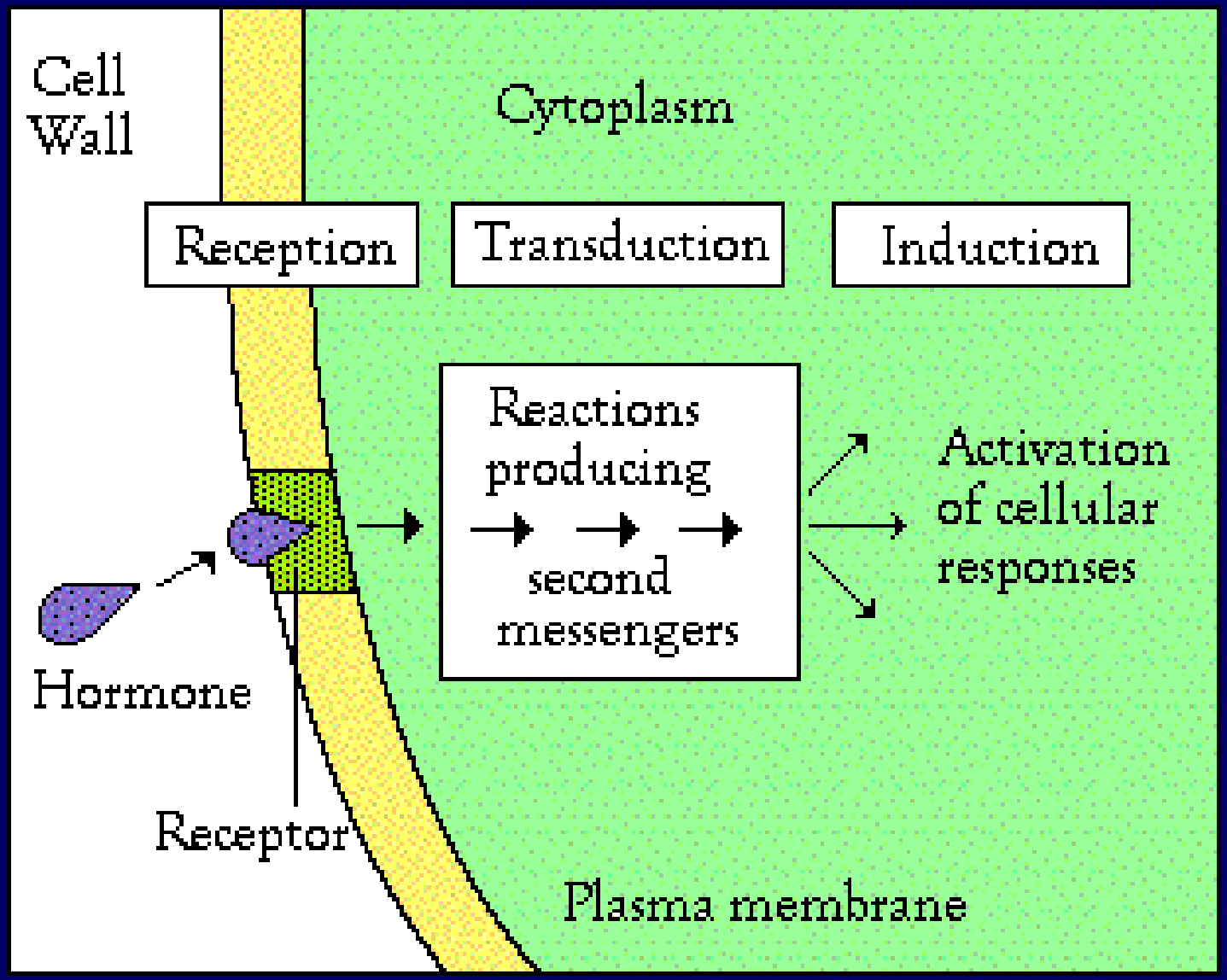
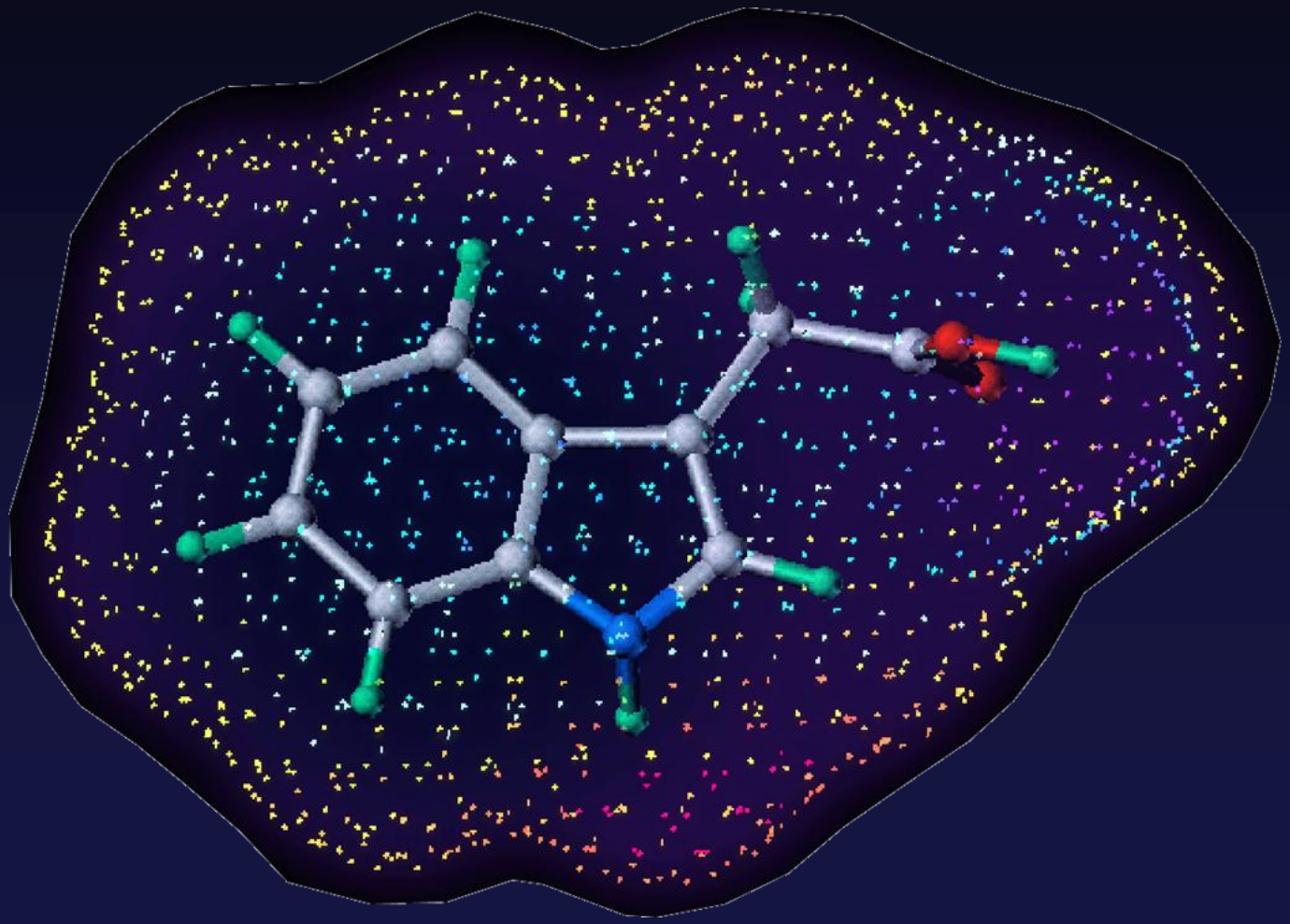
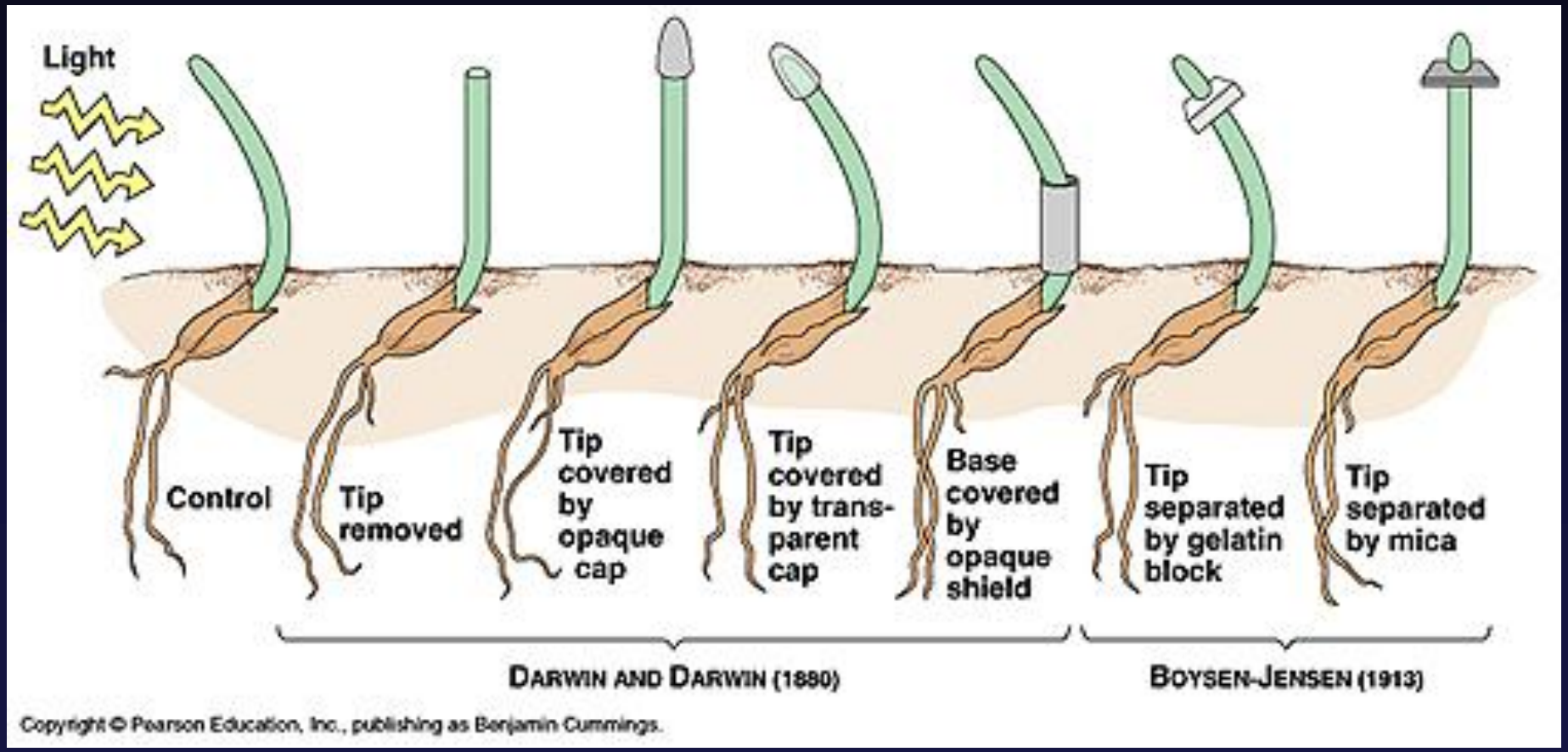
Signal Transduction



Auxin Signaling and Transport

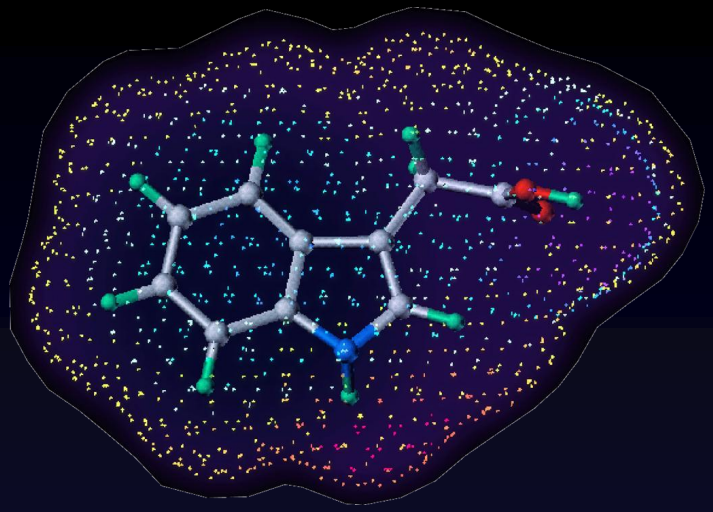


Discovery of the First Plant Signaling Molecule – Auxin and its Transport



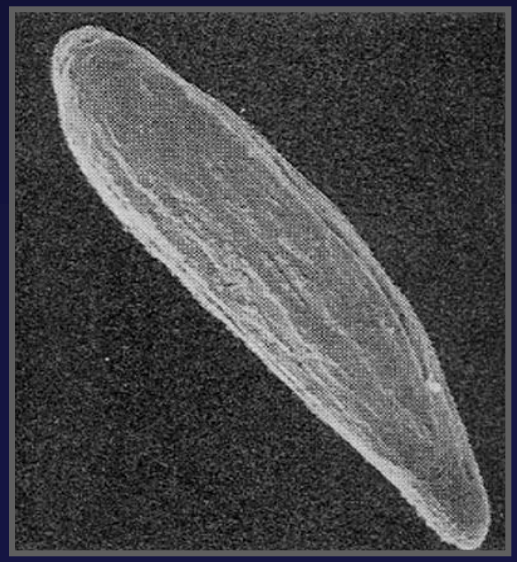
AUXIN

mediates

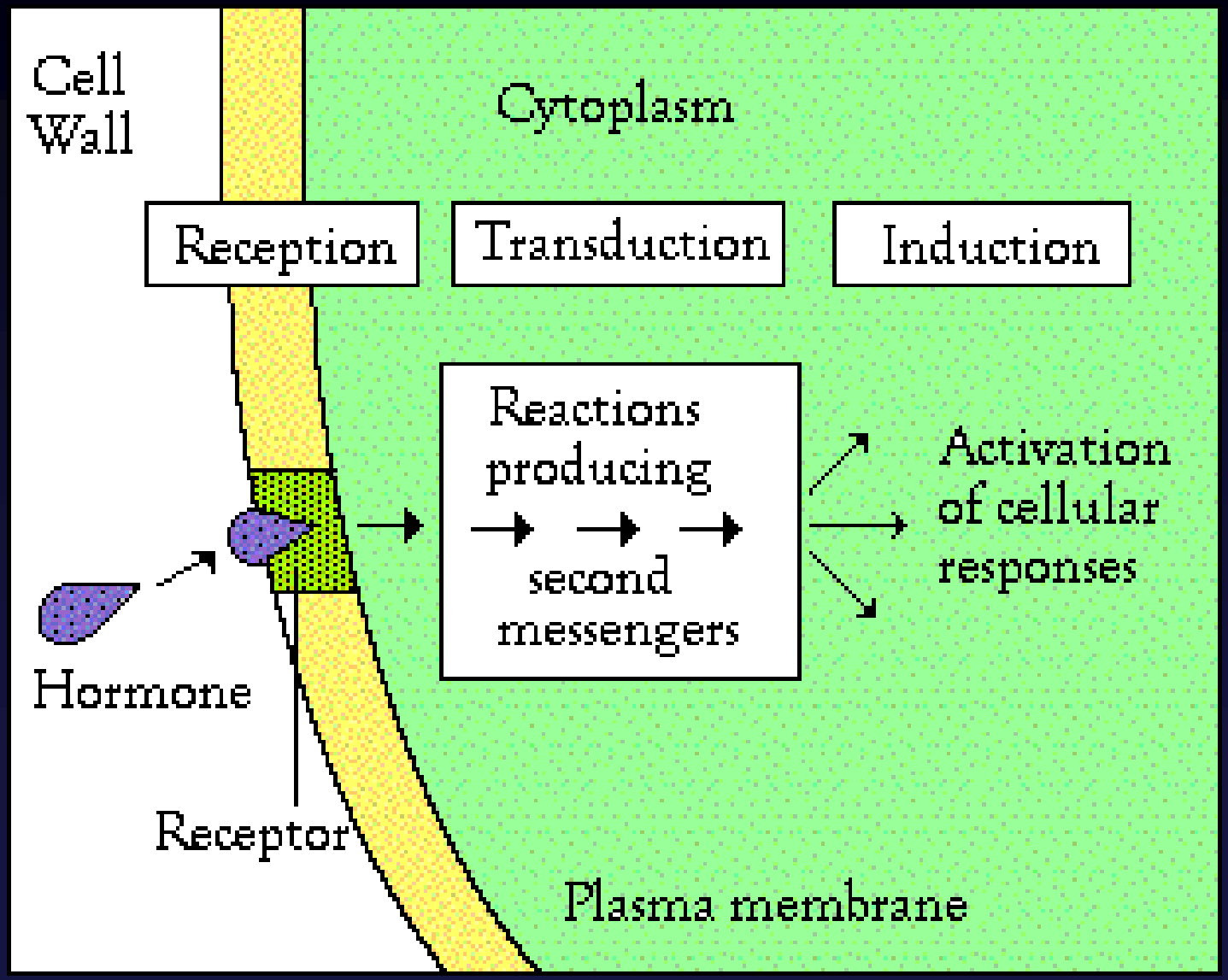


- Embryo development
- Organ initiation and positioning
- Vascular tissue differentiation
- Shoot and root elongation
- Growth responses to light and gravity
- Apical hook formation

embryos



Signal Transduction



Biochemical Approach to Identify Auxin Receptor

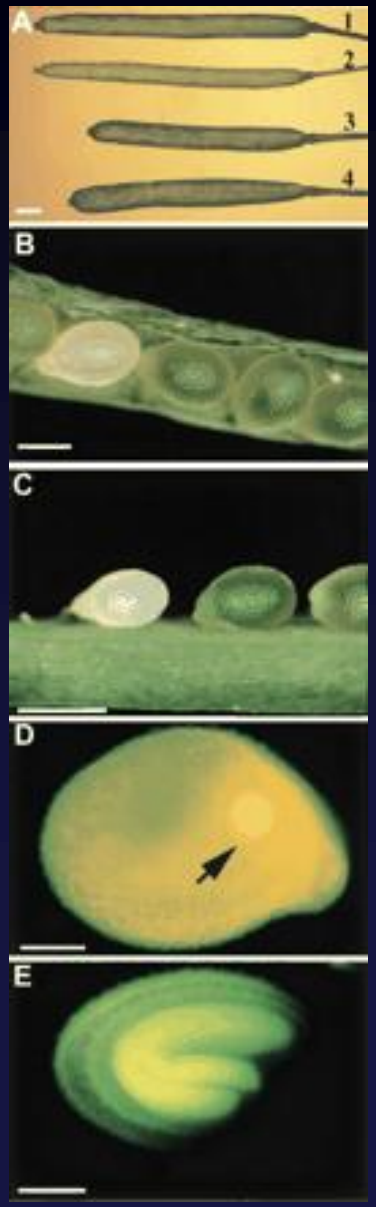
Isolation of auxin binding proteins

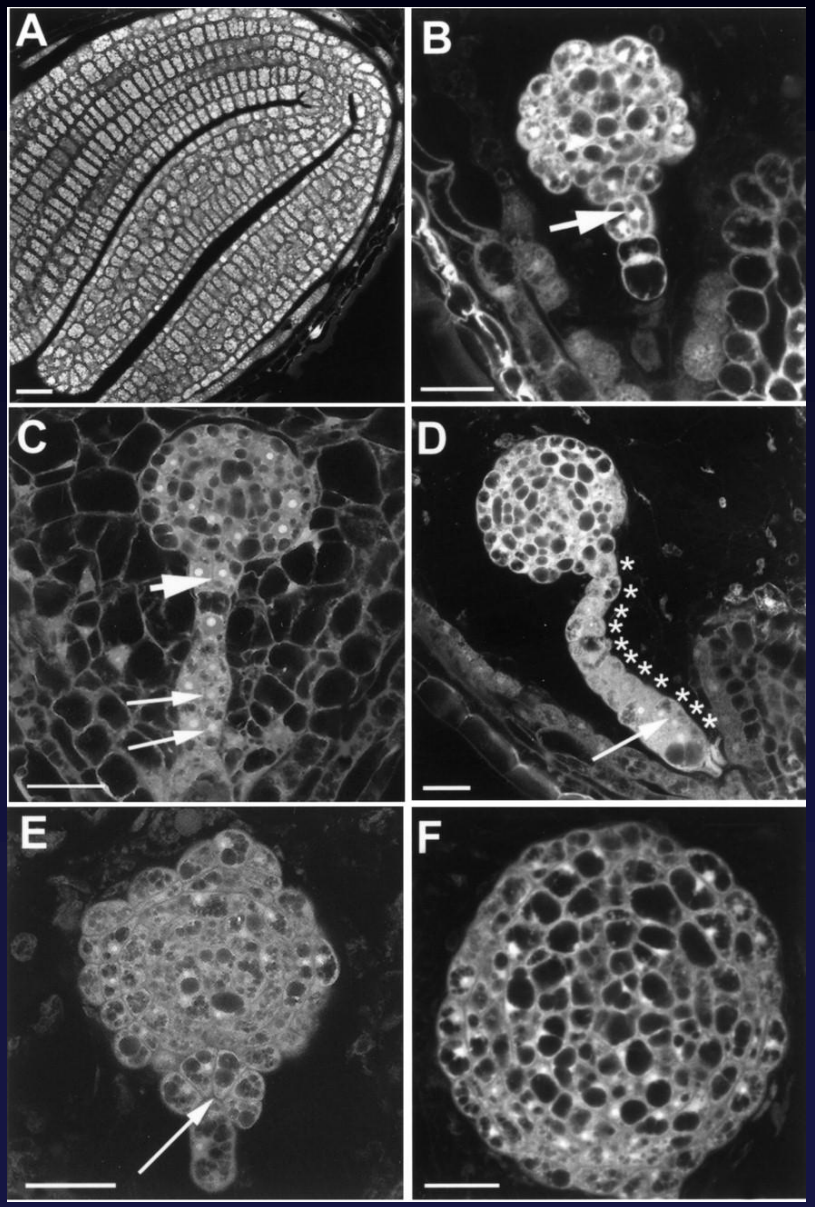
- Azidolabeling
- Affinity chromatography

Protein sequencing, cDNA screening, gene identification

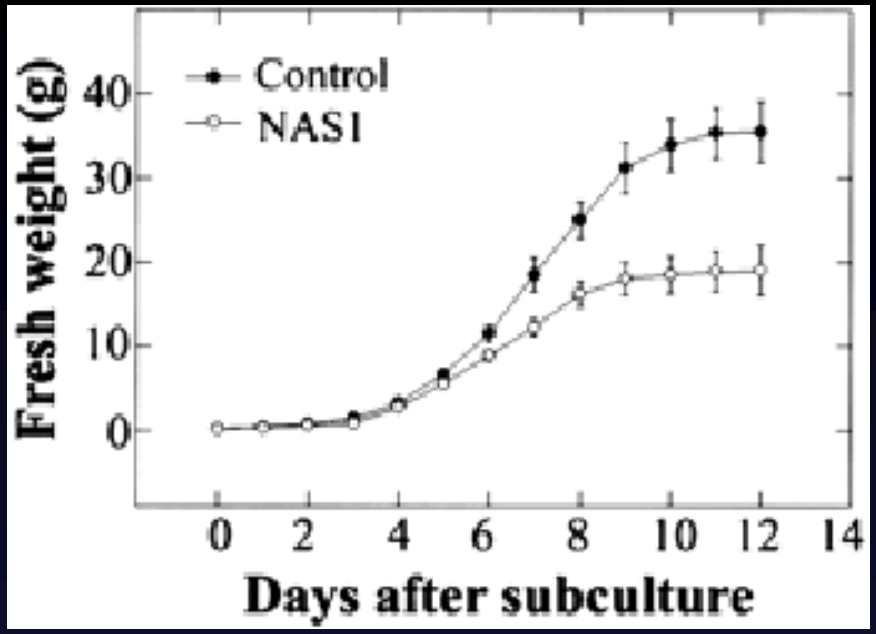
=> Auxin Binding Protein (ABP1)

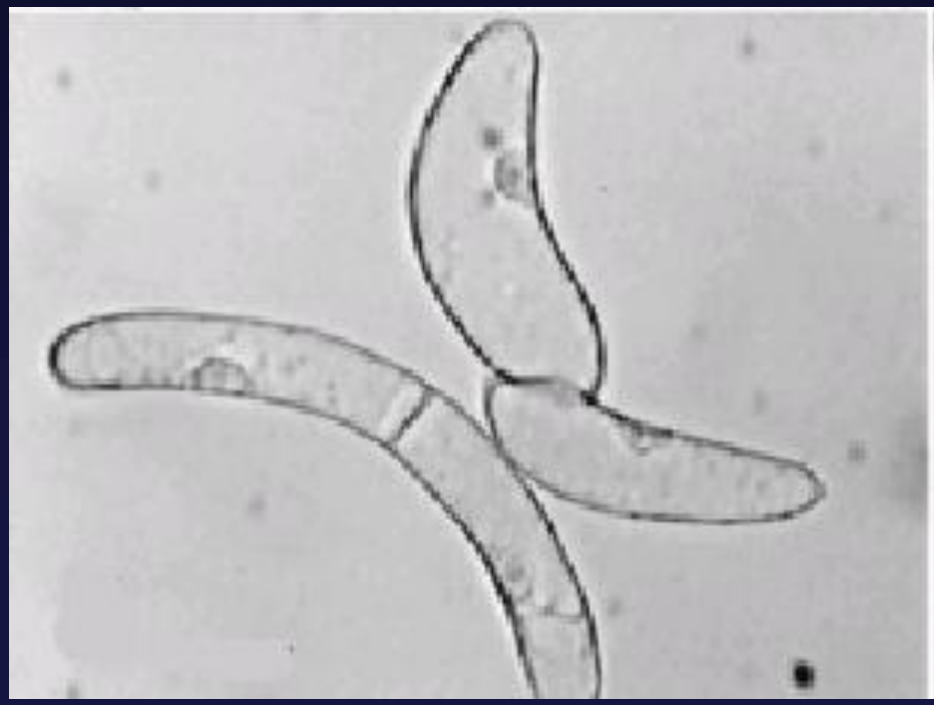
Reverse Genetic – Embryo Lethal *abp1* Mutant



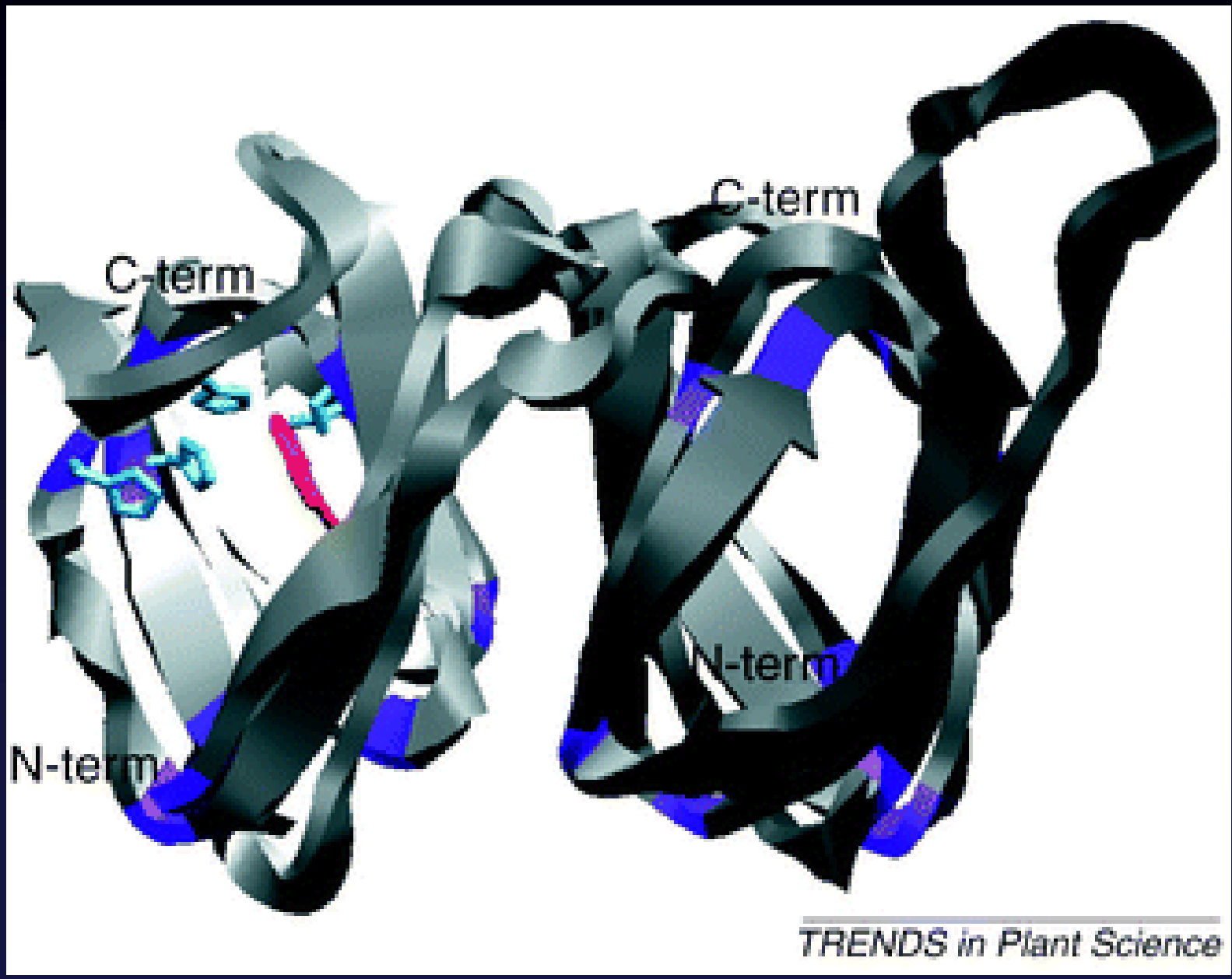


ABP1 Antisense BY-2 Cells Display Defects in Auxin Dependent Cell Elongation

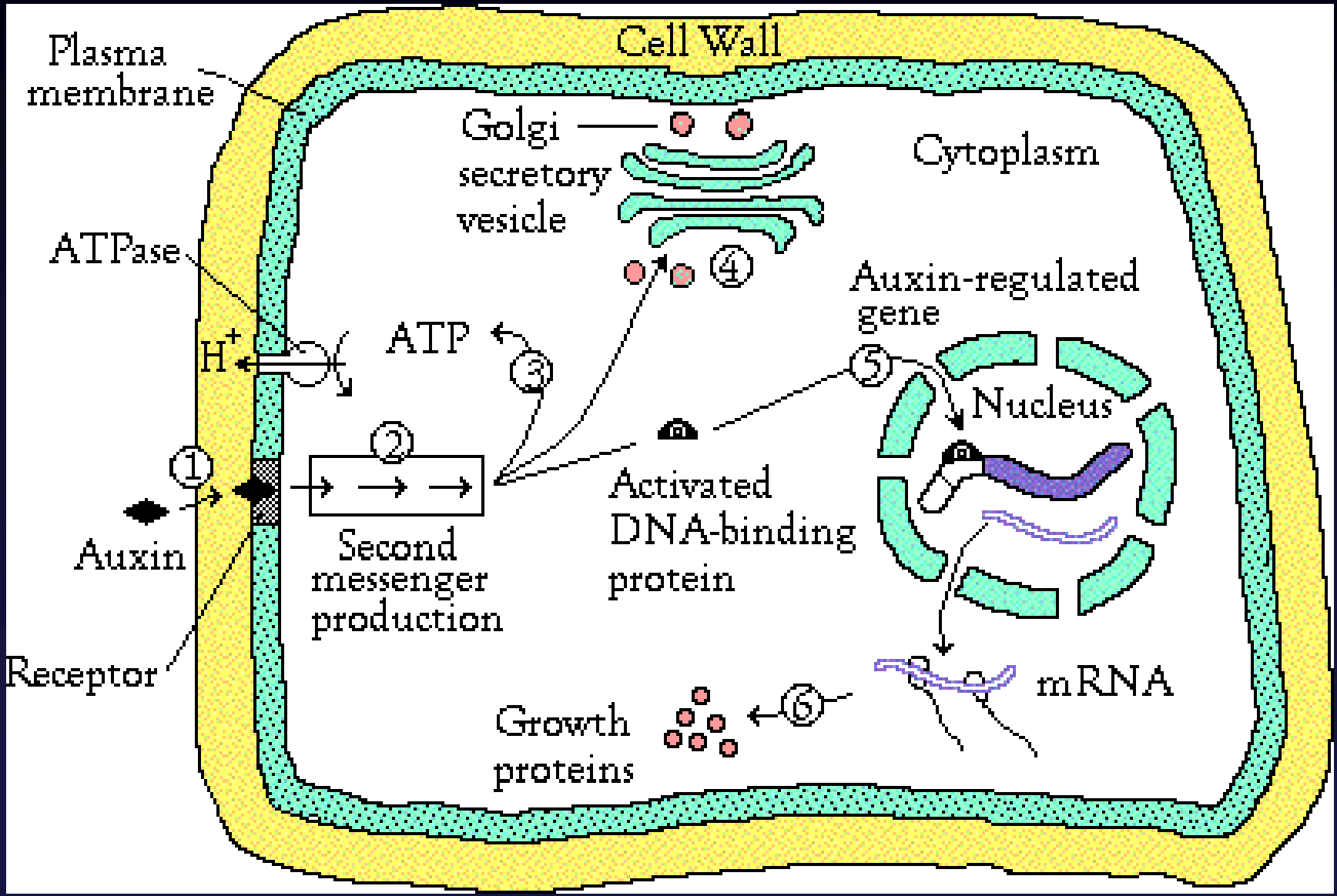




ABP1 – Structure



Optimistic Model for ABP1 Action



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

Molecular Biology Approach to Elucidate Auxin Signaling

Does auxin regulate gene expression?

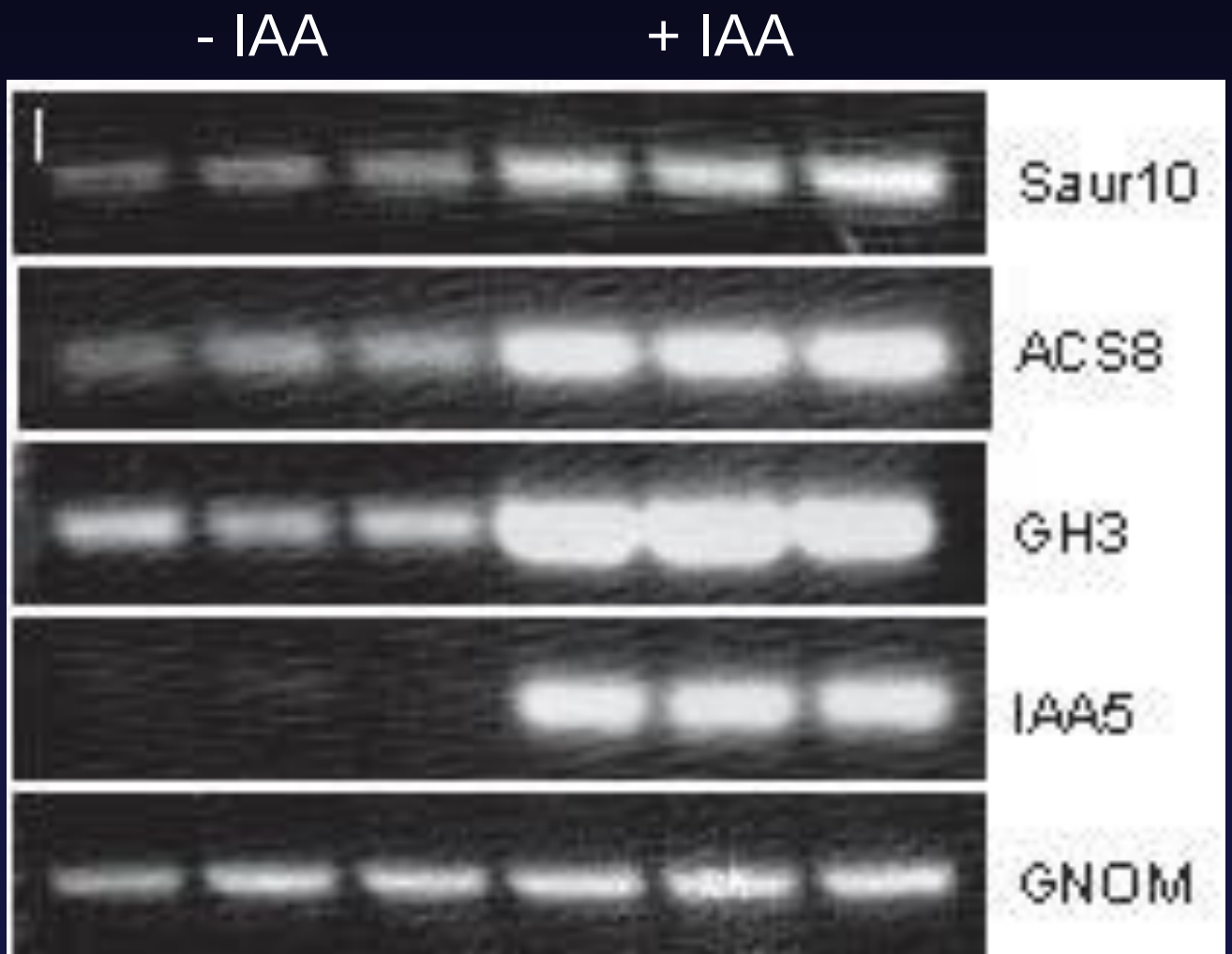
- Rapidly upregulated mRNAs
  (*GH3, SAUR, AUX/IAA* genes)

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

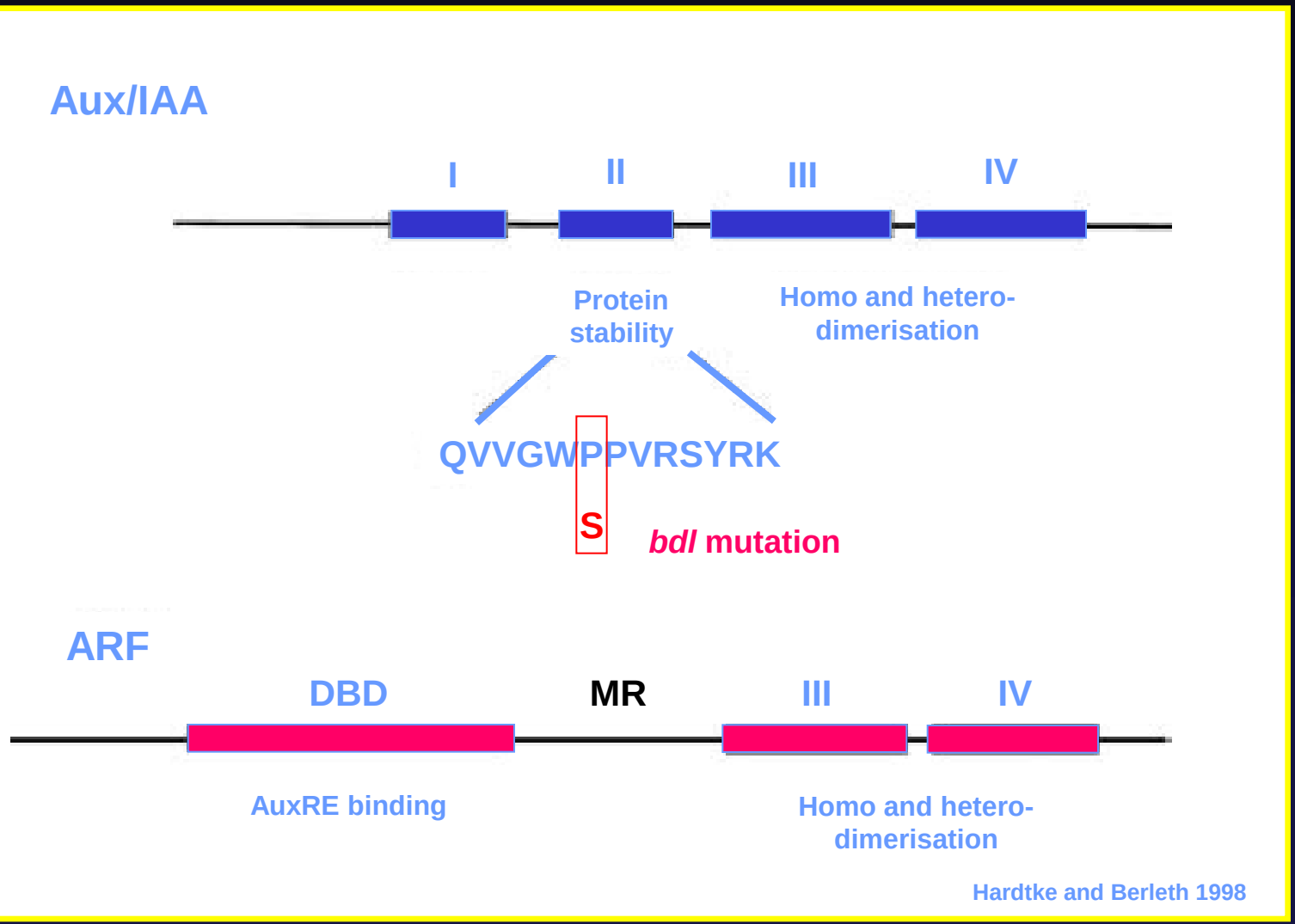
- Two hybrid => AUX/IAAs interact with ARFs

Molecular Biology Approach to Elucidate Auxin Signaling

RT-PCR

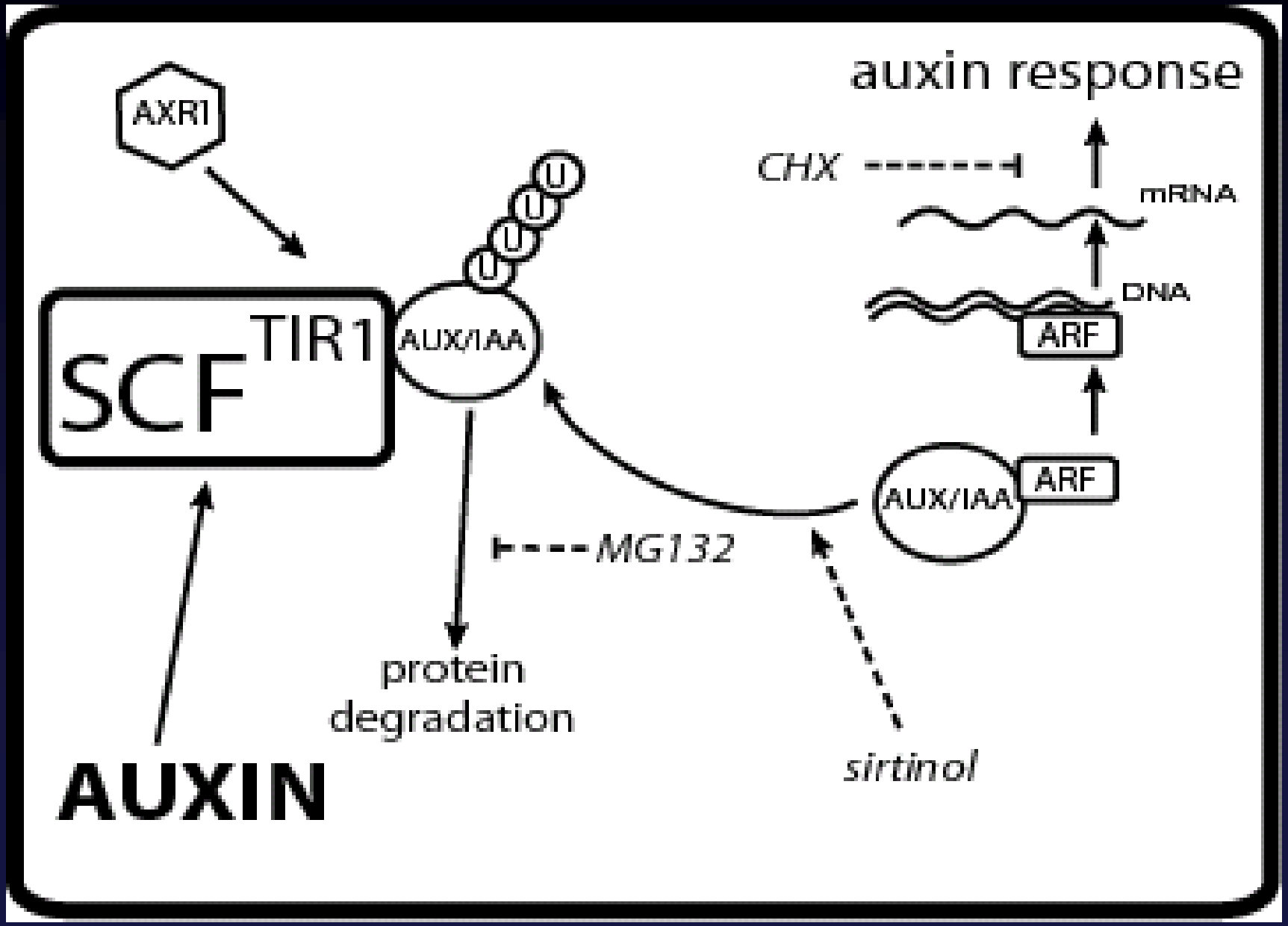


Some ARFs are **Activators**, whereas Aux/IAA **Repressors** of Auxin Response



Hardtke and Berleth 1998

Genomic Auxin Signaling



Summary for Auxin Signaling

Biochemical approach – auxin binding protein ABP1

binds auxin, important in embryogenesis, precise role unclear

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

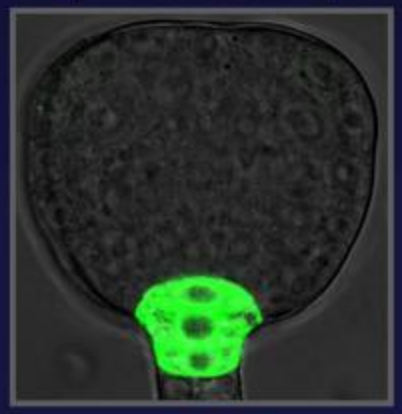
9x inv. *5´ CCTTT* TGTCTC *3´*

Root

Embryos

DR5

anti-IAA

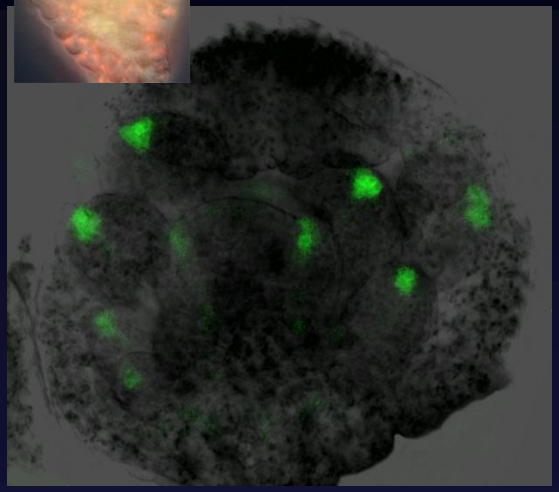


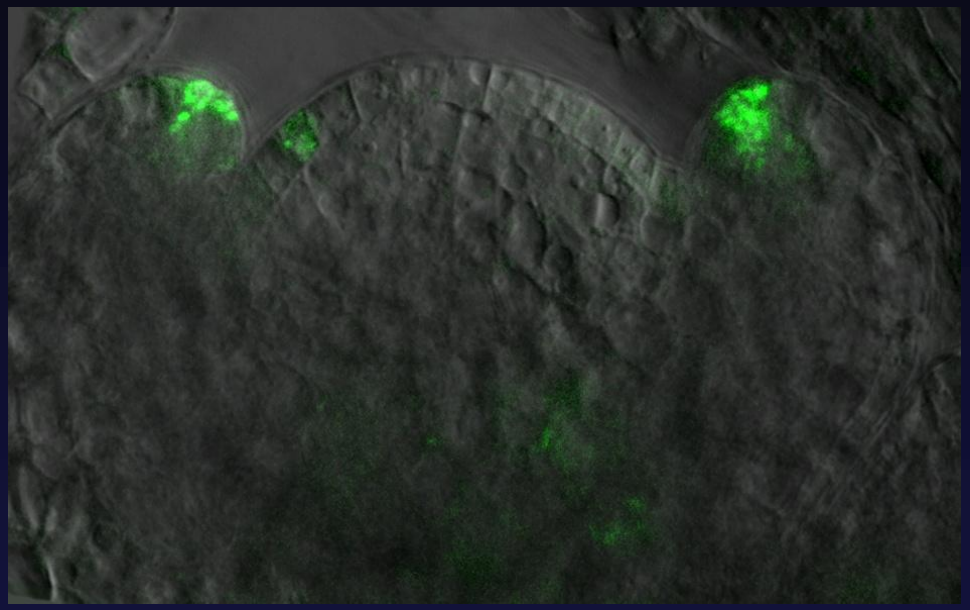
DR5

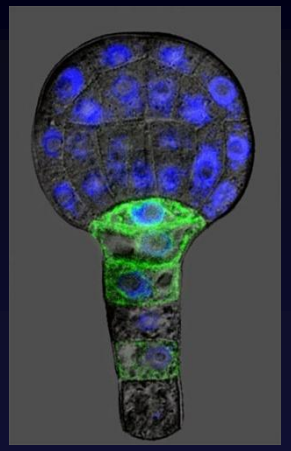
anti-IAA

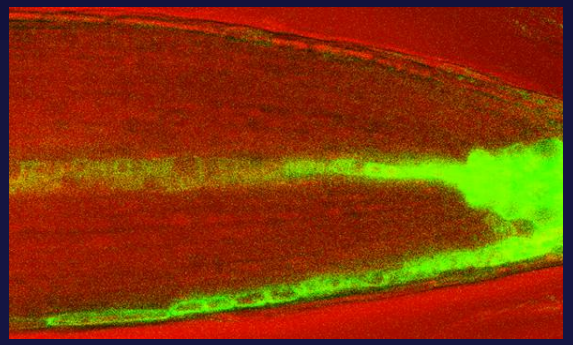
Ulmasov et al. 97; Sabatini et al. 99; Benková et al. 03; Friml et al. 03

Local Auxin Gradients in Plant Development



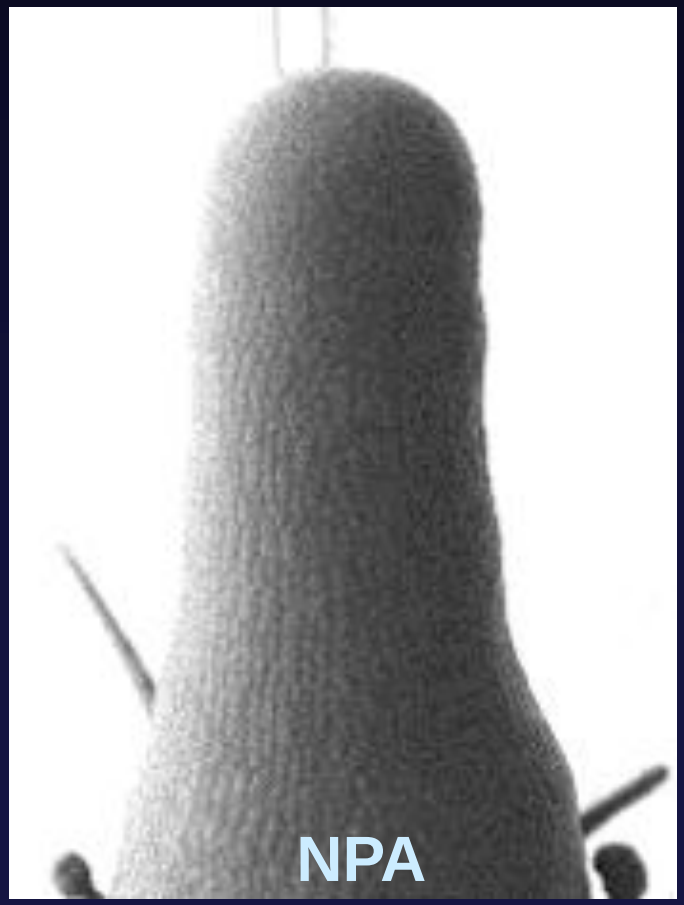


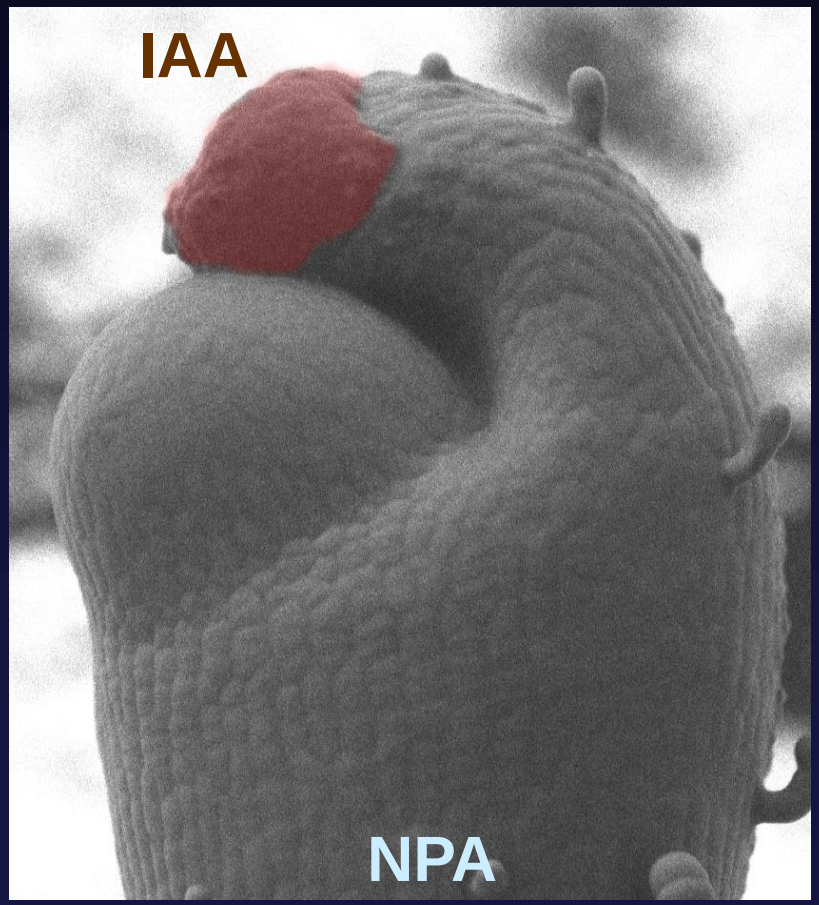




Sabatini et al. 99; Friml et al. 02; Ottenschläger et al. 03; Benková et al. 03; Friml et al. 0

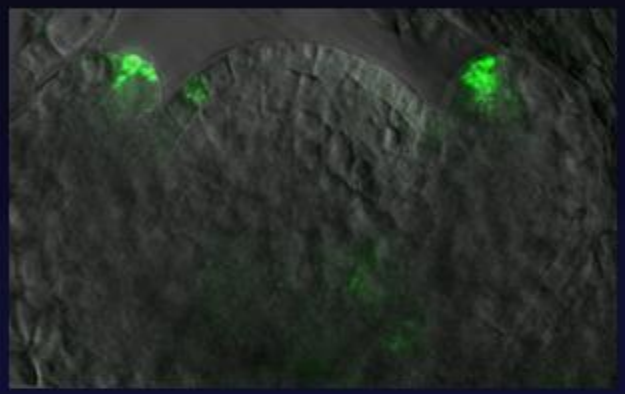
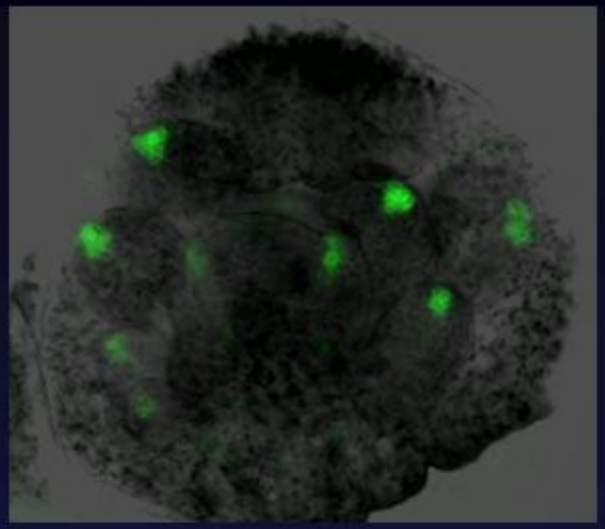
Local Application of Auxin Induces Organ Formation

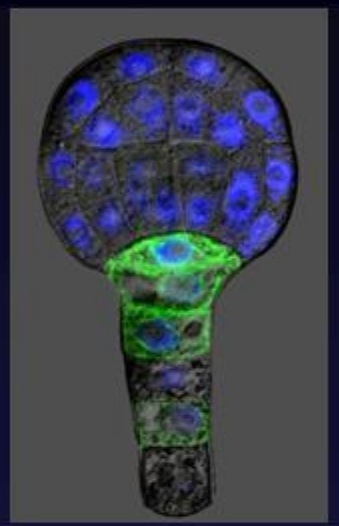
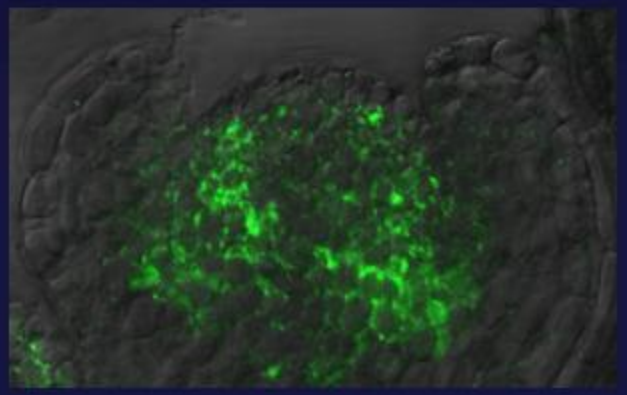
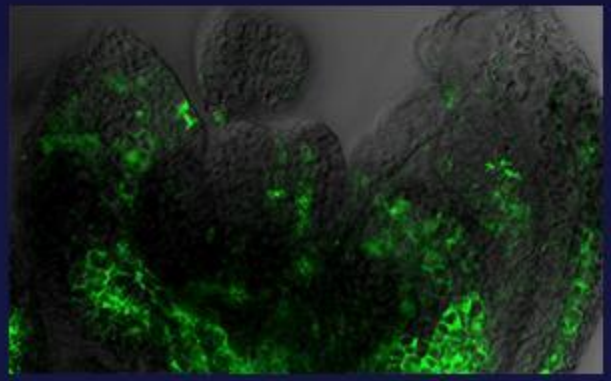




Reinhardt et al. 03

Local Auxin Gradients Require Active Polar Auxin Transport

Auxin Transport

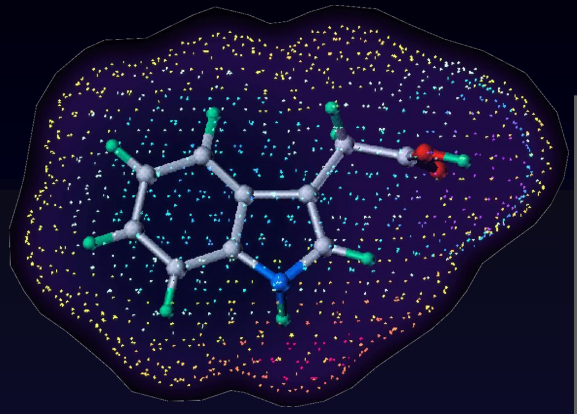
Proteins involved in auxin transport
-PIN proteins (efflux)
-AUX1 proteins (influx)

Role of GNOM dependent vesicle trafficking

PIN proteins cycling and its role

AUXIN TRANSPORT

mediates



- **Embryo development**
- **Organ initiation and positioning**
- **Vascular tissue differentiation**
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- **Growth responses to light and gravity**
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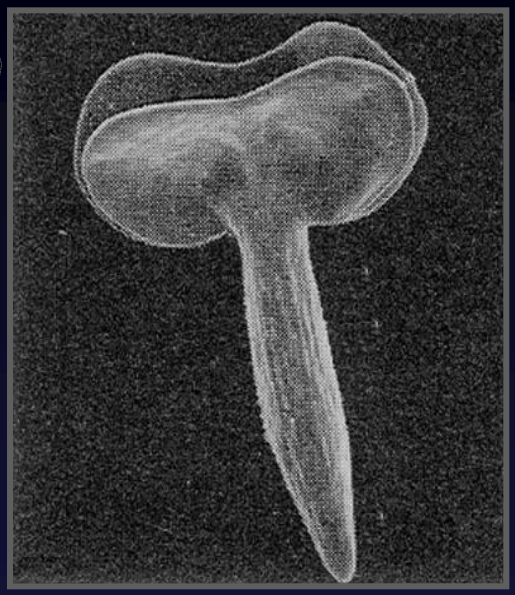
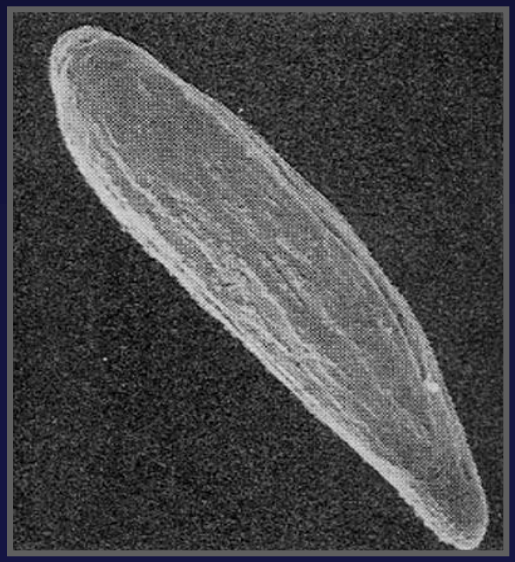
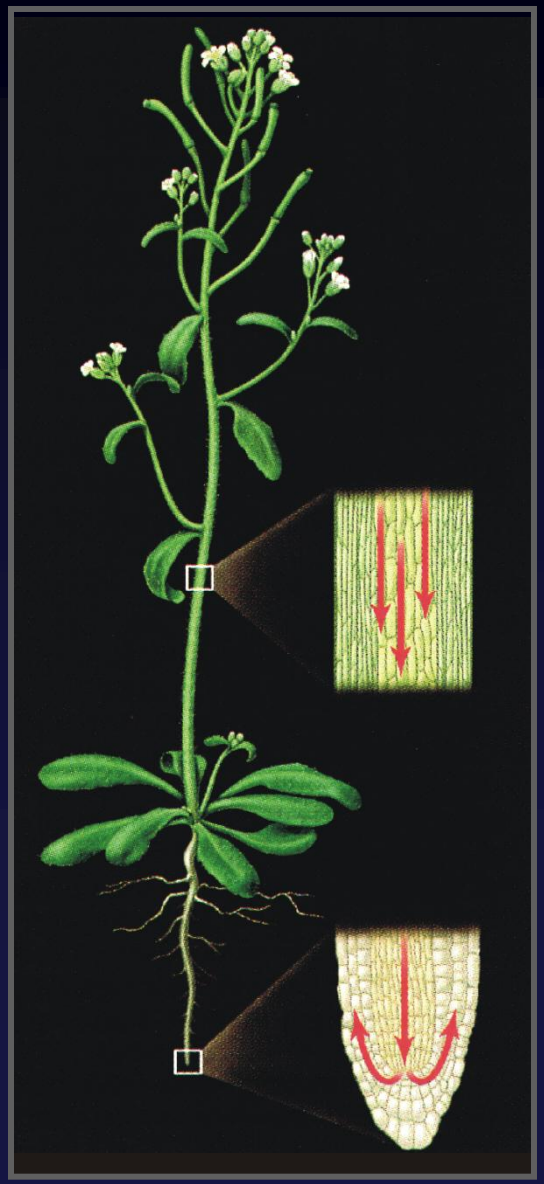
embryos

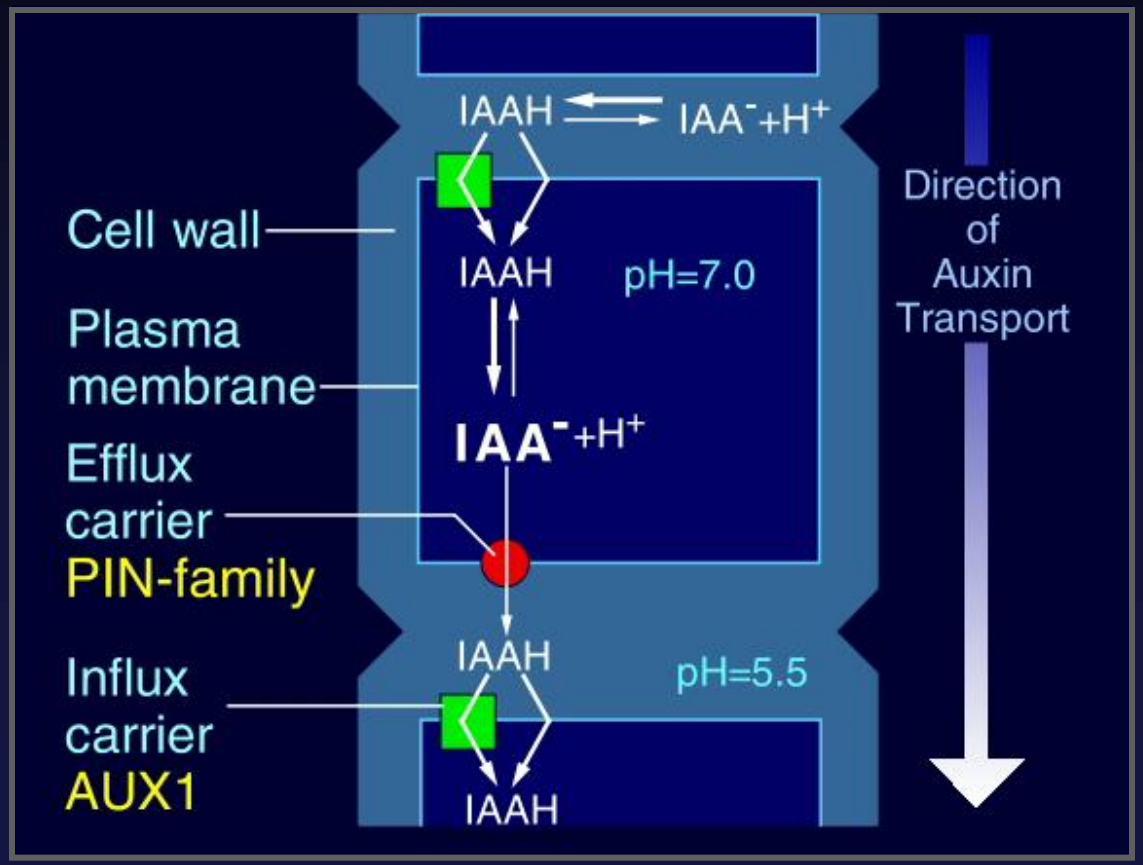




Physiology of Auxin Transport



Chemiosmotic hypothesis

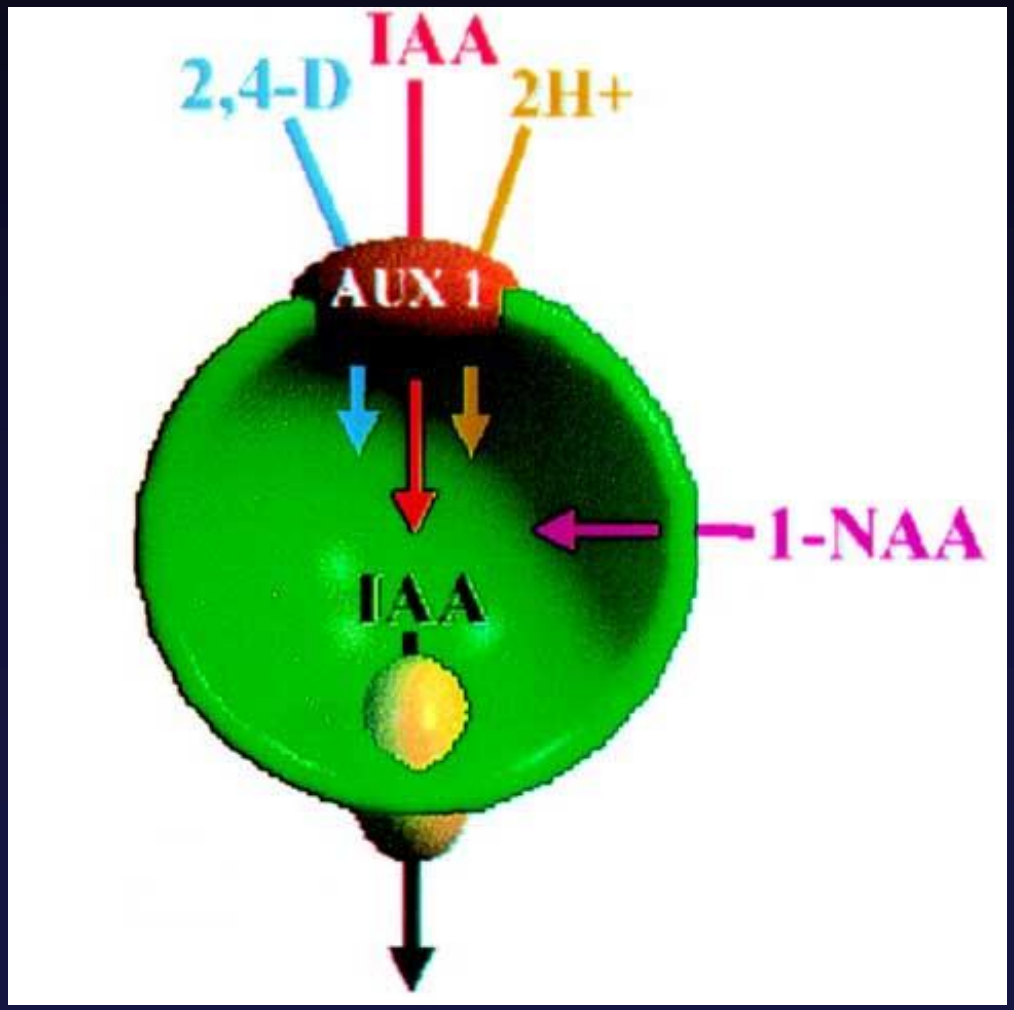


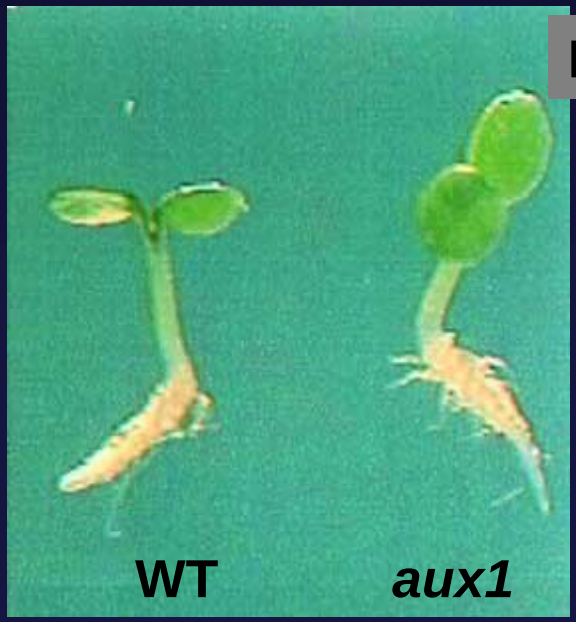
Auxin Influx

aux1 is Resistant to Auxin

***aux1* phenotype**

Transport properties of different auxins





NAA Rescues *aux1* Phenotype

- NAA

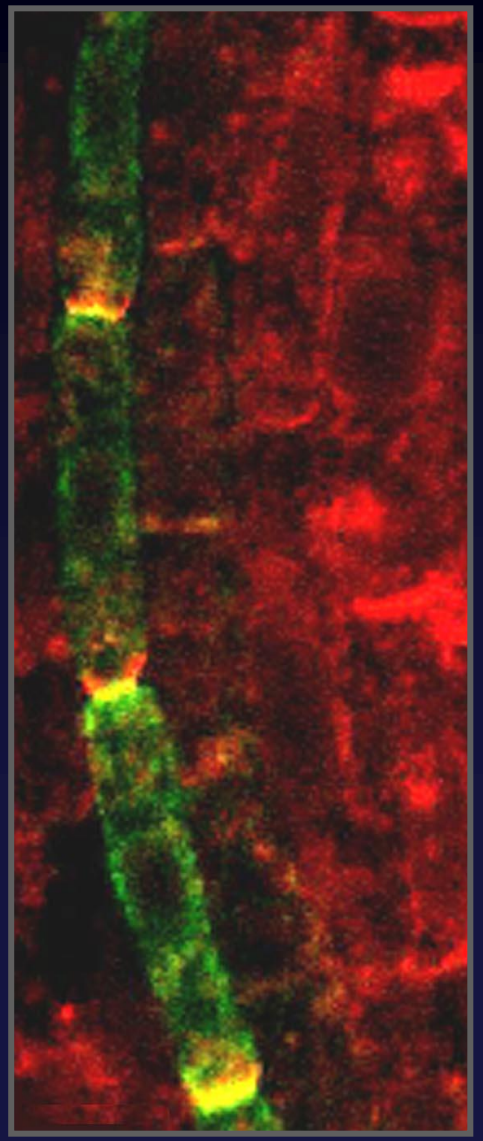
+ NAA

AUX1 – Expression and Localization

AUX1::GUS

AUX1 protein

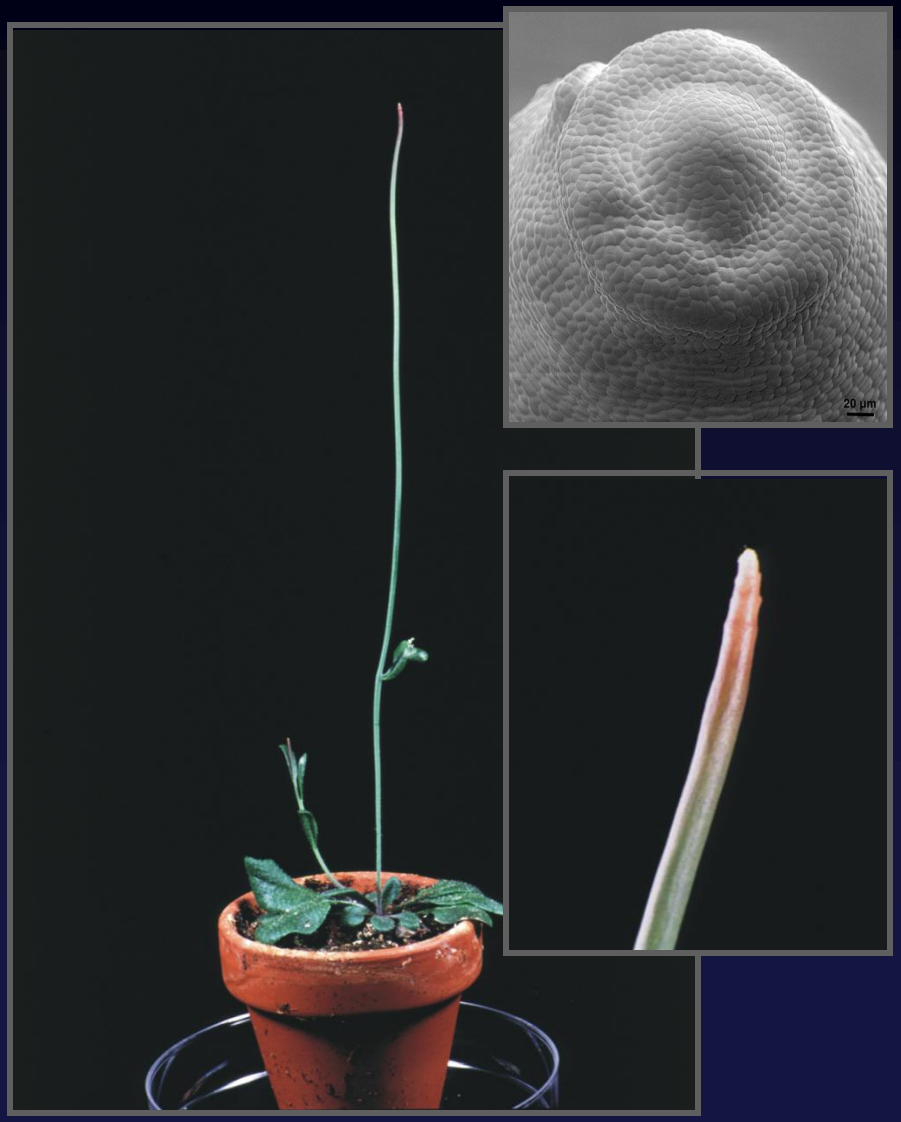
PIN1/AUX1



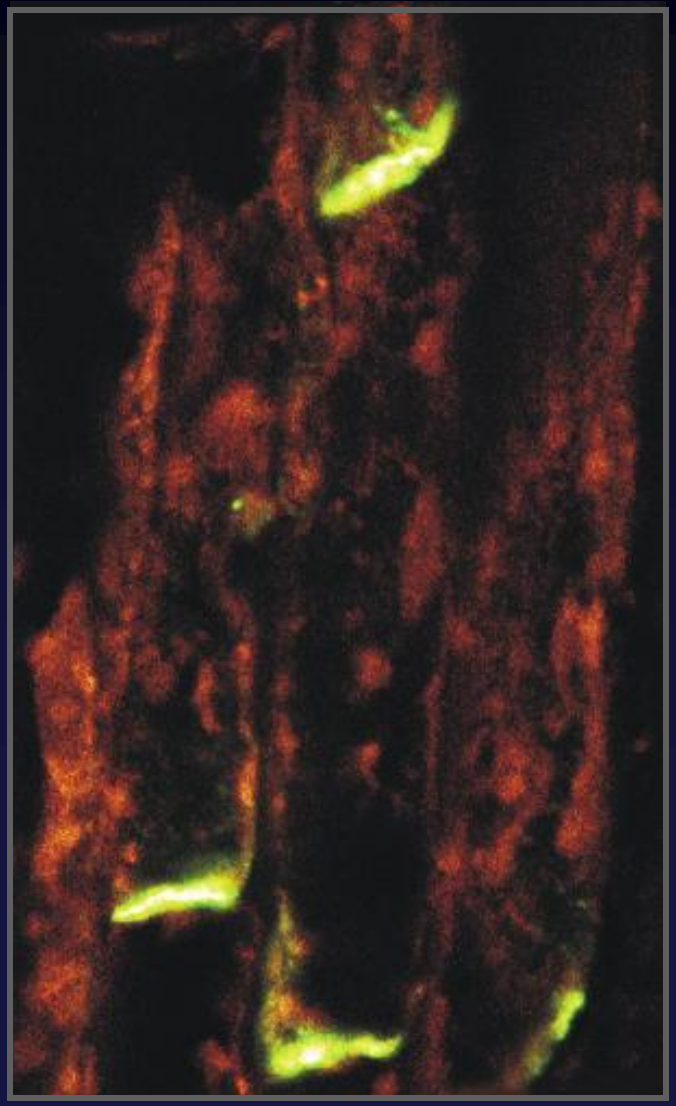
Auxin Efflux

PIN1 – the Auxin Efflux Carrier?

***pin1* mutant**



PIN1 protein



PIN2 – Root Gravitropism

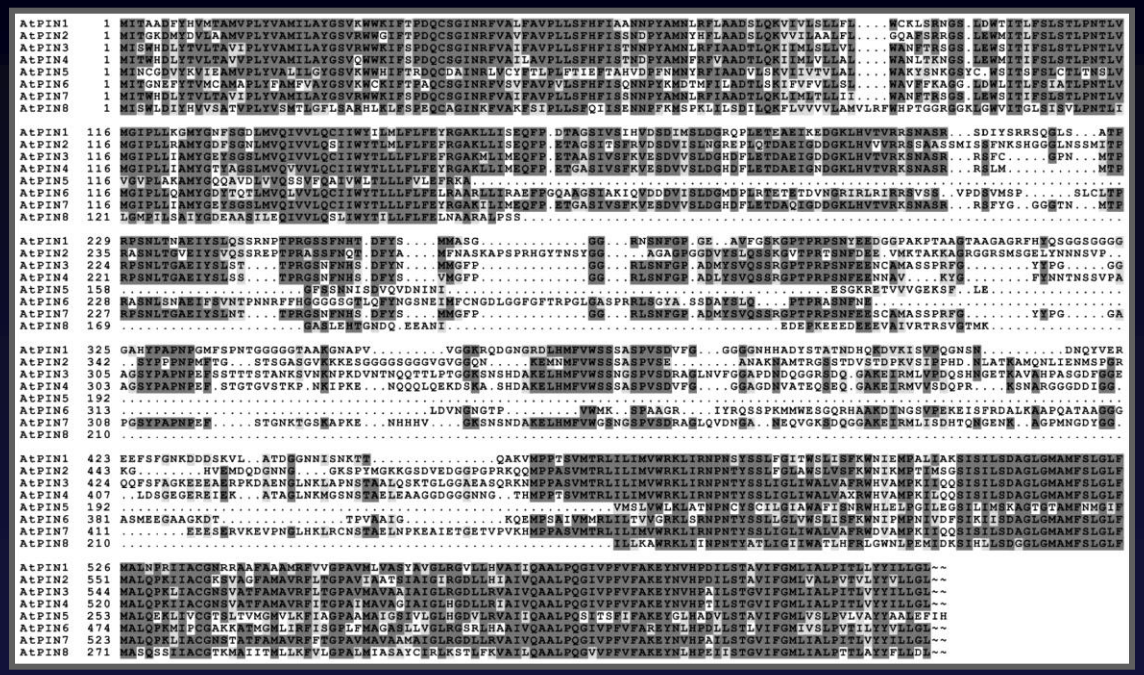
Col-0

pin2

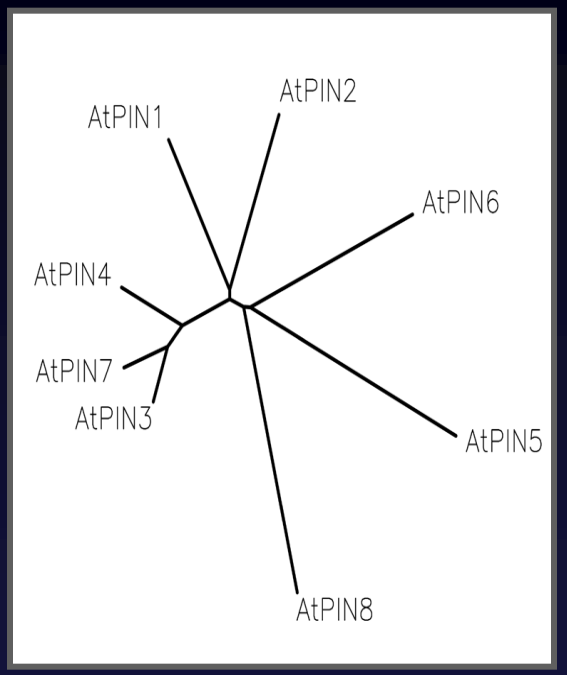
PIN2 protein

The Arabidopsis *PIN* Gene Family

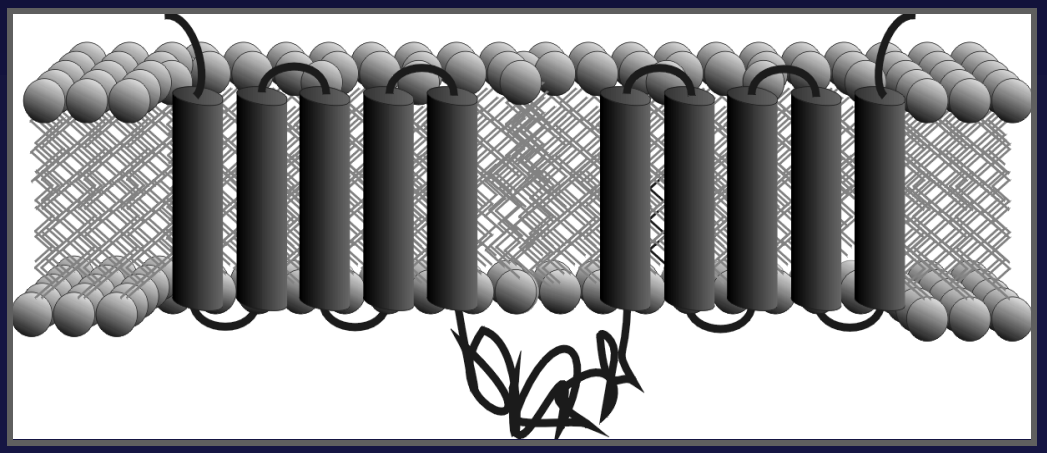
Comparison of *Arabidopsis* PIN proteins



Phylogenetic tree



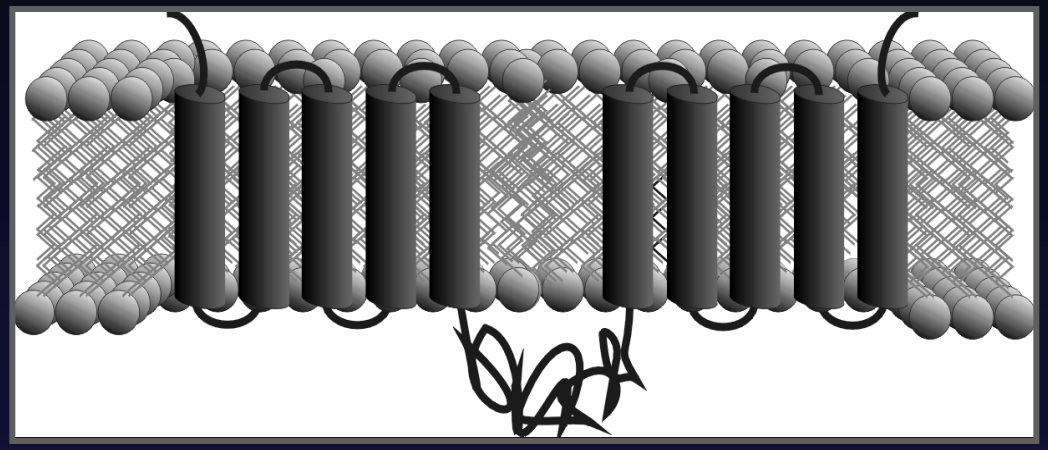
Membrane topology model



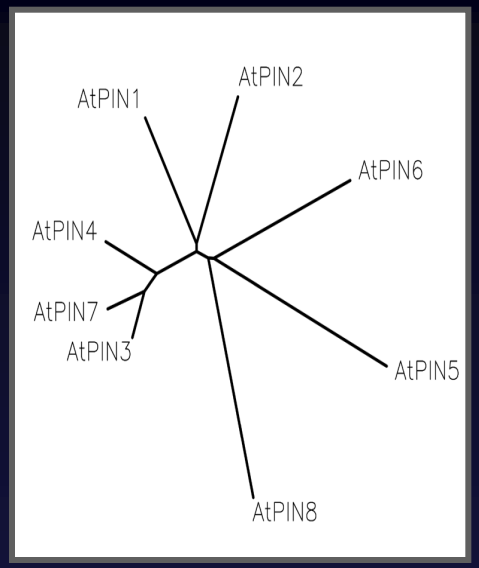
What is Molecular Role of PIN Proteins in Auxin Transport?

PINs Are Essential Components of Auxin Transport

Putative topology of PIN proteins



Phylogenetic tree



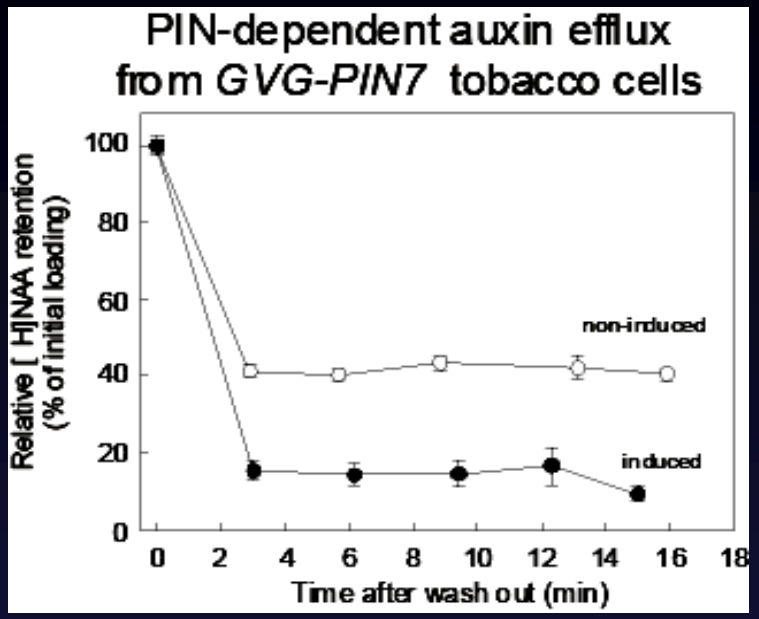
- All defects in *pin* loss-off-function mutants are in auxin transport-dependent processes and can be phenocopied by auxin transport inhibitors

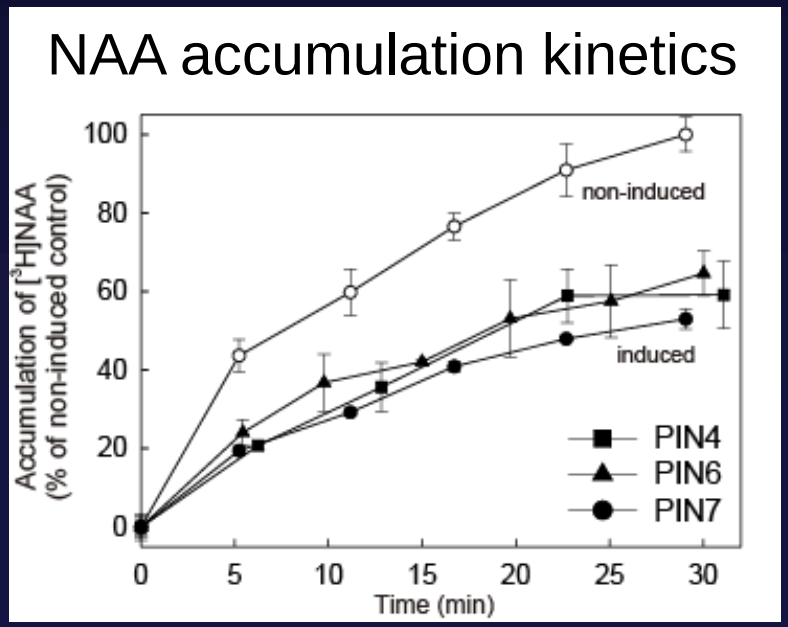
- Local auxin distribution (gradients) are affected in *pin*s

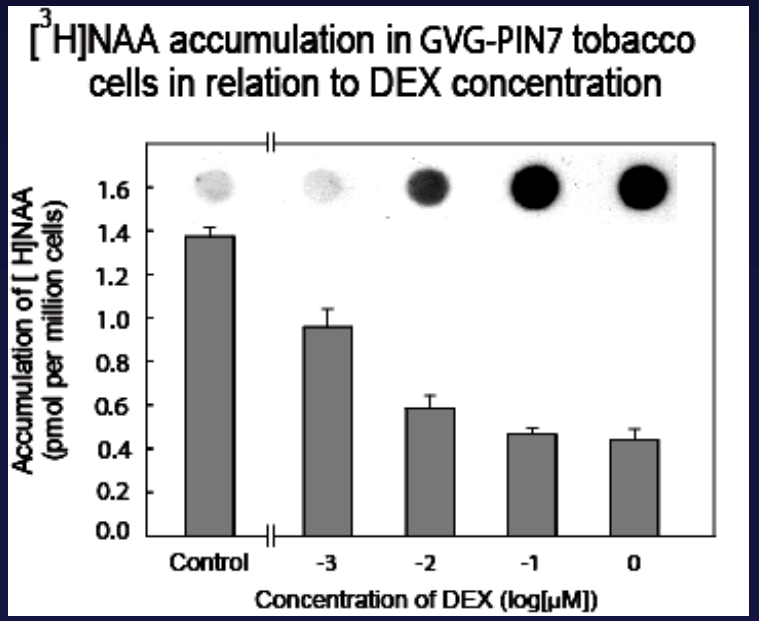
- Polar PIN localization determines direction of auxin flow

PINs Are Rate-limiting Factors in Auxin Efflux

Inducible PIN1 expression

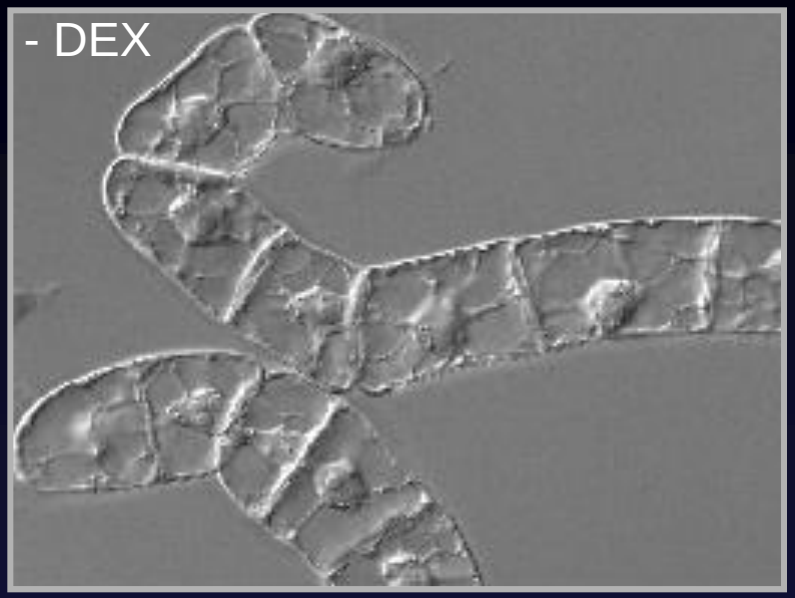


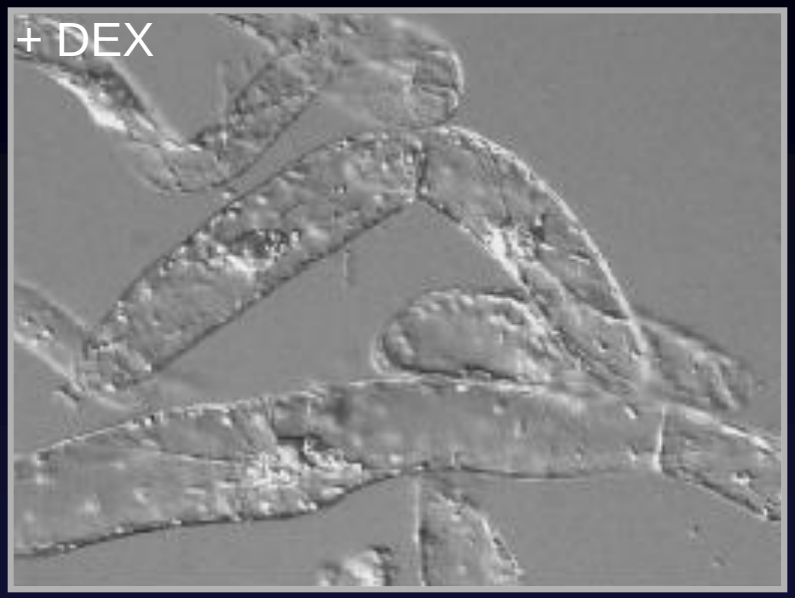


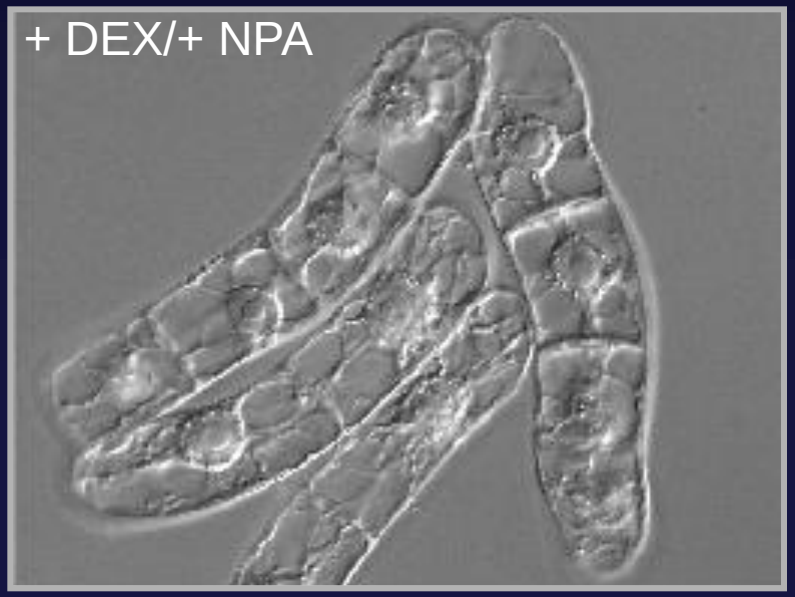


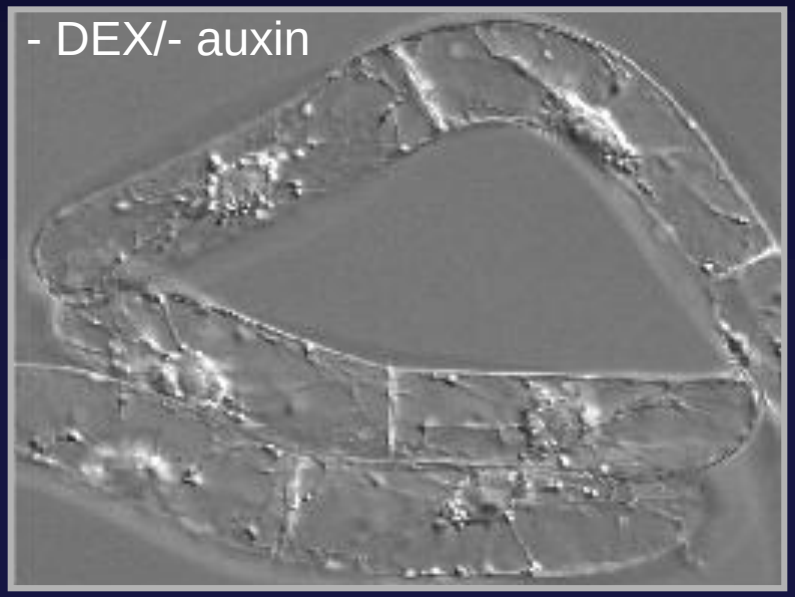
Petrasek et al., 2006

PIN-induced Phenotypes in BY-2 Cells





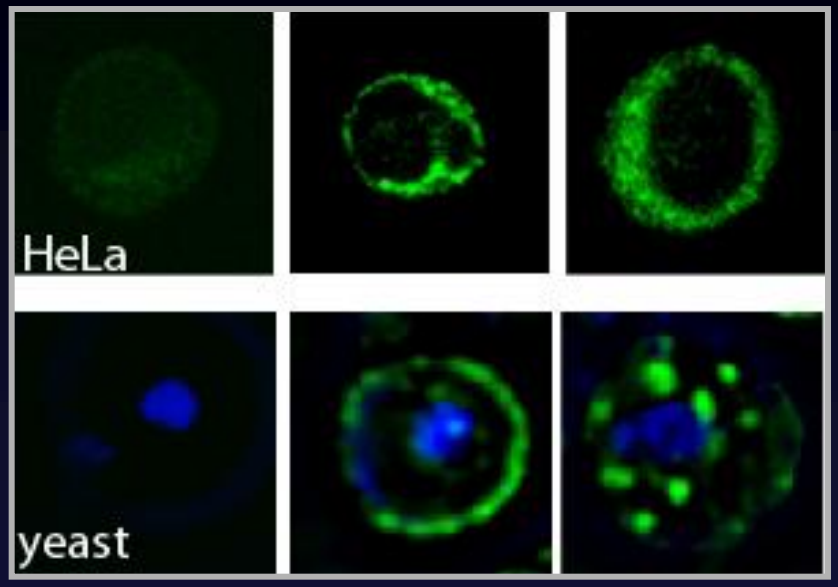




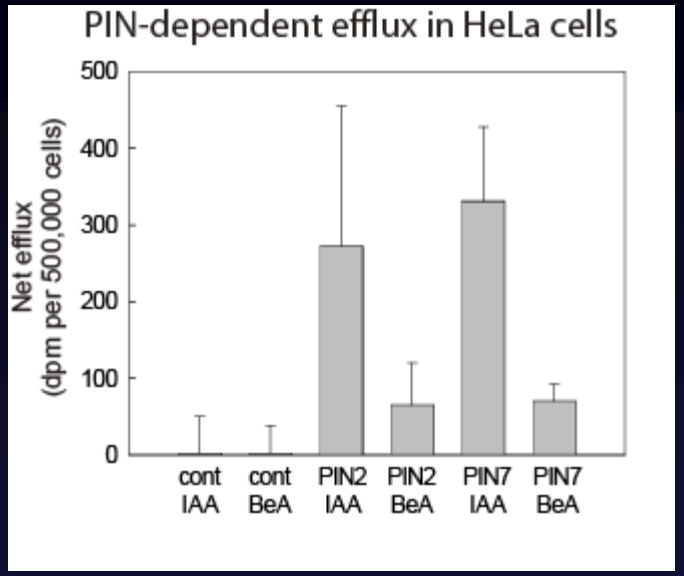
unpublished

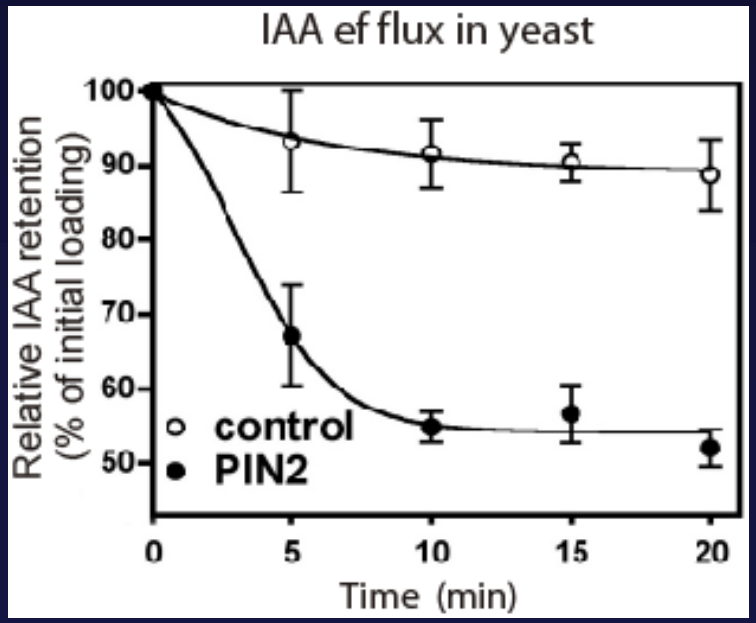
Expression of PINs in HeLa and Yeast

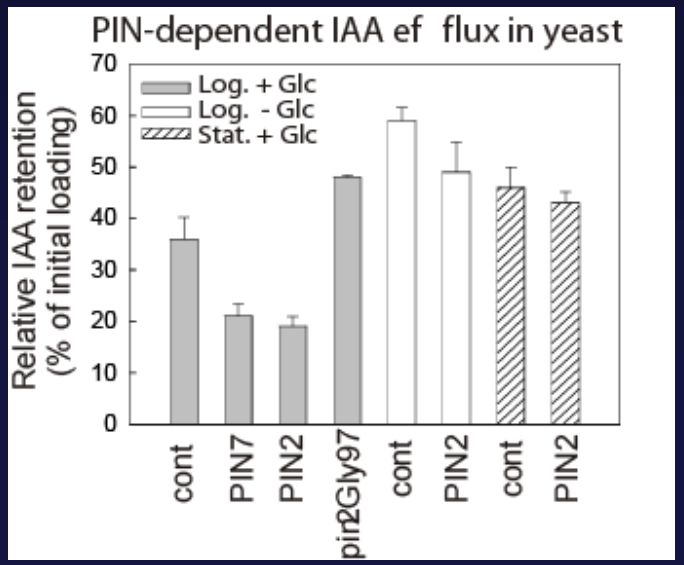
Heterologous PIN2 expression



auxin efflux activity

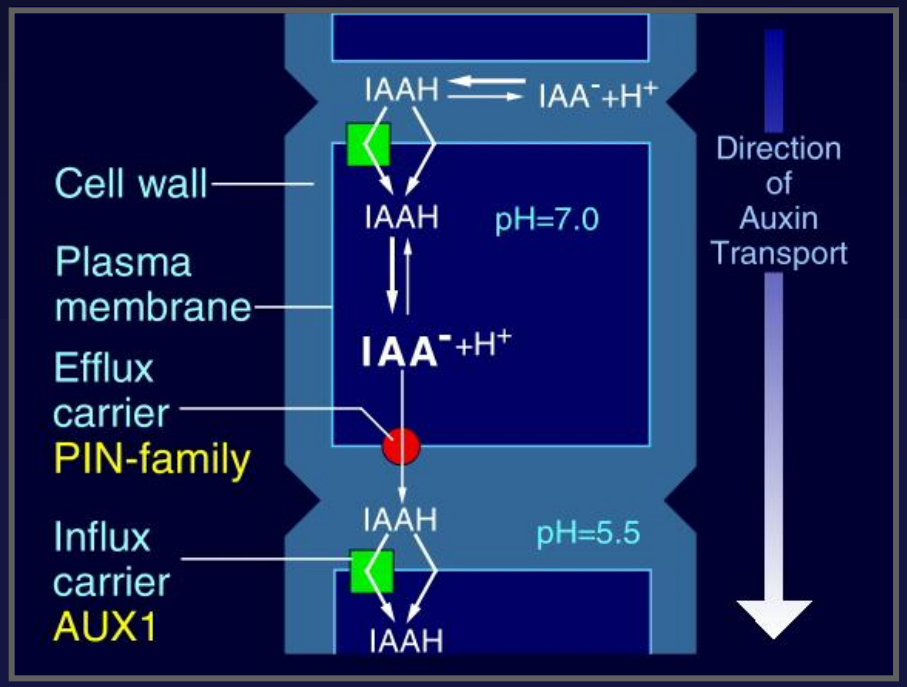


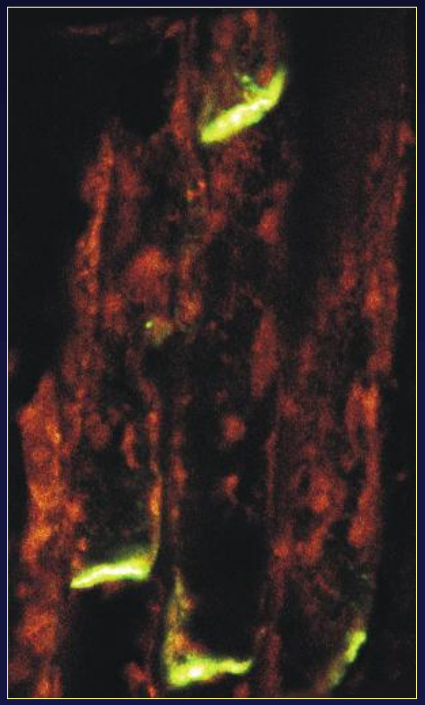




Petrasek et al., 2006

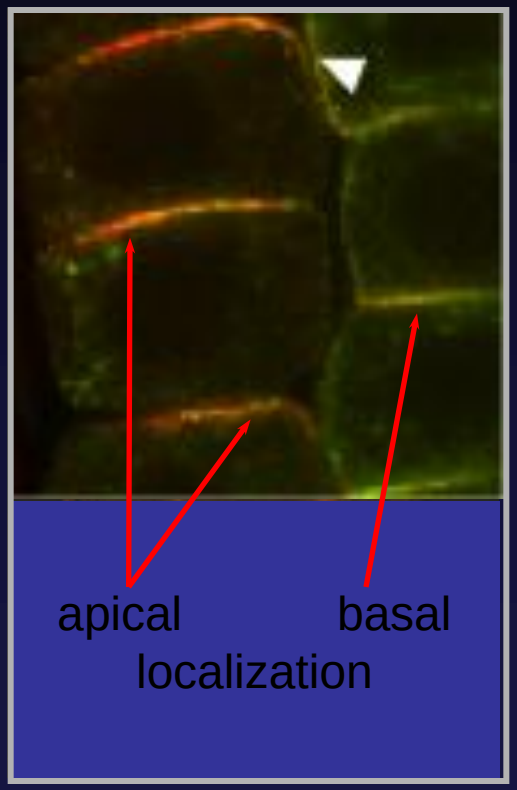
Cellular Polarity of PIN Localization and Directionality of Intercellular Auxin Flow



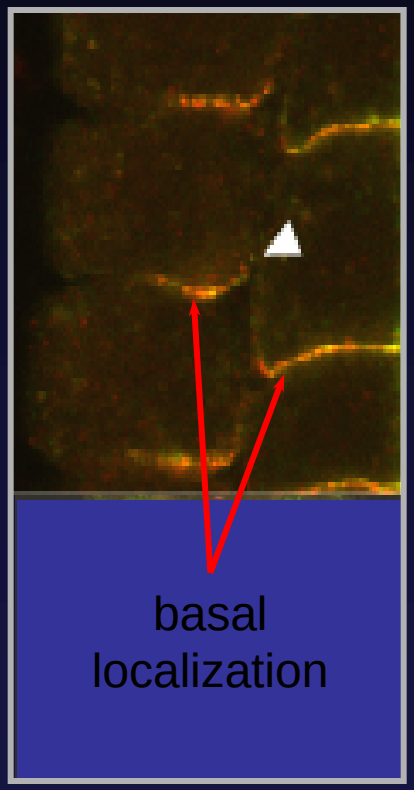


PIN-specific Signals for Polar Targeting

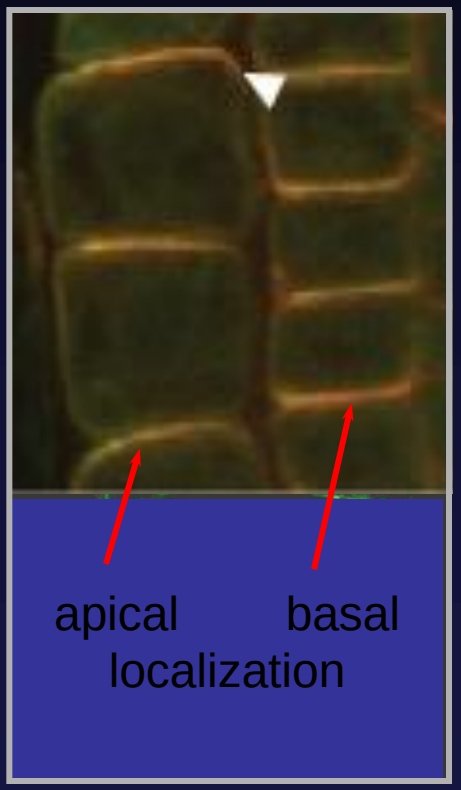
PIN2pr::PIN2:HA



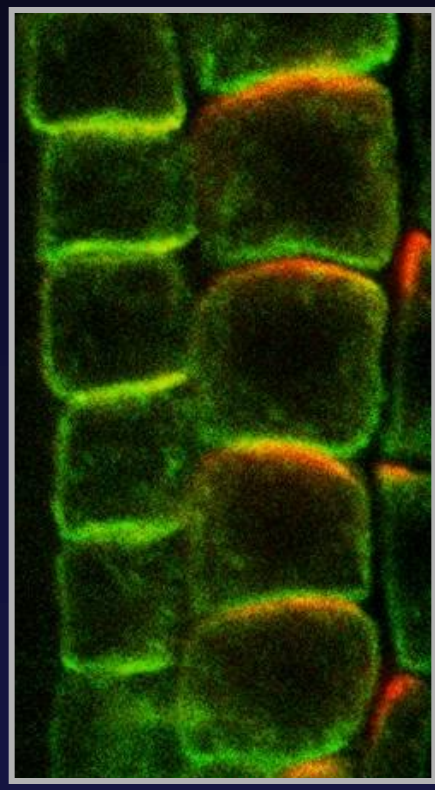
PIN2pr::PIN1:HA



PIN2pr::PIN1:GFP



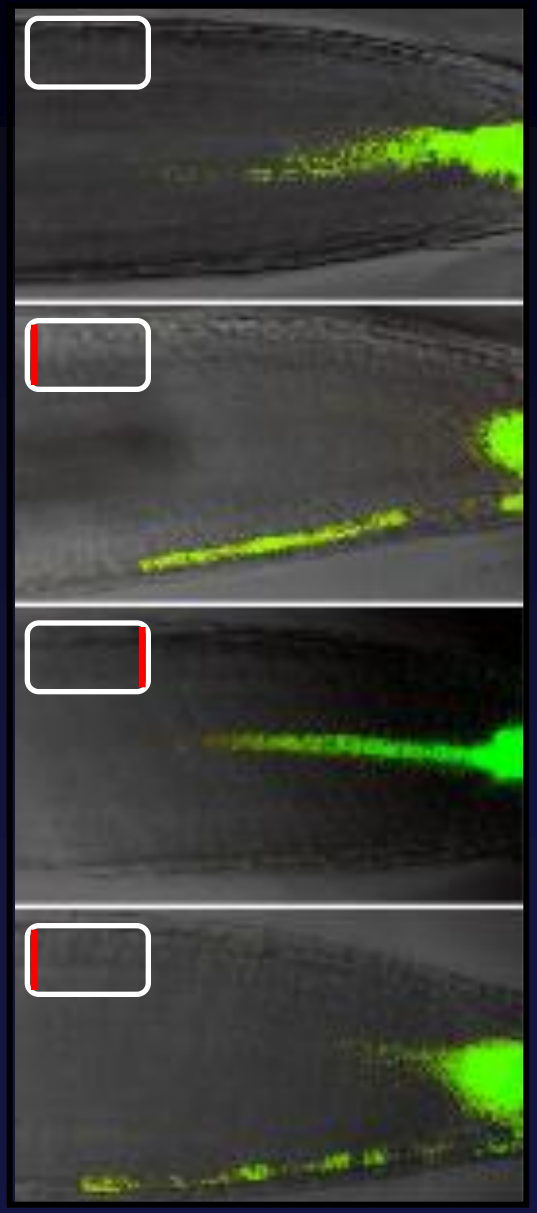
PIN1/PIN1:GFP



Wisniewska et al. 2006

PIN Polarity Determines Direction of Auxin Flow

DR5rev::GFP



pin2 (eir1, agr1)

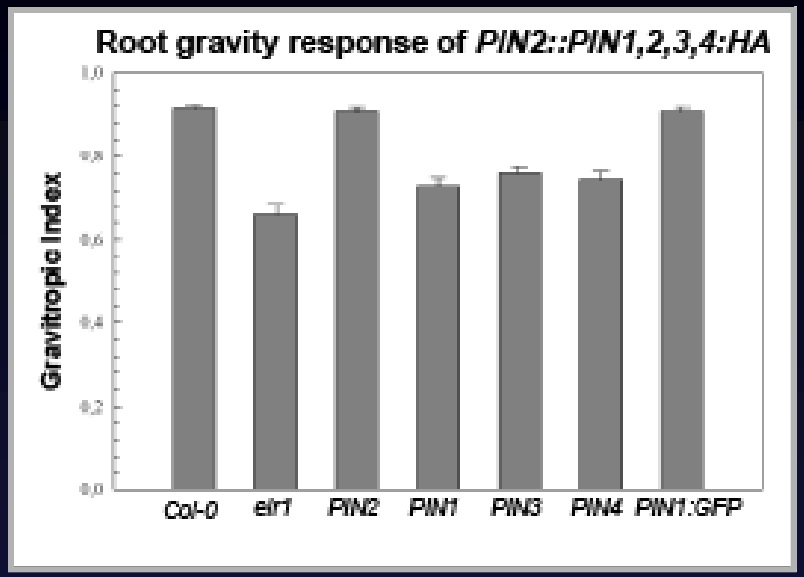
PIN2::PIN2:HA

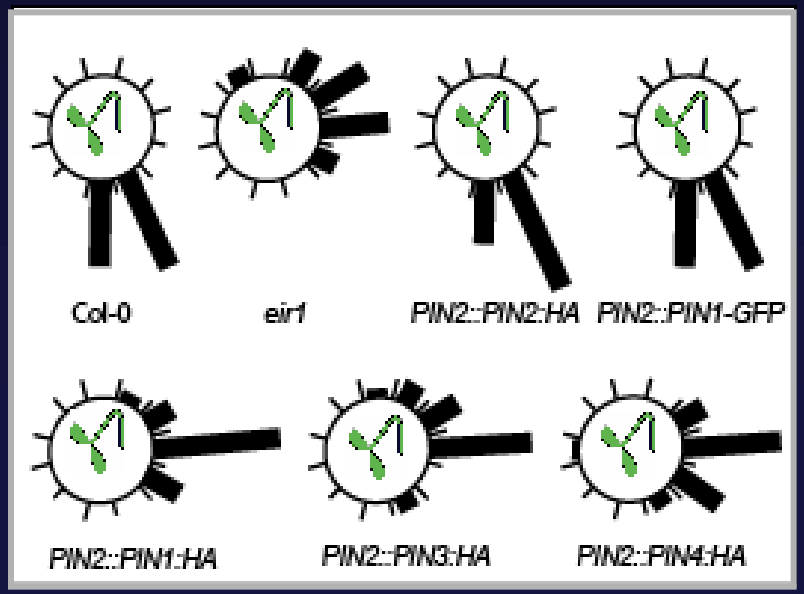
PIN2::PIN1:HA

PIN2::PIN1:GFP-2

PIN2::PIN1:GFP-3

gravitropism





Wisniewska et al. 2006

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

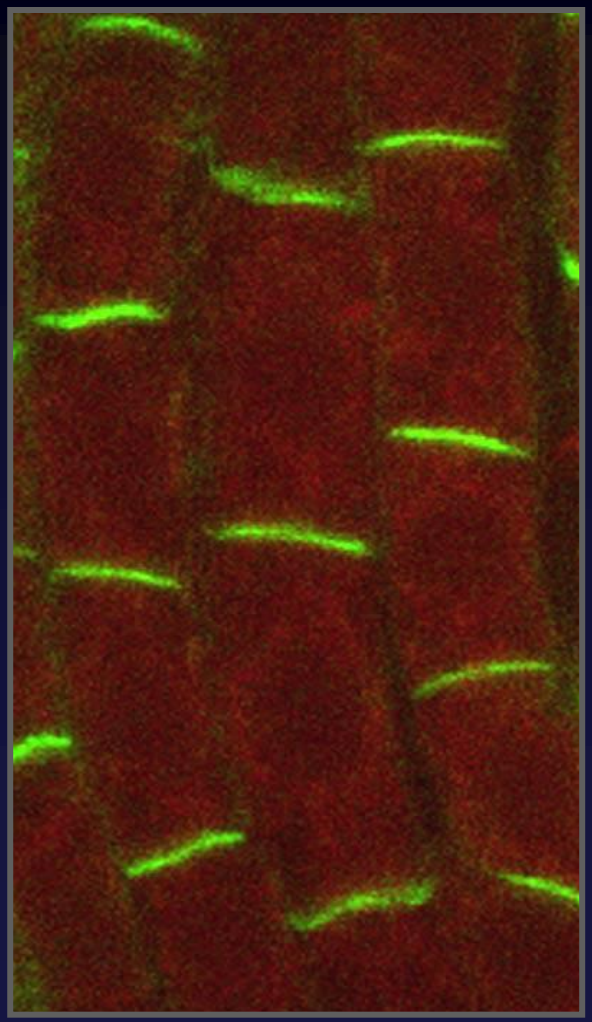
and

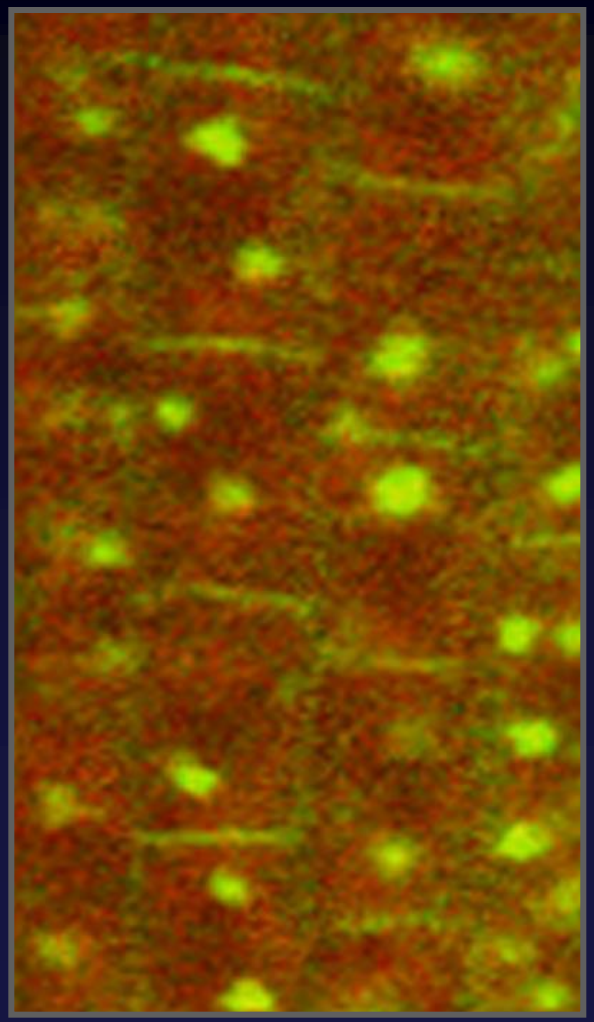
the polarity of their subcellular localization determines direction of intercellular auxin flow

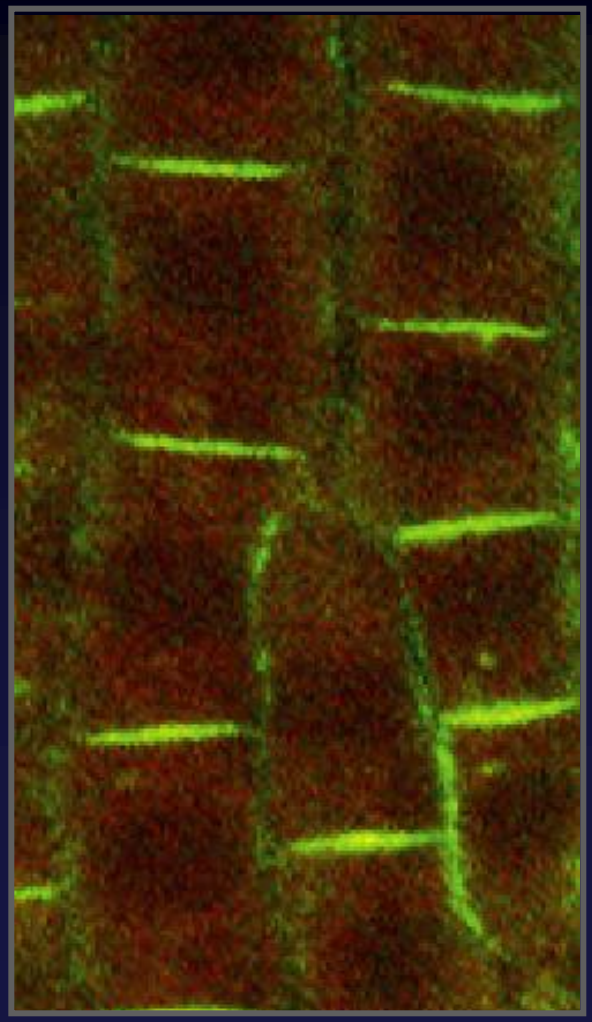
Constitutive Cycling of PINs

PIN1 Subcellular Movement

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

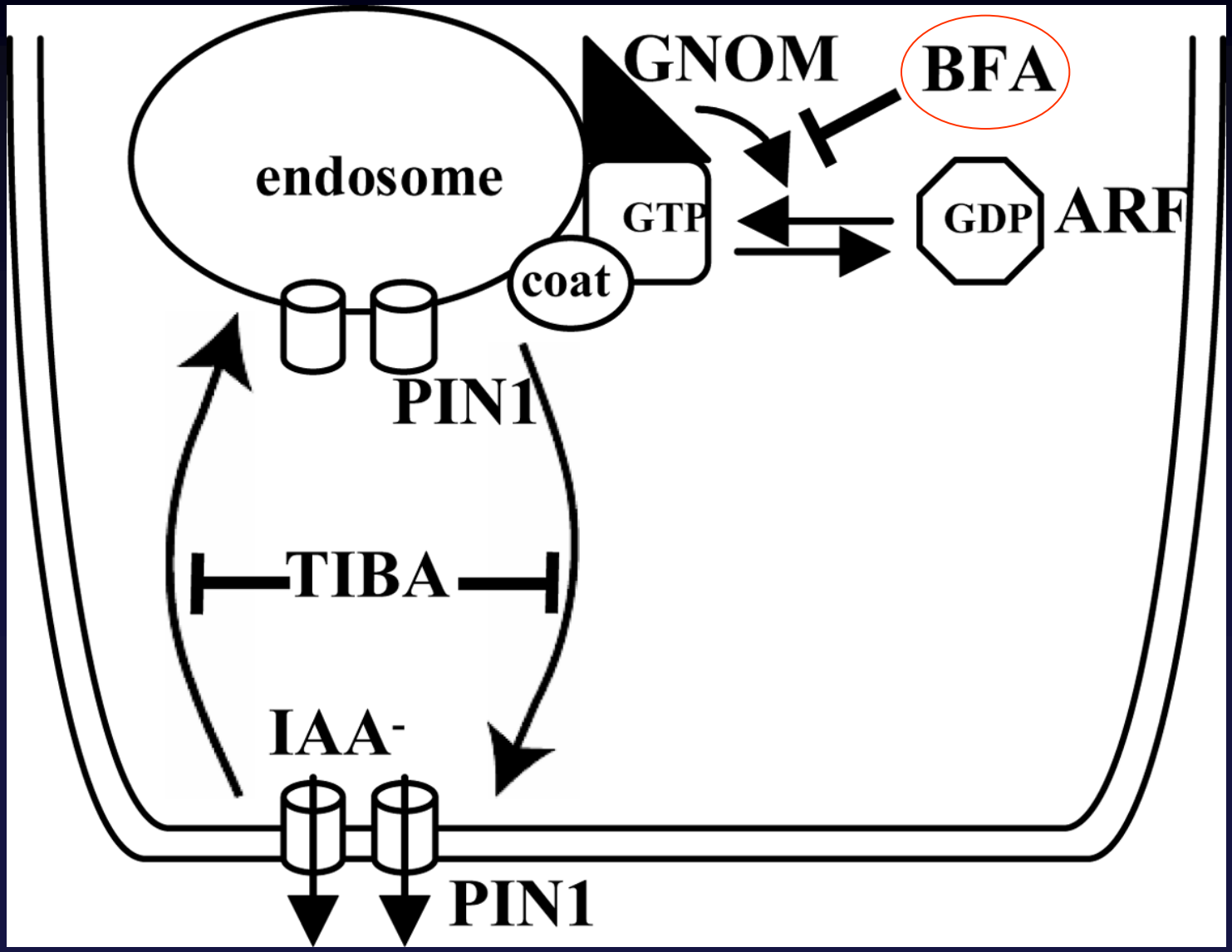




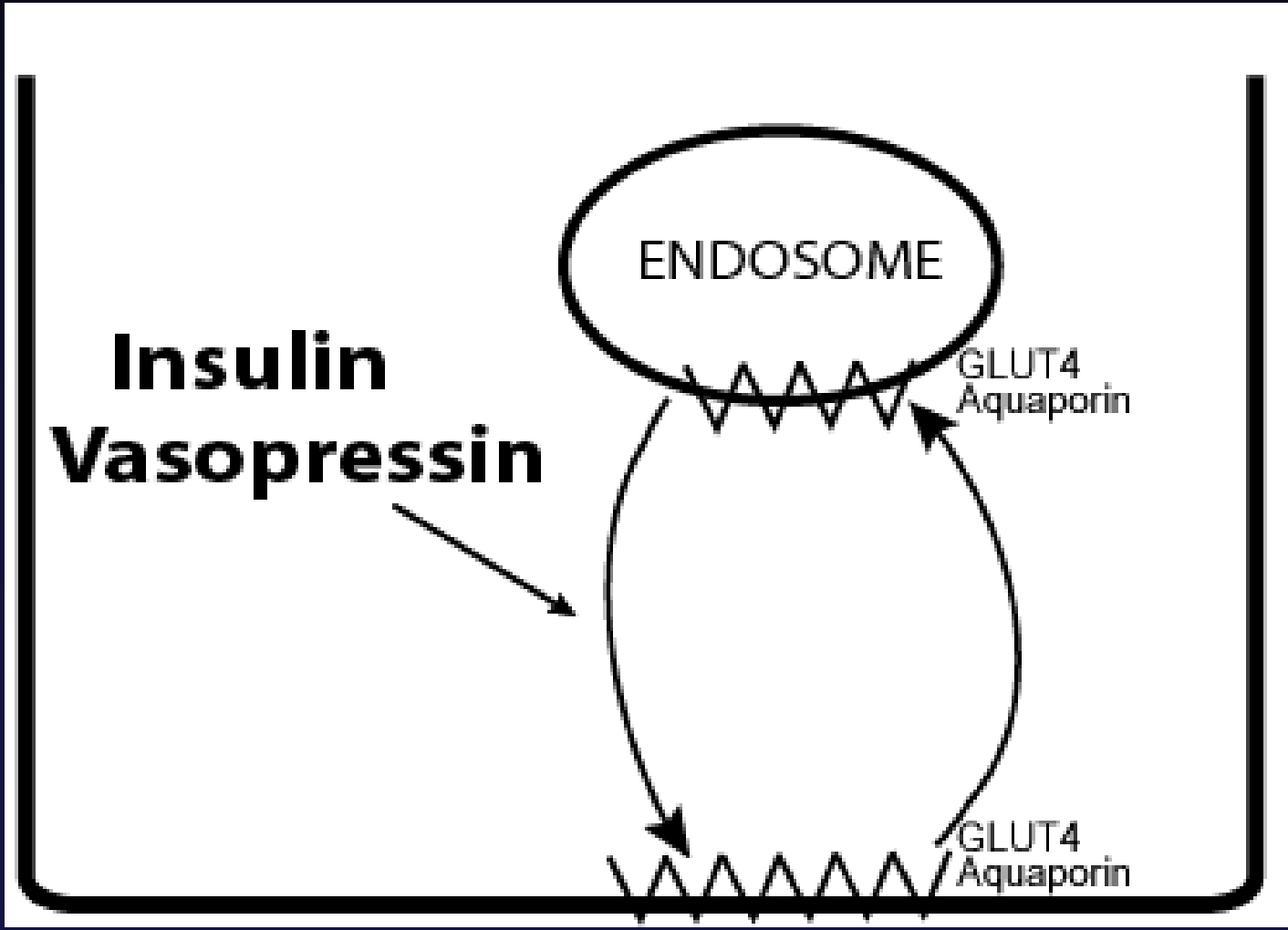


Geldner et al., 2001

Dynamic Movement of PIN Proteins

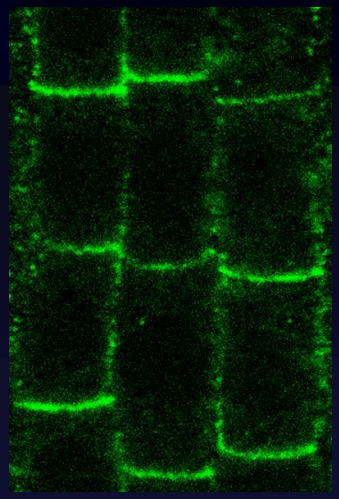


Subcellular Cycling – Means to Modulate Protein Activity?

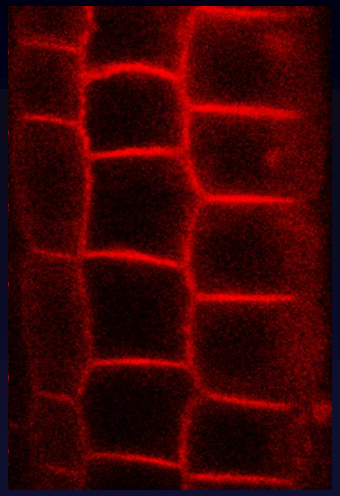


Auxin Inhibits Internalization of Plasma Membrane Proteins

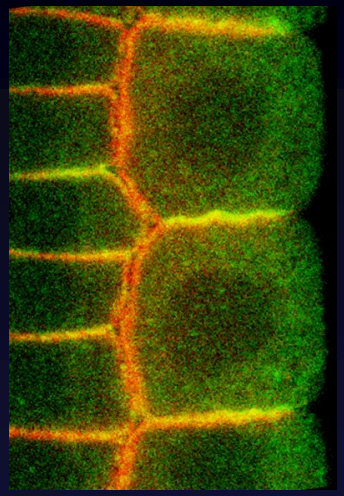
NAA /BFA



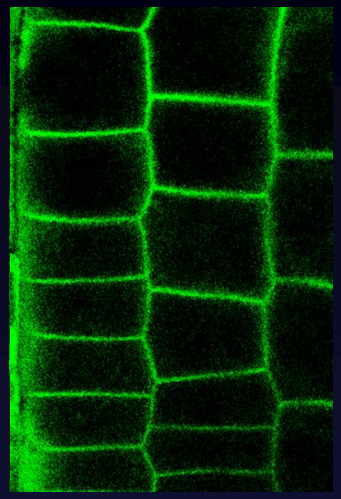
PIN1



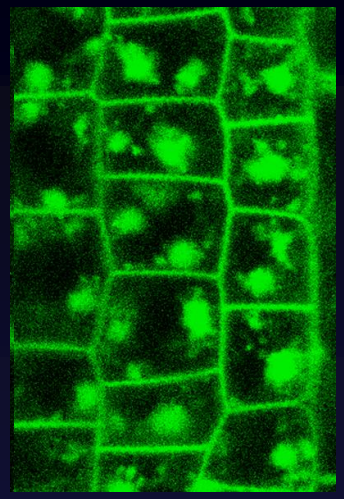
PM-ATPase



PIN2/ATPase

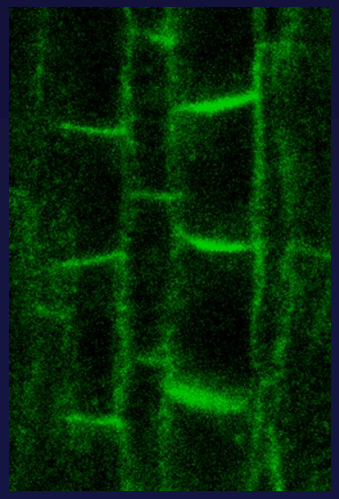


PIP2

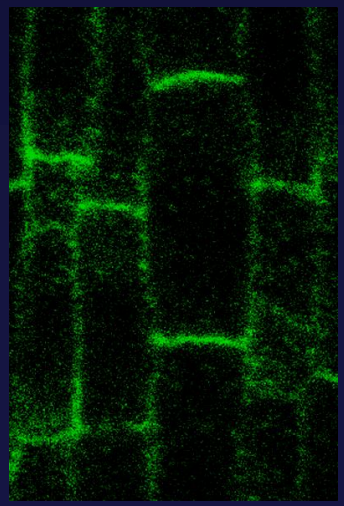


BRI1

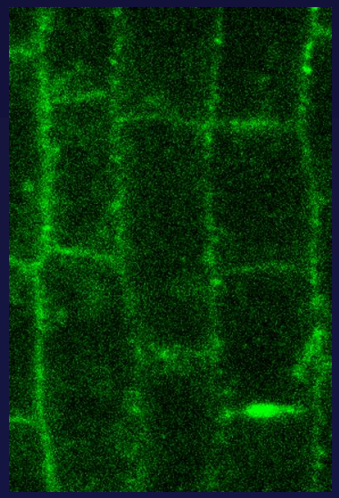
IAA/BFA



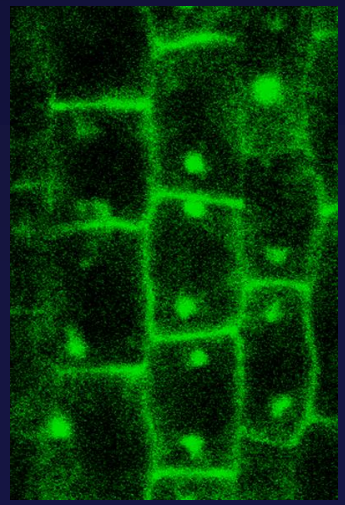
2,4-D/BFA



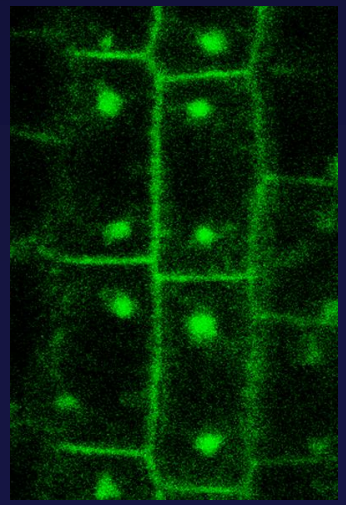
BFA in *sur2*



2-NAA/BFA

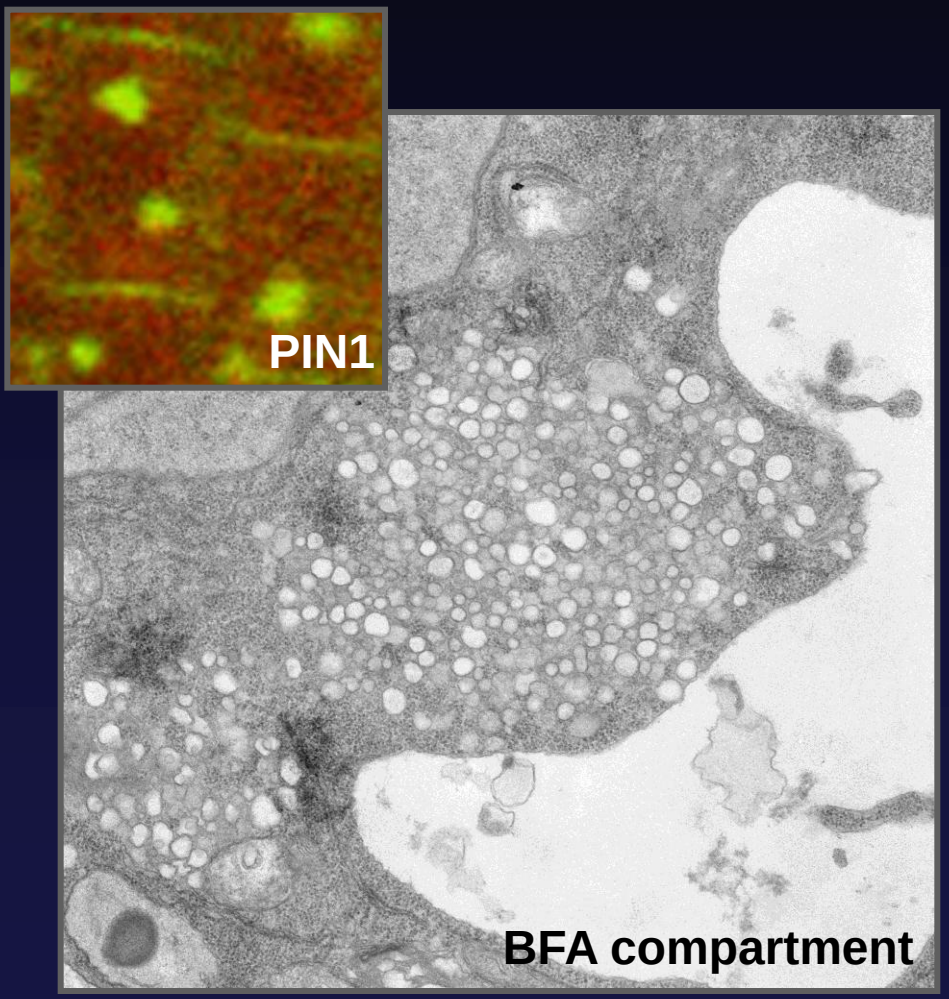


Ethylene/BFA

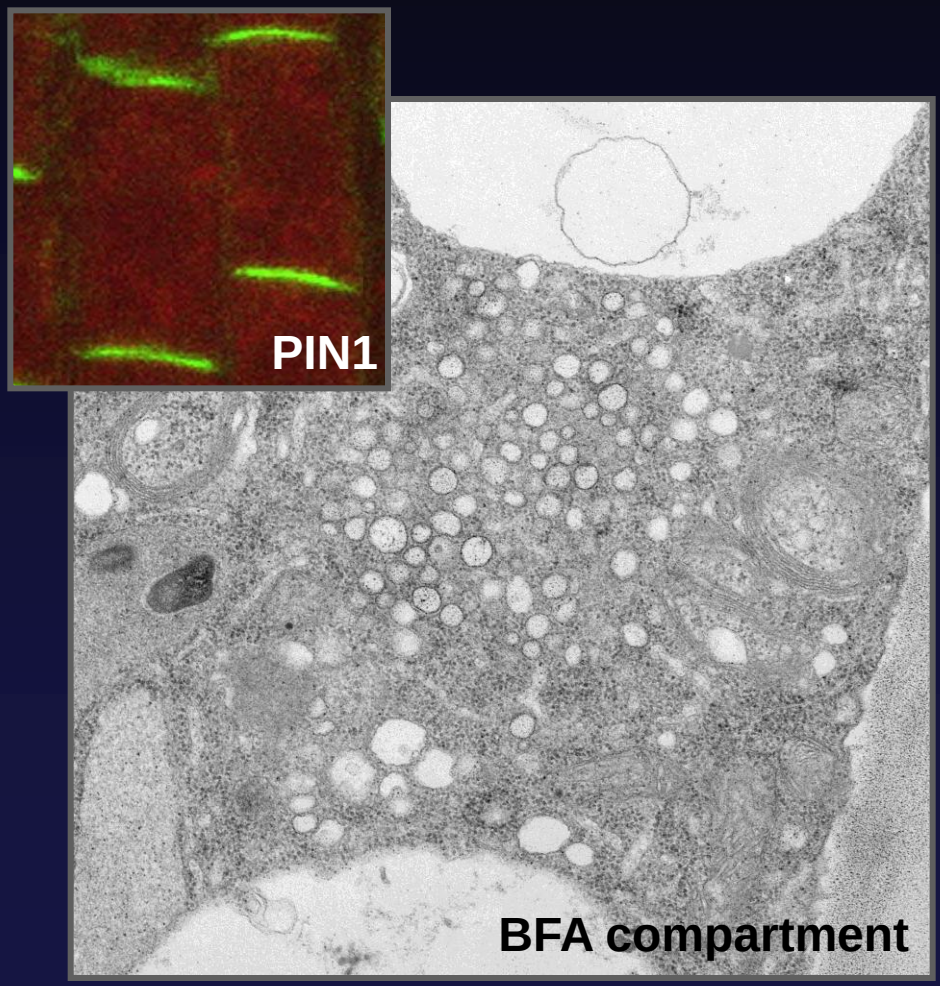


Place of Auxin Action in Protein Cycling

BFA



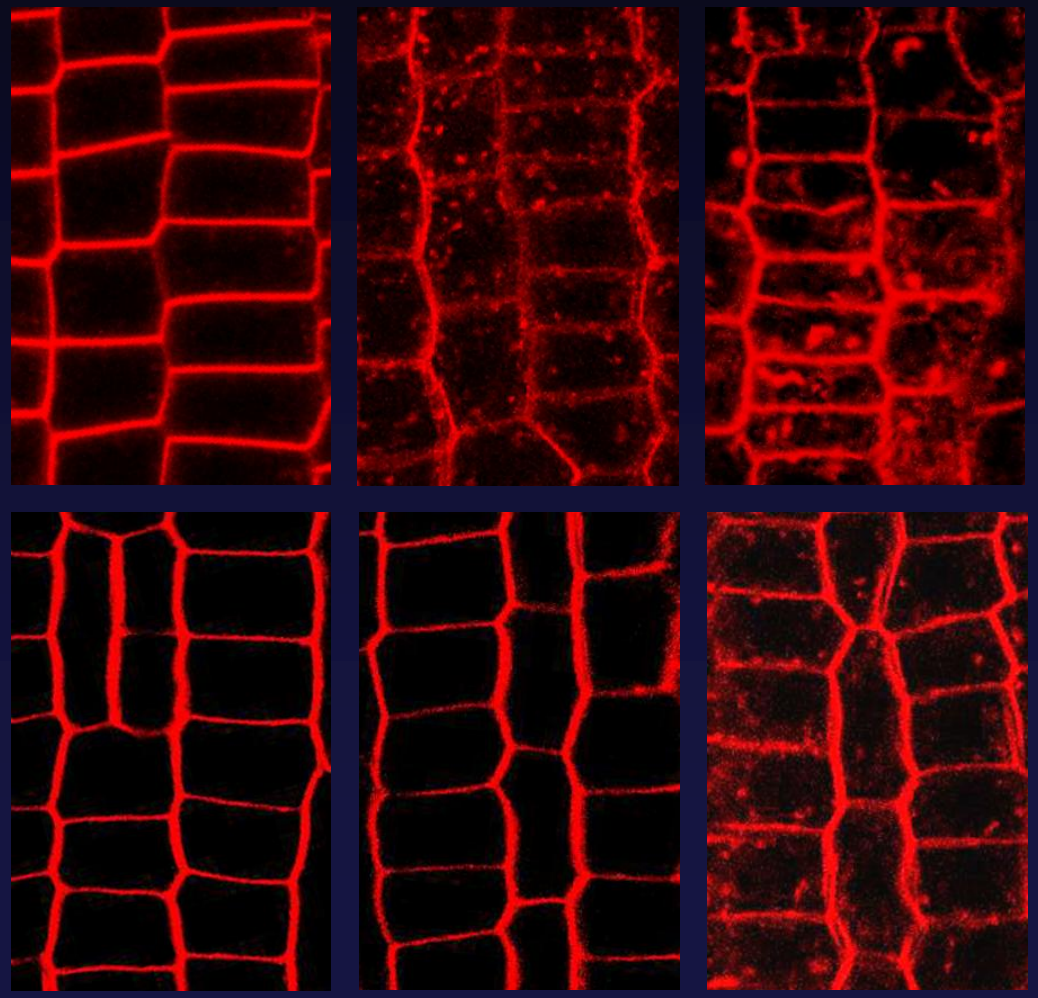
Auxin + BFA



Paciorek et al., 2005

Auxin Inhibits Endocytosis

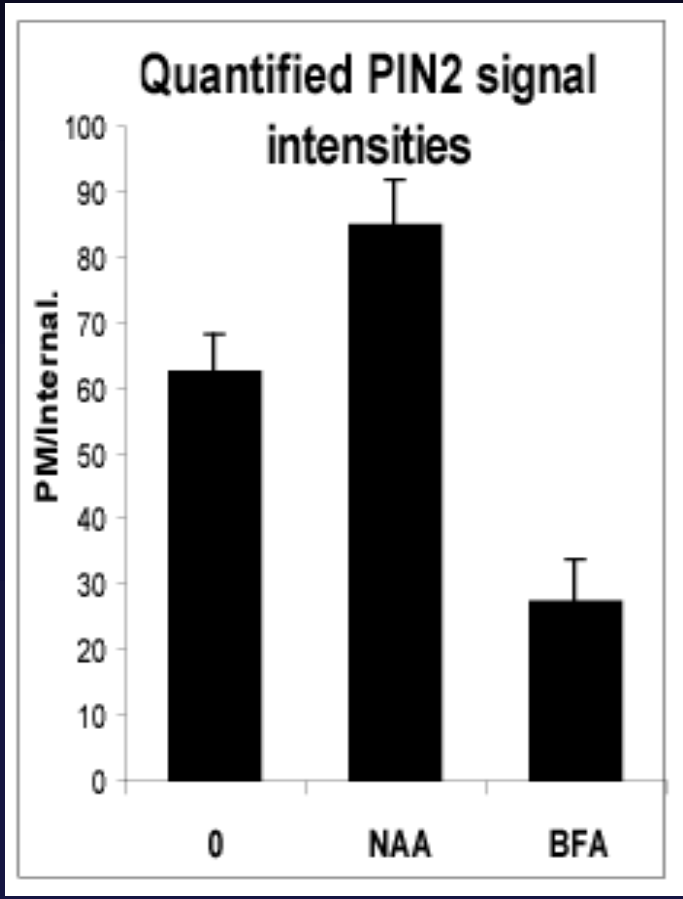
Uptake of endocytic tracer FM4-64



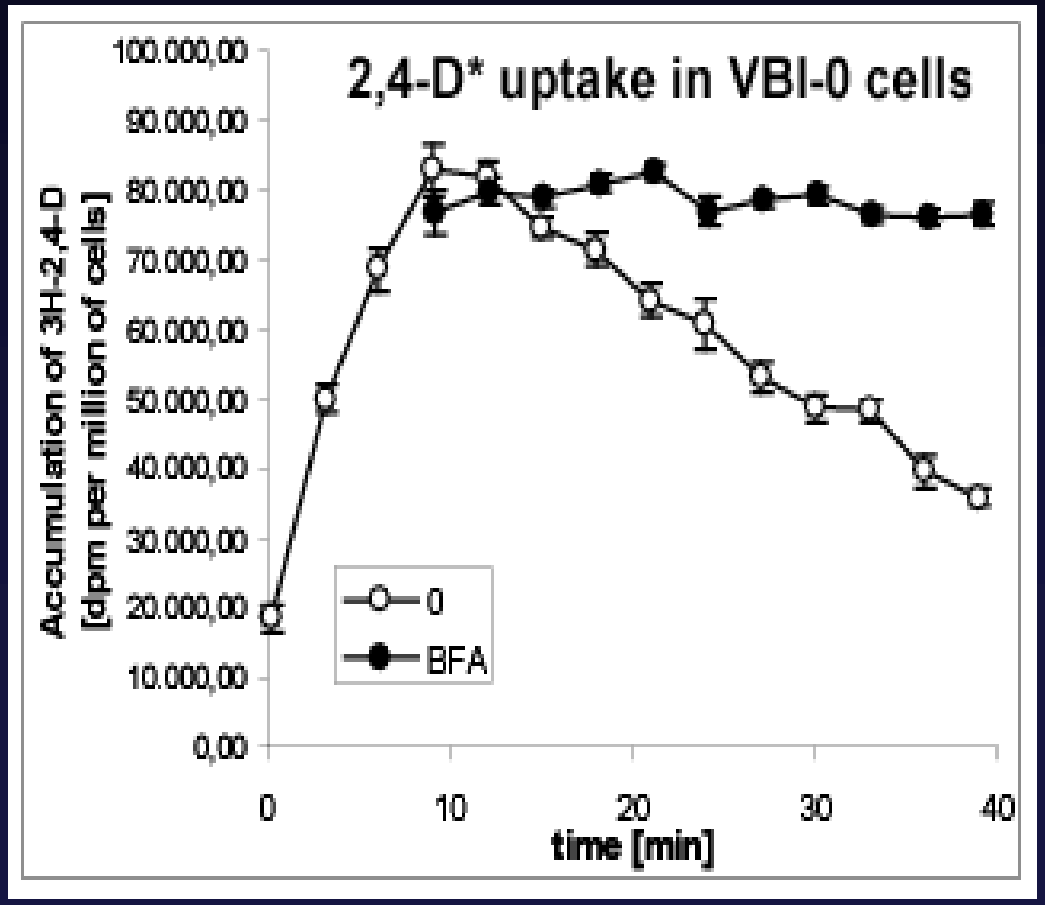
Paciorek et al., 2005

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

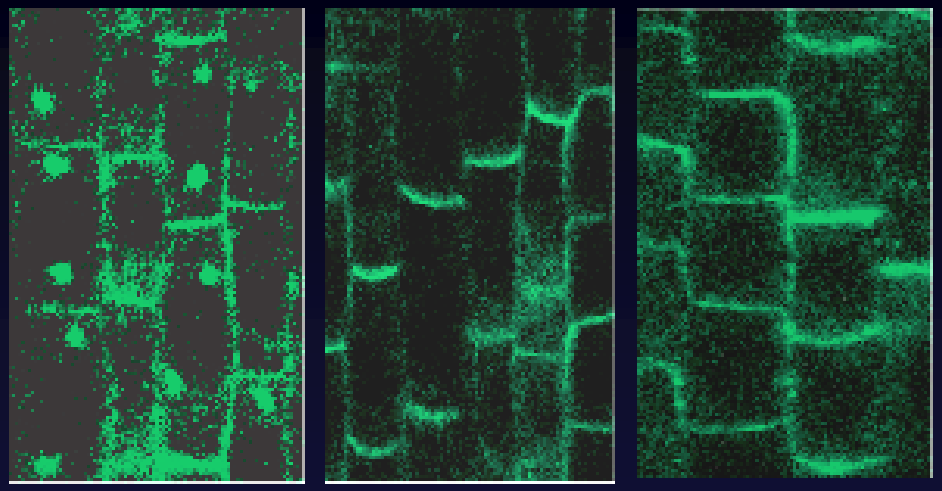
Novel Pathway of Auxin Action

PIN1

BFA

2,4-D/CHX /BFA

2,4-D/MG132 /BFA



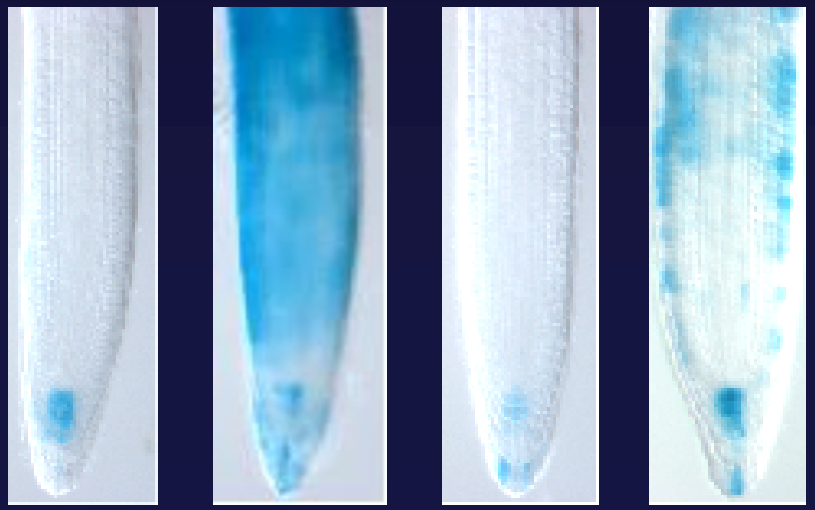
DR5

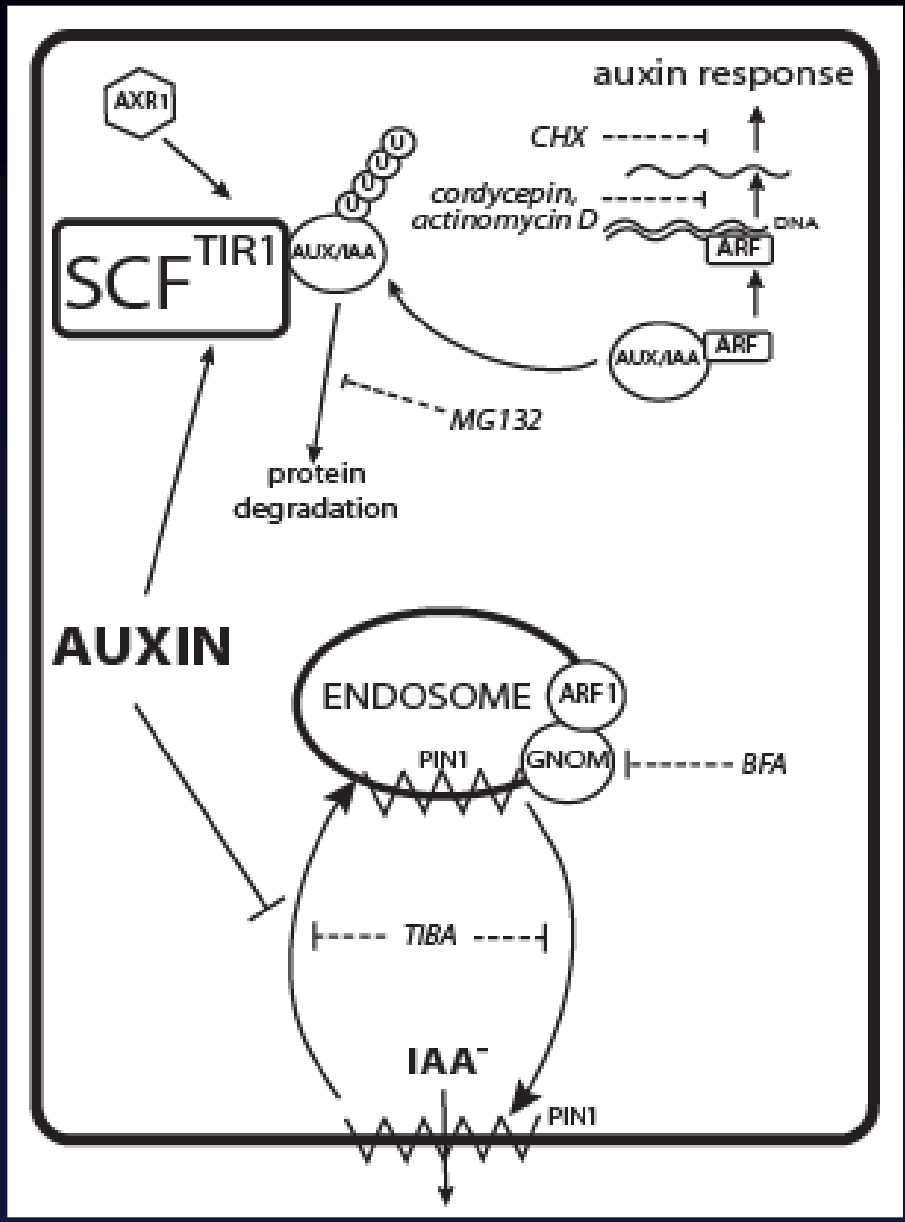
0

2,4-D

2,4-D/ CHX

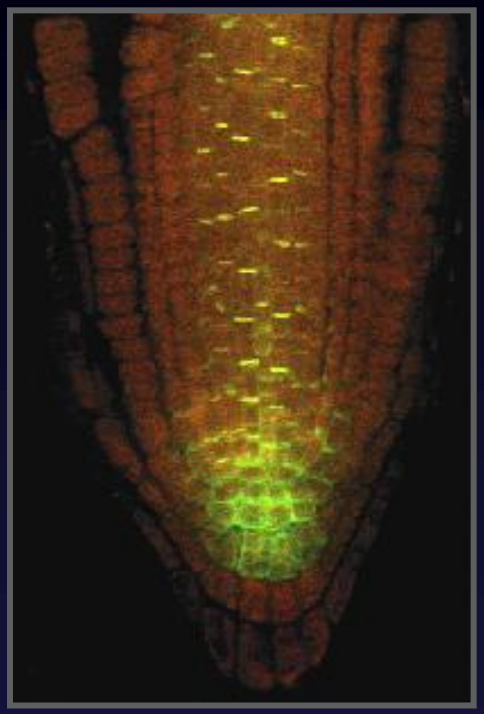
2,4-D/ MG132





unpublished

Mutant Screen for Components of PIN Polarity and Cycling



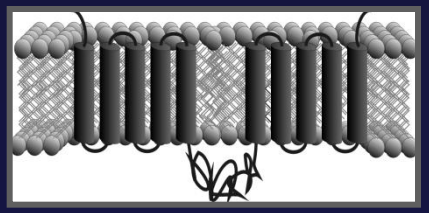
PIN:GFP

EMS mutagenesis. Screening for polarity and cycling defects.

mutant lines

intragenic

extragenic



sequencing

cloning

important residues

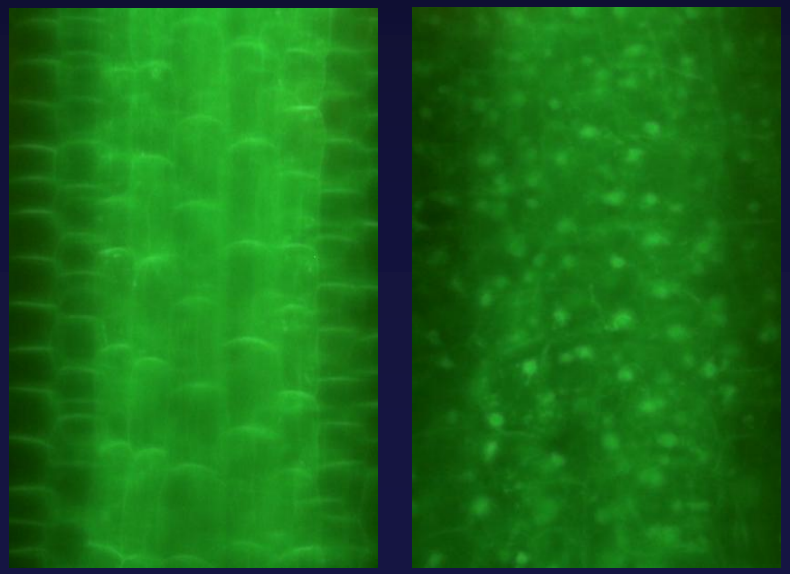
novel genes

unpublished

“Cell Biological” Mutant Screens in Progress:

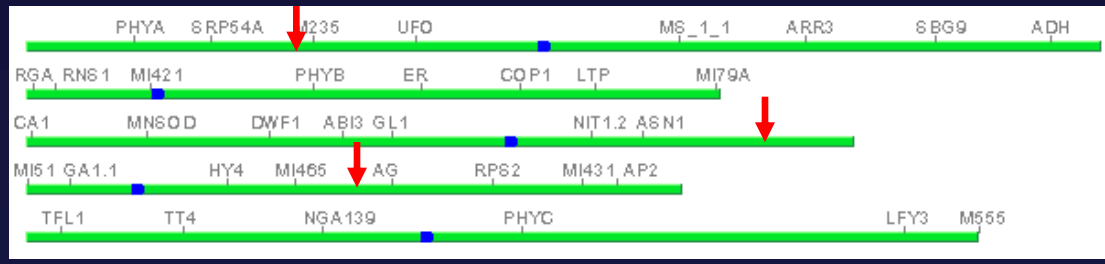
Auxin effect on endocytosis: 3 confirmed mutants

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