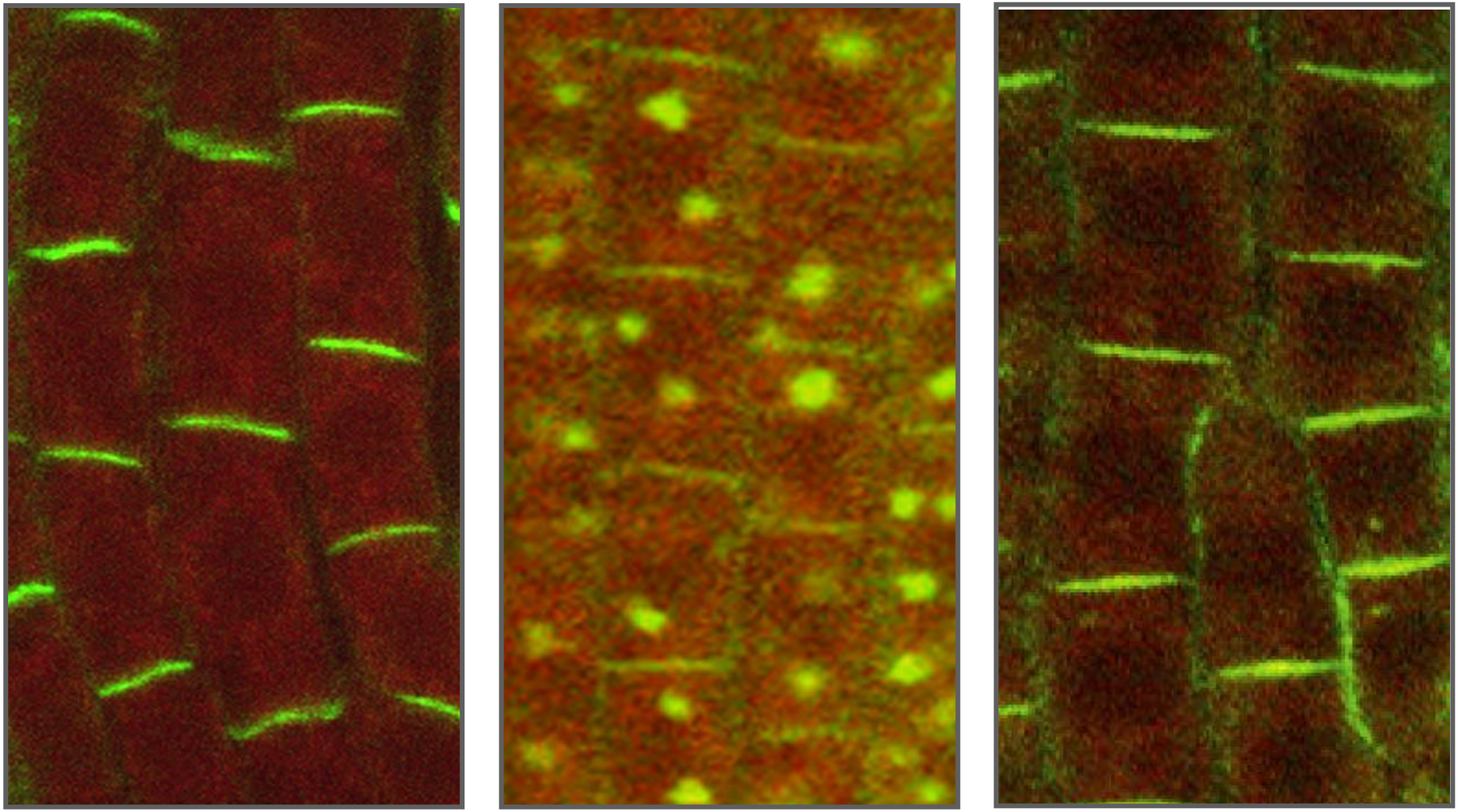
and

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

Constitutive Cycling of PINs

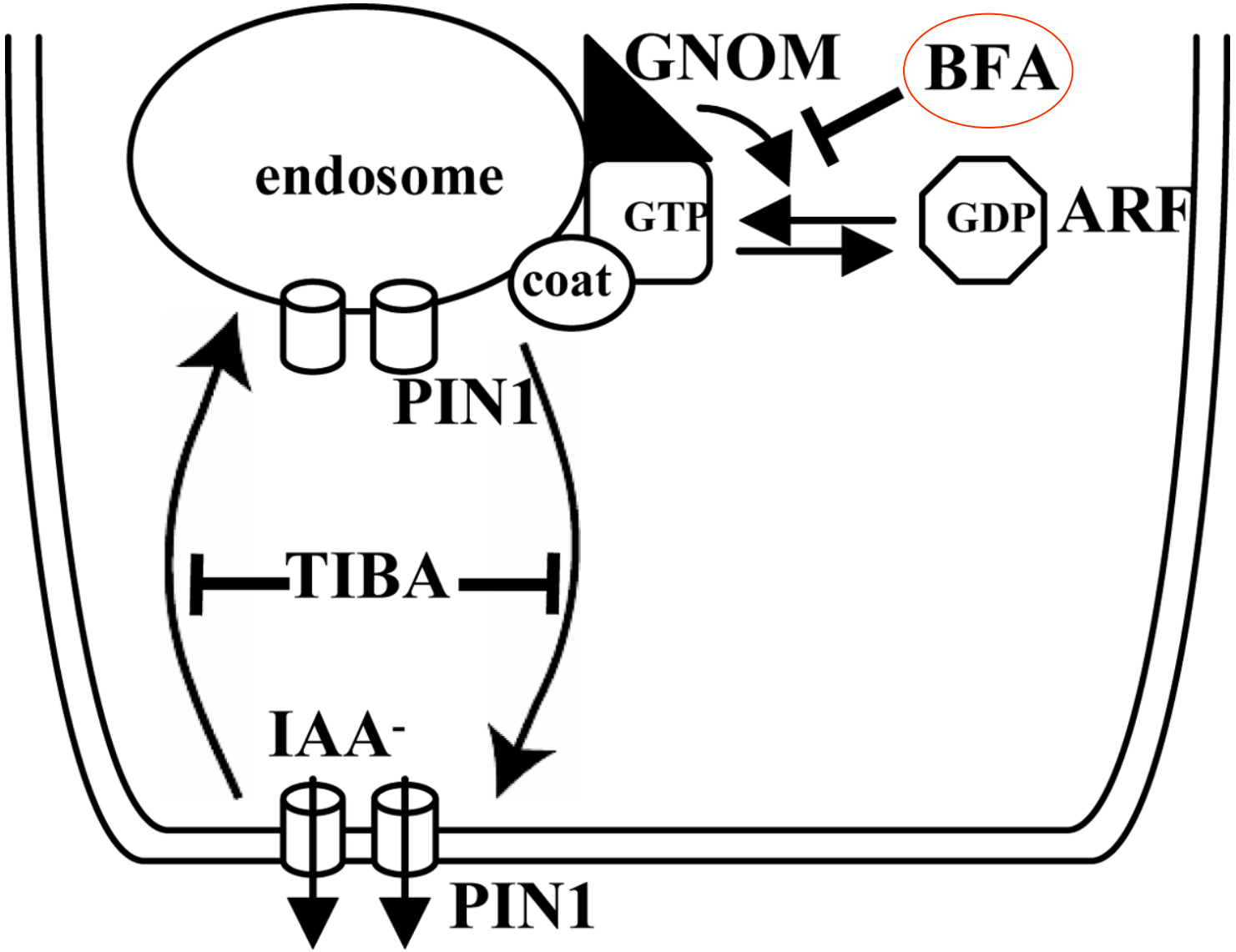
PIN1 Subcellular Movement

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

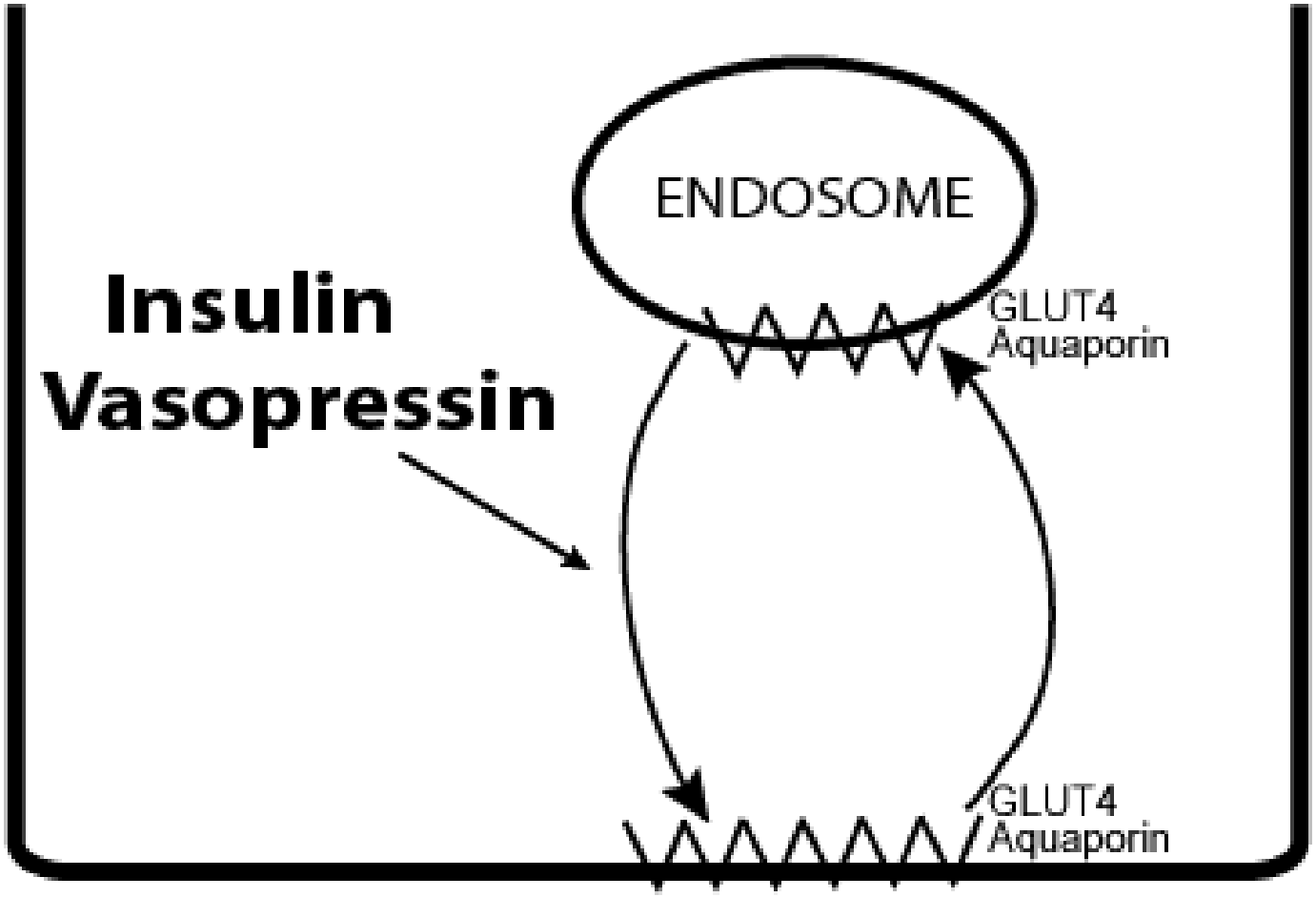


Geldner et al., 2001

Dynamic Movement of PIN Proteins

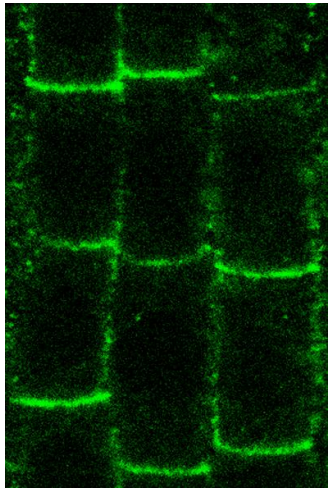


Subcellular Cycling – Means to Modulate Protein Activity?

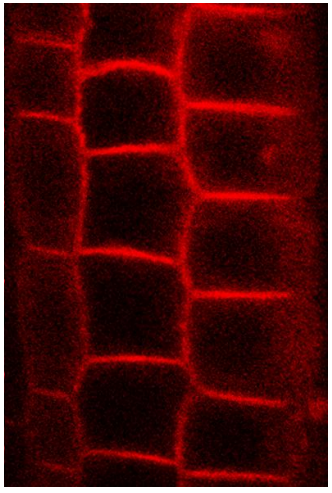


Auxin Inhibits Internalization of Plasma Membrane Proteins

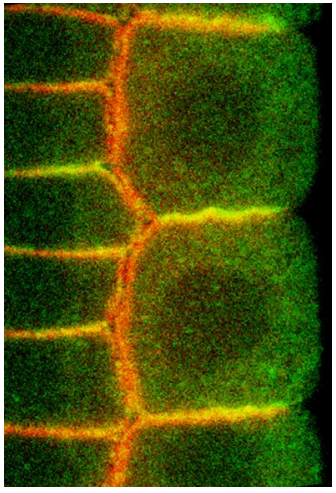
NAA
BFA
/BFA



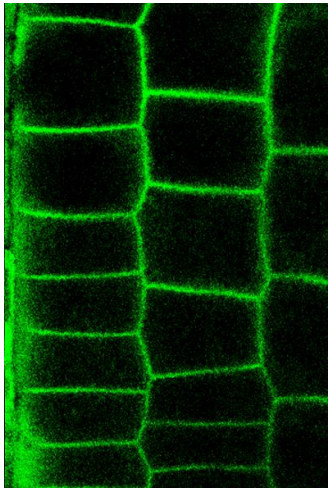
PIN1



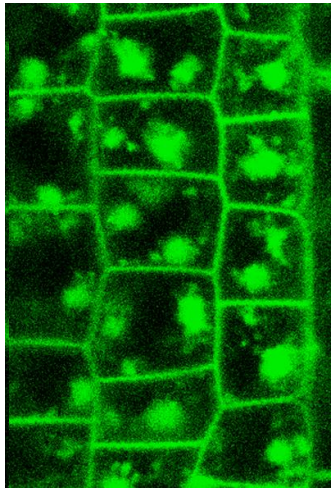
PM-ATPase



PIN2/ATPase

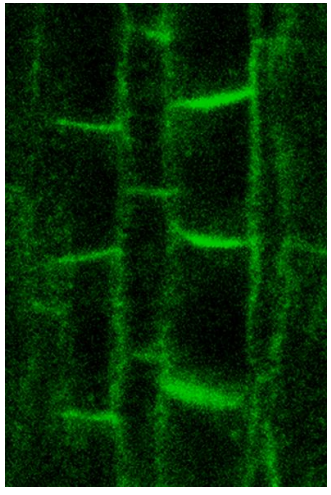


PIP2

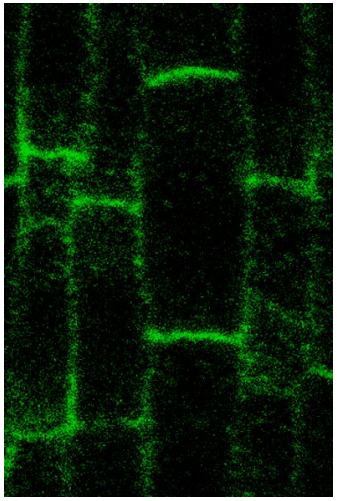


BRI1

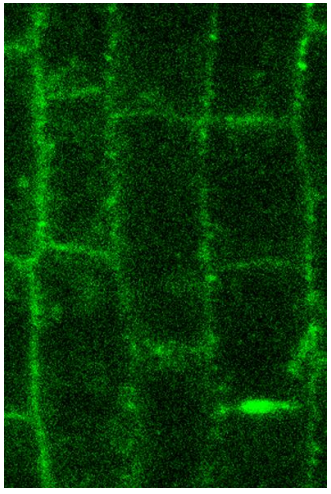
IAA/BFA



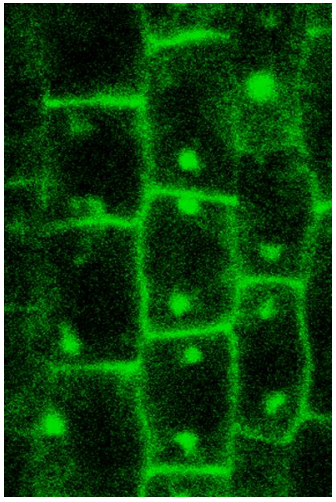
2,4-D/BFA



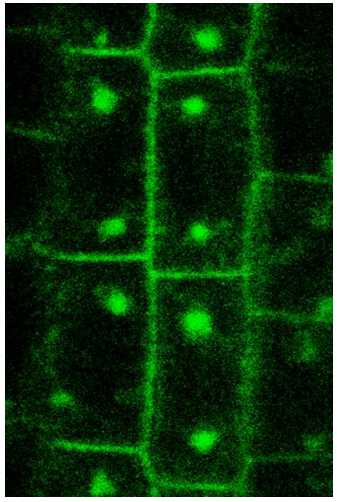
BFA in *sur2*



2-NAA/BFA

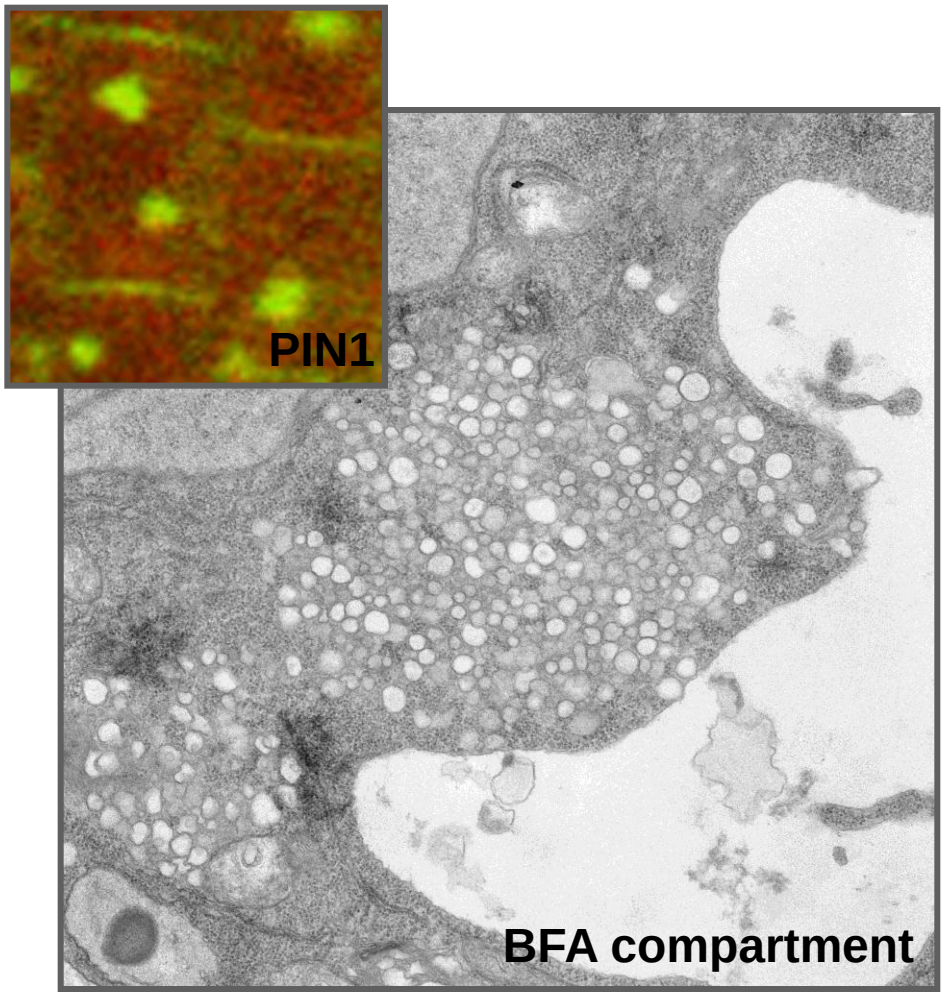


Ethylene/BFA

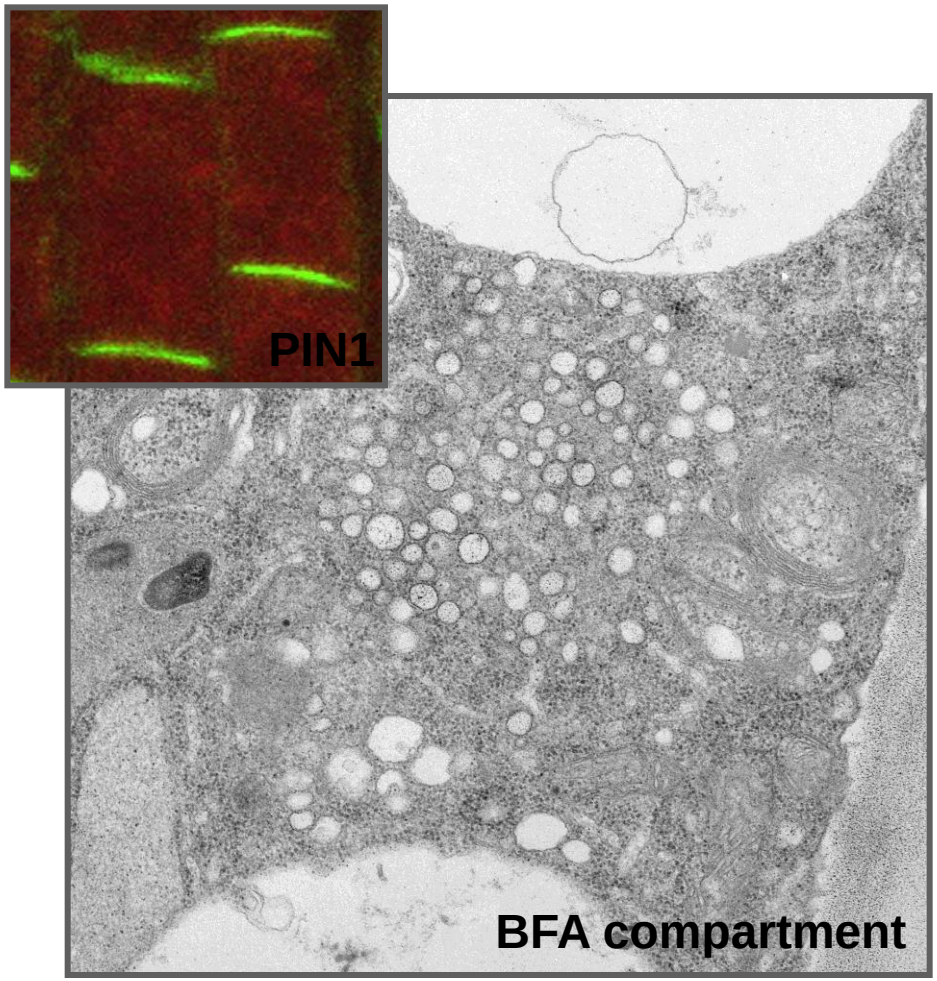


Place of Auxin Action in Protein Cycling

BFA



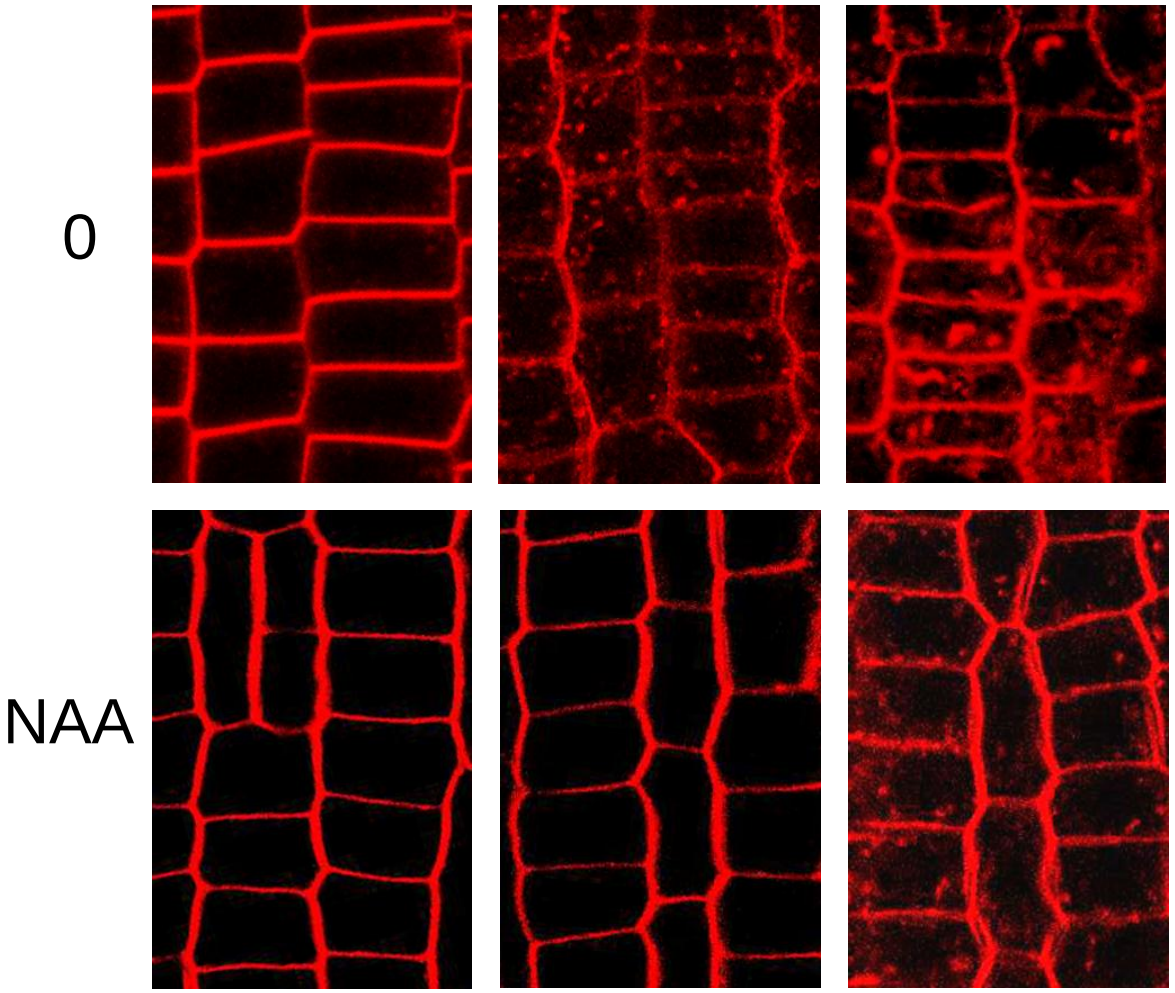
Auxin + BFA



Paciorek et al., 2005

Auxin Inhibits Endocytosis

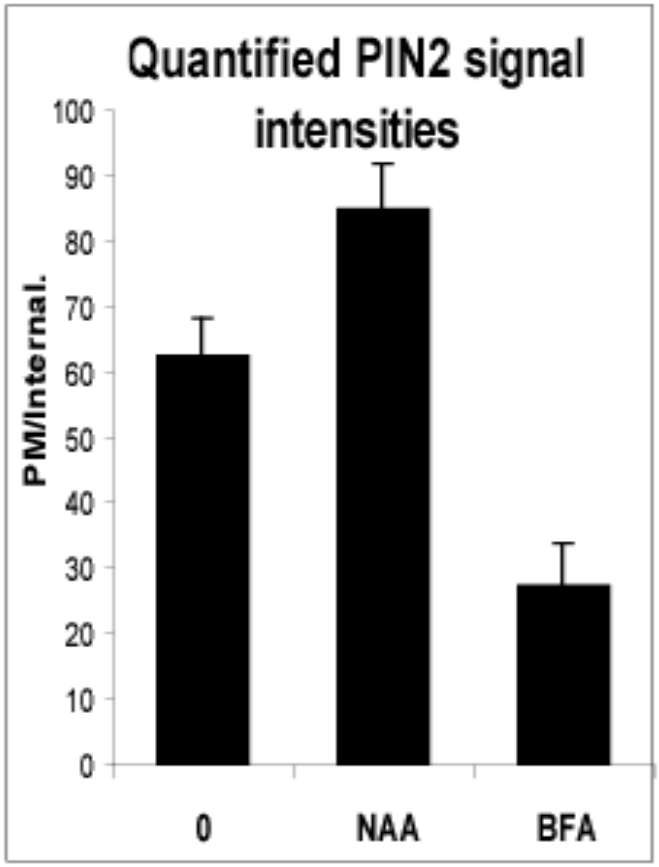
Uptake of endocytic tracer FM4-64



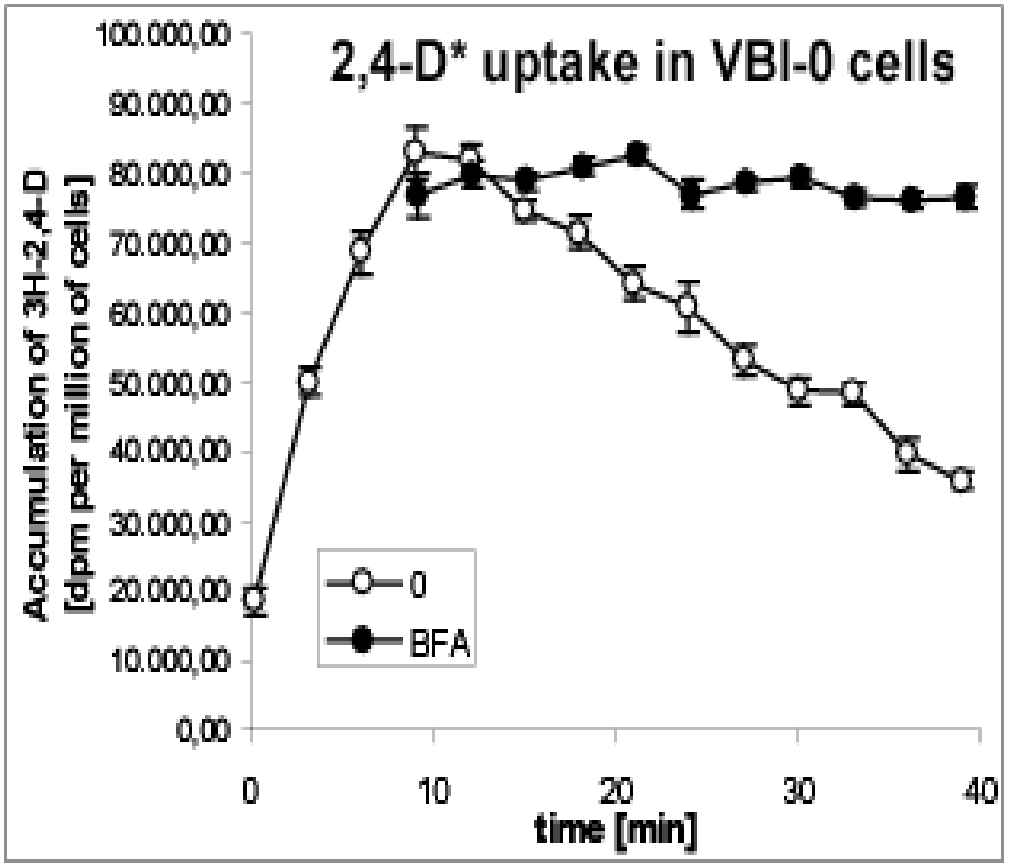
Paciorek et al., 2005

Auxin Increases PIN Levels at Cell Surface and Stimulates its own Efflux

PIN2 levels at PM



Auxin efflux in tobacco cells



Paciorek et al., 2005

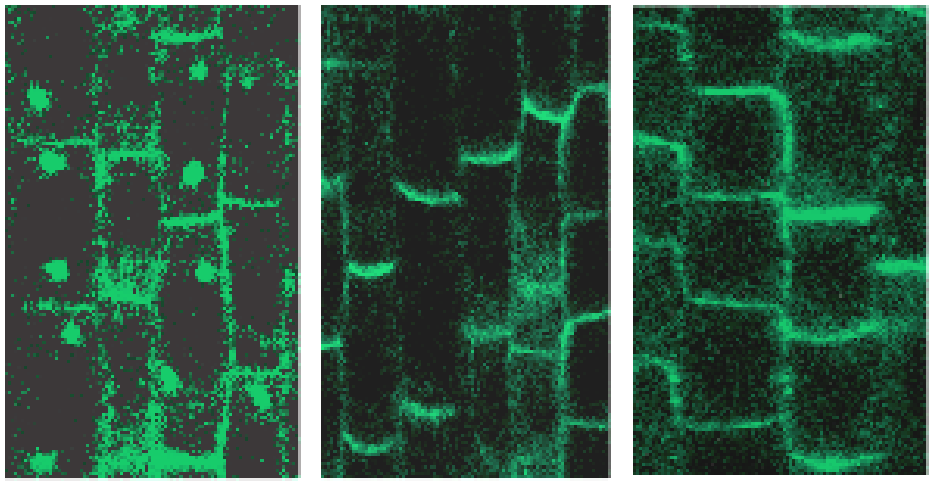
Novel Pathway of Auxin Action

PIN1

BFA

2,4-D/CHX /BFA

2,4-D/MG132 /BFA



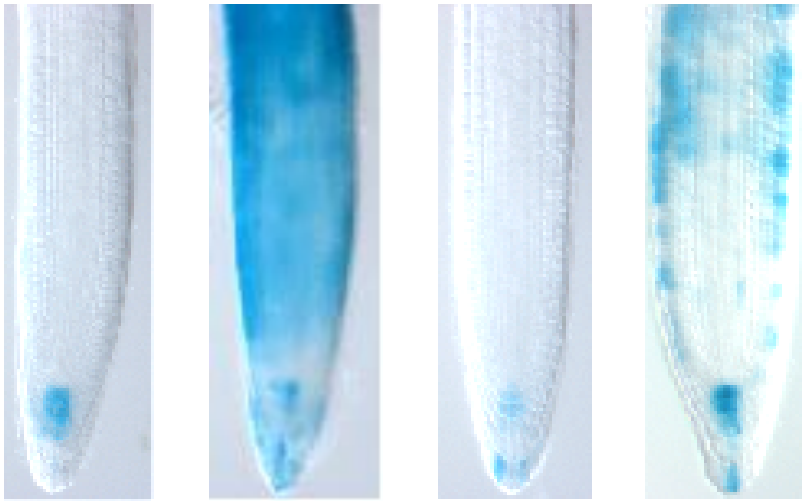
DR5

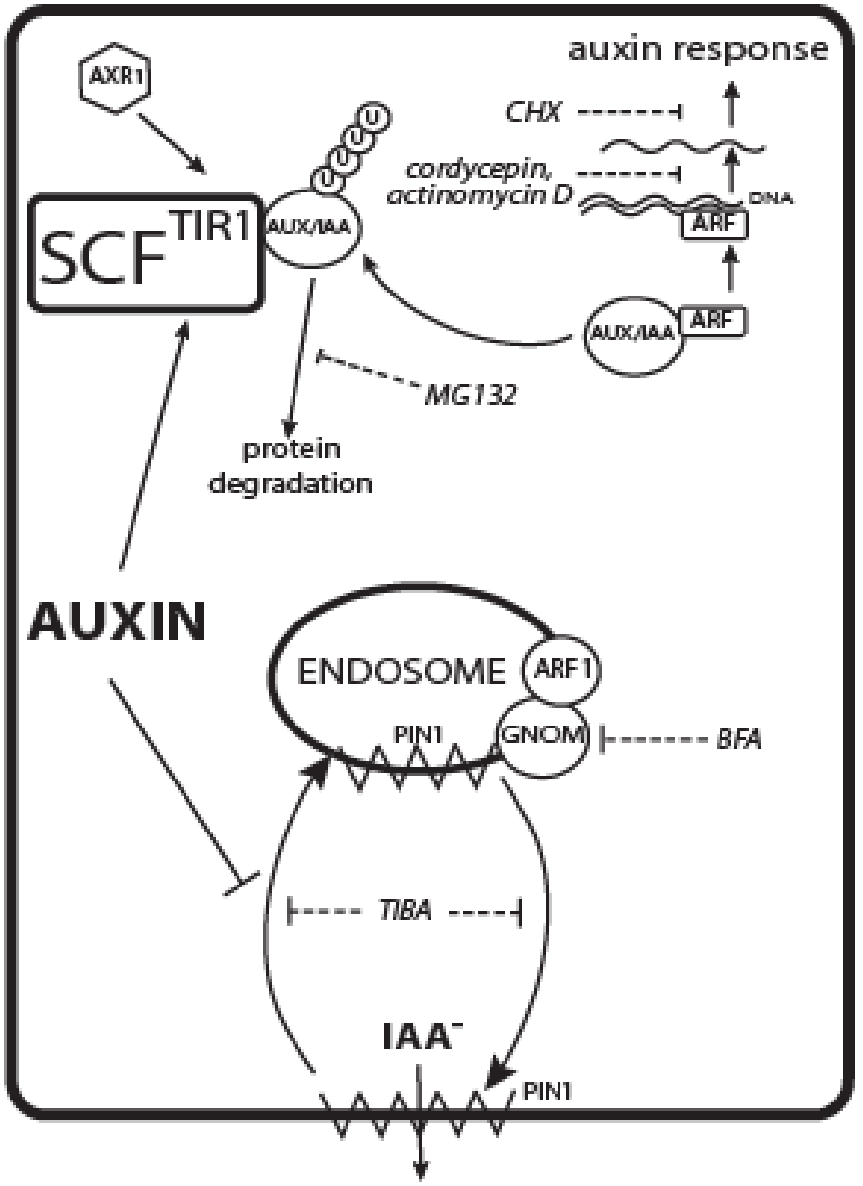
0

2,4-D

2,4-D/ CHX

2,4-D/ MG132

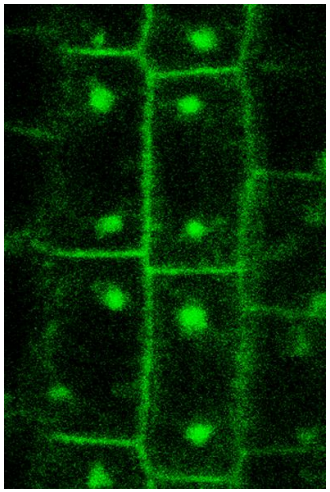




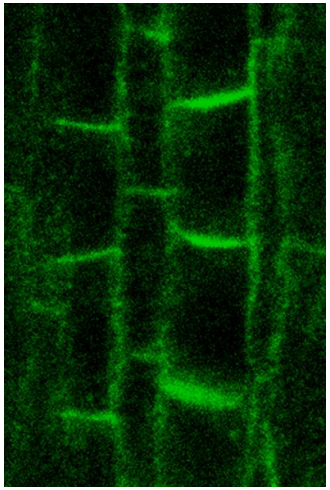
unpublished

Auxin Inhibits PIN Internalization and Stimulates its Efflux

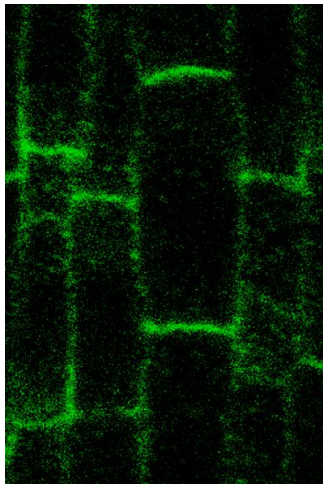
BFA



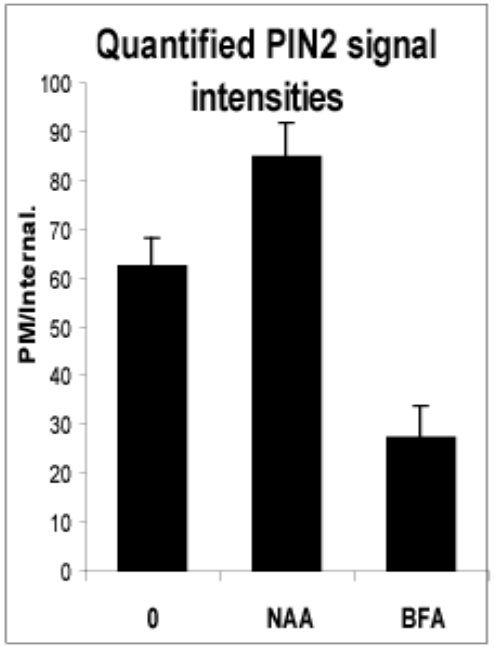
IAA/BFA



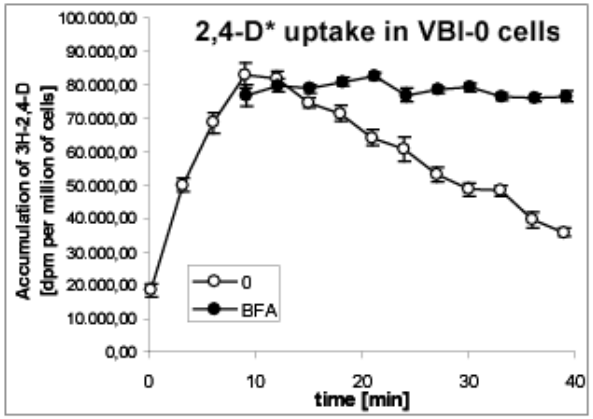
2,4-D/BFA



PIN2 at PM



Auxin efflux

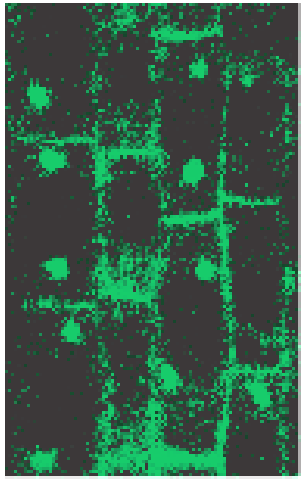


Paciorek et al., 2005

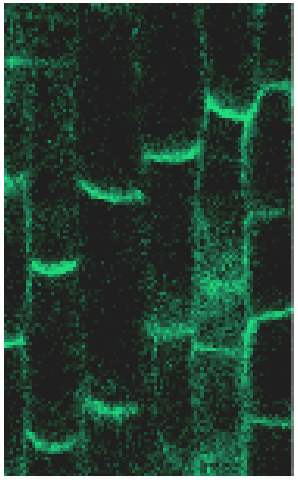
Novel Pathway of Auxin Action

PIN1

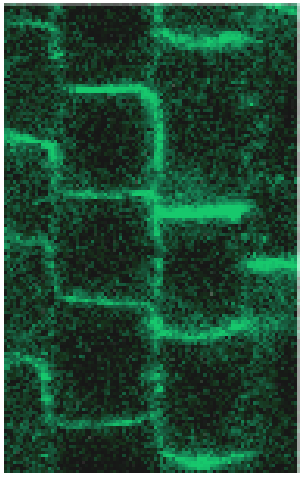
BFA



2,4-D/CHX /BFA

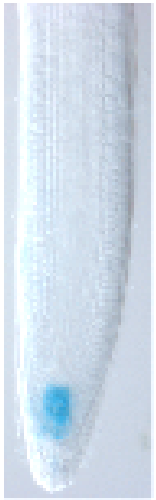


2,4-D/MG132 /BFA



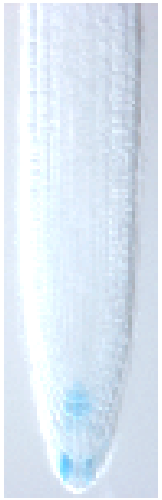
DR5

0

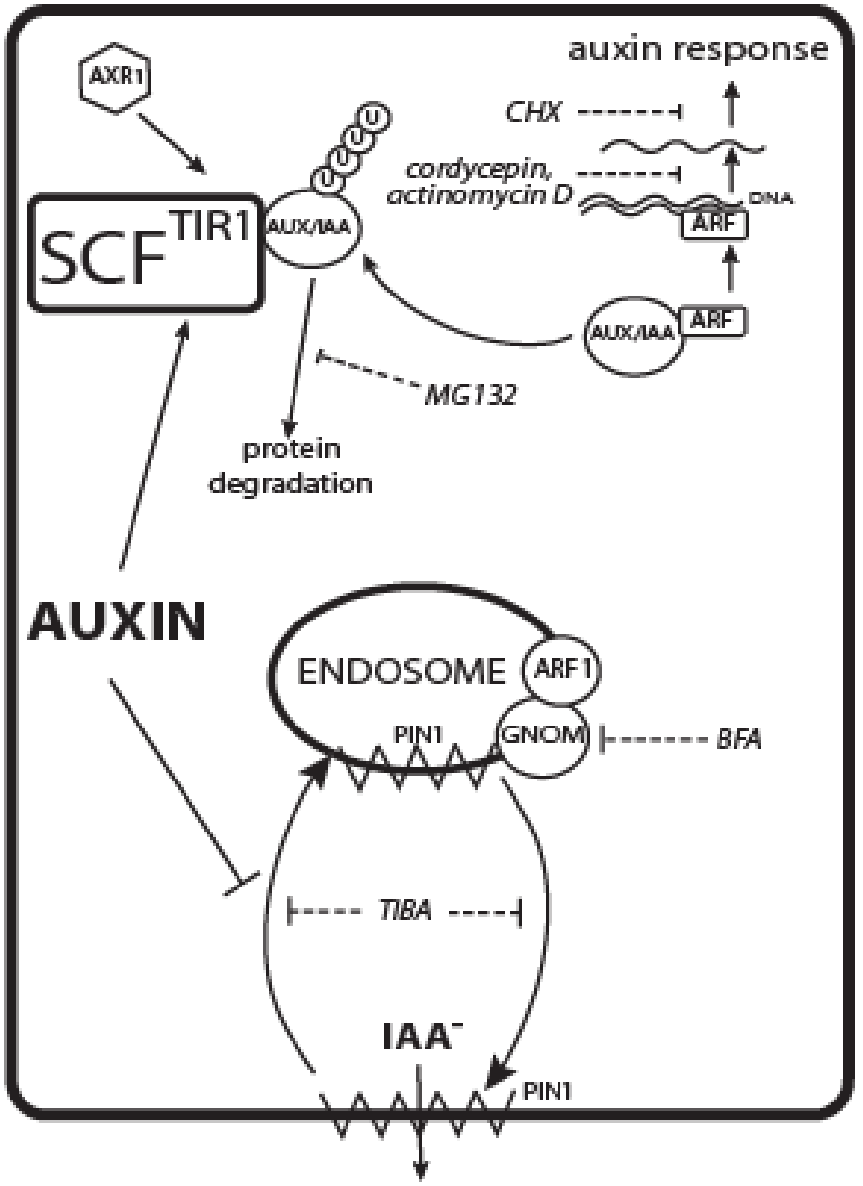


2,4-D

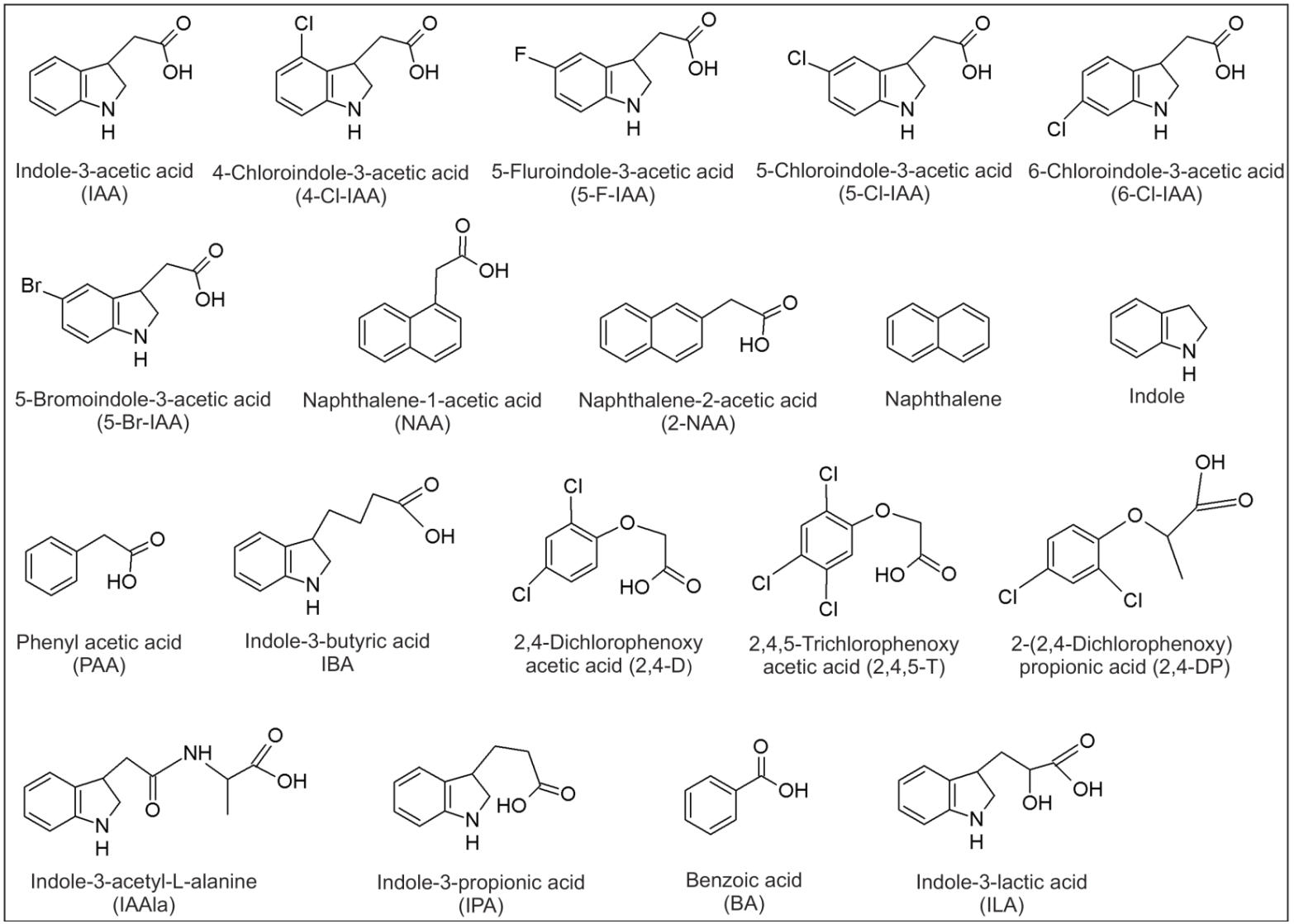
2,4-D/ CHX



2,4-D/ MG132

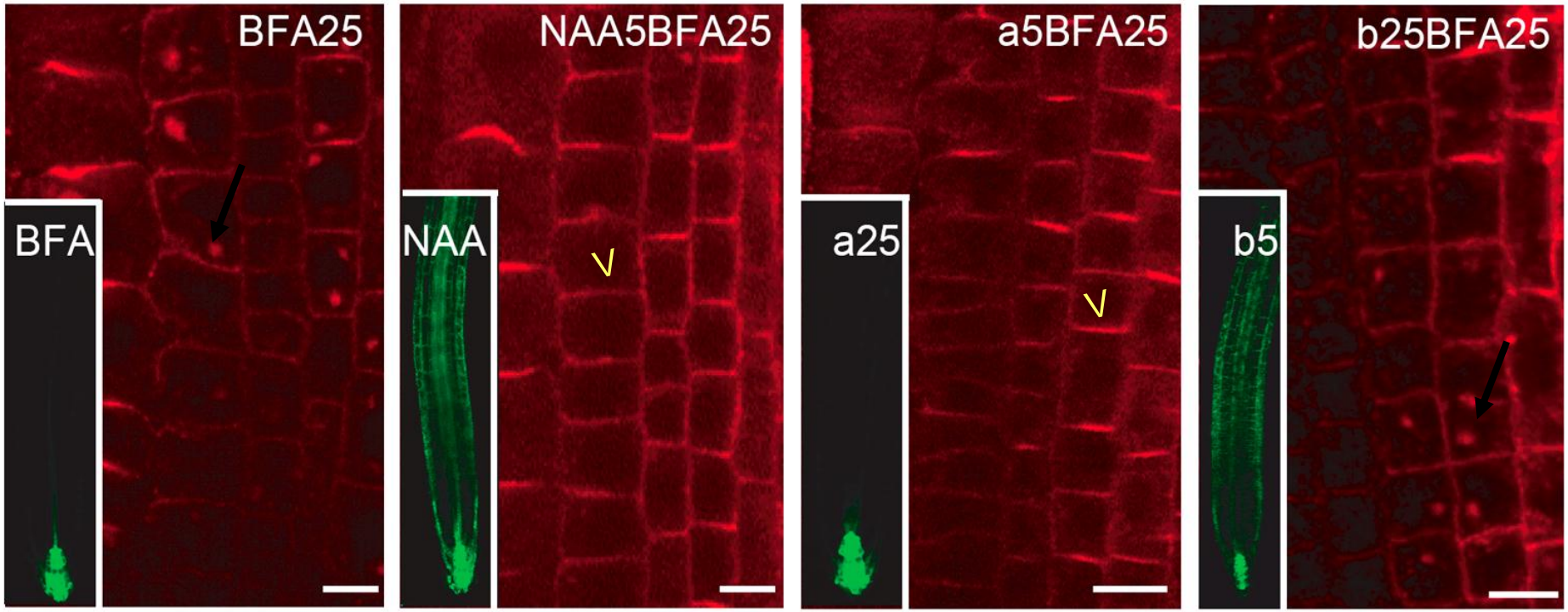


Auxin analogues: mapping the binding sites



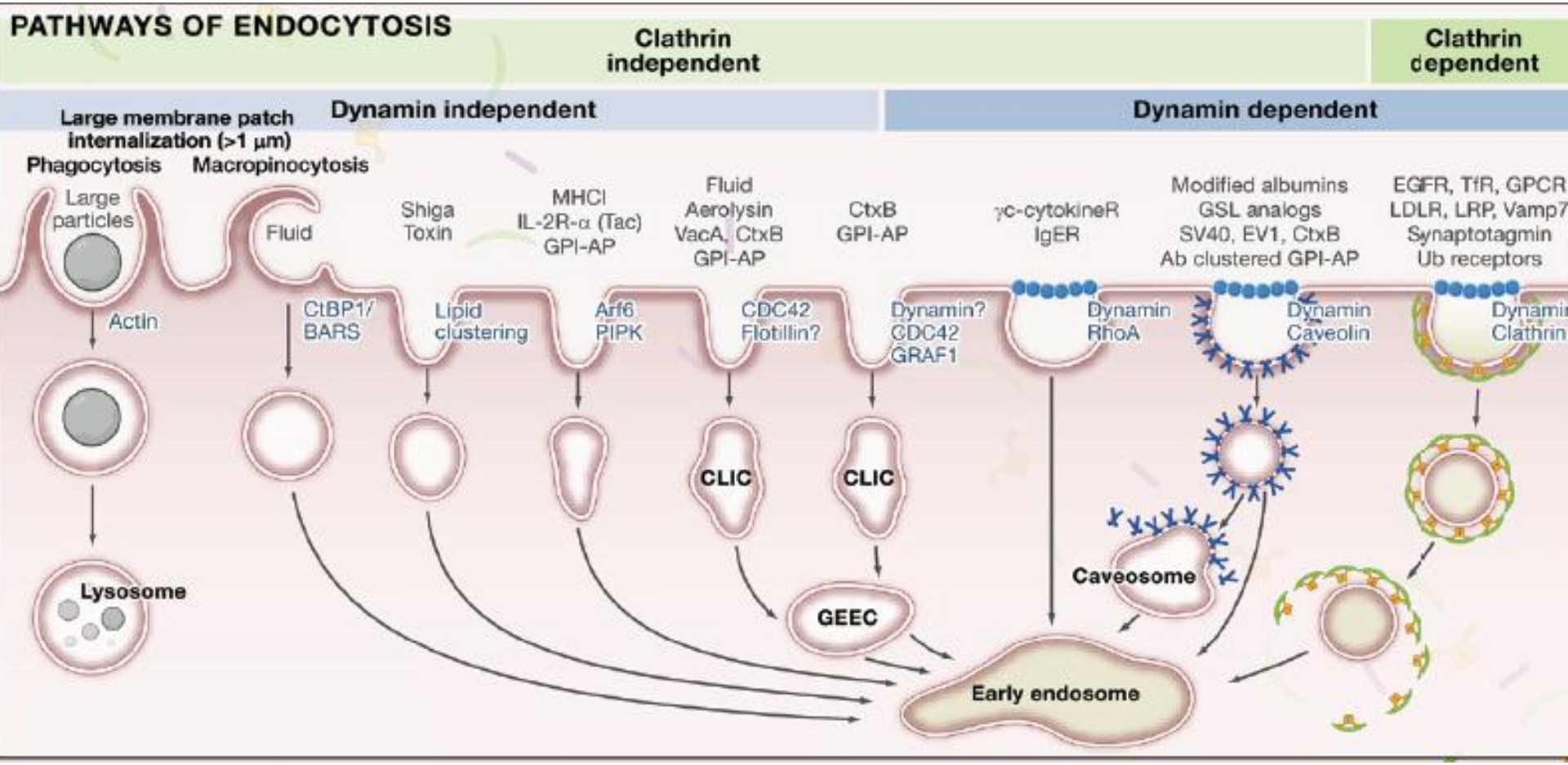
Robert et al., 2010

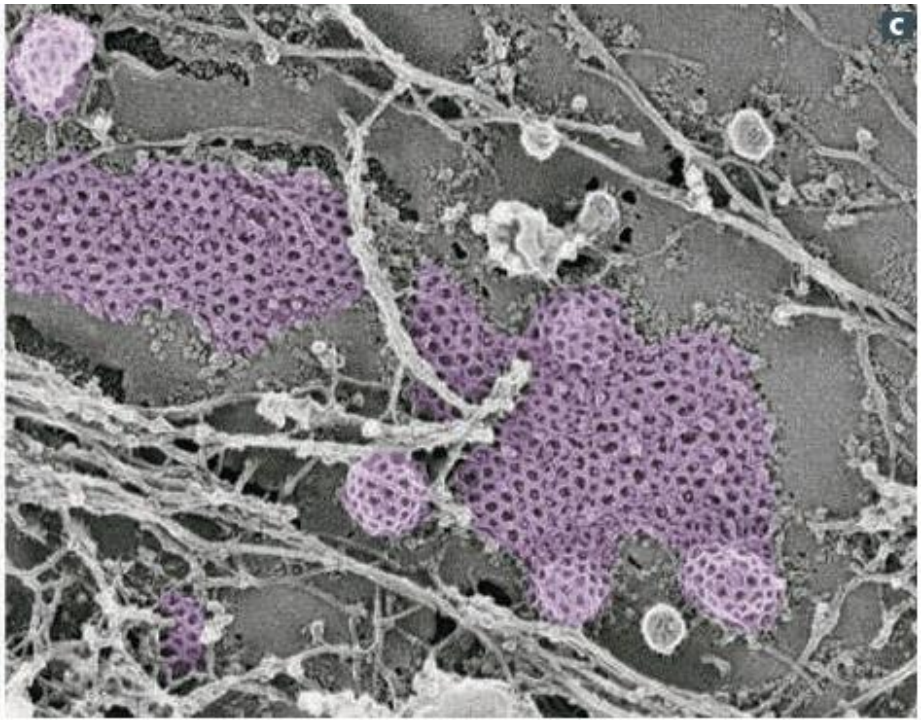
Auxin-mediated regulation of transcription and endocytosis involve different binding sites

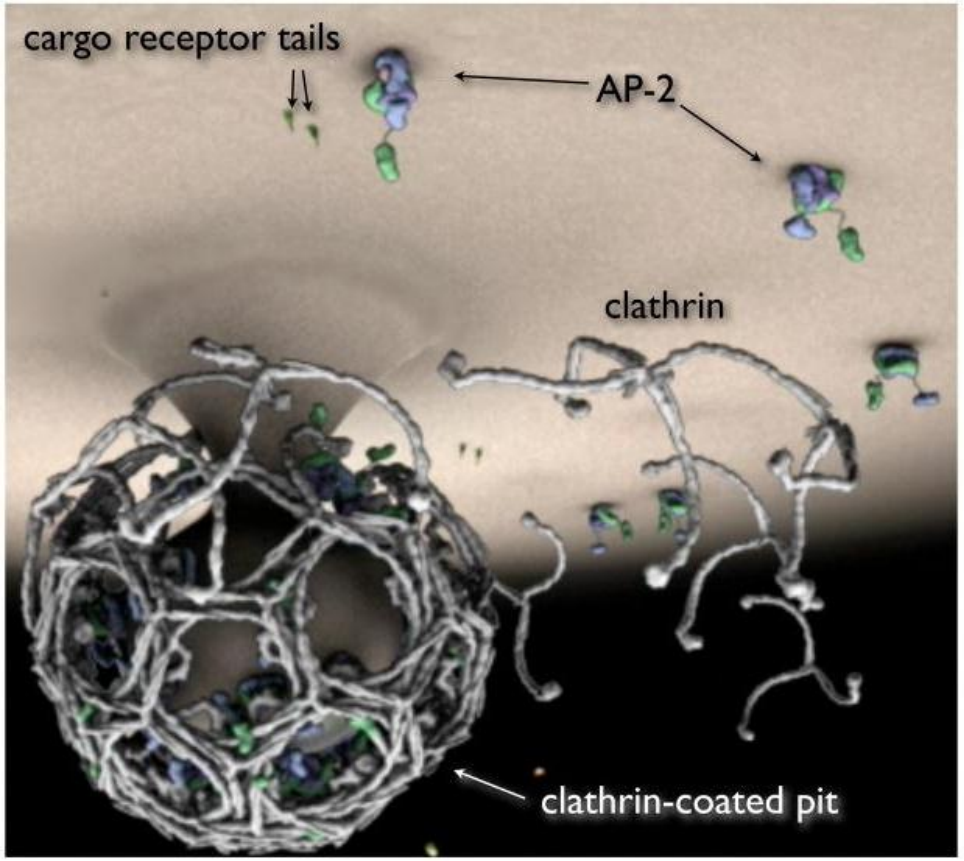


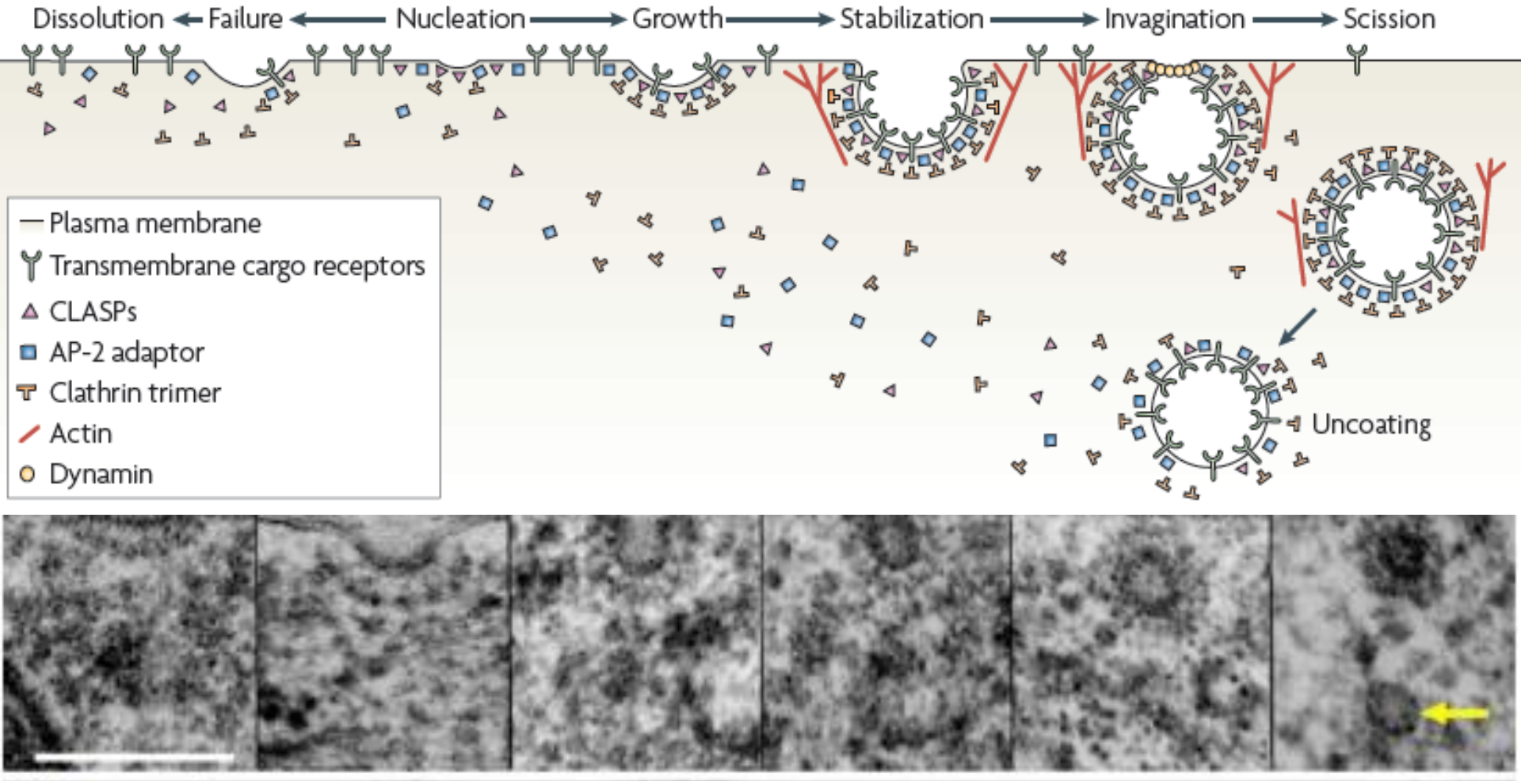
Robert et al., 2010

Mechanisms for endocytosis in animals

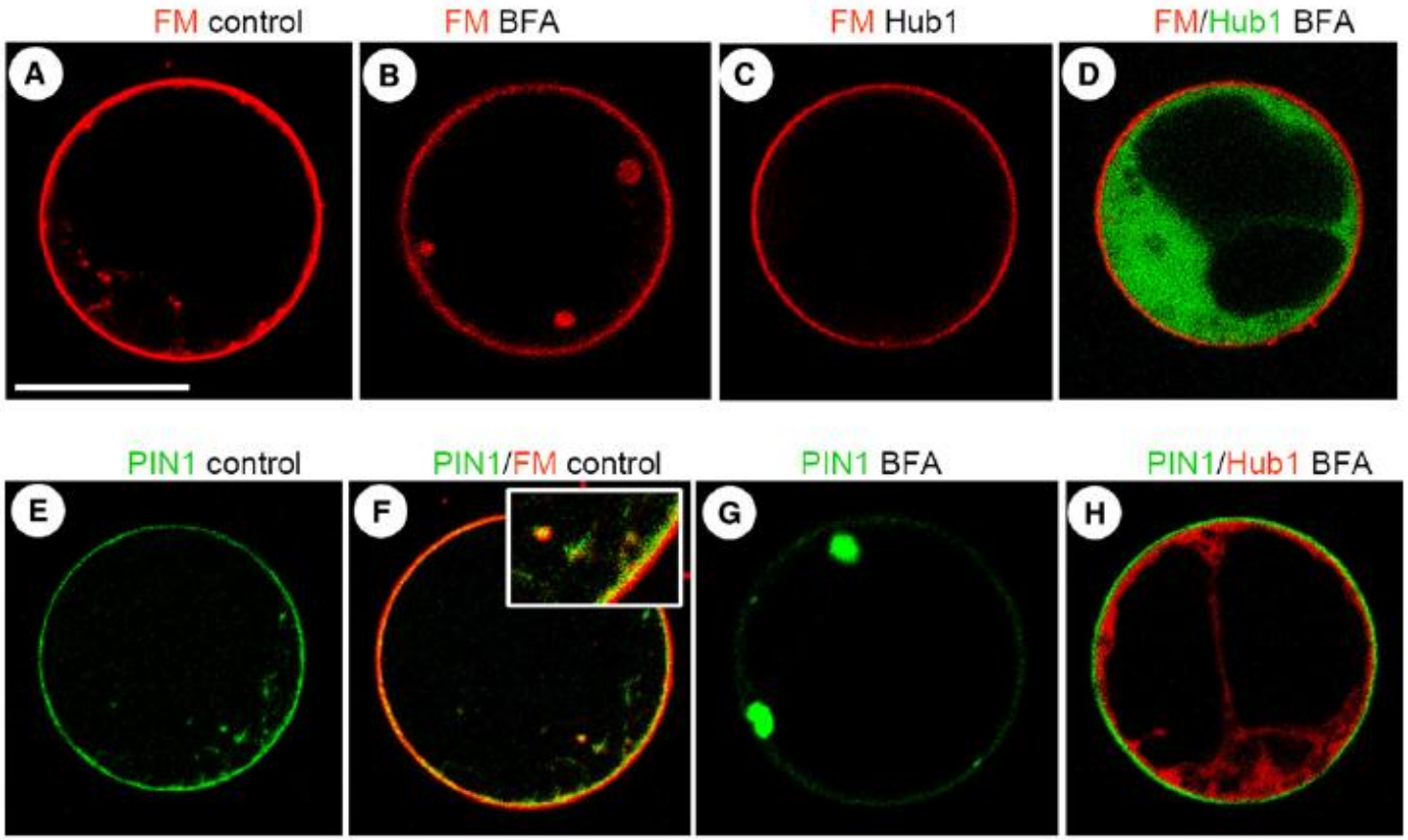



c


cargo receptor tails
AP-2
clathrin
clathrin-coated pit

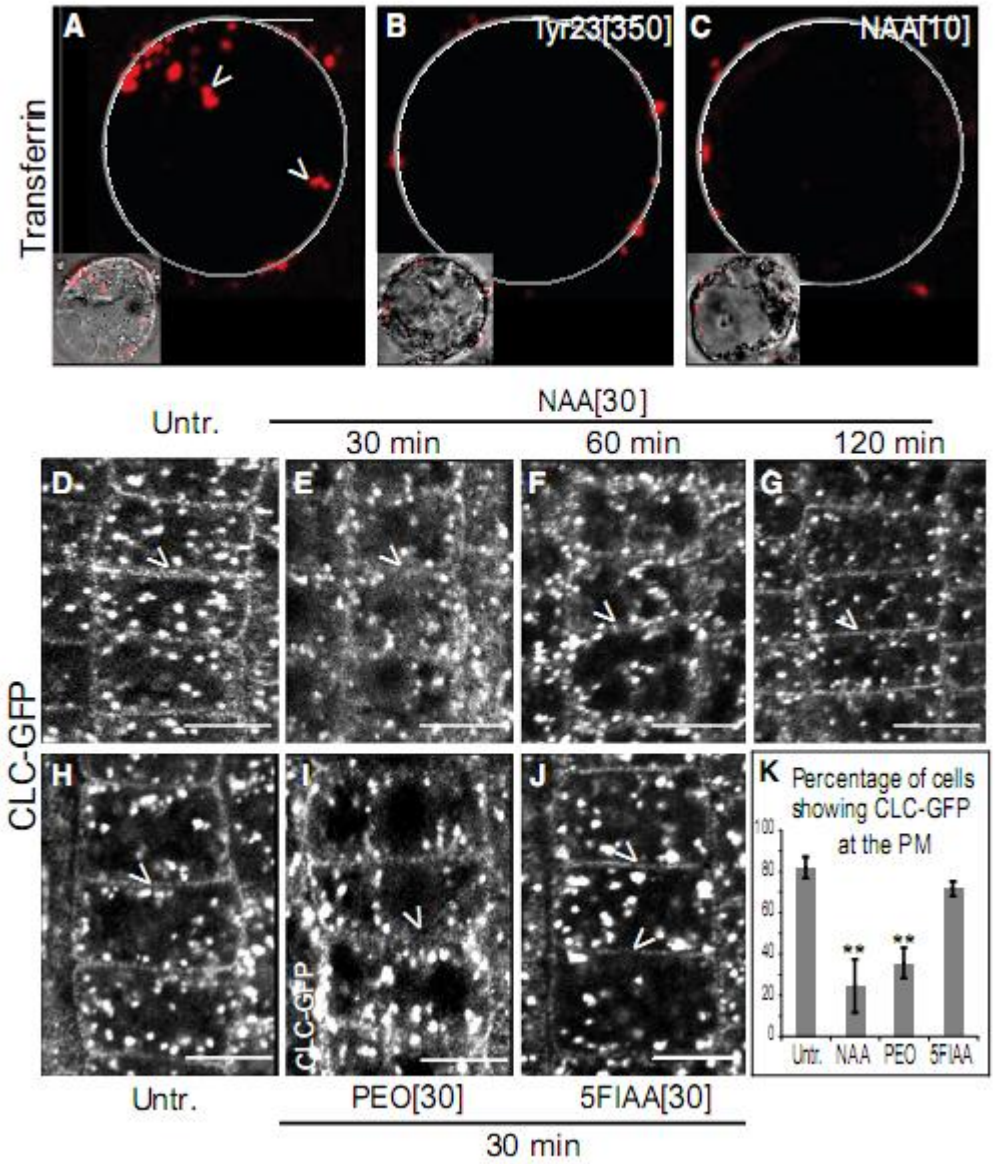

Dissolution
Failure
Nucleation
Growth
Stabilization
Invagination
Scission
Plasma membrane
Transmembrane cargo receptors
CLASPs
AP-2 adaptor
Clathrin trimer
Actin
Dynamin
Uncoating

Clathrin is required for PIN internalization in Arabidopsis protoplasts



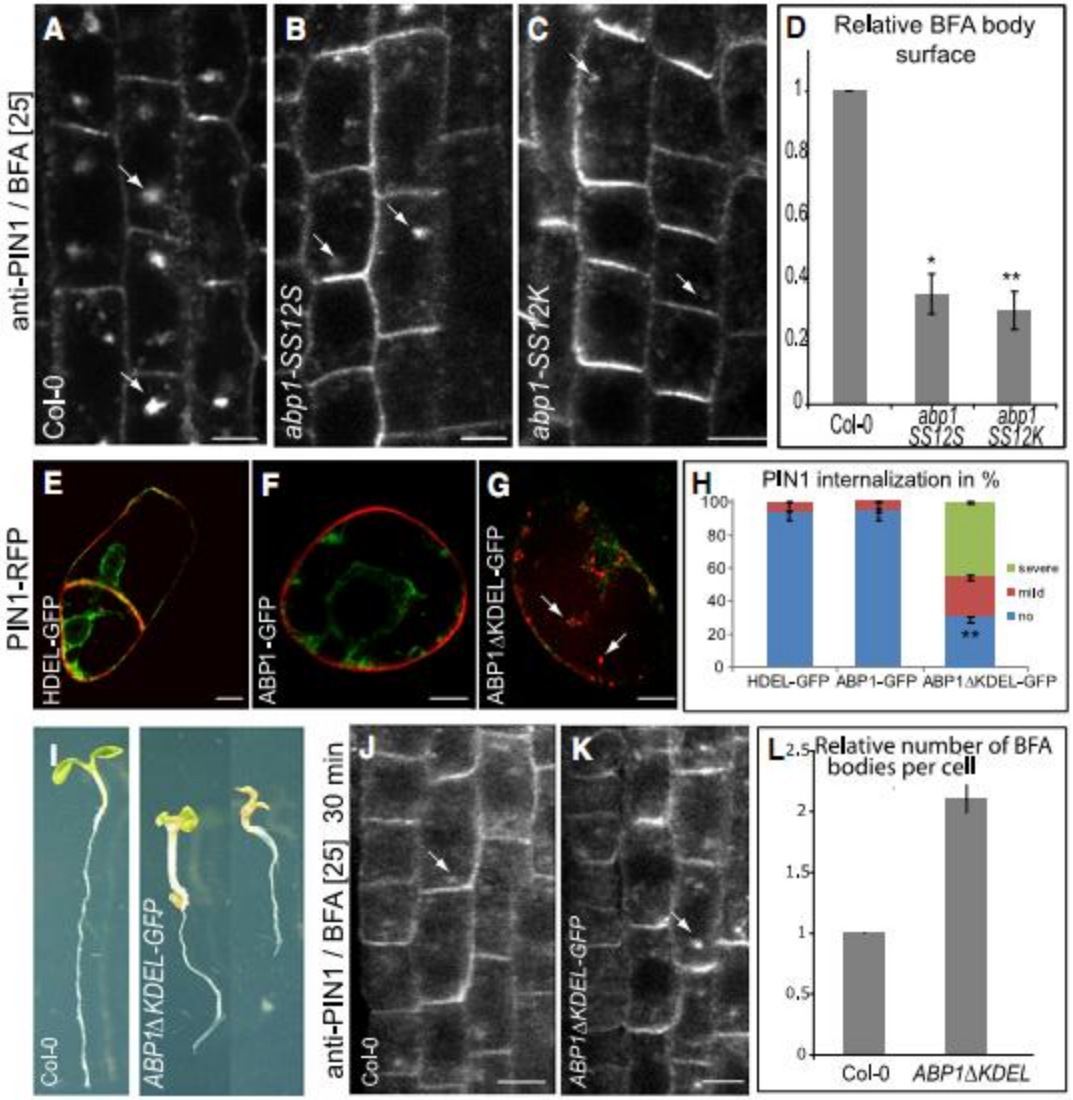
Dhonukshe et al., 2007

Auxin targets clathrin mechanism of endocytosis



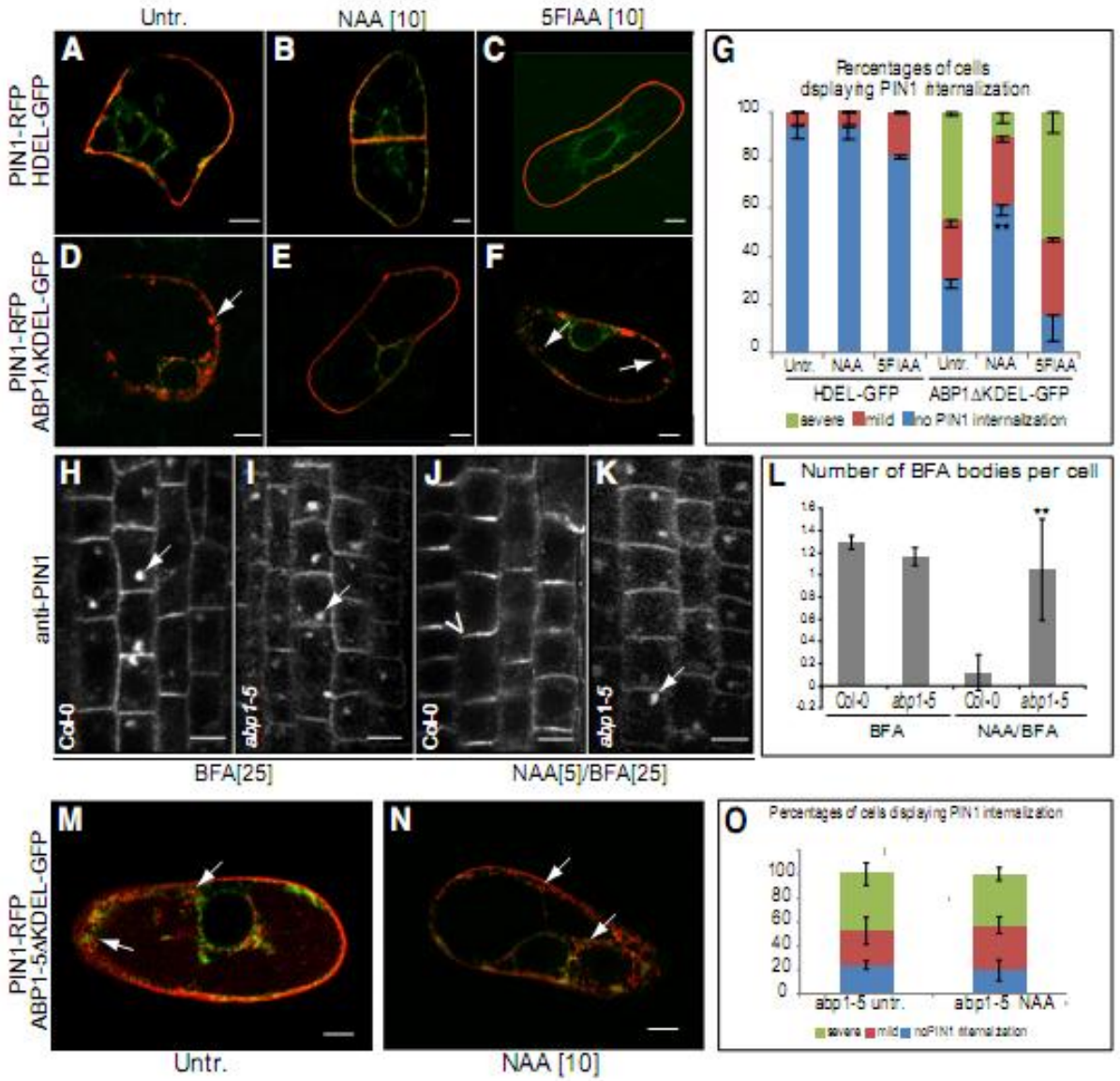
Robert et al., 2010

ABP1 positively regulates endocytosis



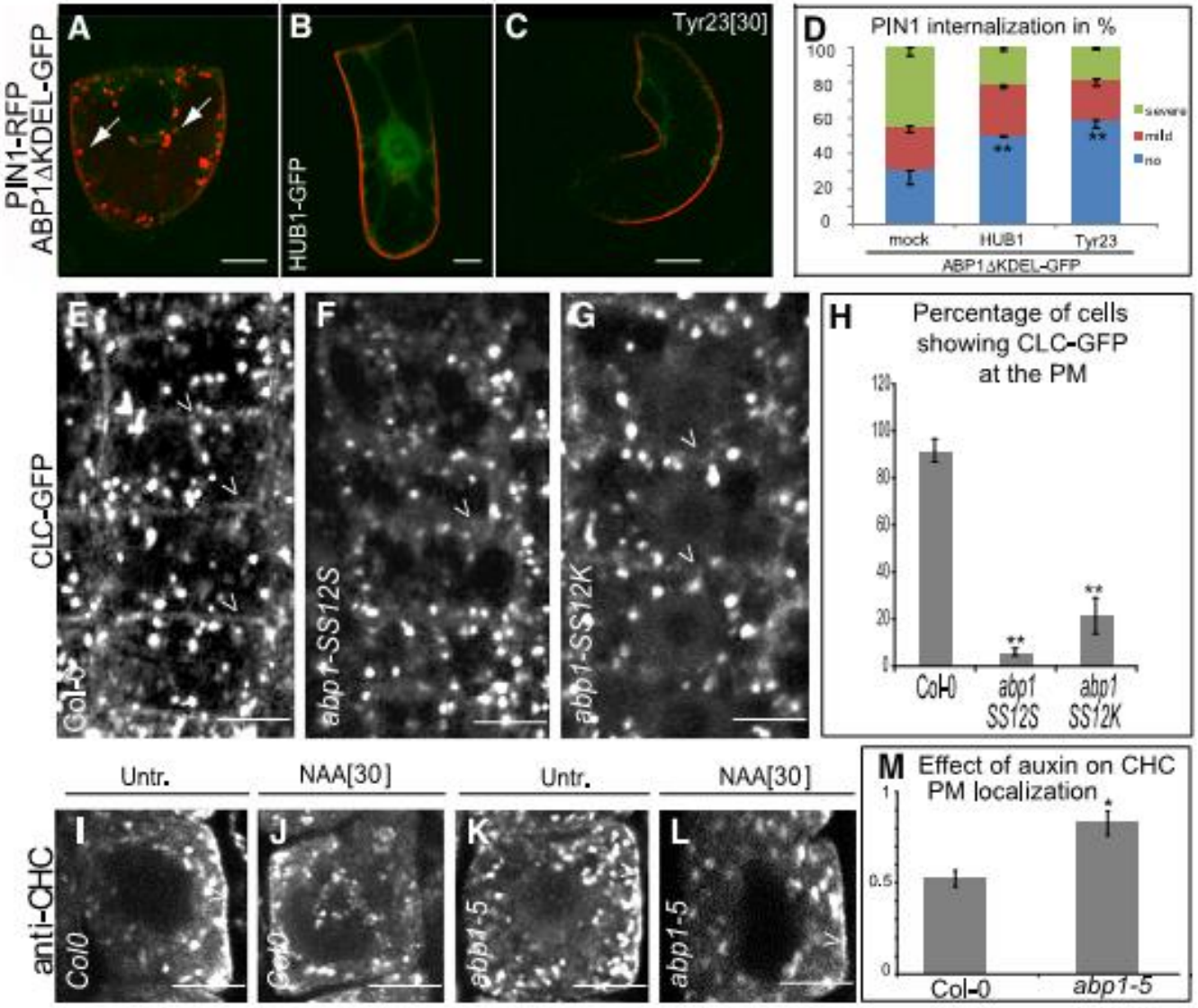
Robert et al., 2010

ABP1 mediates auxin effect on endocytosis



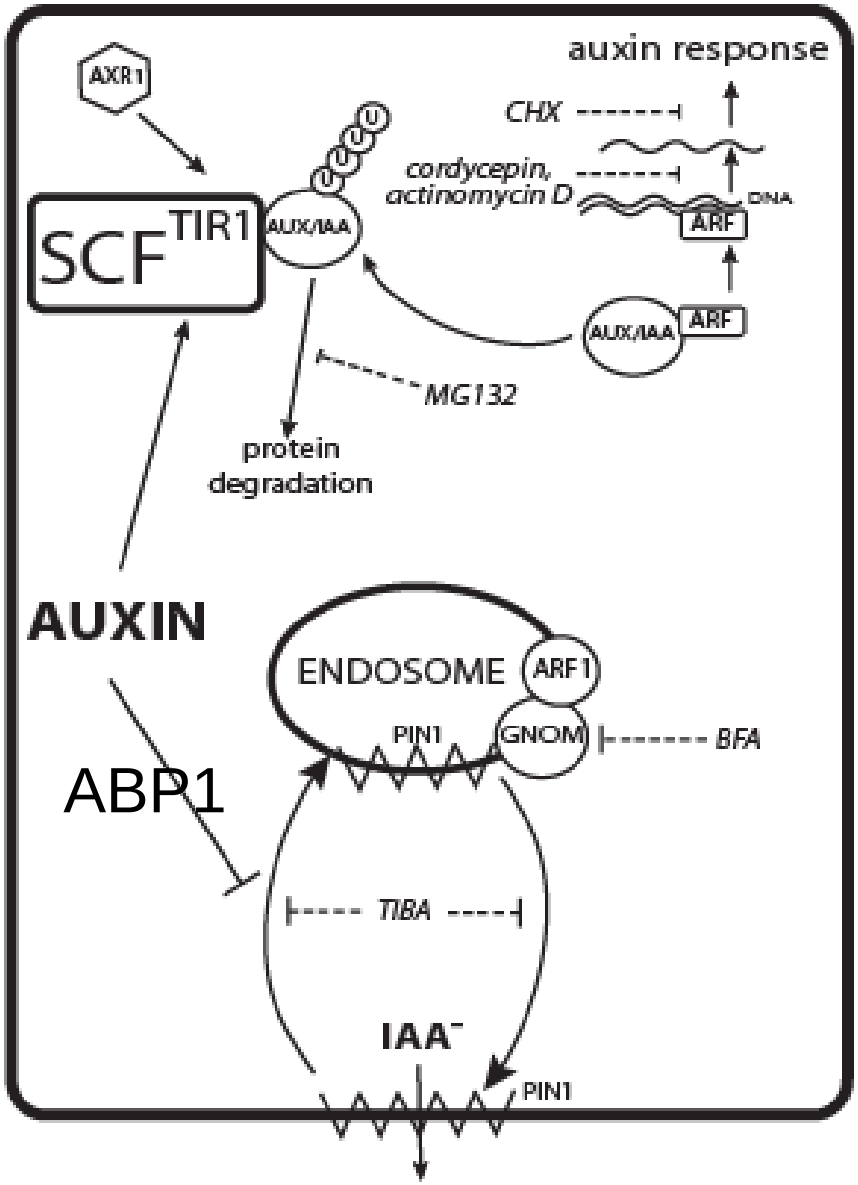
Robert et al., 2010

ABP1 mediates auxin effect on clathrin



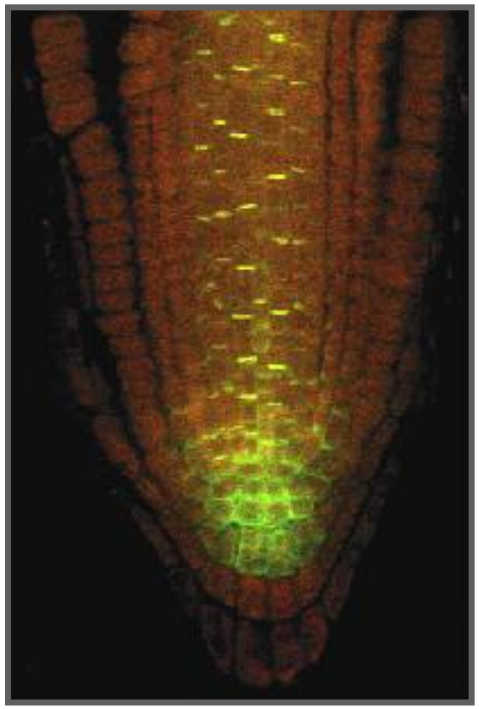
Robert et al., 2010

ABP1- and TIR1-dependent Signaling



unpublished

Mutant Screen for Components of PIN Polarity and Cycling



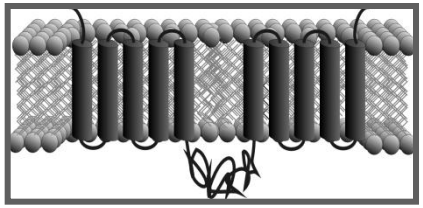
PIN:GFP

EMS mutagenesis. Screening for polarity and cycling defects.

[redacted]

intragenic

extragenic

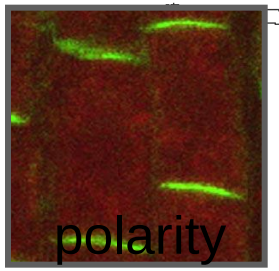


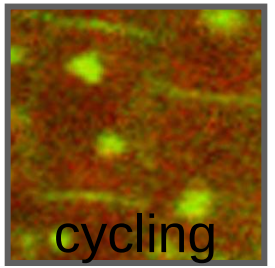
sequencing

cloning

important residues

novel genes



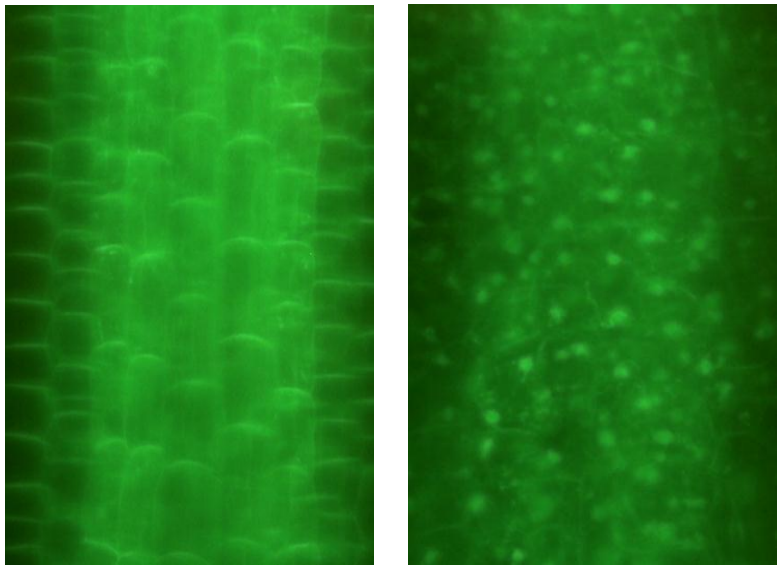


unpublished

“Cell Biological” Mutant Screens in Progress:

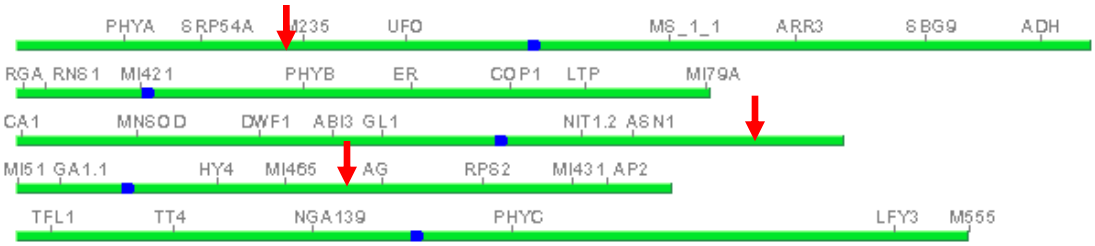
Auxin effect on endocytosis: 3 confirmed mutants

30´ NAA 30 µM/90´BFA 50 µM



wt mutant

Auxin-resistant BFA patches mutants



unpublished

Novel Pathway for Auxin Signaling

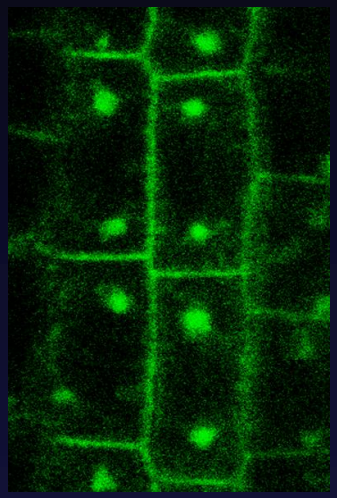
Auxin inhibits endocytosis including internalization of PIN proteins

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

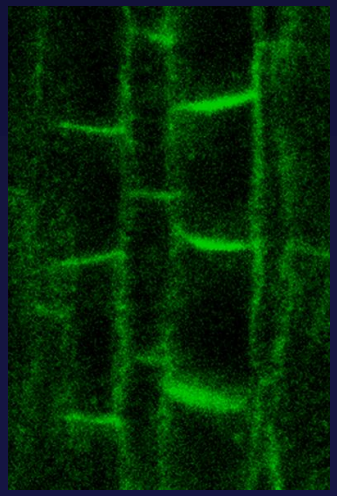
This auxin effect involves novel, genetically tractable auxin pathway

Auxin Inhibits PIN Internalization and Stimulates its Transport

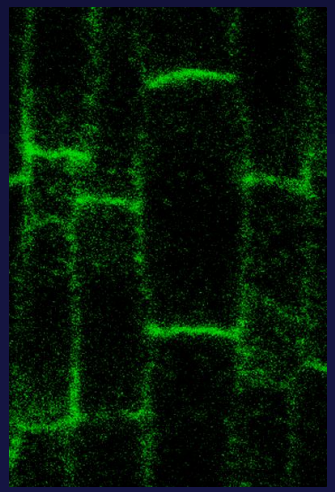
BFA



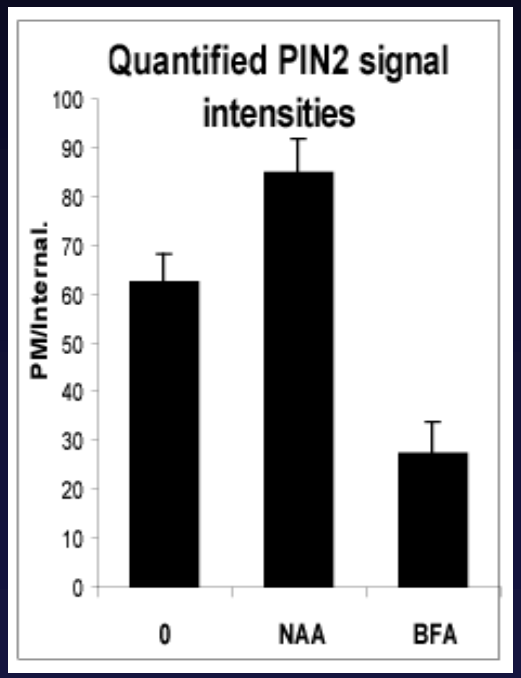
NAA/BFA



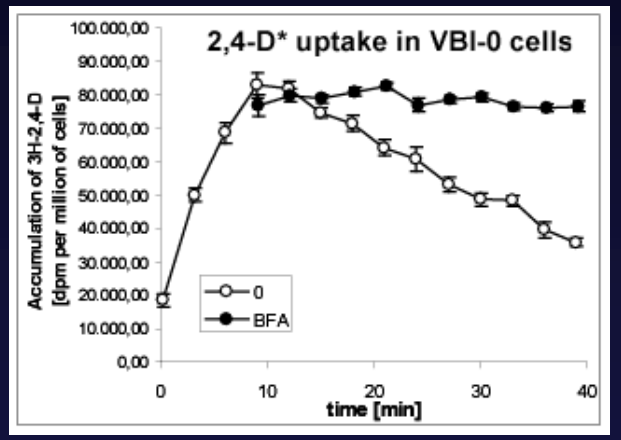
2,4-D/BFA



PIN2 at PM

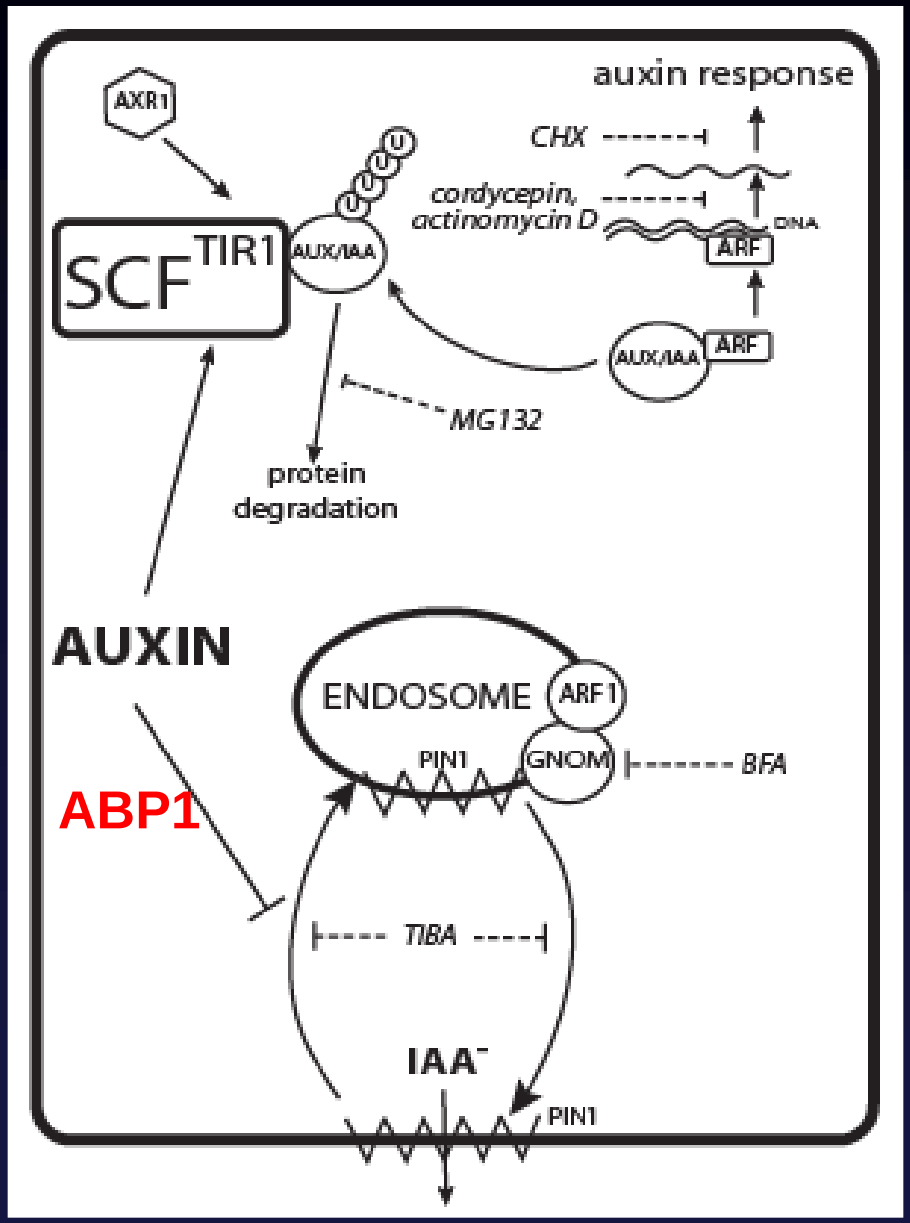


Auxin efflux



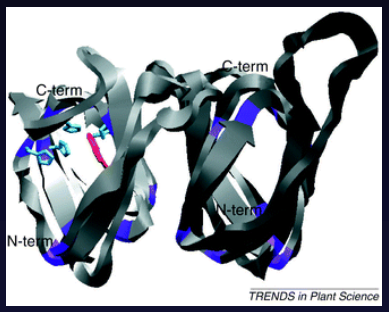
Paciorek et al., 2005

Auxin Signaling for Endocytosis

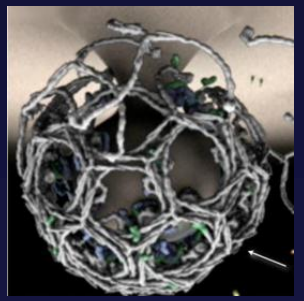


Auxin

ABP1



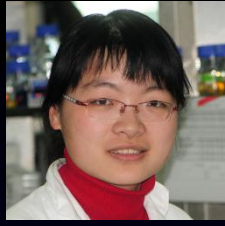
Clathrin

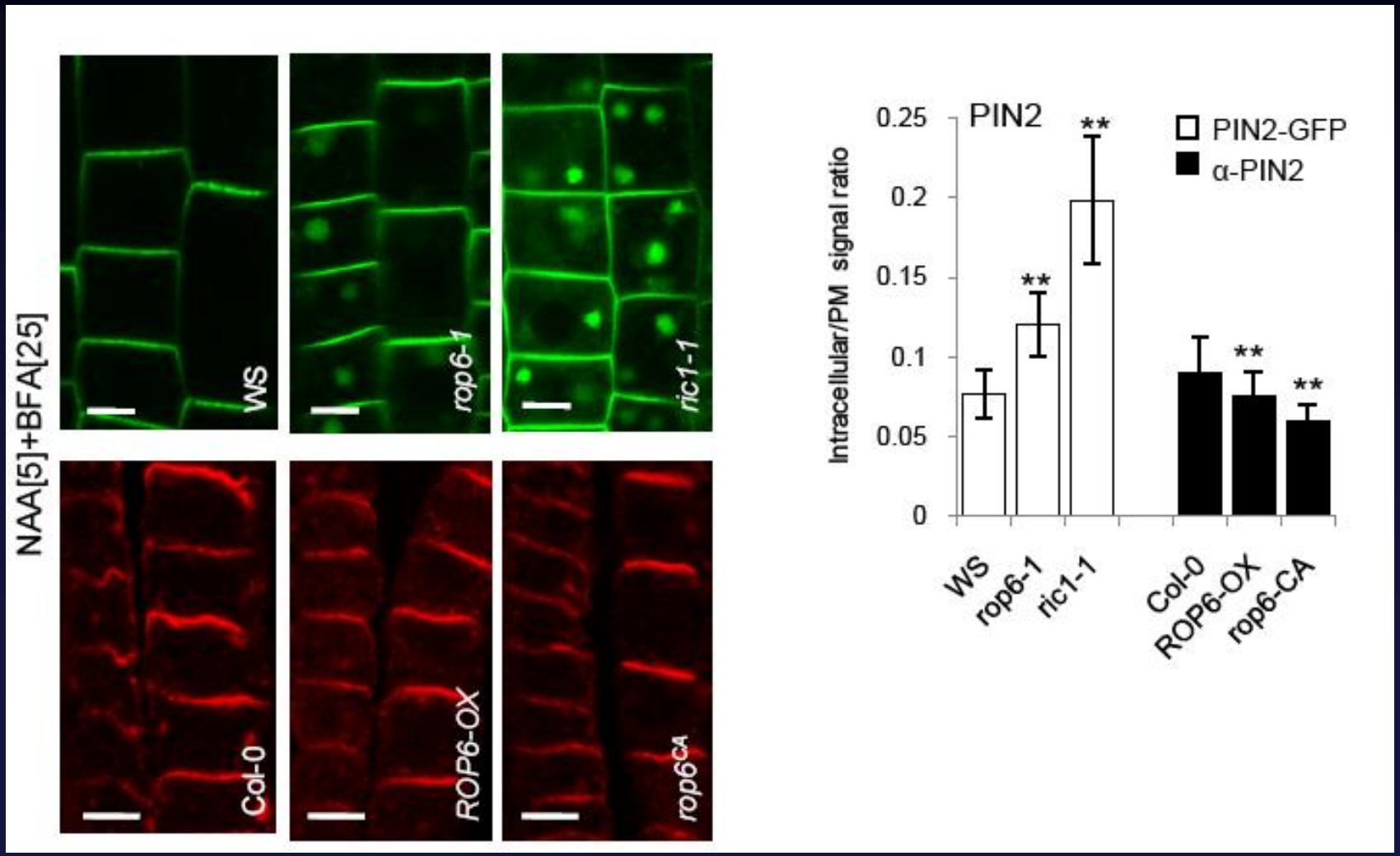


PIN endocytosis

Dhonukshe et al., 2007; Robert et al., 2011; unpublished

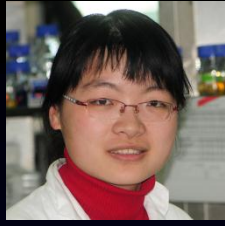
Auxin effect on PIN endocytosis requires ROP6/RIC1 activity

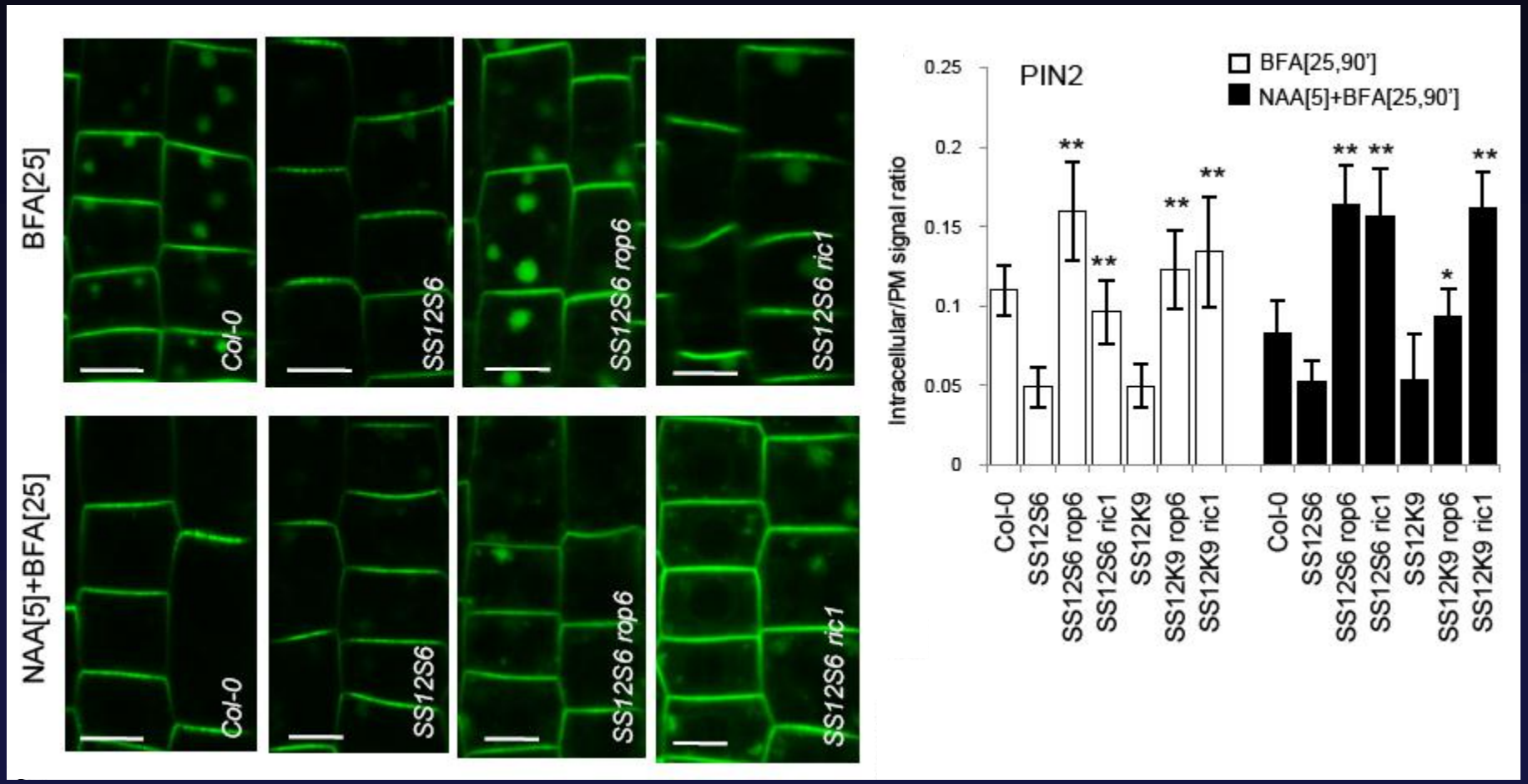




unpublished

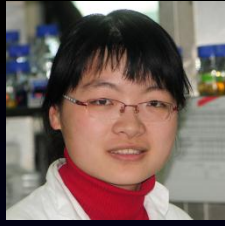
ROP6/RIC1 acts downstream of ABP1 to regulate endocytosis





unpublished

ABP1/ROP signaling for endocytosis and polarity



Auxin

?

ABP1

?

ROP

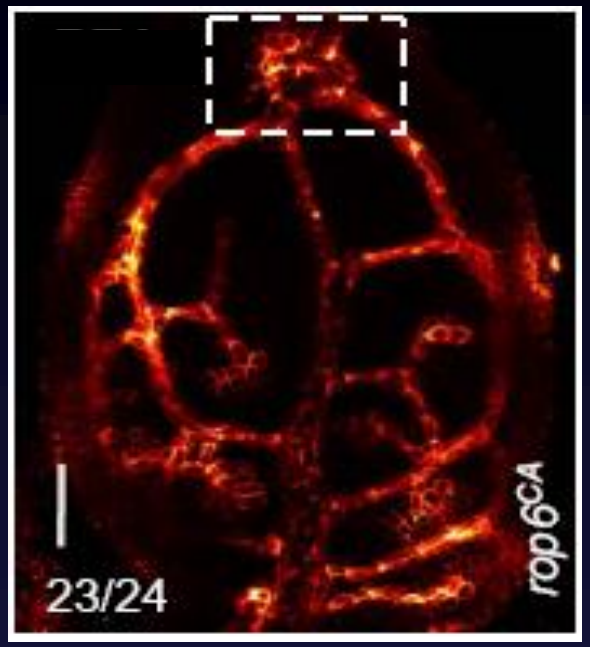
?

Clathrin

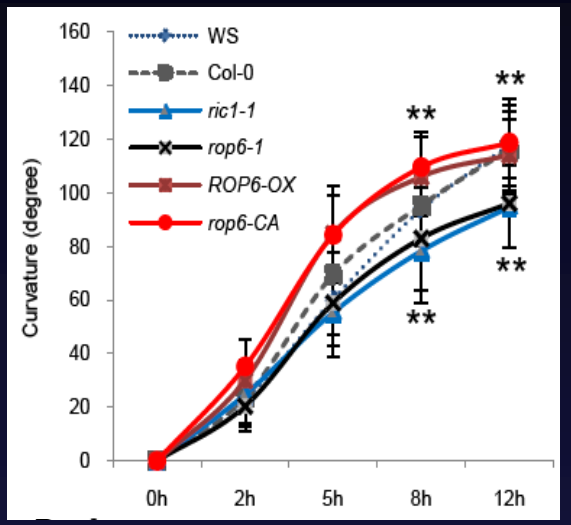
PIN endocytosis

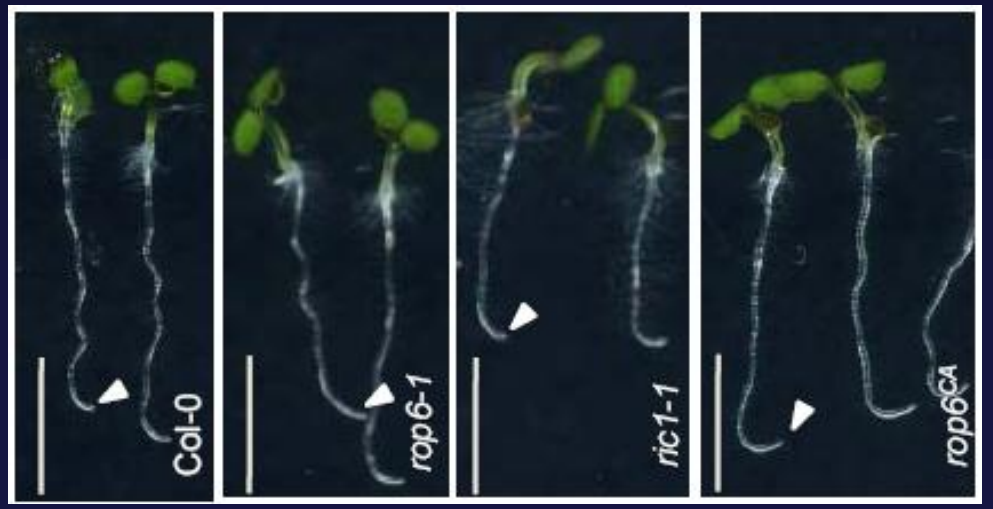
PIN polarity

Vascular tissue formation



Gravitropism





unpublished