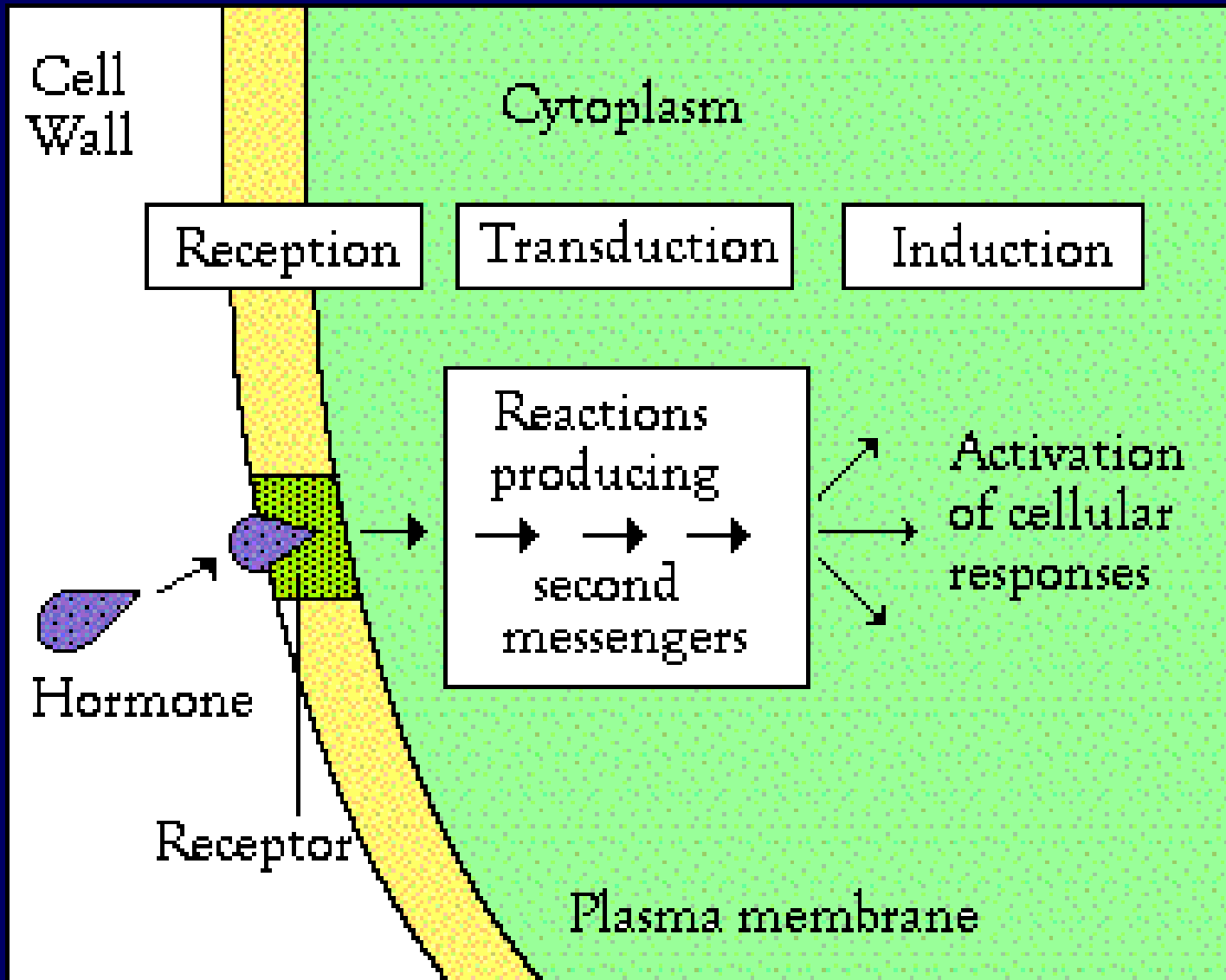
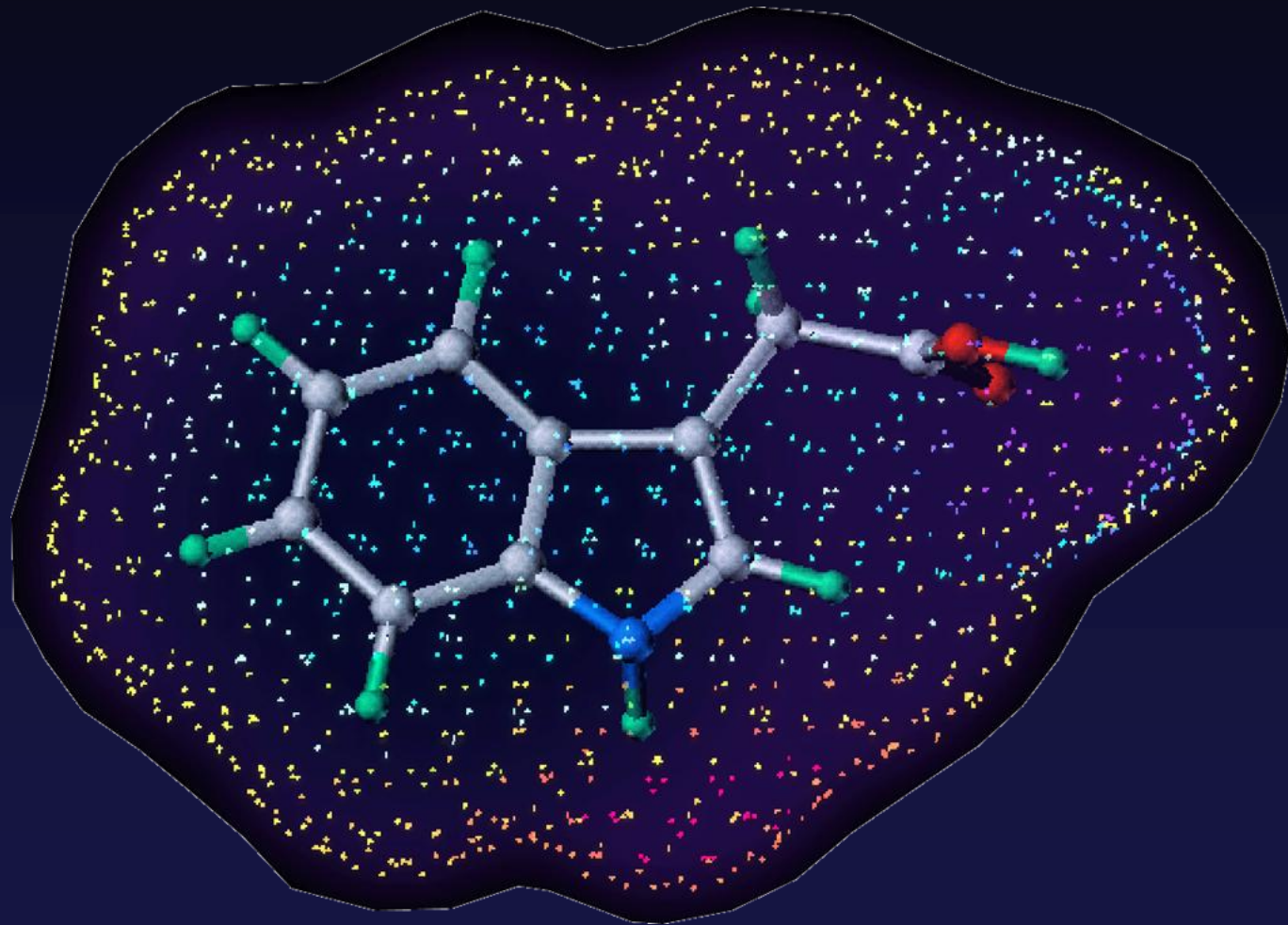


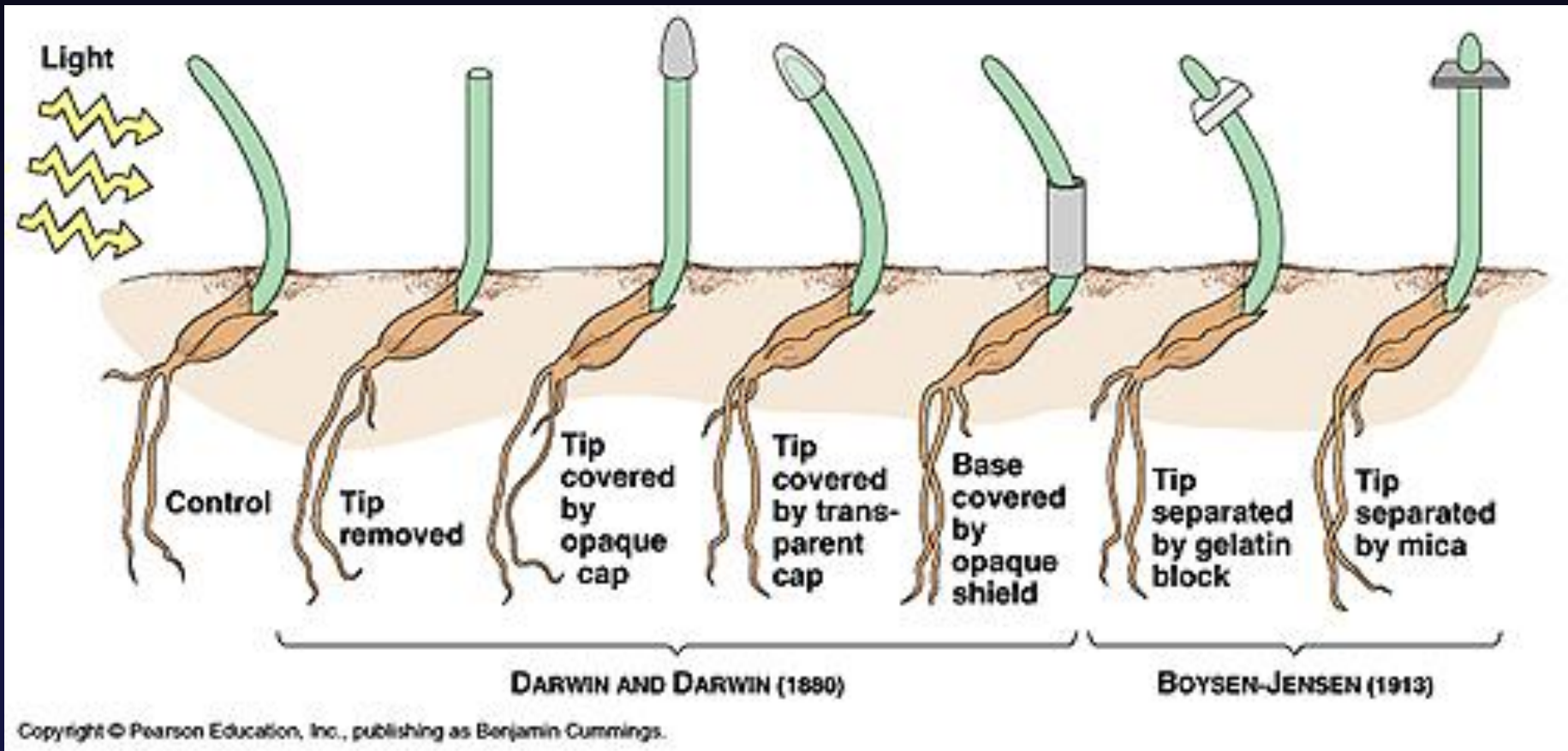
# Signal Transduction



# Auxin Signaling and Transport

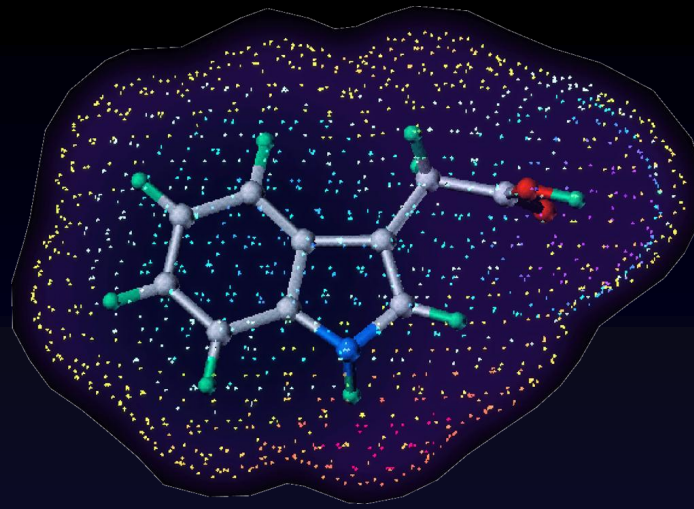


# Discovery of the First Plant Signaling Molecule – Auxin and its Transport



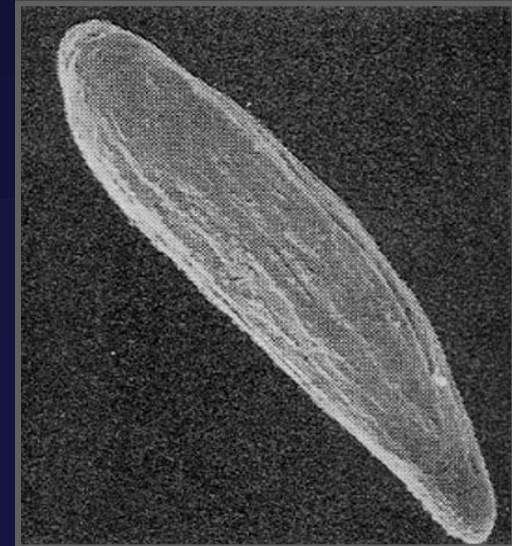
# AUXIN

mediates

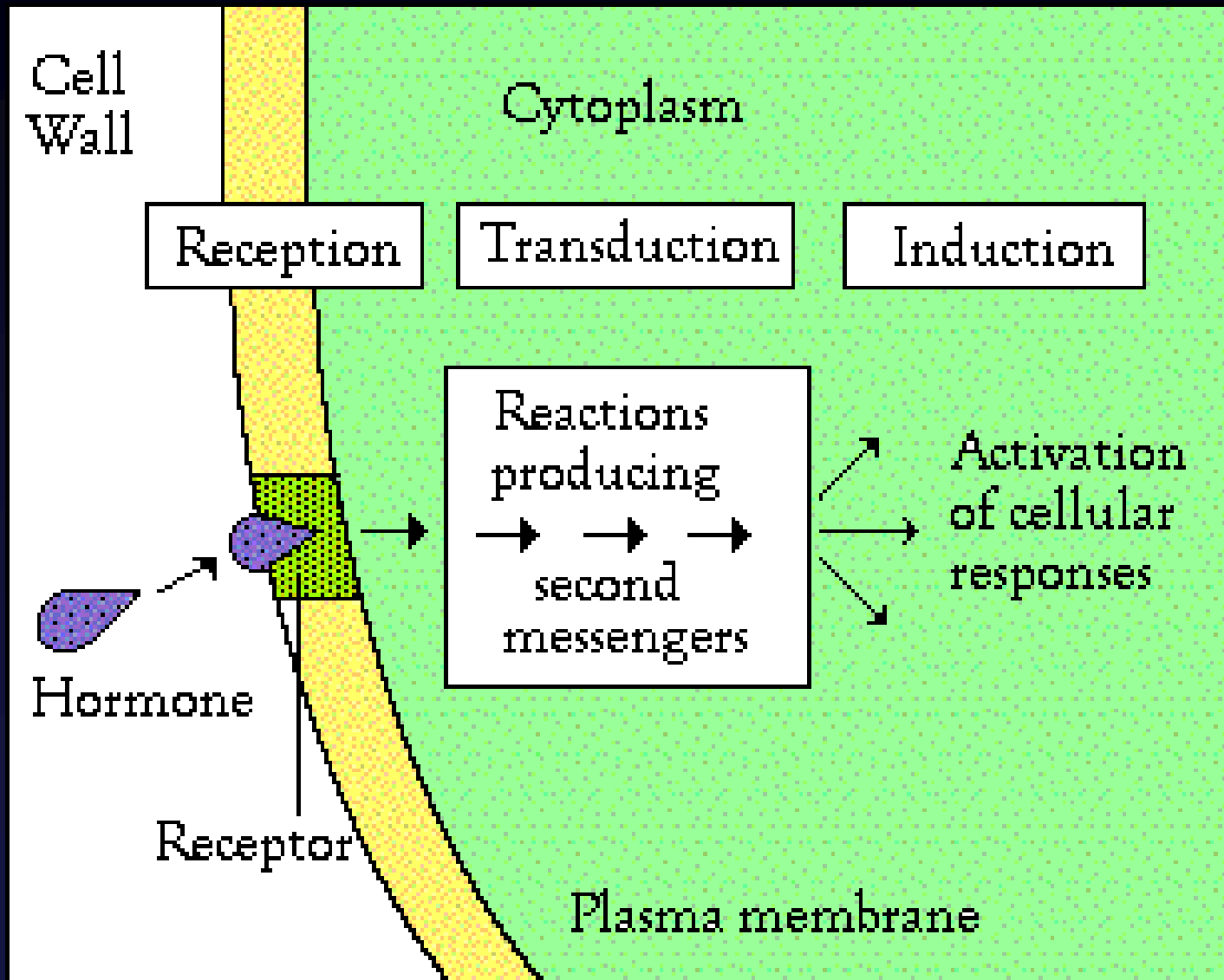


- Embryo development
- Organ initiation and positioning
- Vascular tissue differentiation
- Shoot and root elongation
- Growth responses to light and gravity
- Apical hook formation

embryos



# Signal Transduction



# Biochemical Approach to Identify Auxin Receptor

Isolation of auxin binding proteins

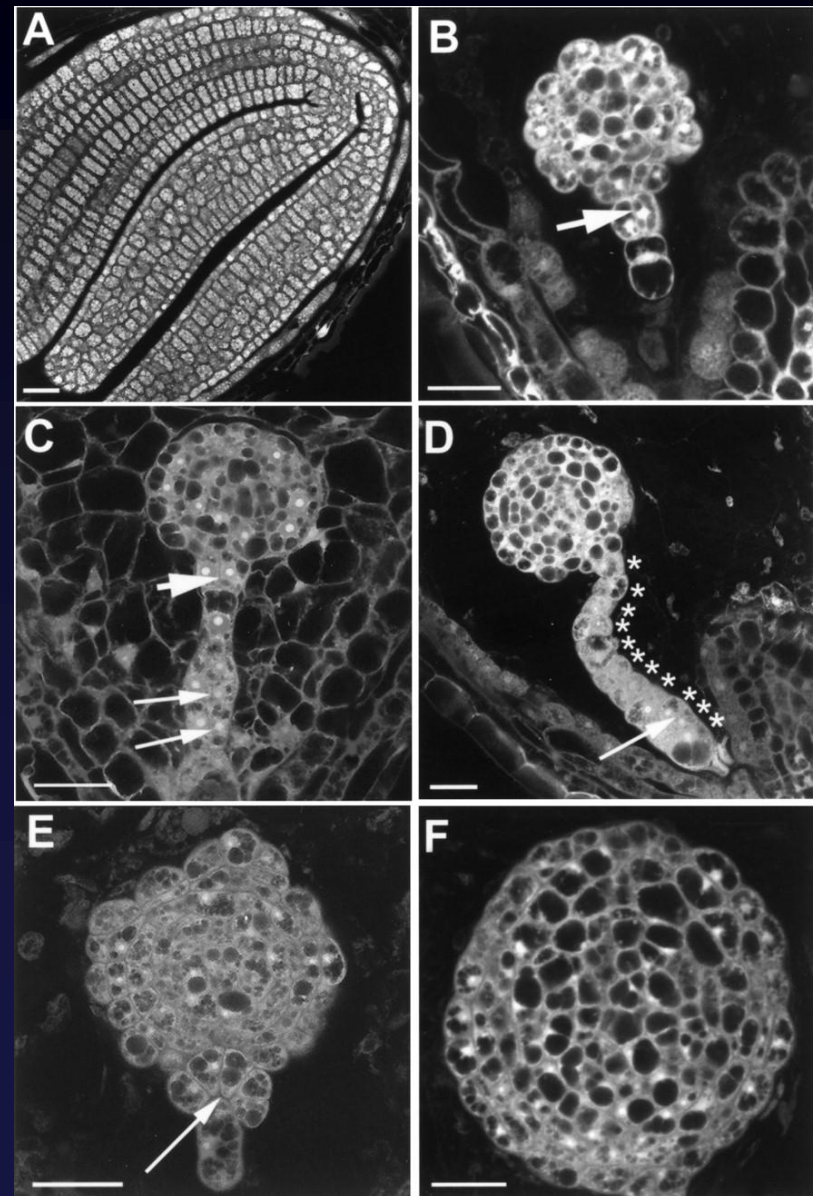
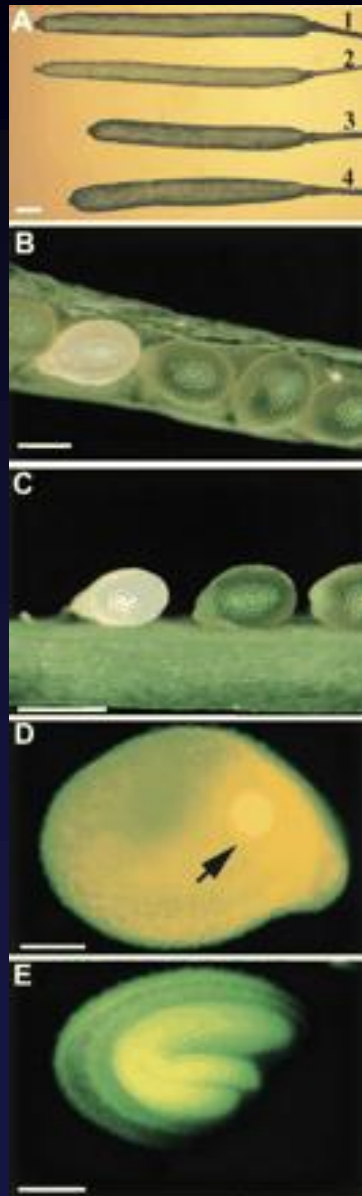
- Azidolabeling
- Affinity chromatography

Protein sequencing, cDNA screening, gene identification

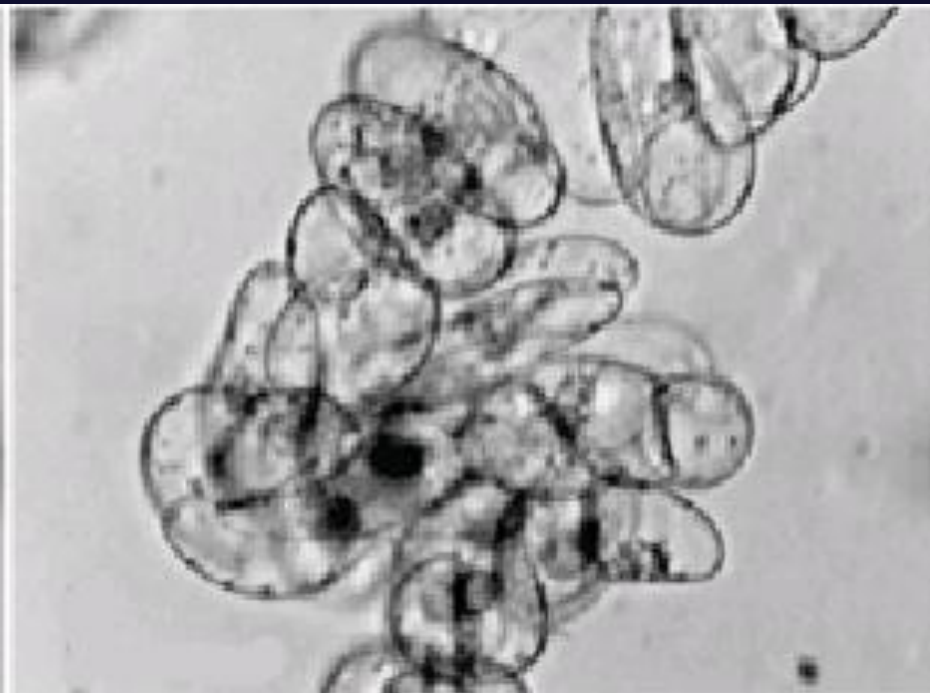
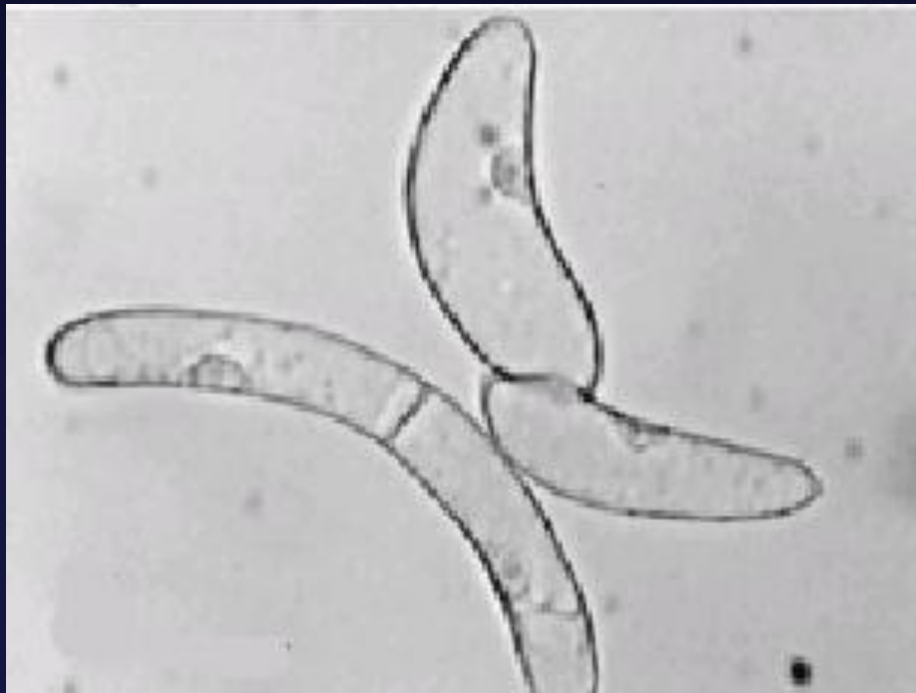
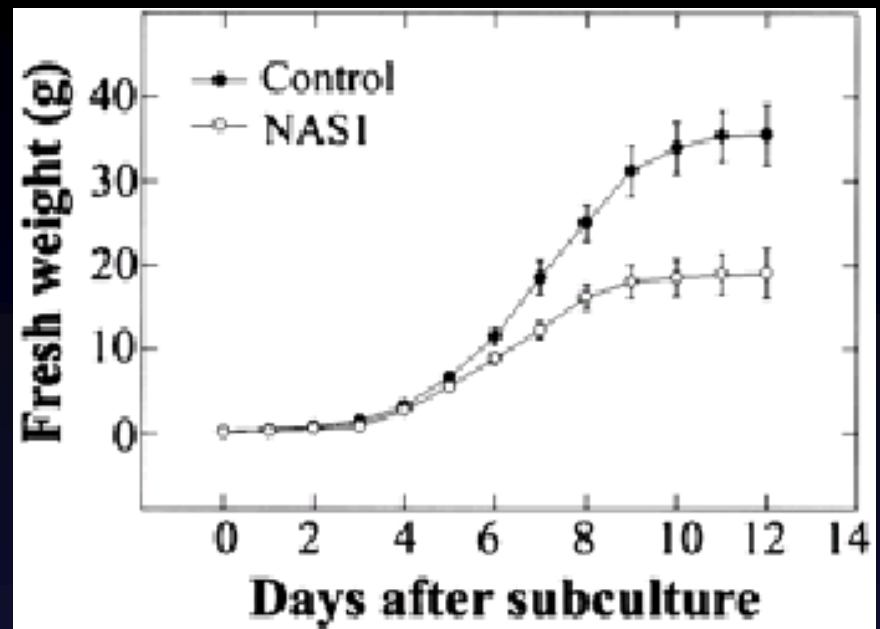
**=> Auxin Binding Protein (ABP1)**



# Reverse Genetic – Embryo Lethal *abp1* Mutant

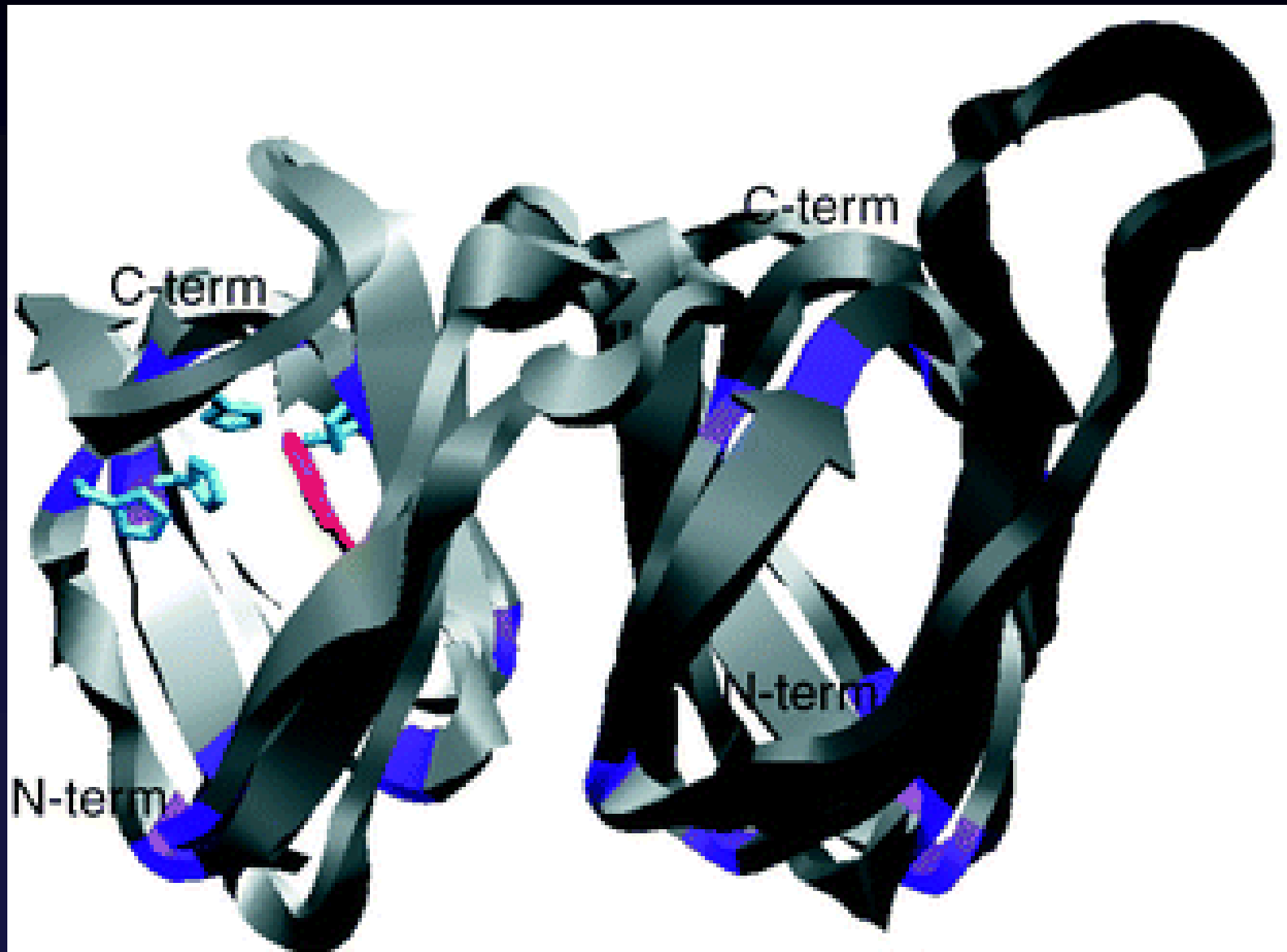


# ABP1 Antisense BY-2 Cells Display Defects in Auxin Dependent Cell Elongation

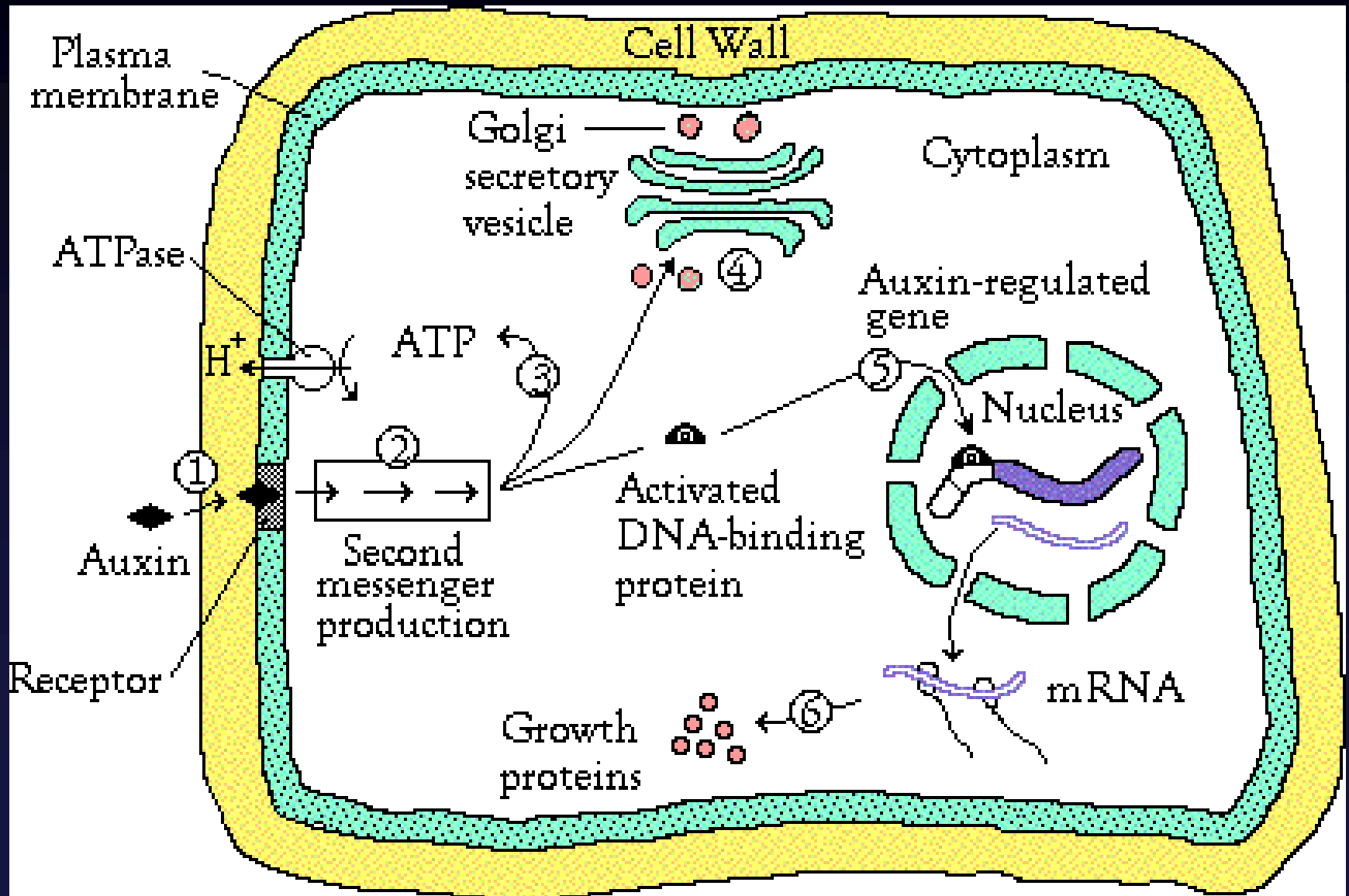




# ABP1 – Structure



# 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?

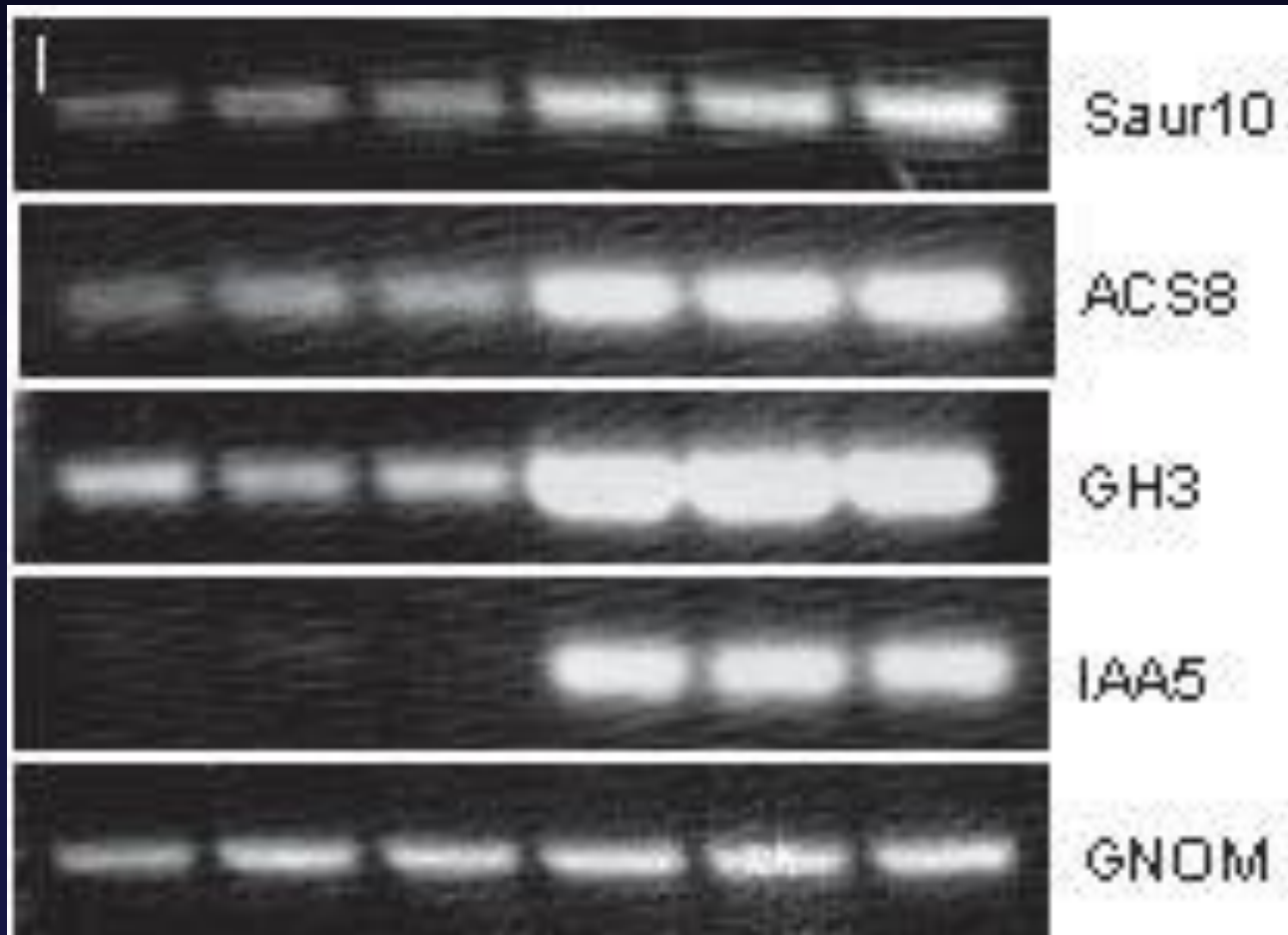
- Rapidly upregulated mRNAs  
(*GH3*, *SAUR*, *AUX/IAA* genes)
- One hybrid screen with Auxin Response Elements  
=> Auxin Response Factors (ARF)
- Two hybrid => *AUX/IAAs* interact with ARFs

# Molecular Biology Approach to Elucidate Auxin Signaling

## RT-PCR

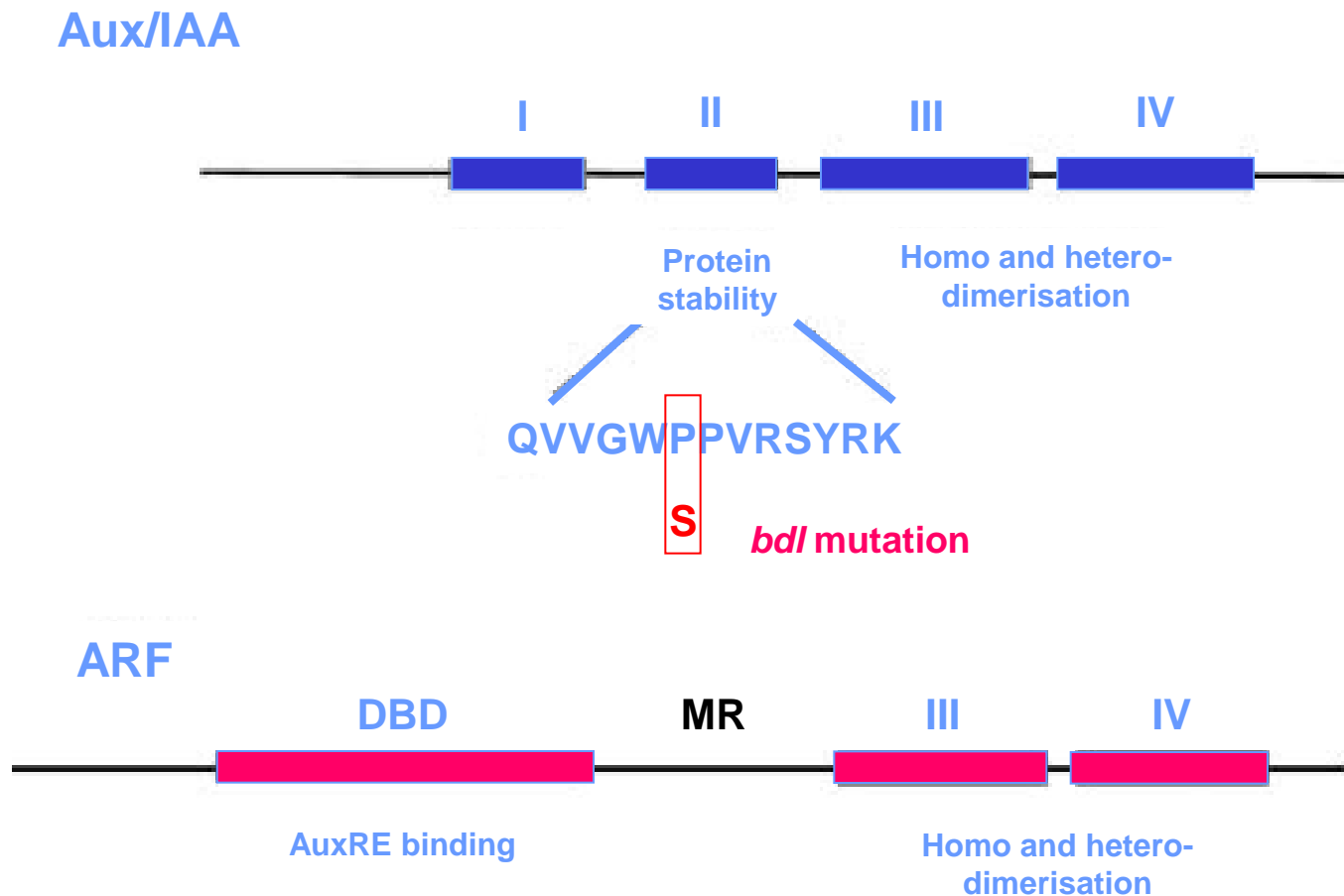
- IAA

+ IAA

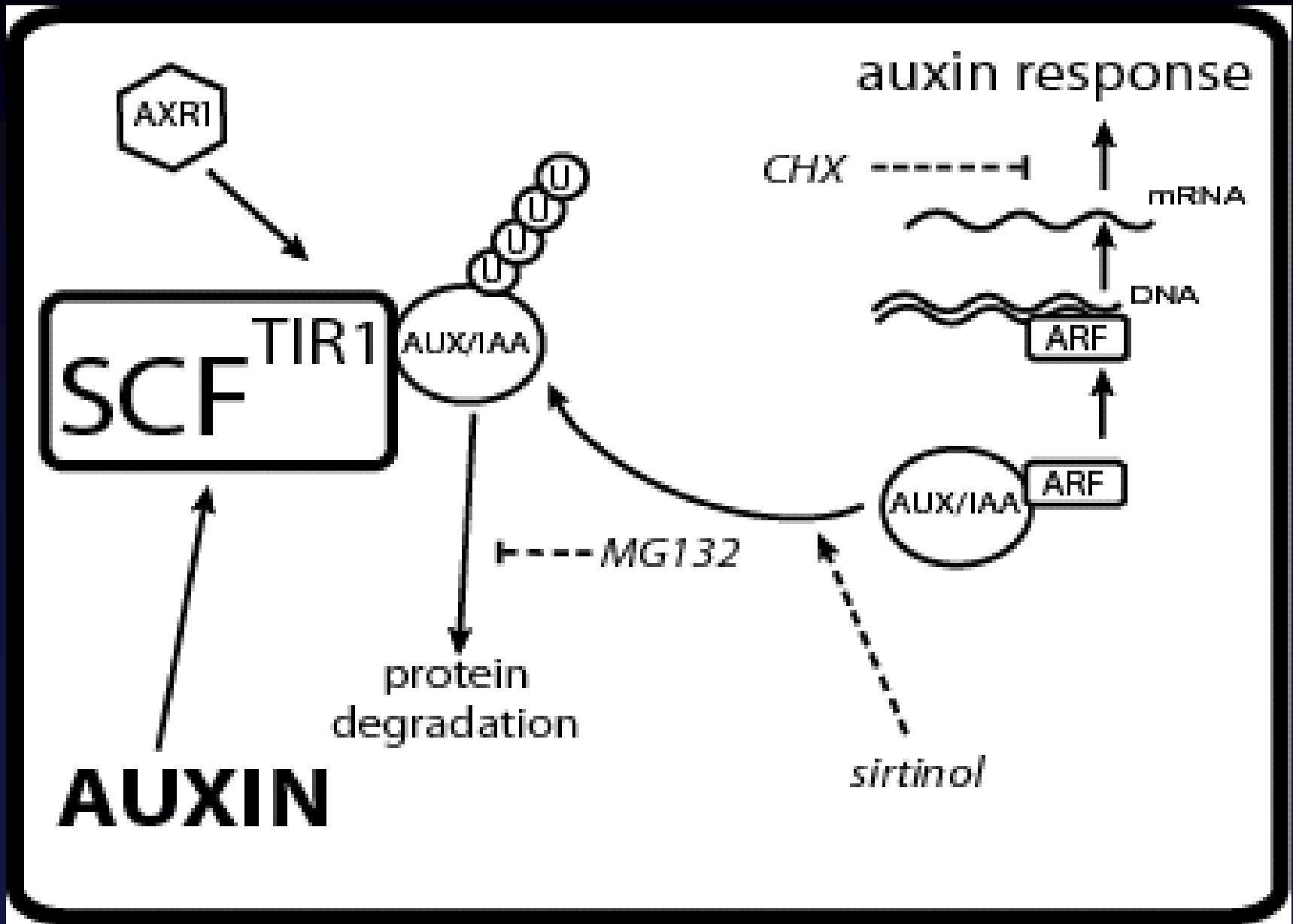




# Some ARFs are **Activators**, whereas Aux/IAA **Repressors** of Auxin Response



# 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*)

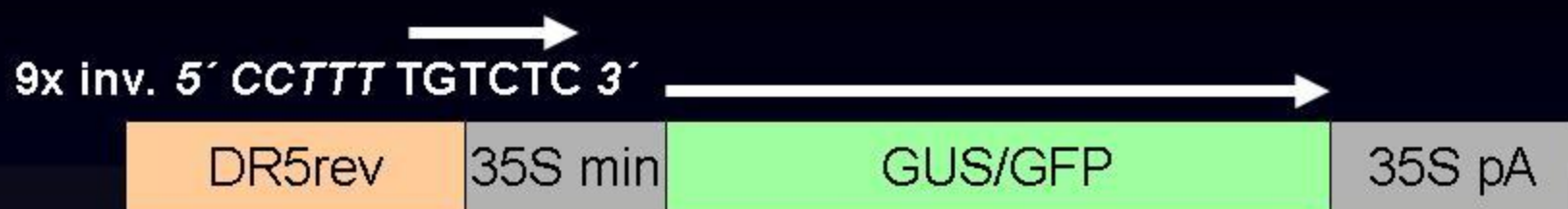
Molecular approach - auxin regulates expression

**ARE** in promoters of auxin regulated genes

**ARF** transcription factors binds to ARE

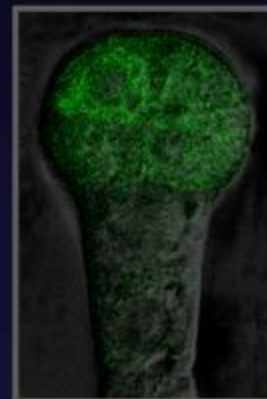
**AUX/IAA** proteins repress ARF and are  
degraded upon auxin signal

# DR5 Auxin Response Reporter



Root

Embryos



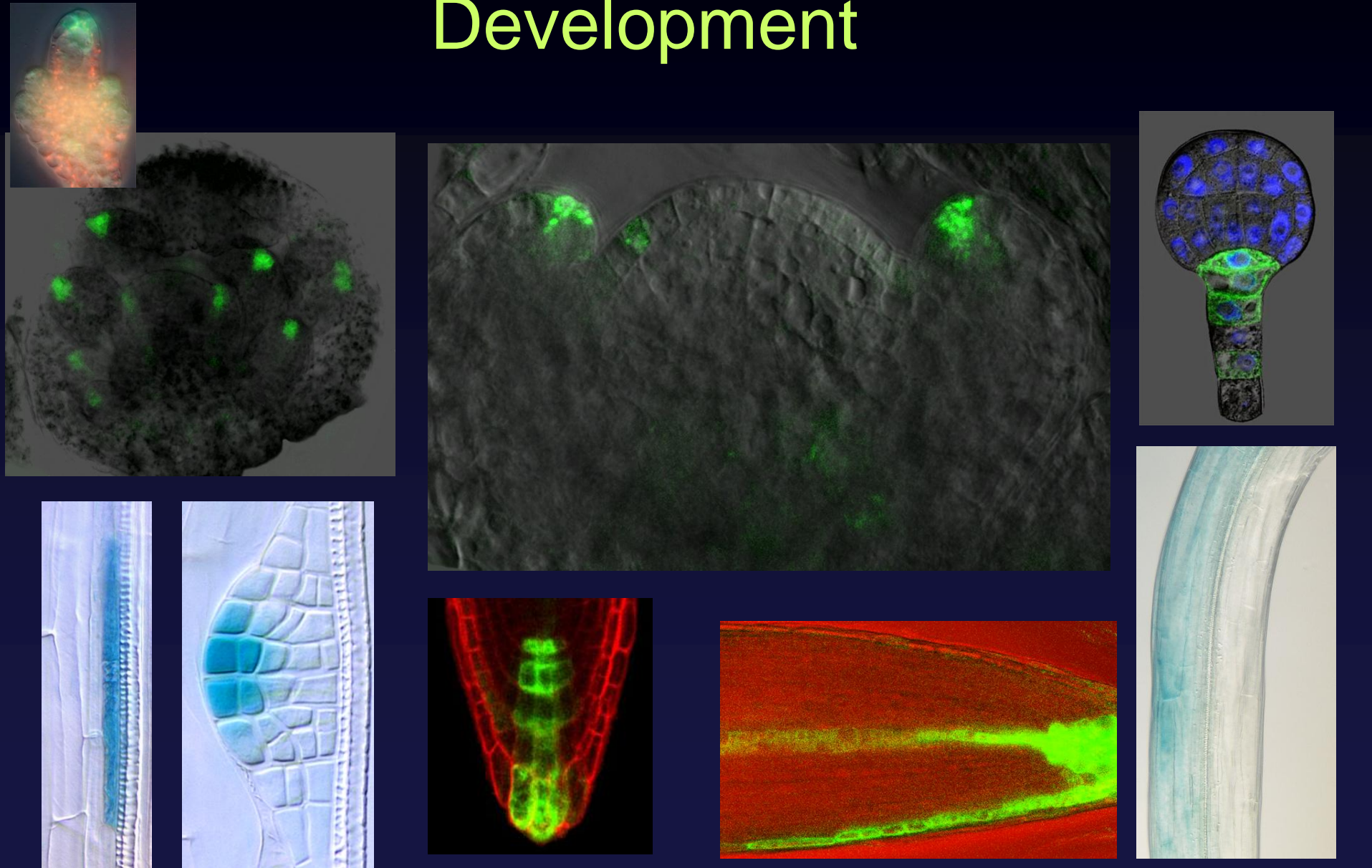
DR5

anti-IAA

DR5

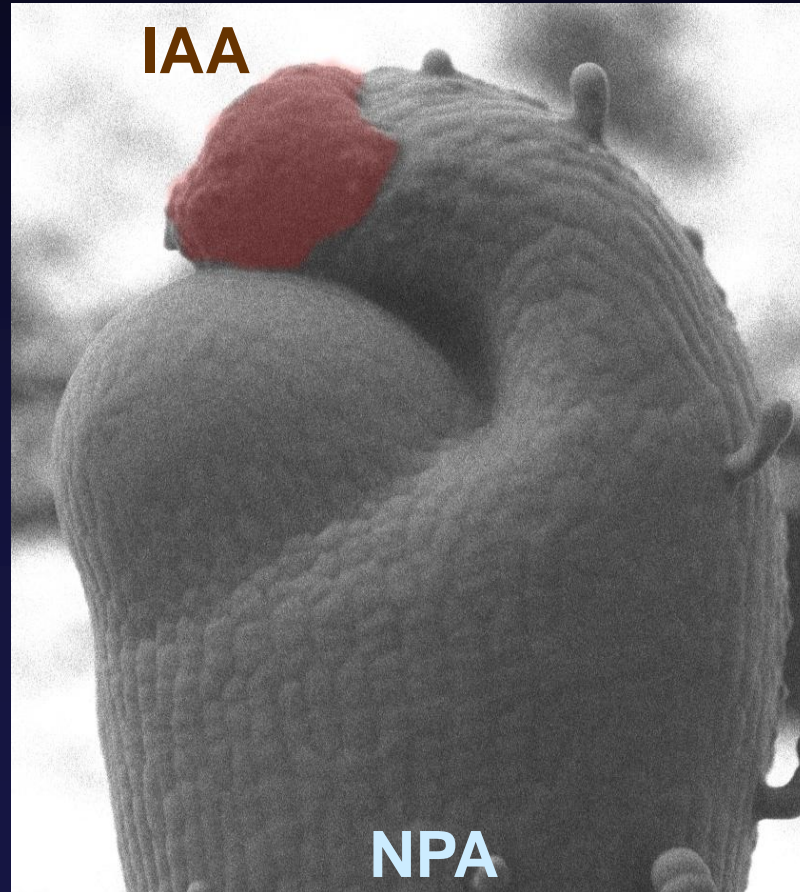
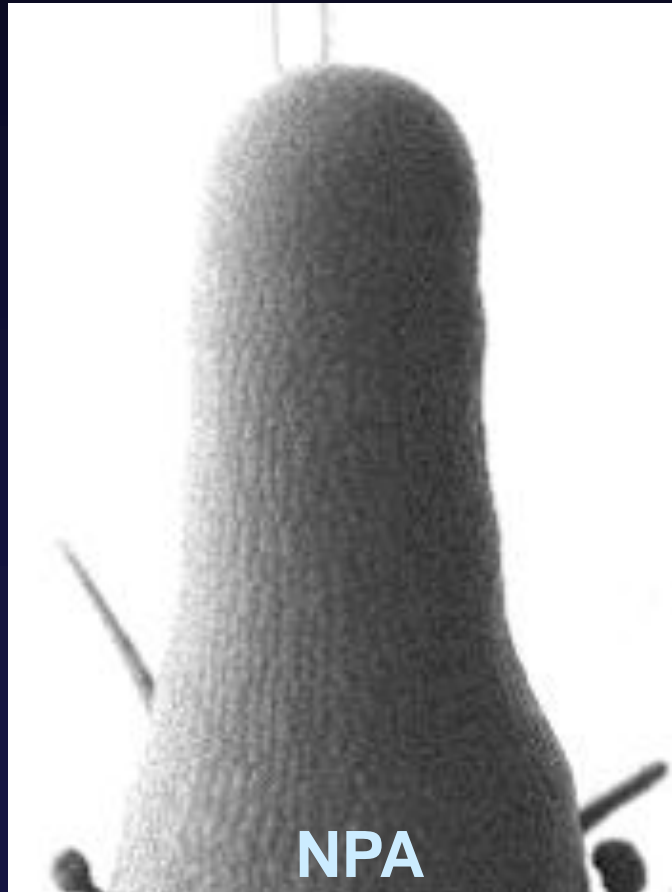
anti-IAA

# Local Auxin Gradients in Plant Development

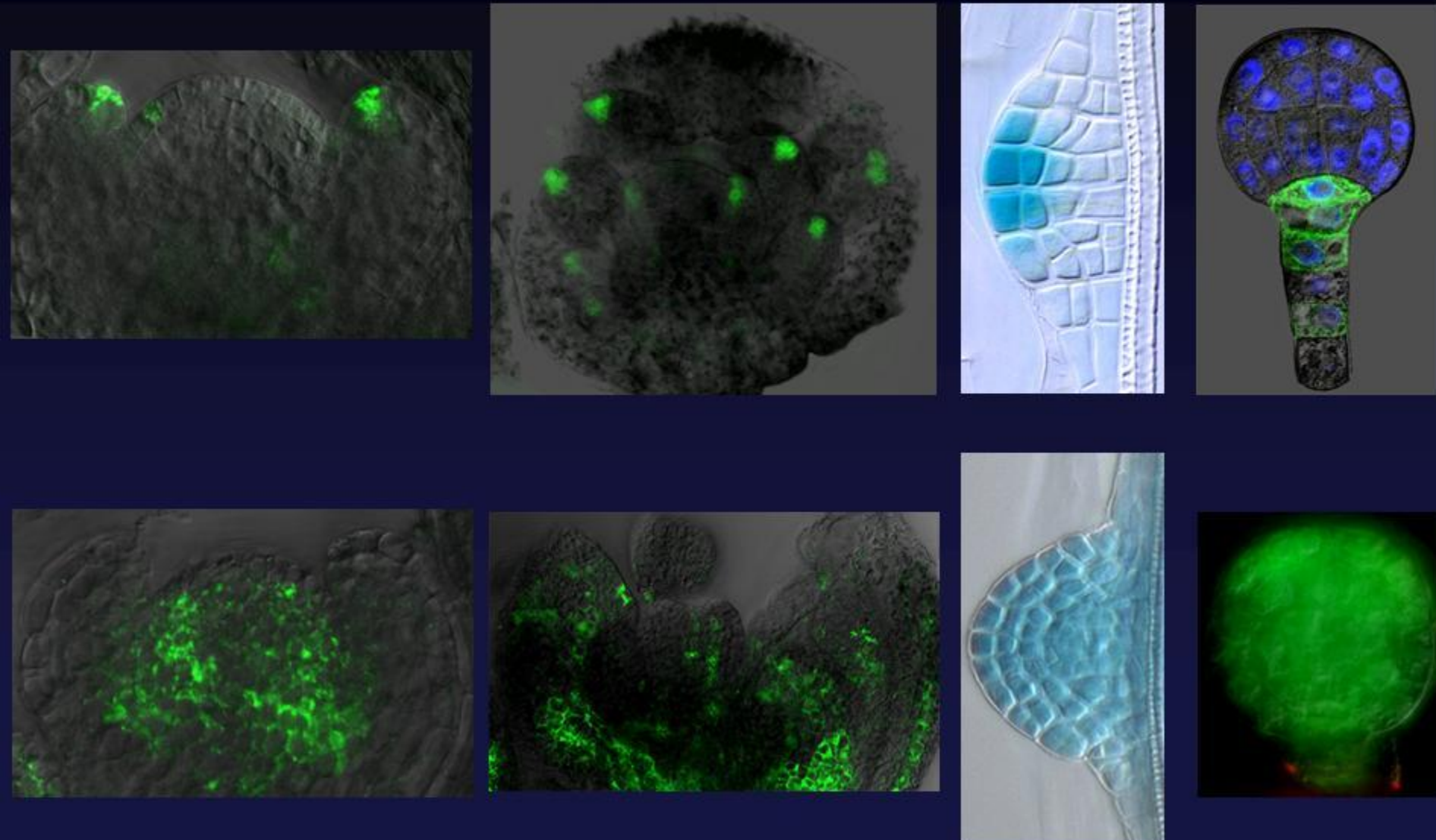




# Local Application of Auxin Induces Organ Formation



# Local Auxin Gradients Require Active Polar Auxin Transport



# Auxin Transport

Proteins involved in auxin transport

- PIN proteins (efflux)

- AUX1 proteins (influx)

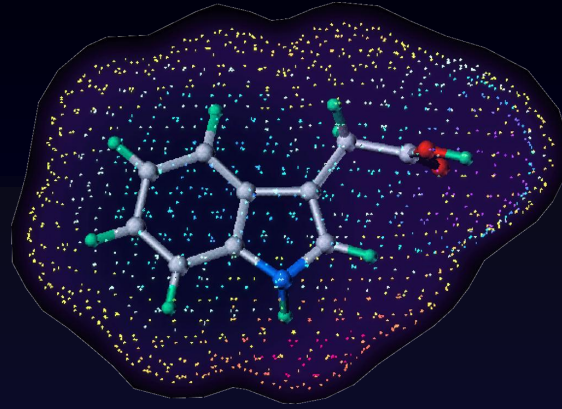
Role of GNOM dependent vesicle  
trafficking

PIN proteins cycling and its role



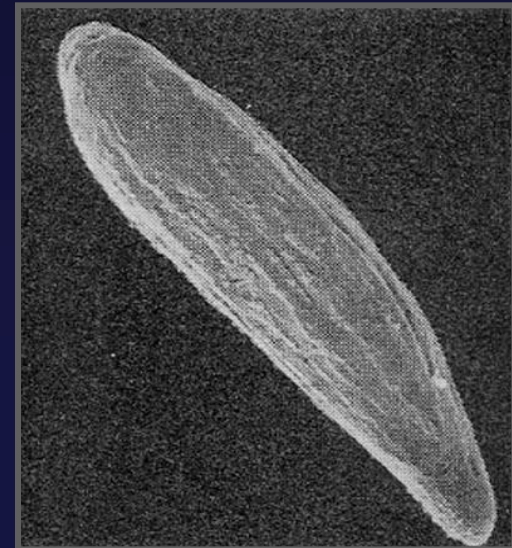
# AUXIN TRANSPORT

mediates

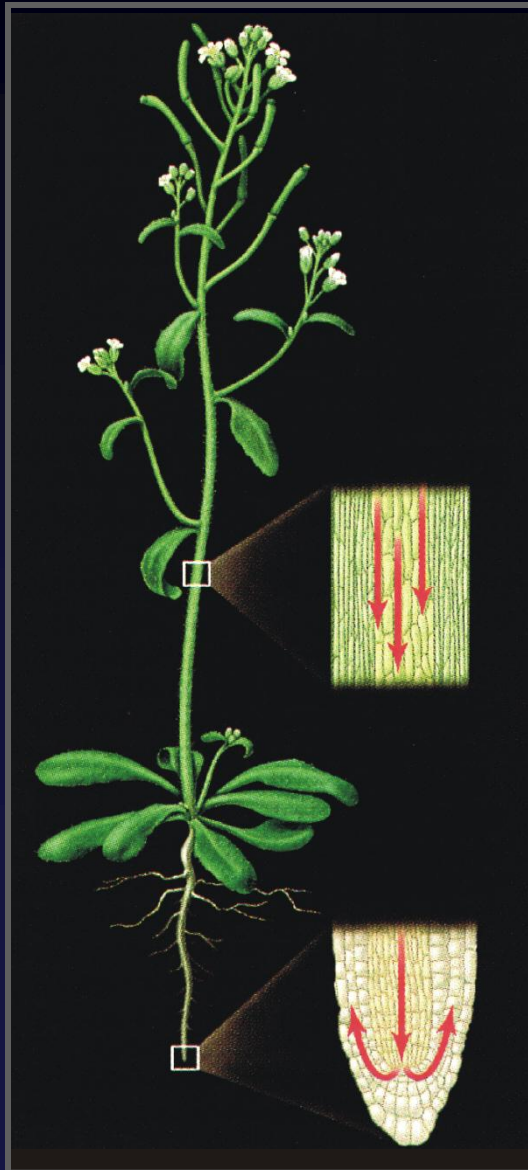


- Embryo development
- Organ initiation and positioning
- Vascular tissue differentiation
- Shoot and root elongation
- Growth responses to light and gravity
- Apical hook formation

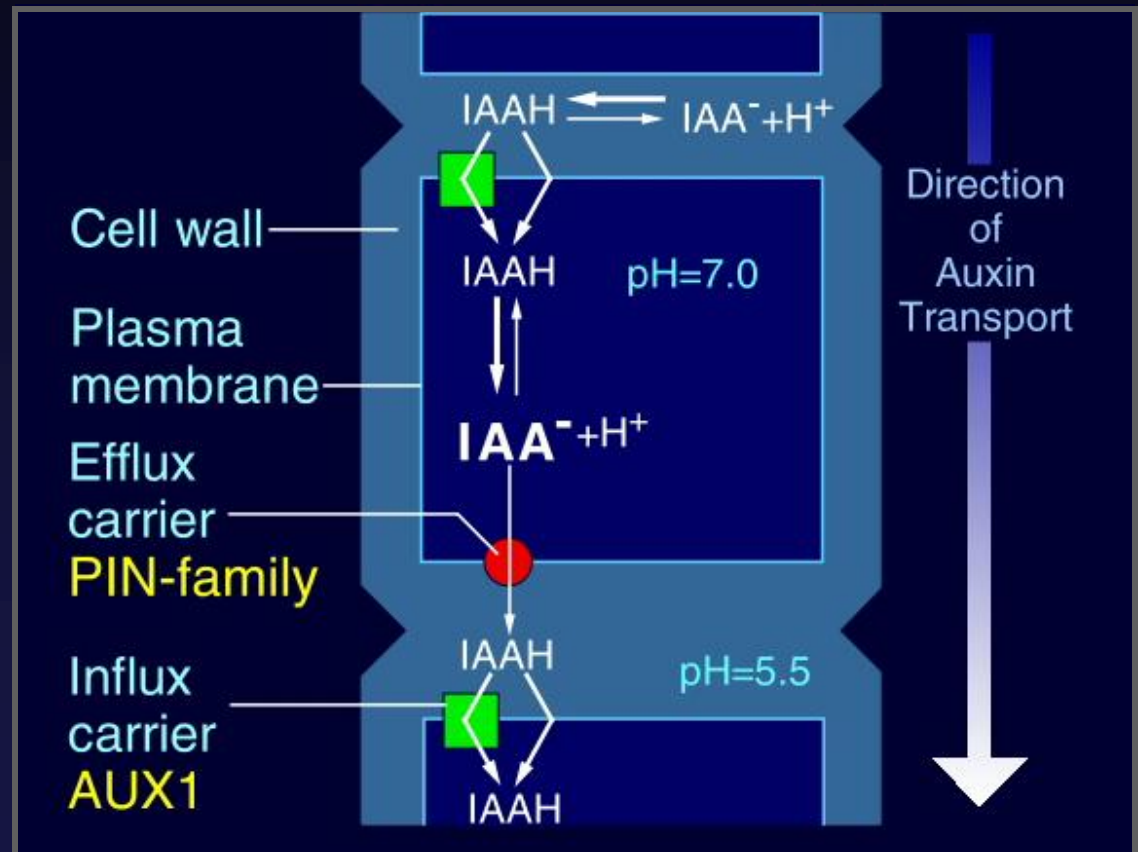
embryos



# Physiology of Auxin Transport



## Chemiosmotic hypothesis



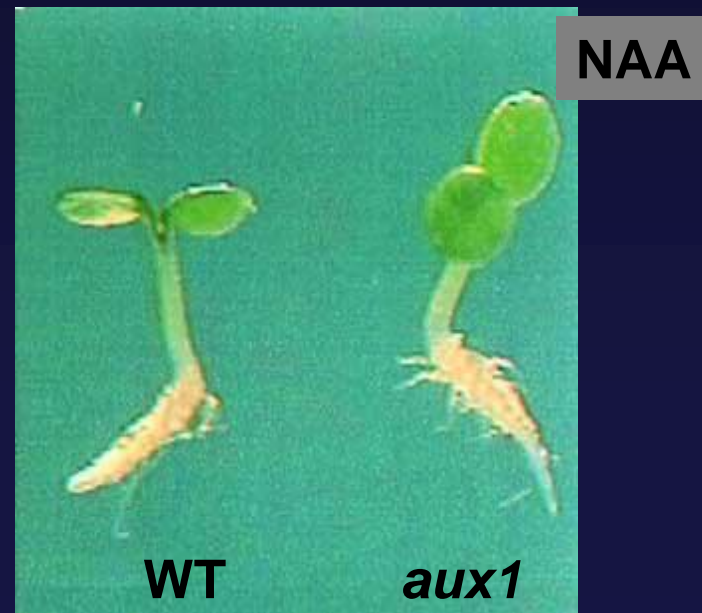
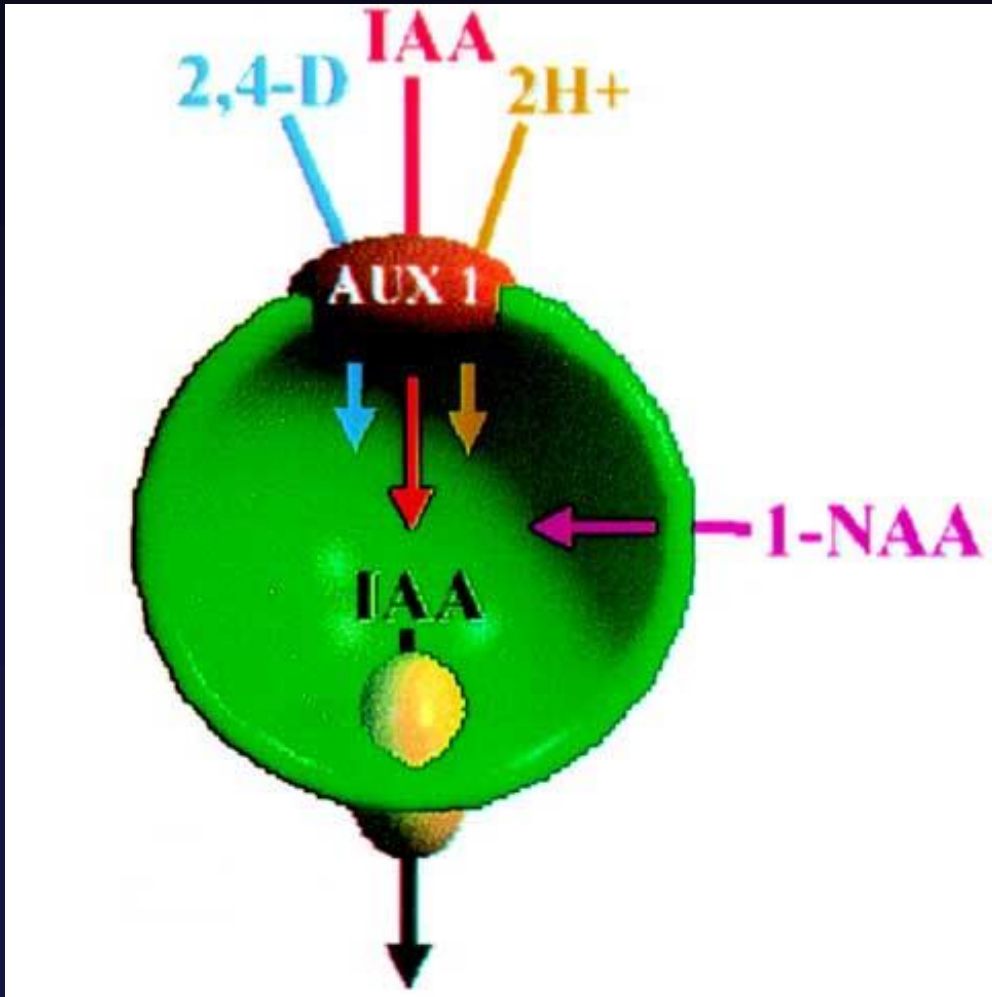


# Auxin Influx

# *aux1* is Resistant to Auxin

*aux1* phenotype

Transport properties of different auxins



# NAA Rescues *aux1* Phenotype

- NAA



+ NAA



# AUX1 – Expression and Localization

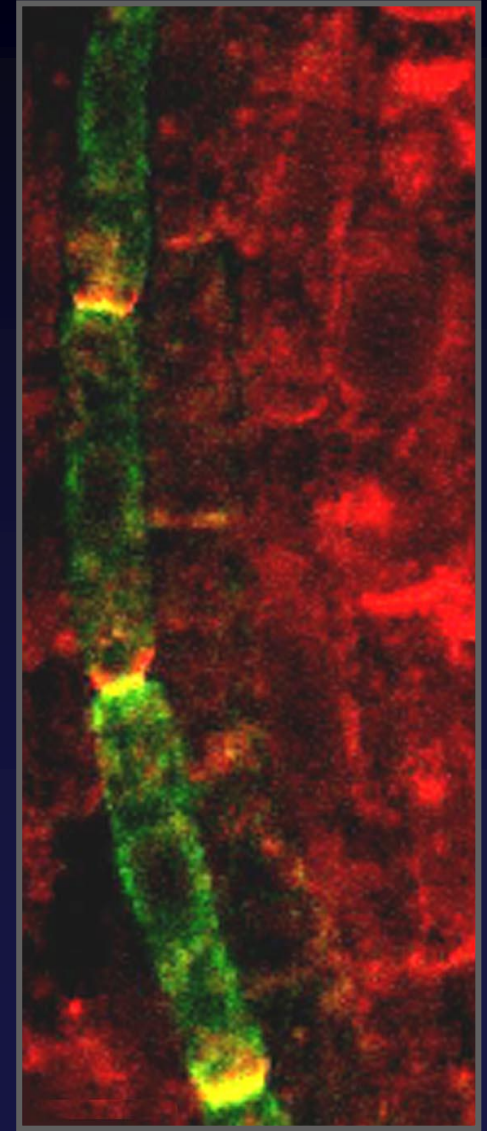
*AUX1::GUS*



AUX1 protein



PIN1/AUX1



# Auxin Efflux

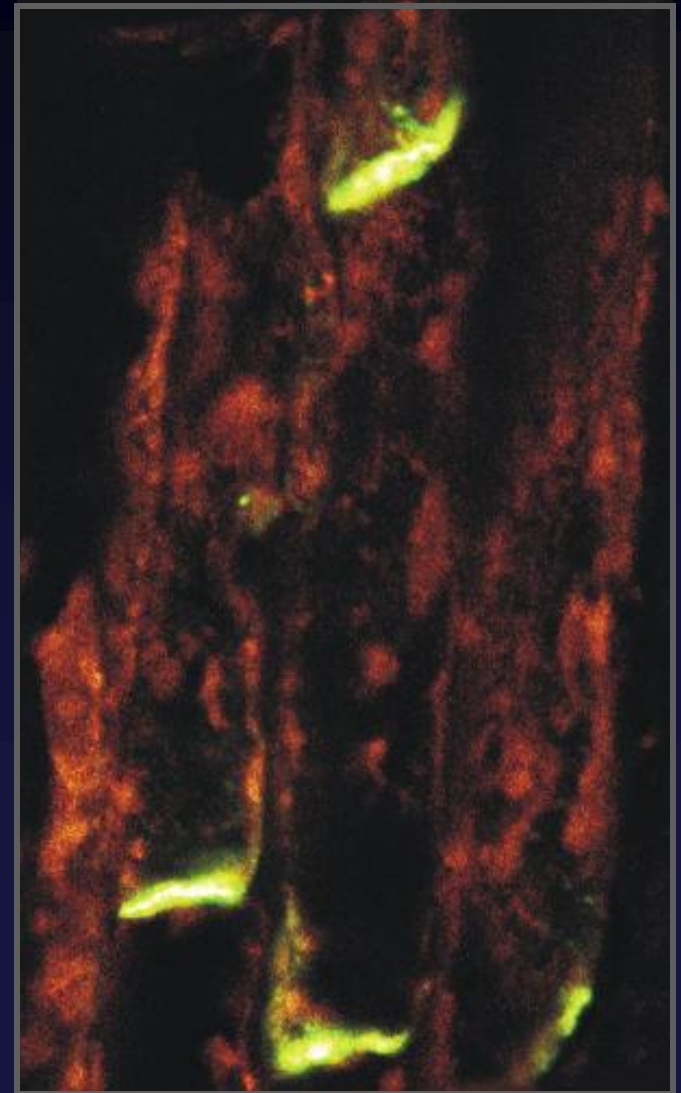


# PIN1 – the Auxin Efflux Carrier?

*pin1* mutant



PIN1 protein



# PIN2 – Root Gravotropism

PIN2 protein

Col-0



*pin2*

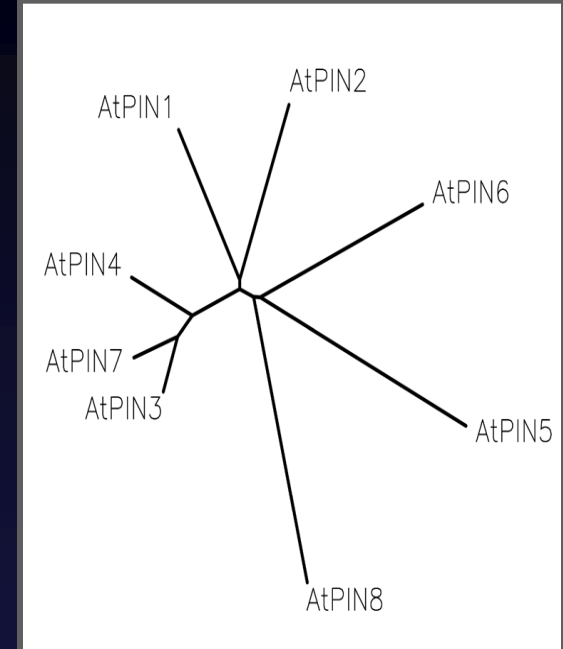


# The Arabidopsis PIN Gene Family

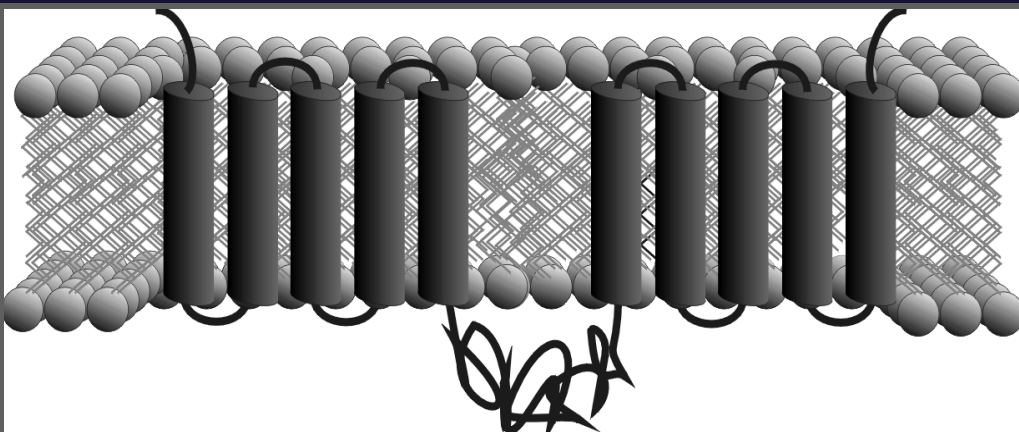
## Comparison of Arabidopsis PIN proteins

AtPIN1	1	MIEAASPTHTMEAVFLVAMILAYGSRVHKEETPDQCSGIRNFVALFAVPLLSFHFIAANNFYAMHLRFEAADSRQKVEVSESEF...ECKLSRNSD...EDMTLFLSLSLPLENTLY
AtPIN2	1	MIEGDMYDVLAAAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAVFAVPLLSFHFISNDPYAMMYHFLAADLQKVVILAAEF...GQAFSRRS...LEWMTLFLSLSLPLENTLY
AtPIN3	1	MISRHDLVTLTAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAIFAVPLLSFHFISTHNFYAMHLRFIAADLQKIMLSLVL...WAFNTRSS...LEWMTLFLSLSLPLENTLY
AtPIN4	1	MISRHDLVTLTAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAIFAVPLLSFHFISTHNFYAMHFRFAADLQKIMLSLVL...WAFNTRSS...LEWMTLFLSLSLPLENTLY
AtPIN5	1	MIEGDMYDVLAAAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAIFAVPLLSFHFISNDPYAMMYHFLAADLQKVVILAAEF...GQAFSRRS...LEWMTLFLSLSLPLENTLY
AtPIN6	1	MIEGDMYDVLAAAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAIFAVPLLSFHFISNDPYAMMYHFLAADLQKVVILAAEF...GQAFSRRS...LEWMTLFLSLSLPLENTLY
AtPIN7	1	MIEGDMYDVLAAAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAIFAVPLLSFHFISNDPYAMMYHFLAADLQKVVILAAEF...GQAFSRRS...LEWMTLFLSLSLPLENTLY
AtPIN8	1	MISRHDLVTLTAVFLVAMILAYGSRVHKEETPDQCSGIRNFVAIFAVPLLSFHFISTHNFYAMHLRFIAADLQKIMLSLVL...WAFNTRSS...LEWMTLFLSLSLPLENTLY
AtPIN1	116	MGIPLLKGMHNFSEDELWQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN2	116	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN3	116	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN4	116	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN5	116	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN6	116	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN7	116	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN8	121	MGIPLLRAMYGDPSGNLMVQIVVLCIWIYTLMLLFLFEEFGAKLLISEQFF...ETAGSITSEFRVDSVISENGREPLETDARIGDDGDKLHVVRSSNAASSMISEFNKSHGGGLNSSMIF
AtPIN1	229	RFSNLTNAEISYQSSRNPFPGSSFNHT...DFYS...MMASG...ENENFGE...GE...AVFCSKGFTEFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN2	235	RASNLTVEIYSVQSSREPFPRASSNQC...DFYA...MNASKAPSPRHGYNYSY...GG...AGAG...GGVVELQSKGVPEPTSNDEE...VMKTKAKRAGGRSMGGLYNNNSVP
AtPIN3	224	RFSNLTGAEIYSST...EPRGNFNIS...DFYN...MGFP...GG...RLSNFGE...ADMEYVQSSRGEPTFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN4	221	RFSNLTGAEIYSST...EPRGNFNIS...DFYS...MGFP...GG...RLSNFGE...ADMEYVQSSRGEPTFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN5	158	RFSNLTGAEIYSST...EPRGNFNIS...DFYS...MGFP...GG...RLSNFGE...ADMEYVQSSRGEPTFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN6	228	RASNLTNAEISYQSSRNPFPGSSFNHT...DFYS...MMASG...ENENFGE...GE...AVFCSKGFTEFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN7	227	RFSNLTGAEIYSST...EPRGNFNIS...DFYS...MGFP...GG...RLSNFGE...ADMEYVQSSRGEPTFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN8	169	RFSNLTGAEIYSST...EPRGNFNIS...DFYS...MGFP...GG...RLSNFGE...ADMEYVQSSRGEPTFRSNEYEDGGPAKPTAA...TAAGAGRFHYSGGGGGGG
AtPIN1	325	GAHYFAPNMGFSPNTGGGGGTAAGNAPV...VGGRRQDNGRDLHMFVSSSAPVSVF...GGGNNHADIATNDHQRDVKISVFGNS...DNQYVER
AtPIN2	342	ETFPENMFTG...STGASGVVKKESGGGGGGGGVGVGQN...KEMMFVSSSAPVSVF...ANAKNAMTRGSDVSDPKVSYPHD...NLATRAMONLNNMSPER
AtPIN3	305	AGHYFAPNMGF...STGTGVSTKP...NNTPE...NQQLQKDSNA...SDAKLWVSSSAPVSVF...GGAGDVATEQEG...GAEERMVSDQPR...SNRGGGDDIGG
AtPIN4	303	AGHYFAPNMGF...STGTGVSTKP...NNTPE...NQQLQKDSNA...SDAKLWVSSSAPVSVF...GGAGDVATEQEG...GAEERMVSDQPR...SNRGGGDDIGG
AtPIN5	192	LDVNGTPT...VWK...EFAAGR...TYRQSSPKMMWESGQRHAKNDNGSWEKEISFRDALRMAPQATAAGGG
AtPIN6	313	LDVNGTPT...VWK...EFAAGR...TYRQSSPKMMWESGQRHAKNDNGSWEKEISFRDALRMAPQATAAGGG
AtPIN7	308	LDVNGTPT...VWK...EFAAGR...TYRQSSPKMMWESGQRHAKNDNGSWEKEISFRDALRMAPQATAAGGG
AtPIN8	210	LDVNGTPT...VWK...EFAAGR...TYRQSSPKMMWESGQRHAKNDNGSWEKEISFRDALRMAPQATAAGGG
AtPIN1	423	EFSFGNKDDSKVL...ATDIGNNISNKT...QAKVHPTVMTKRLILIMVWRKLRNPYSSEFPT...SSEISFRWNIEMALAKSISILSDAGLGMAMFSLGLE
AtPIN2	443	IVEMDQDGN...GKSPYMKKGSVDVEGGPGRQV...VMTKRLILIMVWRKLRNPYSSEFPT...SSEISFRWNIEMALAKSISILSDAGLGMAMFSLGLE
AtPIN3	424	QFSPFAGKEEAEKPKDAENGLKLAPNSALEQSKTGLGGAEASQRKNMPPASVMTKRLILIMVWRKLRNPYSSEFPT...SSEISFRWNIEMALAKSISILSDAGLGMAMFSLGLE
AtPIN4	407	LDSDGEGEREIK...ATAGLNMGNSALEAAGDGGGNGC...THMPTVMTKRLILIMVWRKLRNPYSSEFPT...SSEISFRWNIEMALAKSISILSDAGLGMAMFSLGLE
AtPIN5	192	VMSLWKLKATNPNCYSCILGIAMAFIENRHHLEGLLEGSLIMBRKASTTAMFNMBIF
AtPIN6	381	ASMEGAAKKT...TPVRAIG...KQMEGRIWMBELT...YCKLGNPNYSSELGIWVHSIF...EIPENVDFEIK...IADAGLGMAMFSLGLE
AtPIN7	411	EEESRNVKEVPHLHKLKCSNLELNPKEALETGETVPPKMPFASVMTKRLILIMVWRKLRNPYSSEFPT...SSEISFRWNIEMALAKSISILSDAGLGMAMFSLGLE
AtPIN8	210	LLKAWKRLINPNYATLIGLIWTLHFLGLWNLEEMDKSILHLSGGLGMAMFSLGLE

## Phylogenetic tree



## Membrane topology model

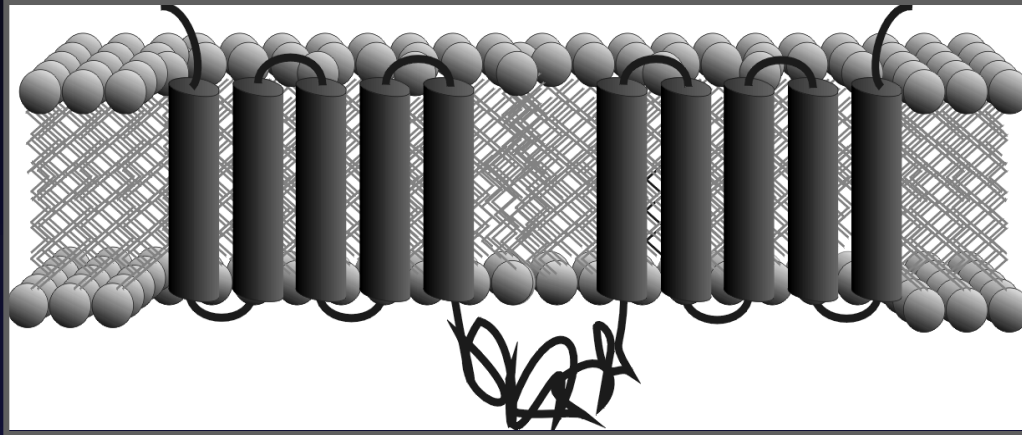


What is Molecular Role  
of PIN Proteins  
in Auxin Transport?

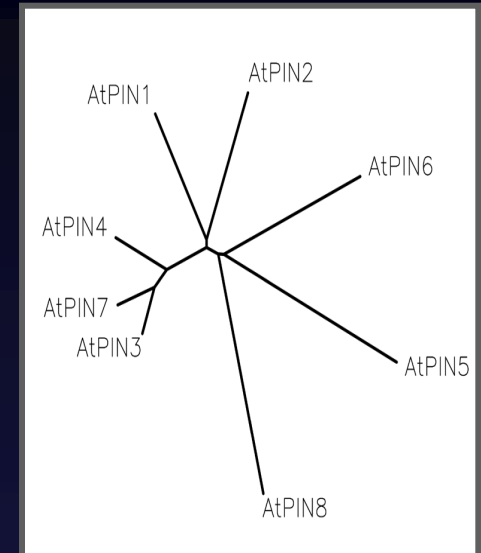


# PINs Are Essential Components of Auxin Transport

## Putative topology of PIN proteins



## Phylogenetic tree

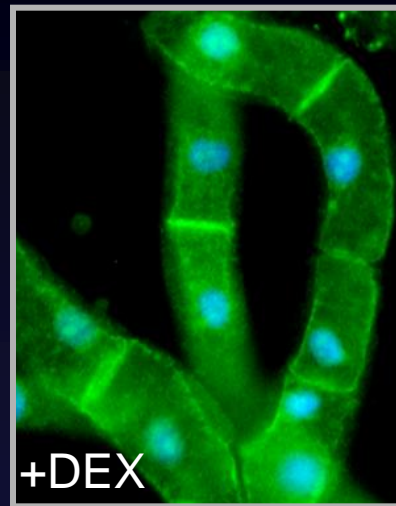
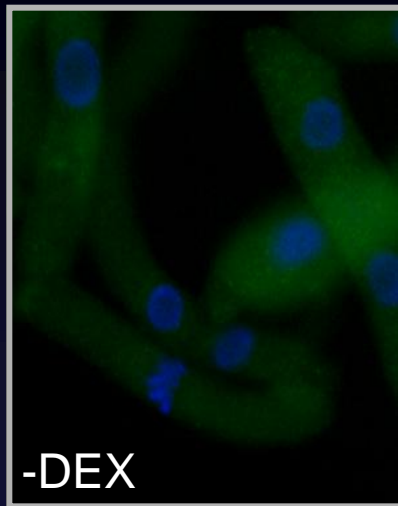


- All defects in *pin* loss-of-function mutants are in auxin transport-dependent processes and can be phenocopied by auxin transport inhibitors
- Local auxin distribution (gradients) are affected in *pins*
- Polar PIN localization determines direction of auxin flow

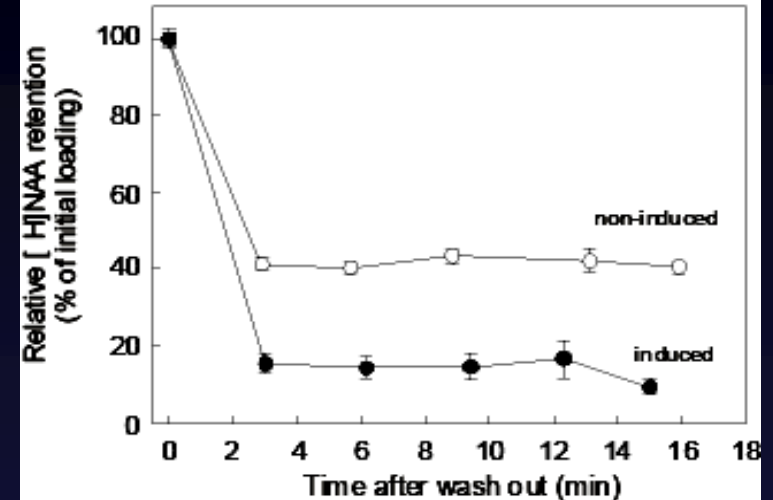


# PINs Are Rate-limiting Factors in Auxin Efflux

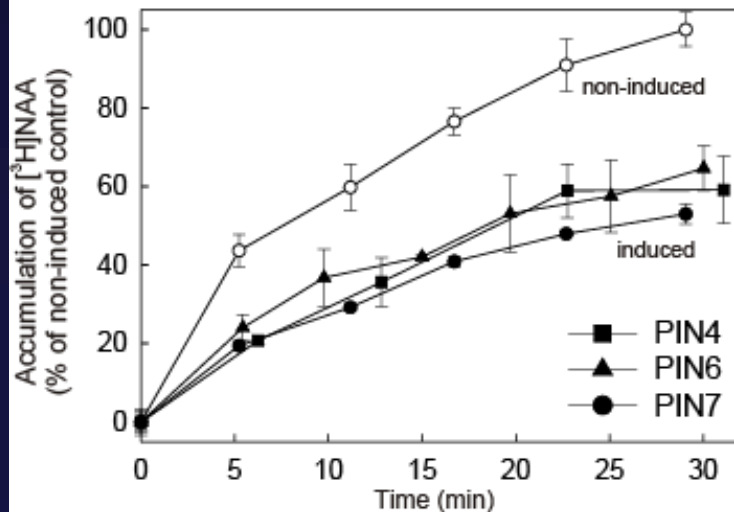
## Inducible PIN1 expression



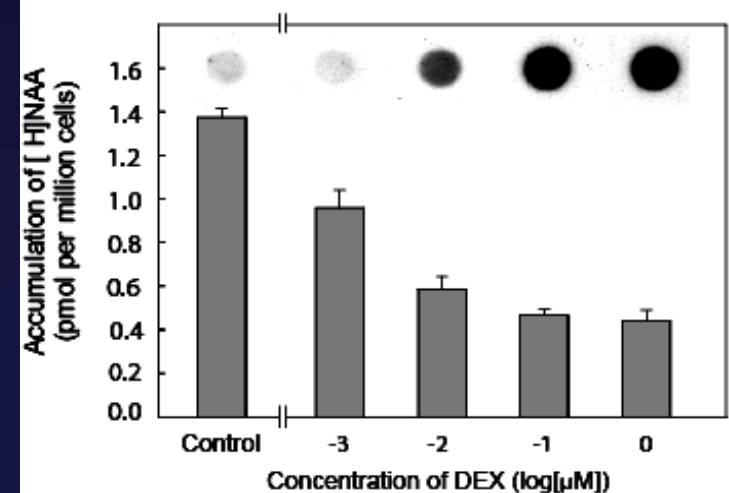
## PIN-dependent auxin efflux from GVG-PIN7 tobacco cells



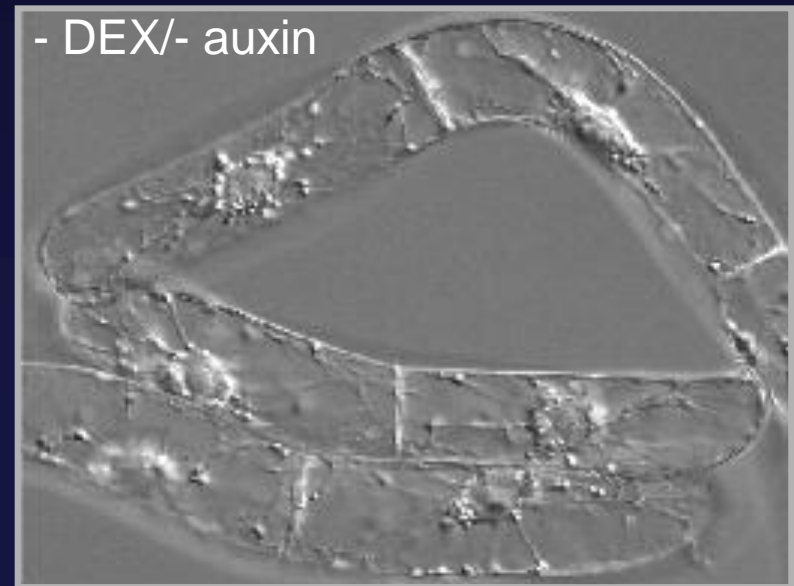
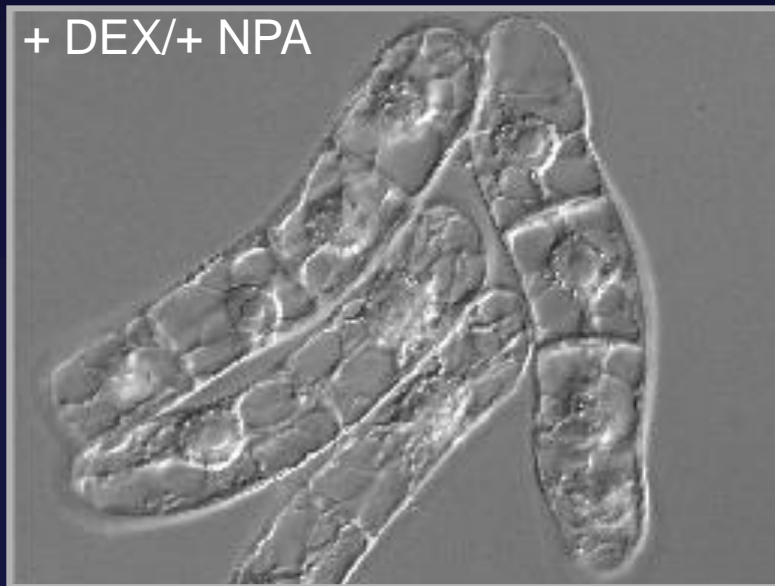
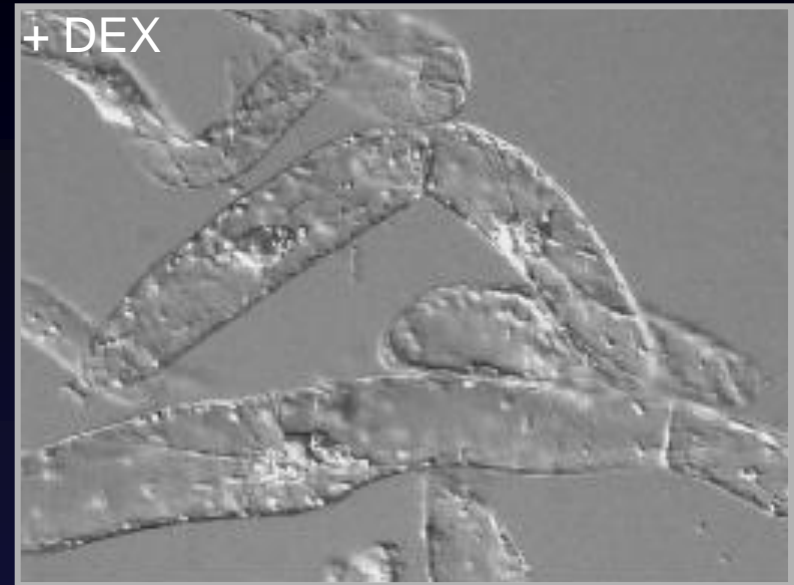
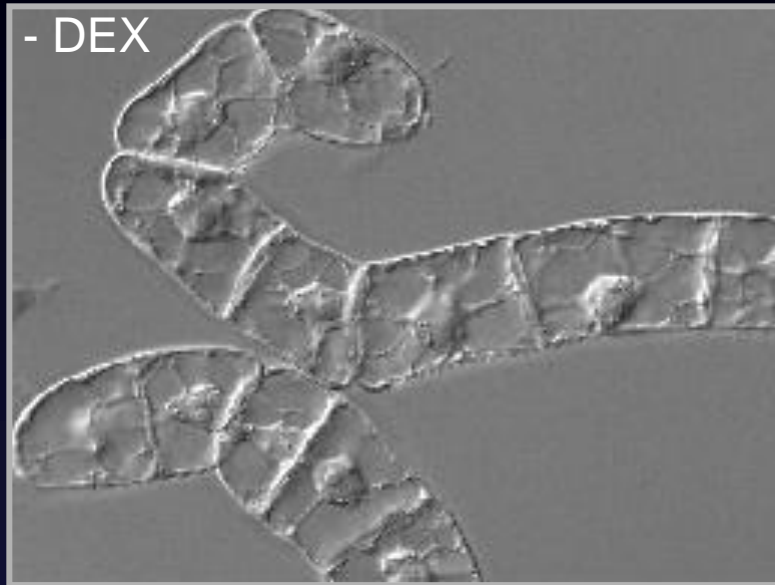
## NAA accumulation kinetics



## [<sup>3</sup>H]NAA accumulation in GVG-PIN7 tobacco cells in relation to DEX concentration

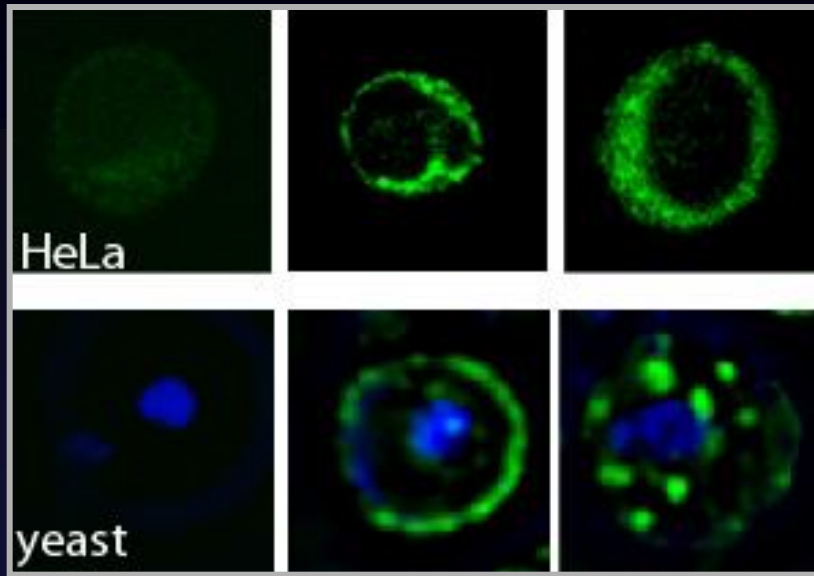


# PIN-induced Phenotypes in BY-2 Cells

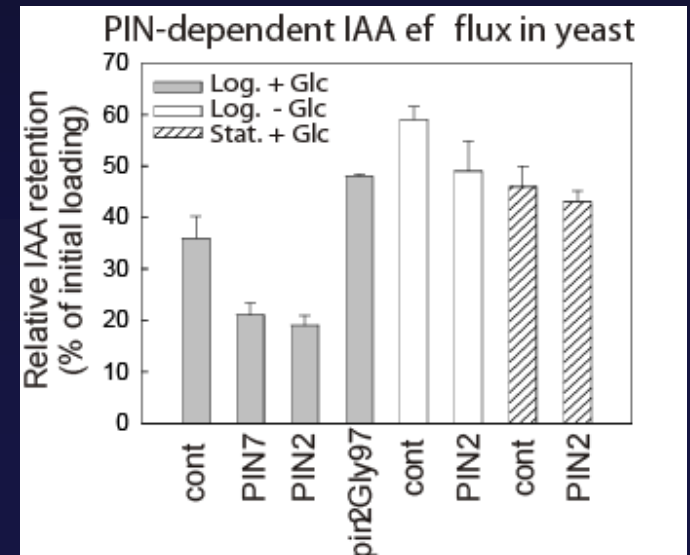
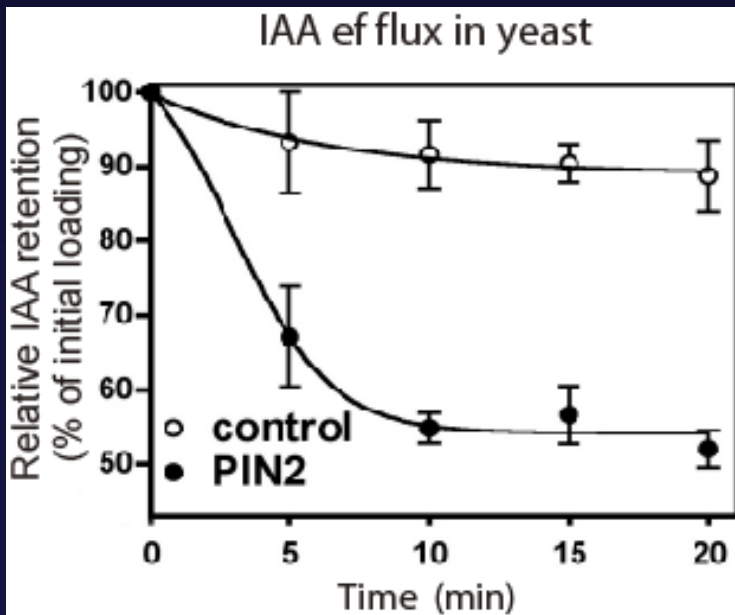
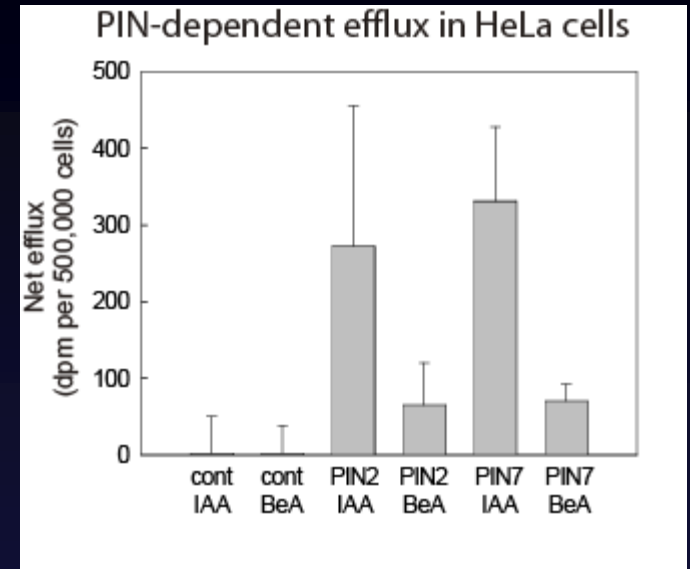


# Expression of PINs in HeLa and Yeast

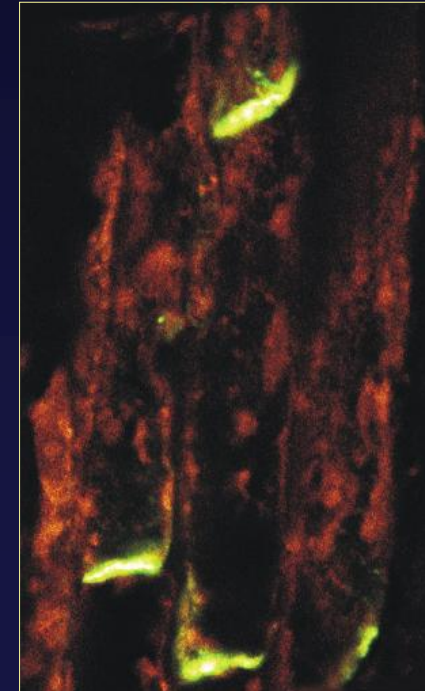
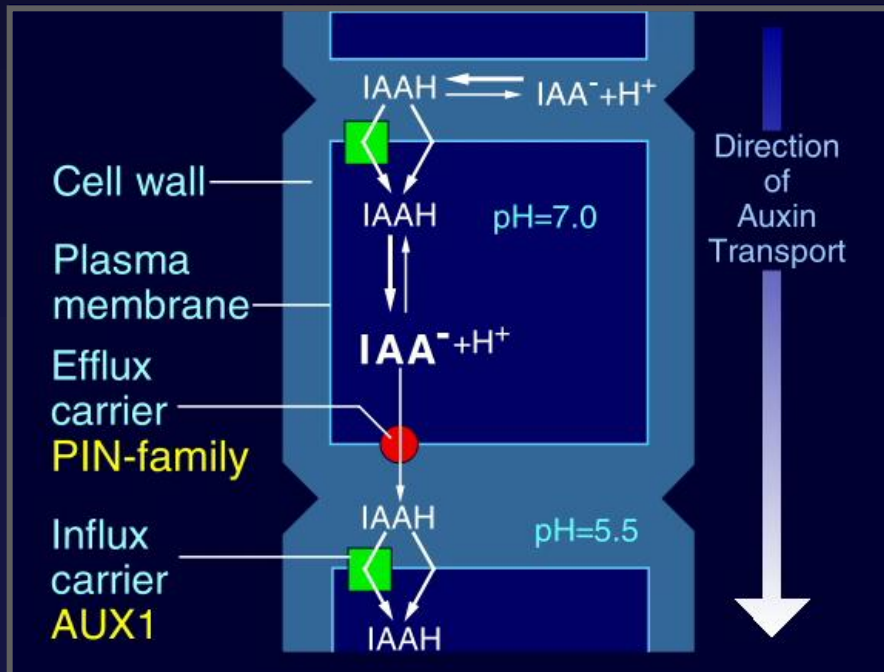
Heterologous PIN2 expression



auxin efflux activity



# Cellular Polarity of PIN Localization and Directionality of Intercellular Auxin Flow



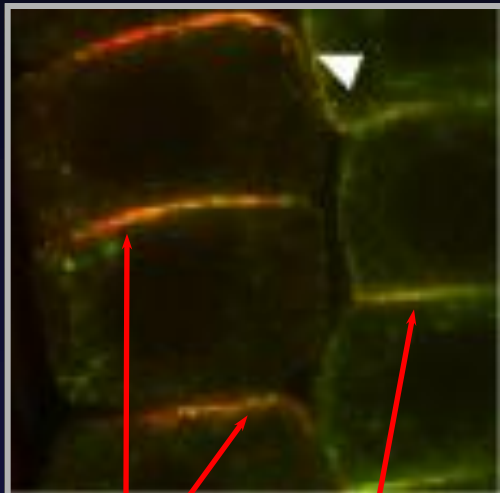
# PIN-specific Signals for Polar Targeting

*PIN2pr::PIN2:HA*

*PIN2pr::PIN1:HA*

*PIN2pr::PIN1:GFP*

*PIN1/PIN1:GFP*



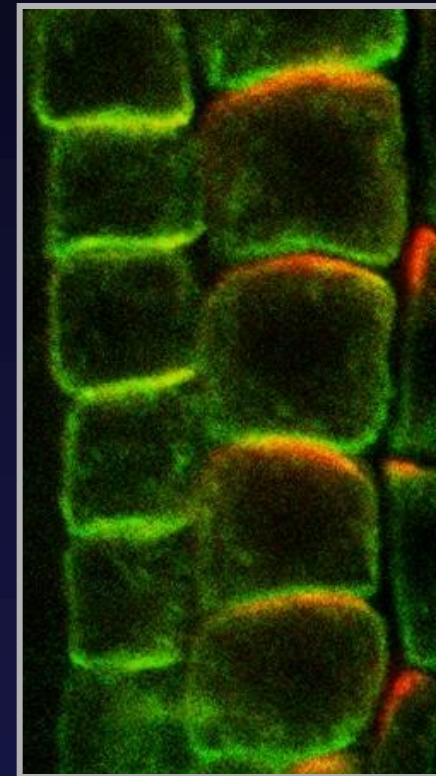
apical  
basal  
localization



basal  
localization



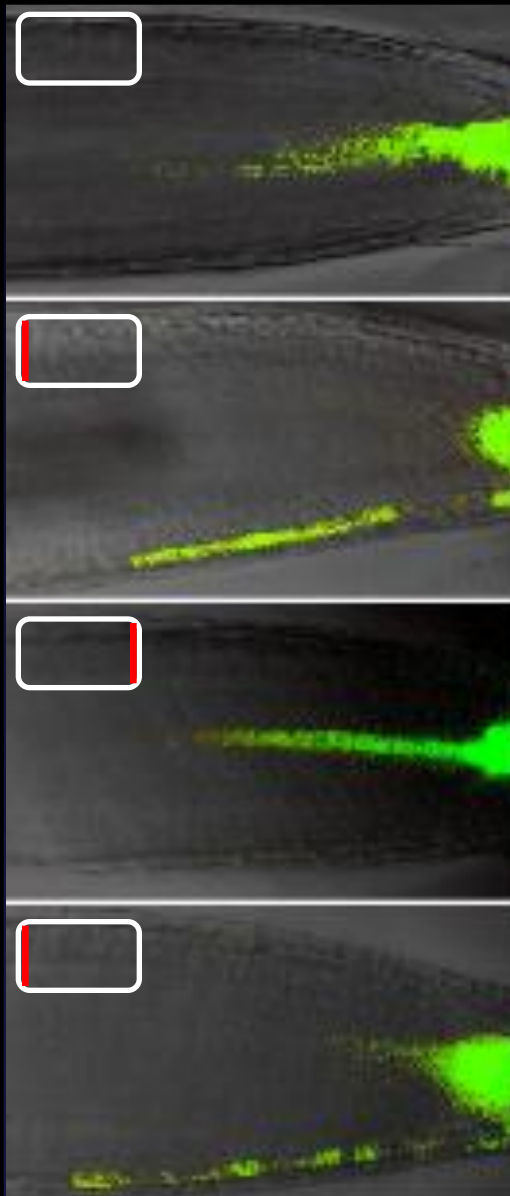
apical  
basal  
localization





# PIN Polarity Determines Direction of Auxin Flow

*DR5rev::GFP*



*pin2 (eir1, agr1)*

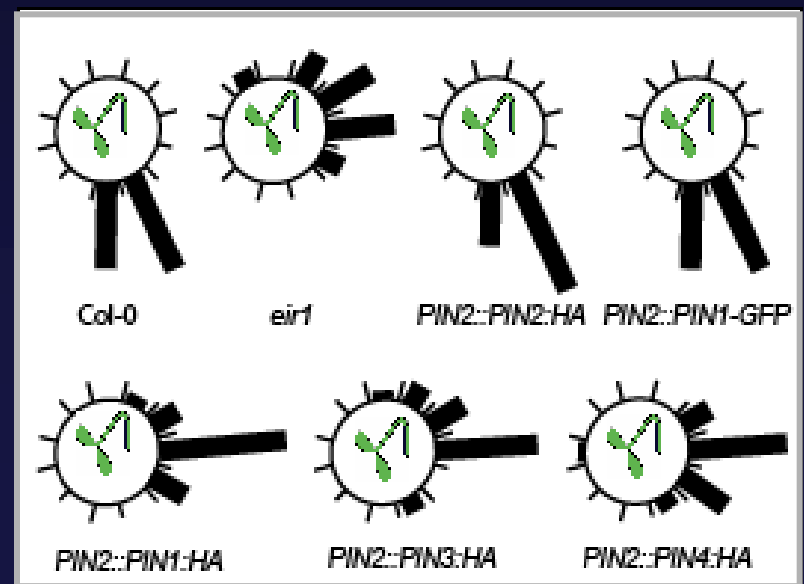
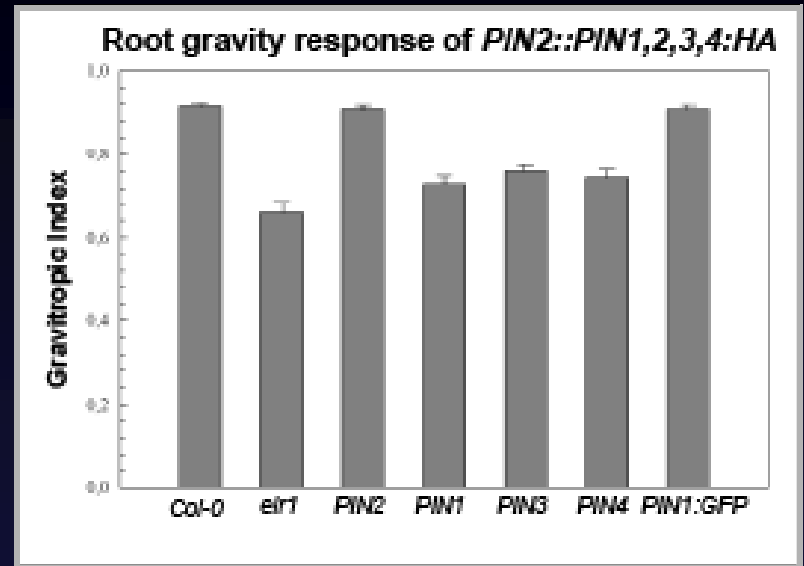
*PIN2::PIN2:HA*

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

*PIN2::PIN1:GFP-3*

## of Auxin Flow

*gravitropism*



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

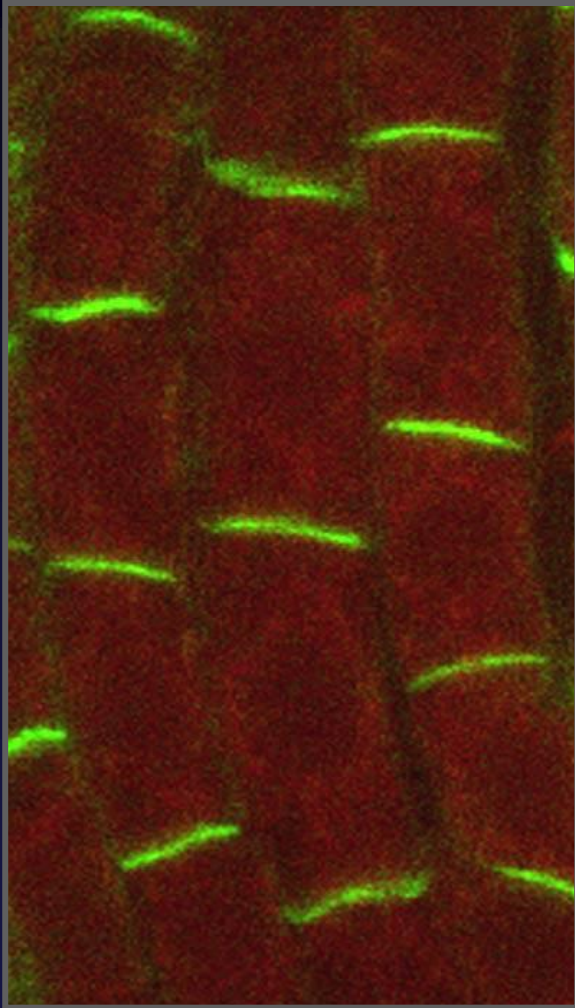
and

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

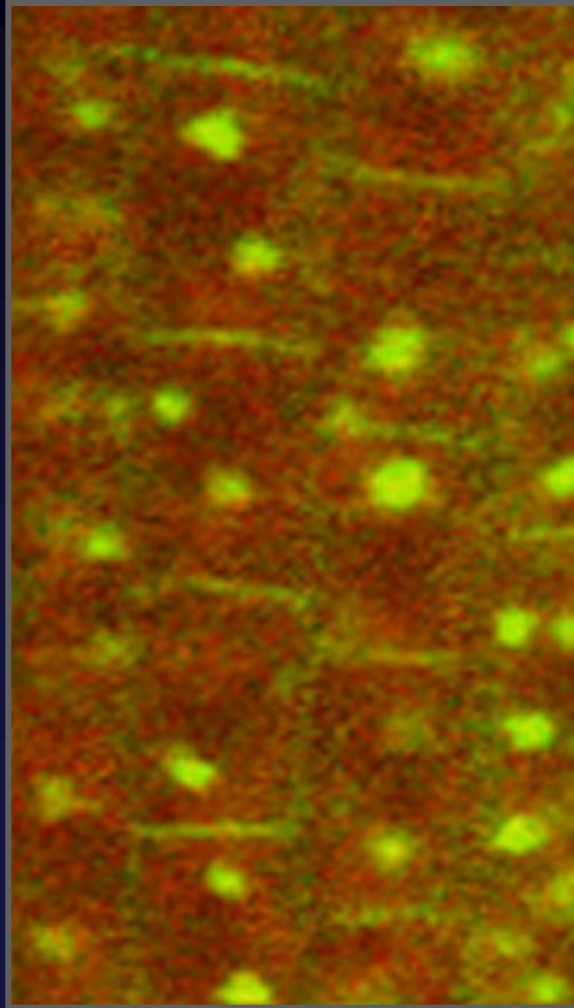
# Constitutive Cycling of PINs

# PIN1 Subcellular Movement

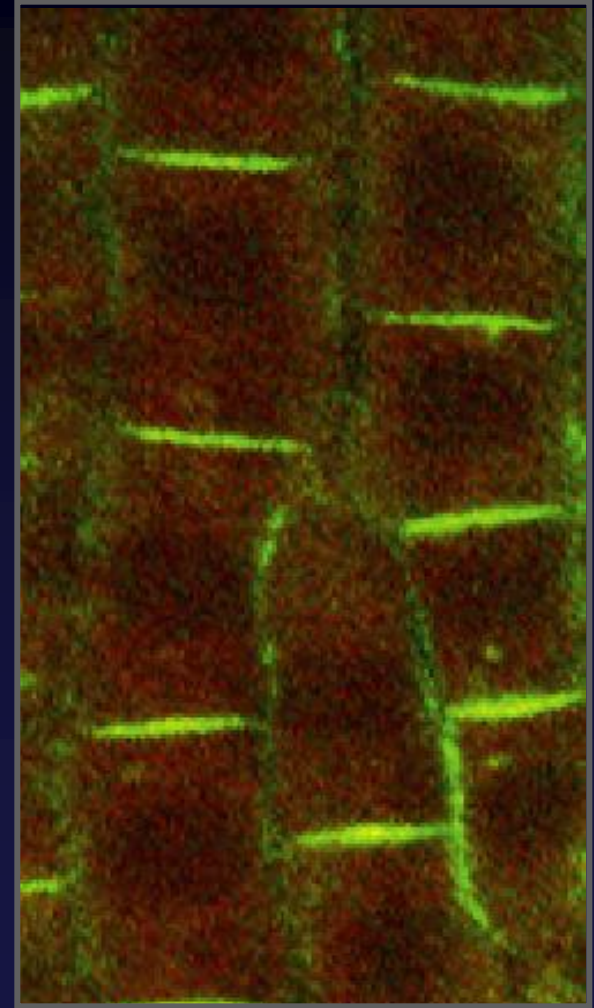
untreated



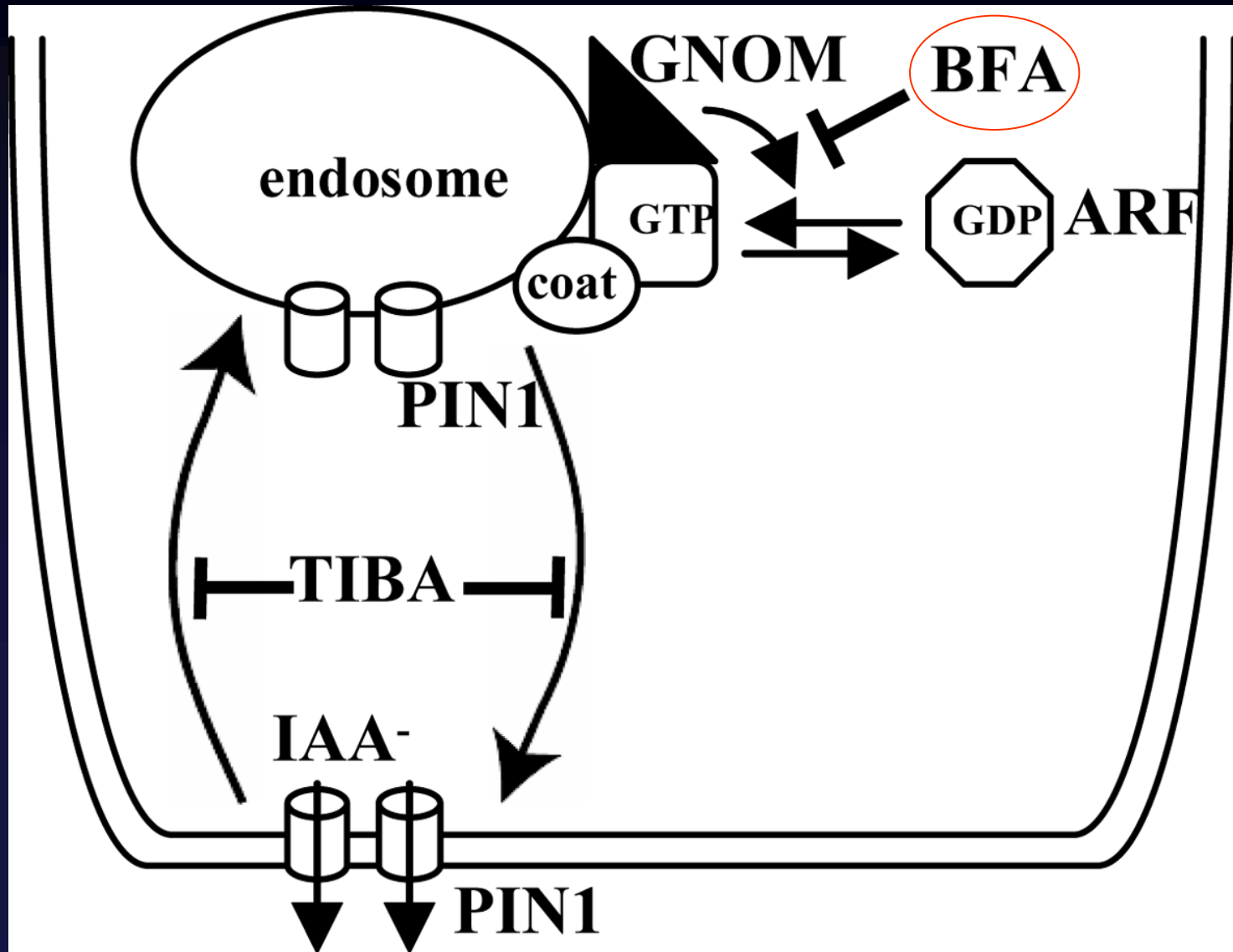
+ BFA



- BFA

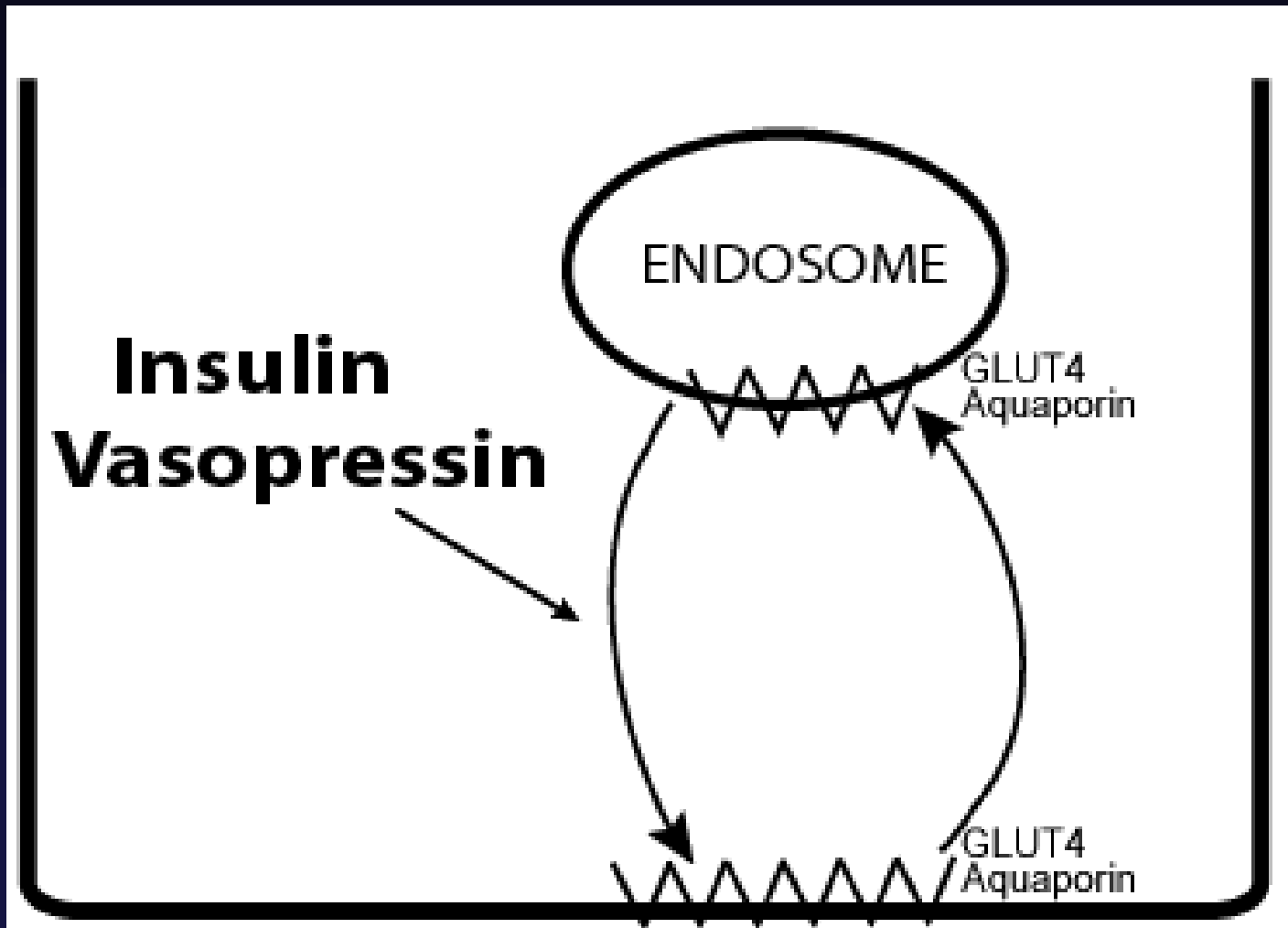


# Dynamic Movement of PIN Proteins



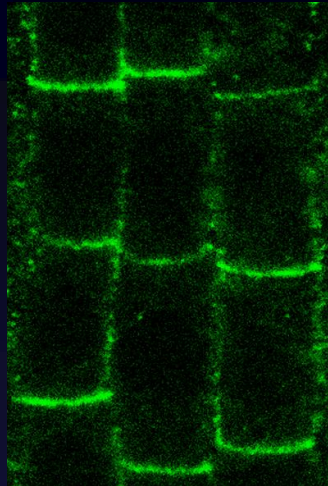


# Subcellular Cycling – Means to Modulate Protein Activity?

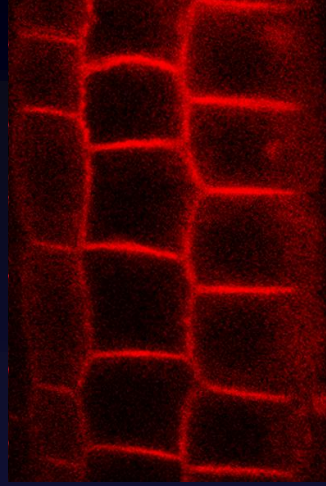


# Auxin Inhibits Internalization of Plasma Membrane Proteins

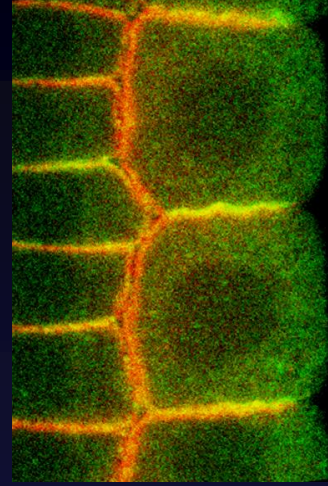
NAA  
/BFA



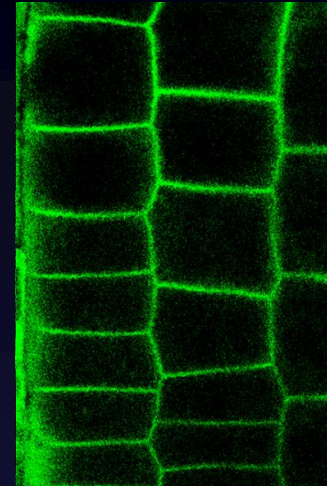
PIN1



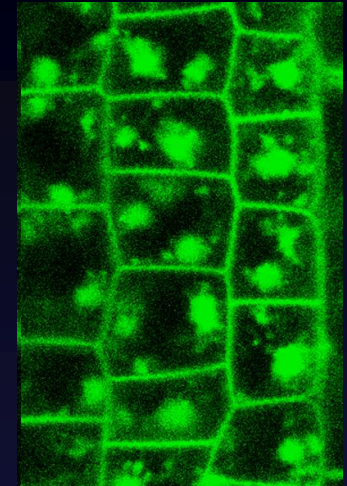
PM-ATPase



PIN2/ATPase

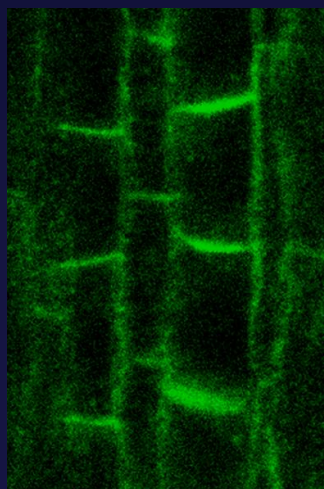


PIP2

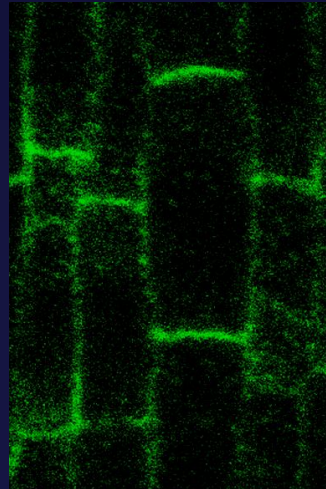


BRI1

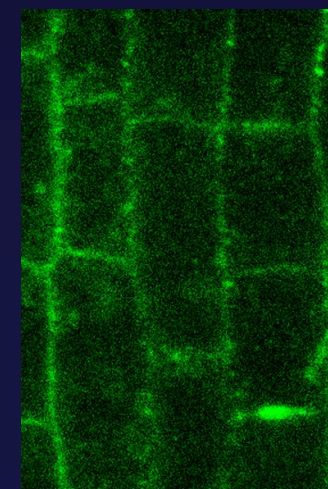
IAA/BFA



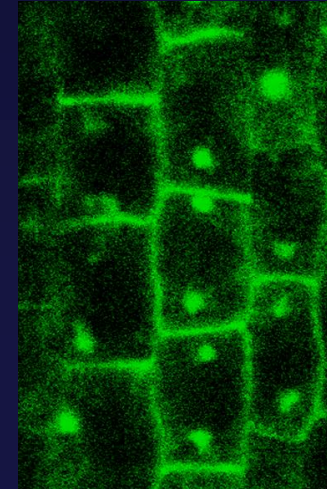
2,4-D/BFA



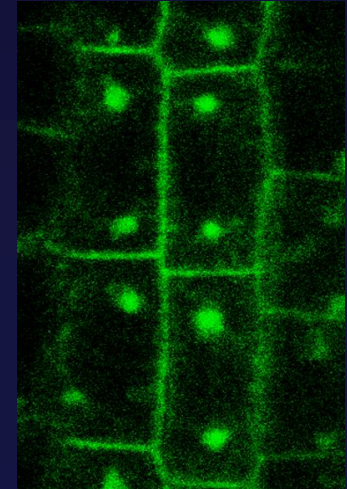
BFA in *sur2*



2-NAA/BFA



Ethylene/BFA

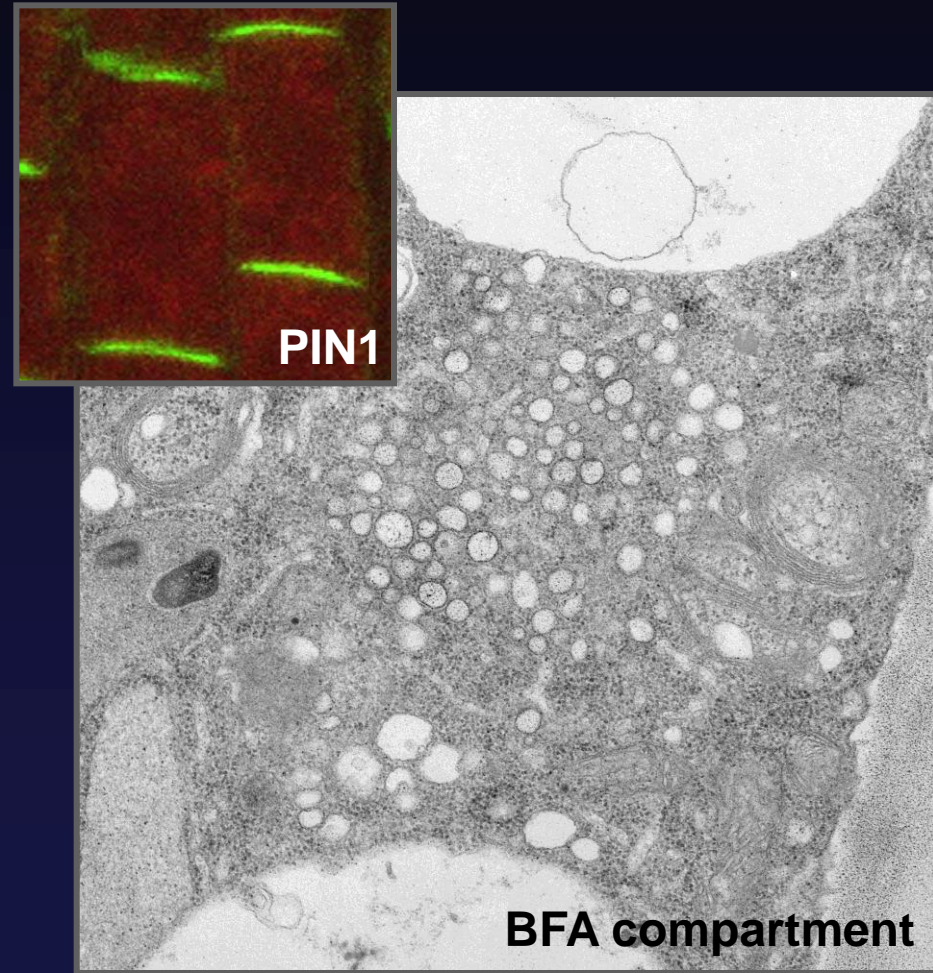
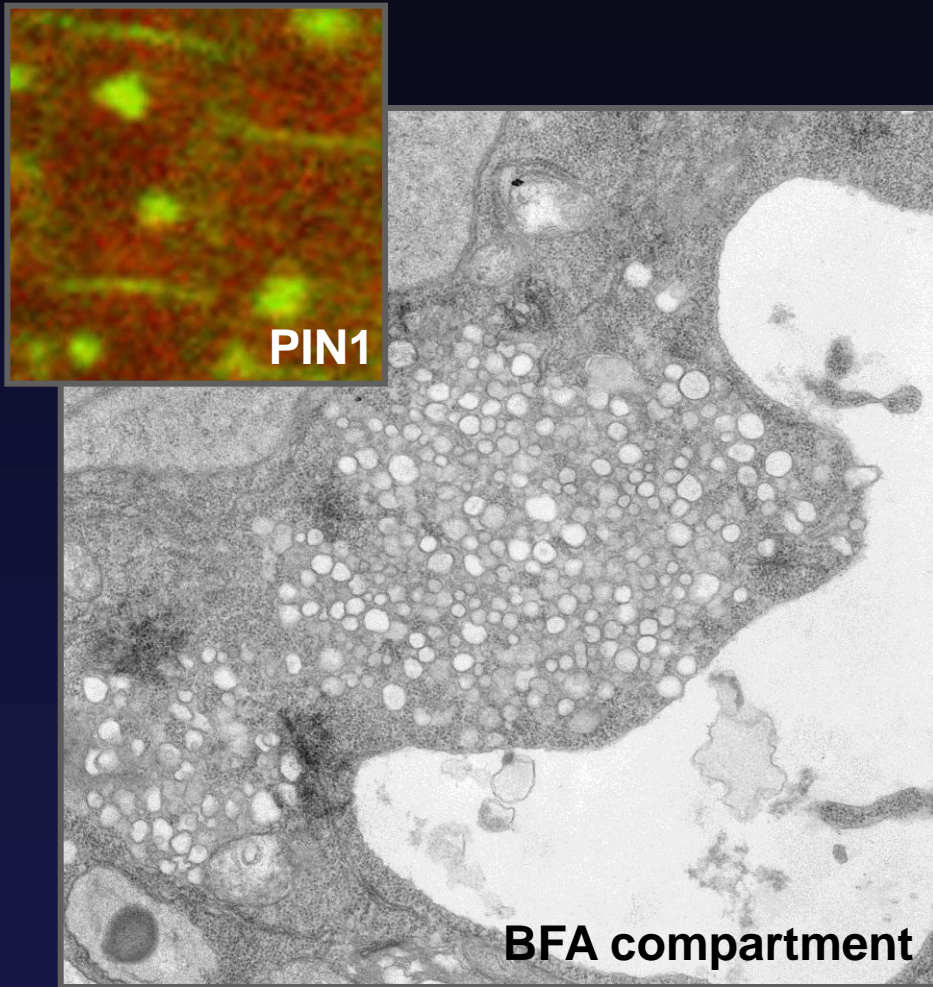




# Place of Auxin Action in Protein Cycling

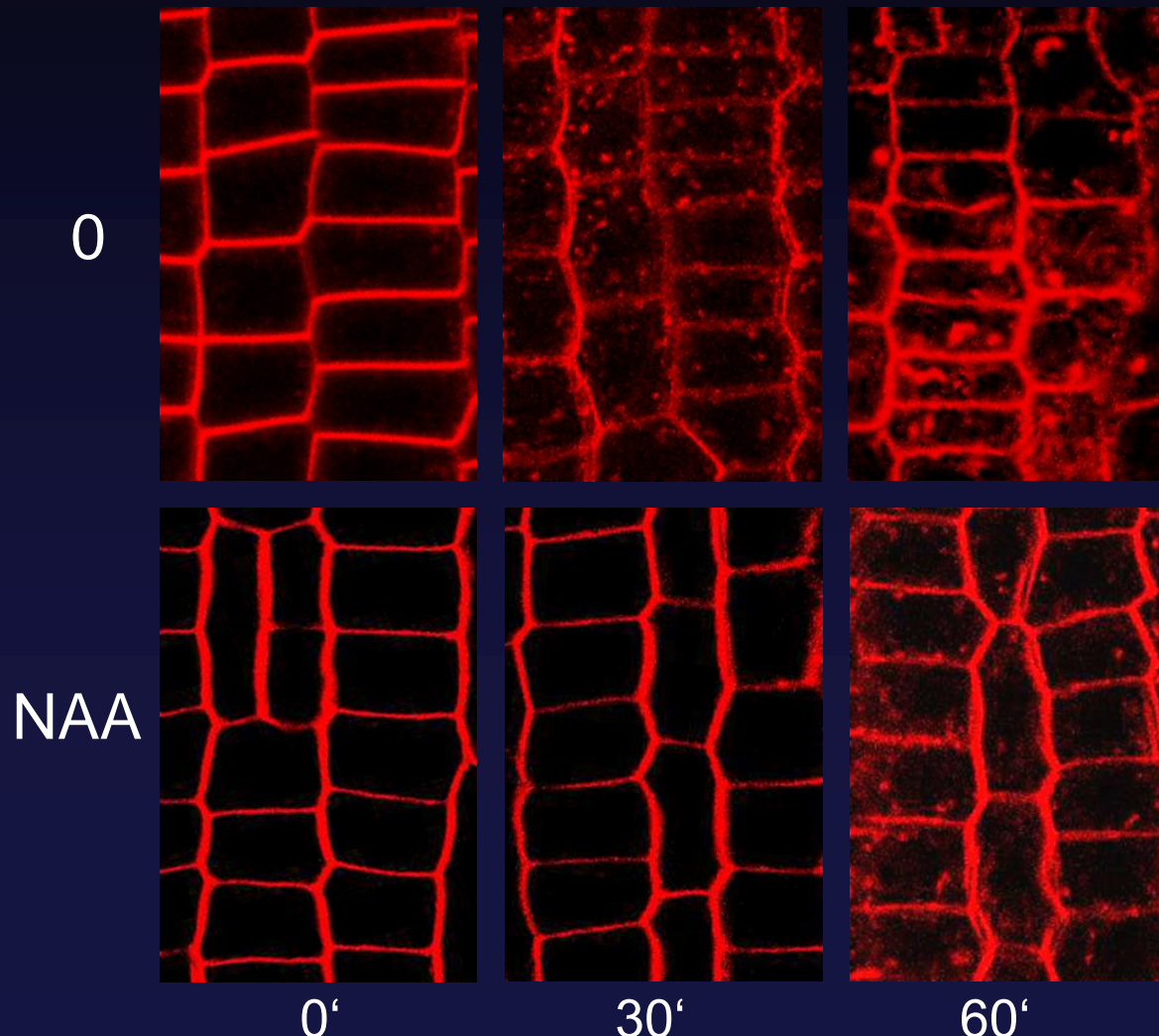
**BFA**

**Auxin + BFA**



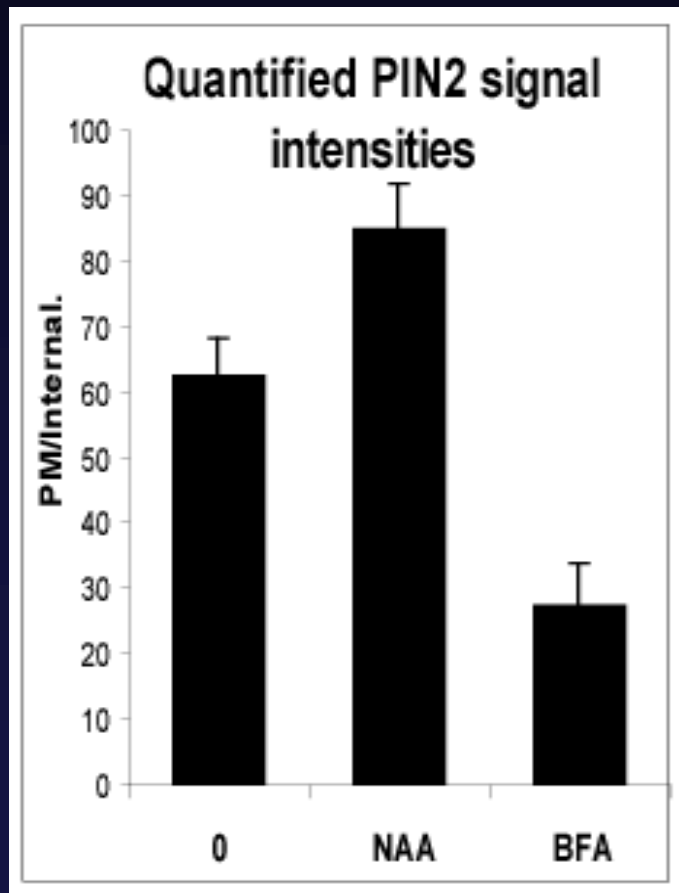
# Auxin Inhibits Endocytosis

Uptake of endocytic tracer FM4-64

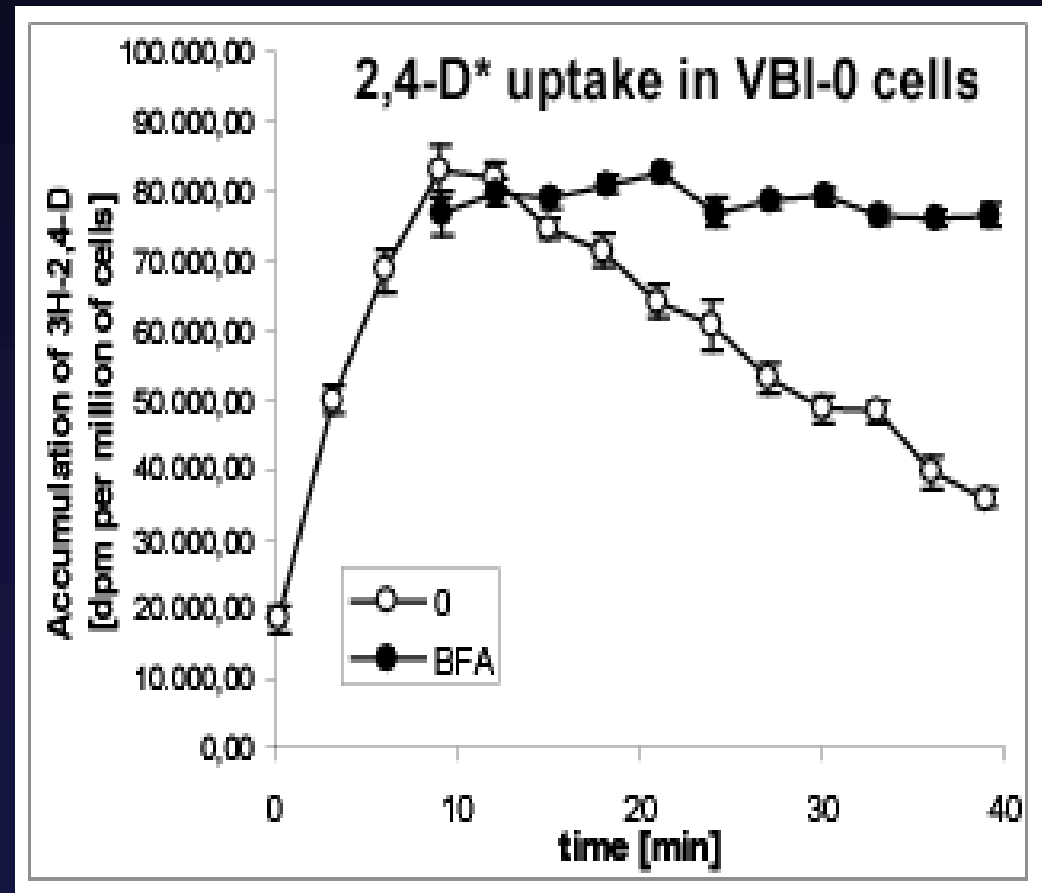


# Auxin Increases PIN Levels at Cell Surface and Stimulates its own Efflux

PIN2 levels at PM

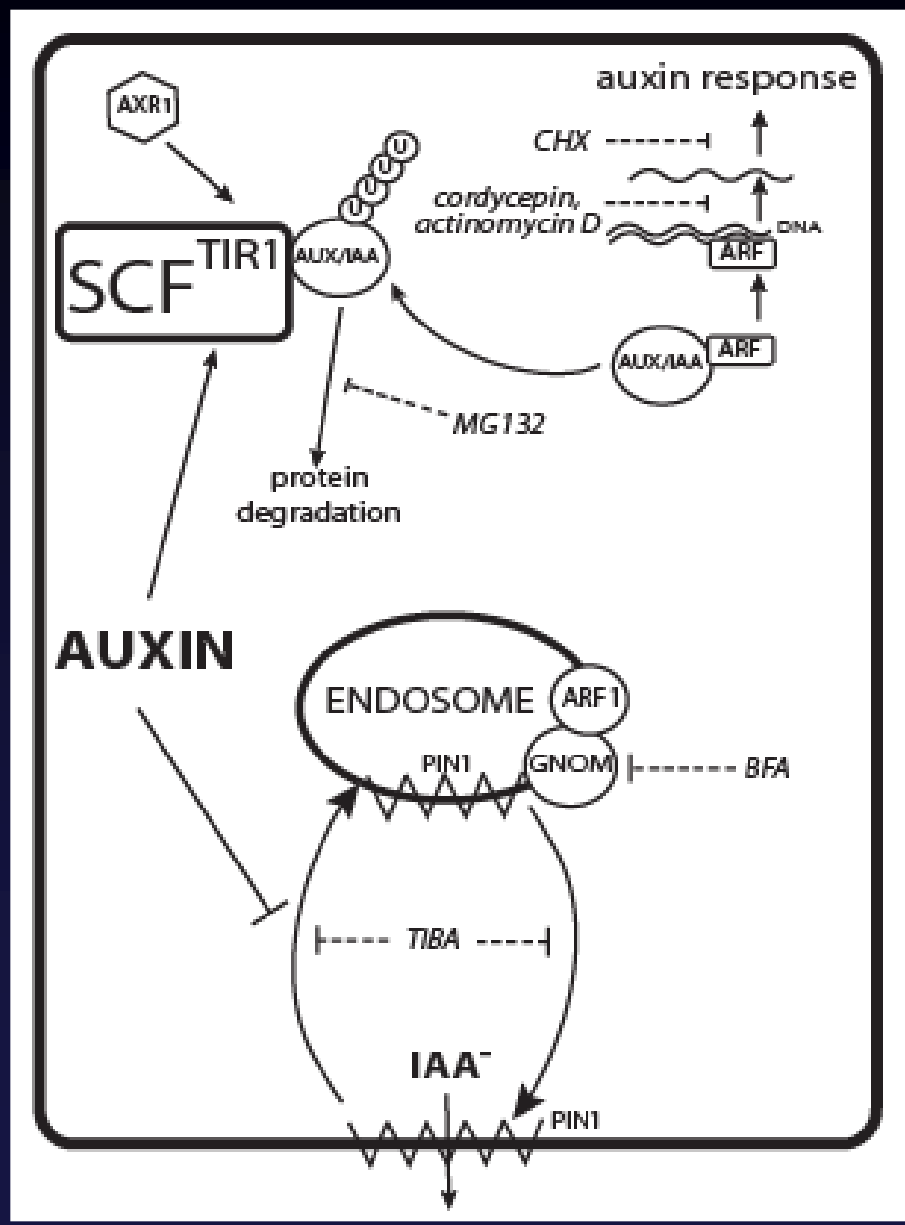
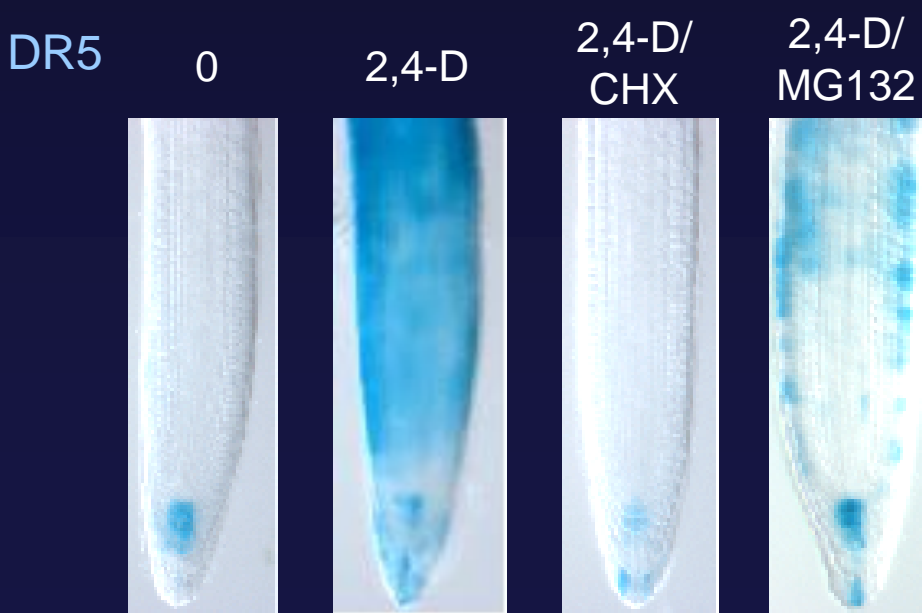
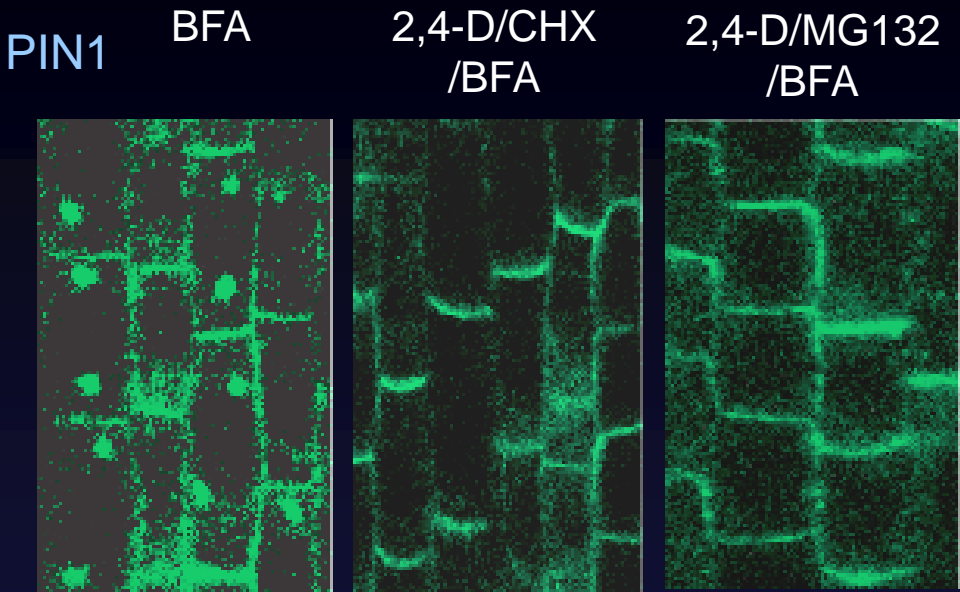


Auxin efflux in tobacco cells

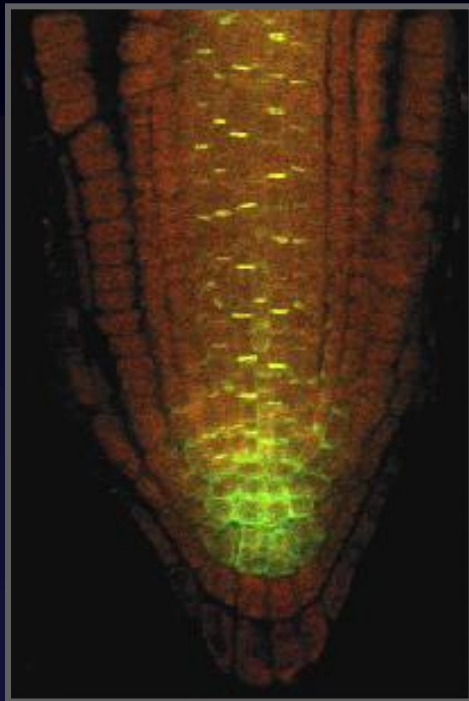




# Novel Pathway of Auxin Action



# Mutant Screen for Components of PIN Polarity and Cycling



**PIN:GFP**

EMS mutagenesis.  
Screening for  
polarity and cycling  
defects.

**mutant lines**

**intragenic**

sequencing

**important  
residues**

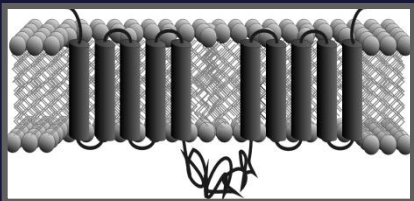
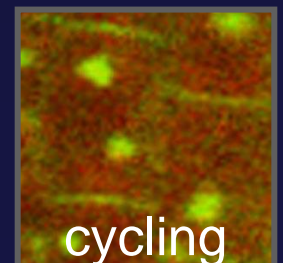
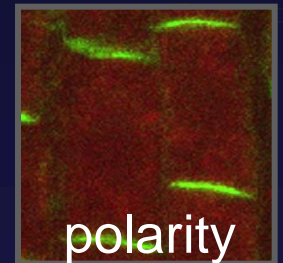
**extragenic**

cloning

**novel genes**

polarity

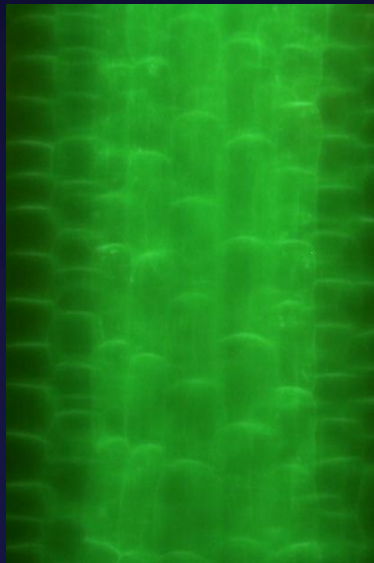
cycling



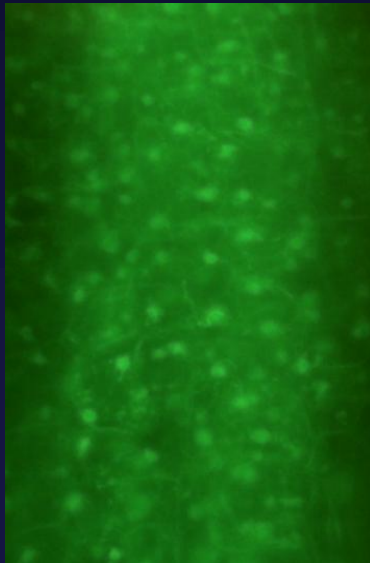
# “Cell Biological” Mutant Screens in Progress:

**Auxin effect on endocytosis: 3 confirmed mutants**

30' NAA 30  $\mu$ M/90' BFA 50  $\mu$ M

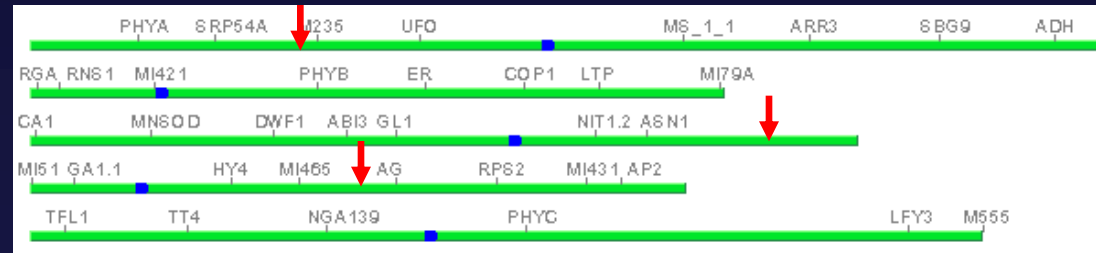


wt



mutant

*Auxin-resistant BFA patches mutants*



# 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